SICKLE CELL DIAGNOSIS FROM PERIPHERAL BLOOD SMEAR IMAGES USING OPEN-SOURCE IMAGE PROCESSING TECHNIQUES

RITSAK PATTANA

Thanyaburi School
E-mail: ritsakpattana@gmail.com

Abstract - Sickle cell disease is a genetic disorder that affects millions worldwide. Its diagnosis often relies on manual examination of blood smear images by experts. This traditional method can be time-consuming and resource-intensive. In this study, we developed an image analysis method using Fiji, an open-source software, to classify red blood cells from peripheral blood smear images into normal, sickle and other cell types. The classification was based on key morphological features, including circularity, aspect ratio and roundness. Our findings showed that aspect ratio and roundness were the most reliable metrics for differentiating elongated sickle cells from rounder normal cells, achieving an accuracy of 76% with balanced sensitivity (0.73) and specificity (0.77). Circularity, however, was less effective, resulting in a high false positive rate. This open-source method offers a cost-effective solution for sickle cell detection, particularly in resource-limited regions. Further improvement with advanced segmentation techniques and machine learning approach could enhance the method's accuracy, making it practical for clinical diagnostics.

Keywords - Image Analysis, Morphological Features, Peripheral Blood Smear, Sickle Cell Anemia.

I. INTRODUCTION

As of 2021, nearly 8 million people worldwide are affected by sickle cell disease (SCD) [1], a genetic disorder that causes red blood cells to assume a rigid, sickle shape, instead of their typical flexible, disk-like form. This abnormality disrupts normal blood flow, leading to a range of symptoms including jaundice, fatigue, weakness, swelling in the hands and feet, and severe pain, particularly in the stomach, chest, and joints. The most severe complications of SCD include stroke, organ damage, other disabilities, and, in some cases, premature death [2]. Therefore, early diagnosis and treatment are critical for improving the quality of life and reducing the mortality rate associated with the disease [3].

Sickle cell anemia can be detected through blood smear analysis, which relies on expert examination for accurate identification. However, there are only limited trained professionals, making it challenging to ensure timely and precise diagnoses. Moreover, quantitative analysis, important for assessing the severity of the disease, involves labor-intensive manual cell counting [4]. Consequently, computerized systems have been developed, offering a more efficient and cost-effective alternative for both qualitative and quantitative detection of sickle cells [5].

In this study, we developed a method using Fiji (ImageJ), an open-source and user-friendly image analysis software, to characterize the morphological features of sickle cells from peripheral blood smear images. Fiji's free accessibility makes it an ideal tool for widespread use by healthcare professionals with varying levels of expertise. We applied these morphological criteria to identify sickle cells in a separate dataset of peripheral blood smear images and assessed the method's accuracy, specificity, and sensitivity to demonstrate its potential for real-world diagnostic applications.

II. METHODOLOGY

A. Datasets

We used erythrocytesIDB database, available athttp://erythrocytesidb.uib.es/ [6], which are peripheral blood smear images from patients with sickle cell disease. The dataset includes individual red blood cell images that were classified by specialists into three categories: circular, elongated and other cells, to establish morphological criteria. A separate dataset of peripheral blood smear images, containing both normal and sickle cells, was used to evaluate the accuracy of our image analysis method.

B. Image Pre-processing

Color images were initially converted to 8-bit grayscale, with pixel intensity values ranging from 0 (black) to 225 (white). To enhance image quality, the brightness and contrast were adjusted by the software to improve visualization of red blood cell structures for the subsequent step. (3 images: color, grayscale, and brightness and contrast adjusted)

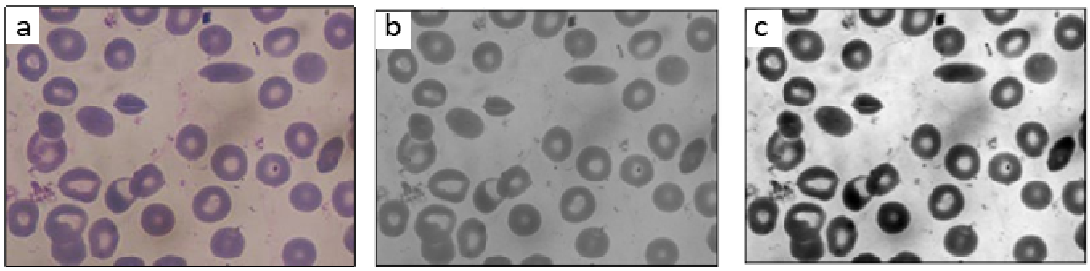


Figure 1: Pre-processing steps for image analysis, showing the conversion of a color image to an 8-bit grayscale image, followed by brightness and contrast adjustments.

