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Breast Cancer Cell Detection Using Digital Image Processing

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Abstract— The paper discusses an image processing method that aims at making more accurate estimates of the population of cancerous cells in the blood sample of a patient suffering from breast cancer. The method aims at providing a reliable, repeatable, and fast alternative that could replace the traditional method of manual examination and subsequent estimation. A marker, known as the Estrogen Receptor (ER), is used to identify cancer cells in the tissue sample. The methods involved in image processing are HSV color conversion from RGB image, Hue, saturation and value based object-background separation, morphological operations such as dilation and closing, and area based filtering for preliminary preparation of image for detailed analysis. Next, 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. Further, intensity based thresholding is performed for identifying and categorizing the cancerous cells into levels of severity of damage done to cells due to cancer. The proposed modified watershed algorithm is compared with the original watershed algorithm and an accuracy of almost 96% was observed and verified.

Keywords—Cancer cells, Estrogen receptor, HSV model based object-background separation, intensity based cancer cell counting, modified watershed algorithm.

I. 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) is one of the most reliable receptor which is 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.

II. 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 (Fig. 1) while non-cancerous cells develop a bluish shade.

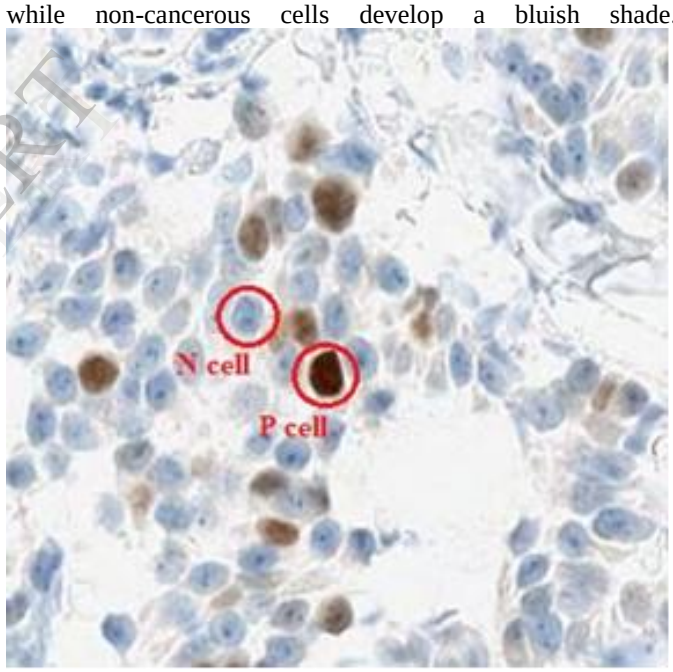

Fig. 1: Progesterone receptor: stained tissue sample showing cancer positive/P (stained brown) and cancer negative/N (stained blue) cells.

A. 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) is of prime importance. Other important receptors frequently used in pathological analysis include progesterone receptor (PR) and HER2 [16] (Table I).

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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

B. 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. Fluorescence-In-Situ-Hybridization (FISH) is another method for analyzing breast cancer tissue for HER2 receptor. Although it is more accurate than IHC method, it tends to be more expensive. Therefore we focus our research on the IHC method only.

III. LITERATURE SURVEY

A number of approaches have been suggested by various authors for developing effective algorithms. In [20], the authors proposed a marker controlled watershed algorithm to avoid the over-segmentation errors while applying the classic watershed algorithm.

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 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.

