ENUMERATING BACTERIAL COLONIES USING WATERSHEDING

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ABSTRACT

There are several methods for enumeration of bacterial colonies. An increased area of focus in Microbiology is the automation of counting methods. Several obstacles need to be addressed for methods that count colonies present, for example on Petri plates. These obstacles include: how to handle confluent growth or growth of colonies that touch or overlap other colonies, how to identify each colony as a unit in spite of differing shapes, sizes, textures, colors, light intensities, etc. This method is designed to provide a degree of accuracy in counting that could be correlated to the counts that would be obtained using a well-trained operator. A colony counter is used to count colonies of bacteria or other microorganisms growing on an agar plate. This method is used to overcome these obstacles are thresholding, segmentation, Watersheding, edge detection and morphology operators. This method provides high degree of accuracy. In the other form this counting problem has vast applications in Pathological & Medical Labs and Fishing, Dairies, Pharma Industries, Testing Assisted Genetic Toxicology, Semi-Automated Grain and Cell Counting etc. Some other examples of technologies that may be used for quantification of microbial growth include: ATP Bioluminescence, Spiral Plating, Membrane Filtration, Direct Epi-fluorescent Filter Microscopy, Membrane Cytometry, Fluorescence Flow Cytometry, and Latex Agglutination.

***Keywords**: Bacteria, Bacterial Colony, Thresholding, Segmentation, Morphology Operators, Watershed.*

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INTRODUCTION

Bacteria colony counting is crucial for quantitative, precise, assessment of pathogen in clinical research & diagnosis. Manually counting of bacteria colony is very difficult. Counting can be affected by numerous parameter related to physical properties of colony i.e. size, shape, overlapped colonies. in biomedical research and clinical diagnosis there is a need to quantify the number of bacteria in the sample manual counting is an error prone process and very time consuming & cost effective. An automated colony counting is time saving & less labor intensive process. To provide the fast & accurate result & to reduce the labor workload colony counting approaches were developed these approaches are classified in two categories. First approach is conventional & another one is automated.

First method is considered an indirect method, desirable due to its low cost. Some examples of technologies that may be used for quantification of microbial growth include: ATP Bioluminescence, Spiral Plating, Membrane Filtration, Direct Epi-fluorescent Filter Microscopy, and Membrane Laser Scanning Fluorescence Cytometry.

Second type is automated approach. In this Bacteria are grown onto filter for 24 to 48 hours to check the contamination level of the sample. 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. Goal of the project is to develop software to save time with accurate results and fast delivery to customers. This project will be extended in such a manner that it will count the colonies after 6 to 8 hours prior, saving a lot more time. In this paper the proposed approaches differentiates the processing of colony images with color information filtering is used for denoising. Color thresholding is used to detect foreground objects in the target region. Problem of counting the total number of bacterial colonies present in a sample (filter) have following issues to handle:

1 Number of non-overlapping colonies.
2 Number of overlapping colonies.
3 Number of edge touching colonies.
4 To subtract the count due to noise.
5 Colonies of different size shape and colors.

