Mobile Application for Automatic Counting of Bacterial Colonies

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Abstract. In the following article it is proposed the design and implementation of a mobile application using a computer vision system that allows to count bacterial colonies in microbial cultures, decreasing significantly the time of quantification and generating a standard counting method for mobile devices running Android OS.

Keywords: Android, mobile application, microbiological count, computer vision

1 Introduction

The microbiological area forms part of the daily activities, everyday the microorganisms are participating in a beneficial and detrimental way, developing different roles. In the biological area, one of the tasks is the isolation and identification. Depending on the type of sample and analysis, it is necessary to know the number of colony forming units (CFU) present, this number meets the standards established under regulations in the area of food, health, water analysis, air and soil, among others. For these analyzes, experts of these areas perform manual counts that consume large amounts of time, yielding results that vary according to who performs the counting.

In this work it is proposed a mobile application to automate the quantification of bacterial colonies on culture plates, using a computer vision system, in which the image is cropped, converted to grayscale, contrast is improved and the image is segmented for counting.

This article is organized as follows: The section 2 describes the traditional counting method used in the microbiology laboratories, as well as some tools to facilitate the task; the section 3 shows the structure of the mobile application, explaining the stages of data acquisition, preprocessing and counting; the section 4 explains the results obtained using simulated and experimental samples of bacterial colonies; and finally the section 5 develops the conclusions of the work.

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2 Traditional Counting Method

It is not only important to know the potential microorganism to cause a severe infection or a beneficial effect, but it is also important to know the number of microorganisms involved [1].

There are different types of methods or techniques to quantify microorganisms, which are described below:

i. Plate count method: This method has the advantage of having a good detection limit, however, it consumes larges amounts of time during the preparation of the plates [2].

   The Mexican official standard NOM-092-SSA1-1994, Goods and Services. Method for aerobic bacteria count in plate [6] suggests using the following tools for counting bacterial colonies:

   - Dark field colony counter, with adequate light, grid plate glass, and magnifying lens: It allows counting colonies per pulse of the counter, recording the result on a digital display.

   - Mechanical or electronic recorder: It combines the function of electronic counter of pressure, with permanent marker labeling, preventing double counting.

ii. Petrifilm system: Its design has a rehydratable film coated with nutrients and gelling agents. Provides results in three steps: inoculation, incubation and counting [3].

3 Structure of the Proposed Application

The application can run in mobile devices with the following minimal characteristics:

- Android OS 4.1+
- 1Gb RAM
- 1.2GHz processor

3.1 General Operating Diagram

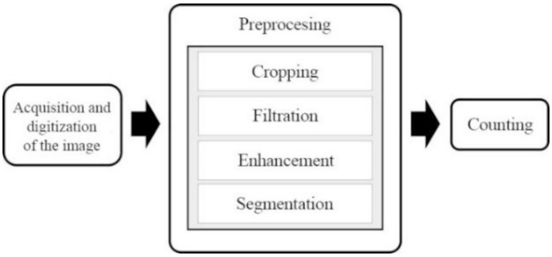


Fig. 1. General sequential operation scheme

3.2 Image Acquisition

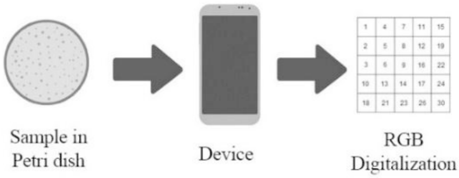


Fig. 2. Schematic operation of the data acquisition stage

In the figure 2 is shown the diagram of the image acquisition process of the application, in which is shown how the device takes the image and digitalizes it to RGB for future preprocessing.

3.3 Preprocessing

This stage involves the orderly implementation of filtering algorithms and improvement of existing images, where each has a well-defined objective.

Description of the preprocessing of the image.

The image conditioning is performed in four steps: cropping, filtering, enhancement and segmentation. Figure 3 shows graphically the order in which the process is performed, later it is explained how each of the stages are performed:

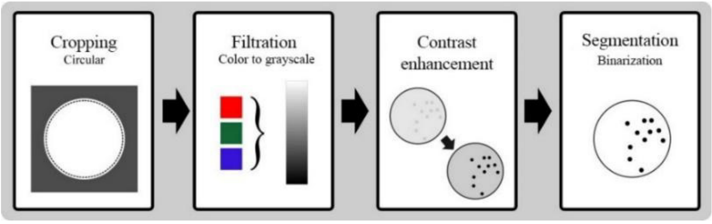


Fig. 3. Diagram of the preprocessing stages.

- Cropping: In the figure 4 is shown an example of an image processed by the mobile application, it can be seen that bacteria are inside a Petri dish, behind the plate can be seen a background which can change color depending of the environment where the photography is taken.

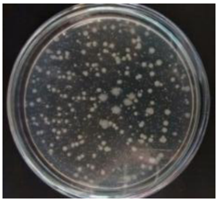


Fig. 4. Image capture by the mobile device

The bacterial colonies to count are concentred only on the content of the Petri dish, therefore, the areas relating to the outside are not useful and have to be excluded to prevent provide information of no interest in the stage of counting. An example of result of the stage of cropping is shown in the figure 5.

