

Spirochaeta Bacteria Detection Using an Effective Semantic Segmentation Technique

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Abstract. Bacterial infections in the blood could have lethal effects if proper medical attention is not provided. Thus, detecting bacteria in the blood would have huge importance for researchers and practitioners. In this paper, we are concerned with the specific spirochaete bacteria. Spirochaete is one of the most common types of invasive bacteria, which causes innumerable diseases in humans such as syphilis, Lyme disease, relapsing fever, and leptospirosis. This research presents a deep learning-based method for detecting Spirochaete in blood, which could have a substantial impact in the medical field. The proposed model was developed on a different U-Net architecture. The model in this paper was fed with 366 Black Field Microscopy images that can identify Spirochaete bacteria, of dimensions 256*256. The trained model in this paper achieved an improved test accuracy of **98.02%**.

Keywords: Spirochaete bacteria, Image Segmentation, Convolutional Neural Network, U-Net, Deep Learning.

1 Introduction

A Spirochaete, also known as a spirochete, is a member of the phylum Spirochaetes, which includes diderm gram-negative bacteria with long, helically coiled cells. Spirochaetes are chemoheterotrophic bacteria with diameters ranging from 0.09 to at least 3 meters and lengths ranging from 3 to 500 meters. Spirochaetes are distinguishable from other bacterial groups by the placement of their flagella, which are

referred to as endoflagella or axial filaments. Endoflagella is fixed at the bacterium's poles and projects rearward into the periplasmic space to prolong the cell's length. These induce the spirochaete to twist, allowing it to move around. Asexual transverse binary fission occurs when a spirochaete reproduces. The majority of Spirochaete are anaerobic and free-living, although there are a few exceptions. Spirochaete bacteria differ in pathogenicity, ecological habitats, and molecular properties such as guanine-cytosine concentration and genome size. The habitats are incredibly diverse, ranging from mud to the mammalian kidney's convoluted tubules.

In humans, the bacteria Spirochaete causes syphilis, yaws, Lyme disease, and relapsing fever. Spirochete genera include Spirochaete, Treponema, Borrelia, and Leptospira. In a liquid environment, spirochetes are common (e.g., mud and water, blood and lymph). Treponema is the same bacteria that causes syphilis and yaws. Borrelia is a bacterial genus carried by lice and ticks. In humans, it causes relapsing fever and Lyme disease. The bacteria Leptospira causes leptospirosis, which is largely a disease of domestic and wild mammals, with humans becoming afflicted as a secondary illness [7].

Darkfield microscopy is a technique that uses oblique illumination to improve contrast in specimens that are difficult to see under normal conditions. Light going through the specimen from oblique angles is diffracted, refracted, and reflected into the microscope objective after an opaque stop in the condenser excludes direct light. This results in a bright image of the specimen superimposed on a black background. Dark Field lighting is the most effective approach to show contours, edges, boundaries, and refractive index gradients. Dark Field lighting is appropriate for miniature living aquatic organisms, diatoms, small insects, bone, fibers, hair, unstained bacteria, yeast, tissue culture cells, and protozoa [6].

2 Literature Review

In this paper, the authors trained a feed-forward network, which receives an image and outputs its segmentation mask [1]. In paper [2], Sundar et al. used four different types of models with slight modifications and achieved the highest accuracy of 96.606% in model number 4 - Optimized architecture with custom loss.

Kulwa et al. offered a review of microorganism image segmentation algorithms in the paper [3], which are divided into traditional and machine learning-based models, which are further divided into subcategories. Out of all the classical based models, they discovered that threshold-based methods are the most utilized because they are suitable for a lot of varied segmentation challenges and the neural networks-based models in machine learning-based models are used most frequently because they can extract the significant relationships between the images without explicit human intervention. They conclude that because these segmentation approaches have comparable image features, they can be successfully applied to various issues such as cervical cancer, cell segmentation, sperm cell segmentation, cytopathology, and histopathology image segmentation.

