Faculty of Engineering

INTEGRATING AN AUTOMATED SORTING MACHINE WITH IMAGE PROCESSING DEVICE FOR COLONY ENUMERATION PROCEDURE

Joel Anak Ngikoh @ Nyikoh

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INTEGRATING AN AUTOMATED SORTING MACHINE WITH IMAGE PROCESSING DEVICE FOR COLONY ENUMERATION PROCEDURE

JOEL ANAK NGIKOH @ NYIKOH

This project is submitted in partial fulfilment of the requirements for the degree of Bachelor of Engineering (Hons) in Mechanical and Manufacturing Engineering

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This thesis is dedicated to my beloved family and friends.

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ABSTRACT

The automation of colony enumeration procedure has been increasingly popular among researchers worldwide. In the field of microbiology, accurate quantitative analysis is important to determine the quality of a tested sample. The technology of powerful imaging processor for this procedure has been developed in recent years to help reduce the amount of manual work require in the laboratories. As time progresses the technology is getting more affordable and inexpensive which allow many of these laboratories to start adopting this technology to their system. However, this technology is only limited in processing the image of samples captured on a cultured petri dish. With the increasing amount of petri dishes to be analyzed, it is necessary to develop a sorting machine that can handle a high number of petri dishes. In this research, an automated sorting machine that can be integrated together with the image processing technology for counting bacterial colony-forming units (CFUs) is developed. The purpose of this research is to fabricate and test the mechanism of the prototype of the machine at a relatively low cost. The machine consists of two systems; a carousel storage system where a few stacks of petri dishes can be stacked on top of it and a petri dish handling system to extract the petri dishes from the storage to the imaging area and back to the storage. The mechanism of the proposed design were developed and tested in the Solidwork CAD software before testing were carried out in full scale prototype. Finally, with further improvement on the electronic implementation of the study, this machine should be capable of processing a high number of petri dishes without human intervention.

ABSTRAK

Sistem automasi dalam prosidur penghitungan sel koloni kini makin kerap dipraktikkan oleh pengkaji serata dunia. Di dalam bidang mikrobiologi, analisis kuantitatif merupakan aspek yang penting untuk menentukan kualiti sampel yang telah diuji. Pengenalan teknologi pemprosesan imej yang bermutu kepada prosidur ini telah membantu untuk mengurangkan kerja-kerja manual di dalam makmal-makmal yang terlibat. Dengan peredaran masa, teknologi ini menjadi semakin mudah untuk diadaptasi dalam sistem makmal yang sedia ada kerana kos teknologi ini semakin berkurang. Namun begitu, teknologi ini mempunyai had yang tertentu di mana fungsi teknologi ini hanyalah untuk memproses imej yang ditangkap di atas piring petri. Apabila bilangan piring petri ini meningkat, kerja-kerja manual untuk menguruskan piring petri ini juga akan bertambah. Oleh itu, sebuah mesin penyusun untuk piring petri ini haruslah diperkenalkan kepada teknologi ini. Dalam kajian ini, sebuah mesin penyusun automatik yang boleh diintegrasikan bersama dengan teknologi pemprosesan imej untuk menghitung koloni-koloni sel yang terbentuk atas piring petri. Tujuan kajian ini ialah untuk membina dan menguji mekanisme yang terdapat dalam prototaip mesin tersebut pada harga yang berpatutan. Mesin ini mempunyai dua sistem iaitu; sistem stor penyimpanan karusel untuk meyimpan piring petri yang akan dianalisa dan sistem pengurusan piring petri untuk mengekstrak petri dish tersebut ke kawasan pemprosesan imej sebelum dihantar semula ke stor peyimpanan karusel tersebut. Mekanisme mesin tersebut telah dicadangkan dan diuji dalam perisian Solidwork CAD sebelum ujian dijalankan pada prototaip berskala penuh. Sebagai pengkakhiran, penambahbaikkan dari segi pelaksanaan komponen elektronik mesin ini dijangka dapat meningkatan jumlah piring petri yang diproses tanpa sebarang gangguan.

