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Integration of Microscopic Image Capturing System for Automatic Detection of Mycobacterium Tuberculosis Bacteria

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Abstract

*The Ministry of Health of the Republic of Indonesia is running a program to eliminate Tuberculosis (TB) by 2030. At the Primary Health Care level, AFB (*acid-fast bacteria*) examination confirms the TB diagnosis. In this process, the patient's sputum is prepared in the form of preparation and observed by the laboratory analyst through the lens of a microscope. The reporting process to establish this diagnosis requires calculating the number of TB bacteria in 100 fields of view per preparation. This manual microscopic observation process is tedious, and the reading results are subjective. This study offers an integrated design for automatic microscopic imaging with a computer-integrated TB bacteria detection system. The process of taking pictures is automatically obtained with the help of a driving motor added to the microscope. With the addition of this motor, the process of taking microscopic images for 100 fields of view takes ±450 seconds. The proposed system integration process can reduce laboratory analysts' work fatigue in conducting microscopic observations manually. The TB bacteria detection system utilizes the working principle of image processing techniques by combining color-deconvolution, segmentation, and contour-detection methods. The comparative value of the TB object detection system with experts resulted in a sensitivity value of 77% and a specificity value of 68%. However, the low detection rate is because the image obtained is still blurry. Thus, further investigation is needed to determine the driving motor's movement rate and the right timing for taking microscopic images so that the resulting image is not blurry. The final result that is the focus of this paper is the successful integration of the system carried out between the motor drive system on the preparation stand and the TB bacteria detection system to become a unified system.*

Keywords: *tuberculosis; sputum; image processing; microscope.*

1. Introduction

TB (Tuberculosis) is a kind of infectious disease caused by a bacteria called MTb (Mycobacteria tuberculosis). That spreads through the air released by the sufferer so that it can be circulated quickly to others [1].

Microscopes are one of the medical devices to carry out the process of analyzing and diagnosing disease [2]. Most Primary Health Care ("PUSKESMAS") in Indonesia use binocular microscopes, which have two observation lenses to facilitate focus on the observed objects. Detection of bacteria using a microscope must be carried out along 100 fields of view, and the observations are still very subjective based on the observers of each laboratory analyst. The results of laboratory observations will affect the diagnosis of diseases experienced by patients. This subjective opinion needs to have an appropriate standard to arrange the result. Moreover, the period of a laboratory worker in detecting TB bacteria is between 3 minutes to 25 to 1 slide of preparation [3].

The problem can be solved by developing a system that detects and classifies TB bacteria automatically. In addition, it can take TB bacteria images along 100 viewing fields by integrating the preparation mount drive motor.

Research on the use of watershed image processing techniques has a significant impact on the results of the identification of TB bacteria object images, the segmentation process carried out also depends on image conditions, so the image color-conversion process will urgently require several morphological operations, such as opening operations and closing operations [4]. Like

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the study entitled "Segmentation of Tuberculosis Bacilli Using Watershed Transformation and Fuzzy C-Means," proposing the use of Watershed Transformation and Fuzzy C-Mean methods to detect TB bacteria, this study shows the value of Cohen Kappa Coefficient 0.838 from the comparison of the results of bacteria detection by three microbiological doctors with the results of system detection [5].

Another study used the multi-SVM classification technique in grouping TB bacteria, using color-conversion, segmentation, and edge-detection techniques. From TB image testing, 95% accuracy was obtained in grouping and identifying TB bacteria [6].

Unlike the case used of the shape features method to obtain data on the size of the object detection area, restrictions can be made on the object to be detected. In addition, liked Polygon Approximation, Concave Point Extraction, Contour Segmentation, and Ellipse Processing can be used to determine TB bacteria objects in a state of overlap between each bacteria on the TB sputum image. This study resulted in an accuracy rate of 88% and a sensitivity of 88.34% to the diagnosed TB bacteria [3]. The study "Mycobacteria Tuberculosis Bacteria Detection System and Determinants of TB Disease Rate" used the Color Deconvolution method to identify the TB bacteria image by dividing two colors on the TB bacteria image. The system produced a precision level of 86.48% and a recall rate of 65.04% [7].

