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Recording Subnanometer Resolution Images of Ice-Embedded Particles with a 4K CCD Camera in a 300 kV Electron Cryomicroscope

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The intermediate voltage electron cryomicroscope has become a routine instrument for imaging ice-embedded particles in structural biology research. For instance, the JEOL3000 SFF liquid helium electron cryomicroscope is well suited for collecting data at subnanometer resolution for 2-D protein crystals, helical arrays and single particles. So far, all the image data used for the subnanometer resolution structures of biological specimens have been recorded on photographic film. Recently, we have reported the feasibility of recording subnanometer resolution images in a JEM2010F instrument operated at 200 kV on a Gatan 4kx4k CCD camera. These images were recorded at a microscope magnification of 60,000x. The data are sufficiently good to allow a 3-D reconstruction of a virus particle where alpha helices of protein components can be clearly resolved (Booth et al., 2004). In this study, the structural resolution of the reconstruction is achieved at about 2/5 of Nyquist frequency of the CCD camera.

For a microscope operated at higher voltage (e.g. 300kV), it is uncertain what practical resolution a commercially available CCD camera can achieve for image data collection of ice-embedded single particles. There have been different design approaches to overcome the relatively large point spread function of the camera when used to detect 300 kV electrons. The Gatan 4kx4k CCD camera with 15 micron/pixel resolution and a 4-port readout, has been designed to optimize the modulation transfer function with an optimal choice of scintillation material and thickness. We have installed this camera in our JEM3000SFF cryomicroscope. We report here the first experimental data that demonstrates its potential applicability to structural studies of ice-embedded single particles aimed at subnanometer resolution.

Epsilon15 phage embedded in vitreous ice suspended across holes was used as a test specimen to evaluate the potential information content in the CCD images. Figure 1 (left) is an example of a 300 kV image of ice embedded phage particles recorded at a defocus of 0.82 microns on the Gatan 4kx4k CCD camera. An effective magnification of 84,000x and a total dose of 25 electrons/Å^2 were used to record this image. The image contrast is rather remarkable, a fingerprint motif, derived from the viral genome in certain projections, is visible in many of the particles. Such contrast is high enough for contrast transfer function determination, particle boxing and orientation determination. Figure 1 (right) shows the power spectrum of the particle images where the CTF rings are apparent beyond 7 Å resolution. Figure 2 is the circularly averaged power spectrum and the estimate of the signal to noise ratio at different spatial frequency according to the procedure described previously (Saad et al., 2001). Based on this data, it is highly likely that this current camera is usable for 300 kV electron image data collection of ice-embedded single particles at subnanometer resolution.

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Saad, A., Ludtke, S.J., Jakana, J., Rixon, F.J., Tsuruta, H. and Chiu, W. (2001) JSB 133, 32-42.

