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# Quantitative phase from defocused intensity by image deconvolution

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## ABSTRACT

We present a method for quantitative phase recovery using the axially defocused intensity information based on the phase optical transfer function in defocused situation. The image formation process is linearized by subtraction of two intensity images with equal and opposite defocus distances and quantitative phase information is separated and extracted by solving an inverse problem with Wiener filtering. Experiments confirm the accuracy and stability of the proposed method outperforms the transport-of-intensity reconstruction method.

**Keywords:** quantitative phase recovery; defocused intensity; optical transfer function; deconvolution; Wiener filtering

## 1. INTRODUCTION

Imaging and recovery of quantitative phase information has received increased interest many fields of physics where either phase imaging or structure retrieval is an issue, such as optics [1], electron- and X-ray microscopy [2, 3], diffraction [4]. Many samples such as optical elements, biological soft tissues, and cells are phase objects which are nearly uniform with little intensity variation under conventional bright-field microscopy. The key feature of interference and holography microscopy is their ability to provide quantitative phase measurements of the wave field with high accuracy and at fast speeds. However, interferometry systems are limited to pure coherent light source, suffer phase ambiguity or unwrapping problems, and cannot offer the highest spatial resolution. A quantitative phase-imaging approach that does not demand the complex optics and high coherence sources needed for holography and interferometry would thus be of value. Direct phase retrieval from intensity measurements using the Transport-of-intensity equation (TIE) [5, 6] has recently gained increasing attention. The TIE follows from the wave equation under paraxial propagation and specifies the relationship between phase and the first derivative of intensity with respect to the optical axis. However, the intensity derivative along the optic axis cannot be directly measured. Conventionally, it is approximated by finite differences taken between two the out-of-focus images, which being recorded symmetrically placed aft and fore of the in-focus image [7-9]. This approximation is valid in the limit of small defocus distances, but experimentally, it is difficult to get good enough measurements of the intensity distribution in two planes sufficiently close to fulfill the small-distance requirement because the discretization and with negligible noise [8, 10-12]. Therefore, a larger defocus step is usually preferred to increase the signal to noise ratio in the intensity difference. The breakdown of this linear approximation induces non-linear errors which reduce the phase resolution.

In this paper, we propose a novel approach, which is able to produce accurate quantitative phase reconstructions under perfect coherent illumination as well as partially coherent light conditions. It has been found that by subtraction of a weak scattering object imaged at two positions on opposite sides of the focal plane, the phase optical transfer function of a defocused optical system can be applied to characterize the intensity image linearly. Quantitative phase information can then be retrieved by solving an inverse problem with Wiener filtering in one-step. Rather than several methods based on a small-defocus assumption [1, 13] which linearized relationship between the defocus distance and phase contrast, our method considers the non-linear effect explicitly and allows the reconstruction of images recorded at a larger defocus distance, rendering stronger phase contrast and therefore increasing the phase retrieval accuracy in the presence of noise. It was shown theoretically and experimentally that such an approach provides simple, accurate, and fast phase retrieval

and yielded a higher precision and more robust against data noise compared with the TIE-based reconstruction method by overcoming its small-defocus restriction.

## 2. THEORY

### 2.1 Phase optical transfer function

The effect of the imaging system on the incident wave can be represented by multiplication of the amplitude and phase of the wave on the back-focal plane by the optical transfer function (OTF)  $T(u, v)$  in Fourier domain, where  $u$  and  $v$  stand for lateral spatial frequency conjugates of  $x$  and  $y$ . The amplitude on the image plane is given by further Fourier transform back to spatial domain. Consider a perfect, aberration free coherent optical system, the optical transfer function is related to the effects of the aperture limitation and defocus value [14]

$$T(u, v) = A(u, v)e^{i\pi\lambda\Delta z(u^2 + v^2)}, \quad (1)$$

$A(u, v)$  is the aperture function which is unity for  $u^2 + v^2 < v_0^2$ . It can be seen the defocus of the lens by an amount  $\Delta z$  is represented by the phase factor  $\pi\lambda\Delta z(u^2 + v^2)$  in the Fresnel approximation. The image intensity distribution is then given by

$$I(x, y, \Delta z) = |U(x, y) * t(x, y)|^2, \quad (2)$$

where  $t(x, y)$  is the Fourier transform of  $T(u, v)$  and is known as the spread function. The spatial space and frequency space are reciprocals so a product is transformed into a convolution. It can be seen the image acquisition process is linear only for the real and imaginary part of the object wave field but is non-linear for the amplitude and phase because of the conjugation process in the image formation. However, by applying the Born (weak scattering) approximation [1, 14, 15], the captured intensity image can be represented by

