Image Segmentation of Ziehl-Neelsen Sputum Slide Images for Tubercle Bacilli Detection

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1. Introduction

Tuberculosis (TB) remains one of the leading causes of death in developing countries and its recent resurgences in both developed and developing countries warrants global attention. Globally, there were an estimated of 9.27 million incident cases of TB in 2007. This is an increase from 9.24 million cases in 2006, 8.3 million cases in 2000 and 6.6 million cases in 1990. Most of the estimated numbers of cases in 2007 were in Asia (55%) and Africa (31%), with small proportions of cases in the Eastern Mediterranean Region (6%), the European Region (5%) and the Region of the Americas (3%). The five countries that rank first to fifth in terms of total numbers of cases in 2007 are India (2.0 million), China (1.3 million), Indonesia (0.53 million), Nigeria (0.46 million) and South Africa (0.46 million). Of the 9.27 million incident TB cases in 2007, an estimated 1.37 million (15%) were HIV-positive; 79% of these HIV-positive cases were in the African Region and 11% were in the South-East Asia Region (WHO, 2009).

Ziehl-Neelsen stain method is one of the common techniques that are being used to diagnose the TB infection. Smear microscopy with Ziehl-Neelsen technique has been the main means of diagnosing TB patients in developing countries. This is because the method is simple, rapid, reproducible, low cost and effective in detecting infectious disease such as TB (Luna, 2004). TB diagnosis is usually being done manually by microbiologist through microscopic examination of sputum specimen of TB patients for pulmonary TB diseases.

However, there are some problems that have been reported with manual screening process, such as time consuming and labor-intensive, especially for screening of the negative slides. (Veropoulus et al., 1998). When reporting the results of the microscopic examination, the microbiologist should provide the clinician with an estimation of the number of acid-fast bacilli detected. If the smear microscopy is clearly positive, very little observation time is needed to confirm the result. The slide is classified as TB positive if at least one tubercle bacilli is found in 300 microscopic fields. The case will then be classified into one of four severity category if the smear on the slide is found to be positive, according to the number of tubercle bacilli found in the slide. For a well-trained microbiologist, it takes 15 to 20 minutes to read and confirm one negative slide, with an average of 25 slides can be read per day. In addition, for some developing countries, there is also a lack of well-trained microbiologist,

which may results in overload, fatigue and reduces the diagnostic performance (Khutlang et al., 2009). Therefore, an automated TB diagnosis is required so that large number of cases can be handled with the same accuracy and speeding up the process while improving the low sensitivity of manual TB diagnosis.

In this research, images of Ziehl-neelsen sputum slide are captured using a digital camera attached to a light microscope and displayed on a computer screen. In order to extract the TB bacilli pixels from the TB slide images, several image processing techniques are required. One of the image processing techniques is image segmentation, which is useful to classify the pixels in the image into two region, TB and background. The sputum specimen that has undergone the process of staining using Ziehl-Neelsen procedure will make the TB bacilli appear red and other cells and organisms in the sputum smear sample will retained blue background. Image segmentation is a part of image processing technique that will help to discriminate between the TB bacilli and background pixels in the digital image.

There are many attempts already been made to enable the image captured by a camera that is attached to the microscope to be viewed through the computer screen. Some image processing algorithms also have been developed in order to carry out automatic TB bacilli detection in the captured image. Forero applied adaptive color thresholding technique to the images that have been captured using fluorescence microscopy (Forero et al., 2003; Forero et al., 2004). Veropoulos used an identification method based on shape descriptors and neural network classifiers (Veropoulus et al., 1998; Veropoulus et al., 1999). Wilkinson proposed a rapid multi resolution segmentation technique based on computing thresholds for different areas in a monochromatic image (Wilkinson, 1996). The studies mentioned above used images captured from fluorescence microscope, which appear different from Ziehl-Neelsen sputum slide images captured under light microscope.

In this study, a method of grey thresholding technique is reviewed, and it is then being adapted to suit with color images. Thus the color thresholding algorithm is expected to be able to discriminate between the pixels that comprises the mycobacterium and sputum in the Ziehl-Neelsen slide images. The outcome of this study should be able to provide a way of getting the suitable threshold values for the images and using the values to achieve the main objective of color thresholding and image segmentation.

2. Image segmentation

Segmentation process subdivides an image into its constituent regions or objects. The level of subdivision depends on the problem being solved, where the segmentation should stop when the objects of interest in an application have been isolated. Image segmentation algorithms generally are based on one of the two basic properties of intensity values: discontinuity and similarity. Thresholding is a method of similarity category. It partitions an image into regions that are similar according to a set of predefined criteria. There are various thresholding techniques and it is also a fundamental approach to segmentation that enjoys a significant degree of popularity, especially in applications where speed is an important factor (Gonzalez & Woods, 2002).

