An Overview of Sickle Cell Anemia- A Special Emphasis on Image Processing of SEM Images

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Abstract
One of the most important components of blood cells is the formed elements called Erythrocyte (Red Blood Corpuscles (RBC)). The shape of the cells during disease varies widely. Hence by visualizing the shape of RBC it is possible to identify diseases like Sickle Cell Anemia (SCA). Computer aided image analysis techniques are useful for such purpose. In the present paper, a survey is conducted and then developed a new algorithm for SCA affected RBC image segmentation and various low level and advanced image processing operations are performed on blood smear images. It is possible to extract much useful information which is helpful for diagnosis and treatment planning of this disease.

Keywords: Sickle cell anemia, Image processing, Red blood corpuscles, Pre-processing, and Image thresholding.

Introduction
Anemia is a condition in which red blood cell is deficient in blood, in haemoglobin or in total blood volume. This diminishes the blood’s ability to carry oxygen and lead to symptoms like fatigue, light headache and shortness of breath. Source forms of this condition are inherited while others are brought on by poor nutrition. There are various types of anemia such as iron deficiency anemia, vitamin deficiency anemia and haemolytic anemia. Body needs iron to produce the haemoglobin which is necessary for red blood cell production. Vitamin B12 and folic acid deficiency also cause anemia. In haemolytic anemia, antibodies produced by the immune system damage red blood cells. Haemolytic anemia can be acquired or inherited. Sickle cell anemia and thalassemia are both inherited type of haemolytic anemia.

Literature Review
This literature review consists of two parts. The first one consists of latest developments that took place in the last five years (i.e. from 2010 to 2015). The second one consists of the developments taken place during the anniversary years (i.e. from 1910 to 2010). The year 1910 is considered as the date of discovery of the sickle cell anemia. During 2010 the world has celebrated the hundredth anniversary of this disease. Over these 100 years several noticeable developments took place and some of the important milestones are summarized below.

A. Latest developments in the last five years (i.e. from 2010 to 2015)
Computerized analysis of sickle cell anemia has got very good attention in recent years and as a result of this many papers were published. A method to recognize sickle shaped cells from blood smear images was developed by Menika Sahu, Amith kumar Biswas et.al. [1].They used fractional dimension to recognize the shape of RBC. An automatic detection method for the detection of sickle cells in blood smear using image segmentation has been developed by Shashi bala and Amit Doeger [2]. They used water-shed segmentation method for RBC extraction. An excellent performance evaluation of different noise removal algorithms in sickle cell images where conducted by Fenwa O.D, Ajala F.A, and Adedeji O.T [3]. A method for the detection of RBC from human blood has also been developed by Rakshavi Desai and H.G. Virani [4]. For the feature extraction they used circular Hough transform. Athira Sreekumar and Ashok Bhattacharya proposed a method for the identification of sickle cells from microscopic blood smear image using image processing [5]. One of the important works in the area of sickle cell disease (SCD) image analysis has been carried out by Manuel Gonzalez Hidalgo, Guerrero-Pena et.al. [6]. They proposed a method for the analysis of the shape of RBC in peripheral blood smear sample of SCD which uses ellipse adjustment and a new algorithm for detecting notable points. Very recently Karl Singer, Amoz I etal [7] demonstrated that haemoglobin obtained from sickle cell erythrocytes differs electrophoretically from normal haemoglobin. They developed a simple technique where an alkaline reagent
destroyed normal haemoglobin completely within one minute. When this procedure was applied to haemoglobin solutions prepared from sickling erythrocytes, a fraction relatively resistant to denaturation was encountered regularly in sickle cell anemia. An activity and school attendance monitoring system for adolescence with sickle cell disease has been carried out by Janani Venugopal, Clark, Brown et al [8]. In this work they designed and prototyped a system called SAM (Sickle school attendance and activity monitoring system) for automatic monitoring of school attendance and daily activity of adolescence with sickle anemia disease.

Few examples of sickle shaped RBC in sickle cell anemia and a normal RBC image is shown below (fig 1, 2, 3, 4).

These images clearly mention the structural difference between RBC and Sickled RBC.

![Figure 1: One sickled RBC and three normal RBC](image1)

![Figure 2: One normal RBC and one sickled RBC](image2)

![Figure 3: 3D image of normal RBC and sickled RBC](image3)

Figure 4: Comparison of normal RBC and sickled RBC

B. Developments took place over the anniversary year (i.e. from 1910 to 2010)

Sickle cell anemia was first described by Dr. James B Herrick in one of the medical journal [9]. It was in 1949[10], Dr. James V Neel who showed the hereditary nature of SCD. During this year two articles appeared independently about sickle cell anemia. One was published by a military doctor Col. E A Beek. His article was in an African medical journal. The other was by Dr. James V Neel who published his article in the prestigious American Journal, Science.

