doi:10.1017/S1431927610056114
Microsc. Microanal. 16 (Suppl 2), 2010

Fringing and Strong Object Effects with the Phase Plate

R. Danev,* K. Nagayama*

* Okazaki Institute for Integrative Bioscience, Okazaki, 444-8787, Japan

Recent advances in the manufacturing and applications of phase plates for transmission electron microscopy demonstrated their ability to roughly double the amount of information in an image [1]. In addition to the improvements, the accumulation of new experimental experience revealed secondary effects which, although not critical, are visually distracting and could hamper the interpretability of the results. The effects are closely related and largely dependent on the design of the phase plate and the properties of the specimen. They may be very obvious in one experimental situation and almost invisible in another.

One such effect is the presence of uniformly spaced fringes around high contrast features in an image acquired using a phase plate (Fig.1a) [1-3]. The effect, also known as "Gibbs phenomenon", is related to the jump discontinuity of the phase plate CTF in Fourier space. The jump is at the so called "cut-on" frequency, corresponding to the position in Fourier space of the edge of the phase plate central hole. At the cut-on frequency the CTF switches from conventional sine-type to phase-TEM cosine-type. The periodicity of the fringes corresponds to the cut-on frequency, while their intensity and falloff depend on the structure of the specimen and the parameters of the phase plate. While visually distracting, the fringes could also lead to density artifacts in tomographic reconstructions (Fig.2). Our simulations show that the fringing can be significantly reduced if the phase plate design is modified to prevent the sudden jump in Fourier space and make the CTF transition gradual. We are working on methods for manufacturing of phase plates with apodized central hole with hope to practically solve the fringing problem. In the meantime, we are using software filtration to smooth the amplitude profile in Fourier space which significantly improves the appearance of images and tomograms (Fig.1b,2).

Another secondary effect is the contrast inversion in phase plate images of strong object specimens. Such specimens introduce large phase variations (larger than $\pi/2$) in the object wave which brings the contrast formation mechanism outside of the "weak phase object" regime. As the phase shift increases the image intensity passes through a minimum and then starts to rise. For strong enough phase objects the intensity in the center of the object can be higher than the background intensity. Experimentally, the contrast inversion effect is often observed in phase plate images of gold nanoparticles used for fiducial markers in electron cryo-tomography (Fig.3). Such artifacts interfere with the software tracking of the gold markers during tomographic reconstruction making impossible the automatic tracking or significantly reducing its accuracy. Our simulations show that the contrast inversion effect depends strongly on the parameters of the phase plate and can be prevented by reducing the phase shift or enlarging the central hole.

In summary, depending on the application, specimen and experimental goals the phase plate parameters must be optimized in order to avoid artifacts and improve performance. The results and discussions in this work are based on experimental data acquired using a thin film phase plate but they are of equal significance and can be easily extended to other phase plate designs. In particular, phase plates that have the flexibility of adjustable phase shift will offer great convenience and the ability to optimize experimental performance in real time.

References
[1] R. Danev, R.Glaeser, K. Nagayama, *Ultramicroscopy* 109 (2009) 312.
[2] R. Danev, K. Nagayama, *Biophysics* 2 (2006) 35.
[3] Y. Fukuda et al., *J. Struct. Biol.* 168 (2009) 476.
[4] This work was supported in part by CREST (Core Research for Evolutional Scienceand Technology), Japan Science and Technology Agency.

