Tracking fluorescent protein transgenic cells using a robot microscope

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Abstract—C.elegans is one of the most important model organism in neuroscience. We can measure neural activity by brightness of cells, and operate each cell activity by light projection. A robotic microscope, which can project light on single cell in C.elegans, has been developed. In this paper, we propose a tracking method on multi cells. The proposed method achieves individually measuring cells brightness in the video. Using a robot microscope, we can also get stage trajectory. Cell brightness depends on it activity, so it can be helpful for analyzing neural activity. In the future work, we will achieve project on multi cells to stimulate and measure each cell brightness and stage trajectory at the same time to analyze its activity.

Index Terms—C.elegans, Optogenetics, Robot microscope

I. INTRODUCTION

In the neuroscience field, *C.elegans* is widely used as model organisms to observe the relationship between neural activity and behavior of living organisms [1]. *C.elegans* have been elucidated its cell lineage from fertilization to adults, the base sequence of the entire genome, and the network of neural circuits.

Optogenetics is the one of methods to manipulate neural activity [3]. In optogenetics, we observe the neural activity when light stimulation is applied to specific neural cells for a specific time. As advantages of optogenetics, it has a superior spatial resolution that can stimulate cells one by one and a time resolution that can be swapped on and off of stimulus in units of milliseconds. Kocabas et al. [4] clarified changes in the direction of progression when photostimulation is given to AIY of *C.elegans* using optogenetics. Tanimoto et al. [5] developed a robotic microscope, Optogenetic Stimulation Associated with Calcium Development of imaging for Behaving Nematode (OSaCaBeN), and clarified correspondence between feeding behavior of *C.elegans* and responses of four dopaminergic neurons. OSaCaBeN can automatically track the moving *C.elegans* cells at high speed and stimulate a specific neural cell by projection
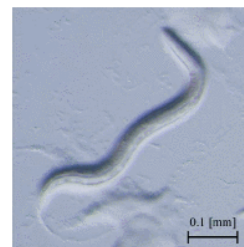

Fig. 1. A bright-field image of C. elegans (wild-type N2 strain) observed by a stereomicroscope (SZX16, OLYMPUS). Magnification of a lens is 8x. [2]

mapping. However, OSaCaBeN can project on only one cell.

In this paper, we propose a tracking method for multiple cells to realize measuring cells brightness. Combining with a robot microscope, we can also get stage trajectory. Our laboratory has worked on tracking animal motion, such as bats [6], and developed a robot microscope system and applied for *C.elegans* [2], paramecium [7] and zebrafish [8]. Fig, 2 shows a schematic diagram of a fluorescent microscope system. Our system has a high-speed CMOS or CCD camera for bright-filed images and an EM-CCD camera for fluorescence images. It is difficult to distinguish multi cells from the shape since fluorescent cells to be tracked have similar elliptical shapes in every cell. In addition, since the brightness of fluorescent cells changes according to the activated state, it is difficult to track using a single template. Therefore, we use features that do not change significantly between 2 successive frames to correspond cells between 2 frames. The features are the area and brightness of each cell, and the geometric features of the polygon whose vertices are the cells to be tracked.

II. PROPOSED METHOD OVERVIEW

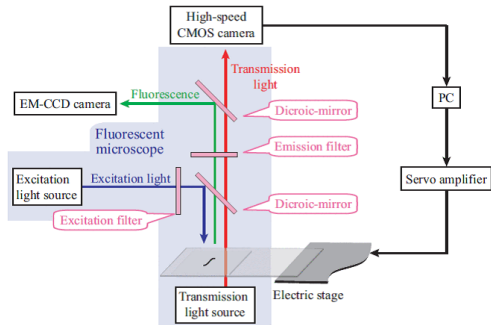In this paper, we use fluorescent protein transgenic cells and an EM-CCD camera. The number of modified

771

Fig. 2. A schematic diagram of a fluorescent microscope system to track a particular region of C. elegans. An excitation light is applied to C. elegans on an electric XY Z stage. The fluorescence images are captured by an EM-CCD camera. We determine a control signal to the XY Z stage based on an image processing result in a PC. [2]
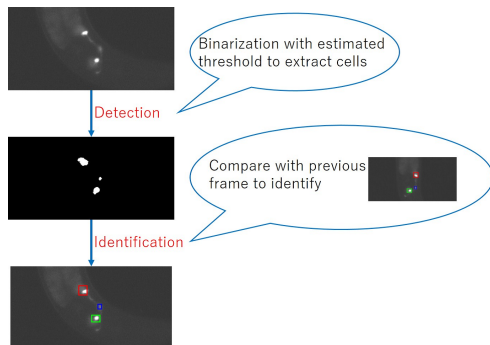

