Bacterial Behaviour Analysis through Image Segmentation using Deep Learning Approaches

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Abstract. Antimicrobial Resistance (AMR) refers to the ability of microorganisms to resist the effects of certain medicines. Medicines that were previously known effective against diseases caused by different types of microorganisms are now incompetent towards the same treatment because of AMR, which also increases the risk of severe illness. By understanding AMR and the potential factors that lead to it, we can see how microorganism behaviour analysis has become a great tool. The limitation of human visual capabilities requires automated image-based solutions to analyse bacterial behaviour effectively. In this paper, we exploit growth stage-based multiple images of bacteria, i.e. *E. coli* (*Escherichia coli*) to Analyse bacterial behaviours to get valuable insight. We have used the Deep Learning algorithms to get segmented images for each of the growth stages. Our objective is to use U-net and StarDist to get bacterial behavioural features and compare their performances in terms of Ground Truth and predicted segmented masks. For both the Ground Truth and predicted segmented mask, we have determined total bacterial cell count, average bacteria volume, central distance from the image center, total area, average aspect, average solidity, average extent, average orientation, average Local Binary Patterns (LBP) and features of Gray-Level Co-occurrence Matrix (GLCM) such as contrast, dissimilarity, homogeneity, energy, and Angular Second Moment for each of the images. Also, we have analysed area change and movement from one frame to another frame, which represents bacterial growth over specific periods. Analysing these features will allow the researcher to identify the best-performing model for each of the calculating features of bacteria. Comparing these features between the actual mask and predicted segmented mask can help to identify valuable insights regarding bacterial behaviour which can be useful to identify factors that contribute towards AMR.

Keywords: Bacterial Behaviour Analysis · Image Segmentation · Deep Learning · Antimicrobial Resistance,AMR.

1 Introduction

Analysis of microorganism images allows healthcare specialists to diagnose infectious diseases caused by bacteria, fungi, or another microorganism [1]. However,

microorganism image analysis is a critical approach because of the cell complexity and structure of microorganisms [2]. Microorganisms play a vital role in our ecological systems. It is estimated that there are nearly 10 million microorganisms can be found in a single drop of water [3]. Bacteria, fungi, parasites, and viruses are essential for the sustainability of our environment [4].

Some microorganisms such as viruses, bacteria, fungi, and others can spread diseases to humans and living animals. These can cause minor infections, severe infections, and even death. In the year 2019, there were 7.7 million people died due to various bacterial infections which refers to the 13.6% of people or 1 in every 8 people in the world on the other hand bacterial human pathogens are increasing very rapidly every year [5].

AMR is a natural phenomenon that happens when microorganisms are exposed to antibiotic drugs [6]. AMR is now considered a global health emergency which makes treatment more and more difficult and requires an urgent global response [7]. Therefore, it is necessary to study AMR and develop new antibiotics through global investments [8]. In this case, AI-based models to analyse behaviours of bacteria can help to identify valuable insight regarding AMR and allow the researcher to fight AMR. It is predicted that AMR could lead 10 million people to death by the year 2050, which is alarming news [9]. We need to identify other root causes of AMR such as overdose and misuse of antibiotics and techniques to control AMR [10] [11].

To study AMR, analysis of bacterial images can be a great tool. Microscopic images of bacteria can be used to identify features and behaviours of bacteria. However, bacterial original images can be misleading in terms of noise generation. Therefore, actual mask image is an excellent alternative to use for analysis. On the other hand, generating an actual mask for each image is not only time-consuming but also costly. Therefore, Deep Learning (DL)-based models can be used to get predicted masks quickly and efficiently. Then those predicted mask images can be used to identify valuable patterns and insights of bacterial behaviours.

Analysing microscopic Images also needs a large number of datasets which is expensive and time-consuming. It is necessary to extract important features from images from small datasets to understand the nature of bacteria and the process of resistance towards available drugs. So that researchers can focus more on advancing the existing antibiotics rather than collecting and evaluating microscopic images. To make their work easier and focused on solving AMR-based challenges we are using Deep Learning based models to do image analysis focusing behaviour analysis of bacterial pathogens.

Figure 1 shows the overview of our explored work. In this study, we have used DL models such as U-Net and StarDist to generate predicted masks from datasets having both original images and actual masks for training and testing. We have trained our U-Net model using original images and actual masks. After that, we tested and measured accuracy over predicted masks.

