

Detection of Abnormal Findings in Human RBC by Analysing Sickle Cells Anemia with MATLAB Image Region Analyzer

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Abstract

Sickle cell disease (SCD) is a blood disorders that referring to the abnormality in the of the oxygen carrying protein, hemoglobin that are found in red blood cells (RBC). This disease is inherited from a parent which can be detected as early or begin from age 5 months and the common attacks or symptom such as anemia, hand and feet getting swelling. The disease can be detected by using blood test and it is recommending to test babies during newborn or at birth, it also possible to run a testing during pregnancy month as the early detection because it just a small percentage of patients of SCD can be cured only be doing a transplant of bone marrow cells. Abnormalities such as sickle cells disease causes the shape of the RBC to be of a crescent nature. The idea of diagnosing digitally and automatically using a simple program will prove very useful and will help the diagnosing process. Each individual red blood cell will be calculated by using the eccentricity formula of which will determine the roundness of specific cell computed by MATLAB. This pre-diagnosis really useful for medical officer who work at pathology and haematology laboratories at the hospital.

Keywords; Image Segmentation, Eccentricity Calculation, Image Processing, MATLAB.

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I. INTRODUCTION

Sickle cell anemia is a genetic blood disorder that occurs when a patient has abnormal hemoglobin on their red blood cell (RBC) called Hemoglobin S (HbS) where normal hemoglobin type is A (HbA).[1] When HbS molecules polymerize inside RBCs, due to lack of oxygen, they affect greatly the shape, elasticity, and adhesion properties of RBCs. Moreover, the RBCs become stiff and more fragile, with vastly heterogeneous shapes in the cell population in which makes this problem an ideal candidate for the examination of morphological heterogeneity.[2], [3] Up to 4.4 million of people detected to have sickle cell disease in year 2015 and 43 million of people diagnosed to have a sickle cell

trait and around 80% of sickle cell diseases are people from Saharan Africa and relatively frequently happens at parts of India region, Arabian Peninsula and resulted about 114800 deaths.[4] Meanwhile in Malaysia round 1% is born with sickle cells anemia, considering Malaysia has the population of slightly over 31 million, it is roughly 310,000.[5][6] The global distribution of sickle cell disease is closely linked to the natural protection against malaria afforded to individuals who are heterozygous for the sickle cell mutation. This selective advantage throughout human history has resulted in the distribution of HbS mutations closely reflecting the global malaria incidence, focused around the tropics.[7]

Sickle cells disease in when your RBCs has abnormalities where the cell itself is shaped like a crescent or sickle as shown in Figure 1. The red blood cells for the person affected by the disease can become rigid and sticky. These abnormalities and the irregular shape will cause the cells to get stuck in small blood vessels, which will result in slow or blockage of blood flow and thus affecting the delivery of oxygen throughout the body. Other characteristic features of this disorder include a repeated infections, and periodic episodes of pain.[8] The severity of symptoms varies from person to person. Some people have mild symptoms, while others are frequently hospitalized for more serious complications. Anemia is referring to the fact that the patient has low RBCs count. Only 50 percent of patient with sickle cell disease was recorded to survive until the age of 50.[9] The pain experienced by the patients are called episode at it was recorded that 0.8 episode per patient-year was the average number of episode pain felt by the patients every year.[8] Current treatment to sickle cell including antibiotics, vitamins, blood transfusions, pain-relieving medicines, other medications and possibly surgery, such as to correct vision problems or to remove a damaged spleen.[4], [10] People with sickle cell disease start to have signs of the disease during the first year of life, usually around 5 months of age. Symptoms and complications of SCD are different for each person and can range from mild to severe. The reason that infants don't show symptoms at birth is because baby or fetal hemoglobin protects the red blood cells from sickling. When the infant is around 4 to 5 months of age, the baby or fetal hemoglobin is replaced by sickle hemoglobin and the cells begin to sickle. The disease gets progressively worse as the patient gets older.

