ANALYSIS OF MICROBIAL IMAGE SEGMENTATION TECHNIQUES – A REVIEW

M. Sivapriya[1], *N. Senthilkumaran[2]

[1, 2] The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Dindigul – 624 302 Tamil Nadu, India.

Abstract: Microorganisms are playing essential roles in many applications fields like biotechnique, medical and industrial domain. Microorganism types are bacteria, fungus, virus, algae. Microorganism counting is required in many different fields for a variety of reasons, including monitoring food and water contamination levels, diagnosing infections in healthcare settings, maximizing industrial processes like fermentation, evaluating environmental health, and studying microbial ecology and evolution. This review analyzes the types of segmentation methods used for microbial image processing and analysis. The traditional methods of segmentation such as Thresolding, Edge-based segmentation, Region-based segmentation, Watershed-based, and Clustering-based, and the advanced techniques such as deep learning techniques likes Convolutional Neural Network (CNN), Object detection algorithms are techniques like Faster RCNN, semantic segmentation algorithms such as Fully Convolutional Network (FCN), U-Net, SegNet, Mask R-CNN, DeepLab are analyzed. Additionally, a summary and analysis of popular image segmentation technique for microorganisms are conducted in order to identify common technological features. This paper analyzes strategies that are available for use by researchers in different domains.

Keywords: Microorganisms, image segmentation, digital image processing, deep learning.

1. INTRODUCTION

Microorganisms are a kind of tiny entities; bacteria can be only seen under a microscope and are not visible to the naked eye. Some bacteria are good for our ecosystem. However, they also can potentially transmit diseases, particularly infectious ones, to people. Therefore, reliable identification of living bacteria is necessary for many applications, such as clinical diagnostics, food production, and evaluation of water quality. Through the use of methods of culture, the features of bacteria can be investigated below a microscope. Traditional clinical laboratory ethnic methods, still, require a great deal of work, mostly manual and physical effort Microorganisms can be a multicellular such as fungi or a unicellular such as bacteria. Microorganisms may live in any kind of environment and are found everywhere. They can also be found in the bodies of various plants and animals. While some bacteria cause diseases, others are beneficial to humans, animals, and plants. Although viruses have simply genetic elements that can only exist in the bodies of other species, they are occasionally categorized as microbes. Examples of microorganisms include viruses, bacteria, fungi, etc [1]. The experts are primarily responsible for identifying and counting bacterial colonies [2]. Furthermore, distinguishing between different species of bacteria can be challenging because they share the same morphology [3]. An automated approach for identification and classification can be developed to deal with these issues [4].

Three kingdoms comprise all living things: Plant, Animal, and Protista. The microbes are a diverse collection of tiny, unique living structures that fall under the kingdom of Unicellular creatures including bacteria, fungi, protozoa, and algae are classified as protozoa. The kingdom Protista has been split into three groups: prokaryotes, eukaryotes, and the most recently described archaebacteria. These divisions are based on variations in cellular organization and biochemistry. Certain bacteria are good for us, but some are bad for us because they break down food, infect people, and cause illnesses. The medical sector made history with the discovery of penicillin, which has prevented many deaths [5]. Yeast is commonly used to produce ethanol, ferment industrial products, and make sustenance for people [6]. Certain microorganisms have a lot of potential for producing renewable resources, can break degraded plastics, and can clean gas and wastewater. In addition, healthy people's intestines are home to a wide variety of microbes that aid in the breakdown and absorption of food and poisons. Industrial production and the human body are negatively impacted by certain bacteria. The human immunodeficiency virus (HIV), for instance, can infect people and impair their immune systems. SARS-CoV-2 is a serious threat to global health since it spreads quickly, has a high death rate, and is incurable [7].

