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Abstract: The digital holographic microscopy can be used for the quantitative and dynamic phase imaging of biological cells, which has a very wide application in the fields of biological and medical sciences. To make the measurement system simple, compact, and low-cost, a common-path configuration based on a beam displacer unit is introduced in the digital holographic microscopy. The simple optical structure and common-path design reduces the system requirement for the light source coherence, realizes the convenient adjustment of the object and reference beams and achieves an excellent system temporal stability of 0.53 nm. The living mouse osteoblastic cells during mitotic phase are quantitatively measured to demonstrate the capability and applicability of the system.

Index Terms: Digital holographic microscopy, biological cells, phase measurement, common-path configuration.

1. Introduction

Phase contrast microscopy, which can be employed to distinguish transparent specimens, has been widely applied in biological, medical and geological sciences. However, its limitation is the difficulty to achieve quantitative description of the specimens’ morphology, especially for biological specimens with low-contrast and blurred boundaries. Transport of intensity equation combined with commercial microscope or the lens-free on-chip microscope is introduced to implement phase imaging of biological specimens [1]–[5]. This technique can achieve phase imaging of pathology slide by a series of digital processing steps to retrieve the original object information. However, it's usually time-consuming due to the complicated calculation.

Digital holographic microscopy (DHM), with the advantages of full-field, non-destructive, real-time, high resolution and three-dimensional (3D) imaging abilities, has enabled to effectively investigate biological specimens including tissues, dry mass and membrane fluctuation by quantitatively measuring the amplitude and phase information of the specimens to acquire its
Typical optical configurations of DHM are based on Mach–Zehnder interferometer or Michelson interferometer, etc. In these traditional configurations, the object and reference beams pass through separated optical path and combine together at the exit of the setup to form digital hologram. Since the two beams travel along different paths, measurement errors occur unavoidably in aligning different optical elements. Meanwhile, any slight mechanical vibrations even tiny airflow disturbances in different interferometer arms introduce instability in temporal domain. The common-path configuration, in which both the object and reference beams pass through the same optical path, is a feasible way to solve above problems [8]–[11]. For example, one of the typical common-path configurations is the use of the parallel glass plate [12]–[15]. Besides, the Michelson or Sagnac interferometer-like configurations are also introduced for reference beam generation in common-path DHM [16]–[20]. However, above techniques suffer from complicated design, cumbersome optical setup, expensive cost, precision adjustment requirement, or the incompatibility to the existing techniques.

In this paper, a simple, compact, and low-cost common-path configuration is proposed for quantitative and dynamic phase imaging of biological cells which is based on a single beam displacer unit in DHM. Living mouse osteoblastic cells during mitotic phase are measured to demonstrate the system’s capability and applicability.

2. Experimental Setup

Fig. 1(a) shows the configuration of proposed common-path DHM based on a single beam displacer unit. A partially polarized laser beam from a fiber-coupled diode-pumped solid-state (DPSS) laser with wavelength $\lambda = 532\,\text{nm}$ is focused by a lens to illuminate the specimen and magnified by a $20\times$ microscope objective. The transmitted light beam travels through a polarizer and enters into the beam displacer. Then, the input beam is separated into two orthogonally polarized beams with a small displacement. The two output beams pass through a polarizer and interfere with each other at the lateral shearing region behind the beam displacer. The corresponding interferogram is recorded by a white-black CCD camera (The Imaging Source DMK41BU02 with a target size of $5.95\,\text{mm} \times 4.46\,\text{mm}$, $1280 \times 960$ pixels, pixel size $4.65\,\mu\text{m} \times 4.65\,\mu\text{m}$).

The used beam displacer (Thorlabs, BD27) is a high grade optical calcite crystal with a size of $11\,\text{mm} \times 11\,\text{mm} \times 26.8\,\text{mm}$ and a nominal beam separation of $2.7\,\text{mm}$. As is shown in Fig. 1(b), after the polarized divergent beam passing through polarizer 1 and entering into the beam displacer, it is divided into two orthogonally polarized output beams with a separation displacement. Behind polarizer 2, the two output beams interfere with each other in the lateral shearing region. The polarization direction of polarizer 2 is set at about $45^\circ$ with the two output polarized beams, respectively. Thus, the two beams interfere with each other in their overlap part to form the off-axis digital hologram. By rotating polarizer 1 and polarizer 2, the intensity of the two orthogonally polarized beams changes, and the contrast of the interferogram changes accordingly. In experiment, the direction of the two polarizers is set to obtain the highest hologram contrast. As is shown in Fig. 1(c), the beam displacer and the two polarizers are assembled together as a low-cost and independent unit. Compared with the configurations using parallel glass plate, this design is more simple and compact.

