Automatic Detection of Escherichia coli Bacteria from Tryptic Soy Agar Image Using Deep Learning Method

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Abstract. Escherichia coli is a normal bacterial flora that lives in the human intestine, is harmless and is part of a healthy digestive tract. However, there are several strains of pathogenic Escherichia coli that can cause infections in the digestive tract, namely diarrhea. Diarrheal disease in Indonesia needs treatment and study because most of the diagnoses are still based on clinical diagnosis. Conventional methods used for the detection of Escherichia coli bacteria include culture methods, biochemical tests, and serological tests. This method has the disadvantage of requiring a long time, a large number of samples, and a relatively high error in reading the results. Therefore, the detection process needs to be done automatically using the Faster R-CNN deep learning method. In this research, we used Faster R-CNN with Inception v2 and ResNet-50 architecture and added augmentation and Image Enhancement to the Tryptic Soy Agar image dataset. The test results show that the addition of Image Enhancement greatly affects model performance and the model that has the best performance and is most appropriate to use is the Faster R-CNN ResNet-50 architecture with the addition of Contrast Stretching and Gaussian Filters to the image dataset. This model has 91% accuracy, 90% precision, 95% recall, and 92% F-1 score.

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INTRODUCTION

Escherichia coli is a normal bacterial flora that lives in the human intestine, is generally harmless, and is part of a healthy digestive tract. However, there are several strains of pathogenic Escherichia coli that can cause infections in the digestive tract such as diarrhea. Infection with pathogenic Escherichia coli bacteria in humans is characterized by a wide range of clinical manifestations ranging from no clinical symptoms or asymptomatic to bloody or bloodless diarrhea (Dutta et al., 2011; Peter et al., 2011). Several factors cause the transmission of Escherichia coli into the human body, including consuming water and food that has been contaminated with Escherichia coli, poor sanitation, and direct transmission from people or animals (Lim et al., 2010).

The World Health Organization (WHO) noted that around 5.6 million children under five died in 2016 and 8.8% were caused by diarrhea (Li et al., 2020). Until now, diarrheal disease in toddlers is still the disease with the second highest mortality rate after pneumonia (Ministry of Health RI, 2020). The Ministry of Health of the Republic of Indonesia has made diarrhea an endemic disease that causes Extraordinary Events (KLB), and reported that diarrhea is a contributor to the mortality rate in children under five in Indonesia. The prevalence of diarrhea in Indonesia, based on the results of Riskesdas (Basic Health Research) in 2018, has decreased by 6.2 percent from Riskesdas in 2013 to 12.3% (Ministry of Health RI, 2018). Even though it has decreased, diarrhea remains the highest cause of infant mortality among other diseases.

Diarrheal disease is still a major problem in Indonesia that needs treatment and study from various aspects because most of the diagnoses made by medical personnel are not based on laboratory examination results but only on clinical diagnosis. For this reason, laboratory tests are very important as a support in examining diarrhea. Several conventional methods are used to determine the presence of pathogenic Escherichia coli bacteria in samples, be it food, drink or in the patient's feces, including culture methods, biochemical tests, and serological tests. This method has the drawbacks of requiring a long time, a large number of samples, and a relatively high error in reading the results (Bakri et al., 2015). In addition, not all Escherichia coli bacteria are present in a sample, for example, Lactobacillus rhamnosus bacteria, which have antioxidant effects that are useful for the digestive tract, and Saccharomyces boulardii bacteria, which have the ability to prevent the formation of wounds in digestive tissue. Because of some of these weaknesses, the detection process must be done automatically. This automatic detection method has many advantages, namely, it can produce accurate, fast, and specific predictions and can be used to overcome the weaknesses of conventional diagnostics.

Automatic detection of pathogenic Escherichia coli bacteria is very important to do to assist doctors in making a diagnosis, one of which is by using machine learning (ML). Research on the automatic detection of Escherichia coli bacteria with ML was carried out by Keeratipibul et al., (2011), which aimed to predict mathematically the relationship between the initial bacterial load, the type of vegetables/fruits, the type and concentration of disinfectants and the residual levels of microorganisms after being sanitized, with application of artificial neural networks (ANN). Escherichia coli and coliform used in this study were isolated from two types of food, and their cultures were activated in Tryptic Soy Broth.

