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Seeing tobacco mosaic virus through direct electron detectors

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ABSTRACT

With the introduction of direct electron detectors (DED) to the field of electron cryo-microscopy, a wave of atomic-resolution structures has become available. As the new detectors still require comparative characterization, we have used tobacco mosaic virus (TMV) as a test specimen to study the quality of 3D image reconstructions from data recorded on the two direct electron detector cameras, K2 Summit and Falcon II. Using DED movie frames, we explored related image-processing aspects and compared the performance of micrograph-based and segment-based motion correction approaches. In addition, we investigated the effect of dose deposition on the atomic-resolution structure of TMV and show that radiation damage affects negative carboxyl chains first in a side-chain specific manner. Finally, using 450,000 asymmetric units and limiting the effects of radiation damage, we determined a high-resolution cryo-EM map at 3.35 Å resolution. Here, we provide a comparative case study of highly ordered TMV recorded on different direct electron detectors to establish recording and processing conditions that enable structure determination up to 3.2 Å in resolution using cryo-EM.

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1. Introduction

The recent introduction of direct electron detectors (DEDs) provide new and exciting possibilities to the field of three-dimensional (3D) electron cryo-microscopy (cryo-EM) (Grigorieff, 2013). When biological samples are rapidly plunge-frozen in a thin layer of vitreous ice using liquid ethane they can be studied in their native structural state (Adrian et al., 1984). Until recently, near-atomic resolution insights have only been possible for molecules that are arranged symmetrically such as two-dimensional crystals, helical arrays or icosahedral assemblies (Henderson et al., 1990; Unwin, 2005; Yu et al., 2008; Zhang et al., 2008). Recent hardware developments have resulted in new DED devices that enabled atomic-resolution single-particle structures of specimens such as the 20S proteasome with lower order symmetry (D7) or the mitochondrial ribosome with no symmetry at all (Amunts et al., 2014; Li et al., 2013a). Resolutions below 3.5 Å allow *de novo* building of atomic models and provide unprecedented insights into the molecular function of biological macromolecules (Allegretti et al., 2014; Amunts et al., 2014; Liao et al., 2013; Voorhees et al., 2014).

The main architectural advantage of new-generation detectors over previously used phosphor-fiber optics CCD cameras or photographic film is the capability to directly capture electrons, thus omitting the intermediate step of signal-to-light conversion (Faruqi and Henderson, 2007). Hence, the latest generation of DEDs has surpassed the performance of film as a high-resolution recording medium. Furthermore, the read-out performance is significantly faster and thus it has become possible to record micrographs in 'movie mode' where multiple frames constitute the traditional micrograph recorded from a single exposure. This feature has opened new means to track individual particles in frames over time and has led to new insights on beam-induced movement (Brilot et al., 2012). Furthermore, frame processing can compensate for beam-induced movement and thus improve the resolution of 3D reconstructions from DEDs (Bai et al., 2013; Campbell et al., 2012; Li et al., 2013a). Currently, five DEDs are commercially available: the Gatan K2 Summit, the FEI Falcon I and II, and the Direct Electron DE-12 and DE-20. The K2 Summit is the only camera that can be operated as an electron-counting device to detect electrons at sub-pixel accuracy in super-resolution mode. Grigorieff and Henderson characterized the performance of these devices by measuring their detective quantum efficiency (DQE) and modulation transfer function (MTF) curves (McMullan et al., 2014; Ruskin et al., 2013) with a focus on the most commonly available voltage of electron microscopes at 200 kV and 300 kV. While these tests provide objective characterization of information transfer, it is less clear how these measurements

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translate into the quality of 3D reconstructions. To address this issue, a biological test specimen of high stability is required so that few micrographs suffice to compute high-resolution 3D structures using a standard image-processing workflow.

Therefore, we turned to tobacco mosaic virus (TMV) that has been extensively studied since the first days of structural biology (Bernal and Fankuchen, 1941). Electron diffraction patterns reveal that the helical order could be measured up to 2.3 Å (Cyrklaff and Kühlbrandt, 1994). With the development of single-particle based helical reconstruction the resolution was pushed to below 5 Å (Sachse et al., 2007) using 200,000 asymmetric units recorded on film. Subsequently, TMV recorded on a US4000 CCD camera gave rise to a 4.6 Å resolution structure (Clare and Orlova, 2009). These structures could be improved to 3.3 Å by using a total of 1,900,000 asymmetric units (Ge and Zhou, 2011) recorded on film. Given the large amount of published structural data on TMV and the known high preservation of helical order, we set out to compare the performance of two commonly available DEDs by testing their effect on the quality of TMV structures determined using a previously published single-particle based helical reconstruction workflow (Desfosses et al., 2014).

In the current article, we compared the performance of the two common DED cameras, K2 Summit and Falcon II, in cryo-EM using TMV as a test specimen. When imaged in the same microscope, micrographs from both cameras give rise to ~4 Å resolution image reconstructions of very similar quality using only 44,000 asymmetric units. A comparison between micrograph-based and segment-based motion correction revealed that both strategies equally lead to significant improvements in the resolution of the TMV reconstructions. Moreover, when investigating the effect of increasing electron dose on the TMV structure we found that radiation damage is side-chain specific and this specificity is very similar to that of X-ray crystallography experiments. Finally, using a total of 450,000 asymmetric units we determined a high-resolution cryo-EM map of TMV at 3.35 Å resolution that contains structural details up to 3.2 Å.

