Comparing marker definition algorithms for Watershed segmentation in microscopy images

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ABSTRACT

Segmentation is often a critical step in image analysis. Microscope image components show great variability of shapes, sizes, intensities and textures. An inaccurate segmentation conditions the ulterior quantification and parameter measurement.

The Watershed Transform is able to distinguish extremely complex objects and is easily adaptable to various kinds of images. The success of the Watershed Transform depends essentially on the existence of unequivocal markers for each of the objects of interest. The standard methods of marker detection are highly specific, they have a high computational cost and they determine markers in an effective but not automatic way when processing highly textured images. This paper compares two different pattern recognition techniques proposed for the automatic detection of markers that allow the application of the Watershed Transform to biomedical images acquired via a microscope.

The results allow us to conclude that the method based on clustering is an effective tool for the application of the Watershed Transform.

Keywords: Segmentation, Digital image processing, Fuzzy logic, clustering.

1. INTRODUCTION

Microscope image components show great variability of shapes, sizes, intensities and textures [1]. Moreover, during acquisition, it is necessary to establish a high number of parameters that result in the presence of noise, non-homogeneous illumination, fuzzy contours and low contrast. This characteristic result in an incorrect segmentation when applying conventional segmentation methods.

Watershed Transform (WT) is a powerful morphological tool to segment texture images into regions of interest. This transform is adaptable to different types of images and capable of distinguishing extremely complex objects.

The WT is a segmentation method based on regions, which classifies pixels according to their spatial proximity, the gradient of their gray levels and the homogeneity of their textures. To avoid over-segmentation a single marker for each object of interest has to be selected [2].

The selection of adequate markers on these kinds of images is a painful and sometimes fruitless task. Hence, the experienced observer defines markers in a semiautomatic way [3][4][5]. The automatic determination of markers is still a difficult goal to achieve. The current determination algorithms are highly dependent on the structure to be segmented [6][7]. Moreover, they have a high computational cost and they determine markers in an effective but not automatic way when processing highly textured images [8].

We developed two algorithms based on fuzzy inference systems and clustering in order to obtain markers that allow robust segmentations in images highly textured.

The first algorithm developed determines texture characteristics and generates a fuzzy inference system that relate them to determine the markers. This algorithm results in correct segmentations but it needs manual intervention to choose the object to be segmented by the user and the previous selection of the texture characteristic to generate the base rule. On the other hand computational times are high. This is an important disadvantage in microscopy image processing because they have a significant spatial resolution in most of the cases.

The second algorithm uses, as input to a clustering algorithm, characteristics determined from the regions resulting from the over-segmentation produced by the WT through regional minima. WT markers are selected as the cluster that represents the objects of interest. Finally WT is applied again over these new markers allowing an effective and robust segmentation of highly textured images. Computational times are lower than the first algorithm's. Furthermore it is not necessary to establish the type of texture characteristics resulting in an automatic method for segmentation.

2. MATERIALS, METHODS AND RESULTS

A. Materials

For this work, microscope images were used in order to evaluate the proposed algorithms due to the great difficulty that their segmentation presents. Bone marrow biopsies were used. In these images we need to segment the trabeculae in order to make a diagnosis. A dye with hematocylin and eosyn was applied to bone marrow tissue to color label the trabeculae. Although the original biopsies are in color, we turned them into gray levels to facilitate their processing. Image adquisition of the samples were made with an optic microscope Medicux-12 and a CCD camera Hitachi KP-C550. Image resolution is 640 x 480 pixels and were saved in Windows bitmap (BMP). Even though the image is formed by different biological structures, the trabeculae are the ones that are most difficult to segment. Thus, they were used to evaluate the algorithms. The images were acquired through a microscope where light, resolution and contrast were established by the lab technician to obtain the best visualization possible.

We also processed other images in order to evaluate the algorithm with microscope images from different applications (bacteria, linfatic nodules).

To segment these images we cannot use a histogram, because we cannot distinguish gray levels characteristic of the trabeculae. Due to the great variability of these conditions it is impossible to automatically define markers with the methods developed so far.

