OVERLAPPING WHITE BLOOD CELL SEGMENTATION AND COUNTING ON MICROSCOPIC BLOOD CELL IMAGES

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Abstract- Overlapping white blood cell identification on microscopic blood cell images is proposed for increasing the accuracy of white blood cell segmentation and counting. The accurate identification of overlapping cells can increase the accuracy of cell counting system for diagnosing diseases. The overlapping cells have different characteristic such as area and shape with a single cell of microscopic cell images therefore the overlapping cell identification based on geometric feature is preferred. As a result, the proposed method identifies and counts the number of overlapping cells similar with manual white blood cell counting. In addition, the proposed method segment nucleus and cytoplasm of white blood cell with average of accuracy 85.22% and 70.27% from the manual segmented respectively. For future work, the results can be extended to separate the identified overlapping cell therefore it can applied for differential white blood cell counting for diagnosing diseases.

Index terms*: white blood cell segmentation, geometric feature, mathematical morphology, microscopic blood cell image.*

I. INTRODUCTION

The number of red blood cells (RBC) or white blood cell (WBC) can provide information about several types of diseases such as leukemia, anemia, and dehydration. The leukemia and anemia decrease the number of red blood cells, in contrast to dehydration increase the number of red blood cells. The accuracy of blood cell counting is a very important factor of disease treatment. The fault diagnosis affects medical treatment to be performed. The counting of different classes of white-blood-cell (WBC) is one of the most frequently performed blood tests and it plays an important role in the diagnosis of diseases such as anemia, leukemia, and HIV [1]. Manual differential counting by an expert is imprecise, difficult to reproduce, and subjective. In addition, it is tedious and time consuming to locate, classify and count WBC types. Therefore, the automatic WBC differential counting system is preferred [2]. How to design a low-cost and reliable recognition system has become a hot topic in the area of image processing [3, 4, 5, 6]. The segmentation process of WBC images is a crucial process in the automatic WBC counting system. Therefore, accurate segmentation method is necessary to obtain the best results for WBC classification [2].

There are some works on white blood cell image segmentation. Combined binary morphology and fuzzy c-means algorithms for WBC image segmentation are used in [7]. Active contour approaches for WBC image segmentation reported in [1, 8]. Recently, there are researches on segmentation of microscopic cell image using color and texture feature approach [9] and combination of the fuzzy morphology and binary morphology for nucleus and cytoplasm segmentation is proposed in [2, 10]. The region finding the algorithms that use the clustering algorithms require considerable computational time and the contour-detection algorithms rely on the discontinuity of image intensities since they are sensitive to noisy images [2]. In addition, due to the characteristics of cell images, in which not all cell boundaries are sharp, it is difficult to extract all the edge information [10]. There are cell images with low-contrast among cell elements and varieties of color that make cell elements difficult to distinguish such as the cytoplasm region may be as dark as the nucleus region or as bright as the background [2]. However, the overlapping cell identification on white blood cell image segmentation is still challenging problem for researchers. The overlapped of cells can decreased the accuracy of segmentation especially for counting the number of cells [2]. There are some works on overlapped cell identification that are available in the literature [11 - 14],

however, the literature are not focus on white blood cell image segmentation. The literatures [11 - 14] focus on fungi spore images, fluorescence microscopy images, immunofluorescence images, and cervical cells images respectively. The literature [11] use fungi spore images and use a combination of graph segmentation technique and thresholding algorithm for cell segmentation and use corner detection algorithm to identify the touching cells. The literature [12] uses the watershed algorithm to segment overlapping and aggregating cells and uses significant concavity points to identify the overlapping/aggregating cells of normal/pathological nuclei cells. In case of overlapping nuclei, the grey level intensity is far higher in the area of overlapping than the mean intensity of the connected component [12]. The literature [13] uses a two stage graph cut based model for segmentation of touching cell nuclei in fluorescence microscopy images. The literature [14] uses an unsupervised Bayesian classification scheme for separating overlapped nuclei of immunofluorescence and cervical cells images. There are two approaches on overlapped cell identification, i.e. region based and boundary based [14]. The region based approaches are based on size or pixel numbers of region. Boundary based are identification based on shape such as curve, the weakness of boundary based is when the point arch unidentified object correctly, nor with boundary based have inaccuracies when the limit (threshold) size of the object does not properly [14]. Due to the characteristic of overlapped white blood cell have large area, ellipse shape, and different intensity on intersect area of overlapping cells. The combination of region and boundary based is used to identify the overlapping cell in this paper.

