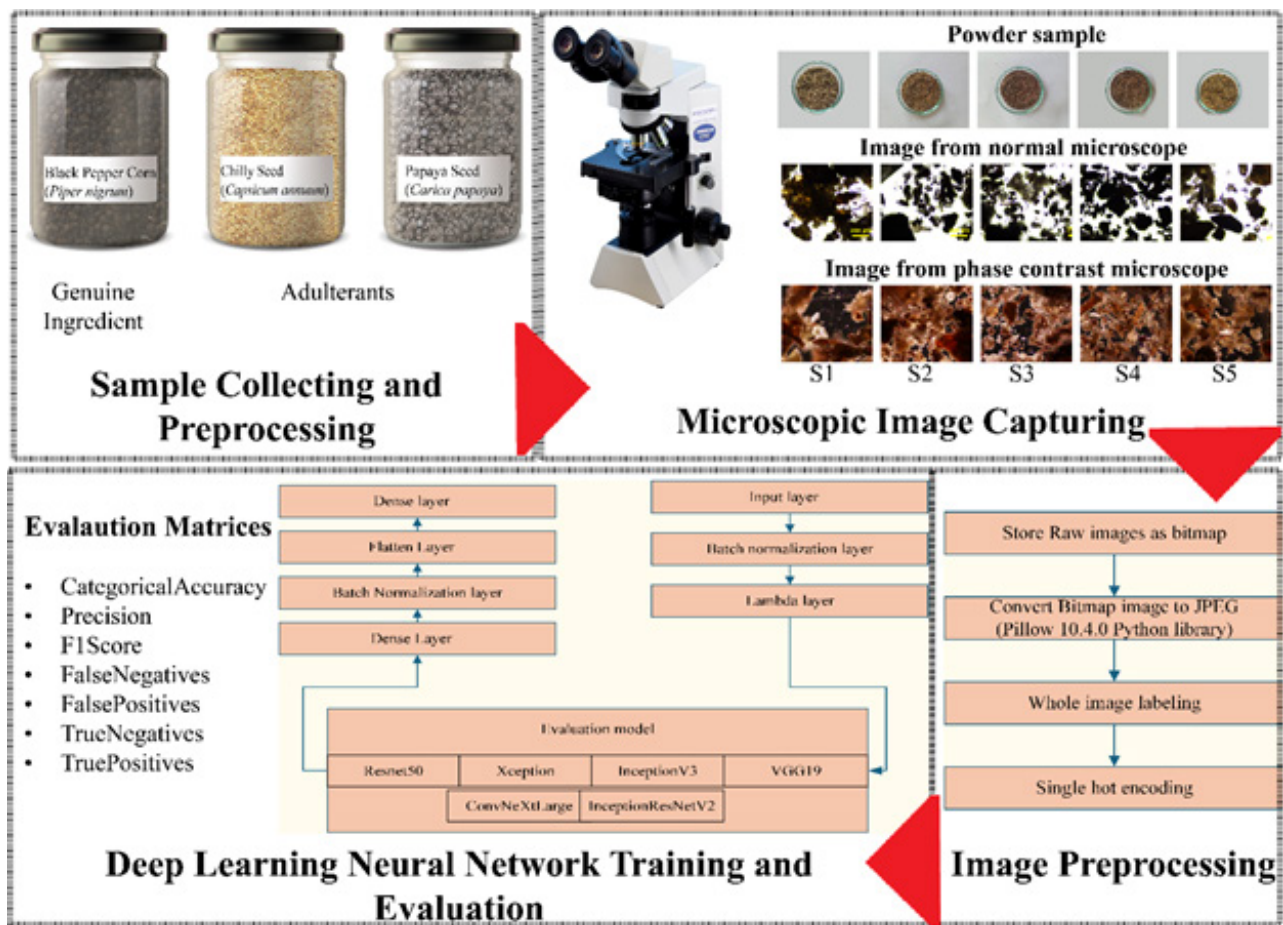


RESEARCH ARTICLE

Detection of Adulterants in Pepper Powder Using Deep Learning-Based Microscopic Imaging: A Cost-Effective Approach for Food Safety

J. K. W. U. D. Karunathilaka, P. C. Arampath, K. S. P. Amarathunga, W. M. K. Fernando and T. Liyanage

Graphical abstract



Highlights

- InceptionV3, Xception & ResNet-50 effectively detect black pepper adulteration
- Dry-mounted phase contrast microscopy provides clear images of pepper adulteration
- Suggested objective method reduces humans' subjective errors in adulteration detection
- The microscopic method is efficient and cost-effective for black pepper adulterant detection

Detection of Adulterants in Pepper Powder Using Deep Learning-Based Microscopic Imaging: A Cost-Effective Approach for Food Safety

J. K. W. U. D. Karunathilaka^{*1}, P. C. Arampath², K. S. P. Amarathunga³, W. M. K. Fernando⁴ and T. Liyanage⁵

¹ Postgraduate Institute of Agriculture, P.O.Box 55, Old Galaha Road, Peradeniya, 20400, Sri Lanka

² Department of Food Science & Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka

³ Department of Agricultural Engineering, Faculty of Agriculture, University of Peradeniya, Peradeniya, 20400, Sri Lanka

⁴ Field Crops Research and Development Institute, Mahalluppallama, 50270, Sri Lanka

⁵ Post-Harvest Technology Division, Department of Export Agriculture, Matale, 21300, Sri Lanka

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Abstract

High-valued black pepper-based products are adulterated with papaya (*Carica papaya*) seeds, and chilli (*Capsicum annum*) seeds. Those are causes of food allergic reactions and food poisonings. Therefore, a cost-effective and efficient system should be developed to identify those adulterants. According to studies, each plant material has its own unique microscopic image fingerprint. Phase contrast microscopes are able to differentiate those features based on their refraction indexes. This research was developed for identifying the best deep learning architecture to differentiate adulterated black pepper powder from genuine black pepper powder by using microscopic images. TensorFlow deep learning models (InceptionV3, Inception ResNetV2, ConvNeXtLarge, Xception, VGG-19, and ResNet-50) were used as the backbone. The Adam optimizer and categorical cross-entropy as a loss function were used in both the evaluation stages and the training stages. Categorical Accuracy, Precision, F1Score, False Negatives, False Positives, True Negatives, and True Positives were used for evaluating the trained models. 0.001 and 0.0001 Fine-tuning with learning rates were used for model training. According to the results of evaluation matrices, InceptionV3, Xception, and ResNet-50 showed superior performances in differentiating genuine black pepper powder from other powder classes. But overfitting was identified when increasing the number of training epochs at lower learning rates. Therefore image segmentation should be introduced for overcoming the challenges in whole image labelling. Selective hierarchical neural network layer fine-tuning and hyper parameter tuning can be implemented for overcoming the model training based issues. According to the findings, those microscopic image based techniques are able to identify genuine black pepper powder from other adulterated black pepper powder.

Keywords: Food Authenticity; Image Classification; Phase Contrast Imaging; Transfer Learning

INTRODUCTION

Black pepper (*Piper nigrum*) is a dominant ingredient not only in cuisine; it spread its flames to cosmetics and medicine (Cvetković et al., 2020; Meenakumari et al., 2024). According to industrial requirements, different processing methods are used in developing huge range of pepper products. White pepper, green pepper, and crushed

pepper-based products are more common and popular in the culinary industry (Baenas et al., 2019; Ravindran & Kallapurackal, 2012). High versatile product ranges provide a clear path to increase the vulnerability to adulterating black pepper. Adulteration can be defined as an act that is performed intentionally or accidentally to lower the quality of the products by adding inferior substances, substituting genuine ingredients, or removing valuable components

*Corresponding Author's Email: udkarunathilaka@gmail.com



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<https://orcid.org/0009-0007-1091-3750>

(Choudhary et al., 2020; Haji et al., 2023). Adulteration activities with black pepper are very difficult to identify due to a lack of efficient scientific methods for identifying adulterants as well as natural variation of the black pepper. Papaya (*Carica papaya*) seeds, dried chili (*Capsicum annuum*) seeds, and the dried fruit of West Indian Lantana (*Lantana camara*) are popular adulterants for black pepper (Essuman et al., 2022; Parvathy et al., 2014; Wilde et al., 2019). These undeclared adulterants can cause food allergic reactions and food poisonings.

