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Colour-Texture based image analysis method for assessing the Hormone Receptors status in Breast tissue sections

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Abstract— **Hormone receptors have been used in prognosis of breast carcinomas and their positive status is of clinical value in hormonal therapy. Determination of this status is based on the subjective visual inspection of the stained nuclei in the specimens. The aim of this study was the assessment of the estrogen receptor's (ER) positive status of breast carcinomas, by means of colour-texture based image analysis methodology. Twenty two cases of immunohistochemically (IHC) stained breast biopsies were initially assessed by a histopathologist for ER positive status, following a clinical scoring protocol. Custom-designed image analysis software was developed for automatically assessing the ER positive status, employing colour textural features and the k-Nearest Neighbor weighted votes classification algorithm. Computer-based image analysis system resulted in 86.4% overall accuracy and in 0.875 Kendall's coefficient of concordance ($p<0.001$), ranking correctly 19/22 cases. Colour-texture analysis of IHC stained specimens might have an impact in the quantitative assessment of ER status.**

I. Introduction

BREAST cancer is considered as the most common malignancy that affects women, according to the World Health Organization [1]. A variety of receptors can be expressed in cancer cells. The abundance of these receptors can provide a means for controlling cell growth through chemotherapeutic agents. The hormone receptor status of the breast cancer cells provides useful information for treatment planning. It has been shown that estrogen receptor (ER) positive cancers will have a better prognosis, thus ER status plays an important role in the development and progression of breast cancer [2, 3]. Recently, significant progress has been made, by means of immunohistochemistry (IHC), in understanding breast carcinogenesis, providing new biological factors [4].

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In daily clinical practice, estimation of ER status is based on the subjective count of the expressed (stained brown) nuclei in each specimen, under the light microscope. Computer-based image-analysis systems have proved to be a means for the quantification of ER status [2].

Previous work, concerning quantitative determination of the ER status is confined to the utilization of mean optical density as a descriptor capable of distinguishing between ER stained and unstained nuclei [5-10]. Regarding texture analysis, it has been extensively used in computer-based image analysis in medical research for both segmentation and classification purposes. To the best of our knowledge, colour-texture analysis approach has not been used for the assessment of ER positive status and it has been implemented in the present work for increasing the accuracy of ER-status quantification.

In the present study, an image-analysis method utilising a custom-designed graphical user interface was developed for the quantitative assessment of ER positive status of breast carcinomas. The image analysis method comprised nuclei segmentation and a pattern recognition algorithm (k-Nearest Neighbours with Weighted Votes classifier/k-NN-WV) based on the Laws texture measures for the classification of nuclei. The proposed method was comparatively evaluated against the results obtained by Adobe Photoshop (Adobe Corp., Mountain view, CA, USA).

II. Material and Methods

Clinical material comprised of twenty-two IHC-stained (employing the Novocasta6F11 monoclonal antibody) for ER biopsy sections were collected by an experienced histopathologist from the Department of Pathology of the University Hospital of Patras, Greece. Diaminobenzidine (DAB) was used as chromogen and Haematoxylin as counterstain. Nuclei having positive ER expression were stained in brown hue by DAB, while those that did not, were stained in blue hue by Haematoxylin. According to recommendations of the American Society of Clinical Oncology (ASCO), the biopsy specimens were assessed for ER positive status, based on a scoring protocol, that takes into consideration the percentage of the number of positive stained nuclei (brown) to the total number of nuclei [5]. The scoring protocol coded 0 for the range 0–5% and 1, 2, 3, 4 for the ranges 6-10%, 11-33%, 34-66%, 67-100%,

respectively. The studied cases had a physician's positivity score assessment ranging between 20%-90%.

For each specimen, a representative region was specified by the histopathologist (R.P). From this region, a number of images were digitized (1300x1030x48bit, 16bits for each RGB channel) at a magnification of x400 using a light microscope (Axiostar Plus – ZEISS; Germany) and a colour video camera (DC 300F - LEICA; Germany).

A. *Nuclei Segmentation*

The original coloured RGB-image was converted to 8 bit greyscale (upper-left image, Fig. 1), for nuclei segmentation purposes. Accordingly, the image was segmented applying the Otsu's global image thresholding method [11], while the physician was able, if necessary, to apply finite corrections by means of a manually adjustable threshold value (Fig. 1) through a graphical user interface developed in Matlab (The MathWorks, Inc.; USA).

