Fuzzy Bio-Image Segmentation with Spatial Covariance

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Abstract

Image segmentation is an important research area in image analysis and its applications to medical and biological imaging. In particular, effective segmentation methods play an essential role in the analysis, classification, and quantification of bioimages for prognosis and preventive treatment. Different segmentation methods based on different criteria of optimality often give different results. This paper introduces a new strategy for modeling image spatial information in the setting of the fuzzy c-means algorithm for segmenting bio-images that are inherently fuzzy. The experimental results have shown the superior performance of the new method over some popular models for the segmentation of cell puncta.

Keywords: Image segmentation, Fuzzy c-means, Geostatistics, Mahalanobis distance.

1 Introduction

The inherent difficulty we often encounter in image segmentation is that there are many background pixels that have similar values as those which belong to the object or vice versa. These pixels are usually found in the proximity of the boundaries between the background and the object. These phenomena are particularly common in many bioimaging problems [1]. As a result, these images produce a vague valley in the histograms, which makes it a challenging task for any threshold-based image segmentation methods. There have been numerous attempts for developing segmentation methods for handling different types of images. Many image segmentation methods can be found in several literature reviews over the last three decades [2]-[13]. Most general approaches for image segmentation are based on thresholding, cluster analysis, edge detection, region growing, and watershed methods. However, a general agreement is that there is no single segmentation method that can be effectively applied to all types of images [7]. Thus, there arises a need for developing new algorithms that may be used for different purposes.

Among numerous image segmentation algorithms, there exist many FCM-based methods for image segmentation where the fuzzy objective function was modified to impose some constraints to incorporate probabilistic modeling, pixel spatial information or entropy [8]-[12]. However, most of these methods were designed to address problems in medical imaging. Analysis of biological images such as fluorescence microscopic images requires different approaches for effective segmentation of cells. It has been realized that the segmentation of bio-images, particularly cells, is effective only if the segmentation process can appropriately model the behavior of the biological objects [14, 15, 16]. For high-content and high-throughput screening, the analysis also requires some fast algorithm for image

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segmentation that serves as a basis for feature extraction and object classification to the demand for down-stream study and practical development such as hypothesis validation and drug discovery.

In this paper, an image segmentation method based on a spatial fuzzy objective criterion is introduced in the setting of the Mahalanobis distance where the covariance matrix is replaced with a spatial covariance matrix. The incorporation of the spatial Mahalanobis distance in the objective function of the fuzzy c-means (FCM) is to sharpen the fuzzy spatial scattering effect on images. The mathematical foundation is based on the theory of regionalized variables [17]. A regionized variable is defined as a random variable that is distributed in space. The spatial variability of the regionalized variables can be characterized by both random and structured aspects: (1) they are considered to be erratic in relation to the surrounding variables, and (2) they are spatially related with respect to the distance separating the variables. This spatial structure is called the variogram which is a geostatistical function that expresses the spatial relation of the regionalized variables. This conceptual framework is highly applicable to image modeling where the pixel values can be thought as being both random and spatially related.

The rest of this paper is organized as follows. Section 2 presents the formulation of a new FCM-based image segmentation method using the concept of a spatial covariance embedded in the Mahalanobis distance measure. Section 3 illustrates and discusses the performance of the proposed and other popular image segmentation methods. Section 4 concludes the research finding and suggests other issues for future development.

2 Spatial Covariance based FCM for Image Segmentation

Let $\mathbf{M}_{fc}$ be a fuzzy c-partition space, $\mathbf{X}$ be a subset of the real p-dimensional vector space $\mathcal{R}^p : \mathbf{X} = \{\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_n\} \subset \mathcal{R}^p$ where $\mathbf{x}_k = (x_{k1}, x_{k2}, \ldots, x_{kp}) \in \mathcal{R}^p$. The fuzzy c-means clustering is based on the minimization of the fuzzy objective function $J_m : \mathbf{M}_{fc} \times \mathcal{R}^{cp} \rightarrow \mathcal{R}^+$, which is defined as [18]

$$J_m(\mathbf{U}, \mathbf{v}, \mathbf{A}) = \sum_{i=1}^{c} \sum_{k=1}^{n} (u_{ik})^m (d_{ik})^2 \qquad (1)$$

where m is the fuzzy exponent, $\mathbf{U} \in \mathbf{M}_{fc}$, $\mathbf{v} = (\mathbf{v}_1, \mathbf{v}_2, \ldots, \mathbf{v}_c) \in \mathcal{R}^{cp}$, and $d_{ik} = ||\mathbf{x}_k - \mathbf{v}_i||_{\mathbf{A}} = (\mathbf{x}_k - \mathbf{v}_i)^T \mathbf{A} (\mathbf{x}_k - \mathbf{v}_i)$ is any inner-product norm metric induced on $\mathcal{R}^p$ by $\mathbf{A}$.

