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Optimization of Digital Histopathology Image Quality

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ABSTRACT

One of the biomedical image problems is the appearance of the bubbles in the slide that could occur when air passes through the slide during the preparation process. These bubbles may complicate the process of analysing the histopathological images. Aims: The objective of this study is to remove the bubble noise from the histopathology images, and then predict the tissues that underlie it using the fuzzy controller in cases of remote pathological diagnosis. Methods: Fuzzy logic uses the linguistic definition to recognize the relationship between the input and the activity, rather than using difficult numerical equation. Mainly there are five parts, starting with accepting the image, passing through removing the bubbles, and ending with predict the tissues. These were implemented by defining membership functions between colours range using MATLAB. Results: 50 histopathological images were tested on four types of membership functions (MF); the results show that (nine-triangular) MF get 75.4% correctly predicted pixels versus 69.1, 72.31 and 72% for (five-triangular), (five-Gaussian) and (nine-Gaussian) respectively. Conclusions: In line with the era of digitally driven e-pathology, this process is essentially recommended to ensure quality interpretation and analyses of the processed slides; thus overcoming relevant limitations.

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1. INTRODUCTION

Histopathology is the field which studies the diseased tissues, that is composed of three Greek terms: histos, pathos and logia, which means “studying diseases suffering tissue” [1]. The pathology lab receives the biopsy that was taken from the patients, which are then stained, placed on glass slides to highlight particular structures [2].

The slides are usually mounted on the tissue sections using mounting media to protect the stained slides from damage. During that process, artefacts may appear and affect the staining results. These artefacts may be any structure that must not be present in the tissue normally [3]. Artefacts may alter the important morphologic and cytological features of the cells leading in sometimes to complete obscure of the image. Air bubbles occur either during tissue floating in the water bath or during covering [4].

Following the era of e pathology, and along with the pioneering initiatives of pathologists who are using digital imaging systems, in this paper, we have focused on removing the air bubbles that distort the vision accuracy and thus impede slide interpretation. Digital pathology is currently considered a very useful aid in assisting diagnosis of suspected cases and enabling exchange of remote consultations among specialists all over the world [5].

Many Problems faced the pathologists during their work; one of them is the appearance of bubble in the digital image slide. The objective of this paper is to design software for removing this artefact and predict the underlying tissue.

The main benefit of fuzzy logic is characterized in having the ability to control nonlinear system, which would be troublesome to demonstrate numerically [6]. Fuzzy control techniques are represented by a matter of fact and trials instead of mathematical models. Fuzzy control systems include an extensive number of inputs; most are related to some conditions which are conducted when the related condition is triggered. Accordingly, minimal extra computational burden is needed to accommodate additional rules [7].

The fuzzy controller has four primary parts as shown in Figure 1: starting with "rule-base" which holds the information, as an arrangement of rules, on the best way to control the system. The "inference mechanism" assesses which control rules are significant at the current time, and later on chooses what contribution ought to be planned. The “fuzzification” interface changes the inputs to be deciphered and matched with the rules available in the “rule-base. Finally, the “defuzzification” interface changes the decision approved by the inference mechanism into the contributions of the planned system [8].

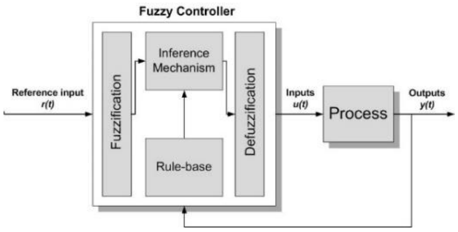


Figure 1. Fuzzy Controller Architecture

2. RESEARCH METHOD

Our system consists of five main parts executed using MATLAB 2016a, starting with input histopathology image, which was corrupted or damaged with bubble, ending with a clear image without the bubbles.

2.1. Input Image

The system starts by accepting any type of histopathological image containing bubbles as an input, as shown in Figure 2.

