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Parallel-quadrature phase-shifting digital holographic microscopy using polarization beam splitter

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ABSTRACT

We present a digital holography microscopy technique based on a parallel-quadrature phase-shifting method. Two $\pi/2$ phase-shifted holograms are recorded simultaneously using polarization phase-shifting principle, slightly off-axis recording geometry, and two identical CCD sensors. The parallel phase-shifting is realized by combining circularly polarized object beam with a 45° degree polarized reference beam through a polarizing beam splitter. DC term is eliminated by subtracting the two holograms from each other and the object information is reconstructed after selecting the frequency spectrum of the real image. Both amplitude and phase object reconstruction results are presented. Simultaneous recording eliminates phase errors caused by mechanical vibrations and air turbulences. The slightly off-axis recording geometry with phase-shifting allows a much larger dimension of the spatial filter for reconstruction of the object information. This leads to better reconstruction capability than traditional off-axis holography.

1. Introduction

Digital holographic microscopy (DHM) has emerged as a non-destructive, full-field, and label-free imaging technique for achieving quantitative phase imaging with high axial resolution and diffraction limited transverse resolution [1–9]. In DHM, interference pattern between the object and reference beam is digitally recorded using a CCD/CMOS sensor and the whole object information (quantitative phase and amplitude) is extracted by performing a numerical reconstruction using a computer [10]. However, the recorded digital hologram contains unwanted dc and twin-image diffracted waves. Several methods have been developed to eliminate these undesired diffracted waves and reconstruct the desired sample field (i.e. object wavefront). In the off-axis DHM, it is achieved by having a large angle between the object and reference waves [3,10,11]. Major advantage with this technique is that a single interferogram is sufficient for numerical reconstruction of the object information making it suitable for live cell dynamic studies. However, due to the large angle between object and reference beams this technique does not completely utilize the available space-bandwidth product (SBP) of the detector. Thus the higher spatial frequency components of the object might be lost [12–14] and hence from an imaging point of view the resolution of the reconstructed image will be lower. The elimination of zero-order intensity and the twin image in off-axis holograms can also be performed by spatial phase-shifting based methods [15]. However such techniques require information concerning the spatial distribution of the phase difference between the object wave and the reference wave in the hologram plane.

This problem can be circumvented by using an in-line geometry in which the angle between the object and reference beam is set to zero. But this "in-line" geometry makes the undesired diffracted waves occlude the desired object field. Hence three or four phase-shifted holograms of the same object are recorded by introducing stepwise phase retardations in the reference beam [3,16]. Although this procedure enables to remove the dc and the twin image diffracted waves, but due to the sequential nature of recording the holograms, it is not suitable for simultaneous measurement of moving objects or dynamic process. In addition, it has been shown that calibration errors in phase-shifting and system fluctuations between the frames degrade the quality of the reconstructed image [17–20]. To overcome this difficulty, different types of parallel phase-shifting methods are proposed which can record the phase-shifted interferograms without any time lag. In one of the methods, four CCD detectors are employed for simultaneous recording of four phase-shifted interferograms [21–25]. Methods based on single CCD detector have also been demonstrated for simultaneous recording of the required number of interferograms by utilizing special optical components such as micro-polarizer array [26–28] and two-dimensional diffraction

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grating [29,30]. The use of four CCD sensors to perform four-step phase shifting leads to complex system configuration and the technique of recording four phase-shifted holograms by a single CCD leads to under-utilization of the field of view of the CCD sensor. Techniques based on micro-polarizer array and phase gratings depend on the effectiveness of these array devices in generating multiple copies of the object and reference beams.

Shaked et al. proposed a two-step phase-shifting interferometry with slightly off-axis configuration where the dc term is successfully suppressed by subtracting one phase-shifted hologram from the other [31,32]. The reconstruction of the object information is based on the Hilbert transformation and hence requires a sinusoidal fringe pattern over the interferogram area. Thus this method is valid only for transparent objects with small thickness or phase modulation. Gao et al. [33] also proposed a different parallel two-step phase-shifting technique, in which two Ronchi gratings are used in tandem to form two parallel copies of the object and the reference beams. The successful operation of the technique would depend on the effectiveness of the phase grating. They followed it by using two cube beam splitters and one cube beam splitter setups where a quarter wave plate (QWP) and half wave plate (HWP) are placed in front of the detector for simultaneous recording of the phase-shifted holograms [34,35]. Since the spatial extent of the two beams that are derived from the cube splitter are generally very close, as they use a single CCD detector, precise size and positioning of the QWP and HWP is very critical. Furthermore, the above mentioned techniques record the two holograms using the same detector and hence place limitation on the size of the object to be investigated.

