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Motile Cell Galvanotaxis Control using High-Speed Tracking System

Naoko Ogawa*, Hiromasa Oku†*, Koichi Hashimoto*† and Masatoshi Ishikawa*
*Graduate School of Information Science and Technology, University of Tokyo
7–3–1, Hongo, Bunkyo-ku, Tokyo 113–8656, Japan
†PRESTO, Japan Science and Technology Agency
4–1–8 Honcho Kawaguchi, Saitama, Japan
E-mail: {Naoko_Ogawa,Hiromasa_Oku,Koichi_Hashimoto,Masatoshi_Ishikawa}@ipc.i.u-tokyo.ac.jp

***Abstract*— We propose a control system of motile cells. Our goal is to construct a large-scale Organized Bio-Modules (OBM) in which microorganisms are controlled as micro-size smart robots in an organized way. For the first step, we have developed a visual feedback control system of *Paramecium caudatum* cells. Tracking method is used for observation of moving cells with high magnification. Cells swim in a chamber and their positions are measured by high-speed vision. The chamber position is visually controlled to track a specific cell. The cell motion is controlled electrically by utilizing the galvanotaxis (intrinsic reaction to electrical stimulus) of microorganisms. Experimental results of region-trapping demonstrate the possibility of the OBM system.**

***Index Terms*—Organized Bio-Modules, Galvanotaxis, Tracking, Control, Paramecium**

I. Introduction

Recent progress in biotechnology has resulted in growing interests and increased demands for measurement and control of the micro- and nano-scale world. However, such measurement and control require high dexterity, practiced skills and long experiences for human operators. Hence automation technologies to assist operators are needed. Yet conventional micromachine technology has many problems to be solved for its practical application due to its poorly developed and relatively unsophisticated capabilities.

Our approach to overcome these problems is to utilize naturally occurring micromachines, or microorganisms. For all living things, detection of changes in the environment and quick reaction are matters of the greatest importance for survival. Therefore microorganisms have acquired sophisticated sensors and actuators through their evolution. If we can develop techniques to control them freely, we can realize multi-purpose and programmable micro-systems superior to existing micromachine systems.

In this paper, we regard an individual microorganism as a "bio-module," a functional unit having sensors and actuators integrated into its body. Our goal is to eventually construct a large-scale microsystem called "Organized Bio-Modules" (OBM), which integrates controlled bio-modules and information processing systems, as illustrated in Fig. 1. By cooperation of bio-modules, we aim to achieve various applications such as cell manipulation, microscopic delivery, smart microsensors, and assembly of MEMS.

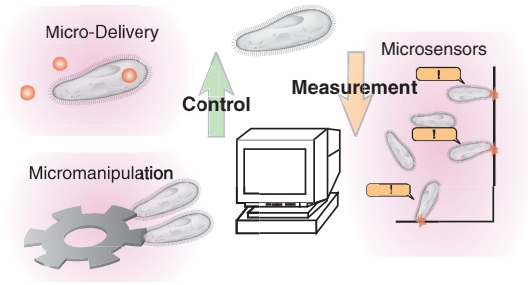

Fig. 1. Concept of Organized Bio-Modules.

Actuation of bio-modules is a key technology for realization of OBM. We focus on "taxis" of microorganisms, an intrinsic locomotor response toward or away from an external stimulus. For example, many protozoa and bacteria exhibit a behavior called galvanotaxis, that is, taxis in response to an electrical stimulus. This implies that it is possible to control their motion by adjusting the electrical stimulus.

There is an essential requirement for realization of OBM; a sufficiently large working area for control of bio-modules. In most conventional micromanipulation systems, however, the working area is limited to within the small visual field of a microscope under high magnification, thus free control is prevented. Itoh achieved the control of the trajectory of a *Paramecium caudatum* cell and actuation to rotate a micro impeller using galvanotaxis [1]. In his system, the working area was limited to within a fixed visual field. In order to realize more advanced actuation for OBM, this constraint must be eliminated.

