Microscope cell color images segmentation by fuzzy morphological reconstruction

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ABSTRACT

There are many different methods to perform gray level segmentation in microscope cell images however, in some circumstances, texture features and roughness are not as relevant as the color for the segmentation task. In many biomedical applications, where these types of images are analyzed, the aim is to segment nuclei for their clinical analysis. In this work, a fuzzy color mathematical morphology reconstruction technique was developed, based on a new locally defined ordering, to achieve microscope cell images segmentation. We show experimental results for this proposed fuzzy color morphological reconstruction, which show that this tool can be efficiently applied in cells segmentation without generating false colors.

Keywords: Microscope cell color image, Color images, Reconstruction, Mathematical Morphology, Fuzzy Mathematical Morphology.

1. INTRODUCTION

Nowadays, technological development has enabled a huge advance in histological knowledge. The use of segmentation algorithms improves the detection and treatment of different diseases. More powerful algorithms will help medical specialists to provide better diagnosis.[1] This paper proposes a new segmentation method, for microscope color images of cells, based on fuzzy morphological reconstruction, where the markers for the cell can be obtained automatically or semi-automatically. Automatically, when an algorithm is used to detect the cells in the image, for example using basic morphological filters. Semi-automatically when the expert manually selects each cell of interest using an interactive software.

The Fuzzy Mathematical Morphology (FMM) is a different approach for the extension of the Mathematical Morphology's binary operators to gray level images, by redefining the set operations as fuzzy set operations, based on Fuzzy set theory.[3–10] FMM is based on a solid theoretical framework, and is proved to be able to solve several problems in image processing and segmentation problems with high texture content.

Although these techniques have been widely studied and applied to gray scale images, currently the use of color medical imaging is growing, due to the additional information they present and the increased computing power available. Traditionally, color images were not used because the capabilities of capturing and processing equipment were limited, as well as the costs and processing times were prohibitive. For that reason, very few disciplines took into account the potential they offer. The growth in computing power, storage capacities and modernization of low-cost systems capture and printing of the images have been fueled a growing interest in the use of color images in the development of many applications.

However, the extension to color or multispectral images is not simple, due to the nature of the data vector and the difficulty of finding a proper order for it. Due to this reason the current approaches of the extension of color Mathematical Morphology (MM) focus on the definition of an order in the color space.[11–13] This difficulty

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mobilized many researchers since the 1990s.[14–18] A relatively recent and extensive work, which has more than 70 references to various methods that make use of the MM color, was written by Aptoula and Lefvre in 2008.[19] Additionally, Hanbury and Serra[20,21] presented a conditional order in the CIELab space, extending the MM to color images. Also, Sartor and Weeksen[22] proposed to combine a distance measure, based on a reference color, and lexicographic order, the latter used to resolve ambiguities. Angulo, Aptoula and De Witte[23–25] also presented proposals based on an order that emerges from a distance function. More recently, in Bouchet et.al.[2] the authors presented the basic operators of the FMM for color images from the definition of a fuzzy order based on fuzzy preference relations.

On the application side, segmentation of cells in microscopic color images still presents a challenge. Automatic or semiautomatic segmentation techniques attempt to improve sensitivity and specificity by detecting, segmenting and classifying the cells, and to determine the shape of the cytoplasm or nuclei.

In this work, a reconstruction technique, based on fuzzy color mathematical morphology, was developed based on a new locally defined ordering.[2] The marker image can be obtained either automatically or semi-automatically. It is obtained automatically when the markers are obtained by an image filter process. For example, automatic selection can be done by successive dilations with a structuring element whose size is related to the size of the nucleus of the cell to be removed. That way the image mask is obtained automatically. It is obtained semi-automatically when the expert manually selects one pixel for each cell of interest.

One of the main advantages of the proposed operators is the no creation of new colors. The final reconstructed image contains the same colors, or less, than the original image. It has been show that this is an important property to maintain the quality and clarity of the processed images, if they are going to be used by specialists in the process of making decisions.

We also show experimental results of applying the proposed fuzzy color morphological reconstruction. We show that this tool can be efficiently applied in cells segmentation.

2. FUZZY MATHEMATICAL MORPHOLOGY IN COLOR SPACE

Once the extensions of different basic morphological operators are defined for color images, the geodesic reconstruction operator is derived from them. This operator is a powerful tools for image segmentation when objects marker are present. Proper treatment of the color information in these types of operations will allow generalization of the geodesic reconstruction in color images using fuzzy operators and, therefore, achieve efficient segmentation for such images. By employing hue as another attribute in identifying objects, they also avoid unpleasant effects of brightness or reflection by the properties of the materials or the lighting conditions in the image acquisition. It is important to note that the attenuation of brightness in an image is desirable because, in a segmentation process, these induce the wrongly detections of regions that are nonexistent in the original image. Therefore, it is of special interest the ability of proper reconstruction in situations of attenuation of brightness and/or chromatic noise.

