Classification of HER2 Breast Cancer with Ensemble of Fuzzy Decision Trees

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ABSTRACT: *In this paper a decision making support system dedicated to histopathology image recognition is considered. The proposed system supports the classification process of histopathology preparations through microscopy image information analysis, with respect to the degree of HER2/neu receptor overexpression. The system combines the output information of ensemble of classifiers – fuzzy decision trees. We propose an aggregation process of the corresponding classifiers results with fuzzy Sugeno integral. What more, we introduce new image fragmentation concept, in order to improve the considered classification process. The proposed approach was tested over real clinical data of HER2 breast cancer histopathology images.*

Keywords: Fuzzy Decision Trees, Image Fragmentation, Fuzzy Sugeno Integral, Histopathology Image Processing, HER2 Breast Cancer

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1. Introduction

Breast cancer is the regarded as the most frequently occurring malignant tumour and comprises approximately 14% of neoplastic diseases. It is estimated that in 2013 more than 230 000 new cases will be diagnosed in the United States and the mortality rate will reach 40 000 cases [19]. Breast cancer is the major cause of death in women 20 to 59 years of age [19]. Breast cancers are typically divided into subtypes based on the expressions of estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2/neu) [7]. These proteins are important predictive factors in breast cancer therapy. Approximately in up to 30% of breast cancers overexpress HER2/neu what is associated with increased tumour growth rate, higher malignancy grade, metastatic spread and high risk of recurrence of such tumours due to poor response to conventional therapy [15, 18]. Therefore, patients with overexpression of HER2/neu have poor prognosis [15, 18]. Several studies have shown that trastuzumab (humanized monoclonal anti-HER2/neu antibody; (Herceptin, Genentech, CA) treatment reduces effectively the risk of recurrence and mortality rates in patients with early, as well as advanced stage breast cancers [25]. In the last years, a routine diagnostic procedure based on immunohistochemical (IHC) and fluorescent in situ hybridization techniques was established to identify potential responders to anti-HER2 based trastuzumab therapy. Both these methods allow to for examination of protein overexpression or gene amplification, respectively [25, 10, 2]. According to the approved guidelines, the IHC evaluation of HER2 expression is based on a visual semi-quantitative examination of membranous cell staining and its intensity in paraffin-embedded breast cancer sections with a light microscope and utilizes a categorical classification system: no staining (0), weak

(1+), moderate (2+), and strong staining and compactness/completeness (+3). Using such approach, cases scored as 3+ are recommended for trastuzumab therapy, whereas 2+ cases are subject to further testing utilizing the costly FISH examination [6, 25, 2]. As the IHC assessment may be susceptible to many factors causing discrepant results (experience of pathologist, staining procedures, amount of tumour material and its heterogeneity in the samples) [22, 23, 24].

Because of the complex and costly procedures FISH examination procedure, it is extremely needed to introduce less complicated and expensive diagnostic process for correct recognition of the corresponding HER-2/neu classes. Recently, solving the above problem, digital computer systems – in particular image analysis systems, have been proposed. Some review and comparison of subjective and digital image analyses can be found in [8, 17]. There are different image processing and analysis concepts that are used in the HER-2/neu problem – based on feature extraction and analysis, data clustering or other techniques [11, 1, 5, 14, 9]. In general, the HER2 classification process can be transformed, in terms of digital image processing, into a problem of cell membrane staining and cell membrane connectivity/completeness recognition. If a considered cell membrane is strong stained and enough compact, then it is very likely to be a 3+ HER-2/neu class. The main problem here is that these cell membrane characteristics are rather fuzzy terms and more, the actual state of HER-2/neu image recognition is based on vague and uncertain information.

Taking the above into consideration, in this paper we propose to use the Fuzzy Sugeno Integral, as an aggregation operator of ensemble of fuzzy decision trees, in order to classify the corresponding HER-2/neu classes. We used three different fuzzy decision trees, build over different image characteristics: colour values, structural factors and texture information. The designed fuzzy trees provided classification information, which we propose to aggregate by using the fuzzy Sugeno integral and so we generate the final medical decision support information.

The article is organized as follows: in section 2 some theoretical background, considering the methods used in the proposed research are described; in section 3 and 4, the suggested classification process is introduced; in section 5 some experiments and results are discussed; in section 6 a new histopathology image segmentation method is proposed in purpose to improve the introduced classification method and finally conclusions are presented.

2. Theoretical Background

In this section, the preliminaries of fuzzy sets [26], fuzzy Sugeno integral [20] and fuzzy decision trees [21] are described.

2.1 Fuzzy Sets

Let $X =_{df.} \{x_1, x_2, \ldots, x_n\} \subseteq \boldsymbol{R}$ be some finite set of elements (domain), then we shall call '*A*' the fuzzy subset of *X*, if and only if: $A =_{df.} \{ (x, \mu_A(x)) \mid x \in X \}$, where μ_A is a function that maps *X* onto the real unit interval [0, 1], i.e. $\mu_A : X \to [0, 1]$. The function μ_A is also known as the *membership function* of the fuzzy set *A*, as its values represents the grade of membership of the elements of *X* to the fuzzy set *A*. Here the idea is that we can use membership functions, as characteristic functions (any crisp set can be defined by its characteristic function) for fuzzy, imprecisely described sets. Let *A* and *B* be two fuzzy subsets of *X*. Then the basic set operations: *union* and *intersection* of *A* and *B*, are defined as follows: $\mu_{A \cup B}(x) =_{df.} \max\{ \mu_A(x), \mu_B(x) \}$, $\mu_{A \cap B}(x) =_{df.} \min\{ \mu_A(x), \mu_B(x) \}$. Additionally, to combine fuzzy values, various *t*- and *s*- norms [3] can be used.

2.2 Fuzzy Sugeno Integral

Sugeno introduced the theory of fuzzy measures and fuzzy integrals [20]. The fuzzy integral is based on the concept of fuzzy measure, which is a generalization of probability measure. The concept is effective in combining information in certain applications.

Consider a finite set $X = \{x_1, x_2, \ldots, x_n\}$ of sources of information. A *fuzzy measure g* is a real valued function $g : 2^X \to [0, 1]$, satisfying the following properties:

i. $g(\varnothing) = 0$; $g(X) = 1$;

ii. $g(A) \leq g(B)$, if $A \subseteq B$; $A, B \subseteq X$.

For a fuzzy measure *g*, let $g^i = g(\{x_i\})$. The mapping $x_i \to g^i$ is called a fuzzy density function. The fuzzy density value is interpreted (often subjective, supplied by experts) as the importance of the i^{th} information source in determining an answer to the particular problem.