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LIST OF ABBREVIATIONS

CFU	-	Colony-Forming Unit
CAD	-	Computer Aided Design
RPM	-	Rotation per Minute
3D	-	3 Dimensional
CNC	-	Computer Numerical Control
DC	-	Direct Current
A	-	Ampere
V	-	Volt
USB	-	Universal Serial Bus
IDE	-	Integrated Development Environment

CHAPTER 1

INTRODUCTION

1.1 Colony Enumeration

Colony enumeration procedure is widely used in the microbiology field to test the quality of a sample. In the food industry, the procedure is used to examine the quality of food and dairy products. The examination is done by counting the number of bacterial colony-forming units (CFUs) in a sample. A bacterial colony-forming unit is a quantity used to estimate the number of viable counts microbes or colony cells present in 1g or 1ml of a sample during the process of colony enumeration. One of the most common methods for counting the CFUs is the viable plate count method. In general, the method is carried by pouring a liquefied sample containing microbes onto petri dishes containing agar, incubating the survived microbes as the seeds for growing the number of microbes to form colonies on the petri dishes. The evaluation is done by examining the survival rate of microbes in a sample. (Chen, 2009).

1.2 Automation in Colony Enumeration

The introduction of automation in microbiology has improved the technique of colony enumeration from time to time. Devices such as the image-processors with built in software which can automatically scan and count the number of CFUs on a plate has reduce the manual efforts of colony enumeration. With the increasing number of samples that need to be processed, the present of imaging-processors alone is not enough to reduce the amount of workload on the laboratories technicians. Hence, a number of sorting machines with integrated imaging-processors and software have been introduced over the last decades. The current technology of the machines are mostly equipped with barcode reader, extracting and retracting mechanisms, robotic gripper, conveyer belts and sensors. These machines have a range of productivity of processing from tens to hundreds of petri dishes at a time without supervision.

1.**3** **Problem Statement**

One of the steps in the colony enumeration procedure requires the laboratory technicians to manually count the CFUs on the petri dish by using a marker. However, there could be thousands of colonies exist on a single petri dish and the method is consider to have a low throughput, time consuming, and labor intensive. (Chen, 2009). Smaller or medium size laboratories such as in higher education institutions and rural healthcare facilities are usually operated with limited budgets and space. Since the manual enumeration of colony is considered to be inefficient and error-prone, then the needs for automation in colony enumeration is essential. The technology of imaging processer has greatly reduced the amount of time needed to perform the procedure. However the petri dishes still needed to be taken out from their storage to the workstation and back again to the storage and this process will be repeated countless of times if there are plenty of petri dishes to be processed. The commercially available machines are rather very expensive. Therefore, it would be impractical for these laboratories to implement the usage of an automated sorting machine for counting CFUs with the amount of resources required. The problem of most of the marketed colony counting machines is the complexity of their design. The common features of these machines often involve a motor and a gripping mechanism such as gripper, a rotating hand or an actuator equipped with multiple sensors to detect the presence of petri dishes on the machines. The more complicated the design of these machines the higher the cost to acquire them.

Furthermore, some of these machines are not portable due to their large size to cope with their high productivity. These laboratories require a machine which could basically perform the same task as the widely marketed machines with more limitation on their technologies for it to be cost-effective and small in size for accommodation without compromising much on the productivity.

1.4 Objectives

The focus of the project is to design and fabricate a prototype of an automated sorting machine which can be integrated to an already existing image processing technology for medium size laboratories such as in universities and facilities in small healthcare institutions with limited budgets. The main objectives are listed below:

i. To design an automated sorting machine for counting bacterial colony-forming units (CFUs) for medium size laboratories with the aid of CAD.

ii. To prove the concept of the design can work by fabricating and testing the mechanism of the prototype of the design.