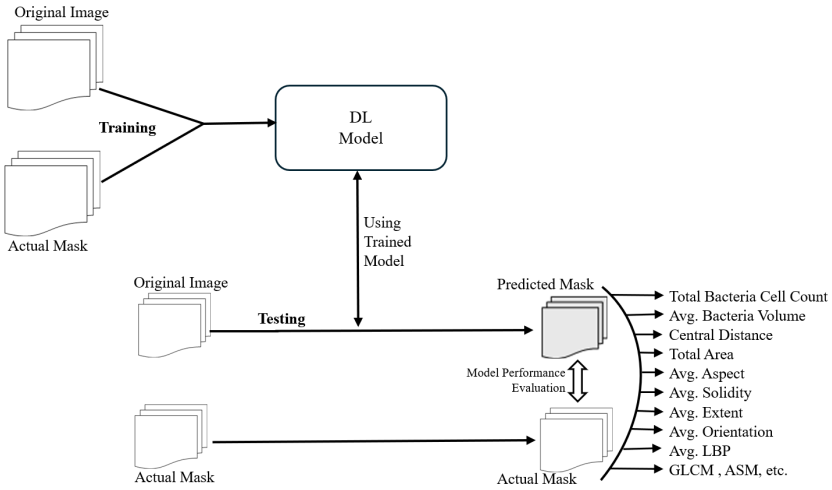

Fig. 1: Overview of our methodology.

Then we have identified some valuable properties of bacteria such as total bacterial cell count, average bacteria volume, central distance from the image center, total area, average aspect, average solidity, average extent, average orientation, average Local Binary Patterns and features of Gray-Level Co-occurrence Matrix such as contrast, dissimilarity, homogeneity, energy, and Angular Second Moment for each of the actual images and predicted images generated from U-Net and StarDist. We have also examined the area changes and movement from one frame of the actual mask and predicted mask to another immediate frame of the actual mask and predicted mask within a specific time duration.

This paper is divided as follows. Section 2 discusses a literature review focusing on both recent and previous works related to bacterial behaviours using AI techniques. Section 3 shows data description and terminologies. Section 4 explains our methodology and Section 5 discusses result analysis. Finally, Section 6 concludes the paper focusing conclusion and future work.

2 Literature Review

In this section, we aim to discuss both recent works and previous works related to bacterial image segmentation, and bacterial behaviours observations focusing on AMR and AI.

In [12], authors focused more on increasing the accuracy of the result of segmentation extending the StarDist algorithm. It also compared the results of classical image processing models and deep learning models for segmentation models. However, it was more focused on single-cell segmentation and it is hard to use in 3D images.They did not analyse the behaviour of the bacteria.

In [13], the authors used DL approaches for Medical Modality Image Segmentation. They have reviewed how Convolutional Neural Networks (CNN), Recur-

rent Networks, Attention Models, and Generative Adversarial Networks (GANs) can be used to perform medical modality image segmentation. However, we did not find any attention towards microscopy image analysis, particularly for microorganisms. Besides, they did not mention any approach to identify image features using these models. In this work, we used various approaches to identify key bacterial behaviours from segmented images after using U-Net and StarDist.

On the other hand, in [14], authors proposed classification methods for phase contrast time-lapse microscopy images using DL to classify four species of bacteria which are (*E. faecalis, E. coli, K. pneumoniae, and P. aeruginosa*), which are relevant to human health. They achieved more than 98% accuracy. But, we did not find any specific comparison among models and which models work better in terms of which type of bacterial behaviours identification. In this work, we have done a vital comparison between two models that are U-Net and StarDist demonstrating their performances in every specific case.

In [15], authors proposed a method using CNN in transmission electron microscope images to identify drug-resistant cells which leads to AMR.They used Pearson's Correlation Coefficient, to investigate the genes which are associated with morphological features.

However, we did not find any explanation for finding bacterial behaviours such as total aspect, total bacteria volume, and others as well as which approach is suitable to identify specific bacterial behaviours.

From [16], we got motivation for this work. We have used a subject of their dataset. The authors proposed DeepBacs for multitask bacterial image analysis using Dl approaches. They showed image segmentation using various techniques, worked on artificial labeling, denoising, enhanced image resolution, and more. But, we did not find any specific approach for determining bacterial behaviours such as central distance from the image centre, average solidity, average extent, and more. In this work, we have analysed various bacterial behaviours which may provide valuable insights towards bacterial study and AMR.