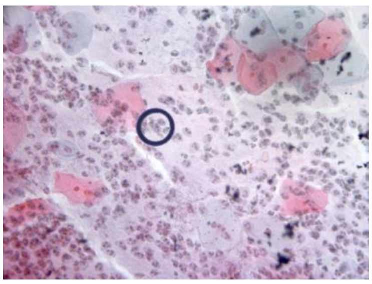


Figure 2. Histopathological image with bubble

2.2. Bubble Detection

The bubble detection process is implemented by two stages; firstly the program will ask the user to select three points on the outer circumference of the bubble. Secondly, the user will be asked again to select three points on the inner circumference of the bubble. The idea behind selecting three points only in each time is explained on the bases that one and only one unique circle will pass through three non-collinear points which is the minimum requirements to define a circle. From the previous selections, the centre point and radius could be defined as below:

$$(x - x_c)^2 + (y - y_c)^2 - r^2 = 0 \quad (1)$$

Where (xc,yc) is the coordinates of the centre, (x,y) is the coordinates for any point that lay on the circle circumference and r is the radius. Thus, it will be easy to draw two circles, one covers the whole bubble (outer-circle) and other covers the inner-circle as shown in Figure 3.

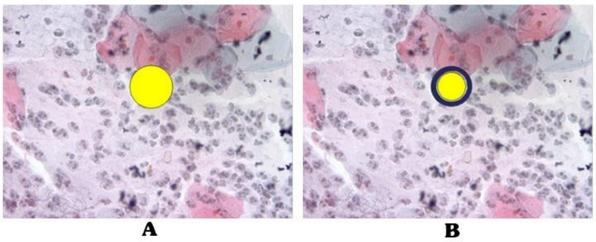


Figure 3. The output after selecting six points by the user. A: After selecting three points from outer-circle. B: After selecting three points from inner-circle

2.3. Removing The Bubble

After the bubble was detected in the previous stage, the importance of removing the bubble takes place at this level. This happens by comparing between the two images shown in Figure 4; the difference between image (A) and image (B) represents the bubble. The output of this stage is a histopathology image without bubbles which will be replaced by transparent pixels as shown in Figure 4-c.

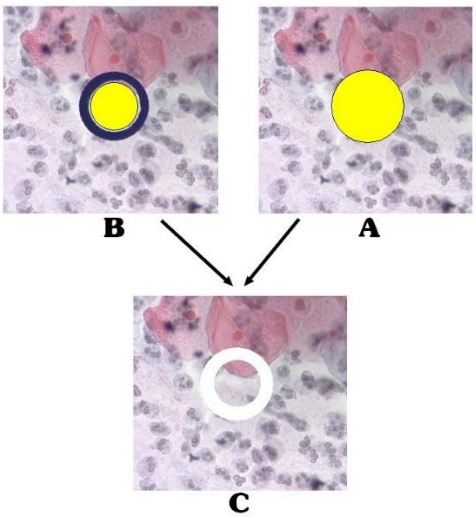


Figure 4. The difference between image (a) and image (b) represents the bubble
(c) Removing bubble from image

Definitely, the accuracy of selecting the six points by the user will improve the process of removing of the pixels that lay on a bubble. The nearest inner and outer boundaries of the circle around the bubble, the more accurate in removing the bubble pixels and preventing loss of pixels that do not lay in the bubble (corrupted pixels).

2.4. Prediction

In this stage, the Fuzzy controller was used to predict pixels that were removed in the previous stage. However, there are still some important preparations which are needed before prediction. First, the bubble region should be divided into four parts (upper left, upper right, lower left, and lower right) as shown in Figure 5.

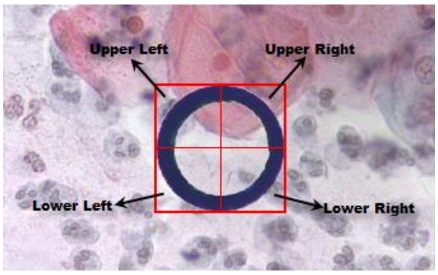


Figure 5. Bubble regions

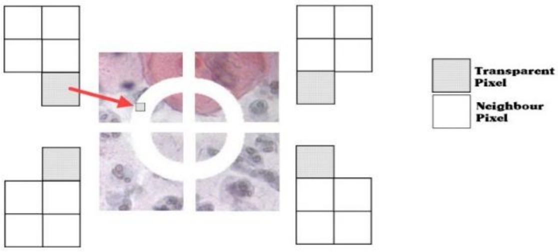


Figure 6. The places of pixel neighbors related to the transparent pixels

Each transparent pixel will take four neighbours according to its region to predict three values (red, green and blue components). The places of neighbouring pixel places differ from region to region; Figure 6 describes the places of these neighbours. This way prevents a colour from separating wildly and to achieve a high percentage of correction in finding the desired pixel colour. In this paper, four tests will be carried out, each one has number and type of membership functions (MF) to make a comparative study and find the best way for prediction, Table 1 describes these four tests.