Therefore we focus on tracking methods; a technique to pursue a target so as to keep it always in the center of the visual field. Since the visual field itself moves according to the target motion, there is in principle no limitation on the working area. Liberation from the constrained small visual field enables us to perform the wider range of operations needed for OBM.

Tracking also has potential for detailed observation of each cell. There is, however, a trade-off relationship between magnification and trackability, because cells swim very quickly and go out of view. As discussed later, use of high-speed vision can effectively overcome this problem.

In this paper, we propose a novel system that can continuously control galvanotaxis of moving cells, using a high-speed tracking system. Experimental results demonstrate the capability of the system using *Paramecium caudatum* cells. This system was constructed for validating feasibility of the single-cell level tracking in OBM, not for implementation of a whole OBM system.

II. Background

In this section, we state the usefulness of OBM, as background of the theme of this paper.

As mentioned in the former section, automation of micromanipulation has become essential in recent biotechnology. Micromachining technology, as represented by Micro-Electro Mechanical Systems (MEMS), is one of the hopes for micro-scale automation. It is difficult, however, to integrate internal sensors which measure the state of the MEMS itself and thus most existing MEMS have only a single function. There seems no report that realized multi-functional and programmable systems with several internal and external sensors, like robots. Moreover, the affinity of MEMS with living bodies is not high enough for biotechnology applications, because such micromechanical and electrical devices are generally too delicate to work in water.

Therefore we focus on microorganisms. We can use microorganisms as smart and autonomous micromachines for the following reasons: (1) they have sensors with high precision, (2) they have wireless and sophisticated actuators (they can move without external power supply), (3) their ease of production (they reproduce themselves), (4) their high affinity with living bodies, and (5) they are large enough for micro-scale operations. In addition, future advances in genetic recombination technology may allows us to even design microorganisms with desired functions. If techniques to control them are developed, we may realize a multi-purpose and programmable micro systems superior to existing MEMS.

Although a number of studies on MEMS have been made [2], and many biosensors using microorganisms have been developed [3], there seem very few studies which have focused on microorganisms as smart motile machines, not as just tools. Arai manipulated yeast cells freely and dexterously by laser micromanipulation [4]. His concept differs from ours, however, in that the intrinsic behavior of microbes was not utilized for actuation. Itoh is a pioneer in using microorganisms as machines and he established a firm basis for this possibility [1]. Now we would like to further improve their capabilities using the high-speed tracking system.

III. Galvanotaxis Control System

A. Related Works on Tracking

Tracking of microorganisms is one of the techniques that many biologists have sought for, because it allows the natural ecology of cells swimming freely to be continuously observed, without fixing them. For instance, Berg *et al.* developed a new microscope which can detect the displacement of a target using six fiber optics fibers and photomultipliers, and succeeded in obtaining the 3-D trajectory of *Escherichia coli* [5]. Thar *et al.* tracked many microorganisms simultaneously with high spatial resolution by using laser beams and CCD cameras [6]. Strickler tracked free-swimming copepods mating by treating them as phase objects and using matched spatial filters [7]. Kuo *et al.* used scattered laser light to track and evaluate the activity of *Listeria* [8]. However most tracking systems reported could detect only a few features of the target, such as its position. To identify a cell and execute intelligent tasks, various types of information about the cell are required. Moreover, each system was specially designed for a specific target, thus lacking versatility. As discussed later, our system has an advantage in that it is versatile and able to extract various features in real-time.

B. Necessity for High-Speed Vision

One major problem in tracking objects with a microscope is the trade-off relationship between magnification and trackability; as the magnification increases, it becomes more difficult to track the target continuously. When the frame rate of the vision system is low, it is difficult to ensure both magnification and trackability.