The overlapping cell of white blood cell image identification is very important for increasing the accuracy of white blood cell counting. Most of overlapped white blood cells have overlapping on nucleus region. Based on this problem, this paper proposes a scheme for white blood cell image segmentation that it includes to identify the overlapping cells. The proposed scheme for white blood cell segmentation are divided three process i.e. nucleus segmentation, overlapped cell identification, and cytoplasm segmentation. The first process is nucleus segmentation because easier to identify the location of white blood cell and overlapped cell using the nucleus. We use fuzzy morphology approach for nucleus segmentation as reference [2]. For overlapped cell identification, the combination of region and boundary based approaches is used. Based on the statistical analysis, the overlapped cells are larger and more oval than the single cells. Therefore the geometry features such as area and eccentricity are used in this paper to identify the overlapping cells. After identify the nucleus and overlapped cells, the final process is cytoplasm segmentation. The binary morphology

using structuring element with size based on the granulometric size distribution of red blood cells is used for cytoplasm segmentation [2]. The final result of white blood cell segmentation is combination the result of nucleus segmentation with identified overlap cell and the result of cytoplasm segmentation. To evaluate the performance of the proposed method, the 7 microscopic blood cell images are used for white blood cell segmentation. The accuracy of white blood cell segmentation is compared with manual segmentation.

The paper is organized as follows. The white blood cell image segmentation scheme is introduced in Section 2. The overlapped cells identification on white blood segmentation based on geometric features is proposed in Section 3. Experiments on white blood cell segmentation are shown in Section 4. Finally, the conclusion of paper in Section 5.

II. THE WHITE BLOOD CELL (WBC) SEGMENTATION

The white-blood-cell (WBC) segmentation is a part of automatic WBC differential counting system. The automatic WBC differential counting system helps a doctor or hematologist in diagnosing diseases such as anemia, leukemia and HIV easily, accurately, and fast [2]. The accurate WBC segmentation on microscopic blood cell images can increase the accuracy of WBC counting system. Our proposed scheme of WBC segmentation is presented in Fig. 1.

The input of the WBC segmentation is a microscopic blood cell image that is usually color images. The microscopic blood cell images are obtained from white blood cell image or leukemia image samples. As shown in Fig. 1, there are three objects on the microscopic cell image i.e. red blood cell (RBC), white blood cell (WBC), and background. The white blood cell objects have dominant colors that are shown in violet color. The white blood cell object contain two regions i.e. nucleus and cytoplasm. Most of overlapped white blood cells have overlapping on nucleus region. Therefore, the first step is nucleus segmentation and the next step is overlapped cell identification. The overlapped cell identification step is used to identify the overlapped cell and the number of cell. The third step is cytoplasm segmentation that it segment cytoplasm of WBC on microscopic blood cell images. And the final step is results combination of nucleus segmentation with overlapped identification and cytoplasm segmentation. The results of WBC segmentation represent each of WBC objects into nucleus and cytoplasm region as presented in Figure 1. The segmented nucleus

region is shown in black and the segmented cytoplasm region is shown in grey. The identified and the number of overlapping cell are shown in red rectangle and blue number.

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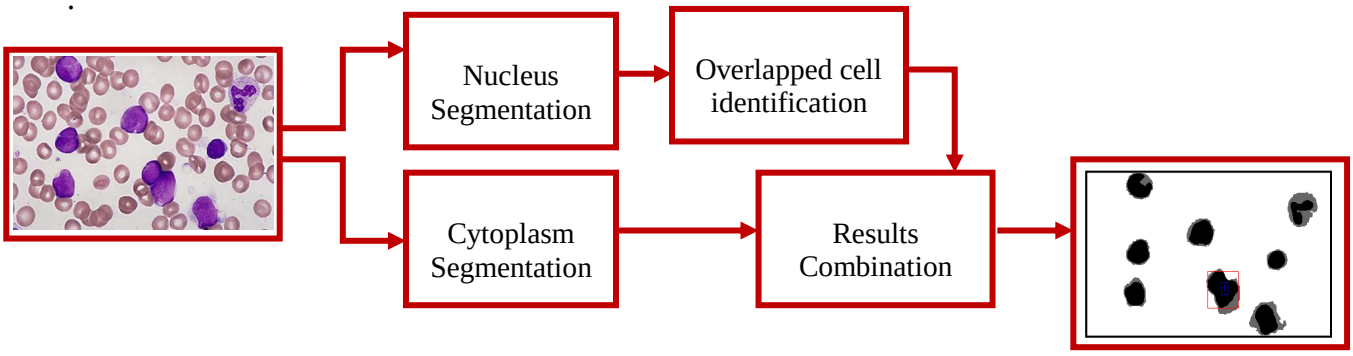


