Superresolution single living cell using digital holography and graphics processing unit

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1. Introduction

Principles of reconstructing a three-dimensional (3D) living cell are based on optical analysis of ultraviolet and visible light when it passes through or reflects from the cell. However, a cell is a small object and its composition includes protein and water, so the intensity of the reflection or pass-through light is very weak, and focal points are often superposed by light out of focus. Consequently, taking a picture of a living cell, especially in reconstructing a 3D living cell, is very difficult and incurs low spatial resolution with the conventional method. To overcome this problem, some methods use confocal laser scanning fluorescence microscopy [1-2].

In this paper, the used reference wave is a laser and the object wave is a light wave that is emitted from a living cell while was dyed with fluorescence which the illumination by a femtosecond laser. The wavelength of emitted light from the living cell is about 300 nm to 600 nm. To satisfy the conditions of the holographic principle, this emission of light should pass through a filter to select the appropriate wavelength to match the wavelength of the reference beam. Thus, each point of emission light that might be inside the living cell or on the surface of the living cell is similarly one object point of a 3D object. So by using the holographic method, we can calculate the distance from this point to the CCD. From this, we can reconstruct the components inside a living cell as well as on its surface in three dimensions.

This paper presents a solution to the problems at previous methods by using the advantage of holographic principles. A proposed method that can reduce the time needed to obtain the full information about the cell compared to the confocal microscopy method. And this method also offer greater depth of resolution of the cell compared to the multi-photon fluorescence light microscopy method by combining the digital hologram method and the principle of multi-photon fluorescence light. According to the holographic principle, with our proposed method, we just need to take a photo of from 3 to 10 layers to reconstruct a cell with full depth of information. Therefore, using the proposed method can reduce the time needed for scanning and increase the resolution of the cell.

2. EXPERIMENT AND RESULT

In this paper, a digital hologram of a cell object, based on the phase-shifting principle using the optical setup as shown in Fig. 9, and the proposed reconstruction method was applied. The femtosecond laser beam of 850 nm wavelength and output power at 2600 mW was expanded in order to reduce power energy per µm, collimated, and turned a plane wave into an object. The emission illuminates a cell placed at a distance of 80 mm $\pm$ 10 µm from the CCD camera. The CCD camera has 1024x768 pixel resolution, with each pixel having an area of 9×9 μm^2, and 8-bit resolution, giving an output video signal with 256 grey levels. Hence each pixel of the captured hologram has 8 bytes of real information and 8 bytes of imaginary information. The object is a B16F10 cell dyed with green fluorescence. The reference beam from a laser beam with a 532 nm wavelength and output power of 30 mW is expanded, collimated and phase-shifted by the retardation plates at amounts of $0, \frac{\pi}{2}, \frac{3\pi}{2}, \pi$ with the femtosecond laser. The object lens is 40x magnification. The method is proposed as follows:

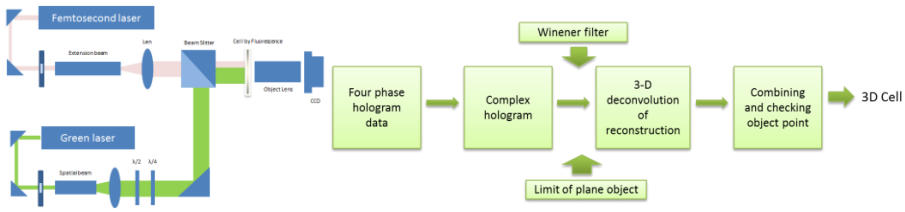
First step: The living cell is dyed with green fluorescence, and a digital hologram is recorded using the optical system shown in Fig. 2 and Fig. 3. The emitted light from the cell is filtered to range of wavelengths from 530 nm to 537 nm. The reference beam of the hologram is set up from a laser with a wavelength of 532 nm, and this laser beam creates the wave plane before hitting the CCD. Four phase holograms with phase shifts of 0, π/2, π, 3π/2 of the reference beam are recorded to yield the amplitude and phase of the object optical field based on the principle of phase-shifting digital holography.

