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DETECTING RED BLOOD CELLS MORPHOLOGICAL ABNORMALITIES USING GENETIC ALGORITHM AND KMEANS

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Dedication

To my loving mother and father
To my brothers and sisters
To my Dear husband and my Precious
Daughter

To all friends.

Acknowledgements

Praise Allah, the Almighty for having guided me at every stage of my life.

This work would not have been possible without the constant encouragement and support I received from Dr.Mohammed A. Alhanjouri, my advisor and mentor. I would like to express my deep and sincere gratitude to him. His understanding and personal guidance have provided a good basis for the present thesis.

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Also, I would like to take this opportunity to express my profound gratitude to my beloved family without whom I would ever have been able to achieve so much and for my own small family : my daughter and my husband whom gave me the energy to survive.

Last, but certainly not least, I want to thank my friends, their moral support during this study.

Faten Abushmmala

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List of Abbreviation

AI	**Artificial Intelligence**
CBC	**Cell Blood Count**
CDF	**Centroid Distance Function**
dl	**Decilitre**
DNA	**Deoxyribonucleic acid**
FBC	**Full Blood Count**
fl	**Femtolitre**
G6PD	**Glucose-6-Phosphate Dehydrogenase**
GA	**Genetic Algorithm**
Hb	**Hemoglobin concentration**
Hct	**Hematocrit**
HDW	**Hemoglobin Distribution Width**
HPLC	**High performance Liquid Chromatography**
MCH	**Mean Cell Hemoglobin**
MCHC	**Mean Cell Hemoglobin Concentration**
MCV	**Mean Cell Volume**
MGG	**May–Grünwald–Giemsa**
NRBC	**Nucleated red Blood Cell**
PCV	**Packed Cell Volume**
pg	**Picogram**
RBC	**Red Blood Cells**
RDW	**Red cell Distribution Width**
RNA	**Ribo-Nucleic Acid**
TAR	**Triangular Area Representation**
WBC	**White Blood Cell Count**

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الملخص

القدرة على الرؤية هي أكثر حواسنا تطورا، لذلك فإنه ليس من المستغرب أن تكون الصور تلعب دور مهم في الإدراك البشري. التشخيص بمساعدة الحاسوب هو تطبيق آخر هام من تطبيقات علم التعرف على الأنماط، ويهدف هذا التطبيق إلى مساعدة الأطباء والعاملين في المجالات الطبية في اتخاذ القرارات التشخيصية. الحاجة إلى التشخيص بمساعدة الحاسوب تنبع من حقيقة أن البيانات الطبية غالبا ما تكون غير سهلة تفسير، حيث ان التفسير يمكن أن يعتمد إلى حد كبير على مهارات الطبيب.او العامل في مجال الصحة عموما.

الكثير من الأمراض التي ليست في الأصل أمراض دم تترك علامات على الدم. CBC (اختبار خلايا عد الدم) على سبيل المثال، لا يزال أول اختبار يتم طلبه من قبل الأطباء أو تصبح في أذهانهم. يمكن أن يكون الشذوذ في الدم إما في خلايا الدم البيضاء أو خلايا الدم الحمراء أو البلازما. في هذه الأطروحة خلايا الدم الحمراء هي المقترح للكشف عن علامات شذوذها. اختبار تعداد كريات الدم لا يمكن من خلاله بسهوله الكشف عن الخلل في إشكال كريات الدم الحمراء حيث أن جهاز تعداد كريات الدم يقوم بإعطاء عدد الكريات والنسب المئوية ولا يقدم وصفا لأشكال خلايا الدم، بما أن عدد خلايا الغير طبيعية للخلايا الطبيعية في عينة الدم تعطي مقياسا لشدة المرض، والكشف عن خلية واحدة مع شذوذ محتمل يمكن أن تعطي إنذار مبكر عن أمراض في المستقبل يمكن تجنبها أو علاجها في وقت مبكر. لا يمكن لمثل هذه الحالات كشفها مبكرا. مشاركة الكمبيوتر في هذه المهمة تساعد في تقليل الوقت والجهد بالإضافة إلى تقليل الأخطاء البشرية.

اسم هذه الرسالة هو **"تحديد أشكال خلايا الدم الحمراء المريضة باستخدام الجينات الخوارزمية والكي مينز"** في هذه الرسالة تم اقتراح نظام جديد.هذا النظام مقسم إلى أربع مراحل. المرحلة الأولى هي مرحلة تجميع البيانات حيث يتم سحب عينات دم من أشخاص أصحاء ومرضى ثم يتم عمل شرائح حافظه لعينات الدم تسمى البلد فلم ويتم معاينة هذه الشرائح تحت الميكروسكوب واخذ صور لها وهي تحت هذا الجهاز. المرحلة الثانية وهي مرحلة التجهيز او ما قبل المعالجة وفيها يتم إعداد الصور للمرحلة القادمة . أما المرحلة الثالثة فهي مرحلة استخراج الصفات الخاصة بكل خلية تحت الدراسة هذه الصفات بعضها في التايم دومين وبعضها في الفريكونسي دومين . المرحلة الرابعة و الأخيرة وهي مرحلة التصنيف وفيها يتم تغذيه المصنف بالصفات ليقوم بعمليه التصنيف. في هذه الرسالة تم الحصول على نتائج ممتازة حيث تحصلت الجينات الخوارزمية على ما نسبته 92.31% كنسبة نجاح بينما الكي مينز تحصل على ما نسبته 94% نسبة نجاح.

Abstract

Vision is the most advanced of our senses, so it is not surprising that images play the single most important role in human perception**.** *Computer-aided diagnosis* is another important application of pattern recognition, aiming at assisting doctors in making diagnostic decisions.

Many diseases which are not blood diseases in origin have hematological abnormalities and manifestation (have symptoms appeared on the blood). CBC (cell blood count test) for instance, is still the first test to be requested by the physicians or become in their mind. Blood abnormality can be in white blood cells, **red blood cells** and plasma. In this thesis, red blood cells are the suggested for detecting it is abnormality. The abnormality of blood cells shapes can't be detected easily, where the CBC (cell blood count) device give a count number and percentages not a description of the shapes of the blood cells, when the blood cells shapes wanted to be known, hematologist asked to view the blood films under the microscope which is time consuming task besides that the human error risk is high. Since the number of abnormal cells to normal cells in a given blood sample give a measure of the disease severity, detecting one cell with potential abnormality can give premature warning for future illness that can be avoided or treated earlier. This case can't be detected by hematologist. Computer involved in such task to save time and effort besides minimizing human error.

This thesis name is "**DETECTING RED BLOOD CELLS MORPHOLOGICAL ABNORMALITIES USING GENETIC ALGORITHM AND KMEANS**". In this thesis, the thesis divided into four phases. First phase data collection where blood samples was drawn from healthy and sick people and then blood films made and viewed under microscope and an images captured for these blood films. Second phase preprocessing phase where the images prepared for the next phase. Third phase feature extraction was executed where these features are spatial domain and frequency domain features. Fourth phase is the classification phase where the features fed into the classifier to be classified. An acceptable detection rate is achieved by the proposed system. The genetic algorithm classifier success rate was 92.31% and the K-means classifier success rate was 94.00%.

Chapter 1: Introduction

1.1 Preface

The abnormality of blood cells shapes can't be detected easily, where the traditional CBC (cell blood count) device gives count number and percentages not a description of the shapes of blood cells. The blood cells shapes examined by the hematologist viewed in the blood films under the microscope. This task is time consuming besides that human error risk is high. Since the number of abnormal cells to normal cells in a given blood sample give a measure of the disease severity, detecting one cell with potential abnormality can give pre-mature warning for future illness that can be avoided or treated earlier. This case can't be detected by hematologist. Computer involved in such task to save time and effort besides minimizing human error.

1.2 Topic Area

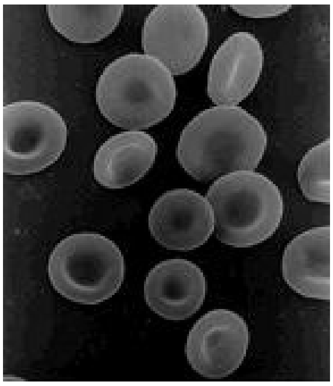


Figure.1.1 Human red blood cells (6-8µm)

Our bodies contain about 5 liters of blood (about 7% of our body). Of average 5 liters of blood, only 2.25 liters (45%) consist of cells [1]. The rest is plasma, which itself consist of 93% water (by weight) and 7% solids (mostly proteins, the greatest proportion of which is alburnin). Of the 2.25 liters of cells, only 0.037 liters (1.6%) are leukocytes (white blood cells). The total circulating platelet volume is even less, about 0.0065 liters or a little over teaspoon (although platelet count is more than leukocytes per cubic millimeter, but their size and volume are much less than leukocytes) [2,3]. The rest of volume is occupied by the **Red Blood Cells** check figure 1.1 to view normal red blood cells shape. The most important terminology in Red blood cells field is Hematology. Hematology is the science/medicine branch which is concerned in the study of blood and blood forming tissues [4] others says that **hematology** is the branch of internal medicine, physiology, pathology, clinical laboratory work, and pediatrics that is concerned with the study of blood, the blood-forming organs, and blood diseases [5]. Hematology includes the study of etiology, diagnosis, treatment, prognosis, and prevention of blood diseases. The laboratory work that goes into the study of blood is frequently performed by a medical technologist. Hematologist’s physicians also very frequently do further study in oncology - the medical treatment of cancer.

Hematologist's should be able precisely gives accurate laboratory results, which are used to diagnose various blood diseases. Blood diseases affect the production of blood and its components, such as blood cells, hemoglobin, blood proteins, the mechanism of coagulation, etc. Many other diseases which are not blood diseases in origin have hematological abnormalities and manifestation. CBC (cell blood count test) for instance, is still the first test to be requested by the physicians or become in their mind. Physicians specialized in hematology are known as hematologists. Their routine work mainly includes the care and treatment of patients with hematological diseases, although some may also work at the hematology laboratory viewing blood films and bone marrow slides under the microscope, interpreting various hematological test results. In some institutions, hematologists also manage the hematology laboratory. Physicians who work in hematology laboratories, and most commonly manage them, are pathologists specialized in the diagnosis of hematological diseases, referred to as **hematopathologists**. Hematologists and hematopathologists generally work in conjunction to formulate a diagnosis and deliver the most appropriate therapy if needed. Hematology is a distinct subspecialty of internal medicine, separate from but overlapping with the subspecialty of medical oncology. Only some blood disorders can be cured. **Red blood cells** (also referred to as **erythrocytes**) are the most common type of blood cell and the vertebrate orga0nism's principal means of delivering oxygen (O_2) to the body tissues via the blood flow through the circulatory system [4]. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries [4,5]. In humans, mature Red Blood Cells are flexible biconcave disks that lack a cell nucleus and most organelles. 2.4 million new erythrocytes are produced per second. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages.

Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells. Red blood cells are also known as **RBCs**, **Red Blood Corpuscles** (an archaic term), **haematids**, **erythroid cells** or **erythrocytes**.

1.3 Thesis Motivation

From the seventies, image synthesis has been undergoing a huge development with its own sub-domains, and obtained results with high visual quality as needed by the image and film industry. In parallel, efforts were made to make these techniques more affordable, using specialized architectures, simulators, and algorithmic research.

Detecting Red blood cells abnormalities using Genetic Algorithm and K-means are the intended topic for this thesis. Identifying specific organs or other features in medical images requires a considerable amount of expertise concerning the shapes and locations of anatomical features. Such segmentation is typically performed manually by expert physicians as part of treatment planning and diagnosis. Due to the increasing amount of available data and the complexity of features of interest, it is becoming essential to develop automated segmentation methods to assist and speed-up image-understanding tasks. Many other diseases which are not blood diseases in origin have hematological abnormalities and manifestation. Blood abnormality can be in white blood cells, **red blood cells** and plasma. In this thesis, red blood cells are suggested for detecting their abnormality.

The subject of using the images of blood films is not a very popular application. This application used in conservative manner and what has been done in this field only scratch the surface. For that and more this subject must be investigated thoroughly and intensively to paves the road for others. Several techniques used for segmenting the objects and for features extraction, Genetic algorithm or k-means tasks are to map these features to the proper case of abnormality. The aim of this thesis is to model this object (shape) and to identify it. Using evolutionary methods (GA) in model-based vision helps to extend the scope of machine vision itself. As image analysis can be defined as the task of rebuilding a model of reality from images taken by cameras, it may be interesting to quote work on the identification of mechanical models from image sequences.

The thesis goal is to develop an automated diagnosis system for detection RBC abnormalities, to obtain this system; the work is divided into four main phases: Data collection, image preprocessing, features extraction and selection/classification, which required broad knowledge in numerous disciplines, such as image processing, pattern recognition, database management, artificial intelligence, and medical practice.

1.4 Problem Definition

The thesis works with images with low resolution these images usually analyzed by human eyes that leaves quite roam for human errors. That is not the only problem where as stated in the section above such thesis idea requires broad knowledge in numerous disciplines and fields, not only this thesis works with medical conditions; the proposed techniques to be used are varied and diverse. All that gives huge number of possibilities and scenarios to be proposed and discussed leading to the best scenario. The previous studies take one side of the thesis but not inclusive as its, meaning image segmentation was applied for such an application as a research as itself where in this thesis such segmentation is merely a preprocessing stage leading to the actual core of this thesis which is the classification stage. Genetic algorithm used in lots of studies as a classifier and as clustering technique, while here is used for the first time as classifier of red blood cells such work is not applied before. As a sum up this thesis problem is to minimize human error besides time and effort.

1.5 Thesis Objective

The main objective of this thesis not just applying the suggested application and raising the bar on cells classification, was also introducing several ideas and techniques in features types that may be not very known or at least not investigated enough. This thesis gives an automatic system that uses an image as an input and gives a set of abnormal red blood cells with a classification of potential types of morphological abnormalities. The thesis goal is to develop a system for detection RBC abnormalities, to obtain this system; the work is divided into four main phases as mention before: data collection, image preprocessing, features extraction and selection/classification.

1.6 Thesis Contribution

This thesis consumes a large amount of time in exploring and surveying techniques to obtain a better result, that itself gives huge value for it. Since this thesis obviously compares techniques among each other and such comparison redirect the search logically. This thesis application is not a very common one. Other authors whom may try and explore this application only scratch the surface on such an important application, and what they done where merely segmenting or cluttering cells, overlapped from non overlapped cells and even such an implementation gives a success rate 95% which is also done in this thesis in the preprocessing phase but here it gives 100% success rate. This is not the only obtained result from the thesis where the genetic algorithm and the K-means used in the classifying phase and both of them gave a success rate exceeded 90%, GA and K-means gave 92.3% and 94% respectively, this result for a classifying a red blood cells using these techniques and others are never obtained before, that makes this thesis state of art search.

1.7 Thesis Methodology

In this thesis the Red blood cells (RBC) isolated (segmented) from other types of cells, each RBC cleaned and ready for the next phase which is the feature extraction phase, after that the final phase came which is the recognition phase. For decreasing the complexity of this thesis the RBC's will be either clustered into two categories (normal/abnormal cells) or to four categories (normal, Teardrop, Sickle and Burr cells). The result mention on this thesis (chapter 6) from using GA and K-means in clustering shapes into two classes (normal and abnormal) and from clustering the shapes into four classes (Normal shape, Teardrop shape, Sickle shape and Burr shape) the chosen shapes are the most common/popular shapes in the RBC morphological abnormalities that is why they were chosen.

The thesis goal as mention before is to develop a system for detection RBC abnormalities, the practical work divided into main four stages: data collection stage, image preprocessing stage, feature extraction stage and selection/classification stage.

*First Stage: D*ata collection stage consists of several procedures:

1. Blood drawing from several people (healthy and sick people).
2. Preparing blood films discussed more thoroughly in appendix B.
3. Preparing the microscope camera and the adapter to capture images of the blood films under the microscope.

Second Stage: Preprocessing Stage:

Red blood cells segmented and clustered away from other cells and then a cell chosen (suitable cells) to work with (for example edge cells or cells at the boundary that has missing parts not suitable to work with).

Third Stage: Feature Extraction Stage:

The cells features (set of features for each cell type: Normal shapes, Teardrop shapes, Sickle shapes and Barr shapes) are extracted. The thesis features are extremely divers', discussed in more details in chapter 5.

Fourth Stage: classification Stage:

In this thesis GA and k-means classification/clustering algorithm are used in the classification phase to determine if the patient has RBC abnormalities or not. Matlab software used to accomplish this task. Good specialized microscope camera is used attached with a microscope to capture the RBC blood films pictures.

1.8 Thesis Organization

The rest of the thesis is organized as follow chapter 2 contains the Literature Review, chapter 3 about human blood mostly Red Blood Cells (RBC), chapter 4 the Theoretical Background of all techniques used in this thesis, chapter 5 discuses the proposed system which is the thesis practical work. The thesis results are presented in chapter 6. Finally, chapter 7 discuses Conclusion with the Future work.

Chapter 2: Literature Review

This thesis has three main significant stages; each of these stages was a goal itself for some researchers, for example image segmentation especially elliptical or circular shapes has extensively applied and studied. Using K-means in image segmentation and clustering has its share of papers. Appling genetic algorithm in clustering also was been investigated intensively in several studies. Several studies used images of red blood cells and other types of cells, for many purposes especially segmentation. For that reason each of which mentioned has its own previous work. In the upcoming section papers that uses genetic algorithm in clustering will be discussed thoroughly. Since this application is the heart of this thesis, papers that used red blood cells as its own application will be discussed too.

2.1 Previous Work

Among researches that has common target application with this thesis, the following papers were worth mentioning, for example Object Localization in Medical Images using Genetic Algorithm [6] is a paper where the red blood cells clustered into two classes: overlapped and non overlapped cells where the proposed system success rate was 94%, this system need a large bunch of parameters to work properly that considered huge disadvantage, but in the next paper which is in On-line Detection of Red Blood Cell Shape using Deformable Templates [7] the red blood cells segmented away from other types of cells using deformable template model this paper give at least 95% success rates which is not the main goal in this thesis, the segmentation is merely a preprocessing step needed to be done before the classification phase began. Other papers like Partial Shape Matching using Genetic Algorithms [8] recognize the red blood cells from other types of cells (ex white blood cells) using genetic algorithm using only two types of features line segments and angle of the line segment where worst case scenarios gives 94% success rates, its considered a very good paper in spits the features simplicity.

For papers that discuses shapes localization in general which is a generalized case of our thesis we have here an algorithm [9] based on the generalized Hough transform (GHT), is presented in order to calculate the orientation, scale, and displacement of an image shape with respect to a template. According to the authors [9] two new methods to detect objects under perspective and scaled orthographic projection are shown. The author also claimed they calculate the parameters of the transformations the object has undergone. The methods are based on the use of the Generalized Hough Transform (GHT) that compares a template with a projected image. This method needs a template of the object to be located which is considered some time hard in our application. Hough transform used by various ways for edge detection and shape modeling [9- 11]. and so GA in image analysis, in this thesis GA will be used on molding an object and identification it, where a fast automatic system for detecting RBC's abnormality will be created, after that the abnormalities are calculated where not every abnormality will be a cause for a red flag, if this abnormality features exceeded certain threshold then we can say safely that this blood film has something need to be look out. The threshold value with more practice will present itself as the best state during this study. This system will allow monitoring and observation during the execution of object identification where it will be used to model different shapes that it may even be hard by ordinary people to recognize and to detect because the huge number of cells per blood film along with the existences of other types of cells. These cells can be overlapped or with low resolution. So many obstacles may exist and will be solved during this study. Other techniques used in similar situation proven their ability, but in the other hand there complexity and time consuming make them less interesting. GA can model any complex model easily.

Segmentation of medical images is challenging due to poor image contrast and artifacts that result in missing or diffuse cells/tissue boundaries. Consequently, this task involves incorporating as much prior information as possible (e.g., texture, shape, and spatial location of organs) into a single framework. A GA [12] presented for automating the segmentation of the prostate on two-dimensional slices of pelvic computed tomography (CT) images. According to the authors [12] the approach is curve segmenting represented using a level set function, which is evolved using a Genetic Algorithm (GA). Shape and textural priors derived from manually segmented images are used to constrain the evolution of the segmenting curve over successive generations in the downside of that search is the time consumed in the operation which make them practically undesired in automatic diagnostic.

2.2 Research Issues

Proposed pre processing techniques used in this thesis are extremely divers. In some stages K-Means used in segmenting the images, image morphological operation where also involved. Using K-Means in image segmentation is not a new topic where it was been used in [13] and in [14] the concept itself its widely popular where clustering and segmentation are in some application gives the same meaning.

Also in the preprocessing phase segmentation and localization of the red blood cells was needed, in the following research Object Detection using Circular Hough Transform [15] the proposed system first uses the separability filter proposed by Fukui and Yamaguchi [16] to obtain the best object candidates and next, the system uses the circular Hough transform (CHT) to detect the presence of circular shape. The main contribution of this work according to the authors is consists of using together two different techniques in order to take advantages from the peculiarity of each of them The highest success rate of the proposed system to detect the objects was 96% and the worst success rate was 80%, keep in mind this system has no noise resistance.

This thesis discusses several techniques for digital image features extraction. Medical imaging is performed in various strategies, automated methods have been developed to process the acquired images and identify features of interest [17], including intensity-based methods, region-growing methods and deformable contour models. Due to the low contrast information in medical images, an effective segmentation often requires extraction of a combination of features such as shape and texture or pixel intensity and shape, although the feature discuss here are extracted from binary and grey level images, some features are in spatial domain and other in frequency domain all that discussed latter in the thesis.

