Preliminary process in blast cell morphology identification based on image segmentation methods

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ABSTRACT

The diagnosis of blood disorders in developing countries usually uses the diagnostic procedure complete blood count (CBC). This is due to the limitations of existing health facilities so that examinations use standard microscopes as required in CBC examinations. However, the CBC process still poses a problem, namely that the procedure for manually counting blood cells with a microscope requires a lot of energy and time, and is expensive. This paper will discuss alternative uses of image processing technology in blast cell identification by using microscope images. In this paper, we will discuss in detail the morphological measurements which include the diameter, circumference and area of blast cell cells based on watershed segmentation methods and active contour. As a basis for further development, we compare the performance between the uses of both methods. The results show that the active contour method has an error percentage 5.15% while the watershed method has an error percentage 8.25%.

Keywords:
Active contour
Blast cell
Developing countries
Image processing
Watershed

1. INTRODUCTION

Identification of blood cells is one of the diagnostic procedures used to identify various diseases. This diagnostic procedure is commonly called the CBC. CBC is a blood test that provides information to doctors about five main parts of blood. The five parts are three types of cells (red blood cells, white blood cells, and platelets) and two types of values (hemoglobin value and hematocrit value) [1]. This CBC procedure is widely applied in developing countries including Indonesia because of limited facilities and resources in the medical field. However, the CBC process still poses a problem, namely that the procedure for manually counting blood cells with a microscope requires a lot of energy and time, and requires a high cost. Blood cells are classified into several types, namely red blood cells, white blood cells often called as leukocytes, and blood platelets. The blood component has specific roles and functions in the circulation process throughout the body. Blood can experience abnormalities that occur mainly in its constituent structures. This disorder results in a disorder or disease in the body. One blood disorder that can occur is acute lymphoblastic leukemia (ALL).

Cancer death rates including leukemia are higher in developing countries compared to developed countries. This difference reflects differences in risk factors and the success of handling detection, as well as the availability of treatment [2]. According to the latest World Health Organization (WHO) data published in 2017 leukemia deaths in Indonesia reached 9,179 or 0.55% of total deaths. The age adjusted death rate is 4.20 per 100,000 of population [3]. According to the fact, it is seen that the presence of blood cell abnormalities such as leukemia is a case that needs serious attention.
As explained in the paragraph above, one of the causes of the high mortality rate of leukemia in developing countries including Indonesia is the limited human resources and health facilities. One alternative to overcome this problem is the implementation of technology that is cheap and easy to use, but also accurate, and one of them is digital image processing. Research on leukemia that involves the use of digital image processing itself is quite a lot. Zhang [3] researched leukocyte detection using digital image processing, specifically a combination of image segmentation and pattern recognition. In the segmentation of the image, he divided the image of leukocytes into several parts. While for the classification he uses support vector machine (SVM). Mohapatra [4] introduced the clustering method using shadowed c-means (SCM) in the segmentation of blood microscopic images. SCM method is used to classify each pixel in 4 clusters. The algorithm is used to separate the nucleus and cytoplasm in each sub-image. For the classification he uses support vector machine (SVM). Carpio et al., [23] presented the PCSeg Tool for segmenting blood plasma cell images. SCM method is used to classify each pixel in 4 clusters. While for the classification he uses support vector machine (SVM).

2. RESEARCH METHOD
2.1. Data acquisition
All the data used in this research came from the Pathology laboratory, Hospital "Dr. Margono Soekardjo," Banyumas, Central Java, Indonesia. Figure 1 shows the stages of the data collection for this research. According to Figure 1, from the pathology laboratory, the medical team examined blood cell smears using a standard microscope. The primary purpose of using this conventional microscope is to create a pilot project so that this can be applied in some rural areas in Indonesia where the district does not yet have a digital microscope facility. Currently, it has only digital microscope facilities, just in large hospitals. In Figure 1, an example of the input image that will be used in this research is also shown, which shows that the image input used has a different illumination level.
2.2. Pre-processing image

2.2.1. Normalization image

The input images used in this research come from several types of cameras, so the resolution of each input image is different. According to this case, we set the size of the existing input image so that the calculation results are according to parameters and do not depend on size, but also to speed up data processing time. In this research, the image is resized with a calculated scale using the formula in (1).

