DETECTION AND COUNTING OF *E. COLI* ON SPECIALIZED TEST PIECE USING YOLO v4

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DETECTION AND COUNTING OF *E. COLI* ON SPECIALIZED TEST PIECE USING YOLO v4

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DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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LIST OF ABBREVIATION

CFU	Colony Forming Unit
CNN	Convolutional Neural Network
mAP	Mean Average Precision
IOU	Intersect Over Union
AC	Autoanalysis Colilert
MTF	Multiple Tube Fermentation
MF	Membrane Filtration
MPN	Most Probable Number
MAE	Mean Average Error

PENGESANAN DAN PENGIRAAN *E. COLI* PADA BAHAN UJIAN KHUSUS DENGAN MENGGUNAKAN YOLO v4

ABSTRAK

Banyak negara masih menghadapi masalah untuk pembekalan air yang bersih dan selamat untuk diminum. Sehingga sekarang masih ada ramai yang sakit akibat memimum air yang tidak bersih dan antara yang paling kerap berlaku ialah cirit-birit. Punca utama penyakit ini ialah bakteria *Escherichia Coli* atau pendeknya *E. Coli*, yang terdapat dalam persekitaran kita. Kehadiran bakteria ini dalam kuantiti yang banyak dalam tubuh manusia boleh menyebabkan penyakit yang serious, malah boleh membawa maut. Lantaran itu, kaedah yang lebih mudah untuk mengesan bakteria ini perlu dikenalpasti dan dikomersilisasikan. Sehingga sekarang, proses untuk mengenalpasti kehadiran bakteria ini masih belum mantap dan memakan masa yang panjang. Kualiti air biasanya diukur dengan meneliti unit pembentukan koloni bakteria dalam setiap 100mL atau ringkasnya CFUs/100mL. Pengesanan bakteria hanya boleh dilakukan dengan pengiraan bakteria tersebut melalui pemerhatian. Kajian yang diusahakan ini melibatkan penggunaan keupayaan pembelajaran mesin, untuk mengenalpasti dan mengira koloni bakteria dengan menggunakan 'kepingan ujian'. Imej bahan uji akan diambil dari makmal yang mempunyai sumber cahaya yang diperlukan. Selepas itu, proses pembesaran atau penambahan akan dijalankan. Imej yang diproses itu akan dihuraikan dengan menggunakan Label Studio dan seterusnya diuji lagi menggunakan YOLO v4. YOLO v4 merupakan rangkaian pengklasifikasian objek yang mengamalkan Convonlutional Neutral Network (CNN). Rangkaian ini telah diprogram untuk mengesan kehadiran E.Coli dalam kepingan ujian tadi. Ini membolehkan pengguna mengetahui 'tahap selamat' kualiti air berdasarkan CFU. Keputusan yang ditunjukkan, dengan hanya menggunakan 50 kepingan ujian sahaja telah berjaya memperolehi ketepatan 91% berdasarkan kiraan ketepatan mAP, dengan skor IOU sebanyak 0.82 dan kelenyapan purata hanya 0.2588 sahaja. Dalam tahap ujian, kerja ini mencatatkan ketepatan 0.9279 ± 0.04195, kebolehan ingat balik 0.9474 ± 0.01831 dan F-score 0.9351 ± 0.0271. Kajian ini merupakan langkah pertama untuk mengesan dan mengira kehadiran bakteria E. Coli secara automatik.

DETECTION AND COUNTING OF *E. COLI* ON SPECIALIZED TEST PIECE USING YOLO v4

ABSTRACT

Supplying clean, safe and drinkable water is still one of the on-going issues faced by the world. To date, people around the still contract sickness and diseases related to unsanitary water. One of the most common sicknesses is diarrhoea and the main contributor to it is *Escherichia Coli* or in short, *E. Coli*. *E. Coli* is a bacterium commonly found in environment and if consumed in moderate and high amounts, may lead to critical illness and death. Therefore, there is a dire need for vision automation in detection of *E. Coli* bacteria. To date, the process of identify the quality of water is still not accurate and is time consuming. The quality of water is measured by colony forming unit per 100mL or in short CFUs/100mL. Only counting of the colonies is possible to obtain that desired value, which even until today, is still counted by sight. This leads to inaccurate *E. Coli* colony reading and inappropriate water treatment procedures. The study includes the usage of machine learning capabilities to detect and count the colony present on the test piece. The sample images obtain from the laboratory is captured under ideal lighting condition and later augmentation process was carried out. The processed images are then annotated using Label Studio and later trained using YOLO v4, an object classifier network that employs Convolutional Neural Network (CNN). The network is being trained to pick up presence of *E. Coli* on the mentioned test piece and provide user the quality of water based on the CFU. The results showed that with only 50 test piece sample images, the model achieve a mAP accuracy of approximately 91%, IOU score of 0.82 and an average loss of 0.2588. During the test phase, this work recorded a precision of 0.9279 ± 0.04195, recall of 0.9474 ± 0.01831 and F-score of 0.9351 ± 0.02718. This research is the first step to automate the *E. Coli* detection and counting process and create a change to world.

CHAPTER 1 INTRODUCTION

1.1 Project Overview

To date, supplying clean, safe and drinkable water remains a challenged for many poverty and developing countries. According to statistics, over 1.1 million people worldwide does not have proper access to safe drinking water [1]. Unsanitary water is the main cause of many illnesses and diseases among mankind. In 2002 alone, diseases associated with water related issue cause an estimated mortality of 2.2 million [1], with children holding the majority. Among the bacteria that is the main cause of the illness is *Escherichia Coli* or in short *E. Coli*. *E. Coli* is one of the main causes in diarrhoea [2] and is commonly found in water environments or food if the proper sanitary steps are not taken. One of the probable reasons that courage this issue to persist of the lack of proper *E. Coli* detection and counting in laboratories.

The quality of water is determined by the amount of *E. Coli* present [3,4]. Presence of *E. Coli* indicates that there is faecal origin in high amount and is harmful to mankind. Therefore, the measure of *E. Coli* with the unit of CFUs/100mL, is the bar standards to identify water sample that may contain traces of contamination. This also emphasize the importance of detecting and counting of *E. Coli* in laboratories. To date, there are many methods developed to effectively detect the presence of *E. Coli.* Among it all, the most common practice done in laboratory is by employing the agar media method [5,6]. This method is suitable for the growth of *E. Coli* as the required nutrient for growth is ideal for metabolism activity. This method is moderately cheaper but the detection of *E. Coli* is made hard for proper counting, thus, leading to inaccurate data analytic [7].

While technology has allowed us as a generation to easily detect *E. Coli* with the innovation of specialized *E. Coli* test piece, laboratory still face plenty of challenges especially when it comes to detecting and counting of *E. Coli.* From a laboratory stand point, counting of the *E. Coli* remains one of the most common problems. Laboratory personnel still employ manual counting technique [8] of the *E. Coli* colony on the specialized test piece. Working under artificial lighting, a magnifying glass and counting using a counter, the process is not only time consuming but, at the same time, increases the risk of incorrect detection or incorrect counting of the *E. Coli.*

Therefore, there is a dire need of automatic detection and counting of the *E. Coli* on the specialized test piece. The use of machine vision and deep learning will be useful and might be crucial in automating the detection and counting process. With the emerging of neural networks, detection *E. Coli* on the specialized test piece is made easier. One of the CNN readily available is YOLO v4, a deep learning object classifier network, can be used to aid in the detection of *E. Coli.* However, to further increase the accuracy of the system, proper image pre-processing work need to be done to further enhance the quality of the sample. Lighting and augmentation process like cropping and contrast adjusting is equally important to improve the quality of the image. This would allow us to apply the readily available technology in developing better detection and counting algorithm of *E. Coli*.

This project aims solve the detection and counting issue of *E. Coli* that is currently faced in laboratory. By using U-Net CNN, image segmentation can be done on sample image to pick up key point that is resemblances of *E. Coli* colony. This would allow for faster detection and with high accuracy of the deep learning algorithm, inaccurate test result can be avoided.

