

A phase retrieval method of interferograms add-subtracting based on two-step phase shifting

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Abstract. A phase retrieval method is introduced in quantitative phase imaging (QPI) based on two-step phase-shifting technique. By acquiring two measured interferograms and calculating the addition and subtraction between them, the quantitative phase information can be directly retrieved. This method is illustrated by both theory and simulation experiments of a ball. The results of the simulation and the experiment of the red blood cell show a good agreement, demonstrating its application for studying cells.

Keywords: Biotechnology, quantitative phase imaging, phase retrieval, two-step phase shifting, addition and subtraction

1. Introduction

Microscopic specimens, such as live cells, are mostly transparent, and can be hardly imaged by conventional bright field microscopy because of inadequate contrast. In order to settle such a serious problem, many methods have been proposed [1,2], among which, the phase detection method provides the possibility for making biological samples visible noninvasively and nondestructively with high sensitivity [2]. Therefore, an increasing number of phase microscopy imaging techniques have been developed, especially the quantitative phase microscopy techniques [3–8]. Retrieving the quantitative phase information is an essential and important procedure in QPI, as it provides useful information related to both cellular structure and dynamic [9,10]. Many researchers apply interferometric methods and their corresponding algorithms to achieve this goal.

In on-axis interference, since the object wave and the reference wave are parallel, the sampled field and undesired field are superimposed. Typically, at least three phase-shifted interferograms are required before applying phase-shifting algorithm to separate the sample field in order to yield quantitative phase information [4,7]. This method is not feasible for real-time imaging due to its long computation time and instability. Therefore, some phase retrieval algorithms from two interferograms have been proposed, for example, in [11] and [12].

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In off-axis interference, by introducing a large angle between the object and reference waves, there is a separation between the sample field and undesired field, so off-axis QPI allows for single shot measurement and fast acquisition rate. Some phase retrieval methods have been proposed and widely used, such as Fourier transform [13,14] and Hilbert transform [5,8]. However, these methods might cause the loss of some high frequency information due to the background filter procedure. Combining the phase shifting technique, Shaked et al. [15] proposed an improved method in which the background intensity is eliminated by computing the difference between two interferograms. Similar to the aforementioned methods, this method also needs heavy computation owing to integral transformations. Recently, some local operators have been used for real-time QPI. A spatial phase-shifting algorithm from Debnath and Park [16] and a derivative method from Bhaduri and Popescu [17] are used to process the measured hologram, respectively. Although these two methods are faster than other approaches, they can only be used in off-axis QPI.

In this paper, based on two-step phase shifting technique, a simple approach for phase extraction in QPI is presented. This method could be performed from two π phase-shifted interferograms by calculating the sum and the difference between them. Nice phase images of a ball and a red blood cell are obtained via the simulations, demonstrating the feasibility of the proposed method in this paper.

2. Method

This method for QPI is based on two-step phase-shifting technique. In the interference system, the intensity at the detector plane can be expressed as Eq. (1) based on the holographic theory,

$$I_1(x, y) = |O(x, y) + R(x, y)|^2 = |O|^2 + |R|^2 + OR^* + O^*R \quad (1)$$

where * denotes the complex conjugation, $O(x, y) = |O|\exp(j\varphi_o)$ and φ_o are the complex amplitude and phase of the object wave, respectively, $R(x, y) = |R|\exp(j\varphi_R)$ and φ_R are the complex amplitude and the initial phase of the reference wave, respectively. The second interferogram can be obtained after shifting the phase of the reference wave by π rather than $\pi/2$. In this case, its intensity can be expressed as

$$I_2(x, y) = |O(x, y) + R(x, y) \cdot \exp(j\pi)|^2 = |O|^2 + |R|^2 - OR^* - O^*R \quad (2)$$

Subtracting Eq. (2) from Eq. (1), then

$$I_1 - I_2 = 2(OR^* + O^*R) = 4|O||R|\cos\varphi, \quad (3)$$

in which, $\varphi(x, y) = \varphi_o - \varphi_R$ is the overall phase difference between two interference waves. In general, it can be described as

$$\varphi(x, y) = \varphi_{OBJ}(x, y) + \varphi_c(x, y) + f_x x + f_y y \quad (4)$$

where φ_{OBJ} is the phase delay induced by the sample, φ_c is the background phase induced by the optical path difference between the sample and reference arms, f_x and f_y are the spatial frequencies of the fringes in the x and y axes, respectively. It can be gotten as below after summing Eqs. (1) and (2),

$$I_1 + I_2 = 2(|O|^2 + |R|^2) \tag{5}$$

When the intensity of the object wave is the same as that of the reference wave, the phase difference $\varphi(x, y)$ can be derived by Eqs. (3) and (5),

$$\varphi(x, y) = \arccos \left[\frac{I_1 - I_2}{I_1 + I_2} \right] \tag{6}$$

In order to simplify the calculation, the background phase φ_c without the sample is usually controlled as zero by adjusting the optical path in real cases. In addition, in on-axis interferometry, both f_x and f_y are zero because the angle between the sample and reference beams is zero. Thus, the phase difference φ and the sample phase φ_{OBJ} are equivalent. In off-axis interferometry, the tilt term [last two terms in Eq. (4)] is required to be removed from the whole phase φ to reconstruct the phase φ_{OBJ} .

