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Analysis of Abnormality based on Size in Red Blood Cells in Peripheral Blood Smear Images

F. Sheeba[1*], T. Robinson[2], J.J. Mammen[3], J.M.S. Philips[4], T. Sathyaraj[5], S.V. Prabhu[6]

[1]Dept. of Computer Science, Madras Christian College, Chennai, India
[2] Dept. of Mathematics, Madras Christian College, Chennai, India
[3]Dept. of Transfusion Medicine & Immunohaematology. Christian Medical College, Vellore, India
[4] PDI Software Solutions, Chennai, India
[5] Jump Start Ninja Technologies, Chennai, India
[6] Jump Start Ninja Technologies, Chennai, India

[*]*Corresponding Author: fsheeba@gmail.com, Tel.: +91-98402-38229*

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***Abstract*—** The diagnostic formulations in patients rest on a tripod consisting of clinical history, physical examination and laboratory investigations. In most of the cases diagnoses are mainly done based on laboratory medicine. Current manual techniques lack precision and reproducibility and hence automated methods where an image of the smear is captured and analyzed offers more precision and accuracy. Accurate analysis of the cells including the red cells in the blood smear images is vital for the diagnosis of various diseases and pathological conditions in patients. This calls for accurate detection and segmentation of the Red Blood Cells (RBCs) prior to analysis. Normal RBCs are biconcave in shape with a central pale area and any deviation in most of the RBCs in their size and ratio of the total surface area of the cell to the central pale area from the normal represents an abnormality. If the size and volume of an RBC is less than a normal cell it is indicative of a pathological process called as microcytosis and on the other hand macrocytosis is the condition where the cell is enlarged. This paper proposes an automated method of analyzing the RBCs in blood smear images for morphological abnormalities, which is an extension of an earlier work focusing on segmentation of all the cells in the blood smear images using Watershed Transform.

***Keywords*—**Segmentation, Watershed Algorithm, Morphological Operations, Mean Corpuscular Volume

I. INTRODUCTION

A peripheral blood smear image has a rich representation of White Blood Cells (WBCs), Red Blood Cells (RBCs) and Platelets. Red cells are the most abundant type of blood cells delivering oxygen to body tissues. Determining the count and haemoglobin content of the RBCs is often the first step in analyzing a patient's pathological condition. The study of the deviation in size of the cell or the ratio of its surface area to the central pale region can lead to detection of abnormalities in patients.

Such abnormalities can be detected by viewing the blood-smear image through a microscope and analyzing the RBCs, which is a time consuming and error-prone process. The alternative is using haematology analyzers, which is highly expensive. Thus there is a need for digital imaging, where the microscopic view of the blood smear image can be captured by a digital camera and the digital image can be used for the study. Using this digital blood smear image, segmentation and analysis of individual Red Blood Cells is made possible.

The proposed study thus aims at analysis of Red Blood Cells based on the surface area of the whole cell and the area of the central pallor, which reflects the amount of hemoglobin present in the RBCs.

Rest of the paper is organized as follows: Section II gives the background of the research paper, Section III explains the methodology that is used in the image analysis, Section IV describes the results and discussion about the study and Section V gives the conclusion of the paper.

II. BACKGROUND

Normal RBCs are biconcave-shaped, approximately 7-8 microns in diameter and 2-2.5 microns in width with a central pale area, which is almost one-third of the cell's area and with an internal volume of 80-96 femtoliters (fL), which is shown in Figure 1.

