Proceedings of Symposium on Ultrasonic Electronics, Vol. 34 (2013) pp. 395-396
20-22 November, 2013

Characterization of Single Cell Properties Using High-frequency Ultrasound

高周波超音波を用いた単一細胞の特性評価

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1. Introduction

Acoustical and mechanical properties of tissues are important for assessment of pathphysiology. These properties are known to change from normal to abnormal tissue.[1)] However, these properties of a single cell or how cells affect ultrasound signals are not well understood.

There have been some efforts to obtain acoustic properties using ultrasound.[2,3)] In this study, we use time-resolved acoustic microscopy analyzing the diferrence between a signal from flat surface of the substrate with or without cells.

O'Brien *et al.* studied acoustic properties of the group of cells which were identical to be investigated in this study, by measuring ultrasound backscatter coefficient (BSC).[2)] It would be important to know the difference between methods for future research of single cells. Also, understanding of single cell properties will be useful for study on iPS cells besides the examining cells of various diseases.

2. Method

2.1 System

The transducer was Sonoscan (Elk Grove Village, IL, USA) SK230/SW which had 150 MHz center frequency and f-number of 0.88. The transducer was connected to a 3-dimentional precision positioning system (Daedal Parker Hannifin Corporation, Irwin, PA, USA) with 1-µm linear accuracy in each direction. Sonoscan SPR500 pulser/receiver was used to driven the transducer. Agilent 10-bit U1065A-002 A/D card (Agilent Technologies, Santa Clara, CA, USA) was used to acquire the analog echo signal. The medium was 0.9 % degassed saline. YSI72 temperature controller (YSI Inc. Yellow Springs, OH, USA) was used to heat the medium up. The experimental system was controlled by LabView.

2.2 Cells

The cell to be scanned was the Fisher 44 Mammary Adenocarcinoma Tumor B III (MAT) cell (American Type Culture Collection, Manassas, VA, USA). This MAT cell had been prepared for 24 hours before the scan on a petri dish. The petri dish has 9 boxes to determine the scanning field.

2.3 Set up

The sampling rate was 2 GHz, number of average per scan line was 500, pulse repetition rate was 1 kHz, and amplitude range was 5 Vpp. The scanning field was 300 µm × 300 µm with 3µm step size. Hence, the scanning field was 101 × 101 points. The medium was heated up to 311 K and kept this temperature by using YSI72 temperature controller. At this temperature, the sound speed in the medium is 1527 m/s.[4)]

2.4 Calculation

Time-resolved acoustic microscopy was used to calculate cell properties, such as sound speed, thickness, and attenuation using RF signals (Fig.1). The red curve shows reference signal, and blue curve shows signal passed through cell. This blue signal was obtained from around center of the cell where signal passed through nucleus and cytoplasm. Attenuation vs. frequency (Hz) curves (Fig.4) were fitted into $\alpha(f) = \beta f^n$ using least square method, where $\alpha(f)$ is in dB/cm, f is in MHz, β and n are fit parameters.

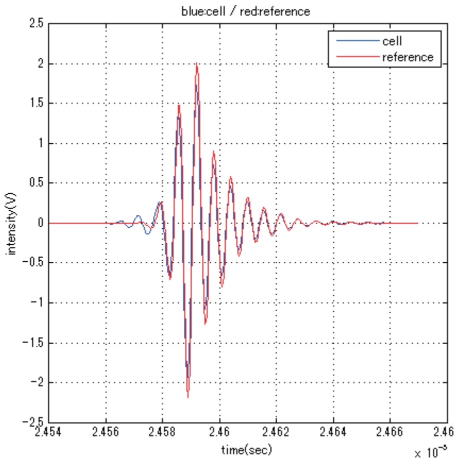

Fig.1 RF signals (blue is of cell, red is reference)

3. Result and Discussion

3.1 C-mode image and Optical image

Fig.2 shows the C-mode image, and Fig.3 shows optical image which was captured before scanning. The scale bars are 30 μm. Each cell can be identified clearly. Also, we can see some cells moved or disappeared from scanning field since some cells were floating on the petri dish. Cells to be calculated their properties were selected comparing these two images. Then, cells in region (a) and (b) in Fig.2, 3 are selected.