Research with similar bacteria objects using a feature of texture image extraction histogram, Gray-Level Co-occurrence Matrix (GLCM), and Principal Component Analysis (PCA) to detect bacteria in X-Ray images by looking for characteristics best of TB bacteria. The research distinguished between normal and abnormal images, the result in a classification accuracy rate using the Histogram method of 81.81%, GLCM of 96.96%, and the PCA method of 81.82% [8].

The use of digital microscopes is also used in research that identifies parasitic worm infectious diseases in cattle, using image processing techniques using the Yolo V3 algorithm to be able to detect egg objects and parasitic worms in cows. This study has a system accuracy of 78.42% for parasites and 80.84% for non-parasites [9].

Automation research for camera focus in reading the field of view of microscopes modifies conventional microscopes by adding digital cameras and stepper motors as a means of focusing view, in addition to the use of the Normalized Variance (F) method used to find good focus values according to humans and computers [10].

A similar study used a motor controller on a microscope tube to move the focus of visibility on the microscope. In addition, the addition of a digital camera to produce image capture and a focus controller program based on the results of the image histogram analysis. Based on measurements of images as much as 20 times the view of TB bacteria, it shows that the image with the best focus has the highest entropy value and an accuracy rate between 81.90% and 100% at a magnification object 1000 times [11].

Similar research focuses on monochromatic light control to detect malaria parasites. The addition of digital cameras and motors as control of the microscope preparation to observe the entire area preparation with only a computer and monochromatic light produces a contrasting image between blood cells, parasites, and a background that reduces the artifact [12].

The development of a microscope using a digital camera based on a smartphone camera is one of the options for displaying microscope images, with a choice of various smartphone camera resolutions. The smartphone camera is good enough to clear the image with a working method, almost like a light microscope. The resulting image is immediately displayed on the smartphone screen [13].

This study will use color-convolution, segmentation, shape descriptors, and contour-detection methods. The selection of the methods was based on the results of a literature review from several previous studies that obtained a high level of accuracy and can apply to TB sputum images. The automatic detection and classification of bacteria will facilitate the analysis and diagnosis of TB disease sufferers without going through objective observations. Furthermore, creating a system integrated with a visual digital and a motor drive of the preparation holder makes it easier for health workers to see objects without having to look directly at the microscope and produce images along 100 viewing fields with the detection of TB bacteria automatically. So that, it will shorten the time in determining the diagnosis results of TB bacteria observation objects.

2. Research Methods

This research will be through several stages. The research starts with problem analysis, system implementation, and system testing. The research stages are in Figure 1.

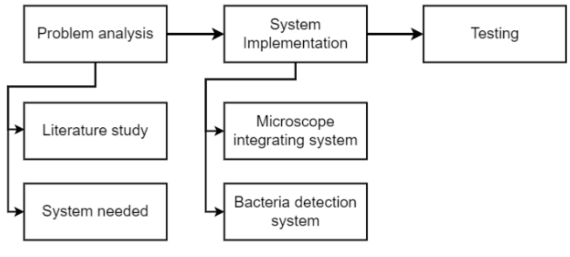

Figure 1. Research stages

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The problem analysis stage in Figure 1 has two steps that will be carried out, namely the literature study and the system need. A literature study stage includes literature search activities that conduct similar research, and guidelines for the managed acid resistance examinations become practical guidelines for Medical Laboratory Experts. The next stage is the collection of data, indicators, and standards needed as inputs, processes, and performance measures of the system that is the focus of research will be carried out.

Furthermore, the implementation stage of the system will utilize one of the microscopes that have been modified with the addition of a camera on the microscope's ocular lens. The movement controller of the preparation holder can be controlled through a computer. Then integration will be carried out on the TB bacteria detection and classification system that can detect TB bacteria objects and classify them by severity using image processing techniques.

The testing phase will be carried out to test the output results of the integration system in detecting bacteria. This test will compare the identification of bacteria by experts with the detection results of the system.

Referring to previous research conducted by the Informatics Engineering team, [5] that there is an FGD (focus discussion group) process with analysts from Temanggung Regency Health Office who stated the detection of TB bacteria throughout the 100 fields of view manually is very tiring to be ab le to diagnose a TB sufferer. The process requires the help of digital tools to observe the number of TB bacteria found in the patient's phlegm along 100 viewing fields—data testing using primary data from sputum samples from patients with TB disease with each severity. The data on TB bacteria sputum was obtained from the Health Office ("DINKES") of Temanggung Regency and Sleman Regency. The number of sputum samples was 125.

