

Feature Article

Parallel Digital Holography

— Three-Dimensional Image Measurement Technique for Moving Cells —

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The author invented and developed a technique capable of three-dimensional image measurement for moving objects. The technique has been called as parallel digital holography and carries out phase-shifting interferometry simultaneously using space-division multiplexing of the reference wave in digital holography. The space-division multiplexing is implemented by a phase-shifting device array. The validity of the technique was confirmed by a preliminary experiment. It was also confirmed that the microscopy using this technique is capable of three-dimensional image measurement of cells.

Introduction

Recently electronics and biotechnology have been drastically advanced. Advanced electronics enables us to process large-capacity information. Biotechnology enables us to discover new phenomenon and to invent new functional materials, in life science, pharmacy, and so on. It is important to achieve welfare and happiness in future human life by combining the two technologies and applying them to medicine. Also, noninvasive means are necessary for measurement of structure and function of living bodies, tissues and cells. Furthermore, image measurement is valuable for living specimens. Conventional optical microscopy, a confocal microscopy, an atomic force microscopy, an optical coherence tomography and so on are usually used to measure the living specimens as noninvasive imaging techniques. Since these techniques need sequential process or scanning to measure three-dimensional (3-D) information of specimen, they are useless for the measurement of dynamically moving specimen. On the other hand, holography^[1] can instantaneously record 3-D information of an object. Conventional holography records interference fringe image, which contains 3-D information of an object, generated by two laser beams on the holographic plate. The holographic plate, a high-resolution photographic plate, is chemically developed, and fixed in the same way as the development process of photographs. Because of the process, conventional holography is useless for 3-D image measurement for

moving object. In recent years, digital holography^[2-4] has been actively studied as a technique for 3-D image measurement of moving objects. This technique records the interference fringe image using an image sensor, and numerically reconstructs 3-D image of an object using a computer. The pixel pitch of an image sensor is, however, too small to resolve fine interference fringe. To record fine interference fringe using an image sensor, in-line digital holography^[3] has been frequently adopted for implementation of digital holography. However, in-line digital holography has such a serious problem that non-diffraction wave and the conjugate image, which are unwanted waves, are superimposed on the desired wave, which is the image of an object. Therefore, the accuracy and precision of measurement decrease. To solve the problem, phase-shifting digital holography was invented^[5]. The phase-shifting digital holography is a technique capable of extracting only the image of an object from the reconstructed image by the in-line digital holography. The technique requires several holograms of which the phases of the reference wave are sequentially changed. The technique is indeed capable of accurate and precise 3-D measurement, but is incapable of measuring a moving object. Then, the author invented parallel digital holography^[6-8] capable of accurate and precise 3-D measurement of dynamically moving objects.