George et al. showed in their study [4] that their model for Brain Magnetic Resonance Images, which uses a Markov Random Field (MRF) hybrid with their biologically

inspired Bacteria Foraging Optimization Algorithm (BFOA), outperforms traditional MRF-GA (Genetic Algorithm). They claimed that their accuracy was higher than that of other algorithms on the market. This is owing to a better understanding of the brain's natural structure.

Research papers on other bacteria and parasites such as *E. coli* and malaria parasites like *Plasmodium* have also been presented and demonstrate the different detection methods. La Spina et al., for example, used Dark Field Microscopy-Based Biosensors to detect *E. coli* in environmental water samples in their study [8]. They used object circularity and size parameters methods to identify the bacteria. Their method was faster and adaptable to infield analyses before the work in their field. Mwanga et al. found malaria parasites with 92 per cent accuracy in another paper [9]. They analyzed the data using mid-infrared spectroscopy and logistic regression analysis.

3 Dataset

The data set used in this paper is available on Kaggle [10]. The total size of the data set is 173.04 MB, there are in total 366 images and respective manually annotated masks of those images. A sample of an image from the data set is attached. As the masks are not visible to human eyes, the masks were scaled to a greater value for visualization. Simple loss functions and classification algorithms cannot be performed on this dataset because it is specifically annotated for semantic segmentation and the dataset is unbalanced.

A sample image, a mask without scaling and one-hot encoding, and a mask of the image after scaling and one-hot encoding from the dataset are shown in the following images.

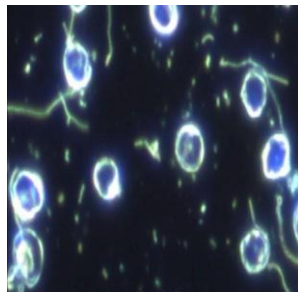


Image 1.1



Image 1.2

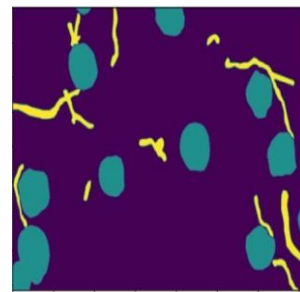


Image 1.3

Image 1 From left to right **Image 1.1** A sample of an image of Black Field microscopy images from the Image data set **Image 1.2** Image of the mask without scaling and one hot-encoding **Image 1.3** Visualization of the label mask of the Image after scaling to a greater value and one-hot encoding was performed.

Yellow-coloured components are the Spirochaete bacteria, and the greenish-coloured ones are the Red Blood Cells in image 1.3 which will be fed to the deep neural network.

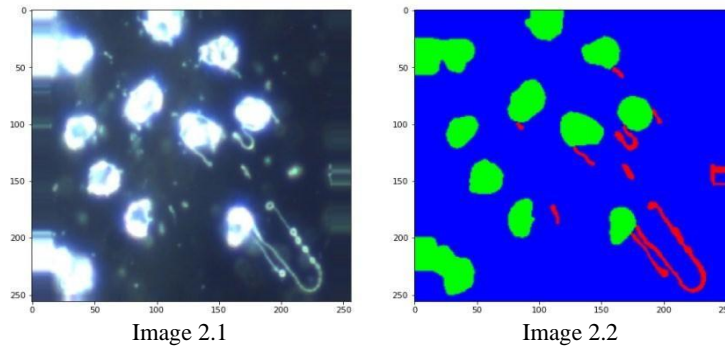


Image 2 From left to right **Image 2.1** A sample dark field image **Image 2.2** Mask of the image becomes visible after scaling to a greater value and one-hot encoding was performed.

The annotated mask image labels, after rescaling, can now help us identify the components that were not visible to the naked eye as witnessed in Image 1.2, and can be used in comparison with the predicted mask that is obtained after carrying out image segmentation on the images and their respective image labels.

4 Methodology

4.1 Data Preprocessing

During the initial analysis, each image in the data set had different dimensions. This paper experimented with different image dimensions and landed with 256x256 dimensions. The data set was split into two parts: 80% for training and 20% for testing. As the machine learning classification algorithms can't handle categorical data, one hot encoding was performed on the masked data. One hot encoding is the process of representing the categorical value in binary form, as a result, a deep learning model could perform better. The total number of classes was 3.