Table 1. Membership functions parameters

	No. of Membership Function	Type of Membership Function
Test one	5	Triangle
Test two	5	Gaussian
Test three	9	Triangle
Test four	9	Gaussian

According to Figure 6, each test has four inputs and one output (four neighbours and one required pixel respectively) that are called MISO (multi-inputs, single output). Each test was executed three times to find red, green and blue values. For example in test one, there are four values for red components taken from the four neighbours to find the red value for the transparent pixel as shown in Figures 7 and 8. A subjective threshold was applied for detachment between 0 and 255, and in this paper, it may be triangle or Gaussian which allows an overlap and gradual changes between MFs; thus allowing fuzzy to find the value that didn’t lie exactly in the centre of MF by comparing among stored input MF.

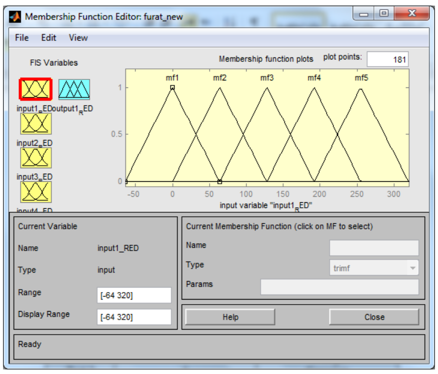


Figure 7. Input membership functions in Test one

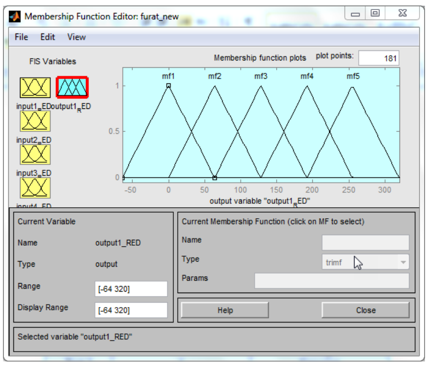


Figure 8. Output membership functions in Test one

The same way has been executed to find green and blue values, and so on for the next pixels. The fuzzy algorithm is based on a collection of logic rules in the form of If-Then statements(IF A and B and C and D THEN Z), where IF part is called the “antecedent” and THEN part is called the “consequent” which merged to produce “Fuzzy Conditional Statements” [9]. Figure 9 shows the rule-base for test one; it is noted that there are four inputs and one output MF. Equation 2 shows an example of a rule in test one which uses Mamdani fuzzy logic control.

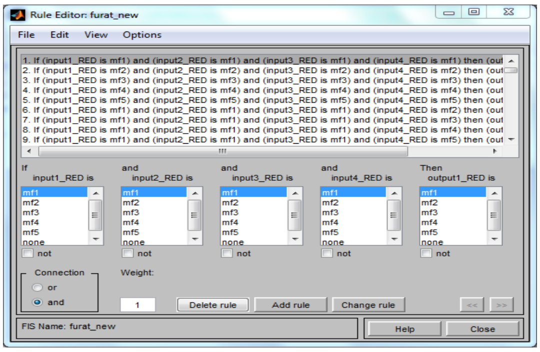


Figure 9. Rule-base for Test One

$$\text{if } \mathrm{input1_{red}} = \mathrm{MF1 \ AND \ input2_{red}} = \mathrm{MF1 \ AND \ input3_{red}} = \mathrm{MF1 \ AND \ input4_{red}} = \mathrm{MF1 \ THEN \ output1_{red}} = \mathrm{MF1} \quad (2)$$

Where input1red, input2red, input3red and input4red are the values of red component for the four neighbour pixels, output1RED is the red value of desire pixel and MF is the membership function.

