Bacteria Colony Enumeration and Classification for Clonogenic Assay

Wei-Bang Chen and Chengcui Zhang
Department of Computer and Information Sciences, University of Alabama at Birmingham, USA
{wbc0522, zhang}@cis.uab.edu

Abstract

Bacteria colony classification and enumeration is an essential step in clonogenic assay, which is often performed manually in many clinical research laboratories. It is known to be time consuming and labor intensive, but also error-prone since the manual counting tends to have more subjective interpretation and largely relies on persistent practice, especially when a large amount of colonies exist in the plate. In this demonstration, we introduce a fully automatic counter for bacteria colony enumeration and classification. The proposed counter accepts digital camera images as its input and performs colony enumeration and classification based on image mining. The proposed counter shows a robust performance in terms of both precision and recall, and is efficient in terms of labor- and time-savings.

1. Introduction

The clonogenic assay is widely used in biomedical examinations, food and drug safety test, environmental monitoring, and public health [1]. In general, this diagnostic assay is achieved by pouring a liquefied sample containing microbes onto agar plates, incubating the survived microbes as the seeds for growing the number of microbes to form colonies (a.k.a. colony forming unit - CFU) on the plates. The evaluation is done by counting the number of viable bacteria as colonies for calculating the survival rate of microbes in a sample. While this assay is very useful, there are two major issues: (1) bacteria colony enumeration, and (2) bacteria colony classification.

The colony enumerating is a low throughput, time consuming and labor intensive process since there might exist hundreds or thousands of colonies on a Petri dish, and the counting process is often manually performed by well-trained technicians. The manual counting is also error-prone since the results tend to have more subjective interpretation and largely rely on persistent practice, especially when a large number of colonies appear on the plate [2]. Thus, having consistent criteria is critical.

Another issue is bacteria colony classification. In many cases, especially in clinic study, there is a need to count colonies of a specific strain in a sample. However, to identify a specific strain of colonies is not a trivial task, even for experienced technicians.

We address the above problems and introduce a cost-effective, software-centered system for detecting as well as classifying bacteria colonies in a fully automatic manner. In Section 2, we briefly describe the framework of the proposed bacteria colony counter (BCC). Section 3 compares BCC with other existing systems. Section 4 summarizes the system.

2. System design

Figure 1 illustrates the overview of the proposed system architecture. As a preprocessing step, the chromatic/color components of the input image are examined first, and a proper processing method is selected, depending on the type of the image (chromatic/achromatic). In the second step, a hierarchical image segmentation step is performed, assuming the image is composed of three layers, including background, container/plate, and colony (see an example in Figure 2). These three layers conceptually form a hierarchical structure in which the colonies (top level) are cultured on the dish/plate (middle level) which is encompassed by the background (bottom level). The proposed approach segments the image layer by layer into background, plate/dish area, and colonies.

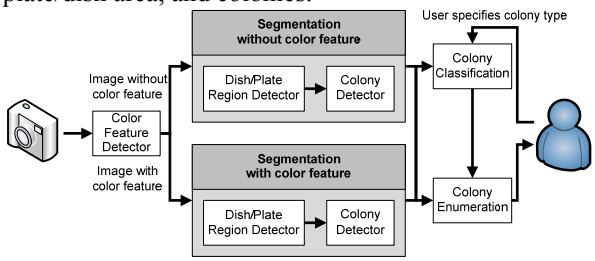

Figure 1. The system overview of BCC

Once all colonies are identified, we examine the morphology of each colony segment. This is an essential step since some colonies may grow close together to form a larger cluster. In order to obtain the accurate colony count, we adopt the Watershed algorithm to further separate those clustered colonies [4]. Once all the colony clusters on the dish/plate have been identified and isolated, we count the number of detected colony segments and use it as the total count of bacteria colonies. Figure 2 exemplifies the result from each major component in Figure 1.

978-0-7695-3454-1/08 $25.00 © 2008 IEEE
DOI 10.1109/ISM.2008.109

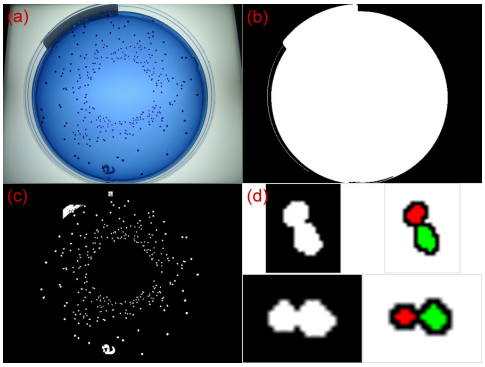

Figure 2. (a) The original image; (b) the plate/dish mask; (c) the colony mask; and (d) colonies separated from aggregated colony clusters.

