



Automated Colony-Forming Unit (CFU) Counting of Bacteria Using Digital Image Analysis Through Computer Vision with Python

Conteo automatizado de Unidades Formadoras de Colonias (UFC) bacterianas mediante análisis de imágenes digitales con visión computacional en Python

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Highlights

- The automated colony-forming unit (CFU) counter emerges as an alternative to manual counting, optimizing time and resources through digital image analysis.
- The hardware design controls photographic conditions, facilitating automation and ensuring high image quality regardless of the imaging device used.
- The proposed automated counting methodology is highly efficient, easily replicable, and accessible to a wide range of microbiological analysts.

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ABSTRACT

Introduction. Quantitative analysis of bacterial growth is essential in microbiological studies, as it enables the evaluation of microbial survival and proliferation rates, facilitating control and manipulation of microbial communities. However, traditional manual counting of colony-forming units (CFUs) on Petri dishes presents major limitations, including high time and resource consumption, and variability linked to the analyst's subjectivity. **Objectives.** This study aimed to develop a Python-based script for efficient and accurate CFU counting using digital images. **Materials and Methods.** Water samples were collected from supraglacial lakes of the Llaca Glacier (Peru) and inoculated on nutrient agar and R2A media using the spread plate method. Cultures were incubated at 5 °C for 30 days and photographed using a custom-built photographic chamber that standardized image capture. **Results.** The script, implemented in Google Colaboratory, follows a three-stage process: preprocessing, segmentation, and colony counting. Gaussian adaptive thresholding was selected for segmentation due to its robustness under variable image conditions. The system's performance was evaluated by comparing automated results with manual counts across 91 images. The method demonstrated high efficiency, achieving an precision of $97\% \pm 0.12$, a recall of $95\% \pm 1.10$, and an F-measure of $96\% \pm 0.10$, with a processing time of only 0.4 seconds per image. **Conclusions.** These results demonstrate that the system offers a reliable, fast, and low-cost alternative for CFU quantification. Its design is simple and adaptable, making it a replicable tool for microbiological laboratories, especially in resource-limited settings.

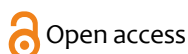
RESUMEN

Introducción. El análisis cuantitativo del crecimiento bacteriano es esencial en los estudios microbiológicos, ya que permite evaluar las tasas de supervivencia y proliferación microbiana, facilitando el control y la manipulación de las comunidades. Sin embargo, el conteo manual tradicional de Unidades Formadoras de Colonias (UFC) en placas Petri presenta limitaciones importantes, como el alto consumo de tiempo y recursos, además de la variabilidad asociada a la subjetividad del analista. **Objetivo.** Desarrollar un script en Python para el conteo eficiente y preciso de UFC a partir de imágenes digitales. **Materiales y Métodos.** Se recolectaron muestras de agua de lagunas supraglaciares del glaciar Llaca (Perú), las cuales se sembraron en medios agar nutritivo y R2A mediante el método de extensión en placa. Las culturas fueron incubadas a 5 °C durante 30 días y fotografiadas utilizando una cabina fotográfica diseñada para estandarizar la captura de imágenes. **Resultados.** El script, desarrollado en Google Colaboratory, siguió tres etapas: preprocesamiento, segmentación y conteo de colonias. Se seleccionó la umbralización adaptativa gaussiana por su robustez frente a variaciones en las condiciones de imagen. El sistema fue evaluado mediante la comparación directa entre conteo automático y manual en 91 imágenes, alcanzando una precisión del $97\% \pm 0.12$, un recall del $95\% \pm 1.10$ y una métrica F-Measure del $96\% \pm 0.10$, con un tiempo promedio de procesamiento de 0,4 segundos por imagen. **Conclusiones.** El sistema demostró ser una alternativa confiable, rápida y de bajo costo para la cuantificación de UFC, fácilmente replicable en laboratorios microbiológicos con recursos limitados.



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INTRODUCTION

Quantifying bacterial growth is a fundamental prerequisite in microbiological research ⁽¹⁾. Determining the survival and proliferation rates of microorganisms is essential for understanding, controlling, and manipulating microbial communities ⁽²⁾, allowing researchers to assess whether their presence is beneficial or harmful ⁽³⁾. To achieve this, the number of microorganisms present in a unit volume of a bacterial broth under specific conditions is quantified ⁽⁴⁾. This quantification can be performed using various methods, including flow cytometry, ATP bioluminescence, spiral plating, membrane filtration, or plate count techniques ^(5,6). Among these, manual colony counting on Petri dishes remains the most common ⁽⁷⁾ and cost-effective method.

Colony-forming unit (CFU) quantification relies on the assumption that each colony originates from a single bacterium ⁽⁸⁾. Although this method appears reliable, it is highly prone to errors due to its manual and subjective nature, in addition to being time-consuming, labor-intensive, and poorly reproducible ^(1,9). As colony density increases, the accuracy of manual counts decreases significantly, often resulting in discrepancies among operators ⁽¹⁰⁾. As an alternative, researchers may count only a portion of the plate and estimate the total via interpolation ⁽¹¹⁾, or reduce bacterial density through dilution—both of which increase costs. In industrial settings, where thousands of samples are processed daily, manual counting imposes a heavy, constant demand on time and effort, and remains highly error-prone ⁽⁵⁾ due to its subjectivity and reproducibility issues.

Technological advancements have significantly enhanced the use of imaging in microbiological research ⁽¹²⁾. What was long considered an ideal—fully automating workflows in microbiology laboratories—is now becoming a reality through the implementation of computer vision and image processing ⁽¹³⁾. Currently, there are numerous automatic colony-counting methods and systems ⁽¹⁴⁾, both open-access and commercial. These systems fall into two main categories: automatic digital counters that require an operator to manipulate a probe to identify and register each colony, and more advanced but expensive systems that rely on specialized imaging hardware, such as ColonyDoc-It and AIDBacSpot for Bacterial Colonies, which require the purchase of compatible equipment ⁽⁹⁾ or additional components to enhance colony detection, such as highly expensive fluorogenic substrates ^(10,15).

