Image Enhancement with Phase Plates in Electron-Phase Microscopy

Kuniaki Nagayama and Radostin Danev, Okazaki National Research Institutes, Okazaki, Japan

INTRODUCTION
Transmission microscopy can be categorized into four classes according to the contrast scheme employed: brightfield contrast, dark-field contrast, Zernike phase contrast (ZPC), and differential interference contrast (DIC). Techniques belonging to the first two categories are perfect as routine tools in the field of transmission light microscopy. The contrast best suited to transmission electron microscopy (TEM), instead, must be the yet unrealized two schemes, ZPC and DIC, since electron scattering by objects exerts only change in phase and not intensity of electron waves in general. This may be the fundamental cause of image contrast always being a major issue in TEM.

Within the context of brightfield contrast, two alternatives are currently known in TEM: scattering contrast and defocus phase contrast (DPC). The former, typical with strong objects such as thick specimens or heavy metal stained samples, uses the principle resembling a shadow picture: namely the stronger electrons are scattered, the darker images become since more electrons are intercepted by the aperture. The latter uses contrast generated by the interference between the primary (unscattered) and the scattered electron waves. This unfamiliar phenomenon makes DPC less accessible to electron microscopists, who rather prefer to use strong objects dominated by scattering contrast. To maintain intact structures in samples, however, thin and non-stained specimens are recommended, which requires DPC.

PHASE-PLATE TECHNIQUES
The aforementioned four contrast schemes can be integrated in image generation by phase-plate techniques which change electron wave phases at the back focal plane, as illustrated in Fig 1. The phase plate shown in Fig 1c manipulates the phases of only the scattered electron waves, an idea which can be traced back to Zernike [1]. The one shown in Fig 1d manipulates the phases of part of the scattered electrons according to the scattering angle, which leads us to the idea of an alternative DIC as shown later.

The Zernike phase plate consists of a uniformly thick film of amorphous carbon possessing a tiny hole in the centre (Fig 1c), which enables the \( \pi/2 \) phase retardation of the scattered waves only [2]. The phase plate for DIC, which can be referred to Hilbert phase contrast (HPC) in the next section, is also made of a carbon film with a half plane shape, which was designed to retard the phase by \( \pi/2 \), for those scattered waves falling in that half of the aperture (Fig 1d) [3].

Images characteristic of the four contrast mechanisms are correspondingly shown in Fig 2. The figures demonstrating a TMV sample were all semi-experimentally obtained by computation based on the exit-wave function of the sample reconstructed from a pair of images actually recorded with DPC and ZPC [4]. As an extension of electron-phase microscopy, one can devise the observation of wave functions in the complex form, which recover the phase and amplitude information directly from objects as has already been reported with quantitative phase microscopy [5]. The complex observation, thus termed in a...
series of publications[4, 6, 7], carries the same sort of virtues and its perfection as an optical measurement is demonstrated in Fig 2 as the reproduction of four images representing four contrast schemes (Fig 1). Even for strong objects such as negatively stained TMV samples, the advantage of the higher contrast in images is obvious in ZPC or HPC (Fig 2, c and d)

THEORY OF PHASE CONTRAST

The traditional magic used in TEM to visualize the phase change arising from scattering is defocusing [8]. Thanks to the extraordinary short length of electron waves, e.g. 0.0047 nm for 100 kV acceleration voltage, the blurring effect due to defocusing can be compensated by the gain in higher visibility. The mechanism of the exchange between the phase and the amplitude is schematically shown in Fig 3a.

The incident plane wave represented by $1$ in Fig 3a is scattered by a phase object characterized with a space-dependent potential $i\phi(r)$ and results in a object wave $e^{i\omega(r)}$. Under the assumption of $i\phi(r) \ll 1$, which corresponds to the condition of the object being very thin (weak phase), the object wave can be divided into two components, the primary wave $(1)$ and the scattered $(i\phi(r))$. They are propagated and Fourier transformed with an objective lens leaving a focused wave which is mathematically expressed as $\hat{i}=F[1]$ (there $\hat{i}$ indicates a delta function and $F[\cdot]$ Fourier transform (FT)) and a spread wave given by $i\phi(r) = F[i\phi(r)]$ at the back-focal plane of the objective. A phase modulation $\gamma(k)$, which manifests lens aberrations and defocus, enters there in the form of a multiplier, $e^{i\gamma(k)}$. The modulated waves are Fourier transformed again with a projection lens to the form of $1+i(\gamma(k)-\pi)\sin(k)\sin(k)+iF[\cos(k)]$ (here $\pi$ indicates convolution) and finally observed at a recording screen through square detection in the form of $1+i(\gamma(k)-\pi)\sin(k)\sin(k)+iF[\cos(k)]$.

The back focal plane to convert the imaginary wave $i\phi$ to a real wave $\hat{i}$ (Fig 3c). This imaginary to real conversion brings about an exchange in the modulated wave as the real wave modulated by $F[\cos(k)]$, which is the PSF arising from the $\cos$-CTF. What is most plausible in the $\cos$-CTF is the maximized contrast transfer around $k=0$ as illustrated in the right-hand side of Fig 3c. In the ideal microscopical condition of no aberration and no defocus ($\gamma(k)=0$), therefore, ZPC gives rise to the strongest contrast (cos=1). The emphasis of the low frequency component characterizes the higher contrast of ZPC.

