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Identification of Bacteria using Digital Image Processing

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Abstract: **- In the field of microbiology, there is no direct method to find out the microorganism and their species of bacteria. Microscopic sample analysis is a common manual technique employed for bacterial detection and identification. After the analysis process, more than 25 biochemical tests are used to determine the bacteria and its species. These tests include gram reaction, motility, shape, spore, biochemical tests and so on. These tests will be time-consuming and is subjected to poor specificity. Hence it requires highly trained personnel for testing. In order to overcome the existing manual problems, digital image processing techniques can be used. The main aim of the proposed work is to use image-processing techniques to identify the bacteria from images. After staining the microorganisms with appropriate dyes, the images of microorganisms were captured using a digital camera attached with an electron microscope. To identify the species, image processing techniques such as preprocessing, segmentation, morphological operations are used.**

Keywords: **Bacteria, microorganism, segmentation, morphological operations.**

I. INTRODUCTION

Bacteria are a very small organism and they cannot be visible by human eyes. To visualize them, electron microscope is used. The most important task in microbiology is to identify the bacteria from the culture plate. To prepare a culture plate, the separation of a dilute mixed population of microorganisms is done by using the spread plate technique. Thus, individual colonies are isolated. Aseptically transfer the loopful of mixed culture on the Nutrient Agar medium. It is spread uniformly with the help of L-shaped spreader on the surface of medium plates. After spreading, it is incubated at 37°C for 24–48 h. After incubation, single colonies will appear on the Nutrient Agar media plates. For further identification staining techniques are applied. A smear of mixed culture of bacteria is deposited on a glass slide and thoroughly air-dried [1]. In general, up to 25 biochemical tests are used to identify the bacteria and its species. These tests will be time-consuming and is subjected to poor specificity. Thus it requires highly trained personnel for testing. To overcome the existing manual problems, digital image processing techniques can be used. The cell structure of a coccus bacterium consists of capsule, nucleoid and ribosome as shown in Figure.1 [2]. The size of a bacterium (measured in micrometers (µm)) is identified by its capsule, which is the outer layer of each bacterium.

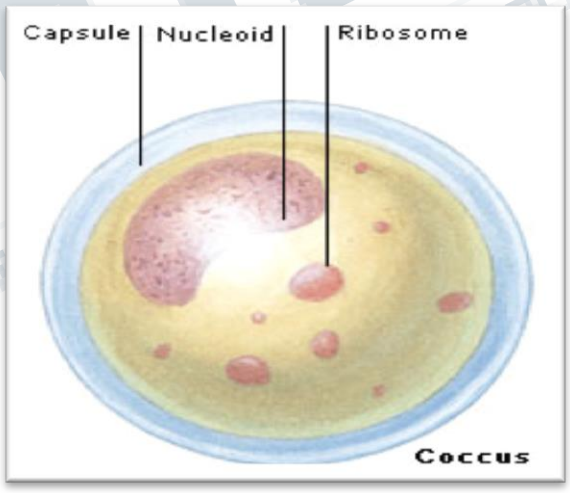

Figure.1.cell structure of a coccus bacterium

Image processing is a technique to perform some manipulations or operations in an image, in order to get an improved image or to extract some useful information from it. At present, image processing is a rapidly growing technology. It forms core research area within engineering and computer science disciplines too [4].

In general, there are two types of image processing methods namely,

- Analog and
- Digital image processing.

Analog image processing can be used for the hard copies like printouts and photographs. Image analysts use a range of essentials of interpretation while using these visual

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techniques. Digital image processing techniques help to perform manipulation of the digital images by using computers.

Image processing includes the following basic steps:

- Importing the image via image acquisition tools.
- Analyzing and manipulating the image.
- Output in which result can be altered image or report that is based on image analysis.

The proposed work is used to identify the spherical shaped (Coccus) bacteria using image processing. After staining the microorganisms (bacteria) with appropriate dyes, the images of microorganisms were captured using a digital camera attached to an electron microscope. To identify the bacteria, image processing techniques such as preprocessing, segmentation, morphological operations are used.

