Fast phase reconstruction in white light diffraction phase microscopy

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In off-axis interferometry, we usually have to deal with the unwrapping process, which is very computationally intensive and prevents us from real time phase reconstruction. The wrapping problem usually occurs when imaging thick objects, which introduce phase shifts of more than 2π radians. However, in off-axis interferometry, the nonzero angle of interference of the two beams creates a ramp in the phase across the image that can produce phase wrapping errors. In this paper, we propose a simple technique that avoids the need for the unwrapping step in reconstructing quantitative phase images in white light diffraction phase microscopy of thin samples. We show that this approach can improve significantly the phase reconstruction speed and allow high impact applications, such as real-time blood testing.

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1. Introduction

Over the past decade, a great deal of scientific attention has been paid to quantitative phase imaging (QPI), which has emerged as one important tool in the area of cell and tissue imaging [1]. QPI can provide label-free imaging of structure and dynamics with nanoscale sensitivity [2–14]. In many applications, including applied physics and biomedicine, it is important to have high throughput, high speed, and real-time phase information. Typically, in order to obtain the pathlength map from an acquired interferogram image, QPI utilizes the Hilbert transform to remove the high-frequency spatial modulation and requires a phase unwrapping algorithm to correctly reconstruct the phase. Phase unwrapping is the process of rebuilding the true phase information from the measured wrapped values, which are between $-\pi$ and $+\pi$. The wrapping problem occurs when imaging thick objects, or if there are large phase variations in the background, which introduce phase shifts of more than 2π radians, thus resulting in phase ambiguities. Wrapping can also occur in the presence of high noise. The phase unwrapping process is computationally intensive and, thus, prevents us from having real time QPI. Recently spatial phase shifting has been proposed to achieve real time phase reconstruction performance [15]. A related method is to use the first and second derivatives of the interferogram image [16]. The authors reported that the two methods take 8 and 27 ms, respectively, to reconstruct the phase map of a 512×512 pixel image. Still, these two methods require phase unwrapping to account for the phase ramp introduced by the off-axis geometry, which takes 30 ms [17] or more depending on algorithms, computer performance, and programming languages used (e.g., our MATLAB implementation of one of the fastest phase unwrapping algorithms, Goldstein algorithm, takes about 70 ms).

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Also, the noise in the reconstructed phase maps in these two methods is larger than that in our new method. This noise reduces the spatial and temporal sensitivities of the system.

In this paper, we propose a simple technique which avoids the need of phase unwrapping when imaging thin objects, i.e., objects that cause phase shifts of less than 2π radians, such as most living cells. Since the phase unwrapping process accounts for most of the time of the phase reconstruction process, this technique enables very fast, real-time phase reconstruction while maintaining the phase sensitivity exactly as in the Hilbert transform based method. In the next section, we will briefly discuss the white light diffraction phase microscopy (wDPM) system and describe our technique. Finally, we demonstrate our method with measurements on human red blood cells.

2. Phase Reconstruction in wDPM

A. wDPM Setup

Diffraction phase microscopy (DPM) [18] is an off-axis and common-path technique which allows fast acquisition rates and high temporal sensitivity [18,19]. These capabilities enabled unprecedented biological studies, especially related to red blood cell membrane dynamics [13,20–22]. However, due to the laser illumination, DPM images suffer from speckles. These speckles degrade phase sensitivity, and thus the applicability to studying subcellular structures is limited. Spatial light interference microscopy (SLIM) solved this problem by using white light illumination in a phased shifting geometry [9,23–25]. However, SLIM requires the acquisition of 4 intensity images for each quantitative phase image; thus the acquisition rate is lower.

In [26], we described a new imaging system called wDPM, which maintains the single shot feature of DPM and low speckle noise associated with white light. Figure 1 shows the wDPM setup, which uses white light illumination obtained from a halogen lamp. The condenser aperture is NA = 0.09, such that the field at the image plane is spatially coherent over the entire field of view. An amplitude grating with a groove density of 110 grooves per mm located at the image plane of a microscope is used to generate diffraction orders, each containing full spatial and phase information of the sample. A mask is projected onto a spatial light modulator (SLM) placed at the Fourier plane to filter the diffraction orders. The SLM was obtained from an Epson Powerlite S5 projector having a pixel size of 13 μm × 13 μm and contrast ratio of 400∶1. The zero-order beam is spatially low pass filtered using a circular mask so that only the DC component is passed through, so that it can be used as a reference beam. The whole +1 diffraction order is passed and is used as the sample beam; all the other orders are blocked. After a second lens L2, the two beams interfere with each other to create the interferogram on the charge-coupled device (CCD) plane. The 4f lens system, with focal lengths of 60 and 150 mm, respectively, gives an additional magnification of so $f_2/f_1 = 2.5$ that the sinusoidal modulation of the image is sampled by 6 CCD pixels per period. Thus, wDPM combines the benefits of high temporal sensitivity of common path and off-axis technique and high spatial sensitivity of white light illumination.

