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OVERLAPPING SICKLE CELLS DETECTION AND SEPARATION USING MARKER-BASED WATERSHED SEGMENTATION

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ABSTRACT

Sickle cells are abnormalities of the Red Blood Cells (RBCs). The focus of this research work is to detect sickle cells, normal RBCs, and to separate the overlapping cells present in a blood film image and also to identify the percentage of each detected cells. The image processing techniques used for the detection of these RBCs consist of five main steps which are, image acquisition and reading, image preprocessing, feature extraction, RBCs classification, and final result. The Marker-Based Watershed Segmentation (MBWS) method was used to separate the detected overlapping cells. To test the accuracy of this system, the system's result was compared to the result of the traditional method of detection and count of RBCs used in the hospital. The identification and separation of the overlapping cells performed by the proposed system made the system includes, inadequate separation of the overlapping cells due to the under segmentation problem of the technique (Marker-Based Watershed Segmentation) used, the quality of images used, and also the cells at the boundary were eliminated together with its diagnostic features which it may possess.

Keywords: Abnormal Cells, Marker-Based Watershed Segmentation, Overlapping Cells, Red Blood Cells, Sickle Cells.

INTRODUCTION

Image processing technique is a signal processing method used to perform certain operations on an image in order to extract valuable information from it. It takes an image as input and yields the image characteristics as output. There are three basic steps of image processing which includes, importing the image through image acquisition tools, studying and manipulating the image, and finally the output (University of Tartu, 2014). Hence image processing can be used to detect several abnormalities of the blood. There are three types of blood which are the RBCs, the white blood cells, and the platelet (Mahmood & Mansor, 2012). Diagnosis of the RBCs gives important information about pathological diseases and condition. Relevant diseases such as sickle anemia can be detected based on the shape and deformability of the RBCs (Tomari et al., 2015). Sickle cell is an abnormality of the RBCs, which could cause sickle cell anemia (Abbas & Muhamad, 2014). Sickle cell anemia is caused by a genetic abnormality in the hemoglobin gene patients that may cause severe pain in the legs, backs, chest, arms, and abdomen (Alotaibi, 2016). The normal or healthy RBCs are usually round in shape while sickle cells have a crescent moon shape (Chintawar et al., 2016). In the laboratory when a blood film is viewed under the microscope for analysis by a medical analyst, the clumsy or overlapped cells are neglected. These overlapped cells may contain important features for diagnosis (Tomari et al., 2015).

During analysis of the sickle cells in the laboratory, at times blood films are prepared and viewed by an analyst under the microscope for its presence. The major issue occurs when massive amounts of blood samples are required to be handled by the analyst (Dalvi & Vernekar, 2016). The time and skills required to process this large volume of blood samples restricts the speed and accuracy with

which the blood samples can be processed and also the discarding of overlapped cells reduces the accuracy of the RBCs count (Abbas et al., 2015). To overcome these problems, image processing technique is increasingly identified as an important method for automated separation and count of overlapped RBCs. Hence this paper has focused on solving the problem of restricted speed and accuracy by developing a user friendly software, which detects, separates, and counts overlapped RBCs using MATLAB.

1. Objectives of the Study

- Identify and classify different red blood cells (normal, overlapped, and sickle) through their shapes and sizes in the blood film.
- Separate overlapping red blood cells.
- Count the number of normal, overlapped and abnormal red blood cells and calculate the percentage of each identified cell present in the blood film image.

