Cell modeling and Cellular Dynamics

A Project Report
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by

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Abstract

Cell Modeling is one of the emerging and challenging areas in our endeavor to model biological processes and indeed entire organisms, areas that are currently being integrated under the banner of Systems Biology. Given that modeling of biological systems is a highly complex task, it is important to start with relatively simpler definitions of A 'system'. A biological cell is a natural fairly self-contained unit, depicting the fundamental unit of living tissue. This project focuses on creating simple models of cells and exploring work in cellular dynamics. While a number of studies have illustrated the design, development and application of metabolic and structural models of the individual proteins and also the proteome, there has not been much work reported in the literature about modeling cell morphologies, analyzing the dynamics of cellular phenomenon focusing on the morphological variations of cellular entities and ultimately relate them to molecular level knowledge. Recent work in the lab,methods that systematically captures data about various morphological features in a cell available through a number of sophisticated cell imaging techniques. The work reported here is an improvement over the previous work in terms of feature extraction from cellular images. An algorithm for efficiently classifying and utilizing this information through the use of machine learning has been developed, learning from successes in the well-established support vector machine. The existing algorithm uses segmentation where as presently developed algorithm uses edge detection techniques.It is semi-automated method. The preliminary models have been developed by generating three-dimensional coordinates; finally a simulation of cellular dynamics has been discussed.

Contents

1 Introduction **1**

1.1 Review of existing work 4

1.2 Objectives 5

2 Overview and Plan of Work **7**

2.1 Feature Exraction 9

2.2 Classification 9

2.3 Cellular Dynamics 9

3 Algorithmic Concept **11**

3.1 Introduction 11

3.1.1 Microscopic Techniques and Cell Images 11

3.2 Morphological operations 13

3.2.1 Erosion 13

3.3 Image Processing 14

3.3.1 Collection of the Images 15

3.3.2 Pre-processing 15

3.3.3 Feature generation 17

3.3.4 Improvement over the existing algorithm 20

3.3.5 Construction of Classification Model 23

3.4 Results and Discussion 24

4 Design and development of in-silico model to study dynamics of Red Blood Cell 2

4.1 Introduction to basic cell modeling 27

4.1.1 Introduction to Sickle cell Anemia 28

4.2 Creating 2D cell model 28

4.2.1 Membrane coordinates extraction and Processing 28

4.2.2 Positioning polymer in membrane 29
4.2.3 Polymer elongation 30
4.2.4 Polymer bending and shape change 30
4.2.5 Force on membrane and membrane shape change 32
4.2.6 Results 34
4.3 Creating 3D model of cell 34
4.3.1 Creating Grid based model 34
4.3.2 Polymerization process inside cell model 36

5 Conclusion and Future Directions **38**
5.1 Conclusion 38
5.2 Future Directions 38
5.2.1 Creating CAD and FEA models 39
5.2.2 Creating ANSYS model 39

Bibliography **44**

List of Figures

1.1 Different levels of hierarchy required for modeling the cellular dynamics of cell. The differ
1.2 Two depictions of the double strand of hemoglobin molecules found in the crystal. 4

2.1 Sickle, Normal and Abnormal Red Blood Cells 7
2.2 Overview of the work 8

3.1 An example of transmission electron microscopy; Blood cell 12
3.2 An example of Fluorescence microscopic image; dermal fibroblast cell 13
3.3 Flowchart of algorithmic steps followed for image processing 16
3.4 Image segmentation and Feature extraction with existing algorithm. . 20
3.5 Processing of sickle cells using algorithm defined above: First after edge detection, Second
3.6 Accuracy for different kernels on whole set of data 25
3.7 Accuracy for different kernels on balanced data set 26
3.8 Accuracy for different kernels when False Negatives are minimum. . . 26

4.1 Cross-section of Red blood cell 28
4.2 2-dimensional model of Red blood cell with polymer inside 29
4.3 Normal and Buckled column 30
4.4 Consecutive points of 2-dimensional space to illustrate vector summation 33
4.5 Polymer stretching after simulation, normal, stretched and deformed. 34

5.1 Model of RBC in 3-dimensional space 41
5.2 FEM based ANSYS model of Red blood cell in front and side view. . 42

Chapter 1

Introduction

It is becoming increasingly clear that in silico modeling of biological systems is a far more complex endeavor than previously imagined [42, 18, 28, 37, 25, 26] mainly because the complexity of biological systems is not amenable to easy, simplistic solutions. In this respect, the biological cell is a natural self-contained unit, of prime importance. The fundamental unit of living tissue, in fact of life itself, is the biological cell. Currently there is enormous interest in in silico modeling of the cell in its many aspects. The cell is, of course, an enormously complex machine which can be understood at many levels, functional, signaling, metabolic, and regulatory and so on. However, there is a growing recognition that understanding its structure and the physical nature of intracellular objects, as well as their three dimensional spatial relationships, can yield significant insights into physiology and functionality [36, 25]. Complex network of interacting bimolecules are responsible for the complexity of cellular phenomenon (metabolic, biochemical, chemical etc.). The dynamics of cell is mainly comprised of biomolecules present at different levels of hierarchy. The (Fig.(1.1)) shows the different levels of hierarchy required to define the complexity of cell.

Although cell modeling in its various aspects is a subject of intense study currently across the globe [42, 28, 37, 36, 2], several questions remain open, warranting further work in this area. One main lacuna is the lack of integrated models that span across cell morphologies to organelle structure, function and dynamics relating ultimately to gene or protein level knowledge. Here we seek to address this issue and have worked towards a framework for such integration, with an emphasis on the cell morphological structures to start with.

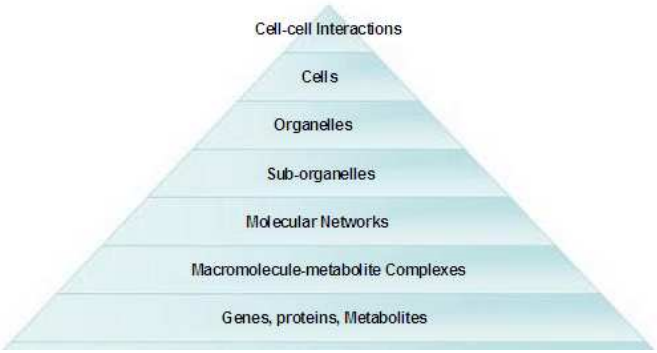

Figure 1.1: Different levels of hierarchy required for modeling the cellular dynamics of cell. The different levels comprises, signaling, behavioral, physical, chemical, divisional mechanisms. The behavior of this complex network depends on genetic and biological parameters (rate constant, equilibrium constant, gene dosage etc.).

Systems biology of red blood cell is complex and interests researchers worldwide. An effort done by Kakniashvili et al. was successful in defining the human red cell proteome [4], similarly the research done by other group indicates that current red blood cell in-silico model includes [8] 36 dynamic, independent variables. The intricacy of erythrocyte has still major issues in order to achieve the complete model. There is still some phenomenon left, in order to extend the existing model. The most important one is deformation of shape of membrane. The main cause of this process is well-known and major area of research. In every red blood cell there are 280 million molecules of hemoglobin. Hemoglobin protein is a long twisted strand of amino acids, having heme disk whose iron in the center attracts, carries and releases oxygen. The structure has been crystallized and its double strand has been described in [13, 12]. Hemoglobin molecule contains four protein chains or globins, among which two strands are "alpha" chains and two are "beta" strands. The four strands assemble together in a way, like a glove, in order to carry oxygen. Sickled cell hemoglobin (Hbs) is mutated and polymerized into long, stiff, rod-like fiber [7]. The genetic mutation in hemoglobin A (HbA) give rise to HbS due to replacement of charged Glu with hydrophobic Val in sixth position of each - chain. The consequences of this mutation results in lose of oxygen by HbS and formation of rigid 14-stranded polymers. This changes the shape of the protein: a small protrusion (or dent) appears on the surface of the proteins. This bump fits exactly into the existing "pocket" on the surface of the next protein. The two proteins "clump" together, then the third clumps. This creates a kind of domino effect, leading to the formation of

long fibers made of many millions of damaged hemoglobin molecules. This seems to happen when the hemoglobin does not have its oxygen. The polymerization of sickle hemoglobin proceeds by two types of nucleation: homogenous as well as heterogeneous. The reaction is enhanced by nucleus formation, which is not any special structure rather a piece of polymer. Therefore, the surface of this polymer can act as stimulation for heterogeneous nucleation. This phenomenon of double nucleation was proposed in 1980 [9], and experimentally observed in 1990 [38]. The model explaining this phenomenon was proposed by Mirchev and Ferrone [32], according to this model same partners are responsible for formation of double strand, i.e., the 6 Val donor and corresponding acceptor regions are critical elements for heterogeneous nucleation. In this way the hemoglobin loses its solubility and clumps into bundles. The long bundled hemoglobins twist in a regular fashion. These bundles self associate into even larger structures, the formation of this stiff, rod like fiber is mainly responsible for deformation, stretching and distortion the cell into a sickle shape. In fig.(1.2) two depictions of the double strand of hemoglobin molecules found in the crystal are shown. On the left, the strand is shown as a transparent molecular surface, with heme groups colored red, and the mutant valine residues blue. In the representation on the right, the protein backbones are shown as white coils, with the color scheme for heme groups and mutant valine residues remaining the same as the left. The axial contacts are located between molecules within a single strand in the vertical direction. Lateral contacts involving the blue valine residues act to associate the two single strands into the double strand.

