HER-2/neu Breast Cancer Diagnosis Procedure, Based on Histopathology Image Analysis

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Abstract. The human epidermal growth factor receptor 2 (HER-2/neu) is a biomarker, recognized as a valuable prognostic and predictive factor for breast cancer. In approximately 20% of primary breast cancers, the HER2/neu protein is over-expressed. The effect of this over-expression is an increase in receptor mediated intracellular signalling, directing the cancer cells into fast proliferation, which results in an aggressive form of breast cancer. By recent clinical research, a treatment procedure, with corresponding monoclonal antibodies specifically designed to target the HER2/neu receptor, was confirmed. Therefore, in modern breast cancer diagnostics, accurate recognition of the HER-2/neu positive breast cancer should be provided. This can be done, first by accurate segmentation of HER-2/neu over-expressed cancer cell membranes and then, by providing corresponding membrane connectivity analysis. This paper is a continuation of a previous research, in which the problem of cell membrane segmentation was considered. In the present study, we propose a HER-2/neu cancer cell membrane connectivity analysis, by examining corresponding shape coefficients. We performed statistical analysis, investigating the significance of the proposed coefficients in terms of HER-2/neu cancer cell recognition process. The proposed research gives a basis for the introduction of a complete HER-2/neu breast cancer recognition procedure.

Keywords: Fuzzy rough sets, Fuzzy set approximations, Histopathology image processing, HER-2/neu breast cancer, FISH test

1 Introduction

Breast cancer poses a serious medical problem worldwide. It is currently the most common diagnosed malignancy in women owing to the highest morbidity in patients aged 20 to 59 years. Abundant lines of evidence point to its heterogeneous character, as several types of this malignancy could be identified using the costly methods of molecular profiling [16]. For routine clinical practice breast cancers are characterized by assessment of the expression of four following markers: estrogen receptor (ER),

progesterone receptor (PR), Human Epidermal Growth Factor Receptor 2 (HER-2/neu) and the Ki-67 antigen. For diagnostic purposes the above mentioned proteins are determined in formalin-fixed paraffin embedded breast cancer tissues utilizing the less costly immunohistochemical (IHC) methods [5]. Of major clinical importance is the assessment of the membrane bound HER-2/neu receptor, which was found to be over-expressed in approximately 20% of diagnosed breast cancer cases [13,15]. Breast cancers characterized by its over-expression are characterized by increased proliferation, invasiveness and metastatic potential resulting in patients poor outcome [13, 15]. The activity of this receptor may be blocked via utilization of monoclonal antibodies, which clinical usefulness was confirmed in clinical trials. One of such agents is the widely utilized trastuzumab (Herceptin, Genentech, CA) [5, 13, 15].

Only patients whose breast cancers are characterized by amplification of HER-2/neu may benefit from such specific agents, therefore strict patients qualification is immensely important [19]. Potential responders to trastuzumab treatment are currently identified in a two-step procedure [19].

The first step is based on examination of membranous cell staining in tumour cells in immunohistochemical sections of breast cancer. The results are categorized according to a semi-quantitative four–grade scale: 0 (no staining), 1+ (incomplete, weak membrane staining regardless of the proportion of tumour cells stained), 2+ (nonuniform complete membrane staining or staining with obvious circumferential distribution in at least 10% of the tumour cells, or intense, complete membrane staining $\leq$30% of the invasive tumor cells), 3+ (intense membrane staining in >30% of the invasive tumour cells) [4, 19]. Cases scored 3+ utilizing this method are regarded as HER2/neu over-expressing cases and qualified for the trastuzumab based therapy. Cases classified as 0 and 1+ are not. Sections scored as 2+ are regarded as unequivocal and require additional testing using a costly fluorescent *in situ* hybridization technique (FISH) for final determination of HER-2/neu amplification status [19].

The major difficulty in the HER-2/neu assessment is the proper assessment of the membranous reaction, in particular its linearity and intensity, which may cause differences among assessing pathologists, which in turn leads to discrepancies between the IHC and FISH final scores. One of methods allowing to overcome the semi-quantitative assessment of HER-2/neu is the computerized assessment of digital images of HER2/neu immunostained sections. This would be of particular importance in cases of sections scored as unequivocal (score +2), as standardized, computerized assessment protocol could limit the number of necessary additional FISH testing. Moreover, a generalized assessment protocol derived from HER2/neu sections, could be also applicable for examination of other membrane bound antigens emerging as new prognostic and predictive factors in breast cancer or other malignancies such as gastric cancer [6, 11].

