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SEGMENTATION OF TISSUE IMAGES STAINED WITH HEMATOXYLIN AND EOSIN USING ARTIFICIAL BEE COLONY ALGORITHM

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SCIENTIFIC ETHICS DECLARATION

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SUITABILITY FOR GUIDE

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ACCEPTANCE AND APPROVAL PAGE

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ABSTRACT

Image segmentation is a very important topic in image processing. There are lots of different algorithms for segmentation of medical images. In this thesis, we study 3 methods for color image segmentation, particularly histological images stained with Hematoxylin and Eosin.

Color image segmentation is a process of extracting information from the images more connected regions satisfying uniformity criterion which is based on features derived from spectral component. An RGB color image is an MxNx3 array of color pixels, where each color pixel is a triplet consists to the red, green and blue components of an RGB image at specific spatial location. Color images provide more information than gray version of it.

In this thesis, 5 images obtained from the literature were segmented by K-means, fuzzy C-means and ABC based iterative Euclidean distance minimization algorithms. Experiments were conducted both on RGB color spaces and Lab color spaces. Experimental results show that methods achieve successful segmentation results.

Keywords: Histological image segmentation, K-means, Fuzzy c-means, ABC (artificial bee colony).

HEMATOKSİLİN VE EOZİN İLE BOYANMIŞ DOKU GÖRÜNTÜLERİNİN YAPAY ARI KOLONİSİ ALGORİTMASI İLE BÖLÜTLENMESİ

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ÖZET

Görüntü bölütleme görüntü işleme için oldukça önemli bir konudur. Tıbbi görüntülerin bölütlenmesi için pek çok farklı yöntem vardır. Bu tez çalışmasında, renkli görüntü bölütlemesi özellikle de hematoksilin ve eozin ile boyanmış histoloji görüntüleri için 3 farklı yöntem çalışılmıştır.

Renkli görüntü bölütleme, bir görüntüden spektral bileşenlerden türetilen öznitelik tabanlı birbirine bağlı ve belirli bir tekdüzelik kıstasını sağlayan bölgeleri çıkartma işlemidir. RGB renkli görüntüler MxNx3 şeklinde bir renk matrisi içerir. Burada her bir renk, ilgili uzaysal koordinattaki kırmızı, yeşil ve mavi bileşenleri ihtiva eder. Bir görüntünün renkli hali, gri-seviyeli haline göre daha çok bilgi içerir.

Bu tez çalışmasında, literatürden elde edilen 5 farklı görüntü, K-ortalama, Bulanık C-ortalama ve Yapay Arı Koloni (ABC) algoritması tabanlı iteratifÖklit mesafesi minimizasyonu yöntemleri ile bölütlenmiştir. Deneyler hem RGB hem de Lab renk uzaylarında gerçekleştirilmiştir. Deneysel sonuçlar, 3 yöntemin de başarılı sonuçlar elde ettiğini göstermiştir.

Anahtar Kelimeler: Histolojik Görüntü Segmentasyonu, K-ortalama, Bulanık C-ortalama, ABC(yapay arı koloni).

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LIST OF SYMPOLS AND ABBREVIATIONS

ABC : Artificial Bee Colony

CT : Computed Tomography

ED : Euclidean Distance

FCM : Fuzzy C-means

H & E : Hematoxylin and Eosin

HVS : Human Visual System

Km : K-means

MRI : Magnetic Resonance Imaging

MSE : Mean Square Error

PSNR : Peak Signal to Noise Ratio

RGB : Red, Green and Blue

SSIM : Structure Similarity Index Metrics

UIQI : Universal Image Quality Index

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INTRODUCTION

Partitioning an image into homogeneous regions is called as image segmentation. Image segmentation is an important task in image analysis, understanding and interpreting such as medical imaging. The objective of image segmentation is to produce a segmented image which is simple and readable. Segmented images are better than the original images, since image segmentation provides practical information for other image analysis tasks. Images become more useful in daily life, such as health care, astronomy etc. Most of medical decisions are based on extracting information from medical images such as physiological, radiological and pathological diagnosis etc. A function of human body is necessary to understand functionality of organs and also their physical condition. The goal of medical image segmentation technique is used to identify different regions, organs and anatomical structures medical images techniques such as Magnetic Resonance Imaging (MRI) and Computed Tomography (CT). Therefore, image segmentation technique is used to help medical doctors to make an accurate decision, through reading and extracting information from segmented images. Nowadays, histology image analysis using computed aided diagnosis system becomes increasingly important. Computed aided diagnosis is very important in histological image analysis as automatically identifying regions of medical interest. In digital pathology, it becomes an important tool for automatic biopsy analysis. The implementation of the different purpose for digital pathology system includes classification, retrieval and segmentation. Using digital pathology system for image segmentation can be categorized into two based on its objective: gland or cell segmentation.

In this thesis, we studied segmentation algorithms for histological image stained with Hematoxylin and Eosin. The main aim is to divide cells into its components like nuclei, surrounded tissue and background. The most essential task to examine histologically

stained image tissue sections is to count the cell nucleus and to detect abnormality of cell nucleus. Staining is used by pathologist to separate cellular components for structural as well as architectural analysis of tissue for diagnosis. The popular staining technique, Hematoxylin and Eosin, is used to highlight or identify cell nuclei, cytoplasm and connective tissue. Cell nuclei is stained blue and cytoplasm and connective tissue is stained pink by Hematoxylin and Eosin.

Using color image is a challenging task in image processing and contains two critical issues; color model to be used and segmentation technique that will be used. Color images consist more attributes as compared to gray images. The color models used in this thesis RGB and Lab color spaces. Lab color space has been considered to have large gamut than RGB color space. Also, we develop a segmentation technique using on Euclidean distance based on iterative clustering and Artificial Bee Colony algorithm.

Image segmentation techniques are categorized into two: discontinuity and similarity. In discontinuity based technique, the partitioning of an image is technique based on same rapid changes in the intensity level of an image. Different methods used for image segmentation based on similarity are as follows:

- Region based techniques.
- Edge based methods.
- Hybrid techniques.

Literature Survey

Pathology analyzes the change in cell structure and tissue distribution by using microscope and then determines if a biopsy is malignant [1]. Image segmentation is difficult and complex task in image segmentation, medical image analysis, and diagnosis and computer pattern. There are many fields that are using image segmentation such as astronomy, computer vision, and medical imaging etc. [2]. Medical image segmentation is a difficuly job and also problematic task due to the intrinsic nature of the images [3].

Hematoxylin and Eosin (H&E) stained histological images are the most difficult and problematic ones to process. H&E images are the most complex due to variability in

staining of nuclei and surrounding tissue. Image segmentation is the most essential technique in medical diagnosis through the analysis of the microscopic images. Today, without implementing image segmentation method it is a difficult task to diagnose the disease correctly. Image segmentation separates objects of interest from background through various methods in image processing such as thresholding where the intensity value is used to separate the regions[4].

Well known image segmentation methods can be grouped as:

- Thresholding algorithm,
- Clustering techniques,
- Compression based methods,
- Histogram based methods,
- Edge detection,
- Region based techniques.

In image segmentation, the gray levels of pixels of the object are very different from the gray levels of the pixels of the background. Thresholding is an effective technique in separating the object from the background of the image. The main aim of thresholding is to choose the gray-level threshold value for discriminating the target object in an image from the background in term of their gray-level distribution. In threshold technique, binary image comes from converted gray level image. The information of the binary image should include the positions and shapes of the objects of interest [5]. The main problem of global thresholding techniques are that they can not distinguish those pixels which share the same gray level but do not belong to the same group [6].

Partitioning an image into similar regions is an image segmentation technique. Group of pixels with similar characteristics or properties are called region. A clustering method is to divide data set at hand into groups are more similar to each other than elements in different clusters. Clustering is a method of grouping a set of patterns into a number of clusters such that similar patterns are assigned to one cluster. Each cluster has well defined centroid. The objective of clustering technique is to measure the similarity or to compute the distance between the respective patterns. Clustering methods can be categorized into two:

- Supervised
- Unsupervised.

Supervised learning based image segmentation algorithms separate the image based on the sample of object colors using prior knowledge. Unsupervised methods are automatic methods they segment regions in feature space that have higher density [7].

An image compression technique is good at representing an approximation of original images with as few bits as possible while controlling the quality of these representations. The compression method implies sending or receiving a smaller of bits. The objective of image compression is to diminish the size of data required to represent an image. In general, there are two ways of image compression techniques, namely Lossless and Lossy Methods. In Lossless compression technique, the data is preserved without any loss. Lossy method data is an efficient method, so it takes short time and less space when it is sending large of amount of data such as image and video. In most of images, there is a common feature which is the neighboring pixels are correlated. Therefore, finding a less correlated representation of image is an essential task. The main idea behind image compression algorithm is to diminish the size of data required to be transmitted or saved. One of the basic concepts in compression is the reduction of redundancy and irrelevancy. This can be done by removing duplication from the image. Images are essential source, by getting more information, however, the modern hardware can be display more different colors, that is why it mostly to have a pixel represented internally as a 24-bit or 3 bytes number, where the percentages of RGB occupy 8 bits each. Such a 24-bit pixel can specify 16.78 million colors [8].

In generall, Histogram based method is simple and well known image segmentation technique and also used in many application. In histogram thresholding method, the best threshold value should be in the valley region. Generally, overall histogram thresholding methods perfom well if the digital image greys level histogram becomes bimodal or nearly bimodal. Even though many digital images are often ill defined which leads to multimodal histogram, so that the usually histogram thresholding methods works poorly or fail. The background of any image it is the grey level occupies most of

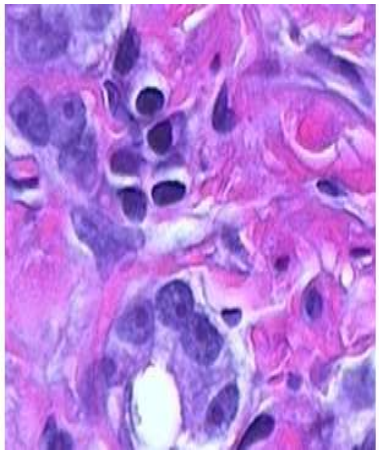
the image. So, its grey level is the large peak in the histogram. The object of the image has another grey level which itself has a small peak in the image objects [9].

Edge based segmentation is the position of pixels of the image pixels, in digital image which represents to the boundries of the object on the image scene. Edge is a boundry of an object then it is closed, number of objects and number boundry are equal in an image. In generally, the outputof image segmentation is a set of an area that entirely covers the all parts of the image, or a set of contours extracted from the image. An edge detection method makes the images to edge images using from the rapit changes of grey tones in the images. Edge is the sign of discontinuity, and ending [10]. Edge image can be found without any changes happened in quality of the main image. Edge, in an image, is a rapidly changing in an image intensity that associated with a lack of discontinuity in the image intensity. Edges are significant local changes of intensity in an image. Really, an edge based detection techniques works very well with high quality image, but often is not good for noisy images because it can not distinguish edges of different significance [11].