This project focuses on the challenges existing in modeling and integrating the information present in literature. The cellular dynamics provide the solution to study the *in-silico*, in order to comprehend the polymerization process. It provides an avenue to observe the effect of perturbation on the cell as the time progresses. The organelles present in cell exert forces on each other and also experience the external forces. Thus time evolution of these cell organelles in the cell can be modeled and after sufficient interval of time the effect on the cell can be seen. Here we propose that a normal RBC can be subjected to cellular dynamics computationally with mutated hemoglobin and its high potential to be associated with other hemoglobins as constraint, so as to simulate the process of sickling. We provide an elementary framework for such a model. Though such an exercise will be computationally expensive it can provide a tool to study the dynamics related to disease in-silico. The features of

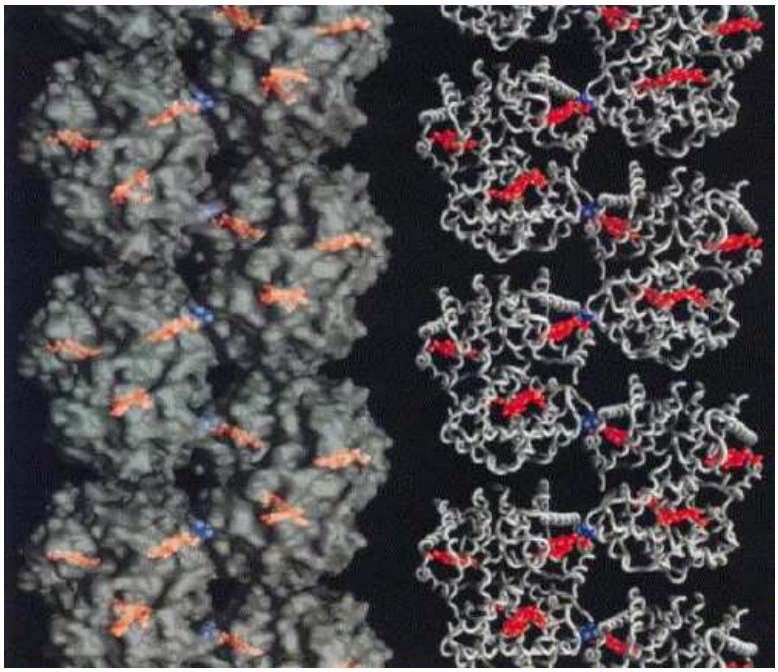

Figure 1.2: Two depictions of the double strand of hemoglobin molecules found in the crystal.

cells present in images are extracted using image-processing algorithm. On the other hand, due to demanding increase in requirement of automated method for classification, a decision support system for diagnosis of red blood cell named Cyto-diagno is developed. This method will help in hassle free identification and classification of altered cells from normal one using images obtained form any source. The extracted features are further used to develop CAD models of sickle and red blood cell.

1.1 Review of existing work

The image processing is very popular all around the globe. It is widely used for diagnosis of different cell types. The primitive methods of diagnosis, evaluating clinical status of cell types by counting and manually analyzing cells have been replaced by automated methods. The reason behind this success is high-resolution images are available with usage of fine quality microscopes [21, 40]. Quantitative image analysis has been utilized before to study and classify red blood cells. Bacus and colleagues had documented the application of various techniques in classifying the red blood cells from patients with various disorders [1, 23, 24]. Contour models (snakes and balloons,

which are initialized during the morphological operators) had been used for segmentation of images [10]. Horiuchi et al. used image analysis for characterizing Wrights stained sickle blood cell morphology [15]. In other study done by Wheeless et al. [44] the metric form factor $(4\pi Area/Perimeter)^2$ was selected as sole feature needed for segregating cells into different classes. A classification approach using eigen images is described in [39]. The description of cell free sickled hemoglobin structure was explained in[6]. An effort to simulate the 2-dimensional model of polymer domains in sickle hemoglobin has been done by[45].

Although, dynamics of sickling of cell in its various aspects is widely popular area of research across the globe, several questions remain open, warranting further work in this area. Similarly, past work done on application of image processing for feature extraction and further classification lacks several assets covered by this work. One main lacuna is problem of variable image quality is not addressed properly. Most of the researchers have used the images generated by them or their collaborators. Here we seek to address this issue and have worked towards an algorithm, which follows crucial steps for proper cell segmentation and edge detection.

1.2 Objectives

Despite the availability of several types of classification, image processing approaches, a automated method to differentiate the sickle and normal cells have not yet been determined, necessitating the exploration of newer concepts and newer algorithms. The in-silico simulation of dynamics of sickle shape formation will provide the new insights into pathology related to this disorder. The main objectives of this work are:

- To develop robust feature extraction methods, in order to remove dependence on manual operation for diagnosis.
- To provide an automated method, which detects the number of cells of a particular morphological type.
- Algorithm for extraction of features and further use for classification in order to study the dynamics of cell.
- To develop hierarchical model considering the morphological changes at different levels.
- To develop cell models in order to describe morphologies of biological cells.

- To carry out simple simulations of Cellular dynamics.
- To develop a framework to study sickling of RBCs.

Chapter 2

Overview and Plan of Work

The work carried out here can be classified into three different modules (a) feature extraction (b) classification (c) model building and (d) cellular dynamics. In order to study the etiology of disease, it is required to differentiate cell on basis of morphology. The variation in shape of normal and altered cells is comprehensive in majority of cases. But the presence of abnormal cells leads to misconception, which ultimately leads to wrong diagnosis. Due to this reason the extraction of features will provide useful insight into diagnosis of disease. Fig. 2.1 shows the difference in morphology of red blood cell, abnormal and sickle cell.

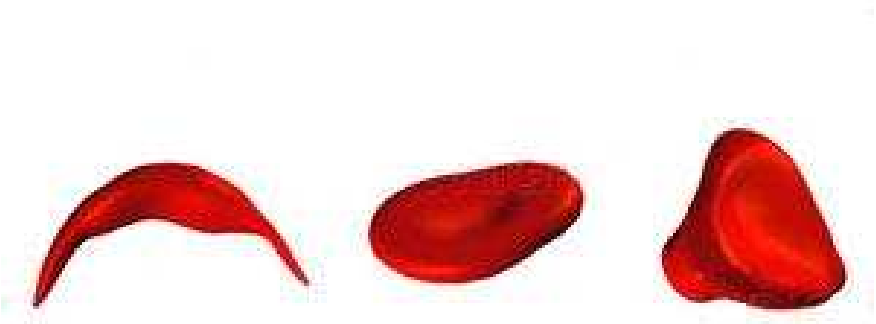

Figure 2.1: Sickle, Normal and Abnormal Red Blood Cells

Microscopic diagnosis is most efficient and reliable technique from past few years,

but the major disadvantages are: resources spent in maintaining and training technicians. On the other hand it is labour intensive and time consuming and accuracy of diagnosis depends on skill, concern and experience of technicians [35]. Thus, in-silico diagnosis will be far better than conventional methods which are labor-intensive along with it requires manual evaluation and enumeration of sickle cells in blood. In this way the automated, quantitative image analysis appears to offer sensitive and lenient classification of cells. Further, using Machine-learning approach we can use these features to classify cells into different types. In order to study the dynamics of this disorder in broader prospective, simulation of sickling of cell will help in unrevealing concealed phenomenon.

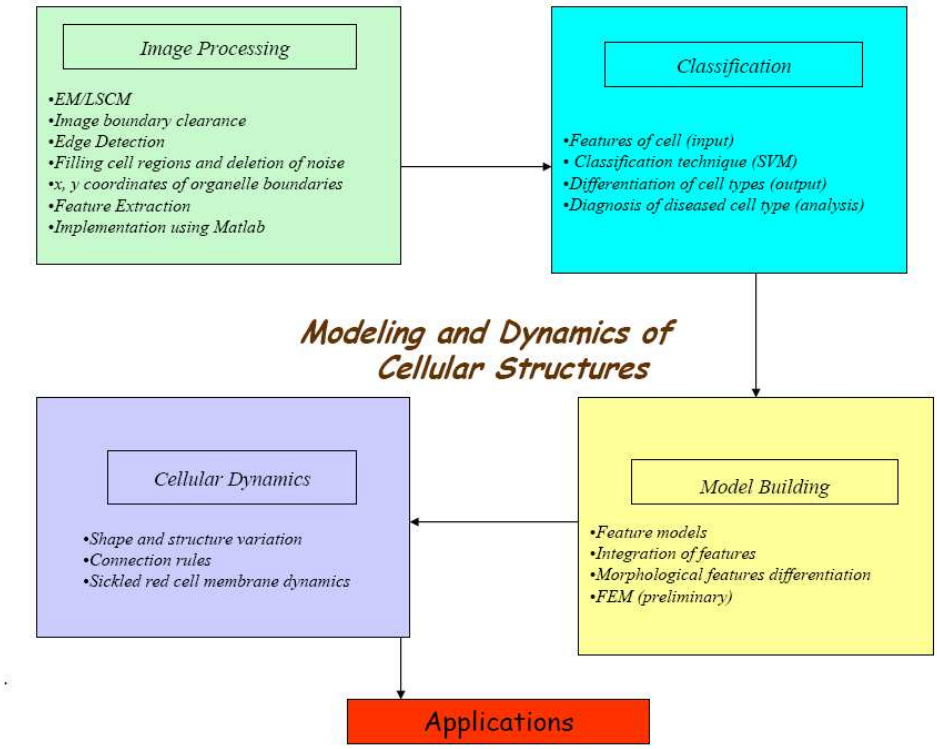

Figure 2.2: Overview of the work

An overview of the work showing the use of image processing, model building and dynamics of cell is shown in the fig.(2.2). Important aspects in each panel are listed. Image processing panel can provide precise definitions of various parameters from real biological images, which can be used for further classification and model building of cell.

