AUTOMATED CELL JUNCTION TRACKING WITH MODIFIED ACTIVE CONTOURS GUIDED BY SIFT FLOW

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ABSTRACT

We present a novel algorithmic approach to track multiple cell junctions automatically in the developing epidermis of the *C. elegans* embryo. 3D cell boundaries are projected into 2D for segmentation using active contours with a non-intersection force, and subsequently tracked using SIFT (Scale-Invariant Feature Transform) flow. Our method achieves MAD (Mean Absolute Distance) less than 3 pixels between all tracked cell contours and ground truth data. Using this method we have generated a quantitative description of epidermal cell movements and shape changes during the process of epidermal enclosure.

Index Terms— C. elegans, cell tracking, SIFT flow

1. INTRODUCTION

Quantitative analysis of cell shape in live samples is an important goal in developmental biology. The nematode worm *Caenorhabditis elegans* is an excellent organism for analyzing fundamental aspects of development because it is simple, easy to grow in bulk populations, and convenient for genetic analysis. *C. elegans* has a fixed number of cells in early embryogenesis, all of which have been individually identified and characterized. We are interested in epidermal development as a model for epithelial morphogenesis.

Tracking cells or subcellular structures in developing embryos is important to understand developmental processes. Computer aided tracking allows quantitative analysis of large numbers of cells or objects. Recently, several automated or semi-automated nuclei tracking algorithms [1][2][3][4] that allow quantitative analysis of nuclear positions in *C elegans* have been developed. However, nuclear positions do not provide direct information on cell shape, size, or cellular contacts. A major remaining challenge is to segment and track cell surfaces or contacts in complex 4D data (Figure 1). The difficulty of tracking cells on surfaces lies in lack of image texture or color information. The recorded data only contains cell boundaries along the surface.

In this paper, we present a new method to automatically segment and track epithelial junctions in 4D data that only requires cell contour signals on the embryo surface. Active contours (also called snakes) [5] with a proposed non-intersection force can precisely segment all epidermal cells in 2D maximum projection images. To handle large displacement of cell movement, we conduct experiments using conventional optical flow and SIFT (Scale-Invariant Feature Transform) flow [6]. Experimental results show our modified active contours with SIFT flow can accurately track epithelial junctions. Our methods yield a quantitative description of the dynamics of epithelial shape changes during epidermal enclosure.

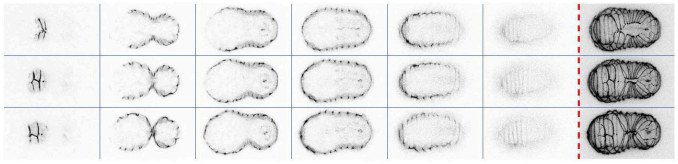

Fig. 1: Dataset snapshots. Columns 1 to 6 show Z-stack images for slices 1,7,13,19,25, and 31. Last column shows maximum projection images. Each row represents acquired data at one time point. Images are inverted for display.

2. DATA ACQUISITION

The *C. elegans* embryo consists of 24 epidermal cells on the surface. We use a Zeiss LSM700 to acquire Confocal Laser Scanning Microscope (CLSM) cell images over 20 minutes to capture the closing of the epidermal cells. Epidermal junctions visualized with DLG-1::GFP form lines at the subapical circumference of differentiated epidermal cells. By changing the focal length, our dataset contains 35 Z-stack image slices that cover the whole *C. elegans* embryo. Columns 1 to 6 in Figure 1 show the Z-stack snapshots for slices $1, 7, 13, 19, 25$, and 31. However, Z-stack images are not informative for visualizing and tracking cell contours. We use a maximum projection image as our input data. The pixel intensity value of each pixel is selected from the maximum pixel value through all Z-stack images. The maximum projection image can better visualize epidermal cells of the *C. elegans* embryo as shown in the last column of Figure 1.

3. PROPOSED FRAMEWORK

We propose a new cell contour tracking algorithm to handle 4D CLSM data. The following sections describe each step.

3.1. Initial Cell Boundary Collection

To track cell boundaries, we first need to acquire initial locations and shapes of each cell. At the initial time point, we manually collect key points (around 7 to 10 points) along the boundary for a cell, and then apply lowpass interpolation to connect those key points into a closed contour. Finally we refine the interpolated cell boundary using snakes [5]. This procedure can generate initial cell boundaries at the first time point quickly with light human effort as shown in Figure 2.

