Motion Control of *Daphnia magna* by Blue LED Light

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Abstract—*Daphnia magna* show strong positive phototaxis to blue light. Here, we investigate the effectiveness of behavior control of *D. magna* by blue light irradiation for their use as bio-micromachines. *D. magna* immediately respond by swimming toward blue LED light sources. The behavior of individual *D. magna* was controlled by switching on the LED placed at 15° intervals around a shallow Petri-dish to give a target direction. The phototaxic controllability of Daphnia was much better than the galvanotactic controllability of *Paramecium*.

Index Terms— *Daphnia*, Bio-micromachine, Phototaxis, Motion Control

I. INTRODUCTION

Studies to use microorganisms as bio-micromachines was first investigated in 1986 as presented by Fearing [1]. We have investigated the control of microorganism motion by "taxes". We have succeeded in controlling the motion of *Paramecium* along a star-shaped route through negative galvano-taxis. The motion of the *Paramecium* could be harnessed to rotate a ϕ 0.5 mm microimpeller, demonstrating the use of a microorganism as a micromachine [2].

We also developed a positioning control system of *Tetrahymena* that utilized the downward flow of the bioconvection. By inducing and controlling the reciprocating movement of a seesaw over a distance of 20 mm, the shapeless actuation system could be realized with a large number of microorganisms [3].

We also developed a motion control method that utilizes phototaxis [4]. A blue laser scanning system was used to illuminate paths in a rectangular, experimental pool that would be followed by *Euglena*. The setting position and angle were adjusted to examine taxis to blue light. By using a large number of *Euglena*, a mechanical assembly could be manipulated to perform the task of inserting the projection into the hole, both of which were made of planar plastic film [5].

Recently, some researchers have attempted to use bacteria as bio-micromachines [6-8]. The microorganisms used in these studies were mainly protests, and similar studies have not yet been conducted on multicelluar motile plankton species. Multicellular motile planktons have larger bodies than protists and are more highly evolved, enhancing their ability to carry out more complex tasks provided that they can be controlled. Tools may be easily attached to their exoskeletons, and the small (body length < 0.5 mm), motile zooplankton or their juvenile instars may have potential as bio-micromachines.

II. TAXES OF MULTICELLULAR MOTILE ZOOPLANKTON

Generally, rearing zooplankton is more difficult than motile protists due to the requirements for also growing natural food (mainly phytoplankton) in culture and to the lack of suitable artificial diets. First, we collected five species, comprising 4 Branchiopoda (*Daphnia magna*, *Moina sp.*, *Bosmina sp.*, *Scapholeberis sp.*), and 1 Copepoda (species: unknown). Continuous cultivation, however, was only achieved for *Daphnia magna*.

First, these 5 zooplankton species were tested for galvano-taxis, and all were found to have no response to the weak voltages that are effective for *Paramecium*.

Next, the 5 zooplankton species were examined for phototaxes. In this experimental set-up, Copepoda did not swim to/from any light source. Branchiopoda, however, immediately moved to light sources, especially blue light. Therefore, it may be possible to control the movement of Branchiopoda by changing the light field, similar to the method used to control *Euglena*.

D. magna, which was selected for use in the following experiments, is a very large member of Branchiopoda, having an adult body length of about 2 to 4 mm. *D. magna* swims by simultaneously moving swimming seta at both second antennae, which are bilaterally symmetrical. Due to the large exoskeleton, their body is heavier than that of ciliates, and swimming is primarily accomplished in an upward direction. *D. magna* tilts the longitudinal axis forward to swim to the forward direction.

III. PHOTOTAXIS OF DAPHNIA

Next, the mechanism of phototaxis in *D. magna* was investigated. In order to make precise observations, we fixed a fine fishing line to the carapace using CA bond,

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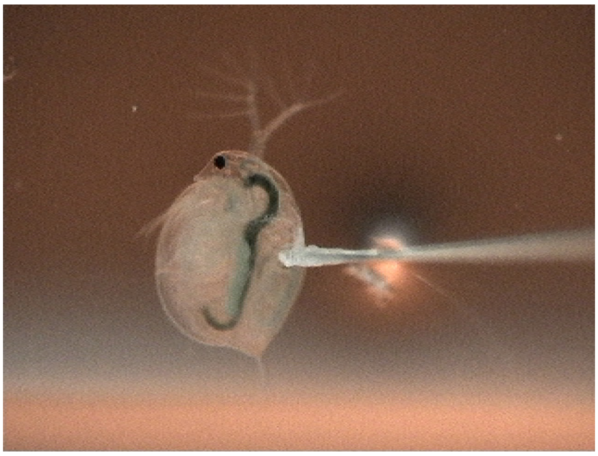


