SJIF Impact Factor: 3.458

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 3.
Issue: 4.
Page N. 38-48
Year: 2019

Review Article www.wjahr.com

AUTOMATIC BREAST CANCER DETECTION BASED ON IMMUNOHISTOCHEMISTRY (IHC) MARKERS

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Received date: 12 April 2019 **Revised date:** 02 May 2019 **Accepted date:** 23 May 2019

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ABSTRACT

The paper discusses an approach involving digital image processing for estimating the extent of cancer in a breast tissue sample. The process aims at providing a reliable, repeatable, fast and cost-effective method that could replace the traditional method of manual examination and subsequent estimation. The markers discussed in the paper are the Human Epidermal Growth Factor Receptor (HER2) and the Estrogen Receptor (ER) that give clear indications of the presence of cancer cells in the tissue sample. ER and PR evaluation, a modified watershed algorithm designed for eliminating errors arising due to over-segmentation in traditional watershed algorithm is proposed to provide comparatively more accurate results. HER2 evaluation, the ratio of extent of staining to the total size of image gives an estimate of the extent of cancer cell spread. An innovative approach of classifying and correlating the makers on single patient, known as three marker method. The statistical result analysis is validated with doctors. IHC marker i.e. HER2 is evaluated, 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. The statistical analysis is given in Table IV.

KEYWORDS: Breast Cancer, marker, Cell Count, Cell membrane staining, Classification and Correlation.

1. INTRODUCTION

Breast cancer cells have receptors on their surface, in their cytoplasm and in the nucleus. Pathologists use external chemical hormones 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 receptors which are actively used in cancer cell analysis and cell population estimation. Traditionally, the analysis is done by manually viewing the sample under microscope. As an alternate to this process, computer aided diagnosis involving image processing can be used to provide a reliable result with minimized errors,[2,3,4] The advantages of employing automation for pathological analysis over manual evaluation of cancer cell population are: Decreased 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 can be made highly accurate and can avoid false positives or misses 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.

2. Traditional Method In Breast Tissue Sample Analysis

The method used currently involves using antigen-antibody reactions due to which cell staining takes place. The cancerous cells are stained in a dark brownish color (Figure 1) while non-cancerous cells develop a bluish shade.

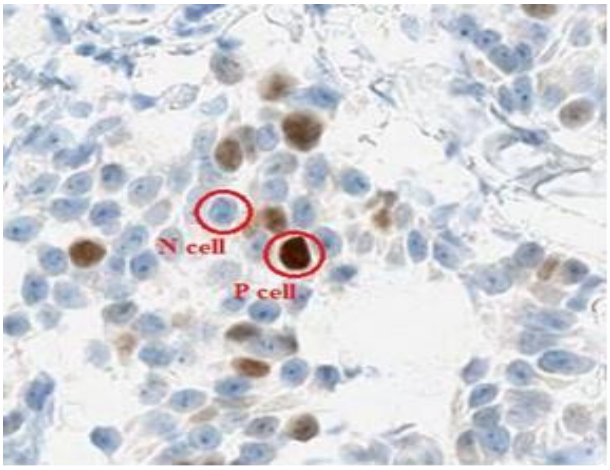

Figure 1: Estrogen receptor: stained tissue sample showing cancer positive/P (stained brown) and cancer negative/N (stained blue) cells.

Receptors and markers

A marker is a predictive indicator that helps to evaluate the response of cancer cells to a particular treatment. Of the many receptors identified,[5-15] the estrogen receptor (ER) and Human Epidermal Growth Factor Receptor (HER2) are of prime importance. Table I gives a summary of the types of receptors and their importance in cancer cell evaluation.

Table I: Various markers and their value in predicting breast cancer.[17]

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 or 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, survivin

Evaluation methods

One of the most popular evaluation methods is the Immuno-histo-chemical or IHC method.[18] This method has a lot of advantages such as its ready availability, relative lower costs per analysis and simple methods for preservation of stained samples.[19]

The IHC method involves visual examination of cell membrane under a microscope. In HER2, evaluation further involves classification of tissue sample into categories of {0, 1+, 2+, and 3+}.[16] depending on the severity of cell damage. In ER, scoring is done on the basis of population of cells and their intensities leading to a score called Allred score ranging from 0 to 8.

