

Interactive/Automated Method to Count Bacterial Colonies

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*“Talvez não tenha conseguido fazer o melhor,
mas lutei para que o melhor fosse feito.
Não sou o que deveria ser, mas Graças a Deus,
não sou o que era antes”*
(Marthin Luther King)

Resumo

O crescimento e manutenção de bactérias em placas de agar (placas de Petri) tem sido uma prática comum em microbiologia. O número de colónias numa cultura é normalmente contado manualmente para calcular a concentração de bactérias, no entanto, este processo é demorado, enfadonho e propenso a erros.

A maioria dos sistemas automáticos de contagem, existentes na literatura; realizam a contagem de forma adequada quando as colónias estão bem espaçadas, são grandes, e de forma circular e têm um bom contraste com fundo. Quando estes pressupostos são violados, sistemas de análise de colónias automáticos podem rapidamente perder a fidelidade, precisão e utilidade.

Para resolver os problemas acima, o objetivo deste trabalho é projetar e implementar um sistema centrado no software de baixo custo que aceita imagens gerais de câmaras digitais, para a deteção, bem como enumerar colónias de bactérias de uma forma totalmente automática. Um sistema interativo também é proposto para ultrapassar qualquer erro do sistema totalmente automático.

Neste estudo foram consideradas 26 imagens, 21 delas obtidas na biblioteca existente e as outras 5 criadas desde o início. Os dois tipos de imagem, têm sistemas de captura diferente, conseqüentemente, os dois tipos de imagens são diferentes. O pré-processamento permite a construção de uma imagem apenas com a placa de Petri, removendo o ruído e o fundo. Esta etapa permite, também, a separação de imagem em duas partes, uma das quais contém a área central e a outra a zona da borda (anel), e prepara-as para a fase de segmentação. A segmentação permite a extração das colónias a partir da área central, como a área do anel. Nas imagens obtidas por este método, esta extração na zona central é realizado por um binarização com um valor fixo para o limiar. Na área de rebordo, é usado um binarização também, combinada com informações sobre o comprimento do eixo maior e menor, excentricidade e média das áreas. Na outra biblioteca, a segmentação é realizada utilizando um bottom-hat filtering,

tanto na área central, como na zona do anel. Informações sobre o comprimento dos eixos maior e menor, excentricidade e áreas também são usados na área do anel. Depois disso, as colónias segmentadas são separados em duas imagens. Uma delas contendo as unidades de colónia e a outra as colónias em cluster. Esta separação é realizada através da excentricidade dos objetos. Para finalizar, o usuário escolhe o método de contagem.

Para separar e contar as colónias em cluster, o sistema automático utiliza a watershed transformation e o sistema interativo utiliza os cliques do usuário. Os sistemas propostos são capazes de reduzir a mão-de-obra e tempo necessário para a contagem de colónias. O sistema automático proposto tem dificuldade em contar colónias na área do anel, fazendo com que o sistema não conte algumas colónias. O método interativo, corrige todos os problemas do método automático, produzindo resultados similares à contagem manual.

Palavras-chave: Contagem de colónias, Unidades formadores de colónias, Segmentação de colónias, *Escherichia coli*, Segmentação de imagem, Métodos interativos, Placa de Petri.

Abstract

The growth and maintenance of bacteria on agar plates (Petri dishes) has long been a common practice in microbiology. The number of colonies in a culture is usually counted manually to calculate the concentration of bacteria, however, this process is time-consuming, tedious and error prone.

Most automatic counting systems, existing on the literature; perform adequately when the colonies are well spaced, large, and circular in shape and with good contrast from the background. When these assumptions are violated, most automatic colony analysis systems can rapidly lose reliability, accuracy and utility.

To address the above problems, the goal of this study is to design and implement a cost-effective, software-centered system that accepts general digital camera images as its input, for detecting as well as enumerating bacterial colonies in a fully automatic manner. An interactive semi-automatic system is also proposed to overcome any error from fully automatic system.

In this study were considered 26 images, 21 them obtained in the existing library and the other 5 created from the beginning. The two types of image, have capturing systems different, consequently the two types of images are different. The pre-processing allows the construction of an image only with the Petri dish, removing noise and the background. This step allows also, the separation of the image in two parts, one of them containing the central area and the other one the rim area, and prepares them to the segmentation stage. The segmentation enables the extraction of the colonies from the central area as the rim area. In the images obtained for this method, this extraction on central area is realized by a binarization with a fixed value for the threshold. In the rim area, a binarization is used too, combined with information about the major and minor axis length, eccentricity and areas from the image objects. In the other library, the segmentation is performed using a bottom-hat filtering in both the central area as in the rim area. Information about major and minor axis length, eccentricity and areas

from the objects are also used in the rim area. After that, the colonies segmented are separated in two images. One of them containing the isolated colonies and the other one the clustered colonies. This separation is performed by the eccentricity of the objects. To finalize, the user chose the counting method. To separate and count the clustered colonies, the automatic system uses a watershed transformation and the interactive system uses the user's input.

The proposed systems are capable to reduce the manpower and time required for counting colonies. The proposed automatic system has difficulty counting colonies in the area of the rim, causing it to have a significant number of non-colonies counted. The interactive method, correct all the problems of the automatic method, producing results similar to the manual count.

Keywords: Colony Counter, Colony Forming Unit, Colony Segmentation, *Escherichia coli*, Image Segmentation, Interactive Methods, Petri Dish.

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Contents

Resumo	v
Abstract	vii
Agradecimentos	ix
Contents	xi
List of figures	xiii
List of tables	xv
Chapter 1	1
Introduction	1
1.1. Motivation	3
1.2. Background	3
Chapter 2	7
Experimental procedure	7
2.1. Bacteria culture	7
2.1.1. Procedure for images of type A.....	8
2.1.2. Procedure for images of type B	9
2.2. Image acquisition	10
2.3. Graphical user interface	11
Chapter 3	13
Colony counting methods	13
3.1. Automatic method.....	13
3.1.1. Preprocessing and segmentation for type A images	14

3.1.2.	Pre-processing and segmentation for type B images	19
3.1.3.	Clustered colony partition	22
3.1.4.	Colony counting	24
3.2.	Interactive method.....	27
3.2.1.	Colony counting	27
Chapter 4	31
Results and discussion	31
4.1.	Methods used in statistical analysis.....	31
4.2.	Results of Automatic method.....	33
4.3.	Results of Interactive method.....	35
4.4.	Evaluation.....	37
Chapter 5	47
Conclusion and future research	47
References	51
Appendix A	53
Appendix B	57
Appendix C	61
Appendix D	63

List of figures

Figure 1: Petri dish with <i>Escherichia coli</i> bacterial colonies.	2
Figure 2: a) Image produced from the beginning for this study (type A) and b) Image obtained from the existing library (type B) [14].	7
Figure 3: Example of an image taken with a dark background and without illumination from below.	10
Figure 4: Image capture system created by Chiang et al [14].	11
Figure 5: Graphical user interface created for the colony counting.	12
Figure 6: a) grayscale result of original image; b) result of the application of median filter.	15
Figure 7: result of binarization using Otsu’s method as threshold.	15
Figure 8:(a) grayscale image of Figure 2(a); (b) mask of Petri dish visualized in (a); (c) image of Petri dish extracted from background obtained as product of (a) and (b); (d) Binarization of (c); (e) mask of central area obtained after hole-filling of (d); (f) image of central area obtained by the product of (c) and (e).	16
Figure 9: image of rim area obtained by subtracting Figure 8 (c) from Figure 8 (f).	17
Figure 10: (a) result of adjusted image intensity values from Figure 8 (f); (b) tresholding results of (a).	18
Figure 11: (a) binarization result of Figure 9; (b) rim elimination results of (a).	18
Figure 12(a) grayscale image of Figure 2(b); (b) mask of Petri dish visualized in (a); (c) image of Petri dish extracted from background obtained as product of (a) and (b); (d) Binarization of (c); (e) mask of central area obtained after hole-filling of (d); (f) image of central area obtained by the product of (c) and (e).	20
Figure 13: (a) result of the application of a close operation on Figure 12(f); (b) result of a difference between Figure 11(f) and (a); (c) thresholding results of (b); (d) final result of elimination of the small non-colony objects of (c).	21
Figure 14: (a) image of rim area obtained by subtracting Figure 12 (c) by Figure 12 (f); (b) results of bottom-hat transform of (a); (c) binarization of (b); (d) final image of rim elimination using properties of objects (eccentricity, major and minor axis length and area).	21
Figure 15(a) original of type A; (b) result of sum of Figure 10 (b) with Figure 11 (b); (c) original of type B; (d) result of sum of Figure 13(d) with Figure 14(d);	22
Figure 16: (a) isolated colonies; (b) clustering colonies.	23
Figure 17: clustered colony.	24
Figure 18: (a) enlarged example of watershed operation; (b) final result after eliminating the small non-colony pixels.	25
Figure 19: (a) example of a clustered colony with a big area (inside the black box); (b) result of watershed operation for the clustered colony inside the black box.	26

Figure 20: final result of automatic count.	26
Figure 21: (a) beginning of interactive method, where the colonies in green are counted; (b) result of interactive count, where the yellow points are the user's input.	28
Figure 22: (a) final result of interactive method of a simple situation; (b) final result of interactive method of a complex situation.	29
Figure 23: result of automatic count.	34
Figure 24: result of automatic count.	34
Figure 25: result of automatic count.	34
Figure 26: result of interactive count.	35
Figure 27: result of interactive count.	36
Figure 28: result of interactive count.	36
Figure 29: a) result of proposed automatic system; b) result of OpenCFU system; yellow boxes represent colonies not counted.	38
Figure 30: a) original image; b) result of binarization.	39
Figure 31: a) result of proposed automatic system; b) result of OpenCFU system; yellow boxes represents colonies not counted.	39
Figure 32: a) and b) are results of interactive system; blue boxes represents the colonies counted that previously on yellow boxes were not counted.	40
Figure 33 :counting times of manual method and interactive method relatively to the 21 images obtained by Chiang et al. [14].	43
Figure 34: counting times of manual method and interactive method relatively to the 5 images obtained for this study.	44

List of tables

Table 1: Table of confusion summarizing colonies counted manually and automatically.	32
Table 2: statistical results of 26 images.	37
Table 3: statistical results od 26 images, comparing the results of central and rim area.	41
Table 4: percentage of time reduction for the 21 images obtained by Chiang et al. [14].	43
Table 5: percentage of time reduction for the 5 images obtained for this study.	44

Chapter 1

Introduction

The growth and maintenance of bacteria on agar plates (Petri dishes) has long been a common practice in microbiology. The Colony Forming Unit (CFU) assay is universally recognized as the gold standard method for measuring the effect of radiation on cell viability, environmental control, food and beverage safety assessment and clinical laboratory exams. A significant example is the monitoring or quality control of drinking water, where bacteria such as *Escherichia coli*, Enterococcus, Cryptosporidium and fecal coliforms are the main indicator of microbiological water quality for human consumption [1]. The culturing process starts by inoculating the specimen to be examined on the agar, thus a solution of the specimen is spread over the agar surface. After inoculation, bacterial cultures are incubated to reproduce good conditions for pathogens bacteria growth. Figure 1 shows a Petri dish with several *Escherichia coli* colonies.

The number of colonies in a culture is usually counted manually to calculate the concentration of bacteria based on the assumption that each colony has raised from one single bacterium (colony forming unit, CFU). However, this process is time-consuming (sometimes, the human who counts the colonies needs to perform the procedure during many hours or even days), tedious (it is a monotonous procedure) and error prone (with the fatigue, the human being has more tendency to fail the evaluation). The obtained counting results depend on the human conducting the count. This variability is one of the sources of error in the colony counting process that, along with methodological differences between different laboratories or even within a laboratory, can result in

considerable fluctuations in results [2]. Due to this, for cultures with high density of colonies, manual counting mostly uses estimation methods, making an extrapolation from a small section of the Petri dish. Automating the detection, counting and analysis of CFU offers significant benefits to eliminate the risk of subjectivity, bias and human.

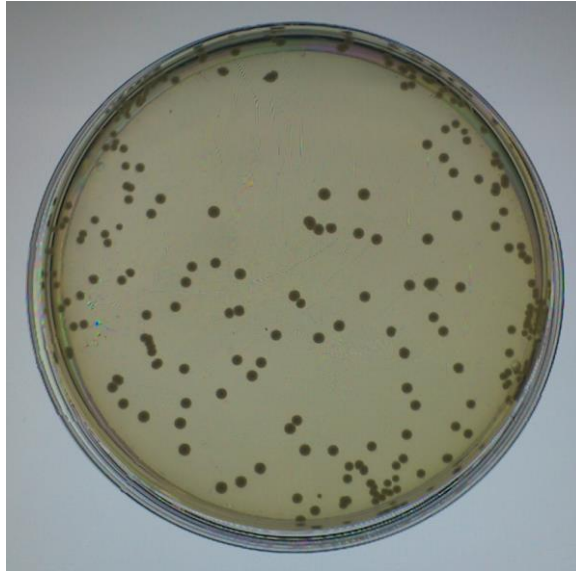


Figure 1: Petri dish with *Escherichia coli* bacterial colonies.

Commercial products exist to facilitate accurate colony counting, ranging from manual counting aids (e.g., counting pens) to all-in-one platforms including image acquisition, processing, and analysis. However, fully automatic counting systems also capable of batch processing multiple images at once can be prohibitively expensive for small labs and large facilities may necessitate multiple counting instruments posing a significant budgetary challenge to many laboratories [3]. Yet, with the development of digital cameras and document scanners alternatives to commercial products has been proposed showing that it is not necessary to use costly hardware and imaging system to easily collect the images of bacterial colonies.

1.1. Motivation

Most automatic counting systems perform adequately when the colonies are well spaced, large, circular in shape and with good contrast from the background. When these assumptions are violated, most automatic colony analysis systems can rapidly lose reliability, accuracy and utility. These obstacles include the need to handle confluent growth or growth of colonies that touch or overlap other colonies; the identification of each colony as a unit in spite of differing shapes, sizes, textures, colors and light intensities; the exclusion of colonies around the periphery of the plate reducing statistical accuracy.

To address the above problems, the goal of this study is to design and implement a cost-effective, software-centred system that accepts general digital camera images as its input, for detecting as well as enumerating bacterial colonies in a fully automatic manner. An interactive semi-automatic system is also proposed to overcome any error from the fully automatic system. The proposed systems are capable to reduce the manpower and time required for counting colonies while producing correct colony counting.

1.2. Background

In different fields of microbiology, immunology and cellular biology, counting colonies of cells growing on agar plates is routine. However, everyone who has already counted colonies knows that this is hard work which takes a lot of time. Many groups have thought about an improvement of the counting system and the cited publications certainly won't cover all attempts, but no method has achieved a widespread use at all.

The colony counting can occur using different approaches, and direct or indirect methods. An indirect method is the traditional plate count method, often preferred because it is cheap. Some technologies can be used for quantification the microbial growth, such as membrane filtration, ATP bioluminescence, direct epi-fluorescent filter microscopy, spiral plating and membrane laser scanning fluorescence cytometry [4]. It

should not be forgotten that the automatic counter methodology will be only an asset, if provides an excellent correlation with the results that would be obtained by a specialist.

The development of automatic counting methods should take into account potential sources of conflict: confluent growth, colonies that touch or overlap the surrounding, and be able to identify and count as being of a different group each colony according the shape, size, texture, color or light intensity. In addition, such methods must be capable of rejecting common artefacts such as imperfections in the agar, dust and edges of Petri dishes. This tool must be designed to deliver a high degree of accuracy in the count, and it is required reliability and reproducibility.

The previously developed automatic colony counting methods relied on various image processing/analysis techniques for automatic detection and counting of colonies on agar media. Corkidi, et al. [5] presented a method that counts bacterial colonies exploring the properties of the surface of microbial colonies. The colonies found in Petri dishes are illuminated so those properties are highlighted. A multilevel threshold algorithm is used to separate and count colonies. This technique does not count colonies on the dish boundary. Marotz, et al. [6] proposed a computer system to detect the microbial colonies in agar Petri dishes with a pre-processing stage composed by the detection of the counting region, image scaling and thresholding. The recognition system is composed by parameter calculation, application of fuzzy logic to determine the local maxima and a measure of goodness to select pixels as potential objects centers, which are used in the final counting. Barber, et al. [7] presented a technique for counting the number of cell colonies when subjected to a certain dose of radiation. The proposed method uses a modified Hough transform, and was designed in order to deal properly with merged or nebulae colonies, which had not been achieved by any other method until then. The results were compared to the counts made by four human experts, and they came to the conclusion that the performances are statistically identical. Dahle, et al. [8] employed a flatbed scanner to count colonies in 12 Petri dishes at a time. After staining, the Petri dishes were put on the specially designed racks used to fix the dishes in the same position from experiment to experiment and decrease shading. Zhang & Chen [9] presented an automatic colony counter for bacterial colony enumeration without any human intervention. Although it has high accuracy in images with coloured colonies, it has problems with those with transparent media. Niyazi, et al. [10] developed the Clono-Counter, which uses three parameters, namely grey level, maximum area of one colony, and grey level distribution within the colony, for colony

counting. Users need to have some experience to find suitable parameters, but some guidelines are provided to speed up the process. Clarke, et al. [11] proposed a low-cost, high-throughput colony counting system consisting of colony counting software and a consumer-grade digital camera or document scanner. The software NICE (NIST's Integrated Colony Enumerator) reads standard image formats, and therefore may be used in conjunction with many imaging systems. Brugger, et al. [12] used a Bayes classifier that is applied to count the final number of bacterial colonies. This step is necessary as some of the colonies are concatenated to form larger groups. Geometric properties such as ratio between major and minor axis of the group are used to verify the number of colonies contained in the group. The colonies that touch the agar boundary were removed. The results were highly correlated with the ones obtained from manual counting. The OpenCFU program created by Geissmann [13] provides control over the processing parameters and can be used to count cell colonies and other circular objects. Chiang, et al. [14] used an image capture system that in order to accentuate the region of interest and provide a good contrast between colonies and background, the plate was illuminated from below by a LED panel light due its uniform illumination. This method could extract colonies from the rim area, using the bottom-hat transformation. To separate clustered colonies, a watershed transformation was performed. The results were highly correlated with the ones obtained from manual counting.

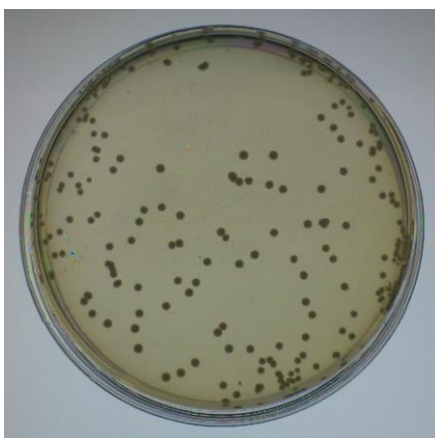
Chapter 2

Experimental procedure

In this chapter, it will be explained how the Petri dishes are prepared, and the importance of this preparation, as well as the way of acquiring them. These are very important steps, because if the images do not have quality, the final result could fail in detecting the number of colonies.

2.1. Bacteria culture

For this study, were considered two types of image: type A, which was produced purposely for this work shown in Figure 2(a) and type B obtained from the existing library shown in Figure 2 (b).



(a)



(b)

Figure 2: a) Image produced from the beginning for this study (type A) and b) Image obtained from the existing library (type B) [14].

2.1.1. Procedure for images of type A

For the first type of image (type A), *Escherichia coli* were selected for the experiments. The technique used for the bacterial culture was the spread plate technique. The spread plate method is a technique to plate a liquid sample containing bacteria so that the bacteria are easy to count and isolate. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

The first step to perform the spread technique was the production of a growth medium. The cultivation and microorganism growth in a laboratory are made in a nutrient material designated by medium culture or growth medium, which should cover the nutritional needs of the growing microorganism which is intended. Generally nutrient sources are necessary, as carbon, nitrogen, minerals and growth factors. These mediums can be prepared or purchased commercially ready for use. Regarding physical state, the growth medium can be solid, semisolid or liquid and can be implemented in petri dishes or test tubes. In this spread technique, the physical condition used was the solid, which are usually obtained by adding a solidifying agent (agar) to a liquid medium. The agar has unique properties; it liquefies at about 100 ° C and maintaining this state until a temperature of 40 ° C. The firm surface of these mediums can enable growth of colonies of individualized way. Therefore, to prepare the growth medium ten Petri dishes were used; each one containing 20 mL of the solution. To produce the solution, 250 mL of growth medium was used (only 200 mL of medium would be needed, but it was used as precautionary measure in case of a mishap). The medium contained 7g of Nutrient Agar in 250 mL of distilled water. It was necessary to adjust the pH of the solution and then to autoclave it for 15min, at 121°C. To complete the procedure, 20 mL of the medium was distributed in each petri dish.

After this procedure, it was required to do the dilution. First, 1 mL of a solution containing *Escherichia coli* was placed in a test tube then the tube was filled with 9 mL of Rengar, so a 1:10 dilution was obtained. Next, 1 mL of this new solution was collected and this was inserted in a new test tube containing 9 mL of Rengar (now, the dilution was of 1:100). The procedure was repeated until a certain dilution, selected by the user, to obtain a number of colonies in Petri dish between 40 and 200. Hill [15], in

his paper affirm that to ensure statistical accuracy, the number of colonies must be between 40 and 200. This number allows to get a number of colonies not too large to count completely and the size of colonies must be large enough to facilitate isolation. Then, using a micropipette 10 μ L of an each dilution was inserted in its respective Petri dish containing nutritive agar (previously prepared). This small amount was spread, with a spreader, until the culture become uniform. The spreading must be done uniformly, in order to colonies in all area of the Petri dish. To finish, the culture was placed in an incubator for 24 h at 37°C.

When it is desired to cultivate a culture of a single microorganism (like in this case) it is important that do not exist contamination from the mediums and materials used in the manipulation of the culture and the environment, that is, that work be executed in sterile conditions. All aseptic precautions must comply with two fundamental principles: not to allow micro-organisms in the lab contaminate samples and not allow the cultures being studied contaminate the lab. Consequently, this technique was performed in a laminar flow hood (machine that provides an aseptic work area while allowing the containment of infectious splashes or aerosols) and all the objects that contacted the cultures were previously sterilized.

2.1.2. Procedure for images of type B

For the second type of image (type B) (Figure 2 (b)), obtained in the literature, the procedure was similar to the explained before. Chiang et al. [14] used *Escherichia coli* as microorganism and the spread technique for culture the bacteria colonies. To obtain a single strain of bacteria from the specimen broth, the specimen broth was first smeared onto a Petri dish. After a period of culturing when the colonies could be observed visually, the target strain was selected and placed in a test tube with nutrients for culturing. The broth was then diluted to a ratio of 1:9. Following repeated dilution, 1 mL of the broth was extracted and smeared with a sterilized spreader on a Petri dish, which was placed within an incubator and cultured for 10 to 15 h at 37 °C.

2.2. Image acquisition

The objective of this subchapter was demonstrate that it is possible count colonies in an image without having complex acquisition software and allows the user, in any situation, to get an image of a Petri dish and get the count of colonies present therein.

Regarding the image acquisition, for the Type A images, it was done by a normal cell phone. The Petri dish was placed over a white light, with the purpose of illuminating this. Then the photo was taken with a 5 megapixel cell phone. The result is shown in Figure 2 (a). Before that, was experimented a dark background without illumination from below (Figure 3).



Figure 3: Example of an image taken with a dark background and without illumination from below.

It is possible observe in Figure 3, that the image presents some reflexes. That would be a problem during the phase of image processing, making the segmentation of the colonies a difficult process.

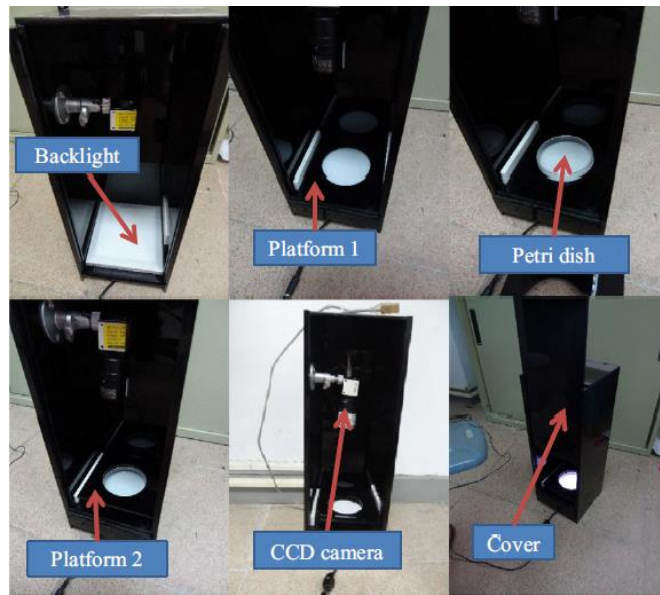


Figure 4: Image capture system created by Chiang et al [14].

