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Alternative color space construction in FISH image analysis for quantification of the HER2 gene amplification

Background

Fluorescent in situ hibridization (FISH) is a valuable method for determining HER2/neu status in breast cancer. The over-expression of HER2 oncogene is presents in approximately 20-40% of breast cancer cases giving an indication to trastuzumab therapy. The analysis of the FISH microscopic images requires the recognition of the separate cell nuclei and green and red dots inside them, represent the 17th centromers (CEP-17) and HER2 genes in the chromosomes. The amplification was considered if the ratio HER2/CEP-17 was >2.0.

The microscopic acquisition of the FISH-stained specimen is realized by registration of the three hybridization signals an appropriate filters set (DAPI for blue, FITC for green and texas red for red channels). These tree signals composed the RGB images in the natural order. The most approaches in the automatic FISH image analysis are based on that RGB space construction. In this paper we propose the alternative color space composition from the registered signals, which enhanced the dots visualization, supporting both manual and automatic quantification of the FISH images.

Materials and methods

The 50 breast cancer cases were retrieved from the archives of Pathology Department of the Military Institute of Medicine, Warsaw, Poland. The images were acquired on the Olympus BX-61 microscope equipped with DP-72 digital camera. Any field of view was represent by the three monochromatic signals images: DAPI, FITC and texas red.

Based on the depicted channels, in the manual specimen analysis, it is constructed the one RGB image (DAPI as blue, FICT as green and texas red as red color components) and the green and red dots are counted in any recognized nuclei. In the automatic image analysis, the DAPI channel is used to nuclei segmentation and FICT or texas red channels are used separately to recognized the appropriate dots. Concludes, the FISH image analysis can be described as a two step process: nuclei segmentation and dots recognition.

To the nuclei recognition task we can find in the other studies many approaches, mainly based on the mathematical morphology [1-3] or neural networks [4]. The main difficulty of this step is the separation of individual nuclei, frequently realized by application of the appropriate distance mask and watershed algorithm.

The dots recognition is a very difficult task, both in manual and automatic image evaluation. In the some specimens/fields the nuclei are not well extracted from the tissue in the specimen preparation process and in the classic-composed RGB image the dots are very poorly visualized. To improve this visualization, we propose the alternative composition of the RGB image from the separate signals. For the dot visualization, we used only FITC and texas red signals, omitted the DAPI. There can be used different combinations of the depicted signals, with one of them get twice. For example, the RGB image can be composed as: texas red – texas red – FITC, FICT – FICT – texas red, texas red – FICT – texas red.

Results

The changes of the classic RGB composition (Fig. 1a) into texas red – texas red – FITC is presented in Fig. 1b. It is evidence that the red dots are now yellow color and originally green are now blue. The color of background and nuclei is filtered closely to grey and visibility of the red dots is significantly increased.

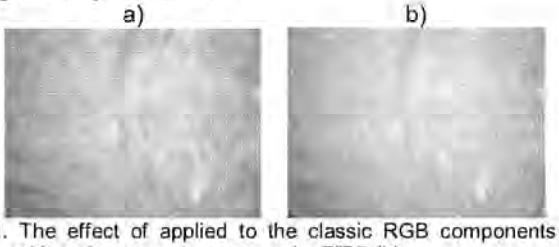

Fig.1. The effect of applied to the classic RGB components (a) composition of texas red – texas red – FITC (b).

For the evaluation of the color effect obtained by the alternative signal composition, the set of well visible red dots and set of poor visible red dots were marked on the example images. Based on the Euler angles, the rotation angles between the average color direction were calculated of both sets for the classic and alternative RBG components. In the experiment, the angle reduction was 3-10° that with increasing contrast gives significant effects to dots detection.

Conclusions

The proposed alternative composition of the acquired signal components into the RGB channels is a effective way to increase visibility of the dots in FISH images. This method reduced noise and enhanced the dots from the background.

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