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Alternative colour space construction *of the stain reaction* in FISH image analysis for quantification of the HER2 gene amplification

Abstract. *The paper presents a study of the different colour representation (intensity of the stain reaction) in FISH images. The better colour visualisation of the centromers and HER2 genes in these methods of the breast cancer stain has a significant influence to correct evaluation of the presence/absence HER2 gene amplification. We taken into account wide range of the image colour representation and propose alternative colour space construction, based on the selected signals in FISH image registration. The proposed approach offers the reduction of the angle between HER2 dots from about 10º to 5.5º.*

Streszczenie. *Artykuł prezentuje analizę różnych reprezentacji barwnych obrazów FISH. Lepsza wizualizacja barwna centromerów i genów HER2 w rozważanym barwieniu preparatów raka sutka ma znaczący wpływ na prawidłowość oceny obecności bądź braku amplifikacji tego genu. W badaniu przeanalizowano wiele reprezentacji barw oraz zaproponowano alternatywny sposób konstrukcji przestrzeni barw, poprzez użycie wybranych sygnałów z rejestracji obrazów FISH. Zaproponowane podejście pozwala zmniejszyć kąt przestrzenny pomiędzy poszczególnymi znacznikami genów HER2 z wartości około 10º do 5.5º.* ***(Alternatywne przestrzenie barw w analizie obrazów FISH w analizie ilościowej amplifikacji genu HER2)***

Keywords: colour representation, FISH, breast cancer.
Słowa kluczowe: reprezentacja barw, FISH, rak sutka.

Background

Fluorescent in situ hybridization (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 three signals composed the RGB (red, green, blue) 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 compare different colour representation at aspect of the stain reaction FISH of HER2 gene evaluation. We also propose the alternative colour 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, FITC as green and texas red as red colour 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 FITC 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 sometimes are very poorly visualized. There exist a significant problem with interpretation of the separate RGB components. On the R or G components not only gene dots have the high signal, but also some parts of the stromal or nucleal elements/areas. This fact make manual or automatic analysis hardness to perform.

In the image analysis topics there are well known many colour image representation gives the wide possibilities of the effective image analysis. The alternative representation, based on luminance, chrominance, saturation or other features, gives the tools to detection the specific regions in the image if that relation exists. In this study we taken into advance the following representations: HSV (hue, saturation, value), YIQ (luminance, hue, saturation), YCrCr (luminance and two difference components), XYZ, L*a*b* (L - psychometric lightness) and Luv (luminance and coordinates).

The alternative approach proposed by us is to compose the RGB image from the different components that it is done in default procedure. The alternative composition of the RGB image from the separate signals should be perform in that manner that improves visualization of the dots. The nuclei recognition can be perform as was proposed in the previous works [1-4], that is based on the DAPI signal only. 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,
- FITC – FITC – texas red,
- texas red – FITC – texas red.

This approach gives the possibility to reduction the influence of the stroma visualization to dot signals. It is done first by omitted the DAPI signal, and second by compensation of the border components with use doubled texas red or FITC signal in RGB image composition.

The final question is a measure the enhancement of the dot signals in the alternative colour representation. This task is hardly to perform because it is a problem with define the appropriate measure gives the comparable results with aspect to manual and automatic image evaluation. At the comparison of the different colour representation we present the human interpretation of changes, that there are tedious and very subjective.

For more precise evaluation of changes, we applied also a measure based on the Euler angles, defined as the rotation angles between the average colour direction of different dots and background. This measure is calculated as

$$r = a\cos\left([x_1, y_1, z_1] \cdot \begin{bmatrix} x_2 \\ y_2 \\ z_2 \end{bmatrix} \right) \quad (1)$$

where: x,y,z – components of the two comparable colours.
We would like find that representation, that gives the smallest angles between dots of the same signal (centromers or HER2 gens) and highest angles in the other cases.

Table 1. The colour identification of the FISH markers

Colour space	centro mers	HER2 genes	nuclei	Stroma area
RGB	green	red	blue	black with green-yellow smudges
HSV	light blue	light blue	dark blue with magenta smudges	green with light blue smudges
YIQ	palish green	red	dark red	black with orange smudges
YCbCr	red	violet	grey	blue with magenta smudges
XYZ	green	palish red	palish blue	black with green smudges
L*a*b*	light magenta	light turquoise	brown	dark turquoise with magenta smudges
Luv, Lu'v'	light blue	light violet	grey-blue	Green
texas red – texas red – FITC	blue	yellow	grey	black with grey smudges
texas red – FITC – FITC	blue	red	grey	black with grey smudges
FITC – texas red – FITC	violet	green	grey	black with grey smudges
FITC – FITC - texas red	yellow	blue	grey	black with grey smudges
texas red – FITC – texas red	green	magenta	grey	black with grey smudges
FITC – texas red - texas red	palish red	green	grey-yellow	black with yellow smudges

Results

The comparison of the different colour representations included identification of the influence to colour visualization of the centromers, HER2 genes, nuclei and stroma. The detailed results are presented in the Table 1.

