

Micro-Structure Measurement and Imaging Based on Digital Holography

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Abstract Advancements in the imaging and computing technology have opened the path to digital holography for non-destructive investigations of technical samples, material property measurement, vibration analysis, flow visualization and stress analysis in aerospace industry which has widened the application of digital holography in the above fields. In this paper, we demonstrate the non-destructive investigation and micro-structure measurement application of digital holography to the small particles and a biological sample. This paper gives a brief description of the digital holograms recorded with this system and illustratively demonstrated.

Keywords: Non-Destructive Investigation, Fourier Transformation, Digital Holography

1. Introduction

Digital holographic interferometry(DHI) is a promising method for non-destructive investigation, micro-structure measurement and its visualization of technical samples, because it can provide not only the intensity-contrast images but also the phase contrast images which have great importance in imaging world (Lee et al., 2006; Maldatue, 2000). Advances in the recent years in holographic interferometry have come largely from the advances in recording materials, recording methods, computer processors and image processing techniques, which has ultimately resulted in digital holography (Knox, 1966, Zhang and Yamaguchi, 1998; Qu et al., 2007). Digital holographic interferometry, which uses electronic detectors and numerical reconstruction, provides full field, non-invasive access to objects and allows high sensitivity and accuracy of measurement of interference phase with high spatial resolution.

Successful applications to problems are many but they still limited to laboratory level because commercial systems are not available and custom instruments are relatively expensive to buy and operate, although, our main interest lies in the non-destructive application of transparent specimens for image visualization.

Several articles have been published in the literature that have successfully attempted to explain the digital holography and its application to non-destructive investigation (Yamaguchi and Zhang, 1997; Matsushima et al., 2003). In this paper, we demonstrate the non-destructive application of digital holography to the some biological samples and small particles detection. This paper gives a brief description of the digital holography and digital hologram reconstruction using basic diffraction theory of light. In our case, the reconstruction of digital hologram is based on the angular spectrum method(ASM). It is also known as plane wave expansion method (Yamaguchi et al., 2001). The

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brief experimental procedure is explained in the following section.

2. Basic Theory

In holography, the superposition of the object wave and reference wave produces a holographic interference pattern that is recorded by a CCD camera. Using the ASM, one can extract information about the wave field that gives rise to the holographic interference pattern (Knox, 1966, Zhang and Yamaguchi, 1998; Qu et al., 2007; Yamaguchi and Zhang, 1997; Matsushima et al., 2003). Suppose that the hologram plane and the parallel image plane are designated by the (x, y, z) and (x', y', z') coordinates, respectively. The two-dimensional(2D) complex wave field $u_z(x', y')$ on the parallel image plane is numerically reconstructed from the complex hologram wave field $u_o(x, y)$ using the ASM, which can accurately reconstruct the complex wave field on the image plane. The angular spectrum is the Fourier transform of the complex hologram wave field:

$$F(k_x, k_y; 0) = \iint_{-\infty}^{+\infty} u_o(x, y) \exp[-i(k_x \cdot x + k_y \cdot y)] dx dy \quad (1)$$

where k_x and k_y are the spatial frequencies. Normally, this spectrum contains the zero-order diffraction and a pair of twin-image components. The real image and virtual image are located symmetrically opposed with respect to the center of the image. The distance from each image to the center depends on the incidence angle of the reference wave, which must be large enough to ensure a complete separation of the zero-order diffraction. The zero-order diffraction and one of the twin image components can be removed by using a numerical band-pass filter (Qu et al., 2007), and the reconstructed wave field can be obtained from the inverse Fourier transform of the filtered angular spectrum:

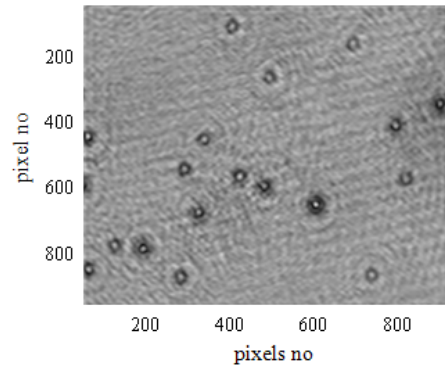
$$\begin{aligned} u_z(x', y'; z) &= \iint_{-\infty}^{+\infty} F(k_x, k_y) \exp[i(k_x \cdot x + k_y \cdot y + k_z \cdot z)] dk_x dk_y \\ &= F^{-1}[F(k_x, k_y; 0) \cdot \exp(ik_z z)] \end{aligned} \quad (2)$$

where $k_z = (k^2 - k_x^2 - k_y^2)^{1/2}$, $k = 2\pi/\lambda$ and $'z'$ is the separation between the image plane and hologram plane. The main advantages of the ASM are consistent pixel resolution, easy filtering of noise and background components, and the possibility of small object-to-hologram distances because the minimum distance requirement does not apply. The computational procedure is no heavier in this case than for the Fresnel approximation or other methods. The numerical diffraction of the hologram results in a reconstructed optical field as a matrix of complex numbers, yielding the amplitude and phase images. One of the advantages of the system presented here is that it does not require physical scanning, as is the case for optical scanning holography. Therefore, the apparatus is not complicated to set-up. Despite its simplicity, the digital holographic method offers an excellent potential for 3D microscopy in biomedical imaging applications.

