"Use of Image Processing Techniques to Automatically Diagnose Sickle-Cell Anemia Present in Red Blood Cells Smear"

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Use of Image Processing Techniques to Automatically Diagnose Sickle-Cell Anemia Present in Red Blood Cells Smear

A Thesis submitted in partial fulfillment of the requirements for the degree of

Bachelor of Technology in "Electrical Engineering"

By

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CERTIFICATE

This is to certify that the draft report/thesis titled "**Use of Image Processing Techniques to Automatically Diagnose Sickle-Cell Anemia Present in Red Blood Cells Smear**", submitted to the National Institute of Technology, Rourkela by **Mr. Siddharth Sekhar Barpanda, Roll No**: **109EE0255** for the award of Bachelor of Technology in Electrical Engineering, is a bonafide record of research work carried out by him under my supervision and guidance.

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Abstract

Sickle Cell Anemia is a blood disorder which results from the abnormalities of red blood cells and shortens the life expectancy to 42 and 48 years for males and females respectively. It also causes pain, jaundice, shortness of breath, etc. Sickle Cell Anemia is characterized by the presence of abnormal cells like sickle cell, ovalocyte, anisopoikilocyte. Sickle cell disease usually presenting in childhood, occurs more commonly in people from parts of tropical and subtropical regions where malaria is or was very common. A healthy RBC is usually round in shape. But sometimes it changes its shape to form a sickle cell structure; this is called as sickling of RBC. Majority of the sickle cells (whose shape is like crescent moon) found are due to low haemoglobin content.

An image processing algorithm to automate the diagnosis of sickle-cells present in thin blood smears is developed. Images are acquired using a charge-coupled device camera connected to a light microscope. Clustering based segmentation techniques are used to identify erythrocytes (red blood cells) and Sickle-cells present on microscopic slides. Image features based on colour, texture and the geometry of the cells are generated, as well as features that make use of a priori knowledge of the classification problem and mimic features used by human technicians.

The red blood cell smears were obtained from IG Hospital, Rourkela. The proposed image processing based identification of sickle-cells in anemic patient will be very helpful for automatic, sleek and effective diagnosis of the disease.

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Chapter 1: Introduction

1.1 Anemia

1.1.1. Anemia: Overview

The cellular part of blood molecule contains several different cell types. One of the most important and the most numerous cell types are red blood cells. The other cell types are the white blood cells and platelets. Anemia is the most common disorder of the blood.

"Anemia", the name is derivative from the ancient Greek word *anaimia*, which means "Lack of Blood". It is possible because of reduction in Red Blood Cells (RBCs) or resulting in lesser than normal quantity of haemoglobin in the blood. However, it can also include decreased oxygen-binding ability of each haemoglobin molecule due to deformity or lack in numerical development.

Anemia is actually a sign of a disease process rather than being a disease itself. It can be either classified as acute or chronic. In chronic anemia, symptoms typically begin slowly and progress gradually; whereas in acute anemia, symptoms can be abrupt and more distressing.

Among many factors, both nutritional (like vitamins and mineral deficiencies) and non-nutritional (like infection and haemoglobinopathies), that contribute to the onset of anemia; Iron Insuffiency and malaria plays a significant role.

For men, anemia is typically defined as hemoglobin level of less than 13.5 g/dl and in women as hemoglobin of less than 12.0 g/dl.

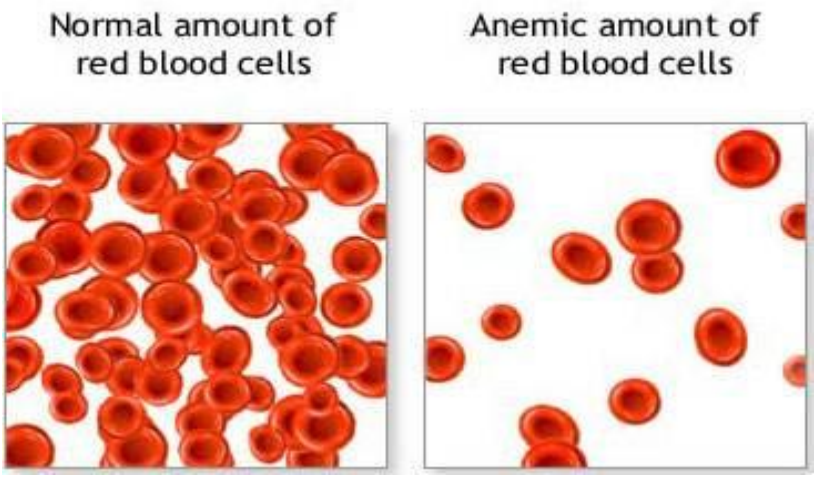


Fig 1.1: (a) Normal Blood Cell Image (b) Anemic Blood Cell Image

1.1.2. Anemia Causes:

Many medical conditions cause anemia. Common causes are:

i) **Anemia from active bleeding:** Loss of blood through heavy menstrual bleeding or wounds can cause anemia. Gastrointestinal ulcers or cancers such as cancer of the colon may slowly ooze blood and can also cause anemia.

ii) **Iron Deficiency anemia:** The bone marrow needs iron to make red blood cells. Iron (Fe) plays an important role in the proper structure of the hemoglobin molecule. If iron intake is limited or inadequate due to poor dietary intake, anemia may occur as a result. This is called iron deficiency anemia. This can also occur when there are stomach ulcers or other sources of slow, chronic bleeding (colon cancer, uterine cancer, intestinal polyps, hemorrhoids, etc). In these kinds of scenarios, because of ongoing, chronic slow blood loss, iron is also lost from the body (as a part of blood) at a higher rate than normal and can result in iron-deficiency anemia.

iii) **Anemia of chronic disease:** Any long-term medical conditions can results to anemia. The exact mechanism of this procedure in mysterious, but any long-standing and continuing medical condition such as a chronic infection or a cancer may cause this kind of anemia.

iv) **Anemia related to kidney disease:** The kidneys release a hormone called the erythropoietin that helps the bone marrow make red blood cells. In people with long standing kidney disease, the production of this hormone is diminished, and this, in turn, shrinks the production of red blood cells, causing anemia.

v) **Anemia related to pregnancy:** Water weight and fluid gain during pregnancy dilutes the blood, which may be reflected as anemia since the relative concentration of red blood cells is lower.

vi) **Anemia related to poor nutrition:** Vitamins and minerals are required to make red blood cells. In addition to iron, vitamin B12 and folate (or folic acid) is required for the proper production of hemoglobin (Hgb). Scarcity in any of these may cause anemia because of insufficient creation of red blood cells. Poor dietary intake is an important cause of low folate and low vitamin B12 levels. Strict vegetarians who do not take sufficient vitamins are at risk to develop vitamin B12 insufficiency.

vii) **Pernicious anemia:** There also may be a problem in the stomach or the intestines leading to poor absorption of vitamin B12. This may lead to anemia because of vitamin B12 insufficiency known as pernicious anemia.

viii) **Sickle cell anemia:** In some individuals, the problem may be related to production of abnormal hemoglobin molecules. In this condition, the hemoglobin problem is qualitative, or functional. Abnormal hemoglobin molecules may cause problems in

the integrity of the red blood cell structure and they may become crescent-shaped (sickle cells). There are different types of sickle cell anemia with different severity levels. This is typically hereditary and is more common in those of African, Middle Eastern, and Mediterranean ancestry. People with sickle cell anemia can be diagnosed as early as childhood depending on the severity and symptoms of their disease.

ix) Other Causes: Thalassemia, Alcoholism, Hemolysis and others related to `medications.