2. Related Literature

A stained smear of peripheral blood shows the morphological features of the RBCs, platelets, and white blood cells (Chourasiya & Rani, 2014). Sickle cell is an abnormality of the RBCs which assume the shape of a crescent moon with one end usually pointed, they can differ in size, but are generally smaller than the normal RBCs. Sickle cells are caused by mutation in the hemoglobin gene (Bala & Doegar, 2015). Karthikeyan and Brharama (2015); Mahmood and Mansor (2012); Mazalan et al. (2013) all used the Hough Circular Transform technique to detect and count the normal RBCs present in the blood film image. But this technique can only detect and count the normal RBCs without considering the abnormal cells. Safca et al. (2018) used the circularity factor, form factor, semiunarity factor, and deviation factor to classify RBCs into abnormal and normal RBCs. Hariharan and Aruna (2016); Aruna and Hariharan (2014) used various edge detection techniques to detect and identify the RBCs and it was concluded that the canny edge detection method was preferable for detection as it gives more details of the

original image. Without considering abnormalities of the blood. Thiruvinal and Ram (2017) have proposed using Otsu's algorithm to segment the RBCs, platelets, and white blood cells and used the four connected components to extract the image features for correct classification. Acharjee et al. (2016) developed a user friendly interface, where the user can specify the dimension of the RBCs by dragging 2 points over the image and afterwards applying the Hough Transform technique to identify the biconcave shape of the RBCs with the specified diameter. A general analysis of blood smear to detect diseases, such as Dengue, Polycythemia vera, Leukemia, Measles, Sickle Cell anemia, Kidney diseases, Hypertension, and Malaria was conducted by Sharma (2017) and Neuro-Fuzzy approach for training the system was adopted by Sharma (2017) to make future prediction of the input diseases. Vignesh and Loganathan (2019); Thanh et al. (2018) detected and classified leucocyte blood cell images using convolutional neural network. Thanh et al. (2018) used convolutional neural network to diagnose leukemia disease at early stage of the disease. But the paper focused only on one type of Leukemia disease, the acute lymphoblastic without considering the other types. While Vignesh and Loganathan (2019) used the convolutional neural network to segment the RBCs into eight categories which are echinocytes, discocytes, elongated, reticulocytes, granular, oval, sickle, and stomatocyte. Dalvi and Vernekar (2016) used 13 geometric features to classify RBCs into four abnormal cells types which are elliptocytes, tear drop, echinocytes, and macrocytes. The decision tree classifier and Artificial Neural Network were used for classification and their proposed method got an accuracy of 96.06% for echinocytes detection, 95.27% for elliptocytes, 85.82% for tear drop cells, and 85.82% for macrocytes. Watershed segmentation technique was adapted by Bala and Doegar (2015) to detect and count the normal and abnormal RBCs, but eliminated the overlapping RBCs. Twelve abnormalities (anemia) of the RBCs were identified by Khawaldeh (2013). However, during identification of the abnormal cells by Khawaldeh (2013), the overlapping and boundary cells were removed from

the image. In order to improve the accuracy of the result and decrease the problem of re-preparation of slides, overlapping cells should be detected and split into single cells for better analyses of their morphological features (Abbas & Muhamad, 2014). Overlapping cells can be spilt using several methods. One of these methods known as the distance transform watershed segmentation algorithm was used by Tomari et al. (2015); Achariya et al. (2014) for separation of the overlapping blood cells into single cells. This separation method used by Achariya et al. (2014); Tomari et al. (2015) has a drawback where the overlapping cells are over-split. Also Abbas et al. (2015) separated overlapping RBCs using boundary analysis. In this method, the convex hull boundaries were identified and split according to the number of RBCs in association to the distance measured. Kaur et al. (2013) used the Marker-Based Watershed Segmentation (MBWS) to split the clustered RBCs. The MBWS which is an in-built MATLAB separation method uses internal marker and external marker to reduce the local minima, hence reducing the over-splitting problem, which occurs when using the distance transform method. From previous research, MBWS method splits overlapping cells more accurately compared to other separation methods, However, MBWS method has the problem of under segmentation or under separation of the overlapping cells.

