REAL-TIME MOTION ANALYSIS OF EUGLENA CELLS SWIMMING IN A MICROFLUIDIC CHIP FOR ENVIRONMENTAL TOXICITY BIOSENSING

K. Ozasa[1*], J. Won[2], S. Song[2], and M. Maeda[1]
[1]RIKEN, JAPAN and
[2]Hanyang University, SOUTH KOREA

ABSTRACT

We developed an automatic categorization algorithm, which distinguishes the motion of micro-algae cells real-time with image processing. The motion of the micro-algae cells, confined in a closed space of microchip (micro-aquarium), was captured, and converted to trace images. The traces were sectioned by rectangular shapes, and categorized to straightforward swimming or on-site rotation, by aspect ratio and filling factor of the rectangular. The automatic categorization enables to detect the abnormal motion of the cells evoked by test chemicals real-time, which is important for sensing the metabolic-disturbing substances quickly.

KEYWORDS: Real-time motion analysis, Micro-organism cells, Chemotaxis, Euglena gracilis

INTRODUCTION

Motile micro-algae cells are attracting bio-materials for chemical sensing [1], since they detect various toxic chemical substances of very low concentrations as low as ppb. Their chemotactic motion is typically escaping from a place with a higher concentration of the toxic substances. They are also low-cost, pathogenicity free, and can be maintained in a microchip for a long time with photosynthesis. The most important advantage of using living micro-algae cells to detect a toxic substance is not their sensitivity, but that they can detect a wide range of chemical substances from a view point of toxicity, without identifying the chemicals. The toxicity analysis of unidentified chemicals is usually a very hard task even modern sensor technologies. Therefore, developing the toxicity monitoring microchips with living micro-algae cells is useful especially for environmental pollution alert.

In this study, we focus on real-time analysis of swimming motion of micro-algae cells. As we previously reported [2], some chemicals induces abnormal behavior of micro-algae cells through heavy metabolic disturbances. If such a type of abnormal behavior can be detected in real-time, it will contribute to rapid toxicity screening of environmental pollution. For this purpose, a new fast image processing algorithm is required, which can categorize the behaviors of micro-algae cells to normal/abnormal real-time. We succeeded to real-time categorization of swimming behavior of *Euglena gracilis* cells exposed to hydrogen peroxide, which changed their swimming style from straightforward to on-site rotation.

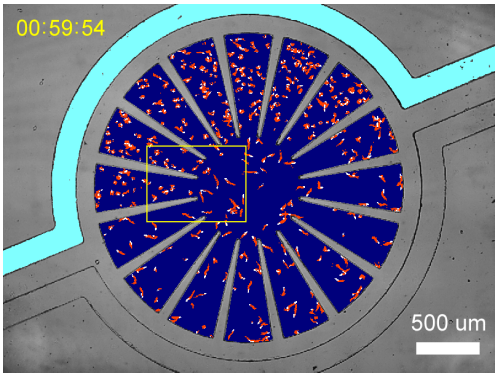

Figure 1: One shot of trace image taken under hydrogen peroxide flow in the upper micro-channel. Real image (b/w) and trace image (inside of the micro-aquarium) were superimposed.

EXPERIMENTAL

Our microchip and observation system were described in our previous report [2], as circular micro-aquarium surrounded by two bypass micro-channels. Approximately 300 cells of *Euglena gracilis*, photosynthetic microbe with a flagellum, were confined in the closed micro-aquarium of 0.7 uL in

volume in the microchip. 1.5% hydrogen peroxide was introduced in one channel, while pure water was flowed in the other. The real image of the whole area of micro-aquarium was captured with a CMOS camera with a frame rate of 16 fps. The images were converted to differentiated, binarized, and superimposed image with a rate of 1.6 fps, which we call trace image [1], as shown in Fig. 1.

The categorization was made by processing the trace image with sectioning, characterization, and distinguishing. As shown in Fig. 2, the categorization proceeded with steps of (1) isolation of traces with rectangular frame, (2) calculation of the aspect ratio of the rectangular and filling factor, and (3) categorization according to an empirically optimized standard shown in Fig. 2. This technique realizes high-speed distinguishing, executable in real-time.

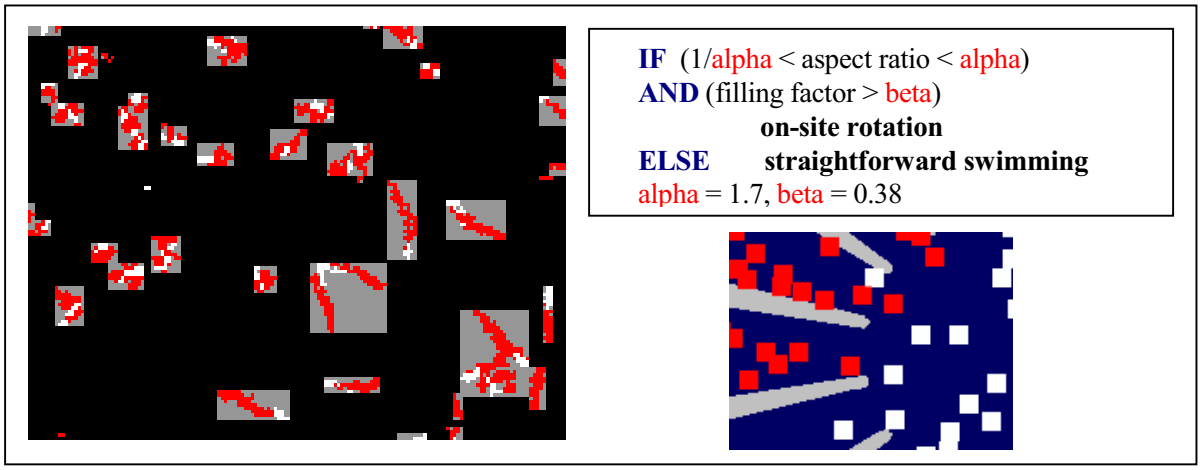

Figure 2: (a, left) A part of trace image taken from Fig. 1, sectioned by rectangular to separate each swimming trace. After the sectioning, aspect ratio and filling factor were calculated for each rectangular. (b, bottom) With the empirically optimized standard for categorization written at upper right, each trace was categorized to straightforward swimming (white square) or on-site rotation (red square).