Despite the variety of available colony counters, none has yet been established as the standard method ⁽¹⁶⁾. The reliability of these technologies depends largely on image quality ⁽¹⁷⁾. Free tools such as NIST's Integrated Colony Enumerator (NICE) or CHiTA, which run on MATLAB, require image processing expertise ⁽⁹⁾. OpenCFU, an open-source tool, although fast and functional for various image types, tends to underestimate colony count and size due to its default settings optimized for specific colony models ⁽¹⁾. Other open-source systems, such as CellProfiler, and the widely used ImageJ platform, rely on custom macros that struggle to analyze different image types, requiring users to manually adjust parameters in a specific order for each reading ⁽¹⁸⁾. In general, these systems lack higher levels of automation ^(1,19) and present two clear limitations: excessive user intervention and lack of statistical relevance ⁽¹⁾.

In this context, an automated script was developed in Python, executable via Google Colaboratory (Colab), for bacterial CFU counting using computer vision with images captured by various devices. The aim was to evaluate the reliability and speed of the system. For this purpose, a low-cost hardware device was also designed to capture standardized, high-quality images—sharp, with uniform lighting, shadow-free, and enhanced colony edge definition.

To assess the reliability of the script, Kumar and Gupta ⁽²⁰⁾ point out that precision and recall are the most commonly used and essential metrics to evaluate the performance of an information retrieval system. These should be complemented by the F-measure, a weighted metric that balances both indicators. However, this metric requires a clear understanding of its inherent biases and the application scenarios in which it is statistically appropriate ⁽²¹⁾. One of its main conceptual limitations is the difficulty in assigning appropriate relative importance to precision and recall depending on the researcher's or user's goals and context ⁽²²⁾. The metric values range from 0 to 100%, with higher values indicating more reliable systems.

In this study, following the approach of Chiang et al. (2015) ⁽²⁾, both metrics were considered equally important. Therefore, false positives, false negatives, and true positives were determined by comparison with manual counting. The integration of low-cost hardware with a flexible, open-source script implemented on a user-friendly platform offers a competitive edge in the biotechnology sector and serves as a practical tool for resource-limited laboratories.

MATERIALS AND METHODS

Following the classification proposed by Pineda et al. (1994) ⁽²³⁾, this was a descriptive study with a mixed-methods approach and an observational, cross-sectional design.

Bacterial Strains and Growth Conditions

High-mountain microorganisms were selected as the study subject. To this end, 1 liter of water was collected from supraglacial lakes of the Llaca Glacier, located in the Llaca Valley, Huaraz, Peru. Since the objective was solely to quantify CFUs, non-selective culture media were used: nutrient agar and R2A, with serial dilutions up to 10^{-2} . The culture process was conducted using the surface-spread plate method, by inoculating 100 μ L of the diluted sample with a micropipette onto plates containing the growth medium, followed by even distribution using a Drigalsky spatula; each condition was tested in triplicate.

The plates were incubated at 5 °C for 30 days, with weekly monitoring to observe bacterial growth. Plates showing contamination by other microbial groups, such as fungi, were excluded from the study. Plates containing CFUs with non-circular or non-oval morphologies were also discarded, as they did not meet the criteria for this proof-of-concept study.

As the study did not involve experiments on humans or animals, ethics committee approval was not required. However, since supraglacial water sampling was conducted within a protected natural area—Huascarán National Park—a research access permit was obtained from the National Service of Natural Protected Areas (SERNANP) of Peru.

Experimental Design

The study was organized into two complementary experimental phases aimed at evaluating and optimizing the automated colony counter. *Phase 1* involved assessing its efficiency using an initial dataset of 14 photographs from five Petri dishes (two with nutrient agar and three with R2A), captured with three different mobile devices. *Phase 2* focused on strengthening and testing system performance by increasing sample variability, analyzing the conceptual capacity of the script, using 77 photographs from 11 Petri dishes (six with nutrient agar and five with R2A), captured with seven different mobile devices.

The devices used are listed in the supplementary materials (Mendeley Data, V1, doi: <https://doi.org/10.17632/psk2k6td6p.1>). It is important to note that the plates analyzed came from a small and specific experiment, and the individuals performing manual counts were not subject to the fatigue typically associated with counting large numbers of densely populated plates.

Image Database

In both phases, images were captured using a custom-built photographic chamber inspired by the simple design proposed by Rodrigues et al. (2022) ⁽¹⁾. The hardware (Figure 1) was developed to enable standardized, high-quality image acquisition of Petri dishes by controlling lighting conditions. The chamber has internal dimensions of 30 × 30 × 40 cm and includes a front access door and two glass shelves positioned 10 cm and 30 cm from the base. The lower shelf, equipped with a reference scale, supports the Petri dish and ensures consistent positioning in the images. The upper shelf holds the imaging device.

The chamber was constructed from black melamine to provide a completely dark environment, enhancing CFU visibility against the background. Illumination was provided by a 24 W LED panel (30 × 30 cm) placed at the bottom of the chamber. Photographs were primarily taken with a 1:1 aspect ratio; however, since the Motorola Moto G5 Play does not support this format, a 4:3 ratio was used instead. Image resolution varied depending on the mobile device model, ranging approximately from 1280 × 1280 px to 3456 × 3456 px. These differences did not pose a limitation to the system, as all images were resized and standardized uniformly during processing.

Image Processing

The automated colony counter was developed in Google Colaboratory (Colab) using Python (v. 3.11.12) ⁽²⁴⁾. The script was structured into three stages: preprocessing, segmentation, and CFU counting, using libraries such as OpenCV (v. 4.11.0) ⁽²⁵⁾, NumPy (v. 2.0.2) ⁽²⁶⁾, and Matplotlib (v. 3.10.0) ⁽²⁷⁾.