A fuzzy measure is a Sugeno measure (or g_λ – fuzzy measure) if it satisfies the following additional condition for some $\lambda > -1$:

For all $A, B \subseteq X, A \cap B = \varnothing$:

$$g_\lambda(A \cup B) = g_\lambda(A) + g_\lambda(B) + \lambda g_\lambda(A)\, g_\lambda(B) \tag{1}$$

The λ value can be calculated regarding to the condition $g(X) = 1$, using the following equation:

$$\lambda + 1 = \prod_{i=1}^{n} (1 + \lambda g^i) \tag{2}$$

The *Fuzzy Integral* (in the literature also called as *Sugeno Integral*) can be perceived as an aggregation operator. Let X be a set of information sources (e.g. features, sensors, classifiers). Let h: $X \rightarrow [0, 1]$, $h(x)$ denotes the confidence value delivered by element x (e.g., the class membership of data determined by a specific classifier). The fuzzy integral of h over A (A is a subset of X) with respect to the fuzzy measure g can be calculated as follows:

$$\int_A h(x) \circ g = \sup_{\alpha \in [0,\,1]} \{\alpha \wedge g\,(A \cap H_\alpha)\} \tag{3}$$

where $H_\alpha =_{df.} \{\, x \mid h(x) \geq \alpha \,\}$.

In a case of finite sets, suppose $h\ (x_1) \geq h\,(x_2) \geq \ldots \geq h\,(x_n)$ (if it is not true, then we can reorder the set of information sources X, so that this relation is true). Then the Sugeno fuzzy integral can be defined as:

$$S_g(h) = {}_{df} \bigvee_{i=1}^{n} \{h\,(x_i) \wedge g\,(H_i)\} \tag{4}$$

with $H_i = \{\, x_1, x_2, \ldots, x_i \}$.

The fuzzy Sugeno integral has been applied successfully in many different research areas as also in the field of bioinformatics [4]. This operator is very powerful in resolving multicriteria decision making problems, where the information that is combined is determined by experts.

2.3 Fuzzy Decision Tree (in short FDT) – FuzzyID3 Algorithm

Knowledge acquisition from data is very important in knowledge engineering. There are some knowledge acquisition methods, one of the most popular is ID3 algorithm proposed by J.R. Quinlan [13, 12], which makes a decision tree for classification from symbolic data. The decision tree consists of nodes for testing attributes, edges for branching by values of symbols and leaves for deciding class names to be classified. ID3 algorithm applies to a set of data and generates a decision tree which minimizes the expected value of the number of tests for classifying the data, basing on concepts of the information theory.

For numerical data, its adjusted algorithms have been proposed, which partitioned a numerical range of attribute into intervals. To make a decision tree flexible and more suitable regarding to practical problems, some algorithms were proposed to fuzzify the interval.

In our research, we used a fuzzy extension of the classical ID3 algorithm, proposed in [21]. The proposed algorithm, called fuzzy ID3 algorithm, is extended to apply to a fuzzy set of data (data with membership grades) and generates a fuzzy decision tree using fuzzy sets defined by a user for all attributes. A fuzzy decision tree consists of nodes for testing attributes, edges for branching by test values of fuzzy sets defined by a user and leaves for deciding class names with certainties. The proposed algorithm is very similar to ID3, except ID3 selects the test attribute based on the information gain which is computed by the probability of ordinary data but fuzzy ID3 by the probability of membership values for data.

The formal specification of the fuzzy ID3 algorithm is omitted here. Comprehensive description of the algorithm and corresponding calculation examples are provided in [21].

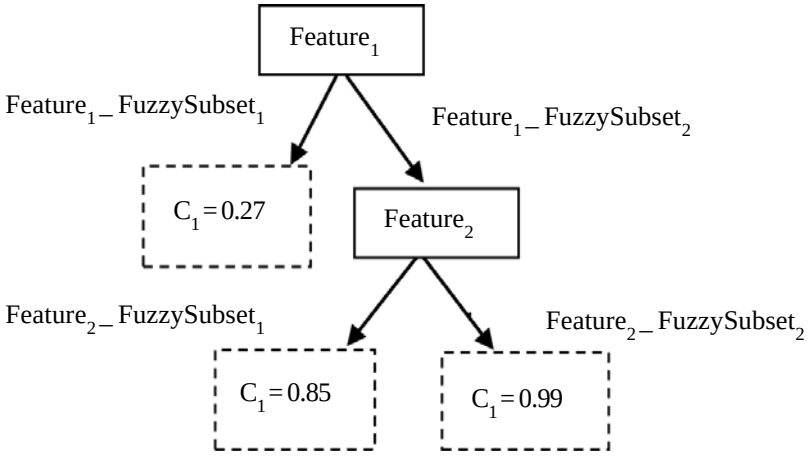
2.4 Fuzzy Decision Trees – reasoning

In Literature, different methods for fuzzy decision trees reasoning can be found. In the approach used [21], we must start reasoning from the top node (Root) of the fuzzy decision tree. Repeat testing the attribute at the node, branching an edge by its

value of the membership function (μ) and multiplying these values until the leaf node is reached. After that we multiply the result with the proportions of the classes in the leaf node and get the certainties of the classes at this leaf node. Repeat this action until all the leaf nodes are reached and all the certainties are calculated. Sum up the certainties of the each class respectively and choose the class with highest certainty.

For better explanation, let consider the following fuzzy decision tree (assuming two decision classes).

Example 1:



For simplicity, let denote $\mu_{i,j}$ to be the membership function value: $\mu_{FuzzySubset}(x)$, $x \in Feature_j$. Let also assume: $\mu_{1,1} = 0.25$, $\mu_{2,1} = 0.75$, $\mu_{1,2} = 0.2$, $\mu_{2,2} = 0.8$. Then, according to the reasoning algorithm used in our research, we have the following decision class probabilities :

$$C_1 = 0.25 * 0.27 + 0.75 * 0.2 * 0.85 + 0.75 * 0.8 * 0.99 = 0.789 \quad (5)$$

$$C_2 = 0.25 * 0.73 + 0.75 * 0.2 * 0.15 + 0.75 * 0.8 * 0.01 = 0.211 \quad (6)$$

(it can be noticed, that $C_1 + C_2 = 1$).

