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Multidimensional measurement by hybrid digital holographic microscopy for biological applications

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Photopolymers are preferred in holography due to their additional advantage of being self-developing along with high resolution. In the present paper, an attempt has been made to review the work relating to the holograms recorded and reconstructed in different types of photopolymers using single/multiple wavelengths. In addition, results of experiments performed on a customized photopolymer with optimized acrylamide concentration and dye concentration for achieving higher diffraction efficiency, are also presented. The results obtained are comparable to the results reported in the literature. A maximum diffraction efficiency of about 88% has been achieved using acrylamide-based photopolymer with Erythrosin B dye

Keywords: Holograms, Photopolymers, Photosensitive Materials, Diffraction Efficiency, Holographic Diffraction Gratings, Diffractive Optical Elements, Holographic Optical elements, Chain Transfer Agent.

1 Introduction

Digital holography is one of the hot research fields in optics. According to Web of Science, more than 200 journal papers have been publishedevery year from 2007. Digital holography is a technique to record the hologram by a digital image sensor and then to reconstruct numerically the original object by calculating the inverse propagation of the lightin a computer [1,2]. By setting the appropriate propagation distance in the numerical reconstruction, the original object is reconstructed. Advantages of the digital holography are quantitative measurement of the amplitude and the phase, numerical focusing in the reconstruction, and simultaneous measurement of multiple physical parameters such as amplitude, phase, spectra, and polarization by hologram multiplexing. There are many attractive applications of digital holography such as particle image velocimetry [3], microscope [4-10], and security [11].These applications are generated by the rapid development of high performance image sensor and image-based calculation by GPGPU. Up to now, fastest recording at a million frames per second was achieved [12]. Visualization of invisible phenomena such as gas flow [13] and voice reproduction [14] by the phase measurement have been presented.

Digital holographic microscopy is one of the successful applications of digital holography and has several advantages compared with conventional optical microscope. One is the quantitative phase measurement. In Ref. 4, the structure change of pancreas tumor cellcaused by anticancer drug was observed by the quantitative measurement. Second is the numerical focusing [15]. In the numerical reconstruction, the sectional images can be reconstructed by changing the propagation distance from the image sensor plane. This results in long depth of field (DOF). For bioimaging, it is possible to measure the moving objects in time and 3D space. Third is the measurement of multiple physical parameters by multiplexing the holograms. Fluorescence imaging and phase contrast imaging are two major imaging methods in bioimaging. Images of the cell nucleus with fine resolution and better contrast can be obtained by a laser scanning confocal microscope. From the phase contrast imaging, the structure of cell walls can be obtained. However, it is difficult to obtain simultaneously both images from a single measurement as well as to obtain 3D information.

In this paper, we review an approach to obtain simultaneously the phase and the fluorescence images by using digital holography.Digital holographic microscopes (DHMs) that can obtain multiple

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physical parameters without any change of the optical setup are calleda hybrid DHM. In Refs. 8 and 9, the combination of the off-axis holography for phase imaging and the conventional fluorescence microscopy has been demonstrated. These approaches give us wide potential use of imaging techniques in biological field.

2 A hybrid digital holographic microscope

A hybrid DHM have been proposed [8,9]. This hybrid DHM consists of two subsystems that are an off-axis DHM and a wide-range 2D fluorescence microscope as shown in Fig 1 [9]. There are two image sensors that capture individually the off-axis holograms and fluorescence images. By selecting the wavelength and the polarization direction, both the object wave passing through the object and the fluorescence light can propagate along the same direction and can be divided into two separated wave fields by dichroic mirrors.

In the off-axis DHM, a He-Ne laser light operated at a wavelength of 632.8 nm is used as a coherent light source. After the expanding of beam diameter, the light passing through a transparent sample put on a movable stage interferes with a reference plane wave by a beam splitter. The reference plane wave is tilted to the optical axis. The interference fringe pattern is captured by the image sensor, Image sensor 2. In the fluorescence microscope, the light emitted from an Nd:YVO_4 laser operated at 532 nm, is used as the excitation light. A defocusing lens is used to illuminate the wide field in a sample plane. The fluorescence light is captured by the image sensor, Image sensor 1, after eliminating unwanted light components such as the excitation light from an Nd:YVO_4 laser and He-Ne laser light by a dichroic mirror and a band-pass filter. We use a microscope objectivelens with NA of 0.6 and 50X magnification. Table 1 shows the parameters in the optical setup. In the following section, the feasibility check of the hybrid DHM was implemented.