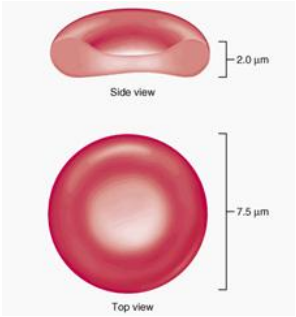


Figure 1. Normal Red Blood Cell

RBCs may vary in size. In terms of the diameter of the RBCs, if the diameter of an RBC is less than 5.0 microns, the abnormality is defined as a microcyte. This is seen to affect a significant proportion of the RBCs in conditions like Iron Deficiency Anemia, Sideroblastic Anemia, Thalassemia and Lead Poisoning. A macrocyte on the other hand, is an enlargement of the red blood cells with a near constant concentration of hemoglobin. The **d**iseases/conditions associated with the presence of macrocytes are Liver Disease, Megaloblastic Anemia (with associated Vitamin B12 or Folate deficiency) and sometimes in aplastic Anemia. The RBCs that contain normal amount of hemoglobin with the central pallor occupying about 1/3rd the surface area are considered to be normochromic. Red cells that have less hemoglobin content than the normal tend to flatten out more n a slide and may appear larger than they actually are. If the central pale area is larger than expected it is considered as ochromic. Lack of central pallor due to increased hemoglobin concentration in the red cell results in a dense, deep staining RBC is called a spherocyte. These are the only cells that are truly hyperchromic. If the Mean Corpuscular Hemoglobin Concentration (MCHC) increases, the likelihood of spherocytes being present increases. The MCHC levels in normal cases should be around 33%. A level much lower than this may be due to anemia or blood loss and higher value may be due to macrocytic anemia, deficiency in folic acid, liver diseases, hereditary spherocytes and vitamin B12 deficiency. MCHC is often accompanied by an MCV (Mean Corpuscular Volume) test, which measures the average volume of a red blood cell. It is reflective of the size of a cell, and thus, higher MCV indicates a large red blood cell and decreased MCV indicates a small red blood cell.

A. Earlier Works of the Author and Related Works

Segmentation of cells in blood smear images was carried out by the primary author using various segmentation methods like intensity based to segment the White Blood Cells (WBCs) [1] and malaria parasites [2], Watershed Transform [3] and Tissue –like P Systems [4] to segment the WBCs and color segmentation to segment Malaria parasites [5]. The author also used color segmentation to segment Tuberculosis bacilli in sputum smear images [6]. The segmentation results suffered from performance degradation due to the presence of many overlapping cells in the blood smear images. Hence the author performed segmentation of such overlapping cells by splitting them using computation of dip points of the cells [7][8] and Active Contour modeling to split overlapping cells and detect Malaria Gametocytes [9]. Also the author carried out a study on detecting overlapping TB bacilli using morphological operations [10].

Analysis of all cells in blood smear images specifically the RBCs are also carried out by various researchers. RBC estimation was done using Hough Transform [11], line operator and watershed algorithm [12**],** masking and watershed algorithm [13] [14] and Discrete Shearlet Transform [15]. Few researches have been carried out in the detection of abnormal RBCs. Certain abnormal RBCs including spiky, sickle, elliptical, tear drop, Rouleaux and bull's eye cells were detected using shape detecting algorithms [16]. Also segmentation of RBCs and shape based detection of abnormal RBCs were carried out by [17]. Certain abnormal cells were detected using Artificial Neural Network [18]. Sickle cells, a half-moon shaped RBCs were detected using Watershed Transform [19]. The proposed study mainly concentrates on the detection of likelihood of abnormality in RBCs based on the variation of their surface areas and the ratio of the central pallor to their surface areas.

III. METHODOLOGY

A. Image Pre-processing

The test images are first converted to binary images using Otsu's thresholding. The objects that are touching the borders are eliminated and holes filled in them. Morphological operations like dilation and erosion are applied onto all the blobs in order to smoothen them and strengthen their borders. The WBCs are eliminated based on their sizes as they are visibly larger than RBCs. The pallor region of the RBCs is normally one third of their total area. All the RBCs which do not fall into this category and have variation in their size have to be detected and if the count of such cells are more than a threshold value, it can be concluded as abnormality. Detection of such varied cells is discussed in the following sub-section.

B. Detection of Abnormal Red Blood Cells

The area of all the blobs in the segmented binary image is obtained. Using granulometric analysis the average area of RBCs in the blood smear image is predicted. A graph is plotted as shown in Figure 2 with the number of blobs in the x-axis and the surface area of the blobs in the y-axis. From the graph it can be found how many blobs are normal in size based on their area.

