Rapid bacterial colony classification using deep learning

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Article Info

ABSTRACT

Bacterial colonies infection is one of the causes of bloodstream disease, and it can be a fatality. Therefore, medical diagnoses require fast identification and classification of organisms. Artificial Intelligence with deep learning (DL) can now be developed as a rapid bacterial classification. The research aims to combine deep learning and support vector machines (SVM). The ResNet-101 model of the DL algorithm extracted the image's features using transfer learning then classified by the SVM classifier. According to the experimental results, this model had 99.61% accuracy, 99.58% recall, 99.58% precision, and 99.97% specificity. The technique presented might enhance clinical decision-making.

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1. INTRODUCTION

Bacterial colonies infection is a prevalent disease that infected human bloodstream and made chiefly mortality in several countries [1]. In past, microbiologist was classified the bacterial species through conventional microscope where needs hard skills and takes more time [2], [3]. However, that approach obviously could be delayed treatment and antimicrobial therapy that can fatality [4]. Therefore, rapid diagnose is significant issues to improve quality of bacterial detection. Current computer-aided diagnosis (CAD) based image processing and machine learning proposed bacterial classification in clinical microbiology accurately. Besides, CAD utilized several machine learning (ML) approach to classify the various bacterial species that used some classifiers such as support vector machine (SVM), backpropagation neural network (BPNN), real AdaBoost, and modest AdaBoost [5]-[13]. However, the CAD process includes traditional methods such as handcrafted features [14]. Recently, deep learning (DL) a subset of machine learning [15]-[17], that have an advantages for medical image analysis[18]-[26]. The beneficial of deep learning is proposed non-handcrafted approach [17]. That utilized numerous convolutional neural network (CNN) layers, it is possible to enhance the classification performance, particularly to improve decision-making. As result, Lopez *et al.* proposed constructing 3x3 convolutional layers for mycobacterium tuberculosis image classification which obtained receiver operating characteristic (ROC) curve of 99% [27]. In addition, high sensitivity score of 83.78% has achieved to classify tuberculosis bacteria images by Kant *et al.* who developed five-layered fully-convoluted neural network architecture combined with a cascading method [28]. Furthermore, Kim *et al.* has proposed an experiment utilizing the CNN model based on the pre-trained DensNet framework, that used to classify bacterial image on optical diffraction tomography (ODT) and is achieved an accuracy of 85% [29]. Therefore, CNN architec-

tures are proven improving the accuracy of classification performances without handcrafted features approach. However, during training the dataset using convolutional layer, that possible generates overfitting and time-consuming [30]-[32]. On the other hand, the experimental conducted by Zielinski *et al.* and Wahid *et al.* who are proposed hybrid DL and ML approach to classify the bacterial species that significant enhance the accuracy rate [33], [34]. The advantages of hybrid method is reduce misclassification and rapid processing. In addition, the success of the classification task, that can be determined by a robust classifier algorithm. Based on the literature, a reliable classifier algorithm is the SVM classifier [8], [34]. Hence, this study proposed to modify the hybrid method for enhancing the accuracy classification performance and prediction score. In addition, all coding generates on graphics processing unit (GPU) it is to optimize the training process [35], [36]. Further-more, this study is also provided comparing performance between the classical transfer learning approach and the proposed method.

2. MATERIAL AND METHODS
2.1. Dataset preparation
The datasets were collected from the Parasitology Laboratory at Universiti Sains Malaysia. During data collection, the microbiologists provided class labeling of the bacterial species. Figure 1 shows image classes of six bacterial organism samples (*Burkholderia pseudomallei sp*, *Hemophilus influenzae sp*, *Klebsiella pneumoniae sp*, *Pseudomonas aeruginosa sp*, *Staphylococcus aureus sp*, and *Streptococcus pneumoniae sp*). The bacterial samples were provided using the gram stain reaction. The samples are included positive cocci, diplococci, and negative bacilli. A Olympus BX40 light microscope series is used to magnify the bacterial object on Gram-stained slides under 40x magnification. The datasets are provided 44,985 images with the pixel size 244x244 pixels size.
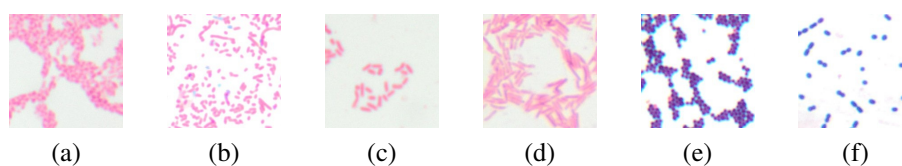

