Detection of *Mycobcaterium tuberculosis* in microscopic images of Ziehl-Neelsen-stained sputum smears LACNEM 2015

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Abstract

Tuberculosis is a disease with a high mortality rate worldwide, but early recognition highly increases the chances of survival. There are several tests to diagnose this disease, however the Pan American Health Organization recommends for its low cost and effectiveness sputum smear microscopy. The count bacilli present in a sputum sample is the core of diagnosis. This procedure requires time and a specialist with a well-trained eye, thus making it prone to error. The techniques used in artificial vision for object detection can detect bacilli, enhancing the performance of bacilloscopies. This paper presents a methodology for detecting bacilli in sputum smear images, implementing adaptive k-mean segmentation clustering, and using artificial neural networks. The database consists in total of 100 images taken from three samples, each with different characteristics. All samples were analyzed by an expert in microbiology, with which the ground truth was obtained. The assessment was done by comparing the segmented image pixel by pixel with the ground truth and calculating the confusion matrix values for specificity, sensitivity, and accuracy. The segmentation succeeded 97.59 % precision. The accuracy of the artificial neural networks reached 98 % using cross-validation. The results show that the proposed methodology is effective, and it can accurately identify the bacilli in the Ziehl-Neelsen-stained sputum smear images.

keywords: bacilloscopy, k-means, tuberculosis, Ziehl-Neelsen

1 Introduction

The bacilloscopy or smear microscopy is a test that is used to diagnose respiratory tuberculosis (TB) by analyzing a sputum sample (lung mucus, saliva) stained using the Ziehl-Neelsen technique under a microscope with a 100x magnification, and counting the number of Mycobacterium tuberculosis present in the smear [1]. This test must be performed by a bacteriologist and can be time-consuming [2], since it is necessary to analyze at least 100 fields of the sample. Furthermore the conditions of the sample can vary by several factors, including the quality of staining or sputum density, which can cause the diagnosis to be imprecise, not to mention the possibility of human error. Smear microscopy has not yet been subject to substantial technical modifications to improve and optimize the process [3]. According to the Manual for the bacteriological diagnosis of tuberculosis by the Pan American Health Organization, it is estimated that approximately one third of the world population is infected with *M. tuberculosis*, the tuberculosis causing bacillus. Of the infected population per year about 8 million people develop the disease and two million do not survive it [3]. *M. tuberculosis* can be latent and affect any organ in the body, but in about 80 % of the cases it infects the lungs, this is due to the large amounts of oxygen required by the tubercle bacillus to reproduce; these cases are called respiratory tuberculosis. To consider a patient TB positive he or she should show at least 5,000 to 10,000 bacilli per milliliter sputum. The counting test could be performed automatically using computer vision techniques to analyze the presence of bacilli in the samples, reducing diagnosis time. Costa et al. [4] propose a method for detecting images of bacilli in smears using adaptive thresholds for segmenting the bacilli's images. The methodology proposed by Costa et al. works very well when the images are quite similar and do not show significant variation in illumination or staining quality. Makkapati et al. [5] present a method where bacilli are characterized using the H channel of the HSV color space, then follows the segmentation using threshold values obtained from the characterization. This method can detect bacilli, but has the disadvantage that the segmentation also obtains elements that are not bacilli. Zhai et al. [6] use experimental threshold operators for segmenting into two color spaces, HSV using the H channel and CIE L*a*b* using the L channel, then the final segmentation is obtained by performing a logical AND operation

with the binary images of the two segmentations. Costafilho et al. [7] proposed a classification by pixel characteristics to segment the bacilli. Using an artificial neural network (ANN) they classified the pixels belonging to the bacilli, and those belonging to the background. The used characteristics of the pixels were components and subtractions of components of the following color spaces: RGB, HSI, YCbCr and CIE L*a*b*. With these features bacilli can be partly detected, but afterwards another discrimination based on other geometric characteristics must be performed. Osman et al. [8] propose a method for detecting bacilli in which they first apply a threshold operator to the picture and then implement filters to remove unwanted elements; after that they use K-means clustering to group pixels belonging to the Bacilli class and finally to refine the segmentation they use region growing algorithms.