Fig. 3. Proposed method overview. There are 2 stages: cell detection and cell identification. In cell detection step, we binarize the image by an appropriate threshold estimated automatically. In cell identification step, we compare a current frame with a previous frame to find cell correspondence between 2 frames.
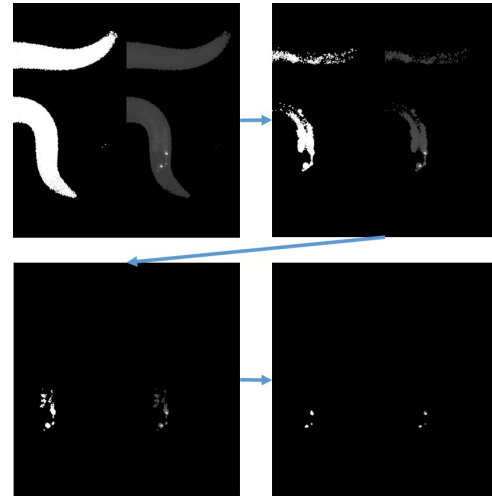

Fig. 4. Determination of brightness threshold. In each image, the left side is a binary image, and the right side is the image extracted only the area having the brightness not less than the threshold. The mean of the brightness of the region remaining after binarization is used as a new threshold, and a threshold for retrieving only cells are extracted.

III. CELL DETECTION

We estimate the brightness threshold suitable for cell detection for gray scale image and perform binarization processing. Areas having a brightness not less than the threshold value is determined to be a detected cell.

Fig. 4 shows how to update the threshold. At first, binarization is performed on all the images in the video with the mean of all pixel brightness. Second, update the threshold with the mean of the brightness of the pixels having the brightness not less than the threshold value. Third, Binarization processing is performed on all the images in the video with the updated threshold value. We repeat the second step and the third step until the number of detected regions reaches the maximum threshold not lower than the known number of cells to get the threshold.

IV. CELL IDENTIFICATION

Fig. 5 shows cell identification overview. To identify cells in the current frame, we compare the reference frame and the current frame. The reference frame has 2 types: a previous frame and a template frame. When two or more cells are detected in a frame 1 time step before the current frame, the previous frame is used as the reference frame. In other cases, the template frame, which is selected by the user, is used. We suppose there are at least two cells detected in one frame. We call these two cells as pivot cells. Our method removes tracking of cells if there are fewer than 2 cells detected. We consider cells as a polygon, each cell corresponds to each vertex.

cells is known. The overview of the proposed method is shown in Fig. 3. In proposed method, tracking is performed in two stages, namely cells detection and cells identification. In cells detection stage, we use binarization by a brightness threshold. The threshold is automatically estimated from the video. In cells identification stage, we compare a current frame, which has no identified cells, with a reference frame, which has identified cells. The reference frame has 2 types: a previous frame and a template frame. The previous frame is 1 time step before the current frame. The template frame is selected by the user. It is used to identify the cells when the tracking is temporarily lost, such as the cell deviating from the field of view. After identification, we can obtain mean brightness of each cell individually. We use a rectangle window to clip a cell. The window size is decided based on cells in the template frame and fixed through the video.
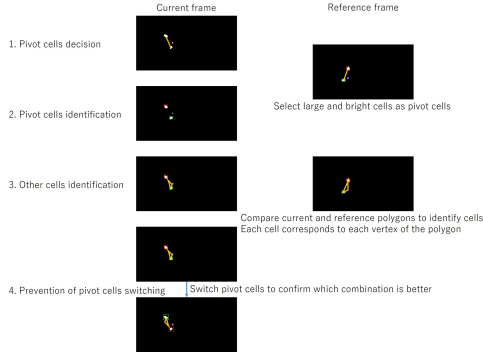
Fig. 5. Cell identification overview. At first, we find and identify pivot cells, which are landmarks to find cells to be tracked. Second, we use a polygon whose vertex are corresponding to cells in the previous frame. By matching the polygon to the pivot cells in the current frame, we can identify cells. Third, we swap the pivot cells on purpose to confirm identification was correct or not.

