International Journal of Electronics Engineering (ISSN: 0973-7383)
Volume 10 • Issue 2 pp. 526-533 June 2018-Dec 2018 www.csjournals.com

Detection of Mycobacterium Tuberculosis in Ziehl Neelsen Stains: An approach of Digital Image Processing

[1]Sandeep Musale, [2]Vikram Ghiye
[1]Dept. of Electronics and Telecommunication, MKSSS's Cummins College of Engg for Women, Pune, India.
[2]Dept. of Electronics and Telecommunication, Anantrao Pawar College of Engg and Research, Pune, India.

Abstract: Tuberculosis (TB) is one of the major diseases at present. One of the methods of TB detection is based on sputum examination microscopically by using Ziehl-Neelsen stain (ZN-stain) method, which is used worldwide. 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. 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 project, an algorithm based on image processing is developed for identification of TB bacteria in sputum.
Keywords: Acid-Fast Bacilli, Segmentation, Threshold Tuberculosis, ZN staining, Sputum.

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.

- Two types of TB are,
- Pulmonary Tuberculosis (PTB).
- Extra Pulmonary Tuberculosis (EPTB).

TB disease most commonly affects the lungs and is referred to as pulmonary TB disease. Extra pulmonary TB (EPTB) refers to TB disease outside the lungs. Clinical specimens are stained using Ziehl-Neelsen (ZN) method to visualize TB Bacilli in the sample. The manual screening of TB is time-consuming and tedious, especially for detecting negative slides. Furthermore, an accurate diagnosing requires an assessment conducted by well-trained personnel. However, in some countries with high TB incidence, the large number of slides must be interpreted and diagnosed by a relatively small number of medical staff. These problems pose a huge obstacle in rapid and accurate result hence efforts to improve the quality of existing methods are necessary. Therefore there is a need of automation of examination and detection of TB Bacteria using digital image of ZN-stain sample. The automated technique can save the time and cost involved, with reduced human error. A complete medical evaluation for TB disease includes the following components.

When conducting a case history, the practitioner ought to raise if any symptoms of TB malady area unit present; if thus, for a way long, and if there has been notable exposure to an individual with infectious TB malady. Equally necessary is getting info on whether or not or not the person has been diagnosed within the past with latent tuberculosis infectious malady infection (LTBI) or TB disease. Clinicians ought to verify if the patient has underlying medical conditions, particularly human immunological disorder virus (HIV) infection or polygenic disease, that increase the chance for progression to TB malady in those latently infected with M. T.B. A physical examination is a necessary a part of the analysis of any patient. It can't be accustomed ensure or rule out TB malady, however it will offer valuable info concerning the patient's overall condition, inform the tactic of identification, and reveal alternative factors that will have an effect on TB malady treatment, if diagnosed.Selection of the most suitable tests for detection of M. tuberculosis infection should be based on the reasons and the context for testing, test availability, and overall cost effectiveness of testing. The tests available are Tuberculin skin test (TST), Interferon-gamma release assays (IGRAs) Chest abnormalities can suggest pulmonary TB disease. Abnormalities seen on chest radiographs may be suggestive of, but are never diagnostic of, TB disease. Chest radiographs is also wont to exclude pneumonic TB unwellness in Associate in Nursing HIV-negative World Health Organization one that one who incorporates a positive TST reaction or IGRA and who has no symptoms or signs of TB unwellness. Examinations of clinical specimens (e.g., sputum, urine, or cerebrospinal fluid) are of critical diagnostic importance. The specimens should be examined and cultured in a laboratory that specializes in testing for M. tuberculosis

- Specimen collection, processing, and review.
- AFB smear classification and results.
- Specimen culturing and identification.
- Drug-susceptibility testing.

