

Multi Classification of Bacterial Microscopic Images using Inception V3

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Abstract

Microorganisms such as bacteria are the main cause of various infectious diseases such as cholera, botulism, gonorrhea, Lyme disease, sore throat, tuberculosis and so on. Therefore, identification and classification of bacteria is very important in the world of medicine to help experts diagnose diseases suffered by patients. However, manual identification and classification of bacteria takes a long time and a professional individual. With the help of artificial intelligence, we can effectively and efficiently classify bacteria and save a lot of time and human labor. In this study, a system was created to classify bacteria from microscopic image samples. This system uses deep learning with the transfer learning method. Inception V3 architecture was modified and retained using 108 image samples labeled with five types of bacteria, namely *Acinetobacter baumannii*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Propionibacterium acnes* and *Veionella*. The data is then divided into training and validation using the k-fold cross validation method. Furthermore, the features that have been extracted by the model are trained with the configuration of *minibatchsize* 5, *maxepoch* 5, *initiallearnrate* 0.0001, and validation frequency 3. The model is then tested with data validation by conducting ten experiments and getting an average accuracy value of 94.42%.

Keywords: Bacterial Classification; Deep Learning; Inception V3; Transfer Learning; Image Processing

Introduction

According to the Food and Agriculture Organization (FAO), the number of victims who die from bacterial infections reaches up to 700,000 people every year. Recognition of bacterial genera and species is necessary because knowledge of the biology of microorganisms is significant in medicine, veterinary medicine, biochemistry, the food industry, and agriculture. Although most microorganisms have a positive impact on various areas of life, they can cause many diseases, including infectious diseases [1] [2].

Biologists identify and classify different types of bacteria with different biochemicals and forms. They used different bacterial attributes for classification. For example, the shape of bacterial cells (spiral, cylindrical and spherical) the size and structure of the colonies formed by bacteria are examined to distinguish bacterial species. Cells of several types of bacteria have different sizes and structures depending on environmental conditions. Several species of bacteria have very similar shapes. Although each bacterial species has its characteristics, the biochemical reactions carried out by bacteria and their metabolic activities together help classify the species. However, the classification of bacterial species is not an easy task even for an experienced specialist [3] [4]. Song, et al. have proposed analysis for Bacterial Vaginosis (BV) images from a microscope. They proposed an automatic method to diagnose BV with several stages, i.e., segmentation, splitting, and classification of overlapping bacteria image cases. Following that, the implementation of the Nugent score criterion was used in their experiment demonstration and achieved high accuracy with computing efficiency [5].

In general, microbiological image analysis using traditional laboratory methods has bacteria recognition errors and requires different experience and long processing time. Therefore, the automatic classification technique of bacterial images is more valuable than traditional visual observations for biologists because of its accurate classification, low cost, and fast diagnosis Previous research related to bacterial classification was carried out in 2018 by Basma, et al. conducted a study by classifying ten types of bacteria using the Bag of words feature extraction and Support Vector Machine (SVM) method with 97% accuracy [6].

Identifying a bacterium is a laborious process. Some of the studies used algorithms to classify with promising alternatives [7]. Kukula, et al. used Convolutional Neural Network based approach paired with Raman spectroscopy to detect and recognize the bacteria class rapidly. The result shows 86% of accuracy with identification speed close to real-time [8]. In 2019 Treesukon, et al. conducted a study by classifying two types of bacteria using a python-based deep learning method and the LeNET architecture with more than 75 percent accuracy [9]. In 2018 Lei Huang used the CNN method with the AlexNET architecture to classify 18 categories of bacteria with an accuracy of 73% [10].

This study has classified bacteria using the CNN deep learning method with the InceptionV3 architecture. InceptionV3 architecture was used because, in previous studies, it was widely used to classify with reasonably high accuracy results, such as the classification of patients with COVID-19 disease [11] and the classification of patients with lung disease [12].