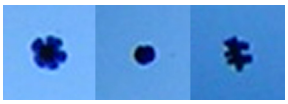

Figure 3. Colonies with different morphologies

For colony classification, a set of shape and color features (including solidity, compactness, and the 1st and 2nd color moments) are extracted for each colony. Three different strain samples are shown in Figure 3. If the user needs the number of colonies of a specific strain, the system prompts the user to select several colony segments of that strain as the training data to train a one-class SVM classifier which classifies all colony segments into two groups – the group of colonies of that specific strain, and all the other colonies (considered outliers to that specific strain) in another group [5]. This allows the system to report the colony count for a specific bacteria strain.

3. Comparisons with related systems

Since the characteristics of the chromatic and achromatic images are quite different, it is more appropriate to discuss the counter performance on them separately. In the experiments, we compared the proposed counter (BCC) with the Clono-Counter (CC) which is reported by Niyazi in 2007 [3].

For chromatic images, we test both counters on 9 images with 2161 colonies. The precision values of BCC and CC are 0.97±0.03 and 0.52±0.19, and their recall values are 0.96±0.04 and 0.99±0.01, respectively. Their F-measure values are 0.96±0.01 and 0.67±0.18, respectively.

To evaluate the robustness of the proposed counter on achromatic images, we test the both counters on 24 achromatic images with 1410 colonies. The precision, recall, and F-measure values of the proposed counter are 0.61±0.29, 0.94±0.06, and 0.69±0.20, while the corresponding values of the Clono-Counter are 0.22±0.25, 1.00±0.00, 0.29±0.31, respectively.

For colony classification, the experimental results show that the proposed counter achieves the highest F-measure value (0.915) when using the combination of solidity and the 2nd color moment features, or the combination of compactness and the 2nd color moment as the colony features. This shows that the combination of color and shape features are quite adequate for classification because of the complementary nature of these two types of features. To our best knowledge, there is no existing tool or software that can perform both counting and classification in one single system.

4. Summary of the system

The proposed automatic bacteria colony counter is capable of recognizing chromatic and achromatic images, detecting the dish/plate regions, isolating colonies on the dish/plate, and further, separating the clustered colonies for accurate counting of colonies. In addition, this counter has the ability to differentiate bacteria colony strains with little user inputs.

The major contribution of this study is that we established a basic framework and implemented a software-based bacteria colony counter. In particular, the proposed counter can handle various kinds of dish/plate. In addition, the counter operates automatically without any human intervention, and the performance is promising, for both color and clear medium. Second, it accepts general digital camera images, which are cost-effective, as its input. The third contribution is that the counter not only can detect colonies on the dish/plate, but also can differentiate colonies of different strains with little user input. These cost-effective features also make our proposed system very attractive to laboratories.

Acknowledgement

The research of Chengcui Zhang is supported in part by NSF DBI-0649894 and UAB ADVANCE program.

References

[1] X. Liu, S. Wang, L. Sendi, and M.J. Caulfield, "High-throughput imaging of bacterial colonies grown on filter plates with application to serum bactericidal assays", *Journal of Immunological Methods*, vol. 292, pp. 187-193, 2004.

[2] C.W. Chang, Y.H. Hwang, S.A. Grinshpun, J.M. Macher, and K. Willeke, "Evaluation of Counting Error Due to Colony Masking in Bioaerosol Sampling", *Applied and Environmental Microbiology*, vol. 60, pp. 3732-3738, 1994.

[3] M. Niyazi, I. Niyazi, and C. Belka, "Counting colonies of clonogenic assays by using densitometric software", *Radiation Oncology*, vol. 2, pp. 4-6, 2007.

[4] V. Luc, and S. Pierre, "Watersheds in Digital Spaces: An Efficient Algorithm Based on Immersion Simulations", *Trans. Pattern Anal. Mach. Intell.*, vol. 13, pp. 583-598, 1991.

[5] B. Schölkopf et al., "Estimating the Support of a High-Dimensional Distribution", *Microsoft Research Corporation Technical Report MSR-TR-99-87,* 1991.