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Enhancement and Segmentation of Ziehl Neelson Sputum Slide Images using Contrast Enhancement and Otsu Threshold Technique

Ainul Kamilah Mohd Yusoff[1*], Rafikha Aliana A Raof[1,2,4], Norfadila Mahrom[1,2], Siti Suraya Md Noor[3], Fazrul Faiz Zakaria[1,4,5], , Phak Len[1,4]

1 Faculty of Electronic Engineering Technology, Universiti Malaysia Perlis, Perlis, Malaysia
2 Sports Engineering Research Centre (SERC), Centre of Excellence, Universiti Malaysia Perlis, Perlis, Malaysia
3 Department of Microbiology, School of Medical Science, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
4 Advanced Computing, Centre of Excellence, Universiti Malaysia Perlis, Perlis, Malaysia
5 Micro System Technology, Centre of Excellence, Universiti Malaysia Perlis, Perlis, Malaysia

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ABSTRACT

Image processing is the most effective method for enhancement and segmentation of tuberculosis bacilli in sputum smear samples. Improper straining can result in poor screening results such as over-staining, under-staining, and blurred images. The goal is to find an image enhancement and segmentation technique that will prepare the image for feature extraction. There are still some shortcomings with existing method when it is implemented on Ziehl Neelson images. In normal images, TB bacilli can be identified easily, but in blur and images with dark background, TB bacilli are sometimes hidden behind the sputum cells. Hence, the basic method of contrast enhancement is not enough to improve the contrast of TB bacilli as the object of interest within the image. In this study, the combination of local and partial contrast enhancement is proposed as the best method for image enhancement. Image segmentation can be accomplished using Otsu thresholding technique. Otsu's method is presented as most suitable image processing techniques in this paper. The goal of the Otsu Threshold is to find a threshold value that distinguishes the object of interest from the background. Experiment shows that the combination of local and partial contrast enhancement followed by Otsu's method achieve an average segmentation accuracy of 98.93% when applied on 50 images of sputum smear.

1. Introduction

The number of deaths from tuberculosis is very high which is 1.3 million in 2021 according to the WHO, but most are preventable if diagnosed and treated early [1]. The most commonly used staining method is Ziehl-Neelson (ZN), which visualises stained sputum smear samples under an optical microscope to determine the presence of tuberculosis bacteria. Images of sputum specimens were automatically captured with a digital camera attached to a light microscope with a motorized stage.

* *Corresponding author.*
E-mail address: ainulkamilah95@gmail.com

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Improper staining procedure and reagent preparation by the microbiologist may result in poor screening results that are caused by over-stain, under-stain, or blurred images [2].

The process of improving the quality of a problematic image so that it can be interpreted by humans is known as image enhancement [18]. Contrast enhancement is one of the most popular image enhancement techniques. Previous researchers identified three types of contrast enhancement techniques: global, local, and partial contrast stretching [2]. These technique have been previously used in the image processing procedure of various type of images [19,20]. However, there are still some shortcomings with the method when it is implemented on Ziehl Neelsen images. In normal images, TB bacilli can be identified easily, but in blur and images with dark background, TB bacilli are sometimes hidden behind the sputum cells. Hence, the basic method of contrast enhancement is not enough to improve the contrast of TB bacilli as the object of interest within the image. This paper suggests that combining local and partial contrast enhancement is the efficient method for improving the image of TB bacilli. This technique is the process of changing the image value distribution to cover a wide range.

Image segmentation's goal is to simplify the image so that it can be easier to be analyzed. Existing image segmentation techniques include threshold, edge-based, region-based, and watershed. However, in this paper, a threshold technique has been chosen to be implemented in the study. Threshold techniques are generally classified as global thresholding or local thresholding. A single threshold value is used for the entire image in global thresholding [3]. Thresholding process will produce a binary image. Pixels with intensities of 1 are considered as objects, while pixels with intensities of 0 are classified as the background [4].

