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Autolabel : Improving Petri Dish Automatic Labels with AI Algorithms

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Summary: The present research aims at improving the accuracy of labels on Petri dish images containing Colony Forming Units using Artificial Intelligence algorithms. Indeed, the labeling methods proposed by classical computer vision software such as ScanStation for example, are prone to errors and the manual correction of these errors is a difficult task. We propose a methodology based on AI models. At first, a YOLO model is trained on the existing labels given by ScanStation. The bounding boxes provided by ScanStation and YOLO are then binarized using the OTSU algorithm to generate semantic labels that are used to train a U-Net. Then, a Xception model is trained to classify all the segments generated by the U-Net as either outlier or colony. For new data, the trained U-Net and Xception models are used to improve the labeling. The results indicate that the proposed approach improves the accuracy of the labeling process without human correction.

Keywords: Deep Learning, Convolutional Neural Network, Yolo, U-Net, Xception, Colony Forming Units, Petri Dish labeling

1. Introduction

Microbial contamination is a pervasive phenomenon that poses a significant threat to human health worldwide. Microorganisms, including bacteria, viruses, fungi and parasites, can cause a wide range of infections, diseases and even death. According to the World Health Organization (WHO) [1], approximately 10% of the world's population, or about 600 million people, become ill each year from eating contaminated food, resulting in 420,000 deaths and 33 million lost years of healthy life.

The best way to prevent a microbial infection is to test samples for the presence of bacteria and other harmful microorganisms. However, their small size does not make them directly visible in a sample, and expensive equipment is often required to accurately detect and quantify these microorganisms.

Petri dishes provide a cheap and simple alternative to this problem. When a sample is placed on a Petri dish containing a nutrient-rich medium, each bacterial cell will begin to grow and divide, forming a colony or 'Colony Forming Unit' (CFU) as shown in Figure 1. A CFU is essentially a visible cluster of identical bacteria that have emerged from a single bacterial cell in the original sample. By counting the number of CFUs on a Petri dish, we can estimate the number of bacteria present in the original sample.



Fig. 1. Growth of a CFU at h+0, h+6, h+12, h+18 and h+24 after incubation (from left to right)

The number of CFU is usually between 30 and 300 in a single Petri dish, usually 90 mm in diameter (Figure 2). Counting CFU's in Petri dishes is an essential process for various industries, especially food but also cosmetics and pharmaceuticals. Accurate CFU counting helps determine the safety and quality of these industrial products for consumption or use, making it a crucial step in the manufacturing process.



Fig. 2. Image of a Petri dish 24 hours later incubation

To evaluate automatic counting software, or to develop a supervised detection model, it is therefore essential to accurately label the images of Petri dishes containing CFUs, i.e., to mark with bounding boxes or masks all CFUs present in the dish.

The traditional labeling method involves manual counting. However, counting CFUs is a time-consuming and laborious process that requires skilled technicians with a keen eye for detail. In addition, visual interpretation can be subjective, as evidenced by the non-reproducibility of accurate labeling between two microbiologists, especially on dishes with many CFUs or clusters. Furthermore, the counting process is affected by various factors such as lighting conditions, colony shape and size, and observer experience. This can lead to variability and errors in labeling, affecting the accuracy and reliability of the analysis.

To overcome this challenge, artificial intelligence (AI) algorithms have been explored in recent years [2, 3]. However, these algorithms require a large, properly labeled dataset. In this study, we propose an AI-based approach called 'Autolabel' to improve the accuracy of labeling images of Petri dishes containing CFUs. Specifically, we use a combination of the YOLO [4] (You Only Look Once), a object detection algorithm that uses a single neural network to simultaneously predict bounding boxes, U-Net [5], a convolutional neural network architecture commonly used for semantic segmentation tasks, and Xception [6], an advanced neural network architecture designed for

efficient and accurate image classification, to generate more accurate and reliable labels.

The methodology of our approach, which consists of three major components: learning strategy (2.1), inference (2.2), and training and test datasets (2.3), is described in detail in Section 2 of this paper. The results of our study are presented in Section 3, while a conclusion on the effectiveness of our approach is drawn in Section 4. Finally, the perspectives of this research as well as possible improvement are discussed in Section 5.

