Microscope Image Processing for TB Diagnosis Using Shape Features and Ellipse Fitting

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***Abstract*—Tuberculosis (TB) is an infectious disease caused by the bacteria Mycobacterium tuberculosis or simply M. tuberculosis. It is primarily an infection of lungs, but it can also affect other parts of the body such as brain, intestine, kidney and spine. TB remains one of the leading cause of death in developing countries, although most are preventable if diagnosed early and treated. Among the available tools, Sputum smear microscopy is the most widely used one for TB detection. It is done manually and is often time consuming; a laboratory technician is expected to spend at least 25 minutes per slide, limiting the number of slides that can be screened. Also any incorrect diagnosis will leads to serious health issues. So a solution is Automatic screening methods. Many attempts have been made to develop automatic approaches to identify TB bacteria from microscopic sputum smear images. In this paper, we present an automatic TB diagnosis technique using morphological features and ellipse fitting. Microscopic images of sputum smear are collected from infected subjects. These images are transformed into HSV color space for a better analysis, which is thresholded using hue range of red pink color. The resultant images will contain TB bacilli along with non-TB objects. In order to identify the TB regions from the non-TB regions, shape features of every identified region is evaluated. Finally a new algorithm that make use of concave contour points as well as ellipse fitting is performed to separate out the overlapping bacilli region and add them to the total count of bacilli.**

***Index Terms*— Tuberculosis(TB), Ziehl-Neelsen (ZN), HSV, Shape Feature analysis, Polygon approximation, Concave point extraction, Contour segmentation, Ellipse fitting.**

I. INTRODUCTION

Tuberculosis (TB) is one of the leading infectious disease causing death in developing countries. It infects primarily the lungs (pulmonary tuberculosis). As per WHO's global tuberculosis report, 2016 [2]; TB is at number two after HIV/AIDS as the top murdering disease worldwide. More than 95% of TB death rate is in middle and low income countries and more than sixty percent TB patients are in Asia, which is increasing rapidly. The key difficulty lies in diagnosing TB. The two principle techniques existing for screening its bacteria (Mycobacterium) are fluorescent microscopy and bright field microscopy or conventional microscopy. In fluorescent microscopy the sputum smear specimens are stained utilizing a stain Auramine.o [19], and in bright field microscopy they are stained with ZN (Ziehl-Neelsen) stain [4]. Bright field microscopes are less expensive and have an easy equipment maintenance as compared to the other one and that is why they are used widely. Fig. 1 shows an example of ZN-stained sputum smear image. The main aim of automation in the context of TB screening is to speed up the screening process and to reduce its reliance on technicians and pathologists.

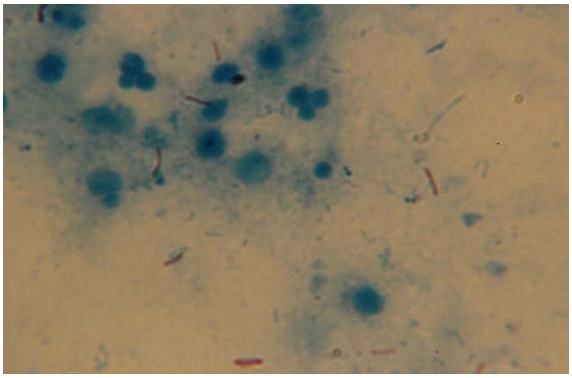


Fig. 1. ZN-stained sputum slide images of TB bacilli

The physical identification and counting of the number of bacilli (M. tuberculosis) through microscopic examination is a strenuous and time consuming task that requires a significant amount of exertion and mental concentration. The expertise of the technicians also have an impact on determining the accuracy of the manual detection. This laborious and time taking process takes about 25 min to 3 h for the complete screening, depending on the count of bacilli present in the sample, expertise of the pathologist, quality of equipment used etc. And the number of bacilli present in a sample in turn will depend upon the stage of infection. Since a technician has to spend more than 25 minutes for screening every single slide that having at least 100 fields for in depth visual observation. There are many advantages to automated methods, like relief from eye strain, greater accuracy. Among them the most promising advantage is patient's record can be stored as soft copy for future reference, thereby allowing better and quick decision making. These stored patients records can be used for sending across the internet for seeking expert's opinion also.