1.2 Problem Statement

By utilizing deep learning technique for the detection of *E. Coli* on the specialized test film, the image that is used for network training is important in order to obtain a high validation accuracy. Images that are used to training and testing has to be under ideal lighting conditions in order to preserve information and enhance the quality of the image. Choosing the correct lighting and lighting technique is crucial in maintaining quality from image to image.

In addition, inconsistent background image test piece is also another concern that have to be overcome. When water sample is tested on the specialized test piece, *E. Coli* bacteria is actively carrying out respiration process, which cause formation of air pocket in the test piece. When viewed under camera, the indicated air pocket might obscure possible colony form below it. Intensity inhomogeneity also leads to inconsistent background. This is usually cause by the water source that might be overly contaminated and make the differentiate of foreground and background harder.

Inconsistent shape or pattern of the *E. Coli* colony formed on the specialized test piece is another issue that needs to be address in this project. Overly contaminated water source leads to multiple overlaps on the test piece. This might cause the network to under estimate the correct *E. Coli* CFU, leading to inaccurate data.

1.3 Objectives

There are three main objectives of this study

i. To develop a deep learning-based YOLO v4 model for the image classifying of *E. Coli* on specialized test piece for the automation of *E. Coli* detection
ii. To evaluate the performance of the model developed on the *E. Coli* samples and evaluate the performance of the network
iii. To perform verification and validation of the *E. Coli* in terms of colony forming unit (CFU) using counting algorithms

1.4 Scope of Project

YOLO v4, an abbreviation for 'You Only Look Once', is an algorithm that detect and recognises various object from a picture [45], is developed via transfer learning method to detect *E. Coli* colony on specialized test piece. Samples were prepared by using sample water obtained from lake surrounding Universiti Sains Malaysia, Engineering Campus and culture at the laboratory. Image that was used to for training and testing were captured using apparatus that setup at fixed parameters to ensure that the field of view (FOV) remains identical from image to image. Augmentation process of the images are carried out with MATLAB to ensure that the input image complies with the input requirements of the network. YOLO v4 network was trained using Python language and various environment was used to run the algorithm. This include both online (Google Colab) and local platforms (MATLAB, Spyder and Anaconda). The accuracy of the training and validation was identified with 2 metrics method namely MAP and IOU. The data obtained from the training is analysed to evaluate the accuracy of the prediction of *E.Coli* colony on test piece matches the original image sample.

CHAPTER 2 LTIERATURE REVIEW

2.1 Conventional Detection Method of *E. Coli*

In laboratory, there are numerous conventional detection methods to identify the levels of *E. Coli* bacteria in water or any other related substance. One of the methods proposed and compared by Stephen C. Edberg and his team [9] is the Autoanalysis Colilert (AC) method or also known as the Defined Subtrate Technology [9-11], where only a hydrolysable substrate is needed as indicator to identify the microbes [10]. This method only requires the adding of powered ingredient to the targeted water source and the change in colour of the solution will indicate present of microbes. *E. Coli* is only detected when the test sample fluoresce under specific lighting. This method is good safety measure of people to determine the presence of potentially harmful microbes in the water. However, there is no way to determine the quality of the water source. Magda M. Abed El-Atty EL- Magharaby claimed that the AC method produce an almost similar to more traditional method like MTF and MF [11], with the AC result more closely related to the MF. He also states that this method also reduces result formation time by 60% when compared to traditional MTF and MF methods.

The MTF method is one of the most conventional methods present to date and is certified by the U. S. Environmental Protection Agency [12]. As the name suggest, the MTF methods utilises multiple tubes in the fermentation techniques [13]. From here, the coliform density is the predicted via the MPN method. The effectiveness and accuracy of the fermentation testing depends on the number of tubes used in the test. A larger sample size is favoured. The MTF method however has major disadvantage [14]. One of it being very time consuming and people might have already been contracted with the disease before the result can be release. Another problem of it is not *E. Coli* specific as it measures the majority types of faecal material.

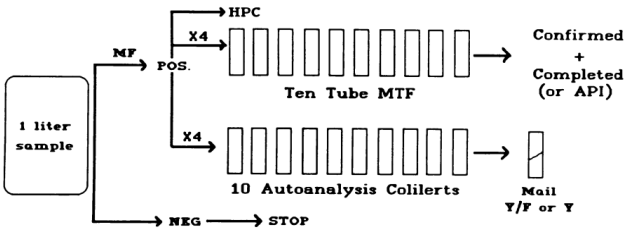


Figure 2.1 Comparison of AC method with standard methods MTF [9]

One successor technique to the conventional detection of *E. Coli* is the MF technique. It was introduced in the late 1950s to replaced the MPN procedure for coliform analysis from targeted water source [15-16]. A thin porous sheet is used to filter test samples and is suck pass it via a vacuum pump. The porous sheet is then culture on agar jelly medium for the detection of *E. Coli.* In a research conducted by Mark Rohit Francis, by applying the MF technique in rural area to filter water with possible *E. Coli* content, he recorded a log reduction of 0.86 to 1.14 for total coliform. [17].

Another conventional method in detecting *E. Coli* is the Plate Count Enumeration Method. As compared to the method mentioned above, this method is the simplest and easiest method to detect *E. Coli*. To get a high specificity of the particular bacteria, the requirement is only to supply the correct nutrient agar. As reported by J. Prats in his study, high percentage of specificity (95.7%) and high regression coefficient [18-19] when specific nutrient agar is used. The plate count enumeration method is effective but detection process is made hard as further processing of the bacteria is needed.

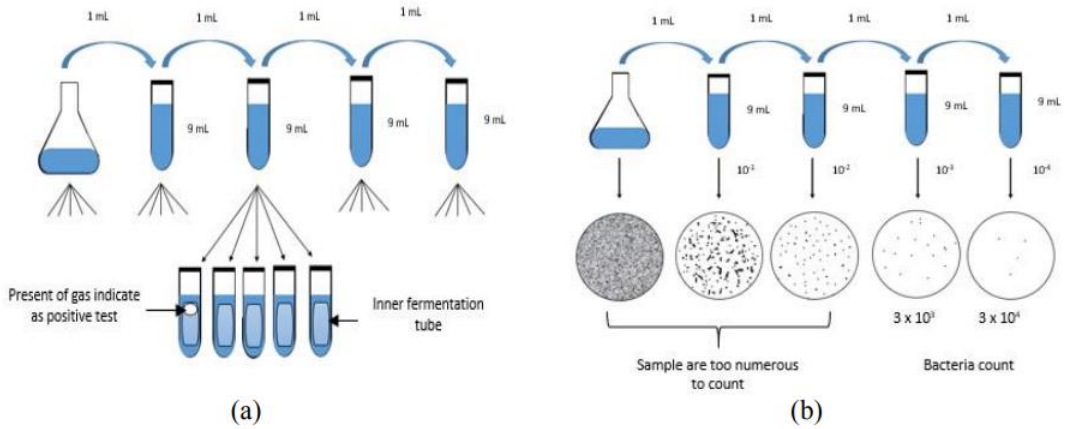


Figure 2.2 Interpolation methods for the determination of concentration of viable *E. Coli.* (a) MTF method; (b) Plate Count Enumeration Method [20]

2.2 Modern Detection Methods of *E. Coli*

In recent years, engineers together with health science personnel started proposing more effective yet faster methods to detect *E. Coli* from water test sites. Proposed by Nicharee Wisuthiphaet is by using genetically engineered bacteriophage T7 [21] to detect the presence of *E. Coli* in beverages. The proposed substance is an alkaline substrate which upon coming in contact with the test sample, reacts with the *E. Coli* which provides an opportunity of time frame to detect signal indicative of presence of *E. Coli* in the beverage. It is claimed that this method can detect presence of *E. Coli* in the beverages as low as 100 bacteria per gram of beverages and it is possible to present a result as fast as 6 hours. This method is actually great for public usage as testing of water source can be fast, greatly reducing the waiting time for result. However, to ensure that a drinkable water source is safe, an ideal water should be in the range of 0-10 CFU/100mL [22], of which this method could not deliver.