3. Method specification

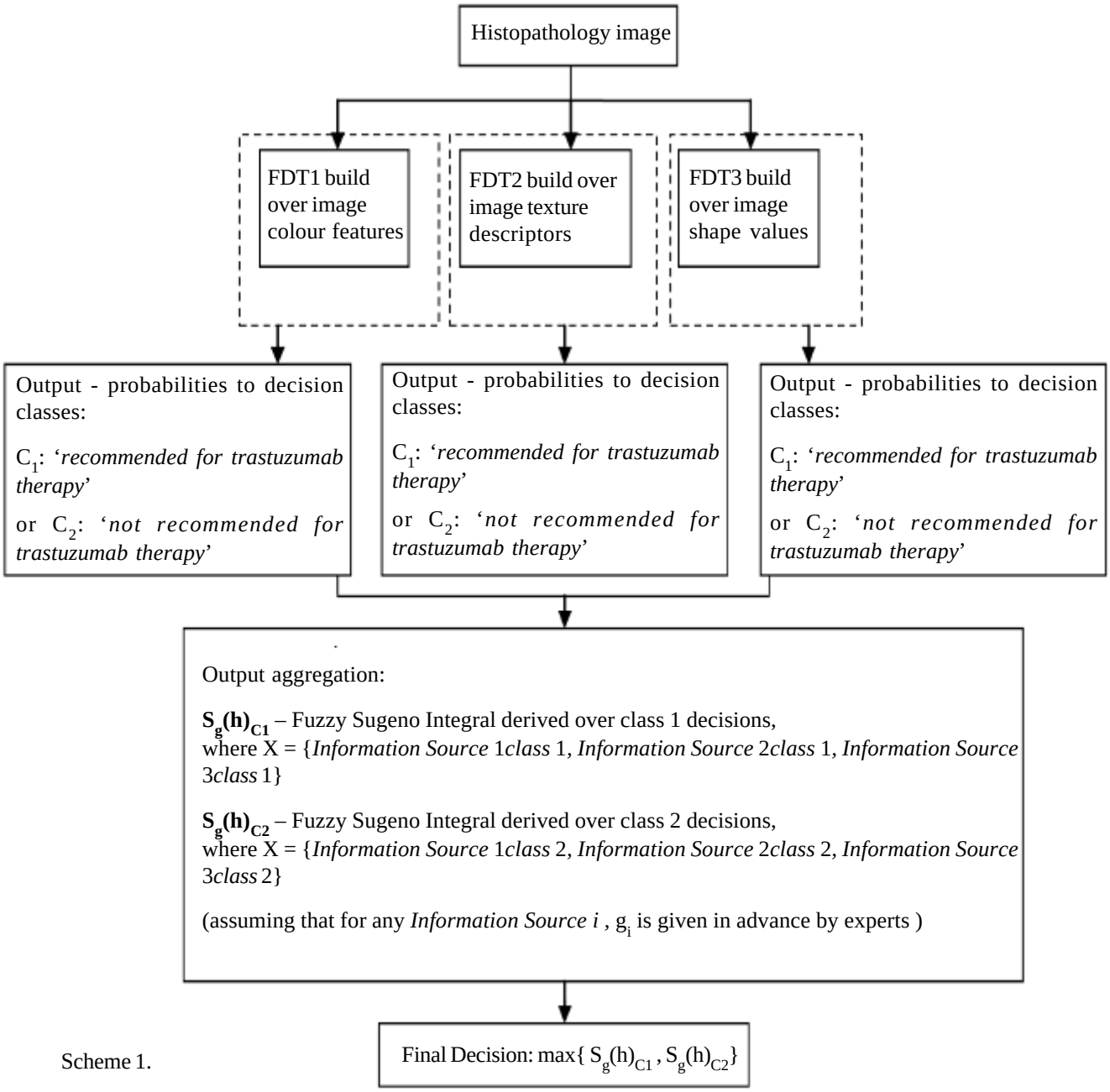
The clue of the classification method presented in this paper, is our proposition to combine ensemble of fuzzy decision trees with the fuzzy Sugeno Integral, as it is prove to be powerful aggregation operator.

What more, as fuzzy decision trees are being combined, their outputs can be naturally interpreted as the confidence values of each classifier (i.e. values of the *h* function – see section 2). To build the complete integral, also the values of the fuzzy measure function (the *g* function – see section 2) should be introduced – in our approach, we assumed that medical experts give these values. The basics of the proposed theoretical conception is presented on the *scheme* 1, below.

For better explanation of the above scheme, let consider the next example.

Example 2: Let suppose that we have the above ensemble of fuzzy decision trees and for a given histopathology image, the following class decision probabilities are calculated (according to the applied fuzzy decision tree reasoning method – see section 2):

Information Source 1 (FDT1)	g$_1$ = 0.3	C$_1$ = 0.56, C$_2$ = 0.44
Information Source 2 (FDT2)	g$_2$ = 0.4	C$_1$ = 0.33, C$_2$ = 0.67
Information Source 3 (FDT3)	g$_3$ = 0.1	C$_1$ = 0.28, C$_2$ = 0.72



Scheme 1.

$X = \{FDT_1, FDT_2, FDT_3\}$ **A** $(A \subseteq X)$	Sugeno fuzzy measure
$\{FDT_1\}$	$g_\lambda(\{FDT_1\}) = 0.3$
$\{FDT_2\}$	$g_\lambda(\{FDT2\}) = 0.4$
$\{FDT_3\}$	$g_\lambda(\{FDT3\}) = 0.1$
$\{FDT_1, FDT_2\}$	$g_\lambda(\{FDT_1, FDT_2\}) = g_\lambda(\{FDT_1\}) + g_\lambda(\{FDT_2\}) + \lambda g_\lambda(\{FDT_1\}) g_\lambda(\{FDT_2\}) = 0.82$
$\{FDT_1, FDT_3\}$	$g_\lambda(\{FDT_1, FDT_3\}) = g_\lambda(\{FDT_1\}) + g_\lambda(\{FDT_3\}) + \lambda g_\lambda(\{FDT_1\}) g_\lambda(\{FDT_3\}) = 0.43$
$\{FDT_2, FDT_3\}$	$g_\lambda(\{FDT_2, FDT_3\}) = g_\lambda(\{FDT_2\}) + g_\lambda(\{FDT_3\}) + \lambda g_\lambda(\{FDT_2\}) g_\lambda(\{FDT_3\}) = 0.54$
$\{FDT_1, FDT_2, FDT_3\}$	$g_\lambda(\{FDT_1, FDT_2, FDT_3\}) = g_\lambda(X) = 1$

and as we assumed:

$$h(FDT_i)_{C1} = \begin{cases} 0.56 & if\ i = 1 \\ 0.33 & if\ i = 2 \\ 0.28 & if\ i = 3 \end{cases} \quad \text{and} \quad h(FDT_i)_{C2} = \begin{cases} 0.44 & if\ i = 1 \\ 0.67 & if\ i = 2 \\ 0.72 & if\ i = 3 \end{cases} \quad \text{then we have:}$$

$S_g(h)_{C1} = \max\{\min\{h(FDT_1), g_\lambda(\{FDT_1\})\}, \min\{h(FDT_2), g_\lambda(\{FDT_1, FDT_2\})\}, \min\{h(FDT_3), g_\lambda(\{FDT_1, FDT_2, FDT_3\})\}\} = \max\{\min\{0.56, 0.3\}, \min\{0.33, 0.82\}, \min\{0.28, 1\}\} = \max\{0.3, 0.33, 0.28\} = 0.33$

$S_g(h)_{C2} = \max\{\min\{h(FDT_3), g_\lambda(\{FDT_3\})\}, \min\{h(FDT_2), g_\lambda(\{FDT_2, FDT_3\})\}, \min\{h(FDT_1), g_\lambda(\{FDT_1, FDT_2, FDT_3\})\}\} = \max\{\min\{0.72, 0.1\}, \min\{0.67, 0.54\}, \min\{0.44, 1\}\} = \max\{0.1, 0.54, 0.44\} = 0.54$

as $\max\{S_g(h)_{C1}, S_g(h)_{C2}\} = S_g(h)_{C2} = 0.54$, we can predict that decision C2 should be taken.