Note that the two output beams are usually parallel to the input beam behind the beam displacer. But in our experimental setup, the input beam is divergent, thus the two output beams have a small inclined angle which helps to form the off-axis hologram. For this reason, the actual separation distance of the two output beams on the CCD target is $2.95\,\text{mm}$, which is a little bigger than the nominal value of $2.7\,\text{mm}$, as shown in Fig. 1(d). The field of view (FOV) is the overlap part of the two beams and the CCD camera is set in this region to record digital hologram.

The main superiority comes from the employment of the beam displacer unit. This simple and practical design of the optical configuration reduces the requirement for the light source coherence, realizes the convenient adjustment of the object and reference beams, and makes the system with an excellent temporal stability. Meanwhile, the beam displacer unit can be easily integrated into the output port of a commercial microscope as an independent compact device. In the system, since
the optical path difference (ΔOPD) between the object and reference beams is less than 1 mm, low coherence light source (i.e., laser diode with coherent length of few millimeters) can be used. This may greatly reduce the cost of the system.

As shown in Fig. 1(e), with a simple upgrade, the commercial microscope can be improved to a digital holographic microscope by combining the beam displacer unit and laser light source easily. In this system, the halogen lamp of the microscope is replaced by a DPSS laser, and the laser beam is converged to illuminate the specimen from top to bottom. The spatial resolution and speed of the system depend on the CCD camera, which is with 1280 × 960 pixels, pixel size 4.65 μm × 4.65 μm and maximum speed of 30 fps. Meanwhile, benefiting from the simple optical structure and common-path design, the excellent temporal stability of the system makes the proposed technique a very promising and potential application in quantitative phase measurement such as fields of biological and medical science.

3. Measurement Principles
In the hologram recording, considering the object wave \( O(x, y) \) and reference wave \( R(x, y) \) interfere on the CCD target plane, the intensity of the interferogram can be given by

\[
I(x, y) = |O(x, y)|^2 + |R(x, y)|^2 + R^*(x, y)O(x, y) + R(x, y)O^*(x, y),
\]

(1)
where, the symbol \( * \) denotes the complex conjugate operation, and \( x, y \) are the rectangular coordinates on the CCD target plane. The hologram can be numerically reconstructed by doing the spatial filtering operation and use of convolution method, and the corresponding reconstructed object wave field \( U(\xi, \eta) \) is expressed as

\[
U(\xi, \eta) = \text{IFFT}\left\{\text{FFT}\left\{I(x, y)\right\} \cdot \text{FFT}\left\{g(\xi, \eta, x, y)\right\}\right\}.
\]  

(2)

where, \( \text{FFT} \) and \( \text{IFFT} \) represent the Fourier and inverse Fourier transform operations, respectively, and \( g(\xi, \eta, x, y) \) is the impulse response function which is expressed as

\[
g(\xi, \eta, x, y) = \frac{i \exp \left[ -\frac{i 2\pi}{\lambda} \sqrt{d^2 + (x - \xi)^2 + (y - \eta)^2} \right]}{\sqrt{d^2 + (x - \xi)^2 + (y - \eta)^2}}.
\]  

(3)

where, \( d \) is the reconstruction distance and \( \lambda \) is the laser wavelength [21].

Assuming that we separately record two digital holograms in the cases without and with cells in some states, and corresponding reconstructed object waves are \( U_1(\xi, \eta) \) and \( U_2(\xi, \eta) \), respectively. Using double-exposure holographic interferometry [22], [23], the phase change \( \Delta \phi \) between \( U_1(\xi, \eta) \) and \( U_2(\xi, \eta) \) can be calculated as

\[
\Delta \phi = \arg\left[ \frac{U_1(\xi, \eta)}{U_2(\xi, \eta)} \right] \pmod{2\pi}.
\]  

(4)

where the function \( \arg[] \) is used to obtain the argument value. This phase change is wrapped due to the argument operation. The data are utilized for quantitative phase measurement after removal of the \( 2\pi \) ambiguity by using phase unwrapping operation [24].

The measured phase difference \( \Delta \phi \) represents the phase change of the reconstructed object waves, or the phase difference between two reconstructed object waves with specimen in different states. For biological cells specimens, \( \Delta \phi \) is dependent on the laser wavelength \( \lambda \), the RI of the surrounding medium \( n_{\text{medium}} \), the actual cell thickness \( d \) and integral mean RI \( n_{\text{specimen}} \). Therefore, the distribution of the corresponding \( \Delta \text{OPD} \) is calculated as

\[
\Delta \text{OPD} = (n_{\text{specimen}} - n_{\text{medium}}) \cdot d = \frac{\lambda}{\pi} \cdot \Delta \phi.
\]  

(5)

It should be emphasized that the \( \Delta \text{OPD} \) is the optical path difference along the light illumination direction while it goes through the cell. It’s an integral effect of the optical path along the optical axis. Different parts of the cell have different RI which results in different optical path. Thus, in a certain sense, the \( \Delta \text{OPD} \) also represents the thickness of the cell.

