SINGLE-CELL LEVEL CONTINUOUS OBSERVATION OF MICROORGANISM GALVANOTAXIS USING HIGH-SPEED VISION

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ABSTRACT

A novel system for measurement of motile microorganism galvanotaxis using high-speed vision is presented. Our goal is to construct a smart microsystem composed of many controlled microorganisms called "Organized Bio-Modules" (OBM). The OBM system utilizes galvanotaxis (intrinsic reaction to electrical stimulus) of microorganisms for actuation. For evaluation of taxis, continuous observation of a moving single cell in a sufficiently large working area without fixation is needed. Using high-speed vision, we developed a system for continuous evaluation of galvanotaxis of freely swimming cells in a large area at the single-cell level. Experimental results demonstrate the continuous measurement of galvanotaxis of a *Paramecium caudatum* cell moving in a 3.5-mm-square area for 18 s with 1 μs precision.

1. INTRODUCTION

Detection of changes in the surroundings and fast reaction against stimulus are matters of the greatest importance for all living things. Thus microorganisms have acquired sophisticated sensors and actuators in their small bodies. If we develop techniques to control microorganisms like micromachines, we can realize a multi-purpose programmable micro system. In this study, we regard an individual microorganism as a "bio-module," a functional unit. Our goal is to integrate a number of bio-modules and computers to construct an "organized bio-modules" (OBM) system. The concept of OBM system is shown in Fig. 1. The OBM system has potential applications such as cell manipulation, microscopic delivery and smart microsensors.

Actuation of bio-modules is a key technology for realizing 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 taxis in response to electrical stimulus (galvanotaxis), which implies the possibility of controlling their motion by adjusting the electrical stimulus.

In order to establish a method for controlling bio-modules, the taxis must be evaluated. Imaging techniques are essential tools for this, due to their noninvasive, non-contact, and precise sensing. Since taxis of microorganisms exhibits significant individuality, identification of each cell and single-cell level observation are important. To be more precise, there are two requirements for measurement in the OBM system: evaluation of the natural states of fast microbes without fixation in a sufficiently large working area, and single-cell level observation with high magnification for detailed evaluation of the individuality. However such requirements are difficult to satisfy in conventional micromanipulation systems.

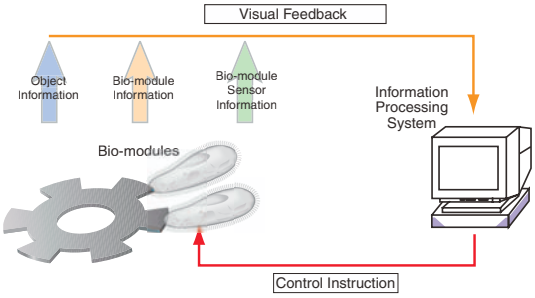

Fig. 1. Concept of Organized Bio-Modules.

In this paper, we propose a system for continuously measuring galvanotaxis of moving cells at the single-cell level without limit on the working area. Our system makes use of high-speed vision.

2. RELATED WORKS

Tracking of microorganisms is one of the techniques that many biologists have sought for, because it allows us to observe the natural ecology of cells without fixation. Though many studies on tracking of microbes have been presented [1, 2, 3, 4], most tracking systems could detect only a few features of the target, such as its position. 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, which is necessary for OBM.

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3. GALVANOTAXIS MEASUREMENT SYSTEM

3.1. Necessity for High-Speed Vision

One major problem in microscope tracking is the trade-off 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. This can be written as

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

where N is the trackability (the number of frames for the target to cross over the visual field), M the magnification (ratio of the target length to the visual field width), f the frame rate of the vision system, and a the target speed (diameters/sec). 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 [1]. Thus, for conventional vision systems with 30 Hz frame rate, we can estimate the value of NM to be 0.6, which is too small for our stated goal, namely compatibility of magnification and trackability. For this reason, we use a high-speed vision system with 1 kHz frame rate, so that NM can be over 20, which is enough for our purposes.

3.2. High-Speed Vision System: I-CPV

In order not to lose the target with high magnification, a high frame rate is required for the vision system. Moreover, an observing method with weak light, such as dark field microscopy, is needed, 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 [5]. CPV is a high-speed vision system developed for robotic use [6]. A block diagram of the I-CPV is depicted in Fig 2. It captures and processes an 8-bit gray-scale image with 128×128 pixels and 1 kHz frame rate. I-CPV has a 128×128 photo detector (PD) array and the same number of programmable processing elements (PEs). The captured image is amplified several thousand times by the image intensifier and transmitted to the PEs in a column-parallel manner. Each PE is based on the S^3PE architecture [7], which adopts a SIMD type program control. I-CPV 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 1ms [6].