2.1 Fuzzy erosion and dilation for color images

We consider a color image as a function $f : D_f \subseteq R^2 \to \tau \subseteq R^3$ where D_f is the domain of the function and τ is a color space.[2] The basic operators of the morphology for a color image f are the erosion and the dilation. Then, combining these operators another more complex can be defined.[26,27] The basic operators are defined as follows:

Definition 2.1. *Let $\tau \subseteq R^3$ be a color space with a structure of complete lattice provided by a total order $\leq_\tau$. Let B be a structuring element, the basic operators, dilation ($\delta_B^{\leq_\tau}$) and erosion ($\varepsilon_B^{\leq_\tau}$), associated to a color image f are defined as follow:*[2]

$$\delta_B^{\leq_\tau}(f) = \sup_{x \in B}\{f \circ T_{-x}\} \tag{1}$$

$$\varepsilon_B^{\leq_\tau}(f) = \inf_{x \in B}\{f \circ T_{x}\} \tag{2}$$

being $T_x : R^2 \to R^2$ the translation function by the element $x \in R^2$, that is, $T_x(s) = s + x$.

Note that for any color image f, any dilation $\delta_B^{\leq_\tau}$ and any erosion $\varepsilon_B^{\leq_\tau}$ are new color images.

2.2 Geodesic morphological operators for gray levels images

The geodesic reconstruction is one of the operators of mathematical morphology that facilitates image segmentation. This operator allows, by using markers, highlighting an image objects of interest, in order to remove them from the rest of the scene. That means geodesic operators are useful when you want to process a subset of the space analyzed. The geodesic reconstruction operator employs successive dilations (erosions) to a marker image whose result is delimited by a mask image.

The definitions of geodesic erosion and dilation are closely related to the geodesic distance.

Definition 2.2. *The geodesic erosion of an image f (called mark), by a structuring element B, conditioned to g (called mask), $f \leq g$, is defined as:*[28]

$$\varepsilon_{B,g}^{(1)}(f) = \varepsilon_B(g) \vee f \tag{3}$$

being $\vee$ the supremum and $\varepsilon_B(g)$ is the erosion of the gray level image g by a structuring element B.

First, the mask image g is eroded. Then the supremum between the eroded image and the mask is calculated. The visual effect of this type of erosion is that the mask retains the marker so that it does not disappear (the contraction of the marker is limited). In this case the geodesic erosion is greater than or equal to the mask, it is also increasing and anti-extensive operation.

Definition 2.3. *The geodesic erosion of size n, with $n > 1$, of an image f, by a structuring element B, conditioned to g, is defined as the iteration of geodesic erosion of increasing size, i.e.:*[28]

$$\varepsilon_{B,g}^{(n)} = \underbrace{\varepsilon_{B,g}^{(1)}(\varepsilon_{B,g}^{(1)}(\ldots(\varepsilon_{B,g}^{(1)}(f))))}_{n\ times} \tag{4}$$

Definition 2.4. *The geodesic dilation of an image f, by a structuring element B, conditioned to g, $g \leq f$, is defined as:*[28]

$$\delta_{B,g}^{(1)}(f) = \delta_B(g) \wedge f \tag{5}$$

being $\wedge$ the infimum and $\delta_B(g)$ is the dilation of the gray level image g by a structuring element B.

The mark acts as the limit of the mask image dilated therefore $\delta_{B,g}^{(1)}(f) \leq f$. The geodesic dilation, like classical dilating, is a growing and extensive operator.

Definition 2.5. *The geodesic dilation of size n, with $n > 1$, of an image f, by a structuring element B, conditioned to g, like geodesic erosion, is defined as the iteration of geodesic dilation of increasing size, i.e.:*[28]

$$\delta_{B,g}^{(n)} = \underbrace{\delta_{B,g}^{(1)}(\delta_{B,g}^{(1)}(\ldots(\delta_{B,g}^{(1)}(f))))}_{n\ times} \tag{6}$$

The geodesic erosion and dilation have the particularity that when they are iterated until stability allows the definition of powerful algorithms of morphological reconstruction. Both the geodesic erosion and dilation converge in a finite number of iterations.

Reconstruction by dilation of a mark image f from a mask image g, both with the same domain $f \leq g$, is define as the geodesic dilation of g conditioned to f until stability and it denotes $\rho_g(f)$.