Figure 1. The white blood cell (WBC) segmentation scheme

The nucleus region of white blood cell object on the microscopic blood cell image usually have dominant color than cytoplasm region and therefore, it is easier to identify the WBC object through identified nucleus. In addition, the nucleus segmentation is an important algorithm for achieving WBC image segmentation accurately [2]. Therefore, the accurate nucleus segmentation is needed to identify the type of WBC. In our proposed scheme, the nucleus segmentation is the first step of WBC segmentation see in Fig. 1. In addition, we assume that it easier to identify the overlapped cell from identified nucleus. Because overlapped cells are generally on nucleus region.

We use fuzzy morphology approach for nucleus segmentation that it has been done by reference [2]. The fuzzy morphology has been proposed by Deng and Heijmans [15] that adopts the fuzzy logic approach to gray-scale morphology and combines it with the concepts of adjunctions. They use notions from fuzzy logic to extend binary morphology to gray-level images. The fuzzy morphological operators can be defined by means of fuzzy logic [16-21]. Let A and B belong to the set of parts of the image. The fuzzy erosion of an image f can be defined by a structuring element B, in a point x:

$$\varepsilon^F(f,B)(x) \coloneqq \inf_{y\in f}\{I(B(y)),f(y)\} \qquad (1)$$

Then, the fuzzy dilation of an image f can be defined by structuring element B, in a point x:

$$\delta^F(f,B)(x) \coloneqq \sup_{y\in f}\{I(B(y)),f(y)\} \qquad (2)$$

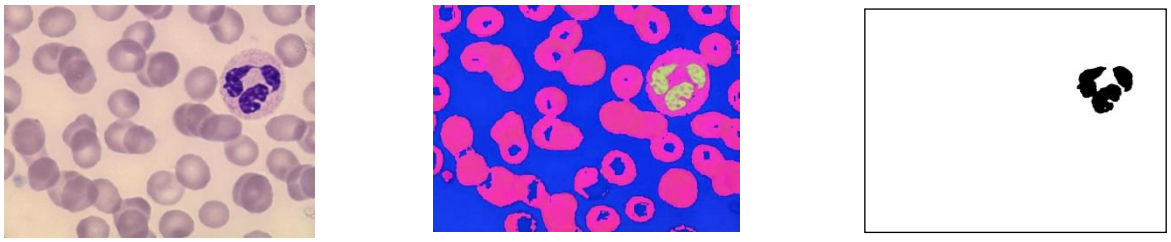
Following the steps of morphological theory, fuzzy opening is expressed as

$$\gamma^F(f,B)(x) \coloneqq \delta^F(\varepsilon^F(f,B),B) \quad (3)$$

and fuzzy closing is expressed as

$$\Phi^F(f,B)(x) \coloneqq \varepsilon^F(\delta^F(f,B),B) \quad (4)$$

The first process of nucleus segmentation is providing an input WBC image and a structuring element image with a size of 3×3 pixels that it taken from nucleus information color are given. Then, an HSV color model is created using the input image and structuring element image. The next process is applying fuzzy dilation-erosion on the HSV color model of the input image by the HSV color model of structuring element. The HSV color model can represent the natural of visual color on images [23]. Then, the fuzzy opening operation is applied to discard small patches or residues [2]. The example result of nucleus segmentation on microscopic blood cell image is shown in Figure 2.



