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Detection of Mycobacterium Tuberculosis in Sputum Sample Images Using Fluorochrome Staining Using the Object Boundaries Method

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Abstract

Mycobacterium tuberculosis is the primary infectious illness that causes tuberculosis. In terms of the total number of TB cases worldwide in 2019, Indonesia comes in third. Experiencing a two-week or longer coughing fit is indicative of tuberculosis symptoms. Treatment for tuberculosis is divided into two stages: the continuation phase, which lasts four or seven months, and the intense phase, which lasts two to three months.

The Mantoux or tuberculin test, the Interferon Gamma Release Assays (IGRA), radiographic examination (X-ray), and microscopic analysis are some of the techniques that can be used to check for tuberculosis. In Indonesia, the Ziehl-Neelsen (ZN) staining method is preferred above other stains for microscopic investigation. This is due to the fact that acid-fast bacteria (AFB) can be easily identified using the simple, affordable, and highly specific Ziehl-Neelsen (ZN) staining procedure. Nonetheless, its sensitivity is comparatively poor. Fluorochrome (auroamine-rhodamine) staining, on the other hand, exhibits higher specificity and sensitivity than the ZN method. Unfortunately, this approach is challenging and expensive to use in Indonesia. Aside from this issue, the existing method of counting tuberculosis germs involves manual counting, which is incorrect and time-consuming. in order to affect the physician's diagnosis and prescription for medicine.

Thus, the purpose of this research was to develop an object boundaries method-based automatic system that can quickly and accurately identify and count bacteria without the need for human assistance.

Keywords: *Mycobacterium tuberculosis, microscopic analysis, Ziehl-Neelsen (ZN), fluorochrome (Auroamine-rhodamine), and boundaries.*

1. Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis, is a direct infectious illness that primarily affects the lungs but can also affect other body areas [1]. These bacteria are rod-shaped, either straight or slightly bent, and lack capsules. The dimensions of Mycobacterium tuberculosis are 1-4 μm in length and 0.3-0.6 μm in breadth. When a person with pulmonary tuberculosis coughs, sneezes, or speaks, microscopic droplets known as droplet nuclei can be released into the air, which can then transfer the germs from one person to another [2].

Meanwhile, India has the highest number of tuberculosis patients (27%), followed by China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%), and South Africa (3%) [3]. These statistics are gathered by the World Health Organization (WHO). In terms of the total number of TB cases worldwide in 2019, Indonesia comes in third place [4].

Coughing up phlegm for two weeks or more is one of the symptoms of tuberculosis. In addition, they may have bloody coughing fits, shortness of breath, weakness, decreased appetite, weight loss, exhaustion, nocturnal sweats without moving, and fever lasting more than a month [5].

There are numerous ways to check for TB, including microscopic examination, the Mantoux test, sometimes known as the tuberculin test, and radiological examination (X-ray). Figure 1 shows the process for finding TB bacteria.

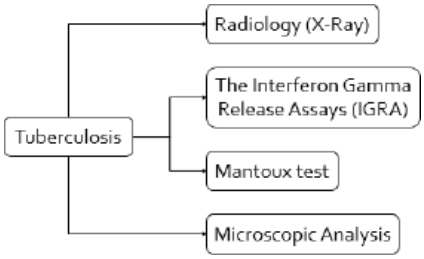

Fig. 1 How to Detect Tuberculosis Bacteria

Because tuberculosis germs affect the lungs in the chest, radiographic examination (X-ray) is the initial method of tuberculosis detection [6]. The second approach involves drawing blood in order to do a TB examination known as the Interferon Gamma Release Assays (IGRA). Not active TB, but latent TB infection is the diagnosis made with this test. [7]. The third technique is the Mantoux test, also known as the tuberculin test, which involves giving a patient who is thought to have tuberculosis an injection of tuberculin into their arm.

Finding tuberculosis using microscopic investigation is the fourth method. In order to do microscopic analysis, a sample of sputum is prepared, dyed, and then reconstituted such that the tuberculosis bacteria are visible and differentiated from phlegm. The amount of tuberculosis bacteria present in the patient's sputum is ascertained by using a microscope to examine the stained preparation after it has been stained [6]. The acid-fast rod bacteria Mycobacterium tuberculosis can be found using three different techniques: fluorochrome staining, Tan Thiam Hok staining, and Ziehl-Neelsen staining [8].