Holography

Holography^[1] was invented as a technique for recording

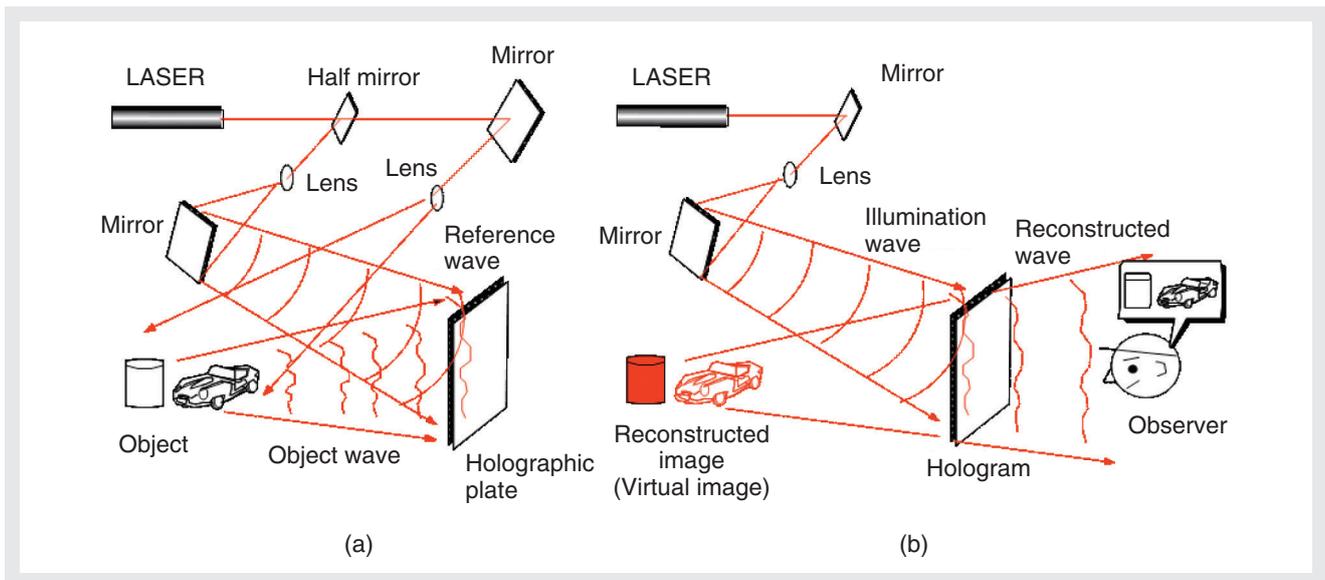


Figure 1 Schematics of holography. (a) Recording, (b) reconstruction.

and reconstructing 3-D image of an object. When light wave irradiates an object, the light wave is scattered from each point of the object. Then, the amplitude and phase of the light wave are modulated by the object, and the wavefront containing 3-D information of the object is generated. To record the wavefront, both amplitude and phase are needed to be recorded. However, a holographic plate and an image sensor can record only the light wave's intensity, which is the same as the amplitude. To record the phase, the phase information is converted into intensity information by using the reference wave and is recorded as form of interference fringe image. Figure 1 shows the schematic diagrams of the recording and reconstruction of hologram. A laser beam is divided into two beams. One beam irradiates the object and is scattered. The scattered wave is called as the object wave.

The other beam does not contain the information of the object and is called as the reference wave. The two waves irradiate the holographic plate, and then generate an interference fringe image on the plate. The medium that records the interference fringe image of an object is called hologram in general. After chemical development process of the plate, the plate is illuminated by the same laser beam as the reference wave, then the reconstructed image appears at the same position where the object was.

Digital Holography

Figure 2 shows a schematic diagram of the concept of digital holography^[2-4]. This technique records the interference fringe image using an image sensor such as CCD and CMOS, and numerically reconstructs 3-D

