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Deep Learning-Based Microorganisms Classification Systems Using Microscopic Images

Baydaa Abdulrazzaq Abed and Jumana Waleed

Department of Computer Science, College of Science, University of Diyala, Iraq

scicompms2207@uodiyala.edu.iq

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Abstract

With enormous amounts of microscopic images created in the research of microbiology, conventional methods of computation have turned more and more difficult to handle. On the contrary, deep learning models are inclined to hold outstanding performance in speed and accuracy. Recently, the microbiologist community embraced deep learning models, thus leading to the appearance of new applications with unprecedented discoveries and perspectives in the research of microbiology. In this paper, various microorganisms classification systems are implemented using various types of microscopic image datasets, including parasites, fungi, bacteria, and viruses. The backbone of these systems is pre-trained convolutional neural networks (CNNs) for detecting microorganisms. This study conducted an exhaustive analysis of various pre-trained CNNs models in the field of microorganism classification, as well as their experimental design and validation and the future extent to which they will present profound perception for the researcher's active in this field. The highest accuracy for parasite classification was achieved by InceptionResNetV2 with a value of 98.44%. While the highest accuracies for fungi, bacteria, and virus classification were achieved by DenseNet201 with a value of 100%, 86.72%, and 77.43%, respectively.

Keywords: Microorganisms Classification, Pre-trained Convolutional Neural Networks (CNNs), Parasites, Fungi, Bacteria, Viruses, Microscopic image datasets.

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نظم تصنيف الكائنات الحية الدقيقة القائمة على التعلم العميق باستخدام الصور الميكروسكوبية

بيداء عبد الرزاق عابد و جمانة وليد
قسم علوم الحاسوب، كلية العلوم، جامعة ديالى

الخلاصة

مع وجود كميات هائلة من الصور المجهرية التي تم إنشاؤها في أبحاث علم الأحياء الدقيقة، أصبحت الطرق التقليدية للحساب أكثر صعوبة في التعامل معها. على العكس من ذلك، تميل نماذج التعلم العميق إلى الأداء المتميز في السرعة والدقة. في الآونة الأخيرة، تبنى مجتمع علماء الأحياء الدقيقة نماذج التعلم العميق، مما أدى إلى ظهور تطبيقات جديدة مع اكتشافات وجهات نظر غير مسبوقة في أبحاث علم الأحياء الدقيقة. في هذا البحث، تم تنفيذ العديد من أنظمة تصنيف الكائنات الحية الدقيقة باستخدام أنواع مختلفة من مجموعات بيانات الصور المجهرية بما في ذلك الطفيليات والفطريات والبكتيريا والفيروسات. العمود الفقري لهذه الأنظمة هو شبكات عصبية تلافيفية مدربة مسبقًا (CNNs) للكشف عن الكائنات الحية الدقيقة. أجرت هذه الدراسة تحليلاً شاملاً لمختلف نماذج CNNs المدربة مسبقًا في مجال تصنيف الكائنات الحية الدقيقة بالإضافة إلى تصميمها التجريبي والتحقق من صحتها والمدى المستقبلي لتقديم تصور عميق للباحثين النشطين في هذا المجال. تم تحقيق أعلى دقة لتصنيف الطفيليات بواسطة InceptionResNetV2 بقيمة 98.44٪. بينما تم تحقيق أعلى درجات الدقة لتصنيف الفطريات والبكتيريا والفيروسات بواسطة DenseNet201 بقيمة 100٪ و86.72٪ و77.43٪ على التوالي.

الكلمات المفتاحية: تصنيف الكائنات الدقيقة، الشبكات العصبية التلافيفية المدربة مسبقًا (CNNs)، الطفيليات، الفطريات، البكتيريا، الفيروسات، مجموعات بيانات الصور المجهرية.

Introduction

Microorganisms represent organisms of microscopy that are existence as single-celled and multi-cellular shapes. It is difficult to see very small-sized microorganisms with the unaided eye; hence microorganisms can be seen under a microscope [1]. Microorganisms have an important part in keeping the biosphere and ecological system of the earth for supportable environmental issues. Moreover, they can be utilized in bio-remediation, biological detection, and pollutant effects mitigation [2]. Besides maintaining the environment, microorganisms have an important part in humans' life. A few categories of microorganisms are useful for humans that can be widely utilized in different actions such as agriculture, food production, fuel production, medicine preparation, sewage treatment, industries, and so on. However, microorganisms can be destructive to the environment and human life. These organisms can be in charge of producing diverse human diseases such as food poisoning, typhoid, cold, cancer, AIDS, and so on. Generally, humans should be conscious of the reality that microorganisms are capable of causing considerable threats to other creatures living on Earth [1].

Our planet is full of microorganisms like parasites, fungi, bacteria, viruses, etc. Parasites have extensive impacts on the public's health of people and produce lots of deaths globally every year [3]. Numerous parasitic diseases have appeared in humans via three fundamental classes: ectoparasites, helminths, and protozoa. Trichomonad, Trypanosome, Plasmodium, Babesia, Leishmania, and Toxoplasma represent the familiar disease-producing protozoan parasites. Malaria represents a deadly disease produced by Plasmodium that works on destroying the cells of red blood. Schistosoma disease represents another main reason for human deaths which takes second only to malaria [4]. Fungi can represent a natural human microbiota component that can produce systemic and surface infections. The main reasons for fungus infections are the weakened immune system's function and the composition of unbalanced microbiota in the body of humans. There are additional reasons for fungus infections, including long-standing antibiotics treatment, steroid treatment, and so on. Candidiasis represents an extensive fungus infection produced via Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida lustianiae, Saccharomyces phylum, Cryptococcus neoformans, and Maalasezia furfur [5]. While bacteria represent a species of prokaryotic microorganism, the structure of its cell is simpler than the cells of other organisms. Some bacterium possesses an additional genetic material circle named a plasmid instead of a nucleus, and this plasmid frequently includes genes that provide the bacteria with a number of advantages over others. For instance, the bacterium may include a gene that enables it to resist a specific antibiotic. Bacteria can be categorized into several categories in accordance with their fundamental forms: spirochaetes (corkscrew), vibrios (comma), spirilla (spiral), bacilli (rod), or cocci (spherical). There is an extensive group of bacteria that are innocuous and even useful in various aspects, while a few are causing diseases for humans and animals and can prove to be fatal as well, for instance, tuberculosis, cholera, foodborne illness, tetanus, and so on [1]. Besides parasites, fungi, and bacteria, other microorganisms named viruses also have extensive impacts on the public's health. By utilizing transmission electron microscopy, the structure of the virus has been observed as a changeably shaped and sized nucleocapsid (including the genetic material) with or without a protective coat of lipoprotein. Viruses are distinguished and categorized into families and groups by considering their chemical composition, replication modes, and morphology [6].

