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The Effect of Image Enhancement Techniques in Segmenting Histopathological Images of Meningioma

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Abstract- *The application of image processing in the medical field is widely used among medical experts since it can help them by providing an earlier detection and treatment stages. Image enhancement plays an important role in medical images especially in diagnosing a tumor or cancer cells in an image. It will improve the visibility and detail of the image, which can help the expert's diagnosis. The Ki67 is a nuclear protein that was widely used among the pathologists to measure the proliferation of tumour cells. This paper aimed to study the performance of different image enhancement techniques in segmenting Ki67 cells. In this study, the proposed system used three image enhancement techniques, which are contrast stretching, histogram equalization, and CLAHE technique. The enhancement techniques were applied to the colored images. The resultant images then were used as the input images to segment the Ki67 cells. The experimental results show that the contrast stretching technique produced higher results compared to other enhancement types.*

Keywords-CLAHE technique, Contrast Stretching, Histogram Equalization, Ki67 cell, Segmentation

1. INTRODUCTION

Meningioma is a type of primary brain tumours. This tumour arises from the arachnoid mater. The arachnoid mater is one of three protective layers besides pia mater and dura mater that surrounds the brain. The previous study had shown the meningioma was the most frequently reported among the central nervous system (CNS) tumours, which account for 36.4%. Bellur and Chandra [1] stated that meningiomas in the diameter range of 0.5 to 2.7 cm are known to be a "small" meningioma, while the "large" meningioma indicated to a tumour cell that has a range of diameter from 2.8 to 10.5 cm. Ki67 is a nuclear antigen used to measure cell proliferation activity. The Ki67 is known as an independent prognostic factor for survival rates, which consist of all stages and grade categories [2]. Generally, pathologists will use the Ki67 labelling index (LI) to measure normal and abnormal cell proliferation.

Image pre-processing is an initial step in digital image processing. This step involves various techniques such as scaling, noise removal, resizing and enhancement. Image enhancement is used to enhance the visualization of the image so that it will be useful for further analysis. Frequently, the captured images from a microscope were lack of contrast and brightness, due to the illumination condition while capturing the image. Due to this problem, image enhancement is required to improve the quality of captured images. Generally, there are two methods of image enhancement, which are spatial domain and frequency domain methods. Spatial domain operates directly on the pixels of the image. Frequency domain method is a method that takes place on the Fourier transform of the respective image. This method operates on Fourier transform, discrete cosine and sine transform of the image [3]. This method can improve the quality of the current image by making changes in the transform coefficient functions.

This paper is organized as follows: Section 2 reviews the literature for image enhancement techniques and an overview of image enhancement techniques that have been proposed from the previous study in segmenting Ki67 cells. Section 3 discusses the procedures and methodology for segmenting the Ki67 cells. Section 4 highlights the results of the segmentation based on image enhancement techniques that were applied to the meningioma images. Section 5 concludes the paper.

2. RELATED WORKS

Image enhancement aims to improve the visualization of an image. Nowadays, the role of medical imaging technologies is growing bigger. The application is not only focusing on the diagnosis and treatment of disease but also in disease prevention, health check-up, major disease screening, treatment effect evaluation, and rehabilitation [4]. Existing methods show the application of enhancement techniques are more concentrated in the areas of the chest radiography, mammography, computed tomography, and other modalities of the medical image diagnosis system [5].

CT images are used to display the internal structure of the human body. With the aid of the image enhancement technique, the CT images can provide good contrast between different soft tissues of the body, which make it useful in diagnosing the brain, muscles, and cancers [5]. Breast cancer is the most frequently diagnosed life-threatening in women. However, the low contrast of mammographic images makes the analysis become complicated especially in identifying signs such as bilateral asymmetry and architectural distortion [6].

Most of the medical images were captured by using a hardware device. The issue that occurred was the output images produced from the device did not give the proper information for analysis of disease. This is due to electrical fluctuations, environmental change, and lack of expertise in using those devices [7]. Here, the enhancement technique will be useful especially in enhancing the low contrast images.

2.1 Image Enhancement Techniques

2.1.1 Contrast Stretching

The contrast stretching was used to improve dark or low contrast objects in an image by applying appropriate tone correction to the objects. The contrast stretching also known as normalization of image. This technique enhances an image by remapping the image intensity values of an image into new values of the data type.

2.1.2 Histogram Equalization

Another method to improve the contrast of an image is by applying histogram equalization. This method adjusts the image intensity values by mapping the histogram of the output image to a specified histogram. It is useful in images with backgrounds and foregrounds that are both bright or both dark [8]. This method is usually applied to the image that is over or under-exposed. In medical applications, this technique commonly used in examining the bone structure in x-ray images.

2.1.3 Contrast-Limited Adaptive Histogram Equalization (CLAHE)

CLAHE is a variant of adaptive histogram equalization to enhance the local contrast of an image [9]. This technique is focusing on small data regions in the image, called tiles. This method contradicts with the histogram equalization method that operates on the whole image. Every tile will be enhanced, resulting in the histogram of each output region approximately matches the specified histogram that determined by the approximation parameters [10]. The 'clip-limit' function is used to apply limit over a noise image.

