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Automatic Detection of Proliferative Cells in Immunohistochemically Images of Meningioma Using Fuzzy C-Means Clustering and HSV Color Space

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***Abstract*—**Visual search and identification of immunohistochemically stained tissue of meningioma was performed manually in pathologic laboratories to detect and diagnose the cancers type of meningioma. This task is very tedious and time-consuming. Moreover, because of cell's complex nature, it still remains a challenging task to segment cells from its background and analyze them automatically. In this paper, we develop and test a computerized scheme that can automatically identify cells in microscopic images of meningioma and classify them into positive (proliferative) and negative (normal) cells. Dataset including 150 images are used to test the scheme. The scheme uses Fuzzy C-means algorithm as a color clustering method based on perceptually uniform hue, saturation, value (HSV) color space. Since the cells are distinguishable by the human eye, the accuracy and stability of the algorithm are quantitatively compared through application to a wide variety of real images.

***Keywords*—**Positive cell, color segmentation, HSV color space, immunohistochemistry, meningioma, thresholding, fuzzy c-means.

I. Introduction

MENINGIOMAS are common neoplasm originating from arachnoid cap cells. Most of these tumors are encountered in middle or later adult life [1], [2], and women are more likely to develop meningiomas.

Meningiomas are categorized in to three grades of malignancy according to proliferative activity histologic features and subtypes [3], [4]. Most of the meningiomas are benign (grade I); however, some (grade II and III) are clinically aggressive tumors. Two of the most important factors in determining the prognosis are the histologic grade and extent of resection [5]. One of the useful criteria in determining histologic grade is counting mitotic figures. Immunohistochemical staining (KI-67, MIB-1) is a technique used for detecting proliferating cells. KI-67 antigen is a nuclear protein present only during active phase of cell cycle [6]. The MIB-1 antibody recognizes the KI-67 antigen on paraffin embedded tissues, by this method any proliferating cell is visualized and can be seen by light microscopy. MIB-1 labeling index was determined and calculated as a percentage of positivity nuclei over the total number of cell nuclei counted. Unfortunately, this type of counting is subjective and time consuming for pathologist physicians. In order to overcome the problem, a good solution is to incorporate digital image processing techniques to automated cell analysis. During the past decade, Computer-Aided Diagnosis (CAD) has become a major research area in medical imaging, diagnostic radiology, mammography, and some other medical specialties such as pathology. The core goal of CAD is to provide a computer output as a second opinion to support the physician in image interpretation by improving the accuracy and consistency of pathological diagnosis and also by reducing the image interpreting time. Possible applications for CAD in immunohistochemically methods in pathology include detecting and classifying cells in digital immunohistochemically images of meningioma, distinguishing between benign, atypical and malignant tumors and quantitative analysis of diffuse meningioma tumors. Cells nuclei segmentation is the key issue in automatic cell images analysis. Pixel based cell segmentation method combining the shape information of the cell nuclei was proposed in [7]. Techniques of active contour [8], neural network [9], mathematical morphology [10], were also used in cell nuclei segmentation. Gray level thresholding techniques are computationally inexpensive methods for partitioning a digital image into mutually exclusive exhaustive regions [11]. In the proposed approach, the color information of the cell image pixels is taken to account and by clustering algorithm the cell image segmentation is performed. Our approach is based on the perceptually uniform HSV color space and color clustering. Accurate segmentation of the nucleus provides valuable prior information regarding cell counts and cell mitotic index detection. This work is part of a larger project in which a reliable CAD will be design for automatic counting each cell type in immunohistochemistry image of meningioma and categorize it in three degree of malignancy.

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II. Image Acquisition

Tissue sections (4-µm thickness) were prepared to immunhistochemically staining and then observing under a light microscope. The positive cells were marked by brown color and the negative ones were violet color. In other words, stained tissue cells are classified into two categories according to their nuclear color Acquisition of microscope image is

usually done using a CCD camera mounted in the optical path of the microscope. The camera usually be full color. The images of meningioma that we will be using here, were captured by using a ZEISS camera plus light microscope. After reviewing and confirming the acquisitioned images by pathologist physician, 150 images were selected for entry to the database.

