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Poikilocyte Cell Detection in Microscopic Images of Blood Smears using Image Processing Techniques

Sumit Kumar Roy[1], Hemlata Sinha[2], Pankaj Mishra[3] and Divya Tyagi[4]
[1]Assistant Professor, Department of Electronics and Communication Engineering, SSIPMT, Raipur, India.
[2]Assistant Professor and HOD, Department of Electronics and Communication Engineering, SSIPMT, Raipur, India.
[3]Professor, Department of Electronics and Communication Engineering, RCET, Bhilai, India.
[4]Assistant Professor, Biochemistry Department, Pt. JNM Medical College, Raipur, India.

(Corresponding author: Sumit Kumar Roy)
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ABSTRACT: Microscopic images of blood smear primarily contains RBCs, WBCs and Platelets, blood count is generally monitored by laboratory test known as CBC i.e. complete blood count, the major cellular component of blood is RBC, which is responsible for gaseous exchange and avails the oxygen for all the organs. In normal physiological conditions, front view and side view of RBC provide circular and biconcave images respectively. In blood borne diseases, generally there is variation in size, shape and in area with respect to normocytic RBCs. Especially in sickle cell anemia which is genetic blood disorder disease, the RBCs after de-oxygenation holds a sickle shape, which as a result causes many diseases. The purpose of this paper; use of Image processing techniques in finding the number of abnormal shaped cell; primary focus is in sickle shaped RBCs in the blood smear image and calculate its percentage and thus highlight the possibility of sickle cell anemia. Early, easy, fast and accurate detection of SCD can save many lives. Results obtained with the experiment are very close to the standard pathological laboratory process results for the blood sample performed by hematologists.

Keywords: Red Blood Corpuscle (RBC), Sickle cell disease (SCD), Digital image processing, Hydroxyurea (HU), Blood smear image, pre-processing, Segmentation, image extraction.

I. INTRODUCTION

SCD (Sickle cell disease) is a generalized term, used for all mutations in the β-globin gene which precipitate the same clinical syndrome; SCD is an autosomal-recessive genetic disorder. Sickle cell anemia (SCA) is caused by homozygosity. SCD is a genetic blood disorder; people from Africa, south East Asia and India are most affected. Chhattisgarh and Rajasthan are the two most widely affected states in India. Hemoglobin present in RBC carries oxygen. The average life time of healthy RBC is approximately 120 days. SCD causes RBC to become crescent (sickle) shaped, which causes them to break easily and the average life time drastically reduces to approximately 20 days only [3]. Symptoms of SCD: Anemia, Severe pain, Chest pain and difficulty in breathing, Strokes, Joint pain, arthritis and bone infractions, Blockage of blood flow in the spleen or liver, severe infection [4].

Diagnosis of SCD: Pathology Laboratory tests include Sickling test, Solubility Test, Hemoglobin electrophoresis, High performance liquid chromatography (HPLC), Deoxyribonucleic acid (DNA) testing. There are associated advantages and disadvantages in context to correctness, time requirement, mobility of the equipment, economy, ease of test, etc. Solubility test and HPLC are taken as (least and most respectively) in context to accuracy. With HPLC test taken as Gold standard, all results are compared with its reference [5].

Types of sickle cell disease: Sickle Cell Anemia (HbSS), sickle Hemoglobin Disease, Sickle beta thalassemia disease (Hb $S\beta^+$), Sickle with hereditary persistence of fetal hemoglobin, Sickle hemoglobin D,E,O disease (HbSD, E, O), Sicklle cell trait (Carrier).

Previous studies extensively included detection of round shaped RBC by using various image processing tools and any irregularity in the round structure were considered as poikilocyte cells. The results derived from this view, deviates from results obtained from std. pathology laboratory results. The major concern with this view is; overlapping of perfectly round and healthy RBCs, also produce irregular shapes in smear images, another drawback from the previous studies; less effort was made in detection of crescent shape (sickle cell) RBCs with respect to detection of round RBCs, also no conclusion were drawn on the basis of percentage of irregularity in comparison to healthy RBCs.

