Hyperchromatic Nucleus Segmentation on Breast Histopathological Images for Mitosis Detection

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***Abstract*—Breast cancer grading is the standard clinical practice for the prognosis and diagnosis of breast cancer development. The Nottingham Histological Grading (NHG) system is widely used in the breast cancer grading. In NHG system, the mitotic count based on histopathological images (i.e. microscopic slide examination) is one of the three criteria that define the overall grade. Image processing techniques such as segmentation could be utilised to detect mitotic cells. This study proposed a new approach to segment hyperchromatic nucleus on the histopathological images based on RGB and HSI colour spaces. The results show that the proposed segmentation technique could provide a promising result in segmenting hyperchromatic nucleus and preserving the ground truth (i.e. true mitotic cells).**

***Index Terms*—Breast Cancer; Hyperchromatic Nucleus; Mitosis; Nucleus Candidates.**

I. INTRODUCTION

Breast cancer is the main cause of mortality that commonly occurred among women. It is a complex, heterogeneous and fatal disease [1]. In the year 2015, the statistic shows that 6.42 deaths per 100,000 people were recorded in Malaysia [2].

The grading of breast cancer plays a vital role in prognosis and treatment planning of cancer. Nottingham Histological Grading (NHG) system is commonly used as a grading system for breast cancer [3]. A patient who is diagnosed with breast cancer is subjected to surgical biopsy. The result from biopsy (i.e., breast tissues) is then examined under a light microscope. In routine histological practice, a pathologist performs breast cancer grading manually using a light microscope. This resulted in a tedious and massive workload which is time-consuming [4]. Besides, manual grading is found to be not reproducible [5 – 7]. The output grading may suffer from inter- and intra- observes variability among pathologist [4]. These issues are then initiated a digitalisation era in pathology labs.

Based on NHG system, the mitotic counts in the histopathological image are one of the three vital factors that define the overall grade. In order to detect mitotic cells, the nucleus should be first segmented from the background. In recent years, some studies have been published on techniques to obtain the nucleus cells in histological images. Yang et al. [8] employed a marker-controlled watershed based on mathematical morphology to segment the cells. The proposed method is based on the watershed and means shift. The results show that the method could obtain 98.8% of segmentation accuracy. Lee et al. [9] implemented two iterative generalised Hough Transforms (GHTs) to segment the nucleus cells. The first GHT obtained the knowledge on the size of the nuclei and the second GHT was used to isolate the nuclei themselves. Nedzved et al. [10] used a fast grey-scale thinning algorithm to detect the cells in the input images. The proposed method analyzed the binary image layers where the one-pixel lines were used to segment the cells. Several studies identified the nucleus features using Gamma-Gaussian Mixture Model (GGMM) [11, 12]. The GGMM is a parametric technique used to estimate the probability density function of the input images. The Gamma function represented the mitotic cells whereas the Gaussian function represented the non-mitotic region. After implementation of GGMM, the context-aware was used as post-processing to reduce the false positive.

Hyperchromatic is one of the mitotic cells features [13, 14]. Therefore, the main objective of this study is to identify the hyperchromatic nucleus areas which could possibly be the mitotic cells. Outputs of the proposed method would be the segmented hyperchromatic nucleus. The segmented hyperchromatic nucleus perhaps could be used to provide a more accurate segmentation and detection of mitotic cells.

II. METHODOLOGY

The flowchart of the proposed method used to obtain nucleus areas is shown in Figure 1. The breast cancer histopathological image was used as the input image. The proposed method starts by marking nucleus in the image. There are two methods (i.e., Method A and Method B) to mark the nucleus. Method A marks hyperchromatic nucleus whereas Method B marks nucleus area in the input image. In Method A, the colour range of the original input image was enhanced using decorrelation stretch [15]. This technique was used to ease features discrimination and to provide better visual enhancement. The colour thresholding method was implemented on the decorrelation stretched image to mark hyperchromatic nucleus present in the image. In Method B, hue (H) channel from HSV colour model was used in the Otsu thresholding [16] to mark nucleus areas. The hyperchromatic nucleus obtained from Method A was superimposed on the nucleus areas obtained using Method B. The nucleus areas (obtained from Method B) remain as nucleus candidates if and only if the hyperchromatic nucleus (obtained from Method A) is an element in the nucleus areas.

