A Robust Multi-Orientation Gabor Based System for Discriminating Touching White and Red Cells in Microscopic Blood Image

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Abstract

In many image analysis systems, segmentation is the first step. So its accuracy impacts on whole system efficiency. In normal human blood microscopic image, which contains white and red blood cells, because of high accumulation of red cells, there exist touch and overlap between these cells. They are two difficult issues in image segmentation which common segmentation algorithms cannot overcome them. Red cell has fine texture while white cell has coarse one, so texture is a very good discriminating feature for segmentation of blood cells. Gabor filters are one of the most powerful methods for texture segmentation. Blood image don't have regular textures. Common Gabor filter banks are suitable for regular texture segmentation. So we have employed a Gabor filter bank including many orientations and few frequencies in combination with sigmoid function for blood image segmentation. Results show that our approach is efficient.

1. Introduction

There are three types of cells in normal human blood: red cells, white cells and blood platelets. Generally, red cells are simple and similar. While white cells contain nucleus and cytoplasm and there are different types of them. White cells are categorized into five groups: Neutrophil, Eosinophil, Basophil, Monocyte and Lymphocyte. Texture, color, size and morphology of nucleus and cytoplasm make differences between theses groups. In a blood smear, the number of red cells is many more than white cells. For example an image may contain up to 100 red cells and only 1 to 3 white cells. Platelets are small particles and are not clinically important.

In laboratories, hematologists analyze human blood by microscope. Their main tasks in this area are: red cell count, differential white cell count and blood disorder detection. Since this is done by humans, it is time consuming and risky. Due to the importance of these processes, an automated system seems necessary and helpful.

In many image analysis systems, segmentation is the first stage. The accuracy of this stage, affects the whole system performance. There are wide variety methods for segmentation. Some of them which has been applied to blood images in previous works are based on automated thresholding[1],[3],[5], unsupervised clustering[2], energy[4], morphology[6], neural network[7], fuzzy[8], model based systems[9].

Many of these methods are not capable segmenting images accurately if there exist touch and overlap between objects. On the other hand, due to the high accumulation of red cells in blood images, there are lots of touches and overlaps among red and white cells. So our focus in this paper is on cell segmentation in blood images in the presence of touch and overlap. There are some methods to resolve mentioned problems like watershed algorithm [10], [11] and Active contours [12], [13]. In this paper we focus on a texture based approach.

Blood Staining in laboratories is not done in a unique and standard way and different chemical materials are used for this purpose. Also microscope type and illumination condition are not the same. Consequently color is not a reliable feature for segmentation and a robust system cannot be constructed based on it. Additionally, in the grayscale image the intensities of red cell and white cell cytoplasm are very close together resulting in week segmentation.

Fig.1 is a sample image of a white blood cell which some red cells contact it. Since nucleus is the darkest part of the image, it can be determined via an adaptive histogram analysis. Thus the goal of white blood cell segmentation is to find boundaries between cytoplasm and red cells. The difference between cytoplasm texture and red cell texture is clearly shown in this figure. Cytoplasm and nucleus have coarse texture but red cell has a very fine texture to the extent that it can be considered it has no texture. Consequently, Texture can be employed to segment white and red cells. Segmentation based on texture in blood images results in a robust system cause in this way system is not dependent on intensity or color of pixels. As mentioned above, these values changes a lot due attention to staining method, microscope type and illumination conditions.

Texture has been used to determine white cell type. Sabino and his students calculated some textural attributes based on gray level cooccurrence matrices (GLCM) as energy, inertia and homogeneity in white blood cell recognition [14].

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Fig 1. A white blood cell with some contacting red cells.

Gabor filters are one of the most powerful methods for texture analysis. They are widely used in image segmentation area. For example, a research has been done to segment some types of medical images based on Gabor filter banks [15]. We have employed Gabor filter bank to segment white and red cells in blood images when they touch each other.

In section 2 we will introduce the Gabor filter briefly and in section 3 some Gabor based features which are useful in segmentation are discussed. In sections 4, segmentation of images based on these filters is considered. Results are outlined in Section 5.

2. Gabor Filter

The Gabor function is implemented as a multi-channel filter. It has properties that make it attractive for computer vision applications. These properties include its appealing simplicity, optimum joint spatial/spatial-frequency localization and ability to simulate the behavior of two-dimensional receptive fields of simple cells in visual cortex [16].

When generating texture features using multi-channel filters, the following two steps should be considered. First, the characterization of the filters must be selected. Second, feature extraction of the raw filter outputs should be performed to improve the feature set.

Spatially a Gabor filter is a Gaussian modulated sinusoid. The complex exponential has a spatial frequency of F and an orientation θ. A complex Gabor filter represented as a 2-d impulse response is:

$$h(x,y)=\exp\left\{-\frac{1}{2}\left[\frac{x^2}{\sigma_x^2}+\frac{y^2}{\sigma_y^2}\right]\right\}\exp\{j2\pi Fx\}$$

A circular Gaussian may be desired so that there is a constant spatial extent in all directions.

