A Common-Path Phase-Shifting Interference-Microscope Using RGB Lasers

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ABSTRACT

We present a near-common-path phase-shifting interference-microscope using RGB lasers for quantitative measurement of living biological cells. In this system, a Wollaston prism is used as both a beam splitter and a phase shifter. Since the object and reference waves pass through almost the same path, the developed system has an extremely high stability. By using RGB lasers as a light source, the sample can be measured at different wavelengths not only one by one but also at the same time. Experimental results for evaluating the system error and for measuring an onion cell are shown.

1. INTRODUCTION

Recently, quantitative measurement of living biological cells is becoming more and more important in biology and medical diagnosis[1]. However, conventional optical transmittance microscopes do not work for a transparent biological cell without a staining procedure that may change the property of the sample. Even a phase-difference optical microscope does not provide quantitative information about the samples. On the other hand, phase-shifting interferometry (PSI) [2] proved to be a very powerful tool for extracting the phase information from a series of interferograms with phase shifts introduced between the object and reference waves. Various type of interference microscope [3, 4] using PSI have been proposed. However, interference microscopes based on Mach-Zehnder or Michelson type interferometers are extremely sensitive to vibration and air turbulence. Moreover all of those systems use a single laser as light source, the color information loses.

In order to make both quantitative measure and microscopic observation with white light illumination possible, we have developed a common-path phase-shifting interference microscope using RGB lasers as quasi-white light source[5~7]. In this system, a Wollaston-prism (WP) located between the objective lens and an imaging lens is used as both a beam splitter and a phase shifter. The WP is laterally moved by a piezoelectric transducer (PZT) to introduce a relative phase shift between the object and reference waves. This interference microscope enables us to measure a living biological cell at RGB wavelength.

2. OPTICAL SYSTEM AND THEORY

2.1 Optical system

A schematic configuration of optical system is shown in Fig.1. The optical system consists of a transmission optical microscope with a WP (CaCO3, separation angle 2°). RGB lasers lasing at 671nm, 532nm, and 453nm wavelengths are used as the light source. The output of the lasers is coupled to optical fibers, and the outputs of these fibers are then combined using a multiplexer to form a quasi-white light source. A polarizer is used to obtain a linearly polarization at 45°. This light beam is then used to illuminate the sample placed in half of the incident beam. The sample is imaged using a microscopic objective lens (Olympus, 20X, NA0.45). The WP splits the incident beam into two orthogonally polarized components. These two components are then interfere with each other after passing through an analyzer. By adjusting the sample position, the wavefront that modulated by the sample can be arranged to overlap with the wavefront unaffected by the sample. The interference fringe pattern is then detected with a C-MOS color camera (Mako G-507C, allied Vision), and then fed to a computer. The WP is mounted on a PZT stage controlled with a computer through GPIB interface. In this way, the WP can be moved in a direction perpendicular to the optical axis to introduce the phase shifts required in phase extraction procedure.

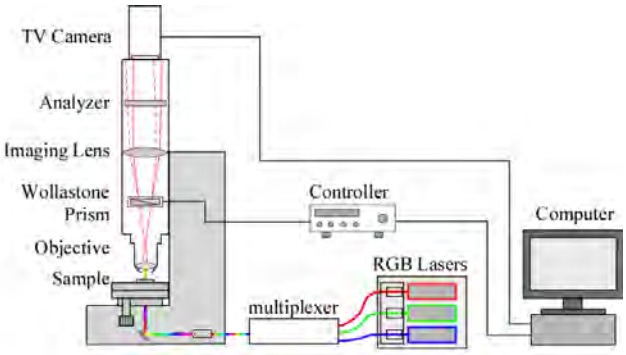

Fig.1. Schematic of the optical system

2.2 The theory of phase measurement

The phase shifting interferometry is used for phase measurements. The intensity of the detected interference fringe pattern is given by

$$I(x,y) = a(x,y) + b(x,y)\cos\left[2\pi f_0 x + \varphi(x,y) + \delta\right]. \quad (1)$$

Where $a(x,y)$ and $b(x,y)$ are bias and modulation, and $2\pi f_0 x$ is tilt wavefront between the object and reference waves. $\varphi(x,y)$ denotes the phase under the measurement. For a sample of thickness t and refractive index n(x,y), $\varphi(x,y)$ is given by

$$\varphi(x,y) = \frac{2\pi}{\lambda} t\left[n(x,y) - n_r\right]. \quad (2)$$

In Eq. (1), δ is phase shift introduced by a small movement of the prism. The phase shift introduced by moving WP by Δy is given by

$$\delta \cong \frac{4\pi}{\lambda}(n_0 - n_e)\tan\alpha \cdot \Delta y. \quad (3)$$

An amount of 15.10 μm movement of the prism corresponds to a 2π phase shift for light of 532nm. Four interference fringe patterns with every $\pi/2$ phase shift were captured. These four interferograms were used to calculate the phase distribution using a 4-step phase extraction algorithm[1]

$$2\pi fx + \varphi(x,y) = \tan^{-1}\left(\frac{I_4 - I_2}{I_1 - I_3}\right) \quad \mathrm{mod}\, 2\pi \,. \qquad (4)$$

The tilt wavefront can then be removed by using least-squares method and a subtraction procedure. For measure the phase distribution at different wavelengths at the same time, a generalized phase extraction algorithm[1] was used.

