Visualization of microscopic morphological characteristics used for determination of infectious molds

Mina Milanović, Aleksandar Milosavljević and Marina Ranđelović

***Abstract*—Invasive fungal infections (IFI) and systemic fungal infections (SFI), caused by molds are on the rise, based on data from literature. Diagnostics of those infections can sometimes be inefficient; they require a longer period of time in laboratory procedures and sometimes may lead to late diagnosis or misdiagnosis, which can result in patient's critical condition or even mortality. The goal of this research is to develop a neural network model that will perform identification of molds, and thus accelerate the process of diagnostics. A classifier has been developed, using an EfficientNet-B1 deep convolutional neural network (CNN) and sample images obtained at the Department of Microbiology and Immunology, Medical faculty, University of Niš, Serbia, archives. We applied Grad-CAM visualization to determine morphological characteristics used by the model to classify samples.**

***Index Terms*—molds identification, fungal infection, convolutional neural networks, deep learning, Grad-CAM.**

I. Introduction

Ability of fungus to start a pathological process in the host organism is as a specific phenomenon, according to numerous authors, because, excluding groups of dermatophyte molds and tropical fungi, these microorganisms does not need pathogenicity for their dissemination and survival in nature [1]. Among 400.000 species of fungi known in the nature, around 50 kinds can cause invasive fungal infections(IFI), that are characterized by very high morbidity (serious clinical case) and mortality. Numerous reasons have contributed to the increase of number of infections among humans, and incidences of IFI caused by molds are constantly growing. The most important reasons are complex procedures and medical interventions, intensive treatments with antibacterial drugs, cytostatics, immunosuppressants; longer lifespan of a humans, increase in the number of patients at high risk due to primary diseases and treatment, the appearance of resistance in fungi and certainly the establishment of mycological analyzes and higher diagnostic efficiency, i.e. more successful diagnostic procedures in a microbiology [2].

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Fungi are eukaryotic microorganisms. In nature, they are widespread, living in soil and water, on organic materials as saprophytes, as symbionts or parasites of animals, plants, or human [3]. Based on the structure, all fungi can be primarily divided into yeasts -unicellular fungi with basal cell blastoconidia (blastospora) and multicellular fungi (molds) with a basic hypha cell. Molds classification is performed on the basis of structure, i.e. macroscopic and microscopic morphological characteristics. Differences between the morphology of molds, hypha structure, production of different conidiae (spores), enable a diagnostic procedure for their identification [4].

This goal of this project is to develop a neural network model that will perform identification of molds, and thus accelerate the process of diagnostics. No similar projects, involving determination of molds or their morphological characteristics, could been found during our research, and beside some rapid tests that can be used only for most common types of infections, whole process of determination is manual and sometimes takes days, so providing an application that can accelerate the process can be very beneficial. During recent years, number of infections caused by more rare species of fungi has drastically increased, which was a motivation for a project like this, which includes classification of so far neglected types of fungi.

Sample collection has contained high resolution images, which needed manual preparation for training, as described in chapter II. Prepared dataset was expanded before training and EfficiencyNet-B1 architecture of convolutional neural network (CNN) has been used for developing and training the model, which makes the core of the classifier, as presented in chapter III. Results and discussion of the results, including visualization of decision making process using Grad-CAM method, have been shown in chapter IV. Conclusion and planned further steps have been described in chapter V.

II. Data

A. Dataset description

Fungi, based on morphology are classified in group of yeasts -unicellular fungi with basal cell blastoconidia (blastospora) and multicellular fungi (molds) with a basic hypha cell. Molds can be primarily divided into dermatophytic and non-dermatophytic fungi [5].

Dermatophytic molds, in other words, dermatophytes, are causative agents of superficial fungal infection of skin, hair and nail with prevalence of 22-25% worldwide.

Other group of molds caused invasive fungal infection (IFI) and in recent years incidence of these diseases has been on the rise [6].

