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Current Opinion in Structural Biology

Expanding the boundaries of cryo-EM with phase plates

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Phase plates have long been considered as a means for improving the performance of cryo-electron microscopy (cryo-EM). But practical limitations, such as a short lifespan or cumbersome usage have prevented their widespread adoption. The recently developed Volta phase plate overcomes most of the practical issues and it is now commercially available. Results from both, electron cryo-tomography (cryo-ET) and single particle analysis (SPA), have demonstrated the benefits of using a phase plate. In CET phase plates have helped to visualize cellular ultrastructure in unprecedented detail. In SPA phase plates allowed to determine the structures of small proteins at near-atomic resolutions. Further improvements in phase plate technology are possible and new designs are already under development.

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Current Opinion in Structural Biology 2017, **46**:87–94

This review comes from a themed issue on **Cryo electron microscopy**

Edited by **Wah Chiu** and **Kenneth Downing**

http://dx.doi.org/10.1016/j.sbi.2017.06.006

Introduction

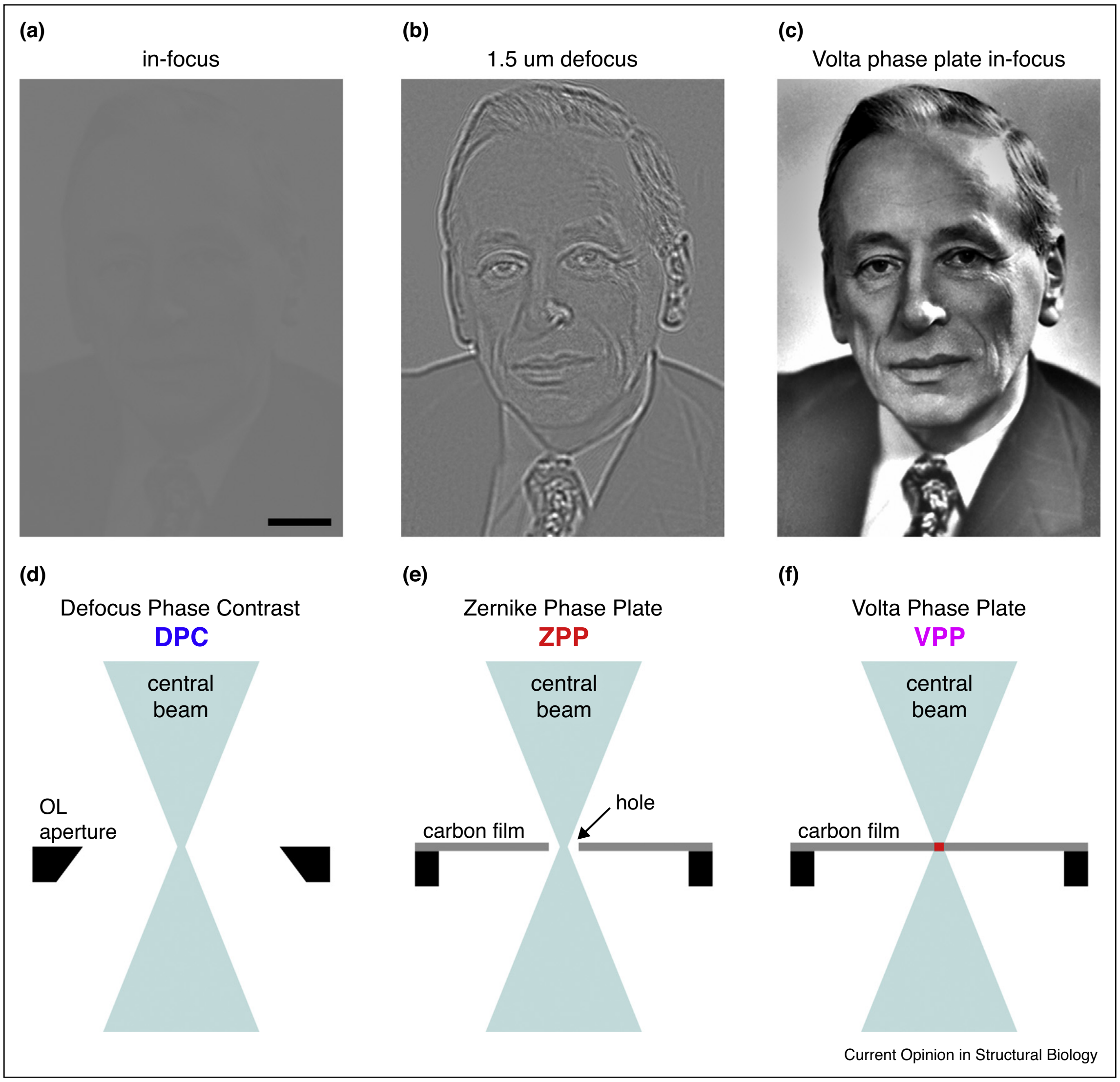
In recent years, in the wake of technological and methodological improvements, cryo-electron microscopy (cryo-EM) has reached a new level of performance; some refer to this as the 'resolution revolution' [1]. And yet, the performance is still fundamentally limited by the radiation sensitivity of biological materials and the need to minimize exposure to the electron beam. As a consequence, signal-to-noise ratio of the images is relatively poor and necessitates the application of averaging to retrieve structural information. The dose limit problem is exacerbated by the low contrast of frozen-hydrated biological samples which consist predominantly of light elements. Electron-optically, they behave like weak-phase objects, that is, they modify weakly the phase of the electron wave but not its amplitude (Figure 1a) [2]. Therefore, phase contrast is essential for extracting a maximum of information from biological samples with a tolerable electron dose.

