

Application of Watershed Segmentation on Biomedical Images as an Educational Tool

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Abstract: Image segmentation is the operation of dividing an image into multiple segments. Watershed segmentation is a widely used region-based segmentation method because of basic and fast features. This segmentation method that is based on morphological operations is used for several areas especially in medical image applications such as magnetic resonance imaging (MRI), computed tomography (CT) and histopathological images. In general, the segmentation problems with these types of images are originated from noise, nonhomogeneity and touching objects. For solving such problems, watershed segmentation is a powerful tool. In addition to all these advantages, if watershed segmentation is applied to image without any additional process, generally it gives an oversegmentation problem. Marker controlled watershed segmentation is improved to overcome this problem. In this article, five different algorithms about marker-controlled watershed segmentation, which each one is modified from five different articles, are applied on a grayscale bone plasmacytoma image and results are compared. An educational platform related with these algorithms is presented by using MATLAB App Designer. Designed graphical user interface (GUI) using App Designer is helpful tool for comparing different algorithms of marker-controlled watershed segmentation. Moreover, image processing learners can use it easily and can observe effectiveness of algorithms. So, the created educational platform has academic and educational characteristics. The advantage of this application is not only comparing different algorithms but also learning different marker-controlled watershed segmentation methods.

Key words: Image processing, mathematical morphology, segmentation, watershed transform, MATLAB GUI.

Havza Bölütlemesinin Biyomedikal Görüntüler Üzerinde Bir Eğitim Aracı Olarak Uygulanması

Öz: Görüntü bölütleme, bir görüntüyü birden fazla bölüme ayırma işlemidir. Havza bölütlemesi, temel ve hızlı özellikleri nedeniyle yaygın olarak kullanılan bölge tabanlı bir bölütleme yöntemidir. Morfolojik işlemlere dayanan bu bölütleme yöntemi, özellikle manyetik rezonans görüntüleme (MRI), bilgisayarlı tomografi (CT) ve histopatolojik görüntüler gibi tıbbi görüntü uygulamalarında birçok alanda kullanılmaktadır. Genel olarak bu tür görüntülerde bölütleme problemleri gürültü, homojen olmama ve dokunan nesnelere kaynaklanmaktadır. Bu tür problemleri çözmek için havza bölütlemesi güçlü bir araçtır. Tüm bu avantajlarının yanı sıra, havza bölütlemesi herhangi bir ek işlem yapılmadan görüntüye uygulanırsa, genellikle aşırı bölütleme problemi verir. Bu problemin üstesinden gelmek için işaretleyici kontrollü havza bölütlemesi geliştirilmiştir. Bu makalede, işaretleyici kontrollü havza bölütlemesi ile ilgili her biri beş farklı makaleden modifiye edilmiş beş farklı algoritma, gri tonlamalı bir kemik plazmasitom görüntüsü üzerinde uygulanmış ve sonuçlar karşılaştırılmıştır. MATLAB App Designer kullanılarak bu algoritmalarla ilgili bir eğitim platformu sunulmuştur. App Designer kullanılarak tasarlanan grafiksel kullanıcı arayüzü (GUI), işaretleyici kontrollü havza segmentasyonunun farklı algoritmalarını karşılaştırmak için yararlı bir araçtır. Ayrıca görüntü işleme öğrenenler bunu kolaylıkla kullanabilir ve algoritmaların etkinliğini gözlemleyebilirler. Dolayısıyla, oluşturulan eğitim platformu akademik ve eğitsel özelliklere sahiptir. Bu uygulamanın avantajı sadece farklı algoritmaları karşılaştırmak değil, aynı zamanda farklı işaretleyici kontrollü havza bölütlemesi yöntemlerini öğrenmektir.

Anahtar kelimeler: Görüntü işleme, matematiksel morfoloji, segmentasyon, havza dönüşümü, MATLAB GUI.

1. Introduction

Image segmentation operation separates all objects from each other and the background. This is used in many image processing fields as pre or main processing stage [1,2]. The main aim of the segmentation is making easier to analyze image for any process. One of the widely used segmentation method is watershed segmentation. The watershed segmentation is a region-based method and derived from mathematical morphology [3]. Watershed segmentation is applied on a gradient form of the image. When the gradient of the image is thought like a topography, dark areas correspond to low altitudes and light areas correspond to high altitudes. For this type of image, there are three meaningful terms; watershed lines (or dams), catchment basins and regional minimums

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(minima) [4]. Watershed lines are created in the image to prevent merging of two or more adjacent catchment basins. Catchment basins are referred to regions of the image and each catchment basin has one regional minimum [5]. Finding watershed lines (watershed ridge lines) is the main aim of the watershed transform. In previous works on watershed segmentation, it is shown that classical watershed segmentation did not segment images correctly because of the over segmentation. The oversegmentation is caused from non-homogeneity or noise in the images [5]. A new watershed segmentation method is improved to overcome this problem and it is called as marker-controlled watershed segmentation [6]. In this way, the markers are calculated and they imposed to the gradient image. Then, the watershed segmentation is applied to the resulted gradient image.

There are different ways to find the image markers based on mathematical morphology in the literature. The watershed algorithm on the basis of mathematical morphology was initially proposed by Digabel and Lantuèjoul [4]. After that, this algorithm was improved by Beucher and Lantuèjoul, and this approach for watershed segmentation is called as flooding process [7]. After several years, Vincent and Soille suggested a watershed algorithm based on immersion, but it had very high complexity because of the sorting step [4]. Bieniek and Moga suggested a watershed algorithm based on connected components, but this algorithm implemented by simulating a raining process also called raining as a new watershed approach [8]. After from these dates, a lot of different works had been performed on classical watershed segmentation. However, the classical watershed segmentation has an oversegmentation problem and to solve this problem a strategy of marker-controlled watershed segmentation had been proposed by Meyer and Beucher [6] taking reference the proposed algorithm by Beucher and Lantuèjoul [7]. Parvati et al. suggested an algorithm for marker-controlled watershed segmentation [9]. They applied this algorithm to MR image, aerial image and high resolution satellite image. Kaur and Jindal used a different marker-controlled watershed algorithm to segment a lung image [10]. Reza et al. used another algorithm and this was applied on an original fundus image [11]. Allaoui and Nasri proposed a marker-controlled watershed algorithm to segment cell, muscle, brain, foot and dental medical images [12]. Kalapala et al. used a different algorithm to segment a satellite image which includes trees [13]. Kaleem et al. applied marker-controlled watershed segmentation on MRI image for tumor segmentation [14]. Koyuncu et al. introduced smart markers for cell segmentation and they were compared this method with some other marker identification algorithms on KATO-3 gastric cancer cells [15]. Yahya et al. get good results by applying watershed segmentation in five stages to accomplish the oversegmentation problem on four different images; rice, fingerprint, cells and grass [16]. Sivagami et al. also studied to overcome the oversegmentation problem by using bit-plane slicing and watershed segmentation together and the advantage of this method was low memory consumption and low computational complexity compared to other methods [17]. Beare et al. proposed a method that is the marker-based watershed scalper (MBWSS) and the method that has no expensive preprocessing steps was applied on T1-weighted MR images to isolate brain [18]. Another study that was introduced by Napoleon et al. was comparison of directly applied watershed segmentation and marker-controlled watershed segmentation on brain images [19]. A technique was proposed by Ravi et al. and the technique solve the oversegmentation problem by applying markers on the gradient image for MRI of brain and mammogram images [20]. Another study on oversegmentation problem was introduced by Bandara et al. and they assumed that the different adjacent segments of an object have similar color distribution [21]. Lahitha et al. studied on different image segmentation methods and the results showed that marker-controlled watershed segmentation is the best and the best solution of oversegmentation problem was bilateral filter [22]. They used Lena image and mentioned that bilateral filter did not reduce the salt and pepper noise. Lu et al. studied on a medical image segmentation algorithm based on gradient edge detection and control marked watershed and they used breast MRI image [23]. They mentioned that in the extraction and segmentation accuracy of breast tumor regions there were some problems and the problem can be solved in the further studies. Sarma et al. focused on a comparison of existing segmentation techniques and new segmentation techniques [24]. The discussed basic techniques are thresholding, edge detection, region-based segmentation, hard and soft type clustering, watershed, partial differential equations, artificial neural network and new techniques are marker-controlled watershed and deep learning techniques. They mentioned that the hybrid combination of existing algorithms gave more accurate results than the single algorithm approaches. In recent years, new approaches of watershed segmentation were proposed. Dai et al. applied their approach on jujubes and trees images [25], and Guo et al. applied their approach on blasted rock images [26]. A marker-controlled watershed algorithm which was proposed by Ghader et al. was used for the detection of Baker's cyst in the knee's joint MRI and axial T2-weighted images first time [27]. Also, Gonzalez and Woods had been given place to watershed segmentation in their famous book named as Digital Image Processing [5].

