Tracking of Active Cells Based on Kalman Filter in Time Lapse of Image Sequences of Neuron Stem Cells

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Abstract - *In multi-cells' tracking of time-lapse image sequences imaged by optical microscope, problem of tracking active cells correctly is still unsolved. It affects tracking accuracy and speed whether an algorithm can predict the position of the active cells or not. The tracking strategy is guided by Kalman forecast in image Cartesian coordinates systems, which may search target cells via minimizing their cost function of characteristics and updating their state equations. Prediction and tracking results from six active cells in three image sequences show that the algorithm can track segmented active cells accurately. And the errors between the tracking estimate values and the practical observation values are no more than 10 pixels.*

Keywords: tracking; active cells; Kalman filter; neuron stem cells; image sequences

1 Introduction

Most of the cells in human body are unregenerate and suffer from various diseases. Some serious damage can't be repaired through natural processes. But with the development of the research in cytology, these damaged cells can be cured by cell therapy. So far, many researchers have made some achievements in the field. Whether to continue the experiments or not depends on correctly extracting some special stem cells for treatment. How to extract stem cells reliably? What internal mechanism may control stem cells growing and proliferating? What are the exact substances to induce their differentiation? How to find the more effective ways to solve these problems?

Along with the development of computer technology and automatic system, more researchers are inspired in the research field. With the digital microscopic image developing, the imaging system can obtain ideal image sequences for the cells living in vitro during a period. Computer vision can establish mathematical models whether the cells in 2D or 3D environments. In this way researchers may find the solution of the above problems.

To track cells in 2D image sequences, main methods are mostly based on overlapping[2,6], where man-machine interaction is usually added to eliminate some tracking errors due to segmentation problem. Mean shift is a directly tracking which tracks cells in original image sequences[1,3,4]. Active contour model[5], auction algorithm[7] and other improved algorithms have also been studied in cells' tracking. However, all these algorithms are not very effective in tracking active cells. Although active cells are very few in one sequence, they have some significance in cells movement analysis. So far no papers have discussed the methods in tracking active cells specially. As the active cells are the cells locomotive distance above one average diameter of cells in two successive images, mean shift[1] which is based on fixed bandwidth is very difficult to find the active cells' centroids. Xiaobo Zhou[10] has studied cancer cells' cycle progression via Kalman filter. However, on Kalman prediction in the first several frames, whether it is artificially operation or automatic recognition, he has not stated in detail. Kalman filter is applied which is focusing on the prediction of the active cells in this paper.

This paper is organized as follows: segmentation of level set combined with average gray threshold has been introduced firstly. In cells' tracking part, all cells have been classified into two categories: inactive cells and active cells. The former is tracked by overlapping firstly. The latter is tracked by Kalman filter. Tracking results and conclusion have been given in the final part.

2 Segmentation

Level set is a particular contour evolution approaching which has good performance in segmentation. In this paper, segmentation of image sequences of neuron stem cells which have been imaged by confocal microscopy is based on level-set combined with local gray threshold[9]. The algorithm can not only solve the problem of focal shift but also separate adherent and clustered cells successfully as well as keeping cells' shape and position.

3 Tracking

3.1 Overlapping

Overlapping being used for the inactive cells' tracking is based on the overlap region between previous frame and current frame to ensure one cell having its own ID till last frame of the image sequence. We can track the inactive cells which moved distance is less than L between previous frame and current frame via overlapping, where L refer to the average diameter of all cells in the sequence.

3.2 Identification of active cells

To the active cells, the overlapping does not work. And in this paper if one of the following three criteria is satisfied, the cells is regarded as active cells.

1. The cell's moving distance is more than L in two adjacent frames;
2. The cell moves in from any edge of an image;
3. The cell emergence is due to over-segmentation.

3.3 Kalman filter tracking

Kalman filter[8] is an optimal recursive data processing algorithm which is extensively applied in tracking and navigation.

In active cells tracking, position and velocity are parameters of motion state. Acceleration is the external input. Although cells' locomotion is chaotic, as sampling interval of image sequences is 10 minutes, the changes of motion state may be very small between two adjacent frames. It is reasonable that the cells motion is uniformly accelerated in two adjacent frames.

