AN AUTOMATED APPROACH FOR BACTERIAL COLONY COUNTER

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Abstract

Counting of bacterial colonies is a complex task for microbiologist. To a large extent, accurate colony counting depends on the ability to see colonies distinctly, whether viewed by the naked eye or by an automated instrument. An increased area of focus in Microbiology is the automation of counting methods. Further in an Industry thousands of such samples are formed per day and colonies on each sample are counted manually, then this becomes a time consuming hectic and error prone job.

We proposed a method to count these colonies to save time with accurate results and fast delivery to customers. This proposed research work will count the colonies after 6 to 8 hours priori, saving a lot more time and this work will more efficient because market range for this is about 10,000 only as compare to prior systems.

Keywords: Automated colony counting, Extraction, Image Processing, Corner Detection, Segmentation, Grayscaling, Thresholding.

Introduction

Bacterial colony in simple words is a group or cluster of bacteria derived from one common bacteria. Many biological procedures depend on an accurate count of the bacterial colonies and other organisms. The enumeration of such colonies is a slow, tedious task. When counts are made by more than one technician, wide variations are often noted.

Colony morphology is largely a result of the characteristics of the growth media and other environmental conditions. To enhance visibility of colonies and enhance the counting accuracy in an even broader range of applications, it is good practice to employ those procedures that form colonies that are counted easily by their improved size, shape, distribution and contrast.

The counting of bacterial colony is usually performed by well-trained technicians manually. However, this manual counting process has a very low throughput, and is time consuming. It is also labor intensive in practice. The existing colony counter devices and software were then developed and commercialized in the market so as to provide consistent and accurate results and improve the throughput. On the other hand, big laboratories may have extremely large counting needs to be accommodated with few automatic counters. Thus, colony counting is a significant budgetary and technical hurdle for laboratories of all sizes.

In this paper, we propose a fully automatic colony counter and compare its performance with manual counting of bacterial colonies.

Our proposed method can significantly reduce the manual labor by automatically detecting the colonies and count of those colonies efficiently. Bacterial colony counting is tedious and laborious work because these colonies are not easily seen by naked eyes. To count these bacterial colonies manually is very hectic because Bacteria’s are grown onto filter for 24 to 48
hours. To count these bacterial colonies microbiologist uses some dyes so that bacterial colonies appear as colored spots and our problem is to count the number of these bacterial colonies.

Further in an Industry thousands of such samples are formed per day and colonies on each sample are counted manually, then this becomes a time consuming, hectic and error prone job.

Goal is to develop software to save time with accurate results and fast delivery to customers. There are so many devices to count these bacterial colonies but these devices ranges about 50,000 to 70,000 according to the Indian currency, that’s why these devices are not so much efficient and economical for daily use.

This proposed research work will count the colonies after 6 to 8 hours priori, saving a lot more time and this work will more efficient because market range for this is about 10,000 only as compare to prior systems.

Intense Testing is required before actual installation, on different images of filters of types:

- Images in which size & shape of bacterial colonies vary.
- Images containing very dense bacterial colonies.
- Images containing different types of bacterial colonies on same filter.

The proposed method will efficiently work for lots of sample of bacteria. Some of the samples images are:

SAMPLE IMAGE

We can design an automated bacterial colony counter which used many image processing algorithms such as grayscaling, thresholding, filtering etc. to count these colonies efficiently.

I. Proposed System

Bacterial colony counting is tedious and laborious work because these colonies are not easily seen by naked eyes. To count these bacterial colonies manually is very hectic and time consuming process because Bacteria’s are grown onto filter for 24 to 48 hours.

To count these bacterial colonies microbiologist uses some dyes so that bacterial colonies appear as colored spots and our problem is to count the number of these bacterial colonies.

Problem of counting the total number of bacterial colonies present in a sample (filter) have following issues to handle:

- Number of non-overlapping colonies.
- Number of overlapping colonies.
- Number of edge touching colonies.
- To subtract the count due to noise.
- Colonies of different size shape and colors.
- And the total count will be the sum of the above five.
II. Block Diagram

To count the bacterial colonies, the block diagram for proposed method is given below:

Image Capturing

Bacterial Colonies are grown onto filter for 24 to 48 hours. Some colored dyes are spread over each filter so that bacterial colonies appear as colored spots. Now this filter is kept on a Petri plate. Background is made of black or white intensity so, it becomes easier to separate the filter from it’s surroundings while processing the image. Petri plate is kept in a box containing a digital camera and light arrangement. Images are then captured using this arrangement.

The collected images are digitized on a computer utilizing image processing software package that has programming capabilities (note: the system works with any of the software packages with these capabilities). The digitized picture is processed using the various procedures described to separate and detect the colonies present.

Extracting the Image content

Information about the image will extract in a single dimensional array using pixel grabber function in JAVA.

Gray Scaling

Brightness of the pixels will be computed using the NTSC (National Television Standards Committee) color – to – brightness conversion factor.

Thresholding

Thresholding can be defined as mapping of the gray scale into the binary set \{0, 1\} that is thresholding essentially involves turning a color or grayscale image into a 1-bit binary image. Thresholding algorithm will be applied to the gray scaled image.

Figure 2: Block Diagram of Proposed Method
Applying Filter
To remove unwanted noise we will use the adaptive median filter which is used for many noises.

Boundary Extraction
Boundaries are linked edges that characterize the shape of an object. They are useful in computation of geometry features such as size or orientation. To extract the boundaries we will use the Dilation and Interior pixel removal method.

Boundary Traversal
Complete image is scanned. It returns the image array containing coordinates of boundary of object and size of image array is equal to the number of points in the boundary.

Counting
Colonies will be count according to the results given by above process for overlapping and non-overlapping bacterial colonies.

III. Results
Out of 50 samples on which algorithm were tested, following are 10 samples with variations in contrast, density, color and noise, following output is occur:

<table>
<thead>
<tr>
<th>Image</th>
<th>Actual Count</th>
<th>Manual Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>91</td>
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<tr>
<td>4</td>
<td>29</td>
<td>29</td>
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<tr>
<td>5</td>
<td>52</td>
<td>45</td>
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<tr>
<td>6</td>
<td>54</td>
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<td>9</td>
<td>197</td>
<td>201</td>
</tr>
<tr>
<td>10</td>
<td>128</td>
<td>122</td>
</tr>
</tbody>
</table>

Table 1: Actual Count & Manual Count

Graph 1: Actual Count versus Manual Count

IV. Conclusion
Bacterial colony in simple words is a group or cluster of bacteria derived from one common bacteria. We can design an automated bacterial colony counter which used many image processing algorithms such as grayscaling, thresholding, filtering etc. to count these colonies efficiently.

Hence, Images with high contrast and low or medium density give accurate or near to accurate count (99-100%). Images with low contrast or high density give less accurate count (95-98%).

The reason thereof is that in case of low contrast after thresholding shape of colony/colonies gets distorted which leads to appearance of high curvature points along the boundary. These high curvature points (corners) get accumulated in count result.

V. Future Enhancement
Bacterial colony counter can be enhanced to:

- Tackle any shape and size of colonies.
- Process the most complex samples and give accurate count.
- Handle all types of noises.
- Detect high curvature points even in very dense overlapping colonies.
- Work on any type of samples i.e. samples with very low contrast.
VI. References


6. SHEN Wei-zheng, WU Ya-chun, ZHAO lie, ZHENG Hui, “Experimental Study for Automatic Colony Counting System Based on Image Processing”, 2010 International Conference on Computer Application and System Modeling (ICCASM 2010)


