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Four-dimensional tracking of spatially incoherent illuminated samples using self-interference digital holography

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ABSTRACT

We present a new method for the four-dimensional tracking of a spatially incoherent illuminated object. Self-interference digital holography is utilized for recording the hologram of the spatially incoherent illuminated object. Three-dimensional spatial coordinates encoded in the hologram are extracted by holographic reconstruction procedure and tracking algorithms, while the time information is reserved by the single-shot configuration. Applications of the holographic tracking methods are expanded to the incoherent imaging areas. Speckles and potential damage to the samples of the coherent illuminated tracking methods are overcome. Results on the quantitative tracking of three-dimensional spatial position over time are reported. In practical, living zebra fish larva is used to demonstrate one of the applications of the method.

1. Introduction

Digital holography (DH) provides unique advantage in digital refocusing of the samples slice by slice through a significant volume. Thus the confined depth of field of classical optical imaging system is considerably expanded by the holographic recording and digital reconstruction procedure in DH. The complex amplitude retrieved from the hologram is propagated to the sample volume by the Kirchhoff–Fresnel equation, makes it possible to perform high-resolution lensless imaging [1], observation of biological samples [2], metrology [3], and other practical applications [4–6]. Among them, 3D tracking of the sample in the entire field of view by DH is of great importance in diverse biologic and bio-technologic context [7]. Recently, many investigations focus on the ability of DH to track particles [8], cells [9] and clinical seminal samples [10] in the microfluidics channel. However, the reconstructed images suffer strong speckles in the highly coherent illuminated systems, as well as the relative high energy and focused laser beam maybe harmful to biological samples. Partially spatial coherent source was used to suppress the speckle noise [10], but the spatial coherence is still necessary to produce holograms with sufficient contrast and size. Thus the applications of the existing methods are isolated from thermal sources illuminated or self-luminous such as fluorescent imaging area, as the light sources or the samples are spatially incoherent.

Since the possibility of recording holograms of object illuminated with spatially incoherent light was proposed [11], the technique has been considered valuable for 3D imaging. The recently developed incoherent digital holographic techniques such as triangular holography [12], Fresnel incoherent correlation holography [13] and others [14–16], make it possible to perform non-scanning 3D reconstruction of the spatially incoherent illuminated or fluorescent samples. Furthermore, such kinds of incoherent holographic imaging system provide a potential superior transverse resolution comparing with conventional coherent or incoherent imaging system [17,18]. Among them, the incoherent off-axis Fourier triangular holography (IFTH) reported in our previous publication [19] provides a convenient and single shot way to retrieve 3D properties of the spatially incoherent objects.

In this paper, we present a new method, first time to the best of our knowledge, for 4D tracking (3D spatial motion over time) of spatially incoherent illuminated samples. In the system, interference patterns are formed by the interference of twin beams originating from the same point on the object. Hologram of the extended object consists of the intensity superposition of all the point source interference patterns. The point sources on each slice of the object volume can be digital refocused independently by propagating the hologram to different distance. Therefore a set of holograms are recorded and the tracking procedure can be performed using related transversal position and focus plane detection algorithms [20–22].

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2. Principle and methodology

The optical setup of the proposed method is shown in Fig. 1. Spatially incoherent and temporally partially coherent light source is generated by an extended white light source and an interference filter. Light scattered from the object is collimated by the lens L0, continues to propagate through a triangular interferometer consist of beam splitter BS, lens L1, L2 and mirrors M1 and M2. Two radial sheared beams interference on the output plane P2, and the interference patterns are captured by the CCD after passing through the 4f system consist of lens L3 and L4. Consider a point source of amplitude A_s located at (x_s, y_s, z_s), a distance z_s from the lens L0. An off-axis diverging spherical wave is induced over the L0 plane. The wave is modulated by the L0, propagates to the input plane P1 located at the front focal plane of both lens L1 and L2. Light is split into two parts by beam splitter BS, continues to propagate through the interferometer by clockwise and counter clockwise directions, respectively. Over the output plane P2 located at the rear focal plane of both lens L1 and L2, the intensity of the interference pattern is:

$$I(x, y; \vec{r_s}, z_s) = |A_s \cdot L(\frac{-\vec{r_s}}{z_s}) \cdot Q(\frac{1}{z_s}) \cdot Q(\frac{-1}{f_0}) * Q(\frac{1}{f_0 + f_1}) \cdot Q(\frac{-1}{f_1}) * Q(\frac{1}{f_1 + f_2}) \cdot Q(\frac{-1}{f_2}) * Q(\frac{1}{f_2}) + A_s \cdot L(\frac{-\vec{r_s}}{z_s}) \cdot Q(\frac{1}{z_s}) \cdot Q(\frac{-1}{f_0}) * Q(\frac{1}{f_0 + f_2}) \cdot Q(\frac{-1}{f_2}) * Q(\frac{1}{f_1 + f_2}) \cdot Q(\frac{-1}{f_1}) * Q(\frac{1}{f_1})|^2 \quad (1)$$

where $Q(b)=\exp[j\pi b\lambda^{-1}(x^2+y^2)]$ represents the quadratic phase factor which is further used to describe the phase transformation of a thin lens and the convolution kernel of Fresnel approximated propagation; $\vec{r_s} = (x_s, y_s)$ and $L(\vec{r_s}) = \exp[j2\pi\lambda^{-1}(xx_s+yy_s)]$ represents the phase of tilted plane wave which is used to describe the transverse position of the point source. Asterisk (*) donates two dimensional convolution, f_0, f_1 and f_2 are the focal length of L0, L1 and L2.