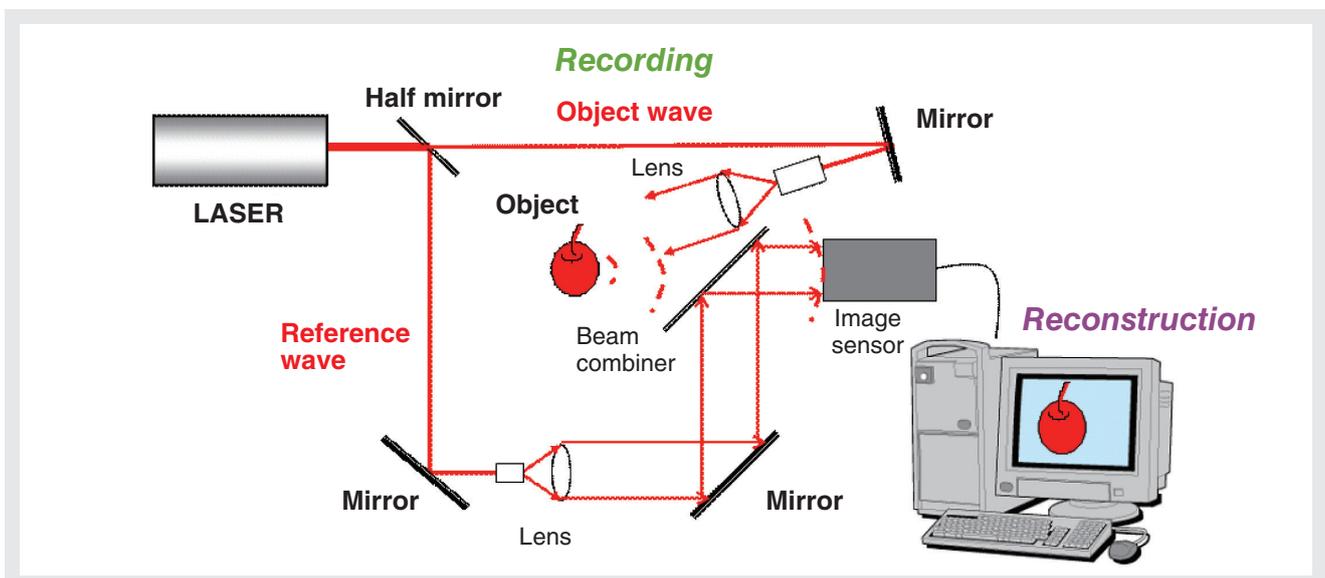


Figure 2 Schematic of digital holography.

image of object by calculating the light wave propagation from the interference fringe image using a computer. The technique has the following attractive features: a chemical process for developing recording media is not required; quantitative evaluation is easy for 3-D images of objects; and focused images of 3-D objects at desired depth can be instantaneously acquired without a mechanical focusing process; available wavelength of laser beam is not limited in comparison to the conventional holography that uses holographic plate.

In-line digital holography has been frequently adopted for implementation of digital holography. In this digital holography, the reference wave orthogonally irradiates an image sensor to enlarge the interval of interference fringe so as to record interference fringe by an image sensor. A Fresnel transform^[3] is generally applied to the hologram recorded on the image sensor to reconstruct the image. The in-line digital holography using the Fresnel transform^[3] alone is the simplest implementation and reconstructs the image of an object from a single hologram. Although this digital holography allows instantaneous measurement, the reconstructed image is inaccurate because both the conjugate image and the non-diffraction wave are superimposed on the image of the object. This is because the pixel size and pixel pitch of image sensors are too large to record fine interference fringes. To obtain only the image of an object, phase-shifting technique has been applied^[5]. The technique requires more than two holograms sequentially recorded using reference waves with different phase shifts. Usually, the phase of the reference wave is sequentially changed by using wave plates or a piezoelectric-transducer mirror. Although the phase-shifting method achieves true image of the object, it is useless for instantaneous measurement. To achieve a phase-shifting method that can perform instantaneous measurement, the author proposed a parallel digital holography.

Parallel Digital Holography

Parallel digital holography^[6-8] can reconstruct amplitude and phase distributions of only the object wave from a single hologram. Figure 3 schematically illustrates an example of the implementation of parallel digital holography and the reconstruction algorithm. This example shows the case that the number of the phase shifts is four. Figure 3(a) shows the principle of the parallel digital holography. As shown in Fig.3(b), a phase-shifting device array is placed in the reference wave of in the conventional in-line digital holography.

The array device is a segmented array with a 2×2-cell configuration which generates the periodical four-step phase distributions, 0, $\pi/2$, π , $3\pi/2$, required for the phase shifting. The array device can be implemented using a glass plate with a periodical four-step thickness. The array device is imaged onto the image sensor by an imaging system so that the phase distribution of the reference wave on the image sensor plane corresponds with the arrangement of pixels in the image sensor. The size of the imaged cell on the image sensor is the same as that of the pixel. Thus, the image sensor captures a hologram recorded with the reference wave containing the four-step phase distributions. Other configurations are possible to implement parallel digital holography. For example, phase-shifting array device can be attached on the image sensor by using polarization technique^[7].