3. LITERATURE REVIEW

An extensive survey of literature, in the context of breast cancer is undertaken and the researchers have developed different methodologies and algorithms.

In,[4] authors proposed a method to overcome the segmentation error for IHC-ER marker by developing marker controlled watershed algorithm.

In,[5] the authors achieved a fairly high accuracy of 80% by marker controlled watershed algorithm. However, the extraction process of IHC markers became difficult due to merging of different objects.[6]

In,[7] authors proposed an algorithm based on "constrained region labeling" for marker extraction but it involved more complexity.

IHC - HER2 marker,[8] 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 IHC assessment and contributes to inter observer variability. 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[9] 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.

In,[10] the authors proposed region growing method which comprises edge detection methods (Canny or Sobel) and intensity based segmentation to extract object of interest. However, these operators were noises-sensitive and intensity based algorithms were taking large computational time. Thus, author adopted region growing method based on intensity and density to improve the accuracy.

TNBC is very much significant as prognosis factor in lymph-node negative breast cancer in Indian women. In,[11] authors have used a database of 36 female patients with triple negative breast cancer. The prognosis techniques to identify the severity of breast cancer by the Estrogen Receptor (ER), Progesterone Receptor (PR) in which counting of cell, other valuable HER2 marker in which the staining of the cell, is measured and the score is evaluated. The triple negative breast cancer (TNBC) identifies the correlation and classification of IHC markers. They are correlated with age, tumour size, histopathological type and conclude that the patients above 40 having triple negative breast cancer (TNBC) were found to be more aggressive.

In,[12] authors studied and performed the analysis of triple negative breast cancer and non- triple negative breast cancer patients. The statistical analysis of results was carried out using lox regression model. The author concluded the Relapse Free Survival (RFS) was significantly shorter with TNBC patients as compared to non -TNBC.

In our algorithm reducing the errors is a higher priority rather than reducing the computation time and therefore we have adopted the intensity based region growing process. HER2 scoring has been proposed in,[16] by membrane staining assessment but involves focusing on a number of region of interests and analyzing each region separately and then combining the results of each regions.

A number of approaches have been suggested by various authors for developing effective algorithms. In,[20] the removal of overlapping redundancies involves the use of Laplace of Gaussian (LoG) edge detection, morphological operation, gradient magnitude and marker controlled watershed algorithm. However, the segmentation is inaccurate due to the inability of identifying the borders of the cells.

Also, in,[21] the authors made use of the marker controlled watershed algorithm which gave a fairly high overall percent correct agreement of 80%. However, the process of extraction of these binary markers is very difficult as too many of these markers cause over-segmentation errors while too few of them cause different objects to merge.[22]

Another technique for marker extraction was proposed by,[23] based on "constrained region labeling" but again this is a complicated process.

In,[24] 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,[25] 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. Image Processing Implementation for ER Eliminating over-segmentation errors

The traditional watershed algorithm was unable to count the accurate number of cells due to intensity variation in the cell. So, a modified watershed algorithm was developed to eliminate the estimation of over segmentation error by introducing the concept of thresholding (equation 1), where if the cell centroid distance between two cells is less than the threshold value, new cell centroid is calculated by averaging the cell centroids in consideration (refer Figure 2).

$$d_j = \sqrt{\left(x_j^2 - x_{j+1}^2\right) + \left(y_j^2 - y_{j+1}^2\right)} \ldots for\ j = 1 \ldots \ldots n, \qquad (1)$$

Where 'j' signifies the present segment of the cells and 'n' denotes the total number of segmented cells.

Figure 2: Conceptual image to eliminate over-segmentation error.

An iterative process was performed using equation (1) until all segments were considered. A conceptual image to eliminate the over segmentation error is shown in Figure 2.

The implementation of image processing on an image of stained tissue sample involves the steps as shown in Figure 2.