1.5 Scope of Project

In completing this project, there are several scopes of work that need to be completed. The scopes are:

i. Design and fabricating the machine which consists of key components such as lower and upper bases and a stationary table.

ii. Selecting the suitable materials such as acrylic sheet for the body frame, and electronic components such as motors and actuator selections to run the machine.

iii. Develop a program to control the mechanism using the Arduino UNO R3 board.

iv. Testing the mechanism of the prototype.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Determination of the number of colonies (colony forming units, CFUs) is a standard method in microbiological analysis to ensure the quality of drinking water. Normally this tedious work is still performed manually (J Moratz, 2001). This is done by liquating a small amount of a liquid culture and plating out several serial dilutions onto culture plates (petri dishes containing medium for growth of microorganisms). After incubation in appropriate conditions for the microorganism of choice, the colonies are counted to determine the number of CFUs (Brugger *et al*, 2012). The concentration of bacteria in the original culture can then be calculated based on the assumption that each colony has raised from one single bacterium (colony forming unit, CFUs). For many, the manual counting is considered to be unreliable and time consuming. The process also become more error prone as the number of samples increases.

2.2 Automated Counting for Bacterial Colonies

Automation of colony counting has been of increasing interest for many decades and these methods have been shown to be more consistent than manual counting (Clark et al, 2011). Herman et al. demonstrated that automated colony counts had significantly less variation when reanalyzing the dishes than those manually determined by individual or multiple observers. Commercial products are available in the market to facilitate accurate colony counting. These products are ranging from manual counting aids to all-in-one platforms including image acquisition, processing, and analysis. However, Clarke et al said that even though these fully automated counting systems are capable of processing multiple images at once, they can be prohibitively expensive for small laboratories and large facilities may necessitate multiple counting instruments.

In 2012 Nagpal proposed a design of an automated bacterial colony counter which used many image processing algorithms such as gray scaling, thresholding, filtering etc. to count these colonies efficiently.

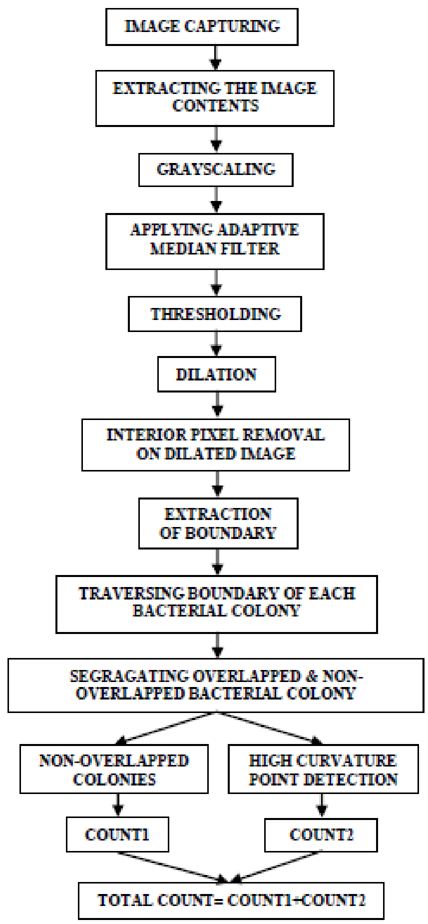

Figure 2.1 Block diagram of proposed method by Nagpal.

The graph below shows that there is a different in manual and actual counting in the result of the method implemented by Nagpal. This clearly suggests that the procedure of colony enumeration should be automated in order to reduce the amount of errors in counting the CFUs.