In [17], authors expressed their opinions about monitoring and investigating microorganisms using AI. The authors showed how researchers can use DL methods to study the classification, detection, segmentation, and quantification of microorganisms. However, we found a clear gap regarding analysing microorganisms' behaviours such as bacterial behaviours and movements in microscopic image segmentation. In this paper, we analysed bacterial movement from frame to frame in specific periods, which may help medicine researchers to examine effects of antibiotics on bacteria that are antimicrobial on microorganisms.

3 Data Description and Terminologies

3.1 Data Description

In this study we have exploited Brightfield Images of *E. coli* bacteria from DeepBacs open-source Dataset[16]. The dataset is a subset of a large dataset that contains different bacteria images in various conditions. We have divided the dataset into three fragments. Each fragment contains several growth-based time

series image data of *E. coli* bacteria. The bacteria cell type is *E. coli* MG1655 wild type strain (CGSC #6300) Here, we have total two types of image data in the dataset which are actual image data and actual mask data. Using our selected models we have generated predicted segmented mask data.

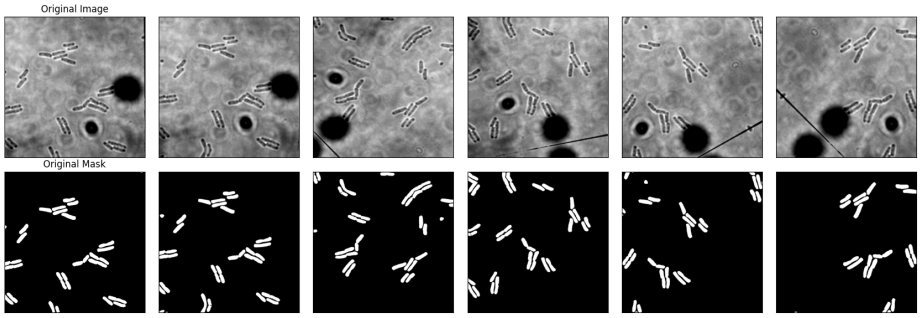

Fig. 2: Several sample image of original images alongside their respective ground truth masks.

The microscopy image data is in 2D, which are recorded at 1 min interval. To capture image data the device was used, the Nikon Eclipse Ti-E, which equipped with an Apo TIRF 1.49NA 100x oil immersion objective. Here image size is 1024x1024 (79nm / pixel), 19/14 individual frames. The generated file format is 8-bit '.tif'. The raw image data were captured in 16-bit mode where image size was 512x512. Figure 2 shows some of the original images and their corresponded actual masks or ground truth from dataset.

3.2 Explored Attributes of Bacteria

Total Bacteria Count: To calculate the total bacteria number in an image we need to follow some steps which include segmentation, contour detection, and counting. We used the adaptive thresholding method for image segmentation and then did Contour detection using OpenCV which uses the Suzuki-Abe algorithm to find contouring in binary images after that we counted the number of contourings considering them as a single bacteria. To enhance the understanding of the behaviour of microbial organisms community total number of bacteria vital parameters for image Analysis. The equation used for Adaptive Thresholding,

$$T(x, y) = \text{mean or Gaussian mean of } I_{\text{local}}(x, y) - C \tag{1}$$

$$I_{\text{segmented}}(x, y) = \begin{cases} 255 & \text{if } I(x, y) > T(x, y) \\ 0 & \text{otherwise} \end{cases} \tag{2}$$

where, $T(x, y)$ is the threshold applied to each pixel (x, y), calculated as the Gaussian-weighted or simple mean of the surrounding pixel values, $\mu(x, y)$, minus a constant C to fine-tune the segmentation.

Average Bacteria Volume: From the contouring, the Bacteria volume is calculated. It assumes that the bacterium is perfectly circulated. The most important reason to calculate this parameter is to Analyse and monitor growth patterns for individual bacteria as in microscopy images it is very hard to accurately differentiate the colony of bacteria. It will be crucial if the AI models are not able to identify it precisely.

Total Area: To calculate the Total Area, which refers to the shape analyzing feature we need three important properties of geometric which are Circularity (understanding the morphological behavior of bacteria cell), Eccentricity (how the bacteria is forming to compare its shape reformation from its original shape which will be very useful to understand Antimicrobial Resistance cell that where is the difference from normal cell) and Convexity (Analyse the physical features and health of bacteria cell).

