Comparison of Hole-Free Phase Plates and Electrostatic Zach Phase Plates for Cryo-Electron Microscopy of Biological Specimens

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The weak contrast of life-science objects in transmission electron microscopy (TEM) has led to a strong interest in the development of physical phase plates (PPs). By inducing a relative phase shift between scattered and unscattered electrons in the objective lens back focal plane (BFP), PPs enable the generation of phase contrast without the need to apply a strong defocus [1]. Here we present results achieved with an electrostatic Zach PP [2] and a hole-free phase plate (HFPP) [3,4]. The HFPP, which exploits the formation of a charged patch on a thin amorphous carbon film induced by the intense zero-order beam, was implemented in the BFP of the objective lens of a JEOL JEM 3200FSC/PP. The Zach PP consists of a micro-structured electronic device which allows to control the phase shift by applying different voltages and was applied in combination with an objective mini lens. To compare phase-contrast formation of these two types of PPs, image series of three standard cryo-TEM samples (T4 bacteriophages (T4), ribosomes and tobacco mosaic virus) as well as cryo-electron tomograms were obtained.

Figure 1 shows the application of the Zach PP on a T4 sample. T4 are embedded in amorphous ice on a holey amorphous carbon film to investigate the T4 in the holes of the film. The Zach PP TEM images show T4 with contracted tails (green arrows) and uncontracted tails (yellow arrows). By applying a negative voltage of -2 V to the Zach PP, the image (Figure 1a) shows strong contrast enhancement of the 250 nm large T4 and their thin tail fibers (red arrows) compared to imaging without applied voltage (Figure 1b). A positive voltage of +2 V (Figure 1c) results in contrast inversion of the T4. Figure 2 shows the application of the HFPP to the previously shown T4 sample. Line scans reveal the contrast enhancement of a T4 head with and without HFPP (Figures 2a,b). This is further illustrated by the intensity line profiles in Figure 2c which also show a reduction of the background grey value due to electron scattering in the HFPP.

In summary, comparable contrast enhancement is achieved with the two PP types and both approaches exhibit advantages and drawbacks [5].

References:

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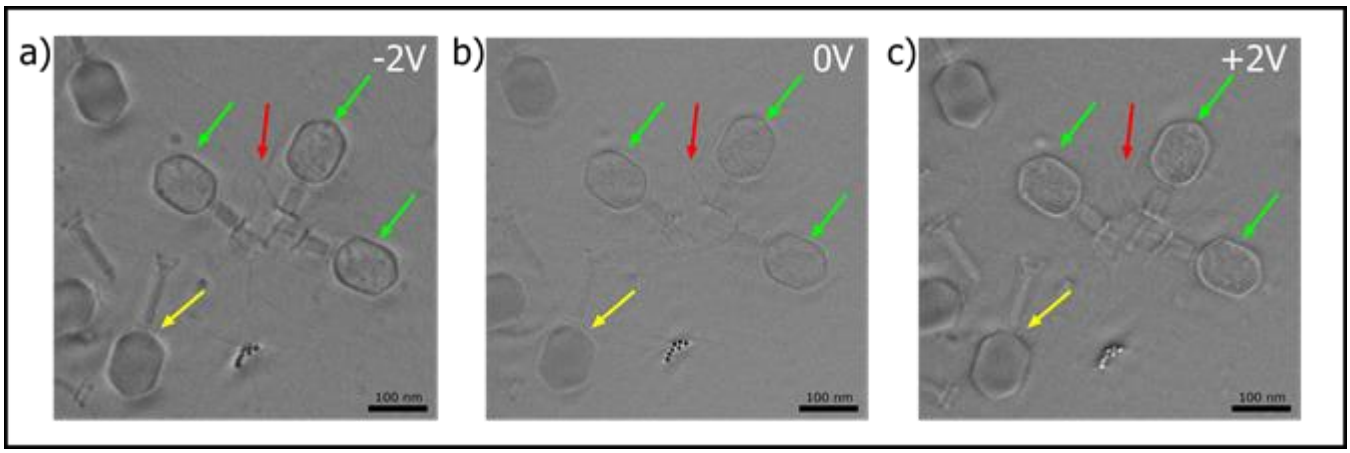

Figure 1. Zach PP TEM images of a T4 sample. (a-c) Images of a voltage series with -2 V, 0 V and 2 V applied to the Zach PP electrode show dark contrast of the T4 for negative voltage and bright contrast for positive voltage.

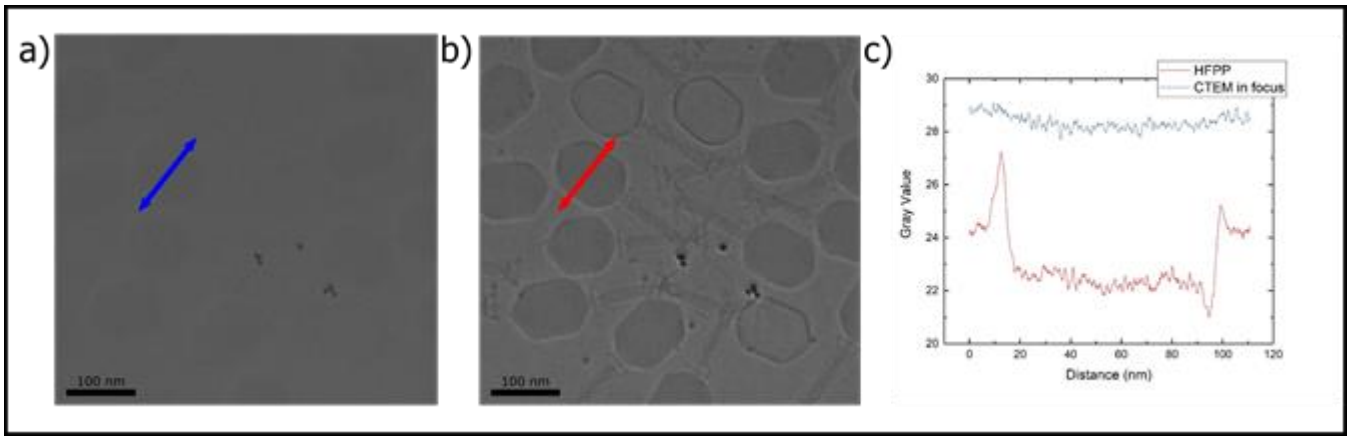

Figure 2. In-focus images of a T4 sample obtained with (a) conventional TEM (CTEM) and (b) HFPP TEM. Line scans across a T4 head in (a) and (b) are shown in (c).