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Abstract
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Microrobotic Visual Control of Motile Cells Using High-Speed Tracking System

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***Abstract*—We propose a visual control system for motile cells. Our goal is to control microorganisms as microscale smart robots for various applications. As a first step, we have developed a visual feedback control system for *Paramecium caudatum* cells. In order to ensure both detailed measurements and a large working space, "lock-on" tracking of a free-swimming cell with a high frame rate is essential. In our system, high-speed (1-kHz frame rate) tracking hardware and software are used for the continuous observation of moving cells with high magnification. Cells swim in a chamber, and their positions and other properties are measured and computed in real time. The chamber position is visually controlled automatically to track a specific cell. The cell motion is controlled electrically by utilizing the galvanotaxis (intrinsic reaction to electrical stimulus) of microorganisms. Experimental results for open-loop control (periodic zigzag motion) and closed-loop control (trapping within a small region that is 1 mm wide) demonstrate the possibility of using microorganisms as micromachines.**

***Index Terms*—Control, galvanotaxis, micromachine, microorganism, Paramecium, tracking.**

I. Introduction

RECENT PROGRESS in biotechnology has resulted in a growing interest in and increased demands for measurement and control of the micro and nanoscale world. However, such measurement and control require human operators with high dexterity, expertise, and much experience. Hence, automation technologies to assist operators are needed. Yet, in conventional micromachine technology, many problems remain to be solved before its practical application becomes realistic; these problems are mainly due to the still relatively young and undeveloped nature of the field and the limited technology available.

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 essential for survival. Therefore, microorganisms have acquired sophisticated sensors and actuators through the course of their evolution. If we can develop techniques to control them freely, we can realize multipurpose, programmable microrobotic systems that are superior to existing micromachine systems. Our goal is to eventually integrate controlled microorganisms and information processing systems as illustrated in Fig. 1. By controlling microorganisms, we aim to achieve various applications, such as cell manipulation, microscopic delivery, smart microsensors, and assembly of microelectromechanical systems (MEMS).

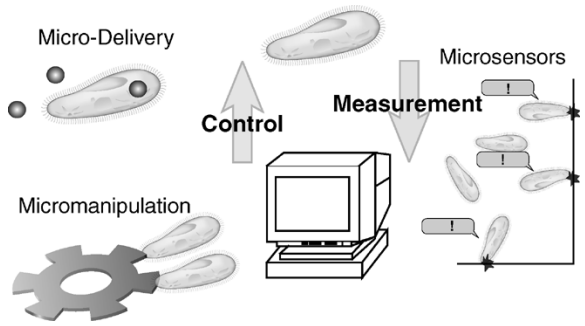

Fig. 1. Concept of our goal: to utilize microorganisms as smart micromachines.

Actuation of microorganisms is a key technology for the realization of our goal. In particular, noncontact and noninvasive methods are desirable. One possible technique, which we focus on here, is to utilize "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.

One essential requirement for microrobotic control of cells is a sufficiently large working area for control of the microorganisms. In most conventional micromanipulation systems, however, the working area is limited to within the small visual field of a microscope under high magnification, thus preventing completely free control. For example, Fearing controlled the trajectory of a *Paramecium caudatum* cell using galvanotaxis [1]. Itoh also controlled a *Paramecium caudatum* cell and actuated it to rotate a microimpeller [2]. In their system, however, the working areas were limited to within their fixed visual fields. In order to realize more advanced actuation of microorganisms, this constraint must be eliminated.

Therefore, we focus on tracking methods; techniques 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 our system. Tracking also has the potential for detailed observation of individual cells.

In microscopic tracking, however, there generally is a tradeoff relationship between magnification and trackability, because cells swim very quickly and go out of view at high

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magnification. As discussed later, use of a high-speed vision system can effectively overcome this problem.

In this paper, we propose a novel system that can continuously control 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 the feasibility of single-cell-level control.

II. Background

In this section, as a background of our research goals, we discuss the usefulness of microorganisms as micromachines.

As mentioned in the previous section, automation of micromanipulation has become essential in recent biotechnology. Micromachine technology, as represented by MEMS, is one of the hopes for microscale automation. It is difficult, however, to integrate internal sensors that measure the state of the MEMS devices themselves and, thus, most existing MEMS systems have only a single function. There seems to be no study that realized multifunction, programmable systems with several internal and external sensors, such as 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 fluids.

