

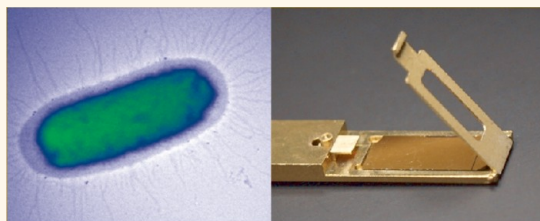
On-Chip Thin Film Zernike Phase Plate for In-Focus Transmission Electron Microscopy Imaging of Organic Materials

Pai-Chia Kuo,^{†,*,§} I-Hui Chen,^{*,§} Chih-Ting Chen,^{*,§} Ku-Pin Lee,^{*,§} Chun-Wei Chen,[†] Chih-Cheng Lin,[†] Shirley Wen-Yu Chiu,[^] Yong-Fen Hsieh,^{||} Yuh-Lin Wang,[^] and Jessie Shiue^{*,§,*}

[†]Department of Materials Science and Engineering, National Taiwan University, Taipei 106, Taiwan, [‡]Research Program on Nanoscience and Nanotechnology and [§]Institute of Physics, Academia Sinica, Taipei 11529, Taiwan, [^]Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 10617, Taiwan, and ^{||}Materials Analysis Technology Inc. (MA-tek) Hsinchu, Taiwan 300

ABSTRACT Transmission electron microscopy (TEM) is a powerful tool for imaging nanostructures, yet its capability is limited with respect to the imaging of organic materials because of the intrinsic low contrast problem. TEM phase plates have been in development for decades, yet a reliable phase plate technique has not been available because the performance of TEM phase plates deteriorates too quickly. Such an obstacle prohibits in-focus TEM phase imaging to be routinely achievable, thus limiting the technique being used in practical

applications. Here we present an on-chip thin film Zernike phase plate which can effectively release charging and allow reliable in-focus TEM images of organic materials with enhanced contrast to be routinely obtained. With this stable system, we were able to characterize many polymer solar cell specimens and consequently identified and verified the existence of an unexpected nanoparticle phase. Furthermore, we were also able to observe the fine structures of an *Escherichia coli* specimen, without staining, using this on-chip thin film phase plate. Our system, which can be installed on a commercial TEM, opens up exciting possibilities for TEM to characterize organic materials.



KEYWORDS: TEM · phase plate · cryo-EM · organic devices · polymer solar cells

Organic devices, such as polymer solar cells and organic light-emitting diodes (OLEDs), have attracted considerable attention in recent years. The efficiencies of these organic devices greatly depend on their nanoscale structures. For example, the nanoscale morphology affects the charge transport in the donor/acceptor blend and hence influences the power conversion efficiency of a bulk heterojunction polymer solar cell.^{1–5} Characterizing nanostructures in organic devices thus has a critical importance in optimizing their performance. Transmission electron microscopy (TEM) has long been used for characterizing nano/microstructures in materials, while its application on organic materials has been limited because the contrast of TEM images for organic specimens is usually poor since they are constituted with light elements, such as C, O, and N. The defocus technique is well-known for enhancing contrast of TEM

images, while it is a tricky technique for not only does it result in a significant loss of resolution, but it also causes some artifacts, consequently complicating the interpretation of the images. In-focus TEM imaging for organic materials with enhanced contrast is therefore a much needed technique, particularly with the rapidly growing interest in organic devices.

Adopting a phase plate in the optical path of a TEM to enhance the contrast of the in-focus image was proposed 60 years ago.^{6,7} Various TEM phase plates have been in development for decades,^{8–16} and some promising results using thin film Zernike phase plates have been presented.^{17,18} Using a thin film phase plate inevitably sacrifices some high-resolution signals, due to the loss of coherence resulting from the interaction between the scattered wave and the carbon film. Such a drawback, however, does not preclude its major advantage in characterizing nanostructures of organic materials

* Address correspondence to yshiue@phys.sinica.edu.tw.

Received for review September 28, 2012 and accepted December 14, 2012.

