



Two-dimensional trajectory analysis of the diatom *Navicula* sp. using a micro chamber

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ABSTRACT

We describe a trajectory analysis of diatom cell locomotion by combining a micro chamber and two-dimensional position coordinate analysis. By shutting cells in a micro chamber, continuous microscopic observation of *Navicula* sp. cells was possible. The trajectory of each cell was visualized once every second by using position coordinate analysis although time resolution of previous papers were range of minutes. Our data revealed frequent change of movement direction. Furthermore, the correlation between the distances moved, the velocity, and the acceleration of the cells was discussed in detail.

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1. Introduction

Motility analysis of microorganisms is an attractive research area because many cells have meaningful and intriguing movement profiles (Harper, 1977). Recently, the progress in nanotechnology has resulted in several breakthroughs in cell motility research. One such breakthrough is the use of a micro chamber used to isolate a cell in Fukuda et al. (2007), Inoue et al. (2001) and Moriguchi et al. (2002). When the movement of a living cell is observed using optical microscopy, the cell can easily escape from the observation field. If a cell is shut into a micro chamber, where the size of the chamber is similar to that of the observation field, continuous observation is available without the concern that the cell may be lost from the observation field. The second breakthrough has been the dramatic improvement in computer performance where currently it is possible to analyze digital videos of cell movements by using a personal computer.

In this paper, we focus on the trajectory analysis of diatom cells. The diatom is one of the major photosynthetic planktons that are found in seas, rivers, and lakes (Falkowski and Raven, 1997; Nelson et al., 1995; Raven and Waite, 2004). As some types of diatom cells can move on solid surfaces, the velocity of cell movements has been investigated since the 1950s (Edgar and Pickett-Heaps, 1984; Gordon and Drum, 1970; Lewin, 1958). In particular, the mechanism

underlying this motility has been intensively studied. For example, Poulsen et al. (1999) proposed that diatom motility is managed by the actin–myosin motility system. The effects of perturbation on diatom motility have also been studied by several research groups (Cohn and McGuire, 2000; Cohn et al., 2003). For example, the requirement for calcium in cell motility was reported by Cooksey and Cooksey (1980). The impacts of electromagnetic fields were studied by several research groups (Clarkson et al., 1999a, 1999b; Parkinson and Sulik, 1992). The influence of photo irradiation was also investigated (Cohn, 1999, 2001; Cohn et al., 2004; Moroz et al., 1999). Furthermore, the effects of the structural properties of the solid surface, to which the diatom cells attach, on cell movement velocity, were also examined (Gordon, 1987; Higgins et al., 2003; Holland et al., 2004; Wigglesworth-Cooksey et al., 1999).

Although the effects of external factors on cell motility have been studied, there are only a few reports regarding trajectory analysis. As a pilot study, the movement of the diatom *Phaeodactylum tricorutum* was reported by Iwasa and Shimizu (1972). In their experiments, cell movements were photographed every 2, 3, or 5 min by 8 mm film on an agar plate. The rolling or rocking of diatom cells has been investigated by several authors since the 1990s (Apoya-Horton et al., 2006; Pickett-Heaps et al., 1991). Although those reports were not specifically trajectory analyses, the movement of the cells was recorded for almost 1 h.

Here we describe a detailed trajectory analysis for individual diatom cells. To achieve this purpose, a micro chamber was employed for microscopic observation of diatom cells for the first time.

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Furthermore, the position of individual cells in the chamber was estimated by the use of two-dimensional video analysis software.

2. Materials and methods

2.1. Sample preparation

Navicula sp. stock was pre-cultured in a petri dish in filtered and sterilized sea water with Daigo IMK (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) and sodium metasilicate (final concentration 1 mM) (Umemura et al., 2007). Cultivation was carried out at 18 °C, under 440 lux of fluorescent light. Inoculation was repeated every week to obtain stable activity of the cells. In the case of cultivation for observing cell motility, a glass slide was put in an empty petri dish, and the dish was filled up with 35 mL of the above mentioned sea water. Subsequently, 0.1 mL of cell suspension was injected into the petri dish. After 7–9 days of cultivation, an acrylic plate (2 cm square) with 9 holes in the center area was put onto the glass slide in the petri dish, following which, a cover slip was put onto the plate. Finally, several to several tens of cells were shut into a micro chamber that was made up of the holes on the plate, the slide glass, and the cover slip.