Automatic systems for identifying and classifying abnormal blood cell types have always been a field of interest to researchers around the world, but no method has been developed yet to replace or improve the classical one. Several method has been

develop over pass decade in detection of sickle cell including implementation image processing system[3], [11], [12],[13] artificial neural network (ANN) [14], [15], spectral detection [16] and other[17], [18]. In this studies we focusing the implementation of image processing in detection sickle cell rather than other method. Image processing method can produce precise and accurate sickle cell counting depend on algorithm that been development. Pranati *et al* reported that manage to identify the aberration in normal parameters of RBCs in an anaemic blood sample by using Weiner filter and Sobel Edge detection where been used to find the boundary of the corpuscles.[3] Several method has been choose in image processing upon detection of sickle cell such as region base and shape base methods. Other recently suggested the automatic identification and classification of RBCs by using binarization, contrast enhancement, noise elimination, morphological operations, labelling and feature extraction[19][20].

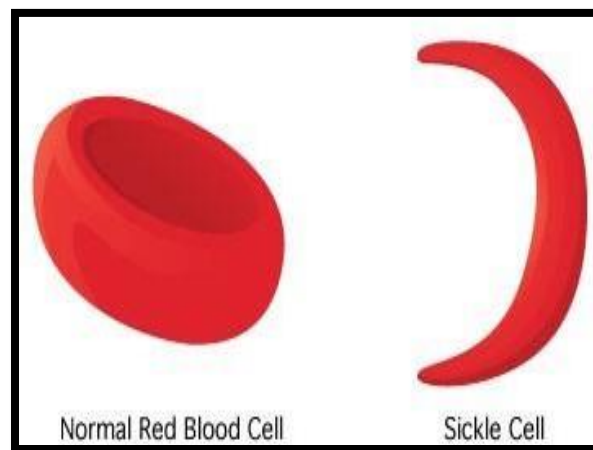


Figure 1: Red Blood Cell

In this research we focusing by using image processing to identify sickle cell abnormally with regional section detection method in MATLABs.

II. METHODS

A. Flowchart

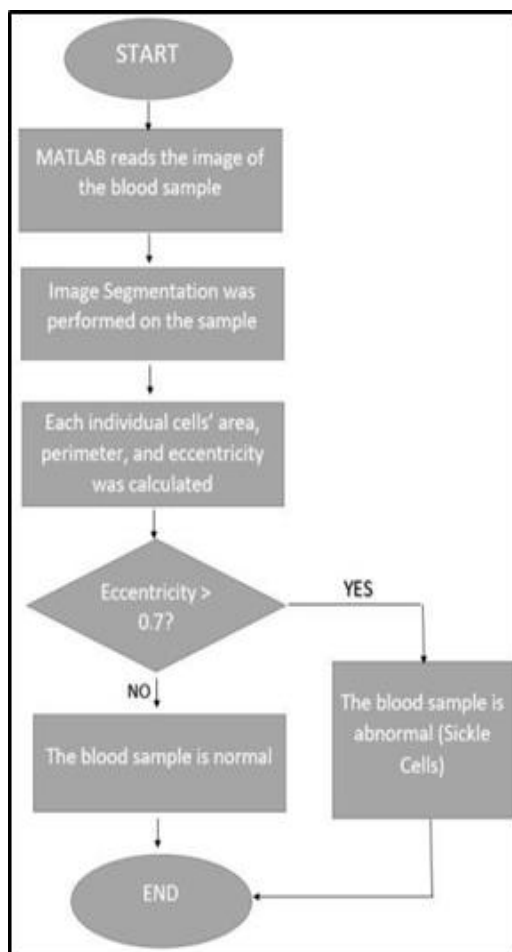


Figure 2: Flowchart

B. Image Processing

There are several steps that must be taken in order to perform image processing to the blood sample and they must be done in a particular order to obtain the outcome desired. The steps are image acquisition, grayscale, threshold, and lastly 'diamond' method.

C. Image Acquisition

In order to read an image from your computer, MATLAB needs to be able to get the path of which your image and in this case the blood sample is located. To do this, the code and the images are grouped under one folder in the desktop. MATLAB will read the pathway that leads to the image file and further action can now be taken.

```

clc;
clear all;
close all;
[fname path]=uigetfile('*.jpg','Enter an Image');
fname=strcat(path,fname);
im=imread(fname);
  
