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Enhancing Accuracy in Mycobacterium Tuberculosis Counting on Acid-Fast Bacteria Sputum through Microscope Camera and Image Processing with Thresholding Method

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ABSTRACT Tuberculosis (TB) is a chronic infectious illness. Pulmonary TB is still a global threat and concern. Currently, TB is among the top 10 causes of death in the world in 2015 and it is estimated that there are around 10.4 million new cases of TB in the world. 6 countries account for around 60% of new cases in the world: India, Indonesia, China, Nigeria, Pakistan, and South Africa. Globally, the TB death rate was around 22% of total world deaths from 2000 to 2015. This study introduces an innovative tool, the "Microscope Camera for Enhanced Mycobacterium Tuberculosis Counting in Acid-Fast Bacteria Sputum through Image Processing with Thresholding Method," designed to revolutionize the examination of tuberculosis (TB) samples. In response to the global threat of Pulmonary Tuberculosis, the tool employs a state-of-the-art CMOS-HD (Complementary metal-oxide-semiconductor High Definition) camera module and leverages the Python application for efficient image processing. The primary objective is to address the limitations faced by conventional diagnostic methods, particularly the challenges associated with the Rapid Molecular Test Method due to its dependence on expensive equipment, thereby making it less feasible for implementation in resource-constrained public health centers. The study underscores the persistent threat of TB as a global health concern, emphasizing its prevalence, with approximately 10.4 million new cases worldwide. Focusing on Ziehl-Neelsen stain sputum samples, the research seeks to elevate the accuracy of TB diagnosis by proposing a streamlined approach. This involves integrating a digital microscope system with a CMOS-HD camera module and utilizing advanced image processing techniques, specifically the Python Thresholding method. In the methodology, the research outlines a comprehensive data collection and analysis process. Mechanical and block diagrams illustrate the design of the module, while the system flow diagram elucidates the sequence of steps involved in TB sample analysis. The data analysis section employs metrics such as average, percentage error, and success percentage, providing a quantitative assessment of the system's performance. The results reveal an encouraging average system accuracy of 85.30%, demonstrating the potential of the developed tool to enhance the diagnostic process. However, the study transparently acknowledges areas for improvement, particularly in addressing discrepancies between manual and system calculations. The conclusion emphasizes the tool's promising role in assisting healthcare workers in detecting and counting TB bacteria in sputum, underscoring the need for ongoing refinements to achieve a definitive 100% accuracy and eliminate the possibility of misdiagnoses.

INDEX TERMS Tuberculosis, Python, Acid-Fast Bacteria

I. INTRODUCTION

Tuberculosis (TB) remains an enduring global health concern, with Pulmonary TB standing as a significant threat. In 2015, TB ranked among the top ten causes of worldwide mortality, estimating a staggering 10.4 million new cases globally. This alarming prevalence is particularly pronounced in six nations—India, Indonesia, China, Nigeria, Pakistan, and South Africa—which collectively account for 60% of reported cases. The TB death rate, constituting around 22% of total global deaths from 2000 to 2015, underscores the urgency for innovative and accessible diagnostic solutions. [1]

The current diagnostic landscape for Pulmonary TB leans heavily on the Ziehl-Neelsen (ZN) method, a microscopic examination of Bacillus Tuberculosis Acid-Fast Bacilli (AFB) in patient sputum. However, the advent of the costly Rapid Molecular Test Method (TCM) poses challenges, particularly in resource-constrained community health centers, limiting its widespread adoption.[2], [3] AFB samples, primarily acid-fast bacteria from patient sputum, play a crucial role in diagnosing Mycobacterium Tuberculosis (MTB) infection—the primary causative agent of TB.[3][4]

Recent studies have significantly contributed to advancing TB detection methodologies. Some research introduced the Otsu auto-thresholding method, coupled with morphological operations and the Adaptive Neuro-Fuzzy Inference System (ANFIS) for categorization.[5] [6] [7] Some researcher explored the Loop-Mediated Isothermal Amplification (LAMP-TB) Technique, emphasizing its molecular diagnostic capabilities. Anaother research introduced a Compressive Sensing and Support Vector Machine system for TB classification based on phlegm samples.[7]–[11]

This backdrop outlines the historical narrative of TB, a disease with a millennia-long existence, known by various names such as skin disease, infectious skin disease,or chronic cough disease. Its endemic prevalence in Europe during the 18th and 19th centuries later escalated globally in the early 20th century.

