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Imaging mechanisms analysis of compact digital holographic microscope for microparticles measurement

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Abstract

Conventional optical microscopy suffers from small depth of focus due to its high numerical aperture and magnification of the microscope objective. In comparison, digital in-line holographic microscopy (DIHM) provides information about the entire 3D volume through numerical reconstruction of the single hologram at several depths. This advantage makes DIHM an effective tool for the measurement of microparticles in suspension. Recently, our group has demonstrated the potential of DIHM for accurate measurement of particles with sizes ranging from 40 microns to a few millimetres. In this paper, the applicability of DIHM is extended for measurement of near-micron sized particles. A compact digital holographic microscope with a single microscope objective is presented. The system imaging mechanisms of the microscope is analyzed first and the recording distance of digital hologram is calculated using spatial frequency analysis. Then the system magnification, lateral resolution and depth resolution are analyzed in terms of the hologram recording distance. Finally, the characterization of microparticles with a diameter of 1 micron and 10 microns is demonstrated with the compact setup. The experimental results show the efficiency and accuracy of this method with a measured error less than 1.55% in the diameter of certified particles.

Keywords: Digital holography; digital in-line holographic microscopy; particle measurement; imaging processing; hologram recording distance

1. Introduction

In digital holography, the hologram is recorded digitally by the CCD or CMOS instead of holographic plate and reconstructed numerically according to optical diffraction theory by which the amplitude and phase information of the recorded optical field can be obtained simultaneously. Digital holography allows full-field, real-time,

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nondestructive and three dimension measurement of samples and it has been widely used in various fields such as object recognition[1], information encryption[2], microscopy[3-4], shape and deformation measurement[5], and so on.

Conventional optical microscopy suffers from small depth of focus due to its high numerical aperture and magnification of the microscope objective. It needs a mechanical motion device along optical axis for automatically scanning the whole three dimensional experimental volume of the sample to image 3D objects. In comparison, digital in-line holographic microscopy (DIHM) provides information about the entire 3D volume through numerical reconstruction of the single hologram at several depths. Thus it has successfully been applied on several domains recently. The 3D location and tracking of particles was reported by Lu et al.[6], Sheng et al.[7], Cheong et al.[8] and Garcia-Sucerquia et al.[9]. The characterization and measurement of micro particles has been published by Coetmellec et al.[10]. Biological applications like cell biology, tracking particles in seawater have been demonstrated by Malkiel et al.[11] and Owen et al.[12]. The microparticles tracking velocimetry with digital in-line holographic microscopy have been shown by Satake et al.[13] and Kim et al.[14].

For the measurement of microparticles in suspension, DIHM is an effective tool. Recently, our group has demonstrated the potential of DIHM for accurate measurement of particles with sizes ranging from 40 microns to a few millimetres[15-16]. However, in lensless DIHM with diverging beam, the magnification ratio of the samples is decided by the different distances from CCD to samples or beam source, which is usually less than 10 times. A microscope objective can be used as a relay lens inserting between samples and CCD to pre-magnify the samples, relay the samples volume at a suitable position and image the hologram on the CCD target in DIHM. With this MO, it's possible to get higher expected magnification ratios and observe small micron size particles. In this paper, the applicability of DIHM is extended for measurement of near-micron sized particles. A compact digital holographic microscope with a single MO is presented. The system imaging mechanisms of the microscope is analyzed first and the recording distance of digital hologram is calculated using spatial frequency analysis. Then the system magnification, lateral resolution and depth resolution are analyzed in terms of the hologram recording distance. Finally, the characterization of microparticles with a diameter of 1 micron and 10 microns is demonstrated with the compact setup. The experimental results show the efficiency and accuracy of this method with a measured error less than 1.55% in the diameter of certified particles.

2. Principles

Figure 1 shows the digital holographic microscope with single MO for micropartilces measurement. A thin laser beam (λ=532nm) is spatially filtered by a 60x microscope objective and a 10μm pinhole, and then illuminates the samples to be measured. Part of this wave is diffracted by the samples at x_0y_0 plane and the remaining part passes the samples without being diffracted and serving as a reference wave. These two parts interfere at the original hologram plane (xy plane) to form the hologram (original hologram). Then a microscope objective is located behind xy plane as a relay lens to image original hologram on the CCD target and a magnified digital hologram is recorded by CCD at the magnified hologram plane ($\xi\eta$ plane). The CCD used in the experiment is an imaging source monochrome CCD (DMK 41BF02, pixel number 1280×960, pixel size 4.65μm×4.65μm).

