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Deep- Image: Automated Identification of Bacteria based on Deep Learning Model

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ABSTRACT

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Received 31 January 2025 Received in revised form 29 June 2025 Accepted 30 June 2025 Available online 10 July 2025 Accurate Classification of bacteria plays a crucial role in microbiology and beyond. It helps to identify infectious agents during epidemiological investigations, food safety monitoring, and detection of biological threat agents. Convolutional Neural Network (CNN) is a deep learning technique that has proven reliable in the field of Classification of medical and biological diseases. In this study, CNNs are utilized to develop a bacterial classification system. Within this system, Classification is subjected to several modifications before the ResNet method is used in order to identify the kinds of Bactria from among sixteen different classes of bacterial images. The model was fine-tuned by training only the last two layers of the pre-trained ResNet101V2 network, which significantly improved the performance. A large-scale dataset and confusion matrix were used to evaluate the model's performance. The experimental results demonstrate that the accuracy rate reached a peak of 98.66%. Moreover, the suggested approach enhances the advancement of automated diagnostic tools for bacterial pictures that surpass the present state-of-the-art models and provide the groundwork for future enhancements in bacterial image classification utilizing CNNs.

Keywords:

ResNet, Bacteria images, Convolution neural network, Classification, Deep learning

1. Introduction

Bacteria are microscopic organisms that need oxygen to live. These organisms are influential in determining their shape and types in many fields, including food safety, health care, and

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environmental monitoring [1][2]. In agriculture and biochemical tests, traditional methods take a long time to identify these organisms. Therefore, artificial intelligence techniques are used through a group of trendy algorithms capable of studying these objects' behaviors and from different data sources to determine the type, benefit, and automation [3]. These models are able to extract significant patterns from microscopic images, mass spectrometry data, and genetic sequences [4]. Bacteria are considered among the most pathogenic organisms that infect humans with diseases through bacterial infections. These infections spread rapidly and cause health problems that affect people at all ages. In recent years, the process of identifying and classifying bacteria has been one of the required tasks in modern healthcare services. Moreover, these infections contribute to increasing deaths because they are able to reproduce on their own in the body and are more dangerous than viruses. Therefore, health institutions seek to benefit from artificial intelligence services to quickly identify types of bacteria, predict and classify them, and help doctors diagnose patients efficiently. The importance of artificial intelligence algorithms is increasing in developing health care services and assisting doctors in diagnosis, prediction, and data analysis, as traditional methods of challenging bacteria, such as microscopic examination, may take time and require the opinions of many doctors and specialists. Therefore, these algorithms significantly impact the study of data patterns and the extraction of features that help determine the appropriate treatment for the patient [5], [6].

There are a wide variety of conditions in which bacteria may flourish. Bacteria are single-celled prokaryotic microorganisms that are tiny. Bacteria exhibit a variety of morphologies, ranging from spheres to rods and spirals, and often measure just a few micrometers in length. Many different environments, including soil, water, radioactive waste, acidic hot springs, and the deepest parts of the Earth's crust, are home to bacteria [7]. Bacteria being microscopic in size (ranging from 0.2 to 20 micrometers) [8], cannot be seen with the naked eye, and hence, the use of microscopes is essential for their observation. There are different types of microscopes, such as optical and electron microscopes. In today's scenario, image processing has become a popular approach in various industries, including medical labs, for fast and efficient microbe classification [9].

When it comes to image classification tasks, such as medical image analysis, CNNs have shown astounding effectiveness. CNNs can automatically learn relevant features from images and provide high accuracy in classification tasks [10]. Several studies have applied CNNs to bacterial image classification, achieving promising results. However, these studies have focused on specific bacterial species or strains, and there is a need for a more comprehensive approach that can classify a broader range of bacterial species [11]. Using convolutional neural networks (CNNs), provide a model for recognizing bacteria based on the composition of region covariance. Using the region covariance model, an input microscope image is segmented in the first step. These portions are then sent to CNN so that any apparent bacterial strains may be identified [12]. The rod-shaped bacteria and the spherical or nearly spherical bacteria were both subjected to experiments. The findings indicate that the suggested technique has a great deal of promise [13-15].