Suppose that a target moves linearly with constant velocity (a times its diameter per second). Let M be the magnification index, i.e. the ratio of the target length to the visual field width ($M = 1$ means that the target fits exactly in the visual field), f the frame rate of the vision system, and N the trackability index, i.e. the number of frames for the target to cross over the visual field. Then the trade-off can be written as

$$NM = f/a. \qquad \text{(const.)} \tag{1}$$

This implies that certain qualities for both magnification and trackability (i.e. sufficiently large M and N) is not ensured by an insufficient frame rate, when the target moves fast (i.e. a is large).

Most bacteria can swim as fast as 50 diameters/sec [5]. For conventional vision systems with around 30Hz frame rate, we can estimate the value of NM to be 0.6, which is too small for our stated goal, namely the compatibility of magnification and trackability. From this reason, we use a high-speed vision system with 1kHz frame rate, so that NM can be over 20, which is enough for our purposes.

C. Overall System Configuration

Configuration of the overall system and its block diagram are illustrated in Figs. 2 and 3, respectively. This system utilizes the microorganism tracking system by Microscopic Visual Feedback (MVF) [9].

An electrical stimulus is applied on cells swimming in a chamber on an electrical stimulus input device mounted on an XY stage. The stage is controlled by a high-speed vision system so as to keep a cell in the center of the view. By reading encoders of the stage, we can obtain the position of the cell. The orientation of the cell is also calculated from image features.

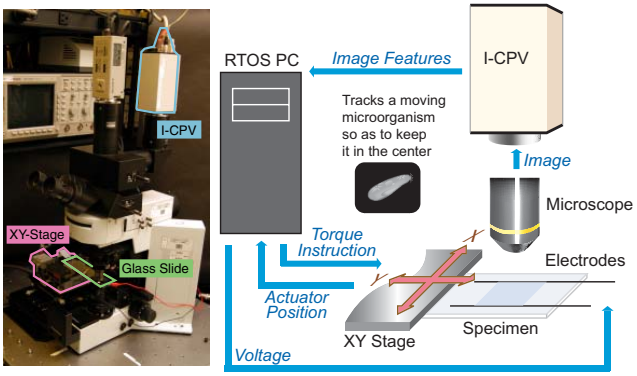

Fig. 2. System configuration.

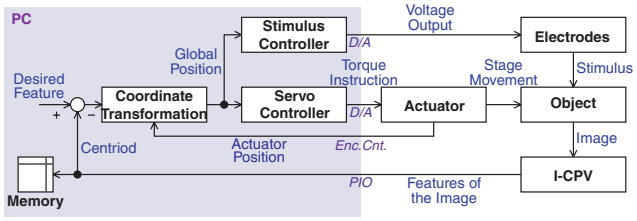

Fig. 3. Block diagram of the system.

D. Vision System and XY Stage

As discussed in III-B, a high frame rate is required for the vision system, in order not to lose the target at high magnification. Moreover, an observing method with weak light, such as dark field microscopy, is needed, so as to prevent heat and light influence on the cells.

To obtain dark images very quickly, we adopted an I-CPV system (Hamamatsu Photonics), which is a Column Parallel Vision (CPV) system with an image intensifier [10]. CPV is a high-speed vision system developed for robotic use [11].

I-CPV captures and processes an 8-bit gray-scale image with 128×128 pixels and 1kHz frame rate. I-CPV has a 128×128 photo detector (PD) array and the same number of programmable general-purpose processing elements (PEs). The captured image is amplified several thousand times by the image intensifier and transmitted to the PEs. Each PE is based on the S^3PE architecture [12], and can process image data completely in parallel. Each PE is connected with a summation circuit, so that it can execute various processes, such as global image feature extraction, edge extraction, embossing and blurring within 1 ms.

In the system proposed here, the I-CPV system is mounted on an upright optical microscope (Olympus, BX50WI) and captures dark-field images. The images are also captured by a CCD camera for convenience of monitoring. From the captured images, the I-CPV calculates image features and sends them to the PC. These features are used for the visual feedback control of an XY stage and microorganisms.

