EFFICIENT CELL SEGMENTATION AND TRACKING OF DEVELOPING PLANT MERISTEM

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ABSTRACT

Analysis of Confocal Laser Scanning Microscopy (CLSM) images is gaining popularity in developmental biology for understanding growth dynamics. The automated analysis of such images is highly desirable for efficiency and accuracy. The first step in this process is segmentation and tracking leading to computation of cell lineages. In this paper, we present efficient, accurate, and robust segmentation and tracking algorithms for cells and detection of cell divisions in a 4D spatio-temporal image stack of a growing plant meristem. We show how to optimally choose the parameters in the watershed algorithm for high quality segmentation results. This yields high quality tracking results using cell correspondence evaluation functions. We show segmentation and tracking results on Confocal laser scanning microscopy data captured for 72 hours at every 3 hour intervals. Compared to recent results in this area, the proposed algorithms provide significantly longer cell lineages and more comprehensive identification of cell divisions.

Index Terms— shoot apical meristems, stem-cell, cell segmentation, cell tracking

1. INTRODUCTION

Proper understanding of the causal relationship between cell growth patterns and gene expression dynamics is one of the major topics of interest in developmental biology. Information such as rates and patterns of cell expansion play a critical role in explaining cell growth and deformation dynamics. The need for quantification of these biological parameters is important to biologists. However manual analysis is extremely tedious because of the high dimensionality and complexity of data.

The subject of this study, the shoot apical meristems (SAMs) also referred to as the stem-cell niche, is the most important part of the plant body plan because cells for all the above ground plant parts are supplied from it. At the same time, the size of the stem-cell niche remains stable in spite of a continuous displacement and diversion of cells into a differentiation program. Understanding this dynamics is a major research thrust for developmental biologists. In this paper, we focus on studying the developing plant meristem with the goal of obtaining very accurate segmentation and tracking. Confocal laser scanning microscopy is used to observe given set of SAMs labeled with plasma membrane localized yellow fluorescent protein (YFP), repeatedly for about 3 days by taking serial images at every 3 hour intervals. At each time point, multiple images are obtained at different depths. Each stack from all time points is registered by method of maximization of mutual information [1, 2]. As a result, the images taken from different time points but at the same slice are registered. Fig. 1 shows an example of the time lapse images. To keep the plant alive for a long period of time, it is necessary to limit its exposure to the laser. This results in poor image quality. This presents significant challenges to image analysis since the segmentation and the tracking needs to be robust to the poor image quality.

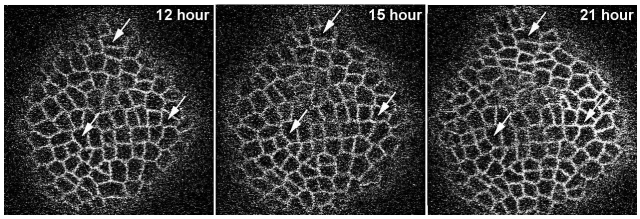

Fig. 1. Example of three images at three time instants. Arrows show same set of cells at time points noted on each panel.

Of late, there has been some work on automated processing of such time lapse images in both plants and animals. The multiple-level-set approach is an active-contour based algorithm, which simultaneously segments cells and also tracks them [3, 4]. However, this method is not suitable for tracking of SAM cells. The method in [5] presents an approach for cell segmentation, but has not been applied to plant cells. In [6] Softassign Procrustes algorithm was used to compute cell lineages, and to detect cell divisions, but its application to plant cells is limited. A recent work [7] addressed the problem of

The authors at University of California, Riverside gratefully acknowledge support from NSF Video Bioinformatics IGERT grant DGE 0903667 and NSF Grants IIS-0712253 and IOS-0820842.

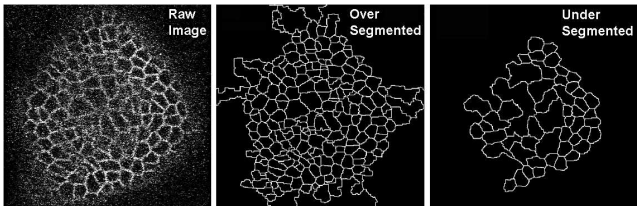

Fig. 2. The detection of cell boundaries varies with the different values of watershed threshold h. First image is the raw input image. Over-segmentation using watershed is shown in the middle image, and under-segmentation in the right most image.

tracking in plant cells and especially the issue of robustness of the trackers. However, they assumed an existing level-set based segmentation method and their performance suffered due to poor quality of the segmentation in many images.