Phase contrast in cryo-EM is traditionally produced by defocusing the objective lens, generating defocus phase contrast (DPC) [2] (Figure 1d). This method provides good information transfer for intermediate to high spatial frequencies but performs poorly at low spatial frequencies. Consequently, DPC images have a high-pass-filter-like appearance and overall low contrast (Figure 1b) which makes them difficult to interpret directly and necessitates various image restoration techniques.

Phase plates are devices which generate phase contrast without the need of defocusing and over a wide range of spatial frequencies (Figure 1c) [3]. They were proposed and theoretically considered already in the early days of transmission electron microscopy (TEM) [4]. Various types of phase plates were tested over time but for several decades no usable device could be built [5]. In the early 2000s a new wave of phase plate experiments started and a promising candidate was developed – the thin film Zernike phase plate (ZPP) (Figure 1e) [6]. It consists of a thin amorphous carbon film with a small hole at the center. For over 10 years the ZPP was tested and used in proof-of-principle applications with some success [7]. Good quality data could be collected, but the ZPP suffered from a number of practical problems [8]. The first issue was the short lifetime of the ZPP. After a few days in the electron microscope the performance degraded and the phase plate had to be exchanged. The second problem was the requirement to precisely center the hole of the ZPP on the beam path. This could not be automated and data collection had to be carried out manually.

A few years ago, a new type of phase plate was proposed – the Volta phase plate (VPP) (Figure 1f) [9]. It is very similar in design to the ZPP but without a central hole. The VPP takes advantage of a phenomenon, which as of today is not yet fully understood; the phase shift is created by the interaction of the electron beam with the continuous carbon film. The current hypothesis is that the beam causes physicochemical changes on the surface of the film which lead to a local change of the work function and in turn to a local surface potential difference. Those changes are temporary and after a few days the surface of the film recovers to its original state by reactions with the residual gasses inside the electron microscope. After their recovery, positions on the VPP can be reused in the following experimental sessions. Consequently, the VPP has a very long practical lifespan and if not damaged mechanically can be used for years. The fact that the phase shift is self-created by the electron beam means that the VPP does not require precise centering and thus can be easily implemented in automated data acquisition schemes.

Figure 1



(a–c) Simulation of a weak-phase object imaging in an electron microscope. The simulation was performed using a portrait of Frits Zernike (1888–1966), the inventor of phase contrast microscopy, as a model object with a maximum phase shift of 0.2π. Scale bar: 20 nm. **(a)** An in-focus image of a weak-phase object shows almost no contrast. **(b)** A defocus phase contrast (DPC) image of a weak phase object has a 'ghost-like' appearance due to the high-pass-filter effect of DPC. **(c)** A Volta phase plate image of a weak-phase object is a close-to-original representation of the model object. (d–f) Phase contrast methods in electron microscopy with their configuration at the back-focal-plane of the objective lens (OL). **(d)** Defocus phase contrast uses an aperture at the back-focal plane. **(e)** Zernike phase plate consists of a thin amorphous carbon film with a small hole centered on the central diffraction beam. **(f)** The Volta phase plate comprises a continuous amorphous carbon film. The phase shift is generated by the modification of the surface properties of the film by the central diffraction beam.

However, the VPP has some limitations. Because it uses a carbon film in the beam path some of the electrons are scattered which leads to ~18% (at 200 kV) signal loss [9]. Additionally, different positions on the VPP could introduce varying amounts of astigmatism due to local variations of the film quality or wrinkling of the film. An ideal phase plate device should not introduce any materials in the beam path to avoid electrostatic charging or scattering effects. Such a device is already under development; in one such design the phase shift is created by a high-intensity focused laser beam [10,11]. If it can be successfully implemented, the laser phase plate could become a permanent phase contrast solution for TEM.

Cryo-tomography with phase plates

Electron cryo-tomography (cryo-ET) is severely limited by the radiation sensitivity of the biological material and the low contrast of ice-embedded biological samples [12].