A Model-Based Hematopoietic Stem Cell Tracker

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Abstract. A better understanding of cell behavior is very important in drug and disease research. Cell size, shape, and motility may play a key role in stem-cell specialization or cancer development. However the traditional method of inferring these values manually is such an onerous task that automated methods of cell tracking and segmentation are in high demand. Image cytometry is a practical approach to measure and extract cell properties from large volumes of microscopic cell images. As an important application of image cytometry, this paper presents a probabilistic model based cell tracking method to locate and associate HSCs in phase contrast microscopic images. The proposed cell tracker has been successfully applied to track HSCs based on the most probable identified cell locations and probabilistic data association.

1 Introduction

One of the most important and common tasks for biomedical researchers is cell tracking, which continues to be undertaken manually. Researchers visually perform cell motion analysis and observe cell movement or changes in cell shape for hours to discover when, where and how fast a cell moves, splits or dies. This task is tedious and painful due to the often corrupted or blurred images, the presence of clutter, fixing eyes for a long time, and repeating the same task for different cell types. Furthermore, with the extent of cell imaging data ever increasing, manual tracking becomes progressively impractical. As a result, automated cell tracking systems are mandatory to further advance the study of biological cells. Such a tracking system will require automatic object tracking, usually under challenging conditions

A variety of semi-automatic or automatic methods have been proposed to segment cell boundaries [1,2,3]. These methods include thresholding, watershed, nearest neighborhood graphs, mean shift procedure and deformable models. Overall, these can be divided into three major categories: *boundary based*, *region based*, and *thresholding*.

Markiewicz et al [4] have used watershed for the segmentation of bone marrow cells. Thresholding methods have been used by Wu [5] and Glasbey [6]. Different

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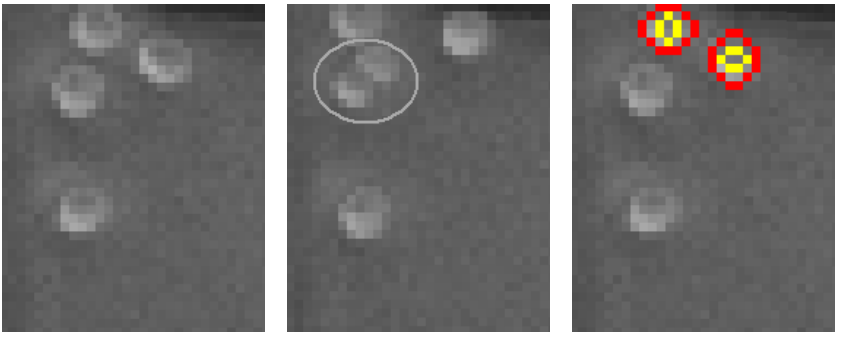

(a) (b) (c)

Fig. 1. (a) Phase contrast microscope image. (b) A mature cell is splitting. (c) Cell model superimposed on original HSC image.

techniques have been used for choosing a suitable threshold, such as calculating the image variance to separate the cell from the background [5], assuming the intensity of the background to be uniform with a low variance while cell intensity variance is high. Comaniciu et al [7] proposed a mean shift procedure method for cell image segmentation for diagnostic pathology. Geusebroek et al [8] introduced a method based on Nearest Neighbor Graphs to segment the cell clusters. Meas-Yedid et al [9] proposed a method to quantify the deformation of cells using snakes.

Due to the large number of cell types having different features such as shape, size, motility, and proliferation rate, designing a universal cell tracking system is impractical. In this paper, we focus on Hematopoietic Stem Cells (HSCs), which proliferate and differentiate to different blood cell types continuously during their lifetime, and are of substantial interest in gene therapy, cancer, and stem-cell research. A novel cell tracking system is developed based on a probabilistic cell model which effectively detect cells and a joint probabilistic data association which associate detected cells over time.

2 Proposed Model-Based Cell Tracking

As a crucial step towards fully automatic cell tracking, an effective cell localization/segmentation method is needed. To keep cells alive and dynamically active, light exposure must be controlled during their life cycle. The limited light exposure and cell transparency both contribute to the very low contrast, moreover most of the cell staining techniques which are used to increase the contrast between cell areas and background undesirably stain different parts of a tissue unevenly, causing inhomogeneity. Fortunately the HSCs in our study have a fairly regular shape and brightness pattern. Hence, a segmentation method which exploits this useful information should be able to perform better than simple thresholding.