1.1.3. Anemia Diagnosis:

The evaluation of anemia can be a complex and difficult endeavor that may not yield a definite diagnosis, even after exhaustive testing, including a bone marrow biopsy. Often, anemia exists in a mileu of chronic organ dysfunction or medical conditions that cloud the diagnosis because of their effect on erythropoiesis or red cell survival. These conditions may also create incongruities in laboratory results, resulting further confusion in the variance diagnosis.

Generally, the diagnosis of certain types of anemia often comprises the application of information derived from a visual assessment of discolored red blood cell types. It also gives an idea about the variations in size and estimates of haemoglobin contents. It has been recognized that better quantification of these determinations would lead to a more detailed characterization and diagnosis of anemia.

Anemia is usually identified, or at least established, by a complete blood cell (CBC) count. A CBC test may be ordered by a physician as a part of routine general checkup and screening or based on clinical signs and symptoms that may suggest anemia or other blood aberrations.

Although the microscopy has good sensitivity and allows species identification, there are some major drawbacks to this technique.

i) Visual inspection of microscopic images is time consuming and exhaustive.

ii) If the detection & counting process is interrupted, the operator has to start from scratch.

iii) There is no quick and easy way of retrieving analyzed images for further reference.

iv) The decision makers may not have access to test results, or may have no experience to deal with a particular rare condition.

v) Emotional problems and fatigue may degrade the expert's performance.

In the past few years, it has become apparent that techniques of microscopic digital image processing some of which have been applied to automated classification to white blood cells could also be applied to the classification of red blood cells.

Research in the early 1970's by Eden & Green and Bentley & Lewis were primarily concerned with measurements of features on normal red blood cells.

In this study, classification method is based on features like Form Factor which differentiate between normal and abnormal red cells in the same blood specimen.

1.2 Sickle-Cell

1.2.1. Sickle-Cell Overview

Sickle-cell disease (**SCD**), or **sickle-cell anaemia** (**SCA**) or **drepanocytosis**, is an autosomal recessive heritable blood disorder with over dominance, categorized by red blood cells that assume an abnormal, stiff, sickle shape. The sickling occurs because of a mutation in the

haemoglobin gene. It follows when a person inherits two abnormal genes (one from each parent) that cause their RBCs to change shape, which is similar to a crescent moon as shown in figure.

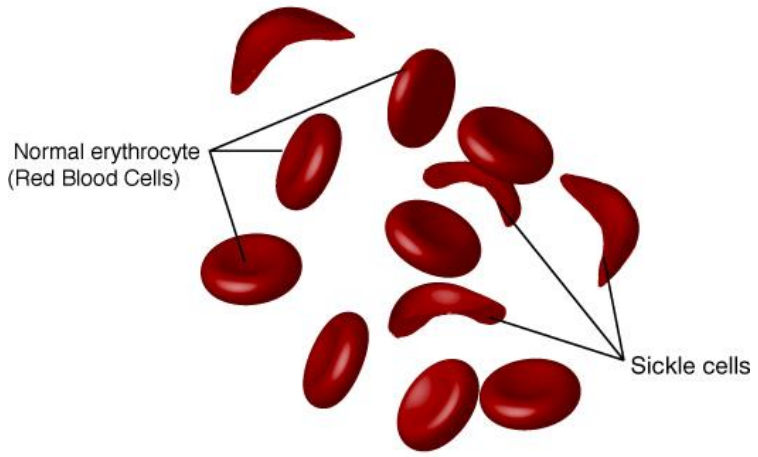


Fig 1.2: Sickle Cells and Normal Red Blood Cells

1.2.2. Causes of Sickle-Cell Disease:

Sickle cell Anemia is caused by an abnormal type of heamoglobin called Haemoglobin S.

- Hemoglobin S changes the shape of red blood cells. The red blood cells become shaped like crescents or sickles.
- The fragile, sickle-shaped cells deliver less oxygen to the body's tissues.
- They can also get stuck more easily in small blood vessels, as well as break into pieces that can interrupt healthy blood flow. These problems decrease the amount of oxygen flowing to body tissues even more.

Sickle-Cell Anemia is inbred as an autosomal (meaning that the gene is not linked to a sex chromosome) recessive ailment whereas sickle-Cell trait is inherited as an autosomal dominant trait. In order for Sickle-Cell Anemia to occur, a sickle-cell gene must be inherited from both father and mother and thus the child grows 2 sickle cell genes.

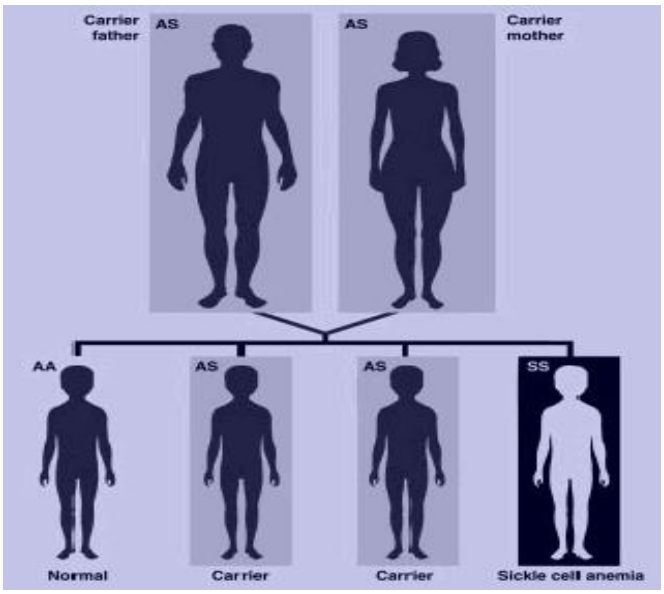


Fig. 1.3: Sickle-Cell Inheritance Chart

The inheritance of just one sickle gene is called sickle cell trait, aka "carrier" state. Sickle cell trait does not cause sickle cell anemia. Persons with sickle cell trait usually do not have many symptoms of disease and have normal hospitalization rates and life expectancies.

Unlike normal RBCs that last about 4 months in the bloodstream, fragile sickle cells break down after only about 10 to 20 days, which usually causes Anemia.

1.2.3. Symptoms of Sickle-Cell Disease:

Virtually all of the major symptoms of sickle cell anemia are the direct result of the abnormally

shaped, sickled red blood cells blocking the flow of blood that circulates through the tissues of the body.

Almost all the patients with sickle cell anemia have painful episodes (called crisis), which can last from hours to days. Some patients have one episode every few years; others have many episodes in a year. The crisis can be severe enough to require a hospital stay.

The most common symptom of Sickle Cell Anemia is fatigue (feeling tired or weak). Other symptoms may include:

- Paleness
- Rapid heart rate
- Shortness of breath
- Yellowing of the eyes and skin (jaundice)
- Dizziness
- Headaches
- Coldness in hands and feet.

Younger children with sickle cell anemia have attacks of abdominal pain. Severe pain is the most common of sickle cell disease emergencies (acute sickle cell crisis).

1.2.4. Complications of Sickle-Cell Anemia:

Sickle cell crisis can affect many parts of the body and cause many complications, such as:

i) Stroke, which can results from a progressive narrowing of blood vessels, preventing oxygen from reaching the brain. Cerebral infractions occur in children and cerebral haemorrhage in adults.

ii) Cholelithiasis (gallstones) and cholecystitis, which may result from excessive bilirubin production and precipitation due to prolonged haemolysis.

iii) Decreased immune reactions due to hyposplenism (malfunctioning of the spleen).

iv) Bacterial bone infection is the most common cause in sickle cell disease.

v) Leg ulcers.

vi) Chronic pain.