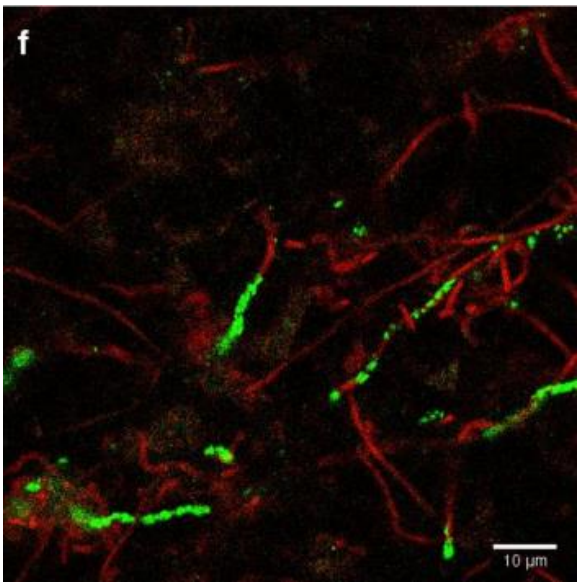


Figure 2.3 Fluorescent images of *E. Coli* 4 hours after adding T7 mixture [21]

Pa. Suriya and her team did a study on the application of the E-Nose system to detect the presence of *E. Coli* in drinking water [23]. E-Nose is apparently a computerised recognition which detect the presence of certain microbes based on their by-products. For example, when *E. Coli* is presence in a water test sample, the by-product of the bacteria is carbon dioxide. E- Nose utilised a gas sensor to detect the presence and determines the amount of *E. Coli* presence by the amount of carbon dioxide is release. For this application, the accepted range based on Indian Standards

IS :10500-1991 [24], drinkable water source should only range from 0 to 51ppm carbon dioxide.

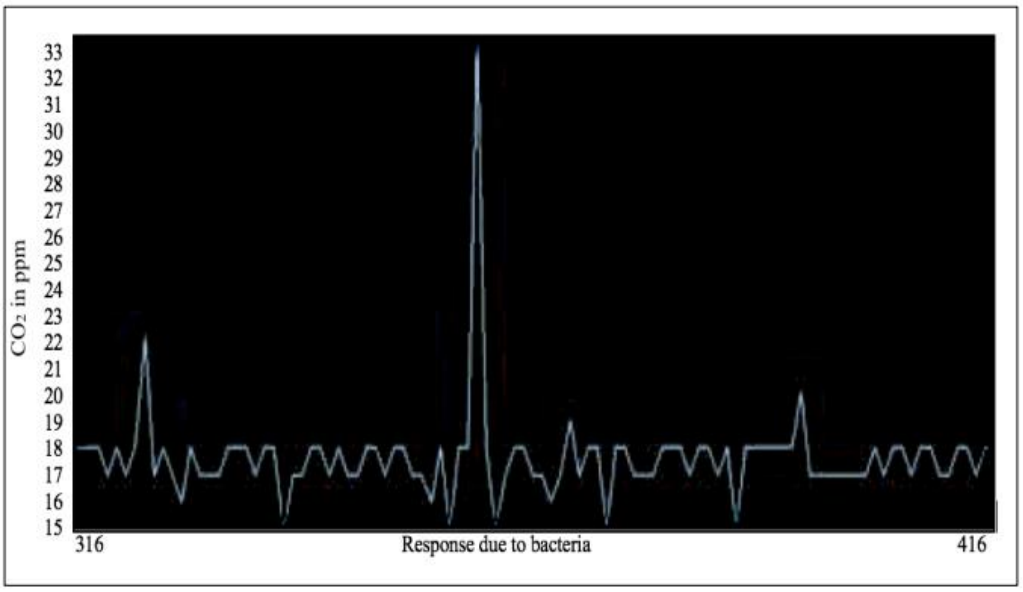


Figure 2.4 Response of carbon dioxide from the *E. Coli* show drinkable water condition

There are also a lot of studies on detection of *E. Coli* using the camera of smartphones. Hongying Zhu reported the use of quantum dots [25] to detect the presence of *E. Coli* in water samples. Using a smartphone camera and lighting emitting diode (LED), the excited *E. Coli* particles are captured on the capillary surface which has been coated with anti-*E. Coli* antibody as solid substrates. However, it is reported that the detection limit of this system is about 5-10 CFU/100mL buffer.

Siti Farah conducted a review on a portable hybrid imaging system to the used to detect *E. Coli* using Surface Plasmin Resonance (SPR) [26]. Based on a study conduction by Zardon, SPR imaging is highly sensitive and label free method that detect the E. Coli or microbes in general by the changes in refractive index [27]. The said device comprises of an array of golden spots which captures the biomolecule of a specific pathogen. Each spot that the *E. Coli* is measured by the area of the gold spot and is analysed using NIH ImageJ software. According to the result, using this method proves an above 90% magnetic bead rate which mean the detect of the *E. Coli* is able to be detect by the SPR method.

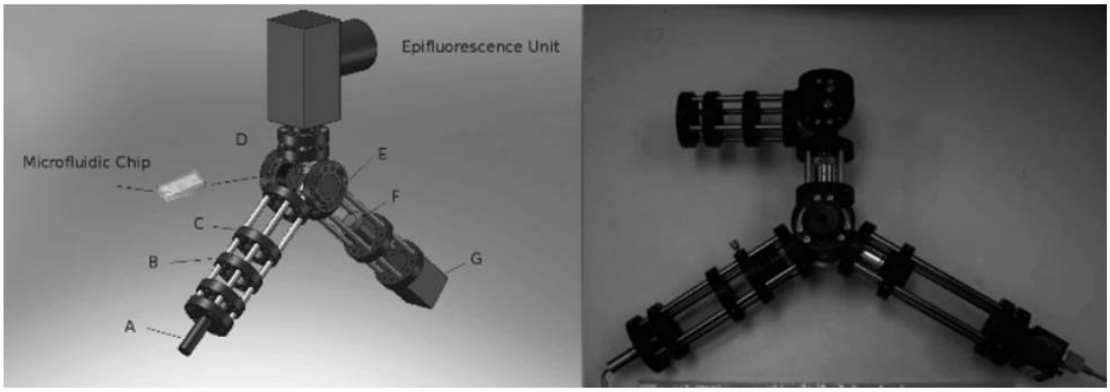


Figure 2.5 Schematic drawing of the portable SPR imaging system

Hoorieh Fallah studied the use of optical fiber [26,28] on the detection of *E. Coli* in polluted water. The tip of optical fibre is coated with a layer gold (Au) nanoparticle for the rapid and sensitive detection *E. Coli.* According to the result obtained by the team, the proposed sensor showed a positive result within 10 second upon contact of the polluted water. Siddharth Kaushik also proposed the use of optical fiber coated with molybdenum disulphide prepared by chemical exfoliation process [29] for the sensitive detection of *E. Coli.* This work by the molybdenum disulphide synthesising the antibodies of the *E. Coli.* The similar claims were also present this research, with rapid bacteria quantification. Sabiha Tok in her research also uses fiber optics mixed in Colilert reagent [35]. According to its research paper, *E. coli* can be easily detected after the first 75 minutes if the intensity exceeds 20%. It is claimed that it can save time as compared to visual inspection, does not require well-trained personnel and most importantly reduces counting error during the counting of the *E. coli* colonies. Among the hardware that is used include the raspberry pi microcontroller, a CMOS sensor-based camera, optical fibers, Plexiglas and UV LED. As for the software, MATLAB software and cross-correlation methods are used during the processing of raw images.