```

Figure 3: Image Acquisition MATLAB code

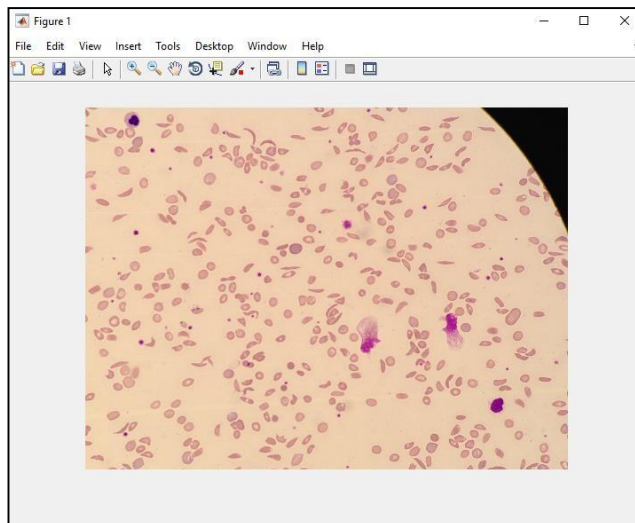


Figure 4: Blood sample displayed

D. Grayscale

```

imshow(im);
im1=rgb2gray(im);
figure
subplot(2,1,1)
imshow(im1);
subplot(2,1,2);
imhist(im1);
  
```

Figure 5: Grayscale MATLAB code

The first line of the code was to display the first image that is shown in figure 5, the blood sample display. The following line was a MATLAB command to grayscale an image. By doing this, the image of RGB nature will be converted into a gradient of black and white image. This part of the process was made to produce a result of which is shown in the next chapter, result and discussion where the image was studied by the program and a histogram will be produced based on this. The command 'imhist' is the MATLAB command to produce a histogram based on the image.

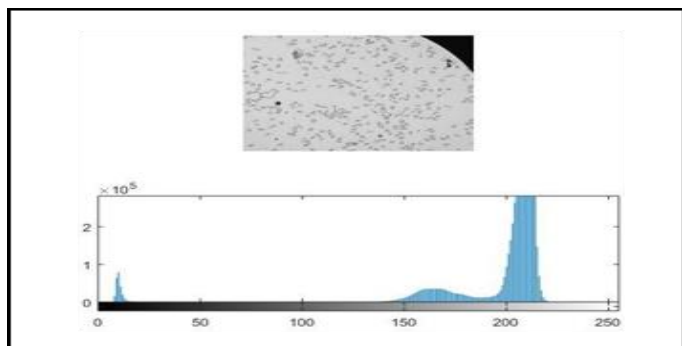


Figure 6: Grayscale Image and Histogram

E. Threshold (Image Segmentation)

Computing the image segmentation was important part in this work. Grayscale Image segmentation was performed through setting up their threshold number. The detailed script of MATLAB is shown at Figure 7.

```
%% Thresholding
imk = im1 > 185;
figure;
imshow(imk);
```

Figure 7: Threshold MATLAB code

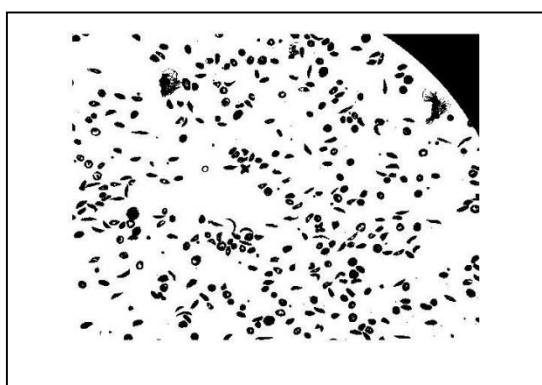


Figure 8: Threshold performed on the sample

The image above shows an image that was thresholding in binary form. Binary form image is crucial in image processing as the MATLAB program will compute the image in a more accurate fashion when dealing with a binary image because they are only two state in the picture, 0 which is completely black and 255 which is completely

white. The image of human sample is not sharp as they are in a small pixel.

