Validation of acoustic cell imaging obtained by Z-scope impedance tomography

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1. Introduction

Currently, it is hard to observe the internal structure of living cells using microscopies noninvasively and without staining. We have proposed an acoustic impedance microscope[1)] and established some methods for noninvasive, stain-free, and continuous visualization of the elastic properties of living cells on the interface of substrates. This acoustic impedance microscopy enables observation with cells and enlarges new cell observation techniques[2)]. Additionally, we have proposed an analytical algorithm as an advanced form of acoustic impedance microscopy to enable three-dimensional elastic imaging of the substrate interface and the three-dimensional cell structure[3)], named the Z-Scope for commercialization. In this study, we report the results of new tomographic images of cells measured with the Z-scope and the comparison and verification of existing ultrasound imaging. Cell images were confirmed with a fluorescence microscope.

2. Experimental methods

2.1 Acoustic Impedance Microscope

Fig.1 shows the acoustic impedance microscope system.

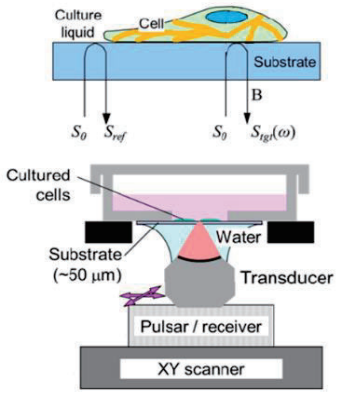

Fig.1 Acoustic Impedance Microscope System

In the acoustic impedance microscopy measurement, cells are cultured in a PS film dish HPS-3805 (Honda Electronics) with a 50μm-thick bottom, and an ultrasonic transducer (Honda Electronics) of center frequency 320 MHz is used to look up at cells from the bottom of the dish. The acoustic impedance of the interface between the cultured cells and the dish is observed [1)].

The acoustic impedance of the interface between cultured cells and the dish is obtained from the following equation.

$$S_{ref} = \frac{Z_{ref}-Z_{sub}}{Z_{ref}+Z_{sub}} \times S_0 \qquad (1)$$

$$S_{tgt} = \frac{Z_{tgt}-Z_{sub}}{Z_{tgt}+Z_{sub}} \times S_0 \qquad (2)$$

S_{ref} is the reflected intensity from the medium, S_{tgt} is the reflected intensity from the cells, Z_{sub} is the intrinsic acoustic impedance of the polystyrene, and Z_{ref} is the intrinsic acoustic impedance of the medium. **Fig.2** shows images of glial cells observed using acoustic impedance microscope.