In this research paper, two orthogonal approaches are evaluated; first approach includes detection of actual healthy and round shaped RBCs. whereas the second approach is to detect only the poikilocyte cells (predominantly sickle cells), results derived from two approaches collectively, which are orthogonal to each other and after careful noise filtering, yields better and more accurate results.

The uniqueness of this research is that, the percentage of sickling is calculated and real time advantage can be taken by the hematologists along with other symptoms of the patient, in determining the dosage of medicine (predominantly Hydroxyurea-HU) [7], the number of healthy RBCs and crescent shaped RBCs are also given for every smear image, which can prove to be very beneficial for further research work.

II. MEDICAL BACKGROUND

Sickle cell disease (SCD) is an autosomal-recessive genetic disorder; SCD is an umbrella term for all mutations in the β-globin gene that precipitate the same clinical syndrome. Sickle cell anemia is caused by homozygosity of the beta-S (β^S), in which GTG is substituted for GAG in the sixth codon of the β-globin gene. This leads to replacement of a hydrophilic glutamic acid residue (Glu) with a hydrophobic valine residue (Val) at the sixth position in the β-globin chain, resulting in a mutated hemoglobin tetramer HbS ($\alpha^2\beta s^2$) in the erythrocytes of individuals with sickle cell anemia. Homozygous inheritance of the β^S mutation (HbSS) or coinheritance of β^S with other mutations such as β^C (HbSC), β^D (HbSD), β^O (HbSO/Arab), β^E (HbSE), or a β-thalassemia allele (HbS/β-thal^0 or HbS/β-thal$^+$) leads to other forms of SCD via multiple interlinked molecular and cellular mechanisms [6, 8].

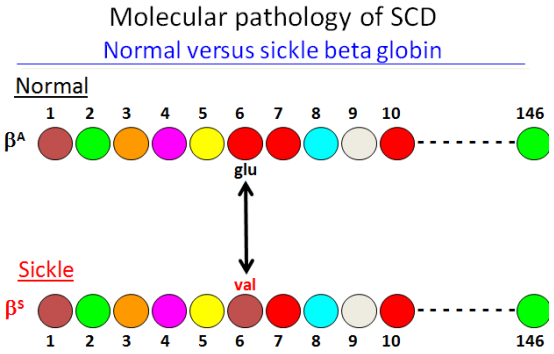

Fig. 1. Genetic Mutation in SCD.

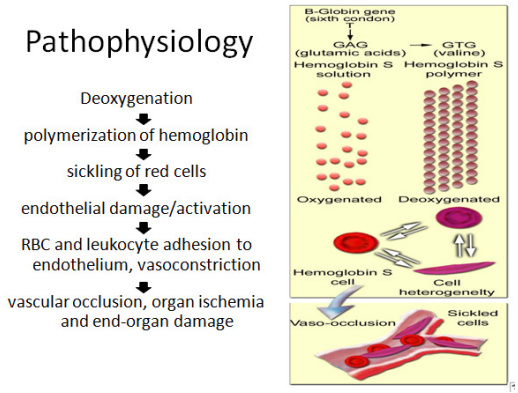

Fig. 2. Pathophysiology of SCD.

III. METHODOLOGY

Methodology is divided in two parts.
— Process to calculate actual RBC count per cu mm.
— Detection of Poikilocyte Cell (pre dominantly sickle cell).
Part I: Process to calculate actual RBC count per cu mm.
Sub division of this process: Image acquisition, pre-processing or Image enhancement, Image segmentation and extraction.
Image acquisition: Blood smear image production (classical way) A blood film is made by pricking pulp of any finger by surgical needle in aseptic condition. Drop of blood which is not larger than a 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 (11 smear images are taken into consideration, some of the examples are displayed).