2. Methodology

2.1. Learning strategy

CFUs labels that are given by professional tools such as ScanStation (an incubator manufactured by Interscience equipped with a computer vision program that analyzes the box throughout its incubation process, allowing to identify growing CFUs), are not perfect and contains some errors, mainly on the edge of the box or in difficult cases such as a high density of CFUs, clusters, low contrasted CFUs or abnormal shaped CFUs. To solve this problem, we proposed the following process, as describe in the diagram in figure 3.

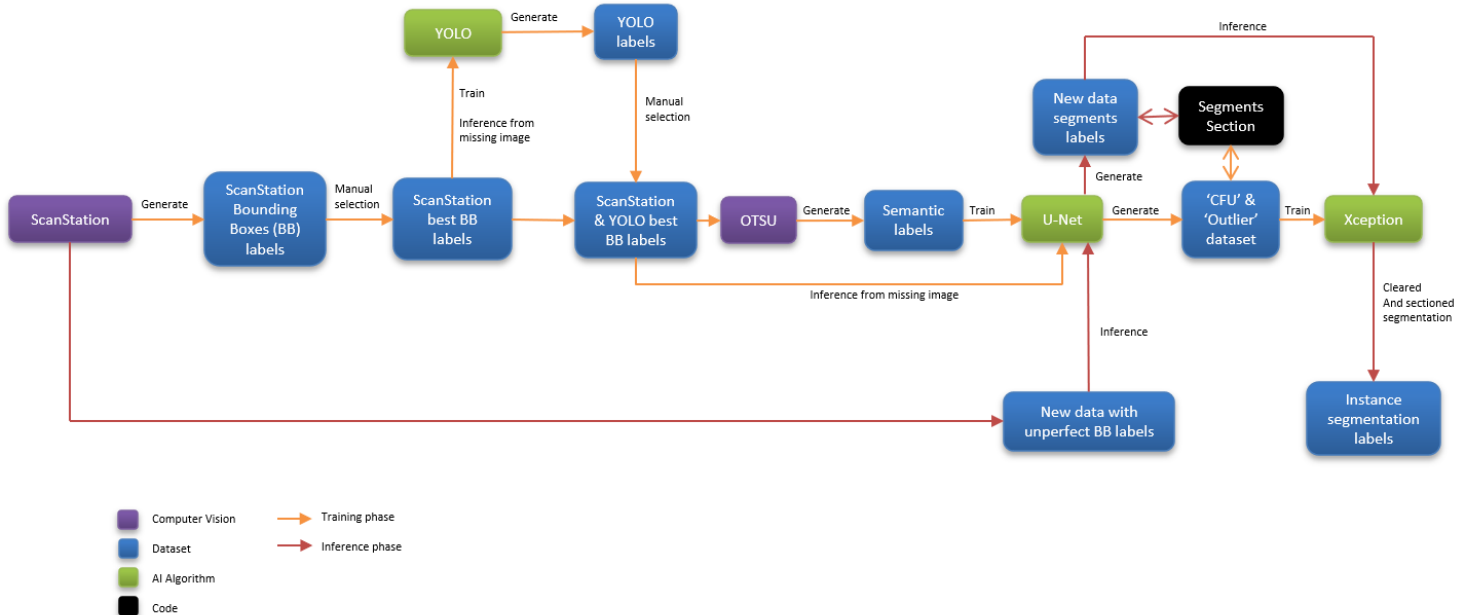


Fig 3. Diagram representing the Autolabel process

The purple boxes encompass Computer Vision algorithms, the green boxes signify AI algorithms, the black box represents a basic program, and the blue boxes indicate datasets or labels. The orange pathway symbolizes the training phase, or learning strategy, while the red pathway corresponds to the inference process.

- 1) The deep learning algorithm YOLO was used to relabel the Petri dish images that ScanStation failed to label well. By manually selecting the best Petri dish images (labeled by ScanStation) to train YOLO, and then using the trained model to label the remaining Petri dish images (Figure 4), a large number of bounding boxes were generated.