Automatic identification of bacilli involves facing several challenges, given that, due to the nature of the samples, the bacilli can be found in different conditions, such as covered by immune cells, or immersed in very dense sputum (see Figure 1). Because of this, the ideal is to have a robust system that can recognize bacilli in most circumstances. In the task of detecting bacilli in images there have been methods proposed to effectively identify bacilli, although they have not tried an adaptive way, i.e., models that are invariant to changes in the sample. The proposed methods are based on the learning of a model that can separate classes; this limits the system, since variations in the conditions render the system inefficient. The invariance method is a crucial aspect that has to be taken into consideration. Hence, there is the need to implement an adaptive method. This paper presents a method for the detection of *M. tuberculosis* in sputum smear images that can diagnose respiratory tuberculosis effectively, via an adaptive segmentation method using an ANN as classifier. In addition to this introduction the manuscript is organized as follows. Section 2 describes the proposed methodology, thus image acquisition (Section 2.1), the method of adaptive segmentation (Section2.2), the selection of cluster centers (Section2.3), feature extraction (Section2.4) and the classifier used (Section2.5). In section 3, the data obtained from the validation results are described; and the section 4 consolidates implementing the proposed methodology.

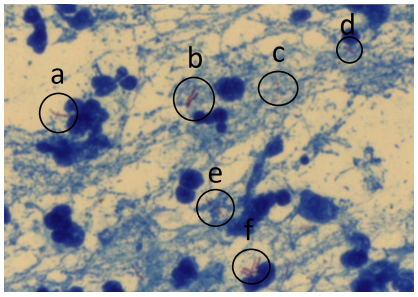

Figure 1: Characteristics of variations in the presentation of bacilli: (a) bacilli in ideal conditions; (b) overlapping bacilli; (c) light bacilli; (d) and (e) bacilli immersed in sputum; (f) grouped bacilli, some underneath cells

2 Materials and Methods

The here proposed detection method is based on a first adaptive segmentation, and then a classification using an artificial neural network. In this section are the steps and operations shown that make up the methodology, from the image acquisition to the classification of segmented elements.

2.1 Image Acquisition

For the smear test a sputum sample of the patient is taken and spread on a glass slide to which then is applied the staining using the Ziehl-Neelsen technique. This makes the bacilli visible in the sample because of turning them color magenta, and the other elements turn blue. After staining, the sample is checked under a microscope at 100x magnification and the number of bacilli is counted, and according to the amount found it is determined whether the patient is infected with *M. tuberculosis*. The sample images were acquired with a Leica ^{TM}DM750M [9] using a 100x/1.25 objective with a Leica camera ^{TM}ICC50 HD (100x magnification, oil immersion). The images were acquired with a resolution of 2048 x 1536 in 24-bit jpg format. The database consists in total of 100 images taken from three samples, each with different characteristics. Two of these three samples were positive, i.e. with bacilli. Of the two positive samples 25 pictures of each were taken, and of the negative sample (without presence of bacilli) 50 pictures were taken. The lighting parameters were varied for each of the 100 images with a total of seven shots per image, as shown in Figure 2,having in total seven hundred images (350 positive and 350 negetive), in order to determine under what image conditions the segmentation method performed the best. All samples were analyzed by an expert in microbiology, with which the *ground truth* was obtained. The processing application was developed using the open source OpenCV library from Intel. The complete sequence of the proposed methodology is illustrated in Figure 3.

2.2 Adaptive k-means segmentation

Segmentation is the step of artificial vision to isolate the elements that are to analyze from those who are not needed. The remaining steps of artificial vision depend on the effectiveness of this step. Numerous previous works [10]–[12] realized the detection of *M. tuberculosis* using fluorescent light microscopy, where the bacilli take a bright green color and the rest of the sample remains black. This way of acquiring images is very efficient, because the bacilli can be seen clearly, but these microscopes are much more expensive compared to normal light microscopes. In the proposed methodology adaptive k-means clustering is used as the method of segmentation. An adaptive method is used since the smear images, depending on the sample quality, show great variation in color intensity and translucency, among other parameters. For this the segmentation must be adaptive, with the aim to differentiate the elements in the sample independently from variations. The *k-means clustering* method has not been used previously to intentionally select the

