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Red Blood Cells Counting by Circular Hough Transform Using Multispectral Images

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Abstract: This study propose a new approach to count red blood cells using multispectral images. The proposed approach first uses the Canny edge filter applied independently in each spectral band and combines them into a single segmented image and next, the system uses the Circular Hough Transform (CHT) to detect the shape of the cells. The main contribution of this study consists in the use of multispectral images in order to take advantage from the peculiarity of CHT. The result obtained, show that the red blood cells detection and counting rate of the proposed approach was 98.07% for 25 samples with regard to manual counting.

Key words: Multispectral images, Circular Hough Transform, Canny edge filter, red blood cells

INTRODUCTION

Malaria is a severe infectious disease caused by a protozoan parasite of the genus *Plasmodium*. Despite it being preventable and curable, it causes more than 1 million deaths arising from approximately 300-500 million infections every year. There are a number of classical techniques used for malaria diagnosis but microscopic diagnosis is the most used. Microscopy diagnosis of malaria is performed by examining Giemsa stained peripheral blood slides using a conventional light microscope. According to a recent WHO report (Moody, 2002), an experienced laboratory specialist can detect parasites on thick films in concentrations as low as 5-10 parasites per μL of blood. However, this depends on the skill of the technician and the time spent for the examination.

The goal of this study is to find a solution for automatic counting of red blood cells to computer-assisted diagnosis of malaria by multispectral imagery using together two techniques, Canny edge filter and CHT. Canny edge filter is an approach to edge detection proposed by Canny (1986) that is optimal for step edges corrupted by white noise. Meanwhile, the CHT is a kind of Hough Transform (HT) that can extract circular objects from an image.

This solution represents a group of methods based on edge segmentation. Many practical segmentation problems need more information that is contained in one spectral band. Color images are a natural example in which information is coded in three spectral bands. Multispectral images may have even more spectral bands. In previous studies (Merdasa *et al*., 2013; Zoueu *et al*., 2009), this study has presented a technology based on multispectral and multimodal LED microscopy as a rapid, low-cost, easy to use and sensitive staining-free thin blood smear malaria diagnosis.

Computer-assisted diagnosis of malaria is a microscopy diagnosis technique. It can be used as an aid or a complete automated diagnosis technique which replaces the manual microscopy examination. A computer-assisted microscopy diagnosis technique can be designed by understanding the microscopy diagnosis expertise and representing it by specifically tailored image processing/analysis and pattern recognition algorithms. However, this study focuses only on automatic counting of red blood cells. There are several problems in detecting and recognizing red blood cells in the image. Primarily, some of the cells overlap between each other and therefore make the recognition process challenging. Moreover, the size of the cells changes and finally, the images itself contain noise that make the recognition process difficult without proper segmentation process.

MATERIALS AND METHODS

Image acquisition: The data acquisition system has been discussed previously (Brydegaard *et al*., 2009). The illumination system which was initially composed with transmission and reflection traditional bright sources, in a metallurgic optical microscope (Brunel compound microscope), were removed and replaced in all modes by a set of 13 LEDs with wavelengths ranging from 375-940 nm. A fiber-ring source was added to the system for scattering mode. The original mechanic eyepieces are similarly substituted by a 12 bit Pixel Depth Monochrome CMOS camera of 5MPix (2592×1944, Guppy-503B, Allied Vision Technology, with a MT9P031 sensor from

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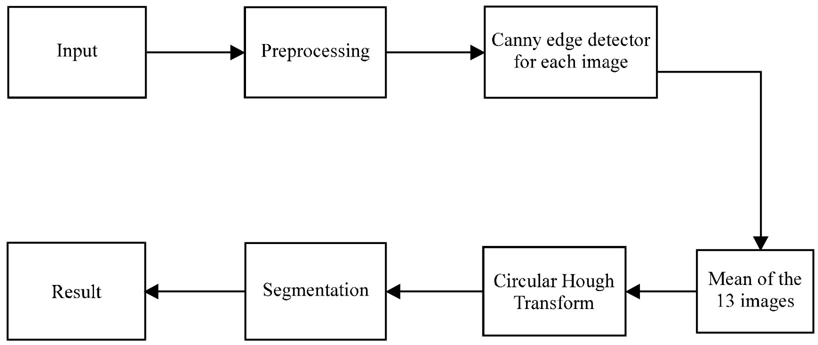

Fig. 1: Red blood cells recognition process

Micron/Aptina) with individual pixel size of 2.2×2.2 μm; this is used to acquire digital monochrome wavelength-dependent images. For transmission, reflection and scattering modes, the system automatically acquire a total of 39 spectral images (13 images per mode), for the same scene, using a data acquisition board (DAQ) coupled to a computer which control the current intensities for calibration purposes. Spectral images are obtained as follow: In the case of the transmission mode (reflection and scattering are performed similarly), the measurements are performed, respectively for the reference I_R (empty slide), the specimen I_S (blood film area) and the dark I_D (light off) and processed according to the equation:

$$I_{Trans} = \frac{I_S - I_D}{I_B - I_D} \quad (1)$$

Proposed approach: The images with 600×800 pixels have been used throughout this study. Figure 1 shows the block diagram of the recognition process.