As mentioned, Gabor is a complex filter. But based on psychophysical grounds, some justification has been proved using real part of Gabor output is sufficient for texture recognition [17].

A Gabor filter bank is constructed by considering several orientations and frequencies. Then filters are being convolved with the original image and some filtered images are produced. These filtered images are fed to a clustering algorithm to segment the image. It is desired to extract some features based on filtered images to achieve better results. Some of these features are introduced in the next section.

3. Feature extracted from Gabor output

Different feature extraction methods can be applied to the Gabor filter outputs. Some of these features which are more common are described in this section [16].

- *Energy:* Texture identification can be performed based on the magnitude of the output of the Gabor functions. Energy of a complex value corresponds to its magnitude.
- *Sigmoid Function:* Jain and Farrokhnia has proposed this function as a nonlinear transform for texture segmentation [17]. This function is defined as follows:

$$\varphi(t)=\tanh(\alpha t)=\frac{1-e^{-2\alpha t}}{1+e^{-2\alpha t}}$$

They have used an empirical value of $\alpha=0.25$ which is a good choice in many images. Formally, the feature image corresponding to the filtered image is given by $\frac{1}{M^2}\sum_{(a,b)\in w_{x,y}} f(a,b)$ where w_{xy} is an $M\times M$ window centered at the pixel with coordinates (x,y).

- *Geometric moments:* Moments based on power spectrum Gabor filter responses can be used. It refers to the squared magnitude response as the local power spectrum. They are defined by

$$m_{pq}=\sum_{i,j}\omega_i^p\omega_j^q\left|g(\omega_i,\omega_j)\right|$$

where p and q are integers which represent the moment order.

- *Mean and standard deviation:* Calculating mean and standard deviation of Gabor outputs in the neighborhood of each pixel yields good features.

4. Blood Image Segmentation by Gabor Filter Bank

Our image dataset contains 30 microscopic images of 5 human normal blood smears. Totally, there are 3000 red cells and 40 white cells in it. Images are taken by an electronic microscope which is equipped with digital camera. Resolution of each image is 500*375 pixels. Image dataset is provided in cooperation with cancer research center of Tehran Imam Khomeini hospital. Fig.2 is a sample blood image.

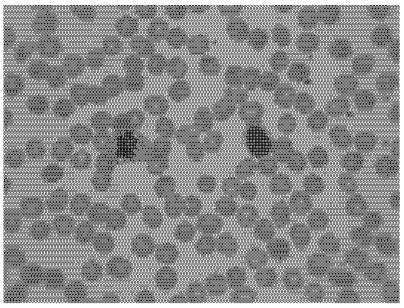
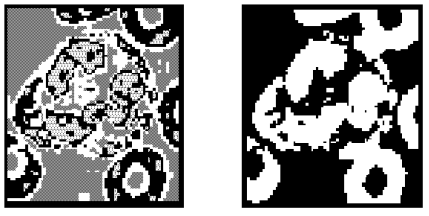


Fig 2. A microscopic blood smear Image

Since nucleus is the darkest part of image, a white cell can be localized by an adaptive histogram analysis. So we first localize white cell via its nucleus. Complete white cell image (with touched red cells) is cropped via a fix size window which is centered at nucleus. Some touched red cells maybe exist in the cropped image. An example of a cropped image is shown in Fig.1. We apply histogram equalization technique to improve contrast of image.

Cropped image sizes which contain white cells are nearly 70×80. According to the previous section, we consider four frequencies in our filter bank which are $\sqrt{2}, 2\sqrt{2}, 4\sqrt{2}, 8\sqrt{2}$ and six different orientations which are 30 degrees apart. Thus, our bank contains 24 filters. Real parts of filters are convolved with white cell image and 24 filtered images are achieved. These filtered images provide 24 dimensional feature vectors which are fed to a clustering algorithm to segment image. Kmeans algorithm is used because of its simplicity and efficiency.

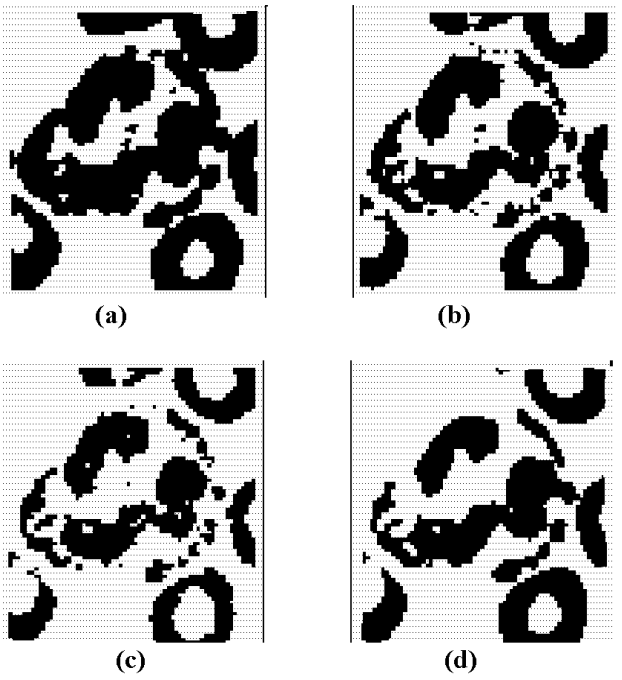
Number of clusters should be determined. Since there are four different objects with different textures in image which are nucleus and cytoplasm of white cell, red cell and background, four clusters were considered first. According to the results, this choice is not appropriate and results in scattered clusters. It seems that designed Gabor bank, tries to find edges between different textures instead of objects. So, we decrease number of clusters to two. Fig.3 illustrates the result of clustering Fig.1 by outputs of Gabor filters with four and two clusters.