3. RESULTS AND ANALYSIS

In this section, it is explained the results of research and at the same time is given the comprehensive discussion. Results can be presented in figures, graphs, tables and others that make the reader understand easily [2], [5]. The discussion can be made in several sub-chapters.

Fifty different histopathological images were executed in the four tests mentioned in Table 1. The way used to find the percentage of correct pixels was measured by taking the red, green and blue values that were produced by the fuzzy controller, and then compared with a reference image (which is the same as input image but before being corrupted by bubble). Then the difference between them was found as below:

$$\text{if } |\mathrm{input_{red}} - \mathrm{original_{red}}| \text{ AND } |\mathrm{input_{green}} - \mathrm{original_{green}}| \text{AND } |\mathrm{input_{blue}} - \mathrm{original_{blue}}| \leq 20 \text{ THEN pixel is correct} = \text{yes} \quad (3)$$

Where inputred , inputgreen and inputblue are the values of red, green and blue respectively for the pixels that were corrupted by the bubble and originalred, originalgreen and originalblue are the values of red, green and blue respectively for the reference pixels.

The idea beyond taking 20 instead of zero is based on the findings that the difference of 20 did not show an obvious effect on the colour degree. That was proved as shown in Figure 10 which illustrates the worst cases if the 20 is taken in consideration for the pixel having 70, 30 and 200 for red, green and blue respectively. After the number of corrected pixels has been calculated, then the percentage of correction should be calculated also by using below equation.

$$\text{Percentage of correction} = \frac{\text{No.of corrected pixels}}{\text{Total no.of pixels inside bubble}} * 100 \quad (4)$$

Table 2 shows the percentage of correction after applying the four tests, we can notice that the higher percentage is in test three (nine MFs with triangle type). As a test samples, final output, the input image (bubble exists) and the original image (bubble didn't exist) are shown in Table 3.

Figure 11 shows two consecutive MFs in test one and two respectively. According to Figure 11(a), the percentage of overlapped area (indexed by yellow colour) from any MF is 25%, measured by dividing the area of overlapped region from the whole triangle.

On other hands, in Figure 11(b), the probability density function for the overlapped normal distribution that is also indexed in yellow colour is less than 25% [10].

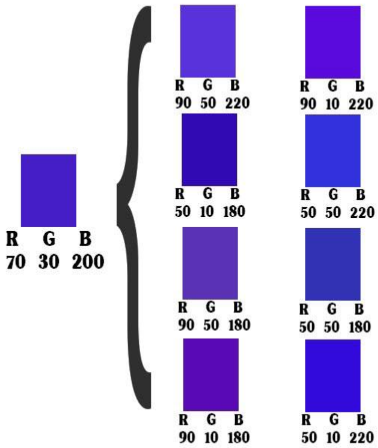


Figure 10. The worst cases if value of 20 is taken for difference

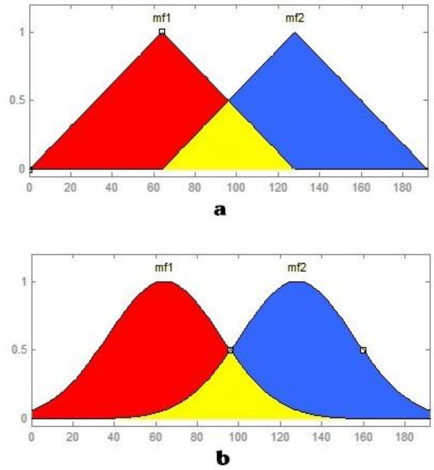


Figure 11. Two consecutive membership functions, (a) for test one, (b) for test two

Table 2. Percentage of correction for the four tests

Test No.	% of correction
One	69.1
Two	72.31
Three	75.4
Four	72

Table 3. Final Results (a) The input image (corrupted by bubble), (b) The original image and (c) Our system output

