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Experimental Analysis of Classical Segmentation Technique for Bacterial Colony Counting

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ABSTRACT

In microbiological analysis, counting colonies is typically performed to determine if samples fit microbial criteria. Although manual counting remains the process as subjective, the gold standard, time-consuming, and tedious. Some automated counting techniques were created to save time and effort. The automated traditional counting techniques are threshold, edge-based, morphological, watershed from image processing, and Support Vector Machine (SVM) from machine learning techniques. This paper analyze the capabilities of the classical image segmentation techniques such as thresholding, edge based, morphological, watershed and SVM classification technique for the segmentation of bacteria colony count in the microbial images. This study reveals that the machine learning technique SVM produced better result in counting the bacterial than the other four traditional methods. However, none of these methods have produced accurate counting compared to manual result. This analyze suggests that the artificial intelligence based method would be effective method for dealing with these kind of problems. *Keywords* — Image Processing, Microbial Image, Machine Learning, Classical Counting Techniques.

I. INTRODUCTION

This In many microorganism researches, bacterial growth is a crucial indicator. To validate findings from studies, microorganism survival rates must be determined when selecting antibiotics, conducting toxicological testing, and assessing food and medicine safety. Counting bacteria in bacterial broth can be done using spectrophotometry, the agar plate method, membrane filtration, and flow cytometry methods. The agar plate method involves distributing the diluted bacterial solution over a suitable medium for culture. Because only living microbes grow and form colonies on the plate, counting the colonies provides the quantity of viable bacteria. The agar plate method is often used to assess the survival rate of microorganisms [1]. Nevertheless, manually counting colonies takes a lot of time and is not very accurate.

The three primary types of quantification currently used in bacterial colony counting are human counting, conventional image segmentation algorithms, and deep neural networks. Image analysis techniques are crucial to achieving accurate counting. Directly culturing the bacterial colonies on solid agar plates makes them challenging to identify due to their high density, low contrast, adherence, and overlap characteristics. Because manual counting is so precise, it remains the gold standard for bacterial colony count. However, it takes a lot of time and cannot be modified for large-scale industry testing [2]. Conventional methods like threshold segmentation, edge-based detection, morphological operations, and watersheds have automation recognition potential, but they struggle to handle images with complex overlap situations and low contrast. Convolution neural networks (CNN)-based deep learning networks, on the other hand, are excellent at handling challenging issues.

This paper discusses traditional methods based on bacterial colony counting. The pre-processing stage of the image is essential for accurate detection. Such as filtering, contrasting colony objects, and cropping the isolated colony [3]. Thresholding based colony counting is mostly used in traditional counting techniques [4]. The majority of methods for counting colonies rely on thresholding, which is a necessary first step in producing binarized images. Through thresholding, pixels are divided into objects in the background and foreground or detection of bacterial colonies. The bacterial colony, a crucial feature in assessing the efficiency of binarization, is also connected to the thresholding success in microbial image processing [5].

The watershed algorithms are used in the count of colonies. This algorithm is used for spatial gradients and spectral markers to segment images and creates internal lines between merged colonies to separate them for b acterial colony counts [6]. A bacterial colony's outer boundary or edge is another name for its margin. Only with a light microscope can margins be seen with magnification [7]. Bacterial colonies might be round, irregular, filamentous, or rhizoid, leading to a morphological analysis for counting. Manually adjusting threshold values in a controlled environment is typical for these strategies [8]. However, accurate bacterial segmentation is still a challenge due to varying colony shapes and close nearest between colonies.

The objective of this work is to develop an automatic realtime counting of bacterial colonies using traditional microbial

image processing techniques. The rest of this paper is structured as follows. In Section II literature review with a focus on conventional techniques, Section III the specifics of the suggested methodology are given. In Section IV results and discussions. Section V is conclusions.

II. LITRATURE REVIEW

In the past, automated colony counting heavily depended on traditional image processing techniques. The traditional colony count techniques are thresholding, edge-based detection, morphological operations, and watershed.

Barber et al., [9] experimented their method with the binary images obtained by thresholding, and the edges were found using the Sobel operator. The centers of circular objects are highlighted using a compact Hough transform. The colonies number and area able to be computed by processing the local area to establish a colony boundary.

