High-resolution Section Recovery Using a Configurable Pupil in a Scanning Holographic Microscopy

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Abstract: We present a sectioning method in optical scanning holography with enhanced depth resolution using a configurable pupil, which is based on spatial light modulator. A depth resolution of 0.7 µm can be achieved by this method.

OCIS codes: (090.1995) Digital holography; (100.3020) Image reconstruction-restoration.

1. Introduction

As one of the 3D digital holography (DH) technique, Optical Scanning Holography (OSH) has already found applications in different ways, such as microscopy, 3D holography display, and remote sensing [1-3]. Although the recorded hologram by OSH contains the 3D information of the object, one may also need to view the individual sections. The step to recover individual 2D planes of the object from the captured hologram is called sectioning. The difficulty of sectioning lies in the suppression of the undesired signals from the other sections, which are known as defocus noise.

In this paper, we propose a sectioning method based on a configurable pupil. By implementing a spatial light modulator as the configurable point pupil in the optical system, two different sets of Fresnel Zone Plate (FZPs) can be generated to scan the same object. With the extra information, a depth resolution of 0.7 µm can be achieved.

2. Principle

The schematic of the OSH system is shown in Fig. 1. The output of a laser source, which is centered at $\omega$, is divided into two parts via a beam splitter (BS1). One part of the light would first pass through a unit pupil ($p_1(x, y)=1$) and then lens 1, which would lead to a spherical wavefront on the object; the other part would first pass through an acousto-optic frequency shifter (AOFS), through which the frequency would be shifted to $\omega+\Omega$. The wavefront of the second part would then be shaped into a plane one by the point pupil ($p_2(x, y)$) and lens 2. The two parts will then be combined by BS2 and shine on the object via a 2D scanning mirror. Lens 3 is used to collect the light from the object. The hologram can be retrieved after some processing of the electrical signal generated by the detector.

In the proposed system, a spatial light modulator (SLM) is used to realize the point pupil $p_2(x, y)$. The SLM can be switched between two states, denoted as $p_2^a(x, y)$ and $p_2^b(x, y)$ respectively, which would lead to two measurements for the same object. With the extra information provided by the second measurement, we expect to have a better depth resolution for sectioning.

For the first measurement, we set $p_2(x, y) = \delta(x, y)$, which would lead to a system impulse response $h_1(x, y; z)$,

$$h_1(x, y; z) = \frac{1}{\lambda z} \exp\left\{ -j \frac{\pi}{\lambda z} (x^2 + y^2) \right\},$$

where $\lambda$ is the wavelength of the laser source, $x$ and $y$ are the transverse coordinates, $z$ is the axial coordinate.

For a given object $\phi(x, y; z)$ with $n$ sections, the generated hologram can be expressed as
where \( z_i \) represents the depth location of the \( i \)-th section.

For the second measurements, the SLM is tuned to the second configuration \( p_{2b}(x, y) = \delta(x-x_0, y-y_0) \), with \( x_0 \) and \( y_0 \) denote the lateral shift of the point pupil. This would lead to a new system impulse response \( h_2(x, y, z) \), as well as a new hologram \( g_2(x, y) \).

By combining the two holograms, we can have,

\[
g(x, y) = \phi(x, y) H(x, y, z_1) + \phi(x, y) H(x, y, z_2) + \cdots + \phi(x, y) H(x, y, z_n) = H \phi + n
\]

where \( n_i \) stands for the respective observation noise. Sectioning means to recover individual section \( \phi_i \) from the hologram \( g \), which is an inverse problem and can be viewed as a minimization problem [4, 5]. To solve this problem, the conjugate gradient (CG) method is used in this work [6, 7].

