Classification of Nuclei in Follicular Lyphoma Tissue Sections Using Different Stains and Bayesian Networks

Kosmas Dimitropoulos[1], Panagiotis Barmpoutis[1], Triantafyllia Koletsa[2], Ioannis Kostopoulos[2], and Nikos Grammalidis[1]

[1] Information Technologies Institute / Centre for Research & Technology Hellas, Thessaloniki, Greece
[2] Medical School / Aristotle University of Thessaloniki, Affiliation, Thessaloniki, Greece

Abstract— **Automated centroblast (CB) detection in Follicular Lymphoma (FL) tissue samples has recently attracted significant research interest. Most of the methods described in the literature are based on the use of Hematoxilin and Eosin (H&E) stain. However, the automated detection of CBs from H&E stained images remains a challenging issue. To this end, this paper presents a novel approach which is based on the use of both PAX5 and H&E stains in tissue sections sliced at the thickness of 1μm. The goal of PAX5 is three-fold: to facilitate the segmentation of nuclei, to remove a number of follicular dendritic cells and finally to extract morphological characteristics of nuclei. Furthermore, the use of H&E stain enables us to extract textural information related to histological characteristics used by pathologists in diagnosis of FL grading. In our method we propose a novel algorithm for the separation of overlapped nuclei inspired by the clustering of large scale visual vocabularies. Finally, aiming to model pathologists' knowledge used in FL grading, we use a Bayesian Network classifier to combine the morphological and textural characteristics. Experiments conducted on a dataset of ten pairs of PAX5 and H&E images demonstrate the potential of the proposed approach providing an average detection rate of 93.46%.**

Keywords— **Biomedical image processing, follicular lymphoma, multi-stains, cell segmentation, overlapped nuclei segmentation, Bayesian networks.**

I. Introduction

Follicular Lymphoma (FL) is one of the most common lymphoma diagnosed in United States and Western Europe [1]. The most commonly used practice for grading of FL, which has also been adopted by the World Health Organization, was proposed by Mann and Berard [2]. According to this method, follicular lymphoma grading depends on the number of CBs that are recognized by a pathologist: Grade I with 0-5 CBs/HPF (high power field), Grade II with 6-15 CBs/HPF and Grade III with more than 15 CBs/HPF [3]. Tissue biopsies of FL are stained with Hematoxilin and Eosin (H&E), which is one of the principal stains in histology, and they are visually inspected by pathologists. In order to account for tissue heterogeneity, the average CB number in ten different HPF images (derived from the same tissue section) is being estimated [4]. Since this manual procedure is highly subjective and requires extensive training, various methods [5][6] for automatic FL grading have been proposed to increase the accuracy and reproducibility of diagnosis, which is directly related to the time and type of therapy.

The main challenge of these methodologies is the accurate segmentation of nuclei and the extraction of a suitable set of features for their classification into CBs or non-CBs. Especially the latter requires the modelling of pathologists' knowledge used in clinical practice, that is, the identification of a number of features, such as morphological characteristics of nucleus (i.e., its size and circularity), the uniformity and brightness of its texture, the number and size of nucleoli or the texture of the cytoplasm in the surrounding area. Thus, either only morphological characteristics of nuclei are used or the textural variation of nuclei is considered along with their morphology. To overcome the problem, some recent studies consider the whole nucleus with its surrounding area as a single feature vector, whose dimensionality is reduced before the final classification step [7], while other researcher efforts have focused on the identification of various texture features [8][9][10]. By taking advantage of the fact that different stains can provide valuable information to aid understanding of the physical or functional properties of tissue [11], we propose the use of PAX5 and H&E stains in tissue sections sliced at the thickness level of 1μm.

Immunostain for PAX5, a transcription factor localized in the nucleus, is expressed by the most B-cells, from B lymphoid progenitors to the mature B-cell stage [12]. The PAX5 gene is essential for B-cell differentiation and has been utilized in the differential diagnosis of undifferentiated malignant neoplasms [13]. The main advantage of PAX5 stain in 1μm sliced tissues of FL is the fact that it facilitates the segmentation of nucleoli from cytoplasm, whilst at the same time it enables the removal of a number of follicular dendritic cells -that appear as blue-coloured nuclei- which could be easily misclassified if only H&E stain was used.

