

Deep Learning Techniques using Automatic Bacterial Colony Counting

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Abstract

Accurate enumeration of bacterial colonies in Petri dish images is critical step in microbiological analysis for applications ranging from antimicrobial testing to food safety and environmental monitoring. Traditional manual counting methods are not only labor intensive and time consuming but also subject to manual errors, particularly when dealing with dense, overlapping, or faint colonies. To address these challenges, we propose a Deep Learning (DL) approach leveraging instance-level segmentation and detection models for automated colony counting. In this study, we focus on the AGAR-bright dataset, which consists of high-resolution Petri dish images organized into three categories such as countable, uncountable, and empty. Our analysis utilizes the countable subset, comprising 2000 images annotated with JSON files detailing individual colony locations. Two advanced Convolutional Neural Network (CNN) architectures namely Faster R-CNN (Faster Region-based Convolutional Neural Network) and Mask R-CNN is employed. The Faster R-CNN detects colonies using bounding boxes; Mask R-CNN extends this functionality by providing pixel-level segmentation, allowing more accurate identification in densely packed regions. Both models are trained using preprocessed and augmented images. Performance evaluation metrics such as Mean Absolute Error (MAE), F1- score, recall, and precision were utilized. According to experimental findings, Mask R-CNN performs better than Faster R-CNN, particularly in challenging scenarios with high colony overlap, due to its instance segmentation capabilities. This study demonstrates that Mask R-CNN can serve as a reliable, scalable tool for accurate and automated bacterial colony counting, reducing manual effort and improving consistency in microbiological analysis.

Keywords: Microorganism, Deep Learning Techniques, Bacteria Colony, Mask R-CNN, Faster R-CNN.

Introduction

Microorganisms are tiny organisms that cannot be seen with the naked eye. These tiny and simple structures are often visible only through a microscope. Bacteria, fungus,

viruses, algae, and other microorganisms exist in many different forms. Microorganisms play a critical role in our ecosystem. They contribute to the monitoring of environmental changes, the breakdown of waste products, the management of wastewater, the digestion of food, and so on. Microorganisms that cause disease can affect humans, plants, and other living things. The spread of fatal diseases like HIV, toxoplasmosis, anthrax, plague, and tuberculosis is their fault. Microorganisms are crucial to clinical microbiology, agriculture, food production, and medical research(1). Among the many advantages of microorganisms is the production of useful compounds like enzymes, antibiotics, and biofuels through biotechnology(2). In agriculture, microorganisms are crucial because they enhance soil quality, promote plant growth, and ward off illnesses and pests. Exact identification and classification of microorganisms is necessary for many applications. Numerous benefits are provided by microorganisms, which are important in agriculture, biotechnology, and environmental monitoring.

Microbial image processing is a set of strategies for improving and analyzing images that employ segmentation, classification, and counting methods. Segmentation methods vary greatly and can be broadly categorized as old and new procedures. Traditional methods for edge detection include thresholding(3), region-based strategies, which are straightforward but may fail with complicated images. In this work Deep Learning based segmentation algorithm namely Faster R-CNN and Mask R-CNN approaches are employed to count the bacterial colonies in the Petri dish images. These approaches can detect and classify microorganisms automatically, even in difficult situations. Counting microorganisms is another important operation that is frequently performed using automated counting algorithms capable of reliably estimating the number of germs in an image.

Classification is the process of grouping microorganisms based on their genetic or physical characteristics. One step in the image classification process is classifying images according to their content(4). To classify objects, the Image Classification system uses a database of predefined patterns. Image classification is a critical and difficult challenge in many applications such as industrial visual inspection, robotic movement, biomedical imaging, vehicle navigation, biometry, remote sensing, and video surveillance(4). However, because species are physically identical, traditional means of examining microorganisms, such as physical microscopy and culturing, can be costly, time-consuming, and frequently useless. It is becoming increasingly vital to build advanced image recognition algorithms that will automate microorganism classification with as little human intervention as feasible(5).