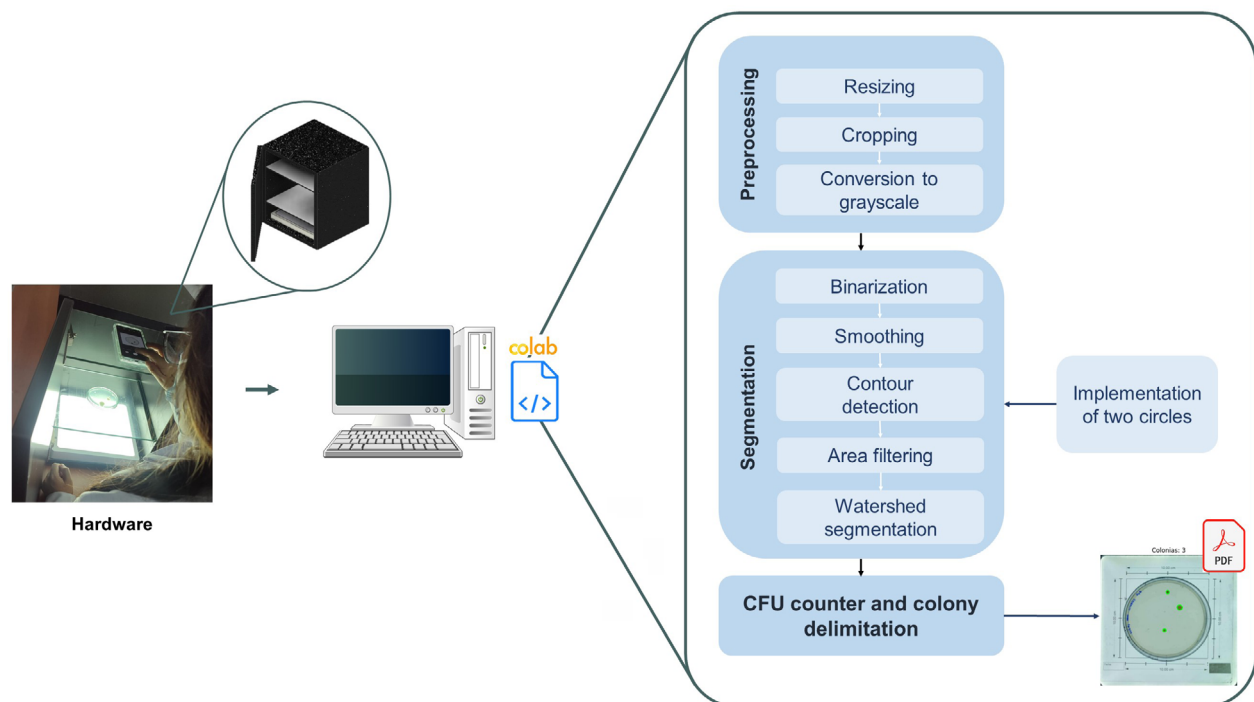


Figure 1. Illustrates the automated CFU counting workflow.

Preprocessing

The initial stage consisted of resizing, cropping, and converting the images to grayscale. All images were resized to 500×500 px while preserving their original proportions. For devices like the Motorola Moto G5 Play, which lack a 1:1 aspect ratio, a single manual cropping procedure was applied based on the reference scale edges (**Figure 2**). To isolate the region of interest, a circular mask with a 150-pixel radius was applied; uniform dish placement allowed the use of a single mask across all images. Finally, all images were converted to grayscale.

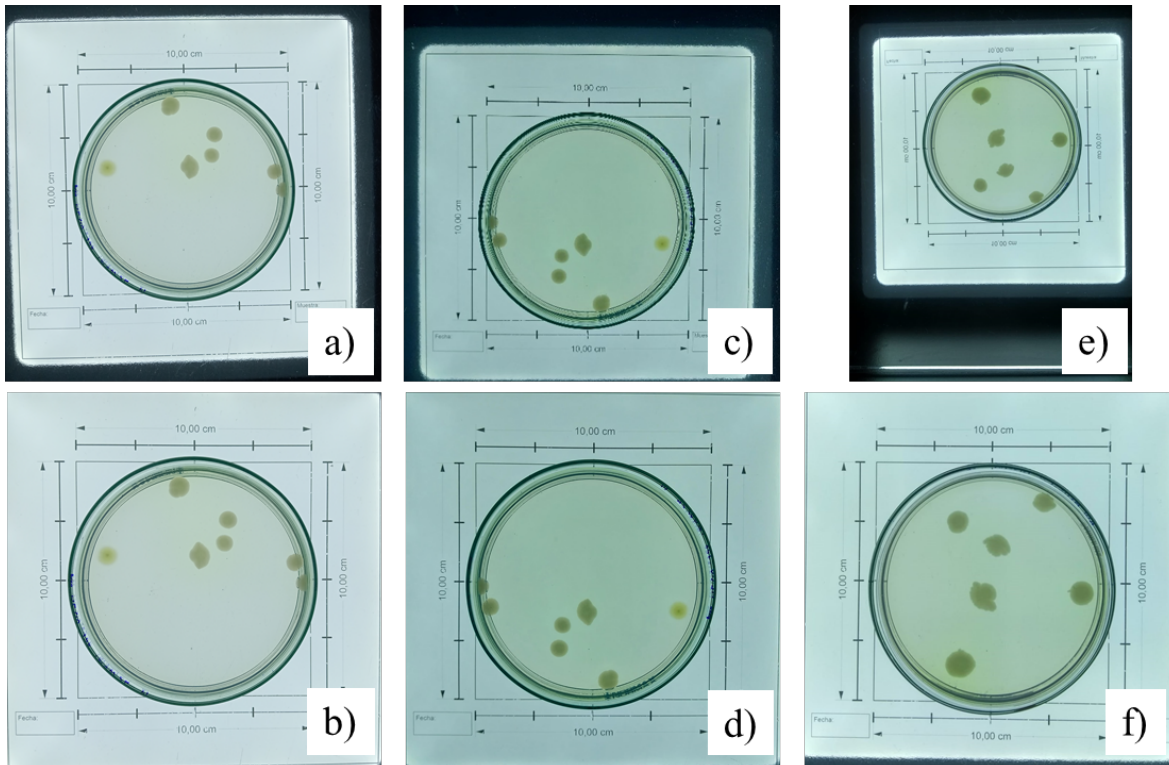


Figure 2. Manually cropped images. Panels (a, b, d and f) show the results of cropping applied to images (a, c, a and e), respectively.