IV. IMAGE PROCESSING IMPLEMENTATION

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

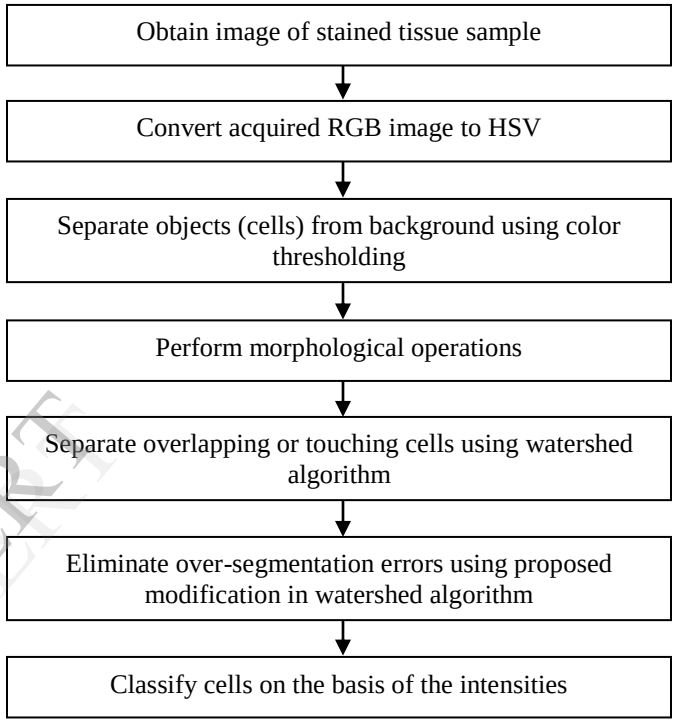


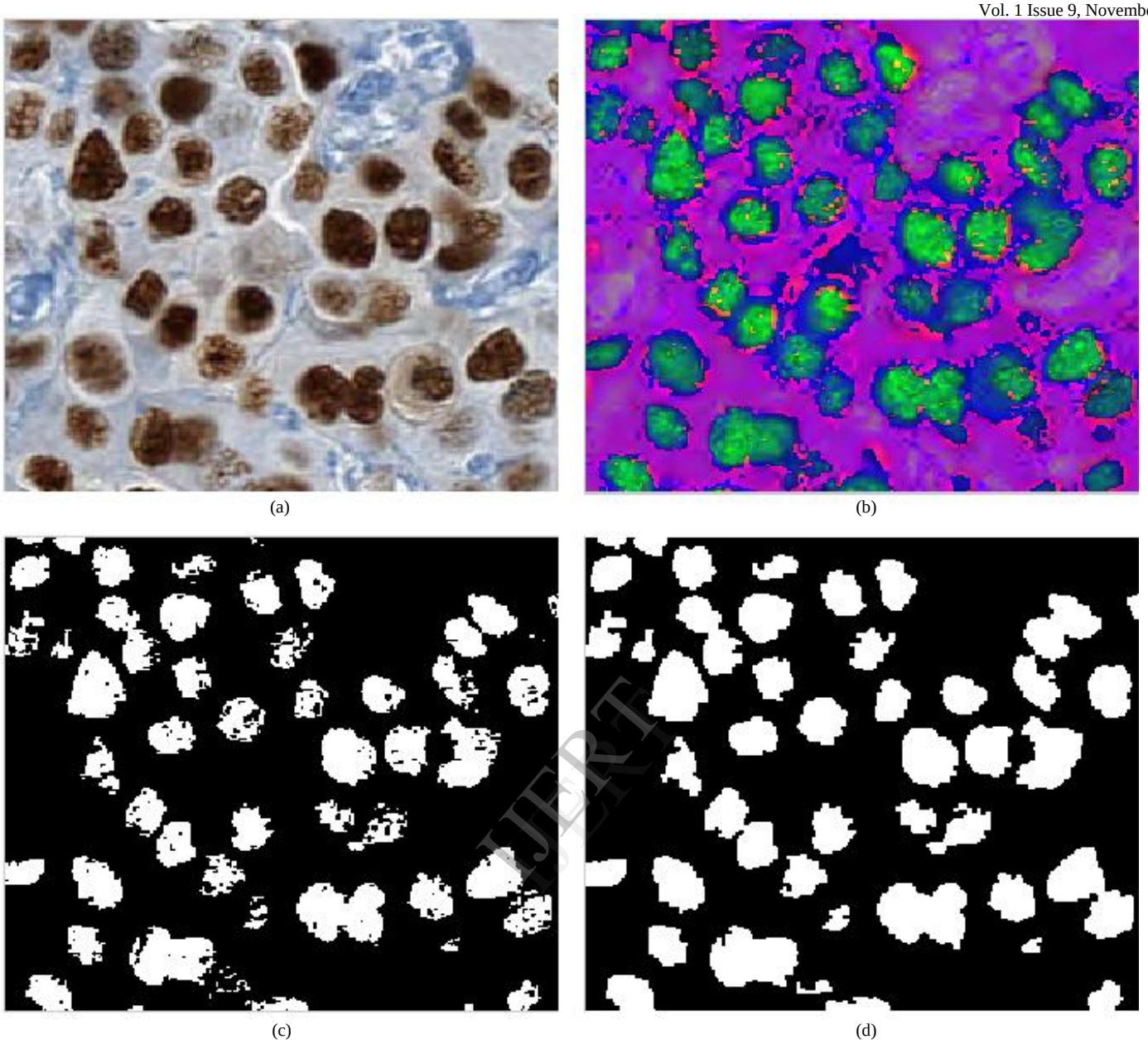
Fig. 2: Flowchart showing the steps involved in image processing implementation.