Therefore, implementing effective and efficient methods for identifying those issues are crucial. The industry has developed several methods for identifying adulterations associated with black pepper and based products. DNA barcoding, polymerase chain reaction (PCR), hyperspectral imaging, gas chromatography-mass spectrometry (GC-MS), near infrared spectroscopy (NIR), and direct analysis in real-time mass spectrometry (DART-MS) are dominant technologies that have been implemented in the industry. But recommending the best techniques for identifying all individual and combined adulterations is a challenge at the moment when comparing technique effectiveness, efficiency, and cost (He et al., 2021).

Among those advanced technical methods, microscopic methods play an undefeated part in identifying adulteration in black pepper-based products (Banti, 2020; Faith et al., 2022; Oliveira et al., 2024). The refraction indices and angular scattering effects for each cell organelle are different and unique. According to the literature, significant differences have been found among those black pepper adulterants and black pepper tissues (Dvořák et al., 2020). Those analyses provide invaluable insights regarding various structures of plant tissues, such as papaya seeds and chilly seeds (Kutlu et al., 2022; Przybył et al., 2021; Xiao et al., 2020). But the quality of those microscopic analyses is mainly dependent on the quality of the microscopic image as well as the microscopic technique. Different wave lengths of the light catch various details of the specimen such as specific chemical distribution within the specimen, processing condition of the specimen, various adulterants in the specimen (Cannaday et al., 2021; Kishimoto et al., 2022; Reiche et al., 2022).

A phase contrast microscope is a light microscope. It is equipped with proper optical filters and the configuration to capture more details than a normal light microscope. It is able to differentiate the light rays that pass through the background and the specimen itself. This is due to the different refractive indices of the background and specimen (Fang et al., 2023). Identifying fine details from the specimen and differentiating adulterants from genuine black pepper is a big challenge at the moment because training a professional to identify specific genuine spices and adulterants is a costly process. It takes a huge time and their judgments are subjective. Even without labelling identifying those adulterants are big challenges at the moment (He et al., 2021; Khan et al., 2024; Kishimoto

et al., 2022; Maturana et al., 2022). This study was targeted to identify the feasibility of a computer vision technique-based deep learning technique to distinguish between genuine black pepper powder samples from other adulterated black pepper powder samples by using phase contrast microscopic images. It will be directly beneficial to small-scale spice exporters in Sri Lanka since it is a rapid, less technical, efficient, and highly accurate method with compare to other methods available in market. That will make ease their approach to the high-end export markets.

MATERIALS AND METHODS

Sample preparation

Black pepper samples were obtained from local collectors in Matale, Sri Lanka. The “Bootawe Rala” black pepper variety was used because of the popularity of the variety. Peppercorns, papaya seeds, and chilly seeds authenticity were verified by the Department of Export Agriculture, Matale. Papaya seeds were collected from fruit processing shops. Chilly seeds were collected from the local chilly grinding mills. Literature was used for identifying the mixing ratios (Dissanayake et al., 2016). Six distinct authentic batches were formulated according to predefined material ratios (Table 1) to represent the five classes in the study. Those batches were subjected to size-reducing operations using the IKA Mills MF 10.2 impact grinding head. Particles that were obtained were sieved by using a W.S. Tyle mesh sieve set. The particles that were passed through the NO 60 (250 µm opening) mesh and retained by the NO 140 (106 µm opening) were used for the analysis. Those samples were packed in resealable polyethylene bags and stored at ambient temperature and in dry conditions throughout the experiment period. 29 unique microscope slides were prepared from those 6 batches.

Sample mounting and image capturing

Standard dry sample mounting techniques were used for mounting the dried and sieved powder to the microscope slide. A transparent colourless polyethylene gum tape was used for securing the mounted samples. According to Figure 1, high clarity can be identified in a phase contrast microscope by using the same light microscopic configuration, by changing the phase contrast lens. Therefore, images were captured using an Olympus CX41 phase contrast microscope equipped with an Olympus DP27 5-megapixel microscope camera. 40X phase contrast objective lens (Olympus PlanC N 40x /0.65. Infinity / 0.17) and PH3 annulus configurations were used as the microscopic configuration. Artificial lighting was used to illuminate the specimen. Standard microscopic practices were followed to mitigate the risk of the model learning slide-specific artifacts rather than true class features. Thus, multiple phase contrast microscopic images were captured from various points across each slide. This methodology ensures that the image set captures a wide representation of the samples' general features. Accordingly, 5625 images

were captured for the above five classes, where 1125 images were captured for each class. The images were captured and stored as bitmaps.

Image preprocessing

The captured images were converted to JPEG images by using the Pillow 10.4.0 Python library. The quality level was encoded as the 100% quality level. Then, the images were uploaded to Google Drive and loaded into the system using Google Drive. Those images were labelled according

to the image directory name, and labels were encoded as a categorical vector. Each captured image was labelled using a hot encoding system. That label defines the image respective sample class, which is mentioned in Table 1. Then, labelled images were converted to float32 by using TensorFlow. The dataset, which had 5,625 images, was partitioned into training (3,375 images; 60%), validation (1,125 images; 20%), and testing (1,125 images; 20%) sets. A stratified splitting method was used to ensure that the class distribution remained consistent across all subsets.

Table 1: Quantification of papaya and chili seeds in adulterated black pepper samples

Sample ID	Ingredients (g)		
	Black pepper	Chilly seeds	Papaya seed
S1	90	0	0
S2	30	30	30
S3	0	0	90
S4	45	0	45
S5	45	45	0