Published online December 26, 2012
10.1021/nn304511p

© 2012 American Chemical Society

which are too vague under conventional TEM. Although Zernike phase plates have been successfully used to reveal the structure of ice-embedded biomolecules,¹⁸ a more reliable phase plate technique that allows phase contrast images to be taken reliably and repeatedly is still in need since the performance of TEM phase plates deteriorates too quickly.^{7,13,16} One of the major causes for the unstable performance of a TEM phase plate is the charging effect.^{13,16} When a physical phase plate is present in the pathway of the electron beam, the buildup of charged particles in some local sites would result in instability and distortion of the image formed. Such a notorious charging effect resulting from phase plates has been known for some time and is still considered an obstacle for TEM phase plate technology to be put into routine use until very recently.^{13,16} Practically, the investigation of nanostructures of organic materials with phase plates can hardly be realized without a reliable and routinely achievable method for taking in-focus phase TEM images.

Here we present an on-chip thin film Zernike phase plate system, operated on a commercial 200 kV TEM, which can effectively release the charging caused by the phase plate, provide a steady performance, and realize the application of in-focus TEM phase imaging by achieving the routine operation of TEM phase plates. Such a reliable system allowed us to recognize and verify an unexpected nanophase in polymer solar cell samples of in-device thickness. Moreover, we were able to observe fine structures of biospecimens without staining using this stable TEM phase imaging technique.

RESULTS AND DISCUSSION

To ensure that the charging is indeed released effectively with our on-chip thin film phase plate system, we investigated the drifting pattern of TEM imaging, using a standard TEM specimen with Au nanoparticles (particle size <5 nm). A standard specimen of Au was used to avoid the image drifting caused by the sample charging itself. It was found that when the phase plate chip was not loaded, the drifting rate is ~ 2 nm/min and toward the same direction, an indication of mechanical drifting of the TEM system itself. Such a drifting rate is within the acceptable mechanical drifting range of this TEM system operated in the environment of our TEM lab. With the on-chip phase plate in position, the imaging drifting rate and pattern were the same as drifting observed without a phase plate. However, the image drifting pattern is rather different while using an “incomplete” chip as the phase plate substrate, which was only coated with a top layer of Au instead of coated with a three-dimensional Au layer. Using this “without 3D Au coverage” phase plate chip, the image drifting rate was found >10 nm/min, and the drifting direction changes randomly, a feature of the charging effect. The TEM images obtained from using the two chips are shown comparably in Figure 1a,b.

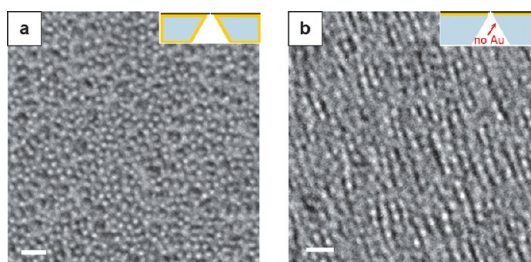


Figure 1. Charging effectively released using a properly made on-chip thin film phase plate. TEM images of a standard TEM specimen of Au nanoparticles obtained (a) using a phase plate chip wrapped around a thin Au layer (inset), (b) using a phase plate chip coated with a surface layer of Au, but no Au coating on the side wall (inset). The image in (b) is distorted as a result of the charging effect. Scale bar: 20 nm. Fast Fourier transforms (FFTs) of the TEM images are provided in the Supporting Information.

Figure 1a shows the image of Au particles obtained using a properly made phase plate chip, and the image of the same specimen obtained using a poorly made chip is shown in Figure 1b. If the chip is not fully covered by Au, even though it has a Au top layer and the metal holder cover touches and grounds the chip surface, the release of charges is still insufficient and results in a distorted image, as shown in Figure 1b. We found that the distortion resulting from such charging is unpredictable and changes direction arbitrarily. The practical problem of the charging effect is that it is unexpected and uncontrollable. It is possible that the image is stable for 1 s, yet distorted in the next. Thus if the charging resulting from all sources is not properly released, it is unlikely to routinely obtain reliable images. Our finding may present a technical challenge for the electrostatic type phase plates,^{5,7} which inevitably need an insulating layer in the pathway of the electrons to generate a phase shifting effect.

