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Importance of observation interval in two-dimensional video analysis of individual diatom cells

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Abstract The effect of the observation interval on two-dimensional trajectory analysis of motility of individual diatom cells of *Navicula* sp. was studied by comparing thinned-out observation data. The trajectory of cell movement was visualized accurately even after thinning the data interval. However, the analysis of velocity fluctuations of individual cells was found to be significantly affected by the data interval. Reproducibility of the results was guaranteed by analyzing many independent cells. In addition, comparison between automatic and manual determination of cell positions proved that automatic determination was a reliable process. Our data indicated that two-dimensional trajectory analysis using a computer can be a powerful technique to study diatom locomotion.

Keywords Diatom · Trajectory · Two-dimensional · Motility · Locomotion

Introduction

The study of locomotion of microorganisms has attracted considerable attention as a research subject because it reveals their complex and curious behavior (Harper 1977). Detailed analyses of cell movement have been made possible by improvements in computer processing ability; this in turn has led to significant breakthroughs in this area of research. Currently, a personal computer capable of processing gigabytes of high-resolution video data can be used to analyze cell movements (Wu et al. 2006).

Diatoms represent a major group of photosynthetic plankton found in rivers, seas, and lakes (Nelson et al. 1995; Falkowski and Raven 1997; Raven and Waite 2004; Armbrust 2009; Bowler et al. 2010). The ability of some types of diatoms to actively slide on a solid surface has led to an increased interest in diatom motilities. For example, it is known that diatom movements are affected by external perturbations by stimuli such as light and ions (Lewin 1958; Gordon and Drum 1970; Cooksey and Cooksey 1980; Edgar and Pickett-Heaps 1984; Clarkson et al. 1999; Moroz et al. 1999; Cohn et al. 2001, 2004; Cartaxana and Serôdio 2008; Perkins et al. 2010a). The behavior of diatom cells in biofilms has been recognized as one of the important research subjects from the viewpoint of biomass production (Underwood and Kromkamp 1999; Consalvey et al. 2004; Perkins et al. 2010b). Structural studies have indicated that the raphe of the diatoms is important for their locomotion (Drum and Hopkins 1966; Edgar and Pickett-Heaps 1982; Edgar 1983; Edgar and Pickett-Heaps 1983). Further, the actin-myosin motility system has been proposed to explain the diatom motility mechanism (Edgar and Zavortink 1983; Poulsen et al. 1999).

Although diatom locomotion has been an active focus area for researchers, there have been few reports of the detailed analysis of diatom movement trajectories. As an example of trajectory analysis, the movement of *Phaeodactylum tricornutum* was reported by Iwasa and Shimizu (1972). In this study, cell behavior on an agar plate was photographed using 8-mm film at 2-, 3-, and 5-min intervals. Subsequently, the studies by Edgar, which have used biophysical approaches to examine motility, have led to a

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significantly improved understanding of diatom motility (Edgar 1979, 1982). In particular, in 1979, she demonstrated the analysis of the velocity fluctuation of diatom cells using a computer. Although her work did not focus on analysis of the 'gliding' of the diatom cells as she mentioned in the paper, she recorded their movement using cine films with a frame rate of 1/20 frames per second for a 10-s period. The data were accumulated on computer punch cards, and the change in velocity over time was analyzed. She found that the velocities of several different species of diatom cells rapidly fluctuated even during 10-s observation. Since the 1990s, several examples of rolling and rocking phenomena occurring during diatom motility have been investigated (Pickett-Heaps et al. 1991; Apoya-Horton et al. 2006). Recently, we reported the trajectory analysis of *Navicula* sp. cells that were enclosed in a microchamber (Murase et al. 2011). In our experiments, the positions of the diatom cells were captured at 1-s intervals, and the data were analyzed using two-dimensional video analysis software.