RESULTS AND DISCUSSION

When *Euglena* cells were exposed to a low concentration of hydrogen peroxide, the cells once escaped to the side of lower concentration. After 15-20 min, however, the cells were trapped at the side of higher concentration of hydrogen peroxide, with their motion of continuous on-site rotation. This behavior indicates that the cells lost the capability of swimming toward the correct orientation, i.e., the control of flagellum motion. Hydrogen peroxide induces a heavy metabolic disturbance for *Euglena* cells, causing uncontrollable and continuous on-site rotation. In the trace images, the straightforward swimming traces have a higher aspect ratio or lower filling factor, whereas the on-site rotating traces have a lower aspect ratio and higher filling factor.

As shown in Fig. 2, the real-time categorization developed in this study resulted in approx. 80% accuracy in categorization. One typical error occurred when the swimming traces of multiple cells were overlapped, and processed as the trace of one single cell (Fig. 2(a)). This resulted in a less number of cells, and in some cases, mis-categorization. The other error occurred when a cell changes swimming direction by occasional one-time tumbling, especially at the wall of the micro-aquarium (Fig. 3(a)). Such one-time tumbling was categorized to on-site rotation, and resulted in the overestimation of on-site rotating cells. These errors might be removed by employing the detailed tracking of cell locomotion, which will take more processing time and not suitable for real-time categorization.

Figure 3 shows the temporal change of the cell number of straightforward swimming and on-site rotating cells, derived with the real-time categorization. Before hydrogen peroxide injection, the cells were swimming mostly straightforward. The ratio of the number of on-site rotating cells to that of total cells was approx. 19% at 20 min in Fig. 3(b).

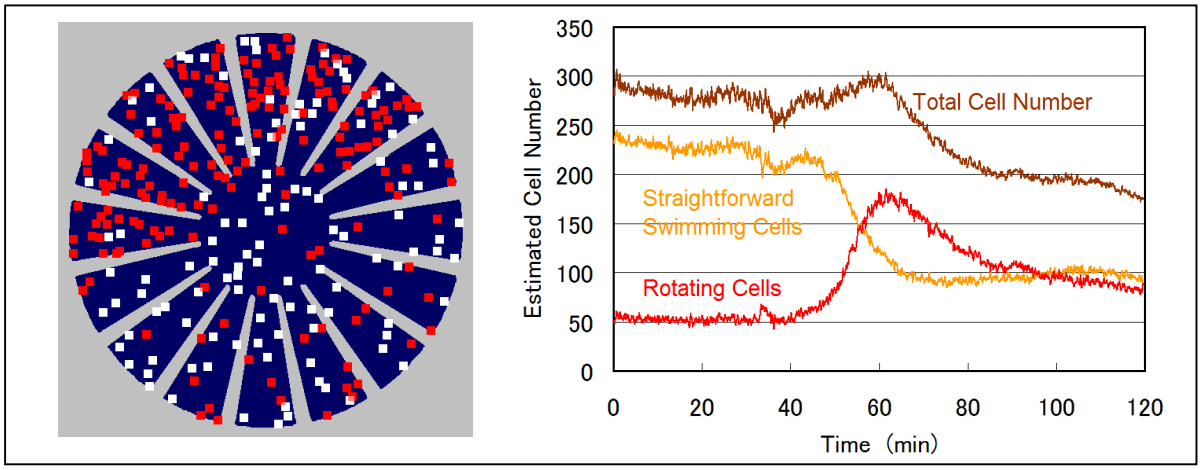

Figure 3: (a, left) Distribution image of categorized cells, corresponding to the trace image in Fig. 1. Straightforward swimming cells are indicated by white square, whereas on-site rotating cells by red. (b, right) Temporal change of estimated cell number for straightforward swimming cells and on-site rotating cells. Hydrogen peroxide was introduced for 30-60 min.

Approx. 3 min after hydrogen peroxide introduction, the cells escaped to a lower concentration side of the micro-aquarium with ordinal chemotaxis. Since the chemotacic escape swimming was straightforward, thus only a small dip/spike was observed in Fig. 3(b). Increase in on-site rotating cells took place approx. 10 min after the introduction of hydrogen peroxide. As the concentration of hydrogen peroxide in the micro-aquarium was gradually increased by H_2O_2 diffusion through PDMS wall, the cells once escaped to the lower concentration side accidentally swan to a higher concentration, and lost their flagellum control, falling into continuous on-site rotation. The on-site rotation ratio reached 59% at 60 min in Fig. 3(b).

CONCLUSION

Real-time categorization of swimming behaviors of *Euglena* cells was developed with trace image processing. The categorization algorithm visualized the distribution of on-site rotating cells with an accuracy of 80%. The continuous on-site rotation of the cells evoked by hydrogen peroxide was analyzed real-time, where the onset of the metabolic disturbance took place at 10 min after hydrogen peroxide introduction.

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CONTACT

* Dr. Kazunari Ozasa; phone: +81-48-462-1111 (ext. 8409); ozasa@riken.jp