3.2. Tracking

Optical flow is a popular feature matching technique that computes motion patterns of two consecutive images. The Lucas-Kanade version of optical flow did not work consistently for our dataset because cells sometimes move rapidly. To handle large displacements for outlier cells, we use a more distinctive image feature representation instead of raw pixel values. SIFT (Scale-invariant Feature Transform) is a robust image feature representation [7]. SIFT image descriptors can encode a histogram of gradient orientations around each interest point, providing more details of image structure around the local area. In 2008, Liu et al. proposed SIFT flow [6] to overcome the disadvantages of traditional optical flow by replacing raw pixel values with SIFT feature descriptors, and then performing a modified optical flow algorithm based on those SIFT features. We use the flow vectors computed from SIFT flow to estimate cell contour movement. The advantages will be demonstrated in Section 4.

3.3. Boundary Refinement

SIFT flow can provide reasonable tracking results between consecutive frames. However, tracked contours might miss subtle details of certain curves. We apply snakes to align the tracked contours with true cell boundaries.

In addition, cell contours in the middle of the embryo present narrow width and therefore can cause contour self-intersection during the iterative snakes algorithm as shown in the left picture in Figure 3. We add a Non-intersection Force (NIF) to the snake objective function [5] to prevent self-intersection:

$$E^*_{snake} = \int (E_{snake}(\mathbf{v}(s)) + E_{NIF}(\mathbf{v}(s)))ds \qquad (1)$$

where $\mathbf{v}(s) = (x(s), y(s))$ is the parametrical position of a snake. Figure 3 illustrates the procedure of E_{NIF} generation. We first extract the cell contour mask, and then generate a skeleton using thinning. The skeleton is used as the seed

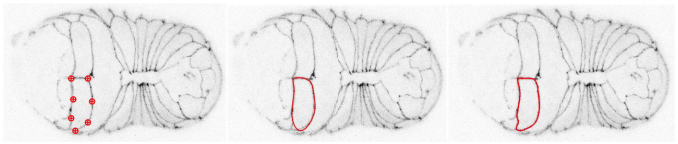

Fig. 2: Procedure of initial contour collection. Left: key points. Middle: interpolated contour. Right: refined contour

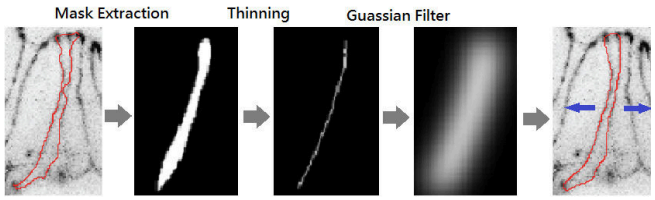

Fig. 3: Illustration of non-intersection force generation.

location to generate the non-intersection force. The magnitude of NIF is determined by a smoothed skeleton image (Gaussian smooth filter). Pixels close to the center line have stronger NIF while pixels far from the center line have weaker NIF. Snakes with NIF can successfully prevent contour self-intersection as shown in Section 4.

4. TRACKING EVALUATION

The proposed cell contour tracking system can produce closed curves for each cell at each time point. To evaluate our tracking results, we use Mean Absolute Distance (MAD) as our metric [8]. MAD measures the distance $e(A, B)$ between two contours $A = \{a_1, a_2, \ldots, a_n\}$ and $B = \{b_1, b_2, \ldots, b_m\}$, where a_i and b_i are points sampled from curves A and B. In [8] MAD is defined as:

$$e(A, B) = \frac{1}{2}\left\{\frac{1}{n}\sum_{i=1}^{n} d(a_i, B) + \frac{1}{m}\sum_{i=1}^{m} d(b_i, A)\right\}$$

where

$$d(a_i, B) = \min_j \|b_j - a_i\|$$

is the distance between point a_i and the closest point b_j on curve B. Here we assign A as any tracked cell contour and B as the corresponding ground truth contour, and this metric computes the average deviation of pixels of the tracked cell contour from the ground truth.

In this experiment we record a 4D CLSM live cell imaging dataset of *C. elegans* embryos for 20 minutes. Each Z-stack has the size of 275×512. We track 24 epidermal cells, and also manually generate corresponding ground truth contours in order to compute MAD. Figure 4 shows the spatial layout of all 24 cells with names. Figure 5 shows the MAD for tracked cells over 20 minutes in projected 2D space. Our system can track all cell contours with MAD less than 3, and achieves MAD less than 2 for the anterior area of the embryo.