Traditionally, one simple way to accomplish thresholding is by defining a range of brightness value in the original image, then selects the pixels within the range as belonging to the foreground and rejects all of other pixels to the background. Such an image is then usually displayed as a binary or two-level image (Sezgin & Sankur, 2004).

The general rule for grey level pixel thresholding is as in Equation (1).

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

where T is the threshold value, $f(x,y)$ is the original pixel value, and $g(x,y)$ is the resulted pixel value after thresholding has been done. Equation (1) specifies 0 and 1 as output values, which will give the result as a true binary image. Equation (1) can be further visualized by Figure 1 as mappings of input grey level to output grey level (Efford, 2000).
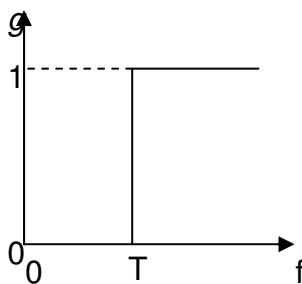
Fig. 1. Thresholding for a single threshold

There could be more than one thresholding value at a time, which change Equation (1), to

$$g(x,y) = \begin{cases} 0, f(x,y) < T_1, \\ 1, T_1 \leq f(x,y) \leq T_2, \\ 0, f(x,y) > T_2. \end{cases} \qquad (2)$$

where $T1$ is the lower threshold value and $T2$ is the upper threshold value.

Figure 2 shows the visualization of how thresholding with a pair of threshold is being done (Efford, 2000).
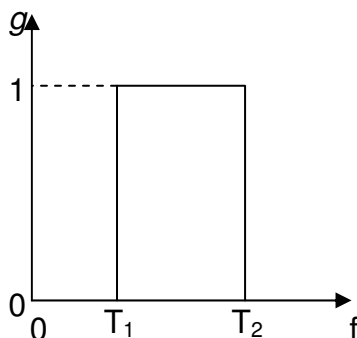

Fig. 2. Thresholding with a pair of threshold

For color images, more than one variable characterises each pixel in the image, which allows multi spectral thresholding (Otsu, 1979). In color imaging, each pixel is characterised by three red, green and blue (RGB) values. However, with multi spectral or multilayer images such as RGB model, it can be difficult to specify the selection criteria. The logical extension of thresholding is simply to place brightness thresholds on each image, for instance to specify the range of red, blue and green intensities.

These multiple criteria are then usually combined with an AND operation (i.e. the pixel is defined as part of the foreground if its three RGB components all lie within the selected range). This logically equivalent to segmenting each image plane individually, creating separate binary images and then combining them with a Boolean AND operator afterward. This color thresholding method is widely used in the image segmentation (Forero et al., 2003; Forero et al., 2004; Mancas-Thillou & Gosselin, 2005).

3. Methodology

In this study, a conventional thresholding method has been adopted to suit with color images of Ziehl-Neelsen sputum slide specimen for TB detection. The method is used to segment the image into two regions, which are TB and background (consists of sputum and other bacilli). Figure 3 demonstrates the steps involved in the proposed image segmentation process.
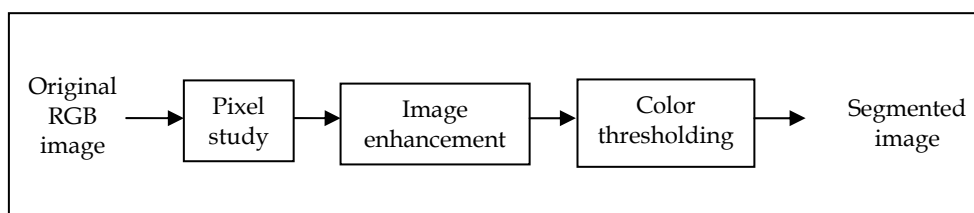

Fig. 3. Block diagram of proposed segmentation process

3.1 Image acquisition

The sputum specimens consist of TB bacilli were obtained from Department of Microbiology and Parasitology, School of Medical Science, Universiti Sains Malaysia, Kubang Kerian. The sputum specimens have been stained using Ziehl-Neelsen staining procedure. The sputum slides were analysed under 40x magnification using LEICA DM-LA microscope and the images were captured using Infinity-2 digital camera attached directly to the microscope. Figure 4 shows few samples of captured sputum slide images consist of TB bacilli. It can be seen that the TB bacilli appear to be red while the sputum background have bluish color.

The original images are images which are directly captured using the digital camera which is attached to the microscope. Since it is manually prepared by the technologist, the thickness of the specimen may vary from one end of the slide to the other end. That is why; it results in the variation in the quality of the images being captured.