In practice, if *C*2 decision class is interpreted as above (i.e.'*not recommended for trastuzumab therapy*'), then the patient related to the corresponding histopathology preparation, should not be recommended for trastuzumab therapy.

Nevertheless, the major problem of the above concept, is the fuzzification process of the input set of features. In our system, we propose the following solution: in general (considering many practical applications), it is sufficient to introduce three basic linguistic variables: '*small*', '*medium*', '*large*'. If there are enough (in a statistical way) available input values for each feature and also assuming Gaussian data distribution, we can define the fuzzy set '*medium*' (over each feature) by interpreting the corresponding Gaussian probability density function as a membership function:

$$\mu_{medium}(x) = {}_{df}\, e^{\frac{-(x-x_0)^2}{2\sigma^2}}, \text{ where } x_0 \text{ is the expected value and } \sigma \text{ is the standard deviation.} \qquad (7)$$

Next, using the μ_{medium} medium membership function, we can define μ_{small}, μ_{large} as well:

$$\mu_{small}(x) = {}_{df} \begin{cases} 1 - e^{\frac{-(x-x_0)^2}{2\sigma^2}} & : x < x_0 \\ 0 : x^3 \geq x_0 \end{cases}, \quad \mu_{large}(x) = {}_{df} \begin{cases} 0 : x \leq x_0 \\ 1 - e^{\frac{-(x-x_0)^2}{2\sigma^2}} & : x \geq x_0 \end{cases} \qquad (8)$$

4. System specification

In purpose to build our system, we have done the following steps:

• Define common input for the system – in the presented system, the input is a set of *histopathology image fragments* (100×100 pixels), derived from histopathological preparation. What more, only the fragments with high entropy value have been considered (the *median* was assumed as a '*cut-off value*'). The last gives the potentiality to avoid any irrelevant image region, such as: background, unimportant cells and so on (see figure 1, below).

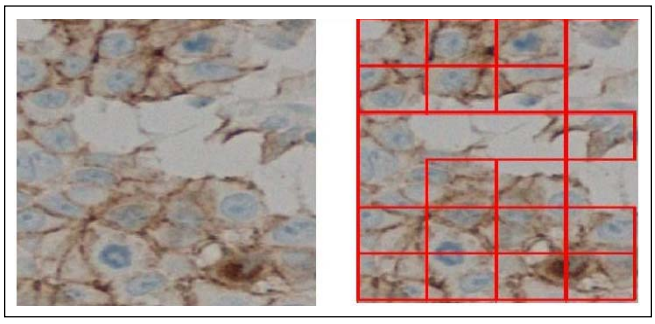


Figure 1. Sample histopathology image (from left) and the corresponding fragmentation

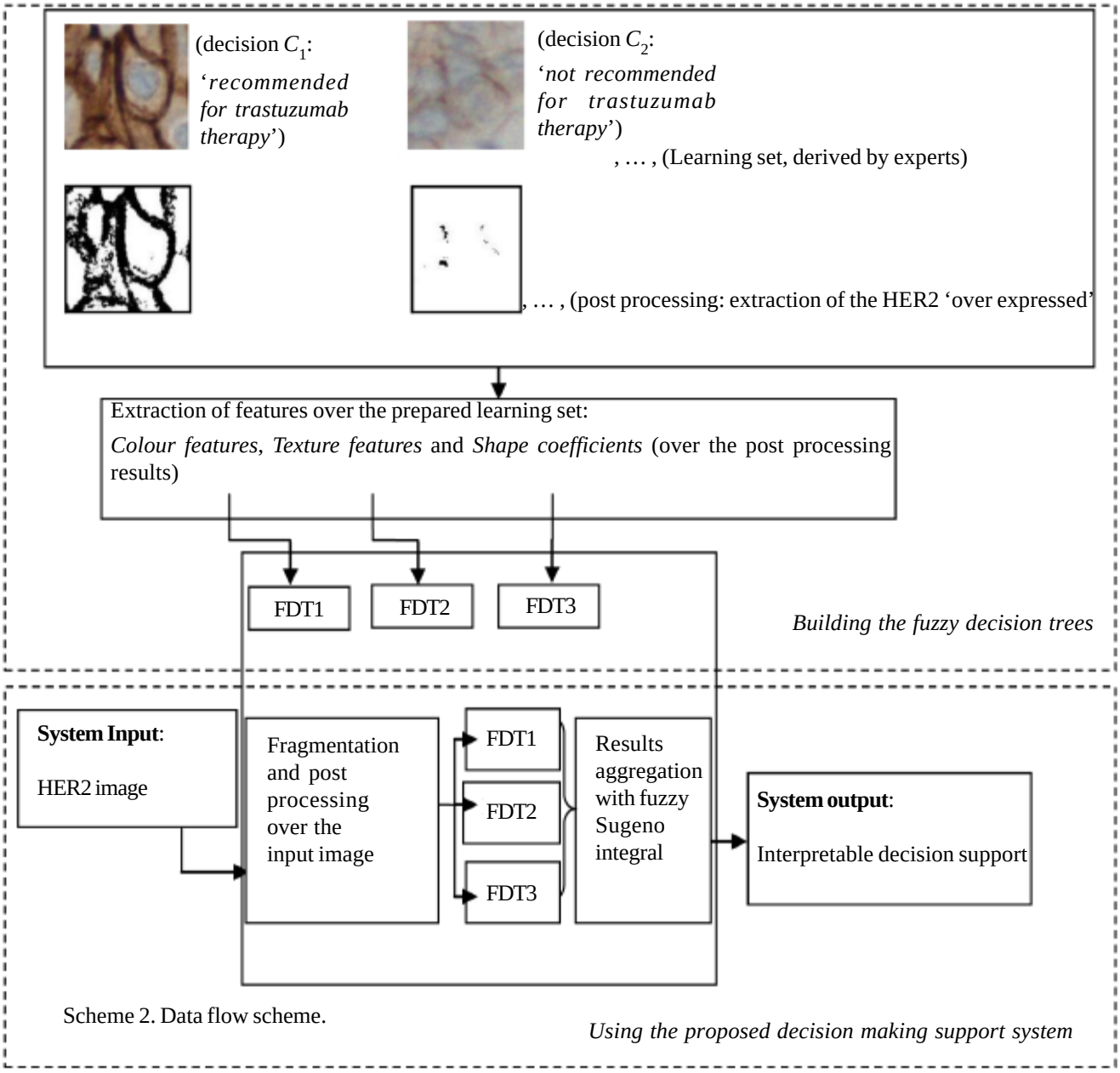
• Define *learning set* – a set of histopathology image fragments, chosen by experts (including decision value).

