Article **Journal of Systematic Innovation**
December 2021, Vol.5 No.17 28-33

Classification and counting system for bacteria in microbiological culture media using image processing

Sistema de clasificación y recuento de bacterias en medios de cultivo microbiológicos mediante el procesamiento de imágenes

GUTIÉRREZ-LEÓN, Diana Guadalupe†*, SERRANO-RAMÍREZ, Tomás, GRANADOS-ALEJO, Vignaud and DE LOS SANTOS-LARA, Pedro Jorge

Universidad Politécnica de Guanajuato, Department of Robotics Engineering, Mexico.

ID 1st Author: *Diana Guadalupe, Gutiérrez-León* / **ORC ID**: 0000-0001-5051-880X, **Researcher ID Thomson:** G-6035-2018, **CVU CONACYT ID:** 443892

ID 1st Co-author: *Tomás, Serrano-Ramírez* / **ORC ID**: 0000-0001-6118-3830, **Researcher ID Thomson:** G-6039-2018, **CVU CONACYT ID:** 493323

ID 2nd Co-author: *Vignaud, Granados-Alejo* / **ORC ID**: 0000-0002-3501-0802, **Researcher ID Thomson:** AAS-4938-2021 y **CVU CONACYT ID:** 211987

ID 3rd Co-author: *Pedro Jorge, De Los Santos-Lara* / **ORC ID**: 0000-0003-4374-1522, **CVU CONACYT ID:** 662527

DOI: 10.35429/JSI.2021.17.5.28.33 Received July 25, 2021; Accepted December 30, 2021

Abstract

Monitoring water quality requires microbiological methods with the aim to provide to population, access to this essential source in appropriate conditions. Currently, conducting microbiological tests involves long periods of time and a high economic investment with the aim to identify and quantitatively determine the microorganisms in the medium. In this work, the use of image processing techniques involving K-means algorithm, Python language and OpenCV library are proposed in order to, through devices such as smartphones or conventional cameras, the samples can be analyzed through images, basing results on the morphological features of microorganisms in a specific growth medium, involving low cost as well as a reduced period of time. Specifically, the results obtained of *Escherichia coli* and *Salmonella Typhimurium* bacteria in Red Bile Violet agar are presented. The developed system was carried out detection and quantification of colonies of these microorganisms correctly. Also, it was possible to identify influencing factors during its operation, which allow to implement improvements to the proposed system.

Processing, Bacterial-counting, Classification

Resumen

El monitoreo de la calidad del agua requiere de métodos microbiológicos, con el propósito de brindar a la población el acceso a este recurso esencial en las condiciones adecuadas. Actualmente, la realización de ensayos microbiológicos involucra largos períodos de tiempo y una alta inversión económica para lograr identificar y determinar cuantitativamente, los microorganismos que se encuentran en el medio. En el presente trabajo, se propone como alternativa, la utilización de técnicas en procesamiento de imágenes que involucran el algoritmo K-means, el lenguaje Python y la librería OpenCV para que, a través de dispositivos tales como teléfonos inteligentes o cámaras convencionales, puedan ser analizadas las muestras a través de imágenes, basando los resultados en las características morfológicas que presentan los microorganismos en un medio de cultivo específico, implicando con ello, una disminución en el costo y un período de tiempo reducido. Concretamente, se presentan los resultados obtenidos para las bacterias: *Escherichia coli* y *Salmonella Typhimurium* en ágar Rojo Bilis Violeta. El sistema desarrollado realiza la correcta detección y el conteo de colonias de estos microorganismos; adicionalmente, se lograron identificar aquellos factores que influenciaban durante su operación, lo cual permitirá implementar mejoras a lo propuesto.

Procesamiento, Conteo-bacteriano, Clasificación

Citation: GUTIÉRREZ-LEÓN, Diana Guadalupe, SERRANO-RAMÍREZ, Tomás, GRANADOS-ALEJO, Vignaud and DE LOS SANTOS-LARA, Pedro Jorge. Classification and counting system for bacteria in microbiological culture media using image processing. Journal of Systematic Innovation. 2021. 5-17:28-33.

* Correspondence to Author (E-mail: dgutierrez@upgto.edu.mx).
† Researcher contributing as first author.

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Introduction

According to experts of United Nations, universal access to water is vital for the human being, in enough quantity and under stablished quality standards (ONU-DAES, 2014). Despite this, in 2019 the World Health Organization declared that, globally, 842,000 people died annually as a result of unsafe water, poor sanitation or inappropriate hygiene during hand washing; additionally, in vulnerable countries it was detected that 22% of hospitals did not have water supply and more than 2 billion people satisfy their needs from contaminated sources containing feces.

