International Journal of Information, Control and Computer Sciences
ISSN: 2517-9942
Vol:10, No:5, 2016

Leukocyte Detection Using Image Stitching and Color Overlapping Windows

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***Abstract*—**Blood cell analysis plays a significant role in the diagnosis of human health. As an alternative to the traditional technique conducted by laboratory technicians, this paper presents an automatic white blood cell (leukocyte) detection system using Image Stitching and Color Overlapping Windows. The advantage of this method is to present a detection technique of white blood cells that are robust to imperfect shapes of blood cells with various image qualities. The input for this application is images from a microscope-slide translation video. The preprocessing stage is performed by stitching the input images. First, the overlapping parts of the images are determined, then stitching and blending processes of two input images are performed. Next, the Color Overlapping Windows is performed for white blood cell detection which consists of color filtering, window candidate checking, window marking, finds window overlaps, and window cropping processes. Experimental results show that this method could achieve an average of 82.12% detection accuracy of the leukocyte images.

***Keywords*—**Color overlapping windows, image stitching, leukocyte detection.

I. Introduction

MEDICAL image processing has become more and more important in health diagnosis with the development of medical equipments and computer algorithms. Huge amounts of medical images are obtained by X-ray radiography, CT, and MRI [1]. However, it is also well known that some medical samples come in the form of microscopy imaging, especially those in pathology anatomy, such as blood cell images, lymphoid images, etc.

Identification of white blood cells (leukocytes) is one of the clinically important tasks as it plays a significant role in the diagnosis of different diseases. The traditional leukocyte detection process is performed manually by experienced laboratory technicians. However, since each video sequence of microscopy images contains a large number of rolling leukocytes (100-1000s), this approach is extremely tedious and time consuming [2]. As an alternative to this traditional technique, many works have been conducted in the area of automatic blood cell detection.

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This work is supported by the Directorate General of Higher Education Research Grants (Hibah Unggulan Perguruan Tinggi 2015-2016), Ministry of Research, Technology, and Higher Education of Indonesia.

There exist several methods for automatic blood cell detection [1]-[7]. Cuevas et al. [1] present an algorithm for leukocyte detection based on the Differential Evolution (DE) algorithm that treats the blood cells as ellipses. It is claimed to outperform the works of [3] which used a method based on boundary support vectors, [4] which implemented an iterative Otsu method on the circular histogram, and [5] with their fuzzy cellular neural network method. Other works proposed by [2] used the Bayesian classification method which depends on a feature score, called the Gradient Inverse Coefficient of Variation (GICOV), while [6], [7] proposed the applied Scale Invariant Feature Transform (SIFT) method for robust translation, which serves to discriminate leukocytes from a cluttered environment. However, all these approaches assumed that the leukocytes can be approximated with an ellipsoid form, so their focuses were using image processing techniques for detecting ellipses. Meanwhile, out of the ideal case, the shape of the blood cells is not perfectly ellipsoidal due to deviations in stage position, microscope lens inaccuracy, camera misalignment, etc.

The main contribution of this paper is the proposal of an accurate leukocyte detection technique that can deal with imperfections of the shape of the blood cells. Moreover, this proposed method, called the Image Stitching and Color Overlapping Windows method, could provide a high quality composite image as an output, which can be used by medical technicians for further analysis.

Image stitching aims to create a composite image by appropriately overlapping individual images acquired at high magnification under a microscope. The composite image must consist of images placed at the right position and the edges between images must be invisible [8]. Based on prior knowledge about the expected overlap when using the motorised stage, it would be straightforward to find the right position in ideal case. However, it cannot be achieved in real life conditions. There are a number of papers that deal with the stitching problem [8]-[10]. Image stitching can be performed using pixel intensity technique (correlation method); in a frequency domain (FFT method); using low level features or using high level features such as parts of objects [10]. Rankov et al. [8] have presented a robust algorithm to do overlap-removal algorithm which is based on the cross-correlation method. Following the stitching method, a gradient blending method is also used to eliminate sharp intensity changes at the image joins.

The approach offered in this paper defines the leukocyte detection process in the following way. First, the preprocessing stage is conducted to do stitching of the existing

microscopic images which were formerly obtained from video framing. The stitching process consists of the determination of the overlapping direction of images, the stitching process, and the blending process of the composite image. Next, the Color Overlapping Windows is performed for the leukocyte detection process. This algorithm starts with color filtering, window candidate checking, window marking, finding window overlaps, and finally do the cropping of the overlapping windows as it contains a leukocyte area.

