Extraction of color features in the spectral domain to recognize centroblasts in histopathology

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Abstract— In this paper, we are proposing a novel automated method to recognize centroblast (CB) cells from non-centroblast (non-CB) cells for computer-assisted evaluation of follicular lymphoma tissue samples. The method is based on training and testing of a quadratic discriminant analysis (QDA) classifier. The novel aspects of this method are the identification of the CB object with prior information, and the introduction of the principal component analysis (PCA) in the spectral domain to extract color texture features. Both geometric and texture features are used to achieve the classification. Experimental results on real follicular lymphoma images demonstrate that the combined feature space improved the performance of the system significantly. The implemented method can identify centroblast cells (CB) from non-centroblast cells (non-CB) with a classification accuracy of 82.56%.

Index—Terms: Follicular Lymphoma, color texture, geometric features, spectral domain, QDA, and PCA.

I. INTRODUCTION

FOLLICULAR lymphoma (FL) accounts for 20-25% of non-Hodgkin lymphomas in the US [1] and affects almost exclusively predominantly adults, particularly the middle-aged and elderly. This lymphoma is characterized by at least a partial follicular or nodular pattern and is composed of lymphoid cells of follicular center origin including small cleaved cells (centrocytes) and larger non-cleaved cells (centroblasts). Grading of FL is crucial for risk stratification, prognosis and treatment and is based on the average number of centroblasts per high power field (40X) in ten representative neoplastic follicles. This method of grading is fraught with inter and intra-observer variability leading to poor reproducibility and prompting the search for a more accurate and efficient method of quantifying centroblasts and more reproducible grading.

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The growing importance of Computer Aided Diagnosis (CAD) can be seen in many fields involved in medical studies [2, 3]. Of interest here are pathological biopsies and their diagnostic and prognostic indicators. In a CAD system, image processing techniques are applied to extract quantitative and precise features and used for further analysis of the images. Afterwards, discriminant techniques, such as parametric and non-parametric statistical classifiers, are employed to classify the images. The selection of the image processing techniques and the classification strategies are important for the successful implementation of any machine vision system. Several statistical, structural and spectral texture approaches for grayscale images have been suggested [4-7].

The main goal of this study is to develop a new method for texture features space computation and use this prior information to reduce inter- and intra-reader variability between pathologists to grade FL. In our previous work, we have used color and texture features and model-based intermediate representations for the grading of follicular lymphoma [8-14].
In this paper we are introducing a new approach that takes into consideration the geometric identification of objects and texture in the color space. A multivariate image analysis technique using principal component analysis (PCA) in the spectral domain is investigated. Instead of gray scale, color spaces are utilized and RGB, Lab, HSI color spaces are explored.

This paper is organized as follows. Section 2, we present the data. In section 3, we describe the proposed method and its methodology. Experimental results and discussion are presented in section 4. Finally, conclusion is offered in section 5.

II. DATA

Since the focus of this study is to classify CB versus non-CB cells, using a supervised classifier, we collected a data set of two populations. The images in the first set are graded by two pathologists as CB and none of the images in the second one is graded by any pathologist as CB. The ground truth is composed of a set 218 CB and 218 Non-CB H&E images. It is important to note that the ground truth data are not manually annotated and the contours of the cells are not marked. Figure 1 illustrates an example of CB and non-CB images. One can notice that CB image contains more objects and higher texture compared to non-CB image. The morphology of the central object and the color texture of the image are considered to classify CB versus non-CB cells.

Figure 1: Examples of (a) a typical CB cell, and in (b) a typical non-CB cell.
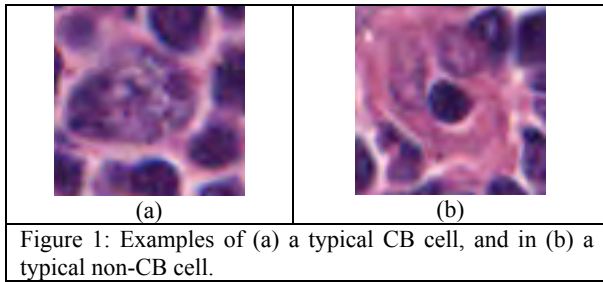
III. DESCRIPTION OF THE METHODOLOGY

The algorithm that we are proposing is based on a supervised quadratic classifier known as quadratic discriminant analysis classifier (QDA). To accomplish the classification, we are considering a set of training features representing some geometric and texture characteristics of the image. The training of the QDA classifier is completed on a set of features combining geometric and color texture characteristics.