The utilization of microscopy imaging enables researchers to determine and observe microorganisms for pathogenesis studies and clinical diagnosis. Regardless of being utilized broadly, human-managed microscopy represents an arduous, inaccurate, and long operation that needs well-trained staff. Taking into account the errors of humans, improper technical foundation-based conclusions can turn arbitrary. Therefore, in numerous resource-limited fields, the microscopic diagnosis of microorganisms remains a challenging operation. Recently, deep learning technology has exhibited its efficiency in all aspects of human life [7], [8], [9], and its implementation in microscopic imaging to classify microorganisms is considerably capable of improving the speed and accuracy of diagnosis [10].

The fundamental contribution of this work is extracting microscopic image features, accurately distinguishing the type of microorganism, and identifying each type. Accordingly, the following objectives have been realized:

1- Implement various recently existing pre-trained CNNs on microscopic images utilizing widespread benchmarks parasites, fungi, bacteria, and viruses datasets.

2- Carry out experiments' series based on deep learning models and present comparisons using the datasets of Microorganisms.

This paper is structured as follows: The first section provides a brief background on common microorganism species and verifies the need for applying deep learning in microscopic imaging, then specifies the fundamental contributions of the implemented models; The next section reviews recent related deep learning-based microorganism classification works; The third section explains the implementation of the pre-trained CNN models in the microorganisms classification systems; The fourth section presents the utilized microscopic images datasets and the results of experiments; The last section condenses this work and depicts its future extent.

Related Work

Microorganisms classification represents a significant issue in the past few years. Various systems have been presented concerning this issue. Moreover, researches on computer-aided microorganism classification emerges increasingly with the development of deep learning models. Recently, various microorganisms classification systems have appeared.

As parasites are a serious and deadly disease, various researchers have been in the throes of this, especially malaria, for a few years. A diversity of deep learning models is carried out for extracting features from microscopic images and classifying or identifying diseases. Alnussairi and İbrahim [11] presented deep-learning systems for improving the diagnostic accuracy of malaria in red blood cell microscopic images. These systems are based on the utilization of three pre-trained models (VGG-19, MobileNet-V2, and ResNet-50) for classifying malaria. These presented systems were evaluated experimentally by utilizing the NIH dataset acquired from the National Institutes of Health. The accuracy achieved was almost 100%. While Bhuiyan and Islam [12] developed a deep-learning system for identifying malaria from microscopic images. In this system, three pre-trained models (VGG-16, VGG-19, and DenseNet-201) were utilized to evolve the accommodative models of the weighted average ensemble. In order to decrease the prediction dispersion, a scheme of the max-voting ensemble was employed along with accommodative models. Furthermore, to decrease the problem of overfitting, the technique of data augmentation was utilized. In this proposed system, the NIH dataset was utilized, and the provided accuracy was 97.92% for classifying uninfected and parasitized cells.

Concerning the issue of fungal disease classification, there are few deep learning-based solutions. Zieliński [13] proposed a system that included several pre-trained CNNs models for classifying various microscopic fungi images. This system was implemented using the Digital Images of Fungus Species (DIFaS) dataset. Since the utilized dataset was small and hence held a large count of parameters, various fine-tuned pre-trained CNNs (AlexNet, ResNet, DenseNet-

169, and Inception-V3) were considered for extracting features. Then, a bag-of-words or Fisher vector was applied for feature aggregation. After that, a support vector machine was utilized for classifying ten species of fungi. In accordance with experimentations, the utilization of the AlexNet model and Ffisher vector with the classifier has provided better results than others, with an accuracy of 82.4%. While Zawadzki [14] attempted to categorize four species of microscopic fungi images from the DIBaS dataset based on various models of pre-trained CNNs. The experimental results depicted that the pre-trained models (ResNet-50 and Xception) outperformed the others with an accuracy of 96% and 88%, respectively.

Accurate classification of bacteria species represents a critical and common issue in diverse domains. Zieliński et al. [15] presented a system in which deep CNN was utilized for obtaining features, then a random forest or support vector machine was utilized for classifying thirty-three species of microscopic bacteria images. In order to evaluate this system, a new dataset named Digital Image of Bacterial Species (DIBaS) was provided, and the recognition accuracy was 97.24%. Furthermore, there are many systems, such as [16], [17], and [18], that have been presented for bacteria species classification. However, there is no improvement to increase the system efficiency has been done yet.

The deep learning-based virus classification using microscopic images attainable from Transmission Electron Microscopy (TEM) assists extremely in the medical domain. Damian J. Matuszewski and Ida-Maria Sintorn [19] compared various deep-learning models to classify the fourteen commonest virus classes and also described and made openly accessible a TEM dataset of virus images. In this system, the DenseNet-201 represented the best fine-tuned pre-trained model with 93.1% accuracy. Ali et al. [20] proposed a system to classify fourteen classes of viruses. The system's performance was compared with various pre-trained CNNs models (DenseNet-201, MobileNet, and XceptionNet). This developed system attained a high result of accuracy reaching 96% which outperformed the other pre-trained CNNs models.

Microorganisms Classification Systems Implementation

The implemented systems comprise two principal phases: firstly the microscopic image pre-processing, secondly extracting features and classification, which are accomplished using the pre-trained CNN models. Figure 1 depicts these phases schematically.