II.BACKGROUND

Bacteria are often maligned as the causes of human and animal disease. The first step is to analyze the bacteria, they were grown in a culture plate like agar. Then, they are stained with appropriate dyes and visualized in the electronic microscope. The images of bacteria was captured through a digital camera attached to an electron microscope[3]. There are three types of bacteria based on their shape (sphere, rod or spiral). The spherically shaped bacteria is known as coccus (cocci). They are usually round, they also may be oval, elongated or indented on one side[2]. Every bacterial cells are clustered together so that, from the original input image a single cocci cell structure is segmented (Figure.2). Then the diameter of the cell is calculated and the result will matched with the database of the bacteria according to its size to specify the bacteria name.

Figure.2.Sample Image of Cocci

III.PROPOSED SYSTEM

There are several ways to find out the bacteria species manually such as (Oxidase, Urease etc.) and this is a complex and continuous task. The main objective of this work is to provide an easiest and efficient way to find out the bacteria in the field of microbiology and this is advantageous in cost and time. This work takes input as an electron microscopic image and performs some image processing techniques. Then finally it will identify the bacteria by its diameter size. The input is the images captured by a digital camera attached to an electron microscope and the result will be based on the feature extraction of the input image. Here, the size of the cell structure will be find out through feature extraction technique.

Table1. Tests and Time required in manual process

S.No	**Test Name**	**Required Time**
1	Gram reaction	57 seconds
2	Motility	2 days
3	Shape	3 days
4	Spore	72 hours
5	Catalase	18 to 24 hours
6	Oxidase	60 to 90 seconds
7	Indole production	24 to 48 hours
8	Methyl red	10 seconds
9	Voges-Prosakauer	15 seconds
10	Citrate	18 to 48 hours
11	Urease	6 days
12	Starch hydrolysis	24 hours
13	Nitrate reduction	12 hours
14	Glucose utilization	19 hours
15	Lactose utilization	8 hours
16	Gelatin	15-30 minutes
17	Hydrogen Sulfide Production	24 hours
18	TSI	4 hours
19	CAMP Test	20 hours
20	DNase Test	18-24 hours
21	Hippurate Test	30 minutes
22	Pottasium Hydroxide Test	45 minutes
23	Hippurate Test	2 hours and 30 minutes
24	Coagulase Test	24 hours10 seconds
25	Lecithinase Test	24 to 48 hours

In microbiology field to identify a bacterium, a personnel should go through various tests which are time consumed as mentioned in the above table (Table.1). The proposed work will be used to avoid these tests for identification.

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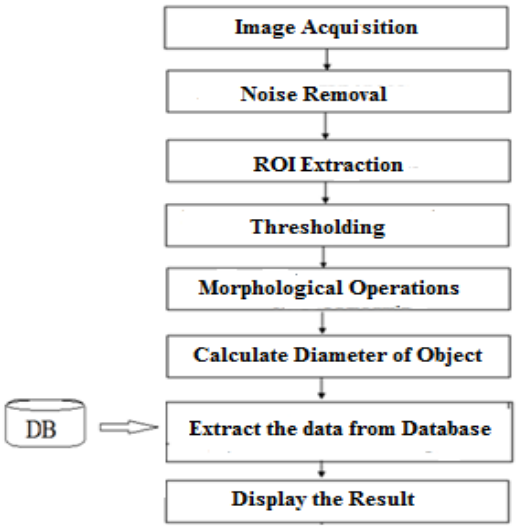
A. Architecture of the work

Image acquisition is the process of capturing image with the help of a digital camera attached with an electron microscope. After this process, the capturing image will have some noises for instance the image may contain some unwanted artifacts which are imperceptible by human eyes. These kind of noise will be removed by applying a particular noise to the original image and this noisy image will be filtered using filtering techniques. Later then, a restored image will be obtained. From the restored image, a particular portion of a cell structure should be choosen. For this purpose a segmentation technique should be used. Segmentation is the process of partitioning an image into multiple regions/parts. Through this the image will be easily analyzed. Here, Region Of Interest (ROI) technique is used for segmenting the image. After the segmentation process, the input image is converted into binary image which consists of 0's and 1's (0 represents black and white represents white), by a threshold method. To remove small blobs in the image, morphological techniques will be used. various features are extracted from the segmented region. In this work, the size of the segmented cell structure will be identified, which is known as feature extraction. After that, the result (size) will be compared with the database and the bacterium which satisfies the criteria (size) will be displayed as by its name.