Method

A convolutional neural network (CNN) is an architecture that can recognize predictive information of an object such as images, text, sound snippets, and so on. CNN is a development of multilayer perceptron (MLP), which is designed to process data in images. CNN is included in the type of deep neural network because of its high network depth and is widely applied to image data. Research on CNN was first conducted by Hubel and Wiesel on the visual cortex in the sense of sight of cats. CNN architecture consists of several layers, namely convolution [13].

Inception V3 uses less computing power by modifying the previous Inception architecture. This idea was proposed in the paper Rethinking the Inception Architecture for Computer Vision, published in 2015. Christian Szegedy co-authored the statement, Vincent Vanhoucke, Sergey Ioffe, and Jonathon Shlens. Compared to VGGNet, Inception Networks (GoogLeNet / Inception v1) is proven to be more computationally efficient, both in terms of the number of parameters generated by the network and the cost of memory and other resources. If any changes are to be made to the Inception Network, they must be carefully made to ensure that the computational advantage is not lost. Thus, the adaptation of the Inception network to different cases turned out to be a problem due to the uncertainty of the efficiency of the new network. In the Inception v3 model, several techniques for optimizing the network have been suggested to reduce the constraints to make model adaptation easier. These techniques include factorized convolutions, regularization, dimension reduction, and parallelized computations [14]. The architecture of Inception V3 can be seen in Fig. 1.

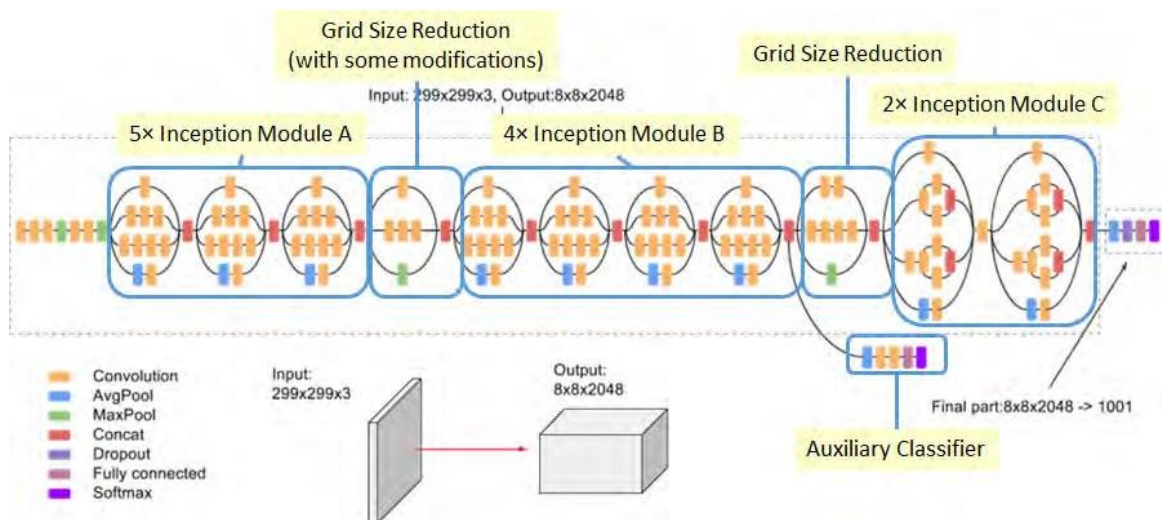


Figure 1. Inception V3 Architecture [15]

A system for classifying bacteria is divided into three essential stages. Before describing the important stages in the development of this system, this study also describes the data sources used. The data were taken in 20 images for each class of bacteria with a resolution of 2048 x 1532. There are 108 images which will later be divided into training and validation data. Bacterial image data obtained through the DIBaS source (<http://misztal.edu.pl/software/databases/dibas/>). This data is an image of bacteria that has gone through the gram staining process and was taken with an Olympus CX31 microscope equipped with an SC30 camera. The data consists of 108 images of five bacteria classes, namely *Acinetobacter baumannii*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Propionibacterium acnes*, and *Veionella*. Examples of training data for each class of bacteria can be seen in Fig. 2.