Marker controlled watershed segmentation is widely used in MRI, CT and histopathological images [28,29]. In this article, five different marker-controlled watershed algorithms were applied on a grayscale bone plasmacytoma image and comparison of algorithm performances is observed. Visuality and application are more

important for image processing training and quick understanding. In this work, MATLAB App Designer is used to improve the quality of understanding. An educational MATLAB App Design is created to make easier the comparison of segmentation results. This educational design is very useful tool for image processing learners because learning algorithms of marker-controlled watershed segmentation takes long time. This tool saves time by visuality and step by step structure of it. MATLAB App Design educational tool provides ease of use by assigning complex and long codes as a single button function. With this property, the GUI becomes a user-friendly tool by establishing direct communication between the student and the computer. It also allows them to improve or reformation the software code by providing easy access to callback files. Additionally, an application on an image makes the subject more understandable by increasing visuality in image processing courses.

The constitution of the other sections of this study is explained as follows. In Section 2, morphological operations for preprocessing and finding image markers are described. In Section 3, the concepts of watershed and marker-controlled watershed segmentations are explained briefly. In Section 4, the details of five marker-controlled watershed segmentation algorithms are given. In Section 5, the results of all algorithms and designed educational MATLAB App Design tool is described in detail and in Section 6, conclusion and future work of this study is explained.

2. Morphological Operations

Morphology is generally defined as a study about shapes and structures. Morphological operators are nonlinear transformations to modify geometric shapes of images [12]. Modification of shapes or structures is made by using structuring element which can be in different shapes and sizes. The shape and size selections of the structuring element affect the result of the desired operation. This effect changes according to requested result and size of the objects in the image. As a general description, morphological operations give a processed image by applying some morphological operations to an image with a suitable structuring element. There are several morphological operations in image segmentation and some of these are explained in the following.

2.1. Grayscale dilation and erosion

Grayscale dilation makes the process of replacing the gray level value at any pixel by the maximum intensity value covered by a flat structuring element. The main purpose is to expand the bright areas in the image and emphasize certain structures. This process thickens the object edges and makes them more prominent. This process is applied on all pixels of the image. The dilation of the grayscale image $I(x,y)$ by a flat structuring element $b(i,j)$ is mathematically defined as (where x,y and i,j are the coordinates (column and row positions) of the image pixels and structuring element, respectively) in Equation 1.

$$(I \oplus b)(x, y) = \max_{(i,j) \in b} \{I(x - i, y - j)\} \quad (1)$$

In contrast to the grayscale dilation, grayscale erosion operation is that the gray level value at any pixel is replaced by the minimum intensity value covered by a flat structuring element. Its purpose is to expand dark (low intensity) regions in the image, narrow the bright areas, and suppress certain structures. This operation can bring details to the fore by thinning the object edges. The erosion of the grayscale image $I(x,y)$ by a flat structuring element $b(i,j)$ is mathematically given as (where x,y and i,j are the coordinates (column and row positions) of the image pixels and structuring element, respectively) in Equation 2.

$$(I \ominus b)(x, y) = \min_{(i,j) \in b} \{I(x + i, y + j)\} \quad (2)$$

In these operations, the structuring element can be different shapes and sizes such that any size is defined as radius for disk and diamond, and any size is defined as width for square and cube. The shape and size of the structuring element is changing according to the image and the desired result from the image.

2.2. Morphological gradient

Gradient form of the image can be obtained by gradient filter and morphological gradient. Morphological gradient of the image is obtained by subtraction of its grayscale erosion result from its grayscale dilation result

[30]. In this process, the same structuring element is used for erosion and dilation operations. Morphological gradient can be described as in Equation 3.

$$\text{grad}(I) = (I \oplus b) - (I \ominus b) \quad (3)$$

2.3. Dilation based morphological reconstruction

The process that repeated dilations of the marker image I with respect to the mask image J is performed until stability is reached and it is called as dilation based morphological reconstruction [31].

The elementary geodesic dilation $\delta_I^{(1)}(J)$ of grayscale image $J \leq I$ “under” I is determined according to Equation 4.

$$\delta_I^{(1)}(J) = (J \oplus b) \wedge I \quad (1)$$

In this equation, \wedge stands for the pointwise minimum and $(J \oplus b)$ is the dilation of J by flat structuring element b [31]. The grayscale geodesic dilation of size $n \geq 0$ is then given as in Equation 5.

$$\delta_I^{(n)}(J) = \delta_I^{(1)} \circ \delta_I^{(1)} \circ \dots \circ \delta_I^{(1)}(J) \quad (2)$$

The grayscale reconstruction $\rho_I(J)$ of I from J is obtained by iterating grayscale geodesic dilation of J “under” I until stability is reached (Equation 6). This operation reconstructs bright regions in grayscale images. In this equation, V refers to the process of taking the supremum, that is, the maximum.

$$\rho_I(J) = V_{n \geq 1} \delta_I^{(n)}(J) \quad (6)$$

2.4. Erosion Based Morphological Reconstruction

The process that repeated erosions of the marker image I with respect to the mask image J is performed until stability is reached is called as erosion based morphological reconstruction [31].

The elementary geodesic erosion $\varepsilon_I^{(1)}(J)$ of grayscale image $J \geq I$ “above” I is given as in Equation 7.

$$\varepsilon_I^{(1)}(J) = (J \ominus b) \vee I \quad (3)$$

where \vee stands for the pointwise maximum and $(J \ominus b)$ is the erosion of J by flat structuring element b [31]. The grayscale geodesic erosion of size $n \geq 0$ is then given as (where n indicates geodesic dilation is repeating n times) in Equation 8.

$$\varepsilon_I^{(n)} = \varepsilon_I^{(1)} \circ \varepsilon_I^{(1)} \circ \dots \circ \varepsilon_I^{(1)}(J) \quad (4)$$

This operation gives opposite results according to dilation based morphological reconstruction. It reconstructs dark regions in grayscale images.

2.5. Opening and opening by reconstruction

When erosion is applied on the image and then dilation is applied on the resulted image, this procedure is called opening operation. Grayscale opening suppresses bright regions smaller than the structuring element in the image while leaving the rest of the image unchanged. The mathematical description of opening is given as in Equation 9 [5].

$$I \circ b = (I \ominus b) \oplus b \quad (5)$$

The opening by reconstruction of size n of the image I is defined as the reconstruction by dilation of I from the erosion of size n of I [5]. The opening by reconstruction of image I can be defined as in Equation 10.

$$O_R^{(n)} = R_I^D[(I \ominus nb)] \quad (6)$$

In this equation, $(I \ominus nb)$ stands for n erosions of I by b , D is given to emphasize dilation operation, n is the size of image I .

2.6. Closing and closing by reconstruction

Closing is described as erosion of the dilation applied image. Grayscale closing operation removes the dark features smaller than the structuring element as an inverse function of grayscale opening. The mathematical definition of closing is given as in Equation 11 [5].

$$I \cdot b = (I \oplus b) \ominus b \quad (7)$$

The closing by reconstruction of size n of the image I is defined as the reconstruction by erosion of I from the dilation of size n of I and it can be defined as in Equation 12 [5].

$$C_R^{(n)}(I) = R_I^E[(I \oplus nb)] \quad (8)$$

In this equation, $(I \oplus nb)$ stands for n dilations of I by b , D is given to emphasize dilation operation, n is the size of image I .

2.7. Multiscale edge detection

The multiscale edge detector is firstly introduced by [4]. Multiscale gradient algorithm is used for greater robustness to noise [9]. The smaller the size of structuring element can increase the ability to detect fine edges while it can decrease the noise removing capacity. The large size of structuring element can remove the noise more effectively but it decreases the ability of detection fine edges [32]. According to these observations, multiscale sizes of structuring element are used for effective results. Analysis of the image with different size of structuring elements supports the meaning of multiscale. The mathematical representation of the multiscale edge detector that gives gradient image is given as in Equation 13.

$$MG(f) = \frac{1}{n} \sum_{i=1}^n [(f \oplus b_i) - (f \ominus b_i)] \oplus b_{i-1} \quad (9)$$

where n is scale and b_i denotes the group of square structuring elements where sizes are $(2i + 1) \times (2i - 1)$ pixels, \oplus stands for dilation operation, \ominus stands for erosion operation.