3. Simulation and analysis

We demonstrated the proposed method with a two-section object, whose section distance is 0.7 \( \mu \)m. Section 1 is located at \( z_1 = 34 \) mm, whereas section 2 is located at \( z_2 = 34.0007 \) mm. Both the two sections are 1 mm\( \times \)1 mm large, and are sampled by 512\( \times \)512 pixels, as is shown in Fig. 2. The wavelength of the laser source is 632 nm. The diameter of the collimated beam is \( D = 40 \) mm, and the focal length of lens 1 and lens 2 is \( f = 50 \) mm. This leads to an \( NA \) equal to 0.3894 (\( NA = \sin(D/(2f)) \)). The configurable pupil \( p_{2a}(x, y) \) is realized by a transmissive Liquid Crystal on Silicon (LCoS) SLM, the pixel size of which is 10 \( \mu \)m. The SLM can be switched between two states: \( p_{2a}(x, y) = \delta(x, y) \), and \( p_{2b}(x, y) = \delta(x-x_0, y-y_0) \), with \( x_0 = 0.5 \) mm, and \( y_0 = 0 \) mm.

The measured FZPs for section 1 with different pupil function \( p_{2}(x, y) \) are shown in Fig. 3, respectively. One can see from Fig. 3 that as the point pupil switching from \( p_{2a}(x, y) \) to \( p_{2b}(x, y) \), the FZP would shift away along the x-axis for 0.5 mm accordingly. The distribution of the opaque and transparent zones of the two FZPs is different, which indicates that the space sampling rate is different as well. By using the different FZPs to scan the same object, extra information can be captured during the second scan, with which we expect to have an enhanced depth resolution.

Fig. 4 shows the real part of the recorded hologram generated by different FZPs.

The sectioning process in this case means to reconstruct image from the hologram for section 1 and section 2 respectively. For comparison, we used three different methods for sectioning: (a) conventional method, (b) CG method with one measurement, and (c) CG method with two measurements using the configurable pupil. The
corresponding results are shown in Fig. 5 (a) - (f). One can see from Fig. 5 that the two sections can not be distinguished using either the conventional method or the CG method with a single measurement. While using the proposed method, the two sections are clearly separated with only small defocus noise. This indicates that with the extra information captured during the second scan, the depth resolution has been enhanced up to 0.7 µm.  

![Fig. 5](image1.png)

**Fig. 5.** Sectioning results using the conventional method for (a) \( z_1 \) and (d) \( z_2 \) planes, using CG method with single measurement for (b) \( z_1 \) and (e) \( z_2 \) planes, using CG method with two measurements by the proposed method for (c) \( z_1 \) and (f) \( z_2 \) planes, respectively.

We also analyze the sectioning effect with different noise levels. The results are shown in Fig. 6. It can be seen from this figure that with higher noise level, the defocus noise becomes larger, which would lead to a reduced depth resolution.

![Fig. 6](image2.png)

**Fig. 6.** Sectioning results with different noise level: SNR = 38.5 dB for (a) \( z_1 \) and (b) \( z_2 \) planes, and SNR = 20 dB for (c) \( z_1 \) and (d) \( z_2 \) planes.

### 4. Conclusion

We proposed a method for sectioning based on a reconfigurable pupil, which can be realized by an LCoS SLM. By switching the point pupil between two states, two different sets of FZPs can be generated to scan the same object. This would provide extra information for the sectional image reconstruction. By combining the two holograms and using the CG method, a better depth resolution can be expected. Simulation results show that the proposed method outperforms both the conventional method and the CG method with single measurement in suppression of the defocus noise, which leads to an enhanced depth resolution of 0.7 µm.

As the reconfigurable pupil is realized by SLM, there is no need to change the experimental setup of the OSH system, which is simple and easy for implementation. It is worth noting that, as two measurements are required in the recording stage, the total acquisition time is doubled as a result. This may limit its application in systems which the data acquisition time is strictly restricted, such as in vivo imaging or other real-time imaging. This problem could possibly be solved with the development of the SLM as well as the scanning mirror.

This work was supported in part by the Research Grants Council of the Hong Kong Special Administrative Region, China, under Projects HKU 7138/11E and 7131/12E, and by the NSFC/RGC under Project N_HKU714/13, and by the National Natural Science Foundation of China under Grant 61107018 and 61361166008, as well as the Fundamental Research Funds for the Central Universities (ZYGX2011J033).

### 5. References