Obviously, once the equality of intensities of two interference waves is guaranteed and two phase-shifted interferograms are acquired, the phase retrieval related to the sample can be achieved. Note that, the wrapped phase is only obtained with Eq. (6) owing to the inverse cosine function, whose measured values range from 0 to π . This is different from the common wrapped phase caused by arc tangent function, whose values are between $-\pi$ to π .

3. Simulation results

In order to demonstrate our algorithm, the phase retrieval of a ball was performed by the simulation calculation. Considering the physical characteristics of blood cells, the radius of the ball, R , is set to be $4\mu\text{m}$, and it has a constant refractive index $n_1=1.59$. In our simulation, the other parameters are as follows: the refractive index of surrounding medium $n_0=1.57$, the wavelength of the laser $\lambda=632.8\text{nm}$, the object and reference waves are normal incident plane waves with unity amplitudes. In the method of on-axis interferometry, assuming that the light propagates along z axis, the 2D phase of the ball can be given by

$$\varphi_{OBJ} = [4\pi(n_1 - n_0)\sqrt{R^2 - x^2 - y^2}] / \lambda . \tag{7}$$

The initial phase of the reference wave is zero and the optical path difference φ_c is zero. According to the above conditions, the complex amplitudes of two optical waves were programmed, and then the interference pattern (1024×1024 pixels) can be obtained with Eq. (1), as shown in Figure 1(a). Figure

1(b) is the phase-shifted interferogram computed with Eq. (2). Figures 1(c) and (d) show the sum and the difference between two interferograms, respectively. Then, according to Eq. (6), the phase of the ball after 2D phase unwrapping is obtained as presented in Figure 1(e). Its thickness distribution can be directly computed from this phase image owing to the uniform refractive index, as shown in Figure 1(f). Through the calculation, the maximum thickness is $7.999\ \mu\text{m}$, and the absolute deviation is $0.0001\ \mu\text{m}$ compared with the theoretical value, which is possibly caused by the phase shifting or the phase unwrapping algorithm. From Figure 1(c), it can be seen that the sum between two interferograms is not a fixed constant, because the phase shifting algorithm introduces the detuning errors, and this conclusion is also demonstrated in [18]. In addition, the phase unwrapping procedure involves heavy computation, so some data may introduce bias.

In off-axis case, the spatial frequencies in the x and y axes, f_x and f_y are set to be $0.194\ \text{rad/pixel}$ and 0 respectively, which are different from those in on-axis case, while other parameters remain the same. Figures 2(a) and 2(b) are two off-axis phase-shifted interferograms of the above ball. Figure 2(c) is the

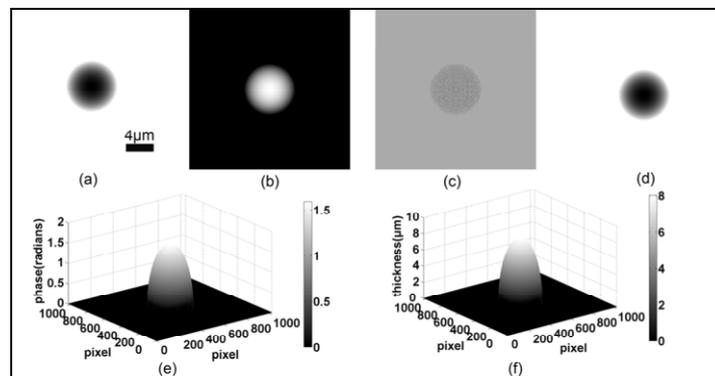


Fig. 1. The ball in on-axis interferometry: (a) and (b) are two phase-shifted interferograms, (c) and (d) are the sum and the difference between (a) and (b) respectively, (e) the reconstructed unwrapped phase, (f) the thickness distribution.

