

Phase contrast transmission electron microscopy with electrostatic phase plate

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The phase contrast mechanism plays an important role in the high-resolution observation of biological specimens with transmission electron microscopy (TEM). Biological specimens are mainly composed of low atomic number elements, which generate only poor contrast in a conventional TEM by imposing small phase shift of electron wave and leaving the amplitude almost unchanged. They are described as weak phase objects. In light optics, the phase contrast is enhanced by the introduction of a Zernike phase plate [1], which is positioned in back focal plane (BFP) of the objective lens. In electron microscopy, an additional phase shift of $\pi/2$ between scattered and unscattered electron waves is required to obtain maximal phase contrast for a weak phase object. Recently, a thin film phase plate [2] was successfully operated in TEM. It consists of a carbon film of specific thickness placed in the BFP of the objective lens; the phase of the scattered electron wave is shifted by $\pi/2$ while the unscattered electron wave is transmitted through a hole in the film. However, inelastic scattering of electrons in the carbon film as well as contamination and charging limit its application. We have fabricated an electrostatic phase plate originally proposed by Boersch [3], based on a weak electrostatic Einzel lens. As shown in the figure 1, the device consists of a ring electrode, shielded by grounded electrodes. The phase shift of the electron wave traveling through the interior of ring is uniform and constant if a low voltage is applied. Since scattered and unscattered electron waves travel through vacuum, inelastic scattering of electrons is avoided.

Fabrication of the phase plate Einzel lens uses two levels of electron beam lithography. First thin Si_3N_4 windows (200nm) are prepared using contact printing on the back and hot KOH etching of the silicon wafer. Next, a thick (1 μm) TOK resist is exposed with 100KeV electron beam lithography to define the active electrode and bonding pad, followed by 5nm Cr and 200nm of Au evaporation for lifted off in acetone. An additional 200nm of Si_3N_4 is deposited over the electrode. A second e-beam level defines the areas of the membrane to open up for etch as well as opening the pad area. The Si_3N_4 window is etched using ICP. A Kapton tape template covering the pad but open over the membrane is placed over the wafer and approximately 200nm of Au is evaporated from both the back and front side. This forms the two outer electrodes of the Einzel lens. Finally, a small circular opening is produced by Focused Ion Beam etching and is shown in the SEM micrographs of figure 2. A prototype of phase plate is tested in a Zeiss LIBRA 200FE microscope, using a objective lens with a focal length of 1.7 mm. Its position in the BFP the objective lens is controlled by a piezo-driven Kleindiek MM3A manipulator which shown in figure 3. The dimensions of the phase plate control the transmittable spatial frequency range. A specific implementation is shown in figure 2. In order to extend the spatial frequency band pass down to $\sim 0.2 \text{ nm}^{-1}$, the radius of central electrode and width of its supporting bars will reduce to optimum size in the future.

