



## Automated Counting of Bacterial Colonies: Simple Contrast Stretching Algorithm

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**Abstract**— *Bacterial colony is a group of bacteria growing on a plate that is derived from one original starting cell. Counting of bacterial colonies is 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. Bacterial colony counting process is usually performed by well-trained technicians manually. However, there might exist hundreds of colonies in a traditional 100mm Petri dish. Therefore, this manual enumeration process has a very low throughput and is time consuming and labor intensive in practice. In addition, the manual counting is an error-prone process since the counting results of the same plate obtained from different technicians might vary, especially when a vast number of colonies appear on the plate. Another possible cause of variation is the judgment of the indistinguishable colony overlaps. Thus, it is important to have consistent criteria for measuring overlapped colonies. To produce consistent and accurate results and improve the throughput, we have worked on the bacteria-colony counter using mat-lab. We proposed a method to count these colonies to save time with accurate results.*

**Keywords**— *Bacterial colonies, contrast stretching, image processing,*

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### I. INTRODUCTION

Bacterial colony in simple words is a group of bacteria derived from one common bacterium. Many biological procedures depend on an accurate count of the bacterial colonies and other organisms. Bacterial colony enumeration has applications in many different assays such as antibiotic screening, and toxicology testing. The number of microorganisms present in food, drinking water has very important consideration. The enumeration of such colonies is a slow, tedious task. When counts are made by more than one technician, wide variations are often noted [1].

It was decided to develop an automated colony counter for three major reasons. Foremost is the speed of automated colony counting, each plate only requires a few seconds to count by computer, much faster than the minutes it may require for a human to count a plate with thousands of colonies [2]. Secondly, the automated counting is more reproducible; the computer uses the same set of parameters for making the decision of classifying a colony, a human operator's judgment may vary within a sample, and moreover the classification between operators differs, resulting in counts that vary by 12% or more [3]. Finally the automated analysis can be extended to other experiments with only minor software changes, allowing the system to have a great versatility. Colony counting is an important step in many lab procedures, and automated counting has been proposed and investigated multiple times before, likewise it is a lucrative market with several companies manufacturing them today. As early as in 1973 an off the market colony counter was tested, found to agree to 89 to 95% of a hand count, and was recommended as useful [4].

Since then several methods have been applied to attempt fast and accurate recognition of uniform bacterial colonies to interestingly shaped mammalian cell colonies. Usually one uses either a scanner [5, 6], or a camera with or without a backlight [3, 7-10] to capture images. A number of novel techniques have been applied ranging from using a distance transform, a Hough transform [7], parameter identification and fuzzy logic [6], and optimizing a Gaussian model of the image [8]. However, the counting range of most of these methods was not verified in cases exceeding 400 colonies / plate. Furthermore, most of the algorithms, while effective, do not see wide circulation. More recently, however, software has been written and released, free of charge; that allows for multiple types of image analysis, including colony counting [11]. Professional colony counters meet many of the challenges to automated colony counting. They are able to obtain colony numbers up to 95% of a hand count [4]. Additionally, the software is proprietary and is not able to be modified by the end user for custom experiments. Furthermore the software must be configured for the parameters of each sample before beginning counting. Many of the algorithms mentioned in the previous paragraph do not suffer from this problem. Although professional colony counters see great use in many settings, they are not idea for all, and have some major drawback that prevents them from seeing prolific use. To complement the advantages that both the academic and professional colony counters see, and to develop a low cost, versatile system that can be put in the hands of the average researcher is the primary goal of this research.

Passionate Testing is required before actual implementation, on different images of bacterial colonies as: the size & shape of bacterial colonies vary. The density of bacterial colonies may vary. Different types of bacterial colonies may be

at the same image. The proposed method will efficiently work for lots of sample of bacteria. Some of the samples images are: Counting cell colonies is a tedious task when performed with the light microscope. Moreover, unless strict double-blind protocols are adhered to, biased counts are difficult to avoid. Presented here is a computer software application that performs accurate, reproducible cell colony counts with a minimum of user generated bias. [12]



Fig. 1 Sample images of bacteria colonies with different colour backgrounds used for the colony counting experiment

In this paper fast and simple blob analysis method with the aid of contrast stretching is described to identify the colonies.

