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Three dimensional acoustic impedance analysis for a cultured cell by 250 MHz ultrasound

250 MHz 超音波を用いた培養細胞の 3 次元音響インピーダンス解析

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1. Introduction

The acoustic impedance of free fatty acids (FFA), which is an important role in NASH progression, could be evaluated as each FFA by scanning acoustic microscopy (SAM), even if they were in solution or absorbed by cells [1]. However, it has been still unknown how the acoustic impedance relates to the microscopic structure and composition in the cells. To preciously understand this relationship, the cultured cells were measured by SAM, and the acoustic impedances of cell organelle (e.g. nuclear) were analyzed in three dimensions (3D), then compared with the 3D structure of the cell visualized by a confocal microscopy.

2. Materials and Methods

2.1. Cell culture

HeLa cells were cultured in 5% CO_2 at 37°C with MEM+10% FBS medium and penicillin-/streptomycin, and were seeded on a dish made of polystyrene thin film with thickness of 50 µm. Laser scanning confocal microscopy (A1R; Nikon) with a Plan Apo 60x oil immersion objective (NA 1.40; Nikon), which has the z-axial resolution of 0.3 µm, was employed for visualization of three dimensional structure in cell. Cellular skeleton (actin fiber) and nuclear could be observed by expressing GFP-actin and mCherry-NLS, respectively [2].

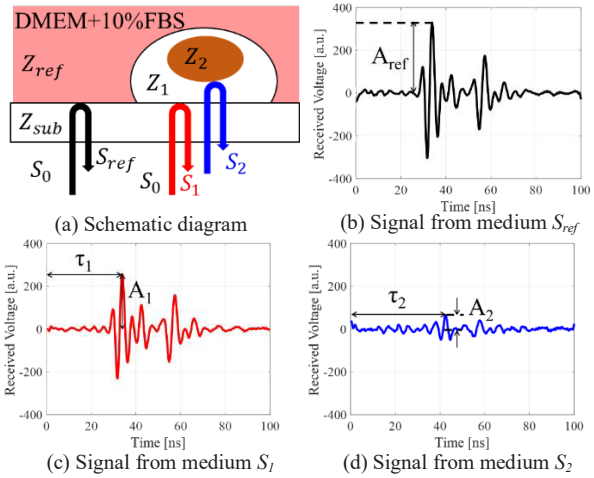
2.2. Acoustic impedance analysis

A scanning acoustic microscopy (modified AMS-50SI, Honda Electronics Co., Ltd, Japan) equipped a 250-MHz center-frequency transducer with the spatial resolution of 7 µm was employed for analyzing the acoustic impedance of the cultured HeLa cells [3]. **Figure 1** illustrates the schematic diagram **(a)** and examples of echo signals from substrate-medium interface S_{ref} **(b)**, from substrate-cell interface S_1 **(c),** and from internal structure S_2 or cell-medium interface **(d)**. The signal from cell is the interference of S_1 and S_2. To analyze the acoustic impedance in cell, S_1 and S_2 must be extracted from the interference signal (received original signal). By subtracting the amplitude-corrected reference signal from the interference signal, the signal from internal structure of cell (S_2) was estimated.

The acoustic impedance (Z_1) of the cell at substrate-cell interface was calculated from the peak amplitudes of a reference signal (A_{ref}) and that of a received signal (A_{ref}) [3]. Assuming that the effect of interference was little at the initial rise (t = 27-37 ns in **Fig. 1**), A_1 was approximately equal to A_{ref}. The acoustic impedance of internal structure of cell (Z_2) was analyzed by comparing the peak amplitude of S_2 with S_{ref} by using a following equation,

$$Z_2 = \frac{1 + \frac{A_2}{A_{ref}} \cdot \frac{Z_{ref} - Z_{sub}}{Z_{sub} + Z_{ref}} \cdot \frac{(Z_1 + Z_{sub})^2}{4Z_1 Z_{sub}}}{1 - \frac{A_2}{A_{ref}} \cdot \frac{Z_{ref} - Z_{sub}}{Z_{sub} + Z_{ref}} \cdot \frac{(Z_1 + Z_{sub})^2}{4Z_1 Z_{sub}}} Z_1$$

where Z_{sub} is the acoustic impedance of the substrate, Z_{ref} is the acoustic impedance of the reference material.



(a) Schematic diagram (b) Signal from medium S_{ref}

(c) Signal from medium S_1 (d) Signal from medium S_2

Fig.1 Echo signal from cell

3. Results and Discussions

Figures 2(a-1) and **2(a-2)** show a C-mode image of normalized envelope amplitude of subtracted signal at 10 µm depth from the substrate and a B-mode image at A-A' line in **Fig. 2(a-1)**,

respectively. Also, solid line at depth = 22 μm in **Fig. 2(a-2)** represents the position of the substrate. **Figure 2(b-1)** shows an acoustic impedance image of Z_2 calculated from the maximum amplitude on each measurement point. **Figure 2(b-2)** shows the acoustic impedance at the depth of substrate (Z_1) and internal structure (or cell-medium interface) (Z_2) at A-A' line in **Fig. 2(a-1)**, where each depth was calculated from time of flight (τ) shown in **Fig. 1**. **Figures 2(c-1)** and **2(c-2)** show confocal images of whole cell and its cross-section at A-A' line in **Fig. 2(a-1)**.

