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Analog feedback in *Euglena*-based neural network computing – Enhancing solution-search capability through reaction threshold diversity among cells

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ABSTRACT

Microbe-based neural network computing, where the reaction of microbial cells to external stimuli is incorporated in the function of virtual neurons, has high potential for developing soft computing based on the survival strategies of the microbe. To utilize reaction-threshold diversity among the cells, we examined analog feedback in *Euglena*-based neurocomputing by solving a simple combinatorial optimization problem. The analog feedback was performed by blue light illumination to *Euglena* cells, where the intensity of the blue light was controlled using the Hopfield-Tank algorithm with a sigmoid function. The solution patterns obtained with analog feedback had greater variations than those with digital feedback, implying that the solution-search capability of *Euglena*-based neurocomputing is enhanced by analog feedback. Moreover, the solutions obtained with analog feedback comprised one stable core-motive selection and additional flexible selections, which are associated with hesitation shown by humans when faced with a frustrated task. The study shows that using analog feedback in *Euglena*-based neurocomputing is promising in terms of incorporating the diversity of photoreactions of *Euglena* cells to enhance the solution-search capability for combinatorial optimization problems and to utilize the adaptive reaction of *Euglena* cells.

1. Introduction

Microbial cells are independent complete units, with functions for nutrition acquisition, metabolic activity, growth and reproduction, adaptation, and evolution. Through evolution and natural selection over several million years, they have developed reasonable and sophisticated reactions to external stimuli as a survival strategy. For instance, *Escherichia coli* has multi-level signal processing of noise reduction and signal amplification allowing them to sense a very small amount of a nutritious substance and to move towards its source [1–4]. *Euglena gracilis* can sense the intensity of blue light allowing them to stay in a moderately illuminated area for photosynthesis by changing their swimming direction [5–7]. A large diversity in photophobic reaction threshold is observed among the cells. They also adapt to external stimuli such as chemical concentration or light intensity by modifying their metabolism [8].

Using living microbial cells in computational processing is, therefore, a fascinating challenge to incorporate their autonomous adaptation and exploration capabilities into a physical computing algorithm. Adaptive computing or explorative solution search may be realized autonomously without creating a complicated computer program by using the reactions of microbial cells to the external stimuli as a function of computational signal processing. Some pioneering works on microbe-based computing have made use of a true slime mold, *Physarum polycephalum*, to study network construction [9] or combinatorial optimization [10,11]. We also studied *Euglena*-based neural network computing by utilizing the photophobic reaction of *Euglena* cells confined in a micro-aquarium [12–15]. In our study, neurocomputing proceeded in an optical feedback system [16] by monitoring the swimming behavior of *Euglena* cells and providing external optical stimuli through the Hopfield-Tank algorithm [17].

One of the essential characteristics of *Euglena*-based neurocomputing identified in our study [14,15] is that a number of solutions are obtained through dynamic transitions among the solutions, derived from the stochastic movements of *Euglena* cells and the existence of photoinsensitive cells. As in conventional

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silicon-based neurocomputing, our previous study used binary states for neuron activities, i.e., firing or non-firing, corresponding to the external stimuli (blue light illumination to *Euglena* cells) being On or Off. However, the photophobic responses of *Euglena* are not identical for all cells. As evidenced by the existence of photoinsensitive cells, the threshold of photophobic reaction varies among the cells, and the frequency of changes in swimming direction depends on the blue light intensity. This diversity among cells creates a new avenue for enhancing the solution search capability of *Euglena*-based neurocomputing by employing analog feedback instead of digital feedback.

In this report, we examine analog feedback in *Euglena*-based neurocomputing and compare its performance with that of neurocomputing with digital feedback. The swimming activity of *Euglena* cells in a micro-aquarium was measured in 16 individual compartments, corresponding to 16 virtual neurons for neurocomputing. The analog/digital feedback was performed by blue light illumination to the 16 compartments according to the Hopfield-Tank algorithm, for a simple combinatorial optimization task involving the selection of certain compartments while avoiding the closest four compartments. The performance differences between the analog and digital feedback neurocomputing were analyzed in terms of the temporal evolution of compartment selection, the number of solution patterns, and the blue light intensity distribution.