Machine learning techniques are rapidly being used in biomedical applications to evaluate and interpret diverse signal sources and medical images. Deep learning (DL) has developed as a strong approach for dealing with enormous amounts of complicated data, such as multidimensional anatomical images and video sequences. DL, a subfield of

Machine Learning (ML), develops algorithms to imitate the structural and functional aspects of the brain using artificial neural networks(6). Bacterial colony counting is the process of determining how many bacterial colonies have formed on agar plates. This technique, which has traditionally been done manually, is prone to error, time-consuming, and subjective. DL provides a robust and automated technique for accurately detecting and enumerating colonies from plate pictures. Models can learn complicated patterns in colony shapes, densities, and arrangements, even in changing lighting circumstances or overlapping (adhesive) colony formations. There is a growing need for deep learning based automated counting systems that offer precision, reproducibility and scalability.

This paper presents a DL based pipeline for accurately counting bacterial colonies from high-resolution Petri dish images using instance segmentation and object detection techniques. The proposed solution leverages transfer learning on Faster R-CNN and Mask R-CNN(7) architectures, both pre-trained on the COCO dataset and fine-tuned for microbiological colony detection. This approach explicitly localizes each colony using bounding boxes and segmentation masks, enabling robust counting even in the presence of clustered or overlapping colonies. To further enhance performance, image annotations in JSON format are converted into bounding box ground truths and masks, facilitating supervised training on real annotated data. The proposed framework attains a high level accuracy, precision and recall while maintaining a low mean absolute error (MAE), even with limited data.

The objective of this work is to develop an automatic real time counting of bacterial colonies using Deep Learning models. The rest of this paper is structured as follows. In Section II review of related works, Section III suggested methodology Faster R-CNN and Mask R-CNN are given. In Section IV results and discussion. Finally, in section V is conclusion.

Literature Review

This section summarizes the current state of image analysis for microbe detection and colony counts, with emphasis on accuracy. Researchers use these approaches in a variety of applications, including food and water sample detection, environmental monitoring, agriculture (RBC, WBC, grain, rice), and microbiology, evaluating medicines and safety of food. Various techniques are used to detect and microbial counts, including as edge detection, thresholding, Hough circle transformation, watershed, and Otsu thresholding. Although these techniques have been helpful in detecting microorganisms, there is still potential for development in segmentation and automation. This can be achieved by increasing the quantity of samples and using other bacterial strains. This has practical benefits for both researchers and academics.

Microbial counting can be performed manually or mechanically. Manual bacterial counting is very subjective and prone to inaccuracy. When the same observer examines

the same samples multiple times, the results may differ. Automated bacterial counting, such as image analysis or machine learning, can provide objective and accurate results with some consistency across time. These techniques distinguish colonies from the background and count regions of interest. Since our study is based on Faster RCNN and Mask RCNN, our review is focus on deep learning algorithms for microbiological analysis.

Ragi et al.,(8) proposed a method to uncover structural properties of G20 bacteria on steel surfaces that induce corrosion. The samples of data include SEM images. Deep Convolutional Neural Network (DCNN) and Mask Region CNN (RCNN) were utilized to segment bacteria and identify clustered bacteria correspondingly. DeepLABv3+, a previously created environment that implemented DL systems. The pre-trained architecture RCNN uses extract image feature maps. The results were compared to expert deductions. Results show that RCNN and DCNN outperform manual and conventional methods in detecting bacteria from biofilms, with an accuracy of 81%.

In (9), the Akbar et al., suggest a image classification method. The authors employ a TL in conjunction with a pre-trained CNN, with all layers save the classifier's weights frozen. Softmax was used as the activation function in the DenseNet 201 model. The model was made up of four convolutional layers, each followed by a dense block with a size varying from 6x to 32x. To speed up calculations, the global max pooling layer was used. The DensNet 201 model attained an accuracy of 99.24%. DensNet performed better than VGG16 and ResNet 18 in terms of accuracy.