Another study was conducted by Lechowicz et al. (2013) with the aim of classifying Escherichia coli strains in terms of susceptibility/resistance to cephalothin based on 109 Uropathogenic Escherichia coli strains in the infrared spectrum. The data is used to classify E. coli strains using a designed artificial neural network. The result is that the most efficient Artificial Neural Network classifies sensitive/resistant Escherichia coli strains with an error of 5%.

Stocker et al., (2022) evaluated several ML models for predicting Escherichia coli in agricultural pond waters. Escherichia coli concentrations, along with 12 other water quality parameters were measured in water samples. The resulting dataset is used to predict Escherichia coli using the Stochastic Gradient Boosting (SGB) engine, Random Forest (RF), Support Vector Machine (SVM), and the k-nearest Neighbor (kNN) algorithm. The performance results of the model predictions were not significantly different and did not substantially increase the prediction accuracy of the evaluated algorithms.

Based on the performance of ML which is only capable of classifying, a derivative of ML was developed, namely Deep Learning (DL). Programs in DL use more complex capabilities in studying, processing, and also classifying data. (Choi et al., 2020). Convolutional Neural Network (CNN) represents one of the most popular DL algorithms for learning with image and video input. CNN consists of many layers to process and extract data features from the input, which are then used to detect objects (Pal SK, 2018). Region based Convolutional Neural Network (R-CNN) is a form of CNN which was introduced in 2014. R-CNN uses selective search as an external proposal to extract features from the input after being normalized with CNN, which then inputs the output to the classifier to get the results classification and bounding box coordinates as output. R. Girshick & Microsoft Research (2015), solved some of the weaknesses of

R-CNN and built a faster object detection algorithm, namely Fast R-CNN. The approach is similar to the R-CNN algorithm which still uses selective search as an external proposed for building proposal regions, but this is computationally burdensome so the process is slow and affects network performance. Therefore, Ren et al., (2017) developed a Faster R-CNN that replaced the external proposed with the Region Proposal Network (RPN).

Deep Learning successfully reduces the need for feature engineering by leveraging large amounts of data to optimize model parameters and find the most important data features. Tissues trained on data sets are irreplaceable in modern imaging diagnostics. Of course, this does not rule out the possibility in the medical field to help make fast and accurate clinical decisions based on medical procedures. Overall, the application of AI provides innovation for medical technology and diagnostics that enables faster and more efficient medical technologies.

Based on this background, the authors will detect Escherichia coli bacteria in Tryptic Soy Agar images using deep learning, namely Faster R-CNN with Inception v2 architecture, and ResNet-50. The author also adds augmentation and Image Enhancement to the image to improve model performance based on parameters of accuracy, precision, recall, and F1-score.

MATERIALS AND METHOD

Dataset Preparation

Dataset

The data used are images of pathogenic faecal Escherichia coli inoculated onto Tryptic Soy Agar (TSA) plates taken from a journal entitled "AGAR a Microbial Colony Dataset for Deep Learning Detection", by Majchrowska et al., 2021. arXiv:2003.13865v3, which can be accessed from the link https://agar.neurosys.com. The initial step of dataset preparation is selecting a dataset of 510 with the file format (.jpg) in the source.

Anotasi

Annotations are performed on pre-selected image datasets. The annotated dataset will then be randomly divided into a test image dataset of 75 files (15%), and a train image dataset of 435 files (85%). Process annotation, export, and sharing of datasets using the ROBOFLOW service.

Map Labelling

Making a data map label that functions to provide text in the bounding box from the detection results. Writing labels according to the class in the file (.csv) that has been created, and the extension format for creating data map labels is protobuftext (.pbtxt). Making map labels using notepad software.

Data Preprocessing

Resize

The resizing process in this study was carried out to standardize the size of the image dataset. The initial image size, which is 2048×2048, is changed to 600×600. The resizing process in this study was carried out using the ROBOFLOW service.

Brightness Adjustment

Brightness Adjustment is a process of adding and decreasing brightness in datasets in the range of -27% to +27% randomly. Brightness adjustment to the Escherichia coli image dataset was carried out using the ROBOFLOW service.

Contrast Stretching

The first Image Enhancement process is Contrast Stretching or increasing the contrast in the image. The Contrast Stretching process is carried out using the ROBOFLOW service.