2.8. Contrast Stretching

Contrast stretching transformation only slightly enhances the darker regions while the brighter regions of the image remain more or less unchanged [33].

3. Watershed and Marker Controlled Watershed Segmentation

Watershed transformation is a region-based segmentation method. Watershed segmentation is generally applied on a gradient image. Gradient of an image refers to measuring how the image's intensity value is changing. Gradient image has two information which are magnitude and direction. The gradient magnitude explains how quickly the intensity value is changing and the gradient direction means the direction in which the information is changing [34]. For watershed transformation, gradient magnitude is needed. Because, gradient form of any image can be thought as a topographic relief. From this point of view, each pixel value of the gradient image corresponds

to the height of this point. In other words, dark pixels indicate low altitudes and light pixels indicate high altitudes [35]. Using the topographical meaning of the gradient image, watershed transform can be explained easily. Watershed transformation can be modeled as flooding (immersed) based or raining based expression.

The valleys, high ridges and hillsides are important descriptions for flooding or immersion-based approach of the watershed segmentation. The valleys, high ridges and hillsides correspond to regional minimas, watershed ridge lines and catchment basins, respectively [36]. This topographical area is slowly immersed into water or in other words, the flooding process is started. Then, from the minimum levels of the valleys (or lowest altitudes) the surface is started to fill. The water will fill up different catchment basins gradually. The watershed (or dam) is created to avoiding mixing of waters in neighboring catchment basins [7]. This watershed ridge lines are created as one pixel width [4]. So, the regions which are separated from each other's by watershed ridge lines are the segmented regions of the image.

The other approach for the watershed segmentation is raining. Raindrops fall on the mountain of the topographical surface and it moves towards to lowest altitude (regional minimum) because of the gravity. For each point, algorithm tracks the path that a droplet of water would follow if it fell at that point [37]. A segment is constituted from all waters that flow to the same regional minimum. When a point has more than one path towards the different steepest surfaces then it can be allocated to any one of the local minimum [38].

One of the drawbacks of watershed algorithm is oversegmentation. Oversegmentation problem is caused by small variations of the gradient image. Markers are used to overcome the oversegmentation problem [4]. Markers are founded by a set of morphological operations [39]. The gradient image is computed by using different ways such as gradient filter or morphological gradient operator. Then, image markers are detected after some operations and the gradient image is modified by superimposing these founded markers into it. After that, watershed segmentation is applied to modified gradient image and correctly segmented image is obtained. Markers are the connected components in an image [3,6].

4. Algorithms

Five different current articles about marker-controlled watershed segmentation are examined. All of them are proposed for different type of images, so different steps are followed for all algorithms. In this study, a grayscale bone plasmacytoma image is used in the comparison of the algorithms. A bone plasmacytoma image is cropped from an image which is downloaded from "www.webpathology.com" [40]. Usage of this data which are obtained from this website, do not require ethics committee approval to the academic researchers. Thus, all of the algorithms are modified for segmenting this image correctly. All steps in the each of the modified algorithms are explained in detail.

4.1. Parvati & Rao & Das algorithm

A study on marker-controlled watershed segmentation is created by Parvatiet al. [9]. In their article, the proposed work is applied on four different images which are two MR medical images, an aerial image and a high-resolution satellite image. The results of standard watershed segmentation and proposed work about marker-controlled watershed segmentation are given in their article.

Firstly, images in color form are converted into grayscale form. Then, multiscale edge detector operation is applied on the grayscale form of the image to obtain the gradient image.

Secondly, morphological image reconstruction is used to create foreground and background markers. Erosion-based grayscale reconstruction and followed by dilation-based grayscale reconstruction are applied on the gradient image. The regional maximas of the reconstructed image are found to get smooth edges for foreground markers. After that, foreground markers are obtained by superimposition of regional maximas into the original image. The superimposed image is converted to binary form and the Euclidean distance of it is calculated to create background markers.

Finally, the morphological reconstruction of the gradient image is created by using foreground and background markers. Then, the watershed transform is applied on the reconstructed gradient image and the segmented image is obtained.

Algorithm 1:

1. Compute the multiscale gradient of the grayscale input image
2. Find erosion-based morphological reconstruction form of the gradient image
3. Calculate the dilation-based morphological reconstruction form of erosion-based reconstructed image
4. Get regional maximas of the dilation-based reconstructed image
5. Compute foreground markers by superimposing the regional maximas into input image
6. Obtain background markers by taking the Euclidean distance of superimposed image
7. Acquire the morphological reconstruction of the gradient image using foreground and background markers
8. Take the watershed transform of the reconstructed gradient image
9. Obtain the RGB labeled form of the watershed segmentation result

4.2. Reza & Eswaran & Dimiyati algorithm

The article of proposed method for marker-controlled watershed segmentation is studied by Wasif Reza et al. [11]. The proposed method can be categorized into three main stages. Initial stage includes preprocessing algorithms to make the bright object features more distinguishable from the background. The subsequent stage is used for detection of image markers to modify the gradient image to control oversegmentation. The last stage includes the watershed segmentation to trace the boundary from the marker modified gradient. All of these stages were applied on an original fundus image in their article. However, some parts are changed for grayscale bone plasmacytoma image and the modified algorithm is described as follows.

In the first stage, averaging filter is applied on the grayscale image to mix the small objects with low intensity variations to the background, while leaving the objects of interest relatively unchanged. The average filter is applied to the image using the Equation 14 [9].

$$f(x, y) = \frac{1}{M \times N} \sum_{i=1}^{M \times N} R_i(x, y) \quad (14)$$

where R is original image, f is filtered image and (x, y) corresponds to pixel location. In this equation, $M \times N$ corresponds to the mask size and the filter is taking the average of all values within the mask. After that, contrast stretching transformation is applied on the filtered image to make the bright object features more distinguishable from the background. Next, negative transformation is applied on the contrast enhanced image and gradient magnitude is obtained by linear filtering (Sobel filter) of negative transformed image. Negative transformation converts dark pixels into light and light pixels into dark by subtracting the pixel values from 255 for an 8-bit grayscale image.

In the second stage, extended minima transformation is applied on the negative transformed image and internal markers are obtained. Internal markers can be associated with objects of interest. Extended minima transform is the regional minima of the H-minima transform. H-minima transform suppresses all minima whose depth is lower than or equal to the given depth, h [41]. While extended minima transform is applied, the given depth is important and it is specified by the user as an input. Then, Euclidean distance of the inverse of internal markers is calculated and watershed segmentation is applied on this distance map image to obtain the external markers. External markers can be associated with background. Euclidean distance transform assigns a number that is the distance between that pixel and the nearest nonzero pixel [5]. The external markers partition the image into regions, and each region contains a single internal marker and part of the background.

The modification of the gradient magnitude using internal and external markers is performed by using the minima imposition. This technique imposes the regional minimas at the marker locations. The modified gradient magnitude only has regional minima where superimposition of internal and external markers is nonzero.

In the last stage, watershed transform of the modified gradient image is created and red green blue (RGB) labeling is applied on the segmentation result.

Algorithm 2:

1. Apply average filter to the grayscale image
2. Find contrast stretching transformation of filtered image
3. Calculate negative transformation of enhanced image
4. Compute gradient magnitude of the negative transformed image using Sobel operator
5. Obtain internal markers using extended minima transformation of the negative transformed image
6. Take the inverse of internal markers
7. Take Euclidean distance transform of the inverse of internal markers
8. Get external markers using the watershed transform of distance transformed image
9. Gain the modified gradient using minima imposition of gradient image with internal and external markers
10. Take the watershed transform of modified gradient and label it by RGB conversion

4.3. Kaur & Jindal algorithm

Kaur and Jindal are proposed a work about marker-controlled watershed segmentation and this proposed work is applied on a Digital Imaging and Communication in Medicine (DICOM) lung image [10]. In their article, firstly, a filter is applied on the grayscale image to reduce the noise. For the grayscale bone plasmacytoma image, median filter is preferred. Morphological gradient operation is applied on the filtered image to obtain the gradient image. After the gradient form is calculated, opening by reconstruction is implemented on gradient image and then closing by reconstruction is implemented on reconstructed image. A flat maxima inside of the each object is created through these two operations and these regional maximas are calculated. Foreground markers are obtained by calculating regional maximas of resulted reconstructed image. After that, thresholding is applied on the resulted reconstructed image and background markers are computed. Using background markers, watershed ridge lines are created by applying firstly distance function on background markers and after that inverse of the watershed segmentation of this distance function is calculated. Then, minimums of foreground markers and watershed ridge lines are imposed to gradient image and modified gradient image is formed. Lastly, watershed segmentation is applied on the modified gradient image and is labeled to RGB. For a better visual, labeled image is superimposed to grayscale image.