In this paper, we propose a single framework that entails segmentation and tracking of plant cell images. Section 2 and Section 3 detail these two parts of the algorithm. Section 4 describes the experiments and results of our methods.

2. SEGMENTATION

The authors in [7] used a level set algorithm for segmentation. Since the input raw images have low signal to noise ratio in the periphery and also in the central regions of the plant stem, the segmentation results of this work are of poor quality.

We used watershed transformation [8] to segment cell boundaries. Watershed treats the input image as a continuous field of basins (low intensity pixel regions) and barriers (high intensity pixel regions), and outputs the barriers, which are the cell boundaries of all the cells in the image. Prior to applying the watershed algorithm, the input image from the confocal microscope undergoes low pass filtering. This Gaussian filtered image is further processed using H-minima transformation in which all the pixels below a certain threshold percentage h are discarded. These two steps minimize the effects of noise in our segmentation. The threshold value h plays a very crucial role in the watershed algorithm. Generally, a higher value of the threshold parameter h performs under-segmentation on the image, and inversely a lower value over-segments it, as shown in Fig. 2. Moreover, if the input image is very noisy, then it becomes extremely important to choose an appropriate threshold value such that only the correct cell boundaries are detected by watershed. One of the main contributions of this paper is a quantitative metric to evaluate the "correctness" of the segmentation. We observe that the area of all the cells in a plant is almost uniform in the image. Thus watershed should ideally produce a segmented image that contains similar sized cells. We use variance in the area of the cells as a metric to measure correctness of segmentation. Thus, the value of h should be chosen such that

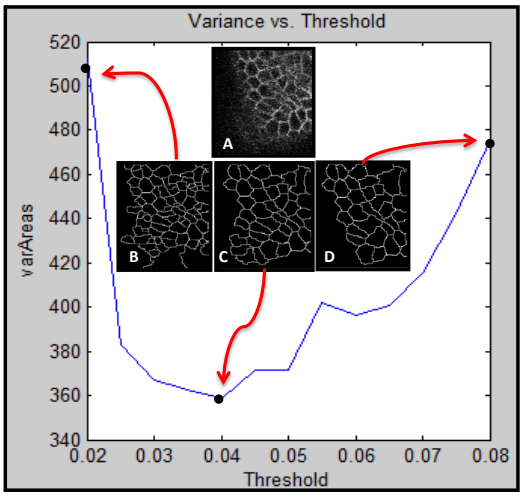

Fig. 3. The graph shows the watershed threshold h in the X axis, and the variance in the area of the segmented cells in the Y-axis. Image A shows the input raw image, images B, C, D show the segmentation results for different values of h. Image B shows over-segmentation, image D shows under-segmentation. The correlation between the minimum variance in the areas of the cells and appropriate segmentation is clearly demonstrated in image C.

variance of area in the segmented image is minimum. Since it is a quantitative metric, the optimal value of h is found automatically without manual intervention. The appropriateness of this metric is also verified through the successful use of these segmented images in tracking with significantly improved results.

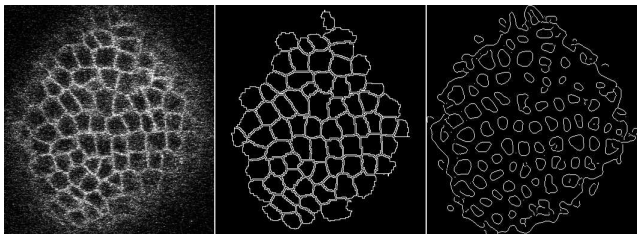

Fig. 4. Raw image in the first column is segmented using both watershed and level-set segmentations. Second column displays watershed segmentation, while the third column is the result of level set segmentation. Qualitatively, watershed far surpasses level-set segmentation as demonstrated in this figure.

Figure 3 illustrates the automatic evaluation of h for the given sample image. The segmented image obtained after watershed segmentation is a binary image.