• Identify the corresponding *feature set* for any fragment – we have used standard features such as: statistical colour values, derived over the HSV and RGB colour models, standard texture descriptors and shape characteristics. The shape coefficients were taken after post processing, which was designed to extract the 'over expressed' cancer cell membrane (detailed description of the proposed post processing is omitted in this paper).

• Define the corresponding fuzzy decision trees, over the developed learning set with respect to the introduced set of features.

• Define common output of the system, interpretable as a decision making support.

For better understanding of the proposed system, within the corresponding data flow, see scheme 2 below.



Scheme 2. Data flow scheme.

5. Experiments and results

Below (figure 2), some system action results and interpretation are shown and the system output interpretation is given (having regard to the limitations of the size of the histopathology images, only chosen HER2 image fragments are considered).

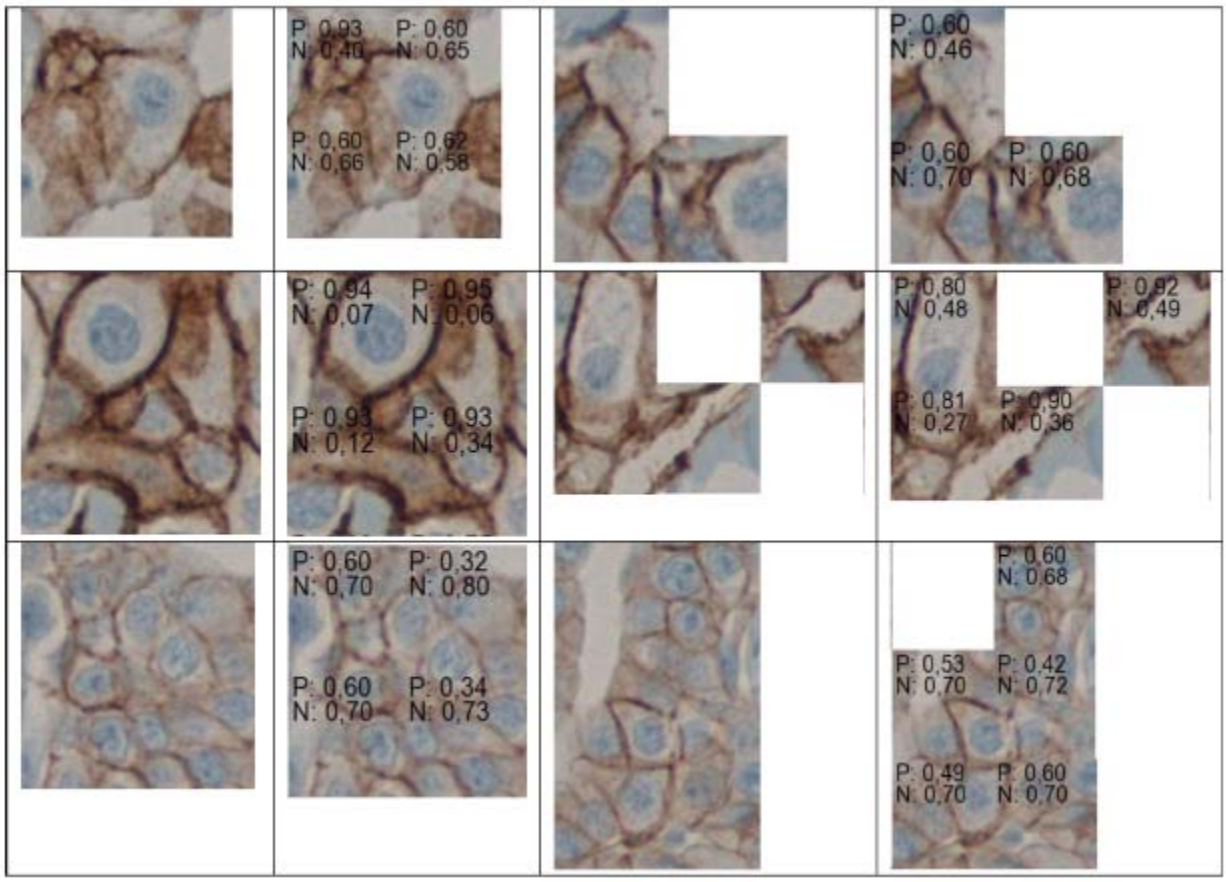


Figure 2. Sample fragments, derived from HER2 images (where: $P \equiv S_g(h)_{C1}$ and $N \equiv S_g(h)_{C2}$). The '*P*' value is considered as positive, in sense of trastuzumab treatment while '*N*', as negative.

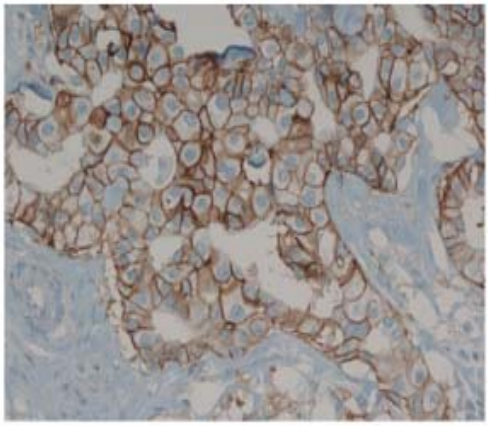
As it is assumed, a processed HER2 fragment '*suggests*' trastuzumab therapy only if $S_g(h)_{C1} \geq S_g(h)_{C1}$. The final decision can be obtained by a quantitative analysis, i.e. if for input HER2 image, fragments that '*suggest*' trastuzumab therapy are more than fragments that '*do not suggest*' it, then the system can recommend trastuzumab therapy (see some decision making support examples, on figure 3 below). The last statement is also the clue of the proposed decision making support process.

We have tested our system under real histopathology data - above 60 patients ('*fairly*' divided: *C*1 class - 31, *C*2 class - 29) with a priori given FISH examination results for the corresponding histopathology preparations.

Due to the complicated acquisition histopathology process and what more, having the limitation that the Fish test examination cannot be processed on the same biopsy section as the section designated for HER2 receptor staining, the considered data set was carefully selected by experts.

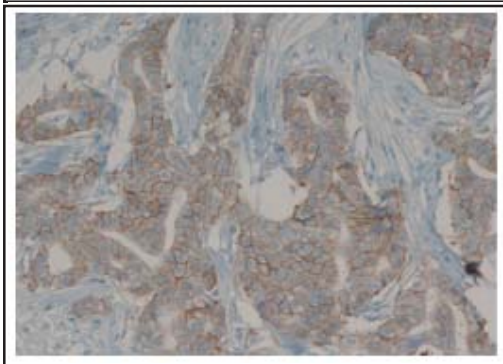
The system accuracy was estimated using the 2-fold cross-validation method, preserving the distinction between the learning set and the test set as: 40% to 60%. It should be noticed, that in our experiments, we assumed large training and testing sets and what more, we performed the learning process on the smallest one.