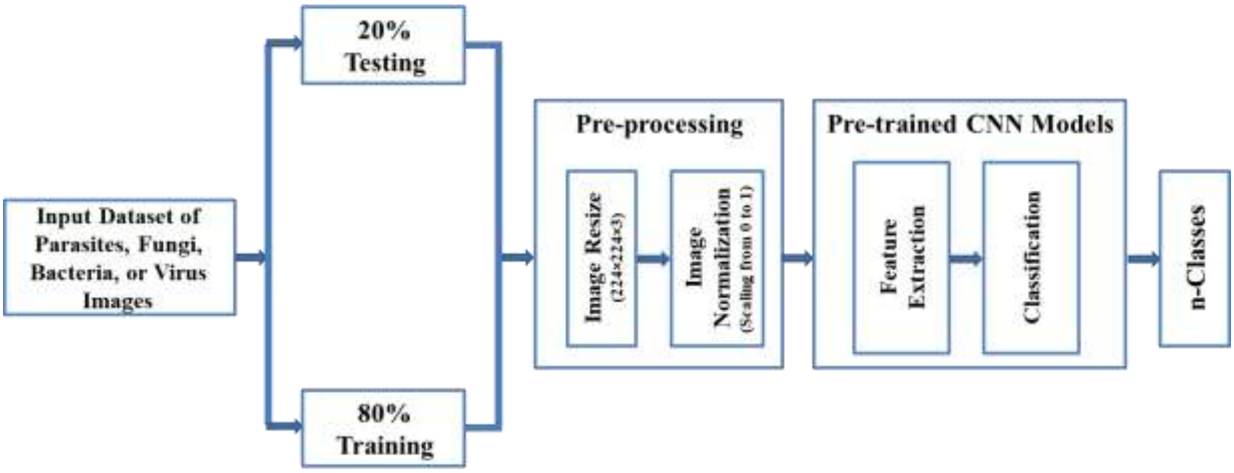


Figure 1: Block diagram of the implemented systems

In the input microorganism dataset, the microscopic images hold various dimensions. The process of increasing image resolution works on improving performance; however, it can exhaust a considerable amount of processing power and memory. Therefore, in any system, a trade-off between computation and precision should be provided. In these implemented systems, all the microscopic images are resized to 224×224×3. Moreover, the process of image normalization works on reducing intensity and color inconsistencies between smudged microscopic images from diverse research laboratories. The utilization of data from non-scaled input images leads to making the process of learning difficult. So, the process of data scaling is accomplished in this phase. In the process of normalization (data rescaling), the data points of the original microscopic images are decreased to be scaled from zero to one via dividing the input microscopic image by 255. Hence, the classification performance is improved in the pre-processing phase.

To effectively extract features and classify microscopic images for microorganism identification, the proposed systems utilize five pre-trained CNN models (DenseNet-201, MobileNet-V2, ResNet-152V2, InceptionResNet-V2, and NasNetLarge). The count of the overall parameters (including trainable and non-trainable) concerning the implementation of pre-trained CNN models using various microorganisms datasets are depicted in Table 1.

Table 1. The count of the overall parameters concerning the implemented pre-trained CNNs models.

MICROORGANISM DATASETS	**PARAMETERS**	**PRE-TRAINED CNNS**				
		DenseNet201	**MobileNetV2**	**ResNet152V2**	**InceptionResNetV2**	**NasNetLarge**
Parasites	**Trainable**	940,810	627,210	1,003,530	384,010	517,450
	Non-trainable	18,321,984	2,257,984	58,331,648	54,336,736	4,269,716
	Total	19,262,794	2,885,194	59,335,178	54,720,746	4,787,166
Fungi	**Trainable**	846,729	564,489	903,177	345,609	465,705
	Non-trainable	18,321,984	2,257,984	58,331,648	54,336,736	4,269,716
	Total	19,168,713	2,822,473	59,234,825	54,682,345	4,735,421
Bacteria	**Trainable**	3,104,673	2,069,793	3,311,649	1,267,233	1,707,585
	Non-trainable	18,321,984	2,257,984	58,331,648	54,336,736	4,269,716
	Total	21,426,657	4,327,777	61,643,297	55,603,969	5,977,301
Virus	**Trainable**	1,411,215	940,815	1,505,295	368,655	776,175
	Non-trainable	18,321,984	2,257,984	58,331,648	54,336,736	4,269,716
	Total	19,733,199	3,198,799	59,836,943	54,705,391	5,045,891

Experimental Results

1. Metrics of Evaluation

It’s a significant assignment for determining which model is the most effective concerning any dataset. Generally, the metrics of evaluation are utilized in this respect. Almost all of these metrics are produced using a confusion matrix with four basic elements: specifically, True Negatives (T_{Negs}), True Positives (T_{Poss}), False Negatives (F_{Negs}), and False Positives (F_{Poss}). Consequently, other typical metrics can be created from these fundamental metrics, like Precision (Pr) recall (Re), accuracy (Ac), and F1-score (F1). All those metrics are in the range [0, 1], and they are efficient for high values [21]. Re is often named sensitivity, and it indicates the rate of TPoss for a class. Ac is utilized to recognize the success rate attained concerning several classes.

$$Pr = \frac{T_{Poss}}{F_{Poss}+T_{Poss}} \tag{1}$$

$$Re = \frac{T_{poss}}{F_{Negs}+T_{Poss}} \tag{2}$$

$$F1 = \frac{2\, Re \times Pr}{Re+Pr} \tag{3}$$

$$Ac = \frac{T_{Negs}+T_{Poss}}{F_{Negs}+T_{Negs}+F_{Poss}+T_{Poss}} \tag{4}$$

2. Microorganism Datasets

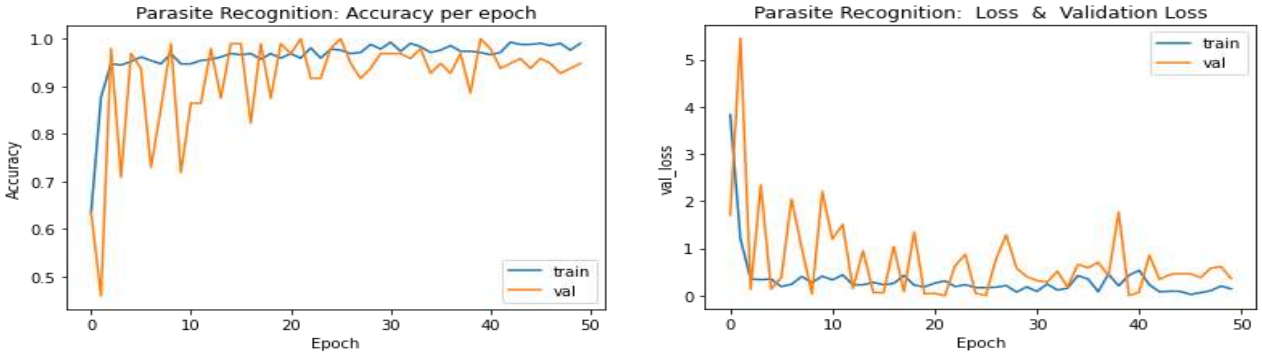
2.1 Parasites

The Parasites Species dataset [22] includes various microscopic parasite images belonging to ten classes. This dataset is separated into 27455 images (80%) and 6863 images (20%) for training and testing, respectively. Table 2 indicates the models' performance for parasite classification.

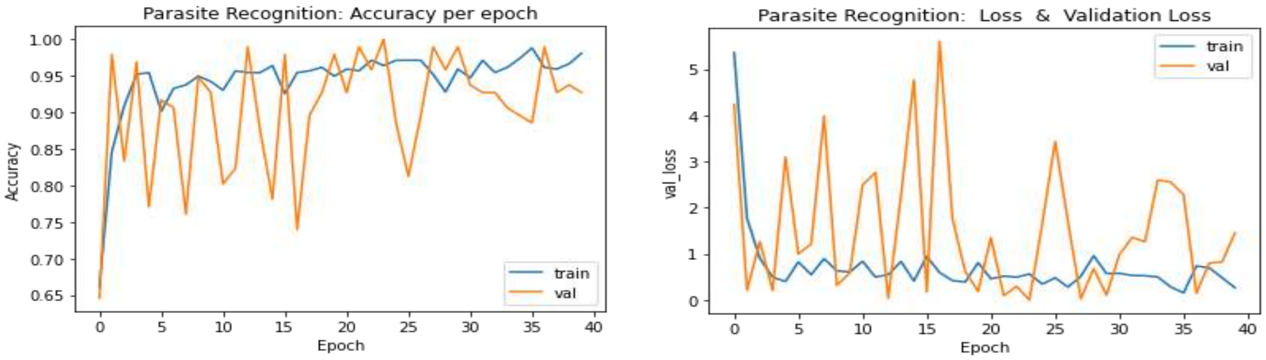
Table 2: Models' performance for parasite classification.

