

## Advantage of Zero-Loss Imaging in Transmission Electron Microtomography

Y. Nishikawa,\* H. Nishioka,\*\* T. Kaneko,\* T. Nishi,\*\*\* H. Jinnai\*

\* Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

\*\* Joint Research Center for Project on Nanostructure Polymeric Materials, Japan Chemical Innovation Institute (JCII), Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

\*\*\* Department of Organic and Polymeric Materials, School of Science and Engineering, Tokyo Institute of Technology, 2-12-1, Ohokayama, Meguro-ku, Tokyo 152-8552, Japan

Transmission electron microtomography (TEMT) is a powerful technique to investigate the three-dimensional structures in nanometer scale, which is based on the transmission electron microscopy (TEM) and computerized tomography (CT) [1]. Various approaches have proposed to improve image quality of TEMT [2, 3]. In this paper, we demonstrate the advantage of electron energy loss spectroscopy (EELS) in the quantitative data acquisition for TEMT.

A poly(styrene-*block*-isoprene) (SI) block copolymer was purchased from Polymer Source Inc., Canada. The number-averaged molecular weight and polydispersity were  $M_n=42,000$  and  $M_w/M_n=1.04$ , respectively. The volume fraction of polystyrene was 0.66. The SI block copolymer formed double Gyroid nanostructure [4]. A polymer film was prepared by solution casting from 5 wt% toluene solution, which was further annealed at 140°C for 24 hrs under vacuum. The film thus obtained was stained by OsO<sub>4</sub> vapor for a day. The ultra-thin section was prepared by a microtome (Reichert Ultracut UCT) at room temperature. TEMT observation was performed by JEM-2200FS (JEOL Ltd., Japan) operated at 200 KV. The energy filter equipped in the TEM was used for eliminating the chromatic aberration to obtain the “zero-loss” images.

Since CT needs a series of projections, TEM image,  $I(x,y)$ , is usually related to the projection,  $p(x,y)$ , by the following equation,

$$I(x,y) = I_0 e^{-p(x,y)}, \quad (1)$$

where  $I_0$  is the intensity of incident electron beam. Eq. (1) is based on the standard concept of transmission,  $\tau = I/I_0 = e^{-\mu\ell}$ , where  $\mu$  and  $\ell$  are, respectively, absorption coefficient and sample thickness. For TEMT, the sample was tilted to collect a series of projections from various directions, so that  $\tau$  should vary with the tilting angle,  $\theta$ , according to the increase of the path length of electron beam as follows.

$$\tau(\theta) = e^{-\mu\ell/\cos\theta}. \quad (2)$$

Tilt series of zero-loss and unfiltered TEM images are shown in Fig. 1. The zero-loss images got darker as increasing the tilting angle, while the average intensity of the unfiltered images did not change significantly. Figure 2 shows the transmission of the TEM images. In the case of the unfiltered images, the transmission was less dependent on the tilting angle except for the extremely high tilting angle. On the other hand, the transmission of zero-loss images lay on the sinusoidal curve, which was excellent agreement with the theoretical line based on eq. (2). This result means

that the zero-loss images are more suitable than the unfiltered (conventional) images in order to obtain the  $p(x,y)$ , which is necessary for quantitative calculation of CT. Figure 3 shows the reconstructed cross-sectional images obtained from zero-loss and unfiltered tilt series, respectively. Comparing to the unfiltered one, the reconstructed image from the zero-loss image had higher contrast and sharper edges. Therefore, zero-loss imaging was turned out to be quite useful for TEMT observation [5].

#### References

- [1] J. Frank ed., *Electron Tomography*, Plenum, New York, 1992.
- [2] U. Skoglund et al., *J. Struct. Biol.*, 117 (1996) 173.
- [3] D.N. Mastrorade, *J. Struct. Biol.*, 120 (1997) 343.
- [4] D.A. Hajduk et al., *Macromolecules*, 27 (1994) 6363.
- [5] This research was supported by NEDO through a Project on “Nano Structured Polymeric Materials” by Ministry of Economy, Trade and Industry, Japan.