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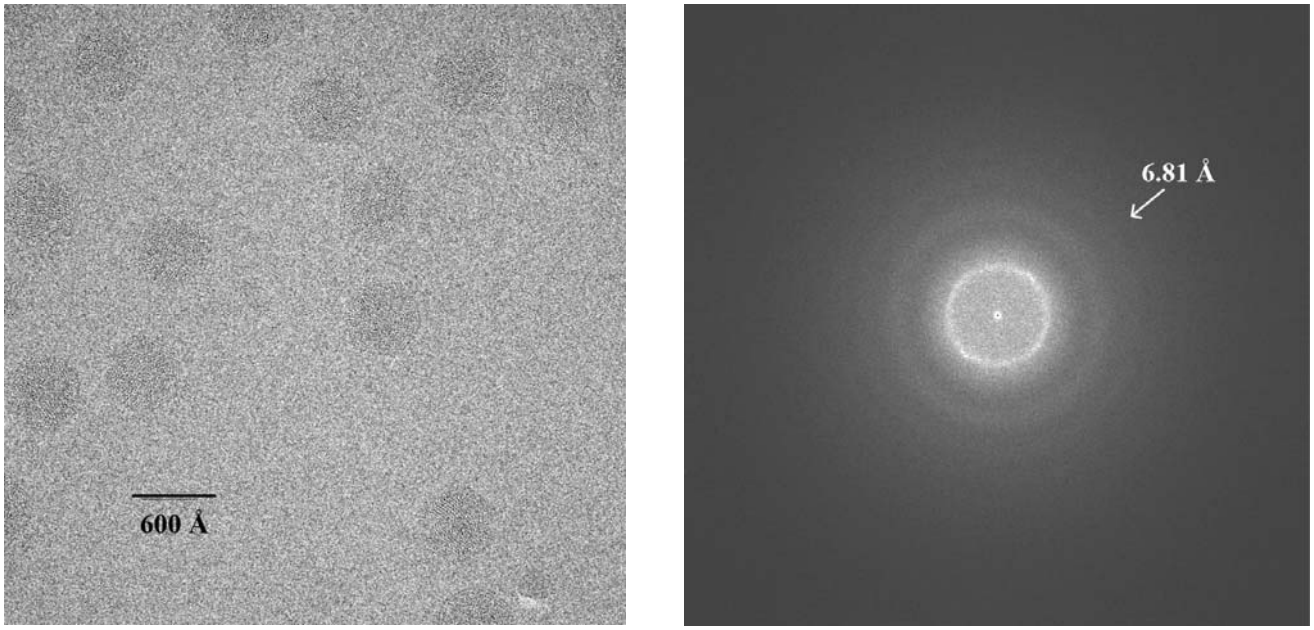

Fig. 1: (Left) 4K CCD frame of an ice embedded image of Epsilon15 phage particle. (Right) Power spectrum of 20 particle images.

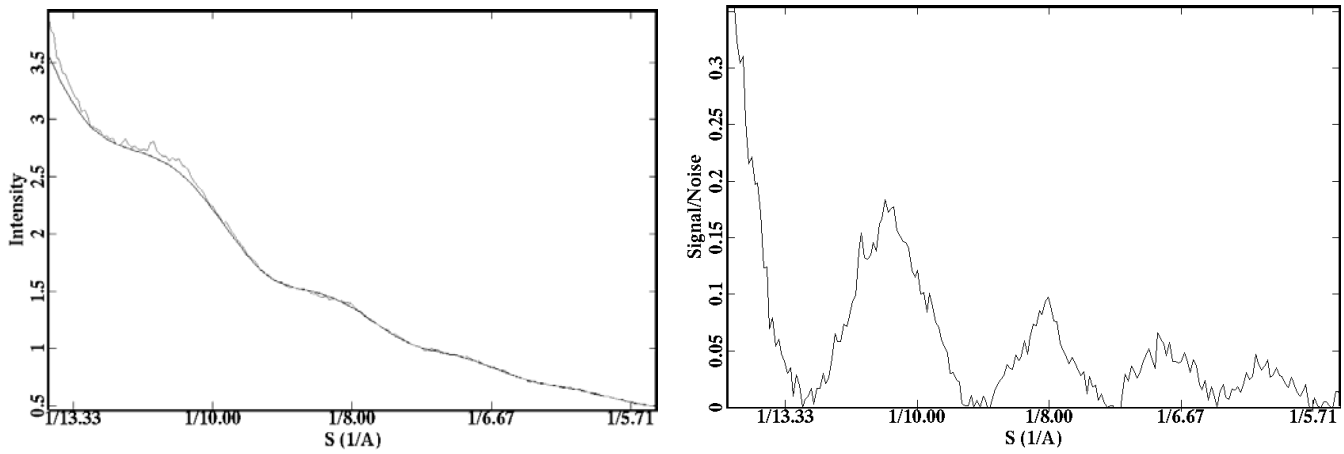

Fig. 2: (Left) Circularly averaged plot of the power spectrum. (Right) Estimated signal to noise ratio of 20 boxed out particle images in Fig. 1.

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