DETECTION OF GRAM POSITIVE AND GRAM NEGATIVE ORGANISMS IN SPUTUM QUALITY TESTING

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JUNE 2012

SUPERVISOR DECLARATION

"I hereby acknowledge that the scope and quality of this thesis is qualified for the award of the Bachelor of Electrical Engineering (Electronics)"

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Date : 21 JUNE 2012

DEDICATION

To my beloved mother and father:
Mrs Azamah bte Ismail and Mr Razi bin Muhammad
My siblings:
Mohd Fazli, Mohd Faizal, Mohd Fahmi Aizzat, Mohd Farhan Azrai
and all my best friends.

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Besides, I would like to take this opportunity to express my sincerely gratitude and appreciation to my family for their supportive, understanding, encouragement and their prayer for me through my university years until the end of degree learning.

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ABSTRACT

Sputum is a mucus that cough up from the lower airways which is a normal body fluid. The sputum consists of squamous epithelial cell, pus cells and bacteria. For this project, it only focus on bacteria organisms which is consists of two type of bacteria such as gram positive and gram negative bacteria. The purpose of this project is to detect and count the quantity for gram positive and gram negative bacteria. At the same time, the grading for both bacteria is identified based on grading criteria. Currently, gram positive and gram negative bacteria is detected and counted manually by human and the grading is identified. Since human might do some mistake in detection and summation for both bacteria and take a long time in doing this process, developing an automatic vision system is necessary to obtain more accurate results and time saving. This automatic vision system developed based on image processing technique which is involve of software simulation only by using MATLAB simulation. In developing this project, some techniques of the image processing is applied into MATLAB simulation such as image analysis, image segmentation, image enhancement, morphological process and other. Then, the results for summation and grading are displayed on MATLAB Graphical user Interface (GUI). Last but not least, the result for grading obtained give similar value compare to validation test from HUSM.

ABSTRAK

Kahak merupakan lendir yang keluar daripada saluran udara yang lebih rendah ketika batuk dan ia merupakan bendalir badan yang normal. Kahak terdiri daripada *squamous epithelial cell, pus cell* dan bakteria. Untuk projek ini, ia hanya memberi tumpuan kepada organisma bakteria yang terdiri daripada dua jenis bakteria iaitu gram positif dan gram negatif. Tujuan projek ini adalah untuk mengesan dan mengira kuantiti untuk gram positif dan gram negatif. Pada masa yang sama, penggredan bagi kedua-dua bakteria dikenalpasti berdasarkan kriteria penggredan. Pada masa kini, bakteria gram positif dan gram negatif dikesan dan dikira secara manual oleh manusia dan penggredan itu dikenalpasti. Memandangkan manusia mungkin melakukan kesilapan semasa mengesan dan mengira untuk kedua-dua bakteria dan mengambil masa yang panjang dalam melakukan proses ini, membangunkan *automatic vision system* adalah perlu untuk memperolehi keputusan yang lebih tepat dan menjimatkan masa. *Automatic vision system* yang dibangunkan berdasarkan teknik pemprosesan imej yang hanya melibatkan simulasi perisian dengan menggunakan simulasi MATLAB. Dalam membangunkan projek ini, beberapa teknik pemprosesan imej dilaksanakan ke dalam simulasi MATLAB seperti analisis imej, segmentasi imej, peningkatan imej, proses morfologi dan lain-lain. Kemudian, keputusan untuk penjumlahan dan penggredan dipaparkan pada MATLAB *Graphical User Interface (GUI)*. Akhir sekali, hasil untuk penggredan yang diperolehi memberi hasil yang hampir sama berbanding dengan ujian pengesahan dari HUSM.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Sputum is a mucus that produced in the lungs and in the lower airways leading to the lungs. It is a normal body fluid, though excessive amounts of sputum often signal pulmonary disease. The colour consistency of sputum provide clues about the health of lungs and airways, though are not reliable diagnostic characteristics by themselves.

This project will focus on detection and counting the quantity of gram positive and gram negative in sputum sample. The image of sputum samples are taken under x100 computerized microscope which are obtained from Hospital Universiti Sains Malaysia (HUSM). Since the human might do some mistake in detection and summation for both bacteria and take a long time in doing this process, developing an automatic vision system based on image processing technique is necessary to obtain more precious results and save the time. Some techniques of the image processing will be used such as image analysis, image segmentation, image enhancement, morphological process and also the other techniques.

This vision system simulation is develop using MATLAB R2010a. All the process of detection and summation will be simulate by MATLAB simulation. Last but not least, the results for summation and grading will be displayed on MATLAB Graphical user Interface (GUI).

1.2 Problem Statement

The process of detection and summation for gram positive and gram negative bacteria in sputum sample nowadays is manually done by human. Since the human might do some mistakes in bacteria detection and summation, and taking a long time in doing this process, an automatic vision system is needed to be developed to avoid that problem occurs. Besides, this automatic system also will obtain more accurate results than previous method which is manually done by human. The grading of the bacteria in the system are calculated properly instead of the normal practice which are just by assumption.

1.3 Objective

The objectives of this project are:

i. To develop the vision system which is able to detect and count the quantity of the gram positive and gram negative bacteria in sputum sample image.
ii. To identify the grading of quantity for gram positive and gram negative bacteria in sputum sample image.

1.4 Scope of Project

This project involves software development only which is the vision system development. This system able to detect and count the quantity of gram positive and gram negative in sputum sample. Various techniques of image processing used for this system which can be applied by using MATLAB R2010a. The image taken from digital microscope under x100 magnification is processed to get the image of sputum which is contains variety bacteria including unwanted objects. So, to obtain the image needed, the associated coding is written on M-File. Then, the final result is

shown through the MATLAB GUI system which is display the number and the grading of gram positive and gram negative based on the final image.

CHAPTER 2

LITERATURE REVIEW

The related literature reviews of this project which have been referred according to this project's needs will be explained in this chapter. Most required literature reviews are focused on the introduction and differences both bacteria, techniques in image processing and how to apply those techniques in this project by using MATLAB simulation.

2.1 Differences between Gram Positive and Gram Negative bacteria

The differences for both bacteria can be determined based on their morphology such as cell shape and size. For cell shape, there are three main shapes which are determined by the molecular properties of cell wall such as spherical, rod-like, spiral and curved forms while pleomorphic form have no defined shape because it lacked a rigid cell wall. Meanwhile, the most bacteria have size in the range of 1-5μ in length [1].

2.1.1 Gram Positive bacteria

Gram positive bacteria have a thick multilayered, peptidoglycan cell wall that is exterior to the membrane. The peptidoglycan in most gram positive bacteria is covalently linked to teichoic acid, which is essentially a polymer of substituted glycerol units linked by phosphodiester bonds. All gram positive bacteria also have teichoic acid in their membranes, where it is covalently linked to glycolipid. The teichoic acids are major cells surface antigens [1]. The cell wall structure and example of gram positive will be shown in Figure 2.1 and Figure 2.2.

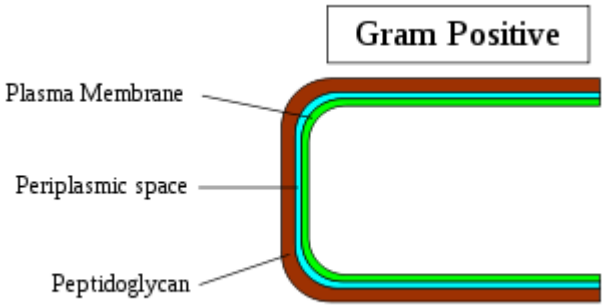


Figure 2.1 Gram positive cell wall structure

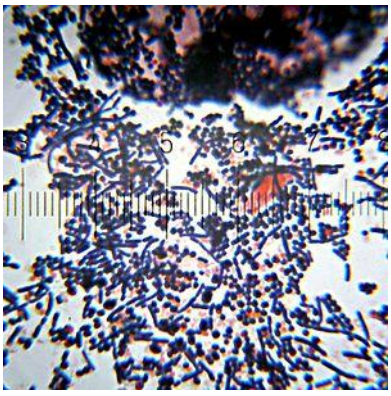


Figure 2.2 Gram-positive bacteria, stained purple, of both the bacillus ("rod-shaped") and cocci (spherical) forms. A few Gram-negative bacteria are also present, stained pink.

2.1.2 Gram Negative bacteria

Gram negative bacteria have two membranes – an outer membrane and inner (cytoplasmic) membrane. Their peptidoglycan layer is located between the two membranes in what is called the periplasmic space. The periplasmic space also contains enzymes and various other substances. In contrast to gram positive cells, the peptidoglycan layer of gram negative is thin, and the cells are consequently more susceptible to physical damage. The outer membrane is distinguished by the presence of various embedded lipopolysaccharides. The polysaccharide portion (O-polysaccharide) is antigenic, and can therefore be used to identity different strains and species. The lipid portion (lipid A) is toxic to humans and animals. Lipid A, because it is an integral part of membrane, is called an endotoxin, as opposed to exotoxins, which are secreted substances [1]. The cell wall structure and the example of gram negative will be shown in Figure 2.3 and figure 2.4.

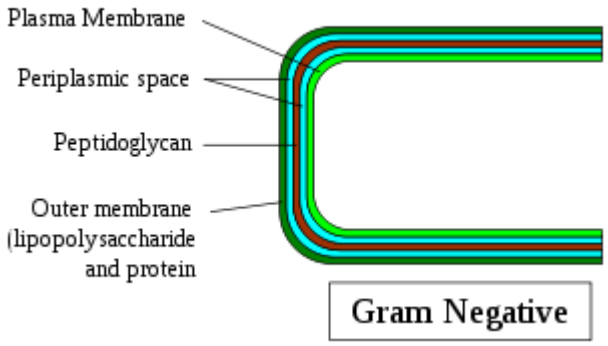


Figure 2.3 Gram negative cell wall structure

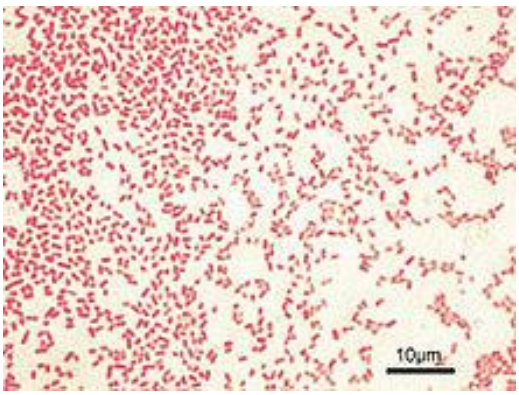


Figure 2.4 Microscopic image of Gram-negative Pseudomonas aeruginosa bacteria (pink-red rods)

Figure 2.5 shows the sputum sample image taken under x10 magnification using digital microscope that used for detection and summation of pus cell and squamous epithelial cells.

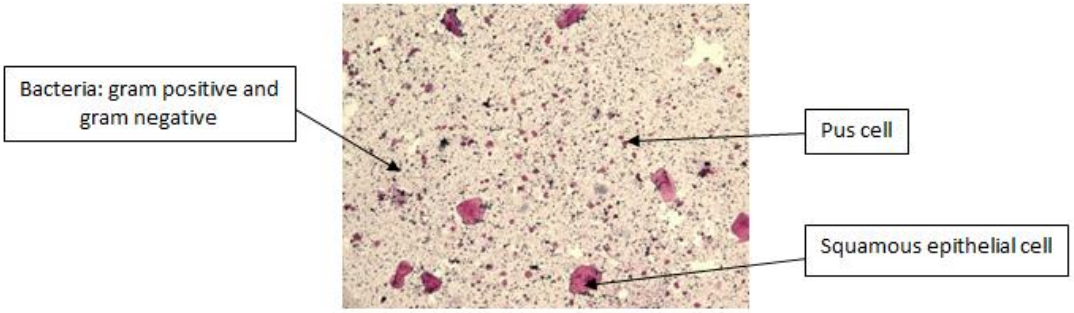


Figure 2.5 Sputum image under x10 computerized microscope

Figure 2.6 shows the sputum sample image taken under x100 magnification using digital microscope that used for detection and summation of bacteria (gram positive and gram negative).

