Automated Identification of Tubercle Bacilli in Sputum

A Preliminary Investigation

Konstantinos Veropoulos, B.Sc., M.Sc., **Geneviève Learmonth,** M.B., B.Ch., B.A.O., EF.Path. (Anat.), S.A., **Colin Campbell,** B.Sc., Ph.D., A.R.C.s., **Bryan Knight,** B.Sc.(Anat.), M.B., Ch.B., M.Med.Path.(Anat.), U.C.T., F.I.A.C., and **John Simpson,** M.B.Ch.B., M.Med.Path.(Micro), U.C.T.

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. (Analyt Quant Cytol Histol 1999;21(4):277-281)

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.[6,22] In developing countries, tuberculosis is responsible for more adult deaths than AIDS and malaria com-

From the Advanced Computing Research Centre, University of Bristol, Bristol, United Kingdom, and the Department of Anatomical Pathology, Medical School, Observatory 7925, University of Cape Town, and South African Institute for Medical Research, Tuberculosis Control Laboratory, Cape Town, South Africa.

Mr. Veropoulos is a Research Student, Advanced Computing Research Center, University of Bristol.

Dr. Learmonth is Honorary Visiting Lecturer, Department of Anatomical Pathology, Medical School, University of Cape Town.

Dr. Campbell is Lecturer, Department of Engineering Mathematics, University of Bristol.

Dr. Knight is Senior Lecturer, Department of Anatomical Pathology, Medical School, University of Cape Town.

Dr. Simpson is Head, Department of Medical Microbiology, South African Institute for Medical Research, and Co-ordinator, Provincial Tuberculosis Laboratory, South African Institute for Medical Research, Western Cape Town Province, South Africa.

Address reprint requests to: Konstantinos Veropoulos, B.Sc., M.Sc., Advanced Computing Research Centre, University of Bristol, Bristol BS8 1TR, United Kingdom (veropoul@cs.bris.ac.uk).

Presented at the Fifth International Conference on the Computerized Cytology and Histology Laboratory, Chicago, March 22-25, 1998, and at the 13th International Congress of Cytology, Tokyo, Japan, May 10-14,1998.

Financial Disclosure: The authors have no connection to any companies or products mentioned in this article.

Received for publication May 8, 1998.

Accepted for publication October 30,1998.

088.4-6812/99/2100-0000/$15.0010 @ The International Academy of Cytology
Analytical and Quantitative Cytology and Histology

bined.[21] 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.[10] 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.[7] There is general consensus that fluorescence microscopy is superior for the identification of tubercle bacilli.[1,18] 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. A*utomated 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.[13] 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 10^4/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.

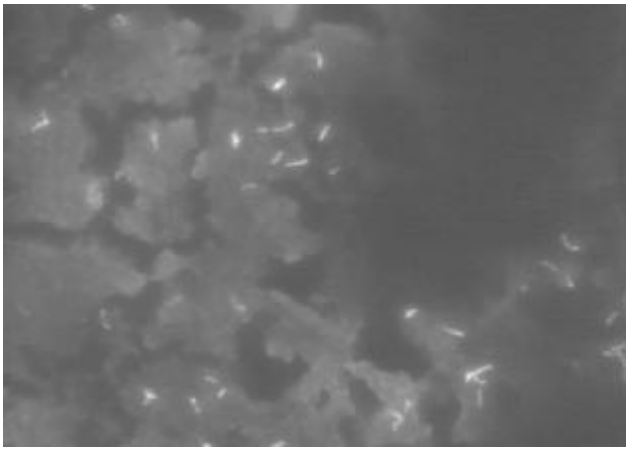

Figure l Image with a number of distinct bacilli present

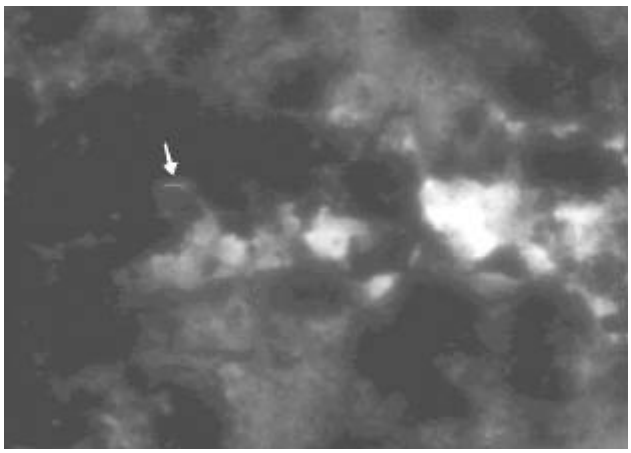

Figure 2 In many instances there may be a few bacilli scattered among a large number of view fields; in this image there is only one bacillus present.

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.[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

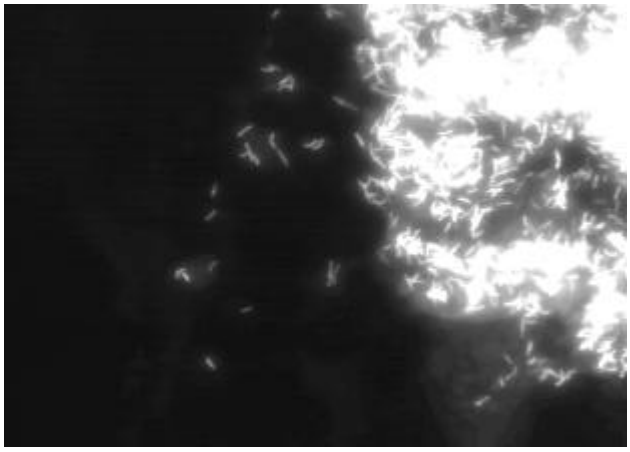

Figure 3 Some images with a very high density of bacilli were excluded from the training and test sets. Due to the dense packing of the bacilli, the recognition rate per bacillus could be artificially lowered. However, these images still contain a sufficiently large number of distinguishable bacilli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