3.2 Pixel study

A study on the color information on digital sputum slide images that consist of the tubercle bacilli and sputum was carried out to get the most suitable threshold values. The study was
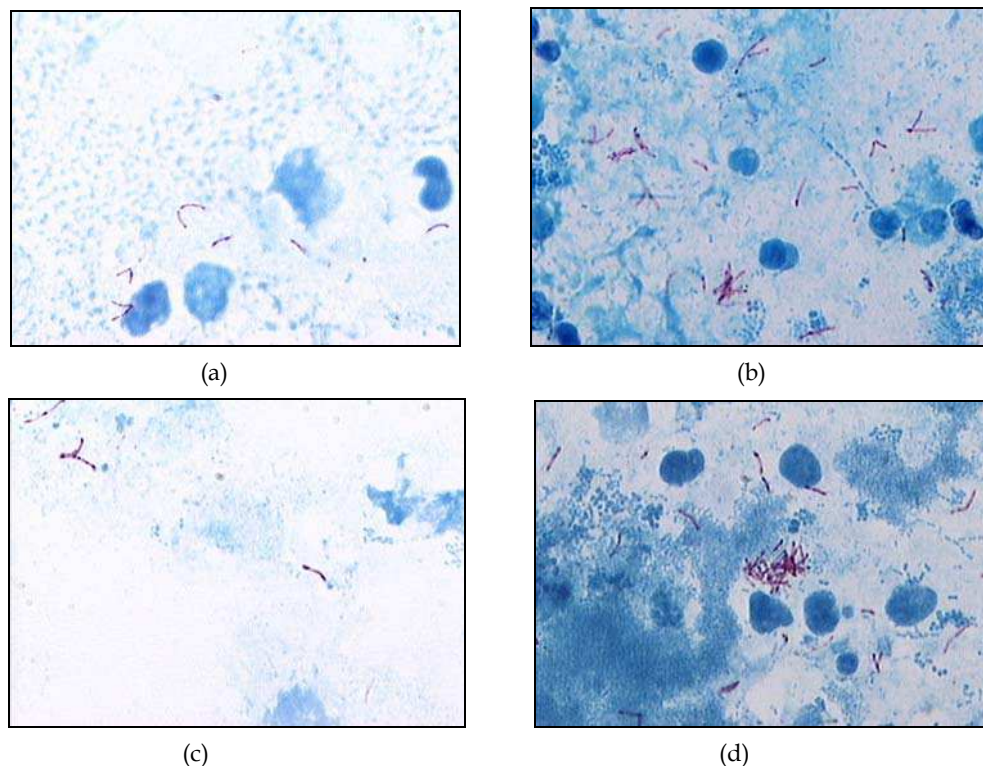
(a)

(b)

(c)

(d)

Fig. 4. Samples of sputum slide images consisting of TB bacilli

carried out on an amount of 244 TB bacilli with more than 2000 pixel samples together with more than 10000 background pixel samples. Sampling is done within five different category of images.

The properties of the RGB pixels are being studied to extract the important features from the image. Based on the color information, the color thresholding algorithm should be able to extract the pixels of tubercle bacilli and reject pixels of other objects. In order to view the important properties of each segment so that necessary features and accurate value of threshold can be obtained from the result, the information is being gathered in a table.

Among the features that are noted are the maximum, minimum and average values for each of the RGB components in tubercle bacilli and sputum respectively. It is found that the featured values are different from one category of images to another. Sample of featured values for normal and dark images are shown in Table 1 and Table 2.

	TB			Background		
	MIN	MAX	AVERAGE	MIN	MAX	AVERAGE
RED	14	255	175	14	255	230
GREEN	8	248	157	15	255	239
BLUE	45	255	196	61	255	252

Table 1. RGB information for images in normal category

From the data in Table 1, thresholding rules have been constructed based on average values. Rules for images from normal category are shown in Equation (3) – (5).

$$g(x,y) = \begin{cases} f(x,\ y), & red(x,y) < 230, \\ 255, & red(x,y) \geq 230. \end{cases} \tag{3}$$

$$g(x,y) = \begin{cases} f(x,y), green(x,y) < 239, \\ 255, green(x,y) \geq 239. \end{cases} \tag{4}$$

$$g(x,y) = \begin{cases} f(x,y), blue(x,y) < 252, \\ 255, blue(x,y) \geq 252. \end{cases} \tag{5}$$

where $red(x,y)$, $green(x,y)$ and $blue(x,y)$ are the pixel values for each of the red, green and blue components respectively.

	TB			Background		
	MIN	MAX	AVERAGE	MIN	MAX	AVERAGE
RED	5	252	155	0	255	160
GREEN	1	235	148	0	255	188
BLUE	38	255	183	33	255	217

Table 2. RGB information for images in dark category

Since the average values between RGB information for images in normal category and dark category are different, separate set of rules has to be constructed for dark images. Rules for images from dark category are shown in Equation (6) – (8).

$$g(x,y) = \begin{cases} f(x,\ y), & red(x,y) < 160, \\ 255, & red(x,y) \geq 160. \end{cases} \tag{6}$$

$$g(x,y) = \begin{cases} f(x,y), green(x,y) < 188, \\ 255, green(x,y) \geq 188. \end{cases} \tag{7}$$

$$g(x,y) = \begin{cases} f(x,y), blue(x,y) < 217, \\ 255, blue(x,y) \geq 217. \end{cases} \tag{8}$$

However, it is found that rules for one category of images are not universal and it can only be used within that particular image category. For example, rules for images in normal category will not give good result when it is applied to images in dark category. In order to overcome this problem, the images are brought through the image enhancement process so that the all the image from various categories are standardized into one same category.

