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Detection of Abnormal Findings in Human RBC in Diagnosing **Sickle Cell Anaemia** Using Image Processing

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Abstract

Red Blood Corpuscles are the major cellular component of human blood which are responsible for gaseous exchange between living cells and external environment. In normal physiological condition, an RBC is circular in front view and bi-concave in side view. In terms of size, it is 7.5 µm in diameter and 2 µm in thickness. This normal morphology of RBC undergoes specific changes as a consequence of different pathological abnormalities. One of such disease is 'Sickle Cell Anaemia' where the RBCs take crescentic 'sickle' like shape. Here in this paper, correct identification of aberration in normal parameters of RBCs in an anaemic blood sample has been presented using different image processing tools and techniques. Here some preprocessing is done using Weiner filter and Sobel Edge detection method is used to find the boundary of the corpuscles. Then using region properties, a metric is formulated to determine abnormal shape of the corpuscles to diagnose the disease. The purpose of this paper is to highlight this medico-technical aspect.

Keywords: Red Blood Corpuscle(RBC); Sickle Cell Anaemia(SCA); Haemoglobin(Hb); Weiner filter; Sobel Edge Detection Operator; Region Selection

1. Introduction

Like all other sectors of medical science, there has been a normal advancement in pathological field too. But still, the current age popular haematological analyzing devices cannot identify morphological abnormalities in blood corpuscles with 100% precision as the detection part may subject to human error [1]. Here comes the helping hand

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of modern engineering science. But till now there are few areas of bio-medical field where engineering science has not shown its magic yet. One of such untouched sectors of medico-technical field is detection of Sickle Cell Anaemia using Image Processing. In this current research work, we have defined a metric and implemented the algorithm to find significant result set which successfully detects the deviation of the shape of human RBCs to diagnose the disease Sickle cell Anaemia.

1.1 Background of SCA:

SCA is a haemoglobinopathy that is hereditary and characterized by presence of structurally abnormal haemoglobin [2] (HbS). In an adult human RBC, there is haemoglobin which is constituted by Haem molecule and 4 globin chains mostly 2 α and 2 β chains. Substitution of the amino acid valine for glutamic acid at the 6^{th} position of β chain produces HbS which is due to point mutation of the gene forming beta globulin chain [3].

(a) (b)

Fig 1. (a) Normal RBC; (b) Sickled RBC

- If both parents are carrying this faulty gene, child is homozygous → child will suffer from full blown SCA → all the adult type Haemoglobin (HbA) is replaced by abnormal haemoglobin(HbS)
- If 1 of the parent is carrying this abnormal gene, the child is heterozygous → (50% HbA is replaced by HbS → child will have sickle cell trait i.e. the child will be a carrier but not sufferer

Sickle cell anaemia is an inherited, lifelong disease. People who have the disease are born with it. They inherit two genes for sickle haemoglobin—one from each parent. People who inherit a sickle haemoglobin gene from one parent and a normal gene from the other parent have a condition called sickle cell trait. Sickle cell trait is different than sickle cell anaemia. People who have sickle cell trait don't have the disease. Like people who have sickle cell anaemia, people who have sickle cell trait can pass the sickle haemoglobin gene to their children.

1.2 Reason behind Sickling of RBC:

When RBC delivers O_2 at the tissues and become deoxygenated, HbS molecule undergoes polymerisation (gelation or crystalisation). This change in physical state of HbS distorts the RBC taking an elongated, cresentic or sickle shaped structure. Initially the sickling of RBC is generally reversible by oxygenation[2]; however membrane damage occurs with each episode of sickling and eventually the RBCs accumulate calcium(Ca++), lose potassium(K+) and water and become irreversibly sickled despite adequate oxygenation. In this way permanent sickling occurs in red cells.

1.3 Fate of sickled RBCs:

As sickled RBCs become rigid, they get trapped in spleenic sinusoid and are destroyed by macrophage which is plentiful in the spleenic chords. This means life span of an RBC is reduced from normal 120 days to only 10 to 20 days. This haemolysis creates anaemia.

