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Hardware Considerations to Optimize Zernike Phase Contrast TEM for Cryo-Tomography and Single Particle Data Acquisition

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Phase contrast TEM imaging with Zernike phase plates has been successfully applied to produce images of vitreously frozen specimens, including cryosections, whole cells and single particles, with optimum contrast at or near true focus [1-7]. Several column designs from 120-400kV have been modified to include a phase plate holder inserted in the back focal plane: Hosokawa et al [8] described a 120kV design incorporating an additional lens doublet and heated phase plate holder, Motoki et al [9] presented high resolution images of amorphous germanium and graphite taken on a 200kV platform with the phase plate positioned at a conjugate back focal plane, the 3.5nm structure of rat TRPV4 cation channel was reconstructed from images taken in a 300kV phase contrast TEM equipped with a liquid helium stage [10], and critical experiments to understand phase contrast optics and improve the quality of phase plates continue [5].

As discussed in Danev et al [5], Marko et al [11] and Armbruster et al [12], the optimal design for phase contrast single particle imaging and cryo-electron tomography requires a thorough review of all optical elements to increase image contrast and automation capabilities to maximize ease of use. Features required in a dedicated column configuration have been incorporated in the JEM-2200FS (Fig. 1a). The JEM-2200FS 200kV TEM features a field-emission gun, a heated phase plate holder, an objective focal length of 5mm or larger to accommodate the phase plate holder, plus a room-temperature aperture between the cryospecimen and phase plate holder to serve as a heat shield. The phase plate holder, shown in Figs. 1b and 1c, includes X and Y piezo drives for precision positioning, as well as continuous heating of the phase plate up to 200ºC to minimize charging and contamination of the carbon film. The phase plate holder accepts multiple phase plate disks, and each disk can have 25 phase plates [4]. The small size of the central hole of the carbon phase plate (Fig. 1d), typically less than 1µm in diameter, requires precise hardware and software control to center parallel illumination in all low dose modes and tilt angles. Zero-loss energy filtering, provided by the in-column Omega filter, doubles the signal-to-noise ratio [13] and is essential for attenuating inelastic scattering when imaging thick samples such as whole cells or vitreous cryosections.

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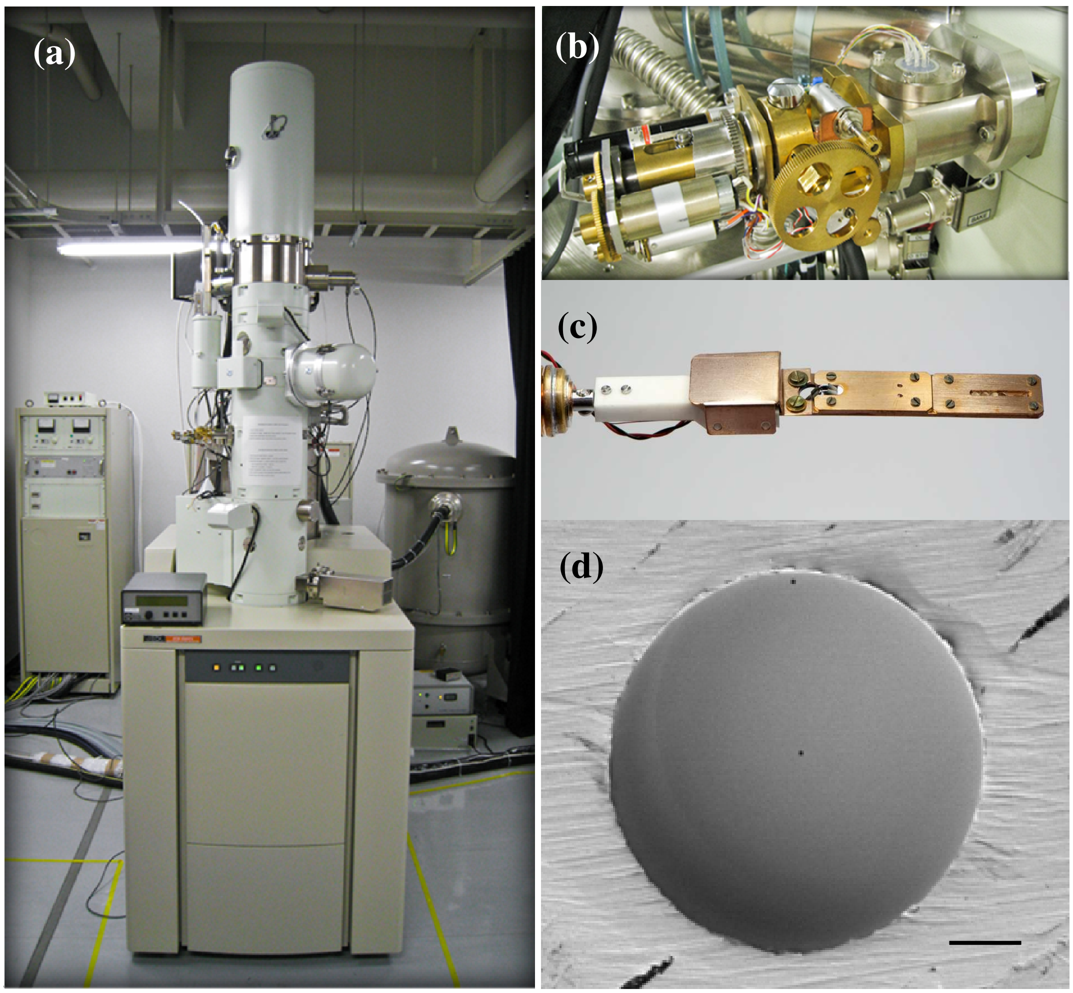

Figure 1. Phase contrast TEM at Okazaki Institute for Integrative Bioscience. (a) The JEM-2200FS includes a dedicated phase plate cryopolepiece and phase plate holder. (b) Phase plate holder motor drive assembly with piezo fine control, cover removed. (c) Tip of holder assembly for heated phase plate incorporates ceramic insulators, heaters and space for multiple phase plate disks. (d) SEM image of a 50µm diameter carbon phase plate with 1µm central hole, scale bar = 10µm.

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