Development of acoustic lens with dual frequency peaks suitable for cell observation

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1. Introduction

Ultrasound is extensively used for industrial and biomedical applications such as imaging[1)], tumor ablation therapy[2)], particle manipulation[3)], and non-destructive evaluation[4)] due to its noninvasiveness and high transparency. The ultrasound transducer is the most important compartment in the ultrasound system to meet the requirements of the applications, and various material strategies and designs have been proposed[5)]. Especially, high-frequency focused ultrasound technique has been used for cell observation using scanninng acoustic microscopy (SAM)[6)]. Their purposes are to evaluate the distribution of the mechanical properties of the cell, so that the transducer of the SAM have been designed to obtain higher resolution images.

On the other hand, Fujiwara *et al.* recently purposed to apply the high-frequency focused ultrasound technique for the localized mechanical stimulation on living cell, not for high resolution imaging[7)]. It is known that cells are constantly subjected to various mechanical forces which greatly affect their morphology and functions[8,9)]. Mechanobiology, which investigates how cells sense the mechanical forces and respond to them, has been attracting much attention, but many of these mechanisms remain unexplored. One of the serious problems in this field is that optimal techiniques to stimulate cells mechanically are still under development. While previous techniques mainly used for localized mechanical stimulation for cells such as atomic force microscopy[10)] and micropipette aspiration[11)] cause cell damage due to physical contact, high-frequency focused ultrasound technique can realize noninvasive stimulation which is expected to play an important role in mechanobiology. Fujiwara *et al.* originally developed a focused ultrasound system for mechanical stimulation on a cell, and demonstrated that ultrasound of a frequency near 100 MHz can stimulate the nucleus selectivly due to its resonance. However, there is no ultrasound transducer designed for effective nucleus stimulation, because previous ones have been designed to excite higher frequencies (~1 GHz) and shorter pulse wave as possible to obtain higher resolution images.

In this study, we develop an acoustic probe which generates ultrasounds with two frequency peaks (near 100 and 160 MHz), being suitable for both nucleus mechanical stimulation and cell observation. Using our original transducer, we can effectively and selectively stimulate only the nucleus by 100 MHz ultrasound, while investigating the mechanical property of the cell in higher resolution by 160 MHz ultrasound. Here, we obtain the spectroscopic absorption images of living cells (myoblast) at 100 and 160 MHz frequencies using the single acoustic probe to demonstrate its usefulness for the two applications. This technique is expected to bring significant progress for mechanobiology.

2. Development of acoustic probe

The acoustic probe consists of a quartz glass rod (10-mm diameter) with a lithium niobate (LN) oscillator on the top of the rod and acoustic lens at the bottom. The LN oscillator was bonded to the top of the quartz-glass rod by an epoxy resin. The acoustic lens is made at the bottom of the rod to focus the ultrasound excited at the LN oscillator to the focal point.

3. Experiment

An electrical signal of 150 V_{pp} with a pulse width of 1.1 ns was sent to the LN oscillator. The generated ultrasonic waves were focused on the culture surface to irradiated the cells. The probe was scanned the cell sample, detecting the ultrasonic echoes from the dish surface by the same probe (**Fig. 1**). The Fourier analysis was performed on the echo, and the spectroscopic acoustic images at the two frequencies were constructed.

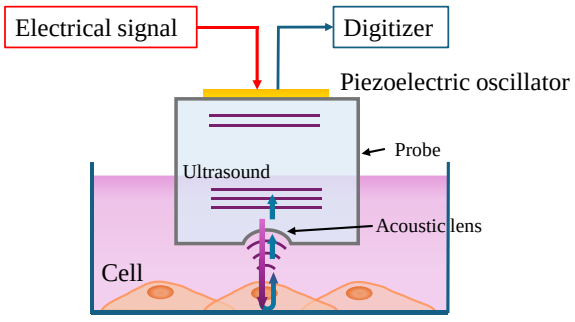

Fig. 1 Experimental setup for obtaining spectroscopic acoustic images of live cells.

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3. Result and discussion

Figure 2 shows an example of the detected waveform, which principally consists of two different frequencies. The former waveform showed a pulse wave with a center frequency of around 100 MHz. On the other hand, the latter waveform showed a burst wave with a center frequency of around 160 MHz. These correspond to the resonances of the system composed of the LN oscillator and the lossy epoxy resin thin layer. Importantly, the burst wave was excited because of no damper on the LN oscillator.

Figure 3 compares an optical image with ultrasonic images at the two specific frequencies. Each amplitude was normalized by the maximum one. Darker points mean lower amplitudes, which indicate more absorption of ultrasound. The 104 MHz image clearly shows the nuclei, indicating that they are resonating at this frequency, and the 160 MHz image shows the cell shape in higher resolution. The spectroscopic images obtain in this study is clearer than those in the previous study[7] even at the same frequency, indicating that the burst-wave excitation caused longer-time vibration to enhance ultrasonic absorption of cells.

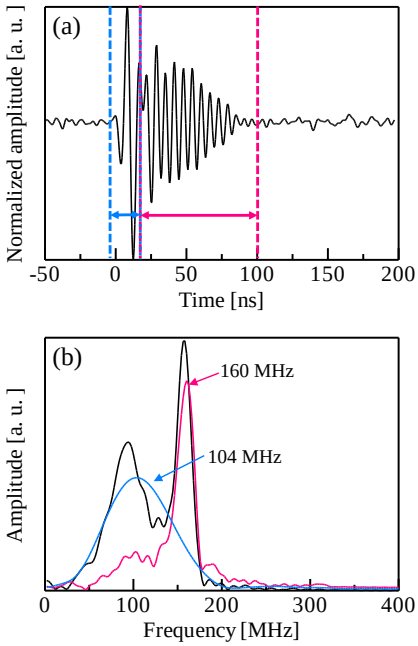

Fig. 2 (a) The representative waveform of reflected wave from the culture surface detected by the self-made acoustic probe, and (b) the corresponding FFT spectra. The blue and magenta spectra are obtained by performing FFT analysis on the waveform in the ranges indicated by the arrows of the same colors as the spectra. The frequency value is the frequency peak on each spectrum.

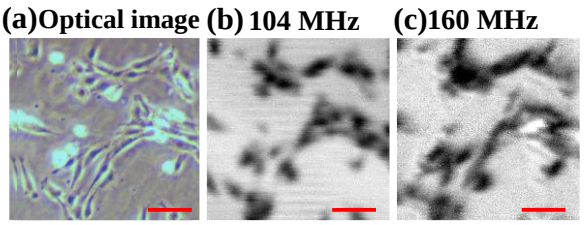

Fig. 3 (a) Optical image and ultrasonic-absorption images of myoblast at (b) 104 MHz and (c) 160 MHz. Scale bars indicate 100 μm.

3. Conclusion

We developed an acoustic probe which generates ultrasounds with two principal frequencies. Ultrasounds with two different frequency peaks allow mechanical stimulation of cells and morphological observation at the same time. The acoustic probe developed in this study is expected to play an important role for mechanobiology studying.

References

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