Diagnostics of infections caused by dermatophytic and non-dermatophytic fungi can sometimes be inefficient; they require a longer period of time in laboratory procedures and sometimes may lead to late diagnosis. In case of SFI (systemic fungal infection) late diagnosis or misdiagnosis can lead to wrong treatment, as well as not implementing measures for preventing the spread of infection [7]. On the other hand late diagnosis or misdiagnosis of IFI can result in patient's condition impairment or even mortality. In our previous paper [8], we considered only fungi genera that cause invasive infections, but we extended the dataset so types of fungi that cause systematic fungal infections are included too.

In both groups molds classification was performed on the basis of structure, i.e. macroscopic and microscopic morphological characteristics. Expert's knowledge and experience are needed for differentiation and identification of isolated fungi in laboratory practice.

B. *Morphologial differences of fungi*

Microscopic morphological characteristics of Dermatophytes are:

i) ***Microsporum*** spp. are characterized by segmented hyphae, numerous macroconidiae that are thick walled, rough, present microconidia;

ii) ***Trichophyton*** spp. are characterized by segmented hyphae, rare macroconidiae that are thin walled and smooth, numerous microconidiae;

iii) ***Epidermophyton*** spp. are characterized by segmented hyphae, numerous macroconidiae that are thin and thick walled, smooth and microconidiae are not formed.

Microscopic morphology of non-dermatophytic genera is characterized by:

i) ***Aspergillus*** spp.: Septate hyphae with unbranced conidiophores which ending with swollen vesicule that is covered with flak-shaped phialides on which are chains of mostly round sometimes rough conidia;

ii) ***Penicillium*** spp.: Septate hyphae with branched or unbrenched conidiophores that have secondary branches known as metulae or prophialides on which are phialides with chains of conidiae (Figure 1);

iii) ***Fusarium*** spp.: Septate hyphae with formation of canoe shaped or sickle shaped multiseptate macroconidia that are produced from phialides on unbranched or branched conidiophores;

iv) ***Alternaria*** spp.: Septate, dark hyphae with septate conidiophpores and formation of large macroconidiae which have transverse and longitudinal septations;

v) ***Mucor*** spp.; Wide and practically non-septate hyphae, speorangiophores are long, often branched and bear terminal round spore-filled sporangia.

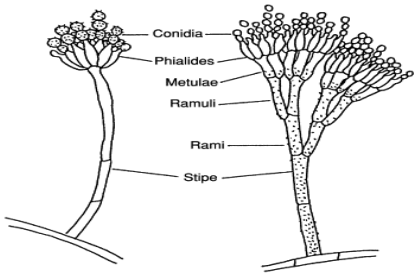

Figure 1. Penicillium morphology

C. *Preparation of dataset images for training*

Machine processes a picture as an array of pixels and numbers, so classification of images can be a rather difficult job, especially in cases where brightness is not the best, position of camera changes or the object is not fully present on the picture, which doesn't present a problem for a person. But, like a human, machine learns in the same manner, with examples of different categories with labels, so it eventually can recognize the patterns on the images.

For our model, we extracted examples of eight fungal genera, which are *Aspergillus* spp., *Fusarium* spp., *Epidermophyton* spp., *Alternaria* spp., *Microsporum*spp., *Penicillium*spp., *Trichophyton*spp. and *Mucorales* spp. (Figure 2). Images have been made at the Department of Microbiology and Immunology, Medical faculty, University of Niš, Serbia, laboratories, where molds have been isolated from patient materials, examined on microscopes and then photographed.

