A SIMPLE TOOL FOR IDENTIFYING OUTER SHAPE OF WHITE BLOOD CELL BASED ON IMAGE PROCESSING TECHNIQUES IN ORDER TO DEVELOP HEALTH FACILITIES IN DEVELOPING COUNTRIES

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A Simple Tool for Identifying Outer Shape of White Blood Cell Based on Image Processing Techniques in Order to Develop Health Facilities in Developing Countries

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Abstract
The shape and size of white blood cells in the blood are often used as indicators of the white blood cell abnormalities. Meanwhile, the diameter of the white blood cells can be used for cell classification and identification if there are abnormalities of white blood cells. During the identification of white blood cells is done manually by medical personnel with the help hemocytometer that calculation results are still dependent on the observer subjectivity. Another way is by hematology analyzer that has a more precise accuracy but at a costly price. Digital image processing is a method of processing an object in a digital image automatically. In this case, it can be an alternative calculation of the s and diameter of blood cells. In this research, the white blood cells identified using Active Contour methods. The results show that this method successfully detects the contours of 200 white blood cells images in a relatively short time.
Keywords: White blood cell, Diameter calculation, Hemocytometer, HSV method, Color segmentation

Introduction
White blood cells or commonly called leukocytes are cells of the immune system that protects the body against infectious disease and foreign particles. All leukocytes are produced and derived from multipotent cells in the bone marrow known as a hematopoietic stem cell. Leukocytes can be found throughout the body, including blood and lymph system. The number of white blood cells in the blood are often used as indicators of the white blood cell abnormalities. If the number of white blood cells is too little, the body will have problems in the fight against infection. In contrast, the number of white blood cells is too much can indicate leukemia, certain infections, or conditions like measles or whooping cough. While the diameter of the white blood cells is useful to classify the white blood cells, as well as indicating abnormalities in the white blood cells if the diameter of abnormal blood cells [1].
In general, the white blood cell count is done manually using a microscope and hemocytometer by a physician or medical personnel health laboratories. But it does have the disadvantage that the subjectivity of the human is very high, which might be due to lack of concentration or misuse of the equipment itself. In addition, it also time consuming. Another way is to use a hematology analyzer. This tool can calculate simultaneously measuring the diameter of blood cells automatically, but the expensive price makes this tool still rarely used. Therefore, it is need other methods as an alternative in the calculation of the number and diameter of blood cells automatically and affordable.

Currently, image processing techniques into one method that is widely used as an alternative to solve the problem. Among them are applicable in the field of health, especially in medical imaging. There are some research that discuss about using image processing techniques in case of blood cell classification. Kaewkamnerd [2] proposed an automated detection and classification of parasites on thick blood films, which contain more numbers of parasite per detection area. The system is based on digital image analysis and featured with motorized stage units, designed to easily be mounted on most conventional light microscopes used in the endemic areas. Walke [3] proposed thermal image processing technique for detecting malaria using General Fuzzy Min-Max neural network. Su [4] proposed a new segmentation algorithm for the segmentation of white blood cells from smear images. Madhloom [5] proposed a new method that integrates color features with the morphological reconstruction to localize and isolate lymphoblast cells from a microscope image that contains many cells. The localization and segmentation are conducted using a proposed method that consists of an integration of several digital image processing techniques. Harbi [6] an application of image segmentation, feature extraction, selection and cell classification to the recognition and differentiation of normal cell from the blast cell. Guo [7] proposed an image analysis of White Blood Cells in bone marrow microscopic images, multispectral imaging techniques with spectral calibration method to acquire device-independent images. Panchbhai [8] proposed a system based on used of RGB color space, G layer processing, and segmentation of Red Blood Cells (RBC) as well as cell parasites by auto-thresholding with offset value and use of morphological processing. Reta [9] proposed a cells separation algorithm to break up overlapped regions. They presented presents a contextual analysis methodology for the detection of acute leukemia subtypes from bone marrow cells images. Koltsov [10] proposed a segmentation method for the microscopy of images of blood cells using edge detection, contour closing, and over segmentation elimination. Avci [11] proposed a new technique based on Adaptive Discrete Wavelet Entropy Energy and Neural Network Classifier (ADWEENN) for recognition of urine cells from microscopic images independent of rotation and scaling. Zhang [12] proposed a novel method for the nucleus and cytoplasm segmentation of WBCs for cytometry. A color adjustment step was also introduced before segmentation. Color space decomposition and k-means clustering were combined for segmentation. Wenhua [13] proposed a method of level-set 3D segmentation for White blood cells using canny. The Level-set segmentation was based on geometric active contour models instead of parameter active contour models. Liu [14] proposed a novel method for segmentation of white blood cells (WBCs) in peripheral blood and bone marrow images under different lights through mean shift clustering, color space conversion and nucleus mark watershed operation (NMWO). The proposed method focuses on obtaining seed points. Ko [15] proposed a new image-resizing method using seam carving and a Saliency Strength Map (SSM) to preserve important contents, such as white blood cells included in blood cell images. Kekre [16] proposed vector quantization technique for segmentation of blast in acute leukemia images. Lei [17] showed an example for the design of automatic image segmentation system by using deep staining of blood cell image. Nguyen [18] proposed a new method for splitting clumped cells into single cells supporting useful information for classification and detection infected cells. The proposed method is mainly focused on rapidly detecting central pointusing the distance transform value. Sushma [19] discussed about the advancement being made in the medical image processing towards an effective diagnosis of the breast cancer from the mammogram image in radiology. Fatichah [20] proposed a fuzzy feature representation for white blood cell differential counting to diagnose types of acute leukemia. The accuracy of diagnosis is higher than that by numerical features by dealing with uncertainty of white blood cell features and inflexibility of diagnosing. Maitra [21] proposed an approach to automatic