All the algorithms were implemented in Matlab® R14. We worked with the standard functions of this language and a specific library called SDC Morphology Toolbox [9] with functions of mathematical morphology..

B. Watershed Transform

A gray scale image can be interpreted as the topographic image of land relief. It can be indicated that the gray intensities of higher amplitude correspond to plains and mountains and the lower intensity ones correspond to valleys and rivers [1][10][11]. Using the characteristics of these images we define a technique of digital image processing called Watershed Transform (WT), which through the flooding of the valleys, is capable of recognizing similar topographical areas, surrounded by mountain ridges. The WT is a segmentation method based on regions, which classifies pixels according to their spatial proximity, the gradient of their gray levels and the homogeneity of their textures [2][12][13].

With the objective of segmenting an image in gray levels, prior to the application of the WT, a gradient image must be obtained, where the levels of the contours of the objects to be segmented represent an area of elevated gray intensity. The areas of low intensity give way to the basins where the water would flow and flood the topography of the image. The elevations in gray levels generated by the contours would remain and give way to the segmentation of the image through the resulting watershed lines. [2][12]. Mathematical morphology allows us to obtain a gradient which is highly adaptable to different kinds of images with a higher precision than conventional algorithms. In this paper we used the morphological gradient to obtain the intermediate image before applying the WT [14][15].

The classic WT floods the gradient image from its regional minima. In non homogeneous or noise embedded images there is not a one to one relation between regional minima and objects of interest. This results in an over segmentation in the majority of images, in other words, after WT each of the objects is represented by more than one region [2][3][4][12][16]. To avoid this over segmentation we resort to the selection of a single marker for each object of interest. Theses markers or seeds initiate the flooding algorithms indicating the sector that gives rise to the basins. Based on these characteristics we can conclude that the success of the WT depends mainly upon the characteristics of the markers.

C. Fuzzy Inference Systems

In this paper we propose to use fuzzy logic as a tool to assist in the determination of markers. This discipline arises from the formalization of imprecise, ambiguous and linguistically expressed knowledge [17][18][19].

A fuzzy inference system processes information from input variables to give output values. It uses the values of the input variables to determine the truth values of the predicates that it will use as antecedents in a rule base. Each value of the input variables is assigned a value of membership to different fuzzy sets defined for each variable. This procedure is done through membership functions that can be, for instance, trapezoid or Gaussian functions. Through an inference process for each rule (determination of consequents) and a following process of aggregation (union of the results of the different rules), we obtain values for the output variables for different values of the input variables.

Nowadays there are segmentation methods that apply fuzzy logic to the entropy function of the histogram of an image with the objective of improving and segmenting it. They define fuzzy membership functions through the image histogram to discriminate objects or regions. However, when it comes to textured images it is not posible to differentiate objects in an automatic manner only using the histograms of the gray intensity levels or its attributes [17][18][19].

The proposed method based on fuzzy logic consists in relating texture features that characterize the different components of the image based on its texture [8][21]. To achieve_this, a fuzzy inference system is generated (Mamdani Fuzzy Inference System) from the indication of the object of interest that is to be segmented by the experienced observer. The proposed system stands out the objects of interest while it attenuates the background and the irrelevant objects. Then a morphological opening and a conventional binarization to obtain the markers are applied. Finally the WT is applied to the gradient of the original image from the obtained markers. The use of fuzzy logic for the definition of markers for the W.T. is adequate because it is not necessary to obtain the complete object, but only an approximation of its interior.

D. Clustering after over-segmentation

We also proposed to apply clustering after over-segmentation. Features extracted from over-segmentation resulting regions, after applying W.T. from its regional minima, are used as input to a clustering algorithm [8][21].

There are different methods that group these regions to segment images; however none uses them to obtain the markers for the WT [22]. The basis for the development of this new algorithm was to reduce the sensitivity of the algorithm to noise and irrelevant objects and to increase its robustness to process biopsies with different features.

We used two clustering algorithms. First the k-means algorithm was used [7][11][22]. This unsupervised algorithm requires the specification of the number of classes in which the data set is going to be partitioned. To each class there is a corresponding cluster center so that the distance of each pattern to its center is minimal. The partition is done by measuring, in an iterative process, the distance between each pattern to its cluster center, and computing again the centers until there is no change. In a second stage the fuzzy k-means algorithm was used [6][11]. This algorithm is based on fuzzy logic and assigns to each pattern a level of belonging to each class instead of offering a unique agrupation of them.

