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Towards In-Focus Phase-Contrast Electron Cryo-Microscopy

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Contrast in electron cryo-microscopy is limited because biological objects scatter electrons weakly and suffer from radiation damage. In conventional cryo-EM, phase contrast is generated by defocusing the objective lens, which causes oscillations of the contrast transfer function and weakens the high-resolution information. In-focus contrast can be generated by a phase plate in the back-focal plane of the objective lens. In collaboration with Zeiss NTS we are developing a 200 kV electron microscope with an in-column energy filter and a C_S corrector, dedicated to in-focus phase contrast electron cryo-microscopy with a Boersch phase plate (PACEM).

Boersch phase plates are microfabricated, solid-state devices of 5 alternating layers of conducting and non-conducting materials, in collaboration with the Caesar Research Institute of the Max Planck Society. An electrode ring with an outer diameter of 3-5 μm and an inner diameter of ~1 μm is supported by thin rods. The unscattered electrons pass through the hole in the ring, where they experience a phase shift that can be adjusted to 90° by applying a voltage (up to a few 100 mV) to the central conducting layer. The electrode ring blocks low spatial frequencies below a certain cut-on frequency. The PACEM has a 1:5 transfer lens, which magnifies the back-focal plane to minimize loss of low-resolution information, while the C_S corrector compensates for the increase in spherical aberration.

We show that the electrostatic phase plate allows free control of in-focus phase contrast in the PACEM. Cryo-EM images of vitrified TMV and purple membrane indicate that a 90° phase shift applied to the unscattered electrons results in an up to 5-fold increase in signal-to-noise ratio at intermediate resolution.

References:

Schultheiß K, Pérez-Willard F, Barton B, Gerthsen D, Schröder RR: Fabrication of a Boersch phase plate for phase contrast imaging in a transmission electron microscope. *Rev. Sci. Instrum.* **77**, 33701-33704 (2006).

Majorovits E, Barton B, Schultheiß K, Pérez-Willard F, Gerthsen D, Schröder RR: Optimizing phase contrast in transmission electron microscopy with an electrostatic (Boersch) phase plate. *Ultramicroscopy* **107**, 213-226 (2007).

Barton B, Rhinow D, Walter A, Schröder RR, Benner G, Majorovits E, Matijevic M, Niebel H, Lacher M, Schmitz S, Holik P, Kühlbrandt W: In-focus electron microscopy of frozen-hydrated biological samples with a Boersch phase plate (2011). *manuscript submitted*