Pathologists identify CB cells from non-CB cells mainly by observing specific, quantifiable structures in the image. In their routine, they compare visually the size of the cells and pick the cells that are larger compared to the rest. A visual scale of measurement typically employed is by comparing to some blood cells. In our case, the geometric characteristics of the large cell and the texture of the image are taken into consideration.
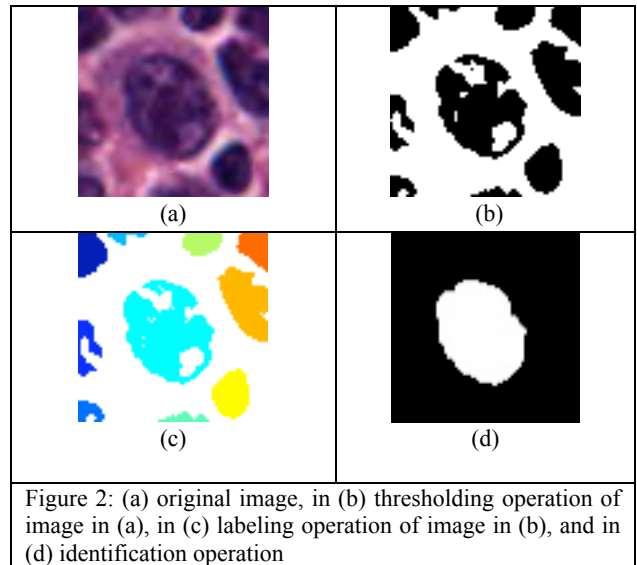
III.1- Cell identification

In order to extract the geometric features of each image of the data set, we have devised a method based on a succession of thresholding, morphological operations and area identification. The sequential processing steps of the method are described as:

1- The RGB image is converted into the Lab space. The Lab space is recognized to be more perceptually uniform in respect to RGB space and present a better overall contrast. The L channel is kept for further processing, while a and b channels are discarded.

2- Otsu thresholding method [15] is then applied on the image obtained from step 1. This operation allows segmenting out the background and therefore extracting the objects of interest present in the image.

3- Opening and closing morphology operations are then applied on the complement of the binary image obtained in step 2. A labeling morphology operation is computed on the resulting binary image to identify each object in the image. The connectivity is chosen to be equal to eight.

4- The area measurement is then calculated for each labeled object. The greatest area, which corresponds to the largest object in the image, is then identified. Figure 2 illustrates the different steps of the processing. This process mimics to some degree the procedure followed by pathologists to identify large versus small cells in the image.

Figure 2: (a) original image, in (b) thresholding operation of image in (a), in (c) labeling operation of image in (b), and in (d) identification operation

Equation (1) summarizes the identification operation:

$$\Omega = \left[A_1, ..., A_n \right]$$
$$A_F = \max(\Omega), \qquad (1)$$

where Ω is the vector containing all labeled regions. A_i is the area of the ith label in the image. A_F is the area of the largest cell in the image.

5- Once the region is identified, an erosion and dilatation operations with a disk-shaped structuring element of radius 3 are applied on the binary image to reconstruct the initial shape of the largest cell.

Along with the area, we have computed also the perimeter of the cell. These parameters and the color parameters are combined to form the feature vector used to train the classifier.

III.2- Texture color features

Expert pathologists describe the CB cells as containing several dark nucleoluses surrounded by bright uniform cytoplasm. On the other hand, the non-CB cells present a homogenous, dense structure. We have also noticed that the color is another criteria that pathologists used in their scoring.

These indications help us to build our model around analyzing the inner color texture of the cells. Therefore, we propose to quantify texture features extracted from several color channels: RGB, HSI, and Lab. We are suggesting that the combination of the three-color spaces can increase significantly the performance of the classifier and therefore improve the accuracy of the extraction of CB versus non-CB images. The contribution of each color space in the performance of the classifier will be further investigated in the future.

Many definitions of the texture have been proposed in the literature [3-7]. They formulate image texture as a function of roughness, coarseness, directionality, homogeneity, spatial frequency, etc. There is no general agreement on one definition. The best definition depends on the particular application.

In this paper, we are interested in the statistical analysis of texture in the Fourier domain. The variation of the power spectrum along the frequency scale can be a good descriptor of the texture. Here, a statistical analysis based on PCA is proposed to quantify this variation. The variance of the first order eigenvector is calculated from the PCA of the power spectrum. This feature is computed in each of the color spaces specified above.

Generally, PCA transforms the data into a new orthogonal coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate axis (called the first principal component), the second greatest variance on the second coordinate axis, and so on. PCA is theoretically the optimum transform for a given data in least squares terms [6].

