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A TEM phase plate loading system with loading monitoring and nano-positioning functions

Jessie Shiue [a,*], Shao-Kang Hung [b]

[a] Institute of Physics, Academia Sinica, Nankang, Taipei 115, Taiwan
[b] Department of Mechanical Engineering, National Chiao Tung University, Hsinchu 300, Taiwan

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ABSTRACT

We present a phase plate loading system developed for a commercial transmission electron microscope (TEM). Our system can be installed without modifying the optical design of the TEM. This system is equipped with a loading monitoring set that allows users to easily and safely locate the phase plate between the pole pieces, and also comes with an airlock that permits quick loading of a phase plate without the need to re-vent the TEM column. The system uses a home-made three-axis nano-positioner to precisely position the phase plate hole at the desired location. Our system has a precision of $\sim$10 nm, an improvement of one order of magnitude compared with the precision of a phase plate holder modified from an objective aperture. We demonstrate the successful installation and the use of the loading system to place a phase plate at the desired position. Our phase plate loading system can be used to accommodate various types of phase plates and thus provides a good way to greatly speed up the development of TEM phase plates.

1. Introduction

The success of a phase TEM adopting a phase plate device is considered to have a high potential to make some impact in the field of structural biology [1–4]. With the proper tool and the appropriate wave reconstruction method, it is possible to reveal bio-structure information which can never be obtained by conventional TEM. Tremendous technical difficulties need to be overcome in order to develop a phase TEM that is applicable for bio-imaging: (1) loading the phase plate into a TEM must not degrade the functions of the TEM; (2) the phase plate needs to be positioned at the back focal plane with nanometer scale precision; (3) fabrication of a reliable phase plate is technically challenging; and (4) cryo-EM and low dose imaging are needed in order for the vulnerable bio-structure to be preserved during the image taking process [5–7].

Various phase plate designs have been proposed, including Zernike-type [8] and Hilbert-type plates [9–12]. Zernike-type plate has been adopted to thin-film style [3,8] as well as to electrostatic style phase plates [13,14], and it is also adoptable to magnetic (vector potential) style phase plate [15]. Hilbert-type plate has been adopted to thin-film style and is adoptable to magnetic style phase plate. In any case, it is essential to load a phase plate in any form into a TEM in order for a phase TEM to be effective. Due to the limited space in the pole piece gap and around the TEM column, previous work that utilized a phase plate in TEM sometimes required modifying the optical design of the TEM, such as to incorporate a transfer lens doublet [16]. The modification of the TEM optical system is certainly not a trivial work, and may not be an option for people looking to upgrade their existing TEM.

All types of phase plates require the plate to be positioned at the back focal plane with nanometer precision so that the electron beam can pass through the device precisely and can be phase shifted. With a fixed focal length, the central hole of a thin film phase plate (or the outer diameter of an electrostatic style phase plate ring) should be small enough (in submicron range) for imaging larger objects [4]. The phase plate holder should therefore have a positioning precision better than that of an objective aperture, since an aperture is generally of micrometer scale. The phase plate holder used in previous work modified from an objective aperture has a precision in the range of hundreds of nanometers [7,16], and may become another barrier to the operation and study of phase plates.

In this work, we present a TEM phase plate loading system which can be installed on a commercial TEM without the need to modify its optical design. Our system offers a loading monitoring function allowing a user to quickly and safely locate the phase plate from a computer screen. It also provides a three-axis

* Corresponding author.
E-mail addresses: yshiue@phys.sinica.edu.tw (J. Shiue), skhung@mail.nctu.edu.tw (S.-K. Hung).

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nano-positioner with <10 nm precision (as measured inside TEM) in three translational directions (x, y, and z), for fine tuning the phase plate position. We demonstrate that this system was successfully installed on a commercial field emission TEM (JEOL JEM-2100F) and was effectively used to place an electrostatic phase plate at the desired position with nanometer precision.

2. System design

2.1. Port choice and loading monitoring

Our loading system is designed to be installed through the port which is usually used for installing an energy dispersive X-ray spectroscope (EDS). Since this TEM is dedicated to bio-imaging, the material analysis function of EDS is not needed in this system. The anti-contamination device (ACD) fin, which is located right between the pole pieces, would however get in the way of inserting the phase plate. The ACD cannot simply be removed because it has to stay in place to avoid the cryo bio-specimen becoming a cold trap for catching the contaminations when the TEM is operated as a cryo-EM. In this work, we slightly trimmed the fin near the EDS port to clear out a path for phase plate loading, and kept the fin in place to serve its function. Fig. 1(a) schematically shows the relative positions of various components, including the fin, the sample holder, and the phase plate holder, around the pole pieces.