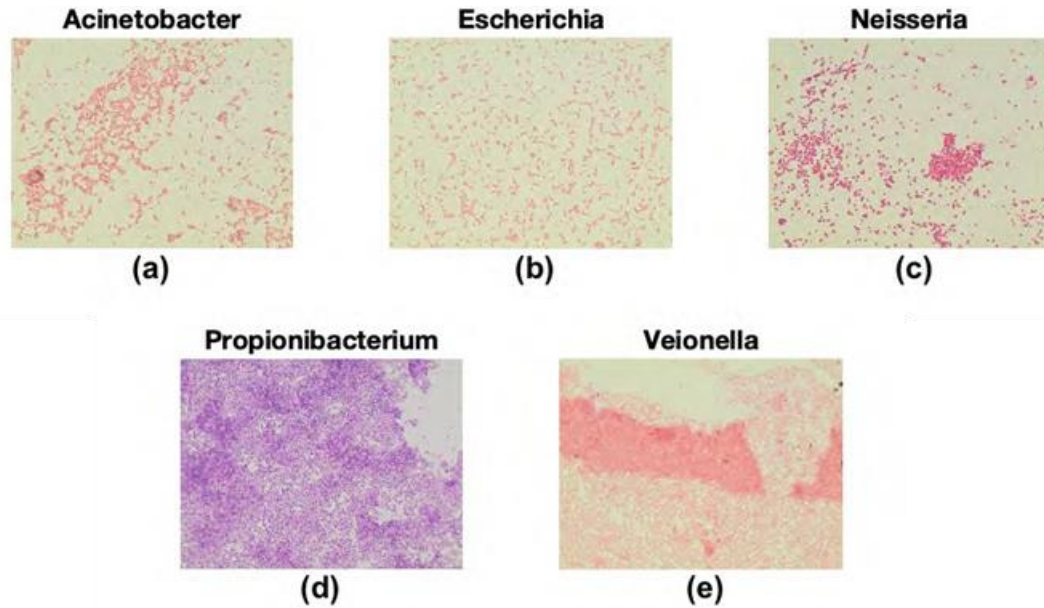


Figure 2. Sample data of (a) *Acinetobacter baumannii*, (b) *Escherichia coli*, (c) *Neisseria gonorrhoeae*, (d) *Propionibacterium acnes*, (e) *Veionella*.

The characteristics or features of each class of bacteria used in the training data are as follows:

- **Acinetobacter.** It has rod-shaped or is included in the bacilli category with a coccobacillus shape (ellipse shape) and has a gram-negative color (reddish-pink).
- **Escherichia.** The shape is included in the bacilli category with the shape of a bacillus (stem) and has a gram-negative color (reddish-pink).
- **Neisseria.** The shape belongs to the cocci category with a diplococci shape (a pair of circles) and has a gram-negative color (reddish-pink).
- **Propionibacterium.** The shape is included in the bacilli category with the shape of a bacillus (rod) and has a gram-positive (violet) color.
- **Veionella** belongs to the cocci category with a diplococci shape (paired circle) and has a gram-negative color (reddish-pink).

After the dataset is ready for use, there are three stages that the system will pass to arrive at the expected classification results, namely the training, validation, and testing processes, as shown in Fig. 3. According to Fig. 3, training and validation process used a preprocessing stage. Before the InceptionV3 pretrained network is used, the last two layers of the architecture, namely the predictions layer and the *ClassificationLayer_predictions* layer, will be adjusted first to classify the dataset used, namely a dataset consisting of five types of bacterial classes. Before the layer modification process is carried out, the two layers to be changed are first searched using the *findLayersToReplace* function. After the layer is found, the predictions layer is modified to match the number of dataset classes used, namely five types of bacterial classes. Furthermore, this *ClassificationLayer_predictions* layer still contains the class label of the pretrained network, so it will be replaced with the *new_classoutput* layer, which does not have a class label. The results of layer adjustments on the InceptionV3 architecture can be seen in Fig. 4.