2.2. Observation

Diatom cells that were shut in a micro chamber were observed using an inverse optical microscope (IX70, Olympus, Tokyo, Japan) for 10 min at room temperature under 5000 lux of light. The observed cell movements were captured as avi files (one frame per second) by a digital camera system (DP72, Olympus, Tokyo, Japan). The tracks for 39 cells were calculated from the avi files using two-dimensional video analysis software (Move-tr/2D 7.0, Library, Tokyo, Japan). The coordinate positions for individual cells in the chamber were estimated by the software, and the velocity, acceleration, and distances moved were calculated from the coordinate data. To avoid the effect of coordinate fluctuation, the velocity, acceleration, and distance values were estimated as an average of 20 s.

3. Results and discussions

Fig. 1a shows a schematic view of a micro chamber. Diatom cells were pre-cultured on a bare glass slide that was placed into a petri dish with 35 mL of culture medium. After 7–9 days of cultivation, an acrylic plate with 9 holes and a glass cover slip was put onto the glass to secure the diatom cells inside. Because the diameter of a hole and the thickness of the plate were 600 μm and 1 mm, respectively, the volume of a chamber was almost 1 mm^3 . In this way, most of the cells in a chamber did not escape during the 10 min observation period.

An example of an optical microscopy image of some diatom cells in a micro chamber is shown in Fig. 1b. The trajectory of a motile cell, which was estimated by the two-dimensional video analysis software, was written over the image. “O” and “E” indicate the original ($t=0$ s) and end ($t=600$ s) positions of the cell, respectively. No.1 to No.4 indicate the turned positions. The cell came into contact with the wall of the chamber at position No.1, and returned to a position just before the wall at position No.3 and No.4. At position No.2, the cell turned, although it did not come into close contact with the wall. If the micro chamber had not been employed, the diatom cell might have escaped from the observation area. Furthermore, because the position coordinates of the diatom cell were calculated every second, the detailed behavior of the cell was reflected in the trajectory.

Fig. 2 shows the trajectories, velocities, and total distances over 600 s for 4 individual cells. One cell turned sharply and frequently (Fig. 2a), another cell turned several times with obtuse angles (Fig. 2b), a third cell traced a similar route twice, and another cell rotated (Fig. 2d). Using the two-dimensional position coordinate

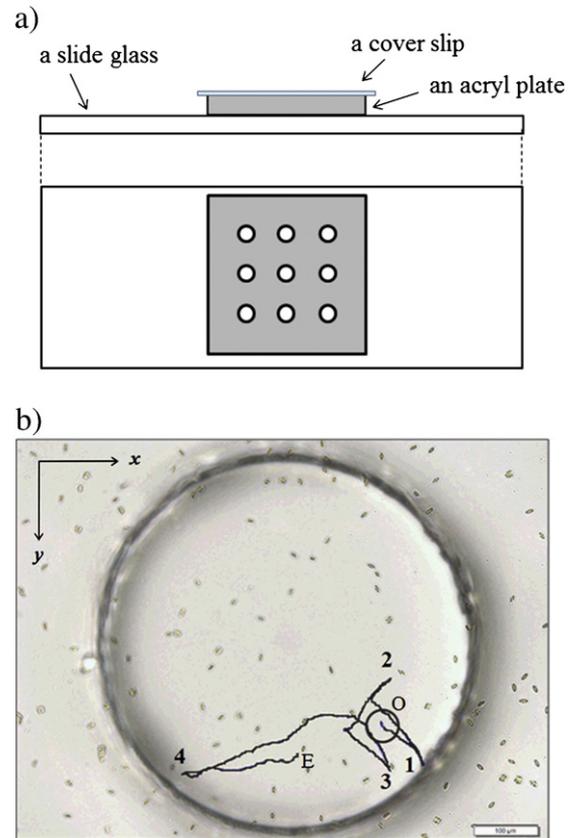


Fig. 1. (a) Schematic view of a micro chamber prepared on a glass slide. (b) A typical micrograph of *Navicula* sp. cells in a micro chamber. The trajectory of a cell over 60 s was over-written. “O” and “E” indicate the origin ($t=0$ s) and end ($t=600$ s) positions. The cell turned at position Nos.1, 2, 3, and 4.

analysis, various movements of the diatom cells were successfully visualized.