This paper is organized as follows. Section II explains the preprocessing step which consists of image stitching and blending method. Section III explains the Color Overlapping Windows methodology for the leukocyte detection system. Section IV gives the results and analysis of the applied algorithm on the blood cell images after the image stitching and detection process. Conclusions are presented in Section V and directions for the future work are defined.

II. Preprocessing: Image Stitching

In this paper, image stitching is performed as a preprocessing stage before conducting a leukocyte detection process. There are three main steps in the preprocessing stage: 1) Determining the overlapping direction of multiple images, 2) Stitching images by finding the best cross-correlation point, and 3) Removing the edge seams by gradient blending.

The process starts by performing the generation of relative positions of the acquired images. The system selects the first and second (neighbouring) images from the stack. The best relative position for these two images is conducted by image translation only. Next, the user has to determine the stitching direction, lower, upper, or left/right stitch.

The second step is a search for the point of best correlation which is performed by sliding adjacent image edges in the determined direction until the best match of edge features is found. The correlation value is ranging between 0 and 1, where 1 is the perfect correlation. The calculation of the cross-correlation method is as follows [8]:

$$CR = \frac{\sum_{x=0}^{L-1}\sum_{y=0}^{K-1}(w(x,y)-\bar{w})(f(x+i,y+i)-\bar{f}(i,j)}{\sqrt{\sum_{x=0}^{L-1}\sum_{Y=0}^{k-1}(w(x,y)-\bar{w})^2}\sqrt{\sum_{x=0}^{L-1}\sum_{y=0}^{K-1}(f(x+i,y+i)-\bar{f}(i,j)^2}} \quad (1)$$

with: $w(x,y)$: pixel intensity of the target image; $\bar{w}$: average intensity value of the target image; $f(x+i,y+i)$: pixel intensity value of the composite image; $\bar{f}(i,j)$: average intensity value of the composite image; K and L: height, width of the target image.

When the best CR is found, the whole stacks were positioned according to the position found from these two images.

Finally, a blending algorithm is performed to eliminate seams after a stitching process. In most cases, the edges of neighboring images show intensity discrepancies which are undesirable. The blending algorithm will remove the seams using gradient blending for the overlapped regions. A search region of approximately 50% of the image gave best results because the adjacent images may have different brightness levels [8]. For RGB images, the calculation of gradient blending is performed separately for each color matrix (Red, Green, and Blue), then finally is combined to create the target image. The calculation of gradient blending is as follows [8]:

$$N(x,y) = \propto I(x,y) + (1-\propto)C(x,y) \quad (2)$$

with: $N(x,y)$: Target (blended) image; $\propto$: Distance from image edge; $I(x,y)$: Stiched image; $C(x,y)$: Original image before stitching process. The final target of this blending algorithm is to minimize the effects of intensity variations, removes the edges, and improves the cross-correlation.

III. The Leukocyte Detection System

The target of a leukocyte detection system is to find the areas where leukocytes exist. Additionally, a cropping process can also be conducted after the area selection step and the output will be in the form of a small-size image which only contains one single leukocyte. Fig. 1 shows the leukocyte detection steps.

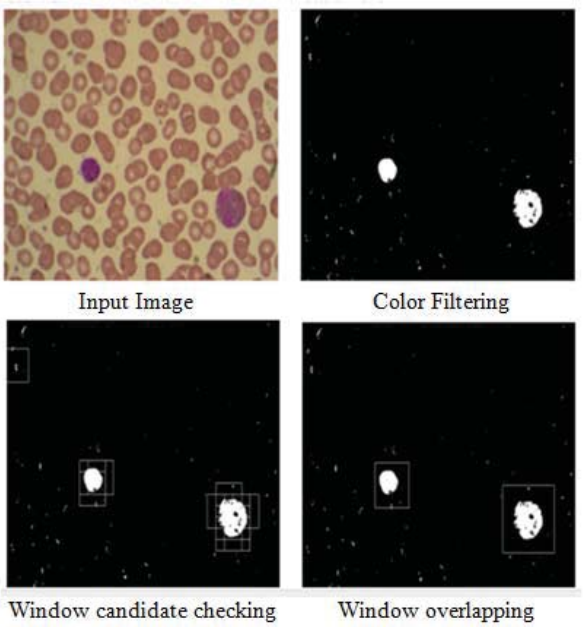


Fig. 1 The leukocyte detection steps

Following the preprocessing stage, the detection of the leukocyte areas is conducted using the Color Overlapping Windows technique. This method is based on color domain and consists of these five steps:

1. Color filtering: In this step the color filtering is performed for a combination of red and blue colors. If the intensity value of a pixel contains Red_value (R) > 150 and Blue_value (B) > 75, then replace the pixel intensity value as (R,G,B) = (255, 255, 255). Otherwise set the pixel value as (R,G,B) = (0, 0, 0).
2. Window candidate checking: From the color filtered images, perform checking of the center of window candidates. The checking is conducted for every 20

pixels. If the pixel values contain (255, 255, 255), then the coordinate will be marked. The value of '20 pixel' is chosen based on the average diameter of the erythrocyte (red blood cell). It is expected that skipping the checking of the center of window candidate for every 20 pixels will reduce the processing time.

3. Window marking: In this step, create a 30x30 pixels window for every marker obtained from step 2 (as illustrated in Fig. 2).
4. Window overlapping: From each constructed windows in step 3, find the overlaps of these windows (as illustrated in Fig. 3). The goal of the overlap findings is to verify that the selected images contain a leukocyte (white blood cell). The largest overlap that can be obtained is 91x91 pixels as the farthest overlap scope is 31 pixels; adding these 31 pixels score with the window size itself will result the 91 pixels.
5. Window cropping: Finally, do the cropping of the overlapping windows in step 4, as it contains a leukocyte area.

IV. Experiments

The methods described above were evaluated on real microscopic blood cell images. Table I shows the samples of input images which consist of the leukocytes (white blood cells) and the erythrocytes (red blood cells). It is shown in Table I that in each blood cell image, there are numerous erythrocytes, but the number of existing leukocyte can only be 1 or 2 cells.

For the experiments, we developed our own database, called the FTI-Untar blood cells database. First, the video of a rolling microscope slides was taken using a camera that is attached to the microscope. Then, we framed the video to images with 10 fps. The images were then resized to 400 x 300-pixel dimension.

We have conducted various experiments with various targets for the proposed leukocyte detection system. First, we evaluated the performance of the image stitching method to combine two blood cell images. Table II shows the samples of image stitching with various stitching directions, i.e. lower stitch, upper stitch, and the right stitch. In the lower stitch process, image#2 will be added at the bottom of image#1, while the opposite happens for upper stitch, where image#2 will be added on top of image#1. For the right stitch, image#2 will be added on the right side of image#1. The determination of these three stitch directions is based on the theory of leukocyte differential count [6], where the microscope slide will be moved downward (lower stitch), then translated to the right (right stitch), and finally goes up (upper stitch). The sliding is never performed to the left side for the leukocyte differential count. Thus, the left stitch experiment is not conducted.

Table II shows the input images for each stitching type and the results (the output images). It is shown in Table II that for each stitching process, the higher the cross correlation value, the lower the percentage of the non-overlap parts; this leads to smaller changes to the original base image (image#1). It can also be seen that the correct stitching positions of images were found, and the seams were not significantly visible. Therefore, the applied image stitching method worked well in preprocessing the input images.

