Optik 125 (2014) 4841–4844

Contents lists available at ScienceDirect

Optik

journal homepage: www.elsevier.de/ijleo

Simulation study on the quantitative phase retrieval method under two-step phase-shifting technique

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ARTICLE INFO

Article history:
Received 3 September 2013
Accepted 5 April 2014

Keywords:
Phase extraction
Quantitative phase imaging
Two-step phase-shifting
Derivative
Cell

ABSTRACT

Based on two-step phase-shifting technique, we present a new derivative method for phase information extraction in quantitative phase imaging (QPI). By acquiring two phase-shifted interferograms and numerically calculating their 1st order derivatives, one can directly obtain the quantitative phase information. We demonstrate the proposed method by comparing our simulated results with the experimental results of the red blood cell and the HeLa cell, respectively. It shows that our method can be generally applied to any QPI, such as on-axis and off-axis interferometry, especially for slightly off-axis interferometry, and it has the feature of efficient utility of camera spatial bandwidth and the ability of fast data processing.

1. Introduction

Over the last decade the quantitative phase imaging (QPI) technique has attracted increasing scientific interest in study of biological cells in virtue of its label-free feature and high sensitivity. In QPI technique, retrieving the quantitative phase information is a key task, because it can offer some structure details to further study the cell's shape and dynamic behavior [1–5]. Generally, the quantitative phase information retrieving process is mainly dependent on the specific interferometric approaches.

Usually, in on-axis interferometry, at least three phase-shifted interferograms are acquired to separate the sample field for yielding the quantitative phase information [6–8]. Thus, it may not be effectively utilized for studying some dynamic imaging processes due to that the whole reconstruct process is time-consuming and the sample may change between frame acquisitions [9,10]. Concerning on this, Meng [11] and Liu et al. [12] proposed the on-axis two-step phase-shifting imaging techniques, but in these cases, the reference wave intensity measurement or estimation is needed. For this reason, Shaked [10] presented a different parallel two-step phase-shifting technique in a single camera exposure without the above measurement based on the wide-field digital interferometry. However, among its phase retrieving, a static phase reference measurement without the sample is required.

In off-axis technique, another interferometeric approach, some phase information retrieval methods have been proposed and successfully applied to cell research based on Fourier transform [13,14], Hilbert transform [15–17], and so on. Only a single image is needed in these methods, which makes it suitable to dynamic research at the expense of losing detector bandwidth. Considering the frequency bandwidth, slightly off-axis interference [18,19] has been put forward, which requires two images. However, similar to other methods, it refers to the integral transformation with the computationally demand, and thus it is difficult to achieve real-time phase information. More recently, a novel derivative method [20] has been proposed, and the speed of its phase retrieval is faster than the current approaches owing to only using the derivative operation. However, it requires that the phase change between adjacent neighborhoods induced by the object could be neglected with respect to the spatial frequency of the carrier fringes.

In this paper, we present an alternative approach for phase extraction, which combines the derivative operation and two-step phase-shifting technique. This method only relies on two phase-shifted interferograms and their 1st order derivatives. It has to be pointed out that it can be applied generally to any quantitative phase imaging, such as on-axis interference, traditional or slight off-axis interference. This method requires neither the knowledge of other parameters compared to two-step on-axis interference, and nor the integral transformation that needed for off-axis interferometry. Besides, in our method, once the two interferograms are

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http://dx.doi.org/10.1016/j.ijleo.2014.04.045
0030-4026/

acquired in a single camera exposure, the data processing speed of its phase extraction is equivalent to that of the derivative method in Ref. [20], and also with a wide bandwidth.

2. Method

In the interference system, two phase-shifted interferograms can be expressed as

$$I_1(x,y) = I_0(x,y) + \gamma(x,y)\cos[\varphi(x,y) + kx], \tag{1}$$

$$I_2(x,y) = I_0(x,y) + \gamma(x,y)\cos[\varphi(x,y) + kx + \alpha], \tag{2}$$

where I_0 (x, y) is a low frequency function that describes background intensity, γ (x, y) is the amplitude modulation factor of the interferogram, φ (x, y) is the spatial phase delay associated with the sample, the most interest quantity in QPI, and k is the spatial frequency of fringes, which is determined by the tilt angle between the sample beam and reference beam. In case of on axis interference, k is zero because the tilt angle is zero. α is the phase shift induced between the interferograms. Note that k and α could be determined by fitting the background interference to a sine wave in the experiment.