2.2 Image Enhancement Techniques in Medical Imaging

Previous studies have shown there were many types of image enhancement technique applied in the medical field, especially in cancer diagnosis. A good image enhancement method could help to improve the quality of the images as well as help the medical experts to detect the tumour area in a short time.

Parameshwarappa and Nandish [11] applied image enhancement techniques and region growing algorithm to segment area of the lung cancer. In the early stage, the input images were compressed, from DICOM into JPEG format and followed by grayscale conversion. The median filter method was applied to remove the noises that existed inside the grayscale images. From the resultant median filtered images, contrast stretching was applied to adjust the contrast of the image by using the histogram shrinking method. The purpose of this method is to highlight some of the relevant intensity values in those images for easier detection. The image histogram was modified into a narrow histogram. Based on the output images, the proposed method was able to segment the tumour cells from the original image. This method has been shown to help the medical research to describe the shape and the location of the cancer cells.

Sheela and Babu [12] investigated the importance of the pre-processing technique for brain tumour detection and segmentation. In this study, three methods were involved in the pre-processing technique before the detection and segmentation of magnetic resonance imaging (MRI) images of the brain tumour. These methods consist of colour space conversion, skull strip removal and histogram equalization. The colour space of the input images was first converted from RGB to grayscale images. The captured images of the brain tumour consist of brain area, scalp, skull, and dura. Next step is to remove the non-brain structure and unwanted portions of the image from the grayscale images. The skull removal was done by implementing the intensity thresholding and followed by a morphological operation to obtain the required tumour detection area. Histogram equalization was used to improve the contrast of the resultant images. In conclusion, the proposed method was able to enhance the quality of the brain tumour images. The resultant images from the pre-processing technique ease the detection and segmentation process since the remaining part was only the brain tumour area after the removal of unwanted objects.

Jamil *et al.* [13] designed an automated pre-processing and segmentation technique for cancerous skin lesions. The proposed method was consists of three major phase. These phases include hair detection and removal, colour space transformation, and image enhancement. At the first phase, the proposed method had detected all the hairs that existed in the original images. After that, the proposed method created a binary mask for all the detected hairs. The detected area was then filled up using a

Neighbourhood Based Region Filling (NBRF) algorithm. The authors had used the 2D Gabor wavelet method to enhance the image pattern and highlight the hair information. The hair pixels were removed since it will affect the system performance with the misdetection of the cancerous skin area. In colour space transformation, the proposed method converted the original images from RGB colour space into L*a*b* colour space. Contrast stretching was used to enhance the illumination variation between the skin and lesion pixels. The proposed yielded a better result with an average value of the total detection rate (TDR) of 97.26%. The percentage of false positive rate (FPR) and the error rate (ER) were 3.52% and 3.01% respectively.
Abdallah *et al.* [14] proposed an image enhancement and segmentation algorithms to improve the performance and detection of breast cancer. The proposed algorithm composed of several techniques such as contrast improvement, noise lessening, texture scrutiny, and partitioning algorithm. In the pre-processing stage, the proposed algorithm applied the colour space conversion from RGB to grayscale colour space. The purpose of the colour conversion is to increase the lesion appearance and recognition of the object. The noise was removed by sharpening the image and applying the Wiener function and median filter technique. The authors applied log transformation to enhance the contrast of the tumour area. The proposed algorithm used K-means clustering technique to automatically segment the breast images. Based on the resultant images, this technique was proven to be practical and applicable for analysing tumour in mammography images.

3. METHODOLOGY

The general goal of the image enhancement technique is to improve the visualization of the original images that will be useful in further analysis. In this paper, three types of image enhancement techniques had been proposed to segment the Ki67 cells. These techniques consist of contrast stretching, histogram equalization, and CLAHE method. Figure (1) shows the general block diagram of the proposed system.

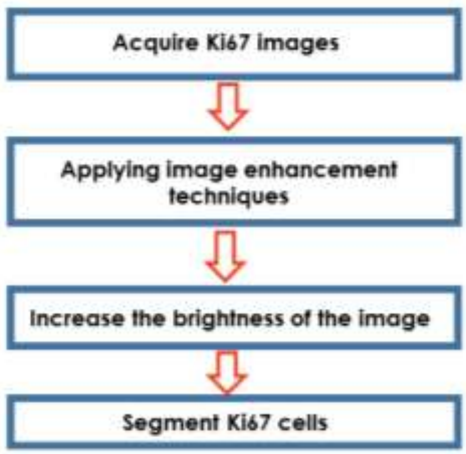


Fig. 1. *General block diagram of the proposed system*

3.1 ACQUIRING KI67 IMAGES

In this study, twelve histopathological images of meningioma were obtained from the Department of Pathology, Hospital Universiti Sains Malaysia (HUSM). The immunostained positive Ki67 nuclei were stained using DAB, while the negative nuclei were counterstained using the haematoxylin. These images were captured under 40x magnification using an Olympus BX51 microscope and Cell^F software that works as an interface to the digital camera that attached to the microscope. The resolution of the captured images were 1360×1024 pixels and the images were saved in (*.jpg) format. Figure (2) shows a sample of immunohistochemical (IHC) stained Ki67 image from a meningioma. The positives Ki67 cells appeared in granular brown colour, while the blue colour was referring to negative Ki67 cells.