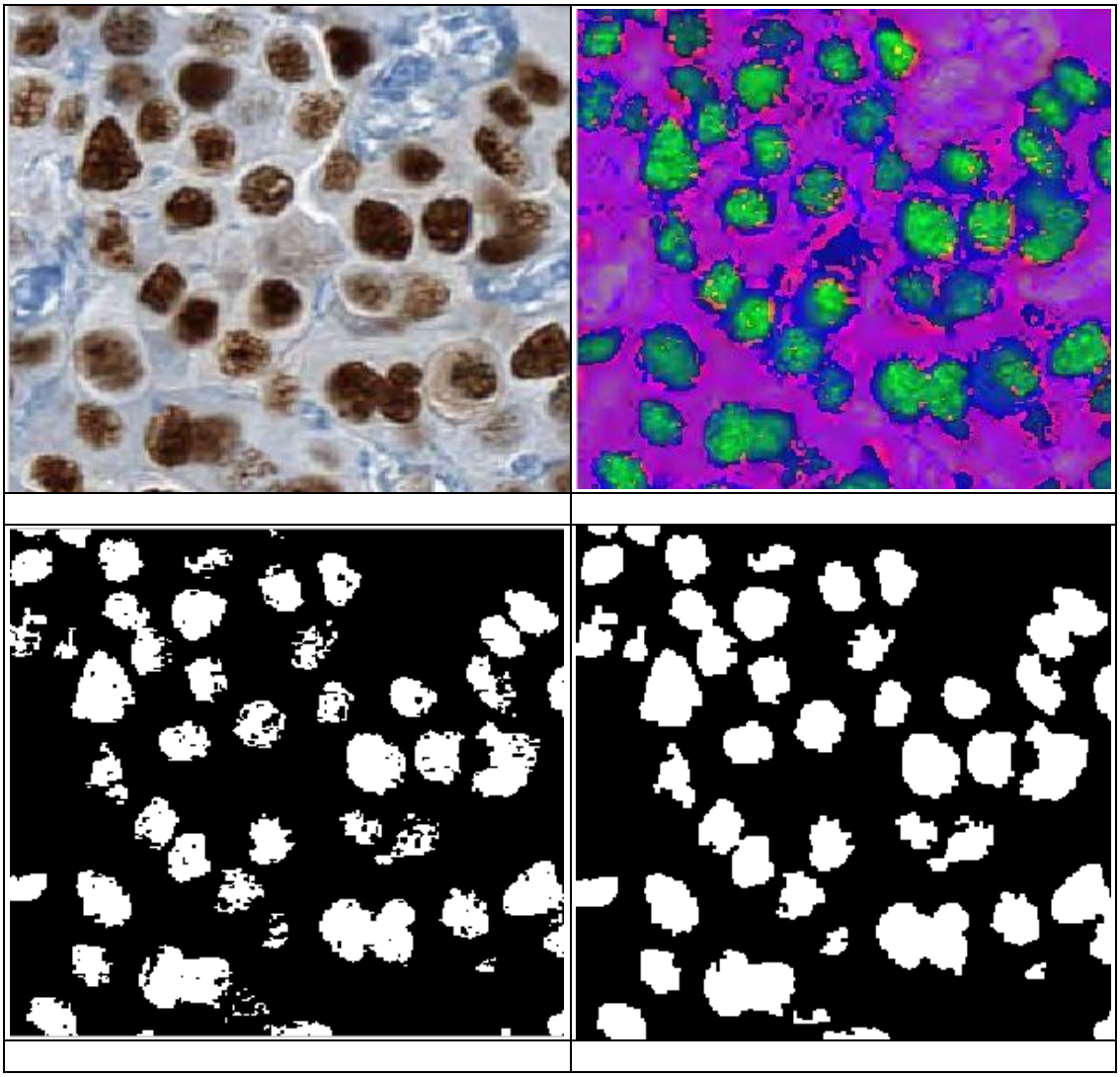

Figure 3: Images showing original RGB image (a), corresponding HSV image (b), after color thresholding (c) and after morphological operations (d).