Fig. 1 An angling fine line as bonded to an individual *Daphnia magna* using CA bond

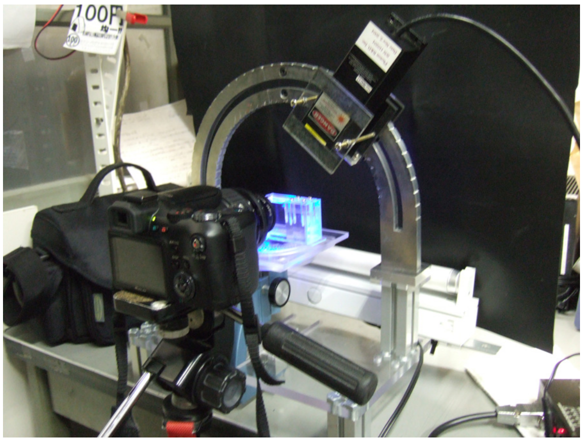


Fig. 2 Movement of the second antenna of *Daphnia magna* was observed following irradiation by a blue laser from a set angle

which is commonly employed for the studies of small crustaceans (Fig. 1). An individual *D. magna* was then set in the observation pool (60 x 22 x 40 mm^3) made of transparent acrylic resin with soaking cultivation water and irradiated by a laser from various angles, as shown in Fig. 2. You can see the transparent rectangular shaped experimental pool at the center and individual D. magna was set in the pool with fixing fishing line. The experiments were done in the room temperature (around 293K). The beating frequency of the second antennae was measured from images captured on high-speed video with a Casio EX-F1 digital camera.

A. Effect of Wavelength

An individual fixed *D. magna* was irradiated with laser beams of the different colors: blue (λ=473 nm, 10 mW, Photon R&D CRB-1010A, Beam diameter=1.49 mm); green (λ=532 nm, 5 mW, LeadLight Technology AGLML1C1-5, Beam diameter=2.00 mm); and red (λ=633 nm, 1 mW, NEC GL5090, Beam diameter=1.83 mm). The reaction of *D. magna* was evaluated using beat frequency in response to the irradiation. As *D. magna* showed little reaction to the red laser, we knew that the blue light was the best for controlling the motion of *D. magna*.

B. Effect of Laser Irradiation Intensity

Irradiation intensity of the blue laser was controlled by ND filters. The *D. magna* was fixed as shown in Fig. 1 and the laser irradiation was oriented to the vertical downward direction over the pool. Irradiation intensity from 100 to 0.1% was tested and beating frequency of the second antennae is shown in Fig. 3. The reaction was increased for laser irradiation intensity at 30% or greater.

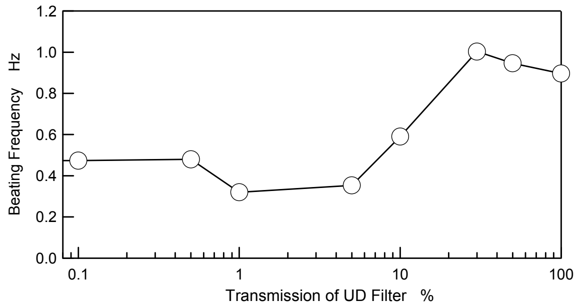
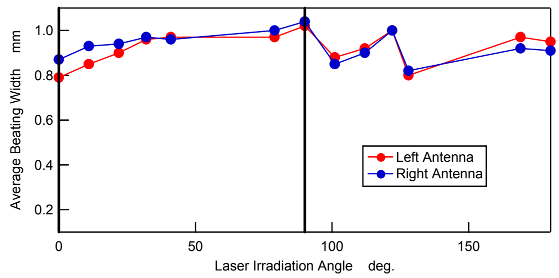


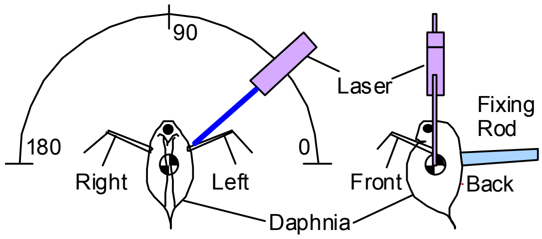
Fig. 3 Relationship between laser beam intensity and the beating frequency of the second antennae of *D. magna*

C. Effect of Laser Irradiation Position

The reaction of an individual fixed *D. magna* to irradiation from different positions was examined using the set-up shown in Fig. 2. The laser was set to the upward position with a vertical beam angle (90° of Fig.4(b)). Irradiation was targeted at different positions relative to the vertical axis of the body. Beating frequency of the second antennae was higher for irradiation closer to the eye.