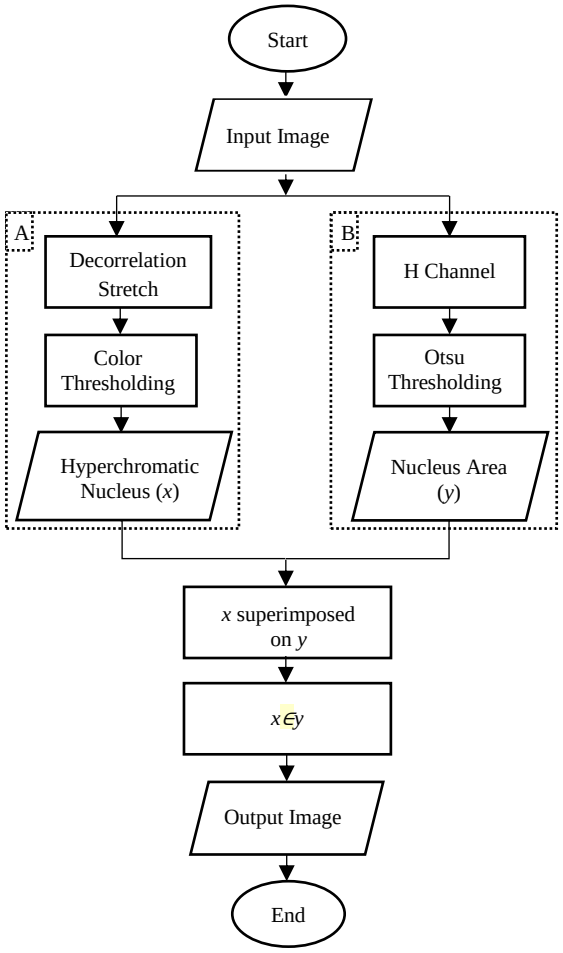


Figure 1: Flowchart of the proposed method.

A. Decorrelation Stretch

The decorrelation stretch was employed on the input image to ease features discrimination and enhance colour differences in the histological image. This enables hyperchromatic nucleus to be more significant in appearance. The details of the decorrelation stretch method can be found in [15].

B. Colour Thresholding

The output from the decorrelation stretch was an RGB colour image with the hyperchromatic nucleus visually appeared green in colour. The conventional thresholding method by defining a single or a range of brightness value in grey scale could not segment the region of interest accurately. Therefore, the conventional thresholding method was modified to make it more adaptive to segment the colour images. In an RGB colour image, each pixel is characterised by three colour bands: red (R), green (G), blue (B) [16]. For colour segmentation purpose, each colour band was processed as an individual component. A single threshold value (T) as shown in Figure 2 was selected in each colour band to overcome the aforementioned problem. For each band, a suitable threshold value that provides the best results for hyperchromatic nucleus segmentation was determined. The obtained red threshold (T_R), green threshold (T_G), blue threshold (T_B) were used to segment the region of interest using Equation (1).

$$g(x,y) = f_R(x,y) \le T_R \ AND \ \ f_G(x,y) \ge T_G \ AND \ f_B(x,y) \le T_B \quad (1)$$

where: $g(x,y)$ = Output pixel
$f_R(x,y)$ = Input pixel in Red band
$f_G(x,y)$ = Input pixel in Green band
$f_B(x,y)$ = Input pixel in Blue band.

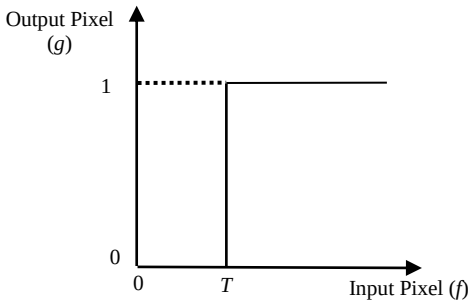


Figure 2: Selected threshold value (T) for thresholding method.