It should be noted that mechanical drifting of a phase plate is also an important factor causing unstable phase plate imaging. This is an issue that was rarely addressed in the past. The correct position of the Zernike phase plate hole, including the centering and the correct focal plane height, is essential for the phase plate to perform well. We found that, right after a phase plate was loaded into the TEM, the drifting rate of the phase plate could be rather high, ~ 100 nm/min, likely a result of relaxation of the O-rings on the phase plate loading path. Such a drifting of the phase plate would result in unstable imaging. However, after the phase plate system was stabilized, the drifting rate was <4 nm/min, giving us sufficient time to take images using our phase plate chip. Computer-controlled phase plate holders to compensate the drifting are currently being developed (Supporting Information). Before such a computer-controlled method is realized, however, an easy way to operate our system is to simply wait for a few hours after a phase plate chip is loaded into the TEM. During such a waiting time, if needed, other imaging

tasks without needing a phase plate can still be conducted by adopting an empty aperture on the chip. Once the phase plate chip is mechanically stabilized, the piezo motor set attached to the phase plate holder can quickly move the chip to the phase plate aperture without disturbing any O-rings. The fine positioning of the phase plate in the three directions, x , y , and z , can also be achieved by using the piezo motor set. The TEM specimens can be changed anytime since the phase plate system is independent of the specimen loading system. Such an operation principle allows us to keep imaging without worrying about the mechanical drifting of the phase plate.

With our on-chip thin film Zernike phase plate, we were able to observe many organic devices, including OLED and polymer solar cell specimens. Figure 2a shows a typical TEM phase image of a photoactive layer of P3HT/PCBM (poly(3-hexylthiophene)/[6,6]-phenyl-C61-butyrac acid ethyl ester) (1:1) blend retrieved from a bulk heterojunction polymer solar cell device (Supporting Information) taken near in-focus conditions. The TEM specimen used in Figure 2 has a thickness of ~ 160 nm and was prepared in the same way as a polymer layer fabricated in a solar cell device (Supporting Information). Some spaghetti-like features

are found in the polymer blend (Figure 2a). These features are invisible in the in-focus TEM image without using a phase plate (Figure 2b). The spaghetti-like phase in the P3HT/PCBM blend is generally believed to be P3HT. With further thermal annealing, P3HT crystallizes better and should thus be easier to be identified under TEM.^{19,20} Our samples, however, did not go through thermal annealing, yet we were able to identify them in the in-focus TEM image using a phase plate. We have investigated numerous polymer solar cell specimens with their in-device thicknesses and found that the P3HT fibers crystallize better in the solvent with a longer drying time, consistent with the common observation in the field.²¹

The significance of the results shown in Figure 2 is the ability to observe these fiber-like features in such a thick (~ 160 nm) specimen. Past work that revealed the nanomorphology of P3HT/PCBM in TEM specimens with a thickness of 50 nm was impressive; using complicated electron tomography, the three-dimensional nanomorphology in a thermally annealed P3HT/PCBM specimen of 100 nm thickness was also nicely achieved.¹⁹ Thicker polymer solar cell samples with such low contrast are difficult to observe with conventional TEM techniques without using a high value of defocus, which inevitably results in difficulty interpreting the image. With our reliable TEM phase plate system, this has become possible.

The striking capability of our system is demonstrated in Figure 3, in which an unexpected nanophase is identified by using an on-chip thin film phase plate. We noticed a nanoparticle phase (5–8 nm) in some P3HT/PCBM specimens (Figure 3a). Such an ambiguous nanofeature with such a low contrast might be mistaken as contaminations under defocused TEM imaging (Figure 3b) and is totally invisible in the conventional in-focus image (Figure 3c). Further defocusing would induce more noise and artifacts, as predicted by the contrast transfer function (CTF), and make the studying of the image in such a high magnification impossible

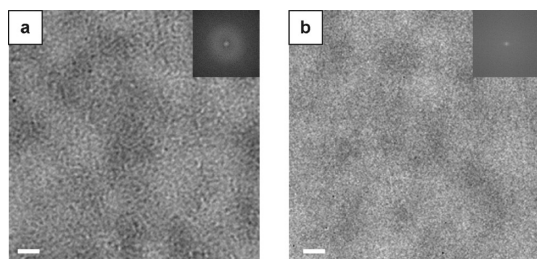


Figure 2. TEM images of a P3HT/PCBM film (160 nm), a photoactive layer of a polymer solar cell device. The image was taken under the condition of (a) in-focus with phase plate, (b) in-focus without phase plate. Insets: Fast Fourier transforms (FFTs) of the corresponding images. Scale bar: 50 nm. Enlarged FFTs of the TEM images are provided in the Supporting Information.