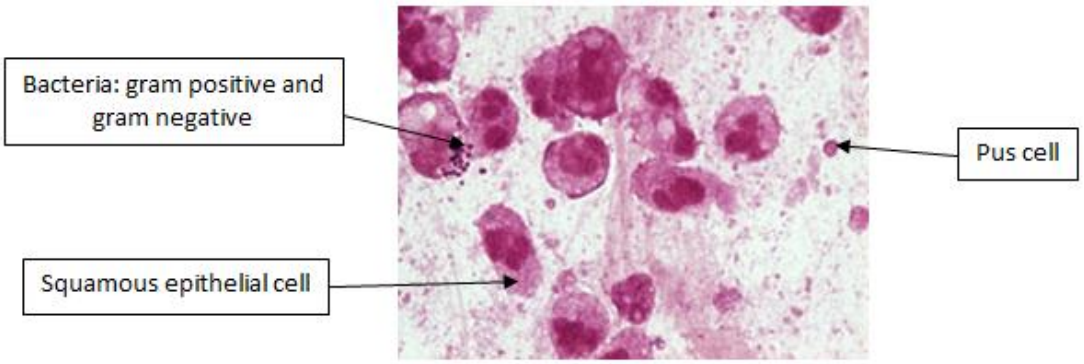


Figure 2.6 Sputum image under x100 computerized microscope

2.2 Staining Properties

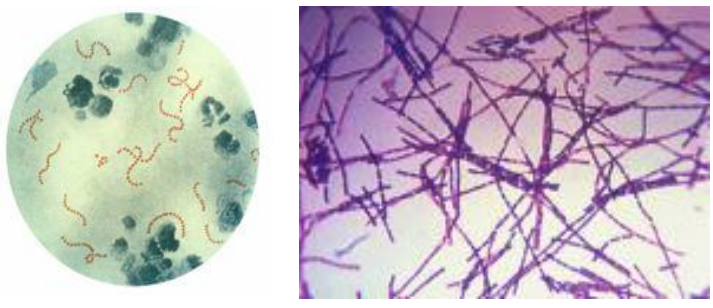
The staining properties are used to differentiate both bacteria through gram stain and counterstain process

2.2.1 Gram Stain

The gram stain is used to classify bacteria on the basis of their forms, sizes, cellular morphologies, and gram reactions. Besides, it is additionally a critical test for the presumptive diagnosis of infectious agents and serves to assess the quality of clinical specimens [2]. In this situation, gram stain is a quick procedure used to look for the presence of bacteria in tissue samples and to characterize bacteria as gram positive or gram negative, based on the chemical and physical of their cell walls [1].

i. Get sputum sample for gram stain.
ii. Add 1 or 2 drop of sputum sample onto glass slide.
iii. Heat fix the smear, by quickly passing it two three times through a flame, or heat it on top of an electric slide warmer.
iv. Flood the smear with crystal violet solution; allow to acts for 1 minute.
v. Rinse the slide, the flood with iodine solution and allow iodine to act for 1 minute. All organisms appear purple, that are gram positive.
vi. Rinse off excess iodine. Decolorize with acetone, approximately 5 seconds (time depends on density of specimen).
vii. Wash slide immediately with water. After acetone decolorization, those organisms that are gram negative are no longer visible.

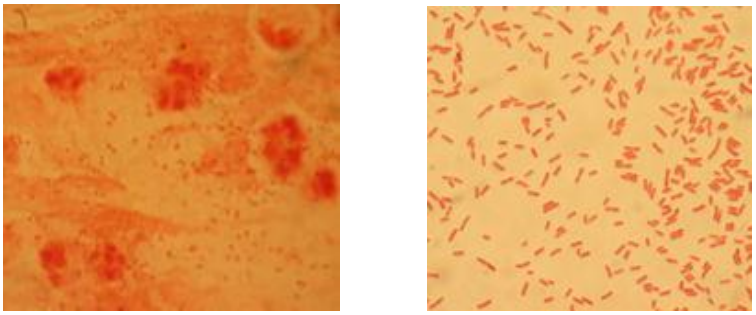
Figure 2.7 shows the examples of gram positive bacteria after doing the gram staining process.



(a) (b)

Figure 2.7 (a) Gram-positive (purple) cocci (round cells) in chains (b) Gram-positive rods

Figure 2.8 shows the examples of gram negative after doing the gram staining process.



(a) (b)

Figure 2.8 (a) Gram-negative diplococci (pink, spherical bacteria appearing as pairs), both inside and outside cells (b) Gram negative rod

2.2.2 Counterstains

In the gram staining procedures, the bacteria cells may be rendered invisible by the decolorization step. Visibility can be restored by using a counterstain that has a color distinctly different from the primary stain. The pink dye, safranin is used in the counterstain procedure. Therefore, gram positive cells are purple (having retained the crystal violet) whereas gram negative cells are pink (having been counterstained with safranin) [1].

i. Apply safranin counterstain for 30 seconds.
ii. Wash in water, blot and dry in air. Gram negative organisms are visualized after the application of the counterstain.

2.3 The Grading of Microorganisms

The grading of microorganisms as shown in Table 2.1 which is used in grading for gram positive and gram negative based on the quantity for both bacteria in a sputum sample.

Table 2.1 The grading of Microorganisms [8]

Occasional	Very few seen
1+	1-5 cells per field / a quarter of the field
2+	5-10 cells per field / half of the field
3+	10-25 cells per field / three quarters of the field
>25 to Numerous	Packed field / the whole field

Table 2.2 shows the example of result based on type of gram positive and gram negative in sputum sample.

Table 2.2 The reported result for bacteria component [16]

Reported results	A Labs	B Labs	C Labs	C1 Labs	Total	Grade
2+ to 4+, >50/oif, abundant gram positive bacilli, ± resembling *Coryne-bacterium* species/coryneforms/diphtheroids	65	5		6	76	4
3+ to 4+ gram positive coccobacilli	3	1			4	3
3+ gram positive bacilli, small, 4+ gram positive coccobacilli/ diphtheroids		1	3		4	3
4+ gram variable coccobacilli, snnp				1	1	3
4+ diphtheroids	2				2	1
4+, >25/oif gram positive bacilli, (± suggestive of coryneforms/ diphtheroids), 1+ to 4+, gram positive cocci	9	4	1	6	20	1
1+ to 4+ gram positive cocci/diplococci +/- refer	2			2	4	1
2+, 30-40/oif gram positive bacilli, ,<1/oif - 1+, 4+ gram negative bacilli +/- 5-10/oif gram positive cocci		1		1	2	0
4+ gram positive bacilli, 1+ yeast, snnp			1		1	0
1+ gram positive cocci, 1+ gram negative bacilli, snnp				1	1	0
2+, 3+ gram negative bacilli				2	2	0
snnp ± refer			2		2	ungraded
Wrong identifier	1	2	1	1	5	0
no report		1	1	2	4	0
Total	**82**	**15**	**9**	**22**	128	

2.4 Image Enhancement

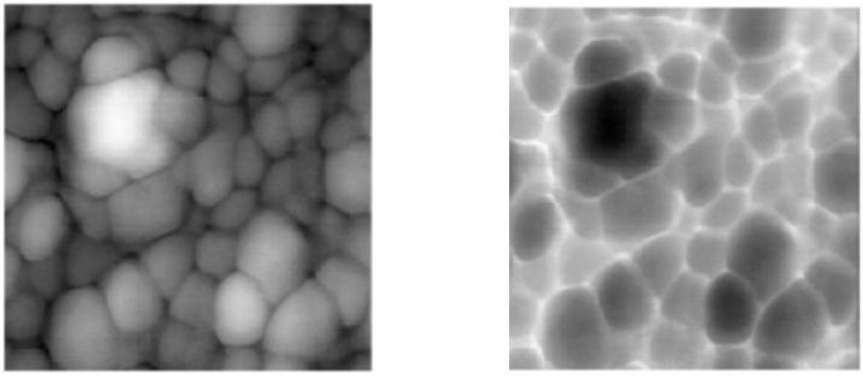
The main purpose of image enhancement is the process of manipulating an image so that the result is more suitable than original image for specific purposes [4][10]. In image enhancement, filtering method is used to enhance desire (structure) information and to suppress undesired (noise) object. Filtering operation is classified into two categories which are enhancing (high-pass filter), wherein desire object is

enhanced hopefully without affecting undesired object, and suppressing (low-pass filter), wherein undesired object is suppressed hopefully without affecting desire object [5].

There are the flows of method used in order to enhance the image:

i. Highlight fine details using Laplacian
ii. Enhance prominent edges using gradient
iii. Mask the Laplacian image using smoothed version of gradient image
iv. Increase the dynamic range of the intensity levels by using an intensity transformation

Thus, the processes image can be easily examined and interpreted. Another purpose of image enhancement is to facilitate printing of images or to allow automatic methods to perform measurements [11]. The result of image enhancement will improve the clarity of images for human viewing. There are many examples of enhancement operations such as removing blurring and noise, increasing contrast and revealing details [12]. Figure 2.9 shows the image after using image enhancement method.



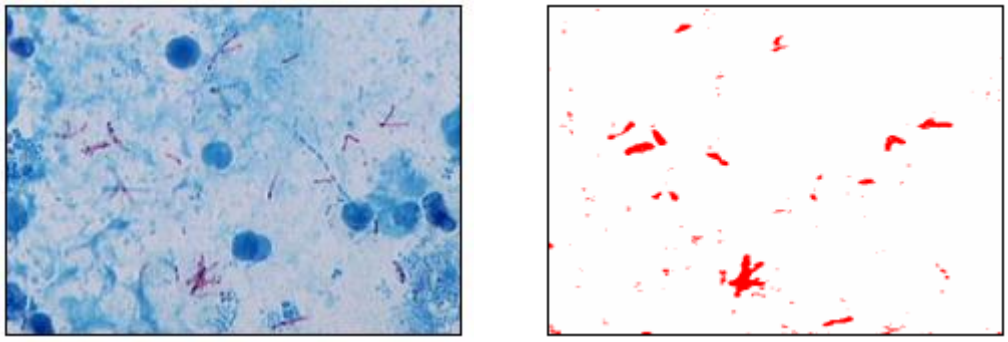
(a) (b)

Figure 2.9 (a) Original image (b) Enhance image

2.5 Image Segmentation by using Colour Thresholding

Segmentation process subdivides an image into its constituent regions or objects. The level of subdivision depends on the problem being solved, where the

segmentation should stop when the objects of interest in an application have been isolated. Image segmentation algorithms generally are based on one of the two basic properties of intensity values such as discontinuity and similarity [6]. Thresholding is a method of similarity category. It partitions an image into regions that are similar according to a set of predefined criteria. One simple way to accomplish Thresholding is by defining a range of brightness value in the original image, then the pixels are selected within the range as belonging to the foreground and all of other pixels are rejected to the background [7]. Figure 2.10 shows the image after use colour thresholding method.

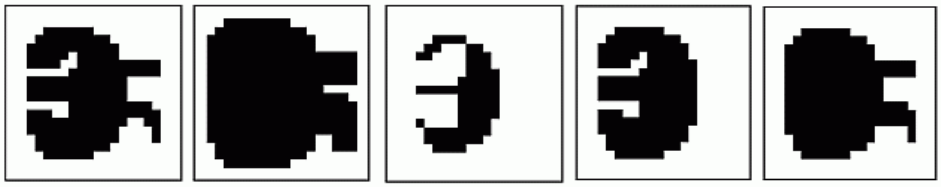


(a) (b)

Figure 2.10 (a) Original image (b) Threshold image

2.6 Morphological Image Processing

To identify the objects within an image is a very difficult task. Therefore, there is one way to simplify the problem which is to change the grayscale image into binary image. This way means that in which each pixel is restricted to a value of either 0 or 1. The morphological image processing is the one technique used on the binary image. Morphological operations have four basics used in the processing of binary images such as dilation, erosion, opening and closing [3]. The example of the techniques in morphological process by using Figure 2.11(a) as an original image as shown in Figure 2.11.



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

Figure 2.11 (a) Original image (b) Dilation image (c) Erosion image (d) Opening image (e) Closing image

2.6.1 Dilation Technique

In dilation image as shown in Figure 2.11(b), every background pixel that is touching an object pixel is changed into an object pixel. This technique makes the objects larger and can merge multiple objects into one.

2.6.2 Erosion Technique

In erosion image as shown in Figure 2.11(c), every object pixel that is touching a background pixel is changed into a background pixel. This technique makes the objects smaller and can break a single object into multiple objects.

2.6.3 Opening Technique

Opening is defined as an erosion technique followed by a dilation technique. As illustrated by Figure 2.11(d), opening removes small islands and thin filaments of object pixels.

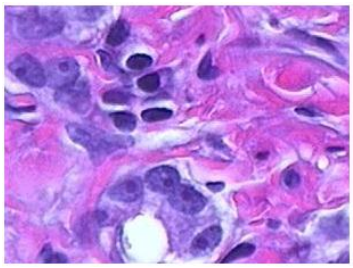
2.6.4 Closing Technique

Closing is defined as a dilation technique followed by an erosion technique. As illustrated by Figure 11(e), closing removes islands and thin filaments of background pixels.

These techniques are useful for handling noisy images where some pixels have the wrong binary value. For instance, it might be known that an object cannot contain hole, or that the object's border must be smooth [3].

2.7 K-Means Clustering

Cluster analysis is a way to organize and represent complex data sets. It is used routinely for data analysis in fields such as bioinformatics. The K-Means problem is to partition data into *k* groups such that the sum squared Euclidean distances to each mean is minimized [9][14][15]. K-Means is an algorithm to classify or to group the objects based on attributes or features into *k* number of group which is *k* is a positive integer number. The grouping is done by minimizing the sum of squares of distances between data and the corresponding cluster centroid. Thus, the purpose of K-Mean Clustering is to classify the data [13]. There is an example for K-Means Clustering method as shown in Figure 2.12.



