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Visualization and Estimation of Contact Stimuli using Living Microorganisms

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***Abstract*— In this paper, we propose a novel microsensing scheme in which intrinsic sensing capability in microorganisms is utilized. Extraction of signals inside the cell and decoding of stimuli received would be an interesting challenge toward development of novel biomimetic sensors, or for potential utilization of a living microorganism itself as a microrobot. As a prototype, we focus on the mechanosensory process in *Paramecium* cells. When a mechanical stimuli is applied, Ca^{2+} ion concentration in the cell rises. By visualizing the Ca^{2+} level rise by using Ca^{2+} fluorescent indicators and input intensity data into PCs, we can extract the sensation perceived by the cell. A simple experiment was performed *in vivo* and contact sensation was successfully extracted and decoded. It can be applied for on-board sensors in cells as microrobots in future works.**

***Index Terms*—Paramecium, microorganism, microrobot, mechanoreceptor, calcium indicator, contact**

I. Introduction

All living things have a great capability to sense external stimuli. Even a tiny unicellular microorganism can sense the light, heat, contact, gravity and chemicals with high precision. Various receptors on the cell membrane detect the external stimuli, and induce chemical or electrical events, or signals, inside the cell. In other words, the received stimuli are encoded as a signal transduction. The signals induce subsequent events, and thus the cell reacts appropriately to the external changes.

The sensing ability of living things are superior to most of the existing artificial microsensors. It would be of great benefit and interest to utilize such sophisticated capabilities built in living cells *in vivo*. As illustrated in Fig. 1, extraction, decoding, and feedback of the sensation received by microorganisms by computers would be an interesting challenge toward development of novel biomimetic sensors, or for potential utilization of a living microorganism itself as a sensor, coupled with robotic maneuvers of living cells that the authors have achieved [1–4]. Technology to link living cells and computers would lead to the cell machine interface, to come next after the forthcoming brain machine interface (BMI) paradigm.

As an example, we introduce *Paramecium caudatum*, a kind of unicellular protozoa, which we have utilized for microrobotic application [1]. A *Paramecium* cell detects a contact stimuli and swims back so as to avoid an obstacle.

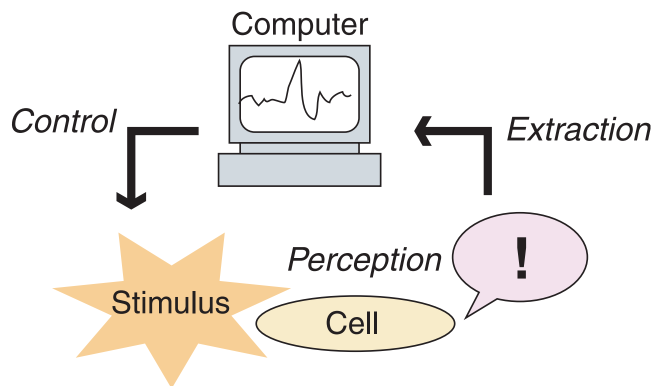

Fig. 1. Our concept: Stimuli perceived by a cell is extracted, taken in computers, and decoded.

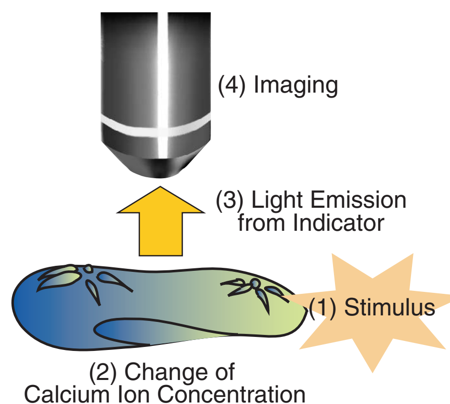

Fig. 2. Concept of visualization of perception by the cell.

During this behavior, the Ca^{2+} ion concentration rises inside the cell. In other words, the stimulus information is coded as a Ca^{2+} signal. If we can extract this mechanosensation signal and decode it, we can estimate what the cell perceived. For extraction of the signal, some visualization methods would be effective. For example, Ca^{2+} indicators can visualize the intracellular Ca^{2+} concentration, as illustrated in Fig. 2.

