Image Processing Based Bacterial Colony Counter

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ABSTRACT

Enumeration of Bacterial Colonies is required in many fields such as in clinical diagnosis, biomedical research for prevention of harmful diseases and pharmaceutical industry to avoid contamination of products. Existing Bacterial Colony counter systems count Bacterial Colony manually which is a time consuming, less efficient and tedious process. Hence, automation for counting of bacterial colony was required. The proposed method count these colonies automatically using image processing techniques. This method will provide a greater degree of accuracy in counting of bacterial colonies. Proposed technique takes an image of bacterial colony and converts it into grayscale. Otsu thresholding is applied for segmentation of the image further its conversion into binary image. After that, morphological operations are applied to clean up the image by removing noise and unnecessary pixels. Distance and watershed transformations are applied on the binary image to create partitions among overlapped and joint bacteria. Region properties and labeling information of segmented image is used for counting of bacterial colony.

Keywords: Bacterial Colony, Thresholding, Morphology, Distance Transform and Watershed Segmentation.

I. INTRODUCTION

Bacterial Colony is defined as a cluster of bacteria derived from one common bacterium. Microbiologists require accurate measure of the bacterial colonies for many biological procedures [1]. Enumeration of Bacterial Colonies is important for obtaining precise assessment of pathogens. Manual counting of bacterial colonies is a tedious and time consuming process. Automation of the process of counting bacterial colonies will save time and labor required for counting of colonies [2]. Image segmentation techniques are used to automate this counting process. Image segmentation changes the image into a form suitable for image analysis. In image segmentation, a digital image is divided into multiple segments. Image segmentation assigns label to each pixel of an image on the basis of visual characteristics. Image segmentation is very used in many areas such as object detection, image retrieval, object-based counting, tissue identification, cell counting and object tracking [3]. Watershed segmentation is widely used for counting microorganisms. It is classified as region-based segmentation approach. It is used to separate two touching objects or overlapping objects. It considers image as a topographic surface in which the gray level of each pixel is considered to be the height on the surface and the two overlapping or touching objects as catchment basins. The motive is to find out watershed ridge lines separating the two catchment basins for separating the two overlapping objects [4] [5].

II. LITERATURE REVIEW

Sethi and Yadav (2012) used the multi- threshold segmentation procedures to count bacterial colonies for separating and detecting the colonies present. Final processed image is used for counting of separated colonies using a conventional single-threshold segmentation procedure. Results depicted the low and medium density bacterial colonies. It have been observed that the proposed technique does not hold good for low contrast images and high density medium of bacterial colonies. In case of low contrast images of colonies gets distorted after thresholding, leading to appearance of high curvature points along the boundary. These high curvature points get accumulated in count result [1].

Uppal and Goyal (2012) developed a system using image segmentation techniques for automatic counting of bacterial colonies. Numerous image processing operations have been applied for segmenting and enumerating the image of bacterial colony. They have applied morphological operations number of times which increased time and space complexity. This proposed technique could not produce accurate results for high density bacterial colonies and low contrast images [2].

Patel and Swaminarayan (2015) proposed an algorithm for automatic counting of bacterial colony using contrast stretching method. In this techni que firstly the image is segmented to obtain a binary image. Then to increase the contrast between the colonies and the medium of their growth morphological operations are applied to the binary image. Blob analysis is used to extract information from colonies such as mean intensity, area, perimeter to calculate the bacterial colonies in the image. This approach works for light and dark background. But for counting colonies from different images modification was required in the approach [6].

Minoi et.al (2016) designed a mobile based computer vision algorithm for automatic counting of bacterial colony. Bacterial colony image has been captured from android mobile and image processing techniques and morphological operations are applied to get the desired results. In this experiment illuminations and shadows lead to inaccurate results. They suggested that the proposed system can be improved by applying appropriate filters to remove noise, by enhancing the acquired images of colonies and by improving camera features of mobile devices [7].

III. METHODOLOGY

The present work builds a system to count bacterial colony automatically using image processing techniques. In the present research work, experimental research method is used. A simulator is designed and executed in MATLAB showing the execution results. The system is built and implemented in MATLAB R2016a. The 'm' script is used to execute the program. MATLAB has various tools which are used in mathematical, scientific, engineering etc. Image processing tool of MATLAB is used for the proposed system. Image of bacterial colony is used as data in the proposed system in RGB format. To carry out image processing, these RGB images are converted into grayscale images and then further processing is carried out to achieve the desired results.

IV. DESIGN OF BACTERIAL COLONY COUNTER

In the proposed technique image of bacterial colony is used for counting bacterial colonies. The image is converted into grayscale image and Otsu thresholding is applied to obtain segmented binary image. The segmented binary image separates the foreground from the background.