This work is a continuation of our previous research [17], here we investigate the possibility of using geometric features that strongly specify the connectivity and linearity of cell membranes. The introduction of such factors, could support the automatic identification of the different classes of HER-2/neu breast cancer cell type and should improve the current diagnostic process of HER-2/neu breast cancer. This article is organized as follows: in section 2 the problem formulation is given, sections 3 and 4

present our previous research on segmentation of HER-2/neu over-expressed cell membrane, section 5 describes the proposed cell membrane connectivity analysis, section 6 shows the method verification by data analysis.

2 Problem Formulation

In terms of digital image processing, the HER-2/neu classification process is a problem of cell membrane staining and the cell membrane connectivity/closure recognition. Over-expressed cell membrane stain connectivity and closure are analyzed and classified by pathologists and are key elements of differentiating the breast cancer cases between scores +2 and +3. This step highly depends on the experience of pathologists and may cause the observed variability of HER-2/neu scoring between observers. Accurate classification of cell membrane to 2+ class causes a big problem and these cases are qualified for FISH test examination, other classes are easier for pathologists as well as for automatic classification. Above difficulty arises from fuzzy nature of the cell membrane. Moreover, the HER-2/neu sections classified by the pathologists as +2 are mostly characterized by the ratio of amplification close to 2 (treatment threshold decision) in the FISH examination [19].Generally, the HER-2/neu histopathology image classification consists of the two following main processes [7, 2]:

- segmentation of the corresponding HER-2/neu over-expressed cell membranes,
- classification of the histopathology preparations on the basis of shape and connectivity analyses by investigating correlation with the FISH test examination.

In our previous research [17], we considered the very important problem of accurate recognition of cell membranes with HER-2/neu over-expression. This is done by analyses of colour image features, with respect to appropriately defined fuzzy set approximations. For clarity, some theoretical basis and our approach to segmentation are briefly explained in two next sections. As a natural continuation of our research, now we investigate the possibility of using geometric features that strongly specify the connectivity and linearity of cell membranes. Geometric features could support the automatic classification of HER2 breast cancer cell type.

3 Basic Notions - Fuzzy Relations, Fuzzy set approximations

In our research we use fuzzy rough sets therefore the basic notions and definitions of fuzzy relations and fuzzy set approximations [3] are given below.

Equivalence relations and orderings are key concepts of mathematics and they play a fundamental role in the areas of fuzzy logic and fuzzy systems [1, 3]. A fuzzy relation or a binary fuzzy relation $\rho(X,Y)$ (or in short: ρ) is the fuzzy subset of the product $X \times Y$, i.e. a function of $X \times Y$ into the closed interval [0,1] [20]. Below some basic properties and operations over fuzzy relations, are presented.

Let $\rho(X,Y)$ and $\sigma(Y,Z)$ be two fuzzy relations. The *composition* of ρ and σ, i.e. $\rho \circ \sigma$ produces a new fuzzy relation $\tau(X,Z)$ defined as follows: $\tau(x,z) =_{df} [\rho \circ \sigma](x,z) =$

$\bigoplus_{y \in Y} \{\rho(x,y) \otimes \sigma(y,z)\}$, where x∈X and z∈Z, ⊕, ⊗ denote *s*- and *t*-norms respectively [9]. We used the Zadeh's *t*- and *s-norms*, i.e. the *min* and *max* operators. Now werestrict our attention only to the case of binary fuzzy relations defined in X (a finite set). The basic properties of the well known (crisp) binary relations over X are extended. A binary fuzzy relation ρ is called *reflexive* if ρ(x,x)=1, for any x∈X. ρ is *symmetric* if ρ(x,y)=ρ(y,x), for any x,y∈X and *t-transitive* if ρ(x,z)≥ρ(x,y)⊗ρ(y,z), for any x,y,z∈X. Any such *t*-transitive relation is said to be a *fuzzy t-equivalence*, i.e. at the same time reflexive, symmetric and t-transitive.