(a)

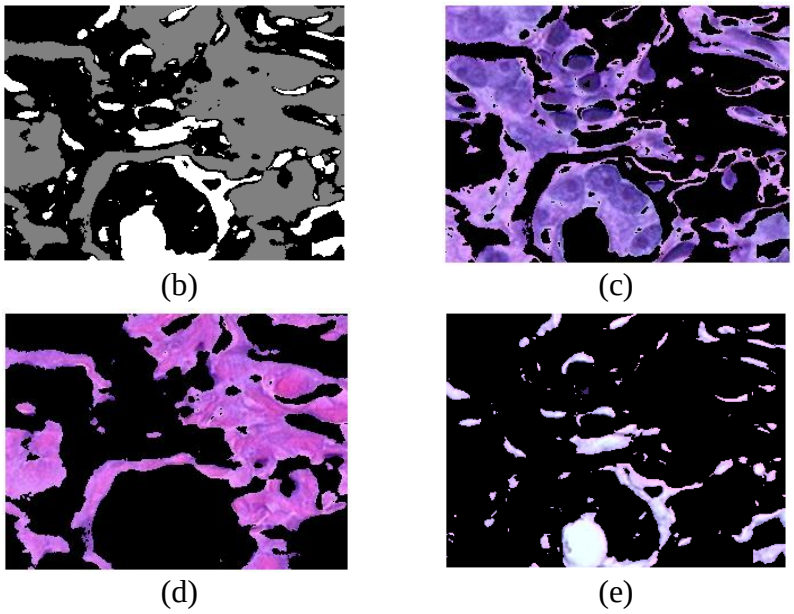
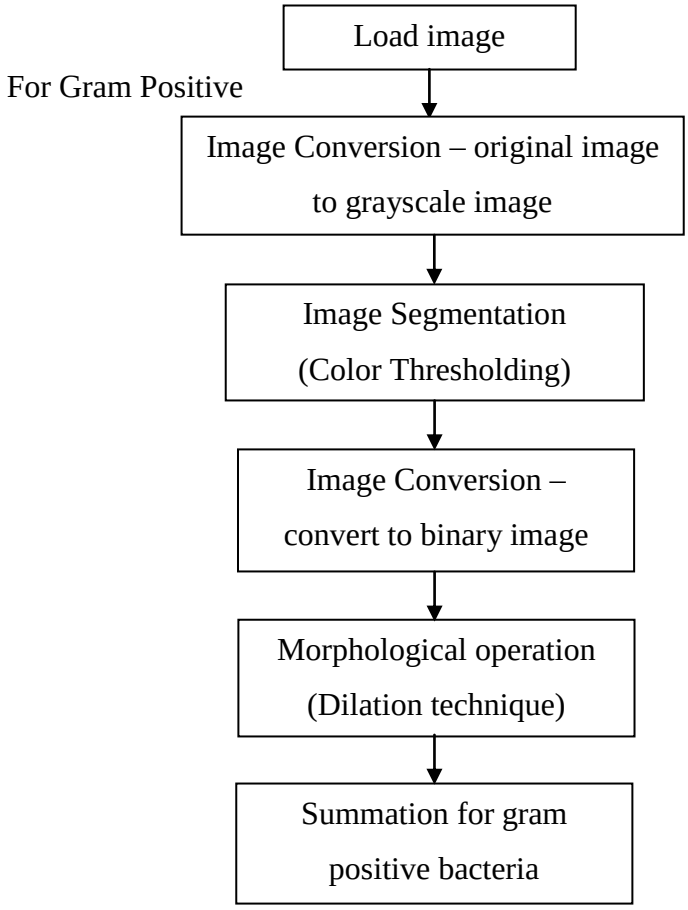


Figure 2.12 K-Means Clustering (a) H&E image (b) Image labelled by cluster image (c) Objects in cluster 1 (d) Objects in cluster 2 (e) Objects in cluster 3

CHAPTER 3

METHODOLOGY

This chapter clearly explain about the approach and methodology used in this project. The image processing techniques are used for this project in detecting and counting the quantity of gram positive and gram negative bacteria by using MATLAB simulation. The program of this project is shown in Appendix A. Then, the result will determined the tendency for both bacteria which is the major and minor in sputum image and the grading for both bacteria will be identified.



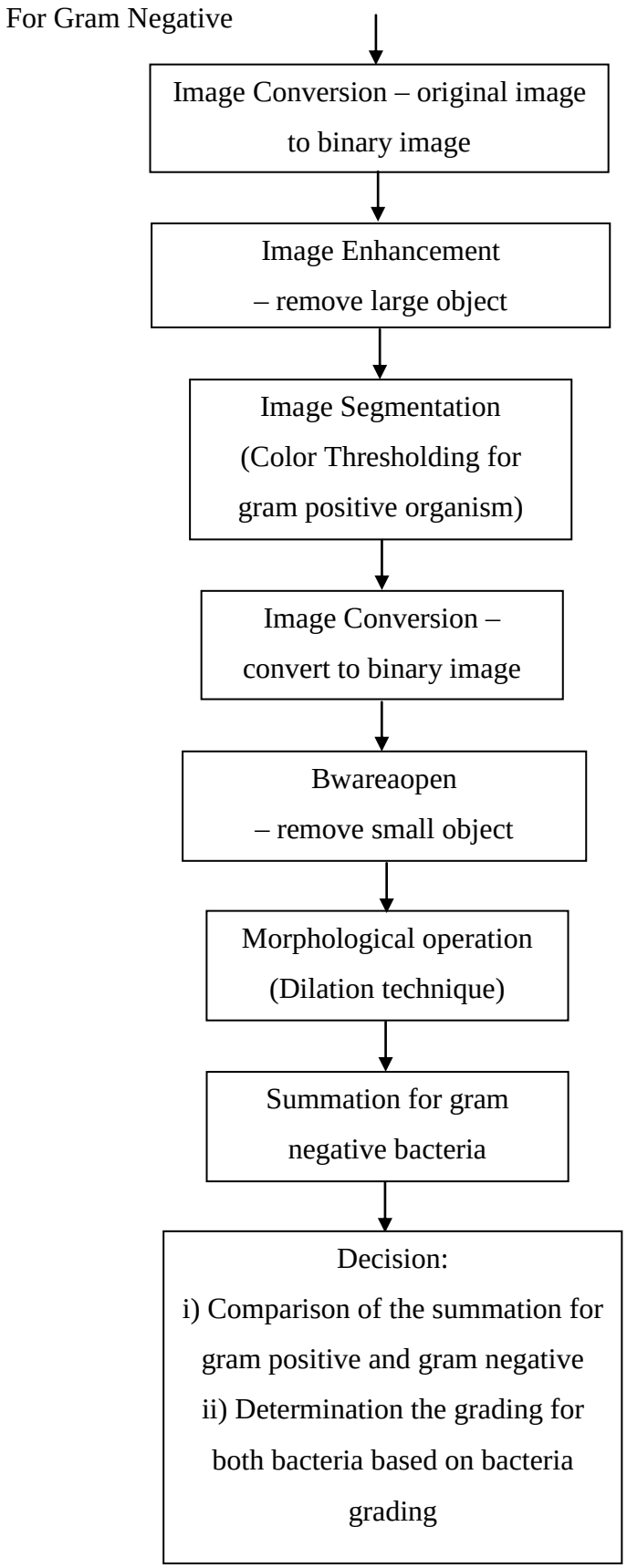
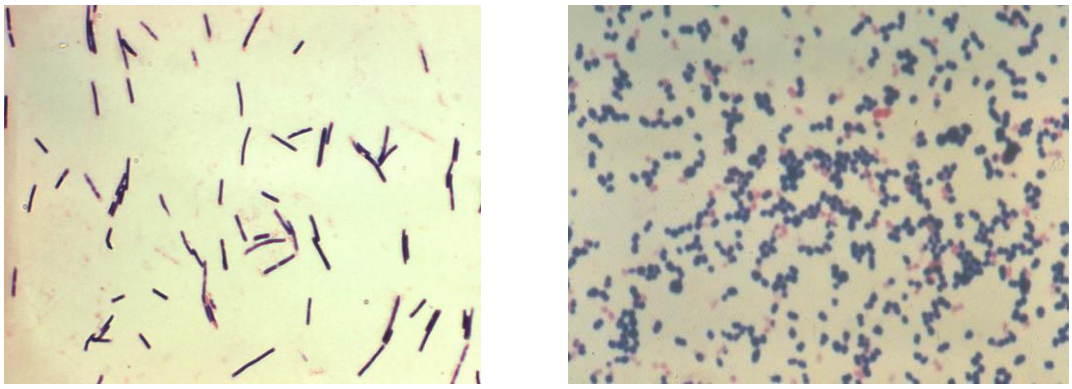


Figure 3.1 Image Processing Technique

3.1 Detection and Summation for Gram Positive bacteria

Gram positive bacteria are dark blue or purple in colour. This bacteria has variety of shape such as bacilli and cocci as shown in Figure 3.1.



(a) (b)

Figure 3.2 (a) Gram positive bacilli (b) Gram positive cocci

3.1.1 Read the image

The original image of the sputum from the file is loaded into the MATLAB software before next step is applied. For example, the original sputum image as shown in Figure 3.3.

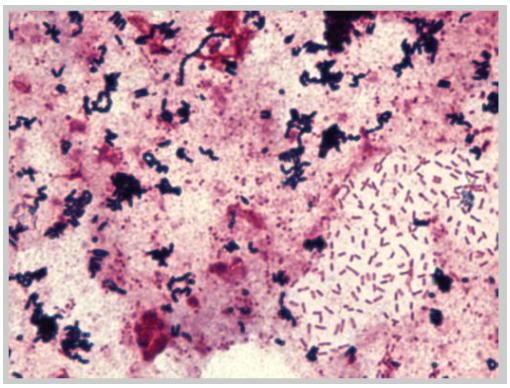


Figure 3.3 Original image

3.1.2 Image Conversion (convert original image to grayscale image)

Then, the original image of sputum is converted to grayscale image by using image conversion before apply the colour thresholding technique. The example of converting original image to grayscale image as shown in Figure 3.4:

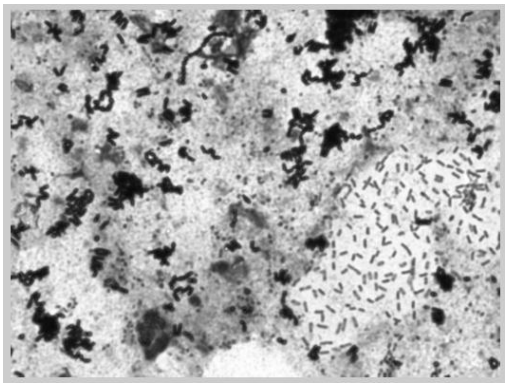


Figure 3.4 Grayscale Image

This is because the colour thresholding technique is applied after the original image converts into grayscale image.

3.1.3 Image Segmentation

Afterward, image segmentation is applied to that image by using Colour Thresholding. Colour thresholding is used to define a range of brightness value in the original image, then selects the pixels within the range as belonging to the foreground and rejects all of other pixels to the background. This technique means to maintain the intensities of gram positive and the other elements will be converted to black elements. The value of colour range in detecting gram positive in sputum image are:

i) $60 < red < 0$

ii) $80 < green < 0$

iii) $150 < blue < 0$

After colour thresholding, sputum image as shown in Figure 3.5 just consist of gram positive bacteria, but however the size of these bacteria become smaller than original image. This is because the effect of using colour thresholding that cannot detect the colour of desired image which is out of range. Hence, dilation technique is needed to enlarge the desired image. This technique is also been applied to gram negative as shown in Figure 3.11.

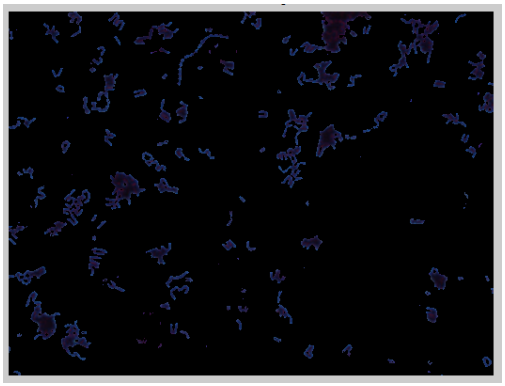


Figure 3.5 Threshold image

3.1.4 Image Conversion (convert to binary image)

Image conversion is applied to convert an image into binary image as shown in Figure 3.6 before undergone morphological process. This technique is also been applied to gram negative as shown in Figure 3.12

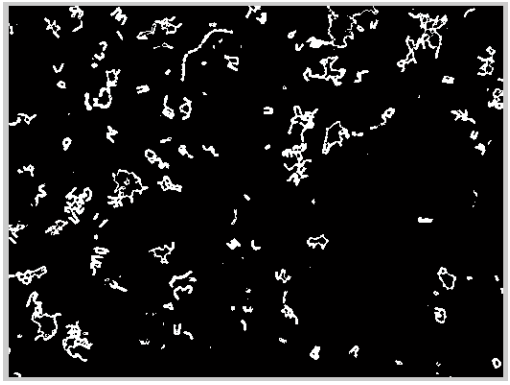


Figure 3.6 Binary image

3.1.5 Morphological Process (dilation technique)

The morphological process is applied on the image by using dilation technique because the desired object in image is very small in size. Dilation technique is used to enlarge the desired objects in the image such as shown in Figure 3.7. This technique is also been applied to gram negative as shown in Figure 3.14.

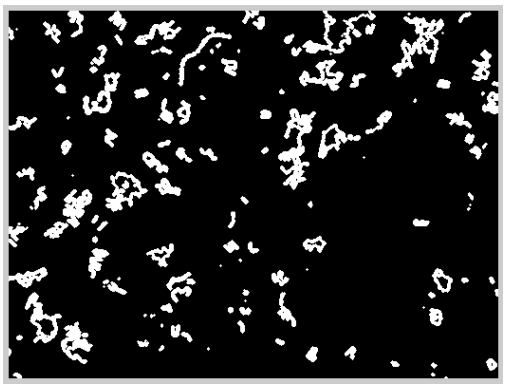


Figure 3.7 Dilate image

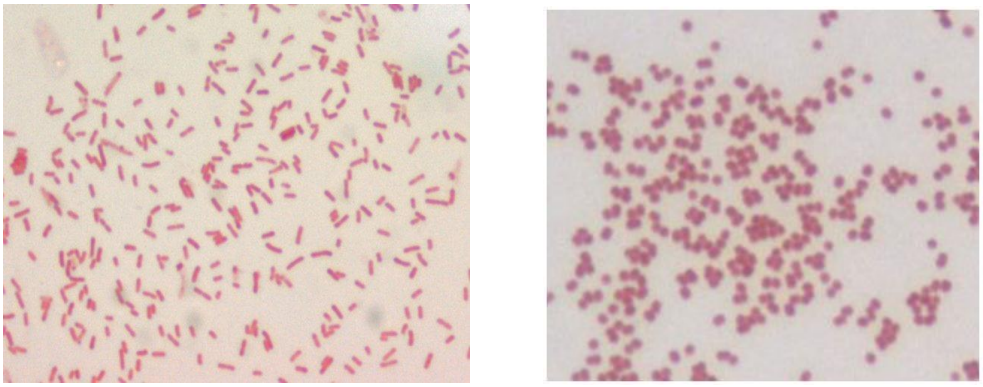
3.1.6 Summation for Gram Positive bacteria

From dilated image, the total area of gram positive will be calculated. Then, the total area obtained will be divided with one size (33pixel) of gram positive to determine the quantity of gram positive in an image. Hence, the quantity of bacteria will be divided into four parts to obtain the average for grading 0, 1+, 2+, 3+ and >25.

i) Occasional (0) – Very few seen
ii) 1+ – 1-5 cells per field / a quarter of the field
iii) 2+ – 5-10 cells per field / half of the field
iv) 3+ – 10-25 cells per field / three quarters of the field
v) >25 – packed field / the whole field

3.2 Detection and Summation for Gram Negative bacteria

Gram negative bacteria are red or pink in colour. This bacteria has variety of shape like gram positive such as bacilli (rod-shaped) and cocci (spherical) as shown in Figure 3.8.