(a) Difference of the beating range of the 2nd antennae measured for blue light irradiated at 0 to 180°.



(b) Experimental setting

Fig.4 Beating width of the right and left second antennas was investigated by targeting laser irradiation from different side angles

D. Effect of Laser Irradiation Angle

Next, the reaction of *D. magna* to laser irradiation angle relative to the mono-sphere compound eye (0°-180°) was investigated. Irradiation was carried out from the front to back and from left to right (Fig. 4(b)).

The beating frequency of the second antennae is not greatly affected by changing the irradiation angle in either orientation.

There was no difference in beating frequency between left and right second antennae, as *Daphnia* always move both second antennae simultaneously. Moving the laser irradiation angle from left to right gave the same movement times for both second antennae, but the swing angles differed with the swinging angle of the antenna of the opposite side of the laser irradiation being increased. These differences of the swing angles may be the gathering mechanism of Daphnia's positive orientation phototaxis. Fig. 4(a) depicts this phenomenon. The measurements of 0° - 180° and 180° – 0° were done using different individual *D. magna*. Therefore, two *D. magna* were used in these experiments

In these experiments, the *D. magna* continued to react to the laser beam for all laser positions. *D. magna* showed gradually decreasing activity. As irradiation was started from 0° (left side) and ended at 180° (right side), the non-symmetrical shape between left side and right side may be attributable to the decrease in activity over time.

The moving times and swing angles of the second antennae did not show any changes with differences in irradiation angle from front to back.

When the position of the *D. magna* was adjusted using the fishing line, the individual swung the second antennae to recover a vertical position in the absence or presence of irradiation by the laser beam.

IV. MOTION CONTROL EXPERIMENT

A. Motion Control Method

As blue light has the strongest phototaxic effect and that of the red light is weakest, we designed the motion control experiment to use blue light as the stimulus and red light to illuminate the experimental field. This is similar to experiments with *Euglena*. The experimental design is shown in Fig. 5. A petri-dish shaped motion control pool was equipped with 24 blue (λ=470 nm) LEDs at intervals of 15° on the vertical wall. The surface of the pool was painted matte black to avoid light reflection. The entire experimental space was illuminated by an array of red LEDs (λ=660 nm). A microprocessor (H8-3048, Renesas Technologies) and a joystick were used to allow manual control of the direction of blue light irradiation.

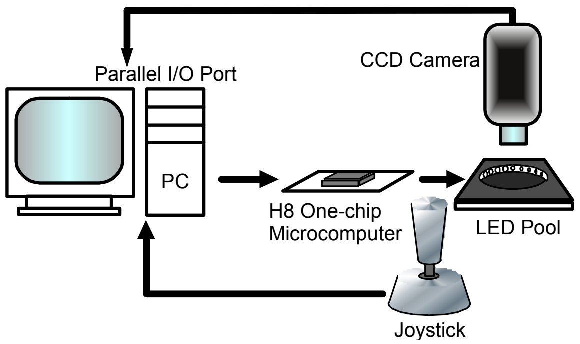


Fig. 5 Schematic diagram of the experimental system

Experimental images were collected by a CCD camera and sent to the PC controller. The position and the body angle of the individual *D. magna* was measured by image processing software by the following method. The center of gravity (COG) of the *D. magna* was determined after the processing of binarization, noise removal, boundary tracing, and detection of the individual area. The COG was calculated and then the body axis was detected. Swimming speed and direction were measured by comparing COG position from images processed at 0.2–s intervals.

An automatic control program, mentioned in the following part, attempted this data to control the movement of individual *D. magna*.

B. Manual Motion Control Experiments

An operator used the joystick to control illumination by turning on individual LEDs. The LED with a oriented in a direction closest to the slant of the joystick was switched on by the program for manual control operation. Since *D. magna* consistently swims directly toward the blue light, its controllability is much better than that of *Paramecium* or *Euglena*. A representative result of a manual control experiment in which the operator gave signals for a star-shaped path is shown in Fig. 6.

