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EVALUATION OF IMMUNOHISTOCHEMISTRY (IHC) MARKER HER2 IN BREAST CANCER

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Abstract

The paper discusses a novel approach involving algorithm implementation and hardware Devkit processing for estimating the extent of cancer in a breast tissue sample. The process aims at providing a reliable, repeatable, and fast method that could replace the traditional method of manual examination and estimation. Immunohistochemistry (IHC) and Fluorescence in situ Hybridization (FISH) are the two main methods used to detect the marker status in clinical practice. FISH is though more reliable than IHC, but IHC is widely used as it is cheaper, convenient to operate and conserve, the morphology is clear. The IHC markers are Estrogen receptor (ER, Progesterone receptor (PR), Human Epidermal Growth Factor (HER2) that give clear indications of the presence of cancer cells in the tissue sample. HER2 remains the most reliable marker for the detection of breast cancer. The Human Epidermal Growth Factor Receptor (HER2) markers are discussed in the paper, as it gives clear indications of the presence of cancer cells in the tissue sample. HER2 is identified based on the color and intensity of the cell membrane staining. The color and intensity is obviously based on the thresholding for classifying the cancerous cells into severity levels in terms of score to estimate the extent of spread of cancer in breast tissue. For HER2 evaluation, the percentage of staining is calculated in terms of ratio of stain pixel count to the total pixel count. The evaluation of HER2 is obtained through simulation software (MATLAB) using intensity based algorithm and same is run on embedded processor evaluation board Devkit 8500. The results are validated with doctors.

Keywords:

Breast Cancer, IHC, HSV Model Based, HER2, Intensity Based Membrane Staining.

1. INTRODUCTION

Breast cancer is the second leading cause of death among women in the world. Pathologists use external chemical hormones antibodies that bind to these receptors [1], and cause visible changes in the cell. The Estrogen receptor (ER), Progesterone Receptor (PR or PgR) and Human Epidermal Growth Factor Receptor (HER2) are the most reliable IHC receptors markers which are mostly used in cancer cell analysis and cell estimation. Traditionally, the analysis is done by the pathologist by manually viewing the sample slide under light microscope. Alternate to this process, automated diagnosis involving image processing system is used to provide a reliable result with minimized errors. The advantages of implementing automation for pathological analysis over manual evaluation of cancer cell decreases time for analysis of pathological samples allowing pathologists to avoid routine scanning and focus on other more complex issues, reduced number of errors, the algorithms and hardware implementation can compare so as to avoid false positives or false negative giving a highly reliable result, faster documentation of results and higher repeatability due to the fact that medical images in digital format can be stored and reused for later analysis and finally minimization of costs as the entire process is automated, the cost per analysis is reduced and can be made portable.

2. METHODS USED TO DETECT MARKERS

To understand the severity of breast cancer and to provide the correct diagnosis, prognosis of the patient and to understand the pace of the cancer development, following test are conducted i.e. biochemical, flow cytometry, Immunoassay, Immunohistochemistry (IHC), Chromogenic in situ hybridization (CISH), Fluorescence in situ hybridization (FISH). Generally IHC and FISH are mostly preferred by the pathologist in clinical testing for the breast cancer patients. FISH testing requires specialized infrastructure and requires high cost equipment and are not easily available in normal pathologist clinic. Thus IHC technique which is widely available, relatively low cost, easy and long preservation of the stained slides is mostly preferred. Both methods IHC and FISH should be used to ensure accurate diagnosis, prognosis and therapy.

2.1 IMMUNOHISTOCHEMISTRY (IHC)

Immunohistochemistry (IHC) has taken over as the major assay method used for assessing markers. This technique is used for detecting in situ a tissue antigen by a specific antibody. An antigen-antibody reaction is visualized by the color development of specific dye and can be seen by the light microscope. The tissue antigen is present at any part of the cell, i.e. cell membrane, cytoplasm or nucleus. Therefore it is a useful technique to quantifiable and standardized measurement to extract protein over expression markers present within the cancer cells. It is of major importance clinically that those undertaking interpretation of predictive markers understand the technical pitfalls and are aware of how expression of a particular marker relates to breast cancer pathology. A false negative or false positive result will impact on the patient management. The Table.2 gives a summary of the types of markers and their importance in cancer cell evaluation. Though there are several markers in the Table II, the markers under Research interest are less likely to be used clinically and are available for researchers in medical field. The markers indicated under Potential for clinical use needs refinement of scoring or antibodies and these markers are not full proof but these markers can aid for prognostic. The markers indicated under Established and in routine clinical are used mandatory in clinical routine for therapeutic decisions i.e. Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor (HER2). The IHC method involves visual examination of cell membrane under a microscope. In HER2,

evaluation also involves classification of tissue sample into estimate score {0, 1+, 2+, and 3+} depending on the extent of spread of cancer in breast tissue [8].

