Derivative method for phase retrieval in off-axis quantitative phase imaging

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We present a method for phase retrieval in off-axis interferometric systems. By numerically calculating the transverse 1st and 2nd order derivatives of the interferogram, we show that one can directly retrieve the quantitative phase image, without the need for Fourier or Hilbert transformations. Because of this, the method is significantly faster than the current approaches. We illustrate our method using biological specimen data from three different off-axis quantitative phase imaging techniques. © 2012 Optical Society of America

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Quantitative phase imaging (QPI) is a rapidly emerging area of study in biomedicine as it provides label-free access to structure and dynamics, quantitatively and with nanoscale sensitivity [1]. Temporal phase shifting based QPI techniques require three or more images for phase extraction and hence are difficult to implement in real time [1,2]. By contrast, off-axis based QPI techniques allow for single shot measurements and, thus, fast acquisition rates [3,4]. Integral operators (Fourier and Hilbert transforms) are established methods for phase reconstruction in off-axis QPI [3–5]. However, because they are integral transformations, these operations are computationally demanding, which makes it difficult to achieve real-time phase extraction. This challenge has been addressed recently by parallelizing the numerical reconstruction [6].

Very recently, spatial phase shifting (SPS) has been used for phase extraction in diffraction phase microscopy (DPM) [7]. Because it uses a local (differential), rather than integral operator, SPS is faster than Fourier and Hilbert transforms for raw phase calculation. However, in SPS the phase shift per pixel needs to be close to $2\pi/3$ rad or the carrier fringe period needs to be 3 pixels, such that the condition of least noise is satisfied [8]. This condition adds constraints to the QPI system design.

In this Letter, we present a *derivative method* for phase reconstructions, which can be applied quite generally to any off-axis interferogram. Our *local* method relies on the first- and second-order derivatives of the recorded image and, thus, is 4 times faster than the Hilbert transform (HT) technique and 10 times faster than Fourier transform (FT) technique for phase extraction. Further, our approach works with fringes sampled by an arbitrary pixel number, N, as long as $N \geq 3$ to satisfy the Nyquist condition for an interferogram (see, e.g., p. 308 in [9]).

In off-axis QPI, the interference pattern can be written as [1]

$$I(x,y) = I_b(x,y) + \gamma(x,y)\cos[\phi(x,y) + kx], \quad (1)$$

where I_b is the background intensity,γ is the modulation factor, ϕ is the phase delay due to the specimen, and k is the spatial frequency of the carrier fringes. The latter is determined by the tilt angle, θ, between the sample and reference beams, that is,

$$k = 2\pi \sin\theta/\lambda, \quad (2)$$

where λ is the wavelength. Typically, the phase is obtained via a spatial Hilbert transform, which provides the complex analytic signal associated with the real interferogram [3–5]. Here we show that, for phase objects, ϕ can be obtained more directly via transverse derivatives of the interferogram. Thus, the first order derivative of Eq. (1) with respect to x can be written as

$$\frac{\partial I(x,y)}{\partial x} = \frac{\partial I_b(x,y)}{\partial x} + \cos[\phi(x,y) + kx]\frac{\partial \gamma(x,y)}{\partial x} - \gamma(x,y)\sin[\phi(x,y) + kx]\left[\frac{\partial \phi(x,y)}{\partial x} + k\right]. \quad (3)$$

For most transparent specimens of interest, i.e., *phase objects*, we can make the following helpful approximations:

$$\frac{\partial I_b}{\partial x} \approx 0, \qquad \frac{\partial \gamma}{\partial x} \approx 0, \qquad \frac{\partial \varphi}{\partial x} \ll k, \quad (4)$$

where we consider that the background intensity, I_b, and modulation factor, γ, are constant over the interferogram and the phase, ϕ, is a slowly varying function. The first two conditions are clearly fulfilled for phase objects, where no intensity modulation is observed. The third assumption, $\partial\phi/\partial x \ll k$, applies because we always adjust the fringe period to be smaller than the diffraction spot of the imaging system, such that the optical resolution is not degraded by sampling [3]. Under these circumstances, over a diffraction spot (or central portion of the point spread function), the phase of the field, $\phi(x,y)$, varies *insignificantly*, but the phase of the fringe, kx, changes by at least 2π. Therefore, from Eq. (3) we have

$$I' = -\frac{\partial I(x,y)}{\partial x} = \gamma k\, sin[\phi(x,y) + kx]. \quad (5)$$

Similarly, the derivative of Eq. (5) with respect to x gives the second-order derivative,

$$I'' = -\frac{\partial^2 I(x,y)}{\partial x^2} = \gamma k^2 \cos[\phi(x,y) + kx]. \quad (6)$$

Using Eqs. (5) and (6), we obtain the phase directly as the argument of the complex function $I'' + ikI'$, with $i = \sqrt{-1}$,

$$\phi(x,y) = \arg(I'' + ikI') - kx. \quad (7)$$

Clearly, this *derivative method*, based on local operations, is faster than the traditional integral operations. The spatial frequency, k, has fixed value over time and throughout the field of view and is determined by the period of the grating. Thus one needs to measure it only once for a particular experimental system. One of the best options to measure k is by detecting the peak position of one of the first orders in the Fourier transform of the interferogram.