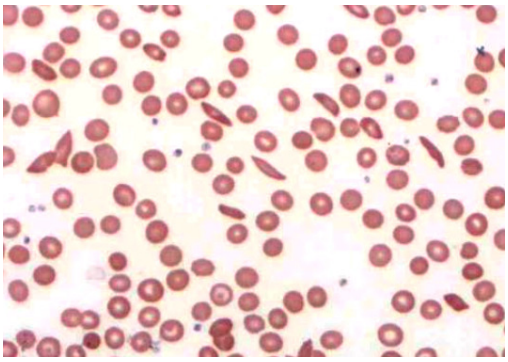

Fig. 3. RGB Image.

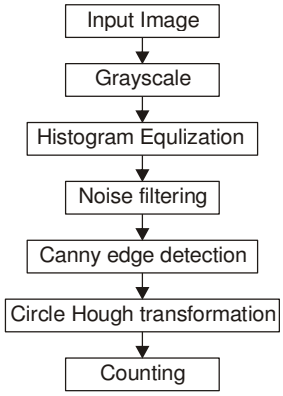

Fig. 4. Flow diagram.

Pre-processing: pre processing or image enhancement is used to raise the quality of the image to be processed. The process involves grayscale conversion followed by histogram equalization and ultimately filtering [10].
Grayscale conversion –True color image is in RGB format which is converted into grayscale which is a numeric array.
Histogram equalization – Histogram equalization is used to enhance the contrast of the image, as image is having close contrast values.
Filtering – Adaptive median filtering method is used to remove noise from the image.

IV. IMAGE SEGMENTATION AND EXTRACTION

Image segmentation is used to locate boundaries such as curves, lines etc. to achieve the process first (a) Canny edge detection technique is used in extraction of important structural information primarily wide range of edges from the image. Followed by (b) Circular Hough Transform is used to determine the circle.
(a) Canny edge detection algorithm: smoothing-finding gradients-non maximum suppression- double thresholding-edge tracking by hysteresis [10].

(b) CHT is a feature extraction technique used to detect circles, CHT includes the following steps:
(i) Accumulator array computation: The solid circle in the figure represents candidate pixel located on the actual circle, the candidate pixels used in voting pattern is shown by dashed lines.
(ii) Center Estimation: the center is estimated by detecting the peaks in accumulator array. Figure shows the center of the circle.
(iii) Radius estimation: the accumulator array is used to calculate radius of the circle as a voting bin [13-16].

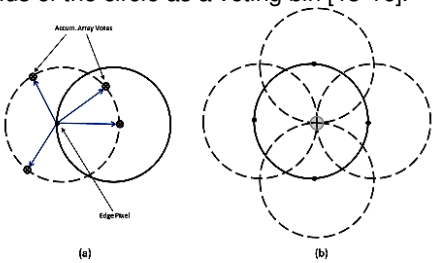

Fig. 5. CHT circle and centre detection.

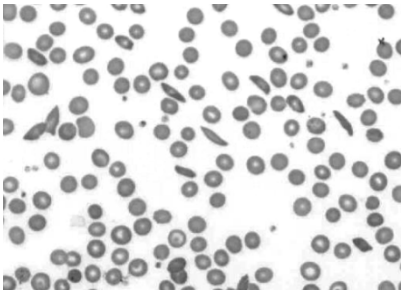

Fig. 6. Grayscale image.

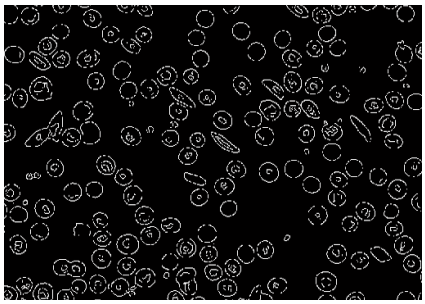

Fig. 7. Canny edge detection.