For these reasons, we focus on microorganisms. Microorganisms for use as smart and autonomous micromachines have the following advantages: 1) they have sensors with high precision, 2) they have sophisticated, wireless actuators (they can move without an external power supply), 3) they can be easily produced (they reproduce themselves), 4) they have high affinity with living bodies, and 5) they are large enough for microscale operations. In addition, future advances in genetic recombination technology may allow us to even design microorganisms with desired functions. If techniques to control them are developed, we may realize multipurpose, programmable microsystems superior to existing MEMS.

Although a number of studies on MEMS have been made [3], and many biosensors using microorganisms have been developed [4], there seem to be very few studies that have focused on microorganisms as smart motile machines, not just as tools. Arai manipulated yeast cells freely and dexterously by laser micromanipulation [5]. His concept differs from ours, however, in that the intrinsic nature of microbes was not utilized for actuation in his work. Fearing and Itoh are pioneers in using microorganisms as machines and they independently established a firm basis for this possibility [1], [2]. Now we would like to further improve their capabilities using a high-speed tracking system. As a first step, here we report on motion control of swimming cells.

III. Tracking of Motile Cells

In this paper, we introduce a high-speed tracking system into the system for actuating cells. We describe the significance of the tracking technique and the system design.

A. Why Tracking?

To realize free and precise control of cells, some requirements for the observation are: 1) continuous observation of quickly swimming cells with no fixation; 2) a sufficiently large working area for free control; and 3) detailed observation of a specific cell with high magnification for precise actuation.

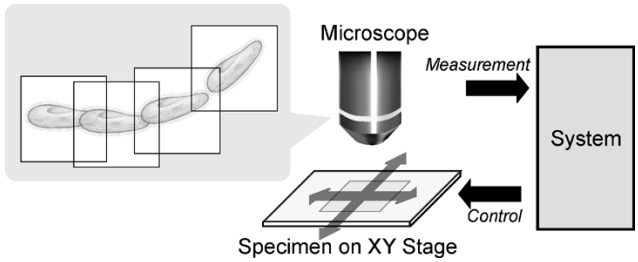

Fig. 2. Lock-on tracking scheme. The camera pursues a target so as to keep it always in the center of the visual field.

We found, however, that most conventional microscope systems could not satisfy these demands: 1) continuous observation was quite difficult, because cells swim very quickly. Instead, physical or physiological fixation had to be performed on the cells; 2) the working area was limited to inside of the visual fieldl; 3) so as not to lose cells, we were compelled to observe them with low magnification, thus preventing detailed observation of the cell properties.

To overcome these obstacles, we adopted a tracking method. In this paper, we mainly use the term "tracking" in the sense that the camera pursues a target so as to keep it always in the center of the visual field (sometimes we also call it "lock-on tracking"), while, in general, the term "tracking" has a wider meaning, such as just locating the position of moving targets or extracting targets from a background. As shown in Fig. 2, a lock-on mechanism can be realized by moving the position of the specimen on a stage so that the camera always keeps the target at the center. Although this can also be achieved by moving the camera and the microscope, we did not adopt this technique because it was much easier to move the stage.

B. Previous Work on Tracking

Lock-on tracking of microorganisms is one of the key techniques that many biologists have sought for, because it allows the natural ecology of cells swimming freely *in vivo* to be continuously observed, without fixing them or slowing them down.

However, examples of lock-on tracking of cells in previous studies are extremely rare, whereas, we can find many works on cell tracking in a broad sense. For instance, Thar *et al.* tracked many microorganisms simultaneously with high spatial resolution by using laser beams and charge coupled device (CCD) cameras [6]. Strickler recorded trajectories of free-swimming copepods mating by using matched spatial filters [7]. Kuo *et al.* used scattered laser light to track and evaluate the activity of *Listeria* [8]. Teunis *et al.* located the three-dimensional (3-D) positions of the moving cilia of protozoa under a microscope [9]. Hasegawa *et al.* constructed a system to record the position of swimming microorganisms automatically [10]. Zimmer *et al.* tracked migrating cells individually using an active contour method [11]. Acton *et al.* detected the position and rotation of leukocytes [12]. These studies, although quite interesting, achieved merely simple positioning, locating, and recording of cells, without pursuing the cells and keeping them in the center