3.3 Image enhancement

Image enhancement process are carried out to overcome the problem risen in the previous stage. This involves the adjustment of brightness, contrast and color in an image so that the pixel values fall into about the same range. Image enhancement technique that is used in

this study is partial contrast. Stretching method is done by requantizing each pixel value to a new value using pre-specified function. Linear stretching will generally improve the overall contrast of an image.

Contrast stretching is a process that applies auto-scaling method, which is a linear mapping function. It is usually used to enhance the brightness as well contrast level of the image. The general mapping function is shown in Equation (9) (Weeks, 1996).

$$p_k = \frac{(\max - \min)}{(f_{\max} - f_{\min})}(q_k - f_{\min}) + \min \tag{9}$$

Referring to Equation (9), $f_{\max}$ and $f_{\min}$ are the maximum and minimum color level in an input image. Variable max and min are the desired maximum and minimum color level in the output image. q_k is the color level of the input pixel while p_k is the color level of the output pixel. The combination of stretching and compressing process is called partial contrast. A part of the intensity level is being stretched to a new range, while other intensity levels left is being compressed to a different new range as well. The stretching and compressing processes are illustrated by Figure 5.
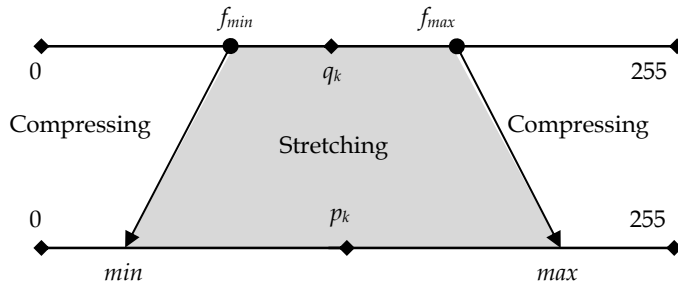
Fig. 5. Illustration of partial contrast process

The process illustrated by Figure 5 can be put into a mathematical function such as in Equation (10).

$$p_k = \begin{cases} \dfrac{\min}{f_{\min}}(q_k) & ;\ for\ q_k < f_{\min} \\[3mm] \dfrac{(\max - \min)}{(f_{\max} - f_{\min})}(q_k - f_{\min}) + \min & ;\ for\ f_{\min} \leq q_k \leq f_{\max} \\[3mm] \dfrac{(255 - \max)}{(255 - f_{\max})}(q_k - f_{\max}) + \max & ;\ for\ q_k \geq f_{\max} \end{cases} \tag{10}$$

3.4 Color thresholding

The color thresholding technique was carried out based on the color information of the bacterium to extract TB pixels from the sputum and other objects. This technique specifies

the range of RGB intensities for thresholding. The objects that lie outside the selection range will be rejected. Therefore, it is very important to determine the selection range because if this threshold cannot acquire a suitable value, the thresholding algorithm will extract pixels other than the expected object.

After image enhancement is done, the process of pixel sampling is done once again using enhanced images and the information is gathered in a table to observe its properties. Table 3 reflect the featured values for all the images that have been enhanced.

	TB			Background		
	MIN	MAX	AVERAGE	MIN	MAX	AVERAGE
RED	0	255	185	0	255	235
GREEN	0	249	161	0	255	246
BLUE	24	255	206	27	255	254

Table 3. RGB information for enhanced images

This time a universal rule for segmentation is produced as in Equation (11) – (13).

$$g(x,y) = \begin{cases} g(x, \ y), & red(x,y) < 235, \\ 255, & red(x,y) \geq 235. \end{cases} \tag{11}$$

$$g(x,y) = \begin{cases} g(x,y), green(x,y) < 246, \\ 255, green(x,y) \geq 246. \end{cases} \tag{12}$$

$$g(x,y) = \begin{cases} 255, blue(x,y) \geq 252, \\ g(x,y), blue(x,y) < 252. \end{cases} \tag{13}$$

From Equations (11), (12), and (13), it can be seen that the original equation that has been mentioned in Equations (1) and (2) have been slightly modified to adopt the method of grey level thresholding to color thresholding. For the new equations, each RGB component is being treated independently. Since there are three components, the thresholding process is being done to one component at a time, and they are then combined into 1 rule using a Boolean AND operator. One more important feature that has been extracted from the information gathered from the study is that in sputum images, the values of green pixels are always greater than the values of red pixels. Therefore, this information also has been adopted as another rule for this thresholding algorithm as in Equation (14).

$$g(x,y) = \begin{cases} 0, green(x,y) < red(x,y), \\ 255, green(x,y) \geq red(x,y). \end{cases} \tag{14}$$

Another modification that has been made is that, the output value is not 0 or 1, but either 255 (white pixel) or retaining the old value of the pixel. This means that if the value of that particular pixel falls in the range of the rule whereby the output value is 255, the original pixel value will be automatically changed to 255, which indicates that it is the area of sputum. However, if it is not fall within that range the original value of the pixel is retained to enable it to go through the next filtering algorithm. Note that since Equation (14) is the last rule for the whole algorithm, then the final value is either 0 (red pixel) or 255 (white pixel).

