Phase plates are promising tools for enhancing contrast, especially in cryo transmission electron microscopy (cryo-TEM). Their advantages for electron tomography of frozen hydrated specimen have been demonstrated recently [1]. Most cryo-samples are phase objects and produce only weak image contrast when analyzed by conventional TEM. Nearly all image information is contained in a phase change of the scattered electron beam. Normally, defocusing enhances contrast of such samples - at the expense of reducing resolution of the recorded images. Alternatively, contrast can be improved in focused images by inserting a phase plate into the back focal plane of the TEM [2]. The phase plate adds an additional phase shift of ideally $\pi/2$ to the scattered electrons and thus enhances image contrast.

Nevertheless, phase plates are not yet tools for routine application. This is mostly due to difficult handling and limited durability of actual phase plate designs.

Here, we present the first installation of a new type of thin film phase plate in an FEI Titan Krios. The KonTEM phase contrast system consists of a piezo-driven nano-positioning device and a new type of thin film phase plate made of silicon instead of carbon. The piezo-driven positioning device allows semi-automated positioning of the phase plate with an accuracy of about 60 nm. The carbon-free thin film is a first step towards a phase plate for long-term application inside the TEM.

Figure 1 shows two images of the KonTEM nano-positioning device. The left image shows the positioning system installed in an FEI Titan Krios for the first time. The right image provides a detailed view of the positioning device, showing its main components: a) the three piezo drives, b) the positioning rod for mounting the phase plates and c) a flange to fit the system to the respective TEM objective port (here adapted for the ZEISS Libra 200 instead of the Titan Krios). Due to the modular design, only the flange and the front part of the positioning rod have to be adapted to the port and column design of the respective TEM model. This allows an easy adaptation of the positioning system to any standard TEM.

The drive-mechanism consists of three piezo leg motors [3]. They allow higher forces along the motor axis compared to standard stick and slip piezo drives. The system has two positions that can be used either for two phase plate chips or one phase plate chip and one standard objective aperture. With the latter set-up, it is possible to also operate the microscope without phase plate. We programmed a small software tool to control the automatic insertion of the mounted objective aperture. This tool replaces the corresponding function in the TEM control software. A second, more sophisticated program is used for positioning of the phase plate chip. Here, more functions like storing positions or manual positioning with nanometer-accuracy were implemented.

Figure 2 shows the first image taken with the KonTEM phase plate installed in an FEI Titan Krios. The phase plate thickness was calculated for an additional phase shift of $\pi/2$ at 300 kV. Figure 2a shows a normal TEM image. 10 nm gold beads were deposited on an amorphous carbon film. Images were taken at 300 KV with a pixel size of 1 Å and a beam current of 1 nA. A Falcon direct electron detector was used for imaging. Figure 2b shows the same image with the phase plate in the back focal plane of the microscope. The change in contrast is clearly visible. The fast Fourier transform (FFT) of the phase contrast image shows phase contrast up to 10 Å. This is a promising first result. Recording of tomography series will be the aim of the following experiments. Here, we will hopefully be able to take advantage of the automation possibilities of the FEI Titan Krios. This would be a great step towards using phase plate for routine work.
References


[4] The authors gratefully acknowledge funding from the German Federal Ministry of Economics & Technology’s grant “EXIST Forschungstransfer” (grant no. 03EFT4NW32). FEI Company is thanked for supporting the installation.

**Figure 1:** a) Image of the piezo-driven phase plate manipulator installed in the objective aperture port (back focal plane) of an FEI Titan Krios. The X-ray shielding has been taken off for photo. b) De-installed phase-plate manipulator (Zeiss adaptation).

**Figure 2.** First image taken with KonTEM phase plate in an FEI Titan Krios: a) Normal TEM image of gold nanoparticles deposited on an amorphous carbon film. b) Image taken under identical conditions with phase plate inserted at the back focal plane. Scale bar = 100 nm. c) FFT of b); yellow circle marks a resolution of 10Å.