BLOOD CELLS DETECTION FROM MICROSCOPIC IMAGES

Remus BRAD and Daniel VOLOVICI
Dept. of Computer Science, "Lucian Blaga" University of Sibiu,
B-dul Victoriei 10, 2400 Sibiu Romania
rbrad@jupiter.sibiu.ro, daniel@jupiter.sibiu.ro

Abstract: In many medical applications, computer vision is required to step behind the image pre-processing level and perform recognition tasks without human interaction. We focused our work on blood tests for leucocyte detection and counting. This test is generally performed manually and required for infection diagnosis. We developed a method for cells classification and counting using images from a laboratory microscope. This is a step forward for the automation of laboratory tests, producing good results without supervision and interaction. A camera calibration is required for the precise determination of the leucocyte formula.

Keywords: *image processing, recognition, counting, blood cells*

1. INTRODUCTION

Processing blood cells images is a practical application of computer vision in the field of medical imaging. The main objectives for this task are to recognize, to classify and to count the various types of cells being present in the image. The results are generally required by biologists or medical doctors performing blood tests, but we can also imagine other kind of applications. In these two particular cases, automation of the laboratory test is necessary for a precise and quick result. The algorithms described in this paper are suited for two types of applications: the first one towards classification and counting the red cells and leucocyte detection and counting for the second one. We focused our work in the second direction, because of the leak of technological support in hospital laboratories. By attaching a video camera to the microscope and a frame grabber to the computer, the process of leucocyte formula determination can be entirely computerized. Using a combination of image pre-processing, segmentation and recognition steps, we are able to decide about the presence of a certain type of cells and count them. Section 2 presents fundamentals of the preprocessing step required for microscopic images. Section 3 describes the unsupervised segmentation methods and section 4, the cell recognition step for classification and counting. In section 5, we present the leucocyte detection and in section 6, the experimental results.

2. IMAGE PREPROCESSING

The image preprocessing is common to both methods and consists of a gray scale transform from the RGB color image to 256 gray levels using the following formula (Parker 1994):

$$Grey = 0.222*Red + 0.707*Green + 0.071*Blue \quad (2.1)$$

and of a Gaussian noise reduction filter sized 3x3 and σ=2 standard deviation (Sonka et al. 1996):

$$G(x, y, \sigma) = \sigma^2 e^{-\frac{x^2+y^2}{2\sigma^2}} \quad (2.2)$$

Filtering is required to reduce the presence of noise, especially the one produced by the low performance CCD camera. The preprocessing step is followed by image segmentation. Due to the low complexity of the image and the real-time analysis, we have used a simple methods based on thresholding.

3. IMAGE SEGMENTATION

The optimal thresholding of the cell image is the essential key for the recognition step and results are directly dependent of the threshold selection. Therefore we have tested several thresholding methods for image segmentation and selected two of them. The first one, used in cells classification is based on the Otsu method (Otsu 1979); a nonparametric threshold selection for maximizing the resulting class separabilty. The Maximum Correlation Criterion (Yen et al. 1995) is the second unsupervised and nonparametric thresholding method tested and applied to the cells counting technique. We shortly introduce both of them.

3.1. The Otsu method

If the gray levels of a given picture are in the range of [1,2, ... ,*L*] then we can denote n_i the total number of pixels at level *i* and *N* the total number of pixels in the image (Otsu 1979, Ng 1996). This gray level histogram can be normalized and regarded as a probability distribution:

$$p_i = n_i / N, \quad p_i \geq 0, \sum_{i=1}^{L} p_i = 1 \quad (3.1)$$

For a given threshold *k*, one can discriminate two classes C_0 and C_1 (for background and objects). The probabilities of class occurrence and class mean levels are given by:

$$\varpi_0 = \sum_{i=1}^{k} p_i = \varpi(k) \quad \varpi_1 = \sum_{i=k+1}^{L} p_i = 1 - \varpi(k) \tag{3.2}$$

and

$$\begin{aligned} \mu_0 &= \sum_{i=1}^{k} iP(i \mid C_0) = \sum_{i=1}^{k} ip_i / \varpi_0 = \mu_k / \varpi_k \\ \mu_1 &= \sum_{i=k+1}^{L} iP(i \mid C_1) = \sum_{i=k+1}^{L} ip_i / \varpi_1 = \frac{\mu_T - \mu(k)}{1 - \varpi(k)} \end{aligned} \tag{3.3}$$

where

$$\varpi(k) = \sum_{i=1}^{k} p_i, \mu(k) = \sum_{i=1}^{k} ip_i \text{ and } \mu_T = \sum_{i=1}^{L} ip_i \tag{3.4}$$

are the zero and first order cumulative moments of the histogram up to the *k*th level and μ_T the total mean level of the image.

