

### 3D acoustic impedance mapping of cultured biological cells

#### 培養生体細胞の3次元音響インピーダンスマッピング

Nur Dalila Binti Jalaluddin<sup>1,†</sup>, Rahma Hutami Rahayu<sup>1</sup>, Kyoichi Takanashi<sup>1</sup>, Tomohiro Kawashima<sup>1</sup>, Sachiko Yoshida<sup>1</sup>, Yoshinobu Murakami<sup>1</sup>, Naohiro Hozumi<sup>1</sup>, Kazuto Kobayashi<sup>2</sup> (<sup>1</sup>Toyohashi Univ. of Technology; <sup>2</sup>Honda Electronics Co. Ltd)

Nur Dalila Binti Jalaluddin<sup>1,†</sup>, Rahma Hutami Rahayu<sup>1</sup>, 高梨 恭一<sup>1</sup>, 川島 朋裕<sup>1</sup>, 吉田 祥子<sup>1</sup>, 村上 義信<sup>1</sup>, 穂積 直裕<sup>1</sup>, 小林 和人<sup>2</sup> (<sup>1</sup>豊橋技術科学大学, <sup>2</sup>本多電子株式会社)

## 1. Introduction

Ultrasonic microscope emits high frequency sound at an object and the reflected sound wave is converted into two-dimensional image of the object [1][2]. Ultrasonic microscope has wide range of usage in industrial, medical, and biological applications.

In the medical and biological field, it is advantageous that the observation can be performed without staining the tissues or cells, that realizes non-invasive and continuous observation.

The purpose of this study is to monitor the changes occurs in internal components of cells by observation and evaluation by using three-dimensional imaging presentation from impulse response waveform [3].

This study also aims for realization of three-dimensional microscale observation by using acoustic impedance mapping. In particular, the effect of anticancer drug onto the cells was monitored by non-invasive, continuous, and quantitative observation.

## 2. Experimental method

C127I cell line (epithelial tumor cell line from mouse murine mammary tumor) distributed from DS Pharma were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% kanamycin, and placed at 37°C in a 5% CO<sub>2</sub> atmosphere. These breast cancer cells were cultured on either 75- $\mu$ m-thick Polystyrene plate (OptiCell™) or 50- $\mu$ m-thick Polystyrene film dish (PS Dish) for a high-resolution acoustic observation. The anti-cancer drug, Nimustine hydrochloride (ACNU) which has the ability to cause DNA fragmentation, inhabitation of protein synthesis and cell death, was applied to cultured cancer cells with 100 $\mu$ g/mL.

Focused ultrasound with center frequency at 300 MHz is transmitted onto the cultured cells, and the reflected waveform is recorded. The signal is re-shaped using filter and deconvolution process.

The signal is compared with the reference signal

from where the substrate is directly in contact with the culture liquid of which acoustic impedance is known, to convert into the acoustic impedance of the target cell [1][2]. Two-dimensional mechanical scan realizes acoustic impedance profile. Fig. 1 shows reflection signal from the cell and deconvolved waveform normalized by the reference signal from where no cell is attached.

Making use of the reflection waveform, three-dimensional cell image in terms of cell thickness was created. The two negative peaks on the left side of Fig. 1(b) represent the positions of substrate and top of the cell. Cell thickness at each point is estimated by assuming the sound speed through the cell as 1550 m/s, which is considered to be a typical number for biological cells.

The cell area is determined from the acoustic impedance image. A viewport that includes only one cell is briefly defined. Number of pixels, of which corresponding acoustic impedance is significantly higher than that of the culture liquid, is defined as the cell area, and normalized by that in the initial stage.

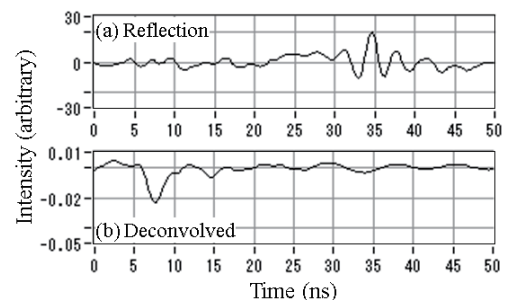


Fig. 1 Reflection from cultured cell (a) and deconvolved waveform referring to the reflection from where no cell is attached.

## 3. Results and Discussion

Fig. 2 shows a typical result of observation. The acoustic impedance image indicates the bottom-view of the cells. It seems that almost no

change in morphology is brought by dosage if they are looked from the bottom. However, a clear change in cell thickness is seen. Therefore, these parameters were statistically analyzed.