The gold standard for early TB identification remains microscopic inspection with the Ziehl-Neelsen (ZN) staining method, which is simple, inexpensive, and highly specific in recognizing acid-fast bacteria (AFB) in sputum [10]. Studies reveal that the sensitivity is actually quite low (20–60%). In addition to the ZN approach, fluorochrome (Auroamine-rhodamine) staining under a fluorescent microscope has been utilized for microscopic investigation in a number of nations. Although prior research has demonstrated that fluorochrome staining is more sensitive and specific than the ZN approach, broad laboratory use of this technique has not been suggested in Indonesia. due to the fact that this approach is challenging and expensive [9].

Counting TB bacteria still requires physical labor at this time. which is inaccurate and takes a long time. Since the concentration of the lab assistant who counts the microorganisms actually determines this. This fact demonstrates the urgent need for an automated system that can quickly and accurately detect and count germs without the need for human assistance [6].

Therefore, the purpose of this research is to identify Mycobacterium tuberculosis bacteria in sputum samples by staining them with fluorochrome (Auroamine-rhodamine) and utilizing the object boundaries approach to compute the quantity of bacteria automatically from the photos of the sputum.

2. Literature reviews

The current research is related to a number of earlier investigations that have been conducted.

A member of the acid-fast bacillus (BTA) group of bacteria, mycobacterium tuberculosis infection typically targets the lungs. Usually, BTA bacterium testing under a microscope is used to diagnose tuberculosis. The Zheil Neelsen microscopic staining technique is typically used in microscopic tests conducted in Indonesia.

According to research (Betty Suryawati, 2018), the fluorochrome (Auroamine-rhodamine) staining method is one staining methodology approach that has higher sensitivity than the Zheil Neelsen method. However, this coloring technique is still carried out traditionally and is not frequently employed, even in Indonesia[9].

Then, based on the primary clinical symptoms that the patient had, Amrin (2019) studied the diagnosis of tuberculosis using the C4.5 algorithm to determine the likelihood of developing tuberculosis. To date, a computerized stage has been used to diagnose tuberculosis in order to facilitate patient diagnosis[11].

The development of research on tuberculosis bacteria was done (Aeri Rachmad, 2020). The research was done using a conventional microscope and digital image processing. The researchers improved phlegm images by using a median filter to remove noise, then cropped and resized the image to equalize the pixel size. The Support Vector Machine (SVM) technique is used to perform the classification. In this instance, scientists present an alternate Zheil Neelsen (ZN) staining technique for categorizing tuberculosis germs[12].

In addition, study was done by Ayu Febriani (2022) using BTA microscopic data using sputum sample data using the Zheil Neelsen microscopic staining method to ascertain the distribution of age and gender features of tuberculosis patients[13].

We are aware that it takes a long time and expensive to diagnose each person with this bacterial illness. The process of collecting phlegm from the lungs is called a biopsy. To detect tuberculosis, this fluid is chemically stained with Ziehl-Neelsen and inspected under a microscope. Therefore, Bob Subhan Riza (2022) carried out research using computer-assisted image processing to help detect germs accurately and fast by developing an application system. Where to create techniques for segmentation methods[14].
The Zheil Neelsen staining method is more commonly utilized in the diagnosis of tuberculosis, despite the fact that the fluorochrome (Auroamine-Rhodamine) staining approach has far higher sensitivity, as shown by a number of prior investigations. This is due to the higher cost associated with fluorochrome staining. As a result, researchers are working to develop a differentiator or alternative for diagnosing tuberculosis bacteria. This differentiator or alternative uses image data obtained from the fluorochrome staining method, which is then used for image segmentation and edge detection (boundaries) to find edges in digital images so that the number of objects in the image can be determined directly. Thus, it is envisaged that in the future, it will be able to recognize tuberculosis bacteria and determine how many are present in the image.

3. Research Methods

Beginning with the design of the study topic, which is how to use the boundaries approach to detect Mycobacterium tuberculosis bacteria in sputum samples using fluorochrome staining, numerous stages of the investigation were carried out, as Figure 2 illustrates.