A. AFB Smear Classification and Results

Finding of acid-fast bacilli in stained and acid-washed smears inspected microscopically may offer the initial bacteriologic evidence of the presence of mycobacteria in a clinical specimen. There are two ways usually used for acid-fast staining:

- Fluorochrome procedure victimization auramine-O or auramine-rhodamine dyes (fluorescent microscopy).
- Carbol fuchsin methods which include the Ziehl-Neelsen and Kinyoun methods (direct microscopy).

B. Sputum Smear Microscopy

Sputum smear microscopy has been the primary method for diagnosis of pulmonary tuberculosis. It is a simple and inexpensive technique which is highly specific in areas with a very high prevalence of tuberculosis. Fig. 2 shows Acid-Fast Bacilli stained in smear.

Disadvantages of sputum smear microscopy,

- Smear examination permits solely the presumptive identification of TB illness as a result of the imperviable bacilli in an exceedingly smear is also imperviable organisms apart from M. TB.
- Fewer ball-hawking technicians, inadequate instrumentation and high caseloads compound the matter of diverse samples at restricted research facilities.

Reading of three initial smears and viewing 100 high power field per slide is recommended. This takes at least 15 mins of experienced staff, is subject to error and the mental concentration and visual strain limits the volume of slides handled per day.

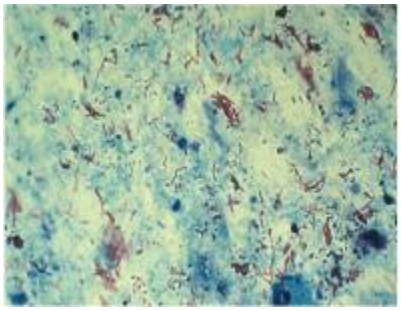


Fig. 2 Acid-Fast Bacilli stained in smear

C. Fluorescence Microscopy

Fluorescence microscopy uses associate imperviable dyestuff dye (e.g., auramine O or auramine-rhodamine) with associate intense light like a group or hard-hitting mercury vapour lamp. microscopy has been attributable with enlarged sensitivity, and, for this reason, has been projected by some consultants to be used in countries with a high prevalence of HIV infection. Disadvantages of C. Fluorescence Microscopy,

- A potential defect of microscopy is that the chance of false-positive results as a result of in organic objects might incorporate dyestuff dyes.
- Cost constraints are major issues with Fluorescence microscopy.
- Frequent burn-out of expensive mercury vapour lamps.
- Continuous power supply necessity, and need of a dark room.

D. Specimen Culture and Identification

Positive cultures for M. T.B. ensure the diagnosing of TB disease but, within the absence of a positive culture, TB sickness may be diagnosed on the idea of clinical signs and symptoms alone. Culture examinations ought to be done on all diagnostic specimens. The commercially offered broth culture systems (e.g., BACTEC, MGIT, Versa TREK, and MBBACT) enable detection of most mycobacterial growth in four to fourteen days compared to three to six weeks for solid media. Laboratories performing arts TB cultures ought to habitually use a broth-based system.

International Journal of Electronics Engineering (ISSN: 0973-7383)
Volume 10 • Issue 2 pp. 526-533 June 2018-Dec 2018 www.csjournals.com

ZN STAINING METHOD

A. Procedure

a) Heat fix and Air dry the smear on to a slide.
b) Primary stain used- Carbolfuchsin is added to the slide and incubated. Excess stain is then washed off with gentle stream of water.
c) Decolonization agent is added to the smear. This removes the stains from non-acid-fast bacteria.
d) The non-acid-fast bacteria are stained with Methylene blue (Second stain).
e) Air dry the slide completely before visualizing Add a drop of oil and visualize under oil immersion lens (x100).

Mycobacteria are acid-fast bacteria which basically means their cell walls can withstand de-colorization with mild acid-alcohol treatments due to presence of certain lipids like mycolic acid in the cell walls. Fig. 3 shows procedure for ZN Staining method. This principle is utilized in ZN staining and will stain the bacteria red. They are rod shaped so what you will see in the sputum smears stained with ZN are red rods. Bacteria other than mycobacteria will be stained blue so they can be eliminated from consideration.

