HER2/CEN17 biomarkers detection in CISH images

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***Abstract*—The article presents an algorithm of HER2/CEN17 genes biomarkers detection in CISH images (Chromogenic in situ hybridization) which supports the diagnosis of the breast cancer. The algorithm is based on morphological transformations, the usage of structural elements (strel objects) and application of distance function. The developed transformation increases the contrast between biomarkers and background which makes possible the use of simple threshold methods for final detection of biomarkers.**

Keywords— CISH; distance function; image processing; mathematical morphology; strel element;

I. INTRODUCTION

The article presents a method for an automatic identification of biomarkers in CISH images (Chromogenic in situ hybridization). CISH is a cytogenetic technique based on immunohistochemical staining (IHC) and in situ hybridization used to detect antigenic substances in microscopic sections. The method uses an antibody against the components of the specimen in the form of insoluble colored substances, seen in a light microscope. This is an alternative method for FISH (fluorescence in situ hybridization), which allows detection of HER2 gene amplification. CISH, the same as FISH, is an in situ technique for the detection of the presence of selected DNA fragments - HER2 gene and HER2 gene chromosome (CEN17). However, CISH method is much faster, easier and cheaper, which makes it a more preferred method used by laboratories and research centers. With this method there is no need for expensive and time-consuming fluorescent staining. For specimen analysis there is also no need to use an expensive fluorescence microscope. An ordinary, bright-field microscope is enough for an observation. Unfortunately, CISH imaging is less accurate than the FISH imaging, and further analysis is required, which makes it more challenging for specialists making the diagnosis [1-5].

II. PROBLEM STATEMENT

Image analysis by specialists relies on the location of HER2 genes (shown as black spots) and CEN17 (shown as red spots). HER2 genes and CEN17 are located in the areas of nuclei. Two common CISH images fragments are shown in the Figure (1).

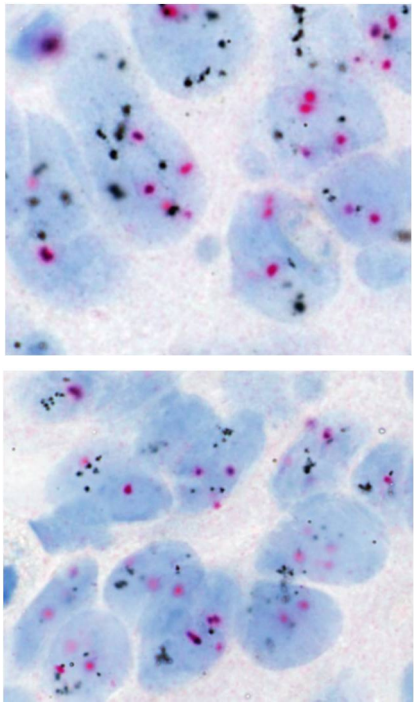

Fig. 1 Two samples of microscopic image in CISH staining

In the images we can observe black (HER2) and red (CEN17) biomarkers. Cell nuclei are marked in blue. In the CISH images two common problems can be specified. The first is that the biomarkers of both types are fuzzy and grouped into stains which makes it difficult to distinguish the contours of the biomarkers. The second problem is related to red spots, which can have such a strong intensity that they can become black. Both problems unable an directly application of similar identification markers solutions, such as the shape analysis [7] or the color analysis methods [8]. The solution proposed in this article is focused on the use of structural objects and distance function increasing the contrast between the markers and the

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background. The developed distance function converts the image into a form where areas with similar intensity are highlighted.

III. IMAGE INVERSION

CISH microscope images are stored in the RGB color space, where three basic channels can be specified: red, green and blue. An analysis of the three-dimensional model of the individual components can show that the black color in RGB image is represented as a hole in each of the channel, while the red color is represented as peak in red channel. Image analysis is more efficient after transformation into complement image [6] using the following conversion:

$$img' = 255 - img \tag{1}$$

The above transformation converts black elements (HER2) into white color (peak in every channel) and elements of the red (CEN17) are visible as a green spots (peak in the green channel).

IV. DISTANCE FUNCTION FROM SIGNIFICANT CHANNEL

In the problem of object recognition leading channels (with provide significant information) and less significant channels can be specified. In complement image, for green spots detection problem, green is a leading channel, while red and blue are less significant channels. A distance function for each point of the image calculates the difference between the leading channel c_k and the less significant channels c_{b1} and c_{b2}. The function has the following form:

$$img(k) = 2 * img(c_k) - img(c_{b1}) - img(c_{b2}) \tag{2}$$

In the case of the biomarker localization, the above transformation can increase the significance of the most significant channel for this type of marker.

V. DISTANCE FUNCTION FROM CENTRAL POINT

Circular structural element (SE) based on radius equal 8 pixels which is the average size of the marker is defined. Each point of the image is combined with central point of the structural element. Then, taking into account the mask of structural element, the following transformation is applied:

$$\begin{matrix} for\ i,j\ \in SE \\ img'(x,y) = \end{matrix} median\left(\sqrt{\begin{matrix} \big(C(R)(x,y) - C(R)(i,j)\big)^2 + \\ \big(C(G)(x,y) - C(G)(i,j)\big)^2 + \\ \big(C(B)(x,y) - C(B)(i,j)\big)^2 \end{matrix}}\right) \tag{3}$$

The above transformation allows to find these areas on the image which have similar size to the marker's size, and each point belonging to the marker is similar (in the meaning of Euclidean distance) to the center point of marker.

VI. DETECTION OF BIOMARKERS USING THE DISTANCE FUNCTION

A complete algorithm for biomarkers detection in CISH images has been developed. First step is to invert an image. The second step is to create a gray scale image (img1), according to the formula (2). The third step is to create a gray scale image (img2), according to the formula (3). The fourth step is to create a difference image: img1-img2 to obtain a new gray scale image.

