Imaging of organic and biological materials by in-focus transmission electron microscopy

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Transmission electron microscopy (TEM) is a powerful characterization tool for imaging nanostructures in materials. However, it is not possible to use the tool for imaging both organic and biological materials due to the intrinsic low contrast of the corresponding TEM images. Organic devices, such as polymer solar cells and organic light-emitting diodes (OLEDs), have generated considerable interest in recent years. The efficiencies of these organic devices depend on their nanoscale structures. Therefore, it is important to characterize nanostructures in organic devices to optimize their performance. However, the contrast of the TEM images obtained for devices made of organic materials is poor as organic materials consist of light elements such as C, O and N. Researchers generally use the well-known defocus technique to enhance the contrast of TEM images. However, this technique reduces the resolution of the images significantly and also creates some artefacts, thus making it difficult to interpret 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.

The use of TEM phase plates developed about 60 years ago to enhance the contrast of in-focus image[1,2]. However, a reliable phase plate technique does not exist due to the rapid deterioration of performance of TEM phase plate, thus limiting the use of this technique in practical applications. Various TEM phase plates have been developed over the decades[3–5], and some promising results using thin-film Zernike phase plates in TEM imaging have been reported recently by two groups[6,7].

Phase contrast microscopy is an optical microscopic technique generally used in biology that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. This technique was invented by Frits Zernike in early 1930s. The phase contrast microscope made it possible for biologists to study living cells and investigate how they grow through cell division. Figure 1 *a* shows the same cells imaged with traditional bright field microscopy (left) and with phase contrast microscopy (right). Figure 1 *b* shows the photograph of a phase contrast microscope.

The use of a thin-film phase plate certainly sacrifices some high-resolution signals of TEM as a result of the loss of coherence due to the interaction between the scattered wave and the carbon film. Such a drawback, however, does not prohibit its major advantage in characterizing nanostructures of organic materials, which are unclear under conventional TEM. Although Zernike phase plates have been successfully used to reveal the structure of ice-embedded biomolecules, a more reliable phase plate technique which allows phase contrast images to be taken reliably and repeatedly is required, as the performance of TEM phase plates deteriorates quickly. One of the major reasons for the unstable performance of a TEM phase plate is the charging effect. When a physical phase plate is present in the pathway of the electron beam, the build-up of charged particles in some local sites would result in instability and distortion of the image formed. Such a charging effect resulting from phase plates is still considered an obstacle for TEM phase plate technology. However, this major drawback has been recently solved by Shiue and his group in Taiwan[8]. They fabricated an on-chip thin-film Zernike phase plate operated on a commercial 200 kV TEM instrument which can effectively release charging caused by phase plate and can provide reliable in-focus TEM images of organic materials with enhanced contrast to be

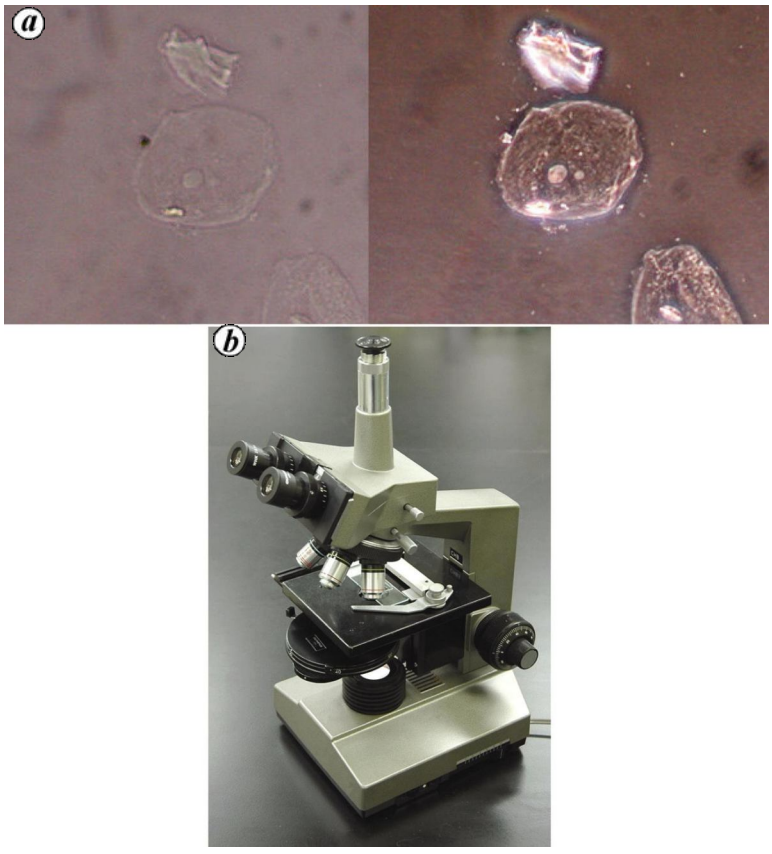

Figure 1. ***a***, The same biological cells imaged with traditional bright field microscopy (left) and with phase contrast microscopy (right). ***b***, Photograph of a phase contrast microscope.

