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An Improvement of The Motion Controllability of Paramecium by The Media Exchange Type Long Electrode Distance Pool

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***Abstract* - We herein propose a new type of motion control pool for Paramecium, in which an electrical field is applied. In order to prevent a pH change, the culture medium in the electrode area is exchangeable. The experimental area and the electrode area are separated by a membrane filter in order to maintain electrical conductivity and to prevent mixing of the media. Two types of pools were constructed: one in which eight electrodes were fixed (as in our previous studies) and one incorporating four rotatable electrodes in order to improve the angular resolution. The controllability of the Paramecium was much improved, and an average control deviation of 0.149 mm was achieved.**

Index Terms* - *Paramecium, Motion control, Long electrode distance pool, Media exchange.

I. Introduction

A. Background

If microorganisms can be used as living micromachines by manipulating them at our will, it may be applicable to medical, engineering, and biological applications that cannot be achieved with current artificial micromachines. Among so many microorganism species, Paramecium is a highly evolved species as a unicellular organism and has excellent motor ability. Therefore, Paramecium is considered to be a very promising species for use as a living micromachine. It will be very important in considering the future mechanical use of microorganisms.

The idea of using protist as bio-micromachine by control their behaviors by using their taxes were first seen in the research of the R. Fearing et.al. [1], who controlled a Paramecium along a square route. However, the controllability was poor.

The present authors have succeeded in automatically controlling a Paramecium along a star-shaped route [2] using a shallow control pool with four pairs of counter electrodes. They also succeeded in rotating a 0.5-mm-diameter micro-impeller using a motion-controlled Paramecium [2] and the same experimental pool.

The success rate for this task was very low due to the poor controllability of Paramecia. Therefore, the present authors attempted to construct an object manipulation system using a motion-controlled Paramecium. However, such a system has not yet been realized due to low positioning accuracy.

The present authors have also investigated the applicability of other microorganisms, such as Tetrahymena [3], Euglena [4], and Daphnia [5]. In the case of individual control of the microorganisms, Daphnia exhibited much better controllability than Paramecia. An automatic object transportation system was developed using a motion control Daphnia [6]. We believe that the difference in controllability between Paramecium and Daphnia might be due to their natural characteristics, and controlling Paramecia with the same degree of ease as Daphnia may not be possible.

B. Relative pool size

In the above mentioned research, a 4-mm octagonal motion control pool was used for the 0.2-mm Paramecium. In the case of the Daphnia, a 130-mm-diameter pool was used for the 3-mm Daphnia. The pool for Daphnia was much larger than that for Paramecium.

Moreover, the results of previous studies have revealed that the controllability of the 0.05-mm Tetrahymena in the 4-mm octagonal pool is better than that of Paramecium [7]. Among Paramecium species, the controllability of the smaller (0.12 mm) *Paramecium tetraurelia* was better, and that of the 0.24-mm *Paramecium multimicro-nucreatum* was worse than that of *Paramecium caudatum* [8]. These results have revealed

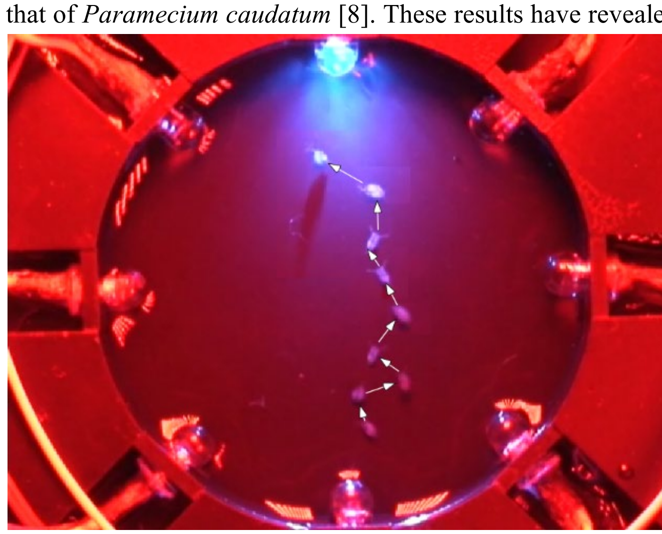

Fig.1 Daphnia's bad controllability in the case of using same relative size and same relative applied electrical field angle resolution control pool of paramecium

that the controllability improves as the relative size of the control pool is increased.