The proposed work of M. Kaur and G. Jindal article is modified for segmentation of a grayscale bone plasmacytoma image and steps are given as follows.

Algorithm 3:

1. Compute the noise reduced or filtered form of the input grayscale image
2. Calculate the gradient image by using morphological gradient
3. Find the opening by reconstruction of the gradient image
4. Obtain the closing by reconstruction of the previous reconstructed image
5. Get the foreground markers by finding regional maximas of the resulted reconstructed image
6. Get the background markers by thresholding the resulted reconstructed image (or input image)
7. Achieve the distance form image of the background markers and then apply watershed on distance form image
8. Obtain the watershed ridge lines by taking inverse of the watershed applied image
9. Gain the modified gradient by imposing minimums of foreground markers and watershed ridge lines
10. Apply the watershed segmentation on modified gradient image and then obtain RGB labeled form of it
11. Show the RGB labeled segmentation result on grayscale input image by using superimposition

4.4. Allaoui & Nasri algorithm

El Allaoui and Nasri are studied a work on marker-controlled watershed segmentation [12]. In their article, the proposed work is applied on six different medical images such as cell, muscle, brain, foot and dental image. Two different results, which are obtained only watershed and marker-controlled watershed, are given in their article. This algorithm is applied on grayscale bone plasmacytoma image in this study and stages are given in detailed as follows.

Mainly, this proposed work can be categorized as morphological reconstruction, markers extraction of the regions and watershed segmentation. All operations from morphological reconstruction to the markers extraction can be thought like preprocessing stage. In this stage, firstly dilation based morphological reconstruction is applied on the grayscale image and the complement of the result is obtained. In case of dilation based morphological reconstruction, shape and size of structuring element are important inputs for dilation and correct segmentation. After that, difference image obtained by subtraction of the complement image from the grayscale image. This difference image can be seen as gradient form in other proposed works.

In markers extraction stage, firstly extended minima transform is applied on difference image and the transformed image is eliminated from the noise. Extended minima and related H-transform are explained in part 4.2. Foreground markers are obtained from resulted image. To obtain background markers, Euclidean distance function is applied on the foreground markers. Euclidean distance gives a number for each pixel which corresponds to the distance from that pixel to the nearest nonzero pixel. The watershed segmentation is applied on background markers. Watershed ridge lines are achieved by applying the superimposition of the resulted image to the background markers.

In the final section, minimums of foreground markers image and watershed ridge lines image are imposed to difference image. After that, watershed segmentation is applied on the modified difference image and correct segmentation result is obtained.

Algorithm 4:

1. Compute the dilation-based morphological reconstruction of the grayscale input image
2. Take the complement of the reconstructed image
3. Calculate gradient image by subtracting the complement image from the grayscale image
4. Obtain the markers by detecting the regional minimums of the difference image
5. Get the cleared markers by using erosion followed by dilation
6. Find modified gradient by imposing the minimums to the difference image using markers
7. Take the watershed transform of the modified gradient image
8. Achieve the RGB labeled form of the watershed segmentation result

4.5. Kalapala & Rao & Srinivas algorithm

A study about marker-controlled watershed segmentation is performed by Kalapala et al. [13]. The proposed method is applied on a satellite image which includes trees. Their algorithm can be categorized into three main steps. First step includes the operations to obtain the gradient image. The second step is performed to find the foreground and background markers. The last step includes the watershed transform. The algorithm is modified for the grayscale bone plasmacytoma image and all steps are given below.

The histogram equalization operation is firstly applied to grayscale image. This method enhances the contrast of the image. After that, Laplacian of Gaussian (LoG) edge detector operation is applied to the histogram equalized image. LoG edge detector operation gives the same effect with gradient operation.

The opening by reconstruction and followed by closing by reconstruction are applied to the edge detected image. Then, regional maximas of the resulted reconstructed image are found and foreground markers are obtained. The background markers are detected by taking the Euclidean distance transform of the foreground markers. Using the foreground and background markers, edge detected image is modified. Modified image only has minima at the foreground and background marker locations.

The watershed transformation is applied on the modified edge detected image. Subsequently, RGB labeling is performed to the segmented image.

Algorithm 5:

1. Compute the histogram equalization of the grayscale input image
2. Apply the LoG edge detector operation to equalized image for obtaining the image that is similar to gradient form
3. Get the opening by reconstruction of edge detected image
4. Calculate the closing by reconstruction from opening by reconstructed image
5. Obtain the foreground markers by finding the regional maximas of the resulted image
6. Find the background markers by taking the Euclidean distance transform of the foreground markers
7. Acquire the modified edge detected image by using the foreground and background markers
8. Apply the watershed transform to the modified edge detected image
9. Achieve the RGB labeled form of the watershed segmented image

5. MATLAB Educational Application Design and Results

MATLAB is a commonly used program for both education and image processing applications [42]. Because, in addition to the many toolboxes, it contains image processing toolbox and App Designer. Image processing toolbox provides functions to analyze the images. App Designer enables the creation of professional applications without requiring advanced software development expertise. Additionally, it allows learners to access the underlying code for all buttons and functions [43]. Through App Designer, learners can not only examine application design elements—such as buttons and functionalities—but also observe the visual effects of the implemented algorithms. It is a user-friendly tool, it creates the contact between user and program by just mouse clicks. MATLAB is both easy to use and easy to teach. Anyone with basic knowledge of programming or coding can learn it easily. MATLAB has also easy accessible design tool; it can be widely used in university labs. Because, it is easy to learn and toolboxes helps to analyze the data and teach the subjects more effectively in many areas, especially in engineering. It will be easier and permanent for the students to comprehend the subject if the lecturers/teachers explain any subject through a sample related App Design.

In this study, A MATLAB App Design is designed as an effective tool for exploring different marker-controlled watershed segmentation techniques. The most important benefit of this design is that the user does not need complex mathematical calculations to use it. The design consists of two stages; entrance screen and application screen. A general flow diagram for the MATLAB App Design is given in Figure 1.

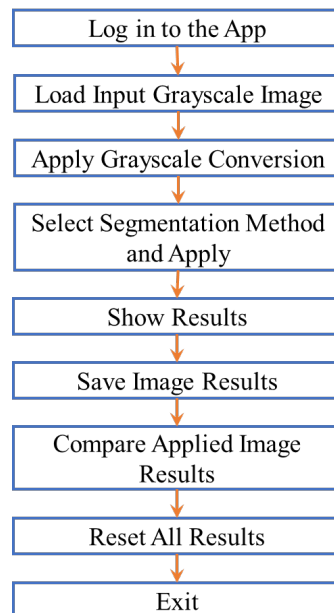


Figure 1. An overview flowchart of the MATLAB App Design process.

The entrance screen is firstly opened and prevents the unauthorized access. After name and password are correctly entered, the application screen is opened. The entrance screen of the design is shown in Figure 2.

The application screen consists of three main stages; initial process stage, methods for segmentation application and options after a method is applied. The opening form of application screen is shown in Figure 3. In the initial process stage, there are two buttons. These are “Load Image” and “Grayscale Conversion”. When “Load Image” button is pressed, a new window will be opened to select and image from the memory and initial directory is set to the current directory of the MATLAB. After an image is selected, the name of the image with its extension is located in a edit field, that is in the near of “Load Image” button. The selected image displayed in axes1 as an “Input Image”. These operations are shown in Figure 4.