The literature is not much rich with the automated techniques for TB detection, a few are proposed which has increased the speed and sensitivity of diagnosis. M.K Osman et al. [8] attempted to find a solution for TB automation, however it has not finished up the detection of bacilli objects. R.A Raof et al. [7] presented a method which explains a grey color thresholding algorithm for finding best threshold value for the segmentation of pixels that comprises TB bacilli from stained Ziehl-Neelsen slide images. CostaFilho et al. [9] work was also based on pixel classification that make use of RGB, HSI, YCbCr and Lab color spaces and subtraction of components of these color spaces are used as input for the feed forward neural network pixel classifier. A new color characteristic, the color ratio is proposed which is used for noise filtering. The best sensitivity achieved in bacilli detection was 91.5%. The extension of this work is proposed in [10] where the feed forward neural network pixel classifier in the previous was replaced with three filters to separate bacilli from artifact : a size filter, a geometric filter and a Rule-based filter that uses the components of the RGB color space, thereby achieved an improved sensitivity.

Vishnu Makkapati et al. [11] was the first who make use of the shape features of M. Tuberculosis bacteria for the identification of TB. They proposed a Hue color component based approach to segment the bacilli by adaptive choice of the hue range. The bacilli are declared to be valid or invalid depending on the presence of beaded structure inside them, thread length and thread width parameters of the bacilli. P. Sadaphal et al. [12] proposed a technique in 2008 and it used 1) Bayesian segmentation to predict the probability of a pixel representing a 'TB object' using prior knowledge of ZN stain colors; and 2) shape/size analysis. Manuel G. Forero et al. technique is based on the heuristic acknowledge resulted from the bacilli shapes [21]. Rethabile Khutlang et al. [15] segment the candidate bacillus objects using a combination of two-class pixel classifiers. Zahoor Jan and Muhammad Rafiq [5] work was on HSV color space rather than RGB, since HSV is well suited for representing colors and color based segmentations. They utilized the color feature of TB bacilli (usually red-pink color) for the detection and OTSU thresholding as well. Shape analysis of identified regions are carry out through the extraction of features eccentricity and aspect ratio. The accuracy is 90 %.

Ebenezer Priya et al. [6] proposed an extension to all the existing TB detection proposals. They introduced a new method for the separation of overlapping TB objects. Shape features like eccentricity, compactness, circularity, tortuosity etc. were examined to distinguish the single bacteria region, overlapping bacilli region and non-bacilli region. Detected overlapping bacilli region were given to the new proposal based on concavities in the region. Since concavities are the indication of presence of overlapping, the deepest concavity point of every concavity is used to identify a best splitting line. The bonus of this Overlapping separation makes the total count of TB bacilli much exact. The suggested concordance is 93.3 %.

II. Proposed Method

The input to the proposed method is microscopic images of ZN-stained sputum smear. It consists mainly three phases. The first phase is the object localization phase, where the main objective is to locate the TB causing bacteria in the image if it is present. The object localization phase further includes three more steps. They are (1) HSV conversion, (2) Thresholding and finally (3) Morphological operations. In the next step shape features of the identified regions are analyzed to select the ROI of the overlapping TB region. In the final step separation of overlapping bacilli, if present, is done using the proposed method of overlapping detection with the aid of concave points and ellipse fitting. The flow chart of the proposed technique is shown in Figure 2.

A. Object Localization

The sputum samples of a TB patient contains the Mycobacterium Tuberculosis, which is the TB causing bacteria. After the ZN-staining procedure of sputum samples, the TB bacilli appears in red-pink color and the non-bacilli region appears in blue color**.** So the microscope images of ZN-stained sputum smears may contain the pink colored bacilli region, blue colored non-bacilli region and the background. In this phase, our objective is to detect the regions having properties of pink

