OPTICAL FEEDBACK CONTROL/ANALYSIS OF PHOTOREACTIVE *EUGLENA* CELLS SWIMMING IN MICRO-AQUARIUMS

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ABSTRACT

We present a two-dimensional (2D) optical feedback system to control/analyze photoreactive flagellate microbes confined in mm-scale microfluidic channels (micro-aquariums). The system uses a liquid-crystal (LC) projector to induce the phototaxis reactions of microbes, thus arbitrary 2D patterns with variable colors and intensities can be projected onto micro-aquariums. We demonstrate that the density and position of *Euglena* cells in micro-aquariums are controlled by the system dynamically and flexibly. Transient steps in the photophobic reactions of *Euglena* were observed with the system with a time resolution of several ten ms, showing a complex photoreaction mechanism of *Euglena*.

KEYWORDS: Flagellate microbes, *Euglena gracilis*, Microfluidics, Phototaxis, Optical feedback control, Micro-aquariums

INTRODUCTION

Photoreactive flagellate microbes such as *Euglena gracilis* have excellent naturally tailored sensors and actuators in their micron-size bodies, which cannot be reproduced artificially even with well-developed nanofabrication/micromachine technologies. The control of movements of photoreactive flagellate microbes enables us to use them as micron-sized robots in microfluidic channels (micro-aquariums) [1] or as an unique media in microbe-based neuro-computing [2]. However, scanning monochrome laser light [1] have not sufficient flexibilities to control the photoreactions of the microbes precisely and dynamically, since the intensity of laser light cannot be changed instantly.

In this study, we develop a closed loop two-dimensional (2D) optical feedback system that enables the precise and dynamic control of *Euglena gracilis* confined in micro-aquariums. The movements of *Euglena* cells were detected as 2D traces in micro-aquariums, and 2D blue-light patterns were produced with feedback algorithms in real-time. The dynamic irradiation of blue-light with arbitrary 2D patterns with spatially-variant intensities were achieved by employing a liquid-crystal (LC) projector. The flexible and dynamic control of the density and position of *Euglena* cells in micro-aquariums was demonstrated, together with ms-time resolved analysis of transient steps in the photophobic reactions of *Euglena*.

EXPERIMENTAL

The 2D optical feedback system was composed with an upright microscope, a video camera, a PC, an LC projector, and optical lenses, as shown in Fig. 1. The images of micro-aquarium containing 20-400 *Euglena* cells were captured through an objective lens of 5X with a resolution of 200 pixel/mm with an acquisition rate of 100 ms/frame. The optical 2D patterns produced by the PC were projected by the LC projector through reduction lenses with the same resolution of 200 pixel/mm. The micro-aquariums used in this study had 100-120 um in depth, which allows *Euglena* cells swim across without collisions [3].

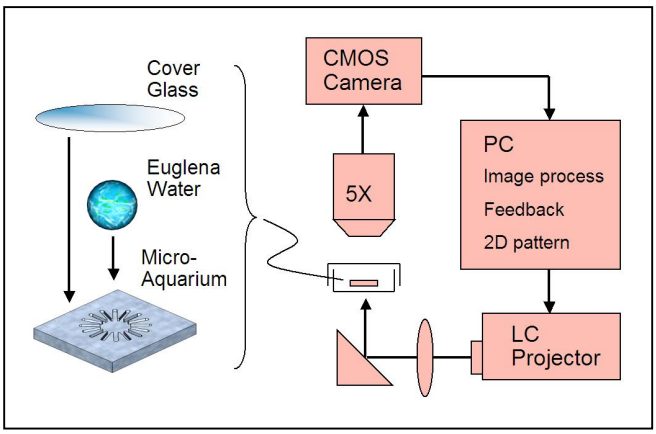

Figure 1: Schematic illustration of 2D optical feedback system.

In order to quantify the movements of *Euglena* cells we defined trace momentum (TM): the number of pixels traced by *Euglena* cells in the sequentially differentiated raw images. TM can be obtained for each arbitrary selected region in the micro-aquarium and represents the activity of *Euglena* cells in the region, i.e., the product of cell number and swimming speed of the cells. Repetition rate of the feedback was approximately 0.5-1.0 Hz.

RESULTS AND DISCUSSION

We used the photophobic reaction of *Euglena* in this study; cells swim out from the region illuminated by blue light. When swimming *Euglena* cells encounter a strong blue light over photosynthesis capability, they turn to escape from the light, while cells captured in a blue light continuously turn in position or migrate with changing swimming direction frequently.