This research is done by utilization of one of the microscopes (Olympus CX31), which has a choice of lenses with a size of 4X, 10X, 40X, and 100X which has been updated with the addition of a camera [14] (Optilab Advance) with a camera resolution of 12MP on microscope lens, and movement control of the preparation mount that can be controlled via computer Figure 2.

This microscope is an object of research from the research team of the Informatics Engineering Study Program, Master Program, Faculty of Industrial Technology, Universitas Islam Indonesia, regarding the microscope automation system.

With the microscope, the integration will be carried out on the TB bacteria detection and classification system that is the object of this research, to take bacteria images in 100 viewing fields and move in horizontally to shoot along the size of the preparation automatically.

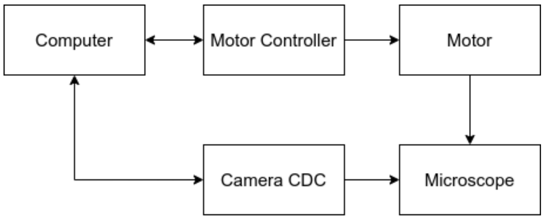

Figure 2. Microscope workflow

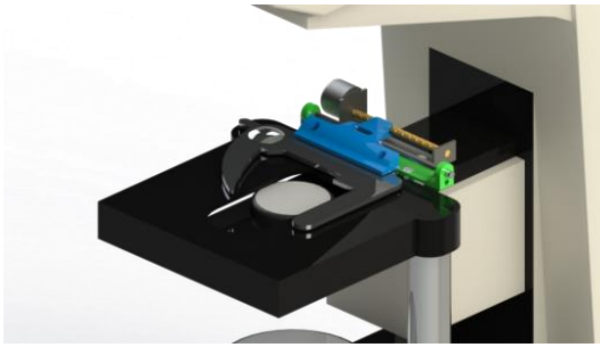

Figure 3. Microscope motor system

The resulting image is a sputum image with a lens magnification of 100 times. The TB bacteria image has a rod-like pattern and criteria with a length of about 2-4 micrometers and a width of 0.2-0.5 micrometers is a magenta result from Ziehl-Neelsen coloring [15]. The determination of severity classification of TB needs to be calculated by how many bacteria are found in one field of view or a single sample [16].

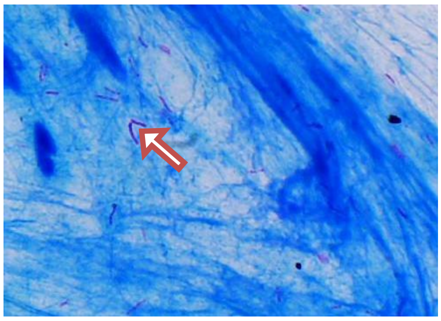

Figure 4. Character and shape of TB bacteria on digital images

Table 1. Scales determining the severity of Tuberculosis bacteria [16]

Diagnosis	Bacteria found
Negative	No visible TB bacteria in 100 Field of View
Doubtful(*scanty*)	1-9 / 100 Free View
1+	10-99 / 100 Free View
2+	1-10 / Viewpoint (min. 50 View)
3+	>10 / Viewpoint (min. 20 View)

Table 1 shows the severity scale of TB sufferers. TB patients will test negative if no TB bacteria are found in the 100 observed sputum field of view, but will it be worth 1 to 9 TB bacteria in 100 fields of view? Unlike the favorable situation, in the patient's sputum will be found a lot of TB bacteria in 100 fields of view.

The color-deconvolution algorithm processes the image into three different types of color, and the process

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results can group the difference in color characteristics between TB bacteria and the color of the surrounding area [17]. Color-deconvolution will help filter objects and back areas in an image [18]. Segmentation methods will clarify the differences in the color-deconvolution results, making it easy to distinguish between TB bacteria and non-bacteria.

To determine whether the object found is a TB bacteria or not, the shape descriptors method is used by determining several criteria or thresholds in the form of area size, length, and width of the object, then the calculation results are entered into the contour-detection method so that this process will label and calculate the number of objects that are included in the threshold of suspected TB bacteria.

