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Express Paper

3D Measurement for Red Blood Cells Using Digital Holography

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Abstract: In these years, for medical analysis, many 3D microscopes have been developed. In the existing 3D microscopes, confocal laser scanning microscope (CLSM) is used mostly. 3D shape of biological tissues can be measured with high-resolution. However scanning, which is necessary during measurement, takes time consuming. So it is difficult to measurement living biological tissues, such as the red blood cells. On the other hand, measurement accuracy of the CLSM is about 1 [μm], so 3D measurement using the CLSM is not high accuracy. To resolve problems in the CLSM, we propose a 3D measurement method using single-shot phase-shift digital holography. Using the proposed method, we also developed a 3D microscopy for 3D measurement of red blood cells with 5.5 [nm] measurement accuracy and 80 [ms] measurement time. This paper describes the detail of the proposed 3D measurement method and the 3D microscopy.

Keywords: digital holography, optical microscopy system, 3D measurement

1. Introduction

In these years, for medical analysis, such as biopsy, demand of 3D microscope is arising. A typical existing 3D microscope is confocal laser scanning microscope (CLSM). Using CLSM, 3D shape of biological tissues can be measured with high-resolution. However, it is necessary to scan the object during measurement, so CLSM is time consuming. Because of the above-mentioned, CLSM cannot be used for 3D measurement of dynamic living tissues. To resolve this problem, 3D microscope using single-shot phase-shift digital holography is proposed in this paper. In proposed 3D microscope, Michelson interferometer and general optical components are used, so reduction in system size and low price can be realized. Simultaneously, using single-shot phase-shift digital holography, 3D measurement can be realized with nano-order measurement accuracy and real-time.

In conventional phase-shift digital holography, at first, for generating four phase-shift interference fringes, optical path difference between reference light wave and object light wave is shifted 1/4 times. Second, using four phase-shift interference fringes captured by CMOS camera, relative phase of object light wave with reference to the absolute phase of reference light wave can be calculated by phase-shift method. Furthermore, using Fresnel's transform, object light wave can be reconstructed by a computer, absolute phase of object light wave can also be calculated from the reconstructed object light wave. Finally, from absolute phase of object light wave, 3D shape of object can be reconstructed by interferometry. However, it is necessary to generate and capture four phase-shift interference fringes during measurement, so the conventional phase-shift digital holography is time consuming. Also, because of vibration of measurement environment, it is hard to capture four phase-shift interference fringes correctly, so that measurement fails often. As the above mentioned, 3D measurement system using the conventional phase-shift digital holography is non-high-speed and unstable. For resolving this problem, it is considered that the single-shot phase-shift is necessary.

In this paper, for realizing a high-speed and stable 3D measurement method, single-shot phase-shift digital holography using Fourier transform method of interference fringe analysis [1] is proposed. In conventional single-shot phase-shift digital holography, off-axis digital holography and parallel phase-shift digital holography are used mostly. In off-axis digital holography, by shifting optical axis of reference light wave and optical axis of object light wave, real component required for measurement can be separated from virtual and DC components. Absolute phase of object light wave can be calculated by the digital holography and 3D shape of object can be reconstructed by the interferometry. However, only the real component is required for 3D measurement, so only 1/3 information captured by CMOS camera can be used. In parallel phase-shift digital holography, using an image sensor on which micro polarizers are attached pixel by pixel, the relative phase of object light wave can be calculated from single interference fringe image. However, the micro polarizers attached on image sensor pixel by pixel are about several million yen expensive, so cost reduction of 3D measurement system is difficult to realize. In proposed single-shot phase-shift digital holography, by applying to phase-shift digital holography the Fourier transform method of interference fringe analysis, which has been applied to interferometry, relative phase of object light wave can be

