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# Toxicity Sensing by Using Chemotactic Reaction of Microbial Cells Confined in Microfluidic Chip

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## Abstract

We developed and examined a toxicity sensing microchip, where 200-300 cells of flagellated microbes (*Euglena gracilis*) were placed and their movements were monitored with a video camera. The microchip had two microchannels for test sample and reference flow, and the cells were confined completely in a separated closed-space (micro-aquarium). The two microchannels run aside of the micro-aquarium, and molecules of test sample permeated into the micro-aquarium from the microchannels via diffusion through porous polydimethylsiloxane (PDMS) wall. The toxicity of test sample was evaluated real-time from the spatial distribution of the cells and swimming motion. Highly quantitative toxicity monitoring was achieved, owing to the complete confinement of microbial cells and sensitive chemotaxis of the cells.

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**Keywords:** Euglena; Chemotaxis; Microfluidic chip; Toxicity sensing

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## 1. Introduction

Environmental toxicity monitoring is increasingly important to maintain safe and natural society. Modern technology has developed spectroscopic sensors with ultra-high sensitivity of ppt levels or fluorescence observation technique with single molecule detection. However, these high sensitivity detection techniques can only be applied when the target substance is identified [1]. For an example, judging whether not-pure water in a glass is good to drink or not is a quite hard task when the substances in the water are unknown. Many water stations still utilize fish

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movement monitoring to secure the supplying water has no biological toxicity. As well, the side effects of newly developed drug cannot be predicted even with high-sensitivity fluorescence observation, and must be elucidated by using mice or rabbits. This is because the metabolic system in living cells or tissues has multi-level structures of complex chemical connections, resulting in unexpected responses by unidentified chemicals. Sensing with fishes and mice requires longer detection time, a larger equipment, and larger cost.

The motivation of our study is to develop a compact, disposable, easy handling, and fast toxicity monitoring device, by using the chemotaxis of microorganisms and microfluidic technology. The flagellated microorganisms have a capability of chemical sensing and locomotion, which can be used as a living sensor/indicator. In order to evaluate the chemotactic movement of microorganisms, we introduced a measure trace momentum (TM), which was derived from the microscopic images of cell distribution. We succeeded in monitoring toxicity by using microorganisms confined a disposal microfluidic chip.

### Nomenclature

PDMS polydimethylsiloxane, transparent, porous, and soft polymer material, used for microfluidic chips  
 TM trace momentum, which represents the product of cell number and swimming speed.

## 2. Experiments

The microorganism we used for our chemotactic device is *Euglena gracilis* Z-strain, which is one of the most common microorganisms living in pure water with photosynthesis [2]. The size of *Euglena* cells is approximately 50–80  $\mu\text{m}$  long and 10–30  $\mu\text{m}$  in diameter (spindle shape), easy to observe with an optical microscope without fluorescence dying. When the cells encounter a chemical gradient, they change their swimming direction to come close to the favorable chemical source (positive chemotaxis), or to escape from the dislike chemical source (negative chemotaxis).

The microfluidic chip we designed has one closed micro-chamber for *Euglena* cell confinement, and two bypass microchannels for test/reference liquid flow, as shown in Fig.1. The diameter of the closed micro-chamber was 2.5 mm, the width of microchannels was 200  $\mu\text{m}$ , and the depth of the structure was 150  $\mu\text{m}$ . The micro-chamber and the microchannels were separated with a 150- $\mu\text{m}$  wall. Small molecules in bypass microchannels permeated through porous polydimethylsiloxane (PDMS) wall into the micro-chamber, where approximately 200 *Euglena* cells were confined. The micro-chamber had 16 walls inside to enhance cell movements along radial direction.

The image of the micro-chamber was observed with an optical microscope processed to obtain TM for each image [3,4]. In the image processing, the traces of swimming *Euglena* cells were obtained by differentiating sequential images. The TM value was calculated by counting the pixels in the trace image for arbitrary area. Here, the TMs for seven compartments close to sample and reference side were summed up individually [5].

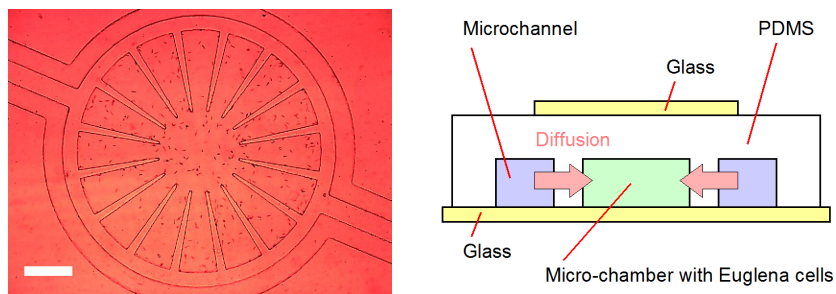


Fig. 1. (a, left) Microfluidic device with *Euglena* cells confined in the micro-chamber. Scale bar indicates 0.5 mm; (b, right) Cross-sectional drawing of our microfluidic device.

### 3. Results and Discussions

#### 3.1. Response to 5%-ethanol

We first examined 5%-ethanol as test sample with pure water as reference for our chemotaxis microchip. Figure 2a shows the typical negative chemotaxis of *Euglena*, where the cells localized at the reference side. *Euglena* cells moved from sample side to reference side when ethanol flowed in the sample (upper) microchannel with pure water in the reference (lower) microchannel. They returned uniform distribution when the ethanol flow was switched back to pure water flow, showing that the cell distribution change evoked by simple chemotaxis was reversible. When ethanol/water was supplied to lower/upper microchannel, the cell distribution was reversed, revealing that our chemotaxis microchip can be used repeatedly.