III. The Selection of the Color Space

Although RGB color models are extremely useful for color representation, color processing and also development of color image processing algorithms, these models are no way similar to the human vision model. One of the major limitations of the RGB color space is that it is a nonuniform color space. Uniform color space is one in which the Euclidean color distance between two color points at any part of the color space corresponds to the perceptual difference between the two colors by the human vision system. In color image processing applications, perceptually uniform color spaces are of great importance. Based on the physiological knowledge of human vision, the nonuniform RGB color space needs to be mapped into new perceptually uniform spaces.

A. HSV Color Space [12]

HSV is one color space, which describes colors as perceived by human beings. In the HSV color space, a color described by values for hue, color saturation and intensity as coordinate axes. By projecting the RGB unit cube along the diagonals of white to black, a hexagon results in that forms the topside of the HSV pyramid. The hue H is indicated as an angle around the vertical axle. Red color determined with $H = 0^{\circ}$or $H = 360^{\circ}$, green with $H = 120^{\circ}$, and so on (Fig. 1).

The saturation is a number between 0 on the central axis and 1 on the sides of pyramid. The brightness value V lies between 0 on the apex of the pyramid and 1 on the base. Some image processing programs as MATLAB contains modules for transforming images between the RGB and the HSV color space.

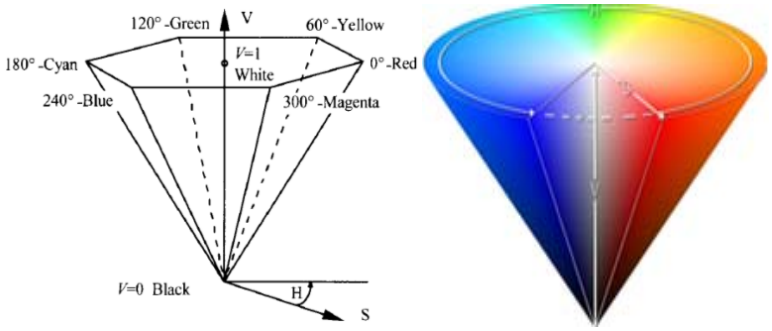

Fig. 1 Perceptual representation of HSV color space

B. RGB to HSV Color Space Transformation

HSV is an alternative color space representation. First, *r, g, b* values are computed by normalizing each pixel such that:

$$r = \frac{R}{R+G+B}\ , g = \frac{G}{R+G+B}\ , b = \frac{B}{R+G+B} \tag{1}$$

Accordingly, the H, S, and V values can be computed as:

$$V = max(r, g, b)$$

$$S = \begin{cases} 0 & if\ V = 0 \\ V - \frac{min(r,g,b)}{V} & if\quad V\rangle 0 \end{cases}$$

$$H = \begin{cases} 0 & if\ S = 0 \\ 60^{\circ} \times \left[\frac{g-b}{S\times V}\right] & if\ V = r \\ 60^{\circ} \times \left[2 + \frac{(b-r)}{S\times V}\right] & if\ max = g \\ 60^{\circ} \times \left[4 + \frac{(r-g)}{S\times V}\right] & if\ max = b \end{cases}$$

$$H = H + 360^{\circ} \qquad if\ H < 0 \tag{2}$$

The results obtained by using either of the above transformations yield reasonably good results.

IV. Color Segmentation via Clustering

Clustering is the methods of classification data samples so that the samples are categorized within each class. The classes are named clusters. Fuzzy C-means clustering treats each object as having a location in space. Fuzzy C-means clustering requires the number of clusters to be partitioned and distance metric measure to quantify how close two objects are each other. In image analysis, clustering can be used to find groups of pixels with similar gray levels, color or local textures in order to discover the various regions in the image. In our algorithm, Fuzzy C-means clustering algorithm is used to cluster the immunohistochemically images of stained tissue of meningioma tumor.