PRE-TRAINED CNNS	PR	RE	F1	TRAINING LOSS	VALIDATION LOSS	TRAINING AC	VALIDATION AC
DenseNet201	0.98	0.98	0.98	0.1441	0.3629	0.9904	0.9479
MobileNetV2	0.97	0.97	0.97	0.2666	1.4530	0.9808	0.9271
ResNet152V2	0.98	0.98	0.98	0.1053	0.4418	0.9904	0.9792
InceptionResNetV2	0.97	0.97	0.97	0.5224	0.1486	0.9700	0.9844
NasNetLarge	0.96	0.95	0.95	0.2859	1.1174	0.9615	0.8854

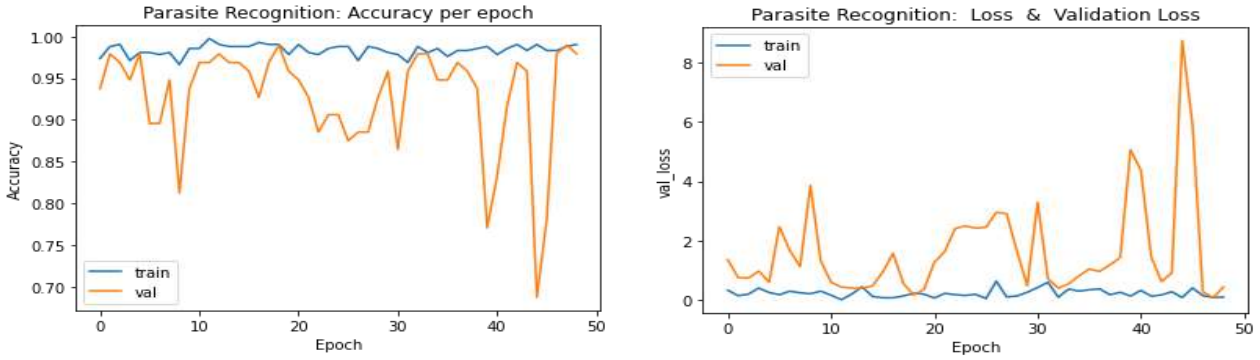
Figure 2 exhibits the accuracy and loss of the implemented pre-trained models on the training and validation parasites dataset per epoch (batch_size = 1024, epochs = 50).



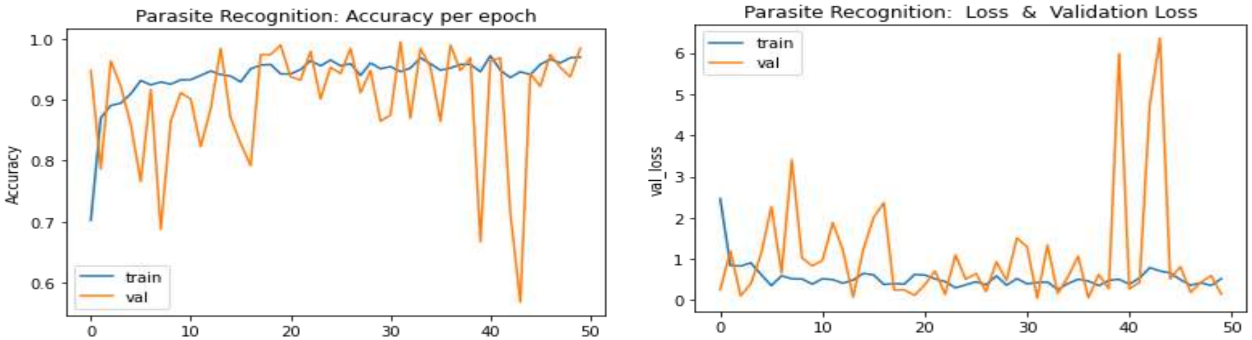
(a) DenseNet201



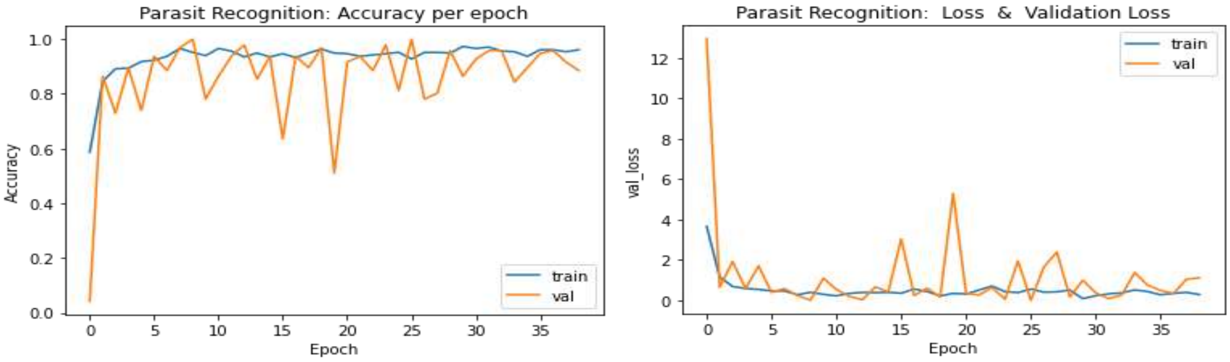
(b) MobileNetV2



(c) ResNet152V2



(d) InceptionResNetV2



(e) NASNetLarge

Figure 2: The accuracy and loss of the implemented pre-trained models on the training and validation parasites dataset per epoch.

In the previous figure (1st column), we notice the instability of the accuracy values concerning the implemented pre-trained CNNs models over the epochs, and the highest accuracy for parasite classification was achieved by InceptionResNetV2 with a value of 98.44%. In the 2nd column, we notice a divergence between the graphs of training/validating losses concerning the implemented pre-trained CNNs models over the epochs.