(a) (b)

Figure 3.8 (a) Gram negative bacilli (b) Gram negative cocci

3.2.1 Image Conversion (convert original image to binary image)

In the same image, after the total of gram positive already counted, image conversion is used to convert original image to binary image as shown in Figure 3.9.

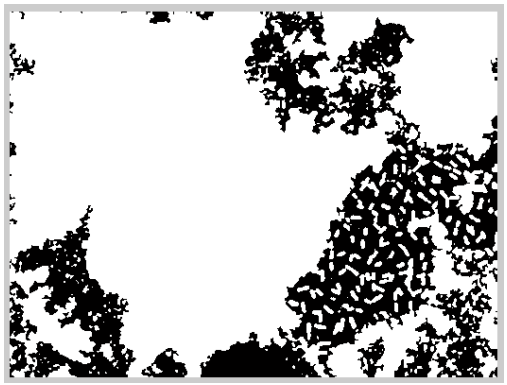
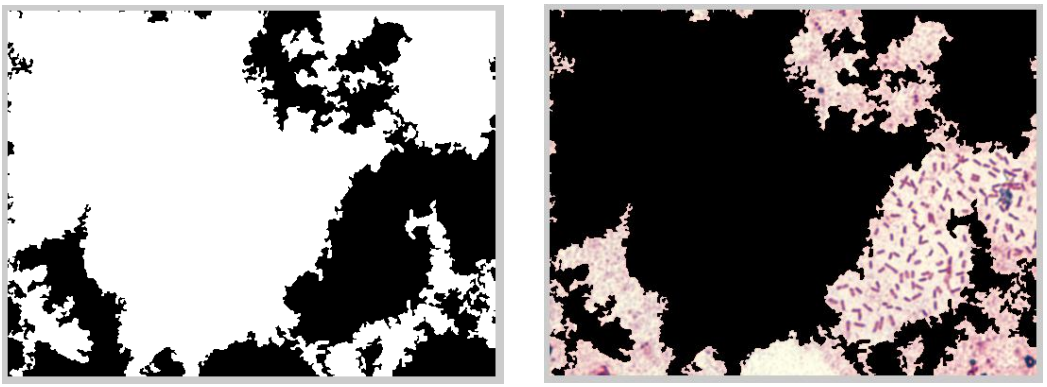


Figure 3.9 Binary image

3.2.2 Image Enhancement (remove large objects)

Image enhancement is applied to enhance the desired object since there are many undesired object having almost the same intensity with the desired (gram negative) objects but undesired objects have large size as shown in Figure 3.10.



(a) (b)

Figure 3.10 (a) Large object detected (b) Substracted image

3.2.3 Colour Thresholding

The image of gram negative using colour thresholding technique is resulted as shown in Figure 3.11.

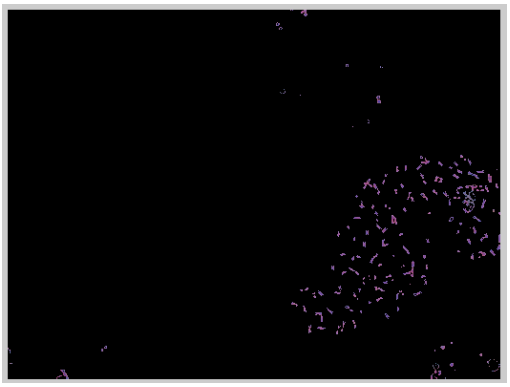


Figure 3.11 Threshold image

3.2.4 Image Conversion (convert to binary image)

The image of gram negative bacteria using image conversion is resulted as shown in Figure 3.12.

Figure 3.12 Binary image

3.2.5 Bwareaopen (remove small objects)

Since, the image still have undesired image which is smaller than gram negative size, bwareaopen technique is used to remove that small image as shown in Figure 3.13.

Figure 3.13 Small objects removed

3.2.6 Morphological Process (dilation technique)

The image of gram negative using dilation technique is resulted as shown in Figure 3.14.

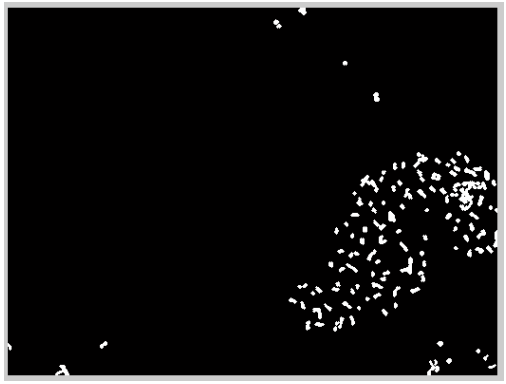


Figure 3.14 Dilate image

3.3 Decision

Thereafter, the comparison of summation for gram positive and gram negative can be conducted to determine which one of these two bacteria is greater. At the same time, the grading of gram positive and gram negative bacteria is identified based on grading of microorganisms.

3.4 Graphical User Interface (GUI)

GUI MATLAB is used in project to make this vision system more user friendly. Figure 3.15 shows the GUI MATLAB used in this project.

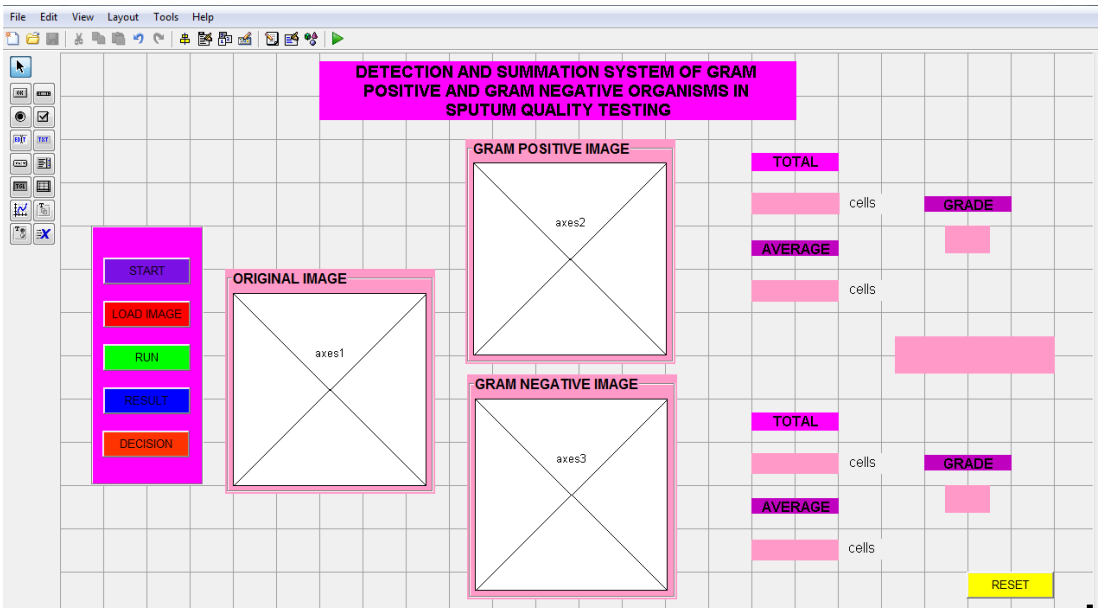


Figure 3.15 Graphical User Interface (GUI)

3.4.1 START Button

The START button is pressed first to run the project. After that, the message box is popup as “Please click on Load Image button to load the image” as shown in Figure 3.16. Then, OK button at message box is pressed.

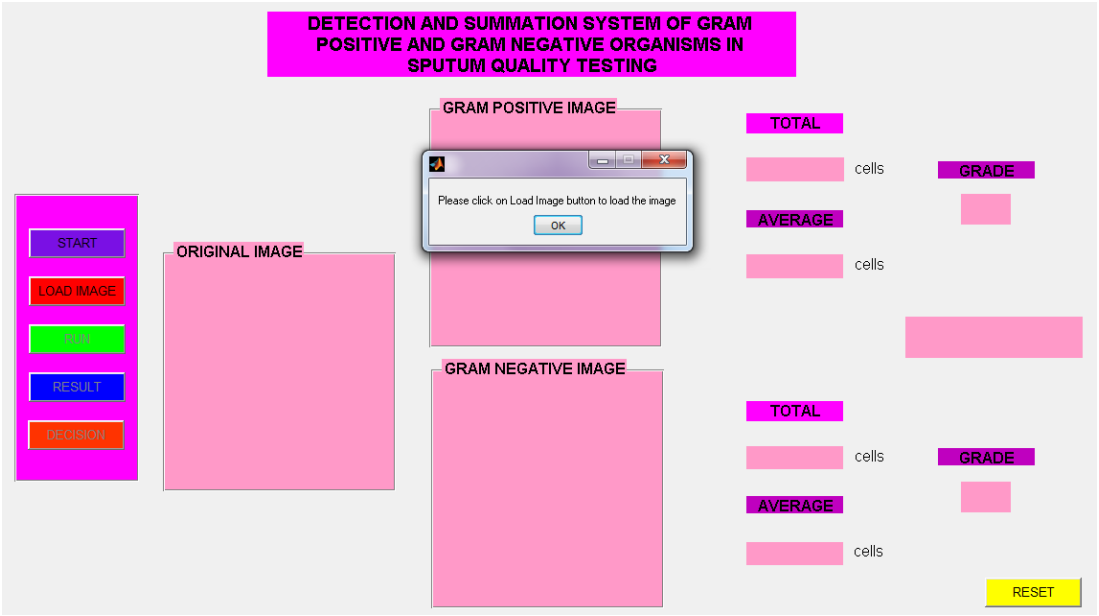
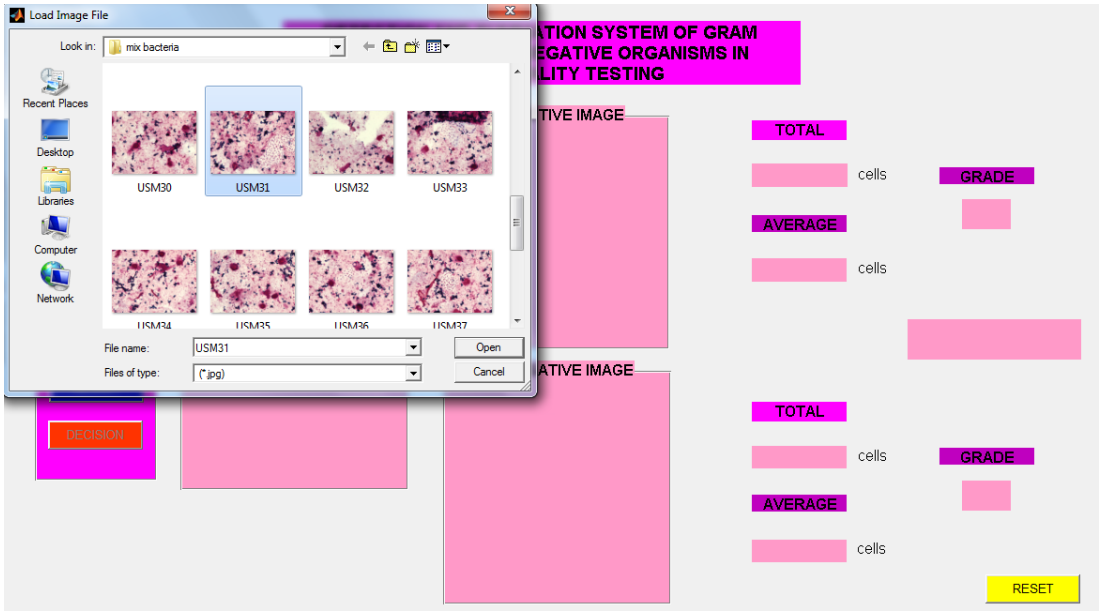


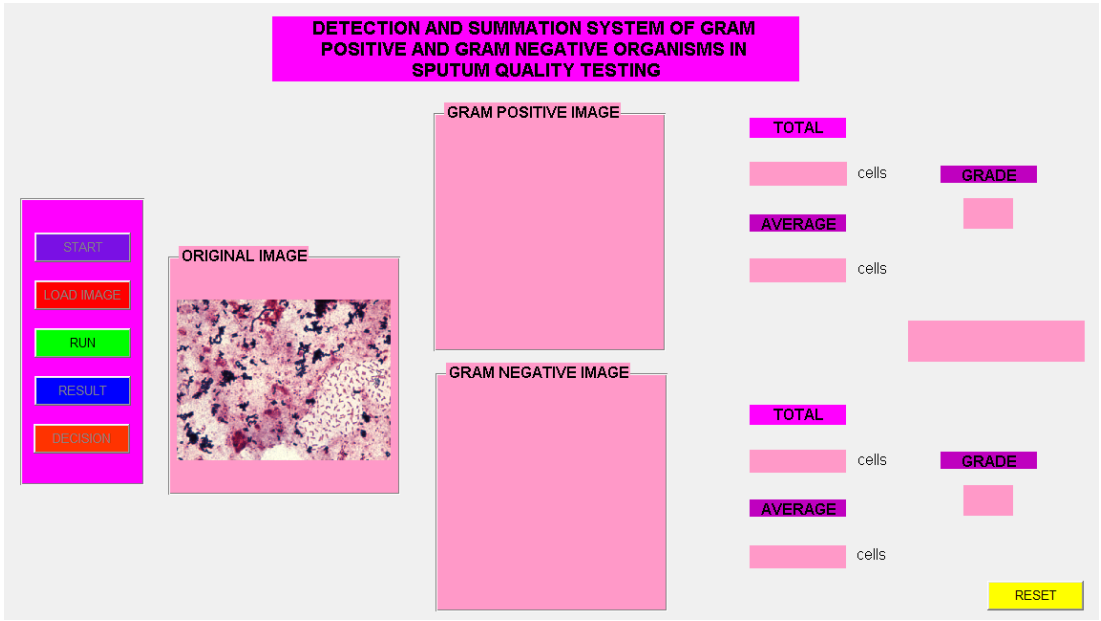
Figure 3.16 START button

3.4.2 LOAD IMAGE Button

LOAD IMAGE button is used to load the image that want to be run in this vision system from the file. The image selected is displayed on GUI MATLAB as shown in Figure 3.17.