The input image has to come across several steps before the proposed technique is performed. The image had to be enhanced using histogram in preprocessing step. Then the edge detection had been employed using Canny edge detector. The edge detection process is very important as the edge information is required for the CHT technique. After application of Canny, all the 13 images are added because low level edge detection operators do not guarantee continuous boundaries of the cells and after the CHT is applied to image. When using the CHT, the radius r is known in advanced because of the cells shape. In this study, the radii for the red blood cells have been set to a range of value from 14-18 pixels.

RESULTS AND DISCUSSION

Canny edge detector: Canny is a filter based on an analytical approach (Canny, 1983). The $g_i(x, y)$ is the input image (i = 1,…,13) corresponding to a spectral range. Each $g_i(x, y)$ is smoothed by using a Gaussian filter with standard deviation σ = 0.2 to reduce the noise.

The local gradient and the direction of edge are calculated in every point:

$$\nabla g = [\left(\frac{\partial g}{\partial x}\right)^2 + \left(\frac{\partial g}{\partial y}\right)^2]^{1/2} \quad (2)$$

Equation 2 is used to measure the local gradient and the Eq. 3 is used to calculate the direction of the edge:

$$a(x, y) = \tan^{-1}(G_x / G_y) \text{ with } G_x = \frac{\partial g}{\partial x} \text{ et } G_y = \frac{\partial g}{\partial y} \quad (3)$$

The determined edge points give rise to peaks in the gradient magnitude image. The algorithm then follows along the top of the ridges and resets of all the pixels that are not actually on the top of the crest, so as to give a thin line in the output. The pixels are stained peaks using two thresholds T1 and T2 with T1<T2. Pixels peaks with higher T2 values expressed strong edge pixels. The ridges pixels with values ranging between T1 and T2 are called weak edge pixels.

Finally, the algorithm performs the connection of the edges by incorporating pixels which have 8 connected neighborhood. The final image is obtained by averaging the gradient images of different spectral bands (Eq. 4 and Fig. 2):

$$f(x, y) = \frac{\sum_{i=1}^{13} \nabla g_i}{13} \quad (4)$$

In Fig. 2, it is noted that the red blood cells are circular and have a diameter variation. The detection algorithm forms the Hough transform is applied to the image by exploiting these two observations.

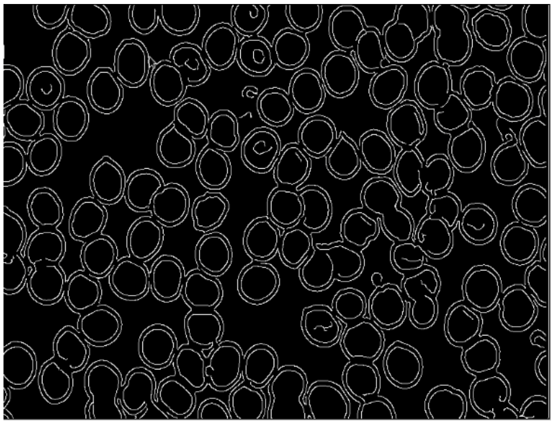

Fig. 2: Image corresponding to the application of he Canny filter

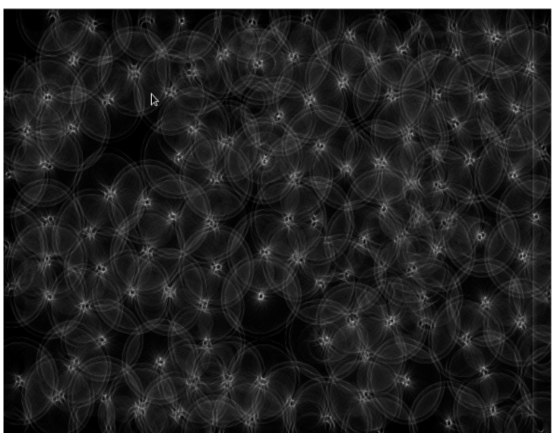

Fig. 3: Image corresponding to the application of the Hough Transform

Circular Hough Transform (CHT): The Hough transform is a robust technique for curve detection. The CHT was sketched by Duda and Hart (1972). The CHT aims to find circular patterns within an image.

The CHT is used to transform a set of feature points in the image space into a set of accumulated votes in a parameter space. Then, for each feature point, votes are accumulated in an accumulator array for all parameter combinations. The array elements that contain the highest number of votes indicate the presence of the shape (Sonka *et al*., 2008). A circle pattern is described by Eq. 5:

$$(x-a)^2+(y-b)^2 = r^2 \quad (5)$$

Where a and b are the coordinates of the center and r is the radius of the circle. An example of result is shown in Fig. 3.