4. Results

The rules that have been formulated are applied to the original raw image of Ziehl-Neelsen sputum slide. Rules for normal images as shown in Equation (3) – (5) have been applied to these images. The result is good for normal images. However, for other types of images, the result is not satisfactorily achieved. Some of the pixels are lost and in some images, too many noises exist. This is reflected in Figure 6.
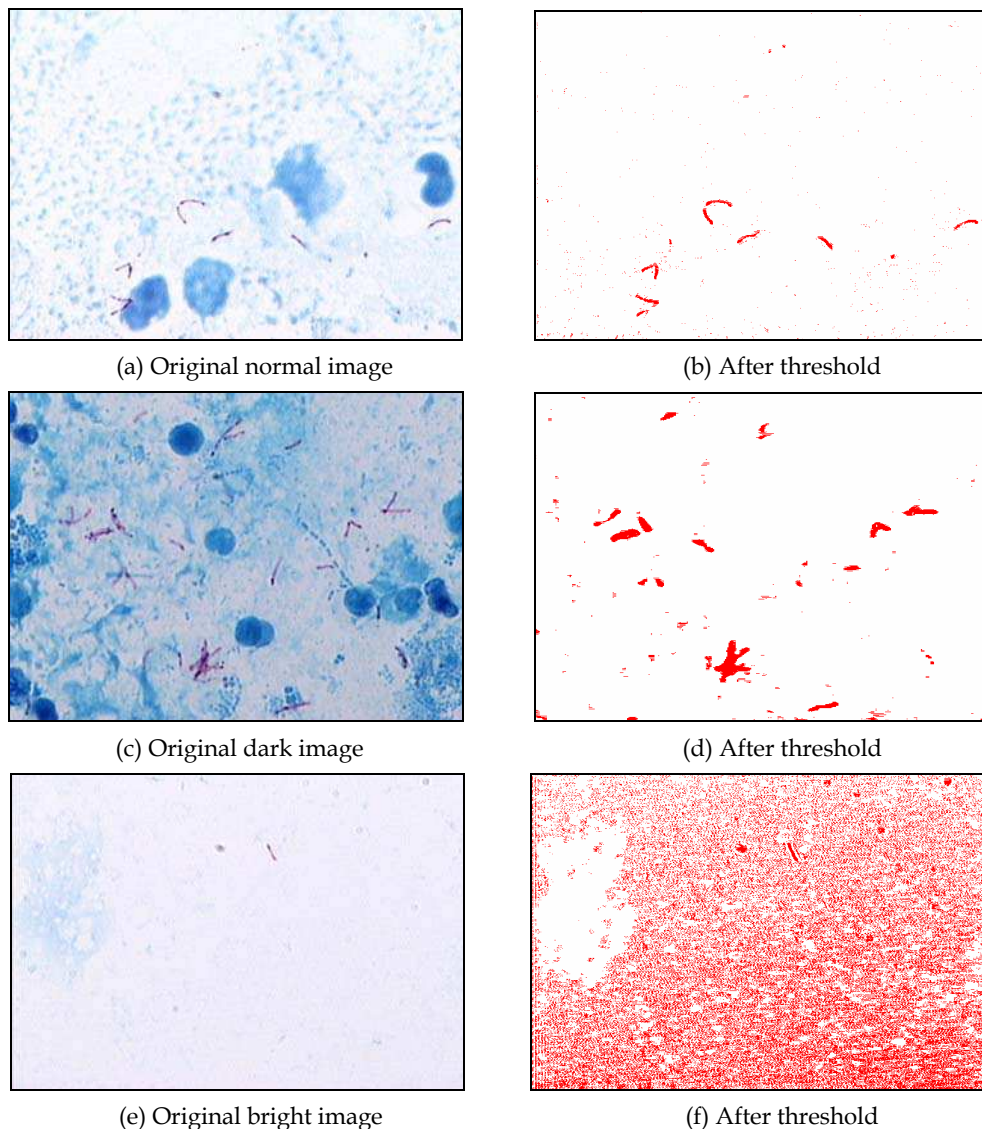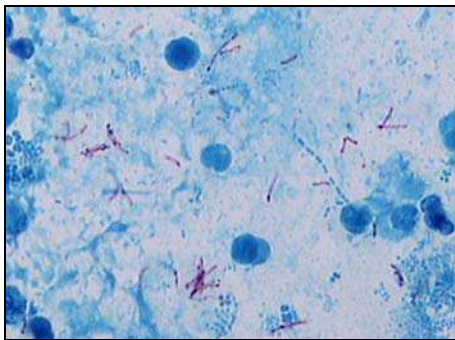