In this study, we report the effect of the data interval of microscopic observation on trajectory and velocity analysis in order to verify the importance of using an observation interval scale of the order of seconds. Although observations were carried out at 1-s intervals, thinned data (5-, 10-, and 20-s periods) were specifically used for the analysis. A personal computer was sufficient to analyze 600-s of video data of many independent cells.

Materials and methods

Navicula sp. cells were prepared by passage culture in seawater using Daigo IMK culture medium (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) and sodium metasilicate (final concentration, 1 mM) in a petri dish (Umemura et al. 2007a).

For observation, a slide glass was placed in the petri dish during the cultivation at 18 °C, and subsequently, cell motility was observed on the slide glass after 7–9 days of cultivation.

Inverse microscopy (IX70, Olympus Co., Tokyo, Japan) and a digital camera system (DP72, Olympus Co., Tokyo, Japan) were employed for the observation of diatom cells. The petri dish was directly placed on the microscope stage, and the cells were observed (without opening the lid of the dish) at 25 °C. The sample was irradiated by a halogen lamp at 5,000 lux during the observation. Images with a resolution of 1,360 × 1,024 pixels were obtained for 600 s at 1-s intervals at an approximate frame rate of 1/30 frames per second, and these images were stored as video (.avi) files. Because the area of the images was 872 μm × 656 μm using a 10× objective lens, one pixel was approximately 640 nm^2 in dimensions. It was not necessary to change the focus plane during the observations.

Trajectory analysis was carried out using two-dimensional video analysis software (Move-tr/2D 7.0, Library, Tokyo, Japan). In the automatic determination of the x and y coordinates of a cell, the centroid mode was employed to determine the center of the cell body. The process of employing the centroid mode is as follows: The target cell was centered in a small square grid, and the cell and the glass surface were separated by binarization. Subsequently, the centroid of the cell was calculated by the software. In the manual process, the center of each cell was determined by visual inspection without any automatic calculation. The video data (20-s periods) of ten independent cells were verified by both the automatic and manual processes.

For observations using scanning electron microscopy (SEM, S-4100, Hitachi, Tokyo, Japan), frustules of the diatoms were purified using a previously established method (Umemura et al. 2007a), and an aliquot of a suspension of the frustules in water was deposited on a slide glass. The sample was coated with platinum prior to SEM observations.

Results and discussion

Figure 1a shows the optical microscope image of the *Navicula* sp. cells used in this study. The density of the cells was optimized to enable application of the automatic calculation by the two-dimensional video analysis software. Figure 1b shows the SEM image of a *Navicula* sp. frustule. The dimensions of the frustule along the major and minor axes were nearly 15 and 3 microns, respectively, as previously reported (Umemura et al. 2007b).

In order to analyze the voluminous data of cell motility obtained by microscopy, the automatic determination of cell coordinates on a surface is considered an effective method. However, it is necessary to evaluate the accuracy of the automatic determination of cell position. Hence, we compared the results of the trajectory analysis by automatic and manual processing of the two-dimensional video analysis software. In the case of manual determination, the center of a cell was directly determined by means of visual inspection at 1-s intervals for 20 s of video data. Therefore, 20 images were displayed on the computer, and the center position of each cell was determined consecutively without any computational calculation. In automatic processing, the centroid mode was employed to determine the center of the cell for the same data. The comparison was carried out for ten independent cells in order to confirm reproducibility.

Figure 2 shows an example of the comparison of the cell trajectory obtained by automatic and manual processing of the same data. The shapes of the trajectories are quite