$$I(x, y, \Delta z) = 1 - 2\mu(x, y) * \mathcal{F}T_A(u, v) + 2\varphi(x, y) * \mathcal{F}T_p(u, v), \quad (3)$$

where  $\mu(x, y) = -0.5 \ln |U(x, y, 0)|^2$ , indicating the amplitude at the zero distance.  $T_A(u, v)$  is amplitude OTF which equals  $A(u, v) \cos[\pi\lambda\Delta z(u^2 + v^2)]$  and  $T_p(u, v)$  is phase OTF which equals  $A(u, v) \sin[\pi\lambda\Delta z(u^2 + v^2)]$ . It can be seen in this case the real and imaginary parts of the OTF have a simple interpretation in terms of image contrast of the amplitude and phase components of the object field. By doing a finite difference between two intensity image with equal and opposite defocus distance,  $\pm\Delta z$ , the phase information can be completely decoupled:

$$\frac{I(x, y, \Delta z) - I(x, y, -\Delta z)}{2} = 2\varphi(x, y) * \mathcal{F}T_p(u, v). \quad (4)$$

For the aperture function  $A(u, v)$  assumed to be unity and  $\Delta z$  sufficiently small to approximate an ideal derivative, we get

$$\mathcal{F}\frac{\partial I(x)}{\partial z} \approx \mathcal{F}\frac{I(\mathbf{r}, \Delta z) - I(\mathbf{r}, -\Delta z)}{2\Delta z} \approx 2\pi\lambda(u^2 + v^2)\hat{\varphi}(u, v). \quad (5)$$

This equation coincides with the Fourier representation of TIE for the case of unit intensity [16]. It can be seen that the TIE neglects the aperture effect and is valid only in the limit of small distances. Experimentally, when the maximum significant frequency in the object wave is well within the aperture (the case for low-to-medium resolution imaging), the aperture effect can be neglected reasonably. However, the effect of defocus distance cannot easily be neglected. We rewrite Eq. (5) into a similar form as Eq. (4)

$$\frac{I(x, y, \Delta z) - I(x, y, -\Delta z)}{2} = 2\hat{\varphi}(x, y) * \mathcal{F}T_{TIE}(u, v), \quad (6)$$

where

$$T_{TIE}(u, v) = \pi\lambda\Delta z(u^2 + v^2). \quad (7)$$

The  $T_{TIE}(u, v)$  stands for the phase OTF implied in the TIE. The form of phase OTF function  $T_p(u, v)$  and  $T_{TIE}(u, v)$  for a single radial variable  $u$ , is shown in Fig. 1 for various defocus values. The observed regulations of the phase OTF can be extended across a range of frequency or defocus values. It is seen that the two functions show good agreement for small  $\Delta z$  the small-defocus regime where TIE is valid. Besides, for a given defocus distance, good coincidence is only achieved at lower spatial frequencies which assumes low phase contrast response value. This means although small-defocus regime gives better theoretical phase accuracy by using TIE, experimentally, however, it is difficult to get good measurements of the intensity distribution in two planes sufficiently close to fulfill the small-distance requirement.

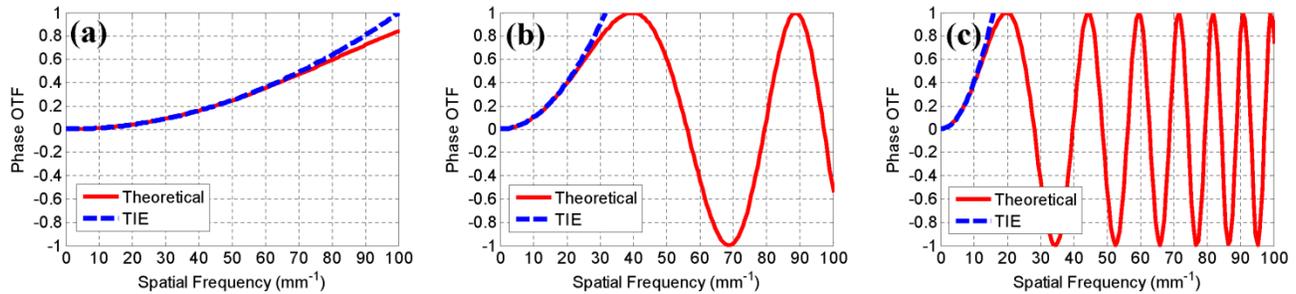


Fig. 1. The theoretical phase OTF  $T_p(u, v)$  (continuous line) and TIE phase OTF (dash line) for  $\lambda = 632.8$  nm. The defocus values  $\Delta z$  are (a) 50, (b) 500 and (c) 2000  $\mu\text{m}$ .