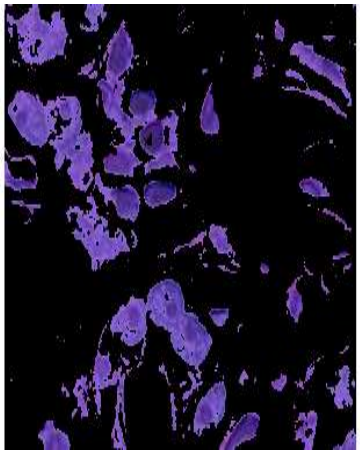
Region based techniques mostly based on an idea that neighboring pixels withing one area or group has similar value. Comparing one pixel with its neighbors that is region based method procedure. The pixel can be membership to the cluster if a similarity criterion is satisfied. The technique of detecting discontinuity property of is an egde based segmentation technique.

The unseeded region growing technique comes from of seeded region growing. Seeded region growing and unseeded growing region techniques the only difference between them is the seed select, unseeded region growing no need seed selection. Because of that unseeded region growing technique can be process automatically; there is no need to return parameters in unseeded region growing.

In image segmentation technique based on similarity criteria and region splitting and merging technique are very similar methods. The main aim of region splitting technique is to identify similarity of the image. Region splittingtechniquechecks the quadrants for the same defined criteria and divides it further into four quadrants if the test result is negative and the process continues till the criteria is satisfied [12].



Hematoxylin and Eosin stained image[22]



Hematoxylin and Eosin segmented image

Figure 1. H & E stained image and its segmented result.

In Figure 1, an example for color based image segmentation using K-means clustering method is shown. In this example using hestained.png, which is an image of tissue stained with Hematoxylin and Eosin, obtained results give pathologist to distinguish different tissue types.

Motivation

Medical imaging becomes part of human life in health care, recently. There are many medical imaging techniques used to produce medical images such as X-rays, ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT). In many years, for diagnosis and analysis, several methods are proposed to use in hospitals and clinics because of that the results become more accurate and fast. When images are segmented perfectly, one can easily extract information from them, and this makes them simple and easy to analysis. That is what makes medical decision more reliable and increases patient safety.

Recently, automatic image segmentation techniques for histological image segmentation become an essential for medical diagnosis and analysis to understand if cell is affected by the cancer or not. Motivation of this thesis is to develop an automatic image segmentation algorithm that can be used to segment histological images stained with

Hematoxylin and Eosin (H&E) to produce an optimum segmented image (result) that makes possible to analyses and detect easily for the cancer.

Aim and Contribution

Medical imaging has transformed medicine and changed diagnosis and treatments very fast and more accurate. Medical imaging is very important for medical conditions and disease. Images are the most important resource that carrying information and it is very important to understand images and extracting information from them. Image segmentation is useful in medical field. It is identifying regions of interest and objects in the images that helps for image analysis.

Aim of this thesis is to simplify representation of pathological images to make the image reliable, more useful and simply to analysis. In generally, pathological section uses an image segmentation technique done by RGB color space. However, Lab color space is better to applay image then the RGB color space. In this thesis,is about histological image segmentation using both RGB and Lab color space, and also simple technique based on Euclidean distance based iterative clustering and Artificial Bee Colony algorithm is developed. Lab color space is color-opponent space with dimensional for lightness L, a and b for color-opponent dimensions, based on non-linearly compressed CIE XYZ color space coordinates.

All methods studied in this thesis, uses both RGB and Lab color spaces. ABC is an algorithm motivated by the intelligent behavior of bees which is used to solve more complex optimization problems. ABC based method is simple and adaptive, because Artificial Bee Colony algorithm works well on finding the better solution of the object function.

Thesis Outline

This thesis is presented in four parts. Firstly, some well-known image segmentation techniques are explained and also, briefly literature survey, motivation of this study and main aim and contribution are given. Chapter one explains color space and segmentation methods. In this thesis used color space such as RGB color space and Lab color space and Segmentation methods such as K-means and Fuzzy C-means. In chapter

two, segmentation method based on Artificial Bee Colony (ABC) algorithm and block diagram of method are explained.In chapter three, details of the implementation and experimental results are presented. In this thesis we used automatic image segmentation for histological images that stained with Hematoxylin and Eosin. In the chapter four, we have discussion, conclusion and recommendations.

The thesis is organizedas follows:

Chapter 1: RGB, Lab color spaces and K-means and Fuzzy C-means methods are described.

Chapter 2: Artificial Bee Colony (ABC) algorithm and block diagram of method are given.

Chapter 3: Implementation details and experimental results are presented.

Chapter 4: This chapter presents discussion, conclusion and recommendations.

CHAPTER 1

COLOR SPACE ANDSEGMENTATION METHODS

In this chapter, firstly RGB and Lab color spaces are introduced, then well-known segmentation techniques such as K-means and Fuzzy C-means algorithms are presented briefly.

1.1. Color spaces

A color space is a systematic way that can be representation of a set of colors. Color space is a technique that can be specified and visualize existing colors. Humans can describe a color by its hue (color), colorfullness and brightness. However, computers define a color by using its amount of RGB phosphor emission required to match colors. Clustering methods can be used to define similarity clusters of elements in the feature space and label cluster as a different region. Color space allows for reproducible representations of color, in analog and digital representations.

1.1.1. RGB Color Space

Color is a sensation produced by the human eye and nervous system. Light is fundamental for color vision. Without light there is nothing to see. Color space is a systematic method for creating a whole range of colors from a set of primary (RGB) color. RGB is most popular color space and commonly used in TV monitors, and digital cameras. Objective of color space is to improve the process of describing color, either between people or between machines. A disadvantage of the RGB representation is that the channels are much correlated, as all of them include a representation of brightness. However, when dealing with real world images, RGB is not an effective method.Thecomponents RGB should have equal band width to perform color within the RGB color cube.

Primary colors space is RGB; addition of primary color RGB produces the secondary colors of light, Cyan, Magenta, Yellow and Key (black). Yellow, magenta, cyan and are used in color printers. Additive color spaces are in systems that uses cathode ray tube (CRT) to display images. Additive color space is the three chromaticity's of RGB additive can produce any exist color [16]. The most of the computer graphics systems uses RGB color; it is not an ideal but it's practical in application. RGB color has red, green and blue components and using these can produce whole existing colors [17].

1.1.2. Lab Color Space

Color image segmentation methods use two basic properties: discontinuity and similarity. In image segmentation objects easily can be identify and extracted from a scene by using colors. Normally, human recognize a color by its properties of saturation, brightness and hue. Color space is systematic way of organization of color. Lab color space has all perceivable colors. It is gamut is larger than the RGB color space.

The Lab space is useful for sharpening images and the removing artifacts in JPEG images or in images from digital cameras and scanners. The Commission Internationaledel'Eclairage (CIE) has been developed this color space. Lab color space is a color opponent space that has three components L (lightness), a (involved red and green colors) and b (controls blue and yellow colors). It is non-linear coordinates and designed to approximate human vision. Also it is used as an interchange format between different devices. Lab model, can only be presented in a three dimensional space [20].

1.2. Segmentation Methods

Partitioning an image into a set of regions that can cover most of the image is called as image segmentation.It is the task of partitioning an image into similar areas or groups. Changing representation and simplifying an image into something useful and easier to diagnose that is the goal of an image segmentation technique. An unsupervised method is an automatic clustering that can be grouping unleveled data set into clusters. Segmentation techniques can be differentiated into the following basic concepts: Pixels-based, Edge-based, Cluster-based, Region-based, Color-based and Hybrid.

1.2.1. K-means Algorithm

K-means is the well-known clustering algorithm. In K-means clustering, the aim is minimizing the sum of squares of distances between data set and their cluster centroid. K-means clustering can be used for color image segmentation which is a type of clustering method. In K-means, the distance function perfoms to calculate the distance between the objects and centroid by using a distance measure such as Euclidean, Manhattan or Mahalanobis, etc. [13]. K-means clustering algorithm performance depends on initial centroid that is why the algorithm cannot guarantee the optimal solution. K-means is an algorithm that clusters the unleveled data point into multiple classes through their inherent distance from each other. K-means algorithm tries to find the natural clustering in them.

Normally, K-means algorithm can be used to perform the natural clusters of pixels in an image. K-means algorithm is simple and very fast algorithm that can be group the data set into *k* cluster. The simplest feature in color images is using the K-means algorithm to partitioning the image pixel colors into *k* cluster. K-means algorithm measures the distances between the data setelementsand elements goes closest center, and inputs goes to the nearest center. Each cluster in the partition is defined by its member objects and by its center.

The objects are clustered around the centroids $\mu i \forall i = 1 \ldots k$ which are computed by minimizing the following objective

$$V=\sum_{i=1}^{n}\sum_{x_{j\in S_i}} X_J - \mu_i \qquad (1)$$

where k is the number of clusters i.e. S*i*, $i = 1, 2, \ldots, k$ and μi is the mean point or centroid of all the points $xj \in Si$. The algorithm requires a color image as input. The algorithm of K-means clustering is as follows

Step 1 Compute the distribution of the intensity values.

Step 2 Using k random intensities initialize the centroids.

Step 3 Repeat the step 4 and step 5 until the labels of the cluster do not change any more.

Step 4 Cluster the image points based on the distance of their intensity values from the centroid intensity values.

$$C^i = \arg min_j \left\| x^{(i)} - \mu_j \right\|^2 \quad (2)$$

Step 5 Compute new centroid for each cluster.

$$\mu_i = \frac{\sum_{i=1}^{m} \{C_{(i)} = j\} x^i}{\sum_{i=1}^{m} 1\{ C_I = J \}} \quad (3)$$

where *k* is the number of clusters, *i* iterates over all the intensity values, *j* iterates over all the centroids (for each cluster) and μi are the centroid intensities [14].

1.2.2. Fuzzy C-means Clustering Algorithm

The objective of fuzzy algorithm is to allow data set elements to all different clusters with some degree of membership. Widely used clustering algorithm is iterative Fuzzy C-means.