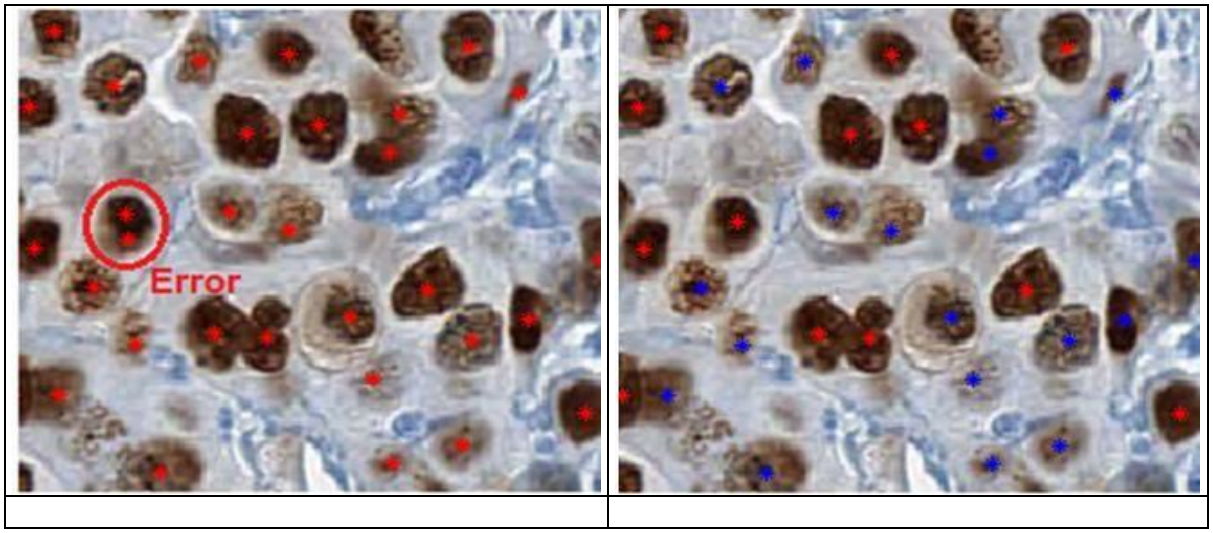

Figure 4: Images showing segmented image with over-segmentation errors (a), and rectified over-segmentation errors as well as classification into high intensity (red) and low intensity (blue) cells (b)

5. Image Processing Implementation for HER2

HER2,[16] evaluation is generally done by estimating the spread of stained region and hence, we can evaluate the sample by training the color pixel classifier to recognize

only the pixels that represent the cell membrane.

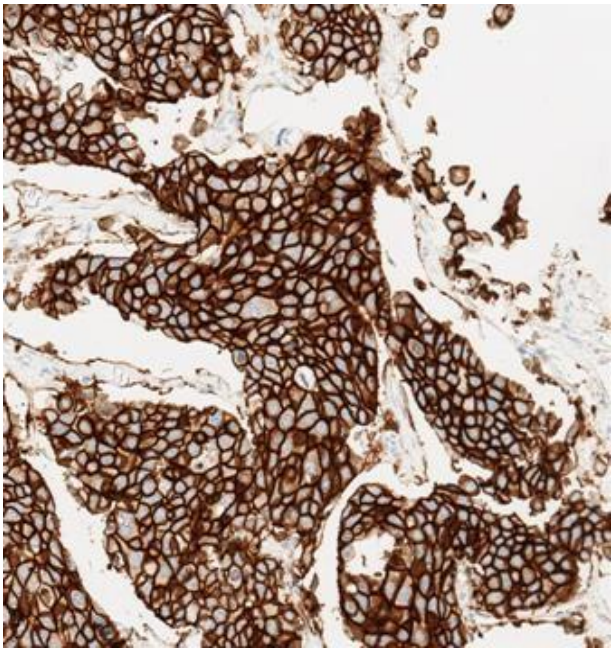

Figure 5: Typical HER2 sample with 3+ staining of cell membranes.

This is done by evaluating the image at various points on the cell membrane and obtaining a threshold for the color pixel classifier. The resulting image after color pixel classification is as shown in Fig. 7.

