Semi-Automatic Labeling and Semantic Segmentation of Gram-Stained Microscopic Images from DIBaS Dataset

Chethan Reddy G.P.  
Dept. of ECE  
NITK, Surathkal  
Mangalore, Karnataka, India  
chethanreddy.201ec216@nitk.edu.in

Pullagurla Abhijith Reddy  
Dept. of ECE  
NITK, Surathkal  
Mangalore, Karnataka, India  
pullagurlaabhijithreddy.201ec148@nitk.edu.in

Vidyashree R Kanabur  
Dept. of ECE  
NITK, Surathkal  
Mangalore, Karnataka, India  
vidyashreerk1992@gmail.com

Deepu Vijayasenan  
Dept. of ECE  
NITK, Surathkal  
Mangalore, Karnataka, India  
deepu.senan@gmail.com

Sumam David S.  
Dept. of ECE  
NITK, Surathkal  
Mangalore, Karnataka, India  
sumam@ieee.org

Sreejith Govindan  
Dept. of Basic Medical Sciences  
MAHE, Manipal  
Manipal, Karnataka, India  
g.sreejith@manipal.edu

Abstract—In this paper, a semi-automatic annotation of bacteria genera and species from DIBaS dataset is implemented using clustering and thresholding algorithms. A Deep learning model is trained to achieve the semantic segmentation and classification of the bacteria species. Pixel-level classification accuracy of 95 percent is achieved. Deep learning models find tremendous applications in biomedical image processing. Automatic segmentation of bacteria from gram-stained microscopic images is essential to diagnose respiratory and urinary tract infections, detect cancer, etc. Deep learning will aid the biologists to get reliable results in less time. Additionally, a lot of human intervention can be reduced. This work can be helpful to detect bacteria from urinary smear images, sputum smear images, etc to diagnose urinary tract infections, tuberculosis, pneumonia, etc.

Index Terms—semantic segmentation, annotation, clustering, thresholding

I. INTRODUCTION

Bacteria are tiny living microorganisms that are found everywhere. Most of the bacteria are beneficial, and very few are harmful. Bacteria are essential to break down the food into nutrients, boost the immune system, etc. Harmful bacteria may cause diabetes, pneumonia, cancer, respiratory and urinary infections, etc. Detection and classification of bacteria based on their genera and species is essential to diagnose these infections and diseases.

Classification of bacteria into various genera and species can be done based on their shape, stain-type, arrangement and size. Manual examination of the bacteria is time-consuming, labor-intensive and costly. Automation of this process will help biologists to get accurate results within a short time. Recently, a lot of work has been happening in biomedical image processing using computer-aided tools and algorithms. Deep learning algorithms are turning out to be a boon in this field of study.

Many researchers in the past have tried to perform classification of bacteria from microscopic images. A lot of data is necessary to perform classification of biomedical images. Abdulla et al. [1] proposed an algorithm to extract color features from Candida, Gram-Negative Bacilli (GNB) and Gram-Positive Cocci (GPC) bacteria appearing in urine smear images for automatic classification of these objects. They used 60 sample images of 100x resolution (20 each from three bacteria types). Andreini et al. [2] created synthetic images from blood agar to classify bacteria. Panicker et al. [3] performed classification of tuberculosis bacteria from sputum smear images.

Zielinski et al. [4] created the a Digital Image of Bacterial Species (DIBaS) dataset, to perform bacterial colony classification. It consists of 20 images of bacteria belonging to 33 genera and species, hence total of 660 images. The authors have made it available for public use and many researchers are using it to perform automatic classification of bacteria from microscopic images. [5], [6] classified bacteria into different shapes, species, etc using this dataset.

Recently, [7]–[10] have used ResNet models to detect bacteria. Costa et al. [11] proposed CNN based deep learning model to segment and identify bacteria type. Dataset was taken from UFAM Pattern Recognition and Optimization Research Group. CNN model was trained using mosaic images and manually annotated labels with a split ratio of 50:25:25. CNN model with Adam optimizer performed best and an F1 score of 98.54% and accuracy of 98.53% was recorded.