The PC controls the position of a chamber fixed on the XY stage by sending instructions to the stage. The XY stage (SMC, LAL00-X070) has two orthogonal axes, X and Y, whose stroke

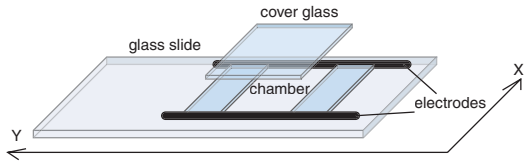

Fig. 4. Electrical stimulus input device.

is 25mm. Each axis has a linear coil actuator to control the stage position. It also has encoders with 1μm precision on each axis. By controlling the stage according to the extracted target position, tracking of the target is achieved.

E. Electrical Stimulus Input Device

Fig. 4 shows the electrical stimulus input device. Two carbon electrodes of 0.5mm diameter are placed in parallel on a glass slide, so that we can control the electrical stimulus in one direction perpendicular to the electrodes. The distance between them is 22mm. Between them, there is a chamber 0.17mm in depth to contain the specimen, The chamber constrains the motion of the cells within the 2-D plane. In order to maintain the chamber depth and to suppress evaporation of the medium, a cover glass is placed on the chamber.

The PC provides a voltage in the range ±10V to the electrodes via a D/A converter board (Interface, PCI-3310). By feedback of the image feature values acquired by the I-CPV, it is possible to control the voltage in real time according to the target status.

The whole system is controlled with a frequency of 1kHz by the PC running a real-time OS (800MHz, ART-Linux). During each cycle, the PC sets the electrical stimulus applied to the microorganisms in the chamber, the I-CPV then captures the image of them and calculates feature values, and the PC sends instructions to the XY stage so as to track the target.

F. Tracking of Cells

We adopted a tracking method called Microscopic Visual Feedback [9].

The I-CPV system provides the 0-th, 1st and 2nd moments of the image every 1 ms. As is well known, the centroid (x_G, y_G) of the target, and its tilt angle θ, shown in Fig. 5, are calculated using a given 0-th moment m_0, 1st moments m_x and m_y, and 2nd moments m_{xx}, m_{yy} and m_{xy} (*e.g.*, see [13]):

$$x_G = m_x/m_0, \quad y_G = m_y/m_0, \tag{2}$$

$$\theta = \frac{1}{2}\arctan\left(\frac{2B}{A-C}\right), \tag{3}$$

where $A = m_{xx} - m_x^2/m_0, \quad B = m_{xy} - m_x m_y/m_0,$
$C = m_{yy} - m_y^2/m_0.$

The 2D position of the XY stage, (X_s, Y_s), is sent from the encoders to the PC. The PC calculates the global target position (X, Y) given by

$$\begin{pmatrix} X \\ Y \end{pmatrix} = -\frac{p}{m}\begin{pmatrix} x_G - \frac{n}{2} \\ y_G - \frac{n}{2} \end{pmatrix} + \begin{pmatrix} X_s \\ Y_s \end{pmatrix}, \tag{4}$$

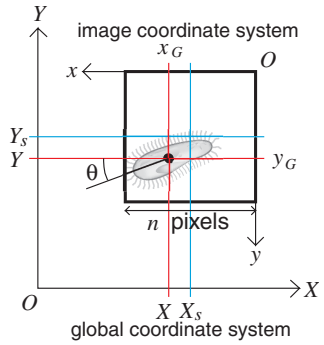

Fig. 5. Relationship between two coordinate systems.

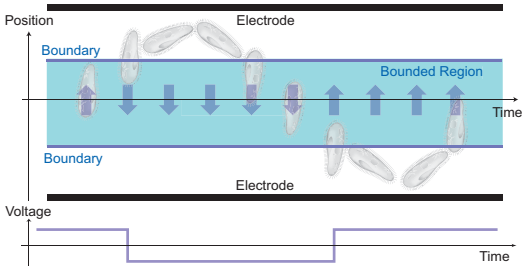

Fig. 6. Trapping of a microorganism within a region.

where p is the pixel pitch, n the number of pixels on an edge of the visual field, and m is the magnification. The minus sign is due to the reversed visual field of the microscope image. Thus the trajectory and the orientation of the target are reconstructed.