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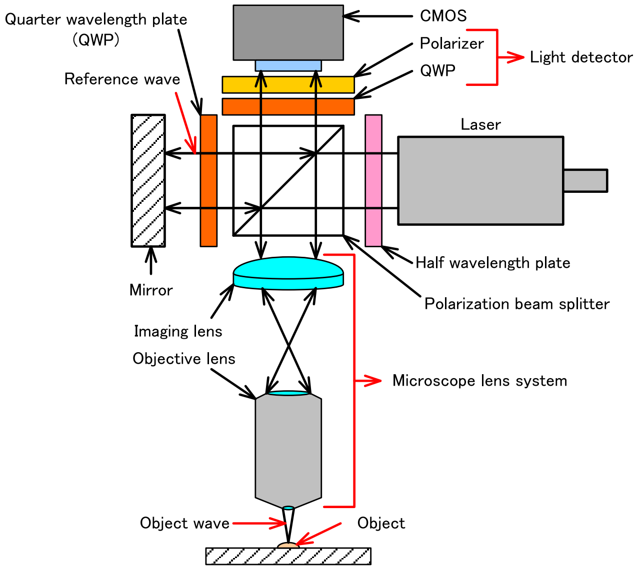

Fig. 1 Structure of proposed 3D microscope.

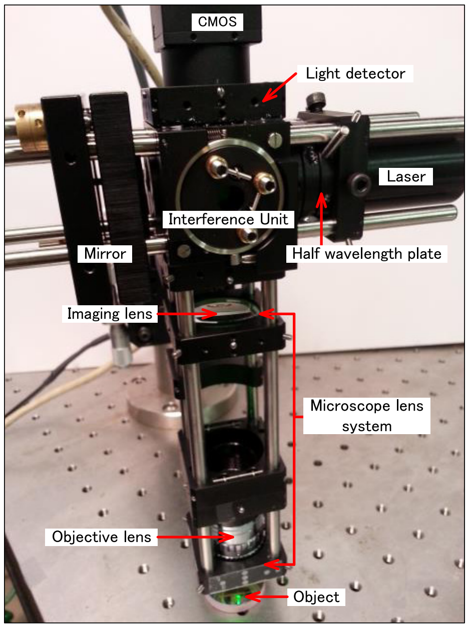

Fig. 2 Appearance of proposed 3D microscope.

calculated from single interference fringe image, and the all information captured by CMOS camera can be used. Simultaneously, general optical components are used in proposed system, so the cost reduction of 3D measurement system can be realized easily. Furthermore, in order to achieve optical resolution of microscope level, lens system constructed by an imaging lens and an objective lens is considered and implemented in proposed system, so that 3D measurement for dynamic living tissues, such as living cells, is possible. In this paper, structure of proposed 3D microscope and proposed measurement method will be explained. Further, by 3D measurement experiment on human red blood cells, it is verified that the proposed 3D microscope can be applied to precision medical analysis in the future.

2. Proposed System

Structure and appearance of proposed system are shown in **Figs. 1** and **2**. In order to increase laser irradiation range, laser beam is expanded by beam expander. The expanded laser beam passes through the half wavelength plate and irradiates the polarization beam splitter. By rotating the half wavelength plate, intensity of object and reference light waves can be adjusted. Laser beam is split into two beams having different polarization plane by the polarization beam splitter. The P-polarized laser beam is emitted toward the mirror, respectively the S-polarized laser beam is emitted toward the object. Thereafter, the S-polarized and P-polarized laser beams become two circularly polarized beams having difference directions of optical rotation after passing through the quarter wavelength plate, and irradiate the mirror and object. After laser irradiating, circularly polarized object light wave reflected from surface of object becomes P-polarized light wave after passing through the quarter wavelength plate, and irradiates the polarization beam splitter. On the other hand, circularly polarized reference light wave reflected from mirror becomes S-polarized light wave after passing through the quarter wavelength plate, and is reflected by the polarization beam splitter. Here, object light wave and reference light wave propagate toward CMOS sensor together, but since polarizations are orthogonal, at output of the polarization beam splitter, interference phenomenon does not occur. Thereafter, the object light wave and reference light wave having orthogonal polarizations become two circularly polarized light waves having difference directions of optical rotation after passing through the quarter wavelength plate of light detector. Because only linearly polarized component of light wave predetermined by the polarization plate can pass through the light detector, interference of reference light wave and object light wave occurs on surface of CMOS sensor, and can be captured and stored as an interference fringe image.