Definition 2.6. *The gray level reconstruction $\rho_g(f)$ of an image g with respect a marker image f, $g \leq f$, is obtained iterating successively geodesic dilation of the gray levels image g, by a structuring element B, until new changes do not occur. That means:*[28]

$$\rho_g(f) = \vee_{n \geq 1} \delta_{B,g}^{(n)}(f) \tag{7}$$

As f acts like constraint, $\rho_g(f) \leq f$, so at the end of the geodesic dilation produces no changes in the image. Reconstruction by dilation is an anti-extensive operation.

Each geodesic dilation of the reconstruction is performed over the result of the geodesic dilation from the previous iteration. Thus the marker reduces progressively its intensity under the mask.

Definition 2.7. *Similarly the dual reconstruction $\rho_g^*(f)$ with respect to an image f is defined $f \leq g$, as:*[28]

$$\rho_g^*(f) = \wedge_{n \geq 1} \varepsilon_{B,g}^{(n)}(f) \tag{8}$$

The idea is to iterate several times to spread the minimum (maximum) value of each component till the entire component is homogenized. That minimum (maximum) represents the gray level of the reconstruction of the object, so that the reconstruction of an image in gray levels will not be perfectly reconstructed, as it happens in the binary case.

2.3 Geodesic morphological operators for color images

In this section, we describe general geodesic operators for color images, based on an order on the color space. For this definition, both basic operators, erosion and dilation, need to be defined.

Definition 2.8. *The color reconstruction $\rho_g^{\leq_\tau}(f)$ of a color image g with respect a color marker image f, $g \leq_\tau f$, is obtained iterating successively geodesic dilation in color space, by a structuring element B, until new changes do not occur. That means:*

$$\rho_g^{\leq_\tau}(f) = \vee_{n \geq 1} \delta_{B,g}^{\leq_\tau^{(n)}}(f) \tag{9}$$

being $\vee$ the supremum given by the order $\leq_\tau$ and $\delta_{B,g}^{\leq_\tau^{(n)}}(f) = \underbrace{\delta_{B,g}^{\leq_\tau^{(1)}}(\delta_{B,g}^{\leq_\tau^{(1)}}(\ldots(\delta_{B,g}^{\leq_\tau^{(1)}}(f))))}_{n \ times}$ where $\delta_{B,g}^{\leq_\tau^{(1)}}(f) = \delta_B^{\leq_\tau}(f) \wedge g$ and $\delta_B^{\leq_\tau}(f)$ is defined in Equation 1.

Similarly, we can define the dual reconstruction as follows:

Definition 2.9. *Similarly the dual reconstruction $\rho_g^{\leq_\tau *}(f)$ with respect to an image f is defined $f \leq_\tau g$, as:*

$$\rho_g^{\leq_\tau *}(f) = \wedge_{n \geq 1} \varepsilon_{B,g}^{\leq_\tau^{(n)}}(f) \tag{10}$$

being $\wedge$ the infimum given by the order $\leq_\tau$ and $\varepsilon_{B,g}^{\leq_\tau^{(n)}}(f) = \underbrace{\varepsilon_{B,g}^{\leq_\tau^{(1)}}(\varepsilon_{B,g}^{\leq_\tau^{(1)}}(\ldots(\varepsilon_{B,g}^{\leq_\tau^{(1)}}(f))))}_{n \ times}$ where $\varepsilon_{B,g}^{\leq_\tau^{(1)}}(f) = \varepsilon_B^{\leq_\tau}(f) \vee g$ and $\varepsilon_B^{\leq_\tau}(f)$ is defined in Equation 2.

3. PROPOSED METHOD

As was seen before, geodesic operators are defined based on the two basic operators of erosion and dilation. In this work we propose to use the fuzzy erosion and dilation based on the fuzzy order defined in Bouchet et.al.[2] as a base for the geodesic color operators.

First, the order defined in Bouchet et.al.[2] is a fuzzy relationship between pixel values. The infimum and supremum of Equations 1 and 2 are computed using this order, so the two basic operators, erosion and dilation, obtained this way are *fuzzy morphological operators*. This order generates an ordering of a set of pixel colors based on the fuzzy "preference criterion". This can be seen as a group decision problem with n alternatives (the n neighbor pixels in this framework) $A = \{a_1, a_2, \ldots, a_n\}$, $(n \geq 2)$ and 3 experts $E = \{e_1, e_2, e_3\}$ (the RGB components). Each expert (RGB component) provides his preference among the set of alternatives (pixels). The goal is to apply a group decision making strategy to identify the pixel most accepted according to their color values. This order is composed by three steps: making uniform the information, aggregating and exploiting the information.

