Quantitative characterization for lymphocyte deformation in image sequences*

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Abstract - A new method is proposed to quantitatively characterize lymphocyte deformation in image sequences. The whole framework includes three steps：cell segmentation, cell tracking, and cell deformation characterization. Firstly, morphological image processing is implemented as a kind of segmentation method to detect cell region. Then, method based on Fourier descriptors is presented to optimize cell contour, which makes cell contour smooth and closer to real contour. Secondly, gradient vector flow (GVF) Snake algorithm is carried out for tracking cell region in image sequences. Finally, the part of cell deformation characterization is accomplished by improved symmetry difference for cell region. Two classes of lymphocyte video database provided by the cooperation hospital, Beijing You'an hospital, are used to verify the proposed approach. Experimental results show that it can quantitatively characterize lymphocyte and produce reasonably good agreement with subjective judgment by experts.

Index Terms - Cell deformation characterization, image sequences analysis, cell image analysis, Fourier descriptors, quantitative characterization.

1. Introduction

Cell is the most basic structural and functional unit of living beings, all organisms are composed of cells. While a live-cell contains tremendous valuable information in vivo, quantitative characterization of cell deformation, cell motility, and cell movement are important to understand the basic physiological processes such as wound healing, tissue repair, differentiation, metastatic potential, chemotaxis and so on [1-2]. In addition, deformation and locomotion of lymphocytes are two essential features of the immune system; therefore measurement of the deformed and locomotor behavior of a lymphocyte is part of its functional analysis [3]. As a result, cell analysis has become one of the most important branches of biomedical field, especially single lymphocyte analysis.

Microscope image processing, an assisting method of clinical information acquisition, has played an important role in facilitating the appropriate diagnosis and treatment against some serious diseases such as cancer, leukemia etc. During the past several years, research on cell image analysis on the basis of static image has attracted significant attention, such as cell segmentation, cell counting, cell classification and so on [4]. On the other side, valuable information obtained from image sequences also plays a significant role in aided diagnosis as it can provide more information than cell shape and cell number in a static image, such as cell deformation, cell motility, and cell movement. For this reason, there has been considerable interest in cell dynamic behavior analysis in cell image sequences [1,3,5-10]. Hendrik et al. studied two methods for the measurement of shape changes in microscopic images of lymphoid cells [3]. Florence Gemain et al. dealt with the spatio-temporal analysis of two-dimensional deformation and motion of cell based on an affine model [1]. Xavier Ronot et al. proposed an approach for quantification of cell motility based on an optical flow method [5]. Cyrus A. Wilson et al. presented a noniterative image cross-correlation approach to track translation and rotation of crawling cells in time-lapse video microscopy sequences [6]. In one word, digital image analysis appears to be attractive tool for characterizing cell deformation, cell motility, and cell movement. Therefore, this paper focuses on the method of quantitative characterization of lymphocyte deformation in image sequences.

In the field of computer vision, cell image analysis is considered as a difficult problem for the following reasons. First, to make sense of the information-rich image sequences by reducing them to simple parameters is not always exact enough to catch the biologically relevant phenomena under investigation. Second, live-cell is a non-rigid body; it makes the classical methods, optimized for rigid transform, unsuitable. Usually, a shape is represented by its bounding contour, or its interior region. Earlier paper discussed quantitative analysis for cellular morphological change based on cell contour [7], while it is found that changes of cell region gives the most direct impression to people too. For binary image after segmentation, cell region in different frames can be assumed as sets of points, which contain the coordinates of cell region pixel points. Therefore, symmetric difference of these sets can be used to analyze cell deformation in image sequences. It is proved to be a reliable parameter to characterize cell deformation, and experimental results show reasonably good agreement with human direct visual evaluation. The whole framework for quantitative characterization of lymphocyte deformation is divided into three steps: cell segmentation, cell tracking, and cell deformation characterization.

2. Cell Segmentation

A. Cell detection

In general, image analysis begins with a segmentation

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process for the purpose of detecting regions of interest. Therefore, cell segmentation is the first step of quantitative characterization for lymphocyte deformation, which aims to detect cell region from image sequences. As indicated above, quantitative characterization of lymphocyte deformation contains three steps; a flow diagram can be seen from Fig. 1(a). Besides, major steps of cell segmentation used in this paper are illustrated in Fig. 1(b). Firstly, morphological grayscale reconstruction, as a simplification step, is used to remove noise and other information that contribute little to subsequent processes. With this operation, region of cell can be extracted fast and effectively. After the procedure of grayscale reconstruction (as shown in Fig. 2(b)), the next problem is to select an optimal threshold for image binarization. As can be seen from the intensity histogram (illustrated in Fig. 2(c)), the largest intensity distribution corresponds to background, therefore the second largest one is selected as optimal threshold, which corresponds to lymphocyte. The binarization result is given in Fig. 2(d). Finally, algorithms of removing small regions and filling holes are implemented to complete the segmentation procedure (as shown in Fig. 2(e)).