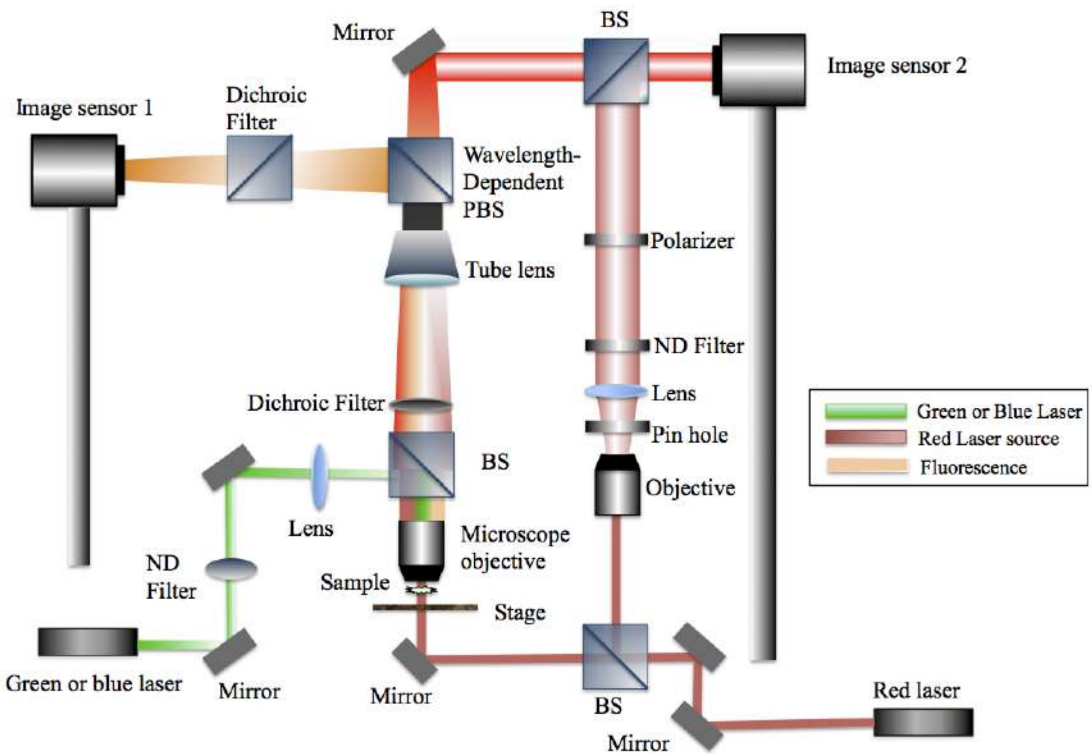


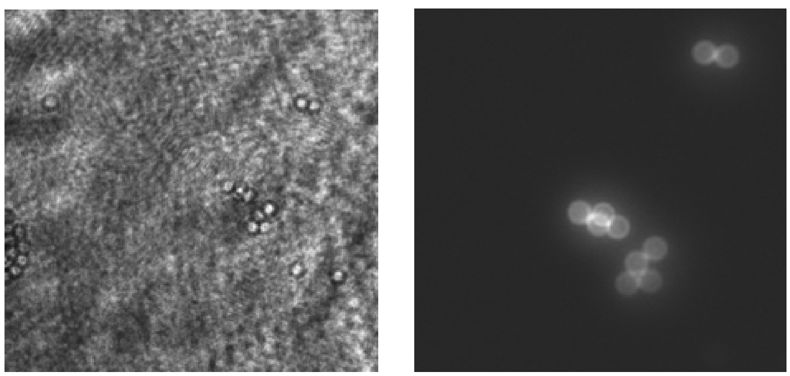
Fig 1. Experimental setup for a hybrid DHM with off-axis DHM and wide-field fluorescence microscope

Table 1. Parameters of the optical setup of Fig 1.

Wavelength of excitation light for fluorescence	532 nm
Wavelength of the laser light for phase imaging	632.8 nm
Image sensor 1	Photometric CoolSNAP KINO 4.54 mm ×4.54 mm
Image sensor 2	POINTGREY Blackfly USB3.0 5.86mm × 5.86 mm
Microscope objective lens	NIKON ELWD 50X NA:0.6

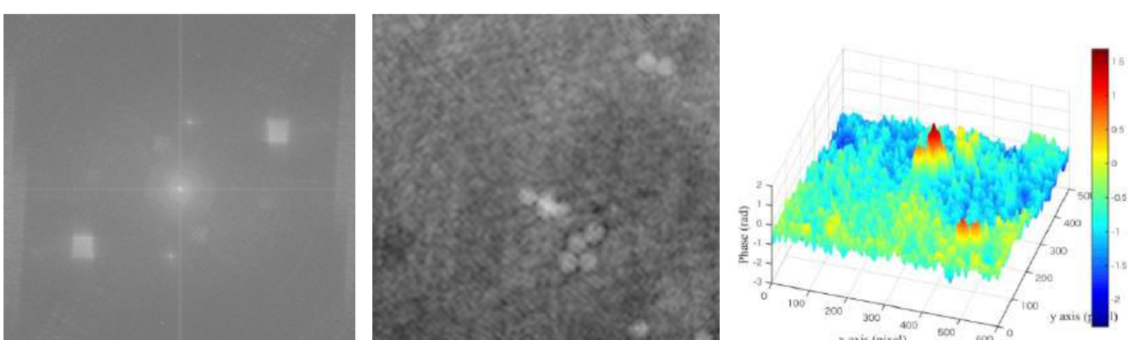
3 Experiments of 3D Phase and 2D Fluorescence Imaging by a Hybrid DHM