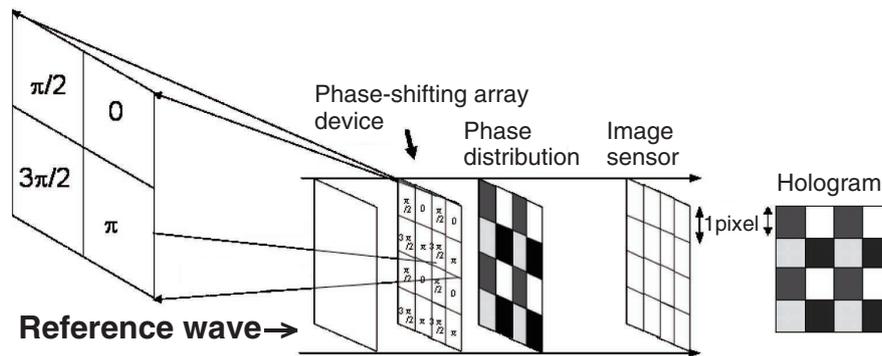
Figure 1(c) shows the processing procedure for reconstruction of the proposed technique. The pixels containing the same phase shift are extracted from the recorded hologram. For each phase shift, the extracted pixels are relocated in another 2-D image at the same addresses at which they were located before being extracted. The values of pixels not relocated in the 2-D image are interpolated using the adjacent pixel values. By carrying out this relocation and interpolation for the four phase shifts, four holograms, $I(0)$, $I(\pi/2)$, $I(\pi)$, $I(3\pi/2)$, are obtained. The complex amplitude of the object wave $U(X, Y)$ can be calculated by applying the same calculation in the conventional phase-shifting digital holography to the four holograms as follows.

$$U(X, Y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \left(\frac{\{I(0)-I(\pi)\} + i\{I(\pi/2)-I(3\pi/2)\}}{4} \right) \exp \left[\frac{2\pi i}{\lambda} \left(Z + \frac{(X-x)^2 + (Y-y)^2}{2Z} \right) \right] dx dy. \quad \dots\dots (1)$$

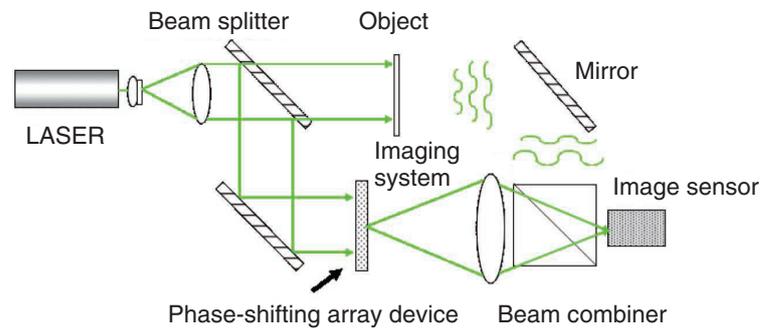
Here, i , λ and Z are the imaginary unit, the wavelength of laser beam, and the distance from the object to the image sensor, respectively.

Preliminary Experiment

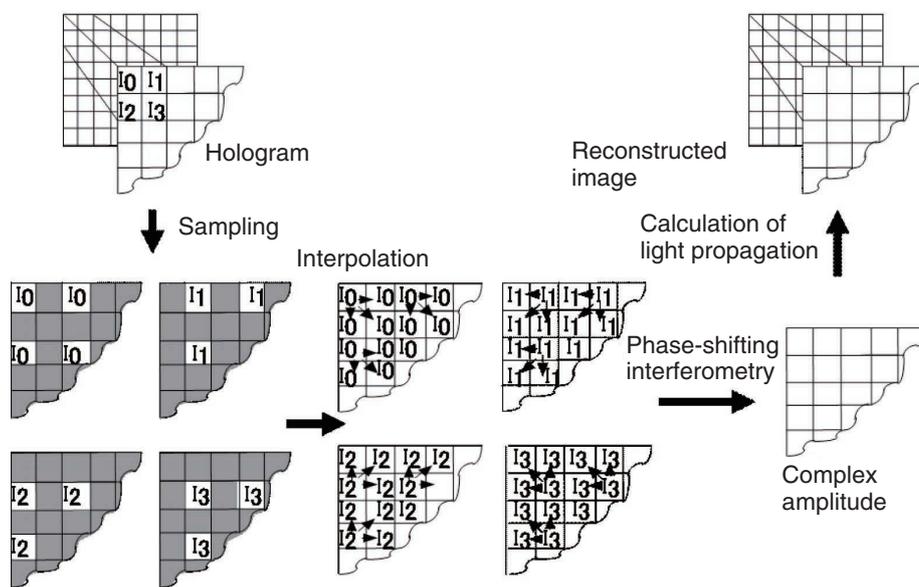
A preliminary experiment was conducted to verify the proposed technique. Since a phase-shifting array device is not commercially available, the author used an optical system employed in conventional phase-shifting digital holography consisting of a half-wave plate and a quarter-wave plate to equivalently produce the hologram to be obtained. A laser operating at 633 nm, serving as the