Segmentation

This process involved four steps: binarization, smoothing, contour detection and filtering, and CFU differentiation. In the binarization step, the goal was to distinguish CFUs from the background by converting the images into a binary format. Several methods and thresholds were tested (Canny, Laplace, fixed, and Otsu), and Adaptive Gaussian Thresholding (31×31 , constant 2) was selected, as it computes a local threshold for each pixel based on its neighborhood. For smoothing (to reduce noise in binary images), a 3×3 median filter was applied, the minimum value to prevent significant distortion.

During contour detection and filtering, potential CFUs were identified using a roundness metric based on area, perimeter, and circularity. Since colonies are not perfectly circular, the function accepted areas with roundness values close to 1 and within the predefined area range. Colonies near the dish edges often had open contours, making detection difficult. To correct this, two concentric circular layers with radii of 136 px and 146 px were added, enabling closure of the open contours (**Figure 3**).

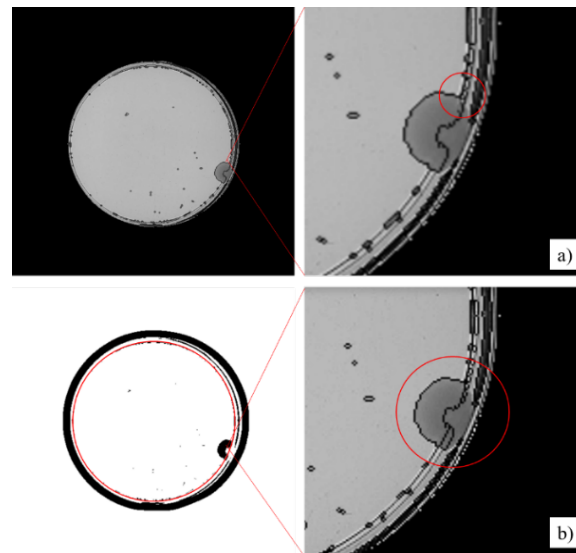


Figure 3. Detection of a CFU located at the edge of the Petri dish before and after the addition of circular layers. (a) shows a colony with an open contour, while (b) displays the result after applying the circular mask, illustrating the closure of the previously open area.

Areas were then filtered using two criteria: *i)* First filter: roundness between 0.8 and 1.2, area between 20 and 90 px, assuming that smaller colonies tend to be more circular; *ii)* Second filter: roundness between 0.25 and 1.2, area between 90 and 2000 px, acknowledging that larger colonies may represent merged CFUs and deviate from ideal circularity. In the fourth step, CFU differentiation, to correct over-segmentation and separate merged colonies, Watershed segmentation was applied using markers derived from the L2 distance transform, with a kernel size of 5 and a threshold of 30% of the transform's maximum value. This allowed separation of continuous colonies along ridge lines.

CFU Counter

The segmented colonies were counted and annotated on the processed images. The script analyzed multiple images in a single run using a for loop, requiring only a linked folder from Google Drive containing the image set. Results were displayed in the interface and automatically saved to a PDF document titled “*Imágenes procesadas*”, including the image filename, the number of detected colonies, and the annotated image.

Execution Environment

Image processing was carried out in Google Colab in a virtualized environment running Python 3. The system operated on an Intel(R) Xeon(R) CPU @ 2.20GHz, with 1 physical core, 2 threads, and approximately 13.2 GB of RAM. No GPU was used, as the implemented processing tasks did not require high computational power.

Data availability

The dataset supporting the findings of this study has been deposited in Mendeley Data and is publicly accessible under the title: Ramirez Panca, Mayte Soledad; Castillo-Vergara, Francisco; Rodriguez-Venturo, Sofia; Alva-Mejía, Holger; Loarte, Edwin; Medina, Katy; Tuya, Eladio (2026), “Automated Colony-Forming Unit (CFU) Counting of Bacteria Using Digital Image Analysis Through Computer Vision with Python - Supplementary Data”, Mendeley Data, V1, doi: <https://doi.org/10.17632/psk2k6td6p.1>

The complete process of the colony counter was described, allowing for a comprehensive understanding of how the script processes and analyzes each image from input to the automatic enumeration of colonies. Each photograph was coded using the format: *Location_SampleType_Device_CultureMedium_Series*. Examples of the results obtained using the script are illustrated in (Figure 4).

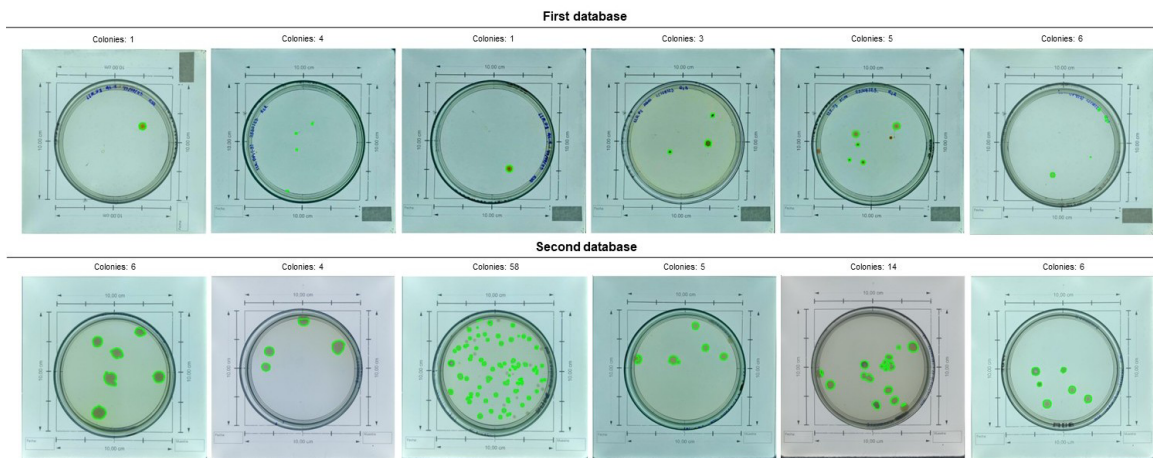


Figure 4. Sample outputs from the image database obtained using the photographic chamber.

