Three-Dimensional Observation of Mitotic Phase Cells Using Ultrasound Microscopy

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1. Introduction

Previously, our research group has proposed acoustic impedance microscopy[1)] as a noninvasive and stain-free method to observe the internal structure of live cells.

Acoustic impedance microscopy provides continuous visualization of the elastic properties of the interface between live cultured cells and the dish.[2, 3)] As a further development of this technique, an analysis algorithm (Z-Scope) has been proposed to calculate not only information on the interface between cultured cells and the dish,[4, 5)] but also three-dimensional elastic imaging of the internal structure of cells, and is being established as a new cell observation technique. However, although two-dimensional information on the interface between cultured cells and dishes and vertical direction tomographic images (vertical slice images) of cells have been observed by using acoustic impedance microscopy, and have yet to be utilized as three-dimensional data. In this study, the results of 3D observation based on 3D information of cultured cells measured by acoustic impedance microscopy, the confirmation of the internal structure of cells using vertical slice images and lateral slice images are reported.

2. Experimental Methods

Figure. 1 shows the acoustic impedance microscopy system.

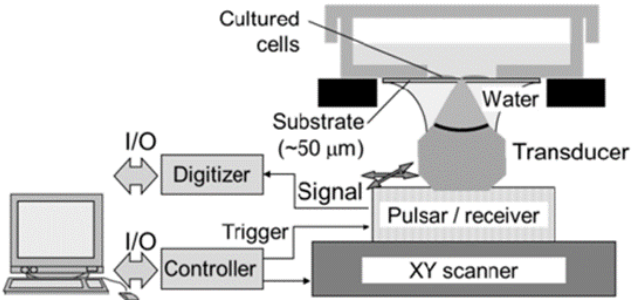

Fig. 1 Acoustic Impedance Microscopy System

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In the acoustic impedance microscopy measurement, cells cultured on a Polystyrene (PS) film dish (Honda Electronics HPS-3805) with a 50 μm-thick bottom are scanned XY direction with an ultrasonic transducer (Honda Electronics HTD400-06) with a focal length of 0.6 mm, aperture diameter of 1.0 mm and center frequency of 320 MHz. The acoustic impedance of cultured cells on PS film is measured by scanning.

To determine the acoustic impedance within the cell, the impedance at the interface of the cell and substrate is calculated by using the PS film dish as a reference (**Fig. 2**). Then the impedance for each internal reflection component, sequentially in the depth direction, can be acquired.

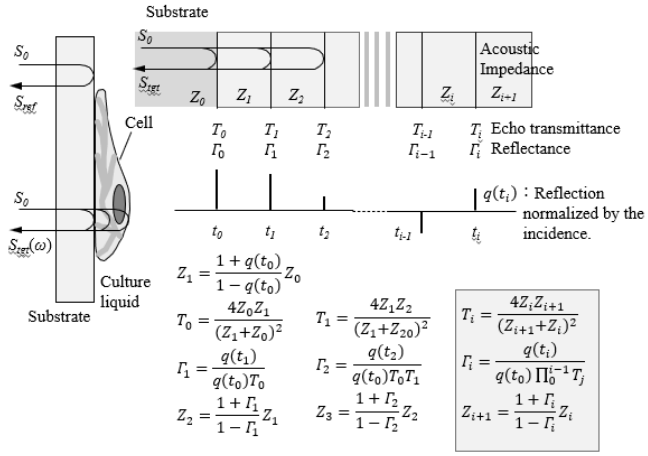

Fig. 2 Acoustic Impedance Calculation Method

3. Verification

3.1 Verification Methods

In this study, three-dimensional data obtained from acoustic impedance microscopy measurements variation of the shape and the internal structure occurs during mitosis of mitotic HeLa cells is used. As a validation, 3D images, vertical slice images and lateral direction tomographic images (lateral slice images) were created and compared. Lateral slice images were created using “ImageJ.”

3.2 Verification Results

Figure. 3 shows an example of a comparison of 3D images, vertical slice images and lateral slice images of cultured cells in the quiescent phase two hours before cell division. In the tomographic image, the cell nucleus was located at the top, and the cytoskeleton supported the nucleus at the bottom of the nucleus.

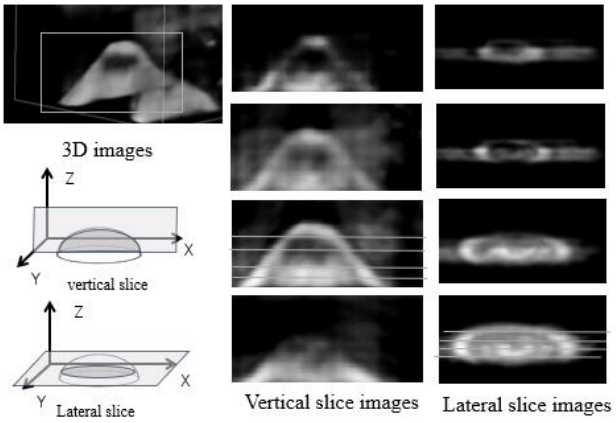

Fig.3 Observation Results (1)
Quiescent phase two hours before cell division.

Figure. 4 shows the results of a comparative study of three-dimensional images of cultured live cells. The cell nuclei divided in the vertical slice image and lateral slice image can be confirmed. The boundary between the two divided cell nuclei was ambiguous in the vertical slice image. By comparing with the lateral slice image of the same cell, it was confirmed that two nuclei existed inside the cell and that nuclear fission was occurring.

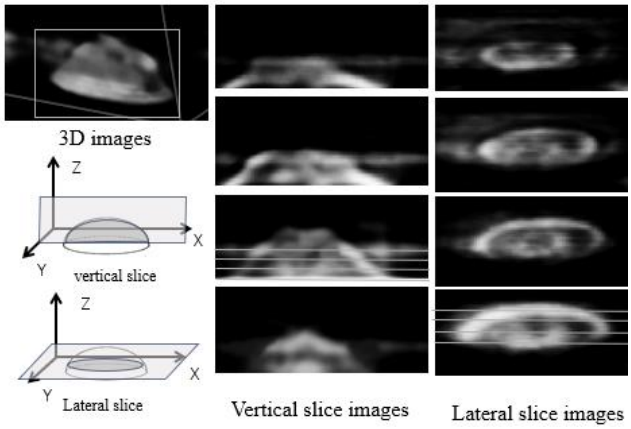

Fig.4 Observation Results (2)
Nuclear division phase.

From the above, it was confirmed that information on the internal structure of cells can be obtained with higher validity by comparing vertical slice image and lateral slice image

4. Conclusions

The results of this study suggest that the construction of lateral slice image from the acoustic impedance obtained by acoustic impedance microscopy can provide useful information on the intracellular structure of cells obtained from tomographic images. In addition, it is important to confirm detailed intracellular structures and shape changes by utilizing 3D data according to the measurement results above.

For future research, the cultured cells analysis will be pursued by obtaining lateral slice image, which was used in this study, to verify the method proposed in this paper.

Acknowledgment

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