Graphical Interface for analyzing HER2 Fluorescent in situ Hybridization Images

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Abstract—**Amplification of HER2 gene occurs in more than 25% of breast cancer cases. A drug named Herceptin is being used in breast cancer treatment for patients with confirmed amplification of this gene, which makes it necessary to determine HER2 status before the start of the treatment. This paper presents a program for automated analysis of HER2 fluorescent in situ hybridization images. Interactive graphical interface allows the user to have an effect on the determined result by manually altering different stages of evaluation process.**

Index Terms—Breast cancer, contrast enhancement, fluorescent in situ hybridization.

I. INTRODUCTION

BREAST cancer is the most frequent malignant disease among women, both in developed and undeveloped countries [1]. HER2 (Human Epidermal growth factor Receptor 2) gene, which controls the production of HER2 protein, is located on centromere of chromosome 17 (CEP17). Amplification of this gene happens with 25-30% of breast cancer cases [2]. This leads to synthesis of excessive quantities of HER2 protein, and it is responsible for rapid cells growth and division. For treatment of patients with HER2 amplification Herceptin (humanized monoclonal antibody) is used since it interferes with HER2 receptor and reduces the growth of malignant cells. Prior to introduction of the therapy, it is important to resolve HER2 status in selection of the patients. In general, HER2 status is found using Fluorescence In Situ Hybridization (FISH) technique.

PathVysion HER2 DNA Probe Kit is an FDA (Food and Drug Administration) approved kit used to determine HER2 status using FISH technique [3]. When applied to the sample, probes (small segments of DNA material) labeled with fluorescent markers bind to their complementary sequences of the HER2 gene or CEP17. Binding of DNA probes causes emission of the light from the fluorescent markers, which further makes HER2 probes visible as red and CEP17 as green dots. DAPI (4',6-diamidino-2-phenylindole) marker is used for coloring of the nucleus body in blue color. HER2 status is determined by finding the

ratio between the number of HER2 and CEP17 markers for 20-60 observed nuclei. Nuclei without present HER2 and CEP17 markers are not used for calculation of the average ratio. From the FDA recommendations HER2 status above 2.2 suggests the amplification of the HER2 gene, a value below 1.8 advises a normal state, and a value in-between these two limits suggests further medical examination [4]. Method for analysis of FISH images used in this paper is consisted of two main steps: segmentation of the nuclei, performed on blue channel of the FISH image, and HER2 and CEP17 dot detection, performed on red and blue channels of the FISH image, respectively.

Interactive graphical interface presented in this paper gives automated results, but also allows the user to manually change the unsatisfying parts of the process by setting contrast enhancement parameter, removing and adding nuclei for evaluation, changing the threshold values for dot detection as well as manually altering number of detected dots for each nuclei. After analyzing one FISH sample the results (red and green valid dots) are stored and after analyzing necessary number of 20-60 samples the HER2 status can be evaluated. This computer aided program facilitates HER2 status evaluation process.

The paper is organized as follows. Section II describes the proposed algorithm and methodology used for calculation of HER2 status. Interactive software for automated segmentation of nuclei and HER2 and CEP17 markers counting is presented in Section III. Conclusion and final remarks are given in Section IV.

II. METHOD FOR EVALUATION OF HER2 STATUS

First step in evaluation of HER2 status from FISH images is nuclei segmentation. For this task, we used algorithm described in [5] whose overview is given below.

Contrast of blue channel of the image is enhanced using Adaptive gamma correction with weighting distribution (AGCWD) [6] defined as:

$$T(l) = 255 \cdot \left(\frac{l}{255} \right)^{\gamma(l)} = 255 \cdot \left(\frac{l}{255} \right)^{1-c_w(l)} \quad (1)$$

where $T(l)$ is the new value of pixels and $\gamma(l)$ is the adaptive gamma parameter based on modified cumulative distribution function c_w calculated using weighting distribution function p_w:

$$c_w(l) = \frac{\sum_{k=0}^{l} p_w(k)}{\sum_{l=0}^{l_{max}} p_w(l)} \quad (2)$$

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$$p_w(l) = p_{\max} \left(\frac{p(l) - p_{\min}}{p_{\max} - p_{\min}} \right)^{\alpha} \qquad (3)$$

where α is the adjustment parameter, p_{max} is the maximum and p_{min} the minimum value of probability density function.