Bacteria are ubiquitous, or present everywhere, single-celled prokaryotes that are essential to food production, the cycling of nutrients, and even the process of assisting digestion in the human gut. Virus Regardless of not being regarded as independent entities, they possess genetic material enclosed in proteins that infect and subjugate host cells, ultimately seizing control of the host. Fungi are eukaryotic organisms, such as yeasts and molds, are essential for breakdown and nutrient recycling. Protozoa is single-celled eukaryotes have a variety of activities and can be helpful or damaging. Algae are photosynthetic organisms, which range in size from microscopic phytoplankton to larger seaweeds, play a crucial role in the creation of oxygen and maintaining aquatic environments. Numerous bacteria can infect humans and crops and cause diseases. Virus diseases are chickenpox, measles, and mumps. Protozoa can spread diseases are malaria, amoebic dysentery. Ringworm and athlete's foot are spread by fungi. Microorganisms are remarkably adaptive; some can survive in harsh conditions such as deep sea or hot springs [8]. Their involvement in activities such as food production through fermentation, antibiotic synthesis, and vaccine development has a significant impact on human life. But since some microbes can spread infectious diseases, it's critical to comprehend and control their functions in ecosystems and public health. Microbiology, the study of microbes, continuously reveals new information about these minuscule living things, influencing our understanding of the microbiological environment and its implications for the broader biological framework [9]. Microbes can be helpful or harmful. A few of the dangerous microbes are listed. The bacteria salmonella is responsible for food poisoning, which manifests as cramping in the abdomen and diarrhea. The respiratory illness tuberculosis is caused by the bacteria Mycobacterium tuberculosis [10]. The most deadly type of malaria in humans is caused by the protozoan parasite plasmodium falciparum. In this paper recent image segmentation techniques are analyzed.

The structure of this paper via follows. Section II describes the image segmentation approach for microbial image. Section III focus on the image segmentation approach for microbial image using deep learning techniques. Finally, Section IV presents the conclusion.

2. SEGMENTATION

An image segmentation technique is used for partitioning an image into those distinct regions. In several disciplines, including environmental science, medical imaging, and microbiology, they are indispensable [11]. Image segmentation methods were used after preprocessing techniques. The preprocessing techniques are used to increase the object particle's contrast with the surrounding area. Images are improved and denoised during the pre-processing stage. When scanning microorganisms through a microscope, noise may be introduced into the image due to information loss during electrical transmission and preparatory procedures like staining. The following segmentation methods are frequently applied when examining the bacteria images.

2.1 Traditional Segmentation

The following Figure1 represents the various types of traditional segmentation methods used for microbial image segmentation

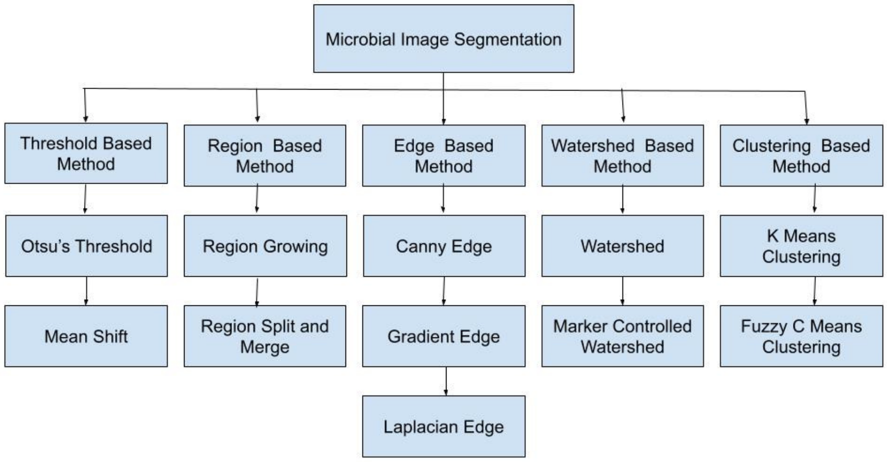


Figure 1. Traditional Techniques for Image Segmentation

A. Threshold-based segmentation

When segmenting a gray image using threshold-based methods, all of the pixels are compared to predetermined thresholds, and the comparison's results are used to identify each pixel said by [12]. Global thresholding is used for separating bacteria from the background by using a single threshold value. Local thresholding apply various threshold values to different areas of the image; this is beneficial for images with uneven illumination. Adaptive thresholding usually adjusting the threshold value automatically following local image attributes. This image dataset is publicly available (https://www.researchgate.net/topic/Plant-Bacterial-Diseases).