The system testing stage will use data from expert observations as a reference to test the accuracy and sensitivity in detecting TB bacteria. The test data used are data from the results of examinations carried out by experts in the amount of 30 data as material for comparing the system results. Figure 5 displays the sputum image labeled by experts and considered TB bacteria.

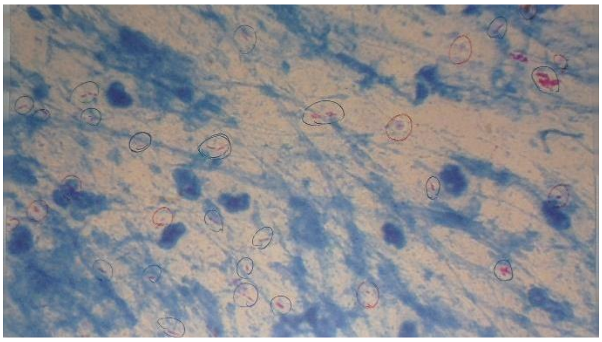

Figure 5. Expert detection and labeling results

Sputum images captured by microscope cameras will be processed using the color-deconvolution method to distinguish the color characteristics of TB bacteria and the color of the surrounding area. Then the process of calculating the number of objects declared as bacteria is carried out. The labeling process of the detected objects will finally determine the level of TB disease based on a comparison of the number of TB bacteria detected by the system with Table 1.

3. Results and Discussions

3.1. Motor Movement Mechanism

The transfer of the preparation holder on the microscope uses 1 DC motor. It is controlled using an Arduino UNO microcontroller with a CNC shield V3 driver motor. The movement of preparation is made horizontally to ensure the bacteria detection process is checked along the preparation.

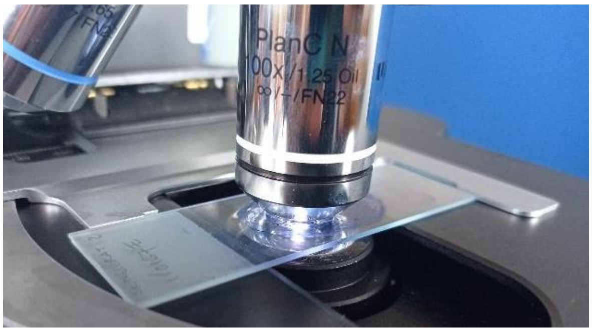

Figure 6. Mounting preparate on a 100x magnification lens

To start using a microscope, the *user* needs to determine the initial position of the preparation to be observed. As shown in Figure 6, this position will make the camera's viewport into coordinates zero of the motor position. Next, the microscope camera will take an image of the intended field of view based on the position of the preparation, as seen in Figure 7.

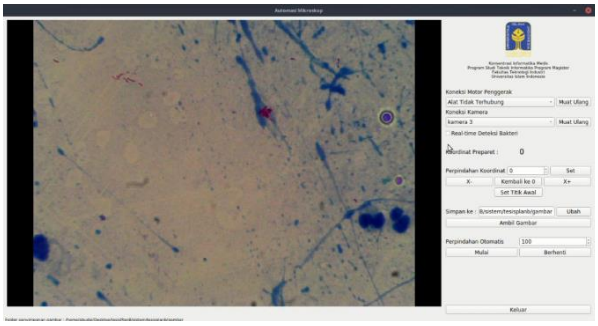

Figure 7. The interface of the bacteria object detection system automatically

The movement of the motor on the microscope will follow the displacement instructions that the user will enter, using serial communication to exchange information between the computer and the microcontroller, with the appropriate workflow on Figure 8 and Figure 9. The computer will send information in coordinates on the microcontroller to move the motor. One transmitted coordinate value has a length of ±0.011mm movement.

3.2. Bacteria Detection

The TB bacteria detection stage is divided into three stages. The first is the image capture stage from a microscope camera, which outputs images with a maximum resolution of 4100 × 3075 pixels. Images with large resolutions will affect the length of time for image analysis, so the process of reducing the image resolution size to 1000 x 750 pixels is carried out, as seen in the results of camera imagery in Figure 4.