A. Fuzzy C-Means (FCM) [13]

Fuzzy c-means is first proposed by Bezdek et al. [12]. The algorithm returns value between 0 and 1 called the partition matrix, which represents the degree of membership of each datum to centers of clusters. It is based on minimizing the following objective function:

$$J_m(U, C) = \sum_{i=1}^{c}\sum_{k=1}^{n} u_{ik}^{m}\, \|X_k - C_i\|^2 \tag{3}$$

where m is any real number greater than one, $X_1, X_2,..., X_n$ are

n data sample vectors. $C = \{C_1, C_2, C_3, \dots, C_n\}$ are cluster center. $U = [u_{ik}]$ is a $c \times n$ matrix,where u_{ik} is the ith membership value of kth input sample $X_k$such that $\sum_{i=1}^{n} u_{ik} = 1$. $m \in [1,\infty)$ is an exponent weight factor that controls the fuzziness of the membership function. $\|*\|$ is any norm expressing the similarities between any input sample and its corresponding cluster center.

By optimizing the above objective function shown above through updating the membership u_{ik}, and cluster center C_i we have:

$$u_{ik} = \frac{1}{\sum_{j=1}^{n} \left\{ \frac{\|X_k - C_i\|}{\|X_k - C_j\|} \right\}^{\frac{2}{m-1}}} \quad (4)$$

$$C_i = \frac{\sum_{k=1}^{n} u_{ik}^{m} X_k}{\sum_{k=1}^{n} u_{ik}^{m}} \quad (5)$$

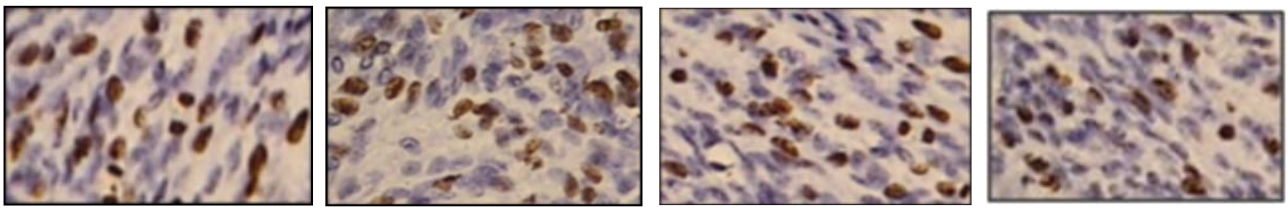

Fig. 2 (a) The original immunohistochemical mages in the RGB color space

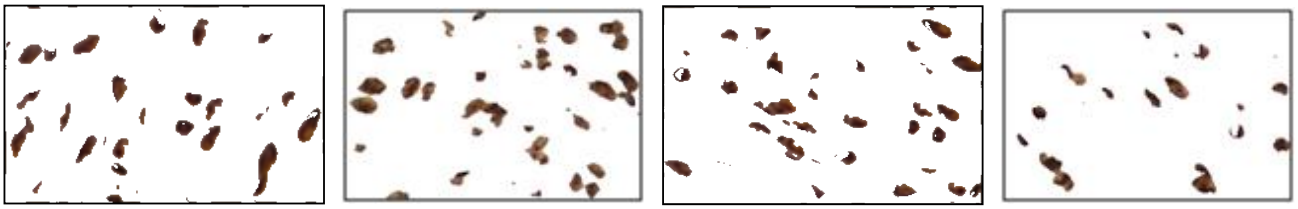

Fig. 2 (b) Positive (Proliferative) cells of immunohistochemical image of meningioma