2.1 Cell Image Model

HSCs must be prepared befor imaging. HSC sample preparation is a two stage process:

1. Extract and process mouse bone marrow.
2. Process and culture the HSCs.

After preparation HSCs are imaged using manual focusing through a 5X phase contrast objective using a digital camera (Sony XCD-900). Images are acquired every three minutes. When a cell division is observed, the progeny are imaged at higher magnification using a 40X DIC objective. A typical HSC phase contrast image is depicted in Fig. 1.

From Fig. 1 we observe that HSCs can be characterized as an approximately circular object with a dark interior and a bright boundary. During splitting, a mature cell is divided to give birth to two new cells, as marked by a circle in Fig. 1(b). The radius of these new cells is slightly smaller than that of their parent. The phase contrast imaging technique leads to an asymmetric cell boundary, one side dark and the other side bright. So rather than a heuristic thresholding approach, the specific, consistent cell attributes observed should allow us to formulate a far more specific model, essentially a matched filter, to be more robust to noise and low contrast. We propose to consider the following criteria: *Cell size*, *Boundary brightness*, *Interior brightness*, and *Boundary uniformity or symmetry*. These criteria are combined to formulate the probability of a cell in image I_k at location (x_c, y_c) and radius r

$$P(x_c, y_c, r|I_k) = P_{cb}(\bar{B}(x_c, y_c, r)) \cdot P_{ic}(\bar{B}(x_c, y_c, \frac{r}{2})) \cdot P_{cdf}(D(cdf(B))) \quad (1)$$

where the meanings of the individual terms P_{cb}, P_{ic} and P_{cdf} will be elaborated in the following sections.

2.2 Probability of Cell Boundary P_{cb}

As depicted in Fig. 1(c), to model a dark region surrounded by a bright boundary, the proposed cell model consists of two concentric circles, with the radius of the internal circle being half of that of the external one. The external circle represents the bright boundary while the internal one represents the dark region inside a cell. Assuming (x_c, y_c) and r as center coordinates and radius of the exterior circle respectively, the continuous circle is discretized spatially as

$$|(x_i - x_c)^2 + (y_i - y_c)^2 - r^2| \leq \epsilon^2, \quad (2)$$

where (x_i, y_i) are coordinates of circle boundary pixels and ϵ is half a pixel. Function $B_i(x_c, y_c, r)$, which is a vector returning the intensity of all boundary pixels, is defined as

$$B_i(x_c, y_c, r) = \{I(x_i, y_i),\ |(x_i - x_c)^2 + (y_i - y_c)^2 - r^2| \leq \epsilon^2 \text{ and } i = 1, 2, ..., N\}, \quad (3)$$

where N is the total number of pixels located on the cell boundary. In our implementation, a rotation angle of 20° is adopted, and the total number of boundary pixels (N) is equal to 18. The probability of cell boundary P_{cb} is assumed to be Gaussian with mean μ_{cb} and variance σ^2_{cb}

$$P_{cb}(\bar{B}(x_c, y_c, r)) \sim N(\mu_{cb}, \sigma^2_{cb}), \tag{4}$$

where $\bar{B}(x_c, y_c, r)$ is the average cell boundary intensity

$$\bar{B}(x_c, y_c, r) = \frac{\sum_{i=1}^{N} B_i(x_c, y_c, r)}{N}. \tag{5}$$

2.3 Probability of Cell Interior P_{ic}

The interior dark region of a cell is represented by the internal circle in the proposed model. Assuming (x_c, y_c) and $\frac{r}{2}$ as center coordinates and radius of the interior circle, it is discretized as

$$|(x_i - x_c)^2 + (y_i - y_c)^2 - \frac{r}{2}^2| \leq \epsilon^2. \tag{6}$$

The probability of dark region inside the cell P_{ic} is assumed to be another Gaussian distribution with mean μ_{ic} and variance σ^2_{ic}

$$P_{ic}(\bar{B}(x_c, y_c, \frac{r}{2})) \sim N(\mu_{ic}, \sigma^2_{ic}), \tag{7}$$

where $\bar{B}(x_c, y_c, \frac{r}{2})$ is the average intensity of cell interior region.

