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Deep Learning Approach for Classifying Bacteria types using Morphology of Bacterial Colony

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Abstract— The significant bottlenecks in determining bacterial species are much more time-consuming and the biology specialist's long-term experience requirements. Specifically, it takes more than half a day to cultivate a bacterium, and then a skilled microbiologist and a costly specialized machine are utilized to analyze the genes and classify the bacterium according to its nucleotide sequence. To overcome these issues as well as get higher recognition accuracy, we proposed applying convolutional neural networks (CNNs) architectures to automatically classify bacterial species based on some key characteristics of bacterial colonies. Our experiment confirmed that the classification of three bacterial colonies could be performed with the highest accuracy (97.19%) using a training set of 5000 augmented images derived from the 40 original photos taken in the Hanoi Medical University laboratory in Vietnam.

I. INTRODUCTION

Rapid identifying bacterial species is a common and critical task in various fields, including medical [1], biochemistry [2], the food industry [3], and agriculture [4]. Traditional laboratory methods for bacterial strain identification based on biochemical or modular biology technologies are costly and time-consuming due to the complex sample preparation. They also frequently need a specialist with extensive knowledge and experience in the field. In bioimage informatics, automating the process of bacteria identification is a promising endeavor to bring highly effective solutions for image analysis tasks including object detection, motion analysis, and morphometric features. Deep learning-based image analysis algorithms create faster, more precise, and less expensive computational approaches for classifying bacterial strains.

Deep CNNs were first employed to determine over 20 basic characteristics of bacteria, such as color [5], shape [6], and cell composition, and then combined with a manual classification process. Scientists have improved CNN's ability to perform bacteria classification tasks with a large number of input images in recent years. Nasip et al. [7] utilized the DIBaS dataset to pretrain deep CNN architectures based on the VGGNet and AlexNet models to classify 33 different bacteria. Images of these species with a high resolution were resized into 227×227 (AlexNet input size) and 224×224 (VGGNet input size) to fit the model inputs. The given dataset had $35,600$ images in total and the classification accuracy of VGGNet and AlexNet was announced as 98.25% and 97.53%, respectively. M. Talo's research [8] and Sanskruti Patel [9] also applied deep learning and a transfer learning method [10] to classify bacterial images into 33 categories. For the ResNet-50 structure in Talo's work, the model's performance was evaluated using five-fold cross-validation for 50 epochs in about 31 minutes 48 second, repeated five times on each validation set to calculate the overall classification performance (99.2%). For a pre-trained VGG-16 model in the Sanskruti study, the last block of this model was replaced by an atrous convolution with a dilation rate of two. These models were tested on 660 images and 33 classification classes from the bacterial colony DIBaS dataset. As a result, 99.25% and 94.85% of the test accuracy were achieved, respectively. Mai et al. [11] presented an efficient approach for reliably detecting and classifying related bacterial species in high-resolution microscopy images. The proposed method has two key stages. Data augmentation techniques are used to avoid overfitting and create a new dataset of species derived from the full DIBaS dataset. Secondly, a compact depthwise separable CNN structure for bacteria recognition was proposed. The recommended detection and classification method performed well on the given dataset, with 96.28% bacterial strain classification. Khalifa et al. [12] introduced a custom CNN architecture based on the AlexNet to classify bacterial names. That paper also utilized a data augmentation-based training and testing strategy to overcome the challenge of training a neural network with a limited dataset. The proposed neural network achieved 98.22% testing accuracy with 6600 augmentation samples. Another work presented by Satoto et al. [13], reached 98.59% (over a subset of the DIBaS dataset, with just four classes) of classification accuracy by using a custom CNN topology and data augmentation techniques. Lei Huang et al. [14] established automated applications for classifying bacterial colonies using two Deep CNN architectures on a custom dataset of 18 classes derived from Peking University's First Hospital. The total number of every bacterial type in this dataset is 4982. The classification accuracy was

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announced to be between 73% and 90%.

This article constructed a deep learning-based system to recognize bacteria strains in the limited dataset collected by Hanoi Medical University (Vietnam). We executed two concurrent steps to process raw data by some recommended methods and evaluate the automatic classification ability of the system. The average classification accuracy was obtained at about 95%. The rest of this paper is structured as follows: Section II describes the proposed methods. Section III discusses the experimental materials and setups. The results are provided in Section IV, and we finally conclude this study in Section V.