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segmentation and counting of red blood cells in microscopic blood cell images using Hough Transform. According to the literatures review above, most of the research using complex method for recognizing blood cell. In our research we proposed a simple method for segmenting white blood cell. Hossen [22] proposed a design and develop an automatic floor cleaning robot that can navigate and clean the floor using software based on Fuzzy algorithm. In this paper, we emphasized our scope about using Active Contour methods for identifying shape and diameter of white blood cell. It motivated our previous research work [23] [24] [25] [26] [27] [28] [29] [30] [31] using a wide variety of image processing techniques in medical image applications. Almost all the results of previous research dedicated to developing countries, especially rural areas. In connection with this research, the pathologist in Indonesia, especially in Banyumas, still observing the contours of the white blood cells manually using a microscope. Observations were done manually is very prone to human error. To minimize this risk, the research aiming to identify the contours of the white blood cells automatically via image processing software based active contour method.

Methodology

Data Acquisition

This research uses image data of white blood cells as many as 200 images obtained from the official website Image Data Base Acute lymphoblastic leukemia (ALL-IDB) University degli Studi de Milano, Italy, as already published in some previous research [32] [33] [34] [35]. Figure 1 shows an example of white blood cell image that used in this research.

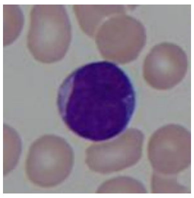


Fig 1. An example of white blood cell image

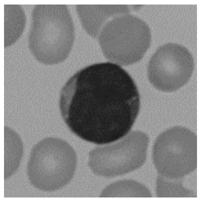


Fig 2. Gray image

(Source: Image Data Base Acute lymphoblastic leukemia (ALL-IDB) University degli Studi de Milano, Italy)

The input image is still a RGB image as seen in Figure 1. However, in the process of developing the algorithm, the image is converted into a Gray image as seen in Figure 2.

Design System

Our system design which will be applied in this research is described in Figure 3. As explained in subsection 2.1, input image is RGB, therefore we have to convert into greyscale image. Then we have to determine an initial masking for detecting white blood cell. In this stage we use an automatic cropping by determining coordinate of initial masking. This is because the input images have similar characteristics. The next step is applying active contour method for detecting outer shape of white blood cell, therefore we could calculating perimeter, diameter and area of white blood cell.