To reduce noise, a median filter is performed first. The Petri dish boundaries are then detected and deleted by Ateş [10]. The circularity ratio is then used to divide the patterns into two categories, like colonies and clusters of colonies. The cluster colonies are then separated into pieces according to watersheds.

To extract candidate colonies Zhu et al., [11] utilized image subtraction to connect them to the agar plate's inner circle. The grayscale is then improved by applying a nonlinear gray modification. The binarized image undergoes a distance transformation before being segmented using a transformation of watershed. Furthermore, the sharp edges created by the transformation of watershed are reduced used morphological opening technique. Next segmentation, Bayes classifier categorizes leftover concatenates groups into one, two, three, or four contained colonies. Finally, colony is enumerated.

In Choudhry [12] experimental six main processes in the edge detection system. To improve contrast and decrease the impact of inconsistent illumination, the backdrop is first eliminated. An empirical method is used to determine the background subtraction radius. The average radius of colonies can serve as a starting point. Finding the edges of the image is the first step in sharpening and improving it. The macro makes use of the Sobel filter. After that, the picture is turned to black and white and smoothed using Gaussian blur. An alternative method is to use successive dilated and eroded processes to smooth the image. After that, the edges close together to create a closed circle. Images with black colonies on a white background are produced by employing hole filling to fill closed objects with black. Closing and filling holes is an extra step that is done to guarantee that every colony is found. To detect colonies whose whole edge beside the perimeter cannot be identified or else, the size of each pixel is enlarged in this case. This brings the detected edges closer to one another. To restore the colony size to its initial values after

filling, the pixel sizes are reduced. Following that, thresholding is used to separate clustered colonies and eliminate tiny particles using denoising and segmentation. Lastly, the items are filtered according to their dimensions, circularity, and size. After that, a brand-new pipeline is created to identify cells and colonies in images.

In Barbedo [13] to count colonies automatically, five digital image processing techniques are suggested and contrasted. After the holes are filled, the connected regions are found and counted using first approach; it uses a Gaussian Laplacian filter to detect edges. In second approach, the canny filter is used in place of the Gaussian Laplacian filter. The third technique uses three thresholding values to segment images. Thresholding is employed in the fourth approach for histogram equalization. Last approach uses segmentation using the region-growing method. The connected colonies can then be divided into a single one by detecting the concave surface between them. Lastly, a count of the colonies is made. The first method's accuracy, which achieves 99% accuracy, performs best.

Hogekamp et al., [14] developed an experimental setup and data processing methods to automatically image and count E. coli DH5 α colonies growing on 110 mm Petri dishes. The method for detecting E.coli DH5 α colonies is detailed, allowing readers to modify it to their needs by reproducing and adjusting the procedures. By developing an automated command sequence, it is possible to evaluate a large number of samples quickly using the technology utilized here. Comparing the final system to manual counting, the time needed for CFU enumeration can be significantly decreased. A database of samples and enumeration results can be created, and CFU counts can be done without subjectivity.

In Ferrari et al.,[15] developed efficient ways to quantify bacterial colonies on microbiological culture plates in clinical microbiology labs. The two machine learning methods that are suggested are based on a Convolutional Neural Network deep learning architecture and on manually constructed morphometric and radiometric features that are taken from a Support Vector Machines solution. To validate the suggested methods, a sizable database of individual and combined bacterial colonies was created and made available to the public. For digital microbiology imaging quantification tasks, the deep learning methodology performed better than the traditional reference technique and the handmade featurebased method, particularly in the developing environment of Full Laboratory Automation systems. To discover extended zones of confluent growth and manage coarse bacterial load quantifications, the suggested deep learning approach needs extra analysis tools, yet it provides accurate counts with dependable outlier rejection.

Zhang et al., [16] in their method proved that a machine learning technique for counting colony forming units (CFUs) in biological research is called CFUCounter. It uses iterative

adaptive thresholding, local-minima-based watershed segmentation, and unsupervised machine learning to interpret digital images and divide up bacterial colonies. In addition to supporting color-based CFU classification and enabling individual plate counting of heterologous colonies, CFUCounter provides an open-source, efficient, and effective solution that beats industry-leading CFU enumeration systems.