Despite TB persisting as a global public health issue, the advent of effective prevention and treatment programs has significantly reduced the death rate over the last few decades. [2], [12]–[14] Building upon prior research, including the Microscope Modification with Digital Magnification Using a Camera System in 2018, the current study introduces the Microscope Camera to Increase Accuracy of Counted Mycobacterium Tuberculosis on Acid-Fast Bacteria Sputum by Image Processing of Thresholding Method. [15]–[17][18][15], [19], [20]

This innovative tool, designed as a digital microscope, not only addresses existing challenges in TB detection but also introduces a practical solution. The tool facilitates the examination of ZIEHL NELSSEN-stained TB samples, allowing analysts to visualize results on an external monitor, thereby enhancing accuracy in TB bacteria counting. With the potential to optimize microscope camera usage, the tool ensures efficient data capture, saving results on micro memory cards or flash disks. [16], [21], [22]

Operational like a conventional microscope, the tool simplifies the analyst's task by securing the Mycobacterium Tuberculosis sample to the camera microscope. Beyond technological innovation, the research's contributions extend to enhancing the overall efficiency and accuracy of TB diagnosis in real-world healthcare settings, marking a significant stride toward combatting this persistent global health challenge. The contributions of this research are:

a. Improved TB detection system.
b. Increased accuracy in calculating the number of bacteria in TB samples
c. Utilization of the threshold method in image processing in the health laboratory sector.

MATERIALS AND METHOD

This research was conducted at the Surabaya Ministry of Health Polytechnic campus. Making a camera microscope tool for analyzing TB samples can provide great practical benefits in diagnosing and treating TB patients. By using this technology, electromedicine can increase diagnostic accuracy, ensure appropriate treatment, and reduce the risk of cross-infection when analyzing TB samples. Apart from that, this research aims to increase the accuracy of calculating *Mycobacterium Tuberculosis* using a CMOS HD camera microscope, for image processing using the *Python* Tresholding method.[23][24]

A. DATA COLLECTION

The system in this research is used to detect and count the number of bacterial colonies automatically so that it can help laboratory staff identify and count the number of bacterial colonies when observing with a digital microscope.

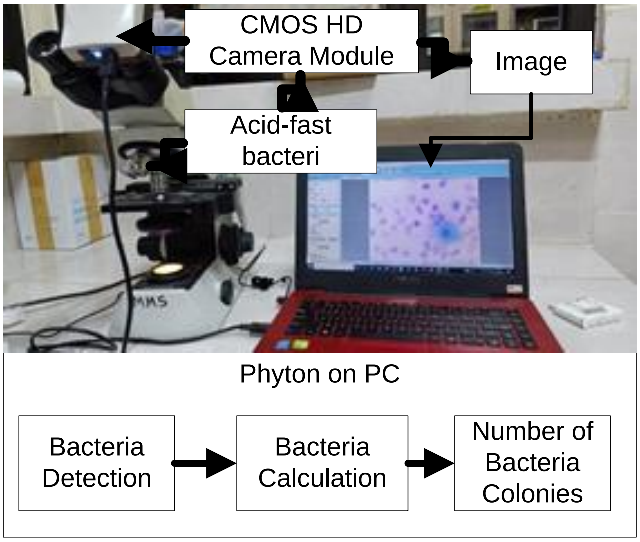

FIGURE 1. **The module design. Consists of a preparation table containing sputum, a microscope equipped with a CMOS HD sensor module, and a laptop. The ACID-FAST BACTERIA sample is placed on a microscope. Images are captured by the camera module, then displayed on the PC, and processed using the application**

The data collection system is as in Figure 1, first, the preparation containing the Acid-Fast Bacteria sample as input is placed on the microscope, and then the image seen in the ocular lens will be captured by the HD CMOS camera module to produce a sample. picture. The image will then be displayed on the PC. After appearing on the PC, the image will be processed using the Matlab application to detect the presence of images of TB bacteria in the image. If bacteria have been detected, the process of counting the number of bacteria can be carried out. In the bacterial counting process, the number of bacterial colonies present will be counted. After the entire process is carried out, the system can produce output in the form of the number of bacterial colonies in a sample. After collecting data in the form of images from Acid-Fast Bacteria samples using a microscope equipped with a CMOS HD Camera module, then the

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microscope is connected to a laptop using a USB cable.Furthermore, the samples taken cover many categories, namely from Negative to 3+.