We obtained different regions by applying the WT to the original image. Then a cluster algorithm was applied. The mean and standard deviation of each region characterize different components in the images. The k-means algorithm applied to the features in those regions determined effectively the internal markers of the objects. The fuzzy k-means did not classify correctly the markers of any kind of regions. We applied morphological operators to the class that represents the objects to generate the internal markers. The markers of the background, external markers, were obtained eroding the internal markers complements. This procedure resulted in adequate segmentations when internal markers sizes are similar to the size of the objects. The gradient of the biopsies images does not have a visible contrast, making it difficult to apply the flooding algorithms. Consequently, by obtaining internal and external markers of a considerable size the results were improved. These properties of the markers were attained due to the use of the algorithm developed from the over segmentation.

E. Proposed Algorithms

The main steps of the algorithms finally implemented are the following:

Fuzzy Systems based algorithm:

Step 1. Characterization of the texture of the image components through co-ocurrence matrices [10][14]. Contrast, mean value and energy were calculated. Masks of a 3x3 size were used.

Step 2. The experienced observer indicates two points of the image to be segmented. The first one corresponds to a point belonging to the object of interest, in this case the trabeculae. The second point belongs to the background of the image.

Step 3. The features values in each point are used to define the membership functions of the fuzzy inference system [5][17][18]. First, we determine the mean value of the selected points found in a space of 9x9 surrounding the points selected by the experienced observer. Then we generate Gaussian membership functions, whose highest membership points corresponded to mean values obtained in the first step. This is done for each characteristic, not only for the background but also for the trabeculae, for each image in particular.

Step 4. A rule base is determined from these membership functions considering the information the experienced observer provided by distinguishing the background from the trabeculae. The output of the inference system has two membership functions, the first one to indicate the presence of the object and the other one the background. This inference system is used for each one of the pixels of the image using the features values corresponding to each pixel.

Next, we present the rules used to define the inference systems:

If the pixel has an energy value that corresponds to the energy of the object of interest, then it is object.

If the pixel has an energy value that corresponds to the energy of the background, then it is background.

If the pixel has a contrast value corresponding to the contrast of the object of interest, then it is object.

If the pixel has a contrast value that corresponds to the background contrast, then it is background.

If the pixel has a mean value that corresponds to the mean value of the object of interest, then it is object.

If the pixel has a mean value that corresponds to the mean value of the background, then it is background.

The rule base proposed made it possible to obtain high levels of gray when the pixel corresponded with the object and low levels of gray when it corresponded with the background.

Step 5. A conventional binarization with a threshold in 128 is applied to differentiate the objects [10].

Step 6. There was a grainy texture in the objects of interest that prevented the use of this result to define markers for the W.T. Therefore, new openings with structuring elements of 3x3 pixels are applied, obtaining unequivocal and homogeneous internal markers for each one of the objects of interest [14].

Step 7. We also need to define the external markers for the background. The latter ones were obtained through the morphological erosion of the complement of the internal markers[20].

Step 8. Finally the W.T. is applied to the gradient image of the original image using the markers obtained in the previous steps [2][8][16][21].

Clustering based algorithm:

Step 1. Application of the WT using the regional minima as markers. The result is a matrix that assigns a label to each pixel indicating the regional minima to which it belongs.

Step 2. The regions resulting from the WT are analyzed to obtain features from each one of them to distinguish the objects of interest based on their texture. Mean and standard deviation are computed from the intensities that are found inside the regions.

Step 3. The features vectors are used as input for the k-means algorithm. The partition of the data set is done in four classes due to the existence of four components of the biopsies (trabeculae, fat, blood cells, and intracellular space).

Step 4. An image is obtained with only the class that distinguishes the objects of interest (trabeculae)

Step 5. Application of the morphological opening and closing operators on the markers to obtain the object markers, or internal markers [14][15]. These operators join adjacent regions because they correspond to the same object and eliminate regions that do not belong to the wanted objects. The previous result is slightly eroded to eliminate the regions that belong to the possible borders of the objects. As a final result we obtain the image that is to be used as the internal marker for the WT.

