

# AUTOMATED REGISTRATION OF LIVE IMAGING STACKS OF ARABIDOPSIS

*Katya Mkrtchyan, Anirban Chakraborty, Amit K. Roy-Chowdhury*

University of California, Riverside, CA 92521, USA

## ABSTRACT

For actively developing tissues, a computational platform capable of automatically registering, segmenting and tracking cells is very critical to obtaining high-throughput and quantitative measurements of a range of cell behaviors, and can lead to a better understanding of the underlying dynamics of morphogenesis. In this work, we present an automated landmark-based registration method to register shoot apical meristem of *Arabidopsis Thaliana* images obtained through the Confocal Laser Scanning Microscopy technique. The proposed landmark-based registration method uses local graph-based approach to automatically find corresponding landmark pairs. The registration algorithm combined with an existing tracking method is tested on multiple datasets and it significantly improves the accuracy of cell lineages and division statistics.

**Index Terms**— registration, shoot apical meristem, live imaging

## 1. INTRODUCTION

The subject of this study, the shoot apical meristem (SAM), is the most important part of the plant body because it supplies cells for all the above ground plant parts and at the same time maintains its stable size. Therefore, a tight spatio-temporal co-ordination between cell division and differentiation of progeny cells into organs is critical to maintain the stability of SAMs. However, the causal link between cell growth and division and how they affect organ formation is not well understood due to the lack of quantitative measurement of growth patterns. This necessitates the development of computational platforms capable of automatically tracking cells and cell division patterns.

The SAM of *Arabidopsis Thaliana* consists of approximately 500 cells and they are organized into multiple cell layers that are clonally distinct from one another. For complex multi layered, multi cellular plant and animal tissues, the most popular method to capture individual cell structures is the Confocal Laser Scanning Microscopy (CLSM) based Live Cell Imaging. By changing the depth of the focal plane,

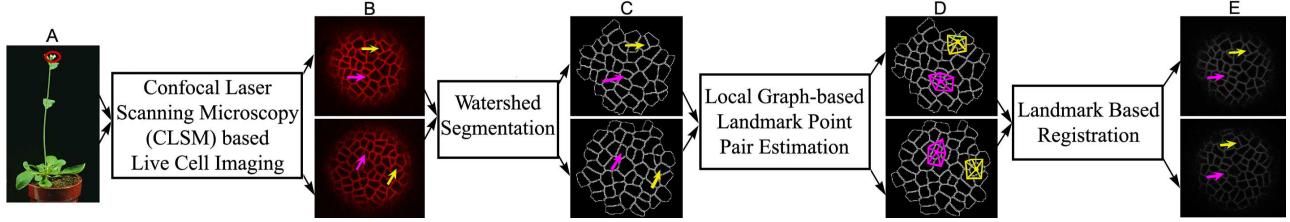
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CLSM can provide infocus images from various depths of the specimen. To visualize cell boundaries of all the cells in the SAM, plasma membrane-localized Yellow Fluorescent Protein (YFP) is used. The set of images, thus obtained at each time point, constitute a 3-D stack, also known as the 'Z-stack'. Each Z-stack is imaged at a certain time interval (e.g. three or six hours between successive observations) and it is comprised of a series of optical cross sections of SAMs that are separated by approx.  $1.5 \mu\text{m}$ , and a standard shoot apical meristematic cell has a diameter of about  $5 - 6 \mu\text{m}$ . In practice, the live cell imaging of *Arabidopsis* SAM comprises of several steps, where the plant has to be physically moved between different places. For normal growth of the plant, it has to be kept in a place having specific physical conditions (temperature of  $24^\circ\text{C}$ ). The plant is moved and placed under microscope at the imaging/observational time points, before it is placed back to the aforementioned place once again. For 72 hours overall, this process is repeated every three hours. Because of this process of replacement of the plant under the microscope and also since the plant keeps growing during these 72 hours, various shifts can occur between two Z-stacks of images taken in consecutive time points, though images in any Z-stack are automatically registered. Fig. 1 (B) demonstrates an example with noticeable shifts between images. So to get the accurate and longer cell lineages and cell division statistics image stacks should be aligned. In this paper we show how to perform automatic image registration for the *Arabidopsis* SAM image stacks.