The objective of this research is to develop a system that is both precise and efficient in its Classification of bacterial images. New image preprocessing algorithms specifically designed for fluorescent microscope images have significantly increased the available training data for neural networks. The CNN developed in this study achieves an impressive accuracy of up to 86% when trained on a dataset of 81 images. Notably, the algorithm provides a total cell count comparable to manual counting but is 10.2 times more consistent and 3.8 times faster [16]. CNNs are efficient techniques for classification problems. In this paper, a ResNet-50 CNN model is employed to classify bacterial images into twenty medically relevant categories. The approach achieves an outstanding accuracy of 99.9% for Classification, outperforming state-of-the-art methods [17]. A novel method proposes automatic bacteria type identification using pattern recognition techniques. The process



involves image segmentation, feature extraction, and Classification. By training a backpropagation neural network on extracted features, the proposed method achieves high efficiency compared to other approaches [18]. Consequently, our contributions can be summarized in the main points as follows.

- 1.We propose a novel model for bacterial recognition, combining region covariance with CNNs.
- 2. In the initial stage, an input microscopy image undergoes segmentation using the region covariance model.
- 3. Subsequently, these segments are fed into the CNN for the recognition of visible bacterial strains.
- 4. Our experiments specifically targeted rod-shaped and nearly spherical bacteria, revealing the high potential of our methodology.

The rest of the paper is arranged as follows: In Section 2, we look at previous research, and in Section 3, we describe the tools and methods used to find bacterial images. In Section 4, we show the results of the experiment, and in Section 5, we talk about what our study found.

2. Related works

In recent years, the intersection of computer vision and machine learning has sparked interest in automating and enhancing bacterial identification processes. Researchers Tumun Shaily and Kala S utilize deep-learning neural networks for bacterial Classification. Variants of ResNet CNN models (such as ResNet-34 and ResNet-50) are employed for feature extraction and Classification. Their approach achieves an impressive 99.9% accuracy in classifying twenty categories of bacteria [19]. M.TALO presents an automatic method based on deep learning for putting digital images of bacteria into 33 different groups. The pre-trained ResNet-50 CNN design and transfer learning work together to get an average classification accuracy of 99.2% [20].

The categorization of bacteria has been investigated using a variety of methodologies in a number of different research papers. Identification of bacteria based on their microscopic appearance was accomplished by the authors of research [21] via the use of a Naive Bayes classifier. By employing a dataset consisting of 500 bacterial images from five distinct species, they were able to reach an accuracy rate of 95%. In the study [22], Classification using Support Vector Machines (SVM) and Random Forest (RF) approaches were also explored. In a study [23], the Bag-of-Words technique was employed for feature extraction in bacterial image classification.

Nie et al., in their study [24], used convolutional deep belief networks, an unsupervised learning method, for bacterial Classification. However, this approach was less efficient for multiple bacterial colonies. Overall, popular CNN models like ResNet[25], AlexNet[26] and VGGNet [27] are widely available in research papers for various classification tasks. In summary, leveraging CNNs and deep learning techniques holds great promise for accurate and efficient bacterial image classification. These advancements contribute significantly to medical research and diagnostics.

2. Methodology

2.1 Classification Methodology

This paper aims to improve the identification of bacterial species by minimizing taxonomic errors associated with human judgment. Additionally, it seeks to facilitate rapid identification of bacterial and other infections for biologists, nutritionists, and doctors. The proposed system comprises four key stages: image preprocessing, dominant feature extraction, feature selection, and Classification of the chosen features using a CNN classifier to arrive at the final decision. Figure 1



illustrates the components of the proposed system. Algorithm 1 describes the pseudo-code of the steps of the Proposed automated identification of bacteria using a deep learning model.