Median Filter

The second Image Enhancement stage is the addition of a Median Filter which is a technique that can be used to reduce noise in an image. The process of adding the Median Filter to the Escherichia coli image dataset is carried out using the Google Colaboratory service.

Gaussian Filter

The third Image Enhancement stage is the addition of a Gaussian Filter which is a low pass filter to reduce noise (high-frequency components) by obscuring the image area. The process of adding the Gaussian Filter to the Escherichia coli image dataset is carried out using the Google Colaboratory service.

Training Preparation

This preparatory stage is a step that needs to be done so that the training process can run smoothly. The first step is to install the required libraries in designing the Faster R-CNN system using the TensorFlow framework. The next step is cloning the TensorFlow model repository to facilitate training according to TensorFlow procedures. After the cloning process is complete, it is necessary to create an Environment, which in a programming context is the place where the code will be executed. It includes all executable code and variables available to the code being executed. After the dataset has been uploaded to the environment that has been created, the next step is to create TFRecords so that the model can read the images along with the labels that were created before. TFRecord is a file format that contains images of datasets and the information contained in them. Making a TFRecord is serialized at the data set level, namely one set of records for training, and one set for testing. The input to this TFRecord is the file (.csv) from the train dataset and test dataset, while the output extension format of the generated file is (.record). Faster R-CNN is one of the many architectural models provided by the TensorFlow Object Detection API. The training process is carried out with the Inception v2 and ResNet-50 architectural models that have been trained on the COCO (common objects in context) dataset. The final step is the configuration process which is performed on the file (.config) in the pre-trained model on the previously downloaded COCO Dataset. The file is a configuration that controls the training process. The configuration derived from the pre-trained model needs to be adjusted to the Escherichia coli image dataset.

Training

At this stage, the Faster R-CNN algorithm is designed using Inception v2 and ResNet-50 architecture to be implemented in the detection system on the prepared dataset. The tool used is Google Colaboratory. Model training is carried out using previously adjusted configurations. The end result of the Faster R-CNN training or training process is the formation of a model and records that are ready to be used for detection. After the training process is carried out, a TensorBoard visualization is performed to determine the level of loss during the training stage. The final step is that the Trained model generated from the training process is a file in the form of a checkpoint (.ckpt), while TensorFlow requires a file in the form of protobuf (.pb) so it is necessary to generate a file. The steps taken are to create a frozengraph using the input config file and the resulting checkpoints. The exported file is named “frozen inference graph.pb”.

Testing

The testing phase is used to test dataset testing using a model that has been decorated at the training stage.

Analisis Data

Data analysis was carried out to determine the results of the detection of Escherichia coli bacteria on Tryptic Soy Agar images using Faster R-CNN as shown in Table 1.

TABLE 1. Variasi Kombinasi Model

	Arsitektur	Augmentasi	Preprocessing
RAW DATASET	**Inception v2**	-	-
Model 1	**Inception v2**	*Brightness* ± 27%	*Resize* 600×600 *Contrast Stretching* ***Gaussian Filter***
Model 2	**Inception v2**	*Brightness* ± 27%	*Resize* 600×600 *Contrast Stretching* ***Median Filter***
RAW DATASET	**ResNet-50**	-	-
Model 3	**ResNet-50**	*Brightness* ± 27%	*Resize* 600×600 *Contrast Stretching* ***Gaussian Filter***
Model 4	**ResNet-50**	*Brightness* ± 27%	*Resize* 600×600 *Contrast Stretching* ***Median Filter***

RESULTS

Anotation result

Object annotation is performed using the ROBOFLOW service. The display of the annotation process can be seen in Figure 1. The annotation results from each image will be combined into a *csv file because the TensorFlow Framework can only read files with that extension.

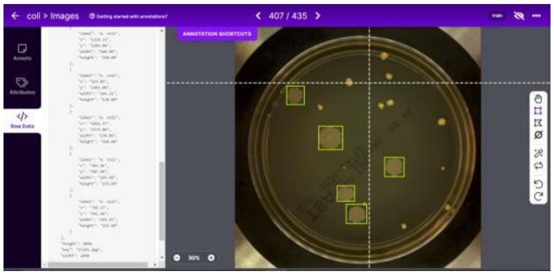

FIGURE 1. ROBOFLOW display

Map Labelling

Label writing is adjusted to the class in the file (.csv) that has been created, and the extension format for creating data map labels is protobuftext (.pbtxt). The process of making a Label Map using Notepad.