1.4 Reason behind generation of Anaemia due to sickling of RBC:

Anaemia is a condition in which blood has a lower than normal number of RBCs. This condition also can occur if red blood cells don't contain enough haemoglobin. Red blood cells are made in the spongy marrow inside the

larger bones of the body. Bone marrow is always making new red blood cells to replace old ones. Normal red blood cells live about 120 days in the bloodstream and then die.
In sickle cell anaemia, the abnormal sickle cells usually die after only about 10 to 20 days. The bone marrow can't make new red blood cells fast enough to replace the dying ones. Thus decreased number of RBCs in blood causes anaemia which is termed as Sickle Cell Anaemia.

2. Methodology

2.1 Preprocessing:

Colored blood smear image is converted into binary image. The image is then complemented. Weiner filter is used to remove noise by adaptive filtering method. Then small unwanted regions are removed from the diagram to obtain a clearer view of the region of interest i.e. only the red blood cells in the blood sample. The obtained image is then used for further processing.

2.2 Edge Detection:

An edge is the demarcation between an object and its background, and points out the boundary between overlapping objects [4]. Edge detection is the process of identifying discontinuities in images. There are several types of edge detectors like Sobel Operator, Robert's Operator, Canny Operator, LoG Operator, Zerocross Operator and Prewitt Operator. Among all these, Sobel Method is described below.

- Sobel Operator:

It is a 3×3 gradient edge detector. The Sobel operator performs a 2-D spatial gradient measurement on an image and so emphasizes regions of high spatial frequency that correspond to edges. It is used to find the approximate absolute gradient magnitude at each point of an input gray scale image. Mathematically, Sobel Operator uses two 3×3 matrix which are convolved with the original image to calculate approximations of the derivatives - one for horizontal changes, and one for vertical changes.

As Sobel operator [5] performs a 2-D spatial gradient measurement on an image and emphasizes regions of high spatial frequency that correspond to edges, it is used to find the approximate absolute gradient magnitude at each point of an input gray scale image. That's why in detection of edge of biomedical images, Sobel operator is used.

2.3 Region Selection:

This measures some properties of image regions. The properties can be a comma-separated list of strings, an array containing strings; the single string 'all', or the string 'basic'. If the property is mentioned as string 'all', Regionprops [7] compute all the shape measurements like Area, Centroid, Bounding Box, ConvexHull, ConvexArea etc. If called with a grayscale image, regionprops also returns the pixel value measurements [8]; like Max Intensity, Min Intensity, Weighted Centroid, Mean Intensity, Pixel Values etc. If 'properties' is not specified or if it is interpreted as the string 'basic', the function 'regionprops' computes only the 'Area', 'Centroid', and 'BoundingBox' measurements. Here extensive use of the property 'Area' has been done. 'Area' is a scalar value which represents the actual number of pixels in the region (This value might differ slightly from the value returned by bwarea, which weights different patterns of pixels differently). Also the property 'Perimeter' is used to calculate the distance around the boundary of the region. The function computes the perimeter by calculating the distance between each adjoining pair of pixels around the border of the bounded region. If the image contains discontinuous regions, 'regionprops' returns unexpected results. Here in this piece of work, Area and Perimeter of each of the connected components are measured to calculate the required metric.

3. Proposed Method

3.1 Data Acquisition:

A blood film is produced by pricking pulp of any finger by surgical needle in aseptic condition. Drop of blood not larger than pin head taken on a grease-free glass slide at half inch from the right side. Another glass slide end held at 45° touching the blood drop is lowered to 35° then pushed gently to the left till blood is exhausted giving a tailing effect. Then the slide is air dried and labeled. The film is stained either by Leishman's stain or Giemsa stain. The stained film is examined under high power oil immersion microscope. This photograph is fed to computer and is ready to be used as the input to the program.