Upon the above assumptions, we have achieved 83% overall system accuracy.

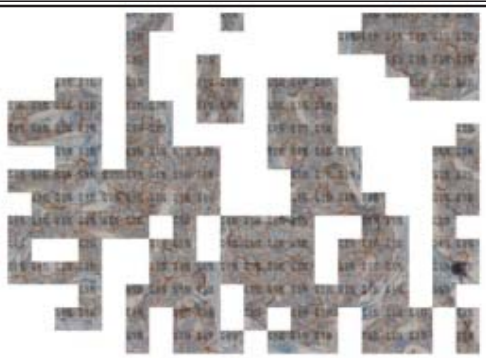


HER2 image 1 (FISH examination result = 2.24; therefore, recommended for trastuzumab therapy)

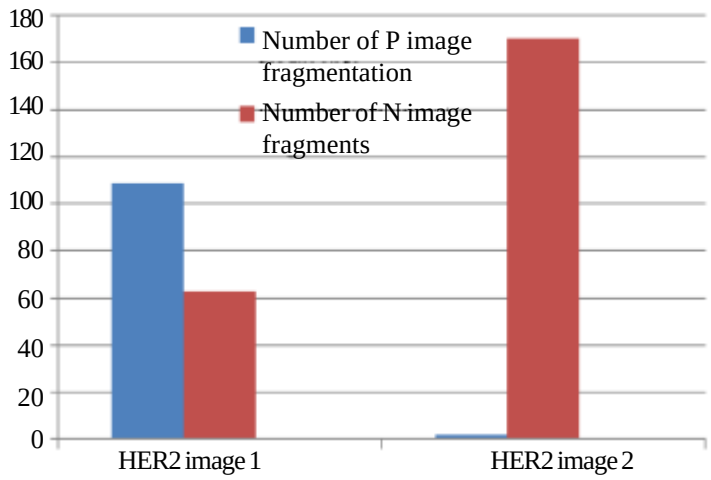
HER2 image 1 fragmentation



HER2 image 2
(FISH examination result = 1.78; therefore, not recommended for trastuzumab therapy)

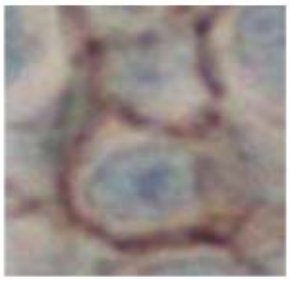


HER2 image 2 fragmentation

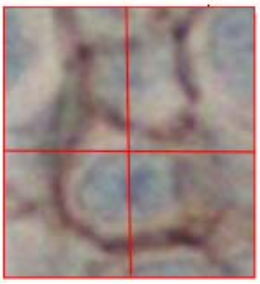


Decisions suggested by the system:
Based on HER2 image 1 – recommended for trastuzumab therapy,
Based on HER2 image 2 – not recommended for trastuzumab therapy.

Figure 3. Decision making support for two sample HER2 images



Sample HER2 image fragment of two cancer cells



Possible partition with simple grid (the considered cancer cells are splitted into four grid elements)

Figure 4. Grid Element

6. Image fragmentation improvement proposition

Under the proposed image partition process (with simple grid, see figure 1), there occurs the problem of inaccurate cell fragmentation – it may occur that single cell is not located entirely in a grid element but is divided into different grid elements (see figure 4, below).

As a solution of the above problem, we propose the following image fragmentation concept:

a) *Find all edges of the image containing cell membranes,*

b) *Locate set of control points,*

c) *Use the located control points to generate set of circle objects,*

d) *Assume that any object 'gives a vote'*(*natural number:* + 1) *for all of its points,*

e) *Accumulate the votes in accumulator matrix and find local maxima values,*

f) *Use the maxima values to identify possible central point of a considered cancer cell.*

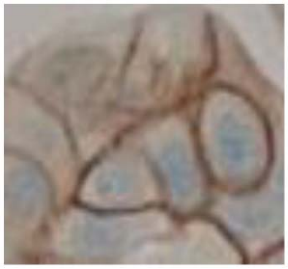
Below, we explain the proposed process in more details:

a) Find all edges of the image containing cell membranes

It can be done, by classic edge detectors.

b) Locate set of control points

Control points **P**, are all '*cross*' or '*end points*' of the edged image generated in step a) A *cross point* is a point common for connected set of edges. *End points* are points located at the both ends of any edge. In order to locate these points, a



HER2 image fragment

Membrane detection

Skeletisation

Control points detection

Figure 5. Points Detection

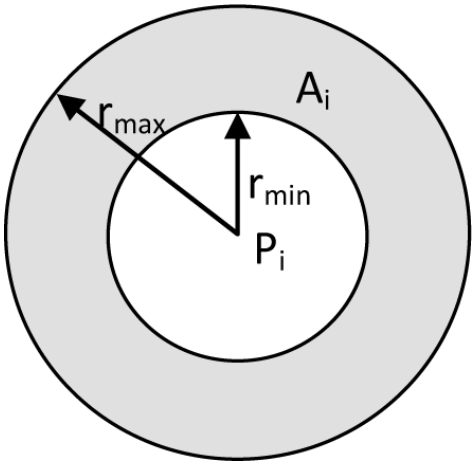
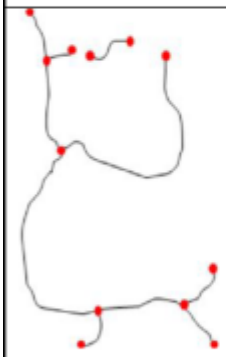


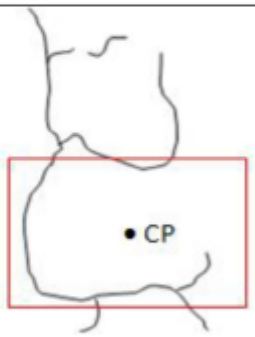
Figure 6. Circle object

(1) Sample HER2 image fragment

(2) Sample edge detection and skeletisation

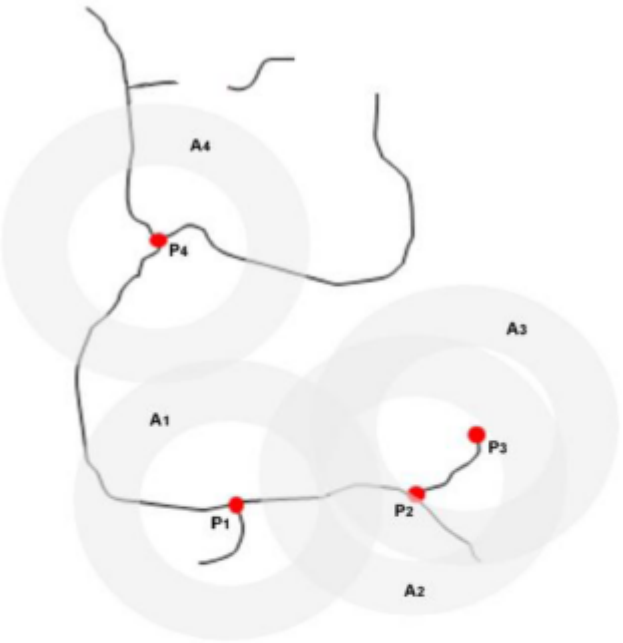


(3) Control points detection



(5) Cell central point (CP) detection as average of all pixels $\in \bigcap_i A_i$, i = 1,...,3.