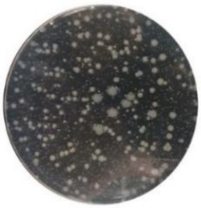


Fig. 5. Resulting image of the cropping stage

- Filtration: Within the capture and digitalization of images, samples are stored in RGB color model [5], the information stored in the different color channels Red, Green and Blue is not necessary to use it fully as it can be translated into excessive processing. In this stage of filtering a scale change is proposed in each pixel values, convert an RGB representation to grayscale. All this help to reduce the amount of information to process and facilities the stage of segmentation. An example in changing levels of color to grayscale is shown in the figure 6.

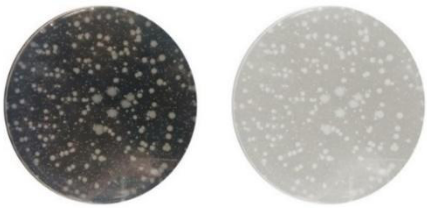


Fig. 6. Original image (left) in RGB format and resulting grayscale image (right)

- Enhancement: This stage is focused on improving the contrast in order to increase the difference between the bacterial colonies and the bottom of the Petri dish, we would expect the generation of a good segmentation stage following the application of contrast enhancement. The technique applied was linear expansion of the histogram [8], this process is based on a transformation of the gray levels, a linear distribution of the values that are within the range of 0 to 255 is performed.

  In the figure 7 is shown an image without the application of the linear expansion and its corresponding histogram of gray levels, later in the figure 8 is shown the result of the implementation of linear expansion (contrast enhancement) and the resulting histogram of gray levels.

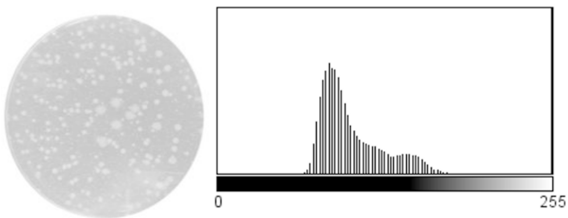


Fig. 7. Original image (left) and its histogram of gray color levels (right)

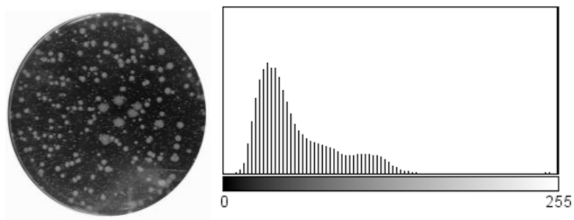


Fig. 8. Resulting image after applying linear expansion of the histogram (left) and its histogram of gray color levels (right)

- Segmentation: Before the counting process it is necessary to fully highlight the bacterial colonies with respect to the bottom of the Petri dish, for this, the Noboyuki Otsu's automatic binarization technique [4] is applied to the resulting image of the enhancement stage, the main goal is to generate sufficient discrimination to detect and segment the colonies. In the figure 9 can be seen the result of the implementation of the automatic binarization.

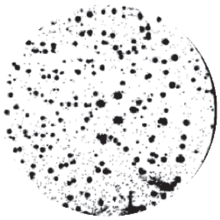


Fig. 9. Result obtained from implementing automatic binarization

3.4 Counting

To count the number of bacterial colonies in the image the Euler's method is used [7], where, the segmented image, is inverted and tagged. In the process of inverting the image, the colonies that were black are transformed to white. Then in the phase of tagging, tags are assigned to all objects found in the inverted image. These new objects will be now the bacterial colonies. The background of the image will not be tagged as a colony because it will have a different color, in this case white. Therefore, the number of colonies will be equal to the number of tags obtained. In the figure 10 can be seen the inverted image.

Fig. 10. Result of the process of invert the image for counting

4 Results

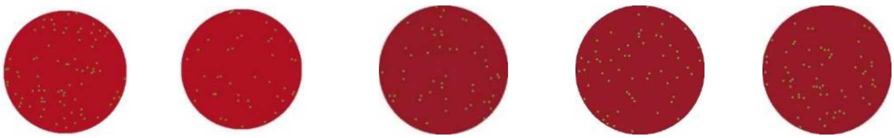
Then the results of the colony counts are presented from experimental and simulated tests achieved with this application called CA-BACT and the following two counting applications selected in the market (Table 1):

Name	Type of Application	O.S.	Counting area
CFU Scope[1] version 1.0.0	Mobile	iOS	Microbiology
AstroimageJ [2] version 2.4.1	Desktop	Windows	Astronomy
CA-BACT version 1.0	Mobile	Android	Microbiology

Table. 1. Comparison of selected applications

4.1 Simulated samples

In the first test, ten images were generated using the simulator (Figure 11) developed to test the application proposed using a sample of *Escherichia Coli* in agar culture medium with eosin methylene blue (EMB):



a)	b)	c)	d)	e)
Dilution 10^7 Plate 1	Dilution 10^7 Plate 2	Dilution 10^7 Plate 3	Dilution 10^7 Plate 4	Dilution 10^7 Plate 5

f)	g)	h)	i)	j)
Dilution 10^8 Plate 1	Dilution 10^8 Plate 2	Dilution 10^8 Plate 3	Dilution 10^8 Plate 4	Dilution 10^8 Plate 5