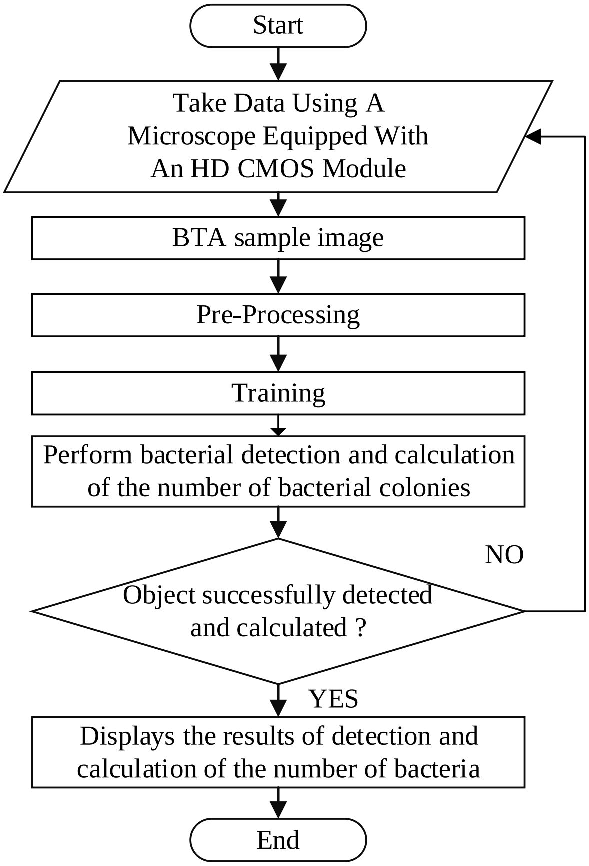

FIGURE 2. System Flow Diagram, After the Acid-Fast Bacteria sample is taken, preprocessing is carried out on the image. To make this system more comprehensive, training was carried out on the application system. Once the data is deemed sufficient, data collection is carried out.

The workflow of this research is shown in Figure 2. After that, the more samples, the better. In this research, 4 samples were used for each category which will be used in the training and pre-processing process. Then, researchers will run the system used to count bacteria from the sample. If the object in the sample can be detected, the researcher can display the detection results and count the number of bacteria from each sample

.In planning object detection and counting, data from the camera's digital microscope is required as input. After researchers enter camera data, the next step is to identify and count objects using image processing. The method used is to improve image quality with one of the image enhancement techniques, namely a high pass filter so that images of bacterial colonies are easier to detect. After that, the next step is to change the RGB image value to an HSV image to get the saturation image value. Then the saturated image is converted into a binary image to perform morphological operations and produce an image according to requirements. After carrying out the morphology procedure, the number of bacterial colonies was counted using an object to determine the number of bacterial colonies in the image.

B. DATA ANALYSIS

The average is the value or outcome of dividing the quantity of data collected or measured by the number of measures taken or collected as in equation 1

$$X = \frac{X1 + X2 + \cdots + Xn}{n} \quad (1)$$

The error is the difference between the intended price's average and measured value. The formula for percentage error is as in equation 2

$$\%Eror = \frac{Xn - \bar{X}}{\bar{X}} x\ 100\% \quad (2)$$

The percentage of system success is used to determine whether the system is what is desired or not. The percentage of system success also aims to evaluate the algorithm and methods used. The formula used in calculating the presentation of system success is where A is the expected value, and B is the value from the test results as in equation 3

$$success\ percentage = \frac{A - |A - B|}{A} \times 100\% \quad (3)$$

II. RESULT

The data required in this research is image data taken using a microscope camera with an objective lens. At least 5 visual field images will be taken for each sample to be used as data. In this study, to determine the severity of Tuberculosis depicted by a sample, researchers need to count how many bacteria are found in one field of view and/or in one sample. Determination of severity levels follows the guidelines issued by the Ministry of Health / International Union Against Tuberculosis and Lung Disease (IUATLD).[25]–[27] The following is a table that will illustrate at Table 1.

TABLE 1
Diagnosis Guidance IUATLD

Diagnosis	Bacteria Found
Negative	0/100 Visual Field
Dubious (*scant*)	1-9/100 Visual Field
1+	10-99/100 Visual Field
2+	1-10/Visual Field (min. 50 Visual Field)
3+	>10/Visual Field (min. 20 Visual Field)

Images of Acid-Fast Bacteria samples are captured with a microscope camera. Detection Threshold which functions to improve the quality of the resulting image. If a camera microscope image is uploaded to the system above, it will immediately read how many bacteria are in one field of view, which then indicates the type or severity of Tuberculosis.