Preprocessing Result

Brightness Adjustment result

This study uses decreasing and increasing brightness in the range of ±27% randomly, so that the images will have various characteristics so that they can train better models. The augmentation process is carried out using the ROBOFLOW service

(a) (b)

FIGURE 2. Brightness Adjustment Results (a) Before (b) After

Contrast Stretching

Contrast stretching or contrast enhancement is done because the Escherichia coli image dataset has a slightly low contrast.

(a) (b)

FIGURE 3. Results of the addition of Contrast Stretching (a) Before and (b) After

Median Filter result

The process of adding the Median Filter to the Escherichia coli image dataset is carried out using the Google Colaboratory service


(a) (b)

FIGURE 4. Results of Adding the Median Filter (a) Before and (b) After

Gaussian Filter result

The process of adding the Gaussian Filter to the Escherichia coli image dataset is carried out using the Google Colaboratory service.


(a) (b)

FIGURE 5. The results of adding Gaussian filters (a) before and (b) after

Model Experiment Results

In the training process it will produce a loss which is a value that shows an indication of how bad the predictions of the model are. The model can be said to be perfect if the loss will be zero, otherwise if the model is bad then the amount of loss will be high. The goal for training a model is to train the model to have the lowest possible loss value. The results of the training process are visualized using the TensorFlow feature, namely the TensorBoard, to find out how much the loss rate is. The more the number of steps in the training process, ideally the loss graph will decrease.

Global step/sec

Global step refers to the number of batches or iterations, while global step/sec refers to the number of batches or iterations that can be performed every one second in the training process. Global step refers to the number Based on the graph generated from the TensorBoard Faster R- CNN, the Inception v2 architecture produces an average Global step/sec value of 7.09, higher than the ResNet-50 architecture which produces an average Global step/sec value of 4,3. These results prove that in the training process the Inception v2 architecture model works faster with a speed of 0.141 step/sec when compared to the ResNet-50 architecture model which works with a speed of 0.232 step/sec. Meanwhile, the difference in the addition of image enhancement only has a slight effect on the number of global step/sec.h batches or iterations, while global step/sec refers to the number of batches or iterations that can be performed every one second in the training process.

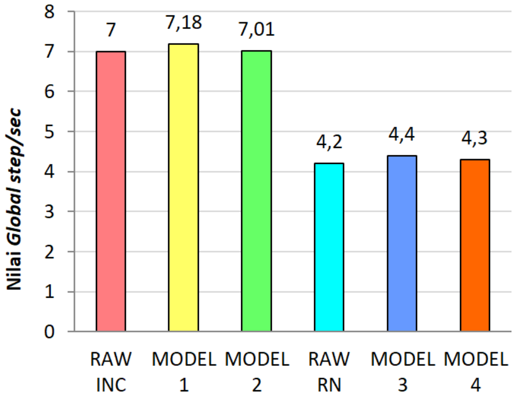

FIGURE 6. Global step/sec results for each model

Classification Loss

Classification Loss is the loss value of the model to classify detected objects into various classes. Based on the graph generated from the TensorBoard, the best Classification Loss value is produced by model 3 which has a value of 0.02 which is a model using image datasets that have been given Image Enhancement in the form of a Gaussian Filter. These results prove that the addition of a Gaussian filter can perform classification with minimal errors when compared to the RAW model.

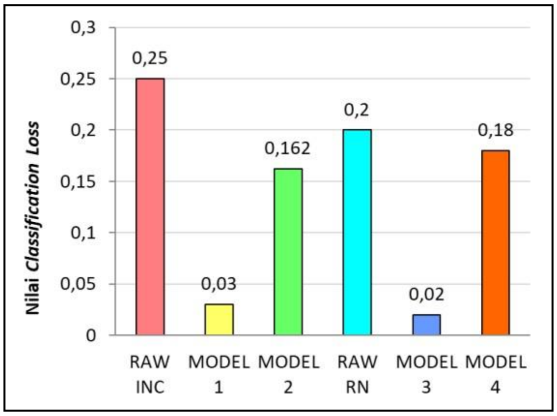

FIGURE 7. Classification Loss results for each model

Localization Loss

Localization loss is also called regression loss which is the error rate of the model to determine the regression box or bounding box containing objects.

