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Interferometric phase microscopy using slightly-off-axis reflective point diffraction interferometer

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ABSTRACT

An interferometric phase microscopy (IPM) is proposed using slightly-off-axis reflective point diffraction interferometry for quantitative phase imaging. A retro-reflector consisting two mirrors is used to generate an angle between the object beam and reference beam, and a 45° tilted polarizing beam splitter is used to split the horizontal and vertical components of the both beams. Two carrier interferograms with $\pi/2$ phase-shift can be acquired in one shot, and the phase distribution of a thin specimen can be retrieved using a fast reconstruction method. The new IPM without loss in the utilization of the input-plane field of view combines the real time and optimizing detector bandwidth measurement benefit associated with slightly-off-axis method, high stability associated with common path geometry, and simplicity in terms of procedure and setup. Experiments are carried out on both static and dynamic specimens to demonstrate the validity and stability of the proposed method.

1. Introduction

Interferometric phase microscopy (IPM) is a powerful label-free tool for studying biological specimens, surface measurements, and micro structures [1–4]. In order to obtain phase distribution of the specimen, many IPM optical geometries have been proposed over the years, including common-path geometries and separated-path geometries. Compared to the separated-path IPMs, the common-path IPMs, such as setup based on point diffraction interferometer (PDI), have caught attention due to their robustness and simplicity [5–8]. To achieve high precision, phase shifting method has also been introduced to common-path PDI which can be defined as phase-shifting point diffraction interferometer (PSPDI) [9–13]. However, due to its common-path configuration, phase shifting has to be done at or near the pinhole with complex pinhole assembles or particular elements [10–13], which makes it difficult to add phase shifts in one beam with respect to the other one for PSPDI. Recently, different on-axis reflective PSPDIs (RPSPDIs) have been proposed using a modified Michelson configuration to solve such problems [14–17]. In the RPSPDI, the frequency spectrum of the input beam is split into two copies by a cube beam splitter so that it is convenient to do any phase shifting operations to either of the copies with simple elements. For example, Guo et al. [14] achieved time-sequent phase shifting with the help of shifting polarization elements. However, it is unsuitable for the measurement of moving objects or dynamic processes. To solve such problems, our group achieved parallel phase shifting using a reflective grating [15]. However, the utilization of the field of view (FOV) of the CCD camera is only 1/3 because three interferograms need to be captured in one shot. Guo et al. [16] realized parallel two-step phase shifting by introducing a 4f imaging system incorporated with a grating and polarization phase-shifting elements. The utilization of FOV of the CCD camera can be then improved to 1/2 because only two interferograms need to be captured in one shot. However, the modulation unit leads to difficulty of alignment and complexity of the setup. To simplify the setup, our group also realized parallel two-step phase shifting by using a 45° tilted polarizing beam splitter to split the horizontal and vertical components of the object and reference beams [17]. However, both the above parallel two-step phase shifting methods need particular requirements for the intensities of the object and reference beams and complex calculations to retrieve the specimen phase. As another attractive method, much work has also been done on off-axis reflective PDI [18,19]. The off-axis reflective PDI can retrieve the phase using only one interferogram, and then achieve a real-time measurement. However, it cannot fully utilize the space-bandwidth of a CCD camera because of the requirement for eliminating the unwanted DC and twin image terms in the interferograms. To provide an intermediate solution between on-axis and off-axis IPM, slightly-off-axis phase shifting IPM (SPSIPM) [20–23] has been proposed to obtain two phase-shifted interferograms with spatial carrier in recent years. The DC term can be eliminated using subtraction of the two interferograms. The SPSIPM

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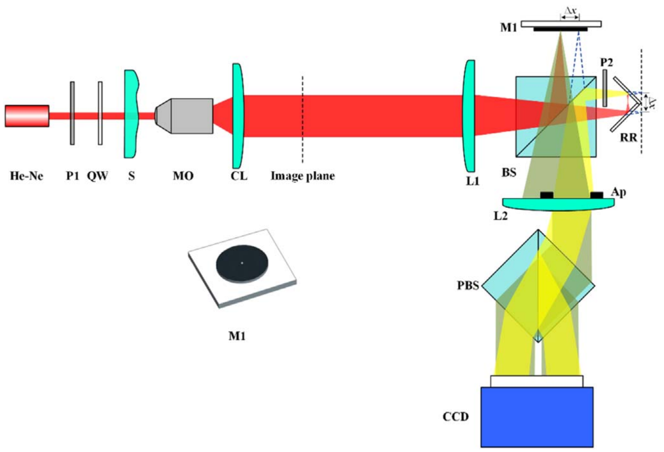

Fig. 1. Experimental setup: P1 and P2, polarizers; QW, quarter-wave plate; MO, microscopic objective; S, Specimen; CL, collimated lens; L1 and L2, lenses with a same focal length; BS, beam-splitter; PBS, polarizing beam splitter; M1, reflective pinhole mirror; RR, retro-reflector; Ap, aperture.

can then require simpler phase retrieving process than on-axis IPM, and lower space-bandwidth of a CCD camera but achieve better reconstruction capability than off-axis IPM. However, most of the setups are based on separated-path geometries. Although Shan et al. [24,25] proposed a common-path slightly-off-axis interferometry with a Ronchi grating placed outside the Fourier plane, the utilization of the input-plane field of view is only 1/2. So, we proposed a slightly-off-axis RPSPDI to realize quantitative phase imaging inspired by previous work [17,25]. The method can achieve real time, optimizing detector bandwidth and high stability measurement, simplicity in terms of procedure and setup but without loss in the utilization of the input-plane field of view.