## 2.2 Phase deconvolution

In order to address the contradiction between noise effect and phase resolution, we propose a phase deconvolution technique to relax the small-distance requirement in TIE. Rather than acquiring two images with very slight defocus values, our technique chooses a defocus distance which maximizes the phase contrast of the acquired images. Ideally, in order to get the best representation of the phase distribution of the object,  $T_p(u, v)$  should be close to +1 or -1 to record a large range of frequencies present on the object. The optimum defocus range can be roughly determined by observing the contrast of defused images. The two defocused images are subtracted and then normalized by the infocused image to remove the effect of average illumination intensity:

$$f(x, y) = \frac{I(x, y, \Delta z) - I(x, y, -\Delta z)}{2I(x, y, 0)}. \quad (8)$$

Ignoring the aperture function, the following relationship can be established.

$$\hat{f}(u, v) = 2\hat{\phi}(u, v)T_p(u, v). \quad (9)$$

The obvious solution to obtain  $\hat{\phi}(u, v)$  would be divide  $\hat{f}(u, v)$  by  $2T_p(u, v)$ . However, there will always be a noise component in  $\hat{f}(u, v)$ , independent of  $T_p(u, v)$ . The direct inversion will inevitably amplify the regions in the spectrum where noise dominates. Furthermore, since the  $T_p(u, v)$  oscillates between -1 and 1, the division will be undefined when  $T_p(u, v) = 0$ . The phase retrieval problem here is analogous to the “inverse problem” in signal recovery and to address this difficulty, Wiener filtering is employed to minimize the noise effect for a given spectral signal-to-noise ratio. After multiplying  $\hat{f}(u, v)$  with the Wiener filter, the estimated phase  $\phi(x)$  can be calculated by inverse two-dimensional Fourier transformation.

Finally, it is worth mentioning that we assume perfectly coherent wavefield with a large numerical aperture. In practice, however, only partially coherent fields are available for the case of bright-field microscope, and performing phase retrieval based on the assumption of a perfectly coherent illuminating field can yield erroneous results. In that case, the three-dimensional OTF theory [1] can be used to analyze image formation under partially coherent illumination in a bright-field microscope. When the intensity images are acquired by a bright-field microscope, the phase can be deconvoluted by substituting the coherent phase OTF with the calculated partially coherent one.

## 3. EXPERIMENTS

We test our method under coherent illumination. The experimental test setup is presented in Fig. 2(a). The test source is a beam from a He-Ne laser ( $\lambda = 632.8\text{nm}$ ) that has been expanded and collimated, then passed through the object under test. The object was reimaged onto a CCD camera with a standard 4-f system - two lenses of focal length  $f = 25$  mm separated by the distance  $2f$ , with the object distance equal to  $f$ . The camera is placed on a translation stage to vary the

virtual distance  $\Delta z$  between the image of the surface and the camera. The phase object under test is a geometric pattern etched on a polymethyl methacrylate (PMMA) substrate, shown in Fig. 2(a). All these images are recorded by a monochrome CCD camera and is processed by MATLAB<sup>®</sup> software.

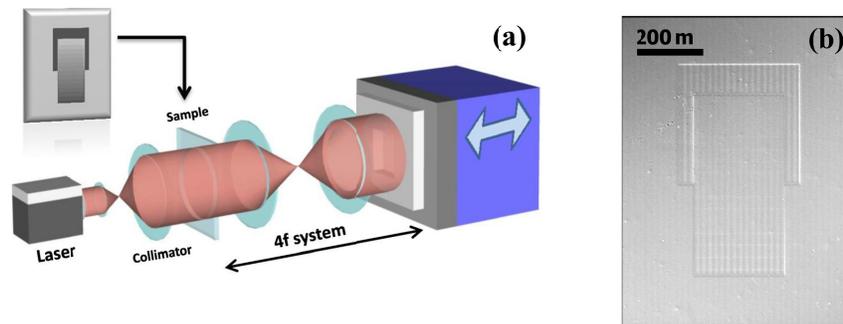


Fig.2. (a) The experimental test setup, and (b) a DIC image of the tested phase sample