For any non t-transitive relation, to ensure the transitive property, the following transitive closure (assuming *transitive max-min closure*) algorithm can be used [10]: *Algorithm 1* - Input: ρ; Output: ρ^+: Begin: (1) Let $\rho' =_{df} \rho \cup (\rho \circ \rho)$; (2) If $\rho' \neq \rho$ set $\rho =_{df} \rho'$ and go to step (1) else ρ´ is the transitive closure of ρ. End. □

Next, we can define approximations of fuzzy sets (originally introduced in [3]). Let assume some domain X, X≠∅, some fuzzy subset 'A' defined in X and ρ: $X^2 \rightarrow [0,1]$ as *max-min equivalence fuzzy relation*. Then, we can define the *lower* and the *upper* fuzzy set approximations as follows:

The lower fuzzy set approximation:

$$\mu_{\underline{A}}(y) =_{df} \inf\{x \in X / \rho(x,y) \Rightarrow_L \mu_A(x)\}, \quad (1)$$

for any x, y ∈ X, where ' $\Rightarrow_L$ ' is the Lukasiewicz's fuzzy implication operator, defined as follows:

$$a \Rightarrow_L b =_{df} \min\{1, 1 - a + b\}, \text{ for any } a, b \in [0,1] \quad (2)$$

The upper fuzzy set approximation:

$$\mu_{\overline{A}}(y) =_{df} \sup\{ x \in X / \rho(x,y) \otimes_L \mu_A(x) \} \quad (3)$$

for any x, y ∈ X, where ' $\otimes_L$ ' is the Lukasiewicz's t-norm, defined as follows:

$$a \otimes_L b =_{df} \max\{0, a + b - 1\}, \text{ for any } a, b \in [0,1] \quad (4)$$

The above defined fuzzy set approximations are direct fuzzy generalizations of the classical rough set theory assumptions [12] and thus allow defining fuzzy rough sets.

4 HER-2/neu Over-expressed Cell Membrane Segmentation Method

The clue of the segmentation process, presented in our recent research [17], was our proposition to use the lower and upper approximations, described in section 3, in purpose to investigate if a certain HER-2/neu cancer cell membrane is close enough (under the interpretation of fuzzy rough sets) to a representative fuzzy set.

The proposed HER-2/neu image analysis can be defined as a composition of the following two processes:

1. Reference fuzzy set generation
2. HER-2/neu over-expressed cell membrane recognition process of a new image

Ad. 1.: The process consists of three steps:

1) *Generation of input set of reference cell membrane pixels* – it is a set of pixels located only on HER-2/neu over-expressed cell membranes, derived by physicians (domain experts).
2) *Extraction of corresponding image colour features* – we use 'rich feature vector' $\overline{\mathbf{v}}$ (but limited to colour features) as a descriptor for any considered image pixel, originated from the UCI Machine Learning Repository[1].
3) *Definition of reference fuzzy set 'Over-expressed Cell Membrane' (in short: OCM)* - we assumed multivariate Gaussian distribution as a membership function of the fuzzy set OCM, defined over the set of a priori given pixels in step 1, with respect to the considered image features (step 2), i.e.:

$$\mu_{OCM}(\overline{\mathbf{v}}) =_{df} \frac{1}{\sqrt{(2\pi)^k \det(\Sigma)}} \exp[-\frac{1}{2}(\overline{\mathbf{v}} - \overline{\mathrm{A}})^T \Sigma^{-1} (\overline{\mathbf{v}} - \overline{\mathrm{A}})] \tag{5}$$

where, $\overline{\mathrm{A}}$ is a k-dimensional mean vector (k is a number of used feature vector), Σ is the corresponding $k \times k$ covariance matrix. □

Ad 2.: The process consists of the five following steps:

1) *Input a histopathology image* – derive a set of image pixels.
2) *Extract corresponding image colour features* – take feature values of vector $\overline{\mathbf{v}}$ for every pixel of the considered image, (denoted in equation (6) below as $\overline{\mathbf{v}}_p =_{df} (v_{p1}, v_{p2}, ..., v_{pk}) \in \mathbf{R}^k$).
3) *Calculate corresponding fuzzy t-equivalence relation* – for every pixel of the input histopathology image define fuzzy t-equivalence relation (ρ), using the *Minkowski distance* (q=2), as follows:

$$\rho(p_i, p_j) =_{\mathrm{df}} 1 - \delta \cdot \left(\left(\sum_{m=1}^{k} \left| v_{p_i m} - v_{p_j m} \right|^q \right)^{\frac{1}{q}} \right), \tag{6}$$

$$\text{where} \quad \delta =_{\mathrm{df}} \frac{1}{\max\{ \left(\sum_{m=1}^{k} \left| v_{p_i m} - v_{p_j m} \right|^q \right)^{\frac{1}{q}} / p_i \neq p_j \}}$$