Using the Mahalanobis distance, $(d_{ik})^2$ is defined as

$$(d_{ik})^2 = (\mathbf{x}_k - \mathbf{v}_i)^T \mathbf{C}_i^{-1} (\mathbf{x}_k - \mathbf{v}_i) \qquad (2)$$

where $\mathbf{C}_i^{-1}$ is the inverse of the sample covariance matrix of the data points in $\mathbf{v}_i$.

The purpose is to introduce the spatial covariance matrix in (2), that is

$$(d_{ik})^2 = (\mathbf{x}_k - \mathbf{v}_i)^T \mathbf{C}(h)_i^{-1} (\mathbf{x}_k - \mathbf{v}_i) \qquad (3)$$

where $\mathbf{C}(h)_i^{-1}$ is the inverse of the spatial covariance matrix of the data points in $\mathbf{v}_i$, and h is a lag variable that spatially separates the data points in $\mathbf{v}_i$.

The determination of the spatial covariance $C(h)$ can be obtained using the theory of regionalized variables and discussed as follows.

In the context of image analysis, pixels can be modeled as regionalized variables [17] developed in geostatistics [19] in the sense that their values are random and they are spatially related. By such hypothesis, the variogram [17, 19] of an image is a function which expresses the spatial correlation of the regionalized variables of the image. In probabilistic notation, the image variogram, denoted as $2\gamma(h)$, can be defined as the expected value of the image intensities spatially distributed apart with a distance h:

$$2\gamma(h) = E\{[x_i - x_j]^2\},\ h_{ij} = h \qquad (4)$$

where x_i and x_j are the intensity values of the pixels located at positions i and j of the image

respectively, and h is the spatial distance that separates x_i and x_j. The values of h can be taken in any directions in a discrete image. In this study, h takes the integer values in the horizontal and vertical directions of the image.

The semi-variogram, denoted as $\gamma(h)$, is therefore half of the variogram. The experimental semi-variogram for lag distance h is defined as the average squared difference of values separated by h:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{(i,j)|h_{ij}=h} (x_i - x_j)^2 \quad (5)$$

where $N(h)$ is the total number of data pairs separated by the distance h.

The behavior of the semi-variogram can be graphically illustrated by the theoretical semi-variogram using the spherical or the Matheron model which is defined as [19]

$$\gamma(h) = \begin{cases} s\left[1.5\frac{h}{r} - 0.5(\frac{h}{r})^3\right] & : \quad h \le r \\ s & : \quad h > r \end{cases} \quad (6)$$

where r and s are called the *range* and the *sill* of the semi-variogram, respectively.

Figure 1 shows the spherical semi-variogram model defined in (6). When $h = 0$, two samples are taken at the same position and the difference between the two must be zero. When $h > 0$, the two samples move a distance apart and some positive difference between the two values can be expected. As the samples move further apart, the differences should increase accordingly. Ideally when the distance becomes very large and reaches r, the sample values become independent of one another. The semi-variogram $\gamma(h)$ will then become constant at s as the result of the calculation of the difference between the pairs of independent samples.

The properties of the semi-variogram can be further explored by again letting h be the distance between two variables x_i and x_j, and by an assumption that the random variables in the random function model has the same

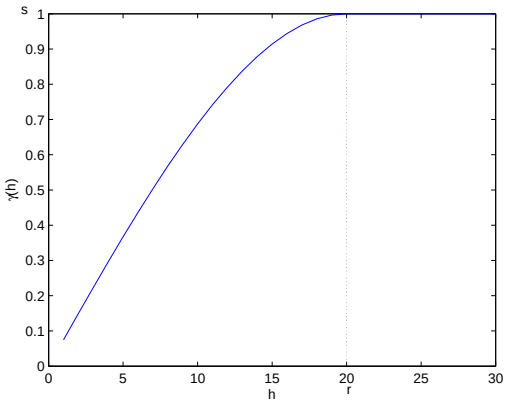

Figure 1: Example of a semi-variogram – the spherical model with $s = 1$ and $r = 20$

mean μ and variance σ^2. These two properties show the relationship between the semi-variogram and the covariance by the following derivation [19]:

$$\begin{aligned} \gamma(h) &= \frac{1}{2}E\{[x_i - x_j]^2\} \\ &= \frac{1}{2}E\{x_i^2\} + \frac{1}{2}E\{x_j\}^2 - E\{x_i x_j\} \\ &= E\{x^2\} - E\{x_i x_j\} \\ &= [E\{x^2\} - \mu^2] - [E\{x_i x_j\} - \mu^2] \\ &= \sigma^2 - C_{ij} \end{aligned} \quad (7)$$

where C_{ij} is the spatial covariance of x_i and x_j.