Fig. 2 Flow diagram of research stages

3.1 Research data

Publicly available digital photos of sputum sample preparations taken under a microscope are used in this study. Sputum samples from patients suspected of having tuberculosis bacteria are placed under the microscope and imaged. The samples are then stained with fluorochrome (Auramine-Rhodamine). Ten open datasets provided the image data.

3.2 Program design

The design of the program was done in two steps. Using the Matlab application's GUI interface, create a program design as the initial step. The created program design is shown in Figure 3. Entering the source code in the meantime allows the program to run at the second step. Figure 4 shows an example of the source code that was used.


Fig. 3 Program appearance design

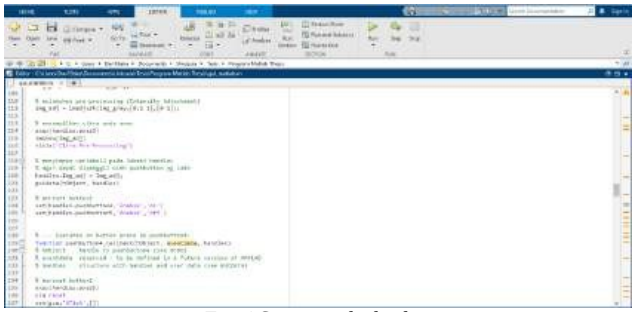
Fig. 4 Source code display

3.3 Data Testing process

As indicated in Table 1 below, testing steps are completed in multiple stages.

Table 1: Research testing steps

a.	*Data input*	:	10 image data points were tested one by one.
b.	*Rgb to grayscale*	:	Transferring color space from RGB to grayscale
c.	*Pre processing*	:	Sharpen the grayscale image with intensity adjustments.
d.	*Image segmentation*	:	Perform thresholding using two methods, namely manual and auto threshold, to separate objects from the background.
e.	*Performs object boundaries*	:	After being able to separate the object from the background using the bwboundaries method
f.	*Evaluation of results*	:	Calculate the number of objects and carry out an analysis of the number of objects.

3.4 Data analysis

Comparing the raw picture data with the processed image data with the naked eye allows for analysis of the image findings. Can all of the objects in the original data be identified and accurately counted. In addition, the accuracy calculation requires the use of the following equation:

$$Accuracy = \left(\frac{Amount\ of\ data\ after\ processing}{Amount\ of\ original\ data\ calculated\ manually}\right) \times 100\% \quad (1)$$

4. Results and Discussion

Image data from microscopic examination with a fluorescent microscope with fluorochrome (Auroamine-Rhodamine) staining, which is obtained publicly via the internet, has an extension format in the form of *PNG (Portable Network Graphics) and has a scale or resolution of 1920 x 1080 pixels. One of the test datasets utilized in this study is shown in Figure 5.

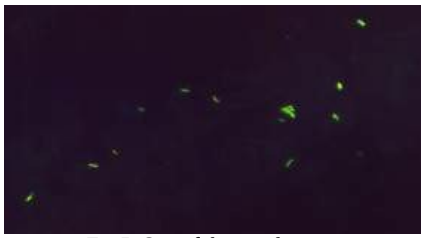

Fig 5. One of the test datasets

The first step in executing a program made in the Matlab application is to input the image that will be used for object detection. The original image in RGB color space will then be displayed in the text edit column along with the file name and extension.

We will convert the RGB color space to grayscale in the following step. This is necessary since the segmentation technique we employ in this study is still predicated on color intensity grouping (degree of gray). And the application will show the outcomes.

Subsequently, the intensity adjustment method is used as part of a pre-processing step to enhance the grayscale image's quality, which will help segmentation yield better results.

Subsequently, a segmentation procedure is executed by merging analogous pixels according to their color intensity (gray degree). In this instance, thresholding automatically and manually are the two alternatives available. By computing the image histogram and estimating the threshold value based on the histogram's features, the threshold is automatically determined. In the meanwhile, the threshold is set manually, that is, by giving a value that corresponds to our desired threshold. You will then be left with a segmentation image where the object is white and the backdrop is black.