C. Otsu Thresholding

The original RGB colour model (i.e., an input image) was converted to HSV colour model. Based on the prior knowledge of histological images, the Hue (H) channel provides a significant characteristic of nucleus components [17]. In this step, Otsu thresholding [16] was applied to the H channel to segment the nucleus area. A pair of thresholding values was selected to perform nucleus segmentation. Equation (2) shows how the Otsu thresholding was implemented.

$$g(x,y) = \begin{cases} 1, & T_1 < f(x,y) < T_2 \\ 0, & otherwise \end{cases} \quad (2)$$

where: $g(x,y)$ = Output pixel
$f(x,y)$ = Input pixel
T_1= Threshold value 1
T_2 = Threshold value 2

D. Super-impose and Segmentation

The marked hyperchromatic nucleus (x) obtained from Method A were superimposed with the marked nucleus area (y) obtained from Method B. In this step, the nucleus areas in Method B were declared as nucleus candidates (ie., final output) if and only if the hyperchromatic nucleus (x) is an element in the nucleus areas (y). The condition is expressed in the following equation.

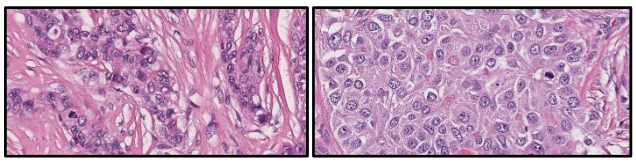
$$C = x \in y \quad (3)$$

where C = Nucleus candidates

III. Results

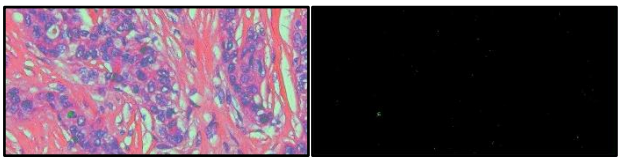
20 images captured from 5 breast carcinoma histopathological slides were used to test the proposed method. The histopathological tissue slides were provided by the Pathology Department, Hospital Tuanku Fauziah, Kangar, Perlis. These tissue slides were stained with Hematoxylin and Eosin (H&E). The digital slides (i.e., whole slide images) were prepared using an Aperio CS2 WSI scanner. Two images namely BC1, Figure 3 (a) and BC2,

Figure 3 (b) were used to present the results of the proposed method. These images were captured from the digital slides under 40x magnification. The resolution for BC1 and BC2 were 614x1264 and 630x1360, respectively. Results from Method A for BC1 and BC2 are shown in Figures 4 and 5 respectively. For each figure, image (a) shows the output of the decorrelation stretch and image (b) shows the result of colour thresholding on decorrelation stretched images. The results from Method B for BC1 and BC2 are shown in Figures 6 and 7 respectively. For each figure, image (a) shows the output image in H channel and image (b) shows the result after implementation of Otsu thresholding. The resultant images from Method A (output from colour thresholding) and Method B (output from Otsu thresholding) were superimposed to produce hyperchromatic nucleus candidates as shown in Figures 8 and 9 for respective BC1 and BC2.



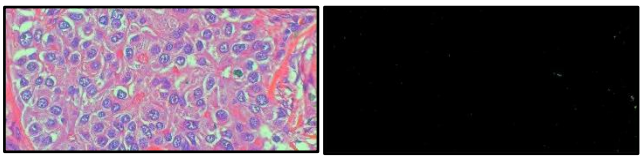
(a) (b)

Figure 3: Original histopathological images, (a) BC1, (b) BC2.



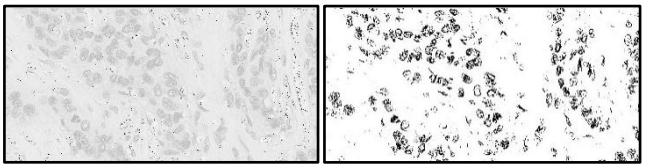
(a) (b)

Figure 4: The results of Method A on BC1, (a) output of the decorrelation stretch (b) result of colour thresholding on decorrelation stretched images.



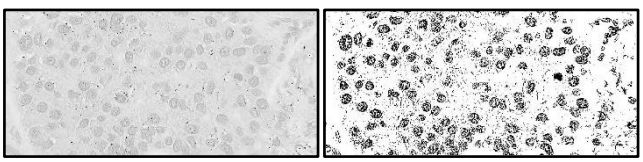
(a) (b)

Figure 5: The results of Method A on BC2, (a) output of the decorrelation stretch (b) result of colour thresholding on decorrelation stretched images.