II. THE PROPOSED METHODS

In this section, we describe the methods that have been utilized in our study. These are assembling of the fundamental data processing knowledge and proposed architecture. All these subsections are showed below:

A. Bacteria ROI segmentation and Preprocessing

Regions of interest (ROI) usually refers to the meaningful and essential areas of the images. The use of ROI [15] can avoid the processing of irrelevant image points and accelerate the processing. The extraction of regions of interest from images is an important and unsolved topic in the image processing area, especially in the biomedical image processing area. In this paper, we applied to extract ROI to get more beneficial information in the original bacteria images.

B. Data Augmentation

The major challenge in developing a robust Computer-Aided Diagnosis (CAD) system is the unbalanced and limited data size. Data augmentation is a technique employed in deep learning to increase the size of the dataset to overcome the issue of limited data size.

From 40 original images, first, we clipped 426 images for bacteria *Escherichia.coli*, 334 images for bacteria *Staphylococcus.aureus*, and 307 images for bacteria *Klebsiella.pneumoniae* by applying the ROI method. After that, 320 E. coli images, 230 images of Coccus bacteria, and 200 images of Kleb bacteria were utilized for data augmentation. We deployed some computer vision functions such as flip, rotate, zoom, and added salt & pepper noise to increase the quantity of the images (Fig. 1). As a result, the number of augmentation images increased to $6,000$ in total.

C. Model Architecture

Our bacteria classification system is depicted in Fig. 2 from the Input images block (Dataset) to the Bacteria Names Classification One (Results), with the $2^n d$ and $3^r d$ stages making significant contributions. In the ROI process and data augmentation step, the raw images are cropped with the beneficial regions, resized to fit each model, augmented with the number of samples for training, and labeled with random weights. Then the new datasets are divided into a training set and a test set with a suitable ratio. Following the data processing step, the input images with a size of $224 \times 224 \times 3$ and $227 \times 227 \times 3$ are fed to Deep Learning Architectures for training, and the model outputs a weight file and the probability of bacteria name (%).

In order to create and evaluate a efficient system for classifying bacterial colonies, we used several fine-tuned neural networks trained on Imagenet. We investigated to perform VGG16, ResNet50, MobileNet v1, DS-CNN [11] and DeepBacteria [12] for recogizing 03 bacteria strains in our dataset.

Table I also defines DS-CNN design based on some specifications including Layers, Filter Shape, Parameters and Multiply-Accumulate (MACs). The total of this architecture's parameters and MACs are 3.23M and 40.1M, respectively.

TABLE I
The DS-CNN architecture specification with input image $224 \times 224 \times 3$

Layer	Type	Filter Shape	Parameters	MACs
Conv1	Conv/stride 2	3 x 3 x 3 x 64	1792	21676032
	Batch Norm	-	256	0
	Max Pooling	Pool 2 x 2	0	0
DS-Conv2	DW-Conv	3 x 3 x 1 x 64	4736	1806336
	PW-Conv	1 x 1 x 64 x 64		12845056
	Max Pooling	Pool 2 x 2	0	0
DS-Conv3	DW-Conv	3 x 3 x 1 x 64	4736	451584
	PW-Conv	1 x 1 x 64 x 64		3211264
	Max Pooling	Pool 2 x 2	0	0
FC4	Fully Connected	1024	3211529	132096
Classifier	Softmax	3	3075	0
Total			3226115	40.1M