## 2. RELATION TO PREVIOUS WORK

Recently, there has been some work done on SAM cells [1], where cells are segmented by watershed algorithm [2] and tracked by local graph matching method. The method in [1] was constrained to focus on datasets that are approximately registered. Therefore, registration is of utmost importance to be able to work with varied datasets. Popular registration method based on maximization of the mutual information [3, 4], fails to provide accurate registration as it uses the pixel intensities to acquire the registration. Pixel intensities in the *Arabidopsis* SAM images are not discriminative features. The landmark-based methods are more suitable to register such images. A recent paper [5] uses SAM images acquired from multiple angles to automate tracking and modeling. For pair



**Fig. 1.** Methodology in Sparse Confocal Image Stacks - A) SAM located at the tip Arabidopsis shoot, B) raw images taken at consecutive time instances, C) segmented images after applying watershed segmentation, D) estimation of the corresponding landmark point pairs, E) bottom image is registered to the top image (the same color arrows represent the same cell)

of images to be registered, the user identified correspondences by pairing a few anchor points (referred as landmark points in this work).

In this work, we present a fully automated landmark-based registration method that can find out correspondences between two images and utilize these correspondences to yield a better registration result. In the experimental results section we show that landmark-based registration is more suitable for noisy and sparse confocal images, than registration based on maximization of the mutual information.

The most common landmark-based registration algorithm is the Iterative Closest Point (ICP) algorithm [6], where a set of landmark point pair correspondences are constructed between two images and then the images are aligned so as to minimize the mean square error between the correspondences. The ICP algorithm is very sensitive to initialization; it provides a good estimate of the correct correspondence when the images are approximately aligned with each other. There are different additions to the basic ICP algorithm, e.g. Iterative Closest Point using Invariant Features (ICPIF) [7], that uses features like eccentricity and curvature to overcome the issue. But in Arabidopsis SAM, because densely packed cells have very similar features, the eccentricity, curvature and other common features such as shape, color, etc. are not discriminative enough to be used for the registration. Thus available landmark-based registration approaches may not be able to properly align the SAM images. This is why we need to develop a novel feature that can be used to register SAM images. The proposed landmark estimation method uses features of the local neighborhood areas to find corresponding landmark pairs for the image registration.

### 3. LANDMARK-BASED REGISTRATION

In this paper, we present automatic landmark-based registration method for aligning Arabidopsis SAM image stacks. The procedure of getting the corresponding landmark point pairs and registering images is the following.

1. Watershed segmentation is applied on the images to get the individual cell information, such as cell position and area.
2. The proposed method is applied on the segmented images; a number of landmark points are selected from the im-

ages and correspondence is established between them.

3. Positions of the corresponding landmark pairs are used to estimate the parameters of the transformation model and the estimated transformation function maps the rest of the points in the input image to the reference image.

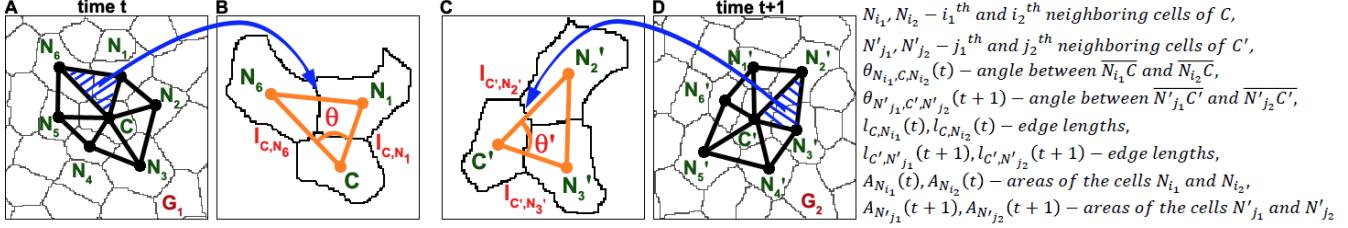
This process is described in Fig. 1. In the rest of this section we call the image that we wish to transform as the input image, and the reference image is the image against which we want to register the input.

#### 3.1. Landmark Identification

As described in Section 1, there is accumulated random shift, rotation or scaling between the images taken at different time points. The performance of tracking is affected by the registration. The quality of the image registration result depends on the accuracy of the choice of the landmark points. As we already described, common features such as shape, color etc. can not be used to choose corresponding landmark pairs. Motivated by the idea presented in [1], we use the relative positions and ordered orientation of the neighboring cells as unique features. To exploit these properties we represent these local neighborhood structures as graphs and select the best candidate landmark points that have the minimum distance between the local graphs built around them.

*Local graphs as features* - Graphical abstraction is created on the collection of cells. Vertices in the graph are the centers of the cells and neighboring vertices are connected by an edge. Neighborhood set  $N(C)$  of a cell  $C$  contains the set of cells that share a boundary with  $C$ . Thus every graph consists of a cell  $C$  and a set of counter-clockwisely ordered neighboring cells (Fig. 2 (A,D)). The ordering of the cells in  $N(C)$  is important because under nonreflective similarity transformation, the absolute positions of the neighboring cells could change but the cyclic order of the cells remains invariant.