(a) (b)

Fig 3. Segmentation of Fig.1 by direct outputs of 24 filter bank considering (a) 4 clusters (b) 2 clusters

The result of clustering Fig.1 by Gabor-based features which has been described in section 3 is illustrated below. Each pixel Neighbor is a 3×3 window. Also the moment order is 2. As it is shown, results which are outlined in Fig. 3 (b) and Fig. 4 are not very good. Especially boundaries between white cell and some red cells are not well-determined.



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

Fig 4. Segmentation of Fig.1 by extracted Gabor features (a)Energy (b)Sigmoid Function (c) Geometrical Moment (d) Mean and Standard Deviation

As mentioned before, a Gabor filter extracts texture features considering its orientation and frequency. If texture contains dominant orientation, very good results will be achieved. But in blood image, object textures don't have orientation as it is shown in Fig. 1. So if the number of orientations is increased, better results would be produced. We consider 12 orientations which are $\frac{\pi}{12}$ distant.

On the other hand, the number of frequencies is decreased to two since higher frequencies tend to extract details of the image which are near to pixel intensities. In fact, this is because of the irregular texture of blood image. Thus filter bank contains 24 filters (twelve orientations and two frequencies).

We test designed Gabor filter bank outputs and all five described features. Best result is achieved by sigmoid function which is shown in the following figure. Boundaries of cells are determined well by this feature. Red cells can be eliminated easily and white cell is segmented as an separated object.

Fig 5. Segmentation of Fig.1 by combination of new designed Gabor filter Bank and Sigmoid function

5. Results

We have applied the designed system on our image dataset. The results show that the performance of the designed system is very good. In the fig. 6, some results are illustrated. The whole white blood cell can be segmented after eliminating red cells which touch to image margins. The results were compared with those of applying features based on gray level cooccurrence matrices (GLCM). It is demonstrated that the applied Gabor based method is much better than the GLCM method.

6. References

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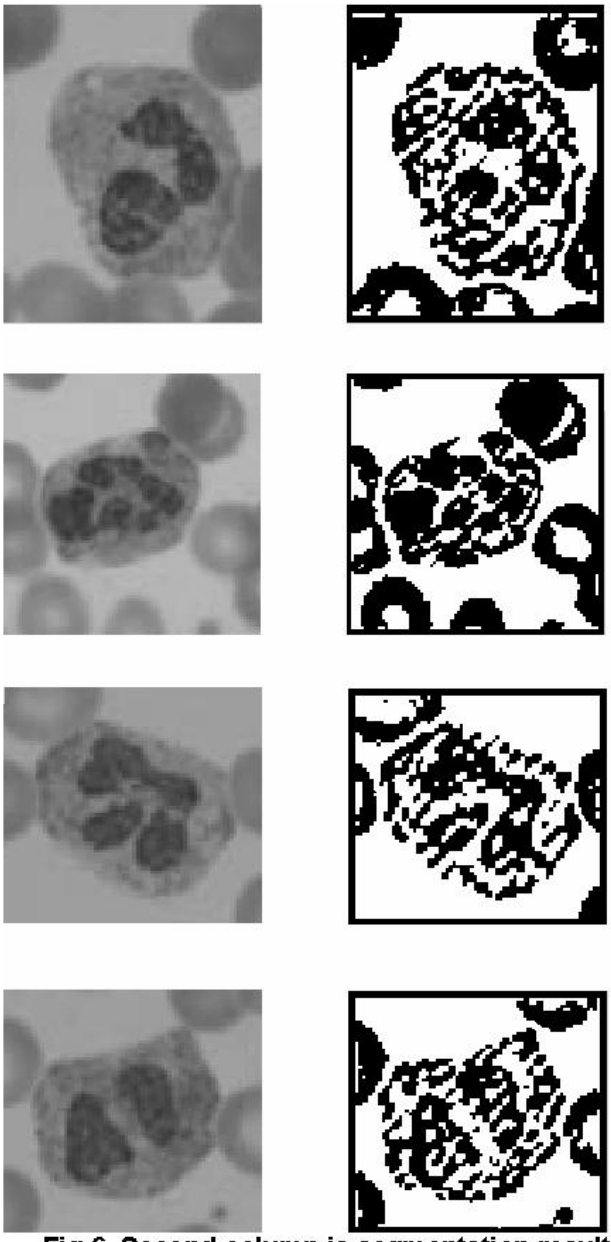

Fig 6. Second column is segmentation result of first column by designed Gabor filter bank.

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