Automated Identification of Tubercle Bacilli in Sputum

A Preliminary Investigation


OBJECTIVE: To use an automated method to detect tubercle bacilli in sputum specimens.

STUDY DESIGN: Using fluorescence microscopy, tubercle bacilli were identified on auramine-stained sputum specimens. Images were then captured with a digital camera and enhanced through imaging processing techniques. The bacilli were recognized using neural network classifiers.

RESULTS: This preliminary investigation demonstrated a sensitivity of 94.1% for the identification of individual bacilli. As there are usually fairly numerous tubercle bacilli in the sputum of patients with active pulmonary tuberculosis, the overall diagnostic accuracy of sputum smear-positive patients can be expected to be very high.

CONCLUSION: Potential benefits of automated screening for TB bacilli are: rapid, accurate, inexpensive diagnosis; the ability to screen larger numbers of people; increased resources to monitor patients; and reduction in health risks to staff.

Keywords: tuberculosis; sputum; image processing, computer-assisted; mass screening; fluorescence microscopy; Fourier analysis; neural networks (computer).

Tuberculosis (TB) kills more young people and adults than any other infectious disease. In developing countries, tuberculosis is responsible for more adult deaths than AIDS and malaria combined.

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In both developed and developing countries the disease is on the increase due to a number of factors, such as multidrug resistant TB and increased incidence of HIV infection. It is a treatable disease, and the key to its control is rapid identification of infected persons, initiation of immediate treatment and monitoring of patients on treatment. World Health Organization guidelines suggest diagnosis of TB by identification of tubercle bacilli in sputum smears using a light microscope for Ziehl-Neelsen-stained specimens or under a fluorescence microscope for rhodamine/auramine-stained sputa. There is general consensus that fluorescence microscopy is superior for the identification of tubercle bacilli. However manual screening for TB is labor intensive and has a high false negative rate.

The automation of screening for tubercle bacilli could offer:

- **Greater accuracy in diagnosis.** In developing countries, stretched laboratory resources necessitate limited screening of sputum smears. Usually not more than 50 high-power fields are examined, resulting in false negative reports.
- **Increase in volume of slides screened.** Automated screening would facilitate the screening of larger numbers of slides; thus, more persons with TB would be discovered, more patients on therapy could be monitored, and multidrug-resistant patients could be identified.
- **Automation of the diagnostic process could reduce the risk of infection in laboratory staff.**

The aim of this preliminary investigation was to explore the automated diagnosis of tuberculosis from sputum samples—the detection of tubercle bacilli using image processing and recognition techniques. Images of tubercle bacilli were captured using a digital camera attached to a conventional fluorescence microscope; these images were then enhanced using a number of image processing techniques. Shape descriptors were derived for each object in the image and used as inputs to a multilayered neural network.

**Materials and Methods**

The data used for this preliminary investigation were drawn from a set of 1,000 randomly chosen auramine-stained slides prepared by the TB Control Laboratory, South African Institute for Medical Research (SAIMR), Cape Town. The sputum specimens had been sterilized using the hypochlorite method. The bacilli were identified first on low-power magnification (×100) and high-power magnification (×400). For image capture, 630× magnification (dry) was used. (Oil immersion is not generally used in screening programs.) The digital camera used for capturing the images contained a CCD chip with a resolution of 720×512 pixels (72 dots per inch).

Figure 1 shows numerous fluorescent tubercle bacilli in a background that is free of debris and contaminants. In many instances, however, there are only a few bacilli scattered over the entire slide, as shown in Figure 2. In this case a false negative diagnosis could be made unless a very large number of fields were viewed. In many cases the bacilli may be faint, occluded, obscured by cells or remnants, or inside macrophages—this imparts a hazy outline to the bacilli, which may cause oversight in recognition. In addition, the background can be complex due to debris and other features in the sputum, making recognition more difficult. During the course of infection the number of bacilli in the sputum may fluctuate close to the estimate of $10^7$/mL needed for a smear-positive result.

The images used for this study were captured from a set of 25 smear-positive slides. A few contained a very high density of bacilli, as shown in Figure 3. These images were discarded from the training and test sets in the investigation. In these images the bacilli are so numerous that they are frequently superimposed on or indistinguishable from each other. This would give an artificially low recognition rate per bacillus. However, this was not regarded as a drawback since these images generally contain a sufficient number of bacilli for reliable identification of tuberculous infection.

![Figure 1 Image with a number of distinct bacilli present](image-url)
In many instances there may be a few bacilli scattered among a large number of view fields; in this image there is only one bacillus present.

The principal steps of the image processing and recognition methods are outlined below. Separate entities in the images, such as tubercle bacilli, are referred to as objects (each object considered as a separate region). These steps are:

1. **Image capture.** Images were captured using the digital camera attached to the fluorescence microscope.

2. **Edge detection.** The captured images were filtered to detect the edges of objects in the image. A number of edge detection algorithms have been investigated.\(^5\,^{12}\) It has been found that the most appropriate edge detector for this problem is the Canny operator.\(^3\,^{16}\) The sensitivity of the Canny filter was adjusted according to amplification gain and resolution used during the original image capture.

3. **Region labeling and removal.** After edge detection was applied, regions were labeled and some removed based on their size. The region removal process enables the system to examine only regions that belong to a certain size range. This way, regions that could not possibly be bacilli, based on size alone, are excluded, and unnecessary processing is eliminated. In this step and in the boundary tracing described below, eight connectivity was used.\(^5\,^{16}\)

4. **Edge pixel linking.** When each region had been identified, edge pixel linking.\(^5\) was applied to objects with possible open edges.