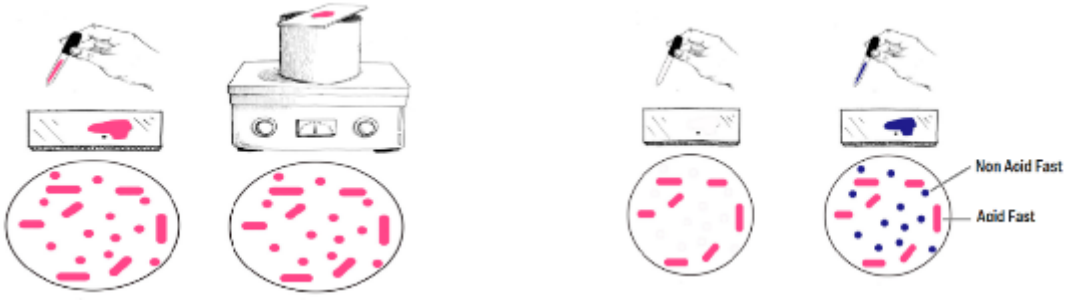


Fig. 3 ZN staining Procedure

B. Results and Interpretation

Acid quick Bacilli: Red, straight or slightly incurved rods, occurring individually or in little teams, could seem beaded.
Cells: inexperienced (malachite green) or Blue (methylene blue)
Background material: inexperienced (malachite green) or Blue (methylene blue)

C. System Architecture

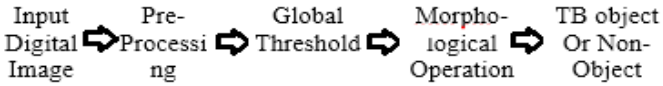


Fig. 4 Block diagram

1) Input Digital Image

ZN stained images are obtained from pathology labs using digital microscope. Fig. 5 shows input digital image, it obtained are colour images and the format of the image will depend upon the microscopic camera and its driver software. For simpler segmentation, clear Mycobacterium Tuberculosis broth images are used to characterize AFB colour.

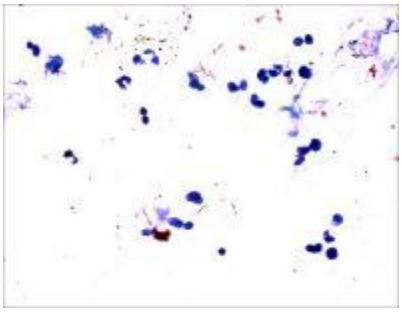


Fig. 5 Input Digital Image

International Journal of Electronics Engineering (ISSN: 0973-7383)
Volume 10 • Issue 2 pp. 526-533 June 2018-Dec 2018 www.csjournals.com

2) Preprocessing

The diagnosis of TB infection in tissue is usually depends on the detection of the bacilli in the ZN-stained tissue slide. Colour is the most useful feature that is utilized in sensing the TB bacilli. During the staining process, Carbolfuchsin dye is used to colour the TB bacilli red, while the methylene blue turns the tissues and backgrounds to the blue colour. Fig. 6 shows RGB to HSV conversion the ZN stain results a good contrast between the bacilli and the background, thus aiding in the detection process.

Hence we use HSV colour space.

RGB to HSV Conversion,

HSV colour model consists of three components: hue (H), saturation (S) and value (V).

- Hue is expressed as a number from 0 to 360 degrees representing hues of red (which start at 0), yellow (starting at 60), green (starting at 120), cyan (starting at 180), blue (starting at 240) and magenta (starting at 300).
- Saturation is the amount of gray from zero percent to 100 percent in the color.
- Value (or brightness) works in conjunction with saturation and describes the brightness or intensity of the color from zero percent to 100 percent.