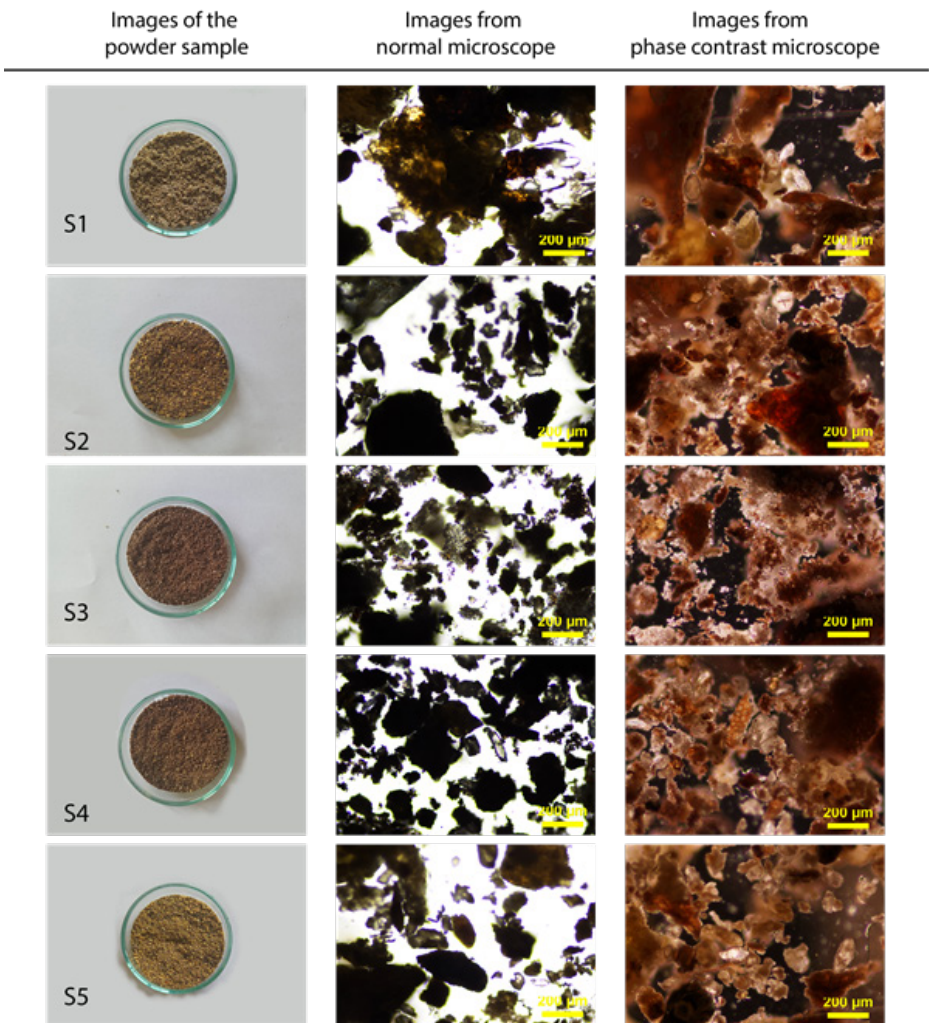


Figure 1: Comparative light microscopy of five powder samples: normal vs. phase-contrast images. S1–S5 represent different black pepper samples with varying proportions of chili and papaya seeds for adulteration testing

Model architecture and training methodology

The deep learning architecture was made according to the following arrangement in Table 2. When compiling the model, the Adam optimizer was used as the optimizer, categorical cross-entropy was used as the loss function, and categorical accuracy was used as the evaluation metric. Six-based models were evaluated. Those models were loaded as the base model layer developed in the model above. The respective pre-processing function was loaded as the lambda layer in the above models. ImageNet pretrained weights were loaded into the respective model. Then the first 30 epochs (Model A) were trained while the base model was not trained. Then the base model layer trainable parameter was trained for 30 epochs (Model B) and another 30 epochs (Model C). While training, loss matrix data for both the validation data set and training data set were collected and saved as a JSON file. Respective learning rates, which are indicated in Table 2, were maintained in those training circles.

Model evaluation

Each trained model was individually evaluated by using the following matrices using a leakage-free test set. Categorical Accuracy, Precision, F1 Score, False Negatives, False Positives, True Negatives, True Positives.

RESULTS AND DISCUSSION

Sample preparation and mounting

People commonly use light microscopes because of their affordability and ease of operation. Brightfield microscopy, a widely used technique, relies on the absorption and scattering of light by the sample. Several factors contribute to capturing a high-quality light microscopic image (Ahmed et al., 2021; Cannaday et al., 2021; Dvořák et al., 2020). Among these factors, sample preparation and mounting steps are crucial.

Rathna, Red Lady, and Sinta are common and popular papaya varieties in Sri Lanka. Seeds from Red Lady were chosen as the papaya adulterant since it is the most popular and highest-cultivated papaya variety in Sri Lanka (Kahawattage et al., 2023). The dried chili seeds were collected from local spice producers. Those difficulties in collecting adulterant materials were not identified during the research period by local communities. Therefore, those adulterants are available for the market for free use.

Every adulterant sample and black pepper sample was cleaned, and authenticity was verified by the Department of Export Agriculture (Matale). Then, the samples were mixed according to Table 1 and subjected to size-reducing activity. The prepared sample set was sieved by using a standard mesh set to ensure that all particles were between 106 μm and 250 μm . This protocol is capable of removing extremely large particles, clumps, and dust. This particle distribution was determined by analysing black pepper

powder samples that were present in the market. The sample that was solely formulated from chili seeds was not used for the experiment. Chillie sample's macroscopic-level visual characteristics were able to introduce biases in the experiment. Because the distinguished colour of that sample showed a significant difference when compared with other prepared samples at the macroscopic level. Therefore, that sample was excluded from the studied samples.

When using a light microscope, staining techniques are frequently used for highlighting and isolating features in the specimen. That process is named as labelling the sample. But those wet preparations can lead to misrepresenting crucial features compared to dry mounting techniques, since liquid absorption in the specimen during wet processing causes alterations to the samples' original and genuine characteristics, such as cell wall structures and surface structures (Ramamoorthy, 2016).

Without those staining techniques, it was a challenge to identify low-contrast features in the specimen. A phase contrast microscope can differentiate low-contrast features in the slide based on different refractive indices of the components. As a result, the phase contrast microscope was used for capturing images, and the dry mounting technique was used as the mounting technique in the research. To ensure consistent image quality and prevent light drift, a standardised image environment was used. A dedicated artificial lighting setup was used, ensuring consistent and uniform illumination for every image captured. Therefore, it is able to overcome the variation that occurred because of the ambient light changes.

Image preprocessing

Images were captured in a bitmap format from the microscope. Then those images were compressed to JPEG using the Pillow 10.4.0 Python library. That ensures minimal data losses during the process and reduces the storage cost. A whole image labelling system was used for the image classification labelling activity. Specific microscopic shapes or orientations were not detected in commodities that had been subjected to size reduction. Therefore, those adulterant particles were not able to be identified separately in the slide, but there is a variation in the pattern when it is considered as a whole slide image.

Then those images were labelled by using a one-hot encoding system. The one-hot encoding system is more robust and flexible than the ordinal encoding system. Those selected categories were not linearly related. Selected categories are not represented in ranks or hierarchies. A one-hot encoding system supports capturing complex patterns and establishing relationships between selected categories. Furthermore, there is a high ability to avoid the false relationships between the categories when comparing the ordinal encoding system (Dahouda & Joe, 2021; Wang et al., 2022a; Wang et al., 2022b; Zhang et al., 2022). Those images were converted to float values by using

TensorFlow's build function. When converting the image to different activities, such as normalization and activation, the processing speed, precision, and flexibility are higher than integer numbers.

Deep learning model architecture

The complexity of a microscopic image makes it difficult for an untrained microscopist to identify. It is difficult to train a microscopist to identify those specific patterns of genuine black pepper samples and adulterant samples. Although there can be subjective errors, even if this is done by an expert microscopist. Furthermore, objective measurements should be established for evaluating those images. According to the literature, a huge image set can make an objective, advanced deep-learning model (Moen et al., 2019; Poostchi et al., 2018; Rivenson et al., 2017).

Both sequential and application programming interface (API) techniques were used to develop the basic architectures of the models. TensorFlow prebuilt layers were used in developing the deep learning models (Pang et al., 2020). The sequence of the layers was according to Figure 2; the input layer was used to ensure all input data would be in the same shape. The following layers were developed based on the input layer.

Batch normalization layers were introduced to ensure several aspects. The batch normalization layer helps to overcome the overfitting problem in the model (Kumar & Shankar Hati, 2021). That is because the batch normalization layer helps to stabilize the distribution of activation. Then, it is allowed to use high learning rates on the model. That normalization layers helped to correct issues associated with vanishing or exploding gradients (Shaw et al., 2020). The lambda function was introduced before each predeveloped model layer. It provides a custom data preprocessing layer before feeding images to the pre-developed models (Paper, 2021). TensorFlow inbuilt The GlobalAveragePooling2D layer helps in transforming a feature map into a fixed-sized vector and making it suitable for classification activity. This is reducing overfitting because of the global averaging activity (Tong et al., 2020; Zhang, 2024).