In this work, input images are captured through a digital camera attached with the electron microscope. These input images are the training dataset for this work as shown in Figure.3.

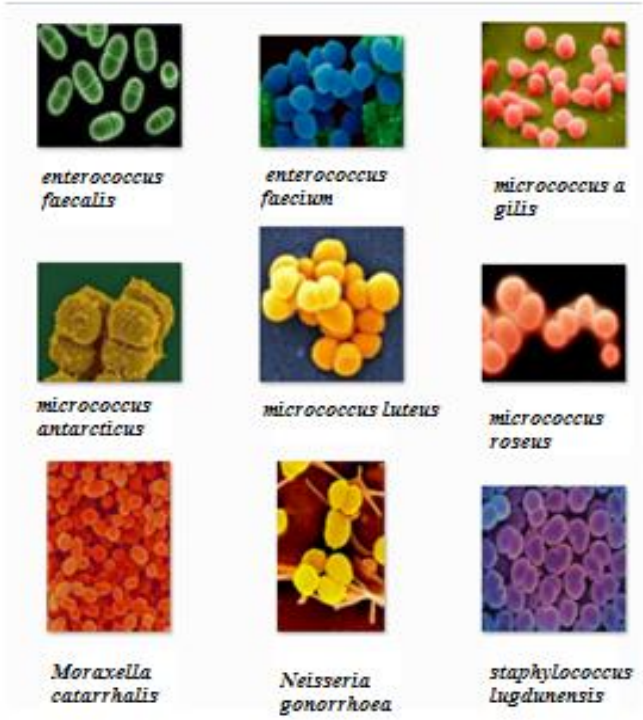


Figure.3.Dataset

These images are then preprocessed to remove noise from the image. When a noise removal technique is applied, the existing noise (during the capturing time) will also be removed. Here, the median filter technique will be applied with 5*5 mask to improve the quality of the image as shown in Figure.4.

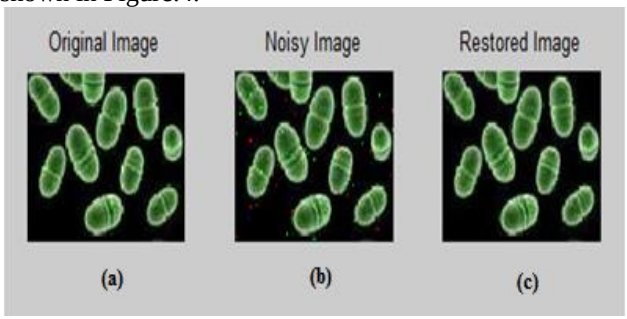


Figure.4.Preprocessed image

(a) Original image captured from an electron microscope (b) Image with noise (c) Restored image without any noise

Using the ROI Segmentation technique a particular cell structure is segmented to identify the diameter of a bacterium. This will be represented in Figure.5.

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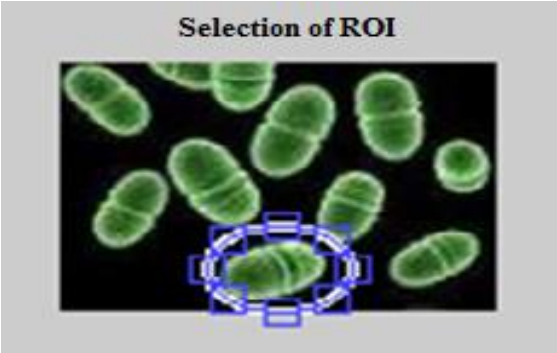

Figure.5.Selection of ROI

Then, each plane (RGB plane) will be extracted from the selected region image. From these images blue plane image is evident in an exact format as shown in Figure.6.