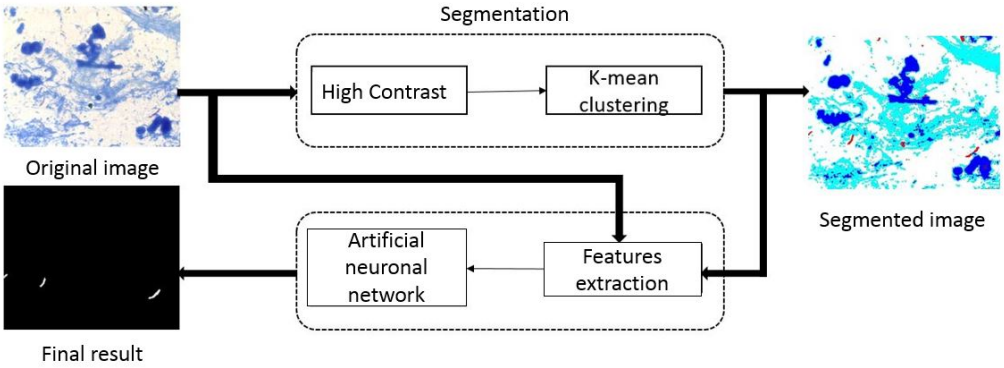

Figure 3: Bacilli detection chart

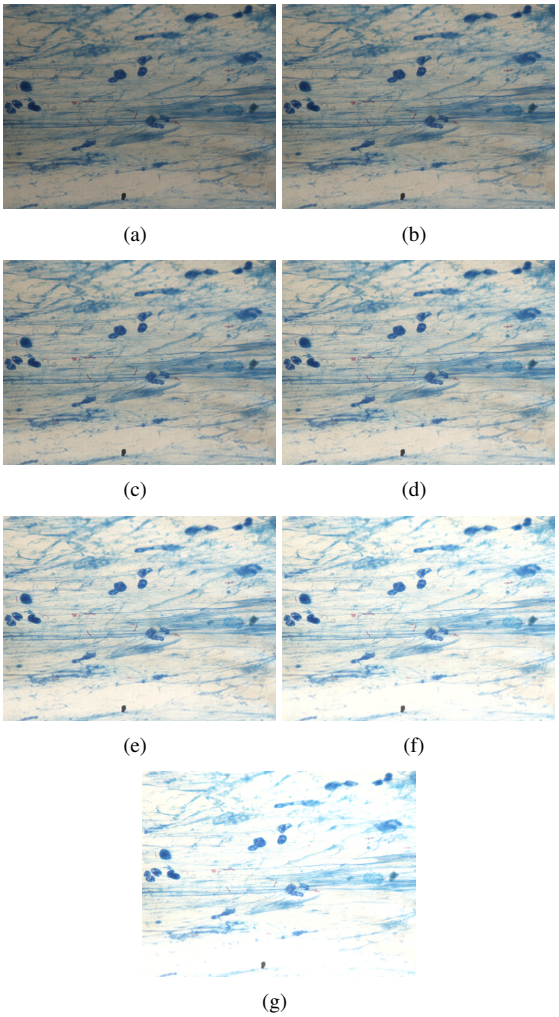

Figure 2: Lighting variations (a) Lighting 1 (b) Lighting 2 (c) Lighting 3 (d) Lighting 4 (e) Lighting 5 (f) Lighting 6 (g) Lighting 7

cluster centers, and that these centers are determined by knowing the characteristic elements you want to group. K-means is a method that brings elements of a set in k number of clusters together; by calculating the distance of each element to a central group, the method assigns an element to a cluster, determining which cluster center is closer. In the proposed methodology four classes (k = 4) are considered corresponding to cells, sputum, background and bacilli. The k-means algorithm assigns each instance (in this case pixel) to a class (cluster) determining to which center there is less distance. Given a group of centers $k = \{m_1^{(t)}, m_2^{(t)} ... m_k^{(t)}\}$ the assignment of each instance X_p to a cluster $S^{(t)}$ is given by the Equation 1.

$$S_i^{(t)} = \{X_p : ||X_p - m_i|| \leq ||X_p - m_j|| \forall 1 \leq j \leq k\} \quad (1)$$

The standard model of the algorithm independently selects the group centers and therefore the clusters. To determine the centroid, random data for each class is selected; the data center uses it as a first center, then groups the clusters, and calculates the distribution of elements updating the centroids. The operations are performed iteratively until it complies with one of two conditions: that the center does not vary or that it meets a limit of iterations. This way of using the clustering method becomes inefficient for the proposed methodology, since the clusters are randomly chosen according to the conditions of the image. As the bacilli are not a relevant pixel number for the area they occupy in the picture, they are not elected as a class (cluster). Therefore the proposed methodology intentionally choses cluster centers, so that it is determined by the conditions of the image, which should be the cluster for each class.