| (a) | (b) | (c) | (d) | (e) | (f) |

Figure 1. Bacterial organism of (a) *Burkholderia pseudomallei sp*, (b) *Hemophilus influenzae sp*, (c) *Klebsiella pneumoniae sp*, (d) *Pseudomonas aeruginosa sp*, (e) *Staphylococcus aureus sp*, and (f) *Streptococcus pneumoniae sp*

2.2. Data augmentation
Recently, DL techniques are most popular in medical fields, such as biomedical imaging [18]. DL needs an enormous amount of dataset for the learning process. If the resulting data were too narrow or too small, it could add sampling bias towards the bulk of each class [32]. Thus, the DL model with low quantities of data can obtain poor performance results [37]. Rachmadi *et al.* and Mikoajczyk *et al.* are described the data augmentation possible training an efficient model and enhance the learning performance [38], [39]. Therefore, this study was conducted data augmentation to enlarge datasets utilizing transformation image techniques such as, translation, rotation, scaling, flipping, and reflection.

2.3. Transfer learning
Recently, DL perform is provided the transfer learning approach. This is significantly improved computational for training and validation [40]-[42]. In CAD, the advantages are utilized to reducing data labeling, and use existing models [43]. The transfer learning technique is utilized the pre-trained CNN network which involved fine-tuning process on convolution layers. That method applied while freeze the first layer and updating weight on each layer [44]. In the end, the fully-connected and softmax layer is used for classification. This study proposed ResNet-101 pre-trained CNN network, that model lead in ILSVRC 2015 classification task [45].

2.4. Deep feature extraction using CNN
DL was performed in the deep feature extraction by utilizing a pre-trained CNN model. It works by reducing the CNN activation dense dimensionality. Activation layers conduct a deep feature extraction in

a pre-trained network as in [44], [46]. This process resulted in feature vectors from the pre-trained weight model. The forward-backward iteration during the training process identifies the correct weights. Hence, this method can minimize binary cross-entropy (misclassification cost). A pre-trained ResNet-101 model is utilized in this study. The proposed method is provided bacterial image classification that it modified the deep feature extraction technique. Figure 2 shows the architecture model. The method is extracted features from the early convolution layers. It showed on conv1 layer that extracted the shallow features. In these case, the feature is tend to be shallower, more localized, and have a better spatial resolution on earlier layers. Furthermore, the max-pooling layer is added to the lower layer to perform subsampling. Finally, multiple feature maps are normalized using local response normalization, later, the new feature was classified. The classification layer on pre-trained network was discard and change by using SVM classifier.
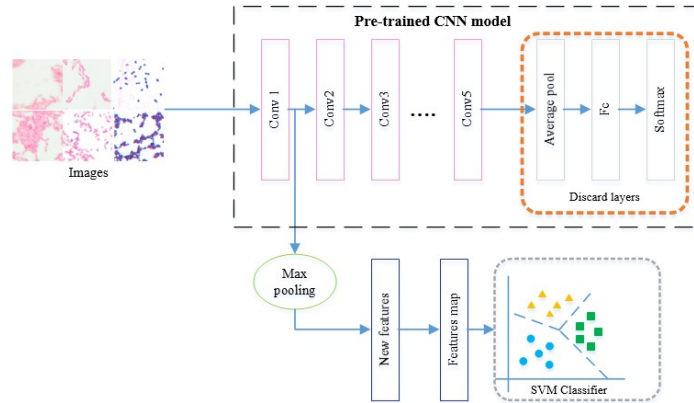