(a) Original normal image

(b) After threshold

(c) Original dark image

(d) After threshold

(e) Original bright image

(f) After threshold

Fig. 6. Original images from normal, dark and bright category with their respective result after applying rules for normal image

However, when the process of thresholding is done to the images with the rules formulated from its own category, the result is satisfying. In Figure 7, samples of dark images are thresholded using rules from dark category as shown in Equation (6) – (8).

In Figure 8, samples of bright images are thresholded using rules from bright category. This result proves that rules for one category of images are not universal and it can only be used within that particular image category. To overcome the poor result, the images are brought through the image enhancement process.

The results of thresholding to the image before the image enhancement process and the results of thresholding to the image after the image enhancement process are shown in Figure 9 to 11 .

(a) Original dark image 1   (b) After threshold
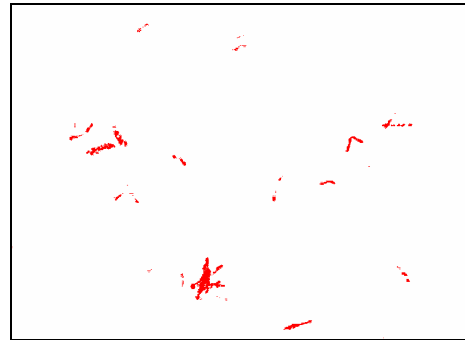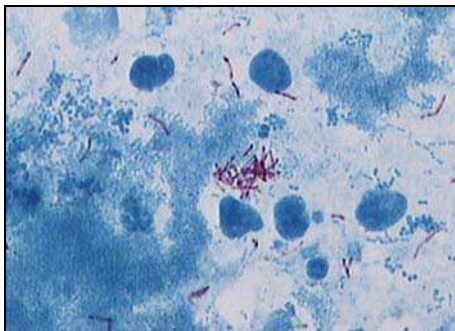
(c) Original dark image 2   (d) After threshold
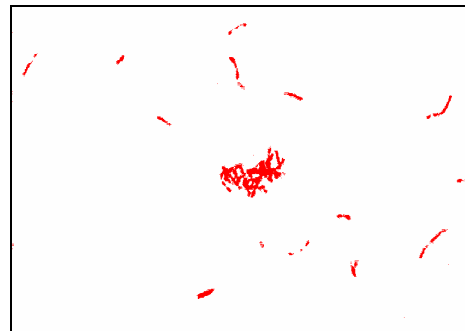
Fig. 7. Original images from dark category with the result after applying rules for dark image
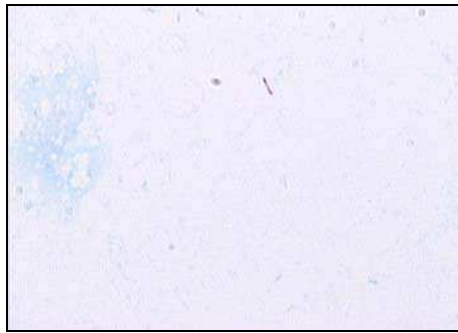
(a) Original bright image 1
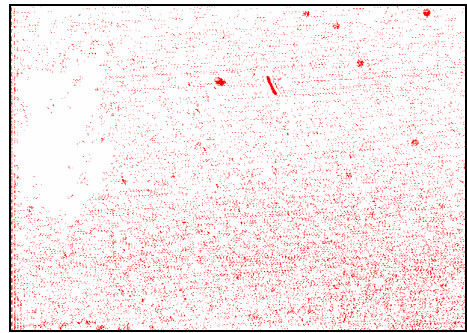
(b) After threshold

Fig. 8. Original images from bright category with the result after applying rules for bright image
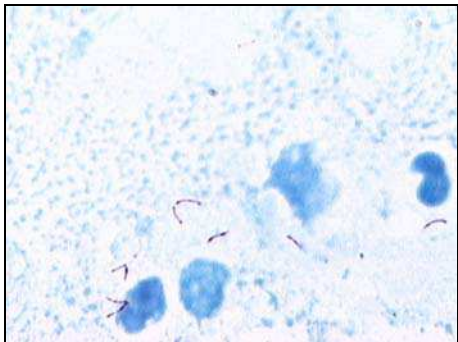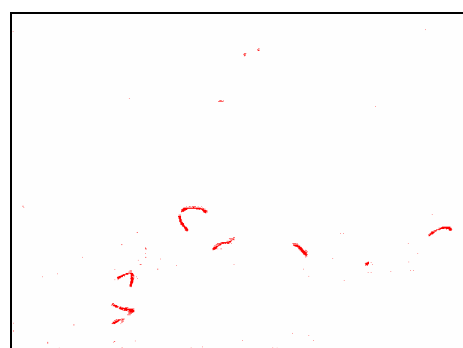