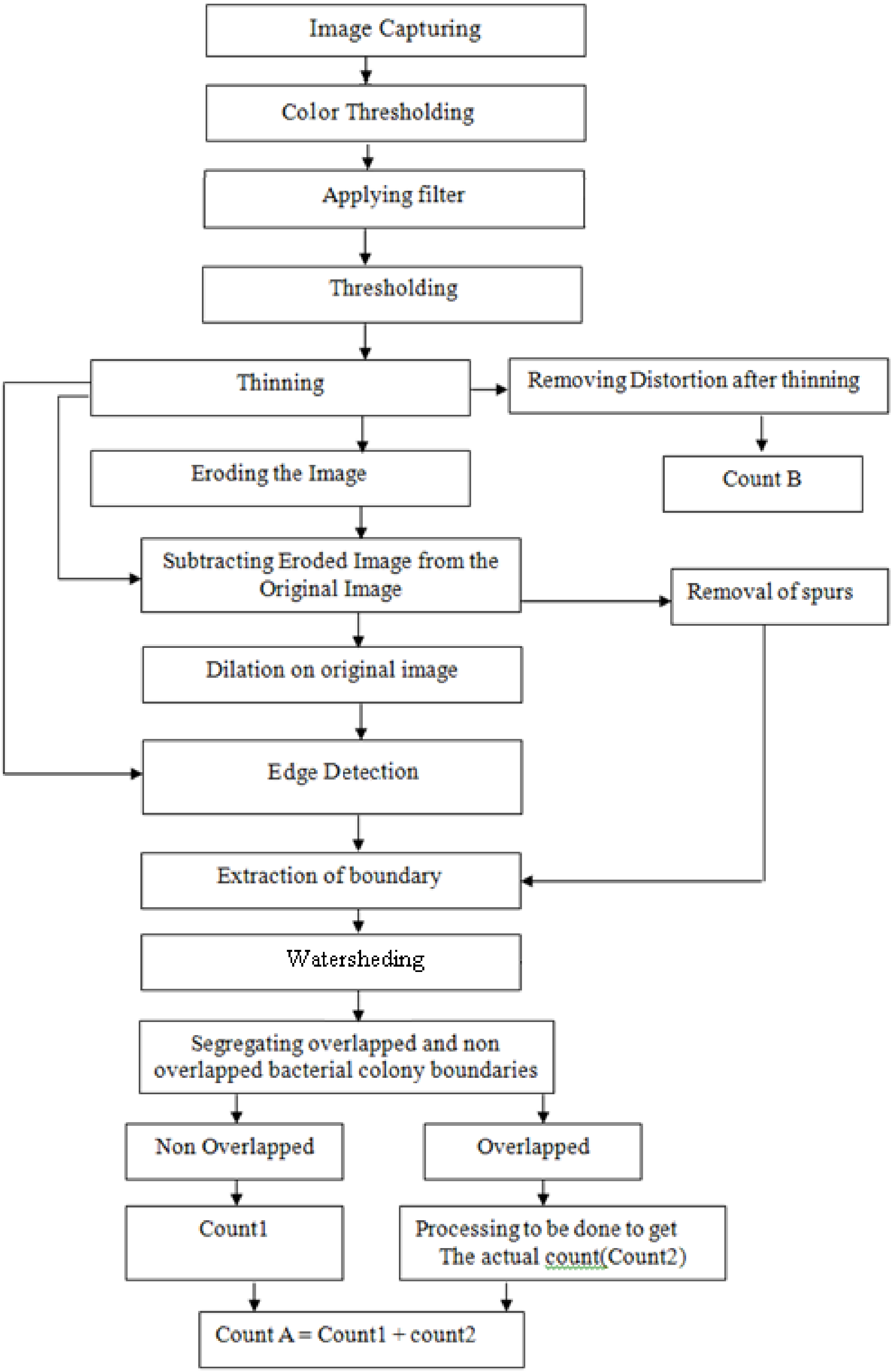

Fig 1 - Work flow

IMAGE CAPTURING

Bacterial Colonies are grown onto filter for 16 to 24 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 a image processing software package that has programming capabilities. The digitized picture is processed using the various procedures described to separate and detect the colonies present.

EXTRACTING IMAGE CONTENTS

Extraction of Bacteria present on petri dish is done using various procedures. Extraction procedure consists of following steps. Each extraction step has its own significance. Given below is the detailed description of each step.

- Conversion of Colored image (RGB) to HSV
- Gray Scaling of Image

APPLYING FILTER FOR NOISE REMOVAL

Adaptive filters adapt or learn the characteristics of the signal. Therefore the adaptive median filtering has been applied widely as an advanced method compared with standard median filtering. The Adaptive Median Filter performs spatial processing to determine which pixels in an image have been affected by impulse noise.

The Adaptive Median Filter classifies pixels as noise by comparing each pixel in the image to its surrounding neighbor pixels. The size of the neighborhood is adjustable, as well as the threshold for the comparison. A pixel that is different from a majority of its neighbors, as well as being not structurally aligned with those pixels to which it is similar, is labeled as impulse noise. These noise pixels are then replaced by the median pixel value of the pixels in the neighborhood that have passed the noise labeling test.

WIENER FILTER

The most important technique for removal of blur in images due to linear motion or unfocussed optics is the Wiener filter. From a signal processing standpoint, blurring due to linear motion in a photograph is the result of poor sampling The inverse filtering is a restoration technique for de-convolution, i.e., when the image is blurred by a known low-pass filter, it is possible to recover the image by inverse filtering or generalized inverse filtering. However, inverse filtering is very sensitive to additive noise. The approach of reducing one degradation at a time allows developing a restoration algorithm for each type of degradation and simply combining them. The Wiener filtering executes an optimal tradeoff between inverse filtering and noise smoothing. It removes the additive noise and inverts the blurring simultaneously. The Wiener filtering is optimal in terms of the mean square error. In other words, it minimizes the overall mean square error in the process of inverse filtering and noise smoothing. The Wiener filtering is a linear estimation of the original image. The approach is based on a stochastic framework.