[1] F. Z. Zernike, *phys* **36** (1934) 848

[2] R. Danev and K. Nagayama, *Ultramicroscopy* **88** (2001) 243

[3] H. Z. Boersch, *Naturforsch.* **2A** (1947) 615

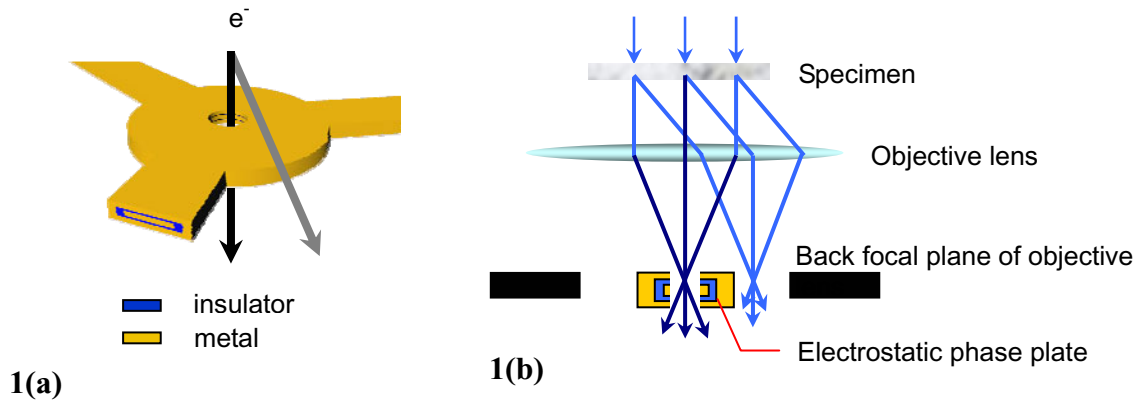


Figure 1
 (a) Schematically shows the design of electrostatic phase plate. The device consists of a ring electrode, shielded by grounded electrodes.
 (b) The electron beam transmitted inside the ring will experience a phase shift relative to scattered electron beams transmitted through the outside.

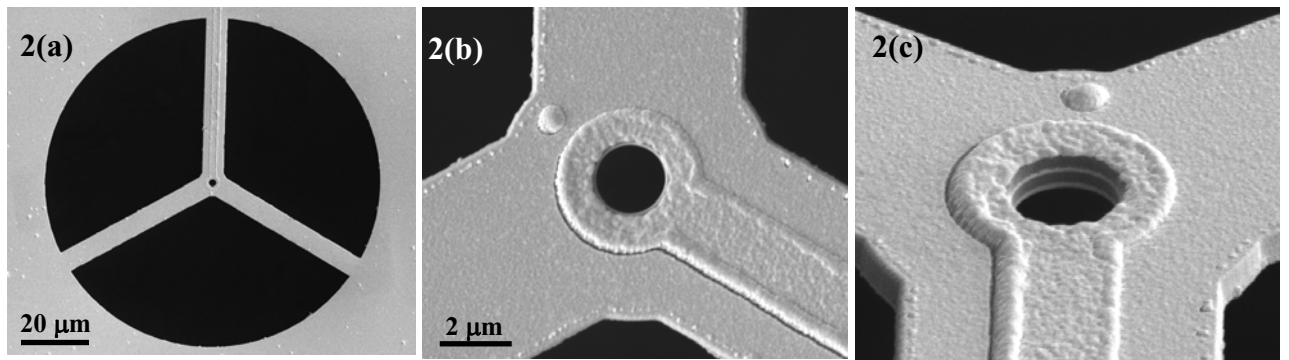


Figure 2
 SEM image of the fabricated electrostatic phase plate

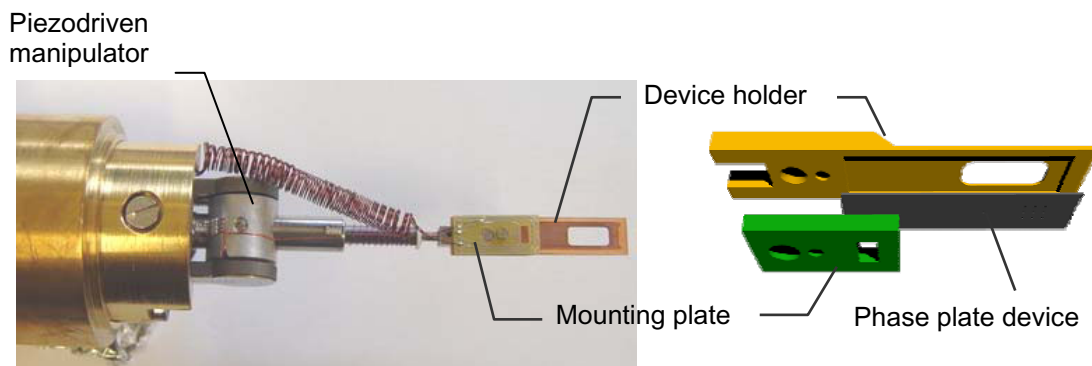


Figure 2
 Driving mechanism of electrostatic phase plate in Zeiss LIBRA 200FE TEM