High-speed extraction and matching of the target between frames are realized by a so-called self-windowing algorithm [14], using the fact that the difference between frames is very small in high-speed imaging.

The error values in displacement of the target are used to obtain the desired position of the XY stage. The servo system for the stage is designed by using the Smith-Davison design rule [15]. The controller compensates for friction and gravity. By controlling the XY stage, the target is always located in the center of the visual field.

G. Control of Cells

As illustrated in Fig. 3, the image features that are fed back are used for stimulation of the cell. When the PC receives feature values from the I-CPV, it adjusts the stimulus voltage applied to the electrodes.

For example, the system can reverse the voltage when a cell goes out of a certain region, as illustrated in Fig. 6. This allows us to trap the cell within the region. This trapping technique will be a powerful tool for advanced actuation in future OBM systems, such as standing-by before an operation or positioning of an object.

IV. Experiments

In this section, we will describe the experimental results to demonstrate the control capability of the proposed system using motile cells.

Fig. 7. Galvanotaxis of *Paramecium caudatum*. They swim toward the cathode.

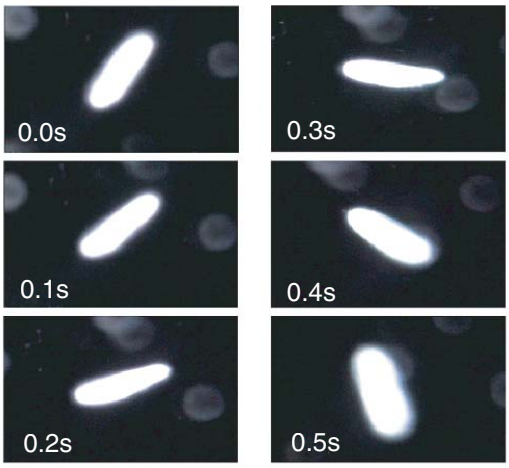

Fig. 8. Sequential photographs of a cell making a U-turn by stimulus reversal (0.1s intervals).

A. Materials

We used *Paramecium caudatum*, a kind of protozoa. *Paramecium* has very strong galvanotaxis; it tends to swim toward the cathode, as shown in Fig. 7. Its strong reaction is suitable for control. The size of a cell is about 200μm in length, enough to be observed easily by an optical microscope.

Wild-type *Paramecium* cells were cultured at 20-25°C in a soy flour solution. Cells grown to the logarithmic or stationary phase (4-10 days after incubation) were collected together with the solution, filtered through a nylon mesh to remove debris, and infused into the chamber.

B. Feedforward Control Experiment

First, feedforward control of cells was performed. Cells were controlled by a stimulus whose pattern was fixed in advance. The electrical stimulus was applied in the X direction, and reversed every 6s. The strength of the voltage gradient was 4.1V/cm (9V across a 22mm gap). A 20× objective lens was used for magnification.

Fig. 8 shows some of the sequential photographs of a cell making a U-turn by stimulus reversal, as captured by the CCD monitoring camera. The time interval between each image is 0.1s. The cell motion was affected by the stimulus and the cell position is kept in the center of the image field.

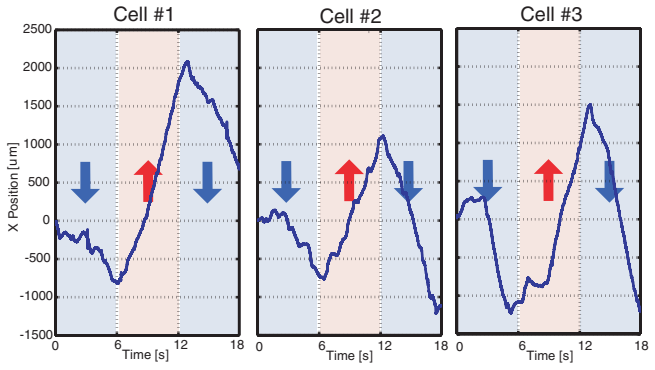

Fig. 9. X position (parallel to the electric field) of cells in feedforward control experiment. Arrows indicate the direction of the applied electric field.