To this end, in this paper we aim to propose a new methodological framework for the automated segmentation and classification of nuclei in FL tissue sections, by making the

E. Kyriacou et al. (eds.), *XIV Mediterranean Conference on Medical and Biological Engineering and Computing 2016*,
IFMBE Proceedings 57,
DOI: 10.1007/978-3-319-32703-7_47

following contributions: a) combine both morphological and textural characteristics of nuclei using respectively PAX5 and H&E stains in tissue sections sliced at the thickness level of 1μm, b) introduce a novel approach for overlapped nuclei splitting and c) design a Bayesian network classifier for the combination of characteristics in order to model the knowledge of pathologists.

The remainder of this paper is organized as follows: Section II describes in detail the different processing steps of the proposed methodology. In section III, experimental results are presented. Finally, conclusions are drawn in Section VII.

II. Methodology

A novel framework is proposed for the segmentation and classification of nuclei based on the use of PAX5 and H&E stains in tissue sections sliced at the thickness of 1μm. By employing PAX5 immunohistochemistry, we facilitate the segmentation of nuclei, while at the same time we can remove a number of follicular dendritic cells, which would be easily misclassified if only H&E stain has been used. On the other hand, the use of H&E stain enables us to extract textural information related to histological characteristics used by pathologists in diagnosis of FL grading, i.e., the number and size of nucleoli as well as textural features of the nucleus and its surrounding cytoplasm.

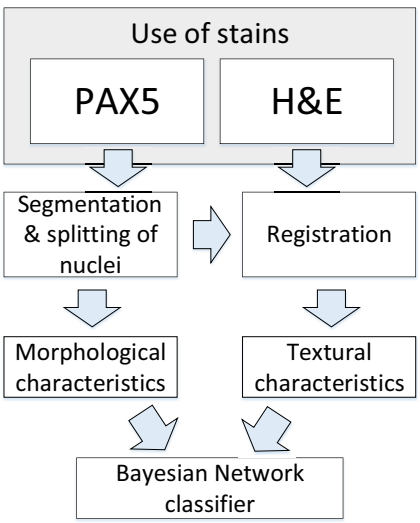

Fig. 1 Methodology of proposed algorithm.

The proposed method for classification of nuclei in FL tissue sections using Bayesian Network is shown in Fig. 1. More specifically, after retrieving pairs of PAX5 and H&E images, for the segmentation procedure of nuclei we use PAX5 stained images. We initially apply an energy minimization technique based on graph cuts and then we propose a novel algorithm for the splitting of clustered nuclei inspired by the clustering of large scale visual vocabularies. For the identification of segmented nuclei in H&E stained images intensity-based rigid image registration is employed and nuclei textural analysis is applied to extract features related to the internal and external texture of nuclei as well as the number and the size of nucleoli. Finally, for the combination of the morphological characteristics of nuclei extracted from PAX5 stained images with their textural characteristics in H&E images, a Bayesian Network classifier is proposed aiming to model pathologists' knowledge used in the diagnostic approach of FL grading. These steps are described in detail in the following subsections.

A. Nuclei segmentation using energy minimization

For the segmentation of nuclei in PAX5 stained images, we apply an energy minimization technique based on graph cuts, which is an unsupervised approach that can be employed efficiently to various image segmentation problems. Specifically, the segmentation procedure is considered as a labelling problem, where the labels in our case represent different cytological components, i.e., nuclei and cytoplasm. In practice, apart from the cytoplasm, the second class also contains the blue-coloured nuclei including follicular dendritic cells. In our experiments, the expansion algorithm [14] was used, which is one of the most efficient algorithms for minimizing discontinuity-preserving energy functions.

B. Separation of clustered nuclei

After the segmentation step, a number of nuclei overlap with each other forming clusters of cells. To overcome this problem, in this section we propose a novel approach, which aims to initially estimate the correct number and location of seeds (nuclei centres) and then to detect each nucleus using an ellipsoidal model.

Initially we aim to identify a list of candidate seeds. We apply a distance transform and we estimate the regional maxima in the generated distance image D. The number of seeds is the result of the only one maximum that can be accepted in each neighbourhood. Towards this end, we apply a regional H-maxima transform [15] in order to suppress all local maxima in its vicinity.