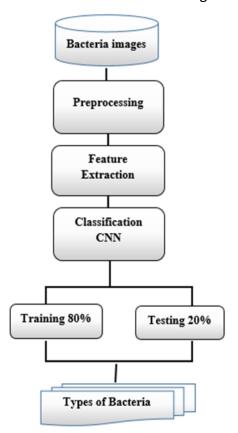


Fig. 1. Proposed Automated Identification of Bacteria using Deep Learning Model

Algorithm 1: Identification of Bacteria based Deep Learning Algorithm

Preprocessing:

- Read the bacterial image (x_0) into x_1 .
- Set the image intensity in x_1 to create x_2 .
- Transform x_2 into a binary image (x_2) .
- Eliminate small artifacts from the binary image (x_3) .
- Fill holes and small gaps in x_4 to obtain x_5 .
- Multiply the pixels of x_5 to create x_6 .

Feature Extraction:

- For each feature extraction operator F_i:
- Extract features (f_i) from x_6 .
- Evaluate features (w_i).
- Select the best features ($\llbracket bf \rrbracket _i$) based on f_i and w_i .

Classification:

- For each classification operator C_j:
- Classify using the selected features (c_i, j) .
- Evaluation:
- For each evaluation operator E_k:
- Evaluate the classification results (e_{j},k) .
- Write the results ($\llbracket bf \rrbracket$ _i, F_i , c_i , c_j , C_j , E_k , e_i , e_j).



Pre-processing Stage

In this study, we employ the histogram thresholding method to isolate individual regions of an image with intensity values exceeding a predefined cutoff from the background pixels. Subsequently, we apply a dilation morphological operation to fill gaps that appear in the image. Any remaining deficiencies are addressed by connecting small objects. As a result, we obtain a binary representation where ones represent the more interesting bacterial area, and zeros represent the background. Figure 2 shows the basic factors in determining image quality. Figure 3 shows pre-processing methods to improve data quality before feeding it into the classification model.



Fig. 2. The basic factors in determining image quality



Fig. 3. The pre-processing methods to improve image quality

• Feature Extraction Method

In this study, we leverage Convolutional Neural Networks (CNNs) to efficiently extract features for classification tasks in the computer vision domain. CNNs, an improved version of classical neural networks, consist of three layers: convolutional, pooling, and fully connected layers [33]. The convolutional layers employ trainable filters that reduce the spatial dimensions of the input. Negative values in feature maps are rectified using the ReLU activation function. Pooling layers further reduce dimensionality, and batch normalization accelerates training. While CNNs are powerful classifiers, they require intensive parameter tuning. To mitigate complexity, we limit the number of



convolutional layers and consider factors like filter size and CT slice dimensions (set to 256×256 pixels).

There are a number of well-known CNN models that have been the subject of lengthy research papers, including as AlexNet [18], VGGNet [19], and ResNet [17]. Among them, Deep Residual Networks, often known as ResNet, have become more popular because to the precision with which they perform classification tasks. When compared to VGGNet, ResNet models have a lower complexity and fewer parameters, even when they include a significant number of layers (up to 1000). The sizes of the filters that are used for convolutions in ResNet designs are quite diverse. In light of the fact that our task involves a wide variety of bacterial classifications, we have decided to use Residual Neural Networks in order to improve the accuracy of species categorization. In this research, we provide ResNet101V2 CNN models for the purpose of tackling the issue of microscopic bacteria picture classification, as shown in Figure 4.

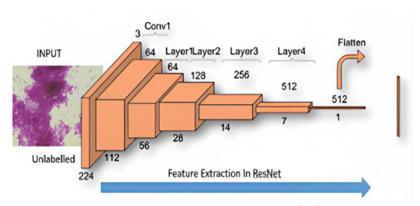


Fig. 4. ResNet101V2 CNN Model [17]

Bacteria Classification based on CNN Model

Bacteria can be classified in various ways based on their characteristics, including morphology, physiology, metabolism, and genetic makeup. Gram-negative bacteria are the two categories that are distinguished by the Gram stain, which contributes to the categorization system that is commonly used. Gram-positive bacteria have a thicker peptidoglycan layer in their cell wall, which allows them to retain the crystal violet stain. Gram-negative bacteria, on the other hand, have a thinner peptidoglycan layer and an outer membrane, which makes them more resistant to certain antibiotics and causes them to stain pink with counterstain (safranin) after decolonization. Other classification systems consider bacterial shape (e.g., cocci, bacilli, spirillum), oxygen requirements (aerobic, anaerobic, facultative anaerobic), and growth conditions (e.g., temperature, pH, nutrients) [28]. Bacterial taxonomy continually evolves with advancements in molecular techniques like DNA sequencing and comparative genomics [29], [30].