Fuzzy C-means clustering algorithm is a clustering technique used to identify an optimal c partition to minimize the cost function defined as follows:

$$J_{FCM}(U,V) = \sum_{k=0}^{n} \sum_{i=0}^{c} u_{ik}^m d^2 (x_k, v_i) \quad (4)$$

where $X = \{x_1, x_2, \ldots\ldots\ldots x_n\}$ is the data set in the *p*- dimensional vector space, n is the number of data items, c is the number of clusters with $2 \leq c \leq n$, $u_{ik} = u_\iota(x_k)$ represents the membership degree of the k^{th} pixels to the i^{th} cluster, the parameter m is the degree of the fuzziness of the clustering process, v_i is the prototype of the center of cluster i, $d^2 (x_k, v_i)$ is a distance measure of similarity between the k^{th} data item and cluster center v_i. The membership function and cluster centers are defined by (5) and (6) in the process of iterations:

$$u_{ik}=\frac{1}{\sum_{j=1}^{c}(\frac{d_{ik}}{d_{jk}})^{2/(m-1)}} \quad (5)$$

$$\text{And} v_i=\frac{\sum_{k=1}^{n} U_{ik}^{m} X_k}{\sum_{k=1}^{n} U_{ik}^{m}} \quad (6)$$

First of all partitioning will be realized randomly. Fuzzy C-means cluster finds for local minimum of the cost function by converges to a clustering solution. This algorithm stops when two successive iterations does not chance [15].

CHAPTER 2

ABC BASED IMAGE SEGMENTATION METHOD

In this thesis, a method based on ABC optimization algorithm is compared with well-known methods. The ABC based method finds nearest neighbors of each centroid to minimize the distance of each pixel to its corresponding centroid. In this chapter, ABC algorithm is introduced firstly and afterwards details of the method is given.

2.1. Artificial Bee Colony Algorithm

Optimization is a method used to find values for a set of parameters that maximize or minimize objective functions to certain constraints. Algorithm as the name implies is an algorithm based on bees searching food sources. ABC is used to solve numerical and engineering design optimization problems. This method has become very popular and is mostly used; because of it has good convergence properties. Artificial Bee Colony hassome advantages such as simple, fast convergence, flexible, and small number of parameters. The segmentation method combines Euclidean distance minimization of each sub-neighbor of the centroids with Artificial Bee Colony optimization clustering method.

Karaboga defined the implementation of the Artificial Bee Colony algorithm that is and algorithm using less parameter compared with others [18]. ABC algorithm compared to other population techniques, ABC demonstrates simplicity and flexiblity. In ABC, food position stands a solution problem and nectar represents the objective function of the solution. Artificial bees are grouped into three: employed bees, onlooker bees, and scouts bees. Each employed bee carries with her information about the associated food source and shares the information to onlookers. Onlooker bees wait in the hive on the dance area for the information about the possible food sources, and then make decision to choose a food source in order to exploit it. Onlooker bees select a food

source because of its quality. Food sources with high quantity of nectar attract more onlooker bees compared to ones with a lower quantity of nectar. A scout bee seeks randomly for a new solution in the neighborhood of the hive. The employed bee whose food source has been exhausted becomes a scout bee which explores a possible promising food source in the neighborhood of the hive. In ABC algorithm, the number of employed or onlooker bees is mostly equal to the number of solutions in the population of solutions and there is one scout. Firstly, the ABC algorithm generates randomly an initial population of SN solutions where SN represents the number candidate solutions that correspond to employed bees or onlooker bees. Each solution (Z_i , $1 \leq i \leq SN$) isa Ddimensional vector, where D is the number of optimization parameters or decision variables. After the initialization phase, the population of candidate solutions undergoes during each cycle, (C = 1, 2, . . . , MCN), search processes conducted by the employed, the onlooker and scout bees. In the employed bees' phase, each employed bee generates a new solution from the neighborhood of the current food source (solution). The new food source (new solution) is calculated using the following expression $U_{i,\ j} = z_{i,\ j} + Q_{i,\ j}$ *($Z_{i,\ j}$ - $Z_{k,\ j}$) (i, K = 1, ..., SN , i ≠ $kandj\ = 1, \ldots., D$) (7)

In the onlooker bees' phase, each onlooker selects a food source with a probability proportional to the nectar amount of that food source (fitness of the solution) which is shared by employed bees. The Probability P_i of selecting the food source zis calculated using the following expression:

$$P_i = \frac{f(Z_i)}{\sum_{j=1}^{Np}(Z_j)}\text{(i=1,....,SN)} \quad (8)$$

Onlooker bee searches around its neighborhood exactly as an employed bee does using (4). It updates the selected food source position when it finds a better solution otherwise it retains the old solution. If a food source position cannot be improved through a predetermined cycles, called limit, it means that the solution has been sufficiently exploited, and it may be abandoned and removed from the population. In this case, the employed bee of that exhaustive food source becomes scout. The scout bee generates a new random food source position using the following equation:

$$Z_{i,\ j} = z^j_{min} + rand(0,\ 1)X\left(z^j_{max} - z^j_{min}\right)\text{(i=1,.....SN and j = 1,...., D)} \quad (9)$$

where theZ_{min}^{j} and $Z_{max}^{j}$are the lower and upper bounds of the j^{th} component of all solutions. A short pseudo–code of the ABC algorithm is given below:

Step 1. Initialize the population of solutions

Step 2. Evaluate the population

Step 3. Cycle = 1

Step 4. Repeat

Step 5. //Employed bee's phase

Step 6. Produce new solutions for the employed bees

Step 7. Apply the greedy selection process

Step 8. //Onlooker bee's phase

Step 9. Calculate the probability values

Step 10. Produce the new solutions for the onlookers

Step 11. Apply the greedy selection process

Step 12. // Scout bees phase

Step 13. Determine the abandoned solution for the scout, and replace it with a new randomly produced solution

Step 14. Memorize the best solution achieved so far

Step 15. Cycle = Cycle+1

Step 16. Until Cycle = Maximum Cycle Number (MCN)[19].

2.2. ABC Based Segmentation Method

K-Means algorithm is the method used to clustering unleveled data set into similar cluster and each input cluster is assigned the nearest cluster. The algorithm assumes that the data features form a vector space and tries to find natural clustering in them. In K-means clustering algorithm, the optimal solution depends on the initial seed selected of cluster and value of K. The most popular used algorithm is Fuzzy C-means. Fuzzy is a technique that can be assigning data set membership levels, and then using them to assign objects more then one cluster.

The proposed algorithm is efficient in term of performance. ABC algorithm is quicker for finding optimal solution then other two algorithms (K-means and Fuzzy C-means).

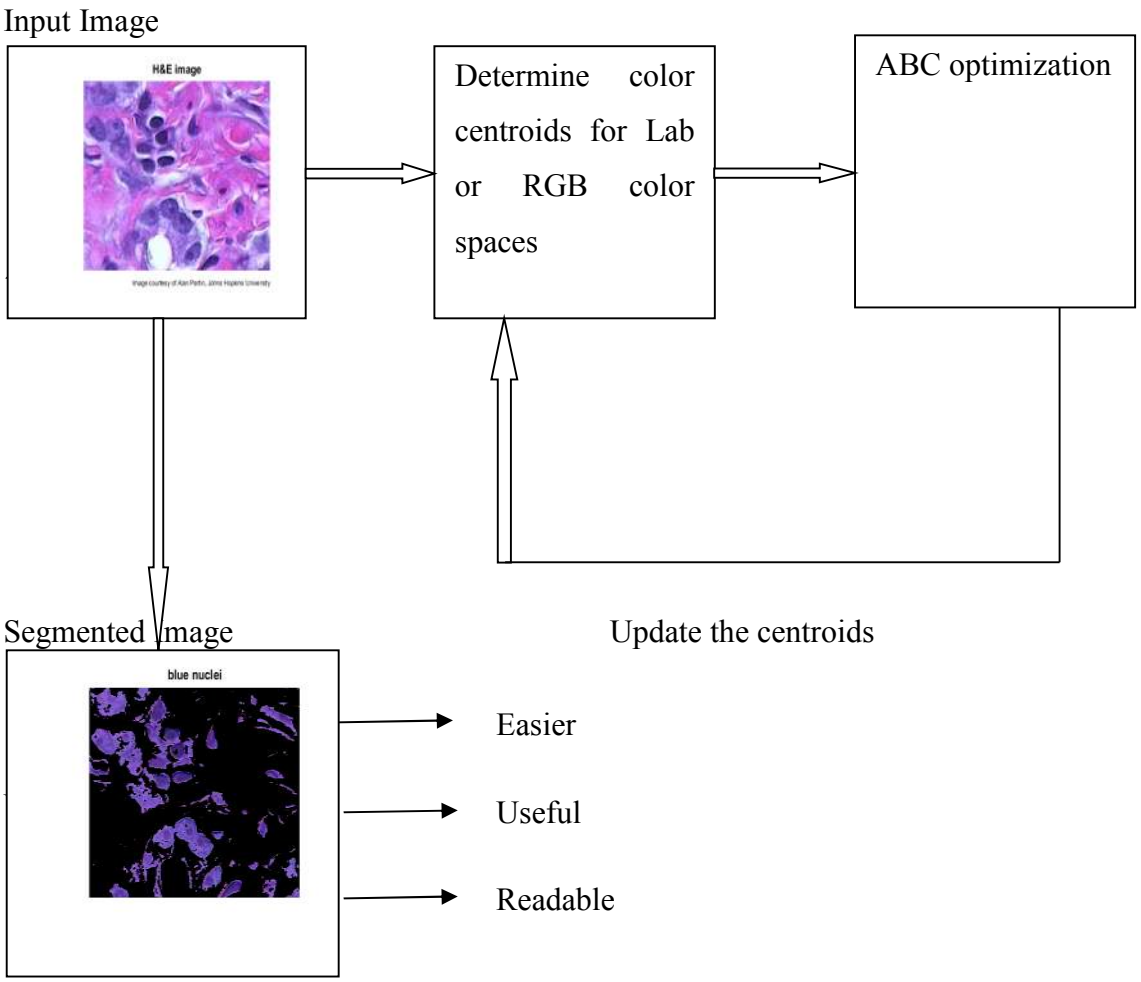


Figure 2.1. Block diagram of the proposed ABC based image segmentation.

An iterative segmentation technique based on optimizing the distance of sub-neighbors of each centroid by using ABC is designed. The method can use both RGB and Lab color values of the pixels. The block diagram of the method is given in Figure 2.1 and the steps of the algorithm as follows:

1) Read RGB image and decomposed R, G and B channels.
2) Convert image from RGB color space to Lab color space and decompose L, a and b channels.
3) Choose either to use RGB or Lab.
4) Segment the pixel values by using ABC:
   a) Generate initial random 3 centroids because of the images are composed of 3 parts: cell nuclei, cytoplasm and the remaining.
   b) Calculate the Euclidean distance of each pixel color value the generated centroids:
   $$D_j = \sum_{i=1}^{N} (P_i - C_j)^2 \quad (10)$$
   where N is the total count of pixels, P is a pixel's color value, C is a generated centroid and j={1,2,3}.
   c) Find the $E_i = \min(D_j)$ for each pixel and assign C_j to L_i where $D_j$is minimum. E is the error vector for all pixels and L is the segmented image.
   d) Minimize the total error of each pixels by updating the centroids C using employed, onlooker and scout bees.
   $$ED = \sum E_i \quad (11)$$
   e) Repeat the steps b), c) and d) until a predefined cycle number is met.
5) Use ED as the error metric and L as the segmented image.