4.2 Data Augmentation

To enhance the available data set and achieve better accuracy for the model, we performed data augmentation on the available data. By performing data augmentation on the available data set, the model will have enhanced data to learn from. To achieve data augmentation in this paper, we performed width shift with a range of 30, height shift with a range of 30, flipped horizontally and vertically, and applied a zoom range of 20%. The generated dataset was used then to train the model and for validation after training.

4.3 Model Architecture

U-Net: Ronneberger et al. developed the U-Net convolutional neural network in 2015 for biological picture segmentation [5]. It has a U-shape because it has two paths: contracting and expansive. The contracting component entails applying convolutions repeatedly, each time with the ReLu activation function and max pooling operation with

strides for downsampling. The number of feature samples is doubled for each downsampling layer. This part is also called an encoder as it encodes images into features. The downsampling process tries to extract more and more useful information from the images by reducing the dimensions in every process. The expansive component functions as a decoder, upsampling the feature map, then performing convolutions to reduce the number of feature channels to half, and finally integrating the feature map from the contracting path with the convolutions. By concatenating pictures from the contracting process, the upsampling process reconstructs the image. The last layer is a convolutional layer that maps the feature vectors to the desired classes. As a result, it is a fully convolutional network (FCN) with no dense layer.

Our Proposed architecture: We are using U-Net: Convolutional Neural Network for Spirochaetes bacteria detection whose architecture and implementation are discussed in detail. The architecture of our proposed model consists of an input convolutional layer, a downsampling process, two middle layers, upsampling process, and the transposed convolution as the classification layer. The basic process of the downsampling process is given below:

$$\text{Conv2D_layer1} \rightarrow \text{Conv2D_layer2} \rightarrow \text{MaxPooling2D}$$

This process was repeated 4 times with the activation function as SoftMax in the first layer and ReLu in the rest of the 3 layers. This was followed by two convolutions in the middle part. The upsampling process was performed after that, whose basic process is given below:

$$\begin{aligned} \text{UpSampling2D} &\rightarrow \text{Concatenate} \rightarrow \text{Conv2DTranspose_layer1} \\ &\rightarrow \text{Conv2DTranspose_layer2} \end{aligned}$$

This process was repeated 4 times with activation function as ReLu in all the layers. The first and the last layers were transposed convolution with SoftMax activation function for classifying the feature vector to desired classes.

The architecture of our proposed model as shown in Fig 1, consists of one input layer, followed by 30 dense layers. The dense layers are a combination of layers having activation of either “Rectified Linear Units (ReLu)” or “Softmax”.

A Rectified Linear Unit (often known as a rectified linear unit or ReLU) features outcome 0 if the inputs fed have a value lower than 0, and raw output if the input value is higher than 0. If the input is larger than 0, the output will be the same as the input. ReLU's functionality is more similar to that of our organic neuron cells. The fundamental equation for ReLu activation function is given by the equation stated below:

$$f(x) = \max(x, 0) \tag{1}$$

ReLU is non-linear and, unlike the sigmoid function, does not have any back-propagation algorithm mistakes. Additionally, for bigger Artificial Neural networks, the efficiency of generating projections based on ReLU is better and quicker than using Sigmoids. Softmax seems to be a fascinating activation function. This activation function, in addition to mapping our product to a [0,1] spectrum, also translates every individual output such that the aggregate amount equals 1. As a consequence, Softmax's result is a probability distribution. In the bottom layer of a Deep Learning based classifier, the softmax layer is typically employed.