F. Diamond Method

Diamond is a MATLAB command that serves as an image enhancer. This command will rule out any unwanted noises in the image and the output of this code will be an image with less noise in order to ease the analysis.

```
%% Cleaning image
seD = strel('diamond',1);
BWfinal = imerode(imk,seD);
BWfinal = imerode(BWfinal,seD);
figure, imshow(BWfinal), title('segmented image');
```

Figure 9: Diamond MATLAB code

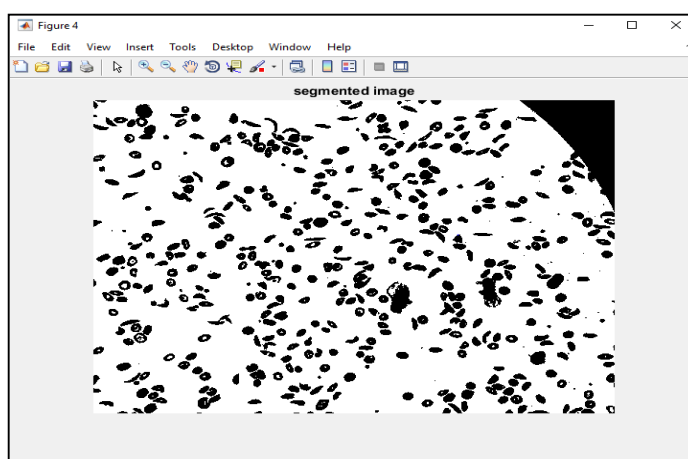


Figure 10: Final Image

Since the image from thresholding was not really clear and further improvement needed to be verified. The method called 'diamond' is to clean the image by clearing out any small particles available in the image and to fill most of the red blood cell to be completely black. Because the red blood cell is usually red in color, but the middle of the red blood cell is usually shaped like a concave and because of this phenomenon, the red blood cell has different shade of red in the center of the red blood cell due to the shape. It is usually a darker shade of red. This method will make the image slightly clearer and cleaner to be processed on next stage.

G. Filter Region

The code stated below image, there are two different code as the second one is a MATLAB function code. Function serves as a call function of which it is located separately from the rest of the code, but a simple one line will call the function to be run as usual. The function was generated automatically by the application available in MATLAB called Image Region Analyzer. The lines of function will filter the image of which in this case, to filter any black dots in the final image and leave it out of consideration. The code to filtering area is called the 'bwpropfilt' method in MATLAB. The code was made to filter out anything under the number of 20 pixels which is basically a dot. By doing this the system will not mistakenly take a single black dot as a 'cell' and will not calculate the rest of the dots in the filtered image. The line of 'regionprops' is to produce a properties table of which will display area, eccentricity, majoraxislength, minoraxislength, and perimeter of each individual cells inside the blood sample.

The Majoraxislength and Minoraxislength is a number of where the cell is located from x-axis and y-axis perspective. The area in the area of each individual cells in pixels. The eccentricity is the shape of the cell whether it is round or slightly crooked. And perimeter is the length of the outline of each individual cells.

```
%% Calculating Area, Perimeter, Eccentricity, And Major/Minor Length
BWend = filterRegions(BWfinal);

function [BW_out,properties] = filterRegions(BWfinal)
%filterRegions Filter BW image using auto-generated code from
imageRegionAnalyzer app.
% [BW_OUT,PROPERTIES] = filterRegions(BW_IN) filters binary image BW_IN
% using auto-generated code from the imageRegionAnalyzer App. BW_OUT has
% had all of the options and filtering selections that were specified in
% imageRegionAnalyzer applied to it. The PROPERTIES structure contains the
% attributes of BW_out that were visible in the App.

% Auto-generated by imageRegionAnalyzer app on 21-Apr-2019
%-----
BW_out = BWfinal;

% Filter image based on image properties.
BW_out = bwpropfilt(BW_out, 'Area', [20, 6551603]);

% Get properties.
properties = regionprops(BW_out, {'Area', 'Eccentricity', 'MajorAxisLength',
'MinorAxisLength', 'Perimeter'});

%The properties in a table.
properties = struct2table(properties)
```

Figure 11: Filter Region MATLAB function

III. RESULT

The result of the work presented in histogram analysis and computation from MATLAB programming.

A. Histogram

A histogram is an accurate representation of the distribution of numerical data. From performing the two steps in image processing that is the image acquisition and also the grayscaling of the image; the outcome of the greyscale version of the image. Grayscale image is the conversion of images of red, green and blue version of the image into a black and white version of the image. The images unnecessarily exist in black and white color, but the images also look gradient of both black and white. Just that the 'color' of the image was being converted in to a gradient of white and black based on the intensity of the pixel.

From the grayscale image, we intensity of each pixel in the image can be converted into a graph that is called the histogram. The number that defines the intensity of each pixel is dependent of the blackness or the lack of color in each pixel, of which is determined by the number 0 for the darkest pixel or black, and 255 is the maximum number of which no color is present in the pixel, or completely white. This is according to the information that each pixel of an image is a number that is stored in an 8-bit integer of which the lowest number is 0 and the highest is 255 or 2^8 .