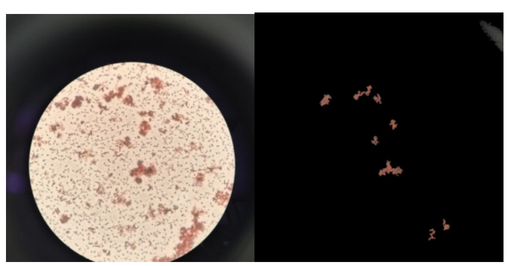


Figure 2. Segmentation by Threshold A) Original Image B) Threshold-Based Segmented Image

B. Region-based segmentation

Using this method, pixels are grouped according to their intrinsic characteristics, like color, texture, or intensity. Region-based segmentation aims to produce logical and significant segments within an image by recognizing areas with similar features. Seed-based Region Growing is used for beginning at seed places and expanding into regions according to predetermined standards like texture or intensity similarity [13]. The following Figure 3 is an illustrative example for region based segmentation of bacterial images.

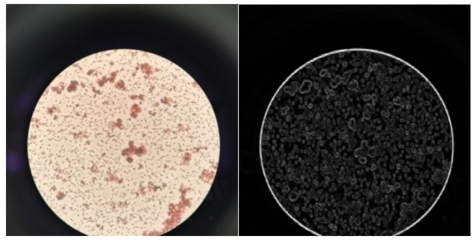


Figure 3. Segmentation by Region A) Original Image B) Region-based Segmented Image

C. Edge-based segmentation

Using different edge detection operators, edges in an image are located and used for edge-based segmentation. These edges identify areas in the image where there is a discontinuity in texture, color, gray levels, and etc. The edges can be located based on the discontinuity. Canny Edge Detection is used for adjusting the threshold value automatically following local image attributes. Sobel and Prewitt Operator's techniques for detecting edges based on gradients this is most frequently employed in image segmentation [14]. Figure 4 illustrate, bacterial image segmentation using edge-based segmentation techniques.

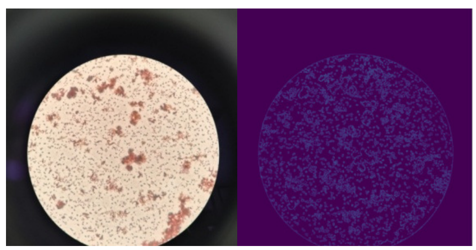


Figure 4. Segmentation by Edge - Based A) Original Image B) Edge-based Segmented Image

D. Watershed-based image segmentation

To recognize the various objects or regions in the image, the watershed method makes use of the borders separating the various colored sections. Tasks like object recognition, image analysis, and feature extraction can be performed with the resulting segmentation [15]. We obtained an output by using these traditional watershed-based image segmentation techniques.

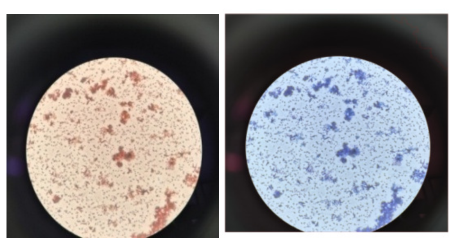


Figure 5. Segmentation by Watershed-based A) Original Image B) Watershed-based Segmented Image

E. Clustering-based image segmentation

This method is used to perform image segmentation of pixel-wise segmentations. K-means clustering is used to cluster pixels according to how similar they are in terms of color or intensity. Mean shift clustering is used to separate bacteria from the background by identifying dense regions in the feature space. Density-Based Spatial Clustering of Applications with Noise a clustering technique that is useful for segmenting bacterial clusters and will group pixels that are closely spaced together [16]. The following Figure 6 is an obtained an output by using the traditional clustering-based image segmentation techniques.