For each of the 4 cells, the changes in velocity over 600 s are shown in Fig. 2e to h. Although microscopic images were obtained once every second to avoid fluctuation of the marker positions during the data analysis, we used values that were averaged every 20 s for quantitative discussion. As a result, the velocities for the 3 cells fluctuated more than twice (Fig. 2f to h). However, these velocities merely fluctuated and did not continuously increase or decrease. These data suggested that the movement of the cells was microscopically non-uniform, yet macroscopically uniform. Fig. 2i to l shows the multiplication of the distances moved for each cell over 600 s. Although the velocities fluctuated, the distances moved were almost linear.

The numerical data for 10 individual cells that were randomly selected are shown in Table 1. The total distances covered during cell movement ranged from shorter than 300 μm to longer than 1000 μm , according to each individual cell. This indicates that the activity for each cell varied continuously not discretely. There are many inactive (immobile) cells also.

The average velocity over 600 s ranged from 1.7 to 0.5 $\mu\text{m}/\text{s}$ for each cell, and the average of the average velocity for the 10 cells was 1.0 $\mu\text{m}/\text{s}$. When we analyzed the velocities of 39 cells, the average velocity was 1.1 $\mu\text{m}/\text{s}$. Thus, the analysis of 10 randomly selected cells was almost consistent with the values obtained from 39 cells. In order to estimate the noise in the experiments, velocity of 10 immobile cells was calculated. The estimated average velocity of the 10 cells for 600 s was 0.11 $\mu\text{m}/\text{s}$. It means that our experimental results included almost 10% noise.

The total distance covered was strongly dependent on the average velocity for each cell. The values for the total distance divided by each

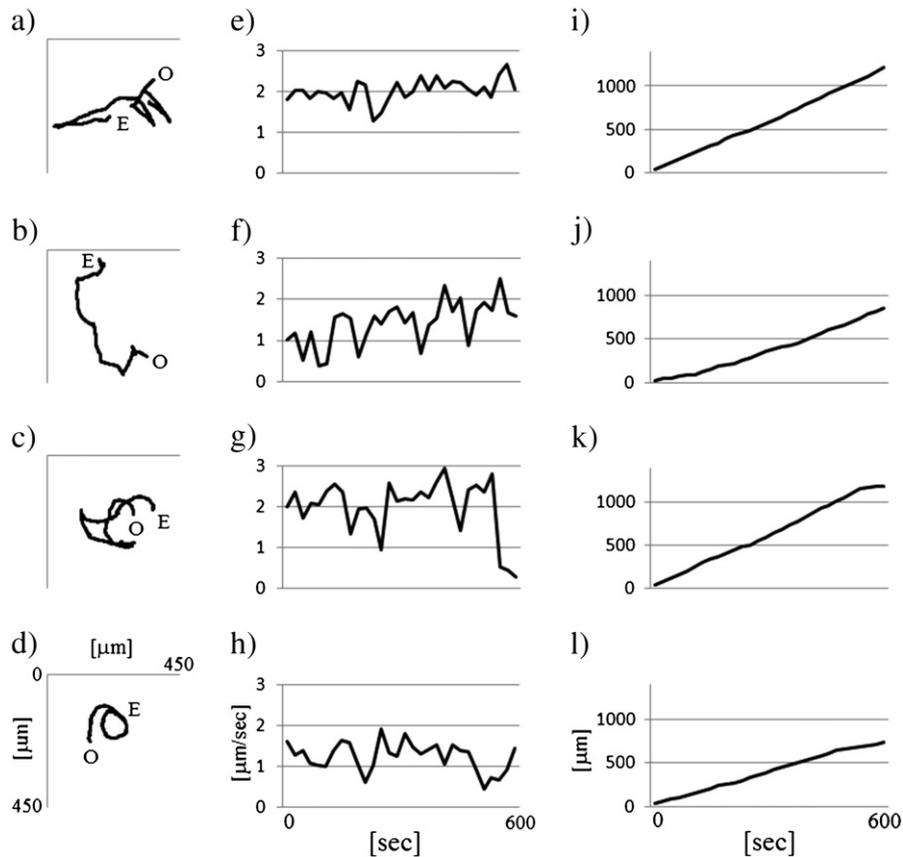


Fig. 2. Examples of the movements of four cells over 600 s. Panels (a) to (d) indicate the trajectories. Panels (e) to (h) show velocity time changes corresponding to (a) to (d), respectively. Panels (i) to (l) show the multiplication of moved distances that correspond to (a) to (d).

average velocity was almost constant (range, 550–590). The result is consistent with the linear profile displayed in Fig. 2i to l.