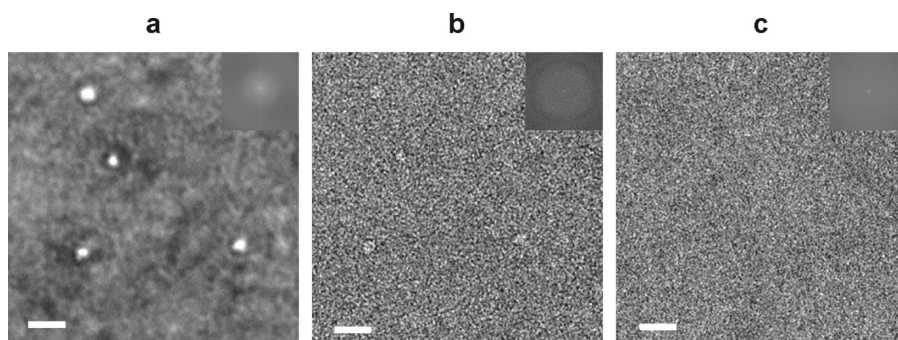


Figure 3. Identification of a nanoparticle phase (5–8 nm) in a P3HT/PCBM specimen. TEM images of a P3HT/PCBM specimen taken under the condition of (a) in-focus with phase plate, (b) defocus (underfocus $\sim 2 \mu\text{m}$) without phase plate, and (c) in-focus without phase plate. These images were taken in the same position of the same specimen. The nanoparticle phase (white dots in image a) is clearly seen in the in-focus phase image, barely seen in the defocus image, and totally invisible in the in-focus image without using a phase plate. Insets: Fast Fourier transforms (FFTs) of the corresponding images. Scale bar: 20 nm. Enlarged FFTs of the TEM images are provided in the Supporting Information.

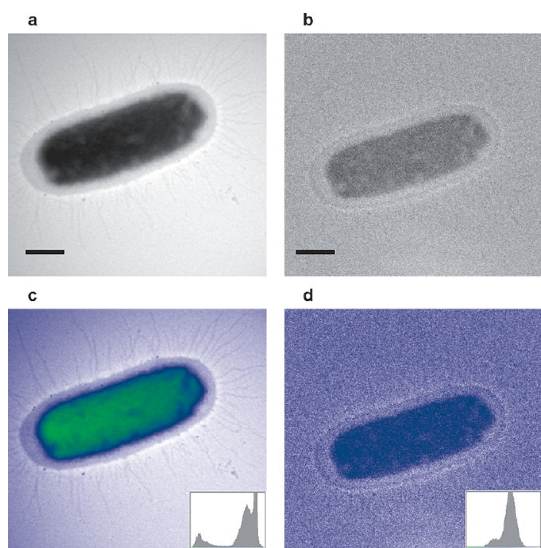


Figure 4. Contrast enhancement of TEM images of “unstained” *E. coli*. The TEM images were taken under the in-focus condition and (a) with a phase plate, (b) without a phase plate. Applying a two-color coding scheme on (a) and (b) resulted in the images in (c) and (d), respectively. Insets: Histograms of the main images with the corresponding color scales. Scale bar: 500 nm. Fast Fourier transforms (FFTs) of the TEM images are provided in the Supporting Information.

(Supporting Information). While using our system, these nanoparticles in the organic matrix of P3HT/PCBM are clearly identified in the in-focus TEM imaging condition, as shown in Figure 3a. We examined many P3HT/PCBM specimens with various fabrication conditions and noticed that this nanoparticle-like phase only exists in specimens prepared in 1,2,4-trichlorobenzene (TCB) with a sufficient drying time. The P3HT/PCBM blend prepared in chloroform (CHCl_3), which dried much more quickly, did not contain such a nanoparticle phase. The nature of such a phase is yet to be investigated and is not the main theme of this work. However, when nanostructures in an organic material with polymer/polymer blends are unknown, the unexpected nanophases are likely to be overlooked under TEM. This is possibly why such a nanoparticle-like phase has never been reported in any P3HT/PCBM work, but with our stable TEM phase plate system, the unexpected nanophase is observed and verified in P3HT/PCBM polymer solar cell specimens for the first time.