B. Cell contour optimization

Cell segmentation is first step of cell image analysis as well as the most important step, since accurate cell contour plays a significant role in subsequent processes. Because coordinates of every pixel in digital image are discrete, coordinates of boundary obtained by segmentation method are discrete too. Though the lymphocyte can be detected exactly; cell contour is not accurate enough.

Fourier descriptor is usually used to describe and recognize object, and its basic idea is to describe shape boundary in terms of its spatial frequency content [11]. A boundary can be represented as the sequence of coordinates $z(n)=[x(n),y(n)]$, for $n=0,1,2\ldots,$N-1. Furthermore, each coordinate pair can be treated as a complex number, so that

$$z(n)=x(n)+jy(n) \tag{1}$$

Although the interpretation of the sequence was recast, the nature of the boundary itself was not changed. Besides, it reduces a 2-D problem to 1-D.

The discrete Fourier transform (DFT) of $z(n)$ is

$$a(k)=\frac{1}{N}\sum_{n=0}^{N-1}z(n)e^{-j2\pi kn/N} \tag{2}$$

for $k=0,1,2\ldots,$N-1. These complex coefficients $a(k)$ are called Fourier descriptors of the boundary. The inverse Fourier transform of these coefficients can restore $z(n)$. That is,

$$z(n)=\sum_{k=0}^{N-1}a(k)e^{j2\pi kn/N} \tag{3}$$

for $n=0,1,2\ldots,$N-1.

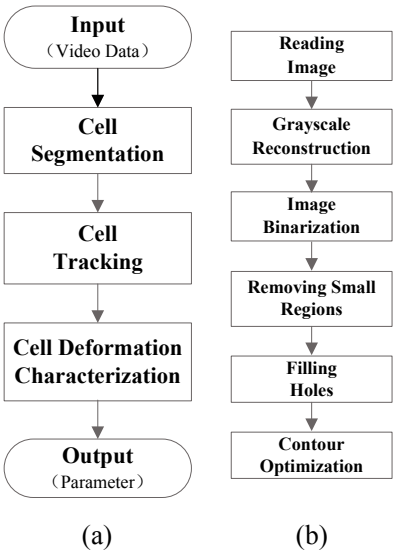


Fig. 1 Procedure of cell deformation characterization. (a) Main steps; (b) Steps of cell segmentation.

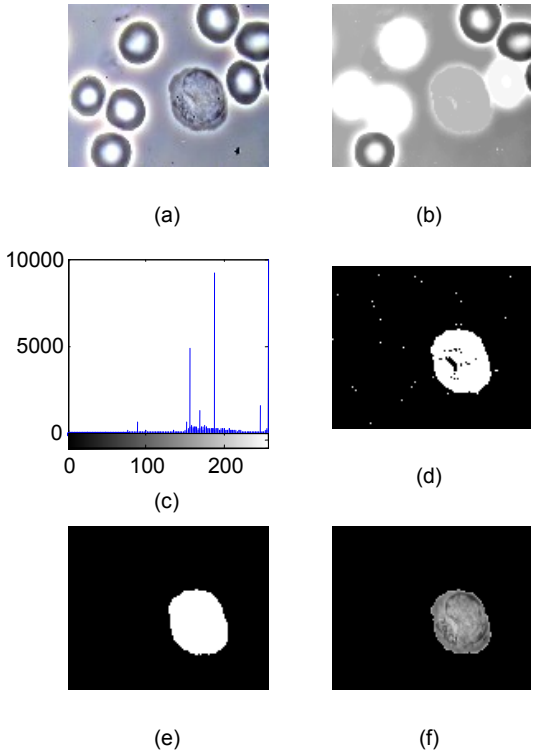


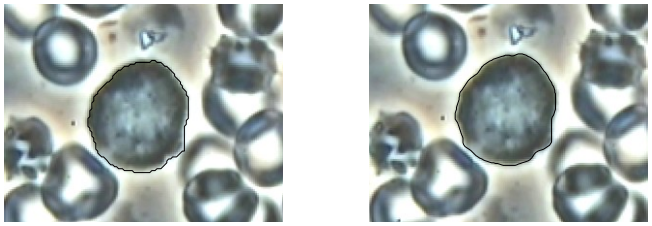
Fig. 2 An example of lymphocyte segmentation. (a) The original image, the biggest cell is lymphocyte, while the others are red blood cells; (b) result of grayscale reconstruction; (c) intensity histogram of (b); (d) result of binarization from (b); (e) result of removing small regions and filling holes from (d); (f) the final result of cell detection.

