Journal of Engineering and Applied Sciences 14 (19): 7138-7141, 2019
ISSN: 1816-949X

An Accurate Computational Analysis Technique for Bacterial Colonies Counting

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Abstract: Bacterial colony counting is one of the fundamental approaches utilized in microbiology for quantifying and isolating various sets of microorganisms. Until now, in most laboratories, the process of counting is achieved manually and this process is very exhausted and consumes time. In this study, an accurate technique based on digital image analysis is proposed for auto-counting of the bacterial colony with minimal time and effort. The results show better reliability by containing the obtained measurements of counting comparing with the manual counting with a total average error equal to 1.22%. The proposed technique outperforms the other traditional techniques, giving a hopeful technique for several related applications.

Key words: Bacterial colonies, auto-counting, digital image analysis, YCbCr color image, technique, applications

INTRODUCTION

Microorganisms such as bacteria and fungi, represent the most abundant living beings on the planet and stand out for their high adaptability to the most adverse environments of temperature, salinity, acidity, pressure and radioactivity found in nature. These microscopic life forms have a decisive influence on human life and terrestrial ecology and transforming all matter. While some microorganisms are of great importance to the food and drug industry, enabling the production of products of commercial value such as cheese, yogurt, vinegar and wine, others are responsible for the proliferation of diseases and deterioration of physical, chemical and biological characteristics of food (Ray and Didier, 2014).

Through research and techniques of food conservation, it is possible to control the rate of microbiological growth, the main determinant of the time in which food will take to deteriorate or how a disease will be proliferated. The control of this rate can be obtained by modifying extrinsic or intrinsic factors such as temperature and pH, respectively or by adding chemical substances such as salt. One of the main ways of calculating the microbiological growth is through plaque replication of Petri with favorable conditions and nutrients development of a given microorganism. During the replicating regions will form visible clumping regions, called Colony Forming Units (CFU) whose format and quantity will depend on the type of study and the time in which the experiment was conducted. The count of these colonies over time allows the calculation of the growth of the micro-organism and makes it possible to the proliferation of microorganisms in situations that will from the manufacturing environment to the final consumer (Brugger *et al*., 2012).

The increase of colonies over time and the difficulty of separating and counting them, usually, in several repetitions can lead to divergence of counting and wrong results. This study presents an accurate technique based on digital image analysis for auto-counting of the bacterial colony with minimal time and effort.

Literature review: Colony bacteria counting is very needful for assessment of pathogens in laboratories which provide a good indicator to doctors for evaluating the status of patients, also to give fast and accurate results to minimize the labor workload (Zhang *et al*., 2008). Recently, more attention has been given by the researchers to develop the programs and vision systems for enhancing the process of counting bacterial cells. Chunhachart and Suksawat (2016) designed a user-friendly program in which the required regions can be chosen with adjustable threshold and colony diameter. The core foodborne pathogens that possess various colony morphology were utilized for colony counting via the proposed system. The experimental results uncover that the average error is 2.13% comparing with the manual counting. An automatic CFU counting technique is presented by Matic *et al*. (2016) which is depending on Hough transform for circles. The results for this technique give an average of falsely detected equal to 3% and the average of undetected CFU equal to 18%. Siqueira and Carvalho (2017) presents good results in identification the microorganism via. improving the images preprocessing. Here, the maximum error rate is 7.69%.

Convolutional Neural Networks (CNNs) has become one of the modern solutions for large-scale object classification and detection applications. Also, CNNs have been applied to different problems of biomedical imaging. In order to improve the performance of automatic counting of bacterial colonies, lots of researchers utilize the CNNs as Ferrari *et al*. (2015, 2017).

The proposed method is to diagnose and count the *Staphylococcus aureus* colonies isolated from skin and food poisoning in order to help the doctor for determining the proper condition of patients with minimum error rate.

MATERIALS AND METHODS

YCbCr color space: In the YCbCr color space, the first component "Y" denotes the luminance, whilst the second and third components "Cb and Cr" denote the chrominance. The conversion process from RGB to YCbCr is presented in Eq. 1:

$$\begin{bmatrix} Y \\ Cb \\ Cr \end{bmatrix} = \begin{bmatrix} 16 \\ 128 \\ 128 \end{bmatrix} + \begin{bmatrix} 65.481 & 128.553 & 24.966 \\ -37.797 & -74.203 & 112.000 \\ 112.000 & -93.786 & -18.214 \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix} \tag{1}$$

Median filter: The median filter is a non-linear https://en.wikipedia.org/wiki/Digital_filter technique, often used to remove noise. Such noise reduction is a typical pre-processing step to improve the results of later processing (for example, edge detection on an image). Median filtering is very widely used in digital image processing because under certain conditions, it preserves edges while removing noise. The pattern of neighbors is called the "window" which slides, entry by entry, over the entire signal. For 2D (or higher-dimensional) signals such as images, more complex window patterns are possible (such as "box" or "cross" patterns). Note that, if the window has an odd number of entries, then the median is simple to define: it is just the middle value after all the entries in the window are sorted numerically (Weiss, 2006).

