

The methods of immunohistochemically stained tissue sections evaluations implemented on the MIAP Platform

Łukasz Roszkowiak¹, Anna Korzyńska¹, Urszula Neuman^{1,4},
Carlos Lopez², Marylene Lejeune², Ramon Bosch³

¹ – Nalecz Institute of Biocybernetics and Biomedical Engineering PAS, Warsaw, Poland.
² – Molecular Biology and Research Section, Hospital de Tortosa Verge de la Cinta, IISPV, URV, Tortosa, Spain.
³ – Pathology Department, Hospital de Tortosa Verge de la Cinta, IISPV, URV, Tortosa, Spain.
⁴ – Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot Oxfordshire .

BACKGROUND

The aim of the MIAP Platform project is the design and implementation of web-based platform for the computer analysis of tissue microscopic images. The MIAP Platform should aid the pathological diagnosis by quantitative image analysis of tissue, stained by the immunohistochemical markers. Selected algorithms developed by our team have been adjusted to be included into the MIAP Platform.

MATERIALS

The method has been validated using several glass slides prepared in:

- Pathology Department of Military Institute of Medicine (MIM, Warsaw) and
- Pathology Department of Hospital de Tortosa Verge de la Cinta, Tortosa

Images to analysis have been digitalized using following digitalization tools:

- Microscope Olympus + ImagePro Premier
- Microscope Nikon
- CoolScope 1 by Nikon
- Pannoramic DESK from 3D Histech
- Aperio AT2 from Leica
- ZEISS Axio Scan.Z1

METHODS

COST Action

The method developed by Anna Korzyńska, Urszula Neuman, Carlos Lopez, Marylene Lejeune, Ramon Bosch in 2010 as Polish-Spanish Cooperative Project on "Development of a Novel Technique for Quantitative Immunocytochemistry" under COST Action [2, 3]. This method is adjusted to follicular lymphoma patients' samples stained against FOXP3 with DAB&H.

Metinus

The method was developed by Anna Korzyńska, Łukasz Roszkowiak, Carlos Lopez, Marylene Lejeune, Ramon Bosch in 2014. It has been developed for lymphoma patients' samples quantification as a consequence of results of investigation of various adaptive threshold methods in nuclei segmentation performed in 2013 [4]. The samples to which this method is adjusted are lymphoma patients' samples stained against FOXP3 with final antigen DAB&H.

MetinusPlus

The method was developed by Anna Korzyńska, Łukasz Roszkowiak, Jakub Zak, Carlos Lopez, Marylene Lejeune, Ramon Bosch in 2015. This method was developed for: "Development of automated quantification methodologies of immunohistochemical markers to determine patterns of immune response in breast cancer", leaded by MB&RS of HTVC. This method is adjusted to breast cancer patients samples from core cancer and from lymph nodes to quantify stained against FOXP3 which marks T-regulatory cells and subpopulation of breast cancer cells [5].

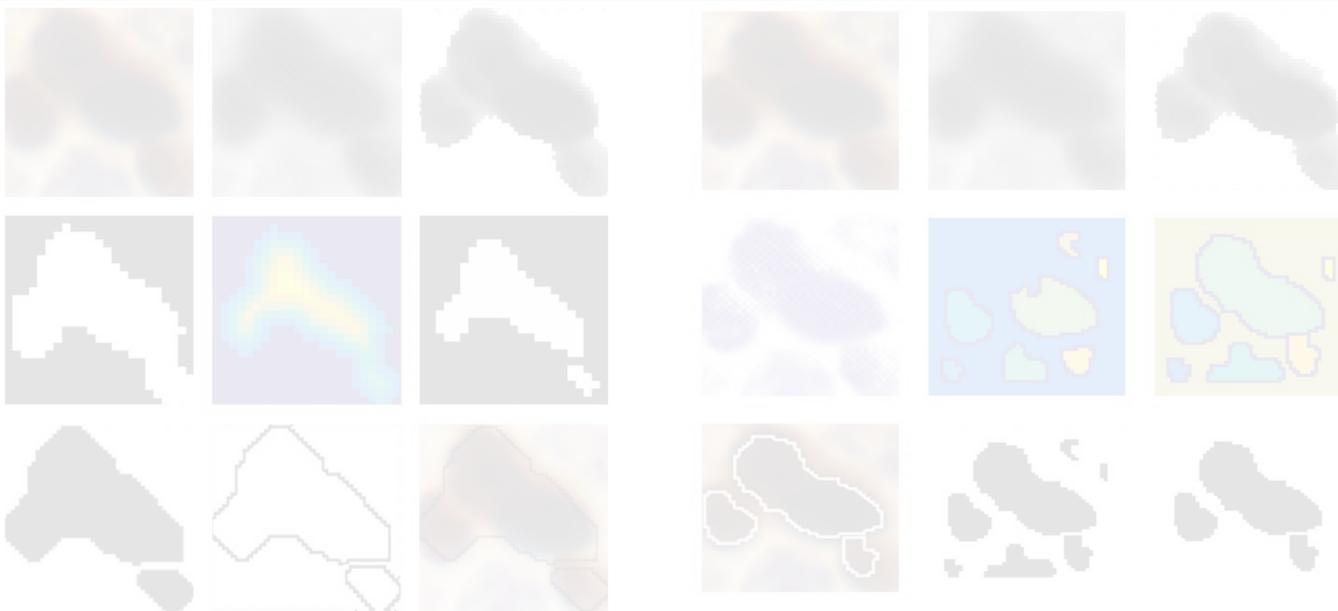
Majority of the algorithms were implemented in MATLAB. The adaptive threshold algorithm is executed in C to speed up this time consuming operation.