Comparison with [7]: Since the tracking algorithm uses cell properties such as area of the cell and its centroid, it is important to retain the shape and structure of cells in the seg-

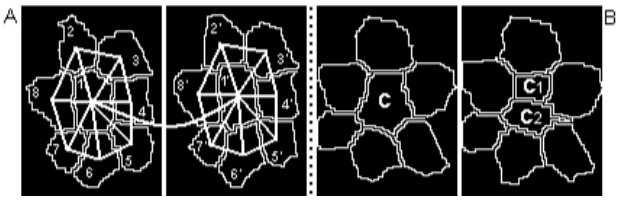

Fig. 5. Image A: The matched local graphs G1 at t and G2 at t + 1 time points, the correspondence of the seed cell pair (1,1'), as well as the correspondences of the neighboring cells, such as (2,2') and (4,4'). Image B: A diagram to describe the relative position of the mother cell and the daughter cells.

mented images. In a noisy image, level-set method fails to preserve these cell properties, which leads to incomplete or faulty tracking of cells. On the other hand, watershed segmentation algorithm finds the geometry and other observed properties of the plant cells more accurately than the level-set, thus producing significantly better correspondence results in tracking (see Fig. 4).

3. TRACKING STEM CELLS

Graphical abstraction is created on a collection of cells in an image. In this process every cell is characterized by a vertex in the graph and neighboring vertices are connected by an edge. The structure of these graphs automatically contains the relative distance between two neighboring cells (the edge length) and the edge orientation. The topology and the geometry of the local graphs should not change if there is no cell division or the images are not noisy. Taking into account above conditions, the correspondences between cells are identified by matching the local graphs (Fig. 5 (A)). To find a correspondence between cells c_i and c_j across different time instants t and $t+1$, the distance measure between two local graphs is defined as follows:

$$D_L(c_i, c_j) =$$

$$\sum_{c_{k_i} \in N(c_i), c_{k_j} \in N(c_j)} \frac{(l_{c_{k_i},c_i}(t) - l_{c_{k_j},c_j}(t+1))^2}{l_{c_{k_i},c_i}(t)^2}$$

$$+\lambda \sum_{c_{k_i} \in N(c_i), c_{k_j} \in N(c_j)} (\theta_{c_{k_i},c_i}(t) - \theta_{c_{k_j},c_j}(t+1))^2$$

$$+\frac{\|P_{c_i}(t) - P_{c_j}(t+1)\|}{\Delta^2}$$

where c_{k_i} is a neighboring cell of c_i, and c_{k_j} is a neighboring cell of c_j, $N(c)$ is the neighboring cell set (set of cells that are within a certain distance around cell c), $l_{c_{k_i},c_i}(t)$ and $l_{c_{k_j},c_j}(t+1)$ are the edge lengths, $\theta_{c_{k_i},c_i}(t)$ and $\theta_{c_{k_j},c_j}(t+1)$ are the orientation angles in radians of the edges measured relative to a horizontal axis, $P_{c_i}(t)$ and $P_{c_j}(t+1)$ are the cell position vectors, and Δ is the average distance between two neighboring cells. If two local graphs match, which is when the distance measure is small, we can say that the central cells

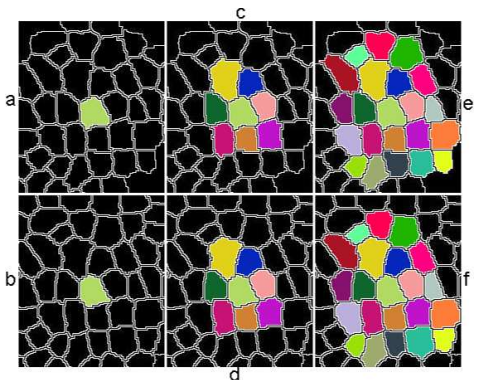

Fig. 6. Growing cell correspondences from a seed to its neighbors. The first column denotes the seed cell pair, the middle column denotes the tracking results after the first step of the recursion of growing the correspondences, while the third column denotes the tracking results after the second step. The same color shows the matched cells.

c_i and c_j are a corresponding cell pair. Overall, tracking of stem cells from any two consecutive cell image stacks using local graph matching [7] consists of the following parts: finding the seed cell pairs, finding the correspondences of the neighboring cells from the seed pairs across time (Fig. 6), and detecting cell divisions (Fig. 5 (B)). Formal description of each procedure is described in [7] in depth.