An example of the FISH image enhancement is given in Figs. 1-3, where original FISH image, its blue channel and enhanced image are shown, respectively.

Binary mask is obtained applying Otsu's algorithm [7] on the enhanced image, after which post-processing steps including filling of small holes and rejection of small objects are performed. Method proposed in [8] is used in [5] for final nuclei segmentation. Method includes hole classification on intra- and inter-nuclei holes, where intra-nuclei holes are being filled. Classification is done based on number of foreground pixels belonging to the perimeter of a circle drawn from each hole. Further steps include applying distance transform, which calculates distance from the nearest background pixel, h-maxima transform for rejection of irrelevant maxima to avoid over-segmentation, and watershed algorithm for nuclei separation (Fig. 4).
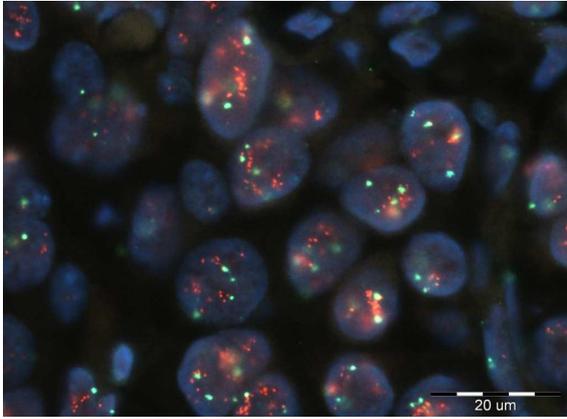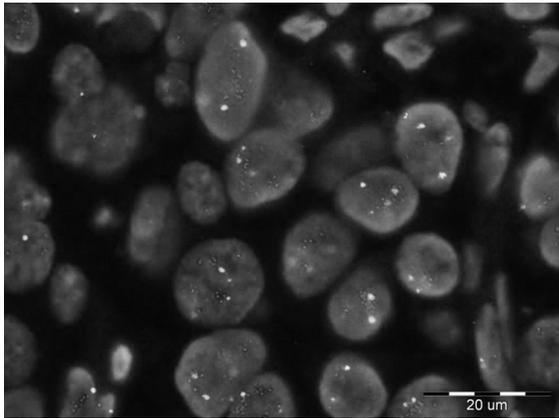

Fig. 1. Original FISH image.

Fig. 2. Blue channel of the FISH image from Fig. 1.

Detection and counting of HER2 and CEP17 dots is carried out in red and green image channel, respectively. Preprocessing of these images comprises of top-hat filtering followed by image normalization to a range of [0,1] by dividing the image with its maximum intensity value. Background regions are removed from further analysis. Each nucleus is individually observed - if its maximum intensity value is smaller than θ_1 (which is set to 0.3) the nucleus does not contain the required dots and the value of all its pixels is set to 0. The pixel values in the remaining nuclei which are less than θ_2 (which is set to 0.2) are set to

0. Figs. 5-7 show red channel of the image, the result after top-hat transformation and the result after thresholding.
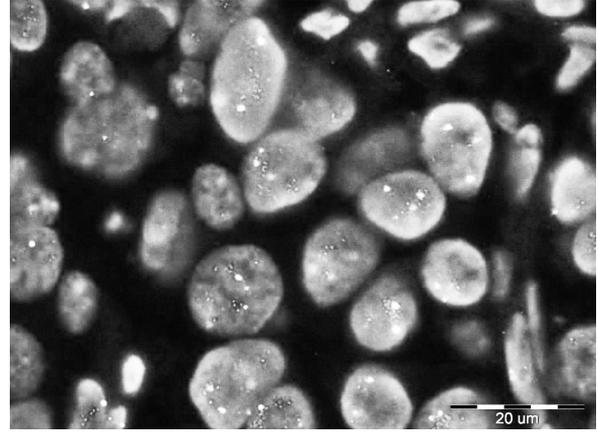