The spatial covariance, $C(h)$, can be determined by [19]

$$C(h) = \frac{1}{N(h)} \sum_{(i,j)|h_{ij}=h} (x_i x_j) - (\mu_{-h}\mu_{+h}) \quad (8)$$

where μ_{-h} and μ_{+h} are the means of all the data values whose spatial locations are $-h$ and $+h$ away from other data points, respectively:

$$\mu_{-h} = \frac{1}{N(h)} \sum_{i|h_{ij}=h} x_i \quad (9)$$

$$\mu_{+h} = \frac{1}{N(h)} \sum_{j|h_{ij}=h} x_j \quad (10)$$

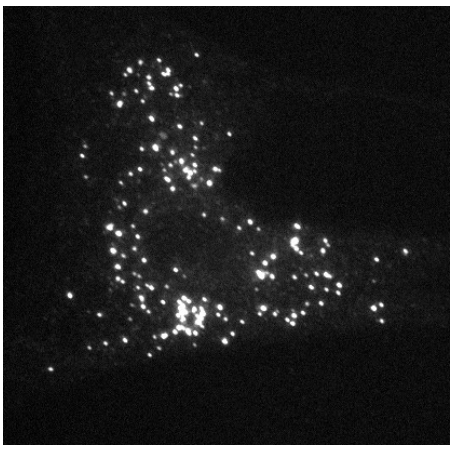

Figure 2: Original image

Figure 3: Segmentation by Otsu thresholding

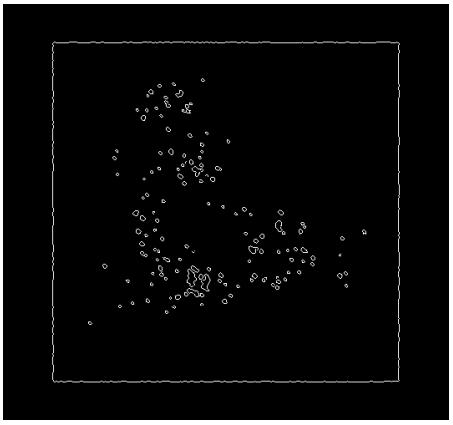

Figure 4: Segmentation by Sobel edge

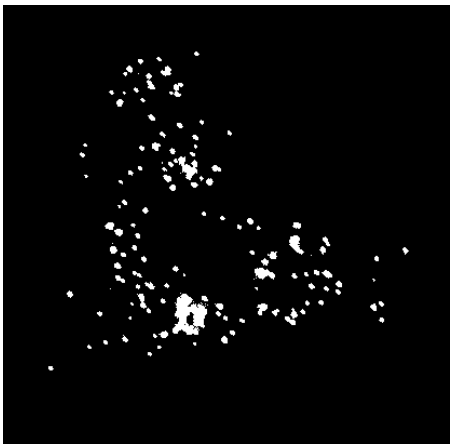

Figure 5: Segmentation by ED-FCM

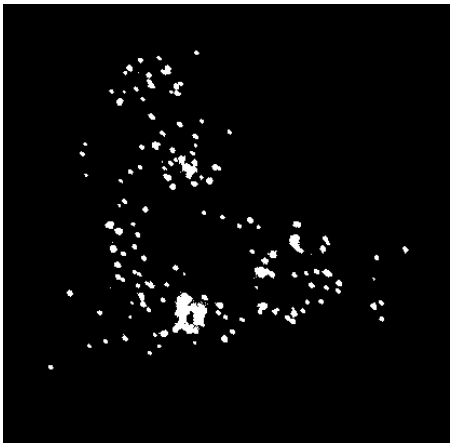

Figure 6: Segmentation by MD-FCM

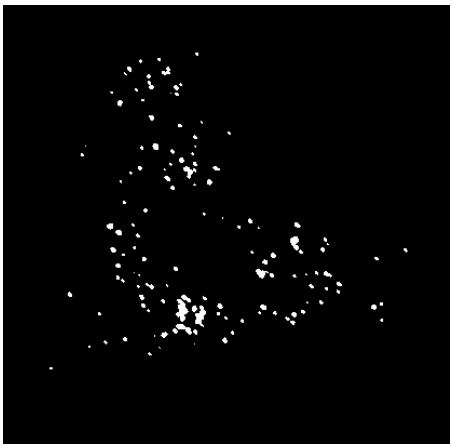

Figure 7: Segmentation by SMD-FCM

An alternative and more popular equation for calculating the spatial covariance is