3.2 Significance of the Proposed Metric:

In Sickle Cell Anaemia, a normal RBC (i.e. circular in front view and bi-concave in side view) is transformed into 'sickle'-like shape and roundness of the RBC is lost. So the confirmatory pathological diagnosis of SCA is obtained by analyzing shape of the RBC. Here a metric has been proposed to identify this change in shape of RBC in order to diagnose the disease. Basically this metric indicates the roundness of a bounded object. It is defined as $(4*pi*area)/perimeter^2$. So for a 2-d circle i.e. round object, metric value is 1. For a 2-d square, it is 0.785. For a free form object value ranges between 0 and 1.That is why for a normal RBC metric value is nearly 1 and for sickled RBC, the value deteriorates from 1 and results in lesser value of metric.

3.3 Algorithm:

The algorithm is described below:

Step 1. Stained and magnified human blood smear image is fed to the program as input and is converted to binary image.
Step 2. The image is complemented and for the ease of further processing, small unwanted spots are removed from it using bwareaopen which removes all the objects in the diagram containing fewer than the number of pixels mentioned in the threshold level.
Step 3. Number of similar components is detected using bwconncomp.
Step 4. Area of each of the components is calculated using regionprops on the connected objects.
Step 5. Detected corpuscles are displayed one by one and then corresponding surface area is displayed in the software command window.
Step 6. Discarding the smaller background objects, only the larger RBCs are considered and pre-processed for going through rest of the steps.
Step 7. Edges of these RBCs are detected using Sobel edge detection algorithm.
Step 8. Small holes inside the objects are covered up using imfill operator to increase precision of further calculation.
Step 9. Boundary of each of the elements are traversed and marked over the objects and perimeter is calculated from the obtained boundary measurement.
Step 10. Metric [i.e. $(4*pi*area) / perimeter^2$] is calculated for each object.
Step 11. By this metric value, any deviation or change in shape of RBC is detected which are helpful in diagnosing some cases of anaemia like sickle cell anaemia.

4. Result and Discussion

MATLAB 7.14.0.739 Software is extensively used for the study of detecting SCA. Here result set for the normal blood sample and an anaemic blood sample are described to distinctly differentiate between the two.

4.1 Result set for Normal Blood smear:

The normal blood smear (figure in the left side) after preprocessing is binarised to get following diagram (right hand side diagram).

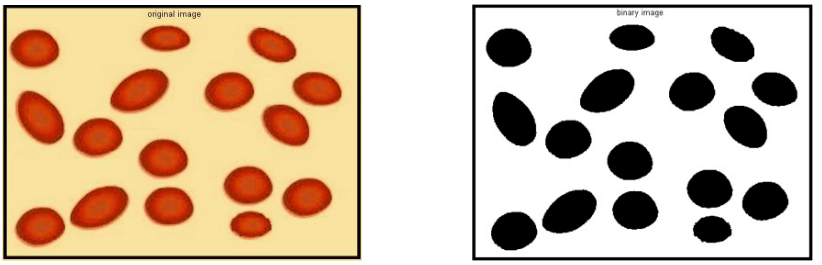

Fig 3: (a) Normal blood smear; (b) Blood smear after binarising

Each connected components are separately displayed and their surface area is calculated. There are 16 elements in the blood smear which are shown one by one. Below only 4 out of these 16 snapshots are shown.

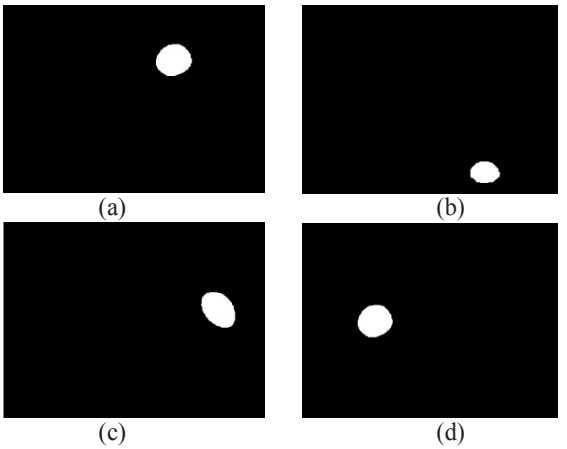

Fig 4 (a) to (d): Snapshot of some of the separated objects

In the left image, larger RBCs are displayed in RGB mode and in the next image foreground larger objects are displayed in green along with smaller cells.