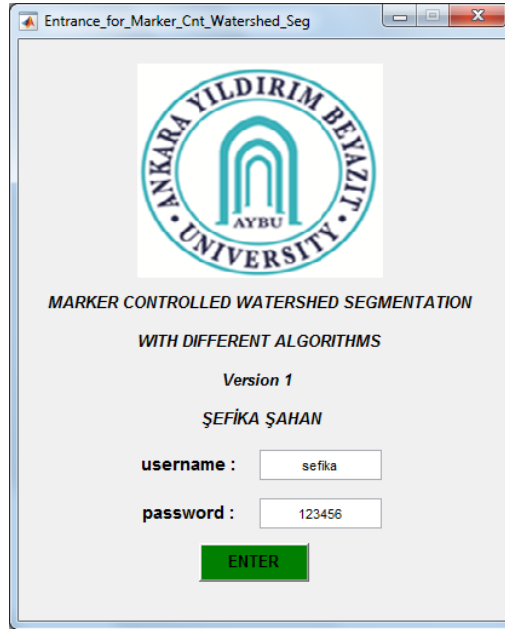


Figure 2. Entrance window.

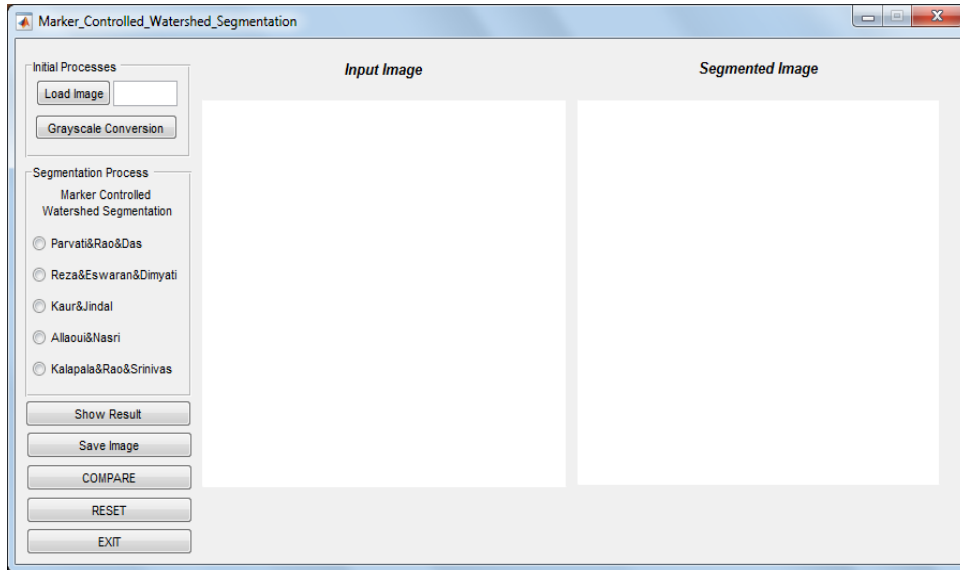


Figure 3. Application window.

If the loaded image is in colored form (RGB), the second button in the initial process stage is pressed to convert it into grayscale form. After pressing this button, color image is changed with the gray scale image and displayed in the axes1. The resulted form of the application screen is shown in Figure 5. As shown in this figure, at the below of the grayscale image two image information is presented. First one specifies the size of the image and other specifies the maximum and minimum grayscale values in the image. This information makes the image more meaningful to the user.

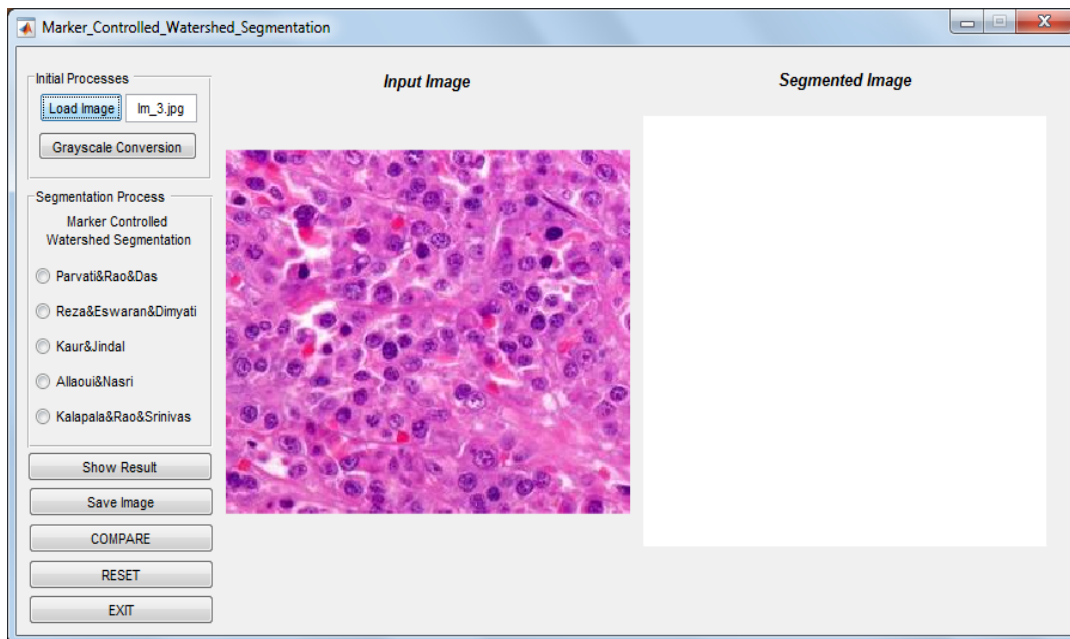


Figure 4. Result of “Load Image”.

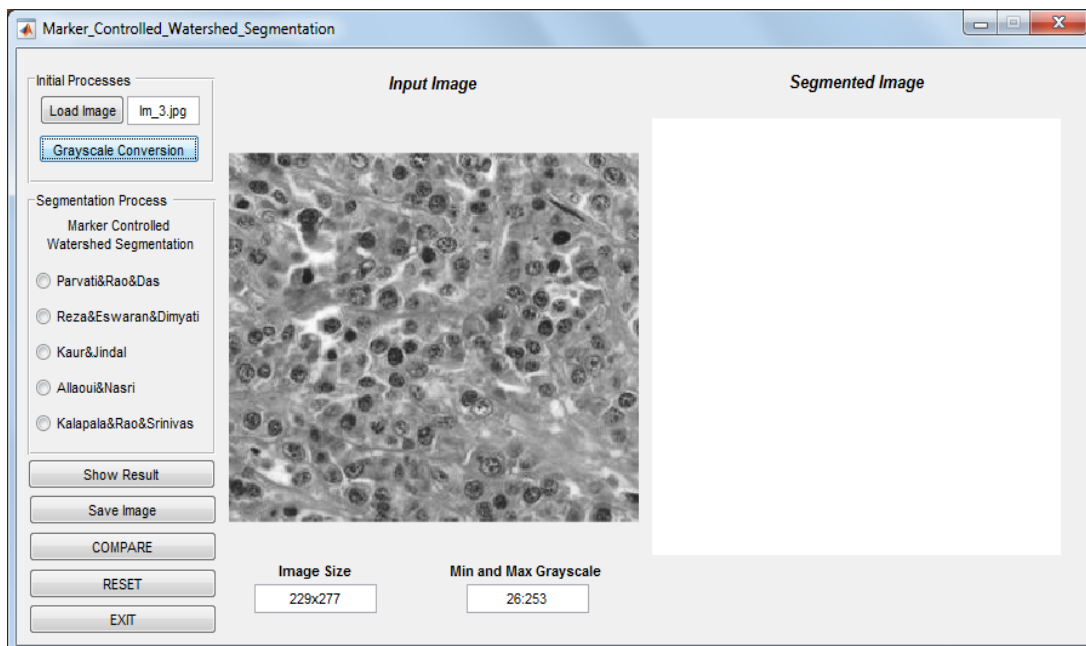


Figure 5. Result of “Grayscale Conversion”.

The other stage is the “Segmentation Process”. There exist five different radio buttons to apply five different algorithms. When one of the algorithms is selected, a new window is opened. If “Algorithm 2” is selected, an opened screen is given in Figure 6.