4) *Calculate fuzzy rough set approximations with respect to the used fuzzy t-equivalence relation and the reference fuzzy set 'Over-expressed Cell Membrane' (OCM)* - calculate the lower and the upper approximation (see equations (1) and (3)).
5) *Output of the proposed recognition process* – decide if a certain pixel represents HER-2/neu over-expressed cell membrane or not, using the following rule:

[1]http://archive.ics.uci.edu/ml/datasets/Image+Segmentation

If $\max\{\left|\mu_{OCM}(\bar{\mathbf{v}}_p)-\mu_{\underline{OCM}}(\bar{\mathbf{v}}_p)\right|,\left|\mu_{\overline{OCM}}(\bar{\mathbf{v}}_p)-\mu_{OCM}(\bar{\mathbf{v}}_p)\right|\}\le\varepsilon$ *Then* (pixel p is recognized as HER-2/neu 'over-expressed') *Else* (pixel p is not recognized as 'over-expressed').

The value of ε is a coefficient of approximation accuracy (given a priori system parameter). □

The proposed cell membrane recognition method requires a priori given system parameter of approximation accuracy ε what is a disadvantage of the method, therefore, we investigated the possibility of determining the parameter value automatically for any histopathology image. We have noticed that it is possible to estimate the approximation accuracy coefficient by examining the intra class homogeneity of pixels that represent over-expressed cell membranes (marked with white colour, in Fig.1:(b), (d)). For example, if we investigate the image entropy value, it will be noticed that there is a 'big jump' in the entropy value, as corresponding pixels are less approximated to the a priori defined fuzzy set OCM.

The above observation gave the possibility to determine the accuracy coefficient for any image (for more details see [17]), which allowed us to develop fully automatic method of HER-2/neu over-expressed cell membrane segmentation.

Below, in Fig 1(a) - (d), some automatically generated segmentation examples are shown.

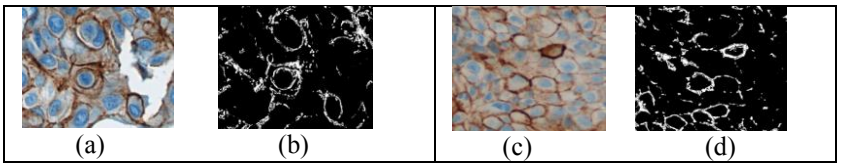


Fig. 1. HER-2/neu image fragments ((a),(c)) and corresponding recognition of the over-expressed cell membranes ((b),(d)).

Fig. 1 presents an example of utilizing the image analysis in selecting the most appropriate cell membranes characterized by an over-expression of HER-2/neu. The segmentation process of the membranes corresponds accurately to the cancer cell membranes indicated by the experienced pathologists which would be considered as 'over-expressed' in histopathological examination.

5 Recognition of cell membrane connectivity

The next phase of our research is the recognition process of membrane connectivity/compactness under the current medical knowledge that HER-2/neu over-expressed cancer cells are defined by both: strong and compact staining, i.e. the cell nuclei is strongly surrounded by HER-2/neu over-expressed cell membrane.

The recognition of the cell membrane connectivity is realized by analysis of the curvature of the membrane, through the inscribing of appropriate geometric 'primi-

tives' in segmented membranes. As a basic geometric object, reflecting the curvature of the membrane, we propose a triangle.
The process of inscribing triangles in cell membranes is done by performing the following steps:

- Post-processing (over the segmented membranes) with classical morphological 'skeleton operator' [14],
- Selection of the longest part of the segmented cell membrane,
- Identification of the points of interest over the selected cell membrane: the set of points of interest consists of points of intersection of the cell membranes and extreme points,
- Generation of the corresponding triangles (see the illustration of the proposed process in Fig. 2(a) - (f) and Fig. 3).