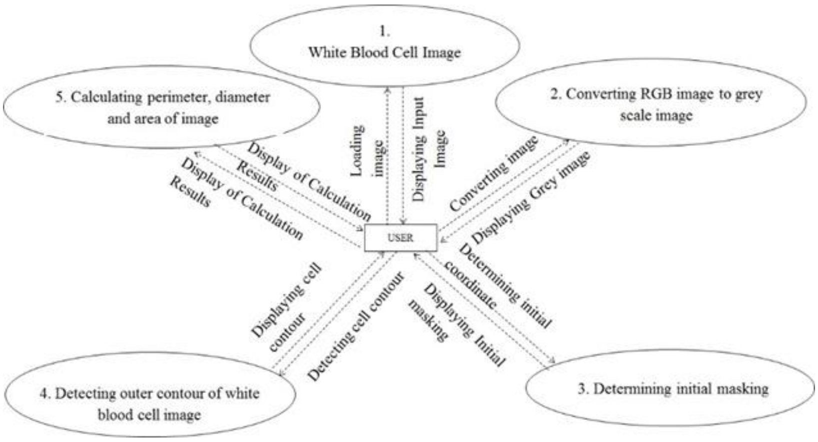
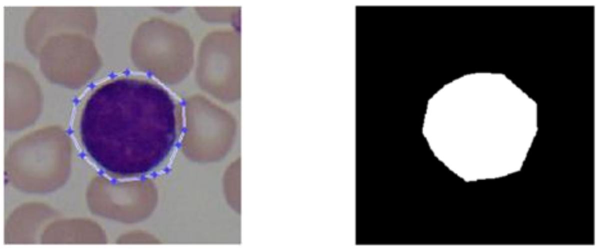


Fig 3. DFD System

Initial Masking

Initial masking is a step of determining the initial curve and manufacture that will be used in the detection of cell contours using active contour method. This initial curve in the form of a closed curve, which means the curve starts from the same point as the point of termination. This stage is important because active contour method will start working at this starting point. Curve was first formed with the same size as the input image but with a value of zero to the entire pixels. Calculations to determine the beginning of the curve is calculated based on the size of the input image. Figure 4 shows an example of initial masking process.



(a) Initial point (b). Initial process result

Fig 4. Initial masking process

Object Segmentation

Segmentation aims to find individual object from an image by dividing an image into sections that are connected and have some similarities and some differences between the areas between the surrounding areas.

This stage runs the contour detection process of white blood cells using active contour method. Curve predefined coordinate is moving narrowed or widened in accordance contour an object, the movement will take a maximum of iterations performed. Phase contour detection, is the step of administering line with a different color on the contour of the image of the white blood cells in the previous stages have been processed using active contour method. Line is placed overlap with the input image so that the image contours can be seen clearly. Figure 5 shows an example of object segmentation result.

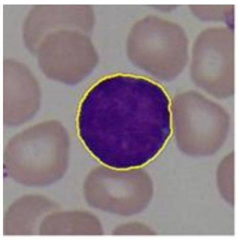


Fig 5. An example of contour image detection results accompanied contour lines

Perimeter, Area and Diameter Calculation

The calculation of the perimeter is done by counting the number of pixels of value 1 from the edges of the image segmentation results were reduced by the image segmentation has been in erosion. When the pixel is found worth 1 then the variable results will increase the value of +1, but if it is not worth 1 then the outcome variable is worth staying or not increased.

Diameter calculation phase is the stage where the program calculating the value of the furthest distance between the contour pixel to another pixel of the image of the white blood cells that have been segmented and obtained its contours.

Results and Discussions

Designing of the system is visualized in the form of Graphic User Interface (GUI) provided by the software Matlab R2013a. GUI itself is a form of interface between systems and users to facilitate the use of systems designed applications that can be easily understood by the user. Graphic User Interface is awakened from a combination of objects graph form (push button), axes (graph), panels, and text that includes static text, and edit text.

In the design of the GUI that we made, there are eight push button, four axes, five edit text, seven static text, and two panels with their respective functions. Four axes each axes1 used to display the original image of the white blood cells and axes2 used for displaying image processing results of each stage, while axes3 and axes4 used to display images and symbols to identify themselves and decorate GUI. Eight push button is useful to load the image, convert to grayscale, initial masking, object segmentation, contour detection, calculating the perimeter and calculate diameter. Five of static text is used to display information related to the program. Two panel serves to unite the six push button and to unite all the GUI objects. Figure 6 shows our design system in GUI.

Data microscopic image of white blood cells used in this experiment were 200 image, the shape of the contours of the white blood cells are manifold. In the process of detecting the contour of the white blood cells using a GUI Matlab R2013a, the researchers conducted several stages which includes the step Load Image or insert the image into the program which will be processed, the stage of conversion to grayscale, the step of determining the curve closed early (initial masking) either manually or automatic, phase image segmentation using active contour, contour detection cell stage, the stage of calculating perimeter, area, and the diameter of the cell, and save the image of the stage. Especially for the save process, can be done at every stage from initial application to the conversion of gray levels until the final stage.