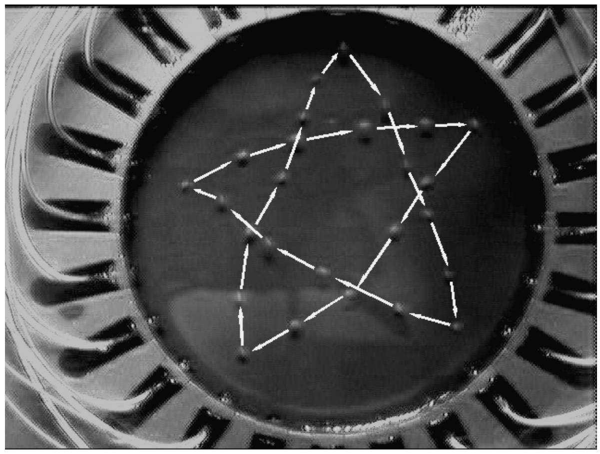


Fig. 6 A representative path taken by *D. magna* in the manual motion control experiment

C. Reaction to LED position change

The manual motion control experiments showed that *D. magna* usually swim directly toward the light. However, there seemed to be considerable delay in the reaction time to the next LED signal. Here, the reaction time was measured for a *D. magna* guided to a LED directly opposite. As the *D. magna* reached the center

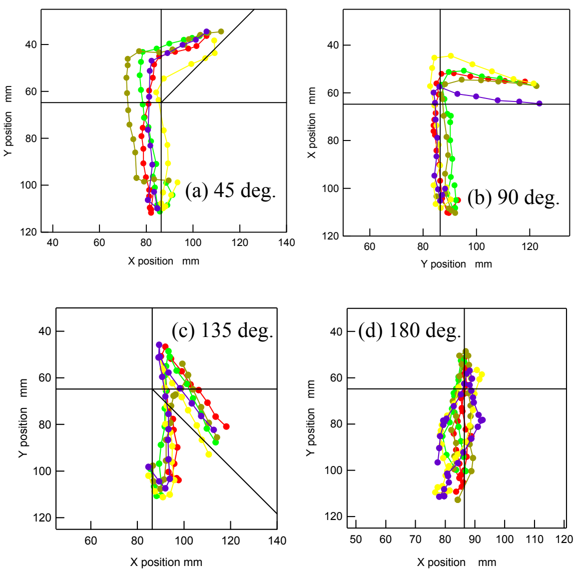


Fig. 7 Change in swimming trajectory in response to the change in LED illumination source for *D. magna*

point of the pool, the LED illumination was switched to an LED of at 45, 90, 135, or 180°, and the recorded swimming trajectory was analyzed using the image processing.

We examined 5 trials for each trajectory. Recorded typical trajectory changes are shown in Fig. 7 (a)-(d). The deviation from the target trajectories clearly shows the reaction delay. However, *Paramecium,* that is guided by the galvanotactic method, show far greater deviation from the target route.

The reaction time to the change in angle of the light source is shown in Fig. 8. For turning angles within ± 90°, the reaction time is estimated to be 0.3 to 0.5 sec. This reaction time is basically considered to be the time needed to make a physiological response to the change in stimulus. The reaction time is determined by the time elapsed between the change in direction of LED illumination and the start of the turning response to the change in stimulus. In the future, we aim to examine the changes in motion more precisely using a motion control pool with fine pitch LEDs.

D. Automated Motion Control Experiments

An automated motion control experiment was set up as follows. These experiments were done using 24LED

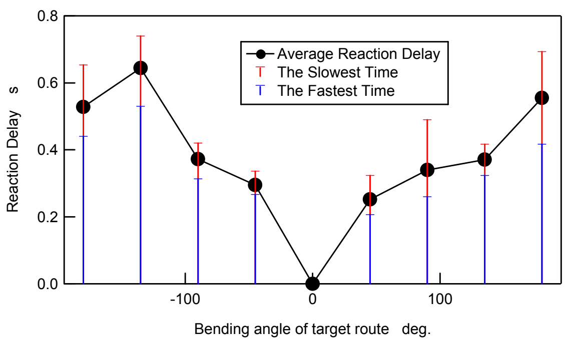


Fig. 8 Reaction time to the change in angle of the light source

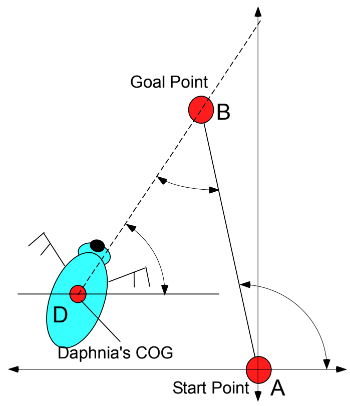


Fig. 9 Schematic for the control equation calculation

pool that is the same to the pool using in the manual control experiments.

