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Visualization of cell structure by optical resolution photoacoustic microscopy with sub-micron lateral resolution

Sub-micron 方位分解能を有する光学分解能光音響顕微鏡による細胞構造の可視化

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Ryo Shintate[1‡], Ryo Nagaoka[2], Kazuto Kobayashi[3], and Yoshifumi Saijo[1] ([1]Graduate School of Biomedical Engineering, Tohoku Univ.; [2]Graduate School of Science and Engineering for Research, Univ. of Toyama.; [3]R&D Division, Honda Electronics Co. Ltd.)

新楯 諒[1†], 長岡 亮[2], 小林 和人[3], 西條 芳文[1] ([1]東北大学大学院 医工学研究科, [2]富山大学大学院 理工学研究部, [3]本多電子株式会社 研究開発部)

1. Introduction

Single-cell biomechanics is important for elucidating the mechanisms of various diseases, and many studies have been conducted to evaluate the acoustic and mechanical properties of cells using acoustic microscopy method[1, 2, 3,4]. However, in this microscopy, there is a trade-off relationship between spatial resolution and attenuation, so it is very difficult to visualize cell structures clearly.

On the other hand, Photoacoustic microscopy (PAM) acquires the photoacoustic (PA) wave generated based on the "photoacoustic effect" when the laser is irradiated[4]. Therefore, it has been attracting attention as a modality that can detect acoustic wave with optical-dependent high contrast in recent years. Optical resolution photoacoustic microscopy (OR-PAM) generates PA waves locally by irradiating a focused laser to the diffraction limit, so the lateral resolution is optically defined[5]. Therefore, OR-PAM has the potential to achieve high cell-level resolution and excellent contrast.

In this study, we developed an OR-PAM system with a sub-micron lateral solution and visualized single-cell structures with high contrast.

2. Materials and Methods

2.1 Experimental setup

As described above, the lateral resolution $R_{lateral}$ of OR-PAM is defined by the optical diffraction limit and can be expressed by

$$R_{lateral} = 0.51 \cdot \frac{\lambda}{NA} \qquad (1)$$

where λ is the optical wavelength and NA is the numerical aperture of the optical objective lens. Therefore, it is theoretically possible to obtain sub-micron resolution by properly selecting the laser source wavelength and the NA of the objective lens.

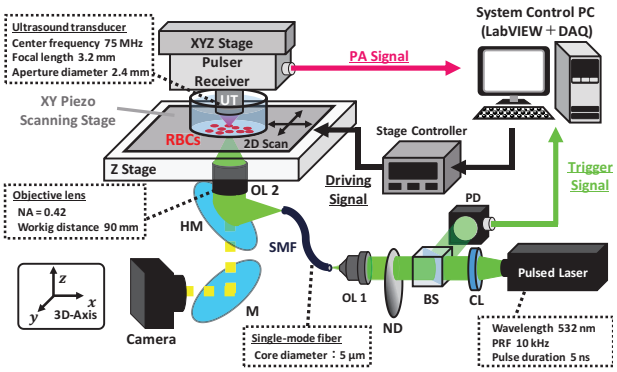

Fig. 1 Schematic of.developed OR-PAM system

Fig. 1 shows the schematic of developed OR-PAM system. This system employs a nanosecond pulsed 532nm laser (HR-G6-N, MegaOpto, Japan) with pulse repetition frequency (PRF) of 10 kHz and pulse width of 5 ns. The laser beam was focused through a 50x objective lens (PAL-50-L-A, SIGMAKOKI, Japan) with NA of 0.42 into the sample. Therefore, the theoretical lateral resolution of the system is 646 nm. The laser power after passing through the objective was measured as ~25 μW using a power meter. The PA waves generated from the sample are detected by a focused high-frequency single element ultrasound transducer with a focal length of 3.2 mm, an aperture diameter of 2.4 mm, a center frequency of 75 MHz. The detected PA signals were amplified by a low noise amplifier (HPR-400F-Ⅲ, HONDA ELECTRONICS, Japan) having ~28 dB gain, and then recorded using a 8 bit high speed digitizer (M4i.2230-x8, SPECTRUM, Germany) at a sampling rate of 5 GS/s. To obtain a better signal-to-noise ratio (SNR), we recorded the PA signals with 100 times averaging. In synchronization with signal acquisition, the XY piezo stage (B17-040, Nano-control, Japan) placed

under the sample is performed raster scanning and PA signals from the whole region of interest is acquired. The all scanning and data acquisition were controlled using LabVIEW software (National Instrument). Also, the obtained PA signal is analyzed and imaged by MATLAB (Math Works, Natick, MA, USA).

