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Dynamic imaging of micro-particles in 3D using lensless in-line digital holographic microscopy

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ABSTRACT

In this paper, dynamic imaging of micro-particles in 3D using lensless in-line digital holographic microscopy (LIDHM) is explored. The methodology presented retains the reconstructed pixels size used for the particles analysis at different depth locations under converging wave reconstruction geometry. Experiments studying the 3D visualization of co-polymer microsphere suspensions in distilled water are presented. The dynamic behavior of microspheres in 1mm cube volume is captured and the numerical reconstruction provides their volumetric flow behavior. From the plot the 3D visualization and the location of each particle, its depth and dynamic behavior can be observed clearly. The proposed work is useful for tracking the 3D dynamic behavior of particles and can be used to predict the motion of the moving particles in volume.

Keywords: Digital holography, lensless microscopy, pixel size, zero-padding, micro-particles, dynamic 3D analysis.

1. INTRODUCTION

The study of motion of micro-objects in volume has significant importance in modern science. Measurements of instantaneous 3D distribution of micro particles dispersed in a flow are required for many applications. The size, position, velocity and 3D distribution of particles within fluid or gaseous media can be determined with holographic techniques [1]. In Digital Holography (DH), optical holograms are recorded digitally using CCD sensors which are then numerically reconstructed, and it can be applied for particles analysis [2]. The potential of DH for measurement of the size, location, speed, and three-dimensional spatial distribution of particles make this technique more popular than the existing ones. Mainly in-line based DH methods were explored for particles analysis. For 3D micro-particles analysis, the reconstruction methodology was presented to slice in-focus particles [3]. For numerical reconstruction, two methods called the Fourier Transform (FT) and the Convolution (CV) algorithms are commonly used [4]. In the FT method of reconstruction, the pixel size of the reconstructed image varies with the reconstruction distance and it also depends on CCD array size and the wavelength used. The CV approach has the advantage that the pixel size of the reconstruction remains constant at different reconstruction depths, but it has certain restrictions to use [5].

Lensless in-line digital holographic microscopy (LIDHM) has been proposed for particles imaging and measurement [6]. In LIDHM, a highly diverging beam illuminates microscopic features of the object in transparent medium and provides the geometrically magnified diffracted object wave which interferes with the undiffracted reference wave. Their interference pattern is recorded by the CCD. LIDHM system provides geometrically magnified images with high resolution in all three dimensions. The geometrical magnification of the system increases with increasing the distance between the object and the CCD. For micro-particles measurement using LIDHM, the amplitude

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of the reconstructed wavefield from particles hologram is used to determine the spatial location and size of the particles. The depth measurement of the particles in 3D space is achieved by measuring the average intensity value of the pixels of the particle along the axial direction and the position. The particle's depth location corresponds to the depth where the intensity value becomes extreme. The size of the particle can be measured from the reconstructed pixels at this distance.

In this paper the advancement using LIDHM is explored for the precise 3D micro-particles study and dynamic analysis. Compared to the existing digital holographic methods, new development presented in this paper is the zero-padding of hologram to keep the pixel size independent of the reconstruction distance for the FT reconstruction for LIDHM. In addition, the methodology of dynamic analysis of micro-particles in 3D is presented. The method of identifying the particles in particular planes based on intensity threshold is used. This method is used to locate dynamic particles in 3D. The results presented from the particles with diameter of $9.6 \mu m$ flowing in water.

2. LIDHM SYSTEM DESIGN AND THEORY

2.1 Optical system

The schematic of LIDHM system is shown figure 1. A highly diverging spherical wave emerges from the point source and the divergence depends on the size of the source. The object is placed inside the cone-shaped spherical diverging wave and the geometrically magnified diffracted/scattered light from the object interferes with the un-scattered spherical reference wave. This interference pattern is digitally recorded using the CCD sensor, called digital hologram, which is numerically reconstructed to provide the desired imaging characteristics of the object. This geometry is suitable only for sufficiently small objects that occupy only a segment of light coming from the point source. If D is the distance between point source and CCD and l is the distance between the point source and object plane, then the geometric magnification of the system is D/l .

Figure 1 Schematic of lensless in-line digital holographic microscope

The lateral resolution of system depends on the numerical aperture (NA) and wavelength, similar to a conventional microscope. The NA of the LDHM system is defined by the range of angle over which the CCD can accept light coming from the point source. According to the Rayleigh resolution criteria, the lateral resolution Δx is defined as,

$$\Delta x \geq \lambda / 2 \cdot (NA) \quad (1)$$

And the depth resolution Δz defined is defined as,

$$\Delta z \geq \frac{\lambda}{2 \cdot (NA)^2} \quad (2)$$

The NA of the LIDHM system is not as high compared to optical microscopic objectives. The depth resolution is also depend on the size of the object. Both the lateral and depth resolution can be improved significantly, by recording the hologram using a CCD with sufficiently larger sensing area, placed close to the point source. Also, the size of pinhole significantly affects the resolution of the system. Smaller pinhole (ideally of the order of wavelength) increases the cone of the emitted light coming through it. This increases the NA of the system and thus improves the system resolution.

