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Detection of Tuberculosis Bacilli using Image Processing Techniques

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Abstract— ***Tuberculosis (TB) is one of the major diseases in developing countries. TB detection is based on sputum examination microscopically by using Ziehl-Neelsen stain (ZN-stain) method, which is used worldwide. This method needs human expertise and intensive examination. The availability of expertise, time and cost are the constraints of the human intervention based examinations. Therefore, there is a need of automation of examination and detection of TB bacteria using digital image of ZN-stain sample. In this work, an algorithm based on image processing is developed for identification of TB bacteria in sputum. The method is based on Otsu thresholding and k-means clustering approach. The performance of clustering and thresholding algorithms for segmenting TB bacilli in tissue sections is compared. The developed automated technique shows good accuracy and efficiency.***

Index Terms— ***Clustering, Image segmentation, Otsu thresholding, Tuberculosis.***

I. INTRODUCTION

Tuberculosis (TB) is a widespread disease caused by infection of bacterium called Mycobacterium Tuberculosis. The disease can affect different parts of body such as lungs, kidneys, liver, bones, brains and central nervous system. Pulmonary TB (PTB) refers to TB disease inside the lungs and is the most common type of TB. Extra pulmonary TB (EPTB) refers to TB disease outside the lungs. According to the World Health Organization's report [1], an estimated of 9.4 million new cases and 1.68 million deaths due to TB were recorded worldwide, making it the second killer disease after HIV/AIDS. TB disease is curable; early detection and treatment are the effective methods to reduce the mortality rate from TB and control the spread of the disease. Manual examination of TB bacilli under the microscope remains the most widely used test for clinical diagnosis of TB. However, shortage of medical facilities and human expertise are the main limitations associated with the early detection. In case of PTB, the diagnosis is based on sputum smear examination, while EPTB is diagnosed by using tissue histology. The task is tedious, time consuming and subject to human error. This suggests the use of computer aided TB detection system to help medical technologists in screening and diagnosing the disease. The diagnosis of TB infection in tissue is usually depends on the detection of the bacilli in the ZN-stained tissue slide. Color is the most useful feature that is utilized in detecting the TB bacilli. During the staining process, carbolfuchsin dye is used to color the TB bacilli red, while the methylene blue turns the tissues and backgrounds to the blue color. The ZN stain results a good contrast between the bacilli and the background, thus aiding in the detection process.

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However, the intensity distribution of TB bacilli and background are often varying from image to image, due to manually ZN preparation by technologist [2]. Fig 1 shows examples of ZN stained tissue slide images which consist of TB bacilli.

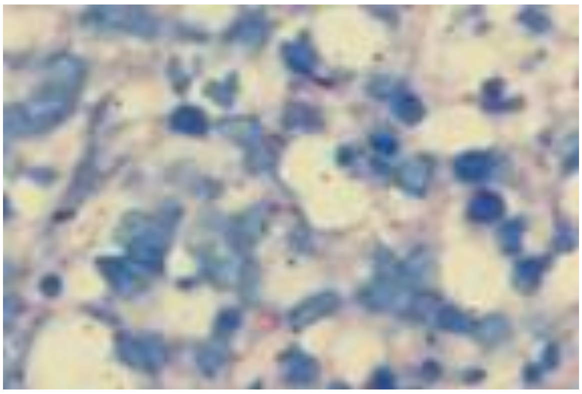


Fig.1 ZN-stained tissue slide images of TB bacilli

The bacilli which have a characteristic of red rod-shape are found scattered irregularly through the tissue images. Some of the bacilli appear stained in deep-red and in some cases they appear in pale-red. The improper reagent preparation and staining procedure may also cause the background to remain red thus make the segmentation process more challenging. Therefore a method for TB bacilli segmentation should be robust with respect to these variations.