At x = 170-210 μm in B-mode (**Fig. 2(a-2)**), it was confirmed that a strong echo was reflected. The distance from the substrate and the reflection source was 6 μm. The value of Z_1 and Z_2 at x = 170-210 μm of the cell were 1.71 ± 0.030 Mrayl and 1.60 ± 0.038 Mrayl, respectively. The value of Z_1 at the other positon (i.e. polystyrene film which cells are adsorbed) was 1.56 ± 0.039 Mrayl. On the other hand, the oval-shaped nuclear is clearly showed on the confocal images. The diameter and thickness of the nuclear observed on the confocal images were 30 μm and 8 μm, respectively.

In comparing of the results of acoustic microscopy with that of confocal microscopy, it was supposed that the strong signals in **Figs. 2(a-1)** and **2(a-2)** were reflected signal from the backside of the cell, possibly nuclear-cytoplasm interface (or from the nuclear-medium interface when the thickness of cytoplasm was very small). Thus, we conclude that Z_1 and Z_2 at x = 170-210 μm were the acoustic impedance of the nuclear. **Figure 2(b-2)** additionally suggested that it was difficult to analyze the acoustic impedance of Z_2 when the incident angle of ultrasound to the object was large (x = 200-210 μm). The acoustic impedance of polystyrene-cytoplasm interface was not visible in **Figs. 2(a)** and **2(b)**. This is because the acoustic impedances of cytoplasm has only little difference with that of polystyrene.

4. Conclusion

In the result of the acoustic impedance analysis of HeLa cell by using SAM with 250 MHz ultrasound, it was found that the nuclear has larger acoustic impedance compared with other portion (cytoplasm). Currently, we are investigating whether finer 3D evaluation including cytoplasm is possible by increasing the accuracy of component separation of echo acquired by SAM.

Acknowledgment

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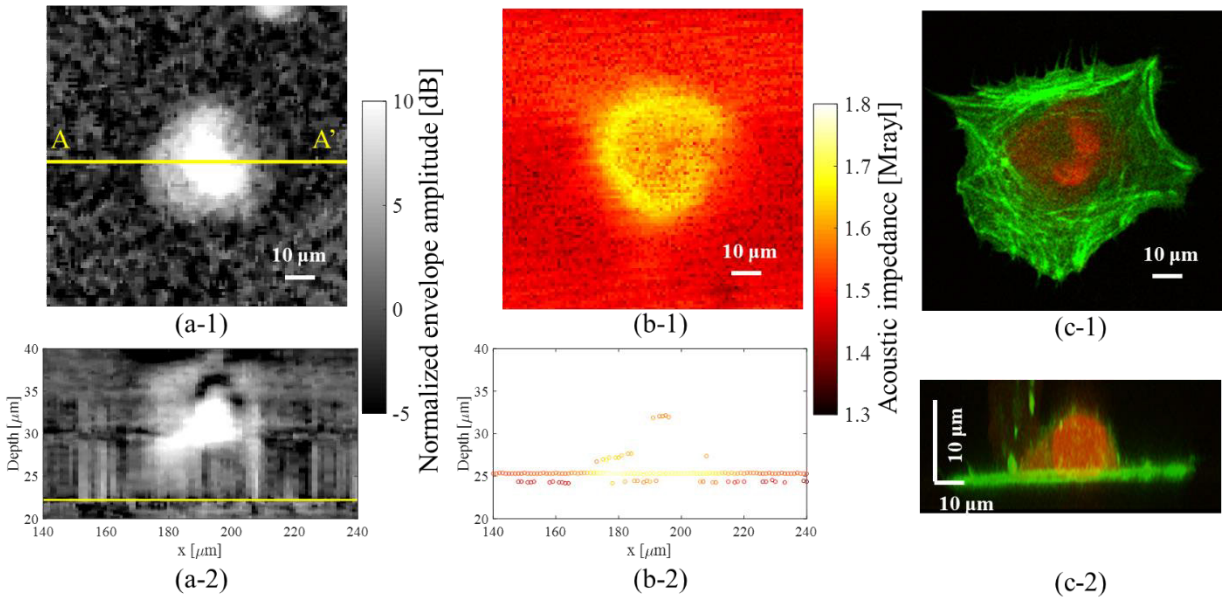

Fig. 2. HeLa cells observed in each modality. (a-1) C-mode image at 10 μm from substrate, (a-2) B-mode image at A-A' line, (b-1) acoustic impedance image of Z_2, (b-2) acoustic impedance of Z_1 and Z_2 at the depth of substrate and refraction source in cell, (c-1) confocal image of cell, (c-2) its cross-sectional image.

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