CHAPTER 3

EXPERIMENTS AND RESULTS

In this chapter, details of the implementation and experimental results are presented. In this thesis, we used automatic image segmentation for histological images that stained with Hematoxylin and Eosin.

K-means, Fuzzy C-means and the ABC based method are used to segment histological images stained with H & E, and we compared their results. Also, we observed their sum of Euclidean distance errors (ED), error between the original image (MSE) and segmented the image and similarity between original images and segmented image (SSIM).

3.1 Image Quality Assessment Metrics

In image processing application an image quality measurement become an essential task. The image quality assessment is subjective study; involved human and aiming at measure their quality perception. Image quality measurement is most important task for most image processing application. The enhancement of video and image quality had become an importantin the design and development of displays. Image quality assessment is a method of measuring quality of image and also is a subjective method. Image quality can be measure into two ways:

1. Subjective
2. Objective assessments.

A real time system subjective is impossible to apply them into an automatic system. Subjective measurements can be used to validate the usefulness of objective measurements. Image quality assessment is the best technique of measuring image quality. Subjective is the commonlymethodthat allow to understanding of the

mechanisms quality perception, produce helpful information for the subsequent modeling stage.

Objective evaluations are automatic and mathematical defined algorithms. The mean squared error (MSE) is the one of most popular objective evaluation algorithms for measuring image quality, peak signal-to-noise ratio (PSNR), and structural similarity (SSIM). The objective measurement there are automatic algorithms for quality assessment that could analyses images and report their quality without human involvement.

Image quality can be measure by comparing it against a reference signal of quality. Measuring between the reference image and the image being evaluated can be calibrated to use as a measure of perceptual quality.

MSE works well if the distortion is caused by effect of the noise. The mean-squared-error is the simplest, and the most popular used, full-reference image quality measurement. MSE works satisfactorily when the distortion is mainly caused by contamination of additive noise.

HVS (Human Vision System) is very difficulty to understand with present psychophysical means, the objective measure reportedly leads to better correlation with the reponse of the human. The HVS is a method that uses human eye as a reference. Universal Image Quality Index (UIQI) breaks the comparison between original and distorted image into three comparisons: luminance, contrast, and structural. Well known method used to measure similarity between two images is structure similarity index measure method. Structure similarity index measure is used to measure an image quality on an initial not uncompressed or not distortion image as refference. Structure similarity index measure is used to improve on method such as peak signal to noise ratio Mean square error, which is proven to be inconsistent with human perception. Structural similarity (SSIM) is designed to improve on traditional methods like peak signal to noise ratio (PSNR) and mean squared error (MSE), which have proven to be inconsistent with human eye perception. The SSIM is designed to improve on traditional metrics like PSNR and MSE, which have proved to be inconsistent with human eye perception. SSIM based on human visual system [21].

3.2. Dataset

We have compared the K-means, Fuzzy C-means and ABC based image segmentation algorithms. The original images in the dataset are summarized in Table 3.1.

Table 3.1. Description of images dataset used in the expriments

Image Number	**Description**
Image #1	Size 200 x 132 Granular cell variant of clear cell renal cell carcinoma on hematoxylin and e stain. Institution Medscape [22]
Image #2	size 586 x 345 Cross-section of multi-cellular bio printed human liver tissue, stained with hematoxylin and Eosin (H&E) [23]
Image #3	Size 303 x 227 Hematoxylin and Eosin stained image[24]
Image #4	Size 480 x 319 kidney stained Hematoxylin and Eosin showing crenation in cells and overstraining [25]
Image #5	Size 583 x 365 H & E staining of tumor shows the characteristics of small cell carcinoma [26]

In Figure 3.1, five test images stained with H&E are shown. These images are used in the experiments to compare the algorithms.

3.3. Experimental Setup

We compared the ABC based clustering method with the K-means and Fuzzy C-Means algorithms. Parameter settings for the methods used in the experiments are given in Table 3.2.

Table 3.2. Parameter settings for different clustering methods.

K-means		**Fuzzy C-means**		**ABC**	
Parameter	Value	Parameter	Value	Parameter	Value
Distance	Euclidean	Exponent for U	2	Population size	32
EmptyAction	Singleton	Max Iter	100	Maximum cycle number	540
Max Iter	100	Min. Amout of Improvement	1e-5		
Start	Plus				

Experiments have been conducted on a desktop computer which have the following specifications: AMD X6 1055 six core CPU, 8GB of RAM and Windows 2008

operating system. Methods were implemented by using MATLAB programming language. Experiments were repeated 30 times due to the usage of stochastic methods.

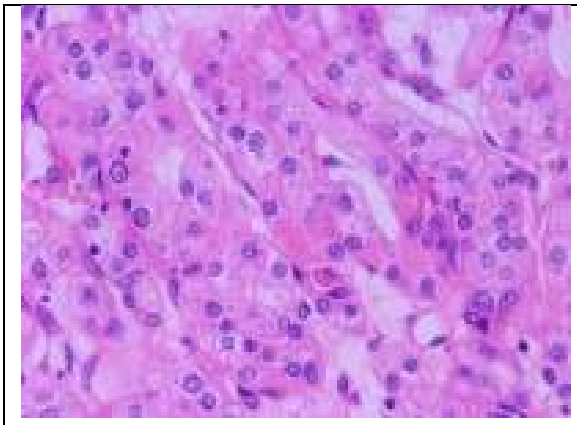


Image #1

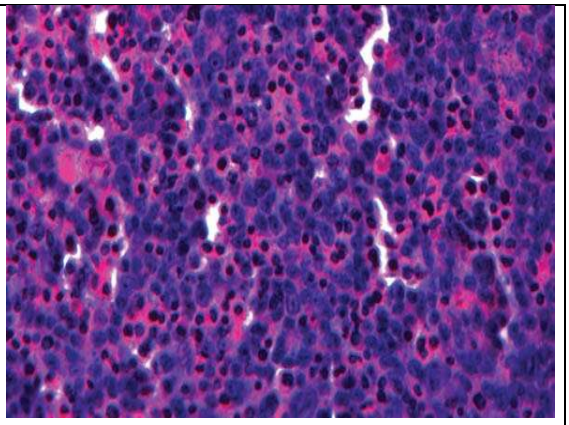


Image #2

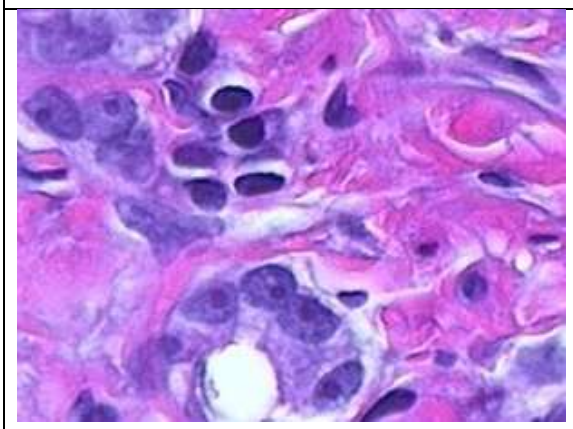


Image #3

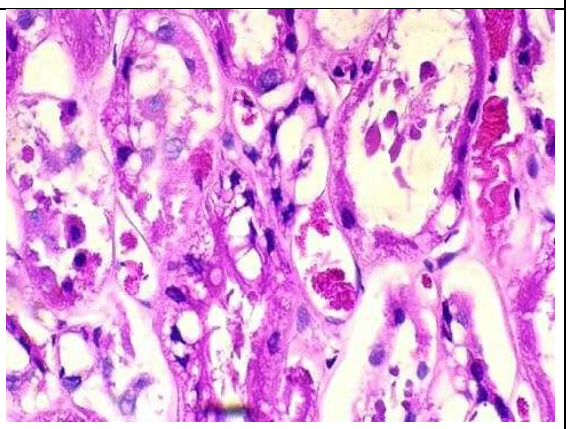


Image #4

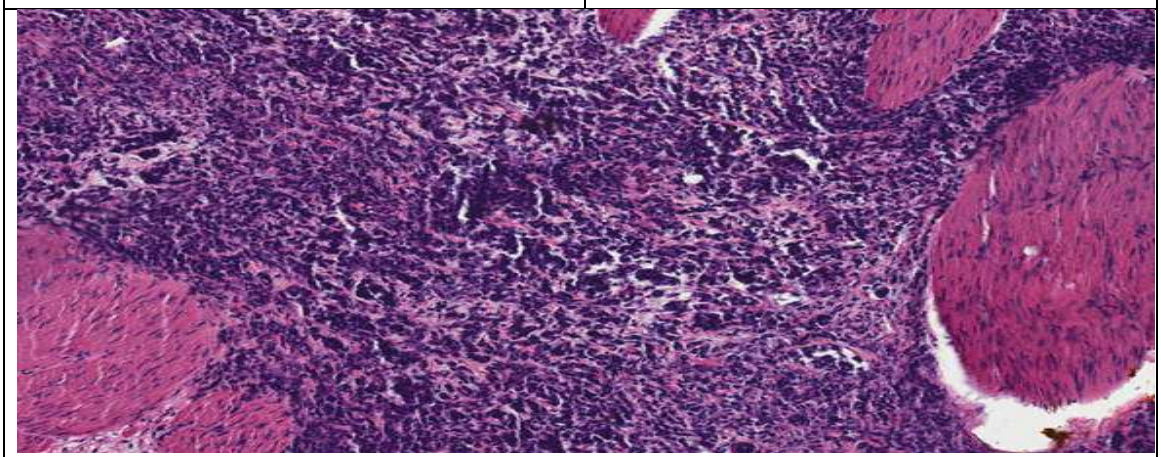


Image #5

Figure 3.1.Images used in the experiments.

3.4 Experiments and Results

The images used are encoded at 24 bit true color RGB. Color image is an M x N x 3 matrix of color pixels and each pixel is triplet corresponding to the red, green and blue components at specific special location. The comparison algorithms clustered the set images into three cluster pixels based on their color. In the experiments, both RGB and Lab channels were used for all methods.