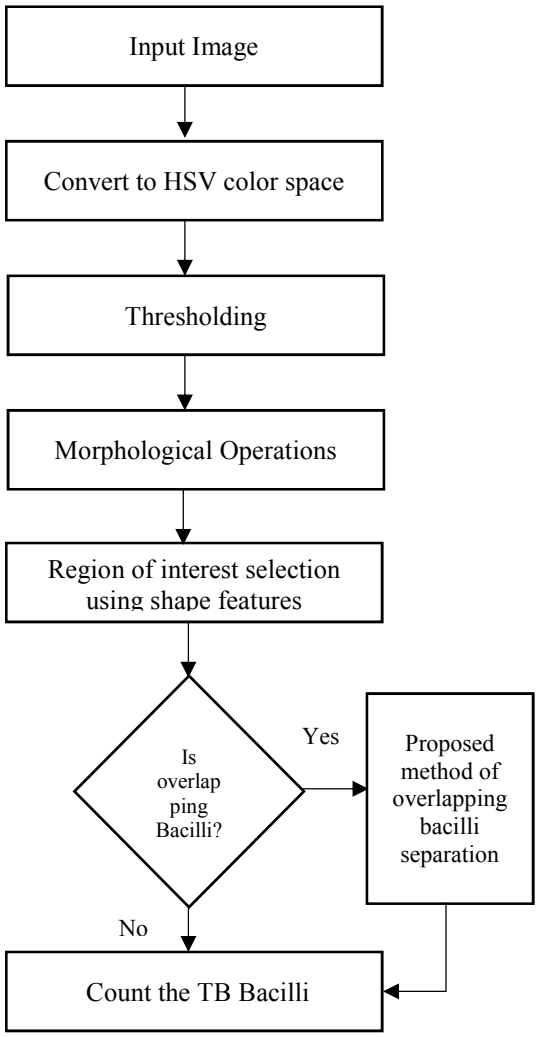


Fig. 2. Flow chart of proposed method

color. For which the image is first converted into HSV color space.

1) HSV conversion

HSV color model is used to replace the straightforward RGB color model. RGB is a combination of three colors, red, green and blue. While HSV or hue saturation model represents three values. The HSV color model is well suited for describing colors in terms that are practical for human interpretation. Any color in HSV color space can be transformed to a shade of gray by sufficiently lowering its Saturation. In HSV, H indicates Hue which is the color attribute that describes a pure color. S is Saturation that is the purity of the color or we can say it defines the purity of hue with respect to a white reference. V indicates Value that represents the different shades of gray.

Here, the bacilli regions in input image have pink color. So we need to localize the regions with different shades of pink color. In RGB image the color information is much noisier. Unlike RGB, HSV separates luma, or the image intensity, from chroma or the color information; i.e. HSV separate color from intensity. In order to detect the pixels with properties of pink color, it is essential to get the chromatic information separated from the pixel intensity information. So the input image is first transformed to the HSV color space. Conversion from RGB to HSV is explained by Gonzales and woods [1], as following.

$$H = \begin{cases} \cos^{-1}\dfrac{\left[\frac{1}{2}[(R-G)+(R-B)]\right]}{\sqrt{\left((R-G)^2+(R-B)(G-B)\right)}}, if\ B \le G \\ 2\pi - cos^{-1}\dfrac{\left[\frac{1}{2}[(R-G)+(R-B)]\right]}{\sqrt{\left((R-G)^2+(R-B)(G-B)\right)}}, if\ B \rangle G \end{cases} \quad (1)$$

$$S = 1 - \frac{3}{(R+G+B)}\left[min[R,G,B]\right] \quad (2)$$

$$\mathrm{V} = \frac{1}{3}(R+G+B) \quad (3)$$

As bacilli appear red pink in color, the main concern in the HSV model is the hue layer. The hue layer is extracted from the HSV format of the image in order to identify the bacilli and the red pink color lies in between angle 0^o and 30^o.

2) Thresholding

It is the process of converting an image into a monochrome or binary image using some threshold value for some constraints. Since we are having Hue layer of HSV image, the hue range of red pink color can be utilized directly in order to isolate the TB bacilli region from the unwanted background. Thresholding leaves the image with white coloured bacilli region and pure black background.

