New technologies for Cryo-EM: Volta Phase Plate and Falcon-III Electron Counting

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1. INTRODUCTION

A) FEI Falcon Direct Electron Detector

It is undeniable, cryo-EM entered a new era recently [1], and this is definitely correlated with the appearance of new CMOS-based detectors, which are able to capture the electrons without the need of intermediate phosphor scintillators and fibre-optic, thus the name direct electron detectors [2].

Detector performance is of particular importance in cryo-EM because biological samples under study are radiation sensitive. Low-dose techniques have to be employed to reduce the beam damage in the sample, but this inevitably results in very noisy images, meaning that their signal-to-noise ratio (SNR) is very poor. In addition to the low signal due to the sample being radiation sensitive, there is noise added by the detector [3]. Ideally the samples would provide a very strong signal, and the ideal detector would not add any noise, such that their ratio would be 1. This ratio is called the detective quantum efficiency (DQE), and it is defined as the square of the ratio of the output signal to noise (SNR_o), to that of the input (SNR_i), or:

$$DQE=[SNR_o]^2/[SNR_i]^2$$

At an operating voltage of 300kV, a typical CCD has a DQE of 0.1 (at half-Nyquist); film plates have a DQE of about 0.3 and newly-developed direct electron detectors have a DQE of 0.6 or above [4], so they are more sensitive than film, and thus the preferred recording medium in a modern cryo-electron microscope. Moreover, the increased speed in readout allows the total electron dose to be fractionated over several frames while the latter are captured and integrated over the exposure time. The single access to the individual frames allows statistical processing, in other words the ability to correct for beam-induced blurring in the image itself during exposure [5]. We will present our new Falcon-III EC (Electron Counting) detector.

B) FEI Volta Phase Plate

Recently the Volta Phase Plate product was introduced after a fruitful collaboration between the Max Planck Institute in Martinsried and FEI [6]. The phase plate is based on a new design taking advantage of a beam-induced Volta potential on the surface of a continuous thin film. The Volta potential is negative, indicating that it is not caused by beam-induced electrostatic charging. The film must be heated to ~200 °C to prevent contamination and enable the Volta potential effect. The phase shift is created "on the fly" by the central diffraction beam eliminating the need for precise phase plate alignment. Images acquired with the Volta phase plate (VPP) show higher contrast and unlike Zernike phase plate images no fringing artefacts. The VPP has a long service life and has been used for more than 6 months without noticeable degradation in performance. The mechanism underlying the VPP is the same as the one responsible for the degradation over time of the performance of thin-film Zernike phase plates, but in the VPP it is used in a constructive way. We will illustrate its usefulness for 3D cryo-ET and also single-particle analysis with a few recent application examples (from data collected on a Titan Krios equipped with Volta phase plate and Falcon-II direct electron detector).

2. RESULTS

2.1 Experimental conditions

All images presented here were acquired on a FEI Titan Krios equipped with a Falcon-II direct electron detector. The Herpes Simplex Virus (HSV-1) samples were kindly provided by Prof. Hong Zhou and Xinghong Dai at UCLA. Direct imaging was performed at 300kV, using a dose flux of 18 electrons/$Å^2$.sec and images were recorded at

1.0 µm underfocus. The FEI Volta Phase Plate was conditioned for ~2 minutes before exposure in a nearby area then two consecutive images were collected on the same field of view, one with the phase plate inserted (VPP IN) and the second one immediately after with a 100-microns objective aperture (VPP OUT). For cryo-electron tomography the tilt series was acquired using FEI Tomography4 software, at a magnification corresponding to a final pixel size of 0.4nm and the sample was exposed to a total cumulative electron dose of 70 electrons/Å^2.