It can be seen in Fig. 4 the last two layers at the bottom have been adjusted to classify five types of bacteria and changed their names to *predictions_softmax* and *new_classoutput*. The image data will then go through a labelling process which is carried out to divide the data into five classes which will later be used in the training and validation process. The amount of data and its labels can be seen in Table 1. After that, the training and validation data splitting process was carried out using the k-fold cross-validation method with k=5. The results of this data division can be seen in Fig. 5. K-fold cross-validation is carried out so that each image in the dataset has the opportunity to become training and validation data so that later we get the best dataset to produce the highest accuracy.

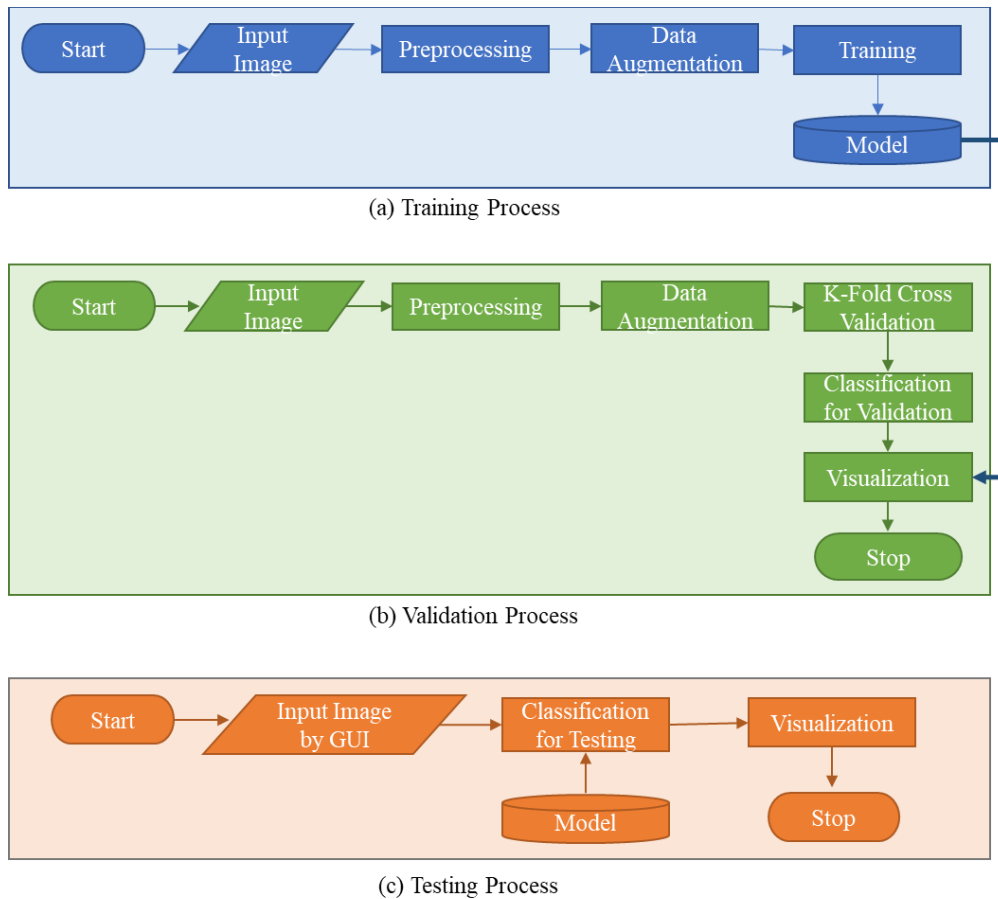


Figure 3. Flowchart of System Design using Inception V3 (a) Training Process, (b) Validation Process and (c) Testing Process

