Cell Segmentation, Tracking, and Mitosis Detection Using Temporal Context

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Abstract. The Large Scale Digital Cell Analysis System (LSDCAS) developed at the University of Iowa provides capabilities for extended-time live cell image acquisition. This paper presents a new approach to quantitative analysis of live cell image data. By using time as an extra dimension, level set methods are employed to determine cell trajectories from 2D + time data sets. When identifying the cell trajectories, cell cluster separation and mitotic cell detection steps are performed. Each of the trajectories corresponds to the motion pattern of an individual cell in the data set. At each time frame, number of cells, cell locations, cell borders, cell areas, and cell states are determined and recorded. The proposed method can help solving cell analysis problems of general importance including cell pedigree analysis and cell tracking. The developed method was tested on cancer cell image sequences and its performance compared with manually-defined ground truth. The similarity Kappa Index is 0.84 for segmentation area and the signed border positioning segmentation error is 1.6 ± 2.1 μm.

1 Introduction

1.1 Living Cells

Study of living cells including cell death, cell motility, measurement of intracellular pro-oxidant species, and the determination of phenotypic changes observed using adenovirus-mediated gene expression systems and many other aspects will help to diagnose and assess the natural course of many diseases.

The need for investigation of cell behavior and movement led to the development of automated systems designed to quantify the reaction of cells in different environments. An automated system, the Large Scale Digital Cell Analysis System (LSDCAS) was designed to analyze large numbers of cells under a variety of experimental conditions in the Real-Time Cell Analysis Laboratory, Department of Biomedical Engineering, University of Iowa, in the previous years. The LSDCAS is capable of monitoring thousands of microscope fields over time intervals of up to one month. LSDCAS was originally designed to study stress-induced

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mitotic catastrophe [1]. Current efforts concentrate on providing imaging and data modeling/visualization technologies that will be useful in many areas of biological research.

1.2 Previous Work and Associated Problems

Corresponding to changing properties of a variety of cells, many distinctive methods have been proposed and employed in the analysis of cellular structure.

A part of the methods which have been widely used in cellular imaging segmentation and tracking are model-free approaches, such as thresholding [2], median filtering [3], watershed segmentation [4], and others. However, these approaches provide no or only poor descriptions of the cellular shape. Even worse, they frequently do not produce closed-object contours and require extensive post-processing steps [5]. These approaches often fail to correctly track multiple cells in sequences exhibiting cell-cell contacts [6].

Model-based techniques, such as active contours [7], are also frequently used. These active contours may be parametric format, geometric, or may use region based models. The methods produce closed and smooth object boundaries, and may provide a first guess through the interactive initialization step. They allow keeping track of object identity in an obvious manner through automatic initialization based on the segmentation of the previous image[8, 5]. With the introduction of the Level Set Methods [9], a powerful mathematical tool to solve the problem of cusps, corners, and automatic topological changes, the active contour methods became well suited to cell image analysis.

It is not sufficient to rely on pure image information for cell analysis. Pattern recognition based segmentation method of cell nuclei in tissue section analysis was utilized in [10]. Neural network [11] and genetic algorithms [12] were applied to cell image analysis. The pattern recognition techniques helped achieve cell analysis objectives in specific cases. However, defining and computing the rules as well as performing the training steps is difficult.

Little effort was devoted to quantitative living tumor cell analysis largely due to its complicated nature: cell shape variability; contact between cells; weak cell boundaries, etc. Many existing methods are only effective in low-density situations and temporal context is frequently not considered.

2 Methods

2.1 Cell Trajectories Extraction

To identify trajectories of moving cells, time is taken as an extra dimension and the level set method-based geometric active contour is directly applied onto a 2D+time image sequence. For each frame, image boundary is taken as the initial location of the moving front. Using the Fast Marching method, the front is moved inward through the gradient defined on the cost image until it stops at the location close to the cell or cell cluster boundary. Next, Narrow Band Level Set method is applied to achieve a more accurate segmentation.