Table 2. The influence of the colour transformation to visualise of the FISH markers related to the standard RGB image

Colour space	centro mers	HER2 genes	nuclei	Stroma area	Useful
HSV	---	-	-	-	nothing
YIQ	-	+ (I component ++)	+/-	+/-	worse colour differentiation
YCbCr	- (Cr component +)	-	--	+/-	worse colour differentiation
XYZ	--	-	-	+/-	worse dots visualization
L*a*b*	--	+	-	-	worse colour differentiation
Luv, Lu'v'	-	-	-	+/-	very worse visualization
texas red – texas red – FITC	+	++	-	+ (more clearly)	better dots visualization
texas red – FITC – FITC	+	++	-	+ (more clearly)	better dots visualization
FITC – texas red – FITC	+/-	++	-	+ (more clearly)	only HER2 dots better visualization
FITC – FITC - texas red	+/-	+/-	-	+ (more clearly)	without significant effects
texas red – FITC – texas red	+/-	+	-	+ (more clearly)	slightly better visualization
FITC – texas red - texas red	-	++	-	+/-	worse colour compensation of the stroma

The influences of the selected colour representation to the depicted objects visualization are described in the Table 2. The baseline to this evaluation was the RGB image, the adjustment of the object visualization was noted by "+", the worse effect was noted by "-". In the fig. 1 there are presented (in zooming) three components of the FISH image (R,G and B), registered in the standard scanning process, the RGB composed image and two alternative colour representations: texas red – texas red – FITC and texas red – FITC – FITC. It should be noted that in the R and G images the high intensity exist not only on the specific dots, but also e.g. closely to the cell membranes. Additionally, dots intensity aren't uniform. On the RGB image can be shown that some dots are very hardly to recognize and there exist yellow-green smudges on the image (rest of the stroma), hinder a counting of the dots in cells. These evaluation problems are reduced in the alternative colour representations, when dots are better recognizable and smudges loss yellow-green colour to the white colour direction.

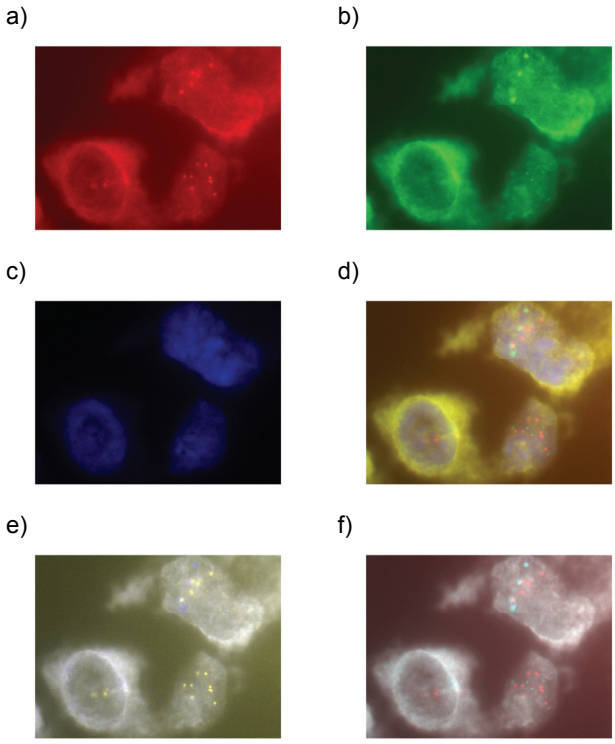

Fig.1. The RGB components of the FISH image (a, b and c), the RGB composition (d), the compositions of texas red – texas red – FITC (e) and texas red – FITC – FITC (f).

The changes of the classic RGB composition (Fig. 2a) into texas red – texas red – FITC for the sample view field in the microscope is presented in Fig. 2b. It is evidence that the red dots are now yellow colour and originally green are now blue. The colour of background and nuclei is filtered closely to grey and visibility of the red dots is significantly increased.

For the numerical evaluation of the colour 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 15 images. Based on the Euler angles, the rotation angles between the average colour direction were calculated of both sets for the classic and alternative RBG components. In the experiment, the mean angle was reduced from 10.2º to 5.5º. The increase contrast obtained in the alternative colour representation gives significant effects to dots detection, in manual and automatic image analysis.

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.

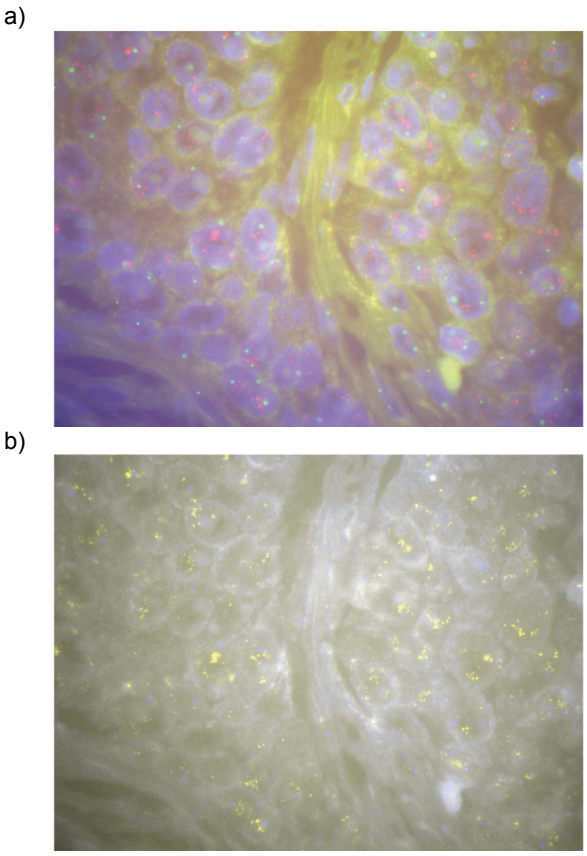

Fig.2. The effect of replaced the classic RGB components (a) by the composition of texas red – texas red – FITC (b) (magnification x1000).

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