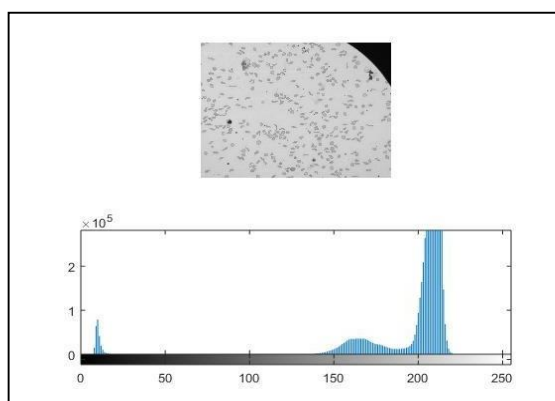


Figure 12: Histogram

From this example, we can clearly see that the grayscale image has a dark or black concave at the top right of the image. This was clearly shown in the histogram generated from the image and shows that the present of black in the image is represented by around 10 in the number of intensity of the pixel and has almost 1×10^5 in the number of frequency as it takes only around 10% of the image. And from the image as well, the number of cells is also on the low side, as patients with sickle cells usually suffers anemia, which means that their number of RBC are on the lower side. The number of intensity of pixels from 200 to around 220 are on the much higher frequency than the rest as the patients lack blood cells and higher amount of plasma in the sample.

B. Image Region Analysis

Second outcome from the research was the set of data gained from the segmented image, and analysis of the data collected. The image that was thresholded, and underwent the process of 'diamond' was further processed in the MATLAB application called Image Region Analyzer. This particular app will acquire the image by pressing the button 'Load Image' and choosing the targeted image. Next the application will select one cell to be selected as the main subject, and in this case, the red blood cell with sickle cell was chosen. The application will then provide the user with the variables that can be controlled manually in the application itself. For this research only the Area, Major Axis Length, Minor Axis Length, Eccentricity and Equivalent Diameter were chosen as the variable shown. Each of these variable is crucial in determining the outcome from the analyzer. This analyzer will display all the information of each individual cells in the image. The area shows the size area of each individual cells and number 1 on the list represents the whole image (not cells), therefore the largest size.

From the image below, the area ranges from as low as 25 pixels and goes as high as 6427070 pixels. The data was then filtered to ignore any cells that are

lower than 100 pixels, as the data that was recorded as a cell is bigger than 100 pixels, and anything lower than that are too small to be considered as a red blood cell. After filtering, the data is then exported into the main workspace. The final data gained is calculated to be around 77 sickle cells detected by the system. The eccentricity data was then transported to be its own numeric array in order to proceed with the next step.

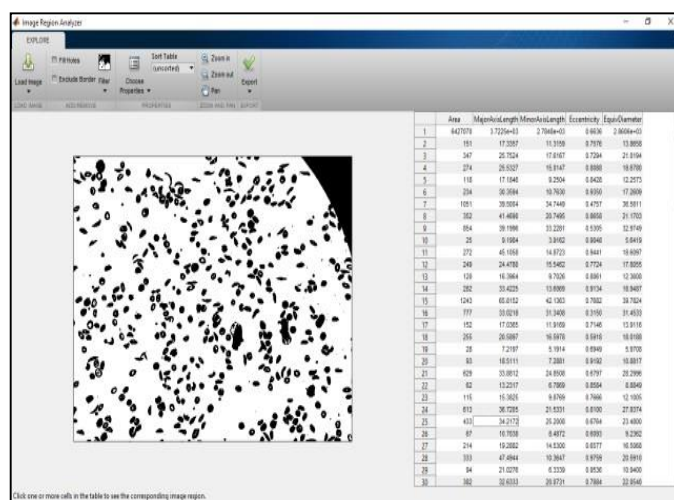


Figure 13: Image Region Analyzer Application

C. Eccentricity Calculation and Diagnosis

From the numeric array created from previous result, the eccentricity is compiled in a single array that can be calculated in groups according to the density of each number of eccentricity from 0 to 1.