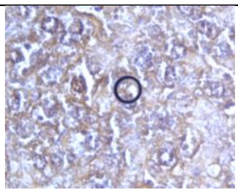
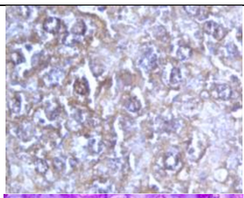
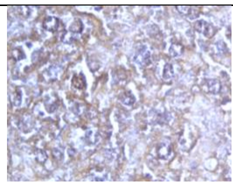
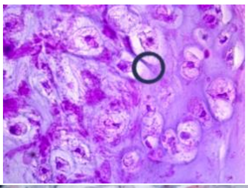
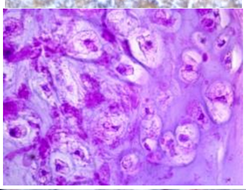
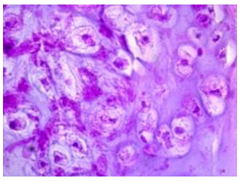
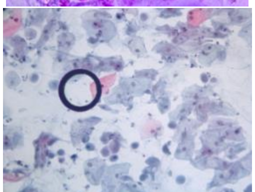
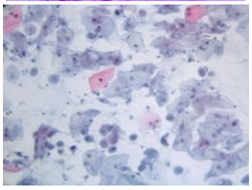
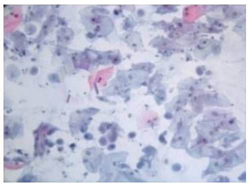
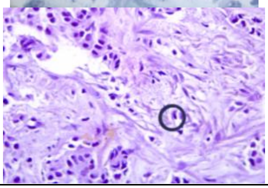
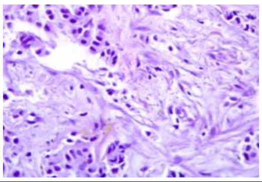
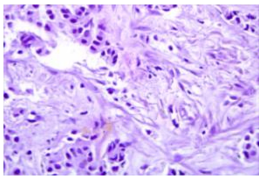
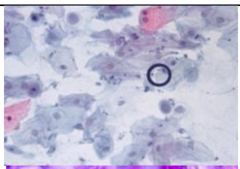
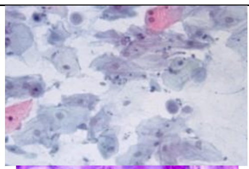
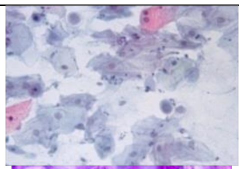
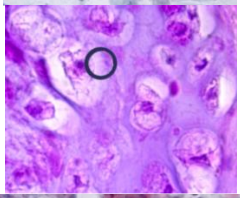
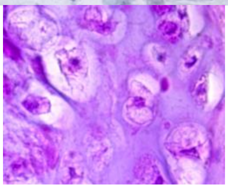
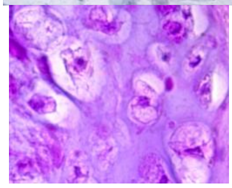
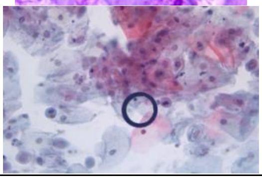
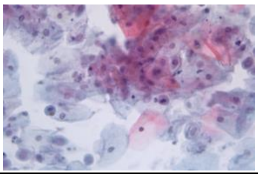
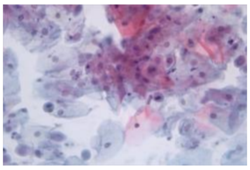
a	b	c
		
		
		
		

Table 3. Final Results (a): The input image (corrupted by bubble), (b): The original image and (c): Our system output

a	b	c
		
		
		

4. CONCLUSION

In our system, we use image processing techniques to detect and remove bubble artefact pixels, and then fuzzy controller is used for pixels predication. Four tests in different types and number of MFs were carried out to find the accurate method. According to our results, the best prediction percentage was displayed when applying nine MFs with triangle type (75.4%). This percentage represents the correct colours of pixels (comparing with the image without bubble) that have been removed in previous step. It has been observed that the increase in the number of MFs lead to higher percentage of correction in the prediction process, while wider overlapping in the area between MF lead to fault prediction in pixel colours, especially if the number of rules is small because it will be far away from getting the desired colour. All the aforementioned results were directly proportional to the increase in the number of MFs. Such applicable technique is specifically recommended when attempting to diagnose stained pathology slides on remote basis. The limitations appear when the size of bubble is too big, that lead to decreasing the prediction percentage.

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