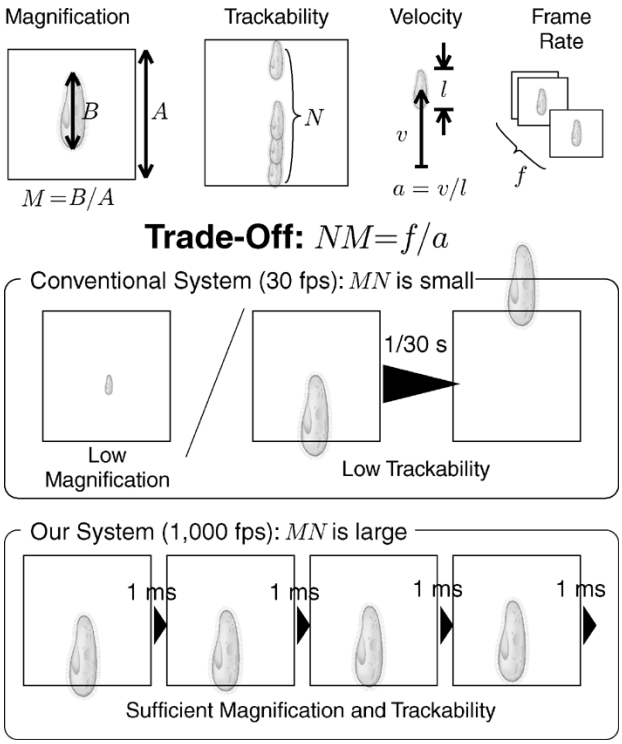

Fig. 3. Tradeoff between magnification and trackability in microscopic tracking.

of view. These systems allowed continuous observation, but not large workspaces and high magnification.

Berg *et al.* realized lock-on tracking by detecting the displacement of a target every 1/12 s using six optical fibers and photomultipliers, and automatically moving the chamber by mounting it on a stage [13]. His group succeeded in obtaining the 3-D trajectory of *Escherichia coli* [14], [15]. Enderlein adopted a similar method by moving the stage, but for tracking of fluorescent molecules, not for cells [16]. Though Berg's system had a remarkable recording ability, it was not suitable for real-time control applications, because it provided the information obtained only in an offline manner, unlike our system, which allows us to acquire information in real time.

Also, most tracking systems, including Berg's one, 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.

C. Necessity for High-Speed Vision

One major problem in tracking objects with a microscope is the tradeoff relationship between the magnification and trackability mentioned above; 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.

As illustrated in Fig. 3, 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 is the frame rate of the vision system, and N is the trackability index, defined as the number of frames for the target to cross over the visual field. In other words, N represents a margin required in order not to lose the target, assuming that the camera stands still. It is desired that both the magnification index M and the trackability index N be large for detailed observation and quick control.

Now we derive the tradeoff relationship. We introduce intermediate parameters: the diameter of the target l (meters), its physical velocity v (meters per second) ($a = v/l$), and the actual width of the visual field observed L (meters) ($M = l/L$). Then the time t (seconds) needed for the target to cross over the visual field with the width L is calculated as

$$t = \frac{L}{v} = \frac{1}{aM}.$$

Because N is the number of frames for this process, we can express N as

$$N = tf = \frac{f}{aM}.$$

Thus, the tradeoff relationship can be written as

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

which is independent of the target size l. This implies that certain qualities for both magnification and trackability (i.e., sufficiently large M and N) are 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/s [13] or 500 μm/s; the frame rates required for these speeds are well beyond the normal video rate and must be analyzed differently as pointed out in [17]. To be more precise, for conventional vision systems with a frame rate of around 30 Hz, we can estimate the value of NM to be 0.6, which is too small for our stated goal, namely ensuring sufficient magnification and trackability. For this reason, we need a vision system with a higher frame rate. As mentioned later in this paper, we used a high-speed vision system with a 1-kHz frame rate, so that NM can be more than 20, which is enough for our purposes.

D. Visual Servoing for Real-Time Control

We need not only the observation of cells; our objective is to control moving cells using the information obtained. For the purpose of real-time control, the vision system must provide high-speed output of information in addition to the high-speed input of scenes. The system must extract information that is useful for control from the images in real time. In other words, observation systems must be embedded in the high-speed servo loop for cell control.

IV. Galvanotaxis Control System

A. Overall System Configuration

The configuration of the overall system and its block diagram are illustrated in Figs. 4 and 5, respectively. This system utilizes a microorganism tracking system known as the microscopic visual feedback (MVF) system [18].