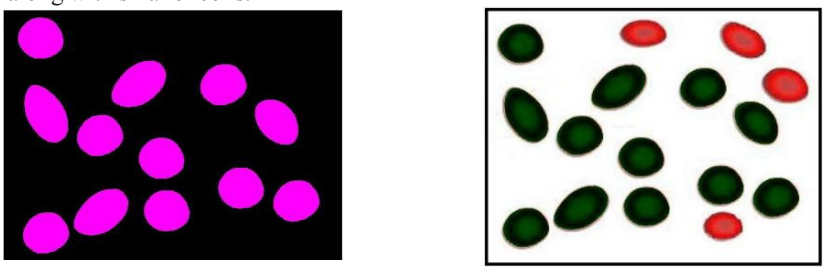

Fig 5: (a) Image converted to RGB; (b) Foreground corpuscles along with the Background smaller ones

Sobel edge detected corpuscles are shown in the left hand side diagram. In the next image, metric values are calculated for each of the RBCs and those are displayed beside each of the objects.

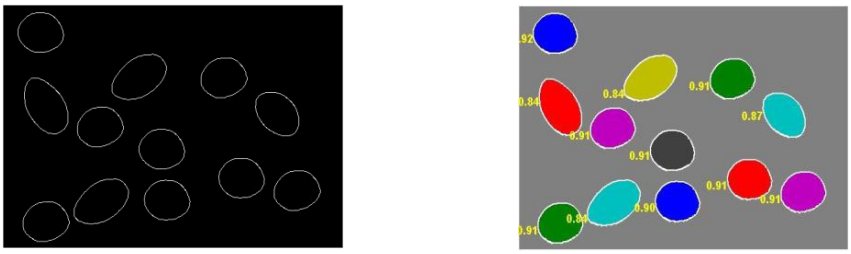

Fig 6: (a) Sobel Edge detected Image; (b) Corpuscles along with Metric value mentioned beside

The metric values for the normal RBCs are: 0.92(1 object), 0.84(1 object), 0.91(6 objects), 0.87(1 object), 0.90(1 object), 0.87(1 object)

4.2 Result set for Anaemic Blood smear:

The anaemic blood smear (left figure) after preprocessing is binarised and the right side diagram is obtained.

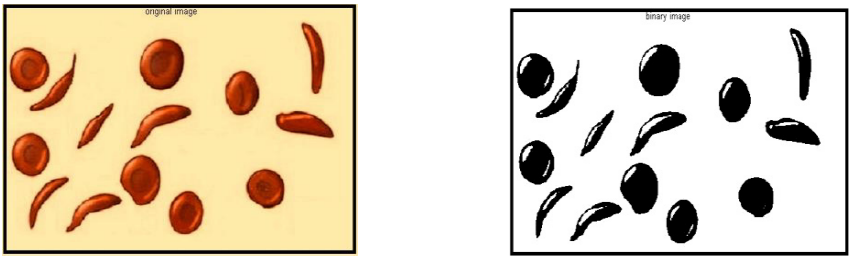

Fig 7: (a) Blood smear of SCA; (b) Binarised blood smears of SCA

Each connected components are separately displayed and their surface area is calculated. There are 14 elements in the blood smear which are shown one by one. Below only 4 out of these 14 snapshots are shown.

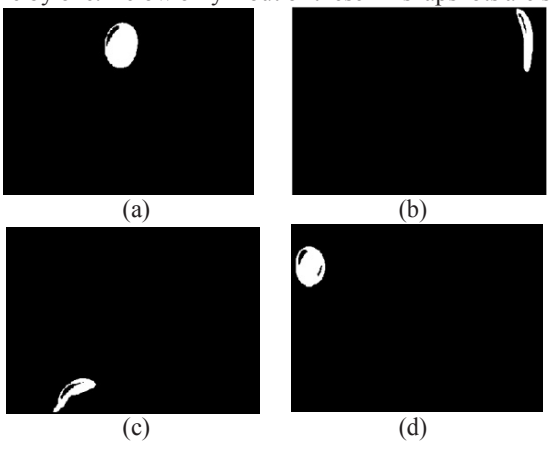

Fig 8 :(a) to (d): Snapshot of some of the separated objects

In next figure larger foreground RBCs are displayed in RGB mode. Foreground larger objects are displayed in green along with smaller cells in the next image.