\[ R = \frac{400}{S} \]  

In (1), \( R \) states the resize scale, \( S \) is the smallest value of the dimensions of the length and width of the image. The value of 400 is used as the basis of the calculation to determine the scale where the smallest \( x \) or \( y \) dimension value is selected as a comparison with the value 400, and the result is used as a scale for the remaining values of \( x \) or \( y \). The above calculation is used so that can adjust the characteristics of images that have different lengths and widths. Moreover, to make it easier to recalculate the original pixel size. Table 1 is a comparison table of original dimensions with resizing dimensions.

| No | Original Dimension | Resize Dimension |
|----|--------------------|------------------|
| 1  | 640 x 480          | 534 x 400        |
| 2  | 960 x 720          | 534 x 400        |
| 3  | 4000 x 3000        | 534 x 400        |
| 4  | 4128 x 2322        | 712 x 401        |
| 5  | 581 x 1032         | 400 x 711        |

2.2.2. Cropping

At this stage, the images obtained are cut using the cropping function in order to obtain the Region of interest (ROI). Cropping is done automatically adjusting the ROI of the image. The process is done by changing the image to binary with a scale of 0.7 then noise cleaning is done by removing objects that have a pixel count of fewer than 4000 pixels. This is done to get objects that only contain ROI. Then scan the object to find the maximum and minimum coordinate values of \( x \) and \( y \). The results are used to calculate the length and width of the object by subtracting the maximum value of the \( x \) and \( y \) coordinates with the minimum value than using the minimum value of the \( x \) and \( y \) coordinates as the initial coordinate of cropping. Figure 2 shows the image before and after cropping.

2.2.3. Thresholding

Thresholding in this system aims to remove other objects other than objects of white blood cells in order to improve accuracy at the stage of segmentation that will be carried out. This step is done by utilizing RGB value differences in white blood cell objects and other objects. Sampling is carried out on five pieces of images that represent the condition of each input image. RGB values are taken from 9 pixels of each image consisting of 3 pixels of white blood cells, 3 pixels of red blood cells, three pieces of background pixels-Table 2 shown this process.
2.3. Active contour segmentation

Active contour is a segmentation method using a closed curve model that can move wide or narrow to adjust the boundary of a segmented object [24]. In this experiment, Chan-Vese model contour was used where this model is a region-based active contour. In this model statistical information is used inside or outside the curve to determine the direction of evolution and the amount of energy used as described in (2).

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\[
(c_1, c_2, C) = \mu.\text{length}(C) + v.\text{area}(C)
+ \int_{\text{inside}(C)} |\mu_0(x, y) - c_1|^2\,dx\,dy + \int_{\text{outside}(C)} |\mu_0(x, y) - c_2|^2\,dx\,dy
\]

(2)

According to (2), the first part defines the length of the curve, the second part of the area in the curve, the third and fourth parts define the difference in intensity of the input image and the average intensity inside and outside the curve. The value of C is the initial masking curve; the values of c1 and c2 are the intensities inside and outside the curve; the value of \(\mu_0(x, y)\) is the input image. The more curves close to the desired object boundary, the smaller the value F. Figure 3 shows an example of using the active contour method in segmenting white blood cell images.

![Chan-vee model active contour method](image)

Figure 3. Chan-vee model active contour method

2.4. Watershed segmentation

Watershed transformation is a transformation that brings the perspective of two-dimensional imagery in a three-dimensional viewpoint. Watershed transformation works on grayscale images by using watershed transformation, the image as if it has a three-dimensional form, x, y, and z. The x and y fields are the original image itself, while the z plane is the level of the ash scale in the form of valleys. The thicker the ash level, the deeper the valley [25]. This watershed segmentation method has the advantage of being able to separate objects which are coiled so that objects can be separated as shown in Figure 4. Referring to Figure 4, the object which had previously coincided can be separated by the watershed segmentation method. This is very useful when calculating objects because multicellular cells that are coiled will count as single cells so that the calculation process becomes wrong.

