Multi-modal Digital Holographic Microscope with Dual-wavelength Excitation and Dual-wavelength Phase Imaging

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1. Introduction

As the development of biomedical researches, microscopic tools with enhanced contrast and information from multiple physical perspectives will be critical. Multi-modal digital holographic microscopy combines epifluorescence microscopy and digital holographic microscopy, and it enables simultaneous detection from a single biological sample. This paper presents utilizing multi wavelengths of fluorescent excitation and hologram recording. This is realized by using a pair of 473 nm and 532 nm lasers as excitation light, and another pair of 632.8 nm and 830 nm as hologram formation. Using two or more kinds of excitation light would increase the number of visible colors, thus multiple marking would be possible. On the other hand, measured phase value is limited to range of $[-\pi, \pi]$ without special phase unwrapping treatment. Using different wavelengths will solve this problem by increasing measurable range. Experiments using plant cells are demonstrated the dual-wavelength excitation and dual-wavelength phase imaging. Quality of phase image affected by diffraction and absorption of intracellular components is also discussed.

2. Multi-modal digital holographic microscopy with dual-wavelength Excitation and Dual-wavelength Phase Imaging

Fig. 1 is a schematic of suggested microscopic system. We use transmittance phase imaging system on the right hand and the epifluorescence microscopy on the left side.

Each laser source has shutter controller and they are synchronized with their own side of recording devices. On the fluorescent side, a blue laser and a green laser are used as a dual-excitation module; on the phase side, a red laser and a near IR laser are used as a dual-phase-imaging module. Recording device 2 records interference patterns that are addition of each individual hologram. Holograms formed by different wavelengths can be separated in Fourier domain.

2. Experimental results

Fig. 2 shows preliminary experimental results using simple micro-beads. From Figs. 2(a) and (b), beads with different fluorescent properties with correspondent wavelengths were identified. The two images also imply that the each bead is placed at different depths. Fig. 2(c) is phase image that is numerically focused on a lower right cornered bead. If apply appropriate reconstruction distances, the other two beads can be recovered. In the dual-phase imaging, recovered phase value is varied by its wavelengths. According to the relationship, we can calculate actual unwrapped phase from a much bigger range. The effect of absorption and diffraction with each wavelength could also be studied by the proposed system.

Fig. 2 Images of micro-beads: (a) Fluorescence excited by 473 nm laser beam, (b) Fluorescence excited by 532 nm laser beam, (c) Phase image of three beads, image is focused on the lower-right bead.

3. Conclusions

Multi-modal digital holographic microscopy with dual-wavelength excitation and dual-wavelength phase imaging is proposed. Preliminary experiments have demonstrated validity of the system. Further discussion on the effect of live plant cell imaging with different wavelengths will be presented.

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