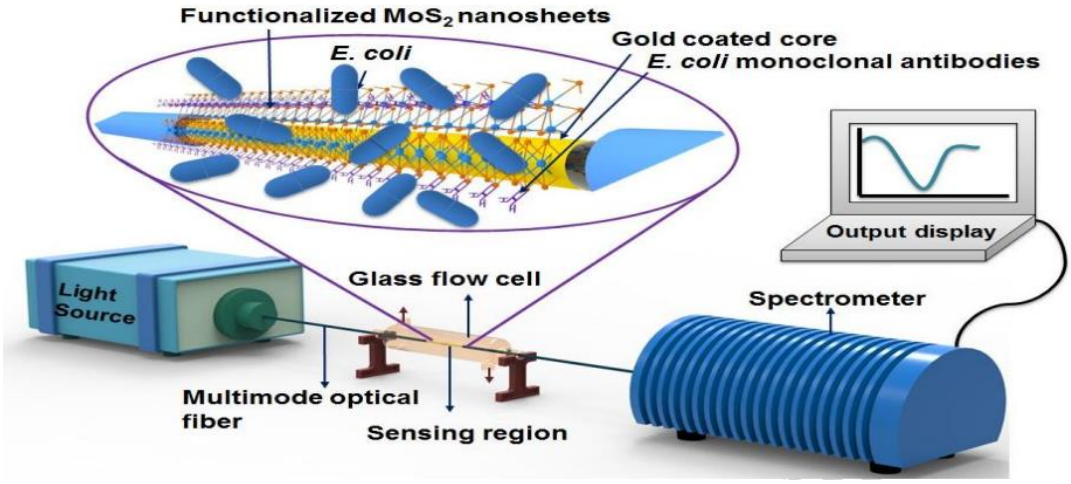


Figure 2.6 Schematic of the fiber optic SPR immunosensor [29]

David S. Liao suggest a fast and highly sensitive detection of *E. Coli* by the means of electric field and micro-Raman spectroscopy [30]. As suggest by the author, this method is able to provide rapid detection of the targeted bacteria based on the water source presented. This occur when the water droplet from the test sample go pass an alternating current (AC) created by a gold microelectrode array on the surface. The cause the water sample to go through electroosmosis and dielectrophoretic process [31] which amplifies the bacteria obtained on the surface. The detection process is then accomplished using the Raman spectra process.

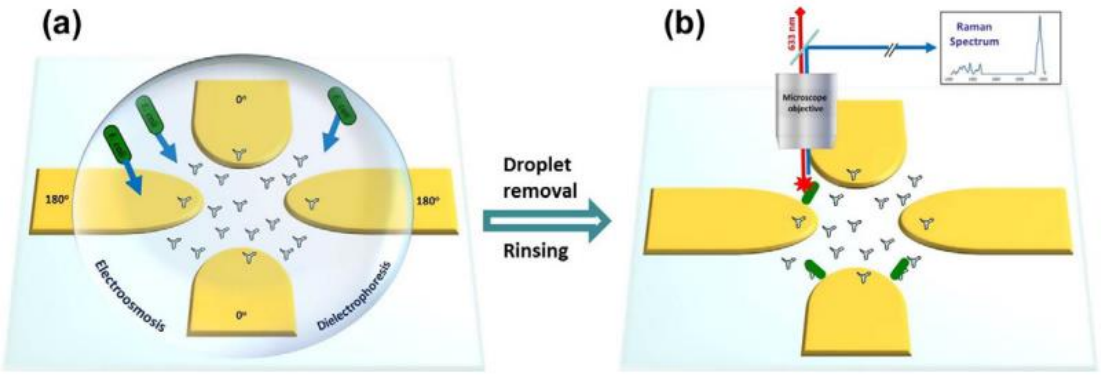


Figure 2.7 Schematic of bacterial capturing and detection process [30]

Dan E. Angelescu and Vaizanne Huynh proposed on a rapid detection of *E. Coli* using autonomous microbiological system or also known as AMAS [32-33]. AMAS is an automatic and remotely-controlled microbiology sensor to detect the presence of *E. coli* and total coliform (TC). The AMAS consist of individual bioreactors which consist of an optoelectronic board that have three kinds of LEDs

with different wavelength (385nm, 430nm and 610nm) as shown in Figure 3. The total coliform is metabolising the ONPG (ortho-nitrophenyl-β-D-galactopyranoside) and resulting ONP (orthonitrophenol) is detected by the optical absorbance measured at a wavelength of 430nm. E. coli on the other hand metabolises MUG (4-methylumbelliferyl-β-D-glucuronide) resulting MUF (methylumbelliferone) create a fluorescence signal and is measure by LED with a wavelength of 445nm. The 3rd LED of wavelength 610nm is to detect turbidity if there is none of the above product interference. Although there were no reports of the accuracy of the applied method, but based on the graph in Figure 2.8, the AMAS is able to pick up high amount of *E. coli* and coliform from the seventh hour mark onwards.

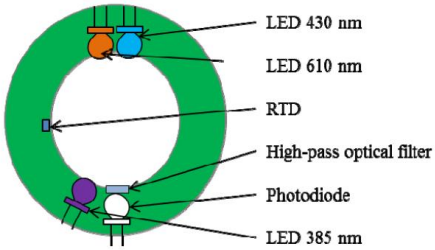


Figure 2.8 Schematic of AMAS optoelectronic board [33]

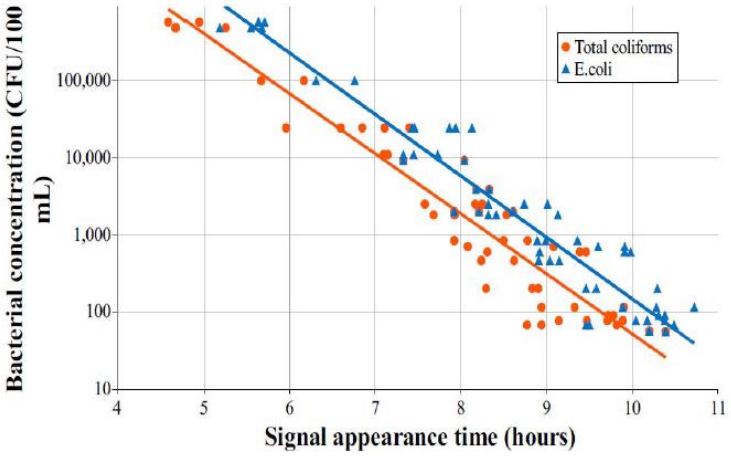


Figure 2.9 Correlation of bacterial concentration and signal appearance time

2.3 *E. Coli* Detection via Deep Learning Approach

With the current access to deep learning method, many engineers together with biological science researcher are now applying deep learning into the detection of *E. Coli* from test samples. Hüseyin Yanik [36] and his team proposed the use of deep learning algorithms to detect *E. coli* bacteria in water. The proclaimed method utilizes a rapid region-based convolutional neutral network (R-CNN) which has a much-improved performance and processing speed. In general, a more advanced CNN detection model to remedy the problem of localizing the object more efficient [36]. In terms of software, Tensorflow is used for the framework for the Faster-R-CNN while an open-source software 'Labelling' [37] is used to identify images which contain a metallic sheen and label them 'bacterial' or otherwise. The labelling of the sample image uses an open-source software. According to them, using the strategy of labelling 'bacterial' when there is a metallic sheen, the algorithm only focuses on this as it is one of the main characteristics of presence of E. Coli bacteria and considering the feature and requirement, a Faster R-CNN model was developed as shown in Figure 2.10. With the applied framework model, the result initially (after 3 hours) started to respond and manage to labelled bacterial medium with an accuracy of about 59% and after 6 hours, the image detection accuracy increases to 83% and after over 9 hours, it became entirely stable and able to detect the E. Coli bacteria of accuracy 99%. The results were also compared when coloured dataset and greyscale dataset were used. From the findings, it was noticed that before the 8-hour interval, greyscale dataset poses a higher accuracy of 55% as compared to coloured dataset of only 30%. But after the 8th hour mark, coloured dataset showed higher accuracy at 99% albeit only 2% higher than the greyscale counterpart.

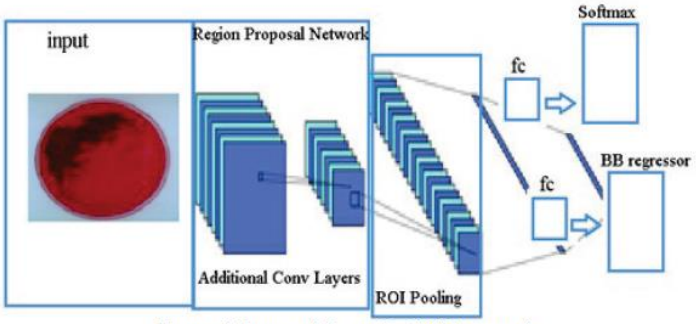


Figure 4 Proposed Faster R-CNN framework

Figure 2.10 Proposed Faster R-CNN framework

Jaroslaw Pawlowski also investigated the usage of deep learning algorithm via transfer style learning to create the generation of microbial colonies on petri dish [38]. Images of microbiological images (can be *E. Coli or Salmonella*) on Petri dish are annotated via a synthetic dataset. The annotated images are then used to be train deep learning model in a supervised fashion [39]. The sample images will first go through a series of image augmentation process to generate a more realistic images before putting in for deep learning training. Their proposed method required significantly fewer dataset while able to achieve good results. The reported a detection MAP of 0.416 and MAE of 4.49. If the same algorithm is put into bigger dataset, higher MAP and MAE scores will be recorded.