The thesis main classifiers are the Genetic Algorithms (GA) and the K-Means. GA provides a learning method motivated by an analogy to biological evolution. GA's generate successor solution for a problem by repeatedly mutating and recombining parts of the best currently known solution [18] .At each step, a collection of solutions called the current population is updated by replacing some fraction of the population by offspring of the fit current solutions. The process gives a generate-and-test beam-search of solutions, in which is different of the best current solutions that are most likely to be considered next. Genetic Algorithms (GA) simulate the learning process of biological evolution using selection, crossover and mutation [19]. The problem addressed by GA's is to search a space of candidate solutions to identify the best solutions [18]. In GA's the "best solutions" is defined as the one that optimizes a predefined numerical measure for the problem at hand. Genetic algorithms are blind optimization techniques that do not need derivatives to guide the search towards better solutions. This quality makes GA's more robust than other local search procedures such as gradient descent or greedy techniques like combinatorial optimization [20] GA's have been used for a variety of image processing applications, such as edge detection [21], image segmentation [22]; image compression [23], feature extraction from remotely sensed images [24] and medical feature extraction [25]. .Genetic algorithms have been used for segmentation by [26- 29].A general-purpose image-segmentation system called GENIE ("Genetic Imagery Exploration") [29,30] GA used in a medical feature-extraction problem using multi-spectral histopathology images [29] Their specific aim was to identify cancerous cells on images of breast cancer tissue. Their method was able to discriminate between benign and malignant cells from a variety of samples. GA is very important algorithm proven it is reliability and efficiency over allots of other algorithms and worth trying and testing in my study. For example it is used for instance in feature extraction according to [31] in image segmentation [32-36] for adaptive image segmentation [37] and for pattern recognition in here [38-41]; it is variant flexibility among other algorithm make it very appealing to be used here.

In this thesis GA used in clustering the data sets of features in interests and this is not the first time that GA used in clustering data sets where in the paper An Efficient GA-based Clustering Technique [42] the propose system is a GA-based unsupervised clustering technique that selects cluster centers directly from the data set, allowing it to speed up the fitness evaluation by constructing a look-up table in advance, saving the distances between all pairs of data points, and by using binary representation rather than string representation to encode a variable number of cluster centers. More effective versions of operators for reproduction, crossover, and mutation are introduced. The development of this algorithm according to the authors has demonstrated an ability to properly cluster a variety of data sets. The experimental results according to the authors show that the proposed algorithm provides a more stable clustering performance in terms of number of clusters and clustering results.

Also GA algorithm used in another paper called Incremental Clustering in Data Mining using Genetic Algorithm [43] this paper according to the authors presents new approach/algorithm based on Genetic algorithm. This algorithm is applicable to any database containing data from a metric space, e.g., to a spatial database. Based on the formal definition of clusters, it can be proven that the incremental algorithm yields the same result as any other algorithm. A performance evaluation of algorithm Incremental Clustering using Genetic Algorithm (ICGA) on a spatial database is presented, demonstrating the efficiency of the proposed algorithm. ICGA yields significant speed-up factors over other clustering algorithms. All these papers used GA in clustering alone and tried to enhance its performance by using different approach and may be add steps before or after, but there is other papers that's tried to enhance GA by concatenating it with another classifier, our thesis do that by applying K-Means before GA starts in attempts to give the initial population to the GA instead of random values, this not the first time GA used with K-Means where it's used in paper called Genetic K-Means Algorithm [44] where the authors proposed a hybrid genetic algorithm (GA) that finds a globally optimal partition of a given data into a specified number of clusters. The hybridize GA with a classical gradient descent algorithm used in clustering viz., K-Means algorithm. Hence, the name genetic K-Means algorithm (GKA). K-means operator defined according to the authors as one-step of K-Means algorithm, and uses it in GKA as a search operator instead of crossover. They also define a biased mutation operator specific to clustering called distance-based-mutation. Using finite Markov chain theory, this GKA converges to the global optimum. It is observed in the simulations that GKA converges to the best known optimum corresponding to the given data in concurrence with the convergence result. It is also observed that GKA searches faster than some of the other evolutionary algorithms used for clustering. Another paper called Genetic K-Means Clustering Algorithm for Mixed Numeric and Categorical Data Sets [45] uses GA with K-Means where the author propose a modified description of cluster center to overcome the numeric data only limitation of Genetic k-mean algorithm and provide a better characterization of clusters. The performance of this algorithm gives a success rate with 100% but with higher number of generations.

system where time plays a critical role.

The following book [46] discuss many interesting topics that was most useful for this thesis, the book gave a survey of existing approaches of shape-based feature extraction and the efficient shape features must present some essential discussed in chapter 4 thoroughly.

Chapter 3: Hematology

3.1. Introduction

Hematology is the science/medicine branch which is concerned in the study of blood and blood forming tissues [47]. Hematology laboratory should be able to supply accurate and precise laboratory results, which are used to differentiate, correlate, and diagnose various blood diseases. Many other diseases which are not blood diseases in origin have hematological abnormalities and manifestation. CBC for instance, is still the first test to be requested by the physicians or become in their mind. The ability to count, size, and classify cells is common to all state-of-art hematology analyzers [48]. Available instruments use different analytical technologies to perform complete blood counts (CBC) and leukocyte differentials. The basic complete blood counts is performed either by measurement of electrical impedance or by measurement of light scatter. These methods provide the conventional RBC, WBC and platelet count, RBC indices and new parameter such as the platelet distribution width (PDW) and hemoglobin distribution width (HDW), which is just beginning to find acceptance in clinical management. The blood consists of plasma platelets, and The Hematic Cells (Red Blood Cells).

3.2. Red Blood Cells (RBC)

Here we discuss RBC color, RBC creation, RBC traffic, RBC counting, RBC size and abnormal RB's in more details.

3.2.1 RBC color

Red blood cells appear red because they consist of a chemical protein called hemoglobin, which is red in color, which makes the red blood cells appear red, and in turn the blood of an animal appears red [49]. If we go into more details we would find out that hemoglobin is the protein which not only imparts the color but it is the protein that helps in carrying oxygen to and from the organs to the heart and vice versa. The oxygen molecules in our heart attach themselves to the hemoglobin and thus are transported to the various organs of the body and then when the hemoglobin has given away all the oxygen particles, the empty hemoglobin particle is taken over by carbon dioxide on its way back to the heart.

3.2.2 The Creation of RBC's

The diameter of a red blood cell is approximately 6 to 8 microns and its thickness is about 1.5 to 1.9 microns [50].The production rate of red blood cells per day is approximately 200 billion and the life span of a red blood cell is approximately 120 to 125 days. The count of red blood cells varies with the sex of the individual. In case of a woman the average count of red blood cell is 4.2 to 6.1 million cells per microliter and in case of men it is approximately 4.7 to 6.1 million cells per microliter. Red blood cells are much smaller than the other cells in the human body and each red blood cell contains approximately 270 million hemoglobin molecules and each carries four groups of heme. Erthropoiesis is the process of creation of red blood cells in human body in which red blood cells are constantly produced in the red bone marrow of large bones and the rate of production per second is approximately 2 million. After the red blood cells are released by the bone marrow in the blood, these cells are known as reticulocytes, which have approximately one percent of circulating blood cells. After the process of creation of red blood cells they continue to mature and as they mature their plasma membrane keeps on undergoing change so that the phagocytes can identify the worn out red blood cell, which would result into phagocytosis. The hemoglobin particles are further broken down to iron and biliverdin. The latter change into bilirubin, which along with the iron particle is released into the plasma and the iron particle, is again circulated with the help of a carrier protein, called transferring. Thus the life cycle of a red blood cell comes to an end by approximately 120 days.

3.2.3 RBC traffic

Transit of elytroid cells through the circulation has been measured precisely using chromium-51-labeld cells [48]. In the adult human, the half-life for a circulating red cell is 120 days. Calculation based on the mitotic indices of red cell precursors in the marrow, the total red cells, and the life span; indicate that there are about 5×10^9red cell precursors/kg of body weight, or in a 70-kg human, 3.5 $\times 10^9$. From the total number of red cells and the circulatory half-life, it can be estimated that the marrow is required to produce 2.1 × 10^9 red cells per day. Given the number of precursors available per day, one can see that there is a minimal reserve of red cells available under normal conditions; that us to say, the daily output of the marrow barely matches the need for red cells. It has been suggested that there is a small reserve of reticulocytes, which can be mobilized upon demand that may take up the short fall in nucleated erythroid precursor.

3.2.5 RBC Counting

The reference method for red blood cell enumeration involves the use of a diluting red cells pipette and the microscopic enumeration of erythrocytes in the hemocytometer loaded with a 1:200 dilution of blood in an isotonic diluting fluid [48]. The number of cells counted within designated red cell areas of the hemocytometer (five center boxes each consisting of 16 small squares) is multiplied by the dilution factors as well as a constant that reflects the volume actually counted (0.02 mm^3). The result is expressed is number per l. thus, if 500 cells were counted, then the number of red cells per l would be:

$$500/0.02 \text{ x } 200 = 5{,}000{,}000/mm^3.$$

The method has two major limitations, namely, variation in the dilution between specimens and the relatively small number of cells actually counted. Both serve to magnify errors because if the large multiplication factors used in the final arithmetic conversions. A coefficient of variation (CV) of + 5% to 10% is common in routine clinical practice.

Red Blood Cell Count

Most RBC counts are performed using automated analyzers that enumerate the RBC count using light scatter or aperture impedance technology [48]. The replicate error is less than 3%, but is also subject to artifacts. Three distinct phenomena cause spurious red cell counts:

3.2.6 RBC Size

Each size of the red blood cells has different name according to [51] Normal size cells are called normocytic, size (6 – 8 micron).Smaller size cells are called microcytic, size (<6 micron). Larger size cells are called macrocytic, size (>8 micron).

3.2.7 RBC Morphologic Abnormalities

The relation between the red blood indices and the diseases discussed in more details in [52]. The table in Appendix B gives details according to [52] of the connection between the red blood cells abnormality shapes and the diseases that related to it.

3.3 Blood Smear

Blood film or peripheral blood smear is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically [52]. Blood films are usually examined to investigate hematological problems (disorders of the blood) and, occasionally, to look for parasites within the blood such as malaria and filarial.

3.3.1 Stains

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image [52] Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells.

In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound. Staining and fluorescent tagging can serve similar purposes. Biological staining is also used to mark cells in flow cytometry, and to flag proteins or nucleic acids in gel electrophoresis. There are several kinds of stains check the Appendix C for it.

3.3.2 Blood Films preparation

Blood films are made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length [52] The aim is to get a region where the cells are spaced far enough apart to be counted and differentiated. The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. The fixative is essential for good staining and presentation of cellular detail. After fixation, the slide is stained to distinguish the cells from each other. Check Appendix B for more.

Chapter 4: Theoertial Background

4.1 Introduction

In this chapter the basic theory of the thesis discussed, where the thesis is one application from many applications of pattern recognition and machine vision.

Machine vision is extremely interesting area. A machine vision according to [53] is a system captures images by a camera and then analyzes them to produce descriptions of what is captured, so under this definition this thesis is classified as a machine vision system. *Computer-aided diagnosis* is popular implementation of pattern recognition which means assisting doctors in making diagnostic decisions. The final diagnosis will lie in the doctor's hands. Computer-assisted diagnosis has been applied to wide range of medical data, be aware that the thesis is not self diagnostic, just narrow down the possibilities. This thesis classifies objects (in images) into classes. This thesis works with images at the preprocessing phase, once when these images at RGB space and other time at LAB space (at segmentation stage), for that we need to know more about these two spaces:

LAB Space

LAB* color space** is a color-opponent space with dimension ***L for lightness and ***A*** and ***B*** for the color-opponent dimensions, a space which can be computed via simple formulas from the *XYZ* space, but is more perceptually uniform than *XYZ*. *Perceptually uniform* means that a change of the same amount in a color value should produce a change of about the same visual importance. When storing colors in limited precision values.

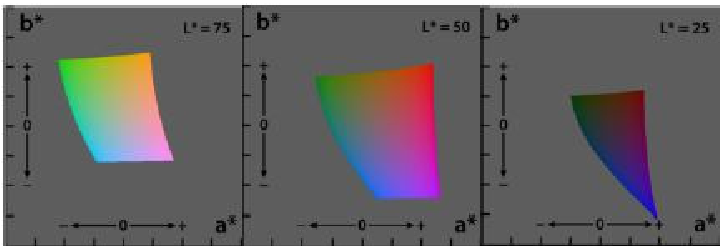


Figure 4.1 LAB Space

Unlike the RGB, LAB color in figure 4.1 is designed to approximate human vision. It aspires to perceptual uniformity, and its L component closely matches human perception of lightness. It can thus be used to make accurate color balance corrections by modifying output curves in the A and B components, or to adjust the lightness contrast using the L component which model the output of physical devices rather than human visual perception, these transformations can only be done with the help of appropriate blend modes in the editing application.

RGB Space

The **RGB color model** is an additive color model in which red, green, and blue light are added together in various ways to reproduce a broad array of colors. The name of the model comes from the initials of the three additive primary colors, red, green, and blue. The main purpose of the RGB color model is for the sensing, representation, and display of images in electronic systems, such as televisions and computers, though it has also been used in conventional photography. Before the electronic age, the RGB color model in figure 4.2 already had a solid theory behind it, based in human perception of colors.

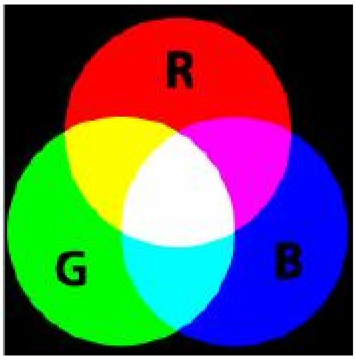


Figure 4.2 RGB Space.

4.2 Digital Image Morphological

The morphology commonly means a branch of biology that deals with the form and structure of animales and plants [54]. Here this word is used to indicate a matmatical morphology as a tool for extracting image components that are useful in the represntation and description of shapes, such as boundries, skeletons and convex hull. We are interseted also in morphological techniques for pre-processing, such as morphological filtering, thining, and pruning. Mathmatical morphology means a set of theores [55]. As such, morphology offers a unified and powerful concpts to numerous image processing problems. Sets in mathmetical morphology represnt objects in an image. Morphology is a wide set of image processing operations that process images based on shapes. Morphological operations apply a structuring element to an input image, creating an output image of the same size. In a morphological operation, the value of each pixel in the output image is based on a comparison of the corresponding pixel in the input image with its neighbors. By choosing the size and shape of the neighborhood, you can construct a morphological operation that is sensitive to specific shapes in the input image. Mathematical morphology provides a number of important image processing operations, including erosion, dilation, opening and closing. These morphological operators take two pieces of data as input. One is the input image; the other is the structuring element. That determines the particular details of the effect of the operator on the image. The structuring element consists of a pattern determined as the coordinates of a number of discrete points relative to some origin [56]. Normally Cartesian coordinates are used and so a suitable way of representing the element is as a small image on a rectangular grid. Basic image morphological according to the author in [54] are:

1. **Logical operation**

The basic logic operation used in image processing are AND, OR and NOT (complemnt) will be discuessed berfly here. Their properties are summarized in table 4.1. these operations are functionally complete meaning they can be compined to form other operations. Logic operation are performed on a pixel by pixle basis between corresponding pixles of two or more images (except NOT, which operates on the pixels of a single image).

Table 4.1 Logic operations.

A	B	A AND B :(A·B)	A OR B :(A + B)	NOT A
0	0	0	0	1
0	1	0	1	1
1	0	0	1	0
1	1	1	1	0

2. **Dilation and Erosion**

The most vital morphological operations are dilation and erosion. Dilation adds pixels to the boundaries of objects in an image, while erosion is the opposite where it removes pixels on object boundaries. The numbers of pixels that added or removed depend on the size and shape of the structuring element used to process the image. In the morphological dilation and erosion operations, the position of any given pixel in the output image is determined by applying a rule to the corresponding pixel and its neighbors in the input image. These operations are elementary to morphological processing. In fact, many of the morphological algorithms discussed here:

Dilation: With A and B as sets in Z^2, the dilation of A by B, denoted $\boldsymbol{A \oplus B}$**.** The dilation of A by B is the set of all displacements, *z,* such that B and A overlap by at least one element. One of the simplest applications of dilation is for bridging gaps.

Erosion: For sets A and B in Z^2 the erosion of A by B, denoted $\boldsymbol{A \ominus B}$, in words the erosion of A by B is the set of all points *z* such that *B,* translated by *z,* is contained in $\boldsymbol{A}$.

3. Opening and Closing

As we have seen, dilation expands an image and erosion shrinks it. Here we discuss two other important morphological operations: opening and closing. Opening generally smoothes the contour of an object, breaks narrow isthmuses, and eliminates thin protrusions. Closing also tends to smooth sections of contours but, as opposed to opening, it generally fuses narrow breaks and long thin gulfs, eliminates small holes, and fills gaps in the contour.

The opening of set A by structuring element B, denoted A ◦ B, is defined as

$$\mathbf{A \circ B = (A \ominus B) \oplus B.} \qquad \mathbf{4.1}$$

Thus, the opening A by B is the erosion of A by B, followed by a dilation of the result by B Similarly, the closing of set A by structuring element B, denoted A • B, is defined as

$$\mathbf{A \bullet B = (A \oplus B) \ominus B.} \qquad \mathbf{4.2}$$

4. The Hit-or-Miss Transformation

The morphological hit-or-miss transform is a basic tool for shape detection. The objective is to find the location of one of the shapes,

$$\mathbf{A \circledast B = (A \ominus \boldsymbol{B_1}) \cap (\boldsymbol{A^c} \ominus \boldsymbol{B_2})} \qquad \mathbf{4.3}$$

$B = (\boldsymbol{B_1}, \boldsymbol{B_2})$, where$\boldsymbol{B_1}$, is the set formed from elements of B associated with an object and $\boldsymbol{B_2}$ is the set of elements of B associated with the corresponding background. Thus, set A ✺ B contains all the (origin) points at which, simultaneously, $\boldsymbol{B_1}$ found a match ("hit") in A and $\boldsymbol{B_2}$ found a match inA^c. The reason for using a structuring element B_1 associated with objects and an element B_2 associated with the background is based on an assumed definition that two or more objects are distinct only if they form disjoint (disconnected) sets. This is guaranteed by requiring that each object have at least a one-pixel-thick background around it. In some applications, we may be interested in detecting certain patterns (combinations) of l's and O's within a set, in which case a background is not required. In such an instance, the hit-or-miss transform reduces to simple erosion.

5. Convex Hull

A set A is said to be convex if the straight line segment joining any two points in A lies entirely within A. The convex hull H of an arbitrary set S is the smallest convex set containing S which is useful for object description.

6. Thinning

The thinning of a set A by a structuring element B, denoted $A \otimes B$, can be defined in terms of the hit-or-miss transform:

$$A \otimes B = A - (A ✺ B) \qquad \textbf{4.4}$$

Is used to remove selected foreground pixels from binary images.

7. Thickening

Thickening is the morphological dual of thinning and is defined by the expression

$$A \odot B = A \text{ U } (A ✺ B) \qquad \textbf{4.5}$$

Thickening is a morphological operation that is used to grow selected regions of foreground pixels in binary images,

8. Skeletons

Morphological skeleton is a skeleton (or medial axis) representation of a shape or binary image, computed by means of morphological operators. The skeleton usually emphasizes geometrical and topological properties of the shape, such as its connectivity, topology, length, direction, and width. Together with the distance of its points to the shape boundary, the skeleton can also serve as a representation of the shape (they contain all the information necessary to reconstruct the shape.

Morphological skeletons are of two kinds:

- Those defined and by means of morphological openings, from which the original shape can be reconstructed.
- Those computed by means of the hit-or-miss transform, which preserve the shape's topology.

9. Pruning

Pruning methods are an essential complement to thinning and skeleton zing algorithms because these procedures tend to leave parasitic components that need to be "cleaned up" by post-processing.

10. Watershed

The notion of watersheds is based on visualizing an image in three dimensions: two spatial coordinates versus gray levels. In such a "topographic" elucidation, we consider three types of points: (a) points belonging to a regional minimum; (b) points at which a drop of water, if placed at the location of any of those points, would fall with certainty to a single minimum; and (c) points at which water would be equally likely to fall to more than one such minimum.

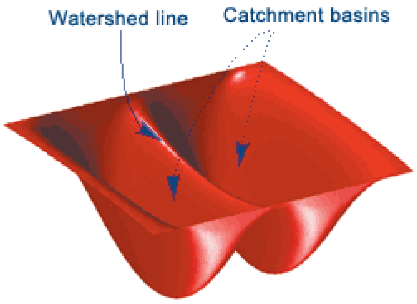


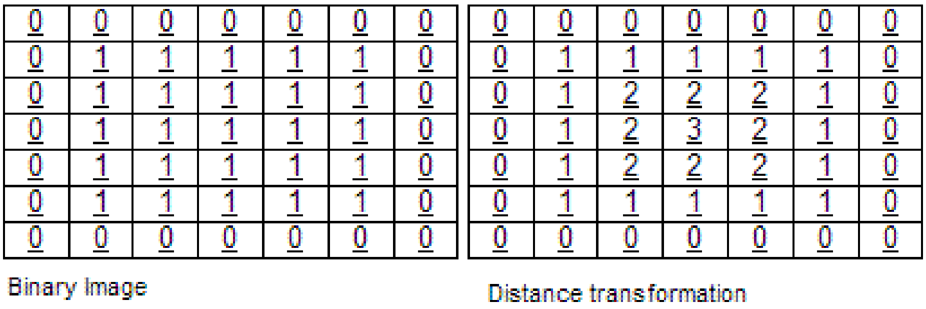
Figure 4.3 Watershed line

For a particular regional minimum, the set of points satisfying condition (b) is called the catchment basin or watershed of that minimum. The points satisfying condition (c) form peak lines on the topographic surface and are termed divide lines or watershed lines. The principal objective of segmentation algorithms based on these concepts is to find the watershed lines check figure 4.3. The basic idea is simple:

Suppose that a hole is punched in each regional minimum and that the entire topography is flooded from below by letting water rise through the holes at a uniform rate. When the Segmentation rising water in distinct catchment basins is about to merge, a dam is built to prevent the merging flooding will eventually reach a stage when only the tops of the dams are visible above the water line. These dam boundaries correspond to the divide lines of the watersheds. Therefore, they are the (continuous) boundaries extracted by a watershed segmentation algorithm. This segmentation based on three principal concepts: (a) detection of discontinuities, (b) thresholding, and (c) region processing. Segmentation by watersheds embodies many of the concepts of the three approaches and, as such, often produces more stable segmentation results, including continuous segmentation boundaries. This approach also provides a simple framework for incorporating knowledge-based constraints.