The class variances are given by:

$$\begin{aligned} \sigma_0^2 &= \sum_{i=1}^{k} (i - \mu_0)^2 P(i \mid C_0) = \sum_{i=1}^{k} (i - \mu_0)^2 p_i / \varpi_0 \\ \sigma_1^2 &= \sum_{i=k+1}^{L} (i - \mu_1)^2 P(i \mid C_1) = \sum_{i=k+1}^{L} (i - \mu_1)^2 p_i / \varpi_1 \end{aligned} \tag{3.5}$$

For "quality" evaluation of threshold *k*, the following discriminant measures are introduced:

$$\begin{aligned} \sigma_B^2 &= \varpi_0 (\mu_0 - \mu_T)^2 + \varpi_1 (\mu_1 - \mu_T)^2 \\ \sigma_T^2 &= \sum_{i=1}^{L} (i - \mu_T)^2 p_i \end{aligned} \tag{3.6}$$

which are the between-class variance and the total variance of levels. The simplest criterion measure for evaluation of the separability of the threshold *k* is the following:

$$\eta = \sigma_B^2 / \sigma_T^2 \tag{3.7}$$

Then, the optimal threshold is the one that maximizes η or σ_B^2. The selection is made by a sequential search using the cumulative expressions in (3.4):

$$\sigma_B^2(k) = \frac{[\mu_T \varpi(k) - \mu(k)]^2}{\varpi(k)[1 - \varpi(k)]} \tag{3.8}$$

and the optimal threshold is:

$$\sigma_B^2(k^*) = \max_{1 \le k < L} \sigma_B^2(k) \tag{3.9}$$

The same criterion can be extended to multilevel thresholding, but it can lose his meaning as the number of classes increase. In the experimental results section we present the cells image segmentation using the Otsu method tresholding.

3.2. The Maximum Correlation Criterion

Identifying multiple classes can be a problem for unsupervised segmentation algorithms. For the particular case of the cell images, the homogenous regions can be discriminated by a multilevel thresholding. Being a nonparametric method, the Maximum Correlation Criterion (MCC), evaluate the optimal thresholds using a cost function (Yen et al. 1995).

If we consider an image *f*(*x*,*y*) of size *N*x*N*, with *L* gray levels and [0,1,...,(*L*-1)] denote the set of gray levels. If n_i is the observed gray level frequencies in image *f*, then the probability of the gray level *i* in image *f* can be computed by:

$$p_i = \frac{n_i}{NxN} \tag{3.10}$$

For a given gray level s, if $0 < \sum_{i=0}^{s-1} p_i < 1$, then the following two distributions can be derived after normalization:

$$\begin{aligned} A &= \left\{ \frac{p_0}{P(s)}, \frac{p_1}{P(s)}, ..., \frac{p_{s-1}}{P(s)} \right\} \\ B &= \left\{ \frac{p_s}{1 - P(s)}, \frac{p_{s+1}}{1 - P(s)}, ..., \frac{p_{L-1}}{1 - P(s)} \right\} \end{aligned} \tag{3.11}$$

where $P(s) = \sum_{i=0}^{s-1} p_i$ is the total probability up to the *s*-1 gray level.

The correlation of a random variable *X* with a finite range $R = \{x_0, x_1, x_2, ...\}$ is defined as:

$$C(X) = -\ln \sum_{i>0} p_i^2 \tag{3.12}$$

where p_i is the probability of *X* being x_i.

The total amount of correlation provided by the two distributions A and B is:

$$\begin{aligned} &\mathrm{TC}(s) = C_A(s) + C_B(s) = \\ &-\ln \sum_{i=0}^{s-1} \left(\frac{p_i}{P(s)} \right)^2 - \ln \sum_{i=s}^{L-1} \left(\frac{p_i}{1 - P(s)} \right)^2 \end{aligned} \tag{3.13}$$

In order to obtain the maximal correlation contributed by the background and the object in the image *f*, we must determine the threshold s^* that maximizes the correlation criterion (3.12):

$$\mathrm{TC}(s^*) = \max_{s} \mathrm{TC}(s) \tag{3.14}$$

The method exposed here can be extended to multilevel, by using a set of thresholds $s_1, s_2, ...$ and using the following criterion:

$$\begin{aligned} &\mathrm{TC}(s_1, s_2, ... s_k) = \\ &-\ln \sum_{i=0}^{s_1-1} \left(\frac{p_i}{P(s_1)} \right)^2 - \ln \sum_{i=0}^{s_2-1} \left(\frac{p_i}{P(s_2)} \right)^2 - ... \\ &-\ln \sum_{i=s_1}^{L-1} \left(\frac{p_i}{1 - P(s_1)} \right)^2 - \ln \sum_{i=s_2}^{L-1} \left(\frac{p_i}{1 - P(s_2)} \right)^2 - ... \end{aligned} \tag{3.15}$$

For the blood cells classification and counting methods we employed the (3.15) criterion using two thresholds, in order to discriminate three classes of interest: the background, the red type blood cells and the leucocytes.