3) Morphological Operations

For bacilli region overlapped with non-bacilli region, there is a chance for forming holes inside. Also there is chance for the presence of very small impurities outside the bacilli region. Inside bacilli region, where impurities are present the object may contain holes which causes the object to be considered as separate regions. In order to get rid of such faulty recognitions morphological operations can be applied. The aim of this step is to remove the impurities and unwanted pixels so as to enhance the quality of the image. Morphological operations like opening, closing, dilation, erosion etc. are applied to fill out the holes and smoothen the edges of the objects.

Subsequently an edge detection is made on the resultant image, because it will minimize the quantity of data in the image while maintaining its structural features. Now the output image and that of canny edge detector are added together to form a single image. Which means the pixel values of each input image is added to the corresponding pixel values of edge detector output. The purpose of this addition is to produce a resultant image with more enhanced boundaries.

At the end of object localization phase a binary image with all bacilli regions isolated will remain.

B. ROI selection using shape features

Now the bacilli regions are isolated. But in cases where overlapping bacilli are present or one or more bacillus is very close to each other, the whole region containing these group of bacilli will be segmented as a single region. So shape feature analysis is done in order to detect the overlapping bacilli region. Shape features are features of a geometrical shape such as area, eccentricity, compactness, circularity etc. They decide the identification of overlapping bacilli from other regions [15]. The rod-shaped bacilli exhibit a high eccentricity and low compactness value providing a better shape characterization of bacilli. The objects with low relative convex area and high circularity are identified as outliers or debris. The regions that have shape features similar to the TB bacilli is counted as the bacilli regions. The regions with features of overlapping case are given to the next phase for the bacilli separation and all remaining regions are neglected. The statistical values of these geometric features are summarized in Table I.

TABLE I

THE RANGE OF VALUES OF SHAPE FEATURES USED FOR BACILLI, NON-BACILLI SEPARATION

Geometric feature	Bacilli	Overlapping Bacilli
Area	≤400	>400
Eccentricity	0.86 – 0.96	0.70 – 0.86
Aspect ratio	2 – 2.5	>0.25
Compactness	0.55-0.75	<0.60
Circularity	0.65 – 0.69	0.30 – 0.40
Roughness	0.75 – 1.25	>1.25

After analysing the shape of bacilli, the ROI for the overlapping TB objects are detected. The detected single bacilli are counted. Separation of overlapping regions are processed using an algorithm, which is detailed in the next section.The resultant image after applying the range of values of shape

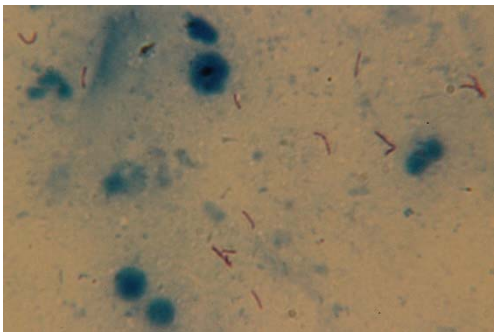
features is shown in Figure 3. Fig. 3(a) is the input image and Fig. 3(b) is the resultant image with overlapping bacilli regions are bounded with a green rectangle.

C. Method of Overlapping bacilli separation

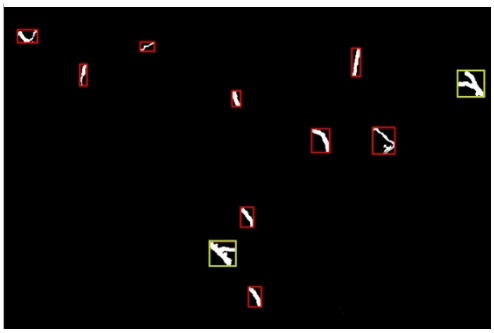
Separating touching objects in an image is considered as the hardest image processing task. When the sputum smear is thick or uneven the number of overlapping bacilli are more which delay the diagnosis. Here the overlapping TB objects are identified by the geometric features and their respective Region of Interest (ROI) chosen. Then separation of overlapping bacilli is carried out for getting the exact count of bacilli present. The new method proposed in this paper for touching bacilli separation is based on concave points and ellipse fitting. The method includes four parts they are: 1) Polygon Approximation, 2) Concave point extraction, 3) Contour segmentation, and finally 4) Ellipse processing.