4.3 Distance Transformer

A distance transform, also known as distance map or distance field, is a derived representation of a digital image. The choice of the term depends on the point of view on the object in question: whether the initial image is transformed into another representation, or it is simply endowed with an additional map or field. The map labels each pixel of the image with the distance to the nearest obstacle pixel. A most common type of obstacle pixel is a boundary pixel in a binary image. See the figure 4.4 for an example of a chessboard distance transform on a binary image.



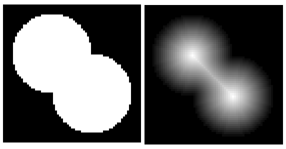
0	0	0	0	0	0	0
0	1	1	1	1	1	0
0	1	1	1	1	1	0
0	1	1	1	1	1	0
0	1	1	1	1	1	0
0	1	1	1	1	1	0
0	0	0	0	0	0	0

Binary Image

0	0	0	0	0	0	0
0	1	1	1	1	1	0
0	1	2	2	2	1	0
0	1	2	3	2	1	0
0	1	2	2	2	1	0
0	1	1	1	1	1	0
0	0	0	0	0	0	0

Distance transformation

Figure 4.4 Distance transformation



(a) (b)

Figure 4.5. Example of applying distance transformation on binary image (a) Binary Image, (b) Distance Transformation of the binary image.

4.4 Hough Transform

The Hough transform is a technique which can be used to isolate features of a particular shape within an image. Because it requires that the desired features be specified in some parametric form, the *classical* Hough transform is most commonly used for the detection of regular curves such as lines, circles, ellipses, *etc.* Due to the computational complexity of the generalized Hough algorithm, The main advantage of the Hough transform technique is that it is tolerant of gaps in feature boundary descriptions and is relatively unaffected by image noise.

4.5 Features Types

As stated before since this thesis classified under this application (improvement of graphic information for human understanding) and since in the mid-level process, which is a description of the objects to reduce them to a form suitable for computer processing, classification and recognition). In this thesis we deal with shape-based recognition so the object description (shape-based features) must present some essential properties according to the authors of [46] such as:

• Identifiability: shapes which are found perceptually similar by human have the same features that are different from the others.

• Translation, rotation and scale invariance: the location, the rotation and the scaling changing of the shape must not affect the extracted features.

• Affine invariance: the affine transform performs a linear mapping from coordinates system to other coordinates system that preserves the "straightness" and "parallelism" of lines. Affine transform can be constructed using sequences of translations, scales, flips, rotations and shears. The extracted features must be as invariant as possible with affine transforms.

- noise resistance: features must be as robust as possible against noise, i.e., they must be the same whichever be the strength of the noise in a give range that affects the pattern occultation invariance: when some parts of a shape are occulted by other objects, the feature of the remaining part must not change compared to the original shape.

- Statistically independent: two features must be statistically independent. This represents compactness of the representation.

- Reliability: as long as one deals with the same pattern, the extracted features must remain the same.

Types of features used in this thesis classified into time domain and frequency domain features, these features defined according to [46] are:

4.5.1 Spatial Domain Features

4.5.1.1 Shape descriptor

In general, shape descriptor is a set of numbers that are produced to represent a given shape feature. A descriptor attempts to quantify the shape in ways that agree with human intuition (or task-specific requirements). Good retrieval accuracy requires a shape descriptor to be able to effectively find perceptually similar shapes from a database. Usually, the descriptors are in the form of a vector. Shape descriptors should meet the following requirements:

- The descriptors should be as complete as possible to represent the content of the information items.

- The descriptors should be represented and stored compactly. The size of a descriptor vector must not be too large.

- The computation of the similarity or the distance between descriptors should be simple; otherwise the execution time would be too long. Shape feature extraction and representation plays an important role in the following categories of applications:

• Shape retrieval: searching for all shapes in a typically large database of shapes that are similar to a query shape. Usually all shapes within a given distance from the query are determined or the first few shapes that have the smallest distance.

• Shape recognition and classification: determining whether a given shape matches a model sufficiently or which of representative class is the most similar.

Shape disruptors more commonly used in image retrieval but in this thesis we used the shape descriptors as one of the set features used in classifying these objects.

Some of the shape descriptors are:

1- Solidity: Scalar specifying the proportion of the pixels in the convex hull that are also in the region. Computed as

$$\frac{Area}{Convex\ Area} \qquad \textbf{4.6}$$

2- 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. (0 and 1 are degenerate cases; an ellipse whose eccentricity is 0 is actually a circle, while an ellipse whose eccentricity is 1 is a line segment.)
Eccentricity's the measure of aspect ratio. It is the ratio of the length of major axis to the length of minor axis. It can be calculated by principal axes method or minimum bounding rectangle method.

3- Circularity ratio represents how much a shape is similar to a circle. There are 3 definitions:
Circularity ratio is the ratio of the area of a shape to the area of a circle having the same perimeter.

$$\mathbf{C1}=\frac{As}{Ac} \qquad \textbf{4.7}$$

Where *As* is the area of the shape and Ac is the area of the circle having the same perimeter as the shape. Assume the perimeter is O, so $As=O^2/4\pi$. Then

$$\mathbf{C1=4\pi.As} = O^2 \qquad \textbf{4.8}$$

As 4π is a constant, we have the second circularity ratio definition.

Circularity ratio is the ratio of the area of a shape to the shapes perimeter square:

$$\mathbf{C2}=\frac{As}{O^2} \qquad \mathbf{4.9}$$

Circularity ratio is also called circle variance and defined as:

$$\mathbf{C3}=\frac{\delta R}{\mu R} \qquad \mathbf{4.10}$$

Where μR and δR are the mean and standard deviation of the radial distance from the centroid (g_x, g_y) of the shape to the boundary points (x_i,y_i), i € [0, Nb-1]. They are the following:

$$\mu R= \frac{1}{N}\sum_{i=1}^{N-i} di \text{ and } \delta R = \sqrt{\frac{1}{N}\sum_{i=1}^{N-i}(di-\mu R)^2} \qquad \mathbf{4.11}$$

Where $$di = \sqrt{(x_i-g_x)^2+(y_i-g_y)^2} \qquad \mathbf{4.12}$$

4- Rectangulrity: the Area of the shape divided by the area of the minimum bounding box Rectangularity represents how rectangular a shape is, i.e how much it fills its minimum bounding rectangle:

$$\mathbf{R}=\frac{As}{AR} \qquad \mathbf{4.13}$$

Where *As* is the shape area, *AR* is the area of the minimum bounding rectangle.

5- Convexity: is defined as the ratio of perimeters of the convex hull $O_{ConvexHull}$ over that of the original contour O:

$$\mathbf{Convexity} = \frac{O_{ConvexHull}}{O} \qquad \mathbf{4.14}$$

The Region R^2 is convex if and only if for any two points P_1, P_2 €R^2, the entire line segmentP_1, P_2 is inside the region. The convex hull of a region is the smallest convex region including it.

6- EllipseRatio: with knowing the next two concepts:

A- Major Axis Length: Scalar specifying the length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.

B- MinorAxis Length: Scalar; the length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.

Calculating the area of an ellipse knowing the major axis length and the minor axis we get *Ae*.

Ellipse Ratio is:

$$\frac{\boldsymbol{As}}{\boldsymbol{Ae}} \qquad \mathbf{4.15}$$

Where *As* is the shape area and *Ae* ellipse area with the same minor axis and major axis of the shape.

4.5.1.2 Shape Signatures

First: Centroid distance function

The centroid distance function is expressed by the distance of the boundary points from the centroid (*gx, gy*) of a shape [56]:

$$\mathbf{r(n)} = [(\boldsymbol{x}(\boldsymbol{nb}) - \ \boldsymbol{gx})^2 + (\boldsymbol{y}(\boldsymbol{nb}) - \boldsymbol{gy})^2]^{\frac{1}{2}} \qquad \mathbf{4.16}$$

Due to the subtraction of the centroid, which represents the position of the shape, from the boundary coordinates check figure 4.6, both complex coordinates and centroid distance representation are invariant to translation.

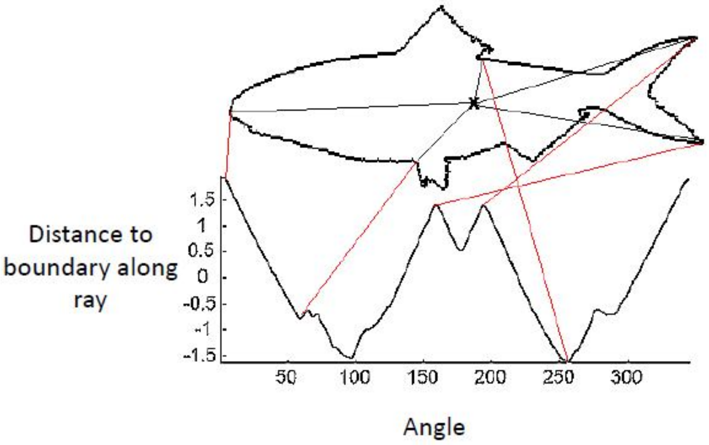


Figure 4.6 CDF (Centroid Distance Function).

Second: Triangle-area representation

The triangle-area representation (TAR) signature is computed from the area of the triangles formed by the points on the shape boundary [57,58].. The curvature at the contour point (x_{nb}, y_{nb}) is measured using the *TAR* as follows. For each three points $P_{nb-ts}(x_{nb-ts}, y_{nb-ts})$, $P_{nb}(x_{nb}, y_{nb})$ and $P_{nb+ts}(x_{nb+ts}, y_{nb+ts})$, where *nb* ∈ [1, *N*b] and *ts* ∈ [1, *Nb*/2 - 1], *Nb* is assumed to be even. The signed area of the triangle formed by these points is given by:

$$\textbf{TAR}\ (\boldsymbol{nb, ts}) = \frac{1}{2}\begin{bmatrix} x_{nb-ts} & y_{nb-ts} & 1 \\ x_{nb} & y_{nb} & 1 \\ x_{nb+ts} & y_{nb+ts} & 1 \end{bmatrix} \qquad \textbf{4.17}$$

When the contour is traversed in counter clockwise direction, positive, negative and zero values of TAR mean convex, concave and straight-line points, respectively. Figure 4.7 demonstrates these three types of the triangle areas and the complete TAR signature for the hammer shape.

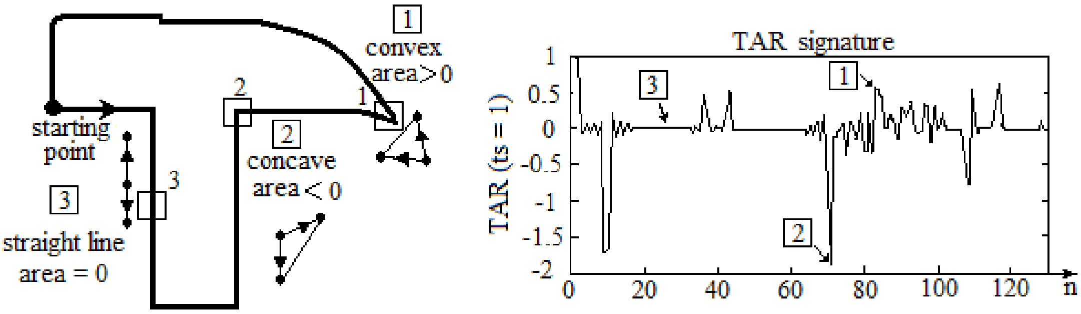
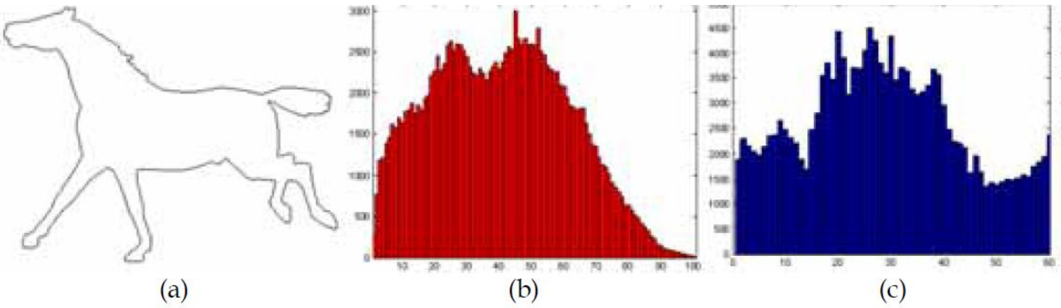


Figure 4.7 TAR (Triangular Area Representation).

Third: Chord distribution



(a) (b) (c)

Figure 4.8 Chord distribution (a) Orginal contour ;(b) chord lengths histogram;(c) chord angles histogram (each stem covers 3 degrees).

The basic idea of chord distribution is to calculate the lengths of all chords in the shape (all pair-wise distances between boundary points) and to build a histogram of their lengths and orientations [59]. The “lengths” histogram is invariant to rotation and scales linearly with the size of the object. The “angles” histogram is invariant to object size and shifts relative to object rotation. Figure 4.8 gives an example of chord distribution.

4.5.2 Frequency Domain Features (Wavelet)

The only frequency domain features used and discussed in this thesis is the wavelet transformation.

Wavelet Transform

A hierarchical planar curve descriptor is developed by using the wavelet transform [60]. This descriptor decomposes a curve into components of different scales so that the coarsest scale components carry the global approximation information while the finer scale components contain the local detailed information. The wavelet descriptor has many desirable properties such as multi-resolution representation, invariance, uniqueness, stability, and spatial localization. In [61] the authors use dyadic wavelet transform deriving an affine invariant function. A wavelet is an elementary function (check figure 4.9) that represents the next logical step: a windowing technique with variable-sized regions [62].Wavelet analysis allows the use of long time intervals where we want more precise low frequency information, and shorter regions where we want high frequency information

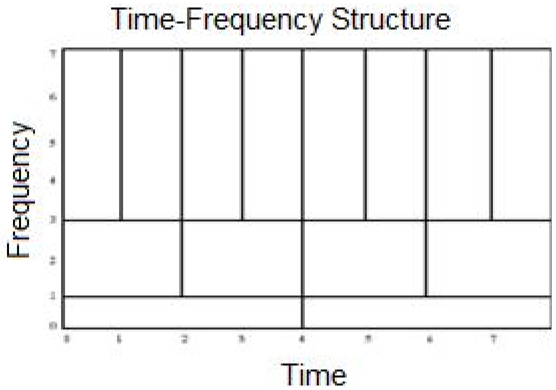


Figure 4.9 Wavelet

Wavelet analysis represents the next logical step: a windowing technique with variable-sized regions. Wavelet analysis allows the use of long time intervals where we want more precise low frequency information, and shorter regions where we want high frequency information.

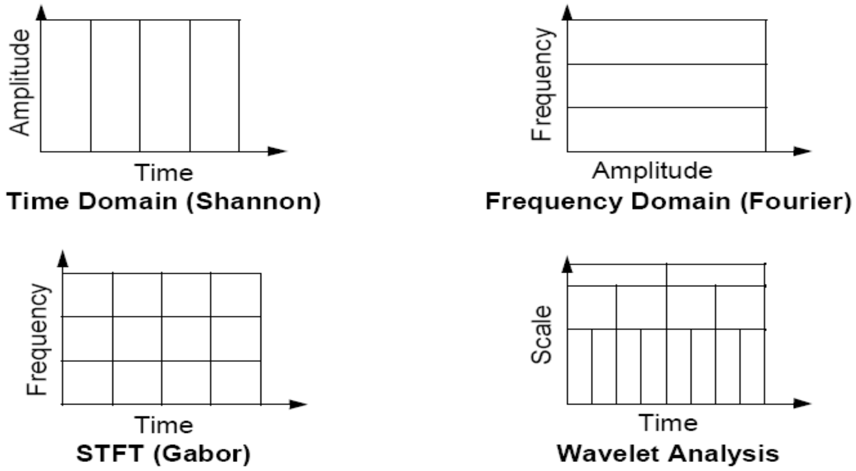


Figure 4.10 Wavelet in contrast with the time-based, frequency-based, and Short time *Fourier* transform (STFT

Figure 4.10 what wavelet looks like in contrast with the time-based, frequency-based, and Short time *Fourier* transform (STFT) views of a signal: Wavelet analysis does not use a time-frequency region, but rather a time-scale region. The wavelet works like this, in figure 4.11.

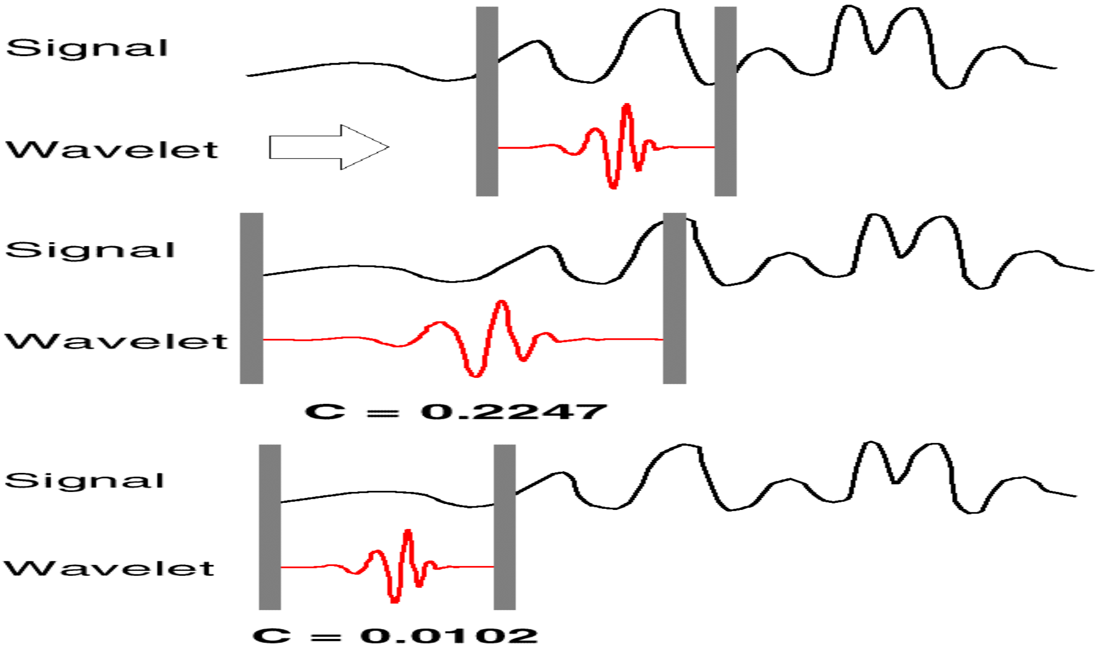


Figure 4.11 The Wavelet Steps

The wavelet done in the following steps (check figure 4.11):

1. Take a wavelet and compare it to a section at the start of the original signal.
2. Calculate a correlation coefficient c
3. Shift the wavelet to the right and repeat steps 1 and 2 until the whole signal covered.
4. Scale (stretch) the wavelet and repeat steps 1 through 3.
5. Repeat steps 1 through 4 for all scales.

The result from this operation is two types of coefficients see figure 4.12, details and approximation. The detailed coefficients (high frequency) the needed for this thesis.

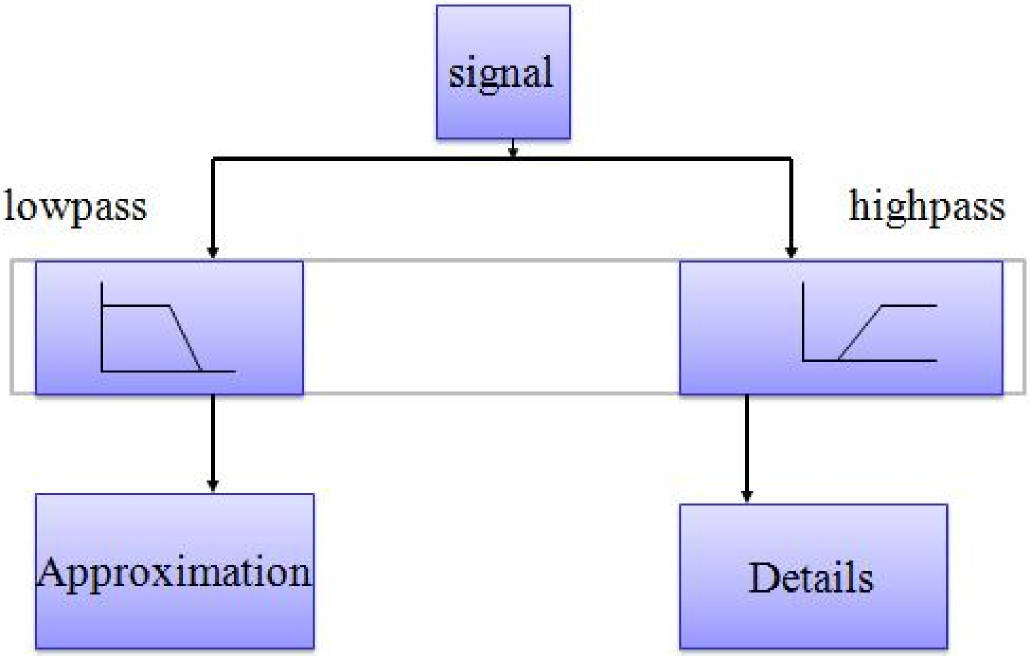


Figure 4.12 Wavelet divides the signal into details and approximation.

