Molecular Structures and High Resolution TEM

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Retrofittable phase plate system for cryo-TEM

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With recent advances in sample preparation and instrumentation, cryo-transmission electron microscopy (cryo-TEM) has become an important and powerful technique in structural biology to analyze specimen in their native state [1]. These frozen, hydrated specimen however show only weak image contrast. Nearly all image information is contained in a phase change of the scattered electron beam. This phase information can be made visible in the image by changing focus. Unfortunately, such defocusing introduces additional zeros to the contrast transfer function (CTF) of the microscope. These zeros are visible in the image as "phase flips", reducing resolution. To compensate this loss in resolution, images at different foci are recorded and the CTF of the microscope is first calculated and then corrected. Unfortunately, cryo-samples are often too beam-sensitive to withstand the recording of multiple images of the same spot which is needed for this compensation. Therefore, particular details or structures might not be visible in these images.

Using a phase plate instead of defocusing can be a viable alternative to gain contrast and preserve resolution. The phase plate is placed at the back focal plane of the objective lens inside the TEM. While passing the phase plate, an additional phase shift is applied to a part of the electron beam. Interference of the two beam parts generates enhanced contrast. Thus, phase plates can show structures that are not visible in the original data gained by defocusing. This holds particular advantage for objects, which cannot easily be averaged [2].

To push TEM phase plates from being used in single experiments towards a routine procedure, three main objectives have to be fulfilled: I) Design of a stable phase plate with high resolution capability that is suitable for commercial production II) Design of a modular, fully encoded, heated and affordable high precision positioning device fitting the most common cryo-microscopes III) Provision of an automated phase plate positioning software and perform its subsequent integration into the TEM and camera software. Here, we present a phase contrast solution whose design was based on the above-mentioned objectives.

I) We developed a new type of Zernike phase plate. It consists of a 5 nm film of amorphous Silicon coated with an additional Chromium layer. The phase shift of pi/2 is controlled by the thickness of the Chromium layer with respect to the desired accelerating voltage. To let pass the unscattered electron beam, a central hole is milled into the film using a focused ion beam system (FIB). Hole diameters vary between 200 and 1000 nm.

Figure 1A shows the currently used array of 65 phase plates to be inserted into the microscope in combination with an objective aperture. Figure 1B shows one of the 65 milled phase plate holes. Figure 2 shows two images of a frozen hydrated mouse retina. Image 2A was acquired without phase plate at 2 µm underfocus. Image 2B was made near focus with the phase plate inserted. The contrast enhancement is clearly visible.

II) We built a piezo-based, encoded positioning device, to exactly align the central hole of the phase plate to the electron beam. A heating element was integrated to reduce contamination of the phase plate. To ensure safe installation and operation, a pole-hit detection device was implemented. The modular design of the positioning device requires that only two parts have to be adapted to the different TEM models. The heart of the drive mechanism consists of three fully encoded piezo-leg motors [3]. In contrast to standard stick-and-slip piezo drives, the leg design allows higher forces along the motor axis. To maximize compatibility, the piezos are located outside the TEM column. The motors allow positioning steps of <1 nm. The encoders ensure a position repeat accuracy of 61 nm. The capabilities of the device were tested on various TEM models. Figure 3 shows the system installed at a Zeiss LEO 922. Other tested models are FEI Titan Krios, JEOL 2200 FS, Zeiss L120 & L200.

III) We developed a software to control all functions of the positioning device (e.g. drive in XYZ direction, changes in motor speed, store and recall phase plate positions, etc.). This allows a user-friendly application in a TEM and is a first step towards a fully automated phase contrast system.

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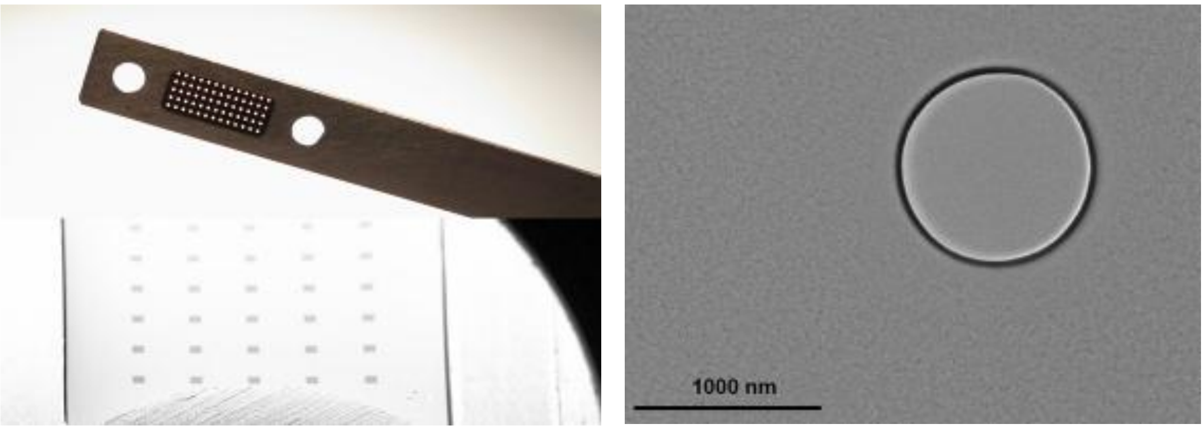
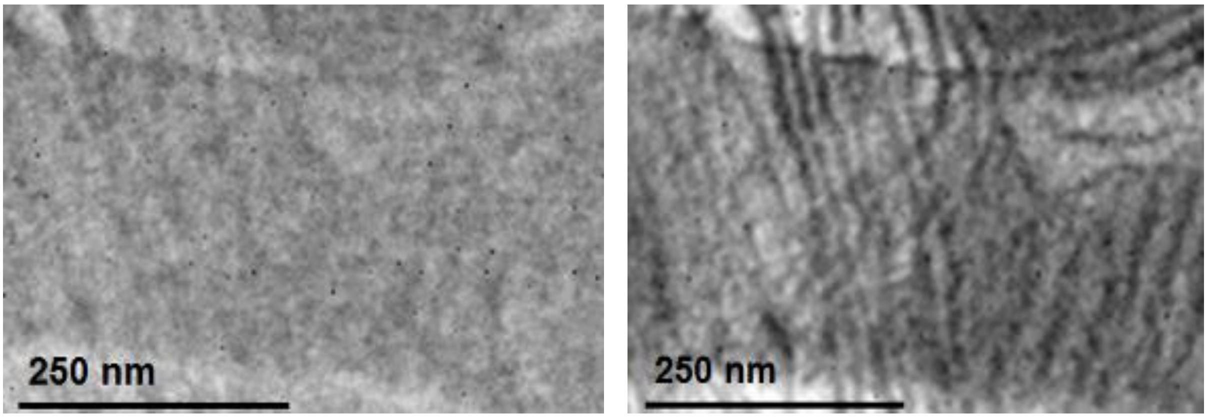


a.) b.)

Figure 1. Phase plate array with 65 phase plates (A) and milled phase plate hole (B)



a.) b.)

Figure 2. TEM micrographs without (A) and with phase plate (B). The images were taken at 2 μm defocus (A) and near focus (B).

Figure 3. The high precision phase plate positioning device installed in a Zeiss LEO 922