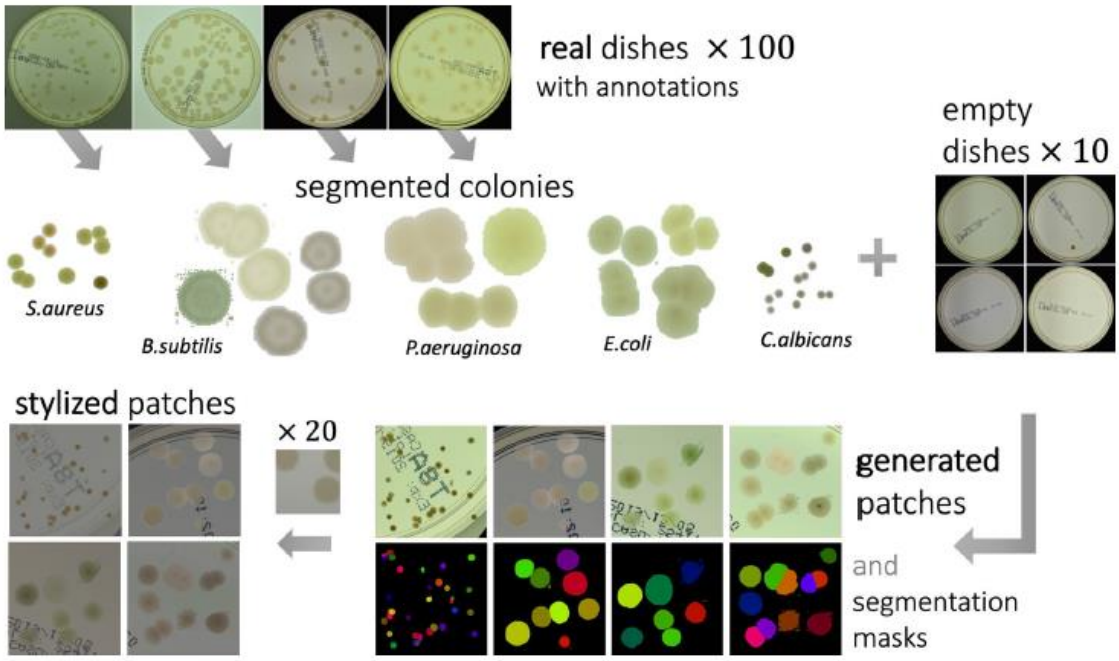


Figure 2.11: The working schematic of deep learning approach [38]

Shaikh Afzal Nehal has proposed the used of deep learning artificial intelligence (AI) algorithm together with the usage of biosensor which are based on Photonic Crystal [40]. He reported a probable accuracy of over 95% when the detection of *E. Coli* is tested. When the tested water source has the presence of *E. Coli* or bacteria in general, the output spectral behaviour of the biosensor changes and the changes in the image is captured then trained in the deep learning algorithm with a slightly optimised input layer. In this research paper, the exact network type is not disclosed but based on the information given by the author, it is assumed to be a fully trained supervised network [39].

Farhan Mohammad Khan also implemented the use of deep learning method for the detection of *E. Coli* in water. The author uses CNN to train image of different water sample and classify them into 2 main group- 'contain *E. Coli*' and 'does not contain *E. Coli*' [41]. The CNN was design using the MATLAB deep learning network toolbox and the training achieve an accuracy of 96% and only a loss of 0.12. The advantages of using this approach are that test images can be determine at a relatively fast rate (reported 458ms). The team also incorporate the deep learning network into a mobile application which can be easily used by the public to determine the quality of the water.

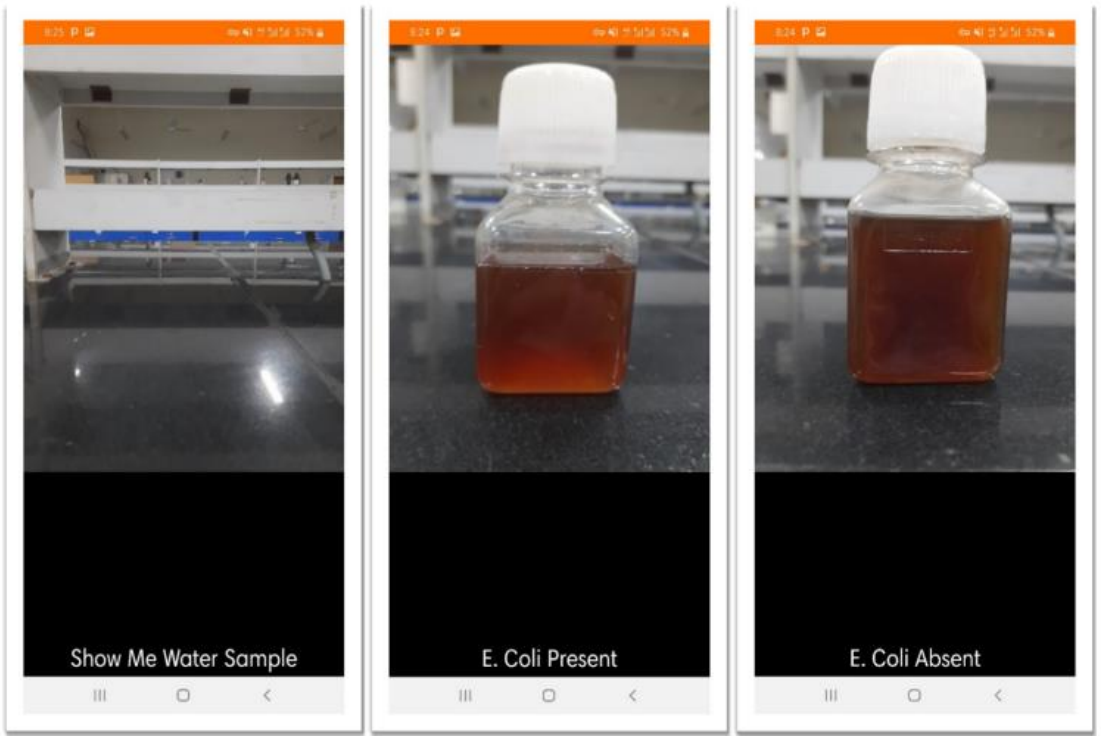


Figure 2.12 *E. Coli* detection app which uses deep learning

CHAPTER 3 METHODOLOGY

3.1 Preparation of Samples

Before the image can be captured for image training and testing, sample is prepared using controlled environment factors to ensure that the result obtained is similar from sample to sample.

3.1.1 Collection of Water Samples

Water sample were collected from water test sites. This includes water samples taken from the lake and drainage inside Engineering Campus, Universiti Sains Malaysia (USM) as well as river along Jalan Transkrian. The water samples were extracted using a dropper and place into bottles provided by the lab.

Figure 3.1 Location for water sample collection (a) Lake inside USM (b Drainage alongside USM hostel (c) Transkrin river

3.1.2 Preparation of Test Piece

The test water collected will be directly send to the preparation lab located at School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia (USM). It is advisable that the water collected should not exceed 24hours from the time of collection. This is to ensure that the *E. Coli* bacteria in it is still active and able to produce a positive result. Once out of the breeding habitat, *E. Coli* only have a lifespan of hours to days [42].

The presence of *E. Coli* is tested using the 3M^{TM} Specialized Test Piece (Model 6404) for *Escherichia Coli & Coliform.* The example of an empty test piece is shown in Figure 3.2. Exactly one millilitre (mL) of the test water is placed on the centre of the circular ring of the aforementioned 3M^{TM} test piece using a calibrated dropper. Ensure that the tip of the dropper is changed from sample to sample to avoid cross contamination. Then carefully close the plastic firm over the area and apply gently and even pressure. This is to ensure the water sample is evenly spread out across the entirely circle.