Shwetha et al.,(10) suggest a deep CNN-based technique for bacilli detection. SqueezeNet, VGG-16and ResNet are utilized for testing and training to determine which bacilli identification is best accomplished using models. The models used TL, as shown by the use of pre-trained architecture. Additionally, K-means clustering and coloring techniques were used to isolate the bacterial images from the background. The 224x224x3 scaled images were fed into the model. Across layers, sigmoid and ReLU activation functions were used. SqueezeNet outperformed other CNN models with a 97% accuracy rate.

Kakishitaet al.,(11) developed a method for segmenting and categorizing six bacterial species. The U-Net architecture was used to split SEM images into foreground and background. The species of bacteria were categorized using VGG16 before the number of cells was counted. For better accuracy and self-learning, the authors divided segmentation and classification. The proposed technique performed better than traditional CNN, with accuracy 95.8%.

Naets et al.,(12) recommended using the Mask R-CNN model to detect and count BVG+ and BVG- bacterial colonies in Petri dishes. The model was integrated into a modern Single-Page Application (SPA), allowing laboratory technicians to upload images, automatically to receive colony count projections, and interact with the results via a user-

10.48047/jocaaa.2024.33.02.46

friendly interface. The customized Mask R-CNN, which was specifically fine-tuned and trained. The model achieved a Mean Average Precision (mAP) of 94% when evaluated at an IoU= 0.5. These findings show the model's robustness and possible application to similar tasks in biological image processing, such as bacterial species detection or viral plaque quantification.

Mohammad Khan et al.,(13)suggests to employ the CNN to classify E. coli and Faster R-CNN model to automate colony counts. The collection included 1,301 samples of groundwater. The faster R-CNN model achieved 97% accuracy with an error loss of 0.10. The system's performance was assessed using F1-score, precision, accuracy, and sensitivity, which demonstrated the model's dependability for counting E. coli colonies.

The Pawłowski et al.,(14) described a synthetic dataset generation method to train deep learning models for microbiological image interpretation with less real data. The technology merged classic computer vision techniques with neural style transfer to provide realistic image augmentation. The authors created a dataset of 100 genuine Petri dish images to train a neural network for localization, segmentation, and classification of five microbial species. The resulting model had a detection mAP of 0.416 and a counting MAE of 4.49, which are comparable to models trained on a considerably bigger real dataset of almost 7,000 images (mAP = 0.520, MAE = 4.31).

Fan Yang et al., (15) developed a deep learning-based technique to solve limitations such as plate contamination, extended culture durations, and a lack of annotated data. Using the AGAR dataset, the scientists used style transfer techniques to increase the training set to 4,000 photos of microbial colonies. For better feature extraction, the study used the Swin Transformer as the backbone in Cascade Mask R-CNN architecture. This model had a mean Average Precision (mAP) of 61.4% across IoU thresholds ranging from 0.50 to 0.95, exceeding the previously most effective Cascade R-CNN with HRNet by 2.2%. Furthermore, trials with YOLOv8x on the AGAR dataset showed an even higher mAP of 76.7%, indicating strong detection performance.

In Zhang et al.,(16), the identification of environmental microorganisms, a novel Squeeze-and-Excitation-based Mask R-CNN (SEM-RCNN) model was developed. While the basic Mask R-CNN extracts features using ResNet, SEM-RCNN improves on this by including SENet to capture inter-channel dependencies and emphasize important features. The model was tested on the EMDS-6 dataset and obtained a mean Average Precision (mAP) of 0.511. Its transferability further evaluated on a blood cell detection work using an more than 17,000 microscopic images.

Methodology

This section describes the suggested methodology for bacterial colony counting, which employs DL based models for detection and segmentation. The total workflow comprises of dataset preparation, annotation parsing, model architecture setup, training

configuration, and performance assessment. Faster R-CNN and Mask R-CNN are two different models used and compared for the task. While Faster R-CNN is used for bounding-box detection and counting, Mask R-CNN is utilized for instance level segmentation, which improves colony separation and enumeration accuracy.