4. CELLS CLASSIFICATION AND COUNTING

In this case, the main goal was to develop an unsupervised algorithm for cell classification and counting. The two operations are performed in

correlation and so, our framework has to deal with both of them. We have investigated two methods: a statistical one, based on area statistics and a structural one who take consideration of the cell shape.

4.1. Statistical Pattern Analysis

The statistical pattern analysis takes in count the fact that cells belonging to the same class must have the same area. In our algorithm, we first mark a cell and then calculate the cell area. Using an autoclustering technique, based on the nearest centroid classifier (Parker 94), we can assign the marked cell to the nearest class. This is performed by computing the distance between the vector of the unclassified feature and all the other centroids (center of mass of a feature collection). The class of the nearest centroid is assigned as the class of the feature.

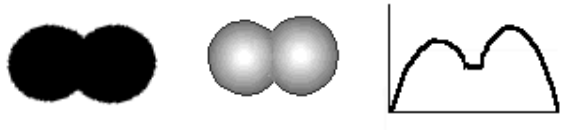
This method was suitable only for a particular case of images. The one that doesn't contain overlapped cells or cells meeting the image boundary. We have developed a method for removing the incomplete cells and modified the original classifier as it can deal with overlapped cells and still can count their precise number from the image. The autoclustering algorithm needs a threshold for class assignment determined by "training", on sample data with a human operator. The decision rule is applied on the area measurement.

- **for** every feature:
  - compute *area*
  - compare *area* with every class mean area:
    - **if** $|area - mean\_class_i| < t$ **then** assign feature to class *i* and recompute parameters of class
    - **else** create a new class for the feature

Finally we will obtain a list of cell classes (one containing singular cells, one containing groups of two overlapped cells, etc...) with the corresponding number of cells.

4.2. Structural Methods

As we have pointed previously, recognition problems can occur when the image contains fragmented or overlapping cells. The algorithm we are proposing take advantage of the cells structure and can discriminate between two joined cells. The basic method is called watershed and has been proposed at the beginning of the '90s (Beucher 94).



a) b) c)

Figure 4.1. The Watershed Method; a) two overlapping cells; b) distance from each pixel to the boundary plotted as gray level; c) axial section in the 3D distance plot.

In figure 4.1.a) we show two overlapped cells after the segmentation step. If the shortest distance between each pixel and the background of the image is computed and plotted as a height, the results can be seen in figure 4.1.b) and c). The peaks determined here can reveal the center of the cell. By using these two points as seed points for a dilatation process, we can recover the joined cells.

Our method performs the following steps:

- make an identification of the feature
  - decide if the feature represents a cell or a noise, based on the area. If its a noise, delete it
  - compute the watershed of the feature and count the peaks. Delete those cells from the image
- continue till no feature is found

The method exposed here has given good results on a large number of test images and the results are shown in the experimental result section.

5. LEUCOCYTE DETECTION AND COUNTING

For the particular case of the leucocyte cells, detection is based on a subjective observation of the microscopic images. These cells have darker gray levels as the red blood cells. This observation is based on a sequence of blood test we have examined at the Sibiu Clinical Hospital Laboratory and lead us to the MCC segmentation process. After the preprocessing and segmentation steps, the resulted binary image is searched for features matching leucocytes cells:

- **do**
  - search for a "black" range pixel
    - make a dilatation process and mark the feature
    - compute *area*
    - **if** *area* > *threshold_area* **then** feature=cell
    - **else** delete the feature
- **while** no other feature found

The *threshold_area* parameter is required to decide if the feature marked is a cell or a noise and can be set after a "training" period. In the next section, the results of the algorithm described above are shown in a series of images, captured all along processing.

6. EXPERIMENTAL RESULTS

Our main objective was to recognize and count the leucocytes from microscopic images. Since the methods we have investigated where also fitted for cell classification, we are introducing these results too.