The Fourier transform of cell contour expands this in a Fourier series and obtains a set of coefficients that capture the shape information. According to the property of Fourier transform, high-frequency components account for fine detail, and low-frequency components determine the global shape. Therefore, if we use an appropriate number of coefficients to

restore $z(n)$, a smooth boundary can be got and the coordinates of boundary is continuous. In other words, setting some of the high-frequency components $a(k)=0$, and using the left coefficients to restore $z(n)$. This process is equivalent to use a low-pass filter to optimize Fourier descriptors. According to numerous experiments, 30% of the total numbers of the $a(k)$ can get a better result for cell images in our research. The approximation to $z(n)$ can be defined as follows:

$$z(n)=\sum_{k=0}^{P-1}a(k)e^{j2\pi kn/N} \quad (4)$$

for $n=0,1,2\ldots,$N-1, and $P=\lfloor 0.3N \rfloor$. Fig. 3 shows the comparison result of detection for lymphocyte before and after using Fourier descriptors. As can be seen from the picture, Fourier descriptors can get a better result that make cell contour smooth and more closer to real contour.



(a) (b)

Fig. 3 Result of cell contour before and after using Fourier descriptors.

3. Cell Tracking

When analyzing cell image sequences, cell segmentation is followed by cell tracking. In this paper, an improved active contour algorithm named gradient vector flow (GVF) Snake [12] is implemented for tracking lymphocyte. Active contour is an energy-minimizing spline guided by external constraint forces and influenced by image forces that pull it toward features such as lines and edges [13]. Traditional active contour has two major shortages. First, the initial contour needs to be close to true boundary; otherwise it may converge to a wrong result. Second, active contours have difficulties in progressing into boundary of concavities. GVF-Snake algorithm improves active contour through introducing a modified external force with gradient vector flow. Fig. 4 gives the result of cell tracking at different times.

4. Characterization of Lymphocyte Deformation

So far, because of rapid development of science technology, many methods with different techniques and devices have been used in cell analysis such as microscopy technique, patch clamp technique, flow cytometry, fluorescence analysis technique and so on [14]. All of these methods can retrieve vivo's physiological and pathological information based on different kinds of properties of one or more cells, whereas microscope has always been considered as the powerful tool for observation of cell. In fact, image sequences obtained by phase contrast microscope have been given sufficient attention for a long time [1-3, 6], since phase contrast microscope does not require staining to view the slide and makes it possible to study cell alive.

As indicated above, from the point of view of computer vision, cell image sequences analysis is a difficult problem. The first reason is that, it is not easy to make sense of information-rich image sequences by reducing them to simple parameters to capture the biologically relevant phenomena under investigation. Usually, object measurement parameters, such as area, perimeter, circularity, elongation are used to characterize single cell for static image. However, image sequences analysis with these parameters often fails to catch dynamic information of the object. The second reason is that classical methods are unsuitable. As we all know, traditional methods are optimized for rigid transform, while lymphocyte is a non-rigid body. Therefore, a suitable method with correct characterization parameters should be proposed for cell deformation characterization.