Therefore, the controllability of Daphnia using the same relative pool size (60 mm) and the same resolution (8) of the Paramecium's motion control was especially investigated in this study using the facilities of the former studies. Figure 1 shows a photograph of the experimental results. The results reveal that Daphnia exhibited worse controllability than Paramecium.

In summary, the poor controllability of Paramecium is not an inherent characteristic, and it may be possible to improve the controllability of Paramecia using a large-scale high-resolution motion control pool.

II. Basic Plans of The New Motion Control Pools

Constructing a large motion control pool for Paramecium is difficult due to the electrolysis that occurs as a result of the applied voltage. A large pool constructed in the same manner as the previous control pool would require a higher voltage in order to realize the same electric gradient in the pool. This higher voltage would result in severe electrolysis around the application electrodes. The pH change of the culture media resulting from the electrolysis rapidly weakens the physiological activity of the Paramecium.

A media-exchange system and a media-separation system between the electrode area and the experimental area was adopted for the new motion control pools. In the present study, the pool size was increased to the diameter of the experimental area (10 mm) in order to maintain the relative dimensions of the 130-mm-diameter pool used for Daphnia.

The experimental area and the area around the electrodes are separated by a membrane filter having micrometer-sized holes. Hence, the electrical conductivity is maintained, whereas the mixture of the media is greatly restricted. The pH-altered medium around the electrodes is exchanged by the micro-tube pump. An experimental pool of similar construction was constructed in order to test the endurance properties of the motion control of the Paramecium by galvanotaxis [9].

Two experimental pools were constructed. The first is a pool with eight fixed electrodes, which is similar to previous pools. However, the thickness of the electrodes is increased in order to apply a relatively homogeneous electric field. The second is a pool with rotatable electrodes. Four wide electrodes, which can be rotated by a servo motor, are placed in this pool. The goal here is to increase the angular resolution of the applied electrical field. The importance of the angular resolution in controlling the motion of the microorganism is described in a previous study on controlling Daphnia [10].

III. Development of New Motion Control Pools

A. Pool with Eight Fixed Electrodes

The first pool developed in the present study has eight fixed electrodes. The diameter of the experimental area of the pool was set to be 10 mm.

The first trial of the control experiment revealed that the applied electric potential gradient in the experimental area was much smaller than the design value. The reasons for this were investigated, and the existence of the membrane filter was found to weaken the potential gradient in the experimental area. The change in the potential gradient between two electrodes is not constant and the gradient near the electrodes is larger than that in the middle area. The membrane filter in the middle area is more affected to weaken the electrical gradient than that near the electrodes. Hence, it is important to place the membrane filter near the electrodes. The first pool has a gap of 2 mm between the electrodes and the membrane filter. Therefore, the gap is reduced to 0.5 mm.

The electrodes in the early pools are planar or concave. The concave electrodes were designed to fit the shape of the round wall of the membrane filter. However, the shape of the electrode was modified to be convex based on the results for the pool with rotatable electrodes described later herein.

The final shape of the 10 mm membrane filter wall pool after modification was such that the distance between electrodes was changed from 14 mm to 11 mm, the thickness of the electrodes was changed from 3 mm to 5.5 mm, and the depth of the pool was changed from 1 mm to 3 mm. The thickness of the electrodes was increased in an attempt to apply a more uniform electrical gradient in the experimental area. An outer view of this pool is shown in Fig. 2.

Fig.2 Media exchange type motion control pool with fixed eight electrodes and membrane filter

The applied voltage on the electrodes was set to 12 V based on the balance between the pH change in the experimental area and the controllability of the Paramecium.