In 1951[11], Dr. Linus Pauling and his colleague Dr. Harvey discovered that haemoglobin had a different chemical structure for the people with sickle cell disease. In 1952[12], Dr. Pauling again hypothesized as the nature of haemoglobin protein may act differently from haemoglobin-A (HbA) and stick together. The details of the abnormality were worked out by Dr. Vernon Ingram in 1956[13]. The association with haemoglobin was discovered by Linus Pauling and Harvey Itano in 1951 and actual amino acid substitution by Vernon Ingram in 1956. In the 1970’s [14,15], more details of how this abnormal structure affects the RBC were revealed and better tests for the detection of the disease were developed.

A work on abnormal cardiac autonomic control in sickle cell disease following transient hypoxia have been performed by Suvimol S, Thomas D.C et.al. [16]. In a different work they conducted a time varying analysis of autonomic control in response to spontaneous sighs in sickle cell anemia[17].

An automatic threshold selection for image segmentation is done by Otsu in [18] which was helpful for the initial segmentation process. Then a faster version of Otsu thresholding is proposed by P-S Liao et.al. [19]. This is to improve the efficiency of computation for optimal thresholds of an image. Combining the survey conducted by P.K. Sahoo [20] we are able to propose a method for multi-level thresholding. J- C Yun et.al. [21], described a new maximum correlation criterion for bi-level thresholding. This method helps to automatically determine the threshold values. Fuzzy thresholding scheme is proposed by C.V. Jawahar et.al. in [23] which was a thresholding scheme based on fuzzy clustering. Cao Binfang et.al. [24], in their work proposed a new algorithm to handle non-extensivity and vagueness of image in segmentation and to reduce the computation time.
The remaining part of this paper is organized as follows. In the next section, we proposed a method for image processing and they are used for the analysis of sickle cell anemia images. Then in next section, a block schematic of the proposed system is shown and a method is described for the analysis of sickle cell anemia. In other sections, the results and discussion about the processed images are given. This is followed by a conclusion.

**Fuzzy Thresholding based Tri level Segmentation**

In the present work, an image segmentation method is proposed in which the region of interest in an image is partitioned and separated out for further analysis. If we want to do some high level image analysis task we have to go for segmentation technique. In this paper we present Fuzzy-based thresholding technique which can be used for the analysis of SCD images. In this approach we first choose 3 thresholds $T_1$, $T_2$, $T_3$ where,

- $T_1$: black pixels (Back ground)
- $T_2$: White pixels
- $T_3$: Global thresholds

Pixels with grey value below $T_1$ and above $T_3$ are considered being pixels with membership values 0 and 1 respectively. Values between $T_1$ and $T_2$ are converted to membership values 0 and $\frac{1}{2}$. Grey value between $T_2$ and $T_3$ are converted in to membership value $\frac{1}{2}$ and 1.

- $T_1$: Minimum grey values of the original image.
- $T_2$: Maximum grey levels values of the original image.
- $T_3$: Minimum grey value of pixels with grey values less than $T_2$.
- $T_4$: Average grey value of pixels with grey values greater than $T_2$.

Consider a grey scale image I with M rows and N columns. This can be viewed as a weighted relation for a set X with M elements into a set Y axis N elements with weight $g(i, j)$ representing grey values are at domain $(i, j)$ in I. The image is divided into segments when the number of segments is decided by the user. The appropriate choice of two thresholds $T_1$ and $T_3$ where $T_1$ corresponds to black values and $T_3$ corresponds to white values. The image is divided into segments where the number of segments is decided by the user $0 < T_1 < T_2 < T_3 < 255$ where $T_2$ is the global threshold.

$$M(i,j)=\begin{cases} \frac{1}{2}(g(i,j)-T_1) : & \text{if } T_1 \leq g(i,j) < T_2 \\ \frac{1}{2}(T_2-g(i,j))+(\frac{g(i,j)-T_2}{T_2-T_1}) : & \text{if } T_2 \leq g(i,j) \leq T_3 \end{cases}$$

The pixels in the original image are partitioned into crisp and fuzzy pixels. The crisp value $M(i,j) = 0$. Corresponding to pixels which are black and white and $M(i,j) \neq 0$ or 1 corresponds to fuzzy pixels. If $M(i,j) < \frac{1}{2}$ corresponds to pixels called somewhat white. Pixels corresponds to $M(i,j) > \frac{1}{2}$ are called maximally fuzzy since they belong to fuzzy sets somewhat black and somewhat white with an equal degree or membership. $M(i,j) = \frac{1}{2}$ are the pixels with grey value $g(i,j) = \text{Global threshold } T_2$.