4.6 Features Enhancement and Manipulations

Not all the extracted features can be helpful in the classification phase sometimes even these features need to be enhanced and may be the shapes itself need to be enhanced before the feature extraction phase began. To enhance the dissimilarity and the differences between the shapes. The features in hand not at equal lengths we used interpolation and histogram to fix this problem.

4.6.1 Interpolation

Interpolation is at the heart of various medical imaging applications [63-65]. In volumetric imaging, it is often used to compensate for nonhomogeneous data sampling. This rescaling operation is desirable to build isometric volumes [66-68]. Another application of this transform arises in the three-dimensional (3-D) reconstruction of icosahedral viruses [69]. In volume rendering, it is common to apply by interpolation a texture to the facets that compose the rendered object [70]. In addition, volume rendering may also require the computation of gradients, which is best done by taking the interpolation model into account [71]. The essence of interpolation is to represent an arbitrary continuously defined function as a discrete sum of weighted and shifted basis functions. An important issue is the adequate choice of those basis functions. The traditional view asks that they satisfy the interpolation property, and many researchers have put a significant effort in optimizing them under this specific constraint [72-77]. Over the years, these efforts have shown more and more diminishing returns. There is traditional interpolation and there is generalized interpolation lets discuss both

Traditional Interpolation:

Let us express an interpolated value $f(\chi)$ at some (perhaps non-integer) coordinate x in a space of dimension q as a linear combination of samples evaluated at integer coordinates $k = (k_1, k_2, \ldots., k_q) \epsilon z^q$

$$\boldsymbol{f(\chi) = \sum_{k \, \epsilon \, z^q} f_k \varphi_{int}(x - k) \quad \forall \chi = (\varkappa_1, \varkappa_2, \varkappa_3, \ldots, \varkappa_q) \epsilon \, \mathbb{R}^q} \qquad \textbf{4.18}$$

The sample weights are given by the values of the function $\varphi_{int}(x - k)$. To satisfy the requirement of exact interpolation, we ask that the function φ_{int} vanishes for all integer arguments except at the origin, where it must take a unit value. A classical example of the basis function φ_{int} is the sinc function, in which case all synthesized functions are band limited.

Generalized Interpolation

As an alternative approach, let us consider the form

$$f(\chi) = \sum_{k \in z^q} c_k \varphi(x-k) \quad \forall \chi \in \mathbb{R}^q \qquad 4.19$$

The crucial difference between the classical formulation (1) and the generalized formulation (2) is the introduction of coefficients c_k in place of the sample values f_k. This offers new possibilities, in the sense that interpolation can now be carried in two separate steps. Firstly, the determination of coefficients c_k from the samples f_k, then secondly, the determination of desired values f(x) from the coefficients c_k. The benefit of this Separation is to allow for an extended choice of basic functions, some with better properties than those available in the restricted classical case where $c_k = f_k$. The apparent drawback is the need for an additional step. We will see later that this drawback is largely compensated by the gain in quality resulting from the larger selection of basic functions to choose from. Some types of interpolation method are discussed in [78- 80].

1- Nearest Neighbor

The simplest interpolation form a computational standpoint is the nearest neighbor, where each interpolated output pixel is assigned the value of the nearest sample point in the input image. This technique is also known as point shift algorithm and pixel replication. The interpolation kernel for the nearest neighbor algorithm is defined as:

$$h(x) = \begin{cases} 1 & 0 \le |x| < 0.5 \\ 0 & 0.5 \le |x| \end{cases} \qquad 4.20$$

The frequency response of the nearest neighbor kernel is :

$$H(\omega) = sinc(\tfrac{\omega}{2}) \qquad 4.21$$

The kernel and its Fourier transform are shown in Figure 4.13.

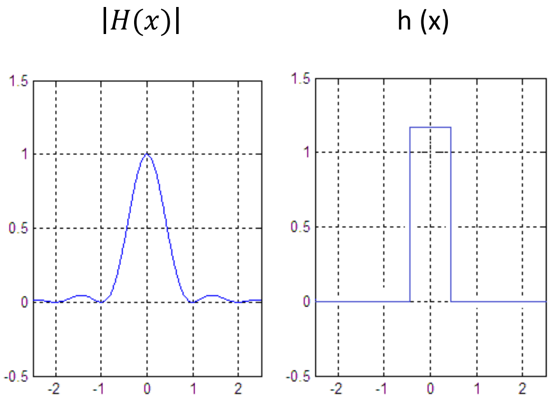


Figure 4.13 Nearest Neighbor Kernel

Convolution in the spatial domain with the rectangle function h is equivalent in the frequency domain to multiplication with a sinc function see figure 4.13. Due to the prominent side lobes and infinite extent a sinc function makes a poor low-pass filter. This technique achieves magnification by pixel replication, and magnification by sparse point sampling for large-scale changes.

2- Linear Interpolation

Linear interpolation is a first degree method that passes a straight line through every two consecutive points of the input signal see figure 4.14. In the spatial domain, linear interpolation is equivalent to convolving the sampled input with the following kernel.

$$\boldsymbol{h(x)} = \begin{cases} \mathbf{1} - |\boldsymbol{x}| & \mathbf{0} \leq |\boldsymbol{x}| < 1 \\ \mathbf{0} & \mathbf{1} \leq |\boldsymbol{x}| \end{cases} \qquad \mathbf{4.22}$$

$$\boldsymbol{H(\omega) = sinc^2(\tfrac{\omega}{2})} \qquad \mathbf{4.23}$$

The kernel is also called triangle filter, roof function or Bartlett window. As shown in Figure 4.14 the frequency response of the linear interpolation kernel is superior to that of the Nears neighbor interpolation function, the side lobes are less prominent, so the performance is improved in the stop band. A pass band is moderately attenuated, resulting in image smoothing.

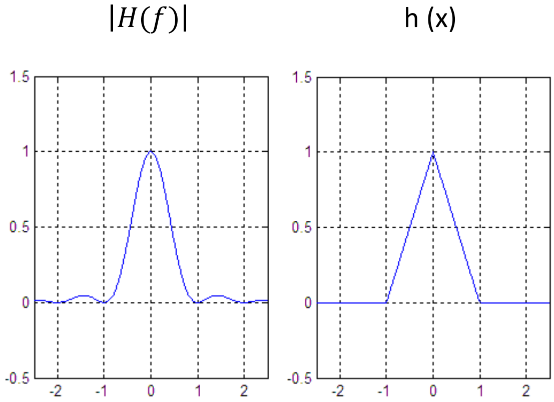


Figure 4.14 Linear Interpolations.

Linear interpolation produces reasonably good results at moderate cost. But for even better performance, more complicated algorithms are needed.

$$\boldsymbol{h(x)} = \begin{cases} \boldsymbol{(a+2)|x|^3 - (a+3)|x|^2 + 1} & \boldsymbol{0} \leq |\boldsymbol{x}| < 1 \\ \boldsymbol{a|x|^3 - 5a|x|^2 + 8a|x| - 4a} & \boldsymbol{0} \leq |\boldsymbol{x}| < 2 \\ \quad \boldsymbol{0} & \boldsymbol{2} \leq |\boldsymbol{x}| \end{cases} \qquad \textbf{4.24}$$

3- Cubic Interpolation

Cubic interpolation (figure 4.15) is a third degree interpolation algorithm that fairly well approximates the theoretically optimum sinc interpolation function. The kernel is composed of piecewise cubic polynomials defined on subintervals (-2,-1), (-1,0), (0,1) and (1,2). Outside this interval the interpolation kernel is zero. For deriving the cubic convolution kernel, we have to solve 8 linear equations with 7 unknown parameters, so the system has one "free" parameter that may be controlled by the user. The kernel is of form:

The frequency response is:

$$\boldsymbol{R(\omega)} = \frac{\boldsymbol{12}}{\boldsymbol{\omega^2}}\left(\boldsymbol{sinc^2}\left(\frac{\boldsymbol{\omega}}{\boldsymbol{2}}\right) - \boldsymbol{sinc\ (\omega)}\right) + \ \boldsymbol{a}\frac{\boldsymbol{8}}{\boldsymbol{\omega^2}}\left(\boldsymbol{3sinc^2(\omega) - 2sinc(\omega) - sinc(2\omega)}\right) \textbf{4.25}$$

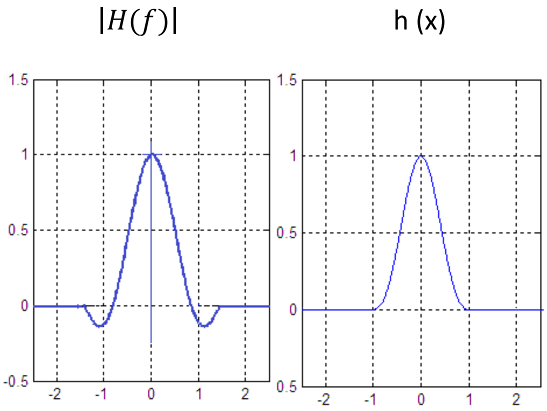

$|H(f)|$ h (x)

Figure 4.15 Cubic Interploation.

Choices for a are a=1. a=0.75 and a=0.5. The performance of the interpolation kernel depends on a, and the frequency content of the signal. For different signals, different values of the parameter a gives the best performance.

4- Piecewise Cubic Hermite (PCHIP) Interpolation

For a given *np* points, interpolation both the function and its derivative with a cubic polynomial on each subinterval. This approach is called **piecewise cubic Hermite interpolation**.

5- B-splines

A *B-spline* of the degree nd is derived through nd convolutions of the box filter, Thus, $B_1 = B_0 * B_0$ denotes a B-spline of degree 1. Yielding the familiar triangle filter. (letter B may stand for basic or basis). The B-spline of dgree 1 is equivalent to the linear interpolation. The second degree B-spline $B_2$is produced by convolving $B_0 * B_1$.

The cubic B-spline $\mathrm{B}_3$is generated from convolving$\mathrm{B}_0 * \mathrm{B}_2$. That is $\mathrm{B}_3 = \mathrm{B}_0 * \mathrm{B}_0 * \mathrm{B}_0 * \mathrm{B}_0$. Figure 4.16 summarize the shapes of these low-order B-splines. The cubic B-spline interpolation kernel is defined as:

$$\boldsymbol{h}(\boldsymbol{x}) = \frac{1}{6}\begin{cases} 3|x|^3 - 6|x|^2 + 4 & 0 \leq |x| < 1 \\ -|x|^3 + 6|x|^2 - 12|x| + 8 & 1 \leq |x| < 2 \\ 0 & 2 \leq |x| \end{cases} \qquad \mathbf{4.26}$$

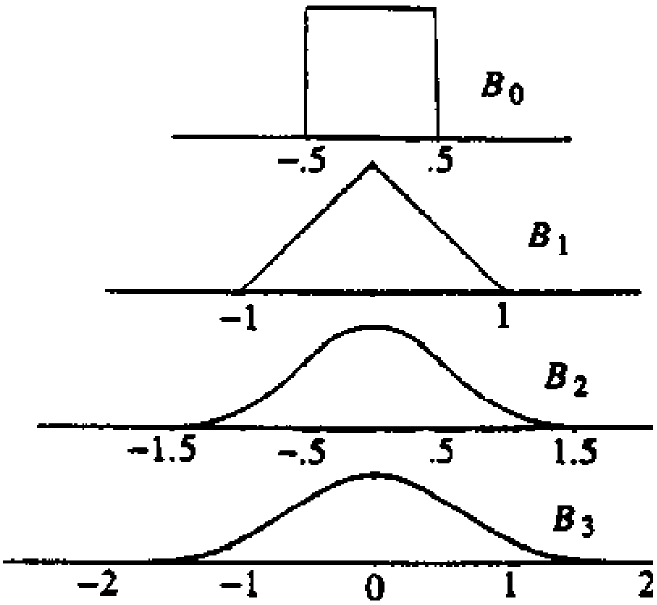


Figure 4.16 B-spline Interpolation.

Unlike cubic convolution, the cubic B-spline kernel is not interpolatory since it does not satisfy the necessary constraint that h(0)=1 and h(1)=h(2)=0. Instead, it is an approximating function that passes near the points but not necessarily through them. This is due to the fact that the kernel is strictly positive application. When using kernals with negative lobes, it is possible to generate negative values while interpolating positive data,. Since negative intensity values are meaningless for display, it is desirable to use strictly positive interpolation kernels to guarantee the passivity of the interpolated image.

4.6.2 Image Rotation

To guarantee rotation invariance, it is necessary to convert an arbitrarily oriented shape into a unique common orientation. First, find the major axis of the shape. The major axis is the straight line segment joining the two points *P*1 and *P*2 on the boundary farthest away from each other. Then we rotate the shape so that its major axis is parallel to the *x*-axis. This orientation is still not unique as there are two possibilities: *P*1 can be on the left or on the right. This problem is solved by computing the centroid of the polygon and making sure that the centroid is below the major axis, thus guaranteeing a unique orientation. So knowing the coordination of the shapes points how we can rotate these points? The answer is by using Matrix rotation.

Matrix Rotation: In linear algebra, a rotation matrix is a matrix that is used to perform a rotation in Euclidean space. For example the Rotation Matrix in the negative sense:

$$\boldsymbol{R} = \begin{bmatrix} \boldsymbol{cos\theta} & \boldsymbol{-sin\theta} \\ \boldsymbol{sin\theta} & \boldsymbol{cos\theta} \end{bmatrix} \qquad \textbf{4.27}$$

This matrix rotates points in the xy-Cartesian plane counterclockwise through an angle θ about the origin of the Cartesian coordinate system check figure 4.17. To perform the rotation using this rotation matrix R, the position of each point must be represented by a column vector v, containing the coordinates of the point. A rotated vector is obtained by using the matrix multiplication Rv. Since matrix multiplication has no effect on the zero vector (i.e., on the coordinates of the origin), rotation matrices can only be used to describe rotations about the origin of the coordinate system. Rotation matrices provide a simple algebraic description of such rotations, and are used extensively for computations in geometry, physics, and computer graphics. Rotation matrices are square matrices, with real entries. More specifically they can be characterized as orthogonal matrices with determinant 1:

$$\boldsymbol{R^T = R^{-1}}, \qquad \det \boldsymbol{R} = \mathbf{1}\,. \qquad \textbf{4.28}$$

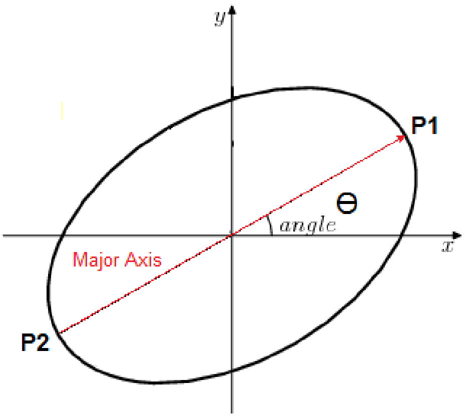


Figure 4.17 Shape rotations.

$$\ominus = \text{arctg}\left[\frac{P2y-P1y}{P2x-P1x}\right] \qquad \textbf{4.29}$$

The Rotation Matrix in the negative sense:

$$R = \begin{bmatrix} cos\theta & -sin\theta \\ sin\theta & cos\theta \end{bmatrix} \qquad \textbf{4.30}$$

4.7 Classification/Clustering Techniques

Pattern recognition can also be seen as a classification process. Its ultimate goal is to optimally extract patterns based on certain conditions and to separate one class from the others according to this there is two types of classification, supervised classification and unsupervised classification. With supervised classification [81], we identify examples of the Information classes (i.e., land cover type) of interest in the image. These are called *"training sites"*. The image processing software system is then used to develop a statistical characterization of the reflectance for each information class. Unsupervised classification [81] is a method which examines a large number of unknown pixels and divides it into a number of classed based on natural groupings present in the image values. Unlike supervised classification, unsupervised classification does not require analyst-specified training data. Unsupervised classification commonly called Clustering defined according to [82] is a method of creating groups of objects, or clusters, in such a way that objects in one cluster are very similar and objects in different clusters are quite distinct. Example of clustering technique K-Means discussed later.

4.7.1 K-Means

The *k*-Means algorithm [82] can be divided into two phases: the initialization phase and the iteration phase. In the initialization phase, the algorithm randomly assigns the cases into *k* clusters. In the iteration phase, the algorithm computes the distance between each case and each cluster and assigns the case to the nearest cluster. The K-Means algorithm, first developed four decades ago [83], is one of the most popular centre-based algorithms that attempts to find *K* clusters which minimize the mean squared quantization error , MSQE. The algorithm tries to locate *K* prototypes (centroids) throughout a data set in such a way that the *K* prototypes in some way best represent the data. We can summarize [84] the K-Means algorithm through the following steps:

Algorithm 4.1: K-Means algorithm

Purpose: clustering data sets into K categories.

Input: matrix of features, each row represents features of cell.

Output: K centroids of the K clusters.

Procedure:

1. Initialization

Step1: Define the number of prototypes (*K*).

Step2**:** Designate a prototype (a vector quantity that is of the same dimensionality as the data) for each cluster.

Step3: Assign each data point to the closest prototype. That data point is now a member of the class identified by that prototype.

Step4: Calculate the new position for each prototype (by calculating the mean of all the members of that class).

Step5: Observe the new prototypes' positions. If these values have not significantly changed over a certain number of iterations, exit the algorithm. If they have, go back to Step 2.

4.7.2 Genetic Algorithm (GA)

Interestingly Genetic algorithms used in both classification types supervised [85] and unsupervised [86- 90]. GA is considered the heart of this thesis and small comparison of GA and K-means is accomplished during it.

Genetic algorithms are a stochastic search algorithm, which uses probability to guide the search. It was first suggested by John Halland [91]; in the seventies. Over the last twenty years, it has been used to solve a wide range of search, optimization, and machine learning. Genetic algorithms are a class of parallel adaptive search algorithms based on the mechanics of natural selection and natural genetic system. It can find the near global optimal solution in a large solution space quickly. It has been used extensively in many application areas, such as image processing, pattern recognition, feature selection, and machine learning [92]. It is a powerful search technique that mimics natural selection and genetic operators. Its power comes from its ability to combine good pieces from different solutions and assemble them into a single super solution [93].Genetic algorithms are initial population of solution called individuals is (randomly) generated, the solutions are evaluated. The algorithm creates new generations of population by genetic operations, such as reproduction, crossover and mutation. The next generation consists of the possible survivors (i.e. the best individuals of the previous generation) and of the new individuals obtained from the previous population by the genetic operations. The best source of information about Gas is Holland's adaptation in natural and artificial systems; Holland uses terms borrowed from mendelian genetics to describe the process: each position in the string is called a gene. The possible values of each gene are called alleles. A particular string is called a genotype. The population of strings also called the gene pool. The organism or behavior pattern specified by a genotype is called a phenotype. If the organism represented is a function with one or more inputs [94] these inputs are called detectors. The algorithm of simple GAs in [95-96]. The following steps show how GA works:

Algorithm 4.2: Genetic algorithm

Purpose: clustering data sets into K categories.

Input: matrix of features, each row represents features of cell.

Output: K centroids of the K clusters.

Procedure:

Step1: Initialization: Generate random population**.**

Step2: Evaluate the fitness f(x) of each element x in the population.

Step3: The best K elements in the population according to f(x) are the K centroid of the clusters.

Step4: Create a new population by repeating following steps until the new population is complete

- Select two parent from a population according to their fitness (the better fitness, the bigger chance to be selected)
- With a crossover probability cross over the parents to form a new offspring (children). If no crossover was performed, offspring is an exact copy of parents.
- With a mutation probability mutate new offspring at each element in the population.
- Place new offspring in a new population

Step5: Use new generated population for a further run of algorithm

Step6: If the end condition is satisfied, stop, and return the best solution in current population. Otherwise go to step 3**.**

Step 3 needs further explanation, for the K best elements each of them considered centroids for K clusters. Each vector in the data set in hand measures the distance between it and the K centroids. The smallest distance to a centroid gives indication that this vector belongs to that cluster with that centroid. According to this the evaluation of fitness function accomplished.

GA parameters:

A. Population

A population consists of n individuals where N is chosen by the designer of the GA's [97]. Every individual has a chromosome which consists of Lg genes. The chromosome is often referred to as the genotype of an individual.

B. Initialization

Initialization means initialize the genes of all individuals randomly or with predefined values it's up to the GA designer. These individuals are the starting points in the search space for the simple GA.

C. Evaluation

Calculate the fitness of each individual by decoding each chromosome and applying the fitness function to each decode individuals. The decoding creates a phenotype based on a genotype.

D. Scaling Function

Scaling function specifies the function that performs the scaling [98]. The options are:

- Rank — scales the raw scores based on the rank of each individual instead of its score. The rank of an individual is its position in the sorted scores. An individual with rank *r* has scaled score proportional to $\frac{1}{\sqrt{r}}$. So the scaled score of the most fit individual is proportional to 1, the scaled score of the next most fit is proportional to $\frac{1}{\sqrt{2}}$, and so on. Rank fitness scaling removes the effect of the spread of the raw scores. The square root makes poorly ranked individuals more nearly equal in score, compared to rank scoring.
- Proportional — Proportional scaling makes the scaled value of an individual proportional to its raw fitness score.
- Top— Top scaling scales the top individuals equally. Selecting Top displays an additional field, **Quantity** variable, which specifies the number of individuals that are assigned positive scaled values. **Quantity** can be an integer between 1 and the population size or a fraction between 0 and 1 specifying a fraction of the population size. The default value is 0.4. Each of the individuals that produce offspring is assigned an equal scaled value, while the rest are assigned the value 0.
- Shift linear — Shift linear scaling scales the raw scores so that the expectation of the fittest individual is equal to a constant multiplied by the average score.

E. Selection

Select specific individuals from the population to be the parents that will used to create new individuals there is many methods are used to choose those parents [97]. The selection method specifies how the genetic algorithm chooses parents for the next generation. The methods are:

- Stochastic uniform —Stochastic uniform, lays out a line in which each parent corresponds to a section of the line of length proportional to its scaled value. The algorithm moves along the line in steps of equal size. At each step, the algorithm allocates a parent from the section it lands on. The first step is a uniform random number less than the step size.