Image segmentation: Image segmentation is dependent on preprocessing. It is necessary to decrease the richness of detail of the images to allow the computer to easily interpret the part of interest and to separate it in this step. To segment the image, binarization algorithms have been implemented that reduce photographs for two color representations, black and white as well as technique and dilation and procedures for edge detection such as the Canny edge detection technique.

The proposed technique: The proposed technique consists of several stages (Fig. 1). Firstly, capturing the image of growing bacteria after 24 h of incubating. Bacterial colonies appear as small colored circles and background of the plat is made of black intensity. Photographs of real environments commonly feature thousands of colors, textures, backgrounds, color problems, contrast, lighting, noise and various aspects that make images almost impossible to process with all the richness of detail. Preprocessing aims to reduce this wealth and make the image easier to segment.

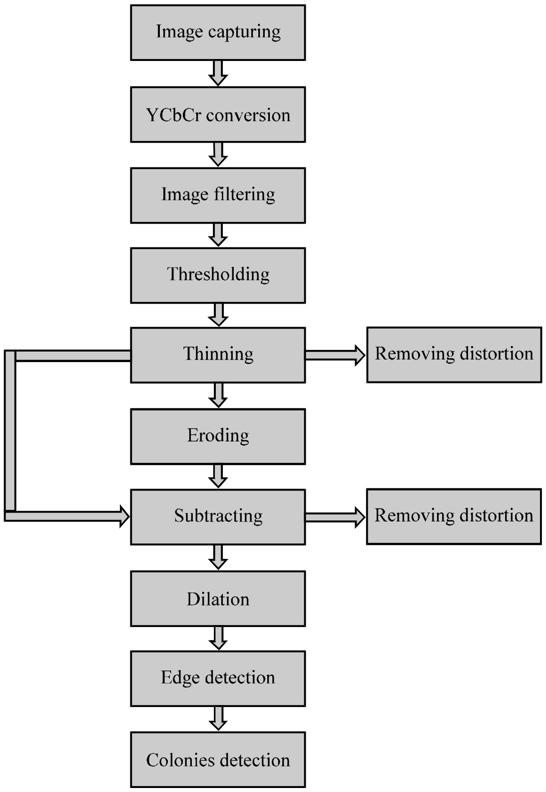

Fig. 1: The general structure for the proposed technique

Secondly, the digital image is transformed from RGB color space to YCbCr and only the Y component is used. Thirdly, performing spatial processing to determine which pixel have been affected by impulse noise. Utilizing the filter to classify pixels as noise due to comparing its surrounding neighbor pixels. This neighborhood is adjustable in size.

Fourthly, utilizing the threshold to isolate the colonies before counting, so, the software can count the colonies based on the deferent between the colors of the colony and the medium. The noise pixels replaced by the value filter of the pixels in the neighborhood pixels that have passed the noise labeling test applied by the filter. Fifthly, thresholding turns a Y component image into a binary image. The main reason that we use thresholding is to discard irrelevant data which curve to classify pixels on the basis of color.

Sixthly, performing the thinning operation to remove the selected pixels like erosion from binary images. This operation reduced thickness of all lines to single pixel.

Seventhly, the eroding process is used to reduce the noise and make the colony appears distinct and clear.

Subtraction from original image neglected the sub-results and the primary task is emphasized which it is count the colony.

Eighthly, performing the dilation to explain the pixels as an input to have a better result with good images. In general, dilation increases the objects sizes, holes and broken areas are filling. Also, it connecting the separated areas with spaces smaller than the structuring elements.

Ninthly, detecting the edges location by sharp changes in the intensity color of the image. The more edge line is thin the more reducing data amount in the image.

Finally, the detection process detects regions in the plate separate overlap colonies and the end gives a report consistent the accurate result of colonies counts in the plate.

RESULTS AND DISCUSSION

Bacteria was isolated from skin and incubated for 24 h. The 30 plates were examined by expert laboratory workers to count the colonies manually as usual. Then, we count the bacterial colony by using the electronic colony counter and the results were too close to the manual method. By applying the algorithm on three different samples with different variation in contrast image resolution noise background colors, the results give a good indicator that the algorithm works better than the electronic device and the manual method to count the bacterial colonies as shown in Fig. 2. Table 1 shows a comparison between the results of manual and automated processes.