Besides characterizing organic devices, our system also has great potential in applications in the biological field. With its stable performance, the imaging of fine structures of biological samples without going through complicated sample preparation process, such as staining, becomes possible. Figure 4a shows the in-focus TEM image of *Escherichia coli* (*E. coli*) without staining (Supporting Information), which was obtained by adopting a phase plate. For comparison, the same unstained *E. coli* specimen was observed using the same TEM imaging conditions but without adopting a phase plate, and the resulting image is shown in

Figure 4b. The pili surrounding the cell envelope can be clearly seen in the phase image (Figure 4a), while they could not be identified in Figure 4b. The image contrast of the cell envelope itself is also enhanced in the phase image.

To further understand the contrast enhancement achieved by using our phase plate system, a color-coding scheme was applied to the *E. coli* images (Figure 4a,b) using computer imaging software (Photoshop, Adobe). The same color-coding scheme was applied to Figure 4a,b, and the resulting color-coded images are shown in Figure 4c,d, respectively, in which the histograms of the corresponding images are shown in the insets. The 256 gray shades of the original image are coded with two colors (green and blue), as demonstrated in the *x*-axis of the histograms (insets in Figure 4c,d), where the *y*-axis of the histograms represents the arbitrary intensity of each color shade in the corresponding image. The *E. coli* image obtained from adopting a phase plate has a bimodal distribution in the histogram and is reflected in the color-coded image (Figure 4c), in which the pili are shown in blue, and the envelope area is shown in green with some fine structures inside. On the other hand, the image obtained without adopting a phase plate does not have a sufficient shading difference, as shown in the inset of Figure 4d, to distinguish the fine structures in the image, and the two-color coding scheme would result in a single-color (blue) image (Figure 4d). The results provided in Figure 4 indicate that observing biological specimens in unstained condition using our reliable on-chip thin film phase plate system is achievable.

It has been reported that another possible source of charging is contamination of the carbon film during the use of thin film phase plate.¹³ In our work, however, we did not find such a problem while using our on-chip thin film phase plate. Our phase plate chip system has been routinely operating for months for observing many organic devices as well as some biological specimens, and no noticeable charging effect was observed. It is possible that our phase plate chip is indeed effective in releasing charging from all sources, and that the contamination coming from our TEM specimens is insignificant. Our phase plate chip is low cost and can be easily loaded and thus can also serve as disposable phase plates if used for cryo-EM where contamination is likely to be severe.

CONCLUSION

We present an on-chip thin film Zernike phase plate system which allows in-focus TEM images with greatly enhanced contrast to be routinely achievable. Reliable in-focus phase imaging is realized by effectively releasing the charging resulting from the phase plate. The on-chip thin film phase plate system presented in this work can be installed in a commercial TEM system without the need to modify the TEM optical design and

