

Single Particle Reconstructions at Subnanometer Resolution from a JEOL 2010F and a 4k x 4k Gatan CCD Camera

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Charge coupled device (CCD) cameras have been widely used in electron microscopy. The modulation transfer function (MTF) of CCD cameras and their smaller imaging area relative to film have limited their routine use for high resolution data collection of ice embedded biological specimens [1]. However, the performance specifications of the current generation of 4kx4k CCD cameras (GatanUS4000) indicated the feasibility for solving structures using electron cryomicroscopy (cryo-EM) and image reconstruction techniques to least 8-10 Å resolution[2]. During our characterization of this CCD camera at 200 kV we made the prediction that usable information could be retrieved up to 2/5 Nyquist frequency[2] (Figure 1, Table 1).

Here we present three reconstructions from single particle cryo-EM and image reconstruction techniques. The first is the reconstruction of Cytoplasmic Polyhedrosis Virus (CPV). Imaging of ice embedded CPV was done at a nominal magnification of 60,000 X. The reconstruction was assessed at 9 Å using the 0.5 threshold of the FSC generated between two half datasets. This resolution assessment was confirmed through the identification of a number of clear sausage like densities indicating alpha helices. The second is the reconstruction of GroEL. Imaging of ice embedded GroEL was done at a nominal magnification of 80,000X. The final resolution was assessed at 7-8 Å resolution, and confirmed by identification of alpha helices and a number of putative beta sheet regions in the final reconstruction. The final reconstruction is that of the Ryanodine Receptor (RYR1). Imaging of ice embedded RYR1 particles was done at a nominal magnification of 60,000X. The final resolution was assessed at 9.5 Å, and though not complete, we have begun to identify alpha helices in this reconstruction.

The current 2/5 Nyquist frequency limit is one which seems to be independent of the algorithm or software package used to perform the particle classification and 3D reconstruction [3, 4]. Although there does appear to be data beyond 2/5 Nyquist frequency, as assessed by looking at the spectral signal to noise ratio (Figure 1), to date it has been exceedingly difficult to recover, unlike film where 2/3 Nyquist is typical. A combination of approaches is going to be necessary to reach higher resolution. Detectors with better noise characteristics, better point spread functions, and finally better use of electron optics to give the highest spectral signal to noise ratio possible for a given specimen at a given dosage. All of these approaches are currently under development at the NCMI in our efforts to reach higher resolution structures.

References:

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Figure 1: Example of the 1D Power Spectrum Used to Calculate the Performance of a given Microscope/CCD combination. This plot was generated from an image of amorphous carbon film at 60,000 X Magnification at a dosage typical for imaging ice embedded biological specimens. Dotted line is data collected using film, solid line is data collected on CCD.

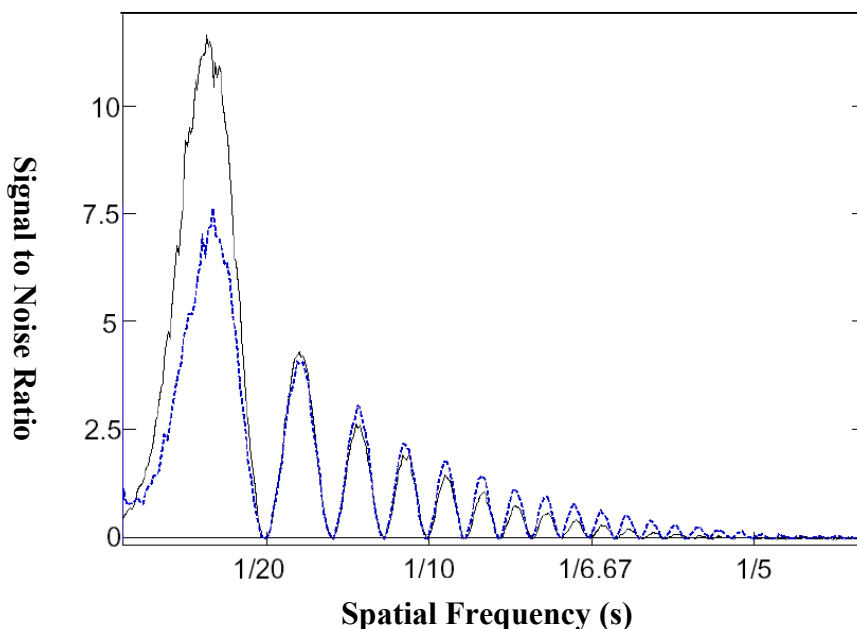


Table 1: A Comparison Between The Nominal Microscope Magnification, CCD Camera Field Of View, and the Expected Resolution On The 4kx4k Gatan CCD Camera At 200 kV with 15 um pixels.

Nominal Microscope Magnification	Magnification On CCD	Å/pixel	Imaging Size on Specimen (nm)	2/5 Nyquist Resolution Limit(Å)
40,000	55,200	2.71	1,110	13.6
50,000	69,000	2.17	886	10.8
60,000	82,800	1.81	738	9.0
80,000	110,400	1.35	554	6.8
100,000	138,000	1.08	443	5.4
150,000	207,000	0.72	295	3.6

Table 2: Comparison of Reconstruction Statistics between Several Different Ice Embedded Single Particles Collected On the Gatan 4kx4k CCD at 200 kV at the Indicated Nominal Magnification

Complex	Number Of Particles	Nominal Microscope Magnification	Expected Resolution (Å)	Final Resolution (0.5 FSC cutoff, Å)	Software Package For Reconstruction
CPV	5,000	60,000	9	9	SAVR
GroEL	8,000	80,000	6.8	7-8	EMAN
Ryr1	29,000	60,000	9	9.5	EMAN
Epsilon Phage	15,000	40,000	13.6	13	EMAN/SAVR