Dataset Description

The studies are carried out utilizing the AGAR-bright dataset(17), which comprises of high resolution Petri dish images taken under bright lighting. The dataset is organized into three categories are countable, uncountable and empty images based on colony visibility and clarity. For this work, we exclusively used the countable subset, comprising 2000 images, each accompanied by a JSON annotation file. These files provide detailed information on the location and dimensions of bacterial colonies using bounding box coordinates. The selected images display a wide range of colony counts and densities, allowing for robust training and evaluation of the detection based counting models. This curated dataset supports the development of accurate and generalizable colony enumeration system.

Data Pre-processing

For the training process to use uniform input dimensions, all images were scaled to 512x512 resolutions. Images were normalized to [0,1] and converted to RGB if not already in that format. Annotation files in JSON format were parsed to extract bounding boxes and masks. Binary masks were created from polygonal annotations using conventional image drawing packages to train Mask R-CNN. For the period of training, techniques of augmentation such as brightness modification, horizontal and vertical flipping was used to improve variability and prevent overfitting.

Model Architectures

In this study, we used two sophisticated deep learning models for effective bacterial colony recognition and counting: Faster R-CNN and Mask R-CNN. Both models capture multi-scale data using a Feature Pyramid Network (FPN) and a ResNet-50 backbone, which is particularly useful for recognizing colonies of varied sizes. A full explanation of each architecture and workflow is provided below.

Faster R-CNN Architecture

Faster R-CNN(14) is a two-stage object identification model that uses bounding boxes to locate objects. The architecture contains the following components

Backbone (ResNet-50 + FPN)

The input image is passed through a deep residual network (ResNet-50), enhanced by a Feature Pyramid Network. The FPN helps to fine-grained spatial features at multiple scales, which is essential for small and dense colony detection.

Region Proposal Network (RPN)

The feature maps generated by the backbone are passed to the RPN, which scans them with anchor boxes of various scales and aspect ratios to propose candidate object regions (ROIs).

ROIAlign + Detection Head

Each proposed ROI is aligned and pooled into a fixed size. These pooled features are then passed to two branches:

A Classification head that predicts the class label (colony or background)

A regression head that refines the bounding box coordinates.

Colony Count Output

After filtering out low-confidence detections using a confidence threshold 0.5, the number of detected boxes in each image is used as the predicted colony count.