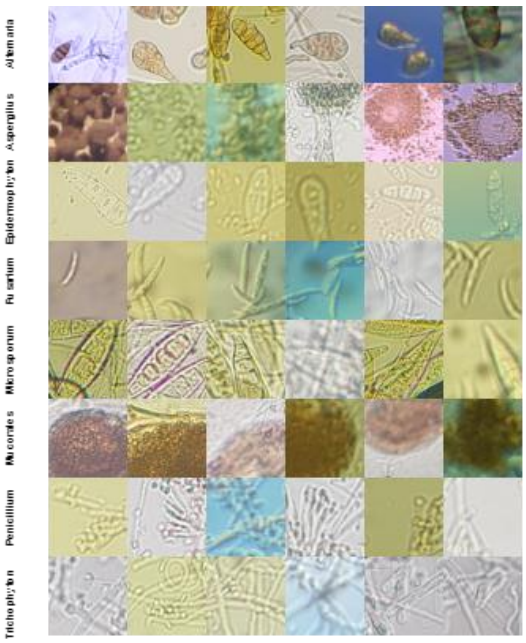

Figure 2. Examples of the dataset images

After preparing the images, which includes manually cutting the high resolution (3024 x 4032 pixels) sample images obtained from Department of Microbiology and Immunology and selecting ones which contain significant

molds parts, it is necessary to determine which percentage of them will be used for training, and which for evaluation, since these sets have to be different so results of evaluation can be regular. After manual preparation, there were 6918 images, from which we used around 80% for training and the rest of the images (20%) for evaluation. In Table I, details of dataset used for training are presented.

TABLE I
Details of used dataset

Number of classes	Number of samples	Number od samples per class	Number of images after preparation	Images used for training	Images used for validation
8	492	50-65	6918	5603	1315

III. METHOD DESCRIPTION

For a neural network to learn to recognize certain patterns in images, it is necessary to create examples so it can learn from them. Sample images of patient materials with molds are high resolution, taken on microscopes, and they have to be cut, because of the GPU limitations when it comes to neural network training, and also to make more examples for network to learn. To obtain small resolution images, it was necessary to cut original images into the set of smaller images, suitable for training. After cutting the images, and manually eliminating the ones that don't contain mold patterns, it was decided to expand the dataset so examples can be more informative.

Operations that are used on the images to widen the dataset and provide multiple examples from one image are called augmentations [9]. Using different brightness, rotation, translation, flipping of the images, etc., we made more examples for training (Figure 3). In the end of this process, dataset became more informative and training could be started.

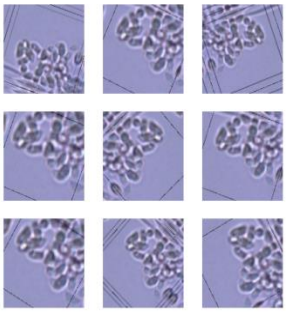

Figure 3. Augmentation of an image gives more images for training

Image classification is a very common problem, present in many different fields of expertise, and traditional approach to this problem is crafting a feature extractor that can be used for training a classifier [10-13]. Earlier solutions used artificial neural networks (ANNs) [14], but major advantages in this area have been made in recent years with development of convolutional neural networks (CNNs) [15]. CNNs represent an aggregation of three architectural ideas, local receptive fields, shared weights and spatial subsampling, which makes them more consistent in terms of translation and distortion [16].

During recent years, many different types of convolutional neural network architectures have been developed, but the one that gave the best result while training our model is EfficientNet. EfficientNet has a family of models (B0 to B7) and during training we tried various variants, where B1 showed the best results, based on accuracy measured. This models, introduced in 2019, by Tan and Le [17], are among the most efficient models, and their innovation lays in heuristic way to scale the model (compound scaling), making them a good combination of efficiency and accuracy [18].

Unlike conventional scaling methods (b-d on Figure 4) that arbitrary scale a single dimension of the network, compound scaling method uniformly scales up all dimensions. In this method, appropriate scaling coefficients are determined with grid search, which discovers relationships between different scaling dimensions. Applying those coefficients to baseline network gets the desired target model size [19].

