Automatic segmentation of morphological structures in microscopic images of lymphatic nodes - preliminary results

RYSZARD GOMOLKA, WLODZIMIERZ KLONOWSKI,
ANNA KORZYNSKA, KRZYSZTOF SIEMION
Nalecz Institute of Biocybernetics and Biomedical Engineering
Polish Academy of Sciences
Ks. Trojdena 4, 02-109 Warsaw
POLAND
wklon@ibib.waw.pl

Abstract: - **Introduction:** Evaluation of microscopic images of lymphatic nodes is time consuming and is usually based on assessment of images stained with multiple types of dyes. **Materials and Methods:** Herein, we propose a supportive method for segmentation of follicles, medulla and cortex in H+DAB-stained microscopic images of lymphatic nodes. The method is based on sampling of pixels intensities distribution in the *RGB* space of the original microscopic image and calculating the feature maps for further segmentation. All calculations are performed in MATLAB and results are compared to regions manually segmented by pathologist. **Results:** Based on 9 sample images of a lymphatic node, our algorithm achieved in average 0.96, 0.45 and 0.47 accuracy in localization of the follicles, cortex and the medulla, respectively. **Conclusions:** Our algorithm achieved good performance in segmentation of follicular regions and moderate performance in segmentation of cortex and medulla in the images of lymphatic node. Ongoing works are aimed to improve segmentation of cortical and medullar regions. These regions are difficult to distinguish without hematoxilin- and eosin-stained images. Validation of our algorithm is planned on a larger data set.

Key-Words: - pixels intensity distribution, *RGB* space, segmentation, lymph nodes, biopsy

1 Introduction

Evaluation of microscopic images of lymph nodes is time consuming and is usually based on assessment of images stained with multiple types of dyes. Single type of staining is mostly insufficient for clear identification of morphological structures - especially in the presence of artifacts.

Herein, we propose a new method for segmentation of follicles, cortex and medulla in H+DAB-stained microscopic images of a lymph nodes. The method is based on analysis of pixels intensity distribution in the *RGB* space of the original microscopic image. The aim of the method is to perform an initial segmentation for further application of automatic tools [1, 2].

2 Materials and methods

We have obtained 4 digital microscopic images of healthy lymph nodes. The images were acquired from standard biopsies of 4 patients admitted to hospital and were subsequently evaluated. For current analysis, a single image of 17173x16732 pixels dimension was chosen. The image was visually assessed by a pathologist (see 2.1). Afterwards, the image was subdivided into 9 regions (*sample images*) of 5578x5725 pixels size, to reduce the amount of operational memory used by the algorithm during a single computation (Fig. 1.).

Separate mask images were performed for the structures present in the image: follicles (including the proliferation, condensation and the mantle zones), cortex, medulla. Each mask was subdivided into the 9 sample images of 5578x5725 pixels size and was subsequently saved into a respective .tiff file format, for further comparison with the algorithm's segmentation results.

All of the 9 sample images were analyzed on a standard PC with Intel i7 dual core processor and 8GB RAM. Algorithm's computations and validation were performed in MATLAB 2016b. Mean ± standard deviation (SD) accuracy was calculated separately for of the results of the follicles, cortex and the medulla automatic segmentation among the 9 sample images.
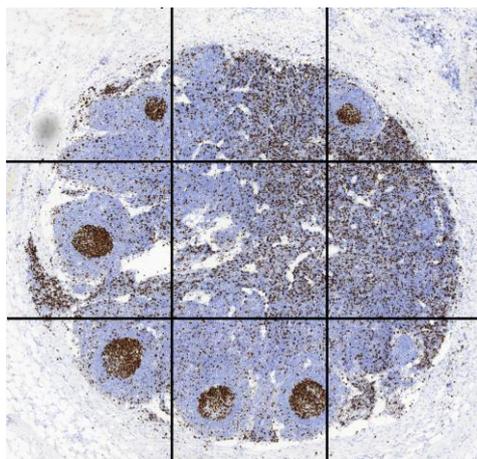
Fig. 1. Microscopic image of a healthy lymph node. Horizontal and vertical lines split the original image into 9 sample images.

Distribution of pixels intensities in microscopic images of lymph nodes is different for H and DAB-stained cells. The difference is clearly visible between mainly DAB-stained structures of follicular proliferation zone and mainly H-stained medulla. However, the difference becomes very subtle if a neighboring structures are compared, for example the cortex and the medulla. We propose a new set of features calculated directly from the original microscopic image.