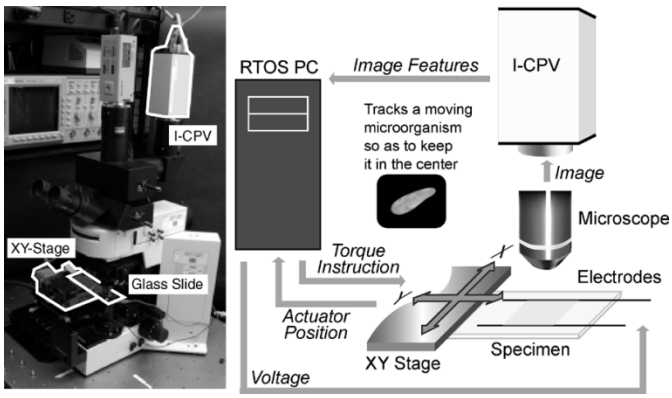

Fig. 4. System configuration.

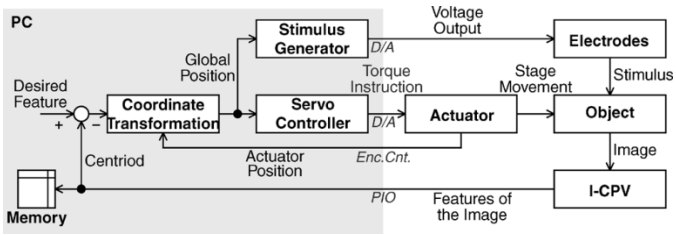

Fig. 5. Block diagram of the system.

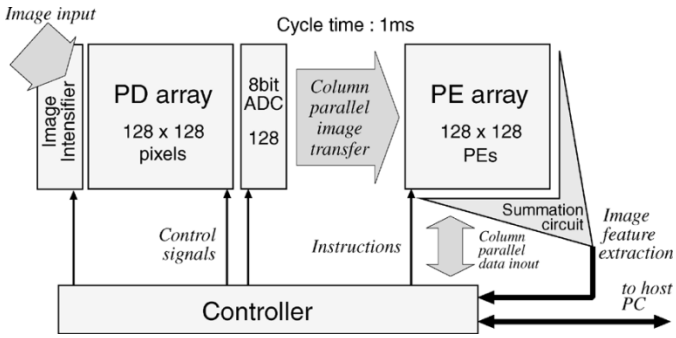

Fig. 6. Block diagram of I-CPV system.

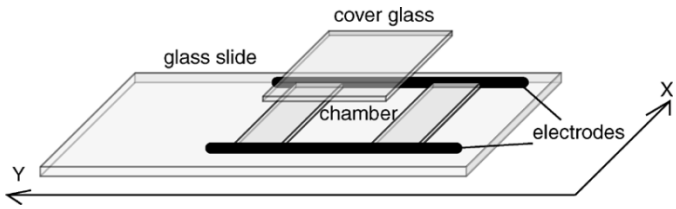

Fig. 7. Electrical stimulus input device.

An electrical stimulus is applied to 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 field of view. By reading encoders on the stage and performing corrdinate transformation, we can obtain the global position of the cell. The orientation of the cell is also calculated from image features. Tracking is based on a dynamic image-based look-and-move structure [19].

B. Vision System

As discussed in Section III-C, 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 a so-called I-CPV system jointly developed by Hamamatsu Photonics K.K. and one of the authors of the present paper (Ishikawa) [20]; this is a column parallel vision (CPV) system with an image intensifier. CPV is a high-speed vision system developed for robotic applications [21].

A block diagram of the I-CPV is depicted in Fig. 6. It captures and processes an 8-b gray-scale image with 128×128 pixels and 1-kHz frame rate. I-CPV has a 128×128 photodetector (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 by a complementary metal-oxide-semiconductor (CMOS) sensor module (consisting of the PDs and analog-to-digital converters) to the PEs in a column-parallel manner. Each PE is based on the $\mathrm{S^3PE}$ architecture [22], which adopts a SIMD-type program control, 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 the 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.