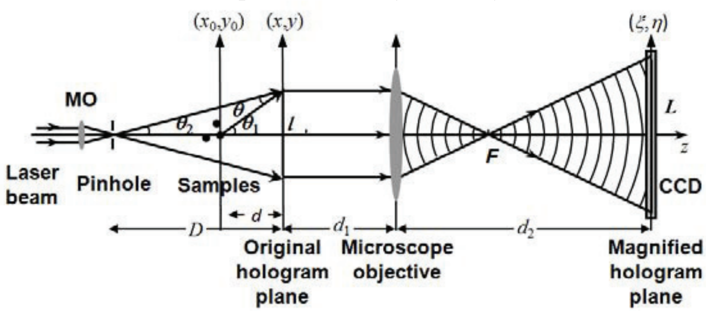

Fig. 1 Schematic of the compact digital holographic microscope with single MO

As is shown in Fig. 1, the laser light passes through the pinhole as a new point source to illuminate the samples. The background wave interferes with the object wave diffracted by samples to from origital hologram $I_0(x, y)$ at xy plane. The distance from xy plane to the phihole and samples is D and d.

Supposing that the object wave field at xy plane is $\boldsymbol{O}(x, y)$ and interferes with a reference wave $\boldsymbol{R}(x, y)$, the intensity of the original hologram $\boldsymbol{I}_0(x, y)$ is given by

$$\begin{aligned}\boldsymbol{I}_0(x,y) &= |\boldsymbol{H}(x,y)|^2 = |\boldsymbol{R}(x,y)+\boldsymbol{O}(x,y)|^2 \\ &= |\boldsymbol{O}(x,y)|^2 + |\boldsymbol{R}(x,y)|^2 + \boldsymbol{R}^*(x,y)\boldsymbol{O}(x,y) + \boldsymbol{R}(x,y)\boldsymbol{O}^*(x,y)\end{aligned} \tag{1}$$

where $\boldsymbol{H}(x, y)$ is the wave field of the interferogram.

Then the original hologram $\boldsymbol{I}_0(x, y)$ is magnified by the MO located behind xy plane at a distance d_1. The intensity distribution $\boldsymbol{I}(\xi,\eta)$ at $\xi\eta$ plane with a distance d_2 to MO can be expressed by

$$\boldsymbol{I}(\xi,\eta) = \boldsymbol{H}\left(-\frac{\xi}{M_2},-\frac{\eta}{M_2}\right)\boldsymbol{H}^*\left(-\frac{\xi}{M_2},-\frac{\eta}{M_2}\right) = \boldsymbol{I}_0\left(-\frac{\xi}{M_2},-\frac{\eta}{M_2}\right) \tag{2}$$

where $M_2=d_2/d_1$ is the transverse magnification of MO. Thus the digital hologram $\boldsymbol{I}(\xi,\eta)$ recorded by CCD at $\xi\eta$ plane is the magnified image of the original hologram $\boldsymbol{I}_0(x, y)$ with a magnification M_2. Considering the discrete features of the CCD, if the digital hologram $\boldsymbol{I}(\xi,\eta)$ has $N\times N$ pixels with a pixel size of Δx_0, the original hologram $\boldsymbol{I}_0(x, y)$ at xy plane can be consided to have $N\times N$ pixels but with a pixels size of $\Delta x_0/M_2$. When the digital hologram $\boldsymbol{I}(\xi,\eta)$ is recorded, it can be reconstructed numerically by convolution method to obtain the reconstructed image of the samples.

The system magnification M in Fig.1 is given by

$$M = \frac{D}{D-d}\times\frac{d_2}{d_1} \tag{3}$$

Part of magnification is given by the diverging wave in the lensless imaging process expressed as $M_1=D/(D\text{-}d)$; the other part is introduced by the magnification of MO equaled to $M_2=d_2/d_1$.