$$C(h) = \frac{1}{N(h)} \sum_{(i,j)|h_{ij}=h} (x_i x_j) - (\frac{1}{n} \sum_{k=1}^{n} x_k)^2 \tag{11}$$

where n is the total number of data points. In (8) the emphasis is on the product of the two different lag means; whereas in (11) the central term is the square of the mean of all the data values.

The determination of the spatial covariance that can be used as a spatial description of the fuzzy objective function has been discussed. The FCM-based image segmentation can be carried out based on the fuzzy membership matrix $\mathbf{U}$ by simply assigning each pixel $\mathbf{x}_k$ of the image to class i if u_{ik} is maximum, that is

$$\text{Assign } \mathbf{x}_k \text{ to class } i^* \text{if } i^* = \arg\max_i u_{ik}$$

3 Experiment

We tested the proposed method with real fluorescent images of peroxisomes contained in cells whose boundaries are inherently fuzzy or imprecise. A typical image is show in Figure 2. The discrimination and measurement of fluorescent-labeled vesicles using microscopic analysis of fixed cells presents a challenge for biologists interested in quantifying the abundance, size and distribution of such vesicles in normal and abnormal cellular situations. Good image segmentation results will allow the precise quantification of changes to the population of a major organelle, the peroxisome, in cells from normal control patients and from patients with a defect in peroxisome biogenesis.

To compare the proposed method with some other segmentation methods, we used Otsu thresholding [20], Sobel edge detection [21], the fuzzy c-mean algorithm using Euclidean distance (ED-FCM), the fuzzy c-mean algorithm using Mahalanobis distance (MD-FCM), and the proposed fuzzy c-mean algorithm using spatial Mahalanobis distance

(SMD-FCM) to carry out the segmentation of the same original image. The original image of fluorescent puncta as shown in Figure 2 where the fluoresecence-stained background between and along the boundaries of the spots makes it a difficult task for extracting the correct sizes of the objects [1].

Figures 3, 4, 5, 6, and 7 show the segmentation results of the original image in Figures 2 obtained by Otsu thresholding, Sobel edge detection, ED-FCM, MD-FCM, and the proposed SMD-FCM methods, respectively. For the SMD-FCM method, $C(h)$ was taken using a lag distance h=1 in both vertical and horizontal directions. The fuzzy exponent $m = 2$ was used for all FCM-based methods.

There has been some effort attempted to investigate the comparisons of different image segmentation methods [22, 23]. Segmentation results are considered favorable if the segmented regions are homogeneous and have smooth and spatially accurate boundaries [24]. In addition, the assessment of the segmentation results of biological images are particularly dependent on the experts when classication does not involve [25]. Using this guideline and by visual obervation of biology experts, the results presented in all the figures show that the proposed method provided the best segmentation. The proposed SMD-FCM method could better recognize the true boundaries of the objects than other methods. Increasing order of over-segmented results can be observed by using the ED-FCM, MD-FCM, and Otsu. The ED-FCM and MD-FCM yielded similar segmentation results. Extraction of the cell puncta by Sobel detection suffers from under-segmentation.

4 Conclusion

A FCM-based method using a spatial covariance function for segmenting fuzzy bioimages has been discussed. The comparative results have shown the promising application of the proposed method to the segmentation of cell puncta in low-contrast and fluorescent-stained images. Effective segmentation of such biological images helps life-science researchers obtain useful imaging information for downstream analysis including disease diagnosis, treatment, and new drug discovery.

What has been successful for the proposed method is that the spatial covariance function at short range, where h is small, is captured in distance measure that in turn has effect on the fuzzy membership functions. Noise effects for various images under present study are similar and mainly due to fluoresence staining. Thus, this short-range spatial information has been appropriately utilized to sharpen the imprecise boundary of the puncta (small objects), where other methods do not address this modeling problem. Incorporating fuzzy membership grades into the spatial covariance function can be expected to yield better results. As another issue, the use of the spatial covariance matrix as a condition for fuzzy shape description [18] is worth considering.

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