C. XY Stage

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

D. Electrical Stimulus Input Device

Fig. 7 shows the electrical stimulus input device. Two carbon electrodes of 0.5-mm 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 22 mm. Between the electrodes, there is a chamber 0.17 mm in depth to contain the specimen. The chamber constrains the motion of the cells within a two-dimensional (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 ± 10 V to the electrodes via a digital-to-analog converter board (Interface, PCI-3310). By feedback of the image feature values acquired by the

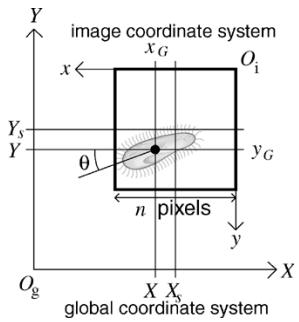

Fig. 8. Relationship between two coordinate systems.

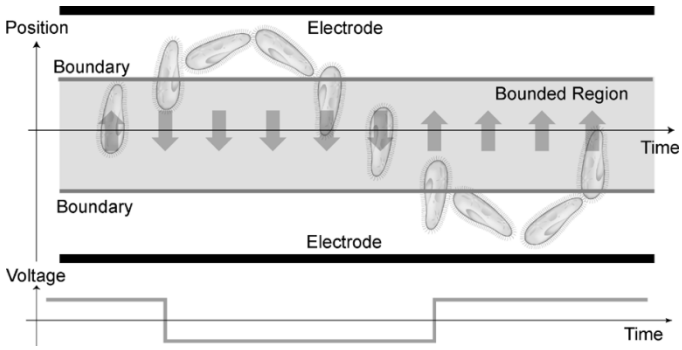

Fig. 9. Trapping of a microorganism within a region.

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 1 kHz by the PC running a real-time operating system (800 MHz, 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 the target and calculates its feature values, and the PC sends instructions to the XY stage so as to track the target.

E. Tracking of Cells

As mentioned above, we adopted a tracking method called MVF [18]. In our system, we extended it by adding a new feature for computing the target orientation to the version previously reported.

The I-CPV system provides the 0th, first, and second moments of the image every 1 ms [23]. As is well known, the centroid $(x_{\rm G}, y_{\rm G})$ of the target, and its tilt angle θ, shown in Fig. 8, are calculated using a given 0th moment m_0, first moments m_x and m_y, and second moments m_{xx}, m_{yy}, and m_{xy} (e.g., see [24])

$$x_{\rm G} = \frac{m_x}{m_0}, \quad y_{\rm G} = \frac{m_y}{m_0} \tag{2}$$

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

where

$$A = m_{xx} - \frac{m_x^2}{m_0}$$

$$B = m_{xy} - \frac{m_x m_y}{m_0}$$

$$C = m_{yy} - \frac{m_y^2}{m_0}.$$

The target point $(x_{\rm G}, y_{\rm G})$ is now expressed in the local, egocentric coordinate system. The PC restores the global, allocentric target position (X, Y) given by

$$\begin{pmatrix} X \\ Y \end{pmatrix} = -\frac{p}{m}\begin{pmatrix} x_{\rm G} - \frac{n}{2} \\ y_{\rm G} - \frac{n}{2} \end{pmatrix} + \begin{pmatrix} X_{\rm s} \\ Y_{\rm s} \end{pmatrix} \tag{4}$$

where $(X_{\rm s}, Y_{\rm s})$ is the XY stage position sent from the encoders to the PC, p is the pixel pitch, n is the number of pixels along 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.

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 [25]. The controller compensates for friction and gravity. By controlling the XY stage, the target is always located in the center of the visual field. At this time, information about the target orientation θ is not used for tracking and control. However, these kinds of higher order information will be necessary for more precise control in the near future.

F. Segmentation and Matching

The tracking method introduced above does not work appropriately when there are two or more cells in the field of view. To solve this problem, segmentation of multiple cells and matching of the target between frames is realized by an algorithm called self windowing [26], using some of the intrinsic characteristics of high-speed imaging, which are described below.

As the frame rate of the vision system becomes higher, the difference between frames decreases. In a vision system with a sufficiently high frame rate, for example, 1 kHz, we can say that the difference between a point in one frame and a corresponding point in the next frame is very small, at most one pixel. Thus, an object in one frame will always be contained within a one-pixel dilation of the object itself in the next frame. In other words, we have only to pay attention to this dilated region for recognition of the target in the next frame, and can ignore the remaining region, including other objects and the background. This dilated region is used as a mask for cutting out the target and for matching between frames. Of course, although this method cannot be applied when two objects are overlapped, the information about their orientation or velocity computed by I-CPV would be useful for distinguishing them.