Local thresholding employs distinct threshold values for the image's partitioned sub-images [3]. It is a pixel in the foreground or background of an image that uses local data from the image. The threshold value (T) could be fixed, but this would only work if the image contained very little noise[4]. Previous researchers have used active contour and Otsu thresholding segmentation algorithms on the BRATS dataset's brain tumour segmentation [5]. The Otsu method is one of a global adaptive binarization threshold-based image segmentation algorithm [6]. The method work by finding the best threshold value between pixel values 0 and 255 by calculating and evaluating their between-class variance (or within-class variance) [7-10].

This paper will present the combination of local and partial contrast enhancement, which is followed by Otsu's method on Ziehl-Neelson sputum slide images to separate the pixels of TB bacilli from the background.

2. Methodology

In this paper, the enhancement and segmentation technique are implemented on ZN sputum slide images [15-17]. According to Figure 1, the input images will be processed by a technique that combines local and partial contrast enhancement, respectively. The original image is 24-bit (colour) image, having the resolution of 800x600 pixels and saved as bitmap files (*.bmp). Then, the image enhancement output will be processed using the Otsu threshold.

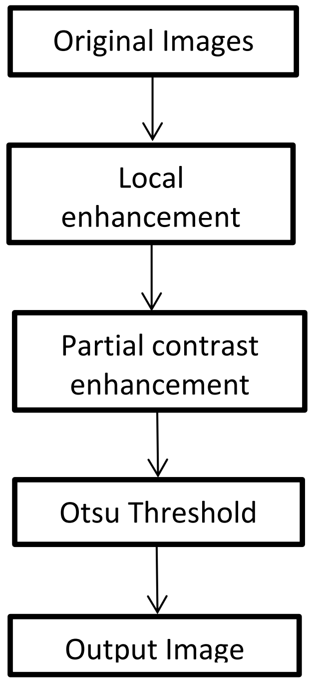


Fig. 1. Overall flow chart of methodology

2.1 Local Enhancement

Local enhancement is to improve the contrast on an image that has variation of contrast within different areas[3]. This algorithm is usually used on images that need to be improved in dark areas and also in the bright areas. According to Eq. (1), the minimum and maximum value for colour level is chosen to get better image[2]. The formula is applied to each pixel within an image.

$$q_k = 255 \text{ x} \left(\frac{I_o - \min}{\max - \min}\right) \tag{1}$$

where
q_k : color intensity for output
I_o : color intensity for input
max : maximum color intensity for input
min : minimum color intensity for input

2.2 Partial Contrast Enhancement

Partial contrast is defined as a linear mapping function that boosts the brightness and contrast of images. First, we determined the majority input pixel value in each colour space. The intensity ranges for red, blue, and green of existing images is determined. The average of the upper and lower colour ranges are then calculated using Eq. (2) and Eq. (3) respectively [2].

$$f_{min} = \frac{\min^{Red} + \min^{Green} + \min^{Blue}}{3} \tag{2}$$

$$f_{max} = \frac{\max^{Red} + \max^{Green} + \max^{Blue}}{3} \tag{3}$$

The minimum colour intensities for each image are identified as $\min^{Red}$, $\min^{Green}$, and $\min^{Blue}$, while the maximum colour intensities are $\max^{Red}$, $\max^{Green}$, and $\max^{Blue}$ for each colour palette. The average number of minimum RGB colour space, f_{min} is given in Eq. (2) whereas the maximum RGB colour space, f_{max} is given in Eq. (3). The mapping procedure will then begin with the usage of min and max. For the pixels transformation, the function in Eq. (4) is utilised.

$$P_k = \begin{cases} \frac{\min}{f_{min}} (q_k) & ;\text{for} \quad q_k < f_{min} \\ \frac{(\max - \min)}{(f_{max} - f_{min})} (q_k - f_{min}) + \min & ;\text{for } f_{min} \le q_k \le f_{max} \\ \frac{(255 - \max)}{(255 - f_{max})} (q_k - f_{max}) + \max & ;\text{for } q_k > f_{max} \end{cases} \tag{4}$$

where
q_k : Color intensity of input
P_k : Color intensity of output
f_{min} : Value of lower threshold
f_{max} : Value of upper threshold
min : New value of lower stretch
max : New value of upper stretch.