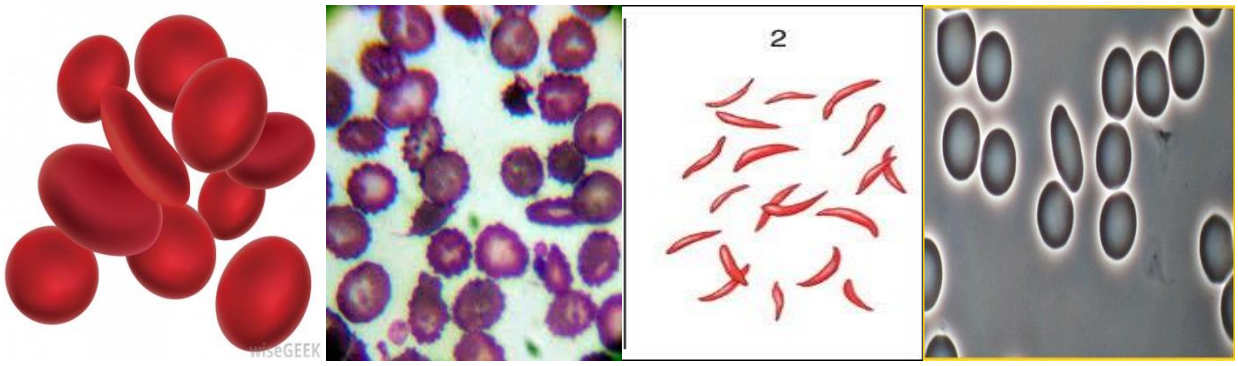
vii) Acute Chest Syndrome: Sudden acute chest pain with coughing up of blood can occur. People with this condition also suffer from fever and shortness of breath.

viii) Acute and painful joint crisis may develop without any significant traumatic history.

1.2.5. Characterization of Sickle-Cell Anemia:

Sickle cell anemia is characterized by the presence of following cells as shown in figure. When the abnormal sickle-shaped cells in the blood are identified, a diagnosis is made.

- Normal Cell: Normally blood cells are round and flexible and flow easily through blood vessels.
- Anisophoikilocytosis: In this case, the red blood cells are of unequal size.
- Sickle Cell: In sickle cell disease, certain red blood cells become crescent-shaped. These abnormal red blood cells, carrying abnormal hemoglobin known as hemoglobin S, are fragile.
- Ovalocytes: It is an inherited blood disorder in which an abnormally large number of the patient's erythrocytes (i.e. red blood cells) are elliptical rather than the typical biconcave disc shape.



(a) Normal RBC (b) Anisopoikilocyte (c) Sickle-Cell (d) Ovalocyte

Fig. 1.4: Different types of Cells

1.2.6. Sickle-Cell Diagnosis:

A simple blood test, done at any time during a person's lifespan, can detect whether he or she has sickle hemoglobin. However, early diagnosis is very important. If the test shows some sickle hemoglobin, a second blood test is done to confirm the diagnosis. The second test should be done as soon as possible and within the first few months of life.

Sickle cells can be seen on a blood smear examined under a microscope. Peripheral blood smear demonstrates moderate to severe degree of anisopoikilocytes. Number of sickle cells is variable.

Thus, the simple blood test is a tiresome and erroneous work which should be replaced by an effective, advanced and accurate tool to successfully diagnose the sickle cell disease. One such tool can be prepared by using Image Processing Technique.

Chapter 2: Literature Survey

2.1 Sickle-cell Anemia Studies:

Sickle cell anemia is the most common form of sickle cell disease (SCD). SCD is a serious disorder in which the body makes sickle-shaped red blood cells. “Sickle-shaped” means that the red blood cells are shaped like a crescent.

Normal red blood cells are disc-shaped and look like doughnuts without holes in the center. They move easily through your blood vessels. Red blood cells contain an iron-rich protein called hemoglobin (HEE-muh-glow-bin). This protein carries oxygen from the lungs to the rest of the body.

Sickle cells contain abnormal hemoglobin called sickle hemoglobin or hemoglobin S. Sickle hemoglobin causes the cells to develop a sickle, or crescent, shape.

Sickle cells are stiff and sticky. They tend to block blood flow in the blood vessels of the limbs and organs. Blocked blood flow can cause pain and organ damage. It can also raise the risk for infection.

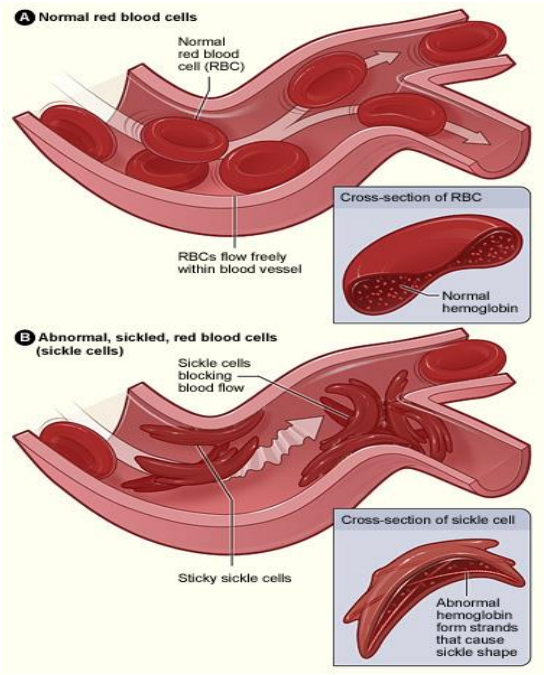


Fig. 2.1 Normal Red Blood Cells and Sickle Cells

Figure shows normal red blood cells flowing freely in a blood vessel. The inset image shows a cross-section of a normal red blood cell with normal hemoglobin. Figure B shows abnormal, sickled red blood cells blocking blood flow in a blood vessel. The inset image shows a cross-section of a sickle cell with abnormal (sickle) hemoglobin forming abnormal strands.

Overview

Sickle cell anemia is one type of anemia. Anemia is a condition in which your blood has a lower than normal number of red blood cells. This condition also can occur if your red blood cells don't contain enough hemoglobin.

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. They carry oxygen and remove carbon dioxide (a waste product) from your body.

In sickle cell anemia, 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.

Sickle cell anemia is an inherited, lifelong disease. People who have the disease are born with it. They inherit two genes for sickle hemoglobin—one from each parent.

People who inherit a sickle hemoglobin 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 anemia. People who have sickle cell trait don't have the disease. Like people who have sickle cell anemia, people who have sickle cell trait can pass the sickle hemoglobin gene to their children.

Outlook

Sickle cell anemia has no widely available cure. However, treatments to improve the anemia and lower complications can help with the symptoms and complications of the disease in both

children and adults. Blood and marrow stem cell transplants may offer a cure for a small number of people.

Over the past 100 years, doctors have learned a great deal about sickle cell anemia. They know its causes, how it affects the body, and how to treat many of its complications.

Sickle cell anemia varies from person to person. Some people who have the disease have chronic (long-term) pain or fatigue (tiredness). However, with proper care and treatment, many people who have the disease can have improved quality of life and reasonable health much of the time.