Fig. 11. Simulated samples

The results achieved by making automatic counts with the three applications were as follows:

[1] http://medixgraph.com/cfuscope
[2] http://www.astro.louisville.edu/software/astroimagej/

Image	Number of CFU simulated	CFU count with CA-BACT	CFU count with CFU Scope	CFU count with AstroImageJ
1)	81	80	31	81
2)	43	42	17	42
3)	53	52	13	54
4)	64	64	16	64
5)	70	69	21	71
6)	28	28	5	27
7)	36	34	11	36
8)	25	25	8	24
9)	30	30	10	30
10)	38	38	22	39

Table 2. Results of different automatic counts from simulated images

In the Table 2 can be seen the counting results of the three applications of which the count efficiency percentage was obtained as follows:

$$\text{Efficiency Percentage} = \frac{\sum_{1..n} CFU\ loss\ percentage}{\text{n}}$$

Where:

n is the number of selected samples

$$\text{CFU Loss Percentage} = \frac{(Simulated\ CFU - CFU\ automatic\ count) * 100}{Simulated\ CFU}$$

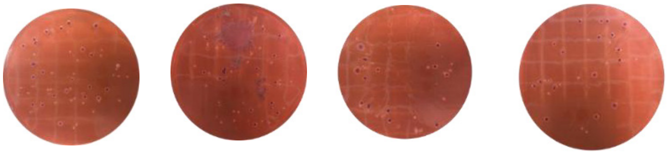
Given that the application CA-BACT has a counting effectiveness rate of 98%, the application CFU Scope has an effectiveness of 32% and the AstroimageJ application has a 100% of effectiveness.

Allowing establish that although the application AstroimageJ obtained a better result with the simulated images, the proposed application CA-BACT has the advantage of being a mobile application which potentiates its usability features, an easy download and installation from the Android Application Store (Google Play Store), more promotion and outreach to potential users.

For all the above it is concluded that the image preprocessing and the counting algorithm of the proposed application is efficient and is developed in a technology that gives preferred use and more visibility to the user.

4.2 Experimental samples

The samples used in these experimental tests were generated in the laboratory of Biology of the Interdisciplinary Professional Unit of Engineering Campus Zacatecas (UPIIZ), having four test samples of effluent dams that contained *Escherichia Coli* in differential culture medium EMB (figure 12), the photographs were taken with a standard device[3] in uncontrolled lighting, background, perspective and reflection conditions, having the following results with reference to the average manual count by four users of the same laboratory, the Table 3 shows the results:



1)Image of Dilution 10^7 Plate 1

2) Image of Dilution 10^7 Plate 2

3) Image of Dilution 10^8 Plate 1

4) Image of Dilution 10^8 Plate 2

Fig. 12. Experimental samples

#	Avg. CFU manual count	Avg. manual count time	Counting with CA-BACT	Counting with CFU Scope	Counting with AstroImageJ
1)	57	3 min	73	18	423
2)	91	4 min	75	43	567
3)	35	2min	22	12	398
4)	27	1 ½ min	21	9	400

Table 1. Manual counting results against automatic counts in the experimental test.

In this test for the counting efficiency of the three applications, the average CFU number of the four manual counts is taken as parameter to compare, having the following results: The proposed application CA-BACT has a counting effectiveness rate of 74%, the application CFU Scope has an effectiveness of 32% and AstroimageJ has a -394% of effectiveness, this negative value shows that the application AstroimageJ it is not feasible with noisy images, because it does not perform any preprocessing to the image.

In the Table 3 can be seen that the times when manual counting is performed in valid samples of 25 to 250 CFU can vary from one minute and a half to about five minutes per sample, having a major error in the manual counting of samples with more number of CFU, so an automatic counting saves almost 98% of time as automatic counting applications presented here take between 2 and 5 seconds to count, and also completely eliminate the difference of counts in the same sample, because

[3] Device with 1GB RAM, 1.2GHz Dual processor and 8Mpx camera

during the experimental tests it was observed that in the manual count of a sample more than once with the same person can produce different results, instead, when the application uses the same image, it always gives the same result.

5 Conclusions

CFU losses in the count with the developed application CA-BACT can be lessened by controlling the shooting conditions of the image that is processed by the application such as lighting, background, perspective and reflections.

In the area of computer vision other techniques can be used for transforming the intensity of the images so it can give better results with the proposed filters in this paper.

A good image preprocessing serves a very important role within the application, as it helps to have better results than if the image was processed as obtained in the acquisition stage.

The proposed mobile application was tested with volunteer users of the biology laboratory at UPIIZ to check its efficiency and effectiveness. It's about to start the implementation of the general use with all users of this laboratory; however, by following norms and standards used in this type of laboratories such as The Mexican official standard NOM-092-SSA1-1994, when counting in culture plates, this mobile application can be a general purpose application in this area.

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