A. Obtaining stained tissue sample image

The pathologists acquire a tissue sample from a patient under test and treat this tissue sample with hormones to produce antigen-antibody reactions causing cells to stain and undergo a change in color. The glass slide is then placed under a microscope capable of magnification up to 80X. A camera mounted on the eyepiece of the microscope can be used to capture the magnified image.

The images used for our research were of ER stained samples in PNG format with an RGB color model and acquired using the process described above.

MATLAB supports the PNG format and the image can be brought into the workspace by simply using the *imread ('filename.png')* command. Fig. 3 (a) shows one of the images loaded into the MATLAB workspace.



(a) (b) (c) (d)

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

B. Converting acquired RGB image into HSV

The HSV color space (Hue, Saturation, and Value) is used because it provides much more details than the RGB color space does.

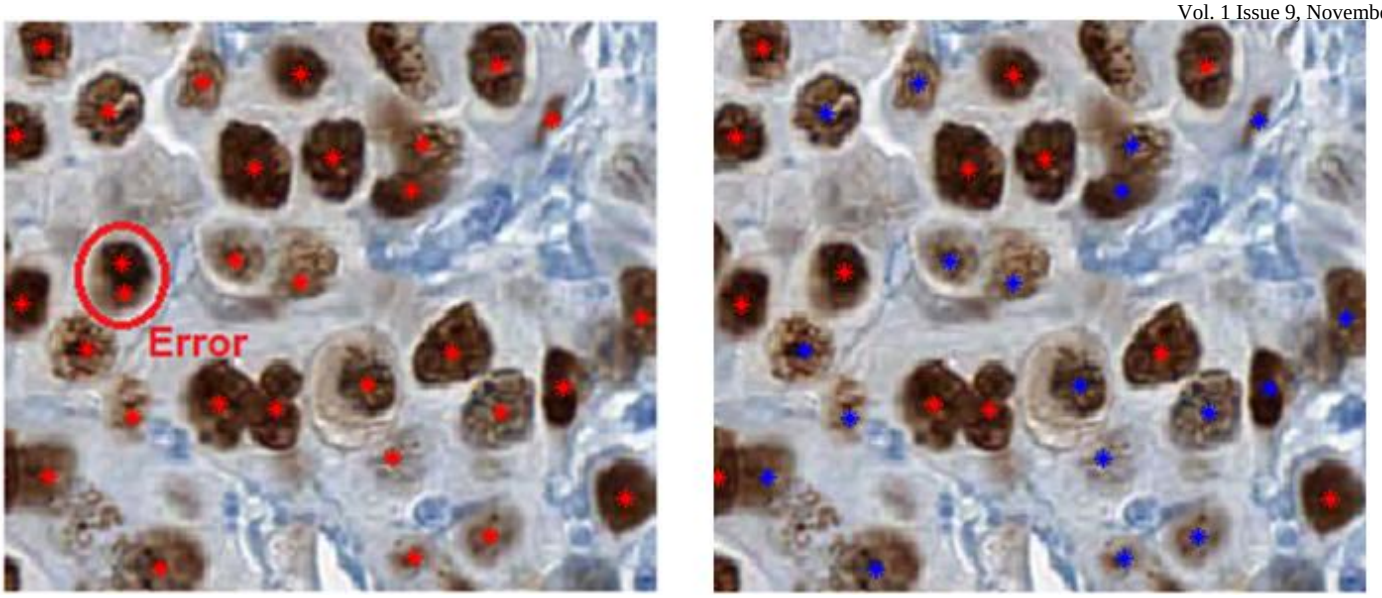
The 'hue' corresponds to all the colors: red, yellow, green, cyan, blue and magenta. With change in the magnitude of 'saturation', the variation from unsaturated (shades of gray) to fully saturated (absence of white component) occurs. The value component corresponds to various levels of brightness. Thus the HSV color model provides additional information regarding color, shade and brightness as compared to RGB color model.