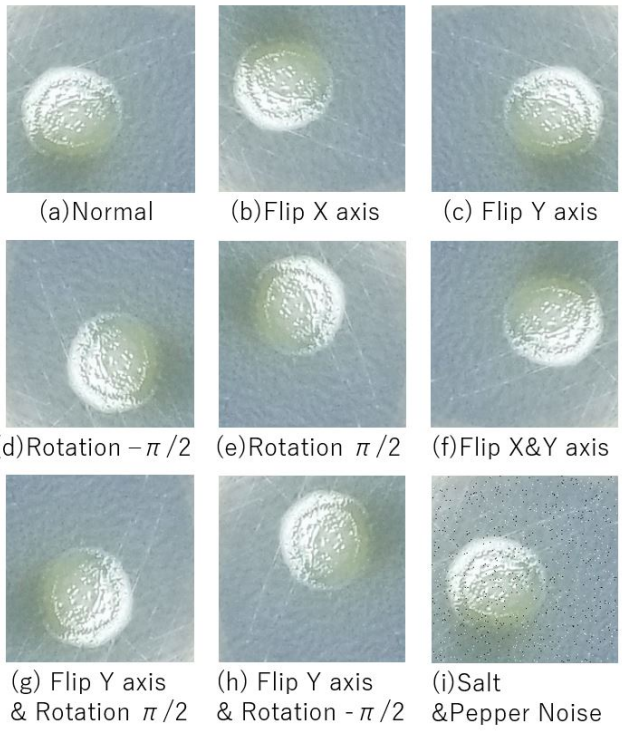

Fig. 1. Data augmentation methods to increase the number of bacteria images from the original dataset.

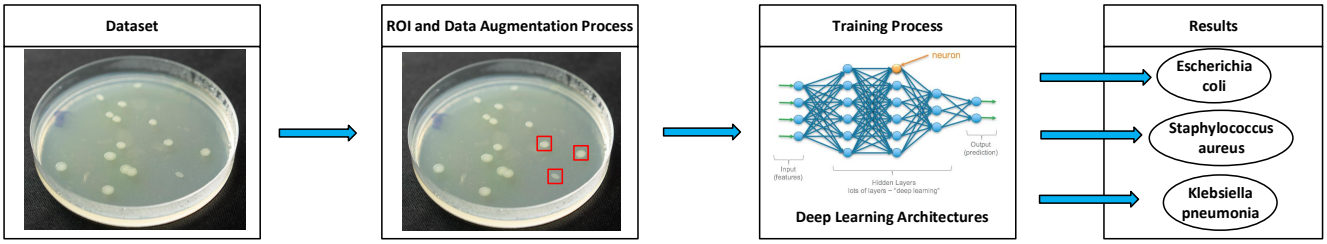

Fig. 2. Suggested stages of bacteria type classification, starting from the original images to bacteria strains recognition.

III. MATERIAL AND EXPERIMENTS SET UP

A. Datasets Description

The bacterial images from Hanoi Medical University (Vietnam) are sRGB images with a pixel size of 3000 × 3000, taken by a Sony ILCE-6000. These images show a petri dish with several bacterial colonies in it. The breakdown of these images was 14 for the *Escherichia.coli* bacteria images, 15 for the *Staphylococcus.aureus* bacteria images, and 11 images of *Klebsiella.pneumoniae*. Several augmented bacteria samples in our dataset are shown in Figure 3.

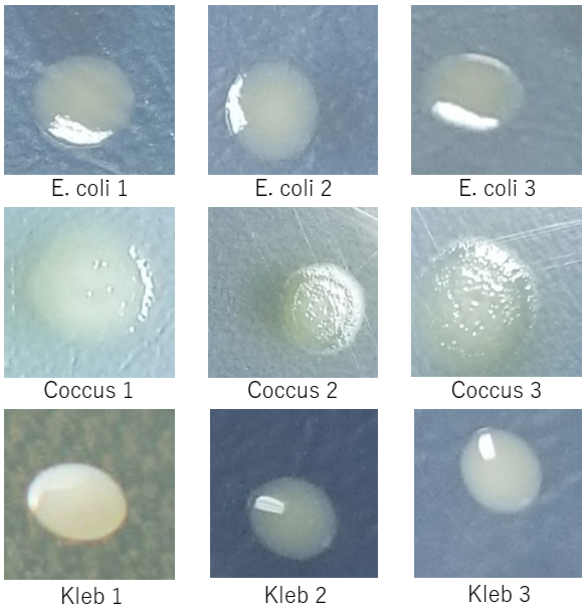

Fig. 3. Augmented samples of bacteria derived from the original dataset.

B. Experimental Setup

1) Computational Resources: The models were trained and tested under the computational specification of Google Colab with the powerful Tesla K80 GPU (12-16 GB RAM). The Python programming language based on Tensorflow framework [16] and Keras libraries [17] are utilized.