3.3. System Configuration

Figures 3 and 4 show the configuration and block diagram of the overall system. An electric stimulus is applied on

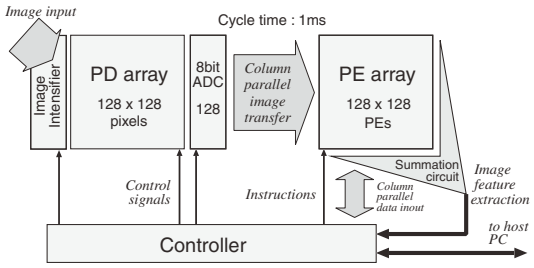

Fig. 2. Block diagram of I-CPV system.

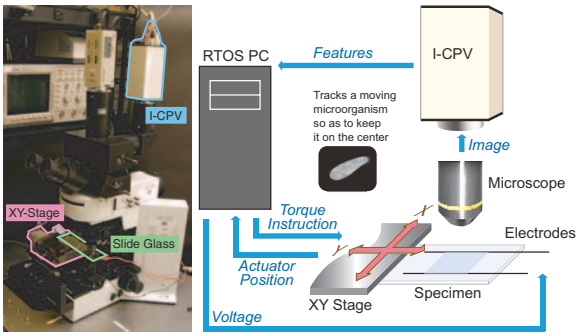

Fig. 3. System configuration.

cells swimming in a chamber on an electrical stimulus input device mounted on an XY stage. The stage is controlled by the I-CPV system so as to keep a cell on the center of the view. By reading encoders of the stage, the position of the cell is obtained. The posture of the cell is also calculated from image features.

In the system proposed here, the I-CPV system is mounted on an upright optical microscope (Olympus, BX50WI) and captures dark-field images. From the captured images, it calculates image features such as summation and moments, and sends them to the PC within 1ms. These features are used for visual feedback control of the XY stage.

The PC controls the position of a chamber fixed on the XY stage. The XY stage (SMC, LAL00-X070) has two orthogonal axes, X and Y, whose stroke is 25 mm. Each axis has an encoder with 1 μm precision. Tracking is achieved by controlling the stage according to the extracted target position.

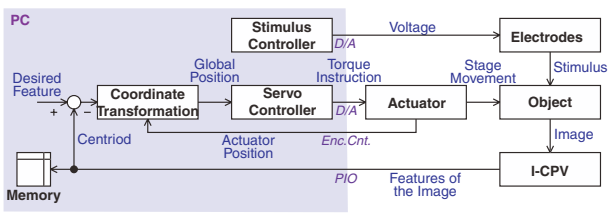

Fig. 4. Block diagram for tracking and measurement.

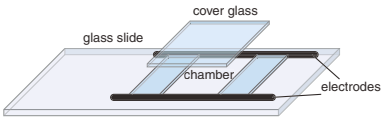

Fig. 5. Electrical stimulus input device.

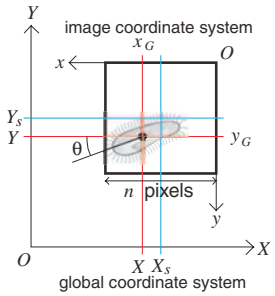

Fig. 6. Relationship between two coordinate systems.

Figure 5 shows the electrical stimulus input device. Two carbon electrodes of 0.5 mm diameter are placed in parallel 22 mm apart on a glass slide, in order to control the electrical stimulus in one direction perpendicular to the electrodes. Between them there is a chamber 0.17 mm in depth to contain the specimen. The chamber constrains the motion of the cells within the 2-D plane. The PC provides a voltage in the range ± 10 V to the electrodes via a D/A converter board (Interface, PCI-3310).

The whole system is controlled at a frequency of 1 kHz by the PC, which runs a real-time OS (800 MHz, ART-Linux). During each cycle, the PC sets the stimulus on the electrodes, the I-CPV captures an image and calculates feature values, and the PC sends instructions to the XY stage to track the target.

3.4. Tracking and Measurement of Cells

We adopted a tracking method called Microscopic Visual Feedback (MVF) [8]. The I-CPV provides 0th, 1st and 2nd moments every 1ms. The centroid $(x_{\mathrm{G}}, y_{\mathrm{G}})$ of the target, and its tilt angle θ, shown in Fig. 6, are calculated using a given 0th moment m_0, 1st moments m_x and m_y, and 2nd moments m_{xx}, m_{yy} and m_{xy}:

$$x_{\mathrm{G}} = m_x/m_0, \quad y_{\mathrm{G}} = m_y/m_0,$$

$$\theta = \frac{1}{2}\arctan\left[\frac{2(m_{xy} - m_x m_y/m_0)}{(m_{xx} - m_x^2/m_0) - (m_{yy} - m_y^2/m_0)}\right].$$