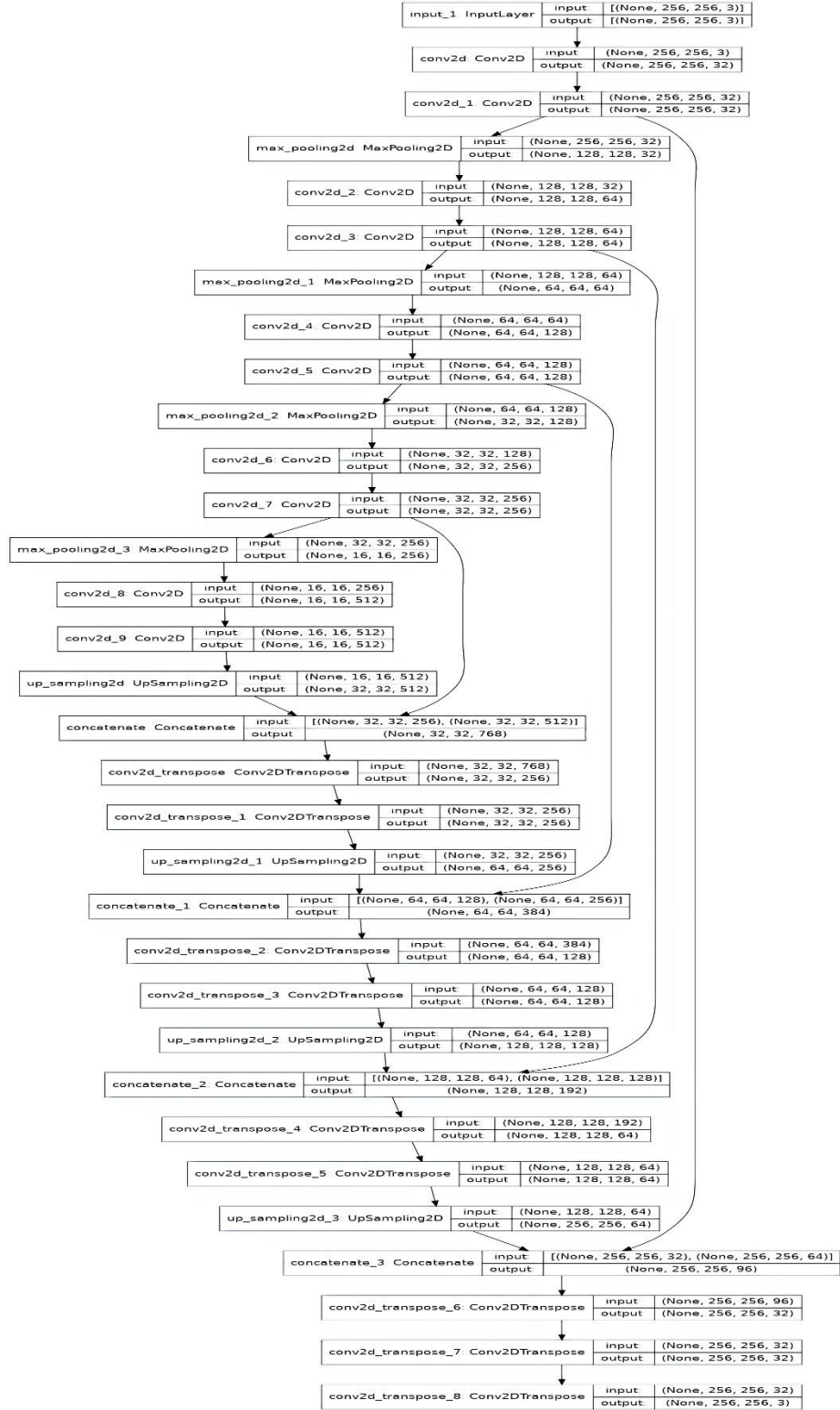


Fig. 1: Proposed Model Architecture consisting of all the convolution layers

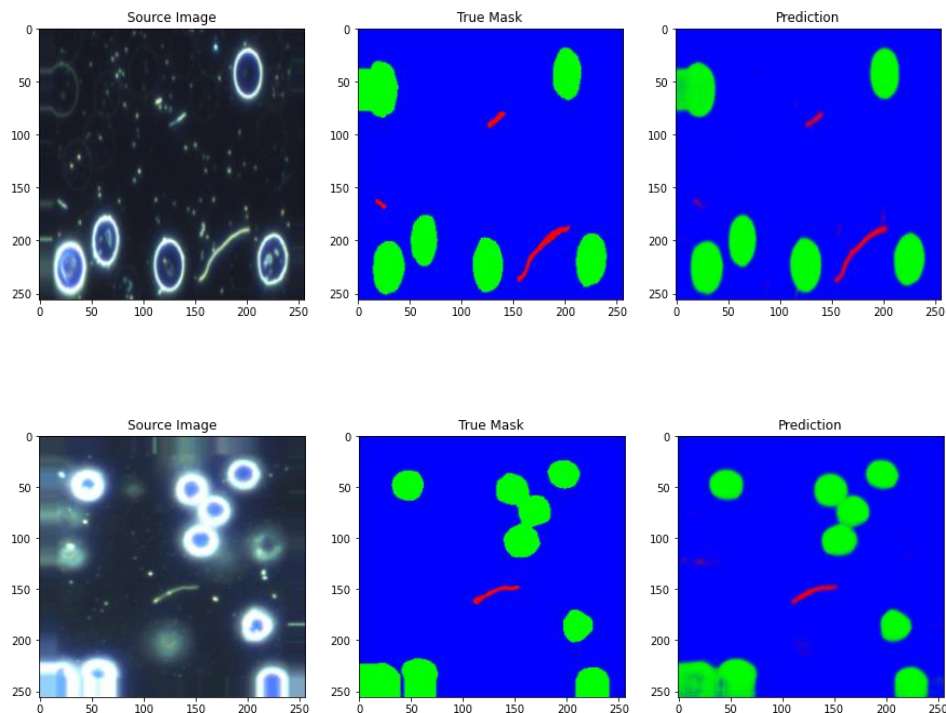
4.4 Model Implementation and Training

We performed exploratory data analysis on the acquired data to understand the data better. The images were divided into batches, each batch containing 16 images. After which, data preprocessing was performed on the data set to make our data suitable for our architecture followed by training the model on the training data set. Finally, it was tested on the test data for performance analysis and plotted further. After trying different optimizers, Adam optimizer was used to reach global minimum value as it was best suited for our model. The loss function used was categorical cross-entropy.

5 Experiment Results and Discussion

In only 15 epochs, we were able to obtain maximum validation accuracy of 98.02 per cent and training accuracy of 96.41 per cent. We employed “SoftMax” as the activation function in the first convolution layer and “Adam” as the optimizer, which improved