Figure 3(b) shows the test phase sample captured in a DIC Microscope. The DIC microscope renders a pseudo three-dimensional relief shading intensity image which is related to the phase gradient of the object. It shows that the test object includes phase ramps of different heights, and more importantly, that it contains some regular periodic vertical line structures with a period of  $25.9 \mu\text{m}$ , corresponding to a spatial frequency of 38.61 cycles/mm, which could be resolved by the 4f system. We acquired five sets of images at defocus distance 50, 100, 600, 1050, and  $1400 \mu\text{m}$ , respectively. Registration of the experimental images was performed beforehand [17] because they are not perfectly aligned when defocus distances are large. The TIE phase retrieval algorithm was applied to reconstruct the phase images using these five images. Besides the traditional TIE, Wiener filter based TIE was performed for comparison. The reconstructed results were shown in Fig. 3. It is shown that while smaller distances improved the theoretical reconstruction accuracy because of their better consistency in phase OTF, they were more sensitive to noise because the intensity variation induced by the phase under test is smaller. The traditional TIE method gave noisy results with low-frequency artifacts superimposed on the acquired phase which obscuring the object of interest itself. As the defocus distance increased, the low-frequency noise reduced but was still comparatively strong. Because all the OTF curves approach zero at very-low frequency, the Wiener filter behaved like a high-pass filter which effectively removed the low-frequency noise. It can be seen that the contrast of the retrieved phase improved significantly when the Wiener filter was applied. However, for small defocus values, the noise cannot be effectively removed due to the low SNR of the difference image. The results with larger defocus values generated much clean phase images. However, as is clearly shown in the magnified area, the fine structural details (e.g. the sharp edges of the objects and the fine periodic structure) were blurred and the resolution became poor especially when the defocus value was large. When the defocus values are 1050 and  $1400 \mu\text{m}$ , the reconstructed phase map shows almost no sign of the periodic structure. Therefore, for TIE method, improper choice of defocus distance can lead to reduction in phase resolution if the defocus distance is large or increase in noise sensitivity if the defocus distance is too small. Usually, a suboptimal defocus distance is chosen to compromise between these opposing trends for the best visibility of a phase-contrast image.

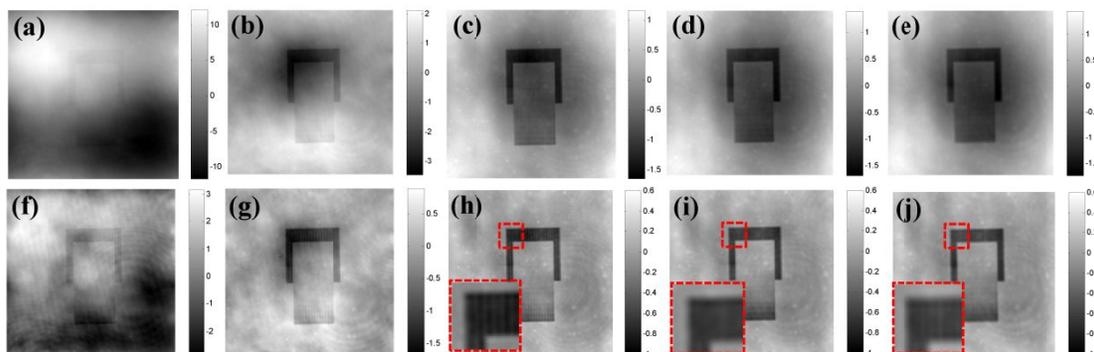


Fig. 3. Phase recovery comparison of a test phase object by traditional TIE (a-e) and wiener-filter based TIE (f-j). The defocus values are (a,f) 50, (b,g) 100, (c,h) 600, (d,i) 1050, and (e,j) 1400  $\mu\text{m}$ . the bottom right red squares show the corresponding enlarged regions.