The values of a pixel within the ranges f_{min} and f_{max} will be stretched to greater range within *min* and *max*, as shown in Figure 2. Compression will be applied to the remaining pixels. As a result, the image's pixels will have a wider range and a brighter intensity. Hence, the contrast of output images for the region of interest are improved.

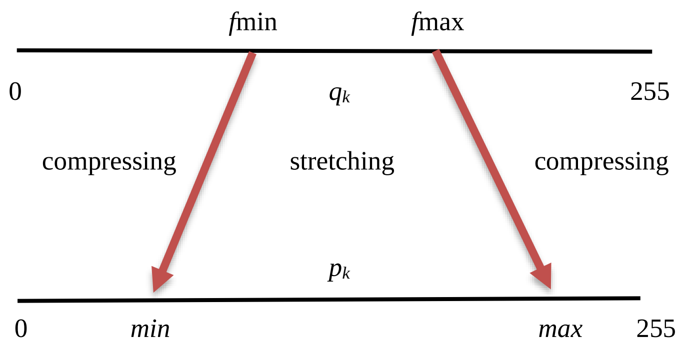


Fig. 2. Partial contrast enhancement process

2.3 Otsu Threshold

Thresholding is one of most popular technique in image segmentation. A single threshold is a value threshold that divides an image into black and white regions. According to Eq. (5), g(x, y) is the output result, and f(x,y) is the current pixel. The output result is generated depending on the current pixel value. If the current value is greater than T, the new value is 1(white). Otherwise, it will become 0(black)[6,11,12].

In Eq. (6), if a green pixel subtracted by a red pixel is greater than 25, the result is 1(white). Otherwise, the colour remains. The threshold value is used to distinguish between TB bacilli and non-TB bacilli. The thresholding method then converts a grayscale image to a binary image by using a threshold value[13,14]. The thresholding algorithm determines the best threshold for image segmentation.

$$g(x,y) = \begin{cases} 1, & f(x,y) \geq T \\ 0, & f(x,y) < T \end{cases} \tag{5}$$

$$g(x,y) = \begin{cases} f(x,y), green(x,y) - red(x,y) \leq 25 \\ white, green(x,y) - red(x,y) > 25 \end{cases} \tag{6}$$

The optimal threshold value level (T), determined by image histogram is chosen using Otsu threshold. When the intensity exceeds T, it turns the image pixels white. In the opposite case, it becomes a black region.

3. Results

According to Figure 3, the original image is captured by a digital camera attach on a light microscope. During the process of image process of image capturing, some condition such as overexposed or underexposed to natural light will affect the quality of images being captured. This explains why the quality of the images captured varies. It is then manually analysed by technologist under light microscope, and it may result in poor screening results. The proposed method has been applied to five categories of images, which are normal, underexposed, overexposed, blur and overlapping TB image. Figure 3 shows the original images from each of the category.