Variables used to accommodate the conversion of the input image into grayscale image is, the variable gray with image conversion using existing toolbox in Matlab R2013a be *rgb2gray*. Table 1 show result examples of conversion RGB image to grayscale image.

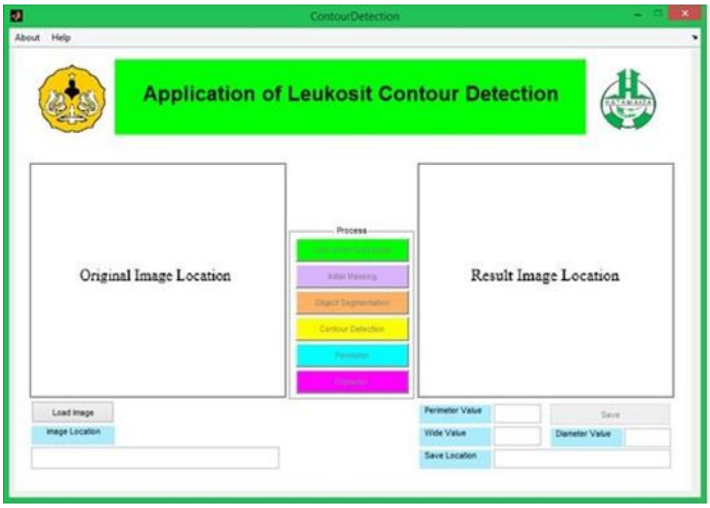
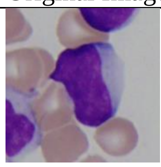
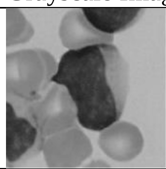
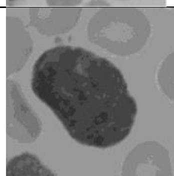
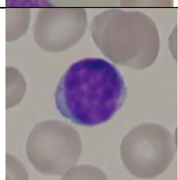
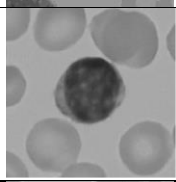
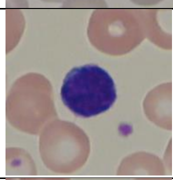
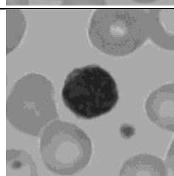
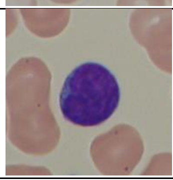
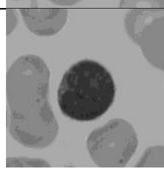


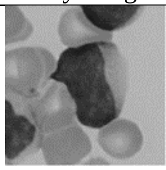
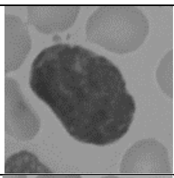
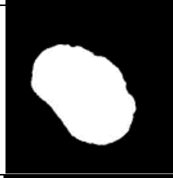
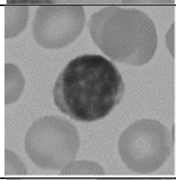
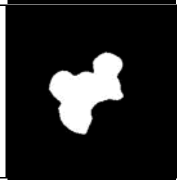
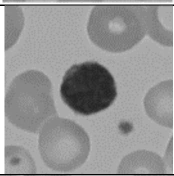
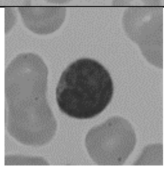
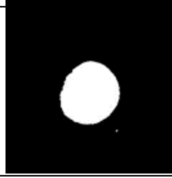
Fig 6 GUI display of White Blood Cells Contour Detection

Table 1. Examples Results of Grayscale Conversion

No	Original Image	Grayscale Image
1		
2		
3		
4		
5		

At segmentation stage, curve predefined coordinate is moving narrowed or widened in accordance contour an object, this movement will take place (maximum) of iterations performed by the program. Examples of image of segmentation using active contour method can be seen in Table 2