A typical example of cell density control is given in Fig. 2, where the eight selected squares were illuminated with blue light to settle the density (represented by *TM*) at a set point (*S*). The intensity of the blue light (*I*) was controlled with proportional- and integral-gain feedback as shown in Eq. (1), where *sTM* is *TM* at time step 0. The set point *S* was changed as 0.3, 0.6, and 0.45, sequentially.

$$I = 0.092\left\{100(TM/sTM - S) + \sum_{timestep}(TM/sTM - S)\right\} \text{ (mW/cm}^2\text{)} \qquad (1)$$

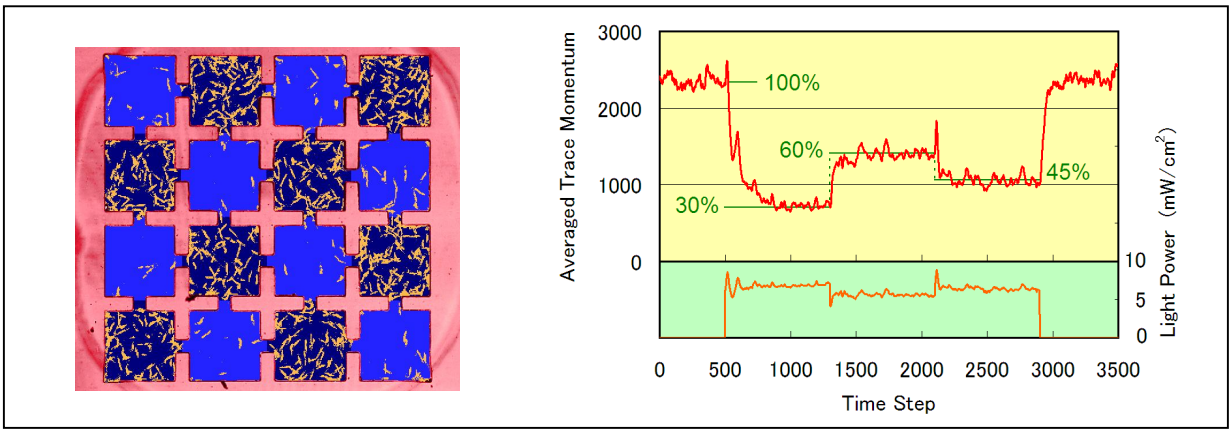

Figure 2: Trace image of Euglena in 16-square micro-aquarium (left) and cell density control by proportional- and integral-gain control (right).

The density of *Euglena* cells for eight squares in Fig. 2 followed the set points well, indicating that our 2D optical feedback system can control the density of photoreactive microbes flexibly and dynamically. Small irregular deviations of TM around the set points observed in Fig. 2 were caused by the natural fluctuation of the movements of *Euglena* cells. The result of density control through blue light intensity reveals that each *Euglena* cell has its own probability/frequency/threshold of the photophobic response (turning from the light) to the blue light.

When we change the position of illumination regions sequentially, we can concentrate *Euglena* cells into a selected region, as shown by a demonstration in Fig. 3. In the demonstration, the number of squares irradiated with blue light (18.8 mW/cm^2, fixed) was increased sequentially with a prescheduled duration of 48 min. At the end of final stage, 72% of spread cells were confined into the two squares. The remaining 28% of the cells were insensitive to blue light, or still migrating from the illuminated squares to the non-illuminated ones.

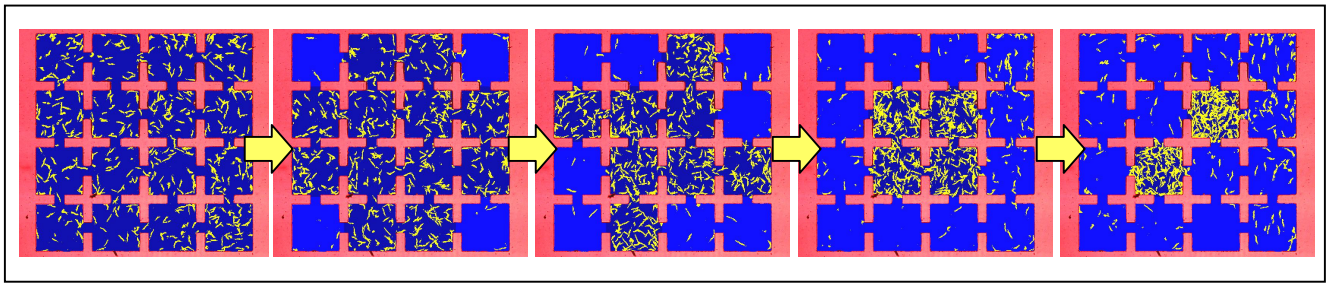

Figure 3: Concentrating Euglena cells by sequential pattern illumination (trace images).