Step 6. Application of the morphological erosion to the complement of the internal markers, to obtain the background markers, or external markers.

Step 7. Application of WT using the internal and external markers computed in the previous steps, over the morphological gradient of the original image.

F. Results

Fig. 1 shows the partial results of the application of the algorithm based on fuzzy logic.

The rule base proposed made it possible to obtain high levels of gray when the pixel corresponded with the object and low levels of gray when it corresponded with the background (Figs. 1-a, 1-b).

A conventional binarization with a threshold in 128 allowed us to differentiate the objects (Fig. 1-c). Openings with structuring elements of 3x3 pixels were applied, obtaining unequivocal and homogeneous internal markers for each one of the objects of interest.

To apply the W.T. it is necessary to mark not only the objects but also the background. We need to define the internal markers for the objects of interest as well as the external markers. The latter ones were obtained through the morphological erosion of the complement of the internal markers.

Finally the W.T. was applied to the gradient image of the original image using the markers obtained in the previous steps (Fig. 1-d). It was not possible to obtain this result with other conventional methods of image segmentation.

The fuzzy inference system generated from the selection of the object of interest, by an experienced observer, allows the correct definition of the internal markers and the ulterior successful application of the W.T. The main advantage of the inference system developed is that the determination of the markers through mathematical morphology, after applying the rule base, is simple and robust. This is not possible to obtain from the original image without the previous application of the fuzzy inference system to the texture features.

Fig. 2 shows the partial results of the application of the algorithm based on clustering.

Figure 2-a) shows two bone marrow biopsies. Figure 2-b) shows the result of the clustering in four classes (trabreculae, fat, blood cells, and intracellular space). This cluster is obtained from the over-segmentation produced by the direct application of the WT using the mean and standard deviation of the intensities of each region. Fig. 2-c) shows an image that contains only the class that distinguishes the objects of interest (the trabeculae).

To obtain the object markers, or internal markers, morphological opening and closing operators are applied. These unite the adjacent regions that correspond to the same object and eliminate the regions that do not belong to the wanted objects. Then the result is eroded to eliminate the regions that belong to the possible borders of the objects. As a result the internal markers for the WT are obtained. This last result is observed in Fig. 2-d).

The background markers or external markers are obtained eroding the complement to the internal markers (see Fig. 2-e).

Finally, the WT is again calculated with the internal and external markers previously determined and the morphological gradient of the original image. Figure 2-f) shows the resulting segmentation.

Trabeculae corresponding to biopsies of different patients and pathologies were successfully segmented. The method based on clustering is malleable and can be applied to all kinds of images. These features make the system adaptable to a particular image without the need to modify the algorithm or its parameters.

The error of the algorithms is determined by a parameter μ based on the symmetrical distance and the Hausdorff distance [23]. The values for this parameter vary from 0 to 1. A value close to 1 means a great difference between the figure segmented by the WT and the segmentation produced by an experienced observer. A value close to 0 means figures that differ in a small number of pixels.

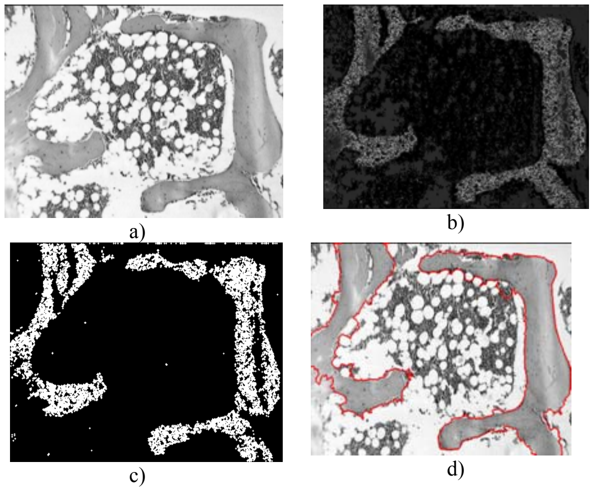

Fig. 1. a) Bone marrow biopsy, b) Result of the fuzzy system, c) Binarization and d) Result of the T.W..