4. EXPERIMENTAL RESULTS

Raw images segmented using watershed improves temporal tracking dramatically. Cell division largely depends on faithful detection of cell boundaries in the raw image. Because watershed produces faithful segmentation, there are less false positives as compared to level-set. We have tested watershed segmentation and tracking on different datasets of SAMs. The experimental results are shown on images obtained from plant cells and observed for 72 hours taken at every 3 hour intervals. Watershed segmentation is applied on images taken from all 24 time points at the same depth level. Then tracking is run on every 2 consecutive segmented images to get information about the cells such as cell lineage, division, area. Fig. 7 compares the results of temporal tracking with level-set and watershed segmentation. Biologists manually verified the accuracy of segmentation results by watershed algorithm. Also in order to verify the overall improvement of the proposed algorithm we do comparison of watershed with level-set algorithm.

The number of correctly tracked cells obtained from tracking images in consecutive time points for some period of time is compared in Table 1. The comparison is done on the different datasets and we can see significant increase in the number of tracked cells obtained from watershed segmentation.

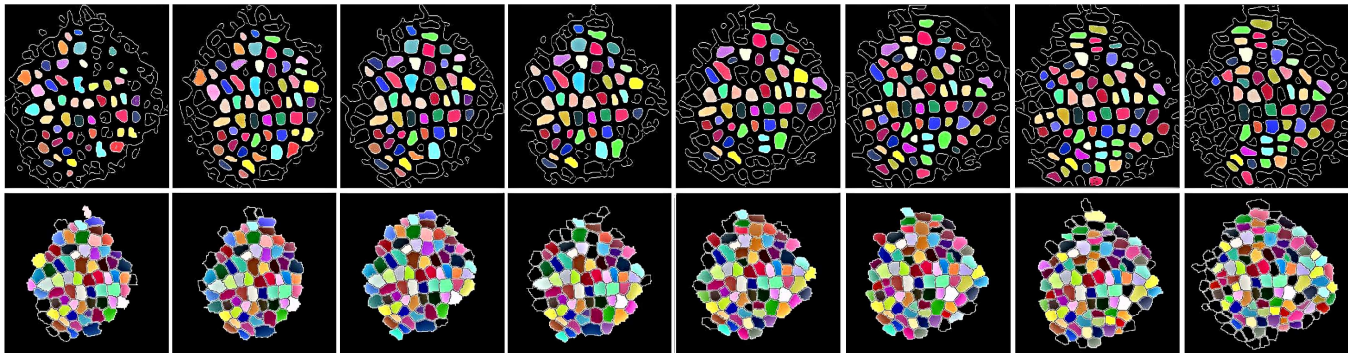

Fig. 7. Improvements in temporal tracking using watershed segmentation. Top row shows temporal tracking using level-set segmentation, whereas the bottom row is obtained using watershed segmentation. Cells are tracked for 24 hours. The same color denotes the same cell.

Table 1. Sum of the number of cells being tracked from watershed segmentation data and level-set segmentation data

Dataset	Time (h)	Watershed segmentation	Level-set segmentation
Dataset1	72	2052 (%90)	1630 (%71)
Dataset2	36	491 (%71)	215 (%31)

One of the important data to obtain is the lineage of a cell. Table 2 shows the comparison of average cell lineage lengths between watershed and level-set algorithms and confirms that watershed segmentation improved those results too.

Table 2. Average lineage length

Dataset	Watershed segmentation	Level-set segmentation
Dataset1	50	25
Dataset2	42	6

5. CONCLUSION

In this paper we proposed a novel algorithm for plant cell segmentation using watershed transformation. Specifically, we proposed a new metric for this application to find the optimal threshold parameter automatically. We show improved results of temporal tracking of cells using the proposed segmentation when compared to recent earlier results of segmentation that uses level set functions. This we believe will significantly benefit the plant biologists to find correct lineages of cell division and death for a more accurate statistical evaluation of plant growth.

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