TensorFlow's inbuilt flattened layer was introduced before the final dense layer. The flattened layer is essential for connecting the convolutional layer to the dense layers (Mondal & Shrivastava, 2022). The dense layer will identify those labels according to the classification. This has happened because it will convert a multidimensional array into a 1D array (Jeczmioneck & Kowalski, 2021). Two dense layers were used in the architecture; those were acting as the training layers in the model (Shang et al., 2020).

Then those were subjected to fine-tuning during the training activities. Two activation functions were introduced. Those were Rectified Linear Unit (ReLU) activation and softmax activation. The ReLU activation function is simple and

effectively trains the neural network compared to other activation functions in dense layers. It effectively helps in gradient propagation. Therefore, it was introduced in the upper layers of the model (Alhassan & Zainon, 2021). The SoftMax activation is used in the final layer output; it effectively helped in identifying the label of the multilabel classifiers. These dense layers are mainly introduced to the neural network for learning complex and high-level relationships between extracted features (Putra & Setumin, 2021; Szandała, 2021).

When compiling the model, optimizer algorithms and loss functions were used. Those optimizers to output the loss function, which adjusts the weights and biases in the model's layers (Liu et al., 2022; Schuetz et al., 2022). The primary goal of that function is to decrease the loss function and enhance the model's performance. TensorFlow inbuilt Adaptive Moment Estimation (ADAM) optimizer and default loss function were used. ADAM can resolve the problem that is associated with getting stuck in local minima and adapting the learning rate based on historical gradient (Chandriah & Naraganahalli, 2021; Melinte & Vladareanu, 2020).

Then, pre-trained and developed applications were used to evaluate each application's performance against the developed image set. The different architectures and technologies were evaluated by using six inbuilt applications (InceptionResNetV2, Xception, ConvNeXt, VGG19, ResNet50, InceptionV3) (Shah et al., 2023). ResNet50 is developed based on residual connections for efficient training (Koonce, 2021). VGG19 is a simple deep architecture with small filters (Mascarenhas & Agarwal, 2021). Exception is an architecture that has depth-wise separable convolution for efficiency (Shaheed et al., 2022). The InceptionResNetV2 is an application that was developed using both inception and resnet architectures, which is a hybrid approach (Thomas et al., 2020). The next is developed in a transformer architecture (Wu et al., 2023). According to the above data, each and every model has a unique architecture to overcome different problems in image classification. Therefore, those models were evaluated to identify the best architecture in microscopic image classification.

Deep learning model training procedure

When evaluating the above-selected models, those are deep convolutional neural networks (CNNs). Small data sets always struggle with training those CNNs (Zhuang et al., 2021). Therefore, a transfer learning system was used as the domain adaptation technique in this research. Models were loaded with pre-trained weights and fine-tuned those models using the captured microscopic image data set (Suwannaphong et al., 2023). This technique helped share features between two knowledge domains, microscopic image domain adaptation, and bridging the gap between large and small image sets (Xue et al., 2020). There are five ways to fine-tune the model: self-tuning transfer learning (STTL), adversarial fine-tuning (AFT), intra-fine-tuning

(IFT), image-specific fine-tuning (ISFT), and learning to reweight (LR). Image-specific fine-tuning techniques were used in the research (Adedigba et al., 2022).

ImageNet-based trained weights were loaded for those selected models. Those trained weights were generated from a large data set. Then, unseen object classes were introduced to the model, and fine-tuning techniques were used to adapt the model to those unseen classes (Morid et al., 2021). When considering microscopic image classification, the ImageNet dataset does not have microscopic images. The model trained its layers, excluding the backbone layer, in the first 30 epochs. The transfer learning strategy employed this approach. At that moment, new layers are trained upon the images. Then the model was trained for the next 60 epochs while training the base model with low learning rates. Two different learning rates were used. Those were 0.001 (used in the first 30 epochs) and 0.0001 (used in the last 60 epochs) for each model category. Those low training rates are caused by a lower overfitting activity because it helped models preserve learned features by avoiding destructive updates. The learning rates were determined by referring to the literature (Aslan et al., 2021; Ghazal et al., 2022; Wang et al., 2022).

Deep learning model evaluation procedure

A leakage-free test image set was used for testing activity. 6 matrices (Categorical Accuracy, Precision, F1Score, False Negatives, False Positives, True Negatives, True Positives) were used for evaluation activity (Hsieh & Tsai, 2020; Vujovic, 2021). False Negatives, False Positives, True Negatives, and True Positives are considered the primary indicators of the evaluation. Based on those indicators, such as Categorical Accuracy, Precision, and F1Score, were calculated to get more details regarding the performance of the models.

True Negatives (TN), True Positives (TP), False Negatives (FN), and False Positives (FP). TP indicates an amount where the predicted label is equal to the actual label (Mohammad et al., 2022). TN indicates an amount where predicted rejection labels are equal to true rejected labels. FP indicates an amount where predicted labels are not matched with the actual label. FN indicates the rejected labels are not the actually rejected labels according to the ground truth (Maxwell et al., 2021). The categorical accuracy matrix is calculated as the percentage of images that matched the predicted label with the ground truth label value. The high

Table 2: Details of deep learning models, training strategies, and hyperparameters

Model ID	Base Model Type	Based Model Training	Category ID	Number of Epochs	Finetuning Rate
Model 001	ResNet50	No	Category A	30	0.001
Model 002	ResNet50	Yes	Category B	60	0.0001
Model 003	ResNet50	Yes	Category C	90	0.0001
Model 004	VGG19	No	Category A	30	0.001
Model 005	VGG19	Yes	Category B	60	0.0001
Model 006	VGG19	Yes	Category C	90	0.0001
Model 007	Xception	No	Category A	30	0.001
Model 008	Xception	Yes	Category B	60	0.0001
Model 009	Xception	Yes	Category C	90	0.0001
Model 010	ConvNeXt Large	No	Category A	30	0.001
Model 011	ConvNeXt Large	Yes	Category B	60	0.0001
Model 012	ConvNeXt Large	Yes	Category C	90	0.0001
Model 013	Inception ResNetV2	No	Category A	30	0.001
Model 014	Inception ResNetV2	Yes	Category B	60	0.0001
Model 015	Inception ResNetV2	Yes	Category C	90	0.0001
Model 016	InceptionV3	No	Category A	30	0.001
Model 017	InceptionV3	Yes	Category B	60	0.0001
Model 018	InceptionV3	Yes	Category C	90	0.0001

accuracy rate indicates the high performance of the model, and the low value shows the low performance of the model (Mohammad et al., 2022).

According to Figure 3, the categorical accuracy for most models has declined with training epochs. But Inception3 has been able to increase the categorical accuracy, and Xception is able to hold the categorical accuracy at a higher level compared to other models. Categorical accuracy provides an indicator of whether the prediction is correct or not. The categorical accuracy was used to evaluate the overall performance of each model. However, it doesn't give any information about the model's confidence regarding predicting an incorrect class. Therefore, precision metrics were calculated according to Figure 4. According to the calculation, all models had low precision, but InceptionV3-based models showed high precision. However, a high precision value does not provide assurance that we will be able to provide high true positive values (Zaidi et al., 2022).

Therefore, precision was not enough to judge models' ability for multi-class classification. Therefore, F1 score metrics were calculated to get more data about the model (Hsieh & Tsai, 2020). Correct predictions for the particular class and total predictions for that class are focused on by the precision metric. Then it calculates the ratio of total correct predictions to total predictions to determine

classes. Furthermore, it is invaluable to determine model performances based on predictions of each class. It was used to evaluate the model's ability to identify each class.