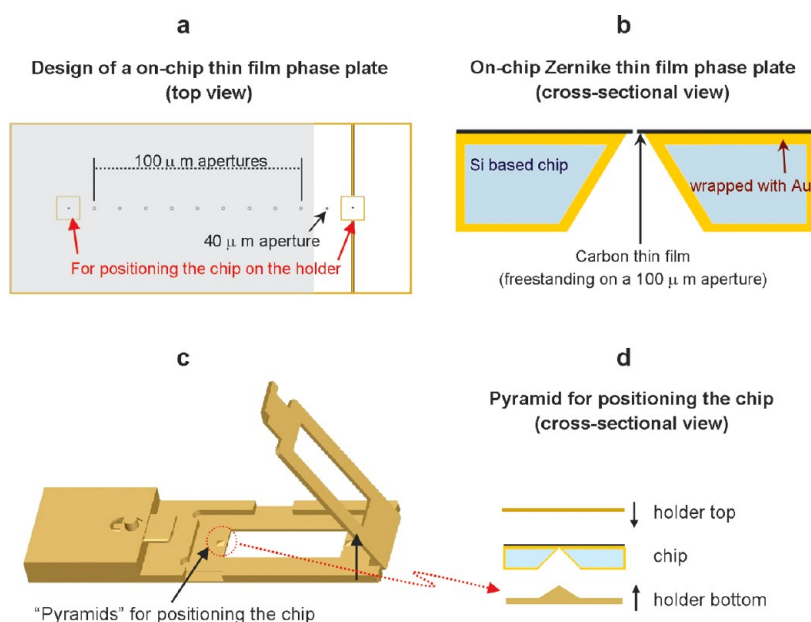


Figure 5. Design of an on-chip thin film Zernike phase plate system. Schematics of (a) a thin film phase plate chip design (top view), and (b) an on-chip Zernike thin film phase plate (cross-sectional view; does not correspond to the real chip dimensions). The thin gold line on the chip is used for identifying the orientation of the chip. The chip has numerous apertures and is partially covered by a thin amorphous carbon film (the light gray area). The film is freestanding on the apertures with small drilled holes, which serve as thin film Zernike phase plates; the uncovered aperture serves as an objective aperture. The chip is wrapped in a thin Au layer for releasing charging. (c) Schematic of a chip holder. (d) Cross-sectional view of the chip positioned by a holder. The chip is positioned on the holder by two “pyramids” which fit the first and the last apertures on the bottom of the chip. The holder top touches the chip surface, which not only secures the chip on the holder but also helps release charging.

is suitable for characterizing organic devices and biological samples. Using this stable system, we were able to identify an unexpected nanophase in a series of polymer solar cell specimens. We were also able to

observe some fine structures in unstained *E. coli* with this system. Our TEM phase plate system thus opens up exciting opportunities for the study of organic materials.

MATERIALS AND METHODS

Our on-chip thin film phase plate design is shown schematically in Figure 5, with a top view (Figure 5a) and a cross-sectional view (Figure 5b, does not correspond to the real chip dimensions). The chip used in this work has dimensions of 14 mm × 6 mm. We find that a series of phase plates and apertures arranged in one single line is easier to operate than arranging them in an array format, thus we chose to use such a design. In this chip, there is one aperture of 40 μm in diameter serving as an objective aperture and nine apertures of 100 μm in diameter which are covered with thin amorphous carbon film serving as phase plates. One can easily change the dimensions of the apertures on the chip, as well as the carbon film coverage area to suit specific needs.

Taking advantage of the surface flatness of a silicon-based chip (the average surface roughness of a commonly used Si wafer is 0.5–1 nm), an on-chip thin film phase plate is likely to be more efficient in releasing charging than the on-grid thin film phase plate used in previous work,⁷ in which the thin film is attached to a metal piece with multiple micrometer sized holes (*i.e.*, phase plate grid). Since the surface of such a metal grid is not microscopically flat, and a surface oxide layer is likely to form locally, this leads to less effective release of charging from the carbon film attached to it. A large contacting area between the Si chip, coated with a thin Au layer (100–150 nm), and the carbon film provides an effective charge release area. A thin Au coating wrapping the chip before the carbon film is attached to the chip is essential because the high conductivity and inert nature of Au are desirable for charge release. Using silicon-based chips to carry thin film phase plates also has the advantages of

low fabrication cost and reproducibility in dimensional precision, which is important for routinely aligning the phase plate.

Note that the chip is “wrapped” with a Au layer before the phase plate film is attached to it, as shown in the cross-sectional view in Figure 5b. The reason that the chip has to be covered by Au in a three-dimensional (3D) manner, rather than simply coated with Au on the top surface, is to avoid charging caused by electrons coming from all directions. These electrons, including scattered and secondary electrons, could build up local fields on any sites along the electrons’ passing path, thus a simple top conducting layer is insufficient to release the charges. After the amorphous carbon film (25–35 nm in thickness) is attached to the chip, phase plate holes of 500 nm to 1 μm in diameter were made on the carbon film using a 30 kV focused ion beam (Supporting Information). The amorphous carbon film used in this work has a uniformly high quality, evidenced by examination under TEM (Supporting Information), which was made possible by loading the chip into the TEM specimen position using a specially made TEM sample holder which can accommodate the chip. The carbon film attaches firmly to the chip substrate, thus no particular handling precautions of the phase plate chip are required.