II. LITERATURE REVIEW

Several computer-aided image diagnosis methods for TB detection have been described in the literature. These methods can be divided into two approaches: using fluorescence and light microscope. Early works focused on using the fluorescence microscope [3] [4]. Images viewed using a fluorescence microscope is more sensitive to TB bacilli and the screening process can be conducted quickly under lower magnification, compared to the light microscope [5]. However, it is expensive and difficult to maintain, thus limiting the use of fluorescence microscope in low and medium income countries. Therefore, recent works in detecting PTB used images acquired from light microscope [6] [7] [8]. The specimens are stained using Ziehl-Neelsen (ZN) staining procedure to visualize the bacilli. The automated technique can save the time and cost involved, with reduced human error. Recent work by Osman et al [2] proposed a method that combined k-means clustering and thresholding algorithm for segmenting the TB bacilli in tissue sections. The work had compared three color models; RGB, HIS and C-Y, and found that better segmentation performance had been achieved by using the saturation component of C-Y color model.

In this work, TB bacilli are segmented from digital microscopic images obtained from hospital. The clustering and thresholding algorithms are used and compared. The motivation behind comparing clustering and thresholding is to find the best method for the bacilli segmentation and to automate the identification of TB bacilli from sputum specimens. Submit your manuscript electronically for review.

III. METHODOLOGY

The images are acquired from slides using digital microscope. The slides are analyzed using Olympus binocular oil immersion light microscope with 100X magnification and their images were captured using Luminera infinity2 digital camera attached to the microscope. The images are of 24 bit color images in size of 512x512 pixels. The steps involved in processing of input images for segmentation of mycobacterium tuberculosis in tissue sections using thresholding and clustering techniques is shown in Fig.2.

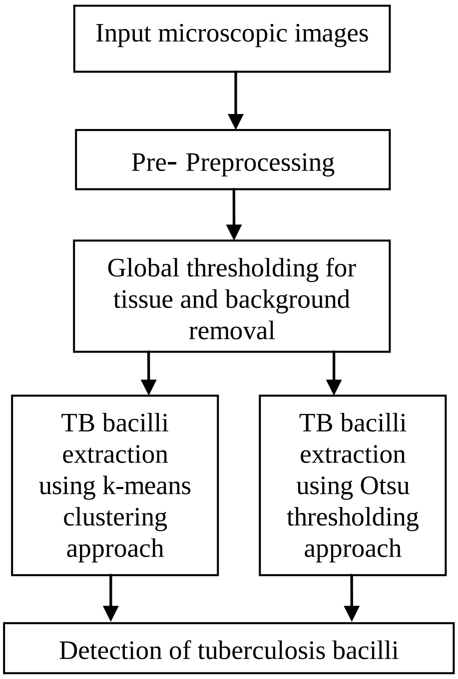


Fig. 2 Steps of segmentation and detection of TB

The present study employs the global thresholding, to remove the tissues and background which were stained by methylene blue dye, in order to initiate segmentation of the TB bacilli. For the second step, k-mean clustering is used to extract the TB bacilli from the remaining background. The third step involves labeling and removing noises and large over stained regions using the region growing method. The study introduces a local thresholding and a median filter, as a fourth step, to refine the segmented regions. In the following sections, the method is presented and discussed in detail.

A. *Thresholding*

Thresholding is an important tool in segmentation. The main objective is to reduce the number of gray levels for gray images or number of color in color images. When thresholding a grey level image or color images, with a threshold T, any point with intensity value larger than T is called an object point candidate, whereas all the other points are called background points [10]. A binary image in black and white is obtained as result of thresholding. A threshold image Pg(x,y) is defined as,

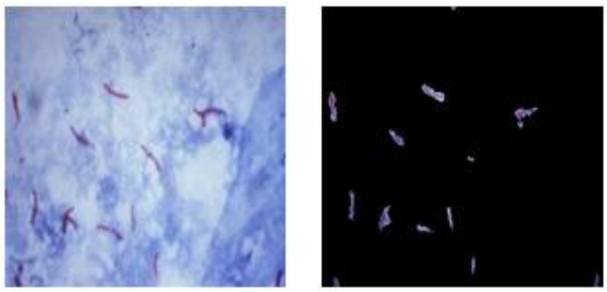
$$p_g = \begin{cases} 1 & f(x,y) > T \\ 0 & f(x,y) < T \end{cases} \qquad (1)$$

T does not have to be one single value for the entire image but can be a function. T= T[x, y, p(x, y), f (x, y)] where, f (x, y) is the grey level of the point, p(x, y) is some local property of the point and x and y are the spatial coordinates. When T depends only on f (x, y) and the same value is set for all the pixels, the operator is called global thresholding. When T depends on both f (x, y) and p(x, y) it is called local.