- Remainder — Remainder selection assigns parents deterministically from the integer part of each individual's scaled value and then uses roulette selection on the remaining fractional part. For example, if the scaled value of an individual is 2.3, that individual is listed twice as a parent because the integer part is 2. After parents have been assigned according to the integer parts of the scaled values, the rest of the parents are chosen stochastically. The probability that a parent is chosen in this step is proportional to the fractional part of its scaled value.
- Uniform — Uniform selection chooses parents using the expectations and number of parents. Uniform selection is useful for debugging and testing, but is not a very effective search strategy.
- Roulette — Roulette selection chooses parents by simulating a roulette wheel, in which the area of the section of the wheel corresponding to an individual is proportional to the individual's expectation. The algorithm uses a random number to select one of the sections with a probability equal to its area.
- Tournament — Tournament selection chooses each parent by choosing **Tournament size** players at random and then choosing the best individual out of that set to be a parent. **Tournament size** must be at least 2.

E. Recombination

Individuals from the set selected-parents are mated at random and each pair created offspring using 1 point crossover or 2-point crossover.

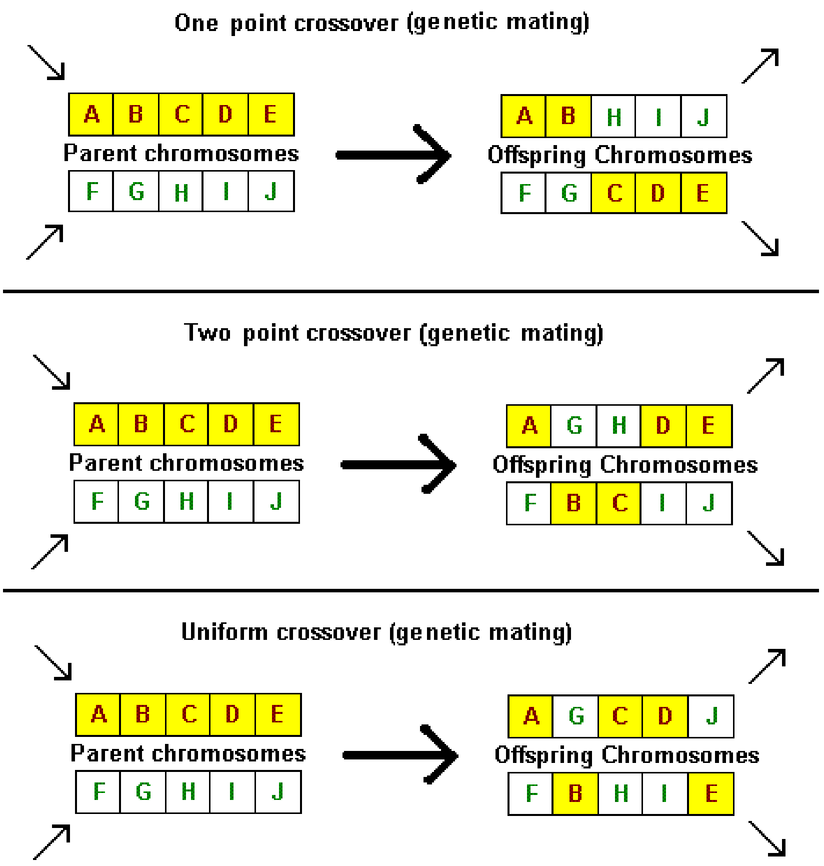


Figure 4.18 Recombination

F. Mutation

Mutation is a random change of one or more genes [97] Every chromosome is simply scanned gene by gene and with a mutation rate Pm a gene is changed/swapped, i.e. 0 1 and 1 0 the probability for a mutation is usually kept small, i.e. Pm = 1/Lg such that we can expect one mutated gene per chromosome.

G. Stop Criterion

Stopping criteria is when the GA should stop. A simple and easy to implement stopping criterion is to stop the simple GAs if no improvement of the best solution has been made for a (large) predefined number of generations, where one generation is one turn through the do-until loop in algorithm in Figure, there are other different stopping criteria: first limited number of generations, limited consumed time during the process. In the next chapters we discuss the practical work of thesis, in other words we apply basic mention theories in this chapter in the next following chapters

Chapter 5: The Proposed System

5.1 Introduction

In this thesis the proposed system consist of four stages check figure 5.1. These stages are: data collection phase, pre-processing phase, feature extraction phase and finally classification phase.

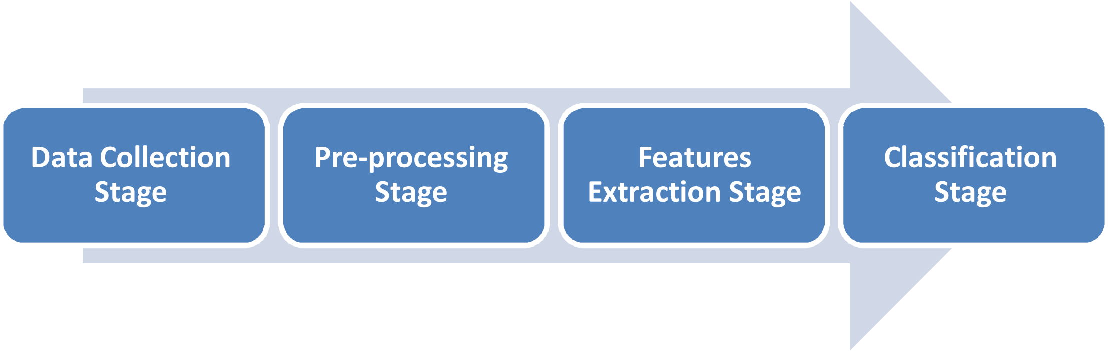


Figure 5.1 The Proposed System.

5.2 Data Collection Stage

In this stage several types of equipments used. First we need equipments and tools to draw bloods and make a blood films from these samples. Second we need equipments and tools for blood films images capturing under the microscope.

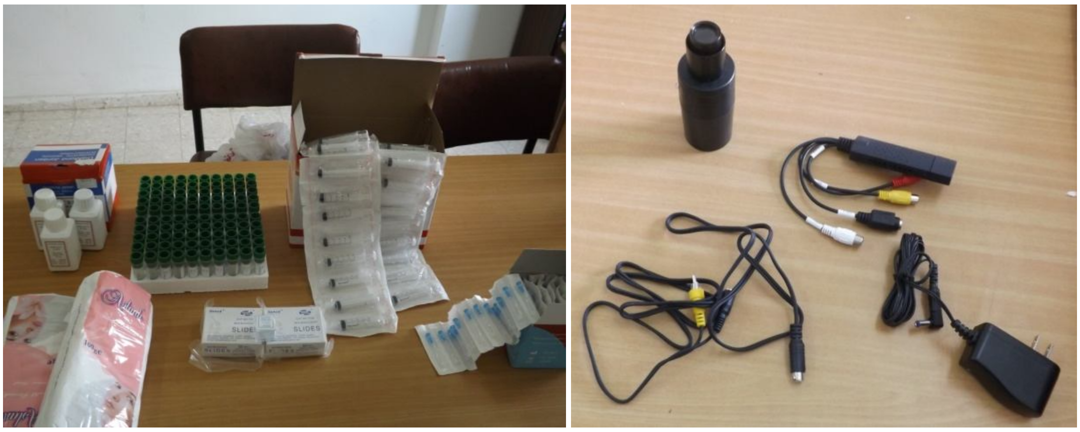


Figure 5.2 The Equipments (a)In the left the equipment needed for blood drawing and blood films making, (b) In the right the needed equipments for blood films

In the left of figure 5.2 the equipment needed for blood drawing and blood films making these equipments in details discussed in table 5.1. The equipment needed and used in this thesis are:

Table 5.1 Blood drawing and Making Equipments

Items	**Amount**
Needles	**100**
Sernges	**100**
Tubes	**100**
White Cotton	**2**
Stains (right stain)	**3 bottles each 125 ml**
Blood Slides	**100**
Blood Slides cover	**100**

These equipments from Needles to Cotton used to draw human blood (sick and healthy people) while the rest are used to make the blood films which is discussed more thoroughly in Appendix B.

In the right of figure 5.2 are the equipment used in the blood films image capturing under the microscope, in table 5.2 discussed in details:

Table 5.2 Blood films images capturing under the microscope Equipments and tools.

Items	Description	Amount
Video Wireless Camera Receiver	USB 2.0 Video Adapter with Audio	1
Electronic Eyepiece	Model: coomo20N	1

Table 5.2 these are the actual tools that used in this thesis where the video camera gives a video stream to what is available under the microscope which is in our case blood films. While the video card is just an adapter so we can connect the video camera to our laptop directly and then we can take images as much as we can. There are an alternative tool can be used here which is a C-mount adapter check figure 5.3 with any kind of camera. In such case the type of C-mount adapter depends very much on the camera and the microscope types. But in our case the video camera we use works very good with any type of microscope. This is what makes it's more appealing.

Figure 5.3 C-Mount Adapter.

The number of obtained samples are hundred (100), for 100 different persons, meaning 100 pictures, these images has 114 shapes for Burr cells, 128 shapes for Normal cells, 59 shapes for Teardrop cells and 118 shapes for Sickle cells.

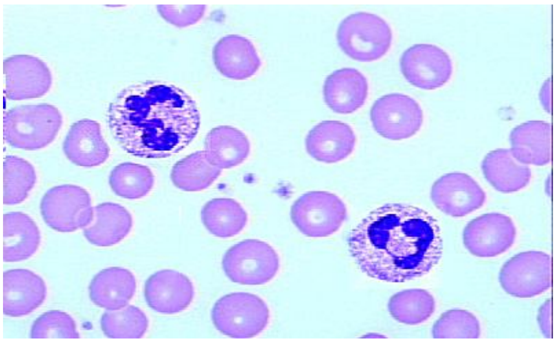


Figure 5.4 Sample of the blood films images in hand.

In the classification phase we classify data to different categories, once into four categories (normal cells, sickle cells, teardrop cells and burr cells), where the last three categories are abnormal cases; second into two categories: normal and abnormal. Example of captures blood films image in figure 5.4. The software we used for such process is Matlab Software where it has Genetic Algorithm and K-Means functions.

5.3 Preprocessing Stage

First we need to split the blood cells from the background. This step is achieved first by using Hough transforms, the resultant image shown in figure 5.5.

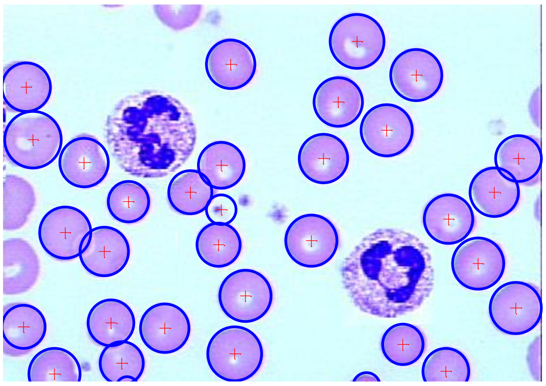


Figure 5.5 Same image with Hough Transformer for cells detections.

As you may notice, sometimes Hough transformer ignores cells or imagines the existence of cells and fails miserably in case of overlapping cells and ignores abnormal cells. Another approach is to use color based segmentation to separate red blood cells from other cells and from the background in a single step. K-means used to cluster the blood films images into 3 cluster, first cluster is red blood cells, second cluster is the background and third cluster is for other cells, repeating clustering three times to avoid local minima. The result from clustering the blood films images in RGB space; each color represents different cluster alone, nuclei of cells and the background in one cluster not quite the wanted result. Each colors in figure 5.6 (a) represents a different cluster.

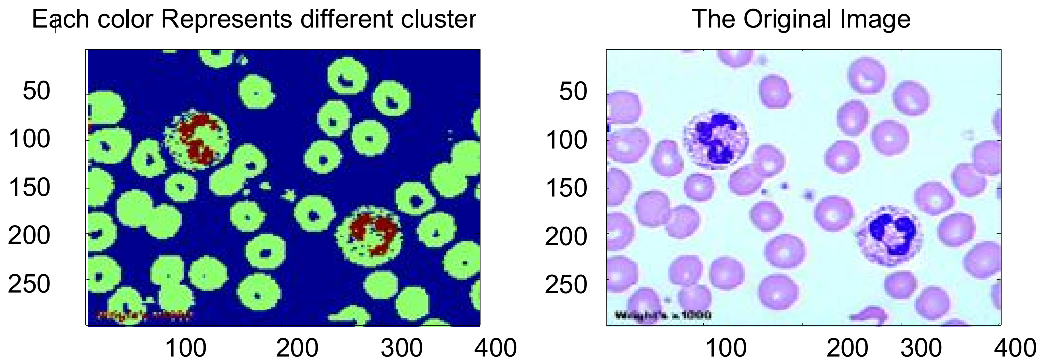


Figure 5.6.Clustering using K-means in RGB space, Figure 5.3 (a) the clustering result, Figure 5.3 (b) the original image.

The features chosen to be fed into K-means (8 features) are:

R: red value for each pixel.

G: green value for each pixel.

B: blue value for each pixel.

x: x- coordinate for each pixels.

y: y- coordinate for each pixels I: intensity value for each pixel.

S: saturation value for each pixel. H: hue value for each pixel.

Using these equations:

$$\mathbf{I} = \frac{\mathbf{1}}{\mathbf{3}}\ (\mathbf{R} + \mathbf{G} + \mathbf{B}) \qquad \mathbf{5.1}$$

$$\mathbf{S} = \mathbf{1} - \left(\frac{\mathbf{3}}{(\mathbf{R+G+B})}\right) \times \mathbf{a}\ \text{, Where } \mathbf{a} \text{ is the minimum of R, G and B}$$

$$\mathbf{H} = \mathbf{cos}^{-1}\left(\frac{\mathbf{0.5}\times((\mathbf{R-G})+(\mathbf{R-B}))}{((\mathbf{R-G})^{2}+(\mathbf{R-B})\times(\mathbf{G-B})^{0.5})}\right) \qquad \mathbf{5.2}$$

Using same clustering techniques (K-Means) and the same features for segmenting/clustering but in the LAB space, each cluster in a separate image alone is shown in figure 5.7.

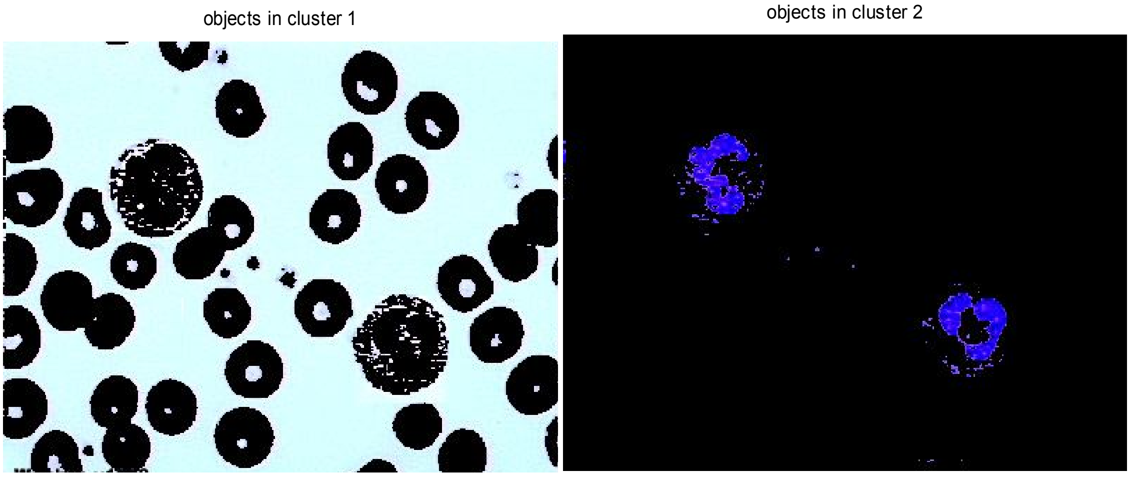


Figure 5.7.Clustering of foreground and using K-means in LAB space.

In figure 5.7, the white cells (large cells) still segmented with the red blood cells, this means that more following steps required; the result form the clustering in the RGB space is much better, since colors manipulation operation are more flexible. In segmenting (clustering) in the LAB space the image entirely fed into K-Means; only pink cells must be in one cluster along with their centers (nuclei).

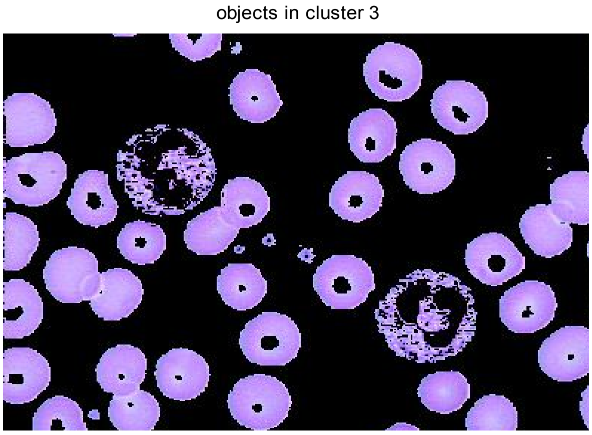


Figure 5.8 Third cluster red cells.

After segmenting/clustering the blood films images using K-means into 3 clusters, first cluster is Pink objects (red blood cells), second cluster is the background and third cluster is for the other cells all that in RGB space, repeating the clustering process three times to avoid local minima. Converting figure 5.8 into binary image is presented in figure 5.9.

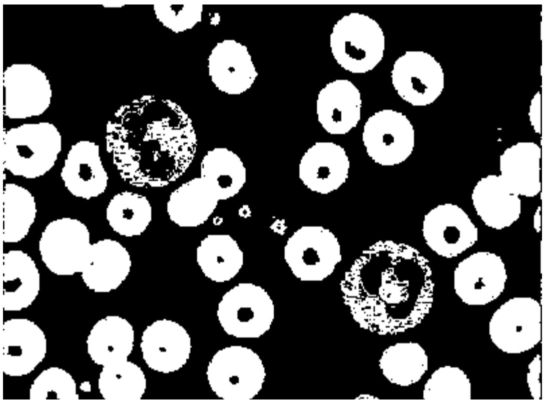
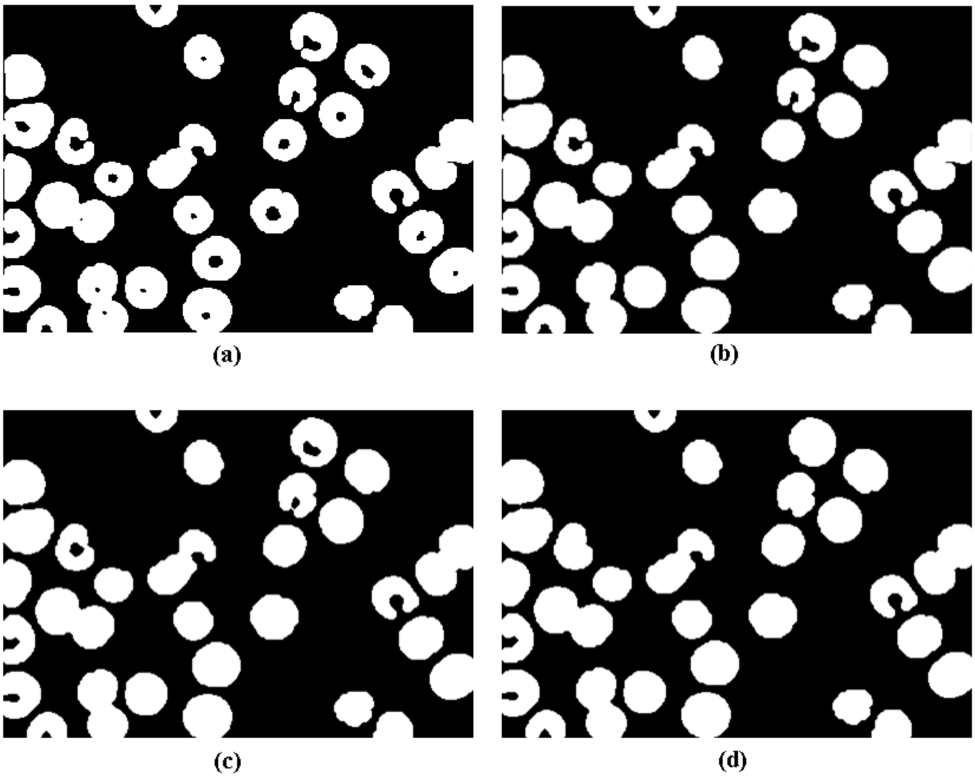


Figure 5.9. Binary image of figure 5.8

For binary image, some morphological operations performed to clean the image from unwanted parts (such as the fractions of white cells). First: opening operation with disk of five pixels radius is done (notice here that the images of the blood films are of the same size).



(a) (b) (c) (d)

Figure 5.10 Some morphological operations: (a) Binary image after opening morphological operation (R=5 pixels). (b) Image filling morphological operation (holes). (c) Opening morphological operation (R=2 pixels). (d) Closing morphological operation

The morphological operations that used in figure 5.10 are necessary to separate the red blood cells alone, these red blood cells is cleaned and ready to be used. Now, the next problem that we faced is the overlapped cells, so the next step is clustering (or separating) the overlapped cells from the non-overlapped cells. The K-Means clustering technique is used also for such purpose:

The suggested features to be fed to the K-means to accomplish such task are:

- The object size (number of pixels in each object).

- The maximum distance between any two pixels (Euclidean distance used).

$$\boldsymbol{Ed}\ (\textbf{Euclidian distance}\ ) = \sqrt{(x_1 - x_2)^{0.5} + (y_1 - y_2)^{0.5}} \qquad \textbf{5.3}$$

Applying equation 5.3 on the final resultant image, after the morphological operations (figure 5.10-d), we have two clusters, shown in figure 5.11, one for overlapped cells and the other for non-overlapped cells, but the results not very stable meaning that it may give different result from run to run.