Chiang et al. [14] did an image capture system to obtain the culture photos (Figure 4). In order to accentuate the region of interest and provide adequate contrast between the colonies and the background, the plate was illuminated from below by a LED panel light, due to its uniform illumination and thin shape. Platform 1 was then used to fix the position of the plate. Since the plate is covered with a lid with a diameter slightly bigger than that of the plate, to take the image inside the plate, platform 2 with the same diameter as the bottom plate was placed just above the lid to exclude the image of the lid.

The main purpose of the design of this apparatus is to obtain the images inside the periphery of the plate, including the edge of periphery. Finally, external light was blocked with the cover, and a CCD camera with 1.3 million effective pixels (1280 (H) \times 1024 (V)) was used to capture the images [14].

2.3. Graphical user interface

To facilitate the interaction between the user and the MATLAB code, a Graphical user interface (GUI) was created (Figure 5). This GUI is created through the GUIDE, which is defined as Graphical User Interface Development Environment. It is a development tool for graphical interfaces by default it runs on Matlab. Creates the code concerning the creation of various objects automatically and allows the call to all functions of Matlab or that the user had built. When saving or run in the figure, are

created by default, two files, .m e .fig. The first contains the code developed interface. The second can be used to change the visual aspect of the application through the GUIDE. For each object a callback function is created. Here it should be put the code that want to run when the object is called.

A main objective of this work was to create software that is easy to use for anyone, be it the expertise about image processing or not. The literature has already colony counting software, such as NICE and OpenCFU, which are adaptive applications, it means, there are certain parameters such as the threshold, which can be adjusted depending on the image displayed. This can be seen as an advantage of the software but if the user does not have a background in image processing may become difficult to use the software. For this study, it was then created non-adaptive software where the user only needs to load the image using the "Load" button, and choose the counting method. If the user presses the "Automatic" button get a completely automatic counting and if the user presses the "Interactive" button then this gets a semi-automatic count, and it will be need the user's input on the image to count the colonies not counted. The result of the count is shown in a text box and to close the application there is a button "close". This makes it easy to use for anyone and can be used by people with no background in image processing.

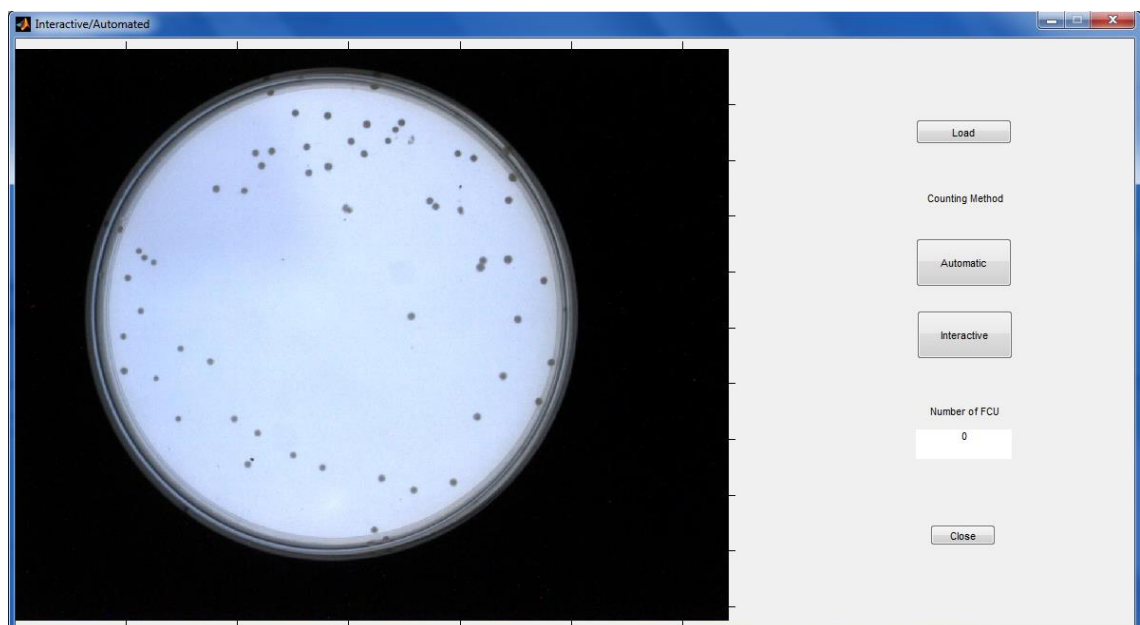


Figure 5: Graphical user interface created for the colony counting.

Chapter 3

Colony counting methods

3.1. Automatic method

The next subchapter represents the methods used to count colonies automatically. After opening the image on the application (Figure 5), the user can opt if they want an automatic count or an interactive count. Choosing the automatic method provides the user to obtain the colonies counted very quickly. Next, it is presented the cluster colony partition, where the isolated colonies are separated from the clustered colonies and the colony counting where it is explained how the automatic method counts.

Before performing the pre-processing, it was necessary to distinguish the two types of images because they had different characteristics. Type A images had a light background shown in Figure 2 (a) and type B had a dark background shown in Figure 2(b). Therefore, the first step of the whole method was to estimate the mean of the 3 components of the image (components RGB). It was noted that the mean of the 3 components for the type A images was bigger than 120 (the grayscale of the images range from 0 to 255). So, this was the parameter chosen to distinguish the two types of images. After the distinction, each type of image had its own pre-processing and segmentation.

Here, the central area and the rim area of image will be separated. This approach performed by Chiang et al obtained results of excellence. To extract colonies around the rim from the background, the required image pre-processing techniques are more

complex than those used for the center. Then, the pre-processing of the image is divided into two parts: the area in the center of the image and the rim area.

3.1.1. Preprocessing and segmentation for type A images

In this study, the RGB image (color images) (Figure 2 (a)) was converted into a grayscale image (Figure 8 (a)). The goal of this method (pre-processing and segmentation) was to divide the images of the Petri dishes into two parts, the central area and the rim area, from the image, and to segment the colonies (in the image where the colonies appear in white and the background in black) and it was based on reasoning used by Chiang et al [14]. Geissmann Q [13] and Clarke et al. [11] in its methods (Open CFU and NICE, respectively) did not separated the image in two parts, they divided the image in sub-regions.

First, masks from the original images were created with the purpose of multiplying them with the grayscale image to remove the background. To get the mask, a median filter was applied, with the goal to remove pixel noise, Clarke et al. [11] used a Gaussian smoothing function and Geissmann Q [13] used a median filter. In the Figure 6, it is possible to the result of the application of the median filter on a) obtained b). Median filtering is a nonlinear operation often used in image processing, because simultaneously reduce noise and preserve edges. The main idea of the median filter is to run through the image pixel by pixel, replacing each pixel with the median of neighboring pixels. The next phase was threshold binarization. To perform this, it used a fixed value of threshold, 0.50, since this worked very well in all 5 images. After some image corrections (a negative was applied, then the borders were cleared and the holes were filled) the mask was obtained (Figure 8 (b)). To remove the background, the mask (Figure 8 (b)) was multiplied by the grayscale image (Figure 8 (a)) yielding Figure 8 (c).

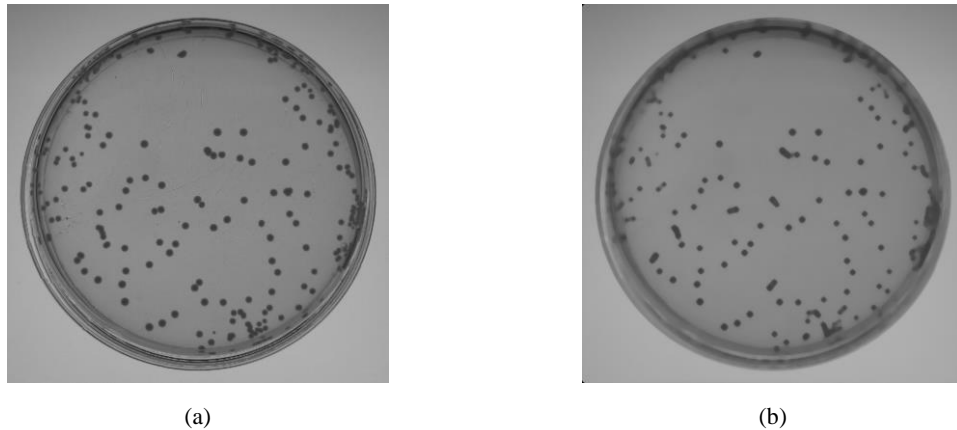


Figure 6: a) grayscale result of original image; b) result of the application of median filter.

The second step of the method was to separate the central area of the Petri dish from the rim area. The grayscale image in Figure 8 (c) shows that the background of the rim area is darker than that surrounding the central area, therefore, threshold binarization can be used to separate the central area from the rim area. The value of threshold used was a fixed value (0.45). This value was chosen because worked well on the all 5 images. In the Figure 8 (c), it is possible to see the image before the binarization, and in the Figure 8 (d) the result of 0.45 as threshold. The Figure 7 shows the result of the binarization if it was used Otsu's method. Figure 8 (f) shows only the central area and it is the result of the multiplication between Figure 8 (c) and Figure 8 (e) (this image, visualized in Figure 8 (e) is the result of the filled holes of Figure 8 (d)).

With the location of pixels in the central area, the rim area can be obtained by subtracting the central area (Figure 8(f)) from the Petri dish area (Figure 8(c)). The resulting image is shown in Figure 9.

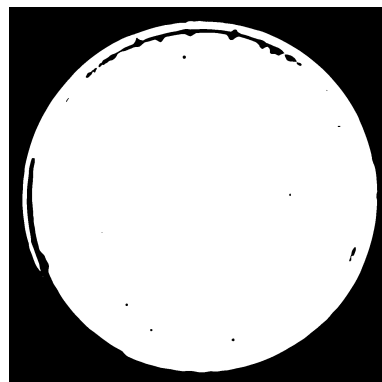


Figure 7: result of binarization using Otsu's method as threshold.

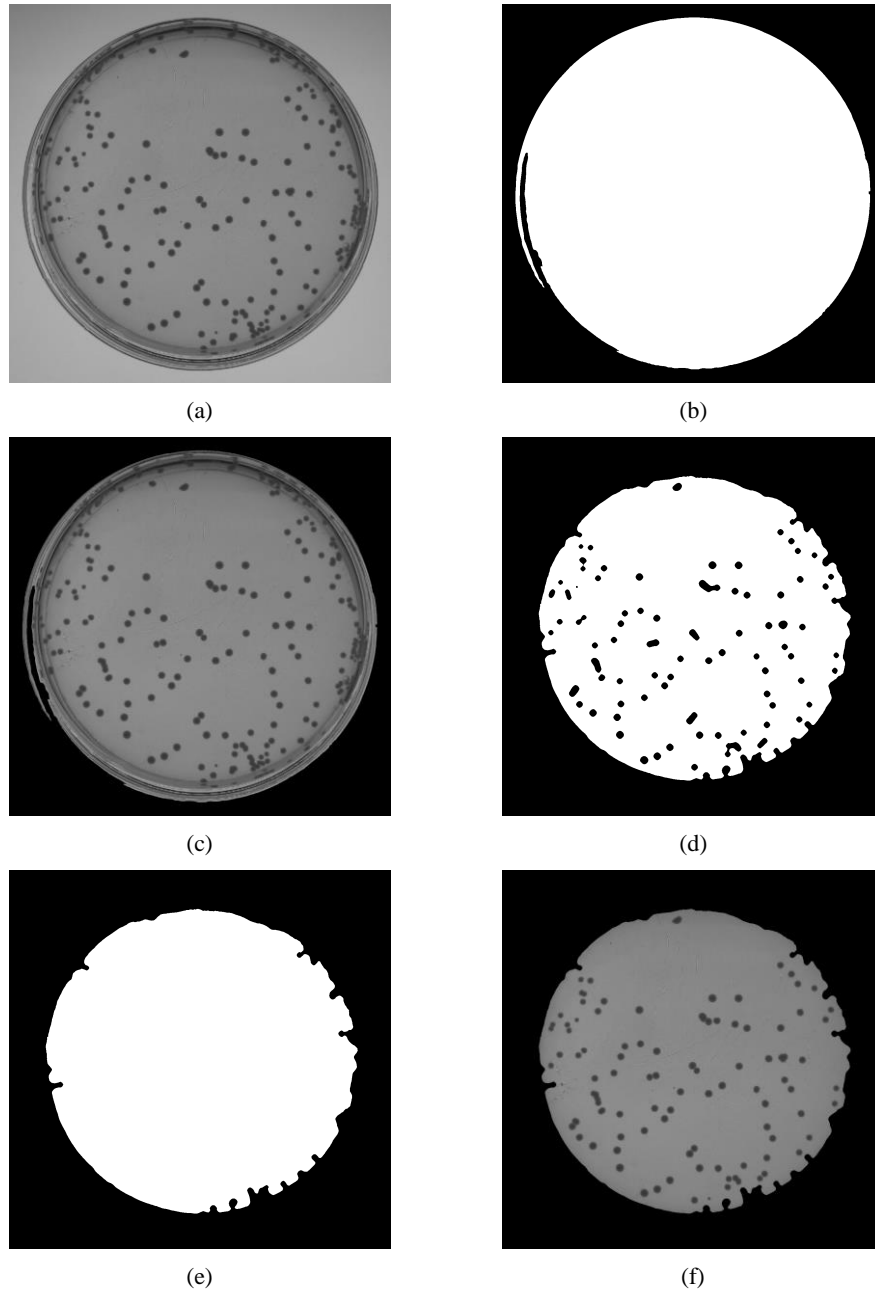


Figure 8:(a) grayscale image of Figure 2(a); (b) mask of Petri dish visualized in (a); (c) image of Petri dish extracted from background obtained as product of (a) and (b); (d) Binarization of (c); (e) mask of central area obtained after hole-filling of (d); (f) image of central area obtained by the product of (c) and (e).

To finish the goal of this step (segmentation of colonies), an intensity adjustment was applied to Figure 8(f), the result is shown in Figure 10(a). Then, a fixed value of 0.3 was used as threshold in a binarization and the borders were cleaned. It was applied an open operation where a 3 pixel radius disk to eliminate small artifacts. To finalize, a close operation with a disk of the same radius was used to add pixels which had previously been removed from the colonies. The image obtained is the final image of the central area, with the colonies in white with a black background (Figure 10(b)). The

Clarke et al. [11] system is an adaptive system, which the user can choose the thresholding method, between Gaussian distribution (standard derivation from 3 to 6), Otsu and manual.

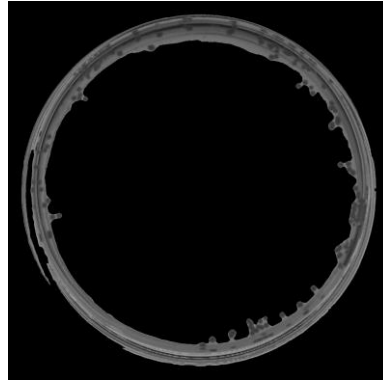


Figure 9: image of rim area obtained by subtracting Figure 8 (c) from Figure 8 (f).

To extract colonies from the rim image, the threshold binarization was applied to Figure 9, with the results shown in Figure 11(a). It was possible to see in Figure 11(a) that the image of the rim is longer and narrower than that of the colonies. Therefore, to obtain Figure 11(b) with only the colonies in white, the mean area, the eccentricity, the major axis length and the minor axis length were calculated for Figure 11(a). Then, the small areas were removed, using the mean area, only areas bigger than $\text{mean} \cdot 0.6$ were considered. An object was considered a colony if the maximum eccentricity of the object was smaller than 0.8 (where 0 is a circle, while an ellipse whose eccentricity is 1 is a line segment) and if the relation between the major axis and the minor axis was less than 1.5. It was noted, after the application of those characteristics that some pixels remained in the image with the same value for major and minor axis. These pixels were not colonies, but artifacts not eliminated by these methods. So, to remove them, every object with same value for major and minor axis was not considered. The result image is shown in Figure 11(b), which is the final image.

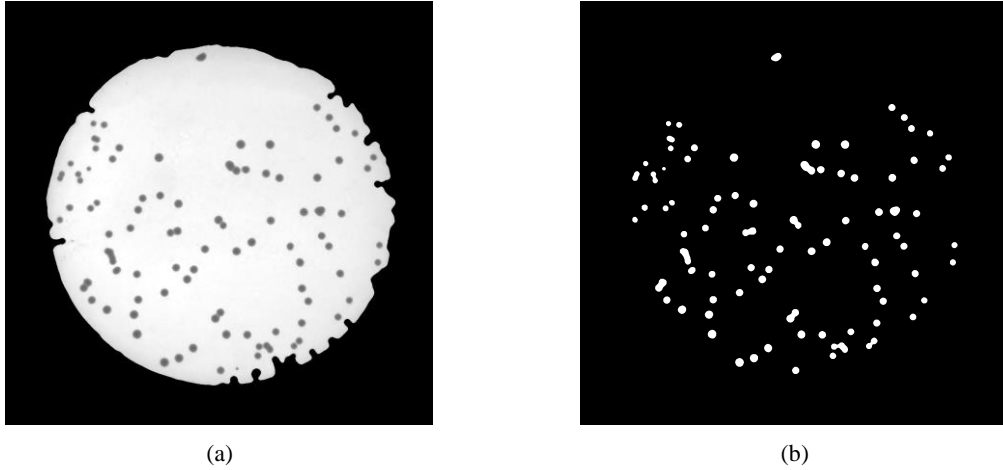


Figure 10: (a) result of adjusted image intensity values from Figure 8 (f); (b) thresholding results of (a).

The main problem of the extraction was the colonies overlapping with the extremities of Petri dish, because in these images, they have the same gray value.

In the NICE method, Clarke et al. [11] combined extended minima and thresholding algorithms to segment the colonies. The extended minima function was used to find the center of colonies which could be considered regional dark points. This regional method allowed identifies colonies based on the local contrast.

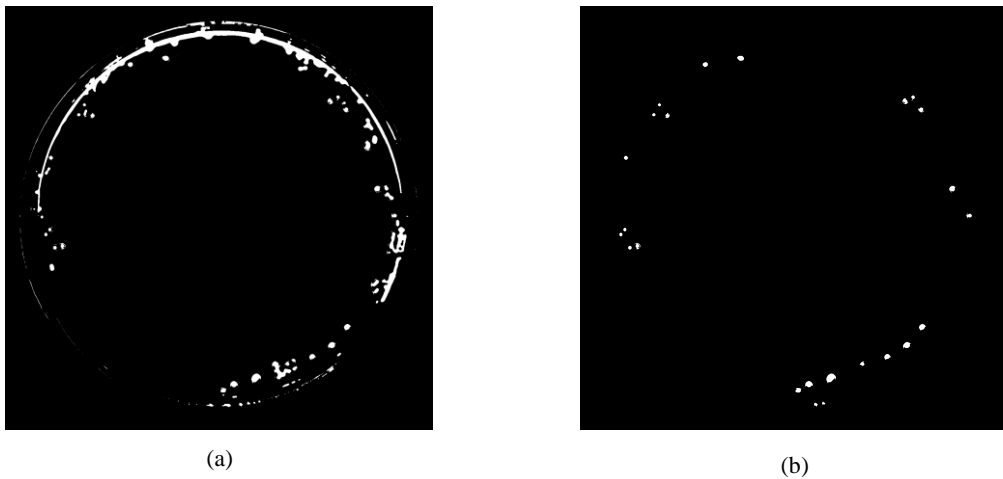


Figure 11: (a) binarization result of Figure 9; (b) rim elimination results of (a).

3.1.2. Pre-processing and segmentation for type B images

The method used in this pre-processing was similar to the previous method, based on the study made by Chiang et al [14].

Firstly, it was necessary to create a mask of the Petri dish, with the purpose of removing it from the background. This mask was created by threshold binarization of Figure 12(a). The value of threshold used was 0.98 and it was a fixed value, because this value worked well in all 21 images. To extract the Petri dish from the background, this mask was multiplied by the image in grayscale (Figure 12(a)), with the result shown in Figure 12(c). As in the previous images, the rim area is darker than the central area, so, to separate the two areas another binarization, with a fixed threshold with the value of 0.7, was applied, in Figure 12(c). The result is shown in Figure 12(d). After filling the holes, the mask of the central area (Figure 12(e)) was obtained. To get the final image of the central area (Figure 12(f)), the mask shown in Figure 12(e) was multiplied by Figure 12(c).

The segmentation of the colonies was different to the previous method. To obtain Figure 13(a), a close operation was applied, with a radius of 15 capable of cleaning the colonies from the central area. The morphological close operation is a dilation followed by erosion, using the same structuring element for both operations. This figure only represents the background of the Petri dish. Therefore, the difference between Figure 13(a) and Figure 12(f) is an image where supposedly only the colonies appear. This image is shown in Figure 13(b). This method is called a Bottom-hat filtering and is the equivalent of subtracting the input image from the result of performing a morphological closing operation on the input image. To get the final image, where the colonies are shown in white and the background in black, a threshold binarization was applied (Figure 13(c) where the pixels between 25 and 170 get the white color and the other ones the black color. Lastly small non-colony objects were eliminated by an erode operation. The resulting image is shown in Figure 13(d).

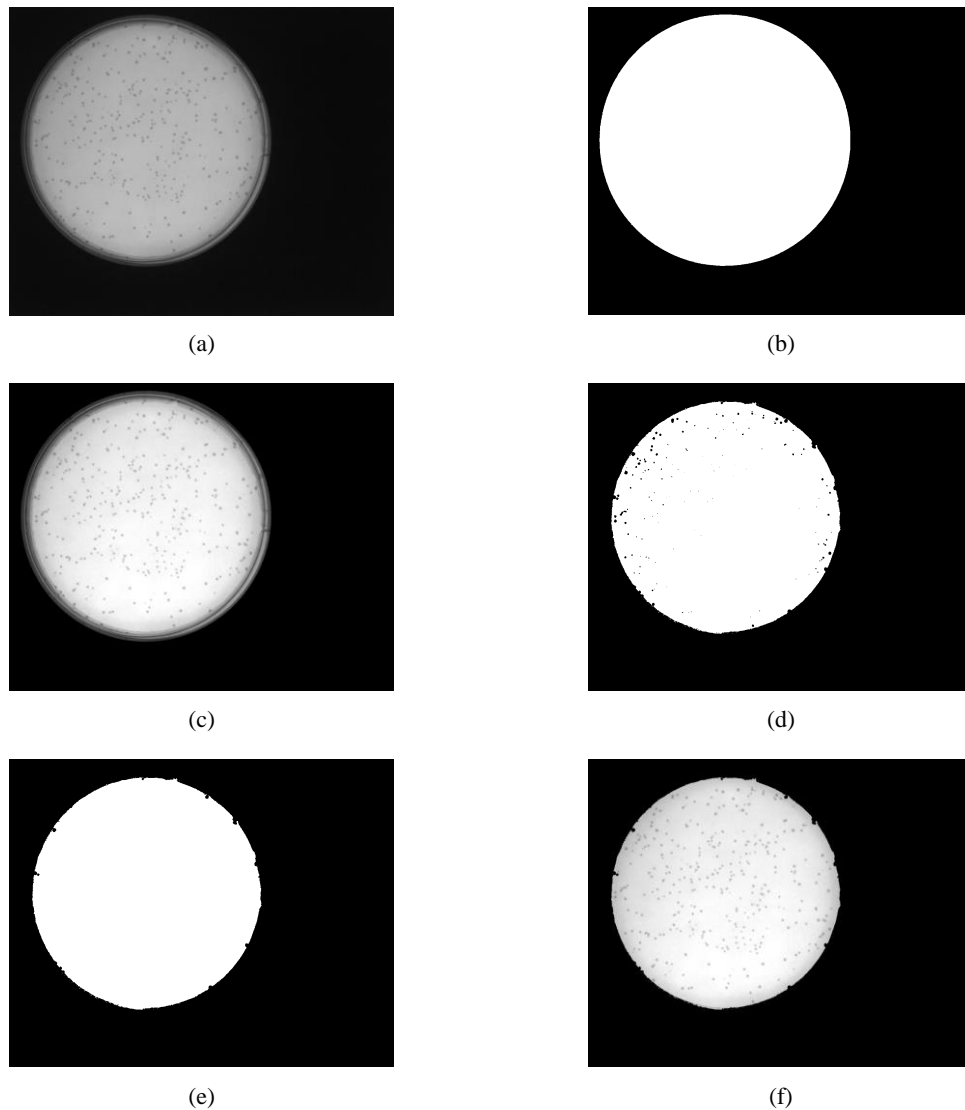


Figure 12(a) grayscale image of Figure 2(b); (b) mask of Petri dish visualized in (a); (c) image of Petri dish extracted from background obtained as product of (a) and (b); (d) Binarization of (c); (e) mask of central area obtained after hole-filling of (d); (f) image of central area obtained by the product of (c) and (e).