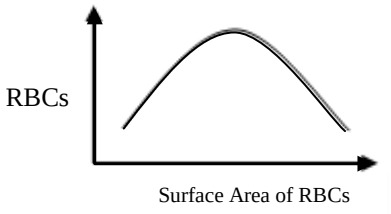


Figure 2 Graph - No. of RBCs against their Areas

In order to find out if the central pale area of the RBC is one third of its total area, the area of the central area (CA) and the total surface area (SA) of the RBC are calculated and it is checked if the CA of each blob is around one third of its SA. This can be analyzed by plotting a graph as shown in Figure 3 with SA in the x-axis and CA in the y-axis.

Figure 3 Graph – Area of Central Pallor of RBCs against their Surface Area

IV. Results and Discussion

500 images were used for the study. The source image, which is a peripheral blood smear image, is shown in Figure 4.

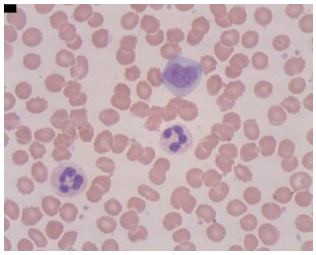


Figure 4. Source Image

The segmented binary image, with the WBCs eliminated and consisting of only the RBCs is shown in Figure 5.

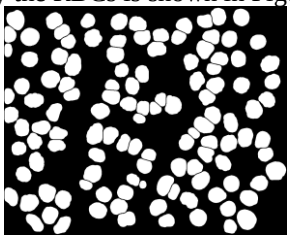
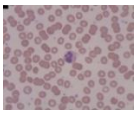
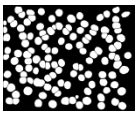
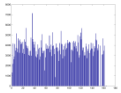
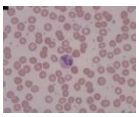
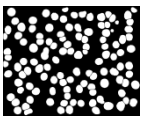
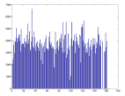
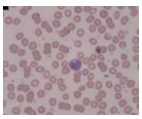
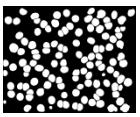
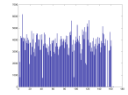
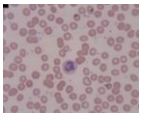
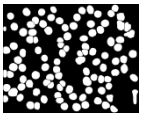
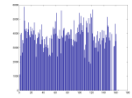
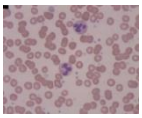
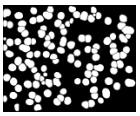
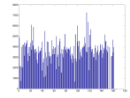


Figure 5. Segmented Binery Image

It is seen that out of 124 RBCs detected 97 were normal in size as their surface area falls in the range of 3000- 4500. The rest of RBCs have areas above 4500 and hence they are concluded as bigger RBCs. For a patient if the majority of the RBCs are bigger in size or the ratio of the central pallor to the total area is not around 33% then it is concluded that there is a possibility of some pathological problem.Table 1 shows five more images, their segmented binary images and bar charts of the central area of RBCs against their surface area.

Table 1. Results of RBC Analysis

Original Image	Segmented Image	Bar Chart
		
		
		
		
		

The second analysis is based on the content of hemoglobin present in each of the RBC. In order to measure this, the surface area of the Central pale Area (CA) of an RBC and the total Surface Area (SA) of the RBC are calculated. All the5 RBCs are normal as their CA is around 33% of SA. All the other RBCs have condensed hemoglobin.

V.Conclusion and Future Scope

It is seen that diagnosis of various diseases is based on laboratory investigations, of which analysis of Red Blood Cells in peripheral blood smear images play a vital role in

obtaining the pathological condition of a patient. In most of the diseases the RBCs vary in size and the area occupied by the central pallor of the cell decides the variations of RBCs. The analysis has to be extended to all the sub-images of a full slide image to conclude the diagnosis of abnormality in a patient. Hence this automated analysis of such variations of RBCs can help a clinician to make quick inference about the condition of a patient with regard to blood smear image analysis specifically the analysis of RBCs. Also this study is only a support to the prediction of abnormalities in RBCs. Only by finding the volume of the red cells and their hemoglobin content the clinicians can conclude the abnormalities of RBCs. Hence the next phase of work includes the computation of the volumes of the RBCs from the 2D images.