Therefore, the F1 value was calculated for each model and class. The F1 value is calculated based on the harmonic mean of precision and recall. The recall tells how many actual positive cases were identified by the model. The F1 value provides a single value that interprets both values. A low F1 value interprets the poor performance of a model. That can be due to the high number of false positives and the low number of true positives for classes (Maxwell et al., 2021).

When considering models in the A category, models that used Resnet50, InceptionV3, and Xception as the base architecture showed a high F1 value according to Figure 5. But that value was reduced in B and C model categories according to figures 6 and 7 (those are subjected to transfer learning activity and trained with more epochs). However, models that were developed by using Xception and InceptionV3 as a backbone showed high precision and categorical accuracy in the evaluated classes when compared to other models in all categories. According to those results, the Inceptionv3 and Xception backbones showed a high resistance against overfitting.

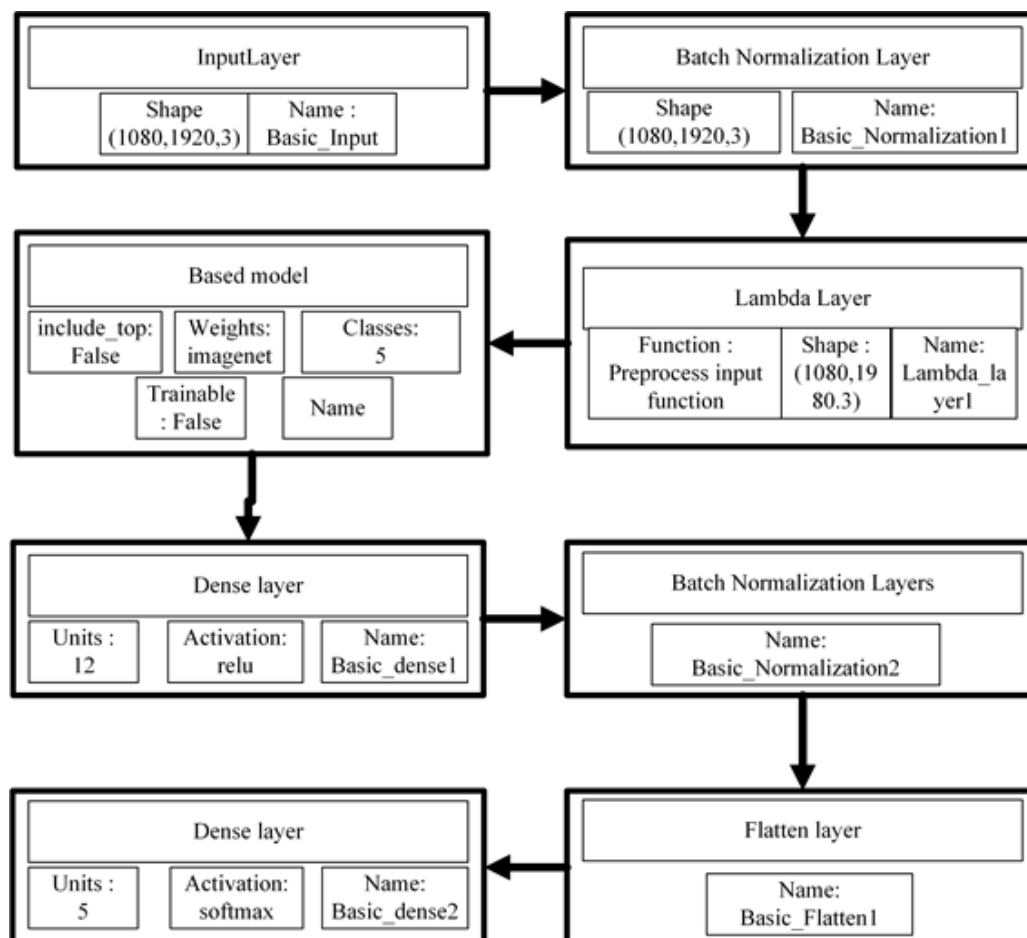


Figure 2: Deep learning architecture showing layer arrangement and evaluated hyperparameters

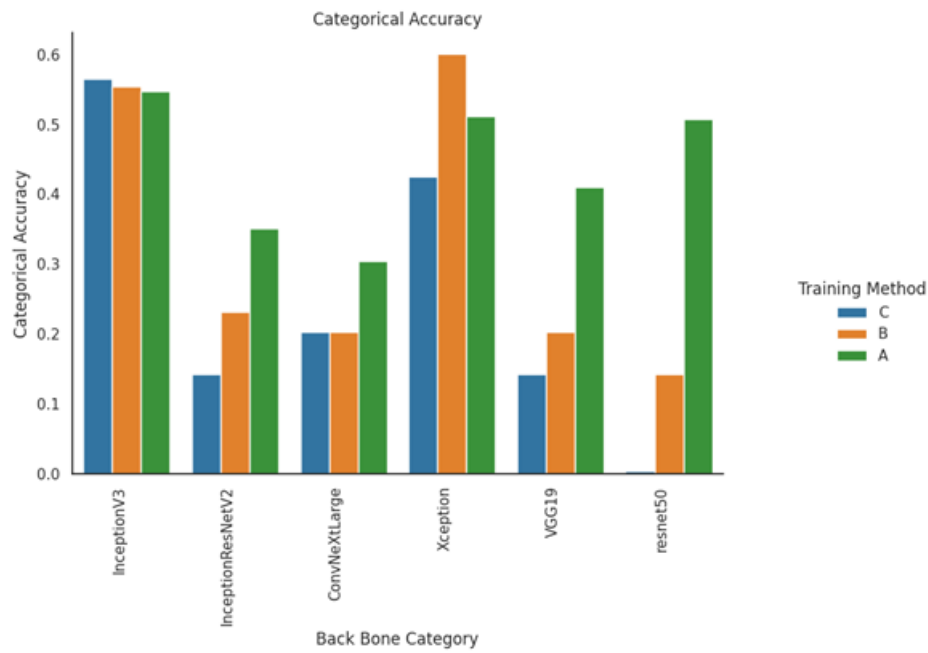


Figure 3: Performance evaluation of deep learning backbone models using categorical accuracy

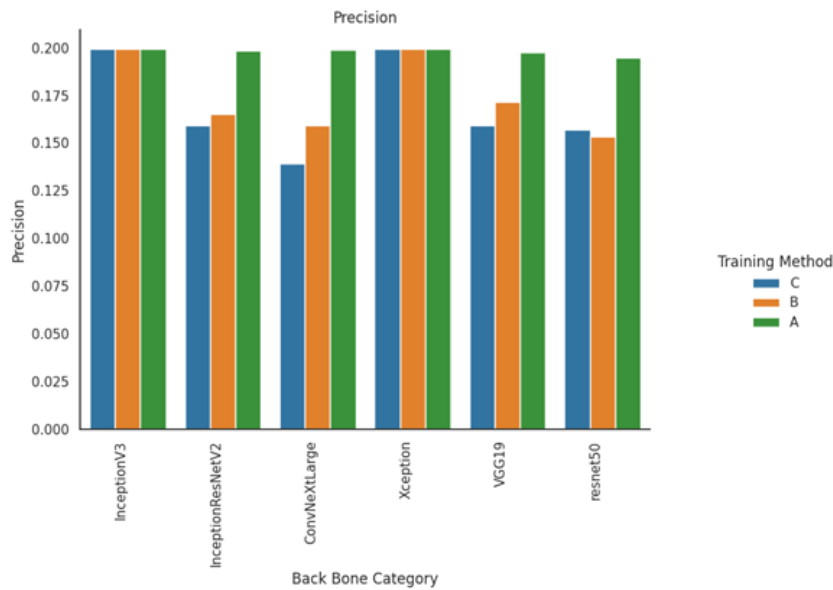


Figure 4: Deep learning backbone model performance evaluated by precision

The InceptionV3 was designed by using a small convolution (3×3) compared to other models (Madanan & Sayed, 2022). It helps to reduce the computational cost while maintaining a high rate of generalized features. This architecture reduced the images in dimensionality before they were subjected to spatial aggregation. That leads to improved processing efficiency without significant information loss. InceptionV3 used auxiliary classifiers, which motivates us to use useful gradients from lower layers. This allows it to effectively capture hierarchical representations of images and visual patterns, which are crucial for accurate classification. It helps to reduce the problem of vanishing gradient. This is very useful when models are trained with more epochs (Wang et al., 2022).