1) Polygon Approximation

Polygon approximation (PA) of the contour is necessary to smoothen the irregular rising and falling of overlapping contour. Usually the region contour is represented by a sequence of points along its boundary. The original contour of a bacilli region may be rough, so that a polygon approximation will reduce the irregularities of the boundary as well as it will be benefitted to detect the exact concave points in the succeeding phase. Moreover PA sufficiently reduces the number of points in the contour boundary and thereby reducing the calculation time in the immediate phases. Because of the less complexity and good performance [1, 16, 17] polygon approximation is an appropriate algorithm for contour smoothing. Fig. 4(b) shows the polygon approximated image of Fig. 4(a).



(a)



(b)

Fig. 3. (a) Input image (b) bacilli and overlapping bacilli separated image.

2) Concave point extraction

Concave points along a region can be identified using the concave property of a region [18, 19]. If $P_c(X_c,Y_c)$, is the current point $P_p(X_p,Y_p)$ is the previous point and $P_n(X_n,Y_n)$ is the next point on the contour after polygon approximation, then the concavity of the contour at P_c can be calculated as:

$$a(P_p, P_c) = tan^{-1}\left(\frac{Y_p - Y_c}{X_p - X_c}\right) \tag{4}$$

$$a(P_n, P_c) = tan^{-1}\left(\frac{Y_n - Y_c}{X_n - X_c}\right) \tag{5}$$

$$Concavity(P_c) = \begin{cases} |a(P_p, P_c) - a(P_n, P_c)|, if\ |a(P_p, P_c) - a(P_n, P_c)| < \pi \\ \pi - |a(P_p, P_c) - a(P_n, P_c)|, \quad else \end{cases} \tag{6}$$

$a(P_p, P_c)$ Is the angle of line joining P_p and P_c and $a(P_n, P_c)$ is the angle of line joining P_n and P_c. *Concavity* (P_c) is the angle between line $\overline{P_p P_c}$ and line $\overline{P_n P_c}$. If P_c is a real concave point, *Concavity* (P_c) should be within a range. The limits of that range must a predefined value which can be obtained through some training programs. But, *Concavity* (P_c) alone is not enough to find concave points. Moreover it is also necessary to examine whether the line $\overline{P_p P_n}$ is belonging to the inside of the bacilli region. If so the point P_c cannot be considered as a concave point. Fig. 5(a) shows the extracted concave points of polygon approximated image in Fig. 4(b).

3) Contour segmentation

Identified concave points are used to divide the contour segment into number of segments. If '*C*' is a contour then, *C* can be represented as:

$$C = L_1 + L_2 + \ldots\ldots + L_m \tag{7}$$

Where '*m*' is the total number of concave points. Each L_i is a segment on *C* having any two adjacent concave points as end point. Now each of these segments can be used for ellipse fitting. Fig. 5(b) shows the image after contour segmentation.

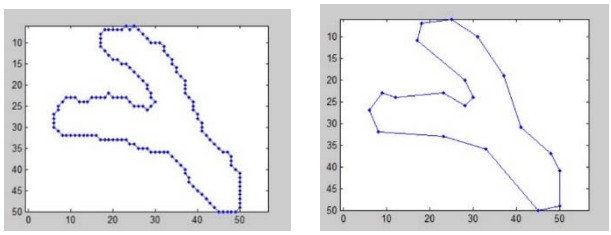
4) Ellipse processing

Ellipse processing assigns all contour segments with similar characteristics to a single bacillus, so that the touching bacilli can be separated. The following are the steps to be performed in ellipse processing:

a) Ellipse Fitting

Initially an ellipse is fitted by using all the points of one segment. Direct least square fitting method [20] is used for ellipse fitting purpose, where an ellipse is fitted by using all the

points of one segment. Consider if L_i is the i^{th} segment and E_i is its corresponding fitted ellipse then, the algebraic distance [20] of all the points on L_i to E_i and the standard deviation σ of these algebraic distances are calculated. Finally, the points, whose algebraic distances are larger than 4σ are deleted. After this procedure, the noisy points which are far away from the real contour of the touching bacilli are removed. Then, the remaining points on the segment are used to fit a new ellipse, which is more precise for the processing in the next step. Figure 5(c) shows the ellipse fitting output of Figure 5(a).



