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Amplitude contrast image enhancement in digital holography for particles analysis

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ABSTRACT

In-line digital holography is presented for the particles analysis in 3-D. The presence of zero order wave, conjugate image wave, and defocused images degrade the image quality in in-line digital holography. In this paper we utilise the numerical reconstruction process to minimise these effects. We present a new subtraction method of reconstructed wave fields for particles analysis. The effects of zero order term, conjugate image wave and more importantly the particles outside the plane of interest (defocused images) are simultaneously minimized, and thus improve the contrast of reconstructed amplitude images. This method is useful to slice in-line digital holograms to display only in-focus particles at any particular plane. Furthermore all this is accomplished from a single hologram with no additional pre or post processing required.

Keywords: Particles analysis, In-line digital holography, CCD, convolution method, real image wave, zero-order wave, defocused image, depth-of-focus, amplitude contrast

1. INTRODUCTION

Holography is an established tool for scientific and engineering studies and has been found a remarkably wide range of applications. With the growing development of charge coupled devices (CCD) and digital computers, digital holography (DH) was explored [1]. It overcomes the problems of classical holography like wet chemical processing of photographic films and vibration isolation requirements during hologram recording. The recently available digital recording devices (CCD sensors) provide flexibility to record the holograms directly in digital form. The reconstruction process is performed numerically giving direct access to the intensity and phase of the wavefront. DH is rapidly developing into an acceptable microscopic imaging modality with growing applications in three-dimensional shape measurement [2], MEMS characterization [3,4], bio-imaging [5], pattern recognition [6] etc. Particles analysis is one of the major applications of digital holography. The potential for measurement of the size, location, speed, and three-dimensional spatial distribution of particles make holographic techniques more popular than the existing techniques, such as particle image velocimetry (PIV), laser-Doppler-anemometry (LDA), phase-Doppler-anemometry (PDA).

The low resolution of the available CCD sensors restricts the angle between object and reference waves to few degrees only. In case of off-axis DH, an offset interference angle is introduced to separate twin image and zero-order term from the real image wave. But in this condition the full area of CCD sensor is not utilized in the real image reconstruction of the objects. While, in in-line digital holography, full area of CCD sensor is utilized for image reconstruction. But because of the in-line geometry, the zero order and conjugate image waves overlap with the real image wave during the reconstruction process. In additions for particles analysis in 3D, the defocused images of object outside the plane of reconstruction degrade the image quality of the reconstructed image. The first two (zero order and conjugate wave) effects have been resolved using various methods [8, 9] and are usually not a major problem. However the third (defocusing) effect has not been addressed and thus the full capability of holography has not been exploited.

Various approaches using image processing, curve fitting and thresholding have been proposed to accurately locate a particle along the axial (depth) direction [10-12]. The accurate axial position of focused particles in in-line DH is obtained by identifying the plane where the variance of the imaginary parts of the reconstructed complex amplitudes is

smallest [10] or by windowing the Fresnel Function [11]. Alternately for transparent particles [12] using a double exposure with one exposure without the specimen and second with the specimen is processed and subsequently fitted to a second order polynomial to accurately locate the axial position has been shown to be very promising. In this paper a method based on subtraction of reconstructed wavefields is proposed to improve the contrast of the reconstructed amplitude image. This method is useful eliminate the defocused particles and display a slice containing only the in-focus parts of the image. Furthermore, only a single hologram is necessary and no other curve fitting or processing is required. The bright background due to zero order wave is also simultaneously minimised and although the twin image is not a major problem for small particles, its effect can be reduced as well. In addition very small particles, which were not visible in the reconstruction, appear in the final image.

2. IN-LINE DIGITAL HOLOGRAPHY

2.1 Theory

Let (x', y') is the object plane and (ξ, η) be the hologram plane. The hologram, which is the interference of the object wave and reference wave recorded by a CCD placed at the hologram plane. Figure 1 shows the coordinate system of recording and reconstruction in DH. The object wave $O(\xi, \eta)$ is defined as the convolution of amplitude transmittance of the object $A_o(\xi, \eta)$ with the impulse response of the coherent optical system and can be written as

$$O(\xi, \eta) = \frac{\exp(ikd)}{i\lambda d} \int_{-\infty-\infty}^{\infty} \int_{-\infty-\infty}^{\infty} A_o(x', y') \exp\left[\frac{i\pi}{\lambda d} \{(\xi - x')^2 + (\eta - y')^2\}\right] dx' dy' \quad (1)$$

where $k = 2\pi / \lambda$, is the wave number and d is recording distance.