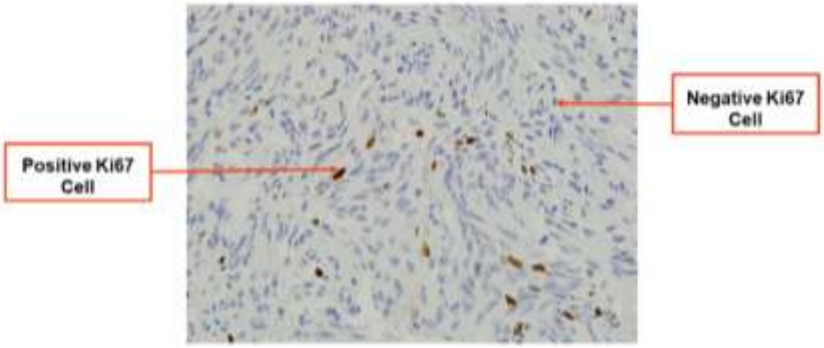


Fig. 2. *A Ki67 image of meningioma using IHC stains*

3.1.1 Applying Image Enhancement Techniques

Sometimes, the captured images from a microscope has several issues that lead to the image imperfections. These issues may be lack of contrast and brightness, the presence of image artefacts, high level of noise and low resolution. These issues can normally be reduced by applying image enhancement techniques.

In this study, the image enhancement method was applied to the Ki67 images to enhance the contrast and brightness of those images. The first step was to implement colour space conversion to the captured images. The Ki67 images which are in RGB colour space have been converted into L*a*b* colour space. The RGB colour space needs to be transformed into CIE XYZ colour space first and followed by the L*a*b* colour space. Equation (1) is used to convert from RGB to CIE XYZ colour space [15].

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} 0.4125 & 0.3576 & 0.1804 \\ 0.2127 & 0.7152 & 0.0722 \\ 0.0193 & 0.1192 & 0.9502 \end{bmatrix} \tag{1}$$

Next is the conversion from CIE XYZ to L*a*b* colour space by using the equations below [16]:

$$L = 116 \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - 16 \tag{2}$$

$$a^* = 500 \left[\left(\frac{X}{X_n}\right)^{\frac{1}{3}} - \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}}\right] \tag{3}$$

$$b^* = 200 \left[\left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - \left(\frac{Z}{Z_n}\right)^{\frac{1}{3}}\right] \tag{4}$$

where X_n, Y_n, and Z_n are the tristimulus value of the reference white. X, Y, and Z describe as any colour that can be perceived by an average human observer. The second step was the selection of luminosity value. The luminosity value needs to be scaled into [0 1] range. In this paper, the luminosity value was selected at 0.85 to get the best segmentation result. The third step was performing image enhancement techniques. As mentioned earlier at the beginning of this section, three enhancement methods were proposed to enhance the Ki67 cells. These enhancement methods operated on the Luminance (L) component of the L*a*b* colour space.

a) Contrast Stretching

The contrast stretching method is commonly used to adjust the contrast of an image. This method remaps the image intensity values to the full display range of the data type. At this moment, the intensity value of each pixel in the raw image was transformed using a transfer function to create a contrast-enhancement image [17]. In this study, the intensity was replaced with the luminance component to enhance the meningioma images. The contrast stretching can be applied by using the equations below [18]:

$$P_k = \frac{q_k - f_{min}}{f_{max} - f_{min}}(max - min) + \min \tag{5}$$

where

P_k = colour level of the output pixel
q_k = colour level of the input pixel
f_{max} = maximum colour level values in the input image
f_{min} = minimum colour level values in the input image
max = desired maximum colour levels in the output image
min = desired minimum colour levels in the output image.

b) Histogram Equalization

The histogram equalization technique modifies the display of an image by controlling the probability density function of its grey levels by the transformation function $T(r_k)$ [19]. This technique will map each pixel in the input image with intensity (r_k) into a corresponding pixel with level (s_k) in the output image [20]. For this study, the grey levels was replaced to luminance levels. The equation below was used to obtain the output image [20] by using histogram equalization.

$$(s_k) = T(r_k) = \frac{(L-1)}{MN}\sum_{j=0}^{k} n_i \qquad k = 0,1,2,\ldots,L-1 \qquad (6)$$

where
$T(r_k)$ = transformation mapping
L = number of possible luminance levels in the image
MN = total number of pixels in the image
n_i = number of pixels that have intensity (r_k)

c) CLAHE method

CLAHE method can be applied either to grayscale or RGB images. This method is an advancement from the adaptive histogram equalization (AHE), where the histogram calculated for the contextual region of a pixel [21]. The procedure below shows the implementation steps for CLAHE method [22]:

i. Acquire the input images.

ii. Get all the input values such as the number of regions in row and columns direction separately, number of bin used in the histogram, cliplimit, and distribution parameter type.

iii. Determine the real clip limit from the normalized value.