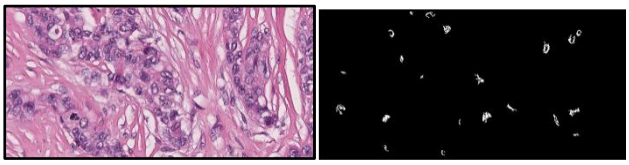
(a) (b)

Figure 6: The results of Method B on BC1, (a) H channel of BC1, (b) the result after implementation of Otsu thresholding.



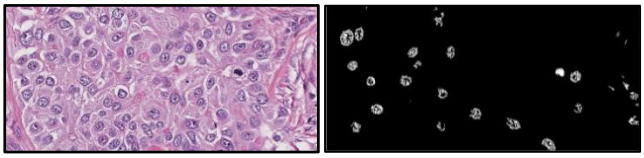
(a) (b)

Figure 7: The results of Method B on BC2, (a) H channel of BC2, (b) the result after implementation of Otsu thresholding.



(a) (b)

Figure 8: (a) BC1, (b) hyperchromatic nucleus candidates on BC1.



(a) (b)

Figure 9: (a) BC2, (b) hyperchromatic nucleus candidates on BC2.

IV. DISCUSSION

The main objective of this study is to identify the hyperchromatic nucleus areas (i.e., nucleus candidates) which could probably be a potential mitotic cell. The nucleus candidates obtained from the proposed methods in this study will be used in the next classification stage to identify true mitotic cells.

Based on prior knowledge of mitotic cells, the hyperchromatic nucleus has the potential to be the mitotic candidates [8, 9]. The hyperchromatic nucleus is heavily stained during the staining process. Thus, the intensity of mitotic cells is more remarkable than the normal nucleus. To ease the detection of nucleus candidates, two methods of pre-processing were employed (i.e., Method A and Method B). Method A marks the hyperchromatic nucleus, whereas Method B marks the nucleus area in the input image. The decorrelation stretch was used to enhance the features of discrimination between the hyperchromatic nucleus and normal nucleus. The nucleus that was heavily stained is visually green in colour after the stretching process. Modified colour thresholding was then applied to segment the hyperchromatic nucleus. A single threshold value was selected for each band (RGB). The selection of the threshold value is based on an analysis of 20 images. The best threshold value for T_R, T_G and T_B are 170, 60 and 60, respectively. The output results of the colour thresholding show that the proposed threshold values were able to segment the green objects (i.e., hyperchromatic objects) in the input image.

Method B marks the nucleus areas in the input image. The input image was converted into H channel from HSV model. H channel was found to be the best channel to display the Hematoxylin stained components on the mitotic cells [17]. Then, a conventional Otsu thresholding [16] was implemented on the H channel image. Two threshold values (i.e. T_1 and T_2) were selected to segment the nucleus area using the conventional Otsu thresholding. The threshold values were selected using a trial and error method. The best threshold values for $T_1$and T_2 are 0.69 and 0.8, respectively. Based on the result of Otsu thresholding, the proposed threshold values were able to segment the nucleus area in the input image.

Due to the different degree of stain absorption during the staining process, a hyperchromatic nucleus may not share the same degree of stain density throughout a single cell. Therefore, Method A does not provide a good segmentation on the whole hyperchromatic nucleus area. In other words,

Method A only marks the area of the possible hyperchromatic nucleus, as shown in Figure 4(b) and Figure 5 (b). In Method B, the whole nucleus areas (including the hyperchromatic nucleus) in the input image were marked. However, not all the marked nucleus areas are the region of interest (i.e., hyperchromatic nucleus). Hence, by combining both outputs from Method A and Method B the nucleus areas that are hyperchromatic were identified, Figure 8 (b) and Figure 9 (b). The results show that the proposed methods can provide a promising segmentation on the hyperchromatic nucleus on breast cancer histopathological image.

V. CONCLUSION

This paper proposed a technique to segment hyperchromatic nucleus areas on the breast cancer histopathological image. Based on the results, the segmentation technique can remove the unwanted background components and remain the hyperchromatic nucleus candidates. The proposed method could be an alternative method to segment the nucleus candidates for mitotic cells on breast cancer images.

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