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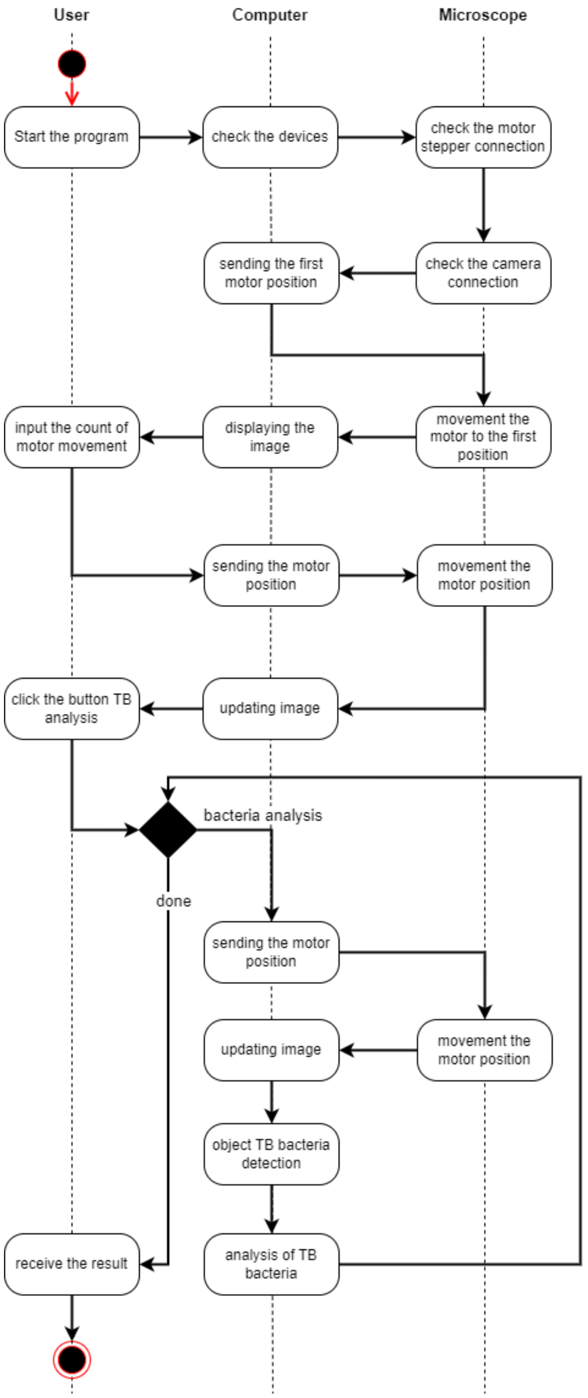


Figure 8. Automatic microscopic shift and shooting workflow

The second stage is the pre-processing stage, which applies the color-deconvolution method to separate several images with dominant colors. This color separation process will result in 3 outers. Among them is the image, which contains the color of the object and the color of the area around the object. Based on the image from Figure 4, the dominant color will appear in two colors, red for TB bacteria objects, blue for parts that are not TB bacteria, and white for the area background of the preparation image, as shown in Figure 10.

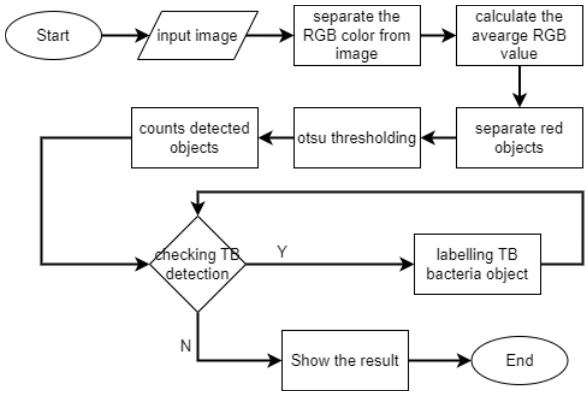
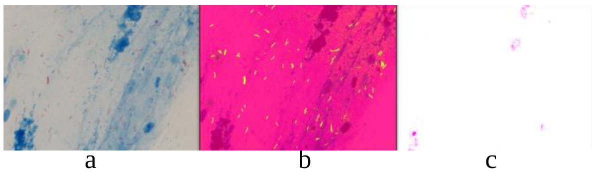


Figure 9. TB bacteria detection flow



a b c

Figure 10. (a) Tb bacteria imagery, (b) The first color-deconvolution result that clarifies the difference in the color of the object and the area around the object, (c) The second color-deconvolution result that displays the object area with a dominant different color on the image

From the results of the color-deconvolution process, the first image results will be used that clarify the appearance of the object and continue with the segmentation process to clarify the object that is suspected to be a bacteria object TB. this process will magnify the appearance of objects that are suspected to be TB bacteria.