The LEDs were manipulated to draw a star-shaped course. The control path was made according to the schematic shown in Fig. 9. The COG of the *D. magna* (D) is tracked. To measure θ_2, the line DB (present position of the D and the goal point) is defined. As D is guided to move along the straight line from vertex (A) to the next vertex (B), the deviation from the target path is calculated from the measured angles θ_1 and θ_2 as an angle $(\theta_1 - \theta_2)$.

Based on the following calculation, we determined the angle of the next LED illumination, θ, with a proportional gain, *Kp*, set from range from 0 to 2:.

$$\theta = \theta_1 + Kp(\theta_1 - \theta_2) \qquad (1)$$

A representative path with *Kp*=0.5 is shown in Fig. 10. Optimal control was achieved for *Kp*=0.5.

The deviation from the target path was greater than that achieved from the manual motion control experiments, suggesting that the arrangement of the LEDs in the automatic motion control experiment was too course to precisely control the swimming path of D.

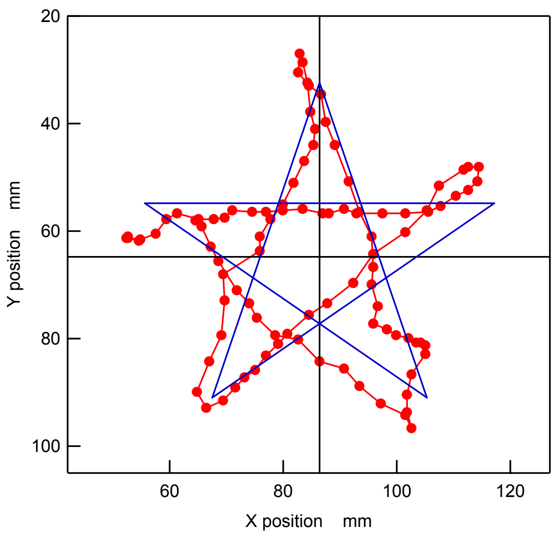


Fig. 10 Representative path with automatic motion control using *Kp* = 0.5

E. Motion Control Using Fine Pitch Pool

Based on the automated motion control results, an experimental pool with 48 LEDs set at intervals of 7.5° was fabricated to achieve more precisely control *D. magna* movement. The automated motion control experiment was run again in the new pool with *Kp*=1.5 for 5 rounds of the target star-shaped route. Control was greatly improved with this pool. A representative trial shows overlapping trajectories that clearly show a star shape (Fig. 11), which is clearer than can be achieved with *Paramecium* guided by galvanotactic control method.

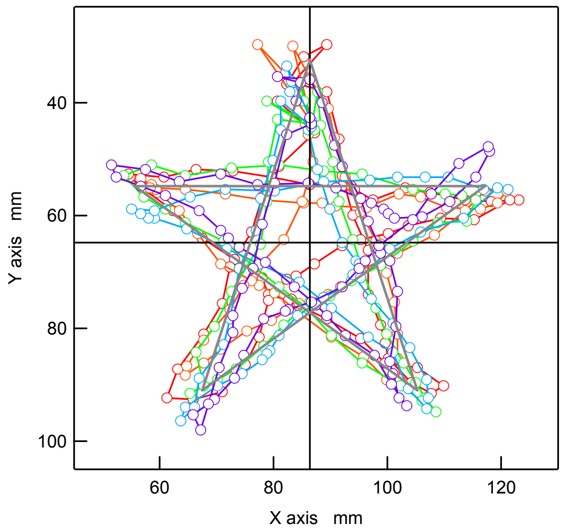


Fig. 11 Representative automated motion control trial with 5 rounds of the star-shaped trajectory using a fine pitch LED control pool and Kp=1.5

The mean control deviations vary with *Kp* as shown in Fig. 12. These values were measured from the trajectories of the 5-round automated experiments such as Fig. 11. There is no clear optimum value because the variation ranges between 4 to 6° over this range of *Kp*. However, the deviation is at a minimum at *Kp*=1.5.