Next, CP is used to generate grid element and so, to segment system inputs



(4) Coverage by circle objects (for simplicity, considering only control points: $P_1 - P_4$). As it can be noticed, there are two intersect areas: $A_1 \cap A_2 \cap A_3$ and $A_1 \cap A_4$. The set of pixels of the first area will get the maximum votes values.

Scheme 3.

morphological operation skeletisation should be proceeded first over the edged image. This was done by classical morphological '*skeleton operator*' [16].

The identification of control points is realized by analysing all separate edge structures and their surrounding areas. A cross point is located only if branching are detected for a considered pixel. The identification of the end points is rather trivial. See some example control points on figure 5, below.

c) Use the located control points to generate set of circle objects

We assumed as a *circle object*, the following structure (see figure 6, below),

where P_i is the i^{th} control point and A_i is the area closed by two circles: with centre point P_i and radius r_{min} and r_{max} respectively (considered as system parameters).

d) Assume that any object 'gives a vote'(+ 1) for all of its points

Any circle object gives vote (+1) for all points of its A_i area. Therefore, if different circle objects intersects, i.e. $A_i \cap A_j \neq \emptyset$, $i \neq j$ this increase the number of votes in the shared area.

e) Accumulate the votes in accumulator matrix and find local maxima values

An accumulator matrix can be used for summing all given votes and next, local maxima can be found. The local maxima values identify image sub-regions which contain possible cancer cells.

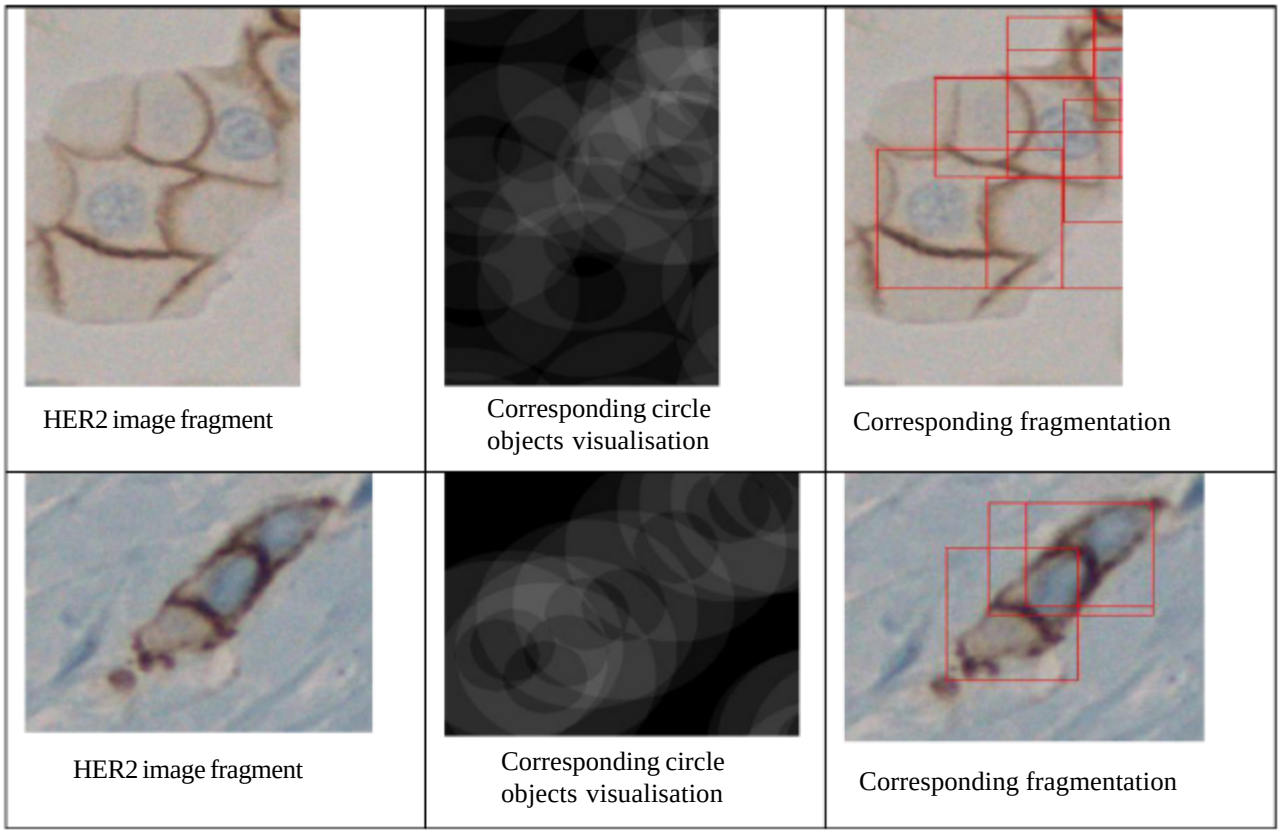


Figure 7. Image Fragmentation

f) Use the maxima values to identify possible central point of a considered cancer cell

Use the derived maxima values to identify target cells central points (in short: CP) and segment the image with respect to the detected central points. If in a certain region, not a single, but a set of point are detected as possible cell central points, then take the average point.

For better explanation of the proposed image fragmentation concept, see the scheme below.

Below (figure 7), some sample image fragmentation, generated on the basis of the proposed concept, are shown.

The proposed HER2 image fragmentation approach increased the number of entire cells in the elements of the grid used and so, contributed to improve the final classification results - we achieved 91,6% of classification accuracy, including:

– true positive: 26 (correctly recommended for trastuzumab therapy),

– true negative: 29 (correctly not recommended for trastuzumab therapy),

– false positive: 0 (incorrectly recommended for trastuzumab therapy),

– false negative 5 (incorrectly not recommended for trastuzumab therapy).