The rim area (Figure 14(a)) was obtained as with the previous method, by subtracting Figure 12(c) by Figure 12(f). To extract the colonies from the rim area, the bottom-hat transform was applied. This method is similar to the one used previously in the extraction of the colonies from the central area. The next step was the binarization in Figure 14(b) with the results shown in Figure 14(c). The threshold used in this binarization was estimated by the Otsu's method. This algorithm assumes that the image contains two classes of pixels following bi-modal histogram (foreground pixels and background pixels), then calculates the optimum threshold separating the two classes. To obtain only the colonies, the same line of thought that was used to extract the colonies from the rim in the previous image was used, where the eccentricity, the

major and minor axis length and the area of each object were used. The results are shown in Figure 14(d)

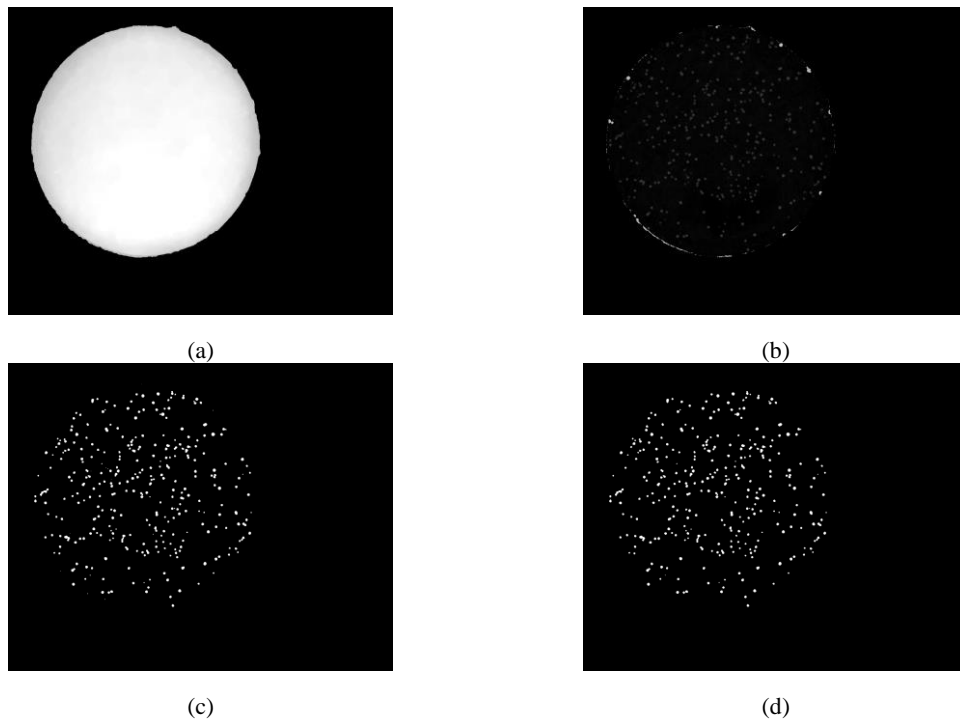


Figure 13: (a) result of the application of a close operation on Figure 12(f); (b) result of a difference between Figure 11(f) and (a); (c) thresholding results of (b); (d) final result of elimination of the small non-colony objects of (c).

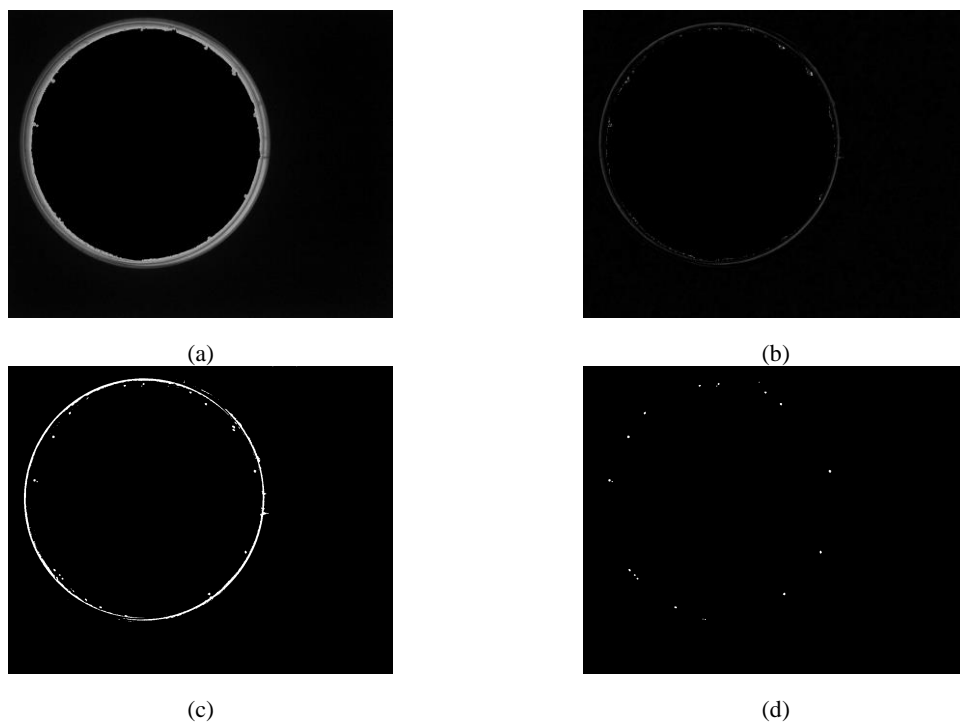


Figure 14: (a) image of rim area obtained by subtracting Figure 12 (c) by Figure 12 (f); (b) results of bottom-hat transform of (a); (c) binarization of (b); (d) final image of rim elimination using properties of objects (eccentricity, major and minor axis length and area).

The images from Figure 15 are the results of segmentation and the corresponding original image. Figures 15 (b) and (d) are the results of the sum of colonies extracted from the central area with the colonies extracted from the rim area. These two images were used in the separation phase, explained in the next chapter.

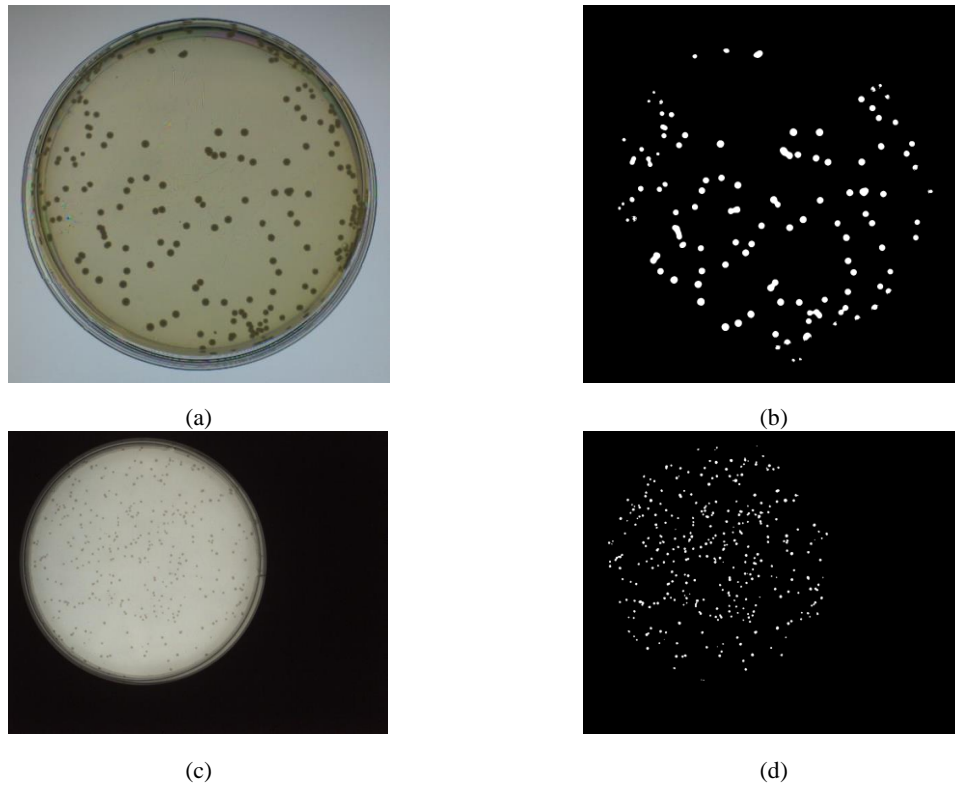


Figure 15(a) original of type A; (b) result of sum of Figure 10 (b) with Figure 11 (b); (c) original of type B; (d) result of sum of Figure 13(d) with Figure 14(d);

3.1.3. Clustered colony partition

In this phase the objective was to create two different images, one of them contains the isolated colonies (Figure 16(a)) and the other containing the overlapping/clustering colonies (Figure 16 (b)).

To obtain these results, the eccentricity and the area of each colony of the segmented image (Figure 15 (b)) were calculated. The line of thought used to separate the colonies was the fact that a colony is almost a perfect circle, so its eccentricity was low. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length and its value is between 0 and 1. An ellipse whose eccentricity is 0 is

actually a circle, while an ellipse whose eccentricity is 1 is considered a line segment. The mean of eccentricity of a normal colony has a value close to 0.25, and normally a clustered colony has a value higher than 0.5. In Figure 17, it is possible to see a clustered colony with a value of eccentricity of 0.8. Normally, the area of a clustered colony is higher than the mean areas of all colonies. Therefore, with these two characteristics, if an object in an image was an eccentricity higher than 0.5 or an area of an object was higher than the mean of the areas plus half of the mean areas, this object was considered a clustered colony, if not the object was an isolated colony.

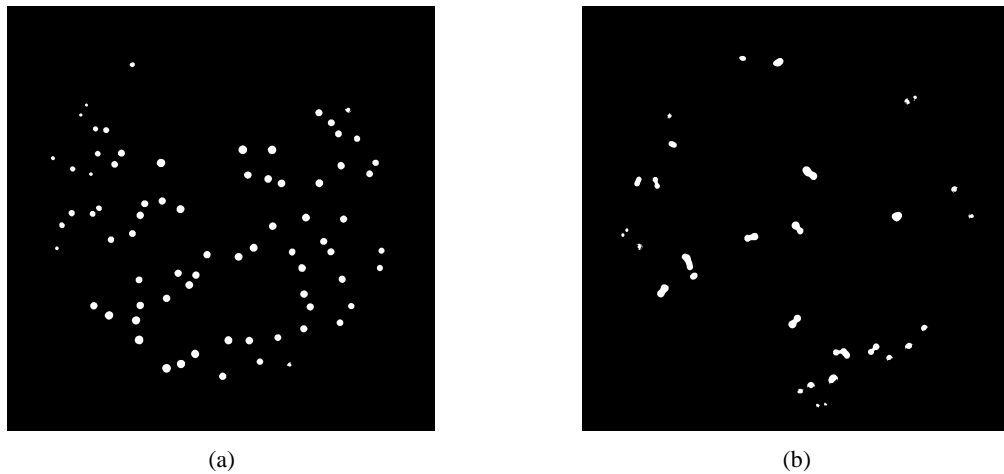


Figure 16: (a) isolated colonies; (b) clustering colonies.

This method that uses the eccentricity worked well, although some isolated colonies have the eccentricity higher than 0.5, appearing then in the image of the clustered colonies. In the case of NICE system, the minima function was used by Clarke et al. [11]. This could distinguish touching colonies using the number of centers in an object, if an object has two centers there is two colonies. The minima identified were dilated and the mean intensity within the minima of each colony is compared against the threshold, if the value was below the object was considered colony.

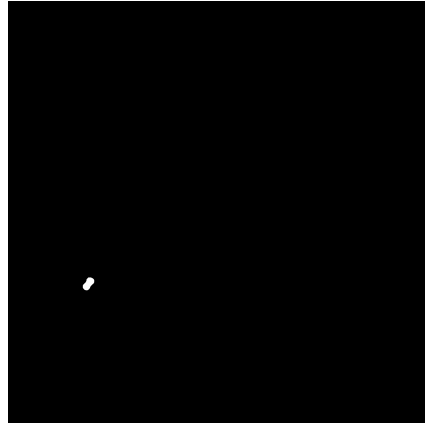


Figure 17: clustered colony.

3.1.4. Colony counting

Brugger et al. [12] to count colonies used a Bayes classifier, which is a probabilistic classifier based on Bayes theorem. It was used some properties such as ratio between major and minor axis to determine how many colonies are in the group. In the other hand, Chiang et al. [14] used watershed transform to separate the clustered colonies and proceeding then to the count. In his study, he obtained great results with this method.

To count the single colonies present in Figure 16 (a), as the colonies are all individual, was used a function that finds connected components in binary image and estimate the number of objects that exist on an image. The result of application of this function shows a structure with 4 elements. The first one is the connectivity of the connected objects, the second one is the size of the image, the third one is the number of objects presents in the image and the last one is a cell array the k th element in the cell array is a vector containing the linear indices of the pixels in the k th object. To obtain the number of objects on an image, it is necessary get the third element referent to the number of objects in an image.

To separate the clustered colonies a method which identifies and divides them was necessary. The method used in this case was the watershed transform used by Chiang et al. [14] in his study. Watershed transform is a classic and effective method for image segmentation. Single pixel holes are pierced at each regional minimum of the activity image which is regarded as topographic landscape. These pixels are calculated through the distance transform. The distance transform is an operator normally only applied to binary images. The result of the transform is a gray level image that looks similar to the

input image, except that the gray level intensities of points inside foreground regions distance are changed to show the distance between that pixel and the nearest nonzero pixel. Monteiro [16], in his PhD thesis explains the watershed transform, and inside this watershed, explains two strands: the immersion watershed and the rainfall watershed. In this study, the watershed used was the immersion watershed and it is when sinking the whole surface slowly into a lake water leaks through the holes, rising uniformly and globally across the image, and proceeds to fill each catchment basin. Then, in order to avoid water coming from different holes merge, virtual dams are built at places where the water coming from two different minima would merge. When the image surface is completely flooded the virtual dams or watershed lines separate the catchment basins from one another and correspond to the boundaries of the regions. The watershed operation computes a label matrix identifying the watershed regions of the input matrix, which can have any dimension. The elements obtained are integer values greater than or equal to 0. The elements labeled 0 do not belong to a unique watershed region. These are called watershed pixels. The elements labeled 1 belong to the first watershed region, the elements labeled 2 belong to the second watershed region, and so on. In Figure 18(a) it is possible to see a result of watershed operation on a clustered colony. The operation divided the object in 3 parts, even though visually the object seemed to have two colonies together. The small object formed after the application of the watershed, was eliminated through the mean area of the isolated colonies (Figure 16(a)). The result is shown in Figure 18(b).

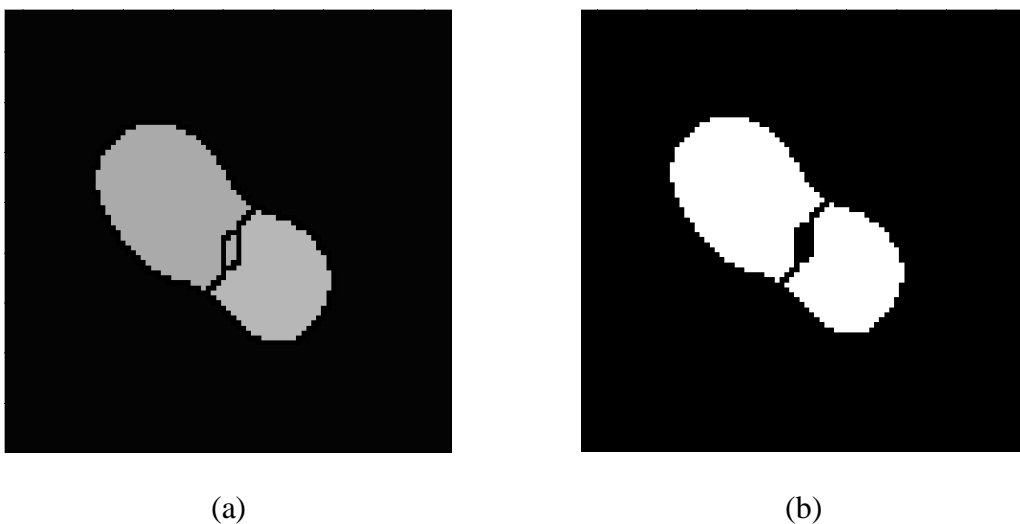


Figure 18: (a) enlarged example of watershed operation; (b) final result after eliminating the small non-colony pixels.

Although the watershed operation worked well for objects with few colonies (2, 3 or 4), when the cluster had a lot of colonies, the watershed did not divide uniformly (Figure 19(b)). To bypass this problem, the mean area of isolated colonies in Figure 16 (a) was calculated. This mean area is the mean area of an isolated colony in the related image so the big areas, like Figure 19 (b) were divided by the mean area obtained in the image with the isolated colonies. These estimated results were summed to the count of the watershed operation, and then summed to the count of isolated colonies. The final result is shown in Figure 20, where all counted colonies appear in green.

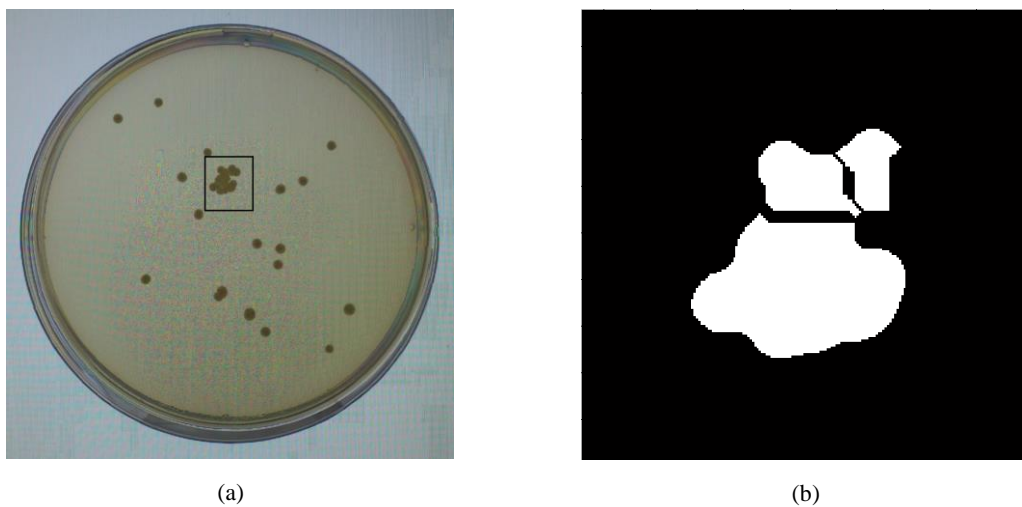


Figure 19: (a) example of a clustered colony with a big area (inside the black box); (b) result of watershed operation for the clustered colony inside the black box.

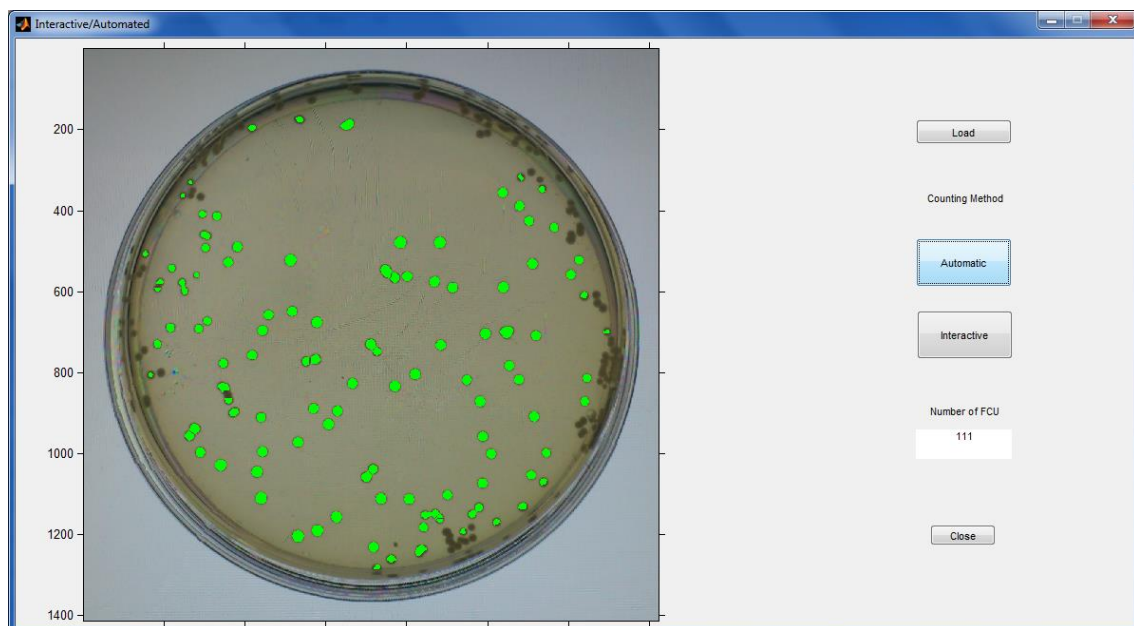


Figure 20: final result of automatic count.

3.2. Interactive method

The next sub-chapter explains the methodology for conducting interactive method. This method combines automatic parts with interactive parts between the user and the system / computer. For this method to be used, the user must press the button of the interactive method presented in the application.

The preprocessing step was the same used on the automatic method, in section 3.1.1 and 3.1.2. To separate the isolated colonies of clustered colonies, was used the methodology explained in section 3.1.3, clustered colony partition, where the eccentricity and the average of the areas of each object was used. If an object had a higher eccentricity than 0.5 or an area of an object was higher than the mean of the areas plus half of the mean areas, then the object was considered as clustered colonies (Figure 16(b)), if not it is considered a single colony (Figure 16(a)).

The followed section represents the methods used to count the colonies interactively.

3.2.1. Colony counting

The result of colony clustered partition shows two images, one of them containing single colonies and other grouped colonies. To count the image of individual colonies, the procedure is the same method used in section 3.4, Colony counting, where was used to function that finds connected components in binary image and estimate the number of objects that exist on an image.

A few seconds after the user's input on the interactive method button, the system displays an image where the single colonies appear in green and are already counted and the clustered colonies are colourless in the image and cannot be labelled (Figure 21 (a)). To count these colonies, the user has to click on the image on the clustered colonies and decide how many colonies are in the cluster. The results are shown in Figure 21 (b) where the yellow points are the user's inputs. The system saved the coordinates of the user's input as a coordinate vector. To know the number of clicks/colonies, the size of the coordinate vector is obtained and summed to the counter. The system also allows the user to delete the previous input by pressing the backspace

or delete keys for the keyboard. The point is eliminated from the picture as the count. To finish the interactive count, the user must press the enter key from the keyboard.