Precise mounting of the chip and its repeated delivery to the desired position in the TEM are important for the system to work. Figure 5c schematically shows the design of our chip holder. The holder is made of copper with a holder bottom and a thin cover, which touches the chip surface and helps to further release the charges from the chip. Note that there are two positioning “pyramids” on the holder bottom. Since pyramid-shape-like holes form on the Si wafer during the KOH (potassium hydroxide)

etching procedure needed for chip fabrication, we took advantage of such uniquely shaped holes on the chip for positioning, as shown schematically in Figure 5d. The two pyramids on the holder are used to fit the first and the last apertures, consequently making sure that the chip is always well aligned on the holder, and thus making the positioning of the phase plate quick, easy, and repeatable. Such a design, integrating the chip and the chip holder, could overcome the operating difficulty for locating the phase plate hole, effectively releasing the charging, and enabling the routine operation of such an on-chip thin film phase plate. The phase plate holder is attached to a loading system, similar to the one shown in the previous work,²² and operated on a JEOL JEM-2100 LaB₆ TEM (Supporting Information). All TEM images presented in this work were taken under the condition that the TEM was operated at 200 kV, and all phase plate assisted images (phase images) were taken using a 1 μm phase plate hole.

Conflict of Interest: The authors declare no competing financial interest.

Acknowledgment. We thank Dr. W.-H. Chang and Dr. Y.-M. Wu for knowledge on carbon film fabrication, Dr. M.-J. Chen, L.-W. Nien, and Y.-C. Yeh for help with film thickness measurement, and C. K. Lin for help on the early stage of the fabrication of the phase plate loading system. Technical support from NanoCore, core facilities at Academia Sinica, is acknowledged. This work was partially funded by a research program on nanoscience and nanotechnology at Academia Sinica and National Science Council in Taiwan.

Supporting Information Available: Additional experimental details, TEM system information, operation of the on-chip phase plate system, and Fast Fourier transforms of all TEM images are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