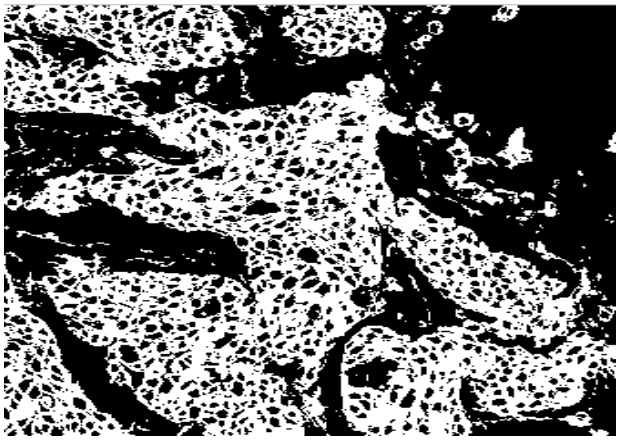

Figure 6: Image after color pixel classification. The region in white corresponds to the cell membrane of cancer affected cell.

The sizes of the image used are constant and equal to 1024 x 1024 pixels. The ratio that determines the extent of cancer is given by Equation (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. The scoring is done as indicated in Table II.

Table II: Percentage staining and corresponding score for HER2 evaluation.

Staining percentage	Score	Indication
>20%	3+	Positive
>5% and <20%	2+	Mildly Positive
>1% and <5%	1+	Mildly Negative
<1%	0+	Negative

Table III: Summary of results of modified watershed algorithm obtained for 30 Estrogen and stain calculation of HER2.

HER2 Image ID number	Percentage staining as calculated by algorithm (%)	Corresponding score by algorithm	Score by histo-pathologist	ER Image ID number	Number of cells detected by algorithm	Number of cells counted by histo-pathologist	Percent accuracy (%)
HER2_1	0.0101	0+	0+	ER_1	382	402	95.02
HER2_2	0.0027	0+	0+	ER_2	453	490	92.45
HER2_3	1.1605	1+	1+	ER_3	782	852	91.78
HER2_4	0.0464	1+	1+	ER_4	861	914	94.20
HER2_5	0.0105	0+	0+	ER_5	256	275	93.09
HER2_6	33.4243	3+	3+	ER_6	333	344	96.8
HER2_7	40.4417	3+	3+	ER_7	461	498	92.57
HER2_8	37.7324	3+	3+	ER_8	968	881	109.88
HER2_9	44.3087	3+	3+	ER_9	901	830	108.55
HER2_10	0.0000	0+	0+	ER_10	913	1022	89.33
HER2_11	0.0013	0+	0+	ER_11	1081	1004	107.67
HER2_12	0.0016	0+	0+	ER_12	858	816	105.15
HER2_13	0.0015	0+	0+	ER_13	485	447	108.50
HER2_14	0.7220	1+	1+	ER_14	52	59	88.14
HER2_15	0.1758	1+	1+	ER_15	435	389	111.83
HER2_16	0.5336	1+	1+	ER_16	620	663	93.51

HER2_17	0.5695	1+	1+	ER_17	470	492	95.53
HER2_18	0.1162	1+	1+	ER_18	423	464	91.16
HER2_19	0.0006	0+	0+	ER_19	473	428	110.51
HER2_20	0.0215	1+	1+	ER_20	363	336	108.04
HER2_21	0.0059	**0+**	**1+**	ER_21	451	403	111.91
HER2_22	6.8357	2+	2+	ER_22	306	324	94.44
HER2_23	3.6821	2+	2+	ER_23	201	208	96.63
HER2_24	4.0943	2+	2+	ER_24	180	189	95.24
HER2_25	5.8999	2+	2+	ER_25	659	615	107.15
HER2_26	3.7085	**2+**	**1+**	ER_26	670	639	104.85
HER2_27	8.9539	2+	2+	ER_27	932	957	97.39
HER2_28	4.7415	**2+**	**1+**	ER_28	558	522	106.90
HER2_29	0.1017	**1+**	**0+**	ER_29	625	605	103.31
HER2_30	1.5159	1+	1+	ER_30	740	785	94.27

Table IV: Statistical analysis for HER2 results.

Algorithm	Dr		Total
	Positive	Negative	
Positive	20	1	21
Negative	1	8	9
Total	21	9	30

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 III above. 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. 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. THE TABLE IV statistical analysis.

6. Classification and Correlation of IHC Markers

The results obtained by the Cell Count algorithm for IHC ER/PR markers in estimating the severity of the breast cancer score is based on the cell count. The Stain Measure Algorithm for IHC - HER2 marker estimates the extent of cancer based on the breast tissue sample, as shown in Table V and its statistical analysis is shown in Table V.