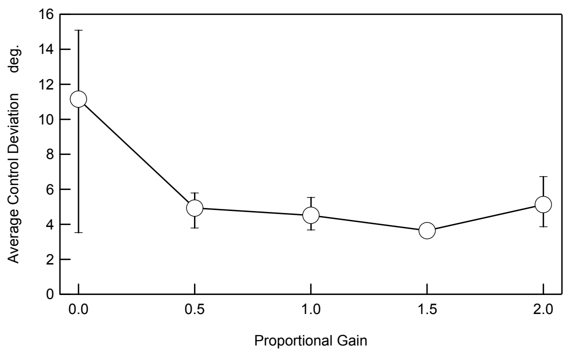


Fig. 12 Mean control deviations for the *Kp* values tested

V. FORCE MEASUREMENT

To measure the force generated by *D. magna*, we tethered polycaprolactone thermoplastic polymer plastic balls of different diameters (5-10mm) to the carapace with fine polypropylene string (<10 μm diameter) as shown in Fig. 13.

The maximum diameter of the ball that adult *D. magna* could pull was 8 mm. If we neglect the resistance of the string and consider only the viscos drag of the plastic ball, we can calculate the force generated by *D. magna* by the following equation:

$$F = 3\pi d \mu U \tag{2}$$

where F is the generating force, d is the diameter of the ball; μ is the viscosity of the medium, and U is the velocity of the ball. The representative calculated value was 0.28 μN.

This value may be too small considering the body size of *D. magna*. Additional measurements by other methods will be necessary to confirm the force generated by *D. magna*.

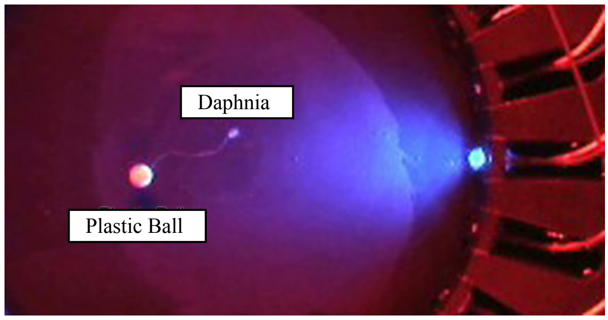


Fig. 13 Photograph taken during the force measurement experiment

VI. CONCLUSION

This study clarifies that the performance of the Branchiopoda tested in this study is much better suited for use as a bio micromachine ciliates and flagellates. In the case of Branchiopoda, we can attach special tools to their exoskeletal shell, making it is much easier to equip them for special functions. As members of Branchiopoda molt every 3 days, the effect of any special equipment to the health of the organism is limited.

VII. FUTURE WORK

The Branchiopoda used in this study, *D. magna*, is too large for use as a bio-micromachine. However, small species of Branchiopoda such as *Bosmina* may be suitable.

Juveniles may also be suitable. *Bosmina* juveniles are much smaller (body length < 100 μm) than *Paramecium*. We have already confirmed that the *Bosmina* juveniles also show strong positive phototaxis to blue light.

In the future work, we must refine the control methods in consideration of reaction times in order to improve controllability. Then, we can consider what special tools can be attached to Branchiopoda.

VIII. ACKNOWLEDGEMENTS

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REFERENCES:

[1] R.S. Fearing, Control of a Micro-Organism as a Prototype Micro-Robot, *2nd International Symposium on Micromachines and Human Sciences, (1991)*, pp.1-15.

[2] A. Itoh, Motion Control of Protozoa for Bio-MEMS, *IEEE/ASME Transaction on Mechatronics*, Vol.5, No.2, (2000), pp.181-188.

[3] A. Itoh and H. Toida, Control of Bioconvection and Its Mechanical Application, *IEEE/ASME International Conference on Advanced Intelligent Mechatronics AIM'01,* (2001), pp.1220-1225.

[4] A. Itoh, Eulgena Motion Control by Local Illumination, *Bio-mechanisms of Swimming and Flying* (2004), Chapter 2, pp.13-26, Springer-Verlag Tokyo.

[5] A. Itoh and W. Tamura, Object Manipulation by a Formation-Controlled Euglena Group, *Bio-mechanisms of Swimming and Flying* (2007), Chapter 4, pp. 41-52, Springer-Verlag Tokyo.

[6] T. Matsunaga and T. Tanaka, Nano-Actuator Using Magnetotactic Bacteria, *Jidokagijutu*, vol.29, no.4, (1997), pp.53-57.

[7] Y. Uno et al., Metal Biomachining of Bacteria, *JSME Int. J. Ser. C*, vol.39, no.4 (1996), pp.837-842.

[8] Y. Hiratsuka, et al., A Microrotary Motor Powered by Bacteria, *Proc. the National Academy of Science of the United States of America*, Vol. 103, No.37, (2006), pp13618-13623.