3. LITERATURE SURVEY

A number of approaches have been suggested by various authors for developing effective algorithms. In [9] evaluating criteria such as intensity and uniformity of staining and estimating the percentage of stained cells is a subjective process that affects the accuracy of Immunohistochemistry (IHC) assessment and contributes to inter observer variability. Observer variability associated with quantifying expression levels using color-grading approaches is well documented. A recent study on the evaluation of HER2 by five observers reported complete agreement in 48% of HER2 cases (22 out of 46). There is clearly a need for quantitative methods to improve the accuracy and reproducibility in the assessment of IHC staining. In [13] the paper presents an automated method about the quantitative assessment of HER2 expression of IHC stained images. The proposed system efficiently extracts nuclei of interest including positive stained nuclei and negative stained nuclei. By applying a series of images processing including color pixel classification nuclei segmentation cell membrane extracting, measures of cell membrane staining intensity and completeness, HER2 expression can be assessed. This evaluation provides pathologist significantly with a good reference for diagnosis and prognosis. Another technique for marker extraction was proposed by [15] based on "constrained region labeling" but again this is a complicated process. In [17], the authors proposed edge detection as well as intensity based extraction of objects of interest from background (also known as region growing). However, the edge detection method using techniques like Sobel and Canny are sensitive to noise while intensity based algorithms are computationally time consuming as each pixel's intensity is scanned in the image. In [23], the author proposed hardware implementation on DSP TMS 320C6713 and is achieved successfully, for developing the malignant and benign cancer and run on MATLAB for the medical images obtained from the radiologist. Jordan and Elman network has achieved top result.

4. IHC BIOMARKER HER2

Epidermal growth factor receptor (EGFR)/erb-B family of receptor plays very important role for in cancers including lung, breast, gastric, colon etc. HER2 biomarker expresses protein as indicator for Herceptin therapy in both metastatic and pre-metastatic patients of breast cancer. HER2 over expressed in approximately 18-20% of invasive breast cancers, which has both prognostic and predictive markers in breast cancer.

HER2 over expression and amplification are recognized as very important marker for aggressive disease and are target for specific therapies, such as transtuzumab and Lapatinib. Both these are approved by Food and Drug Administration (FDA) for the treatment of HER2 positive breast cancer and are clearly associated in the improved clinical outcomes in metastatic spreading of breast cancer.

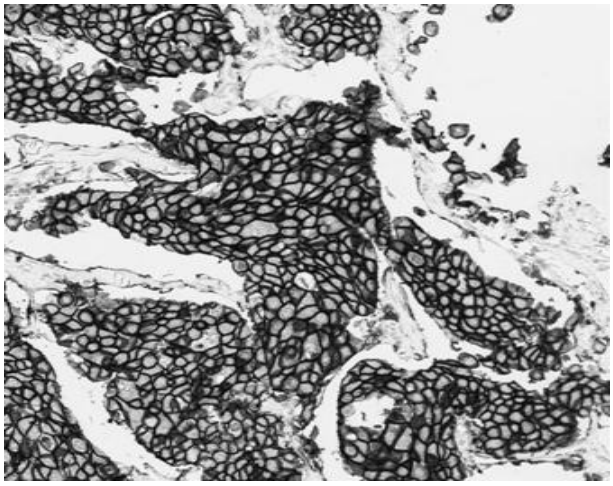

Fig.1. Typical HER2 sample with 3+ staining of cell membranes

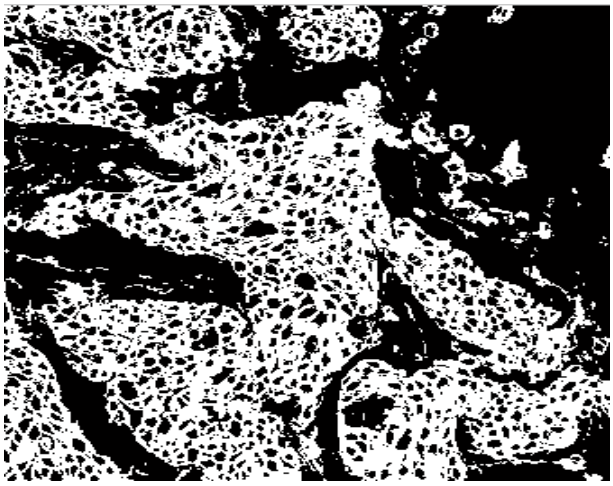

Fig.2. Image after color pixel classification. The region in white corresponds to the cell membrane of cancer affected cell

5. IMAGE PROCESSING IMPLEMENTATION FOR HER2

IHC marker HER2 evaluation is generally done by estimating the membrane stained region based on color and intensity of cell membrane thus the evaluation of HER2 images classified into four categories of {0, 1+, 2+, 3+} scoring assay of ASCO/CAP is as per the Table.1.