iv. Create luminance level mapping and clipped histogram. Basically, the region numbers of pixels are equally divided in each luminance levels. Therefore, the average number of pixels is calculated as follows:

$$N_{avg} = \frac{N_{CR-XP} \times N_{CR-YP}}{N_{lum}} \qquad (7)$$

where
N_{avg} = average number of pixels.
N_{lum} = number of luminance level in the contextual program.
N_{CR-XP} = number of pixels in the X direction of the contextual region.
N_{CR-YP} = number of pixels in the Y direction of the contextual region.

v. Calculate the actual cliplimit using the equation below:

$$N_{CL} = N_{clip} \times N_{avg} \qquad (8)$$

Later, the output image is converted back to the RGB colour space for adjusting the brightness of the image. The purpose of brightness correction is to improve the visibility of Ki67 cells so that the cells can be correctly segmented in the next process. In this step, a constant value is selected to be added to each pixel of the image. The selected value must be greater than 0 to get a brighter image. For this study, the best value was decided at 10 to get the best segmentation result. If the constant value is higher or lower than 10, it will degrade the segmentation results. The selection of luminosity and brightness value was done manually. After performing analyses on twelve Ki67 images, it has been found the selected value able to detect most of the Ki67 cells.

4. SEGMENTING KI67 CELLS

In this study, the segmentation process was done automatically. At the first stage, the proposed system will segmenting the positive Ki67 cells and followed by negative Ki67 cells. The resultant images from the enhancement methods were used as the input images in segmenting Ki67 cells. Fig. 3 shows the flowchart for segmenting Ki67 cells.

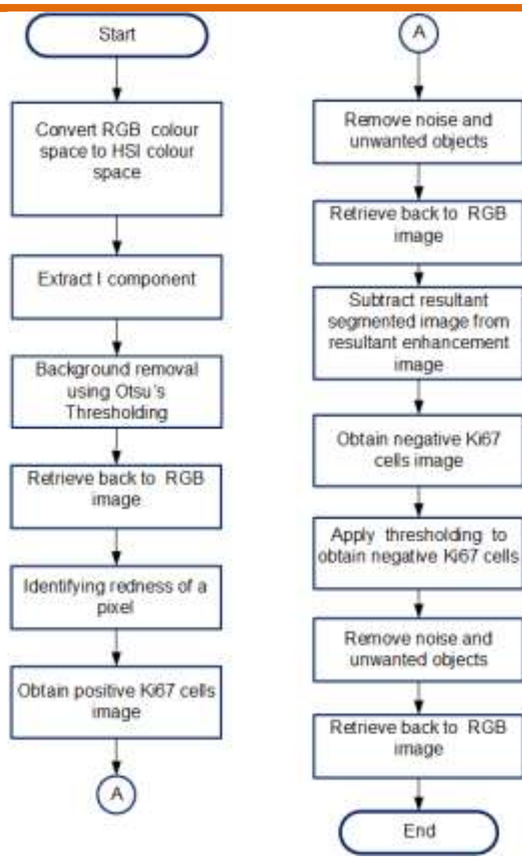


Fig. 3. *Flowchart for segmenting Ki67 cell*

The first step to segment the Ki67 cells was colour space conversion. The colour space conversion was applied to the resultant image from the enhancement technique by converting RGB colour space into Hue, Saturation, Intensity (HSI) colour space. The process of colour space conversion was carried out using the following equations [23]:

$$H \text{ (Hue)} = \begin{cases} \theta & \text{If } B \leq G \\ 360-\theta & \text{If } B > G \end{cases} \tag{9}$$

with the angle θ is defined as

$$\theta = \cos^{-1}\left\{\frac{\frac{1}{2}[(R-G)+(R-B)]}{[(R-G)^2+(R-B)(G-B)^{1/2}}\right\} \tag{10}$$

The saturation (S) is defined by

$$S \text{ (Saturation)} = 1 - \frac{3}{(R+G+B)} \tag{11}$$

and the intensity (I) is given by

$$I \text{ (Intensity)} = \frac{1}{3}(R+G+B) \tag{12}$$

The second step is to extract the Intensity (I) component from the HSI colour space. Global thresholding was applied to I component to remove the background of the image. At this moment, the remaining objects in the image will be Ki67 cells only. Background removal was performed using Otsu's technique, which is a type of global thresholding. The reason for using this technique is because this method is a widely known method to segment an image by using a histogram. Global thresholding can be defined as [23]:

$$g(x,y) = \begin{cases} 1, & f(x,y) \geq T \\ 0, & f(x,y) < T \end{cases} \tag{13}$$

where the $g(x,y)$ is the resultant threshold image, $f(x,y)$ is the test image and T is the threshold value. Once the background is removed, the resultant image was retrieved back into the RGB image to perform the segmentation technique toward Ki67 cells.The segmentation of Ki67 cells was divided into two stages, where the segmentation process was performed for positive Ki67 cells and followed negative Ki67 cells. Since the positive Ki67 cells appeared in red-brownish colour, the pixel value at the red channel was high compared to the green and blue channel. The equation to obtain the positive Ki67 nuclei was calculated as follows [24]:

$$\text{Redness} = \text{double}(R) - \max(\text{double}(G), \text{double}(B)) \qquad (14)$$

where R, G, and B, indicates each red, green and blue component from the retrieved image after applying Otsu's thresholding technique. The noise and unwanted regions were removed using the area opening method. Area opening method is a filter that removes components (object) with an area smaller than the parameter (λ) from binary images [25]. The three separate channels were recombined by concatenating the arrays of R, G, and B components along specified dimensions.