A fuzzy relation $M$ from a set X into a set Y is a fuzzy subset of the Cartesian product $X \times Y$ ie, $M(x,y) \in [0, 1] \text{ for all } (x,y) \in X \times Y$ (3)

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A fuzzy relation $M$ from X into Y, two fuzzy sets $\sigma_x$ and $\sigma_y$ as X and Y respectively

$$\sigma_x : X \rightarrow [0,1], \quad \sigma_x(a) = \bigvee_{y \in Y} \mu(y,a)$$

$$\sigma_y : Y \rightarrow [0,1], \quad \sigma_y(b) = \bigvee_{x \in X} \mu(x,b)$$

Where $\mu(a,b) \leq \sigma_x(a)$ and $\sigma_y(b)$ it follows that $\mu(a,b) \leq \sigma_x(a) \land \sigma_y(b)$

A fuzzy set $\sigma$ as a set X and a fuzzy set Y, a fuzzy relation $\mu$ from X and Y can be defined as:

$X \ast Y \rightarrow [0, 1]$ such that $\mu(x,y) = \sigma_x \land \sigma_y$

From this fuzzy relation $\mu$ we can write $\sigma_{min} X = \sigma_{max} \ast \sigma_{min} Y, \sigma_{max} \ast Y$ on $Y$

$$\Rightarrow \left \{ \begin{array}{ll} \sigma_{min} X \sigma_{max} Y, \sigma_{max} \ast Y \end{array} \right.$$}

The minimums and maximums are computed over the fuzzy values in each segment, that is’ where $0 < \mu(i,j) < 1$.

The minimum and maximum values are computed over the fuzzy values in each segment, where $\mu(i,j)<1$. We now define the membership values of the nth compliment $\mu^{nc}(i,j)$ of the fuzzified image as follows:

$$\mu^{nc}(i,j) = \begin{cases} 1 & \text{if } \mu^{nc}(i,j) \leq 0 \\
\frac{1}{2}[\sigma_{max}(i) \sigma_{min}(j)] & : \text{if } \mu^{nc}(i,j) < 1 \\
\frac{1}{2}[\sigma_{max}(i) \sigma_{min}(j)] & : \text{if } \mu^{nc}(i,j) < 1 < \frac{1}{2} \\
0 & : \text{if } \mu^{nc}(i,j) = \frac{1}{2} \end{cases}$$

where the first compliment is defined as
\[ \mu^{CC}(i, j) = \begin{cases} 1; & \text{if } \mu(i, j) = 0 \\ \sigma_{max}X(i)\Lambda\sigma_{max}Y(j); & \text{if } 0 < \mu(i, j) < \frac{1}{2} \\ \sigma_{max}X(i)\Lambda\sigma_{max}Y(j); & \text{if } \frac{1}{2} < \mu(i, j) < 1 \\ 0; & \text{if } \mu(i, j) = 1 \end{cases} \quad (7) \]

\[ \mu^{CC}(i, j) = \begin{cases} 0; & \text{if } \mu(i, j) = 0 \\ \sigma_{max}X(i)\Lambda\sigma_{max}Y(j); & \text{if } 0 < \mu(i, j) < \frac{1}{2} \\ \sigma_{max}X(i)\Lambda\sigma_{max}Y(j); & \text{if } \frac{1}{2} < \mu(i, j) < 1 \\ 1; & \text{if } \mu(i, j) = 1 \end{cases} \quad (8) \]

\[ g^{CC}(i, j) = \begin{cases} g(i, j); & \text{if } \mu^{CC}(i, j) = 0 \text{ or } 1 \\ 2\mu^{CC}(i, j)(T_2 - T_1) + T_1; & \text{if } \mu^{CC}(i, j) < \frac{1}{2} \\ 2\mu^{CC}(i, j)(T_3 - T_2) + (2T_2 - T_3); & \text{if } \mu^{CC}(i, j) > \frac{1}{2} \\ T_2; & \text{if } \mu^{CC}(i, j) = \frac{1}{2} \end{cases} \quad (9) \]

After the entire operation, the modified image is an enhancement of the original image.