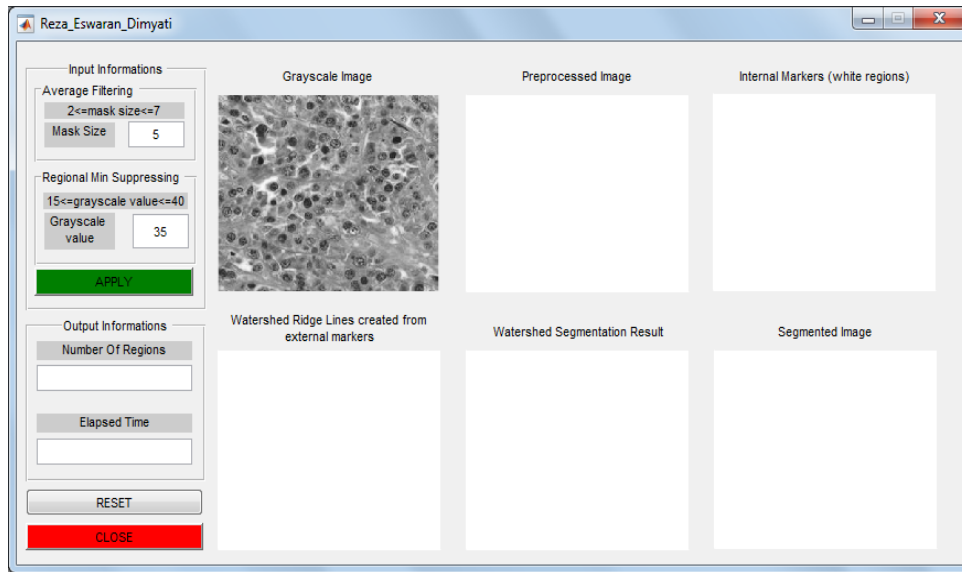


Figure 6. Result of “Reza&Eswaran&Dimyati”.

In this screen, mainly there are three stages; input information, output information and options. There are two input values for Algorithm 1 which should be entered by user. One of them is a mask size value of the average filter and another is a grayscale value for regional minimum suppressing operation. There is information at the above of inputs. This information show valid range of input values. The default values of the mask size value and grayscale value are given as 5 and 35, respectively. After the input values are entered, “APPLY” button is pressed and the images which are computed as a result of Algorithm 1 are shown. All the images are shown in the axes which are related with the names. They show important intermediate stages of segmentation and they make the process easier to understand for education. In addition to the segmented images, the number of segmented regions and elapsed time are presented below of these. After pressing the “APPLY” button, created images and results with respect to the given input values are presented in Figure 7.

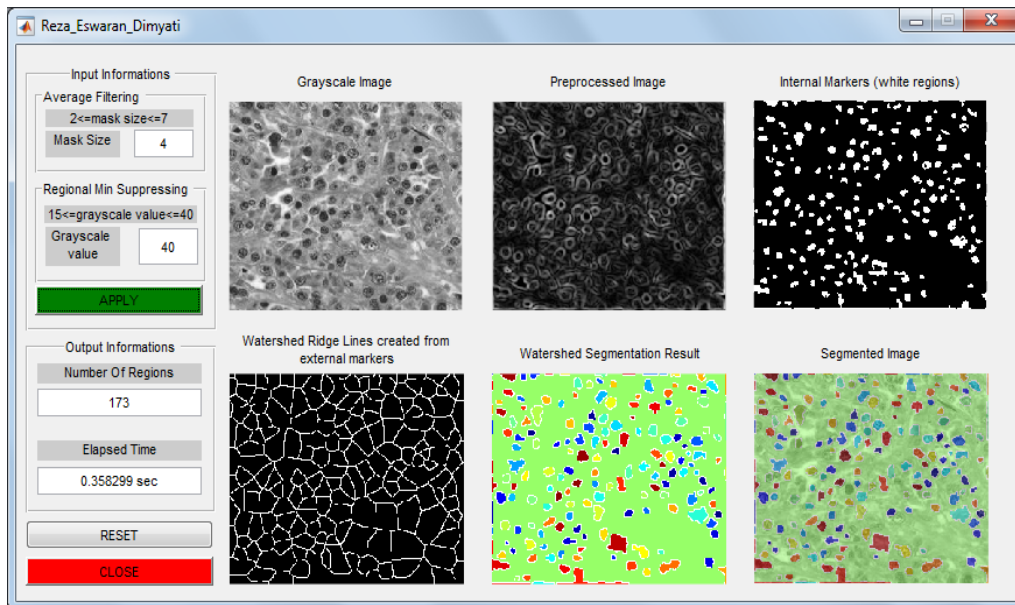


Figure 7. Result of “Apply” Button.

To perform a new test with different parameters, “RESET” button is pressed and all results (images and output information) are cleared and input values are replaced with default ones. For closing this screen and returning the application screen, “CLOSE” button is pressed. After the “CLOSE” button is pressed in the application screen, the last selected algorithm with the results can be seen on the application screen.

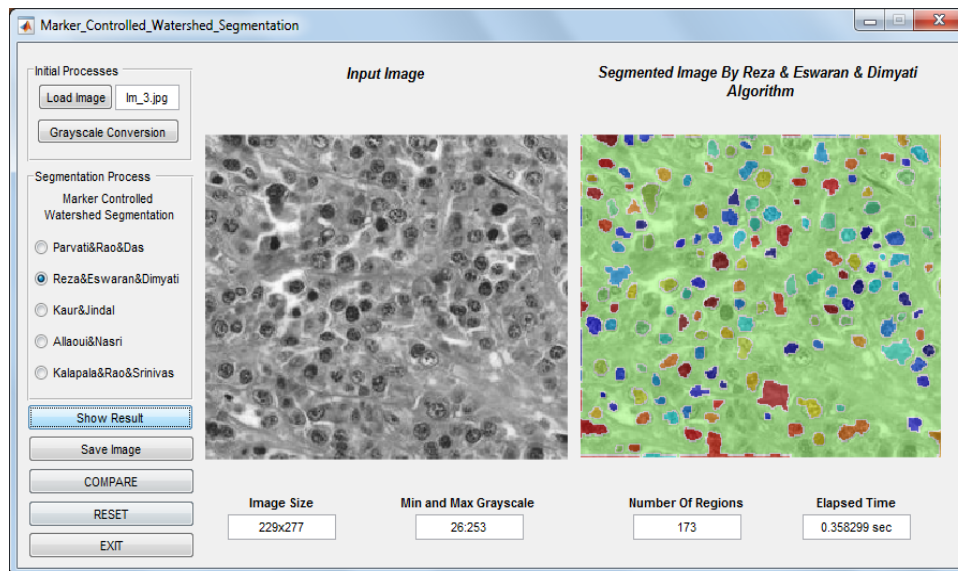


Figure 8. Result of “Show Result” Button.

Options stage is created to make the design more convenient. Since, it enables to operations in the resulted image. “Show Result” button is pressed for comparing input image and the segmented image. The result of this button is presented in Figure 8. The output information in the screen of Algorithm 1 come at the below of the segmented image. This option helps to improve the visual appearance and the quality of education.

For saving the segmented image, “Save Image” button is pressed and a new window is opened. In this new window, name of the image and directory for saving can be changed by the user. Then, format type of the segmented image is selected. After that, “Save” button is pressed and the window is closed.

After each algorithm is applied, “Show Result” and “Save Image” buttons should be pressed, respectively, to observe the result of the “COMPARE” button. If two or more algorithms have been applied by following the sequence described up to now, the “COMPARE” button can be used. For comparing two saved segmented images, “COMPARE” button is pressed and a new window is opened as presented in Figure 9.

“Select First Image” button is pressed for selecting a segmented image. Then, the selected image, its name and output information are placed to the axes1 as shown in Figure 10(a). All steps for the “Selected First Image” button are valid for “Select Second Image” button. The results of this button is placed to the window as given in Figure 10(b). After the comparison is completed, “EXIT” button is pressed. This window is closed and the application window appears.

The remaining two buttons in the application window are “RESET” and “EXIT”. If “RESET” button is pressed, the window is cleared and it returns the initial form as shown in Figure 3. For closing the application window, “EXIT” button is pressed.