(a) (b) (c)

Figure 2. The result of nucleus segmentation (a) original image (b) HSV color space and (c) segmented nucleus

The cytoplasm area is around the nucleus of white blood cell. The literatures [2, 5] use the binary morphology to segment cytoplasm of WBC. The important thing of mathematical morphology method is how to determine the structuring element especially size. The size of structuring element is usually depending on the problem. Therefore, many researches are still focus on how to automate

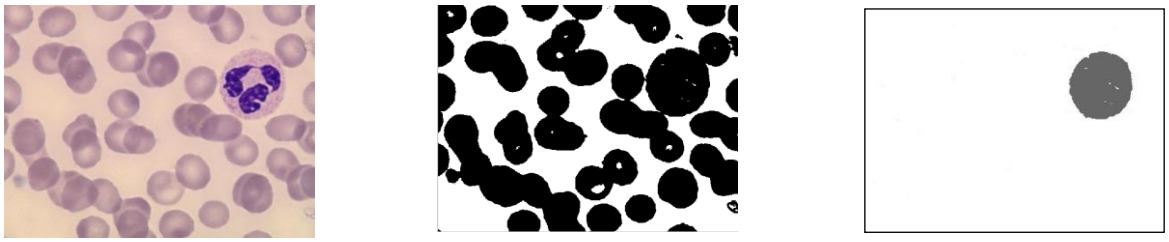
the size of structuring element if there are varieties of data [24]. In this paper, the binary morphology using structuring element with size based on the granulometric size distribution of red blood cell is used [2]. The sizes of WBC are usually larger than the sizes of red blood cell (RBC), therefore the size of structuring element is based on the granulometric size distribution of RBC. The granulometric size is defined by granulometry and a size distribution concept that is called size distribution function. The size distribution function of size r indicates the area ratio of the regions whose sizes are greater than or equal to r [2]. The size distribution function of image X by structuring element B can be expressed as follows:

$$F_{X,B}(r) = A(X_{rB}) / A(X), \tag{5}$$

where r is the size and $A()$ indicates the area of image objects. rB is defined by the Minkowski set addition as follows:

$$rB = B \oplus B \oplus \ldots\ldots \oplus B \text{ ((}r\text{-1) times)} \tag{6}$$

The first process of WBC segmentation computes the granulometric size distribution of RBC from the binary image. The next process creates a binary structuring element with the size distribution of the red blood cell to get the cytoplasm object accurately. The final process is main process of cytoplasm segmentation that it applies the opening operation on the binary input image through binary structuring element. The results of cytoplasm segmentation are whole area of white blood cells. The example result of cytoplasm segmentation on microscopic blood cell image is shown in Figure 3.



(a) (b) (c)

Figure 3. The result of Cytoplasm segmentation (a) original image (b) binary image and (c) segmented cytoplasm

III. THE OVERLAPPED WHITE BLOOD CELL (WBC) IDENTIFICATION BASED ON GEOMETRIC FEATURES

Some works on overlapped cell identification are available in the literature [11-14], however, they are not focus on white blood cell image segmentation. The literature [11] use fungi spore images and use corner detection algorithm to identify the touching cells. The literature [12] uses the watershed algorithm to segment overlapping and aggregating cells and uses significant concavity points to identify the overlapping/aggregating cells of normal/pathological nuclei cells. The literature [13] uses a two stage graph cut based model for segmentation of touching cell nuclei in fluorescence microscopy images. The literature [14] uses an unsupervised Bayesian classification scheme for separating overlapped nuclei of immunofluorescence and cervical cells images. There are varieties of overlapping cell of white blood cell on microscopic blood cell images that are shown in Figure 4. Most of overlapping cells of white blood cell object are around nucleus area. To identify the overlapped cell, a combination of boundary-based and region based approach is used in this paper.

The overlapped cells are usually larger than single cells and have eccentricity close to ellipse. Because of size varieties of white blood cell on microscopic blood cell images, we determine the size and the eccentricity of single cell from input image. Based on the analysis of single cell samples, the single cell have differences in eccentricity between 0 - 0.65 while the overlapped cells have eccentricity above 0.65. To determine the size or area of overlapped cell based on the analysis that the overlapped cells have relatively large area compared to the average single cell. The analysis of area and eccentricity of overlapping cells obtain rules to detect overlapping region of cells. The rules are defined as follows:

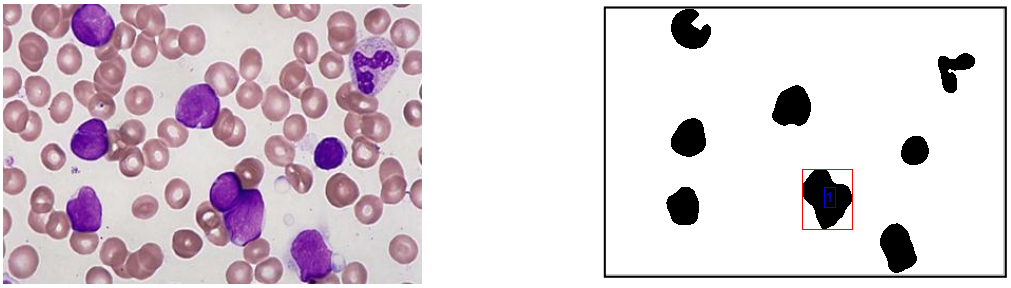
a. Region which have an eccentricity > 0.65

b. Region which have area > threshold, with threshold = mean (area) - (0.3 * standard deviation (area)).

Based on the analysis above, the size of overlapped cell are obtained from aggregrating the size of single size. Therefore, we calculate the number of cell of overlapped cell are defined as follows.

$$n = A \,/\, (\text{mean}(B) - 0.5 * \text{standard deviation}(B)) \quad (7)$$

where n is number of cells of overlapped cell, *A* is area of overlapped cell and *B* is area of single cell.



(a) (b)

Figure 4. Overlapped WBC Identification (a) original image (b) overlapped cell identification

To evaluate the accuray of number of cells on overlapped cell, the result of proposed method are compared with the manual counting. To calculate the accuracy of white blood cell segmentation, the region of overlapped and non overlapped cells are calculate to compare with the white blood cell manual segmentation. The evaluation procedure and the analysis of result are presented in the next section.

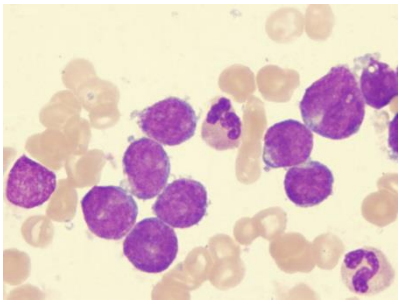
IV. EXPERIMENTAL RESULTS ON WHITE BLOOD CELL SEGMENTATION

To evaluate the performance of the proposed method, a sample of 7 images of the microscopic white-blood-cell (WBC) images and microscopic leukemia image samples are used. The segmentation results of the proposed method are compared with manually segmented images. The manually segmented image is considered to be the correct segmentation result. Manually segmentation of an image is done by marking the nucleus and cytoplasm area on microscopic blood cell images. Figure 5 shows samples of microscopic blood cell image and corresponding manually segmented images. By manual segmentation, the accuracy rate can be quantifiably calculated.

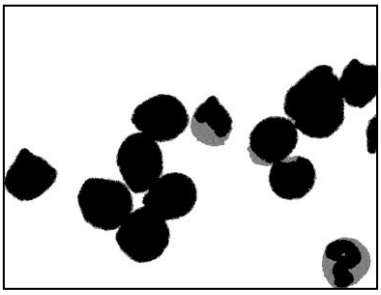
The metrics for evaluating the segmentation accuracy use Precision, False Positive (FP) rate, and False Negative (FN) rate. Precision is calculated as the ratio of the number of pixels that are correct (True Positive/TP) to the number of pixels classified as nucleus or cytoplasm (False Positive/FP). FP is calculated as the ratio of the number of pixels that are incorrect) to the number of pixels classified as nucleus or cytoplasm. FN is calculated as the ratio of the number of pixels that are

incorrectly classified as background to the number of pixels classified as background. The experimental results of WBC image segmentation and the detail information of segmentation results (precision, true positive and false negative) are presented in Table. 1.

The experimental result shows that the highest accuracy is 93.16% for nucleus segmentation and 79.87% for cytoplasm segmentation, respectively. From all result of microscopic blood cell images, we can calculate the average accuracy of nucleus and cytoplasm segmentation. The average accuracy of nucleus segmentation is 85.22% and the average accuracy of cytoplasm segmentation is 70.27%.