Table.1. ASCO/CAP score of HER2

Staining Percentage	Score	Indication
>30%	3+	Positive
>10% and <30%	2+	Mildly Positive
>1% and <10%	1+	Mildly Negative
<1%	0+	Negative

Table.2. Various types of Biomarkers

Established and used in routine clinical analysis	Potentially useful for clinical use; require refinements	Research interest, less likely to be used clinically
Estrogen Receptor (ER)	Epidermal Growth Factor Receptor (EGFR / HER1)	P53
Progesterone Receptor (PgR)	Ki-67 (MIB-1)	Cyclin E, Cyclin D1, p21, p27
Human Epidermal Growth Factor Receptor (HER2)	Topoisomerase II alpha	Bcl2, bax, bcl-x, surviving

The flow chart implementation using MATLAB area based algorithm for the HER2 image is as shown in Fig.3. This is done by evaluating the image at various points on the cell membrane stained and obtaining a threshold for the color pixel classifier. The resulting image after color pixel classification is as shown in Fig.2.

$$ratio = \frac{number\ of\ stained\ pixels}{total\ number\ of\ pixels} \times 100 \quad (1)$$

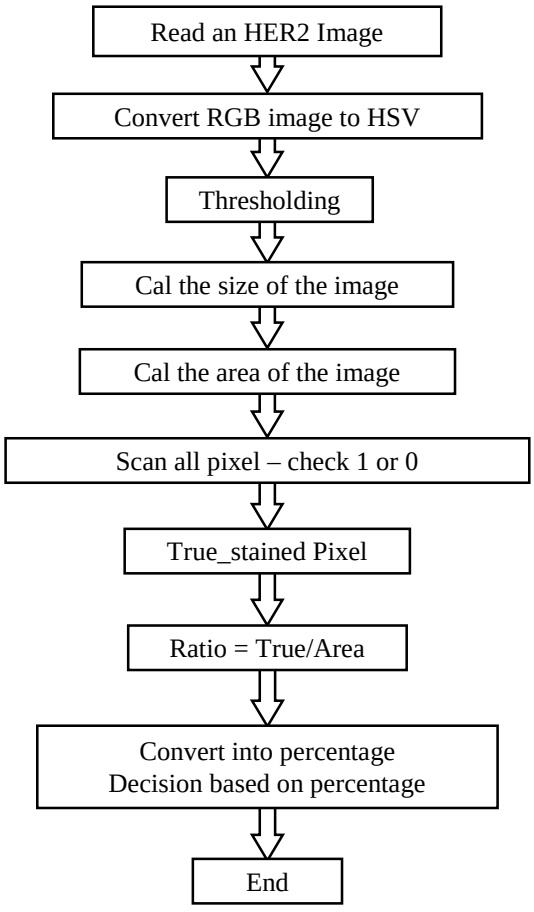

Fig.3. Flow Chart using Image Processing for HER2

The original HER2 image as shown in Fig.1. The ratio of the stained pixel to the total pixel is calculated as per the Eq.(1). The scoring of 30 HER2 images is as shown in Table.1.

6. HARDWARE IMPLEMENTATION FOR HER2

Evaluation board Devkit 8500 is a hardware and software platform with the Texas Instruments Davinci DM 3730 Digital media Processor; also it supports high level Operating system such as Linux, WIN CE and Android. The programmable DSP engine allows multiple signals processing task such as Image Processing and analysis, which requires large amount of data processing. The software platform used is open cv. Open CV is an IP library created for C, C++ and Python. It is open source software free of cost, easy to use and install, The IHC marker HER2 images evaluation is implemented on the Digital media Processor and software language using C++. The evaluation board implemented for the HER2 IHC biomarker is shown in Fig.4.