Centralization Distance from Image Center: Distance of the center of the image to the bacterial centroid. Helps to understand the movement and clustering behaviour of bacteria. To understand the dynamics of colony formation of bacteria and how and using which process bacteria become resistant to antibiotics.

Average Aspect Ratio: This ratio represents the average of all detected bacteria in the image. During bacterial infections, some bacteria change their shape. To understand is stages of pathogenic bacterial infection Aspect Ratio is required.

Average Extent: Collecting data to take microscopic images some bacteria can be seen in irregular shapes due to stress or in the process of dying. To Analyse the behaviour of bacteria it is very necessary to understand which are important descriptors for Bacteria morphology.

Average Orientation: It helps to understand the interaction between bacterium, how it responses toward the environment it is surrounded by and most importantly analyse the development direction of bacterial tissue.

Local Binary Pattern (LBP): It is used to extract the important features from an image[18]. It generates accurate grouping result for different bacterial types in automatic image processing program.

Frame to Frame Area and Movement change: This Analysis play an major role to find out insight about antimicrobial cure. By this exploration of changes we can also monitor the bacterial growth stages according to AMR.

GLCM Features: GLCM is used for image analysis to understand the texture feature in the image and extract it. Contrast, Dissimilarity, Homogeneity, Energy, and ASM are the statistical measures that we extract from GLCM.
Contrast: It is defined as,

$$\text{Contrast} = \sum_{i,j=0}^{L-1} P(i,j) \times (i-j)^2 \tag{3}$$

where, $P(i,j)$ is the GLCM, L is the number of gray levels, and (i,j) represent pixel intensities.
Homogeneity and Energy: The Homogeneity is defined as,

$$\text{Homogeneity} = \sum_{i,j=0}^{levels-1} \frac{P(i,j)}{1+(i-j)^2} \tag{4}$$

where, $P(i,j)$ is the element at the i^{th} row and j^{th} column of the GLCM, and $levels$ represents the number of intensity levels in the image. The Energy is defined as,

$$\text{Energy} = \sum_{i,j=0}^{levels-1} P(i,j)^2 \tag{5}$$

To analyse bacterial phenotype behaviour, to understand structural patterns GLCM is important. Contrast is used for determining imbalances in bacterial cell walls, while Dissimilarity measures the differences in the pathological state of bacteria. Homogeneity helps cluster different types of bacteria. Energy focuses on regular or normal bacteria that do not exhibit any irregularities, particularly those influencing AMR, analyzing these can reveal which features protect the bacterial cell wall that antibiotics cannot breach. Finally, ASM is employed to study the density of bacterial colonies and identify areas where active bacteria growth is observed.

4 Methodology

We divided our methodology into two parts. In the first part, we generated a predicted mask using U-Net [19] and StarDist [20] from original bacterial images and ground truth masks. We have separated the test input original images and their ground truth masks into three divisions which contain continuous frames in specific time delays. In the training part, we used a complete training dataset containing both original and ground truth bacterial images. After training our

U-Net model and StarDist, we tested our model using three-division original image data. Here, U-Net and StarDist models generated predicted mask images for each of the original corresponding images. Then we determined the accuracy of the U-Net model and StarDist by comparing generated predicted masks and their actual masks. Figure 3 shows the flow chart of the first part, which is generating predicted masks using U-Net and StarDist.

