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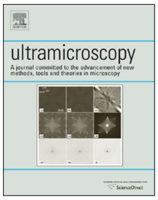

Towards an optimum design for thin film phase plates

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ABSTRACT

A variety of physical phase plate designs have been developed to maximize phase contrast for weak phase objects in the transmission electron microscope (TEM). Most progress towards application in structural biology has been made with Zernike PPs consisting of a ~30 nm film of amorphous carbon with a central hole. Although problems such as beam-induced deterioration of Zernike PPs remain unsolved, it is likely that thin film phase plates will be applied routinely in TEM of ice-embedded biological specimens in the near future. However, the thick carbon film of thin film PPs dampens high-resolution information, which precludes their use for single-particle electron cryo-microscopy at atomic resolution. In this work, an improved design for a thin film phase plate is proposed, combining the advantages of Zernike PPs and 2D materials, such as graphene. The improved design features a disc of phase-shifting material mounted on an ultrathin support film. The proposed device imparts a phase shift only to electrons scattered to low angles, whereas contrast at high resolution is generated by conventional defocusing. The device maximizes phase contrast at low spatial frequencies, where defocus contrast is limiting, while damping of information at high spatial frequencies is avoided. Experiments demonstrate that the fabrication of such a device is feasible.

1. Introduction

In contrast to high-resolution transmission electron microscopy (TEM) in materials science, where a broad transfer band up to high spatial frequencies is generated at Scherzer defocus, structure determination of phase objects such as biological macromolecules depends on contrast at low-resolution, which is indispensible for the detection and proper alignment of particles. In conventional TEM, phase contrast is generated by defocusing the objective lens up to several micrometers. The contrast transfer function (CTF) for TEM imaging of a phase object is given by

$$CTF = \sin(-\pi/2(2\Delta z\lambda k^2 - C_s\lambda^3 k^4)\cdot E_{damping} \quad (1)$$

Δz is the defocus; λ is the wavelength; k is the spatial frequency; C_s is the spherical aberration; $E_{damping}$ is the damping due to spatial and temporal coherence.

It was recognized early on that defocus contrast for low spatial frequencies is poor and generates image artefacts due to CTF oscillation. In his original proposal, as early as 1947, Boersch proposed two designs for physical phase plates that enable in-focus TEM imaging of phase objects when placed in the back focal plane of a TEM [1]. One design, referred to as the Boersch phase plate (BPP), consists of an electrostatic einzel lens shifting the phase of the unscattered electrons. An einzel lens is an electrostatic lens, which does not alter the energy of the propagating electrons. But Boersch suggested also a much simpler device, consisting of a thin amorphous carbon film with a central hole shifting the phase of the scattered electrons by 90° [1]. This PP type is today referred to as Zernike PP. In the last decade, BPPs, Zernike PPs, and a variety of other PP designs have been fabricated and implemented into TEMs [2–12]. Although generation of in-focus contrast for ice-embedded biological specimens has been demonstrated [3,8,13,14], progress has been slow. Electrostatic charging of physical PPs has so far prevented their routine use in electron cryo-microscopy (cryo-EM) of biological specimens [15–17]. Due to their complex multilayer design, electrostatic PPs are more prone to charging than thin film PPs [16]. More progress has been made with the simple design of Zernike PPs. Zernike PPs have been successfully used solely for electron cryo-tomography of large biological structures, such as viruses [18], phages [19], and cells [20]. Recently, a new thin film phase plate has been developed, the so-called Volta PP, which is superior to the Zernike PP with respect to lifetime and reliability [21,22]. Therefore, it is very likely that thin film phase plates will be routinely used for PP cryo-EM of biological specimens in the near future. It is worth noting that current hole-free thin film PP designs are closely related to a PP proposed and fabricated by Nigel Unwin as early as 1970 [23]. In his original proposal, Unwin generated phase contrast by beam-induced electrostatic charging of a gold-coated spider thread mounted in the back focal plane of a TEM [23].

Inelastic scattering of electrons propagating through the ~30 nm thick carbon film causes loss of high-resolution

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information. Currently, the resolution achieved with Zernike PPs is worse than 8 Å [19]. To facilitate cryo-EM of small single particles at atomic resolution, the high signal-to-noise ratio of direct electron detectors [24–26] should be combined with in-focus imaging using a thin film PP. The main function of a physical PP is to enhance contrast at low spatial frequencies, and thus to facilitate particle picking and alignment or sub-tomogram averaging with cryo-EM. In this work, a new type of thin film phase plate is proposed that imparts a phase shift only to electrons scattered to low angles, thereby maximizing contrast transfer at low spatial frequencies and avoiding damping of high-resolution information. Furthermore, the fabrication of such a device is discussed.

2. Results and discussion

2.1. A spatial frequency-selective thin-film PP

Fig. 1A shows the proposed design of a thin film PP imparting a phase shift only to electrons scattered to small angles. This small angle PP (SAPP) consists of a disc of amorphous material with a central hole mounted on a thin support film, which is virtually transparent to the electron beam. The phase shift depends on the thickness of the amorphous material and its inner potential. A ~30 nm thick carbon film imparts a 90° phase shift to the scattered electrons at an acceleration voltage of 300 kV [2].

