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Optical/Photoacoustic Hybrid Microscopy with Deconvolution Processing for Visualizing Morphology and Composition of Cells

光学・光音響ハイブリッド顕微鏡とデコンボリューション処理を用いた細胞イメージング

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1. Introduction

Optical Resolution Photo-Acoustic Microscopy (OR-PAM) is a promising photoacoutic (PA) imaging tool that can achieve high lateral resolution of optical diffraction limit (< 1 μm), and visualize optical absorber distribution at the single cell level [1]. Thus, it is expected to characterize precise cell dynamics such as interactions with drugs coupled with photoacoustic contrast agents [2,3].

However, visualization of such intracellular microenvironments may require breaking through the diffraction-limited resolution of OR-PAM, which may be achieved by deconvolution processing. Especially, blind deconvolution can recover blurred images without prior knowledge of the point spread function (PSF) of the system, it is suitable for the OR-PAM with a very small PSF that is difficult to obtain. Also, because the PA signal images only the light absorption distribution, it lacks the cell morphology information, which usually imaged by optical microscopy, impeding the biological interpretation of the acquired PA properties.

In this paper, for overcoming these problems, we devolop optical/PA hybrid microscopy that combines deconvolution processing for detailed investigation of the intracellular microenvironment.

2. Materials and Methods

2.1 Experimental Setup

Fig. 1 shows the schematic of developed hybrid optical/photoacoustic microscopy. In PAM part, this system employs a pulsed laser (532 nm, 6 ns, 10 kHz,) for irradiation. The laser beam was focused through an objective lens (50x, NA=0.42) into the target with the laser power ≦25 μW. The generated PA waves were detected by a focused single element ultrasound transducer (50 MHz), amplified, and then recorded by data acquisition card

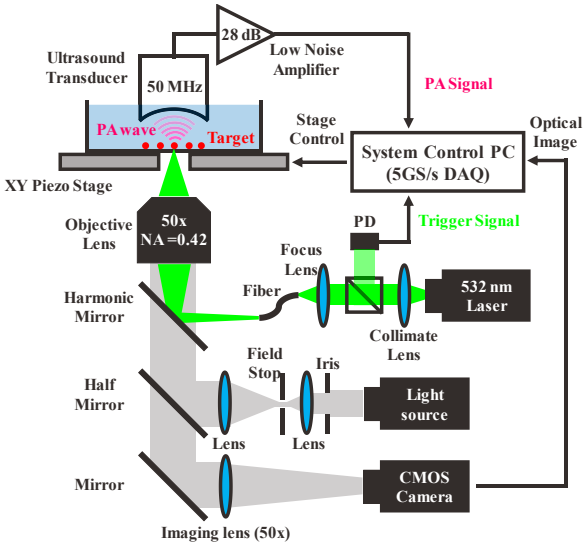

Fig. 1 Schematic of hybrid microscopy system

(5 GS/s). To obtain a better SNR, we recorded the PA signals with 100 times averaging. In synchronization with signal acquisition, the XY piezo stage under the target is moved to acquire PA signals from the whole region of interest. These acquisition sequences were controlled by LabVIEW, and the obtained PA signal was analyzed, filtered and displayed as the maximum amplitude distribution (MAP) image by MATLAB. In the optical imaging part, a CMOS camera (1536 px×2048 px, pixel size = 3.45 μm) and a white light source was devised under the PAM and the axis was adjusted coaxially with the axis of laser irradiation and ultrasonic detection.

To test the system performance, lateral resolution measurement was performed by imaging USAF1951, and applying edge spread function (ESF). Also, bovine red blood cells (RBCs) were imaged as more practical experiment. Optical images were acquired just before the PA imaging sequences.