2.1 Feature Exraction

Much of the information on cellular structures at various levels of detail that we have today has been obtained from different types of cellular imaging techniques. The most prominent of these techniques being, electron microscopy and fluorescence microscopy. Converting the qualitative data into quantitative type is required to model the cell as well as to study the morphological details. Image processing and computer vision techniques help to convert the qualitative information in cell images into quantitative information using the features extracted and further can be used for classification on basis of them.

2.2 Classification

The 'Cyto-diagno' software is used for classification. The final classification of red blood cells as sickled or not is basis for the classifier. The major dimensionality problem is that with the fixed sample size accuracy of classification decreases when we increase the features present [11]. It implies that the more number of features will reuire large training data set in order to give accurate and reliable results [5]. Cell classification is very old concept that initially started with utilizing the quadratic decision rule [34], minimum Bayes error [14], and scoring systems [41]. The classification problem is resolved by using Support vector machines (SVMs) classifier to differentiate red blood cells.

2.3 Cellular Dynamics

Cell models can be utilized for several purposes and form the starting point for many subsequent investigations. Studying the dynamics of cells, similar in concept to that of molecular dynamics, is one such possibility that has been largely untapped as yet. Availability of cellular models will enable exploring this area. As a case study, simulating the conversion of a normal RBC to a sickled RBC has been attempted. there are many challenges in this area, which have not been addressed thoroughly in this project, given that they comprise separate research areas in themselves. For example, there is no formal approach available to evaluate the consequence of the change in shape of one organelle within the cell, let alone estimating the structural or energetic feasibility of cell-cell interactions. However, attempts have been made

to carry out preliminary work in this regard, which provides further directions to fill the numerous gaps we have in this regard. One essential part and hurdle is the force fields for substructure interactions. Novel force fields are needed to evaluate these interactions.

Chapter 3

Algorithmic Concept

3.1 Introduction

The automated image processing algorithm used by us is basically designed for classification of red blood cells in order to help pathologists to diagnose the differences between normal and altered cells to detect diseases as soon as possible. In order to achieve this aim, algorithm works on the entire available image format and converts them to grayscale. It finds outs the edges of cells present and on the basis of features extracted differentiates the data into normal and altered forms. The algorithm design is type of classification problem and thus involves the pattern recognition and classification. It consists of mainly four stages: collection of images, image pre-processing, feature generation and classification [41].

3.1.1 Microscopic Techniques and Cell Images

Transmission Electron Microscopy (TEM): The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. What can be seen with a light microscope is limited by the wavelength of light. TEMs use electrons as light source and their much lower wavelength makes it possible to get a resolution thousand times better than with a light microscope. One can see the objects to the order of a few Angstrom units. For example, one can study small details in the cell or different materials down to molecular levels. The possibility for high magnifications has made the TEM, a valuable tool in medical, biological and as well as in materials research. Fig.(3.1) shows the blood cell. Here we see the cell substructures with the clarity required.

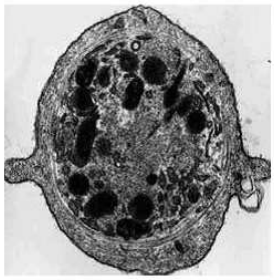

Figure 3.1: An example of transmission electron microscopy; Blood cell

Fluorescence Microscopy: In fluorescence microscopy, the sample to be studied is itself the light source. The technique is used to study specimens, which can be made to fluoresce. The fluorescence microscopy is based on the phenomenon that certain materials emit energy detectable as visible light when irradiated with the light of a specific wavelength. The sample can either be fluorescing in its natural form like chlorophyll and some minerals, or treated with fluorescing chemicals (see fig.(3.2). Other microscopic techniques such as 3D EM [22, 29] and Laser Scanning Confocal Microscopy (LSCM) are important considering the 3D reconstruction required for modeling.

Algorithm 1 : Histogram Equalization Algorithm

Require: Image having G gray levels and of size N X M

Ensure: Histogram equalized image

$H \leftarrow 0$

for all pixels in the image **do**

H[gray value of pixel] = H[gray value of pixel] +1

end for

for p=1 to G-1 **do**

H[p] = H[p-1] + H[p]

end for

T[p] = round ((G-1) x H [p] / NM)

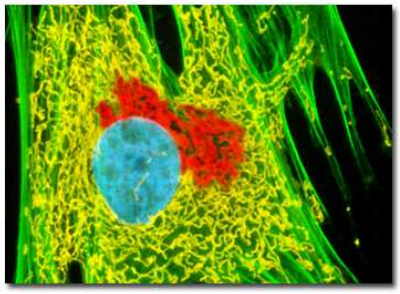

Figure 3.2: An example of Fluorescence microscopic image; dermal fibroblast cell

rescan the image and write an output image with gray levels Q

3.2 Morphological operations

3.2.1 Erosion

Erosion is a morphological operation that shrinks or thins objects in a binary image. The manner and extent of shrinking is controlled by a structuring element. Let us consider an example. Consider the following matrix :

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0
Let the structuring element be a vertical line, i.e. [1 1 1]'. The result of erosion is shown below :
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 1 1 1 1 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

Erosion is a process of translating the structuring element throughout the domain of the image and checking to see where it fits entirely within the foreground of the image. The output image has a value of 1 at each location of the origin of the structuring element such that the element overlaps only 1-valued pixels in the input image(does not overlap any of the image background). Mathematically erosion of A by B is defined in Equation.(3.1).

$$E(A,B) = \Theta(-B) = \bigcup (A - \ \beta) \quad \beta \in B \tag{3.1}$$

3.3 Image Processing

Much of the information on cellular structures at various levels of detail that we have today has been obtained from different types of cellular imaging techniques. The world wide popular and most regularly used of these techniques being EM and fluorescence microscopy. It helps in converting the qualitative data into quantitative

type that is required to study the complexity of cell. Image processing and computer vision techniques help to convert the qualitative information in cell images into quantitative information using the features extracted and use them in the design of mathematical models as well as study of dynamics related to cell. Fig.(3.3 illustrates the algorithmic steps followed for processing of images for extraction of features.

3.3.1 Collection of the Images

The images were collected form the internet. The images available in various formats were processed, and further classified.

3.3.2 Pre-processing

Our main aim was to extract the features present in the image in order to use them for classification. The images were processed in order to remove the noise and other effects from the images. As the protocol used for extracting features stress on using the gray scale images for processing, therefore we had converted other formats (RGB, indexed) to gray scale. In general there are three types of images:

- Gray scale images
- RGB images (Jpg and Jpeg images)
- Indexed images (Gif images)

The protocol followed to convert the other format into gray scale is as follows:

Converting RGB to gray scale: To convert RGB image to grayscale image we have used command "rgb2gray" present in image processing toolbox of MATLAB. This converts RGB images to grayscale by eliminating the hue and saturation information while retaining the luminance.

Converting Indexed to gray scale: For the conversion of indexed image we have used command ind2gray present in same image processing toolbox of MATLAB. Indexed image contains indexed color scale. According to the color of image pixel corresponding index value will be given to that location in image matrix. Using this color map and indexed image matrix the function converts the image to an intensity image and then removes the hue and saturation information from the input image

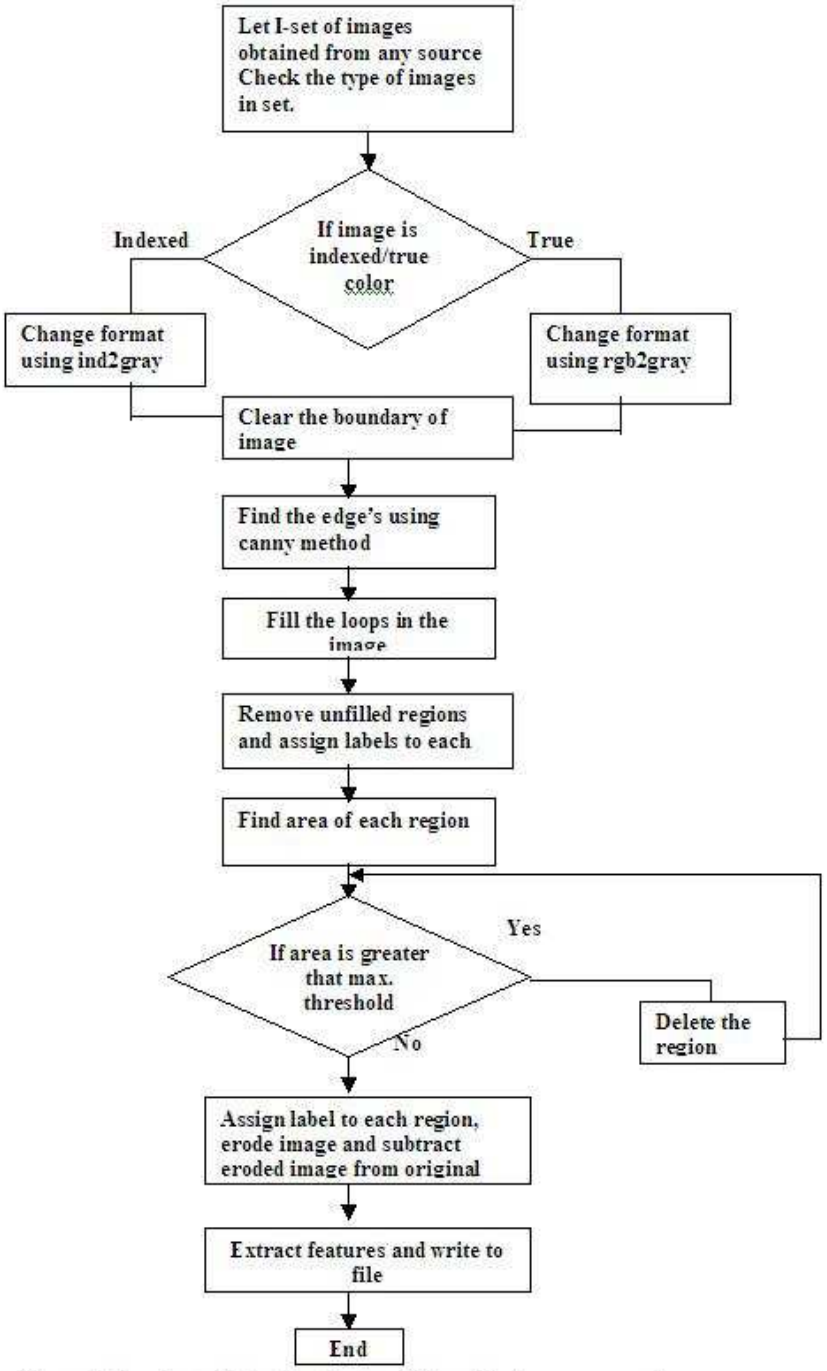

Figure 7 Flowchart of algorithmic steps followed for image processing

Figure 3.3: Flowchart of algorithmic steps followed for image processing

while retaining the luminance.