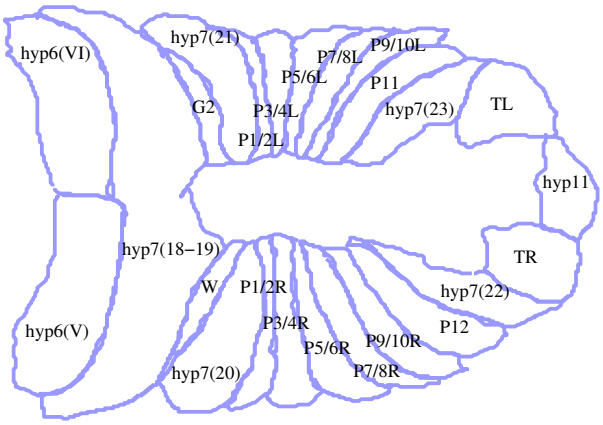

Fig. 4: Illustration of individual cell names.

Optical flow can track most of the cell contours reasonably. However, our approach aims at performing accurate and reliable automatic cell contour tracking for all cells over the whole video dataset. Table 1 illustrates the importance of the proposed SIFT flow tracking and non-intersection force for outlier cells. The top half of the table clearly shows that SIFT flow tracking outperforms traditional optical flow for cells *hyp6(VI)* and *hyp6(V)*, which present rapid movement during the closing operation. The bottom half of the table compares MAD between SIFT flow tracking with and without the non-intersection force (NIF). Tracking with NIF significantly reduces MAD for outlier cells (*P9/10R*, *P9/10L* and *P7/8L*) due to the narrow width of the cell. SIFT flow tracking with NIF can not only capture fast moving cells but prevents self-intersection for narrow cells. Figure 6 shows examples of tracked cells with ground truth at different time points.

Table 1: MAD for Outliers in Pixel Unit

Method	hyp6(VI)	hyp6(V)	P9/10R	P9/10L	P7/8L
Optical Flow	3.57	4.52	-	-	-
SIFT Flow	**0.71**	**0.65**	-	-	-
SIFT Flow	-	-	1.95	3.12	2.18
SIFT Flow+NIF	-	-	**1.22**	**1.51**	**0.69**

5. BIOLOGICAL FEATURE EXTRACTION

The proposed cell contour tracking method can estimate accurate and reliable cell contour locations and shapes, and the tracking result can also be used to compute biological features. We focus on cell contour lengths computation and cell motility visualization.

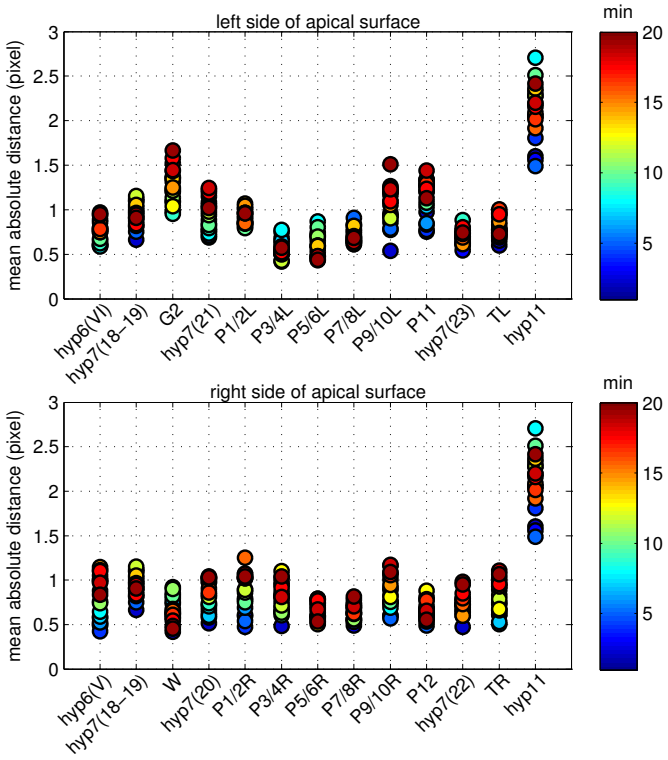

Fig. 5: MAD for tracked cell contours.