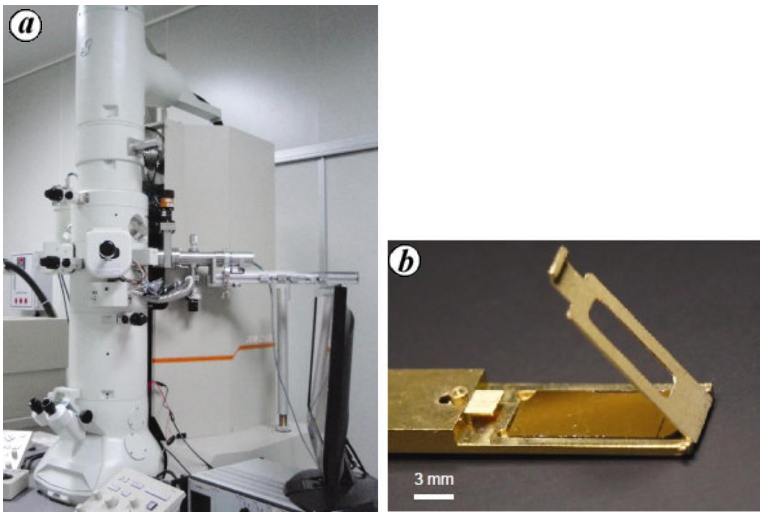

Figure 2. ***a***, Photograph of the commercial TEM machine (JEOL JEM-2100) equipped with the phase plate system introduced by Shiue and his group. ***b***, Photograph of an on-chip thin-film phase plate loaded on a phase plate holder. (From Kuo *et al.*[8], reprinted with permission from American Chemical Society.)

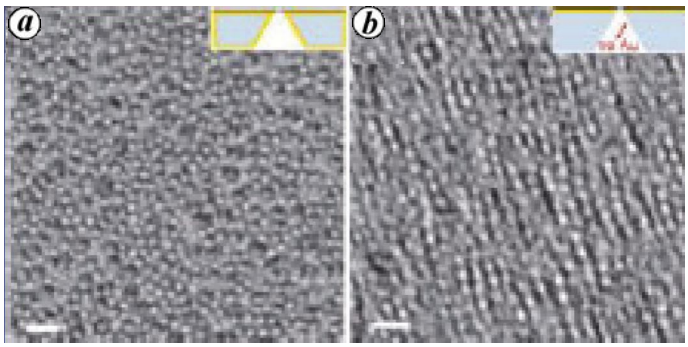

Figure 3. TEM images of a standard specimen of Au nanoparticles using two different chips used as phase plate system. ***a***, Phase plate substrate coated with a three-dimensional Au layer. ***b***, Phase plate substrate coated with a top layer of Au without a three-dimensional Au layer. (From Kuo *et al.*[8], reprinted with permission from American Chemical Society.)

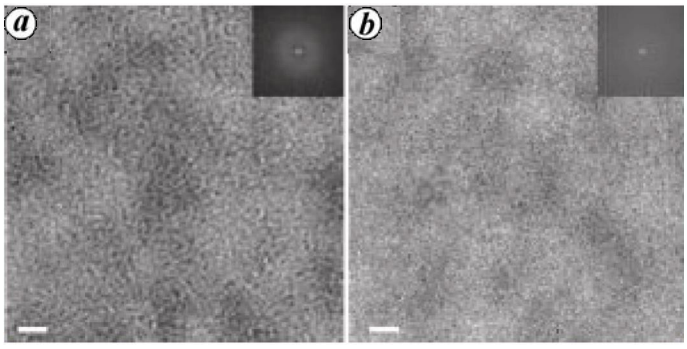

Figure 4. TEM images of a P3HT/PCBM film (160 nm), a photoactive layer of a polymer solar cell device. The image was taken under the condition of (***a***) in-focus with phase plate, (***b***) in-focus without phase plate. (From Kuo *et al.*[8], reprinted with permission from American Chemical Society.)

routinely obtained. Figure 2 *a* and *b* shows the commercial TEM machine (JEOL JEM-2100) equipped with phase plate system and an on-chip thin film phase plate loaded on a phase plate holder respectively.

To ensure that the charging is released effectively with their on-chip thin-film phase plate system, they 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 was approximately 2 nm min^{-1} and towards the same direction, an indication of mechanical drifting of the TEM system itself, and the drifting rate was within the acceptable mechanical drifting range of the TEM system. With the on-chip phase plate in position, the image drifting rate and pattern were the same as that observed without a phase plate. However, the image drifting pattern was quite different while using an 'incomplete' chip as the phase plate substrate, which was only coated with a top layer of Au instead of a three-dimensional Au layer. Using this 'without 3D Au coverage' phase plate chip, the image drifting rate was found > 10 nm min^{-1}, and the drifting direction changed randomly, a feature of the charging effect. Figure 3 *a* and *b* illustrates the TEM images obtained using the two chips as the phase plate substrate.