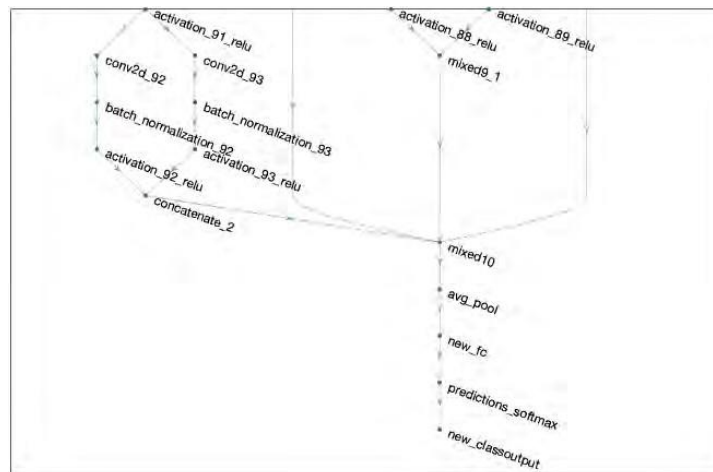


Figure 4. The illustration of Inception V3 Layer Adjustment

Table 1. The Quantity of Datasets based on Five Classes

Class	Label	Count
1	<i>Acinetobacter baumannii</i> ,	20
2	<i>Escherichia coli</i> ,	20
3	<i>Neisseria gonorrhoeae</i> ,	20
4	<i>Propionibacterium acnes</i>	20
5	<i>Veionella</i>	20

```

i = 1
Numberoftraindata = 86
Numberofvalidationdata = 22
i = 2
Numberoftraindata = 87
Numberofvalidationdata = 21
i = 3
Numberoftraindata = 86
Numberofvalidationdata = 22
i = 4
Numberoftraindata = 86
Numberofvalidationdata = 22
i = 5
Numberoftraindata = 87
Numberofvalidationdata = 21

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Figure 5. Distribution of training and validation data using k-fold cross validation

Data Augmentation provides more variety of data in the network by performing various random transformation processes such as *RandXReflection*, *RandXTranslation*, and *RandYTranslation* on each training data and giving it to the network, so that later the network contains multiple dataset variances. As for the amount of data, data augmentation produces the same amount of data as the process without data augmentation. Hence, this augmentation data will provide more data variance. In the training process, each epoch iteration will provide various data variances transformed randomly with *RandXReflection*, *RandXTranslation*, and *RandYTranslation*. In addition, a resizing process is also carried out to change the image size to 299x299 pixels. This is done because the InceptionV3 architecture *InputLayer* requires an image size of 299x299 pixels. The results of the data augmentation preview (eight images) can be seen in Fig. 6.

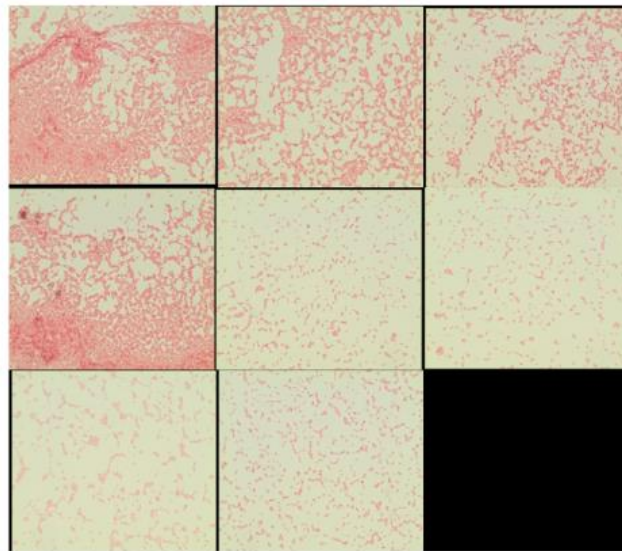


Figure 6. Augmentation results with different parameters

Inception modules are the core block of this transfer learning model. There are 11 inception modules, ranging from *mixed0* to *mixed10*. Two hundred fifty-six channel features with a resolution of 35x35 are generated by the inception module in the *mixed0* layer. Furthermore, the convolution process will further develop along with the increase in layers to extract more abstract features. This convolution layer will produce 768 and 2048 channel-feature maps of 17x17 and 8x8. Finally, all feature extraction results are combined into a one-dimensional vector with an output of five neurons according to the number of data classes, namely five bacterial classes. The neuron with the highest probability will be the class label of the bacterial image used as test data.

A. Training Process

The training process is carried out using the transfer learning method with a *minibatchsize* configuration of 5, epoch 5, and a learning rate of 0.0001. A lot of training and validation data is used following the distribution of the

dataset through a k-fold cross-validation process with k=5 so that it will be repeated five times. The explanation of the parameters used in the configuration of the training process:

- *MiniBatchSize*

This parameter is a measure of the mini-batch that is used in each iteration of the training process. The mini-batch is the part of the training process used to evaluate the gradient of the loss function and update the weights.

- *MaxEpoch*

This parameter is the maximum number of epochs used in the training process. Iteration is the step taken by the gradient descent algorithm to minimize the loss function with batches. The epoch is the full path of the training algorithm across the training set.

- *InitialLearnRate*

