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Sub-tomogram Averaging in RELION

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Electron cryo-tomography (cryo-ET) and sub-tomogram averaging allow structure determination of macromolecular assemblies in their natural microenvironment e.g. in reconstituted biochemical systems, inside viruses, in cellular organelles or within whole cells [1-3]. Sub-tomogram averaging is also gaining in popularity to provide initial models for single-particle analysis.

We have developed a sub-tomogram averaging algorithm for processing cryo-ET data using a regularized likelihood optimization approach [4] in the RELION software [5, 6]. The aim of this conference paper is to introduce our sub-tomogram averaging approach and highlight some recent results that we have obtained.

First, we will give an overview of the sub-tomogram averaging algorithm within RELION. This algorithm uses a novel three-dimensional (3D) model (Figure 1) to compensate for the contrast transfer function (CTF) and the missing wedge of each sub-tomogram for macromolecular structural refinement from cryo-ET data. This 3D CTF model is reconstructed by successively placing the 2D CTF of each image in the tilt series into a 3D volume. Using known information about data collection parameters used at the microscope, this 3D CTF model takes into account the increase of noise in images collected at higher tilts due to increased effective ice thickness, as well the destruction of the higher resolution information in images as the specimen is irradiated during data acquisition.

As example applications, we present analyses of purified hepatitis B capsid particles and *S. cerevisiae* 80S ribosomes. In both cases, we show that initial classification of the data may be conveniently conducted by projecting each sub-tomogram onto a 2D image followed by 2D classification of the projected images. This classification allowed us to select the best data, which was used for *de novo* generation of initial models (Figure 2A-B) by sub-tomogram averaging.

Next, we used the obtained initial models to conduct refinements with differing parameters. We found that using the 3D CTF model significantly improved the final resolution obtained compared to refinements where this model was not used. The obtained resolution was further improved by using the tilt-dependent and the dose dependent weighting in the 3D CTF model.

Finally, using the best selected data and the optimally weighted 3D CTF model we obtained high-resolution maps of both our test specimens where secondary structure elements were resolved (Figure 2C-D) and where higher resolution atomic structures could be unambiguously fitted into the sub-tomogram averaging maps.

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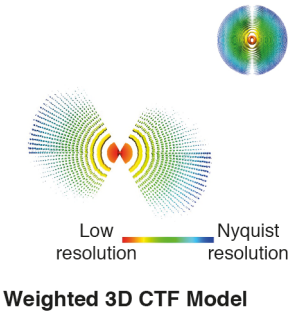

Figure 1. A combined model for the CTF and missing wedge.
A slice through the combined model for the CTF and missing wedge used in RELION for sub-tomogram averaging. This model is weighted according to the actual tilt of the stage and the dose applied on the specimen. The model is defined in reciprocal space - low resolution is red while high resolution is blue. Inset – the 3D CTF model in an orthogonal orientation.

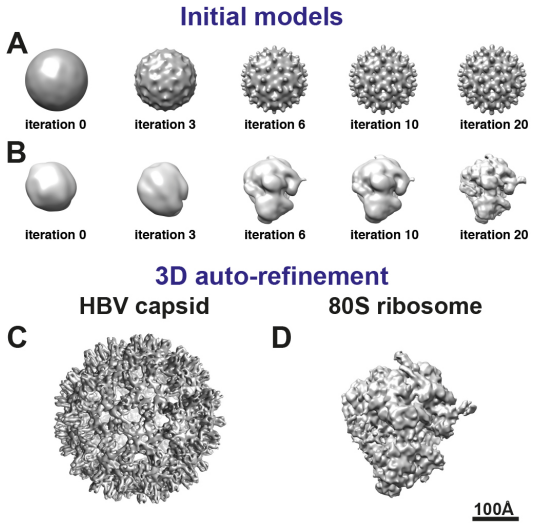

Figure 2. Initial model generation and automatic refinement using RELION.
(A,B) Generation of initial models of Hepatitis B virus (HBV) capsid particles and *S. cerevisiae* 80S ribosomes. (C) Final refined ~9 Å resolution map of the HBV capsid. (D) Final refined ~13 Å map of the 80S ribosomes.

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