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High density cell tracking with accurate centroid detections and active area-based tracklet clustering

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ABSTRACT

Accurate cell tracking and lineage construction under microscopy has played an important role in analyzing cell migration, mitosis and proliferation. In the last decade, this labor-intensive manual analysis was gradually replaced by automated cell tracking methods; however, they are often limited to cells with certain morphologies or staining. In this paper, we propose a novel hierarchical tracking framework (Hift), which does not have these limitations. To keep the robustness and feasibility with different cell densities, we concluded several cell motion events into different tracking stages, including entry, exit, division, merge, fast motion, etc. And the fusion of global and local information is applied in both detection and tracking modules, to ensure the flexibility and expansibility of cell detection. To get a full-time cell lineage, we first introduce a conservative distance limit to obtain tracklets with high reliability in the tracking stage. Then motion events are recognized with local information for further corrections. At last, trajectories are linked and completed based on an active search area estimated by the established tracklets. The hierarchical framework designed in Hift enhances the ability of cell detection and tracking by combining the global assignment and local optimization in spatial-temporal dimension. Experimental results of Hift on three large-scale datasets with high cell densities and four sparse datasets demonstrate its efficacy. Hift is available at: http://www.csbio.sjtu.edu.cn/bioinf/Hift/.

1. Introduction

Quantitative analysis of molecule morphology and motion characteristics plays an important role in revealing complex mechanisms of microtubule, stem cells, embryo and other organisms in the micro-world [1,2]. For instance, to understand how drug affects cells, or study the propagation process of embryo cells, even analyze cell cycle variation, accurate cell detection and tracking is critical [3,4]. To achieve this goal, manual analysis is naturally a simple and straightforward method to track moving cells. However, because of the large cell population and long cell cycle, it is very difficult to accomplish tracking these moving cells by human power, since the work is tedious and subjective. Automated cell detection and tracking with intelligent algorithms can provide an alternative solution for this problem. With the development of hardware facilities in last decades, the cost for generating cell image sequences has been significantly reduced in terms of both money and time. Efficient cell tracking algorithms are highly desired to analyze time-lapse microscopy videos in the field of biomedicine and wet-lab cell culture. It is not an easy task, however, because the imaging of cell microscopy is complicated, and the morphology and motion of cells are hard to be modularized.

One of the major challenges for designing highly accurate automated cell tracking algorithms comes from the imaging step. The phase contrast microscopy (PCM) and fluorescence microscopy (FM) are two major techniques used in cell imaging. They both have advantages and disadvantages. For example, with PCM, we can implement long time tracking without damaging cells; but the generated microscopy images have low contrast and halo around cells, resulting in difficulties in next cell segmentation and recognition, especially in the case of high dense cell population. For FM, the frame sampling rates are often limited by the phototoxicity, and the appearance of cells may not be steady due to the change of fluorophore intensity and distribution [5]. In addition to PCM and FM, there is another cell imaging technique called oblique illumination microscopy (OIM), which can increase specimen contrast and highlight otherwise invisible features, but the images sometimes suffer from uneven illumination.

The other major challenge in methodology design is from complex cell morphology and motion characteristics. Because cells are non-rigid, the shape and size will change in different periods of cell cycle and get easily influenced by surrounding environment

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Table 1
Summary and comparison of three categories of cell tracking methods.

Cell-tracking methods	Advantages	Problem unsolved	Representative algorithms
Detection-based association algorithms	Flexibility and fast computing	Cell mitosis, poor portability as the cell diversity	Graph matching [19]; Minimum cost flow [13]
Model-based evolution algorithms	Efficiently solving cell division event	Cells entry/exit the field of view, high computation complexity	Parametric active contour [24]; Level set methods [5,25]
Filtering-based algorithms	High accuracy	The computational complexity increased dramatically with the cell population	Particle filtering [22]; Mean-shift [26]; Kalman filtering [27]

and interactions with other cells, which makes it more difficult for cell detection. During the cell proliferation process, the population of cells can increase to hundreds or even more from much less original ancestor cells. On the one hand, the high population of cells makes the recognition of independent cells difficult since multiple cells can cluster and overlap together. On the other hand, simultaneously tracking of extensive targets in a high population density is difficult especially when considering the cells have a great diversity of behaviors, including cell migration, mitosis, merge, exit and entry in the field of view, etc. With division of cells, the density will be further enhanced, which can bring more challenges for automated tracking.