(a)



(b)

Figure 3.17 LOAD IMAGE button (a) Image selection (b) Image selected

3.4.3 RUN Button

Then, RUN button is pressed to run the system which is display the gram positive and gram negative image on GUI MATLAB as shown in Figure 3.18.

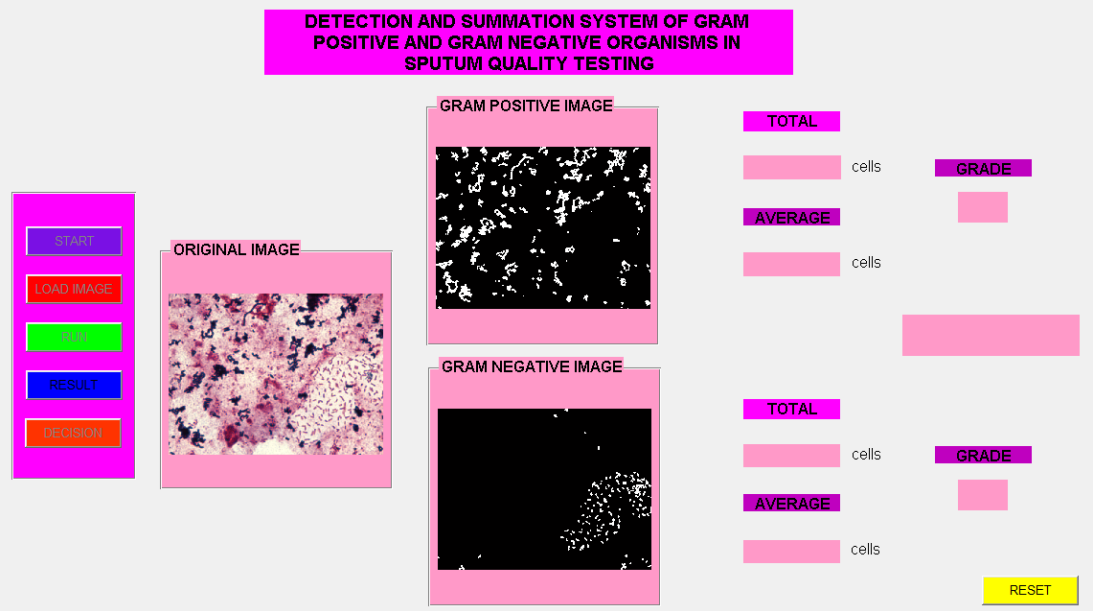


Figure 3.18 RUN button

3.4.4 RESULT Button

Hence, RESULT button is pressed to get the total and average of gram positive and gram negative in a sputum image. From that, the total value of gram positive and gram negative bacteria is obtained. Meanwhile, the average value of both bacteria used to identify the grading for gram positive and gram negative bacteria. Figure 3.19 shows the total and average for both bacteria.

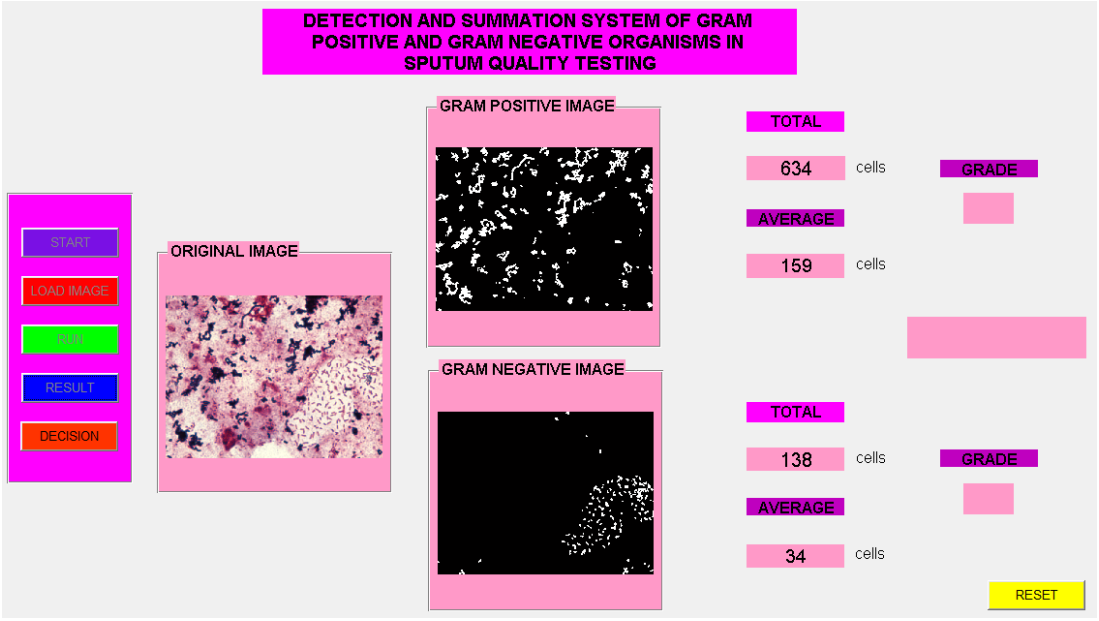


Figure 3.19 RESULT button

3.4.5 DECISION Button

Finally, the DECISION button is pressed to find out which one is major and minor in sputum image. At the same time, the grading for gram positive and gram negative bacteria is identified. The result is displayed as shown in Figure 3.20.

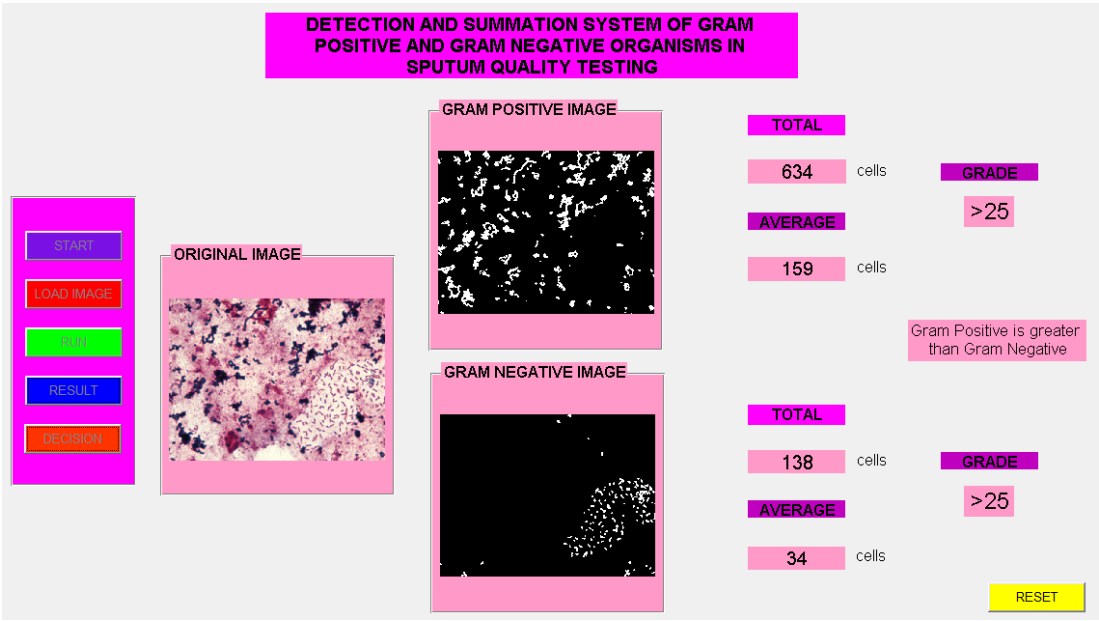


Figure 3.20 Decision button

3.4.6 RESET Button

Then, RESET button is used to clear all the previous process and return to normal or initial condition as shown in Figure 3.21.

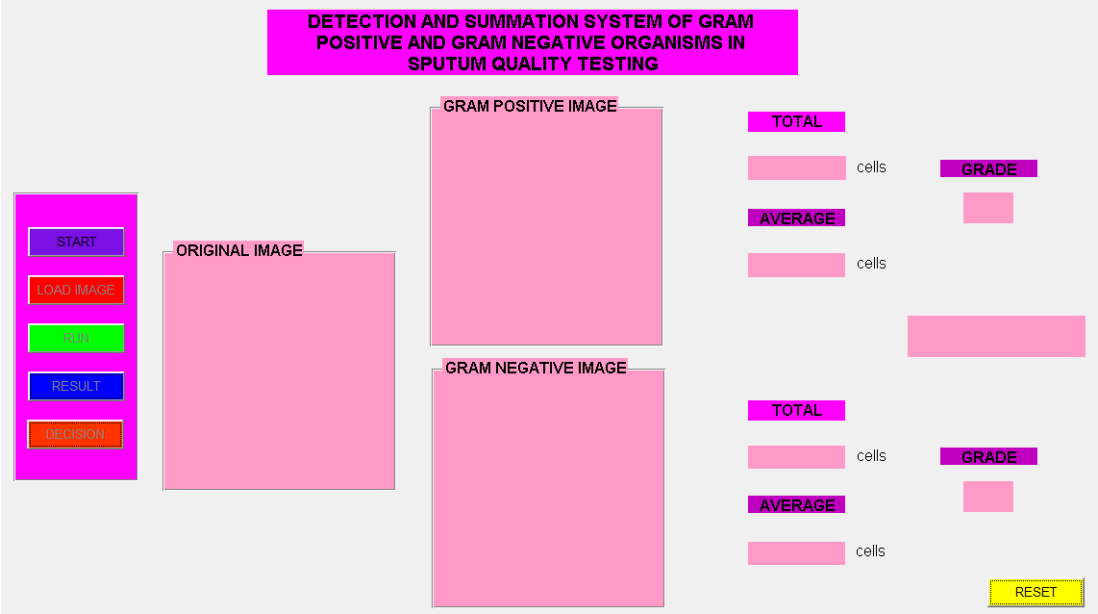


Figure 3.21 RESET button

CHAPTER 4

RESULT AND ANALYSIS

This chapter discuss on the result of this project. There are the results for two methods used in this project which is by using colour thresholding (Method 1) and combination of K-Means Clustering and colour thresholding (Method 2). The analysis of the results are clearly describes and the outcomes will be discussed.

4.1 Result and Analysis

1. Method 1 (Colour Thresholding) – Gram positive and gram negative bacteria is detected using colour thresholding method.
2. Method 2 (combination of K-Means Clustering and Colour Thresholding) – Gram positive is detected using K-Means Clustering followed by colour thresholding whereas gram negative is detected using colour thresholding method.

The first method used to detect and count the gram positive and gram negative bacteria was using colour thresholding. By using this method, the range of the value for gram positive and gram negative need to determine based on the range of colour for both bacteria.

At first, it is easier to determine the range of colour value for gram positive bacteria because their colour which is dark blue or purple colour is totally different with pink or red colour of background and other undesired object in the sputum image. But, there has a problem in determining the range of colour value for gram negative bacteria because their colour is almost the same with background and other undesired objects which is pink colour. Otherwise, if used the image detection method based on shape, there still has a problem because the shape and size almost the same with noise.

Meanwhile, another method has been found such as K-Means Clustering. K-Means Clustering is a method of cluster analysis which aims to partition *n* observations *k* cluster with the nearest mean. Besides, it is also easy to detect gram positive bacteria because there has a cluster for dark blue or purple colour which is same with gram positive bacteria colour. Anyhow, there still has a small problem when proceed to the next step which is some colour of gram positive bacteria cannot be detected. This is because the effects of using K-Means make some colour of gram positive bacteria changed to dark colour which is same with background colour. While for gram negative, it is difficult because it has no specific cluster for pink or red colour but will produce some clusters to be selected. This means, one of the clusters produced which has a lot of pink or red colour should be chosen. However, the results for gram negative bacteria are not accurate because some of gram negative bacteria are lost during this process

Besides, the combination of colour thresholding and K-Means Clustering method (Method 2) in one system also used for detecting both bacteria. Since, there has a problem in detecting gram positive colour, K-Means Clustering method is applied first and followed by colour thresholding method. After colour thresholding applied, there has a problem in detecting completely for blue or purple colour because the colour of gram positive has changed after use K-Means Clustering. This problem is the same with applying K-Means Clustering method only for gram positive. But, this method can be proceeds so that it can be compared to other method. While for gram negative bacteria, the Method 1 is applied for detecting these bacteria but with some other steps needed to remove undesired image. The

results for area, number of bacteria, average, grading and the tendency for both bacteria were obtained as shown in Table 4.1. All sample images used for this method can be seen in Appendix B.