Figure 2. The proposed method modified CNN deep feature extraction

2.5. Classifier
2.5.1. Softmax classifier

The last step of the generated CNNs model was to extract vectors in fully connected layers. Additionally, the fully connected layers affected the performance of CNN models with a linear feature variable was present by [47]. Thus, those features were necessary to be classified using a softmax classifier as a linear classifier. On the other hand, a softmax classifier could also be used for multi-class classification in binary classification tasks that generalized the logistic regression model. In hinge loss functions describes by [48], a mapping function h is denoted as the dot product of x_i as data input and weight matrix of w, as (1):

$$h(x_i, w) = w.x_i \tag{1}$$

besides, output values were represented as unnormalized log probabilities to class tags, unlike in the hinge loss function. Those output values were changed with the cross-entropy loss function as shown in (2) and the output in (3).

$$E_i = -log\frac{e^{k_{yi}}}{\sum_j e^{k_j}} \tag{2}$$

$$k = h(x_i, w) \tag{3}$$

2.5.2. SVM classifier

An effective classifier is the SVM classifier. Goals for separating of multi-class with the selection of a hyperplane significantly increase margin using optimization explained by [27], SVM consists of finding the solution:

$$
\begin{aligned}
&\min_{w,b,\zeta_i} F(\vec{w}, \vec{\zeta}) = \frac{1}{2}w^T w + C\sum_{n=1}^{N} \zeta i \\
&st.y_i[w^T \varphi(x_i) + b] \geq 1 - \zeta_i \\
&\zeta_i \geq 0, n = 1, ..., N
\end{aligned}
\tag{4}
$$

where N, x_n is a training vector with labels y_i. Thus, the weight vector, bias, and slack variable are $w, b,$ ζ_i. Meanwhile, $C \geq 0$ is a constant error rate, then φ is the non-linear kernel function. At the same time, SVM intends to minimize the classification error and maximize the margin size by determining an optimal hyperplane. As known, Lagrange multipliers can resolve the optimization issues by modifying the primal space to dual space, as (5):

$$L(a) = \sum_{n=1}^{n} \alpha_i - \frac{1}{2} \sum_{n=1}^{n} \sum_{n=1}^{n} \alpha_i \alpha_i y_i y_{ji} x_i^T x_j \tag{5}$$

which subjects to:

$$\sum_{n=1}^{n} \alpha_i y_i = 0, 0 \geq \alpha_i \geq c, i = 1, 2, ..., n \tag{6}$$

in a non-linearity state, $\varphi(x_i)$ as a mapping function can replace x into a high dimensional feature. For the kernel function, SVM can use $k(x_i x_j) = \varphi(x_i x_j)$ to increase the optimization process. Hence, it can be defined as in (7).

$$L(a) = \sum_{n=1}^{n} \alpha_i = \frac{1}{2} \sum_{n=1}^{n} \sum_{n=1}^{n} \alpha_i \alpha_i y_i y_j k(x_i x_j) \tag{7}$$

2.6. Computational hyperparameters

The domain of hyperparameters is considered to train the dataset using pre-trained CNN models. The Adam optimization algorithm as an optimizer is used to optimized for training the models. Instead of the classical stochastic gradient descent procedure, the Adam optimizer possibly creates efficient computational power and utilizes little memory that showed by [49]. The training of the dataset was conducted by using MATLAB software and supported by RTX 2070 Super GPU series with 32 GB memory. Table 1 shows setup the hyperparameters setting in proposed models.

Table 1. Selected hyper-parameters

Parameter	Optimizer	Learning rate	Epoch	Bacth size
VGG16	Adam	0.1^{e-5}	15	32
VGG19	Adam	0.1^{e-5}	15	32
InceptionV3	Adam	0.1^{e-5}	15	32
ResNet-101	Adam	0.1^{e-5}	15	32

2.7. Evaluation performance

There are numerous efforts in deep learning studies, not all researchers report computational cost, but entirely depend on model assessment. Besides, with the confusion matrix explained of [50], that can assess each model's performance. To determine the percentage of each organism that was correctly classified with statistical parameters, such as recall (positive sample correctly classified), specificity (negative sample correctly classified), and precision (positive class were actually positive). The metric evaluation as follows:

$$recall = \frac{TP}{TP + FN} \tag{8}$$

$$specificity = \frac{TN}{TN + FN} \tag{9}$$

$$precision = \frac{TP}{TP + FP} \tag{10}$$

in (8)-(10) explained the number of predictions in which the classifier correctly predicts. TP is defined the number of cycles correctly classified as true bacterial, and TN denotes the number of cycles correctly classified as false bacterial. FP denotes the number of cycles wrongly correctly classified as false bacterial, and FN denotes the number of cycles wrongly correctly classified as true bacterial.