We denote the state vector $X(k)=[xs_k \quad xv_k \quad ys_k \quad yv_k]^T$ and the system external input vector $U(k)=[xa_k \quad ya_k]^T$.We assume *xs*, *xv*, *xa*, *ys*, *yv*, *ya* are cells' position, velocity and acceleration on *x* axis and *y* axis respectively. Let the interval time of two adjacent frames is *T*. In *T*, state transition matrix $A(k)$, system parameters $B(k)$, and observation matrix $H(k)$ are defined in equation (1)~(3).

$$A(k)=\begin{bmatrix}1 & T & 0 & 0\\ 0 & 1 & 0 & 0\\ 0 & 0 & 1 & T\\ 0 & 0 & 0 & 1\end{bmatrix} \tag{1}$$

$$B(k)=\begin{bmatrix}0.5T^2 & 0\\ T & 0\\ 0 & 0.5T^2\\ 0 & T\end{bmatrix} \tag{2}$$

$$H(k)=\begin{bmatrix}1 & 0 & 0 & 0\\ 0 & 0 & 1 & 0\end{bmatrix} \tag{3}$$

If active cells, say C_2, has been located in the $(t\text{-}1)^{th}$ frame, we track C_2 in the t^{th}, $(t+1)^{th}$, $(t+2)^{th}$ frames using the following method. The searching area is defined as five times of the diameter of C_2. If the cells are considered as elliptic shape, its long axis, short axis, angle between long axis and *x*-axis, which is called azimuth, eccentricity and centroid can be regarded as six parameters to describe itself. The cell having the smallest parameters changes is the matching cell of C_2 in the next frame. After C_2 has been tracked in the t^{th}, $(t+1)^{th}$, $(t+2)^{th}$ frames, we can calculate the initial value of $P(0/0)$, $\hat{X}(0/0)$ and $Z(1)$ in Kalman filter. After $T>(t+2)$, the matching area is estimated via Kalman filter firstly, then local searching is used to save searching time.

Kalman filter is to predict active cells' location form the $(t+3)^{th}$ frame. Searching area is square. Those cells falling into the square are called candidate cells. The best matching one can be found by association operation.

In association operation, we set up a cost function using the above six parameters to show the changes of cells' movement. The smaller the cost function is, the higher probability confidence the matching has.

We assume $\{B_j, j=1, 2, \ldots, J\}$ as the candidate cells' centroids set in the t^{th} frame. If $\tilde{A}$ is the best prediction of cell *A*, the cost function is in equation (4):

$$\begin{aligned}&COST(A,B_j)=\alpha*D(A,B_j)+\beta*K(A,B_j)+\\&\gamma*H\left(A,B_j\right)+\sigma*G\left(A,B_j\right)+\\&\lambda*S\left(A,B_j\right)+\delta*Q\left(A,B_j\right)\end{aligned} \tag{4}$$

Where, $D\left(A,B_j\right)=\dfrac{distance\left(\tilde{A},B_j\right)}{\max\left(distance\left(\tilde{A},B_j\right)\right)}$,represents for distance; $K\left(A,B_j\right)=\dfrac{Vc_j}{\max\left(Vc_j\right)}$, represents for velocity, $V_j=distance\left(\tilde{A},B_j\right)/T$, $V_{cj}=|V\text{-}V_j|$; $H\left(A,B_j\right)=\dfrac{|Area_A-Area_{B_j}|}{\max\left(|Area_A-Area_{B_j}|\right)}$,represents for area; $G\left(A,B_j\right)=\dfrac{|Dia_A-Dia_{B_j}|}{\max\left(|Dia_A-Dia_{B_j}|\right)}$,represents for diameter;

$S(A,B_j)=\dfrac{|Ecc_A-Ecc_{B_j}|}{\max(|Ecc_A-Ecc_{B_j}|)}$, represents for eccentricity; $Q(A,B_j)=\dfrac{|Azi_A-Azi_{B_j}|}{\max(|Azi_A-Azi_{B_j}|)}$, represents for azimuth.

α、β、γ、σ、λ and δ are the coefficients in cost function. And the sum of them equals to 1. Find the cell's centroid in candidate cells having the minimal cost function. Then the cell is considered as the best matching one of A in the next frame. Thus the observation vector and the external input vector can be obtained. Kalman filter can be iterated till target cells can not be found, it means the cell having moved out of observation vision or changing its locomotive characteristics.