With the phase plate in place, the auto-controlled objective aperture set installed from the back of this TEM would have to be removed. To keep the function of the objective aperture, the aperture can be incorporated into the phase plate set, or another objective aperture set can be installed from the front of the TEM. We take advantage of the emptied aperture port on the back of the TEM, and design a monitoring set used for monitoring the coarse moving of the phase plate, as shown in Fig. 1(b). Practically, the monitoring system allows users to observe the phase plate moving between the pole pieces on a computer screen, and thus greatly decreases the chances of breaking the vulnerable phase plate and the holder, and also significantly speeds up the searching for the phase plate ring (or phase plate hole). It should be noted that the lighting from outside the aperture port should be carefully chosen so that it can shine through the view port, which has a diameter of 2 cm, to provide adequate illumination for the video camera to capture the inside of the TEM chamber.

2.2. Airlock

Our loading system comes with an airlock design for the phase plate to be quickly pre-pumped before entering the TEM chamber. The airlock chamber has to be small enough to fit into the limited space around the EDS port. The small airlock chamber is designed to connect with the sample pre-pumping system of the TEM, and thus no extra pumping station is needed for our loading system. The position of each component attached to the airlock can be found in "Standard operating procedures" shown as supplementary materials. The airlock chamber not only serves for quickly loading the phase plate but also provides a resting space for the phase plate while it is not in use. It has been reported that when a thin film type phase plate is used for observing a biological sample, the charging effect resulting from the contaminations, which come from the bio-specimen, could be severe, and thus it is necessary to heat the phase plate [7,16]. The charging effect may vary on different types of phase plates, and in any case the airlock chamber can provide a place where the used phase plate can be baked; so the heating of the phase plate does not need to take place in adjunction to the vulnerable cryo-specimen.

2.3. Nano-positioner: suitable for short and long range movements

In the current design, we use a home-made three-axis nano-positioner set, which provides translational motion in three directions: x, y, and z. The nano-positioner is attached on a loading rod, which can be easily loaded into the TEM. The often used micrometers are not included in our loading rod design for efficient use of the limited space around the TEM chamber.

The phase plate loading rod in this work utilizes piezoelectric motors; it is conceptually different from the well developed piezo-driving probe used on a TEM sample holder [17,18]. A piezo-driving probe uses micrometers for the motions at millimeter scale and takes advantage of the piezoelectric element for the probe to move in a short range (in nanometer to micrometer scale). A piezoelectric element provides a range of movement of tenths of a micrometer at maximum, and thus is not suitable for millimeter range movement. A "piezoelectric motor" takes advantage of the converse piezoelectric effect of the piezoelectric element, and converts it into an inertial motor, which consequently drives forward a component attached to the piezoelectric element.

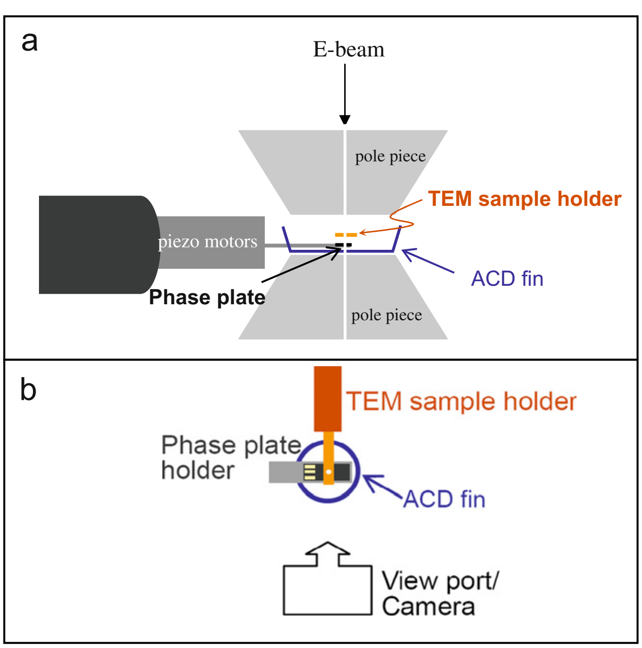

Fig. 1. Schematic of the relative positions of various components around the pole pieces: (a) side view; and (b) top view, where a monitoring set including the view port and a camera can be installed on the emptied aperture port.