(a)



(b)



(c)

Figure 3 Schematics of the parallel digital holography. (a) Principle, (b) an example of optical implementation of parallel digital holography, (c) algorithm for reconstructing the image of object.

light source, and a CCD camera with 768(H)×493(V) pixels of pixel size 11 μm (H)×13 μm (V) were used. Four holograms, $I(\vartheta)$ ($\vartheta = 0, \pi/2, \pi, 3\pi/2$), were optically obtained in sequence using the half-wave plate and quarter-wave plate. Here, ϑ represents the phase shift of the reference wave.

For the hologram recorded using each phase-retardation, the pixels corresponding to the same phase retardation by the phase-shifting device were extracted at 1-pixel intervals. The extracted pixels for the four holograms were relocated in a single 2-D image at the same addresses at which they were located before being

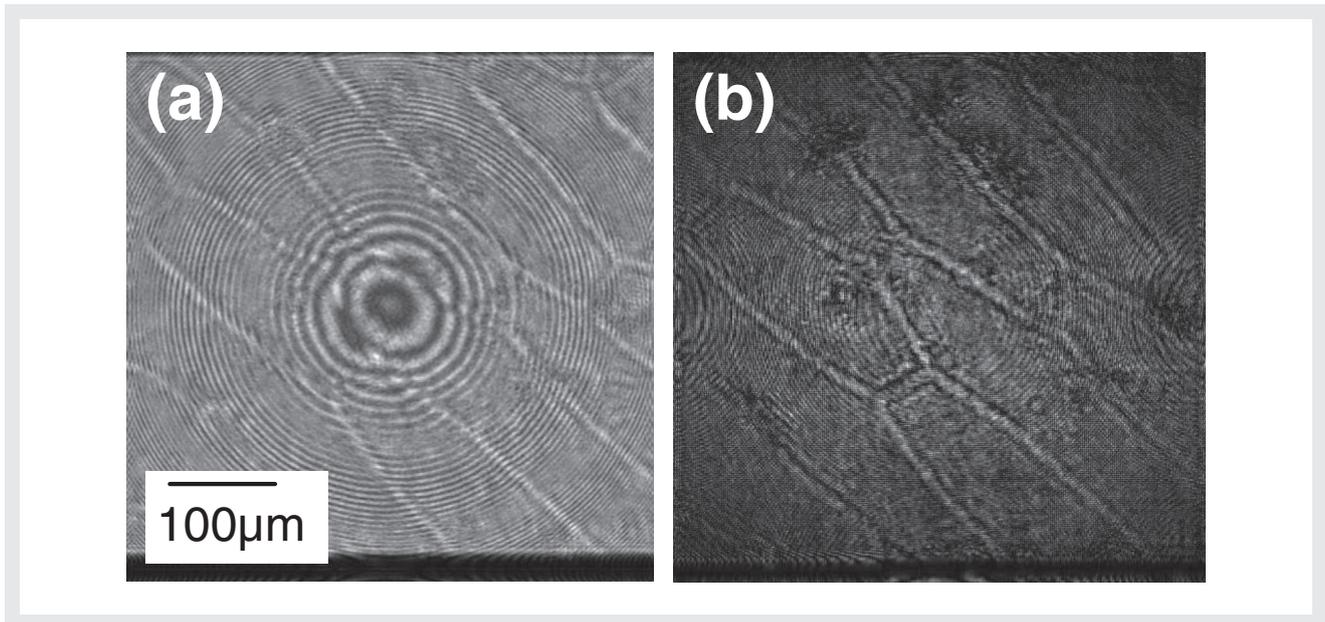


Figure 4 Reconstructed images in the preliminary experiment. The objects were cells of onion pear. (a) Fresnel transform alone technique, (b) parallel digital holography. Each image consists of 512×512 pixels.