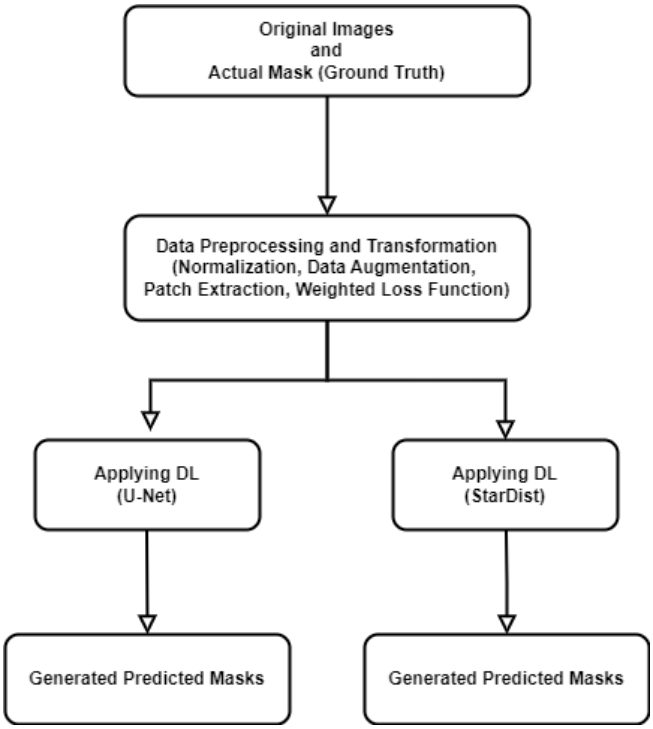

Fig. 3: Flow chart of the first part: generation of predicted masks using U-Net and StarDist.

In the second part of our work, we used all the generated predicted mask images and their corresponding actual mask images to determine bacterial behaviours and features. For each of the actual masks, we calculated all the mentioned bacterial behaviours. Also, for each of the generated predicted masks, we calculated all the mentioned bacterial behaviours.

Table 1: Detailed Workflow of Bacterial Behaviour Analysis, Segmentation, and Temporal Growth Dynamics.

Stage	**Description**
Input	Image Data: Series of grayscale images containing bacterial colonies.
Output	Bacterial Behaviour Metrics: Cell count, average volume, distance from image center, total area, texture features. Temporal Growth Metrics: Changes in area and centroid movements between consecutive frames.
Part 1: Image Processing and Segmentation	
Data Preprocessing and Transformation	Normalize images, apply data augmentation, extract image patches, and use weighted loss functions for training data.
Deep Learning (DL) Models	Utilize U-Net and StarDist architectures for segmenting bacterial colonies in the images.
Bacterial Segmentation	Generate predicted masks from the DL models.
Part 2: Bacterial Behaviour Analysis	
Feature Extraction	Detect contours, calculate cell count, volume, centroid distance, and shape descriptors (aspect ratio, solidity, extent, orientation). Apply LBP for texture and compute GLCM features (contrast, dissimilarity, homogeneity, energy, ASM).
Data Aggregation	Average the extracted features across all bacteria within an image to provide a summary statistic per frame.
Part 3: Temporal Growth Dynamics	
Frame-to-Frame Analysis	Calculate area change and centroid movements between frames to assess bacterial growth dynamics.
Visualization	Generate visual outputs to display original and processed images with highlighted bacterial contours and centroids.

Then we compared and checked similarities and differences in behaviours between outputs from actual masks and generated predicted masks. Manually creating actual masks from original bacterial images is both time-consuming and costly. Therefore, our comparison may justify using generated masks instead of actual masks for bacterial behaviours analysis. Figure 4 shows the flow chart of second part which is determining bacterial behaviours from actual masks and generated predicted masks. Table 1 shows the Detailed Workflow of Bacterial Behaviour Analysis, Segmentation, and Temporal Growth Dynamics.

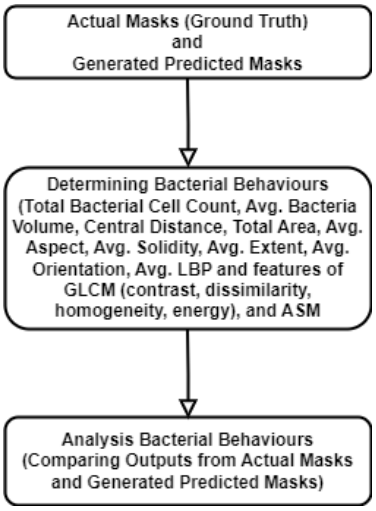

Fig. 4: Flow chart of second part: determining bacterial behaviours from actual masks and generated predicted masks.

5 Result and Analysis

In this section, we discussed the performance of U-Net and StarDist approaches in our work. Here Figure 6 shows some actual image and their corresponding ground truth images. Besides it also shows generated predicted masks using U-Net and generated predicted masks using StarDist.