B. Pool with Rotatable Electrodes

When we control the motion of the Paramecium, it is better to apply the electrical field using the wider electrodes in order to generate a more uniform electric potential gradient. However, it is better to increase the angular resolution of the applied electrical field in order to control the Paramecium more precisely. When we used the pool with eight fixed electrodes, multiple electrodes were used to apply the electrical field from the pseudo angle.

It may be better to apply the electrical field by means of real counter electrodes in order to rotate the Paramecium to the setting angle. The rotatable-electrode pool was designed in order to realize the abovementioned concept. This pool has two pairs of counter electrodes (four electrodes), which are rotatable using a servo motor. The uniform electric field distribution created by a wide counter electrode is the most suitable to control behavior of Paramecium. Therefore, in an electrode rotating pool, when an electric field is applied to only one of the two counter electrodes and if the movable range goes out, the electrode switch to the other pair.

Since the first pool was constructed using monolithic construction, the maintainability of this pool was very poor. In particular, the membrane filter, which divides the experimental area and the electrode area, is very brittle and is very easily broken. Therefore, we changed its structure. The modified pool is divided into a number of blocks, each of which is exchangeable.

Next, we investigated the structure of the motion control program for the rotatable-electrodes pool. In the case of the rotatable-electrodes pool, we can set both the applied voltage and the angle of the application electrodes. The setting flexibility is higher than that of the conventional fixed-electrodes pool. After our investigation, we decided to use a voltage-application method in which a pair of counter electrodes was used within an angle of 90deg. Moreover, if the angle must be made to exceed 90deg, the pair of application electrodes is changed.

We used a fixed value as the applied voltage. The voltage applied to each electrode is such that the electrode located on the target angle received the lowest applied voltage, the counter electrode received the highest applied voltage, and the remaining two electrodes received the average applied voltage. We used an H8-3048 one-chip microcomputer for the sub-controller, which produced a PWM signal to control the PS-050 servo motor (Tonegawa Seiko Inc.) for the radio control system. We added an H8 circuit to four 10-bit D/A converters, and the voltages applied to a pair of electrodes are also controlled by the H8 circuit. The main PC controller provides the angle of the electric field to H8, and the H8 sub-controller controls the servo motor and D/A converter. The servo motor is connected to the rotatable electrodes by the timing belt through a 1:2 ratio pulley. Therefore, the controllable angle area is approximately 270deg. The maximum rotational speed of the electrodes is approximately 400deg./s. The structure and the outer view of this pool are shown in Fig. 3.

Using this control program, we attempt to manually control the behavior of a Paramecium along a star-shaped route and the control was successful.

Next, we investigated the effect of the electrode shape. The wider electrodes can be used for the rotatable electrodes

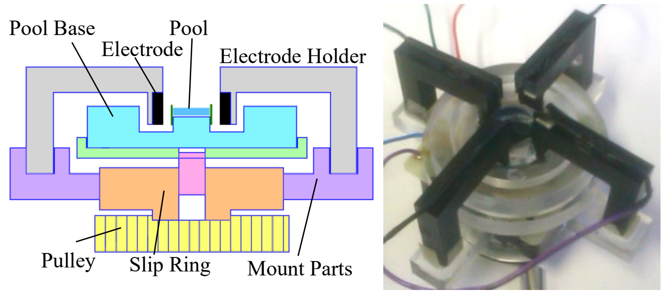

Fig.3 A schematic diagram and an outer view of the electrodes rotatable type motion control pool for paramecium

as compared to the eight fixed electrodes. Therefore, the flexibility of the shapes of the electrodes is increased. The following electrode types were primarily investigated:

1) Concave electrodes

These electrodes are depicted on the left-hand side of Fig. 4. The tip shape of the electrodes matches that of the wall of the membrane filter. The width of the electrodes is 10 mm, and the distance between electrodes is 12 mm.