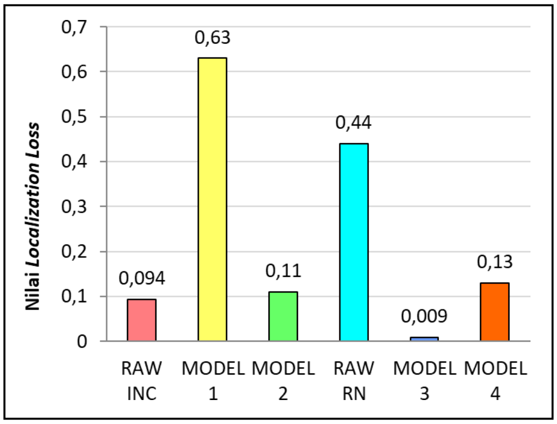

FIGURE 8. Classification Loss results for each model

Results of the Testing Process

The testing process is carried out using a test image dataset of 75 images. This process produces an image that is equipped with a bounding box and labels that have been made before. The image will be analyzed at the data analysis stage to determine the performance of each model.

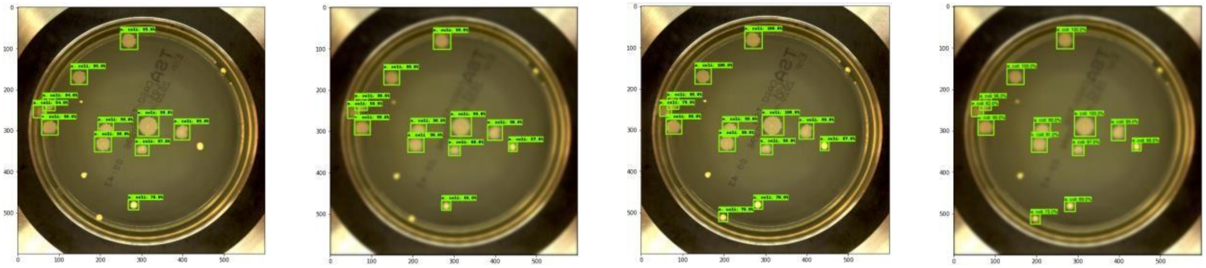

FIGURE 9. The results of the testing process from one of the test images

Model Experiment Analysis

Analysis of the performance of each model using the Confusion Matrix by calculating True Positive, True Negative, False Positive, and False Negative. The calculation of the four components is calculated manually by comparing the ground truth box with the bounding box produced by the model. The Confusion Matrix will tell you how well the model is made. (Nugroho, 2019) True Positive is the success of the system to detect objects as Escherichia coli bacteria, while False Positive is a failure of the system to detect Escherichia coli bacteria so that it will regard other objects as Escherichia coli bacteria. This might be caused by objects that are not Escherichia coli bacteria having similar characteristics to Escherichia coli bacteria so that the system will assume it is correct.

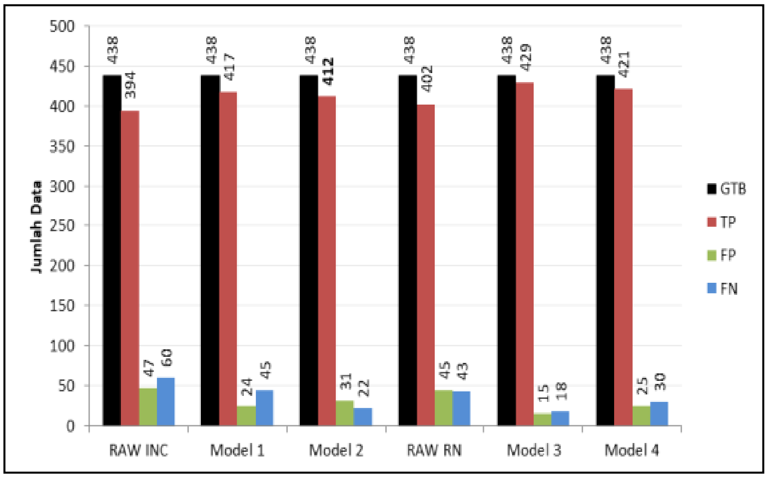

FIGURE 10. Calculation results of the Confusion Matrix Components

True Negative cannot be valuable because in this study only used 1 class, as a result the specificity of the model cannot be calculated. Therefore the performance of the model is determined through accuracy, precision, recall and F1-Score.