2. Results

2.1. Performance comparison of K2 Summit and Falcon II recorded on the same microscope

The recently published results (Amunts et al., 2014; Bai et al., 2013; Campbell et al., 2012; Fernandez et al., 2013; Li et al., 2013a; Liao et al., 2013) were obtained using different commercially available DED devices. In this context, it is of urgent interest to the scientific community to objectively measure their performance and in particular to compare their direct effect on the final 3D image reconstruction of biological macromolecules. Therefore, we set out to compare the quality of TMV 3D image reconstructions from electron cryo-micrographs acquired with a Gatan K2 Summit and a FEI Falcon II detector using a recently described and publicly available standardized single-particle based 3D helical reconstruction workflow from the software suite SPRING (Desfosses et al., 2014).

When comparing DED performance with existing high-resolution 3D structures, the main concern is that the data sets were recorded on different electron microscopes. Moreover, different samples and preparation protocols are being used, so that results may not be generally conclusive about DED performance. Therefore, we recorded two small data sets from the same EM grid of TMV on the MRC-LMB Titan Krios that is equipped with both K2 Summit and Falcon II DED devices. Here, the Falcon II is in the pre-GIF position while the K2 Summit is attached to the end of a Gatan Quantum energy filter. The data was acquired with the energy slit retracted. In order to record data at comparable physical pixel sizes the exact imaging conditions in the microscope had to be varied due the different position of the cameras. We collected micrographs in movie mode with 2–3 $e^-/Å^2$ dose per frame accumulating to a total of 22 or 16 frames and 43 $e^-/Å^2$ for the K2 Summit and Falcon II camera, respectively. Optimal performance of the K2 Summit and the Falcon II requires the use of significantly different dose rates at 5–10 e^-/pixel/s (Li et al., 2013b) vs. 50 e^-/pixel/s, respectively. To make our studies comparable we chose the typical dose rates that were used in a number of high-resolution structure studies from the K2 and Falcon II camera (Amunts et al., 2014; Bai et al., 2013; Li et al., 2013a; Liao et al., 2013).

For the initial analysis of the raw data without subject to any structural refinement, we generated approximately 690 overlapping segments with a step size of 90 Å and applied only the in-plane rotation as determined from the interactive picking of the viruses (Fig. 1A). The sum of the power spectra from overlapping segments represents an unbiased measure of the order and the recorded information content of the helical segments (Fig. 1B). The collapsed power spectra profiles where pixel rows along the layer lines were averaged from the two data sets revealed that peaks from meridional layer lines were clearly detectable up to 4.6 Å (Fig. 1C). Using approximately 44,000 asymmetric units, we determined two cryo-EM maps at a resolution of 4.0 and 3.7 Å (FSC 0.143 cutoff) for the K2 Summit and Falcon II respectively including motion correction (see below and in Fig. 1D). The FSC curve from the K2 Summit reconstruction had slightly stronger low-resolution and weaker high-resolution signal when compared with the Falcon II curve. Visual inspection of the two maps revealed almost identical quality of the density. At this resolution, the β-strands within the small twisted β-sheet at higher outer radius position are clearly separated in both maps (Fig. 1E and F). Given the similarity in the achieved resolution, we further assessed whether one of the detectors requires significantly less data to achieve a particular resolution. Therefore, we computed six reconstructions for each data set by approximately doubling the number of asymmetric units from 1500 to 44000. The FSC resolution cutoffs showed a similar behavior for both detectors starting from 6.7 and reaching 4.0/3.7 Å (Fig. 1G). Comparison of FSC resolution curves between the K2 Summit and Falcon II detectors (Supplementary Fig. S1) confirmed that there were no significant differences in the amount of data required to achieve a particular resolution.

2.2. Comparison of segment-based and micrograph-based motion correction

The cryo-EM maps (Fig. 1) described above were obtained with frame processing depositing a total dose of 21.5 $e^-/Å^2$ for the Falcon II and the K2 data set. As exposures are collected in movies as an image series over time, frame processing has become an important factor to compensate for movement during the exposure and to produce maps of highest resolution (Bai et al., 2013; Campbell et al., 2012; Li et al., 2013a). In principle, two methods of motion correction have been put forward. First, in case of large icosahedral particles with high signal, the motion correction was directly based on the alignment of individual particles within a subset of frames (Campbell et al., 2012). In practice, this requires the starting parameters of the frame sum to perform a local refinement of *x–y* shifts and Euler angles to compensate the translational and rotational movements during the exposure. This approach has been extended with additional restraints such as running averages of frame sets on viruses and Bayesian statistics on images of ribosomal particles (Bai et al., 2013). Second, in the case of smaller particles, the motion is tracked using the signal from a field of multiple particles, i.e. the frames from larger sub-regions of the micrograph or entire micrographs (Li et al., 2013a; Scheres, 2014;

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