The pandemic caused by Covid-19 has shown that, availability of water source is essential for the consumption and hygiene of everyone and at the same time, it represents an important vulnerability factor in health. Water problem was significantly accentuated due to confinement; for those who lacked an adequate water supply, the situation became extremely complex because this resource was required to attend to basic preventive measures such as hand washing and ensuring cleanliness of spaces in order to reduce the contagion from Covid-19 to the maximum extent possible (Maganda, 2020). Additionally, those who used contaminated water trying to prevent virus were exposed to diseases caused by other pathogenic microorganism contained in it (UN, 2020).

In this respect, microbiological monitoring plays a determining role in water quality and, it is important to ensure that supply of the source does not imply a risk health for population. Its implementation requires different stages among which, experimental tests are carrying out for identification of microorganisms contained in water and their corresponding concentration (Colony Forming Units, CFU).

Figure 1 presents the scheme to follow the implementation of the plate count technique applied to water (Sánchez *et al*., 2017). In it, from a water sample of interest, 1:10 serial dilutions are prepared. Subsequently, from each dilution, 100 µL volume of aliquot is extracted, emptied and distributed into a Petri dish containing the solidified culture medium, in order to carry out the inoculation through the uniform spreading of liquid.

Then, Petri dish is inverted and entered into the incubation process. After the period under appropriate conditions, qualified personnel must manually, with the support of a counting camera, quantify hundreds of bacteria colonies, as well as verify their characteristics in order to contribute to the emission of a reliable microbiological diagnosis (Chen and Zhang, 2009). It is important to consider that, for each dilution, the inoculation in culture medium must be carried out in triplicate.

The microbiological procedure in general, requires long periods of time and a significant economic investment in terms of reactants and material requirements, it is therefore necessary to guarantee that the results generated are safe and reproducible.

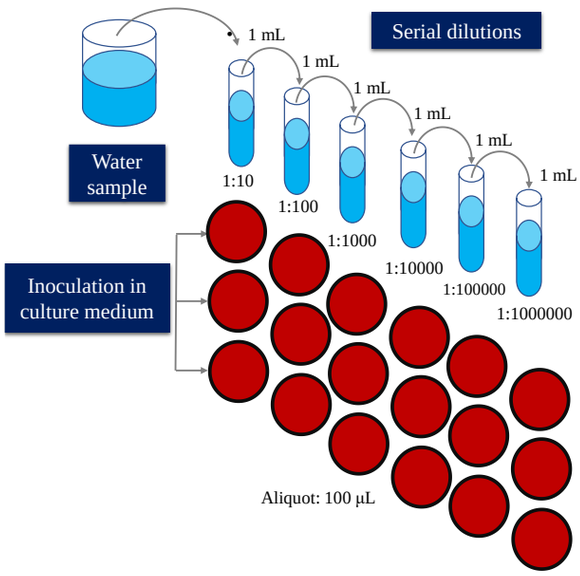

Figure 1 Implementation in plate count technique to a water sample

Currently, there are equipment on the market that has the technology to perform the task of detecting microorganisms and counting them; however, their cost is high, they require specialized maintenance as well as the allocation of a space under certain conditions and, are designed to operate only under stablished standards; eg. The size of Petri dish (Chen y Zhang, 2009; Sánchez *et al*., 2017).

Considering the above, the objective of this work is to contribute to the implementation of a system for detecting, classifying and counting bacterial colonies through image processing using K-means algorithm, Python language and the OpenCV library.

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The tests carried out in this study were based on the growth on the culture medium Red Bile Violet Agar (RBV), of the bacterial microorganisms detected in a water sample generated from poultry activities: *Escherichia coli* (*E. coli*) and *Salmonella Typhimurium* (*S. Typhimurium*).

Stages for system development

The counting, morphological analysis and the growth response in a specific medium of bacterial colonies found in laboratory cultures, are repetitive and demanding tasks that require significant time to perform, even for the expert human eye.

This work focuses on the implementation of a system for the segmentation and counting of bacterial colonies through the use of image processing techniques, applied in color photographs, taken to bacterial cultures using smartphones or any conventional camera.
The first stage consists of testing a set of segmentation techniques to separate the bacterial colonies from the rest of the image sections that are not of interest. These techniques include: thresholding, edge extraction by Canny algorithm and clustering by K-means.

The second stage consists in the implementation of an automated bacterial colony detection and counting system that indicates in the original image the contour found for each of the colonies and, in turn, displays the final count of their number.