3. Methodology

In this work, three tools were used which are, the microscopic image of the stained blood sample viewed under the microscope by an analyst, a personal computer and the Matrix laboratory (MATLAB) IDE. MATLAB was chosen because it has an inbuilt image processing toolbox that provides set of reference-standard algorithms and workflow apps for image processing, which helps oneto automate common image processing workflows (Karthikeyan & Brharama, 2015; Mahmood & Mansor, 2012; Bala & Doegar, 2015). The image processing methods used for the detection and separation of the overlapping RBCs are discussed below (Alotaibi, 2016; Hariharan & Aruna, 2016; Sreekumar & Bhattacharya, 2014; Rexcy et al., 2016).

3.1 Data Collection

Before an image can be acquired, a blood film has to be prepared and stained. The blood film images used in this work were collected from Plateau State Hospital in Nigeria. The blood film images were captured directly under the microscope using a camera connected to the computer system via a USB.

3.2 Image Acquisition and Reading

The captured photo microscopic image of the blood film were saved on the computer system where it was obtained and read as a digital image in any format supported by MATLAB.

3.3 Image Preprocessing

Image preprocessing was used to detect the object of interest. The following are the image preprocessing techniques used (Figure 1).

3.3.1 Grey Scale Conversion

The acquired image usually in RGB format was converted to a gray scale image format in order to separate the object of interest from its surrounding.

3.3.2 Image Enhancement

Image enhancement was used to distinguish the localized regions from the study image. Image enhancement increases the contrast of the image and enhances the color difference between the objects and its background. This method was used to extract the objects boundaries. The "IMADJUST" built in MATLAB function was used to carry out this enhancement (Patil & Khot, 2015).

3.3.3 Conversion of Enhanced Grey Scale Image to Binary Image

After enhancing the image, the next step was to convert the grayscale image to a binary form (either white or black) in order to increase readability. Before the image was converted to a binary form, a threshold value was required.

The threshold value was acquired by using the MATLAB ADAPTHISTEQ function. This function performs contrastlimited adaptive histogram equalization, hence automatically providing a suitable threshold for the



Figure 1. System Architecture

image conversation. By default when the image is converted to binary form, the background is white and the objects of interest is black and this makes the boundary tracing difficult. Therefore, the binary image was converted to its complement (background is black and the object of interest is white) for a better boundary tracing.

3.3.4 Filling Holes of Region

To avoid an error or an incorrect result, the holes in the objects had to be filled. MATLAB provides the inbuilt 'IMFILL' function for this purpose.

3.3.5 Filtering (Removal of Unwanted Noise and Object)

In this phase, unwanted noise and small objects were removed in order to make the image better, clearer and easier for analysis. Instead of using the well-known filtering methods such as the median and Gaussian, the (MathWorks, 2016c). MATLAB inbuilt BWAREAOPEN function was used to remove the unwanted noise and small objects that has area less than the specified value (70 pixels) from the binary image. The BWAREAOPEN function was used because it does not affect the image objects and edges quality by blurring the image, unlike most filtering methods which removes the image noise and reduces the image quality by blurring the image (Hariharan & Aruna, 2016). The BWAREAOPEN function removes all connected objects that have fewer value pixels than the objects of interest, hence producing another image without unwanted objects/noise. The default connectivity is 8 for two dimensions, 26 for three dimensions and conndef (ndims (Cleared), 'maximal.) for higher dimensions (MathWorks, 2016a).

3.3.6 Removing Incomplete Cells at the Boundary of the Image

Most RBCs at the boundary are incomplete hence this

boundary cells do not carry any useful information.

However to increase the efficiency of the result, this boundary cells were removed from the image using the 'IMCLEARBORDER' function.

3.3.7 Shrinking of Objects

The sizes and areas of the RBCs were reduced using the erosion function (IMERODE). This function helps in separating some overlapping RBCs which helps reduce the problem of under-separation which is a drawback of using the watershed segmentation method to separate overlapping cells.

3.3.8 Identification and Separation of Overlapping Cells

After using the erosion method to shrink and separate some overlapping RBCs, the remaining unseparated overlapping cells were identified and separated using the MBWS method. Although not all overlapped cells were separated after implementing the MBWS method. However, this method was adapted because it reduces the effect of severe over-segmentation caused by using just the watershed transformation method. The MBWS technique follows these basic procedures for segmentation (Kaur et al., 2013; Bala, 2012).