Based on the hypothesis that nuclei can be spatially modelled as ellipsoids, the pixel coordinates in each cell are modelled using a Gaussian distribution. More precisely, a Gaussian mixture model is applied with the number of clusters, k, being equal to that of candidate seeds. The unknown parameters of the Gaussian mixture, i.e., $\Theta = \{\pi_i, \mu_i, \Sigma_i\}$,

where μ_i, and Σ_i are the mean value and the covariance matrix respectively of the distributions of pixel coordinates in each nucleus and π_i are the mixing coefficients, are estimated using the expectation maximization (EM) algorithm.

After estimating the ellipsoidal models of nuclei for all seeds, we need to identify if there is a spurious cell from the list of candidate seeds in order to estimate the correct number of nuclei. To do so, we attempt to compute an overlap measure for the estimated clusters. Based in [16] we try to identify the *weak* seed in the cluster and then we propose a validation process in order to decide whether a cluster is redundant or not. We can say that $\hat{\gamma}_f(p_g) = \hat{\gamma}_{fg} \in [0,1]$ is the generalized responsibility of component *g*, for component *f* and, in general, that $\hat{\gamma}_{ii}$ is the responsibility of component *i* for itself, with each component of the mixture corresponding to a unique seed. In this paper we propose the identification of the *weak* seed based on the criterion of the following equation:

$$r_{i,\hat{k}} = \frac{\hat{\gamma}_{ii}}{\hat{\gamma}_{ii} + \sum_{j \in \hat{k}} \hat{\gamma}_{ij}}, \text{ with } i \notin \hat{k} \qquad (1)$$

That is, the seed whose cluster has the lowest $r_{i,\hat{k}}$ among all the mixture components in the clustered nuclei is considered as the *weak* seed, s_w:

$$s_w = \arg\min_i (r_{i,\hat{k}}) \qquad (2)$$

The above indication, however, is not enough for purging seed s_w from the list, therefore, a cluster validation process is needed in order to make the final decision. Similar to [17], we use a validation criterion, which requires the clusters to be well-separated and with a compact structure, and also introduce a "Fitness score" which aims to measure how well the ellipsoidal models fit to the elements of their clusters:

$$V(\hat{k}) = \frac{Sep(\hat{k})}{Comp(\hat{k}) \cdot Fitness(\hat{k})} \qquad (3)$$

The Fitness score $Fitness(\hat{k})$ is defined as the sum of the normalized fitness scores of each individual cluster, which are computed as the sum of distances of all elements in set $W_i = E_i \oplus X_i$ (where sets E_i and X_i represent the elements of ellipsoidal component *i* and the elements of the corresponding cluster respectively and $\oplus$ is the symmetric difference operator) from the centroid of cluster *i*, normalized by the maximum distance within the cluster from its centroid. For the estimation of the separation and compactness measures *Sep* and *Comp* respectively, the reader is referred to [17].

After that, we can easily compute the validation index *V* of the estimated clusters from equation (3). Then, we can simply claim that if a new EM solution $\hat{\Theta}$ increases the validity index *V*, then the weak seed s_w can be considered as a spurious seed and is deleted from the list. The same procedure is repeated for the identification of other spurious seeds in the list until the validity index cannot be further increased.

C. Nuclei classification

After the splitting of nuclei, some dendritic cells exhibit similar morphological characteristics (they are large and round cells) [18] with CBs and could be easily misclassified if only H&E stain was used. Moreover, endothelial cells would be difficult to discriminate (they are relatively large and sometimes elongated cells) from CBs cells if only PAX5 image was used.

In order to assess the shape of nuclei in PAX5 stained images, the perimeter of each nucleus is extracted and the best fitting ellipse is estimated using the Orthogonal Distance Regression (ODR) algorithm [19]. Subsequently, ellipse residual is being estimated as the average geometric distance of the pixels in the perimeter from the ellipse. This feature is referred to as nuclear regularity, since it estimates the regularity of the shape of the nucleus.

In order to extract textural information from the corresponding H&E stained images, the registration of H&E and PAX5 stained images is needed. Towards this end, we apply an intensity-based rigid image registration [22] to determine the locations of the corresponding nuclei in H&E stained images. Textural analysis can then be applied to extract features related to the internal and external texture of nuclei as well as the number and the size of nucleoli in their interior, as proposed in [20].