The first step in segmentation of the TB bacilli in ZN-stained tissue slide images involves removing the tissues and background. Median filtering is done as preprocessing operation to remove noise. Global thresholding is used in order to eliminate pixels with methylene blue counter stained. The purpose of this step is to find the sub images that contain only pixels with red color, so that later it will facilitate the process of bacilli extraction. Since the color property is defined by hue, color segmentation can be performed based on a simple hue space [2]. A global thresholding based on hue space is given in equation (2)

$$P_g(x,y) = \begin{cases} p(x,y) & if\, 0 < \theta(x,y) < \frac{2\pi}{3} \\ 0 & otherwise \end{cases} \qquad (2)$$

Where P(x, y) and (x, y) are the intensity value in RGB and the hue value at a point (x, y) in an image, respectively. Fig 3(b) shows applying global thresholding to a ZN-stained tissue slide image.



(a) (b)

Fig.3 (a) Input image (b) Global threshold image

It is observed that the process had eliminated most of the tissues and background in the image by removing pixels other than red color.

B. *K-means Clustering*

Cluster analysis or clustering is the assignment of a set of observations into subsets (called clusters) so that observations in the same cluster are similar in some sense. Clustering is a method of unsupervised learning, and a common technique for statistical data analysis used in many fields, including machine learning, data mining, pattern recognition, image analysis, information retrieval, and bioinformatics. In this work, Partitional (k-means) clustering is done using Euclidean distance measure for performing image segmentation. Compare to hierarchical clustering, Partitional clustering is found to be simple and efficient. In

Partitional clustering it creates one set of clusters that partitions the data into similar groups, whereas in Hierarchical it finds successive clusters using previously established clusters. Partitional is more efficient because it just needs to do distance calculation, whereas in hierarchical need to do full inverse distance weight where efficiency will get reduced. K-means clustering is an iterative technique that is used to partition an image into K clusters [2]. This algorithm aims at minimizing an objective function, in this case a Euclidean distance measure. The objective function

$$J = \sum_{j=1}^{k} \sum_{i=1}^{n} \left\| x_i^{(j)} - c_j \right\| \qquad (3)$$

Where $x_i^{(j)} - c_j$ is a chosen distance measure between a data point $x_i^{(j)}$ and the cluster centre c_j is an indicator of the distance of the n data points from their respective cluster centers. The algorithm is as follows

1. Pick K number of clusters either manually, randomly or based on some heuristic.
2. Generate K clusters and determines the cluster's center
3. Assign each pixel in the image to the cluster that minimizes the distance between the pixel and the cluster center
4. Re-compute cluster centers by averaging all of the pixels in the cluster.
5. Repeat steps 3 and 4 until some convergence criterion is met.

The second step involves extracting the TB bacilli from the remaining background with the red color. However, the intensity distribution of TB bacilli and background are often varying from image to image, due to manually Z-N preparation by technologist. It is also observed that most of the TB bacilli have appeared more deep red compared to the background. Therefore an adaptive solution is required for segmenting the bacilli. An adaptive pixel segmentation based on k-mean clustering is implemented on the color intensities of pixels and grouped the pixels into two clusters; correspond to the TB bacilli and the remaining background.

C. Otsu Thresholding

The Otsu's thresholding[13] algorithm is one of the most referenced thresholding methods to partition images by automatically selecting threshold values from the histogram of the image. To find threshold values, the Otsu's algorithm utilizes the variance property of the image because variance is the measure of uniformity; the greater value of variance represents the greater difference between the background and the object. Initially, two regions are separated by the intensity threshold, and then the optimal threshold is determined by minimizing the within-class variance or maximizing the between-class variance.