The 2D position of the XY stage, $(X_{\mathrm{s}}, Y_{\mathrm{s}})$, is sent from the encoders to the PC. The PC calculates the global target position (X, Y) given by

$$X = -\frac{p}{m}\left(x_{\mathrm{G}} - \frac{n}{2}\right) + X_{\mathrm{s}}, \quad Y = -\frac{p}{m}\left(y_{\mathrm{G}} - \frac{n}{2}\right) + Y_{\mathrm{s}},$$

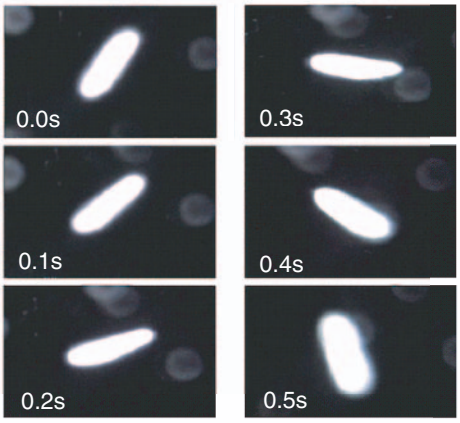

Fig. 7. Sequential photographs of a cell making a U-turn by stimulus reversal (0.1 s interval).

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 every 1ms.

High-speed extraction and matching of the target between frames are realized by a so-called self-windowing algorithm, using the fact that the difference between frames is very small in high-speed imaging [9].

The error values in displacement of the target are used to obtain the desired position of the XY stage. The servo system of the stage was designed using the Smith-Davison design rule [10]. By controlling the XY stage, the target is always located in the center of the visual field.

4. EXPERIMENTS

We used *Paramecium caudatum*, a kind of protozoa exhibiting very strong galvanotaxis; it tends to swim toward the cathode. 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.

We measured the galvanotaxis of cells by tracking each cell. A $20\times$ objective lens was used for magnification. The 4.1 V/cm (9 V across a 22 mm gap) stimulus was applied in the X direction, and reversed every 6 s.

Figure 7 shows sequential photographs of a single cell making a U-turn by stimulus reversal, as captured by a CCD monitoring camera (0.1 s intervals). The cell motion was affected by the voltage and the cell position is kept in the center of the image field.

Figure 8 shows examples of the trajectory of a cell and its sequence of orientations (small arrows) reconstructed with high fidelity. It also indicates that both of high magnifi-

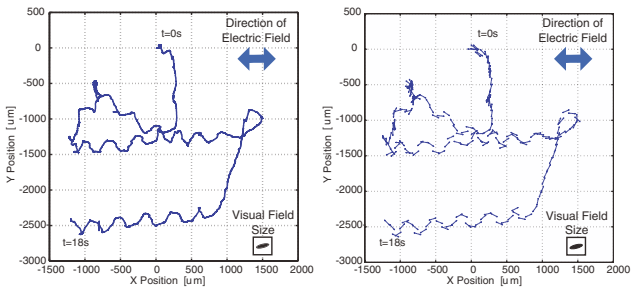

Fig. 8. Reconstructed trajectory (left) and postures (right) of a cell.

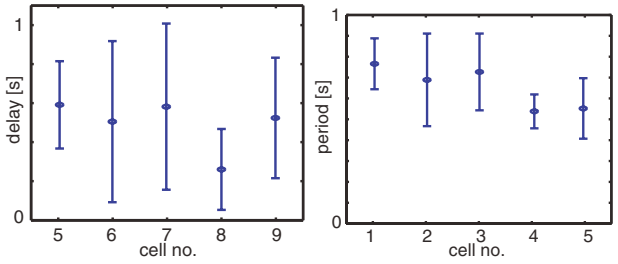

Fig. 9. Individuality seen in U-turn delay (left) and wriggling period (right).

cation and good trackability in a large working area were achieved.

Based on the data obtained, individuality of the cells was roughly evaluated. Figure 9 shows the individuality seen in the U-turn delay time from the point of voltage reversal (left), and wriggling period for Y direction (right), respectively, for several cells. Each error bar indicates the sample variance of each cell. A substantial amount of individuality was seen in the delay time, while that for the wriggling period was relatively small. There would be external factors (*e.g.* congestion of cells) and internal factors (*e.g.* fluctuation of membrane potential [11]) for these variances.

In this paper, the motion of the cells was restricted within the focal plane, owing to the inevitable drawback of conventional microscopic imaging methods, namely, their shallow depth of field. This constraint is an obstacle for understanding the natural ecology of microorganisms, and it also reduces the potential for OBM system. Thus we are developing a variable-focus lens with 1 kHz bandwidth suitable for 3-D measurement [12].

5. CONCLUSION

For continuous and single-cell level observation of galvanotaxis without limit on the working area, a measurement system using high-speed vision was developed. Continuous measurement of galvanotaxis of *P. caudatum* cells at the single-cell level was achieved.

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