The experimental arrangement is shown in figure 2. Copolymer micro-spheres, $9.6 \mu m$ in diameter are studied using this LIDHM. The particles are flowing in water placed inside a cuvet. The laser beam with wavelength $0.405 \mu m$ is focused on a $1 \mu m$ size pinhole, and the sample is placed in the path of the diverging beam coming from the pinhole. The hologram of the particle system is recorded with the CCD having the array size 1280×960 of pixels with size $4.65 \mu m \times 4.65 \mu m$. The CCD is placed at a distance of $35 mm$ from the pinhole.

Figure 2 Experimental arrangement of LIDHM system

2.1 Recording and reconstruction methodology

The reference wave propagates along the same optical axis. One part of the diverging laser beam coming from the point source (figure 1) is the un-scattered spherical reference wave and the other part is scattered by the object is the object wave. Consider (x, y) be the hologram plane and the object and reference waves denoted by the $O(n_x, n_y)$ and $R(n_x, n_y)$ at the CCD plane. Here $n_x = 0, 1, \dots, N_x - 1$ and $n_y = 0, 1, \dots, N_y - 1$ are the pixel indices of the camera,

and $N_x \times N_y$ is the size of the CCD sensor in pixels. The hologram is the interference of the object wave reference waves and can be written as,

$$\begin{aligned} h(n_x, n_y) &= |O(n_x, n_y) + R(n_x, n_y)|^2 \\ &= |O(n_x, n_y)|^2 + |R(n_x, n_y)|^2 + O^*(n_x, n_y)R(n_x, n_y) + O(n_x, n_y)R^*(n_x, n_y) \end{aligned} \quad (3)$$

Here O^* and R^* are the complex conjugate of O and R , respectively. The CCD, placed at the hologram plane, records the interference pattern as given in eqn. 3. The first two terms create the undiffracted DC term in the real interference term that is represented by the other two terms. This background light (DC term) is due to the undiffracted light (zero-order term) during the reconstruction process. One possible method to suppress the zero-order term is the subtraction of the reference wave from hologram before reconstruction [7].

The recorded pattern is converted into a two-dimensional array of discrete signals by using the sampling theorem. Let pixel size of the CCD is Δ_x and Δ_y , then the digitally sampled holograms, can be written as,

$$H(n_x, n_y) = [h(n_x, n_y) \otimes \text{rect}(\frac{x}{\alpha\Delta_x}, \frac{y}{\beta\Delta_y})] \times \text{rect}(\frac{x}{N_x\Delta_x}, \frac{y}{N_y\Delta_y}) \text{comb}(\frac{x}{\Delta_x}, \frac{y}{\Delta_y}) \quad (4)$$

Where $\otimes$ represents the two-dimensional convolution and $(\alpha, \beta) \in [0,1]$ are the fill factors of the CCD pixels.

Figure 3 Reconstruction with converging reference wave

For the reconstruction of hologram and to achieve greater depth resolution and for a correct representation of the reconstruction, a converging reconstruction beam along with the FT method is preferred. The reconstruction geometry is shown in figure 3. The reconstruction wave impinges normally onto the CCD. The spherical reconstructed wave is focused at a distance equal to the distance between the pinhole and the CCD sensor used to be during the recording of the hologram. For a distance D between the pinhole and the CCD, the converging reconstruction wave can be

numerically approximated as $R(n_x, n_y) = A \exp[-ik(n_x^2 + n_y^2)/2D]$, where A is the amplitude, $k = 2\pi/\lambda$ is the wave vector and λ is the wavelength of the laser. The numerically reconstructed wavefield $U(n_{x'}, n_{y'})$ at the image plane (x', y') at distance d from the hologram plane is obtained by using the discrete FT method [4],

$$U(n_{x'}, n_{y'}) = \frac{e^{ikd}}{i\lambda d} e^{i\pi\lambda d (\frac{n_{x'}^2}{N_x^2 \Delta_x^2} + \frac{n_{y'}^2}{N_y^2 \Delta_y^2})} \times \sum_{n_x=0}^{N_x-1} \sum_{n_y=0}^{N_y-1} H(n_x, n_y) R(n_x, n_y) e^{[\frac{i\pi}{\lambda d} (n_x^2 \Delta_x^2 + n_y^2 \Delta_y^2)]} e^{[-2\pi i (\frac{n_x n_{x'}}{N_x} + \frac{n_y n_{y'}}{N_y})]} \quad (5)$$

where $n_{x'} = 0, 1, \dots, N_x - 1$ and $n_{y'} = 0, 1, \dots, N_y - 1$ are the discrete special coordinates at the image plane. By using Eq. (5), the calculation of the reconstructed wavefield can be done using the fast Fourier transform (FFT) algorithm.