Capturing bacterial cell images involves using microscopes or other imaging devices, such as scanning electron microscopes or transmission electron microscopes. Different techniques, such as bright-field microscopy, dark-field microscopy, phase-contrast microscopy, fluorescence microscopy, and confocal microscopy, can be employed based on the bacteria type and desired resolution and contrast [31]. Once captured, these images can be digitized and stored as digital data. Image processing techniques can then extract relevant features for classification purposes. Analyzing bacterial images aids in understanding cell morphology and structure, benefiting the diagnosis and treatment of bacterial infections [32].

In the context of bacterial image classification, popular CNN models include AlexNet, VGGNet, and ResNet. Among these, ResNet has gained prominence due to its accuracy. In comparison to



VGGNet, it has a lower number of parameters and a lower level of complexity, and its layer count may reach up to one thousand. In this network, the filter sizes that are used for convolutions are quite diverse. Due to the wide variety of bacterial classifications, we make use of the Residual Neural Network (ResNet) in order to achieve a higher level of precision. In this research, we offer the ResNet-34 and ResNet-50 CNN models as a solution to the issue of microscopic bacteria picture categorization.

Machine learning includes CNN as one of its subsets. A number of different kinds of artificial neural networks are used for a variety of applications and kinds of data, and this particular kind is one of those sorts. A CNN is a kind of network architecture that is used for deep learning algorithms. Its primary use is in image recognition and other tasks that need the processing of pixel data [35].

CNN is constructed up of three layers: Fully connected (FC) layer, a pooling layer, and a convolutional layer. The FC layer comes after the convolutional layer. The CNN gets more complicated as you move from the convolutional layer to the FC layer. This rising level of difficulty is what lets CNN spot bigger and more complicated parts of an image until it finally finds the object in its entirety [36].

- 1. The convolutional layer, which is the fundamental component by which a CNN is constructed, is where the vast bulk of calculations take place. It is possible for the first convolutional layer to be followed by a second convolutional layer. When performing the process of convolution, a kernel or filter that is included inside this layer will move across the receptive fields of the picture in order to determine whether or not a certain feature is present in the image. The kernel crosses the whole image across many repetitions. In every iteration, a dot product is computed between the filter and the input pixels. A feature map or convolved feature is the resultant output from the sequence of dots. In this layer, the picture is ultimately transformed into numerical values so that the CNN may analyse it and derive pertinent trends.
- 2. The pooling layer, like the convolutional layer, also sweeps a kernel or filter across the input image. However, the pooling layer, in contrast to the convolutional layer, minimizes the number of parameters in the input and leads to a certain amount of information loss. Positively, this layer enhances the efficacy of the CNN and reduces its complexity.
- 3. Fully connected layer, In the CNN, the FC layer is where image classification takes place. This Classification is based on the characteristics that were retrieved from the layers that came before it. In this context, the term "fully connected" refers to the situation in which all of the inputs or nodes from one layer are connected to each activation unit or node of the layer below it.

CNN is a multilayer approach that includes both convolutional and fully connected layers. CNNs trace their origins back to the 1960s and are built upon three key concepts: local perception, weight sharing, and time or space sampling. Local perception efficiently detects local aspects of data, extracting basic features relevant to visual objects (e.g., angles or arcs of animals). One advantage of CNNs is their ability to achieve good performance with fewer parameters compared to fully connected networks. The CNN architecture consists of two main layers: convolution and pooling layers, which collaborate to process features. However, pooling layers can sometimes lead to confusion by focusing on unimportant feature locations. Mean pooling computes the neighborhood average of feature points, while max pooling computes the maximum value. The size limitation of the neighborhood may cause a feature extraction error. This error arises due to the estimated parameter and variance errors in the convolution layer. Mean pooling helps preserve information about the image background, while max pooling preserves information related to image texture [36-38][40-46].