Color-coding of computer images most often uses RGB system - the color of a pixel is expressed as a triplet, *(r,g,b)* i.e. *(read, green, blue)*, each component of which can vary from *0* for the darkest one to *255* for the brightest one. Instead of intensity-based segmentation, the follicles, the cortex, and the medulla are delineated based on the percentiles ratio of the pixels intensities distribution in the *RGB* space. The features are computed in 100x100 pixels windows, displaced iteratively by 10 pixels across the image. For every window the 1st, 5th, 25th, 75th, 95th and 99th percentiles *(P)* and mean values *(μ)* of pixels intensities are calculated from the distributions. These quantities are denoted $R(P)$, $G(P)$, $B(P)$ and R_μ, G_μ, B_μ, respectively. Afterwards, the following features *F* are calculated for every part of the image in 100x100 pixels windows:

$$F_1 = R(5) / [B(5)+G(5)] \tag{1}$$

$$F_2 = B_d / [R_d + G_d], \tag{2}$$

where

$$B_d = [B(75) - B(25)] / [B(75) + B(25)] \tag{3}$$

$$R_d = [R(75) - R(25)] / [R(75) + R(25)] \tag{4}$$

$$G_d = [G(75) - G(25)] / (G(75) + G(25)] \tag{5}$$

$$F_3 = B_{dm} / [R_{dm} + G_{dm}] \tag{6}$$

where

$$B_{dm} = B_d \cdot B_\mu \tag{7}$$

$$R_{dm} = R_d \cdot R_\mu \tag{8}$$

$$G_{dm} = G_d \cdot G_\mu \tag{9}$$

$$F_4 = B(95) / [R(95) + G(95)] - B(5)/[R(5) + G(5)] \tag{10}$$

Features maps are generated separately for each of the F_1 - F_4 features (cf. Fig. 2.). Subsequent pixel-by-pixel brightness filtration of the features maps is a basis for segmentation of the follicles, the cortex and the medulla in the original image.
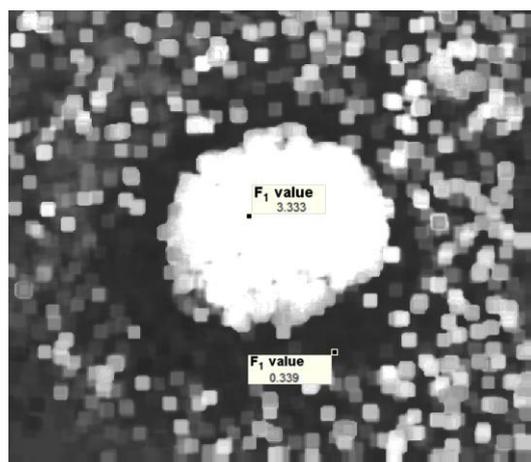

Fig. 2. F_1 feature map for a part of a sample image.

The segmentation is performed in 3 consecutive steps: firstly, the pixels belonging to the follicles are delineated from the microscopy image, based on coordinates of the pixels fulfilling the criteria in the feature maps:

$$F_1 >= 2 \ AND \ F_2 >= 0.5 \ AND \ F_2 <= 1 \ AND \ F_4 >= 0 \tag{11}$$

Secondly, the cortex is delineated from the pixels not belonging to the follicles, based on criteria:

$$F_1 >= 0.5 \ AND \ F_3 >= 0.5 \ AND \ F_4 >= 0 \tag{12}$$

And the medulla is segmented from the remaining pixels as filling the conditions:

$$F_1 >= 1 \text{ AND } F_2 < 0.5 \text{ AND } F_4 < 0 \qquad (13)$$

To differentiate the follicles, the cortex and the medulla in the original image, features main threshold points for the brightness filtration are chosen as *0.5*, *1, 2*. The values indicate the relative differences in skewness of pixels intensity distributions between the nodal structures.

3 Results

In comparison with the segmentation performed manually by the pathologist our method achieved 0.96 ± 0.03, 0.46 ± 0.13 and 0.47 ± 0.12 accuracy in localization of the follicles, the cortex and the medulla among 9 sample images analyzed (Figs. 3-5). Time of computation was 670 ± 211 seconds for the images of 5578x5725 pixels size.