Figure 3.2 shows segmented images results. We used K-means, Fuzzy C-Means and ABC based clustering method for both RGB and Lab channels. Therefore, there are 6 results for each image shown in Figure 3.2. Segmented image of all 3 methods (Km, FCM and ABC) using two different (RGB and LAB) color spaces were considered as successful.

Table 3.3 illustrates the results found during segmentation of the five original images. The bold results show which method has better result then the other methods. In table 3.3 we declared results of Euclidean distance (ED), Mean Square Error (MSE) and structural similarity image quality (SSIM) of all algorithms that we used our experiments. For ED and MSE, small values are better. For SSIM higher values are better. Quantitative results show that 3 methods gave similar results however ABC based method is slightly better than the others. In the result table the numbers in the parenthesis indicades the standard deviation of 30 runs.

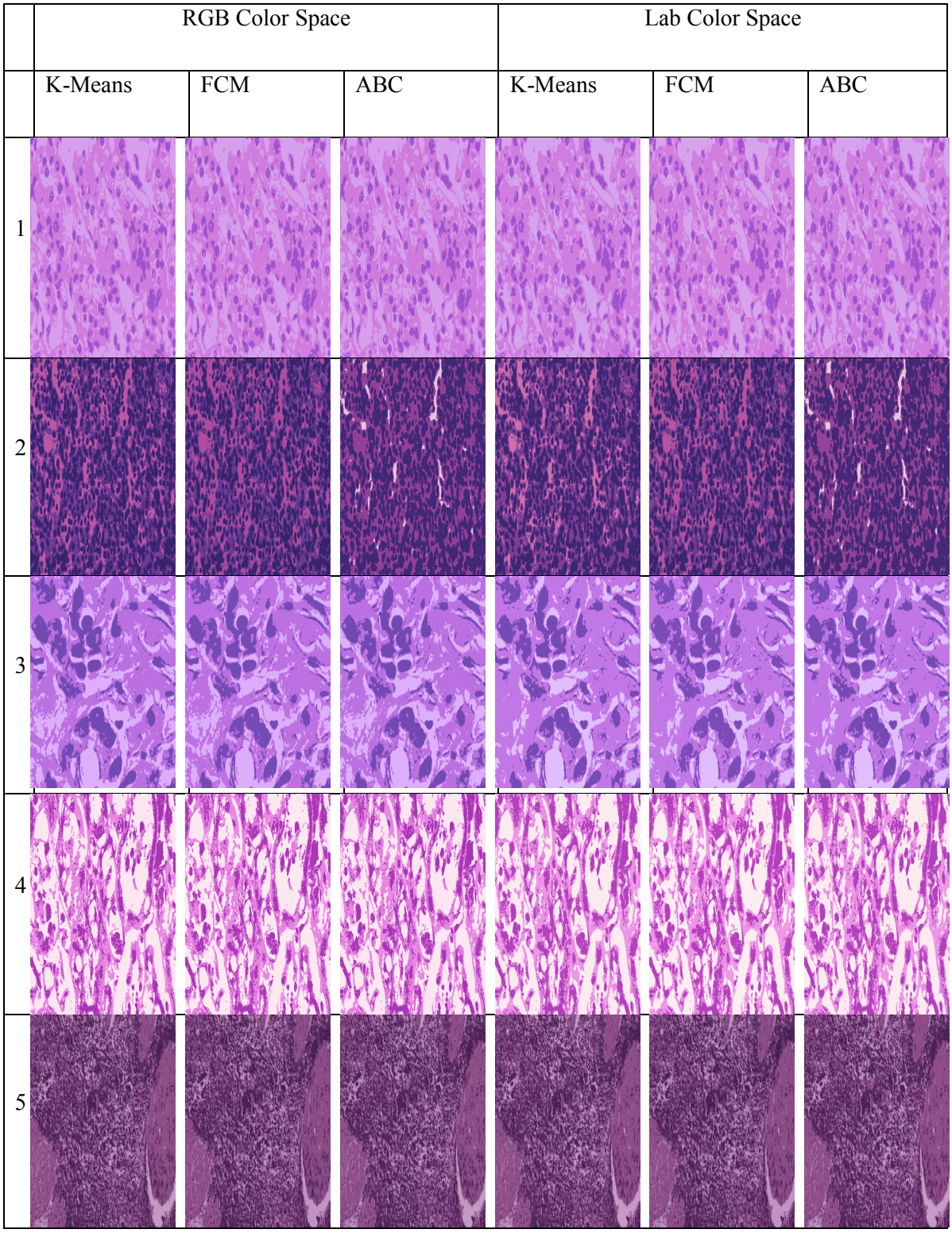


Figure 3.2. Comparison of segmented images obtained with K-means, Fuzzy C-means and ABC methods

Table 3.3. Quantitative results of the segmentation experiments.

Image #1		K-means	Fuzzy C-means	ABC-ED
RGB	ED	141.309(0.000)	141.593(0.000)	**141.308(0.001)**
	MSE	226.850(0.071)	228.047(0.000)	**226.809(0.068)**
	SSIM	1.364(0.000)	1.361(0.000)	**1.365(0.000)**
LAB	ED	353.887(0.000)	354.344(0.000)	**353.886(0.000)**
	MSE	226.340(0.000)	**225.953(0.000)**	226.340(0.009)
	SSIM	1.389(0.000)	1.387(0.000)	**1.390(0.001)**

Image #2		K-means	Fuzzy C-means	ABC-ED
RGB	ED	505.498(12.684)	530.581(0.000)	**479.843(0.001)**
	MSE	941.319(16.260)	**923.256(0.000)**	974.617(0.597)
	SSIM	0.989(0.026)	**1.050(0.000)**	0.936(0.001)
LAB	ED	1558.065(0.000)	1582.742(0.000)	**1544.946(0.040)**
	MSE	**907.836(0.006)**	927.132(0.000)	940.597(0.299)
	SSIM	1.044(0.000)	**1.065(0.000)**	0.934(0.001)

Image #3		K-means	Fuzzy C-means	ABC-ED
RGB	ED	451.565(0.001)	452.633(0.000)	**451.564(0.001)**
	MSE	786.791(1.302)	786.264(0.000)	**785.626(0.723)**
	SSIM	1.074(0.002)	**1.083(0.000)**	1.075(0.001)
LAB	ED	1257.528(0.001)	1260.536(0.000)	**1257.526(0.021)**
	MSE	**760.841(0.046)**	761.558(0.000)	760.939(0.195)
	SSIM	1.150(0.000)	**1.153(0.000)**	1.150(0.000)

Image #4		K-means	Fuzzy C-means	ABC-ED
RGB	ED	546.424(0.000)	548.356(0.000)	**546.423(0.001)**
	MSE	887.173(0.000)	891.081(0.000)	**886.629(0.371)**
	SSIM	1.171(0.000)	1.169(0.000)	**1.172(0.001)**
LAB	ED	**1418.789(0.000)**	1423.136(0.000)	1418.825(0.095)
	MSE	863.953(0.005)	**863.481(0.000)**	864.120(0.647)
	SSIM	1.222(0.000)	1.221(0.000)	**1.223(0.001)**

Image #5		K-means	Fuzzy C-means	ABC-ED
RGB	ED	451.535(0.000)	453.829(0.000)	**451.532(0.000)**
	MSE	**741.019(0.023)**	743.272(0.000)	741.020(0.024)
	SSIM	1.371(0.000)	**1.378(0.000)**	1.371(0.000)
LAB	ED	**1255.493(0.000)**	1259.710(0.000)	1255.494(0.002)
	MSE	**735.895(0.001)**	738.413(0.000)	735.900(0.011)
	SSIM	1.385(0.000)	**1.389(0.000)**	1.385(0.000)

Segmentation results are visualized in Figure 3.3 – 3.12. Each row of Figure 3.3 – 3.12 indicades the different parts (segments) of the images like cell nuclei, cytoplasm or the remaining. Three segments of each image using 3 segmentation methods for both Lab and RGB color spaces are given. In Figure 3.3 and 3.4 results of image #1 is given for Lab and RGB color spaces, respectively.

As shown in Figure 3.3 and 3.4, Lab and RGB spaces results are similar. Also, results of 3 methods are similar.

In Figure 3.5 and 3.6 results of image #2 is given for Lab and RGB color spaces, respectively.

As shown in Figure 3.5 and 3.6, Lab color space is better than RGB color space. A result of ABC method is better than K-means and Fuzzy C-Means methods.

In Figure 3.7 and 3.8 results of image #3 is given for Lab and RGB color spaces, respectively.

As shown in Figure 3.7 and 3.8, Lab and RGB spaces results are similar. Also, results of 3 methods are similar.

In Figure 3.9 and 3.10 results of image #4 is given for Lab and RGB color spaces, respectively.

As shown in Figure 3.9 and 3.10, Lab and RGB spaces results are similar. Also, results of 3 methods are similar.

In Figure 3.11 and 3.12 results of image #4 is given for Lab and RGB color spaces, respectively.

As shown in Figure 3.11 and 3.12, Lab and RGB spaces results are similar. Also, results of 3 methods are similar.

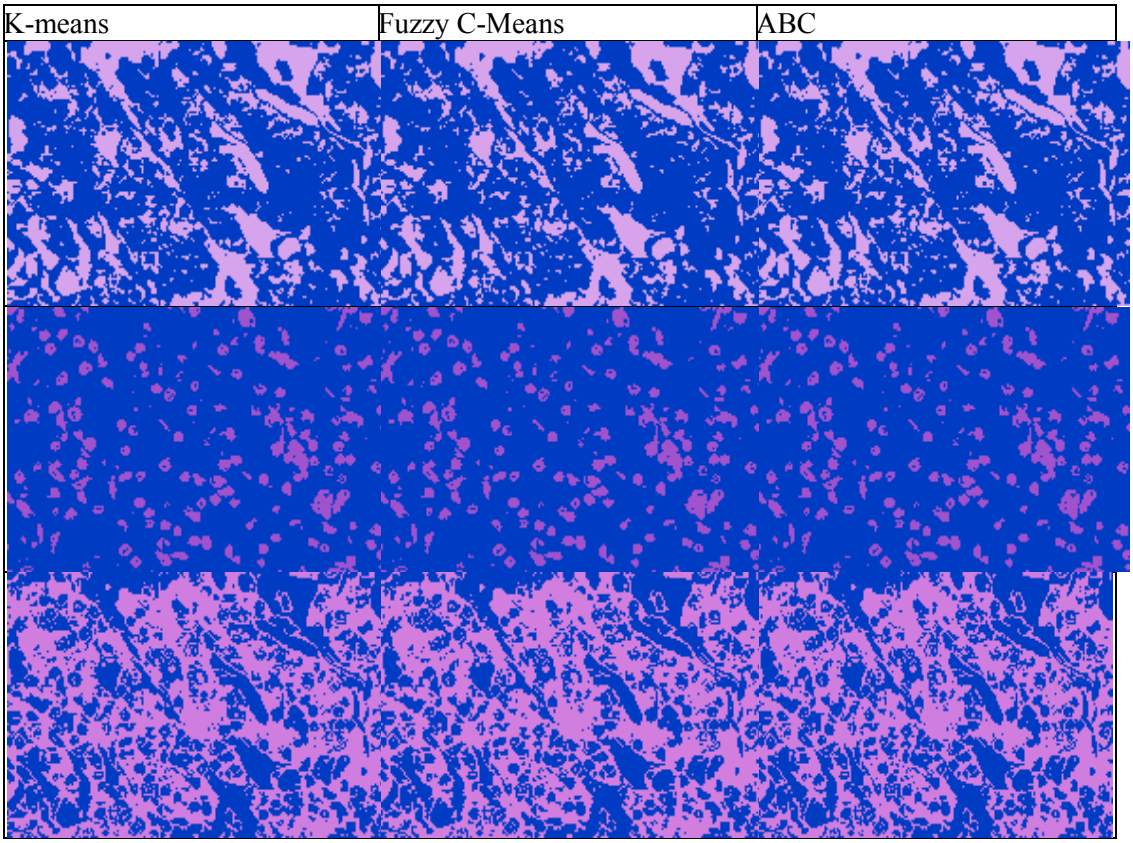


Figure 3.3. Visual segmentation results of Image #1 using Lab color space.