When evaluating the Xception model architecture, it is developed based on three main parts. Those were the entry flow, the middle flow, and exit flow (Mehmood et al., 2023). Skip connections were established on those flow layers. Mainly, that architecture was used for depth-wise separable convolutional (DSC) layers. DSC is responsible for splitting input data into individual channels, convolving each channel, producing multiple outputs, and concatenating the outputs from those channels. It doesn't mix information like a regular convolution layer (Phukan & Gupta, 2022).

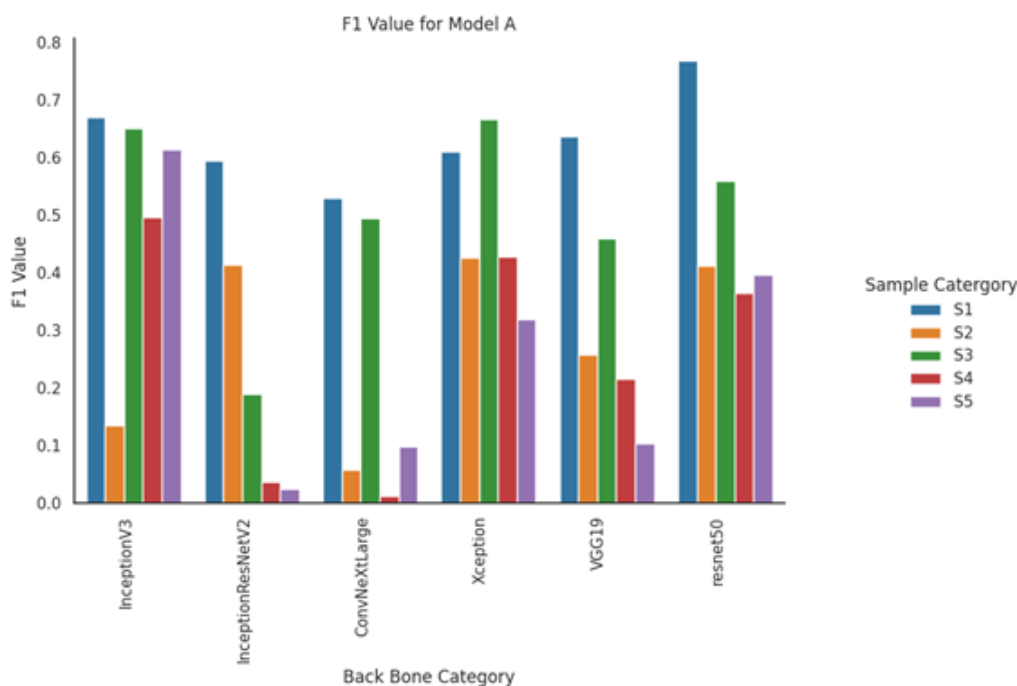


Figure 5: Variation of F1 values in Category A deep learning models across image classes

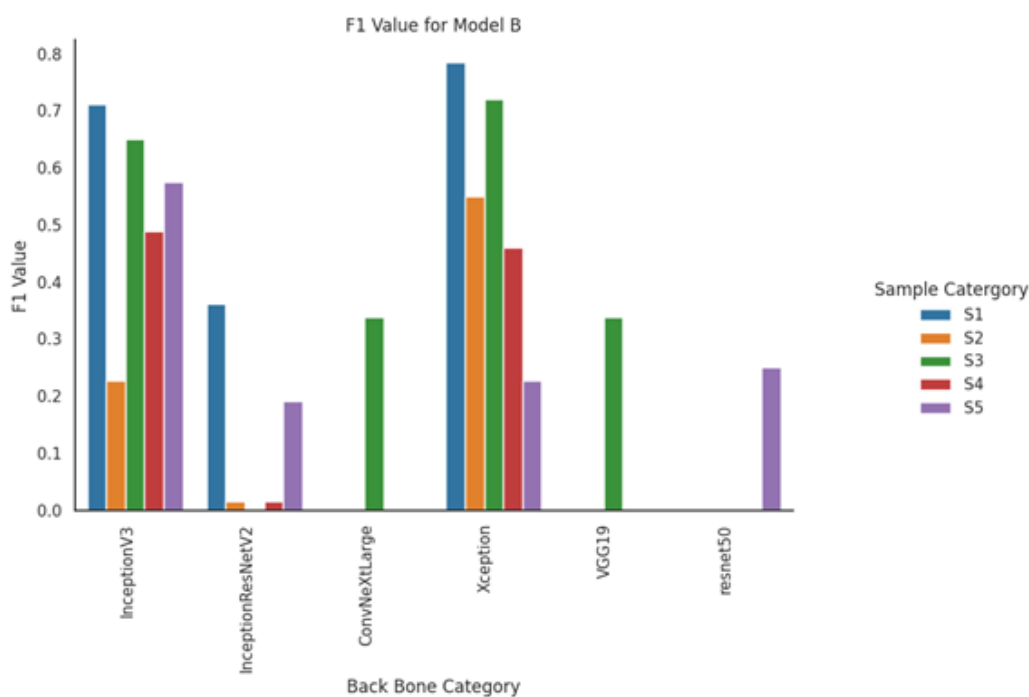


Figure 6: Variation of F1 score (harmonic mean of precision and recall) in Category B models across image classes

Beyond the aforementioned arrangements, the Xception model architecture employs several regulatory configurations. Weight-decaying configuration and dropout configuration are the main configurations. Default rates were used in the above configuration (weight decay: $4e-5$, dropout configuration: 0.5). The weight decay promotes the learning of weights from similar neurons (Hardjadinata et al., 2021). Several neurons are selected randomly, and the output is set to zero in the dropout when overfitting occurs. As a result, new, small, and multiple neural networks. Therefore, it stops neurons from depending on

each other and encourages them to learn independent and diverse features from the image set. Therefore, the neurons can generalize the features across the network, which in turn reduces the likelihood of overfitting (Sathya et al., 2024).

The Hybrid models, such as Inception-ResNet v2 and ConvNeXtLarge models, showed less F1 value in genuine black pepper and precision value when compared to other models (Jena et al., 2021; Zhang et al., 2021). Those are robust architectures.