G. Control of Cells

As illustrated in Fig. 5, 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. 9. This allows us to trap the cell within the region. This trapping technique will be a powerful tool for advanced actuation in our future microsystems, such as standing by before an operation or positioning of an object.

Fig. 10. Galvanotaxis of *Paramecium caudatum*. They swim toward the cathode.

V. Experiments

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

A. Materials

Paramecium caudatum, a kind of protozoa, was used in our motile cell control experiments. The size of a cell is about 200 μm in length, large enough to be observed easily by an optical microscope. *Paramecium* has very strong galvanotaxis [27]; when the electric field is applied, it tends to swim toward the cathode, as shown in Fig. 10. We can say that their motion toward the cathode is not caused by electroosmosis or other nonbiological phenomena, because we confirmed that dead cells do not move in the presence of the electric field. It is a byproduct of the electrophysiological nature of the cell. The ciliary motion of *Paramecium* is determined by the membrane potential and the accompanying changes in ion concentration in the cell. When an external electrical stimulus is applied, it modifies the membrane potential and alters the ciliary movements, consequently exerting an influence on the cell motion. Strong galvanotaxis of *Paramecium* makes it suitable for external control.

Wild-type *Paramecium* cells were cultured at 20 °C–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. Open-Loop Control Experiment

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

Fig. 11 shows some 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 0.1 s. The cell motion was affected by the stimulus and the cell position was always kept in the center of the image field.

Fig. 12 shows 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 motion, whereas the motion for the Y direction seemed

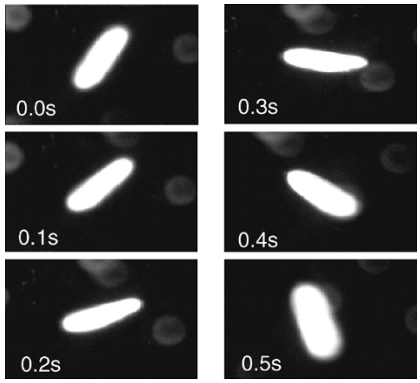

Fig. 11. Sequential photographs of a cell making a U-turn by stimulus reversal (0.1-s intervals).

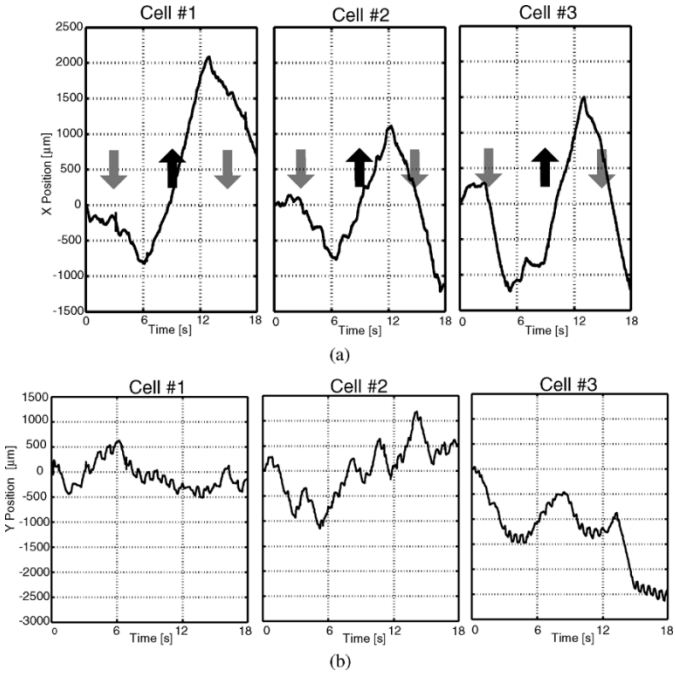

Fig. 12. (a) X position (parallel to the electric field) of cells in the open-loop control experiment. Arrows indicate the direction of the applied electric field. (b) Y position (perpendicular to the electric field) of cells in the open-loop control experiment.

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 Section IV-E, we can reconstruct the trajectory and the orientation of the target from the features extracted by the I-CPV system. Fig. 13 shows the trajectory of cell #3 presented in Fig. 12, and its orientation (small arrows), 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 high magnification and good trackability over a large working area were achieved.