In microscope lens system, an achromatic doublets lens with 80 [mm] focal length, and a 50X objective lens are used. According to this structure of microscope lens system, 3D measurement range is set to 500×500 [μm], resolution of interference image is 0.17×0.17 [μm]. According to the appearance, size of proposed system is 480×240 [mm].

3. Proposed Method

In this paper, a new single-shot phase-shift digital holography 3D measurement method is proposed by using Fourier transform method of interference fringe which was used in interferometry.

3.1 Problem of the Conventional Method

In conventional phase-shift digital holography, four phase-shift interference fringes are necessary to generate and capture for 3D measurement. Because the generation and capturing of four phase-shift interference fringes is time consuming, so the 3D measurement using the conventional method is difficult to realize the real-time measurement. Furthermore, as shown in **Fig. 3**, because of vibration of measurement environment, four phase-shift interference fringes always cannot be captured correctly. In Fig. 3, because of vibration of measurement environment, phase difference of four phase-shift interference fringes is not 90-degree, so that phase of a flat mirror is a curve, not a straight line when phase calculated correctly.

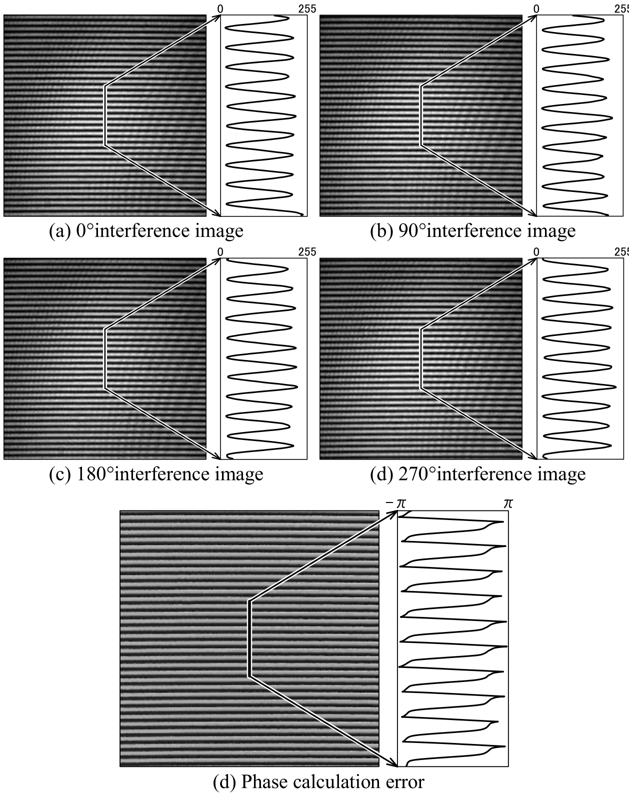

Fig. 3 Phase calculation error (object is a flat mirror).

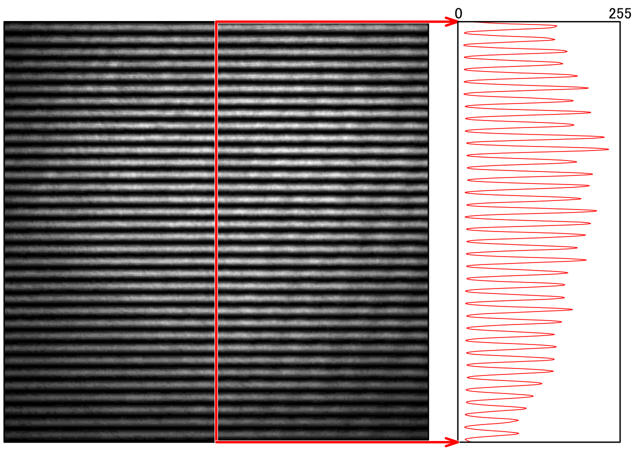

Fig. 4 Single interference fringe of a flat mirror.