Subsequently, data from manual counts were compared with those obtained using the automated system. A perfect correlation ($R^2 = 1.00$) was observed between manual and automated colony counts, indicating full agreement between both methods, as illustrated in (Figure 5a). The correlation between the two counting approaches is summarized in (Figure 5). When comparing by culture medium, the system achieved a correlation of 0.90 for nutrient agar and 1.00 for R2A (Figure 5b), demonstrating good performance across both media. Regarding the type of imaging device, all correlations were high, with a coefficient of determination above 0.97 (Figure 5c).

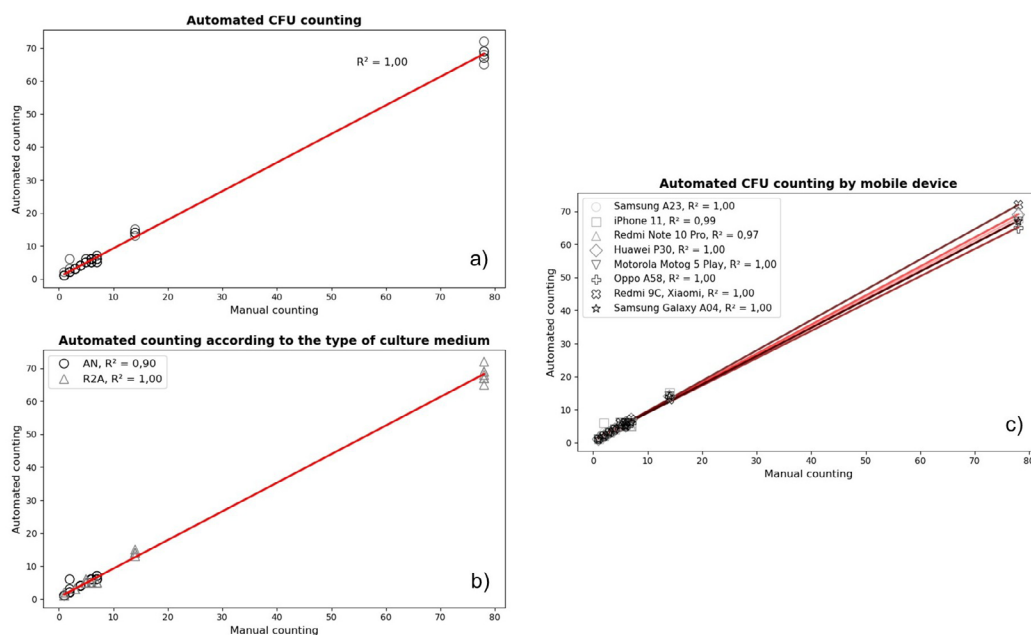


Figure 5. Correlation between manual and automated colony counts.

(a) Shows the overall correlation between manual and automated counts. (b) displays the correlation by culture medium, and (c) presents the correlation by mobile device used in both datasets.

To estimate the system's efficiency, three standard evaluation metrics were used: precision, recall, and the F-measure. For their calculation, values of false positives (segments identified as CFUs), false negatives (CFUs not detected), and true positives (correctly detected CFUs) were determined from the processed images. Chiang et al. (2015) ⁽²⁾ provided the following formulas:

$$\text{Precision} = \frac{\text{True positives}}{\text{True positives} + \text{False positives}}$$

$$\text{Recall} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}}$$

$$\text{F-Measure} = 2 \times \frac{\text{Precisión} \times \text{Recall}}{\text{Precisión} + \text{Recall}}$$

Precision was defined as the ratio between the number of true positives and the sum of true positives and false positives. This metric indicates the proportion of correctly identified colonies among all those detected by the system. *Recall* was calculated by dividing the number of true positives by the total number of actual colonies present, which includes both true positives and false negatives. It reflects the system's ability to detect all relevant colonies in the image. *F-measure* (or F1-score) was determined as the harmonic mean of precision and recall. This metric provides a balanced evaluation of the system's performance by considering both over-segmentation (false positives) and under-segmentation (false negatives). *Processing performance* was measured as the total processing time (in seconds) divided by the number of images analyzed, reflecting the computational efficiency of the script.

Since the aim was to design an accessible and widely usable system, a variety of mobile devices with differing photographic capabilities were employed. This allowed for testing the script's performance under different conditions. The average values of precision, recall, and F-measure were calculated for each imaging device and culture medium used in the analysis, as summarized in (Table 1).

Table 1. Average precision, recall, and F-measure by mobile imaging device and culture medium.

