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Context aware spatio-temporal cell tracking in densely packed multilayer tissues

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ABSTRACT

Modern live imaging technique enables us to observe the internal part of a tissue over time by generating serial optical images containing spatio-temporal slices of hundreds of tightly packed cells. Automated tracking of plant and animal cells from such time lapse live-imaging datasets of a developing multicellular tissue is required for quantitative, high throughput analysis of cell division, migration and cell growth. In this paper, we present a novel cell tracking method that exploits the tight spatial topology of neighboring cells in a multicellular field as contextual information and combines it with physical features of individual cells for generating reliable cell lineages. The 2D image slices of multicellular tissues are modeled as a conditional random field and pairwise cell to cell similarities are obtained by estimating marginal probability distributions through loopy belief propagation on this CRF. These similarity scores are further used in a spatio-temporal graph labeling problem to obtain the optimal and feasible set of correspondences between individual cell slices across the 4D image dataset. We present results on (3D + t) confocal image stacks of Arabidopsis shoot meristem and show that the method is capable of handling many visual analysis challenges associated with such cell tracking problems, viz. poor feature quality of individual cells, low SNR in parts of images, variable number of cells across slices and cell division detection.

1. Introduction

In developmental biology, the causal relationship between cell growth patterns and gene expression dynamics has been one of the major topics of interest. A proper quantitative analysis of the cell growth and division patterns in both the plant and the animal tissues has remained mostly elusive so far. Information such as rates and patterns of cell expansion and cell division play a critical role in understanding morphogenesis in a tissue. The need for quantifying the cellular parameters such as average rate of cell divisions, cell cycle lengths, cell growth rates etc. and observing their time evolution is, therefore, extremely important.

Towards this goal, with the advancements in microscopy and other imaging techniques, time lapse videos are being collected to quantify the behavior of hundreds of cells in a tissue over multiple days. For visualizing the cells over time within a densely packed multilayer tissue, one such in vivo time-lapse microscopy technique is confocal laser scanning microscopy (CLSM) based *Live Cell Imaging*. With this technique, optical cross sections of the cells in the tissue are taken over multiple observational time points to generate spatio-temporal image stacks. For high-throughput analysis of these large volumes of image data, development of fully automated image analysis pipelines are becoming necessities, thereby giving rise to many new automated visual analysis challenges.

Automated cell tracking with cell division detection is one of the major components of all such pipelines (such as Fernandez et al., 2010) that analyze the live cell imaging data. A review of current cell tracking imaging methodologies can be obtained in (Kircher et al., 2011). The computational challenges related to a robust design of cell tracker come from multiple sources such as variable number of cells in the Field of View (FoV), deformation of cell shapes, complex topologies of cell clusters, low SNR in the images, etc. In this paper, we present an automated visual tracker for cells tightly packed in developing multilayer tissues. This calls for developing strategies for *temporal* associations of the cells. Moreover, since at every time point of observation a cell could be imaged across multiple spatial images, the tracking method must be capable of finding correspondences in the *spatial* direction as well. Beyond these, the tracker has to be able to detect cell divisions, detect new cells as the deeper layers of the tissues are imaged, differentiate between cells in a close neighborhood sharing similar physical features and generate correct matches in

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presence of low SNR. These challenges are evident in the sample CLSM image stack of a live Arabidopsis shoot meristem, as shown in Fig. 1.

1.1. Related work and our contributions

There has been some work on automated tracking and segmentation of cells in time-lapse images, for both plants and animals. One of the well-known approaches for segmenting and tracking cells is based on evolution of active contours (Dzyubachyk et al., 2010; Li and Kanade, 2007; Li et al., 2008; Padfield et al., 2009; Dufour et al., 2005). However, this method is not suitable for tracking where all the cells are in close contact with each other and share very similar physical features, nor is there any reported result on spatial correspondence. In fact, in spatio-temporal image stacks where the cells are arranged in compact multilayer structure, slice of a new cell can legitimately appear at the exact same spatial location as that of a different cell located in the layer just above it. This characteristic, along with the fact that these tightly packed cells are mostly stationary, can force the active contour based tracker to generate false spatial tracks.

The Softassign method uses the information on point location to simultaneously solve both the problem of global correspondence as well as the problem of affine transformation between two time instants iteratively (Chui and Rangarajan, 2000; Gor et al., 2005). However, these methods are more suitable for aligning global features than finding correspondences between non-uniformly growing individual cells. Although (Gor et al., 2005) present a sample result on SAM shoot meristem without validating against ground truth, it is not enough to evaluate the accuracy of this method on a typical 4D confocal data.