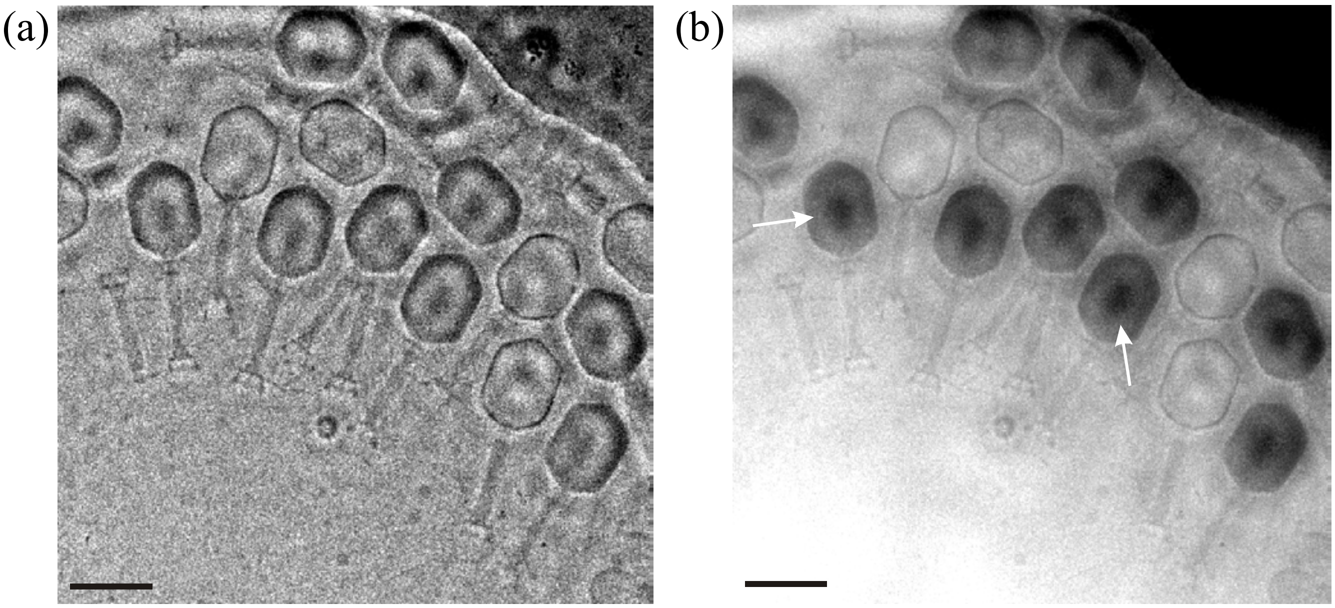

Fig. 1. (a) Phase plate image of ice-embedded T4 bacteriophage and (b) the same image after software filtration. Arrows indicate density artifacts in the middle of the phage capsid that remain even after smoothing of the Fourier space amplitude profile. Scale bars = 100 nm.

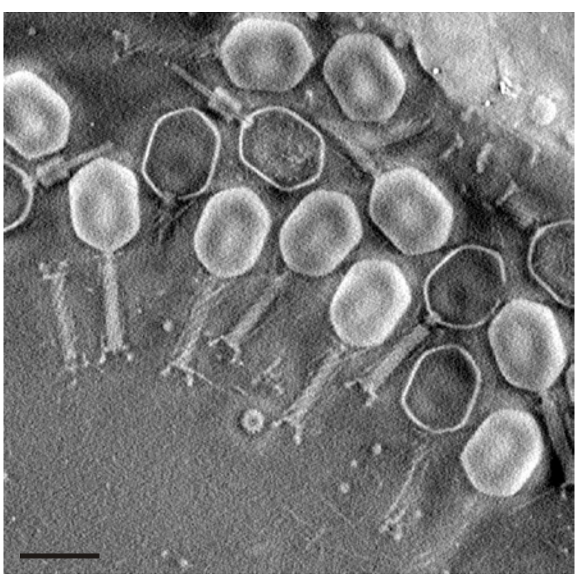

Fig. 2. A slice through a tomogram reconstructed from the filtered dataset illustrated in Fig. 1b. Scale bar = 100 nm.

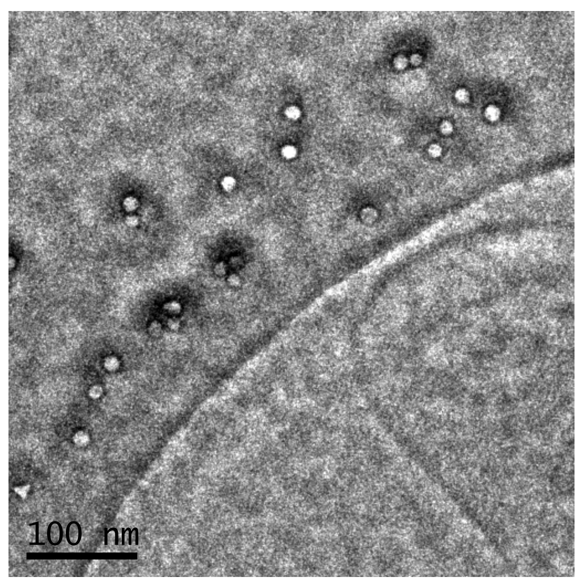

Fig. 3. White spots in the middle of gold nanoparticles due to strong phase object effects.

https://doi.org/10.1017/S1431927610056114 Published online by Cambridge University Press