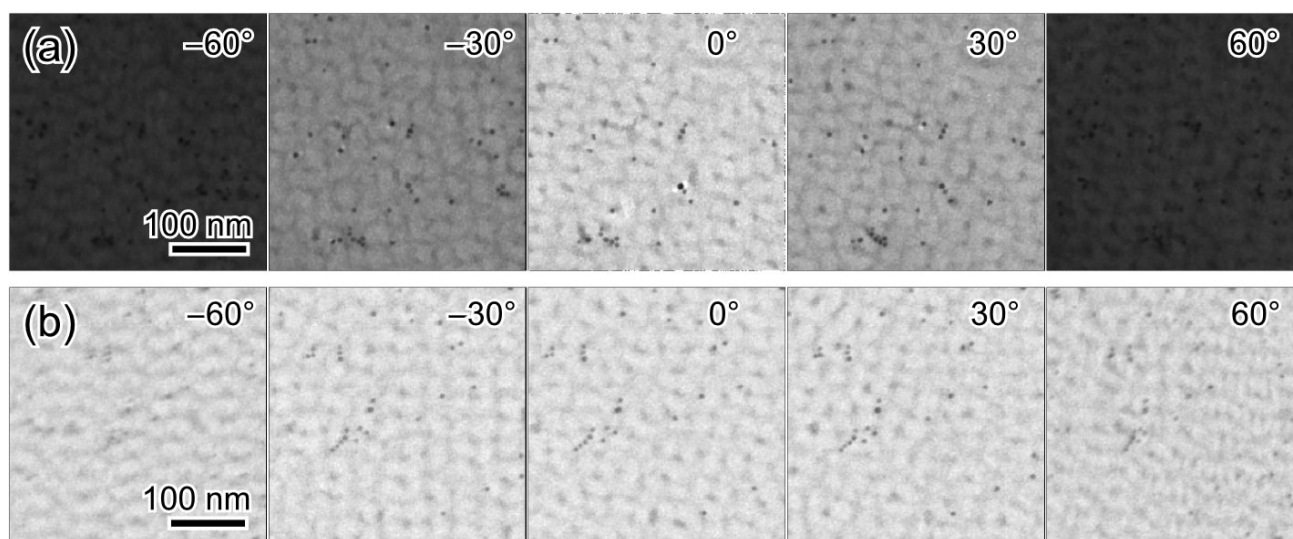


Fig. 1 Tilt series of (a) zero-loss and (b) unfiltered TEM images. The number placed in the upper right corner of each image corresponds to the tilting angle.

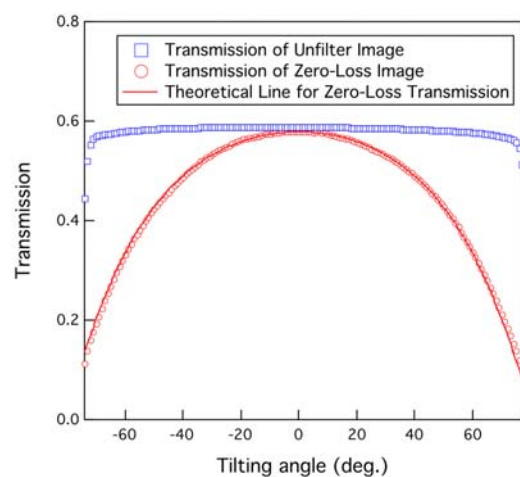


Fig. 2 Transmission of tilt series.

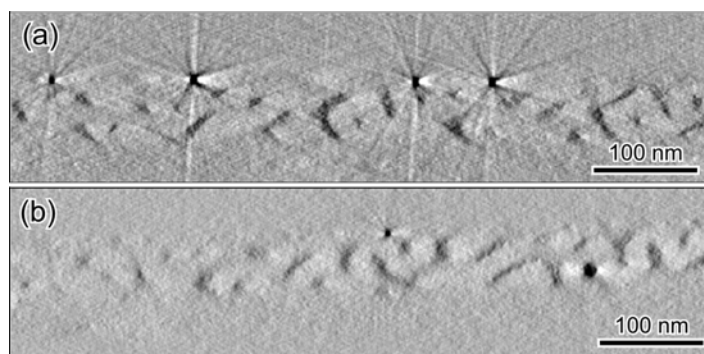


Fig. 3 Reconstructed cross-sectional images obtained from (a) a series of zero-loss images and (b) a series of unfiltered images.