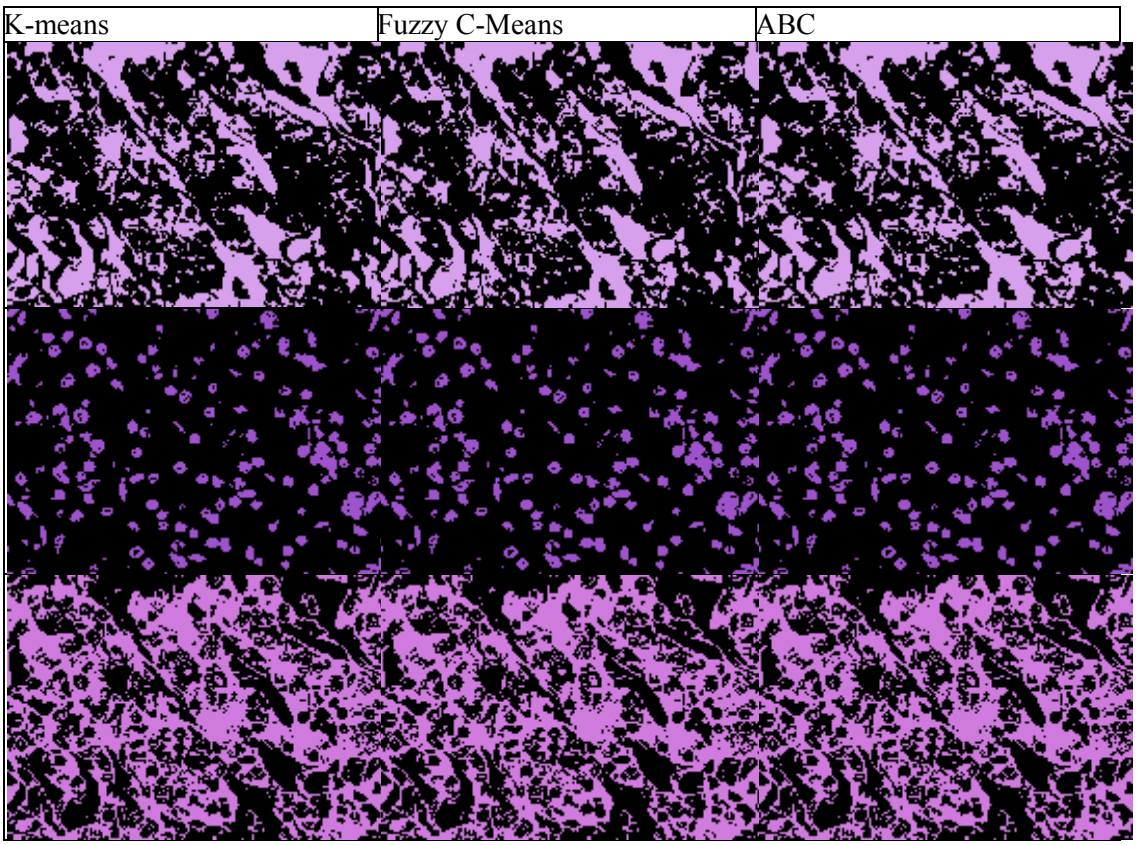


Figure 3.4. Visual segmentation results of Image #1 using RGB color space.

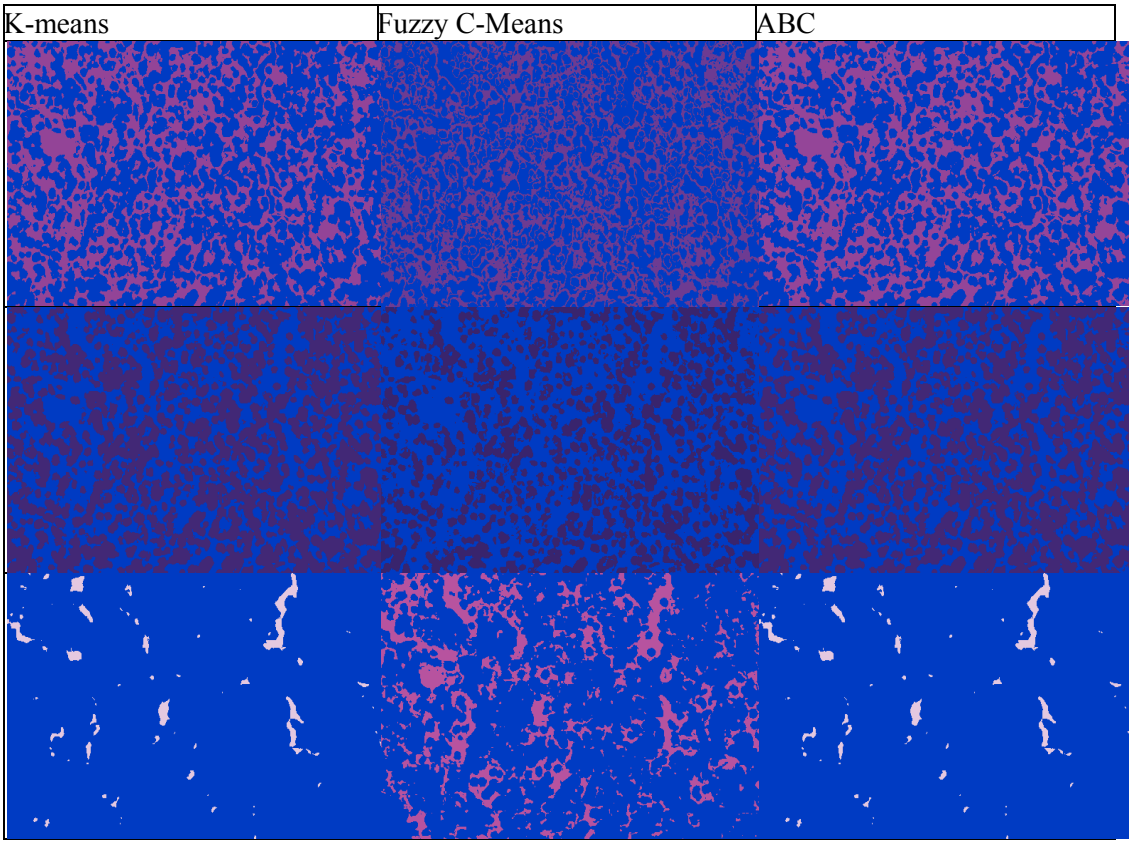


Figure 3.5. Visual segmentation results of Image #2 using Lab color space.

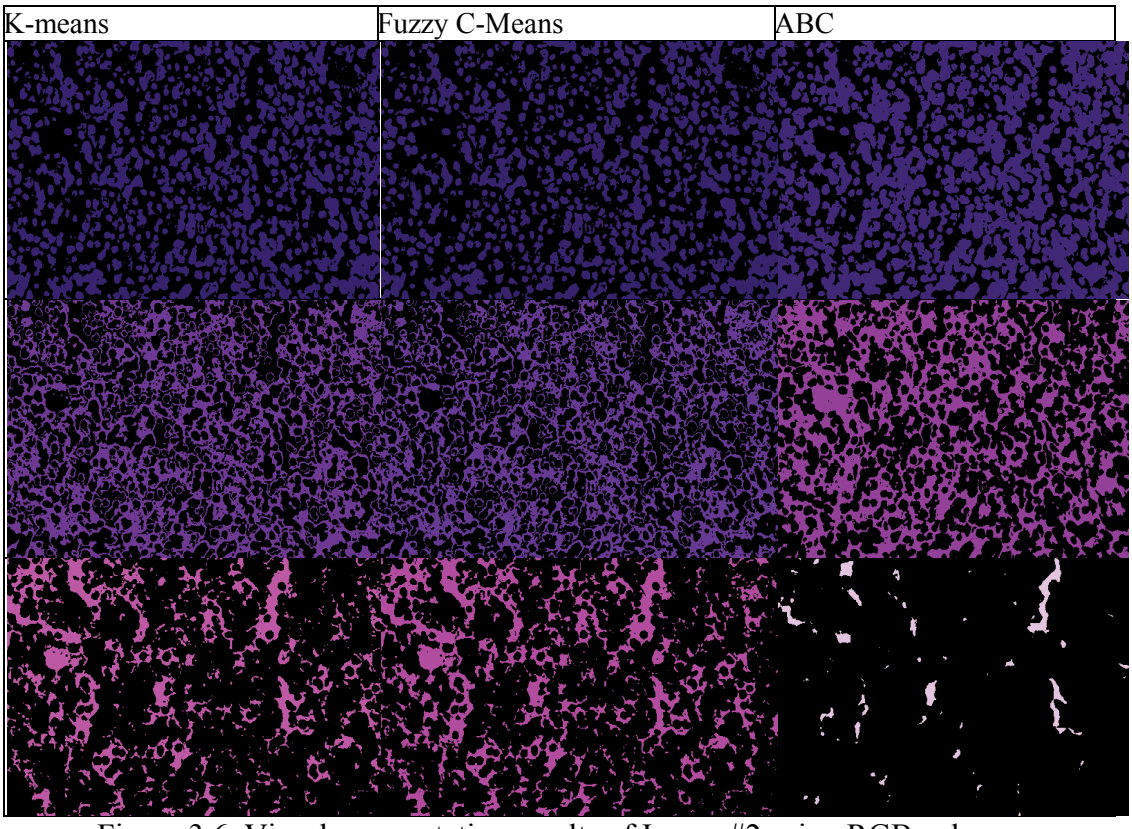


Figure 3.6. Visual segmentation results of Image #2 using RGB color space.