Based on the two new fuzzy erosion and dilation operators, and the order in color space defined in Bouchet et.al.,[2] we can now define the new *fuzzy geodesic morphological operators*, from Equations 9 and 10. The new geodesic operators have the great advantage that no new colors are created in the process, so that the final reconstructed image contains only the colors present in the new image.

Finally, the proposed method to segment the cells can be summarized in the following four steps:

Step 1: Defining fuzzy erosion and dilations based on the new order in the RGB color space.

Step 2: Defining the fuzzy geodetic operators for color images based on the basic operators defined in step 1.

Step 3: Determining the marker image manually for the cells to be segmented.

Step 4: Applying the fuzzy geodesic reconstruction using the marker image of the previous step.

Figure 1 shows the proposed color fuzzy reconstruction applied to a synthetic image. The marker image was obtained by a manual selection. It can be seen that both in the area of gray levels as in the area of color, the reconstruction works satisfactorily.

Figure 1. Result of the propose method applied to a synthetic image. (a) Original image. (b) Marker image. (c) Reconstructed image.

4. RESULTS

As an example of application, we applied the proposed color segmentation method to cell images from different sources.

Below we present different examples of the results of the proposed color morphological reconstruction in cells image segmentation. Figure 2 shows an example of an image of avian erythrocyte,[29] or red blood cells, which also show their nuclei. In this case, the marker image was obtained by manual selection of the blood cells markers. Figure 3 shows frog red blood cells, called also erythrocytes. These cells, in contrast to mammals red blood cells, contain a nucleus, which is visible as a dark purple dot in the center of each cell.[30] For this image, semi-automatic and automatic markers were used. Finally, figure 4 shows an example of nasal mucosa in allergic rhinitis obtained from a nasal cytology,[31] where the markers were obtained semi-automatically and automatically.

In many biomedical applications, where these type of images are analyzed, the aim is to segment nuclei for their clinical analysis. The color fuzzy reconstruction operator defined in this paper is an useful tool to achieve such kind of segmentations. While the choice of marker can be automatic, this paper shows the results with a manual selection of markers, since it allows the expert to perform a separation of the nuclei of interest.

The behavior of the color fuzzy reconstruction operator in these images shows a similar performance to occurs in the binary case in which the selected object it is rebuilds accurately unlike what happens in gray levels images.[32] The color of objects in the image in many cases can be associated with a pathology. The results are interesting because the color of the object is not changed with respect to the original image.

Two of the orders most used in the literature to extend the mathematical morphological operators to color images are the marginal order and the lexicographic order. The extension obtained using the marginal order generates false colors in the resulting image.[2] The extension of lexicographical order is strongly dependent on the choice of the main component. The third approach used here computes maximum and minimum, in the neighborhood, based on the three channels with no differentiation between them, using a fuzzy approach to sort the color values. The preliminary results show a good behavior relative to the resulting color reconstruction. Since it is a preliminary work, for future works we plan to evaluate quantitatively the differences against the other methods, in order to validate the proposed algorithm.

Figure 2. Result of the propose method. (a) Original image. (b) Semi-automatic selection of markers. (c) Reconstructed image.