The required image properties can be stated as under:

- Dark background to increase image contrast
- Illumination of lights should be non-reflective (i.e. diffused light source)
- Variation of dish position in an image
- Varying diameter of the dish in image
- Varying intensity of the dish background
- As well as the colonies have different types of morphologies and colors.
- They might be overlapped

Considering the fulfillment of the above requirements we have proposed the algorithm.

## 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. The first difficulty in detecting the colonies was developing an accurate method to threshold the images, and a metric which could alert the user upto what extent the threshold should be good. To further equalize any intensity fluctuations from lighting, as well as to increase the contrast between the colonies and the plate medium, morphological operations like, dilation followed by erosion have been carried out.

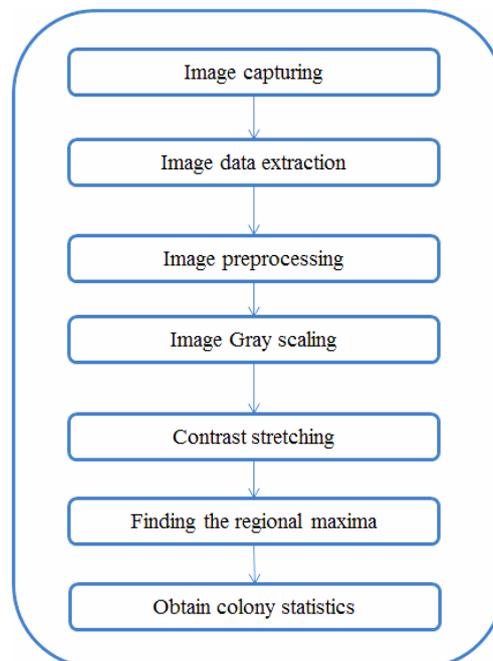


Fig. 2. Proposed algorithm for the colony isolation

The intensity threshold limit was then applied to the image, extracting the image having the intensities more than the applied threshold limit and thus colonies. The extracted image was then complemented and converted to binary image and Blob analysis on the extracted colonies has been carried out to extract the parameters like Mean intensity, Area, Perimeter, Centroid, Diameter were calculated. For different image conditions few modification to the procedure are required.

### III. RESULTS AND DISCUSSION

All Identifying the proper threshold to use proved to be the most challenging aspect of designing software that would reliably, and with further operator input, count a sample. Operator have to set the limiting the threshold value which can control the colony identification process. This kind of operation was selected as no method was found suitable for the correct counting of different intensities of plates and background and colonies. The algorithms, as described above have been implemented in a counting too. In the background this tool performs contrast stretching of the image, localization of the dish and counting of colonies as shown in the Fig.3.

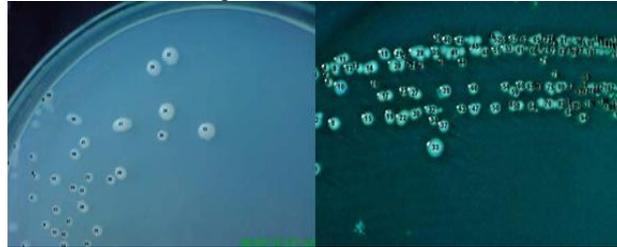


Fig. 3 Bacterial colony counted by using the proposed algorithm.

### IV. CONCLUSIONS

The In the paper, we have presented the method for counting of bacterial colonies based on contrast stretching followed by blob analysis. This algorithm performs well in both light and dark backgrounds. It also omits the text written on the image or petri-dish. This is satisfying the requirements of bacterial colony counting. Although, in this method threshold needed to be modified in every case, especially with the smaller colonies. It works effectively with different colored background. In future a simple user friendly program will be developed which can allow user to modify the background of the images and thus the appreciable threshold values in addition to that automatic and adequate contrast stretching algorithm also requires the attention.

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