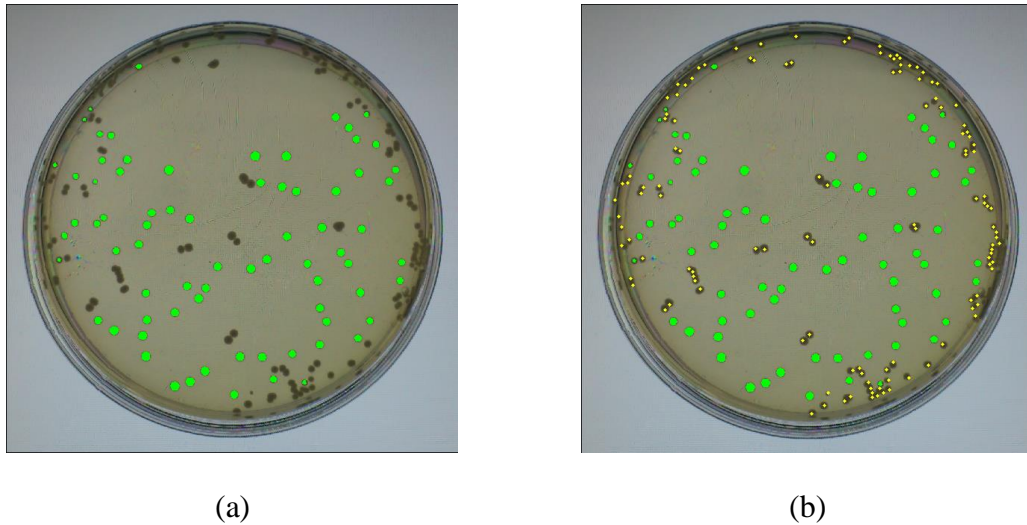
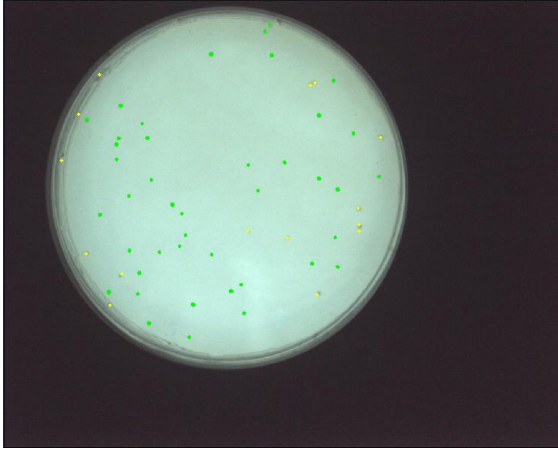


Figure 21: (a) beginning of interactive method, where the colonies in green are counted; (b) result of interactive count, where the yellow points are the user's input.

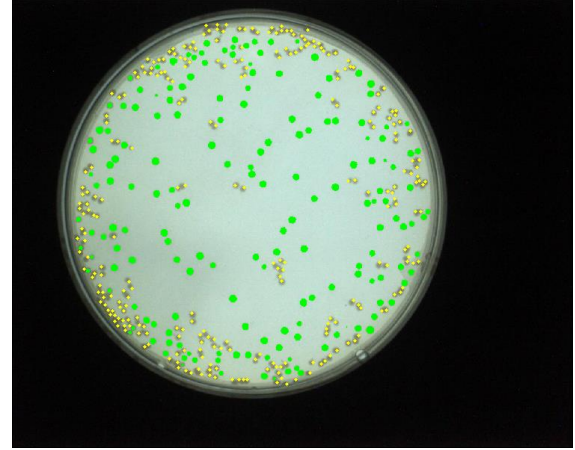
Figure 22 shows two interactive count results, being (a) considered the result of a simple count and (b) considered the result of a complex count.

In Figure 22 (a) it is observed that most of the colonies are already counted by the automatic part of the interactive method. It is observed that there are few clustered colonies in the image being constituted mainly of single colonies. It is also possible to verify that there are a reduced number of colonies at the edge of the Petri dish. In this case, the use of interactive method becomes a simple case because in 65 colonies, the user only needs to count, by click, about 15. This causes a reduction in 50% of count time, compared to manual counting.

In Figure 22 (b), there is the opposite; there are a high number of colonies per count (over 50%). This is because there are a large number of clustered colonies. Furthermore, there are also a high number of colonies at the edge. This situation causes the user had interactively to count a large number of colonies. In this case, the counting time is only reduced by 24%.



(a)



(b)

Figure 22: (a) final result of interactive method of a simple situation; (b) final result of interactive method of a complex situation.

Chapter 4

Results and discussion

The practical application of an image segmentation algorithm requires that we understand how its performance varies in different operating conditions. Evaluating algorithms allows researchers to know the strengths and weaknesses of a particular approach and identifies aspects of a problem where further research is needed [16].

In this evaluation, 26 images of colonies in Petri dishes were used. These images were counted automatically by the proposed systems and also manually by Biomedical Engineering students. To evaluate the performance of the two proposed methods, the results obtained were compared with two other automatically counting systems (NICE and Open CFU).

4.1. Methods used in statistical analysis

The number of colonies counted manually ranged from 29 to 687 and the number of colonies counted automatically ranged from 24 to 598. The counting results obtained manually were taken as the real situations, with True (colonies) and False (non-colonies) cases. The counting results obtained by the proposed automatic system, NICE and Open CFU were the outcomes of colony identification, with Positive (identified as colonies) and Negative (identified as non-colonies) cases. The four possible situations are shown in table 1, where true positive represents the correct result, false negative means a result was missing, false positive represents a result that was not supposed to appear and true negative that represents a correct absence [14].

Chiang et al. [14] in his study used the statistical results of precision, recall, f-measure and APE to evaluate his results. To compute precision and recall, it is necessary to determine which true positive pixels are correctly detected, and which detections are false or missing. The precision is related with the false positive values, and in this case they are colonies that the system identifies but that do not exist. The recall is related with the false negatives and represents the colonies that were not identified. In probabilistic terms, precision is the probability that the result is valid, and the recall is the probability that the ground truth data was detected. A low recall value is typically the result of under-segmentation and indicates failure to capture salient image structure. Precision is low when there is significant over-segmentation, or when a large number of boundary pixels have greater localization errors than some threshold [16].

Table 1: Table of confusion summarizing colonies counted manually and automatically.

Table of confusion		Manual counting	
		True	False
Automatic counting	Positive	True Positive (correct result)	False positive (unexpected result)
	Negative	False negative (missing result)	True negative (correct absence)

The equations used to obtain the precision (measure of exactness) and recall (measure of completeness) [14], are defined as:

(1)

$$\begin{aligned} \text{Precision} &= \frac{\text{Number of colonies retrieved}}{\text{Total number of components retrieved}} \\ &= \frac{\text{True positive}}{\text{True positive} + \text{False positive}} \end{aligned}$$

(2)

$$\text{Recall} = \frac{\text{Number of colonies retrieved}}{\text{Total number of existing colonies}} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}$$

The aim of the proposed systems is to get both high precision and high recall

A measure that combines precision and recall known as F-measure, is defined as:

(3)

$$F - \text{measure} = \frac{\text{precision} \cdot \text{recall}}{\alpha * \text{precision} + (1 - \alpha) * \text{recall}}$$

where α determines the relative importance of each term. Following Martin et al [17], α is selected as 0.5 expressing no preference for either.

The absolute percentage of error (APE) is the most appropriate information about average percentage errors which are used to a great extent in reporting accounting results. Makridakis S. [18] defined a new equation to obtain the absolute percentage of error, and it is defined as:

(4)

$$APE_t = \left| \frac{A_t - F_t}{(A_t + F_t)/2} \right| * 100$$

where A_t is the actual value and it is represented as the manual counting obtained and F_t is the forecast value, which is the counting obtain by the automatic methods.

4.2. Results of Automatic method

The functioning of automatic method depends largely on the type of image. Features such as the number of colonies at the edges of the Petri dish, the number of clustered colonies, and the contrast in the image interfere with the performance of the method.

In Figure 23, is shown result of the automatic counting for a relatively simple image. It is possible to observe that the colonies present spread throughout the area and widely spaced. Furthermore, there are few colonies on the edge, which facilitates the automatic counting. Another feature of this picture is that there are very few clustered colonies. In this case, the value of APE is about 0.6%, a very low, almost close to perfect.

The other results of automatic count are shown in Appendix D.

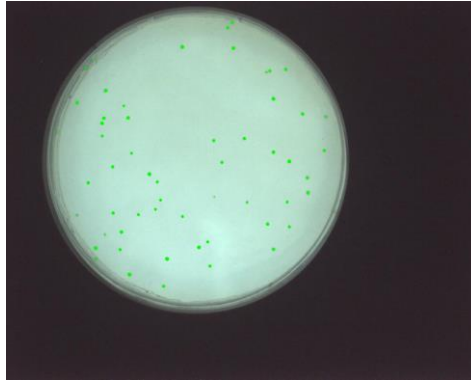


Figure 23: result of automatic count.

Figure 24 shows an image with an average degree of complexity. In this image the automatic method has an APE of 17%. This happens due to little contrast existing in the image. It presents a considerable number of clustered colonies, making it difficult to hit in the count. In this case, the number of colonies in the edge of Petri dish is low.

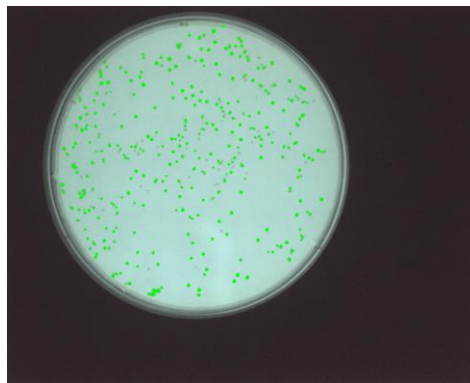


Figure 24: result of automatic count.

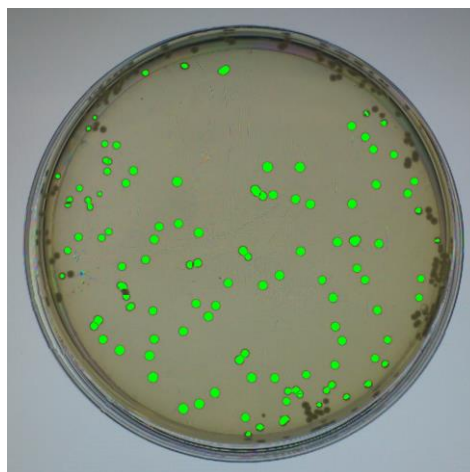


Figure 25: result of automatic count

Figure 25 shows the result of automatic counting for an image with a high degree of complexity. It is possible to observe that about 50% of the colonies are not counted by this method and APE in this case around 50%. In this image, there are many clustered colonies also are at the edge. The difficulty of the automatic method is counting colonies on the edge.

4.3. Results of Interactive method

One goal of this work was to create a method to reduce the counting time but had a percentage great success.

The interactive method, the APE is always very close to zero. So the complexity of the counts is measured as the time that each delay.

Figure 26 shows an image in where the interactive method don't present a big reduction of time. Although, it is possible to observe that the automatic part of the interactive method left a small number of colonies per count, which acilitates the work of the user. This is because there are no colonies on the edges and besides there are only two groups of clustered colonies. The results for the 26 images are presented in Appendix D.

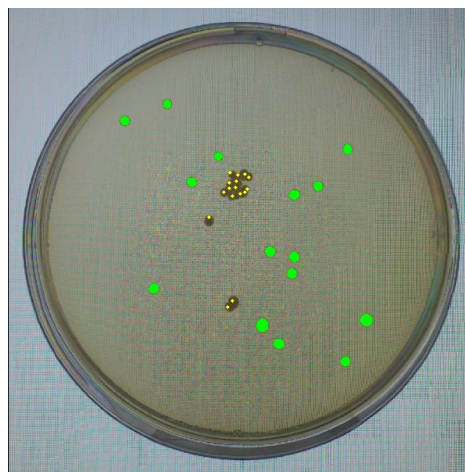


Figure 26: result of interactive count.

In Figure 27, it is possible to observe an image in which the complexity is of a medium level. While there are no colonies on the edges, automatic phase of the

interactive method provides some colonies for the user select. These colonies are mainly clustered colonies.

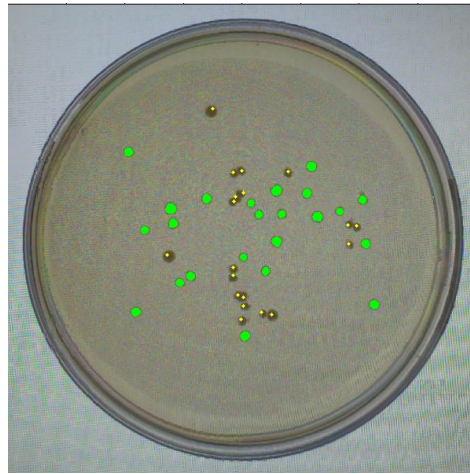


Figure 27: result of interactive count.

When the number of clustered colonies is high, is presented to the user a high number of colonies to count. This causes the small reduction of the counting time. In the Figure 28, there is shown the result of an interactive count, where a large number of clustered colonies. In this case, the user had to rely manually 366 colonies. Although a relatively large number, the user achieves a 36% reduction in the count time while maintaining accuracy.

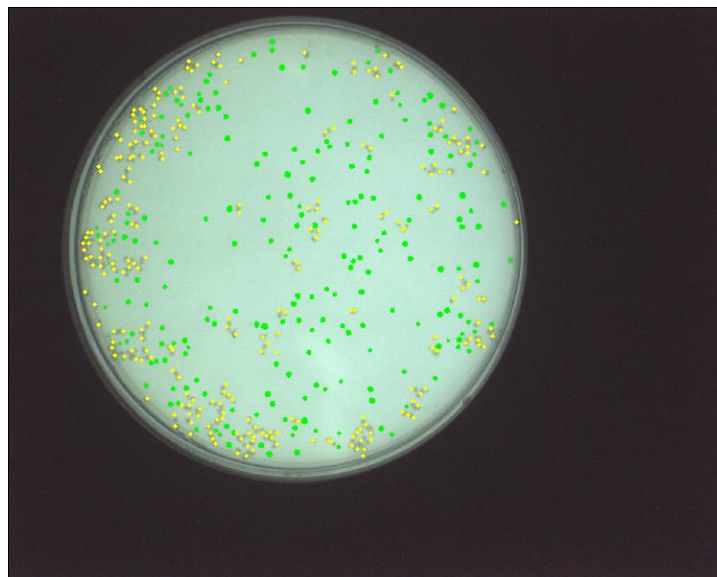


Figure 28: result of interactive count.

4.4. Evaluation

In this subchapter, it will be compared all the methods used in this study. Measures relatively to the statistical results and also time will be studied.

The Table 2 shows the results of Precision, Recall, F-measure and APE for each method, approached in this study, in relation to the 26 images. In the Appendix B it is shown the individualized results for the 26 images for the 4 methods studied (Table B1 the automatic results, Table B2 interactive results, Table B3 OpenCFU results and Table B4 NICE results). The first step to obtain these statistical values was the manual count. This was performed by different students of Biomedical Engineer. The results were obtained by comparing each manual count with the other 2 counts. To calculate the values for the other 4 methods, the automatic count of each method was obtained for the respective software. Then, this count was compared with the 3 different manual counts. The individualized results for the 26 images are shown in Appendix A. The results shown in table 2 are the mean of the calculated values.

Table 2: statistical results of 26 images.

Measure	Method				
	Manual	Automatic	Interactive	NICE	OpenCFU
Precision	0.9879	0.9848	0.9808	0.9607	0.9915
Recall	0.988	0.8698	0.9937	0.878	0.918
F-measure	0.9876	0.9188	0.9869	0.9055	0.9514
APE (%)	2.4937	16.3128	2.3272	18.914	9.7067

Observing the table 2 results, it is possible to affirm that in relation to Precision, the results are similar, although the NICE method has the lowest value. This means that the colonies counted normally are correct, being the number of false positives a low value.

In relation to the Recall the values are different. The method which presents the best result is the Interactive (0.9937), followed by the OpenCFU (0.918). Both the NICE as well as the proposed automatic method have values below of 0.90 (0.878 and 0.8698, respectively). Being the Recall the measure related with the false negatives, it is

possible to conclude that these 2 methods fail in the identification of some colonies. The OpenCFU method has an acceptable result for the recall with a higher than 0.90. In contrast to these three methods, the Interactive presents a value close to the manual value.

Figure 29 shows the results of the proposed automatic method and the OpenCFU. It is possible to observe in the yellow box that both methods fail in the detecting some colonies in the rim area of Petri dish. These failures, contribute to the high number of false negatives and thus the low Recall value. It is also possible to see that OpenCFU detects a higher number of colonies in the rim area than the proposed automatic system. The explanations for these detection failures are the fact that some of the colonies are eliminated in the pre-processing step due to its color which is similar to that of the edges of the Petri dish. An example it is shown in Figure 30, where it is possible to see the original image and the image after a binarization. The colonies that appear in the yellow box in a) are attached to the edge of the Petri dish (Figure 30 b)).

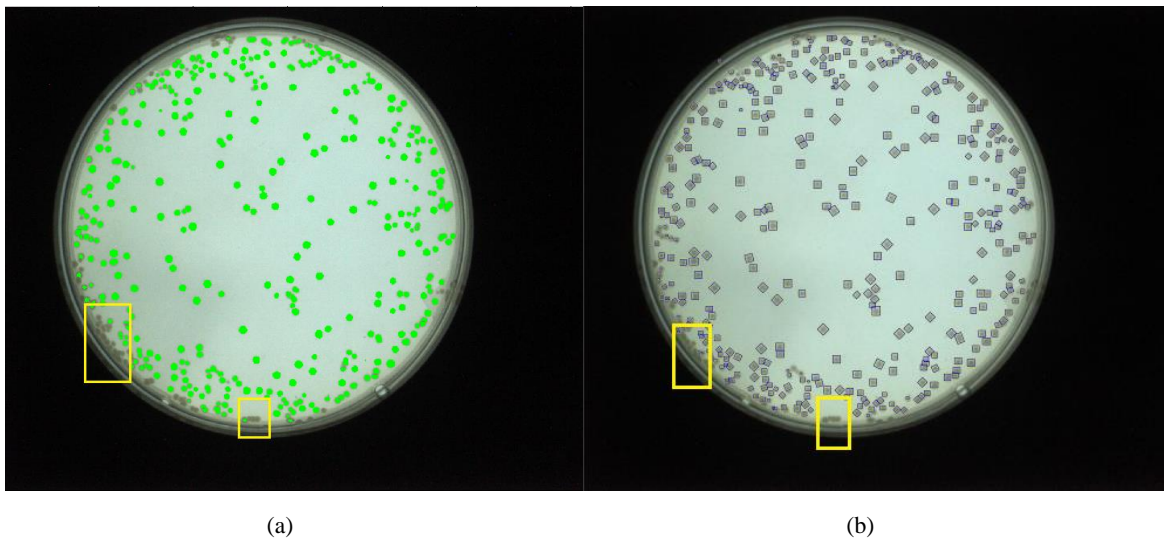


Figure 29: a) result of proposed automatic system; b) result of OpenCFU system; yellow boxes represent colonies not counted.

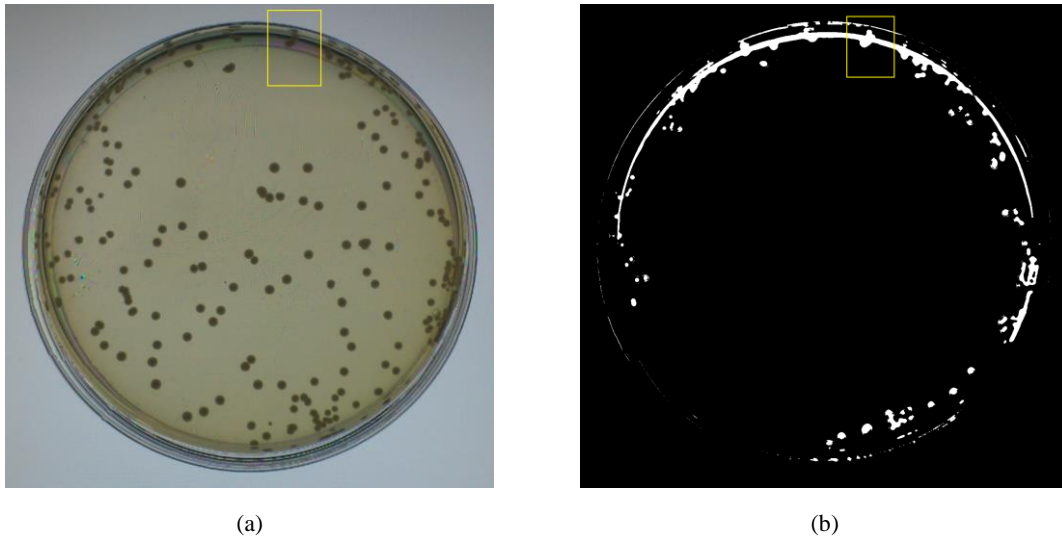


Figure 30: a) original image; b) result of binarization.

Another failure that contributes to the low Recall values of the proposed automatic system and also observed in the OpenCFU method, is the fact that in some cases, the system does not identify some colonies in the central area (observed in the yellow boxes), shown in Figure 31. Analyzing the images, it can be concluded that these problems exist due to the low contrast in the image. The non-identified colonies are practically the same color as the background, which makes it difficult to segment them. To improve this method and avoid these problems, the contrast has to be increased thereby facilitating the identification of these colonies.

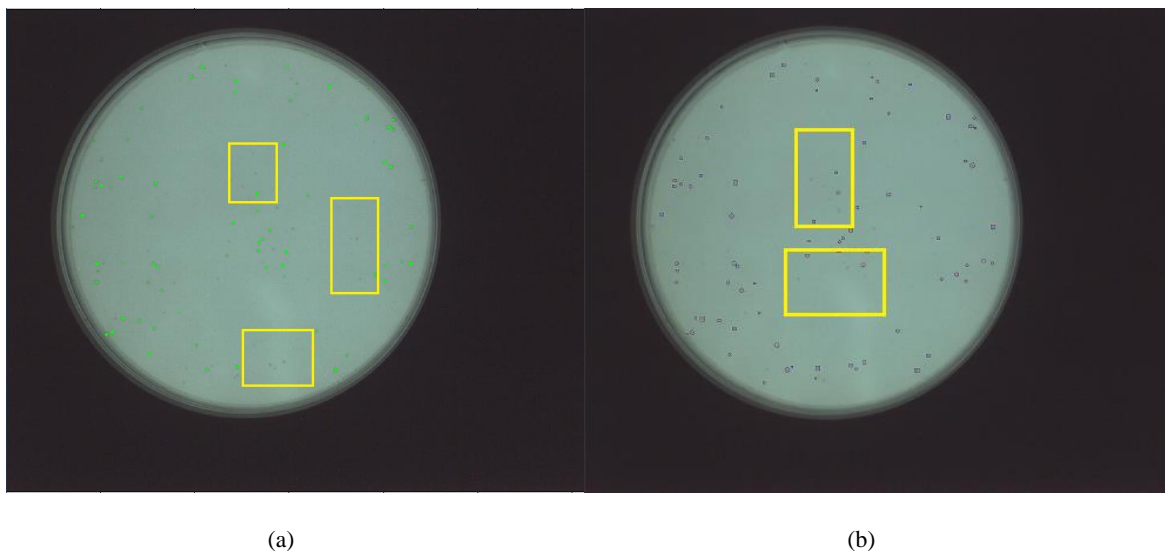


Figure 31: a) result of proposed automatic system; b) result of OpenCFU system; yellow boxes represents colonies not counted.

Another possible error is related to the fact that the separation watershed not always provides the best results. As explained earlier, the watershed works well for cluster with a small number of colonies. When this cluster is too large (Figure 19 (b)), the number of

colonies is estimated by averaging the areas of isolated colonies. This estimate does not always get the best results, counting down the number of colonies. This problem also influences the false negative numbers.

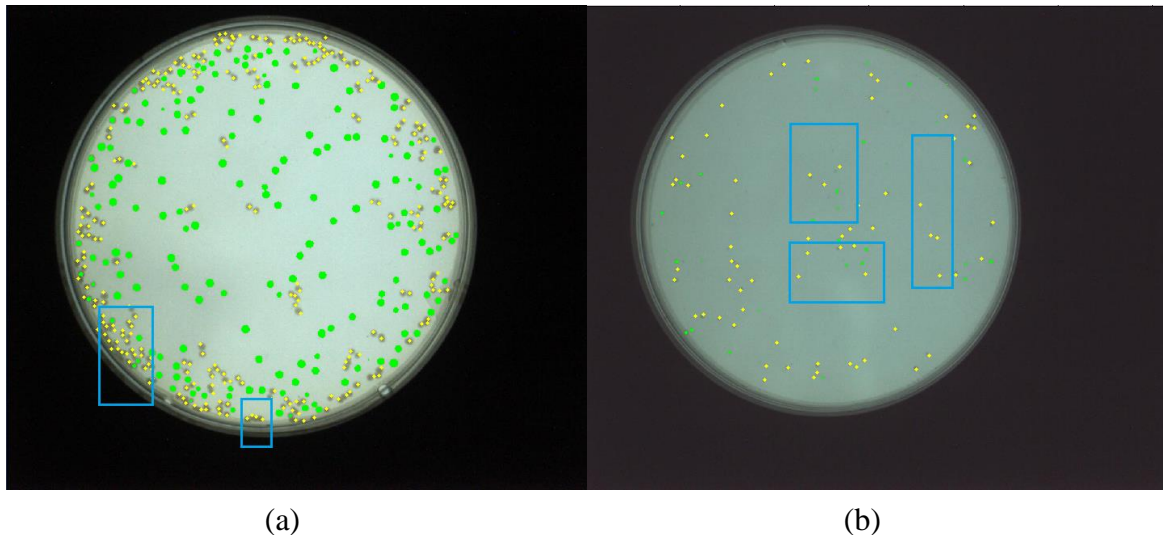


Figure 32: a) and b) are results of interactive system; blue boxes represents the colonies counted that previously on yellow boxes were not counted.