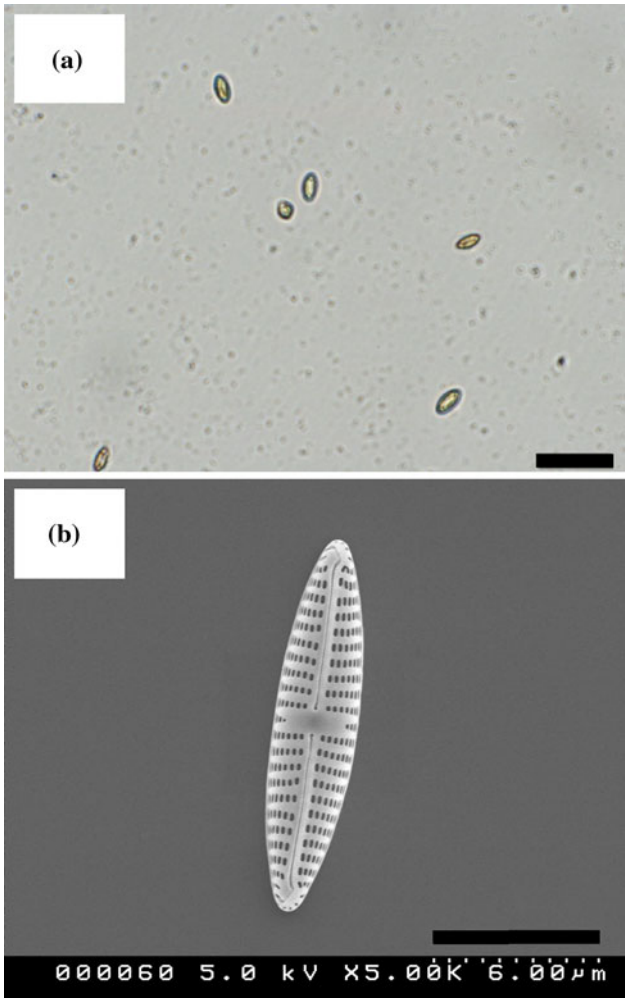


Fig. 1 **a** Optical microscope image of a *Navicula* sp. cells on a glass surface. *Scale bar* is 50 µm. **b** Scanning electron microscope (SEM) image of *Navicula* sp. frustule. *Scale bar* is 6 µm

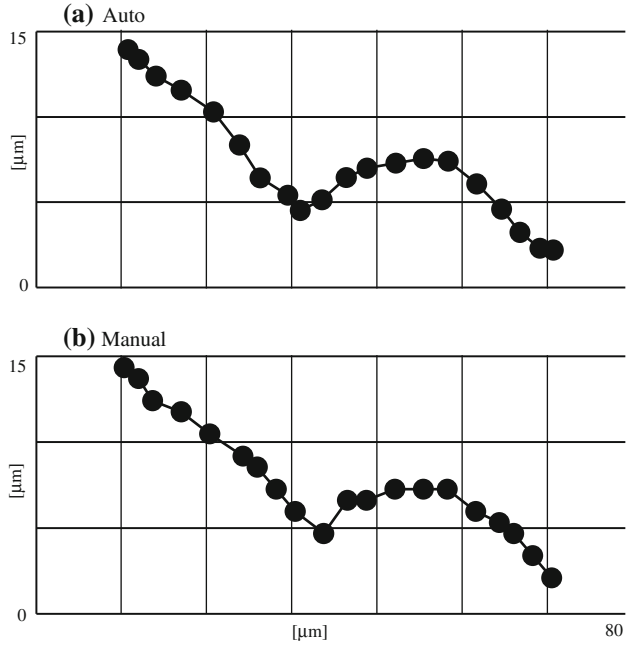


Fig. 2 Trajectories of a *Navicula* sp. diatom cell. The coordinates of the cell were determined by **a** automatic and **b** manual processing using the two-dimensional video analysis software

similar. To verify the result quantitatively, the difference of the x and y coordinate values as determined by automatic and manual processing was calculated for ten independent cells. As a result, the average difference of the x and y coordinate values ($|\Delta x|$ and $|\Delta y|$) between the automatic and the manual processing methods was 0.36 ± 0.28 µm and 0.62 ± 0.44 µm, respectively. The difference of the absolute distance $\left(\Delta d = \sqrt{(\Delta x)^2 + (\Delta y)^2}\right)$ as determined by automatic and manual processing was 0.77 ± 0.55 µm. The difference between the automatic and manual determination was in the range of the pixel size of the image (0.64 µm), and therefore, we assumed that automatic determination of the cell position was sufficiently accurate for our analysis. On the other hand, Δd is <5 % of the length of the major axis of the cell (around 15 µm). Because the cell moves along the major axis of the cell body, the calculation error (0.77 µm) in visualizing the trajectory by the automatic process was nearly 5 %. Even in the case of the width, the Δd value was nearly 24 %. We concluded that the automatic determination of the cell position was as accurate as manual visual determination. Automatic determination of cell position was used in all the subsequent analyses.