As seen from the literature, many researchers have proposed CNN models to perform the classification and detection of bacteria but semantic segmentation of the bacteria is not attempted. Semantic segmentation of bacterial images will aid the biologists to determine the morphological features.
such as shape, colour, stain-type and arrangements of bacteria. However, there is no annotated dataset of bacteria genera and species which is available for open access. Therefore, in this work, we have annotated images from the DIBaS dataset and used it to perform semantic segmentation of the bacteria. This dataset can be used to perform detection and classification of bacteria from microscopic images to diagnose urinary tract infections, tuberculosis, pneumonia, etc.

Standard algorithms which are popularly used for semi-automatic image segmentation are discussed in the next section. After that ResUNet++ architecture for semantic segmentation of microscopic images is explained in section III. The training procedure and the results are illustrated in section IV. Conclusion is drawn in section V.

II. SEMI-AUTOMATIC LABELLING

Deep learning models need large datasets and their corresponding annotated labels for training. Annotations have to be carried out by experienced biologists. Segmentation of bacteria at pixel level will help the biologists to get deep understanding of their arrangement, shape and stain-type. Also, this will be helpful to recognize the bacteria genera and species. Fig 1 shows the flow chart of the annotation process. k-means clustering and Otsu thresholding algorithms are employed to separate the bacteria from the background of each image. Morphological operation is performed on the resulting images to remove staining and noise artifacts. Detailed discussion of the three operations is provided in the sections to follow.

A. k-means clustering

k-means clustering is an unsupervised segmentation algorithm to segment the dataset into k clusters. It tries to group data points with similar properties into a single cluster. Centroids with the least squared distance between the data points are computed. The data points are assigned to the closest centroid, and data points assigned to the same centroid are treated as a single cluster. For an RGB image, the k-means clustering algorithm is used with RGB pixel values as data points, and the resulting labels of each pixel can be used for image segmentation.

Due to staining, bacteria have a significantly different color than the background. Stain is applied uniformly for all the bacteria, which makes the color of bacteria uniform throughout the image. The fact that bacteria color is distinct and uniform can be exploited because pixels of bacteria form a cluster in a 3D RGB space. The k-means clustering algorithm can label the cluster to segment bacteria in the images. Fig. 2 shows an example of performing k-means clustering on a microscopic image.

B. Otsu thresholding

Otsu algorithm is widely used to perform image segmentation. It generates a threshold value based on a weighted variance of the foreground and background classes, and this threshold helps distinguish the foreground pixels from the background class.

The k-means algorithm works when bacteria have a uniform color in an image, but it is not the case for the pictures of vionella bacteria in the DIBaS dataset. The color of the stain varies significantly throughout the image; hence otsu thresholding is used to segment the bacteria from the image. Fig. 3 shows an example of applying otsu thresholding to image on left to get its segmented mask as shown on right.

C. Morphological operations

Morphological closing can be formed by two basic mathematical operations, namely, dilation and erosion. The noise artifacts in the bacterial images get stains same as that of the bacteria; hence, the stain artifacts get clustered with bacteria.
in the k-means algorithm. But they are significantly smaller when compared to bacteria, so morphological closing can be used to remove the stain artifacts. This is illustrated in Fig. 4. The image on the left has some small patches of noisy regions. When a morphological closing operation is applied on this image using circular kernel, these artifacts are reduced as seen in the right image.

## III. SEMANTIC SEGMENTATION OF BACTERIAL IMAGES USING RESUNET++

Automatic segmentation of bacteria is quick, free of human intervention and also it gives accurate results. For instance, automatic detection of bacteria and pus cells from gram-stained urine smear images will aid the microbiologists in the diagnosis of urinary tract infection. Urine culture test is performed to diagnose urinary tract infections and also grade its intensity by noting the number of bacteria and pus cells present in the smear images. Urine culture needs atleast 48 hours to give the results and most of the time bacteria and pus cells will not be available in the culture and the patient has to wait unnecessarily for 48 hours to get the results. Microscopic examination can be performed prior to culture to check if there are bacteria and pus cells present in the smear images and this entire process can be automated using machine learning and deep learning approaches. Urine culture can be carried out only when bacteria and pus cells are present in urine smear images. This will reduce the time, cost as well as the labour involved in culture examination and testing. Also the results are available in less time. Therefore, we perform automatic semantic segmentation of bacteria from DIBaS dataset.