This parameter indicates the *initiallearnrate* used in the training process. Three kinds of solvers can be used, namely *sgdm*, *rmsprop* and *adam*, the solver used in this training process is the *sgdm* solver. The training process will take longer when the learning rate is too low. On the other hand, when the learning rate is too high, the training process will be faster, but the training results may give less than optimal or distorted results.

- *ValidationFrequency*

This parameter indicates the number of iterations performed for the network validation process in each epoch.

- *Model*

The output of the training process is a model that will later be used for the validation process using data validation that has been divided through the k-fold cross-validation process.

B. Validation Process

This validation process is carried out to see the loss value and model performance resulting from the classification in the training process. The classify function will generate matrix scores of 21x5, which contains the accuracy of the five dataset classes. The highest accuracy class will be used as a data label from validation data that has been classified and then stored in the *ypred* matrix with a size of 21x1.

When executed, the classify function will classify the validation data in the *uugimdsvalidation* cell using a network net. This cell contains 21 data validation images. A label is a matrix of size N-of-1 where N is the number of validation data, and scores is a matrix of size N-of-C, where N is the number of validation data and C is the number of classes of the dataset.

The process of calculating the probability score for multiclass using the classify function in Equation 1 below.

$$P(c_r | x, \theta) = \frac{P(x, \theta | c_r) P(c_r)}{\sum_{j=1}^k P(x, \theta | c_j) P(c_j)} = \frac{\exp(a_r(x, \theta))}{\sum_{j=1}^k \exp(a_j(x, \theta))} \quad (1)$$

Where $0 \leq P(c_r | x, \theta) \leq 1$ dan $\sum_{j=1}^k P(c_j | x, \theta) = 1$

In addition, a_r is the conditional probability of the sample by class r and $P(c_r)$ is the probability of the previous class. The probability score calculation process is in the range 0 – 1, and the class with the highest probability will be the label of data validation.

C. Testing Process

- *Input Image*

The data used for the testing process differs from the training and validation data dataset. For each session of the testing process, the maximum number that can be classified is one image of bacteria.

- *Classification*

The classification stages similar with data validation process

- Visualization

The classification results will display the original image, the Grad-CAM visualization, and the prediction and accuracy results of the testing data input.

- GUI (Graphical User Interface)

The GUI in this system plays a role in facilitating the user in classifying bacteria. The user inputs an image of the bacteria classified by clicking the "browse" button, then clicking the "prediction" button. The system will classify bacteria based on the network that has been trained, and will provide output in the form of prediction results, percentage accuracy, and Grad-CAM visualization.

D. Metric Evaluation

In measuring the performance of the bacterial classification system, it can be done by calculating the prediction performance made by design on the input image data in the training process using the help of a confusion matrix. The Confusion Matrix consists of four parts, namely:

- True Positive (TP). Represents the number of correct predictions from positive data.