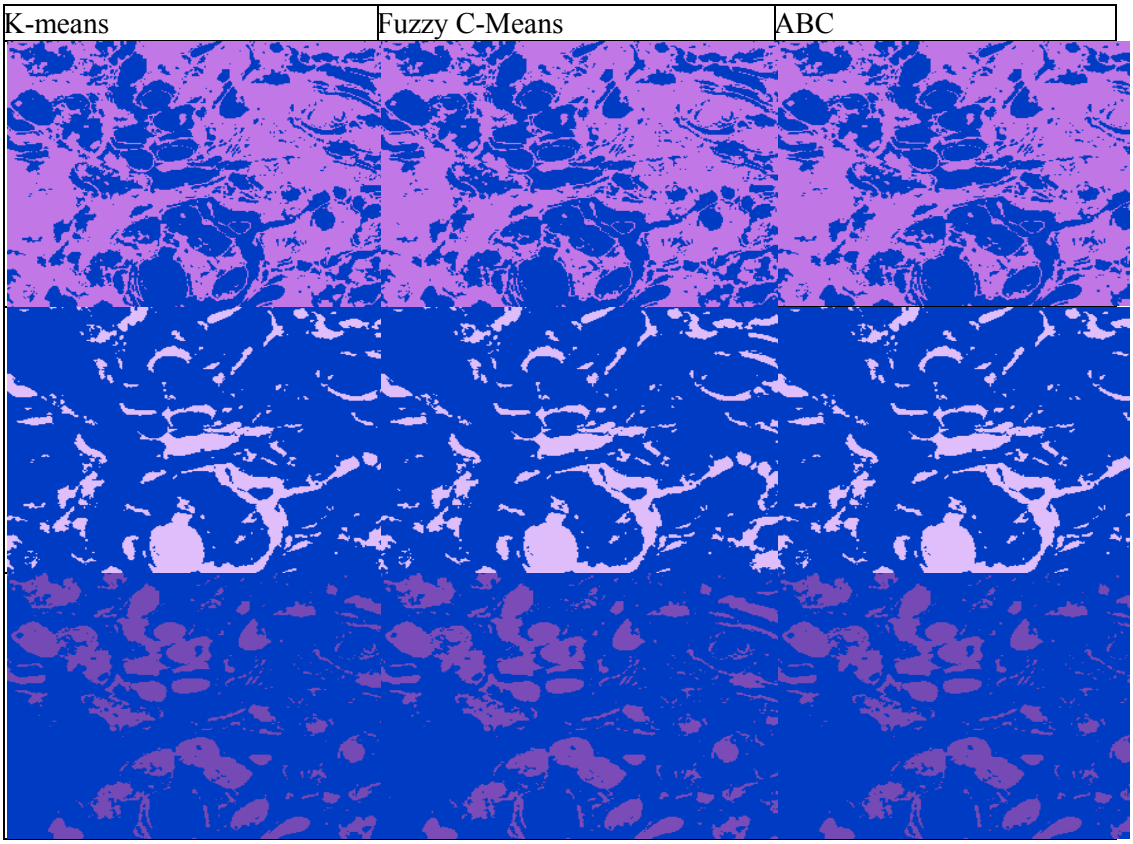


Figure 3.7. Visual segmentation results of Image #3 using Lab color space.

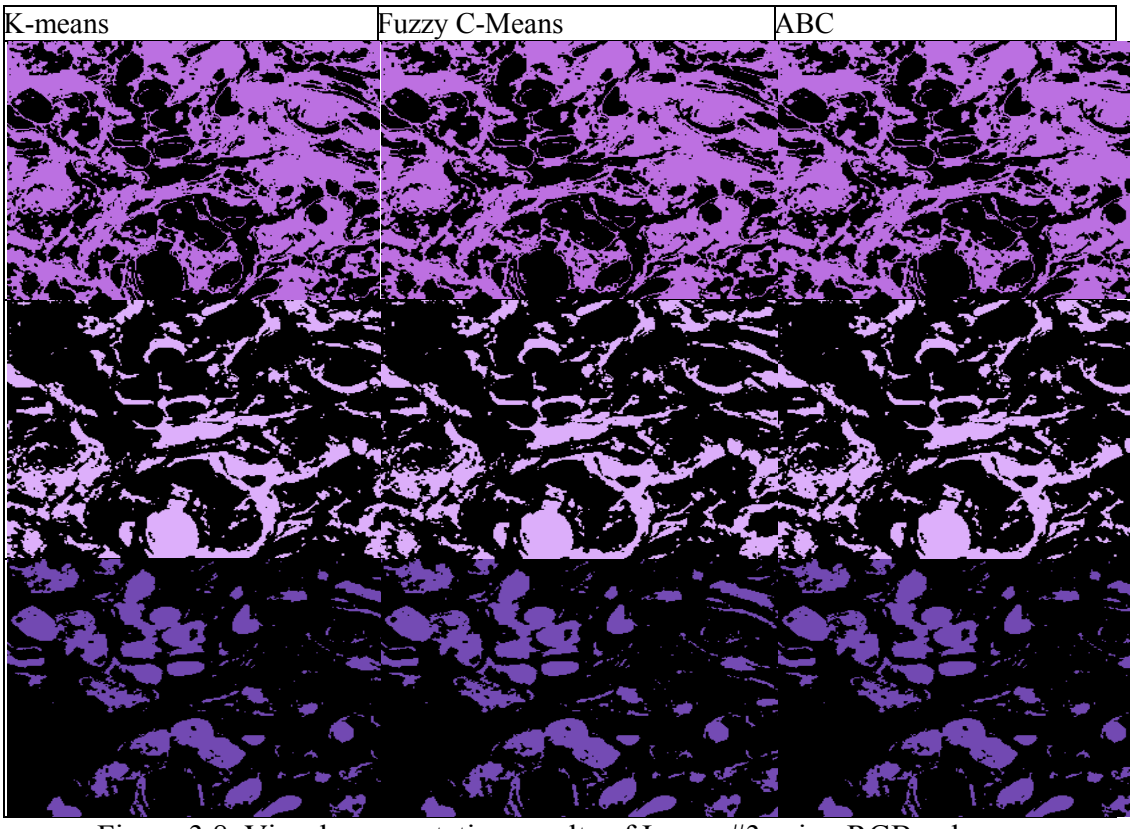


Figure 3.8. Visual segmentation results of Image #3 using RGB color space.

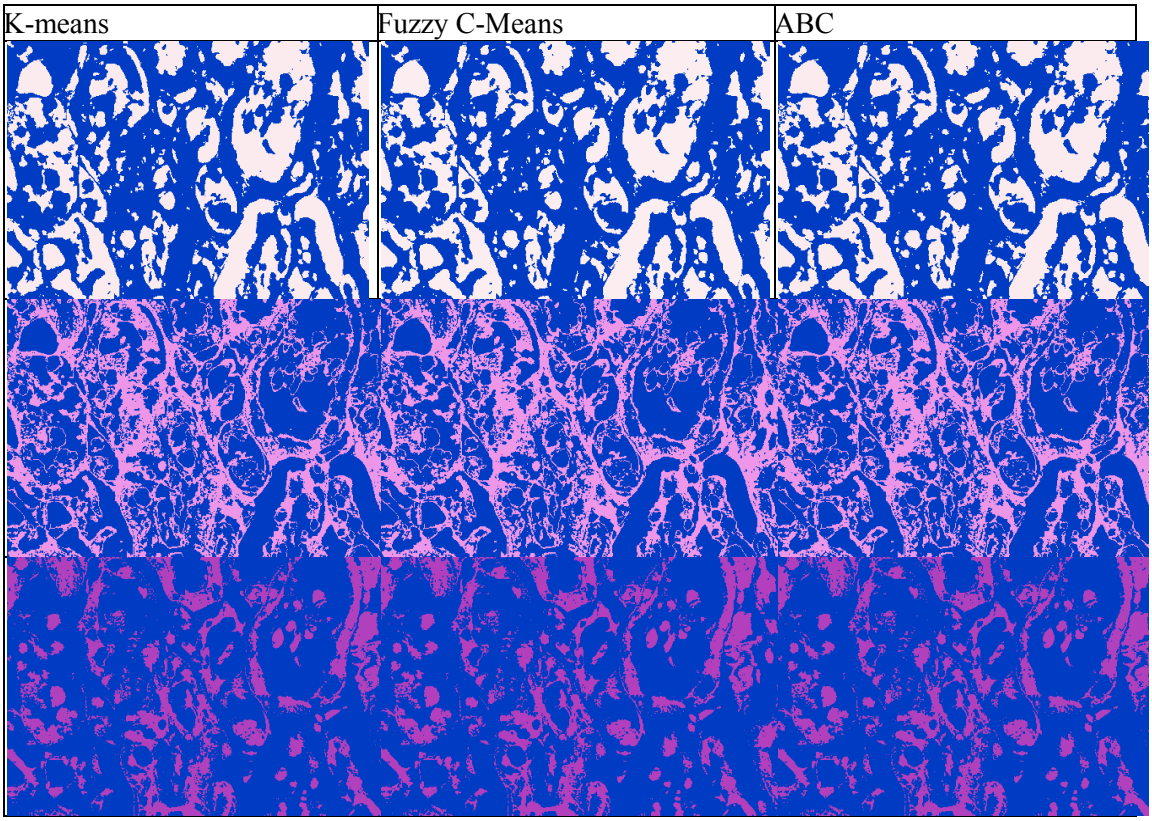


Figure 3.9. Visual segmentation results of Image #4 using Lab color space.