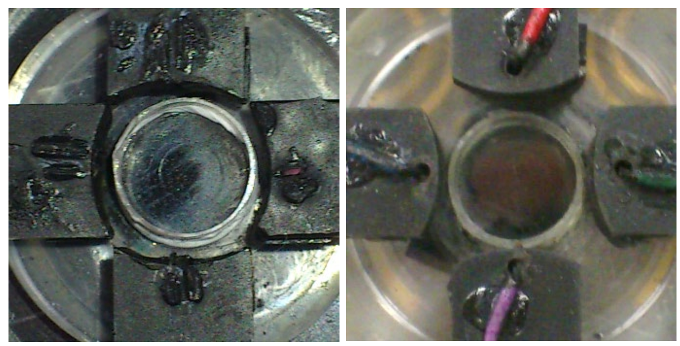

Fig.4 Concave (Left) and convex (Right) electrodes of the electrodes rotatable type experimental pool

The experiments revealed that when several Paramecia entered the pool, the Paramecia swam toward the nearest electrode, rather than toward the center of the electrodes. As a result, the swimming direction varies greatly in the neighborhood area of the electrodes. This property is disadvantageous for precise control of the Paramecium's motion.

2) Convex electrodes

Based on the above results, we changed the design principle and investigated the electrode shape in order to collect the Paramecium at the center of the electrode. An outer view of the convex electrodes is depicted on the right-hand side of Fig. 4. The width of the electrodes is 10 mm, and the center of the electrode is the closest point to the membrane filter by the convex shape.

We also conducted experiments by placing several Paramecia and found that, in the case of this electrode, all of the Paramecia swam to the center of the negative voltage application electrode. Therefore, in the present study, we adopted this design for the control pools. The electrode distance is 11 mm, which is the same as for the pool with eight fixed electrodes.

C. The Voltage-Application Circuit

In the present study, the larger pool was used. Therefore, we must prepare a voltage application circuit that can apply a higher voltage. We first planned to increase the voltage in proportion to the electrode distance. However, the reaction of the Paramecium was very weak.

The electrical potential gradient in the experimental area was investigated, and we found that if we apply a voltage of -9.5 V between the counter electrodes, the potential gradient in the experimental area is 0.25 V/mm, much smaller than the assumed value. The effect of the membrane filter to decrease the potential gradient was very large. Therefore, we increased the applied voltage to -13.5 V. Under this condition, the potential gradient was increased to 0.45 V/mm, and we were able to control the Paramecium well.

However, under this condition, the potential gradient was gradually decreased. This is due to the electrolysis near the electrodes. Therefore, it is necessary to continuously exchange the culture medium around the electrodes in order to maintain the application condition of the electric field.

D. The effect of flow rate on pH change

The pH-altered culture medium outside the filter wall was exchanged by a micro-tube pump in order to prevent the pH change of the culture medium inside the filter wall. In this case, if the flow rate is too high, the effect of the exchanged flow appeared in the culture medium of the experimental area. If the flow rate is too low, the pH inside the filter wall will change. Therefore, the proper flow rate was investigated.

A bromothymol blue solution was poured into the culture medium of the experimental area, and the relationship between the flow rate and the pH change was investigated.

As a result, the pH of the experimental area was maintained at the neutral condition within 15 minutes with a flow rate in the range of 100 to 150 ml/h. Both the feeding valve and the drain valve must be adjusted during the operation of the pump. This adjustment is difficult at flow rates higher than 150 ml/h. At a flow rate of less than 100 ml/h, the pH change in the experimental area was observed. Therefore, we decided to set the flow rate to between 100 and 150 ml/h.

IV. Investigation of The Control Algorithm

A. Control Experiments in The Fixed Eight Electrode Pool

The control experiment involving the media-exchange motion control pool became practicable after the abovementioned refinement. Therefore, automatic motion control experiments were conducted in the next stage. The control experiments were conducted in order to guide the Paramecium along the star-shaped target route based on the information regarding the experimental image processing. This process is closed loop control to feedback the detected Paramecium's position. In the case of this pool, the pool shape is about 6 times larger than the old pool in the area and thereby the average pixel number those were composed the Paramecium were much less than that our old system. Hence we had to newly make a control program especially image processing routine.