The CTF for the SAPP is given by Eq. (2).

$$CTF\ (SAPP) = \left(\begin{array}{ll} \cos(-\pi/2(2\Delta z\lambda k^2 - C_s\lambda^3 k^4)\cdot E_{damping} & k_{cut-on} < k < \dfrac{D_{phase}}{2\lambda f} \\ \sin(-\pi/2(2\Delta z\lambda k^2 - C_s\lambda^3 k^4)\cdot E_{damping} & k \geq \dfrac{D_{phase}}{2\lambda f} = k_{cut-off} \end{array} \right) \tag{2}$$

D_{phase} is the diameter of the disc of amorphous material shifting the phase of the unscattered beam; k_{cut-on} is the cut-on frequency, determined by the diameter of the central hole; f is the focal length; $E_{damping}$ is the damping due to spatial and temporal coherence.

The SAPP imparts a phase shift only to electrons scattered to spatial frequencies $< k_{cut-off}$, thus generating optimum contrast transfer for low spatial frequencies where defocus contrast is low, whereas contrast at higher resolution is generated by conventional defocusing. Only low amounts of defocus are required to generate contrast at high spatial frequencies. Due to the low thickness of the film supporting the phase-shifting material, damping at high resolution is avoided. The phase shift can be adjusted by the thickness of the phase-shifting material. Note that the phase-shifting material protects the thin support film from electron beam-induced damage.

Eq. (2) was used to calculate CTFs and to simulate SAPP TEM images of ice-embedded specimens (Fig. 2) using MATLAB programs developed previously [16]. Electron-optical parameters of a phase contrast aberration-corrected microscope (PACEM) [8] were used. The radius of the central disc of phase-shifting material was chosen to match 10 µm (Fig. 2B) and 20 µm (Fig. 2C), corresponding to cut-off frequencies of 0.26 nm^{-1} and 0.52 nm^{-1}, respectively. Compared to conventional defocusing (Fig. 2A), the SAPP generates substantial contrast at low spatial frequencies, thus enhancing the visibility of particles (Fig. 2B, C) at 500 nm defocus. To avoid sudden changes in the CTF (Fig. 2B), the phase shift imparted by the SAPP and the defocus should be carefully tuned. Fig. 2C demonstrates that the SAPP CTF is smoother for a phase shift of 45°, a cut-off frequency of 0.52 nm^{-1} and a defocus of 500 nm. Classical CTF correction can be applied to high-resolution information of SAPP TEM micrographs.

The design of the SAPP can be easily modified to obtain an improved Volta PP [21] or hole-free PP [10]. Fig. 1B shows the improved design of a hole-free PP, referred to as spot-type hole-free PP, that avoids contrast loss due to inelastic scattering of electrons by a thick amorphous carbon film. Analogous to the SAPP, a spot of amorphous material is deposited onto a mechanically stable thin support film, which is transparent to the electron beam. The diameter of the spot is chosen to match the dimension of the unscattered beam in the back-focal plane.

In contrast to the SAPP, the phase shift is imparted to the unscattered beam and depends on the inner potential and the thickness of the deposited amorphous material and is furthermore influenced by beam-induced charging [21]. The spot-type PP can be fabricated from a variety of materials without compromising the high-resolution signal. An advantage of the Volta PP is that a Volta potential can by created anywhere in the film. To increase the lifetime of the spot-type PP, a pattern of spots could be deposited in the centre of the thin support film. Depending on the performance of the PP, the electron beam is moved to the next spot.

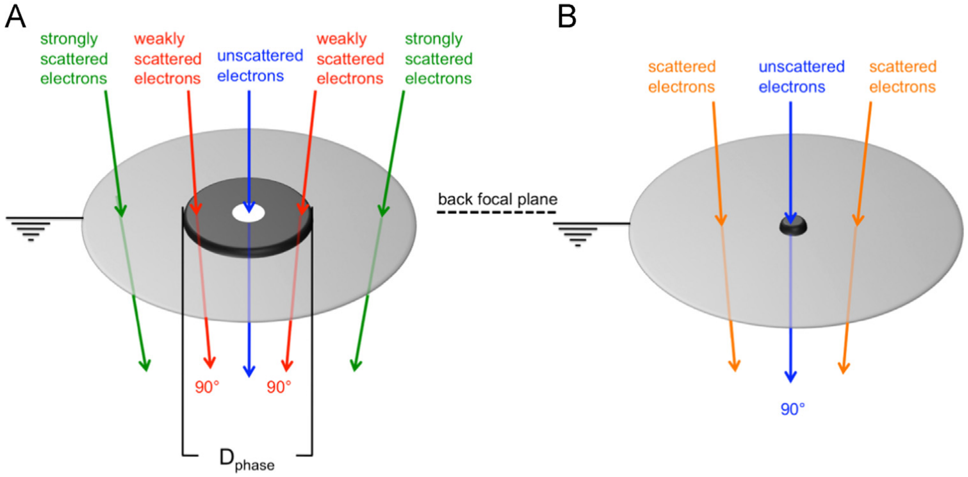

Fig. 1. New design for thin film PPs. (A) The proposed SAPP shifts only the phase of electrons scattered to low angles. The SAPP consists of an ultrathin support film made from a mechanically stable 2D material such as graphene. A central disc of phase-shifting material is mounted in the centre of the film. Finally, a hole is drilled in the centre of the disc. The phase shift imparted to the scattered electrons depends on the thickness of the material. In this example the phase shift is 90°. (B) Improved design for a hole-free PP [10]. The unscattered beam propagates through a spot consisting of phase-shifting material. The phase shift imparted to the unscattered beam depends on the thickness of the material and on beam-induced electrostatic charging of the spot.