accuracy from 96 to 98 per cent. When the model was trained with 256*256 picture dimensions, the accuracy was significantly higher than when the model was trained with 128*128 image dimensions.

To test the model, we fed the test images into the network and the model predicted the bacteria very well which is evident from the given prediction and can be visualized in the below images:



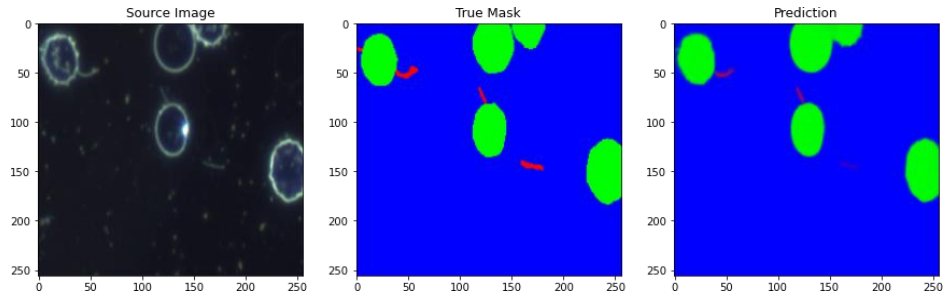


Image 3: From left to right in every image: Source image, True mask, and the Prediction image from the proposed model.

The Spirochaete bacteria are indicated in red in the rightmost plots in each image. Every image has a source image, a true mask, and the bacteria forecast may be seen. The images above demonstrate that the model properly identified Spirochaete bacteria and performed perfectly.

Further, it is evident from the below graph in Fig 2 that the model has performed well and is highly accurate. As the model accuracy neither started declining nor stagnated at any particular point it clearly shows that model didn't face overfitting.