Table 4.1 Data Analysis for Gram Positive and Gram Negative in Sputum Sample by using Method 2 (combination of K-Means Clustering and Colour Thresholding method)

IMAGE	AREA (pixels)	NO. OF BACTERIA	AVERAGE (no. of bacteria/4)	GRADING (based on average)	RESULT (based on no. of bacteria)
Image 1 1) Positive 2) Negative	 2772 1285	 84 39	 21 10	 3+ 2+	+ve > –ve
Image 2 1) Positive 2) Negative	 1351 3153	 41 95	 10 24	 2+ >25	+ve < –ve
Image 3 1) Positive 2) Negative	 1010 1269	 33 38	 8 10	 2+ 2+	+ve < –ve
Image 4 1) Positive 2) Negative	 7978 792	 242 24	 60 6	 >25 2+	+ve > –ve
Image 5 1) Positive 2) Negative	 510 1121	 15 34	 4 8	 1+ 2+	+ve < –ve
Image 6 1) Positive 2) Negative	 9 3511	 0 106	 0 27	 0 >25	+ve < –ve
Image 7 1) Positive 2) Negative	 0 308	 0 9	 0 2	 0 1+	+ve < –ve
Image 8 1) Positive 2) Negative	 130 1039	 4 31	 1 8	 1+ 2+	+ve < –ve
Image 9 1) Positive 2) Negative	 96 3425	 3 104	 1 26	 1+ >25	+ve < –ve
Image 10 1) Positive 2) Negative	 739 3617	 22 110	 6 27	 2+ >25	+ve < –ve
Image 11 1) Positive 2) Negative	 307 1221	 9 37	 2 9	 1+ 2+	+ve < –ve
Image 12					+ve >

1) Positive 2) Negative	11252 2317	341 70	85 18	>25 3+	–ve
Image 13 1) Positive 2) Negative	11555 4541	350 138	88 34	>25 >25	+ve > –ve
Image 14 1) Positive 2) Negative	7747 1706	235 52	59 13	>25 3+	+ve > –ve
Image 15 1) Positive 2) Negative	15994 4103	479 124	120 31	>25 >25	+ve > –ve
Image 16 1) Positive 2) Negative	12412 4559	376 138	94 35	>25 >25	+ve > –ve
Image 17 1) Positive 2) Negative	11667 5610	354 170	88 43	>25 >25	+ve > –ve
Image 18 1) Positive 2) Negative	12321 3887	373 118	93 29	>25 >25	+ve > –ve
Image 19 1) Positive 2) Negative	15148 1735	459 53	115 13	>25 3+	+ve > –ve
Image 20 1) Positive 2) Negative	11666 3541	354 107	88 27	>25 >25	+ve > –ve

There is an example that shows the problem occur in detecting of gram positive by using this method.

Figure 4.1 shows the sputum image loaded into the vision system.

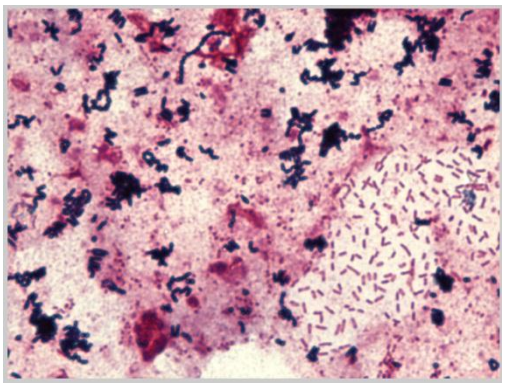
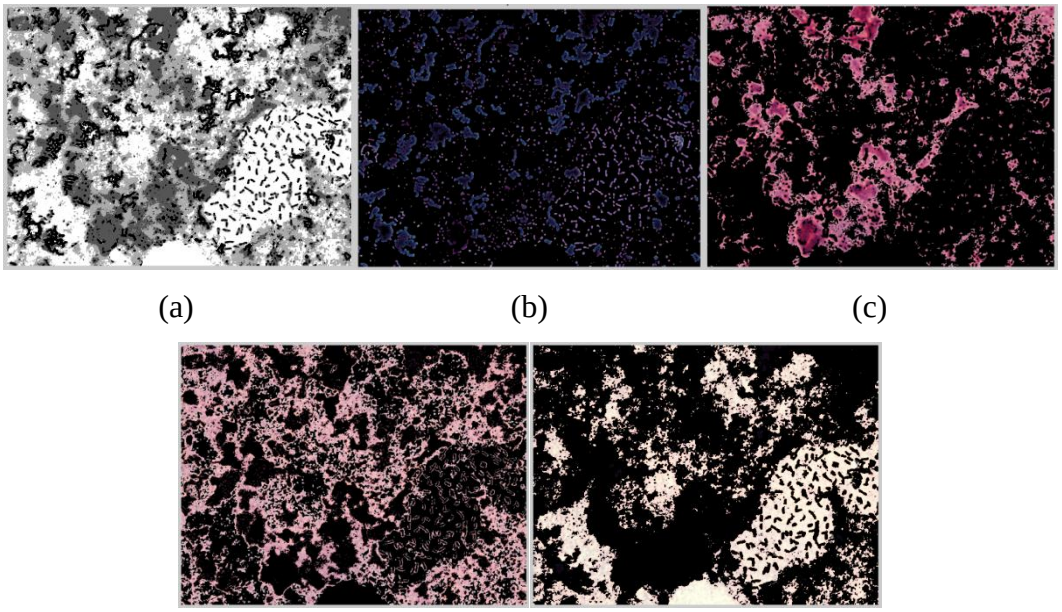


Figure 4.1 Original Image

Figure 4.2 shows the clusters produces from K-Means Clustering method. The suitable cluster based on gram positive colour is selected. From this clusters, Figure 4.2 (b) is chosen because there has more colour related with gram positive colour which dark blue or purple colour. But, at the same time, there has gram positive bacteria which is cannot be detected.



(a) (b) (c)

(d) (e)

Figure 4.2 (a) Image labelled by cluster index (b) Object in cluster 1 (c) Object in cluster 2 (d) Object in cluster 3 (e) Object in cluster 4

Then, colour thresholding is applied to that selected cluster as shown in Figure 4.3.

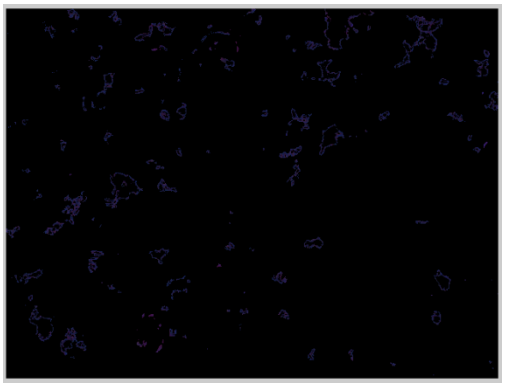


Figure 4.3 Threshold image

After that, the image is converted to binary image before applying the dilation technique as shown in Figure 4.4.

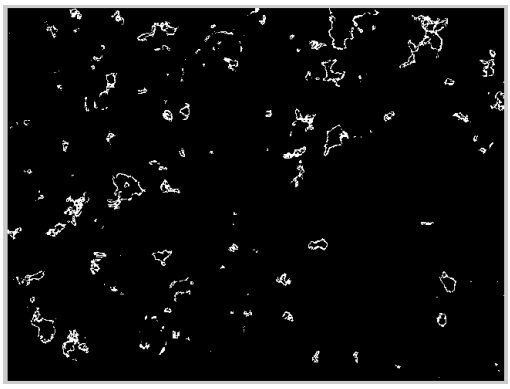


Figure 4.4 Binary image

Hence, the morphological image is applied to this image by using dilation technique as shown in Figure 4.5. From this image, the total of gram positive detected can be seen the differences with the original image because of using K-Means Clustering.

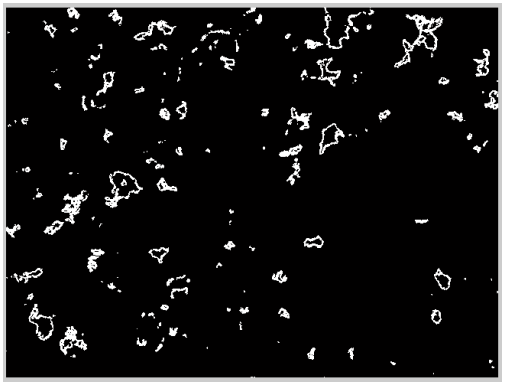


Figure 4.5 Dilate image

Since the gram positive cannot be detected properly using Method 2, the Method 1 is the better method to apply for this bacteria. This method is using colour thresholding only which is can detect all gram positive colours in sputum sample. At the same time, colour thresholding also use to detect gram negative followed by another step to remove the undesired objects in small and large size compared to gram negative. The results for area, number of bacteria, average, grading and the tendency were obtained for this method as shown in Table 4.2. All sample images used for this method can be seen in Appendix B.

Table 4.2 Data Analysis for Gram Positive and Gram Negative in Sputum Sample by using Method 1 (Colour Thresholding method)

IMAGE	AREA (pixels)	NO. OF BACTERIA	AVERAGE (no. of bacteria/4)	GRADING (based on average)	RESULT (based on no. of bacteria)
Image 1 1) Positive 2) Negative	 4207 1285	 127 39	 32 10	 >25 2+	+ve > –ve
Image 2 1) Positive 2) Negative	 3147 3153	 96 95	 24 24	 3+ 3+	+ve > –ve
Image 3 1) Positive 2) Negative	 3872 1269	 117 38	 29 10	 >25 2+	+ve > –ve
Image 4 1) Positive 2) Negative	 9747 792	 295 24	 74 6	 >25 2+	+ve > –ve
Image 5 1) Positive 2) Negative	 2258 1121	 68 34	 17 8	 3+ 2+	+ve > –ve
Image 6 1) Positive 2) Negative	 33 3511	 1 106	 0 27	 0 >25	+ve < –ve
Image 7 1) Positive 2) Negative	 18 308	 1 9	 0 2	 0 1+	+ve < –ve
Image 8 1) Positive 2) Negative	 1071 1039	 32 31	 8 8	 2+ 2+	+ve > –ve
Image 9 1) Positive 2) Negative	 1234 3425	 37 104	 9 26	 2+ >25	+ve < –ve
Image 10 1) Positive 2) Negative	 4863 3617	 147 110	 37 27	 >25 >25	+ve > –ve
Image 11 1) Positive 2) Negative	 1832 1221	 56 37	 14 9	 3+ 2+	+ve > –ve
Image 12 1) Positive 2) Negative	 13809 2317	 418 70	 105 18	 >25 3+	+ve > –ve
Image 13 1) Positive 2) Negative	 20931 4541	 634 138	 159 34	 >25 >25	+ve > –ve
Image 14 1) Positive 2) Negative	 9654 1706	 293 52	 73 13	 >25 3+	+ve > –ve
Image 15					+ve >

1) Positive 2) Negative	23788 4103	721 124	180 31	>25 >25	–ve
Image 16 1) Positive 2) Negative	 17629 4559	 534 138	 134 35	 >25 >25	+ve > –ve
Image 17 1) Positive 2) Negative	 17837 5610	 541 170	 135 43	 >25 >25	+ve > –ve
Image 18 1) Positive 2) Negative	 16479 3887	 499 118	 125 29	 >25 >25	+ve > –ve
Image 19 1) Positive 2) Negative	 18802 1735	 570 53	 142 13	 >25 3+	+ve > –ve
Image 20 1) Positive 2) Negative	 14601 3541	 442 107	 111 27	 >25 >25	+ve > –ve

Figure 4.6 shows the comparison of gram positive bacteria grading using Method 1, Method 2 and validation test (APPENDIX C) from HUSM. From this figure, detection of gram positive by using Method 1 more accurate compared than Method 2. Therefore, Method 1 is preferred to use in detecting gram positive bacteria.