In this paper, we present a new parallel-quadrature phase-shifting DHM (PPS-DHM) based on a polarization beam splitter (PBS). A circularly polarized object beam is interfered with 45° polarized reference beam in the slightly off-axis geometry and the two quadrated phase-shifted holograms are recorded simultaneously at the transmitted and reflected beam paths of the PBS. We present reconstruction results of both amplitude and phase objects, and also the effects of the size of frequency plane aperture on the reconstructed object information. The advantage of this technique, compared to other techniques that are discussed above, is that the system configuration is simple and does not require any special optical elements. Furthermore, since we use two identical CCD sensors to record the two holograms, the size of the object to be investigated is only limited by the CCD detector area. Additionally, the two quadrature phase-shifted holograms are recorded simultaneously without any time lag, thus offering the possibility to reduce phase errors caused by mechanical vibrations and air turbulence that occurs in temporal phase-shifting holography. Moreover, another important advantage of the proposed parallel-quadrature phase-shifting holography is that it is built with simple polarization based optical components.

2. Experimental setup

An optical wave is in quadrature with another wave if they are matched in frequency and amplitude but differ in phase by 90°. The quadrature detection technique has been used to measure the amplitude and phase of an unknown signal by mixing with two reference signals that are in quadrature. We utilize this quadrature detection in digital holographic microscopy to measure the amplitude and quantitative phase information of microscopic objects. The schematic of the PPS-DHM with slightly off-axis geometry is shown in Fig. 1. Linearly polarized light from Ar–Kr laser (λ=0.488 μm) is used in a Mach–Zehnder interferometer. This linearly polarized beam is initially converted into a circularly polarized beam by using a QWP. The optical beam is then expanded and collimated. A non-polarizing beam splitter ($NPBS_1$) divides the beam into reference and object beam paths. The specimen is placed in the object beam path and the transmitted light is collected by a 20× microscope objective (MO) and collimated by lens L_2. A linear polarizer oriented at 45° relative to the $\vec{x}$ and $\vec{y}$ basis vectors is placed in the reference beam path to ensure that the reference beam is polarized at 45° before mixing with the object beam. In order to achieve slightly off-axis configuration, a very small angle between the object and reference beam is introduced by adjusting the mirror M_1 or beam splitter $NPBS_2$. The non-polarizing beam splitter ($NPBS_2$) is used to recombine the 45° linearly polarized reference beam with the circularly polarized object beam. The output from the $NPBS_2$ travels through a polarizing beam splitter (PBS) to separate into the quadrature components. The quadrature phase shift between the two interferograms is explained as follows: In the object beam arm the horizontal and the vertical components of the light has 90° phase difference between them. On the other hand, in the reference arm there is no phase difference between the horizontal and the vertical components. So, when the beams are combined by PBS, there is α=90° shift between the interference pattern formed by the horizontal components and the interference pattern formed by the vertical components. Two identical CCD sensors (IDS UI1488LE-M, pixel size 2.2 μm, frame rate: 6 fp s) are used to record these quadrature phase-shifted holograms in parallel without any time lag. We used a LabVIEW VI to capture the two holograms from the two CCD sensors without any time lag. For the alignment purposes, the two CCDs are placed in x–y–z translation stages together with 360° rotation stages. The rotation axis is along the direction of propagation of the object beam. At first the distances between the PBS and the two CCDs are carefully adjusted to be equal. Direct images of an amplitude object are then grabbed by the two CCDs. The z micrometer translation stages are used to correct the defocusing error between the two images. Any lateral shift between the two images are also removed by using the combination of x and y micrometer stages. Finally, the alignment is checked by subtracting the images one from the other. This alignment procedure between the two CCD images is very essential for error-free reconstruction of the object information.

3. Reconstruction method

The two recorded quadrature phase-shifted holograms can be expressed as

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

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

where, $O(x,y)$ and $R(x,y)=A_r \exp(i2\pi qx)$ represent the object and reference beam complex amplitudes. q denotes the spatial carrier frequency introduced by the angle between the object and the reference waves. This spatial carrier frequency is kept the same for the both the holograms. The square terms are the dc components from the irradiance of the individual object and reference beams.

The dc terms $|O|^2+|R|^2$ from the two holograms can be eliminated by subtracting Eq. (1) from Eq. (2) which gives rise to $I(x,y)$ as follows,

$$I(x,y) = I_2 - I_1 = [\exp(i\pi/2) - 1]O^*R + [\exp(-i\pi/2) - 1]OR^* \quad (3)$$

Next, the real image OR^* is separated from the twin image O^*R by standard technique of Fourier plane spatial filtering [11]. We perform a Fourier transform on $I(x,y)$. The frequency spectrum of