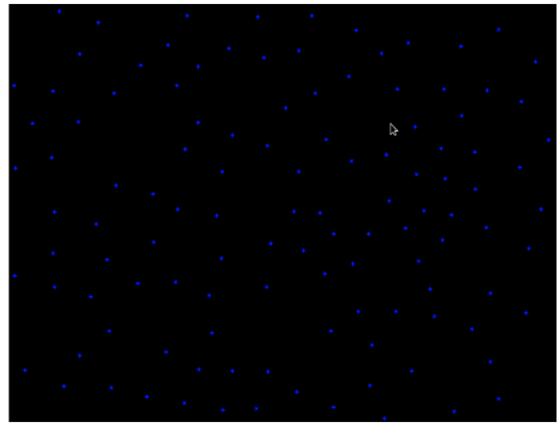

Fig. 4: Image segmentation of local maxima

Table 1: Result manual counting and automatic counting of 25 samples

No samples	Manual counting	Automatic counting	Error (%)
1	1440	1412	1.94
2	1840	1780	3.26
3	1800	1808	0.44
4	2612	2628	0.61
5	1432	1368	4.46
6	1436	1416	1.39
7	2604	2648	1.68
8	1824	1868	2.41
9	1816	1892	4.18
10	1468	1428	2.72
11	1856	1816	2.15
12	2020	2032	0.59
13	1416	1412	0.28
14	2616	2708	3.51
15	1816	1788	1.54
16	2232	2248	0.71
17	1768	1744	1.35
18	1844	1832	0.65
19	1832	1792	2.18
20	1848	1892	2.38
21	1424	1356	4.77
22	1816	1788	1.54
23	1864	1824	2.14
24	1868	1844	1.28
25	2636	2644	0.30
Mean error		1.93	

In Fig. 4, it is observed that the local maxima correspond to the intensities center cells and subsequently segment centers. The best segmentation is obtained for a threshold higher than 37.5 based on a study of the histogram of the pixel intensities (Fig. 4).

Experiments were made by using 25 samples to evaluate the performance of the proposed approach. The lower bound and the upper bound on the radius of the cells in the input image were set to 14 and 18, respectively. The algorithm was compared with manual counting.

Table 1 shows the results of the counting, Fig. 5 show the correlation between automatic and manual counting and Table 2 show the goodness of the interpolation.

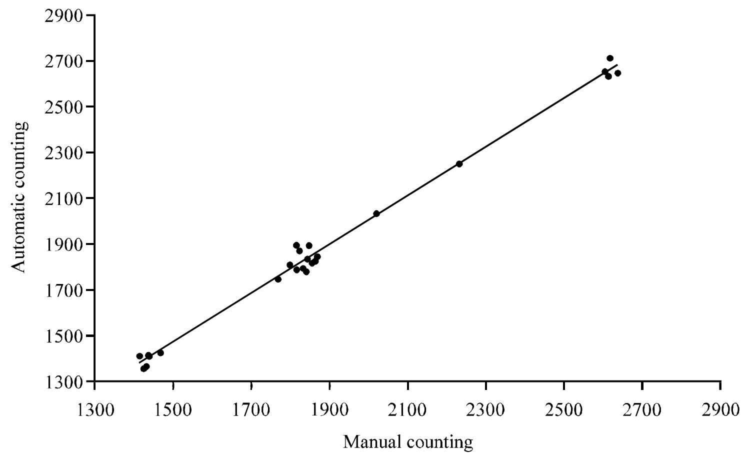

Fig. 5: Linear interpolation automatic counting vs. manual counting

Table 2: Statistics parameters

Goodness of fit	Vlaues
SSE	2.907e+04
R-square	0.9928
Adjusted R-square	0.9925
RMSE	35.55

The aim of this study has been to investigate the potential of performing manual microscopy counting of red blood cells without human intervention and to provide an objective, reliable and efficient tool to do manual microscopy diagnosis. A new thin blood film image segmentation method has been presented by using multispectral images. This method defines a new Circular hough transform tool and applies it to perform an initial segmentation. The fact to use multispectral images introduces a solution for the over-segmentation problem associatied with the classical circular hough transform such as edges corrupted by withe noise. Experiments were made by using 25 blood smears not marked to evaluate the performance of the proposed system. As the result of the experiments, 1.93% of error is done with comparison to manual counting.

CONCLUSION

Automated microscopy diagnosis of malaria essentially requires a function which can differentiate between regular blood components (also artefacts) and parasites. The significance of this study is that it defines the computerized counting of red blood cells directly and thoroughly. It proposes a novel method to perform this fundamental function. Future study in progress includes the automatic parasitemia estimation.

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