(a)



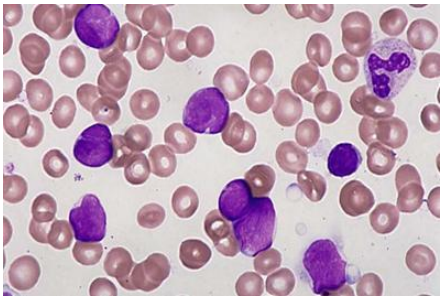
(b)

Figure 5. Samples of Manual Segmented Microscopic Blood Cell Image (a) original image (b) WBC manual segmented

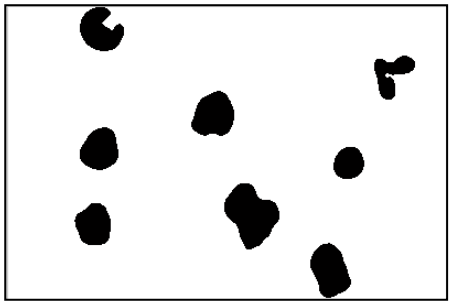
Table 1. Accuracy of WBC segmentation on microscopic blood cell images

Images	**WBC segmentation accuracy (%)**					
	Nucleus			**Cytoplasm**		
	Precision	**FP**	**FN**	**Precision**	**FP**	**FN**
Image #1	86.17	13.83	0.44	74.63	25.37	0.94
Image#2	92.16	7.84	0.25	63.19	36.81	1.63
Image#3	83.32	16.68	0.17	64.42	35.58	0.56
Image#4	93.16	6.84	0.25	75.19	24.81	1.63
Image#5	89.21	10.80	0.21	79.87	20.13	0.72
Image#6	71.29	28.71	0.13	56.46	44.54	0.79
Image#7	82.25	17.75	0.15	78.19	22.81	0.73

The experimental result of each process or phase of WBC image segmentation is visually presented in Figure 6 (a) – (e). The Figure 6 (a) is original image of microscopic blood cell image. Figure 6 (b) is the result of nucleus segmentation and Figure 6 (c) is the result of overlapped cell identification. Figure 6 (d) is the result of cytoplasm segmentation and the final result is WBC segmented by proposed method shown in Figure 6 (e). In addition, the proposed method identifies and counts the number of overlapping cells similar with manual white blood cell counting as shown in Table 2. From all of microscopic blood cell images, there are two images that did not obtain 100% correctly counting.



(a)



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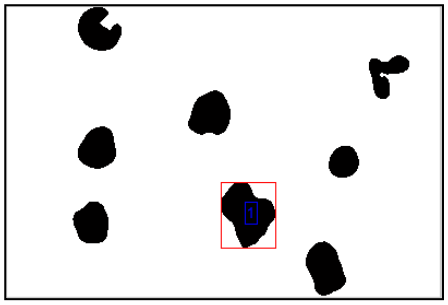
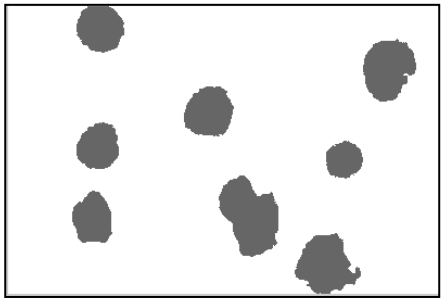
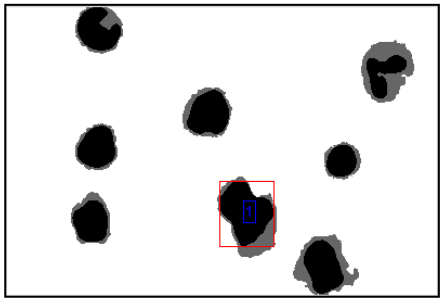


(c)



(d)



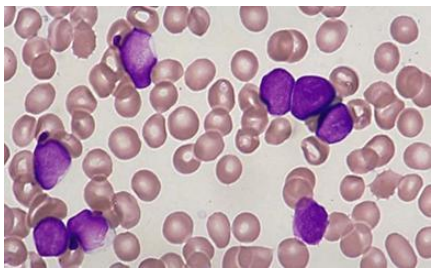
(e)

Figure 6. The experimental results of WBC Segmentation on Microscopic Blood Cell Image (a) original image (b) segmented nucleus (c) identified overlapped cell (d) segmented cytoplasm (e) WBC segmented by proposed method