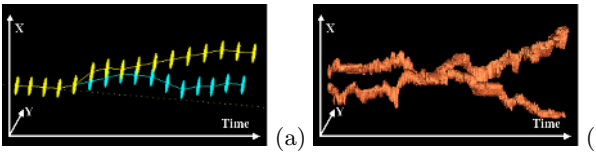


Fig. 1. Trajectory model.(a) The cell trajectory model in 2D + time space, each disk represents a cell or cell compound at a single time and the model is showing a normal cell division. (b) A cell trajectory, with 2 cells in the field, the total number of frames being 300, the time span between frames was 5 minutes and the total time span was 1500 minutes.

The cell trajectory model behind this method is shown in Fig. 1. An example of the real cell trajectory segmented from the LSDCAS data set is also shown.

In this step, the multi-channel cost image is used for level set segmentation, which is $f(intensity) + g(localvariance)$, a combination of the different kinds of information from the original image. $f()$ is the function of the image intensity and $g()$ is the function of the local variance.

A special-designed edge enhancement step is included to prevent the moving front evolving into the internal portion of the cells which have weak boundary areas. Let $\theta(x, y) : R^2 \rightarrow R$ and the arc be defined as: $d\theta = \frac{\partial \theta}{\partial x}dx + \frac{\partial \theta}{\partial y}dy$. Based on Riemannian geometry standard notation, we have:

$$\lambda_{\pm} = \frac{s_{xx} + s_{yy} \pm \sqrt{(s_{xx} - s_{yy})^2 + 4s_{xy}}}{2} \tag{1}$$

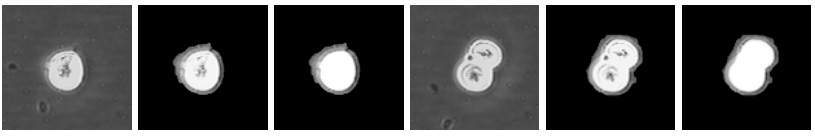
where second order derivative $s_{xx} = (\frac{\partial \theta}{\partial x}).(\frac{\partial \theta}{\partial x})$, $s_{yy} = (\frac{\partial \theta}{\partial y}).(\frac{\partial \theta}{\partial y})$, $s_{xy} = s_{yx} = (\frac{\partial \theta}{\partial x}).(\frac{\partial \theta}{\partial y})$. These derivatives are approximated using forward difference method and in 0 - 1 scale. In the image, the maximal and minimal changes at a specific location are provided by the eigenvalues $\lambda_{\pm}$. In our approach, we define the driving force for the moving front as $F = e^{-\rho * (\lambda_+ - \lambda_-)}$.

2.2 Mitosis Detection

In the life cycle of living cells, mitosis (cell division) may provide extra information. Mitosis typically involves a series of steps consisting of prophase, metaphase, anaphase, and telophase, and results in the formation of two new nuclei each having the same number of chromosomes as the parent nucleus. Mitotic cells tend to be circular and have a larger percentage of bright pixels than non-mitotic cells. Once mitosis is detected, the change of the number of the cells in current field is observed. In a long temporal sequence, the frames between mitotic states have the same number of cells (not considering cells moving in or out of the current field of view).

The mitosis detection is done according to the following procedure:

1. Apply an optimal threshold method [13] to remove the effect from non-mitotic cells in the cell compound identified in the cell trajectory and to get the Region of Interest (ROI) for the mitotic candidates.



(a) (b) (c) (d) (e) (f)

Fig. 2. Mitosis detection. (a) The original image at frame 12. (b) The profile of the cell trajectory at frame 12. (c) After threshold and movement under curvature via level set methods at frame 12, the ROI properties are $P = 144$, $A = 1540$, $AI = 237$, $C = 0.933$. (d) The original image at frame 13. (e) The profile of the cell trajectory at frame 13. (f) After threshold and curvature-based smoothing via level set methods at frame 13, the ROI properties are $P = 176$, $A = 2072$, $AI = 236$, $C = 0.841$. (P and A in pixels).

2. Apply Level Set Methods based on moving under curvature [9] to smooth the ROI boundary.
3. Compute ROI properties: area, perimeter, circularity, etc.
4. In adjacent frames t and $t+1$, only the ROI whose area A increases, perimeter P increases, circularity C decreases, and average intensity AI remains unchanged are considered a mitosis.