The concentration of *Euglena* cells demonstrated with sequential-pattern-change opens unexplored uses of microbes in microfluidic channels, such as microbe-driven particle transport or separation of photo-sensitive/insensitive microbes. By employing 2D optical feedback control onto flow-type microfluidic channels with multi inlet/outlet ports, we may be able to switch a flow of individual *Euglena* cells arbitrary through blue light irradiation.

New transient steps in the photophobic reaction of *Euglena* were found by the quantitative analysis of TMs with a time-scale of ms. Figure 4 shows the photophobic reactions induced by periodic blue light illumination with 47.3 mW/cm^2 onto the whole area of a micro-aquarium. At switching blue light On (Off), TM shows a faster decay with a time scale of 30-150

(300-390) ms followed by a slower decrease (increase). This finding reveals that the photophobic reaction of *Euglena* is composed with multiple steps of flagellate movements.

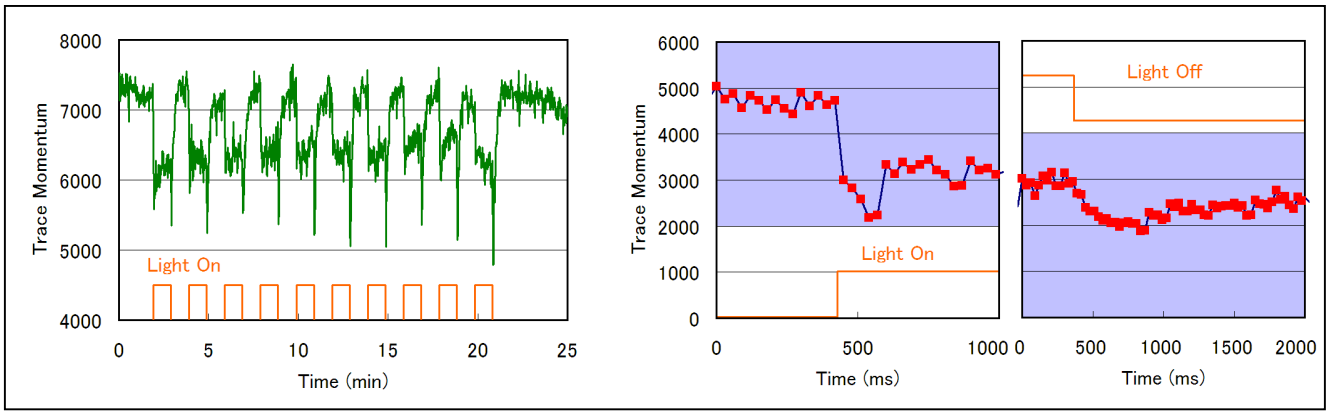

Figure 4: Time-resolved analysis of photophobic reactions of Euglena cells measured as TM. Periodic blue light illumination with 47.3 mW/cm^2 (left) and typical transient behavior at blue light On and Off (right).

The results obtained in this study clearly show that our 2D optical feedback system is suitable for the detailed study on microbiology as well as for microbe-based biosensors [4] or optical cell controlling [5]. For instance, rapid toxicity detection will be achieved by monitoring the photoreactions of *Euglena* cells under exposure to test samples like wastewater.

CONCLUSION

We succeeded in controlling the density and position of *Euglena* cells in micro-aquariums with the 2D optical feedback control system, employing a LC projector to achieve a flexible and dynamic blue light irradiation. The density of *Euglena* cells can be settled to set points with proportional- and integral-gain control of blue light intensity. The position of the cells can be controlled sequential pattern change with a fixed blue light intensity. Further, the photoreactions of *Euglena* cells were observed/analyzed quantitatively with a time resolution of several tens ms, showing that multiple transient steps in the photoreactions. The study showed that 2D optical feedback has a high potential for applications including microbe biological study, microbe-driven particle transportation, and microbe-based environmental biosensors.

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REFERENCES

[1] S. Hori and A. Itoh, "Micro Parts Assembly by Formation Controlled *Euglena* Group Using Their Phototaxis" ASME International Mechanical Engineering Congress and Exposition IMECE2008-66600 1-2 (2008) 1.

[2] K. Ozasa, M. Aono, M. Maeda, and M. Hara, "Simulation of neurocomputing based on the photophobic reactions of *Euglena* with optical feedback stimulation" Biosystems, 100 (2010) 101-107.

[3] K. Ozasa, J. Lee, S. Song, M. Hara, and M. Maeda, "Two-dimensional optical feedback control of *Euglena* confined in closed-type microfluidic channels" Lab Chip 11 (2011) 1933-1940.

[4] D. L. Englert, M. D. Manson, A. Jayaraman, "Investigation of bacterial chemotaxis in flow-based microfluidic devices" Nat. Protocols, 5 (2010) 864.

[5] B. Kaehr, J. B. Shear, "High-throughput design of microfluidics based on directed bacterial motility" Lab Chip 9 (2009) 2632.

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