As shown in **Fig. 3**, the cell thickness decreases after the application of ACNU. In addition, as shown in **Fig. 4**, it seems that the cell contact area increases after the application, although the tendency is only a little.

These findings suggest the destruction of intracellular structures surrounding the nucleus. Although ACNU is supposed to attack chromosome inside the nucleus, the above result indicates that the drug is affecting the region that surrounds the nucleus as well. As chromosome is closely in connection with microtubules, ACNU would destabilize microtubules, and finally lead to the destabilization of organelles that surround the nucleus.

These changes inside the cell would lead to the morphological change in three-dimensional structures. Recently it is able to observe three-dimensional cell image using the optical method, with which in many cases cells are exposed to strong light emission, and may damage cell functions. On the contrary, it is advantageous that ultrasonic observation can be performed non-invasively, as the strength of the employed pulse sound is very small. In addition, as the spatial resolution along the depth is much smaller than wavelength, it is expected that as small as sub-micrometer in resolution would be realized.

It would be possible to estimate internal acoustic impedance distribution by tracing the deconvolved waveform like one in **Fig. 1 (b)**<sup>[3]</sup>. However in this series of data acquisition the waveform was not stable to realize such an attempt, as significant error was seen. Improvement of both hardware and software is required.

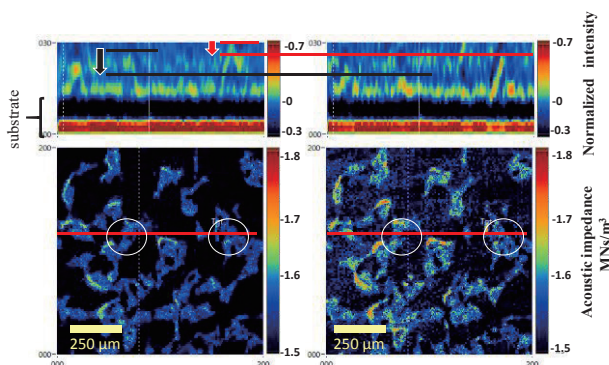


Fig. 2 Typical result of observation.

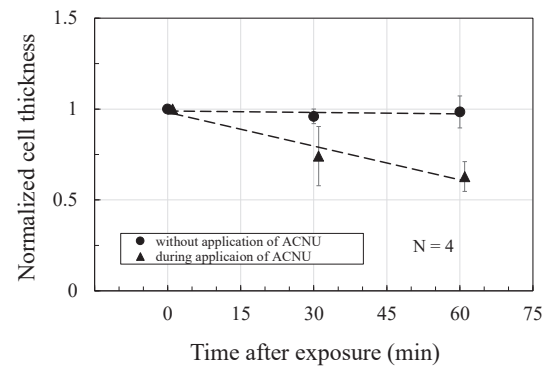


Fig. 3 Change in time of cell thickness after the exposure to ACNU.

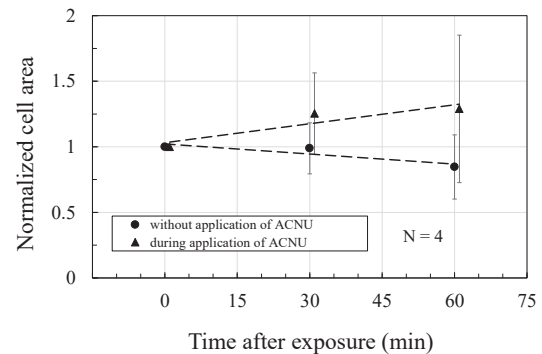


Fig. 4 Change in time of cell area after the exposure to ACNU.

#### 4. Conclusion

Non-invasive cell assessment by means of acoustic microscope has been performed. The acquired waveform that represents the reflection from the cell was subjected to the signal processing that can separate the waveform into reflections from the bottom and top of the cell. The two reflections are interpreted into the cell thickness assuming a certain expected sound speed through the cell. In addition, areal extension of each cell was estimated from the two-dimensional acoustic profile. The assessment was applied to cultured breast cancer cells that were being exposed to ACNU anti-cancer drug. It was suggested that the cell thickness significantly reduced after the exposure. As the areal cell extension increased after the exposure as well, the cells seemed to be flattened as the result of drug application.

#### References

1. Naohiro Hozumi et al. 2013 Joint UFFC, EFTF and PFM symposium, 1212-1215(2013)
2. Agus Indra Gunawan et al. Ultrasonics 61, 79-87(2015)
3. Tan Wei Chean et al. Jpn. J. Appl. Phys. 56, 07JF18 (2017)