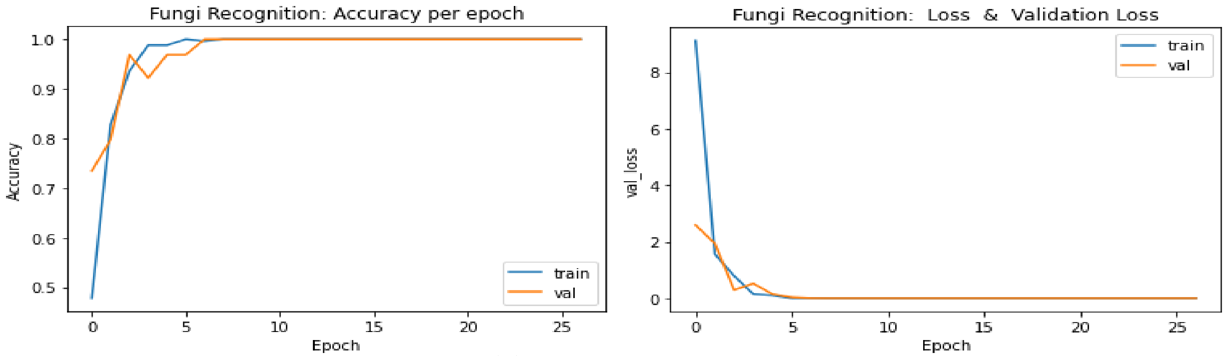
2.2 Fungi

The DIFaS dataset [14] includes various fungus images belonging to nine classes. This dataset is separated into 281 images (80%) and 71 images (20%) for training and testing, respectively. Table 3 indicates the models' performance for fungi classification. The highest accuracy for fungi classification was achieved by DenseNet201 with a value of 100%.

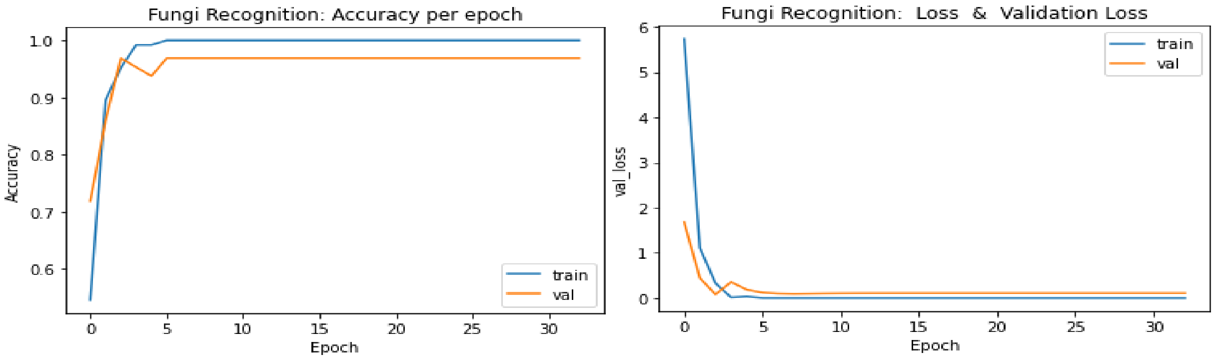
Table 3: Models' performance for fungi classification.

PRE-TRAINED CNNS	PR	RE	F1	TRAINING LOSS	VALIDATION LOSS	TRAINING AC	VALIDATION AC
DenseNet201	1.00	1.00	1.00	4.5133	5.2903	1.0000	1.0000
MobileNetV2	0.97	0.97	0.97	7.0757	0.1054	1.0000	0.9688
ResNet152V2	0.93	0.92	0.92	3.9691	0.8969	1.0000	0.9062
InceptionResNet V2	0.91	0.89	0.88	2.9238	1.3343	1.0000	0.8750
NasNetLarge	0.95	0.94	0.94	1.0484	0.4279	1.0000	0.9375

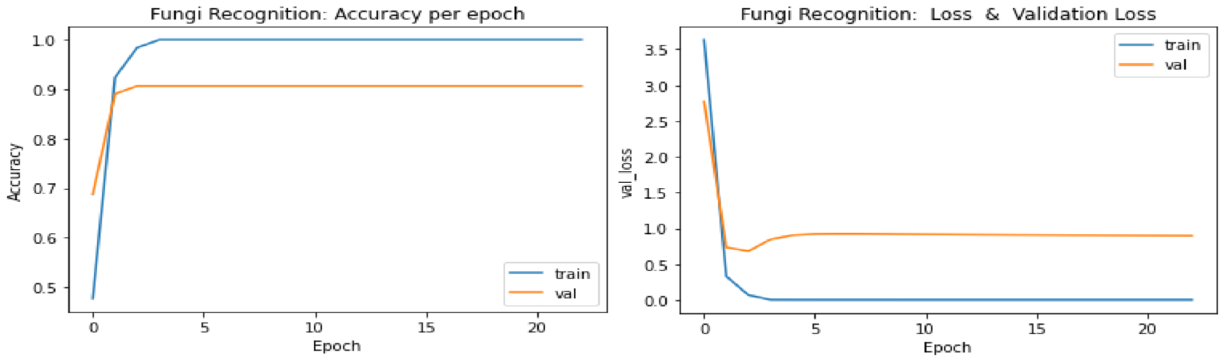
Figure 3 exhibits the accuracy and loss of the implemented pre-trained models on the training and validation fungi dataset per epoch (batch_size = 32, epochs = 50).