2. Experimental setup and computational algorithm

2.1. Experimental setup

The transparent micro-aquarium used in this study was 0.12 mm deep and comprised 16 equal compartments with an outer diameter of 2.5 mm, as shown in Fig. 1a. The compartments were located around a center circle, 0.8 mm in diameter. Approximately 160 cells of *Euglena gracilis* (spindle shaped cells, 50–80 μm long, 10–30 μm in diameter, and with 0.1–0.4 mm/s swimming speed) were confined in the micro-aquarium by placing a droplet of water containing *Euglena* cells on the micro-aquarium and capping it with a cover glass. The micro-aquarium was sealed in a glass-bottom dish and placed on the stage of an optical microscope (Olympus, BX51). The optical feedback system we developed [16] can project two-dimensional (2D) optical patterns of size $5.1 \times 3.8\ \mathrm{mm}^2$ from a liquid crystal projector (Sanyo, LP-XU87) onto the micro-aquarium. The 16 compartments, labeled A to P as shown in Fig. 1b were illuminated individually by blue light according to the neural network algorithm described in the next section. Blue light induces a reduction in cell density in the compartment through the photophobic reaction of *Euglena* cells [16]. Real images of the *Euglena* cells were taken with a video camera (Trinity, IUC-200CK2) through a 5× object lens with an observation area of $4.0 \times 3.0\ \mathrm{mm}^2$. The resolution of pattern projection and image capture was 200 pixel/mm. The real images taken with a frame rate of 0.16 s were converted into trace images of swimming *Euglena* cells by subtracting, thresholding, binarization, and superimposition of 10 images. Through this conversion, trace images were produced with a repetition rate of 0.63 Hz, as shown in the example in Fig. 1b.

2.2. Neural network algorithm

Fig. 2a depicts a chart giving the calculation steps for *Euglena*-based neurocomputing with digital/analog feedback. The swimming movement of *Euglena* cells in individual compartments was evaluated using trace momentum (TM), i.e., the number of pixels in a compartment in the trace image. As shown by the numbers in Fig. 1b, a higher TM means that the compartment has a greater number of swimming *Euglena* cells with a higher swimming speed.

Each compartment corresponds to one neuron as illustrated in Fig. 2b, and the TM of the compartment is treated as the output

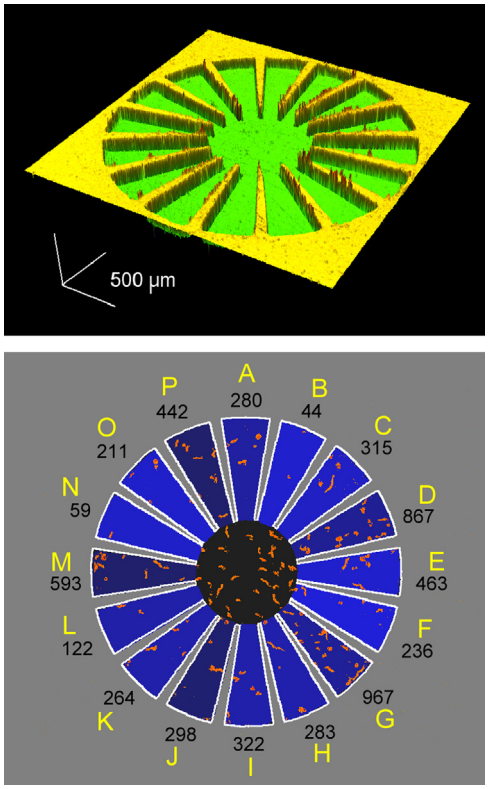

Fig. 1. (a, Top) 3D image of 16-compartment micro-aquarium observed with a confocal microscope. The micro-aquarium was made of polydimethylsiloxane. (b, Bottom) Example of trace image of swimming *Euglena* cells in the micro-aquarium. Letters outside the micro-aquarium denote the indices of the compartments, while numbers outside the compartments represent the TM values obtained by counting the pixels of traces in the individual compartments. TM values are high for compartments with larger numbers of swimming *Euglena* cells. Illumination intensity for each compartment is indicated by the brightness of the compartment's color.

signal $\{x_j(t)\}$ of the neuron. All 16 output signals $\{x_j(t)\}$ are sent to the other neurons as the input signals for the next time step. The input signals $\{x_j(t)\}$ are integer values typically in the range of [0..1000]. In order to fit input signals $\{x_j(t)\}$ to the Hopfield-Tank algorithm [17], the values $\{x_j(t)\}$ are converted into real values in the range [0..1] by the sigmoid function given in Eq. (1).

$$\sigma(x) = 1/(1+\exp(-b(x-c))) \tag{1}$$

Parameters b and c in Eq. (1) are calculated by Eqs. (2) and (3), respectively, which were derived empirically as a function of the photoreaction ratio γ. The photoreaction ratio γ is defined by the ratio between the 10-latest averaged TMs without blue-light (av_TM0) and with blue-light (av_TM1), as given by Eq. (4).

$$b(\gamma) = (0.72\gamma + 0.246) \times (22.0/av\_TM0) \tag{2}$$

$$c(\gamma) = (32.1\gamma^2 - 2.79\gamma + 11.9) \times (av\_TM0/22.0) \tag{3}$$

$$\gamma = av\_TM1/av\_TM0 \tag{4}$$

Details of the parameter determination in Eqs. (2)–(4) have been reported separately [14,15]. The photoreaction ratio γ and parameters b and c were refreshed every 10 time steps, i.e., every 16 s.

After calculating $\sigma(x_j(t))$with Eq. (1)–(4), a weighting summation was obtained for each neuron by the Hopfield-Tank algorithm given in Eq. (5) with a weight matrix w_{ij}.

$$y_i(t+\Delta t) = f\left(\sum_j^N w_{ij}\sigma(x_j(t))\right) \tag{5}$$