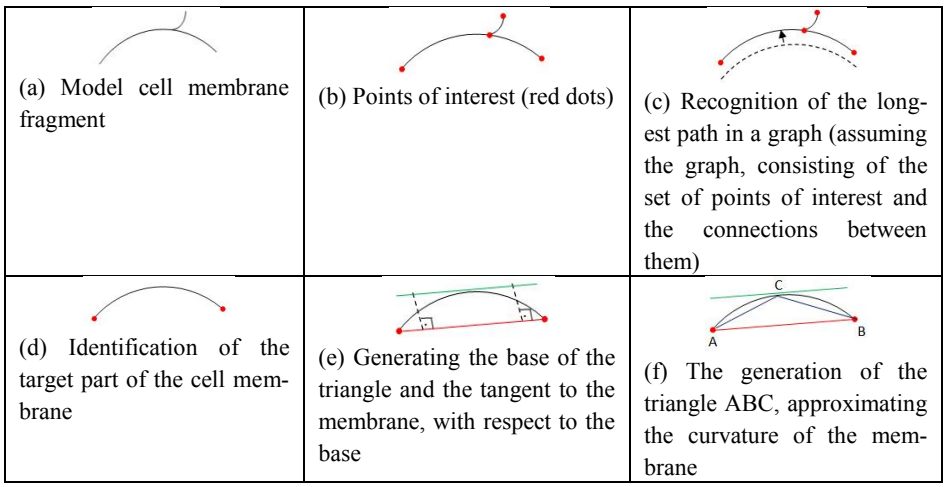


Fig. 2. The process of inscribing triangles for a model cell membrane fragment

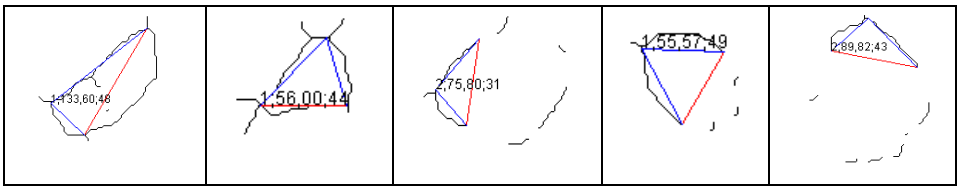


Fig. 3. Some examples of triangles inscribed for cell membranes (both 'open' and 'closed'), segmented from histopathology images. For 'closed' membranes (cancer cells fully surrounded by over-expressed cell membrane), the inscribed triangle is defined as the triangle with maximum surface area.

The so defined (inscribed) triangles are used to determine a set of features that describe the curvature of the membranes and so affect the connectivity/compactness of the over-expressed cell membranes. In our study we assumed that the degree of membrane compactness is changing from line shape to ellipsoidal. The more the cell

membrane is closed (tends to ellipsoidal shape) the more it is likely to be HER2 over-expressed cancer cell. The last assumption is absolutely consistent with the corresponding medical knowledge.

Below, in Fig. 4**,** the basic shape information that can be gain from simple triangle are presented, since they constitute the set of features, mentioned above.

$Feature_1$	α - angle at the vertex of the triangle opposite to the base
$Feature_{2-3}$	β, γ - the base angles
$Feature_{4-6}$	$\sin(\alpha),\sin(\beta),\sin(\gamma)$ – values of the sine function
$Feature_{7-9}$	$\cos(\alpha),\cos(\beta),\cos(\gamma)$ – values of the cosine function
$Feature_{10-12}$	$tg(\alpha),tg(\beta),tg(\gamma)$ – values of the tangent function
$Feature_{13}$	W/H- the ratio between the length of the base of the triangle and the height
$Feature_{14}$	H - the height of the triangle
$Feature_{15}$	W - the length of the base of the triangle
$Feature_{16}$	A - surface area of the triangle

Fig. 4. Set of features gathered from triangle and used for further analysis

6 Method verification

The objective of this study is to analyze the possibility of varying the type of tumor cells grades (HER2 over-expressed, relative to a not over-expressed). Only the features that differentiate these two classes of cells, can be used for further investigation – searching for numeric coefficient which correlates with the value of the FISH test.

We have performed our study on real clinical histopathology data of invasive ductal breast cancer HER2/neu sections scored as 2+. These sections pose the main clinical issue in patients selection for trastuzumab administration. What more, experienced pathologists manually selected two groups of cancer cells - HER2/neu positive (over-expressed) and negative (detailed information on the analyzed data set are given in Table 1). These image data were used for testing of the possibility of varying over-expressed cells, relative to not over-expressed, by corresponding statistical analysis.