Figure 3.2 Sample of unused 3M^{TM} *E. Coli* Test Piece (Model 6404)

3.1.3 Incubating the Test Sample

The prepared test piece containing the water sample is then placed into the incubation oven for incubation process. The suitable incubation temperature is 37℃ [43], which is the ideal environment for *E. Coli* to grow. As per the manufacturer recommendation, the incubation process should be about 18 to 24 hours and should not exceed 48 hours. The testing period for this research is set at exactly 24 hours and is taken out thereafter to prevent further reproduction of the *E. Coli* bacteria.

Figure 3.3 Incubation oven set at 37℃

3.2 Image Acquisition

The image acquisition step is crucial to ensure that each image of the sample achieve the same level of details, quality and orientation. Lighting used has to be ensured that the details of the information require is enhanced further making it easier to be train later on the CNN.

3.2.1 Setting up of Equipment

The equipment to capture the image of the test sample is set up in the Machine Vision Lab situated inside Metrology and Precision Lab at the School of Mechanical Engineering, Universiti Sains Malaysia. The setup was done as shown in Figure 3.4 and 3.5. An 8MP auto focusing camera (Model: KYT-U800-R2AF01M by Kayeton Technology) with a resolution of 3264×2448 was used to capture the image of the

test piece and is connect to the computer. The working distance between the camera and the test sample was fixed at 10cm to ensure equal size of the picture from image to image and is held in placed by a tripod stand.

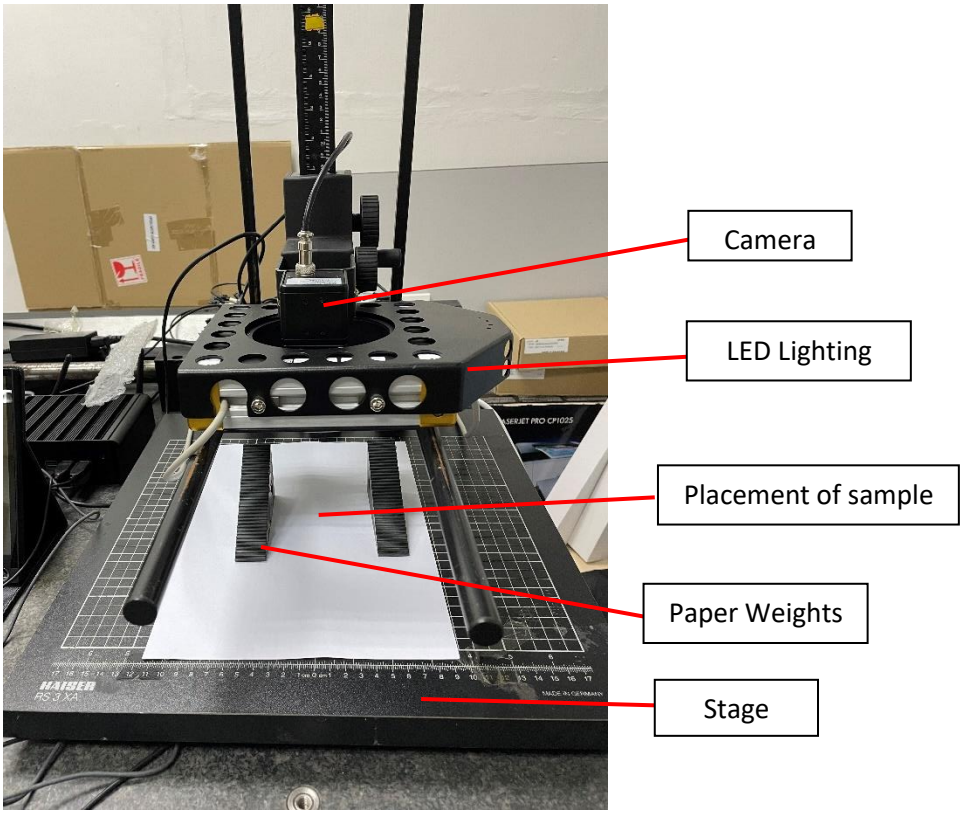


Figure 3.4 Setup of apparatus in lab

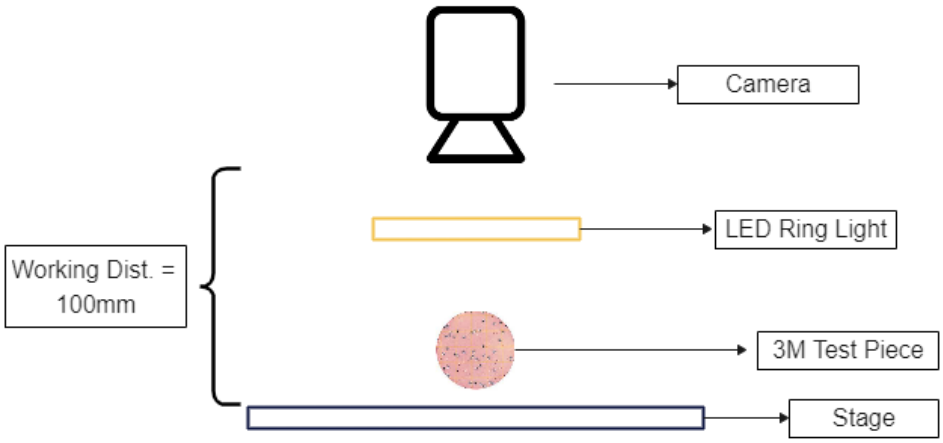


Figure 3.5 Schematic of the setup apparatus

3.2.2 Determination of Lighting Type

Deciding the lighting colour, intensity and type of lighting need to be thoroughly research as this may greatly impact the quality of the image which in return may affect the accuracy in the model training phase. The criteria when choosing the lighting colour depends on the ability of it differentiating the foreground and the background of the image. The intensity value which ranges from 0 to 255, is the measured parameter. The greater value in the ratio between the foreground and background, the better the lighting colour is able to differentiate it.

The test of the is done using MATLAB where 7 samples were randomly picked from the 40 piece of image capture. The foreground and background of the particular image is then randomly picked using the pinpoint tool. The value of the background and foreground was notarised in a Microsoft Excel Spreadsheet and the ratio between it was obtained. The test was run 3 times per image and the value was obtained.

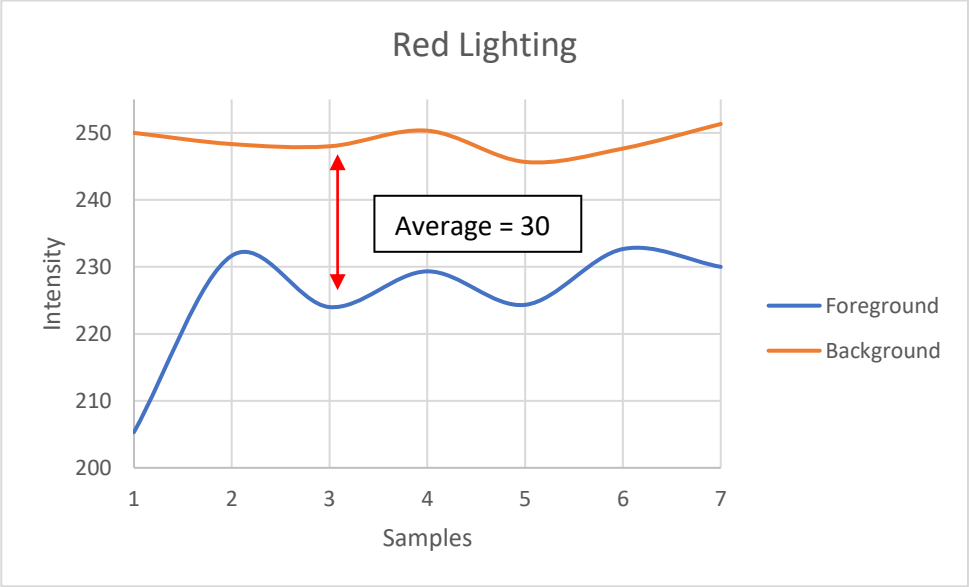
The intensity ratios were calculated using the formula listed below and then tabulated in Table 3.1. Based on the Table 3.1, it is observed that the red lighting on the 3M test piece has a higher intensity ratio (highest = 0.95454), evident by the higher ratio between foreground and background. White lighting on the 3M test piece does not show any big difference between the foreground and background (highest obtained = 0.5). Therefore, red colour lighting was chosen as the ideal lighting colour type of this research