Figure 1- Optical system for digital holography

The interference between the object wave $O(\xi, \eta)$ and reference wave $R(\xi, \eta)$ is recorded in the hologram plane (ξ, η) . The complex amplitude of the interference pattern at the hologram plane is

$$U(\xi, \eta) = O(\xi, \eta) + R(\xi, \eta) \quad (2)$$

A CCD sensor records this interference pattern in the form of intensity distribution $h(\xi, \eta) = U(\xi, \eta)U^*(\xi, \eta)$, which is stored in the computer as the digital hologram. Thus,

$$h(\xi, \eta) = |O(\xi, \eta)|^2 + |R(\xi, \eta)|^2 + O^*(\xi, \eta)R(\xi, \eta) + O(\xi, \eta)R^*(\xi, \eta) \quad (3)$$

The recorded whole optical wavefield can be reconstructed during the reconstruction process [13]. The hologram is multiplied by reconstruction wave (R') and the reconstructed complex wave field at a distance d' is obtained using the Fresnel's diffraction integral. The reconstructed wavefield at the image plane by using Fresnel approximation can be written as,

$$U(x, y) = \frac{e^{ikd'}}{i\lambda d'} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} h(\xi, \eta) R'(\xi, \eta) \exp\left[\frac{i\pi}{\lambda d'} \{(x - \xi)^2 + (y - \eta)^2\}\right] d\xi d\eta \quad (4)$$

The impulse response $g(\xi, \eta)$ of the coherent optical system at reconstruction plane can be defined as [14]

$$g(\xi, \eta) = \frac{e^{ikd'}}{i\lambda d'} \exp\left\{\frac{i\pi}{\lambda d'} (\xi^2 + \eta^2)\right\} \quad (5)$$

For space invariant systems, wavefield is reconstructed by convolution (CV) approach. This approach is useful if the pitch of the reconstructed image has to be independent of the reconstruction distance and used when object size is much smaller than the CCD array, thus most suitable for particles analysis. The reconstructed wave field for space invariant system can be obtained as:

$$U(x, y, d') = \{h(\xi, \eta) R'(\xi, \eta)\} \otimes \{g(\xi, \eta)\} \quad (6)$$

For reconstruction of the real image, the reconstruction wave should be an exact replica of the complex conjugate of the reference wave ($R' = R^*$). At the reconstruction distance $d' = d$, the real image wave becomes a real quantity and forms the image of the object. The pixel size of the reconstructed image is same as the pixel size of CCD and does not vary with reconstruction distance, i.e.

$$\Delta x' = \Delta \xi, \Delta y' = \Delta \eta \quad (7)$$

The reconstructed wave field contains mainly three parts, (i) zero order wave, (ii) real image wave, and (iii) conjugate image wave. The presence of the zero order wave and conjugate image wave results in degradation of the quality of the reconstructed image. In addition for a 3-D distribution of particles, the defocused images of objects lying outside the focus plane also appear in the final reconstructed image.

2.2 Image slicing

In the proposed method the property that only the in-focus reconstructed image wave is a real quantity, is used to minimise the out-of-focus and other terms effects, and thus provide an image slice with only the focused parts of the object visible. The depth of focus of the system limits the isolation of the slice. The depth of focus depends on the numerical ($N.A.$) that is related to the particle size. If θ be the angle made by particle at CCD plane then $N.A. = \sin \theta$ and depth of focus Δz becomes [15],

$$\Delta z = \frac{\lambda}{2 \cdot (N.A.)^2} \quad (8)$$

The $N.A.$ of digital particle field is limited to less than 0.1 because of small interference angle because of small interference angle [10]. This results the large depth of focus during reconstruction process.