The last stage is clustering or sorting the candidates for bacteria objects into two parts, the candidate objects with criteria resembling TB bacteria and objects with criteria as TB bacteria. The criteria that become a benchmark for determining TB bacteria are based on the detected object's color, length, and area. After finding the TB bacteria object, the system will mark the objects, as shown in Figure 11.

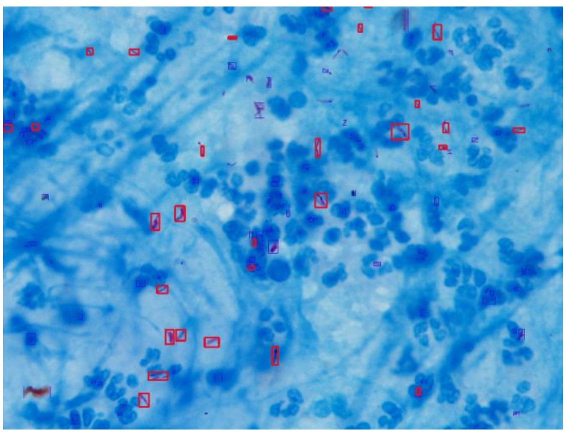


Figure 11. Results of bacteria detection and labeling

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3.3. Integration of Microscope System with TB Bacteria Detection System

Integrating the microscope motor system with the TB bacteria detection system is carried out by connecting computers and devices in the microscope, including motor control devices and cameras, using a USB cable Figure 12. The mounting position of the camera needs to follow the viewpoint of the lens. Therefore, the camera's mounting position will affect the image's display, whether inverted or not, with the results of the manual observation lens Figure 13.

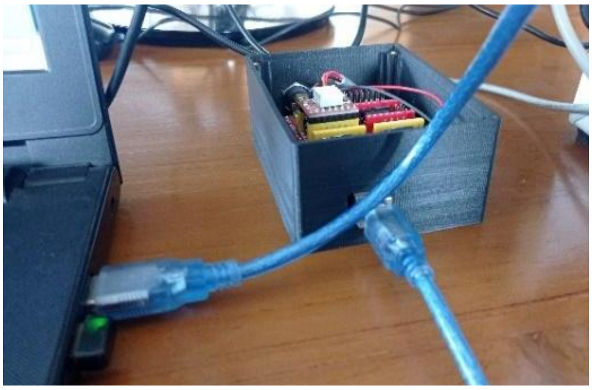

Figure 12. Connecting the control motor to a computer

The system will automatically detect the connection of these hardware devices in Figure 14. Then, for the first time, the system will send a command to the control motor to move to the initial coordinate point. It is this so that the locking of the preparation holder is not in the middle position of the thread but rather is in the initial position of the thread of Figure 3, so that the initial position of the thread will be defined as the coordinate '0' by the system, besides that the user can also confirm the initial coordinate point of the motor.

Figure 13. Camera position on the microscope lens

Figure 14. Integration of control motors, cameras, and computers

The system will then detect the camera device and display the captured imagery of the camera. Integrating the microscope motor system and the TB bacteria detection system works by means that the system will send commands to the motor control to move the coordinate. Then, the system will update the image from the camera and perform image processing to detect TB bacteria from the latest image. The results of this image processing will be stored on a computer with the image output labeled TB bacteria and the count of bacteria detected in the viewport. This process will be performed repeatedly during the large number of viewpoints to be observed by the *user*.

3.4. Evaluate the quality of the Shooting Image

The speed of movement of the preparation mount influences the image quality of microscope shooting results in Figure 15, which shows the effect of rpm speed (rotation per minute) on the degree of clarity of the image displaying the object of TB bacteria. The best image is tested with three speeds, namely 10rpm, 100rpm, and 200rpm. The selection of these three speeds is based on the ability of the motor to rotate at the lowest speed, average speed, and maximum speed.

This evaluation is carried out to find the most optimal image shooting results to detect bacteria TB bacteria, making it easier for the system to recognize TB bacteria objects in the image. The test was carried out by shooting ten images from each speed so that there were 30 TB bacteria image samples.

