Abstract Submission Form: ASEAN IVO Forum 2021

I. Title of presentation: Optical segmentation on E. coli colonies formed on petrifilms – for the development of IoT devices for water quality monitoring.

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III. Abstract

(Describe the purpose, background, objectives, content, plans for connected projects, etc. This abstract should be no more than 3 pages and must be saved as a PDF.)

Purpose

This work aims to design and develop a portable machine vision system for automatic counting of E. coli colonies on petrifilms for water quality monitoring in project – IoT for water reuse in developing cities. The focus of this work is the optical segmentation of E. coli colonies formed on petrifilms.

Background

Supplying clean useable water has been an increasing problem globally due to limited water resources, the rising urban water demand, and climate change. Water reuse after proper water treatment appears to be the best option to tackle this problem [2]. The quality of the treated water, especially the total *Escherichia coli (E. coli)* count become one of the major concerns among the end-users [1,2]. This is because that improper treatment can cause severe diarrhea or even death. Therefore, E. Coli colonies count is one of the critical measurements to determine the quality of the treated water [1,2,4]. However, the process of colonies counting is tedious, and it requires highly trained personnel for its hazardous in nature. The process is typically started from the identification of a reuse water source, where water samples were collected and then sent to well-equipped laboratories for testing and analysis. Alternatively, the water samples could be then dripped on petrifilms for incubation at least 24 hours before one could count the *E. Coli* formed under a light microscope manually. *E. coli* colonies count is determined in colony forming unit (CFU/100ml) [2]. In general, the counting process is labour intensive and time-consuming. Sometimes, the accuracy is subjected to human error in specimen handling and results.

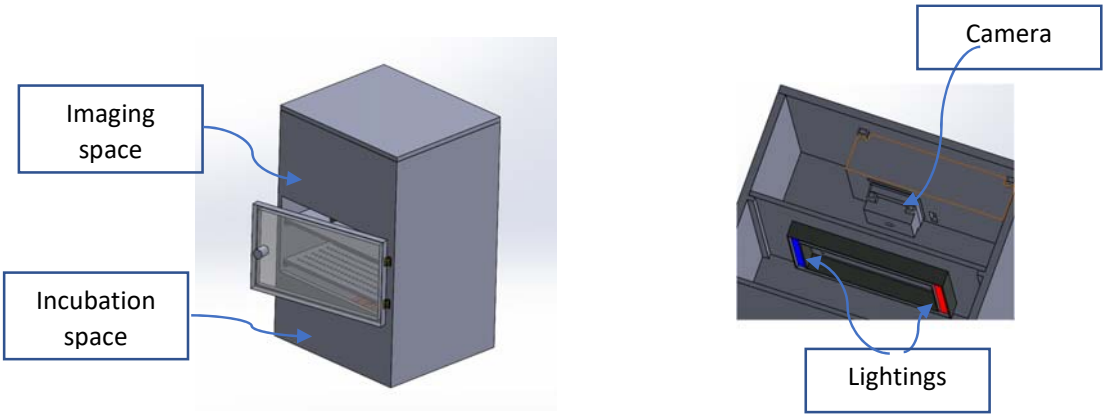
Objective

Hence, we aim to enable the machine vision design and development for automated colonies counting using an appropriate light source and lighting configuration for enhancing the images of *E. coli* colonies on petrifilms captured prior to image processing and analysis. The specific objectives of this work, therefore, includes the following:

- To investigate lighting effect on the colonies formed on the petrifilm.
- To segment the colonies from the background of the petrifilm optically before image processing and analysis.

Content

In this work, we propose a simple, functional yet cost-effective solution to determine the total E. coli content of the water sample. A portable machine vision system with a low cost and low power microprocessor resided in an enclosure assembly. This device has a few sectioning spanning across its structure, featuring space for incubation with integrated controlled environmental conditions and space for image capturing. An image processing algorithm will be developed for counting and hosted in a Raspberry Pi microcomputer. The conceptual design of the enclosure is shown in **Figure 1**.



(a) (b)

Figure 1: (a) Conceptual design of the enclosure and (b) closeup on the camera and lighting used in machine vision.

E. coli petrifilm is an all-in-one plating system that allows E. coli to be cultured for detection and counting [5]. The presence of the E. coli colonies would be stained with purplish color as the petrifilm contains Violet Red Bile (VRB) nutrients. The color intensity, saturation as well as spreading radii of the colony stains formed are related to the quantity of the E. coli colonies of the water samples tested. Based on the physics of color and light, a proper selection of LED lightings and a well-designed configuration of the imaging system, the stained colonies on the petrifilm could be segmented optically for further counting and analysis using image processing techniques. Therefore, investigation of lighting effects on the colonies formed on the petrifilm becomes the main interest of this work. The wavelengths of the LED lighting used included 375 nm (UV light), 460 nm (blue light), 525 nm (red light), 630nm (red light) as well as 850 nm (IR light).

Among all LED lights were tested, it can be noticed that the red-light illumination gave the best visualization of the stained E. coli colonies when compared to other colored lights. **Figure 2** compares the images of stained colonies on the same petrifilm with three different colored illuminations, white, red, and blue LED lights. The white light illumination served as a benchmark image. It can be observed that E. coli colonies can be visualized clearly and sharply under the red-light illumination, Figure 2(b). At the same time, other information, such as texts and grids, which are not of our interest, was not seen. While under the blue-light illumination, Figure 2(c), the colonies were unseen but the texts and grids remained clearly in the image.

(a) (b) (c)

Figure 2: Petrifilm under (a) white (normal) light illumination, (b) red-light illumination (c) blue-light illumination

The observation in the preliminary result indicates that a proper selection of the LED colored lights for illumination could help to segment the stained colonies optically. *E. coli* detection for counting can be

made more accurate. This result also suggested a positive finding that can facilitate the development of image processing and analysis algorithms for automated E. coli detection and counting.

Plans for connected projects

Moving forwards, a thorough analysis will be carried out on the lighting effect better E. coli colonies visualization using ANOVA analysis. Also, the development of the image processing algorithm will be commenced to automate the image analysis for the device fully. The success of the development of a portable machine vision system for automatic counting on *E. coli* colonies would bring positive impact to not only our main project – IoT for water reuse in developing cities, but also benefits to everyone who wishes to investigate the *E. coli* contamination in their water sources, as we can analyze the water samples remotely and with literally no technical requirement for hazardous sample handling [3].

Acknowledgment

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