A huge advantage of the interactive system is the ability to fix these failures, which occur in the proposed automatic system and in the OpenCFU. After the automatic count, the user can correct the missed colonies. Figure 32 shows the result of the interactive count, and comparing it to Figure 29 and 31, it is possible to observe that in the interactive method, the colonies that were missing in the rim and central areas, shown in the yellow box are marked and counted (blue box).

Analyzing the F-measure (table2), the relation between Precision and Recall, as expected, the Interactive method presents the best value, close to the manual count, due to its great values of Precision and Recall. The OpenCFU method has an acceptable result through its good Precision result. The NICE presents the worst result due to its low recall value and the automatic proposed system, despite the low recall value has an F-measure higher than the NICE.

Observing the APE, which is the relation between the manual count and the results of the count methods, automatic proposed system and NICE shows a big percentage of error (16.3128 and 18.914, respectively) mainly due to the big number of false negatives. OpenCFU system presents an error of 9.7067%, which is a good value for the APE. In contrast, the Interactive system presents a value of APE similar to the manual value.

Table 3: statistical results of 26 images, comparing the results of central and rim area.

Measure	Method			
	Manual	Automatic		
		All area	Central area	Rim area
Precision	0.9879	0.9848	0.9659	0.9615
Recall	0.988	0.8698	0.9064	0.636
F-measure	0.9876	0.9188	0.9288	0.7248
APE (%)	2.4937	16.3128	14.233	55.0449

One of the first steps of the proposed automatic method was the separation of the original image in two images, one of them containing the central area of the Petri dish and the other one containing the rim area. As the proposed automatic method has different results to the manual count (Table 2) an individualized study of these two different areas was necessary, to determine whether the method fails in the central, rim or in the whole area. In Table 3, the first column refers to the manual count, column 2 shows the results of the automatic count analyzing the image as a whole, and column 3 shows the results of the central area and the last column the rim area.

In relation to the Precision values, it is possible to observe that both in the central area as well as in the rim area the results are lower comparing with the results of the automatic count of the whole area. As the values of the central and rim areas are similar, it is not possible to confirm where the automatic system works better.

Analyzing the Recall value, of the automatic system, the central area has a value greater than the rim area. In this case, the rim area has a really low value, around 0.636. Observing the result of the recall to the whole area, around 0.8698, it is possible to conclude that this low value is influenced by the result of the value of the rim area. The false negatives on the rim area have a high value, comparing to those of the central area.

Being the F-measure the relation between Precision and Recall, the results observed in Table 3 are the expected.

In relation to the absolute percentage of error, due to the high number of false negatives, the rim area presents a high value (55.0449%). On the other hand, the central area shows a similar result, although smaller, to the value of the automatic count of the whole area.

To improve the results of the automatic count, the method applied to the rim area needs to be modified in order to reduce the number of false negatives. Even the system applied to the central area needs to be modified to improve the results. It is possible to conclude that the system fails mainly in the rim area but the central area can be improved too.

As previously explained, the manual count is a time-consuming process, which can at times take up many human hours; for example, the time needed to count all 26 images is around 71 minutes. The manual counting time depends on the experience of the researchers, the number of colonies present in a Petri dish and the number of aggregated colonies. The counting time of the interactive method of this study depends on the number of aggregated colonies, the number of colonies that part of this method can automatically count and the speed and system performance. One of the main goals of this study was the time reduction, to protect the human against tedious work.

Figures 33 and 34 show the box plots of the time that the manual method and the Interactive method took to count each image. A box plot is a way of graphically depicting groups of numerical data through their quartiles. Box plots may also have lines extending vertically from the boxes indicating variability outside the upper and lower quartiles. Observing the graphics in Figures 33 and 34, the blue box represents 50% of all analyzed values. The base of the box is the lower quartile and represents 25% of the values, the top of the box is the upper quartile and represents the 75% of the observed values and the line presented in the middle of the box is the average and divides the bottom half from the upper half of the sample. The straight vertical line connects the top of the box to the highest value observed and the other line connects the bottom of the box at the lowest observed value, this segment is called Whisker. Box diagram is a tool for outlier detection, which are represented by the black points.

Figure 33 represents the time that each method took to count the 21 images obtained by Chiang et al. [14] and Figure 34 represents the time that the methods took to count the 5 images created for this study. The time of study of the two different libraries was divided because these had different pre-processing methods which caused a significant difference in time. The reduction of time that each method allows in each image is shown in the Appendix C.

Firstly, examining the graph of Figure 33, it is possible to observe that a significant reduction of the time exists. Whereas the box plot of the manual method is situated between 90 and 250 seconds, the box plot of the interactive method is situated between

50 and 150 seconds. The median observed in the two boxes is different too, while the median of the manual method is 129 seconds, the median of interactive method is 81 seconds. It is also observed that two outliers exist above the box. These values are from the count of the images with more colonies. In the interactive method, this time is reduced too.

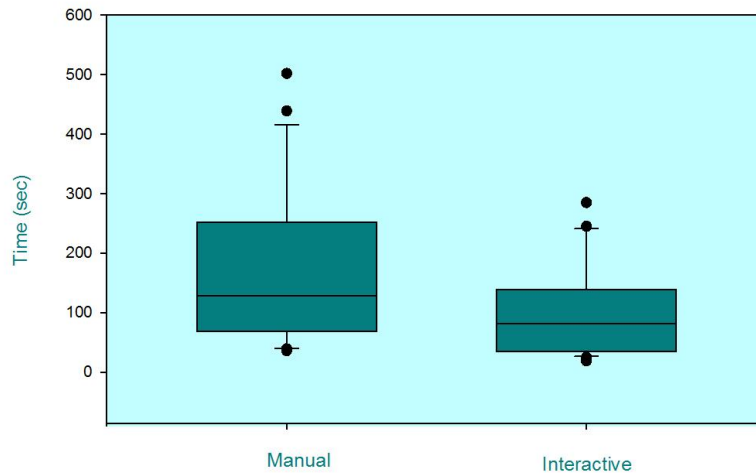


Figure 33 :counting times of manual method and interactive method relatively to the 21 images obtained by Chiang et al. [14].

Table 4 shows the total time reduction for the 21 images of each of the proposed methods, automatic and interactive, in relation to manual counting. The average of the automatic count is about 6 seconds, hence a 94.14% reduction in count time. This is a huge time reduction, enabling the researcher a quick count. Regarding the interactive method, this presents a significant reduction in time, around 40%, which allows for relieving the user's workload.

Table 4: percentage of time reduction for the 21 images obtained by Chiang et al. [14].

Method	Time reduction (%)
Interactive	40.10
Automatic	94.14

Considering the graph in Figure 34, obtained from the time of study of the 5 images created for this work, it is possible to observe that there is a reduction of the time to the interactive method to the manual method. The median is also smaller in the case of the interactive method. In this case, there are no outliers in both the manual and the interactive. It is observed in the manual method box that there is a picture that takes 200

seconds to be counted. This time is the maximum for the manual method. For this same image, watching the interactive method, the time is reduced to 110 seconds (maximum of the box).

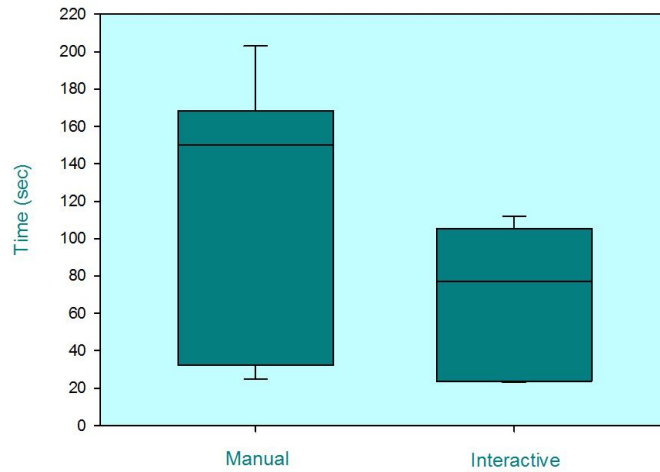


Figure 34: counting times of manual method and interactive method relatively to the 5 images obtained for this study.

Looking at table 5 it can be concluded that in the case of the automatic method the time reduction for the count of the 5 images is 83.73% and 33.46% in the case of the interactive method. These results are worse than the results for the 21 pictures, due to the fact that these images present an inferior quality to those obtained by Chiang et al. [14]. These have a higher amount of noise, a higher number of colonies on the edge of the Petri dish and a larger quantity of aggregated colonies, requiring different and harder pre-processing work, making the system slower.

Table 5: percentage of time reduction for the 5 images obtained for this study.

Method	Time reduction (%)
Interactive	33.46
Automatic	83.73

Summarizing and comparing all the results it is possible to conclude that there are advantages and disadvantages to all the proposed and studied methods. The main advantage of the fully automatic methods is that they are able to reduce the count time. The interactive method also has the advantage of reducing the time, although smaller than that of the automatic methods. For the case of images 21 obtained by Chiang et al. [14], the proposed automatic method has a time reduction of around 94% and the interactive

method a reduction of around 40%. In the images obtained for the purpose this study, the reduction is less (about 83% for the proposed automatic method and about 33% for the interactive method). This is due to the fact that the pre-processing is different. As the obtained images had a higher noise level a stronger pre-processing was done, which slowed down the system.

Analyzing all the methods, it is possible to observe that the main advantages of the interactive method are the precision values, the recall, the F-measure and the APE. These values are really close to the manual counts. This method has the advantage of counting colonies that the other methods do not count; fixing failures such as correctly identifying how many colonies exist in the aggregate count, the overlapping colonies on the edge of the Petri dish and counting the colonies even when the contrast is not too big.

In this study, two counting methods stand out, for their reliability, quality and ease of use. These are the OpenCFU and the Interactive method showing tremendous advantages and few disadvantages. Choosing the method it is now up to the user and will depend on the type of count that will be required. If the count requires a great precision method, where the number of false positives is low, meaning that the identified colonies are all correct, any method works well. All of them have a low number of false positives and a large number of colonies counted. If the count requires a method in which the number of false negative is low, then the manual or the interactive method is recommended. The fully automatic methods have some failures in identifying colonies along the edges of the Petri dish as well as properly separating an aggregate thereof. Besides that, in some images, the contrast is not high or there is a high noise. However, the counting time also impacts considerably on the user's decision. If they want a quick count even if the absolute percentage error is a little high the preferred method is the OpenCFU. Of all the automatic methods studied, the OpenCFU is the one with fewer failures with acceptable count values and a high execution speed. The automatic method proposed, despite a high speed does not give the best count guarantees, mostly featuring a high number of false negatives. The same applies to the NICE method, which despite its speed can have worse results than the proposed automatic method. If the user needs an accurate count with a low false negative value and a low absolute percentage error, the best method is still the manual one. But if a considerable counting time reduction with similar values to the manual counting is required, the interactive method is the one with these characteristics; it is a

big time saver, in some cases exceeding 50% while maintaining the robustness and reliability of the count.

The user's decision should be considered in the choice of the software. Features like the way the results are presented, the complexity of use and esthetics are important in the choice of the software. In this case, as the users of these applications are typically researchers in chemistry and microbiology, the software must be easy to understand, because normally they do not have much background in image processing. In this perspective, the proposed method is easy to use where only the choice of the counting method is required. If the chosen method is the automatic one the software then returns the value and the colonies appear marked in green while if the chosen method is the interactive one, the count has to be completed by counting the unmarked colonies. The OpenCFU software is also easy to use, automatically counting the colonies and presenting them in a little blue box, having the advantage that if the user has image processing concepts, to be an adaptive software where you can choose between three thresholds (normal, inverted and bilateral), as well as drawing areas of interest. If the user does not have image processing background the software becomes more complex. Analyzing NICE, it is a difficult to use software, where the selection of the threshold value (manual or by one of the software methods) requires the user to have image processing knowhow. Additionally, colonies appear unmarked, thereby making it difficult to identify software failures.

The colony counting increasingly acquires an importance to public health. Used for controlling the quality of water, the environment, food and beverage safety assessment and for improving clinical laboratory tests. Commercial products exist to facilitate colony counting, although accuracy is not always the required. This proposed automatic method did not show the best results; there are methods in the literature with better quality. On the contrary, the created interactive method allows for an accurate count, with values close to manual counts, with a significant time reduction, which can be easily used by anyone.

Chapter 5

Conclusion and future research

This dissertation focuses on simplifying bacterial count problems using two methods, a fully automatic one and the other interactive. Another two methods (NICE and OpenCFU) were studied in order to compare them to the created methods.

Normally, this tedious, time-consuming and error-prone method count is performed manually by researchers. Automatic methods have been studied and created to facilitate the task of researchers. A user-friendly GUI was also developed for the proposed method.

The proposed automatic method presented some good results in the quantification of *E. coli* colonies. It presents a high precision value of around 0.98, the number of false positives detected was low, although the recall value was relatively low with a high number of false negatives. The reasons for the high number of false negatives are, namely: the low contrast present in some images, which is the reason for the elimination of the colonies in the pre-processing and separation steps; the colonies on the rim that have the same color or are overlapping it, which makes their separation a huge challenge and also the division of clustered colonies, that in some cases are not completely separated by the watershed transform, making it is necessary to divide the resulting colonies by the mean area of the isolated colonies, which sometimes does not produce the correct result. The advantages of this proposed automatic method is the huge time reduction of around 94 % for the 21 Chiang et al.[14] images, and 83 % for the other 5 images. Another advantage is that the system created for this job is a non-adaptive system.

The NICE system presents the worst results, mainly because of the high number of false negatives, which makes the recall a bad value, around 0.878. With this study, it can be concluded that NICE system is not recommended for researchers who do not have image processing knowledge, because apart from the low results, it is a difficult to use system, where the user needs to choose the threshold and the counted colonies do not appear marked, making it difficult for the user to identify the uncounted colonies.

The OpenCFU is the fully automatic method that produces the best results, with a high precision value, an acceptable value of the recall measure and an absolute percentage of error of around 9%. It is a system that reduces the counting time and is user-friendly.

The interactive method was found to be capable of counting the number of *E. coli* colonies within an acceptable absolute percentage of error, 2.3272%. The mean values of precision, recall, and F-measure are all bigger than 0.98. The Interactive method shows similar results to those of human count because it allows for the correction of the false negatives of the other methods. This method is capable of reducing the counting time by 40% for the 21 images obtained by Chiang et al. [14] and by 33% for the 5 images created for this dissertation. The human interaction with the system is also a simple task, since the user only has to click on the unmarked colonies and these are added to the previously counted ones.

The choice of the method is up to the user; if you need a count where accuracy is more important than the time spent, the best method in this case is the interactive one. If accuracy is not as important as the time spent, then the method that produces better results with a large reduction in time is the OpenCFU.

The results obtained in this study, suggested that there are many problems to be solved in the proposed automatic bacterial count and some improvements to be made in the interactive system.

In the image acquisition, the researcher has to be careful to obtain images with the least possible noise and maximum possible contrast. The acquisition method of Chiang et al. [14] works fairly well, although sometimes the image presents little contrast. In a future study, it would be more interesting to have pictures from day to day cameras, like cell phones, and work with them in a system. Although this study presents images taken with a mobile phone, high noise is present.

Regarding the proposed automatic system, there are many improvements to be made. Improving the preprocessing and working the image as a set of areas instead of

separating it into two. Thus, it would be possible to identify colonies more easily, providing it is a small area, even if the contrast is low, identification is possible. Another issue to be considered in the future is if it would be better to have an adaptive counting system or not. The system created for this job is a non-adaptive system. The big advantage is that it is easy to use, even by researchers without expertise in the image processing area. The problem is that it works well for 26 images, but if you add a different image it no longer works. I think the future is to create a non-adaptive system but that works well for almost all types of images and colonies.

The work presented in this dissertation provides a new approach, the Interactive count system, is based on the interactivity between user and system. This method presents some good results, although it can be improved. The interactive method displays an image containing the counted isolated colonies and colonies grouped or that are unidentified or uncounted in the rim area. In some images, the number of colonies per count is relatively high, leading the user to spending little time counting. In future work, it would be interesting to develop a more accurate, automatic method, where only at the end of the automatic count would the interactive part act, correcting the false negatives and discounting false positives. Thus, the time required for the interactive method would be reduced and would it be possible to further improve the results.

This work resulted in two publications, RECPAD 2015 - Portuguese Conference on Pattern Recognition [19] and EJI 2015 – Encontro de Jovens Investigadores [20]. It was also submitted to be part of the book Handbook of Research on Human-Computer Interfaces, Developments and Applications, 2015 [21].

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Appendix A

Table A1: statistical results for the first manual count.

1st Count	Manual			True Positive		False Positive		False negative		Precision			Recall			F-measure			APE		
	1	2	3	2	3	2	3	2	3	2	3	Average	2	3	Average	2	3	Average	2	3	Average
1	63	66	61	63	61	3	0	0	2	0,9545	1	0,9773	1	0,9683	0,9841	0,9767	0,9839	0,9803	4,6512	3,2258	3,9385
2	44	44	44	44	44	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
3	87	85	86	85	86	0	0	2	1	1	1	1	0,9770	0,9885	0,9828	0,9884	0,9942	0,9913	2,3256	1,1561	1,74083
4	56	55	56	55	56	0	0	1	0	1	1	1	0,9821	1	0,9911	0,991	1	0,9955	1,8018	0	0,9009
5	115	113	114	113	114	0	0	2	1	1	1	1	0,9826	0,9913	0,9870	0,9912	0,9956	0,9934	1,7544	0,8734	1,31387
6	171	178	175	171	171	7	4	0	0	0,9607	0,9771	0,9689	1	1	1	0,9799	0,9884	0,9842	4,0115	2,3121	3,1618
7	325	348	337	325	325	23	12	0	0	0,9339	0,9644	0,9491	1	1	1	0,9658	0,9819	0,9738	6,8351	3,6254	5,23022
8	187	193	196	187	187	6	9	0	0	0,9689	0,9541	0,9615	1	1	1	0,9842	0,9765	0,9804	3,1579	4,6997	3,92882
9	94	94	94	94		0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
10	145	156	151	145	145	11	6	0	0	0,9295	0,9603	0,9449	1	1	1	0,9635	0,9797	0,9716	7,309	4,0541	5,68151
11	205	203	204	203	204	0	0	2	1	1	1	1	0,9902	0,9951	0,9927	0,9951	0,9976	0,9963	0,9804	0,489	0,73469
12	460	470	465	460	460	10	5	0	0	0,9787	0,9832	0,9840	1	1	1	0,9892	0,9946	0,9919	2,1505	1,0811	1,61581
13	351	352	352	351	351	1	1	0	0	0,9972	0,9972	0,9972	1	1	1	0,9986	0,9986	0,9986	0,2845	0,2845	0,2845
14	552	563	558	552	552	11	6	0	0	0,9805	0,9892	0,9849	1	1	1	0,9901	0,9946	0,9924	1,9731	1,0811	1,52709
15	103	95	99	95	99	0	0	8	4	1	1	1	0,9223	0,9612	0,9417	0,9596	0,9802	0,9699	8,0808	3,9604	6,0206
16	713	661	687	661	687	0	0	52	26	1	1	1	0,9271	0,9635	0,9453	0,9622	0,9814	0,9718	7,5691	3,7143	5,64171
17	306	295	301	295	301	0	0	11	5	1	1	1	0,9641	0,9837	0,9739	0,9817	0,9918	0,9867	3,6606	1,6474	2,65401
18	257	247	236	247	236	0	0	10	21	1	1	1	0,9611	0,9183	0,9397	0,9802	0,9574	0,9688	3,9683	8,5193	6,24376
19	393	397	396	393	393	4	3	0	0	0,9899	0,9924	0,9912	1	1	1	0,9949	0,9962	0,9956	1,0127	0,7605	0,88656
20	410	421	416	410	410	11	6	0	0	0,9739	0,9856	0,9797	1	1	1	0,9868	0,9927	0,9897	2,6474	1,4528	2,0501
21	575	583	579	575	575	8	4	0	0	0,9863	0,9931	0,9897	1	1	1	0,9931	0,9965	0,9948	1,3817	0,6932	1,03747
22	27	31	29	27	27	4	2	0	0	0,871	0,931	0,9010	1	1	1	0,931	0,9643	0,9477	13,793	7,1429	10,468
23	42	42	42	42	42	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
24	193	196	195	193	193	3	2	0	0	0,9847	0,9897	0,9872	1	1	1	0,9923	0,9948	0,9936	1,5424	1,0309	1,28667
25	286	268	277	268	277	0	0	18	9	1	1	1	0,9371	0,9685	0,9528	0,9675	0,984	0,9758	6,4982	3,1972	4,84768
26	188	189	189	188	188	1	1	0	0	0,9947	0,9947	0,9947	1	1	1	0,9973	0,9973	0,9973	0,5305	0,5305	0,5305

Table A2: statistical results for the second manual count.

2st Count	Manual			True Positive		False Positive		False negative		Precision			Recall			F-measure			APE		
	2	1	3	1	3	1	3	1	3	1	3	Average	1	3	Average	1	3	Average	1	3	Average
1	66	63	61	63	61	0	0	3	5	1	1	1	0,9545	0,9242	0,9394	0,9767	0,9606	0,968687	4,6512	7,874	6,26259
2	44	44	44	44	44	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
3	85	87	86	85	85	2	1	0	0	0,977	0,9884	0,9827	1	1	1	0,9884	0,9942	0,991262	2,3256	1,1696	1,74759
4	55	56	56	55	55	1	1	0	0	0,9821	0,9821	0,9821	1	1	1	0,991	0,991	0,990991	1,8018	1,8018	1,8018
5	113	115	114	113	113	2	1	0	0	0,9826	0,9912	0,9869	1	1	1	0,9912	0,9956	0,993411	1,7544	0,8811	1,31772
6	178	171	175	171	175	0	0	7	3	1	1	1	0,9607	0,9831	0,9719	0,9799	0,9915	0,985722	4,0115	1,6997	2,85559
7	348	325	337	325	337	0	0	23	11	1	1	1	0,9339	0,9684	0,9511	0,9658	0,9839	0,974883	6,8351	3,2117	5,02337
8	193	187	196	187	193	0	3	6	0	1	0,9847	0,9923	0,9689	1	0,9845	0,9842	0,9923	0,988249	3,1579	1,5424	2,35016
9	94	94	94	94	94	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
10	156	145	151	145	151	0	0	11	5	1	1	1	0,9295	0,9679	0,9487	0,9635	0,9837	0,973584	7,309	3,2573	5,28315
11	203	205	204	203	203	2	1	0	0	0,9902	0,9951	0,9927	1	1	1	0,9951	0,9975	0,996321	0,9804	0,4914	0,7359
12	470	460	465	460	465	0	0	10	5	1	1	1	0,9787	0,9894	0,9840	0,9892	0,9947	0,99195	2,1505	1,0695	1,61003
13	352	351	352	351	352	0	0	1	0	1	1	1	0,9972	1	0,9986	0,9986	1	0,999289	0,2845	0	0,14225
14	563	552	558	552	558	0	0	11	5	1	1	1	0,9805	0,9911	0,9858	0,9901	0,9955	0,992837	1,9731	0,8921	1,43258
15	95	103	99	95	95	8	4	0	0	0,9223	0,9596	0,941	1	1	1	0,9596	0,9794	0,969489	8,0808	4,1237	6,10226
16	661	713	687	661	661	52	26	0	0	0,9271	0,9622	0,9446	1	1	1	0,9622	0,9807	0,971433	7,5691	3,8576	5,71335
17	295	306	301	295	295	11	6	0	0	0,9641	0,9801	0,9721	1	1	1	0,9817	0,9899	0,985815	3,6606	2,0134	2,83699
18	247	257	236	247	236	10	0	0	11	0,9611	1	0,9805	1	0,9555	0,9777	0,9802	0,9772	0,978692	3,9683	4,5549	4,26156
19	397	393	396	393	396	0	0	4	1	1	1	1	0,9899	0,9975	0,9937	0,9949	0,9987	0,996838	1,0127	0,2522	0,63243
20	421	410	416	410	416	0	0	11	5	1	1	1	0,9739	0,9881	0,9810	0,9868	0,994	0,990395	2,6474	1,1947	1,92108
21	583	575	579	575	579	0	0	8	4	1	1	1	0,9863	0,9931	0,9897	0,9931	0,9966	0,994825	1,3817	0,6885	1,03508
22	31	27	29	27	29	0	0	4	2	1	1	1	0,8710	0,9355	0,9032	0,931	0,9667	0,948851	13,793	6,6667	10,2299
23	42	42	42	42	42	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
24	196	193	195	193	195	0	0	3	1	1	1	1	0,9847	0,9949	0,9898	0,9923	0,9974	0,994865	1,5424	0,5115	1,02696
25	268	286	277	268	268	18	9	0	0	0,9371	0,9675	0,9523	1	1	1	0,9675	0,9835	0,975498	6,4982	3,3028	4,90047
26	189	188	189	188	189	0	0	1	0	1	1	1	0,9947	1	0,9974	0,9973	1	0,998674	0,5305	0	0,26525

Table A3: statistical results for the third manual count.