The mitosis detection uses a classifier trained on an independent training set. An example of the mitosis detection procedure is shown in Fig. 2.

2.3 Cell Separation and Segmentation

The next step is extraction of the cell trajectory between the already identified mitotic frames. If there is no mitosis detected from the trajectory, the whole trajectory has a fixed number of cells (possibly more than 1 if cell compounds are present). Backward and forward adjacent frame separation and segmentation are attempted for the trajectories which have cell compounds exhibiting cell-cell contact.

A special data structure is utilized which helps to generate an effective description of the trajectory. Importantly, it sets up a connection graph through the trajectory. Each node stores the information for the individual region corresponding to each time frame. The information includes: area, perimeter, average intensity, father identifier (the connected individual region in the previous frame), father label, son number (the connected individual region at next frame), son label.

We applied marker-based watershed method for the cell separation. The watershed method is implemented via rain falling simulation [14]. The separation is directional because, in addition to the number of cells, the separating cell border in adjacent frame helps to segment and separate the cell cluster in the current frame.

For a non-mitosis example, in Fig. 3 (a) and (b), which are the connection graph and the separation result, node 13 is the first cluster to be separated using nodes 8 and 9 as the reference markers. The processing direction at node 13 is forward. Node 53 will be the second to be separated using nodes 57 and 58 as a reference and the processing direction here is backward. Basically, the separation

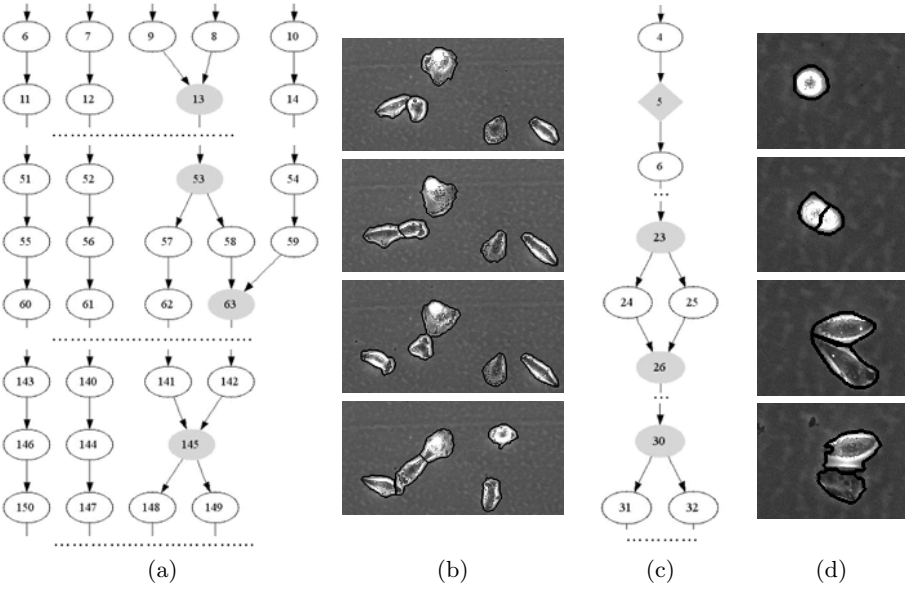


Fig. 3. Cell connection graph and cell segmentation/separation. (a) No mitosis detected. The arrow in the graph is representing the time change and links the connected node at the adjacent frame. The gray circles are the very first clusters to be separated, where cell contact happens. (b) The cell separation corresponding to (a). The first image is corresponding to frame at the first gray circle 13. The second image is for the frame at gray circle 53. The third image is for the frame at gray circle 65. The fourth image is for the frame at gray circle 145.(c) With mitosis detected. The gray diamond in this graph represent the labeled mitotic cell. The merging in this graph with gray circle is due to the cell contact and the cell trajectory generated by the level set methods was not able to separate them. (d) The cell separation corresponding to (c). The first image is for the frame at gray diamond 5, which is a mitotic cell. The second image is for the frame just after mitosis detected, at circle 6. The third image is for the frame at gray circle 23. The fourth image is for the frame at gray circle 26.

strategy is from known to unknown, from large number of cells or cell clusters per frame to a smaller number.