The CNN architecture consists of multiple layers. Each layer contains multiple maps, and within each map, numerous neural units share the weight of the convolution kernel. These convolution



kernels act as features, allowing access to image edges. Image data, represented as input, can be effectively controlled for deformation. The size of the convolution kernel and its parameters contribute to multi-scale image feature extraction, creating distinct information angles within the feature space.

2.2 Bacterial Images Dataset

The dataset consists of 16 different classes of bacterial images. Free public diBas datasets are provided and used in our study [39]. The dataset consists of 17877 images; 14296 of them are used as training set, while 3581 are used for testing. An example of a bacterial images dataset is shown in Figure 5.

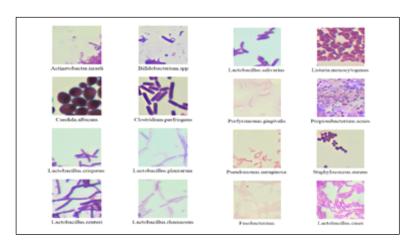


Fig. 5. Images of 16 Cases of Bacterial Classes.

3. Experimental Results and Discussion

This section contains the setup parameters of the experiment as well as the results of the suggested systems based on the categorization of the images of the bacteria. The following are the two primary subsections that are presented:

3.1 Experiment Setup

For the purpose of this research, we make use of the pre-trained ResNet101V2 CNN architecture using transfer learning in order to categorize bacterium species into sixteen different categories. The convolutional and pooling layers used in the ResNet101V2 model are transferred to the new model that we have developed. For the purpose of species categorization, the fully connected layers of ResNet101V2 are eliminated and replaced with a new, completely connected layer. This layer generates a 16-unit tensor and employs the Softmax activation function.

During the training process, we also add two dropout layers to prevent the garment from being very tight. An example of overfitting is when a model is able to remember training samples but is unable to generalize to data that it has not seen before. The rates of first and second dropouts are set at 25% and 50%, respectively, in the first dropout rate. Among the 17877 photos that make up our dataset, 80% (14296) are designated for training purposes, while the remaining 20% (3581) represent validation. A random value of 1e-3 is assigned to the learning rate hyper parameter, which controls the rate at which parameter updates are performed. If set too low, classification



performance proceeds slowly; if too high, the model may miss local minima and diverge. We use the RMSprop optimizer algorithm for parameter updates during training. The Adam optimizer is used, which is a popular optimizer for training deep neural networks. The categorical cross-entropy loss function is used, which is commonly used for multiclass classification problems. We implement the training and testing using the Python-based Keras library and the TensorFlow framework and follow the steps:

Step 1: Define the parameters for training the model: train_data, test_data, target_size, batch_size, class mode, optimizer, loss function, and metrics.

Step 2: Instantiate ImageDataGenerator objects for train_datagen and test_datagen.

Step 3: Create train and test generators using the flow_from_directory method of ImageDataGenerator objects and the parameters defined in Step 1.

Step 4: Import ResNet101V2 architecture from Keras applications. resnet_v2 and set its layers as untrainable.

Step 5: Create a Sequential model and add ResNet101V2 architecture to it, followed by a global average Pooling2D layer, a Dense layer with 128 units and 'ReLU' activation function, a Batch Normalization layer, and a Dense layer with 16 units and 'SoftMax' activation function.

build the sequential model by adding the layers in the following order:

- i. ResNet model
- ii. Global Average Pooling 2D layer: This layer pools the feature maps produced by the ResNet model by averaging each feature map across its spatial dimensions. This results in a single feature vector for each image.
- iii. Dense layer with 128 neurons and ReLU activation
- iv. Batch Normalization layer: This layer normalizes the inputs to the next layer in order to reduce the internal covariate shift problem during training.
- v. Dense layer with 16 neurons and SoftMax activation, which is the output layer of the model.
- Step 6: Compile the model using the optimizer, loss function, and metrics defined in Step 1.