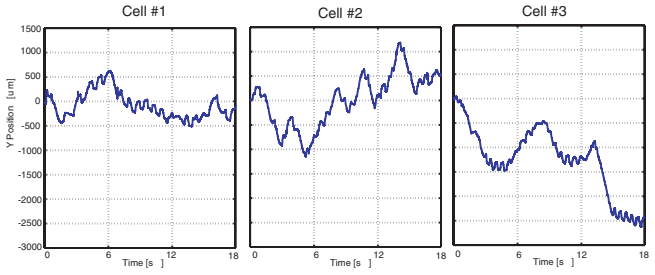

Fig. 10. Y position (perpendicular to the electric field) of cells in feedforward control experiment.

Fig. 9 and Fig. 10 show the time sequence of the positions parallel (X) and perpendicular (Y) to the electric field, respectively. Arrows indicate the direction of the electric field. For the X direction, the cells reacted sensitively to the stimulus and showed almost uniform motions, whereas the motion for the Y direction seemed almost independent of the stimulus. Small fluctuations in Y position were observed, which were due to the spiral wriggling movement of the cells.

As discussed in III-F, we can reconstruct the trajectory and the orientation of the target from the features extracted by the I-CPV system. Fig. 11 and Fig. 12 show the trajectory of the cell # 3 presented in Fig. 9 and Fig. 10, and its orientation (small arrows) respectively, where the direction of the electric field is horizontal. The spiral path and the ever-changing orientation of the cell were reconstructed with high fidelity. It also indicates that both of high magnification and good trackability in a large working area were achieved.

C. Feedback Control Experiment

In order to confirm the ability of visual feedback control of cells, we performed a simple trapping experiment, as described in III-G; the stimulus was adjusted in real time according to the target status. The width of the trapping region was set to 1mm. The voltage was reversed when the cell moved out of the boundaries. Other conditions were the same as those of the feedforward control experiment described above.

Fig. 13, Fig. 14 and Fig. 15 demonstrate results of the control experiment. Fig. 13 shows the time sequence of both

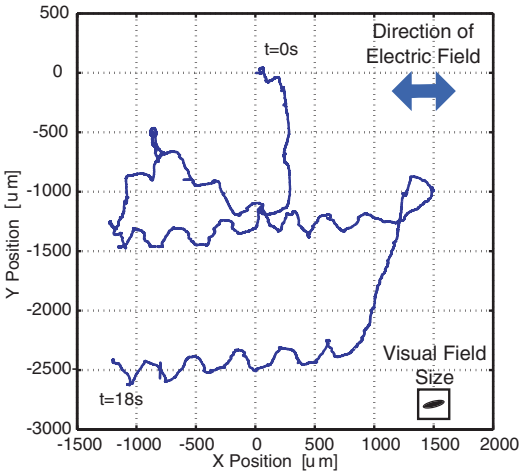

Fig. 11. Trajectory of a cell in feedforward control experiment.

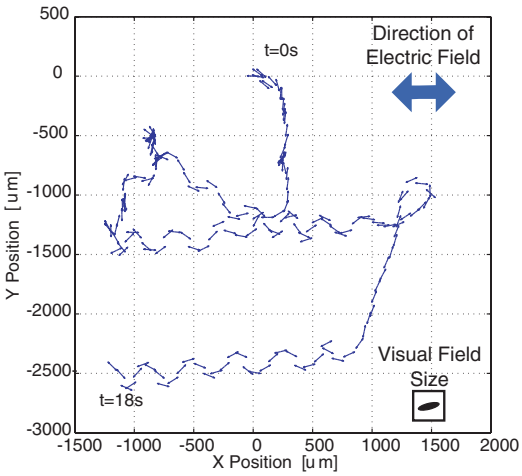

Fig. 12. Orientation of a cell (small arrows) in feedforward control experiment.

the voltage and the position parallel to the field (X). Similarly, Fig. 14 shows the position perpendicular to the field (Y). The voltage was reversed when the cell went out the region. Consequently the cell swam back and forth in the region. The trajectory of the cell is shown in Fig. 15. These figures indicate there is a time delay in U-turn motions of the cell. To achieve more precise control, a motion model of a cell and a model-based control will be required.