4 Simulation

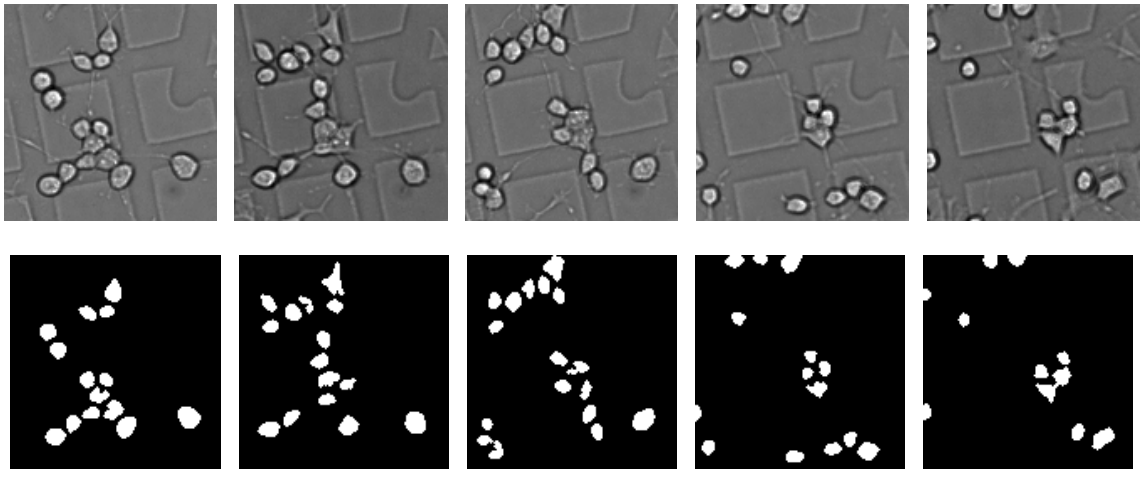
The presented algorithm has been tested in three image sequences. The image and cells' information are listed in Table 1. M_1 is the number of the cells which moving distance is greater than L between the adjacent image sequences. M_2 is the number of the cells which move to the edge of image. M_3 is the number of cells which moving distance is greater than L caused by over segmentation between the adjacent frames. M_4 is the number of frames that the cells' moving distance are greater than L. M_5 is the number of the cells which moving distance are greater than L and frame number must be greater than 2. According to the identification conditions of active cells in 3.2, $M_6=M_1-M_2-M_3-M_4+M_5$ is the number of the active cells in the three image sequences.

(1) From Table 1, we know that there are three active cells in sequence I. Because one of them exists only in five frames, and then moves out of the image sequences, we did not simulate it. We track the other two separately. In Fig.1 (a) ~ (e), it shows 1, 10, 20, 40 and 60 frames in the image sequence I respectively, and the corresponding segmentation images. For active cell 1, it has moved out of the image in the 45th frame. So we can track the active cells between the 1st frame and the 44th frame. And it costs 10.625 seconds. For the active cell 2, it is moved into the image in the 46th frame, so we can track the active cells between 46th frame and the 70th frame.

(2) In Fig. 2 (a) ~ (e), it shows 1, 10, 20, 40 and 60 frames in the image sequence II, and the corresponding segmentation images respectively. For the only one active cell, it has moved in the image in the 4th frame. Therefore, we can track it between the 4th frame and the 70th frame. It costs 13.985 seconds.

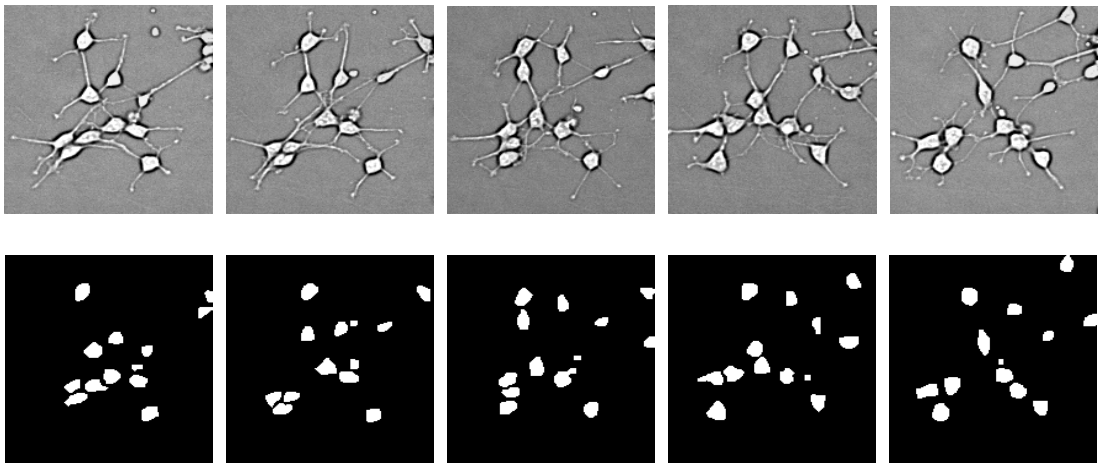
Table1 Numbers of active cells in the image sequences