Simultaneous measurement of the 3D phase and the 2D fluorescence images was demonstrated. First, fluorescence beads with a diameter of 4 μm are used. Figures 2(a) and (b) show the off-axis hologram and the fluorescence images, respectively. Figure 3(a) shows that the spatial frequency distribution is obtained by taking the Fourier transform of Fig 2(a). The signal processing to extract the object wave is applied and then the phase distribution is reconstructed as shown in Fig 3(b). By comparing Fig 3(b) with Fig 2(b), similar images are obtained. At the lower right in Fig 3(b), two beads can be seen. However, there is no bead at that area in Fig 2(b). This is because the illuminated area of the exciting light for fluorescence is smaller than that of the phase imaging. Figure 3(c) shows the 3D profile of Fig 3(b). From Fig 3(c), it can be observed as quantitative phase measurement. In this case, there is no phase wrap.



(a) (b)

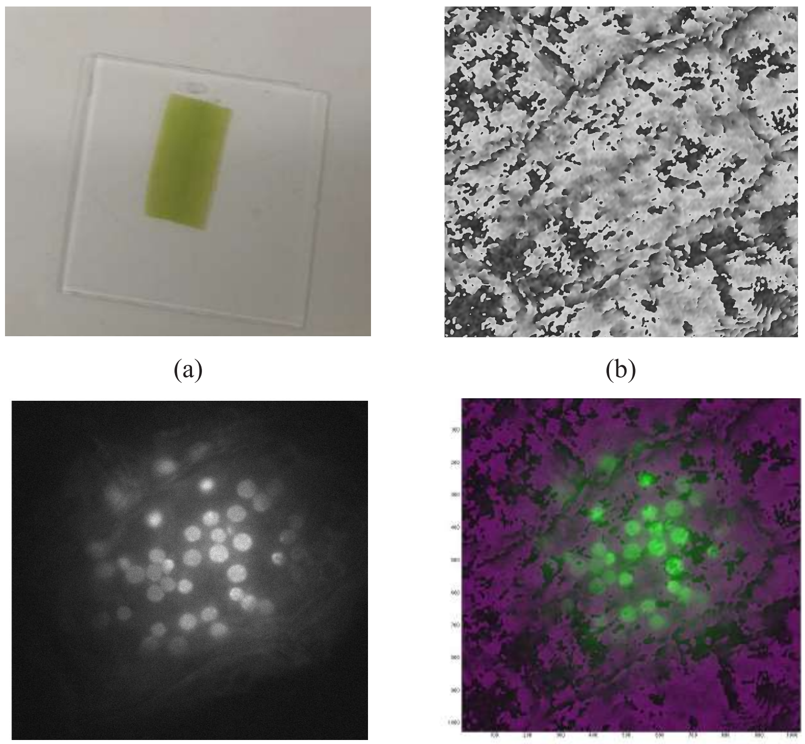
Fig 2. Off-axis hologram and fluorescence image obtained by a hybrid digital holographic microscope. (a) Off-axis hologram and (b) fluorescence image.



(a) (b) (c)

Fig 3. Reconstructed phase distribution from Fig 2(a). (a) Spatial frequency distribution, (b) reconstructed phase image, and (c) 3D profile of (b).

Next, the measurement of living cells was demonstrated. Here, *Egeria densa* as shown in Fig 4(a) is used. There is two-layer structure. Fluorescence light is generated by Chlorophyll in chloroplast by excitation light from Nd:YVO_4. Figures 4(a) and (b) show the phase and the fluorescence distributions, respectively. In the phase imaging in Fig. 4(b), the cell walls were extracted as well as the structure of chloroplasts. Figure 4(d) shows the synthesized image of the phase and the fluorescence distributions. This result also indicates the high potential of the proposed system.



(a) (b) (c) (d)

Fig 4. Experimental results of simultaneous measurement of Egeria densa: (a) Picture, (b) phase image, (c) fluorescence image, and (d) synthesized image of the phase and the fluorescence images.

4 Conclusions

We have presented hybrid DHMs for the simultaneous measurement of the phase and the fluorescence distributions. The measurement of multiple physical parameters such as the phase and the fluorescence is one of the big advantages of the digital holography compared with the conventional optical microscope. Experimental results are promising for fabricating the hybrid DHMs.

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