C. Image Processing

1. Thresholding

Thresholding is a fundamental image processing technique used to separate objects of interest from the background. This process converts a grayscale image into a binary image, classifying each pixel as either part of the foreground (the object of interest) or the background based on its intensity value. In this study, the thresholding value for each image was automatically selected by the software.

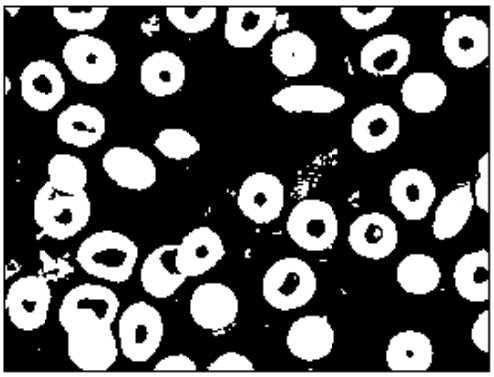


Figure 2: Thresholding process converts a grayscale image to a binary image.

2. Erosion

Erosion is a morphological operation applied to binary images in image processing to shrink or thin objects within an image by eroding the boundaries of the foreground objects, which are typically represented by white pixels (value 1). As a result, the background, usually represented by black pixels, expands.

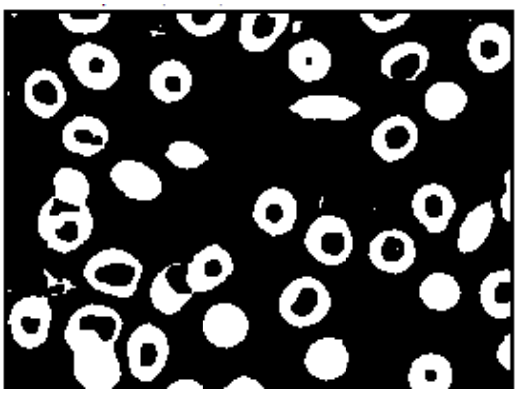


Figure 3: Erosion process helps reduce noise and refine the shape of the detected cells.

3. Watershed

The watershed algorithm is a technique used in image processing for image segmentation, which involves partitioning an image into different regions or objects. In this study, the software automatically applied watershed processing to separate adjacent red blood cells in the images.

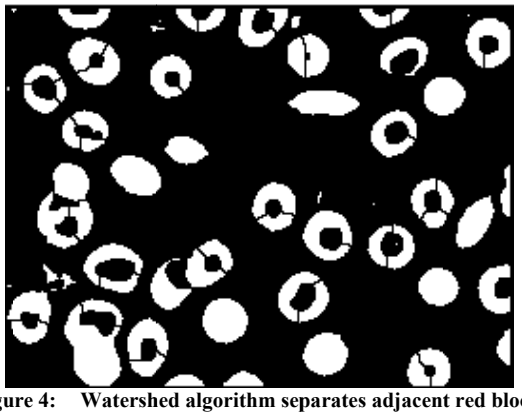


Figure 4: Watershed algorithm separates adjacent red blood cells.

D. Characterization of Morphological Features

Fiji was used to analyze the morphological features, including circularity, aspect ratio and roundness of normal and sickle cells, as well as other cells that did not fit into either category.

1) Circularity

Circularity (CR) is a measure used to quantify how close the shape of an object is to a perfect circle, with a value of 1 indicating a perfect circle. Circularity was calculated based on (1).

$$CR = \frac{4\pi A}{P^2} \qquad (1)$$

A= the area of the shape.
P = the perimeter of the shape.