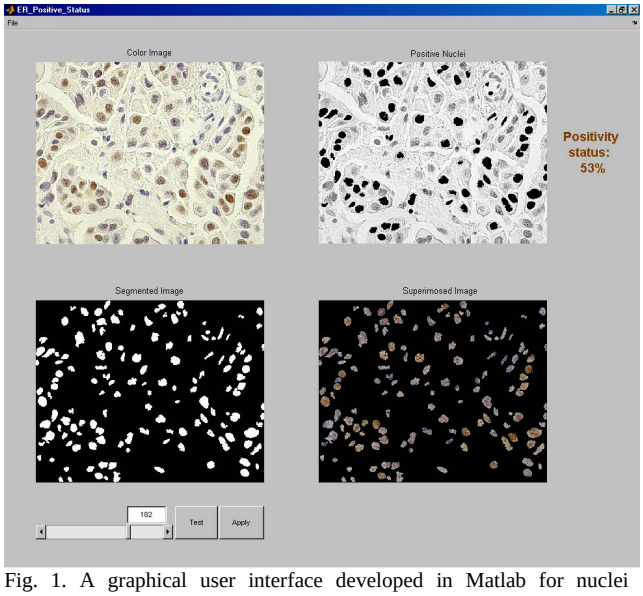

Fig. 1. A graphical user interface developed in Matlab for nuclei segmentation purposes. Top Left: Original RGB image. Bottom Left: segmented nuclei. Bottom Right: The segmented image mathematically processed by the AND operator with the original RGB image. Top Right: The positive nuclei after classification (dark coloured). The static text displays the result of computer-based assessmentis abbreviated.

Subsequently, the image was further processed by morphological operations for eliminating noisy regions. The latter comprised image closing and hole-filling, with disk shaped structuring element of three and five pixels respectively. Next, size filtering operation was performed on the image for sustaining nuclei larger than 300 pixels (Fig. 1, bottom-left image). Parameters of morphological operations were fixed for the particular image acquisition employed. The segmented image was then mathematically processed by the AND operator with the initial image (Fig. 1 bottom-right image). The same procedure was repeated for each image, in order to collect an adequate number of nuclei for classifier training process (see section II.C).

B. *Textural Features Extraction*

Laws' texture energy measures were used for textural features generation [12]. Five kernels, accounting for Level, Edge, Spot, Wave, and Ripple, were considered for analysis. Those masks are derived by convolving the three basic vectors L3= (1, 2, 1), E3= (-1, 0, 1) and S3= (-1, 2, -1) with each other representing smoothed local averaging, symmetric first differencing for edge detection and second differencing for spot detection respectively. The examined kernels were: L5=[1, 4, 6, 4, 1], E5=[-1,-2, 0, 2, 1], S5=[-1, 0, 2, 0,-1]; W5=[-1, 2, 0, -2, 1] and R5=[1, -4, 6, -4, 1]. Multiplying column vectors by row vectors, twenty five two-dimensional (5x5) convolution kernels were generated and were used to convolve the nuclei images. Next, a 15x15 averaging filter was applied on the convolved images to produce 25 texture energy images. Accounting for rotational invariant features, 15 texture energy measures images remained and from each one the mean value, standard deviation, skewness and kurtosis were calculated, providing sixty features for each RGB channel. Subsequently, the image's red, green, and blue channels were combined into a single-colour-channel for squeezing in one feature the combined colour and intensity texture information. The colour Laws features (CLF), were calculated as:

$$CLF_i = CLF_i^R \times CLF_i^G \times CLF_i^B \quad (1)$$

where i=(1,.., 4) are the examined features. Hence the finally 60 colour texture energy measures were regarded as the feature vector.

All features were normalized to zero mean value and unit variance [13] according to the relation

$$\tilde{x}_i = \frac{x_i - m}{s} \quad (2)$$

where x_i and $\tilde{x}_i$ are the feature vectors prior and after the normalization, m and s are the mean value and standard deviation of each feature respectively. Normalization was necessary to ensure that features with large variations from the mean value did not dominate during the classifier's training.

C. *Feature Selection and Classification*

To reduce the problem's dimensionality as well as the computational time demands for classifier design, features were reduced by means of the Wilcoxon statistical non parametric test. Accordingly, the first 15 most significant features (in least correlated manner, $p>0.1$) were fed into the k-Nearest Neighbours with Weighted Votes' classifier (k-NN-WV). The k-NN-WV is an extension of the k-Nearest Neighbours classifier, which classifies the unknown feature to the class with the largest sum of weighted votes [14]. Each weighted vote is a decreasing function of the distance

of each one of the k nearest points from the unknown pattern [14]. The weighted vote function employed was

$$w = \left(1/a\sqrt{2\pi}\right) e^{-\frac{d^2}{2a^2}} \quad (3)$$

where d is the distance of the unknown pattern from each class pattern, and α a tuning parameter (experimentally determined and set to $\alpha = 0.01$) [14]. Feature selection was performed by means of the exhaustive search algorithm [13], according to which all possible features combinations were used to design and evaluate the classifier. For the evaluation process one-third of the segmented nuclei were chosen as training set while the rest of them were used to test the accuracy of the k-NN-WV in classifying nuclei as brown (stained) or blue (unstained). Those are the two colour hues of nuclei staining according to the IHC protocol employed.

D. Positivity Status Assessment

In order to assess the ER positivity status of a new image, the computer-based method follows two sequential steps: a/ nuclei segmentation as explained in section II.A and b/ classification of nuclei into brown and blue based on the designed classification model (see section II.C). Finally, according to the aforementioned scoring protocol, the ER positive status was calculated and displayed (Fig. 1 upper-right image).