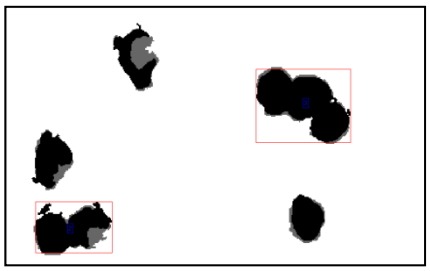
Table 2. WBC Counting Accuracy of Proposed Method

Images	Manual Counting	Proposed Method	Accuracy
Image #1	19	19	100%
Image #2	9	9	100%
Image #3	8	8	100%
Image #4	12	12	100%
Image #5	13	13	100%
Image #6	14	13	92%
Image #7	13	12	92%

The experimental result of some samples of WBC image are visually presented in Figure 7 that (a, c, e) are original images and (b, d, f) are WBC segmented images by proposed method. The red rectangles on WBC segmented images indicate the overlapped cells and the number of cells in the overlapped cells is shown with the blue rectangle.



(a)



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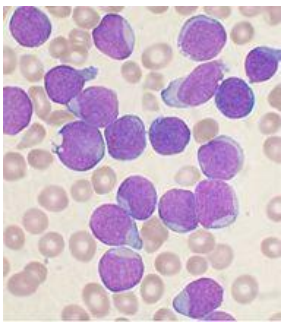

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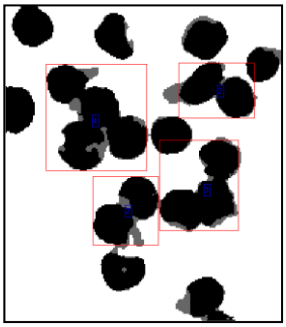
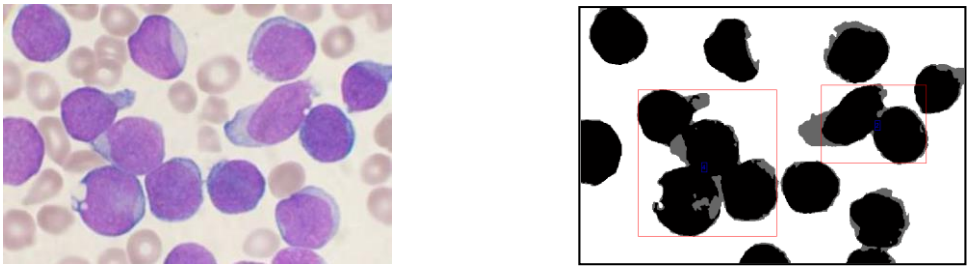


(d)



(e) (f)

Figure 7. The experimental results of WBC Segmentation on Microscopic Blood Cell Image (a, c, e) original images (b, d, f) WBC segmented images by proposed method

V. CONCLUSIONS

Overlapping cells identification on microscopic blood cell images is proposed for increasing the accuracy of white blood cell segmentation. The accurate identification of overlapping cells can increase the accuracy of cell counting system for diagnosing diseases The overlapping cells have different characteristic such as area and shape with a single cell of microscopic cell images therefore the overlapping cell identification based on geometric feature is preferred. The proposed scheme for white blood cell segmentation are divided three process i.e. nucleus segmentation, overlapped cell identification, and cytoplasm segmentation. The first process is nucleus segmentation because easier to identify the location of white blood cell and overlapped cell using the nucleus. Based on the statistical analysis, the overlapped cells are larger and more oval than the single cells. Therefore the geometry features such as area and eccentricity are used in this paper to identify the overlapping cells. After identify the nucleus and overlapped cells, the final process is cytoplasm segmentation. The final result of white blood cell segmentation is combination the result of nucleus segmentation with identified overlap cell and the result of cytoplasm segmentation

To evaluate the performance of WBC image segmentation, the 7 samples of the microscopic blood cell image samples are used. The segmentation results from the proposed method are compared with manually segmented images. The manually segmented image is considered to be the correct segmentation result. As a result, the proposed method identifies and counts the number of overlapping cells similar with manual white blood cell counting. In addition, the proposed method segment nucleus and cytoplasm of white blood cell with average of accuracy 85.22% and 70.27%

from the manual segmented respectively. For future work, the results can be extended to separate the identified overlapping cell therefore it can applied for differential white blood cell counting for diagnosing diseases.

The WBC image segmentation is the most important task for WBC classification in the automatic WBC different counting system. High accuracy results of WBC image segmentation are needed to get the best performance of WBC classification for WBC type differential counting.

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