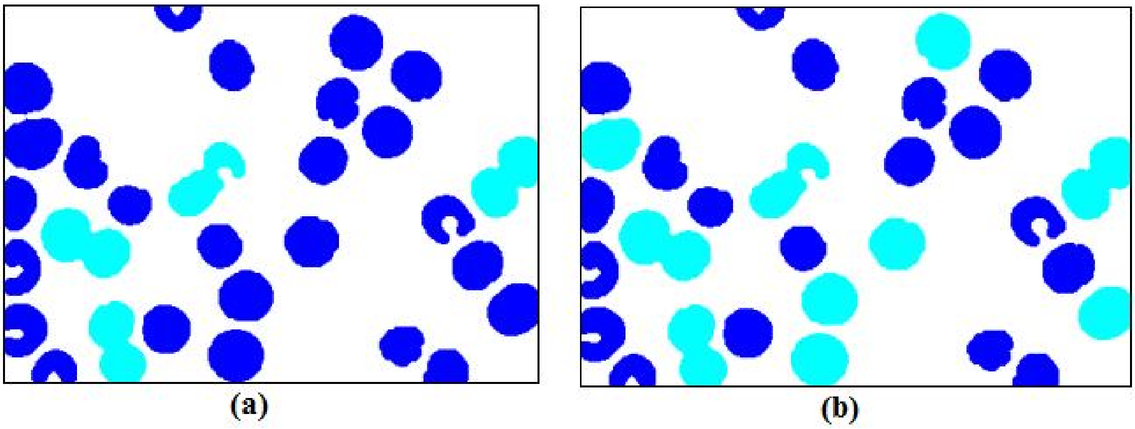


Figure 5.11 Two cases for clustering of overlapped/non-overlapped cells. (a) is good results. (b) is bad results.

To solve this critical problem, another feature must be included in order to separate both groups more exclusively. So new feature added to the previous set of features which is:

-The maximum distance between the median point (centroid point of the object) and any other pixel in the object (Euclidian distance used).

Be aware that each shape (object) is resized to fix size before the previous features are taken and before clustering operation is done. The result from such modification produces stable result after executing the code at any number of times, i.e. the stability is achieved, as shown in figure 5.12, no matter how many times the code is run or whatever the blood film (abnormal or normal cases) the result is the same. The success rate for such clustering was 100%.

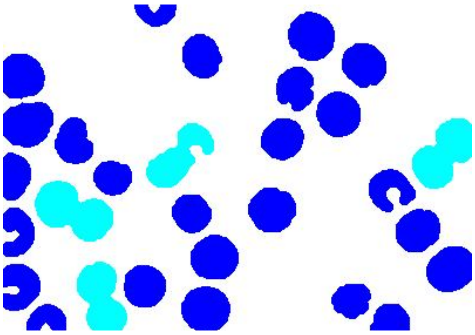


Figure.5.12 The final result from clustering overlapped / non-overlapped cells.

The number of cells in a particular area is important feature for doctors and hematologists. So for counting the objects (cells) in image correctly, the overlapped cells should be separated into the actual number of cells that overlapped. Ordinary counting will count the overlapped cells as one object. So we apply the watershed morphological operation at overlapped cells to segment it to the approximate number of cells that overlapped. Figure 5.13 show the overlapped cells in part (a), while part (b) separate each cell from the overlapped cells. This is done by first applying distance transformer to the objects then on the result applying the watershed, which gave good result.

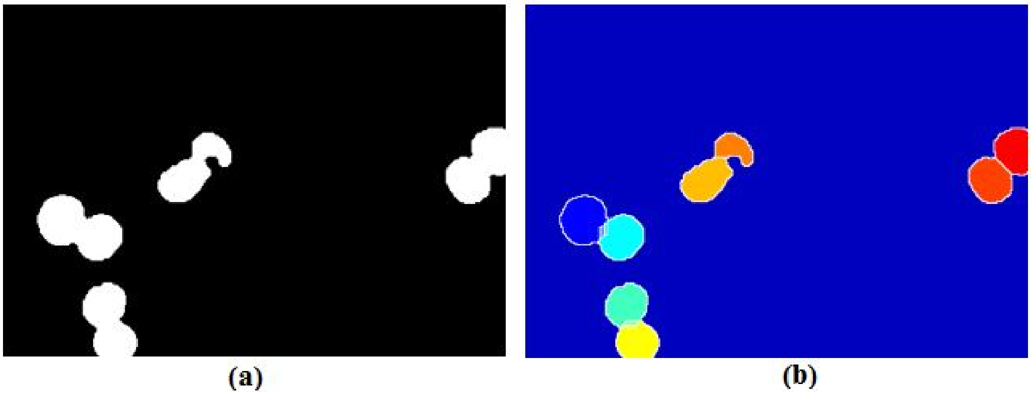


Figure 5.13. Watershed morphological operation: (a) overlapped cells. (b) Segmented overlapped cells using watershed.

By zooming in for one overlapped cells object, figure 5.14, we will find that there are several segmentation lines in between the two overlapped cells, which indicate improper segmentation. The solution for such problem is to enhance (or more accurate filter) the image in the grey level before converting it to binary image in the first place.

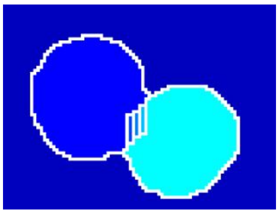


Figure 5.14 Two Overlapped cells.

The image filtered using smoothing edge filter then watershed applied which is give much better result as illustrated in figure 5.15.

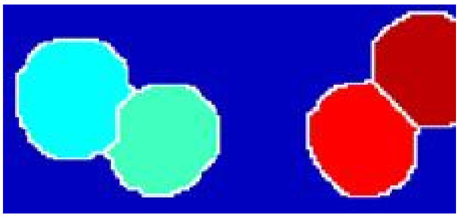


Figure 5.15 Overlapped cells after smoothing filter

Previous phase results gave each red blood cell alone as required to count the number of cells, but the produced binary image lost a lot of it is properties due to enhancement and morphological operations usage. To solve this problem, we located the centroid for each object in the binary image in hand and convert the **original image (the first blood films images)** to binary image, and use the centroids as flags to locate the wanted objects as shown in figure 5.16.

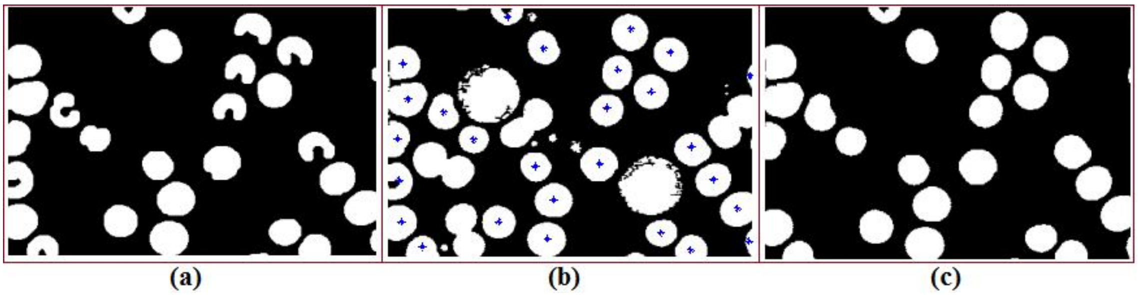


Figure 5.16 Cell Segmentation (a) The binary image (non-overlapped cells). (b) The centroids of cells in binary image as flag in the original binary image. (c) The resultant

Getting the red blood cells alone then getting these cells from the original image with all their details intact, clustering the overlapped and non-overlapped cells and removing the boundary object, with all that done the preprocessing phase is finished. This can be summaries in the following steps:

1- Resize images to one size (Average size among image sizes).

2- Clustering the image based on color. (Features were: red, green, blue, the coordination, saturation, intensity and hue), we are only concerned with the pink objects (the red blood cells).

3- Filtering the pink cluster with smoothing filter then convert it into binary image.

4- Applying opening morphological operation (R=5 pixels) on the binary image.

5- Applying image filling morphological operation (holes) on the result from the third step.

6- Applying opening morphological operation (R=2 pixels) on the result from the fifth step.

7- Applying closing morphological operation (holes) on the result from the sixth step.

8- Clustering the result from sixth step based on these features:

   - The object size (no of pixels in each object).

   - The maximum distance between any two pixels (Euclidean distance).

   - The maximum distance between the median point (object centroid) and any other pixel in the object (Euclidian distance).

This is step done in order to get the overlapped cells alone and non-overlapped cells (worked as intended).

9- Applying watershed morphological operation on the cluster that contains non overlapped cells.

10- The object exist in result of sixth step is the only object we concerned in, but they lose their distinguishing, so the centroids of each object in the binary image from the sixth step made as flags to the binary image of the original image, mapping the original image by centroids (flags) to locate object needed with all their details.

5.4 Feature Extraction Stage

After the isolation of the red blood cells from the image, and clustering the overlapped cells away from the non-overlapped cells, the feature extraction phase is come next, to extract the unique features of the individual cells which distinct each cell. The suggested methodology in this phase is to extract the features in time domain and frequency domain. Figure 5.17 demonstrates the methods that been used to extract the features which needed to be fed into the classifier and their relationships.

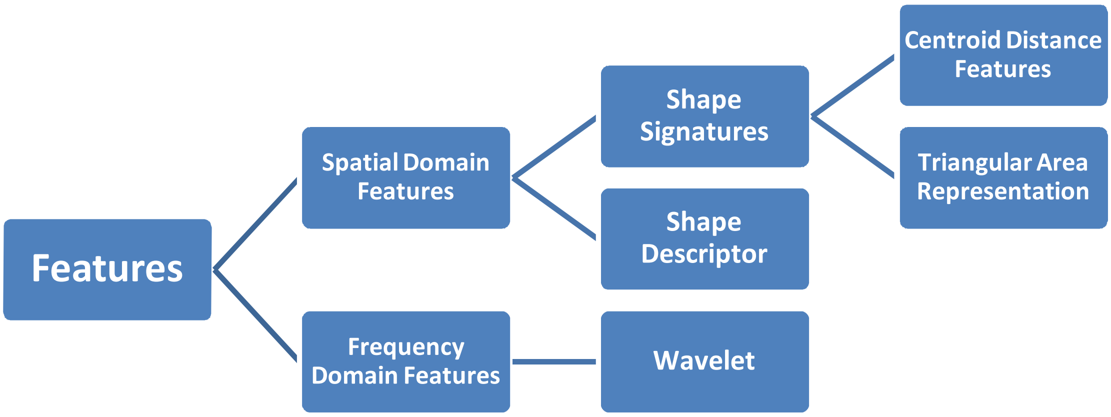

Features
Spatial Domain Features
Frequency Domain Features
Shape Signatures
Shape Descriptor
Wavelet
Centroid Distance Features
Triangular Area Representation

Figure 5.17 Methods that used to extract features.

5.4.1 Spatial Domain Features

In this thesis, several functions in time domain were been applied to extract suitable red blood cells features. These features were fed to our classifier to achieve best results as much as possible. As explained in chapter 4, Centroid Distance Function (CDF), and Triangular Area Representations (TAR) are used in this phase, but there are some pre-processing steps should be achieved before extracting the CDF and TAR features or even frequency domain features for each individual cell. These steps in time domain are as follows.

1- Bounding rectangle (BR).

This means containing the object (in our case each RBC cell) in a bounding rectangle.

2- Fixing the orientation.

Unifying the orientation angle for all shapes (cells), this is done by using the following procedures:

1) Measuring the angle between the major axis and the x-axis of the shape (cell) and called the angle θ.

2) Multiplying each point’s coordination by R.

Explanation: the shapes (cells) need to have common orientations angle; orientation angle is the angle between the major axis of shape with the x-axis; So in order to make all shapes (cells) have a common orientation angle which in our case is zero (meaning the major axis lying on the x-axis) we need to know the current orientation and multiply the current coordination of the shape (cell) pixels to R matrix that described in equation 5.6.

$$\theta = \mathbf{arctg}\left[\frac{\mathbf{P2y-P1y}}{\mathbf{P2x-P1x}}\right] \qquad \mathbf{5.4}$$

The rotation matrix in the negative sense:

$$\mathbf{R} = \begin{bmatrix} \mathbf{cos\theta} & \mathbf{-sin\theta} \\ \mathbf{sin\theta} & \mathbf{cos\theta} \end{bmatrix} \qquad \mathbf{5.5}$$

Where θ is the angle used to rotate the shapes (cells) in (clock wise or in the negative sense); in our case θ is the current orientation angle of the shape (to make the orientation zero).

3- Resize the cell size to fix size.

Using interpolating which discussed thoroughly in the previous chapter.

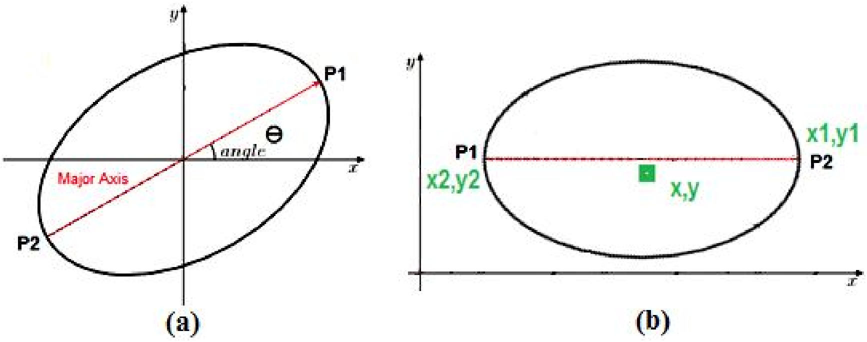


Figure 5.18. Fixing the orientation. (a) Before Shape rotation. (b) After shape rotation

4- Rotate the image around the centroid.

To guarantee rotation invariance, it is necessary to convert an arbitrarily oriented shape (in our case cells) into a unique common orientation. First, find the major axis of the shape. The major axis is the straight line segment joining the two points P_1 and P_2on the boundary farthest away from each other. Then we rotate the shape (cell) so that its major axis is parallel to the *x*-axis. This orientation is still not unique as there are two possibilities: P_1 can be on the left or on the right. This problem is solved by computing the centroid of the polygon (shape or cell) and making sure that the centroid is below the major axis, thus guaranteeing a unique orientation. Let us now consider scale and translation invariance. We define the bounding rectangle (BR) of a shape as the rectangle with sides parallel to the x and y axes just large enough to cover the entire shape (after rotation). Note that the width of the BR is equal to the length of the major axis. To achieve scale invariance, we proportionally scale all shapes so that their BRs have the same fixed width (pixels). Since major axis of the shape laying on the x-axis (after fixing the orientation) the shape will looks like figure 5.18 (b).

So $\mathcal{Y}_1 = \mathcal{Y}_2$ and length of the major axis is $\mathcal{X}_1 - \mathcal{X}_2$

After finding the centroid of the shape $(\mathcal{X}, \mathcal{Y})$; if $\mathcal{Y} > \mathcal{Y}_1$ or if $\mathcal{Y} > \mathcal{Y}_2$ then the centroid is above the major axis else the centroid below the major axis. In the graph, the $\mathcal{Y}$ is below the major axis, an example is presented in figure 5.19

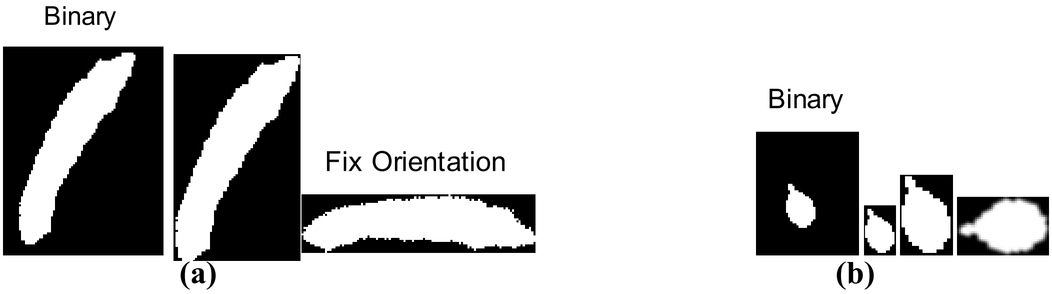


Figure 5.19 Shape rotation for: (a) RBC Sickle. (b) RBC Teardrop.

Each shape with its CDF values and TAR representations values the lengths of each representatives features is variant than others and all that discussed significantly obvious in the data description tables.

SD (Shape **D**escriptor**):**

Features = [Solidity, E, Circle Ratio, Rectangularity, Convexity, Ellipse Ratio, Extent, Ecc, R1, R2];

7- Solidity: Scalar specifying the proportion of the pixels in the convex hull that are also in the region. As stated in chapter 4 computed using the equation 4.6.

8- Eccentricity (**E**): 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. (0 and 1 are degenerate cases; an ellipse whose eccentricity is 0 is actually a circle, while an ellipse whose eccentricity is 1 is a line segment.) .Eccentricity's the measure of aspect ratio. It is the ratio of the length of major axis to the length of minor axis.

9- Circularity ratio represents how much a shape is similar to a circle. Circularity ratio is the ratio of the area of a shape to the area of a circle having the same perimeter. Check chapter 4 for equations 4.7 to 4.12.

10- Rectangularity: the Area of the shape divided by the area of the minimum bounding box. Rectangularity represents how rectangular a shape is, i.e how much it fills its minimum bounding rectangle see equation 4.13 in chapter 4.

11- Convexity: is defined as the ratio of perimeters of the convex hull over that of the original contour, check equation 4.14 in chapter 4.

12- Ellipse Ratio: first with knowing the next two concepts:

A- Major Axis Length: Scalar specifying the length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.

B- Minor Axis Length: Scalar; the length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.

Where *Ellipse Ratio:* is ratio of the shape area and *the* ellipse area with the same minor axis and major axis of the shape.

7. Extent: Scalar that specifies the ratio of pixels in the region to pixels in the total bounding box. Computed as the Area of the shape divided by the area of the bounding box.

8. Ecc: first let's understand the following definition:

Longest Length: is a variable we made to measure the distance between farthest two points at the boundary.

So, $$\textbf{Ecc} = \frac{\textit{Minor Axis Length}}{\textit{Longest Length}} \qquad \textbf{5.6}$$

First we calculate distance of the farthest point on the boundary and the closest point on the boundary to the centroid.

D1= the longest distance (Centroid to boundary).

D2= the shortest distance (Centroid to boundary).

9. R2 is the ratio of longest distance of the boundary to the centroid to the major axis length of the shape

$$R1=\frac{D1}{Major\ Axis\ length} \tag{5.7}$$

10. R2 is the ratio of shortest distance of the boundary to the centroid to the major axis length of the shape

$$R2=\frac{D2}{Major\ Axis\ length} \tag{5.8}$$

Spatial Domain Features Data sets

After applying all what is mention before we obtained several data sets, each of these data sets described in a table where the maximum value of the features and the minimum value of the features also the maximum length of the features and the minimum length of these features, where in front of each cell type these values recorded and the number of cells in question. First the CDF:

CDF Data Set Description

Table 5.3 Data Set Description for Centroid Distance Function (CDF) features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	Number of Cells
Burr	1	8.4582e-004	1515	864	**114**
Sickle	1	0.004	962	254	**118**
Tear drop	1	0.0048	1271	499	**59**
Normal	1	0.0029	1540	990	**128**

As mention before this table (table 5.3) is a description for the Centroid Distance Function (CDF) features. These data sets contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features not equal. For example the first raw is for Burr cells where the maximum feature value for this type of cells is 1 while the minimum is 8.4582e-004, in the other hand the maximum feature length for this cell type is 1515 while the minimum feature length is 864. Other rows (cell types) read in the same manner.

TAR Data Sets Description

Table 5.4 Data Set Description for Triangular Area Representation (TAR) features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	Number of Cells
Burr	1	-17.5135	1515	864	**114**
Sickle	1	-14.4444	962	254	**118**
Tear drop	1	-43	1271	499	**59**
Normal	1	-9.5172	1540	990	**128**

This table (table 5.4) is a description for the Triangular Area Representation(TAR) features. This data set contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features not equal. For example the fourth raw is for Normal cells where the maximum feature value for this type of cells is 1 while the minimum is -9.5172, in the other hand the maximum feature length for this cell type is 1540 while the minimum feature length is 990. Other rows (cell types) read in the same manner.

The following two tables (table 5.5 and table 5.6) are also for TAR features, but here the TAR parameter **ts** which discusses in chapter 4 manipulated. Where in the table 5.4 ts have the value 3, while in table 5.5 ts equals half of the number of boundary points, and in table 5.6 ts equals quarter the number of boundary points.

Table 5.5 Data Set Description for Triangular Area Representation (TAR) features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	Number of Cells
Burr	1	-1.4722	1515	864	**114**
Sickle	1	-2.7845	962	254	**118**
Tear drop	1	-2.0551	1271	499	**59**
Normal	1	-1.4419	1540	990	**128**

This table (table 5.5) is a description for the Triangular Area Representation(TAR) features (ts=1-N/4; N=number of boundary points). This data set contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features not equal. For example the fourth raw is for Normal cells where the maximum feature value for this type of cells is 1 while the minimum is -2.0551, in the other hand the maximum feature length for this cell type is 1540 while the minimum feature length is 990. Other rows (cell types) read in the same manner.

Table 5.6 Data Set Description for Triangular Area Representation (TAR) features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	Number of Cells
Burr	1	-1.7321	1515	864	**114**
Sickle	1	-2.4783	962	254	**118**
Tear drop	1	-1.6558	1271	499	**59**
Normal	1	-1.2620	1540	990	**128**

This table (table 5.6) is a description for the Triangular Area Representation (TAR) features (ts=1-N/2; N=number of boundary points). This data set contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features not equal. For example the second raw is for Sickle cells where the maximum feature value for this type of cells is 1 while the minimum is -1.6558, in the other hand the maximum feature length for this cell type is 1271 while the minimum feature length is 499. Other rows (cell types) read in the same manner.

