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Use of the Zernike Phase Plate for Electron Tomography of Frozen-Hydrated Specimens

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In electron tomography, the resolution attainable with frozen hydrated specimens is ultimately limited by radiation damage, due to the need to record many images in a tilt series. Improvement in the signal-to-noise ratio makes it possible to increase resolution without increasing the electron dose. Reduction of radiation damage by cooling the specimen to liquid helium temperature [1,2], and optimization of the signal-to-noise ratio by the use of zero-loss energy-filtered imaging and an optimal underfocus setting [3,4], have been explored. Further improvements in the signal-to-noise ratio will be obtained by the use of new camera systems [5,6]. Beyond these efforts, progress may require electron optical improvements such use of phase plates and aberration correction.

The high underfocus typically used for phase-contrast imaging of frozen-hydrated specimens produces an oscillating contrast-transfer function (CTF), and only narrow bands of spatial frequencies in the specimen are represented by strong image contrast. The electron phase plate replaces the aperture in the back focal plane of the objective lens, and creates an appropriate phase shift of $\pi/2$ between unscattered electrons and those scattered by the specimen. Thus, images can be recorded close to focus, and the CTF stays high and without oscillation over a wide band of spatial frequencies. This produces an image with optimum contrast. The avoidance of CTF oscillation allows more straightforward interpretation of the images, and simplifies computation of faithful representations of the specimen in 3-D reconstructions from both tomography and single-particle data.

Since Boersch introduced the idea in 1947, many different types of phase plates have been tried. Representative references are listed below [7-12]. Here we show results from the simplest to make and use type, the Zernike phase plate, recently re-introduced by Nagayama [11]. The improved design and automation capability of modern TEMs may make it practical to use phase plates in routine cryo-TEM. [13]

References

[1] G. Jensen *Microsc Microanal.* 10 (Suppl 2) (2004) 1186CD.
[2] J. Plitzko, personal communication.
[3] R. Grimm et al. *J. Microsc.* 190 (1998) 339.
[4] B. McEwen et al. *J. Struct. Biol.* 138 (2002) 47.
[5] B. Bailey et al. *Microsc Microanal.* 10 (Suppl 2) (2004) 1204.
[6] K. Downing and P. Mooney *Microsc Microanal.* 10 (Suppl 2) (2004) 1378.
[7] H. Boersch *Z. Naturforsch*. 2a (1947) 615.
[8] W. Hoppe *Phil. Trans. Roy. Soc. Lond. B*. 261 (1971) 71.
[9] G. Balossier and N. Bonnet *Optik* 58 (1981) 361.
[10] T. Matsumoto and A. Tonomura *Ultramicroscopy* 63 (1996) 5.
[11] R. Danev and K. Nagayama *Ultramicroscopy* 88 (2001) 243.
[12] E. Majorovits, and R. Schröder *Microsc Microanal.* 8 (Suppl 2) (2002) 540CD.
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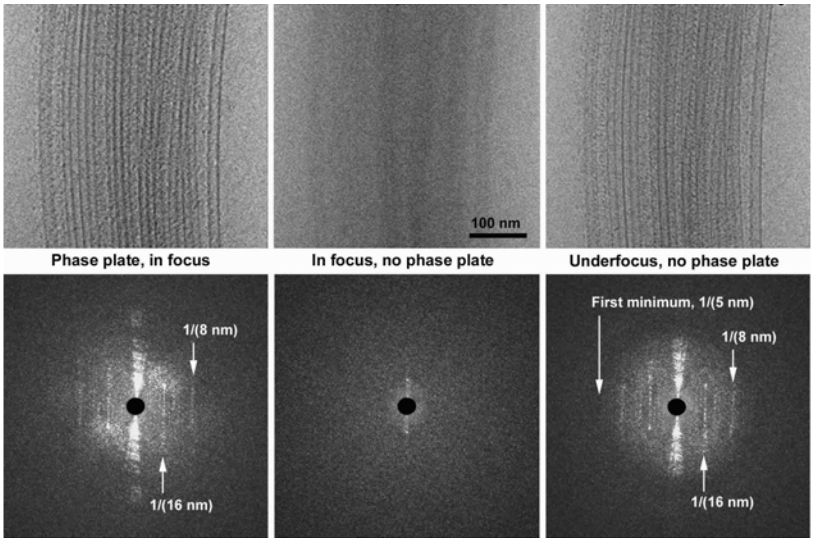