Fig 4. CFUs detection by YOLO

- 2) In order to obtain semantic labels, the OTSU thresholding method was used to binarize the content of the bounding boxes from ScanStation and YOLO. The bounding boxes and OTSU semantic results are shown in Figure 5.

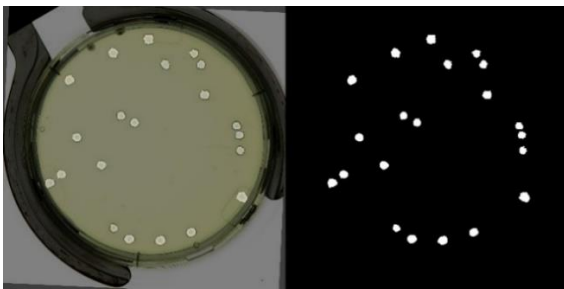


Fig 5. CFUs segmentation with OTSU

- 3) These binarized bounding boxes combinations are then used as semantic labels to train a U-Net model, a convolutional neural network used for image segmentation.

The primary purpose of employing U-Net lies in two aspects. Firstly, it aims to reduce the possibility of undetected CFU by generate segments that may not contain bounding boxes.

Secondly, it seeks to establish semantic labels for all CFUs. This form of labeling can be utilized to train an instance segmentation model like Mask-RCNN [7], facilitating more accurate and comprehensive CFU detection. The output segments of the U-Net model can be seen in Figure 6.

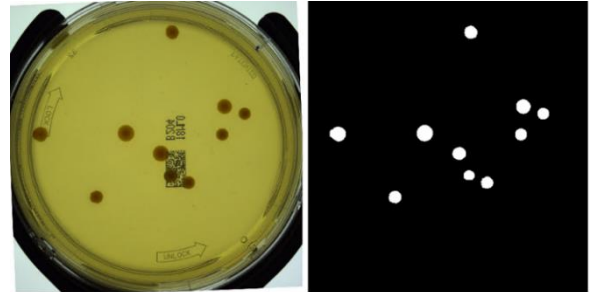


Fig 6. Output of U-Net

- 4) Several outputs of the U-Net are then classified as 'UFC' or 'outlier'. For this purpose, a model based on Xception, commonly used for image classification was trained with two datasets (one per class). For the 'UFC' class, we used the segments given by U-Net which were identified inside a bounding box given by ScanStation and YOLO. For the 'Outlier' class, we took the segments given by U-Net from boxes without CFU (before colony growth but with all artifacts like bubbles, annotations, impurities, etc.).

If a segment contains multiple bounding boxes, meaning it's a cluster, we separated the segment according to the distance from the center to the bounding box limit. The results of this segment sectioning can be seen in Figure 7, and an example of segments removed by Xception (bubble) is shown in Figure 8.



Fig 7. Section of two agglomerate segments



Fig 8. Classification of an outlier (bubble)

2.2. Inference

Once learning is complete, inference is performed by injecting the new data into the U-Net model for segmentation followed by Xception for classification. As a consequence, the imperfect labels of bounding boxes from ScanStation are transformed into instance segmentation, incorporating certain missed CFUs while eliminating identified outliers that could have been detected. Figure 9 shows the results of our improved Autolabel labeling algorithm. Individual colonies are identified by different shades of purple.

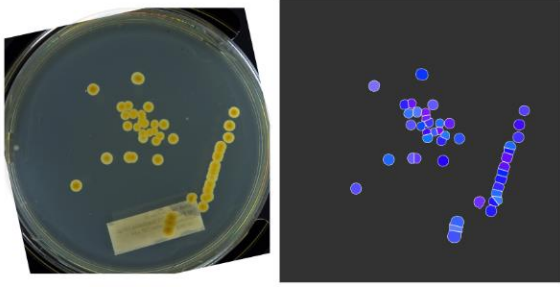


Fig 9. Result of our method

To compare the results with the original method, we transformed the individual segments into bounding boxes.