In contrast, the distance between the origin ($t=0$) and end points ($t=600$) was roughly, but not strictly, related to the total distance. For example, the total distances for cell Nos. 8, 9 and 10 were rather short, and the distances between the origin and the end points were also short. This suggests that the 2 distance values were not independent. However, for cell Nos. 1, 2, and 3, there is no clear dependency on the 2 factors.

The averaged absolute values of the X and Y components of the velocity (V_x , V_y) for the 10 cells were randomly varied. Both the average values of V_x and V_y were $0.6 \mu\text{m}/\text{s}$. These results are expected because there is no specific anisotropy in the micro chamber. In addition, the average values of V_x and V_y , including plus minus information, were almost zero (the averages of V_x and V_y were 0.03 and $0.05 \mu\text{m}/\text{s}$, respectively, these data were not included in Table 1).

The calculation of the averaged absolute values of the X and Y components of acceleration (A_x , A_y) showed a unique result. The averages for A_x and A_y were 0.6 and $0.5 \mu\text{m}/\text{s}^2$, respectively. It is interesting that the velocities and acceleration values are almost similar. This suggests that changes in velocity for the cells were drastic.

Fig. 3 shows the V_x and V_y analysis of 2 cells that turned in response to other objects. In Fig. 3a, a cell moved from left to right, and then turned at point No. 1 just before the edge of the micro chamber (point No. 2). The time changes for V_x and V_y for this movement are shown in Fig. 3c. The turning was clearly reflected as the inversion of the V_x and V_y values. Estrangement of V_x and V_y was increased after the turning. In our experiments, we observed no stopping of the cells in front of the edge of the chamber. On the other hand, some of the cells moved along the edge for a few seconds, but quickly separated from the edge. In general, the cells changed directions in front of the edge without decrease in the velocity.

Table 1

Two-dimensional trajectory analysis for ten cells.

Cell no.	Total distance [μm]	Distance between O and E [μm]	Average velocity [$\mu\text{m}/\text{s}$]	Average V_x [$\mu\text{m}/\text{s}$]	Average V_y [$\mu\text{m}/\text{s}$]	Average acceleration [$\mu\text{m}/\text{s}^2$]	Average A_x [$\mu\text{m}/\text{s}^2$]	Average A_y [$\mu\text{m}/\text{s}^2$]
1	1008	151	1.7	1.4	0.9	1.0	0.7	0.7
2	877	217	1.5	0.8	1.1	1.2	0.8	0.7
3	862	108	1.5	1.1	0.8	1.2	0.9	0.6
4	567	165	1.0	0.5	0.7	1.2	0.7	0.9
5	541	294	0.9	0.6	0.6	1.0	0.7	0.6
6	459	165	0.8	0.6	0.4	0.5	0.3	0.2
7	380	117	0.7	0.4	0.5	0.5	0.3	0.4
8	364	91	0.6	0.4	0.4	0.5	0.3	0.3
9	345	75	0.6	0.4	0.4	0.6	0.4	0.4
10	276	75	0.5	0.3	0.3	0.4	0.3	0.3
Average	568	146	1.0	0.6	0.6	0.8	0.6	0.5