- False Positive (FP). Represents the number of false predictions from positive data.
- True Negative (TN). Represents the number of correct predictions from negative data.
- False Negatives (FN). Represents the number of false predictions from negative data.

To calculate the system accuracy value, it can be done with the following Equation 2:

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (2)$$

Results and Discussion

This deep learning bacterial classification system uses a transfer learning-based model with the Inceptionv3 architecture. The data used are 108 images consisting of five classes of bacteria, namely *Acinetobacter baumannii*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Propionibacterium acnes* and *Veionella*. This data is then divided into training and validation data using the k-fold cross-validation method with the number of folds k=5, producing 87 images for training data and 21 for data validation. Before the data is trained, the data augmentation process is carried out with the transformation of *RandXReflection*, *RandXTranslation*, and *RandYTranslation*. The training process will provide more variety of datasets into the network.

In this study, three testing scenarios were carried out, starting with the first experiment using the Inceptionv3 architecture with k-fold cross-validation. The second experiment used the Inceptionv3 architecture without k-fold cross validation then divided the training validation data with a percentage of 70:30. The third experiment uses the inceptionv3 architecture with k-fold cross validation without augmentation. The last is a comparison of the performance of the inceptionv3 architecture with seven other architectures, namely *googlenet*, *vgg16*, *vgg19*, *resnet101*, *inceptionresnet2*, *squeezenet* and *xception*.

The training configuration in each experiment uses the same parameters, namely *minibatchsize*, *maxepochs*, *initiallearnrate*, and *validationfrequency*. The values used for each of these parameters are *minibatchsize* 5, *maxepoch* 5, *initiallearnrate* 0.0001, and *validationfrequency* 3. Each experiment was trained five times to see the average performance and tested with data testing.

A. First Scenario

In the first experiment, the system was built using the Inceptionv3 architecture with k-fold cross-validation. The results of the five-time trial training can be seen in Table 2.

Table 2. *InceptionV3 with K-fold cross validation*

Training	Time	Accuracy
1	7 minutes 8 seconds	95.45%
2	7 minutes 23 seconds	90.48%
3	7 minutes 39 seconds	95.45%
4	7 minutes 39 seconds	95.45%
5	7 minutes 29 seconds	95.29%
Average of Time Training		7 minutes 27 seconds
Average of Accuracy		94.42%

B. Second Scenario

In the second, the system was built using the Inceptionv3 architecture without k-fold cross-validation. The training and testing data is divided by the percentage of 70:30. The results of the five-time trial training can be seen in Table 3.

Table 3. *InceptionV3 without K-fold cross validation*

Training	Time	Accuracy
1	8 minutes 3 seconds	90.91%
2	7 minutes 54 seconds	93.94%
3	7 minutes 1 seconds	93.94%
4	7 minutes 42 seconds	84.85%
5	8 minutes 11 seconds	81.82%
Average of Time Training		7 minutes 49 seconds
Average of Accuracy		89.09%

C. Third Scenario

In the last experiment, the system was built using the Inceptionv3 architecture with k-fold cross validation without the augmentation process. The results of the five-time trial training can be seen in Table 4.

Table 4. *InceptionV3 without augmentation*

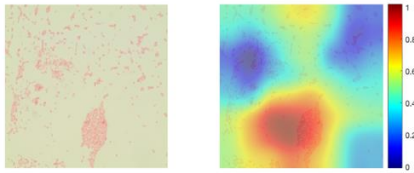