2.4 Probability of Uniformity of Cell Boundary P_{cdf}

Despite having an asymmetric boundary, both dark and bright sides of the cell boundary maintain almost uniform intensities. To maximize the likelihood of cell detection, an empirical cumulative density function (CDF) is calculated to discriminate uniform background from the cell boundary. The CDF on cell boundary pixel intensities is computed by

$$cdf_n(\bar{B}(x_c, y_c, r)) = \frac{\sum_{i=1}^{n} B_i(x_c, y_c, r)}{N \cdot \bar{B}(x_c, y_c, r)}, \ n \in 1:N \tag{8}$$

A distance function $D(cdf)$ is defined to find the maximum non uniformity of cell boundary, i.e., the maximum cumulative distance of cell boundary intensities from local mean:

$$D(cdf) = \underset{n \in [1:N]}{max} |cdf_n - \frac{n}{N}| \tag{9}$$

An exponential function $P_{cdf}(D)$ is used to penalize the non uniformity in cell boundary:

$$P_{cdf}(D) = \exp\{-2 \cdot N \cdot D(cdf)\} \tag{10}$$

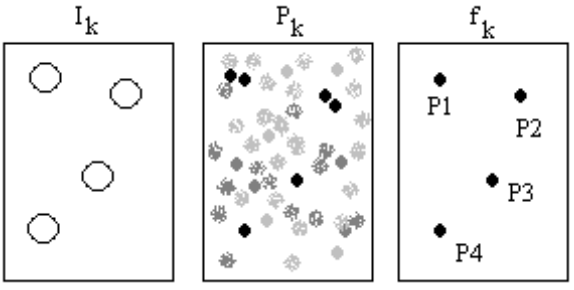

Fig. 2. Measurement hypothesis for frame k which is generated based on image frame k (I_k) by thresholding the local maxima map

2.5 HSC Tracking

A joint probabilistic data association (JPDA) method is proposed to solve the object tracking problem. A probability map is obtained by applying the cell model to the microscope image. The measurements are inferred from the input microscope image by finding the local maxima in the probability map and thresholding the local maxima map. As depicted in Fig. 2 the local maxima which are at least δD apart in the probability map P_k are located and the generated local maxima map is thresholded to obtain a set $S_{\tau h}$ of local maxima which are δD apart and are greater than a threshold τ_h

$$S_{\tau_h} = \begin{cases} \text{Location}: & x^h_{k,1},\ x^h_{k,2},\ ...,\ x^h_{k,q} \\ \text{Probability}: & P_k(x^h_{k,1}),\ P_k(x^h_{k,2}),\ ...,\ P_k(x^h_{k,q}) \end{cases}$$

This set is considered as HSC centre candidates. To track the HSCs over time, each detected cell in the current frame must be associated to the proper one in the previous frame as depicted in Fig. 3. To achieve this goal a distance matrix D is generated to determine the validation gate of each cell as

$$D = \{d_{j,i},\ j \in [1, M_{k-1}]\ and\ i \in [1, M_k]\} \tag{11}$$

where M_{k-1}, the number of rows is equal to the number of cells in previous frame $k-1$ and M_k, the number of columns is equal to the number of detected cells in the frame k. Each element $d_{j,i}$ shows the euclidian distance between detected cell j in frame k and identified cell i in frame $k-1$.

The displacement of HSCs over time can be considered as a random walk, hence a probabilistic validation gate is obtained by considering a Gaussian motion. A Gaussian function $N(0, \sigma)$

$$Pd_{j,i} \sim N(0, \sigma) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{d^2_{j,i}}{\sigma^2}\right) \tag{12}$$

is applied on the distance matrix to obtain a Gaussian probability distance matrix. Each element $P_{j,i}$ of this matrix shows the probability of associating

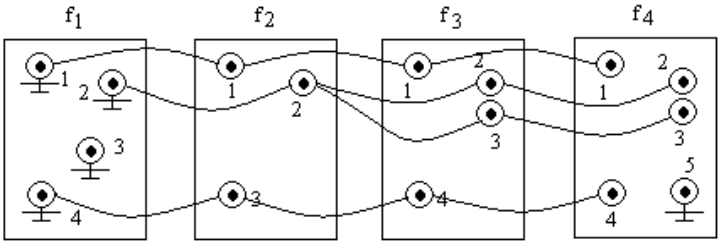

Fig. 3. Cell centre association over time

detected cell j in frame k to identified cell i in frame $k-1$ and is higher if they have smaller distance.