Testing techniques for segmentation

In this work, color images taken from a proprietary database were analyzed with cultures of *E. coli* and *S. Typhimurium* bacteria on RBV agar, whose coloration is reddish. This culture medium is characterized by being selective and differential, allowing only the growth of gram-negative and enteric bacteria. It is differential because it allows the type of bacteria to be distinguished by the color tones acquired by the colonies, which ranging from intense red through pink to colorless.

In the particular case of *E. coli* bacteria, the colonies are red or pink in color, while for *S. Typhimurium* colorless colonies are obtained.

Figure 2 shows an image belonging to the own database with a culture of *E. coli* and *S. Typhimurium* bacteria in 150 mm diameter of Petri dish using RBV agar.

The first method implemented for the separation of bacterial colonies is thresholding, corresponding to the simplest way of performing a segmentation process. The thresholding carried out consists of converting a RGB image to grayscale, performing an analysis of the frequency of intensities by means of the histogram in order to determine the possible range in which the intensities of the objects to be segmented are found. Once this has been done, a threshold is determined. If the intensity of a pixel exceeds the threshold, it will take the value of 255 (white); on the other hand, if it is below the threshold, it will take the value of 0 (black). Each pixel has an intensity set between 0 (black) and 255 (white) in a grayscale image.

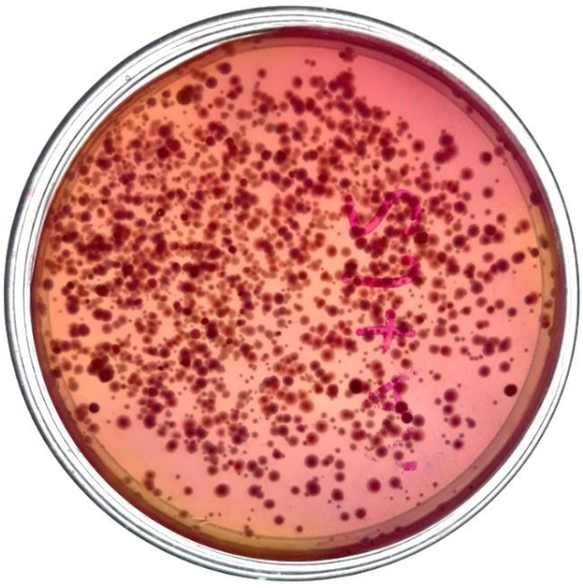

Figure 2 *E. coli* and *S. Typhimurium* bacteria culture.

The thresholding carried out consists of converting a RGB image to grayscale, performing an analysis of the frequency of intensities by means of the histogram in order to determine the possible range in which the intensities of the objects to be segmented are found. Once this has been done, a threshold is determined. If the intensity of a pixel exceeds the threshold, it will take the value of 255 (white); on the other hand, if it is below the threshold, it will take the value of 0 (black). Each pixel has an intensity set between 0 (black) and 255 (white) in a grayscale image.

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The algorithm was applied to a subset of the culture database, resulting in the generation of a significant number of false positives, produced by bubbles, shadows, reflections, labeling marks, among others. The results are in agreement with those reported by Chen and Zhang (2009). Elements of different class, but with similar intensities in the histogram, can be easily segmented as of the same type.

Another important issue is the lighting conditions, which can considerably affect the performance of this approach. Additionally, color information is also lost when converting to grayscale, preventing the subsequent separation of different types of bacteria. Figure 3 shows the binary image produced by the thresholding algorithm applied to the culture in Figure 2.

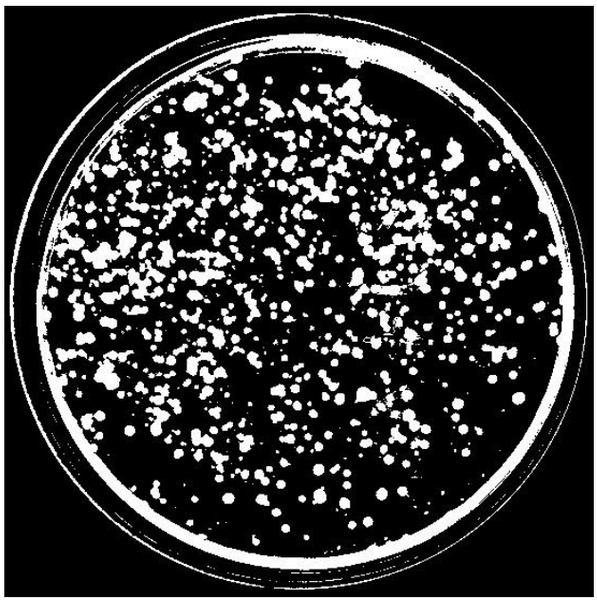

Figure 3 Segmentation produced by the thresholding algorithm applied to the culture in Fig. 2.