REFERENCES AND NOTES

- Vanlaeke, P.; Swinnen, A.; Haeldermans, I.; Vanhoyland, G.; Aernouts, T.; Cheyins, D.; Deibel, C.; D'Haen, J.; Heremans, P.; Poortmans, J.; *et al.* P3HT/PCBM Bulk Heterojunction Solar Cells: Relation between Morphology and Electro-optical Characteristics. *Sol. Energy Mater. Sol. Cells* **2006**, *90*, 2150–2158.
- Wang, T.; Dunbar, A. D. F.; Staniec, P. A.; Person, A. J.; Hopkinson, P. E.; MacDonald, J. E.; Lilliu, S.; Pizzey, C.; Terrill, N. J.; Donald, A. M.; *et al.* The Development of Nanoscale Morphology in Polymer: Fullerene Photovoltaic Blends during Solvent Casting. *Soft Matter* **2010**, *6*, 4128–4134.
- Chang, C.-Y.; Wu, C.-E.; Chen, S.-Y.; Cui, C.; Cheng, Y.-J.; Hsu, C.-S.; Wang, Y.-L.; Li, Y. Enhanced Performance and Stability of a Polymer Solar Cell by Incorporation of Vertically Aligned, Cross-Linked Fullerene Nanorods. *Angew. Chem., Int. Ed.* **2011**, *50*, 9386–9390.
- Staniec, P. A.; Parnell, A. J.; Dunbar, A. D. F.; Yi, H.; Pearson, A. J.; Wang, T.; Hopkinson, P. E.; Kinane, C.; Dalgliesh, R. M.; Donald, A. M.; *et al.* The Nanoscale Morphology of a PCDTBT:PCBM Photovoltaic Blend. *Adv. Energy Mater.* **2011**, *1*, 499–504.
- Chu, T.-Y.; Alem, S.; Tsang, S.-W.; Tse, S.-C.; Wakim, S.; Lu, J.; Dennler, G.; Waller, D.; Gaudiana, R.; Tao, Y. Morphology Control in Polycarbazole Based Bulk Heterojunction Solar Cells and Its Impact on Device Performance. *Appl. Phys. Lett.* **2011**, *98*, 253301–253303.
- Nagayama, K.; Danev, R. Phase-Plate Electron Microscopy: A Novel Imaging Tool To Reveal Close-to-Life Nanostructures. *Biophys. Rev.* **2009**, *1*, 37–42.
- Nagayama, K. Another 60 Years in Electron Microscopy: Development of Phase-Plate Electron Microscopy and Biological Applications. *J. Electron Microsc.* **2011**, *60*, S43–S62.
- Unwin, P. N. T. Phase Contrast and Interference Microscopy with the Electron Microscope. *Philos. Trans. R. Soc., B* **1971**, *261*, 95–104.
- Parsons, D. F.; Johnson, H. M. Possibility of A Phase Contrast Electron Microscope. *Appl. Opt.* **1972**, *11*, 2840–2843.
- Danev, R.; Nagayama, K. Transmission Electron Microscopy with Zernike Phase Plate. *Ultramicroscopy* **2001**, *88*, 243–252.
- Barton, B.; Joos, F.; Schroder, R. R. Improved Specimen Reconstruction by Hilbert Phase Contrast Tomography. *J. Struct. Biol.* **2008**, *164*, 210–220.
- Barton, B.; Rhinow, D.; Walter, A.; Schroder, R.; Benner, G.; Majorovits, E.; Matijevic, M.; Niebel, H.; Muller, H.; Haider, M.; *et al.* In-Focus Electron Microscopy of Frozen-Hydrated Biological Samples with a Boersch Phase Plate. *Ultramicroscopy* **2011**, *111*, 1696–1705.
- Danev, R.; Glaeser, R. M.; Nagayama, K. Practical Factors Affecting the Performance of a Thin-Film Phase Plate for Transmission Electron Microscopy. *Ultramicroscopy* **2009**, *109*, 312–325.
- Danev, R.; Nagayama, K. Optimizing the Phase Shift and the Cut-On Periodicity of Phase Plates for TEM. *Ultramicroscopy* **2011**, *111*, 1305–1315.
- Buijsse, B.; van Laarhoven, F. M. H. M.; Schmid, A. K.; Cambie, R.; Cabrini, S.; Jin, J.; Glaeser, R. M. Design of a Hybrid Double-Sideband/Single-Sideband (Schlieren) Objective Aperture Suitable for Electron Microscopy. *Ultramicroscopy* **2011**, *111*, 1688–1695.
- Walter, A.; Muzik, H.; Vieker, H.; Turchanin, A.; Beyer, A.; Golzhauser, A.; Lacher, M.; Steltenkamp, S.; Schmitz, S.; Holik, P.; *et al.* Practical Aspects of Boersch Phase Contrast Electron Microscopy of Biological Specimens. *Ultramicroscopy* **2012**, *116*, 62–72.
- Fukuda, Y.; Fukazawa, Y.; Danev, R.; Shigemoto, R.; Nagayama, K. Tuning of the Zernike Phase-Plate for Visualization of Detailed Ultrastructure in Complex Biological Specimens. *J. Struct. Biol.* **2009**, *168*, 476–484.
- Murata, K.; Liu, X.; Danev, R.; Jakana, J.; Schmid, M. F.; King, J.; Nagayama, K.; Chiu, W. Zernike Phase Contrast Cryo-Electron Microscopy and Tomography for Structure Determination at Nanometer and Subnanometer Resolutions. *Structure* **2010**, *18*, 903–912.
- van Bavel, S. S.; Sourty, E.; de With, G.; Loos, J. Three-Dimensional Nanoscale Organization of Bulk Heterojunction Polymer Solar Cells. *Nano Lett.* **2009**, *9*, 507–513.
- Agostinelli, T.; Lilliu, S.; Labram, J. G.; Campoy-Quiles, M.; Hampton, M.; Pires, E.; Rawle, J.; Bikondoa, O.; Bradley, D. D. C.; Anthopoulos, T. D.; *et al.* Real-Time Investigation of Crystallization and Phase-Segregation Dynamics in P3HT:PCBM Solar Cells during Thermal Annealing. *Adv. Funct. Mater.* **2011**, *21*, 1701–1708.
- Li, G.; Yao, Y.; Yang, H.; Shrotriya, V.; Yang, G.; Yang, Y. Solvent Annealing Effect in Polymer Solar Cells Based on Poly(3-hexylthiophene) and Methanofullerenes. *Adv. Funct. Mater.* **2007**, *17*, 1636–1644.
- Shiue, J.; Hung, S.-K. A TEM Phase Plate Loading System with Loading Monitoring and Nano-positioning Functions. *Ultramicroscopy* **2010**, *110*, 1238–1242.