6.1 The histopathology data used

In Table 1, the histopathology data used in our research are presented, with the following assumptions:

- We derived data from histopathology preparations, with FISH test examination values $\in$ [0.83, 3.47], which is considered as highly complex for histopathology recognition, if the recognition is based only on digital image information;
- For analysis we divided the considered objects into eight groups with respect to the a priori given FISH test examination values, analysing concentration points and at the same time, if possible, trying to preserve equinumerosity of the groups: $Group_1$ - $Group_8$.

Table 1. The histopathology data used

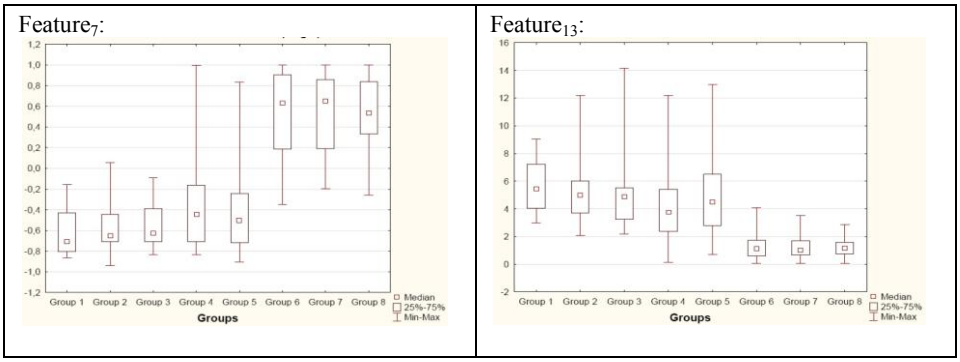
Number of selected HER2/neu cancer cells	714
$Group_1$: Number of selected cancer cells recognized as not HER2/neu over-expressed, selected from images with FISH test examination values ∈ [0.83 - 0.95]	26
$Group_2$: Number of selected cancer cells recognized as not HER2/neu over-expressed, selected from images with FISH test examination values ∈ [1 - 1.22]	186
$Group_3$: Number of selected cancer cells recognized as not HER2/neu over-expressed, selected from images with FISH test examination values ∈ [1.25 - 1.45]	120
$Group_4$: Number of selected cancer cells recognized as not HER2/neu over-expressed, selected from images with FISH test examination values ∈ [1.52 - 1.55]	65
$Group_5$: Number of selected cancer cells recognized as not HER2/neu over-expressed, selected from images with FISH test examination values ∈ [1.6 - 1.89]	98
$Group_6$: Number of selected cancer cells recognized as HER2/neu over-expressed, selected from images with FISH test examination values ∈ [2.01 - 2.49]	77
$Group_7$: Number of selected cancer cells recognized as HER2/neu over-expressed, selected from images with FISH test examination values ∈ [2.51 - 2.89]	90
$Group_8$: Number of selected cancer cells recognized as HER2/neu over-expressed, selected from images with FISH test examination values ∈ [3.06 - 3.47]	52

The corresponding histopathology preparations were performed using the Pathway® HER2 (4B5) Kit.

6.2 Data analysis

The following statistics analysis were performed with the data analysis software: STATISTICA 10 (provided by StatSoft Inc.).

The basis of the proposed data analysis was to provide robust statistics, concerning the graphical visualization of the assumed data groups, concerning each feature through their quartiles, with corresponding box-and-whisker plots. The best achieved results, with respect to the assumed research problem, are presented in Fig. 5. We have assumed the *median* along with the quartiles, as robust statistics replaces the mean by other measures of central tendency and the most common of those is the median [8].