In this paper, we propose a novel scheme to extract stimuli perceived by living cells. As a prototype, we consider visual-

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ization of contact stimuli using *Paramecium caudatum* cells. When a paramecium cell receives a contact stimulus, a rise in Ca^{2+} concentration occurs inside the cell, which can be visualized by Ca^{2+} indicators. We will introduce an indicator into cells by microinjection, visualize the signal transduction, and estimate the timings of when the cell detected contact stimuli. Although this is a preliminary study, it would be a significant step for biomimetic sensors or utilization of microorganisms as smart microrobots.

II. Background Theory

A. Mechanical Receptors as Sensors

Paramecium caudatum is an ellipsoidal unicellular protozoa that inhabits freshwater [5]. The size of a cell is about 200 μm in length, large enough to be observed easily by an optical microscope. It swims by waving cilia on its body; thousands of cilia beat the water backward to yield forward propulsion by means of a reaction force [6]. The cilia therefore act as tiny actuators.

One of the most researched characteristics of paramecium cells is the "avoiding reaction" [7]. This is a phenomenon commonly seen when a freely swimming paramecium's anterior end hits an obstacle. During such an occurrence, it swims backward for a while and then change its direction and swims forward again. Hence, it literally avoids an obstacle in making its way forward. In 1970s, Eckert and his colleagues revealed that mechanoreceptors, a kind of receptor that is scattered throughout on the cell membrane, play a role as contact sensors [8], [9].

The receptor is a special membrane protein, and identified as a kind of ion channel, a microscopic gate to transport several ions [10]. When a stimulus is applied, the channel opens and specific ions flow in or out through it. This leads to a change of the membrane potential, followed by some reaction of the cell against the stimulus. We can say that the receptor is a sensor to transform physical values to electric signals.

As illustrated in Fig. 3, a *Paramecium* cell has mechanosensitive Ca^{2+} channels (mechanoreceptors) localized in the anterior side of the soma [11–13]. The sequence for the reaction of a paramecium cell against a mechanical reaction is as follows:

1) When a contact stimuli is applied at the anterior side, the membrane is deformed.
2) Mechano-sensitive Ca^{2+} channels detect deformation of the membrane and open their pores. Ca^{2+} ions flow in, causing a depolarization (a rise of the membrane potential).
3) Subsequently, it evokes an activation of depolarization-sensitive Ca^{2+} channels localized on the cilia membrane, and much more Ca^{2+} ions enter [14]. This two-step mechanism amplifies faint signals by a positive feedback.
4) The depolarization causes the ciliary beating reversal and the cell swims backward. Macroscopically, the cell shows an avoiding reaction.
5) When the Ca^{2+} concentration exceeds some threshold, the depolarization-sensitive Ca^{2+} channel closes and the Ca^{2+} inflow stops. Ca^{2+} pumps on the membrane transport the Ca^{2+} ions away from the cell to restore the ion balance [15], [16].

This is a very brief explanation; many other processes are omitted here for the sake of simplicity. In this paper, we will not consider or discuss each process. Instead, we deal with the process as a black box and concentrate on the relationship between contact stimuli and the rise in Ca^{2+} concentration.

B. Extraction of Sensation from Cell by Ca^{2+} Indicator

As described above, a mechanical stimulation activates Ca^{2+} channels, which induces a rise in Ca^{2+} level. Thus, we can estimate the stimulus information from a change in Ca^{2+} level. It is a kind of inverse problems.

Among several existing methods for the measurement of Ca^{2+} levels, visualization of the Ca^{2+} concentration by some UV-excitable indicator reagents is a feasible and promising method for our purpose. Under UV radiation, Ca^{2+} indicators emit a fluorescent light whose intensity varies according to the Ca^{2+} concentration [17]. By loading indicators into the cell body, we can visualize a Ca^{2+} concentration map. Such a Ca^{2+} imaging method is suitable for our goal, because it is a non-contact, non-invasive measurement appropriate for experiments with mechanical stimuli, and it has high feasibility.

III. Materials and Methods

We performed an *in vivo* experiment to verify the feasibility of our concept.