Analogous to the derivative method [20], we apply the local transformation to the interferograms. Thus, the first order derivatives of each interferograms with respect to x can be expressed as

$$I_1^{(1)} = \frac{\partial I_1(x,y)}{\partial x} = \frac{\partial I_0(x,y)}{\partial x} + \frac{\partial \gamma(x,y)}{\partial x}\cos[\varphi(x,y) + kx] + \gamma(x,y)\{-\sin[\varphi(x,y) + kx]\}\left[\frac{\partial \varphi(x,y)}{\partial x} + k\right], \tag{3}$$

$$I_2^{(1)} = \frac{\partial I_2(x,y)}{\partial x} = \frac{\partial I_0(x,y)}{\partial x} + \frac{\partial \gamma(x,y)}{\partial x}\cos[\varphi(x,y) + kx + \alpha] + \gamma(x,y)\{-\sin[\varphi(x,y) + kx + \alpha]\}\left[\frac{\partial \varphi(x,y)}{\partial x} + k\right]. \tag{4}$$

For most transparent phase objects such as biological cells or tissues, both the background intensity I_0 and the amplitude modulation factor γ (x, y) are constants over the interferogram approximately [20], so we can make the following favorable approximations:

$$\frac{\partial I_0(x,y)}{\partial x} \approx 0, \quad \frac{\partial \gamma(x,y)}{\partial x} \approx 0. \tag{5}$$

Thus, Eqs. (3) and (4) can be transformed to

$$I_1^{(1)} = \gamma(x,y)\{-\sin[\varphi(x,y) + kx]\}\left[\frac{\partial \varphi(x,y)}{\partial x} + k\right], \tag{6}$$

$$I_2^{(1)} = \gamma(x,y)\{-\sin[\varphi(x,y) + kx + \alpha]\}\left[\frac{\partial \varphi(x,y)}{\partial x} + k\right]. \tag{7}$$

If the phase shift α is a special value, such as $\pi/2$, Eq. (7) can be simplified as

$$I_2^{(1)} = \gamma(x,y)\{-\cos[\varphi(x,y) + kx]\}\left[\frac{\partial \varphi(x,y)}{\partial x} + k\right]. \tag{8}$$

Based on Eqs. (6) and (8), the phase distribution induced by the specimen can be extracted by

$$\varphi(x,y) = \tan^{-1}\left[\frac{I_1^{(1)}}{I_2^{(1)}}\right] - kx. \tag{9}$$

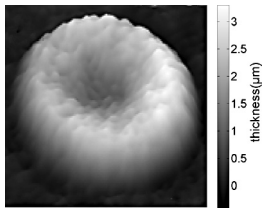

Fig. 1. The thickness distribution of the RBC obtained from Ref. [21].

In case of on-axis interference, $k = 0$, the quantitative phase image φ (x, y) can be simply determined by

$$\varphi(x,y) = \tan^{-1}\left[\frac{I_1^{(1)}}{I_2^{(1)}}\right]. \tag{10}$$

Note that, once the two interferograms are acquired simultaneously, the phase information associated with the sample can be computed from them, and the fast phenomena can be visualized.

3. Simulation results

To demonstrate the applicability of our proposed method, we firstly imaged a simulated red blood cell (RBC) in the cases of on-axis interference and off-axis interference by applying MATLAB software, and then compared the thickness distributions obtained from both reconstructed phase maps with the original thickness distribution of the simulated RBC. The thickness distribution of the simulated RBC is obtained from Ref. [21], as shown in Fig. 1.

Generally speaking, the RBC can be regarded as a homogenous phase object owing to the absence of intracellular organelles such as the nucleus. Therefore, the refractive index of RBC, n_{cell}, is uniform, which can be set a constant, $n_{cell} = 1.4$ in our simulation. The refractive index of the cell medium, n_{medium}, is 1.34 and the wavelength, λ, is 514 nm, which both are same as the experiment value for comparison. In addition, in order to simplify the calculation, both the sample wave and reference wave are considered as plane waves, and their amplitudes are assumed to be 1 in our simulation.

At first, in case of off-axis interference, the tilt angle between the sample and reference beams is set in our simulation. According to the above parameters, we can obtain two off-axis interferograms (512 pixels × 512 pixels) with a phase shift of $\pi/2$, as shown in Fig. 2(a) and (b) respectively, and through the numerically calculation, their corresponding first order derivatives with respect to x are shown in Fig. 2(c) and (d). For the situation of on-axis interference, the tilt angle is zero, the relative results are shown in Fig. 2(e)–(h). Once k is known, which is determined by the tilt angle, according to Eqs. (9) and (10), the 2D reconstructed phases after phase unwrapping in cases of off-axis and on-axis interference are extracted, which are presented in Fig. 2(i) and (j), respectively. We have compared these results in both cases, and obtained good agreement. According to the definition of image similarity that reflects the correlation degree between two images [22], the similarity between them is calculated to be 0.9981, and the shape distribution of these two maps is similar to the original thickness distribution (Fig. 1) owing to the homogenous object, which is consistent with the theory.

To demonstrate the validity of this method further, we analyzed the thickness distributions of the cell. Fig. 3(a) and (b) display the 2D thickness distribution maps of the simulated RBC in cases of off-axis and on-axis, respectively, which are obtained from the above corresponding quantitative phase maps with the relationship $h = (\varphi\lambda/2\pi)/(n_{cell} - n_{medium})$, where h indicates the thickness. For further comparison and demonstrating the accuracy of our method, we have calculated the similarity between these two thickness maps