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Three-dimensional mapping of fluorescence point sources using self-interference digital holography with space-division matching method

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Fluorescence microscope is a well-known important tool in biology for visualizing the locations of biological molecules and fluorescence nanomaterials. Chemical reactions in life processes are localized in three-dimensions in contrast to those that take place in test tubes. Therefore, to understand the spatiotemporal dynamics of biological chemical reactions, the three-dimensional (3D) mapping of points where the fluorescence originates is indispensable for analyzing biological phenomena. A self-interference digital holography can obtain the 3D fluorescence image with a small number of images without a laser scanning and a sample movement. The 3D mapping of one or several fluorescence point sources was performed [Opt. Lett. **40**, 3312 (2015).].

In this paper, we demonstrate the 3D mapping of many fluorescence point sources using the space division matching method.

Figure 1 shows the experimental setup composed of a fluorescence digital holographic microscope and optical tweezers. In the fluorescence digital holographic microscope, excitation light was irradiated to a sample from an ultraviolet light emitting diode (UV-LED). The fluorescence was given to self-interferometer composed of two concave mirrors with the focal lengths of 400 and 500 mm for the axial shearing and the interference image was imaged on an electron-multiplying charge-coupled device image sensor. The hologram was obtained with the phase-shifting method and the diffraction was performed by the angular spectrum method. In the optical tweezers, the beam from a Yb-fiber laser with a wavelength of λ = 1070 nm was collimated and focused in a sample solution using a 60× oil-immersion microscope objective lens (OL) with a numerical aperture of NA = 1.25. The sample was fluorescence nanoparticles with a diameter of 500 nm fixed in gelatin.

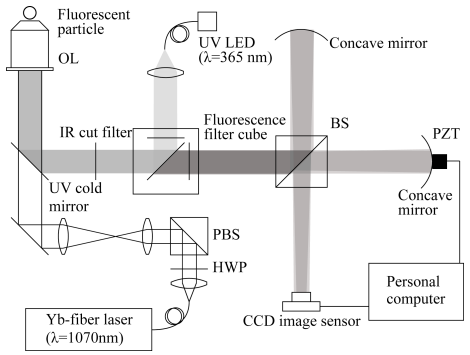

Fig. 1. Experimental setup. PBS; polarized beam splitter, HWP, half-wave plate; BS, beam splitter; OL, objective lens; PZT, piezoelectric transducer

The space-division matching method to measure 3D position of many fluorescence sources was proposed. The procedure is performed by 3 steps as follows. Step 1: The whole observation space shown in Fig. 2(a) was equally divided to the search subspace shown in Fig. 2(b). Step 2: The peak fluorescence point was searched in the search subspace. Step 3: The measurement subspace put the point having the strongest fluorescence intensity on the center is made for each fluorescence source, as shown in Fig. 2(c). In the measurement subspace, the 3D positions of fluorescence sources was decided by the Gaussian fitting.

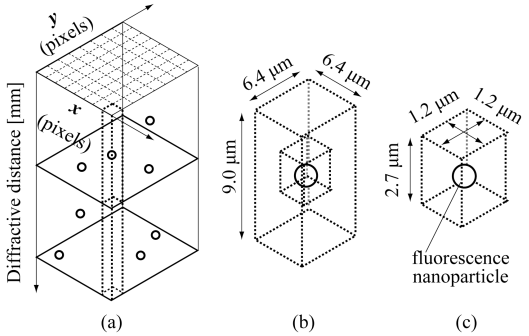

Fig. 2 (a) Whole observation space of the holographic imaging. (b) Search subspace for searching the strongest fluorescence light source. (c) Measurement subspace for detecting the 3D position.

Figure 3 shows the 3D mapping of the fluorescence nanoparticles. By using the space division matching method, the 12 fluorescence nanoparticles were searched. The mapping was obtained in the area with the radius of ~30 μm, because the fluorescence lights reflected from two concave mirrors with difference focal lengths were laterally deviated in the outside of this area. The fluorescence lights from nanoparticles located around the focus plane had no interference according to the above same reason.

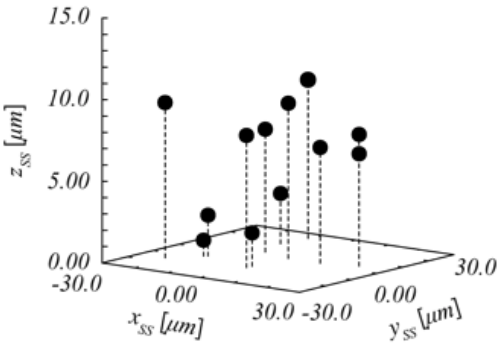

Fig. 3 Mapping of fluorescence point sources .