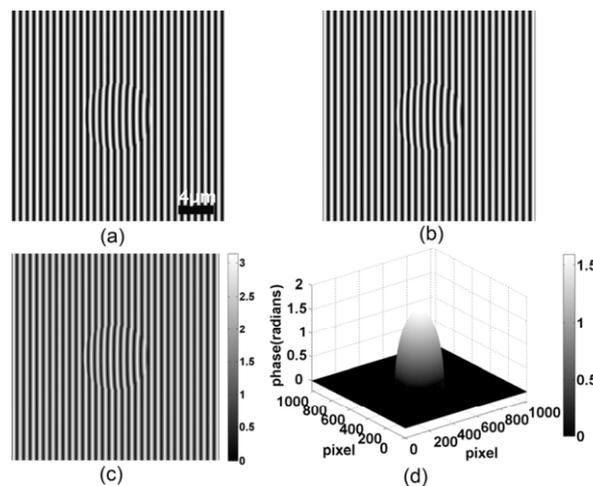


Fig. 2. The ball in off-axis interferometry: (a) and (b) are two phase-shifted interferograms, (c) and (d) are the wrapped and unwrapped phases, respectively.

wrapped phase without removing the tilt term, and its values are between 0 to π . After unwrapping, the real phase information is reconstructed, as shown in Figure 2(d).

Moreover, in order to demonstrate the capability of our method for faster phase imaging, the execution time for the phase calculation was compared among the cases of using our method, Hilbert transform and Fourier transform. Our method takes 0.108s when two phase-shifted interferograms are acquired simultaneously while Hilbert transform and Fourier transform methods take 1.264s and 1.929s respectively. The phase reconstruction was performed on a desktop computer with an Intel Core Pentium(R) Dual-E2180 CPU (2.00 GHz) in the MATLAB environment.

A red blood cell (RBC) was considered in order to verify the flexibility of this method in quantitative analysis for cells. Firstly, a RBC phase model is built according to the data of the thickness and the refractive index. Figure 3(a) shows the thickness distribution of a RBC from Popescu *et al.* [19]. In general, a matured RBC is regarded as a homogenous phase object with a uniform refractive index. In our simulation, the refractive indices of the RBC and surrounding medium, n_{cell} , and n_{medium} are set to be 1.4 and 1.34 respectively, and the wavelength is 514 nm. These parameters are the same as the experiment values for the following comparison.

The simulation process of the RBC is the same as the above ball in the case of on-axis. The reference wave phase was taken as 0 and π for the first and the second interferograms, respectively. Figures 3(b) and 3(c) show these two interferograms with the size of 512×512 pixels, respectively. Then both the phase and thickness distributions are reconstructed from both interferograms, which are presented in Figures 3(d) and 3(e), respectively. From Figure 3(e), the biconcave morphology of the RBC is clearly displayed. Moreover, it can be seen that there is no significant difference between Figures 3(a) and 3(e). For further comparison to demonstrate the accuracy of our method, the horizontal thickness profiles of them were made as shown in Figure 3(f). These two curves are almost superimposed, in which relatively large deviations occur only at the ends of the curves. Through the calculation, the average deviation of the calculated thickness (solid line) with respect to the original one in the simulation (circle line) is $0.0174\mu\text{m}$, the relative average deviation is 11.33%, and the mean squared error (MSE) is 0.0064.

4. Conclusion

A simple phase extraction method in QPI is presented. By acquiring two interferograms with a phase shift of π and calculating the sum and the difference between them, the quantitative phase image

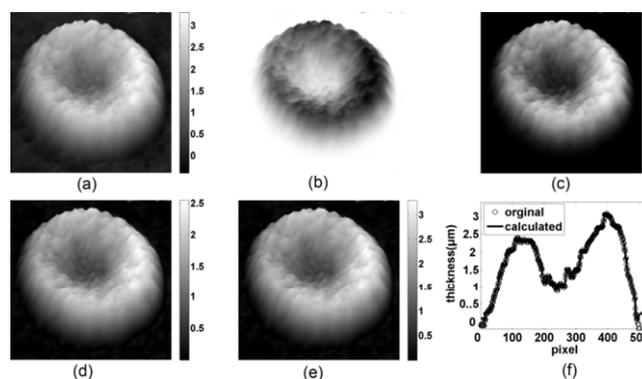


Fig. 3. The simulated red blood cell: (a) the thickness distribution from Popescu *et al.* [19], (b) and (c) are two phase-shifted interferograms, (d) the unwrapped phase, (e) the thickness distribution, (f) the horizontal thickness profiles of (a) and (e).

can be obtained. Compared with the traditional phase-shifting algorithms in the case of on-axis interferometry, this new method requires less measurement. Moreover, once two interferograms are obtained simultaneously, a faster processing speed can be achieved. These results demonstrate that this method can be applied as a powerful tool for the analysis of fast phenomenon of biological specimen.

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