Consider an image consists of N pixels with their intensity levels [0, 1, 2…… L-1], and having the frequency of h(i) at intensity level i. The probability distribution is a normalized intensity histogram, and is shown in Fig. 4.

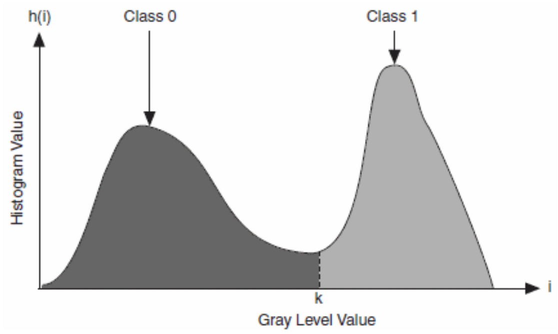


Fig. 4 Normalized intensity histogram

Where, *i* represent the gray level value of pixel. *k* represents the gray level value chosen as the threshold. h(i) represents the number of pixels in the image at each gray level value. N represents the total number of gray levels in the image (256 for an 8-bit image). n represents the total number of pixels in the image.

Use the automatic thresholding techniques to determine the threshold pixel value k such that all gray-level values less than or equal to k belong to one class 0 and the other gray level values belong to another class 1.

$$P(i) = h(i) / N \qquad (4)$$

The zeroth, the first order cumulative moments of the histogram up to the t-th level and the total mean level can be determined using(5) (6) (7)respectively.

$$\omega(k) = \sum_{i=0}^{k} p(i) \qquad (5)$$

$$\mu(k) = \sum_{i=0}^{k} i\, p(i) \qquad (6)$$

$$\mu_T(k) = \sum_{i=0}^{L-1} i\, p \qquad (7)$$

The threshold value is the pixel value k at which the following expression is maximized and is given by eq(8)

$$\sigma_B^2(k_{otsu}) = \frac{[\mu_T(k)\,\omega(k) - \mu(k)]^2}{\omega(k)[1-\omega(k)]} \qquad (8)$$

D. Region Growing Method

Region growing is a procedure that groups pixels into regions taking into account the neighborhood of each pixel according to selected properties and pre-defined similarity criteria including texture, brightness and color or gray level of individual elements. Pixels having similar properties form a region and are joined together. The aim of region-based segmentation method is to extract the homogeneous sectors from the given input image, i.e. to partition an image into regions. It is the process of merging neighboring areas of the image into larger regions based on the similarity of pixels and works by selecting seed pixels as starting point. The selection of region of interest is determined based on its size. If such a region is supposed to occupy the size within the minimum and maximum of predefined size, A_{min} and A_{max} respectively, then the region is preserved by restoring its original intensity of the pixel. Otherwise, the region will be eliminated from the image. In this work, A_{min} = 30 and A_{max} =500 were found suitable for the ZN stained images and were obtained through observations. In Fig 5(a) and 5(c) shows results of k-means

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clustering Otsu thresholding, 5(b) and 5(d) shows results of region growing applied to the both methods and it can observed that most of the unwanted regions and noise are eliminated.

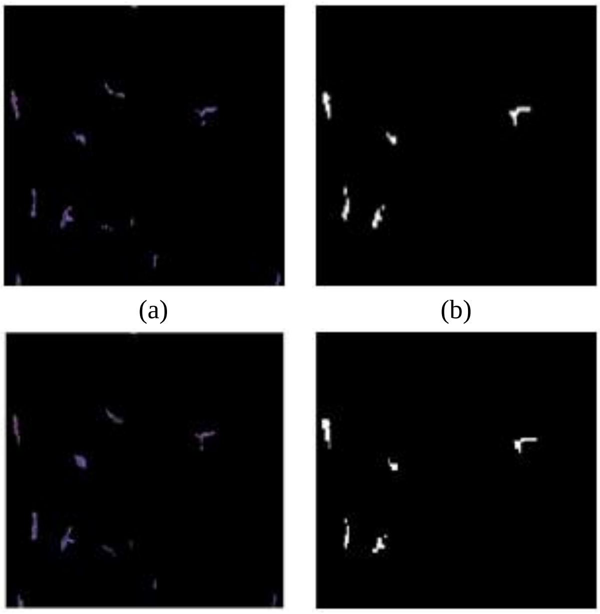

Fig. 5(a) K-means clustering (b) region growing after K-means clustering (c) Otsu thresholding (d) region growing after Otsu thresholding