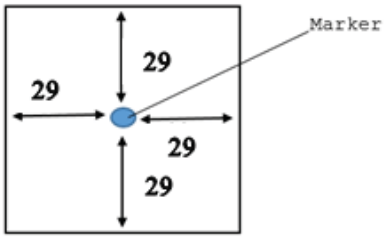


Fig. 2 Creating a 30x30 pixels window with a marker

1	1	1	1	1
1	255	1	1	1
1	255	255	255	1
1	1	1	255	1
1	1	1	1	1

Fig. 3 Map the overlapping windows

TABLE I
Samples of Blood Cell Images

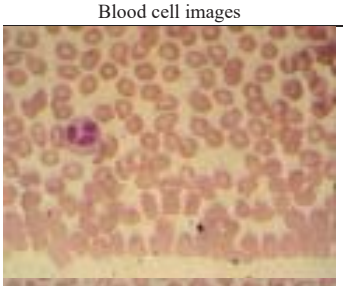
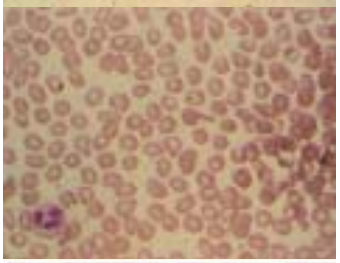
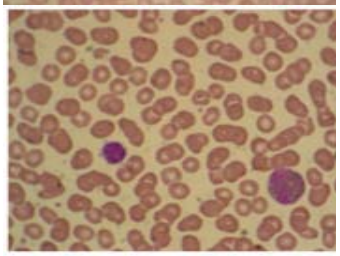
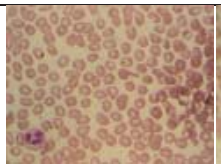
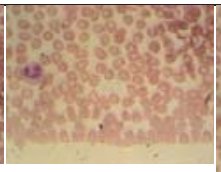
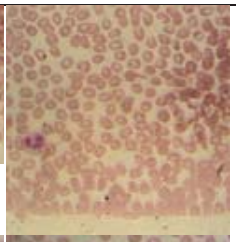
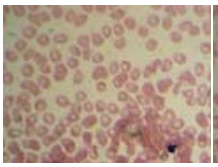
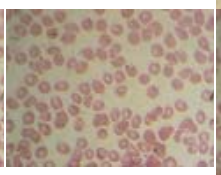
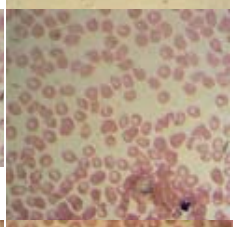
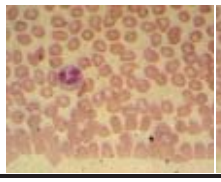
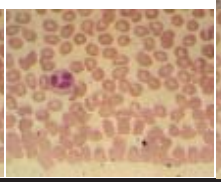
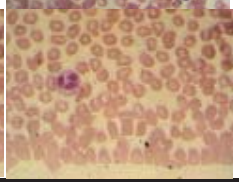
Blood cell images	# of Leukocyte
	1
	1
	2

TABLE II
SAMPLES OF IMAGE STITCHING

Stitch Type	Input Image Size	Image #1	Image #2	Output Image	Cross Correlation	Non-over-lap (%)	Output Image Size
Lower Stitch	400 x 300				0.64	41.33	400 x 423
Upper Stitch	400 x 300				0.79	27.5	400 x 381
Right Stitch	400 x 300				0.80	3.75	414 x 300

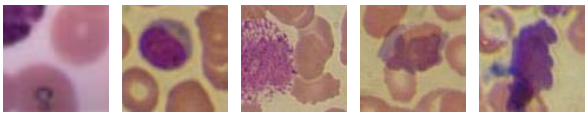
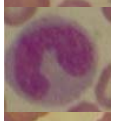
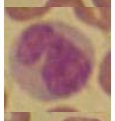
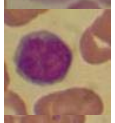
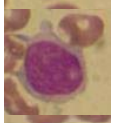
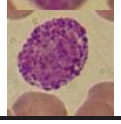
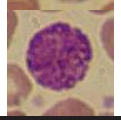


Fig. 4 Samples of images of the failed-detection category

TABLE III
SAMPLES OF THE CROPPED LEUKOCYTE AREA

Leukocyte Type	Sample Image 1	Sample Image 2
Neutrophil		
Monocyte		
Lymphocyte		
Eosinophil		
Basophil		

The next experiment focuses on evaluating the accuracy of the leukocyte detection system. After obtaining the output image from the preprocessing stage by image stitching method, the detection system was performed to locate and crop the leukocyte area. The samples of the cropped leukocyte area for each leukocyte type are shown in Table III. As shown in Table III, the dimension of each leukocyte area was different from one type to another. Even for the same leukocyte type, the size of a leukocyte cell might be different.

The experiment for the detection system was performed to locate leukocyte areas from 1130 blood cell images which consist of 400 neutrophils images, 360 lymphocytes images, 170 monocytes images, 50 eosinophils images, and 150 basophils images. The detection results were classified into three classes, i.e. S, M, L, and one 'failed-detection' category. The detection result was classified into the S class when only 0-30% of the correct leukocyte area is detected. The M class category was determined when 31-70% of the correct leukocyte area is successfully detected, while L class was counted when more than 70% of the leukocyte area is correctly detected. Finally, when a non-leukocyte image was cropped and assumed as a leukocyte area, we classified the result as the failed-detection category. The samples of image of the failed-detection category are presented in Fig. 4. There were many conditions that might cause misdetection of the leukocyte images, such as the use of different coloring technique when preparing the microscope slides, using different background lighting when capturing the leukocyte images, too many overlapping erythrocyte areas in the image, capturing images from a "dirty" slide, etc. Moreover, Table IV shows the detection results of the leukocyte images. It can be seen in Table IV that the detection system obtained only 9.7% of failed detection in average, while 8.18% and 82.12% were achieved for detection results in M and L category, respectively. This shows that the proposed system worked well and could achieve satisfactory accuracy for the leukocyte detection. Based on those experimental results for each