Consider an RGB image with R', G' and B' represent their normalized red, green and blue components respectively. The conversion is given by,

$$Cmax = \max(R', G', B')$$

$$Cmin = \min(R', G', B')$$

$$\Delta = Cmax - Cmin$$

Hue Calculation:

$$H=\begin{cases} 60° \times \frac{(G'-B')}{\Delta} mod 6, & Cmax = R' \\ 60° \times \frac{(B'-R')}{\Delta} + 2, & Cmax = G' \\ 60° \times \frac{(R'-G')}{\Delta} + 4, & Cmax = B' \end{cases}$$

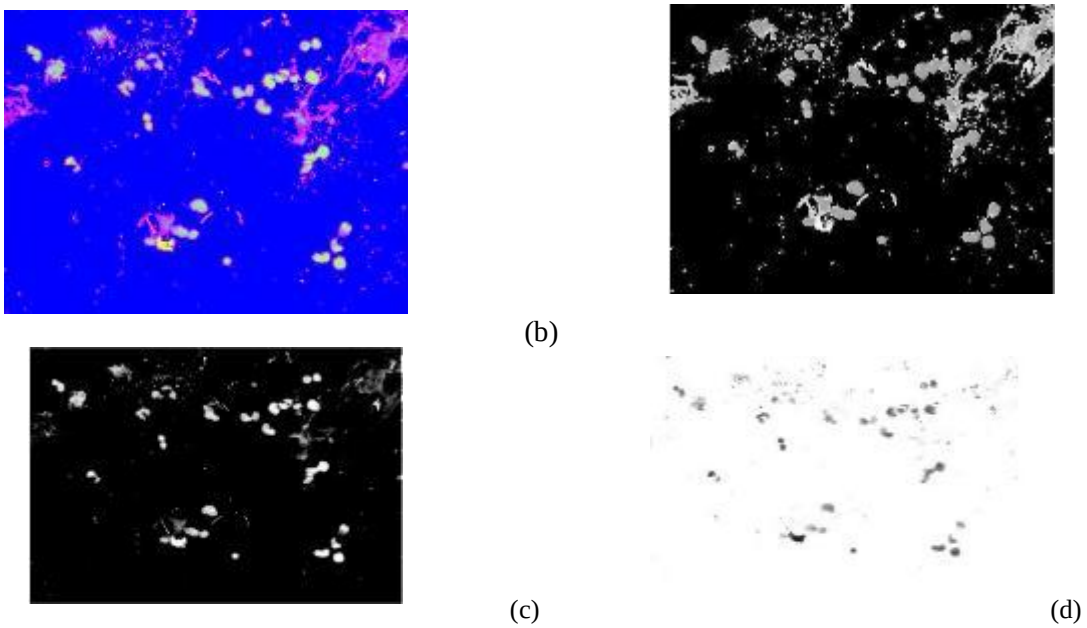
$$H = 0° \ for \ \Delta = 0°$$

Saturation Calculation:

$$S = \begin{cases} 0 & , \quad Cmax = 0 \\ \frac{\Delta}{Cmax} & , \quad Cmax \neq 0 \end{cases}$$

Value Calculation:

$$V = Cmax$$



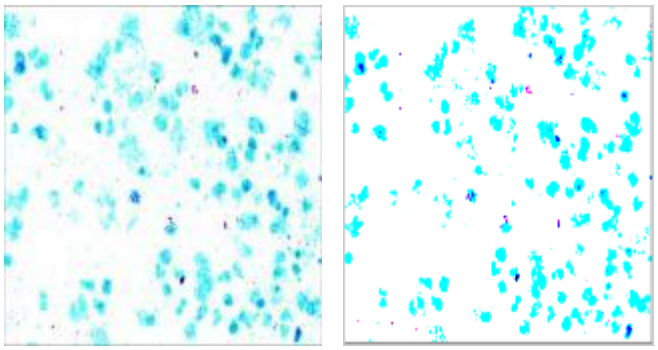
(a) (b) (c) (d)

Fig. 6 RGB to HSV conversion
(a) HSV Image (b) Hue image (c) Saturation image(d) Value image