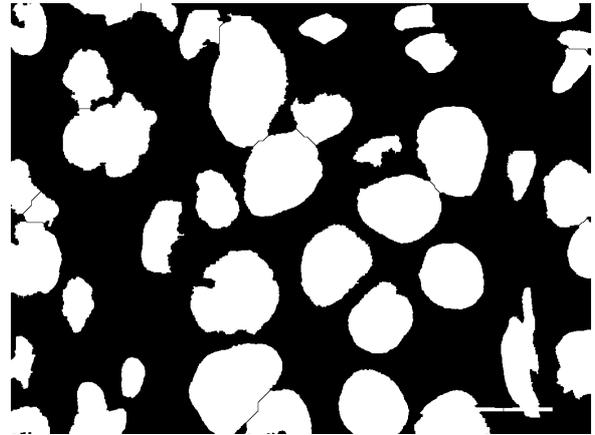Fig. 3. Image from Fig. 2. enhanced using AGCWD.

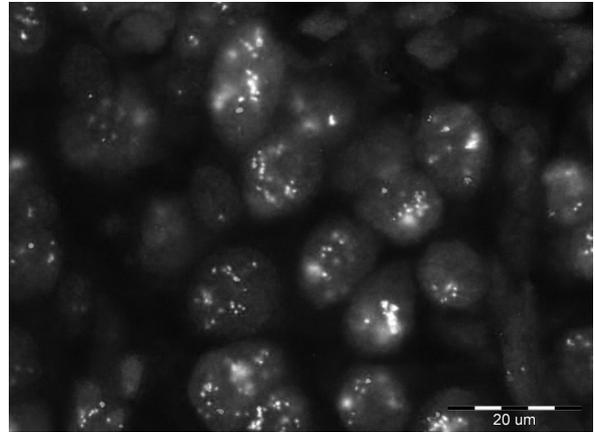Fig. 4. Result of segmentation of nuclei from the image from Fig. 3.

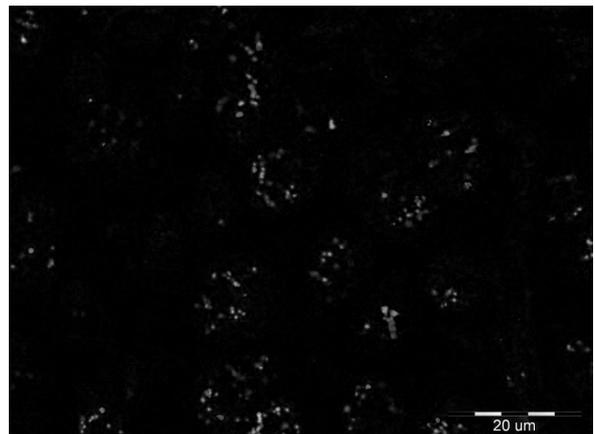Fig. 5. Red channel of the FISH image from Fig. 1.

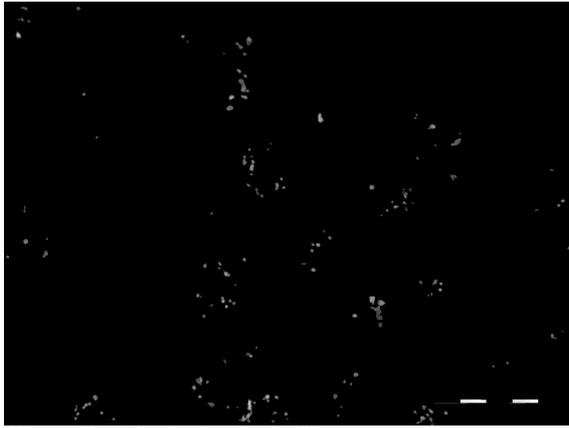Fig. 6. Image from Fig. 5. filtered using top-hat transformation.