The image quality of microscope shooting results was evaluated by 25 Medical Laboratory Technology Experts of the Sleman Health Office. The experts will assess the image of each speed by three levels, i.e., The image cannot be assessed, the image is still appraised, and the image is easy to judge. However, there are some obstacles when printing and shooting images. For example, there are differences in the use of digital color standards and physical printing so that the brightness level is changed to digital imagery to produce prints that are close to digital images. In addition to that, there is also a reduction in image sharpness from image prints

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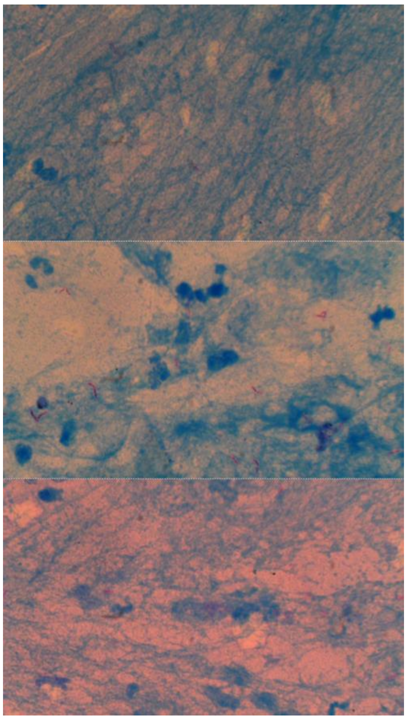


a

b

c

Figure 15. (a) 10rpm motor speed result image, (b) 100rpm motor speed result image, (c) 200rpm speed result image

Figure 16. FGD process with the experts for evaluation of shooting image quality

Table 2. Evaluation of the quality of image shoot

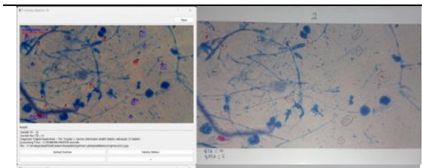
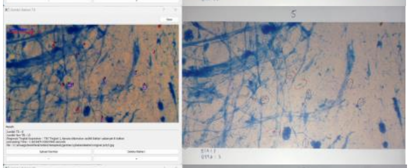
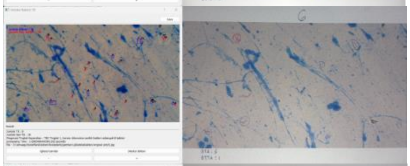
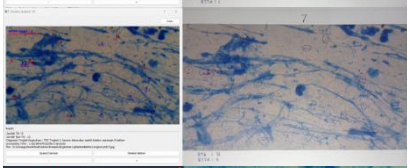
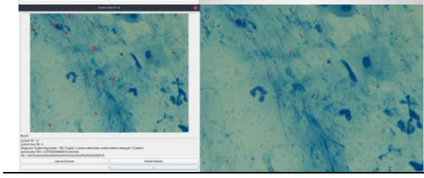
	10rpm	100rpm	200rpm
Image cannot be assessed	7	4	5
Imagery can still be assessed	3	2	4
Imagery is easy to judge	0	4	1

Table 2 shows that the image is easy to judge at a speed of 100rpm. Therefore, this speed will be the ideal speed in the movement of the preparation mount for producing shooting imagery.

3.5. Evaluation of Detection Accuracy

To ensure that the bacteria detection results of the system are as expected, accuracy testing is carried out by comparing the results of the system with experts who have previously marked the bacteria object on the related image in Figure 5 so that the development of this system involves competent experts in their fields. Some samples will be used to compare the detection results of the system with the results of expert observations.

Table 3. Comparison of system detection results with experts

Comparison of diagnosis results	Number of Bacteria Detection
	System: 10 Experts: 10
	System: 21 Experts: 20
	System: 8 Experts: 7
	System: 8 Experts: 6
	System: 9 Experts: 13
	System: 13 Experts: 18

Most of the results of Table 3 shows the detection of TB bacteria by the system close to the results of expert observations. The detection of TB bacteria results based on the sensitivity of the limitation of the criteria values applied to the system. This results in the number of TB bacteria candidate objects not being considered bacteria.

Another criterion that appears in the image of TB bacteria is the overlapping bacteria form, as seen in Figure 17. The large number and gathering of bacteria in an area result in overlapping events between bacteria often occurring, set display a form that does not enter the characteristics of TB bacteria.

