Bacterial colony counting could be rapid, adaptive and automated

Minghua Zheng[1][*], Na Helian[1], Peter Lane[1], Yi Sun[1] and Allen Donald[2]

[1]University of Hertfordshire
[2]Synoptics Ltd
corresponding author: m.zheng2@herts.ac.uk

Although many attempts have been made to automate bacterial colony counting, little has tackled the counting of clustered colonies and adaptations to handle different bacteria species. In this work, we explore the counting by density estimation method via few-shot learning. We have avoided the difficult localisation and detection of clustered colonies by estimating a density map from the input image. We have also exploited exemplars provided by users to make the method agnostic and adaptive to different bacteria species. Our experiments show that using the counting by density estimation method via few-shot learning results in a promising accuracy from the data set provided by Synoptics Ltd.

Keywords: Bacterial colony counting, automation, clustered colonies, density estimation, few-shot learning

Introduction

Counting the number of colonies on an agar plate is a widely used technique in both industry and research laboratories to estimate the number of viable bacteria (total viable count) present in a test sample in a wide range of applications; these include quality control, environmental monitoring, immunological studies and medical testing. The number of colonies can be used as an important indicator of the cleanliness of a surface, the sterility of a product or the present of a bacterial infection.

Traditionally, bacterial colony counting is performed manually, which is time-consuming and prone to human error [1]. Many attempts have been made to automate it with traditional image processing methods or machine learning algorithms. But they are designed specifically for certain bacteria species without identifying and counting clustered colonies where a group of colonies are close together, touching and overlapping. This research aims to address this research gap. We investigate the impact of counting by density estimation via few-shot learning on clustered colonies and its adaptations to handle different bacteria species. The difficult localisation and detection of clustered colonies are avoided by estimating a density map where the sum of values indicates the colony count. The counting algorithm only learns from three exemplars (examples of the target colony) provided by users in the few-shot learning fashion so that it is adaptable to different bacteria species.

Methodology

FamNet [2] is used in this work due to its outstanding ability to count many different types of object. As shown in Figure 1, users are required to provide three exemplars by drawing three bounding boxes in the input plate image. These three exemplars are the support set. The plate image is the actual query image. FamNet consists of a multi-scale feature extraction module and density map estimation module.

The first module is ResNet-50 [3] whose parameters are frozen during both training and inference phase so that it only acts as a feature extractor. It takes the query image as input and returns two feature maps from the third block and fourth block in ResNet-50 respectively. Features inside the region of interest (ROI), which is the exemplar specified by the bounding box, in the feature map are used to convolve the two feature maps to form two new feature maps which are later concatenated into a new feature map. The former is called ROI pooling and the latter is called feature correlation. To recognise similar colonies at different scales, the ROI pooling and feature correlation are repeated with the three exemplars rescaled at 0.9 and 1.2. The two new resulting feature maps are concatenated with the previous feature map to form a final feature map.

The density map estimation module is a regression model that uses the correlated feature map to predict a density map. It consists of five convolutional layers where the first, second and third convolutional layer is followed by an upsampling layer. The fifth convolutional layer is convolved by 1 x 1 kernel to predict the density

map whose shape is the same as that of the input image. The kernel parameters for the five convolutional layers are 7 × 7 × 196, 5 × 5 × 128, 3 ×3 ×64, 1 × 1 × 32 and 1 × 1 × 1 respectively. The Rectified Linear Unit (ReLU) is used as activation function in each convolutional layer.

The data set used in this work is a collection of 128 plate images provided by Synoptics Ltd. The resolution of each plate image is 3 x 1040 x 1040. The ground truth annotations contain density maps and coordinates of the three bounding boxes. Exemplars specified by the bounding box have a different colony species based on different images in the data set. The whole data set is divided into training set, validation set and test set with a ratio of 6:2:2. The training method is the same as used in [2]. The optimiser is Adam. The learning rate is 10^{-5}. Batch size is 1. Epoch number is 1500. The loss function is mean square error. However, the adaption loss used in [2] is not included in this work because it has been proven ineffective based on our experiments. The results are measured by mean absolute error (MAE) and root mean square error (RMSE).
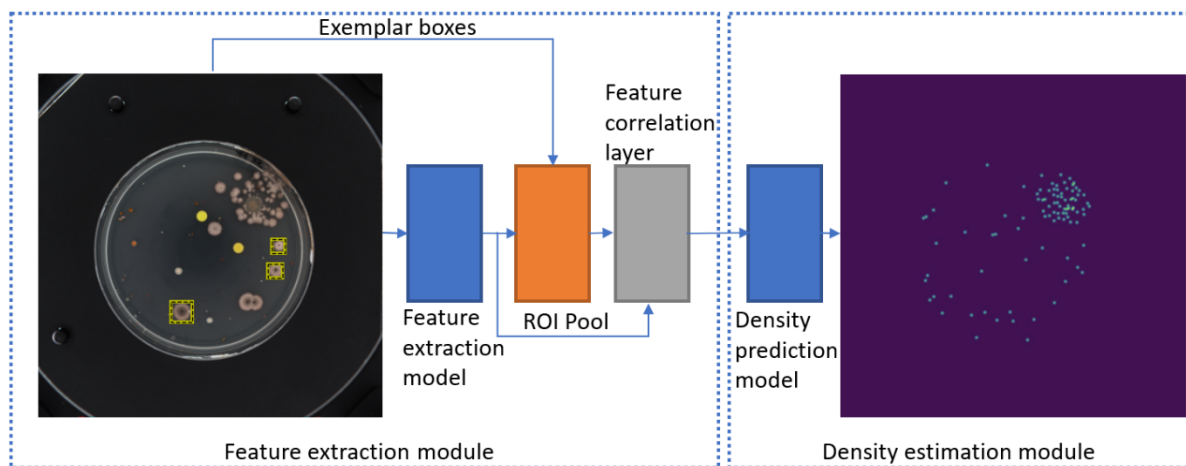

