Estimation of Stimuli Received by Cell Through Signal Visualization

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I. INTRODUCTION

This paper presents the author’s research on the estimation of sensory information of a cell by using signal visualization. As a single-celled creature, the signal transduction between Paramecium’s interior and its surroundings through the plasma membrane is controlled by a logical sequence. The objective of this research is to develop a method in which this signal transduction within an organism, in this case a Paramecium, can be estimated through signal visualization.

The use of microorganisms, and Paramecium in particular, as microactuators has already attracted several researchers such as Itoh [3], Fearing [2] and Ogawa [6] in the area of dynamic motion control.

Many microorganisms that can barely be seen with the naked eyes are surprisingly well equipped in terms of actuators and sensors. A Paramecium has actuators and sensors all-in-one inside its 150µm length, 40µm diameter semi-elliptic volume.

II. BACKGROUND THEORY

When a Paramecium experiences external stimulation, such as a mechanical stimulation, the intracellular calcium ion concentration ([Ca^{2+}]_{i}) changes [5]. Through the determination of the relationship between the force applied on Paramecium’s anterior part and the emitted fluorescence intensity, realization of force measurement sensor is viable in the future applications.

There are several different kinds of protein signal transmitting receptors but the focus here have been on the mechanoreceptor. Cytosolic [Ca^{2+}]_{i}, level of a Paramecium rises when its anterior membrane is stimulated mechanically. The relationship between [Ca^{2+}]_{i} and depolarization of the membrane potential has been extensively researched. This led to a proposition that states that the [Ca^{2+}]_{i} level rise is dependent on mechanical intensity applied [1]. The whole system inside and outside of a microorganism is quite sophisticated. There are different kinds of Ca^{2+} channels located at the ciliary and somatic membrane that act differently. Here the attention is on the estimation of Ca^{2+} channel activated by a mechanical stimulus, located at the anterior somatic membrane.

III. MATERIALS AND METHODS

A. Ca^{2+} indicator and microinjection

The calcium indicator to be injected was prepared according to Iwadate [4]. The injection medium was composed of 1 mmol^{-1} Calcium Green-1 dextran 10000MW (Molecular Probes, Inc., Eugene, USA), 150 mmol^{-1} KCl, 0.5 mmol^{-1} Pipes (pH 7, adjusted using 1 mmol^{-1} NaOH), and will be referred to as “Ca-Green medium.”

Microinjection capillaries were prepared by pulling a glass tube (G-1; Narishige) using a puller (PN-30; Narishige) and forming an angle by a microforge (MF-900; Narishige). The final diameter of injection capillary was about 0.5-1µm.

Using a microloader (Eppendorf), Ca-Green medium was placed inside the injection capillary through backfilling method. The volume of solution injected into the cell body corresponded to about 10% of the cell body.

B. Microscopy and digital imaging

To examine the relationship between the intensities of mechanical stimuli and the cytosolic calcium ion concentration ([Ca^{2+}]_{i}), a fluorescent dye was introduced inside the cell. By illuminating the compound at its absorbing wavelength and then viewing it through a filter that allows only the light of the emitted wavelength to pass, the compound is seen to glow against a dark background.

An inverted microscope (IX 71; Olympus) equipped with a xenon lamp (AH2-RX-T; Olympus) was used to illuminate through an excitation filter. The filter set (XF104-2; Omega Optical) compatible to Calcium Green-1 dextran was chosen to illuminate excitation wavelength and to pick up the emission wavelength. The images of the emitted cell were detected with a cooled CCD camera (Retiga 2000R; QImaging). In order to determine the timing of stimuli, a regular CCD camera (Hitachi) was used as a monitoring camera.

IV. EXPERIMENT

A. Microinjection

Loading of dye into cells was carried out by means of microinjection. The Ca-Green medium was injected into Paramecium under conventional light microscopy. A successfully injected cell appeared healthy; i.e., the cell resumed regular ciliary beating and no extrusions of intracellular components were observed after the injection.
B. Ca$^{2+}$ level

The cell loaded with fluorescent indicator was confined to a drop of 2% methylcellulose, surrounded by 100:1 silicon oil. The cell was left to swim freely where avoidance and escape reaction were repeated.

Fluorescent emission was captured with cooled CCD camera. Simultaneously, a conventional CCD camera was used to record the non-emission wavelength to enable monitoring the cell movement.

V. RESULTS

From the experiment, there were three results. First, the calcium imaging of a Paramecium was successfully recorded through emission and excitation wavelengths. Second, transient change in fluorescence intensity was visually recorded. Third, estimation of anterior collision points were made using a very simple algorithm.

Pseudocoloured fluorescent image along with that monitored with a regular CCD camera is shown in Fig. 1.

![Fig. 1. Pseudocoloured image of a Paramecium after a few minutes of indicator injection and repeating avoidance reaction and image monitored with Hitachi CCD camera.](image)

Estimation of the duration of received stimuli sensed is shown in Fig. 2. Top graph shows the pixel count of intensity above a threshold. An estimation of stimuli sensed is shown in the second graph. Third graph shows the timing of anterior collision estimated. The last graph is the actual cell movement.

Comparison of sensation received estimated and the actual cell movement shows that the estimation can be used to determine cell’s intracellular sensing information.

VI. CONCLUSION

In this paper, a method to recognize the sensor information in Paramecia was proposed. Unicellular cells are equipped with built-in actuators and sensors, hence they are considered as an excellent work force. The goal of visualizing the estimation of signal information in a Paramecium when it received stimuli was met.

In order to determine the sensing information received by the cell, the visualization of [Ca$^{2+}$] level is necessary. To visualize [Ca$^{2+}$], a fluorescent indicator was loaded to the cell through the method of microinjection. The indicator in the cell was excited and the fluorescence emitted according to the intensity of [Ca$^{2+}$]. In analyzing the recorded image on cooled CCD camera, pseudocolour was used in highlighting pixel intensities.

Although the microorganism used in this research was limited to Paramecia, similar characteristics and methods should be applicable with many other single-cells as well.

REFERENCES