Table 2 shows the performance metrics of DL models that is U-Net and StarDist in this case. For each of the divisions (D1, D2, D3, D4 and D5) the table shows Intersection over Union (IoU), Dice, Accuracy, Precision, recall, F1 Score, Matthews correlation coefficient (MCC), Sensitivity, Specificity, Area Under the Curve - Receiver Operating Characteristic (AUC-ROC), and Area Under the Curve - Precision-Recall (AUC-PR). According to Table 2, we can notice that U-Net consistently shows better performance in all metrics, especially in IoU, Dice, Accuracy, Recall, F1 Score, MCC, and AUC-ROC, While StarDist has better Specificity and high Precision. However, Stardist lower scores in IoU, Dice, Recall, F1 Score, and AUC measures indicate that it is less effective at segmenting compared to U-Net from our selected dataset.

Table 3 represents that StarDist perform better to calculate Central Distances, Avg. Aspect ratio, and Avg. Solidity than U-Net. U-Net outperforms better in Total Bacteria Count, Avg. Bacteria Volume, and Avg. Orientation. Even if StarDist does not generate good result for Total bacteria count like U-Net, it is good at spotting bacteria until it get strong similarities between Ground Truth and Predicted Masks. Other features generate almost similar result for both models. Here, average LBP is 0.10 for Ground Truth, U-Net and StarDist.

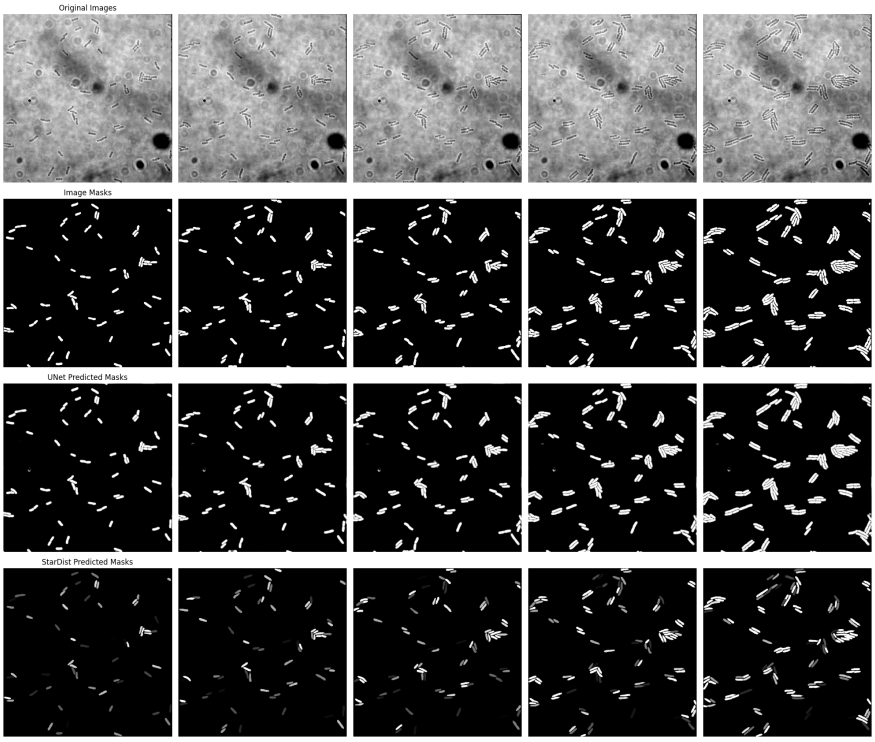

Fig. 5: Predicted Masks for U-net and Stardist

Table 2: Performance Metrics of DL Models

DL Model	**IoU**	**Dice**	**Accuracy**	**Precision**	**Recall**	**F1 Score**	**MCC**	**Sensitivity**	**Specificity**	**AUC-ROC**	**AUC-PR**
U-Net (D1)	0.85	0.92	0.99	0.87	0.98	0.92	0.92	0.98	0.99	0.99	0.92
U-Net (D2)	0.78	0.87	0.99	0.89	0.86	0.87	0.87	0.86	1.0	0.93	0.88
U-Net (D3)	0.84	0.91	0.99	0.88	0.95	0.91	0.91	0.95	0.99	0.97	0.92
StarDist (D1)	0.45	0.62	0.97	0.95	0.46	0.62	0.65	0.46	1.0	0.73	0.72
StarDist (D2)	0.29	0.43	0.98	0.94	0.29	0.43	0.51	0.29	1.0	0.64	0.63
StarDist (D3)	0.46	0.62	0.97	0.95	0.47	0.62	0.65	0.47	1.0	0.73	0.72
Avg. U-Net	0.82	0.90	0.99	0.88	0.93	0.90	0.90	0.93	0.99	0.96	0.91
Avg. StarDist	0.40	0.56	0.97	0.95	0.41	0.56	0.60	0.41	1.00	0.70	0.69

Table 3: Comparison of Bacterial Behaviour for Frames 1-5.