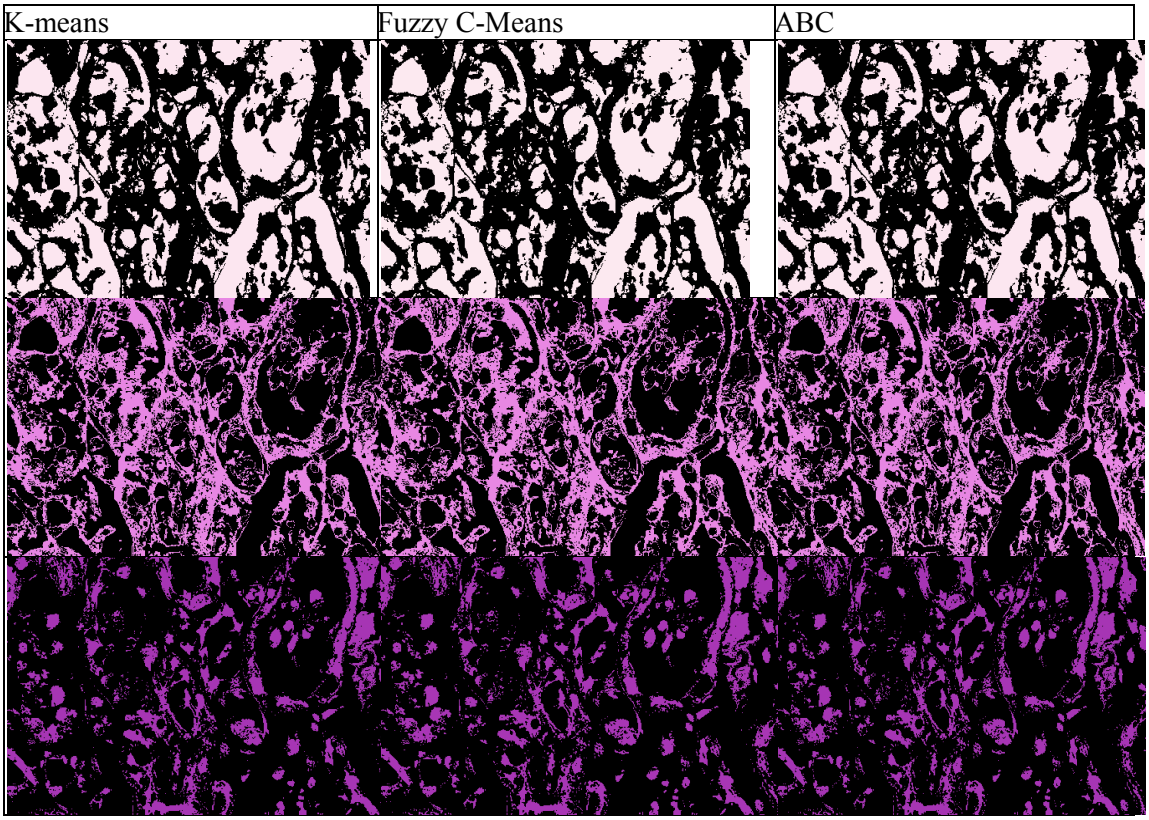


Figure 3.10. Visual segmentation results of Image #4 using RGB color space.

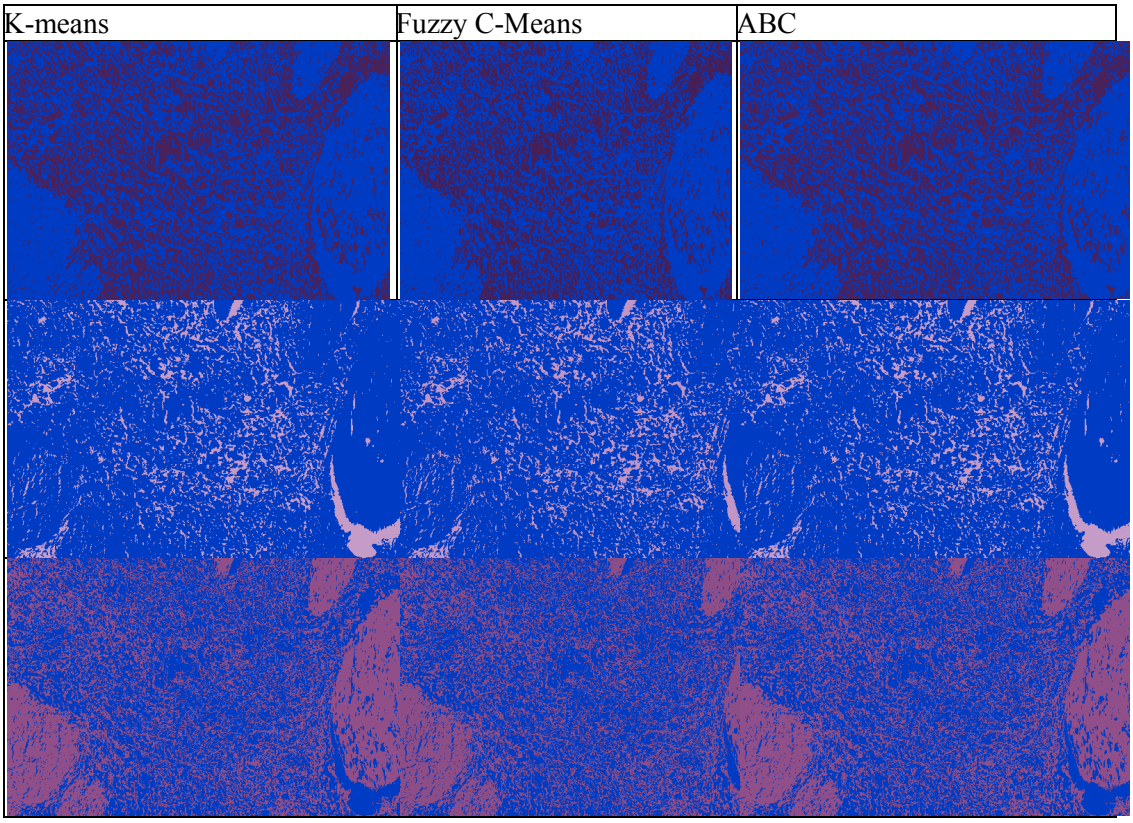


Figure 3.11. Visual segmentation results of Image #5 using Lab color space.

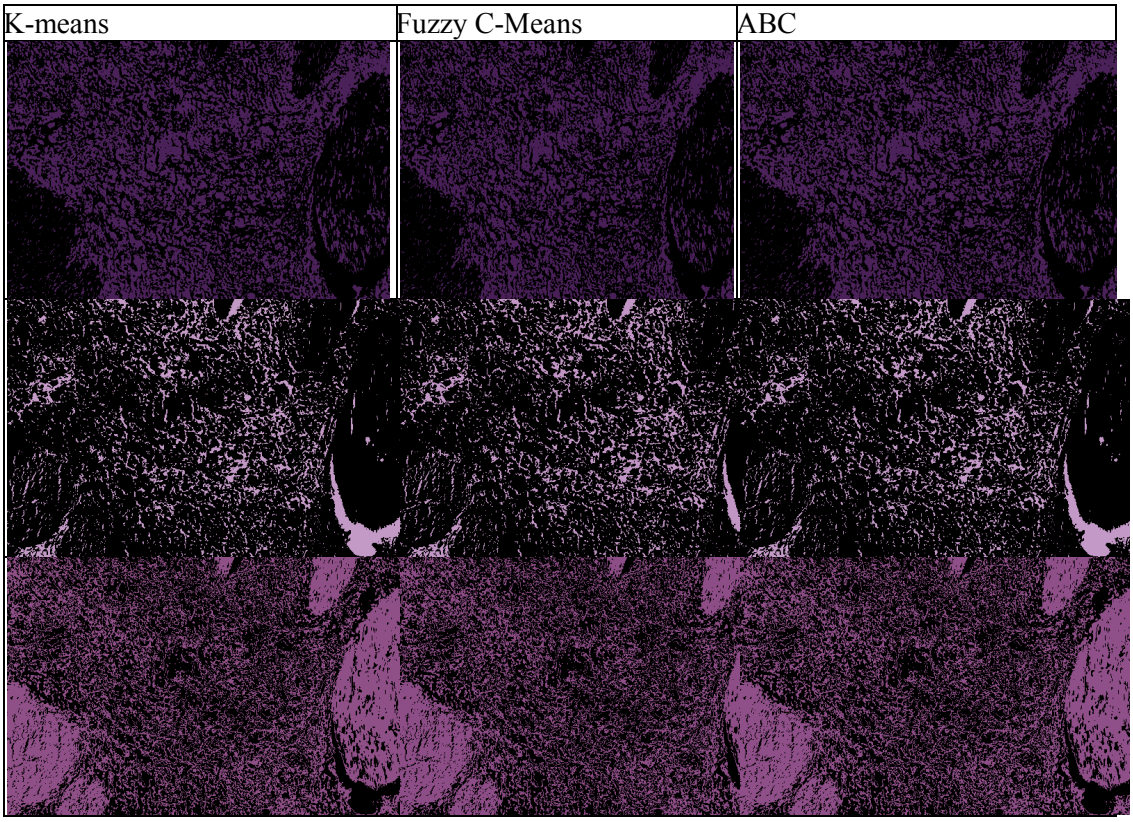


Figure 3.12. Visual segmentation results of Image #5 using RGB color space.

CHAPTER 4

CONCLUSION

4. Conclusion

Cancer has become major problem of the human health and the development and enhancement of techniques for use in its diagnosis and treatment has increasingly become a focus of research. In this thesis, we have made comparison of the three clustering techniques Fuzzy C-means, K-means and ABC optimization. In this thesis, we developed an iterative segmentation technique based on optimizing the distance of sub-neightbors of each centroid by using ABC is designed. Image segmentation is the task of processing an image into its constituent objects. Recently, considerable work on histological segmentation has focused on fully automated approaches that rely on machine learning techniques. Color based features are important in histology applications since biologists stain tissues to highlight special structures. The color difference classifies specific objects to be investigated and finds relationship among them. The aim to use image segmentation technique is to find the areas of interest depending on the image and its characteristics.

They allow for large amounts of data to be processed. Based on descriptive features of data, intresting patterens are discovered. The extraction of features from histological images has been addressed in.

ABC based segmentation aims to find nearest neighbors of each centroid to minimize the distance of each pixel to its corresponding centroid. This method is used to improve an efficient of Euclidean distance based by using ABC optimization algorithm. The comparative study of algorithms shows that the results are similar in most cases. However, in particular cases ABC is slightly better than Fuzzy C-means and K-means.

Histology is the anatomical study of the microscopic structure of tissues. It is regarded as a gold standard for clinical diagnosis of diseased tissue and for the identification of therapy effects. The manual work is however very time consuming and prone to subjective interpretation. The digitized tissue histopathology has now become an important application of computer image analysis as well as machine learning techniques. The role of Computer Aided Diagnosis algorithms in medical imaging has escalated to a point where algorithms have been developed for disease detection, diagnosis, and prediction of prognosis that complements the opinion of the pathologist. Digital pathology takes traditional pathology specimens – tissue samples mounted on glass slides and transform them into digital images that can be shared electronically by care providers.

Therefore, computer-assisted diagnosis (CAD) systems are becoming crucial in histology analysis, as they could automatically identify regions of medical interest. Their main advantage is the ability to provide immediate results in a consistent and objective manner, thereby reducing the workload of the medical experts.

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