Segmented binary image may contains some noise and small elements of no interest. Morphological operations are applied on this segmented binary image to remove small elements and noise from it. Opening morphological operation is performed on the segmented binary image using square structuring element. Structuring element gets fit into the foreground regions in the image and remove the foreground pixels where it cannot fit. It removes small unwanted white pixels from the image and clean up the image for proper segmentation. The image is then complemented resulting the foreground pixels as black and background pixels as white.

Distance transform is applied to this complemented binary image producing a distance matrix to compute distance of every pixel from nonzero-valued pixels. Watershed transform is applied after the distance transform that produces a labeled matrix as output. Labeled matrix contains zero values for watershed ridge lines that separates the two overlapping bacteria and nonzero-values for catchment basins (overlapping bacteria). Distance and watershed transformations is called as watershed segmentation that divides the overlapping and joint bacteria into segments. The region properties are extracted using blob analysis from the image and the count of bacterial colonies in the image is displayed.

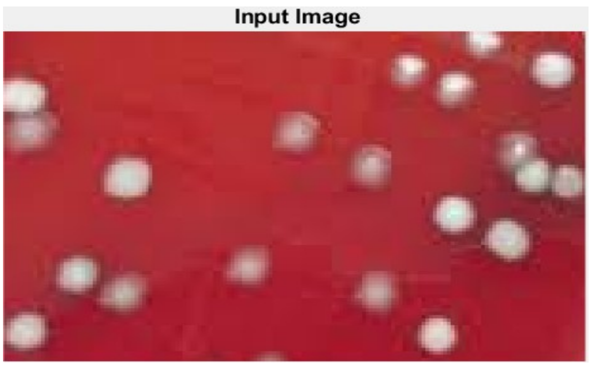
V. PROPOSED ALGORITHM OF PROPOSED SYSTEM

The proposed algorithm for the proposed system is as follows:

1. The image is read in variable im.
2. Image is converted into grayscale and stored in imgray variable.
3. Thresholding is applied on the grayscale image stored in imgray variable using matlab graythresh() function and resultant binary image is stored in thresh variable.
4. Opening operation is applied on the segmented binary image in thresh variable using square structuring element and stored in variable iopen.
5. The opened image in iopen is complemented so that foreground is black in color and background is white in color.
6. Distance transform is computed of the complemented image that finds out distance of every pixel from the nearest non-zero pixel values.
7. Watershed transform is computed taking distance transform as parameter which returns zeros for the watershed ridge line and non-zero values for the catchment basins. It gives output as labeled matrix.
8. The labeled matrix obtain in step 7 is given color in RGB space to distinguish between the segments.
9. Region properties are extracted from the labeled matrix and number of bacteria in the image is displayed.

VI. EXPERIMENTAL RESULTS

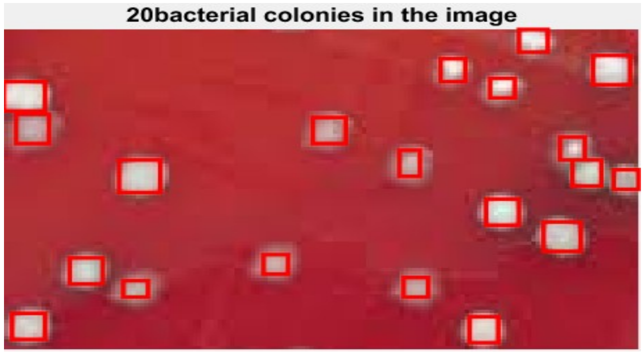
A. Input to the proposed system



Snapshot 1 Image of bacterial colony

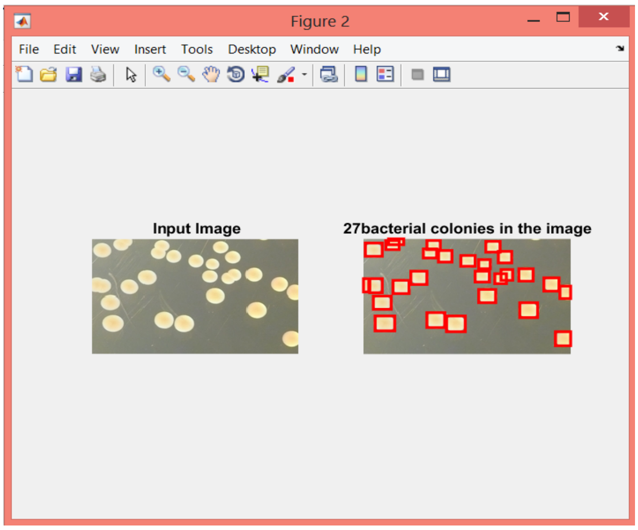
The image of bacterial colony in RGB format shown in snapshot 1 is used for counting of bacterial colonies. Image has been downloaded from the internet. It is converted into appropriate form before applying operations on it for the counting process of colonies. In this proposed method image is first converted into grayscale image and then into binary image before carrying out further processing.