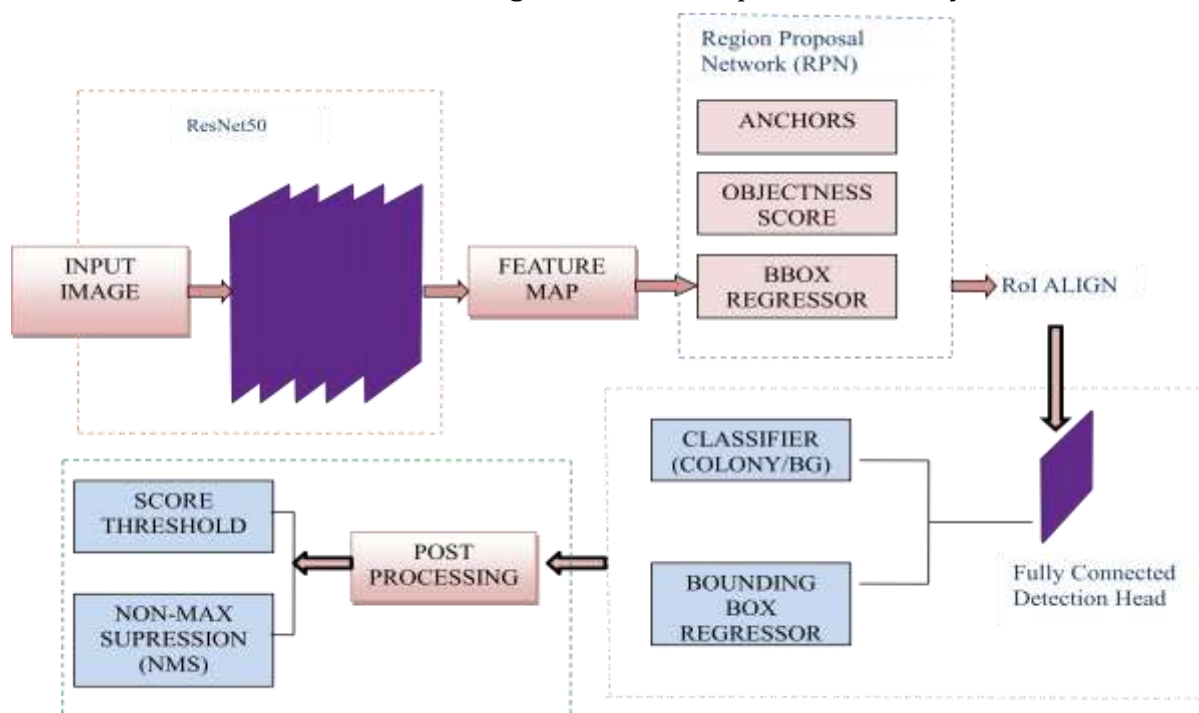


Figure 1: Workflow of Faster R-CNN architecture

The implemented pipeline is based on the Faster R-CNN object detection architecture. The task is to automatically detect and count bacterial colonies in agar plate images. The process begins with a custom PyTorch dataset class that reads image files and their associated annotation in JSON files. Each JSON file includes bounding box coordinates for colonies and a label (*B.subtilis*), (*C.albicans*), (*E.coli*), (*P.aeruginosa*), and (*S.aureus*). These annotations are converted into the format required by the Faster R-CNN model as a dictionary with keys for bounding boxes, labels, and image ID.

The backbone network used is ResNet-50 with a FPN, which is capable of extracting multi-scale features from the input image. The Faster R-CNN model is pretrained on the COCO dataset, and the final classification head is modified to detect just one foreground

10.48047/jocaaa.2024.33.02.46

class (the colony) and the background. The model includes three main components are a Region Proposal Network (RPN), which produce proposals for candidate objects (regions likely to contain colonies), next RoI Align layer, which extracts fixed size features for each proposal, and a Detection Head, which classifies each regions as colony or background and refines the bounding box predictions.

The model is trained using optimizer Stochastic Gradient Descent (SGD) and a standard multi-task loss combining classification and bounding box regression. During training, images are fed in batches, and the model learns to detect colonies through supervised learning over multiple epochs. After training, the model is evaluated on a validation set. Predictions undergo post-processing, where low-confidence detections are filtered out using a score threshold, and overlapping boxes are refined using Non-Maximum Suppression (NMS).

Mask R-CNN Architecture

Mask R-CNN extends Faster R-CNN by adding a third branch for instance segmentation. This addition allows the model to predict precise binary masks for each detected object, improving performance in densely clustered regions.

Backbone (ResNet50 + FPN): Same as in Faster R-CNN Multi-scale characteristics are extracted from the images using this component.

Region Proposal Network (RPN)

Proposes potential regions of interest containing colonies.

ROIAlign + Multi-Branch Heads:

A classification branch predicts the presence of a colony.

A bounding box regression branch refines coordinates.

A mask prediction branch outputs a pixel wise binary mask for each detected object.

Segmentation Output

Detection comes with both a bounding box and a segmentation mask, which helps to separate overlapping colonies more effectively.

Colony Count Output

Similar to Faster R-CNN, valid detections above a threshold is counted. The masks are also used to visualize and verify the accuracy of each predicted colony instance.