The author Chen et al., [17] deviced a bacterial colony counter that is completely automated and capable of classifying and counting colonies. It takes as input images from a variety of digital cameras and can distinguish between chromatic and achromatic images. As a classifier, the counter employs a one-class Support Vector Machine (SVM) with a Radial Basis Function (RBF). The counter is reliable and effective, and its precision and recall performance is encouraging. In this article, we analyzed the state of art image processing techniques for its applicability in bacterial colony counting problem.

III. METHODOLOGY

The automatic counting system must pre-process the collected images to facilitate image segmentation and increase processing accuracy. Image preprocessing techniques such as median filtering, image gray-oriented, contrast enhancement, and others are used to improve and eliminate noise from the images because the threshold value selection for image segmentation is sensitive to noise [18]. Since a lot of microorganisms are colorless, staining techniques must be used before capturing images. The different staining techniques provide varied color images, the color characteristic is inappropriate for counting microorganisms automatically. To address the issues and get ready for image segmentation, pre-processing techniques should be used because of the illumination and the inhomogeneity of the image noise. Bacteria can be distinguished from other microorganisms in the image or from the backdrop using image segmentation techniques. Features from the regions of interest can be retrieved and utilized for categorization after they have been segmented.

The first step is to convert RGB images to grayscale by modifying the ratios of red, green, and blue channels to lessen the impact of various colors for image segmentation. It is also possible to convert RGB to HSI (Hue-Saturation-Intensity) color space to help detect boundaries of colony. When processing colored images, HIS be able to modify intensities other than does not affect original image's color characteristics. Moreover, it can finally correspond to the outcome of color perception and indicate the key attribute of color perception; it is useful to subsequent segmentation.

Second, the uneven lighting may cause shading and a nonuniform background. These can typically be fixed via low pass filtering, linear grayscale transformation, and background subtraction. Thirdly, one of the most important aspects of preprocessing is noise reduction. In this section, denoising techniques that are simple to use and effective are the median filter and the Gaussian filter. Halos that show up during imaging can be eliminated using the morphological opening and closing techniques.

Lastly, images may not have a strong contrast and require improvement for image segmentation. The most well-known technique for improving contrast in a global field that is also simple to use is the gray-level equalization of histograms [19]. The goal of this work was to use method to create an automated system for counting the colonies of S.aureus and Salmonella bacteria.

A. Classical colony counting methods

This section described automatic bacteria colony counting traditional methods like thresholding, edge-based detection, morphological operations, and the watershed, Support Vector Machine.

1) Thresholding based colony counting method

Segmented images were used for thresholding based colony count. As a kind of image segmentation, thresholding involves changing an image's pixels to facilitate analysis [20]. The process of thresholding involves transforming a color or grayscale image into a binary image, which is only black and white. To improve segmentation, a Gaussian blur is used for smoothing the image and minimizing noise. The image is automatically thresholded and converted to a binary representation using Otsu's approach. White patches on a black background are colonies. Next morphological opening and closing operation is applied. Opening operations are used for small noises and are removed by degrading small items, and then their size. Closing operations are used to fill minor gaps in the colonies. Finally, contours are used to identify the colony's boundaries. The following Fig.1 shows the bacterial colony counting based on thresholding approach.



Fig.1 Thresholding based colony count

2) Edge-based method for colony counting

Edge detection is applied for colony count. Identifying object boundaries and brightness discontinuities in images are two

applications of edge detection. In fields like computer vision, machine vision, and image processing, this method is employed for data extraction and image segmentation [21]. To improve edge identification, a Gaussian blur using a kernel size of (5, 5) is used for smoothing the image and minimizing noise. Then, to locate edges in the image, the canny edge detection technique is used. 50 and 150 threshold values are applied to this technique. Using a list of points that define the boundaries, the contour functions are used to identify the contours in the edge-detected image. A process that determines if each contour meets the image boundary after retrieving the image dimensions. A contour is disregarded if any of its points contact the boundary. The image's entire outlines are the only ones taken into account. By doing this, colonies that are partially outside the image bounds are eliminated and count number of colonies. In Fig.2 shows edge-based colony count.