U-Net [13] is a popular deep learning architecture for semantic segmentation of biomedical data. It provides reliable results with relatively smaller dataset. ResUNet++ [14], [15] architecture is a modified version of U-Net and it provides state-of-the-art results. ResUNet++ architecture [16] contains one stem block followed by three encoder blocks, Atrous Spatial Pyramid Pooling(ASPP), and three decoder blocks. This architecture takes advantage of the residual blocks, the squeeze and the excitation block, ASPP and the attention block. Batch normalization unit, A Rectified Linear Unit (ReLU) activation unit and convolutional layers are present in every residual block. Thus this block helps to build a deeper neural network with less parameters to address degradation issues found in encoder units of other UNet models. Also the model provides accurate results in less time. Therefore, we choose ResUNet++ model for segmenting bacteria from DIBaS dataset.

## IV. EXPERIMENTS AND RESULTS

This work includes the development of labels of images from DIBaS dataset. Various semi-automatic labelling schemes such as k-means clustering, otsu thresholding and morphological closing operations are explored to generate groundtruth labels. The images with the generated labels are used to train a semantic segmentation system. ResUNet++ model is employed for this purpose and this is a completely automated process.

### A. Dataset

Gram-stained images of different bacterial genera and species have been taken from Digital Images of Bacteria Species(DIBaS) dataset [4]. The dataset consists of 33 genera and species of bacteria with each species having 20 images of size 2048x1532 and 100x magnification and they are available for public access here.

### B. Semi-automatic Annotation

Most of the images in the dataset have different textural features, colour and noise artifacts. To address these issues, we try to segment foreground from background using different unsupervised approaches like k-means clustering and Otsu thresholding. K-means clustering is employed to exploit the color based features of the bacterial images. Each image in the dataset is segmented using k=2. Some of images have stain artifacts. To reduce the staining artifacts, segmentation is performed with k=3. For few species of bacteria, k-means did not give good segmentation due to their closely spaced morphology. So, Otsu thresholding is employed to segment such species. In order to remove noise artifacts, all the images are then subjected to morphological closing operation using circular kernel. Bacteria species have different sizes, so best kernel size specific to each bacteria species is selected by manually examining various kernel sizes. Few sample images are shown in Fig. 5. These annotated labels are validated by an expert microbiologist and they can be accessed from https://github.com/nitksmile/DIBaS_Annnotations.

### C. Training using ResUNet++

The ResUNet++ model is implemented on Tensorflow’s Keras platform. DIBaS dataset has nearly equal number of
samples in each class and hence, the training is performed by randomly splitting the dataset into train:validation:test as 60:20:20. The images in the training dataset are converted into overlapping patches while those in validation set are converted to non-overlapping patches of size 512x512. Data augmentation is performed using rotation, random flipping, and shifting. A batch size of 4 is chosen. Categorical accuracy, F1 score and IoU are used as performance metrics. Categorical cross-entropy is used as a loss function. RMS prop optimizer is used, with the learning rate set to 0.0001 for 40 epochs and decreased to 0.00001 for later epochs. The model was trained for 50 epochs. The loss and training accuracy over 54 epoch is depicted in Fig. 6.

The training was stopped after 50 epochs as the loss started to increase after that and the change in loss was minimal after 34 epochs. Genera and species-wise accuracy, F1 score and IoU scores are tabulated in Table I. Confusion matrix of the predicted labels versus the actual groundtruth labels can be seen in Fig. 7. The numbers on the horizontal and vertical axes of the matrix represent the class IDs for each bacteria species as assigned in Table I, and the background class is assigned a class ID of 34.

From the confusion matrix, it is seen that large number of false predictions are made in Escherichia coli, Lactobacillus johnsonii, rhamnosus and reuteri, Porphyromonas gingivalis and Staphylococcus epidermidis classes. This can be observed in Table I where their corresponding F1 scores are less