Fig.4. Hardware implementation of HER2

However as the modified version tends to remove the error segments, the time saved is much greater in the intensity based cell classification stage as a fewer number of cells have to be evaluated. This fact is evident from Table.3. The time taken is proportional to the number of cells recognized in the image as well as to the size of the image: in a larger image, a higher number of pixels have to be analyzed in the region growing process. A fixed size of image is preferred. The sizes of the image used are constant and equal to 1024 ×1024 pixels. The ratio that determines the extent of cancer is given by Eq.(2). For the above image, number of pixels in stained region is 424062. The area of the image is 1048576 pixels. The ratio of the two is 0.4044 or 40.44 percent.

$$Sn\ (\%) = \frac{True\ Positives\ (TP)}{True\ Positives\ (TP) + False\ Negatives\ (FN)} \times 100 \quad (2)$$

$$Sp\ (\%) = \frac{True\ Negatives\ (TN)}{False\ Positives\ (FP) + True\ Negatives\ (TN)} \times 100 \quad (3)$$

$$PPV\ (\%) = \frac{True\ Positives\ (TP)}{True\ Positives\ (TP) + False\ Positives\ (FP)} \times 100 \quad (4)$$

$$NPV\ (\%) = \frac{True\ Negatives\ (TN)}{False\ Negative\ (FN) + True\ Negatives\ (TN)} \times 100 \quad (5)$$

$$Acc(\%) = \frac{TP + TN}{TP + FP + TN + FN} \times 100 \quad (6)$$

Further, in HER2 evaluation, the size of the image directly affects the ratio which determines the extent of cancer. Thus a fixed size of image is preferred. The results are also affected by the quality of the image being analyzed. Images, in which tissue samples are folded, blurred or non-uniformly illuminated, produce erratic results. The solution to this is to have a proper image normalization or de-blurring process prior to image analysis, as the case may be done.

7. RESULTS AND DISCUSSION

The cell membrane staining obtained by the Area Ratio algorithm for HER2 was compared to the staining obtained by manual analysis by the histo-pathologist for each image. The HER2 30 images were shown to the multiple pathologists for scoring. The Table.3 shows a summary of the results obtained for 30 HER2 images comparison with the algorithm, Hardware implementation and the results provided by histo-pathologist. Sensitivity (Sn), Specificity (Sp), Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Accuracy are calculated for 30 Images of HER2 IHC marker using the data in Table.2 and the Eq.(2), Eq.(3), Eq.(4), Eq.(5) and Eq.(6). The Statistical analysis of MATLAB algorithm, Hardware results and Doctors results is shown in Table.4.

Table.3. Result of HER2 Images-Evaluation of Matlab, Hardware implementation and Doctors truth reality

S. No. Image	MATLAB Algorithm Score	Hardware Score	Dr. AJ	Dr. KD	Dr. KMK
1	0+	0+	1+	0+	0+
2	0+	0+	0+	0+	0+
3	2+	2+	2+	2+	2+
4	0+	0+	1+	0+	1+
5	0+	0+	1+	1+	0+
6	3+	3+	3+	3+	3+
7	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+
9	3+	3+	3+	3+	3+
10	0+	0+	0+	0+	0+
11	0+	0+	0+	0+	0+
12	0+	0+	0+	0+	0+
13	0+	0+	0+	0+	0+
14	0+	0+	1+	1+	1+
15	0+	0+	1+	1+	1+
16	2+	2+	2+	2+	2+
17	2+	2+	2+	2+	2+
18	2+	2+	2+	1+	2+
19	0+	0+	0+	0+	0+
20	0+	0+	1+	1+	1+
21	0+	0+	0+	1+	0+
22	3+	3+	3+	3+	3+
23	3+	3+	3+	2+	3+
24	3+	3+	3+	3+	3+
25	3+	3+	3+	3+	3+
26	3+	3+	2+	3+	3+
27	3+	3+	3+	2+	2+
28	3+	3+	3+	2+	3+
29	0+	0+	1+	0+	0+
30	2+	2+	2+	2+	2+

8. CONCLUSION

The paper presents a blended investigation of estimation of spread of cancer in breast tissue using HER2 biomarkers thus employing software algorithm and hardware. The results are validated with Doctors and presented in Table.4. The statistical analysis shows that either results (software algorithm and hardware) concurs with Doctors prognosis with minimum 75% accuracy.

9. FUTURE WORK

The threshold level chosen for algorithm is manually taken for the evaluation of HER2 images based on the magnification which remain fairly constant. For different magnitude levels algorithm will be with a new threshold value needs to be manually evaluated. The process of calculating an auto optimal value of the threshold can be automated for a more generalized implementation of the algorithm. The size of the image directly affects the ratio which determines the extent of cancer. Thus a fixed size of image is preferred. Further many more biomarkers can be identified which are having the potential for clinical analysis also the algorithm and hardware implementation can be extended to identify the biomarkers as per Table.2.

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Table.4. Statistical Analysis

MATLAB and Doctors						Hardware and Doctors					
Dr.	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**	**Dr.**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
AJ	100	50	69.56	100	75.86	**AJ**	100	69.57	50	100	76.67
KD	100	64.28	76.19	100	83.33	**KD**	100	64.28	76.19	100	83.33
MAK	100	69.23	80.95	100	86.67	**MAK**	100	69.23	80.95	100	86.67

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