2.3 Calculation of cluster centers

The cluster centers are the average values of pixel intensity corresponding to suspected regions of each class. For this, images are used in high contrast determined by Equation 2 for each image channel, where $g(x, y)$ is the resulting image and $f(x, y)$ is the original image. From the picture with high contrast the pixels which were red (255,0,0) are taken as bacillus class, the blue (0,0,255) ones as cell class, the cyan (0, 255, 255) ones are taken as sputum class and the white (255 , 255, 255) pixels as

the background class. They are taken as a mask and the intensity values of the classes are averaged in the HSV color space to determine the cluster centers.

$$g(x,y) = \begin{cases} 0 & if \quad f(x,y) \leq 128 \\ 255 & if \quad f(x,y) > 128 \end{cases} \tag{2}$$

2.4 Feature extraction

The extraction of descriptors should ensure that they are exclusive to guarantee that the classifier can discriminate between classes. For the selection of features Balu Toolbox Matlab [13] was used, which allowed extracting 700 features regarding morphology and texture in the RGB, HSV, L* a* b* color spaces. Once extracted, Balu Toolbox Matlab makes a selection of the most discriminating features. This process resulted in 20 features, which are listed below, where the first letter refers to the color space channel, where the feature is extracted (abbreviated: g-Grya, R-red, G-green, B-blue).

Geometric features: Eccentricity, Solidity, Roundness, R-Humoment-int 1.

Color features: R-Tx 8 d 4(range), g-Tx 2 d 3(range), g-Gabor(2,7), g-Intensity Skewness, R-Gabor(2,6), R-Tx12,d 1(mean), Fourier-des 7, g-Mean Boundary Gradient, R-Mean Laplacian, R-Gabor-J, g-Gabor-max, g-Gabor-min.

Contrast features: g-contrast-K1, R-contrast-K1, g-contrast-K, g-HOG.

2.5 Clasifier

The Balu Toolbox Matlab performance was tested with several classifiers, where the most efficient methodology was found to be a neural network. Neural networks are classification systems that mimic the most fundamental process of reasoning. Each neuron receives signals that are interlinked with the weight of that signal. This means that the signal is very important for the decision process, and generates an output that depends on its activation function. Before the neuron is able to differentiate classes it is necessary to subject it to a learning process. For this training examples, belonging to the Bacilli class and others who do not, were entered. A neuron with 20 inputs and one output with binary activation function was implemented. This step gives the system the ability to distinguish which ones of the targeted elements are bacilli.

3 Results

The adaptive segmentation algorithm was applied to all images in the database. As a first step the high contrast image was generated obtaining the results shown in Figure 4(b). After calculating the cluster centers, this image is applied to k-means segmentation and the results show Figures 4(c). After separating the classes, the bacilli class is discriminated. An example of a bacilli segmentation for each of the intensity levels is shown in Figure 5. This assessment was done by comparing the segmented image pixel by pixel with the *ground truth* (Figura 6) and calculating the confusion matrix values for specificity, sensitivity and accuracy. The results are shown in Table 1. The specificity index refers to the ability of the method to recognize a negative element in the sample as negative; the sensitivity index indicates the ability of the method to recognize a positive element as positive, while precision refers to the ability of the method to find more accurately the actual value of the sample These indices ideally have 100 %, in this case the highest precision value is chosen, related to the highest value of specificity. The lighting condition that meets the above mentioned is the condition 3.

	Sensitivity	**Specificity**	**Accuracy**
Lightin 1	87,85%	45,47%	87,88
Lightin 2	94,72%	55,32%	94,75%
Lightin 3	97,58%	55,28%	97,59%
Lightin 4	97,15%	44,29%	97,19%
Lightin 5	97,95%	28,92%	98,01%
Lightin 6	99,62%	10,81%	99,69%
Lightin 7	99,89%	4,27%	99,97%

Table 1: Results of k-means segmentation

For the characterization de *ground truth* images were used to obtain the features of the bacilli and to characterize the non-bacilli classes the segmented images were subtracted from the *ground truth* images. Thus all false positives of the segmentation were identified, which are the elements to be eliminated ultimately. A simple perceptron with 20 entries was used. It was trained by adding a feature and measuring the performance to find those features with which it performed best. The results found with this operation are shown in Figure 7.

4 Conclusions

This paper presents a method for detecting *M. tuberculosis* in smear images. The methodology was evaluated with images with various conditions. The segmentation approach based on k-means clustering presents results of 97.59 % precision. The implementation of the ANN can effectively discriminate the elements which were wrongly classified in the segmentation step. The accuracy of the ANN is 98 % using cross-validation. Thereby the system can detect the presence of *M. tuberculosis* in a sputum sample stained with Ziehl-Neelsen with high effectivity.