3. RESULT AND DISCUSSION

Recent years, Lamy *et al.* explained the golden standard used blood culture gram-stained classification based on the shape and color using a microscope [51]. However, those producing are incorrect gram stain interpretation from the reader error of 9% to 45% using standardized criteria evaluation [3]. Besides, due to the limitation influencing delayed diagnosis and treatment, thus that needs an other approaches to handle the problem for expert use. This study develops an AI-based algorithm using deep learning CNN-based for rapid bacterial specimen identification. The proposed method is consist to enhance the classification performance and reduce time-consuming of diagnosis. In the experiment, the recommended pre-trained models such VGGNet (VGG16, VGG19) [33] and InceptionV3 [52] are tested to compare the result between the proposed method using ResNet-101 model. On the other hand, the datasets were divided 70% and 30% for data training and data testing. The first approach, the transfer learning method is used training and testing the datasets. During training, overfitting can be decreased accuracy performance [53]. Hence, the cross-entropy loss function was used to minimize loss and optimizing the classification model [48], [50]. The result of the first approach illustrates in Figures 3-7, which is shown the training period of accuracy and loss graphs from four pre-trained CNN models VGG16, VGG19, InceptionV3, ResNet101. The proposed method in second approach, this study utilized convolution layers to conduct modified deep extraction feature technique, which is the extracted features from the early convolution layers will proceed on SVM classifier. Furthermore, Table 2 shows the accuracy achievement comparison that obtained from three pre-trained CNN model. The high accuracy performance was achieved the proposed method of 99.61% by using ResNet-101 model, which modified deep feature extraction technique. In addition, the best processing time in the first approach is 79.20 m/s, but the proposed method is faster than the first approach, which is 9.19 m/s (see Table 3).
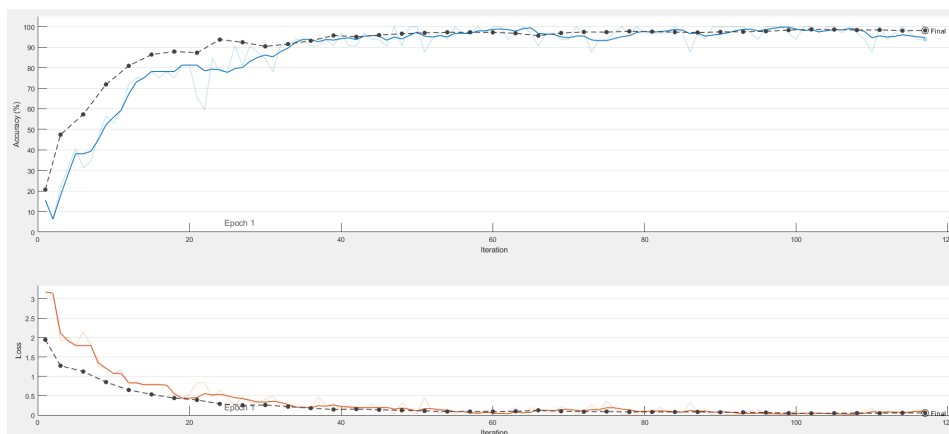