Traditional segmentation methods can temporarily solve a detection or counting problem under controlled conditions, always taking into account changes in the initial conditions and their calibration. Changes in brightness, agar type, bacterial class can lead to erroneous results if not taken into account.

There are more advanced techniques that enter the field of pattern recognition and artificial intelligence, capable of grouping pixels by their similarity of characteristics and proximity, without any a priori knowledge. Such algorithms fall into a type of learning known as unsupervised.

The image of a bacterial culture can be divided into an undetermined number of groups of pixels, related to each other by their nature. These groups may represent the type of bacteria or colony, background, culture medium, container vessel or some other class.

K-means corresponds to one of the simplest and most robust unsupervised learning clustering techniques used in data analysis. Because of this, it was chosen to be implemented in this work with the objective of separating the bacterial colonies from the rest of the image and not only that, to separate the colonies by the type of bacteria that make them up.

K-means clustering algorithm attempts to separate an anonymous data set without any class information, into a fixed number of *K* groups. The letter *K* corresponds to the number of centroids which are points at the center of each cluster.

Automatic system for classifying and counting bacteria

The results obtained by the K-means algorithm, allowed the segmentation of two different bacteria types, as shown in Figure 4 b) and c). Item b) corresponds to the segmentation of colonies for *S. Typhimurium* and c) corresponds to *E. coli*.

These images clearly separate both groups and therefore will be used in the implementation of the automatic colony counting and classification system, although they still need to be processed to be used effectively in this task.

The first step consists of converting images 4 a) and 4 b) into binary type images to be used as masks. For this purpose, a thresholding algorithm is implemented, whose threshold is set to a very low value.

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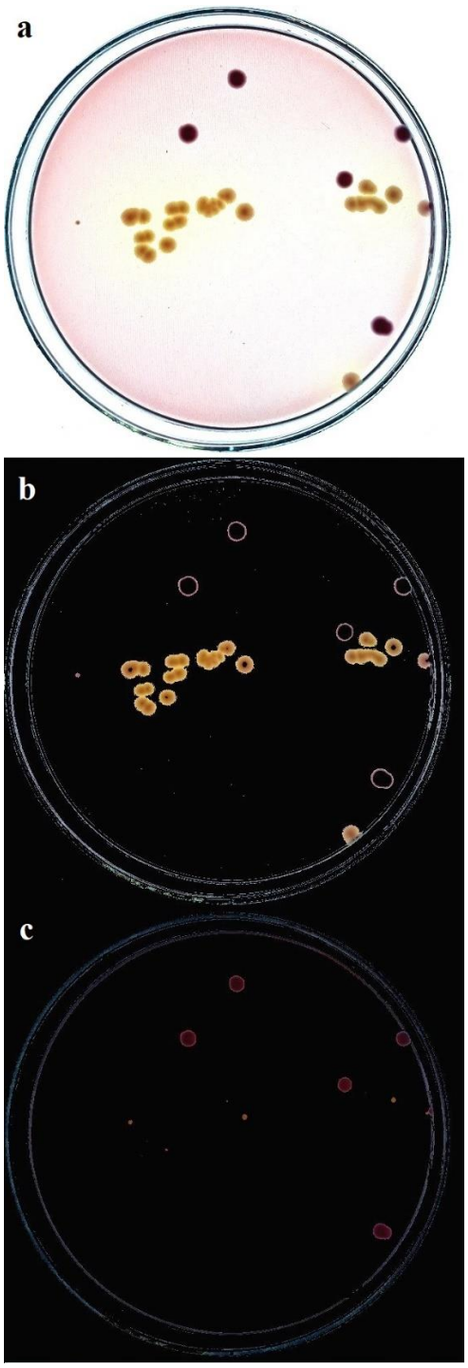

Figure 4 Segmentation produced by the K-means algorithm, showing a clear separation between colonies of a) *S. Typhimurium* and b) *E. coli*

The result can be seen in Figure 5. The masks obtained in the previous step contain noise generated in the classification process by K-means or even due to noise generated by the contours of the Petri dish, reflections and imperfections of the agar. That is why the masks need to undergo a filtering process, which is performed by morphological operations: erosion, dilation, opening and closing.

