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# Polarization phase shifting in digital holographic microscopy

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ABSTRACT

In the present work we have made use of polarization phase shifting in digital holographic microscopy (DHM) for three dimensional phase profiling of transmissive and reflecting microscopic samples. The Mach–Zehnder arrangement with proper polarizing elements (polarizer-masked cube beam splitter, quarter wave plate and a linear polarizer) is used for recording the phase-shifted digital holograms. The suggested procedure is simple and accurate and obviates the need of piezo devices for phase shifting. The phase profile of the specimen is reconstructed from the holograms by using standard phase shifting algorithms.



## 1. Introduction

Quantitative measurement of phase in microscopic samples is an important task in diverse areas such as biology, microelectronics, and micro electromechanical systems engineering. Digital holography [1–3], adapted to microscopic samples, has the potential to render microscopic structures visible with an axial resolution of the order of nanometers. The basic optical configuration for digital holographic microscopy (DHM) recording is the standard Mach–Zehnder interferometer with single microscope objective in the sample arm [4]. In this arrangement the finer structures of the retrieved phase map of the reconstructed wavefront is masked by the unwanted phase introduced by the objective lens, resulting in a significant loss of phase resolution. It is therefore essential to explore ways and means to compensate the effects of wavefront curvature and residual aberrations introduced by the microscope optics. Different techniques have been proposed towards this end [4–13].

The in-line configuration of the DHM makes use of the full pixel count in forming the holographic image, but the zero-order and the twin image terms are superposed on the image. A very effective method of removing these terms was introduced by Yamaguchi and Zhang [14], where the complex field at the hologram is obtained by phase-shifting interferometry. The reconstructed wavefront from phase-shifted in-line holograms is resulting in a marked improvement in the fidelity of the reconstructed image. Phase shifting in digital holography is essentially implemented by translating one of the interferometer mirrors attached to a pre-calibrated piezo actuator.

In the present study we propose a polarization phase shifted DHM setup based on Mach–Zehnder interferometer where polarization phase shifting is achieved by the use of a polarizer-masked cube beam splitter, quarter wave plate and a linear polarizer. A series of four phase shifted interferograms are recorded by the CCD. The proposed arrangement a single microscopic objective lens critically positioned outside the interferometer arms so as to intercept both the sample and the reference beams [13]. Reconstructions from transmissive and reflective samples using Fresnel and image-plane digital holograms are demonstrated.

## 2. Experimental arrangement

The recording geometry for transmissive and reflective samples is shown in Fig. 1. Spatially filtered, beam expanded and collimated light beam from He–Ne laser forms the input to a standard Mach–Zehnder Interferometer (MZI), employing two mirrors $M_1$ and $M_2$ and cube beam splitters (CBS) CBS1 and CBS2 of side 10 mm. In order to render polarization phase shifting capability, the two input faces of CBS2 is masked with two linear polarizers $P(0^\circ)$ and $P(90^\circ)$ so that the transmitted and reflected components from the interfacing layer of the CBS2 are linearly polarized and orthogonal to each other. The input polarizer $P_1$ is initially oriented such that the intensities of the transmitted and reflected components from CBS2 are almost equal. Long working distance (LWD) infinity corrected microscope objective (MO2), of working length ∼20 mm,

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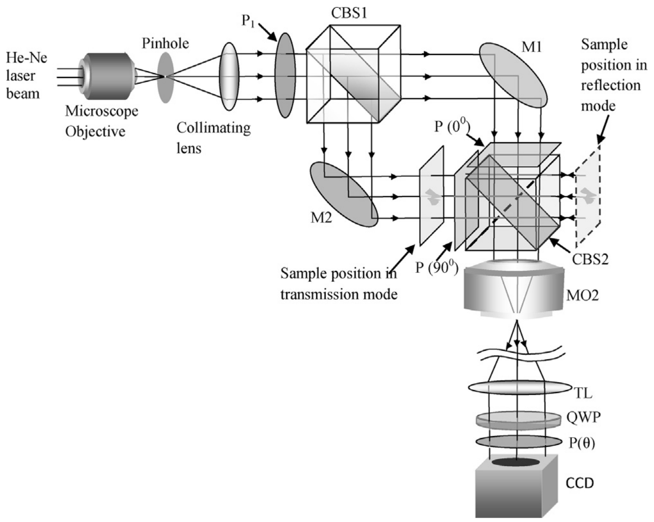


**Fig. 1.** Schematic of the experimental arrangement.

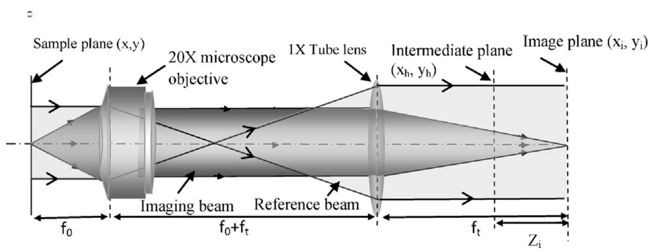


**Fig. 2.** Unfolded ray path showing microscopic image formation and passage of the reference beam.

is placed immediately after CBS2 with the sample placed at the focal plane of MO2 in as shown in Fig. 1. With this arrangement both the sample and reference beams are allowed to pass through the same microscope objective and wavefront defects that may be introduced by the microscope optics is present in both the sample and the reference beams.