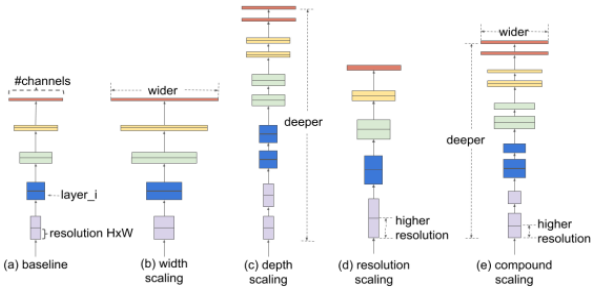

Figure 4. Comparison of different scaling methods [17]

Programming language Python [20] and library Keras have been used for training the model. Keras library [21], implemented in Python, has an interface which can be used for creating and training neural network models, including EfficientNet family. Keras is a deep learning API, running on top of the machine learning platform TensorFlow [22]. They were developed with a focus on enabling fast experimentation.

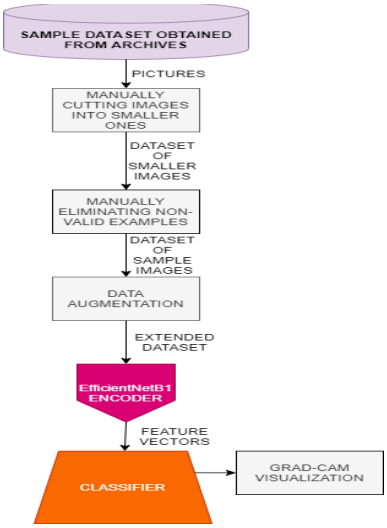

Figure 5. Solution diagram

Model has been compiled with ***RMSprop*** algorithm [23] for optimization (optimizers module),

sparse_categorical_crossentropy type of error (losses module), and the only parameter of metric during learning has been set as accuracy.

EfficientNet-B1 architecture model makes the core of this solution. After training of this model, feature vectors are obtained, which are then used to form a classifier. Classifier can then be used to determine which of 8 classes of molds new input images belong to. Diagram of current solution is shown in Figure 5.

Adjusting parameters of Keras functions and starting the training with different number of epochs, results at these phase of the project show that the trained model after twenty one epochs gives the best results, with 95,74% validation accuracy in classification of images (Figure 6). In our previous paper [8], with a slightly different (including only invasive fungi infections) and drastically smaller dataset, we got the accuracy of around 92%, which shows that we reached a very good improvement with new model. Also, in our previous work we haven't tried EfficientNet neural networks, which gave the best accuracy for our, now expanded, dataset.

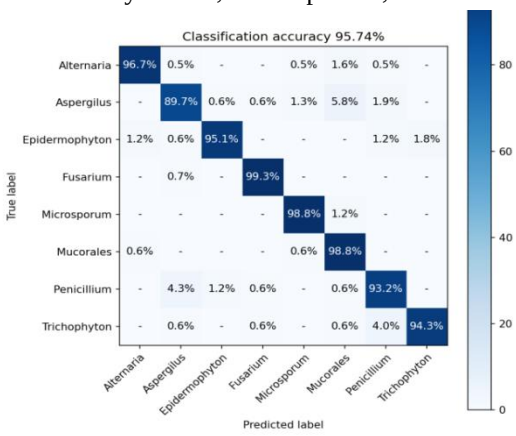

Figure 6. Confusion matrix showing accuracy in %

IV. RESULTS AND DISCUSSION

Because of specific nature of the dataset and sample making, model has not been compared and tested with other datasets or models. In Table II, average results for each fungi genera have been presented.

TABLE II

Results for different fungi genera

Fungi genera spp.	Accuracy [%]	Samples placed correctly/samples per class
Alternaria	96,7	177/183
Aspergillus	89,7	139/155
Epidermophyton	95,1	155/163
Fusarium	99,3	144/145
Microsporum	98,8	166/168
Mucorales	98,8	161/163
Penicillium	93,2	151/162
Trichophyton	94,3	166/176

After validation of the model, it has also been tested manually, showing that the results for most images are accurate. Figure 7 shows confusion matrix, which contains accuracy results per classification class, showing problematic areas too. The most misclassifications happened for *Apergillus* spp. genera, for which we had the least number of clear images, which points out that more images have to be obtained or existing images should be sharpened, so better accuracy can be achieved.