| Class ID | Bacteria Class      | F1 score | IoU   | Accuracy |
|----------|---------------------|----------|-------|----------|
| 1        | Acinetobacter baumanii | 0.81     | 0.68  | 0.97     |
| 2        | Actinomyces israeli  | 0.98     | 0.95  | 0.97     |
| 3        | Bacteroides fragilis | 0.78     | 0.64  | 0.97     |
| 4        | Bifidobacterium spp  | 0.81     | 0.69  | 0.96     |
| 5        | Candida albicans     | 0.96     | 0.92  | 0.96     |
| 6        | Clostridium perfringens | 0.95   | 0.91  | 0.98     |
| 7        | Enterococcus faecalis | 0.95     | 0.84  | 0.97     |
| 8        | Enterococcus faecium  | 0.91     | 0.84  | 0.97     |
| 9        | Escherichia coli     | 0.97     | 0.99  | 0.96     |
| 10       | Fusobacterium spp    | 0.89     | 0.80  | 0.96     |
| 11       | Lactobacillus casei  | 0.86     | 0.75  | 0.97     |
| 12       | Lactobacillus crispatus | 0.94    | 0.89  | 0.97     |
| 13       | Lactobacillus delbrueckii | 0.59    | 0.42  | 0.78     |
| 14       | Lactobacillus gasseri | 0.93     | 0.87  | 0.98     |
| 15       | Lactobacillus johnsonii | 0.61    | 0.44  | 0.95     |
| 16       | Lactobacillus casei  | 0.30     | 0.18  | 0.97     |
| 17       | Lactobacillus paracasei | 0.71    | 0.55  | 0.97     |
| 18       | Lactobacillus plantarum | 0.82    | 0.20  | 0.97     |
| 19       | Lactobacillus reuteri | 0.01     | 0.00  | 0.97     |
| 20       | Lactobacillus rhamnosus | 0.49    | 0.33  | 0.97     |
| 21       | Lactobacillus salivarius | 0.93    | 0.87  | 0.97     |
| 22       | Listeria monocytogenes | 0.99    | 0.98  | 0.97     |
| 23       | Micrococcus spp       | 0.98     | 0.95  | 0.97     |
| 24       | Nesseria gonorrhoeae  | 0.90     | 0.82  | 0.94     |
| 25       | Porphyromonas gingivalis | 0.41    | 0.26  | 0.97     |
| 26       | Propionibacterium acnes | 0.90    | 0.82  | 0.98     |
| 27       | Proteus               | 0.83     | 0.71  | 0.97     |
| 28       | Pseudomonas aeruginosa | 0.83    | 0.71  | 0.98     |
| 29       | Staphylococcus aureus | 0.65     | 0.48  | 0.88     |
| 30       | Staphylococcus epidermidis | 0.37     | 0.23  | 0.86     |
| 31       | Staphylococcus saprophyticus | 0.74    | 0.58  | 0.87     |
| 32       | Streptococcus gaiactiae | 0.96    | 0.92  | 0.97     |
| 33       | Veillonella           | 0.87     | 0.76  | 0.87     |

| Average Scores | F1 score | IoU | Accuracy |
|----------------|----------|-----|----------|
| 0.77           | 0.64     | 0.95 |

Authorized licensed use limited to the terms of the applicable license agreement with IEEE. Restrictions apply.
than 50%. The sample images of some of the misclassified bacteria species are shown in Fig. 8. The major reason for misclassification is the similarity in the shape and arrangement of the bacteria belonging to different species. The model is also influenced by intra-species similarity. The misclassification error can be reduced by incorporating stain correction algorithms at the preprocessing stage and it will be taken up in the future.

Most of the researchers [4], [5], [7], [8] have performed only image level classification of bacteria from DIBaS dataset. Highest image level classification accuracy of 99% is achieved. Semantic segmentation of bacteria from this dataset is not attempted. Semantic segmentation of bacteria colony was attempted by Andreini et al. [2]. However, they only segmented foreground regions from the background. Species of bacteria not considered. In proposed method, 33 different species and genera of bacteria are semantically segmented. As per authors’ best knowledge, this is the first attempt to segment the image from DIBaS dataset and segmentation accuracy of 95% is achieved. The annotated labels for this dataset is available for public use. This model can be used for semantic segmentation of images containing different species of bacteria and for detection of gram-type, shape and arrangement of bacteria.

V. CONCLUSION

A deep-learning model for semantic segmentation and classification of bacteria genera and species from DIBaS dataset has been implemented. The images from the dataset are annotated using k-means clustering and otsu thresholding. Morphological closing is performed using circular kernels of varying sizes. Semantic segmentation is performed by ResUNet++ model. An average classification accuracy of 95%, IoU score of 64% and F1 score of 77% is achieved. To the best of the authors knowledge, semantic segmentation of bacteria from microscopic images is being attempted for the first time. We aim to use this model as a baseline to perform classification, segmentation of bacteria for other biomedical applications such as gram-stained urine smear images, sputum-smear images and so on.