3.3.3 Feature generation

Edge Detection: The first and most vital step for generating features is detection of edges. There are so many algorithms defined in literature [17, 16, 20, 27, 19] for detecting edges of solid boundaries. But we have used Canny method for detection of edges due to its appropriateness for our analysis. 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 multi-step 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.

$$g(m,n) = G_\sigma(m,n) * f(m,n) \tag{3.2}$$

where

$$G_\sigma = \frac{1}{\sqrt{2\pi\sigma^2}} \exp -\frac{m^2+n^2}{2\sigma^2} \tag{3.3}$$

Now gradient g (m, n) is computed using gradient operators as follows:

$$M(m,n) = \sqrt{g_m^2(m,n) + g_n^2(m,n)} \tag{3.4}$$

and

$$\theta(m,n) = \tan^{-1}[g_n(m,n)/g_m(m,n)] \tag{3.5}$$

Calculate the M as follows:

$$M_T(m,n) = \begin{cases} M(m,n) & if M(m,n) > T \\ 0 & otherwise \end{cases} \tag{3.6}$$

where T is chosen that all edge elements are kept while most of noise is suppressed. The non-maxima pixels are suppressed in edges in M_T, obtained from the above to

thin the ridges of the edge (as the edges might have been broadened before). In order to do so, a test is performed to check whether the each non-zero $M_T(m,n)$ is greater than its two neighbors along the gradient direction $\theta(m,n)$. If this is the case $M_T(m,n)$ remains unchanged, otherwise is set to 0. Threshold the previous result by two different thresholds τ_1 and τ_2 (where $\tau_1 < \tau_2$) to obtain two binary images T_1 and T_2 now link edges segments in T_2 to form continuous edges. To do so, trace each segment in T_1 to its end and then search its neighbors in T_1 to find any edge segment in T_1 to bridge the gap until reaching another edge segment in T_2. The method uses two thresholds, to detect strong and weak edges, and includes the weak edges in the output only if they are connected to strong edges. This method is therefore less likely than the others to be fooled by noise, and more likely to detect true weak edges. This produces black and white image.

Filling cell with white: In order to obtain the area of cell the area inside the edges should be filled. To perform this operation we have used an image filling technique it assumes that white is "pixel on" and black is "pixel off". Afterwards, using 4-way connectivity check whether pixel is on or off, if the current pixel is on we cross verify neighborhood pixel else we move to further step. While traversing if we came back to visited pixel then these all pixels form a loop and area inside this loop is called as hole.
Example:

bw1 =
0 1 0 0 1 0 0 0 0 1 1 0
1 0 1 0 1 0 1 0 1 1 0 1
1 0 0 1 0 1 0 1 0 0 1 0
0 1 0 0 1 0 0 0 1 0 0 0
0 0 1 1 0 0 1 0 0 1 0 0
0 1 0 1 1 0 0 0 0 1 0 0
0 0 1 0 0 0 1 0 1 0 1 0
1 0 0 1 0 0 0 0 0 0 0 1
Pixels (1,2),(2,1),(2,3),(3,1),(3,4),(4,2),(4,5),(5,3),(5,4) forms a loop. So, after filling the hole inside the loop resultant matrix is
bw2 =

0 1 0 0 1 0 0 0 0 1 1 0
1 1 1 0 1 0 1 0 1 1 1 1
1 1 1 1 0 1 0 1 0 0 1 0
0 1 1 1 1 0 0 0 1 0 0 0
0 0 1 1 0 0 1 0 0 1 0 0
0 1 1 1 1 0 0 0 0 1 0 0
0 0 1 0 0 0 1 0 1 0 1 0
1 0 0 1 0 0 0 0 0 0 0 1
This way we can fill all loops.

Delete the edges that dont form loop: To perform this operation we have to first understand how the edge formation takes place. If we take an edge for each on pixel of the edge the neighbor row pixels or column pixels must be off. For example if we consider above situation, each on pixel's neighbor pixels either in row or in column are off. so, we can use this property. After filling the image if we take mode as pixel value for each three consecutive pixels the resultant value is off pixel.
Example:
If above matrix bw2 is our input then the output will be
bw3 =
0 1 0 0 0 0 0 0 0 1 1 0
1 1 1 0 0 0 0 0 0 1 1 1
1 1 1 1 0 0 0 0 0 0 0 0
0 1 1 1 0 0 0 0 0 0 0 0
0 0 1 1 0 0 0 0 0 0 0 0
0 1 1 1 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
At this point we have filled regions but some regions may come as a result of noise present in the image. These regions will be very small comparing to cell regions so, by finding area of each region and deleting smaller regions from image by applying some threshold we can get proper cell regions.

Give labels to find area of cells region: Here for each region we give a label. After giving labels to regions we calculate no of pixels belongs to each region

this is considered as the area of the region. We will delete some regions that are having lesser value than threshold value. Now in our images there may be overlapping cells. To delete this we need an upper threshold value.

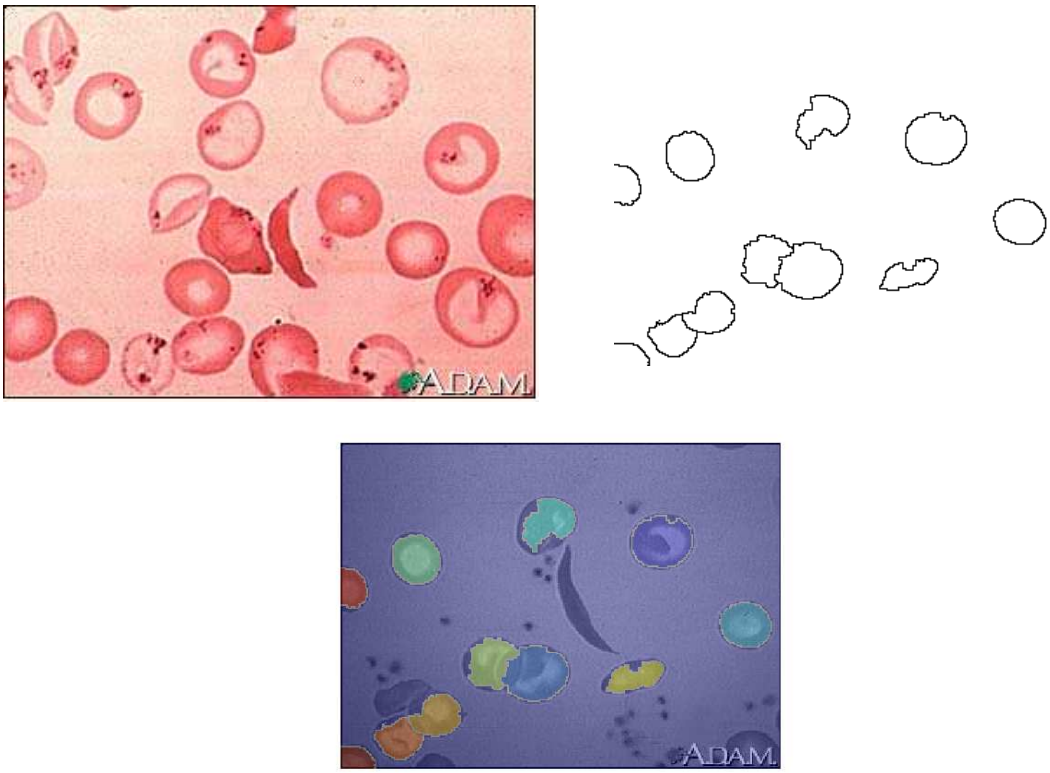

Figure 3.4: Image segmentation and Feature extraction with existing algorithm.

3.3.4 Improvement over the existing algorithm

- It is clearly seen in the images that the segmentation using newly developed algorithm is more accurate.
- The sickle cell present in the original image is not segmented with existiing algorithm whereas it is perfectly segmented using newly developed algorithm.
- With the existing algorithm we need to extract features of each cell present in the image by finding its label manually. The newly developed algorithm uses minimum threshold and maximum threshold to delete the noisy regions. For single scaled images we can fix these threshold values. If the images are not single scaled then we have to run the code twice or thrice for each image to fix threshold value for that image.

