IRES²プロジェクト研究

# 酵素光デバイスと 超音波顕微鏡を用いた発達期神経細胞障害の生理機能評価と医療への応用

プロジェクトメンバー：ダイバーシティ推進センター吉田祥子

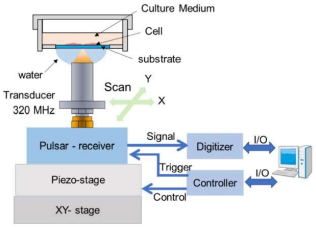
## Materials and Methods

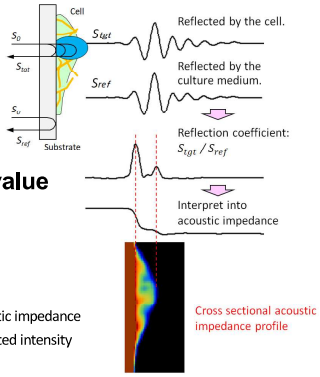
### Scanning Acoustic Microscopy (SAM)

#### SAM's measuring system

Ultrasound waves, whose central frequency is 320 MHz, are sent to the target applying a voltage to the transducer and received the reflection of the waves as signals using the same device. The signals are defined as the reflected intensities.

We obtained the reflected intensities of the target by scanning the transducer two-dimensionally under the dish, whose bottom surface is made of a 50 µm thick polystyrene (PS) film.




#### Calculation of Acoustic Impedance value

$$Z_{tgt} = \frac{1+\frac{S_{tgt}}{S_0}}{1-\frac{S_{tgt}}{S_0}} Z_{sub} = \frac{1-\frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}}}{1+\frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}}} Z_{sub}$$

$Z$ : Acoustic impedance
$S$ : Reflected intensity

#### Z-scope analysis and Three-dimensional image

The acoustic impedance of the interface between cultured cells and substrate can be calculated from the above formula. *Sref* and *Stgt* are the reflected intensities from the medium and cells, respectively. *Zsub* and *Zref* are the specific acoustic impedances of the substrate and medium, respectively. The multiple signals *Stgt* reflected from the cell include reflections due to the internal structure of the cell. Using the reflected component *Sref* from the medium, the reflection coefficient of the locally reflected wave can be calculated as shown in the figure on the right. From the obtained reflection coefficients, the specific acoustic impedance of the structure are calculated one after another, and a tomographic image of the cells is constructed.

### Microglia (MG) and Granule Cell (GC)

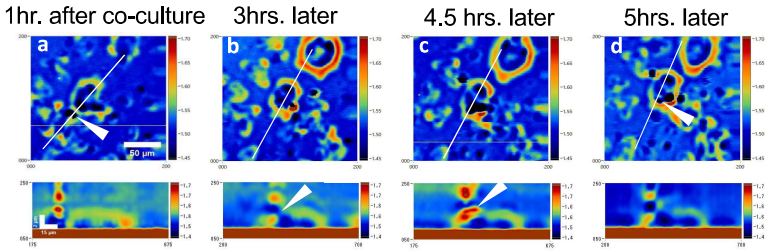
Microglia (MG) is one of glial cells, which are brain immune cells, and granule cell (GC) is one of neurons. MG's roles are promoting synaptogenesis, protecting neurons and phagocytosis. Currently, excessive phagocytosis resulting from abnormal activation of MG is known to be one of the causes of neurodegenerative diseases such as Alzheimer's disease. However, the dynamics and three-dimensional structure of phagocytosis are not known. Therefore, targets for phagocytic control have not been identified.

## Purpose

**To consecutively observe specific dynamics and three-dimensional cell-cell interactions during phagocytosis.**

## Consecutively observation

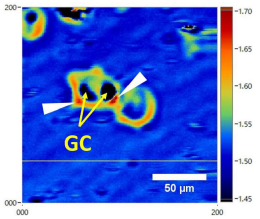
### The interaction between MG and GC



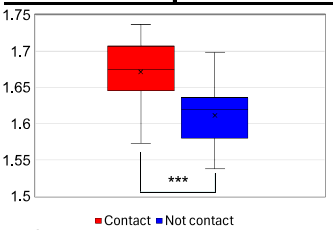

XY-plane image of **figure a** showed out of MG and GC's plasma membranes were contacting 1hour after co-culture. Cross-sectional image of **figure b** showed MG's plasma membrane opened 3hours later. Cross-sectional image of **figure c** showed MG's plasma membrane closed and there is high acoustic impedance area four and a half hours later. XY-plane image of **figure d** showed acoustic impedance decreased of MG's plasma membrane and there was GC under MG 5hours later. This consecutive observation suggests GC is taken up through the MG membrane.

## Difference of Acoustic Impedance

### XY-plane




### Acoustic Impedance value

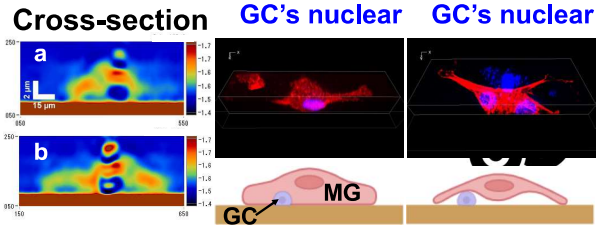



Contact: N = 43, Not contact: N = 106
*** P value < 0.005

The acoustic impedance value around GC increased significantly inside MG when MG contact with GC. The difference of acoustic impedance value reflects the difference of densities of structure. Therefore, indicates that the structure, which have stiffness, trap GC within MG as shown in the schematic on the upper right.

## Acoustic Impedance distribution

### Cross-section




- Scanning acoustic microscopy is useful to visualize three-dimensional cell-cell interaction in co-culture condition non-invasively and consecutively.
- The acoustic impedance value from the signals obtained with scanning acoustic microscopy reflects changes intracellular stiffness.
- This study propose that the cytoskeleton is a potential target for controlling phagocytosis.

Sachiko Yoshida syoshida@tut.jp