**Proposed System**

The proposed image processing system consists of few image processing steps. First the colour images are converted into grey scale images. It is further followed by some image enhancement operation such as contrast stretching and unsharp masking. This is followed by an edge detection operation in which the edges are enhanced more. Contrast stretching and un-sharp maskings are two common image processing operations which are useful for improving the quality of image. However they are low level computer vision techniques. Edge detection is also an enhancement as well as edge highlighting technique, which usually provides valuable information as far as image processing research is concerned. Human visual system always concentrates on the edges which are places of maximum intensity variation. This is one of the reasons, by which edge detection techniques got significant importance in any image analysis system.

**Results and Discussions**

The ultimate aim of this work is to find out whether any clinical information can be drawn from the images after performing various image processing operations. Few image processing operations are performed on blood smear images starting with some simple low level vision techniques and then implemented some advanced image analysis methods and several outputs are obtained. The various output images thus obtained are studied by us and also that was given to the medical experts who are involved in the study, diagnosis and treatment of sickle cell anemia. Also we have proposed some new and advanced high level vision techniques using image transformation based on soft computing techniques. We have varied various parameters responsible for improving the image quality and different outputs are obtained for visualization. Encouraging results are obtained for confirming whether RBCs are SCA...
affected or not. Also experiments were conducted to extract information from images to check whether any diagnostic clues can be drawn from these output images as a result of this newly developed method. Some of the results which we obtained are reported on the following paragraphs.

**Figure 6**: Blood sample of normal and sickled RBC

The proposed system is implemented on SCA affected blood smear image and various processing operations as shown in the block schematic are performed. The output image obtained is shown and discussed in section V. In fig.6 two RBC’s are shown, which are affected by sickle cell anemia. This is an RGB image and its gray scale version is shown in fig.7. Edges of the above images are enhanced and are shown in fig 8. Un-sharp masking operation has been carried out on fig 6 and the output is presented in fig 9. Similarly in fig 6 contrast stretching has been carried out and shown in fig 10.

**Figure 7**: Gray scaled image of fig.6

**Figure 8**: Edge detected with sobel

**Figure 9**: Unshaped masking

**Figure 10**: Contrast Stretching applied to fig. 6

Another set of sickle cell anemia affected blood samples is taken and two RBC’s are shown in fig 11. The various image pre-processing operations as shown above are performed on fig11 and the output images obtained are shown in fig 12, 13, 14 and 15 respectively.

**Figure 11**: Another sample of sickled and normal RBC

**Figure 12**: Gray scaled image of fig.11

**Figure 13**: Edge detected output of fig.12

**Figure 14**: Unsharp masking of fig. 11
Fig 16 is a sickle cell anemia affected blood smear image. Various image processing steps as shown in the block schematic of the proposed system are performed on fig 16 and the output thus obtained are shown in fig 17, 18, 19 and 20. Using the pre-processing operations it is found that there is not much clinical information’s which can be drawn from the output images.

Hence we further go for some high level image processing strategy which is a fuzzy based tri level image segmentation operation, as proposed in this paper. Encouraging results are obtained as explained below. Fig 21 is the original image of two RBC’s, one of which is affected by sickle cell disease. We have varied the threshold values of this image to $T_1$, $T_2$ and $T_3$ and the results are shown in fig 23, 24 and 25. (Fig 22 is the grey scale version of fig 21.) From fig 25 it can be observed that one of the image characteristic features of the sickle cell anemia has come out. After observing this output image, we can see that some sticky objects are appeared on the outer surface of these RBC’s, which causes the RBC to stick together, and block the small blood vessels. This is very important clinical information which can only be bought out by high level image segmentation techniques, with the help of grey level imprecision with uncertainty.

Fuzzy based systems are quite famous and suitable for such natural image processing operations. We have developed the code for the entire operation explained in section III & IV and the software is specifically turned to bring out the specific nature of sickle cell anemia.
The above mentioned same procedure is adopted on blood sample of fig 26 and the threshold is varied from $T_1$ to $T_3$. It can be seen that in fig 30 in the outer periphery of the RBC’s the sticky objects can be seen clearly which causes the RBC’s to stick together. It can also be seen that there is a considerable reduction in the number of RBC’s as compared to the sample image of RBC.

Conclusion
Sickle cell anemia is a disease which is severely affected in many parts of the world. Sickle cell anemia is a type of anemia in which red blood corpuscles take the shape of sickle, half moon shape or crescent shape. This shape has been demonstrated in the present paper. Here an extensive literature review of sickle cell anemia is presented for about one hundred and five years. This disease is specially found in a group of tribes in Idukki district, Kerala, South India. Sickle cell disease affected blood smear images are taken from the Government Hospital of Idukki district. Image processing operations are performed on these image and the outputs are obtained. These outputs are analyzed with the
help of medical professionals involved in treatment and research of sickle cell anemia.

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