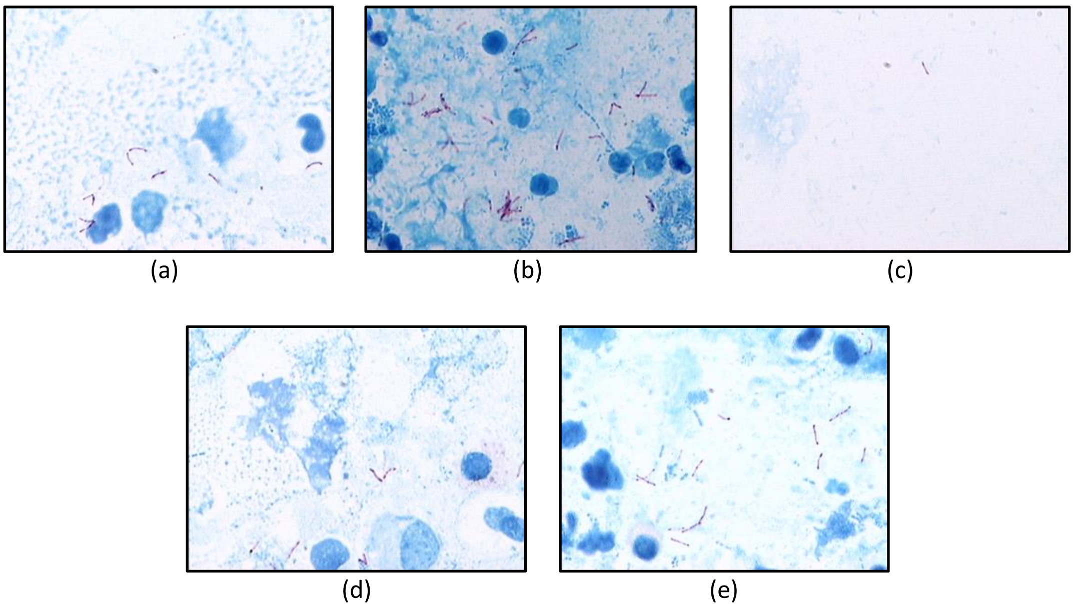
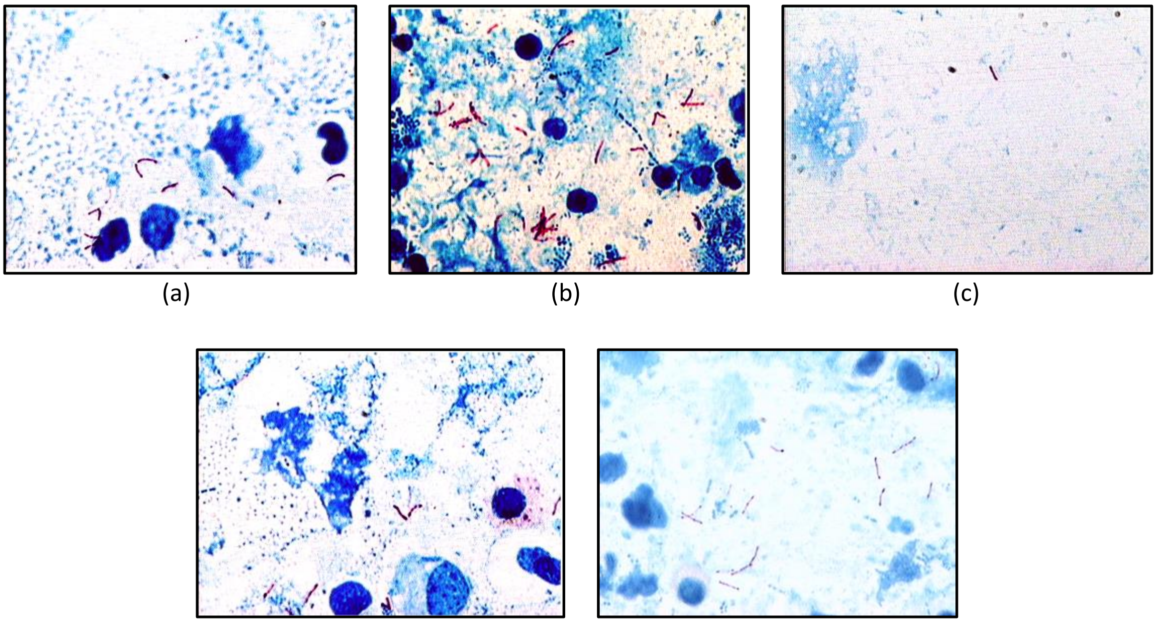


Fig. 3. Original image of TB bacilli (a) Normal (b) Underexposed (c) Overexposed (d) Blur (e) Overlapping

Original images must go through an image enhancement process to improve poor screening results. As shown in Figure 4, the enhancement process will adjust the image's intensity and brightness to achieve a better result. The bacilli appear more red, making it easier to distinguish between TB bacilli and non-TB bacilli.