IHC markers ER, PR and HER2 are evaluated individually. The correlation of ER, PR and HER2 for one patient is a predictive and prognosis evaluation of the breast cancer patient. The correlation of ER, PR and HER2 identifies a three-marker method.

Table V: Comparison of IHC – Markers and Doctors score.

Sr. No. One Patient	ER Algm	ER Dr AJ	PR Algm	PR Dr AJ	HER2 Algm	HER2 Dr AJ
1	-ve	-ve	-ve	-ve	-ve	-ve
2	-ve	-ve	-ve	+ve	+ve	-ve
3	+ve	+ve	-ve	-ve	+ve	+ve
4	+ve	+ve	+ve	+ve	+ve	+ve
5	+ve	+ve	-ve	+ve	+ve	+ve
6	+ve	+ve	-ve	-ve	+ve	-ve
7	-ve	-ve	+ve	+ve	+ve	+ve
8	-ve	-ve	+ve	-ve	+ve	+ve
9	+ve	-ve	+ve	+ve	-ve	+ve
10	+ve	+ve	+ve	+ve	-ve	-ve
11	+ve	+ve	+ve	+ve	+ve	+ve
12	+ve	-ve	+ve	-ve	+ve	+ve
13	+ve	+ve	+ve	+ve	+ve	+ve
14	+ve	+ve	+ve	+ve	-ve	+ve
15	-ve	-ve	+ve	-ve	+ve	+ve
16	+ve	+ve	+ve	+ve	-ve	+ve
17	+ve	+ve	+ve	+ve	+ve	+ve

+ve: Positive, -ve: Negative, Algm: Algorithm

Classification: The correlation of the IHC markers ER, PR and HER2 and its prognosis classified by three marker methods is shown in Table VI. In addition to this, correlation of the IHC markers such as ER, PR, and HER2 is also evaluated for 17 - single breast cancer patient.

Table VI: Classification Three Marker Method One Patient.[14]

SUB TYPE	ER and /or PR	HER Over expression
Luminal A Subtype	+ve	-ve
Luminal B Subtype	+ve	+ve
HER2 Subtype	-ve	+ve
Unclassified Subtype	-ve	-ve

Table VII: Statistical Analysis of IHC-ER, PR and HER2 markers.

Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ER	83.33	100.00	77.78	71.42	88.23
PR	75.00	60.00	81.81	50.00	70.58
HER2	83.33	40.00	76.92	50.00	70.58

The accuracy is calculated as shown in Table VIII based on the results of IHC markers on single patient along with the comparison of classification algorithm and Doctors score is as shown in the Table IX and its graph is plotted as shown in Figure 6.

Table VIII: Correlation of IHC – ER, PR, HER2 on Single Patient.

Subtype	ER/PR	HER2	EGFR-Ck5/6	Algorithm	Dr. AJ	Accuracy %
Luminal A	+	-	- Or +	04	04	100
Luminal B	+	+	- Or +	10	09	90.00
HER2	-	+	- Or +	02	03	66.66
Unclassified	-	-	-	01	01	100

Correlation of IHC - ER PR and HER2

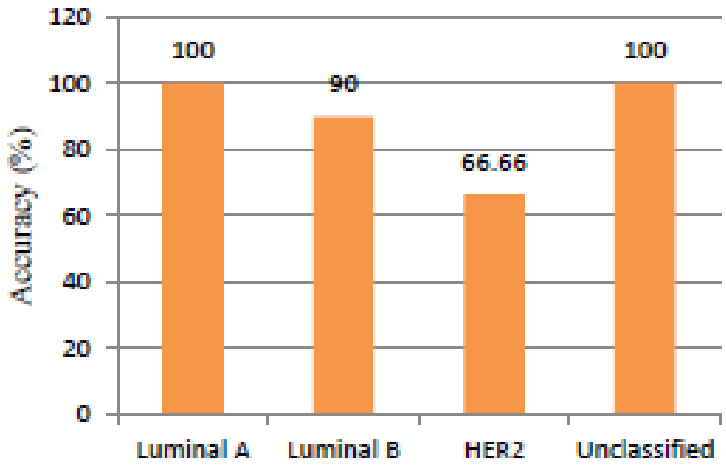

Figure 6: Result for Correlation of IHC - ER, PR, HER2 Markers.