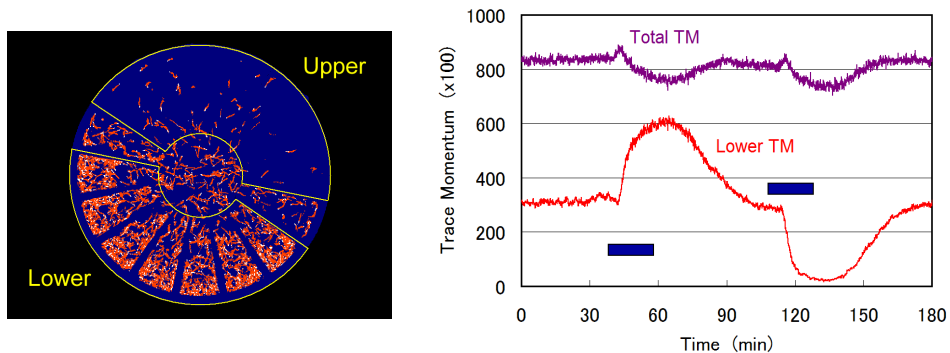


Fig. 2. (a, left) Cell distribution observed when 5%-ethanol was supplied in the upper microchannel at the timing of 59 min in Fig. 2b; (b, right) Temporal change of total and lower TM observed with 5%-ethanol supply in the upper/lower microchannel as indicated by squares.

The temporal change of cell distribution was evaluated by TM, as shown in Fig. 2b. Soon after ethanol supply started, the cell movement in the lower compartments was increased, and it started decreasing seven min after switching ethanol to pure water. The inverted response was observed when ethanol was supplied to the lower microchannel at 108 min. The total TM exhibited small fluctuation by the supply of ethanol as can be seen in Fig. 2b. The decrease in total TM was caused by the overlapping of the traces of individual cells when the cells gathered at one side. The TM was recovered when ethanol was removed from microchannels, indicating that the response of *Euglena* cells to 5%-ethanol was simple negative chemotactic response.

#### 3.2. Response to $H_2O_2$

Some chemicals induce metabolic disturbance in the cells through the production of radical oxygen species (ROS). As a type of these metabolic disturbance substances, we examined the effects of hydrogen peroxide on *Euglena* chemotactic behavior. When 1.5%- $H_2O_2$  was supplied in a microchannel as a test sample with pure water as reference, the reaction of *Euglena* differed from that for 5%-ethanol. As shown in Fig. 3a, the cell density became lower at the sample side first, but it increased after 37 min and became higher than that at the reference side. The motion of the cells observed at 55 min was straightforward swimming at the reference side, but continuous rotation at the sample side. The motion suggests that radical oxygen species released from  $H_2O_2$  disturbed the metabolic balance in the cell body, and the cells at the sample side lost flagellum control. After 51 min, the total TM was decreased to 30% or lower of its original, showing that many *Euglena* cells stopped swimming.

In order to analyze the difference of cell motion, i.e., rotating cells and straightforward swimming cells, we developed real-time motion analysis based on the trace image processing. The trace of each *Euglena* cell was sectioned by a surrounding square, and the aspect ratio and filling factor were calculated for each square. When the aspect ratio was higher than a fixed threshold level ( $\alpha$ ), or the filling factor was lower than another fixed threshold level ( $\beta$ ), we judged that the motion of the cell was straightforward swimming. Two threshold levels  $\alpha$  and  $\beta$  were

determined empirically as  $\alpha = 1.7$  and  $\beta = 0.38$ . An example of cell motion distribution obtained by the real-time motion analysis was given in Fig. 3b, where the rotating cells localized at the upper microchannel side for  $\text{H}_2\text{O}_2$  flow.

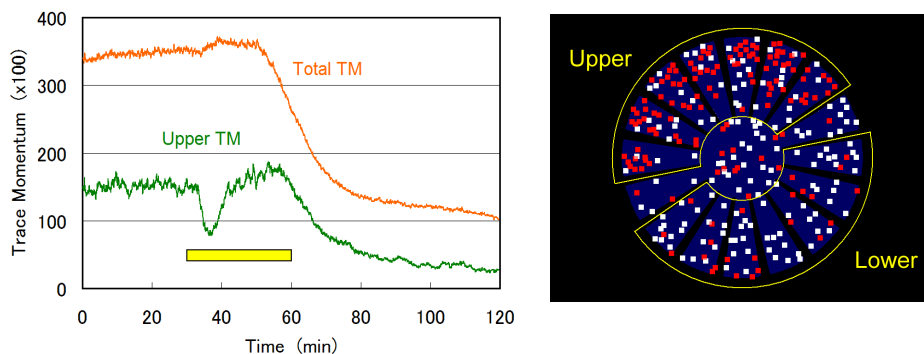


Fig. 3. (a, left) Temporal change of total and lower TM observed with 1.5%- $\text{H}_2\text{O}_2$  supply in the upper microchannel as indicated by square; (b, right) Cell distribution at the timing of 55 min in Fig. 3a. Red/white squares represent rotating/straightforward-swimming cells.

#### 4. Conclusion

This study elucidated that the toxicity sensing microchip we have developed by utilizing the chemotaxis of *Euglena* cells is useful for environmental toxicity monitoring for a long term. The cells in our microchip are confined completely in the closed micro-chamber, leading to highly quantitative measurements. The chemical gradient of test sample was formed in the closed micro-chamber by molecular diffusion through porous PDMS from separated bypass microchannels. The cell distribution and cell motion were analyzed in real-time monitoring. Our device can also detect the metabolic disturbance effect on living cells or tissue, as demonstrated for hydrogen peroxide, which will be important for the side-effect screening of newly developed drugs.

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