A. Specimens and Medium

As a specimen, *Paramecium caudatum* 27aG3 stock was cultured at 25 ℃ in Dryl's solution [18] containing lettuce juice. The stock 27aG3 is a mutant which does not eject trichocysts (a kind of microscopic spears for defense), and its nature is convenient for the operator to perform microinjection. Cells grown to the logarithmic or stationary phase (4-10 days after incubation) were collected together with the solution and infused into the dishes. To decelerate the swimming velocity, cells were dispersed in a highly viscous medium containing 2% methylcellulose.

B. Loading of Indicators into Cells

In order to visualize the Ca^{2+} concentration inside the cell, we have to load some Ca^{2+} indicators into it. Unlike typical cells, the protozoa have a hard layer called pellicle under the membrane, which disables the fluorescent indicators from permeating a cell naturally [19–21]. Therefore, we used microinjection method for loading.

The Ca^{2+} indicator to be injected was prepared according to Y. Iwadate [20]. We chose Calcium Green-1 dextran for the indicator, because its effectiveness for *Paramecium* cells is established [20], [22–24]. The injection medium was composed of 1 mM Calcium Green-1 dextran 10000MW (Molecular

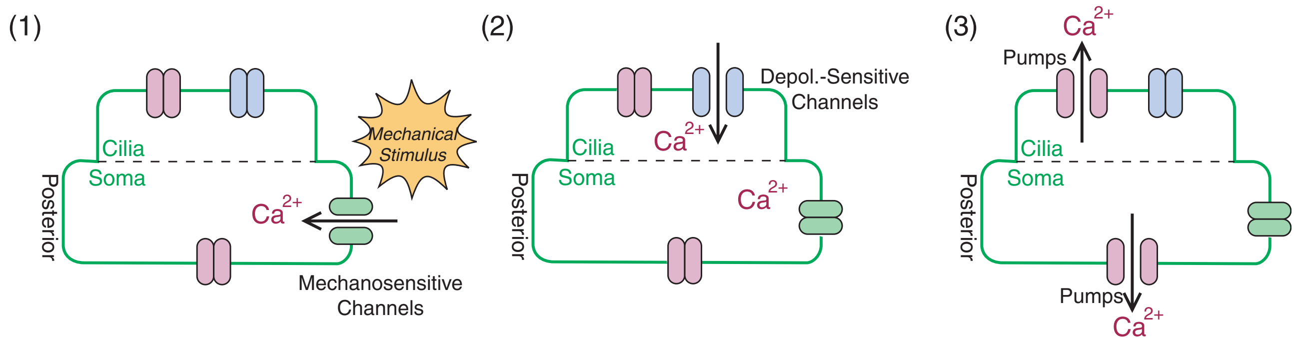

Fig. 3. A simplified scheme for mechanosensory process in *Paramecium* cell. (1) A mechanical stimulus activates mechanosensitive channels localized on the anterior side of the soma, and Ca^{2+} ions flow in. (2) Inflow of Ca^{2+} causes depolarization, which evokes activation of depolarization-sensitive channels, and the Ca^{2+} level rises significantly. (3) When the Ca^{2+} level surpasses the threshold, channels are inactivated and Ca^{2+} ions are exported by the Ca^{2+} pumps.

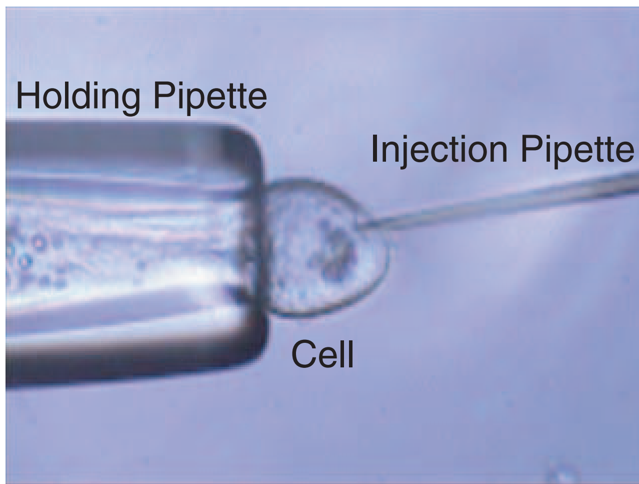

Fig. 4. Loading of indicators into a cell by microinjection.