(a) (b) (c)

(d) (e)

Fig. 4. Result of enhancement image (a) Normal (b) Underexposed (c) Overexposed (d) Blur (e) Overlapping

According to Figure 5, the main goal is to remove the sputum (blue pixels) while keeping the TB bacilli (red pixels). The RGB image is then converted into binary image by using a threshold value. The thresholding algorithm determines the best threshold for segmenting the image. When the value of green pixel subtracted by red pixel is greater than 25, the output is set to change into white pixels. It is used to separate TB bacilli from sputum smears. If green pixel subtracted by red pixel is equal or less than 25, the original colour remains. Therefore, the only objects left are TB bacilli in red colour.

From Figure 5, it can be seen that the result in category of normal, underexposed and overexposed which are shown in Figure 5 (a),(b) and (c) image are satisfactory. However, the outcome images in (d) and (e) are unsatisfactory. This is due to pixel loss in (d) and some noise in (e). To summarise, if the image has less noise, the Otsu threshold method is more successful in clearing all blue pixels while retaining red TB bacilli. The method has been applied on 50 images of ZN sputum specimen. Segmentation accuracy of 98.93% has been achieved, which is better than previous studies by other researchers that obtained an average of 90% accuracy.

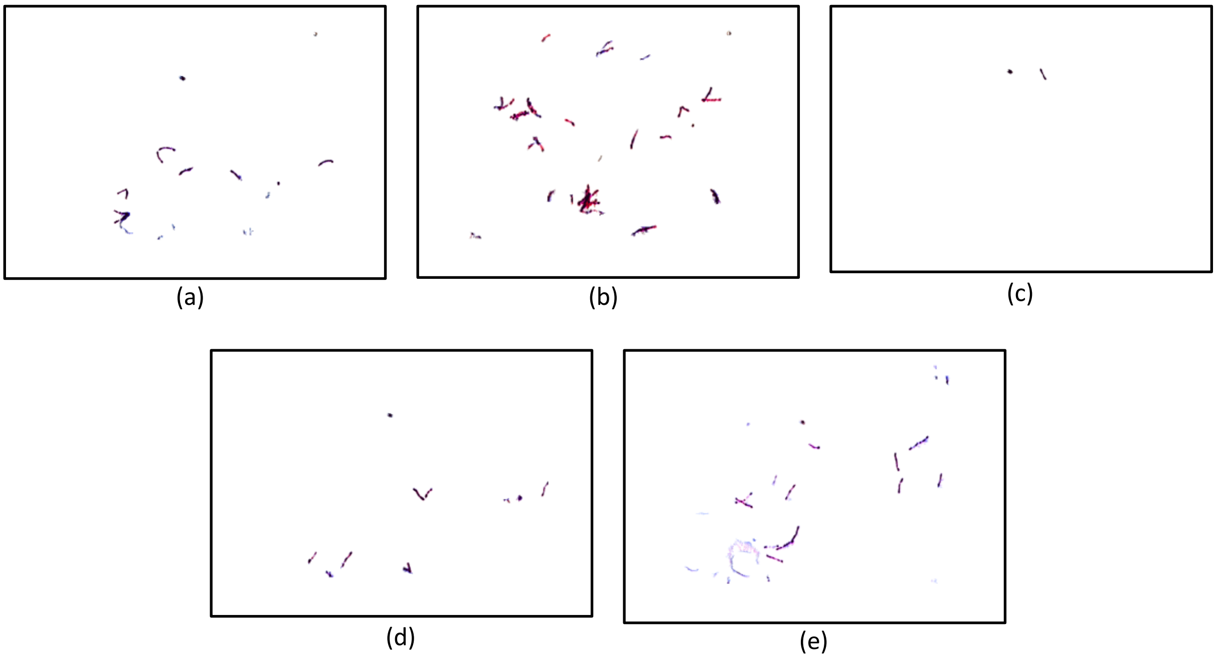


Fig. 5. Result of Otsu threshold (a) Normal (b) Underexposed (c) Overexposed (d) Blur (e) Overlapping

4. Conclusions

Image enhancement using local and partial contrast technique made the segmentation process easier, whether TB or non-TB. The TB bacilli turn redder. For sputum slide images, an image segmentation technique based on Otsu thresholding method was presented. The technique successfully separated the sputum using the selected threshold value.

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