C. Closed-Loop Control Experiment

In order to confirm the ability of closed-loop visual feedback control of cells, we performed a simple trapping experiment as described in Section IV-G; the stimulus was adjusted in real time according to the target status. The width of the trapping

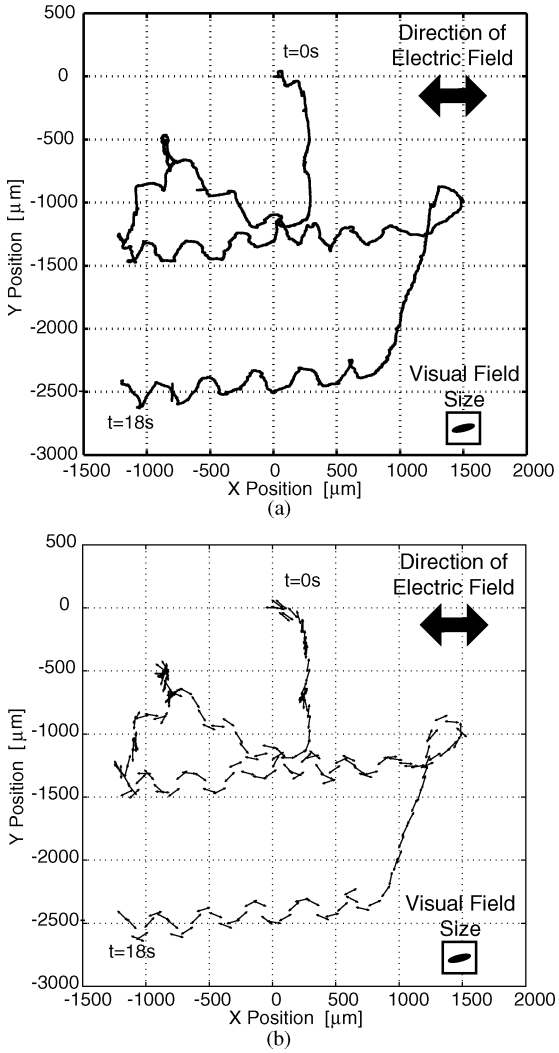

Fig. 13. (a) Trajectory of a cell in the open-loop control experiment. (b) Orientation of a cell (small arrows) in the open-loop control experiment.

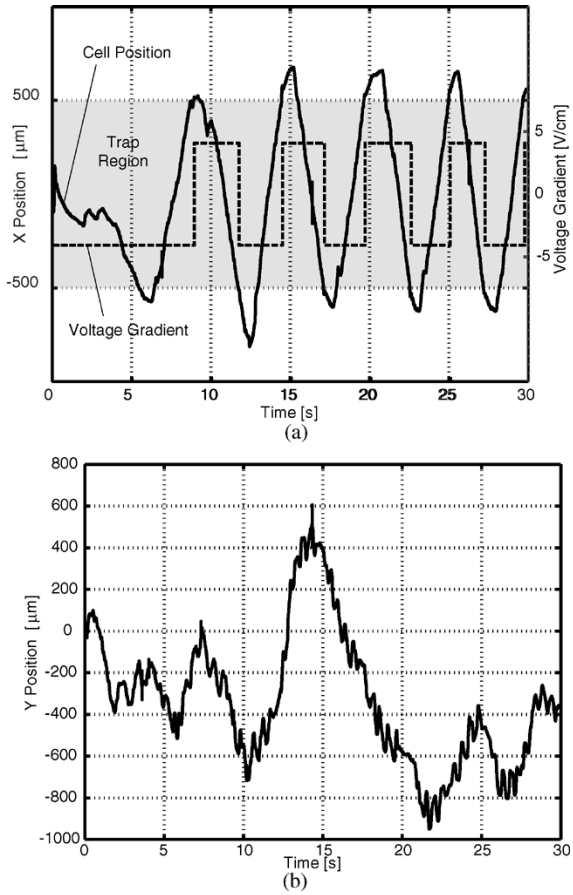

Fig. 14. (a) Applied voltage (dashed line) and X position (parallel to the electric field) of a cell (solid line) in the closed-loop control experiment. The shaded region is the bounded region for trapping. (b) Y position (perpendicular to the electric field) of a cell in the closed-loop control experiment.

region was set to 1 mm. The voltage was reversed when the cell moved out of the boundaries. Other conditions were the same as those of the open-loop control experiment described above.

Figs. 14 and 15 demonstrate results of the control experiment. Fig. 14 shows the time sequence of both the voltage and the position parallel to the field (X), and the position perpendicular to the field (Y). The voltage was reversed when the cell went out of the region. Consequently, the cell swam back and forth in the region. The trajectory of the cell is shown in Fig. 15.