For comparing our result with that in [7], we can rewrite Eq. (7) as:

$$\phi(x,y) = \tan^{-1}\left[\frac{kI'}{I''}\right] - kx, \quad (8)$$

where the $\tan^{-1}$ is calculated over 4 quadrants. Equation (2) in [7] (also Eq. (5) in [8]) can be rewritten using spatial separation between pixels,δx as

$$\phi(x,y) = \tan^{-1}\left[\tan\left(\frac{k\delta x}{2}\right)\frac{I'_2 + I'_1}{I'_2 - I'_1}\right] - kx, \quad (9)$$

where I_i, $i = 1, 2$ are the spatially phase shifted intensities, $\delta x = 1$, k is the phase shift between adjacent pixels, and

$$I_1 - I_3 = -(I_3 - I_2 + I_2 - I_1) = -(I'_2 + I'_1), \quad (10)$$

$$-I_1 + 2I_2 - I_3 = -(I_3 - I_2) + (I_2 - I_1) = -(I'_2 - I'_1). \quad (11)$$

In Eqs. (10,11), $I_2 - I_1 = I'_1 = \partial I_1/\partial x$ and $I_3 - I_2 = I'_2 = \partial I_2/\partial x$. Note that in derivative method (DM) [Eq. (8)] we calculate *locally*, using consecutive pixels, while in SPS [Eq. (9)], the calculations are spread over 3 pixels. The noise associated with the SPS approach has been studied by Servin and Cuevas and it was found that $k\delta x$ should be equal to $2\pi/3$ rad for best noise rejection and 3×3 averaging is necessary in order to remove a large quantity of noise even with $k\delta x = 2\pi/3$ rad [8]. By contrast, our method works equally well with any values of k.

In order to demonstrate our algorithm, we applied the derivative method to three distinct diffraction grating based off-axis QPI techniques: diffraction phase microscopy (DPM) [4], white light DPM (wDPM) [10], and instantaneous spatial light interference microscopy (iSLIM) [11]. DPM is a common path, off-axis QPI method [4] where the zero and first order diffraction orders from a grating placed at the image plane of a microscope interfere at some angle to produce stable carrier fringes. We

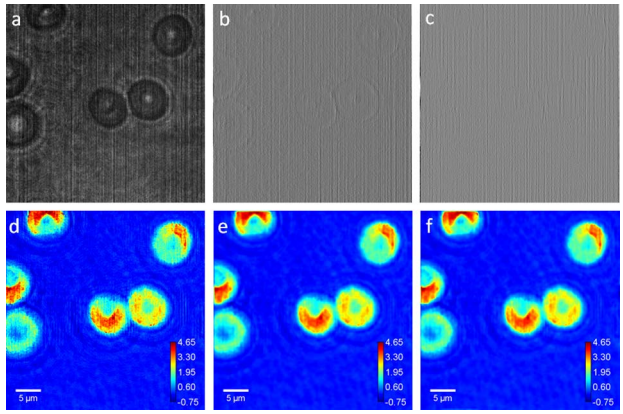

Fig. 1. (Color online) Derivative method for phase calculation: (a) original DPM image, (b) 1st order derivative of (a) w.r.t. x, (c) 2nd order derivative of (a) w.r.t. x, (d) the reconstructed unwrapped phase, (e) 3×3 average filtered phase of (d), (f) phase obtained by HT; the color bars show the phase in rad.

illustrate the phase reconstruction procedure in Fig. 1. The interferogram (512 pixels × 512 pixels) associated with red blood cell (RBC) specimen [Fig. 1(a)] has a spatial frequency, $k = 1.8285$ rad/pixel. The numerically calculated first and second derivatives of the interferogram are shown in Figs. 1(b) and 1(c), respectively. Since we know k, we can calculate the phase from the two derivative images and remove the tilt due to the carrier fringes [last term in Eq. (7)]. In calculating the derivatives and the phase, we used MATLAB, but, of course, our method can be implemented with any computing platform.

Figure 1(d) shows the reconstructed phase after 2D phase unwrapping. The color bar indicates the phase in radians. Some pixel noise is visible because the derivative calculations act as high-pass filters and may introduce high-frequency noise in the image. Fortunately, our images are oversampled to preserve optical resolution (as explained earlier) and it is expected that below the optical resolution, noise is dominant. Therefore, filtering is permissible over an area up to the diffraction spot size, i.e. at least 3 pixels. Figure 1(e) shows the filtered version of Fig. 1(d), where a 3 pixel × 3 pixel window average was used. Further, we have compared this result given by our DM with that given by the Hilbert transform [3] [Fig. 1(f)] and obtained excellent agreement. Standard deviation of the difference of phases between Fig. 1(e) and Fig. 1(f) is only 0.026 rad, which represents 0.56% of the maximum phase value. We compared the execution time for the phase calculation in both the cases: the Hilbert transform method takes 89.9 ms whereas our DM takes only 21.7 ms which is more than 4× faster. The phase extraction was performed on a desktop computer (Intel Core i7-960 CPU, 3.20 GHz) in the MATLAB environment.