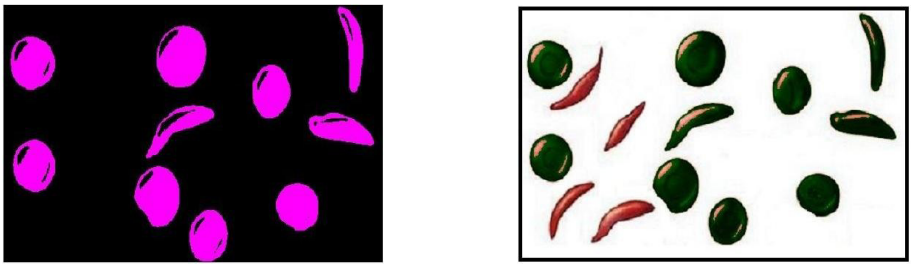

Fig 9: (a) Blood smear of SCA after converting it to RGB; (b) Foreground corpuscles along with the Background smaller ones

Sobel edge detected corpuscles are shown in the next figure. RBCs along with metric value calculated beside each object are shown in the right hand side diagram.

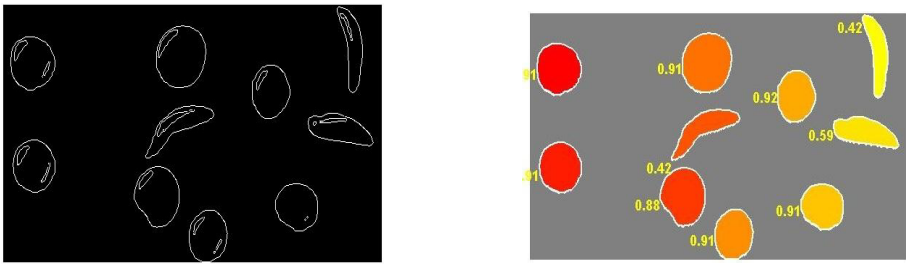

Fig 10 a) Sobel Edge detected Image of SCA; (b) Corpuscles along with Metric value mentioned beside

The metric values for the normal RBCs are: 0.92(1 object), 0.91(5 objects), 0.88(1 object), 0.59(1 object), 0.42(2 objects)

4.3 Mathematical Analysis:

Here metric M is defined as

$$\mathbf{M = (4*pi*area)/perimeter^2}$$

I. M is used to measure roundness of an object.
II. For a perfect circle, value of M is 1. As the shape of object is distorted from a circle to a free-form body, value of M deviates from 1 and tends towards 0.
III. Thus, range of M is: $0 < M < 1$ for all connected components in an image.
IV. In normal blood sample value of M ranges between 0.84 and 0.92; signifying healthy RBCs (in reference with **Result set for Normal Blood smear**)
V. In sample of sickle cell anaemia, some of the cells have M = 0.42, 0.59 whereas rest of the cells have M = 0.88, 0.91, 0.92 (in reference with **Result set for Anaemic Blood smear**)
VI. As normal RBCs are circular in front view and in blood smear most of the time the front view is seen, we will consider that a round shaped cell is healthy and normal.
VII. That's why in normal blood smear value of M is nearly 1 and in diseased blood smear, value of M is far less than 1.