Table 2. Examples of Segmentation Result

No	Gray Image	Segmentation Result
1		
2		
3		
4		
5		

Phase contour detection, is the step of administering line with a different color on the contour of the image of the white blood cells which in previous stages have been processed using methods of active contour function. This line laid stacked with the original image so that it can be seen clearly the contours of the image. This phase will begin when the program is run by the user pressing the "Contour Detection" in the view GUI application designed. The program executed syntax is as follows.

```
global seg img
imshow(img);title('The Contour of Image');
hold on
contour(seg, [0 0], 'y','LineWidth',2);
```

In a piece of the program, the variable seg, and img, given the right of public access so that its value will be consistent wherever these variables used in the program. Seg variable is a variable that has previously been established and has a value of image segmentation active contour method, while the img variable is a variable that has previously been initialized as a variable that holds the value of the original image. Images in the img variable, is displayed using the function imshow on axes2 (graph 2), entitled "The Contour of Image". Display image in axes2 retained alongside the appearance of contour lines. The command hold on will hold the image in the img variable persists in axes2 and contour command (Seg [0 0], 'y', 'linewidth', 2) will display the contour lines in yellow and a thickness of 2 pixels thick contour lines. Figure 7 shows the GUI of the image that has been displayed in axes2 (graph 2).

After successfully detect the contours of the white blood cells, then the system performs the calculation of the perimeter, diameter and area of the white blood cells. Perimeter and area value calculation will be made when user push the button "perimeter" that will cause the program to execute the following syntax.

```
global seg K
seg2 = seg;
H = ones(2);
G = erosi(seg2,H);
K = seg2-G;
[height, width] = size(K);
result = 0;
for p = 1 : height
    for q = 1 : width
        if K(p, q) == 1
            result = result + 1;
        end
    end
end
perim = result;
wide = area(seg2);
set(handles.editperim,'string',perim);
set(handles.editwide,'string',wide);
```

Variable seg and K in pieces over the program, given the public access rights so that this variable can be accessed anywhere in the program and have a consistent value wherever the variable is used. Perimeter calculation is done by counting the number of pixels is 1 of the edge image segmentation results were reduced by the image segmentation has been erosion using matrix ones (2). Seg2 variables are variables that are made to existing backup image value in a variable seg, with the aim that the original value of the image in the variable seg not bothered changes were made in the operation of this stage. If Perim is the variable used to hold the value of the circumference of the image segmentation of the wide variable is the variable used to accommodate the broad value of image segmentation.

```
function result = area(BW)
[height, width] = size(BW);
result = 0;
for p = 1 : height
    for q = 1 : width
        if BW(p, q) == 1
           result = result + 1;
        end
    end
end
```

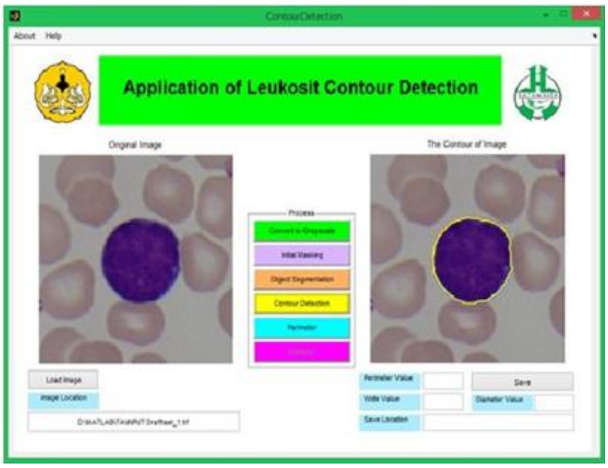


Fig 7. An example of contour detection result in our GUI

Perimeter and area value has units of pixels, and will be displayed in the GUI in the two text edit field. Perimeter value will be displayed in the text edit field named *editperim* and area will be displayed in the text edit field named *editwide*.
Diameter calculation phase is the stage where the program calculating the value of the furthest distance between the contour pixel to another pixel of the image of the white blood cells that have been segmented and obtained its contours.

```
global seg img
imshow(img);title('Image Contour With The Diameter');hold on;
contour(seg, [0 0], 'y','LineWidth',2);
[d,x1,y1,x2,y2] = get_diameter(seg);
X = [x1,x2]; Y = [y1,y2];
line(X,Y, 'Color', 'r')
set(handles.editdiam,'string',d);
```

Variable *img seg* and in pieces above program is a variable that has previously been formed. *Img* is the variable used to hold the original image when the image while *seg* Load stage is a variable that is used to hold the value of image segmentation using active contour method. Diameter will be obtained from the largest value obtained and stored in the variable *distance_max* were finally transferred in a variable diameter. Original image previously stored in the variable *Img* will be shown to axes2 (graph 2) using the function *imshow* and maintained appeared to then stacked in a straight line bright red longitudinal of pixels at coordinates (x1, y1) to pixels at coordinates (x2, y2). Diameter has a value in pixels and will be displayed in the GUI on the text edit field named *editdiam*. Figure 8 shows an example of calculation results in our GUI.