B. Output of the proposed system



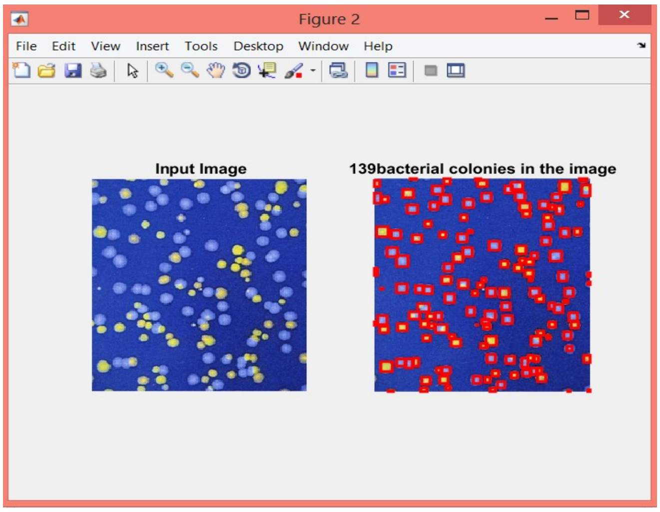
Snapshot 2 Output of count of bacterial colony

In snapshot 2 the count of bacterial colonies is shown as output for the input in snapshot 1. The output is obtained by applying watershed segmentation that separates the overlapping bacterial colonies. Region properties of image are extracted from the segmented image to count the separated bacterial colonies.



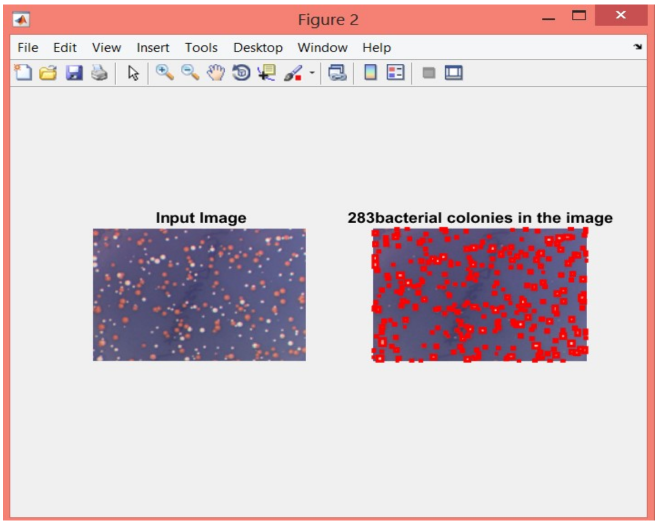
Snapshot 3

Snapshot 3 contains two windows. In one window an image of bacterial colony is shown and in other window the output displaying the count of bacterial colony is shown. The output shown is obtained by executing the proposed system. Red color bounding boxes in the output distinguishes the bacterial colonies.



Snapshot 4

In snapshot 4 another image of bacterial colony and its output displaying the count of bacterial colony is shown. The increased number of bacterial colonies in the image is inputted to the simulator. The simulator gives accurate count of bacterial colonies as output.



Snapshot 5

In snapshot 5 the number of bacterial colonies in the image is increased almost twice the previous image in snapshot 4. This image is given as input to the simulator. The proposed system is then executed on the image to obtain the accurate count.

Results of the simulator are summarized in table 1 as under:

TABLE 1
Summary of output of simulator

Sr.No	Proposed Count	Manual Count
1	20	20
2	27	27
3	139	139
4	283	283

The above table 1 shows the result of count of bacterial colonies by using different images for the proposed method. Table shows the count obtained by the proposed method and the manual method.

VII. CONCLUSION

From the results we conclude that the "**image processing based bacterial colony counter**" is better than the traditional method of manual counting of bacterial colonies. It is clearly evident from the Table 1 that the proposed system produces accurate results. Difference between manual count and automated count is zero. Four results are shown in the table in Table 1. The bacterial colony with 20, 27, 139, 283 bacteria are counted using automated process and the result of automated process is same as manual count method, thereby producing accurate results. Manual counting of colonies is a tedious and time consuming task. The proposed system has eases this task. Chances of errors are also reduced. In proposed system few morphological operations are used since morphological operation parameters has to be varied manually according to the image, so excessive use of it is undesirable. The proposed system can even work for high density bacterial colonies images efficiently.

VIII. FUTURE SCOPE

Image segmentation is a very extensive and prominent field which requires extensive research and hard work. The proposed work can be enhanced as:-

1. This work can be used to count bacterial colonies of different shape and size.

2. Excessive overlapping in the bacterial colony can still be improved.

IX. REFERENCES

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