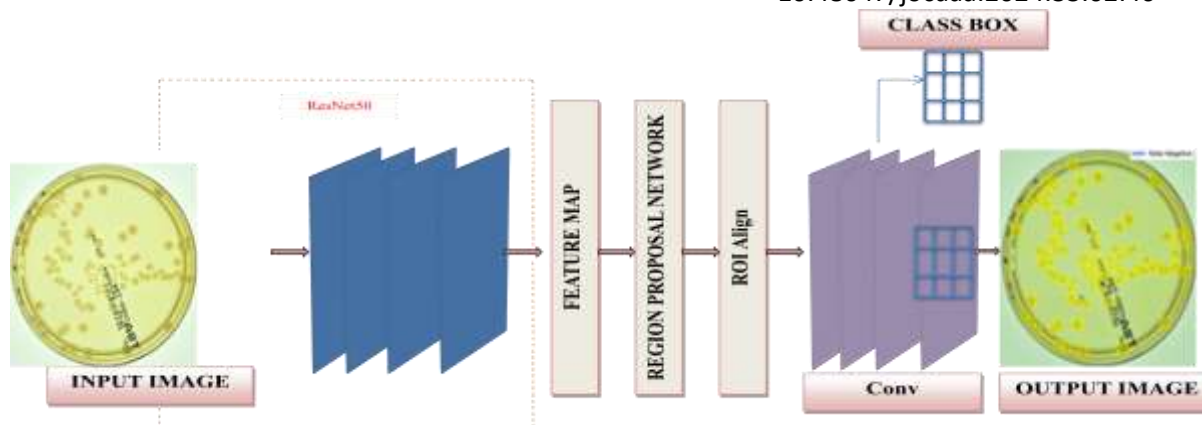


Figure 2: Workflow of Mask R-CNN Architecture

This work presents a robust and scalable instance segmentation pipeline for the automatic detection and enumeration of bacterial colonies in agar plate images. The model is based on Mask R-CNN with a FPN pre-trained on the COCO dataset is combined with the ResNet50 backbone. The implementation is carefully adapted and optimized to handle the unique challenges to biomedical images, particularly the visual similarity and overlapping of bacterial colonies. To incrementally enhance learning throughout the training phase, a mini-batch Stochastic Gradient Descent (SGD) optimizer was employed in conjunction with a step based learning rate scheduler. Following training, the anticipated segmentation masks were post-processed to calculate the number of colonies in each image. The performance was thoroughly tested utilizing measures such as MAE for accuracy, precision, and recall, F1-score for detection performance. The results showed that Mask R-CNN offers a reliable, high-precision pixel-level solution for bacterial colony enumeration, overcoming bounding box only approaches such as Faster R-CNN, especially in challenging cases involving touching or overlapping colonies.

Evaluation Metrics

To evaluate the performance of detection of object models in bacterial colony enumeration, several quantitative metrics were employed. These metrics assess detection accuracy, mask segmentation quality and counting performance.

Mean Absolute Error (MAE)

MAE measures the average absolute difference between the anticipated number of colonies and the ground truth count for each image. It provides insight into the accuracy of the models counting ability, especially in high density colony regions. A lower MAE indicates better performance and more reliable enumeration.

Mean Absolute Error

$$(\text{MAE}) = \frac{1}{N} \sum_{i=1}^N |\hat{y}_i - y_i| \quad (1)$$

N is total number of images, $\hat{y}_i$ predicted colony count, y_i ground truth colony count.

Precision

To evaluate the detection capability of the model. Precision refers to the proportion of correctly detected colonies (True Positives) among all detected colonies (True Positives + False Negatives).

$$\text{Precision} = \frac{TP}{TP+FP} \quad (2)$$

TP – True Positives (correct detections) and FP – False Positives (incorrect predictions)

Recall

The recall quantifies the proportion of approximately detected colonies among all real colonies (True Positives + False Negatives).

$$\text{Recall} = \frac{TP}{TP+FN} \quad (3)$$

FN –False Negatives (Missed Colonies)

F1-Score

The F1-Score, being the harmonic mean of precision and recall, offers a balanced measure when the dataset is imbalanced or when both false positives and false negatives are important to consider(18).

$$\text{F1-Score} = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad (4)$$

The Faster R-CNN is lightweight and effective for general object detection and colony counting when colonies are sparse. The Mask R-CNN is more powerful in separating overlapping of touching colonies, making is suitable for dense Petri dishes. By training and evaluating both models on the AGAR- bright countable dataset, we can compare effectiveness of bounding box detection versus instance segmentation in bacterial colony enumeration tasks.