To associate the cell centers, a zero scan joint probabilistic data association is considered as

$$P(f_k|\ f_{k-1}) = \prod_{j\in[1,M_{k-1}]} max(P_{j,i}, P_F) \cdot \prod_{N} max(P_{j,i}, P_F) \cdot \prod_{\sim F\cap N} P_{age} \quad (13)$$

where P_F is probability of false alarm and is assumed as a constant, P_{age} is age penalty which means cell split may not occur sooner than a minimum age, $S = \sim F \cap N$ is the set of splitted cells, F and N are

$$\begin{aligned} F\ &: \{i|\ i \in \{False\ alarm\}\} \\ N\ &: \{i|\ i \in \{New\ detected\ objects\}\} \end{aligned} \quad (14)$$

3 Results

By applying our proposed model based tracking method to the phase contrast microscopic images, first a probability map of cell centers is obtained for each frame. To further identify the cell centers, the probability map is thresholded and local maxima are located. Eventually the detected cell centers are associated based on proposed JPDA method.

Fig. 4(a) shows the detected cell centers which is obtained by applying the proposed probabilistic cell model, locating the local maxima in the probability map and thresholding the local maxima map. Results obtained by applying the proposed tracking method are depicted in Fig. 4(b). As can be observed from Fig. 4(b), by applying our probabilistic model based tracking to depicted HSC image sequence, it is able to identify and associate all cell centers correctly not only in the non splitting case but also in the more challenging splitting case. Color coding is used to high light associated cell centers such that different colors show the association of cell centers over time.

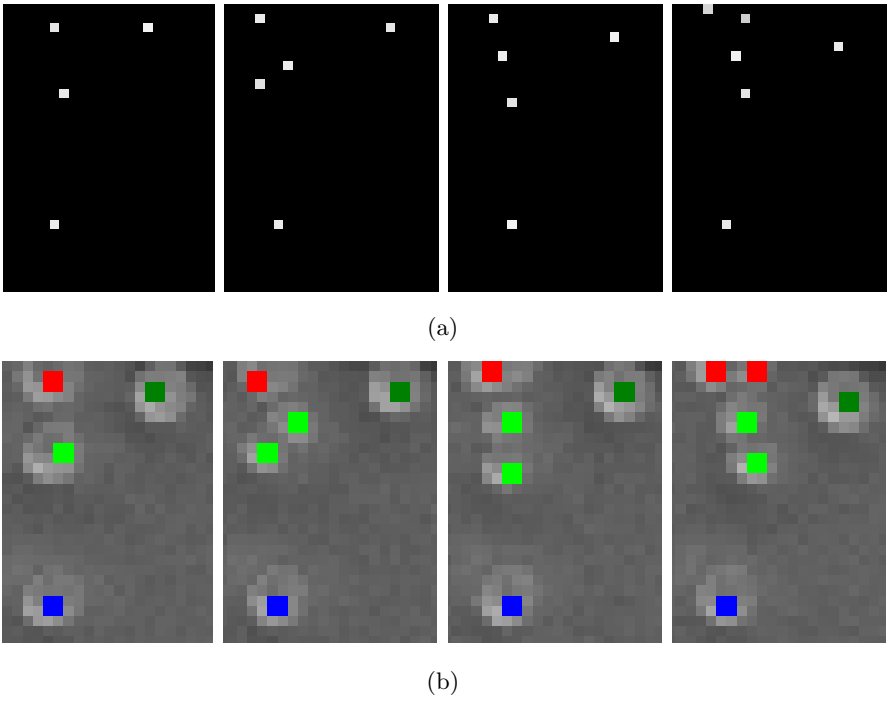

Fig. 4. (a) Detection of non splitting and splitting cell centers. (b) Associated cell centers superimposed on original HSC image such that each color shows a different cell track over time (from left to right).

4 Conclusions and Discussions

The fields of bioinformatics and biotechnology rely on the collection, processing and analysis of a huge number of bio-cellular images, including cell features such as cell size, shape, and motility. This paper presents a probabilistic model based cell tracking method to locate and associate HSCs in phase contrast microscopic images. Our statistical cell model, which is constructed after carefully observing HSCs in typical image sequences, captures the key properties of HSCs. By matching the image data with the cell model, a probability map of cell centers is generated for each frame. Cell centers are located by further thresholding the probability map and locating the local maxima. Cell association is accomplished based on a joint probabilistic data association in which random walk is considered to model the cell motion. It can be seen from the previous section that such a probabilistic model- based cell tracking method has produced very promising results.

Our future work includes further improving the cell model to more accurately reflect unique properties of the cells under different conditions and to fuse information from adjacent image frames to make the method more robust to noise

and clutters. Designing a parametric cell shape with more degrees of freedom has also been considered as future work to adapt the proposed model to other cell types.

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