As can be seen in Fig. 3 (b), only the brown cancer positive cells have been highlighted by green, cell edges in blue and all cancer negative cells as well as the background in pink. The *rgb2hsv (variable)* command returns the HSV image to the user.

C. Separating cells from background

Now, the primary task is to extract only those regions that need to be considered for analysis, namely those regions where the cancerous cells are present. On observing the HSV image, (Fig. 3 (b)), we see that the green shaded regions correspond to the cells which need to be evaluated for counting.

We separate the HSV image into its three channels namely hue, saturation and value and pixels that do not satisfy the "green" shaded regions are removed. The cell boundaries, as observed in Fig. 3(b), are represented in blue color. Thus by eliminating (by thresholding also known as region-growing based on intensities), [1] pink and blue regions and retaining only the green ones, separation of touching cells becomes an easier task in further analysis. This is an advantage of the HSV color scheme over the RGB color scheme [25], [26]. This process of classifying pixels to belong to a cell or background



(a) (b)

Fig. 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).

generates a binary image with pixels satisfying the color range marked by logical ones (white) and the rest marked as logical zeroes (black). This returned image (Fig. 3 (c)) contains irregular object edges as well as holes which need to be removed using further morphological operations.

D. Perform morphological operations

The thresholding process returns the regions of cells but there is a problem of rough cell edges and holes in the bodies of the cells. During further analysis of the sample, these problems cause inaccurate results. Thus it is necessary to use the following morphological operations [27]: (1) Dilation, causing the surfaces to develop smoother edges and (2) Closing, causing holes in the surface of the cells to be filled in. Fig. 3 (d) shows the morphologically modified version of the original image.

In MATLAB, operations such as *imdilate (variable)* and *imclose (variable)* are used for dilation and closing respectively.

E. Separating touching or overlapping cells

The tissue sample will always contain a few cells that either overlap or just touch each others' boundaries. Such cells may be evaluated to be a single cell; giving inaccurate results. A solution to this problem is to use the watershed algorithm [28]-[38] which, in simpler words, searches for regions with sudden changes in intensities and identify these as boundaries. The word watershed is a geographical term often used to refer to the region between two highlands where water collects during monsoon.

Mathematically, the watershed algorithm is implemented by applying the distance transform [27]. The distance transform involves calculation of the separation of points in the image. In MATLAB, the *bwdist (variable)* function calculates the distance between each pixel whose value is zero and the nearest nonzero pixel for binary images.

F. Eliminating over-segmentation errors

As seen in Fig. 4 (a), there are a few errors due to over-segmentation. These errors arise when a single cell is divided into multiple regions due to intensity variations within the cell. Thus a single cell might be counted more than once due to these multiple segments.

A simple algorithm was designed by us that calculated the distances between any two centroids of segments (Fig. 5). A minimum distance threshold value was calculated and all calculated distances were compared to this threshold (Equation (1)). The distances that were below this threshold value were identified as erroneous segments and were eliminated. The new centroid was calculated by averaging the coordinates of the error centroids.

$$d_i = \left(\sqrt{\left(x_i^{\,2} - x_{i+1}^{\,2} \right) + \left(y_i^{\,2} - y_{i+1}^{\,2} \right)} \right)_{i=1}^{i=n} \qquad (1)$$

Where 'i' represents the current segment under consideration and 'n' represents the total number of segments.

Fig. 5: Representative image showing the concept of elimination of over-segmentation errors

Using (1), the distances were calculated in a number of iterations till all segments have been considered. Fig. 5 expounds, pictorially, the idea behind this algorithm.

TABLE II
Summary of results of modified watershed algorithm obtained for 7 Estrogen receptor images as compared to the classic watershed algorithm.