Figure 3 shows the trajectories of a moving cell during a 600-s period. The trajectory in Fig. 3a was drawn using all the x and y coordinate data. The movement of the cell was visualized in detail. The trajectory clearly shows that the cell frequently changed its direction of movement. Some cells were found to move in the opposite direction, although the example shown in the figure does not include such inverse turning. In such a case, the trajectory should be overwritten before and after the turning.

In order to verify the effect of the measurement period, the data were thinned out on the purpose. In Fig. 3b–d, the coordinate data that were used in Fig. 3a were plotted for every 5, 10, and 20 s, respectively. It was found that the shape of the trajectory was not significantly affected by this thinning treatment. The arrows in Fig. 3 indicate the characteristic sections of the trajectory curve to facilitate comparison. The crossed position and the turned position did not change because of the thinning. Moreover, we examined the averaged values for 5, 10, and 20 s $\left(x = \frac{1}{n}\sum_n x_i, y = \frac{1}{n}\sum_n y_i\right)$ Even with this treatment, the only difference was that the trajectory curve became

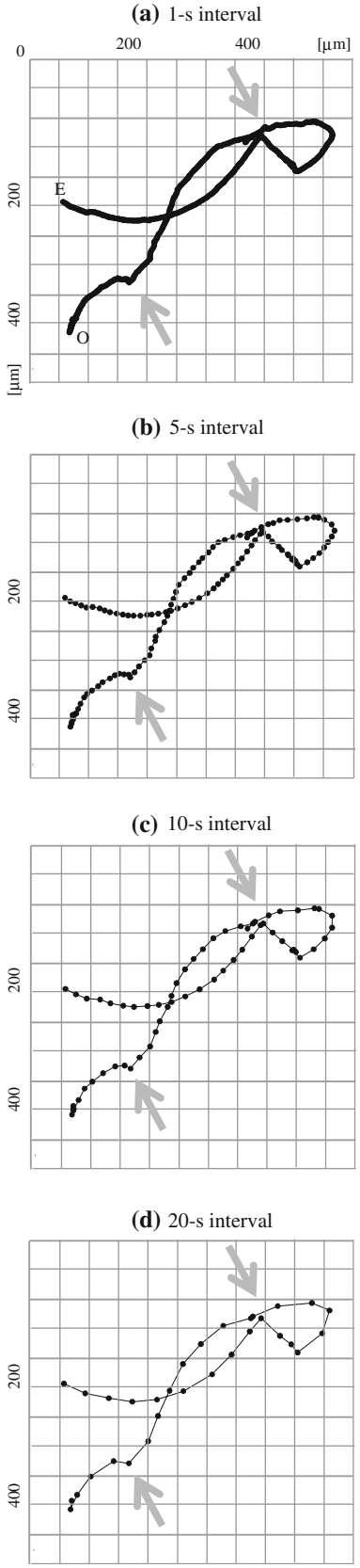


Fig. 3 Trajectories of a diatom cell measured for 600 s. The data intervals are 1, 5, 10, and 20 s in (**a**), (**b**), (**c**), and (**d**), respectively

slightly smoother; therefore, no significant change was observed (data not shown).