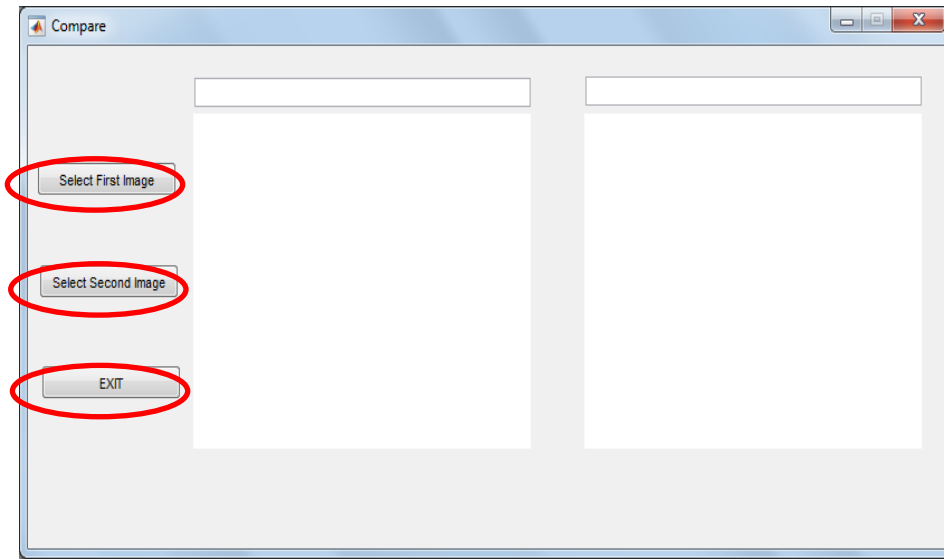


Figure 9. New Window After “COMPARE” Button.

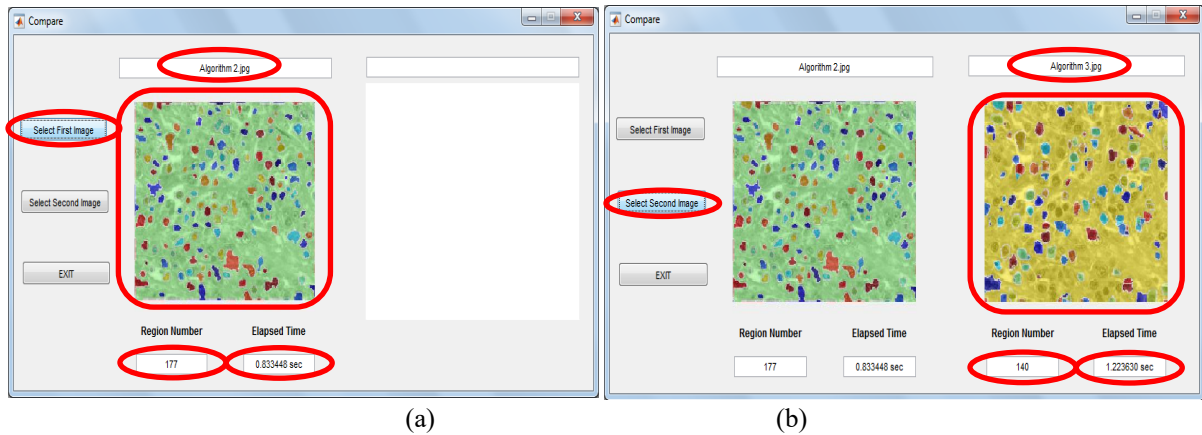


Figure 10. (a) Result of “Select First Image” (b) Result of “Select Second Image”.

Segmentation results of the different methods, which are applied to the grayscale bone plasmacytoma image, are shown in Figure 11. The results of all algorithms with their output data information are shown in Table 1. The best segmented result was obtained from Reza & Eswaran & Dimyati algorithm and the fastest segmentation was computed by Allaoui & Nasri algorithm according to Table 1. The differences between the results of the all algorithms can be defined as preprocessing stages and how to obtain gradient form of the image. The preprocessing stage is important for correct segmentation because gradient of the enhanced input image gives better results than gradient of the unenhanced image. Also, the way how to obtain the gradient form is important because it affects the image markers and image markers also affect the segmentation performance.

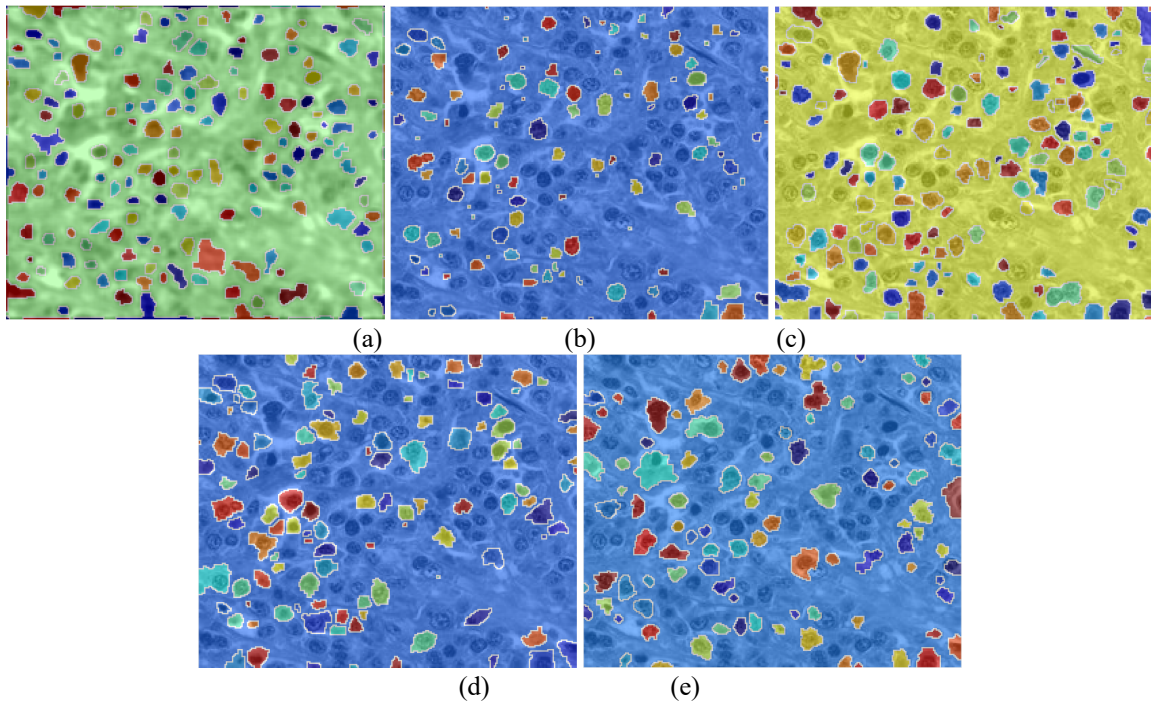


Figure 11. Segmentation results of (a) Reza&Eswaran&Dimiyati, (b) Parvati&Rao&Das, (c) Kaur&Jindal, (d) Kalapala&Rao&Srinivas, (e) Allaoui&Nasri algorithms.

Table 1. Output data information of all algorithms.

Algorithm	Property	Region Number	Elapsed Time (sec)
Reza & Eswaran & Dimiyati		177	1.083245
Parvati & Rao & Das		116	3.473332
Kaur & Jindal		151	1.393245
Kalapala & Rao & Srinivas		112	0.577348
Allaoui & Nasri		99	0.250725

6. Conclusion

In this study, some preprocessing morphological operations and five different published marker-controlled watershed algorithms were expressed clearly. An effective and educational GUI tool was designed using MATLAB App Designer for making comparison easier and quick understanding. This tool is very instructional for both pathology and image processing students. This was obtained by means of easy usability and extensive functionality of MATLAB App Designer. MATLAB is also a widely used and highly functional tool for many educational and scientific subjects based on mathematical calculations. By using functionality of MATLAB, a grayscale bone plasmacytoma image was segmented by applying all algorithms and the results were compared. In comparison of five different algorithms, the most effective and accurate segmentation was obtained by applying

Reza & Eswaran & Dimiyati algorithm for this image. This study also shows that marker-controlled watershed segmentation can be applied in different ways according to the way of marker determination, preprocessing stage and the way of the gradient image determination.

This study can be improved by adding more published algorithms about marker-controlled watershed segmentation to compare more effectively. Also, a new and more appropriate algorithm can be created to increase the accuracy of the watershed method. In addition to all improvements, these published algorithms can be applied to different images and the best one can be observed as more accurately. Consequently, designed GUI using MATLAB App Designer can be used as an educational tool in lessons to teach and to make comparison of the different watershed algorithms.