In DIHM, original hologram plane is located close to the samples to record high frequency information and obtain a higher magnification M_1, but the low CCD cutoff frequency (only 100 line pairs/mm) restricts the hologram recording ability. The maximum spatial frequency $f_{\max}$ to be resolved is determined by the maximum angle $\theta_{\max}$ between the reference and the object wave.

$$f_{\max} = \frac{2\sin(\theta_{\max}/2)}{\lambda} \tag{4}$$

where λ is the laser wavelength. Corresponding maximum resolvable spatial frequency can be calculated by $f_{\max}=1/2\Delta x$ and Δx is its sampling period. We consider the sampling of hologram at the original hologram plane (xy plane). Considering the CCD size is $L=N\Delta x_0$ with N pixels and a pixel size of Δx_0 and the recorded digital hologram at $\xi\eta$ plane has a magnification ratio M_2 relative to the original hologram. So the sampling period Δx in xy plane is equal to $\Delta x_0/M_2$, which should be greater than or equal to Rayleigh distance due to the Rayleigh limit. So we have $\Delta x\geq 0.61\lambda/\text{NA}$, where NA is the numerical aperture of the microscope objective. For a wavelength λ=532nm and NA=0.8, the sampling period Δx is greater than 0.406μm.

As shown in Fig. 1, the maximum angle $\theta_{\max}$ are decided by CCD size $l=N\Delta x_0/M_2$ and hologram recording distance d (the distance from object to hologram). For a greater l and a smaller d, the angle will be greater and the sampling requirement will increase. Assuming that the maximum spatial frequency diffracted from the sample reach the edge of CCD target and can be recorded, we have

$$\frac{2\sin(\theta_{\max}/2)}{\lambda} = \frac{M_2}{2\Delta x_0} \tag{5}$$

where

$$\sin\left(\frac{\theta_{\max}}{2}\right) = \sqrt{\frac{\sqrt{d^2+(l/2)^2}\cdot\sqrt{D^2+(l/2)^2} - d\cdot D + (l/2)^2}{2\sqrt{d^2+(l/2)^2}\cdot\sqrt{D^2+(l/2)^2}}} \tag{6}$$

Defining

$$c_1 = 1 - \frac{N^2 M_2^2 \lambda^2}{8l^2} \tag{7}$$

$$c_2 = (l/2)^4 - c_1^2 \cdot (l/2)^2 \cdot \left(D^2 + (l/2)^2\right) \tag{8}$$

and taking Eqs. (6) - (8) into Eq. (5), then the hologram recording distance d can be expressed as

$$d = \frac{-2D\cdot(l/2)^2 + \sqrt{4D^2\cdot(l/2)^4 - 4\cdot c_2\cdot\left(D^2 - c_1^2\cdot\left(D^2+(l/2)^2\right)\right)}}{2\cdot D^2 - 2\cdot c_1^2\cdot\left(D^2+(l/2)^2\right)} \tag{9}$$

Here d is the minimum hologram recording distance. In DIHM, the samples and original hologram distance should be greater than d. In Eq. (9), the original hologram size l is given by $l=N\Delta x_0/M_2$, hence d is a function of distance D, pixel size Δx_0, pixels number N and MO magnification M_2. High frequency information will not be hold on at the CCD edge if d is smaller than the value calculated in Eq. (9).

For a laser with wavelength λ=532nm, point source distance D=10cm and CCD with 512, 1024 or 2048 pixels and pixel size 5 or 10μm, the relationship of minimum hologram recording distance d and MO magnification M_2 is shown in Fig. 2. When MO magnification is 20x, the minimum hologram recording distance is about 0.43mm. Because CCD sampling period Δx should be greater than or equal to Rayleigh distance, when M_2 is greater than 24.6, it's helpless to decrease the recording distance to obtain a higher reconstruction resolution for a CCD with 1024 pixels and a pixels size of 10μm. Here 24.6 is the critical magnification for the given CCD parameters. If MO's magnification is greater than this critical value, the recording distance will be still equal to the minimum distance d decided by Eq. (9), otherwise the sample's high frequency information will not be lost.