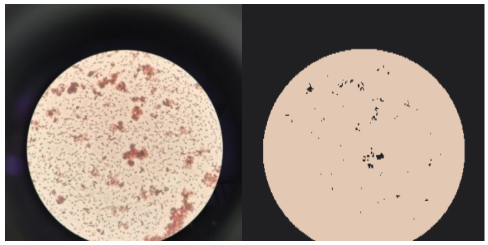


Figure 6. Segmented By Clustering-Based A) Original Image B) Clustering-Based Segmented Image

3. DEEP LEARNING-BASED SEGMENTATION

Machine learning in artificial intelligence refers to techniques that increase the accuracy of software programs while forecasting results. Deep learning is a kind of machine learning that has become a helpful computing methodology in the past ten years to help in the resolution of numerous challenging computer vision problems. Deep learning using kinds of layers input layer, hidden layer, output layer. The input layer is used to get the input data and hidden layer is used to receive the input data from the input layer and finally output layer is used to receive the feature-extracted data from the hidden layers and then produce the output [17]. The use of deep learning techniques to tackle image segmentation-based applications has become increasingly popular because of advancements in their large processing and memory capacity. Here is an explanation of the well-known deep-learning models that are used for image segmentation.

A. Fully convolutional network

A convolutional neural network that converts input image pixels to pixel categories is called a fully convolutional network (FCN). In classification challenges, segmentation involves the prediction of a class for each pixel. Segmentation adds the location of the input in the input image, whereas classification concentrates on what is in the input. The design of a fully convolutional network is illustrated in Figure 7.

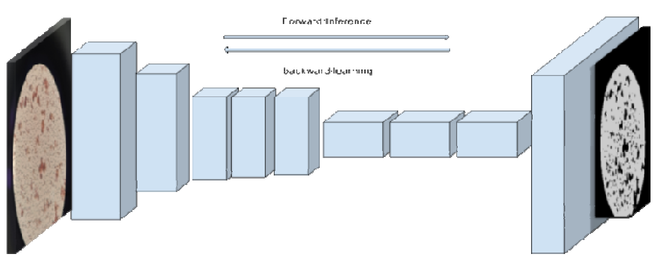


Figure 7. Fully Convolutional Network

Classical convolutional neural networks for image classification run with an input image via a series of convolutional layers and fully connected layers, which reduce the image size. CNN needs to go deeper to retrieve the deep features, even though this results in the loss of spatial position data. Up-sampling must be done to have an output size that is comparable to the input size. Finally, element-wise addition is utilized in FCN to fuse the output, producing an improved result. For that, a transposed convolution layer is used to return the height and breadth of the intermediate layer feature map to the dimensions of the input image [18].

B. U-Net

A fully convolutional network called U-Net is intended for the segmentation of biomedical images. It is made up of a symmetric expanding path to allow for exact localization and a contracting path to record context. With minimal training data, U-Net's architecture enables the effective segmentation of high-resolution images [19]. A typical U-Net architecture is shown in Figure 8.