In the early experiments, there were numerous misrecognitions due to dust and scratches. Therefore, we confirmed the Paramecium image on the computer. The image processing method used in the automatic motion control experiments involves 1) capturing the image using a camera, 2) Gaussian smooth filtering, 3) binarization, 4) labeling objects larger than a threshold value based on raster scanning, 5) boundary tracking, and 6) recognition of the Paramecium. Misrecognition was confirmed to have occurred by such as the dust in the pool or the reflection of illumination.

Therefore, we decided to use the feature of the Paramecium's slender oval shape, and the average aspect ratio was used to identify the Paramecium. Using this recognition algorithm, the misrecognition ratio was decreased.

Next, we first used the simplest control algorithm, in which the electrical potential gradient was applied to the direction between the present position of the Paramecium and the target apex of the star shape without any correction. Fig. 5 shows an example. The swimming route of the Paramecium was plotted by dark dots as a control result. We could control Paramecium along the star-shaped target route using this simplest algorithm. The result, however, was not good and the influence of the rapid turning around the apex was significant.

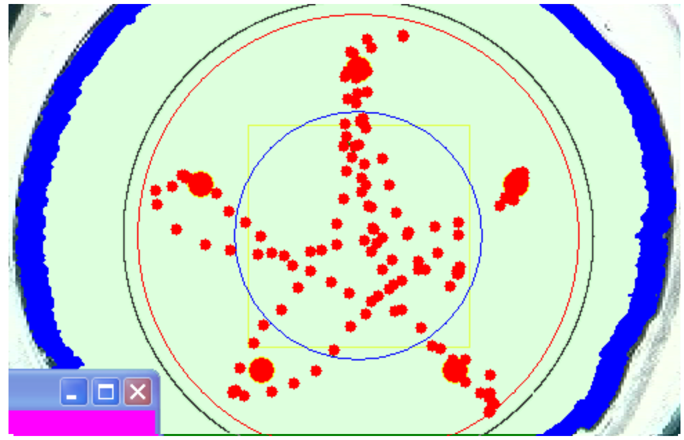

Fig.5 An example of the automatic motion control experiments using simple control algorithm

B. Correction by Proportional Control

Next, an angle-correction algorithm using the proportional control method was adopted. This algorithm was also adopted in our previous motion control program. In this algorithm, the application angle of the electrical field was corrected to the direction of the guide route in order to follow the guide route. The correction amount was calculated by the proportional control method.

As a result, this algorithm sometimes worked well if the Paramecium existed in the proper range. For the case in which the Paramecium becomes more distant from the target route, the correction angle becomes too large, and in the worst case, the program guided the Paramecium in the opposite direction.

C. Algorithm Using Internal Division Ratio

A new control algorithm was proposed to solve the abovementioned problem and to guide Paramecium smoothly along the guide route. A schematic diagram of this algorithm is show in Fig. 6. In this algorithm, a perpendicular line was drawn from the present position of the Paramecium to a line of the guide route between the previous target and the current target. The temporary target was then decided by the internal division ratio of the intersection point of the perpendicular line with the line of the guide route and the current target. Paramecia were guided to this temporary target.

In the case of this control algorithm, over-correction of the swimming angle did not occur. We investigated the optimal internal division ratio. The experimental results are

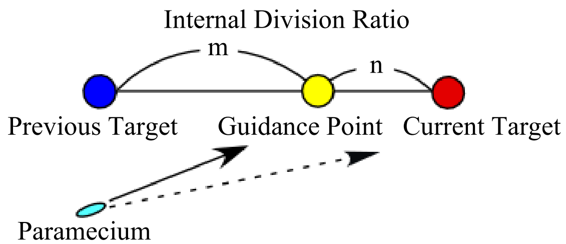

Fig.6 An angle correction algorithm by internal division ratio

summarized in Fig. 7. The results reveal that the ratio (m:n) = 7:3 provided the minimum average control deviation. Therefore, the ratio of 7:3 was adopted for this control algorithm.