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Authors Profile

Ms. F. Sheeba pursed Bachelor of Science from Madurai Kamraj University, S.India in 1987 and Master of Science from Anna University, Chennai, India in 1990. She is currently pursuing Ph.D. and working as Associate Professor in Department of Computer Science, Madras Christian College, Affiliated to the University of Madras, Chennai, India since 1995. She has published 4 research papers in reputed international journals including Springer-LNCS and conferences including IEEE and are also available online. Her main research work focuses on Medical Image Analysis, Pattern Recognition and Digital Image Watermarking She has more than 25 years of teaching experience and 8 years of Research Experience.

Dr. T. Robinson pursed Bachelor of Science and Master of Science from University of Madras, Chennai, India in 1986, Ph.D. (Computer Mathematics) from University of Madras, Chennai, India in 2001 and fPh.D (Disability Science) from the University of Tsukuba, Japan in 2007. He is currently heading the Department of Mathematics, Madras Christian College, Chennai, India. Tamil Nadu. He has received the Tamilnadu Innovation Award from the Confederation of Indian Industries (CII), Chennai in the year 2009, the National Award from Min. of Social Justice & Empowerment, Govt. of India in the year 2005 and Friend of Blind award from the Welfare Foundation of the Blind, Chennai, India. He has designed the Tactile Graphic Tool, Universal Kolam Cube, Tactile Kolam sheets:and the Tactile Sudoku Puzzle for the visually impaired people. He has published more than 10 research papers in reputed international journals including Springer-Verlag Berlin Heidelberg and Springer – LNCS and conferences including IEEE and are also available online. His main research work focuses on Membrane Computing (Bio-Inspired Computing), Medical Image Analysis, Robotic Self-assembly, Pattern Generation, Tactile Geometry (Disability Science) and Inclusive Education (Special Needs Education). He has more than 25 years of teaching experience and 15 years of Research Experience.

Dr. J.J. Mammen pursed MBBS and MD (Pathology) from Christian Medical College, Vellore, India in 1999. He was the Pathology Informatics Fellow for 2 years (July 2006 – June 2008) and Bio-scientific Staff for Pathology Informatics for 2 years (Jan 2007 – May 2009). He is currently the Professor and Head of the Department of Transfusion Medicine & Immunohaematology. Christian Medical College, Vellore, India. His main research work focuses on Laboratory and Healthcare informatics, Digital imaging in Pathology (acquisition, management and analysis), Error reduction & tracking (Barcoding solutions for the clinical lab), Lab decision support (Algorithms for automated cell counter area), Patient care (Nosocomial infection - active surveillance) and National External quality assessment (haemostasis & transfusion medicine). He has more than 25 years of teaching experience and 15 years of Research Experience.

Mr. J.M.S. Philips pursed Bachelor of Computer Applications and Master of Computer Applications from University of Madras, Chennai, India in 2018 and is currently working as a Software Trainee in PDI Software Solutions, Chennai, India. He has been involved in research in Medical Image Inalysis for the past one year and has published research papers in two International Conferences.

Mr. T. Sathyaraj pursed Bachelor of Science in Barathiyar University, Trichy, India in 2015 and Master of Computer Applications from University of Madras, Chennai, India in 2018. He is currently working as a Software Developer in Jump Start Ninja Technologies, Chennai, India. He has been involved in reseach in Medical Image Inalysis for the past one year and has published research papers in two International Conferences.

Mr. D.V. Prabhu pursed Bachelor of Science in Thiruvalluvar University, Tirupattur, India in 2015 and Master of Computer Applications from University of Madras, Chennai, India in 2018. He is currently working as a Software Developer in Jump Start Ninja Technologies, Chennai, India. He has been involved in reseach in Medical Image Inalysis for the past one year and has published research papers in two International Conferences.