For the proposed method, we use two dataset with defocus distance 600 and 1400  $\mu\text{m}$  because they give complete phase information with high SNR. The final results are shown in Fig 5. Because the nonlinearity error caused by relatively large defocus is considered explicitly, our methods provided sharp phase results with good noise performance in both cases. To better assess the accuracy of the phase measurements, we use a confocal microscope with a 50x objective (NA=0.8) to independently measure the surface profile of a small area corresponding to the magnified area shown in Figs. 4 and 5. Fig. 6(a) shows the inverse height distribution and one line profile was extracted for better quantitatively evaluation (Fig. 6(b)). The same line from Figs. 4(h,j) and Figs. 5(a,b) were also extracted, plotted in Fig. 6(c), converted to inverse height (refractive index  $n_{\text{PMMA}}=1.49$ ). The offsets of the four curves were adjusted to the same level. The confocal gave 136 nm for the height of the large step and 32.1 nm for the average peak-to-valley height of the small periodic structure. The TIE method produced a step height of 133 nm and underestimated the height of the small variations by about half (15.9 nm) when  $\Delta z = 600 \mu\text{m}$ . For large defocus value  $\Delta z = 1400 \mu\text{m}$ , the step height estimate reduced to about 121 nm and the small variations almost fattened. When  $\Delta z = 600 \mu\text{m}$ , our method provided a more faithful result with 134 nm step height and 29.2 nm of average peak-to-valley height of periodic structure. And when  $\Delta z = 1400 \mu\text{m}$ , our method can still recover the fine details of the structure reliably (29.4 nm) with a slight underestimate of the large step (122 nm).

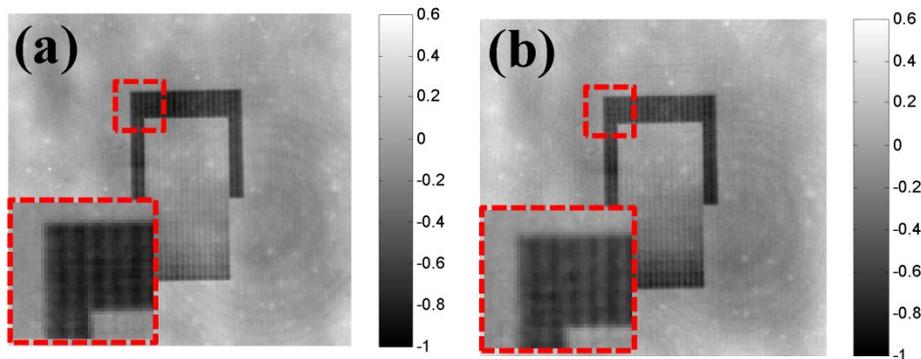


Fig. 4. Phase recovery result of a test phase object by the proposed method. The defocus values are (a) 600  $\mu\text{m}$ , and (b) 1400  $\mu\text{m}$ .

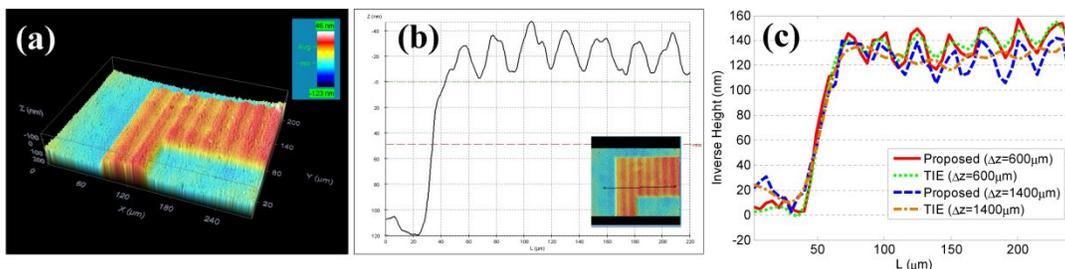


Fig. 5. Quantitative phase comparison of different methods. (a) The 3D topography characterization of the test sample by a confocal microscope shown in inverse height. (b) Phase profile obtained along the line. (c) Comparison of line profiles between TIE and proposed method.

#### 4. CONCLUSION

In summary, we have proposed a novel phase retrieval approach to reconstruct by deconvoluting the intensity difference using the theory of phase OTF. Traditional TIE approach is restricted to small-defocus regime because it assumes a linear relationship between the defocus distance and phase contrast, while our method relaxes this restriction by consider the nonlinearity of the phase OTF which allows the reconstruction of images recorded over a larger focal range. The advantage of taking images further away from the sample lies in stronger phase contrast with larger propagation distances, which therefore increases the accuracy in the presence of noise. The experimental results show that our proposed method yielded a higher precision and was more robust against data noise compared with the TIE-based reconstruction method. It is expected that this new phase retrieval method will help improve quantitative phase contrast imaging and find significant material or biomedical applications.

## 5. ACKNOWLEDGEMENT

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