(a) Original Image
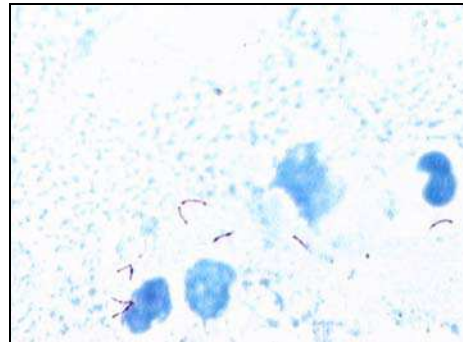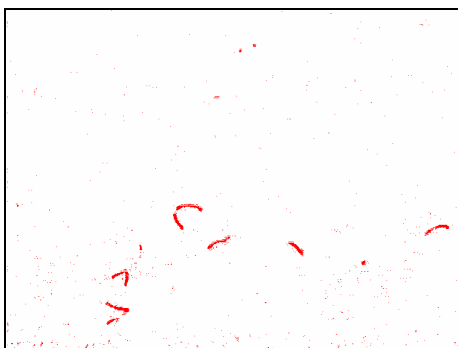
(b) Enhanced image

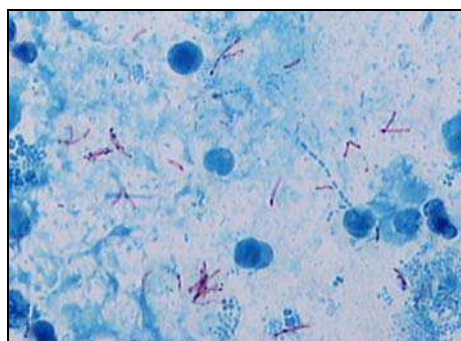(c) Result of thresholding original image

(d) Result of thresholding enhanced image

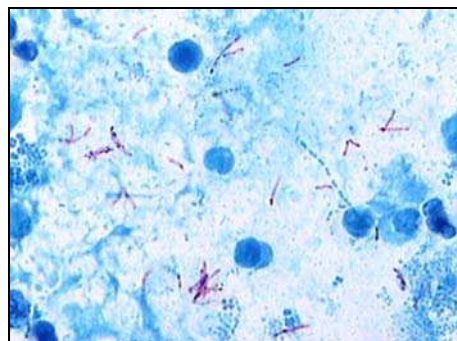Fig. 9. Segmentation result of normal image

In order to determine whether the thresholding method that has been carried out is successful or not, it relies solely on human intervention. Therefore, the threshold value need to be varied until acceptable results are achieved, based on the human observation. That is why, in carrying the color thresholding procedure, it may be necessary to do a few level of thresholding in order to get the best results.

The thresholding procedure must be done to the red, green and blue components. The thresholding on the three colors may be combined into one complete rule using the Boolean AND operator or it may also be separated into two or more rules. If more than one rule is being created, then it is considered to be done on a few level of thresholding. In this study, the process of thresholding is being done automatically based on the predetermined threshold value.

In the case of sputum slide images, it can be seen from the original images that most of the pixels appears to be blue in color. The main objective is to filter out those blue pixels and retain the reddish pixels, which are the tubercle bacilli. Therefore, the first level thresholding has been carried out, which involves pixels of RGB components which have been combined using Boolean AND operator, as mentioned earlier in the methodology part. The second level filtering which involves the difference between green and red pixel is carried out, resulting in a binary image, in which the tubercle bacilli finally appears red, while the background which are originally blue, turn out to be white.
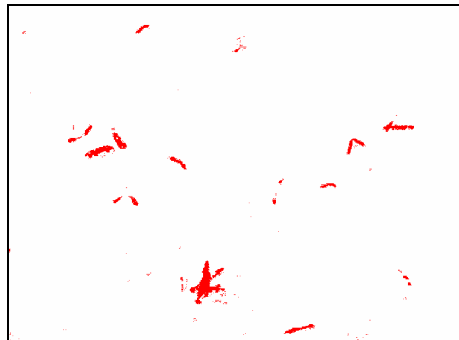