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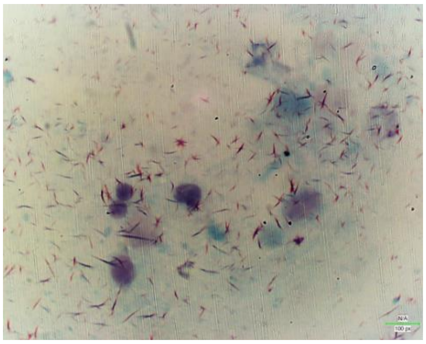

Figure 3. Sample Image Before Calculation

The system will read and detect bacterial patterns based on matching the shape of previous training results. For example, if training is carried out on the shape of rice, whether the rice is large or small, as long as it is shaped like rice it will be detected in the green box. This is also influenced by the proximity of the object shape to the training data. The closer the shape, the greater the resulting value, which means the object will be read or detected as a training object. The following is an example of an image of one field of view before measurements are made using the system in Figure 3 and after measurements are made in Figure 4.

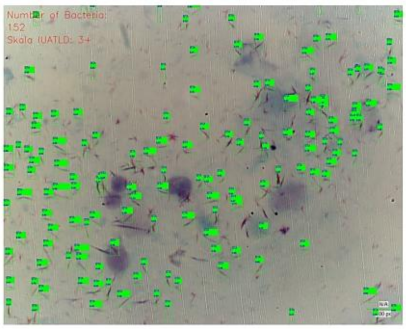

Figure 4. Sample Image After Calculation

Several findings were acid-fast bacteria when researchers tested the detecting technology and calculated Tuberculosis bacterial colonies. There are some images with a high success rate and there are some images with a low success rate. The las result of this research can see at table 2.

TABLE 2
The result of Sample Data

number of samples	26
the smallest value calculated	23
the highest value calculated	178
smallest accuracy	62.92%
highest accuracy	97.50%
average accuracy	85.30%

Testing was carried out on 26 images and the average percentage of success of the detection system and the counting of the number of bacteria was 85.30%.

III. DISCUSSION

Researchers have created a microscope module using a CMOS-HD microscope camera to detect and count the number of bacteria in the sputum of TB sufferers. Researchers used the Python programming language to design a system for detecting and counting bacteria in a single field of view. To get the accuracy of the results, the module readings are compared with manual readings. From the results data shown in Table 2, it can be seen that the samples that have been measured are 26 samples. Of these samples, the data with the smallest value was 23 and the lowest value was 178 bacteria in one field of view. In samples that have a smaller number of bacteria, the system appears to be more accurate. However, this statement cannot be stated as accurate because it does not apply linearly. The difference in the level of bacteria calculations between manual calculations and system calculations is caused not only by the system's inability to detect several bacteria in one field of view but could also be due to errors during manual calculations. This incident occurred because there was a possibility of bacteria stacking 2 or 3, so there were errors in detection and manual calculation.

Another thing that has an influence is the procedure for processing the Acid-Fast Bacteria data itself, the procedure for taking images with a microscope camera, and the lighting used. When processing Acid-Fast Bacteria, good bacteria to observe are those that are 20 - 24 hours old. More than 24 hours the shape will appear less clear when observed. [1][27]

It can be seen in the table above that the biggest difference between using the module and manual calculations is 37.08% or has an accuracy value of 62.92%. This incident was a sample that had a bacterial count of 89 according to the module and 56 according to manual calculation. The smallest difference between using the module and manual calculations is 2.5% or has an accuracy value of 97.5%. This incident was a sample that had a bacterial count of 40 according to the module and 39 according to manual calculations.

The weakness of this research is the use of manual calculations as a comparison tool to state its accuracy. This means that the standards used still have the possibility of human error. On the other hand, the results of this research have not yet reached the point of detecting samples that are positive and negative whether the samples contain TB bacteria or not. This is due to a lack of information about the samples used.

However, looking at the results of experiments and data collection compared with other studies, this study can show more details about the presence of TB bacteria.[28]

It would be very good if the system proposed by researchers could be used as a starting point for developing bacterial detection. The research developed will help health workers detect and count bacteria in tuberculosis sufferers' sputum. Apart from that, the accuracy between manual reading and reading by the system can be considered quite good.

IV. CONCLUSION

This study introduces an innovative tool, the "Microscope Camera for Enhanced Mycobacterium Tuberculosis Counting in Acid-Fast Bacteria Sputum through Image Processing with Thresholding Method," designed to revolutionize the examination of tuberculosis (TB) samples.

The finding in this research is a tool with a system that can read and has good sensitivity. This is shown by the average accuracy of measurements and readings of this equipment in detecting the number of TB bacteria which has a fairly high value, around 85%. This is a good start for developing a detection system for these bacteria.

However, there is a difference in the bacterial calculation value between manual calculations and calculations carried out by the system, it is due to the system that does not detect some bacteria in one field of view. Training with lots of samples will make this system better. In future research, it would be better to use machine learning that is better at image processing and recognizing bacterial shapes.[29], [30]

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