# Automated Procedure for the Microbial Analysis of Contact Plates using Deep Learning

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## Introduction

The counting of bacteria colony-forming units (CFUs) is a common way of auditing the microbiological life present in places such as microbiology labs, clean rooms or other clinically related environments. The automatic counting of CFUs on contact plates has been investigated in the past relying on computer visions methods. For example, studies by Hogekamp et. al utilise Local Darkest Spot Maxima [1], while Alves and Cruvinel use Hough Transform to identify the circular-shaped CFUs on the plate [2]. However, these studies, as well as many others, are calibrated for homogeneous bacteria colonies, which differs from the heterogeneous CFU formation found in most clinical contact plates.

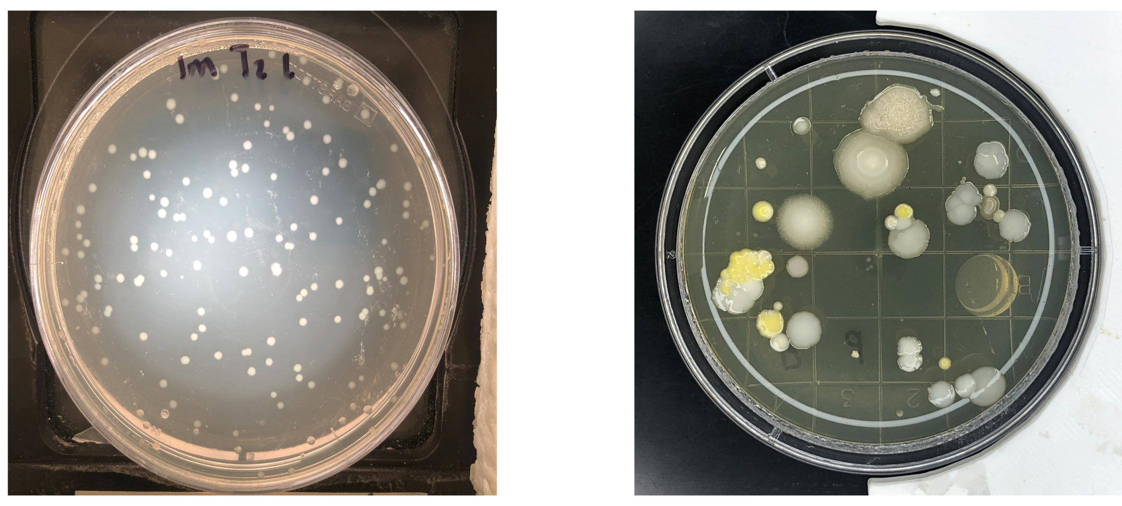

Fig. 1: Homogeneous colony formation (left) and heterogeneous colony formation (right)

**Aim of the project:** Investigate the use of deep learning for automatically counting heterogeneous colonies on a contact plate

## Methodology

**Dataset:**

This study started with an image set of 145 high-quality images of contact plates. These images were pre-processed using Python and the OpenCV python library [3] to allow for faster processing during deep learning training. The image set was duplicated to create two datasets; "CFU Dataset," which containted locations/labels of individual bacteria colonies for each plate, and "Cluster Dataset," which contained locations/labels of bacteria clusters. These datasets were used to create deep-learning models which can be implemented as an object detector and classifier. All labelling was done manually using LabelImg [4].

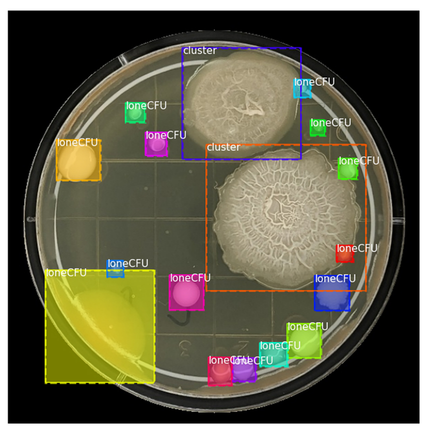

Fig. 2: Example of image with corresponding object labels

**Object Detection and Classification Training:**

Deep learning (DL) allows computers to learn from experience to understand the world around it. Two separate DL models were trained using Mask R-CNN. Mask R-CNN provides easy training and implementation for object detection and classification [5]. Each dataset was trained for their respective objects; "individual CFUs (71 images)" and "colony clusters (47 images)," which created models with 90% and 67% accuracy respectively.

**Final Counter System:**

Using the DL Models, Mask R-CNN was used to create CFU count predictions on new images. If an "individual CFU" was detected, the prediction increases by 1. If a "cluster" is detected, a prediction of the amount of CFUs in the cluster was made using watershed segmentation [6] and added to the final count if it was equal to two or more.