Because of improved treatments and care, people who have sickle cell anemia are now living into their forties or fifties, or longer.

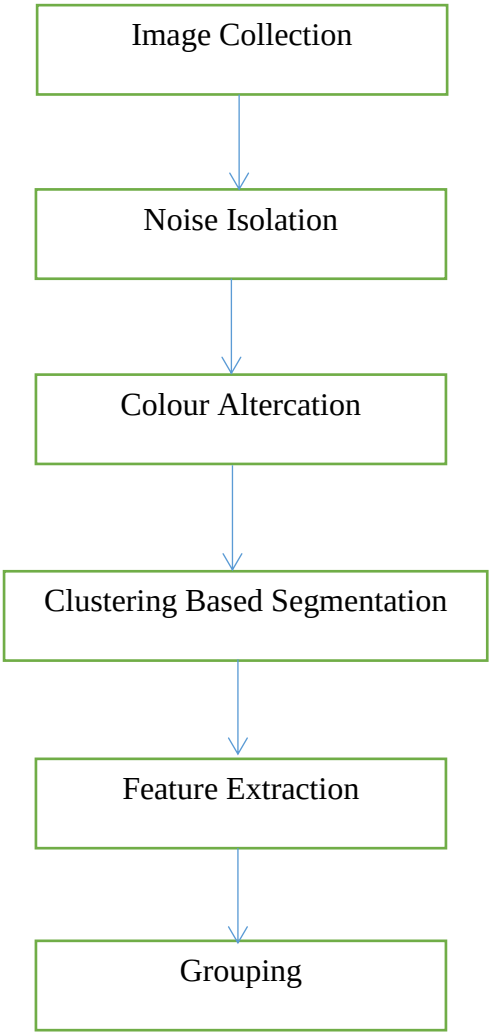


Fig. 2. 2. Basic System Overview

2.2 Image Representation

There are five types of images in MATLAB.

1. Grayscale. A grayscale image *M* pixels tall and *N* pixels wide is represented as a matrix of double datatype of size *M*×*N*. Element values (e.g., MyImage(m,n)) denote the pixel grayscale intensities in [0,1] with 0=black and 1=white.

2. Truecolor RGB. A truecolor red-green-blue (RGB) image is represented as a three-dimensional *M*×*N*×3 double matrix and each pixel has red, green and blue components along the third dimension with values in [0,1], for example, the colour constituents of pixel (m,n) areMyImage(m,n,1) = red, MyImage(m,n,2) = green, MyImage(m,n,3) = blue.

3. Indexed. Indexed (paletted) images are represented with an index matrix of size *M*×*N* and a colormap matrix of size *K*×*3*. The colormap holds all colors used in the image and the index matrix represents the pixels by referring to colors in the colormap. For example, if the 22nd color is magenta MyColormap(22,:) = [1,0,1], then MyImage(m,n) = 22 is a magenta-colored pixel.

4. Binary. A binary image is represented by an *M*×*N* logical matrix where pixel values are 1 (true) or 0 (false).

Grayscale is usually the preferred format for image processing. In cases requiring color, an RGB color image can be decomposed and handled as three separate grayscale images. Indexed images must be converted to grayscale or RGB for most operations.

2.3 Image Segmentation

Blood cell segmentation and identification is a vital in the study of blood as a health indicator. A complete blood count is used to determine the state of a person's health based on the contents of the blood in particular the white blood cells and the red blood cells. The main problem arises when massive amounts of blood samples are required to be processed by the hematologist or

Medical Laboratory Technicians. The time and skill required for the task limits the speed and accuracy with which the blood sample can be processed.

Image segmentation is one of the primary steps in image analysis for object identification. The main aim is to recognize homogeneous regions within an image as distinct and belonging to different objects. Segmentation stage does not worry about the identity of the objects. They can be labeled later. The segmentation process can be based on finding the maximum homogeneity in gray levels within the regions identified. There are several issues related to image segmentation that require detailed review. One of the common problems encountered in image segmentation is choosing a suitable approach for isolating different objects from the background. The segmentation doesn't perform well if the gray levels of different objects are quite similar. Image enhancement techniques seek to improve the visual appearance of an image. They emphasize the salient features of the original image and simplify the task of image segmentation. The type of operator chosen has a direct impact on the quality of the resultant image. It is expected that an ideal operator will enhance the boundary differences between the objects and their background making the image segmentation task easier. Issues related to segmentation involve choosing good segmentation algorithms, measuring their performance, and understanding their impact on the scene analysis system.

Some of the recent types of methods in segmentation shared are:

1. Thresholding – based on histogram characteristics of pixel intensities of image.
2. Morphological Operation – Continuity based techniques which involve the processing of shapes, to segment the red blood cell images.
3. Colour Image segmentation – allow more reliable image segmentation than greyscale images and applying of hue feature.
4. Model-based contour tracing – to overcome the problem of automatically segmenting a Scanning Electron Microscopic image of red blood cells that have high number of overlapping cells and relatively smooth contour.
5. Tabu Search – a method for finding elliptical cell boundaries.
6. Matlab – to overcome the problem of counting overlapping red blood cells by applying new algorithm by using the method.

7. Fluroscent Microscopy Images – can remove object with poor contrast and distance transformed watershed segmentation.

Some of the recent types of methods in classification shared are:

1. Multilayer perceptron – Classifying various types of blood cells.
2. Support Vector Machine – Analyses data and recognize patterns which could be used for classification and regression analysis.
3. Learning Vector Quantization – A type of artificial neural network and can be applied to multi-classification problem is a natural way. E.g. in classifying blood cells and bone marrow.

2.4 Feature Extraction

In pattern recognition and in image processing, **feature extraction** is a special form of dimensionality reduction.

When the input data to an algorithm is too large to be processed and it is suspected to be notoriously redundant, then the input data will be transformed into a reduced representation set of features. Transforming the input data into the set of features is called *feature extraction*. If the features extracted are carefully chosen it is expected that the features set will extract the relevant information from the input data in order to perform the desired task using this reduced representation instead of the full size input.

Feature extraction involves simplifying the amount of resources required to describe a large set of data accurately. When performing analysis of complex data one of the major problems stems from the number of variables involved. Analysis with a large number of variables generally requires a large amount of memory and computation power or a classification algorithm which over fits the training sample and generalizes poorly to new samples. Feature extraction is a general term for methods of constructing combinations of the variables to get around these problems while still describing the data with sufficient accuracy.

2.4.1. Geometrical Features

1. 'Area' — Scalar; the actual number of pixels in the region.

2. 'Centroid' – 1-by-Q vector that specifies the center of mass of the region. Note that the first element of Centroid is the horizontal coordinate (or *x*-coordinate) of the center of mass, and the second element is the vertical coordinate (or *y*-coordinate).

3. 'Eccentricity' — Scalar that specifies the eccentricity of the ellipse that has the same second-moments as the region. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length. The value is between 0 and 1.
4. 'EquivDiameter' — Scalar that specifies the diameter of a circle with the same area as the region. Computed as sqrt(4*Area/pi).
5. 'Extent' — Scalar that specifies the ratio of pixels in the region to pixels in the total bounding box. Computed as the Area divided by the area of the bounding box.

6. 'MajorAxisLength' — Scalar specifying the length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.

7. 'MinorAxisLength' — Scalar; the length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.

8. 'Orientation' — Scalar; the angle (in degrees ranging from -90 to 90 degrees) between the *x*-axis and the major axis of the ellipse that has the same second-moments as the region.