- Compute a segmentation function (This is an image whose dark regions are the objects we are trying to segment).
- Compute foreground markers (These are connected blobs of pixels within each of the objects).
- Compute background markers (These are pixels that are not part of any object).
- Modify the segmentation function so that it only has minima at the foreground and background marker locations.
- Compute the watershed transform of the modified segmentation function.

The above basic procedures includes various morphological operations such as finding the regional maxima and marking the foreground objects for image reconstruction. After reconstructing the image we superimposed it with the original image, cleaned the edges of the segmented image, and computed the background markers. Finally, the watershed transformation was then applied to the distance transform of the image to segment the overlapping cells.

3.4 Feature Extraction

After image preprocessing, the next phase is the feature extraction phase. In this phase, important information are gotten from the objects of interest. Their features are extracted by computing the geometric shape features (Major/Minor Axis length, Area, Circularity, Center, and Bounding Box) of each cell. The geometric shape features were used to identify the various types of cells. These geometric shape features were calculated using the MATLAB 'regionprops' function, which measures properties of image regions (MathWorks, 2016a).

3.5 Red Blood Classification

After the cells features were extracted, the RBCs were sorted out as normal, overlapping, abnormal and sickle RBCs using their form factors, areas, and perimeters.

3.5.1 Form Factor Calculation

Normal red blood cell, form factor > 0.94, but less than or equal to 1, Abnormal RBCs, form factor < 0.94 and Sickle cells, 0.15 < form factor < 0.60. The form factors were calculated using the properties of the RBCs. In order to calculate the form factors, the objects of interest were labelled. Then the form factor of each labelled object was calculated using the formula "Form factor=4* pi * area/ (perimeter* perimeter)" (Patil & Khot, 2015). Hence the form factor range was decided from the calculated values of the form factors displayed on the labelled objects. The area and perimeter of each objects was calculated using the MATLAB "REGIONPROPS" Function (MathWorks, 2016b).

4. Result and Discussion

To check the accuracy of the proposed system, test was conducted on 14 images (12 taken of real blood films and 2 gotten from journals). Eventually the results were acceptable and comparable with the traditional visual counts conducted by two analysts and the detection result gotten by two researchers (Alotaibi, 2016; Sreekumar & Bhattacharya, 2014) for im7 and im8.

The results of the analyzed images shown in Table 1 are

discussed below.

The table consists of nine columns. The first column lists the name of the images used while the remaining eight columns report the number of various RBCs identified in each image. The second column consists of the total number of identified normal RBCs in each image, the third column consists of the total number of abnormal RBCs identified, fourth column consists of the number of identified overlapping RBCs, fifth column reports the total number of overlapping RBCs which the proposed system was able to separate, sixth column shows a count of the identified single sickle cells while the seventh column reports the count of the separated overlapping sickle cells after applying the marker-based watershed segmentation for separation of the overlapped cells, the eight column reports the total count of identified sickle cells (i.e. identified single sickle cells + the separated overlapped sickle cells) and lastly the ninth column gives the total count of the identified RBCs (normal + abnormal RBCs).

From the image in Figure 2, the green line cells indicate the sickle cells and the black line cell shows the sickle cells identified after separating the overlapping cells. In Figure 1, the 0% on the normal RBC count shows that all the identified RBCs on the image are abnormal. This is because the software is able to classify a cell as a normal RBCs if the threshold is between the range of 0.94 and 1 (considering that a normal RBC is circular in shape the form factor used calculates the circularity of the cell) and if the area is greater than 300 pixels but less than 850 pixels. Hence an RBC outside these form factors and area range is counted as an abnormal RBC.