B. Phase Reconstruction

Figure 2 illustrates the steps involved in phase reconstruction. The intensity distribution of the interferogram [Fig. 2(a)] at the detector plane takes the form (in the absence of noise)

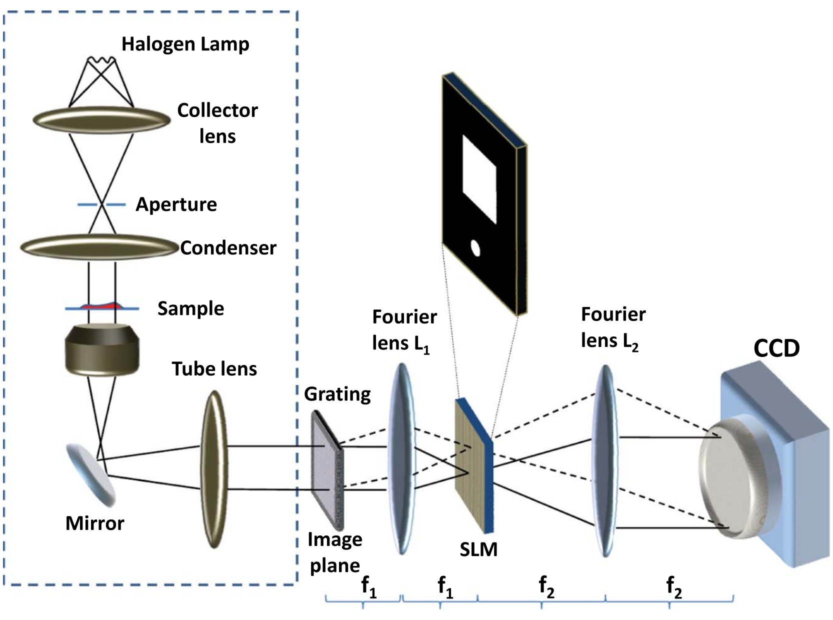

Fig. 1. (Color online) Experimental setup of wDPM.

$$I(x,y) = |U_i(x,y)|^2 + |U_r|^2 + 2|U_r| \cdot |U_i(x,y)| \cdot \cos[kx + \phi(x,y)]. \quad (1)$$

$U_i(x,y)$ is the imaging field (the first-order diffraction field) and U_r is the reference field (the zeroth-order diffraction field), k is the period of the grating, and $\phi(x,y)$ denotes the phase delay induced by the sample.

In the Hilbert transform based method, the interferogram is first Fourier transformed to get the power spectrum [Fig. 2(b)]. The next step is to move one of the first orders to the center [Fig. 2(c)] and use a filter [the red circle in Fig. 2(c)] to remove the modulation (kx) term. We then perform the inverse Fourier transform and calculate its angle to obtain the phase image $\phi(x,y)$ [Fig. 2(d)]. The next step uses an unwrapping algorithm to obtain the unwrapped phase image [Fig. 2(e)]. We can easily see that the final unwrapped image still has many unwanted patterns, which are caused by dirt on the grating and other optical components of the system. Therefore, in this method, we also capture a single background calibration image, which we finally subtract from the unwrapped phase map. The final result is shown in Fig. 2(f).

We propose a simple method to reconstruct quantitative phase images in wDPM. Our approach allows very fast reconstruction because it bypasses unwrapping, which is responsible for most of the processing time. When taking into account the background and the noise, Eq. (1) becomes

$$I(x,y) = |U_i(x,y)|^2 + |U_r|^2 + 2|U_r| \cdot |U_r(x,y)| \cdot \cos[kx + \phi(x,y) + \varphi_{bg}(x,y) + \varphi_n(x,y)], \quad (2)$$

where φ_{bg} is the phase shift caused by the background and φ_n is the noise. When there is no sample in the field of view, Eq. (2) becomes

$$I(x,y) = |U_i(x,y)|^2 + |U_r|^2 + 2|U_r| \cdot |U_r(x,y)| \cdot \cos[kx + \varphi_{bg}(x,y) + \varphi_n(x,y)]. \quad (3)$$

We Fourier transform Eq. (3), shift the first order back to the center, and perform the inverse Fourier transform. The result is the term

$$2|U_r| \cdot |U_i(x,y)| \cdot \exp[i(\varphi_{bg}(x,y) + \varphi_n(x,y)]. \quad (4)$$

When there is a phase object in the field of view, we follow the same process and obtain

$$2|U_r| \cdot |U_i(x,y)| \cdot \exp[i(\Delta\phi(x,y) + \varphi_{bg}(x,y) + \varphi_n(x,y)]. \quad (5)$$