2.3. Training and testing datasets

In the training phase, we used a dataset consisting of 11,900 annotated Petri dish image sequences acquired from ScanStation. Each sequence includes several images illustrating the incubation and growth stages of CFUs. We selected a total of 989 Petri dishes based on the best visually apparent ScanStation results on the last image of the sequence. For each selected Petri dish, we extracted images containing CFUs at 2-hour intervals, resulting in a dataset of 11760 images for training the YOLO model. The U-Net dataset contained 7584 images, each from the last image of the sequence and selected based on the best OTSU binarization result from the bounding boxes generated by YOLO and Scanstation.

For training the Xception classifier, we provided an equal number of segments from both classes (13,027). We performed the test on a separate set of 166 images captured by a system from the same manufacturer as the training data. The manually corrected results from ScanStation were used as ground truth.

It should be noted that, unlike the training dataset which contained only the best visual results from ScanStation, the test dataset used to evaluate our method contained mostly images of Petri dishes with difficult counting tasks, clumps, and/or high CFU numbers.

3. Results

The classical measure for evaluating object detection algorithms is the Intersection over Union (IoU) measure. However, given the non-reproducibility of our data, the delimitation of bounding boxes is very subjective, which can lead to low IoUs, even for close detections. We therefore propose to adopt a metric based on a distance criterion (between the predicted center and the ground truth center of the colony) and on a shape criterion (average radius of the predicted colonies and the ground truth colonies). This new metric called 'Distance Normalized by Mean Radius' (DNMR) is defined as follows (eq. 1):

$$DNMR = 1 - \frac{dist(C_{GT}, C_{Pred})}{avg(Rad_{GT}, Rad_{Pred})} \quad (1)$$

where GT is the ground truth, $Pred$ is the prediction either by ScanStation or our method, C is a center in a 2D spatial position, and Rad is the radius.

With this metric, we decided to apply a threshold of 0.5 to define if a colony is detected or not compared to the ground truth. As CFUs have a low degree of overlapping the threshold was set to 0.5, we ensured that any score beyond this value would provide a reliable indication of successful detection. This allows us to evaluate our method using the classical criteria: Precision, Recall and F1 score.

Table 1 shows the precision, recall and F1 score obtained by ScanStation and our labeling method Autolabel on the Petri dish images of the inference base.

Table 1. Precision, Recall and F1 Score comparison

Label	Precision	Recall	F1 Score
ScanStation	0.622	0.590	0.606
Autolabel	0.808	0.683	0.740

The results clearly show that the Autolabel method performs better than a conventional ScanStation-type method in terms of precision, recall and F1 score. However, we found that some CFUs are still not detected, especially very small ones, due to the U-Net resolution limit imposed by the memory size of the GPU we used (RTX 2080Ti, 11gb VRAM).

4. Conclusion

Our research propose a novel approach to train machine learning models without using manually labeled images in the training phase. Instead, we relied on imperfect labels generated by an automated process (ScanStation). Despite this unconventional approach, our results demonstrated significant improvements in precision (+18%), recall (+9%), and F1 score (+14%). It should be noted that the testing dataset utilized a different labeling approach, a manual one, which

posed some challenges in replicating the same type of bounding boxes. To address this issue, we employed the 'Distance Normalized by Mean Radius' metric.

Overall, this study highlights the potential of using AI algorithms to improve detection accuracy before a manual correction phase, which is typically a time-consuming and labor-intensive task. Our approach leverages the power of AI generalization to improve the labeling accuracy of Petri dish images, using multiple cascaded algorithms, resulting in improved detection of CFUs. Although the results of our study demonstrate a significant improvement in the accuracy of Petri dish labeling, it is important to note that there is still tremendous potential for improvement.

Furthermore, our approach has the potential to be applied beyond CFUs detection and used to test large datasets of interpretative labels, which can help highlight errors made by both human and automatic labeling processes. This can be particularly useful in scenarios where manual labeling is not feasible due to resource constraints or time limitations. By utilizing machine learning to identify errors in interpretative labels, we can improve the accuracy and reliability of data-driven applications in various domains.

5. Perspectives

In future research, we plan to apply the proposed approach to improve the label accuracy of a larger dataset of Petri dish images. We aim to use the generated labels to train a detection model such as YOLO or instance segmentation model like Mask R-CNN. Our ultimate goal is to develop a robust and accurate detection model that can be used for a variety of applications, including bacterial colony detection and classification.

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