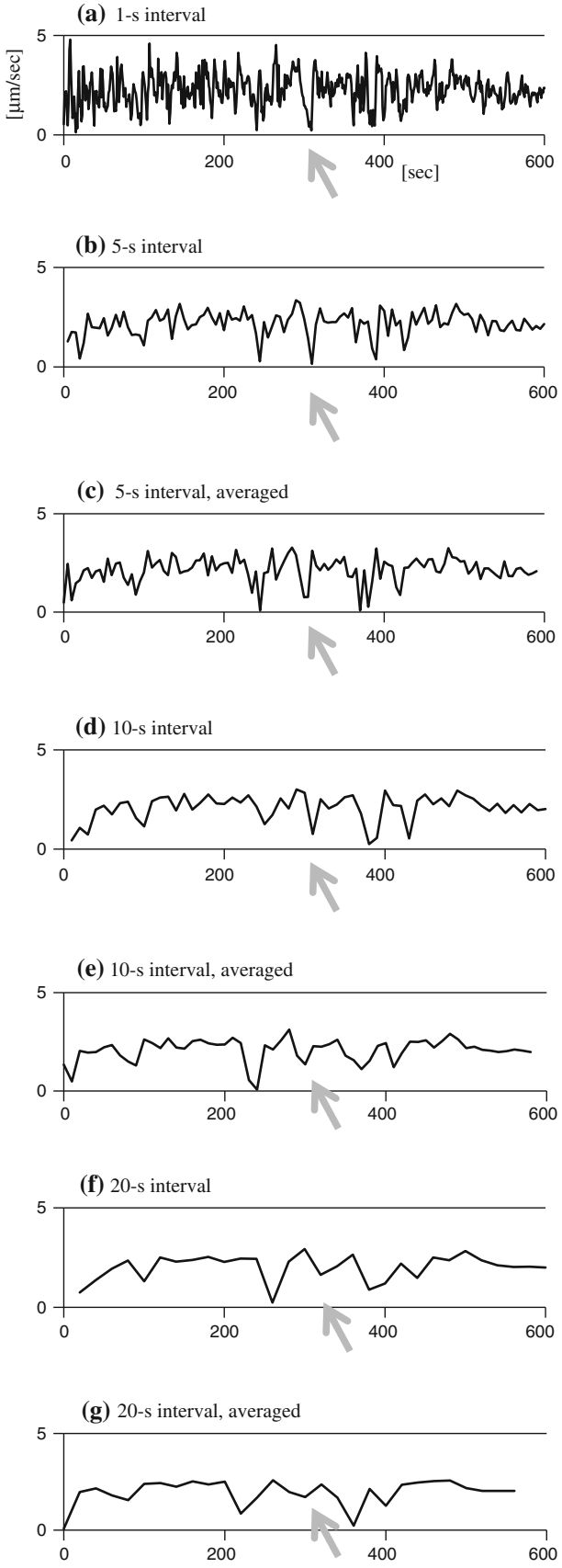
In contrast, analysis of velocity fluctuation of the cells was drastically affected by the data interval. Figure 4a shows the velocity fluctuations of a cell for 600 s as plotted with the original data (1-s interval). The profile shows a significant fluctuation in the velocity. For a quantitative assessment, we focused on the velocity decrease (the convex shape in the top curve in Fig. 4a). We found that the velocity changed 309 times during the 600-s period. This does not directly indicate the change in the direction of the cell movement. There were 37 instances of large decreases in velocity ($\geq$1 μm/s) among the 309 observed changes in velocity. On the other hand, in our previous study, we reported that the apparent fluctuation of immobile cells caused by the instrumental noise or other artificial reasons was 0.11 μm/s, while the average velocity of active cells was 1.0 μm/s (Murase et al. 2011). Therefore, the noise level was close to 10 %. This suggests that the number of changes shown in Fig. 4a is fairly accurate, although the data include the noise.

We compared our experiments with those of Edgar (10 s of observation) in 1979; our observation period was much longer (600 s of observation). The data interval was 0.1 s for Edgar's experiment, whereas in our experiment it was 1 s. This difference in the experimental conditions suggests that the objective of Edgar's analysis was slightly different from ours; our objective was the verification of the effects of data thinning. In addition, since the time of Edgar's experiment in 1979, rapid advancements in computer technology have enabled long-term analysis of diatom motility. In our study, we were able to compare the motilities of many independent cells, as shown in the following analysis.