Fig. 5. A cell trapped within a droplet. Monitored with a color CCD camera (Hitachi, KP-D).

Probes, Inc.), 150 mM KCl, 0.5 mM Pipes (pH 7, adjusted using 1 mM NaOH). It was then microinjected into the cell by using the microinjection system (Narishige), as shown in Fig. 4. The volume of solution injected corresponded to about 10% of the cell body. We confirmed that the cell remained vibrant after the microinjection and had sufficient time to settle down after the injection stimulus.

C. Trapping, Stimulation and Imaging

In order to trap the cell within a small region for the ease of observation, the cell was moved from the injection environment to a restricted droplet with 40 $\mu\ell$-volume methylcellulose medium surrounded by highly viscous wall of 100 cs silicon oil, as shown in Fig. 5. This method has the advantage of keeping the cell in a relatively small area without unnecessary stress on the cell. Inside the droplet, when the head of the swimming cell collides with the wall of the droplet, it starts to swim backward (avoiding reaction). Then its posterior side collides with the wall, and the cell switches to forward swimming again. Thus the cell swims back and forth. We regarded these collisions as mechanical stimuli, and visualized the signal transduction within the colliding cell.

Fig. 6 illustrates the system configuration. The cell was observed via an inverted microscope (Olympus, IX71). Simultaneous use was made of a cooled monochrome CCD (Qimaging, Retiga 2000R) camera that was used to capture the movie of the faint fluorescent images while a normal color CCD camera (Hitachi, KP-D) was used for monitoring by humans Several dichroic mirrors and filters were mounted in the microscope to separate the excitation light from a xenon lamp and the emitter light from probes and halogen illumination. The frame interval of Retiga 2000R was set to 982 ms, and each frame was exposed to UV light for 100 ms. The images were 587×859 and 12-bit grayscale. Pseudo-color fluorescence images were processed and displayed in real-time using the imaging software QCapture Pro (Qimaging). The images were also stored and analyzed by the numerical calculation software MATLAB (MathWorks) offline.

IV. Results and Discussion

Fig. 7 shows an example of the captured image sequence of the detected fluorescence. The images were pseudo-colored

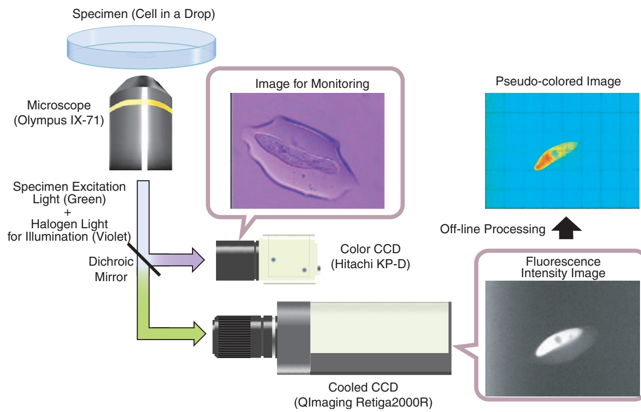

Fig. 6. System configuration. The cell on a plastic dish was observed via an inverted microscope (Olympus, IX71). The movie was captured by a cooled monochrome CCD camera (Qimaging, Retiga 2000R) and a normal color CCD camera (Hitachi, KP-D). Pseudo-color fluorescence images were processed and displayed in realtime

using the QCapture Pro, where the color ranges from blue to red, and red indicates higher Ca^{2+} levels. After stimulus application, one can find that the Ca^{2+} level increased rapidly and then decayed slowly. Note that the indicator, Calcium Green-1, indicates only relative concentration of Ca^{2+} ions, and more quantitative evaluation can be performed by using indicators with two excitations and rationing method. It is also noteworthy that this is the world's first report on visualization of Ca^{2+} level in *Paramecium* cells against contact stimuli as far as we know, though there are reports on imaging during exocytosis [21], [25], [26], calcium oscillations [27], artificial loading of caged Ca^{2+} [23], [28] and attack by *Didinium* [20], or other species such as *Vorticella* [29].

From the intensity of the psuedocolored image sequences in Fig. 7, we retrieved the information on whether or not the head of the cell made a contact, i.e., on/off 1 bit information. In the future, when the method of calibration is improved, the contact intensity of the cell should be discernible.