With this stable system, Shiue and his group were able to characterize many polymer solar cell specimens and accordingly identified and verified the existence of an unexpected nanoparticle phase. Figure 4 *a* shows a typical TEM phase image of a photoactive layer of P3HT/PCBM (poly(3-hexylthiophene)/[6,6]-phenyl-C61-butyric acid ethyl ester) (1 : 1) blend retrieved from a bulk heterojunction polymer solar cell device taken near in-focus conditions. The TEM specimen used in Figure 4 has a thickness of ~ 160 nm and was prepared in the same way as a polymer layer fabricated in a solar cell device. Some spaghetti-like features are found in the polymer blend (Figure 4 *a*). These features are invisible in the in-focus TEM image without using a phase plate (Figure 4 *b*). 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 identify under TEM. The significance of the results shown in Figure 4 is the ability to observe these fibre-like features in such a thick (~ 160 nm) specimen. Figure 5 shows the identification of an unexpected nanoparticle phase (5–8 nm) in some P3HT/PCBM speci-

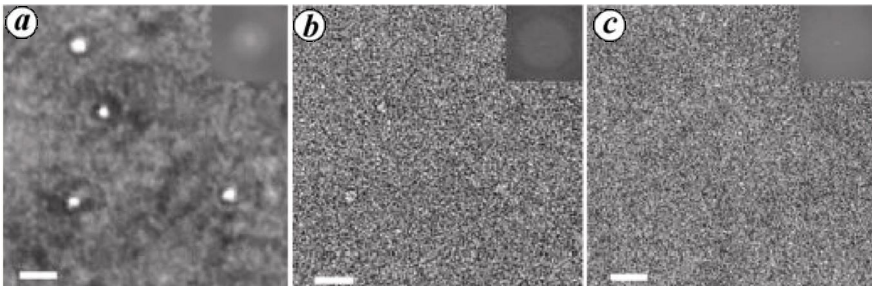

Figure 5. Identification of a nanoparticle phase (5–8 nm) in a P3HT/PCBM specimen. TEM images of the specimen were taken under the condition of (***a***) in-focus with phase plate, (***b***) defocus (under focus ~ 2 μm) without phase plate, and (***c***) in-focus without phase plate. (From Kuo *et al.*[8], reprinted with permission from American Chemical Society.)

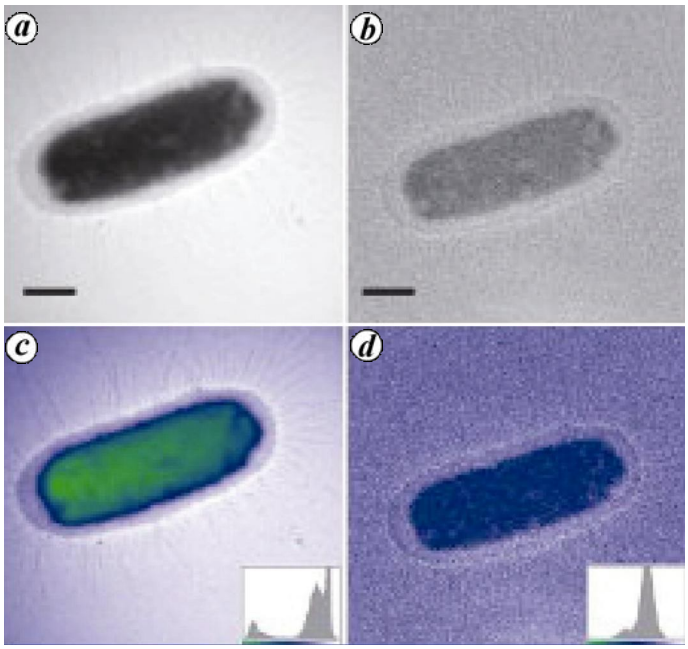

Figure 6. Contrast enhancement of TEM images of 'unstained' *Escherichia coli*. TEM images were taken under the in-focus condition and (***a***) with a phase plate, (***b***) without a phase plate. Applying a two-colour coding scheme on (***a***) and (***b***) resulted in the images in (***c***) and (***d***) respectively. (From Kuo *et al.*[8], reprinted with permission from American Chemical Society.)

mens using an on-chip thin film phase plate. Such an ambiguous nanofeature with low contrast might be mistaken as contamination under defocused TEM imaging (Figure 5 *b*) and is totally invisible in the conventional in-focus image (Figure 5 c).

Shiue and his group were also able to observe the fine structures of an *Escherichia coli* specimen, without staining, using this on-chip thin film phase plate (Figure 6). The *E. coli* image obtained from adopting a phase plate has a bimodal distribution in the histogram and is reflected in the colour-coded image (Figure 6 *c*), 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 sufficient shading difference (Figure 6 *d*), to distinguish the fine structures in the image, and the two-colour coding scheme would result in a single-colour (blue) image (Figure 6 *d*). The results provided in Figure 6 indicate that biological specimens can be observed in unstained condition using this reliable on-chip thin film phase plate system.

The on-chip thin film phase plate system developed by Kuo *et al.*[8] can be installed in any commercial TEM system without modifying the TEM optical design and is suitable for characterizing organic devices and biological samples. It is expected that this kind of TEM phase plate system will open up exciting opportunities for the study of organic materials in the near future.

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