For reflective samples, CBS2 needs to be reoriented so that the beam splitting interface is along the dotted line and the sample is placed as shown in Fig. 1. Here too, the sample plane is the front focal plane of MO2.

The remaining optics follows the principle of infinity corrected microscopes. The sample is imaged on the CCD through a 1× tube lens. The unfolded ray diagram showing the passage of the sample and reference beams are shown in Fig. 2.

Light beams emerging from the infinity-focused objective and tube lens are collimated, allowing quarter wave plate (QWP) and polarizer ($P(\theta)$) to be introduced into the space between the tube lens and the CCD. The QWP with its fast axis at an angle 45° generates two mutually orthogonal circularly polarized output beams and then the output polarizer, is used to produce interference pattern. The recorded image on the CCD is the digital hologram. Phase shifting is effected by rotation of the polarizer $P(\theta)$.

## 3. Theory

For a phase object given by $e^{i\delta(x,y)}$, from the output cube beam splitter (CBS2) the object and reference waves are given by $e^{i\delta(x,y)}\begin{pmatrix}0\\1\end{pmatrix}$ and $\begin{pmatrix}1\\0\end{pmatrix}$ respectively.

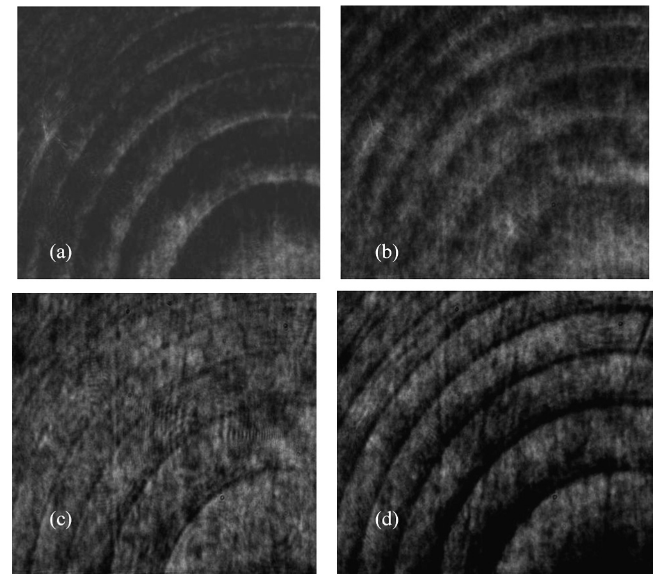


**Fig. 3.** Phase shifted digital holograms (a) $\theta=0°$, (b) $\theta=45°$, (c) $\theta=90°$ and (d) $\theta=135°$.

On the image plane, the object beam, $E_o$ and reference beam, $E_r$ can be written as

$$E_0(x_i,y_i)=P(\theta)W\left(\frac{\lambda}{4},45°\right)e^{i\delta(x_i,y_i)}\begin{pmatrix}0\\1\end{pmatrix} \quad (1)$$

and

$$E_r(x_i,y_i)=P(\theta)W\left(\frac{\lambda}{4},45°\right)\begin{pmatrix}1\\0\end{pmatrix} \quad (2)$$

where, $W(\lambda/4, 45°)$ is the Jones matrix of a quarter wave plate with its fast axis oriented along 45° to the reference $x$ axis, and is given by, $W\left(\frac{\lambda}{4},45°\right)=\frac{1}{\sqrt{2}}\begin{pmatrix}1 & i\\ i & 1\end{pmatrix}$ and $P(\theta)$ is the Jones matrix of a polarizer with the transmission axis oriented along $\theta$, and is given by, $P(\theta)=\begin{pmatrix}\cos^2\theta & \cos\theta\sin\theta\\ \cos\theta\sin\theta & \sin^2\theta\end{pmatrix}$

The intensity distribution at the image plane of the sample is:

$$I(x_i,y_i)=\left|E_R(x_i,y_i)+E_o(x_i,y_i)\right|^2 \quad (3)$$

$$I(x_i,y_i)=1+\cos\left(\frac{3\pi}{2}+\delta(x_i,y_i)-2\theta\right) \quad (4)$$

In cases where the CCD is not the image plane, the two dimensional intensity distribution on the CCD is defined as

$$I(x_h,y_h)=\left|E_R(x_h,y_h)+E_o(x_h,y_h)\right|^2 \quad (5)$$

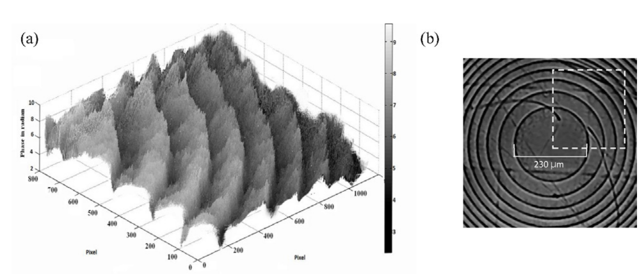


**Fig. 4.** (a) Phase reconstruction of Fresnel lens and (b) microscopic image of the sample, the reconstructed area bordered.