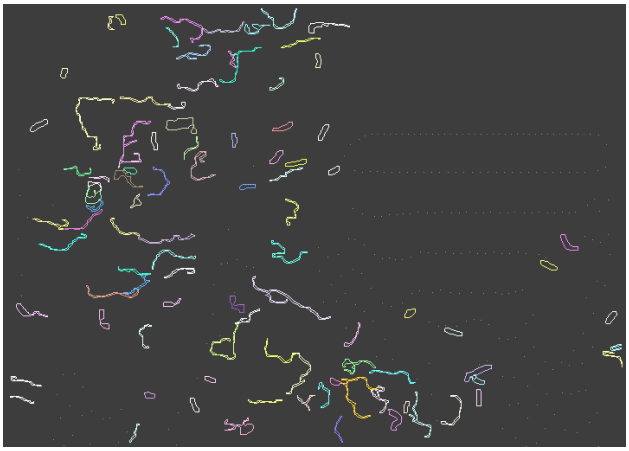

Figure 4 Image in Figure 1 after edge detection, region labeling/removal, edge pixel linking and boundary tracing have been applied.

Fourier descriptors was found to be sufficient to represent each object.[5,11,14,16]

(7) 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.[2,8,9,15] 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 softwarel7 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 nonbacilli) 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.[19] 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.

Table I *Expected and Detected Classes*

	Detected class	
Expected class	**1 (Bacillus)**	**0 (Nonbacillus)**
1 (Bacillus)	94.1%	5.9%
0 (Nonbacillus)	2.6%	97.4%

The expected class is the category assigned to regions by manual identification. The detected class is the category found by the computer.

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.[4] 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.[20]

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.[20] 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.

Acknowledgements

The authors thank Dr. Mark Jepson and the staff of the Cell Imaging Facility, Biomedical Sciences School, Bristol, and Chief Technologist Marlein Bosman, Valentine Horne and the staff at SAIMR (Cape Town) for their technical assistance.

References

1. Bailey TM, Akhtar M, Ali MA: Fine needle aspiration biopsy in the diagnosis of Tuberculosis. Acta Cytol 1986;29:732-736
2. Bishop CM: Neural Networks for Pattern Recognition. Oxford, Clarendon Press, 1995
3. Canny JF: A computational approach to edge detection. IEEE Trans Pattern Anal Machine Intell 1986;8:679-698
4. Collins CH, Grange JM, Yates MD: Organization and Practice in Tuberculosis Bacteriology. London, Butterworths, 1985, p 42
5. Conzalez RC, Woods RE: Digital Image Processing. Reading, Massachusetts, Addison-Wesley, 1992
6. Kumar P, Clark M (editors): Clinical Medicine: A Textbook for Medical Students and Doctors. London, Bailliere Tindall, 1994, p 683
7. Kupper TH, Steffen K, Wekle G, Richartz G, Pfitzer P: Morphological study of bacteria of the respiratory system using fluorescence microscopy of Papanicolaou stained smears with special regard to identification of Mycobacteria in sputum. Cytopathology 1995;6:388-402
8. Lachenbruch PA: Discriminant Analysis. New York, Harner Press, 1975
9. Mitchic D, Spiegelhalter DJ, Taylor CC: Machine Learning: Neural and Statistical Classification. New York, Ellis Horwood, 1994
10. Mushtaque A, Chowdhury AMR, Chowdhury 5, Islam MN, Islam A, Vaughan P: Control of Tuberculosis by community health workers in Bangladesh. Lancet 1997;350:169-172
11. Persoon E, Fu K: Shape discrimination using Fourier descriptors. IEEE Trans Systems Man Cybern 1977;7:170-178
12. Phillips D: Image Processing in C: Analyzing and Enhancing Digital Images. Lawrence, Kansas, R&D Publications, 1994, p 213
13. Rattan A, Kishora K, Singh 5, jaber M, Immaculata X, Ramesh K: Evaluation of a safe sputum processing method for detecting tuberculosis. J Clin Pathol 1994;47:411-413
14. Reeves AP, Prokop RJ, Andrews SE, Kuhl FP: Three-dimensional shape analysis using moments and Fourier descriptors. Proceedings of the 7th International Conference on Pattern Recognition, Montreal, Quebec, Canada, July 30-August 2, 1984, pp 447-450
15. Rojas R: Neural Networks: A Systematic Introduction. Berlin, Springer, 1991
16. Sonka M, Hlavac V, Boyle R. Image Processing, Analysis and Machine Vision. London, Chapman and Hall, 1993
17. Stuttgart Neural Network Simulator v4.1. Institute for Parallel and Distributed High Performance Systems, University of Stuttgart, Stuttgart, Germany, 1995
18. Uribe-Ribero MD, John G, Prichard MD, Kaplwitz HW: Bone marrow in HIV infection: A comparison of fluorescent staining and cultures in the detection of mycobacteria. Am J Clin Pathol 1989,91:313-315
19. Veropoulos K: The Probabilistic Neural Network. Bristol, University of the West of England, Intelligent Computer Systems Center, 1995, p 33
20. Wilkinson D, De Cock KM: Opinion: Tuberculosis control in South Africa: Time for a new paradigm? S Afr Med j 1996;86: 33-35
21. WHO Fact Sheet No 104: Tuberculosis. Geneva, WHO, 1998
22. WHO: The World Health Report 1998-Life in the 21st Century: A Vision for All. Geneva, WHO, 1998
23. WHO Tuberculosis Diagnostics Workshop: Product Development Guidelines. Edited by J Foulds. Geneva, WHO, 1997