Finding cell region features: After all now we have proper cell regions with

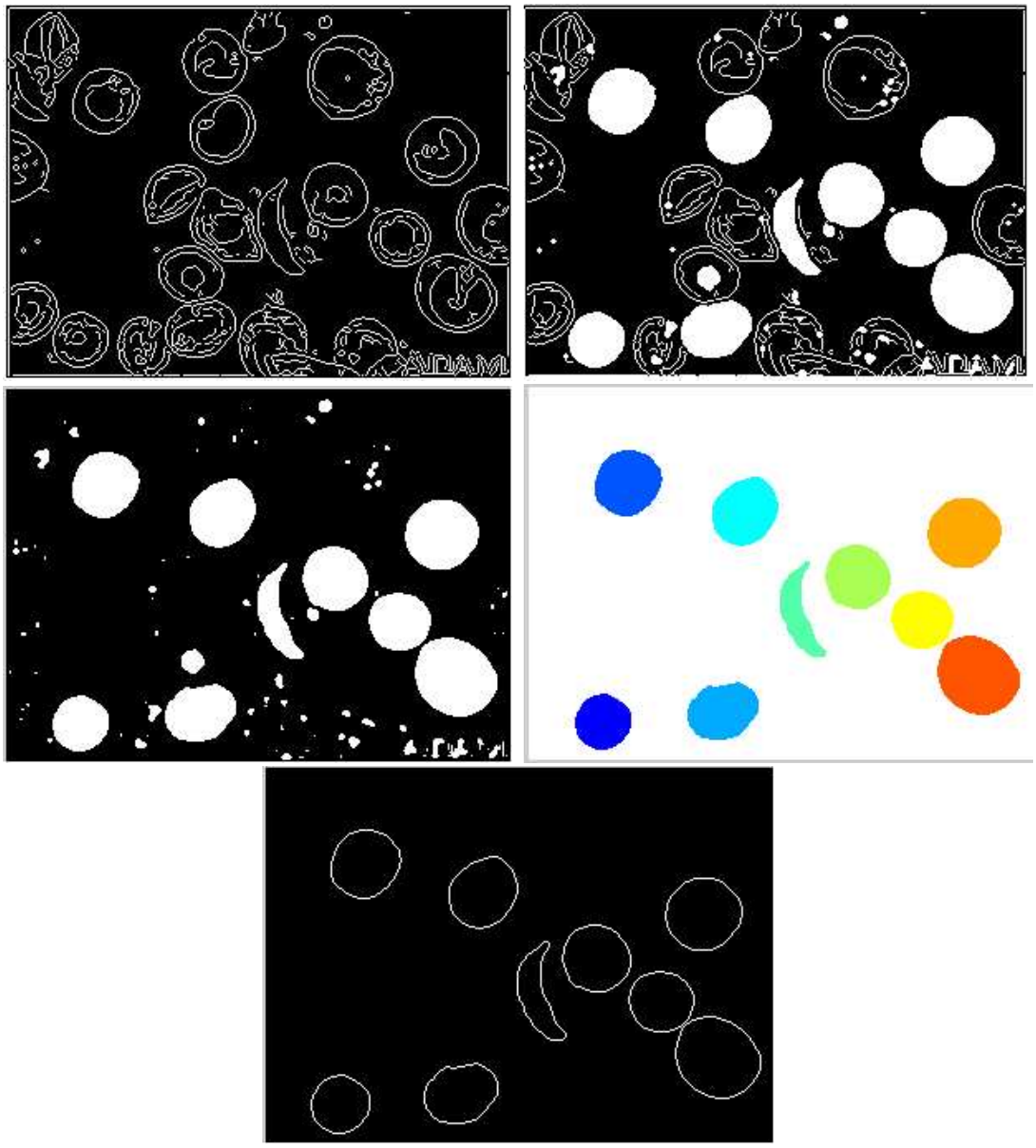

Figure 3.5: Processing of sickle cells using algorithm defined above: First after edge detection, Second image is after filling, Third image shows filtered regions which forms loops,Forth image which displays regions with clearly defined and Fifth image shows edges without noise.

us. The extracted features used for differentiating the cells are given in the Table

S.No	*Feature*	*Explanation*	Use in Classification
1.	Area	Describes the actual number of pixels in the region	Sickle cells area will be much less than normal cell. This variation provides us some information in classification
2.	Eccentricity	Ratio of distance between the foci of the ellipse and its major axis length	Eccentricity of sickle cell will have some higher value than normal cell
3.	Euler Number	Number of objects in region minus the number of holes in those objects	This is same for sickle cell and normal cell so, this feature is not useful in this case but is useful for some other disease cells like malaria
4.	Major axis length	Length (in pixel) of major axis of ellipse that has the same normalized second central moments as the region	Since sickle cell grows in one direction after sickling its major axis length will have higher value than normal cell
5.	Orientation	Angle between the x-axis and major axis of the ellipse	
6.	Equiv diameter	Diameter of a circle with the same area as the region	Sickle cells area is lesser than normal cells. so, diameter also small for sickle cells.
7.	Solidity	Proportion of pixels in the convex hull that are also in the region	Since sickle cells shape is concave the convex hull that bounds sickle cell is larger than normal.
8.	Minor axis length	Length (in pixel) of minor axis of ellipse that has sane normalized second central moments as the region	Sickle cells minor axis will have lesser value than normal cells minor axis.

Table 1.Features and their use in classification

3.3.5 Construction of Classification Model

The features computed for the compounds in the training sets were in turn used to construct the classification model using the support vector machines. The LIBSVM was used to build the SVM classifier. The different kernel functions, that is, linear, polynomial, sigmoid and gaussian, which were available as part of LIBSVM package were examined in order to build the classification model. The linear kernel was found to give a good performance.

Support Vector Machines

Support Vector Machines (SVMs) represent the learning technique that by following principles from the Statistical learning theory [43], presents the high generalization ability in several domains and robustness to high dimensional data [3]. This technique looks for a hyper plane that separates the data from classes +1 and 1 with a maximal margin, for a given dataset with the n samples (x_i, y_i). Each x_i is an input sample and $y_i \in -1, +1$ corresponds to x_is label. In equation 1, w is the normal vector to the hyper plane and b is an offset.

$$w.x + b = 0 \tag{3.7}$$

Margin maximization is the equivalent to minimize the norm of w, such that SVMs solve the following optimization problem [23]:

Minimize:

$$\|w\|^2 + C \sum \xi_i \tag{3.8}$$

Resrictions:

$$\xi \geq 0 \tag{3.9}$$

$$y_i(w.x_i + b) \geq 1 - \xi_i \tag{3.10}$$

where C is a constant that imposes a trade-off between training error and generalization and the ξ_i are slack variables. These variables relax the restrictions imposed on the optimization problem, and consequently allow some patterns to be within the margins, which yields some training errors. The resulting decision frontier is given

by the equation 3.11.

$$F(x) = \sum_{x_i \in SV} y_i \alpha_i x_i . x = b \tag{3.11}$$

where the constants α_i are called Lagrange multipliers and are determined in the optimization process. Here, SV corresponds to the set of support vectors, patterns for which the associated Lagrange multipliers are larger than zero. These samples are those closet to the optimal hyperplane. For all other patterns, the associated Lagrange multipliers are null, so they do not contribute to the determination of the final hypothesis. The classifier represented in equation 3.11 is still restricted by the fact that it performs only a linear separation of data. Mapping the input samples to high dimensional space, also named feature space, where they can be efficiently separated by a linear SVM, can solve this. This mapping is performed with the use of Kernel functions that allow the access to spaces of high dimensions without the need of knowing the mapping function explicitly, which usually is very complex. The Kernel Functions compute dot products between any pairs of patterns in the feature space. Thus, the only modification necessary to deal with non-linearity is to substitute any dot product among the patterns by Kernel product. The main advantage of the SVMs is their precision, usually good even in high dimensional problems.

3.4 Results and Discussion

After getting features of all cells we have done classification of these cells using support vector machine classifier. Considered cell features for this classification are :

- Area
- Eccentricity
- Major Axis Length
- Minor Axis Length
- Orientation
- Equiv Diameter
- Solidity
- Extent

Since any other feature that can classify normal and sickle cells is not available the classification process is done using this set. Classification is done using features

of 688 cells. In this 577 are normal and 111 are sickled.

Does this entire set is needed for good classification accuracy?

We can get answer to the above question by taking all possible combinations of eight features and performing classification process.

No of possible combinations: $2^8 - 1 = 255$

The classification is done for each combination. The data is classified using each combination with 4 different kernels and using k-fold cross validation for k = 4, 8 and 12. So, totally the classification is done for 1536 times. Using the last five features it gave 93% of accuracy for balanced data (111 class one type data and 111 class two type data) and using all features except Area it gave 96% of accuracy for whole data set. Here we have to consider one thing that a normal cell classified as sickle cell may not be a big problem but a sickle cell shouldnt be classified as a normal cell. To avoid this problem we should calculate the no of wrong classified cells and no of correct classified cells. To do this Let we assume Normal cells are true and sickle cells are false. Correct classification is positive and wrong classification is negative. It gave the minimum no of false negatives when the linear kernel is used with features 1, 3, 7 and 8.