Using Ca^{2+} level data, we estimated the sensation the cell perceived, and the timings of contact. The estimation was performed off-line by MATLAB. The sequence is as follows:

1) Each image was binarized with the threshold of 430 and total "on" pixels were counted, as shown in Fig. 8 in blue bars.
2) We regarded the period during which the pixel count was over 25 as the sensation duration (green areas).
3) We regarded the rising edges of areas estimated above as the starting points for contact (red arrows).

Note that we realized a simple on-off contact sensor because the indicator was not suitable for rigorous quantitative evaluation, and estimation of the magnitude of the stimuli is to be performed by using indicators with two excitation wavelengths in the future. Although the retrieved information is not substantial, we have succeeded in the extraction of the perception in this experiment.

We also analyzed the actual contact timings from video sequences captured by Retiga 2000R to verify our estimation (blue arrows). Fig. 8 shows four contacts occurred in 30s were successfully estimated with a few seconds of delays. The cause of the variable time-delay from the instant of the actual contact and the estimated contact delays are due to the latency of series of physiological reaction. Although we would like to consider in our future works how to deal with these delays, along with how to determine thresholds, this is the first step in our attempt to extract sensory information from cells. As challenges for the future, the problems regarding the latency, calibration and individuality still remains to be done.

V. Conclusion

In this paper, we proposed a novel microsensing scheme in which intrinsic sensing capability in microorganisms is utilized. As a prototype, we chose the mechanosensory process in *Paramecium* cells. A simple experiment was performed in order to verify its feasibility, and we succeeded in extracting and decoding the contact sensation from the cell.

One promising application of our concept would be active and smart microsensors organized by living microorganisms. Microorganisms are known to have sophisticated sensors and actuators. Therefore, if we can develop techniques to control them freely, we can realize multi-purpose, programmable microrobotic systems that are superior to existing man-made micromachines. From this viewpoint, we have achieved motion control of *Paramecium caudatum*, along with micromanipulation by a cluster of cells, and modeling of dynamics from the viewpoint of a robotic framework [1–4]. On-board sensor function is a next important step for autonomous microrobotic systems to interact properly with the environment.

In the experiments reported above, we succeeded in visualization of contact stimuli. However, problems regarding the latency, calibration or individuality were also revealed, which are to be investigated more closely in future works for microsensor applications.

Acknowledgment

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References

[1] N. Ogawa, H. Oku, K. Hashimoto, and M. Ishikawa, "Microrobotic visual control of motile cells using high-speed tracking system," *IEEE Trans. Robotics*, vol. 21, no. 4, pp. 704–712, Aug. 2005.

[2] J. Yamane, N. Ogawa, H. Oku, K. Hashimoto, and M. Ishikawa, "A current controlled electrostimulation device for the motion control of Paramecia," in *Proc. 2004 JSME Conf. Robotics & Mechatronics (Robomec'04)*, Jun. 2004, pp. 1A1–H–28, in Japanese.

[3] K. Takahashi, N. Ogawa, H. Oku, and K. Hashimoto, "Organized motion control of a lot of microorganisms using visual feedback," in *Proc. 2006 IEEE Int. Conf. Robotics & Automation (ICRA2006)*, May 2006, pp. 1408–1413.

[4] N. Ogawa, H. Oku, K. Hashimoto, and M. Ishikawa, "A physical model for galvanotaxis of paramecium cell," *J. Theor. Biol.*, 2006, (in press).

[5] H.-D. Görtz, Ed., *Paramecium*. Springer-Verlag, 1988.