Training	Time	Accuracy
1	7 minutes 9 seconds	72.73%
2	7 minutes 4 seconds	85.71%
3	7 minutes 10 seconds	95.45%
4	7 minutes 9 seconds	90.91%
5	7 minutes 16 seconds	95.24%
Average of Time Training		7 minutes 9 seconds
Average of Accuracy		88.01%

Based on the results of the three scenarios, it can be seen that in the first experiment, the average accuracy was higher than in the second experiment. Due to the influence of k-fold cross-validation, it increased the system's accuracy. By using k-fold cross-validation, every data in the dataset has the opportunity to become training and validation data so that the best dataset is obtained that produces the highest accuracy.

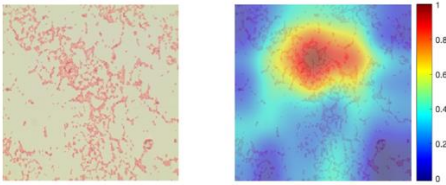
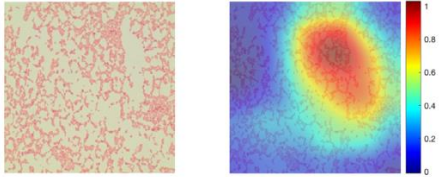
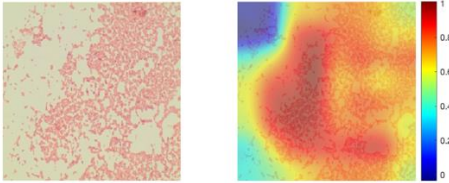
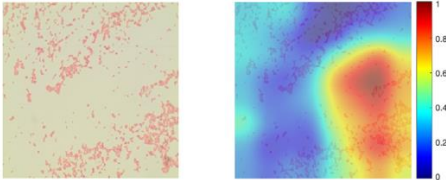
Finally, in the third experiment, it can be seen that if you don't use augmentation on the system, the accuracy of the system will decrease due to the lack of data variation in the training process so that the resulting network is not optimal. The system in these three experiments was made to classify four types of bacteria: *Acinetobacter baumannii*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Propionibacterium acnes*, and *Veionella*.

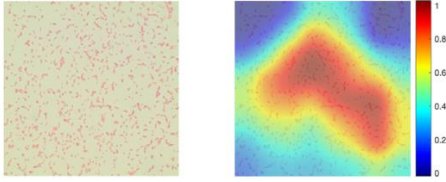
In the first experiment, it can be seen that in the confusion matrix, there is one false positive or misclassification, namely in the data validation classification of *Escherichia coli* bacteria, which then the system classifies as *Acinetobacter baumannii* bacteria. The two bacteria have similar characteristics, namely gram-negative colour and have a similar shape, namely the shape in the bacilli category. This wrong classification process can be seen with the Grad-Cam visualization in Table 5. As for the visualization of the results of the correct bacterial classification, it can be seen in Table 6.

Tabel 5. Classification Error Results

Data Validation	Visualization
8	<p style="text-align: center;">Acinetobacter</p> 

Tabel 6. The Correct Classification

Data Validation	Visualization
1	<p style="text-align: center;">Acinetobacter</p> 
2	<p style="text-align: center;">Acinetobacter</p> 
3	<p style="text-align: center;">Acinetobacter</p> 
4	<p style="text-align: center;">Acinetobacter</p> 

Data Validation	Visualization
5	<p style="text-align: center;">Escherichia</p> 

Conclusion

This study has carried out the analysis of bacterial classification using deep learning. It can be concluded that of the three experiments that have been carried out, the bacterial classification system with the InceptionV3 architecture with k-fold cross-validation and augmentation has the highest average accuracy. This system can classify five types of bacteria, namely *Acinetobacter baumannii*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Propionibacterium acnes* and *Veionella*. The experimental results of the bacterial classification system with the InceptionV3 architecture with k-fold cross-validation and augmentation using the *minibatchsize* 5, *maxepoch* 5, *initiallearnrate* 0.0001, and *validationfrequency* 3 training configurations get an average validation accuracy value of 94.42% with an average training time of 7 minutes 27 seconds.

In the future, the system can be developed by adding the types of classified bacteria to classify more types of bacteria. In addition, the system that has been made can also be modified to classify other objects to overcome different problems.

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