Type	Frame	Total Bacterial	Avg. Bacteria Volume	Central Distance	Total Area	Avg. Aspect	Avg. Solidity	Avg. Extent	Avg. Orientation
Ground Truth	1	43	1504.43	29.91	64690.50	0.63	0.92	0.90	46.31
	2	44	1810.74	47.41	79672.50	0.58	0.90	0.91	48.24
	3	40	2392.96	41.09	95718.50	0.56	0.88	0.88	54.88
	4	41	2876.85	47.95	117951.00	0.56	0.87	0.87	56.49
	5	39	3791.56	67.66	147871.00	0.49	0.84	nan	53.24
U-Net	1	48	1461.14	33.08	69711.00	0.43	0.74	0.90	57.00
	2	49	1706.69	25.41	83536.50	0.39	0.71	0.91	58.37
	3	49	1961.80	33.20	100314.50	0.38	0.72	0.89	59.20
	4	63	1787.39	111.43	116945.00	0.38	0.70	0.88	55.95
	5	97	1468.16	26.77	146129.50	0.38	0.72	0.88	58.97
StarDist	1	78	653.37	45.35	50962.50	0.55	0.85	0.91	41.93
	2	79	808.13	146.90	63842.50	0.51	0.83	0.92	48.97
	3	85	890.91	41.68	75727.00	0.49	0.82	0.90	52.76
	4	63	1600.79	52.03	100850.00	0.48	0.82	0.88	56.62
	5	63	1975.81	52.89	124476.00	0.46	0.84	0.88	53.38

Table 4: Comparison Area Change and Movement for U-net and Stardist.

Area Change and Movement	Ground Truth	U-Net	StarDist
Frame 1 to Frame 2	(14982.00, 253.17)	(13825.50, 317.08)	(12880.00, 439.70)
Frame 2 to Frame 3	(16046.00, 340.24)	(16778.00, 341.95)	(11884.50, 448.11)
Frame 3 to Frame 4	(22232.50, 244.30)	(16630.50, 343.72)	(25123.00, 336.89)
Frame 4 to Frame 5	(29920.00, 222.79)	(29184.50, 423.46)	(23626.00, 378.94)

According to Table 4, StarDist generated better result for movement between frames. On the other hand, U-Net shows better performance in Area Change between frames. Here, we used only U-Net and StarDist in this work. We selected U-Net because it excels at detailed localization for creating accurate masks. On the other hand, StarDist's shape-based optimization is ideal for distinguishing overlapping bacteria.

6 Conclusion

Studying bacterial behaviours from microscopic images using AI can save both time and effort. Also, medicine specialists could get valuable insights regarding bacterial behaviours from any datasets within a short time after using DL approaches, which eventually will allow them to develop new medicines to fight AMR. Finding to stop and delay AMR could save millions of lives worldwide. This research work focused to identify bacterial behaviours from microscopic images from both actual mask images and predicted mask images after using U-Net and StarDist. We identified some of the vital bacterial behaviours such as average bacteria volume, central distance from the image center, total area, average aspect, average solidity, average extent, average orientation, and LBP. Also, we determined other features of GLCM such as contrast, dissimilarity, homogeneity, energy, and ASM for each of the actual mask images and predicted mask images. This analysis may provide valuable insight to researchers to identify patterns and ways of experimenting with new antibiotics. Also, we also analysed bacterial area changes and movement from one frame to another considering certain periods. This may help medicine specialists to identify the optimal point for administering antibiotics. Moreover, we provided comparisons between U-Net and StarDist, focusing on which approach performs better for specific behavior identification.

In our future work, we aim to increase dataset and data variability i.e. we will work with other types of microorganisms. Also, we aim to use other approaches such as V-Net, SegNet, and LinkNet and demonstrate a large comparison for more precise recommendations. Finally, we will increase the number of way to study bacterial behaviours and analyse how these behaviours contribute towards Antimicrobial Resistance (AMR).

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