Image sequences	Image size and number of frames /(pix * pix * frame)	M_1	M_2	M_3	M_4	M_5	M_6
I	127*127*70	60	34	18	7	2	3
II	184*169*70	36	12	16	8	1	1
III	256*256*50	152	20	119	5	2	10



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

Fig.1 Segmentation of frame 1、10、20、40 and 60 in sequence I



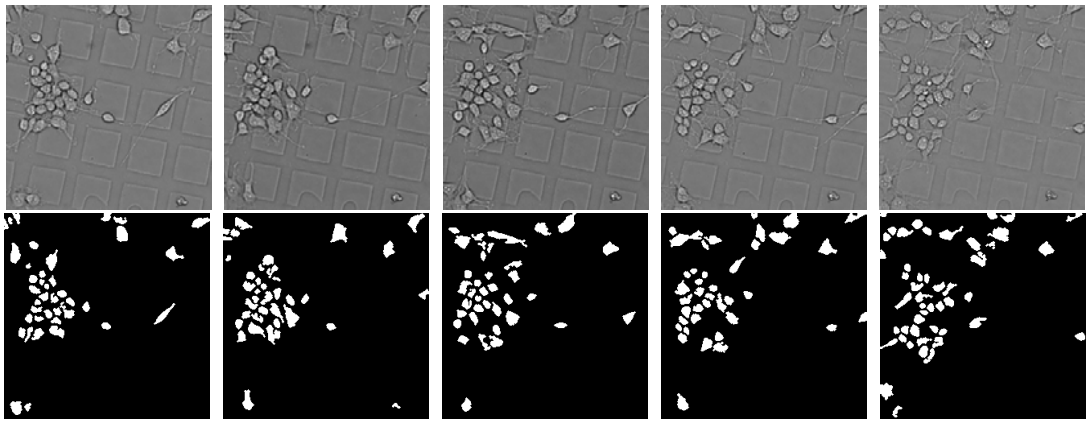
(a) (b) (c) (d) (e)

Fig. 2 Segmentation of frame 1、10、20、40 and 60 in sequence II

(3)There are clustered cells and under segmentation cells in image sequence III, which can lead to tracking error. From Table 1, we know that there are 10 active cells in it. As the majority of them results from under segmentation, 3 active cells should be tracked by Kalman filter actually.

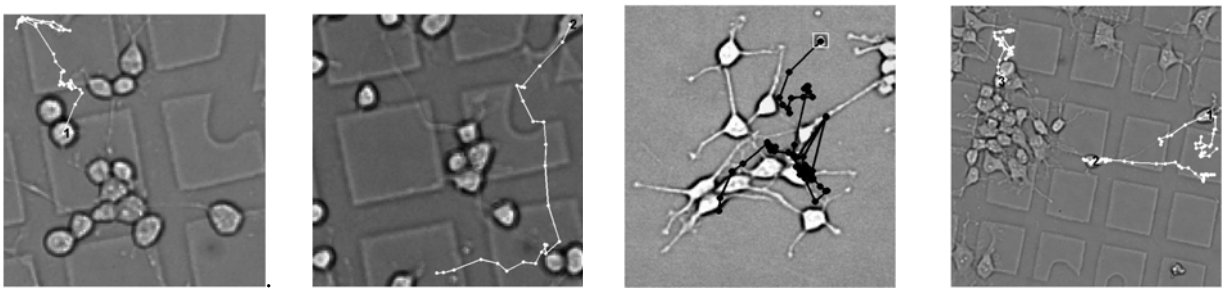
In Fig. 3 (a) ~ (e), it shows 1, 10, 20, 40 and 60 frames in image sequence III respectively, and the corresponding segmentation images. To active cell 1, it has moved out of the image in the 31st frame. So we can track it from the 1st frame and the 30th frame. It costs 17.125 seconds. For the active cell 2, we can track it from the 1st frame and the 50th frame. It costs 27.016 seconds. For the active cell 3, we can track it from the 9th frame and the 50th frame. It costs 22.563 seconds.

In Fig. 4(a), it shows the trajectory of active cell 1 in image sequence I which is labeled in black "1" to represent its initial position in the first frame. "." represents its centroid. "-" represents its tracking trajectory. below is same. In Fig. 4 (b), it shows the trajectory of active cell 2 in the same sequence which is labeled in black "2" to represent its position in the 46th frame. In Fig. 4(c), it is the active cell's tracking trajectory in image sequence II. We use a white square to represent the cells' starting position in the first frame. In Fig. 4(d), it is the three active cells' trajectories in image sequence III. We use black "1", "2" and "3" to label the cells' starting positions in the 9th frame respectively.