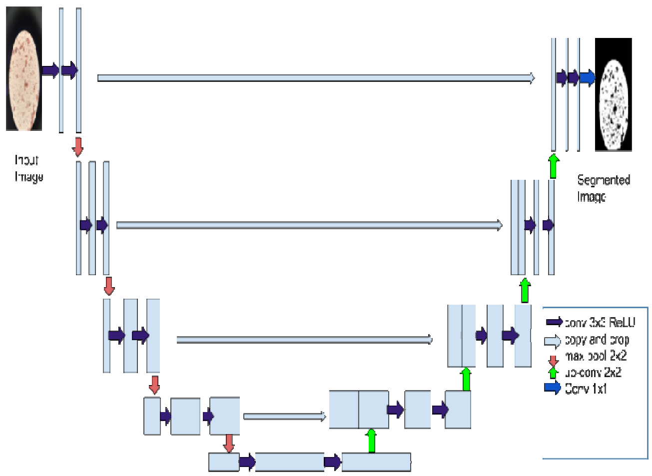
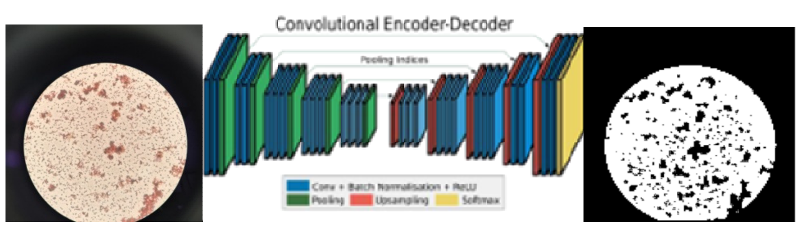


Figure 8. U-Net Architecture

C. SegNet

Another well-liked CNN-based model for semantic segmentation is called SegNet. It makes use of an encoder-decoder architecture, in which the decoder upsamples to produce pixel-wise segmentations after the encoder captures spatial data [20]. SegNet works well in applications that need fine-grained segmentation, like item detection and analyzing bacteria shapes.



Input image **Segmented image**

Figure 9. Illustration of SegNet Architecture [20]

D. Mask R-CNN

The region-based convolutional neural network, or R-CNN, group of algorithms is a popular class of two-stage object detection models. It starts by determining which areas inside an image are most likely to contain an object. Next, it categorizes the object according to each region. The Faster R-CNN network serves as the foundation for the Mask R-CNN. As a result, it gives along with the bounding box coordinates and class label for each object, there is also a segmentation masks to each region that contains an object. Mask R-CNN thus adds an enhancement for predicting an object mask to Faster R-CNN, making it comparable to the current branch for bounding box identification [21].

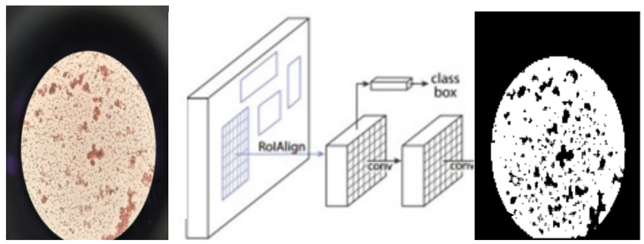


Figure 10. R-CNN for Instance Segmentation [21]

E. DeepLab

DeepLab is designed by Google to carry out semantic segmentation. DeepLab is used to lower the quantity of samples with data that the system processes to conduct image segmentation. The popular version of DeepLab in DeepLab V3+ is illustrated given below in Figure 11.

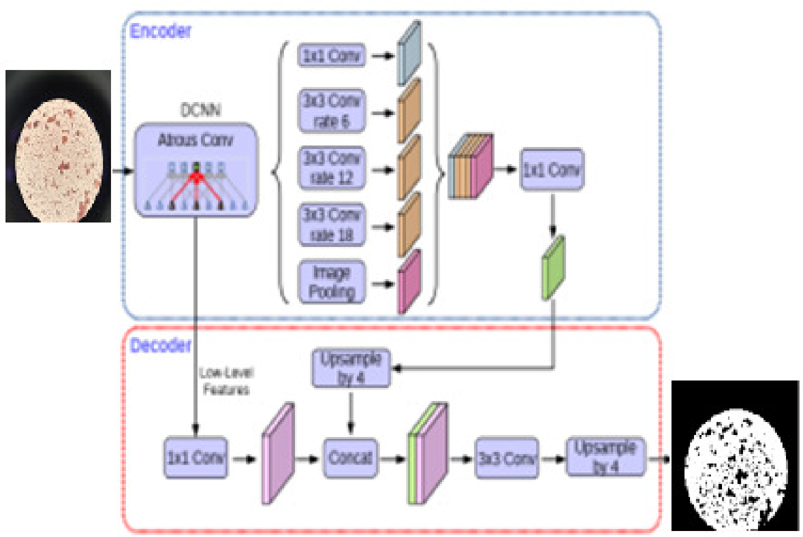


Figure 11. DeepLab V3+ Model [22]

