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Assessing the capabilities of a 4kx4k CCD camera for electron cryo-microscopy at 300 kV

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Abstract

CCD cameras have numerous advantages over photographic film for detecting electrons; however the point spread function of these cameras has not been sufficient for single particle data collection to subnanometer resolution with 300 kV microscopes. We have adopted spectral signal to noise ratio (SNR) as a parameter for assessing detector quality for single particle imaging. The robustness of this parameter is confirmed under a variety of experimental conditions. Using this parameter, we demonstrate that the SNR of images of either amorphous carbon film or ice embedded virus particles collected on a new commercially available 4kx4k CCD camera are slightly better than photographic film at low spatial frequency (<1/5 Nyquist frequency), and as good as photographic film out to half of the Nyquist frequency. In addition it is slightly easier to visualize ice embedded particles on this CCD camera than on photographic film. Based on this analysis it is realistic to collect images containing subnanometer resolution data (6–9 Å) using this CCD camera at an effective magnification of ~112 000× on a 300 kV electron microscope.

Keywords: Cryo-EM; CCD camera; 300 kV; Electron cryo-microscopy

1. Introduction

Available detectors in electron microscopy include photographic film, image plates and charge coupled device cameras (CCDs). They differ in the levels of noise, the linearity, the dynamic range, point spread function (PSF) and detective quantum efficiency (DQE) (Samei et al., 1998; Janesick, 2001). Choosing the most appropriate detector for an experiment depends on the resolution goal of the project and the cost-effectiveness in terms of number of micrographs or frames for the entire project. For example, if a single particle project is targeted at 6 Å resolution using electron cryo-microscopy (cryo-EM), the detectable signal that can be extracted from a low dose image at that resolution is the biggest concern. In contrast, data collected in electron diffraction would span many orders of magnitude. In this case, the dynamic range and linearity of the signals become the most important factors in choosing a detector.

Subnanometer resolution structures by cryo-EM are becoming increasingly common (Chiu et al., 2005). The specimen preparation, data collection and image reconstruction have gradually been streamlined for biological end-users. Despite their numerous advantages, the biggest limitation of CCD cameras for the purpose of cryo-EM is the attenuation of high resolution information in images due to the point spread function (PSF) of the CCD camera. In addition, the PSF and its Fourier transform, the modulation transfer function (MTF), are thought to become worse at higher electron accelerating voltage (Downing and Hendrickson, 1999; Meyer and Kirkland, 2000). Recently, a 4kx4k CCD camera (Gatan US4000) has been demonstrated to be feasible for direct data collection with a 200 kV electron microscope and structural determination of biological single particles to subnanometer resolution

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where long α-helices of proteins are clearly resolved (Booth et al., 2004; Ludtke et al., 2005; Chiu et al., 2006). It remains unknown if CCD based detectors can be used to collect cryo-EM data for structural determination to subnanometer resolution at higher accelerating voltages. To evaluate the performance of a new CCD detector, a comparison with photographic film provides a benchmark against which new detectors can be measured.

Traditionally, the MTF has been used to describe the attenuation of signal at high spatial frequency. There are a wide variety of techniques used to estimate the MTF for a CCD camera, including, the use of knife edges, interferometer and statistical methods (de Ruijter and Weiss, 1992). Typically, MTF curves are normalized to unity at the origin. The MTF is therefore useful for describing the width of the PSF, but it does not indicate the amount of noise present at a particular spatial frequency. For example if the MTF of a detector decays by 50% at half of the Nyquist frequency, this does not provide any indication if the signal at that frequency can be reliably recovered using standard image processing techniques.

Spectral signal to noise ratio (SNR) has been a common measurement made from images of an amorphous carbon film or an ice embedded single particles (Saad et al., 2001). It is an operational metric which can be influenced not only by the detector but also the microscope performance and the specimen stability. In addition, there are concerns that such a measurement may be dependent on the detector dose, or the size of specimen area to be analyzed. To experimentally establish the robustness of SNR as a detector quality metric this paper describes the effect that different imaging conditions can have on SNR estimation. Images of amorphous carbon are then used to estimate SNR from CCD and photographic film to assess the performance of the current Gatan 4kx4k CCD camera in a JEM3000SFF operated at 300 kV. Finally SNR calculated from real biological specimens is used to estimate a plausible resolution that can be expected from data collected on such a camera.

2. Materials and methods

2.1. Specimens

Calibration using amorphous carbon and graphitized carbon was done using commercially available grids (Electron Microscopy Services, Ft. Washington, PA). Epsilon15 and T7 bacteriophages were used as biological test specimens. Freshly isolated T7 samples were flash frozen in liquid ethane on washed, electron-beam pre-irradiated (Miyazawa et al., 2003) and glow discharged Quantifoil 400 mesh R2-2 holey carbon grids (Quantifoil Micro Tools GmbH, Jena, Germany). Epsilon15 phage was prepared in the same manner, but a layer of amorphous carbon (~100 Å thick) was deposited on the grid prior to vitrification. Samples were then stored in liquid nitrogen until they could be transferred to the electron cryo-microscope for imaging.

2.2. Cryo-electron microscopy

All images were acquired using a JEM3000SFF electron microscope (JEOL Inc, Tokyo, Japan) operated at 300 kV. This microscope was equipped with the JEOL telemicroscopy software package called FasTEM (JEOL USA Inc, Peabody, MA); with a JEOL top entry cryo-stage operated at liquid helium temperature (4.2 K), and a Gatan US4000 4Kx4K CCD camera (Gatan, Pleasanton CA). The specimen dose rate was estimated from the current density readout in the viewing screen in the microscope. All images were acquired on a Gatan US4000 CCD camera or Kodak SO160 films. The photographic films were developed in full strength D19 at 20 °C.

2.3. Collection of images on CCD

JAMES is a custom software package developed at the NCMI for automation of microscopy in JEM2010F microscope (Booth et al., 2004). JAMES has been installed on the JEM3000SSF and was used for data collection. Briefly, this package co-ordinates the communication between the microscope, CCD camera and database (Ludtke et al., 2003) to eliminate the need for dealing with multiple computers and software simultaneously during the data collection. JAMES also provides the optional capability to immediately process the CCD frames collected on the microscope and provide feedback on the quality of the images acquired during a microscopy session.

2.4. Magnification calibration

The magnification of images recorded on the CCD camera were calibrated by calculating the Fourier transform of an image of graphitized carbon (Electron Microscopy Services, Ft. Washington, PA) with diffraction peaks at 1/3.44 $Å^{-1}$. From the distance, in pixels, that these peaks were located from the origin in the Fourier space, the fold increase of the effective magnification on the CCD camera over that of photographic film was determined to be 1.41×. In this estimate, we assumed the pixel size of the camera to be 15 μm as specified by the manufacturer. Unless otherwise specified, all magnifications reported in this work are effective magnification based on this measurement.

2.5. Estimating spectral signal to noise ratio

Estimation of the spectral SNR was done as described previously (Booth et al., 2004). Briefly, under low dose conditions an area of thin amorphous carbon film was recorded consecutively on Kodak SO163 film and on the CCD camera. For all images collected on photographic film and CCD, the dosage was held constant on the specimen for both detectors. Images recorded on photographic films were scanned on a Nikon scanner (Nikon Inc., Melville, N.Y.) at a step size of 6.35 μm/pixel. The 2-D power spectrum of each image was estimated by incoherently averag-