2) Aspect ratio

Aspect ratio (AR) is a measure used to describe the proportional relationship between the width and height of a shape or image. For normal cells, the aspect ratio is close to 1, while for sickle cells, their values are greater than 1. Aspect ratio was calculated based on (2).

$$AR = \frac{a}{b} \quad (2)$$

a = major axis
b = minor axis

3) Roundness

Roundness (RN) describes how closely the shape of an object to a perfect circle (roundness = 1) and can be calculated according to (3).

$$RN = \frac{4A}{\pi a^2} \quad (3)$$

E. Accuracy, Sensitivity and Specificity

To evaluate the performance of our image analysis method for detecting sickle cells, we calculated accuracy, sensitivity and specificity, which are standard metrics used in diagnostic testing.

Accuracy evaluates how well the method can correctly identify normal cells, as shown in (4).

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \quad (4)$$

Where TP is true positive, the number of correctly classified as sickle cells. TN is true negative, the number of correctly classified normal cells. FP is false positive, the number of incorrectly identified sickle cells. FN is false negative, the number of sickle cells that were incorrectly identified as healthy cells.

Sensitivity measures the ability of the method to correctly identify sickle cells using (6).

$$Sensitivity = \frac{TP}{TP + FN} \quad (5)$$

Specificity evaluates how well the method can correctly identify normal cells, as shown in (6).

$$Specificity = \frac{TN}{TN + FP} \quad (6)$$

III. RESULTS AND DISCUSSION

Table 1 presents the morphological characteristics—circularity, aspect ratio, and roundness—for normal cells, sickle cells, and other cells. As expected, normal cells exhibited the highest mean circularity, followed by other cells, with sickle cells showing the lowest value. The mean circularity of normal cells is less than 1, possibly because the image processing step might have slightly distorted the cell boundaries, causing the typically round normal cell to deviate from a perfect circle. Aspect ratio, on the other hand, provided a more distinct separation between each cell type. Normal cells, with an aspect ratio close to 1, indicate their nearly perfect circular shape. In contrast, sickle cells showed a significantly high aspect ratio, reflecting an elongated shape. This finding is consistent with previously reported values [7], validating our analysis. Similarly, the roundness values followed the same pattern as circularity, but the differences between the roundness of each cell type were more pronounced, offering a clearer distinction between normal and abnormal cell morphologies.

	Normal cells	Sickle cells	Other cells
CR	0.81 ± 0.08 (0.60 - 0.91)	0.56 ± 0.08 (0.34 - 0.74)	0.66 ± 0.13 (0.25 - 0.84)
AR	1.10 ± 0.08 (1.00 - 1.47)	2.96 ± 0.68 (1.90 - 5.24)	1.50 ± 0.30 (1.03 - 2.83)
RN	0.90 ± 0.05 (0.67 - 1.00)	0.35 ± 0.07 (0.19 - 0.52)	0.69 ± 0.11 (0.35 - 0.96)

Table 1: the values of CR, AR, and RN of normal cells, sickle cells, and other cells (the values represent mean ± standard deviation and min-max in parenthesis)

The performance of the classification model using three key morphological features—circularity, aspect ratio, and roundness—is summarized in Table 2. A total of 647 cells from 30 peripheral blood smear images, comprising normal, sickle, and other cell types, were analyzed to evaluate the accuracy, sensitivity, and specificity of each morphological feature in distinguishing between different cell types. The results indicate substantial variation in the model's ability to accurately classify normal, sickle, and other cells based on these features.

	CR	AR	RN
TP(cells)	133	101	101
FP(cells)	497	119	119
TN(cells)	12	390	390
FN(cells)	5	37	37
Accuracy	0.22	0.76	0.76
Sensitivity	0.96	0.73	0.73
Specificity	0.02	0.77	0.77

Table 2: Performance metrics for classifying normal, sickle and other cell types based on different morphological features, evaluated from a total of 647 cells.

Circularity showed the lowest performance, with an overall accuracy of 0.22. While its sensitivity was 0.96, indicating that circularity was sensitive to identifying sickle cells, its specificity was 0.02, implying a very poor ability to correctly classify normal cells, leading to a high number of false positives (FP = 497). This finding suggests that circularity is not an effective feature to distinguish between sickle and normal cells.