True Positives: 92

True Negatives: 109

False Positives: 19

False Negatives: 2

Accuracy: 90.54 %

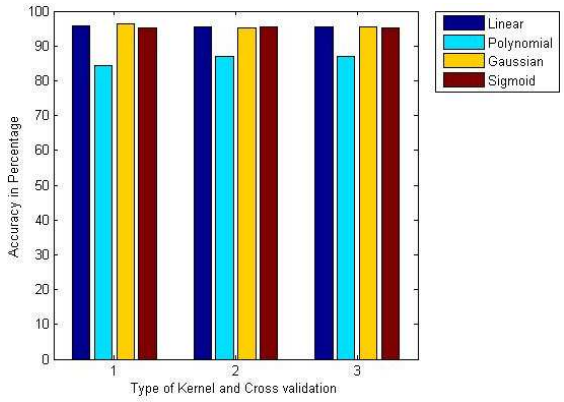

Figure 3.6: Accuracy for different kernels on whole set of data

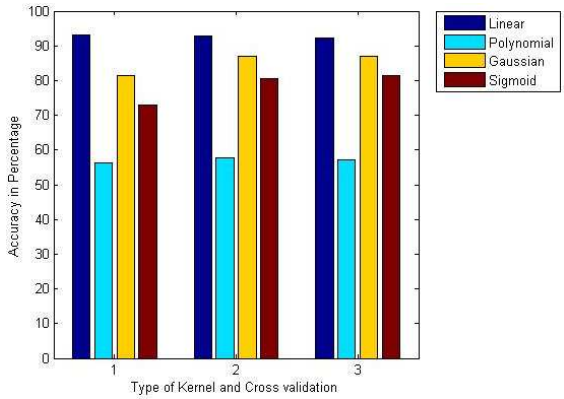

Figure 3.7: Accuracy for different kernels on balanced data set

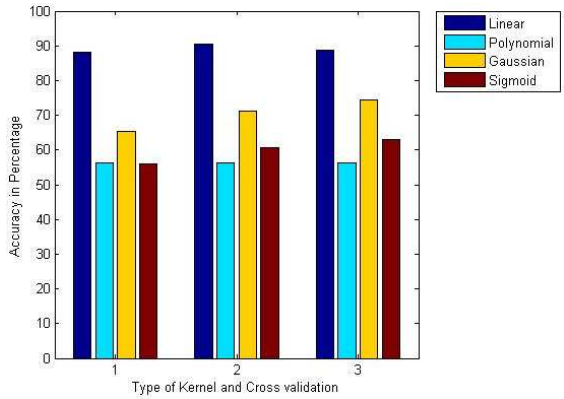

Figure 3.8: Accuracy for different kernels when False Negatives are minimum.

Chapter 4

Design and development of in-silico model to study dynamics of Red Blood Cell

4.1 Introduction to basic cell modeling

Cell Modeling is one of the emerging and challenging areas to model biological processes and indeed entire organisms, areas that are currently being integrated under the banner of Systems Biology. Given that modeling of biological systems is a highly complex task, it is important to start with relatively simpler definitions of A 'system'. A biological cell is a natural fairly self-contained unit, depicting the fundamental unit of living tissue. In order to model various aspects of a cell it is required to integrate knowledge encoded at different levels of abstraction, with cell morphologies at one end to atomic structures at the other. While a number of studies have illustrated the design, development and application of metabolic and structural models of the individual proteins and also the proteome, there has not been much work reported in the literature about modeling cell morphologies, visualizing dynamics of processes and ultimately relate them to molecular level knowledge. As a first step, methods have been developed in this work to systematically capture data about various morphological features in a cell available through a number of sophisticated cell imaging techniques. This helps in capturing the morphological properties of certain cell type or different types of cell, which ultimately leads to extraction of features morphologically. The dynamics of cell can be further understood by simulating the process

in-silico. This study will help in apprehension of peculiarities of cellular dynamics.

4.1.1 Introduction to Sickle cell Anemia

Sickle cell disease is a blood condition seen most commonly in people of African ancestry and in the tribal peoples of India. It is an inherited blood disorder characterized primarily by chronic anemia and periodic episodes of pain. It is caused by the hemoglobin variant Hb S. In this variant, the hydrophobic amino acid valine takes the place of hydrophilic glutamic acid at the sixth amino acid position of the normal hemoglobin polypeptide chain. This substitution creates a hydrophobic spot on the outside of the protein structure that sticks to the hydrophobic region of an adjacent hemoglobin molecule's beta chain. This clumping together (polymerization) of Hb S molecules into rigid fibers causes the "sickling" of red blood cells[30].

4.2 Creating 2D cell model

4.2.1 Membrane coordinates extraction and Processing

This process started with image processing to get morphological features of general cells. From that 2D surface coordinates obtained. One model cell was taken, which built by a system biology research group(http://gcrg.ucsd.edu/organisms/rbc.html) and processed it to get surface coordinates. But this won't give the coordinates along the surface curve sequentially. Instead of traversing along the surface curve it just gives the pixel values of surface in the following manner. Suppose our coordinates along the surface curve is as shown in the (fig.(4.1)). In the above image (fig.(4.1)),

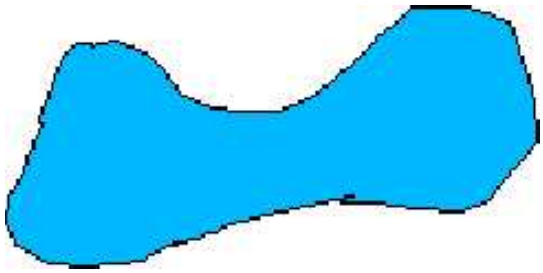

Figure 4.1: Cross-section of Red blood cell

we suppose to get first left top curve coordinates next middle and then right top curve. But from image processing we get first left top next right top then middle one.

The points to be arranged so that these all are consecutive on the curve. One point is taken(Let it be A) on the curve initially and found the point closest to it (Let it be B). Next the process started with B and found closest point to it. Like that all points on the curve arranged sequentially.

4.2.2 Positioning polymer in membrane

Actually the process should start the simulation with Hemoglobin monomers and polymerize them. The entire simulation should give us a polymer in membrane that is having contacts with cell membrane on both sides to supply forces to membrane. Because it is known that the cytoplasm inside cell is almost all liquid so, it can't supply forces from polymer to membrane. Instead it makes space to polymer by self-adjusting it self. So,the polymer should be in membrane like the figure shown below (see fig.(4.2)). But since getting it is tricky job and the main intension is to do

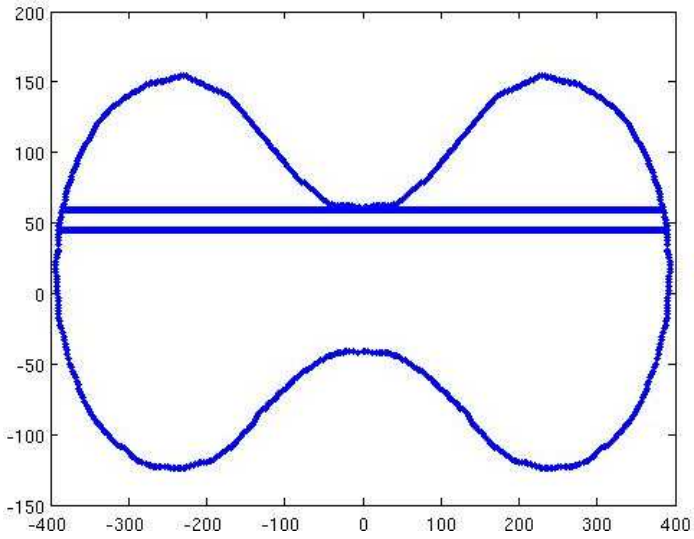

Figure 4.2: 2-dimensional model of Red blood cell with polymer inside

simulation to get sickle cell from normal cell so,polymer inside membrane manually is taken. This is done by taking 4 points on membrane and generating other points on polymer. Initially this polymer looks like a rectangle but when it bends it gets curve shape.

4.2.3 Polymer elongation

Here some assumptions are made:
Since the polymer is fit inside membrane its length can't be increased in its axis direction. So, It is assumed that there should be some kind of heterogeneous polymerization with other small polymers or monomers of hemoglobin at polymer and membrane contact points. This can happen on either sides of the polymer. It is taken on upper side of the polymer both sides.
Now let us assume that polymer has enough stiffness so that it won't bent by force of membrane on it. Then even the polymer elongates its elongation takes place in its axis direction then we can't get sickle shaped cell.

4.2.4 Polymer bending and shape change

This gives us some basic understanding that even if it applies force on membrane and causes change in its shape; polymer also should bend to some extent. So, what shape will it get when bent.
Let us suppose assume that the force on both ends is equal and opposite in direction. We know that polymers cross-section mean radius 110A is very much lesser than the polymers length 10(micro meters approx). Here we are applying forces from both sides and equally. The resultant shape can be seen in the Fig.(4.3)). In the above

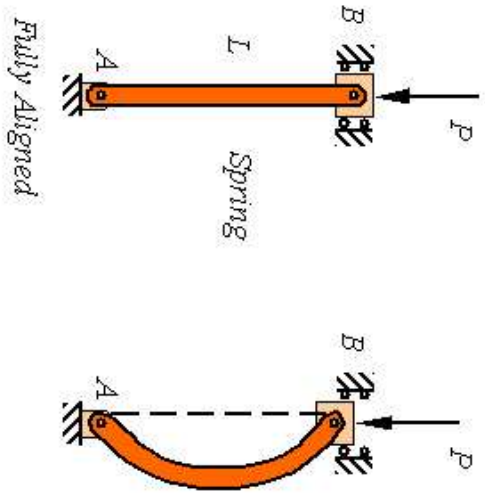

Figure 4.3: Normal and Buckled column

image take starting point as x =0 and ending point as x = L and vertical axis as

y-axis. The force applying from right side. Let the force is applied for unit time and then the condition is maintained then the force traverses along the column and it happens that from the middle point of the column equal distances will have equal forces on it.
This condition is same as half of the force is applied from either side of the column. We can find the critical load(Force) and the deflected shape of the buckled column using following equation.