Besides the aforementioned approaches, tracking based on association between detections such as (Kachouie et al., 2006; Kirubarajan et al., 2001) has shown good performance on time-lapse images. In (Bise et al., 2011), the authors proposed a cell tracking method on phase contrast time-lapse images that performs a global association of tracklets generated by frame-by-frame detection based tracking. Many other algorithms that have been successfully applied to single molecule localization and 2D movement tracking have been reviewed in (Kalaidzidis, 2007). Delibaltov et al. (2012) and Karthikeyan et al. (2012) describe probabilistic framework for joint detection and tracking of melanosomes. In (Liang et al., 2013), the authors have proposed a multiple hypothesis based framework that can be applied to solve particle tracking and 3D cell segmentation problems, which include splitting and mergings. In (Yang et al., 2005), the authors presented a method for tracking large number of particles undergoing dense motion by integrating motion models at particle, local and global levels. However, these methods perform well when the feature quality or the underlying motion model is reliable. In fact, for many applications such as the one presented in this paper, there is no motion information available and hence it cannot be exploited for tracking.

We are looking at a more challenging problem, where the features extracted from each cell may not be reliable enough for accurate data association. As an example, in this paper the experiments are performed on confocal time lapse image stacks of plant shoot apical meristem, where hundreds of cells are tightly clustered in a multi-layered architecture and only the boundary of each cell is visible. Thus the features extracted for each cell could only be the shape and area, which could often be non-discriminating between cells even from a local neighborhood. The cell tracking problem is targeted to obtain association between cell slices along both space and time. One possible solution approach could be to begin with 3D segmentation (such as 3D watershed) at each time step and then associate them across time. This approach would fail for the problem at hand, where because of extremely low z-resolution (3–4 slices/cell) the horizontal cell walls (x–y plane) are

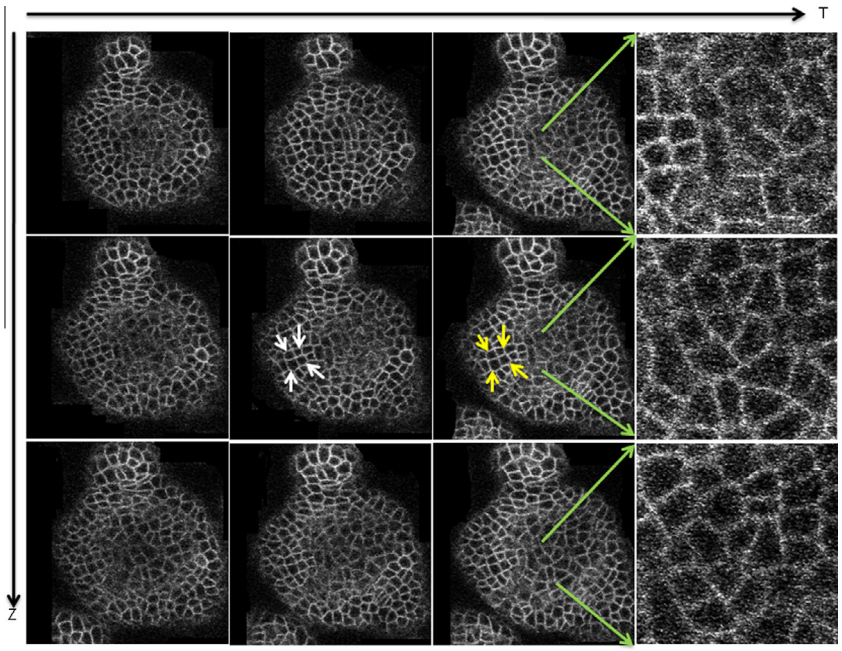

Fig. 1. A typical 4D (X–Y–Z–T) live-imaging data. A live Arabidopsis shoot meristem tissue is imaged using a confocal laser scanning microscope at multiple time points. The plasma membranes of the cells are stained with fluorescent proteins and that is why the cell walls are the only visible parts. Each of the first three columns of images presents Z stack of image slices, i.e. the cross sections of the tissue imaged at various depths of it. When such images are collected over time to capture the growth of the tissue along with that of individual cells in it, it forms a 4D image stack. As can be seen from the figure, there are various challenges associated with the problem, viz. growth/deformation of the cells in the tissue, stereotypical cell shapes in the tissue and hence less discriminative physical features (as an example, four cells from a close neighborhood are marked with white and yellow arrows respectively in two consecutive time points which have very similar shapes and sizes), minor shifts between images and low SNRs in the central regions of the tissue. We have zoomed into these low SNR regions in the 4th column of the figure. As seen, it is really difficult to even manually mark the boundaries of a number of cells in these regions.