When we examined 11 independent cells, the average number of instances of decreases in velocity was 301 per cell during the 600-s period. The maximum and minimum number of instances of decreases in velocity were 309 and 285 for all 11 cells, respectively. The number did not significantly fluctuate among the individual cells.

The average number of instances of large decreases in velocity ($\geq$1 μm/s) for the 11 cells was 84 per cell. The maximum and minimum numbers of instances of large decreases in velocity were 141 and 37, respectively. The data suggest that the acceleration behavior of each cell varies, although the frequency of the velocity change does not fluctuate among the individual cells.

A similar velocity analysis using thinned-out data was also performed to confirm the benefits of making observations at 1-s intervals. Table 1 summarizes the analysis with the thinned-out data for the 11 cells. For example, in the case of the 5-s data interval, 120 data points were employed among the original 600 data points (obtained at



◀ **Fig. 4** Number of instances of changes of velocity of a diatom cell. The data intervals are 1, 5, 5, 10, 10, 20, and 20 s in **a**–**g**, respectively. In **c**, **e**, and **g**, averaged data for 5, 10, and 20 s were employed for velocity calculation, respectively

1-s intervals). In the case of averaging cell coordinates over 5 s $\left(x = \frac{1}{5}\sum_5 x_i, y = \frac{1}{5}\sum_5 y_i\right)$, the coordinates were calculated and employed in the analysis.

The number of instances of decreases in velocity over 600 s was found to be inversely proportional to increases in the data interval. For example, in the case of the 5-s interval, the number of instances of decreases in velocity was 61 for 120 data points. In all cases (1-, 5-, 10-, and 20-s intervals), the ratio of the number of instances of decreases in velocity against the data interval was almost 50 %.

In contrast, the number of instances of large decreases in velocity (≥1 μm/s) was significantly reduced with increased data intervals. This number was 84 when using the original 1-s data among 600 measurements; this number represents 14 % of the total data. However, the number was only 2 for the 20-s interval, representing 7 % of the total data. Similar results were obtained when averaged data were employed. The results clearly indicate that the measurement interval strongly affects the quantitative results of motility analysis.

Figure 4b–g shows the velocity profiles of a cell for different observation intervals (5-, 10-, and 20-s intervals) using variously thinned data. The arrows in the figure indicate a concave below a point at approximately 300 s. It is clear that the convex shape is gradually flattened out because of the increase in the data interval. Furthermore, a decrease in the convexity of the curve according to the increased data interval is clearly visible.

Although diatom motility has been studied by many different research groups, the importance of the interval of

Table 1 Velocity decrease for the 600-s observation period. The data interval was varied from 1 to 20 s

Data interval [s]	Number of data point (A)	Velocity decrease [times] (B)	Velocity decrease ≥1 μm/s [times] (C)
1	600	301 (50 %)	84 (14 %)
5	120	61 (51 %)	14 (12 %)
5	120 (Avg.)	59 (49 %)	11 (9 %)
10	60	30 (50 %)	6 (10 %)
10	60 (Avg.)	29 (48 %)	4 (7 %)
20	30	15 (50 %)	2 (7 %)
20	30 (Avg.)	14 (47 %)	2 (7 %)

The percentage number in parentheses in the third column indicates the ratio of (B)–(A)

The percentage number in parentheses of the fourth column indicates the ratio of (C)–(A)

observation has not been examined. In our previous study, we demonstrated the trajectory analysis of diatom cells enclosed in a microchamber using two-dimensional video analysis software (Murase et al. 2011). In this study, we report the importance of the data interval as a factor to be considered in the trajectory analysis of diatom cells. When we can carefully verify the analyzed results obtained by computer analysis, the detailed trajectory analysis of diatoms can be considered an accurate technique to study diatom motility.

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