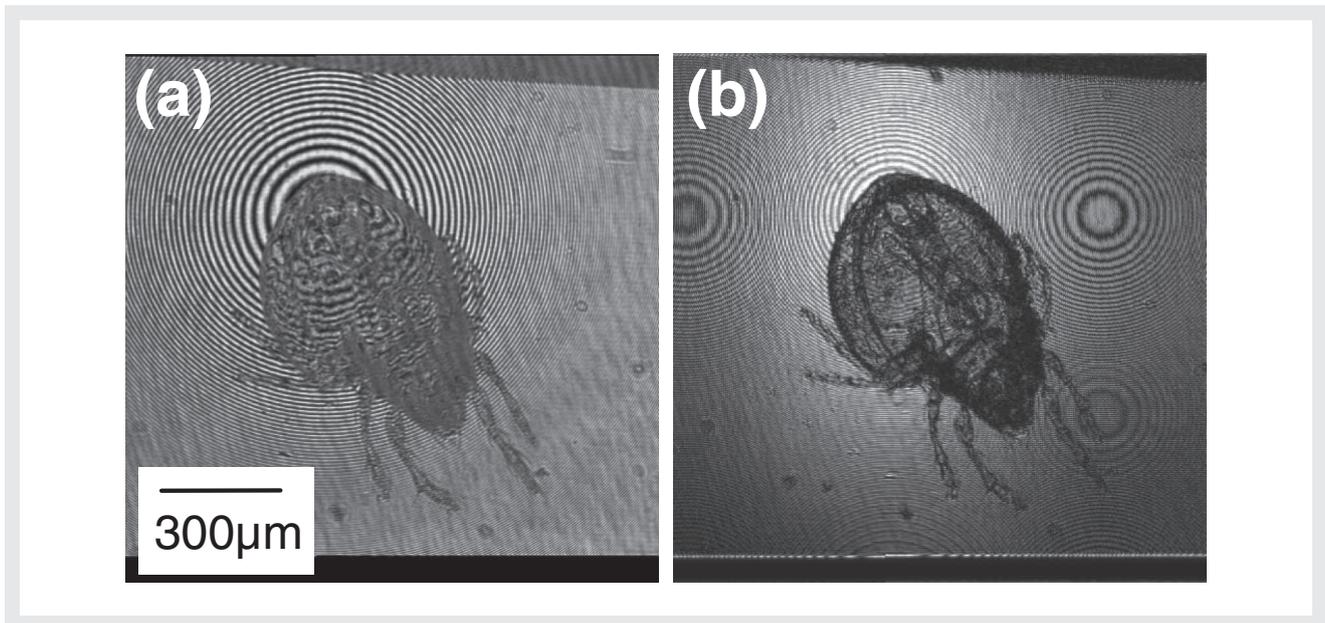


Figure 5 Reconstructed images in the preliminary experiment. The object was a water flea. (a) Fresnel transform alone technique, (b) parallel digital holography. Each image consists of 512×512 pixels.

extracted. Thus the hologram to be recorded using the array device was equivalently generated.

The reconstructed images by the conventional single-shot in-line digital holography using Fresnel transform alone and the parallel digital holography are shown in Figs. 4(a) and (b). Cells of onion skin were chosen as an object. When a water-flea was used as an object, the reconstructed images obtained by the Fresnel transform alone technique and the parallel digital holography are shown in Figs. 5(a) and (b), respectively. Internal

structure of the object has been clearly seen only from Fig. 5(b). Both experimental results show that the parallel digital holography reconstructs the object much more accurately than the Fresnel transform alone technique.

Conclusion

Parallel digital holography has been introduced as a technique capable of noiseless instantaneous measurement of 3-D objects. The results of a preliminary experiment have shown the validity of the proposed technique. The technique will contribute to the measurement of dynamically moving 3-D objects, such as those of interest in biology, structure of a living cell, spatial distribution of cells, flow inside a cell.

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