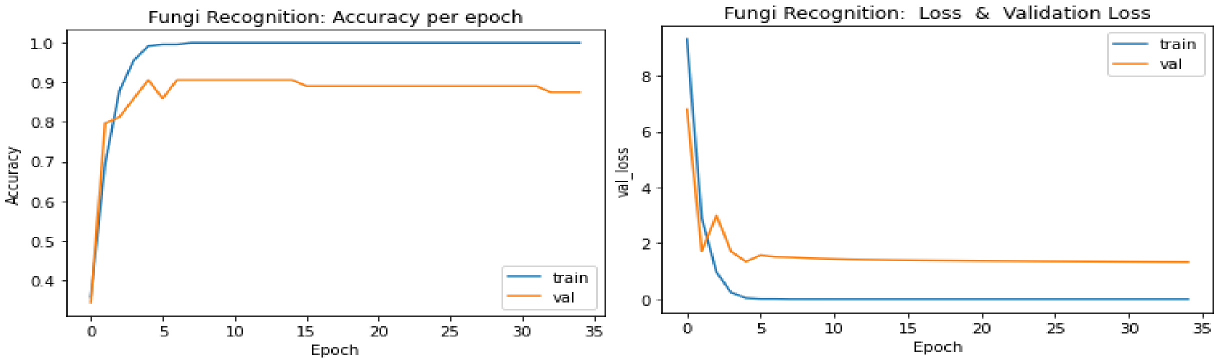
(a) DenseNet201



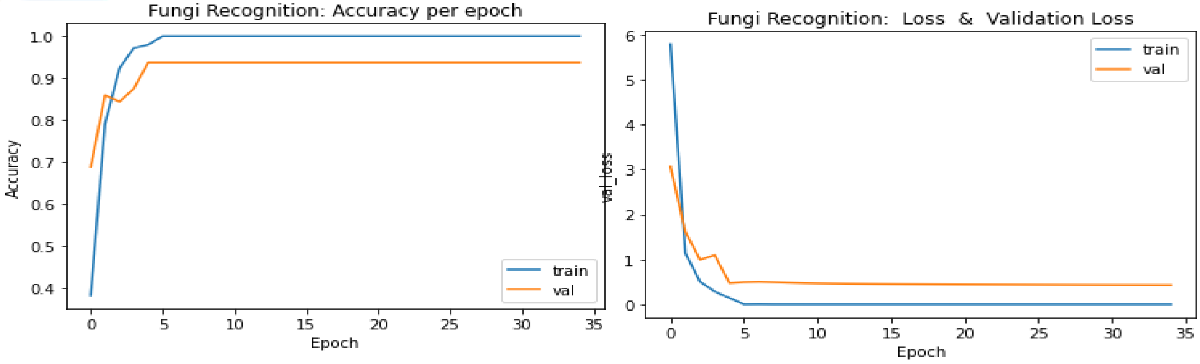
(b) MobileNetV2



(c) ResNet152V2



(d) InceptionResNetV2



(e) NASNetLarge

Figure 3: The accuracy and loss of the implemented pre-trained models on the training and validation fungi dataset per epoch.

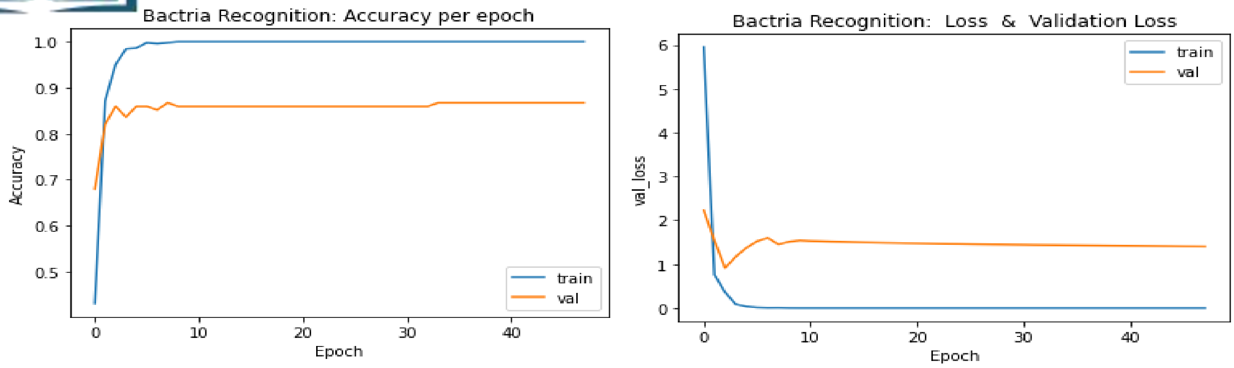
2.3 Bacteria

DIBaS dataset [15] includes various species and genera of bacteria images belonging to thirty-three classes. Here, three fundamental forms (spiral, spherical, and cylindrical) are distinguished. This dataset is separated into 538 images (80%) and 143 images (20%) for training and testing, respectively. Table 4 indicates the models' performance for bacteria classification.

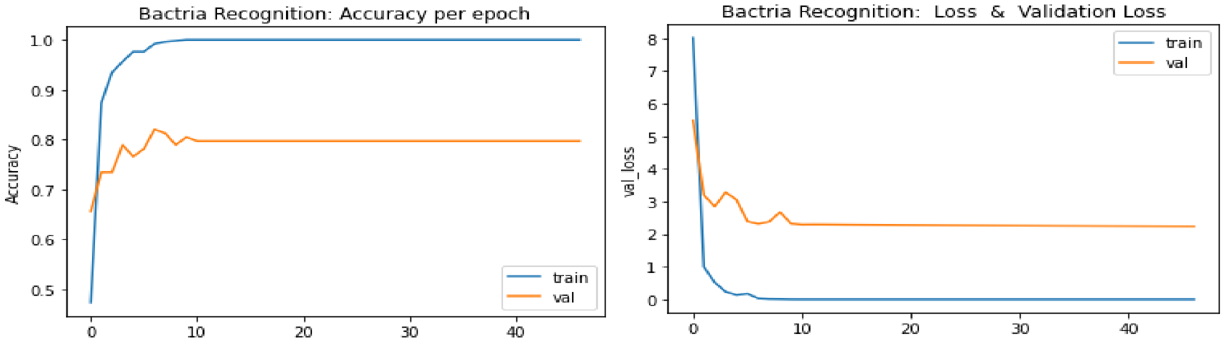
Table 4: Models' performance for bacteria classification.

PRE-TRAINED CNNS	PR	RE	F1	TRAINING LOSS	VALIDATION LOSS	TRAINING AC	VALIDATION AC
DenseNet201	0.93	0.87	0.87	3.9628	1.4022	1.0000	0.8672
MobileNetV2	0.84	0.77	0.77	1.3433	2.2382	1.0000	0.7969
ResNet152V2	0.75	0.72	0.71	1.2682	1.7199	1.0000	0.7422
InceptionResNetV2	0.76	0.73	0.73	2.0291	1.2120	1.0000	0.7422
NasNetLarge	0.79	0.76	0.75	1.5481	1.6723	1.0000	0.7734

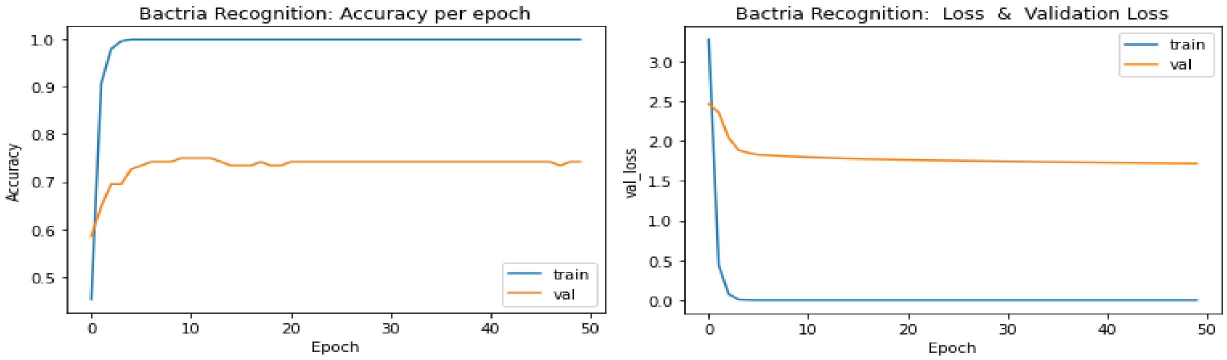
Figure 4 exhibits the accuracy and loss of the implemented pre-trained models on the training and validation bacteria dataset per epoch (batch_size = 32, epochs = 50).