3st Count	Manual			True Positive		False Positive		False negative		Precision			Recall			F-measure			APE		
	Images	3	1	2	1	2	1	2	1	2	Average	1	2	Average	1	2	Average	1	2	Average	
1	61	63	66	61	61	2	5	0	0	0,9683	0,9242	0,9462	1	1	1	0,9839	0,9606	0,9723	3,226	7,874	5,550
2	44	44	44	44	44	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
3	86	87	85	86	85	1	0	0	1	0,9885	1	0,9943	1	0,9884	0,9942	0,9942	0,9942	0,9942	1,156	1,170	1,163
4	56	56	55	56	55	0	0	0	1	1	1	1	1	0,9821	0,9911	1	0,991	0,9955	0	1,802	0,901
5	114	115	113	114	113	1	0	0	1	0,9913	1	0,9957	1	0,9912	0,9956	0,9956	0,9956	0,9956	0,873	0,881	0,877
6	175	171	178	171	175	0	3	4	0	1	0,9831	0,9916	0,9771	1	0,9886	0,9884	0,9915	0,9900	2,312	1,700	2,006
7	337	325	348	325	337	0	11	12	0	1	0,9684	0,9842	0,9644	1	0,9822	0,9819	0,9839	0,9829	3,625	3,212	3,419
8	196	187	193	187	193	0	0	9	3	1	1	1	0,9541	0,9847	0,9694	0,9765	0,9923	0,9844	4,700	1,542	3,121
9	94	94	94	94	94	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
10	151	145	156	145	151	0	5	6	0	1	0,9679	0,984	0,9603	1	0,9801	0,9797	0,9837	0,9817	4,054	3,257	3,656
11	204	205	203	204	203	1	0	0	1	0,9951	1	0,9976	1	0,9951	0,9975	0,9976	0,9975	0,9975	0,489	0,491	0,490
12	465	460	470	460	465	0	5	5	0	1	0,9894	0,9947	0,9892	1	0,9946	0,9946	0,9947	0,9946	1,081	1,070	1,075
13	352	351	352	351	352	0	0	1	0	1	1	1	0,9972	1	0,9986	0,9986	1	0,9993	0,284	0	0,142
14	558	552	563	552	558	0	5	6	0	1	0,9911	0,9956	0,9892	1	0,9946	0,9946	0,9955	0,9951	1,081	0,892	0,987
15	99	103	95	99	95	4	0	0	4	0,9612	1	0,9806	1	0,9596	0,9798	0,9802	0,9794	0,9798	3,960	4,124	4,042
16	687	713	661	687	661	26	0	0	26	0,9635	1	0,9818	1	0,9622	0,9811	0,9814	0,9807	0,9811	3,714	3,858	3,786
17	301	306	295	301	295	5	0	0	6	0,9837	1	0,9918	1	0,9801	0,9900	0,9918	0,9899	0,9908	1,647	2,013	1,830
18	236	257	247	236	236	21	11	0	0	0,9183	0,9555	0,9369	1	1	1	0,9574	0,9772	0,9673	8,519	4,555	6,537
19	396	393	397	393	396	0	1	3	0	1	0,9975	0,9987	0,9924	1	0,9962	0,9962	0,9987	0,9975	0,760	0,252	0,506
20	416	410	421	410	416	0	5	6	0	1	0,9881	0,9941	0,9856	1	0,9928	0,9927	0,994	0,9934	1,453	1,195	1,324
21	579	575	583	575	579	0	4	4	0	1	0,9931	0,9966	0,9931	1	0,9965	0,9965	0,9966	0,9965	0,693	0,688	0,691
22	29	27	31	27	29	0	2	2	0	1	0,9355	0,9677	0,9310	1	0,9655	0,9643	0,9667	0,9655	7,143	6,667	6,905
23	42	42	42	42	42	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0
24	195	193	196	193	195	0	1	2	0	1	0,9949	0,9974	0,9897	1	0,9949	0,9948	0,9974	0,9961	1,031	0,512	0,771
25	277	286	268	277	268	9	0	0	9	0,9685	1	0,9843	1	0,9675	0,9838	0,984	0,9835	0,9838	3,197	3,303	3,250
26	189	188	189	188	189	0	0	1	0	1	1	1	0,9947	1	0,9974	0,9973	1	0,9987	0,531	0	0,265

Appendix B

Table B1: statistical results for the automatic count.

Image	Manual			Automatic	True Positive			False Positive			False negative			Precision				Recall				F-measure				APE			
	1	2	3		1	2	3	1	2	3	1	2	3	1	2	3	Average	1	2	3	Average	1	2	3	Average	1	2	3	Average
1	63	66	61	59	59	59	59	0	0	0	4	7	2	1	1	1	0,9365	0,8939	0,9672	0,932553	0,9672	0,944	0,9833	0,964849	6,5574	11,2	3,3333	7,030237	
2	44	44	44	43	43	43	43	0	0	0	1	1	1	1	1	1	0,9773	0,9773	0,9773	0,977273	0,9885	0,9885	0,9885	0,988506	2,2989	2,2989	2,2989	2,298851	
3	87	85	86	85	85	85	85	0	0	0	2	0	1	1	1	1	0,9777	1	0,9884	0,988461	0,9884	1	0,9342	0,994175	2,3256	0	1,1696	1,165057	
4	56	55	56	56	56	55	56	0	1	0	0	0	0	1	0,9821	1	0,93405	1	1	1	1	0,991	1	0,996937	0	1,8018	0	0,600601	
5	115	113	114	97	97	97	97	0	0	0	18	16	17	1	1	1	0,8435	0,8584	0,8509	0,850921	0,9151	0,9238	0,9194	0,919445	16,981	15,238	16,114	16,11099	
6	171	178	175	168	168	168	168	0	0	0	3	10	7	1	1	1	0,9825	0,9438	0,96	0,962092	0,9912	0,9711	0,9796	0,980614	1,7699	5,7803	4,0816	3,877297	
7	325	348	337	306	306	306	306	0	0	0	19	42	31	1	1	1	0,9415	0,8793	0,908	0,90962	0,9699	0,9358	0,9518	0,952486	6,0222	12,844	9,6423	9,502842	
8	187	193	196	163	163	163	163	0	0	0	24	30	33	1	1	1	0,8717	0,8446	0,8316	0,849283	0,9314	0,9157	0,9081	0,918412	13,714	16,854	18,384	16,31754	
9	94	94	94	88	88	88	88	0	0	0	6	6	6	1	1	1	0,9362	0,9362	0,9362	0,93617	0,967	0,967	0,967	0,967033	6,5934	6,5934	6,5934	6,593407	
10	145	156	151	127	127	127	127	0	0	0	18	29	24	1	1	1	0,8759	0,8141	0,8411	0,843675	0,9338	0,8975	0,9137	0,915006	13,235	20,495	17,266	16,99873	
11	205	203	204	173	173	173	173	0	0	0	32	30	31	1	1	1	0,8439	0,8522	0,848	0,848053	0,9153	0,9202	0,9178	0,917776	16,931	15,957	16,446	16,44476	
12	460	470	465	372	372	372	372	0	0	0	88	98	93	1	1	1	0,8087	0,7915	0,8	0,800062	0,8942	0,8836	0,8889	0,88891	21,154	23,278	22,222	22,21799	
13	351	352	352	326	326	326	326	0	0	0	25	26	26	1	1	1	0,9288	0,9261	0,9261	0,927016	0,9631	0,9617	0,9617	0,962125	7,3855	7,6696	7,6696	7,574919	
14	552	563	558	484	484	484	484	0	0	0	68	79	74	1	1	1	0,8768	0,8597	0,8674	0,867958	0,9344	0,9245	0,929	0,929297	13,127	15,091	14,203	14,14053	
15	103	95	99	74	74	74	74	0	0	0	29	21	25	1	1	1	0,7184	0,7789	0,7475	0,74829	0,8362	0,8757	0,8555	0,855796	32,768	24,852	28,902	28,84072	
16	713	661	687	598	598	598	598	0	0	0	115	63	89	1	1	1	0,8387	0,9047	0,8705	0,871284	0,9123	0,95	0,9307	0,930993	17,544	10,008	13,852	13,80131	
17	306	295	301	231	231	231	231	0	0	0	75	64	70	1	1	1	0,7549	0,7831	0,7674	0,768465	0,8603	0,8783	0,8684	0,869028	27,933	24,335	26,316	26,19445	
18	257	247	236	212	212	212	212	0	0	0	45	35	24	1	1	1	0,8249	0,8583	0,8983	0,860502	0,9041	0,9237	0,9464	0,924742	19,19	15,251	10,714	15,05153	
19	393	397	396	332	332	332	332	0	0	0	61	65	64	1	1	1	0,8448	0,8363	0,8384	0,839813	0,9169	0,9108	0,9121	0,912929	16,828	17,833	17,582	17,41422	
20	410	421	416	351	351	351	351	0	0	0	59	70	65	1	1	1	0,8561	0,8337	0,8438	0,844526	0,9225	0,9093	0,9153	0,915684	15,506	18,135	16,949	16,86326	
21	575	583	579	403	403	403	403	0	0	0	172	180	176	1	1	1	0,7009	0,6913	0,696	0,69605	0,8241	0,8174	0,8208	0,820783	35,174	36,511	35,845	35,8434	
22	27	31	29	24	24	24	24	0	0	0	3	7	5	1	1	1	0,8889	0,7742	0,8276	0,830223	0,9412	0,8727	0,9057	0,906521	11,765	25,455	18,868	18,69573	
23	42	42	42	54	42	42	42	12	12	12	0	0	0	0,7778	0,7778	0,7778	0,77778	1	1	1	1	0,875	0,875	0,875	0,875	25	28,571	25	26,19048
24	193	196	195	170	170	170	170	0	0	0	23	26	25	1	1	1	0,8808	0,8673	0,8718	0,873324	0,9366	0,929	0,9315	0,932369	12,672	14,208	13,699	13,52615	
25	286	268	277	333	286	268	277	47	65	56	0	0	0	0,8589	0,8048	0,8318	0,83183	1	1	1	1	0,9241	0,8918	0,9082	0,908038	15,186	23,466	18,361	19,00405
26	188	189	189	111	111	111	111	0	0	0	77	78	78	1	1	1	0,5904	0,5873	0,5873	0,588343	0,7425	0,74	0,74	0,740825	51,505	52	52	51,83501	

Table B2: statistical results for the interactive count.

Image	Manual			Interactive	True Positive			False Positive			False negative			Precision				Recall				F-measure				APE			
	1	2	3		1	2	3	1	2	3	1	2	3	1	2	3	Average	1	2	3	Average	1	2	3	Average	1	2	3	Average
1	63	66	61	64	63	64	61	1	0	3	0	2	0	0,9844	1	0,9531	0,979167	1	0,9697	1	0,989899	0,9921	0,9846	0,976	0,984247	1,0582	3,0769	4,8	2,978375
2	44	44	44	49	44	44	44	5	5	5	0	0	0	0,898	0,898	0,898	0,897959	1	1	1	1	0,9462	0,9462	0,9462	0,946237	7,5758	10,753	10,753	9,693711
3	87	85	86	86	86	85	86	0	1	0	1	0	0	1	0,9884	1	0,996124	0,9885	1	1	0,996169	0,9942	0,9942	1	0,996124	0,7692	1,1696	0	0,646274
4	56	55	56	54	54	54	54	0	0	0	2	1	2	1	1	1	1	0,9643	0,9818	0,9643	0,97013	0,9818	0,9908	0,9818	0,984821	2,4096	1,8349	3,6364	2,626955
5	115	113	114	109	109	109	109	0	0	0	6	4	5	1	1	1	1	0,9478	0,9646	0,9561	0,956189	0,9732	0,982	0,9776	0,977692	3,5398	3,6036	4,4843	3,875911
6	171	178	175	173	171	173	173	2	0	0	5	2	2	0,9884	1	1	0,996146	1	0,9719	0,9886	0,986827	0,9942	0,9858	0,9943	0,991398	0,7797	2,849	1,1494	1,592718
7	325	348	337	347	325	347	337	22	0	10	0	1	0	0,9366	1	0,9712	0,96926	1	0,9971	1	0,999042	0,9673	0,9986	0,9854	0,983734	4,5128	0,2878	2,924	2,574856
8	187	193	196	196	187	193	196	9	3	0	0	0	0	0,9541	0,9847	1	0,979592	1	1	1	1	0,9765	0,9923	1	0,989596	3,2086	1,5424	0	1,583658
9	94	94	94	96	94	94	94	2	2	2	0	0	0	0,9792	0,9792	0,9792	0,979167	1	1	1	1	0,9895	0,9895	0,9895	0,989474	1,4184	2,1053	2,1053	1,876322
10	145	156	151	155	145	155	151	10	0	4	0	1	0	0,9355	1	0,9742	0,969892	1	0,9936	1	0,997863	0,9667	0,9968	0,9869	0,98346	4,5977	0,6431	2,6144	2,618389
11	205	203	204	203	203	203	203	0	0	0	2	0	1	1	1	1	1	0,9902	1	0,9951	0,995114	0,9951	1	0,9975	0,997547	0,6525	0	0,4914	0,38131
12	460	470	465	462	460	462	462	2	0	0	8	3	0,9957	1	1	0,998557	1	0,983	0,9935	0,992176	0,9978	0,9914	0,9968	0,995337	0,2899	1,7167	0,6472	0,884614	
13	351	352	352	351	351	351	351	0	0	0	0	1	1	1	1	1	1	1	0,9972	0,9972	0,998106	1	0,9986	0,9986	0,999052	0	0,2845	0,2845	0,189663
14	552	563	558	560	552	560	558	8	0	2	0	3	0	0,9857	1	0,9964	0,994048	1	0,9947	1	0,998224	0,9928	0,9973	0,9982	0,996115	0,9662	0,5343	0,3578	0,619416
15	103	95	99	101	101	95	99	0	6	2	2	0	0	1	0,9406	0,9802	0,973597	0,9806	1	1	0,993528	0,9902	0,9694	0,99	0,983195	1,3029	6,1224	2	3,141794
16	713	661	687	703	703	661	687	0	42	16	10	0	0	1	0,9403	0,9772	0,972499	0,986	1	1	0,995325	0,9929	0,9692	0,9885	0,983545	0,9394	6,1584	2,3022	3,133308
17	306	295	301	302	302	295	301	0	7	1	4	0	0	1	0,9768	0,9967	0,99117	0,9869	1	1	0,995643	0,9934	0,9883	0,9983	0,993346	0,8753	2,3451	0,3317	1,184002
18	257	247	236	256	256	247	236	0	9	20	1	0	0	1	0,9648	0,9219	0,96224	0,9961	1	1	0,998703	0,9981	0,9821	0,9593	0,979836	0,2597	3,5785	8,1301	3,98945
19	393	397	396	397	393	397	396	4	0	1	0	0	0	0,9899	1	0,9975	0,995802	1	1	1	0,9949	1	0,9987	0,997892	0,6785	0	0,2522	0,310249	
20	410	421	416	418	410	418	416	8	0	2	0	3	0	0,9809	1	0,9952	0,992026	1	0,9929	1	0,997625	0,9903	0,9964	0,9976	0,994788	1,3008	0,7151	0,4796	0,831855
21	575	583	579	579	575	579	579	4	0	0	4	0	0	0,9931	1	1	0,997697	1	0,9931	1	0,997713	0,9965	0,9966	1	0,997697	0,4638	0,6885	0	0,384079
22	27	31	29	29	27	29	29	2	0	0	0	2	0	0,931	1	1	0,977011	1	0,9355	1	0,978495	0,9643	0,9667	1	0,976984	4,9383	6,6667	0	3,868313
23	42	42	42	43	42	42	42	1	1	1	0	0	0	0,9767	0,9767	0,9767	0,976744	1	1	1	1	0,9882	0,9882	0,9882	0,988235	1,5873	2,3529	2,3529	2,097728
24	193	196	195	195	193	195	195	2	0	0	0	1	0	0,9897	1	1	0,996581	1	0,9949	1	0,998299	0,9948	0,9974	1	0,997429	0,6908	0,5115	0	0,400785
25	286	268	277	300	286	268	277	14	32	23	0	0	0	0,9533	0,8933	0,9233	0,923333	1	1	1	1	0,9761	0,9437	0,9601	0,95997	3,2634	11,268	7,9723	7,501093
26	188	189	189	192	188	189	189	4	3	3	0	0	0	0,9792	0,9844	0,9844	0,982639	1	1	1	1	0,9895	0,9921	0,9921	0,991242	1,4184	1,5748	1,5748	1,522682

Table B3: statistical results for the OpenCFU count.

Image	Manual			Open CFU	True Positive			False Positive			False negative			Precision				Recall				F-measure				APE				
	1	2	3		1	2	3	1	2	3	1	2	3	1	2	3	Average	1	2	3	Average	1	2	3	Average	1	2	3	Average	
1	63	66	61	62	62	62	61	0	0	1	1	4	0	1	1	0,9839	0,994624	0,9841	0,9394	1	0,974507	0,992	0,9688	0,9919	0,984	1,6	6,25	1,626	3,158672	
2	44	44	44	47	44	44	44	3	3	3	0	0	0	0,9362	0,9362	0,9362	0,93617	1	1	1	1	0,967	0,967	0,967	0,967	6,5934	6,5934	6,5934	6,593407	
3	87	85	86	86	86	85	86	0	1	0	1	0	0	1	0,9884	1	0,996124	0,9885	1	1	0,996169	0,9942	0,9942	1	0,996	1,1661	1,1696	0	0,77522	
4	56	55	56	60	56	55	56	4	5	4	0	0	0	0,9333	0,9167	0,9333	0,927778	1	1	1	1	0,9655	0,9655	0,9655	0,963	6,8966	6,8957	6,8966	7,496252	
5	115	113	114	109	109	109	109	0	0	0	6	4	5	1	1	1	1	0,9478	0,9646	0,9561	0,956189	0,9732	0,982	0,9776	0,978	5,3571	3,6036	4,4843	4,481684	
6	171	178	175	158	158	158	158	0	0	0	13	20	17	1	1	1	1	0,924	0,8876	0,9029	0,904825	0,9605	0,9405	0,9489	0,95	7,9027	11,905	10,21	10,0059	
7	325	348	337	331	325	325	331	6	0	0	0	0	17	6	0,9819	1	1	0,993958	1	0,9503	0,9822	0,977496	0,9909	0,9745	0,991	0,985	1,8293	5,0074	1,7964	2,87768
8	187	193	196	176	176	176	176	0	0	0	0	11	17	20	1	1	1	1	0,9412	0,9119	0,898	0,917018	0,9697	0,9539	0,9462	0,957	6,0606	9,2141	10,753	8,675795
9	94	94	94	89	89	89	89	0	0	0	5	5	5	1	1	1	1	0,9468	0,9468	0,9468	0,946809	0,9727	0,9727	0,9727	0,973	5,4645	5,4645	5,4645	5,464481	
10	145	156	151	140	140	140	140	0	0	0	5	16	11	1	1	1	1	0,9655	0,8974	0,9272	0,930035	0,9825	0,9459	0,9622	0,964	3,5088	10,811	7,5601	7,29324	
11	205	203	204	192	192	192	192	0	0	0	0	13	11	12	1	1	1	1	0,9366	0,9458	0,9412	0,941192	0,9673	0,9722	0,9697	0,97	6,5491	5,5696	6,0606	6,059782
12	460	470	465	392	392	392	392	0	0	0	68	78	73	1	1	1	1	0,8522	0,834	0,843	0,843076	0,9202	0,9095	0,9148	0,915	15,962	18,097	17,036	17,03202	
13	351	352	352	340	340	340	340	0	0	0	11	12	12	1	1	1	1	0,9687	0,9659	0,9659	0,966826	0,9841	0,9827	0,9827	0,983	3,1838	3,4682	3,4682	3,373403	
14	552	563	558	523	523	523	523	0	0	0	29	40	35	1	1	1	1	0,9475	0,929	0,9373	0,937897	0,973	0,9632	0,9676	0,968	5,3953	7,3665	6,4755	6,412439	
15	103	95	99	90	90	90	90	0	0	0	23	15	19	1	1	1	1	0,7767	0,8421	0,8081	0,808962	0,8743	0,9143	0,8939	0,894	25,137	17,143	21,229	21,16951	
16	713	661	687	617	617	617	617	0	0	0	96	44	70	1	1	1	1	0,8654	0,9334	0,8981	0,898967	0,9278	0,9656	0,9463	0,947	14,436	6,8858	10,736	10,68602	
17	306	295	301	253	253	253	253	0	0	0	53	42	48	1	1	1	1	0,8268	0,8576	0,8405	0,841652	0,9052	0,9234	0,9134	0,914	18,962	15,328	17,329	17,20647	
18	257	247	236	221	221	221	221	0	0	0	36	26	15	1	1	1	1	0,8599	0,8947	0,9364	0,897033	0,9247	0,9444	0,9672	0,945	15,063	11,111	6,5646	10,91281	
19	393	397	396	357	357	357	357	0	0	0	36	40	39	1	1	1	1	0,9084	0,8992	0,9015	0,903052	0,952	0,9469	0,9482	0,949	9,6	10,61	10,359	10,18955	
20	410	421	416	370	370	370	370	0	0	0	40	51	46	1	1	1	1	0,9024	0,8789	0,8894	0,890241	0,9487	0,9355	0,9415	0,942	10,256	12,895	11,705	11,61877	
21	575	583	579	403	403	403	403	0	0	0	172	180	176	1	1	1	1	0,7009	0,6913	0,696	0,69605	0,8241	0,8174	0,8208	0,821	35,174	36,511	35,845	35,8434	
22	27	31	29	29	27	27	29	2	0	0	2	0	0	0,931	1	1	1	0,977011	1	0,931	1	0,977011	0,9643	0,9643	1	0,976	7,1429	6,6667	0	4,603175
23	42	42	42	44	42	42	42	2	2	2	0	0	0	0,9545	0,9545	0,9545	0,954545	1	1	1	1	0,9767	0,9767	0,9767	0,977	4,6512	4,6512	4,6512	4,65163	
24	193	196	195	182	182	182	182	0	0	0	11	14	13	1	1	1	1	0,943	0,9286	0,9333	0,93497	0,9707	0,963	0,9655	0,966	5,8667	7,4074	6,8966	6,723542	
25	286	268	277	241	241	241	241	0	0	0	45	27	36	1	1	1	1	0,8427	0,8993	0,87	0,870649	0,9146	0,947	0,9305	0,931	17,078	10,609	13,9	13,86215	
26	188	189	189	162	162	162	162	0	0	0	26	27	27	1	1	1	1	0,8617	0,8571	0,8571	0,858663	0,9257	0,9231	0,9231	0,924	14,857	15,385	15,385	15,20879	

Table B4: statistical results for the NICE count.