Next step was to obtain the negative Ki67 image. The negative Ki67 image was acquired by subtracting the resultant image from the enhancement technique with the output image from the thresholding technique. Figure (4) represents the steps to find negative nuclei.

(a) Resultant Ki67 image after applying image enhancement

(b) Image of the Ki67 positive nuclei, after implementing the thresholding technique

(c) The resultant image after subtracting image (b) from the image (a)

Fig 4. *Methods to acquire negative Ki67 image*

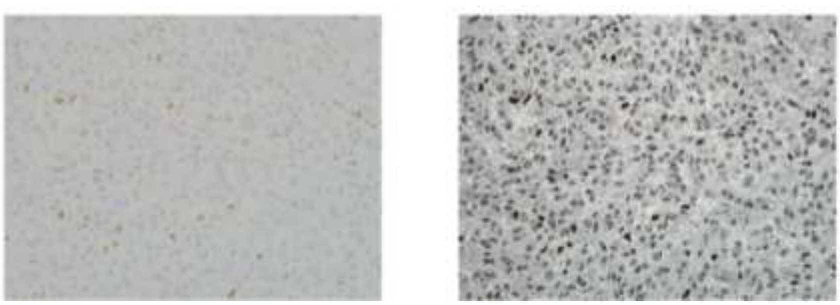
The figure above shown the resultant image of the enhancement method, Figure 4(a) that has similar pixel values with the output image from the colour thresholding technique, Figure 4(b) will be converted into a white pixel, which subsequently created a new image, Figure 4(c). The final step was applying Otsu's method to segment the negative Ki67 cells. Noises and unwanted objects were filtered out using the area opening method.

5. EXPERIMENTAL RESULTS AND DISCUSSION

The proposed system was developed using the MATLAB software version 2018. A personal computer which runs on an Intel Core i7-5500U, 2.4 GHz, processor with 16.0 GB RAM that operates on Microsoft Windows 10 Pro was used to develop the system. In this paper, the proposed system was tested on 12 histopathological images on meningioma.

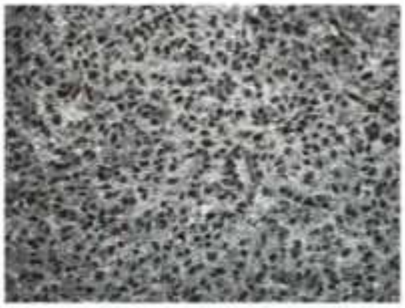
5.1 Image Enhancement

This study used three different image enhancement techniques to improve the Ki67 images. The resultant images from the enhancement techniques were used to segment the Ki67 cells. The segmentation results were calculated and compared to find the appropriate enhancement technique to segment Ki67 cells. Figure (5) shows resultant Ki67 images after implementing three different types of image enhancement techniques.

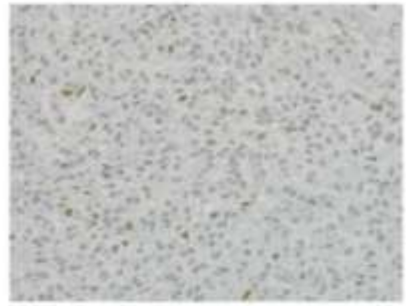


(a) Original image

(b) Ki67 image after contrast stretching



(c) Ki67 image after histogram equalization

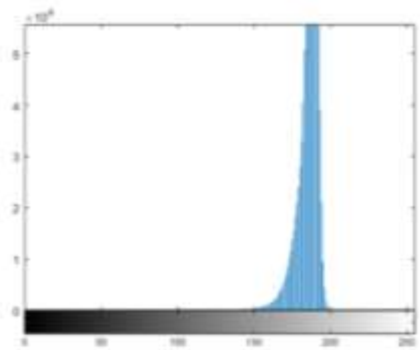


(d) Ki67 image after CLAHE technique

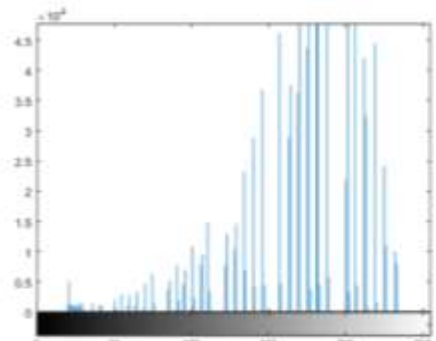
Fig. 5. *Outputs of Ki67 images after applying image enhancement techniques*

Fig. 5(a) shown an example of a low contrast Ki67 image. Some of the Ki67 cells appeared to have a dull colour issue. In this way, the accuracy of the system might be low due to misdetection of the cells. Generally, the resultant images have improved the contrast of the Ki67 cells. The Ki67 cells that were not visible in the original image were revealed after applying the enhancement techniques.