E. Local Thresholding

Another problem with global thresholding is that changes in illumination across the scene may cause some parts to be brighter (in the light) and some parts darker (in shadow) in ways that have nothing to do with the objects in the image. The uneven intensity of TB bacilli makes segmentation process difficult. Due to this reason, performing thresholding and clustering based on global image information is occasionally inadequate to segment the bacilli properly. In order to overcome the problem, a local adaptive thresholding based on local statistic of a region is proposed [2]. In local adaptive thresholding technique, the aim is to compute a threshold value T_n for each region in the image. Consider a segmented color image in which P(x, y) be the intensity of a pixel at location (x, y) and has N regions. Also, suppose that an inadequate segmentation of a region is indicated by poor uniformity value of a region. The method starts by calculating the uniformity of each region in the image, as give by,

$$U_n = 1 - \frac{s_n^2}{\bar{x}_n^2} \qquad n=1, 2\ldots..N \qquad (9)$$

Where S_n^2 and $\bar{x}_n$ are the variance and mean intensity of the n^{th} region. If a region has uniformity less than the minimum allowable uniformity value, U_{min}, then the local adaptive thresholding are applied according to:
The threshold value T_n is computed using the mean, x_n and standard deviation, σ^2_n of the region intensity, and is given by

$$T_n = \bar{x}_n - k\,\sigma_n^2 \quad k>0 \qquad (10)$$

Where *k* is a parameter which controls the value of the threshold. The value of U_{min} and *k* are set to 0.95 and 0.80, respectively and are used throughout this paper. The intensity of image P(x, y) is chosen to be similar to that previously used color component in the k-mean clustering process. The presence of blurring, non-uniform illumination, over staining and under staining of tissue slides will result in regions with unsmooth boundary and increase the segmentation error. It is also realized that some features which are sensitive to noise can be quite complicated to describe an object with unsmooth boundary. In order to avoid this problem, a median filter is introduced. Filter has successfully smoothed the boundary without altering the region color information. Fig.6 (a) and (c) shows result after k-mean clustering and thresholding (b) and (d) shows result is further improved by applying local adaptive thresholding.

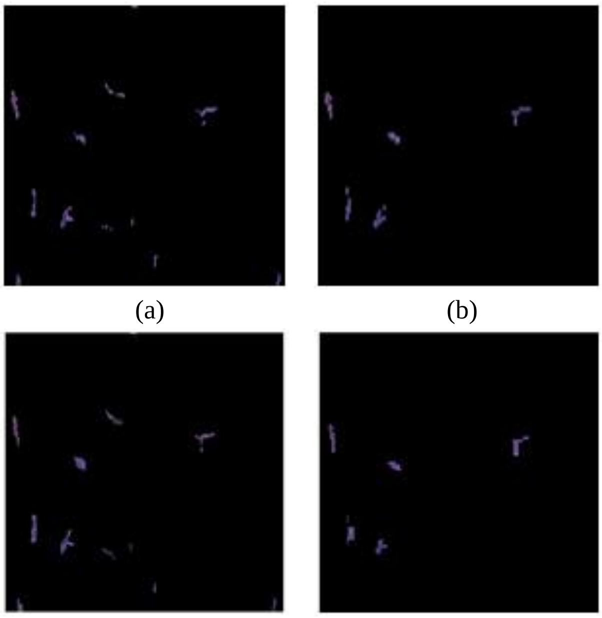

Fig.6(a) K-means clustering (b)local thresholding after k-means clustering (c)Otsu thresholding (d)local thresholding after Otsu thresholding

It is observed that the method yields a better segmentation result by removing the under-staining pixels.