9. 'Perimeter' — Scalar; the distance around the boundary of the region. regionprops computes the perimeter by calculating the distance between each adjoining pair of pixels around the border of the region.

2.4.2. Textural Features

Gray-Level Co-Occurrence Matrices (GLCM):

A **co-occurrence matrix** or **co-occurrence distribution** is a matrix or distribution that is defined over an image to be the distribution of co-occurring values at a given offset. Mathematically, a co-occurrence matrix **C** is defined over an **n × m** image **I**, parameterized by an offset **(Δx,Δy)**, as:

$$C_{\Delta x,\Delta y}(i,j) = \sum_{p=1}^{n}\sum_{q=1}^{m} \begin{cases} 1, & \text{if } I(p,q) = i \text{ and } I(p+\Delta x, q+\Delta y) = j \\ 0, & \text{otherwise} \end{cases}$$

where *i* and *j* are the image intensity values of the image, *p* and *q* are the spatial positions in the image **I** and the offset **(Δx,Δy)** depends on the direction used θ and the distance at which the matrix is computed *d*. The 'value' of the image originally referred to the grayscale value of the specified pixel, but could be anything, from a binary on/off value to 32-bit color and beyond.

1. Homogeneity, Angular Second Moment (ASM):

Returns a value that measures the closeness of the distribution of elements in the GLCM to the GLCM diagonal. Range lies between [0 1]. Homogeneity is 1 for a diagonal GLCM.

.

2. Contrast:

Returns a measure of the intensity contrast between a pixel and its neighbor over the whole image. Range = [0 (size(GLCM,1)-1)^2]. Contrast is 0 for a constant image.

3. Entropy:

Inhomogeneous scenes have low first order entropy, while a homogeneous scene has high entropy.

4. Correlation:

Returns a measure of how correlated a pixel is to its neighbor over the whole image. Range = [-1 1]. Correlation is 1 or -1 for a perfectly positively or negatively correlated image. Correlation is NaN for a constant image.

5. Energy

It returns the sum of squared elements in the GLCM. Range lies between [0 1]. Energy is 1 for a constant image.

2.4.3. Circles Detection

Detect circular shapes in a grayscale image using Circular Hough transform based on the gradient field of an image.

INPUT Parameters: (img, radrange, grdthres, fltr4LM_R, multirad, fltr4accum)

img: A 2-D grayscale image (NO B/W bitmap)

radrange: The possible minimum and maximum radii of the circles to be searched, in the format of [minimum_radius , maximum_radius] (unit: pixels).

Grdthres: The algorithm is based on the gradient field of the input image. A thresholding on the gradient magnitude is performed before the voting process of the Circular Hough transform to remove the 'uniform intensity' (sort-of) image background from the voting process.

fltr4LM_R: The radius of the filter used in the search of local maxima in the accumulation array. To detect circles whose shapes are less perfect, the radius of the filter needs to be set larger.

multirad: In case of concentric circles, multiple radii may be detected corresponding to a single center position. This argument sets the tolerance of picking up the likely radii values. It ranges from 0.1 to 1.

fltr4accum: Filter used to smooth the accumulation array. Depending on the image and the parameter settings, the accumulation array built has different noise level and noise pattern. The filter should be set to an appropriately size such that it's able to suppress the dominant noise frequency.

OUTPUT Parameters: [accum, circen, cirrad, dbg_LMmask]

accum: The result accumulation array from the Circular Hough transform. The accumulation array has the same dimension as the input image.

circen: Center positions of the circles detected. Is a N-by-2 matrix with each row contains the (x, y) positions of a circle.

cirrad: Estimated radii of the circles detected. Is a N-by-1 column vector with a one-to-one correspondance to the output 'circen'.

dbg_LMmask: Mask from the search of local maxima in the accumulation array.

2.5 Feature Classification

There are many techniques that can be used to classify the cells in the blood smear effectively and within a short time period. One such technique is Multilayer perceptron (MLP) (trained with back propagation learning algorithm) but it's drawback is it takes large computational time. The complexity of the network increases as the number of layers and number of nodes in layers increases. Further, it is also very difficult to decide the number of nodes in a layer and the number of layers in the network required for solving a problem a priori.

Chapter 3: Image Processing Algorithm & Outcomes

The automated image processing algorithm is designed to diagnose sickle-cell disease in much the same way as a human operator performing microscopy. To do this, the algorithm finds and identifies red blood cells and sickle-cells present in a microscopic field of a thin blood smear. Based on the results found, the program makes a diagnosis as to whether or not sickle-cell is present, and if present, it determines the species of the infection. The system must have a high degree of sensitivity. It must also have good specificity to be useful as a clinical tool. The algorithm design is essentially an image classification problem, and thus takes the form of a standard pattern recognition and classification system. Basically, it consists of five stages: image acquisition, pre-processing, segmentation, feature generation and classification, and the performance of the system is then evaluated. A morphological method used to identify sickle-cell in Giemsa-stained blood slides is used as a starting point for the algorithm, from which many of the pre-processing and image segmentation steps are derived.

3.1 Clustering Based Segmentation:

3.1.1 Acquisition:

Before examine the structure of RBCs, the images can be recorded with the help of glass slides and images get captured using microscopes. Images are acquired from IG Hospital, Rourkela

using a 3.34 megapixel Nikon Coolpix 995 digital camera (Nikon Corporation, Tokyo, Japan). The camera, using full 4· optical zoom, is connected to a light microscope with 1,000· magnification. The blood smear image slides are examined under oil immersion. Images are captured in the JPEG format at the maximum resolution of the camera, 2,048·1,536 pixels.

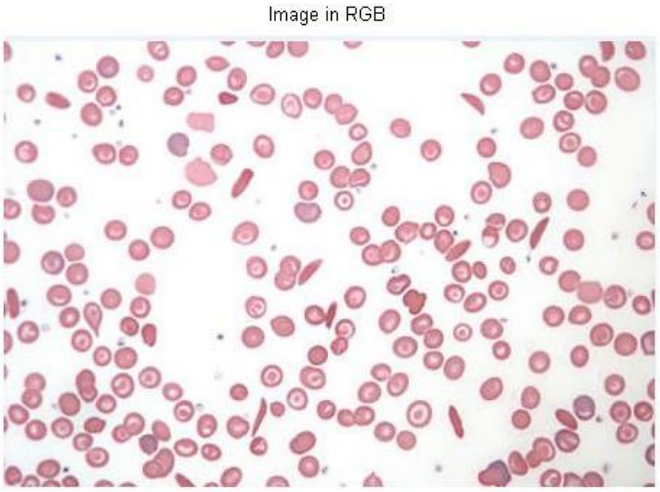


Fig.3.1. Blood cell image in RGB color space

3.1.2 Pre-Processing:

The purpose of the pre-processing stage is to remove unwanted effects such as noise from the image, and transform or adjust the image as necessary for further processing. The resolution of the image is reduced by a factor of four to 512·384 to speed up performance of the system.

Also, the test images will be subjected to selective median filtering and unsharp masking to isolate noise which may have been accumulated during image acquisition and due to excessive staining.

3.1.3 Color Transformation:

Typically an image can be represented with the help of three colour componenets. Images generated by the digital microscope are usually in RGB color space which is visually difficult to segment. For better colour based segmentation we map the RGB image to L*a*b (LAB) colour space. The LAB space consists of luminosity layer L*, chromaticity layers a* and b*. Since all the colour information is in the chromaticity layers, we use these two componenets for colour based Red Blood Cell segmentation.