(a) (b)

Fig. 4. (a) Original contour representation of an overlapping bacilli region. (b) Polygon approximated image.

b) Ellipse Selection

After ellipse fitting, each segment of the contour has a fitted ellipse. If the fitted ellipse does not satisfy the following two conditions, the corresponding segment will be removed from the candidate ellipses for ellipse combination step and will be processed at the ellipse refinement step.

CASE 1

- Calculates the Mean algebraic distance $dis(E_i; L_i)$
- **If** $dis(E_i;Li) < disTh$
  Selects the corresponding ellipse.
- *disTh* should be selected following prior knowledge or some training programs.

CASE 2

The shape of TB bacteria is too slender. So, the ratio of the minor axis to major axis of the fitted ellipse should be very small. Let *MaxA* and *MinA* represent the lengths of major axis and minor axis of the fitted ellipse respectively. The ratio of *MinA* and *MaxA* is defined as follows:

$$eRatio = \frac{MinA}{MaxA} \quad (8)$$

- **if** $eRatio < eTh$
  Selects the corresponding ellipse.
- *eTh* should be selected following prior knowledge or some training programs.

c) Ellipse Combination

This step is to combine the candidate ellipses whose corresponding contour segments belong to same bacillus. For which different cases of the touching bacilli should be analysed. In order to find the ellipses belong to same bacillus, every pair of ellipses fitted to the segments of the current contour are combined to generate a newly combined ellipse and then verifies the newly fitted ellipse through the procedure of ellipse selection. If it is an appropriate ellipse for the selected pair of ellipses then newly fitted ellipse and the two previously fitted ellipses are verified using different rules under various cases. The cases are described below:

CASE 1

- Explicit touching bacilli should be separated [18]. In order to identify these explicitly touching bacilli the following conditions can be verified.
  1. The distance between the center of the newly fitted ellipse (C_{new}) and the centers of the two previously fitted ellipses should be larger than $d\text{Min}Th$.
     $||C_1 - C_{new}|| > d\text{Min}Th$
     $||C_2 - C_{new}|| > d\text{Min}Th$
  2. The distance between the centers (C_1 and C_2) of the two previously fitted ellipses should be very large.
     $||C_1 - C_2|| > (2.5 \sim 4.0)d\text{Min}Th$.
- *dMinTh* is usually close to the length of the minor axis of the smallest bacillus.

CASE 2

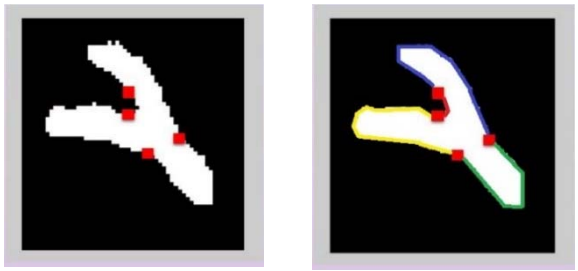
- In the case of complicated touching bacilli, the touching bacilli may have more than two bacillus touching each other.
- Consider two segments L_i and $L_j$and their ellipses E_i and E_j. Consider also, a segment $L_{ij} = L_i \cup L_j$ and its ellipse E_{ij}.
- **If (the segments L_i and L_j belong to the same bacillus) then:**
  $dis(E_{ij}; L_{ij}) \approx dis(E_i; L_i) \approx dis(E_j; L_j)$
- If this occurs, the segments should be combined.

If the selected pair of ellipses satisfy CASE 1 and do not satisfy CASE 2, no need to combine the ellipses otherwise the selected pair of ellipses should be combined. After ellipse combination, the touching bacilli are separated and each separated bacillus is represented by an ellipse.

d) Ellipse Refinement

The ellipses which are not selected as the candidate ellipses are processes here. Each unprocessed segment is combined into one existing ellipse to generate a new ellipse. The fitting errors of the new ellipses are calculated and compared. The unprocessed segments are combined with ellipse to which it has the least fitting error.

The Fig. 5(d) shows the output image after ellipse combination and refinement. Fig. 6.Shows some sample outputs.