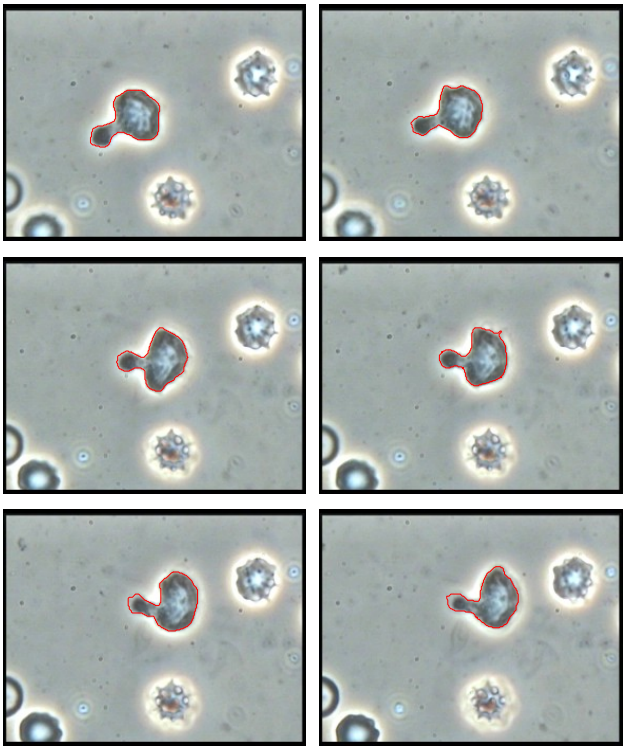


Fig. 4 Result of cell tracking at different times (1s, 8s, 25s, 28s, 33s, 38s).

During the process of cell deformation characterization, the serious problem after cell shape detecting is to represent it effectively. Shape is multifaceted, in that it involves a range of "dimensions". Several researchers have investigated that using bounding contour and interior region to represent shape [15]. Besides, in lymphocyte image sequences, changes of cell region and cell contour give the most direct impression to people. Earlier paper discussed quantitative analysis for cellular morphological change based on cell contour [7],

which is a kind of shape representation method. While in this paper, another shape representation method, cell region is used for cell deformation characterization.

Cell's motion always contains rotation, translation and deformation. We can ignore rotational motion, since lymphocyte scarcely rotate in these cell image sequences according to our observation. Besides, by taking the centroid of cell region as the origin of coordinates, translation motion does not need to be taken into consideration. In other words, it makes the centroid of cell region of different frames coincide, so translation can be ignored. For the binary image after segmentation, cell region in different frames can be assumed as sets of points, which contain the coordinates of cell region pixel points. Then, symmetric difference of these sets can be used to analyze cell deformation in image sequences after the above preprocess.

In mathematics, symmetric difference of two sets is the set of elements which are in one of the sets, but not in both. This operation is the set-theoretic kin of the exclusive disjunction (XOR operation) in Boolean logic [16]. The symmetric difference of sets A and B is commonly denoted by $A\Delta B$. It is the set of all x such that either $x \in A$ or $x \in B$ but not both. $A\Delta B$ is defined as:

$$A\Delta B := (A \cup B) \setminus (A \cap B) \tag{5}$$

In Fig. 5 a schematic diagram of symmetric difference of sets A and B is given. Obviously, we can assume that, set A is cell region in previous frame, set B is cell region in the later frame. According to the definition of symmetric difference, symmetric difference of sets A and B can characterize cell deformation after taking the centroid of cell region as the origin of coordinates. However, the point need to be emphasized is that cell is different in shape and in size. Therefore, cell deformation characterization is reasonable to normalize as a dimensionless parameter P_{SD}:

$$P_{SD} = \frac{A\Delta B}{A} \tag{6}$$

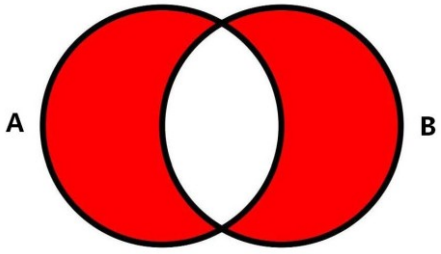


Fig. 5 Schematic diagram of symmetric difference of the sets A and B.

Furthermore, cell image sequences is an (2+1)-dimensional (space and time) data, hence time factor should be considered in cell image sequences analysis. Usually, cell deforms a lot within a period of time, and return to its original shape at the last fewer frames. In that case, it is not proper to calculate P_{SD} with the beginning and the ending frames for characterizing cell deformation. In fact, several frames at cell deforming time point should be picked up for image sequences analysis. In this paper, we use a fixed time interval $\Delta t = 1s$ for picking up frames (images for calculating P_{SD}) from image sequences. Furthermore, in order to make sense of the information-rich image sequences by reducing them to simple parameters, mean value of all the P_{SD} is characterized as the final result. This makes comparison of cell image sequences at different length feasible. Assume that, an image sequences has N checked frames, cell region in each frame is defined as C. n is the ordinal number, and $n+1$ frame is the image picked up after Δt in same image sequences. Then, cell deformation characterization parameter (mean value of P_{SD}) is defined as:

$$MP_{SD} = \left(\sum_{n=1}^{N-1} \frac{C_n \Delta C_{n+1}}{C_n}\right) / (N-1) \tag{7}$$

Moreover, P_{SD} value of two adjacent frames can be calculated by binary image of cell region. Fig. 6 shows binary image $b(i, j)$, which is the result of symmetric difference of two adjacent frames picked up at a time interval $\Delta t = 1s$. Assume that, the image size is L by K, SD value of two adjacent frames can be calculated as follows:

$$P_{SD} = \sum_{l=1}^{L} \sum_{k=1}^{K} b(l,k) \tag{8}$$

where $b(l,k) = \begin{cases} 1, & (l,k) \in SD \\ 0, & (l,k) \notin SD \end{cases}$.