*Landmark point pair estimation from local graphs* - Cell divisions happen throughout the 72 hour intervals but at the consecutive images, taken every three hours apart, only several cell divisions are present. Ideally, in the areas where there is no cell division, the local graph topology should not change (segmentation errors will circumvent this in practice). We



**Fig. 2.** A,D) The local graphs  $G_1$  and  $G_2$  at time  $t$  and  $t+1$  with the central cells  $C$  and  $C'$  respectively, and counter-clockwisely ordered neighboring cell vertices  $N_1, \dots, N_6$  and  $N'_1, \dots, N'_6$ , B,C) two enlarged triangle subgraphs with indicated features

exploit these conditions to find the corresponding landmark pairs in two images. Let,  $G_1^{(t)}$  and  $G_2^{(t+1)}$  be two local graphs constructed around the cells  $C$  and  $C'$  in consecutive temporal slices (Fig. 2). For each subgraph of the local graph  $G(t)$ , we define feature vector the following way;

$$F^C(t) = [f_1 \ f_2 \ f_3 \ f_4 \ f_5]^T, \text{ where} \\ f_1 = \theta_{N_{i_1}, C, N_{i_2}}(t), \\ f_2 = l_{C, N_{i_1}}(t), f_3 = l_{C, N_{i_2}}(t), \\ f_4 = A_{N_{i_1}}(t), f_5 = A_{N_{i_2}}(t)$$

We define the distance between two triangle subgraphs as

$$D_{TS} \left( F_i^C(t), (F_j^{C'}(t+1)) \right) = \sum_{k=1}^5 \left( \frac{f_k - f'_k}{f_k} \right)^2, \\ \text{where } f_k \in F_i^C(t), f'_k \in F_j^{C'}(t+1).$$

To ensure that our landmark estimation method takes care of the rotation of the local area, we consider all cyclic permutations of the counter-clockwise ordered neighbor set  $\{N'_1, N'_2, \dots, N'_m\}$  of the cell  $C'$  from the input image. The cyclic permutations of the set  $\{i_1, i_2, \dots, i_m\}$  can be written in terms of the shift  $k$  ( $k = 0, 1, \dots, (m-1)$ ) as the set  $\{(i_1+k-1)_{mod(m)}+1, \dots, ((i_m+k-1)_{mod(m)}+1)\}$ . As an example, if  $(1, 2, 3)$  is the given sequence, then possible values of the shift  $k = 0, 1, 2$  and all the cyclic permutations of the sequence  $(1, 2, 3)$  will be  $(1, 2, 3), (2, 3, 1), (3, 1, 2)$  for  $k = 0, 1, 2$ . We consider all cyclic permutations of the counter-clockwise ordered neighbor set  $\{N'_1, N'_2, \dots, N'_m\}$  of the cell  $C'$  from the input image and define the distance  $D(G_1, G_2^k)$  between two local graphs  $G_1$  and  $G_2$  based on the chosen permutation corresponding to shift  $k$  as

$$D(G_1, G_2^k) = \sum_{\{i,j\}} D_{TS} \left( F_i^C(t), (F_j^{C'}(t+1)) \right) \\ \forall i \in \{1, 2, \dots, m\}, \quad j = [(i+k-1)_{mod(m)} + 1]$$

for  $k \in \{0, 1, 2, \dots, (m-1)\}$ . We compute the sum of the distances between each of the ordered pairs of triangle subgraphs for each permutation  $k$ .

The distance  $D^*(G_1, G_2)$  between two graphs  $G_1$  and  $G_2$  corresponding to cells  $(C, C')$  for all permutations  $k$  is

$$D^*(G_1, G_2) = D(G_1, G_2^{k^*})$$

where  $k^* = \arg_k \min D(G_1, G_2^k)$ ,  $k \in \{0, 1, \dots, (m-1)\}$ . This guarantees that our landmark estimation method is invariant of the rotation in the local area.

For all cell pairs  $C_i, C'_j$  and corresponding graphs  $G_i, G_j$  from two consecutive images, we compute the distance  $D^*(G_i, G_j)$ . Then the cell pairs are ranked according to their distances  $D^*$  and the top  $q$  cell pairs are chosen as landmark point pairs. The choice of  $q$  is described later.