(a) (b)

(c) (d)

Fig. 5. (a) Extracted concave points. (b) Image after contour segmentation. (c)Ellipse fitted for every segments in the contour. (d) Output after ellipse combination and refinement step.

III. RESULT ANALYSIS

The aim of our study was to detect TB-bacilli in images of ZN-stained sputum smears that helps for the automatic diagnosis of TB. Performance of the proposed framework is tested using a dataset, which consists of 176 images. The smear slides were prepared at the Bacteriology Department of NIRT, Chennai. An example image is shown in Figure 1.

Quantitative analysis

The performance of the proposed method is evaluated using criteria such as sensitivity, specificity and accuracy. For this, the number of true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN) are estimated and compared with the ground truth, which is shown in Table II. The average sensitivity, specificity and accuracy of the method for the set of 176 images, is found to be 88.34%, 76% and 88% respectively.

From 176 images analysed, a total of 412 true bacilli (B) have been identified by the human annotator. The number of detected objects resembling the human annotated bacilli (A) is found to be 393. Thus the percentage of concordance is obtained as:

$$\frac{A}{B} \times 100 = 95.4\%$$

The proposed method is a combination of TB detection and counting the TB bacilli after overlapping bacilli separation. Table III shows the comparison of proposed method with the existing methods using TB detection only. Table IV shows the comparison of proposed method with existing technique using overlapping bacilli separation also.

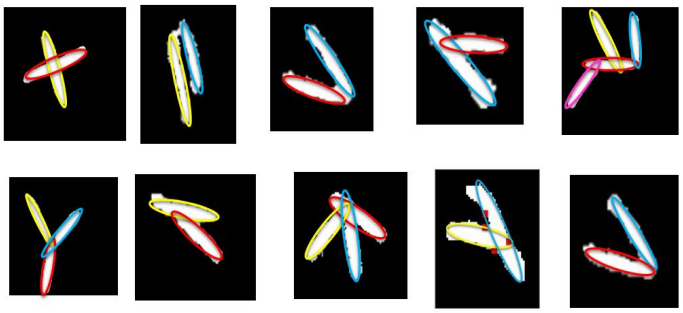


Fig. 6. Some of the results.

TABLE II

ACCURACY, SENSITIVITY AND SPECIFICITY CALCULATION

Image	TP+ TN	FP+ FN	Accuracy (%)	Sensitivity (%)	Specificity (%)
1	12	2	85	91	75.3
2	14	2	88	84	77.6
3	11	2	87	92	76.6
4	8	1	88	91	75.8

TABLE III

COMPARISON WITH TECHNIQUES USING DETECTION ONLY

	Accuracy (%)	Sensitivity (%)	Specificity (%)
Proposed Method	91.5	90.18	76.3
CostaFilho et al. [9]	91.49	91.53	Not Reported
Zahoor Jan et al. [5]	90	Not Reported	Not Reported
Osman et al. [8]	86.32	Not Reported	Not Reported
Osman et. Al [3]	75.46	Not Reported	Not Reported
Sotaquira et al. [13] (2009)	85.7	90.9	~100

TABLE IV

COMPARISON WITH TECHNIQUES USING OVERLAPPING ALSO

	Accuracy	Sensitivity	Specificity	Concordance
Proposed method	88%	88.34%	76%	95.4%
Priya et al.[6]	Not Reported	Not Reported	Not Reported	93.3%

The results show that the method is simple and computationally efficient and is a promising approach towards the automation of TB diagnosis.

IV. Conclusion

Microscope image processing is the area which process, analyse and present images obtained from a microscope. It is mainly used for the analysis of cell structures and microorganisms. Tuberculosis (TB) remains one of the death-dealing disease in the world. Sputum smear microscopy is the traditional method used for the quick identification of the TB. Automatic screening systems for the diagnosis are in need to avoid the reliance on laboratory technician. In TB diagnosis, digital image processing techniques are applied for the detection and classification of the TB bacilli. In this work a framework is proposed to count the number of bacilli from the images of ZN-stained sputum smears which are obtained using a DSLR camera attached to a conventional light microscope. The specificity, sensitivity and accuracy of the method is estimated by comparing the results with manually segmented images. The results show that the method is simple and computationally efficient and is a promising approach towards the automation of TB diagnosis.

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