International Journal of Electronics Engineering (ISSN: 0973-7383)
Volume 10 • Issue 2 pp. 526-533 June 2018-Dec 2018 www.csjournals.com

3) Image Enhancement:

In image enhancement as shown in fig 7, the original image is processed so that the resultant image is more suitable than the original image for specific applications. Image enhancement is a purely subjective processing technique. Specifically, the enhancement of color images is to process the luminance and color information so as to obtain a better contrast between the TB bacilli (red) and the background (blue). Hence contrast stretching facilitates better segmentation results. The idea of distinction stretching in gray scale pictures is extended to paint pictures by applying identical methodology one by one to Red, inexperienced and Blue elements of the RGB color intensity values of the image.

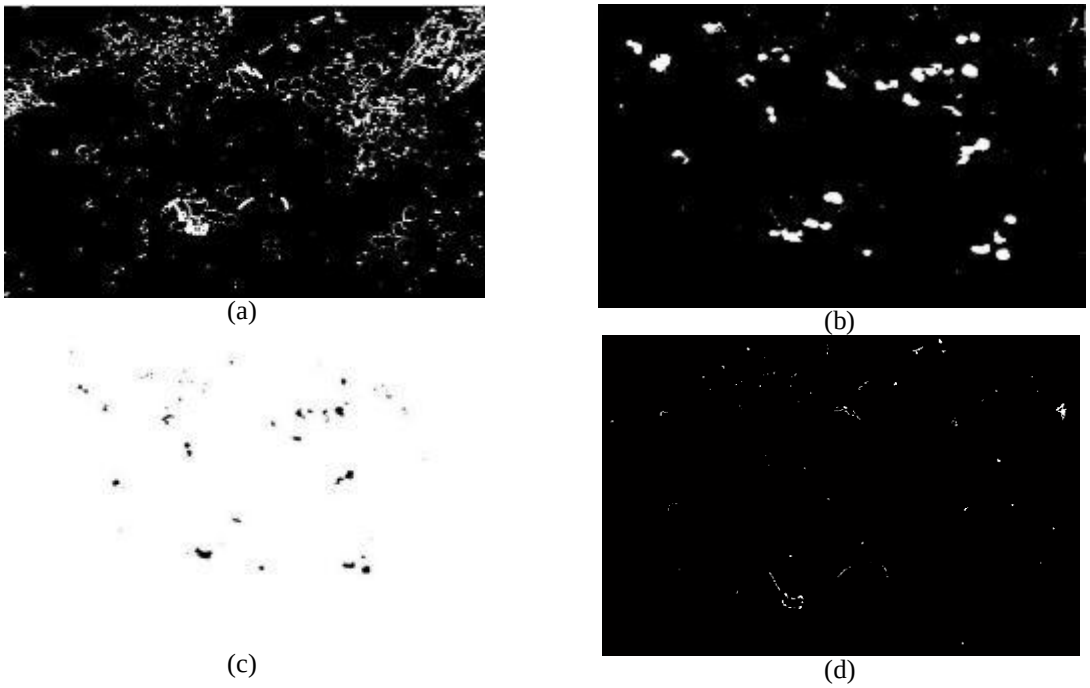


(a) (b)

Fig. 7 Image enhancement (a) Original image (b) Contrast stretched image.

4) Global Thresholding

Global thresholding is used to distinguish Mycobacteria from Background. Global thresholding is shown in fig 8. Due to the presence of certain lipids like mycolic acid in the cell walls, Mycobacteria can withstand de-colourisation with mild-acid alcohol treatments. Due to this acid-fast organisms (including Mycobacterium) will appear red while all the other bacteria will be stained blue (methylene blue) so they can be eliminated from the consideration. Separate threshold values are decided for Hue, Saturation and Value taking into account different shades of red colour. These values were decided through observation using standard values. The pixels which satisfied the threshold conditions were used to create a binary mask. Hence a separate Hue, Saturation and Value mask was obtained. These three masks were combined to form a single mask.



