A PROBABILISTIC LIVING CELL SEGMENTATION MODEL

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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 from image sequences manually is such an onerous task that automated methods of cell tracking and segmentation are in high demanded, especially given the increasing amount of cell data being collected. In this paper, a novel probabilistic cell model is designed to segment the individual Hematopoietic Stem Cells (HSCs) extracted from mice bone marrow cells. The proposed cell model has been successfully applied to HSC segmentation, identifying the most probable cell locations in the image on the basis of cell brightness and morphology.

1. INTRODUCTION

The field of bioinformatics and biotechnology is growing rapidly and relies more and more on advances of software and hardware computer technology to collect, process and analyze ever-increasing amount of data. More closely related to the community of image and video researchers, digital cytometry [1] has been recently introduced to adapt and extend image processing techniques to analyze and extract cell properties from microscopic cell images. By applying advanced techniques in digital image processing and pattern recognition to a huge number of bio-cellular images, digital cytometry can improve our understanding of cellular and inter-cellular events so that significant progress and new discoveries in biological and medical research may be achieved.

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 it moves, splits or dies. This task is tedious and painful due to the often corrupted or blurred images, the presence of clutters, 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 a computer to perform automatic object tracking, usually under challenging conditions, which also presents a very attractive yet difficult research problem for researchers in computer vision and digital image processing. 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. As a crucial step towards fully automatic cell tracking, an effective cell localization/segmentation method is developed based on a novel probabilistic cell model that incorporates key properties of HSCs.

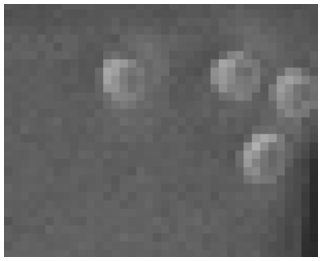

Fig. 1. Phase Contrast Microscopic Image.

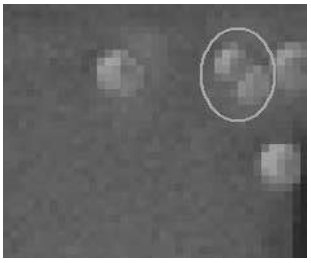

Fig. 2. A mature cell dividing into two new cells.

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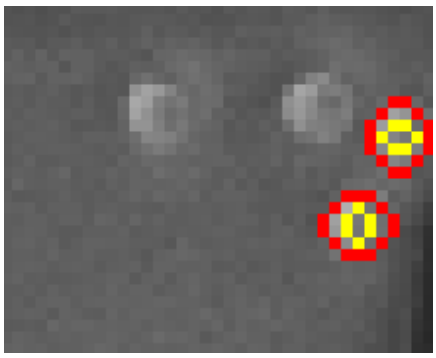


Fig. 3. Cell model superimposed on original HSC image.

2. BACKGROUND

A variety of semi-automatic or automatic methods have been proposed to segment cell boundaries [2, 3, 4]. These methods include thresholding, watershed, nearest neighborhood graphs, mean shift procedure and deformable models, which can be divided into three major categories:

1. Boundary based, which generally employ deformable models, such as snakes to find the cell boundaries.
2. Region based, such as split & merge, morphological operators, watershed and region growing methods.
3. Thresholding, in which the cell classification is achieved based on a threshold which is applied to some extracted image feature.

Thresholding methods have been used by Otsu [5] and Wu [6]. Different techniques have been used for choosing a suitable threshold, such as calculating the image variance to separate the cell from the background [6], assuming the intensity of the background to be uniform with a low variance while cell intensity variance is high. Markiewicz et al [7] have used watershed for segmentation of the bone marrow cells. Comaniciu et al [8] proposed a mean shift procedure method for cell image segmentation for diagnostic pathology. Geusebroek et al [9] introduced a method based on Nearest Neighbor Graphs to segment the cell clusters. Meas-Yedid et al [10] proposed a method to quantify the deformation of cells using snakes.

3. MATERIALS

HSC samples must be extracted and processed before imaging. HSC sample preparation is a two stage process:

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

The cells were imaged using manual focusing through a 5X phase contrast objective using a digital camera (Sony XCD-900) connected to a PC computer by a 1394 connector. Images were acquired every three minutes. When a cell division was observed, the progeny were imaged at higher magnification using a 40X DIC objective.