![Separation of Objects Coinciding with Watershed Segmentation](image)

Figure 4. Separation of Objects Coinciding with Watershed Segmentation

2.5. Post processing

The post-processing stage is a stage that aims to improve the quality of the second stage image after the segmentation process is carried out. This stage is done because the results of segmentation still allow producing small objects or noise due to the lack of or excessive process of segmentation. Image improvement must be made with good and clean image quality. The operations performed at the post-processing stage are morphological operations, objection elimination on the border edge, elimination of unusual objects, and object names.
3. RESULTS AND DISCUSSION

In this experiment, the variables to be measured in segmentation with the two methods are the detection of the number of leukocytes in each image, area, maximum diameter, minimum diameter and circumference of leukocyte cells. The first experiment was carried out by exploring information using the results of segmentation of the active contour method. Calculation of cell numbers is done by separating cell objects. The process of separating objects is done by calculating the distance transformation on the object to determine the intersection of objects that are overlapped. Distance values are calculated for each nonzero pixel. The model used in this distance transformation is a city block model. The city block model measures distance based on four neighborhoods. Where if the pixel is in area 4, the distance is calculated by one but if it is not then counted two. In order for getting the best result, the distance in the distance transformation needs to be adjusted by removing the small minima locale on the object. Figure 5 shows the results of the separation of objects in this method.

The next process is noise elimination, which functions to eliminate other objects other than the leukocyte cell object. In this process, three parameters are used for three different functions adjusting to the size of the object of the white blood cell. The parameters used are 10000, 3000, and 300 pixels. Then remove the object that is tangent to the edge of the image. Finally, which image is selected is an unusual object and which is not to be removed. The trick is to calculate the degree of roundness of each object with (3).

$$R = \frac{4\pi A}{P^2}$$  \hspace{1cm} (3)

Referring to (3), then R states the Degree of Roundness, A states Area, and P states Circumference. The roundness used in this experiment is 0.78 so if there is an object with a degree of roundness less than that it will be eliminated. Figure 6 shows some examples of the image results of segmentation by active contour method.

Referring to Figure 6, the number of leukocyte cells can be segmented using the active contour method. So that based on the segmentation display, the number of leukocyte cells can be calculated automatically. For measurements of diameter, circumference, and area, we refer to (2) above. In (2) above, $C_1$ and $C_2$ are two constants which are the average intensity inside and outside the contour, respectively. Moreover, $\mu_0$ is the input image. On active contour without the edge, it will minimize term fitting and add some term regulations, such as the length of the C curve and the area of the region in C. From the equation, the length of the curve can be measured by (4). While the area is formulated in (5).

$$\int_{C} \sqrt{1 + (y')^2} dx$$  \hspace{1cm} (4)

$$L = \frac{1}{2} \int_{C} (xy' - y)dx = \frac{1}{2} \int_{C} (xy' - y)dx$$  \hspace{1cm} (5)
In other hand, the watershed segmentation method is a series of methods consisting of watershed transformation, distance transformation, morphological operations, and other related operations. The process of identifying white blood cells in this study is divided into several stages, namely: color threshold, pre-processing, segmentation, post-processing, calculating object parameters. After going through pre-processing as described above, noise is eliminated. Noise removal aims to clean binary images from small objects that are not perfect color thresholds. Also, repairs to the edges of the object are made to be neater and smoother. This operation can be done using image morphology operations. As explained above, watershed segmentation also includes distance transformation. This distance transformation function will produce a set of matrices that contain the transformation value of the distance of each pixel. Distance values are calculated for each nonzero pixel. The chosen model is very decisive towards the results of segmentation because it can allow over segmentation when using the incorrect distance transformation method. According to our experiments using Euclidian, City block, Chess board, Quasy-Euclidian models, and most objects in the form of round objects are not perfect. Therefore, we need a model that has similar shapes or characteristics with the aim that the distance transformation is smooth and later does not cause over-segmentation. With that consideration, it can be seen that the city block method matches the characteristics of the object to be transformed, besides that this model can produce the right segmentation output, while other methods produce over segmentation output.

Referring to (3), properties that can be measured using this function include area, diameter, circumference of the object. This operation of the elimination of abnormal objects can be done by utilizing the degree of roundness of the object. In order to determine the roundness value of the object, an analysis of the value of the roundness of the object is carried out. Figure 7 shows an example of the property of the degree of roundness of an object. Based on Figure 7, the value of the degree of roundness of strange objects is lower than the standard object. The highest value of the degree of a strange object in the data is 0.53. Therefore, the degree of roundness is taken at a value of 0.6. Figure 8 is an example of an image that has experienced abnormal object elimination.