V. Discussion

The experimental results suggest that the individuality among cells is substantial and not negligible. A system for screening might be needed for bio-modules to provide co-ordinated motions. Additionally, for future studies, a detailed evaluation of the individuality can also be an interesting topic.

In this paper, the control of microorganisms was restricted within the focal plane. This is due to the inevitable drawback of conventional microscopic imaging methods, where only objects on the focal plane are observable. This constraint is an

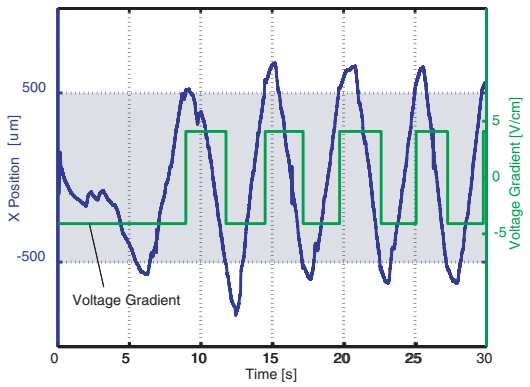

Fig. 13. Applied voltage (green) and X position (parallel to the electric field) of a cell (blue) in feedback control experiment. The shaded region is the bounded region for trapping.

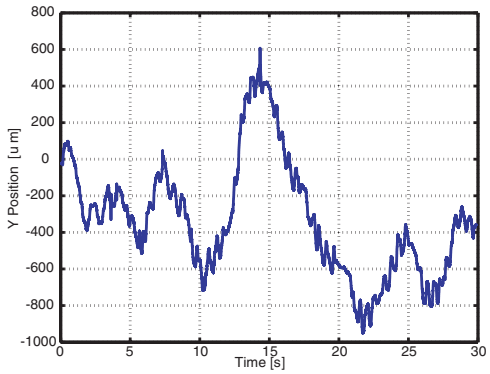

Fig. 14. Y position (perpendicular to the electric field) of a cell in feedback control experiment.

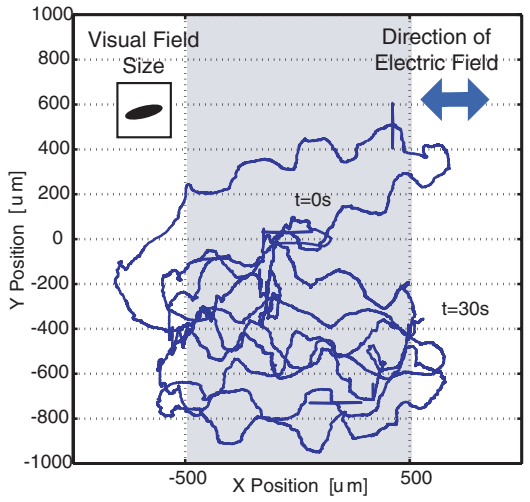

Fig. 15. Trajectory of a cell and its orientation in feedback control experiment (small arrows). The shaded region is the bounded region for trapping.

obstacle for understanding the natural ecology of microorganisms, and it also reduces the potential for OBM systems. Thus we are developing a variable-focus lens with 1kHz bandwidth suitable for 3-D control [16].

VI. Conclusion

We proposed a system for control of motile cell galvanotaxis without limitation on the working area by using high-speed tracking. This is a first step for realization of "Organized Bio-Modules," a large-scaled microsystem composed of controlled microorganisms.

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