Figure 3. The training and validation graph of accuracy and loss per epoch on VGG16
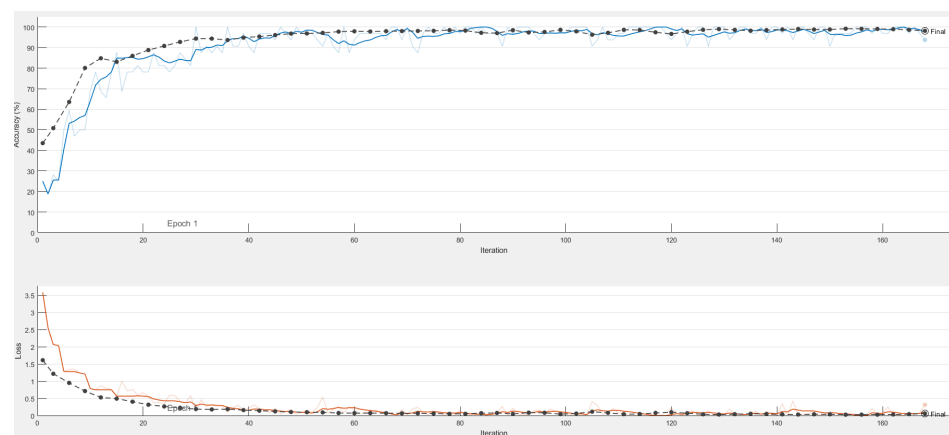

Figure 4. The training and validation graph of accuracy and loss per epoch on VGG19 model
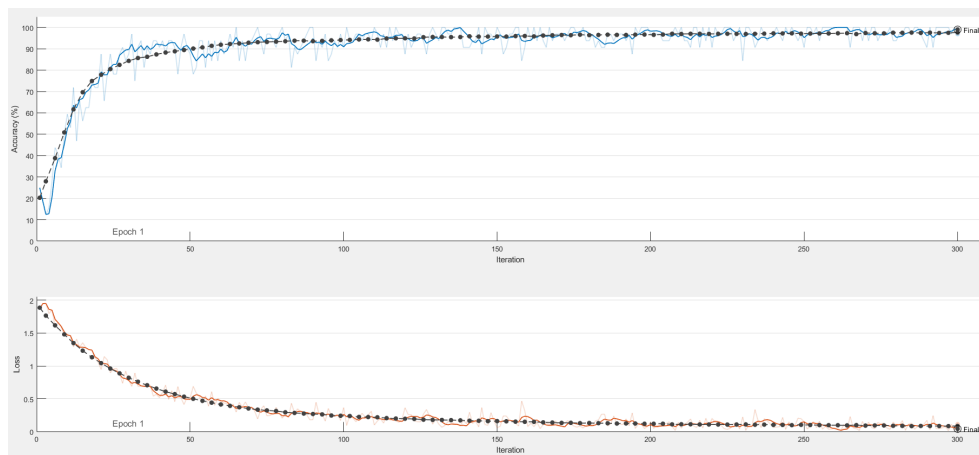
Figure 5. The training and validation graph of accuracy and loss per epoch on InceptionV3 model
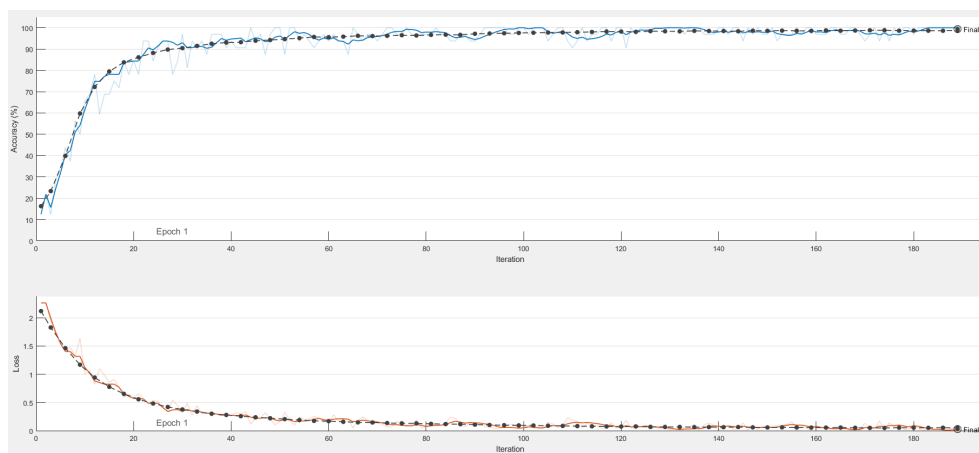