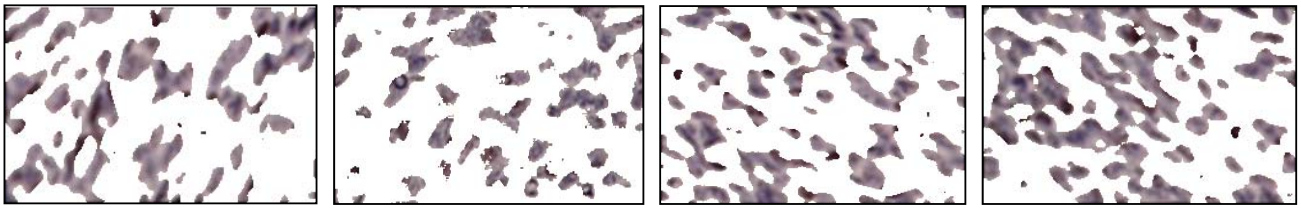

Fig. 2 (c) Negative (Normal) cells of immunohistochemical image of meningioma

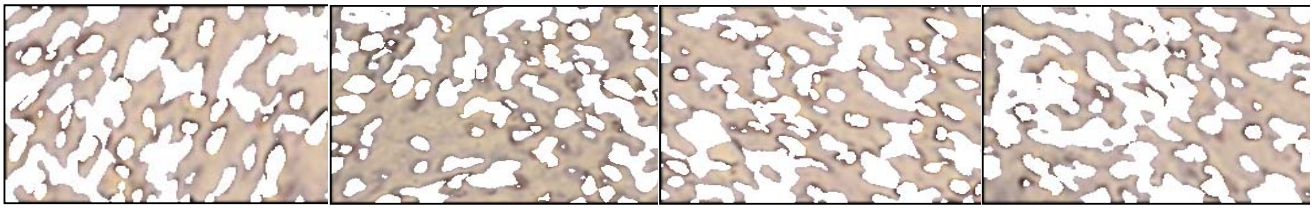

Fig. 2 (d) Background of immunohistochemical image of meningioma

B. Clustering Pixels in an Immunohistochemical Image of Meningioma

We can use Fuzzy C-means to cluster pixels color in an image into three clusters. By using fuzzy c-means, we are able to find the positive (proliferative) and negative cells for each cluster where some useful features could be extracted from them. Assume that size of the given color image is $m \times n$, then convert to a matrix with $(m \times n)$ rows and three columns. Each row is feature vector of a pixel color. With color images, we can use a 3-dimensional feature vector per pixel. Run the Fuzzy C-means algorithm with input vector of colors and assign each pixel, the color of the cluster it is assigned to. Fuzzy C-means put pixels onto C groups based on color similarities. The result is a set of regions in an image, where each region is relatively homogeneous in terms of pixel color. Fuzzy C-means clustering requires that we specify the number of clusters to be partitioned and distance metric to quantify how close two objects are to each other. In our proposed algorithm, cityblock distance measuring method is applied in HSV color model. Since the processing of

immunohistochemical images of meningioma relies mostly on the assumption that we have three distinctive regions on image, so color segmentation of the immunohistochemical images is distinguished between three classes of pixels: positive cells, negative cells and background. Figs. 2 (a)-(d) show the result of segmentation of four samples image of meningioma by FCM algorithm base on HSV color space.

V. Experimental Results and Conclusion

In this paper, we have presented a fast and accurate fully-automated approach to the immunohistochemically meningioma cancer tissue image segmentation. The proposed method is based on clustering method to extract the positive (proliferative) and negative (normal) cells which overcome the accuracy and sensitivity limitations of current solutions. Experimental results on a large dataset extracted from real immunohistochemically meningioma tissue images with positive reaction at the KI-67 antibody, demonstrated the effectiveness of the proposed approach when compared to much slower manual approaches that rely on the skill of experienced operators. Images are acquired from samples with ×400 enlargement factor.

Performances of the method are shown on different type of cell images, with both regular and irregular patterns, and overlapping cells and they perform satisfactorily well. Furthermore, the algorithm can be used for different markers and different tissues.

Since automatic cell segmentation and tracking in optical microscope plays a very important role in the study of the meningioma cancer type through mitosis index detection, the method can be further extended to a CAD system for assist pathology physicians.

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