(a) (b)

(c) (d)

Fig. 8 Global Thresholding
(a) Hue mask (b) Saturation mask (c) value mask (d) combined mask

.

International Journal of Electronics Engineering (ISSN: 0973-7383)
Volume 10 • Issue 2 pp. 526-533 June 2018-Dec 2018 www.csjournals.com

5) Morphological Operations:

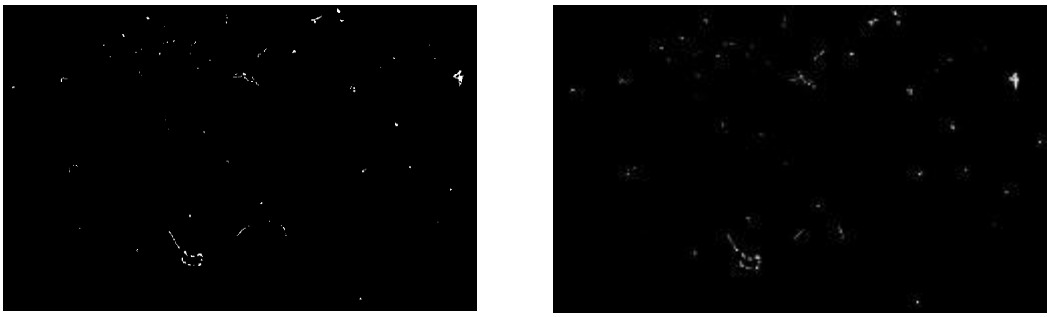
Morphology is used as a tool for extracting image components that are useful in representing various regions and shapes. The morphological operations used are, Closing, Region Filling and Dilation.

a) Closing

Theoretically Closing operation is dilation followed by erosion it shown in fig 9. It is denoted as,

$$A \cdot B = (A \oplus B) \ominus B$$

Closing is used to fuse narrow breaks and eliminate small holes in bacteria structure or the clusters formed.



(a) (b)

Fig. 9 closing operation
(a) Combined mask (b) Closed mask.

b) Region Filling:

Region filling is used to fill holes in the binary image. As shown in fig. 10, a hole is a set of background pixels that cannot be reached by filling in the background from the edge of the image.

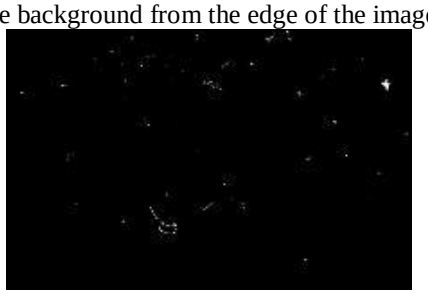


Fig. 10 Region Filled mask

c) Dilation

Dilation adds pixels to the boundaries of objects in a picture. The quantity of pixels superimposed depends on the scale and also the form of structuring part. The structuring part chosen may be a disk of radius one. The radius of the disk is unbroken tiny to incorporate less background pixels within the mask.

Theoretically, dilation may be outlined as,

$$A \oplus B = \{Z|[(B^{'})z \cap A] \in A\}$$

A is the image and B is called the arranging element. (B') z only means enchanting the reflection of B about its source and shifting it by Z. Hence, dilation of A with B is a set of all dislodgments, Z, such that (B') and A overlap by at smallest one section.

The dilated mask is shown in fig 11.