For the classification of cells into CBs and non-CBs we propose the design of a classifier which is based on probabilistic graphical models, such as Bayesian Networks [21], in order to better model the decision making procedure of pathologists. The classifier combines strengths of both the morphological characteristics of nuclei in PAX5 images and the textural characteristics of nuclei in H&E images.

More specifically, the proposed BN classifier (Fig. 2) receives as input a feature vector containing nine individual features (two morphological and seven textural), extracted after the identification of nuclei in both PAX5 and H&E stained images. Finally, the decision for the classification of

a nucleus is taken based on the conditional probabilities of the parent nodes in the second level of DAG, which quantify different properties of nuclei associated with their morphology, internal texture, nucleoli characteristics and external texture.

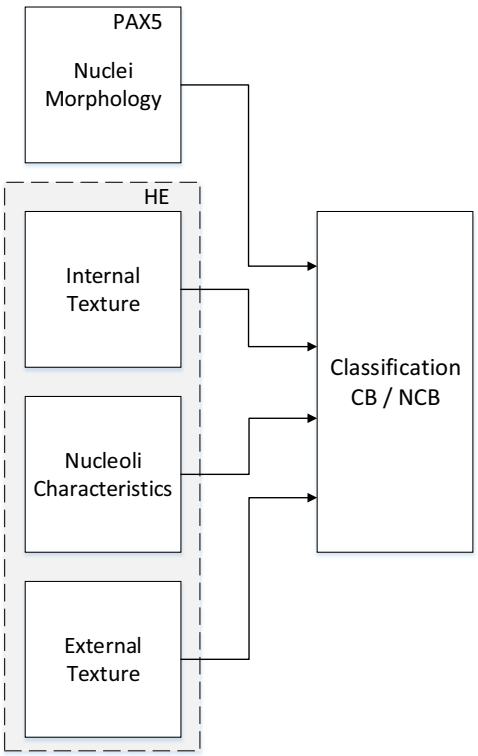

Fig. 2. The structure of the proposed BN classifier

III. Experimental Results

The performance of the proposed method for automated nuclei segmentation and classification was evaluated using a dataset consisting of ten pairs of PAX5 and H&E stained HPF images of 1280x960 pixels using a Nikon Eclipse E600W microscope and a Nikon DS-Fi1 digital camera. CB and non-CB cells from three additional image pairs were used for the training of the BN classifier.

Regarding the classification results, Fig. 3 and Fig. 4 present detailed evaluation results for each tissue slide. Specifically, Fig. 3 presents the True Positive (TP) rate and Fig. 4 presents the False Positive (FP) rate, which is the number of non-CB cells erroneously detected as CBs divided by the number of annotated non-CBs in each slide. As shown in Fig. 3, the proposed method provides an average TP rate of 93.46% and the average FP rate (Fig. 4) is 2.46%.

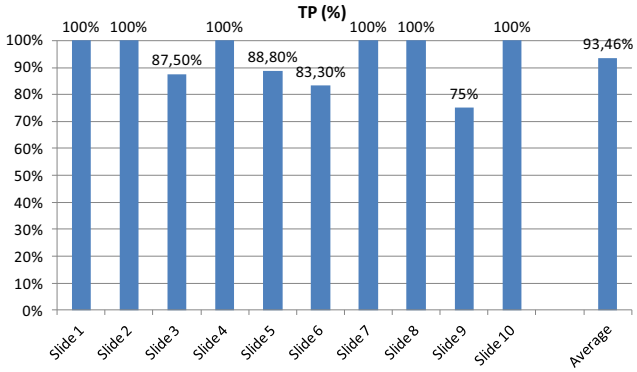

Fig. 3 True Positive (TP) per slide and average rate

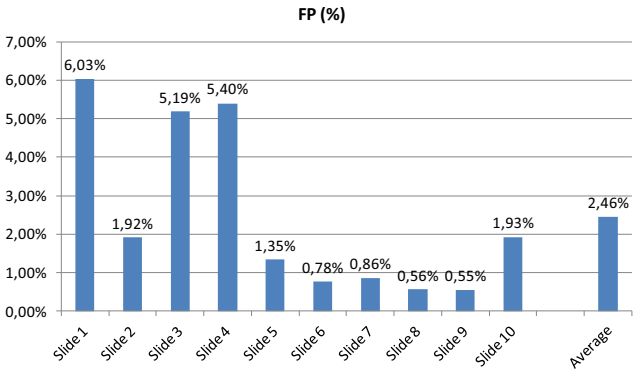

Fig. 4 False Positive (FP) per slide and average rate.