(a) Original Image
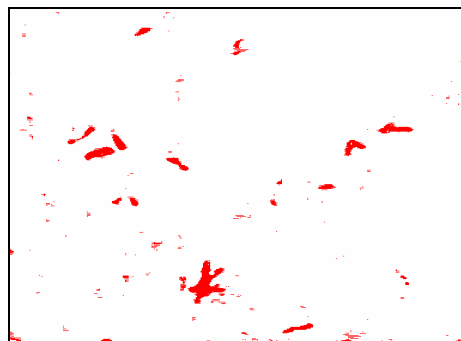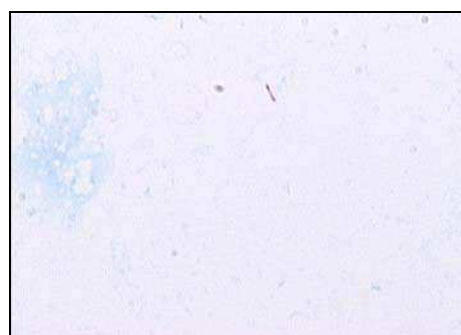

(b) Enhanced image

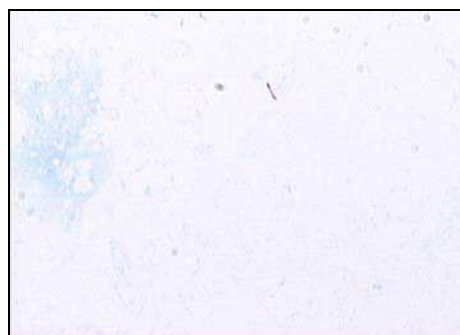
(c) Result of thresholding original image

(d) Result of thresholding enhanced image

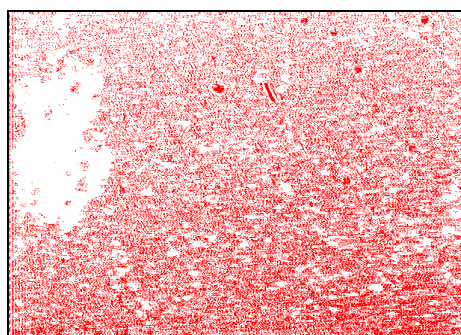Fig. 10. Segmentation result of dark image

From the resulted images that have been presented, it can be said that this technique could be an alternative solution for the image segmentation of TB bacilli, and to further helps the process of TB bacilli identification as well as classification in sputum samples. Most of the research that have been done previously used fluorescence images of sputum (Veropoulos et al., 1998; Veropoulos et al., 1999; Forero et al., 2003; Forero et al., 2004), whereby this research concentrates on the Ziehl-Neelsen stained images of sputum. Hence, it provides another option of TB bacilli identification especially for developing countries which are still sticking to this method for TB detection.
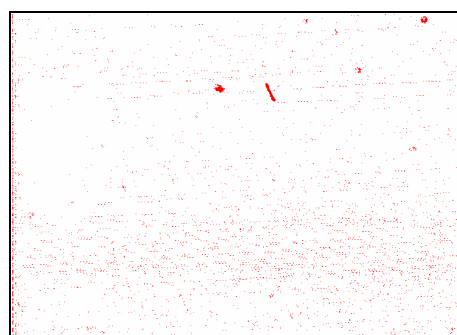

(a) Original Image

(b) Enhanced image

(c) Result of thresholding original image

(d) Result of thresholding enhanced image

Fig. 11. Segmentation result of bright image

5. Conclusion

A technique of image segmentation by conducting a thresholding method for sputum slide images has been presented. The segmentation allows the elimination of a great amount of unwanted pixels, and retained only those pixels characterised to have similar color to the TB bacilli. The key to this method is to conduct a study on the color attribute of the tubercle bacilli in order to get the basic rules of selecting the most accurate threshold value. The resulted images satisfactorily showed that after the image enhancement process, and by using the selected threshold values, the image segmentation method has been able to filter out the sputum images from the tubercle bacilli images.

6. References

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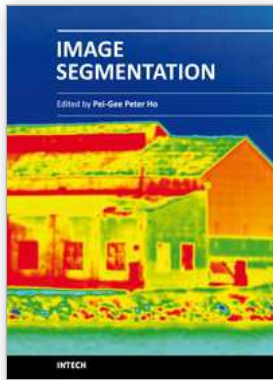
Image Segmentation

Edited by Dr. Pei-Gee Ho

It was estimated that 80% of the information received by human is visual. Image processing is evolving fast and continually. During the past 10 years, there has been a significant research increase in image segmentation. To study a specific object in an image, its boundary can be highlighted by an image segmentation procedure. The objective of the image segmentation is to simplify the representation of pictures into meaningful information by partitioning into image regions. Image segmentation is a technique to locate certain objects or boundaries within an image. There are many algorithms and techniques have been developed to solve image segmentation problems, the research topics in this book such as level set, active contour, AR time series image modeling, Support Vector Machines, Pixon based image segmentations, region similarity metric based technique, statistical ANN and JSEG algorithm were written in details. This book brings together many different aspects of the current research on several fields associated to digital image segmentation. Four parts allowed gathering the 27 chapters around the following topics: Survey of Image Segmentation Algorithms, Image Segmentation methods, Image Segmentation Applications and Hardware Implementation. The readers will find the contents in this book enjoyable and get many helpful ideas and overviews on their own study.

How to reference

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