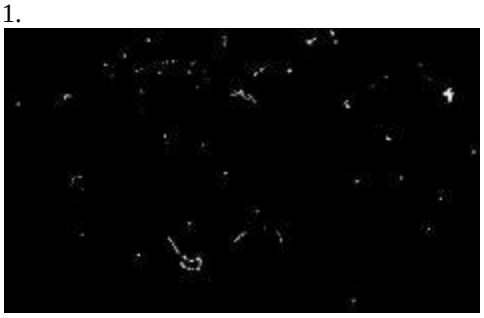
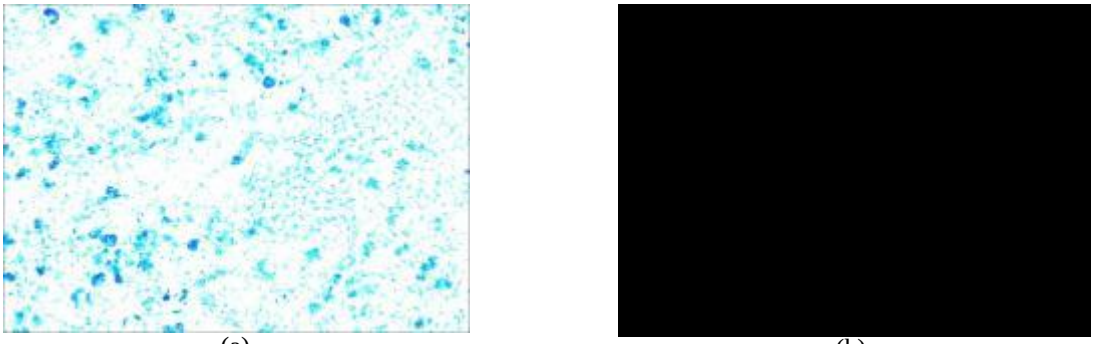


Fig. 11 Dilated mask

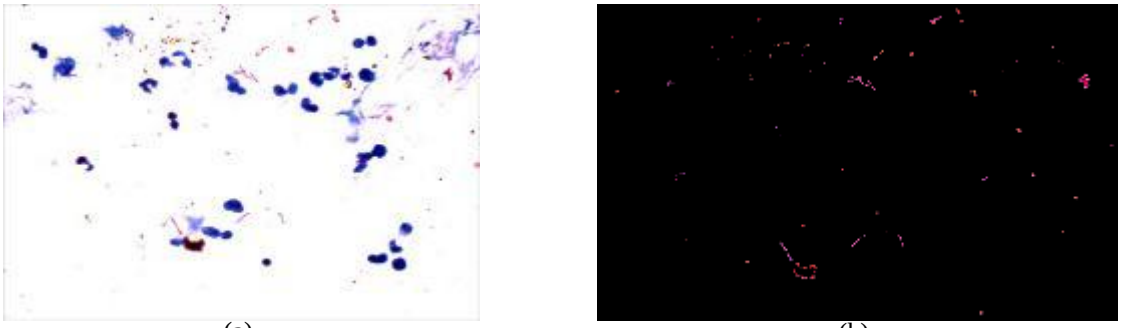
International Journal of Electronics Engineering (ISSN: 0973-7383)
Volume 10 • Issue 2 pp. 526-533 June 2018-Dec 2018 www.csjournals.com

RESULT



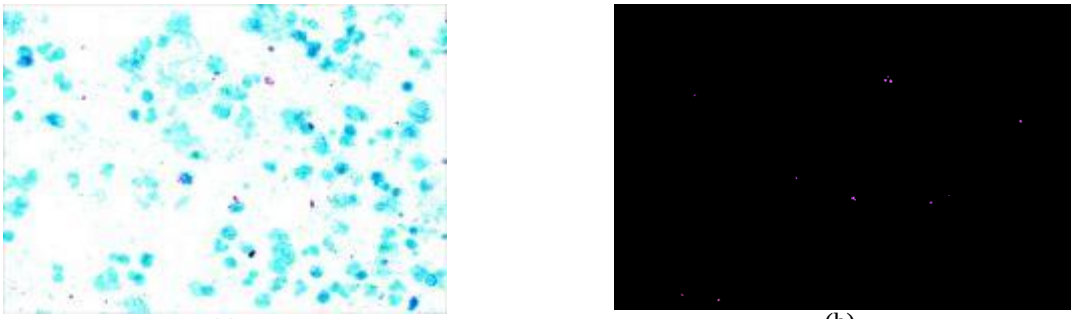
(a) (b)

Fig. 12 Result for TB Object absent (a) Input digital image (negative) (b) Output image.



