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Automated counting of bacterial colonies by image analysis

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ABSTRACT

Research on microorganisms often involves culturing as a means to determine the survival and proliferation of bacteria. The number of colonies in a culture is counted to calculate the concentration of bacteria in the original broth; however, manual counting can be time-consuming and imprecise. To save time and prevent inconsistencies, this study proposes a fully automated counting system using image processing methods. To accurately estimate the number of viable bacteria in a known volume of suspension, colonies distributing over the whole surface area of a plate, including the central and rim areas of a Petri dish are taken into account. The performance of the proposed system is compared with verified manual counts, as well as with two freely available counting software programs. Comparisons show that the proposed system is an effective method with excellent accuracy with mean value of absolute percentage error of 3.37%. A user-friendly graphical user interface is also developed and freely available for download, providing researchers in biomedicine with a more convenient instrument for the enumeration of bacterial colonies.

1. Introduction

Bacterial growth is an essential indicator in many studies on microorganisms. The selection of antibiotics (Van Doorn et al., 2000), toxicology tests (Chen et al., 2004), and the evaluation of food and drug safety (Itoh et al., 1998) require the determination of microorganism survival rates to verify research achievements. This usually involves counting the number of bacteria in a unit volume of bacterial broth using various methods, including flow cytometry, spectrophotometry, membrane filtering, and the agar plate method. Flow cytometry (Macey, 2007) combines the use of bacterial properties with various fluorescent substances. Bacteria are placed in a flow cytometer, in which the fluorescent substances they carry are excited by lasers set to particular frequencies for the generation of optical signals. Filters of various wavelengths convert these signals into electronic signals to enable the counting of bacteria. Spectrophotometry (Schmidt and Schmidt, 2004) is a quantitative measurement of the optical transmission of a bacterial suspension as a function of wavelength. The amount of light that passes through the suspension is indicative of the concentration of certain bacteria that do not allow light to pass through. The membrane filter method (Inatomi, 2003) involves passing suitably diluted samples through a membrane filter with pore diameters smaller than those of the microorganisms. The microorganisms remain on the membrane, which is then placed on a culture medium. The total number of bacteria in the original sample can then be calculated according to the number of colonies that form on the membrane filter. The agar plate method (Barbosa, 1995) involves smearing the diluted bacterial suspension on an appropriate culture medium. Since only surviving microbes grow and form colonies on the plate, by counting the number of colonies, the number of viable bacteria can be obtained. Of these methods, the agar plate method is commonly used to assay the survival rate of microbes.

However, the manual counting of colonies is time-consuming and imprecise. To save time and prevent inconsistencies, a number of image processing software programs, such as ImageJ, have been developed. ImageJ is a freeware image analysis program that can be used for many image processing and analysis. However, for users who do not have deep knowledge of the image processing, more efforts and familiarity with the language are required to obtain satisfactory results. In addition, Clarke et al. (2010) 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, called "NICE" (NIST's Integrated Colony Enumerator), reads standard image formats, and therefore may be used in conjunction with many imaging systems. The program (OpenCFU) created by Geissmann (2013) that provides control over the processing parameters can also be used to count cell colonies and other circular objects. Niyazi et al. (2007) developed Clono-Counter, which uses three parameters, namely gray levels, maximum size of one colony, and gray 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. Zhang and Chen (2007) proposed an automatic colony counter for bacterial colony enumeration without any human intervention, which has been proven to be more accurate than Clono-Counter. Although it has high accuracy in images with colored media, it has problems with those with transparent media. Chen and Zhang