Image ID number	Total number of cells	Percentage accuracy of classic watershed algorithm	Percentage error for classic watershed algorithm	Percentage accuracy of modified watershed algorithm	Percentage error for modified watershed algorithm
1	42	47.62%	52.38%	97.62%	2.38%
2	120	14.17%	85.83%	91.67%	8.33%
3	130	60.00%	40.00%	98.47%	1.53%
4	210	54.29%	45.71%	98.58%	1.42%
5	213	64.79%	35.21%	97.66%	2.34%
6	440	36.60%	63.40%	95.50%	4.50%
7	565	69.92%	30.08%	95.58%	4.42%
Total/Average %	1720	49.63%	50.37%	96.44%	3.56%

G. Categorizing cells on the basis of their intensities

Pathologists often require the cells to be classified into categories on the basis of the intensities. This can be done by obtaining the RGB information at the center and around it for each cell followed by thresholding, Fig. 4 (b). In MATLAB, the regionprops (variable) command is used to obtain coordinates of recognized segments. Then the impixel (variable, coordinates) is used to obtain RGB information at that point from the original image. The RGB information is then compared to fixed thresholds corresponding to different intensity levels.

V. RESULTS AND DISCUSSION

The count obtained by the modified watershed algorithm was compared to the count obtained by manual analysis by the pathologist. Table II shows a summary of the results obtained when the modified watershed algorithm was implemented on seven of the images that were provided as well as the results of the original watershed algorithm implemented on the same set of images. An average accuracy of 96.44% can be obtained in this way. The table also shows the results for the implementation of the classic watershed algorithm. The percentage accuracy in this case is less than 50% indicating a high number of errors due to over-segmentation. There is a possibility where our technique may fail. If a cell of smaller size is on the periphery of a cell containing the over-segmentation error or in the case of an elongated cell (Fig. 6), the algorithm might wrongly interpret the first situation as an error due to small inter-centroid distance and the second situation having no error due to larger inter-centroid distance, both instances causing an unacceptable error in count.

Fig. 6: Incorrectly interpreted distances

A remedy to this is to involve the user in a review process that allows him/her to either confirm or reject the result produced for each instance.

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.

Fig. 7 shows the graphical summary of implementation of the modified watershed algorithm on Image ID number 5.

VI. IMPACT

As prescribing the treatment to a cancer patient is a matter of great responsibility, the results presented to the pathologists must be accurate and reliable. An error due to a miss or a false positive result may be catastrophic if the wrong medication is administered to the patient.

It will still be a while before a pathologist will completely trust an automated algorithm to make decisions on his/her behalf. There may be a possibility in the future when the algorithm might perform even better than a pathologist in making decisions on the basis of numerical data instead of the pathologist's instinct.

The algorithm is, and will always be, only a tool used by pathologists to help them in the tedious work of counting cell population and not a replacement of the pathologist. The time saved by pathologists can be better utilized to address even greater problems in the field of bio-medical sciences.

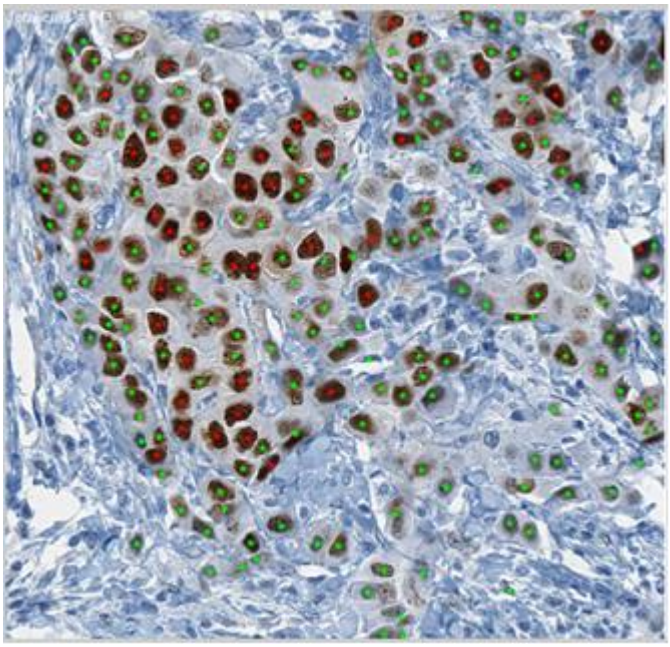


Fig. 7: The breast cancer tissue showing the implementation of modified watershed algorithm as well as intensity based categorization. The cells marked in green are lower in intensity while the cells marked in red are of higher intensity.

VII. FUTURE WORK

The threshold level for minimum distance used in the algorithm is manually chosen for now as this set value satisfies the images when factors such as magnification remain fairly constant. For the algorithm to work with different magnification levels, a new threshold value needs to be manually evaluated. This process of calculating an optimal distance can be automated for a more generalized implementation of the algorithm.

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