3.2 Fourier Transform Method

For realizing a real-time and stable 3D measurement using phase-shift digital holography, Fourier transform method of interference fringe analysis is used for calculating relative phase of object light wave, which is calculated by phase-shift method in the conventional method. According to interference theory, interference fringe captured by CMOS camera is sine wave shown in **Fig. 4**. Because negative, positive and zero phase components exist in single sine wave, in digital holography, virtual, real and DC terms exist in single interference fringe image. For measuring 3D shape of object, the relative phase of object light wave can be calculated only from the real term, so the virtual and DC terms must be removed as noise. Using Fourier transform method, the real term can be extracted easily by band pass filter in frequency domain as shown **Fig. 5**. In Fig. 5, the interference fringe data in

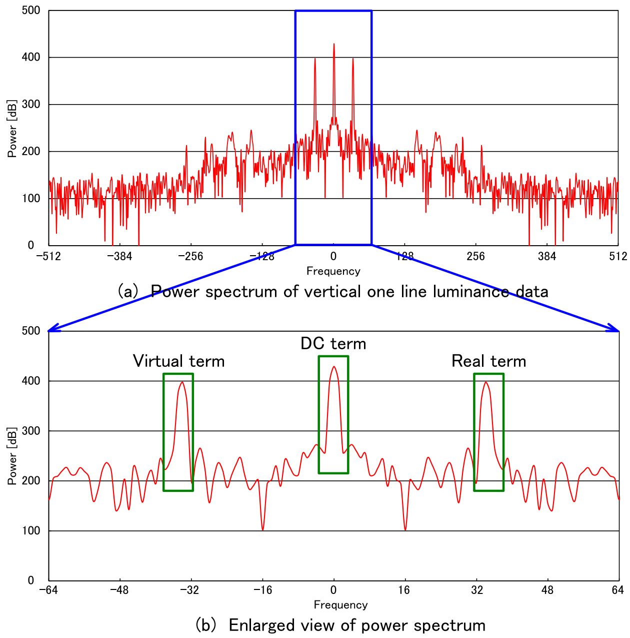

Fig. 5 Power spectrum of luminance data in vertical single line.

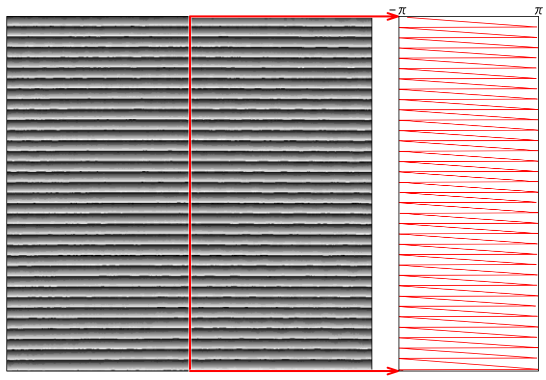

Fig. 6 Result of Fourier transform method.

vertical one line can be transformed to frequency domain. Using band pass filter, only the real term can be left. The relative phase of object light wave can be calculated using Eq.(1) after inverse Fourier transform.

$$\Delta\varphi(x, y) = \tan^{-1}\left\{\frac{\mathrm{Im}[I'(x, y)]}{\mathrm{Re}[I'(x, y)]}\right\}, \tag{1}$$

Here, $I'(x, y)$ is result of inverse Fourier transform of real term extracted by band pass filter. (x, y) are coordinates of image, Im and Re are imaginary and real part of $I'(x, y)$. $\Delta\varphi(x, y)$ is relative phase of object light wave. Result of phase calculation using Fourier transform method is shown in **Fig. 6**.

3.3 Single-shot Phase-shift Digital Holography

Using Fourier transform method of interference fringe analysis, relative phase of object light wave can be calculated, so the complex amplitude of object light wave with relative phase can be described as in

$$E_O(x, y) = \exp\{i\Delta\varphi(x, y)\}, \tag{2}$$

$E_O(x, y)$ is the complex amplitude of object light wave with relative phase, $\Delta\varphi(x, y)$ is relative phase of object light wave calculated by Fourier transform method of interference fringe analysis.