REFERENCES

[1] S. U. Abdulla, T. G. Hridya and V. V. Nair, “A general approach for color feature extraction of microorganisms in urine smear images,” 2015 Fifth International Conference on Advances in Computing and Communications (ICACC), pp. 338-341, 2015.
[2] P. Andreini, S. Bonechi, M. Bianchini, A. Mecocci and F. Scarselli, “A deep learning approach to bacterial colony segmentation”, Artificial Neural Networks and Machine Learning – ICANN 2018, pp. 522–533, 2018.
[3] R. O. Panicker, K. S. Kalmady, J. Rajan and M.K.Sabu, “Automatic detection of tuberculosis bacilli from microscopic sputum smear images using deep learning methods,” Biocybernetics and Biomedical Engineering, vol. 38, no. 3, 2018, pp. 691-699, 2014.
[4] B. Zielinski, A. Plchta, K. Misztal, P. Spurek, M. Brzychczy-Wloch and D. Ochońska, ”Deep learning approach to bacterial colony classification”, 27th International Conference on Artificial Neural Networks, Rhodes, October 4–7, 2018 Proceedings, Part III, 2018.
[5] B. A. Mohamed and H. M. Afify, ”Automated classification of bacterial images extracted from digital microscope via bag of words model,” 2018 9th Cairo International Biomedical Engineering Conference (CIBEC), pp. 86-89, 2018.
[6] D. Polap and M. Woźniak, ”Bacteria shape classification by the use of region covariance and Convolutional Neural Network,” 2019 International Joint Conference on Neural Networks (IJCNN), pp. 1-7, 2019.
[7] T. Shailey and S. Kala, ”Bacterial image classification using Convolutional Neural Networks,” 2020 IEEE 17th India Council International Conference (INDICON), pp. 1-6, 2020.
[8] M. Talu, ”An automated deep learning approach for bacterial image classification,” International Conference on Advanced Technologies, Computer Engineering and Science (ICATCES2019), pp. 1-5, Apr 26-28, 2019 Alanya, Turkey.
[9] Iida R., Hashimoto K., Hirata K., Matsuoka K. and Yokoyama S., "Detection system of gram types for bacteria from gram stained smears images," Proc. 9th International Conference on Pattern Recognition Applications and Methods (ICPRAM 2020), pp 477-484, 2020.

[10] A. Borowa, D. Rymarczyk, D. Ochońska, M. Brzychczy-Włoch and B. Zielniński, "Deep learning classification of bacteria clones explained by persistence homology," 2021 International Joint Conference on Neural Networks (IJCNN), pp. 1-8, 2021.

[11] M. K. M. Serrão, M. G. F. Costa, L. B. Fujimoto, M. M. Ogusku and C. F. F. C. Filho, "Automatic bacillus detection in light field microscopy images using Convolutional Neural Networks and mosaic imaging approach," 2020 42nd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC), pp. 1903-1906, 2020.

[12] Costa MG, Costa Filho CF, Kimura Junior A, Levy PC, Xavier CM and Fujimoto LB. "A sputum smear microscopy image database for automatic bacilli detection in conventional microscopy," Annu Int Conf IEEE Eng Med Biol Soc. 2014, pp. 2841-2844, 2014.

[13] Ronneberger O., Fischer P. and Brox T., "U-net: convolutional networks for biomedical image segmentation," Proceedings in Medical Image Computing and Computer-Assisted Intervention (MICCAI 2015), pp. 234-241, 2015.

[14] A. Khanna, N. D.Londhe, S.Gupta and A. Semwal,"A deep residual u-net convolutional neural network for automated lung segmentation in computed tomography images," Biocybernetics and Biomedical Engineering vol. 40, no. 3, pp. 1314-1327, 2020.

[15] Siddique N., Paheding S., Elkin C.P. and Devabhaktuni V.,"U-net and its variants for medical image segmentation: A review of theory and applications," IEEE Access, vol. 9, pp. 82031-82057, 2021.

[16] D. Jha et al., "ResUNet++: An Advanced Architecture for Medical Image Segmentation," 2019 IEEE International Symposium on Multimedia (ISM), pp. 225-2255, 2019.