Results and Discussion

This part presents experimental results and performances evaluation of the anticipated models Faster R-CNN and Mask R-CNN for automated bacterial colony counting using the AGAR- bright dataset. The dataset used comprises 2000 countable Petri dish images annotated with colony coordinates in JSON format. To assess the effectiveness of both models, we employed a comprehensive set of evaluation metrics such as MAE, Precision, Recall, and F1-Score. The qualitative results below show in Figure 3 depict the comparisons between ground truth annotations and models predictions for both models.

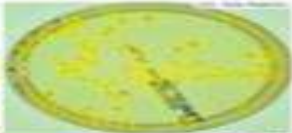
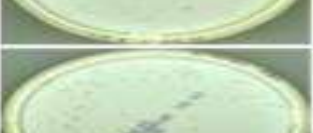
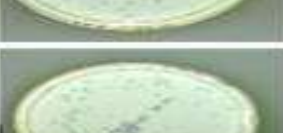
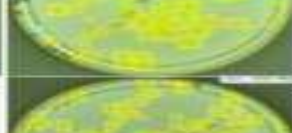
S.No	Original Images	FasterR-CNN	Mask R-CNN
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
	a)	b)	c)

Figure 3: Example of Output Images of Bacterial Colony Counting a) Original Images b) Faster R-CNN Images c) Mask R-CNN Images

Table1: Bacterial colony counting values of manual, Faster R-CNN and Mask R-CNN

Images	Manual	Faster R-CNN	Mask R-CNN
1	151	135	148
2	139	125	137
3	232	208	228
4	116	104	114
5	150	135	147
6	161	144	158
7	126	113	124
8	121	108	119
9	243	218	239
10	123	110	121

Table 1 presents the bacterial colony counts obtained from ten representative agar plate samples using manual enumeration, Faster R-CNN, and Mask R-CNN. Manual counts serve as the basis for evaluating model performance. Across all samples, both models approximated the manual counts to various degree of accuracy. The Faster R-CNN model often underestimated colony counts, with variations ranging from 11 to 25 colonies when compared to the manual technique. This undercounting impact was more prominent in examples with greater colony densities, such as sample 3 (manual 232, Faster R-CNN 208) and sample 9 (manual 243, Faster R-CNN 218), indicating that the model struggled to distinguish between densely packed or overlapping colonies.

In contrast, Mask R-CNN, on the other hand, gave counts that were far closer to the manual values, with no more than two colonies separating the samples. In sample 3, Mask R-CNN reported 228 colonies versus the manual count of 232, and in sample 9, it reported 239 versus 243. Such high agreement suggests that Mask R-CNN instance segmentation capabilities enabled more exact delineation of separated colonies, even in the presence of significant overlap. Overall, these findings demonstrate that, Faster R-CNN provides a good estimate of colony counts, Mask R-CNN approaches near human performance, providing more reliability automated bacterial colony enumeration. This increased accuracy can be due to its capacity to build exact pixel level masks, which aid in the separation of nearby colonies that object detection based techniques may combine or ignore.

