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Editorial Board

Title

AN AUTOMATIC BACTERIAL COLONY COUNTER

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Abstract:

Bacterial colony counting is useful in many areas e.g. Disinfectants are used to kill harmful microorganismsin many different applications. They are used in veterinary practice to help to preventthe spread of infectious diseases such as foot and mouth and avian influenza. They are used in food production and catering premises,as well as in the pharmaceutical industry, to prevent contamination of products with harmful bacteria. In the medical area, hospital acquiredinfections (e.g. caused by Staphylococcus aureus MRSA) can be controlled by employing a rigorous regime of cleaning and disinfectingwith appropriate bactericidal products [1, 2].To count these bacterial colonies manually is very hectic and time consuming process 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. An increased area of focus in Microbiology is the automation of counting methods. Counting of bacterial colonies is complex task for microbiologist. We proposed a method to count these colonies to save time with accurate results and fast delivery to customers.

Keyword: Bacterial colony, corner detection, image processing, thresholding,

I. Introduction:

Bacterial colony in simple words is a group or cluster of bacteria derived from one common bacterium. 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.

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. 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 and labor intensive in practice. To provide consistent and accurate results and improve the throughput, the existing colony counter devices and software were then developed and commercialized in the market. 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 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 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. 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 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.

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

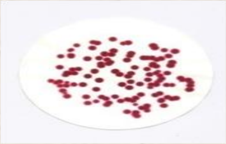


Figure 1.1: Sample Input Image

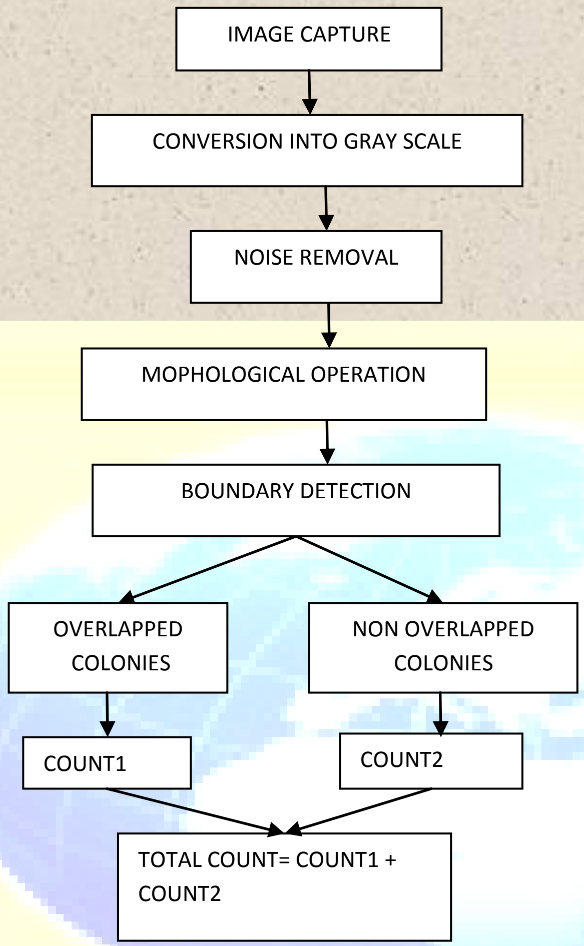
There are various devices available in market to count these are various devices available in market to count these colonies but those devices are very costly. We can design an automated bacterial colony counter which used many image processing algorithms such as grayscaling, thresholding, filtering etc. to count these colonies efficiently.

II. 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.



A. Block Diagram

To count the bacterial colonies, the block diagram for proposed method is given below(Figure 1.2):

Figure 1.2: Block Diagram of Proposed Method

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 its 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.

Gray Scaling

Brightness of the pixels will 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.

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.

Counting

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

II Related Work:

Shen Wei-zheng and WU Ya-chun[3] developed a new automatic colony counting system, which makes use of image-processing technology to feasibly count white bacterial colonies in clear plates according to the RGB color theory. It has been proved that the method greatly improves the accuracy and efficiency of the colony counting and the counting result is not affected by shape or size of the colony.

Chengcui Zhang and Wei-Bang Chen[4] proposes a fully automatic colony counter and compare its performance with Clono-Counter, an existing automatic colony counter reported by Niyazi et al. This proposed method can significantly reduce the manual labor by automatically detecting the dish/plate region and extracting and counting colonies.

Wei-Bang Chen & Chengcui Zhang[5] proposed a fully automatic yet cost-effective bacterial colony counter. In this paper, the proposed method can recognize chromatic and achromatic images and thus can deal with both color and clear medium. In addition, the proposed method is software-centered and can accept general digital camera images as its input. The counting process includes detecting dish/plate regions, identifying colonies, separating aggregated colonies, and reporting colony counts. The proposed method is robust and efficient in terms of labor-and time-savings.

Hong Men, Yujie Wu, Xiaoying Li, Zhen Kou, Shanrang Yang [6] proposed a method of rapid determination of Heterotrophic bacteria in industrial cooling water is developed.

Heterotrophic bacteria is detected and counted by National Standard Method of China at present, i.e. flat dish numberation, which has the disadvantages of subjectivity, big error and low efficiency because of relying on manual counting.

The method greatly improves the accuracy and efficiency of the heterotrophic bacteria counting and the counting results are not affected by shapes or sizes of the colony.

Wen-Lin Liu, and Chi-Bang Chen et. al[7] introduce a fully automatic bacterial colony counter. In this paper, proposed method can recognize chromatic and achromatic images and can deal with both color and clear medium. In addition, proposed method can also accept general digital camera images as its input. The whole process includes detecting dish/plate regions, identifying colonies, separating aggregated colonies, and finally reporting consistent and accurate counting results.

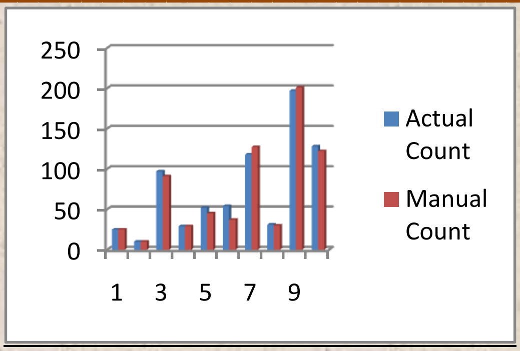
Sigal Trattner, Hayit Greenspan[8]has been developed an automatic tool to identify microbiological data types using computer vision and statistical modeling techniques. The statistical methodology presented in this paper, provides for an automated, objective and robust analysis of visual data, along with the ability to cope with increasing data volumes.

III. Result:

Out of 50 samples on which algorithm were tested, following are 10 samples with variations in contrast, density, color and noise, following output occurred as shown in TABLE 1.1 & GRAPH 1.1:

Image	Actual Count	Manual Count
1	25	25
2	10	10
3	97	91
4	29	29
5	52	45
6	54	37
7	118	127
8	31	30
9	197	201
10	128	122

Table 1: Actual Count & Manual Count



Graph 1.1: Actual Count versus Manual Count

IV. Conclusion:

Bacterial colony in simple words is a group or cluster of bacteria derived from one common bacterium. 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 will be enhanced to:

- Process the most complex samples and give accurate count.
- Work on any type of samples i.e. samples with very low contrast.
- Tackle any shape and size of colonies.
- Handle all types of noises.

- Detect high curvature points even in very dense overlapping colonies.

Take the shape of a product accepting different samples and giving count of total number of colonies present, which can be used in various biotech and other areas/ industries.

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