2) Parameters Selection: The datasets after preprocessing have a total of 6000 bacteria images; after that, 5000 samples and 1000 images are allocated for the training and validation sets, respectively. The process of updating weights is called the "training process." This training process is done by a method called backpropagation. The Categorical Cross Entropy is employed to calculate and generate the error as a loss function. The SGD optimizer [18] is used for updating the weights with the learning rate (0.0001) and with a batch size of 64. The most significant hyper-parameter to tune is the learning rate. The high learning rate can lead to the convergence problem of the training algorithm. Oppositely, a minimal learning rate may get the algorithm stuck in a local minimum with lousy generalization. Also, for the last stage, we apply a dropout technique [19] after the Fully Connected (FC) layer by 25%. The networks were trained for 100 epochs.

IV. RESULTS AND DISCUSSION

Our experiments were deployed with some scenarios where five CNN models are used to train with the number of epochs of 100. Table II presented the parameters,

TABLE II
CNN architectures specifications when training our bacterial dataset

Model	Layers	Parameters (M)	MACs (G)	Accuracy (%)
ResNet50	50	25.63	3.52	97.19
Tuned VGG - 16	16	14.78	15.34	96.88
MobileNet	28	4.28	0.57	94.17
DS-CNN [11]	5	3.22	0.04	94.66
DeepBacteria [12]	6	5.6	0.71	95.16

MACs utilization, as well as the validation accuracy of various popular CNN models when trained on our custom dataset. It is evident that we could apply the lightweight CNN architectures to classify bacteria strains with high performance. Although each of five deep learning models utilized the number of parameters less than 26M, such as 25.63M (ResNet-50) and 14.78M (Turned VGG16), that classification performance could be obtained over 96%. In particular, the DS-CNN design was the least complicated (5 layers and 3.22M parameters) and could achieve an accuracy that was quite good compared to some other state-of-the-art models.

The testing results of 600 images for three strains of bacteria: E. coli, Staphylococcus, and Klebsiella using VGG-16 are described in Table III. The system could

TABLE III
Test Results of the Tuned VGG-16 model

	E.coli	Staphylococcus	Klebsiella
No. Test Images	200	200	200
Incorrect	14	3	2
Sensitivity (%)	93.0	98.5	99.0

operate well to classify two bacteria, Staphylococcus and Klebsiella (about 99%); the performance was lower in terms of E. coli bacteria. Fig 4 shows the best validation accuracy of the VGG-16 network on the custom dataset derived from the limited dataset of Vietnam when training the model with 100 epochs.

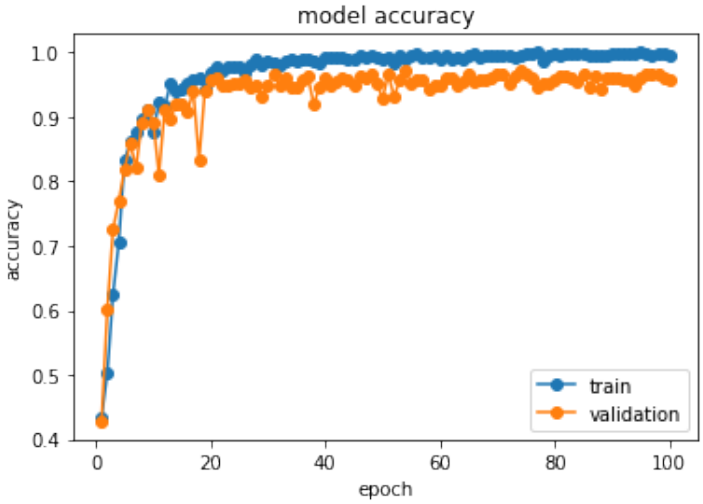

Fig. 4. Validation accuracy using SGD optimizer in Tuned VGG-16 model.

An exciting and substantial data-related contribution: our recommendation of data augmentation and image processing methods led all mentioned architectures to obtain a tremendous potential improvement. This technique could help some designs achieve high performances as good as those networks that used transfer learning or support better scores for networks with low capacity in the medical field.

V. CONCLUSION

This paper investigated and discussed several popular CNN architectures for bacteria classification. The results of these proposed methods showed how to classify three types of bacteria, including Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus, with a classification accuracy of over 92% and three of five models using low resources. This work also supplied robust evidence that lightweight CNN networks can approach roughly state-of-the-art outcomes and thus be highly effective at solving this problem in the real world, especially in embedded implementations or mobile applications for automatic bacterial recognition.

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