These results indicate that we achieved continuous open-loop and closed-loop control of galvanotaxis of motile cells by target tracking. It is a first step for realizing a microrobotic system composed of microorganisms.

D. Discussion

First, we discuss the control precision, including visual tracking precision and positioning precision. The precision of the XY stage is 1 μm, one pixel in an image corresponds to 2 μm, and the disposition of a cell in one frame is estimated to be about 0.5 μm. Thus, the visual tracking precision is 2 μm. On the other hand, positioning precision is several hundred micrometers and it leads to the time delay of control, although it was more precise than the trapping results by Fearing [1]. The plots of trajectories indicate that there is a delay in the U-turn motions of the cell and it sometimes ran slightly over the boundary. We assume that this is caused by the physiological response time of the cell body, and/or the stabilization time of the electrical field in the conductive liquid. Anyways, to achieve more precise control, a motion model of a cell and model-based control will be required.

The experimental results also suggest that the individuality among cells is substantial, and cannot be ignored. A system for screening microorganisms might be needed to provide coordinated motion. For instance, cells with high sensitivity to stimulus or with fast motion will be collected and cultured for quick actuation. To that end, inheritable properties in microorganisms have to be investigated.

Additionally, for future studies, a detailed evaluation of the individuality is also an interesting topic [28]. Investigation of diversity and variety among cells will be essential in not only understanding the sophisticated function of living things from a biological point of view, but also in screening of cells with desired characteristics for practical use. However, previous studies in biology have mainly focused on the statistical nature of cells as a large population, lacking the ability to observe in detail

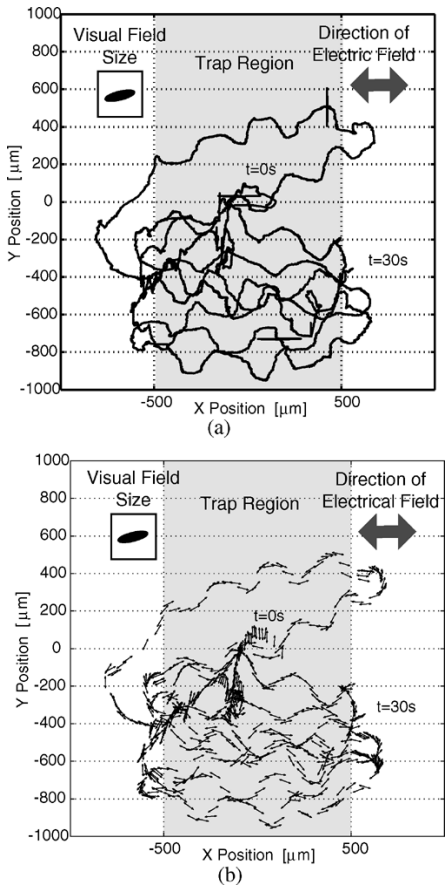

Fig. 15. (a) Trajectory of a cell in the closed-loop control experiment. (b) Orientation of a cell in the closed-loop control experiment (small arrows). The shaded region is the bounded region for trapping.

single cells *in vivo*. Our tracking system will be of some help in such single-cell level analysis.

Also to be noted in this paper is that 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 obstacle for understanding the natural ecology of microorganisms, and it also reduces the potential for our goal of 3-D free actuation of microorganisms in a large space. Thus, we are developing a variable-focus lens with 1-kHz bandwidth suitable for 3-D cell control [29]. The lens transforms its shape rapidly using the liquid pressure generated by a piezo stack actuator and shifts its focal plane to within 1 ms. Using such a device and some transparent electrodes, 3-D tracking and control of cells in truly free space will be realized.

Finally, one might doubt that our approach can indeed be scaled up to a larger scale. Indeed, the proposed system is not directly applicable for practical use yet, but we believe that it can be a testbed for a more practical system to come, as one of the alternatives to existing microrobots. We think that using centralized visual control is not the sole solution, but an important element for future large-scale systems. Tracking enables us to obtain both high-resolution information and sufficient observation space/time, both of which are essential for the evaluation of intercellular and intracellular diversity and individuality for tailored robotic control of cells.

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 a large-scale microsystem composed of controlled microorganisms. Continuous open-loop and closed-loop control of galvanotaxis of *Paramecium caudatum* cells was achieved.

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