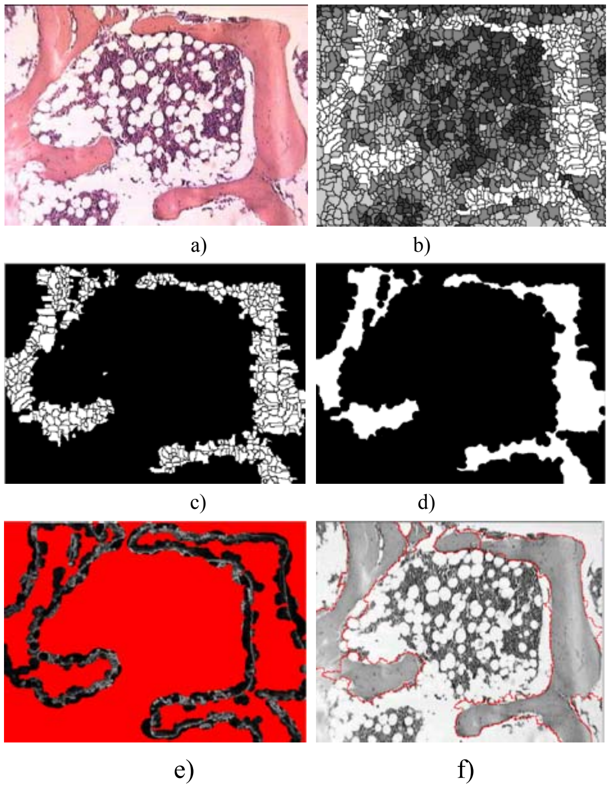

Fig. 2. Intermediate stages in the application of the algorithm. a) two bone marrow biopsies, b) classification in four classes (trabeculae, fat, blood cells, and intracellular space), c) only the class that distinguishes the objects of interest (the trabeculae), d) internal markers for the WT, e) external markers f) the resulting segmentation.

Figure 1 and figure 2 show successful trabeculae segmentation by applying the proposed algorithms. Fifty six images belonging to fourteen different patients were processed. Trabeculae segmentation error is usually bounded above 1% and below 13% in the case of the algorithm based on clustering and between a range of 1% and 18% in the algorithm based on fuzzy logic (see Table 1).

On the other hand, as it can be seen in Table 1, the selected regions were not enough to segment correctly the trabeculae, it was necessary to apply the WT again using the markers produced by these regions. Fig. 3 shows the variation of the µ parameter when using clustering. The mean value was 0.065 and the standard deviation was 0.198.

Figure 4 shows the images resulting from the application of the clustering algorithm. The trabecuale's successful segmentation can be seen. It was possible to analyse biopsies of different patients with different pathologies that could not be segmented with other algorithms of marker detection. Samples of bacteria and linfatic nodules images were also processed with a clustering algorithm (see Fig. 4).

TABLE 1

EXAMPLE OF VARIABILITY OF µ FOR DIFFERENT BIOPSIES, INCLUDING BEST AND WORST CASES.

# Trabéculae	Fuzzy Logic	K-means	K-means + W.T.
1	0.09	0.18	0.13
2	0.18	0.14	0.07
3	0.12	0.10	0.04
4	0.16	0.14	0.12
5	0.13	0.10	0.07
6	0.14	0.10	0.12
7	Incorrect_Segmentation	0.08	0.08
8	Incorrect_Segmentation	0.18	0.07
9	Incorrect_Segmentation	0.10	0.08
10	0.08	0.09	0.09
11	0.09	0.12	0.05