In contrast, aspect ratio and roundness performed significantly better, each having an accuracy of 0.76. Both features demonstrated a balanced performance in identifying sickle cells and normal cells, with similar values for sensitivity (0.73) and specificity (0.77). The aspect ratio and roundness appear to be effective in classifying normal and sickle cells, as these features reflect the distinct elongated shape of sickle cells compared to the near-circular shape of normal cells.

The higher specificity of aspect ratio and roundness, as compared to circularity, suggests that these features are more reliable in correctly identifying normal cells (TN = 390) while still maintaining a high sensitivity for detecting sickle cells. These features are more suited for practical diagnostic applications, as they minimize false positives, while maintaining high sensitivity for detecting sickle cells.

The performance metrics from this study are in line with those reported in other studies, which typically range between 0.50–0.95for classification accuracy [8]. To further improve the classification performance, more advanced image segmentation methods could be employed. For example, using more sophisticated machine learning algorithms or deep learning-based segmentation may improve the identification of cell boundaries and feature extraction, leading to higher classification accuracy [9].

IV. CONCLUSION

In this study, we developed and evaluated an image analysis method using Fiji to classify red blood cells from peripheral blood smears into normal, sickle and other cell types based on key morphological features, including circularity, aspect ratio, and roundness.

Our findings demonstrate that aspect ratio and roundness are more reliable metrics for the classification of sickle cells, providing a balance between sensitivity and specificity, as they clearly differentiate between the elongated sickle cells and the rounder normal cells. In contrast, circularity was less effective due to its high rate of false positives, making it unsuitable as a standalone metric for classification.

This implementation of this method in Fiji, a free and open-source software, offers potential for widespread application, particularly in resource-limited settings. Future improvements, such as incorporating advanced segmentation techniques or machine learning approaches, could further enhance the accuracy of this classification model, making it practical for real-world diagnostic use.

REFERENCES

[1] Thomson, Azalea M et al. The Lancet Haematology, Volume 10, Issue 8, e585 - e599.

[2] https://www.hematology.org/education/patients/anemia/sickle-cell-disease#:~:text=Complications%20of%20sickle%20cell%20disease,in%20some%20cases%20premature%20death.Accessed on August 23, 2024

[3] Olaniyan HS, Briscoe C, Muhongo M, Pascoal R, Armando A, Santos B, McGann PT. Early diagnosis of sickle cell disease at birth hospitals and vaccination centers in Angola using point-of-care tests. Blood Adv. 2023, 7(19):5860-5867.

[4] Alvarez O, Montague NS, Marin M, O'Brien R, Rodriguez MM. Quantification of sickle cells in the peripheral smear as a marker of disease severity. Fetal PediatrPathol. 2015 Jun;34(3):149-54. doi: 10.3109/15513815.2014.987937. Epub 2014 Dec 17. PMID: 25517885.

[5] Acharya V., Prakasha K. Computer Aided Technique to Separate the Red Blood Cells, Categorize them and Diagnose Sickle Cell Anemia. J. Eng. Sci. Technol. Rev. 2019;12:67–80. doi: 10.25103/jestr.122.10.

[6] Gonz.lez-Hidalgo, M., Guerrero-Pena, F., Herold-Garc.a, S., Jaume-i-Cap., A. & Marrero-Fern.ndez, P. D. Red blood cell cluster

[7] separation from digital images for use in sickle cell disease. IEEE J. Biomed. Health Informatics 19(4), 1514–1525 (2015).

[8] T. S. Chy and M. A. Rahaman, "Automatic Sickle Cell Anemia Detection Using Image Processing Technique," 2018 International Conference on Advancement in Electrical and Electronic Engineering (ICAEEE), Gazipur, Bangladesh, 2018, pp. 1-4.

[9] Rubio, J.M.B., Moyà-Alcover, G., Jaume-i-Capó, A. et al. Crowdsourced human-based computational approach for tagging peripheral blood smear sample images from Sickle Cell Disease patients using non-expert users. Sci Rep 14, 1201 (2024).

[10] Roopa B. Hegde, Keerthana Prasad, Harishchandra Hebbar, I Sandhya,Peripheral blood smear analysis using image processing approach for diagnostic purposes: A review, Biocybernetics and Biomedical Engineering, 38(3), 2018, p 467-480.

★★★