4. METHODS

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 of typical microscopic cell images. 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 fairly regular shape and brightness patterns. Hence, a segmentation method which exploit these useful information should be able to perform better than simple thresholding methods.

Looking at microscope images in Figs. 1 and 2, we can 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. 2. The radius of these new cells is slightly smaller than that of their parent. Although HSCs can be identified by a bright boundary, 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. The model considers Cell size, Boundary brightness, Interior brightness, and Boundary uniformity (symmetry). These criteria are combined to formulate the following probabilistic cell model

$$\begin{aligned} P(I_k|x_c,y_c,r) = P_{cb}(\bar{B}(x_c,y_c,r)) \cdot \\ P_{ic}(\bar{B}(x_c,y_c,\frac{r}{2})) \cdot \\ P_{cdf}(D(cdf_{n\in 1:N}(B(x_c,y_c,r)))) \end{aligned} \quad (1)$$

as a function of cell center locations (x_c, y_c) and radius r, and the meaning of the individual terms P_{cb}, P_{ic} and P_{cdf} will be elaborated in the following sections. The probability density functions and their parameters are obtained by close investigation of ground truth results. Ground truth have been produced by manual cell segmentation and extracting the cell boundary and interior intensities on several image frames of different image sequences.

4.1. Probability of Cell Boundary P_{cb}

As depicted in Fig. 3, 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

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

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

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

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 μ_{cb} and σ^2_{cb} are estimated over several frames by experiment and $\bar{B}(x_c, y_c, r)$ is the average cell boundary intensity

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

4.2. 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 μ_{ic} and σ^2_{ic} are estimated over several frames by experiment and $\bar{B}(x_c, y_c, \frac{r}{2})$ is the average intensity of cell interior region.

4.3. 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

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

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:

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

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

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

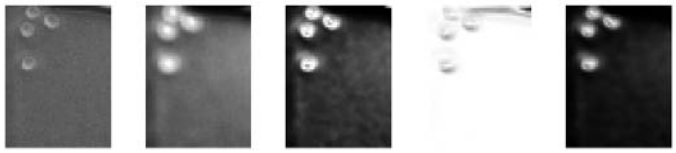
5. RESULTS

By applying our proposed probabilistic cell model (1) to the phase contrast microscopic images, a probability map of cell centers is obtained for each frame, which characterize the HSCs having circular shape with an average radius of about 2.5 pixels. In the generated probability map, to further identify the cell centers, the probability map is thresholded and local maxima are located.

Fig. 4(a) and (b) show the original cell images and the results obtained by applying the proposed model. The non splitting mature HSCs are depicted in Fig. 4(a), while Fig. 4(b) shows a splitting case. The identified HSC centers after thresholding the probability map and locating local maxima are depicted in Fig. 4(c). As can be observed from Fig. 4(c), by applying our probabilistic cell model to two typical HSC images, it is able to identify all cell centers correctly not only in the non splitting case but also in the more challenging splitting case.

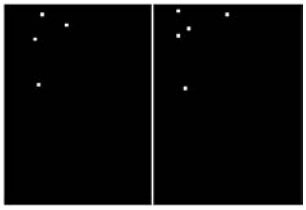
6. CONCLUSIONS AND DISCUSSIONS

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 cell segmentation method to locate HSCs in phase contrast microscopic images, which is the main stage of a fully automatic cell tracking system. 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



(a) Mature HSCs

(b) Splitting HSC



(c) Located cell centers

Fig. 4. From left to right: (a) and (b); original image, probability of the cell boundary, probability of the inside cell, probability of the uniformity of the cell boundary and the probability map, i.e., the product of all three probabilities. (c)Located non splitting and splitting cell centers.

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. It can be seen from the previous section that such a probabilistic cell segmentation method has produced very promising results.

However, our current method is not without limitations, e.g., the final result is somewhat sensitive to the threshold used in processing the probability map, especially when cells are splitting since at that time they don't have a fixed circular shape. 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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