Based on the mathematical calculation procedure presented in [19], Eq. (1) can be rewritten and simplified as follows:

$$I(x, y; \vec{r_s}, z_s) = A_s^2(|c_1|^2 + |c_2|^2) + \{c_1 c_2^* A_s^2 Q(\frac{f_1^4 - f_2^4}{f_1^2 f_2^2} \frac{f_0 - z_s}{f_0^2}) \cdot L[\frac{\vec{r_s}(f_1^2 - f_2^2)}{f_0 f_1 f_2}] L_c(\vec{r}, \vec{r_s}) + c.c.\} \quad (2)$$

where $L_c(\vec{r}, \vec{r_s})$ is the linear phase function introduced by the tilted mirror M1, c_1 and c_2 are constants; *c.c.* is the complex conjugate of the left term inside the brace. For spatially incoherent illuminated object, only the twin beams originating from the same point source is coherent. Thus the recorded hologram $H(x, y)$ for the extended object $g(x_s, y_s, z_s)$ is simply the intensity summation over all the point source contributions:

$$H(x, y) = \iiint_g I(x, y; \vec{r_s}, z_s) dx_s dy_s dz_s \quad (3)$$

The intensity distribution in Eq. (2) is similar to Fresnel zone plate, and the hologram can be reconstructed as follows:

$$p(x, y, z_r) = F^{-1}[H(x, y)] * Q(\frac{1}{z_r}) \quad (4)$$

where F^{-1} donates the 2D inverse Fourier transform and z_r is the reconstruction distance. For the special case of the point object located at the front focal plane of lens L0, $z_s=f_0$ and the value of quadratic term Q in Eq. (2) equals to one. Thus the form of point source hologram is inferred to be cosine interference fringes. Therefore Fourier hologram is recorded and can be reconstructed by using inverse Fourier transform. For general case of $z_s \neq f_0$, the reconstruction procedure is implemented by calculating the inverse Fourier transform and an additional Fresnel propagation. If a Fourier transforming lens of focal length f_r is used for reconstruction, the propagation distance z_r, based on Eqs. (2) and (4), equal to:

$$z_r = \frac{f_r^2(f_1^4 - f_2^4)(f_0 - z_s)}{f_1^2 f_2^2 f_0^2} \quad (5)$$

According to the Eqs. (2), (3) and (4), the reconstructed $p(x, y, z_r)$ includes three terms: the zero order, the point source image and its twin. Assuming a sufficient separation of the three terms by implementing an appropriate tilting of M1, the point source image can be extracted from other two terms by a filter window. Thus tracking of the depth position of the object point is possible by finding an appropriate z_r that makes the point source image in-focused.

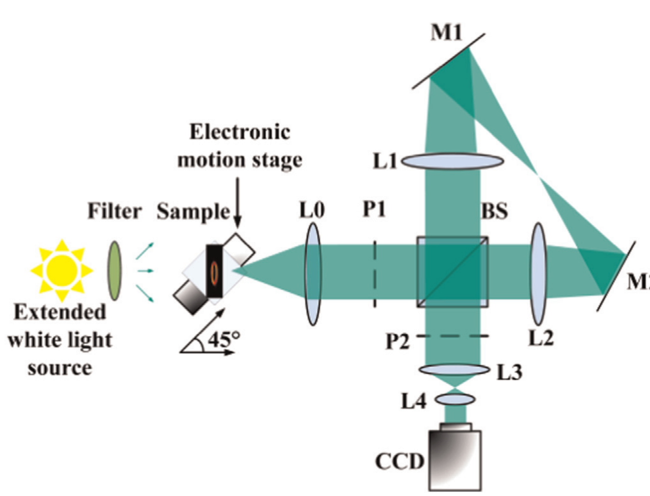

Fig. 1. Schematic of IFTH recorder: P1 and P2, input and output plane of the interferometer; BS, beam splitter; L0, L1, L2, L3 and L4, lens; M1 and M2, Mirror; CCD, charge coupled device.

3. Experiments

The optical system as shown in Fig. 1 is implemented using white light source (Newport Oriel research arc lamp 66477), digital camera with 1280 × 1024 pixels and 4.65 μm pixel pitch (Thorlabs DCU224M) and an interference filter with 532 nm central wavelength and 10 nm bandwidth (Newport 10BPF10-532). Other parameters are: $f_0=150$ mm, $f_1=150$ mm, $f_2=175$ mm, $f_3=100$ mm, $f_4=55$ mm. A transmittance target chart with a character "0" of about 1 mm width and 2 mm height is placed at a distance of $z_s=146$ mm to the lens L0. A hologram is captured and reconstructed by inverse Fourier transforming the hologram and then performing a distance of propagation based on angular spectrum Fresnel diffraction method. One of the recorded holograms is shown in Fig. 2(a). Reconstructed images corresponding to three different z_r are shown in Fig. 2(b)–(d). In Fig. 2(e), the intensity distribution along the cross line (red line in Fig. 2(c)) for