Dividing Eqs. (5) and (4), we end up with the term

$$\exp[i\Delta\phi(x,y)]. \quad (6)$$

Finally, in contrast to the original approach, which performs the tangent operations of Eqs. (4) and (5), unwraps and subtracts them from each other to get the final phase image, we take the angle of Eq. (6) to

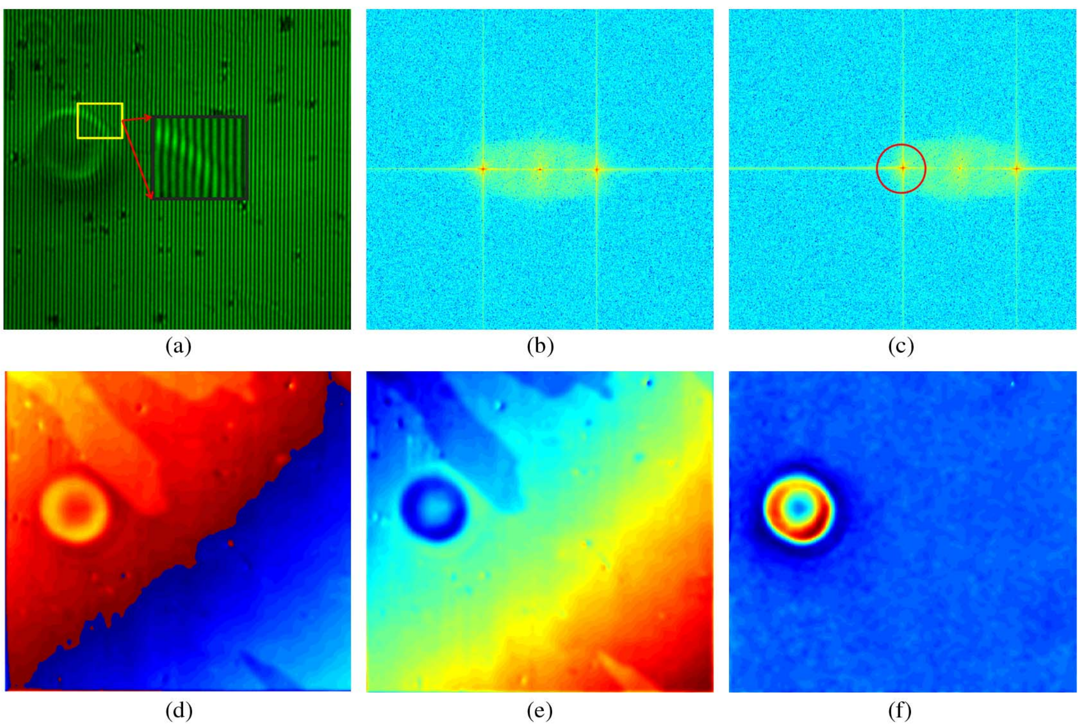

Fig. 2. (Color online) Phase reconstruction in wDPM. (a) Interferogram from the CCD camera, (b) power spectrum of (a), (c) circular shifted power spectrum to move the first-order to the center, (d) wrapped phase image, (e) unwrapped phase images, (f) background subtracted phase image.

retrieve the phase map. The key point here is that the tangent operation is very sensitive to the noise. Thus, the proposed technique avoids this noise by correcting the images *before* performing the tangent operation, which results in phase images without wrapping problems.

In our experiment, the phase of the background is a property of the optical system and, thus, does not change for a particular specimen-holder combination. Therefore, we only capture the background interferogram once, at the beginning of the measurement. This background information is stored and used in all subsequent images.

3. Results

Here, we present the result of our proposed phase reconstruction technique and a comparison with the original approach. We imaged live red blood cells diluted with Coulter LH series diluent (Beckman Coulter). Figures 3(a) and 3(b) show the phase maps obtained, respectively, by using the original approach (which requires phase unwrapping) and our new approach. The experimental results show the excellent agreement. Figure 3(c) shows a profile plot across the red blood cell as indicated by the yellow line in Figs. 3(a) and 3(b). Figure 3(d) shows the phase image achieved with the original approach but without using phase unwrapping algorithm in the final step. This operation obviously gives wrapping problems in the resulting image.