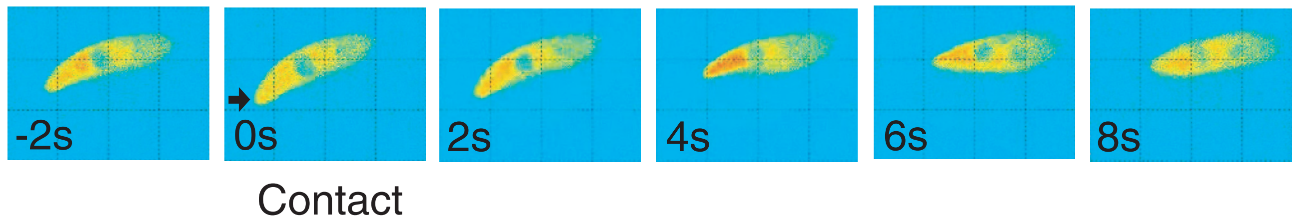

Fig. 7. Pseudocolored image sequences of the detected fluorescence. The color ranges from blue to red, and red indicates higher Ca^{2+} level. The approximate contact point is indicated by the arrow in the 0s frame. After stimulus application at 0s, the Ca^{2+} level increased rapidly and then decayed slowly.

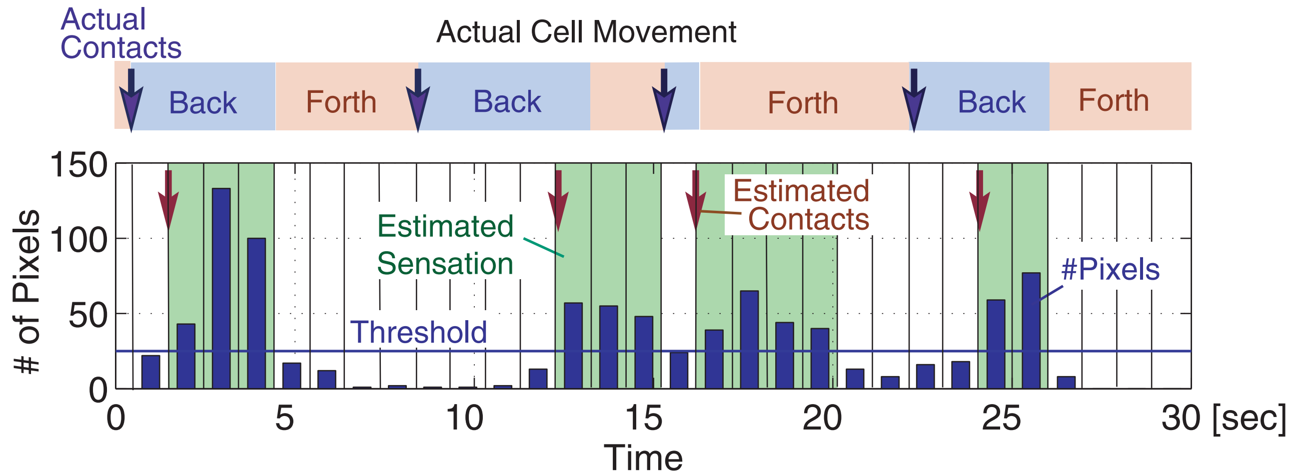

Fig. 8. Estimated sensation sequence and the actual stimuli. Blue bars: the number of pixels whose intensity is over 430. Green areas: estimated duration for contact sensation. Red arrows: estimated start points for contact. Blue arrows: actual contact moments. Words "Back" and "Forth" indicate the direction of swimming. Contacts were successfully estimated with a few seconds of delays.

[6] Y. Naitoh and K. Sugino, "Ciliary movement and its control in *Paramecium*," *J. Protozool.*, vol. 31, no. 1, pp. 31–40, 1984.

[7] H. S. Jennings, *Behavior of the Lower Organisms*. Columbia University Press, 1923.

[8] R. Eckert, Y. Naitoh, and K. Friedman, "Sensory mechanisms in *Paramecium*: I. two components of the electric response to mechanical stimulation of the anterior surface," *J. Exp. Biol.*, vol. 56, pp. 683–694, 1972.

[9] Y. Naitoh and R. Eckert, "Sensory mechanisms in *Paramecium*: II. ionic basis of the hyperpolarizing mechanoreceptor potential," *J. Exp. Biol.*, vol. 59, pp. 53–65, 1973.

[10] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*, 4th ed. Garland Science, Mar. 2002.

[11] A. Ogura and H. Machemer, "Distribution of mechanoreceptor channels in the *Paramecium* surface membrane," *J. Comp. Physiol. · A*, vol. 135, pp. 233–242, 1980.

[12] H. Machemer and A. Ogura, "Ionic conductances of membranesin ciliated and deciliated *Paramecium*," *J. Physiol.* , vol. 296, pp. 49–60, 1979.