Image	Manual			NICE	True Positive			False Positive			False negative			Precision				Recall				F-measure				APE			
	1	2	3		1	2	3	1	2	3	1	2	3	1	2	3	Average	1	2	3	Average	1	2	3	Average	1	2	3	Average
1	63	66	61	67	63	66	61	4	1	6	0	0	0	0,9403	0,9851	0,9104	0,945274	1	1	1	1	0,9692	0,9925	0,9531	0,97161232	6,1538	1,5038	9,375	5,677535
2	44	44	44	51	44	44	44	7	7	7	0	0	0	0,8627	0,8627	0,8627	0,862745	1	1	1	1	0,9263	0,9263	0,9263	0,92631579	14,737	14,737	14,737	14,73684
3	87	85	86	89	87	85	86	2	4	3	0	0	0	0,9775	0,9551	0,9663	0,966292	1	1	1	1	0,9886	0,977	0,9829	0,982835	2,2727	4,5977	3,4286	3,433
4	56	55	56	61	56	55	56	5	6	5	0	0	0	0,918	0,9016	0,918	0,912568	1	1	1	1	0,9573	0,9493	0,9573	0,95426859	8,547	10,345	8,547	9,146282
5	115	113	114	104	104	113	104	0	0	0	11	9	10	1	1	1	1	0,9043	0,9262	0,9123	0,914286	0,9498	0,9617	0,9541	0,95520075	10,046	8,2949	9,1743	9,171635
6	171	178	175	164	164	164	164	0	0	0	7	14	11	1	1	1	1	0,9591	0,9213	0,9371	0,939185	0,9791	0,9591	0,9676	0,96857348	4,1791	8,1871	6,4897	6,285305
7	325	348	337	328	325	325	328	0	0	0	0	20	9	1	1	1	1	1	0,942	0,9733	0,971774	1	0,9701	0,9865	0,98553847	0,9188	5,9172	2,7068	3,180921
8	187	193	196	159	159	159	159	0	0	0	28	34	37	1	1	1	1	0,8503	0,8238	0,8112	0,828442	0,9191	0,9034	0,8958	0,90608629	16,185	19,318	20,845	18,78274
9	94	94	94	90	90	90	90	0	0	0	4	4	4	1	1	1	1	0,9574	0,9574	0,9574	0,957447	0,9783	0,9783	0,9783	0,97826087	4,3478	4,3478	4,3478	4,347826
10	145	156	151	138	138	138	138	0	0	0	7	18	13	1	1	1	1	0,9517	0,8846	0,9139	0,916749	0,9753	0,9388	0,955	0,95635261	4,947	12,245	8,9965	8,729478
11	205	203	204	175	175	175	175	0	0	0	30	28	29	1	1	1	1	0,8537	0,8621	0,8578	0,857857	0,9211	0,9259	0,9235	0,92348714	15,789	14,815	15,303	15,30257
12	460	470	465	357	357	357	357	0	0	0	103	113	108	1	1	1	1	0,7761	0,7596	0,7677	0,767801	0,8739	0,8634	0,8686	0,86863457	25,214	27,328	26,277	26,27309
13	351	352	352	327	327	327	327	0	0	0	24	25	25	1	1	1	1	0,9316	0,929	0,929	0,929859	0,9646	0,9632	0,9632	0,96365469	7,0796	7,3638	7,3638	7,269062
14	552	563	558	505	505	505	505	0	0	0	47	58	53	1	1	1	1	0,9149	0,897	0,905	0,905618	0,9555	0,9457	0,9501	0,95045618	8,8931	10,861	9,9718	9,908765
15	103	95	99	86	86	86	86	0	0	0	17	9	13	1	1	1	1	0,835	0,9053	0,8687	0,869634	0,9101	0,9503	0,9297	0,93001963	17,989	9,9448	14,054	13,99607
16	713	661	687	634	634	634	634	0	0	0	79	27	53	1	1	1	1	0,8892	0,9592	0,9229	0,923735	0,9414	0,9792	0,9599	0,96012687	11,73	4,1639	8,0242	7,974626
17	306	295	301	239	239	239	239	0	0	0	67	56	62	1	1	1	1	0,781	0,8102	0,794	0,795078	0,8771	0,8951	0,8852	0,8857935	24,587	20,974	22,963	22,8413
18	257	247	236	221	221	221	221	0	0	0	36	26	15	1	1	1	1	0,8599	0,8947	0,9364	0,897033	0,9247	0,9444	0,9672	0,94543596	15,063	11,111	6,5646	10,91281
19	393	397	396	342	342	342	342	0	0	0	51	55	54	1	1	1	1	0,8702	0,8615	0,8636	0,865109	0,9306	0,9256	0,9268	0,9276722	13,878	14,885	14,634	14,46556
20	410	421	416	351	351	351	351	0	0	0	59	70	65	1	1	1	1	0,8561	0,8337	0,8438	0,844526	0,9225	0,9093	0,9153	0,9156837	15,506	18,135	16,949	16,86326
21	575	583	579	382	382	382	382	0	0	0	193	201	197	1	1	1	1	0,6643	0,6552	0,6598	0,659779	0,7983	0,7917	0,795	0,79501439	40,334	41,658	40,999	40,99712
22	27	31	29	63	27	31	29	36	32	34	0	0	0	0,4286	0,4921	0,4603	0,460317	1	1	1	1	0,6	0,6596	0,6304	0,63000308	80	68,085	73,913	73,99938
23	42	42	42	31	31	31	31	0	0	0	11	11	11	1	1	1	1	0,7381	0,7381	0,7381	0,738095	0,8493	0,8493	0,8493	0,84931507	30,137	30,137	30,137	30,13699
24	193	196	195	87	87	87	87	0	0	0	106	109	108	1	1	1	1	0,4508	0,4439	0,4462	0,446936	0,6214	0,6148	0,617	0,61776361	75,714	77,032	76,596	76,44728
25	286	268	277	221	221	221	221	0	0	0	65	47	56	1	1	1	1	0,7727	0,8246	0,7978	0,798396	0,8718	0,9039	0,8876	0,88774352	25,641	19,223	22,49	22,4513
26	188	189	189	227	188	189	189	39	38	38	0	0	0	0,8282	0,8326	0,8326	0,831131	1	1	1	1	0,906	0,9087	0,9087	0,90777726	18,795	18,269	18,269	18,44455


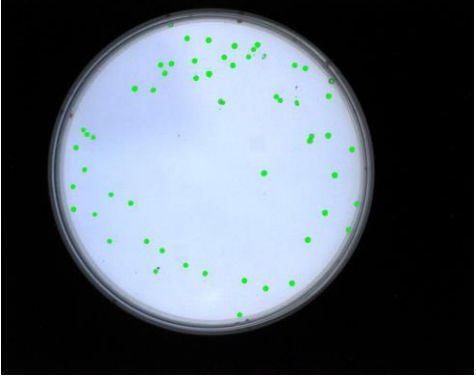
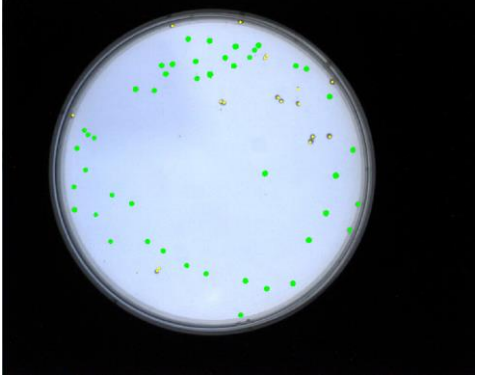
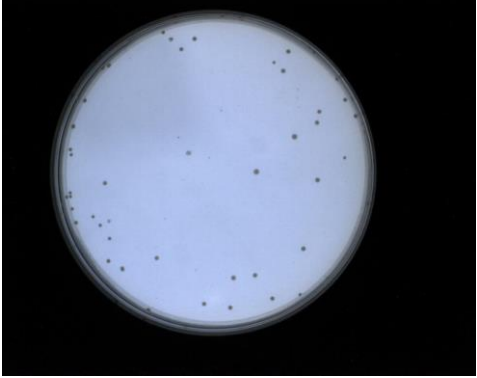
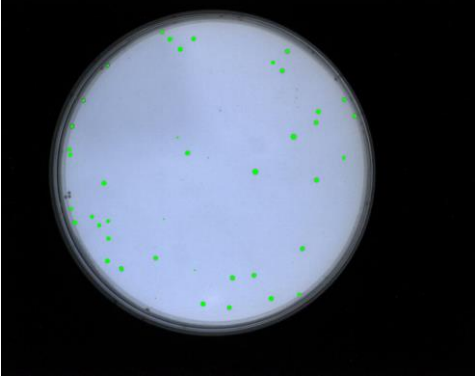
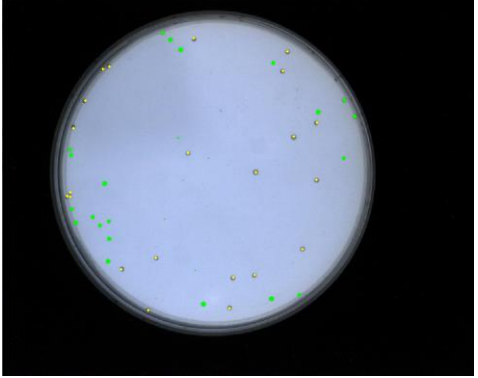
Appendix C

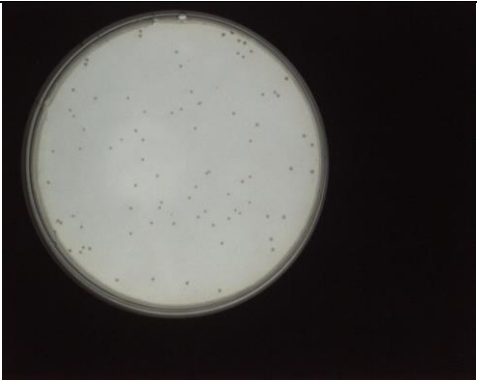
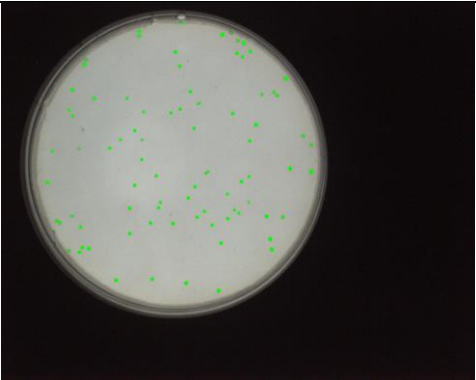
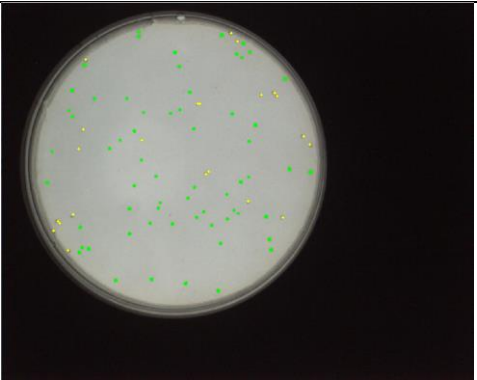
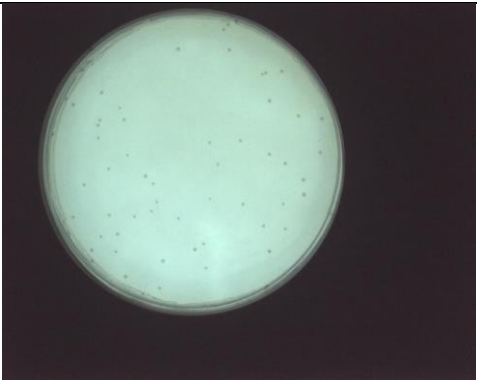
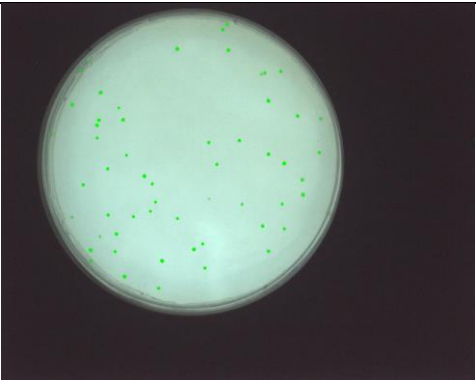
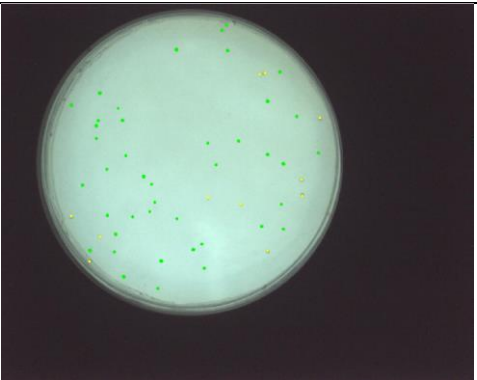
Table C1: times reduction for the automatic and interactive method.

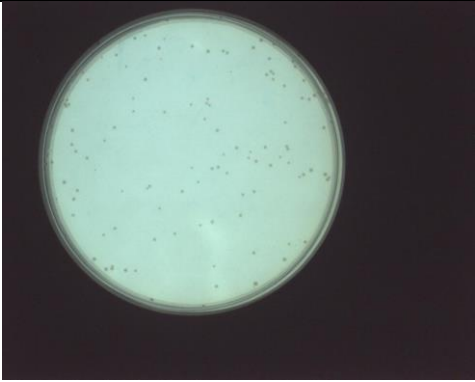
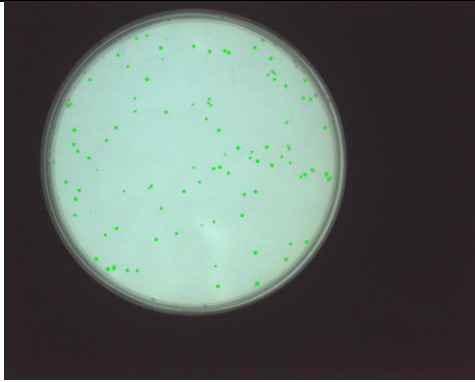
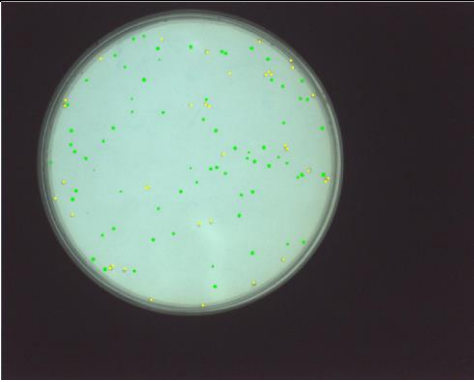
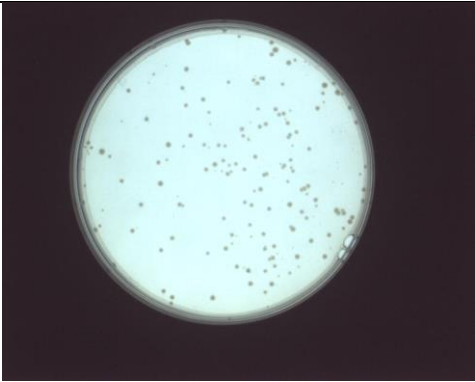
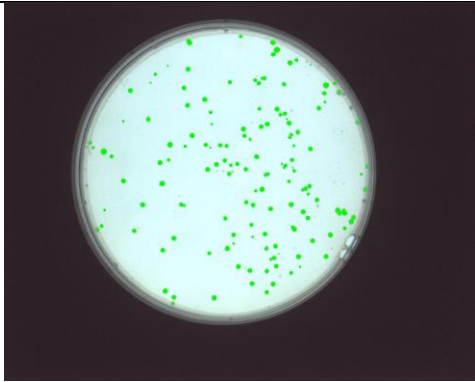
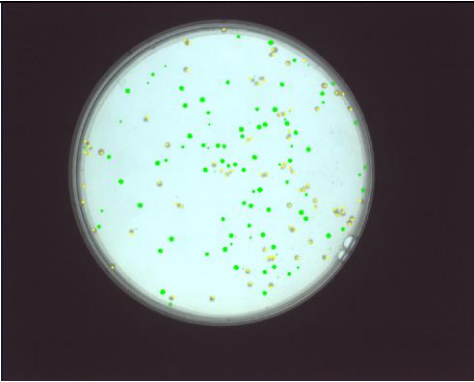
Image	Time					Number of colonies counted				
	Manual	Automatic	(%) Reduction	Interactive	(%) Reduction	Interactive	Automatically in the Interactive method	(%)	Number of the clicks	(%)
1	45	6	0,86666667	25	0,44444444	64	46	71,875	18	28,125
2	36	5	0,86111111	33	0,08333333	49	26	53,0612	23	46,93877551
3	55	6	0,89090909	32	0,41818181	86	63	73,2558	23	26,74418605
4	39	6	0,84615385	19	0,51282051	54	41	75,9259	13	24,07407407
5	69	6	0,91304348	34	0,50724637	109	73	66,9725	36	33,02752294
6	102	6	0,94117647	75	0,26470588	173	97	56,0694	76	43,93063584
7	250	7	0,97200000	114	0,544	347	188	54,1787	159	45,82132565
8	120	6	0,95000000	63	0,475	196	100	51,0204	96	48,97959184
9	69	5	0,92753623	36	0,47826087	96	50	52,0833	46	47,91666667
10	120	6	0,95000000	81	0,325	155	87	56,129	68	43,87096774
11	129	6	0,95348837	74	0,42635658	203	122	60,0985	81	39,90147783
12	323	8	0,97523220	245	0,24148606	462	173	37,4459	289	62,55411255
13	241	6	0,97510373	144	0,40248962	351	222	63,2479	129	36,75213675
14	502	7	0,98605578	285	0,43227091	560	257	45,8929	303	54,10714286
15	75	5	0,93333333	54	0,28	101	34	33,6634	67	66,33663366
16	439	8	0,98177677	226	0,48519362	703	363	51,6358	340	48,36415363
17	162	6	0,96296296	93	0,42592592	302	132	43,7086	170	56,29139073
18	140	6	0,95714286	93	0,33571428	256	145	56,6406	111	43,359375
19	244	7	0,97131148	133	0,45491803	397	201	50,6297	196	49,37027708
20	253	6	0,97628458	122	0,51778656	418	230	55,0239	188	44,97607656
21	306	7	0,97712418	194	0,36601307	579	213	36,7876	366	63,21243523
22	25	8	0,68000000	23	0,08	29	15	51,7241	14	48,27586207
23	35	10	0,71428571	24	0,31428571	43	24	55,814	19	44,18604651
24	150	12	0,92000000	77	0,48666667	195	102	52,3077	93	47,69230769
25	203	13	0,93596059	112	0,44827586	300	120	40	180	60
26	157	10	0,93630573	103	0,34394904	192	74	38,5417	118	61,45833333


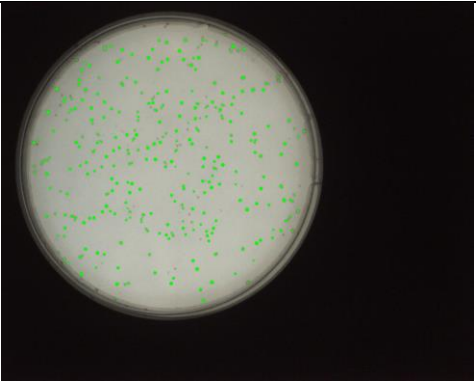
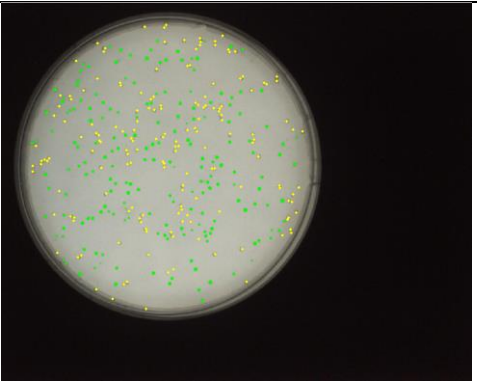
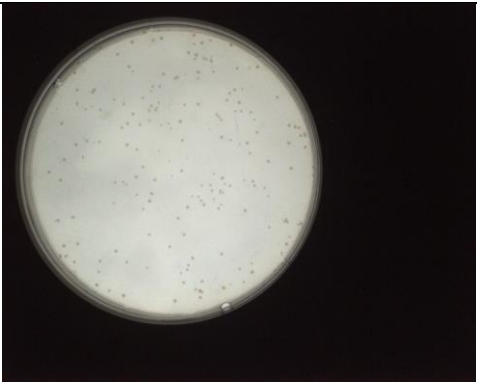
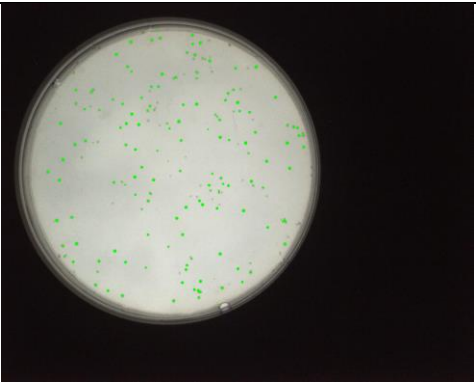
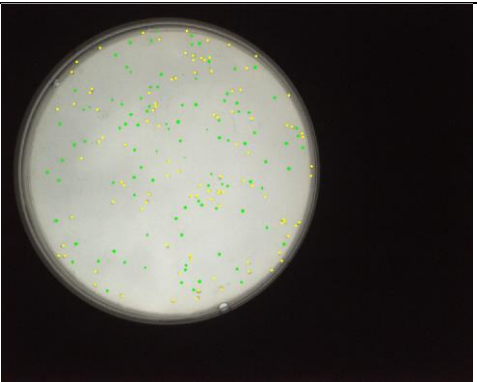
Appendix D

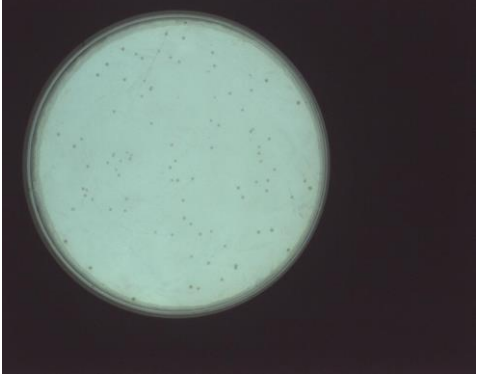
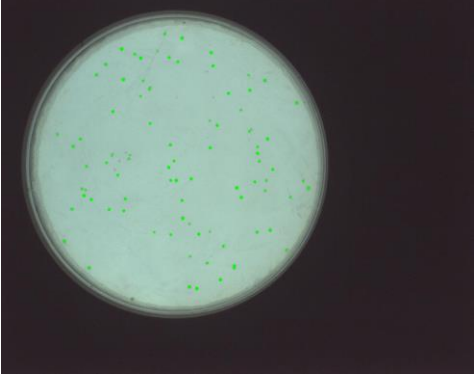
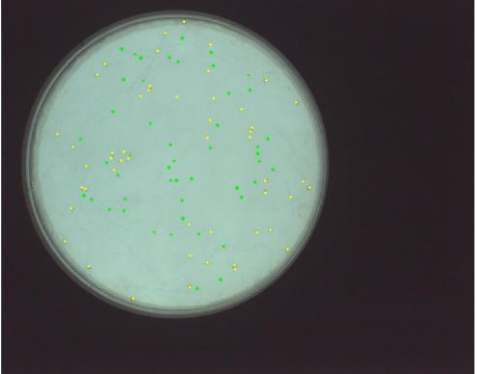
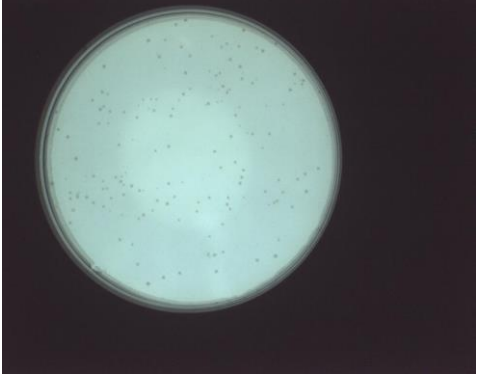
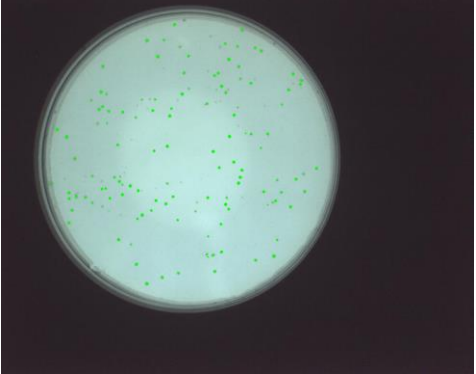
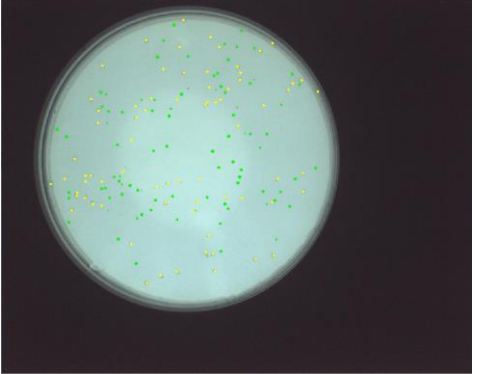
Results of Automatic and Interactive methods for the 26 images.