$$EIv'' = -M \tag{4.1}$$

in which v is the lateral deflection in the y direction.E is modulus of elasticity,I is 2nd moment of inertia and M is bending moment [31].
Since

$$M = -Pv \tag{4.2}$$

$$EIv'' + Pv = 0 \tag{4.3}$$

The solution gives v as a function of x. Let

$$k^2 = \frac{P}{EI} \tag{4.4}$$

then

$$v'' + k^2 * v = 0 \tag{4.5}$$

The general solution of this equation is

$$v = C_1 * sinkx + C_2 * coskx \tag{4.6}$$

By applying boundary conditions

$$v(0) = 0 \tag{4.7}$$

$$and \tag{4.8}$$

$$v(L) = 0 \tag{4.9}$$

it gives

$$kL = n\pi \tag{4.10}$$

where n = 1,2,3,...
then

$$P = \frac{n^2\pi^2 EI}{L^2} \tag{4.11}$$

So, the deflected curve equation is

$$v = C_1 sin\frac{n\pi x}{L} \tag{4.12}$$

4.2.5 Force on membrane and membrane shape change

Now the elongated polymer applies force on membrane. This force will be applied at contact points. The amount of force applied at each point can be calculated using the equation (4.13).

$$F = k * x \tag{4.13}$$

Here k is stiffness of the membrane or polymer and x is displacement. After getting the actual value of k for red blood cell membrane and polymer we can replace it. Initially each contact point should move till the end of polymer. Now keeping the extended points as constant the other points are moved on the membrane.
Here is one example (see fig.(4.4)), which explains about this movement. Let A, B, C are three consecutive points on membrane. Suppose the force is applied in BA direction. Then in BA direction we have two forces stiffness of the membrane and force of the polymer. In BC direction we have only force of stiffness of membrane. The addition of these two vectors will give the resultant force direction i.e. BA + BC

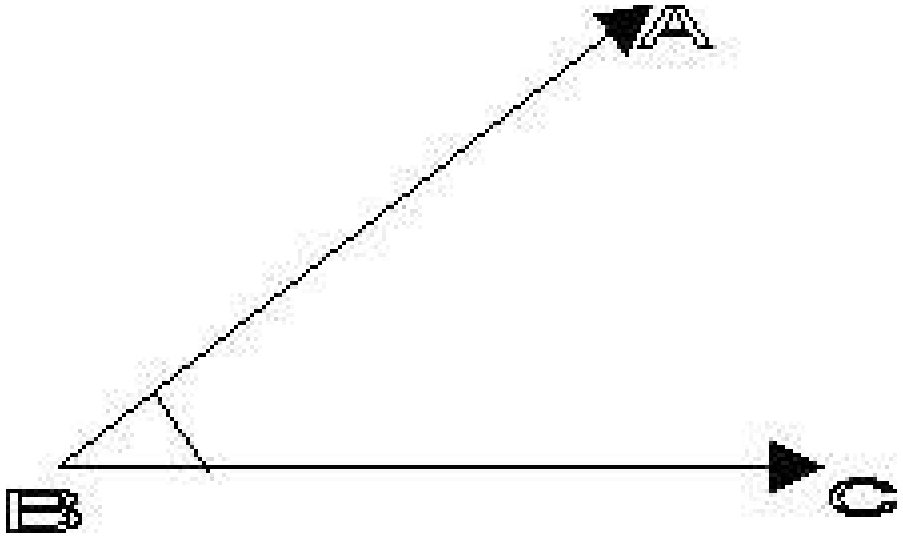

Figure 4.4: Consecutive points of 2-dimensional space to illustrate vector summation

is the resultant force direction.

Suppose A, B, C, D, E, F, G, H, I are consecutive points on membrane and we applied force on point E. movement in E forces the other points to move in the direction of E. Now suppose angle between EFG is some theta degrees. If we apply force on E by fixing G at the same place then point F moves towards the line joining EG. If the extreme force is applied then these three points become on the same line. After that whatever the force applied on that portion will result in elongation of membrane till the membrane can bear shear force applied, extra force application will lead to bursting of membrane. Using this fact point F is moved on to the line joining EG keeping G fixed and E moved already by the polymer. The point F remains its distance ratio with E and G. After finishing this FGH, GHI, EDC, DCB and CBA were selected respectively and moved the middle point on to the line joining the extreme points.

Here it has been checked whether the entire membrane length is out of range. If so, then the it informs about it and exits from running code. As a further step it has been checked whether any pair of points violated their distance range, If so, then that points were readjusted to its max or min distance according to its violation type.

All the time the polymer will be inside the cell. So, to keep the polymer inside the cell the x axis is divided into small intervals and checked in each interval if there is a polymer and membrane intersection if so, then the present point is noted down and searching process continued for the other intersection point of the polymer

and membrane and given 75% moment to the membrane and 25% moment to the polymer opposite to their crossing so that both polymer and membrane join between the crossing points.Actually this membrane and polymer adjustment depends on their stiffness which we don't have now.

4.2.6 Results

The above entire process undergoes one iteration. By performing more iterations (approx. 600) the shape of membrane changes to sickled shaped cell (see fig.(4.5)). In further iterations it got stretched and at one point shear force leads to bursting of membrane.

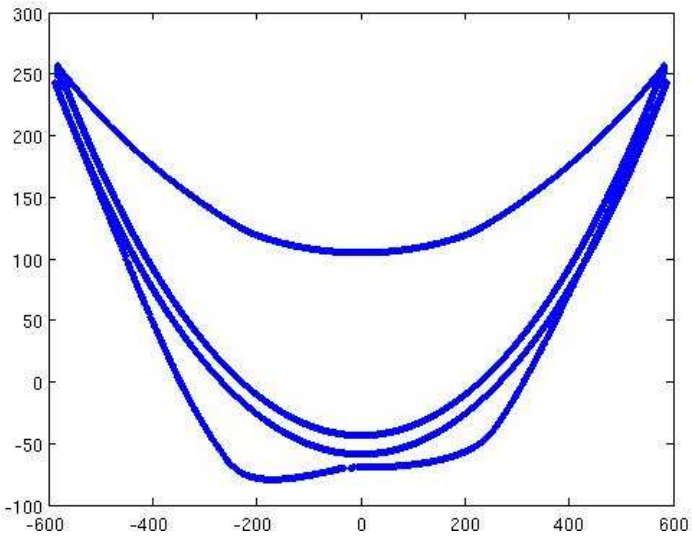

Figure 4.5: Polymer stretching after simulation, normal, stretched and deformed.

4.3 Creating 3D model of cell

4.3.1 Creating Grid based model

What is Grid based model?
It is a model build using 3D points of the membrane. Keeping the whole cell in a cuboid, The cuboid is divided into a mesh of small cubes with edge length 1. The vertices of cuboid are:
A(-400,-130,-400) , B(-400,-130,400), C(-400,130,-400), D (-400,130,400), E (400,-130,-400), F (400,-130,400), G (400,130,-400), H (400,130,400).

After making setup the traversal started from vertex A to vertex H. This traversal is done to setup membrane inside the cube by defining each cube value of grid as

- 1 if the cube is inside the membrane.
- 2 if the cube is outside the membrane.
- 0 if the cube is on the membrane.

Then how to find out which point is where. For this it is taken care when the points for membrane were generated. For each pair of consecutive points the max coordinate distance between them is 1. For example if (x, y, z) is a point on the membrane then the next point on the membrane can have maximum (x +1,y + 1, z + 1). i.e. If we take a pair of consecutive points then in any direction they wont have distance more than 1.

Now since the taken membrane is symmetric to XZ plane and for each side its like a single valued function for y i.e. y = f (x, z) is single valued on each side of the plane So, when the traversal goes through the grid-keeping x and z coordinates fixed and y varying. It will touch the membrane at most twice and there will not be any loopholes.
Advantages of This setup:

- We can generate hemoglobin monomer points inside the membrane with out putting much effort. i.e. just check whether the box value is one or not if it is 1 then generate point inside the cube.
- Assuming all monomers are having Brownian motion we can easily check whether a hemoglobin monomer is inside or outside of the membrane after its moment to next step. Accordingly we can set its position.
- Polymerization process can be carried out inside the membrane easily.

Disadvantages of this setup:

- Extra memory space required. Even after using 3D char datatype array to represent the grid. It took 177 MB extra space. which is more considerable amount.

- Handling membrane deformation is difficult.

The actual red blood cell contains approximately 270 millions of hemoglobin monomers. But generating this many hemoglobins takes much memory space. In the present system, in which simulation process is being done is not capable to generate and maintain these many points.
The 1 million points was generated inside the membrane and taken into consideration for simulation initially. In this simulation, first we considered that each monomer is behaving as a polymer and then some of the monomers get polymerized. These polymers get elongated when some other hemoglobin monomers or polymers joined with them. If the process will go on like this then at one stage if the number of polymers are less than the specified threshold number of polymers then polymerization will halt.

Polymerization process has initially carried out in 2-D with out considering membrane[29].

4.3.2 Polymerization process inside cell model

The steps involved in this process are
- Each hemoglobin monomer will have Brownian motion i.e. in each time unit it moves one step in random direction.
- A threshold is specified on membrane and monomer distance to maintain all monomers inside the membrane. Whenever a monomer takes movement it is checked whether the monomer is inside or not. If it is going outside of membrane then it goes the specific threshold distance closer to the membrane and stops there.
- Once two monomers get polymerized then from that step they move together in the same direction and same step size unless these polymer goes outside of membrane.
- At each time unit the closeness of two monomers will be observed and if it is much closer than the threshold distance then these two will be moved back on the line joining them.

Optimizations in code :
- We need to calculate distance between each pair. This will be an $O(n^2)$ job. But here all points are sorted on x coordinates of points and distance is calculated only

for all the monomers who's x coordinate distance itself with in threshold distance for polymerization.