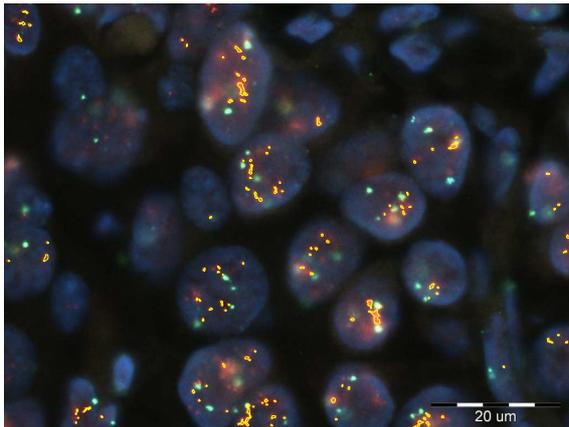Fig. 7. Image from Fig. 6. after thresholding.

Fig. 8. Labeled HER2 markers.

For detection and counting of markers, 3x3 pixel neighborhood is observed. If the value of the current pixel is within a certain range (between *max* and 0.75·*max*, wherein the *max* is the local maximum value of the neighborhood), it is said that the pixel belongs to a marker. If its closest pixel already belongs to a particular marker, observed pixel joins the same marker, otherwise, it belongs to the new marker. Markers with more than five local maxima are discarded because they are assumed to represent noise. HER2 and CEP17 markers are visible in both red and green RGB channels. CEP17 has a greater intensity value in the green channel, while HER2 has a greater intensity value in the red channel. Hence new restrictions are introduced to avoid misclassification of red markers as green and vice versa. When the point is marked, the sums of its red and green channels are compared. For red dots, only those markers in which the sum of the red channel is at least 10% greater than the sum of the green channel are retained. Fig. 8. shows the detected red markers highlighted in yellow. The same procedure is applied to the green channel of the image. HER2 status is calculated as the average value of the ratio of HER2 and CEP17 markers in the nuclei.

III. Software description

Upon opening of the image and by clicking the button "SEGMENT NUCLEI" result of nuclei segmentation is shown on the right side of the panel (Fig. 9). Click on the "REMOVE NUCLEI" option rejects marginal objects and objects whose roundness factor is less than 0.4 as well as those objects whose area is less than 50% of the average area (Fig. 10). Option "ALPHA" enables user to change the

α parameter for AGCWD algorithm and thus by clicking nearby button "SEGMENT NUCLEI" change initial segmentation result.

Option "UPDATE NUCLEI" allows user to remove unwanted nuclei from further analysis by simple clicking anywhere inside the desired nuclei. By activating the "DRAW NUCLEI" button, user can manually label edges of nucleus (Fig. 11). Option "SAVE" allows user to save the image with labeled nuclei.

By clicking "DETECT HER2 AND CEP17" a new window is opened. Image with labeled nuclei, as well as images with labeled HER2 and CEP17 markers are shown. An example using result from Fig. 10 is shown in Fig. 12. Table with number of HER2 and CEP17 markers detected in each nuclei is also visible. Program allows user to change θ_1 and θ_2 parameters for detecting HER2, as well as θ_3 and θ_4 parameters for detecting CEP17 markers. By clicking "UPDATE" option in θ panel images with newly detected markers will be shown. Options "UPDATE" above images with labeled markers allow user to remove incorrectly detected markers by simple clicking on them. Number of detected markers can be altered manually in table and by clicking the "UPDATE" the data will be updated. Options "SAVE" allow saving the table with data as Microsoft Excel document shown in Fig. 13a where valid nuclei are labeled in green, and the rest in red color, as well as saving images with labeled markers. More than one image is often needed for evaluation of HER2 status. Hence, data should be saved as a single Microsoft Excel document for each analyzed image. By clicking the option "HER2 status" a new window is opened (Fig. 13b) which allows user to load multiple Microsoft Excel files. From these files program will calculate HER2 status as ratio of all HER2 and CEP17 markers found. Only nuclei with at least one marker of each color are taken into consideration. As an example, we divided results from Fig. 13a in two files. Results for nuclei 1-7 are put in one, while results for nuclei 8-14 in second file. An example of evaluated HER2 status is showed in Fig. 14a. Here, number of HER2 and CEP17 markers denotes number of those markers found in valid nuclei. Right side of the window lists all files used. By clicking the file name a table with data from that file is showed (Fig. 14b). Results can be saved as Microsoft Excel document (Fig. 15) where data from multiple files will be sequentially combined.