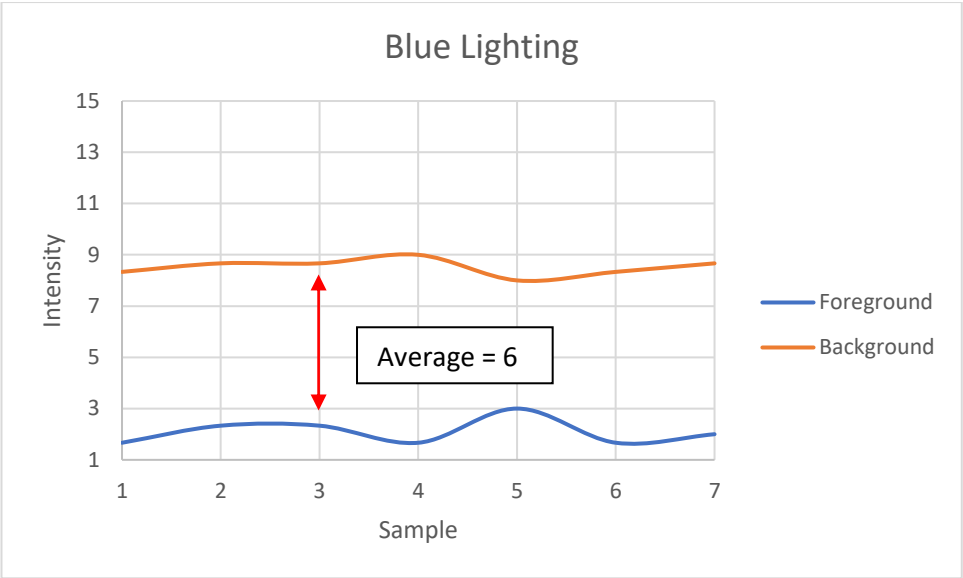
$$Intensity\ Ratio = \frac{Foreground}{Background}$$

Table 3.1 Table of comparison of intensity ratio between red lighting, blue lighting and white lighting

Sample (With Date)	Red Lighting			Blue Lighting			White Lighting		
	Intensity			Intensity			Intensity		
	Foreground	Background	Ratio	Foreground	Background	Ratio	Foreground	Background	Ratio
1 (13th Dec 2021)	204	248	0.822580645	1	8	0.12500	91	179	0.50838
	200	253	0.790513834	1	10	0.10000	87	185	0.47027
	212	249	0.851405622	3	7	0.42857	75	186	0.40323
2 (10th Jan 2022)	229	248	0.923387097	4	8	0.50000	89	206	0.43204
	231	250	0.924000000	1	8	0.12500	87	197	0.44162
	235	247	0.951417004	2	10	0.20000	83	185	0.44865
3 (10th Jan 2022)	225	249	0.903614458	3	8	0.37500	80	185	0.43243
	217	252	0.861111111	1	10	0.10000	92	200	0.46000
	230	243	0.946502058	3	8	0.37500	81	179	0.45251
4 (10th Jan 2022)	231	250	0.924000000	1	8	0.12500	83	181	0.45856
	231	251	0.920318725	2	9	0.22222	86	181	0.47514
	226	250	0.904000000	2	10	0.20000	53	185	0.28649
5 (17th Jan 2022)	222	247	0.898785425	4	7	0.57143	68	177	0.38418
	220	248	0.887096774	2	9	0.22222	87	175	0.49714
	231	242	0.954545455	3	8	0.37500	83	175	0.47429
6 (17th Jan 2022)	238	253	0.940711462	1	8	0.12500	84	178	0.47191
	231	249	0.927710843	2	9	0.22222	82	175	0.46857
	229	241	0.950207469	2	8	0.25000	92	181	0.50829
7 (17th Jan 2022)	231	251	0.920318725	1	8	0.12500	77	173	0.44509
	231	253	0.913043478	2	8	0.25000	94	175	0.53714
	228	250	0.912000000	3	10	0.30000	102	179	0.56983

The light intensity was set at 80 over the maximum 255 on all sets of the image taken. The main criteria for the light intensity is that the lighting must be the lighting cannot be on the extreme ends of the brightness. If the lighting is too bright, it might cover up the dots (presences of *E. Coli*) on test piece, while too dim will limit the ability of differentiating the background and foreground.





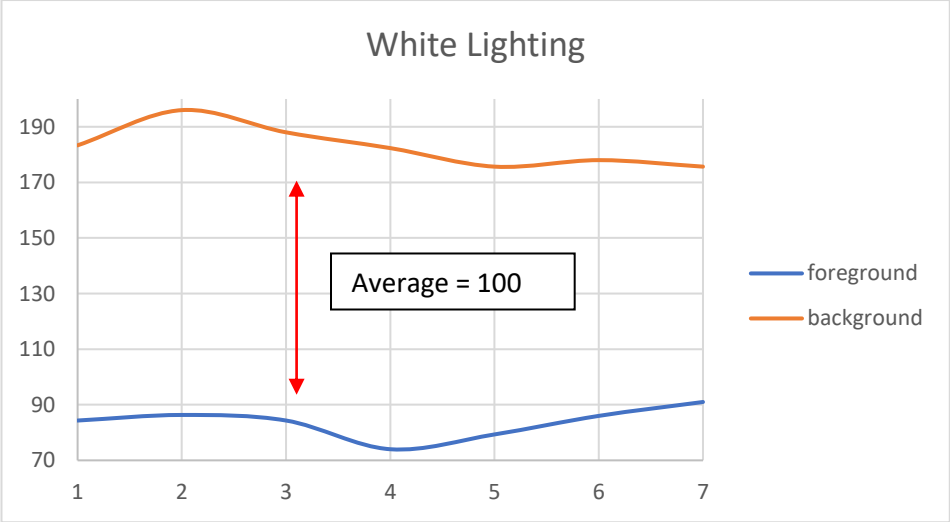


Figure 3.6 Graph showing the ability of (a) red lighting, (b) blue lighting and (c) white lighting in differentiating foreground and background

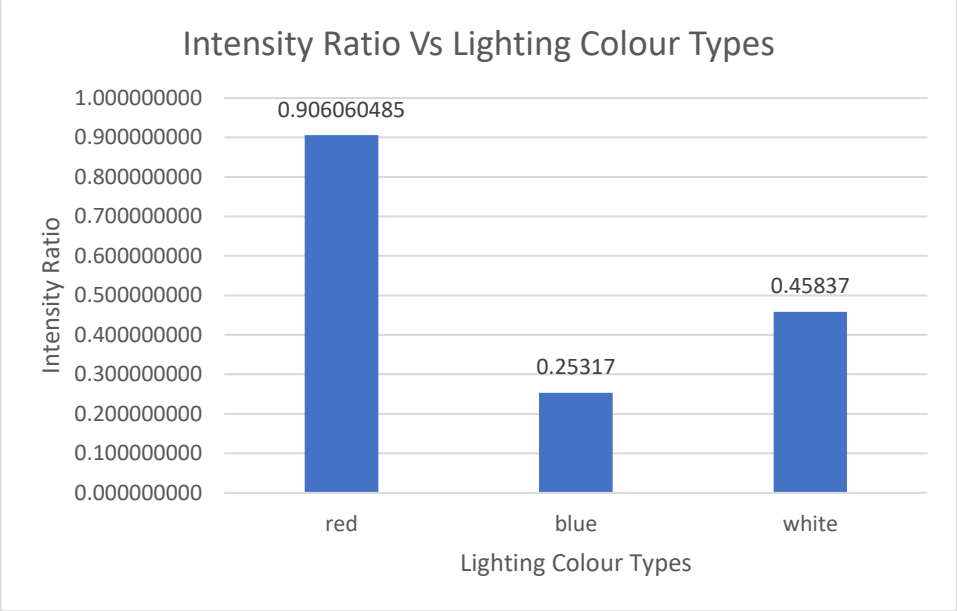


Figure 3.7 Graph showing the average intensity ratio of different lighting colour types

From Figure 3.6, when the average intensity value of the foreground and background were taken from each image sample, we can observe that white colour lighting used indeed shows better differentiating between the foreground and background. The difference in the intensity value has an average of approximately 100 which betters the blue lighting which have an average of approximately 6.