- For sorting purpose heap sort is used. which is best if we have millions of points.Since the simulation is still in progress results were not given here.

Chapter 5

Conclusion and Future Directions

5.1 Conclusion

Use of automated methods in disease analysis got great importance today due to reduction in human dependency.Sickle cell anemia is one disease that caused by change in cell morphologies. In this present work a semi-automated method is developed to analyse the morphology of a Red Blood Cell, whether it is diseased or not.We have used image processing techniques to acquire cell features automatically.Ultimately classification method SVM(Support Vector Machines) is used to classify and find the existence of the disease in terms of number of Sickle cells present in the blood sample. As a further step in this work the surface coordinates, which found using image processing techniques has been processed and used to build a simple model cell in 2D and 3D. Using this model an attemp is made to carry out simple simulations of cellular dynamics. The simulations mainly focused on polymerization process of hemoglobins and sickling of RBCs.

5.2 Future Directions

The present work can be further improved to detect other diseases, which caused by change in cell morphologies. The sickling of RBCs is done in 2D this can be extended further to 3D and can add more properties of polymer and cell membrane. A robust cell model should be developed to carry out simulations of cellular dynamics. An attempt is made to develop and use a mechanical model it is given in next subsection.

5.2.1 Creating CAD and FEA models

The use of mechanical engineering CAD/FEA software to develop mechanical models of cells has been attempted here,though no FEA simulations have been performed. The CAD/FEA models should aid the understanding of the behaviour of these cells in the long term. However, it must be emphasized that benchmark studies and the use of appropriate elements and non-linear solution techniques are essential. CAD models can be used in conjunction with SVM to reconstruct from images, especially since we have used 2D models so far. Developing 3D models and constructing images from these can have distinct advantages in behaviour simulation. In this thesis, an attempt has been made to create a CAD and FEA model of the red blood cell to study the feasibility of using widely available FEA software like ANSYS.

5.2.2 Creating ANSYS model

Creating a fem model needs the points in a particular format. The points are called as nodes and group of nodes (3 to 24) forms elements. In FEM (Finite Element Method) the deformation will be calculated on each element. To create ANSYS model we should provide all commands starting with nodes first then elements and then material property commands and so on. Here is the example file with needed commands and description.

/PREP7: This command is used to start creating the new model.

As we already seen that FEM model contains Elements. These elements are of different types. One of such element is SHELL63. The following is the description of SHELL63.

SHELL63

The geometry, node locations, and the coordinate system for this element are good enough to build our model. The element is defined by four nodes, four thickness each applied at one node. Since thickness of rbc varies this gives flexibility in assigning various thickness values. The thickness is assumed to vary smoothly over the area of the element. Moreover since the membrane is also having elasticity property we need an element which maintains elasticity property and with SHELL63 we can do

it. Other properties like stiffness of membrane and bending energy an important property in building a proper model, we can achieve this also. The element coordinate system orientation is as described in Coordinate Systems. Since polymer and membrane have different properties we can create two models each having their own properties.

Commands:

ET, 1,SHELL63

ET, 2,SHELL63

The above command means the element which ones reference no 1 or 2 is SHELL63 type element. The SHELL63 has the required properties so; I have used this shell for my modeling purpose.

N, num, x coord, y coord, z coord: This command defines a node with number "num" and with coordinates xcoord, ycoord and zcoord. Here after whenever we refer to this point we can refer it using this "num".

Presently we have only 2D model to get 3D model for this modeling purpose I Rotated each point around Y- axis and got the 3D coordinates. Pattern to specify elements: E, I, J, K, L, M, N, O, P.

Here the first character E refers to the command for an element. I,J etc. refers to nodes which forms this element.

Defining Material properties: To define material properties we have command MP.

Example: MP, EX, 1,1E9

In the above the young's modulus of material whose reference no 1 is 1E9. EX denotes command for young's modulus. MP, PRXY, PRXY denotes command for poison's ratio.

Defining Membrane variable thickness at various nodes To do this we have RTHICK command usage:

RTHICK, Par, ILOC, JLOC, KLOC, LLOC Par is the Array parameter that expresses the function to be mapped for example; func (17) should be the desire shell thickness at node 17. Here ILOC, JLOC etc. are Positions in real constant set for thickness at node I or J of the element.

Defining stress of membrane:

ISTRESS Sx, Sy, Sz, Sxy, Syz, Sxz, MAT1, MAT2, MAT3, MAT4, MAT5, MAT6
Sx, Sy etc. are initial stress values. MAT1, MAT2 etc. are materials to which the initial stress should apply. If these Materials are not specified then the stresses apply to all materials.

Defining Bending stiffness:
SSPD, D1, D2, D3
This command specifies a pre integrated bending stiffness for shell sections. D1, D2 etc. are bending stiffness components.

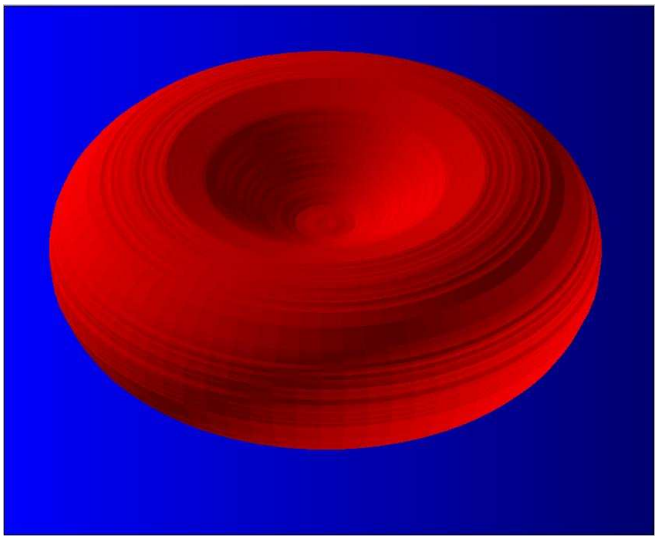

Figure 5.1: Model of RBC in 3-dimensional space

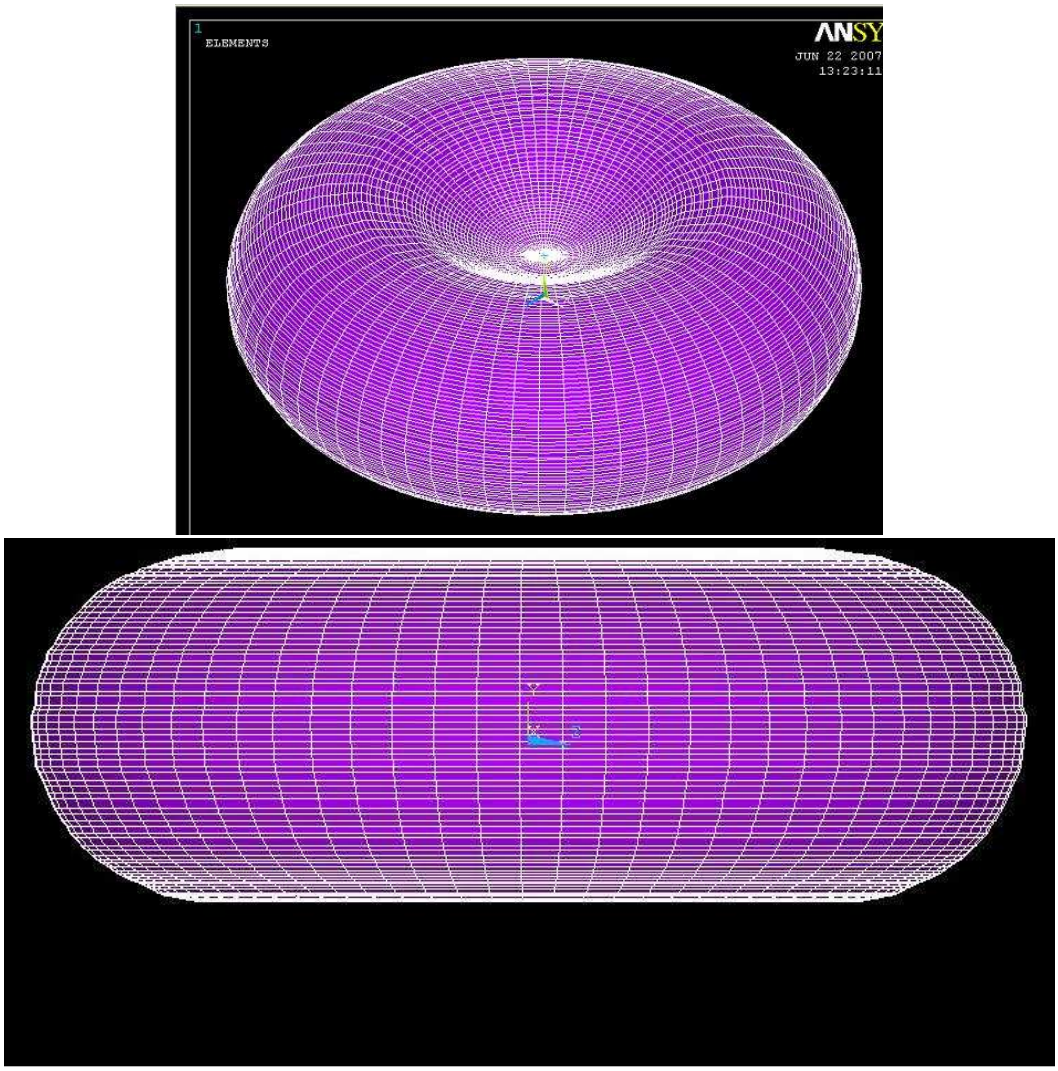

Figure 5.2: FEM based ANSYS model of Red blood cell in front and side view.

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