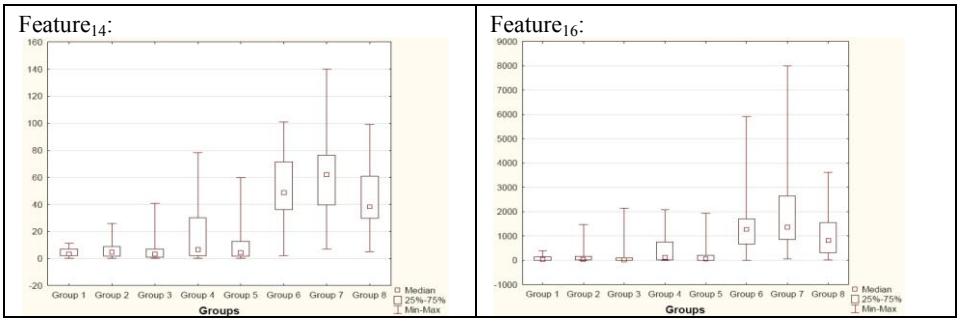


Fig. 5. Visual presentation of data used – best results

As it can be noticed, taking into consideration the visual presentation of the quartiles, the following set of features can be used for recognition of the type of tumor cells with respect to HER-2/neu over-expression:

- $Feature_7$, with the following recognition rule: *If* ($Feature_7(Object) \geq -0.2$ (approximately)) *Then* (Object is considered as HER-2/neu over-expressed) *Else* (Object is considered as not HER-2/neu over-expressed),
- $Feature_{13}$, with the following recognition rule: *If* ($Feature_{13}(Object) \leq 2.3$ (approximately)) *Then* (Object is considered as HER-2/neu over-expressed) *Else* (Object is considered as not HER-2/neu over-expressed),
- $Feature_{14}$, with the following recognition rule: *If* ($Feature_{14}(Object) \geq 20$ (approximately)) *Then* (Object is considered as HER-2/neu over-expressed) *Else* (Object is considered as not HER-2/neu over-expressed).

To handle with the inaccuracy of the threshold value, some fuzzy techniques can be used. For example, the corresponding threshold decision values, can be interpreted as fuzzy numbers.

6.3 Results discussion

The above data visualization, received by corresponding data analysis, provides the solid basis to accept the hypothesis, that it is possible by image processing of HER-2/neu histopathology preparations to recognize fully automatic the HER-2/neu over-expressed and not over-expressed cancer cells. This would give the basis to develop new standard of HER-2/neu histopathology diagnosis. Of course, if we look at the plots in Fig. 5, we will see especially for cell selected from images with FISH test examination value in the range of [1.5 - 2), that there is an overlap (lack of the correct recognition) for about 25% of the data used. This means that in the mention range, cell that are not HER-2/neu over-expressed are recognized as over-expressed. But, still we have the following observations that positively influence presented concept:

- the mentioned problem concerns almost explicitly images with FISH test examination values in the range of [1.5 - 2),
- the mentioned problem concerns rather the smaller part of the considered data (about 25% of the data in Groups that overlap)

- the mentioned problem does not negatively affect the patients in sense of their treatment procedure – for example, for feature 7 it is rather a 'false positive' issue, which mean that patients who does not have to receive necessarily trastuzumab treatment will get it (in medical sense – does no harm to the patient),
- the requirement, that it is better to get 'false positive' than 'false negative', can be easily enhanced by appropriate modification of the threshold values,
- the presented analysis concerns the considered features separately, it is very likely that the combination of the selected above features throughout corresponding machine learning techniques would improve the results.

7 Conclusions and further work

In this research, we propose the basis for a diagnostic procedure, that can be used for breast cancer cell recognition with respect to the HER-2/neu cell membrane over-expression. The procedure assumes the following major steps:

1. automatic segmentation of HER-2/neu over-expressed cancer cells from histopathology images and next,
2. the classification process based on appropriately selected geometric factors, that affect the compactness of the segmented cell membrane structures.

Using real clinical data, we were able to verify the proposed approach.

The novelty of this research lies on introducing geometric features, assuming segmentation process of HER2/neu over-expressed cell membrane for automatic recognition of HER-2/neu over-expressed cancer cells. Nevertheless, the data analysis process was performed on manually selected cancer cells, as the main research problem, concerns the introduction of geometric coefficients that distinguish over-expressed cell with respect to not over-expressed. Thus, to complete the process of recognition, as a further work, we will also automate the process of cell segmentation. We have done recently some research, using points of interest (see section 5, Fig. 2 (b)) as a nodes of corresponding relative neighbourhood graphs (the cycles of so defined graphs very well segment individual cancer cells) [18]. Thus, our current research is concentrated on the developing of an automatic computer system which will take as an input histopathology image itself and as an output gives accurate diagnostic decision - apply trastuzumab treatment or not.

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