SD Data Set Description

Table 5.7 Data Set Description for Shape Descriptor (SD) features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	Number of Cells
Burr	0.9912	0.1754	12	12	**114**
Sickle	1	0	12	12	**118**
Tear drop	1	0.1485	12	12	**59**
Normal	1	0.1630	12	12	**128**

This table (table 5.7) is a description for the Shape Description (SD) features this data set contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features are equal. For example the third raw is for Teardrop cells where the maximum feature value for this type of cells is 1 while the minimum is 0.1485, in the other hand the maximum feature length for this cell type is equal the minimum feature length which is 12. Other rows (cell types) read in the same manner.

5.4.2 Frequency domain Features (Wavelet Transformation)

In this domain, wavelet transform is applied into binary shapes to extract frequency features, another idea was to apply distance transformer on the binary shapes (cells) which give more information about the shape then apply wavelet to the result. As discussed in chapter 4 the distance transformer is labeling of each pixel of the object by the distance to the closest point in the background.

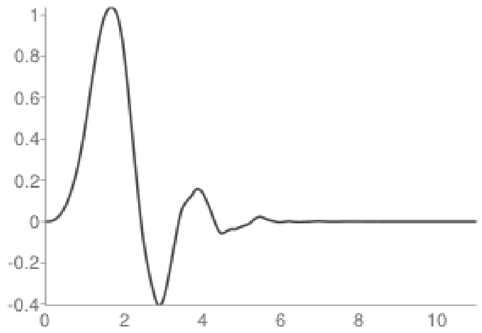


Figure 5.20 The Wavelet Daubechies 6 (db6)

For applying wavelet to the shapes (cells) the wavelet applied till the level three with Daubechies 6 **(**db6) check figure 5.20. This signal p**roperties are:** asymmetric, orthogonal, biorthogonal, all that makes it is the perfect one to be used with shapes in general especially cells. The Obtained results (Data sets) are the detailed coefficients (high frequency) of this process.

Wavelet Data Sets

Table 5.8 Data Set Description for Wavelet features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	**Number of Cells**
Burr	10.3508	-3.1039	15	15	**114**
Sickle	10.4481	-3.9622	15	15	**118**
Tear drop	10.8553	-2.6596	15	15	**59**
Normal	10.8359	-3.0436	15	15	**128**

This table (table 5.8) is a description for the wavelet features this data obtained from applying the wavelet on the cells in the binary form. The data set contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features are equal. For example the third raw is for Teardrop cells where the maximum feature value for this type of cells is 1 while the minimum is -2.6596, in the other hand the maximum feature length for this cell type is equal the minimum feature length which is 15. Other rows (cell types) read in the same manner.

Table 5.9 Data Set Description for Wavelet features for each type of cells

Type of The Cell	Maximum Features Value	Minimum Features Value	Maximum Features Length	Minimum Features Length	**Number of Cells**
Burr	129.8418	-10.2216	15	15	**114**
Sickle	90.2513	-12.2198	15	15	**118**
Tear drop	128.8718	-9.8820	15	15	**59**
Normal	149.4929	-11.8421	15	15	**128**

This table (table 5.9) is a description for the wavelet features this data obtained from applying the wavelet on the cells in the binary form. The data set contains features for four different types of cells the number of cells for each type of cell is variant and the lengths of these features are equal. For example the first raw is for Burr cells where the maximum feature value for this type of cells is 1 while the minimum is -10.2216, in the other hand the maximum feature length for this cell type is equal the minimum feature length which is 15. Other rows (cell types) read in the same manner.

First: Interpolating

There are five interpolation methods used to do this work, they are: nearest, linear, spline, cubic, pchip:

Nearest: Nearest-neighbor interpolation; the output pixel is assigned the value of the pixel that the point falls within. No other pixels are considered.

Linear: linear interpolation; the output pixel value is a weighted average of pixels in the nearest 2-by-2 neighborhood

Cubic: cubic interpolation; the output pixel value is a weighted average of pixels in the nearest 4-by-4 neighborhood.

Pchip: applying cubic interpolation on subintervals gives pchip.

Second: Histogram

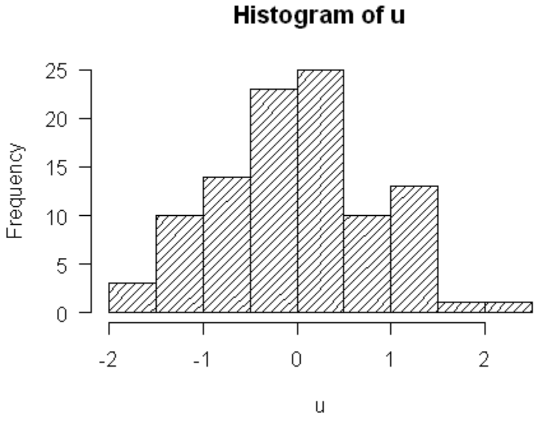


Figure 5.21 Histogram of the feature vector into fixed size of bins.

Histogram in figure 5.21 plots the data values to their frequency on the data (y-axis). That can be done with fixed size where the numbers of data values (sub-intervals) are fixed in order to unify the size of the features set.

5.5 Classification and Selection Stage

To use a genetic algorithm, we must represent a solution to our problem as a genome (or chromosome). The genetic algorithm then creates a population of solutions and applies genetic operators such as mutation and crossover to evolve the solutions in order to find the best solutions. The most important aspects of using genetic algorithms are: (1) definition of the objective function, (2) definition and implementation of the genetic representation, and (3) definition and implementation of the genetic operators. For the Genetic algorithm which is the heart of this thesis, GA used in unsupervised learning (Clustering) the data. The objective function

Elements of the population $x = (x_x, y_x)$;

Centroid of the cluster i is (x_i, y_i)

Centroid of the cluster j is (x_j, y_j)

$$Sim_i = \left(\frac{1}{|C_i|} \sum_{x \in C_i} \left\| (x_x, y_x)_x - (x_i, y_i)_i \right\|_2 \right) \qquad \textbf{5.9}$$

$$Re_i = Max\left\{\frac{Sim_i+Sim_j}{d_{ij}}\right\}_{j,j\neq i} \quad \textbf{5.10}$$

Where $d_{ij} = d(C_i, C_j) = \left\|(x_i, y_i)_i - (x_j, y_j)_j\right\|_2$ **5.11**

$$D = \frac{1}{k_r}\sum_{i=1}^{k_r} Re_i \quad \textbf{5.12}$$

$$\text{Fitness (x)} = \frac{1}{D}$$

We need here to obtained good results to maximize the difference between clusters and minimize the difference between elements among one cluster and this is accomplished by minimizing the Re and maximizing the Sim, Meaning maximizing the Fitness function.

Genetic Algorithm Parameters

The default chosen parameters for GA during this thesis are:

1- Initailization of population is random.

2- Populatition size is 20.

3- Maximum number of generation is 100.

4- Maximum time limit is infinity (no limit).

5- Crossover fraction percentage is 80%.

6- Mutation Function Fraction is: 0.2.

7- Crossover Function: scattered.

8- Migration Direction: Forward.

9- Function tolerance: 1e-6.

10- Selection function is: stochastic.

11- Scaling function is: Rank.

Chapter 6: The Proposed System Results

6.1 Introduction

In this chapter the results from the proposed system in chapter 5 is discussed. The categories are four: normal cells, Sickle cells, Burr cells and Tear drop cells. In other cases the data (set of features) classified into two categories: normal cells and abnormal cells. Note that Sickle cells, Burr cells and Teardrop cells considered abnormal cells. We applied the designed GA with the previous objective function and with the previous parameters mentioned in last of chapter 5.

6.2 Spatial Domain Results

The results obtained with the spatial domain data sets are discussed in the following two sections, first CDF and the second TAR:

6.2.1 Centroid Distance Function (CDF) Results

The CDF features have different lengths for different cases and they are not equal each others, to address these problem two solutions were suggested:

First using interpolation:

Interpolation works by using known data to estimate values at unknown points. For example: if we wanted to know the temperature at noon, but only measured it at 10AM and 12:30PM, we could estimate its value by performing a linear interpolation: In this case two problems presented themselves first we must choose the length of data as a parameters for the interpolation process (what is the appropriate length?), second problem there are several types of interpolation methods (which one is the best in our application?). A suggested solution for these two problems a study made for three possible lengths (minimum, mean and maximum length) and for five interpolation methods:

Table 6.1 Result for different scenarios to classify CDF data into four categories (Normal, Teardrop, sickle and Burr)

Type of interpolating	GA	K-Means		GA	k-Means		GA	k-Means	
Nearest	20.1	19.0	**CDF max 1500**	27.6	27.50	**CDF min (100)**	26.02	19.97	**CDF mean (800)**
Linear	24.07	0.8		28.15	36.41		23.20	20.125	
Spline	33.87	32.62		26.73	42.72		37.12	29.00	
Pchip	44.33	26.7		24.83	36.82		51.00	29.66	
Cubic	20.62	26.7		47.87	27.06		30.34	29.66	
V5cubic	24.02	0.8		32.67	36.41		43.78	19.9	

The table 6.1 contains in the first column the type of interpolation method used to unifies the features lengths. The second column is the success rate of GA and the third column is for the success rate result of K-Means. All these results when the data CDF lengths 1500 point. Reading the rest of columns is the same. The test was made by measuring the success rate in classifying the data in K-Means and GA with different interpolating method and different data lengths listed in table 6.1. Best result for CDF features was at mean length (the average) and at pchip interpolating methods. The 51.00% success rate not very good result any way!

6.2.2 Triangular Area Representation (TAR) Results

Table 6.2 Result for different scenarios to classify data TAR into four categories (Normal, Teardrop, sickle and Burr)

Type of interpolating	GA	K-means		GA	k-Means		GA	k-Means	
Nearest	27.3	25.2	**TAR max 1500**	29.03	26.22	**TAR min (100)**	27.94	23.958	**TAR mean (800)**
Linear	25.5	26.1		26.80	25.16		25.26	21.898	
Spline	28.48	28.61		26.52	25.35		42.46	30.29	
Pchip	33.55	28.60		26.11	26.03		28.88	28.389	
Cubic	30.91	28.60		26.24	25.577		28.24	27.33	
V5cubic	24.04	4.60		5.52	26.44		26.97	24.633	

The table 6.2 contains in the first column the type of interpolation method used to unifies the features lengths. The second column is the success rate of GA and the third column is for the success rate result of K-Means. All these results when the data TAR lengths 1500 point. Reading the rest of columns is the same. As you may notice the Best result according to table 6.2 was at mean length (average length), spline interpolation method.

Second: using Histogram

Histogram is another way used to unifies the data lengths (certain values, and there frequencies). So we have three scenarios: one using interpolating, second using histogram and third using both methods together first interpolation then histogram. We need to make the data or the set of features informative as possible. The result was:

First for CDF features data using GA classifier:

Table 6.3 Result for different scenarios to classify data into four categories (Normal, Teardrop, sickle and Burr)

Interpolating Method	Using histogram	Data size	K-means	Success Rate
Yes-Pchip	Yes	1010	No	**53.0977**
Yes-spline	Yes	1010	No	**36.0318**
Yes-Pchip	Yes	505	No	**55.9611**
Yes-spline	Yes	505	No	**35.3888**
Yes-Pchip	No	1010	No	**51.00**
Yes-spline	No	1010	No	**37.12**
No	Yes	1010	No	**42.909**
No	Yes	505	No	**26.1391**
Yes-Pchip	Yes	1010	Yes	**41.5775**
Yes-spline	Yes	1010	Yes	**26.3009**
Yes-Pchip	Yes	505	Yes	**40.9270**
Yes-spline	Yes	505	Yes	**36.9181**
Yes-Pchip	No	1010	Yes	**28.8999**
Yes-spline	No	1010	Yes	**31.6587**
No	Yes	1010	Yes	**42.909**
No	Yes	505	Yes	**27.5927**

The table 6.3 interpretation is for the first raw → the first columns the interpolation method name and its type pchip, second columns yes histogram implemented on the resulted data and third columns the size of the histogram (or resulted data) is 1010 and no we do not used the K-means to give the initial population for the Genetic algorithm classifier. We can conclude from this table that using interpolating with histogram without using the k-means to give the initial estimation of the population and with data size 505 gave the best success rate till now which is 55.9611%.

For TAR Data using GA classifier:

Table 6.4 Result for different scenarios to classify data into four categories (Normal, Teardrop, sickle and Burr)

Interpolating Method	Using histogram	Data size	K-means	Success Rate
Yes-Pchip	**Yes**	1010	**No**	**36.2362**
Yes-spline	**Yes**	1010	**No**	**33.0748**
Yes-Pchip	**Yes**	505	**No**	**36.2362**
Yes-spline	**Yes**	505	**No**	**33.7807**
Yes-Pchip	No	1010	**No**	**35.8497**
Yes-spline	No	1010	**No**	**28.6703**
No	**Yes**	1010	**No**	**25.4237**
No	**Yes**	505	**No**	**28.4322**
Yes-Pchip	**Yes**	1010	**Yes**	**26.7395**
Yes-spline	**Yes**	1010	**Yes**	**25.9012**
Yes-Pchip	**Yes**	505	**Yes**	**25.9012**
Yes-spline	**Yes**	505	**Yes**	**37.7565**
Yes-Pchip	No	1010	**Yes**	**35.8720**
Yes-spline	No	1010	**Yes**	**42.3877**
No	**Yes**	1010	**Yes**	**28.0519**
No	**Yes**	505	**Yes**	**25.4237**

In this table 6.4 the first column demonstrate the used interpolation method along with yes or no to demonstrate if the interpolation process performed or not. In this table we see that best success rate was 42.3877% which is happen when using interpolation without following it with histogram operation, along with k-means to lead the GA classifier and gives the initial set of population and with data size 1010. Using GA to cluster the data into two categories (Normal, Abnormal) where the features (CDF and TAR) represented using the histograms operation only check the following table 6.5:

Table 6.5 CDF and TAR signatures in histogram form.

Data Type	Data Length	The Result
CDF	5	68.7218
CDF	10	68.1594
CDF	100	67.8157
CDF	1000	68.1594
TAR	5	58.1830
TAR	10	52.9626
TAR	100	52.9317
TAR	1000	60.7751

In the table 6.5 the furst column shows the data feature type (CDF or TAR), the second column is the data length of these features and the last column demonstrate the success rate (Result). In this table we are only interested in classifying the data into two groups normal and abnormal, best success rate given at CDF feature data and this happens when the data size is 5. So we have learned from this that the five as a length is enough to represents the CDF as data set that informative as possible.

For TAR signature be aware that there is one parameter were fixed during the past work, in this paragraph we manipulates it as possible this parameters called ts, for each three points $P_{nb-ts}(x_{nb-ts}, y_{n-ts})$, $P_{nb}(x_{nb}, y_{nb})$ and $P_{nb+ts}(x_{nb+ts}, y_{nb+ts})$, where $nb \in [1, N\text{b}]$ and $ts \in [1, Nb/2 - 1]$, Nb is assumed to be even. The signed area of the triangle formed by these points is given by the equation 4.17 in chapter 4. So why we do not change ts and sees which value will give better results. Previously ts were equal 3, what if we change it to variable value:

Table 6.6 Results from using TAR data to classify the data into four categories (Norma, Teardrop, Sickle and Burr cells)

ts	Success Rate
3	21.34
$\frac{Nb}{2} - 1$	32.9134
$\frac{Nb}{4} - \frac{1}{2}$	33.8407

The table 6.6 first column shows the ts values, the second column demonstrate the success rate. The last case in table 6.6 gives the best success rate.

6.2.3 Shape Descriptor (SD) Results

Using Genetic Algorithm in clustering the Shape Descriptor (SD) features the initial success rate was 80.1%, but for k-means was 86.74% when k-means used to give the initial population estimation of the GA population, the resultant classifier (Combined GA and K-means (GAK)) gave a success rate 89.2%. For clustering the same data into two categories (normal and abnormal) the k-means alone gave 83.16% but the GA alone gave 99.4845%.

6.3 Manipulating the GA parameters

In case of changing population size from 20 to 200 (20, 50, 100 and 200) for the Shape descriptor (SD) features the success rate do not change significantly, for changing the selection function to all types, the Roulette and stochastic both of them gave the best success rate which is (89.2%), For the Reminder the success rate was

88% but for the Uniform the success rate was 89.1% and For the Tournament the success rate was 88.6%, the following table 6.7 demonstrates these results.

Table 6.7 Scaling function manipulations.

Selection function	Result
Roulette	89.20%
Stochastic	89.20%
Reminder	88.00%
Uniform	89.10%
Tournament	88.60%

Table 6.7 proposes variant selection function in attempts in testing all scenarios, the second columns demonstrates the success rate,

Spatial Domain Results (summary)

After multiple trials for different data lengths for CDF features data combined with SD features the best success rate was at CDF with 5 as a length, represented in histogram operations all that combined with the SD features, the success rate were 89.47%, but for clustering data into two categories (**Normal and Abnormal)** CDF lengths in histogram form combined with the SD features the success rate were 81.34%, but with CDF in 5 as a length for it, representative in interpolation spline form combined with SD feature the success rate was 97.1408%. For CDF in interpolating pchip form and 5 as a length for it with SD features combined with them, the success rate was 80.2405%. Now doing all that but with TAR instead of CDF, the result were worse for that we don't use it, but in case adding two elements of TAR (TAR features of length two) in the histogram form and CDF in the histogram form with length 5 combined with SD features the result was 89.47% which is the best success rate in this thesis till now.

Result Comments and Justification

The result of TAR was worse than the result from CDF, the main reason is that most of the shapes (cells) we deal with are either elliptical (or almost elliptical) or circular. Expressing them in TAR does not give good description for them. Even CDF which measures the distances from the centroid to the boundary points. The made features which is the shape descriptor were much expressive and give more information with higher classification success rate.

6.4 Frequency Domain Results

Wavelet transformation for the binary shapes, two ways were used to address this binary shapes, first convert the binary shapes directly into wavelet coefficients, the second convert the distance transformation of the binary shapes into wavelet coefficients. The first called data1 and the second called data2, we do not know yet which contain more information from the other. For this we make the following table 6.8:

Table 6.8 The wavelet Result.

Data	Size	Classifier	Success Rate
data1	5	GA	25
data1	5	K-means	41.6580
data1	10	GA	25
data1	10	K-means	44.3979
data1	100	GA	25
data1	100	K-means	26.3468
data1	200	GA	25
data1	200	K-means	26.1278
data2	5	GA	25
data2	5	K-means	44.6428
data2	10	GA	25
data2	10	K-means	50.7926
data2	100	GA	25
data2	100	K-means	42.9350
data2	200	GA	25
data2	200	K-means	42.2440

Table 6.8 the first column to indicates the data (features) type, data1 as mention before is the wavelet result obtained after applying the wavelet db6 to the third level on the binary cells with taking high frequency values only which is the detailed form of the cells. Data 2 is the same but the binary cells converted to grey cells with distance transformer then wavelet (db 6) applied to the third level and the high frequency values were obtained.

Result Comments and Justification

The result of wavelet was as expected but that can be justified when we examine normal cells and burr cells shapes. In some cases normal cells due to noise or cells capturing positions shows few curves on the cells boundaries which make the obtained features for its similar to burr cells, even the burr cells can demonstrates some rare cases where the cells are almost circular that also can make their features close to the normal cells features.

6.5 Final Result

Combining frequency domain and spatial domain features together gave the following results:

Table 6.9 Final result.

Classifier	data1 size	data2 size	CDF size	SD	Result
K-means	5	5	0	Yes	93.9786
GA	5	5	0	Yes	92.2854
K-means	5	5	5	Yes	90.4323
GA	5	5	5	Yes	87.8419
K-means	2	2	2	Yes	92.7297
GA	2	2	2	Yes	91.6961
K-means	1	1	2	Yes	91.1872
GA	1	1	2	Yes	90.5921
K-means	*5*	*5*	*2*	*Yes*	*94.0009*
GA	*5*	*5*	*2*	*Yes*	*92.3168*

Best result in this thesis for both K-means and GA with success rate percentage 94.0009% and 92.3168% respectively. In the case of K-means and GA the feature set that gave best results 94.00% and 92.3168% percentage respectively is formed from wavelet of the binary shape with 5 as a length for it and wavelet of the distance transformation of the binary image with 5 as a length for it and CDF with a 2 as a length for it all that with all SD (length 12).

Chapter 7: Conclusion and Future Work

7.1 Summary and Conclusion

This thesis discuses an image processing application, this application concerns blood films, the reason behind choosing such application that along with reasons mentioned before is the richness of these images with different types of object and there distinguishing features where it is hard sometimes to distinguish object form another even for experience eyes, all that leave us with no easy application allows us to try different techniques, methods and features, even the long work done here, this thesis covers only parts of Red Blood cells abnormalities where Red Blood cells abnormalities is part of the blood abnormalities. This state of art work opens the door for complementary work in any future work under this application

Sometimes the objects in hand is slam dunk cases where cells can be categories right away, but in other cases it's hard for doctors or even hematologist (blood lab specialist) to differentiates these object from each other. All that put us in different kinds of crossroad and moves our imagination and helps in extending our thoughts beyond and outside the box and what is familiar.

The techniques used sometimes helped in the classification process and in other times make situation worse, any way Tom Watson, Sr., the founder of IBM once said, "The way to accelerate your rate of success is to double your failure rate." And other says the heart of creativity is trial and error. Thomas Edison's the discoverer of the light bulb said "I have discovered a thousand things that don't work."

Best Result in this thesis for both K-means and GA with efficiency percentage 94.0009% and 92.3168% respectively.

In the case of K-means and GA the feature set that gave best results 94.00% and 92.3168% percentage respectively, is formed from wavelet of the binary shape length 5 and wavelet of the distance transformation of the binary image length 5 and CDF with length 2 with all SD (length 12). This result doest detracts the effort done in this thesis or minimize it. The features and the parameters that manipulated to reach this result it's not small and discussed thoroughly in chapter 5.