6.1. Cells classification

As we have previously suggested, the proposed approach was tested to classify red cells or groups of red cells and count them. Image preprocessing is common to all methods and consists of a gray scale transform and a gaussian filter of σ=2 for noise cleaning (described in section 2). The segmentation step is based on the Otsu

method, as presented in 3.1 and the results are shown in figure 6.1.b). The recognition is processed by autoclustering using the nearest centroid. As we have pointed in section 4.1., cells meeting the image boundary are not recognized, as show in figure 6.1 c). The algorithm count 22 cells in the image, discriminating classes of one (9 cells), two (3 groups), three (1 group) and four (1 group) cells.

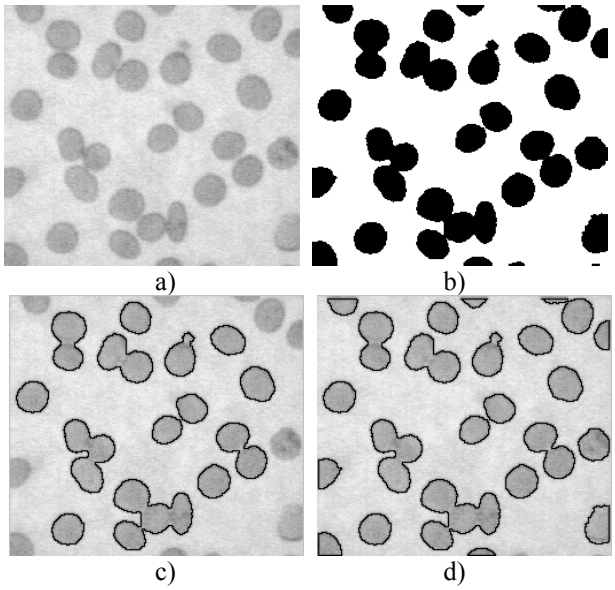


Figure 6.1. a) original red cells image; b) result of segmentation; recognized and marked cells using c) nearest centriod classifier and d) watershed method

Due to weak recognition performance, we have turn to structural classification, as presented in section 4.2. After preprocessing and segmentation, the watershed is employed to recognize singular and overlapped cells. The results are show in figure 6.1. d). The algorithm successfully recognized all 32 red cells, even if cells are meeting the image boundary. On a large number of test images (noisy or faulty focused images), this method have given very good results.

6.2. Leucocyte detection and counting

In this particular case, in the microscopic image two kinds of cells are being present: red cells and leucocytes. The last ones appear in darker shades of gray, as in figure 6.2.a). The image preprocessing consist in a two pass gaussinan filtering of standard deviation σ=0.66. The process of segmentation is based on the multilevel MCC, as pointed in section 3.2. Two levels were employed and the results are show in figure 6.2.b). Using the algorithm presented in section 5, we are able to discriminate between red and white cells as shown in figure 6.2.c). Figure 6.2.d) show the results of cell marking. Using an appropriate calibration of the optical system microscope-camera and the classical leucocyte formula, the software developed could give the precise result of blood test.

The proposed approach of cell recognition, classification and counting was tested on a large number of images grabbed from a laboratory microscope and a professional video camera. All the tasks where successfully performed and the total recognition rate was 98%. We are looking for further improvements of cells classification methods suggested by other applications.

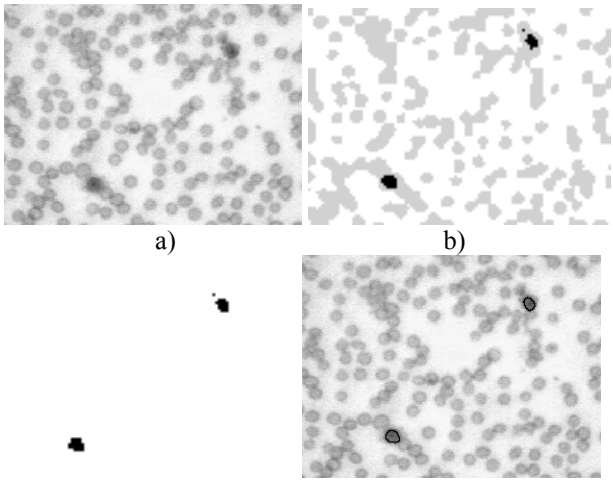


Figure 6.2. a) original blood cells image; b) results of MCC segmentation; c) features detected as leucocytes; d) marked leucocytes on original image

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BIOGRAPHY

Remus Brad was born in Sibiu Romania in 1967. He received the Engineer Diploma degree in Automation and Computer Science from the University "Lucian Blaga" of Sibiu Romania and the M.S. degree from Université "Pierre et Marie Curie" Paris France in Artificial Intelligence. From 1994 he has joined the Department of Computer Science at the Faculty of Engineering from Sibiu. His current research interests include image processing, motion estimation and prediction and biomedical imaging. Remus Brad is a member of the IEEE Signal Processing Society.