By calculating the ratio of pivot cells distance in the reference frame and the current frame, we can get similar polygons. Matching the polygon in the reference frame with the polygon in the current frame, we estimate the position of cells in the current frame. Moreover, in order to prevent the pivot cell from swapping, it is judged which is more appropriate as compared with the case where pivot cells are intentionally swapped.

A. Pivot cells decision

Pivot cells candidates are combination to take two cells from the current frame. At first, remove the candidates which has larger pivot cells distance than a threshold. Among left pivot cell candidates, a candidate in which the sum of the area and brightness of the normalized cells is the largest is regarded as pivot cells. Pivot cells in the current frame are written as

$$P_{\mathrm{cur}} = \arg\max_{\{k,l\}} \frac{a_k + a_l}{\max\limits_{i \in Q_{\mathrm{cur}}} a_i} + \frac{b_k + b_l}{\max\limits_{i \in Q_{\mathrm{cur}}} b_i}, \qquad (1)$$

$$|d_{kl} - d_{\mathrm{ref}}| < d_{\mathrm{thresh}}, k \neq l$$

where Q_{cur} is the set of cells in the current frame, a_i is area size and b_i is brightness of cell $i \in Q_{\mathrm{cur}}$ and d_{kl} is the distance between cell k and cells l, d_{ref} is the distance between the pivot cells in the reference frame, d_{thresh} is a constant value.

B. Pivot cells identification

By Calculating a cost to correspond the reference cells and the current cells and minimizing the cost, we identify the pivot cells in the current frame.
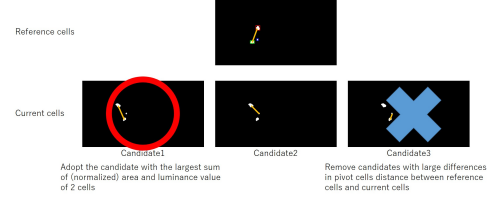

Fig. 6. Pivot cells decision. We find pivot cells in the current frame according to the pivot cells in the previous frame, using 3 features: i) a distance between pivot cells, ii) area size, iii) brightness.

1) When the reference cells are cells of the previous frame: Calculate combinations that minimize the weighted sum of the three types of costs. The three kinds of costs C_1, C_2, C_3 are calculated based on the moving distance of the cell, the difference of area and the difference of brightness, respectively. Fig. 7 shows an example of pivot cell identification.

Let k be a cell belongs to the pivot cell in the reference frame and l be a cell belongs to the pivot cells in the current frame. The cost C_1 about cell k and cell l, based on moving distance, is written as

$$C_1(k,l) = \frac{d_{kl}}{\max\limits_{i \in P_{\mathrm{ref}}, j \in P_{\mathrm{cur}}} d_{ij}} \qquad (2)$$

where P_{ref} is the pivot cells in the reference frame, P_{cur} is the pivot cells in the current frame and d_{ij} is the distance between cell i and cell j.

The cost C_2 about cell k and cell l, based on difference of area, is written as

$$C_2(k,l) = \frac{|a_k - a_l|}{\max\limits_{i \in P_{\mathrm{ref}}, j \in P_{\mathrm{tar}}} |a_i - a_j|} \qquad (3)$$

Where a_i is an area size of cell i.

The cost C_3 about cell k and cell l, based on difference of brightness, is written as

$$C_3(k,l) = \frac{|b_k - b_l|}{\max\limits_{i \in P_{\mathrm{ref}}, j \in P_{\mathrm{tar}}} |b_i - b_j|} \qquad (4)$$

Where b_i is a brightness of cell i.

The cost to correspond cell k with cell l is written as

$$C(k,l) = w_1 C_1(k,l) + w_2 C_2(k,l) + w_3 C_3(k,l) \quad (5)$$

Where w_1, w_2, w_3 are constant values.

2) When the reference cells are cells of the template image: Calculate combinations that minimize the cost C_2. Since the area of the cell does not change so much but brightness changes greatly, only area size is used as a feature to identify the pivot cell.

