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Pseudo-transmission imaging of cultured cells by using focused ultrasound reflectometry

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1. Introduction

Ultrasound microscopy is recognized as a powerful observation device with high resolution for nondestructive and non-staining observation of thin biological tissues such as skin and cells(1). It is possible to realize not only two dimensional obserbation but also three dimensional, by analyzing the data set obtained from the scan area. The acoustic impedance microscope for biological use proposed by the authors takes into account of reflectance from the target, and converts into acoustic impedance(2).

The 2-D observation mode is based on the reflected intensity of focused ultrasound waves transmitted through the polymeric substrate to the biological sample(1). As the obtained images are mainly composed of reflection coefficients at the interface between the biological tissue and the substrate, it is unlikely to represent the information across the entire thickness range. Therefore, we newly propose a method to separate the obtained signals into reflection components from the substrate surface and inside of the biological samples, and to display the images as pseudo-transmission components. In this report, the results of a fundamental study using microglial cells cultured on the substrate is described.

2. Measurement system

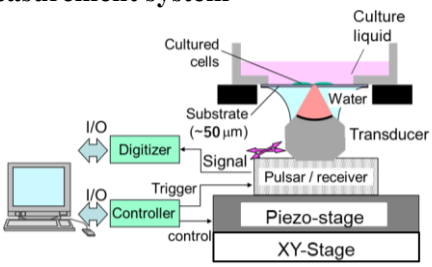

Fig.1 Ultrasonic Microscope System

Figure 1 shows the ultrasonic microscope system. The focused pulsed ultrasound waves with a center frequency of 320 MHz were transmitted from an ultrasonic transducer with an aperture diameter of 0.9 mm and a focal length of 0.6 mm. The measurement targets were microglial cells cultured on a polystyrene film substrate with thickness of 50 μm. The pure water was employed as the coupling medium between the transducer and the substrate.

3. Measurement method and Signal processing

3.1 Measurement and conversion to reflection coefficient

The pulsed ultrasound focused on the contact interface between the substrate and the sample surface was transmitted, and the reflection from the back of the substrate was received while scanning the transducer along the plane. The spectrum $S_{tgt}(\omega)$ obtained from the signal reflected from the object (target) was normalized (deconvolved) by the reflection signal $S_{ref}(\omega)$ from the interface between the substrate and the reference material. In addition, the incident signal S_0 can be obtained from the $S_{ref}(\omega)$ as:

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

$$S_0 = \frac{Z_{ref}+Z_{sub}}{Z_{ref}-Z_{sub}} S_{ref} \quad (2)$$

The reflection coefficient $R_{tgt}(\omega)$ of the object can be calculated as:

$$R_{tgt}(\omega) = \frac{S_{\mathrm{tgt}}(\omega)}{S_0} = \frac{Z_{ref}-Z_{sub}}{Z_{ref}+Z_{sub}} \cdot \frac{S_{\mathrm{tgt}}(\omega)}{S_{\mathrm{ref}}} \quad (3)$$

where Z_{ref} , Z_{sub} are the acoustic impedance of the reference material and substrate, respectively.

3.2 Pseudo-transmission of cells

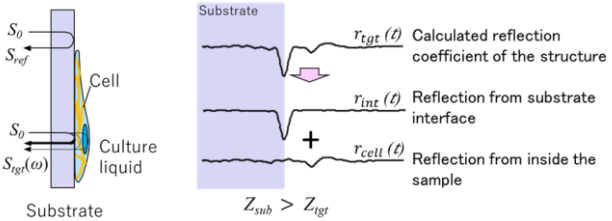

Fig.2 Superimposition of reflectance coefficients from the substrate and cells

The difference between acoustic impedances of substrate and biological samples is significant in comparison with the reflection from the internal structure of the cell. Therefore, the reflection from internal structure of the sample is very small relative to reflection from the substrate interface (**Fig. 2**). The C-mode intensity image of the sample measured in planar observation mode can only show the thin part of the cell in the vicinity of the substrate. On the cntrary, we proposed a method to describe the entire

thickness of the biological sample in the planar observation mode. The reflection component from the substrate interface and the inside of the biological sample were separated, and the latter was enhanced in the signal processing.