Step 7: Train the model using the fit method of the model object and the generators created in Step 3, for the number of epochs specified in Step 1.

Step 8: Evaluate the trained model on the test data using the evaluate method of the model object and print the test accuracy. The ResNet101V2 model, with the following parameters:

- i. include_top=False means that the fully connected layers at the top of the model are not included, as we will be adding our own layers later.
- ii. weights='imagenet' means that the pre-trained weights on the ImageNet dataset will be used.
- iii. input_shape= (224, 224, 3) specifies the shape of the input images.

3.2 Bacteria Classification Results

In this section, we present the classification results for the bacteria image identification system. These results are based on experiments using CNN. We evaluate the performance of the model using accuracy, sensitivity, and specificity as indicators. The classification outcomes for the proposed system are summarized in Table 1.

The model was trained on a dataset of 16 classes using ResNet101V2 architecture with transfer learning. The model achieved a training accuracy of 98.66% and a sensitivity of 93.55%. And specificity of 93.54%. The training and validation accuracy and loss were plotted using Matplotlib, and it was observed that the model achieved a high accuracy and a low loss on both the training and validation sets, as shown in Figure 6. Finally, a confusion matrix was created using the test set,



and it was plotted using Matplotlib. The confusion matrix, as shown in Figure 7, illustrated the number of true positives, true negatives, false positives, and false negatives for each class, indicating how well the model performed in classifying each class. Overall, the model achieved high accuracy and performed well in classifying the different classes. In Table 2, we present the evaluation scenario alongside other benchmarked studies to ensure a fair comparison; our experiments utilize the same dataset and hyper parameter tuning settings.

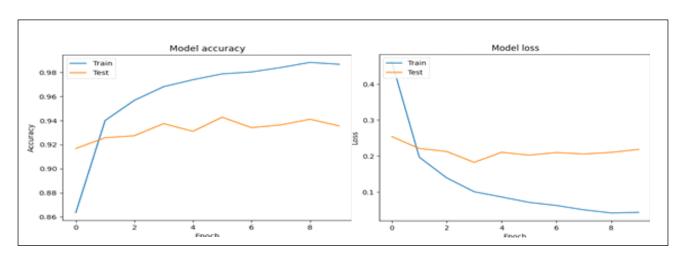
Table 1Deep Learning Model Results

Deep Learning Models	Accuracy %	Sensitivity%	Specificity%
CNN	98.66%	93.55%	93.54%

Table 2Comparison with benchmarked Studies

(a)

Study	Method	Accuracy (%)	Sensitivity (%)
[18]	CNN- Naive Bayes	95.5%	N/A
[19]	CNN, SVM and	97.24%	97.24%
	Random Forest		
[20]	Bag-of-Words and SVM	97%	N/A
Our proposed method	CNN	98.66%	93.55%



(b) **Fig. 6.** (a) Model accuracy, (b) Model loss



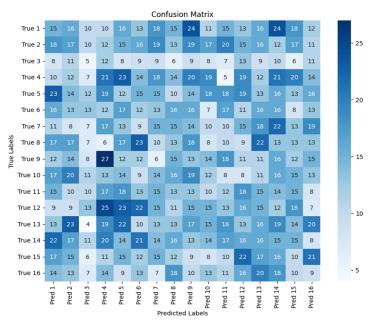


Fig. 7. Confusion matrix results

4. Conclusion

In our study, we propose a computer-aided approach for bacterial Classification to aid in disease diagnosis. We employ deep-learning neural networks to tackle this classification problem. Specifically, we utilize deep residual networks, including ResNet101V2, for feature extraction and Classification., we validate its performance using a confusion matrix. Our approach achieves an impressive accuracy of 98.66%, a sensitivity of 93.55% and a specificity of 93.54% in classifying 16 bacterial categories using the ResNet101V2 CNN model. Comparative analysis with existing works demonstrates the superior accuracy of our approach.

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