2. Experimental setup

The proposed experimental setup is depicted in Fig. 1. A non-polarizing He-Ne laser with a wavelength of λ is used as the light source. The input light is linearly polarized by polarizer P1 through 45° with respect to the horizontal axis. A quarter-wave plate QW oriented 0° along the horizontal axis is used to transform the linearly polarized beam into circularly polarized beam. Specimen S is placed in the focal plane of microscopic objective MO. The magnified object wave is collimated by lens CL. Two lenses L1 and L2 with a same focal length of f are positioned in a reflective 4f configuration to execute Fourier transformation. The microscopic image of the specimen is Fourier transformed by L1, and split into two copies using a beam splitter BS. One copy used as the reference beam RB is low-pass filtered and reflected by M1 with a pinhole diameter of d_p, which is under the limitation of $d_p < 1.22\lambda f/D$ (D is the aperture size of the CCD camera [15]). The other copy used as the object beam OB is reflected by a retro-reflector RR, which contains two mirrors attached to each other in a right angle. The RR can be used to shift the actual Fourier plane to create an angle between the OB and RB [18]. The object beam is transformed to 45° linearly polarized beam again by a polarizer P2. Both beams are combined by BS again and Fourier-transformed by L2 to the output plane to interfere. A CCD camera is placed in the output plane, and a 45° tilted polarizing beam splitter PBS with its semi-reflecting layer parallel to the propagation direction of the incident RB is placed before the CCD camera. The PBS is used to split the horizontal components (P-pols) and vertical components (S-pols) of the both beams [17]. To guarantee each interferogram smaller than half FOV of the CCD camera, a rectangular aperture Ap is used to adjust the aperture of L2. Two mirror-reversed spatial carrier interferograms with $\pi/2$ phase shift can be then captured by the CCD camera in one shot.

To demonstrate the design of aperture Ap, the ray tracing of the RB

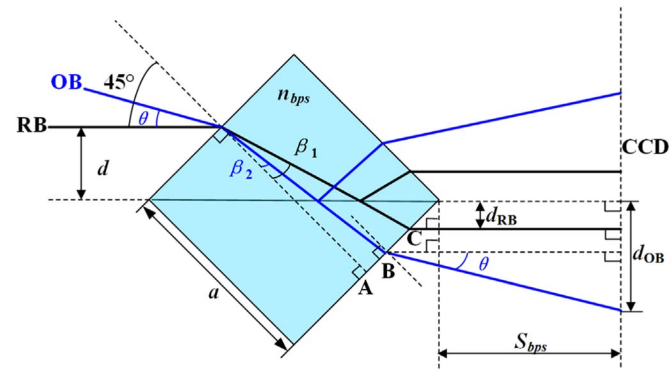

Fig. 2. Explanation for the arrangement of PBS using ray tracing of the object and reference beams through the PBS.

through the 45° tilted PBS is shown in Fig. 2, where d is the distance of the incident RB between the upper bound incident point and the semi-reflecting layer of PBS, and d_{RB} is the corresponding bound distance in the output plane. As the size of the distance d is related to that of the aperture Ap, we firstly analyze the distance d. For parallel with the semi-reflecting layer, the RB has an incident angle of 45°, and generates a refractive angle of β_1 and then an exit angle of 45° after passing through the PBS with a refractive index of n_{pbs}. Assuming one side size of the PBS is a, one expression can be obtained by following the geometry relation in Fig. 2 as shown below:

$$\sqrt{2}a = d + d\tan(\beta_1 + 45°) + d_{RB} + d_{RB}\tan(\beta_1 + 45°) \tag{1}$$

where β_1=arcsin($1/\sqrt{2}n_{pbs}$).

Let us rewrite Eq. (1), in such a way we can obtain the distance d_{RB} as:

$$d_{\mathrm{RB}} = \frac{\sqrt{2}}{2}\left(1 - \frac{1}{\sqrt{2n_{bps}^2 - 1}}\right)a - d \tag{2}$$

To realize beams replication without crosstalk, we must make the value of d_{RB} positive. But it can be seen from Eq. (2) that once a and n_{pbs} of the PBS is determined, the value of d_{RB} is inversely proportional to that of d. So when we design aperture Ap to guarantee each interferogram smaller than half FOV of the CCD camera but with higher utilization, we should make an appropriate choice between d and d_{RB}. To make it clear, we choose a PBS with a=25.4 mm and n_{pbs}=1.515, and then obtain a relation between d and d_{RB} as shown in Fig. 3. It can be seen from Fig. 3 that to make d_{RB} positive, the value of d can be chosen in the range of (0, 8.48 mm). Therefore, the side size of the aperture Ap can also be chosen in the same range with taking the utilization of FOV of the CCD camera into consideration.

On the other hand, the OB and RB will separate from each other in the output plane due to the effect of the small angle θ between them which is generated by the retro-reflector RR. However, to access two

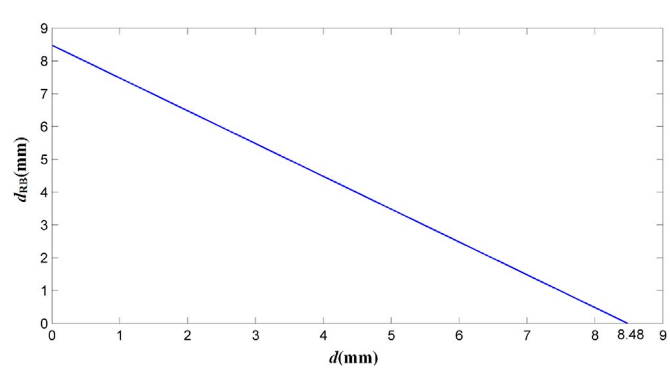

Fig. 3. The relation between d and d_{RB} when a=25.4 mm and n_{bps}=1.515.

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