2.2 Imaging HSV-1 with the FEI Volta Phase Plate

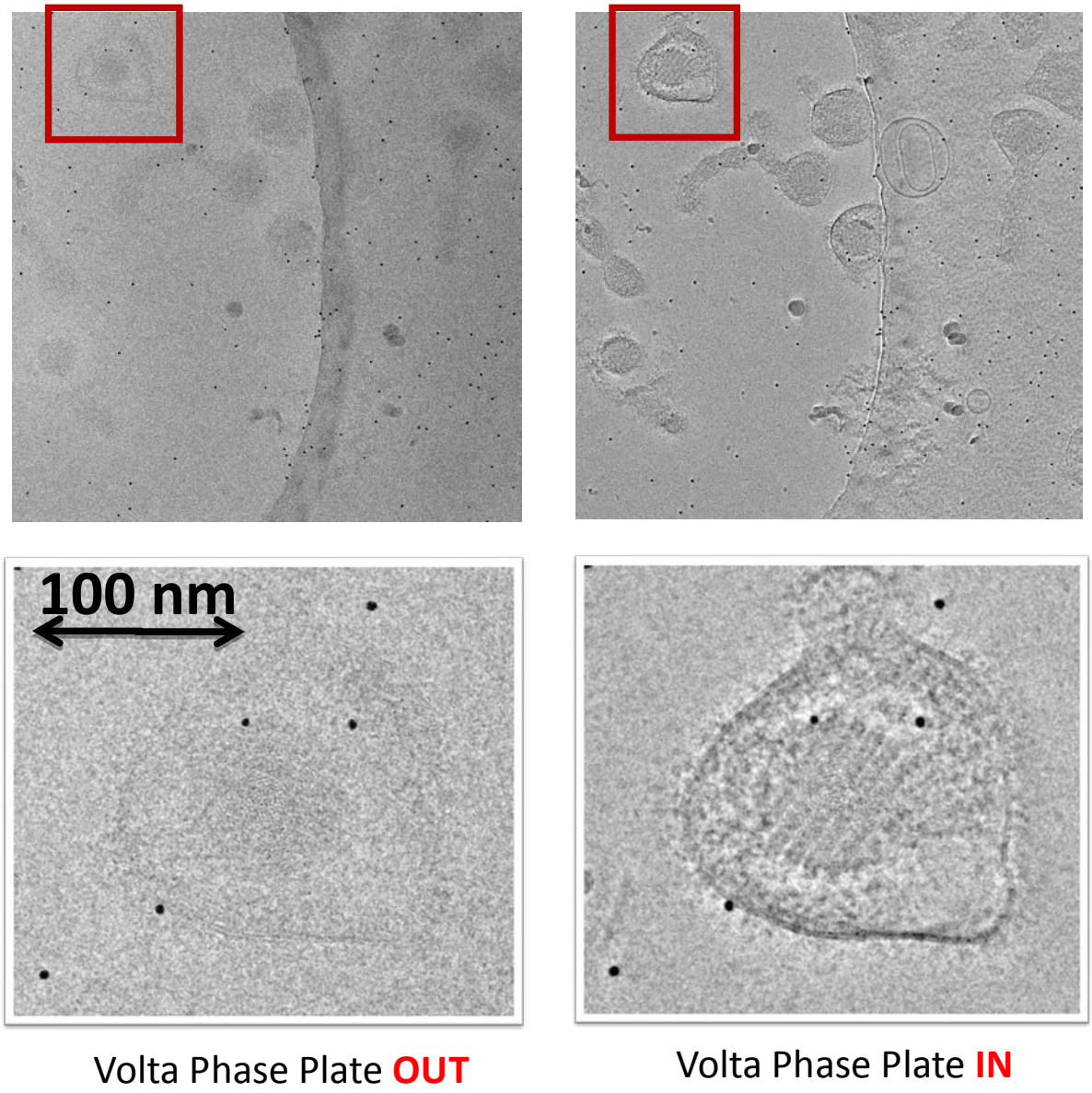

Figure 1. Cryo-EM of HSV-1 imaged at -1.0µm defocus with a 100-µm objective aperture (left) or with the new FEI Volta Phase Plate (right). Insets: enveloped viral particle, same field of view, as shown with gold markers, near focus without the Volta phase plate (left) and with the same illumination conditions but with the Volta phase plate inserted in the back focal plane.

2.2 Cryo-electron tomography of HSV-1 with the FEI Volta Phase Plate

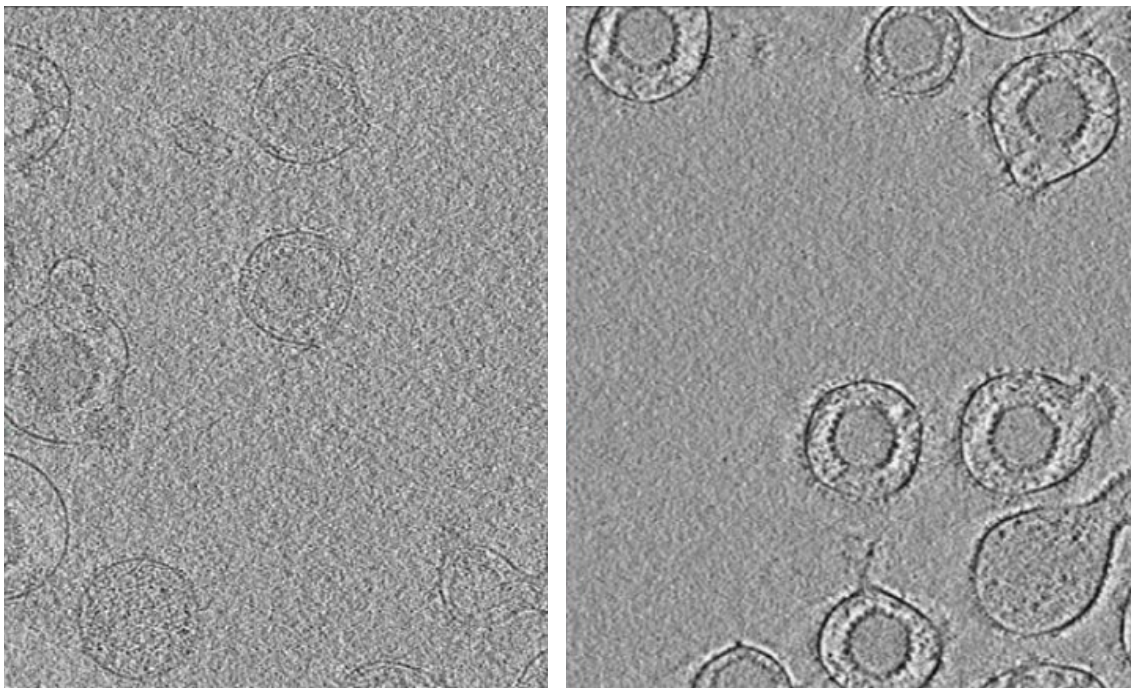

Figure 2. Cryo-Electron Tomography of HSV-1 imaged at -5.0µm defocus with a 100-µm objective aperture (left) or near-focus with the new FEI Volta Phase Plate (right). The still images are representative slices from the tomographic reconstructions after alignment of the tilt series acquired with same illumination parameters besides the defocus, as indicated.

3. REFERENCES

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