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Single-shot quantitative phase microscopy with the transport-of-intensity equation

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ABSTRACT

We present a single-shot experimental configuration for quantitative phase microscopy recovery based on the transport-of-intensity equation (TIE). The system can simultaneously capture two laterally separated images with different amounts of defocus using only one digital camera. The defocus distance can be adjusted by varying the free space propagation transfer function on a phase only spatial light modulator. The intensity derivative along optics axis can thus be estimated optimally. In contrast to the state of the art techniques, this configuration requires no mechanical moving parts. Furthermore its single-shot property allows potential application for measuring fast moving objects or dynamic processes. Validation experiments are presented.

Keywords: quantitative phase microscopy; transport-of-intensity equation; single shot; spatial light modulator

1. INTRODUCTION

Over the past decade, a great deal of scientific attention has been paid to quantitative phase imaging (QPI), which has emerged as an important tool in 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]. In the area of cell and tissue imaging, quantitative phase imaging is also vital because biological soft tissues and cells are phase objects which are nearly uniform with little intensity variation in the case of conventional bright-field microscopy. Transport-of-intensity (TIE) phase imaging is a promising tool for QPI because of its unique advantages over interferometric methods such as: non-interferometric approach, does not require phase unwrapping, and not strict requirement on coherent illumination [1, 5].

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. It can be approximated by finite differences taken between two close separated images [6-8]. To acquire the two images with slight defocus, either the camera or the object has to be mechanically translated, which limits the applicability of the technique to static objects. However, in many applications, including applied physics and biomedicine, it is important to have high throughput, high speed, and real-time phase information.

In this paper, we propose a novel experimental configuration for quantitative phase microscopy, which is able to achieve simultaneous capture of two laterally separated images with different defocusing degrees, using only one digital camera. The defocusing distance can be adjusted by changing the free space propagation transfer function on a phase only spatial light modulator (SLM). Thus the intensity derivative along optics axis can be estimated optimally. Since no mechanical adjustment is required during the recording process, this provides quantitative phase images that are inherently stable and at an acquisition speed limited only by the detector.

2. THEORY

Figure 1 shows the experimental configuration, wherein a partially coherent white light source with green interference filter (central wavelength $\lambda = 550$ nm) is used for illumination. A commercial inverted bright field microscope (Olympus IX71), which itself is composed of a collector lens, aperture diaphragm, condenser lens, objective, reflective mirror (M1), and tube lens, produces a magnified image of the specimen at the camera output port (image plane). In order to balance the spatial coherence in white light illumination and the image resolution, the aperture diaphragm is set to about 35% of the maximum size (NA of the objective). From the image plane of the microscope, two lenses L1 and L2 (focal length $f_1 = f_2 = 150$ mm) form a 4f system. Additionally, the non-polarizing cube beam splitter (NPBS), mirror (M2), and SLM are sandwiched between the two lenses. The SLM is a reflective liquid crystal phase only panel supplied by Holoeye Photonics AG. It has a pixel pitch of $8\ \mu\text{m}$ and was configured to provide full 2π phase modulation with a linear electro-optical characteristic. The image beam is split by the NPBS into a reflection and a transmission beam. The two beams are reflected by the SLM and M2 respectively at their Fourier planes.

Fig. 1. Experimental setup; NPBS, non-polarizing beam splitter; M1, M2, mirrors; L1, L2, lenses; SLM, spatial light modulator

Considering the Fourier transform property of the first lens L1, the complex amplitude of the wave field across the Fourier plane $U(\xi, \eta)$ is proportional to the Fourier transform of the complex amplitude incident in the image plane, where vector $(\xi, \eta) = (u/\lambda f, v/\lambda f)$, and (u, v) represents spatial frequencies in two-dimensional image space. The normal of mirror M2 has a small angle with respect to the optical axial direction, thus the reflected beam is laterally shifted with angular offsets α and to the optical axis.

$$G_r(\xi, \eta) = U(\xi, \eta) \exp(-i2\pi\xi \sin \alpha / \lambda) \quad (1)$$

The SLM is loaded with a designed phase pattern, enabling another laterally shifted beam with angular offsets $-\alpha$ to the optical axis. Furthermore, this wave field is also axially-displaced by Δz [9]

$$G_t(\xi, \eta) = U(\xi, \eta) \exp(i2\pi\xi \sin \alpha / \lambda) H_{\Delta z}(\xi, \eta) \quad (2)$$

where $H_{\Delta z}(\xi, \eta)$ is the angular spectrum transfer function in the spatial frequency domain

$$H_{\Delta z}(\xi, \eta) = \exp\left(-i2\pi\Delta z \sqrt{1 - (\lambda\xi)^2 - (\lambda\eta)^2} / \lambda\right) \quad (3)$$