FIG 1. Images and power spectra of frozen-hydrated axoneme. Note increased overall contrast with phase plate compared to underfocus, where transfer drops off sharply beyond the first minimum. Specimen thickness 250 nm. Imaged at 400 keV with zero-loss filtering (JEOL JEM4000FX). Underfocus 15 µm. Phase plate is a 50 µm objective aperture covered with a 34 nm thick carbon film, in which a 1µm central hole was made using a focused ion beam.

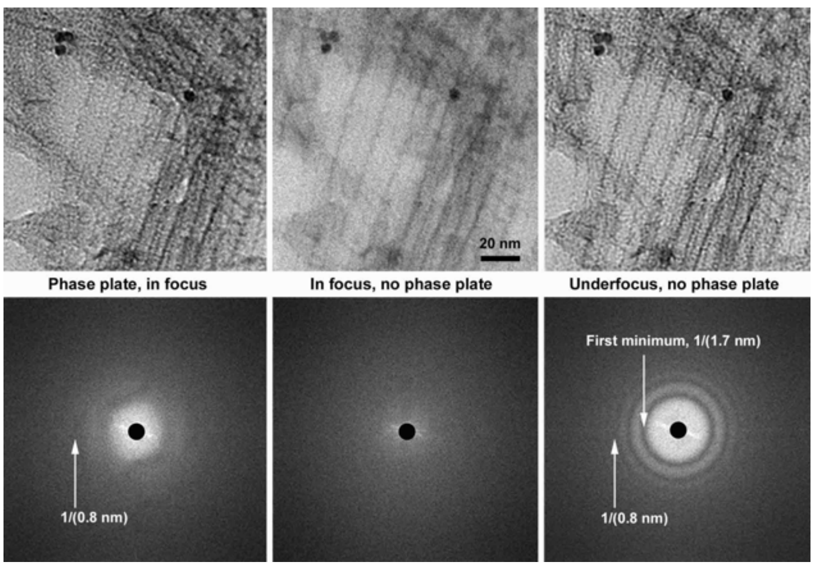

FIG 2. Similar experiment at higher magnification, using TMV with light negative stain. By comparison with the amplitude-contrast image in the center, phase plate imaging more faithfully represents mass differences, and gives higher contrast than the underfocus image. Note that some features are darker, and others lighter, in phase plate compared to underfocus images. Underfocus 2 µm.

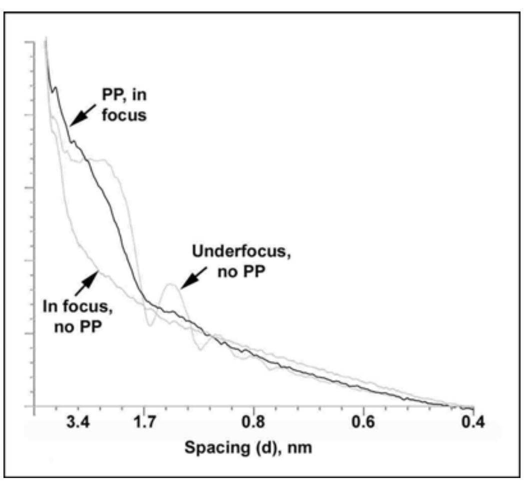

FIG 3. Azimuthally-averaged plots of the power spectra in Fig. 2. Phase plate imaging avoids the effects of CTF oscillation, yet provides strong phase-contrast transfer. Note increased transfer for spacings larger than 3.4 nm (at upper arrow). The calculated CTF for imaging close to focus with this phase plate has constant, maximum transfer over a spatial frequency range corresponding to spacings of about 2-20 nm, which is ideal for tomography of frozen-hydrated specimens. Figs. 1 and 2 confirm that these frequencies are transferred. Transfer is expected to be reduced about 15% due to scattering within the carbon film.

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