![Figure 7. An example of property of roundness object](image)

![Figure 8. Elimination of abnormal objects](image)
The way to calculate the circumference of this function is to use eight neighbors; each pixel that does not have neighbors will be counted as a circumference of the object. Calculation of diameter is done by calculating the longest distance and the shortest distance from the edge of the object. The results of diameter properties in this study will get two values for the diameter, namely the maximum diameter and minimum diameter. The extensive calculation is done by counting all pixels on the object. Table 3 shows the results of the calculation of the diameter, circumference and area of leukocytes using the active contour and watershed methods. Referring to Table 3, we make a graph of analysis that shows the relationship between the results obtained in each method as shown in Figure 9.

Referring to Table 3 and Figure 9, in general, the results of the measurement of the two methods give almost the same results for each measurement variable. However, for morphological calculations using watershed segmentation, calculation of properties which include Amount, Diameter, Area, and circumference of leukocyte images. The results of the calculation are in the form of a matrix $n \times 1$, where $n$ is the number of objects detected. The way to calculate the circumference of this function is to use eight neighbors; each pixel that does not have neighbors will be counted as a circumference of the object. Calculation of diameter is done by calculating the longest distance and the shortest distance from the edge of the object. The results of diameter properties in this study will get two values for the diameter, namely the maximum diameter and minimum diameter. The extensive calculation is done by counting all pixels on the object.

| Img | Active Contour | Watershed |
|-----|----------------|-----------|
|     | Min | P  | Max | Min | P  | Max | A    | Min | P  | Max | A    |
| C1  | 147 | 582 | 20329 | 146 | 179 | 564 | 20729 | P9  | 91 | 105 | 323 | 7417 |
| C2  | 186 | 189 | 22245 | 157 | 191 | 569 | 23058 | P10 | 92 | 104 | 325 | 7533 |
| C3  | 167 | 184 | 23815 | 139 | 164 | 494 | 17548 | P11 | 92 | 101 | 320 | 7257 |
| C4  | 154 | 200 | 28873 | 189 | 201 | 672 | 29715 | P12 | 96 | 99 | 323 | 7494 |
| C5  | 163 | 174 | 19454 | 143 | 173 | 546 | 19350 | A1  | 60 | 89 | 258 | 4029 |
| C6  | 139 | 165 | 17486 | 133 | 161 | 475 | 16635 | A2  | 73 | 90 | 272 | 3150 |
| C7  | 126 | 158 | 14504 | 124 | 158 | 455 | 15167 | C1  | 138 | 164 | 515 | 17221 |
| C8  | 125 | 158 | 14504 | 124 | 158 | 455 | 15167 | C2  | 143 | 157 | 516 | 17393 |
| C10 | 112 | 122 | 10551 | 112 | 121 | 377 | 10483 | A4  | 84 | 216 | 677 | 12338 |
| M1  | 26  | 30  | 90   | 26  | 29  | 85  | 583  | P1  | 78 | 88  | 283 | 4057 |
| M2  | 24  | 27  | 83   | 24  | 27  | 78  | 505  | P2  | 30 | 33  | 102 | 763  |
| M3  | 26  | 34  | 98   | 26  | 34  | 92  | 672  | P3  | 26 | 32  | 88  | 203 |
| M4  | 23  | 32  | 89   | 23  | 31  | 85  | 567  | A5  | 78 | 88  | 283 | 4057 |
| M5  | 28  | 41  | 117  | 28  | 41  | 112 | 856  | A6  | 84 | 216 | 677 | 12338 |
| M6  | 27  | 37  | 105  | 27  | 37  | 99  | 750  | A7  | 23 | 31  | 86  | 510  |
| M7  | 23  | 32  | 91   | 23  | 32  | 90  | 621  | A8  | 18 | 29  | 76  | 409  |
| M8  | 21  | 32  | 90   | 21  | 32  | 90  | 510  | P  | 29 | 36  | 106 | 815  |
| M9  | 28  | 33  | 98   | 28  | 33  | 112 | 981  | A9  | 32 | 36  | 110 | 912  |
| M10 | 23  | 24  | 74   | 23  | 24  | 71  | 427  | A10 | 30 | 30  | 95  | 708  |
| M11 | 29  | 36  | 111  | 29  | 37  | 106 | 779  | A11 | 23 | 31  | 90  | 536  |
| M12 | 26  | 34  | 99   | 26  | 34  | 93  | 685  | A12 | 22 | 28  | 80  | 477  |
| M13 | 37  | 42  | 134  | 37  | 42  | 129 | 1132 | A13 | 22 | 28  | 78  | 477  |
| M14 | 26  | 31  | 92   | 26  | 31  | 87  | 609  | A14 | 22 | 28  | 78  | 477  |
| P1  | 95  | 103 | 329  | 95  | 103 | 315 | 7601 | A15 | 22 | 28  | 78  | 477  |
| P2  | 84  | 109 | 323  | 84  | 108 | 308 | 6929 | A16 | 22 | 28  | 78  | 477  |
| P3  | 80  | 84  | 280  | 78  | 83  | 267 | 5051 |
| P4  | 82  | 102 | 306  | 82  | 101 | 295 | 6433 |
| P5  | 87  | 105 | 322  | 87  | 105 | 307 | 7099 |
| P6  | 89  | 96  | 305  | 89  | 96  | 292 | 6678 |
| P7  | 93  | 96  | 305  | 93  | 96  | 293 | 6093 |
| P8  | 84  | 97  | 307  | 84  | 97  | 295 | 6392 |
| P9  | 56  | 70  | 221  | 56  | 70  | 220 | 3049 |