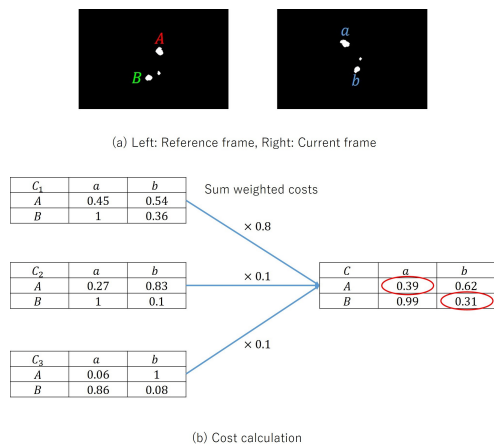(a) Left: Reference frame, Right: Current frame

(b) Cost calculation

Fig. 7. Pivot cells identification. There are 2 candidates to correspond cells in the reference frame with cells in the current frame. One is $\{(A,a),(B,b)\}$ and the other is $\{(A,b),(B,a)\}$. We adopt the combination has smaller cost. In this example, $\{(A,a),(B,b)\}$ is suitable
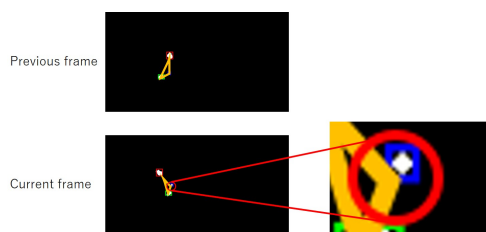

Fig. 8. Cells identification. Adopt a polygon whose vertex are the cells in the reference frame to the current frame and search nearest neighbor cell of the vertex.

C. Cells identification

Calculate the ratio of the pivot cells distance in the reference frame to the pivot cells distance in the previous frame, and create a similar polygon having cells in the reference frame at the vertex. Let this polygon be a reference figure. Place the reference figure so that the vertex corresponding to the pivot cells in the reference figure coincides with the pivot cells in the previous frame. By searching the vicinity of the vertex of the placed reference figure, identification other than the pivot cell is performed. If the nearest neighbor cell of the vertex of the reference figure is within the threshold distance, the cell is identified as being identical to the cell corresponding to the vertex of the reference figure. When there are no cells within the threshold pixel, the position of the apex is estimated as the position of the cell. Fig. 8 shows an example of cell identification.
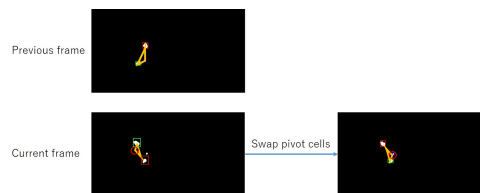

Fig. 9. Prevention of pivot cells swapping. In order to prevent pivot cells swapping, when we couldn't find cells except for the pivot cells, we swap the pivot cells on purpose and search cells again.

D. Prevention of pivot cells swapping

In order to avoid the pivot cells identification failure and swapping of the two pivot cells, if the number of cells could not be found is larger than the number of cells found near the vertex, we swap the pivot cells to apply the reference cells and search for cells near the vertex. However, even if swapping pivot cells, if there are many numbers that could not be found than the number of cells found near the vertex, adopt the state before swapping. Fig. 9 shows an example of pivot cells swapping.

V. RESULT

Cell tracking was performed on seven videos. Some of the results are shown in Fig. 10 and Fig. 11. Table II shows the tracking success rate of cells in each video by visual evaluation. A frame in which there is no error in detection and identification of cells to be tracked within one frame is regarded as a successful frame and the ratio of the number of successful frames to the total number of frames of the moving image is taken as the tracking success rate. Except for video 2, we can Successfully determine the threshold by automated binarization and achieve high tracking success rate. Propose method failed tracking ,especially in video 2, when the brightness of the entire image is low except for one cell, and tracking success rate is low because it is difficult to separate foreground and background.

Fig. 12 shows mean brightness of each cell in each video. After identification, we can get these data. Some of the data may not be correct because of tracking failure. We use a rectangle window to clip a cell. The window size is decided based on cells in the template frame and fixed over the video. Using a robot microscope [2], stage trajectory is also available as you can see in Fig. 14. The stage was controlled to cancel *C.elegans* motion and keep its head under the microscope. Thus, stage trajectory represent *C.elegans* motion. Raw data has time-stamps for every plots. At present, we couldn't correspond brightness data with trajectory data.