To demonstrate the accuracy of our method, we have imaged a 2.9 ± 0.14 μm diameter polystyrene bead immersed in immersion oil (Zeiss) using wDPM which is an off-axis common path QPI that uses *plane wave* white light illumination [10]. Figure 2(a) shows such a phase image; the color bar shows the height in μm. The measured height is 2.95 μm at 550 nm (center wavelength of the source), which agrees very well with the given value. In

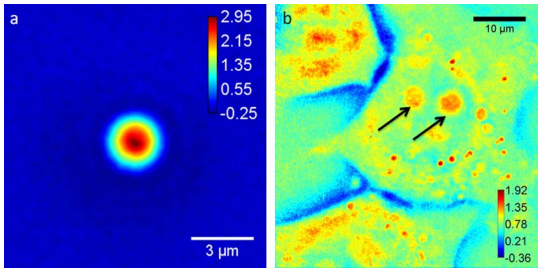

Fig. 2. (Color online) Quantitative phase imaging with wDPM setup: (a) Microbead immersed in oil; the color bar shows the height in μm, (b) Live Hela cells; the color bars show the phase in rad. The arrows indicate nucleoli.

wDPM the spatial frequency, $k = 1.0267$ rad/pixel. Further, our technique is used to measure the phase of a wDPM image of HeLa cells (ATCC, CCL-2), a human cervical epithelial cell line, 24 h after the cells were plated onto a glass bottom dish. Figure 2(b) shows the QPI image of HeLa cells where arrows point to the nucleoli; the color bar represents the phase in radians. The arrows indicate nucleoli.

Next we have used our method to evaluate the phase of iSLIM image. iSLIM is also an off-axis common path QPI methods that uses white light *phase contrast* illumination [11]. In the iSLIM setup, $k = 0.801$ rad/pixel, which is much smaller than $2\pi/3$. We compared our reconstruction method with the SPS method [7], as follows. We imaged live red blood cells (RBCs) diluted with Coulter LH series diluent (Beckman Coulter) to a concentration of 0.2% whole blood in solution. Figures 3(a) and 3(b) show the QPI images of RBC obtained with DM and SPS methods, respectively without any filtering; the color bar represents the phase in radians. The higher range in the color bar of Fig. 3(b) is due to noise spikes. Figure 3(c) shows profiles across a RBC in Figs. 3(a) and 3(b) (dashed lines). As can be seen, the line profile for SPS is much noisier than DM. Figure 3(d) shows profiles across the same dashed lines in Figs. 3(a) and 3(b) after filtering the phase images through a 3×3 window average filter. The profile for SPS is noisier even after the filtering. DM gives less noisy phase compared to SPS at any k and it is expected to give approximately the same performance when k is close to $2\pi/3$ [8]. Note that the execution times for DM and SPM are approximately the same.

It is noteworthy to mention that the work presented by Joenathan and Khorana also uses fringe derivatives for phase calculation [12]; however, it requires two interferometric fringe patterns instead of the one required in our case. Thus, our method avoids the need for the temporal phase shifting used in [12]. Further, fringes in our work always orient along the x direction; thus the possible generation of artifacts like Moire fringes is avoided. The effect of shearing operations in differentiation is negligible as only one pixel is needed to be sheared for differentiation, compared to 24 pixels in [12].

In summary, we presented a DM for phase retrieval in off-axis quantitative phase imaging. By numerically calculating the 1st and 2nd order derivatives of the interferogram, one can calculate the quantitative phase without the use of integral transformations. We anticipate that this approach will be useful in achieving real-time QPI using various off-axis methods including digital holography [13–15]. Importantly, all the derivative calculations can be parallelized and performed extremely fast.

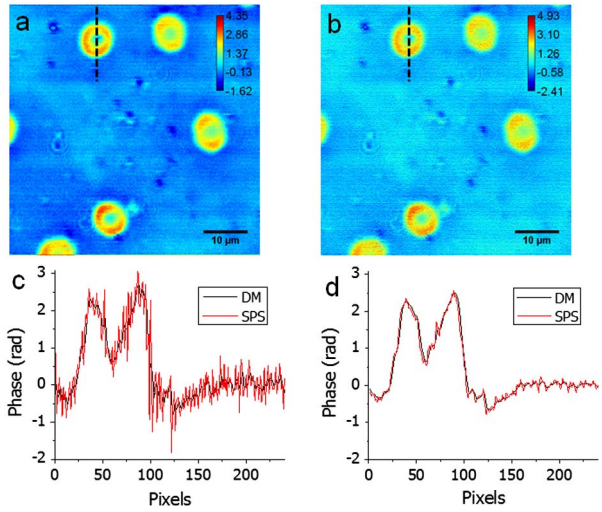

Fig. 3. (Color online) Comparison between DM and SPS. Quantitative phase image of red blood cells processed from an interferogram obtained by iSLIM without filtering using: (a) the DM method, (b) SPS method; the color bars show the phase in rad; (c) Profile of the dashed lines in (a) and (b); (d) Profile of the same dashed lines after filtering the phase images using 3×3 average filtering.

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