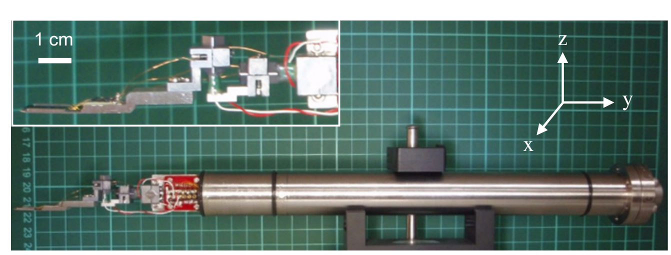

Fig. 2. The phase plate loading rod. The inset shows an enlarged photograph of the three-axis nano-positioner connected with a phase plate holder.

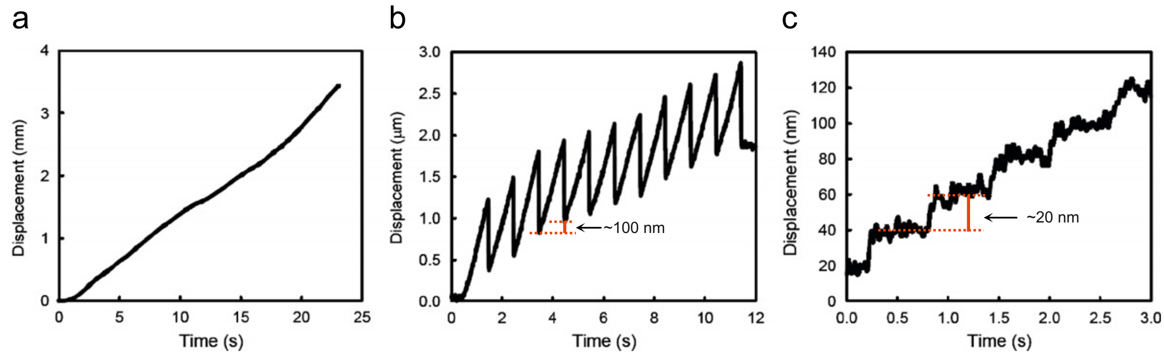

Fig. 3. The displacement of the nano-positioner as a function of time: (a) moving in millimeter range; (b) moving in micrometer range with ~100 nm average displacement in each step; and (c) moving in nanometer range with ~20 nm average displacement in each step.

Fig. 2 shows a photograph of our home-made phase plate loading rod. The inset shows an enlarged photo of the three-axis nano-positioner, which is connected with a phase plate holder at the front through an L-shaped extension. The holder has dimensions of 28 mm × 8 mm and a bottom thickness of 0.25 mm. The positioner can carry the load of the phase plate with the holder and move it in three directions with millimeter moving range while having nanometer-scale precision. The positioner is controlled by a commercial controller (Attocube, ANC 150), which is connected with an external voltage source to provide better precision control. Technical details of the positioner (the piezo motor set) can be found in supplementary materials.

3. Results

The motion of the nano-positioner was detected in an ambient environment using a high speed laser displacement sensor (Keyence Corp.) and was recorded as a function of time. The data shown in Fig. 3 represent typical moving patterns in all directions. Fig. 3(a) shows the displacement of the positioner in millimeter range, operated at 70 V and 800 Hz. As shown in the figure, the component can move several millimeters within tenths of a second, which is fairly efficient, while used in loading a phase plate. Fig. 3(b) shows the average displacement of the piezo motor in each step when the motor was directly controlled by the commercial controller, operated at 50 V and 1 Hz. It has a precision of ~100 nm/step. Fig. 3(c) shows the average displacement of the motor while it was controlled by the controller connected with an external voltage source, which can be tuned to precisely control the moving of the phase plate down to ~20 nm in each step. As shown in Fig. 3(c), the noises are obvious at nanometer scale. These noises resulting from the noisy environment, such as the air turbulence caused by human activities, were hard to be avoided in ambient even with the use of a shielding and an anti-vibration station. Such noises were not observed when the device was operated in the vacuum of the TEM and thus are of no concern for its performance.