Table IX: Classification of IHC Markers.

One Patient	ER_Algm	ER_Dr	PR_Algm	PR_Dr	HER2 Algm	HER2 Dr	Classification Algorithm	Classification by Dr
1	-ve	-ve	+ve	-ve	-ve	-ve	Unclassified	Unclassified
2	-ve	-ve	-ve	+ve	+ve	+ve	HER2	Luminal B
3	+ve	+ve	-ve	-ve	+ve	+ve	Luminal B	Luminal B
4	+ve	+ve	+ve	+ve	+ve	+ve	Luminal B	Luminal B
5	+ve	+ve	-ve	-ve	+ve	+ve	Luminal B	Luminal B
6	+ve	+ve	-ve	+ve	+ve	+ve	Luminal B	Luminal B
7	-ve	-ve	+ve	+ve	+ve	+ve	Luminal B	Luminal B

8	-ve	+ve	+ve	-ve	+ve	+ve	Luminal B	Luminal B
9	+ve	+ve	+ve	+ve	+ve	+ve	Luminal A	Luminal B
10	+ve	+ve	+ve	+ve	-ve	-ve	Luminal A	Luminal A
11	+ve	+ve	+ve	+ve	+ve	-ve	Luminal B	Luminal A
12	+ve	-ve	+ve	-ve	+ve	+ve	Luminal B	HER2
13	+ve	-ve	+ve	-ve	+ve	+ve	Luminal B	HER2
14	+ve	+ve	+ve	+ve	-ve	+ve	Luminal A	Luminal B
15	-ve	-ve	-ve	-ve	+ve	+ve	HER2	HER2
15	+ve	+ve	+ve	+ve	-ve	-ve	Luminal A	Luminal A
17	+ve	+ve	+ve	+ve	+ve	-ve	Luminal B	Luminal A

7. Hardware Implementation For HER2 IHC Marker

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 Figure (7).

Figure 7: 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 X. 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 x 1024 pixels. The ratio that determines the extent of cancer is given by Equation (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.

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.

Table X: Result of HER2 Images – Evaluation of MATLAB, Hardware implementation and Doctors truth reality.

Sr. 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+

Table XI: Statistical Analysis.

MATLAB and Doctors						Hardware and Doctors					
Dr.	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NP V (%)**	**Accuracy (%)**	**Dr.**	**Sensi-tivity (%)**	**Speci-ficity (%)**	**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

CONCLUSION

The IHC marker ER and PR cell count is obtained by the modified watershed algorithm for ER and PR and is compared to the count obtained by manual analysis by the histo-pathologist for each image. The HER2 images were shown to the same pathologist for scoring. Table III shows a summary of the results obtained for 30 Estrogen Receptor images and 30 HER2 images as compared to the results provided by histo-pathologist. The evaluation of ER had an accuracy of 99.86%, PR had an accuracy of 94.33% and for HER2 had an accuracy of 86.67%. The correlation of IHC markers ER, PR and HER2 and its prognosis classified by three marker method and results of the same are validated with the Doctors and presented in the result Table IX. The statistical analysis shown in the result table has more than 75% accuracy. The HER2 30 images are evaluated by the algorithm and implemented by hardware as shown in Table X. The statistical analysis of MATLAB and Doctors ad Hardware and Doctors are as shown in the Table XI.

ACKNOWLEDGMENT

The authors gratefully acknowledge the guidance of Dr. Aparna Joshi, MD pathologist, consultant histopathologist, at KEM hospital, Pune, India, in the field of immuno-histo- chemical analysis of tissue samples, The authors gratefully acknowledge the guidance of Dr. Mayur Kulkarni, MD radiologist, Pune, and Dr. Kavita Kulkarni, MD Pathologist, Pune, India. The authors thank the BCUD, University of Pune for the funding for the research grant and Principal and faculty of Matoshri College of Engineering and Research Centre, Nashik and Sinhgad COE Research Center, Pune for providing an opportunity to conduct this research. The author Dr. Prasanna Shete is very grateful, thankful to his guide Dr. G.K.Kharate and also to those who has directly or indirectly supported, motivated in completion of the research work.

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