Similarly for a mitosis example, in Fig. 3 (c) and (d) in which a mitotic cell is detected at node 5, the whole trajectory is divided into 2 parts. The first part starts at node 1 (not shown in the image) and ends at node 5. The second part of the trajectory starts at node 6. For each new trajectory, the same method was applied as was used for the trajectory where there was no mitosis detected.

3 Experimental Methods

Image Data

The performance of our cell sequence analysis was tested in field images acquired using the LSDCAS. All the cells were U87-MG cells.

Validation

The manually identified independent standard was defined using a user-friendly border tracing program. Automated segmentation was obtained for all the cells in each of the data sets. The independent standard was used to determine the signed (bias) and unsigned mean border positioning errors as well as the maximum border positioning errors.

Positioning error: For each pixel along the automatically-identified (AI) border of individual cell, a nearest counterpart is found on the manually-identified (MI) border of the same cell and the distance between these two pixels is computed. If the counterpart is inside of the AI contour, we define the distance as positive. Otherwise the distance is negative. For each cell, the average value of the absolute distances was taken as the unsigned positioning error and the average value of the signed distances is taken as the signed positioning error.

Area error: Let A denote the AI segmentation area, M the MI segmentation area representing the ground truth. Ratios of overlapping areas were assessed by applying the similarity Kappa Index (KI)[15] and the overlap, which are defined as:

$$KI = 2 \times \frac{A \cap M}{A + M} \quad ; \qquad overlap = \frac{A \cap M}{A \cup M}$$

4 Results

The reported method successfully identified the individual cells in 50 2D + time microscopy data sets collected from the LSDCAS system. A total of 3368 frames containing 6654 cells was analyzed. There are totally 26 mitoses among all the data sets and our method successfully made the detection and recorded the mitosis time and location of all of them.

The analysis errors obtained with no manual interaction or editing are shown in the following table.

Table 1. Segmentation and separation errors

validation indices	in pixel	in μm
Mean absolute positioning errors	4.2 ± 2.8	2.6 ± 1.7
Mean signed positioning errors	2.6 ± 3.4	1.6 ± 2.1

The area error KI was 0.84 ± 0.09 and the overlap index was 0.74 ± 0.12.

5 Discussion and Conclusion

Quantitative analysis of living cells is a very complex task. It has strict requirements on hardware for data acquisition as well as on software for advanced quantitative analysis. All the processing modules, including preprocessing, segmentation, detection of cell splits, etc. are of great importance to the overall result. In

this paper, we introduced a new fully automated method for cell segmentation, separation and spatial cell state detection. Our approach helps solving one of the most crucial problems facilitating quantitative analysis of living cells.

The connection graph, as a by-product, gives a complete description of the cell-dividing temporal data set. Each cell trajectory can be directly used to describe behavior of a specific cell. For the cell trajectory with mitotic events, a pedigree tree can be generated by combining all nodes on each branch of the connection graph.

Under some circumstances involving abnormal cancer cells, atypical cell divisions may happen when the cell reaches the mitotic state. Compared to normal cell division when the cell begins as a single round entity and then splits into two completely separate objects, the abnormal cell division states include a multipolar state (when one cell moves into 3 or more distinct circular shapes), fuse state (which is defined as a cell which attempts to divide but is interrupted internally and re-forms as a single cell), and cell death (cell ceases to continue intracellular activities). These abnormal situations bring additional difficulty to the trajectory analysis since the a priori information used in our approach cannot be reliably extracted using the presented mitosis-detection method. A more powerful classifier combining cell trajectory analysis with cell status assessment is under development, which is based on Hidden Markov Models (HMMs) using time varying information from bright object sequences found in the cell compounds. The robustness of the HMM-based classifier shall provide reliable information helping to guide the segmentation of cancer cells in the image sequences.

Overall, the reported method is a substantial improvement in comparison with most existing cell analysis approaches. The temporal information is fully utilized, yielding more robust segmentation results compared to the previous frame-by-frame approaches [4].

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