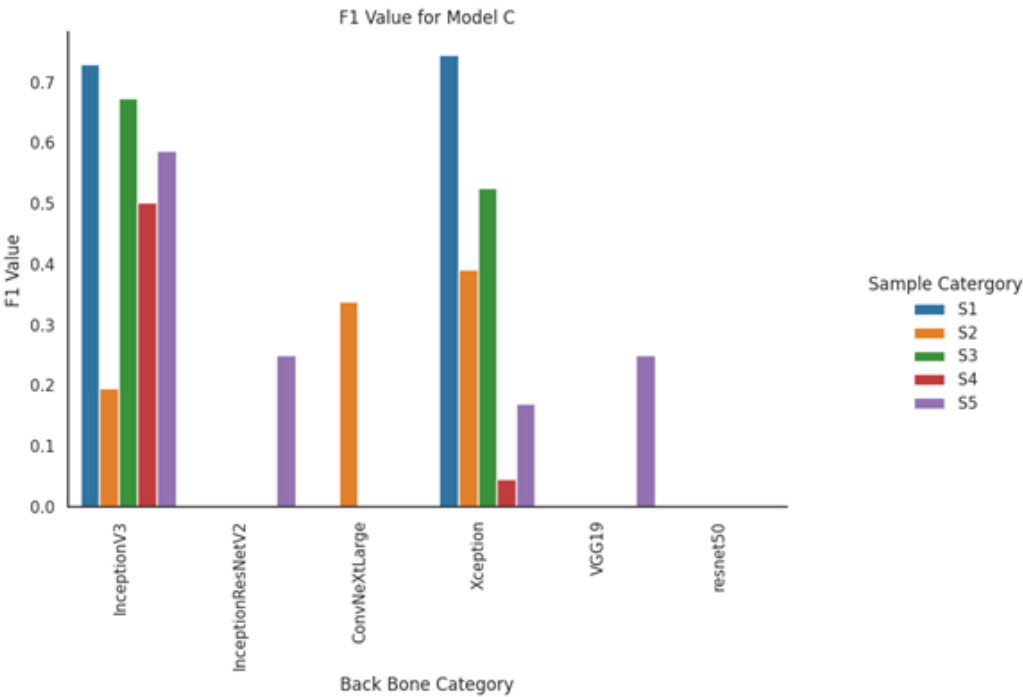


Figure 7: Variation of F1 score (harmonic mean of precision and recall) in Category C models across image classes

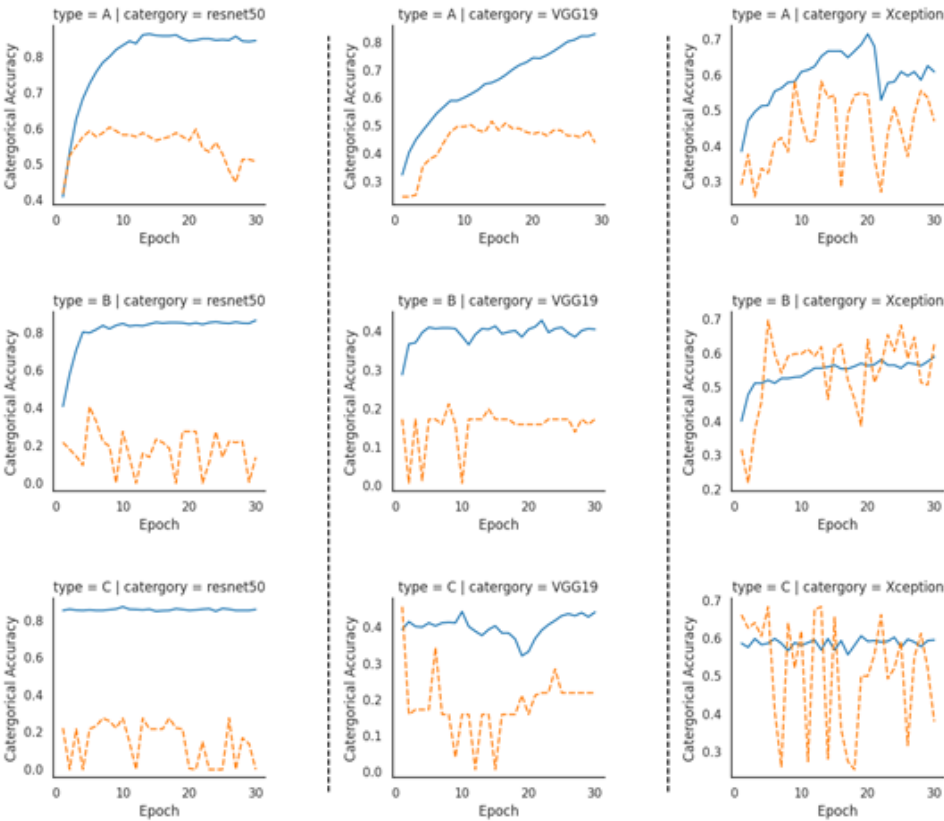


Figure 8: Changes in categorical accuracy over training epochs for ResNet50, VGG19, and Xception models. Blue line: training accuracy; orange line: validation accuracy

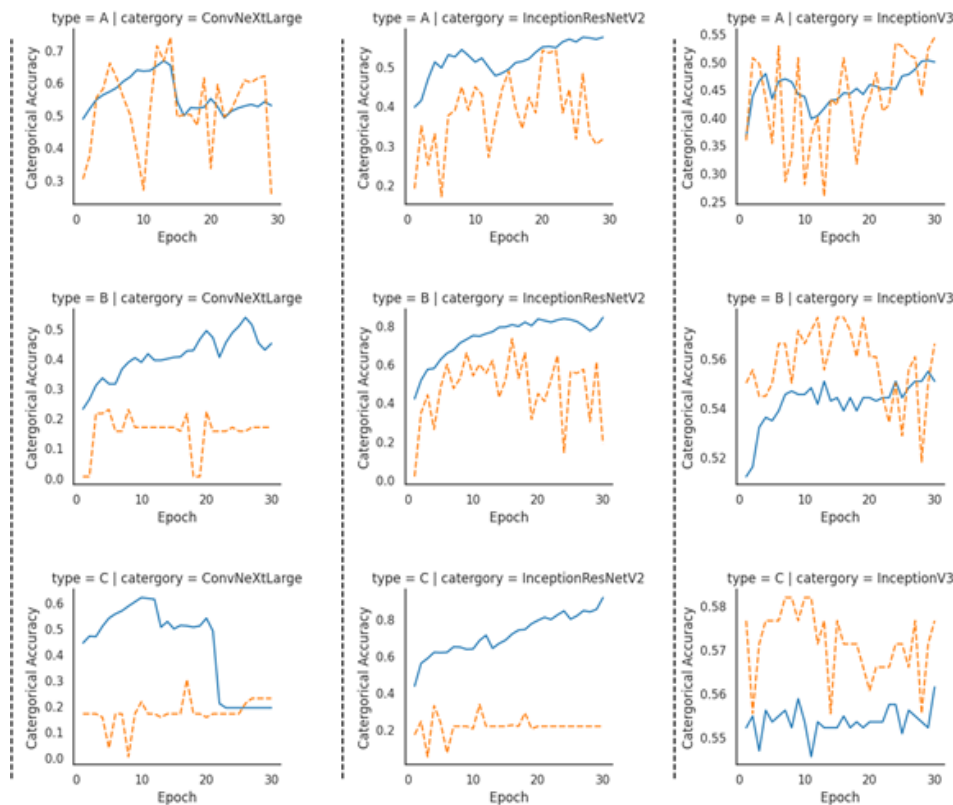


Figure 9: Categorical accuracy over training epochs for ConvNeXt Large, Inception ResNetV2, and InceptionV3. Blue: training accuracy; orange: validation accuracy

Table 3: Confusion matrix results of deep learning models across categories

Model ID	Model Category	Base Architecture	True Positives	True Negatives	False Positives	False Negatives
Model 001	A	ResNet-50	92	851	87	95
Model 002	B	ResNet-50	27	776	161	161
Model 003	C	ResNet-50	1	750	187	187
Model 004	A	VGG-19	76	834	102	113
Model 005	B	VGG-19	39	788	148	150
Model 006	C	VGG-19	26	777	161	161
Model 007	A	Xception	60	895	43	127
Model 008	B	Xception	39	936	2	148
Model 009	C	Xception	35	924	13	153
Model 010	A	ConvNeXt Large	45	879	59	142
Model 011	B	ConvNeXt Large	39	786	150	150
Model 012	C	ConvNeXt Large	39	787	151	148
Model 013	A	InceptionResNetV2	51	864	73	137
Model 014	B	InceptionResNetV2	42	811	127	145
Model 015	C	InceptionResNetV2	27	776	161	161
Model 016	A	InceptionV3	62	909	28	126
Model 017	B	InceptionV3	68	906	31	120
Model 018	C	InceptionV3	76	898	32	119