Dataset	Imaging Device / Culture Medium	Recall	Precision	F-measure
By imaging device				
First dataset	iPhone 11	0.81	0.72	0.72
	Redmi Note 10 Pro	0.93	0.88	0.88
	Samsung A23	0.89	0.93	0.90
	Huawei P30	0.97	1.00	0.99
Second dataset	iPhone 11	0.96	0.99	0.98
	Motorola Moto G5 Play	0.96	0.98	0.97
	Oppo A58	0.96	1.00	0.98
	Xiaomi Redmi 9C	0.99	1.00	0.99
	Samsung Galaxy A04	0.96	1.00	0.98
	Samsung A23	0.96	1.00	0.98
By culture medium				
First dataset	Nutrient Agar	0.82	0.65	0.69
	R2A	0.90	0.94	0.91
Second dataset	Nutrient Agar	0.98	1.00	0.99
	R2A	0.94	0.99	0.97
Summary	Average by imaging device	0.94	0.95	0.94
	Average by culture medium	0.91	0.90	0.89
	Overall average (91 images)	0.95	0.97	0.96

As shown in (Table 1), from the 91 images analyzed, the system achieved an average precision of 0.97 ± 0.12 , a recall of 0.95 ± 0.10 , and an F-measure of 0.96 ± 0.10 . Practically, this indicates that the system is 97% accurate, with a 95% detection capability, and an overall 96% balance between correctly identified and missed colonies.

These results show that, despite differences in device image quality and culture media characteristics, the system's performance remained consistent. Both false positives and false negatives were observed across cases, confirming the effectiveness of the photographic chamber in standardizing images regardless of device or medium. The full dataset is available in Table S2 in the supplementary material.

Although overall performance was satisfactory, the first dataset showed lower metric values compared to the second. In the first set, manual CFU counts ranged from 1 to 7 per plate, and the primary limitation identified was over-segmentation caused by plate labeling—an issue absent in the second dataset due to the lack of labels. This over-segmentation, particularly affecting nutrient agar due to its transparency and high contrast with both CFUs and labels, resulted in reduced precision due to an increased rate of false positives. Consequently, the F-measure was also affected. Nonetheless, a strong correlation between manual and automated counts was maintained.

In contrast, the second dataset included a broader range of CFU presence, from 1 to 78 colonies per plate. In most cases, recall was lower than precision. Since recall relates to the detection of false negatives, this indicates that the system had difficulty identifying some CFUs—particularly those lacking circular or quasi-circular morphology. These colonies, not meeting the predefined shape criteria, were excluded during processing. Additional limitations were noted in detecting CFUs in low-contrast media, where colonies were less distinguishable from the background.

DISCUSSION

The positive results demonstrate that the design not only enabled the acquisition of high-quality, non-professional photographs, but also provided adequate contrast between colonies and the background, facilitating their differentiation. However, certain aspects were identified that could be optimized: the glass shelves, being transparent, are susceptible to accumulating dirt, which can lead to over-segmentation and reduced image quality. As a solution, it is recommended to include openings in both platforms to allow clear access for the Petri dish and the camera lens. Additionally, increasing the distance between the upper shelf and the ceiling could benefit the use of a wider range of devices, expanding the chamber's applications to more specialized studies—such as detailed observations of microorganisms using more advanced imaging systems at the microscopic level.

The importance of image quality and uniform lighting has led to the development of cylindrical automated counting systems housed in aluminum casings, incorporating circular LED arrays and light-diffusing filters to homogenize illumination across the area of interest⁽⁴⁾. The presence of shadows can interfere with detection and lead to data loss; therefore, closed systems for image acquisition have been implemented in several designs^(1,5,28). More advanced and sophisticated models have also been proposed^(2,8), and some approaches have even leveraged shadow projection as a method for separating overlapping colonies⁽²⁹⁾.

The success of an automated counting system largely depends on image characteristics and the algorithms applied. A common concern among authors is the elimination of excessive information. In this study, a difficulty arose due to inconsistency in Petri dish placement, attributed to the use of devices lacking a 1:1 aspect ratio. To address this, a single manual cropping step was implemented. Following extraction of the region of interest, images were converted from RGB to grayscale. An alternative approach involved converting RGB images to HSV, which minimized image size and increased the robustness of the system against lighting variations ⁽³⁰⁾.

Binarization, or segmentation, is the most critical step in colony counting, as it enables the distinction between CFUs and the background. Although most approaches begin with Otsu's method, alternative techniques such as Laplacian and Adaptive Gaussian Thresholding were evaluated in this study, with the latter showing the best performance. While some methods rely solely on Watershed segmentation, others have implemented two separate binarization steps ^(1,2,8). For future replication of the system, the parameters used throughout the image processing pipeline would likely require adjustment depending on the specific chamber conditions, particularly the intensity of the LED illumination.

A recurring challenge in automated counting is the detection of CFUs located at the edges of Petri dishes, which are often excluded from analysis due to open contours. This issue has been addressed by dividing the plate into two distinct regions: central and peripheral ⁽²⁾. In the present study, two circular layers were implemented to close the gaps associated with potential edge colonies.

The culture medium is another relevant factor, as the literature highlights the influence of properties such as transparency, color, thickness, and overall quality. Some systems have reported limitations when using non-transparent media ^(1,2), whereas others claim adaptability to any type of culture medium and microorganism ⁽⁸⁾. Additionally, sample conditions—such as the absence of degradation or contamination—are essential for efficient image processing ^(2,8,31).

Although the proposed system addresses most of the issues identified in the literature, full automation is not viable for all types of images—particularly those not captured using the standardized hardware. Tools such as AutoCellSeg ⁽¹⁸⁾ and OpenCFU ⁽¹⁹⁾, while intuitive and versatile, still require human intervention. The application of deep learning and other artificial intelligence (AI) methods represents a promising direction toward complete automation. Recent developments have demonstrated their effectiveness; one approach integrated a thin-film transistor-based image sensor array with two deep neural networks for CFU detection ⁽³²⁾, while another introduced an automatic colony counting system assisted by U²-Net—a convolutional neural network (CNN) specialized in image segmentation—that significantly improved precision ⁽³³⁾.

Programming is becoming increasingly accessible, thanks to a wide array of courses and online resources. Today, numerous platforms and intelligent assistants facilitate learning and optimize code development, allowing users to enhance their skills and streamline projects more efficiently. Training models on diverse image sets—such as those managed by the Segment Anything Model (SAM)—could further improve CFU counting accuracy. It may even be possible to classify and identify bacterial groups or species, similar to a system that employed transfer learning in deep learning ⁽³⁴⁾. Moreover, an interactive analysis interface could be developed to enable dynamic functionality and expand analytical capabilities.