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Figure 17. TB bacteria with overlapping conditions

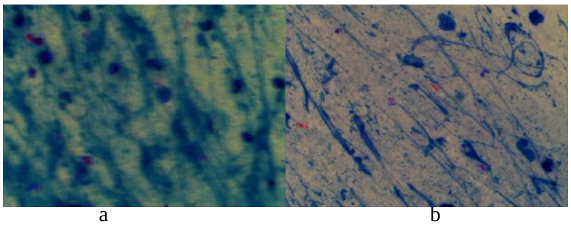
Table 4. Confusion matrix comparison system with experts

True Positive	True Negative	False Positive	False Negative
240	230	70	106

Table 4 shows the confusion matrix results from comparing the number of bacteria detected by the system with experts. Based on these results, 106 objects were not successfully detected. However, the hidden objects are objects that should be considered TB bacteria. In addition to that, as many as 70 objects should not be included in the bacteria object but are declared TB bacteria by the system. As many as 240 True positive objects and 230 objects True Negative state that objects detected by the system and experts are the same as TB bacteria. These results obtained a sensitivity value of 77% and a specificity value of 68%.

The value obtained by the system is relatively low. In addition to the influence of the sensitivity of the specified object detection criteria, the image quality of the bacteria object also affects the results of TB bacteria detection by the system. The movement of the motor displacement on a micrometer scale produces vibrations against the preparation holder; this impacts the microscope's quality of view and the mount preparation's position. These vibrational preparation mounts occasionally produce an image of TB bacteria that is unclear.

Vibrations resulting from the displacement of motor movement have an impact on the visibility of the observation lens (camera) with the preparation mount, resulting in an unclear image in Figure 16 (a), with the results of this unclear imagery will significantly affect the detection of objects by the system based on predetermined bacteria object criteria.



a b

Figure 16. (a) Show an unclear image due to vibration of motor displacement movement, (b) Show a clear image

3.6. Evaluation of System Time

Python programming language version 3.8.10 with a compute specification of a 1.70GHz Core i3 processor, and 8Gb RAM is used to build the TB bacteria detection system. The image resolution that this research uses as test data is 1600 x 1200 pixels. Table 5 shows the average result of a process of 4,869 for each test data image from the results of the Detection Accuracy Evaluation of 30 test samples.

Table 5. System compute time

Process Stages	Average time per second
Preprocessing	3.587 ± 5.613
Clustering	0.006 ± 0.027
Total	3.603 ± 5.646

3.7. Differences with Other Studies

This research is a development of the TB bacteria detection system previously carried out by the Informatics Study Program team of Universitas Islam Indonesia in detecting TB bacteria from sputum images [5][7] and digitization of ordinary microscopes with even cameras and driving motors, previously the detection of TB bacteria was only processed per-image without being connected to a microscope.

The researchers carried out this continuous development to integrate the TB bacteria detection system with a digitized microscope. The goal is to detect and automatically accumulate the number of TB bacteria found in the sputum of patients with TB. Similar research developed the digitization of ordinary microscopes using computers to control the cameras and driving motors [9] and focusing on observing malaria bacteria and clarifying the imagery of malaria bacteria. However, unlike this study, it focuses on the detection of TB bacteria and the classification of TB bacteria automatically by moving autonomously along 100 fields of view areas. The results of the detection and classification of the system in the form of the number of batteries detected throughout the 100 fields of view become a laboratory benchmark to determine the severity of the patient TB sufferers.

4. Conclusion

The TB bacteria detection system has sensitive parameters in determining objects, with a comparative result of sensitivity of 77% and a specificity value of 68% through the test results. Therefore, future works need to observe the criteria for recognizing TB bacteria objects even based on some objects that often appear, including overlapping criteria and abnormally sized ones. Furthermore, the success of the integrated system with the movement of the motor displacement also affects the results of TB bacteria detection; vibration and motor displacement impact some of the images produced and display bacteria objects which are

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obscure and vague. From this case, it is necessary to update the drive motor that does not have a vibrational impact to not change the view distance of the observer lens with the preparation mount.

Acknowledgment

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Thanks to the Temanggung Regency Health Office and the Sleman Regency Health Office for the availability of secondary data on TB patients; with data collection development, the system will be unrestricted.

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