5.1. Cell Contour Length

Epidermal cell contour length provides one way to analyze the cell activities. Figure 7 shows contour lengths of all tracked cells over 20 minutes. In this figure we discover that cells *TL*, *TR* and *hyp11* have obvious contour length increases because these cells demonstrate a closing activity toward the center of the embryo while other cells have decreasing contour lengths because they transform into narrower shapes along the time sequence. In addition, we find a larger variation for cells G2, W, hyp7(21), hyp7(20) over time because these cells undergo an extreme shape transformation (from wide to narrow shape).

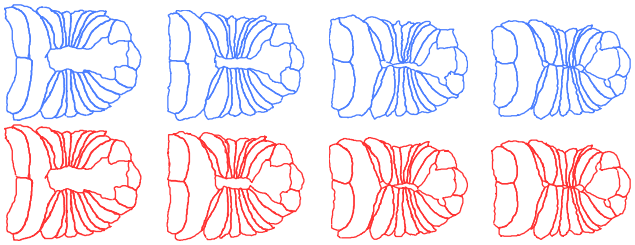

Fig. 6: Example of tracked cell contours and corresponding ground truth at time points 1,7,14, and 20 from left to right. Blue: tracked cells. Red: ground truth.

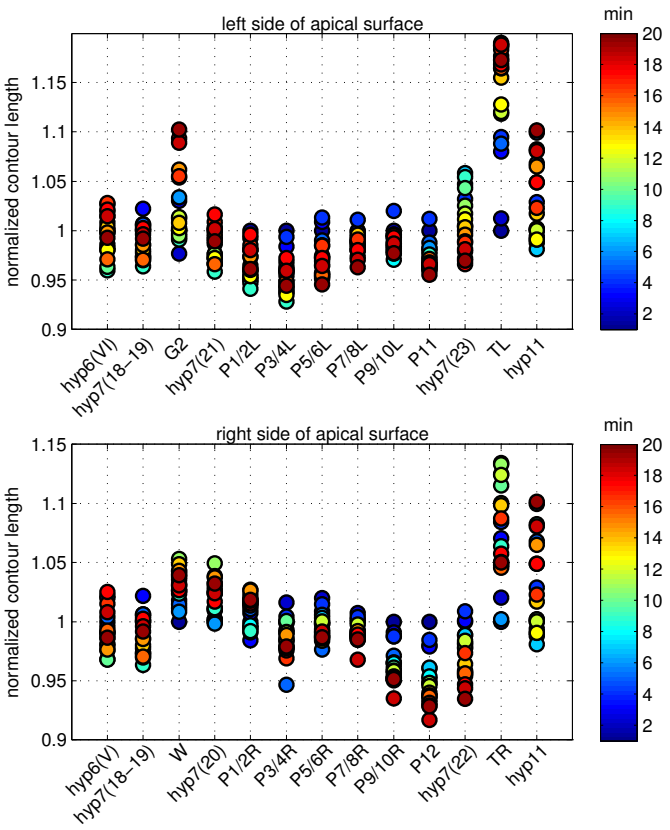

Fig. 7: Normalized cell contour lengths.

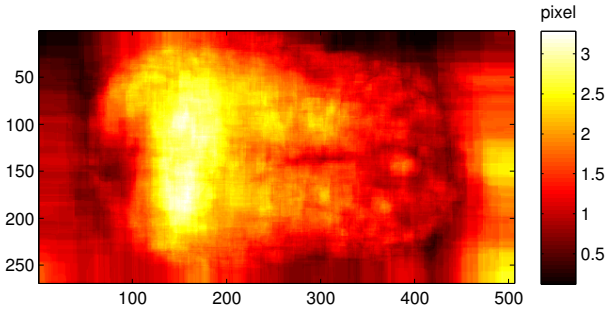

Fig. 8: Average motility map over the whole dataset.

5.2. Motility Map

Cell motility patterns can give essential understanding of cell dynamics. We generate an average motility map using SIFT flow velocity computed during the cell tracking step. Figure 8 demonstrates the average velocity of pixel movement in 2D space. We can see active area in the anterior which matches the closing behavior in the anterior cells.

6. CONCLUSIONS

In this paper, we proposed an automated cell contour tracking method for developing epidermis of the *C. elegans* embryo and generate a quantitative analysis of cell contour lengths over time, and a visualization of cell motility. Future work includes more biological feature extraction and comparison of mutant and wild type worms.

7. REFERENCES

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