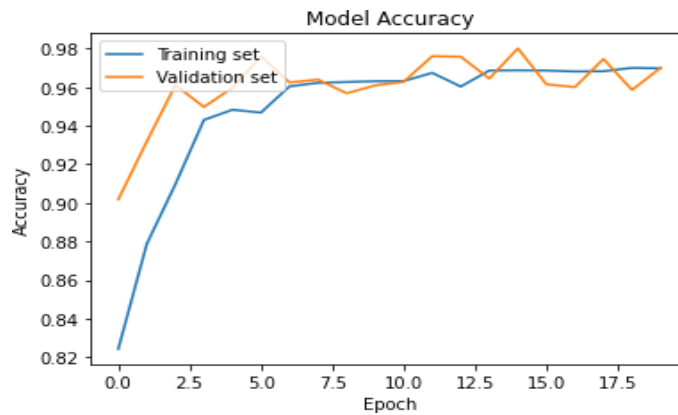


Fig. 2: On the training and validation sets, a graph of model accuracy is shown.

The proposed model didn't face underfitting as the model performed well on the validation data as seen in the following Fig 3.

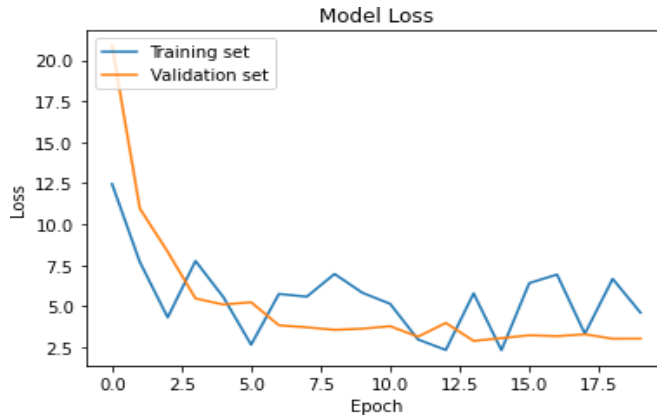


Fig. 3: On the training and validation sets, a graph of model loss is shown.

In the comparison table shown below, it is evident that our proposed architecture is more efficient and has delivered better results.

Table 1: Comparison of accuracies of different models on the similar works

Approach Implemented	Method/Model used	Best Accuracy
Detection of malarial parasites in human blood [9]	Logistic Regression with mid-infrared spectroscopy	92%
Detection of harmful bacteria in blood [12]	U-Net architecture with customized loss	96.60%
Automatic brain tumor detection and segmentation [16]	U-net based fully convolutional neural networks	77%
Classification of white blood cells [17]	AlexNet with Gaussian filter ResNet50 with Gaussian filter DenseNet 201 with Gaussian filter GoogleNet with Gaussian filter	82.16% 80.02% 83.44% 75.21%
Identification of bacteria in 3D microscopy datasets [18]	Convolutional Neural Networks; Transfer learning	75% (approx.)
Detecting Spirochaete bacteria using efficient deep learning technique U-Net (Our proposed model)	Our Proposed model	98.02%

6 Conclusion and Future Scope

In the science of computer vision, semantic segmentation is a crucial domain. Spirochaete bacteria have a symbiotic relationship with insects, commensals, termites, ticks, lice, and parasites, and cause diseases like syphilis and relapsing fever in humans. The ability to detect these microorganisms correctly and precisely will be extremely beneficial in the medical industry. The goal of this research is to use semantic segmentation to accurately detect Spirochaete bacteria in a given image. The model used in the paper was adapted and developed on the prior U-Net architecture and had a significant difference in accuracy when the image dimensions were altered from 128*128 to 256*256 and when the SoftMax activation function was used in the first convolutional layer of downsampling layers. The accuracy achieved by our model is greater than the highest accuracy achieved i.e., 96.02% in the paper [2] out of the four models which use the same dataset.

In future works, we will try to achieve more accuracy and minimize loss value using the U-Net++ model and by including dropout layers. We will also focus on implementing and identifying other bacteria and pathogens in blood, water, food, and other common sources from where they might infect us, thereby enhancing the ubiquity of our model.

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