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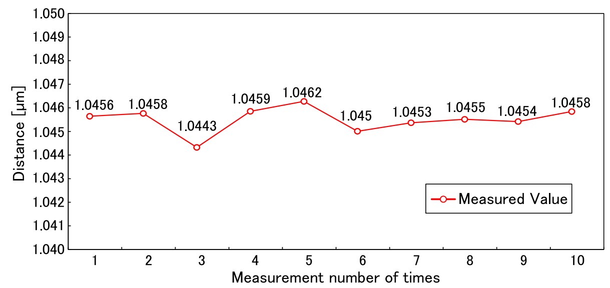


Fig. 7 Result of measurement accuracy examination.

$$
\begin{aligned}
\Gamma(\xi,\eta) &= \Im^{-1}\left\{\frac{\Im\{E_O(x,y)\}}{\Im\{g(\xi,\eta,x,y)\}}\right\},\\
\varphi(\xi,\eta) &= \tan^{-1}\left\{\frac{\mathrm{Im}[\Gamma(\xi,\eta)]}{\mathrm{Re}[\Gamma(\xi,\eta)]}\right\},\\
g(\xi,\eta,x,y) &= \frac{i\exp\left(-i\dfrac{2\pi}{\lambda}\rho\right)}{\lambda\rho},\\
\rho &= \sqrt{(x-\xi)^2+(y-\eta)^2+d^2}.
\end{aligned}
\tag{3}
$$

$\Im$ is Fourier transform. $\Im^{-1}$ is inverse Fourier transform. Im and Re are imaginary part and real part of the complex amplitude respectively. $\varphi(\xi,\eta)$ is absolute phase of object light wave.

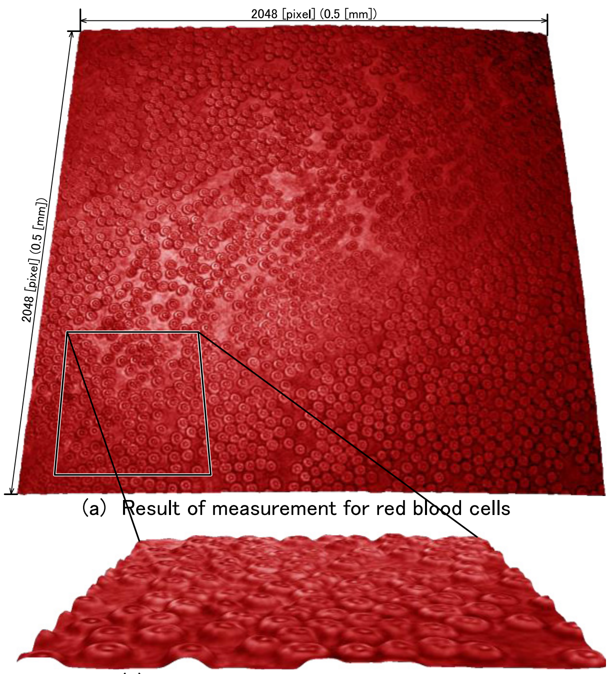
After phase unwrapping process [2], the 3D shape of object can be calculated by interferometry [3] from unwrapped absolute phase of object light wave.

4. Measurement Accuracy

In order to evaluate measurement accuracy, 1 [μm] step place on the step master (Mitsutoyo NO. 616-498) is measured by 10 times. Average error between the measurement value and the true value (1.04 [μm]) is calculated as result of measurement accuracy evaluation shown as **Fig. 7**. According to the result of measurement accuracy evaluation, It is can be confirmed that the measurement accuracy is 5.5 [nm]. Simultaneously, time of measurement process is 80 [ms] when CPU Intel core i7 3.20 [GHz], GPU NVIDIA Tesla C2050, RAM 18 [GB] and Windows7 64-bit Operating system are used.

5. 3D Measurement of Red Blood Cells

Using proposed system, living human red blood cells are measured. Result of 3D measurement is shown in **Fig. 8**. According to this result, several thousand red blood cells can be measured by real-time and nano-order accuracy. Simultaneously, average volume of all cells can be calculated from measurement result, so variation of cells' average volume can be calculated by measuring an hour shown as **Figs. 9–12**. In Figs. 9–11, because water environment is changed in an hour, vibratory pressure of red cells is changed, so 3D shapes of red cells are changed from expansion to contraction. The 3D shape of hypotonic red blood cell is shown in Fig. 9, 3D shape of isotonic red blood cell is shown in Fig. 10, 3D shape of hypertonic red blood cell is shown in Fig. 11. The variation of cells' average volume is shown in Fig. 12. Because the average volume of red blood cells is a standard of human blood test, so it can be considered that proposed 3D microscope can be applied to precision medical analysis in the future.