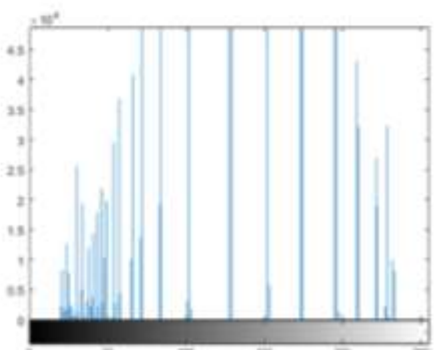
A histogram is used to determine whether the image is bright or dark. In image processing, the histogram of an image is indicating to the histogram of the pixel intensity values. Fundamentally, an image with its pixels value clustered at the right side of the histogram corresponds to the white and light area, while on the left side of the histogram represents the black and dark area. The horizontal axis is referring to the tonal variations while the vertical axis is labelling as the number of pixels in that particular tone. Figure (6) illustrates the intensity histogram of the resultant images after applying enhancement techniques. The intensity histogram was obtained by converting the colour space of Ki67 images from RGB to grayscale colour space.



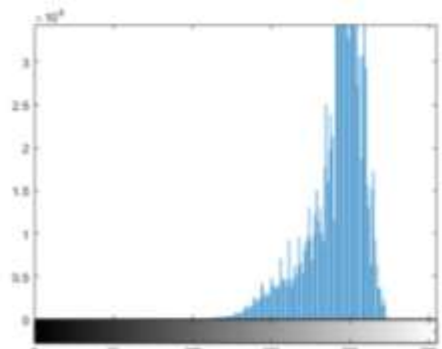
(a) Intensity histogram of the original Ki67 image



(b) Intensity histogram of Ki67 image after applying contrast stretching



(c) Intensity histogram of Ki67 image after applying histogram equalization



(d) Intensity histogram of Ki67 image after applying the CLAHE method

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Fig. 6. *Intensity histogram after applying image enhancement techniques*

From the figures above, the intensity histogram for all original images had shown there are no values below 150 or above 200. The implementation of image enhancement techniques can improve the image by remapping the data values to fill the entire intensity range [0, 255]. The intensity histogram of these images also shown an improvement, where the histograms are spreading wider compared to the original image.

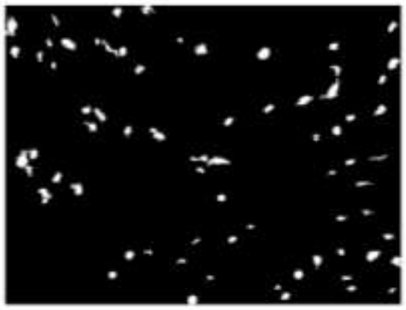
6. IMAGE SEGMENTATION

In order to test the performance of the segmentation method, the proposed segmentation algorithm was compared with manual segmentation. The manual segmentation was carried out using an open source image analysis software known as Image J [26]. This software was used to segment the Ki67 cells manually and the resultant images were validated by HUSM pathologists. In the manual segmentation process, the images were enhanced automatically at first by using the Image J software. Then, the images were converted from RGB to grayscale colour space. Automatic global thresholding was applied to the images for segmenting the Ki67 cells. The noise removal and fill regions process of target structures on the images were done manually using the software. Figure (7) illustrates the comparison between the manually segmented image and the resultant image by using the proposed system.

(a) Manual segmentation using Image J software

(b) Automatic segmentation of Ki67 image without using enhancement technique

(c) Automatic segmentation using contrast stretching technique.



(d) Automatic segmentation using histogram equalization technique.

(e) Automatic segmentation using CLAHE technique.

Fig. 7. *Comparison of the segmented image between manual segmentation and automatic segmentation*

Fig. 7 shows the segmentation results of the Ki67 images. In Fig. 7(b), there were few Ki67 cells that cannot be detected. This was due to the Ki67 cells that may have a dull colour, which makes the system difficult to identify the cells. Fig. 7(c) shows a good segmentation result since most of the Ki67 cells can be recognized using the proposed system. In Fig. 7(d) and 7(e), the image shows the Ki67 cells can be detected using the proposed system. However, there were noises and some unwanted objects also existed in the image.

Quantitative analysis was performed to measure the performance of the proposed system in segmenting Ki67 cells. The evaluation of the performance depended on the calculation of segmentation accuracy, sensitivity, and specificity. These analyses are calculated by:

$$Accuracy = \left(\frac{TP\ + TN}{(TP + TN + FP + FN)}\right) \times 100\% \qquad (15)$$

$$Sensitivity = \left(\frac{TP}{(TP + FN)}\right) \times 100\% \qquad (16)$$

$$Specificity = \left(\frac{TN}{(TN + FP)}\right) \times 100\% \qquad (17)$$

where TP was defined as the number of positive Ki67 cells that were correctly segmented. FP indicated as the number of cells that incorrectly segmented as positive Ki67 cells. TN was referred to the number of cells that were correctly segmented as a negative Ki67 cell, while FN signified the number of cells that incorrectly segmented as negative Ki67 cells. Table I showed the comparison of Ki67 cells segmented results using three different image enhancement types with manual segmentation.