References

- [1] Yumuş M, Apaydın M, Değirmenci A, Kaplanoğlu H, Kesikburun S, Karal Ö. Deep Convolutional Neural Networks Using SegNet for Automatic Spinal Canal Segmentation in Axial MRI. In: 2023 Innovations in Intelligent Systems and Applications Conference (ASYU); 11-13 October 2023; Sivas, Türkiye: IEEE. pp. 1-6.
- [2] Kilic C, Degirmenci A, Karal O. Segmentation of the Area Between Anterior and Posterior Vertebral Elements in Axial MR Images Using U-Net. In: 2024 Innovations in Intelligent Systems and Applications Conference (ASYU); 16-18 October 2024; Ankara, Türkiye: IEEE. pp. 1-6.
- [3] Beucher S, Meyer F. The morphological approach to segmentation: the watershed transformation. In: *Mathematical Morphology in Image Processing*. 1st ed. Boca Raton, FLA, USA: CRC Press, 1993.
- [4] Vincent L, Soille P. Watersheds in digital spaces: an efficient algorithm based on immersion simulations. *IEEE Trans Pattern Anal Mach Intell* 1991; 13(06): 583-598.
- [5] Gozalez RC, Woods RE. *Digital Image Processing*, 3rd ed. NY, USA: Pearson, 2009.
- [6] Meyer F, Beucher S. Morphological segmentation. *J Visual Commun Image Represent* 1990; 1(1): 21-46
- [7] Beucher S. Use of watersheds in contour detection. In: *Proc. Int. Workshop on Image Processing*; 17-21 September 1979; Rennes, France. pp. 17-21.
- [8] Bieniek A, Moga A. An efficient watershed algorithm based on connected components. *Pattern Recognit* 2000; 33(6): 907-916.
- [9] Parvati K, Prakasa Rao BS, Mariya Das M. Image Segmentation Using Gray-Scale Morphology and Marker-Controlled Watershed Transformation. *Discrete Dyn Nat Soc* 2008; 2008(1): 384346.
- [10] Kaur M, Jindal G. Medical image segmentation using marker controlled watershed transformation. *IJCST* 2011; 2(4): 548-551.
- [11] Reza AW, Eswaran C, Dimiyati K. Diagnosis of diabetic retinopathy: automatic extraction of optic disc and exudates from retinal images using marker-controlled watershed transformation. *J Med Syst* 2011; 35(6): 1491-1501.
- [12] El Allaoui A. Medical image segmentation by marker-controlled watershed and mathematical morphology. *IJMA* 2012; 4(3): 1-9.
- [13] Kalapala M, Rao VS, Srinivas K. Robust Tree Crown Delineation using Novel Marker Controlled Watershed Segmentation Algorithm. *Int J Eng Res Technol* 2012; 1(7):1-11.
- [14] Kaleem M, Sanaullah M, Hussain MA, Jaffar MA, Choi TS. Segmentation of brain tumor tissue using marker controlled watershed transform method. In: *International Multi Topic Conference*; 28-30 March 2012; Berlin, Heidelberg: Springer. pp. 222-227.
- [15] Koyuncu CF, Arslan S, Durmaz I, Cetin-Atalay R, Gunduz-Demir C. Smart markers for watershed-based cell segmentation. *PloS one* 2012; 7(11): e48664.
- [16] Yahya AA, Tan J, Hu M. A novel model of image segmentation based on watershed algorithm. *Adv Multimedia* 2013; 2013(1): 120798.
- [17] Beare R, Chen J, Adamson CL, Silk T, Thompson DK, Yang JYM, Andersan VA, Seal ML, Wood AG. Brain Extraction Using the Watershed Transform From Markers. *Front Neuroinf* 2013; 7(32): 1-15.
- [18] Sivagami M, Revathi T. Marker Controlled Watershed Segmentation Using Bit-Plane Slicing. *IJIPVS* 2013; 1(3):179-183.
- [19] Napoleon D, Santhoshi R, Shameena A. Verdict of Objects in Medical Images Using Marker-Controlled Watershed Image Segmentation. *Int J Comput Appl Technol* 2013; 975: 8887.
- [20] Ravi S, Khan AM. Bio-medical Image Segmentation Using Marker Controlled Watershed Algorithm: A Case Study. *IJRET* 2014; 3: 26-30.
- [21] Bandara, R. Image segmentation using unsupervised watershed algorithm with an over-segmentation reduction technique. *arXiv preprint arXiv:1810.03908* 2018.
- [22] Lahitha K, Amrytha R, Stafford M, Shivakumar DrM. Implementation of Watershed Segmentation. *International Journal of Advanced Research in Computer and Communication Engineering* 2016; 5(12): 196-199.
- [23] Lu Y, Jiang Z, Zhou T, Fu S. An Improved Watershed Segmentation Algorithm of Medical Tumor Image. In *IOP Conference Series: Materials Science and Engineering*; December 2019; 677 (4): IOP Publishing. pp. 042028.

- [24] Sarma R, Gupta YK. A comparative study of new and existing segmentation techniques. In IOP Conference Series: Materials Science and Engineering; January 2021; 1022(1): IOP Publishing. pp. 012027.
- [25] Dai Y, Meng L, Wang S, Sun F. A marker-controlled watershed algorithm for the intelligent picking of long jujubes in trees. *Forests* 2022; 13(7): 1063.
- [26] Guo Q, Wang Y, Yang S, Xiang Z. A method of blasted rock image segmentation based on improved watershed algorithm. *Sci Rep* 2022; 12(1): 7143.
- [27] Ghaderi S, Ghaderi K, Ghaznavi H. Using marker-controlled watershed transform to detect baker's cyst in magnetic resonance imaging images: A pilot study. *J MED SIGNALS SENS* 2022; 12(1): 84-89.
- [28] Salman SD, Bahrani AA. Segmentation of tumor tissue in gray medical images using watershed transformation method. *Int J Adv Comp Techn* 2010; 2(4): 123-127.
- [29] Goshal D, Acharjya PP. MRI image segmentation using watershed transform. *Int J of Emerging Technology and Advanced Engineering* 2012; 2(4): 373-376.
- [30] Solomon C, Breckon T. *Fundamentals of Digital Image Processing: A practical approach with examples in Matlab*. 1st ed., NJ, USA: John Wiley & Sons, 2011.
- [31] Vincent L. Morphological grayscale reconstruction in image analysis: applications and efficient algorithms. *IEEE Trans Image Process* 1993; 2(2): 176-201.
- [32] Chanda B, Kundu MK, Padmaja YV. A multi-scale morphologic edge detector. *Pattern Recognition* 1998; 31(10): 1469-1478.
- [33] Abdul-Nasir AS, Mashor MY, Mohamed Z. Modified global and modified linear contrast stretching algorithms: new colour contrast enhancement techniques for microscopic analysis of malaria slide images. *Comput Math Methods Med* 2012; 2012(1): 637360.
- [34] Jacobs D. Image Gradients. www.cs.umd.edu/~djacobs/CMSC426/ImageGradients.pdf Published 2005. Accessed 05 June 2023.
- [35] Beucher S. The watershed transformation applied to image segmentation. *Scanning microscopy* 1992; 1992(6): 299-314.
- [36] Sun H, Yang J, Ren M. A fast watershed algorithm based on chain code and its application in image segmentation. *Pattern Recognit Lett* 2005; 26(9): 1266-1274.
- [37] De Bock J, Philips W. Line segment based watershed segmentation. In: *International Conference on Computer Vision/Computer Graphics Collaboration Techniques and Applications*; March 2007; Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 579-586.
- [38] Ruparelia S. Implementation of Watershed Based Image Segmentation Algorithm in FPGA. 2012. Master's Thesis.
- [39] Maheshwari D, Shah AA, Shaikh MZ, Chowdhry BS, Memon SR. Extraction of Brain Tumour in MRI Images Using Marker Controlled Watershed Transform Technique in MATLAB. *J Biomed Eng Med Imaging* 2015; 2(4): 9-16.
- [40] www.webpathology.com/ Accessed 13 May 2023.
- [41] Jung C, Kim C. Segmenting clustered nuclei using H-minima transform-based marker extraction and contour parameterization. *IEEE Trans Biomed Eng* 2010; 57(10): 2600-2604.
- [42] Chowdary PRV, Babu MN, Subbareddy TV, Reddy BM, Elamaram V. Image processing algorithms for gesture recognition using MATLAB. In: *Proceedings of 2014 IEEE International Conference on Advanced Communication, Control and Computing Technologies*; 08-10May 2014; ICACCCT 2014: IEEE. pp. 1511-1514.
- [43] Çankaya İ. *MATLAB App Designer ile GUI Tasarımı ve Uygulamaları*. Ankara, Türkiye: Seçkin Yayıncılık, 2024.