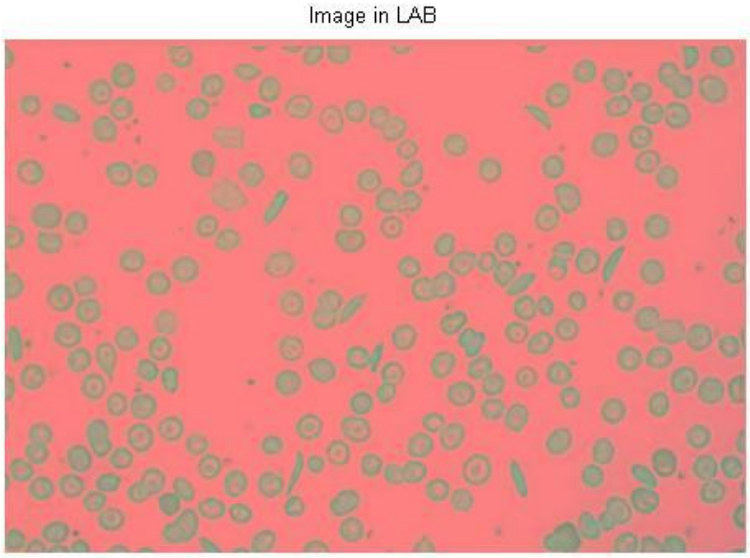


Fig.3.2. Blood cell image in LAB color space

3.2 Segmentation Techniques

Segmentation refers to the process of partitioning a digital image into multiple segments (sets of pixels, also known as superpixels). The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze. Image segmentation is typically used to locate objects and boundaries (lines, curves, etc) in images. More precisely, image segmentation is the process of assigning a label to every pixel in an image such that pixels with the same label share certain visual characteristics. The anemia blood smear will be segmented by appropriate segmentation methods.

Primarily those studies that are based on finding object regions in gray-level images has been studied. We also mention couple of studies that deal with color segmentation to highlight how this has been used for outdoor scene analysis. Image segmentation has been approached from a wide variety of perspectives.

- Edge Detection:

Edges in images are regions with very high contrast in intensity of pixels; detection of edges reduces the amount of data, filters useless information and preserves important structural details. This method is multistep procedure; it first finds edges by looking for local maxima of the gradient of image. The gradient is calculated using the derivative of a Gaussian filter which smoothes the image in order to reduce noise and unwanted details as well as textures. There are an extremely large number of edge detection operators available, each designed to be sensitive to certain types of edges. Variables involved in the selection of an edge detection operator include Edge orientation, Noise environment and Edge structure. The geometry of the operator determines a characteristic direction in which it is most sensitive to edges. Operators can be optimized to look for horizontal, vertical, or diagonal edges. Edge detection is difficult in noisy images, since both the noise and the edges contain high frequency content. Attempts to reduce the noise result in blurred and distorted edges. Operators used on noisy images are typically larger in scope, so they can average enough data to discount localized noisy pixels. This results in less accurate localization of the detected edges.

- Clustering Methods:

Clustering based methods have been widely used in segmentation of gray level images. Since the clustering methods are either directly applicable or easily extendable to higher dimensional data, their application in segmentation of colour and multispectral images is a natural choice. Some of the clustering techniques rely on knowing the number of clusters apriori. In that case the algorithm tries to partition the data into the given number of clusters. K-means and Fuzzy C-means clustering are of that type.

The accuracy in segmentation of a colour image depends not only on the algorithm used but also on the colour space selected. Moreover, the psycho-visual response in deciding the accuracy of segmentation results, especially in the case of colour images, seems to vary a lot from person to

person. This makes an objective evaluation of segmentation results all the more necessary in the analysis of colour images.

The first technique is *K-means* clustering (or *Hard C-means* clustering, as compared to *Fuzzy C-means* clustering). This technique has been applied to a variety of areas, including image and speech data compression, data preprocessing for system modeling using radial basis function networks, and task decomposition in heterogeneous neural network architectures. This algorithm relies on finding cluster centers by trying to minimize a cost function of dissimilarity (or distance) measure.

The second technique is *Fuzzy C-means* clustering, which was proposed by Bezdek in 1973 as an improvement over earlier Hard C-means clustering. In this technique each data point belongs to a cluster to a degree specified by a membership grade. As in K-means clustering, Fuzzy C-means clustering relies on minimizing a cost function of dissimilarity measure.

3.2.1 K-Means Clustering

The K-means clustering, or Hard C-means clustering, is an algorithm based on finding data clusters in a data set such that a cost function (or an objection function) of dissimilarity (or distance) measure is minimized.

K-means clustering is a partitioning method. The function k-means partitions data into k mutually exclusive clusters, and returns the index of the cluster to which it has assigned each observation. Also, k-means clustering operates on actual observations (rather than the larger set of dissimilarity measures), and creates a single level of clusters.

K-means treats each observation in the data as an object having a location in space. It finds a partition in which objects within each cluster are as close to each other as possible, and as far from objects in other clusters as possible. You can choose from five different distance measures, depending on the kind of data you are clustering.

Each cluster in the partition is defined by its member objects and by its centroid, or center. The centroid for each cluster is the point to which the sum of distances from all objects in that cluster

is minimized. K-means computes cluster centroids differently for each distance measure, to minimize the sum with respect to the measure that you specify.

K-means uses an iterative algorithm that minimizes the sum of distances from each object to its cluster centroid, over all clusters. This algorithm moves objects between clusters until the sum cannot be decreased further. The result is a set of clusters that are as compact and well-separated as possible. You can control the details of the minimization using several optional input parameters to k-means, including ones for the initial values of the cluster centroids, and for the maximum number of iterations.

Algorithm:

1. Place K points into the space represented by the objects that are being clustered. These points represent initial group centroids.
2. Assign each object to the group that has the closest centroid.
3. When all objects have been assigned, recalculate the positions of the K centroids.
4. Repeat Steps 2 and 3 until the centroids no longer move. This produces a separation of the objects into groups from which the metric to be minimized can be calculated.

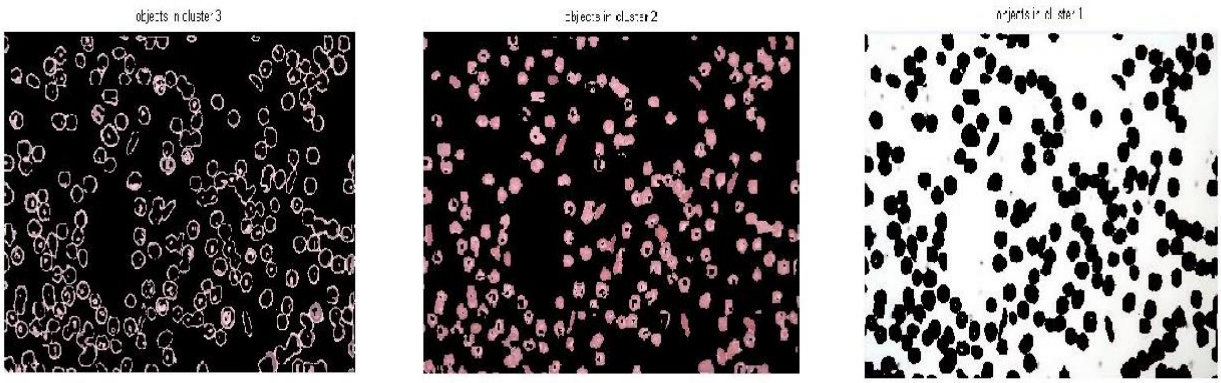


Fig.3.3. Objects in cluster after K-Means Clustering based segmentation

3.2.2 Fuzzy C-Means Clustering

Fuzzy c-means (FCM) is one of the commonly used methods for image segmentation and its success is mainly due to the introduction of fuzziness for the belongingness of each image pixels. Compared with crisp or hard segmentation methods, FCM is able to retain more information from the original image. However, one disadvantage of FCM is its sensitivity to noise and other imaging artifacts. The reason may perhaps stems from the non-unimodal property of its membership functions and the use of the squared Euclidean distance.