leukocyte type, it can also be seen that Neutrophil, Lymphocyte, and Basophil were the leukocyte types that could be easily detected by the proposed system with less than 10% failed classification. Meanwhile, Eosinophil was the most difficult leukocyte type to be detected correctly with 25% failed detection result.

TABLE IV
THE DETECTION ACCURACY OF THE LEUKOCYTE IMAGES

No.	Leukocyte Type	Detection Result (%)			Failed (%)
		S	M	L	
1	Neutrophil	0	7.32	90.24	2.44
2	Monocyte	0	25	62.5	12.5
3	Lymphocyte	0	8.57	82.86	8.57
4	Eosinophil	0	0	75	25
5	Basophil	0	0	100	0
	Average	**0**	**8.18**	**82.12**	**9.70**

TABLE V
PROPERTIES OF LOWER STITCHING ALGORITHM

# of Images	Non-overlap (%)	Processing Time (Second)	Output Image Size
2	49.667	14	400 x 448
3	35.491	37	400 x 588
4	34.824	63	400 x 682
5	35.19	97	400 x 741
6	19.03	127	400 x 897
7	18.364	163	400 x 1055
8	20	214	400 x 1144
9	18.757	250	400 x 1320
10	13.18	328	400 x 1445

TABLE VI
PROPERTIES OF UPPER STITCHING ALGORITHM

# of Images	Non-overlap (%)	Processing Time (Second)	Output Image Size
2	32.64	13	400 x 396
3	28.66	38	400 x 503
4	40.024	53	400x 599
5	32.542	82	400 x 697
6	38.486	110	400 x 781
7	18.364	148	400 x 1021
8	20.25	197	400 x 1112
9	18.58	235	400 x 1300
10	13.2	271	400 x 1441

TABLE VII
PROPERTIES OF RIGHT STITCHING ALGORITHM

# of Images	Non-overlap (%)	Processing Time (Second)	Output Image Size
2	28.75	19	514 x 300
3	25.292	43	529 x 300
4	36.862	76	594 x 300
5	36.7	108	617 x 300
6	15.64	137	704 x 300
7	23.648	158	866 x 300
8	24.57	191	1055 x 300
9	0.25	230	1057 x 300
10	4.125	273	1099 x 300

Finally, we conducted experiments for image stitching with more than 2 images. The image stitching algorithm was performed recursively up to ten sequential images. The process was started from stitching the first two images with the same size, then was continued by performing another stitching process between the previously combined image with another new image, and so on. The experiments were performed for various stitching directions, i.e. the lower stitch, the upper stitch, and the right stitch. The starting images have the dimensions of 400x300 pixels.

Table V shows the properties of lower stitching algorithm, while Tables VI and VII show the properties of upper and right stitching algorithm, respectively. For the lower stitching experiments, it can be seen from Table V that the processing times increased along with the increment of the dimension of the output images. Meanwhile, the dimension of the output image was highly dependent on the percentage of the non-overlap properties. The same phenomena were also found in the upper stitch and right stitch algorithms in Tables VI and VII, respectively.

V. CONCLUSION

A leukocyte detection system using Image Stitching and Color Overlapping Windows has been presented. The proposed algorithms consist of an accurate leukocyte detection technique that can deal with imperfect shapes of the blood cells and could provide a high quality composite image which can be used by medical technicians for further analysis. Two methodologies are applied in the proposed leukocyte detection system, i.e. the Image Stitching method as the preprocessing step and the Color Overlapping Windows method as the leukocyte detection step. The presented method is fast and effective; as shown in the experimental results where the average leukocyte detection accuracy reached 82.12%.

Future work will include an algorithm for both recognition and counting of the detected leukocytes. Automation of the whole process will also form the part of the future work.

ACKNOWLEDGMENT

The authors thank our research assistants at the Pattern Recognition Research Laboratory, Faculty of Information Technology, Tarumanagara University, Jakarta, Indonesia, for their help in preparing the experiments.

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