After passing through lens L2, the complex amplitude distributions of the two split beams are given by

$$u_r(x, y) = u(x + \sin \alpha f, y) \quad (4)$$

$$u_l(x, y) = u_{\Delta z}(x - \sin \alpha f, y) \quad (5)$$

Thus the two images in the CCD plane I_t and I_r are laterally separated by $2\sin \alpha f$. To prevent overlapping of the two images in the CCD plane, a rectangular aperture is set just behind the image plane. Besides, to ensure the SLM is working in the linear region and to maintain the intensity balance between the two images, three linear polarizers are added in the reflection beam (M2 side), and the transmission beam (SLM side), and the combined beam (CCD side) respectively (not shown in Fig. 1).

For the phase reconstruction step, the phase map without 2π discontinuity is directly calculated using the two images acquired within single shot. Accurate registration of the two experimental images (I_t and I_r) should be performed beforehand [10]. Once the axial shift between the two images is obtained, no other calibration procedure is needed for the successive measurement unless the setup is changed. First the intensity derivative is estimated by

$$\frac{\partial I(x, y)}{\partial z} \approx \frac{I_t(x, y) - I_r(x, y)}{\Delta z}, \quad (6)$$

Then the phase map is directly calculated by solving the TIE

$$-k \frac{\partial I(x, y)}{\partial z} = \nabla \cdot [I(x, y) \nabla \phi(x, y)], \quad (7)$$

Mathematically, the smaller the defocus distance, more accurate the approximation in Eq. (2) will be. However, real measurements yield data with noise and discretization which does not allow Δz to be very small. The optimal Δz should be chosen by balancing the high-order (or nonlinearity) error and the noise effect [11, 12], which can be easily adjusted by changing the phase pattern loaded on the SLM.

3. RESULTS

An experiment was conducted to verify the validity of the proposed method. In the experiment, the microscope objective used has a magnification 40x with 0.8NA. A plano-convex linear microlens array with $30 \mu\text{m}$ pitch from SUSS MicroOptics was used as the test sample. Fig. 2(a) shows the raw images of the microlens array obtained in both halves of the camera in a single-shot. The right-side shows the best focus image, while the left-side depicts the defocused one controlled by the SLM. The defocus distance between the two planes was chosen as $8.5 \mu\text{m}$. After registration, the intensity derivative can be obtained, as shown in Fig. 2(b). Fig. 2(c) shows the recovered phase and Fig. 2(d) shows the unwrapped phase with the range within 2π . For comparison, the same sample was also measured using a digital holography microscopy (DHM) system (laser wavelength 650 nm, magnification 43x), and the wrapped phase is shown in Fig. 2(e). The three-dimensional profile of one single lens (indicated by the black square region in Fig. 2(d)) by converting the phase to the physical height of the lens. One cross-section corresponding to the red solid red line in Fig. 2(d) are shown in Fig. 4(g). One line profile for DHM result is also shown in Fig. 2(h). Given the height profile of the lens, the radius of curvature (ROC) can be calculated by

$$ROC = h/2 + D^2/8h, \quad (8)$$

where h is the height of the microlens, and D is the diameter of the microlens. Considering the fill factor of the microlens array, the diameter of the microlens is $27 \mu\text{m}$. The average height h of the microlens are measured $2.371 \mu\text{m}$

for our method and 2.464 for DHM. Thus, the calculated ROC are $396\text{ }\mu\text{m}$ for our method and $382\text{ }\mu\text{m}$ for DHM, which both correspond reasonably well to the ROC value provided by the supplier ($390\text{ }\mu\text{m}$).

Fig.2. The experimental results for a lens array. (a) raw images obtained in both halves of the camera in a single-shot. (b) intensity derivative. (c) recovered phase. (d) rewarped phase from (c). (e) DHM result. (f) 3D profile for one single lens. (g) and (h) are two crosssections corresponding to the lines indicated in (d) and (e), respectively.

4. CONCLUSION

We have presented a new configuration for quantitative phase microscopy based on TIE. The experimental results show that the proposed method can reconstruct phase of the object accurately with a one-shot measurement. This offers the possibility to imaging moving objects and dynamic scenes, e.g. in-vivo cell imaging.

5. ACKNOWLEDGEMENT

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