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

Fig. 3 Segmentation of frame 1、10、20、40 and 60 in sequence III



(a) active cell 1 in I (b) active cell 2 in I (c) the active cell in II (d) the active cell in III

Fig. 4 Cells' trajectory in three image sequences

5 Conclusions

The curves shown in Fig. 5 are the best estimate in x and y directions of the active cells 1 and 2 in image sequence I via Kalman filters which are compared with the actual observation values of the two cells. Fig. 6 shows the two pairs of comparison curves of active cells in the image sequence II. Fig. 7 shows the three pairs of curves of the active cell 1、2 and 3 in image sequence III. From Fig. 5-7, we can see that the differences between the best estimate values and the actual observed values are less than 10 pixels both in x and y directions, which satisfies the local search criteria.

Aimed at tracking the active cells in confocal microscopy image sequences, this paper has proposed a tracking algorithm based on active cells' characteristics via Kalman filter. The cost function may reflect individual differences among the active cells. The results show that Kalman filter can track the active cells if we preset some appropriate parameters in cost function.

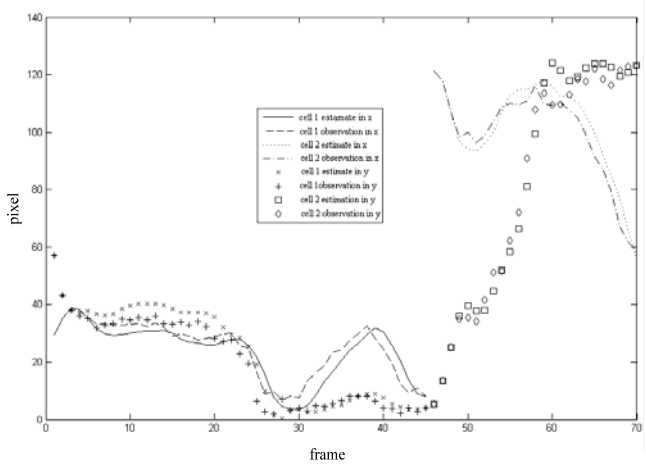


Fig. 5 Kalman filter estimating curve and the observing curve in image sequence I

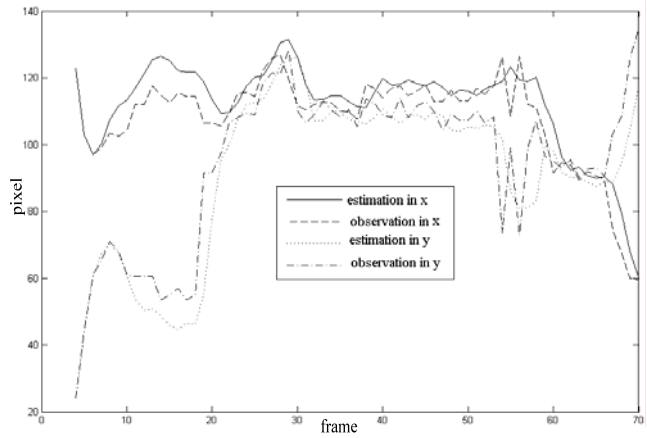


Fig. 6 Kalman filter estimating curve and the observing curve in image sequence II

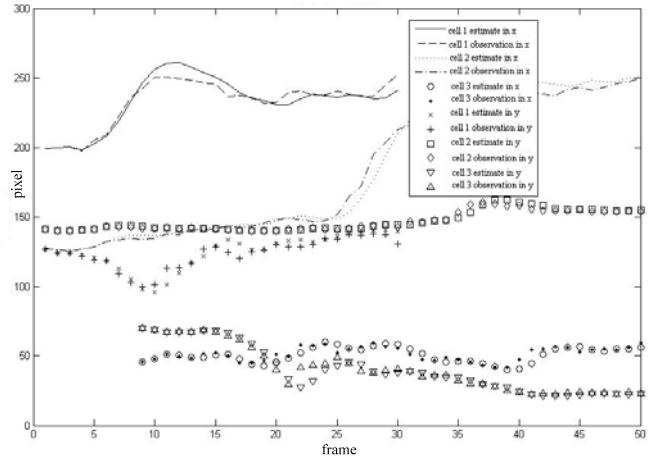


Fig. 7 Kalman filter estimating curve and the observing curve

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