Typically, these advances demand more technical expertise, time, and computational resources, limiting their accessibility. In this study, however, a simplified approach was adopted through standardization of photographic conditions, enabling consistent image capture and the development of a system that does not rely on complex models. As a result, CFU counting can be achieved using only the essential steps of preprocessing, segmentation, and counting—facilitating comprehension, execution, and reproducibility.

CONCLUSIONS

The automated colony counter was evaluated through the analysis of 91 images, achieving a precision of $97\% \pm 0.12$, recall of $95\% \pm 1.10$, and an F-measure of $96\% \pm 0.10$. These results demonstrate the high reliability of the script for colony detection, regardless of the imaging device used. In addition, the system showed good robustness against interfering elements such as dust, lint, and text markings on the plates, avoiding their misidentification as CFUs.

Regarding its limitations, the system is designed for detecting colonies with circular or quasi-circular morphology. Therefore, its performance may be affected when analyzing organisms that form irregular, elongated, or diffuse-edged colonies, as these do not meet the geometric criteria defined during filtering. Similarly, in plates subjected to prolonged incubation times—where colonies tend to merge and form aggregates—the loss of circularity prevents the system from identifying them individually. In such cases, additional processing adjustments will be necessary.

In terms of performance, batch processing allowed each image to be analyzed in an average time of 0.4 seconds, significantly optimizing the time required compared to manual counting.

In conclusion, the system developed represents an accessible, accurate, and efficient alternative for CFU quantification, with high potential for application in microbiological laboratories. Its simple design allows for easy replication by any technician or laboratory in need of such a tool.

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ETHICAL CONSIDERATIONS

This study was conducted as part of the basic research project “*Microbial communities of debris-covered glaciers in the Cordillera Blanca – Peru (MicroDebris-Glacier)*” – RCUR-703-2022-UNASAM. The collection of supraglacial water samples was carried out in a protected natural area (Huascarán National Park), with prior authorization obtained from the National Service of Natural Protected Areas of Peru (SERNANP), Head Resolution No. 006-2023-SERNANP-JEF, ensuring compliance with all applicable national regulations and institutional guidelines.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCIAS

1. **Rodrigues PM, Luís J, Tavaría FK.** Image Analysis Semi-Automatic System for Colony-Forming-Unit Counting. *Bioengineering* [Internet]. 2022;9(7):271. Available from: <https://doi.org/10.3390/bioengineering9070271>
2. **Chiang PJ, Tseng MJ, He ZS, Li CH.** Automated counting of bacterial colonies by image analysis. *J Microbiol Methods* [Internet]. 2015;108:74–82. Available from: <https://doi.org/10.1016/j.mimet.2014.11.009>
3. **Corral-Lugo A, Morales-García YE, Pazos-Rojas LA, Ramírez-Valverde A, Martínez-Contreras RD, Muñoz-Rojas J.** Cuantificación de bacterias cultivables mediante el método de “Goteo en Placa por Sellado (o estampado) Masivo.” *Rev Colomb Biotecnol* [Internet]. 2012;14(2):147–56. Available from: <http://www.revistas.unal.edu.co/index.php/biotecnologia/article/view/37416/40417>
4. **Martinez-Espinosa JC, Cordova-Fraga T, Vargas-Luna M, Ortiz-Alvarado JD, Pablo AIR, Cisneros MT, et al.** Nondestructive technique for bacterial count based on image processing. *Biol Eng Med* [Internet]. 2016;1(1):1–6. Available from: <https://doi.org/10.15761/BEM.1000103>
5. **Uppal NK, Goyal R.** Computational approach to count bacterial colonies. *Int J Adv Eng Technol* [Internet]. 2012;4(2):364–72. Available from: <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=5f04eb14873221475956488c09198360724cbdda>
6. **Zhang J, Li C, Rahaman MM, Yao Y, Ma P, Zhang J, et al.** A comprehensive review of image analysis methods for microorganism counting: from classical image processing to deep learning approaches [Internet]. Vol. 55, *Artificial Intelligence Review*. 2021. 2875–2944 p. Available from: <https://doi.org/10.1007/s10462-021-10082-4>
7. **Ramírez J, Parra J, Alvarez-Aldana A.** Análisis de técnicas de recuento de microorganismos. *Mente Joven*. 2017;6:10–6. https://doi.org/10.18041/2323-0312/mente_joven.0.2017.3665
8. **Brugger SD, Baumberger C, Jost M, Jenni W, Brugger U, Mühlemann K.** Automated counting of bacterial colony forming units on agar plates. *PLoS One* [Internet]. 2012;7(3):1–6. Available from: <https://doi.org/10.1371/journal.pone.0033695>
9. **Choudhry P.** High-Throughput method for automated colony and cell counting by digital image analysis based on edge detection. *PLoS One* [Internet]. 2016;11(2):1–23. Available from: <https://doi.org/10.1371/journal.pone.0148469>
10. **Ateş H, Gerek ÖN.** An image-processing based automated bacteria colony counter. 2009 24th Int Symp Comput Inf Sci Isc 2009 [Internet]. 2009;18–23. Available from: <https://doi.org/10.1109/ISCIS.2009.5291926>
11. **Hogekamp L, Hogekamp SH, Stahl MR.** Experimental setup and image processing method for automatic enumeration of bacterial colonies on agar plates. *PLoS One* [Internet]. 2020;15(6):1–17. Available from: <http://dx.doi.org/10.1371/journal.pone.0232869>
12. **Mallard F, Le Boulrot V, Tully T.** An Automated Image Analysis System to Measure and Count Organisms in Laboratory Microcosms. *PLoS One* [Internet]. 2013;8(5):1–10. Available from: <https://doi.org/10.1371/journal.pone.0064387>

