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

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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_0), 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.

Recently the Volta Phase Plate product was introduced after a fruitful collaboration between the Max Planck Institute in Martinsried and FEI [6]. 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).

[1] Smith MT, Rubinstein JL (2014). Structural biology. Beyond blob-ology. *Science* **345**, 617-619.
[2] Faruqi AR, McMullan G (2011). Electronic detectors for electron microscopy. *Q. Rev. Biophys*. **44**, 357-390.
[3] McMullan G, Chen S, Henderson R, Faruqi AR (2009). Detective quantum efficiency of electron area detectors in electron microscopy. *Ultramicroscopy* **109**, 1126-1143.
[4] McMullan G, Faruqi AR, Clare D, Henderson R (2014). Comparison of optimal performance at 300kV of three direct electron detectors for use in low dose electron microscopy. *Ultramicroscopy* **147**, 156-163.
[5] Scheres SHW (2014). Beam-induced motion correction for sub-megadalton cryo-EM particles. *eLife* **3**,e03665.
[6] Danev R, et al (2014). Volta potential phase plate for in-focus phase contrast transmission electron microscopy. *PNAS* **111**, 15635-40.