Figure 1: FamNet takes the query image (plate image) as input along with three bounding boxes representing the target colony and predicts a density map. The support set is the three exemplars provided by the bounding boxes. The colony count is obtained by summing all density (pixel) values in the predicted density map.
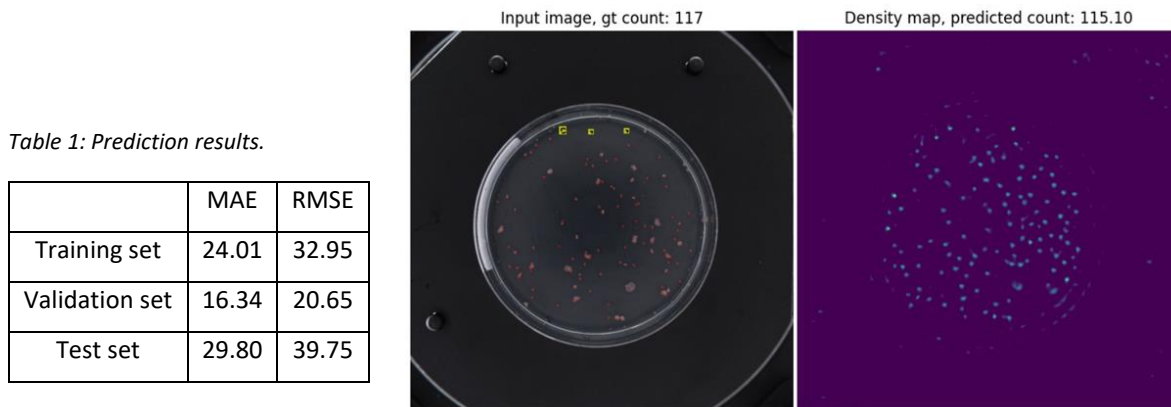

Table 1: Prediction results.

	MAE	RMSE
Training set	24.01	32.95
Validation set	16.34	20.65
Test set	29.80	39.75

Figure 2: Predicted density map.

Results and discussion

As shown in Table 1, FamNet achieves 29.80 in MAE and 39.75 in RMSE on the test set. It has not shown the sign of overfitting because the results from the test set are close to the results from the training set despite that FamNet performs better on the validation set. FamNet can predict 115.1 colonies from a plate image that has 117 colonies as shown in Figure 2. FamNet is also able to handle different colony species because it can learn from the three exemplars. The predicted density map also contains spatial information of colonies. The time used to make a single prediction is less than a second because images are batched and the computation is shared.

Conclusion

We have investigated the impact of density estimation via few-shot learning on the clustered colonies and its adaptations to handle different colony species. The FamNet used in this work achieves 29.80 in MAE and 39.75 in RMSE on the test set. Our experimental results show this method is adaptive to different colony species because of the user-specified exemplars. In a real-world application, these three exemplars can be stored in the system and reused for other data to ensure a high level of automation. The experimental results also show the prediction is very rapid because plate images are batched and the computation is shared. However, there are still rooms to improve the prediction results by fine tuning the model or modifying the design of two modules in FamNet. Future work also concerns a deeper analysis of the counting error and FamNet.

Reference list

[1] Brugger, S. D. et al. (2012). Automated Counting of Bacterial Colony Forming Units on Agar Plates, *PLoS ONE 7(3)*, e33695.
[2] He, K., Zhang, X., Ren, S., and Sun, J. (2015). Deep Residual Learning for Image Recognition. *2016 IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, 2016-Decem, 770–778.
[3] Ranjan, V. et al. (2021). Learning To Count Everything. *2021 IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR)*, 3393.