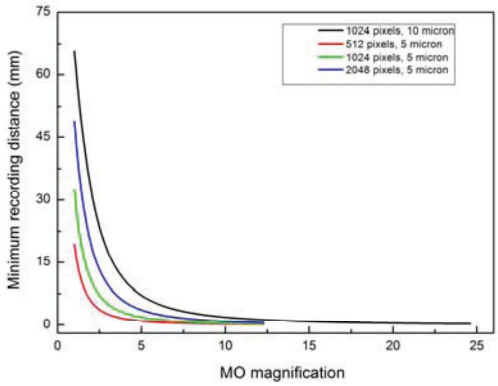

Fig. 2 Relationship of minimum recording distance and MO magnification

Thus the lateral resolution of the system δ_{xy}, which defines the minimum resolved sample size in reconstruction, depends on the used laser wavelength λ, the original hologram size l and hologram recording distance d. Thus it can be expressed as

$$\delta_{xy} = \lambda\sqrt{\left(\frac{d}{l}\right)^2 + \frac{1}{2}} = \lambda\sqrt{\left(\frac{M_2 d}{N\Delta x_0}\right)^2 + \frac{1}{2}} \tag{10}$$

The lateral resolution δ_{xy} is about 0.51μm for N=1024, Δx_0=10μm and a 20x MO.

The intensity of the reconstructed image along optical axis in the holographic microscopic system is described by the variation $(\mathrm{sin}g)^2/g^2$ with $g = \pi\lambda\Delta d/2\lambda d^2$. Considering a 20% loss in the intensity, this consideration yields to the total depth of focus δ_z of the system, which is also depends on the laser wavelength λ, the original hologram size l and hologram recording distance d. It can be written as

$$\delta_z = \frac{\lambda}{2\cdot\left(\frac{l}{2d}\right)^2} = \frac{2\lambda M_2^2 d^2}{N^2 \Delta x_0^2} \tag{11}$$

The depth resolution δ_z define the minimum resolved distance of the samples along z axis in the system. When a 20x MO is used, depth resolution δ_z is about 0.74μm for N=1024 and Δx_0=10μm.

3. Experiment results and discussions

To demonstrate the capability of DIHM, several experiments are presented to verify the theory analysis. Polymer microsphere opaque particles manufactured by Duke Scientific Corporation with diameters of 10μm and 1μm are suspended in water solution to make the particles suspensions sample and then the suspensions are measured separately with 10x, 40x and 60x MO by using the compact digital in-line holographic microscope shown in Fig. 1.

All the parameters for present experiments and measured results are shown in Table 1.

Table 1: Parameters for experiments and measured results

Certified average size of microspheres (μm)	Power of MO	System magnification	Measured size (μm)	Measured error
10±0.05	10x	10.01	10.32±0.55	3.20%
1.034±0.020	40x	39.66	1.05±0.03	1.55%
	60x	60.76	1.05±0.02	1.55%

To measure 10μm microparticles suspension, a 10x MO is used in the digital in-line holographic setup in Fig. 1 to image the hologram on CCD target and the hologram is captured by the CCD with a rate of 1 frame/s. The system magnification M is 10.01 calculated by Eq. (3) according to the distance parameters in experiment. Figure 3(a) shows one of the recorded holograms. The interference patterns in the hologram are very clear. In the motion of the 10μm particles suspensions, several holograms are captured. Then the holograms are reconstructed at several depths covering the volume to be measured. Figures 3(b)-3(d) show the reconstructed images located at d=14.4mm, 26.2mm, and 42.0mm from the hologram plane. The focused particles in the reconstructed images have been separately marked with red circle line. It's clear that the edges of the focused particles are very sharp. Canny edge detection is used to identify particles on each reconstruction. The intensity of each identified area is compared to the mean intensity of the reconstruction and only dark areas are considered to avoid considering areas of the background, which might happen to be surrounded by strong edges. Also areas touching the edges of the reconstruction are