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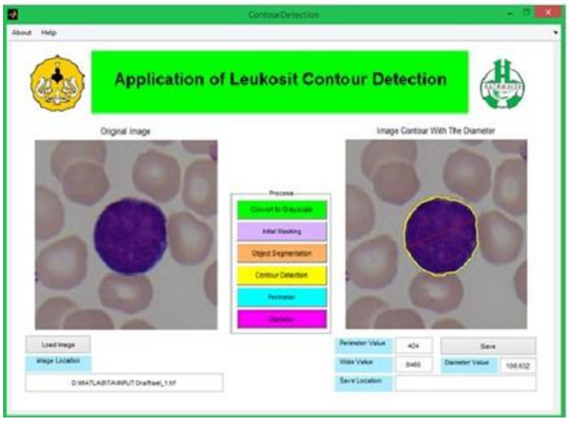

Fig 8. An example of calculation result displayed in our GUI

As for the recapitulation of the calculation of the area and perimeter of the white blood cells are shown in Figure 9. Meanwhile diameter calculation results are shown in Figure 10.

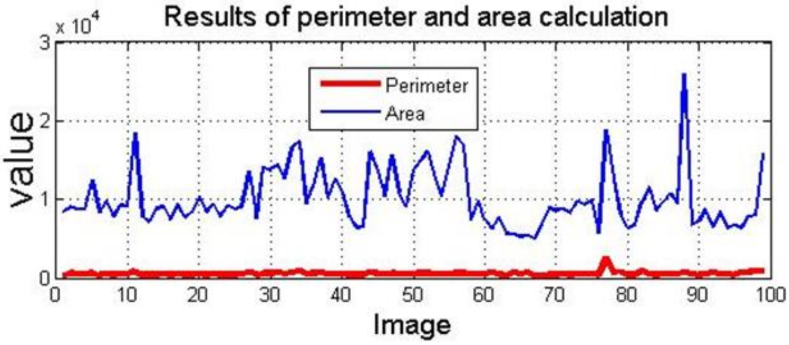

Fig 9. Results of perimeter and area calculation

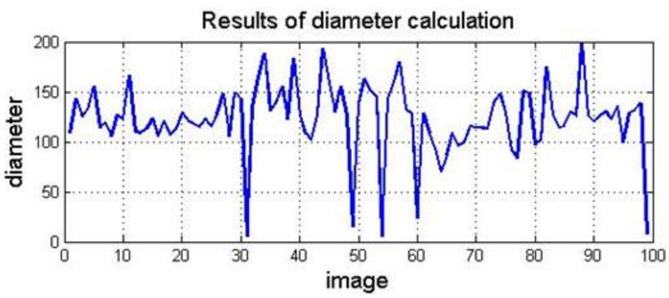

Fig 10. Results of diameter calculation

Some of the records obtained in this experiment are: (i) outer contour detection system of white blood cells using active contour method capable of recognizing the outer contour of the white blood cells properly. Every contour of 200 images of white blood cells is able to be obtained with a relatively short time, ie less than 10 seconds. (ii) Initial masking done manually or automatically. Initialize the curve manually done by determining the coordinate points on the original image through mouse / pointer while the automatic initialization is done by calculating the value of the existing image in the system which refers to the size of the input image as a determinant calculation coordinate initial value masking. (iii) Graphic User Interface (GUI) that is designed to use digital image processing application Matlab can run stably throughout the operation and able to perform image recognition of research in large quantities. (iv) Each image of the white blood cells have a different value of perimeter, area, and diameter. So it can be information for the user to later classify independently according to their requirement.

Conclusions

This research has been able to recognize the outer contour of the white blood cells with a square image size using active contour method as well, using the masking initial both set manually or automatically. According to the performance of active contour method that is reliable in recognizing the shape or contour, on further research we will try to apply this method in the microscopic images taken under uncontrolled conditions. So that it is hoped will be applied to areas that are deficient health facilities.

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