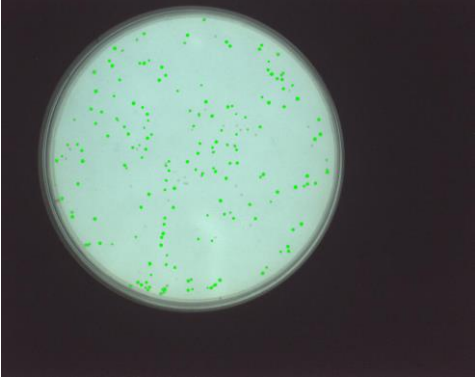
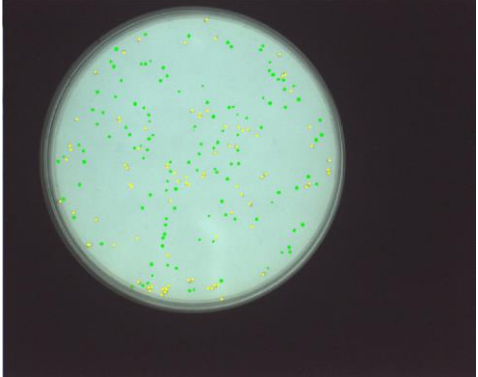
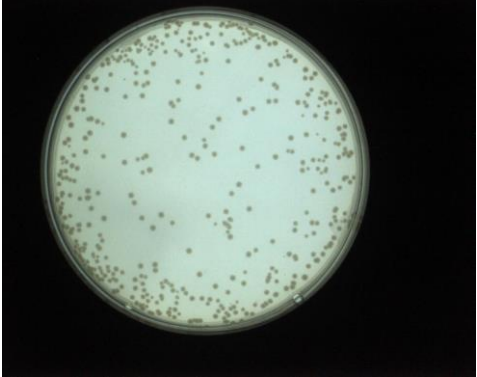
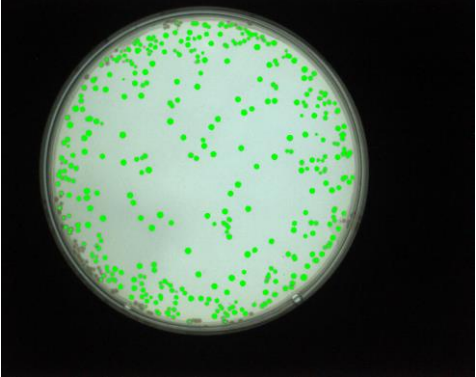
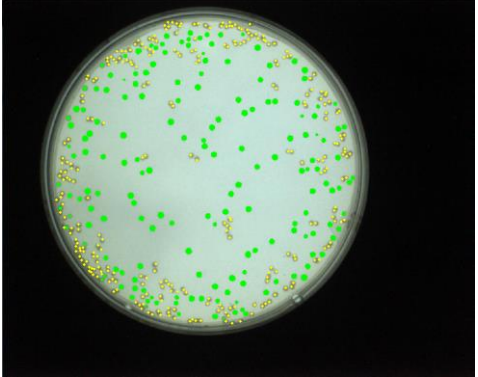
Original	Automatic method	Interactive method
		
		


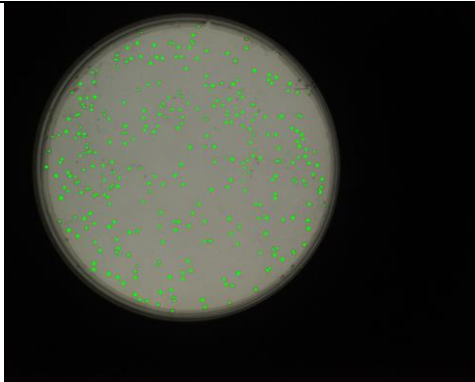
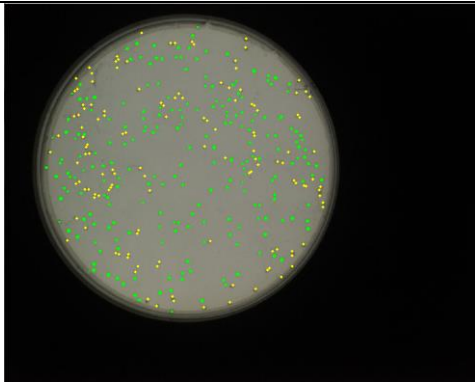
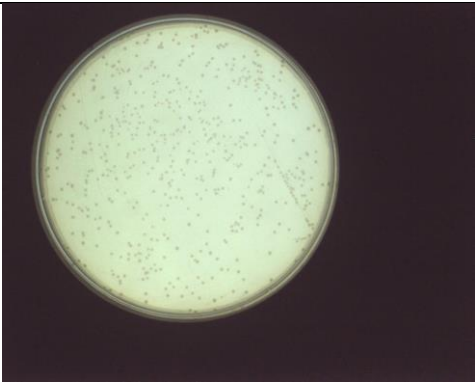
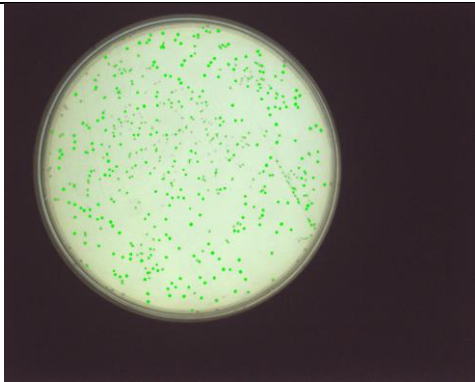
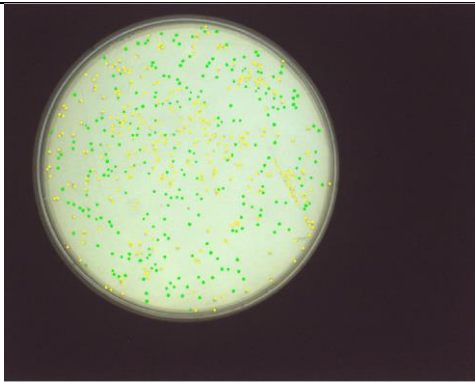
Original	Automatic method	Interactive method
		
		

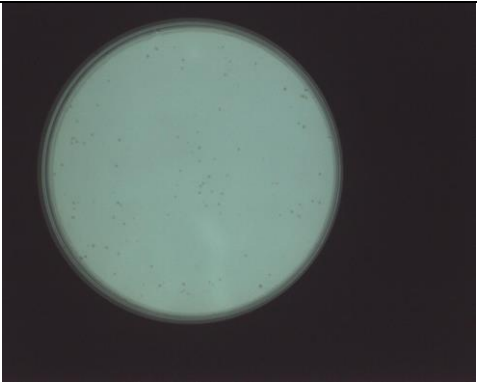
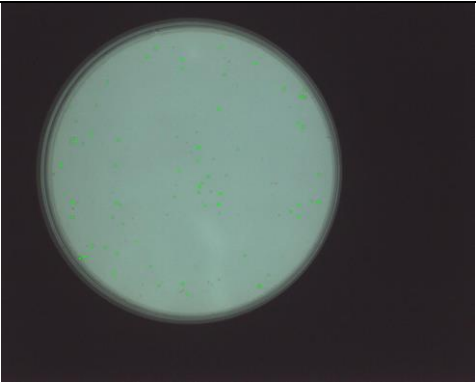
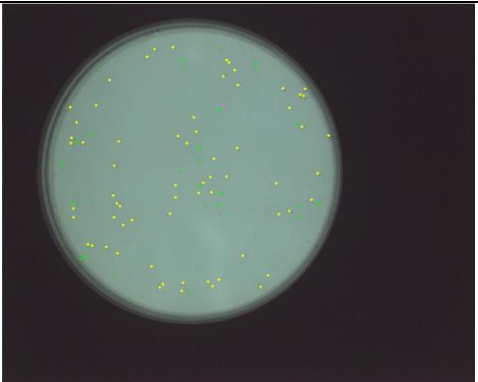
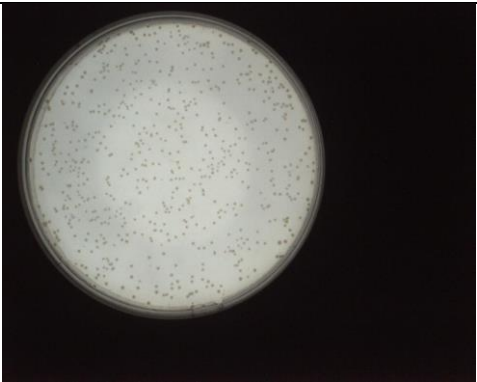
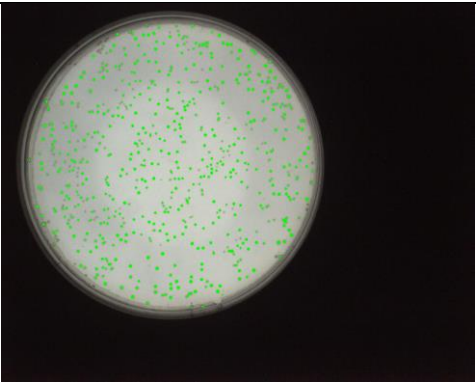
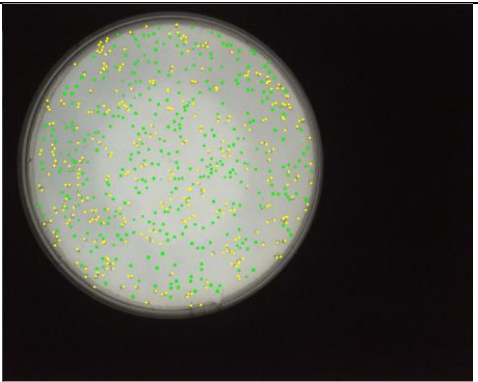
Original	Automatic method	Interactive method
		
		


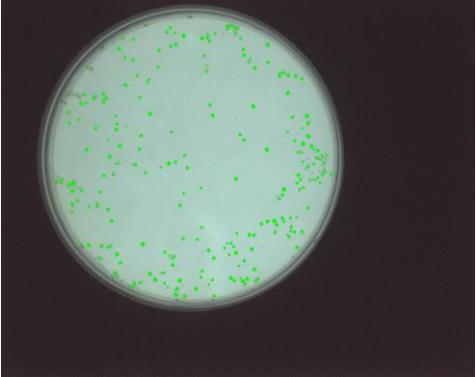
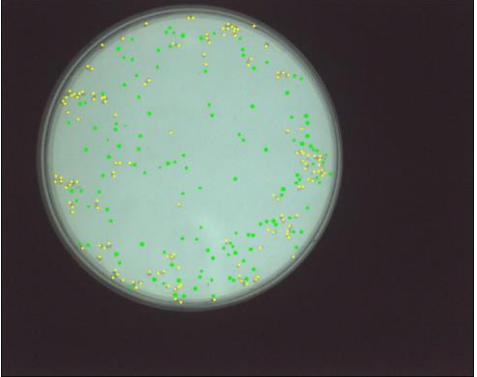

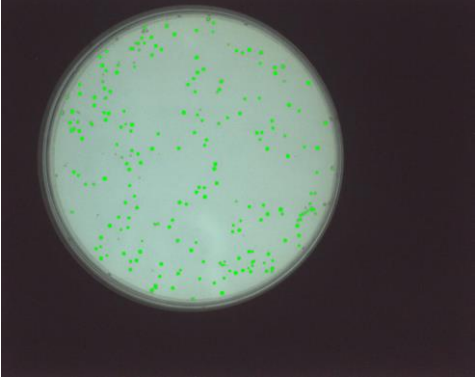
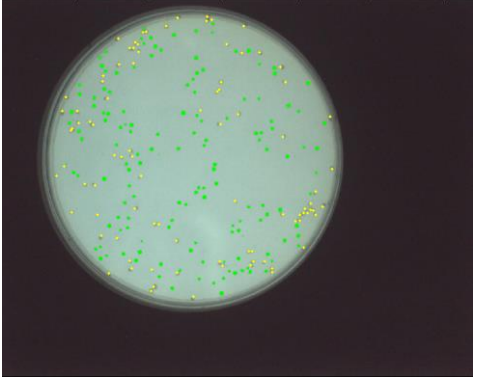
Original	Automatic method	Interactive method
		
		

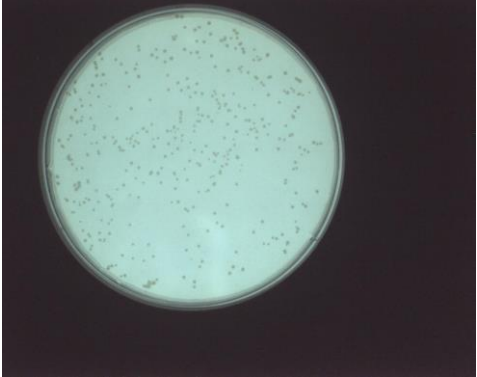
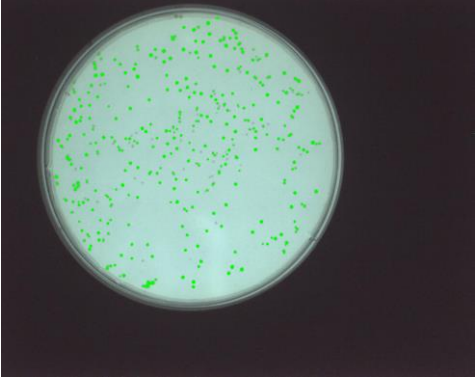
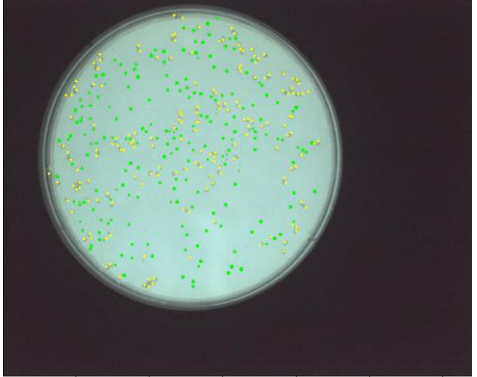
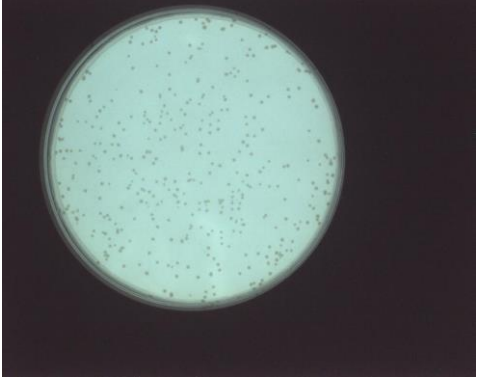
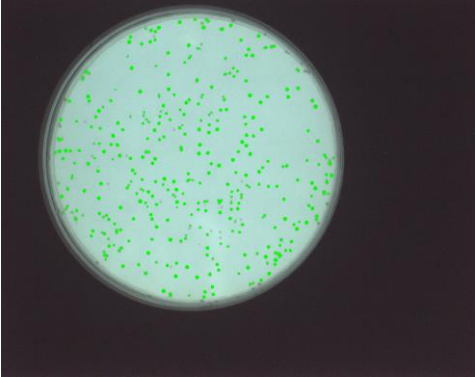
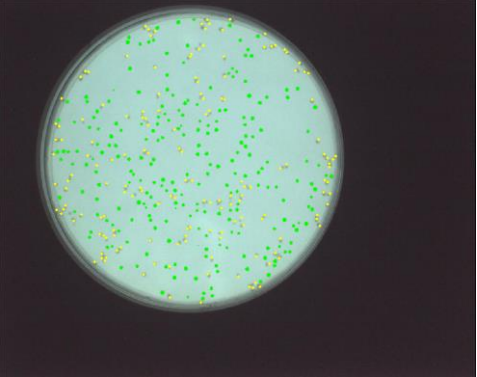
Original	Automatic method	Interactive method
		
		

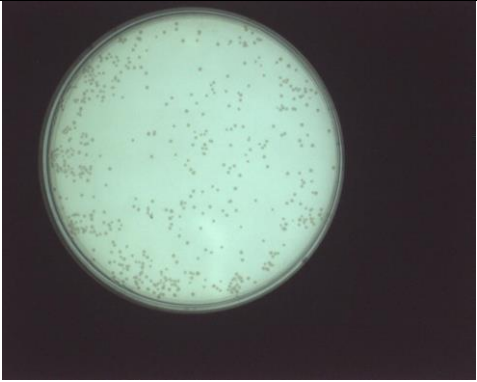
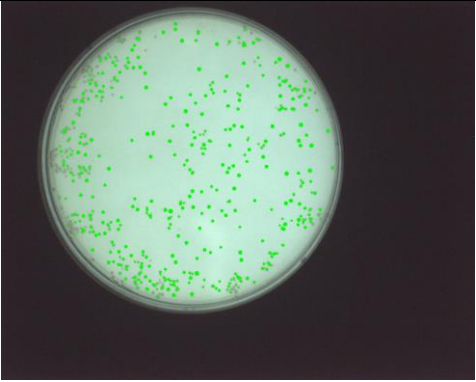
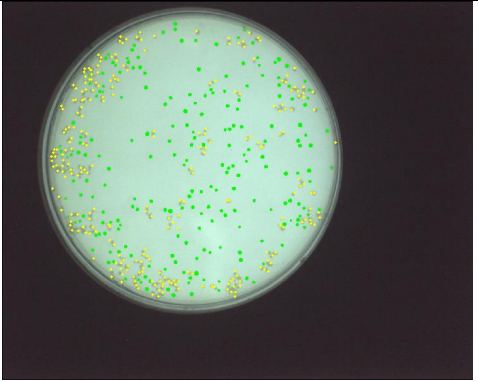

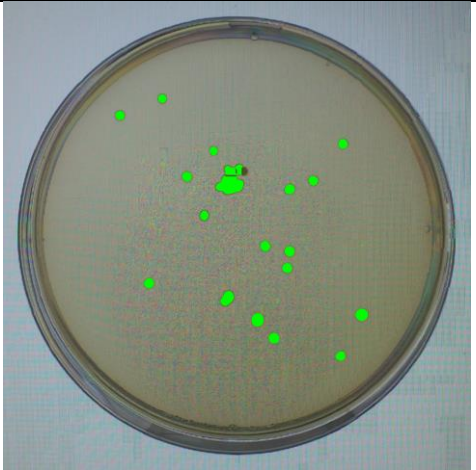
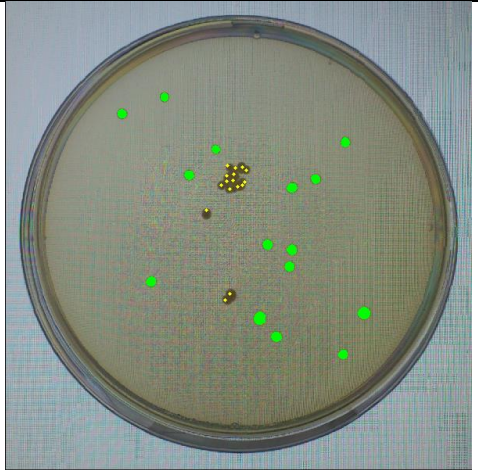
Original	Automatic method	Interactive method
		
		

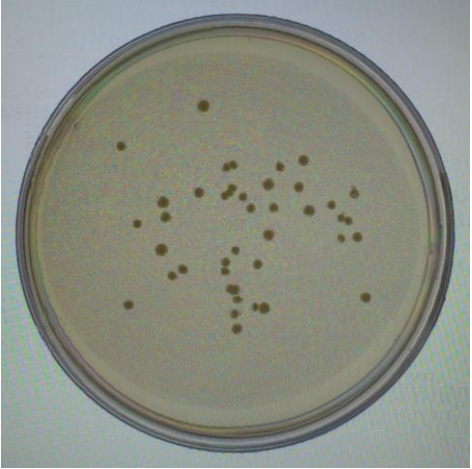
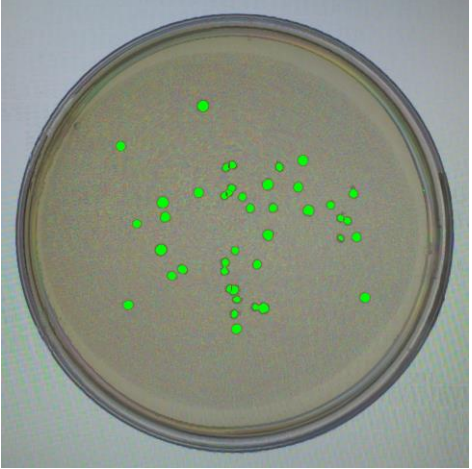
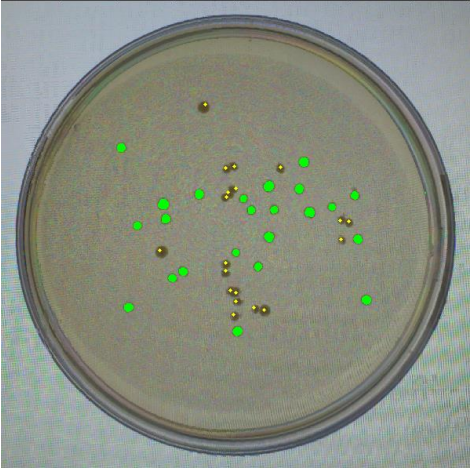
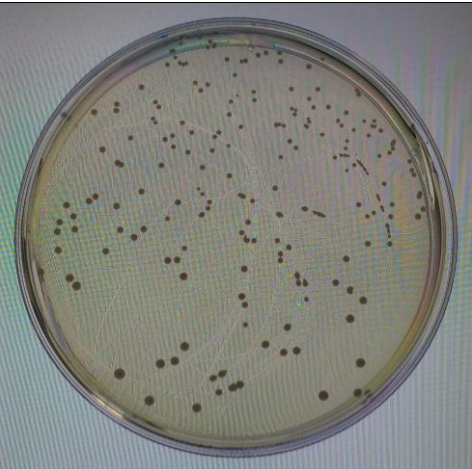
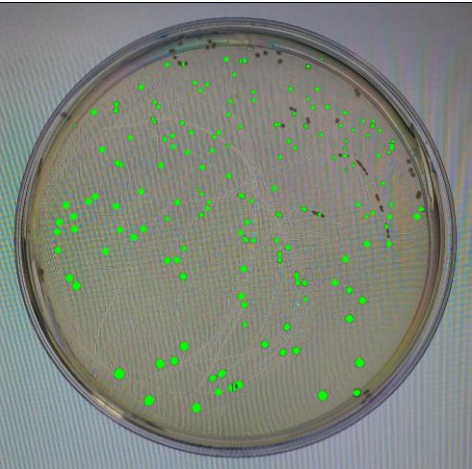
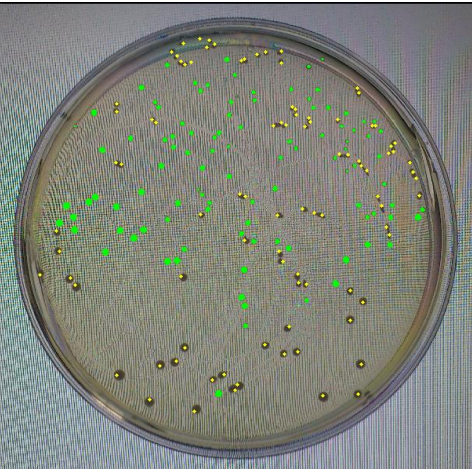
Original	Automatic method	Interactive method
		
		

Original	Automatic method	Interactive method
		
		

Original	Automatic method	Interactive method
		
		

Original	Automatic method	Interactive method
		
		

Original	Automatic method	Interactive method
		
		

Original	Automatic method	Interactive method
		
		

Original	Automatic method	Interactive method