3. EXPERIMENTS AND RESULTS

Several experiments were carried out to demonstrate the performance of the developed system.

3.1 Linearity of phase measurement and system error

To check the linearity of the proposed system, the tilt wavefront were measured. The results for the red laser is shown in Fig. 2, where line profiles of the tilt wavefront and the phase noise were plotted against pixel across the horizontal direction. Since the object and reference beams pass through the almost the same path, the developed system can measure the phase with a very extremely stability. Therefore, the system error can be removed by subtraction of the background phase error. After such a procedure, the rms phase error was evaluated as small as λ/1000.

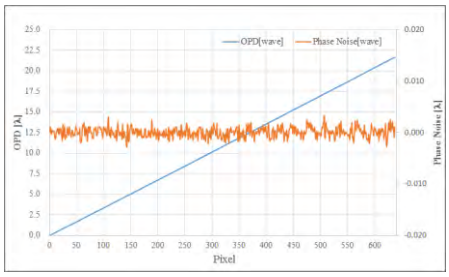

Fig. 2 Experimental results for evaluating the linearity and the residual noise of phase measurements

3.2 Measurement of biological cells

To demonstrate the ability of the developed system for quantitatively measuring the small phase objects such as a living biological cell, an onion-skin cell was measured. To prepare the specimen, a small piece of epidermis was peeled from an onion, and then was put on a slide glass.

For the phase measurement, 4 color interference fringe patterns were taken with every $\pi/2$ phase shift. The obtained experimental results are shown in Fig.3. Fig. 3(a) is an RGB interference pattern. (b) extracted green channel image, (c) masked interferogram, (d) 3D plot of the measured phase distribution. The phase distribution can be easily transformed to the refractive index distribution.

The developed system can also be used to measure a biological sample at red, green, blue laser wavelength at the same time. Four color interferograms with phase shifts were taken with the color camera, and then separated to RGB channels. Since the phase shift has different value for each wavelength, a phase shift estimation method was developed and a generalized phase extraction algorithm was then used to used. The experimental results for measuring an onion cell at RGB wavelengths are shown in Fig. 4.

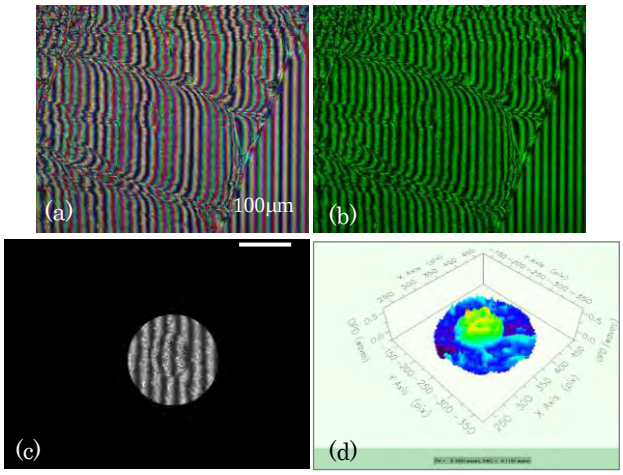

Fig. 3 Experimental results for measuring the nucleus of an onion skin cell: (a)a color interferogram, (b) green-channel image, (c) masked interferogram, (d) 3D plot of the measured phase

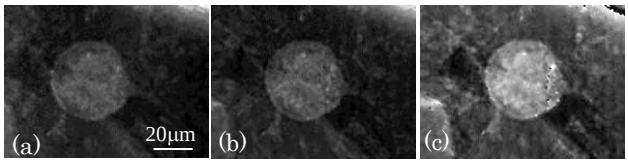

Fig. 4 Experimental results of simultaneously measuring an onion living cell: (a)~(c) show the phase image at red, green, and blue laser respectively.

4. Conclusions

A common path phase shifting interference microscope using RGB lasers is presented. Since the object and reference beams pass through almost the same path, this system is insensitive to vibration and air turbulence, and the rms phase error is as small as λ/1000 . This method enables us to quantitatively measure the phase distribution of transparent objects such as a biological cell at RGB laser wavelengths. The usefulness of the developed system has been demonstrated by measuring the index distribution of living cell. This system may be used for cancer cell identification.

5. Acknowledgment

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6. REFERENCES

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