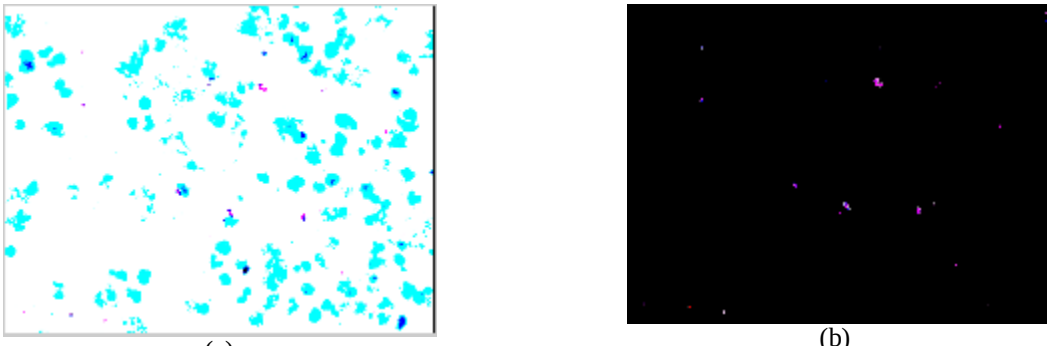
(a) (b)

Fig. 13 Result for TB Object present (a) Input digital image (positive) (b) Output image.



(a) (b)

Fig. 14 Result for without enhancement image (a) Input digital image (b) Output image.



(a) (b)

Fig. 15 Result for enhanced image (a) Input digital image (b) Output image

FUTURE SCOPE:

- The process of capturing the images could be automated so as to facilitate complete automation of detection of TB bacilli. For this purpose standard values should be decided for contrast and brightness settings.
- Our technique could also potentially quantify TB bacilli in ZN smears for future use so as to classify the smears as 1+, 2+ and 3+.
- Shape and Size analysis can be used to identify Mycobacterium Tuberculosis from other acid-fast organisms (e.g. Nocardia).

CONCLUSION

ZN staining technique algorithmic rule detects the TB bacilli mechanically. This machine-controlled system reduces fatigue by providing pictures on the screen and avoiding visual review of microscopic pictures. The system aims to own higher speed in police investigation TB bacilli. The strategy is easy and cheap to be used in rural/remote areas.

In the typical cytosmear image, the algorithmic rule can notice most of the one bacilli; also as overlapping bacilli. It can also be concluded that increasing the contrast of the image makes segmentation easier and helps in providing better results.

REFERENCES

[1] Sadaphal P., Rao J., Comstock G. W., Beg M. F. (2008). Image processing techniques for identifying Mycobacterium tuberculosis in Ziehl-Neelsen stains. Int. J. Tuberc. Lung Dis. 12 579–582.
[2] Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infectious Diseases 2006;6(9):570-81.
[3] DhurbaGiri in Bacteriology, Microbiology.
Ziehl-Neelsen Stain (ZN-Stain): Principle, Procedure, Reporting and Modifications. January 16, 2016.
[4] Muhammad Khusairi Osman, MohdYusoffMashor and HasnanJaafar. Combining Thresholding and Clustering Techniques for Mycobacterium tuberculosis Segmentation in Tissue Sections. Australian Journal of Basic and Applied Sciences. 5(12): 1270-1279, 2011.
[5] Wilbur C.K. Wong and Albert C.S.Chung. Bayesian image segmentation using local iso-intensity structural orientation IEEE transactions on image processing, Vol 14, no. 10, Oct 2005
[6] http://www.tbfacts.org/tb-tests/
[7]http://web.uconn.edu/mcbstaff/graf/Student%20presentations/Mtuberculosis/Mtuberculosis.html
[8] https://en.wikipedia.org/wiki/Ziehl%E2%80%93Neelsen_stain