7. Conclusions

In this paper, a quantitative histopathology image analysis method is proposed, which takes into consideration the features normally taken into account during routine histopathological assessment of HER2 IHC slides by pathologists. Although, the sample size was relatively small, the high accuracy of the presented method in predicting HER2 expression status may become a valuable tool for standardizing HER2 assessment and could assist patients diagnosis treatment management in the future. In our work, we use the fuzzy Sugeo integral as it can be interpreted in different ways, regarding to the analyzed problem. This is due to the interpretation of the *h* function (see section 2) and the fuzzy density function. Therefore, the aggregator can be evolved and what more, fits in medical decision making domain, as takes into consideration the subjectivity of medical experts opinion, by the possibility of defining appropriate fuzzy density function. The proposed concept of image processing may be found applicable not only for HER2 assessment in whole tissue sections of breast cancer, but may be also potentially introduced to for testing for specimens with little diagnostic material (such as core needle biopsies). Moreover, the growing amount of newly identified membrane cell antigens of breast cancer cells of potentially therapeutic significance, may require the estimation of new therapy cut-off points for the recently developed anticancer agents.

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References

[1] Avoni, M. (2008). Image analysis methods for determining HER2neu Status of breast cancers, BME MSc., DTU – Technical University of Denmark, Copenhagen, Denmark, in collaboration with Visiopharm A/S, Hørsholm, MATLAB User Conference, Denmark.

[2] Bartlett, J. M., Starczynski, J., Atkey, N., Kay, E., O'Grady, A., Gandy, M., Ibrahim, M., Jasani, B., Ellis, I. O., Pinder, S. E., Walker R. A. (2011). HER2 testing in the UK: recommendations for breast and gastric in-situ hybridisation methods. *Journal Clin Pathol*, 64 (8) 649-653.

[3] Bronstein, I. N., Semendjajew, K. A., Musiol, G., Mühlig, H. (2001). Taschenbuch der Mathematik, Verlag Harri Deutsch, p.1258.

[4] Dong, X., Keller, M. J., Popescu, M., Bondugula, R. (2008). Applications of fuzzy logic in bioinformatics. *Advances in Bioinformatics and Computational Biology*.

[5] Doyle, S., Agner, Sh., Madabhushi, A., Feldman, M., Tomaszewski, J. (2008). Automated grading of breast cancer histopathology using spectral clustering with textural and architectural image features. *IEEE International Symposium on Biomedical Imaging*, Paris, France.

[6] Gavrielides, M. A., Masmoudi, H., Petrick, N., Myers, K. J., Hewitt, S. M. (2008). Automated evaluation of HER-2/neu immunohistochemical expression in breast cancer using digital microscopy, 5th IEEE International Symposium on Biomedical Imaging From Nano to Macro, p. 808-811.

[7] Goldhirsch, A, Wood, W. C., Coates, A. S., Gelber, R. D., Thürlimann, B., Senn, H. J. (2011). Panel members. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer. *Ann Oncol*. Aug. 22 (8) 1736-47.

[8] Gurcan, N. M., Laura, E., Boucheron, A. C., Madabhushi, A., Rajpoot, N. M., Bulent, Y. (2009). Histopathological Image Analysis - A Review. *IEEE reviews in biomedical engeneering*, V. 2.

[9] Joshi. A. S., Sharangpani, G. M., Porter, K., Keyhani, S., Morrison, C., Basu, A. S., Gholap, G. A., Gholap A. S., Barsky, S. H (2007). Semi-Automated Imaging System to Quantitate Her-2/neu Membrane Receptor Immunoreactivity in Human Breast Cancer, *Cytometry A.*, 71 (5) 273 – 85.

[10] Kallioniemi, O. P., Kallioniemi, A., Kurisu, W., Thor, A., Chen, L. C., Smith, H. S., Waldman, F. M., Pinkel, D., Gray, J.W (1992). ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. Proc Natl Acad Sci USA , 89, 5321-5325.

[11] Kuo, Y-L, Ko, Ch-Ch, Lai, J-Y. (2010). Automated Assessment in HER-2/neu Immunohistochemical Expression of Breast Cancer, *International Symposium on Computer, Communication, Control and Automation*, 3CA 2010, 585 – 588.

[12] Quinlan, J. R. (1979). Discovering Rules by Induction from large Collections of Examples, in D. Michie (ed.): Evert Systems in the Micro Electronics Age, Edinburgh University Press.

[13] Quinlan, J. R. (1986). Induction of Decision Trees, Machine Learning, 1, p.81-106.

[14] Rexhepaj, E., Brennan, J. D., Holloway, P., Kay, W. E., McCann, H. A., Landberg, G., MDuffy, J. M., Jirstrom, K., Gallagher M.W. (2008). Novel image analysis approach for quantifying expression of nuclear proteins assessed by immunohistochemistry - application to measurement of oestrogen and progesterone receptor levels in breast cancer, *Breast Cancer Research*, 10.

[15] Ross, J. S., Fletcher, J. A. (1998). The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy, *Stem Cells*, 16: p. 413-428.

[16] Serra, J. (1988). Image Analysis and Mathematical Morphology, Volume 2: Theoretical Advances, Academic Press.

[17] Skaland, I., Øvestad, I., Janssen, M. A. E., Klos, J., Kjellevold, H. K., Helliesen, T., Baak, A. P. J., Pathol, C. J. (2008). Comparing subjective and digital image analysis HER2neu expression scores with conventional and modified FISH scores in breast cancer, *Journal of Clinical Pathology*, 61 (1).

[18] Slamon, D. J., Clark, G. M et. al. (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER2/neu oncogene, *Science*, 235, 177-182.

[19] Siegel, R., Naishadham, D., Jemal, A. (2013). Cancer statistics. *CA Cancer J Clin*. 63, 11-30

[20] Sugeno, M. (1977) *Fuzzy measures and fuzzy integrals - A survey*. North-Holland, p. 89-102, Amsterdam.

[21] Umanol, M., Okamoto, H., Hatono, I., Tamura, H., Kawachi, F., Umedzu, S., Kinoshita, J. (1994). Fuzzy decision trees by fuzzy ID3 algorithm and its application to diagnosis systems, IEEE World Congress on Computational Intelligence. *In*: Proceedings of the Third IEEE Conference on Fuzzy Systems, p. 2113 - 2118 vol.3.

[22] Umemura, S., Osamura, R.Y., Akiyama, F., Honma, K., Kurosumi, M., Sasano, H., Toyoshima, S., Tsuda, H., Ruschoff, J., Sakamoto, G. (2008). What causes discrepancies in HER2 testing for breast cancer? A Japanese ring study in conjunction with the global standard. *Am J Clin Pathol*, 130, 883-891.

[23] Wojnar, A., Drozdz, K., Podhorska-Okolow, M., Pudelko, M., Szuba, A., Zabel, M., Dziegiel, P. (2009). Differentiated expression of estrogen receptors (ER) and progesterone receptors (PgR) in ductal breast cancers. *Folia Histochem Cytobiol*, 47, 55-60.

[24] Wojnar, A., Pula, B., Podhorska-Okolow, M., Dziegiel, P. (2013). Discrepancies between HER2 assessment from needle core biopsies and surgical specimens of invasive ductal breast carcinoma. *Adv Clin Exp Med*, 22, 27-31.