THINNING

It is a morphological operation that is used to remove selected foreground pixels from binary images, somewhat like erosion or opening. It can be used for several applications, but is particularly useful for skeletonization. In this mode it is commonly used to tidy up the output of edge detectors by reducing all lines to single pixel thickness. Thinning is normally only applied to binary images, and produces another binary image as output.

THRESHOLDING

It is one of the methods of image segmentation. This method is based on a clip-level (or a threshold value) to turn a gray-scale image into a binary image. 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. The key of this method is to select the threshold value (or values when multiple-levels are selected). Several popular methods are used in industry including the maximum entropy method, Otsu's method (maximum variance), and et al. k-means clustering can also be used. & the main purpose of thresholding is to discard irrelevant data and keep only the important segments of data which lie above threshold curve to Classify pixels on the basis of colour or a local property. To provide a bi-level picture that distinguishes objects from background. To represent the text and diagrams in binary formats.

MORPHOLOGICAL IMAGE PROCESSING

It describes a range of image processing techniques that deal with the shape of features in an image. The technique was originally developed by Mat heron and Serra Des Moines in Paris in 1964.The mathematical morphology simplifies image data, preserves essential shape characteristics and eliminates noise. Morphological operations are based on simple expanding and shrinking operations.Morphological operations are typically applied to remove imperfections introduced during segmentation. Erosion generally decreases the sizes of objects and removes small anomalies by subtracting objects with a radius smaller than the structuring element. Dilation generally increases the sizes of objects, filling in holes and broken areas, and connecting areas that are separated by spaces smaller than the size of the structuring element.

EDGE

It is a part of an image that contains significant variation. The edges provide important visual information since they correspond to major physical, photometrical or geometrical variations in scene object.

EDGE DETECTION

It is a process that detects the presence and location of edges constituted by sharp changes in colour intensity (or brightness) of an image. . For a good edge detection, the edge line should be thin and with few speckles. The purpose of edge detection in general is to significantly reduce the amount of data in an image, while preserving the structural properties to be used for further image processing.

The SOBEL OPERATOR

It is used in image processing. For edge detection. The Sobel edge detector uses a pair of 3x3 convolution masks, one estimating the gradient in the x-direction (columns) and the other estimating the gradient in the y-direction (rows). A convolution mask is usually much smaller than the actual image. As a result, the mask is slid over the image, manipulating a square of pixels at a time the operator consists of a pair of 3×3 convolution kernels as shown in Figure below.

-1	0	+1
-2	0	+2
-1	0	+1

Gx

+1	+2	+1
0	0	0
-1	-2	-1

Gy

One kernel is simply the other rotated by 90°.

Gradient magnitude is given by:

$$|G| = \sqrt{(Gx^2 + Gy^2)}$$

Typically, an approximate magnitude is computed using:

$$|G|=|Gx|+|Gy|$$

PREWITT OPERATOR

This is similar to the Sobel operator and is used for detecting vertical and horizontal edges in images. The Prewitt edge detector is an appropriate way to estimate the magnitude and orientation of an edge.

1	1	1
0	0	0
-1	-1	-1

Gx

-1	0	1
-1	0	1
-1	0	1

Gy

Canny's aim was to discover the optimal edge detection algorithm. Sobel and Prewitt which uses first derivative have very simple calculation to detect the edges and their orientations but have inaccurate detection sensitivity in case of noise.

WATERSHED

This is method of image processing segmentation. There is a need to split the colony to get correct colony counts. To separate the connected colonies intensity are considered. Intensity gradient image as a topological surface, thus Watershed algorithm can be applied to divide the clustered colonies in the image just as water fold in the topological surface. The gradient magnitude of an image is considered as a topographic surface for the watershed transformation. Watershed lines can be found by different ways. The complete division of the image through watershed transformation relies mostly on a good estimation of image gradients. The result of the watershed transform is de-graded by the background noise and produces the over-segmentation. Also, under segmentation is produced by low-contrast edges generate small magnitude gradients, causing distinct regions to be erroneously merged.

RESULTS

S.NO	FIG. NAME	TOTAL COUNT MANUALLY	OVERLAP	NON-OVERLAP	TOTAL AUTOMATED
1	1.jpg	27	17	10	27
2	2.jpg	19	6	13	19
3	3.jpg	17	6	11	17
4	4.jpg	29	16	13	29
6	6.jpg	29	14	15	29
7	7.jpg	35	14	21	35
8	8.jpg	27	16	11	27
9	9.jpg	28	12	16	28
10	10.jpg	28	8	20	28

RESULTS – TEST 1 (Processed image of Non Overlapped Bacterial Colonies)