All these methods are based on adaptive thresholding, adjusted for the images of stained tissue fragments proposed in [2]:

$$B(x, y) = \begin{cases} \text{object} & \text{if } (I(x, y) \leq T_w \text{ and } C_w > F) \text{ or } (I(x, y) \leq T_s \text{ and } C_w \leq F) \\ \text{background} & \text{otherwise} \end{cases}$$

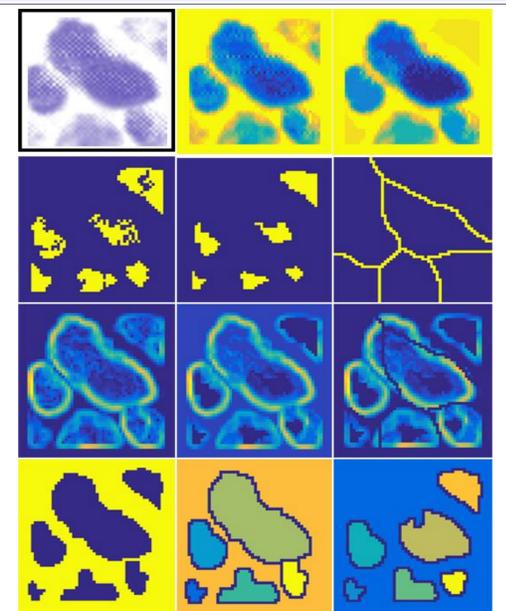
T_w – mean of values from the 30x30 window,
 C_w – absolute difference of maximum and minimum in the same window,
 F – threshold corresponding to typical noise levels (in 15 shades of gray);
 T_s – constant threshold, indicating either object or background, set to 205.

RESULTS



(I) The watershed based on the distance transform is used for separation of cell nuclei from their cluster. Top left – a fragment of an original image, bottom right – superimposition of resulting cell contour on the original image, images between (in rows) present: blue channel after colour modification and Gaussian pyramid, result of adaptive thresholding of blue channel, object map with an object of the same size as the cluster, object map's distance transform, result of watershed on distance transform, object map after Gaussian pyramid and object contours.

(II) **Metinus** – The watershed based on the constructed as combination of results of typical (center image) and proposed by authors (middle right) markers construction methods applied to the monochromatic image of haematoxylin intensity (middle left).
Top row: fragment of an original image, intensity of blue channel, result of adaptive thresholding of blue channel.
Bottom row (from left to right): original image with superimposed contours; an intermediate objects map adjusted as a "sum" of the results of both watersheds; final objects map after objects from intermediate classification based on intensity of DAB dispersion in object region.



(III) **Metinus Plus**
First row (left to right): (1L) haematoxylin intensity with space to add a frame; (1M) haematoxylin intensity with a frame in pseudo-colours; (1R) image after morphological filtration;
Second row: (2L) local minima calculated based on 1R; (2M) local minima from 2L with their shape smoothing; (2R) background marker with its division calculated based on watershed after the distance transform of smooth minima map 2L;
Third row: (3L) Sobel gradient of haematoxylin intensity calculated based on 1M; (3M) Sobel gradient with minima superimposed; (3R) Sobel gradient with minima and background division superimposed;
Fourth row: (4L) the final objects map adjusted based on the next two images; (4M) result of the watershed of image 3M; (4R) result of the watershed of image 3R.

CONCLUSIONS

All proposed methods are available for pathologists and researchers as standard tools. The analysis will address both single-frame field of view and for the areas identified by an expert or software [6] in whole slide images as regions of interests.

References:

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