Figure 6. The training and validation graph of accuracy and loss per epoch on Resnet-101 model
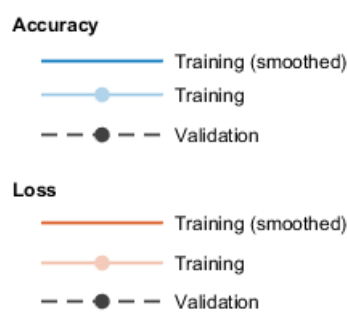

Figure 7. The training and validation curve of accuracy and loss per epoch of each model curve line description

Other evaluated performance parameters were recall, precision, and specificity scores. Table 4 shows the comparison of both method performances. As shown in Table 4, the first proposed method accomplished 99.30% recall, 99.37% precision, and 99.88% specificity. Improved performance was shown in the implementation result of the second proposed method, which increased up to 28% of recall, 26% of precision, and 9% of specificity more than the performance results of the first proposed method. The second proposed method could

reduce the number of false-negative detections in clinical diagnosis to reduce excessive anxiety in the patient being treated. To performance comparison, the accuracy assessment in image classification was used to evaluate the proposed training validation set and the CNN model. The proposed models were compared with other models which utilized gram-stained images. We evaluated the CNN model with various layers configuration. The performance results did not show any significant divergence between models which were previously tested in this study. For instance, this stage are tested the dataset using the method proposed [34], which resulted in only a slight difference in the accuracy performance, which was 0.74%. Nevertheless, different datasets and hyperparameters settings might influence the divergent performance results. Table 5 shows the comparison of proposed model with other methods. the proposed methods performed superiority over other models. Hence, it could serve as an aided tool for bacterial classification based on Gram-stained images.

Table 2. The accuracy of classification

CNN Models	Transfer learning (Acc %)	Deep feature + SVM (Acc %)
ResNet-101	99.34	99.61
InceptionV3	98.98	99.44
VGG19	98.14	98.99
VGG16	97.78	99.05

Table 3. Processing time

CNN Models	Transfer learning (m/s)	Deep feature + SVM (m/s)
ResNet-101	118.28	9.19
InceptionV3	177.00	14.39
VGG19	147.00	48.20
VGG16	79.20	47.24

Table 4. The comparison classification result between CNNs model

2-7 CNN Models	Transfer learning (%)			Deep feature + SVM (%)		
	Recall	Precision	Specificity	Recall	Precision	Specificity
ResNet-101	99.30	99.37	99.88	99.58	99.58	99.97
InceptionV3	98.90	99.00	99.83	99.42	99.15	99.91
VGG19	98.22	98.35	99.70	98.87	98.88	99.85
VGG16	97.62	97.82	99.63	99.02	99.03	99.84

Table 5. The comparison of accuracies performance between the proposed method and other methods

Author	Methods	Accuracy (%)
H. Sajedi, *et al.* [54]	Gabor Feature, XGboost classifier	90.28
K.P. Smith, *et al.* [52]	InceptionV3	94.90
B. Zieliński, *et al.* [33]	VGG, SVM classifier	97.24
F. Wahid, *et al.* [34]	InceptionV3, SVM classifier	98.70
Proposed method 1	ResNet-101, softmax classifier	99.34
Proposed method 2	ResNet-101, SVM Classifier	99.61

4.   CONCLUSION

In this study, rapid classification based deep learning method has been proposed to improve the prediction result and fast recognition of bacterial colony species. The datasets are provided of six bacterial species that caused patient acute infection symtomps. The proposed method is modified deep feature extraction technique using the pre-trained CNN layers, which is extracted features on shallow convolution. Then the classification process conducted using SVM classifier. The proposed method utilized the ResNet-101 CNN architecture for trained and tested the datasets and compared with three CNN models. As results, the classification performance obtained high accuracy of 99.61% rather then use the transfer learning approach. The prediction score involved three matrix parameter measurement that achieved 99.58% recall, 99.58% precision, and 99.97% specificity. Besides, the classification time touched fastest time processing of 9.19 m/s. Hence, this study has been proven to be successful in enhancing classification accuracy and less time consuming. Furthermore, the bacterial colony detector's success in rapid bacterial species classification set this study apart from other

transfer learning studies. In future works, the architecture will be applied for real-time various microorganism species detection.

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