4.4 Statistical Analysis:

From Figure 10(b), Result set can be tabulated as follows [9]:

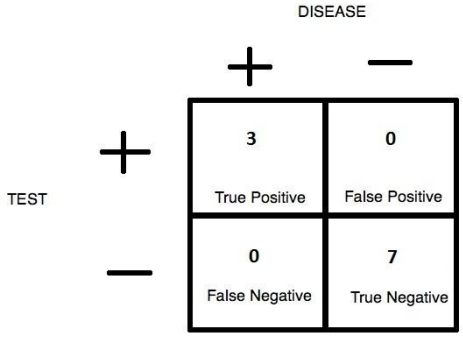

Fig 11. Statistical analysis of the anaemic blood sample

Here, **True Positive:** SCA is present and the test result is +ve. Here in this sample, number of actually diseased RBCs in Fig 10(b) i.e. 3

False Positive: SCA is not present and the test result is +ve. It is the number of normal RBCs mistakenly diagnosed as the diseased one. The count is 0 in this case.

True Negative: SCA is not present and the test result is -ve. Here in this sample, number of normal RBCs in Fig 10 (b) i.e. 7.

False Negative: SCA is present and the test result is –ve. It is the number of diseased RBCs which are not properly diagnosed. Here in the sample count is 0 in this case.

5. Conclusion

This present work on hematology is innovative of its kind where change of shape of RBCs of a blood sample is detected. The paper also presents a method to measure accuracy of the detection of anaemia of several samples by reviewing the feedback from hematologists. Following chart describes the fact [9].

Table 1. Result set for different blood samples

Sample	RBC count in the sample	True +ve (a)	True -ve (b)	False -ve (c)	False +ve (d)	Accuracy (a+b) / (a+b+c+d)* 100	Overall accuracy of the system
1	10	3	7	0	0	100%	
2	29	9	17	1	2	89.66%	
3	17	6	10	0	1	94.11%	95.8%
4	21	4	16	0	1	95.23%	
5	14	4	10	0	0	100%	

From the above chart it is seen that 5 samples are analyzed using the proposed system and in 95.8% cases the diagnosis of SCA is correct and goes at par the result detected by hematologists.

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References

1. "Basic Pathology" by Kumar, Cotran, Robbins 6th edition; Published by Harcourt Asia Saunders; Reference pages 345-347
2. "Davidson's Principles and Practice of Medicine" 20th edition; Published by Churchill Livingstone (Elsiever); Edited by Nicholas A. Boon, Nicki R. Colledge, Brian R. Walker, John A. A. Hunter; Reference pages 1035 - 1037
3. "Review of Medical Physiology" by William F. Ganong (International edition); 18th edition; Published by Prentice-Hall International, Inc. Reference page 499
4. www.mathworks.com/matlabcentral/fileexchange/9303-sobel-edge-detection
5. "Study and Comparison of Various Image Edge Detection Techniques" by Raman Maini, Dr. Himanshu Aggarwal
6. http://www.nhlbi.nih.gov/health/health-topics/topics/sca
7. http://blogs.mathworks.com/steve/2011/09/02/area-opening-terminology-question/
8. www.mathworks.in/help/images/ref/regionprops.html
9. http://www.usmlesuccess.net/answering-biostatistics-questions-on-the-usmle-step-1-and-step-2-ck-exams
10. http://en.wikipedia.org/wiki/Sickle_cell_anaemia
11. "An Improved Sobel Edge Detection" by Wenshuo Gao, Lei Yang, Xiaoguang Zhang, Huizhong Liu
12. Tanmay Bhattacharya, Nilanjan Dey,S. R. Bhadra Chaudhuri "A Novel Session Based Dual Steganographic Technique Using DWT and Spread Spectrum", International Journal of Modern Engineering Research , Vol.1, Issue1, pp- 157-161
13. http://en.wikipedia.org/wiki/rbc
14. Mamta Juneja, Parvinder Singh Sandhu "Performance Evaluation of Edge Detection Techniques for Images in Spatial Domain" International Journal of Computer Theory and Engineering, Vol. 1, No. 5, December, 2009
15. R.C.Gonzalez and R. E. Woods "DigitalImage Processing" 2nd edition Prentice Hall, 2002.
16. http://www.sicklecell.howard.edu/ABriefHistoryofSickleCellDisease.htm
17. http://serendip.brynmawr.edu/biology/b103/f01/web3/rosas.html