IV. Conclusion

An interactive graphical interface for automated analysis of HER2 FISH images is presented. The method is comprised of two main steps. First step refers to automated nuclei segmentation. If the user is not satisfied with results he can alter them by changing parameter for contrast enhancement, or by manually removing or adding nuclei for further evaluation. Marker detection is the second step of the method where user can also alter the results by changing threshold parameters or number of detected markers for each nucleus. Results can be saved as Microsoft Excel document and program allows using one or more of these files for HER2 status evaluation, since multiple images are often used for this task.
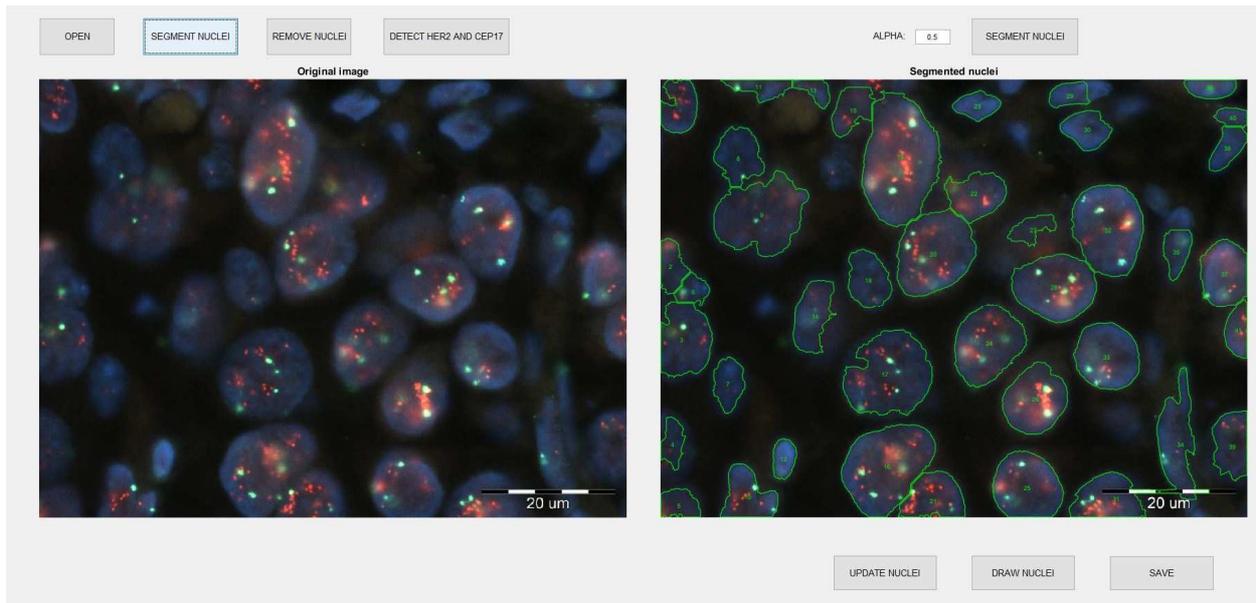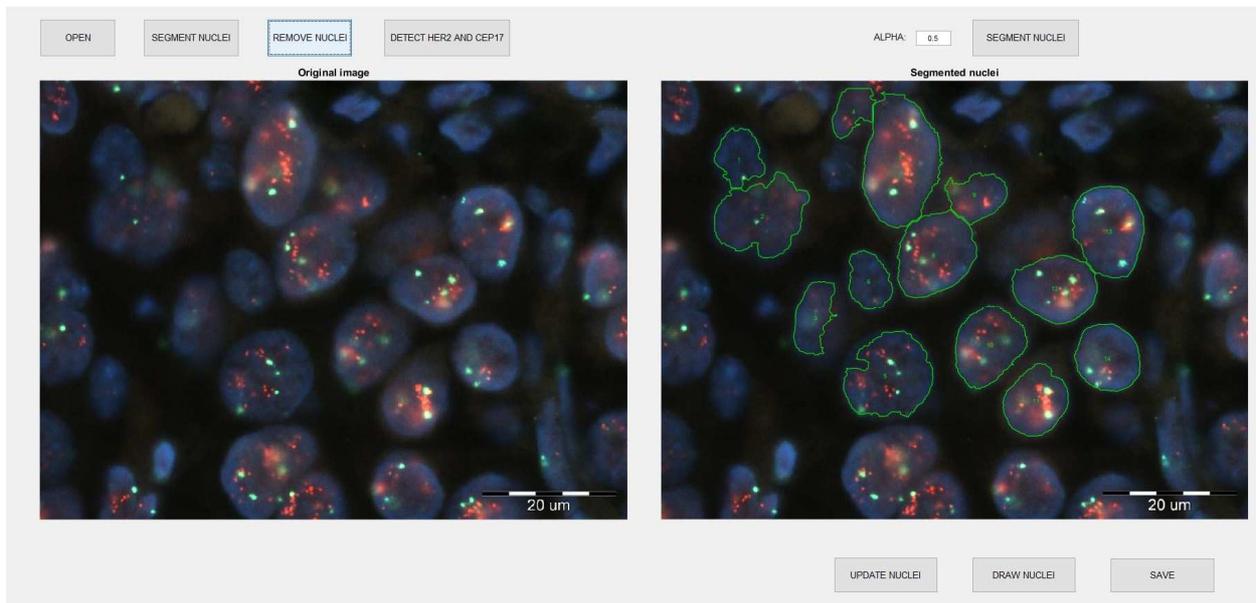
Fig. 9. Initial segmentation result.
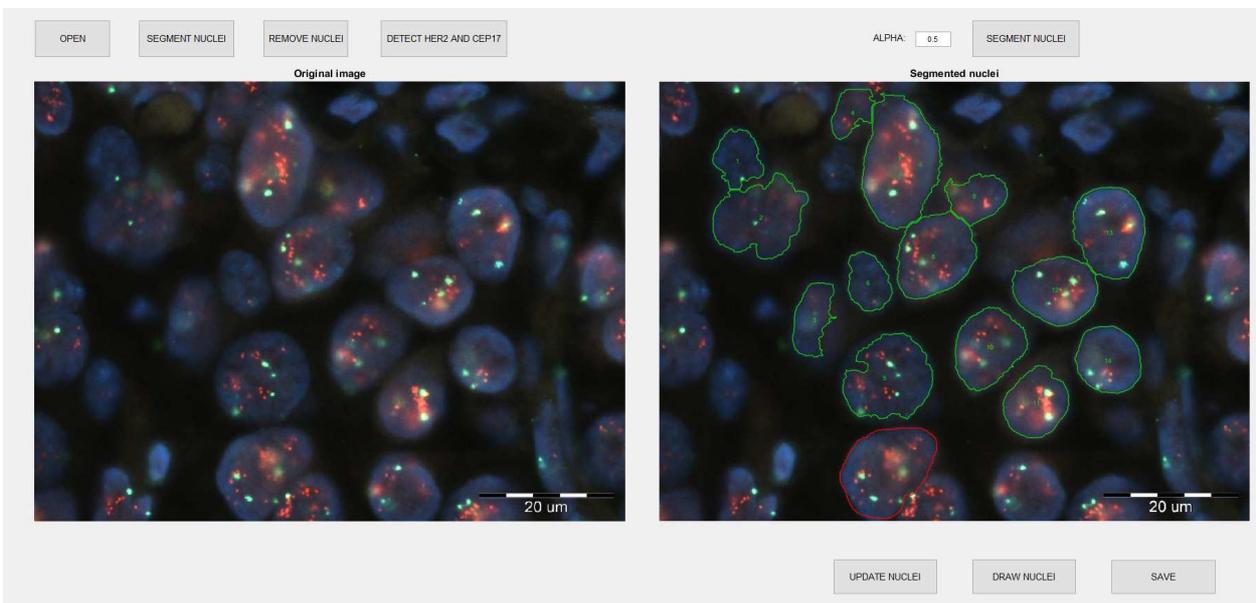