D. Overrun Suppression Algorithm

An overrun around the target point was often observed in the motion control results along the star-shaped target route, as shown in Fig. 5. This might be caused by the reaction delay of the Paramecium. Therefore, an overrun suppression algorithm was created in order to change the target in anticipation of the reaction delay time from the prediction based on the

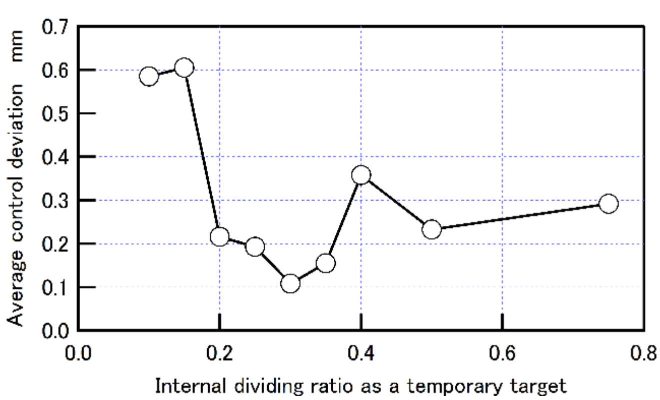

Fig.7 The effect of the internal division ratio as a temporary target on the average control deviation

swimming speed, position, and direction of the Paramecium.

If the estimated approach time to the target is shorter than the average reaction delay time, then the target is changed to the next target. The average reaction delay is approximately 700 ms. A schematic diagram of this algorithm is shown in Fig. 8. This algorithm worked very well on average.

Some Paramecia, however, has a different reaction delay from the average delay. Therefore, the program was modified in order to determine the delay time from the previously measured delay time. This modified program was more stable than the previous program

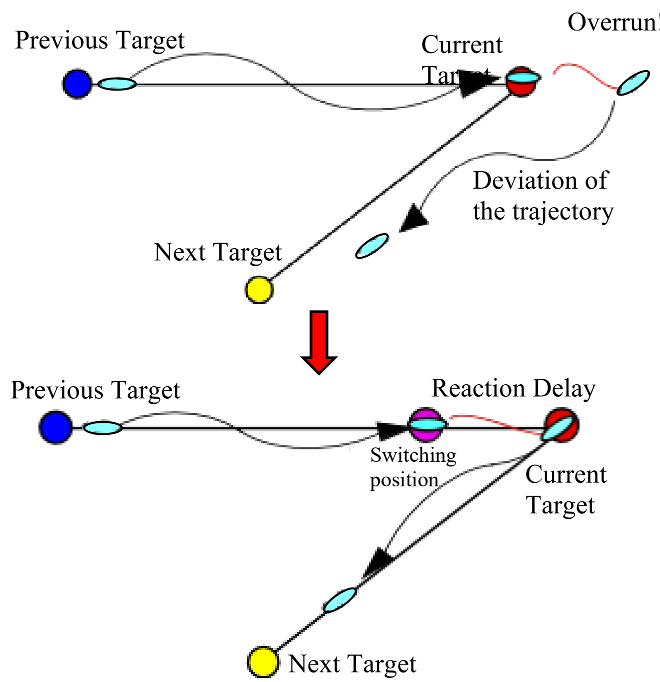

Fig.8 Schematic diagram of the overrun suppression control algorithm

E. Motion Control Results after Improvement

Paramecium motion control experiments were performed using the abovementioned modified control algorithm. The results showed that the controllability using the refined pool with eight fixed electrodes and the modified program was much better than that of the previous system.

The average control deviation was recorded to be 0.152 mm, which indicates that the use of the long-electrode-distance pool is very effective in order to improve the motion controllability of the Paramecium.

The results for the average control deviation of each control line, however, indicate the variation of each line. Fig. 9 shows the relationship between the deviation angle of the swimming route from the nearest electrode pair and the average control deviation. The control results were good if one couple of electrodes were mainly used to apply the electrical field.