The reconstruction geometry of the particles slicing is shown in figure 2. At the focus plane of the object, let U_I^* and U_I represent the real and conjugate image waves respectively, U_{II}^* and U_{II} be the real and conjugate image waves due to all other out of focus objects and U_D is the direct wave. Since U_I^* becomes a real quantity ($real(U_I^*)$) at the focus plane then the reconstructed complex wave fields at in-focus plane (reconstruction distance d) and out-of-focus plane (reconstruction distance $d + \Delta d$) can be written as

$$U(x, y, d) = real(U_I^*) + U_{II}^* + U_{I+II} + U_D \quad (9a)$$

$$U(x, y, d + \Delta d) = U_I^* + U_{II}^* + U_{I+II} + U_D \quad (9b)$$

Figure 2- Reconstruction geometry for particles slicing

U_{I+II} is the sum of conjugate image waves due to object (U_I) and all other out-of-focus objects (U_{II}). The amplitude of conjugate image waves, defocus object waves and the direct wave does not change significantly for small values of Δd , while the amplitude of the real image wave changes drastically at these two reconstructed planes. If opaque particles are the object and A_0 represents their amplitude transmittance, then the subtraction of $U(x, y, d)$ and $U(x, y, d + \Delta d)$ represent mainly the real part of U_I^* , and for amplitude image this difference represents only amplitude of the focused particles (at reconstruction distance d) i.e.

$$U(x, y, d) - U(x, y, d + \Delta d) \approx A_o^* \quad (10)$$

Thus subtraction of the two reconstructed waves eliminates the direct wave and the defocused object waves simultaneously.

3. EXPERIMENT

Experimental set-up as shown in figure 3 was used to show the subtracted wavefield effect for particles analysis. A single mode optical fiber with a core diameter about $10\mu m$ coupled to a $532nm$ frequency doubled diverging Nd-YAG laser. The diverging beam was used to illuminate a 4-mm thick glass slide, which had a few particles on either

face. The size of the particles varies from $5\mu m$ to $15\mu m$. The glass slide was placed at a distance of 40 mm from end of the fiber. The diverging beam is used to get magnified object beam which results to resolve the particles size smaller than the CCD pixel size. The magnification factor depends on the divergence of the beam and the distance of the object plane from the fiber and CCD.

Figure 3 - Experimental set-up for particles analysis

The beam diffracted from the particles interferes with the undiffracted beam to form the hologram at the CCD plane. In-line Fresnel digital hologram was recorded on a CCD placed at distances of 64mm and 68mm from the faces of the slide. The CCD had 1280 x 720 square pixels, $6\mu m$ in size.

The numerical reconstruction is performed using MATLABTM on Pentium IV computer.

4. RESULTS AND DISCUSSION

The recorded digitally sampled hologram is shown in figure 4. The pre-processing of the holograms did not require with the proposed method.

Figure 4 - Digitally sampled hologram

Figure 5 (a)- Reconstructed amplitude image of front side of glass plate

Figure 5(b)- Reconstructed amplitude image of back side of glass plate

The reconstruction process is performed by using convolution approach. Figure 5 (a) and (b) show the amplitude images at reconstruction distances 64mm and 68mm. In the reconstructed image the bright background is because of the zero-order wave. The twin image is not a problem in such type of geometry because its effect appears as the background signal in the reconstructed image which is negligibly small. The out-of-focus images of the particles on the other side of the glass plate appear as hollow circles in the reconstructed image. These defocusing effects are minimised with the proposed method of subtraction of reconstruction wavefields. The subtraction method simultaneously suppresses the effect of the zero order wave as well.

Figure 6(a)- Subtracted image for front side of glass plate

Figure 6(d) Subtracted image for back side of glass plate

The choice of subtraction distances (Δd) is limited on the one hand by the depth of focus of the particles and on the other hand by the effect of influence from other waves which are simultaneously present with the real image wave. In the current experiment, since a diverging beam is used to resolve particles in two planes which are smaller than the pixel size of the CCD sensor, there are a variation in the $N.A.$ for different planes and hence affect the depth of focus. The depth of focus depends on the particles sizes and their distances from the CCD during recording process. For the current set-up, the particles range in size from $5\mu m - 15\mu m$ and their distance is also varies, this results a range of depth of

focus for corresponding reconstruction planes. The subtraction distances (Δd) for which best effect is observed are 0.7mm and 1.1mm for front and backside of glass plate respectively.