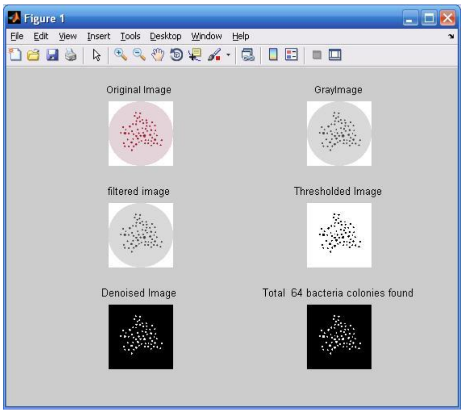

Figure 1.1 – Processed image of Non Overlapped Bacterial Colonies

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Calulating the Number of OverLapping
Number of OverLapping Regions
0
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Figure 1.2 – Total number of Overlapped Bacterial Colonies found

RESULTS – TEST 2(a) (Processed image of Overlapped Bacterial Colonies)

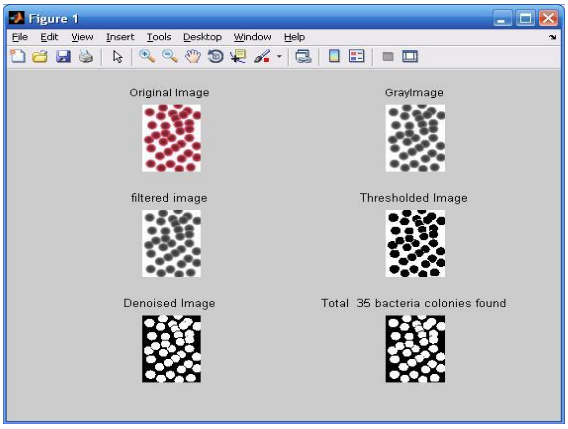

Figure 2.1 – Processed image of Overlapped Bacterial Colonies

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Calulating the Number of OverLapping
Number of OverLapping Regions
14
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Figure 2.2 – Total number of Overlapped Bacterial Colonies found.

RESULTS – TEST 2 (b) (Processed image of Overlapped Bacterial Colonies)

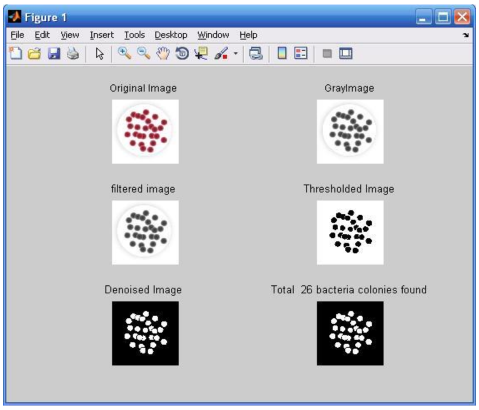

Figure 2.3 – Processed image of Overlapped Bacterial Colonies

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Calulating the Number of OverLapping
Number of OverLapping Regions
14
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Figure 2.4 – Total number of Overlapped Bacterial Colonies found

RESULTS – TEST 3 (Processed image of colored Bacterial Colonies)

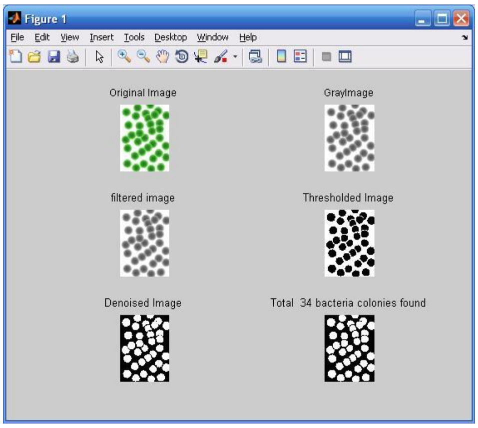

Figure 3.1 – Processed image of Colored Bacterial Colonies

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Calulating the Number of OverLapping
Number of OverLapping Regions
14
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Figure 3.2 – Total number of colored Bacterial Colonies found.

CONCLUSION

From the results and comparison of the different methods of edge detection, it is concluded that the "**Computational Automated Bacterial Colony Counter**" is better than the traditional method of manual counting of bacterial colonies on petri dish. .In these work, overlapped and Non-overlapped Bacterial colonies are counted using Watersheding. Edges of images using mathematical morphology and traditional methods like Sobel and Canny method has been studied.

FUTURE SCOPE Image processing is very advanced and extensive field which needs extensive research and hard work. Following are the suggestions for future work.

- This work can be extended to run on different types of micro biological bodies in order to count and evaluate their different parameters like shape, density etc.
- To analyze any shape and size of colonies having complex structure.

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