Fuzzy c-means (FCM) is a data clustering technique in which a dataset is grouped into n clusters with every datapoint in the dataset belonging to every cluster to a certain degree. For example, a certain datapoint that lies close to the center of a cluster will have a high degree of belonging or membership to that cluster and another datapoint that lies far away from the center of a cluster will have a low degree of belonging or membership to that cluster.

Algorithm:

Assumptions: Image transformed into feature space, number of clusters *c*, stop condition ε, fuzziness parameter *m*.
Step 1: Cluster image in feature space, with next conditions: number of clusters is *c*, fuzziness index is *m* and stop condition is ε .
Step 2: Repeat for each pixel a*ij* of image *I*.

Step 2.1: Find out, into which cluster *Cl* belongs pixel a*ij* at most.

Step 2.2: Find out, whether in the closest surroundings of pixel a*ij* exists segment *Rk*, which points belong to same cluster *C*.

Step 2.3: If such segment *Rk* exists, than pixel a*ij* add to segment *Rk*, else create new segment *Rn* and add pixel a*ij* to new segment *Rn*.

Step 3: Merge all segments, which belong to one cluster and are neighbors.
Step 4: Arrange borders of all segments.

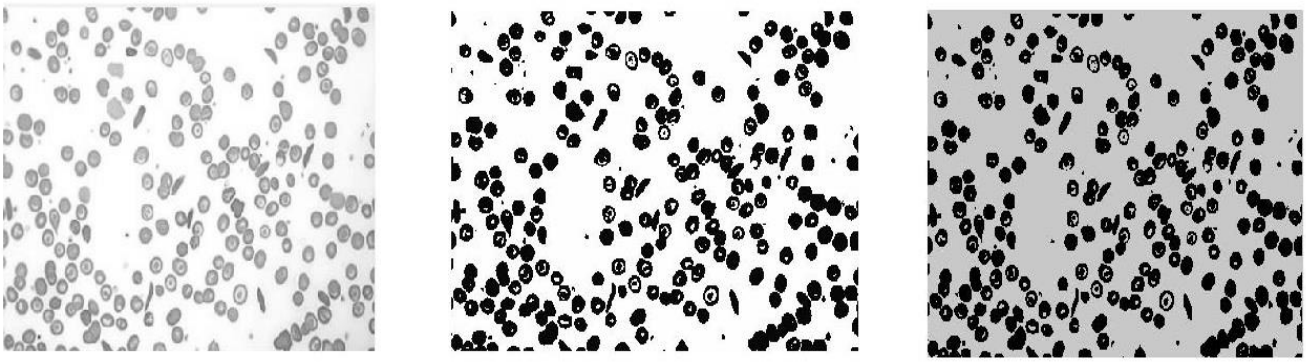


Fig.3.4. Objects in cluster after Fuzzy C-Means Clustering based segmentation

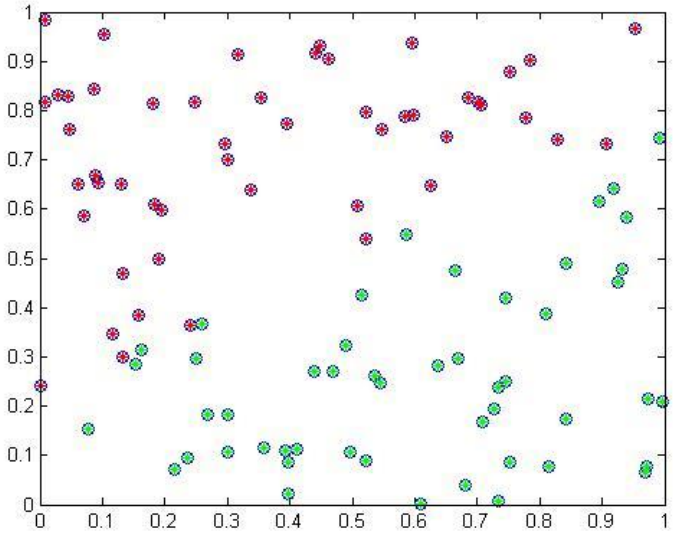


Fig.3.5. Clustering graph of samples in Fuzzy C-Means Clustering.

3.3 Feature Generation Processes

3.3.1. Geometrical Features Extraction

For unit cluster of k-means clustering based segmentation

Geometrical Features	Normal RBC	Sickle-cell type-I	Sickle-cell type-II	Anisopoikilocyte/Ovalocyte
Area	274	268	183	212
Perimeter	62.14	85.11	131.64	62.1
Centroid	15.6, 14.8	16, 17.2	15.4, 24.9	14.5, 15.0
Eccentricity	0.43	0.94	0.95	0.8
Major Axis Length	19.8	32.91	32.8	21.9
Minor Axis Length	17.8	11.1	10.2	13.1
Orientation	11.34	57.6	-74.5	-28.37
Equiv Diameter	18.7	18.47	15.3	16.4
Solidity	0.97	0.83	0.6	0.86
Extent	0.84	0.48	0.4	0.66
Mean	48.9	42.6	28.1	44.5
Aspect Ratio	0.9394	1.1935	1.6333	0.9667
Standard Deviation	76	68.5	54.6	69.6
Form Factor	0.9526	0.7149	0.6834	0.4872

Tab 1. Geometrical Features of K-Means Clusters

For unit cluster of fuzzy c-means clustering based segmentation

Geometrical Features	Normal RBC	Sickle-cell type-I	Sickle-cell type-II	Anisopoikilocyte/Ovalocyte
Area	420	512	708	506
Perimeter	78	88	110	86
Centroid	10.4, 11	10.4, 13.2	10.2, 19	12, 11.5
Eccentricity	0.3133	0.63	0.84	0.29
Major Axis Length	24.2	30	43.4	26.5
Minor Axis Length	23	23.1	23.9	25.4
Orientation	90	-88.7	89.6	0
Equiv Diameter	23.2	25.5	30	25.4
Solidity	1	0.98	0.96	1
Extent	1	0.98	0.96	1
Mean	210.45	219.2	226	220
Aspect Ratio	1.05	1.3	1.85	0.9565
Standard Deviation	37.8	44.4	42.2	31.73
Form Factor	0.9528	0.6535	0.7737	0.3628

Tab 2. Geometrical Features of Fuzzy C-Means Clusters

3.3.2 Textural Features Extraction:

GLCM Results:

For unit cluster of k-means clustering based segmentation

Textural Features	Normal RBC	Sickle-Cell I	Sickle-Cell II	Ovalocyte/ Anisopoikilocyte
Contrast	7.94e-02	9.03e-02	9.64e-02	1.08e-01
Correlation	8.05e-01	7.57e-01	5.72e-01	7.2e-01
Energy	5.18e-01	5.45e-01	6.87e-01	5.16e-01
Homogeneity	9.6e-01	9.54e-01	9.51e-01	9.45e-01
Entropy	0.34	0.28	0.39	0.28