By using the proposed approach, we do not have to perform phase unwrapping, which requires most of the processing time. We performed a comparison in MATLAB between the two methods, using the same image of size, 512 × 512 pixels. The results revealed that the original Hilbert transform based method takes 0.6 s to process, while the new method takes only 0.046 s. Furthermore, the new method only requires Fourier transform and point-by-point matrix multiplication, which are highly parallelizable operations. Using CUDA [11] will allow much higher phase retrieval rates. Our estimates indicate

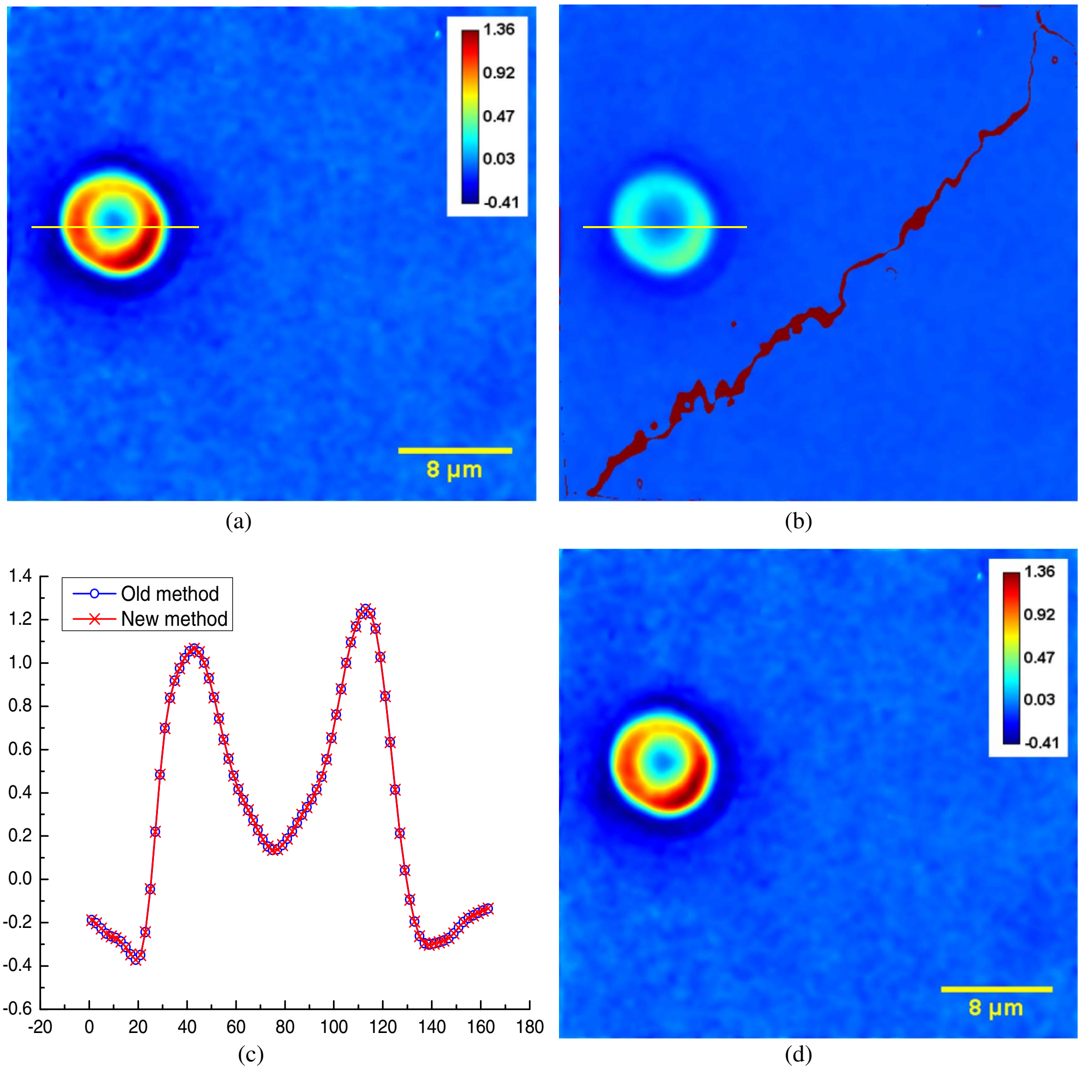

Fig. 3. (Color online) Comparison of the phase reconstructions of Hilbert based method and the proposed method: (a) Phase map of a red blood cell obtained by the original Hilbert based method, (b) phase map of a red blood cell obtained by the new method, (c) profile plots at the lines across the cell in (a) and (b), (d) subtraction method without using phase unwrapping.

that if implemented in CUDA, our phase reconstruction would take merely a few milliseconds, which will result in ~100s of frames/second. In essence, bypassing the unwrapping and using CUDA should allow us to reconstruct quantitative phase images in real time, limited only by the camera acquisition time.

4. Conclusion

In this paper, we present a simple technique for reconstructing phase image from the wDPM system. By a simple division of a background image from the captured image, we can cancel the noise caused by the background and all the optical components of the system. Thus we avoid the need for phase unwrapping when imaging thin objects, i.e., specimens that cause less than 2π phase shifts. The experimental results show that the new approach gives exactly the same result as the original Hilbert transform based approach while performing much faster. This technique, though very simple, will be very useful in real-time off-axis QPI systems. All the operations can be done in parallel using CUDA, which can achieve extremely fast performance.

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