Based on the results, all three enhancement techniques are able to segment the positive Ki67 cells. The table showed that the contrast stretching and CLAHE techniques give higher accuracy with more than 90%. The contrast stretching technique showed a high sensitivity value compared to the histogram equalization and CLAHE technique with 92.46%, 65.09% and 88.00% respectively. Thus, it shows that these three image enhancement techniques can be applied to segment the positive Ki67 cells.

Table I shows the sensitivity values are relatively low. This indicates that the FN values are high. This is may be due to the “leftovers” or remaining pixels after segmenting the positive Ki67 cells. The color thresholding algorithm should be able to detect the entire positive cells. However, there are some pixels that were not detected by the algorithm, which then be transferred to the negative Ki67 cells images and cause false detection. Secondly, some of the images are low quality, where some of the cells could not be identified by the proposed system. This occurs when the color of the images are blurry, which makes the system difficult to identify the Ki67 cells. Thirdly, some of the cells were overlapped and touched by each other.

As a result, it will disturb the circularities values, since the proposed system was not able to determine the shape of the cell. Next is the uncertainties objects or noises that present in microscopic images. This may occur during surgical removal fixation, staining procedures, processing, embedding and microtomy [27].

Table 1: Segmentation Performance for Ki67 Cell Images

Type of Image Enhancement	Input Image	Accuracy (%)	Sensitivity (%)	Specificity (%)
Contrast Stretching	Cell 01.bmp	97.79	96.34	99.34
	Cell 02.bmp	97.13	95.37	99.03
	Cell 03.bmp	98.03	97.12	98.97
	Cell 04.bmp	95.93	93.04	99.25
	Cell 05.bmp	95.23	92.06	98.91
	Cell 06.bmp	95.57	92.63	98.95
	Cell 07.bmp	98.38	97.44	99.35
	Cell 08.bmp	97.95	96.75	99.21
	Cell 09.bmp	98.44	97.58	99.34
	Cell 10.bmp	91.19	85.62	98.84
	Cell 11.bmp	90.62	85.12	98.17
	Cell 12.bmp	87.08	80.40	97.51
Average		**95.28**	**92.46**	**98.91**
Histogram Equalization	Cell 01.bmp	70.00	62.76	96.15
	Cell 02.bmp	68.28	61.55	93.82
	Cell 03.bmp	66.80	60.43	93.24
	Cell 04.bmp	73.54	67.35	86.61
	Cell 05.bmp	74.91	71.86	78.95

	Cell 06.bmp	74.07	68.93	83.05
	Cell 07.bmp	71.35	65.46	84.49
	Cell 08.bmp	71.34	64.85	87.91
	Cell 09.bmp	70.56	63.95	89.06
	Cell 10.bmp	72.70	69.48	77.21
	Cell 11.bmp	72.70	68.04	80.61
	Cell 12.bmp	58.34	56.41	61.93
Average		**70.38**	**65.09**	**84.42**
CLAHE Technique	Cell 01.bmp	96.90	94.90	99.10
	Cell 02.bmp	95.91	93.40	98.73
	Cell 03.bmp	96.07	93.90	98.48
	Cell 04.bmp	95.17	91.83	99.09
	Cell 05.bmp	93.76	90.43	97.70
	Cell 06.bmp	93.31	89.23	98.25
	Cell 07.bmp	93.10	88.81	98.46
	Cell 08.bmp	92.18	87.42	98.33
	Cell 09.bmp	91.90	87.01	98.28
	Cell 10.bmp	86.54	81.91	92.75
	Cell 11.bmp	86.72	81.65	93.70
	Cell 12.bmp	82.10	75.57	93.11
Average		**91.97**	**88.00**	**97.17**

7. CONCLUSION

This paper studies the effect of three different image enhancement techniques in segmenting Ki67 cells. The enhancement types are consist of contrast stretching, histogram equalization, and CLAHE method. The performance of the proposed algorithm in detecting the positive and negative Ki67 cells has been calculated by conducting some quantitative analyses. Based on the results, it showed that the contrast stretching technique delivered the best results among other enhancement techniques. Hence, this promising method may be an excellent technique to segment Ki67 cells.

REFERENCES

[1] S. N. Bellur and V. Chandra, "Meningioma Size: Its Relationship to Other Diseases," *Arch. Neurol.*, 1981.

[2] L. T. Li, G. Jiang, Q. Chen, and J. N. Zheng, "Ki67 is a promising molecular target in the diagnosis of cancer (Review)," *Mol. Med. Rep.*, vol. 11, no. 3, pp. 1566–1572, 2015.

[3] P. Janani, J. Premaladha, and K. S. Ravichandran, "Image Enhancement Techniques: A Study," *Int. J. Sci. Technol.*, vol. 8, no. 22, 2015.

[4] S. Fu, M. Zhang, C. Mu, and X. Shen, "Editorial Advancements of Medical Image Enhancement in Healthcare Applications," *J. Healthc. Eng.*, vol. 2018, 2018.

[5] S. Singh and P. Pal, "Contrast Enhancement of Medical Images : A Review," *J. Heal. Sci. Nurs.*, no. 4, pp. 32–35, 2016.