2.2 Deconvolution processing

Richardson-Lucy blind deconvolution method

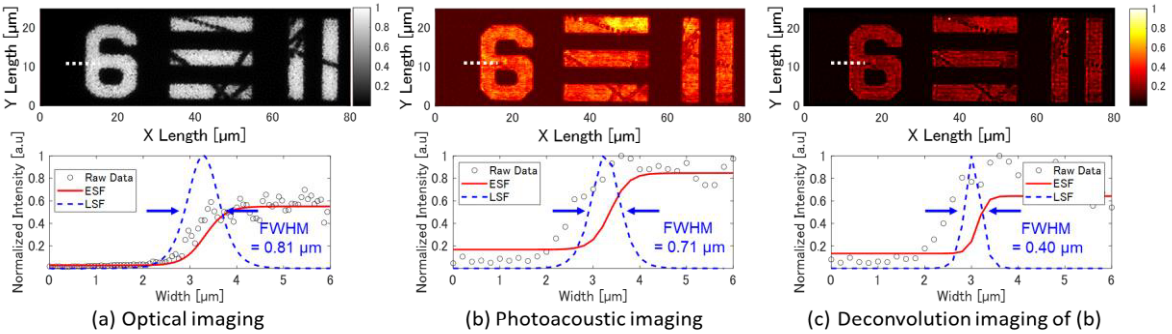

Fig. 2 Images of USAF1951 (upper) and the lateral resolution by ESF (downer) in each imaging methods.

was applied to acquired PA image [4]. This method iteratively estimates the system PSF and the deconvolved image from an original PA image as shown in following equation.

$$h_k'(x,y) = \left[\frac{g(x,y)}{h_{k-1}'(x,y) * o_{k-1}'(x,y)} * o_{k-1}'(-x,-y)\right] h_{k-1}'(x,y)$$

$$o_k'(x,y) = \left[\frac{g(x,y)}{o_{k-1}'(x,y) * h_k'(x,y)} * h_k'(-x,-y)\right] o_{k-1}'(x,y)$$

where $h'(x,y)$ is the estimated PSF of OR-PAM, $o'(x,y)$ is the deconvolved image, and $g(x,y)$ is the original PA image. The iteration was continued until the normalized adjacent mean square error (AMSE) of deconvoluted images converged.

3. Results and Discussions

Fig. 2 shows the result of lateral resolution measurement. In Fig. 2(a) and Fig. 2(b), the lateral resolution of optical and PA imaging, which is FWHM of the line spread function (LSF) calculated by the differential of ESF, was measured as 810 nm / 710 nm, respectively. These values are limited to the optical diffraction limit of OR-PAM system (~650 nm). On the other hand, as shown in Fig. 2(c), the lateral resolution after deconvolution is 400 nm, which is much better than the diffraction limit.

Fig. 3 (b) shows the imaging result of bovine RBCs. The optical image in Fig. 3(a) visualized the shape of RBCs, and the PA image in Fig. 3(b) visualized the optical absorption distribution of the hemoglobin. In addition, the deconvolution image in Fig. 3(c) visualized clearly the distribution of hemoglobin due to the biconcave shape of RBCs due to the higher resolution than optical diffraction limit.

4. Conclusion

In this paper, we developed an optical/PA hybrid microscopy with deconvolution processing. The system performance was evaluated by lateral resolution measurement and RBCs measurement. Optical imaging visualized the cell shape, and deconvoluted PA imaging visualized the hemoglobin distribution at high resolution that exceeded the diffraction limit. These results indicate that the developed system may visualize both morphology (optical imaging) and constitution (PA imaging) of cells that will facilitate single-cell level analyses.

References

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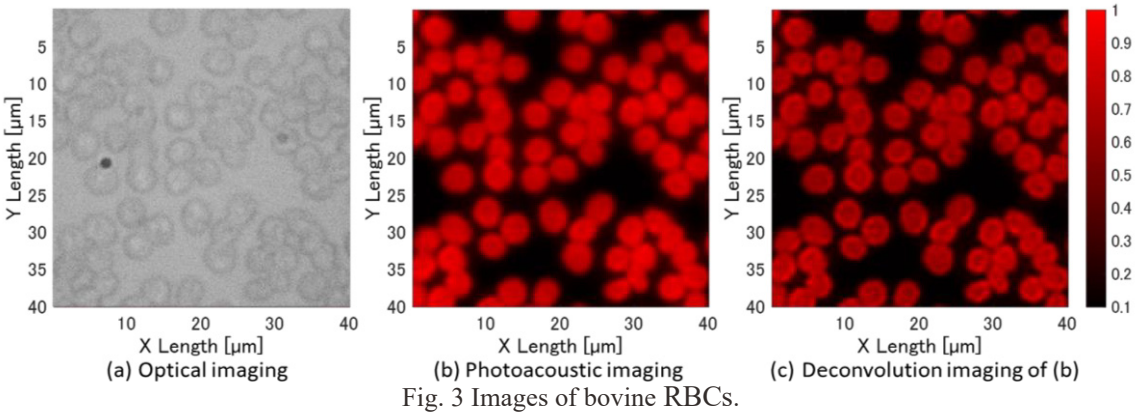

Fig. 3 Images of bovine RBCs.

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