The eigenvectors e_i and the corresponding eigenvalues λ_i are the solutions of the equation:

$$C_x e_i = \lambda_i, \quad i = 1,......,n \qquad (2)$$

In our case, the covariance matrix C_x is defined in the spectral domain as follows:

$$C_x = E\left[\left(S - \mu_p\right)\left(S - \mu_p\right)^T\right], \qquad (3)$$

where S is the power spectrum matrix, and μ_P is the mean of the matrix S.

We assume that the λ_i are distinct. These values can be found by solving the following equation:

$$\left|C_x - \lambda I\right| = 0 \qquad (4)$$

Where I is the identity matrix having the same order as C_x and $|\ |$ denotes the determinant of the matrix.

Reordering the eigenvectors and corresponding eigenvalues in a decreasing order; the first component corresponding to the greatest eigenvalue is selected from the rest of the components. This mode is suggested to carry most of the texture variations in the image compared to the rest of the modes. We are limiting the quantification to the first mode in order to filter out the noise from the texture. It is suggested that the noise is located in higher modes. The variance of the first component is calculated to quantify these variations. It is defined as follows:

$$Var(X) = E\left[\left(X - \mu\right)^2\right] \\ \mu = E(X) \qquad (5)$$

Where X is the first eigenvector and μ is the mean of the same vector.

In our model, we are proposing the variance as quantification of the texture color feature. This value is calculated in each proposed space and used as feature in the training process. The union of the geometrical features vector and texture color features vector define the final features vector.

III.3- Design of the classifier

To demonstrate a proof of concept of our approach, we are suggesting a statistical classification. The classification is based on quadratic discriminate analysis (QDA). QDA is used in machine learning and statistical classification to separate measurements of two or more classes of objects by a quadratic surface [16,17]. It is closely related to linear discriminant analysis (LDA), where it is assumed that there are only two classes of points (0 and 1).

Unlike the LDA classifier, for the QDA classifier, the correct solution is supposed to be quadratic, and the predicted class y from a training set of observations x is derived from the following:

$$y = x^T A x \qquad (6)$$

Where x is the observation vector and A is the features matrix.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

In order to train the classifier we have divided CB and Non-CB data into training and testing sets using an 80%-20% ratio. The 20% of each population used as testing set are chosen randomly. The residual 80% of CB and non-CB images allocated for training are divided again into 90% for training and 10% for testing using the "Hold-out K-folds" cross-validation approach. Empirically, K is set to 10. This operation is repeated 10 times to select the best training set. The training set producing the highest performance is picked as the final training set. The final testing is then applied on the testing set obtained from initial 80%-20% allocation. Table 1 demonstrates the 90%-10% training approach using the supervised QDA classifier. The same rule is used in training and testing operations. In this example, the training set corresponding to the highest performance of the classifier, is identified as set 6. Its classification accuracy is equal to 88%. This set is then selected as the training set for further testing of the classifier. The testing of the QDA classifier is completed on the 20% remaining images (88 images). The average classification rate of the classifier obtained from the testing is equal to 82.56%. QDA classifier is preferred because it showed a higher classification rate in respect to LDA and K-nearest classifiers.

In order to demonstrate the usefulness of the system, we have enlisted two experienced pathologists to complete two different experiments. The experiments are realized on the testing set data. In the first experiment the pathologists

graded the images without CAD assistance.

Table 1: The 90%-10% training methodology

Set	Performance
1	0.70
2	0.62
3	0.85
4	0.71
5	0.76
6	**0.88**
7	0.62
8	0.71
9	0.79
10	0.85

In the second experiment, we displayed the results of the CAD system on the same data set and asked them if either they agreed with the results or not. In case there is a disagreement of judgment between the pathologist and the CAD, the pathologist is free to change the grading. It is important to note these experiments are administered on different days for each pathologist.

Table 2 demonstrates some comparison between the grading of the two pathologists. Inter- and intra-reader variability are shown. One can notice that the pathologists are not in agreement in all cases. Inter- and intra-reader variability errors are introduced during their subjective reading. Although the performance of the algorithm is around 80%, it should be noted that the intra- and inter-reader variability is more than 25%.

Table 2: Inter and intra variability reading

	Inter variability	Intra variability
Pathologist 1	-	25.00%
Pathologist 2	26.13%	28.40%

V. CONCLUSION

In this paper, we have demonstrated a new quantitative methodology to diagnosis CB versus non-CB cells in follicular lymphoma disease using geometric and color texture features in the spectral domain. The goal of this study is to highlight inter- and intra-reader variability reading that the pathologists introduce during their reading and propose a method to reduce these errors. In our future work, we will further develop the segmentation algorithm by taking into account the variations in staining and by using other color texture features with the model-based intermediate representation. The current classification rate obtained by the classifier (82.56%) is very encouraging considering the inter and intra-reader variability of the pathologists which is over 25.00%.

VI. REFERENCES

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