Let $r_{tgt}(t)$ be the inverse Fourier transform of the reflection coefficient $R_{tgt}(\omega)$ corresponding to the structure. The first reflection component taken from $r_{tgt}(t)$ is defined as the reflection $r_{int}(t)$ from the substrate interface. The reflection from the substrate interface and the reflection $r_{cell}(t)$ from inside the biological sample can be separated as shown in Figure 3. The acoustic impedance Z_{int} of the area in contact with the substrate is:

$$R_{int} = \frac{Z_{int}-Z_{sub}}{Z_{int}+Z_{sub}} , \quad (4)$$

$$Z_{int}(\omega) = \frac{1+R_{int}}{1-R_{int}} Z_{sub} . \quad (5)$$

The incidence into the cell structure is calculated as:

$$T_{int} = \frac{4Z_{int}Z_{sub}}{(Z_{int}+Z_{sub})^2} . \quad (6)$$

The reflection from inside the biological sample is small because the acoustic impedance values of the polymeric substrate and the biological sample are very different. For this reason, the polymeric substrate is virtually replaced by a substance (culture medium) that has a similar acoustic impedance to that of the biological sample (**Fig. 3**).

So the reflection coefficient $R'_{int}(\omega)$ at the substrate interface can be expected as:

$$R'_{int}(\omega) = \frac{Z_{int}(\omega)-Z_{ref}}{Z_{int}(\omega)+Z_{ref}}, \quad (7)$$

The incidence into the cell structure is calculated as:

$$T'_{int} = \frac{4Z_{int}Z_{ref}}{(Z_{int}+Z_{ref})^2}. \quad (8)$$

And the reflection from inside of the sample is:

$$R'_{cell}(\omega) = R_{cell}(\omega)\frac{T'_{int}}{T_{int}} . \quad (9)$$

The apparent reflection coefficients is shown as:

$$R'_{total}(\omega) = R'_{int}(\omega) + R'_{cell}(\omega) . \quad (10)$$

This reduces reflections at the culture medium interface and allows the calculation of apparent reflection coefficients weighted to the information inside the biological sample.

The energy transmission rate T'_{total} is:

$$T'_{total} = 1 - {R'_{total}}^2(\omega). \quad (11)$$

The sound pressure reaching behind the target can be estimated as:

$$\sqrt{1 - {R'_{total}}^2(\omega)} . \quad (12)$$

In this way, a two-dimensional pseudo-transmission image is obtained by calculating the transmitted sound pressure at each scanning point using the reflection coefficient.

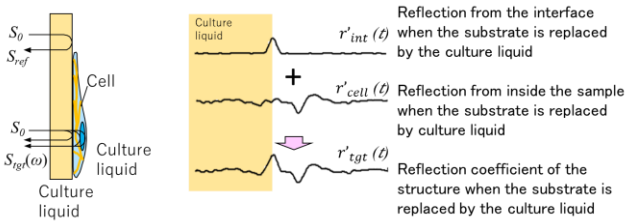

Fig.3 Superimposed reflection coefficients when the substrate is replaced by the culture liquid.

4. Results

A pseudo- transmission image of microglial cells is shown in **Fig. 4**. Fig. 4(a) is the reflection intensity image from which the cell in contact with the substrate are clearly visible. In Fig. 4 (b), the nucleus of microglial cells are clearly identified by pseudo-transmission. The nucleus are black in color with weak intensity because the signal is difficult to penetrate in it. This pseudo-transmission image is similar to the results of optical microscopy of the same type of cells, as shown in **Fig. 5**.

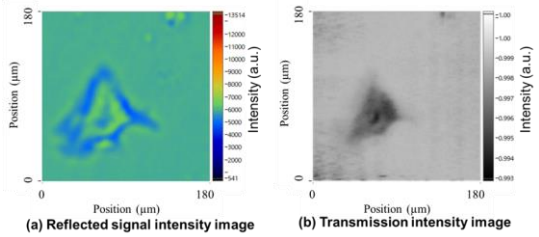

Fig.4 Reflection and transmission images of microglial cells

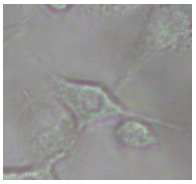

Fig.5 Optical microscope image of microglial cells

5. Conclusion

In the planar acoustic impedance image based on the reflection, it is usually difficult to find the position of the nucleus that is not perfectly in contact with the culture surface. It was shown that the proposed method produces a profile similar to a transmission optical microscope image. It is possible to relate the acoustic impedance distribution to the position of the nucleus for the same cell without the optical and acoustic images.

References

1. Naohiro Hozumi et al.,Ultrasonics, Vol.99, November 2019.
2. Edo Bagus Prastika et al.,Japan Society of Applied Physics, Volume 59, Number SK, July 2020.