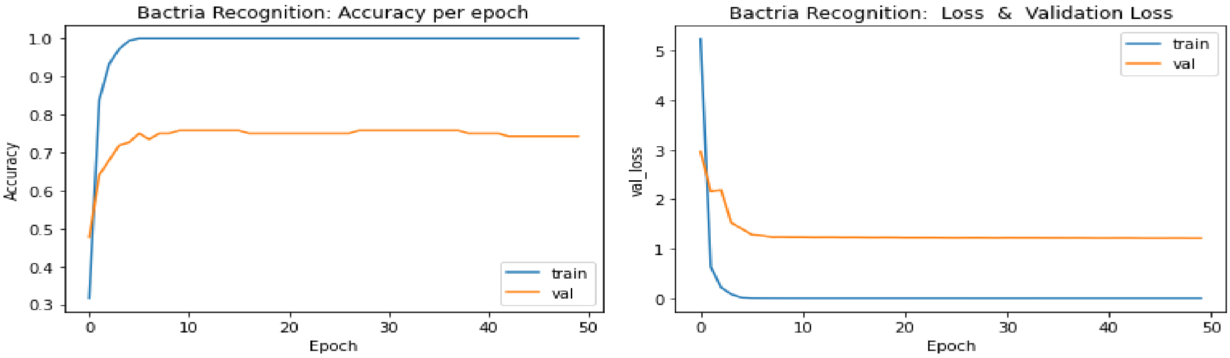
(a) DenseNet201



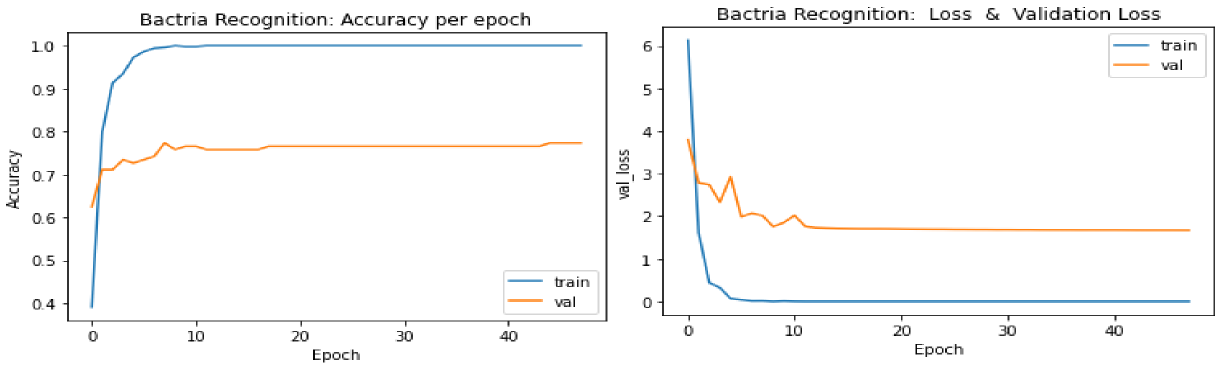
(b) MobileNetV2



(c) ResNet152V2



(d) InceptionResNetV2



(e) NASNetLarge

Figure 4: The accuracy and loss of the implemented pre-trained models on the training and validation bacteria dataset per epoch.

In the previous figure (1st column), we noticed a divergence between the graphs of training and validating accuracy concerning the implemented pre-trained CNNs models over the epochs, and the highest accuracy for bacteria classification was achieved by DenseNet201 with a value of 86.72%. In the 2nd column, we also noticed a divergence between the graphs of training and validating loss over the epochs.

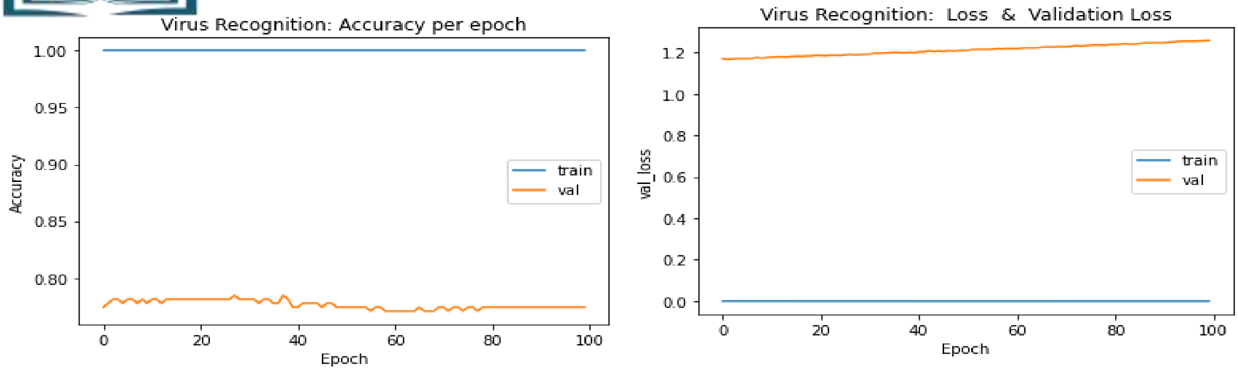
4.2.2 Virus

The Virus Texture dataset [23] includes transmitted electron microscopy images belonging to fifteen classes of viruses. This dataset is separated into 1200 images (80%) and 300 images (20%) for training and testing, respectively. Table 5 indicates the models' performance for virus classification.

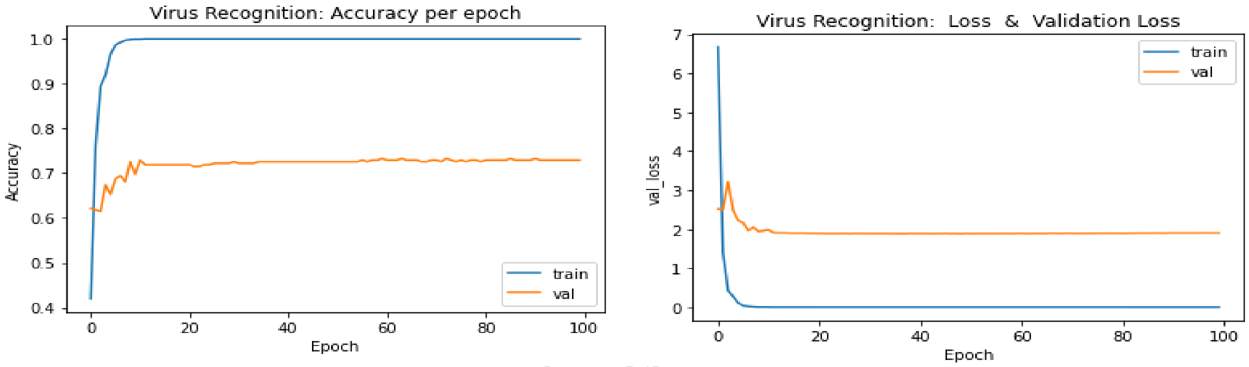
Table 5: Models' performance for virus classification.