To overcome the difficulties aforementioned, plenty of work has been carried out and made substantial progresses [6]. Most reported algorithms focus on specified cell data with specific morphological characteristics in order to achieve better tracking results [7,8]. Existing tracking methods can be generally classified into three groups (Table 1): (1) detection-based association, (2) model-based evolution, and (3) filtering-based sampling [7].

The detection-based association methods are implemented in two stages: at first, all cells in each frame will be detected, and then in association stage, lineages of cells are constructed in adjacent frames. This type of framework is widely used in cell tracking tasks due to its flexibility and robustness, and can detect the entry, exit and abrupt motion accurately [9–11]. The improvements for this type of framework are on the two stages of detection and association, respectively. Many cellular features have been extracted to describe the morphology of cells in detection stage. Besides the intuitionistic characteristics like cell location and intensity [4,12,13], shape fitting is also a common feature to describe the cell shape. For instance, the overlap of convex hulls and the fitting ellipse are often used as models of cells [14,15]. In several reported methods, different features are combined together. For instance, orientation, area, eccentricity, and major axis length of the fitting ellipse are combined into one vector in [16]. In association stage, lineage construction is often regarded as a linear optimization problem like graph matching [13], or minimum cost flow [17]. Most association methods need an additional cell mitosis detection procedure since the linkage between adjacent frames can be one-to-many or many-to-one while the graph matching is one-to-one [18].

Different from the first category of methods, model-based evolution methods detect and track the cells simultaneously. A representative method is active contour model [19,20]. The cell boundaries are represented by a parametric curve in the first frame, and then the curve evolves with change of cell boundaries to minimize the energy function in each frame. Another method to describe the curve without parameters is level set method, which also needs to initialize the curve manually. One of the typical merits of model-based tracking methods is the convenience to handle topological changes like cell division [3]. Despite its merits, as this type of methods is often sensitive to the location of initial curve and could not detect cells accurately, many efforts have been done to improve this framework. For instance in [5], a modified energy function and a random transformation was introduced to improve the robustness and applicability. Previous studies also show that abrupt motion of cells may reduce the efficiency of model-based algorithms, and cause missing detection when the cells have high density or irregular shapes [21].

The third category of cell tracking methods is filtering-based sampling approach. The key idea of this type of methods is to establish a model for each cell to predict the next status by obtained ground truth dataset. So many algorithms in this category need post-processing to correct tracking errors or manual initializations. One typical algorithm is particle filtering method within Bayesian framework proposed in [22], which is able to track multiple cells. In [23], the authors proposed to combine Kalman filtering with the modified mean shift algorithm to predict the position and shift in current frame. In general, this type of methods works well when the objects can be modeled within a small scale but it is also not very effective to handle complex events like cell division.

In this work, we develop a new cell tracking system named Hift, aiming to further improve the efficacy of detection-based association cell tracking algorithms. In this framework, a critical part in the first stage is accurate detection of proper cell characteristics for recognition purpose. The main challenge in cell detection is under-segmentation of the adherent cells. In the proposed Hift, we use a hierarchical protocol that firstly segments all cell regions by a global thresholding approach, and then recognizes the under-segmentation regions by morphological features, where the cell cluster regions will be further segmented with a local wavelet-based method. Different types of cells have distinct features, which is one of the main factors to hamper the universality of this category of methods. To solve this problem, we propose a new simple but effective hierarchical tracking method using cell centroid information as the only input for the tracking module, which can be applied to track different types of cells. We use an adaptive distance limit to estimate the possibility of association of cells in adjacent frames. During the linking process, we detect and handle cell motion events in different tracking levels. The linking limitation can be adjusted based on the established short tracklets. In this way, the temporal information will also be used to analyze the linkage possibility, which is demonstrated capable of enhancing the robustness of linkage construction.

2. Materials and methods

The hierarchical method of cell tracking developed in this study is called Hift, which consists of two function modules: detection and tracking. The input of the system is a synthetic microscope video or a sequence of raw images. Due to the low contrast and noise of raw images, a pre-filter method is used in Hift to enhance the contrast and eliminate the noise. In the detection part, cell images will be segmented and detected orderly. Then the centroid of each cell will be used as the only feature to represent the cell in the tracking step. In this way, a cell can be abstracted as a point (centroid), which will help reduce the difficulty for representing a cell with different morphology in a highly dynamic environment. Thus, the proposed Hift is a more general framework that can be applied to track different types of cells.

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