The result of the above transformation is shown in the fig. 2

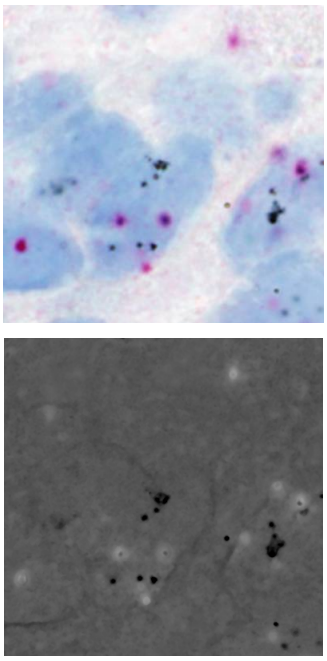

Fig. 2 The result of transformation RGB image into a gray scale image

The resulting grayscale image significantly increases the contrast between the two types of biomarkers. Black markers (HER2) are still black while red (CEN17) are now light. The background values are situated in the middle of gray scale. Recognition of the markers in new grayscale image is much easier and it can be achieved by application of an appropriate threshold filter – another for HER2 and CEN17.

VII. MATERIALS

The examples of the breast cancer used in the tests were obtained from the Department of Pathomorphology in Military Institute of Medicine in Warsaw. All cases represent different status of HER2 gene amplification, mainly classified into immunohistochemical HER2 examination for 2+ and direct to futher in-situ diagnosis based on FISH/CISH. The CISH staining was prepared with the use of ROCHE/VENTANA INFORM HER-2 Dual ISH DNA Probe Coctail, ultraView Red ISH DIG and SISH DNP Detection Kits. The staining procedure was performed on the VENTANA Autostainer.

VIII. TESTES AND RESULTS

In order to assess the efficiency of the evaluated algorithms the tests using 12 CISH microscope images were performed. An expert marked HER2 and CEN17 genes for each image. As

a result he created two masks for each image: HER2 and CEN17. Then a degree of compliance between the system and the expert was calculated for each image in the following way:

$$\begin{gathered}\mathrm{Img}(x,y) = \\ (\mathrm{expert}(x,y) \in \mathrm{maker\ and\ system}(x,y) \in \mathrm{marker}) \text{ or} \\ (\mathrm{expert}(x,y) \notin \mathrm{maker\ and\ system}(x,y) \notin \mathrm{marker})\end{gathered} \quad (4)$$

$$\mathrm{Compatibility} = \frac{\mathrm{sum}(\mathrm{Img}(x,y)==\mathrm{True})}{\mathrm{dimX}*\mathrm{dimY}}$$

Where dimX and dimY are dimensions of the image and $\mathrm{sum}(\mathrm{Img}(x,y) == \mathrm{True})$ is the sum of the points for which there was a full compatibility between the expert and image system.

For markers recognition following parameters in threshold were used: 25 for detection HER2 and 100 for detection of CEN17, in 8-bit gray scale. If pixel intensity after transformation was below 25 then it was classified as a HER2 gene fragment. If pixel intensity after transformation was above 100 it was classified as a CEN17 gene fragment.

In the Table 1 results are presented showing the degree of compliance of the result obtained by the system with the result of an expert.

TABLE 1. DEGREE OF COMPLIANCE OBTAINED BY THE SYSTEM COMPARED TO EXPERT'S RESULTS.

Results obtained with automated system		
case	***HER2***	***CEN17***
1	82,76	61,27
2	79,66	78,37
3	82,07	89,14
4	82,75	87,37
5	75,34	84,54
6	86,54	86,69
7	73,79	77,04
8	89,27	65,50
9	85,32	83,26
10	79,64	62,74
11	88,08	79,62
12	73,41	85,50
Avg.	81,55	78,42

Figure 3 shows the result of detection of HER2 and CEN17 markers by system is shown.

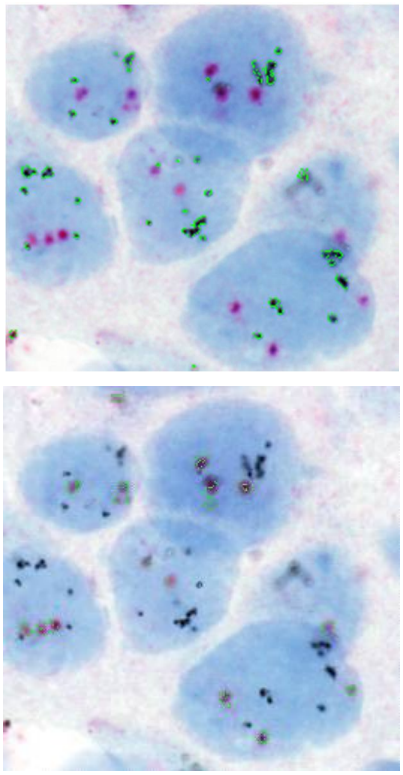

Fig. 3 The result of automatic detection of HER2 (above) and CEN17 (below)

IX. CONCLUSION

Automatic detection of biomarkers in images CISH is much more difficult than in similar images based on FISH - fluorescence staining. In both cases, the aim is to localize the markers. In the FISH images markers are rarely connected with others while in the CISH images biomarkers are often seen as stains (representing group of genes). Therefore, it is not possible to use methods that are based on 3-D shape reconstruction. In CISH images markers are often grouped into stains and have complex, non-standardized shapes. Despite the great difficulty we managed to get a satisfactory result of recognition of the markers areas. The proposed algorithm can be a starting point for segmentation of a stains of markers.

CISH is a practical and often used method in routine diagnostics [9]. Automatic support for image analysis can help to faster achieve diagnosis results.

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