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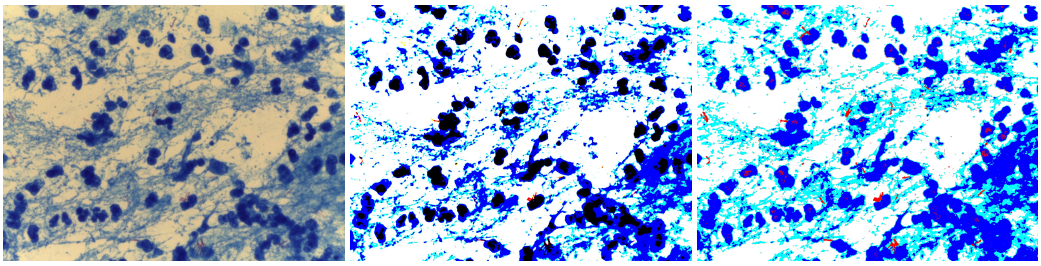
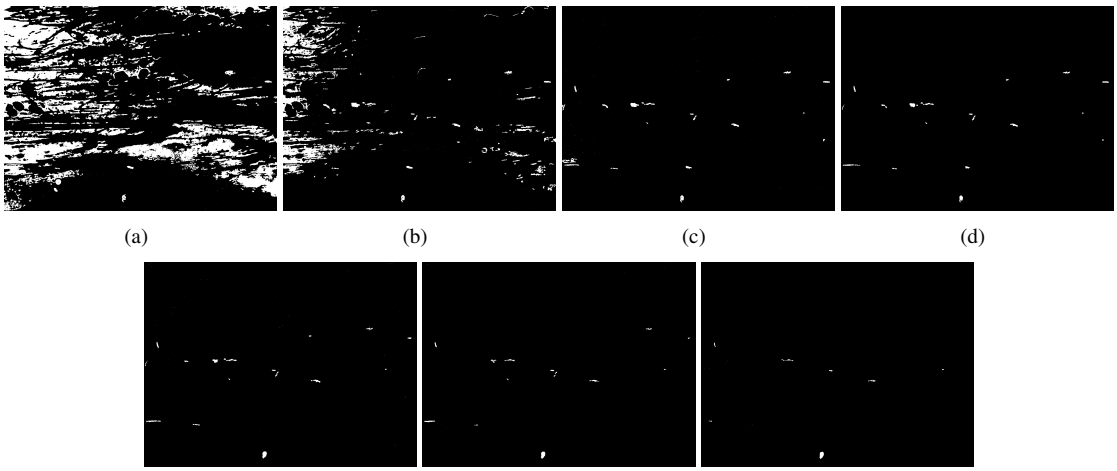


(a) (b) (c)

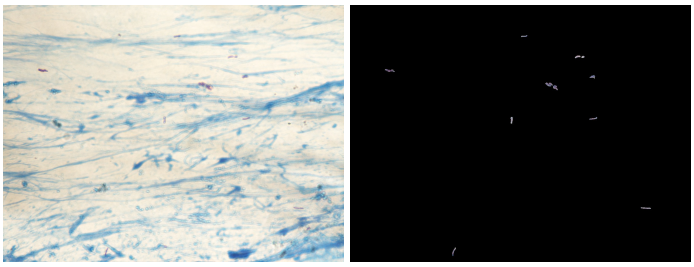
Figure 4: (a) original image (b) high contrast image (c) k-means segmentation



(a) (b) (c) (d)

(e) (f) (g)

Figure 5: Segmentation under different lighting conditions (a) Lighting 1 (b) Lighting 2 (c) Lighting 3 (d) Lighting 4 (e) Lighting 5 (f) Lighting 6 (g) Lighting 7



(a) (b)

Figure 6: Ground truth

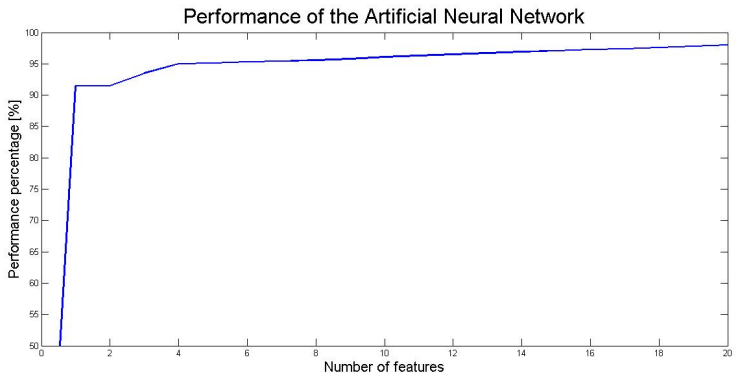


Figure 7: Performance of the Neural Network.

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