a) Sickle Cell Anemia Sample

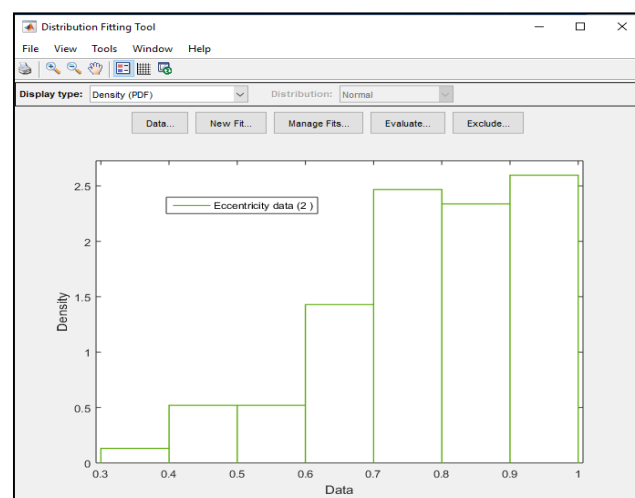


Figure 14: Eccentricity Graph

Based on the figure above, we used an application in the MATLAB distribution fitting tool. This particular application will group the data into groups and distribute a plotting of density against data. In this case, the data was eccentricity that was taken from the numeric array that was created by the end of the last result. The data was sorted to a 0.1 interval groups and in this result from 0.3 to 1 because there are no data under the value of eccentricity of 0.3 and no data above the number of 1. Based on the blood sample, the patient is suffering from sickle cell anemia and the result shows the same. The relation between healthy and abnormal red blood cells are really obvious in this sample because the highest density in the end result showed the highest number are above 0.7 eccentricity.

b) Healthy Blood Sample

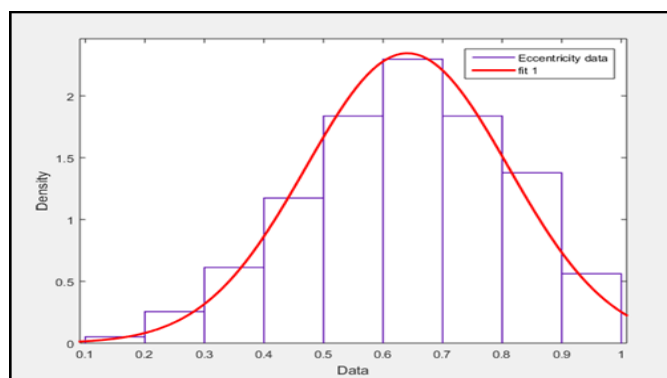


Figure 15: Eccentricity Grap

Based on the second example, this is a blood sample from a healthy patient without sickle cell anemia. The blood sample clearly shows the roundness of each individual cells and there are no visible sickle cell observed. The difference between two example shown are clear, whereas the sickle cell patient blood sample clearly shows and increased density in the higher end of the eccentricity data rather than this sample that shows that the data is widely spread but the most density is between 0.6 to 0.7 eccentricity. The 'fit' line representation was inserted to present the sine wave version of the graph.

From the result from over 30 samples provided by Ampang Hospital, Selangor, only 4 of them were

diagnosed with sickle cell anemia. We could presumed as the samples are most dense eccentricity result at the above the value of 0.7, then most likely treated as sickle cell anemia. If the sample shows the highest density of eccentricity is below 0.7, the patient is most likely not with sickle cell anemia.

IV. CONCLUSION

However, in a healthy person the hemoglobin is smooth, round and more flexible that give the red blood cell go easily in your body blood stream meanwhile for SCD patient the shape of hemoglobin is not in normal shape that are develop of forms rods and clump together that will cause the blood cells rigid and become curved. In which the abnormal or odd shape of cell will block the flow and it is dangerous that can cause extreme pain.

Diagnosis of the disease was usually made by visual inspection of trained professional dealing with blood diseases and by this program, diagnosis of the disease can be made digitally just by providing a blood sample to the MATLAB and then the outcome of the image processing will determine whether the blood sample is healthy or with sickle cell anemia.

The result section of this report explained more on the outcome obtained throughout the research period and the goal was met but the accuracy of the calculation using eccentricity was not accurate compared to metric formula. The pros of using eccentricity is that the program managed to calculate each individual cells under the blood sample and obtain their relative eccentricity values. Whereas when using metric formula, the program needs to calculate manually and calculation may really useful to get quick and robust pre-diagnostic.

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