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## Results and Discussion

**Results:**

The root-mean squared error (RMSE) calculates the error between actual amounts and predicted amounts. The RMSE of the counter system was calculated across three sets of sample images; "Minimal Clustering," "Heavy Clustering," and "Empty Plates." Fig. 3 shows the equation for RMSE and Fig. 4 highlights what the counter system detects in an image.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(Predicted_i - Actual_i)^2}{N}}$$

Fig. 3: Equation for RMSE

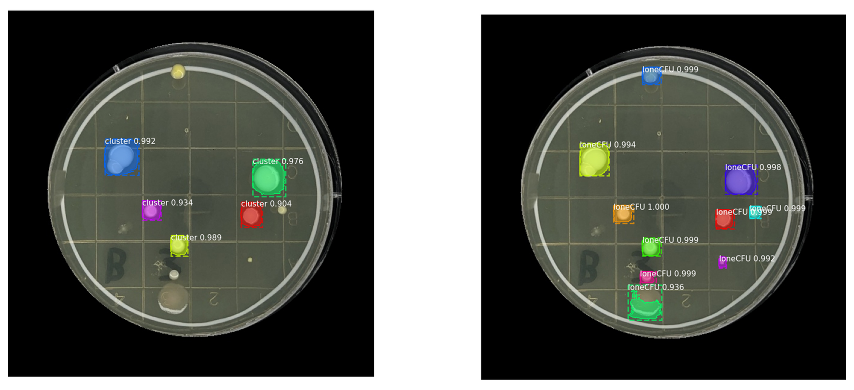

Fig. 4: "Individual CFU" and "Colony Cluster" detection

The RMSE of the counter system was compared to OpenCFU [7], a common software package for CFU counting and another counter based on deep-learning by Albaradei et. al [8]. Additionally, the frequency of over-counting and under-counting was also recorded across the "Minimal Clustering" and "Heavy Clustering" image sets.

| | Counter System | OpenCFU | Albaradei |
|---|---|---|---|
| Minimal Clustering | **5.98** | 9.24 | 25.37 |
| Heavy Clustering | **95.47** | 100.92 | 103.50 |
| Empty Plates | 7.16 | **4.19** | 7.16 |

Fig. 5: Comparison of RMSE between sample image sets (bold represents best results)

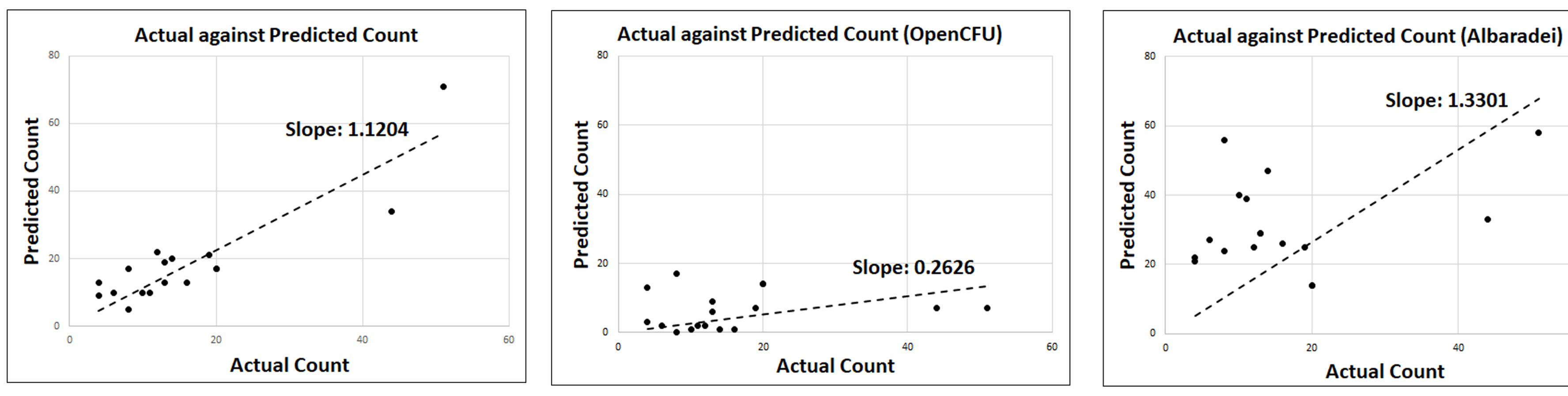


Fig. 6: Actual amount of CFUs on contact plates plotted against predicted amount of CFUs for corresponding plates. Slope values greater than 1.0 indicate "over-counting," while values less than 1.0 indicate "under-counting"

**Discussion:**

As seen from the results, the counter system created for this project had the best performance regarding plates with confirmed bacteria growth present. It produces the lowest RMSE compared to current software packages, with a more diminished over-count compared to the alternative deep learning model. It should be noted that tuning for OpenCFU was not performed prior to testing, which may have improved its accuracy.

There were instances where the counter system detected a CFU as both an "individual CFU" and "colony cluster." For a majority of these instances, having a "two or more" threshold on the watershed segmentation algorithm reduced the likelihood of further over-counting. Additionally, the watershed algorithm breaks down when light defects are present on the plate as the system detects them as "clusters." This causes severe over-counting particularly in the "Empty Plates" image set. The system also loses accuracy when presented with an image with very different lighting conditions compared to the lighting in the training dataset.

## Conclusion

As a result of this project, a colony counter has been created using deep-learning and object detection, and provides promising results regarding the automated counting of heterogeneous bacteria colonies. It shows that even a small dataset can generate relatively accurate models which can be used in a practical setting. If the project was to be continued, future work will involve creating a larger dataset of contact plate images, which will improve the accuracy of all object detection models used in the system. Additionally, further developments will also include creating a user-interface that is easily deployable, intuitive, open-source, and will allow users to upload their own labeled images for more accurate predictions.

## References

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