4.1 Comparison of Classification: System vs. Traditional Method

In the traditional method, RBCs are counted manually by viewing a slide prepared with a patient's blood under a microscope. The benefit of this method is that it is straightforward and does not need expensive instruments. The drawback of this technique is that it is tedious and is more prone to errors, especially when there are a lot of blood samples to examine and the overlapping cells are neglected (Sreekumar & Bhattacharya, 2014). It also needs the dilution of blood samples to avoid sticking together of the cells and reduce overlapping and it does not accurately count the overlapped cells. The chart in Figure 3 presents the comparison between the traditional method and the proposed system, which was conducted on 14 blood film images. In this chart, the orange line represents the system results and the blue line represents Traditional method results.

From the result it can be seen that the number of sickle cells detected by the computerized system is more than those detected through the traditional method and this is because of the addition of the separated overlapping cells.

4.2 Comparison of Classification: Proposed System vs. Method Proposed by a Related Work

lmage Name	Number of Normal RBCs	Number of other Abnormal RBCs	Total Number of Overlapped RBCs	Total Number of Separated Overlapping RBCs	Number of Single Sickle Cells	Number of Separated Overlapped Sickle Cells	Total Number of Sickle Cells	Total Number of RBCs
lm1	0	31	5	12	5	2	7	31
lm2	0	44	8	16	6	0	6	44
lm3	2	32	7	15	2	0	2	36
lm4	1	44	6	14	4	1	5	50
lm5	1	22	1	3	5	0	5	28
lm6	2	34	7	12	4	0	4	40
lm7	15	9	5	8	3	1	4	24
lm8	18	7	2	4	2	1	3	25
lm9	13	25	3	6	3	0	3	38
lm10	5	12	5	8	1	0	1	17
lm11	10	20	7	12	1	1	2	30
lm12	7	11	4	4	0	1	1	18
lm13	20	5	2	4	1	0	1	25
lm14	5	14	5	10	0	2	2	19

Also the result of the sickle cell count gotten by the

Table 1. Result of Test conducted on 14 Images



Figure 2. Result Screen with an Examined Blood Film Image





Figure 3. Result of Comparison for Classification between System and Traditional Method

proposed system was compared with those gotten by Alotaibi (2016). The results for four images are as follows.

From the result in Table 2 it can be seen that the some of the count gotten by the proposed method had a higher number of sickle cells than that proposed by Alotaibi (2016) because the proposed method considered the overlapped sickle cells while the method proposed by Alotaibi (2016) neglected this overlapped sickle cells. Hence from the result gotten from the comparison between the traditional method, the related work and the proposed system, it can be deduced that the system provided a more accurate count than both methods due to consideration of the overlapped RBCs.

Conclusion

The proposed system was able to give a better count than the traditional method because in the traditional method

Alotaiba (2016) (Number of Sickle Cells identified)	Proposed System (Number of Sickle Cells identified)
4	6
3	4
4	5
5	5
6	8
4	4

Table 2. Comparison of Sickle Cells Count

the overlapped RBCs were discarded without considering their features and importance to the analysis but the proposed system considered identifying and separating this overlapped cells for improved analysis as this overlapped cells could contain very important features for diagnosis. This study identified that separation of overlapped RBCs improved the analysis result.

Therefore a software was developed to detect, count, and separate this identified overlapping sickle cells in blood film images using image processing technique that were based on calculated form factors and separation based on MATLAB improved Marker-Based Watershed Segmentation method.

The software used green and black lines to mark the detected sickle cells. Hence this system solves the problem of reduced speed and accuracy encountered by the analyst because of the massive amount of blood samples to be processed and reduced accuracy due to negligence of overlapped RBCs.

Future Work

The technique used for the separation of the overlapping cells in this paper has a drawback of undersegmentation, where only few of the overlapping cells were separated. Hence in future, a better technique of separation can be adapted to solve the problem of under-segmentation which will result to a more accurate separation/result. Also the higher the image quality the better the result, therefore it is advisable to get the blood film images from a photo-microscope.

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