Fig. 10. Result after nuclei removal.

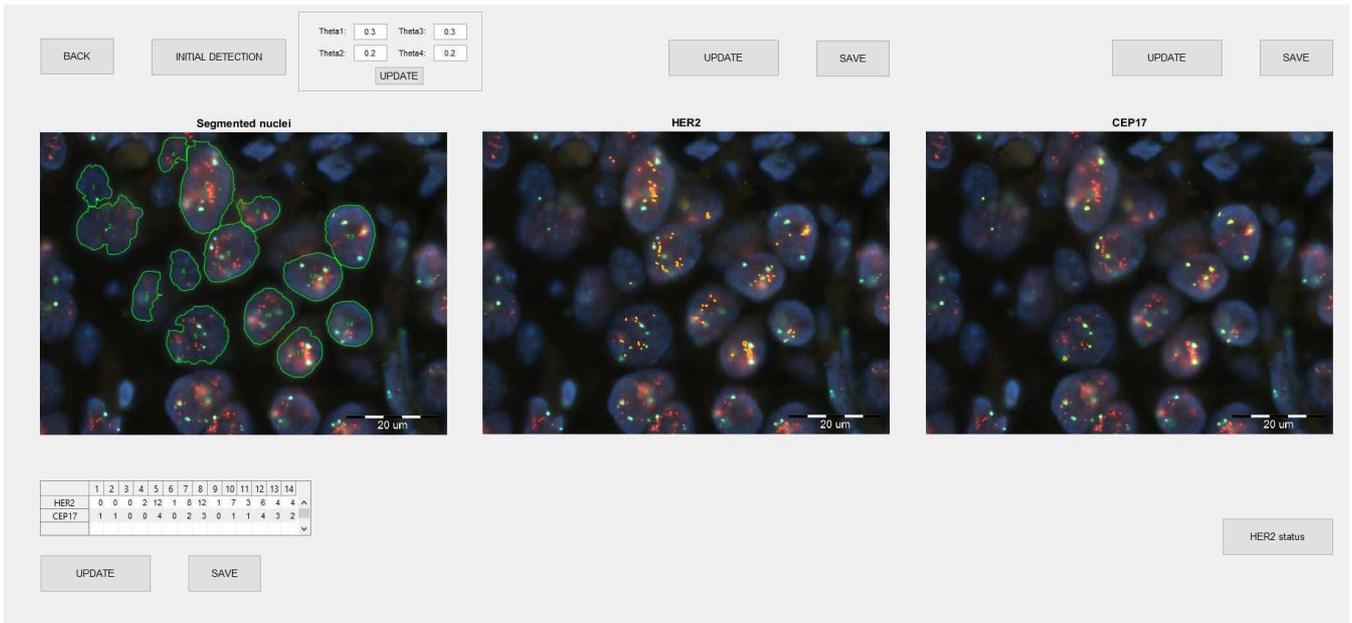Fig. 11. Manually labeled nuclei.
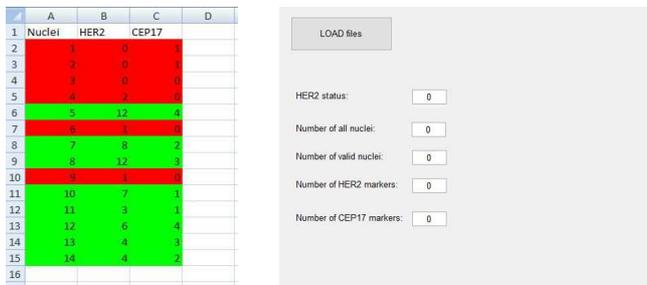
Fig. 12. Detected HER2 and CEP17 markers.

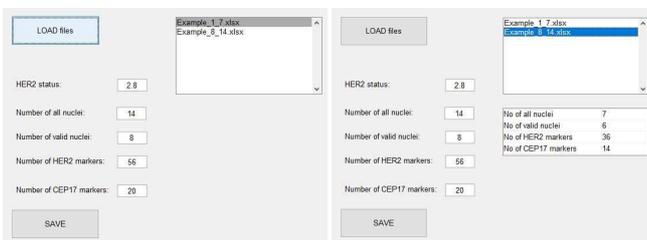Fig. 13. a) Example of saved data b) Window for HER2 status evaluation

Fig. 14. a) Evaluated HER2 status b) Data for selected file Example1.xlsx

Fig. 15. Example of saved final results

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