[6] P. Spandana, K. M. M. Rao, B. V. V. S. N. Prabhakar Rao, and Jwalasrikala, "Novel image processing techniques for early detection of breast cancer, mat lab and lab view implementation," in *IEEE EMBS Special Topic Conference on Point-of-Care (POC) Healthcare Technologies: Synergy Towards Better Global Healthcare, PHT 2013*, 2013.

[7] Y. Bangalore, "Review of Various Histogram Based Medical Image Enhancement Techniques," in *ICARCSET '15 Proceedings of the 2015 International Conference on Advanced Research in Computer Science Engineering & Technology (ICARCSET 2015)*, 2015.

[8] R. Dorothy, R. M. Joany, R. J. Rathish, S. S. Prabha, and S. Rajendran, "Image enhancement by Histogram equalization," *Int. J. Nano Corros. Sci. Eng.*, vol. 2, no. 4, pp. 21–30, 2015.

[9] S. M. Pizer *et al.*, "Adaptive histogram equalization and its variations," *Comput. Vision, Graph. Image Process.*, vol. 39, no. 3, pp. 355–368, 1987.

[10] A. Namdeo and S. S. Bhadoriya, "A Review on Image Enhancement Techniques with its Advantages and Disadvantages," *Int. J. Sci. Res. Technol.*, vol. 2, no. 5, pp. 171–182, 2016.

[11] V. Parameshwarappa and S. Nandish, "Segmentation of Lung Cancer using Image Enhancement Techniques and Region Growing Algorithm," *Int. J. Eng. Res. Technol.*, vol. 3, no. 2, pp. 482–485, 2014.

[12] Sheela.V.K. and S. S. Babu, "Pre-Processing Technique for Brain Tumor Detection and Segmentation," *Int. Res. J. Eng. Technol.*, vol. 02, no. 03, pp. 1208–1212, 2015.

[13] U. Jamil, M. U. Akram, S. Khalid, S. Abbas, and K. Saleem, "Computer Based Melanocytic and Nevus Image

Enhancement and Segmentation," *Biomed Res. Int.*, vol. 2016, 2016.

[14] Y. M. . Abdallah, S. Elgak, H. Zain, M. Rafiq, E. A.Ebaid, and A. A. Elnaema, "Breast Cancer Detection Using Image Enhancement and Segmentation Algorithms," *Biomed. Res.*, vol. 29, no. 20, pp. 3732–3736, 2018.

[15] A. Kaur and B. . Kranthi, "Comparison between YCbCr Color Space and CIELab Color Space for Skin Color Segmentation," *Int. J. Appl. Inf. Syst.*, vol. 3, no. 4, pp. 30–33, 2012.

[16] M. Tkaleie and J. F. Tasie, "Colour spaces - Perceptual, Historical and Applicational Background," *IEEE Reg. 8 EUROCON 2003. Comput. as a Tool.*, vol. 2, pp. 304–308, 2003.

[17] H. Kueh, E. Marco, M. Springer, and S. Sivaramakrishnan, "Image analysis for biology," *Image analysis for biology MBL Physiology course 2008*, 2008. [Online]. Available: http://www.rpgroup.caltech.edu/courses/PBoC GIST/files_2011/Matlab/Image Analysis with Matlab.pdf.

[18] A. R. Weeks, *Fundamentals of Electronic Image Processing*. Wiley, 1996.

[19] O. M. Miljkovi´c, "Image Pre-Processing Tool," *Kragujev. J. Math*, vol. 32, pp. 97–107, 2009.

[20] R. C. Gonzalez, R. E. Woods, and B. R. Masters, "Intensity Transformation and Spatial Filtering," in *Digital Image Processing*, 3rd ed., Prentice Hall, 2008, pp. 126–215.

[21] E. D. Pisano *et al.*, "Contrast limited adaptive histogram equalization image processing to improve the detection of simulated spiculations in dense mammograms," *J. Digit. Imaging*, vol. 11, no. 4, pp. 193–200, 1998.

[22] G. Yadav, S. Maheshwari, and A. Agarwal, "Contrast limited adaptive histogram equalization based enhancement for real time video system," *Proc. 2014 Int. Conf. Adv. Comput. Commun. Informatics, ICACCI 2014*, pp. 2392–2397, 2014.

[23] R. Gonzalez and R. Woods, "Image Segmentation," in *Digital Image Processing*, 3rd ed., Prentice Hall, 2008, pp. 760–764.

[24] Z. Fegyver, "A simple image segmentation example in MATLAB," *matlab tricks.com*, 2014. [Online]. Available: http://matlabtricks.com/post-35/a-simple-image-segmentation-example-in-matlab.

[25] L. Vincent, "Grayscale Area Openings and Closings: their Applications and Efficient Implementation," *EURASIP Work. Math. Morphol. its Appl. to Signal Process.*, no. May, pp. 22–27, 1993.

[26] M. D. Abràmofff, P. J. Magalhães, and S. J. Ram, "Image processing with ImageJ Part II," *Biophotonics Int.*, vol. 11, no. 7, pp. 36–43, 2005.

[27] S. Jimson, L. Malathi, G. M. Kailash Kumar, and N. Balachander, "Artifact in histological section," *Biomed. Pharmacol. J.*, vol. 9, no. 2, pp. 843–845, 2016.