Inserting a half plane $\pi$-phase plate at the back focal plane is mathematically equivalent to a multiplication of the sign function ($\text{sgn}(x)=1, x>0; -1, x<0$) to the scattered wave $i\phi(k)$ (Fig 3d). When Fourier transformed, this multiplication becomes the convolution of $i\phi$, which plays two roles in the contrast transfer; the imaginary to real conversion by $i$ and the Hilbert transform by $H[f]=i\hat{f}$. The latter is the origin of the differential image, a resemblance of DIC and the former is the cause of the exchange of CTFs from sine to cosine in the final images, similar to ZPC. The differential feature can be easily understood from the function form of PSF shown in the right-hand side of Fig 3d, which approximates the real dif-

![Figure 2](image215x529.png to 470x787.png)

Figure 2: TMV images representing four contrast schemes. (a) A defocus phase contrast image. (b) A dark field contrast image. (c) Zernike phase contrast image. (d) A Hilbert phase contrast image. These images were obtained through computer simulation using a complex image experimentally obtained as an object wave function (refer to text).

![Figure 3](image215x140.png to 552x363.png)

Figure 3: The contrast generation mechanisms, contrast transfer functions and point spread functions for weak phase objects for four contrast schemes. (a) Defocus phase contrast generation. (b) Dark field contrast generation. (c) Zernike phase contrast generation. (d) Hilbert phase contrast generation. (e) CTF of the optimum resolution for DPC with the Scherzer defocus. This function is rotationally symmetric and hence defined for the modulus of $k$. (f) CTF is not well defined as the dark field contrast is out of the CTF theory. (g) CTF of the optimum resolution for ZPC with a defocus nearly zero. This function is also rotationally symmetric. (h) CTF of the optimum resolution for HPC with a defocus nearly zero. It is defined in one direction and contrarily to (e) and (g). The negative direction in the $x$-axis has a true meaning. (i) PSF obtained by the numerical Fourier transform of the CTF shown in (e). This function is rotationally symmetric and hence defined for the modulus of $k$. (j) PSF is not well defined. (k) PSF obtained by the numerical Fourier transform of the CTF shown in (g). This function is also rotationally symmetric. (l) PSF obtained by the numerical Fourier transform of the CTF shown in (h).
ferential operation given by $\delta(x-\Delta) - \delta(x+\Delta)$. The differential contrast generated with a phase plate shown here may be termed as Hilbert phase contrast in order to emphasize the distinction from conventional DIC, which demands Wallaston prisms in the real space.

**ZERNIKE PHASE CONTRAST IMAGES OF FERRITIN**

Negatively stained ferritin molecules were subjected to a contrast comparison between Zernike phase contrast and defocus phase contrast [2]. Figure 4 demonstrates the very high contrast for the image taken with ZPC (Fig 4a) and the defocus dependent contrasts for images taken with DPC (Fig. 4b-d). The Scherzer defocus (-120 nm in Fig 4d) is successful in yielding a high resolution (about 1 nm) comparable to that of ZPC (Fig 4a) as judged from the cut-off frequency given by CTFs (refer to insets) but revealing its weakness in contrast. Higher contrast is achieved with a deeper defocus (-4,050 nm) as visible in Fig 4b. But that virtue seems to be cancelled by the strong modulation in images, which appears as the peculiar white-out in the centre of the ferritin molecules (compare with Fig 4a). Judging from the cut-off frequency given by the CTF (refer to the inset of Fig 4d), one should not discuss structural details smaller than 6 nm with the deep defocus in DPC.

**HILBERT PHASE CONTRAST IMAGES OF A CELL SECTION**

A section of a plastic-embedded cell prepared from a mouse kidney was another subject for our study of the contrast comparison between HPC and DPC (Fig 5) [3]. To emphasize the distinct high contrast in HPC as compared with DPC, heavy metal staining with uranium or lead was omitted in during specimen preparation. The HPC image in Fig 5b is characterised not only by the high contrast but also the topographical feature, which is characteristic of DIC. As is well known in conventional DIC, the feature never represents the real surface topography as it arises purely from the mathematical operation of the differential. Owing to the higher topographic contrast, however, detailed structures inside the cell are more pronounced in the HPC image, which may provide more information on the cell function.

**CONCLUSIONS**

A classical scheme using the Zernike phase plate has been proven to dramatically enhance TEM image contrast without any image deterioration. The scheme is based on one proposed by Zernike 60 years ago for light microscopy but has not to date been implemented into TEM due to demanding issues of fabricating a tiny hole smaller than 1 µm in diameter and phase-plate charging. Relying on the nanofabrication technology developed in the semiconductor field and a newly developed anti-charging holder, we could overcome these difficulties and solve it half a century after the TEM innovation. These core technologies in phase plated electron-phase microscopy will be reported elsewhere.

Two kinds of electron-phase microscopy, Zernike phase-contrast and differential-interference-contrast, the former using the Zernike phase plate and the latter a half plane $\pi$-phase plate, will be useful particularly in biology by their high contrast as demonstrated with biological samples.

**REFERENCES**


©2003 Rolston Gordon Communications.