5. **Boundary tracing.** Boundary tracing techniques were used to find the boundaries of each object in each image. Having found the regions and their boundaries (Figure 4), shape descriptors can be derived from the boundary curve of each region. In this project the inner boundary tracing algorithm was used.\(^5\,^{16}\)

6. **Shape description.** Shape descriptors are a set of parameters that gives a numerical representation of each region. A particular bacillus boundary must give rise to the same set of shape descriptors irrespective of its location and orientation in the image; hence, moment invariants or Fourier descriptors can be used. After experimentation, a total of 15

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**Figure 2** In many instances there may be a few bacilli scattered among a large number of view fields; in this image there is only one bacillus present.

**Figure 3** Some images with a very high density of bacilli were excluded from the training and test sets. Due to the dense packing of the bacilli, the recognition rate per bacillus could be artificially lowered. However, these images still contain a sufficiently large number of distinguishable bacilli for reliable detection of tuberculous infection.

**Figure 4** Image in Figure 1 after edge detection, region labeling/removal, edge pixel linking and boundary tracing have been applied.
Fourier descriptors was found to be sufficient to represent each object. Image recognition. The shape descriptors were fed into a classifier to identify any relevant regions as bacilli. A number of classification techniques can be used at this stage-for example, discriminant methods from statistics, neural networks and decision tree algorithms. However, we decided to use a fully connected multilayer feedforward neural network. Several different networks containing varying numbers of processing units were constructed using general-purpose neural network software and executed on a Silicon Graphics O2 workstation (Mountain View, California, U.S.A.) running IRIX 6.4 (Silicon Graphics). Two different learning rules were used for experimentation: the back propagation of error and the scaled conjugate gradient. The latter finds a more-direct route to a minimum error than a standard back propagation, but at higher computational cost.

Results

The number of features used to represent each example object (15 Fourier descriptors, referred to in step 6) determined the number of input units in the input layer. A single output unit was used for binary "bacillus" and "nonbacillus" decisions. The number of units in the remaining middle layer was chosen by experimentation. A training set consisting of 800 image objects, a validation set of 100 objects and a test set of 247 objects (bacilli and non-bacilli) were used to train and test the neural networks. Ninefold cross-validation was used with the training and validation data. The optimal overall performance on the validation set was achieved using the standard back propagation learning rule and four hidden units. The performance on the test set is shown in Table 1.

Table 1 shows the diagnostic sensitivity (94.1%) and specificity (97.4%), which are two important factors that may be used in conjunction with the overall accuracy of the method to determine the performance of a neural network on a medical application. Sensitivity is the ratio of true positive decisions to the number of positive objects. Specificity is the ratio of true negative decisions to the total number of negative objects. Overall accuracy expresses the ratio of correct decisions to the total number of objects.

It is also possible to shift the decision boundaries in the hidden units to achieve an alternative balance between sensitivity and specificity. For example, the specificity could be increased to 98.2% (on the test set used), but with a sensitivity of 88.2%. Table 1 shows the detection rate for individual bacilli present. However, on most positive smears, there will be more than one bacillus present. Positive diagnosis of TB is not usually made unless three or more bacilli are detected. Thus, the diagnostic accuracy of these smears will be very high.

Discussion

Based on this preliminary study, the automated detection of tubercle bacilli in sputum stained by auramine stain and screened using a fluorescence microscope appears to be feasible and practical. It could enhance current TB screening programs without dismantling existing infrastructures. This is an important socioeconomic and practical point.

The feasibility of introducing automated detection of tubercle bacilli in epidemic areas appears to hinge on one issue: its commercial viability. Initially, it is hoped that automation would be introduced in the Western Cape Province, South Africa, which has one of the highest recorded incidences in the world, about 700/100,000. Statistics from three SAIMR laboratories in the Western Cape (Worcester, Paarl, Cape Town) reveal that the number of slides processed in the three-month period January- March 1998 was 38,152 (approximately 500 slides per day). A conservative estimate for a dedicated system is $15,000 based on component values, though the continued rapidly decreasing cost of components, such as microprocessors and CCD chips, means that this could be an overestimate. The current cost per slide is $3, and this figure, together with the above throughput of slides, suggests that the approach is cost-effective. The method would also be time-effective because the image processing and recognition can be easily performed in parallel on separate processors, thereby reducing the processing time per field to an acceptable speed, such as three seconds per field.

There are other factors for and against automa-
tion. It would facilitate the screening of a much larger number of people at little extra cost, without additional staff. In fact, the facility to increase screening of a population for a highly contagious disease may be a better argument for automation than an attempt to cheapen current practice. With automation a larger number of fields can be examined, resulting in improved accuracy. It has been reported that current sputum screening tests result in a 33-50% false negative rate. 23

A disadvantage of automation is that it may lead to centralization of the diagnostic process. Due to the inconvenience of travel, patients are more inclined to bring sputum samples to their local clinic rather than a district hospital. However, this may not be a restriction. For example, in the Western Cape, medical technologists take mobile clinics into townships and rural areas where there are no clinics. The technologist performs on-site screening and diagnosis of urgent cases, while other specimens are collected and transported to one of the main laboratories for manual screening.

In summary, this preliminary investigation has shown that image processing and recognition techniques can be successfully used for the identification of tubercle bacilli in sputum smears. It is widely accepted that the sputum test is more specific (up to 98-99%) than other diagnostic tests currently available. The introduction of automation would radically improve and augment this cornerstone of TB diagnosis.

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