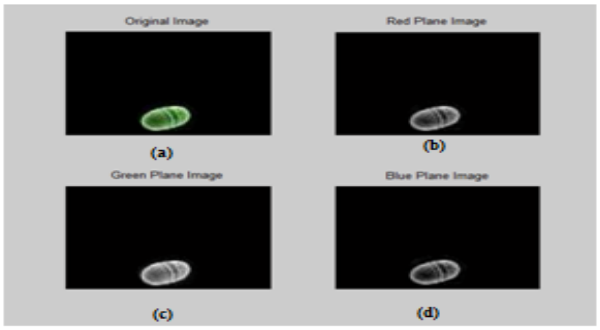

Figure.6.Extracted Image
(a) Original image (After applying ROI)
(b) Red plane image (c) Green plane image
(d) Blue plane image

After this extraction, a threshold image will be obtained. Using the fill, open and clear border morphological operations the unwanted structures of the blue plane will be removed as shown in Figure.7. Figure.7.d will contain the image which is to be used in feature extraction, to identify the size of the region selected region segmented in Region Of Interest (ROI) technique.

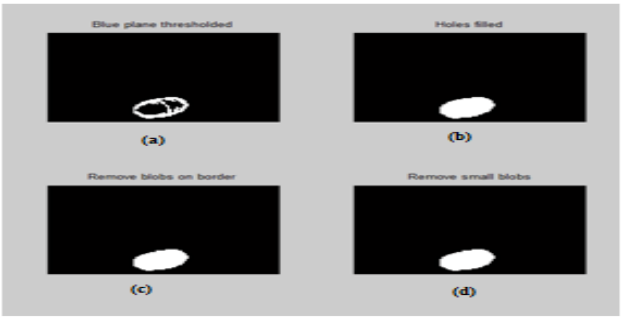

Figure.7. Resultant Image

(a) Threshold image of blue plane (b) Holes filled in the threshold image (c) Remove blobs on border of the image (d) Remove small blobs of the image

Then, the diameter of the particular cell structure (spherical) will be calculated.

IV.RESULT

The diameter is compared with the database which contains bacteria name and their size. After that, the result will be fetched from the database if it matches with the resultant size. Finally, the name of the bacterium will be known in an easier way without much manual work. This work identifies coccus bacteria by its size represented in a database. Normally, these bacteria are in the range of size from 0.5-2µm approximately as shown in the Table.2. Using this work, the much manual work has been reduced to identify the bacteria and so the time and cost (include the cost of chemicals, a trained personnel etc.,) used for the existing manual process will be reduced. The medical and the microbiology field are correlated together in this work.

Table.2.Bacteria and their Size

S.No	Name of the bacterium	Size of the bacterium (µm)
1	*Cryptococcus neoforman*	0.634
2	*Enterococcus faecalis*	0.792
3	*Enterococcus faecium*	0.632
4	*Micrococcus agilis*	0.923
5	*Micrococcus antarcticus*	0.597
6	*Micrococcus luteus*	0.543
7	*Micrococcus roseus*	0.529
8	*Moraxella catarrhalis*	1.609
9	*Neisseria gonorrhoea*	0.854
10	*Neisseria gonorrhoeae*	0.965
11	*Neisseria meningitis*	0.578
12	*Staphylococcus epidermidis*	0.762
13	*Staphylococcus aureus*	0.961
14	*Staphylococcus capitis*	0.564
15	*Staphylococcus haemolyticus*	0.734
16	*Staphylococcus hominis*	0.828
17	*Staphylococcus lugdunensis*	0.632
18	*Staphylococcus saccharolyticus*	0.975
19	*Staphylococcus saprophyticus*	0.543
20	*Staphylococcus warneri*	0.872
21	*Staphylococcus xylosus*	0.643
22	*Streptococcus agalactiae*	0.853
23	*Streptococcus lactis*	1.921
24	*Streptococcus mutans*	0.756

35	*Streptococcus pneumoniae*	0.831
26	*Streptococcus pyogenes*	0.749
27	*Streptococcus salivarious*	0.925
28	*Streptococcus sanguis*	0.854
29	*Streptococcus thermophilus*	0.798

V.CONCLUSION

In medical domain, the diseases are predicted by analyzing the samples collected from the patients. The samples contain microorganisms which are harmful to the human. To detect the particular microorganism, the medical domain uses the microbiology biochemical tests. Because of this work, the microbiology field should be enriched. This work is designed based on Digital Image Processing (DIP) is used to identify the coccus bacteria in an easier and efficient manner with less manual work. In future, this work will be extended for the other two kinds of bacteria (rod and spiral) to find out their species name based on the same parenthesis (size).

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