3.2 Thickness

Calculated value of the thickness of 13 cells was 13.4 ± 0.9 μm. This value is considered as reasonable because the cell diameter is around 15 micron by the observation of optical image. However, the previous study by O'Brien *et al.* showed the radius is diameter 16 μm.[2)] Considering the cell is actually not sphere, the value in this study is considered more accurate since they used concentric sphere model to estimate.[2, 5)]

3.3 Sound speed

Sound speed in cell was 1591 ± 35 m/s, which was faster than sound speed in the medium. This value is similar to the value in previous study of MAT cells.[5)]

3.4 Attenuation

Fig.4 shows attenuation vs. frequency (Hz) curves of each cell, and black curve shows mean curve. This mean curve was fitted into $\alpha(f) = \beta f^n$, and then, $\beta = 1.6 \times 10^{-10}$ and n = 1.69 were obtained.

4. Conclusion

Thickness, sound speed and attenuation of single cells were obtained using time-resolved acoustic microscopy. These values were different from the result of previous work[2)], which means these properties could be different with methods applied to measurement.[1-3)] The result in this study will be one of keys to understand living cell properties.

References

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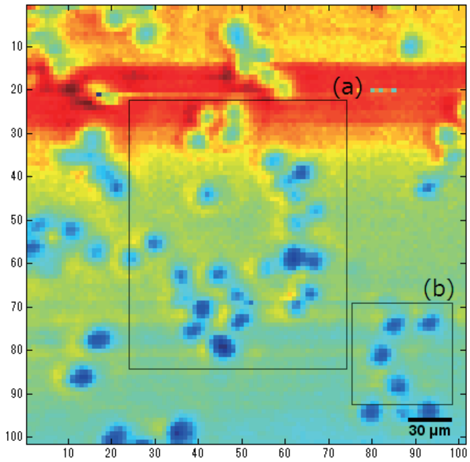

Fig.2 C-mode ultrasound image (scale bar is 30 μm)

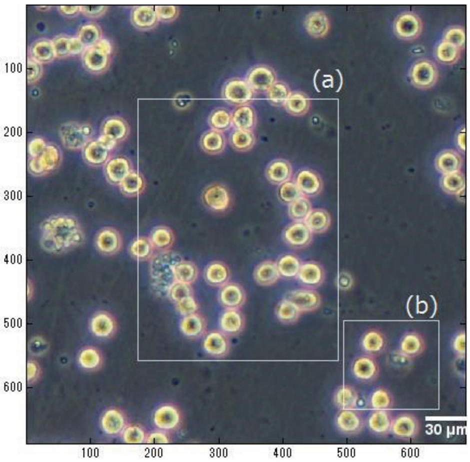

Fig.3 Optical image (scale bar is 30 μm)

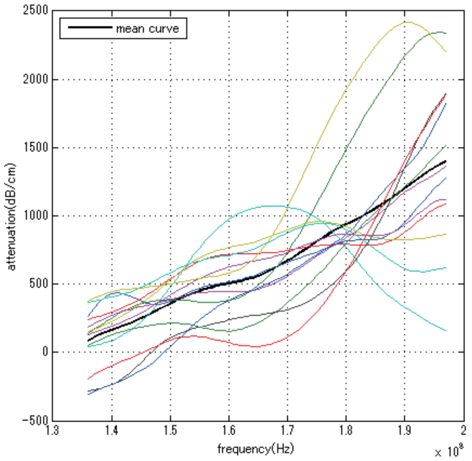

Fig.4 Attenuation vs. frequency curves (black is mean curve)