Finally, two different examples of CB detection are presented in Fig. 5. More specifically, in the first case Fig. 5(a) contains a CB and a dendritic cell, while in Fig. 5(b) only one CB exists in the selected Region of Interest (ROI). As we can see from Fig. 5(c), the dendritic cell appears in blue colour in the corresponding PAX5 stained image, so the segmentation algorithm can easily discard it. As seen in Fig. 5(c-d), CB cells are correctly identified in both cases. In Fig. 5(e-f), the final classification result is shown.

IV. Conclusions

In this paper, we have proposed a new method for automated for nuclei segmentation and classification in FL tissue sections using Bayesian Networks. The proposed method combines morphological characteristics of nuclei in PAX5 images with textural characteristics of the corresponding nuclei in H&E stained images. Experimental results using ten HPF images showed that the proposed methodology can achieve high detection rates while the number of false positives is kept relatively low. Future work may be focused on applying different stains and using different classification methods (e.g., deep learning).

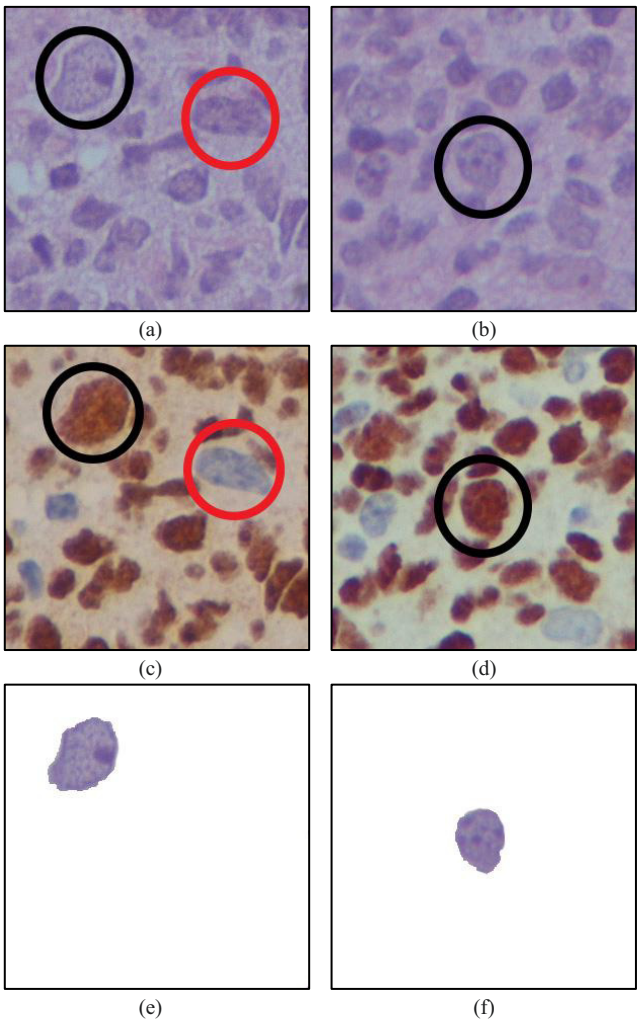

Fig. 5 Two examples of CB detection (black circles indicate CBs, while red circles indicate dendritic cells). a-b) the H&E stained images, c-d) the corresponding PAX5 stained images, and e-f) the two classification results.