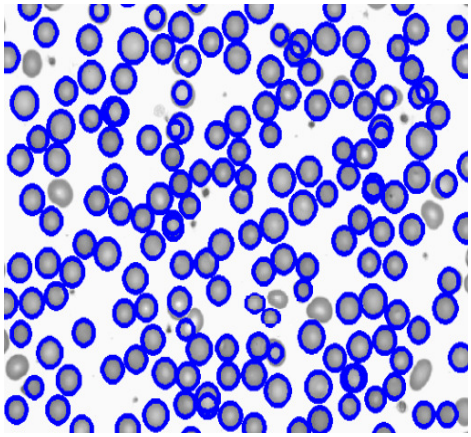

Fig. 8. Normal RBC smear.

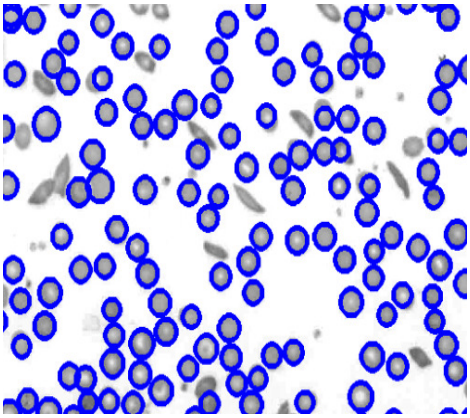

Fig. 9. Sickle cell RBC smear.

Blood Cell Count: The number of RBC per cu mm is based on following assumptions, as in standard medical blood smear formation, the blood film layer is 0.1 mm thickness, which allows maximum two layers, magnification under microscope and dilution of with anticoagulant liquid are taken under obvious consideration.

$$\text{Actual RBC count per cu mm} = \frac{\text{CHT result giving RBC count}}{0.1\text{ mm} * \frac{\text{blood smear image area}}{\text{magnification in X axis} * \text{magnification in Y axis}}} \times \text{factor of dilution}$$

Table 1: Representation of total number of cells, normal and Abnormal RBC.

Sample no.	Total no. of count of cells in cropped and magnified smear image	RBC (Normal) From Algorithm (CHT)	RBC (Abnormal)
1	312	308	4
2	279	271	8
3	321	314	7
4	302	297	5
5	297	296	1
6	281	275	6
7	291	288	3
8	303	302	1
9	337	336	1
10	299	291	8
11	271	269	2

Part II: Detection of Poikilocyte Cell (pre dominantly sickle cell) [17-21].

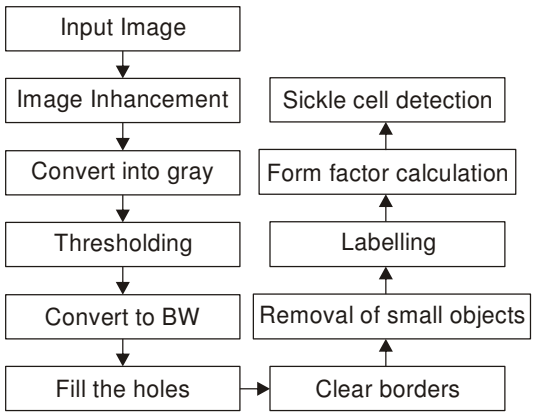

Fig. 10. Design flow.

Some MATLAB functions used [source: Mathworks.com] For Gray scale conversion: rgb2gray Black and White conversion: graythresh, Filling of holes: imfill, Clearing the borders: imclearborder
Small objects removal: bwareaopen.

$$\text{Form Factor (FF)} = \frac{\text{Major axis length}}{\text{Difference between major and minor axix}}$$

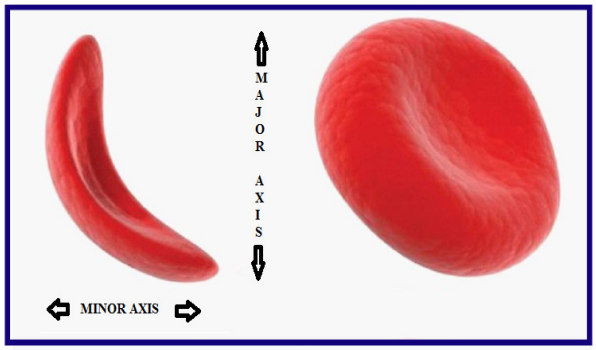

Fig. 11. (Major and minor axis representation in sickle cell).