(a) Result of measurement for red blood cells

(b) Enlarged image of measurement result

Fig. 8 3D measurement result of red blood cells.

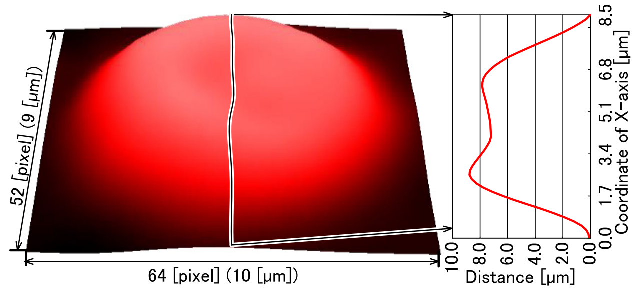


Fig. 9 3D shape of hypotonic cell.

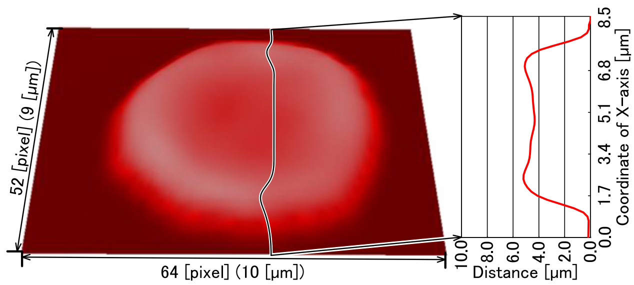


Fig. 10 3D shape of isotonic cell.

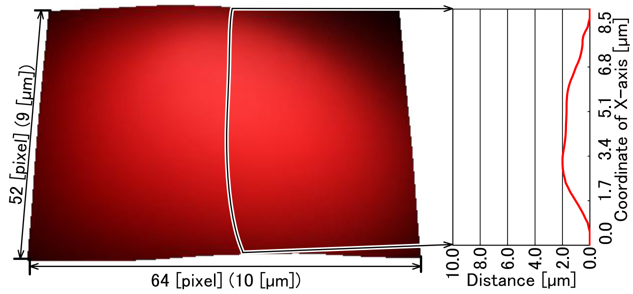


Fig. 11 3D shape of hypertonic cell.

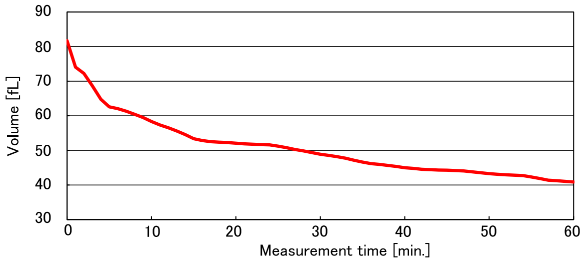


Fig. 12 Change of red blood cells' volume in an hour.

6. 3D Measurement of Red Blood Cells

In this paper, a 3D microscope using single-shot phase-shift digital holography was proposed. In proposed system, the measurement range is $500 \times 500\,[\mu\text{m}]$, measurement accuracy is 5.5 [nm], measurement processing time is 80 [ms]. According to measure experiment, 3D shapes of red blood cells can be measured with nano-order accuracy and in real-time. So it can be concluded that proposed 3D digital holography microscope can be used in precision medical analysis.

References

[1] Takeda, M., Ina, H. and Kobayashi, S.: Fourier Transform method of fringe-pattern analysis for computer-based topography and interferometry, *J. Opt. Soc. Am*, 72, (1982).

[2] Barseghyan, Y. and Sarukhanyan, H.: Laplacian Based LF Quality Map for Phase Reconstruction, *CSIT*, *Pattern Recognition and Image Processing*, pp.332–335 (2009).

[3] Kronrod, M.A., Merzlyakov, N.S. and Yaroslavski, L.P.: Reconstruction of holograms with a computer, *Sov Phys-Tech USA*, Vol.17, No.2, pp.434–444 (1972).

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