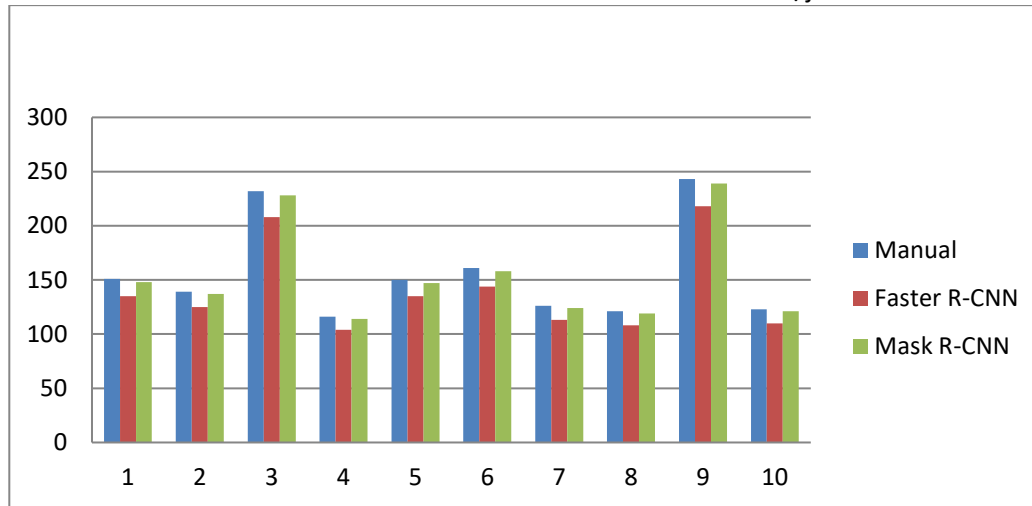


Figure 4: Enumeration Comparison Results of Manual and DL Models

Table 2: Shows the evaluation metrics using Faster R-CNN and Mask R-CNN

Metrics	Faster R-CNN	Mask R-CNN
MAE	3.40	2.70
Precision	0.93	0.98
Recall	0.94	0.99
F1-Score	0.93	0.99

The findings in Table 2 reveal that the Mask R-CNN demonstrates clear improvements over Faster R-CNN, especially in scenarios involving overlapping or dense colonies, due to its instance segmentation capability.

This improvement in performance by Mask R-CNN can be attributed to its dual ability to detect and segment individual colonies, even in complex overlapping regions. In contrast, Faster R-CNN struggles to separate closely packed colonies, leading to undercounting. Moreover, the relatively high F1-Score and Recall of the Mask R-CNN model suggest its robustness in both detection and classification tasks across diverse sample conditions in the AGAR-bright dataset. This demonstrates the effectiveness of using instance segmentation techniques for CFU detection and highlights the potential for integration into automated microbiological workflows.

Conclusion

This study demonstrates how DL based models detect and segment bacterial colonies in Petri dish images accurately and efficiently. We tested the Faster R-CNN and Mask R-

CNN under identical pre-processing and training setup using the countable portion of the AGAR bright dataset. Mask R-CNN consistently beats Faster R-CNN, especially in images with dense, overlapping or irregularly shaped colonies, owing to its pixel level segmentation capabilities. Performance improvements were visible across numerous evaluation parameters, including lower MAE, improved accuracy, and higher F1-Scores. The automated counting architecture given here has substantial advantages over manual methods, saving time, effort, and the risk of human error while retaining high precision and reliability. These findings indicate that Mask R-CNN may be implemented into microbiological processes as a scalable and robust colony quantification method, with applications in antimicrobial testing, food safety, environmental monitoring, and other microbiological research fields. Future research could look into lightweight model variants for faster deployment on edge devices, as well as the use of advanced post-processing techniques to refine counts in challenging visual conditions.

Future Enhancement

Future enhancements of this study aim to extend the proposed deep learning framework toward real-time and large-scale microbial image analysis. The integration of advanced architectures such as Vision Transformers (ViT), Swin Transformers, and Hybrid CNN-Transformer models can significantly improve feature extraction, particularly for complex and overlapping colony structures. Moreover, expanding the dataset to include a broader range of bacterial species, agar media types, and illumination conditions will enhance the model's robustness and generalization across diverse experimental settings. In addition, incorporating multi-task learning strategies can enable the model to perform colony counting, bacterial species classification, and antimicrobial activity prediction simultaneously, thereby creating a unified and intelligent microbial analysis system. Such advancements will ultimately facilitate the development of automated, interpretable, and deployable frameworks for routine laboratory applications and industrial microbiology.

Abbreviations

CNN: Convolutional Neural Network, Faster R-CNN (Faster Region-based Convolutional Neural Network), DL: Deep Learning, ML: Machine Learning, JSON: Java Script Object Notation, MAE: Mean Absolute Error.

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Author Contributions

Sivapriya: Analysis, Model design, Data Collection, Formulation, Testing. Senthilkumaran: Analysis, Testing, Validation.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this research work.

Ethics Approval

This study was conducted in accordance with the ethical guidelines and principles.

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