III. Results

Twenty-two images were processed and 921 nuclei were finally segmented; 307 (155 brown and 152 blue) nuclei constituted the 'gold standard' and were used as the training set that was different from the test set. The number of neighbours of the k-NN-WV classifier was experimentally determined for optimum performance to be equal to 5. The classification accuracies and corresponding feature vectors for the exhaustive search experimentation are showed in Table I.

Table II shows the mean error of the percentage ER positive status evaluation, as derived from the computer-based system, and its accuracy per scoring scale against the physician's estimation.

Figure 2 depicts the results of the proposed system in discriminating brown and blue nuclei, and the results of the Photoshop following the methodology by Lehr *et al*. The mean error of the Photoshop package was 9.4±1.3%, 11.6±2.7% and 8.7±2.6% for the 3, 4 and 5 scoring scale respectively.

IV. Discussion and Conclusion

The highest discrimination accuracy of the k-NN-WV classifier was 86.8%, for combinations of CLF consisting of the mean value, standard deviation, skewness and kurtosis. The Wilcoxon rank test revealed that no significant differences existed between the physician and the proposed system ($p>0.1$). Computer-based image analysis system resulted in 86.4% overall accuracy, ranking correctly 19/22

TABLE I
Classification Performance And Best CLF Feature Vector

Number of Feature Combinations	Accuracy (%)	Feature vector
2	82.73	$R5E5^{m}$, $R5S5^{sk}$
3	83.06	$E5E5^{s}$, $R5S5^{sk}$, $W5L5^{k}$
4	83.99	$R5E5^{s}$, $W5L5^{s}$, $R5S5^{sk}$, $W5L5^{k}$
5	84.49	$R5E5^{s}$, $W5L5^{s}$, $E5E5^{s}$, $R5S5^{sk}$, $W5L5^{k}$
6	86.81	$R5E5^{s}$, $E5E5^{s}$, $R5S5^{m}$, $R5S5^{sk}$, $R5S5^{k}$, $W5L5^{k}$
7	84.41	$R5E5^{s}$, $R5S5^{sk}$, $E5E5^{s}$, $R5S5^{m}$, $R5S5^{k}$, $S5L5^{s}$, $W5L5^{k}$

m, s, sk, and k: mean value, standard deviation, skewness, and kurtosis

TABLE II
Mean Errror Of The ER Positive Status Evaluation

Scoring Scale	Number of cases	Mean Error of the computer-based percentage evaluation (%)	Accuracy per scoring scale of the computer-based performance (%)
3	4	7.2 ± 1.2	75
4	5	8.6 ± 2.9	80
5	13	7.6 ± 2.8	92.3

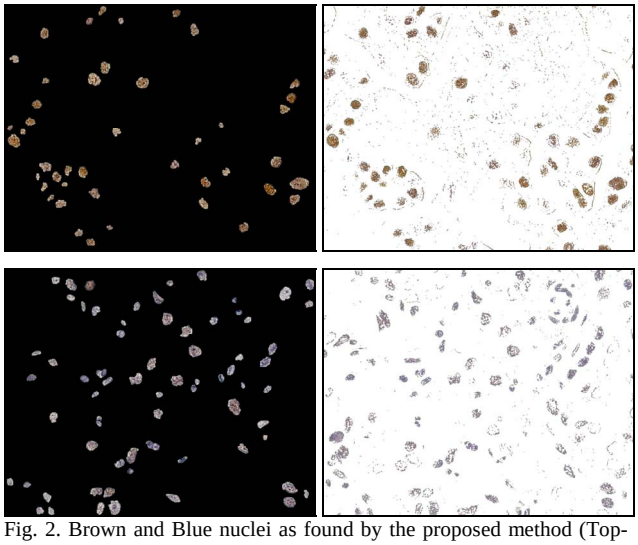

Fig. 2. Brown and Blue nuclei as found by the proposed method (Top-Bottom left) and Brown and Blue nuclear area as obtained using Photoshop (Top-Bottom right).

cases outperforming Photoshop's ranking (17/22). Kendall's coefficient of concordance (KCC) was used to determine the level of agreement between assessments [15]. KCC revealed a high level of agreement among the histopathogist and the computer-aided system ($W=0.875$, $p<0.001$).

A diversity of image analysis methods (commercially available packages and off-the-shelf algorithms) have been studied for quantification of IHC sections [6, 7, 9, 10, 16]. Recently, Mofidi *et al.* [6] and Schnorrenberg *et al.* [7] have used image analysis methodologies but a different scoring protocol with the presented study, reporting significant correlation of ER status between manually and image analysis assessment. The mean optical density was implemented as the key feature in specifying colour nuclei. In contrast, the present study introduces a computer-based classification approach, employing the Laws colour-textural features extracted from nuclei.

Measures of ER status for the examined cases, showed a high level of agreement between the clinical assessment of the pathologist and the proposed computer-based image analysis method.

Colour-texture analysis of IHC stained specimens might have an impact on the quantitative assessment of ER status. Combining colour-texture based pattern recognition algorithms with more sophisticated methods for segmentation, such as unsupervised clustering [17], may contribute to the prognostic outcome in every day clinical routine as a second opinion tool, providing an objective assessment to the pathologist.

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