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Figure 9. Graphs of comparison of measurement results based on active contour and watershed methods, (a) minimum diameter, (b) maximal diameter, (c) perimeter, (d) area
Based on the table above, it can be seen that actual cell count: 97 and total cell deviation calculation: 8. In order to calculate the percentage of errors can use the following formula as described in (6).

$$\text{Error percentage} = \left[ \frac{\text{Total Deviation}}{\text{Actual Cell Number}} \right] \times 100\%$$  \hspace{1cm} (6)

Using (6) above, the following results are obtained

$$\text{Error percentage} = \left[ \frac{8}{97} \right] \times 100\% = 8.25\%$$

Based on the results of watershed segmentation and cell count calculations, it can be concluded that some of the causes of this experiment failure are: (a) Overlapping objects have an irregular shape so that the system does not detect leukocyte cells, so they are not segmented. (b) Other objects besides leukocytes can be detected as leukocytes. This is because the object has characteristics that are similar in color and shape.

In the morphological calculation using the active contour segmentation method, the way to calculate the circumference of this function is to use eight neighbours, each pixel that does not have neighbours will be counted as a circumference of the object. Calculation of diameter is done by calculating the longest distance and the shortest distance from the edge of the object, so in this experiment, there will be two values for the diameter. Extensive calculations are carried out by counting all pixels on the object. In general, the calculation method is the same as the calculation on the watershed segmentation method. We did the morphological calculation using (6) the error percentage can be calculated from morphological calculations based on the segmentation method of active contour.

$$\text{Error percentage} = \left[ \frac{5}{97} \right] \times 100\% = 5.15\%$$

Based on the results of segmentation using the active contour method and cell count calculations, it can be concluded that some causes of failure in the experiment are: (a) Overlapped objects have irregular shapes so that over-segmentation occurs or the object is not even segmented. (b) Cells that are truncated or not intact remain detected because they have the size of an object that is almost the same as an intact cell object.

### 4. CONCLUSION

In this experiment, it was found that both for the use of the watershed segmentation method and the active contour segmentation method, for each image that has different characteristics it will require different treatments. Objects other than leukocyte cells can be detected as white blood cells because they have the same intensity of color and shape. Image segmentation using the Watershed Segmentation method has the advantage of being able to separate the blood cells that are huddled together. While for the segmentation of active contour, overlapped object separation can use the distance transformation method. In order to achieve a high percentage of segmentation success, an image that has uniform characteristics and variable control are needed. In this experiment, the calculation based on the active contour method has a lower error percentage than using the watershed segmentation method.

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