We compare propose method with DeepLabCut (DLC) [9], which is a toolbox for markerless pose

TABLE I
IDENTIFICATION SUCCESS RATE

	Number of frames	Identification success rate (%)
Video1	149	100
Video2	59	62.7
Video3	59	100
Video4	59	91.5
Video5	79	100
Video6	79	100
Video7	59	96.6
Total	543	94.7

TABLE II
COMPARISON BETWEEN OUR METHOD AND DLC

	Number of frames	DLC success rate (%)	Our method success rate (%)
Video1	109	81.7	100
Video2	43	93.0	76.7
Video3	43	88.4	100
Video4	43	100	90.7
Video5	58	86.2	100
Video6	58	94.8	100
Video7	43	58.1	100
Total	397	85.6	96.5

estimation of animals performing various tasks using deep learning. We use 149 images from 7 videos to train DLC and compare propose method with DLC on 397 images, which are not used for train DLC. Table **??** shows comparison between our method and DLC. Definition of success is same as Table II. While our method fail because binarization failed, DLC successfully track. However, our method achieve better result than DLC about total success rate.
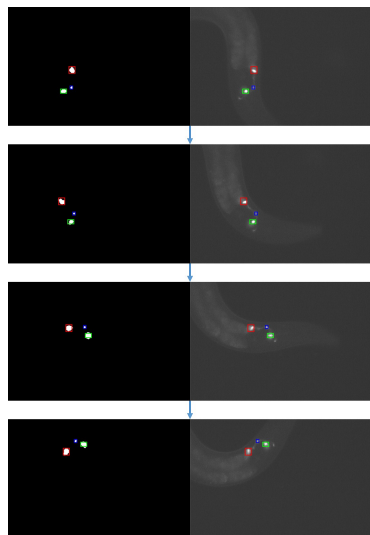

Fig. 10. A part of a result in Video1. It worked successfully.
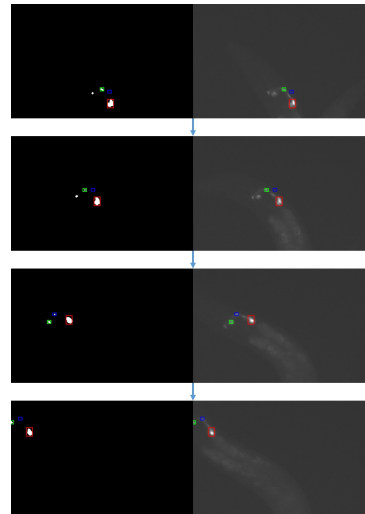

Fig. 11. A part of a result in Video2. Tracking failed on the top two images. Since the brightness of the entire image is low except red cells and it is difficult to separate foreground and background., tracking success rate is low.

Fig. 12. Mean brightness of each cell in each video. Cell A/B/C are corresponded to red/green/blue windows in Fig, 10 and Fig. 11.

Fig. 13. x axis and y axis on the stage.

Fig. 14. Stage trajectory in each video. Raw date has time-stamps for every plots.

VI. CONCLUSION

In this paper, we proposed a tracking method for tracking fluorescent cells of *C.elegans*. By using features that do not change with the passage of time, such as the geometrical positional relationship between cells, we achieved tracking distinguishing between cells that change in shape and brightness. By binarizing the image using the threshold estimated from the video, the cell was detected and tracking was performed by using the two cells that are reliably detected and the polygon made by the cell. We succeeded in cell tracking except when the tracking target goes out of the screen or when it is difficult to separate the foreground and background by binarization. After identification, we can get mean brightness of each cell and stage trajectory.

In the future work, we will experiment with actual *C.elegans*, project light on each cell to stimulate and measure their brightness and analyze *C.elegans* activity.

ACKNOWLEDGMENT

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[9] A. Mathis, P. Mamidanna, K. M. Cury, T. Abe, V. N. Murthy and M. Weygandt, DeepLabCut: markerless pose estimation of user-defined body parts with deep learning, Nature N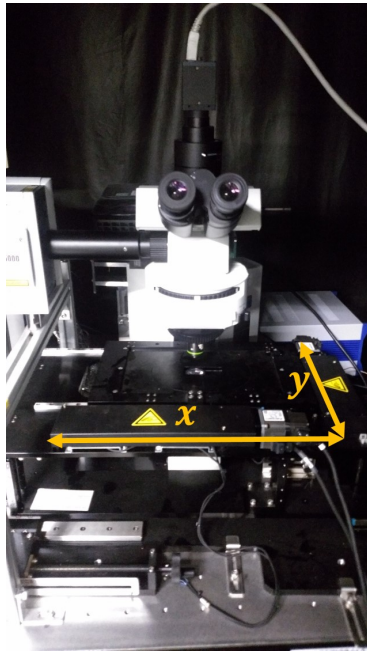euroscience, vol. 21, pp. 1281–1289, 2018.