Table 1: Comparative result between manual and automated processes

Plates images	Manual count	Output count	Percentage error
1	69	69	0.00
2	73	71	2.73
3	30	29	3.33
4	74	73	1.35
5	103	103	0.00
6	56	58	3.57
7	98	98	0.00
8	30	30	0.00
9	42	42	0.00
Total average error			1.22

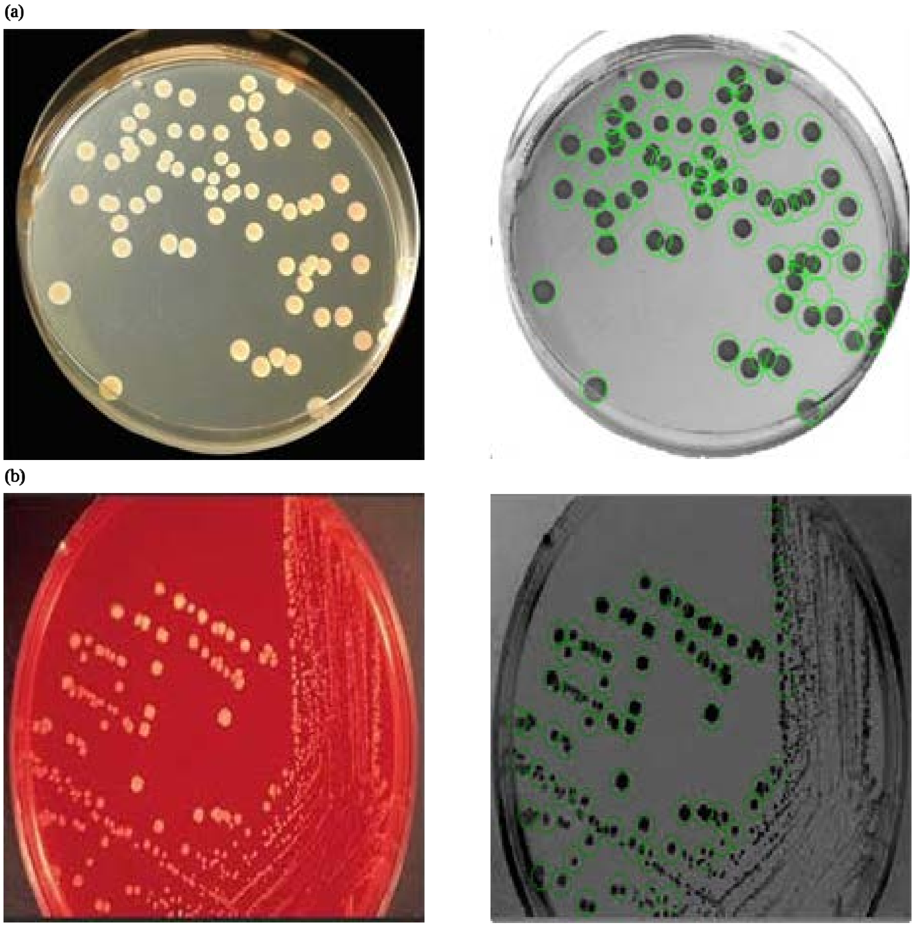

Fig. 2: Continue

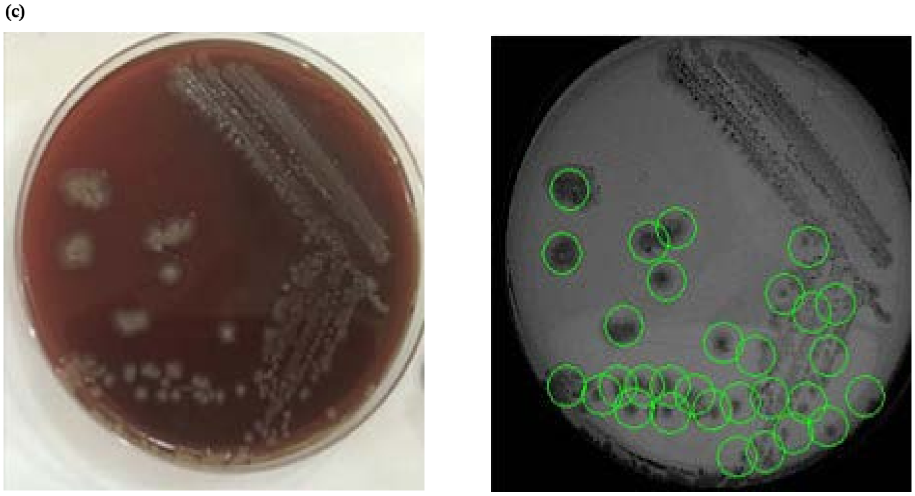

Fig. 2: The obtained results: a) Count equal to 69; b) Count equal to 71 and c) Count equal to 29

CONCLUSION

The identification of microorganisms by computational processing of photographs proved viable, practical and with a high rate of accuracy. The small difference between the human count and the count made by the proposed technique, together with the possibility of the user complementing and correcting the count through the program interface, make the process completely accurate and reliable. The proposed technique can be used in other practical situations involving image processing and that the time saved by the ease and speed of the counting, coupled with the fact that the program is free and has a user-friendly interface, may help in the activities of professional's microbiology, immunology and cell biology. New efforts are being made to improve the functional blocks of software, especially, segmentation and identification of objects, to make it more reliable and recognize other types of colonies and patterns.

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