TAB LE 2

EJECUTION TIME OF TE DIFFERENT STEPS OF THE ALGORITHM

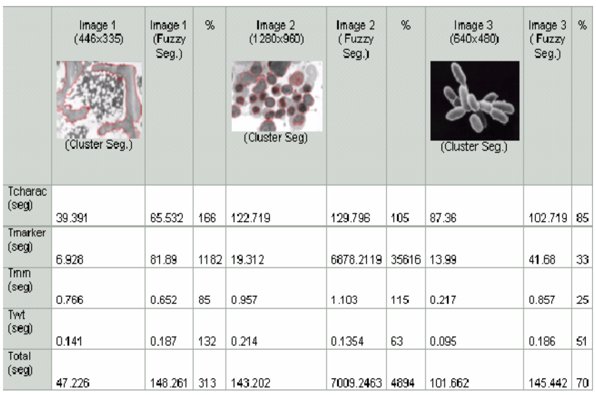

	Image 1 (446x335) (Cluster Seg.)	Image 1 (Fuzzy Seg.)	%	Image 2 (1280x960) (Cluster Seg)	Image 2 (Fuzzy Seg.)	%	Image 3 (640x480) (Cluster Seg.)	Image 3 (Fuzzy Seg.)	%
Tcharac (seg)	39.391	65.532	166	122.719	129.796	105	87.36	102.719	85
Tmarker (seg)	6.928	81.89	1182	19.312	6878.2119	35616	13.99	41.68	33
Tmm (seg)	0.766	0.652	85	0.957	1.103	115	0.217	0.857	25
Twt (seg)	0.141	0.187	132	0.214	0.1354	63	0.095	0.186	51
Total (seg)	47.226	148.261	313	143.202	7009.2463	4894	101.662	145.442	70

Fig. 3. Error analysis for clustering algorithm

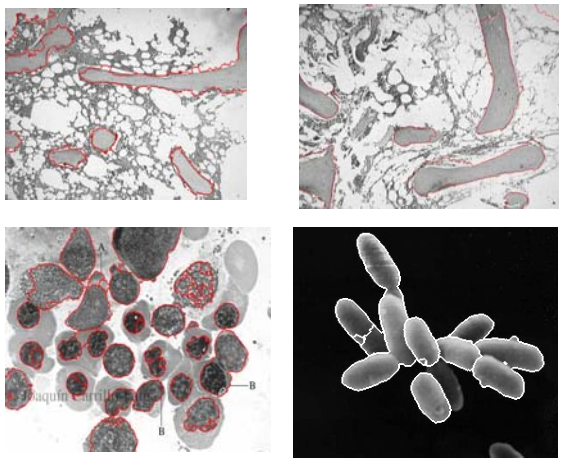

Fig 4. Resulting segmentation for other sample images. The upper biopsies correspond to other patients and the lower images correspond to lymphatic nodules and bacteria.

Table 2 shows the ejecution time for both algorithms. This time is divided in four parts: the determination of the features time, the definition of markers time, the morphological operations time and finally the W.T. time. It can be seen that the morphological operations and WT have a minimal computational cost.
On the contrary, the characterization of texture and marker definition determines the total ejecution time. In all the images processed the clustering algorithm last lesser than the fuzzy algorithm.

CONCLUSIONS

Comparing with the algorithm based on fuzzy logic, clustering is not only automatic but it is also able to segment the trabeculae with a lower error. Moreover, it presents smaller computational costs than the algorithm based on clustering.

The chosen method based on clustering correctly groups the regions produced by the WT through regional minima. The different classes correspond to the components of the images: trabeculae, fat, blood cells, and intracellular space. These classes allow us to define effective markers to segment the trabeculae through WT but they can also be used to segment the other components of biopsies that are equally important from the point of view of the diagnosis of illnesses.

The first grouping of pixels using the classical WT reduces the noise in the resulting images and consequently makes it possible to define unique, homogeneous and larger markers in contrast with the other existing methods. It also makes possible to reduce the computational cost of the algorithm. Biomedical images have a high spatial resolution and the computational cost of the markers detection is added to the cost of the gradient computation and WT. That is why the computational cost of the algorithm has to be taken into account during its development.

The error analysis shows that the segmentation done with the proposed algorithm is precise, simple and robust. It adapts automatically to the different features of the biopsies without having to change the process of biopsies for different patients.

The proposed method is an effective tool to obtain internal markers for the watershed transform automatically. It is possible to apply it on highly textured images, like the microscopic images, which processing is complex and cannot be done trough conventional methods.

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