Fig.2 Edge-based colony count

3) Colony counting based on Morphological approach

Every pixel in an image corresponds to the value of every other pixel nearby in a morphological action [22]. We can create a morphological operation that is sensitive to particular shapes in the input image by selecting the neighborhood pixel's size and shape. Initially we load the specific images and that image converted grayscale image. Secondly, the grayscale images are applied to Gaussian blur. The Gaussian blur is used to reduce the image noise and enhance the image thresholding accuracy. Third, Otsu thresholding is applied for converting the images to a binary format where the foreground colonies are black and the background is white. Fourth the morphological operations are applied. These operations are closing, erosion, and dilation. The closing is used for closing tiny gaps within colonies. The erosion operations are used to remove tiny noise. And the dilation operations are used for expanding colonies, bringing them closer. Finally, contours are used to identify the colony boundaries in the processed images and display the number of colonies counted. In Fig.3 depicts the bacterial colony count using morphological approach.



Fig.3 Morphological based colony count

4) Watershed based approach colony counting

A traditional method for segmenting images, the Watershed Algorithm is founded on the idea of watershed transformation. Similarity with neighboring pixels in the image will be used as a crucial reference by the segmentation process to link pixels with comparable grayscale values and spatial positions [23]. The input image is converted to a grayscale image. The grayscale image is blurred with a Gaussian filter. Gaussian blur is used to minimize noise. The Otsu method is used to convert a grayscale image to a binary image, which appears as colonies of black regions on a white background. After performing morphological operations, closure is performed to remove noise and close gaps among colonies. To determine the foreground by dilating the cleaned image to identify the places those are certainly part of the colony. The distance transform is used to measure the distance to the closest zero pixel for each pixel in the foreground colony. Thresholds the output to produce a binary image of the specified background. After locating the unknown region, subtracting a specific foreground from the backdrop reveals any unnamed regions that may occur between colonies. Label markers are used to indicate related label components in the foreground. We set unidentified regions to zero and alters the marker image to distinguish the colonies from the background. The Watershed method is used to find and segment overlapping colonies. The original image's watershed boundaries are highlighted in red. Uses contour functions to determine the contours of the foreground. Builds an empty list to hold colonies which are totally within the image and copies original image for contour drawing. It checks to see whether any contour points touch the images edge as it iterates through the contours. A contour is added and rendered in green if it does not touch the boundary and displays the number of colony counts. In Fig.4 shows the bacterial colony count using watershed approach.



Fig.4 Watershed based colony count

B. Machine Learning based colony counting methods

Artificial intelligence, which is often described as a machine's ability to mimic intelligent human behavior, includes machine learning as a subfield. The following are the three categories into which machine learning algorithms are divided according to the learning system's capacity for learning [24]. In Fig. 5 depicts the machine learning types.



Fig.5 Machine Learning Types

By training the labeled data samples, the supervised learning algorithm creates a model. Then the created model to predict the class of the real data. The accuracy of the model's predictions is used to gauge its effectiveness.

Unsupervised learning discovers data patterns on its own by learning from the real data without drawing any conclusions.

It functions by using the features that were taken out of the data to create a class. There is no model for these algorithms to make predictions. Using the characteristics of the images, it learns to recognize the classes [25]. The combination of supervised and unsupervised methods is called semisupervised. A limited percentage of the data is used for learning by the labeled class or model, while the remainder is used for learning independently. The three most well-known and extensively used machine learning methods are regression, clustering, and classification. The process of image classification involves taking characteristics or qualities out of the input images and classifying them according to those features or attributes. Pixel-wise, sub-pixel-wise, and objectbased image classification are the three different categories [26]. Traditional machine learning techniques like Support Vector Machines (SVM), Random Forests or K-Nearest Neighbors (K-NN) can then be trained with these extracted features to classify data.