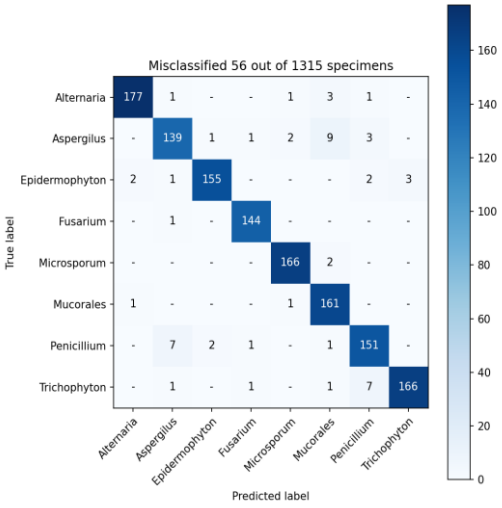

Figure 7. Confusion matrix

Taking into consideration that neural networks learn from examples, from which they learn patterns, and that some sample molds images contain not only significant parts used for diagnostics, but also other parts of materials (for example plain parts of the branches, end of slides on the microscope, different base colors) it is important to verify those learned patterns to be sure that classification, and later diagnostics, performed by the model is valid.

Grad-CAM method is a technique used for visualization of decisions from CNN models, making the decision making process transparent and understandable [24]. This method uses gradients of a target concept (in our cases molds) flowing into final convolutional layer in a network, so it can highlight regions of significance. This way, part of the image which had lead to decision of the classifier is highlighted.

Based on the majority of heat maps got from Grad-CAM method, decisions made by our classier have been done on significant parts of mold samples. Figure 8 shows the examples.

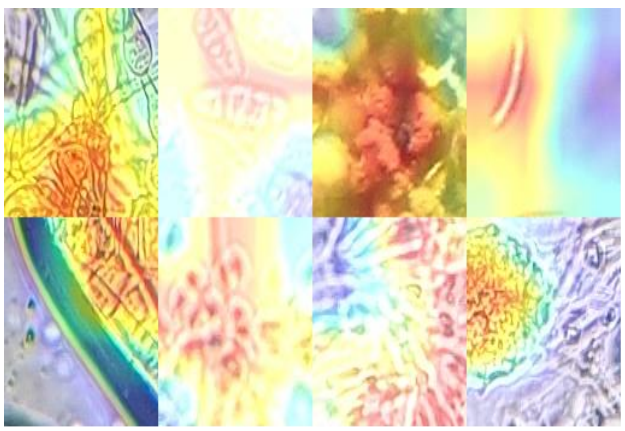

Figure 8. Examples of good pattern recognition visualization

Grad-CAM method is very useful in terms of concluding which of the test images have been misclassified because of the wrong pattern recognition in wrong part of the image (Figure 9). In our case, most of the misclassification happened because of poor quality of input images, because some of them are taken by mobile phones brought close to the microscope oculus, which can result in blurry image. In this way, visualizing the decision making process pointed out that maybe images should be sharpened before processing.

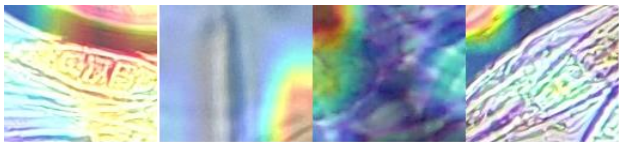

Figure 9. Examples of bad pattern recognition on blurry samples

V. CONCLUSSION

In this paper, we described developing a identification model which, based on accuracy results and testing, presents a solid base for developing an application that can be used in practice and drastically accelerate the process of diagnostics.

Grad-CAM method used to visualize the decision making process has proven to be a very efficient method of evaluation of the model, not only in terms of validating the "thinking" process of the classifier, but to point out flaws and cases where errors happen.

Future development of the model and application will involve developing an algorithm that can reach the decision based on high resolution photo, from which number of smaller images will be cut, and then classification will be performed on each of the small sample images. This approach will increase precision of the diagnostics, since the decision will be a ruling of the mayor, rather than determination based on one small sample.

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