The average control deviation was greatly increased on the route along which two electrode pairs were evenly used to

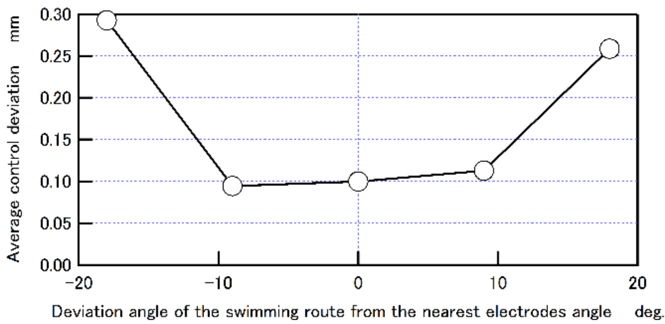

Fig.9Relationship between deviation angle of the swimming route from the nearest electrodes and the result of the average control deviation

apply the electrical field. These facts indicate that the effect of the angular resolution of the applied electrical field was low.

F. *Experiments Using Electrode Rotatable Type Pool*

Next, the motion control experiments were performed using the pool with rotatable electrodes and the abovementioned modified control program in order to compare the results to those of the pool with eight fixed electrodes.

Our first control program to drive the electrodes was that every electrode pair is used in the field of 90deg. The results of the initial experiments, however, revealed that the algorithm in which one electrode pair is used only in the field of 90deg. causes numerous changing action of the application electrode pair during control along one line. The time lag of the movement of the electrodes changes, and the discontinuity of the applied electrical field disturbs the smooth guiding of the Paramecium.

Therefore, the voltage application method was changed so that one electrode pair is used over an angle of 160 deg. by overlapping both sides by 35deg. This application method is effective in order not to change the application electrode pair during control along one line. The application electrodes are usually changed when the target point is changed. The electrodes are changed to the nearest electrode pair to the new application angle.

The control experiment was conducted again after this modification. A great improvement in the results was observed. An example of the five loop traces of the motion control experiments along a star-shaped target route is shown in Fig. 10. The stability was much better than that for the previous control system.

The average control deviation was 0.149 mm, which is 28% of the best result for Daphnia. Hence, by using the newly developed control system, the motion control accuracy of Paramecia exceeded the control accuracy of Daphnia, which we assumed has much better controllability than Paramecia.

V. Conclusion

The controllability of Paramecium was greatly improved by the development of a large motion control pool. The adaptation of the high angular resolution of the application of

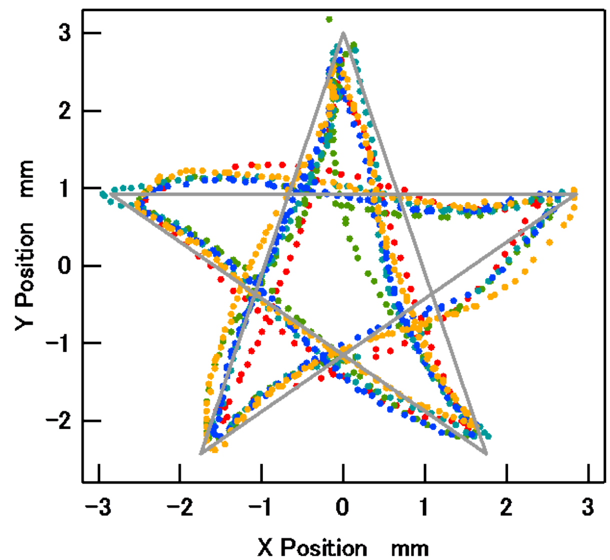

Fig.10 An example of the five loop traces of the motion control experiments using electrodes rotatable pool and refined motion control algorithm

the electrical field was also effective for improving the controllability of the Paramecium.

In the future, we intend to develop an automatic object transportation system using motion controlled Paramecia. This has been achieved for Daphnia [6]. The average control deviation obtained in the present study is smaller than the length of the body of the Paramecium. Therefore, the probability of a collision with a transportation object will be greatly increased, and our goal may be achieved using the newly developed control system.

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