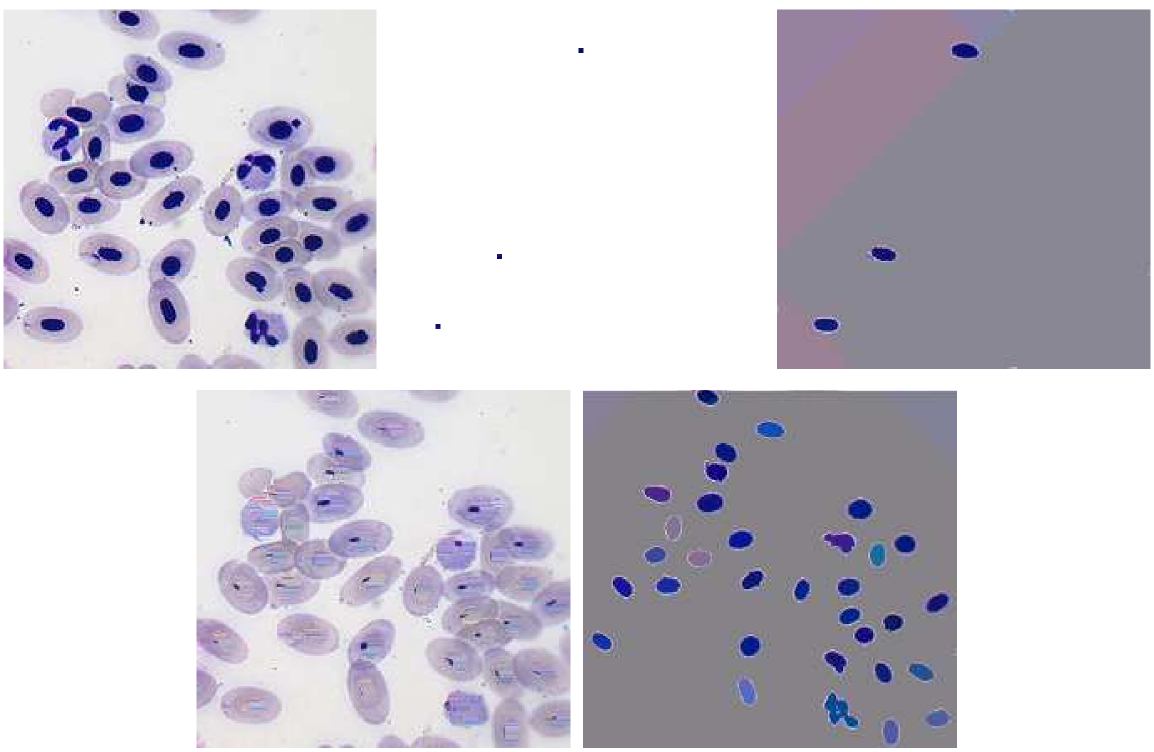

Figure 3. Result of the propose method. (a) Original image. (b) Semi-automatic selection of marker. (c) Reconstructed image with semi-automatic marker. (d) Automatic selection of marker. (e) Reconstructed image with automatic marker.

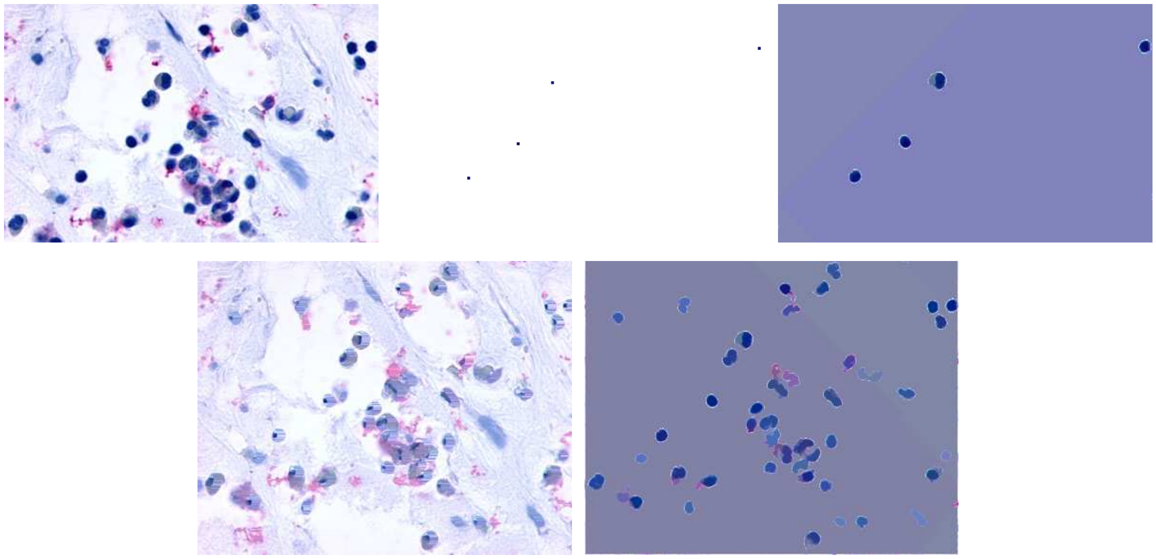

Figure 4. Result of the propose method. (a) Original image. (b) Semi-automatic selection of marker. (c) Reconstructed image with semi-automatic marker. (d) Automatic selection of marker. (e) Reconstructed image with automatic marker.

5. CONCLUSIONS

We proposed a new method for color image segmentation based on fuzzy color reconstruction, with application to the segmentation of cells images. The algorithm works directly on color images, avoiding the need to convert them to gray scale images, with the loss of information that it produces, and the final results are color images of the fully selected cells, reconstructed from original markers. Another advantage of this approach is that, because of the use of the new fuzzy color image operators, no false colors are created in the process, and the resulting image contains exactly the same colors present in the original image. As we can see in the results, the reconstruction algorithm can perform properly in different situations, where the individual cell nucleus were identified manually. Future work will be focused on the evaluation of the proposed method with more complex images, as low contrast ones, or with non homogeneous intensities.

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