13. **Williams REO, Trotman RE.** Automation in diagnostic bacteriology. *J Clin Pathol* [Internet]. 1969;S2-3(1):8–13. Available from: <https://doi.org/10.1136/jcp.s2-3.1.8>
14. **Putman M, Burton R, Nahm MH.** Simplified method to automatically count bacterial colony forming unit. *J Immunol Methods* [Internet]. 2005;302(1–2):99–102. Available from: <https://doi.org/10.1016/j.jim.2005.05.003>
15. **Zhang C, Chen WB, Liu WL, Chen CB.** An automated bacterial colony counting system. *Proc - IEEE Int Conf Sens Networks, Ubiquitous, Trust Comput* [Internet]. 2008;233–40. Available from: <https://doi.org/10.1109/SUTC.2008.50>
16. **Marotz J, Lübbert C, Eisenbeiß W.** Effective object recognition for automated counting of colonies in Petri dishes (automated colony counting). *Comput Methods Programs Biomed* [Internet]. 2001;66(2):183–98. Available from: [https://doi.org/10.1016/S0169-2607\(00\)00128-0](https://doi.org/10.1016/S0169-2607(00)00128-0)
17. **Wong CF, Yeo JY, Gan SKE.** Republication – APD Colony Counter App: Using Watershed Algorithm for improved colony counting. *Sci Phone apps Mob Device* [Internet]. 2019;5(5):5–7. Available from: <https://doi.org/10.30943/2019/c23122019>
18. **Khan AUM, Torelli A, Wolf I, Gretz N.** AutoCellSeg: Robust automatic colony forming unit (CFU)/cell analysis using adaptive image segmentation and easy-to-use post-editing techniques. *Sci Rep.* 2018;8(1):1–10. <https://doi.org/10.1038/s41598-018-24916-9>
19. **Geissmann Q.** OpenCFU, a New Free and Open-Source Software to Count Cell Colonies and Other Circular Objects. *PLoS One* [Internet]. 2013;8(2):1–10. Available from: <https://doi.org/10.1371/journal.pone.0054072>
20. **Kumar S, Gupta P.** Comparative Analysis of Intersection Algorithms on Queries using Precision, Recall and F-Score. *Int J Comput Appl.* 130(7):28–36. <https://doi.org/10.5120/ijca2015907042>
21. **Powers D.** Evaluation: From Precision, Recall and F-Factor to ROC, Informedness, Markedness & Correlation. *Mach Learn Technol.* 2007;2:37–63.
22. **Hand D, Christen P.** A note on using the F-measure for evaluating record linkage algorithms. *Stat Comput.* 2018;28:539–47. <https://doi.org/10.1007/s11222-017-9746-6>
23. **Pineda, Alvarado D, Canales D.** Metodología de la investigación. Manual para el desarrollo de personal de salud. Segunda ed. Organización Panamericana de la Salud; 1994. 81–86 p.
24. **Van Rossum G, Drake F.** Python 3 Reference Manual. Scotts Val CA Creat [Internet]. 1995;Python ver. Available from: <https://www.python.org/>
25. **Bradski G.** The OpenCV Library. Dr Dobb's J Softw Tools [Internet]. 2000;OpenCV ver. Available from: <https://opencv.org/>
26. **Harris CR, Millman KJ, van der Walt SJ, Gommers R, Virtanen P, Cournapeau D, et al.** Array programming with NumPy. *Nature* [Internet]. 2020;585(7825):357–62. Available from: <https://doi.org/10.1038/s41586-020-2649-2>

27. **Hunter J.** Matplotlib: A 2D graphics environment. *Comput Sci Eng.* 2007;9(3):90–5. <https://doi.org/10.1109/MCSE.2007.55>
28. **Kis B, Unay M, Ekimci GD, Ercan UK, Akan A.** Counting bacteria colonies based on image processing methods. *TIPTEKNO 2019 - Tip Teknol Kongresi [Internet].* 2019;1–4. Available from: <https://doi.org/10.1109/TIPTEKNO.2019.8895213>
29. **Corkidi G, Diaz-Uribe R, Folch-Mallol JL, Nieto-Sotelo J.** COVASIAM: An image analysis method that allows detection of confluent microbial colonies and colonies of various sizes for automated counting. *Appl Environ Microbiol [Internet].* 1998;64(4):1400–4. Available from: <https://doi.org/10.1128/AEM.64.4.1400-1404.1998>
30. **Peña C, Peña L, Moreno G.** Sistema de visión artificial para el reconocimiento y el conteo de Unidades Formadoras de Colonias (UFC). *Rev Colomb Tecnol Av.* 2011;1(17):9–15.
31. **Naranajo A.** Conteo de Colonias de Bacterias en Cápsulas de Petri [Internet]. Universidad de Talca; 2017. Available from: https://dspace.otalca.cl/bitstream/1950/12105/2/naranajo_espinoza.pdf
32. **Li Y, Liu T, Koydemir HC, Wang H, Riordan KO, Bai B, et al.** Deep Learning-enabled Detection and Classification of Bacterial Colonies using a Thin Film Transistor (TFT) Image Sensor. *ACS Photonics.* 2022;9:1–18. <https://doi.org/10.1021/acsp Photonics.2c00572>
33. **Cao L, Zeng L, Wang Y, Cao J, Han Z, Chen Y, et al.** U2-Net and ResNet50-Based Automatic Pipeline for Bacterial Colony Counting. *Microorganisms.* 2024;12(1):201. <https://doi.org/10.3390/microorganisms12010201>
34. **Albaradei SA, Napolitano F, Uludag M, Thafar M, Napolitano S, Essack M, et al.** Automated counting of colony forming units using deep transfer learning from a model for congested scenes analysis. *IEEE Access [Internet].* 2020;8:164340–6. Available from: <https://doi.org/10.1109/ACCESS.2020.3021656>