IV. RESULTS

The fully automatic TB bacilli segmentation method was tested using 25 positive tissue slides. All the slides were prepared at the pathology department of hospital. 24 bit color images, in size of 512 ×512 and were stored in JPEG format. All the segmentation methods were implemented using MATLAB 7.6 version.

Throughout the visual inspection and comparison with the manual segmentation image, it can be seen that all of the algorithms are able to segment TB bacilli. Increasing the number of cluster from 2 to 3 had lead to over segmentation and had tendency to reduce the TB segmentation rate. As the main objective is to detect the presence of bacilli, the higher TB segmentation rate is important to avoid missed segmentation of TB bacilli during performing the task. Therefore, k-mean clustering algorithm with k = 2 is chosen, as it produced the highest TB segmentation rate. In clustered image of Otsu thresholding, it is observed that pixels are grouped into two clusters; corresponds to TB bacilli and background

Performance comparison of thresholding and clustering algorithm is evaluated by comparing the results of segmented images against a manual segmentation image. Manual segmentation images are prepared through manually tracing the TB bacilli and removing the background. For quantitative evaluation of each method, a pixel based comparison between the resultant image and manual segmentation is performed. The numbers of false positives, false negatives, true positives and true negative were obtained. From these four values, other three indicators were formulated to measure the quality of segmentation; accuracy, TB segmentation rate and background segmentation rate, and are defined as follows:

$$\textit{Accuracy} = (TP+TN))/ ((TP+TN+FP+FN)) \times 100 \quad (11)$$

$$\textit{TB segmentation rate} = TP/ ((TP+FN)) \times 100 \quad (12)$$

$$\textit{Background segmentation} = TN/((TN+FP)) \times 100 \quad (13)$$

True positive (TP): a pixel that belongs to the expert segmented region and was detected as “object-of-interest” by the algorithm;

True negative (TN): a pixel that does not belong to the expert segmented region and was detected as “non-object-of-interest” by the algorithm;

False positive (FP): a pixel that does not belong to the expert segmented region and was detected as “object-of-interest” by the algorithm;

False negative (FN): a pixel that belongs to the expert segmented region and was detected as “non-object-of-interest” by the algorithm.

The accuracy measures the number of correctly segmented TB and background pixels. The TB segmentation rate refers to the sensitivity of segmenting of TB pixels, while the background segmentation rate is the ability of the segmentation in rejecting the background pixels. For these measurements, their values range between zero and one, with higher measurement number indicates better segmentation performance. Table 1 shows segmentation performance of clustering and thresholding algorithms.

Table 1 Segmentation performance of clustering and thresholding algorithms

Segmentation method	Accuracy	sensitivity	Specificity
K-means clustering	98.91	99.22	98.73
Otsu thresholding	98.36	99.02	98.02

It is very evident from Table1 that, the clustering and thresholding algorithms are able to segment the TB bacilli well, with an accuracy of up to 98.00%. The performance of the K-Means algorithm is found to be better than Otsu thresholding for identification of bacilli in ZN stain sputum images because it gives clean clusters. Otsu method is very sensitive to image contrast, hence not a stable method for different contrast level images.

V. CONCLUSION

The developed algorithm detects the TB bacilli automatically. This automated system reduces fatigue by providing images on the screen and avoiding visual inspection of microscopic images. The system has a high degree of accuracy, specificity and better speed in detecting TB bacilli. The method is simple and inexpensive for use in rural/remote areas in the emerging economies. Segmentation algorithm is developed to automate the process of detection of TB using digital microscopic images of different subjects. A performance comparison of clustering and thresholding algorithms for segmenting TB bacilli in ZN-stained tissue slide images is carried out. The results presented showed that a more convincing segmentation performance has been achieved by using the clustering methods, as compared to the thresholding method. These results also suggest that k-mean clustering is the best method for segmenting the bacilli, as it is highly sensitive to the TB pixels.

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