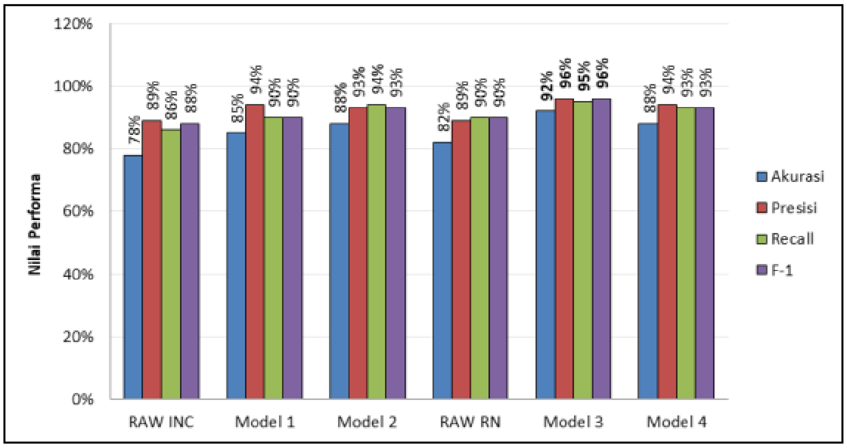

FIGURE 11. Calculation results of the Confusion Matrix Components

Based on the parameters of the table, the model that has the highest Accuracy, Recall, Precision, and F1-Score will be selected. So the most suitable model to be used to detect Escherichia coli bacteria automatically is model 3, namely Faster R-CNN ResNet-50 architecture by adding Image Enhancement in the form of Gaussian Filter to the dataset.

Discussions

The accuracy value is the most important parameter when conducting a study to detect diarrheal disease because True Positive and True Negative are parameters that will be used as a diagnosis for further treatment. If the accuracy value is too low, the model does not have the ability to distinguish between right and wrong objects, so there is no advantage and will cause harm to sufferers. Model 3 has an accuracy value of 92%, so model 3 is the model that has the highest accuracy value among the other models.

The recall value is the second important parameter because it is the value of the comparison between True Positive and all true actual data. In conducting a study on the detection of diarrhea, the appearance of False Negatives was not expected. False negatives or Escherichia coli bacteria that are negatively detected will be detrimental to the patient because it causes the bacteria to be considered as not Escherichia coli bacteria or considered as another object so that the patient will be considered fine by the doctor. The relationship between recall values and False Negatives in equation 2.12 is inversely proportional, so that the fewer the number of False Negatives, the higher the recall value. Model 3 has a recall value of 95%, so model 3 is the model that has the highest recall value among the other models.

The precision value is an important parameter number three because it is the value of the comparison between the True Positive and all the detection results that the model considers true. In accordance with equation 2.11, if there are fewer False Positives, the precision value will be greater. Even if it is a False Positive or other object that is considered to have a large number of Escherichia coli bacteria, it will actually not be detrimental for sufferers of diarrhea because the patient will immediately be given further treatment by a doctor. Model 3 has a precision value of 96%, so model 3 is the model that has the highest precision value among the other models. and recall, which will be used as an option when the difference between the precision and recall values is too great.

CONCLUSION

An object detection model designed using Faster R-CNN with Inception v2 and ResNet-50 architecture has been tested and successfully detects Escherichia coli bacteria in Tryptic Soy Agar images. The addition of Image Enhancement in the form of Median Filter and Gaussian Filter can increase the True Positive value so that it affects the Accuracy and Recall values which are used as important parameters in the detection results of Escherichia coli bacteria in Tryptic Soy Agar images using Faster R-CNN. Faster R-CNN with ResNet-50 architecture has better performance in terms of accuracy, precision, recall, and F1-Score compared to Inception v2 architecture, although Inception v2 architecture has the advantage of being faster in the training process. The most appropriate model for detecting Escherichia coli bacteria in Tryptic Soy Agar images is model 3, namely Faster R-CNN with Res-Net50 architecture with the addition of Image Enhancement in the form of Gaussian Filter which produces 92% accuracy, 96% precision, 95% recall, and F-1 score 96%. F1-Score is a special option if there is an imbalance between False Positive and False Negative in a model. This can result in if the precision value is too high then the recall value will be low, and vice versa. F1-Score is the harmonic mean between precisions.

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