### 4. Experimental Results and Discussions

Firstly, a resolution target (USAF 1951) is tested using the proposed common-path DHM configuration. Fig. 2(a) shows the recorded digital hologram with a size of 5.95 mm \( \times \) 4.46 mm. The inset represents the enlarged image of the hologram in the blue box, in which the vertical distributed fringes generated by the lateral shearing in the horizontal direction [see Fig. 1(d)] can be clearly seen. As a result, the \( \pm 1 \) orders of the spatial spectrum separate in Fourier domain of the hologram, as shown in Fig. 2(b). By rotating the beam displacer, we can control the shearing direction and thus adjust the direction of interference fringes and position of the \( \pm 1 \) orders. Meanwhile, by controlling the divergence angle of the input beam, we can adjust the period of interference fringes and the distance between \( \pm 1 \) orders and zero order. To reconstruct the holographic image of resolution target, the +1 or −1 order of the spatial spectra are filtered out and then the holographic image can be obtained by use of convolution method [25], [26].

Fig. 2(c) shows the reconstructed intensity image of the resolution target. The test region is the Group 5 grating of resolution target. We can find overlapping images of number 5 in the reconstructed image because of the beam displacement depicted in Fig. 1(d). For small or sparse
distribution specimens, i.e., microlens, biological cells, the problem of overlapping images will be absent. An unaffected image can be obtained by moving the specimen to a particular position where it's clean in the reference beam part. Or we can move the specimen with high cell density to one side of the FOV, and the other side of the FOV acts as the reference beam. In our experiment, the actual beam separation displacement is 2.95 mm. The magnification of the system can be calculated and calibrated to be $19.0 \times$ according to the gratings period and the pixel size of the CCD. Thus, the actual measurement region of our system is $2.95 \, \text{mm} \times 4.46 \, \text{mm}$ and the system FOV is $155.3 \, \mu\text{m} \times 234.7 \, \mu\text{m}$. To further improve the FOV, we can reduce the MO magnification, use a larger size CCD camera or change a big size beam displacer with a bigger beam separation.

It should be noted that the illumination light source of the microscope has changed as DPSS laser and the location of the CCD camera may deviate slightly from its design location in our experimental setup. This may lead to the imaging aberrations, especially phase aberrations. It can be corrected in the process of hologram numerical reconstruction with double-exposure digital holographic interferometry. Fig. 2(d) shows the reconstructed wrapped phase map of the resolution target, in which slight phase aberration is surrounded by background phase stripes. The reason is due to the illumination system and the object and reference wavefront curvature radius. As is shown in Fig. 1(b), after the diverging beam traveling through the beam displacer unit, the optical path of the two generated beams are different, resulting in the difference of the wavefront curvature radius [14]. In the experiment, by recording two holograms with and without the specimen in the FOV, the phase aberration can be removed using double-exposure digital holographic interferometry.

After accomplishing the calibration, the proposed DHM system can be used for quantitative and dynamic phase imaging of biological cells. Living mouse osteoblastic cells are measured as the specimen, which are first placed in the cell incubator. Then it's taken out and placed in room temperature environment for measurement. The mouse osteoblastic cells IDG-SW3 are cultured in Alpha minimum essential medium ($\alpha$MEM, gibco by life technologies). The RI of the $\alpha$MEM is $n_{\text{medium}} = 1.3377$, measured by an Abbe refractometer. Fig. 3 shows the measurement results of the mouse osteoblastic cell. One of the recorded digital holograms is shown in Fig. 3(a). For transparent cells, we cannot clearly observe their contours in the holograms. After numerical reconstruction and phase aberration correction, it's found that the mouse osteoblastic cells are in spreading state with four cell synapses in the wrapped phase.
Fig. 3. Recorded digital hologram of the mouse osteoblastic cell and its reconstructed results. (a) Recorded hologram; (b) wrapped phase map; (c) 2D unwrapped phase map; (d) 3D phase distribution.

map, as shown in Fig. 3(b). This phase map represents the \( \Delta \text{OPD} \) caused by the cell thickness. Due to the lateral shearing effect of the beam displacer, there are two cell images in the phase map in which the phase value of the cell in the left side is opposite to that of the right side. By operating phase unwrapping process, the contours of the cells become very clear as shown in the two-dimensional (2D) unwrapped phase map of Fig. 3(c). It can be found that, compared with the synapses surrounding the cell, there is a biggest phase value in the central part which represents a maximum cell thickness. By assuming a constant and homogeneous cellular RI \( n_{\text{specimen}} = 1.375 \), we can estimate that a phase difference of 1 rad corresponds to a cellular thickness of 2.27 \( \mu \text{m} \) according to Eq. (4). For the cell in the left side, the measured length of the cell is about 125 \( \mu \text{m} \) and the measured phase difference is between \([-5, 0]\) which can be equivalent to a cellular thickness of 11.35 \( \mu \text{m} \). Meanwhile, considering the magnification and FOV of the DHM system, the maximum length of cells can be measured in lengthwise direction of FOV is 234.7 \( \mu \text{m} \). Fig. 3(d) shows the 3D phase distribution of the osteoblastic cell. In its 3D phase view, the cell synapses are more easily observed.