Figure 6(a) and (b) shows the corresponding subtracted images for front and backsides of glass plate. The wavefields used for subtraction were reconstructed at 63.3mm & 66.9mm and then subtracted from the wavefields reconstructed at 64mm & 68mm respectively. It is clear that the direct beam, the conjugate image and the out-of-focus images are eliminated and only the in-focus particles are visible. In addition, certain smaller particles not visible in the earlier image appear in this image. Also large objects are not completely eliminated as their depth of focus is large and hence they also appear in the subtracted image. Furthermore, what appear as single particles in the original reconstructed hologram are indeed two particles. Thus the subtraction approach allows each slice of the hologram to be viewed with more clarity.

5. CONCLUSIONS

In summary, the proposed method is useful to get reconstructed image free from zero order term and the out-focus particles twin image. This method is most effective for particle analysis, because of small depth of focus. The best effect is observed for smaller subtraction distances and when the separation between the subtracted planes of the objects is more than the depth of focus of the system. For DH, depth of focus depends on the particle size amongst other parameters and hence this approach is best for very small particles analysis in volume.

REFERENCES

1. U. Schnars, and W. Jüptner, "Direct recording of holograms by a CCD target and numerical Reconstruction", *Applied Optics*, Vol. 33, No. 2, pp. 179-181, 1994.
2. B. Javidi, and E. Tajahuerce, "Three-dimensional object recognition by use of digital holography", *Optics Letters*, Vol. 25, No. 9, pp.610-612, 2000.
3. L. Xu, X. Peng, A. Asundi, and J. Miao, "Hybrid holographic microscope for interferometric measurement of microstructures", *Optical Engineering*, Vol. 40, No. 11, pp. 2533-2539, 2001.
4. G. Coppola, P. Ferraro, M. Iodice, S De Nicola, A Finizio, and S. Grilli, "A digital holographic microscope for complete characterization of microelectromechanical ststems", *Measurement Science and Technology*, Vol. 15, pp. 529-539, 2004.
5. P. Marquet, B. Rappaz, P. J. Magistretti, E. Cuhe, Y. Emery, T. Colomb, and C. Depeursinge, "Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy", *Optics Letters*, Vol. 30, No. 5, pp. 468-470, 2005.
6. F. Dubois, C. Minetti, O. Monnom, C. Yourassowsky, J.C. Legros, and P. Kischel, "Pattern recognition with a digital holographic microscope in partially coherent illumination", *Applied Optics*, Vol. 41, No. 20, pp. 4108-4119, 2002.
7. M. Adams, T. M. Kreis, and W. P. O. Jüptner, "Particle size and position measurement with digital holography, in Optical Inspection and Micrommeasurements", *Proceedings of SPIE*, Vol. 3098, pp. 234-240, 1997.
8. T.M. Kreis, and Werner P.O. Jüptner, "Suppression of DC term in digital holography", *Optical Engineering*, Vol. 36, No. 8, pp. 2357-2360, 1997.
9. Y. Takaki, H. Kawai, and H. Ohzu, "Hybrid Holographic Microscopy Free of Conjugate and Zero-Order Images", *Applied Optics*, Vol. 38, No. 23, pp. 4990-4996, 1999.
10. G. Pan, and H. Meng, "Digital holography of particle fields: reconstruction by use of complex amplitude", *Applied Optics*, Vol. 42, No. 5, pp. 827-833, 2003.
11. C. Fournier, C. Ducottet, and T. Fournel, "Digital in-line holography: influence of the reconstruction function on the axial profile of a reconstructed particle image", *Measurement Science and Technology*, Vol. 15, pp. 686-693, 2004.
12. W. Xu, M.H. Jericho, I.A. Meinertzhagen, and H.J. Kreuzer, "Digital in-line holography of microspores", *Applied Optics*, Vol. 41, No. 25, pp. 5367-5375, 2002.
13. S. Grilli, P. Ferraro, S. De Nicola, A. Finizio, G. Pierattini, and R. Meucci, "Whole optical wavefields reconstruction by Digital Holography", *Optics Express*, Vol. 9, No. 6, pp. 294-302, 2001.
14. J. W. Goodman, "Introduction to Fourier Optics", *McGraw-Hill Companies, Inc.*, New York, 1996.
15. M. Born, and E. Wolf, "Principles of Optics", (*Sixth ed.*) Oxford: Pergamon Press, 1980.