4 Conclusions

Presented method is based on sampling of pixels intensity distribution in the original RGB space of the microscopic image. The method allows to segment the follicles, cortex and the medulla without an overlap of segmented areas. Moreover, our method does not require color translation or thresholding [3] but performs calculation in the original image. The method is dedicated to microscopic images of lymph nodes, however its statistical principle is applicable to other, even low contrast images [4]. The method is currently in development and does show encouraging results. Further research on the algorithm might include segmentation of lymph node capsule or lymph vessel wall [5].
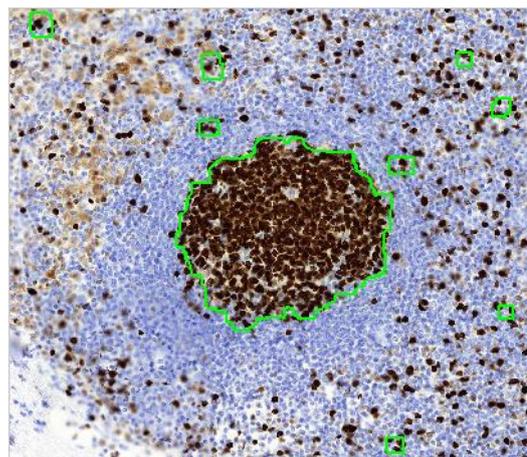
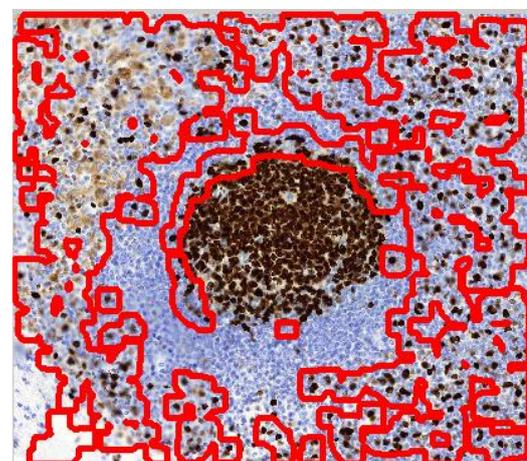Fig. 3. Follicular region of lymphatic node segmented in the part of sample image.

Fig. 4. Cortical region of lymphatic node segmented in the part of sample image. The segmentation properly excluded follicular mantle zone.
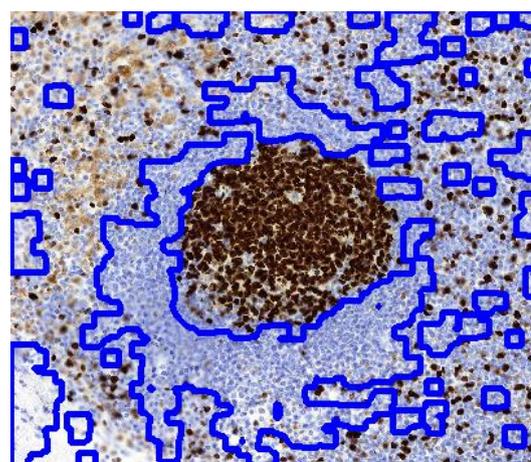

Fig. 5. Mantle follicular zone and the regions similar to paracortex or medulla segmented in the part of sample image.

References:

[1] A.Korzynska   et al. Validation of various adaptive threshold methods of segmentation applied to follicular lymphoma digital images stained with 3,3'-Diaminobenzidine & Haematoxylin. *Diagnostic Pathology*, vol.8:48, 2013, Doi: 10.1186/1746-1596-8-48.

[2] Z.Swiderska et al. Comparison of the manual, semiautomatic, and automatic selection and levelling of hot spots in whole slide images for ki-67 quantification in meningiomas. *Analytical Cellular Pathology*, vol.2015, 2015, pp.1-15.

[3] A.C.Ruifrok, Quantification of immunohisto-chemical staining by color translation and automated thresholding, *Analytical and Quantitative Cytology and Histology*, vol.19, 1997, pp. 107-13.

[4] Kayser K et al. How to measure image quality in tissue-based diagnosis, *Diagnostic Pathology*, vol.3, 2008, pp. S11.

[5] J.L.Jones et al. Automatic segmentation of lymph vessel wall using optimal surface graph cut and hidden Markov Models, Conference Proceedings, *37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, vol.2015, 2015, pp. 2403-6.