2.2 Lateral resolution test and RBCs imaging

The experimental lateral resolution was measured by imaging USAF1951 resolution target (R1DS1P, Thorlabs) in the XY ranges of 200 μm × 200 μm with the step size of 0.25 μm, and using ESF (Edge Spread Function) method to the edge, as shown in **Fig. 2**[6].

In addition, we imaged bovine red blood cells (RBCs) with a diameter of about 3 μm to confirm that the cell structure could be visualized with the developed OR-PAM system. RBC blood smears were prepared in glass-based dishes, filled with saline and placed in the microscopy. In this experiment, imaging range was 10 μm × 10 μm and scan step size was 0.2 μm.

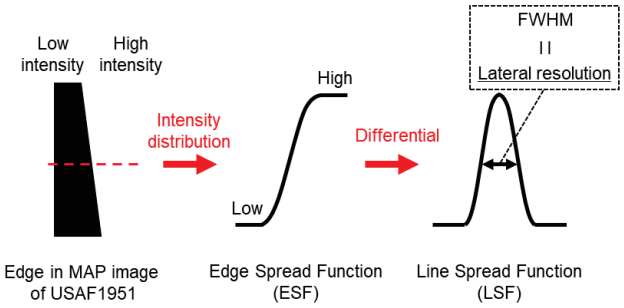

Fig. 2 Edge spread function (ESF) method

3. Results and discussions

Fig. 3 (a) shows the measurement results of lateral resolution by applying the ESF method. The experimental lateral resolution obtained from FWHM of LSF was calculated to be 685.71 nm, which was very close to the theoretical value (646 nm) due to the optical diffraction limit. From this result, it can be said that the developed OR-PAM achieved the sub-micron high resolution system.

Fig. 3 (b) shows the maximum amplitude projection (MAP) image of bovine RBCs. Each red blood cells could be visualized with the high resolution of the system, and the biconcave shape typical of red blood cells was reproduced.

4. Conclusions

In this paper, we developed an OR-PAM system to enable cell imaging and demonstrated the performance. The lateral resolution was very close to the optical diffraction limit and achieved sub-micrometer resolution, and it was confirmed that bovine RBCs could be visualized with the individual shapes. The results indicated that the developed microscope could be visualized cells with high resolution and high resolution.

References

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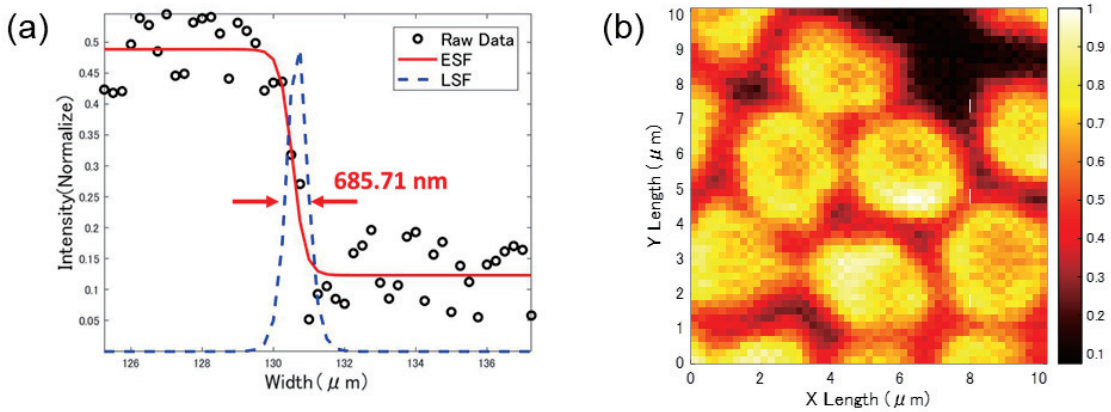

Fig. 3(a)Experimental lateral resolution by ESF method. (b)MAP image of bovine RBCs

ryo.shintate.r4@dc.tohoku.ac.jp