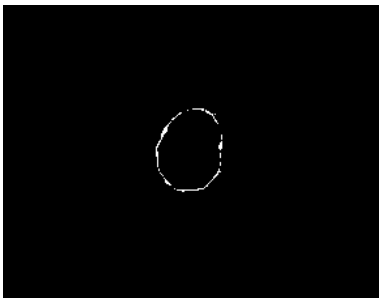


Fig. 6 Binary image b(l,k) result of symmetric difference of two adjacent frames.

As can be seen from Fig.6, SD region shows the difference of cell region between two adjacent frames. In fact, it is the area of cell deformation, and P_{SD} value is a reliable parameter to characterize it. A similar idea is proposed that area of the non-overlapping parts divided by the average cell area is used as a shape change factor [17]. However, this paper presents it from the point of view of set, and improves cell boundary based on Fourier descriptors. In addition, we propose the normalized value P_{SD} and mean value MP_{SD} for comparison of cell image sequences at different length. It is worth

emphasizing that choosing a better time interval Δt is needed to investigate according to image sequences, since deformation of cell occurs at random time. In this paper, based on numerous experiments, time interval Δt is fixed at 1s. While frames should be picked up at the time which cell begins deformation or cell finishes deformation, an unfixed time interval can make the cell deformation characterization parameter more accurate.

5. Experiments

To verify the efficiency of the proposed method, we conducted the experiment with 42 lymphocyte videos clips (image sequences) taken from our cooperation hospital---Beijing You'an Hospital. Materials： Clean healthy Balb/c male mice as host; clean healthy C57BL/6 male mice as donor, 6-8 weeks old, 20-22 g. Lymphocyte in peripheral blood, 7 days after the skin transplantation were observed. All observations were made using inverted optic phase contrast microscopy with magnification 16x1 000. These video clips are divided into two classes by experts according to the degree of cell deformation. Cell deformation of the first class is drastic, while the second is slight.

Fig. 7 illustrates the experimental results. It is observed that, the two class data are classified according to the MP_{SD} value. Values of drastic data are higher than the slight one. Furthermore, values of slight class are relatively stable, and values of drastic class are not, just as the experts observed. It is proved to be reliable parameter of cell deformation, and the experimental results show reasonably good agreement with human direct visual evaluation about the degree of cell deformation.

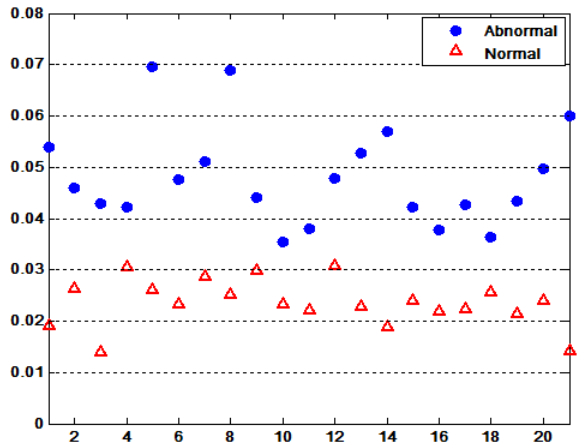


Fig. 7 Result of MSD for the two sets of data.

6. Conclusions

In this paper, we focus on quantitative characterization of lymphocyte deformation based on digital image processing. Morphological image processing is used to segment cell region, and Fourier descriptors based method is presented to optimize cell contour. And then cell tracking is carried out by GVF-Snake algorithm in image sequences. Cell deformation characterization is conducted with symmetry difference of cell region. Experimental results show that the proposed method can produce reasonably good agreement with direct visual evaluation by experts. Furthermore, this task has profound significance for further study of dynamic information of cell in image sequences. And it can facilitate the appropriate diagnosis and treatment against some serious diseases, because dynamic cell image can reflect the activity level of the captured cell, as well as the corresponding organism.

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