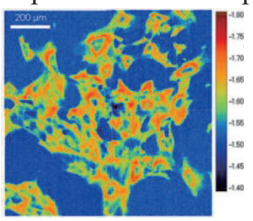

Fig.2 Intrinsic acoustic impedance image of glial cells

2.2 Principle of Elastic Imaging of Cell Interiors

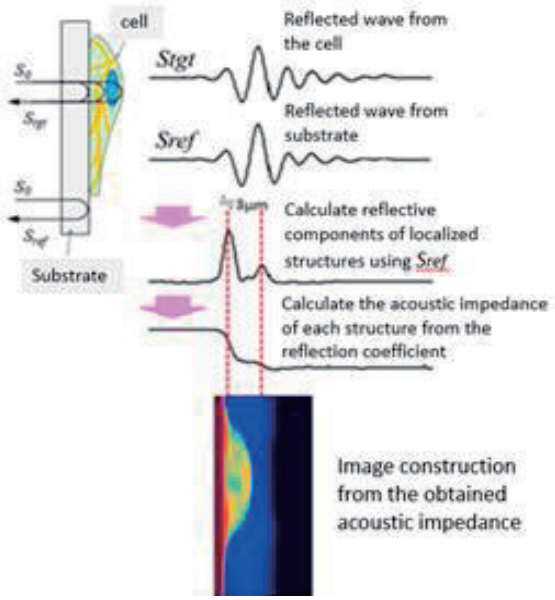

Fig. 3 Calculation method of three-dimensional acoustic Impedance

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The acoustic impedance of the cell interior is basically obtained from the same equations (1) and (2) as in the previous section. In the reflected wave S_{tgt} from the cell, there are reflections caused by the internal structure of the cell. Using these reflection components S_{ref}, the reflection coefficient of the localized reflection wave is obtained(**Fig.3**). From the obtained reflection coefficients, the reflection coefficients of the structure are calculated sequentially, the intrinsic acoustic impedance of the structure is obtained, and a tomographic image of the cell is constructed from the obtained intrinsic acoustic impedance[4].

3. Verification

3.1 Verification Methods

In this study, the following two points were verified.

(a)To confirm the changes in cell shape, HeLa cells with different mitotic cycles seeded at a concentration of 1×10^4 cells/ml in PS film dishes were observed in two dimensions (C-mode) and three dimensions (Z-scope).

(b)To validate the cross-sectional images of HeLa cells obtained with the acoustic impedance microscope Z-scope, the same cells in a living state were observed using a fluorescence microscope BZ-X800 (KEYENCE) after biofluorescence staining with Hoechst 33342 and PlasMem Bright Green.

3.2 Results

The results of the validation are shown below.

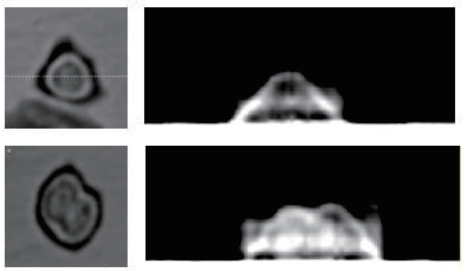

Fig.4 Comparison of tomograms of HeLa cells with different cycles

(a)**Fig.4** shows an example of a comparative study of tomographic images of a quiescent cell and a cell in division. The nucleus is observed in the middle part of the cell, but the acoustic impedance of the nucleus is low when the nucleus is not dividing. The acoustic impedance of the nucleus during cell division is high, and the situation where the nucleus divides into two cells is also observed. The reason for the high value of the acoustic impedance of the nucleus in the tomogram during cell division may be due to the aggregation of chromosomes during cell division.

(b)The same HeLa cells were measured by ultrasonic microscope and fluorescence microscope. After the acoustic impedance images were acquired, the cells were fluorescently stained and measured while they were still alive, so the size and location of the nuclei were confirmed, although some shape changes were observed over time(**Fig.5**).

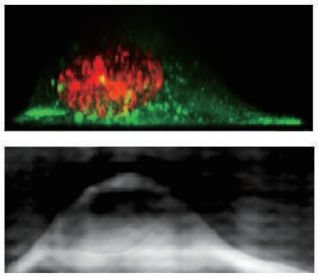

Fig5. Fluorescence microscope image of the same HeLa cells fluorescence microscope image (top) and Z-Scope image (bottom)

The results correlate with the cell tomogram measured by fluorescence microscope. From the above, it was confirmed that the acoustic impedance image acquired by Z-scope analysis correlates with the fluorescence microscope image, and that the physical property changes inside the cell due to the mitotic cycle are observed in the living state.

4. Conclusion

It is suggested that the three-dimensional elasticity imaging method (Z-scope), which can display the acoustic impedance distribution inside the cell, can provide useful information on changes in cell shape and physical properties inside the cell, such as nuclear division. In addition, the position of the nucleus imaged in the Z-scope cell tomogram was confirmed by comparison with a fluorescence microscope image of the same cell, and its validity was confirmed.

However, since the cell measurement data obtained in this study were only for ectodermal HeLa cells, we will increase the number of cell types (endoderm, mesoderm, and ectoderm) to be measured in the future and continue the verification of the data.

References

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