The reconstructed pixel size along the x , and y directions changes according to the reconstruction distance. The method to control the pixel size for LIDHM system is presented here. Without loss of generality, equations for the x dimension are presented. The pixel size of the reconstructed image using FT method as given in eqn. 5 becomes,

$$\Delta_x' = \lambda d / N_x \Delta_x \quad (6)$$

It is required that the pixel size of the reconstruction should be constant at different depths, especially for 3D particle analysis and tracking where the co-ordinates of a particle used for studying the intensity of the reconstruction around the region of the particle. It has been proposed in past that to equalize the pixel size at reconstruction distances d_1 and d_2 ($d_2 > d_1$), the hologram size has to be increased before the reconstruction by zero padding as $N_x' = (d_2 / d_1) N_x$ and consider the central $N_x \times N_y$ portion of the propagated wavefront after reconstruction [8]. Zero padding fictitiously increases the hologram size thus compensating the pixel resizing effect of the FT reconstruction. In LIDHM, a diverging reference wave is used to achieve magnification. So, zero padding as above cannot retain the correct pixel size because the use of the converging reconstruction wave affects the pixel size too. Hence, in order to maintain the same image size at distances d_1 and d_2 , the hologram size needs to be zero-padded more. To compensate the converging reconstruction wave effect, the exact zero-padding of the hologram is calculated from the optical system geometry as,

$$N_x'' = \frac{D - d_1}{D - d_2} \frac{d_2}{d_1} N_x \quad (7)$$

Thus by doing this zero-padding of hologram and using the eqn. 5 for reconstruction, the pixel size at different reconstruction depths remains constant and the correct magnification is observed.

4. DYNAMIC 3D ANALYSIS OF MICRO PARTICLE FLOW

The dynamic analysis of copolymer micro-spheres with $9.6 \mu\text{m}$ in diameter are studied with LIDHM system as shown in figure 2. The micro-spheres were suspended in distilled water within a cuvet of internal size $10 \text{ mm} \times 10 \text{ mm} \times 100 \text{ mm}$. The cuvet was placed 15 mm from the CCD plane, thus the particles were suspended within a

volume located at 15 mm to 25 mm from the CCD sensor. The method of hologram zero-padding in conjunction with thresholding the reconstructed intensity [7] is incorporated for dynamic analysis of micro-particles in 3D.

The methodology for dynamic 3D analysis the particles has been developed in MATLAB environment. The following algorithm steps were used:

- (i) Reading of the number of frames for reconstruction;
- (ii) Subtract the reference beam from each frame and set the reconstruction parameters;
- (iii) Selected reconstruction depth range and divide into number of steps;
- (iv) Perform zero-padding of holograms (eqn. 7), to avoid shifting of the pixels;
- (v) Calculate the particles intensity (eqn. 5), and select a threshold for the fully reconstructed image intensity. All intensity values higher than threshold are consider as a particle;
- (vi) Reconstruct for depth steps and find the exact position of each particle. The particles depth position is calculated as the depth where the average intensity of pixels become maximum;
- (vii) Calculate the number of identified particles corresponding to each frame;
- (viii) Display the reconstruction of each frame corresponding to all reconstruction distances;
- (ix) Display the particles position in x-y, y-z and in 3D.

An experimental investigation has been performed. Six holograms were recorded, at the intervals of one second. The behavior of the particles within 1mm of depth were analyzed, with a reconstruction step of 20 μm . The results are shown in Figures 4, 5 and 6. Figures 4 and 5 show the movement of the particles in the x-y and y-z planes, respectively. Each sub-figure (a)-(f) corresponds to a frame and only in-focus particles are shown. The observed motion of the particles is shown as lines in the direction of the particles' movement.

Figure 4 x-y plots of six frames of micro-particles during motion

Figure 5 y-z plots of six frames of particles during motion

Figure 6 3D plots of six frames of particles during motion

A 3D view of the reconstructed volume is shown in Figure 6. This plot provides a better visualization of dynamic motion of particles in 3D. The location of each particle within the investigated volume and its dynamic behavior can be clearly observed. Hence this analysis is can be used to study dynamic 3D behavior of particles and to predict their motion in 3D. Any microscopy or a particle image velocimetry (PIV) [9] system cannot provide such images.

5. CONCLUSIONS

We have presented a LIDHM system and the methodology for its application for dynamic imaging and measurement of micro-particles in 3D. The method presented, avoids the pixel resize caused by FT reconstruction method of digital hologram using converging wave for reconstruction. The method can control the shifting of reconstructing particles image pixels by appropriately zero pad the hologram prior to reconstruction. Experimental results are presented for the 3D visualization study of co-polymer microsphere suspensions in distilled water. The dynamic behavior of microspheres in 1mm cube volume is captured and the numerical reconstruction provides their volumetric flow behavior. The presented system and methodology is useful for accurate measurement of the 3D dynamic behavior of particles and can be used to predict the motion of the moving particles in volume.

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