Threshold of form factor (FF): Based on continued comparison with microscopic true values, sickle cells can be detected: if the form factor is less than 1.81 then the cell will be counted as sickle cell, higher the value of FF lesser are the characteristics of sickle shape in the RBC.

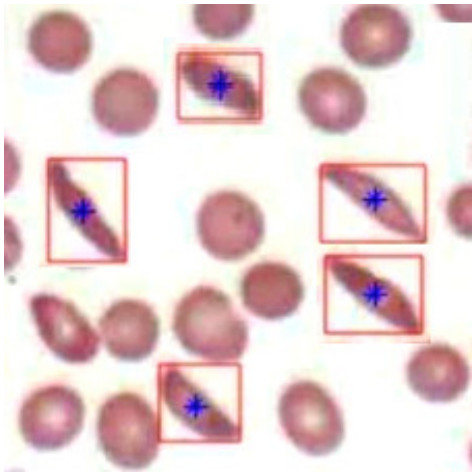

Fig. 12. Detection of sickle cell after running algorithm.

Table 2: Experiment carried out on the same sample for different FF.

Sample No.	True no. of sickle cells (expected to be detected)	Reading of no. of sickle cells (algorithm detected) FF=1.65	Reading of no. of sickle cells (algorithm detected) FF=1.74	Reading of no. of sickle cells (algorithm detected) FF=1.81	Reading of no. of sickle cells (algorithm detected) FF=1.91
1	4	2	3	4	5
2	7	4	5	6	7
3	6	3	4	6	8
4	5	3	4	4	6
5	1	0	0	1	1
6	6	2	3	5	4
7	3	1	1	3	2
8	1	1	1	1	1
9	1	1	1	1	1
10	6	4	5	6	9
11	2	1	1	2	3

Statistical Result: Form Factor of 1.81 proves to yield more accurate results.
Statistical result: Collective efficiency = 92.86% with FF = 1.81. Experimental Results Percentage of Sickle cells present in the smear image, the results obtained prove to be better in context to recent investigations [19-21].

Table 3: Percentage of Sickle cells present in the smear image.

Sample No.	RBC (Normal) From Algorithm (CHT) (from part 1)	Reading of no. of sickle cells (algorithm detected) FF=1.81	Percentage of sickling in RBC (%)
1	308	4	1.29
2	271	6	2.21
3	314	6	1.91
4	297	4	1.34
5	296	1	0.33
6	275	5	1.80
7	288	3	1.04
8	302	1	0.33
9	336	1	0.29
10	291	6	2.06
11	269	2	0.74

V. CONCLUSION AND FUTURE SCOPE

– Microscope illumination, staining of the plate and other process included in preparation of blood smear image is very important.
– The image cropping must be done by the specialist, since cells at other region of the plate are usually deformed.
– Many assumptions were taken regarding preparation of blood smear as preparation requires human interventions, which may give deviation from the results.
– The test results were obtained for few patients (three samples per patient) who were not suffering from any other type of infection at that time; thus the results may deviate from the percentage of accuracy obtained, when tests are conducted for more no. of patients.
– Results obtained from first method may include false positives of sickle cells as other abnormal RBC (non circular shape) is counted.
– Machine learning algorithm can be used to further train and improve the obtained results.
– Real time implementation to assist hematologist and doctors in analysis of present situation of the patient; in context to percentage of poikilocyte cells w.r.t. healthy RBCs and decide on the dosage of drug such as Hydroxyurea-(HU).

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