[13] R. Eckert and Y. Naitoh, "Bioelectric control of locomotion in the ciliates," *J. Protozool.*, vol. 19, no. 2, pp. 237–243, 1972.

[14] K. Dunlap, "Localization of calcium channels in *Paramecium caudatum*," *J. Physiol.* , vol. 271, pp. 119–133, 1977.

[15] J. L. V. Houten, W. Q. Yang, and A. Bergeron, "Chemosensory signal trandsuction in *Paramecium*," *J. Neutrition*, vol. 130, no. 4, p. 946S, Apr. 2000.

[16] B. Martinac and E. Hildebrand, "Electrically induced Ca^{2+} transport across the membrane of *Paramecium caudatum* measured by means of flow-through technique," *Biochim. Biophys. Acta*, vol. 649, no. 2, pp. 244–252, Dec. 1981.

[17] G. Grynkiewicz, M. Poenie, and R. Y. Tsien, "A new generation of Ca^{2+} indicators with greatly improved fluorescence properties," *J. Biol. Chem.*, vol. 260, no. 6, pp. 3440–3450, Mar. 1985.

[18] S. Dryl, "Effect of adaptation to environment on chemotaxis of *Paramecium caudatum*," *Acta Biol. Exp.*, vol. 19, pp. 83–93, 1959.

[19] J. Pernberg and H. Machemer, "Fluorometric measurement of the intracellular free Ca^{2+}-concentration in the ciliate *Didinium nasutum* using Fura-2," *Cell Calcium*, vol. 18, pp. 484–494, 1995.

[20] Y. Iwadate, K. Katoh, H. Asai, and M. Kikuyama, "Simultaneous recording of cytosolic Ca^{2+} levels in *Didinium* and *Paramecium* during a *Didinium* attack on *Paramecium*," *Protoplasma*, vol. 200, pp. 117–127, 1997.

[21] N. Klauke and H. Plattner, "Imaging of Ca^{2+} transients induced in *Paramecium* cells by a polyamine secretagogue," *J. Cell Sci.*, vol. 110, pp. 975–983, 1997.

[22] Y. Iwadate and T. Suzaki, "Ciliary reorientation is evoked by a rise in calcium level over the entire cilium," *Cell Motil. Cytoskeleton*, vol. 57, pp. 197–206, 2004.

[23] Y. Iwadate, M. Kikuyama, and H. Asai, "Photolysis of caged Ca^{2+} induced trichocyst discharge in *Paramecium caudatum*," *Protoplasma*, vol. 206, pp. 11–19, 1999.

[24] Y. Iwadate, K. Katoh, M. Kikuyama, and H. Asai, "Ca^{2+} triggers toxicyst discharge in *Didinium nasutum*," *Protoplasma*, vol. 206, pp. 20–26, 1999.

[25] C. Erxleben, N. Klauke, M. Flötenmeyer, M.-P. Blanchard, C. Braun, and H. Plattner, "Microdomain Ca^{2+} activation during exocytosis in *Paramecium* cells. superposition of local subplasmalemmal calcium store activation by local Ca^{2+} influx," *J. Cell Biol.*, vol. 136, no. 3, pp. 597–607, Feb. 1997.

[26] N. Klauke and H. Plattner, "Caffeine-induced Ca^{2+} transients and exocytosis in *Paramecium caudatum* cells. a correlated Ca^{2+} imaging and quenched-flow/freeze fracture analysis," *J. Mem. Biol.*, vol. 161, pp. 65–81, 1998.

[27] M. Prajer, A. Fleury, and M. Laurent, "Dynamics of calcium regulation in *Paramecium* and possible morphogenetic implication," *J. Cell Sci.*, vol. 110, pp. 529–535, 1997.

[28] Y. Iwadate, "Photolysis of caged calcium in cilia induces ciliary reversal

in *Paramecium caudatum*," *J. Exp. Biol.*, vol. 206, pp. 1163–1170, Apr. 2003.
[29] K. Katoh and M. Kikuyama, "An all-or-nothing rise in cytosolic [Ca^{2+}] in *Vorticella* sp." *J. Exp. Biol.*, vol. 200, pp. 35–40, 1997.