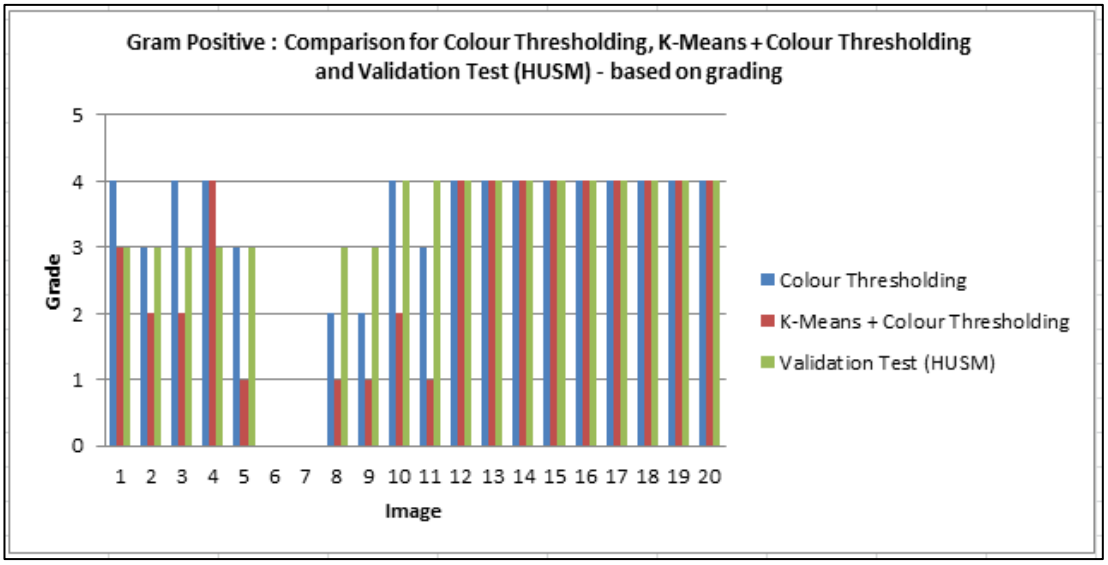


Figure 4.6 Comparison between both method and validation test for gram positive

Figure 4.7 shows the comparison of gram negative bacteria grading using Method 1, Method 2 and validation test (APPENDIX C) from HUSM. From this figure, the result of Method 1 is the same with Method 2. This is because both methods used in detecting gram negative are same technique which is colour thresholding technique. The result from colour thresholding technique almost the same with validation test from HUSM.

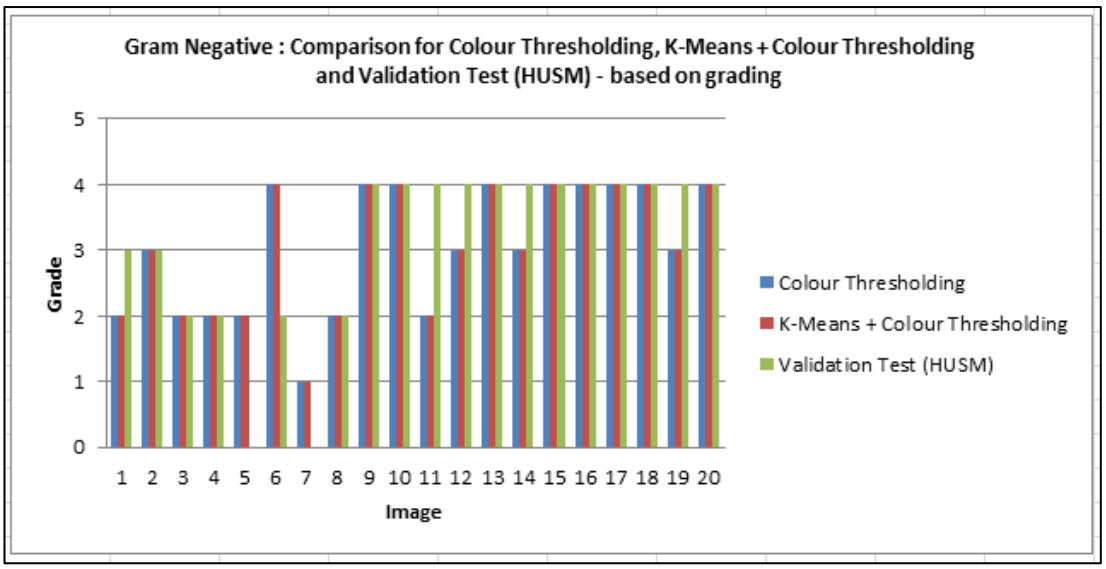


Figure 4.7 Comparison between both method and validation test for gram negative

After comparison between both methods has been made, Method 1 is chosen for the better method. This is because of more accurate result obtained from Method 1 compared to Method 2. Figure 4.8 shows the percentage error between the system (Method 1) and validation test from HUSM for gram positive bacteria. From this figure, the result obtained from the system is almost the same compared to validation test which is 70%. The detection of gram positive bacteria is easier compared to gram negative bacteria.

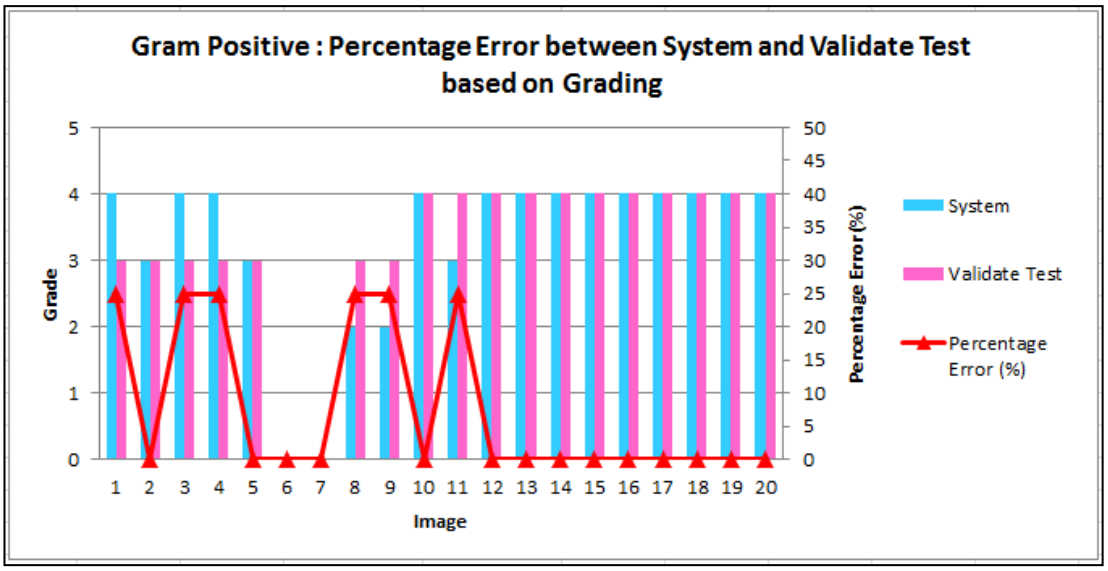


Figure 4.8 Percentage error between system and validation test for gram positive

While Figure 4.9 shows the percentage error between the system (Method 1) and validation test from HUSM for gram negative bacteria. From this figure, the result obtained from the system is less accurate compared to validation test which is 60%. This is because, it difficult to detect gram negative bacteria compared to gram positive bacteria.

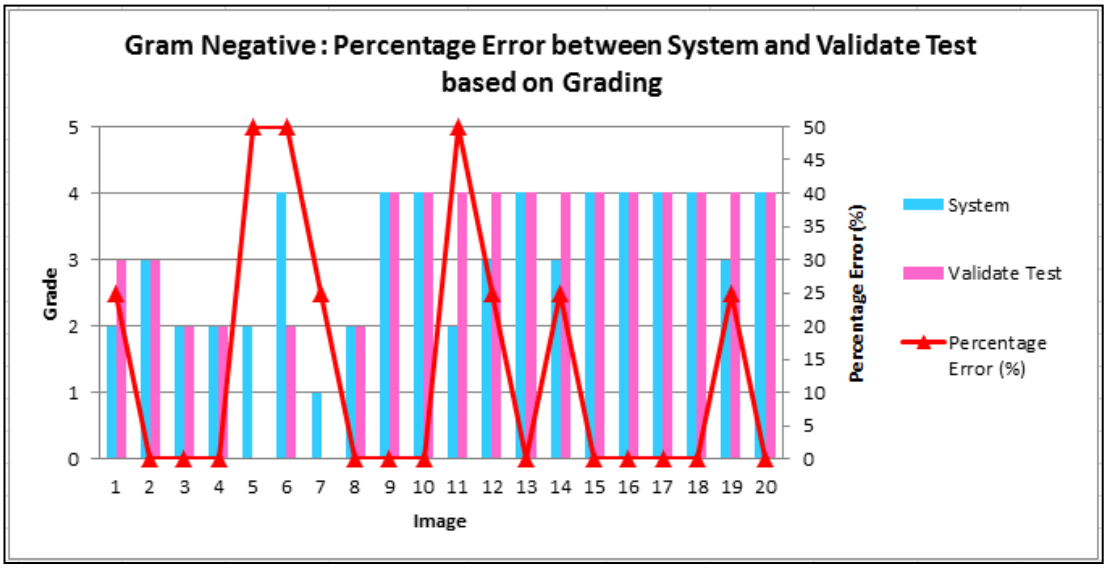


Figure 4.9 Percentage error between system and validation test for gram positive

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The detection of gram positive and gram negative bacteria in sputum sample is important to determine the grading for both bacteria. This grading will be used to identified the diseases exist from that sputum sample such as pneumonia, tuberculosis and others. Currently, the detection of both bacteria in sputum sample is manually calculated by human. Nevertheless, the disadvantages of human's work might do some mistakes during calculation process. In the meantime, human's work also take a long time to detect and count the quantity for both bacteria. Therefore, to avoid the mistake from human and save the time in detecting and counting the bacteria, an automatic vision system is developed. This automatic vision system only consists of software simulation using MATLAB. This vision system is used to detect and count the quantity of gram positive and gram negative without wasting time. Besides, the grading for both bacteria is identified automatically from this vision system. All the results obtained is displayed on MATLAB GUI system. The main purpose of using MATLAB GUI system in this project is to make this project more user friendly.

5.2 Recommendation

This project is only to detect and count the quantity of gram positive and gram negative bacteria in sputum sample. For recommendation, this project should be upgrade to detect and count the types for both positive and negative bacteria such as bacilli, cocci and others. So, more accurate grading can be obtained for these types of bacteria.

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[16] CMPT Clinical Bacteriology Program

APPENDICES

APPENDIX A

```
%get image from file
ori_img=imread ('F:\mix bacteria\USM13.JPG');
image=imresize(ori_img,0.4);
image1=image;
image2=image;
figure(1), imshow (image1), title('original image');

%Detection and summation for gram positive bacteria

%convert to grayscale image
red=image1(:,:,1);
green=image1(:,:,2);
blue=image1(:,:,3);
image_gray=rgb2gray(image1);
figure(2), imshow(image_gray), title('gray-level image');

%threshold image for gram positive
[r c]=size(image_gray);
for loop1=1:r
    for loop2=1:c
        if red(loop1,loop2)>60||red(loop1,loop2)<0|| ...
           green(loop1,loop2)>80||green(loop1,loop2)<0|| ...
           blue(loop1,loop2)>150||blue(loop1,loop2)<0
           image1(loop1,loop2,:)=0;
       end
    end
end
figure(3), imshow(image1), title('threshold image');

%convert to black & white image
BW=im2bw(image1,0.1);
figure(4), imshow(BW), title ('binary image');
```

```
%dilate image
se=strel('disk',1);
BW1=imdilate(BW,se);
figure(5), imshow(BW1), title('dilate image (gram positive image)');

%total area of gram positive
area=bwarea(BW1)
total=area/33
average=total/4

R = eval(int2str(total));
R3 = eval(int2str(average));

%grading for gram positive
if R3 > 25
   disp ('grade = >25');

else if R3 > 10 && R3 <= 25
        disp ('grade = 3+');

        else if R3 > 5 && R3 <= 10
                disp ('grade = 2+');

            else if R3 > 0 && R3 <= 5
                    disp ('grade = 1+');

                else
                     disp('grade = 0');

            end
        end
    end
end


%Detection and summation for gram negative bacteria
gray =rgb2gray(image2);
figure(14), imshow(gray);
thresholdValue = 200;
```

```
binaryImage = gray < thresholdValue;
binaryImage = imfill(binaryImage,'holes');
figure(6), imshow(binaryImage)
img = bwareaopen(binaryImage,2000);
figure(7), imshow(img)

[hg, map] = gray2ind(img,65534);
RGBk = ind2rgb(hg,map);
RGBk = im2uint8(RGBk);
figure(8), imshow(RGBk), title('RGBk img');

image3 =imsubtract(image2,RGBk);
figure(9), imshow(image3),

%threshold image for gram negative
red=image3(:,:,1);
green=image3(:,:,2);
blue=image3(:,:,3);
image_gray=rgb2gray(image);

[r c]=size(image_gray);
for loop1=1:r
    for loop2=1:c
        if red(loop1,loop2)>180||red(loop1,loop2)<80|| ...
           green(loop1,loop2)>120||green(loop1,loop2)<60|| ...
           blue(loop1,loop2)>180||blue(loop1,loop2)<120
           image3(loop1,loop2,:)=0;
       end
    end
end
figure(10), imshow(image3), title('threshold image');

%convert image to black & white image
BW=im2bw(image3,0.1);
figure(11), imshow(BW), title ('binary image');

%remove small object
BW2=bwareaopen(BW,5);
figure(12),imshow(BW2), title('bwareopen image');
```

```
%dilate image
se=strel('disk',1);
BW3=imdilate(BW2,se);
figure(13), imshow(BW3), title('dilate image (gram negative
image)');

%total area of gram negative
area2=bwarea(BW3)
total2=area2/33
average2=total2/4

R2 = eval(int2str(total2));
R4 = eval(int2str(average2));

if R4 > 25
   disp ('grade = >25');

else if R4 > 10 && R3 <= 25
        disp ('grade = 3+');

        else if R4 > 5 && R4 <= 10
                disp ('grade = 2+');

            else if R4 > 0 && R4 <= 5
                    disp ('grade = 1+');

                else
                     disp ('grade = 0');

            end
        end
    end
end
```