Tab 3.Textural Features of K-Means Clusters

For unit cluster of fuzzy c-means clustering based segmentation

Textural Features	Normal RBC	Sickle-Cell I	Sickle-Cell II	Ovalocyte/ Anisopoikilocyte
Contrast	0	2.91e-02	6.5e-02	0
Correlation	NaN	1.1e-01	2.4e-01	NaN
Energy	1	9.3e-01	8.5e-01	1
Homogeneity	1	9.8e-01	9.6e-01	1
Entropy	2.76	2.76	2.76	2.76

Tab 4. Textural Features of Fuzzy C-Means Clusters

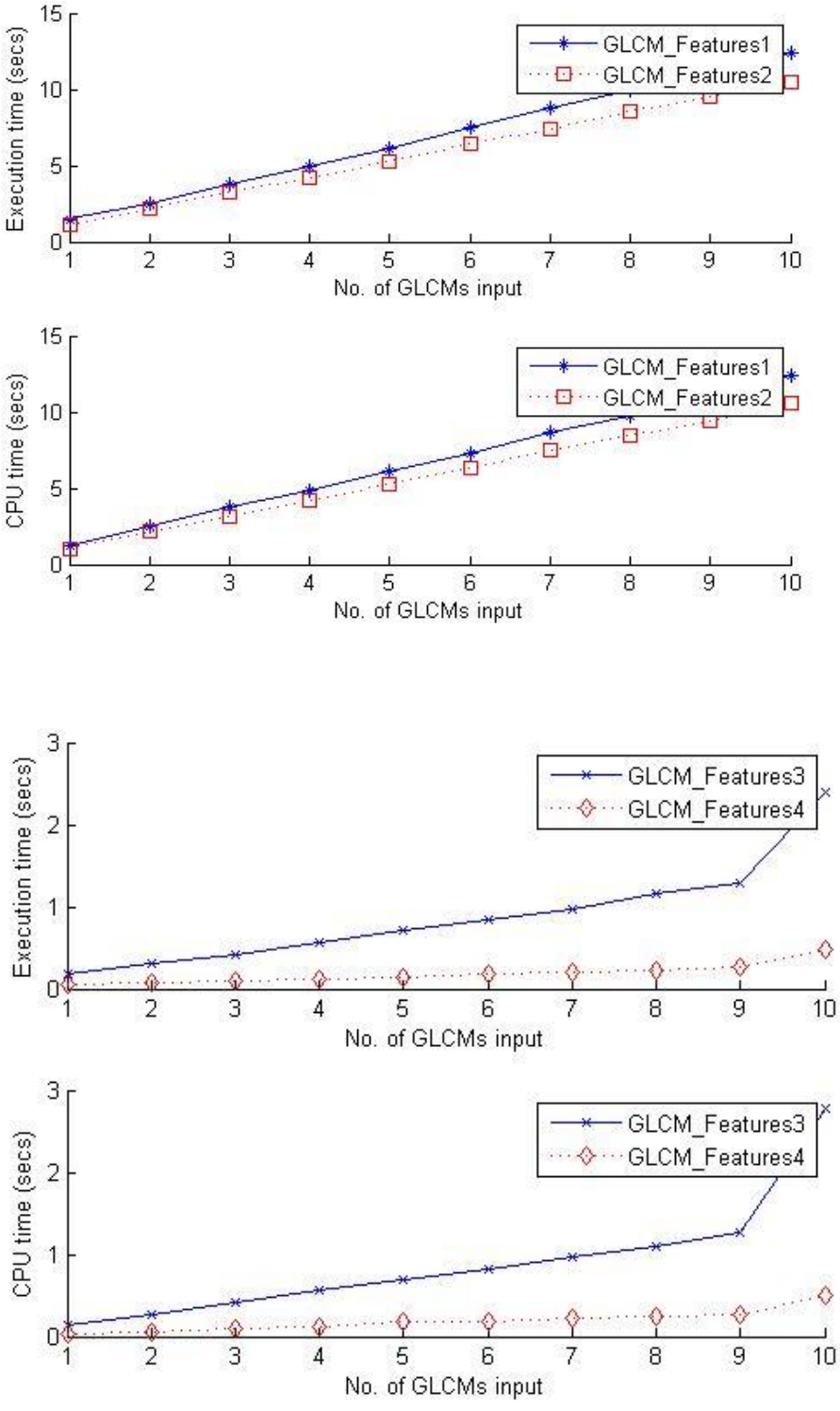


Fig.3.6. GLCM Features Graph

3.3.3. Detecting Circles in an Image

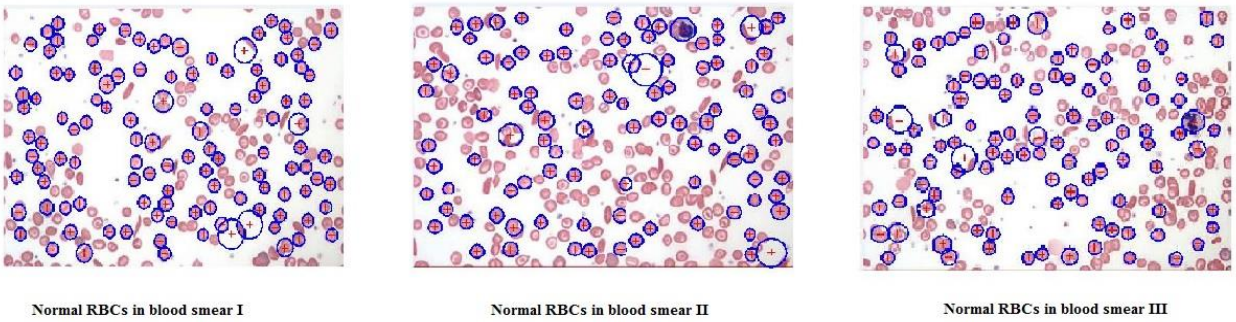


Fig.3.7. Circles Recognition

3.4 Classification

After the various kinds of features were generated from the previous step, now they are used for classifying the different kinds of red blood cells (RBC) present in the blood smear image. After using proper grouping technique, we find that the form factor values extracted from the geometrical features is of our great interest. For different values of form factor, cells are sorted out as normal cell, sickle-cell, ovalocyte and anisopoikilocyte. Their numbers and the total number of blood cells present in the smear are also calculated. For a normal blood cell, the value of Form Factor nearly equals one.

Consequently, the cells in blood smear are categorized as follows:

- Normal Cell Form Factor > 0.95
- Sickle-Cell 0.6< Form Factor < 0.8
- Ovalocyte/Anisopoikilocyte Form Factor < 0.5

Chapter 4: Conclusions and Future Study

The image processing techniques used in this project, which includes color altercation and clustering based image segmentation has helped us to better understand the sickle-cells present in Red Blood Cells (RBCs) in case of sickle-cell patient.

Using the image segmentation technique and the following sub-imaging technique, I can obtain the images of particular affected RBCs, i.e. Sickle-cells, Anisopoikilocytes and Ovalocytes and further apply feature extraction process to determine the characteristics of affected RBCs and thus make an artificial neural network to automatically diagnose sickle-cells disease affected person.

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Road Map

Work List	**Deadline**
Literature Review and System Overview	10th March, 2013
Color Altercation and Clustering based image segmentation	5th May, 2013
Feature Extraction Process	30th May, 2013
Classification using Artificial Neural Network	15th June, 2013