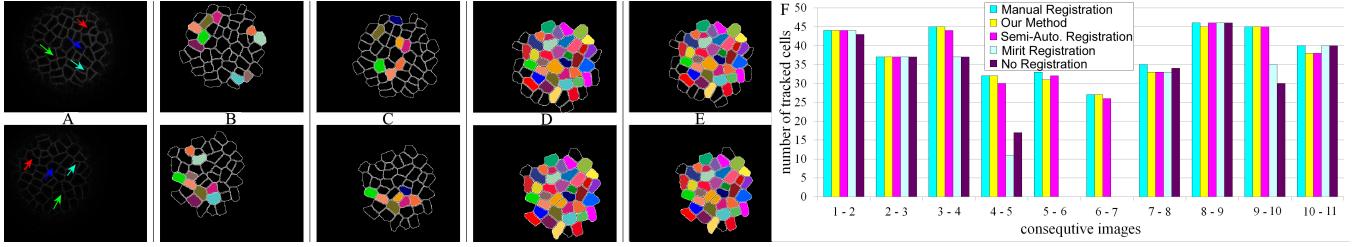
### 3.2. Image Registration

Once we have the landmark point pairs corresponding to the reference and input images, we find the spatial transformation between them. Finding the nonreflective similarity transformation between two images is a problem of solving a set of two linear equations. As mentioned before, for better accuracy of transformation parameters the top  $q$  landmark point pairs are used in a least square parameter estimation framework. The choice of  $q$  depends on the quality of the input and base image as choosing more landmark point pairs generally increases the risk of having more false positive landmark point pairs. In our experiments we choose four, five or six landmark pairs depending from the dataset image quality.

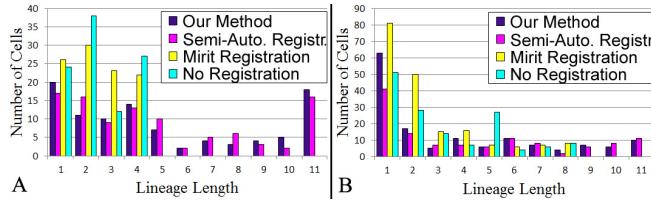
## 4. EXPERIMENTAL RESULTS

We have tested our proposed automatic landmark-based registration method, combined with the watershed segmentation [2] and local graph matching based tracking [1], on two different datasets. We compared tracking results of the proposed method with results obtained without registration, with semi-automated registration (the landmark pairs are chosen manually, the transformation is obtained automatically) and with MIRIT software ([4]).

**Pairwise Tracking** - Fig. 3 (A-E) shows cell tracking results from two consecutive images ( $30^{th}$  and  $36^{th}$  hour). The results with MIRIT registration and without registration show incorrect cell tracks. Whereas the proposed method and semi-automated registration correctly registered two images with 100% correct tracking results. Detailed results for the same dataset are shown in Fig. 3 (F). We can see that from 33 and 27 cells, present in the images at time points 5 ( $30^{th}$  hour) to 6 ( $36^{th}$  hour) respectively, none are tracked by the tracker



**Fig. 3.** A) Raw consecutive images (the same color arrows represent the same cells), tracking results obtained B) without registration C) with MIRIT registration, D) with manual registration, E) with proposed automatic registration. The same colors represent the same cell. F) Number of tracked cells across two consecutive images.



**Fig. 4.** Length of cell lineages for two different datasets A,B.

run on the images registered with the MIRIT software and not registered images (as in Fig. 3 (A-E)). The same result is seen for the tracking results in images at time points 6 to 7. But the tracking results obtained with proposed and semi-automated methods provided very close to manual results.

**Table 1.** Total Number of Cell Divisions/Ground Truth

Data	Our Method	Manual	MIRIT	No Registration
1	28/34	30/34	23/34	25/34
2	17/21	17/21	11/21	12/21

**Lineage Analysis -** Fig. 4 shows lengths of the cell lineages calculated with the proposed method, semi-automated registration, MIRIT registration and without registration. We can see that in tracking without registration and after registration with MIRIT software, there are no cells that have lineage lengths greater than four (Fig. 4 (A)) and greater than eight (Fig. 4 (B)), as opposed to the case with the proposed and semi-automated registration, where cells have lineages for the entire 72 hours. The reason for such results is that there is a big shift between two images from consecutive time points in the middle time points. Without proper registration the tracking algorithm is not able to provide correct cell correspondence results, which interrupts the lineage of the cells. Fig. 4 (A) result can be also related to Fig. 3 (F) since they are representing statistics from the same dataset. Since no cells have been tracked in frames five to six and overall there are eleven frames, then no cell can have a lineage life with the length greater than or equal to five.

Table 1 shows the number of cell divisions in 72 hours. We can see that the semi-automated and the proposed registration provide results that are close to the manual results as opposed to without registration and MIRIT software.

## 5. CONCLUSION

Automated image analysis such as registration, segmentation and tracking of cells in actively developing tissues can provide high-throughput and quantitative spatiotemporal measurements, which will lead to a better understanding of the underlying dynamics of morphogenesis. In this paper, we have described an automated landmark-based image registration method. The novel contribution of the work lies in its ability to automatically estimate corresponding landmark point pairs in densely packed SAM tissue to register CLSM images. We tested our method on multiple datasets of SAM cells and showed that the described method significantly improves the cell lineage and division statistics.

## 6. REFERENCES

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