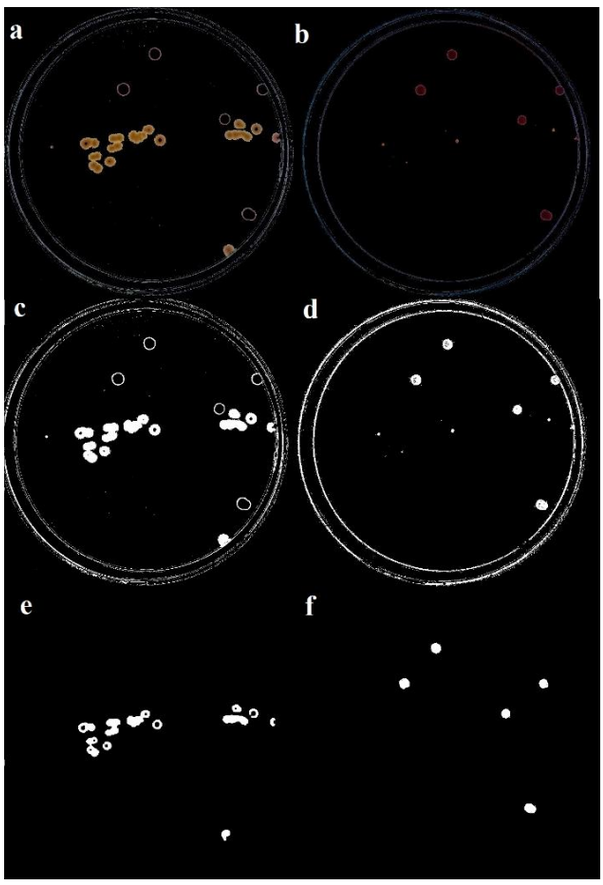

Figure 5 Implementation of binary masks: a) and b) K-means results. c) and d) Thresholding. e) and f) Application of morphological operations: erosion, dilation, opening and closing

The binary masks shown in Figure 5 e) and f) will be used for the classification of bacterial colonies corresponding to *S. Typhimurium* and *E. coli* in the original image, as well as allowing the separate counting of each type of colony. This was implemented using a contour detection and counting tool provided by the vision library: open CV.

Figure 6 shows the result of the classification and counting of bacterial colonies. Colonies belonging to *S. Typhimurium* were enclosed in a blue outline, while those belonging to *E. coli* were indicated by a green outline. The count of each colony type is displayed in the upper left part of the image, achieving a compatibility similar to manual counting.

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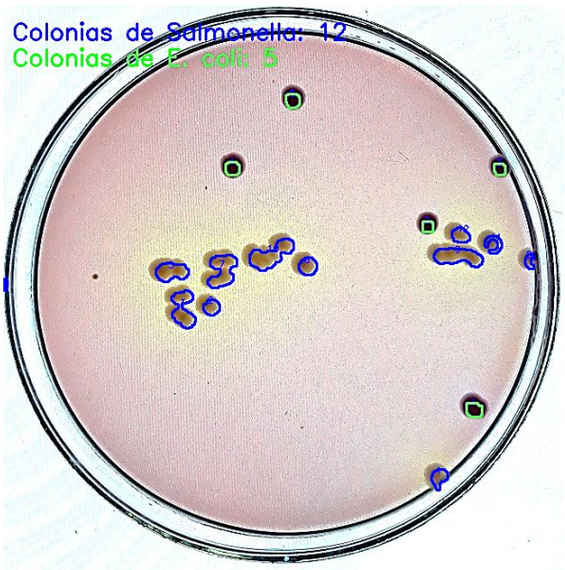

Figure 6 The culture image is submitted for analysis. The system draws an outline around each bacterial colony and distinguishes between two types of existing colonies, blue corresponding to *E. coli* and green to *S. Typhimurium.* The system counts each type of colony separately and displays the result on the image.

Acknowledgments

The authors would like to thank the Department of Robotics Engineering and Subdirección de Investigación Posgrado e Internacionalización, Universidad Politécnica de Guanajuato for its support in the publication of this work.

Conclusions

In this work, a classifier and counter of bacterial colonies in laboratory cultures was successfully performed by processing images obtained with a smartphone.

The implementation of an unsupervised learning algorithm allowed, without prior training, to separate the bacterial colonies from the other elements of the image such as: the Petri dish, the agar, the background objects and the artifacts generated by the capture conditions. It was even possible to successfully segment the types of existing colonies, allowing their classification and counting by type.

The system was implemented using open source software, employs low-cost hardware and can be easily scaled for real-time operation through video analysis.

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