Table 4: Comparative evaluation of established industrial techniques and the proposed deep learning microscopic method for black pepper powder adulteration detection

Methodology	Cost (Setup & Running)	Throughput (Samples/hr)	Limit of Detection (LoD) / Sensitivity	Operational Complexity
GC-MS	Very High: Requires expensive gases, columns, and high maintenance (Catarro et al., 2025; Xu et al., 2021)	Low: 1–2 samples (requires lengthy extraction/derivatization) (Foguet et al., 2022; Jonsson et al., 2005)	Superior: Can detect trace chemical contaminants (ppm/ppb level) (Schanzmann et al., 2025; Shankar et al., 2022)	Very High: Requires a highly skilled chemist (Catarro et al., 2025; Schanzmann et al., 2025)
FTIR (Fourier Transform Infrared Spectroscopy)	Moderate: Mid-range equipment cost; minimal consumables (Kotaska et al., 2023; Valente et al., 2025)	High: 10–20 samples (fast spectral scanning) (Kotaska et al., 2023; Yang et al., 2022)	Moderate: Good for bulking agents (>5%); poor for trace chemicals (Kotaska et al., 2023; Valente et al., 2025)	Moderate: Needs chemometric software and model calibration (Kotaska et al., 2023)
NIR	Low to Moderate: Portable units available; no reagents needed (Beć et al., 2022a)	Very High: 30+ samples (instant non-destructive scanning) (Beć et al., 2022a)	Moderate: Best for screening high-volume adulteration (>10%) (Beć et al., 2022a,b)	Low: Simple "point-and-shoot" once models are trained (Beć et al., 2022b; Wang, 2024)
DART-MS	High: Very expensive mass spec hardware (Couch et al., 2023; Wang, 2024)	Very High: 40+ samples (no sample prep; "ambient" ionization) (Gupta & Samal, 2022; Sisco & Forbes, 2021)	Excellent: High sensitivity for volatile organic markers (Couch et al., 2023; Sisco & Forbes, 2021)	High: Requires expert interpretation of complex mass spectra (Gupta et al., 2022; Sisco & Forbes, 2021)
DNA Barcoding	Moderate to High: Requires DNA extraction kits and sequencing (Dawan & Ahn, 2022; Guo et al., 2022)	Very Low: Takes 24–48 hours for a full batch (Antil et al., 2023; Dawan & Ahn, 2022)	Superior: Perfect for species identification (Antil et al., 2023; Dawan & Ahn, 2022; Guo et al., 2022)	High: Requires molecular biology lab and bioinformatics (Antil et al., 2023; Chac & Thinh, 2023)
PCR	Moderate: Requires thermal cycler and specific primers (Chac & Thinh, 2023; Dawan & Ahn, 2022)	Moderate: 10–15 samples (batch processing) (Chac & Thinh, 2023; Dawan & Ahn, 2022; Guo et al., 2022)	Superior: Highly sensitive to specific biological adulterants (Chac & Thinh, 2023; Guo et al., 2022)	Moderate/High: Requires clean-room protocols to avoid contamination (Chac & Thinh, 2023; Dawan & Ahn, 2022)

According to the literature, they can grab the image sets general features. When the model gets complex and deeper, those parameters' weights vanish or explode during the training. High-quality input should be used to overcome this issue in deeper architectures (Rasool et al., 2022). The whole image labelling was used in the research. When considering captured microscopic images, focus areas, less focus areas, and background areas were identified. Furthermore, a segmented labelling system will be able to provide more efficient and accurate models (Jaouedi et al., 2020).

The model, using ancient architectures (VGG19, ResNet50, InceptionV3, Xception) as its backbone, demonstrated the highest F1 value when it came to classifying genuine black pepper powder compared to other powder classes in the Model A category. However, the genuine black pepper powder F1 value was decreased with fine-tuning activity, which is due to the overfitting of the data. According to the confusion matrix in Table 3, the ResNet50- A-category shows a higher true-positive value and a lower false-negative value when compared to other models. According to figures 8 and 9, when the architecture gets deeper, training the following layers is a challenge to overcome overfitting. The ResNet 50 introduced residual blocks as a solution for that (Zhang et al., 2023). This method is able to add input directly to the output of the layer. These connections allow gradients to follow early layers by avoiding vanishing gradient problems. Moreover, it is able to learn more complex and hierarchical data compared to other models. But its resistance to overfitting is less in comparison to the exception. When evaluating F1 values, the single ingredient item showed a high value when compared to the adulterated sample. According to data, genuine samples can be identified by using these deep learning models more than identifying a single adulterant or multiple adulterants from the blend.

To overcome the overfitting problem, the layers' learning rates should be carefully selected. According to the rule of thumb, small learning rates should be assigned to early layers and large learning rates to the following layers (Iman et al., 2023). Early layers learn primitive features of the images, but the following layers will learn complex features from the domain. But the fine-tuning rates for each layer change with the domain of training. Therefore, it should be determined by using an experimental pathway. More than adjusting learning rates, image segmentation and labelling can provide a huge impact in overcoming overfitting issues in those models (Ghiasi et al., 2022; Qureshi et al., 2023). The process of labelling an entire image is a powerful classification technique. But it does not give image classification information at the pixel level. When image segmentation is used, it is able to identify boundaries and general and specific data for particular objects. When whole image labelling is used, it will be difficult to do (Greenwald et al., 2022).

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According to data of the table 4, the deep learning-based microscopic method provides a balanced performance profile that is able to address the limitations of the high-end industrial technologies. Unlike high-cost chemical and molecular methods, this microscopic method, which cooperates with a deep learning model, is able to overcome expert chemical or molecular knowledge, expensive analysis methods, high operational expenses, and low throughput. Therefore, the developed method can be used to identify the adulterants cost-effectively and efficiently.

CONCLUSION

In conclusion, the application of scientific evidence in deep learning techniques accurately identified adulterated black pepper powder based on phase-contrast microscopic images. The clarity of phase-contrast images was higher than that of normal bright-field microscopic images at the same magnification. According to the results, the ResNet50 architecture provided the best performance in classifying these images. Hybrid and deeper architectures, such as InceptionResNetV2 and ConvNeXt, tended to overfit with increasing training epochs. However, traditional architectures, including Xception, VGG19, ResNet50, and InceptionV3, showed greater resistance to overfitting. To address overfitting, optimization of both image classification and transfer learning techniques can be applied. Segmentation-based classification can reduce labeling noise and enhance the quality of input data for the model. Additionally, a layer-wise transfer learning approach can further improve model performance. Overall, this research scientifically confirms that deep learning architectures can effectively replace subjective microscopic analysis with objective, automated techniques for identifying adulteration in black pepper powder.

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DECLARATION OF CONFLICT OF INTEREST

The author declared that there is no conflict of interest

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AUTHOR CONTRIBUTION

Conceptualization, funding acquisition, model development, and data collection: **J.K.W.U.D.K.** Supervision and review

of the study: **P.C.A., K.S.P.** Provision of phase-contrast microscope resources: **W.M.K.F.** Verification of sample authenticity: **T.L.** All authors have accepted responsibility for the content of the manuscript, reviewed all results, and approved the final version.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to ongoing follow-up studies addressing limitations identified in this research. However, they are available from the corresponding author upon reasonable request.

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