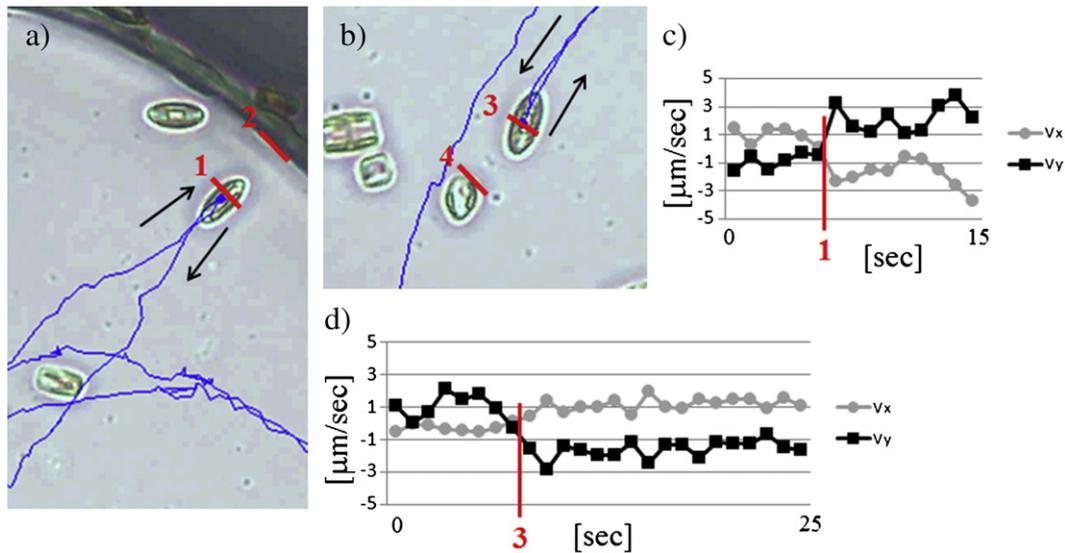


Fig. 3. Examples of turned cells. (a) A cell turned in front of the wall of the micro chamber. (b) A cell turned in front of another cell. (c) Change at time points V_x and V_y corresponding to the trajectory for (a). (d) Change at time points V_x and V_y corresponding to the trajectory for (b).

Fig. 3b shows the turning of a cell in front of another cell located at point No.3. In this case, values of V_x and V_y were clearly changed before and after the turning. Although it is not clear whether the cells recognized the chamber wall and another cell at this moment, detailed analysis is available by our way.

4. Conclusion

Our experiments demonstrated that a detailed trajectory analysis was available by combining a micro chamber and two-dimensional video analysis on a computer. We believe our results will be a basis of understanding diatom motility and of applying the intelligence of the cell movement to new technologies such as bio computers.

References

Apoya-Horton, M.D., Liang, Y., Underwood, G.J.C., Gretz, M.R., 2006. Movement modalities and responses to environmental changes of the mudflat diatom *Cylindrotheca closterium* (Bacillariophyceae). *J. Phycol.* 42, 379–390.

Clarkson, N., Davies, M.S., Dixey, R., 1999a. Diatom motility: the search for independent replication of biological effects of extremely low-frequency electromagnetic fields. *Int. J. Radiat. Biol.* 75, 387–392.

Clarkson, N., Davies, M.S., Dixey, R., 1999b. Diatom motility and low frequency electromagnetic fields—a new technique in the search for independent replication of results. *Bioelectromagnetics* 20, 94–100.

Cohn, S.A., 1999. High energy irradiation at the leading tip of moving diatoms causes a rapid change of cell direction. *Diatom Res.* 14, 193–206.

Cohn, S.A., McGuire, J.M., 2000. Using diatom as an indicator of environmental stress: effects of toxic sediment elutriates. *Diatom Res.* 15, 19–29.

Cohn, S., 2001. In: Hader, D.P., Lebert, M. (Eds.), *Photomovement*. Elsevier Science Pub, B.V. Amsterdam, pp. 375–401.

Cohn, S.A., Farrell, J.F., Munro, D.J., Ragland, R.L., Weitzell, R.E., Wibisono, B.L., 2003. The effect of temperature and mixed species composition on diatom motility and adhesion. *Diatom Res.* 18, 225–243.

Cohn, S.A., Bahena, M., Davis, J.T., Ragland, R.L., Rauschenberg, C.D., Smith, B.J., 2004. Characterisation of the diatom photophobic response to high irradiance. *Diatom Res.* 19, 167–179.

Cooksey, B., Cooksey, K.E., 1980. Calcium is Necessary for Motility in the Diatom *Amphora coffeaeformis*. *Plant Physiol.* 65, 129–131.