However, red lighting was chosen instead of white. This is be due white lighting has a wider colour spectrum and provides more glare which makes differentiating the fore and background harder. From Figure 3.7, the average intensity ratio between the foreground and background were calculated between the 3 lighting colour types. We can observe that red lighting colour once again shows the highest intensity ratio when compared to the white and blue lighting. Red lighting with an intensity ratio of almost 1 (0.906060485) shows that it has better differentiating power between the foreground and background of the image. Therefore, with all parameter considered, red lighting is chosen with this work.

As for the light setup, the one used in the research project is direct lighting with an active diffusor before the lights hits the test piece. This is to prevent glare on the test piece as commonly the test piece will come with a plastic protective sheet to prevent the spreading of bacteria during examination stage.

3.2.3 Placement of Test Piece on Stage

The placement of test piece on the stage is placed on the same designated spot each time an image is taken. This is to ensure that each picture that was taken remain the same, and this will help in the image processing part later on. The dimension of the test piece on the stage is shown in Figure 3.7 below.

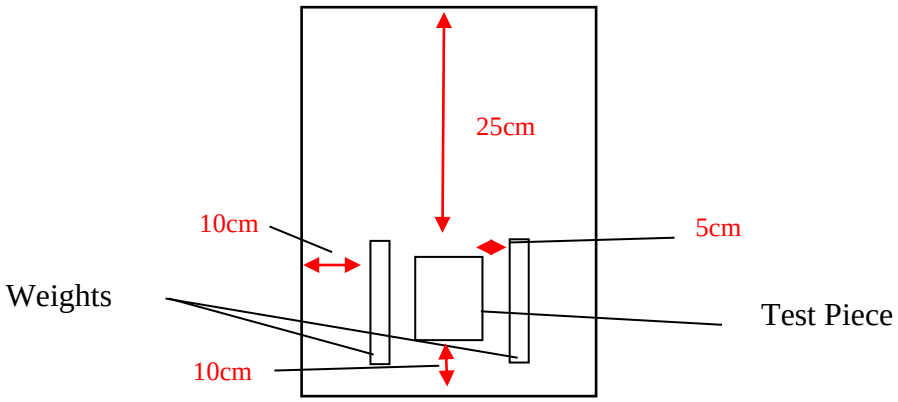


Figure 3.8 Schematic of the stage with dimensions

3.3 Model Architecture

For the identification of *E. Coli* bacteria on the 3M test piece, YOLO v4, the fourth version of YOLO, is used. Published by Alexey Bochkovsky in 2020, YOLO v4 is a single stage object detector in a given image, which prioritizes on inference speeds. In a single stage object detector, region of interest (ROI) will not be used, instead, bounding box and IOU is used for the complete image predicted.

For the YOLO v4, the architecture consists of various parts, namely the backbone network and the neck. CSPDarknet 53 CNN is used as the backbone network and it functions to concatenate previous inputs with the incoming input before entering dense layers. The neck is where features from the backbone is mix and combined to prepare for the next step. In the neck region, PANet or a feature aggregator network is present to increase the receptive field and separate the significant feature in the image [46].

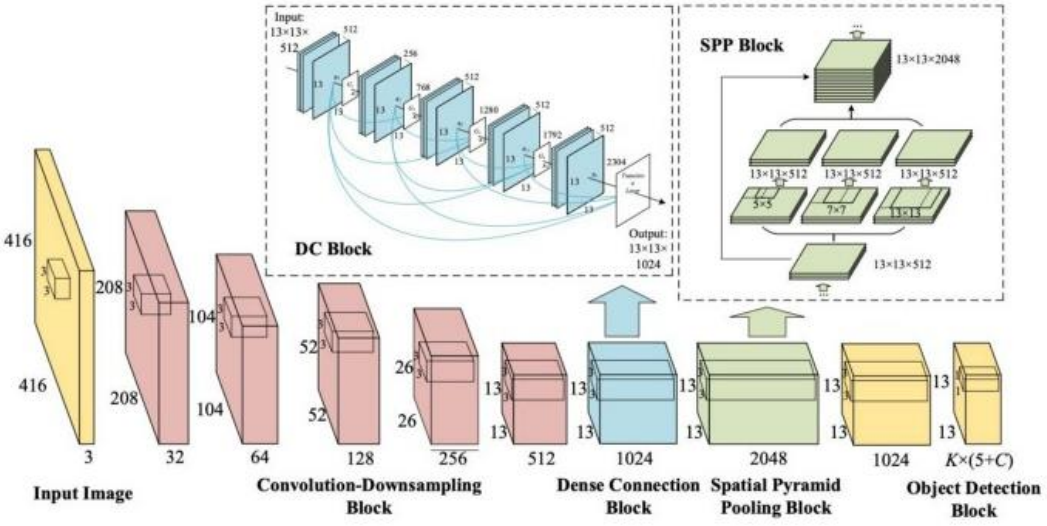


Figure 3.9 YOLO v4 Architecture

Layers	Parameters		Output	Layers	Parameters		Output
	Filters	Size / Stride			Filters	Size / Stride	
Conv 1	32	3×3/ 1	416×416×32	**DC Block**	**1024**	**3×3 / 1 ×4**	**13×13×2304**
Maxpool 1		2×2 / 2	208×208×32	**Conv 14-21**	**256 or 512**	**1×1 / 1**	
Conv 2	64	3×3 / 1	208×208×64	**Conv 22**	**1024**	**3×3 / 1**	**13×13×1024**
Maxpool 2		2×2 / 2	104×104×64	**Conv 23**	**512**	**1×1 / 1**	**13×13×512**
Conv 3	128	3×3 / 1	104×104×128	**SPP Block Maxpool 6-8**		**5×5 / 1**	**13×13×2048**
Conv 4	64	1×1 / 1	104×104×64			**7×7 / 1 Concat**	
Conv 5	128	3×3 / 1	104×104×128			**13×13 / 1**	
Maxpool 3		2×2 / 2	52×52×128	**Conv 26**	**512**	**1×1 / 1**	**13×13×512**
Conv 6	256	3×3 / 1	52×52×256	Conv 27	1024	3×3 / 1	13×13×1024
Conv 7	128	1×1 / 1	52×52×128	Reorg Conv13		/ 2	13×13×256
Conv 8	256	3×3 / 1	52×52×256	Concat -1, -2			13×13×1280
Maxpool 4		2×2 / 2	26×26×256	Conv 30	1024	3×3 / 1	13×13×1024
Conv 9-12	512	3×3 / 1 1×1 / 1 ×2	Conv 31	Conv31	*K**5+*C*	1×1 / 1	13×13×(*K**5+*C*)
Conv 13	512	3×3 / 1	26×26×512	Detection			
Maxpool 5		2×2 / 2	13×13×512				

Figure 3.10 An overview of YOLO v4

3.3.1 Deep Transfer Learning Approach

To ease the image training process, instead of retraining a new YOLO network, a pre-trained network is obtained from GitHub [47]. By applying deep transfer learning, much processing time is saved as the network was previous used by certain user to train their work on something similar to this research. Since YOLO recognises object within an image and in this research to detect the presence of *E. Coli*, the pre-trained network was previously used to recognises mould forming on food surfaces. This is somewhat similar to our research and can be used to training.

3.3.2 Image Dataset for Training

The test piece image dataset for training and testing purposes consist of a total of 60 test piece image samples was prepared. A total of 40 out of 60 test piece image were selected randomly as training dataset images. For the 40 images used for training, 20% of it (8 images) were selected at random and used for validation purpose during the training phase. The remaining 20 images were use as test dataset. The images were also crop to $896\ pixel \times 896\ pixel$ as per the requirement of the network which the input image needs to be of a multiple of 32 for the horizontal region.