Performing the object boundary detection method, which involves converting the image to a binary image to facilitate object detection, is the last step. In this study, a Matlab programming tool called bwboundaries was used to determine object borders in binary pictures. The pixel coordinates that define the boundaries of the object are returned by this function. To locate and characterize an item's edges, edge tracking algorithms monitor variations in pixel values or changes in intensity between the object and the backdrop. Applying the bwboundaries function to a binary picture causes it to begin

looking for the contour's beginning point by identifying unvisited pixels with value 1. Next, it moves on to nearby, unvisited pixels that are likewise contiguous and have a value of 1. Until the contour returns to its beginning, this process is continued until a closed line encloses the object. Once you have the ability to recognize things, you may use the object boundaries to automatically calculate objects. Figure 6 displays the outcomes of the developed and executed program.

Fig. 6 The results of programs run with a GUI

The findings of the analysis are compared between the initial image results prior to and following object edge detection image processing. An RSUD employee with expertise in laboratory analysis manually determined the number of objects from the raw data using only their eyes. as indicated by H. Badaruddin Kasim Kab. Tabalong in the tables below:

Table 2. Image comparison results using manual threshold values

Data	*Manual Threshold Value*	*Number of Bacteria*		*Accuracy*
		Manual	*Computing*	
001	0,6 × 255 = 153	12	11	91,67
002		22	25	88
003		1	1	100
004		15	13	86,67
005		9	9	100
006		43	68	63,24
007		91	231	39,39
008		41	29	70,73
009		31	43	72,09
010		109	108	99,08
	Average accuracy			81,09

Table 3. Image comparison results using the otsu threshold value (automatic)

Data	*Otsu Threshold Value*	*Number of Bacteria*		*Accuracy*
		Manual	*Computing*	
001	Automatic	12	11	91,67
002		22	25	88
003		1	980	0,1
004		15	12	80
005		9	9	100
006		43	102	42,16
007		91	1156	7,87
008		41	53	77,36
009		31	419	7,40
010		109	100	91,74
	Average accuracy			58,63

Table 4. Image comparison results using manual threshold values based on object values

Data	*Manual Threshold Value*	*Number of Bacteria*		*Accuracy*
		Manual	*Computing*	
001	0,3647 × 255 = 93	12	11	91,67
002	0,99 × 255 = 252	22	23	95,65
003	0,7 × 255 = 179	1	1	100
004	0,12 × 255 = 31	15	15	100
005	0,4686 × 255 = 119	9	9	100
006	0,8 × 255 = 204	43	43	100

007	0,954 × 255 = 243	91	90	98,9
008	0,535 × 255 = 136	41	39	95,12
009	0,742 × 255 = 189	31	29	93,55
010	0,536 × 255 = 137	109	109	100
	Average accuracy			97,49

From the results of comparing the original image with the image that has been processed, this method with object boundaries has a drawback in that the results still depend on the threshold value to be able to determine between the object and the background. It can be shown from the table above that the level of accuracy obtained depends on the threshold value. that is given. Because the characteristics of image data are different, the threshold value given according to the object value, as in Table 4, is better than automatically providing a threshold value, as in Table 3, and the threshold value, which is equated, as in Table 2.

Apart from that, for single objects this method can still be used, but if there are overlapping objects, he can still recognize the object, but only by counting one object. So this affects the calculation value of the number of objects detected. However, this object boundary method can be used for detecting and calculating the number of objects.

5. Conclusions

Based on the results and discussion of research on "Detection of Mycobacterium Tuberculosis in Sputum Samples with Fluorochrome Staining Using the Object Boundaries Method," it can be concluded that using this method can detect objects based on color intensity (degree of gray) because grouping is according to color.

The advantage of this research is that it produces an image that can detect and count the number of objects automatically, so it can help improve the quality of reading sputum preparations.

Meanwhile, the weakness of this research is that the object boundary method is still based on color intensity (degree of gray), resulting in having to be able to determine a threshold value that is appropriate to the background and object you want to observe. If the object values are close together, it will be difficult to compare the object and background. Apart from that, with this method, the bacteria that accumulate can still be read by one object.

So further research is needed to add other methods that can help detect objects within object boundaries to get better results.

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