APPENDIX B

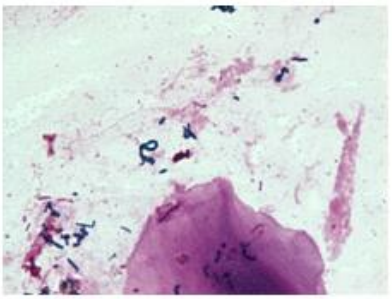
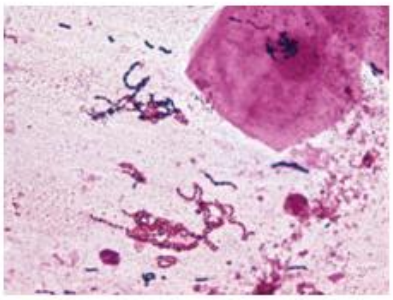
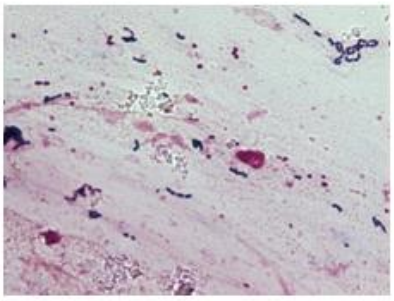
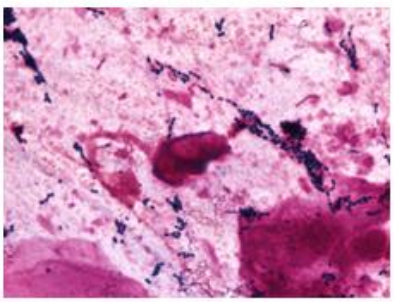
IMAGE	ORIGINAL IMAGE	COMMENT (based on original image)
Image 1		Gram positive is greater than Gram negative
Image 2		Gram positive is equal to Gram negative
Image 3		Gram positive is greater than Gram negative
Image 4		Gram positive is greater than Gram negative

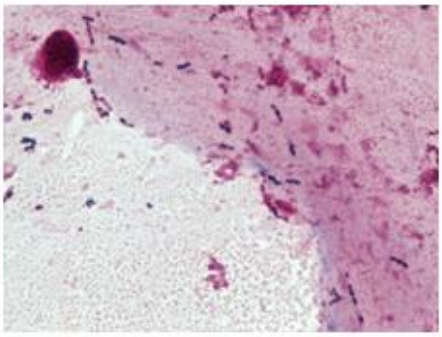
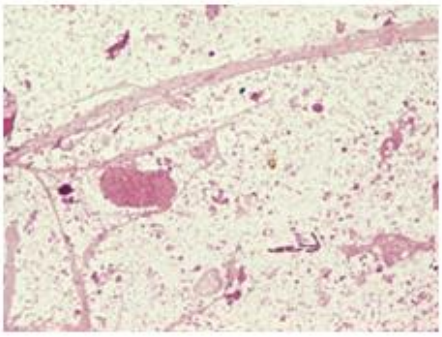
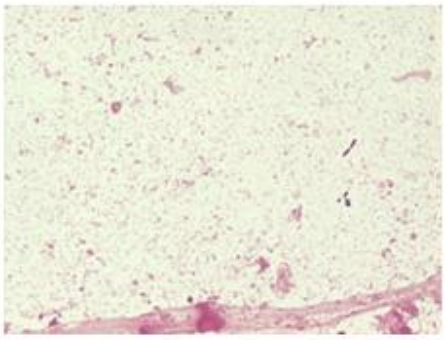
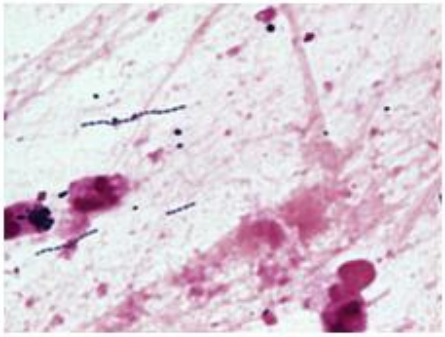
Image 5		Gram positive is greater than Gram negative
Image 6		Gram positive is less than Gram negative
Image 7		Gram positive is less than Gram negative
Image 8		Gram positive is greater than Gram negative

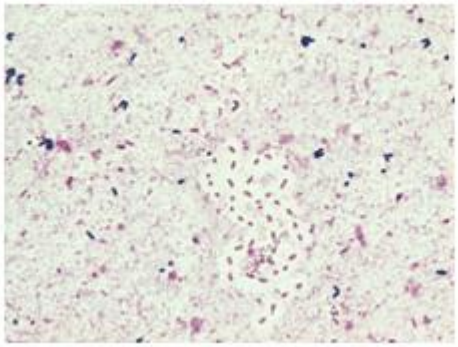
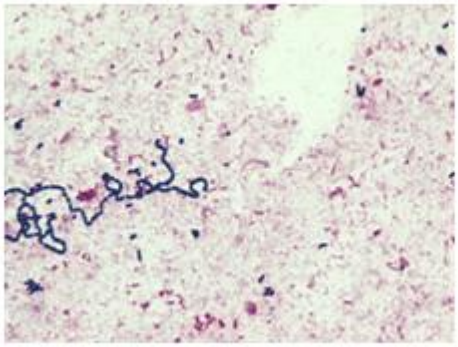
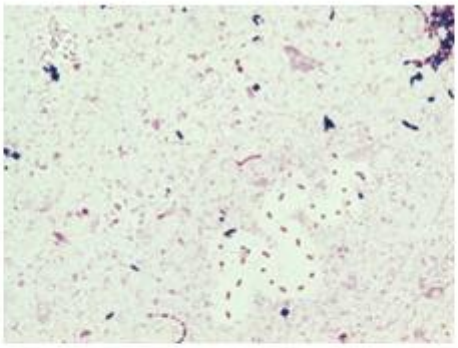
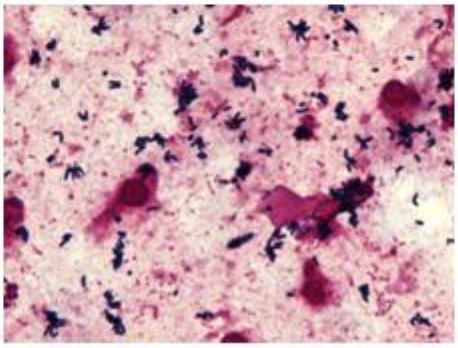
Image 9		Gram positive is less than Gram negative
Image 10		Gram positive is greater than Gram negative
Image 11		Gram positive is greater than Gram negative
Image 12		Gram positive is greater than Gram negative

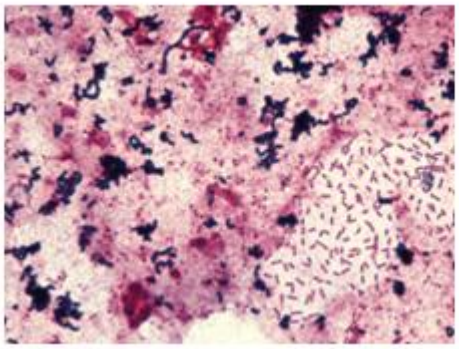
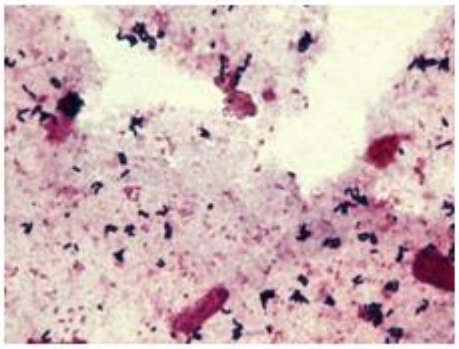
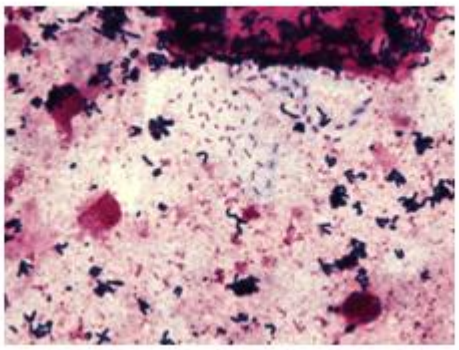
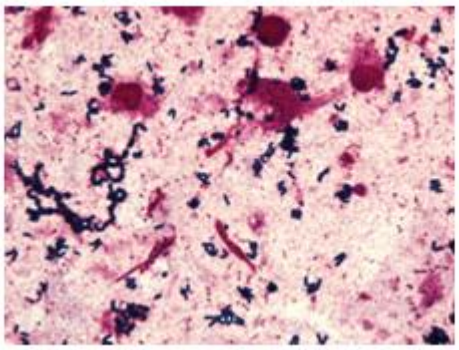
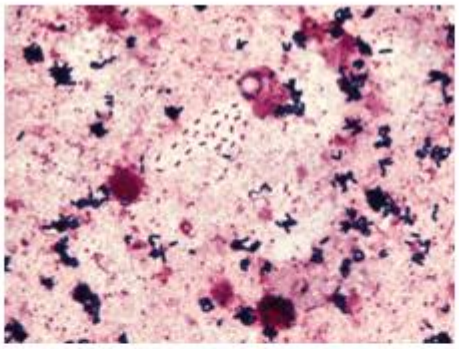
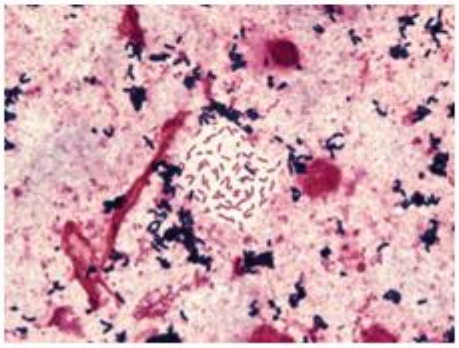
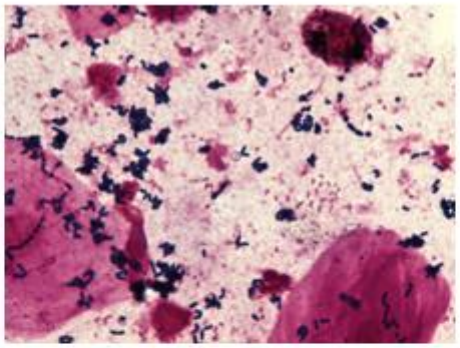
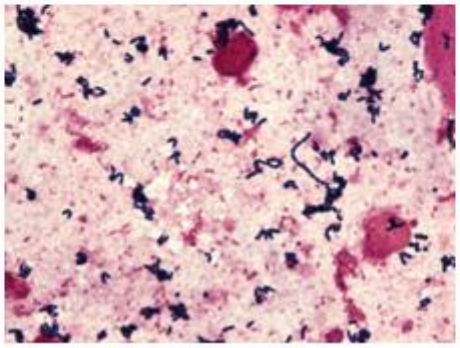
Image 13		Gram positive is greater than Gram negative
Image 14		Gram positive is greater than Gram negative
Image 15		Gram positive is greater than Gram negative
Image 16		Gram positive is greater than Gram negative

Image 17		Gram positive is greater than Gram negative
Image 18		Gram positive is greater than Gram negative
Image 19		Gram positive is greater than Gram negative
Image 20		Gram positive is greater than Gram negative

APPENDIX C

IMAGE	Colour Thresholding		K-Means Clustering + Colour Thresholding		VALIDATION TEST
	Grade	Result	Grade	Result	
Image 1 1) Positive 2) Negative	>25 2+	+ve > –ve	3+ 2+	+ve > –ve	3+ 3+
Image 2 1) Positive 2) Negative	3+ 3+	+ve > –ve	2+ 3+	+ve < –ve	3+ 3+
Image 3 1) Positive 2) Negative	>25 2+	+ve > –ve	2+ 2+	+ve < –ve	3+ 2+
Image 4 1) Positive 2) Negative	>25 2+	+ve > –ve	>25 2+	+ve > –ve	3+ 2+
Image 5 1) Positive 2) Negative	3+ 2+	+ve > –ve	1+ 2+	+ve < –ve	3+ 0
Image 6 1) Positive 2) Negative	0 >25	+ve < –ve	0 >25	+ve < –ve	0 2+
Image 7 1) Positive 2) Negative	0 1+	+ve < –ve	0 1+	+ve < –ve	0 0
Image 8 1) Positive 2) Negative	2+ 2+	+ve > –ve	1+ 2+	+ve < –ve	3+ 2+
Image 9 1) Positive 2) Negative	2+ >25	+ve < –ve	1+ >25	+ve < –ve	3+ >25
Image 10 1) Positive 2) Negative	>25 >25	+ve > –ve	2+ >25	+ve < –ve	>25 >25
Image 11 1) Positive 2) Negative	3+ 2+	+ve > –ve	1+ 2+	+ve < –ve	>25 >25
Image 12 1) Positive 2) Negative	>25 3+	+ve > –ve	>25 3+	+ve > –ve	>25 >25
Image 13 1) Positive 2) Negative	>25 >25	+ve > –ve	>25 >25	+ve > –ve	>25 >25
Image 14 1) Positive 2) Negative	>25 3+	+ve > –ve	>25 3+	+ve > –ve	>25 >25
Image 15 1) Positive 2) Negative	>25 >25	+ve > –ve	>25 >25	+ve > –ve	>25 >25
Image 16 1) Positive 2) Negative	>25 >25	+ve > –ve	>25 >25	+ve > –ve	>25 >25
Image 17 1) Positive 2) Negative	>25 >25	+ve > –ve	>25 >25	+ve > –ve	>25 >25
Image 18 1) Positive 2) Negative	>25 >25	+ve > –ve	>25 >25	+ve > –ve	>25 >25
Image 19 1) Positive 2) Negative	>25 3+	+ve > –ve	>25 3+	+ve > –ve	>25 >25
Image 20 1) Positive 2) Negative	>25 >25	+ve > –ve	>25 >25	+ve > –ve	>25 >25