The motion tests of the nano-positioner were done in the ambient environment as described above, and the operation of the whole loading system on a commercial TEM is demonstrated in the following. Our phase plate loading system was successfully installed on a JEOL JEM-2100F TEM. Fig. 4 shows a photograph of the installed phase plate loading system. The airlock chamber is relatively small and can be fitted well on the TEM without interrupting the operating of the pumping system installed on the back of the TEM, as shown in Fig. 4. A guiding rail is attached on

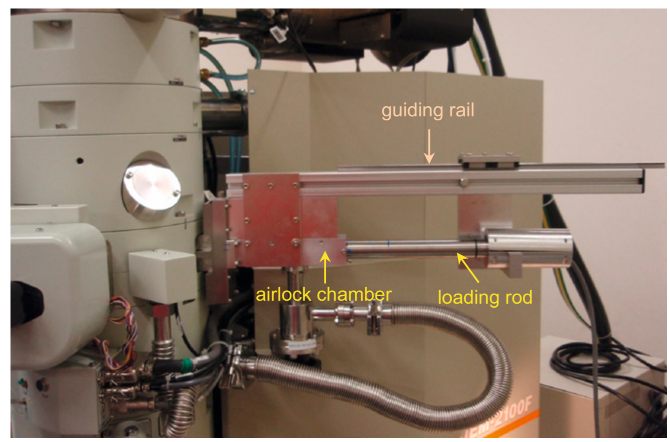

Fig. 4. The phase plate loading system installed on the JEM-2100F TEM.

top of the airlock chamber to guide the loading rod into the TEM chamber. This is a safety design so that the risk of damaging the phase plate holder during loading and removing is greatly reduced, as the guiding device hooked on the rail limits the rotating and translational forces that a user may manually apply to the rod. Further details can be found in the supplementary materials.

After the installation, we tested the pumping efficiency during phase plate loading. The pumping time needed for loading a TEM sample without phase plate operation generally takes ~20 min. The time needed for replacing a phase plate and reloading it into the TEM chamber and for the system to reach the vacuum condition for operating ($<4\times10^{-5}$ Pa) can be accomplished within 30 min. This is quite efficient and useful for developing a phase TEM, considering the phase plates are still under development and often need to be replaced and reloaded to test their effects.

A photograph demonstrating the phase plate holder precisely inserted between the TEM sample holder and the ACD fin is shown in Fig. 5. A video is provided as the supporting information to show the moving of the phase plate holder in the *y* and *z* directions in the pole pieces gap. This video was taken using the loading monitoring system, and can represent how our system is used to monitor the phase plate position on a computer screen and how to quickly and safely locate the phase plate ring (or phase plate hole). It also shows the smooth moving motion of our home-made piezo motors while traveling in millimeter scale.

The details of the loading procedures are provided as supplementary materials.

After locating the phase plate device, the fine tuning to position the hole for the electron beam to pass through should be operated while accompanied with TEM observation. Fig. 6(a) shows the scanning electron microscope (SEM) image of an electrostatic phase plate used in this work for testing the loading system. This phase plate device has a simplified structure that will be further studied and reported in the future. The inset of Fig. 6(a) shows the central hole of this phase plate. The hole with a diameter of 670 nm had to be precisely positioned where the electron beam can pass through. A carbon film was used as a TEM sample simply for testing purposes. We targeted a feature on the carbon film (a hole), which has a feature size of ~650 nm. Fig. 6(b) shows how a phase plate hole is precisely positioned to align with the desired feature on the TEM sample. The dark shadow on the image is the cantilever of the phase plate device, as shown in Fig. 6(a). The beam was set as parallel illumination while taking this image. The inset of Fig. 6(b) shows the TEM image taken before the phase plate was in place. When the holder was operated inside TEM, the minimum step of < 10 nm can be achieved. The displacement of one step was measured by comparing the relative positions of the phase plate ring shown in two TEM images taken before and after applying a voltage to the piezoelectric element. The nano-positioner indeed served its function for positioning the phase plate hole exactly at the desired position with precision at nanometer scale.

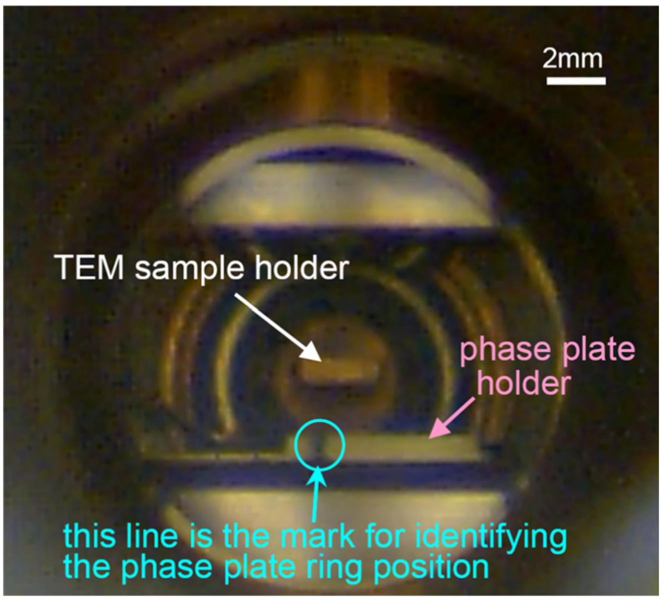

Fig. 5. The phase plate holder is precisely positioned between the TEM sample holder and the ACD fin. This photo was taken using the loading monitoring system.