1) Support Vector Machine (SVM)

By executing optimal data transformations that establish boundaries between data points based on predefined classes, labels, or outputs. The supervised learning models enable support vector machines (SVMs), a type of machine learning algorithm, to solve challenging classification, regression, and outlier detection problems [27]. Using a threshold, the preprocessed segmenting algorithm converts grayscale images into binary images (black and white). By doing this, the backdrop is guaranteed to be black (0) and the colonies white (255). The binary image's contours are found using the extract colony features. After that, it determines three characteristics for every colony found, such as the colony's area. The colony's perimeter serves as its boundary length. A shape descriptor called compactness measures how compact a colony is by comparing its size to its perimeter. To train the SVM classifier, it uses labeled data (such as area, perimeter, and compactness). After dividing the dataset into training and testing subsets, the classifier is trained, and its accuracy is assessed. A new image is subjected to the previously specified procedures in the colony count image: preprocessing, colony feature extraction, classification using the trained SVM model, and display of the expected colony count. In Fig.6 shows the support vector machine for bacterial colony count.



Fig.6 Support Vector Machine Architecture

IV. RESULTS AND DISCUSSIONS

This section discusses traditional automatic bacterial colony count methods using a dataset available from the public [28]. The input image is subject to preprocessing techniques like noise removal, filtering, and contrast on the image. After preprocessed images, selected method is applied for segmenting the bacterial colony. Finally, the colony counting methods like thresholding, edge detection, morphological, watershed, and Support Vector Machine are utilized. Next, the counting values computed through the metrics are recall and F1-Score. In Fig.7 details about original images, ground truth images, and traditional counting techniques are threshold, edge-based, morphological, watershed, and SVM counting. In Table1 displays the counting values from traditional methods. The F1-Score and recall served as the evaluation metrics for colony counting. True Positive correctly predicts the positive class, and True Negative correctly predicts the negative class, False Positive incorrectly predicts the positive class, and False Negative incorrectly predicts the negative class. To put it simply, the F1-Score is the harmonic mean of precision and recall [29].

$$Recall = \frac{TruePositives}{TruePositives + FalseNegatives}$$
(1)

$$Precision = \frac{TruePositives}{TruePosotives + FalsePositives}$$
(2)

F1-Score =
$$2X \frac{Precision X Recall}{Precision+Recall} \times 100\%$$
 (3)

			Segmentation Methods for Bacterial Colony Counting					
S.N o	Original	Ground truth	Threshold	Edge	Morphologic al	Watershed	SVM	
1			(<u>j</u>	(Q),		Q.	(A).	
2								
3							1.12 1.24 1.12 1.12 1.12 1.12 1.12 1.12 1.12	
4								
5					131 144			
6								
7				•••••• •••			09,090 6 ⁰ 0	
8					•			
9								
10				•••••				
	a)	b)	c)	d)	e)	f)	g)	

a)b)c)d)e)f)Fig.7 Segmentation of bacterial colony images a) Original b) Ground Truth c)Thresholdd) Edge-based e) Morphological f) Watershed g) SVM

artificial intelligence based approaches to get exact count values.

Table1. Counting values of manual and the selected traditional method thresholding, edge, morphological, watershed, and SVM method

Bacterial count value using the manual and traditional method										
Images	Manual	Thresh old	Edge	Morpho logical	Watersh ed	SVM				
1	137	51	43	37	47	63				
2	145	59	49	27	53	67				
3	164	42	30	17	39	48				
4	98	45	41	28	43	48				
5	139	35	28	19	33	38				
6	143	52	38	18	45	56				
7	67	25	25	22	27	31				
8	50	33	33	28	32	35				
9	55	39	38	28	39	42				
10	51	17	16	13	17	19				



methods

The above Fig. 8 is used for comparing the bacteria colony count values using recall and F1-Score metrics. From this experimental analysis, it is found that, the state-of-art method SVM have produced better colony counting results than the threshold, edge-based, morphological, watershed approaches. Also, it is observed that the traditional method unable to count the bacterial colonies accurately, thus it is suggested to use

V. CONCLUSIONS

This paper made an heap to count the bacteria colonies in microbial images. Traditional methods are used for preprocessing the input images. The preprocessed images are used for automated bacteria colony counting techniques such as thresholding, edge-based, morphological, watershed, and SVM methods. Finally, compared the counting values are compared using recall and F1-Score. Compared to the experimental traditional methods, SVM have produced better result.

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