The output of the final convolutional layer is upsampled while pixel-by-pixel loss computation is done. DeepLab uses atrus, sometimes known as dilated, convolutions with upsampling instead of standard convolutional operations. A helpful decoder module has been added to the most recent version of DeepLabV3+, which helps to enhance segmentation outcomes, particularly along object borders [22].
The summary of the existing segmentation approaches on traditional and deep learning based techniques are tabulated in table 1.

Table 1. Literature Review of Image Analysis-based Segmentation for Microorganisms

S. No	Author and Year	Types of Microorganisms	Preprocessing	Segmentation
1	Tucker et al. [23]	Fungal	Circularity test	Thresholding, Edge detection, and skeletonization
2	Viles and Sieracki [24]	Picoplankton	Global thresholding	Edge detection, filter, and MarrHildreth
3	Sieracki et al. [25]	Heterotrophic bacteria	No preprocess	Edge detection, filter, MarrHildreth method
4	Bloem et al. [26]	Microorganisms	Image sharpening and convolutional filtering	Quantity 570
5	Shabtai et al. [27]	Fungal	No preprocess	Multilayer neural networks, Self-organizing
6	Sándor et al. [28]	Fungi	Square root transformation	Quanitmet 570
7	Dias et al. [29]	Protozoan	Wiener filter	Thresholding and size filter
8	Cross and Kenerley [30]	Fungi	Square root transformation	Line detection, thinning, and Thresholding
9	Zeder et al.[31]	Cyanobacteria	No preprocess	Thresholding and gray level intensities maximizing
10	Dazzo and Gross [32]	Colony	Image calibration	Grid overlay, Quadrate size segmentation
11	Mazzei et al. [33]	Bioluminescence microorganisms	No preprocess	Thresholding and food-filling
12	Gabriel M et al. [34]	Bacteria	Laplacian filter	Otsu Thresholding, Edge detection, Hough transform
13	Masubuchi et al. [35]	Microscope images	No preprocess	Neural Network, RCNN
14	Golnaz Moallem et al. [36]	Red blood cell	No preprocess	Binary mask, Mean shift clustering, gradient vector flow(GVF)
15	Christoph Spahn et al. [37]	Bacteria	No preprocess	StartDist, U-Net
16	Jürgen Niedballa et al. [38]	Canopy images	No preprocess	U-Net and U-Net+
17	Joshua Stuckner et al. [39]	Microscope images	No preprocess	UNet and DeepLabV3+, ImageNet,MicroNet
18	P. Ajay et al. [40]	Microorganisms	No preprocess	K-median clustering, FCM
19	Saqib Qamar et al. [41]	TEM images	No preprocess	CNN, Random Forest, AdaBoost, XGBoost, and SVM

4. CONCLUSION

This research proposes a review of image analysis techniques based on image segmentation for microorganisms. The various segmentation techniques and other microbes are compiled and categorized according to the kinds of microorganisms. The techniques such as thresholding, edge detection, and deep learning are analyzed according to segmentation approaches. The use of traditional techniques like edge detection, the watershed algorithm, and thresholding technique, region growing, and clustering based technique demonstrates the rapid advancement with digital image processing methods of microorganism investigation. Deep learning techniques Convolutional Neural Network, Object detection algorithm, U-Net, SegNet, Mask RCNN, and DeepLab are demonstrates. The findings of the microbe segmentation have been carried out with excellent accuracy thanks to the advancement of deep learning. In conclusion, there is a great deal of room for research in this area segmenting microorganisms.

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