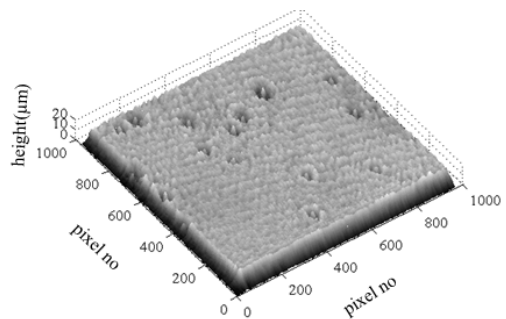
3. Experiment and Results

An experiment is performed to demonstrate the micro-measurement and non-destructive investigation for the application of digital holography. A schematic of the holographic system based on a Mach-Zender interferometer is shown in the Fig. 1. It consists of two mirrors (M1 and M2) and two beam splitters (BS1 and BS2) form the Mach-Zender interferometer. The laser beam generated by the He-Ne laser is split into two beams (reference and object beams). All the specimens are illuminated with a collimated He-Ne laser beam (by collimating lens, L) of wavelength 632.8 nm. Special filters (SF1 and SF2) are used for reducing noise and aberrations present

in the beam. The off-axis geometry is considered, the separation of the real image and virtual image can be controlled by second beam splitter(BS2). Zero- order and one of the twin image components can be removed by using a numerical band-pass filter. The holograms formed by the interference between the object and the reference beams were captured by a CCD camera with the pixel size $N_x \times N_y = 960 \times 960$, pixel pitch $4.65 \mu\text{m}$ and transmitted to a computer by means of a Frame Grabber card. The diffraction patterns for the small particles are shown in the Fig. 2. We obtained the complex hologram of three-dimensional object consisting of slide of bacteria of size about $10 \mu\text{m} \sim 15 \mu\text{m}$ and small particles of size vary from $20 \mu\text{m}$ to $30 \mu\text{m}$. Small particles are produces by spray nozzles on slide glass. The reconstructed images of small particles is shown in the Fig. 3(a) and the



(a) Small particles



(b) 3D prospective of small particles

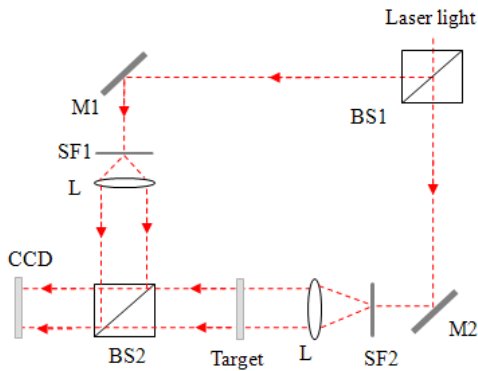
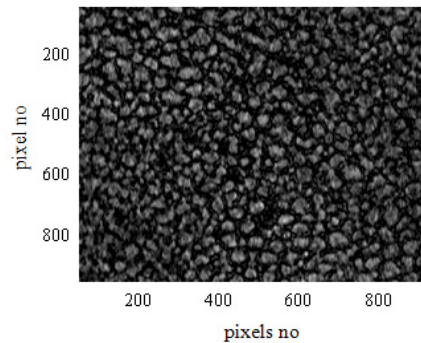
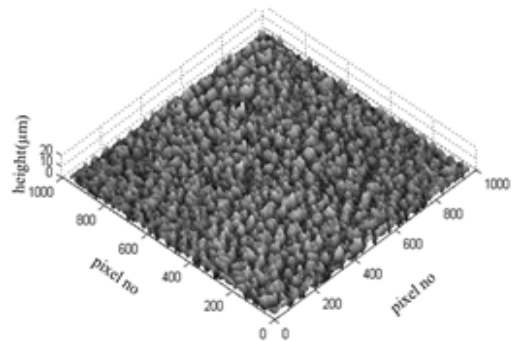


Fig. 1 Set-up of the digital holographic system



(c) Bacteria



(d) 3D prospective of bacteria

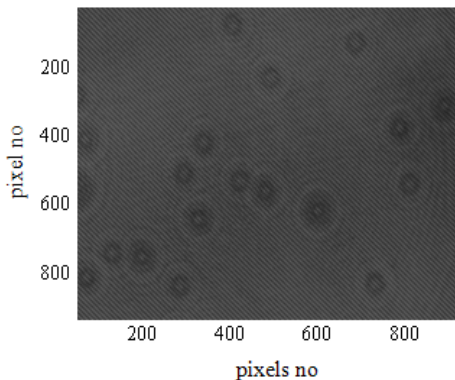


Fig. 2 Diffraction patterns of small particles

Fig. 3 Micro-structure images of small particles & bacteria and its 3D prospective

Fig. 3(c) shows the *Candida Albican* bacteria, both specimens have micro-structure, which is easily obtained by this digital holographic technique.

In this technique focusing can be adjusted numerically to reconstruct images at arbitrary positions after unfocused holograms are acquired. These bacteria used as the specimen was grown directly on a cover glass in a culture solution. The cover glass was put on a slide glass with the living cells between them. The object beam entered the specimen from the slide glass side. One can also obtain the three-dimensional perspective of these specimens using this technique, these reconstructed images contain 3D information in the form of two-dimensional complex matrix. The three-dimensional perspective of these specimens are shown in the Fig. 3(b) and 3(d) respectively.

4. Conclusion

The digital holographic technique is used for recording and reconstructing digital holograms and generating holographic interferograms for non-destructive testing. The applications of digital holography, especially in the field of nondestructive investigations and micro-structure measurement of technical samples are of particular importance. Another superiority of digital holography is that focusing can be adjusted numerically to reconstruct images at arbitrary positions after unfocused holograms are acquired. However the applicability of this technique was limited to the laboratory environment due to the cumbersome optics and requirement of vibration isolation and controlled ambient lighting. In this study we obtained the images of some micro-structure specimens for non-destructive evaluation.

Acknowledgement

This work was supported (in part) by the research funds from Chosun University, 2009.

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