PRE-TRAINED CNNS	PR	RE	F1	TRAINING LOSS	VALIDATION LOSS	TRAINING AC	VALIDATION AC
DenseNet201	0.76	0.76	0.76	3.1232	1.2571	1.0000	0.7743
MobileNetV2	0.73	0.72	0.72	2.9974	1.9048	1.0000	0.7292
ResNet152V2	0.71	0.70	0.70	4.8375	2.5772	1.0000	0.7118
InceptionResNetV2	0.76	0.73	0.73	0.1659	3.6464	0.9786	0.7500
NasNetLarge	0.72	0.73	0.72	2.3674	1.8897	1.0000	0.7257

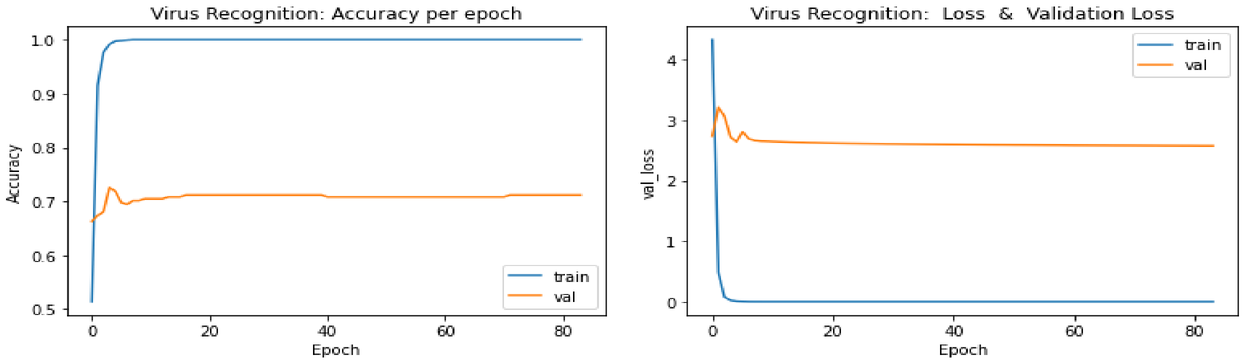
Figure 5 exhibits the accuracy and loss of the implemented pre-trained models on the training and validation virus dataset per epoch (batch_size = 32, epochs = 100).



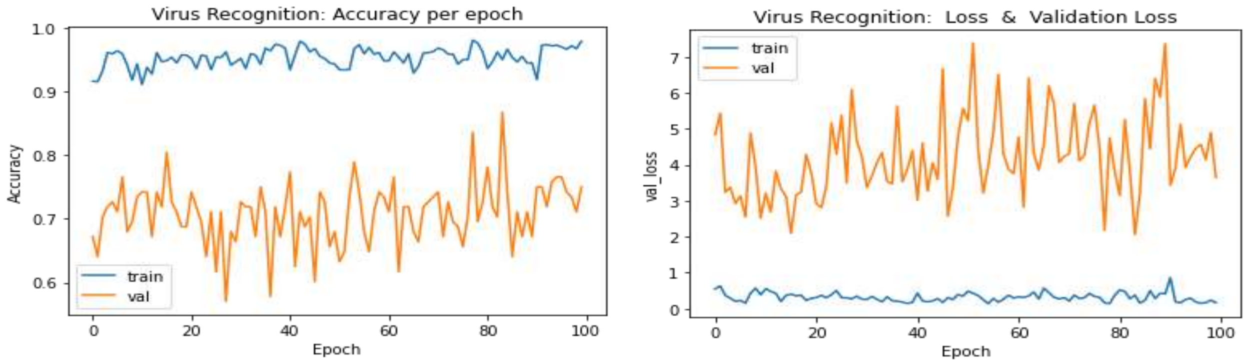
(a) DenseNet201



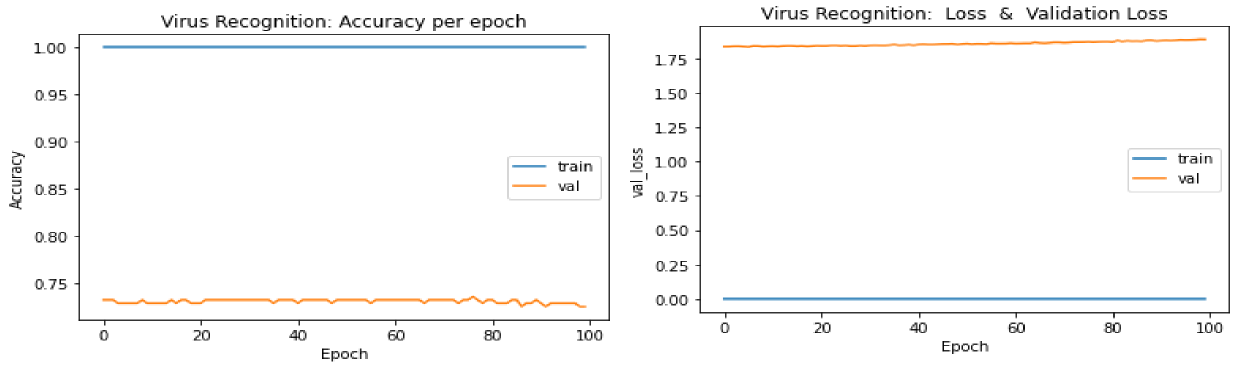
(b) **MobileNetV2**



(c) ResNet152V2



(d) InceptionResNetV2



(e) NasNetLarge

Figure 5: The accuracy and loss of the implemented pre-trained models on the training and validation virus dataset per epoch.

In the previous figure (both columns), we noticed a very large divergence between the graphs of training/validating (accuracy or loss) concerning the implemented pre-trained CNNs models over the epochs, and the highest accuracy for virus classification was achieved by DenseNet201 with a value of 77.43%.

Conclusion

Taking into consideration the accessibility of public benchmark datasets and the expanding count of freely accessible programming frameworks, toolboxes, and libraries, it is acceptable that the technology of deep learning-based microorganisms classification systems will be further useful and user-friendly for the microbiologist's community. Moreover, deep learning schemes are capable of taking benefit of the increased speed of computer processing for observing and advising microbiologists in actual time, providing further accurate and fast microorganisms classification. As a consequence of the attained results, it can be concluded that the implementation of the recent effective pre-trained CNNs was able to provide high indicators of performance with a high extent of conviction. The future grade of the implemented models is to explore system performance by using the technique of data augmentation to decrease the problem of overfitting, or using other datasets of microorganism images.

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