Second step: From four holograms recorded by the CCD camera, we have a complex hologram at a constant distance. This complex hologram is the hologram's plane of the cell that has the focal point of the object lens. Therefore, the hologram recorded in this case is a hologram of the object points on the plane's focal point or around this plane with a distance of $\Delta\eta$. In this paper, to increase resolution of the reconstructed hologram, we chose $\Delta\eta = 0.7\mu m, 0.8\mu m, \text{and } 1.2\mu m$. It is described in Fig. 8.

Third step: For each complex hologram, we reconstructed a 3D object using the hologram principle and reduced depth of focus method, as described in the Section 2.3 method, to reduce noise and some object-point errors on each plane. The result is a whole object point on the plane that has a 2 µm thickness.

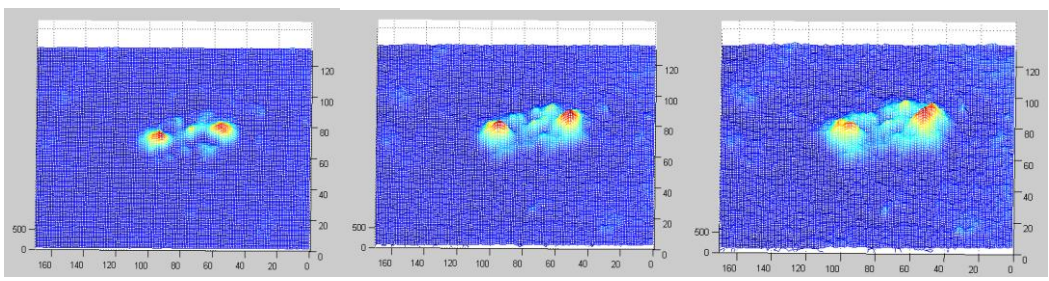
Fourth step: Each thick plane of object points is actually a layer of the living cell, and by combining whole 3D layers and checking object points on the border of the layers and inside layers, we reconstruct a living cell including both the interior and the exterior of the cell.

Finally, as shown in Figure 5. These figure show that the reconstructions of layers represent the real cell. For Fig. 2 a) and b), which show 2 layers and 5 layers of the reconstructed hologram respectively, the object plane of two layers is located at 50 mm, 50 mm + 0.7 µm. And the object plane of five layers is located at 50 mm, 50 mm + 0.7 µm, 50 mm + 1.4 µm, 50 mm + 2.1 µm and 50 mm + 2.8 µm. For Fig.2 c), which show 7 layers of the reconstructed hologram that include 5 layers as above and two layers as 50mm + 3 µm and 50mm +4.2 µm. With Fig. 2 d), 12 layers of reconstructed hologram, the distance from CCD to object for each layers are spaced 0.3 µm. Figure 13 confirms the variations of the reconstruction quality according to various numbers of layers, which were reconstructed from the four-phase-shifting hologram process described above. The processing of calculating by CUDA for GetForce GTX 460 Graphic Card



a) **b)**

Fig. 1. a) Setup principle of proposed method. b)The proposed method for reconstructing a living cell.



a) **b)** **c)**

Fig. 2. Reconstruction of the cell with a) 2 layers of the hologram, b) 5 layers of the hologram, c) 7 layers of the hologram

3. Conclusion and Discussion

A method combining the photon fluorescence method and the holographic method is proposed. The performance of the proposed method was analyzed by varying three parameters; the distance between two layers, the different excited wavelengths, and the number of layers in the reconstruction. Changes in these parameters show the ability for reconstruction with the proposed method. In addition, the method can reduce the number of layers while still providing the full depth information of the object. In future work, the proposal method such as a reconstruction technique instead of scanning fluorescence microscopy will be used for our research. It will be a useful method to acquire 3-D information of the microscope object without mechanical movements.

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