V. References

1. S H Swerdlow et al., Classification of Tumours of Haemaopoietic and Lymphoid Tissues, Fourth Edition ed. Lyon: World Health Organization, 2008.
2. R B Mann and C W Berard, "Criteria for the cytologic subclassification of follicular lymphomas: a proposed alternative method," Hematl. Oncol., vol. 1, no. 2, pp. 187-192, 1983.
3. A Freedman, "Follicular Lymphoma: 2012 Update on Diagnosis and Managemet," Am. J. Hematol., vol. 87, no. 10, pp. 988-995, September 2012.
4. O Sertel et al., "Histopathological Image Analysis Using Model-Based Intermediate Representations and Color Texture: Follicular Lymphoma Grading," Signal Processing Systems, vol. 55, no. 1-3, pp. 169-183, April 2009.
5. K Belkacem-Boussaid, O Sertel, G Lozanski, A Ahana'aah, and M Gurcan, "Extraction of color features in the spectral domain to recognize centroblasts in histopathology," in Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Minneapolis, USA, 2009.
6. O Seterl, G Lozanski, A Shana'ah, and M Gurcan, "Computer aided detection of centroblasts for follicular lymphoma grading using adaptive likelihood-based cell segmentation," IEEE Trans. Biomed. Eng., vol. 57, no. 10, pp. 2613-2616, 2010.
7. E N Kornaropoulos, M Niazi, G Lozanski, and M N Gurcan, "Histopathological image analysis for centroblasts classification through dimensionality reduction approaches," Cytometry Part A, vol. 85, no. 3, pp. 242-255, 2014.
8. O Sertel et al., "Histopathological Image Analysis Using Model-Based Intermediate Representations and Color Texture: Follicular Lymphoma Grading," Signal Processing Systems, vol. 55, no. 1-3, pp. 169-183, April 2009.
9. K Belkacem-Boussaid, O Sertel, G Lozanski, A Ahana'aah, and M Gurcan, "Extraction of color features in the spectral domain to recognize centroblasts in histopathology," in Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Minneapolis, USA, 2009.
10. O Seterl, G Lozanski, A Shana'ah, and M Gurcan, "Computer aided detection of centroblasts for follicular lymphoma grading using adaptive likelihood-based cell segmentation," IEEE Trans. Biomed. Eng., vol. 57, no. 10, pp. 2613-2616, 2010.
11. Y Song et al., "Unsupervised content classification based non-rigid registration of differently stained histology images," IEEE Trans. Biomed. Eng., vol. 96, no. 1, pp. 96-108, Janouary 2014.
12. M Horcher, A Souabni, and M Busslinger, "Pax5/BSAP Maintains the Identity of B Cells in Late B Lymphopoiesis," Immunity, vol. 14, pp. 779-790, 2001.
13. K C Jensen et al., "The utility of PAX5 immunohistochemistry in the diagnosis of undifferentiated malignant neoplasms," Modern Pathology, vol. 20, no. 8, pp. 871-877, August 2007.
14. Y Boykov, O Veksler, and R Zabih, "Fast Approximate Energy Minimization via Graph Cuts," IEEE Transactions on Pattern Analysis and Machine Intelligence, vol. 23, no. 11, pp. 1222-1239, 2001.
15. P Soille, Morphological Image Analysis: Principles and Applications. Berlin, Germany: Springer-Verlag, 1999.
16. Y Avrithis and Y Kalantidis, "Approximate Gaussian Mixtures for Large Scale Vocabularies," in European Conference on Computer Vision, Florence, Italy, 2012.
17. Mohamed Bouguessa, Shengrui Wang, and Haojun Sun, "An objective approach to cluster validation," Pattern Recognition Letters, vol. 27, no. 13, pp. 1419-1430, 2006.
18. Jos B.T.M. Roerdink and Arnold Meijster, "The Watershed Transform: Definitions, Algorithms and Parallelization Strategies," Fundamenta Informaticae, vol. 41, pp. 187-228, 2001.
19. W Gander, G H Golub, and R Strebel, "Least-square fitting of circles and ellipses," BIT, vol. 34, no. 4, pp. 558-578, 1994.
20. Kosmas Dimitropoulos, Emmanouil Michail, Triantafyllia Koletsa, Ioannis Kostopoulos, and Nikos Grammalidis, "Using adaptive neuro-fuzzy inference systems for the detection of centroblasts in microscopic images of follicular lymphoma," Signal, Image and Video Processing, vol. 8, no. 1, pp. 33-40, December 2014.
21. P Kevin Murphy, "The Bayes Net Toolbox for MATLAB," Computing Science and Statistics, vol. 33, 2001.
22. MathWorks. (2015, June) R2013a imregister. [Online]. http://www.mathworks.com/help/images/ref/imregister.html