Edgar, L.A., Pickett-Heaps, J.D., 1984. Diatom locomotion. *Prog. Phycol. Res.* 3, 47–88.

Falkowski, P.G., Raven, J.A., 1997. *Aquatic photosynthesis* Malden. Blackwell Science.

Fukuda, T., Kato-Murai, M., Suye, S.I., Ueda, M., 2007. Development of high-throughput screening system by single-cell reaction using microchamber array chip. *J. Biosci. Bioeng.* 104, 241–243.

Gordon, R., Drum, R.W., 1970. A capillarity mechanism for diatom gliding locomotion. *Proc. Natl. Acad. Sci. U. S. A.* 67, 338–344.

Gordon, R., 1987. A retaliatory role for algal projectiles, with implications for the mechanochemistry of diatom gliding motility. *J. Theor. Biol.* 126, 419–436.

Harper, M.A., 1977. *The Biology of Diatoms*. In: Werner, D. (Ed.), Blackwell Scientific Publications, Oxford, pp. 224–249.

Higgins, M.J., Molino, P., Mulvaney, P., Wetherbee, R., 2003. The structure and nanomechanical properties of the adhesive mucilage that mediates diatom-substratum adhesion and motility. *J. Phycol.* 39, 1181–1193.

Holland, R., Dugdale, T.M., Wetherbee, R., Brennan, A.B., Finlay, J.A., Callow, J.A., Callow, M.E., 2004. Adhesion and motility of fouling diatoms on a silicone elastomer. *Biofouling* 20, 323–329.

Inoue, I., Wakamoto, Y., Moriguchi, H., Okano, K., Yasuda, K., 2001. On-chip culture system for observation of isolated individual cells. *Lab. Chip* 1, 50–55.

Iwasa, K., Shimizu, A., 1972. Motility of the diatom, *Phaeodactylum tricornutum*. *Exp. Cell Res.* 74, 552–558.

Lewin, J.C., 1958. The taxonomic position of *Phaeodactylum tricornutum*. *J. Gen. Microbiol.* 18, 427–432.

Moriguchi, H., Wakamoto, Y., Sugio, Y., 2002. Takahashi K., Inoue I., Yasuda K., An agar-microchamber cell-cultivation system: flexible change of microchamber shapes during cultivation by photo-thermal etching. *Lab. Chip* 2, 125–132.

Moroz, A.L., Ehrman, J.M., Clair, T.A., Gordon, R.J., Kaczmarek, I., 1999. The impact of ultraviolet-B radiation on the motility of the freshwater epipelagic diatom *Nitzschia linearis*. *Global Change Biol.* 5, 191–199.

Nelson, D.M., Treguer, P., Brzezinski, M.A., Leynaert, A., Queguiner, B., 1995. Production and dissolution of biogenic silica in the ocean: revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biogeochem. Cycles* 9, 359–372.

Parkinson, W.C., Sulik, G.L., 1992. Diatom response to extremely low-frequency magnetic fields. *Radiat. Res.* 130, 319–330.

Pickett-Heaps, J.D., Hill, D.R.A., Blaze, K.L., 1991. Active gliding motility in an araphid marine diatom, *Ardissonea* (formerly *Synedra*) crystalline. *J. Phycol.* 27, 718–725.

Poulsen, N.C., Spector, I., Spurck, T.P., Schultz, T.F., Wetherbee, R., 1999. Diatom gliding is the result of an actin-myosin motility system. *Cell Motil. Cytoskeleton* 44, 23–33.

Raven, J.A., Waite, A.M., 2004. The evolution of silicification in diatoms: inescapable sinking and sinking as escape? *New Phytol.* 162, 45–61.

Wigglesworth-Cooksey, B., van der Mei, H., Busscher, H.J., Cooksey, K.E., 1999. The influence of surface chemistry on the control of cellular behavior: studies with a marine diatom and a wettability gradient. *Colloids Surf. B Biointerfaces* 15, 71–80.

Umemura, K., Yamada, T., Maeda, Y., Kobayashi, K., Kuroda, R., Mayama, S., 2007. Regulated growth of diatom cells on self-assembled monolayers. *J. Nanobiotechnol.* 5, 2.