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0167-7012/

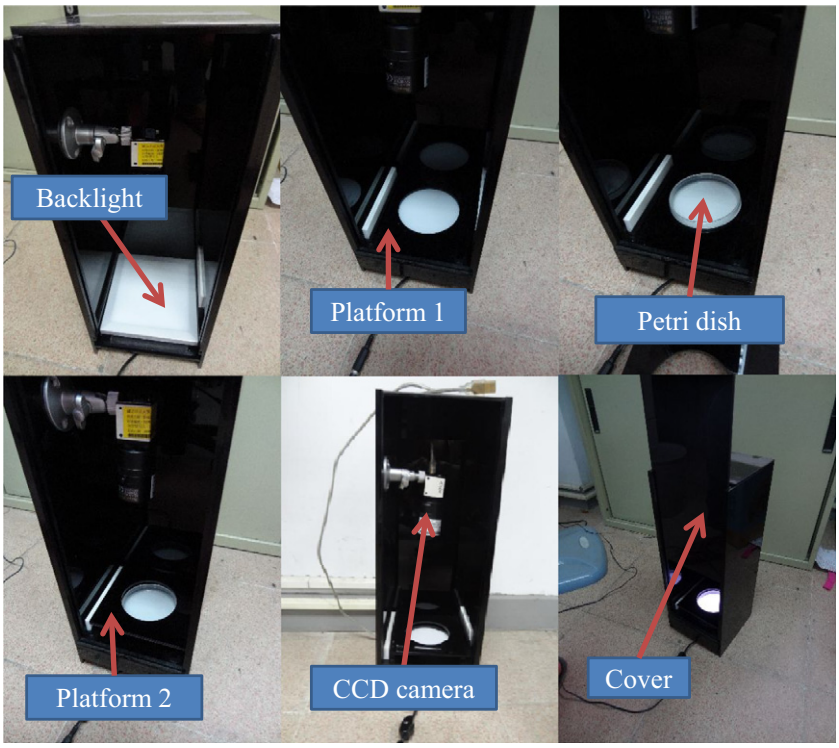

Fig. 1. Apparatus for image capture.

(2009) proposed a method to eliminate the differences in color resulting from culturing in various culture environments, allowing it to be applied to Petri dishes of various media. Reasonable performance for images with colored and clear media was demonstrated. Men et al. (2008) eliminated the rims of Petri dishes by assuming that they are perfect circles despite the adherence of colonies on the rims. Through a number of iterations, they identified threshold values capable of isolating colonies from the background, employed the distance transform and a watershed algorithm to divide the colonies, and then used the compactness ratio to remove noise prior to counting. Hong (2008) proposed a support vector machine (SVM)-based method for colony identification. Their approach used six parameters, namely area, perimeter, equivalent diameter, shape factor, length, and width, to identify colonies, with recognition rates of over 96%. Ates and Gerek (2009) proposed a method that required users to first select three points. Using the fact that three points can define a circle, they then semi-automatically identified the rims of Petri dishes and used the compactness ratio to determine the existence of clustered colonies that require dividing. Brugger et al. (2012) used one CCD to capture images of colonies. The boundary of the dish was viewed as a perfect circle. The colonies that touch the boundary were removed. The results were highly correlated with the ones obtained from manual counting. Dahle et al. (2004) employs a flat bed 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. Bae et al. (2009) proposed a microbiological instrument integrating a colony locator, a forward scatterometer and a 2D motion controller that not only counts and locates the bacterial colonies but also automatically measures the forward-scattering signature to identify the species of the bacterial colony under investigation. Ogawa et al. (2012) proposed time-lapse shadow image analysis (TSIA), which involves capturing one image of the Petri dish each hour and then analyzing the differences according to shadows. This technique enables observers to identify the locations of colonies. Other novel techniques such as the distance transform (Mukherjee et al., 1995), Hough transform (Barber et al., 2001), and

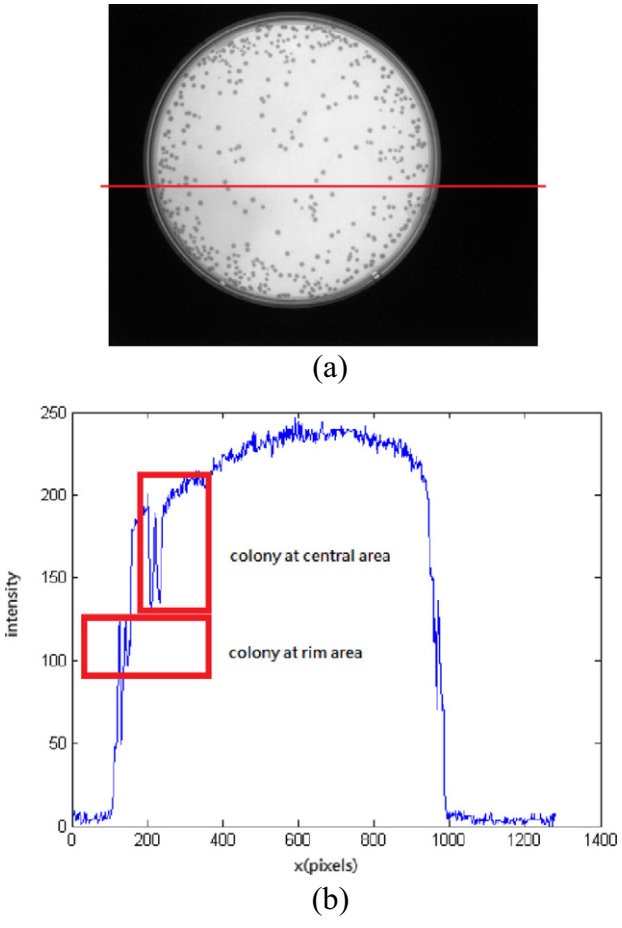

Fig. 2. (a) Gray image of Petri dish with bacterial colonies; (b) the horizontal intensity profile of the line shown in (a).