High Contrast Digital Holographic Microscopy by use of Femtosecond Pulse Light

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1. Introduction

The study of coherent noise suppression in digital holographic microscopy (DHM) is an important issue in fields of both engineering and biological science [1]. In usual DHM, optical sources with long coherent lengths such as He-Ne laser has been widely used to feature the sample. Because of the high degree of coherence of the He-Ne laser light, harmful coherent noise often arises [2]. This noise affects on the quality of the holograms and hence leads to error in phase measurement. Optical sources with short coherent lengths such as LEDs were proposed in order to avoid the harmful coherent noise [3]. However, the limited coherence length of LED and its insufficient brightness hinders its application in an off-axis DHM. In this paper, we present an off-axis DHM configuration using femtosecond pulse light with ultrashort coherent length.

The experiment is conducted to feature a biological sample. A typical configuration to place a fresh, sliced biological specimen in DHM is shown in Fig. 1(a), where the specimen is sandwiched between two thin glass plates to avoid dehydration. Investigating such biological samples using conventional DHM with long coherent He-Ne laser light is challenging because of existence of the harmful coherent noise (speckle and spurious). Photograph of the investigated sample taken by phase contrast microscope is shown in Fig.1(b).

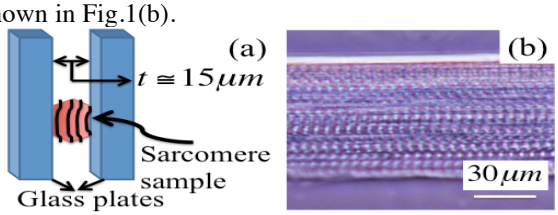

Fig. 1. (a) Sliced biological specimen mounted in between two thin glass plates to avoid dehydration, and (b) photograph of the investigated sample taken by phase contrast microscope.

2. Experiment

A mode-locked Er-doped fiber laser light (center wavelength λ_c = 1550 nm, spectral bandwidth $\Delta\lambda$ = 73 nm, pulse duration $\Delta\tau$ =100 fs, mean power P_{mean} = 380 mW, and repetition frequency f_{rep} = 250 MHz) was focused on a periodically-poled-lithium-niobate (PPLN) crystal to convert the wavelength by second harmonic generation (SHG) into the operating wavelength region of a charged coupled device (CCD) camera used. The extracted SHG light has λ_c of 777.8 nm, $\Delta\lambda$ of 10 nm, $\Delta\tau$ of 120 fs, and P_{mean} of 14 mW. The output power was sufficient to illuminate the sample and produce off-axis holograms with high contrast in the entire field of the CCD camera. The off-axis hologram was transferred via a microscope lens MO3 (20x, NA=0.1) to a black and white CCD camera (640 pixel by 480 pixel, pixel size = 4.3 μm).

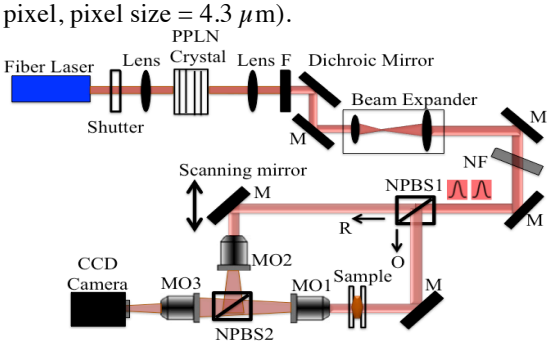

Fig. 2. Experimental setup. PPLN, periodically poled lithium niobate crystal; F, band-bass filter at 775nm; M, mirror; NF, neutral density filter; NPBS1 and NPBS2, nonpolarizing beam splitters; MO1, MO2, and MO3, microscope objectives with (50x, NA = 0.45), (50x, NA = 0.45), and (20x, NA =0.1), respectively.

Figures 3 shows 3D pseudocolor reconstructed phase-contrast images of the sarcomere sample with (a) He-Ne laser light and (b) femtosecond pulse light, respectively.

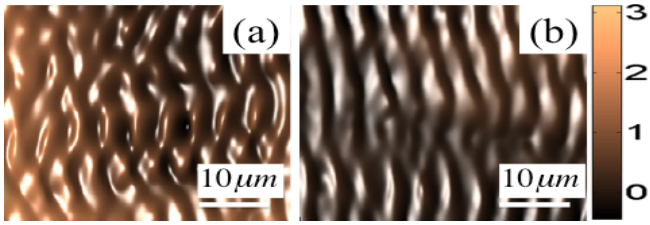

Fig. 3. 3D pseudocolor reconstructed phase-contrast image of stripy sarcomere with (a) He-Ne laser light, and (b) femtosecond pulse light.

3. Conclusions

The experimental results show that the contrast of the phase-image of the proposed technique is improved 2 to 3 times better than a He-Ne laser-based result.

References

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