Fig. 4 shows the profile map along with the dash lines A, B and C in Fig. 3(c), the profiles demonstrate different thickness distributions of the cells. The left axis in the figure represents \( \Delta \text{OPD} \) while the right one represents phase difference. The maximum \( \Delta \text{OPD} \) value along line B is about 460 nm. This can be translated to an cell thickness of 12.33 \( \mu \text{m} \) according to (4). Because the central part contains the cell nucleus, it has the highest altitude. Meanwhile, the second maximum \( \Delta \text{OPD} \) value is 180 nm along line A which is equivalent to an cell thickness of 4.83 \( \mu \text{m} \).

Furthermore, the mouse osteoblastic cells in different states are measured. Fig. 5 shows the reconstructed 2D and 3D phase distributions of three different cells. The cell in Fig. 5(a) and (d) is in contracted state, it shrinks into a group with an obvious altitude difference. The tiny synapses on the cell are vaguely visible. The cell in Fig. 5(b) and (e) is spindle-shaped and has two long synapses. Fig. 5(c) and (f) show that the cell is in spread state with five synapses. Compared with Fig. 5(d) and (e), the cell in Fig. 5(f) has a more gentle height change, the change range is less than 3 rad, which can be translated to an cell thickness of 6.81 \( \mu \text{m} \).

Fig. 6 shows different moments of the mouse osteoblastic cell during the mitotic phase. In Fig. 6(a), the cell is in the anaphase which becomes an irregular ellipse, and the cytoplasm is uniformly distributed in the whole cell. After 80 s, the cell nucleus is divided into two closely parts
in Fig. 6(c) and the cell wall pinches in to form two new daughter cells in the telophase. In Fig. 6(f), the cell division is finished and two daughter cells are reproduced and separated completely. In fact, the duration of the cell cycle varies greatly from one cell type to another. For this mouse osteoblastic cell, a cell cycle usually continues several hours and it only shows the anaphase, telophase and subsequent cytokinesis in Fig. 6 [27]. The evolution of the cell during mitotic phase processes about 280 s, and it can be observed clearly in the movie (Visualization 1). By recording a series digital hologram and reconstructing them rapidly, the quantitative measurement of the morphological changes can be realized dynamically.

As discussed in Section 2, the simple optical structure and common-path design of the proposed DHM system make it present an excellent temporal stability. To evaluate this, the experiment is implemented in the condition without specimen. 225 times measurements are continuously carried out at a rate of 7.5 fps for 30 seconds. The standard deviation of ΔOPD at one pixel provides the fluctuation of the selected point. A random point is selected from the reconstructed phase maps. The temporal ΔOPD fluctuation associated with the selected point is given in Fig. 7(a). The standard deviation of the ΔOPD fluctuation has a value of 0.51 nm, which indicates that the ΔOPD
sensitivity with sub-nanometer level can be obtained in the proposed experimental setup with a long-term stability.

The mean value of the fluctuations for each pixel provides another characterization method of the temporal stability of the system. Therefore, 80 pixels \( \times \) 80 pixels region is selected from the series of phase maps and the fluctuations of all the 6400 points are calculated. Fig. 7(b) shows the histogram of the fluctuations with a mean value of 0.53 nm, which indicates that, the temporal stability of the proposed setup reaches 0.53 nm. This is very important for the tiny specimen measurement, such as living biological cells that are of the order of several nanometers. Compared with other common-path configurations, the proposed common-path DHM exhibits better temporal stability and performance [18], [28]. In the meanwhile, a high stability light source will help further improve the stability of the system.
5. Conclusion

We have demonstrated a simple, compact, and low-cost common-path DHM based on a single beam displacer unit and its application in realizing the quantitative and dynamic phase imaging of biological cells. In the proposed system, the input beam is separated into two orthogonally polarized components with a small displacement, and these two components interfere with each other at the lateral shearing region. The beam displacer unit can be easily set up in the output port of a commercial microscope as a compact independent device to make the system with an excellent temporal stability of 0.53 nm. Living mouse osteoblastic cells are quantitatively measured with the system to demonstrate its capability and applicability. The results show that the proposed technique has very promising and potential applications in the fields of biological and medical science.

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