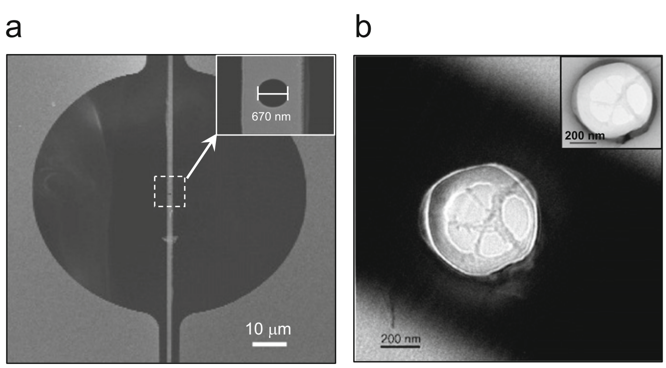

Fig. 6. (a) SEM image: the electrostatic phase plate used for testing the loading system. The inset shows that the central hole of the phase plate has a diameter of ~670 nm. (b) TEM image: the central hole of the phase plate can be precisely positioned to align with the desired feature on the TEM sample. The inset shows the TEM image of this featured area before the phase plate was in place.

Fig. 7 shows a series of Fourier-transformed images of a carbon test sample obtained when a phase plate device was inserted and applied with various voltages. The changes of the Thon rings verified the phase shifts were induced by the tunable phase plate. The contrast enhancements are similar to the results obtained from electrostatic phase plates reported by Alloyeau et al. [19]. The study of the phase plate effect on the TEM images is not the main theme of this work, while the results shown in Fig. 7 confirm a phase plate device with a central hole diameter < 1 μm can be effectively operated using our loading system.

4. Conclusion

We developed a phase plate loading system which can be installed on a commercial TEM system through an EDS port without modification of the optical system of the TEM. This loading system was successfully installed on a field emission TEM (JEOL JEM-2100F). Our system comes with a loading monitoring set for users to monitor the moving of the phase plate holder on a computer screen, and thus allows the phase plate device to be driven safely and quickly into the desired position. The loading set uses a three-axis nano-positioner, which takes advantage of piezo motors for the phase plate to travel in millimeter range while having a translational precision < 10 nm. The replacing and reloading of a phase plate can be accomplished within 30 min through the airlock that comes with this loading system. Our loading system therefore provides an efficient and effective way to speed up the studies and development of various phase plates utilized on a TEM. With some alternations, the functions of auto-loading and computer control of phase plate alignment can be incorporated into our system. Similar loading system designs can be slightly modified with dimensions to fit different commercial TEM systems.

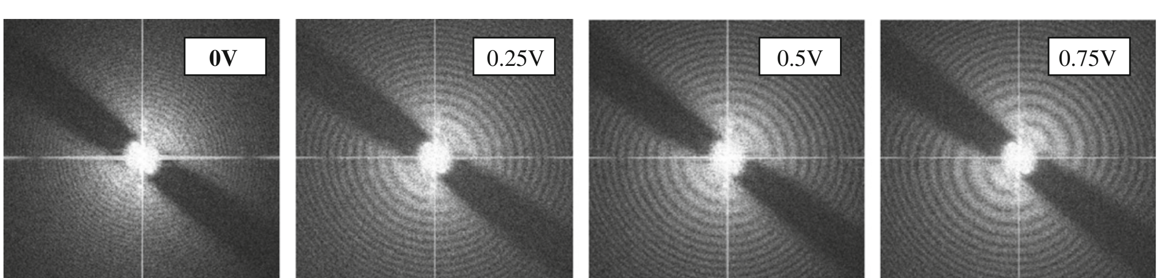

Fig. 7. Fourier-transformed images of a carbon test sample obtained with phase plate inserted and applied with various voltages.

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Appendix A. Supplementary Material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ultramic.2010.05.005.

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