7.2 Future Work and Recommendation

There are many ideas to evolve and develop this thesis, some of these ideas are:

- Makes the population of the GA to be the coordination of the centroids of the needed cluster these coordination can either be double or binary data:

The idea behind this is to accelerate the classification phase and may b to coverage much faster with better success rate. This idea implemented among the work done for this thesis but not tested yet due to the time factor involving finishing this thesis.

- Using GA in supervised learning (learning phase then classification phase) and a comparison of using GA in supervised and non supervised learning (clustering).

Also this idea implemented among the work done for this thesis but gave miserable results; first guess of the reason of failure is the terrible execution of this idea.

- Work with curvelet and compare curvelet with wavelet and check if curvelet can help increase the efficiency of the classifier.

Also this idea implemented among the work done for this thesis but gave feature is considerably long and consumes huge amount of time, may be some trimming to these features is needed to make working with such feature possible, of course without distrusting there essence.

- Implementing Chord distribution signature and testing whatever this signature is informative or not and compare it with other two signatures used here (CDF and TAR).

The reason behind that why using this idea may help is that such a signature gave tow set of features vectors one for lengths and other of angles and both of these features are invariant to translation. May be such features contain information that other set of features discussed in this thesis does not have.

- Applying tree classification; since separating normal than abnormal has very high success rate, clustering the abnormal cases to three categories directly.

  Highest success rate obtained in this thesis came from classifying the cells into just two categories (normal and abnormal) may be after this classification, classifying abnormal cell into specific abnormal morphological gave better result.

- Besides the classifying normal, abnormal (sickle cell, burr cell and Teardrop) increase the number of abnormal morphological in hand to includes other shapes.

  The four categories/ classes mentioned and targeted in this thesis is just the start, blood morphological abnormalities shapes has no limit. May be working with third dimension help in identifying some abnormalities where the cells for example have spherical shapes which cannot be detected easily in two dimension images. Finding ways to capture 3-dimention images under the poor equipments found in here is discovery by itself.

Appendix

Appendix A. Classification of RBC Morphologic Abnormalities

The following table [52] gives details of the connection between the red blood cells abnormality shapes and the diseases that related to it.

Table A.1 Classification of RBC Morphologic Abnormalities

RBC Abnormality	Description of Cells	Associated Conditions
Normal erythrocytes ("Discocytes," "normocytes")	Round to slightly ovoid biconcave disks, approximately 7 m in diameter. Less hemoglobin in center of cell (zone of pallor). Regular in size and shape.	Normal individuals.
Acanthocyte ("Spur cells")	Spheroid RBCs with few large spiny projections. 5-10 spicules, irregular spacing and thickness (must be differentiated from echinocytes).	Abetalipoproteinemia, postsplenectomy, alcoholic cirrhosis and hemolytic anemia, microangiopathic hemolytic anemia, autoimmune hemolytic anemia, sideroblastic anemia, thalassemia, severe burns, renal disease, pyruvate kinase deficiency, McLeod phenotype, infantile pyknocytosis, post-splenectomy.
Autoagglutination	Irregular RBC agglutination/clumping resembling Chinese letters.	Anti-RBC antibody, paraprotein. Cold agglutinin disease, autoimmune hemolytic anemia, macroglobulinemia, hypergammaglobinemia
Basophilic stippling	Fine, medium, or coarse blue granules uniformly distributed throughout RBC. Fine stippling - polychromatophilia. Coarse stippling - Impaired erythropoiesis.	Heavy metal poisoning (e.g. lead and arsenic), hemoglobinopathies, thalassemias, sideroblastic anemias, pyrimidine-5'-nucleotidase deficiency
Bite cells	RBCs with peripheral single	Oxidant stress. Normal individuals

("Degmacytes")	or multiple arcuate defects. Usually associated with spherocytes and blister cells.	receiving large quantities of aromatic drugs (or their metabolites) containing amino, nitro, or hydroxy groups. Individuals with red-cell enzymopathies involving the pentose phosphate shunt (most notably G6PD deficiency.
Blister cells	RBCs with vacuoles or markedly thin areas at periphery of membrane.	Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Other oxidant stress.
Codocytes **("Target cells")**	Thin, hyopochromatic cell. Round area of central pigmentation.	Splenectomy, thalassemia, hemoglobinopathies (hemoglobin SS, SC, CC, EE, AE, sickle cell-thalassemia), iron deficiency anemia, liver disease, postsplectomy, familial lecithin-cholesterol acyltransferase (LCAT) deficiency.
Dacrocytes **("Tear drops")**	Cell in shape of tear drop. Usually accompanied by microcytosis and hypochromia	Myelophthisic anemia (particularly myelofibrosis with myeloid metaplasia), magaloblastic anemia, b-thalassemia, anemia of renal failure, tuberculosis, Heinz body disease, hemolytic anemias, hypersplenism.
Drepanocytes **("Sickle cells")**	Irregular, curved cells with pointed ends	Hb S hemoglobinopathies (sickle cell anemia, hemoglobin SC disease, hemoglobin S-beta-thalassemia, hemoglobin SD disease, hemoglobin Memphis/S disease), other hemoglobinopathies (especially Hb I, Hb CHarlem, HbCCapetown).
Echinocytes **("Sea urchin cells, crenated cells, burr cells")**	RBC with many tiny spicules (10-30) evenly distributed over cell	Post-splenectomy, uremia, hepatitis of the newborn, malabsorption states, after administration of heparin, pyruvate kinase deficiency, phosphoglycerare kinase deficiency, uremia, HUsS.

Elliptocytes	RBCs with elliptical or oval shape	Hereditary elliptocytosis, thalassemia, sickle cell trait, Hb C trait, cirrhosis, decreased erythrocyte glutathione, glucose-6-phosphate deficiency, iron deficiency anemia, megaloblastic anemia, myelophthisic anemia, hereditary hemorrhagic telangiectasia, mechanical trauma.
Howell-Jolly bodies	Small (1 mm), round, dense, basophilic bodies in RBCs.	Splenectomized patients, megaloblastic anema, severe hemolytic processes, hyposplenism, myelophthistic anemia.
Hyperchromia	Increased RBC hemoglobin concentration (MCHC > 36 g/dL). Usually associated with spherocytosis	Hereditary spherocytosis, immune hemolytic anemias.
Hypochromia	Decreased RBC amount (MCH) and concentration (MCHC). Expanded central zone of pallor	Iron deficiency, other hypochromic anemias.
Keratocytes ("Horn cells")	Helmet forms	Mechanical damage to red blood cells from fibrin deposits (DIC, microangiopathic hemolytic anemia, thrombotic thrombocytopenic purpura), prosthetic heart valves, severe valvular stenosis, malignant hypertension, or march hemoglobinuria, normal newborns, bleeding peptic ulcer, aplastic anemia, pyruvate kinase deficiency, vasculitis, glomerulonephritis, renal graft rejection, severe burns, iron deficiency, thalassemias, myelofibrosis with myeloid metaplasia, hypersplenism

Macrocyte	Large RBCs (> 8.5 mm, MCV > 95 fL). Normal MCH	Accelerated erythrocytosis. Macrocytic anemia (B12 or folate deficiency)(oval macrocytes)
"Thin" macrocyte	Increased diameter, normal MCV. Usually hypochromic	Liver disease, postsplenectomy
Microcyte	Small RBCs (< 7.0 mm, < 80 fL). Normal or decreased Hb	Iron deficiency, thalassemias, anemia of chronic disease, lead poisoning, sideroblastic anemia
Nucleated red blood cells ("NRBCs")	Immature RBCs, basophilic nucleus.	Acute bleeding, severe hemolysis, myelofibrosis, leukemia, myelophthisis, asplenia.
Poikilocytosis	Variation in RBC shape.	Many disorders.
Polychromasia ("Polychromatophilia")	Blue-gray coloration of RBCS. Due to mixture of RNA and hemoglobin.	Increased - Increased erythropoietic activity. Decreased - Hypoproliferative states.
Rouleaux	Linear arrangement of RBCs,"coinstack." Increased fibrinogen, globulins, or paraproteins (compare with autoagglutination, above).	Acute and chronic inflammatory disorders, Waldenstroms macroglobulinemia, multiple myeloma.
Schistocytes ("Fragmented cells")	Fragmented RBCs (compare with keratocytes, above)	Mechanical damage to red blood cells from fibrin deposits (DIC, microangiopathic hemolytic anemia, thrombotic thrombocytopenic purpura), prosthetic heart valves, severe valvular stenosis, malignant hypertension, or march hemoglobinuria, normal newborns, bleeding peptic ulcer, aplastic anemia, pyruvate kinase deficiency, vasculitis, glomerulonephritis, renal graft rejection, severe burns, iron deficiency, thalassemias, myelofibrosis with myeloid metaplasia,

		hypersplenism
Spherocytes	RBCs with spheroidal shape. Usually dense, small (< 6.5 mm) RBCS with normal or decreased MCV, and absent central pallor	Hereditary spherocytosis and hemolytic anemias (isoimmune or autoimmune), microangiopathic hemolytic anemia, hypersplenism and post-splenectomy, myelofibrosis with myeloid metaplasia, hemoglobinopathies, malaria, liver disease, older population of transfused cells, artifact. Microspherocytes in severe burns and hereditary pyropoikilocytosis.
Stomatocyte **(Fish mouth cell")**	Uniconcave RBC, slitlike area of central pallor	Hereditary or acquired hemolysis. Hereditary stomatocytosis, alcoholic cirrhosis, acute alcoholism, obstructive liver disease, malignancy, severe infection, treated acute leukemia, artifact.

Appendix B. Blood Films Preparation

Blood films are made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length [52]. The aim is to get a region where the cells are spaced far enough apart to be counted and differentiated. The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. The fixative is essential for good staining and presentation of cellular detail. After fixation, the slide is stained to distinguish the cells from each other.

MATERIALS

- Sterilized lancets or needles
- 20 clean microscope slides and coverslips
- Canada balsam or other medium for permanent preparations
- 95% ethyl or methyl alcohol
- Distilled water
- Giemsa stain or other.
- Low containers or Petri dishes.
- Microscope which magnifies 200 times at least

Taking the Blood

Cleanse a finger. With a sterile lancet, make a puncture on a fingertip. keep all the materials needed ready and protected from dust, particularly the clean microscope slides.

Making the Smear

Place a small drop of blood near an end of a slide. According to figure 7, bring the edge of another slide in contact with the drop and allow the drop to bank evenly behind the spreader. The angle between the two slides has to be 30-40 degrees. Now, push to the left in a smooth, quick motion. The smear should cover about half the slide. It is important that the quantity of blood is not excessive; otherwise the red cells could hide the leukocytes. So, if you succeed in making a gradual transition from thick to thin in your smear, you should get a zone with a satisfactory distribution of cells. With a single drop of blood, you can make several smears.

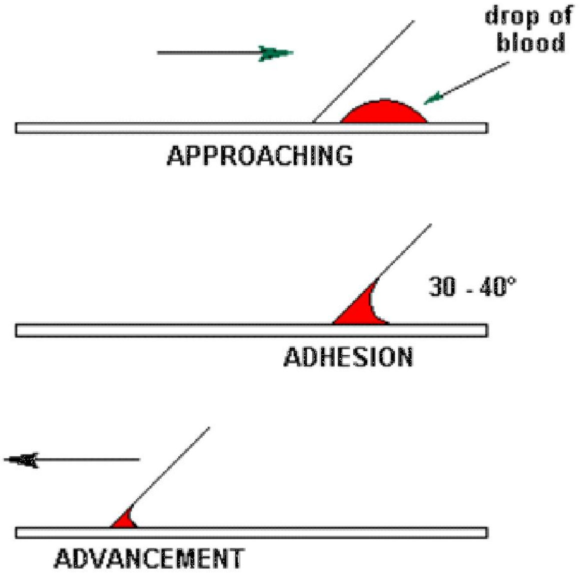


Figure B.1. Prepare blood smear

In fact, to make a smear, it is enough to leave a spot of blood of 3 mm about in diameter on the slide. It is useful to perform many smears. In fact, not always they are successful, and with some attempts, it is easier to get one well prepared. To avoid producing clots, you must make each smear with fresh blood and straight after having deposited it. To this purpose, it is useful to be helped by another person where one deposits the blood, and the other makes the smears. With the microscope, you should observe the smears to check that some of them are properly made. The red cells must not overlap each other, nor be so scarce as to be too spread out.

Fixing

If you apply the stain to a smear without having fixed it beforehand, the cells will explode because of the so-called **osmotic** or **hypotonic shock**. This happens because the saline concentration inside the cells is much higher than that of staining fluid which is diluted in distilled water. In the attempt to equal the internal saline concentration to the values of the external one, the cells undergo swelling by **osmosis**. To attain the same saline concentration of the external liquid, the cells should swell more than their membrane allows, in fact they explode. The cell contents are released, and the preparation becomes unusable. To avoid this, before staining, you have to **fix** the smear. This operation hinders the inflation of the cells which keep sound when they are stained. A simple and effective fixing technique consists of dipping the smear in a vessel containing 95% ethyl or methyl alcohol for 3-5 minutes. In order to put alcohol on the smear, you can also use a dropper or a bottle dispenser.

Staining

If you observe the smear as it is after fixing, you will see very little because all cells are very transparent. The erythrocytes are slightly visible, but the leukocytes are too pale, almost invisible and you will not see anything inside them. To be able to observe and recognize the different kinds of leukocyte, you must stain them. For this purpose, normally **Giemsa stain** is used. It is a mixture of stains, based on methylene blue and eosin. It is cheaply available commercially in volumes of 100 cc. It consists of a concentrated solution which you have to dilute in the proportion1/10, that is one part of Giemsa in nine of distilled water, or buffer solution (pH = 6,8-7,2). You can buy the stain in a store of chemicals and laboratory equipment.

To stain a smear, take a slide with a fixed and dry smear. Put on the slide a drop of stain until it is fully covered. Stain for about 16 minutes, renewing the stain about four times. Then rinse the slide with distilled water at room temperature. Drain off the water and leave the slide to dry.

Checking

With the microscope, verify that the cells are well stained. If necessary, apply the stain for a few more minutes. If you were planning to mount the slide with Canada balsam, the staining has to be stronger.

Cover-Slipping

At this point, your smear is ready to be observed, but if you want to keep it for a long time, you should make the preparation permanent. To this purpose, after drying the slide, place a drop of **Canada balsam** or another medium mountant on the smear, then mount the coverslip. If the balsam is too viscous, you may heat a few of the slides (but not over 40 degrees C) to help the balsam flow between the slide and coverslip.

Observation

A magnification of 200 times is enough to allow you to observe and identify the different types of cells. If you use a higher power, you can also see the cells details better. You can examine either with dry objectives or with the oil immersion technique. In this last case, if you have put on a coverslip, you must wait a day to allow the balsam to set, otherwise, when you move the slide, oil will displace the coverslip.

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قسم هندســـــة الحاســـــوب
كلية الـهندســـــــــة
عمادة الدراســـــــات العليا
الجامعة الإسلامية ـ غزة
فلســـــــــطين

تحديد أشكال خلايا الدم الحمراء المريضة باستخدام الجينات الخوارزمية والكي مينز

رسالة مقدمة كجـــــزء من متطلبـــات نيل درجة الماجســـتير في كلية الدراســـات العليا
قســـم الهندســـة تخصص هندســـة الحاســـوب

اعداد:

المهندســـة/ فاتن فرج ابوشمالة

اشـــــــراف:

الدكتور.المهندس / محمـــــد احمد الحنجوري

غزة، فلسطين

(1433هـ ,2012)

إهداء

إلهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلا بطاعتك.. ولا تطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك .. ولا تطيب الجنة إلا برؤيتك

اهدي هذا البحث

إلى من كلل العرق جبينه.. وشققت الأيام يديه

إلى من علمني أن الأعمال الكبيرة لا تتم إلا بالصبر والعزيمة والإصرار

إلى والداي أطال الله بقاءهم، وألبسهم ثوب الصحة والعافية، ومتعني ببرهم ورد جميلهم،

أهديهم ثمرة من ثمار غرسهم

والى ابنتي الحبيبة لين بارك الله لي فيها والى زوجي العزيز

وإلى من زرعوا التفاؤل في دربنا وقدموا لنا المساعدات والتسهيلات والأفكار والمعلومات، ربما دون أن يشعروا بدورهم بذلك فلهم منا كل الشكر، وأخص منهم:

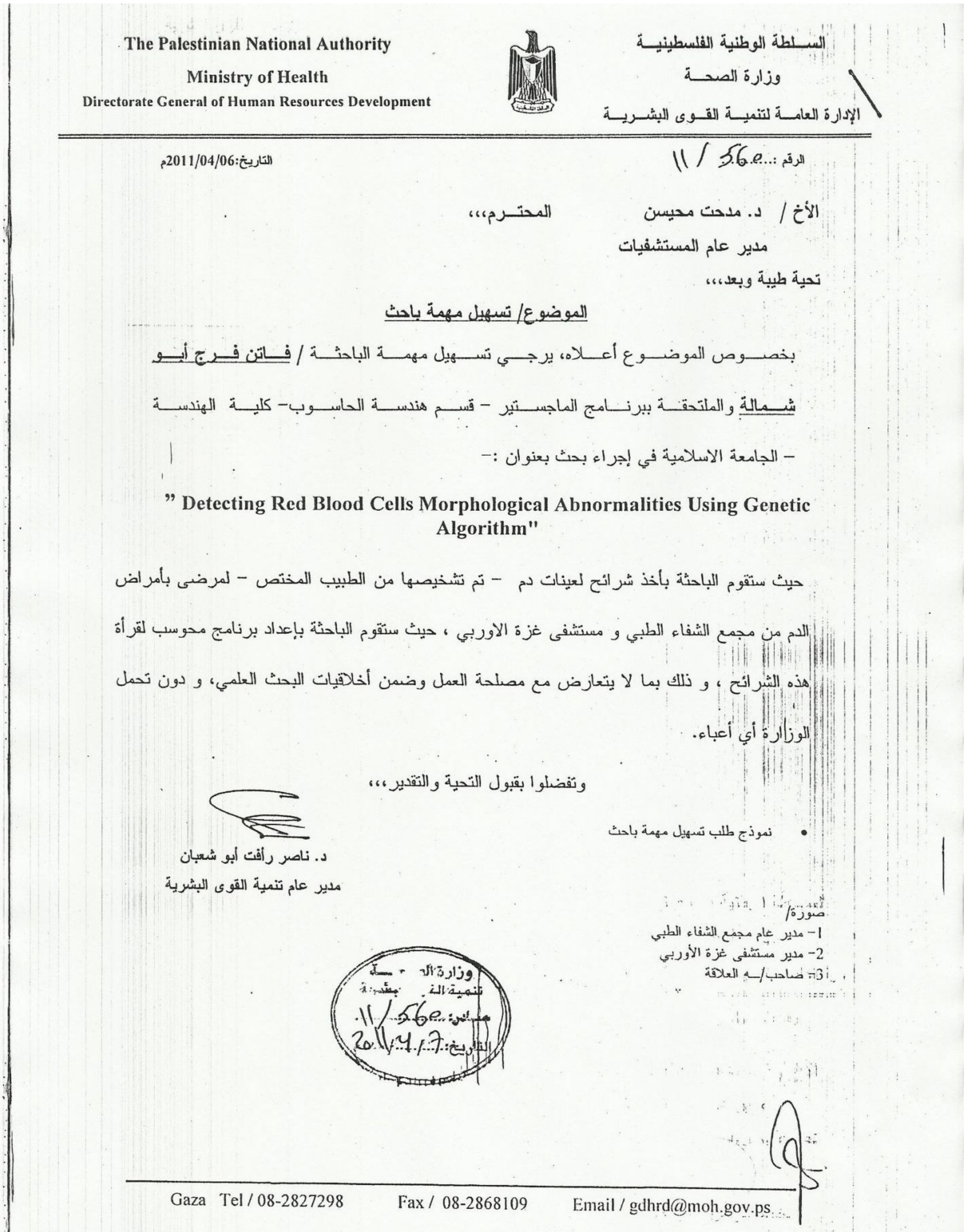
الدكتور مسعود ابوحليمة

الدكتور وسام عاشور

والأستاذ الدكتور إبراهيم أبوهيبة

والى الدكتور/ محمد احمد الحنجوري

الذي تفضل بالإشراف على هذا البحث فجزاه الله عنا كل خير فله منا كل التقدير والاحترام ..



The Palestinian National Authority
Ministry of Health
Directorate General of Human Resources Development

السـلطة الوطنية الفلسطينيـة
وزارة الصحـة
الإدارة العامـة لتنميـة القـوى البشـريـة

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الأخ / د. مدحت محيسن المحتـرم،،،
مدير عام المستشفيات
تحية طيبة وبعد،،،

الموضوع/ تسهيل مهمة باحث

بخصوص الموضوع أعلاه، يرجي تسهيل مهمة الباحثة / **فاتن فرج أبو شمالة** والملتحقة ببرنامج الماجستير – قسم هندسة الحاسوب- كلية الهندسة – الجامعة الاسلامية في إجراء بحث بعنوان :-

" Detecting Red Blood Cells Morphological Abnormalities Using Genetic Algorithm"

حيث ستقوم الباحثة بأخذ شرائح لعينات دم – تم تشخيصها من الطبيب المختص – لمرضى بأمراض الدم من مجمع الشفاء الطبي و مستشفى غزة الاوربي ، حيث ستقوم الباحثة بإعداد برنامج محوسب لقرأة هذه الشرائح ، و ذلك بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، و دون تحمل الوزارة أي أعباء.

وتفضلوا بقبول التحية والتقدير،،،

- نموذج طلب تسهيل مهمة باحث

د. ناصر رأفت أبو شعبان
مدير عام تنمية القوى البشرية

صورة/
1- مدير عام مجمع الشفاء الطبي
2- مدير مستشفى غزة الأوربي
3- صاحب/ـه العلاقة

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