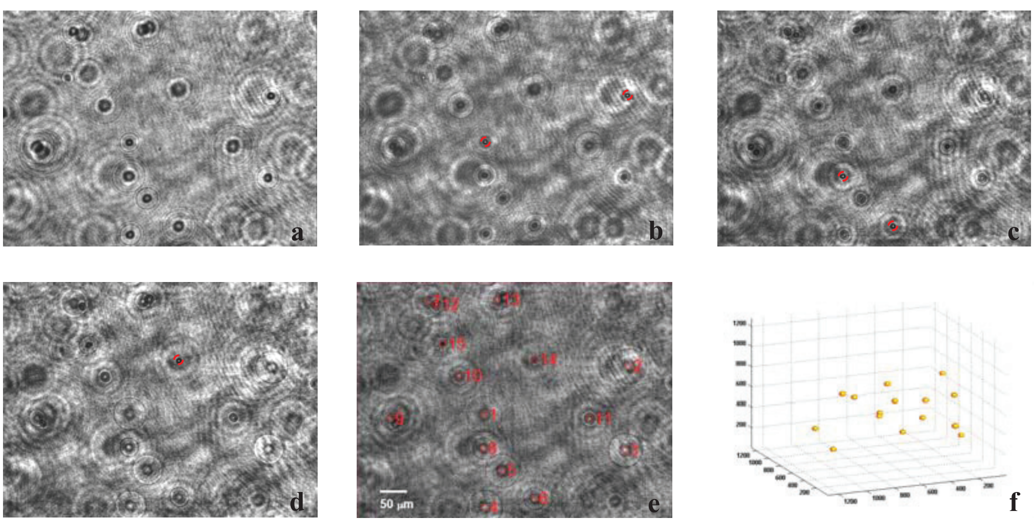

Fig. 3 Digital hologram and the reconstruction (10μm particles, 10x MO). a) Hologram with 10x MO; b)-d) reconstructed image located at d=14.4mm, 26.2mm, and 42.0mm from hologram plane. The focused particles has been marked with red line; e) the position of measured particles in the hologram; f) 3D distribution of the particles in solution.

neglected to avoid erroneous size measurements. By using digital image process approach, we can get the size and spatial position of focused particles at different reconstruction depths. Figures 3(e) and 3(f) show all the detected particles in 2D and 3D distribution. Here the measured depth of focus is about 2.8mm and the spatial location of every particle is marked in Fig. 3(f).

In order to measure particles diameter accurately, 27 holograms have been captured in the movement of the micron size particles suspension in experiment. All the 27 holograms are processed and 320 particles are identified with a mean particle size 10.32μm±0.55μm while the actual size is 10.00μm±0.05μm. Figure 4(a) shows the measured particles size distribution. Compared with the actual particle size, there is an error about 0.3μm.

For 1μm polymer microspheres, a higher magnification MO, like 40x or 60x, is used to record the hologram. The calculated system magnification with 40x and 60x MO are 39.66 and 60.76 in experiment separately. Then 20 and 10 holograms are captured separately with 40x and 60x MO. These holograms are reconstructed and processed to obtain the particles size distributions which are shown in Figs. 4(b) and (c). In the case with 40x MO, 1015 particles in suspension are identified with a mean particle size 1.05μm±0.03μm while the actual size is 1.034μm±0.020μm. The error is about 1.55% in the measurement. And for the case with 60x MO, the measured size of particles is 1.05μm±0.02μm which error is also 1.55%. As is seen from Fig.4 and Table 1, DIHM by introducing a single MO can be used for measurement of near-micron sized particles, and the measurement error is only 1.55% for 1μm samples which shows its efficiency.

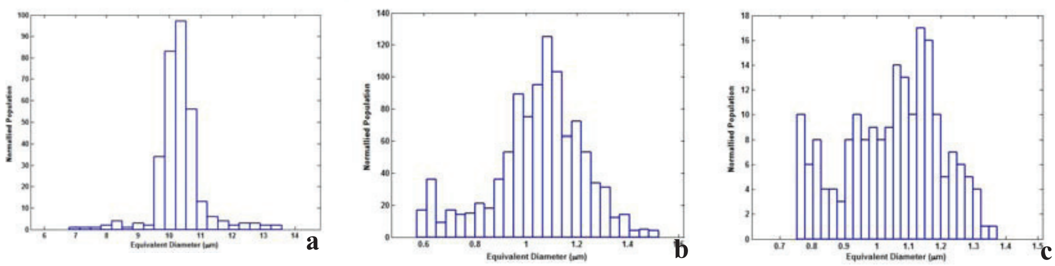

Fig. 4 Measured particles size distribution. a) 10μm particles and 10x MO; b) 1μm particles and 40x MO; c) 1μm particles and 60x MO.

4. Conclusions

In the paper, a compact in-line holographic microscope with a single MO is presented to quantitatively measure the size and 3D distribution of micron size particles in solutions. The characterization of microparticles with a diameter of 1 micron and 10 microns is demonstrated with the setup. The experimental results show the efficiency and accuracy of this method with a measured error less than 1.55% in the diameter of certified particles.

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