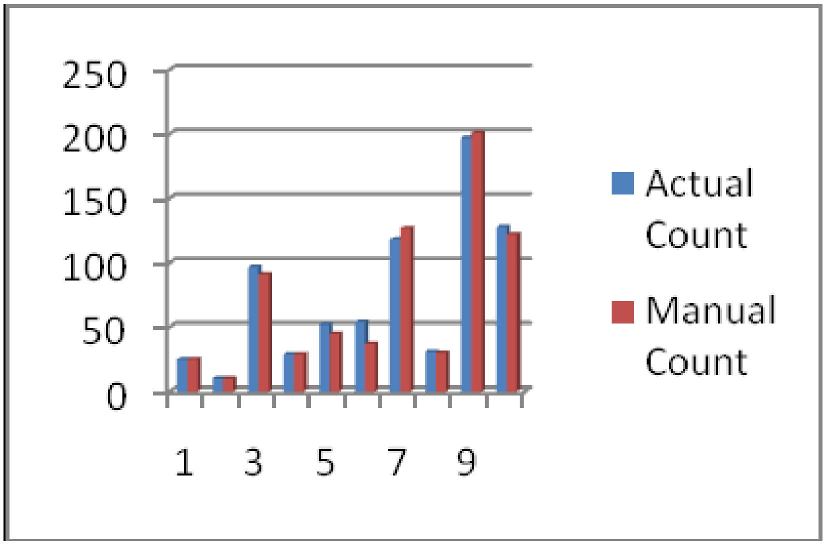

Figure 2.2 Comparison between actual and manual count

Similarly, in 1998 Corkidi introduced the confluent and various sizes image analysis method (COVASIAM), an automated colony count technique that uses digital imaging technology for detection and separation of confluent microbial colonies and colonies of various sizes growing on petri dishes. The proposed method exploits the optical properties of the surfaces of most microbial colonies. Colonies in the petri dish are epi-illuminated in order to direct the reflection of concentrated light coming from a halogen lamp towards an image-sensing device. At the same time, a multilevel threshold algorithm is proposed for colony separation and counting. The results show that COVASIAM estimated an average of 95.47% of colonies scored by manual counting with 8.55% of error estimated. Corkidi stated that these results prove that this method to be very effective in detecting and counting different kinds of colonies regardless of their size and degree of confluence.

2.3 Laboratory Automation

Total laboratory automation has been developed since the 1980s. Although robotics has been in place in the industrial setting for over two decades, it represents relatively new technology to the clinical laboratory (Felder, 1990). However, the increasing number of samples and workload in the laboratories has increased the demand for total laboratory automation. Laboratories today face increasing pressure to automate their operations as they are challenged by a continuing increase in workload and samples, need to reduce cost of operation, and difficulties in recruitment of experienced technical staff (Lam, 2012). The first step in introducing automation in laboratory can be traced back to the early 1950s. It was in 1956 when the first mechanized clinical laboratory was introduced (Sarkozi, 2003). In 1957 the first auto analyzer was described and it was a major leap in technology (Skeggs, 1957). At the time, the instruments included an auto analyzer which could assay blood urea nitrogen at the rate of 20 samples per hour (Streitberg, 2009).

Automation in clinical laboratories reached its height when total laboratory automation was introduced. The systems have grown dramatically in the United States, Europe, and Japan during the 1990s (Hawker, 2000). Since 1997, the efforts to standardize total laboratory automation has been increased to reduce the cost of implementing the systems. Hawker also said that there are five aspects that were considered for the standardization of laboratory automation. These aspects are bar code labels, specimen containers and carriers to electromechanical, computer devices between devices, automation systems, and information systems. Currently, there are four different types of total laboratory systems available in the market (Greub, 2011). They are the Walk Away Specimen Processor (WASP), Previ-Isola, Innova, and Inoqula-FLA.

2.4 Sorting Machine for Colony Counting

The most important aspect in the effort of automating the counting of a high-volume colony forming units are in the functionality of the sorting machines. Clinical laboratory automation technology derives its usefulness from functionality, where “functionality” is defined as functions performed or supported by the technology (S. Markin, 2000). For this particular design, the functions required of the sorting machine are to provide a storage and a petri dish handling systems for the process of CFU counting in order to increase the number of samples to be processed in laboratories. In 1998, ARUP Laboratories implemented an automated transport and sorting system in their large laboratories. Since implementation, the volume of work transported and sorted has grown to over 15 000 new tubes and over 25 000 total tubes per day. Median turnaround time has decreased by an estimated 7 h, and turnaround time at the 95th percentile has decreased by 12 h (Hawker, 2002).

A high-volume yet compact storage system in the design will influent the portability and size of the machine. This type of system allows the machine to be accommodated in smaller laboratories. In 2000, Inaba stated that the traditional method in which a rack is employed to accommodate large supply of petri dishes is unreliable as the rack has limited amount of storage capacity. The rack could be made into a taller size to increase its capacity but that would make the rack become unstable during transportation. Many lab automations are currently employing a rotating carousel storage system. This system consists of multiple columns and each column consists of a stack of petri dishes. For example, The WASP instrument has nine columns, each of which holds 42 dishes, and one to nine types of media can be loaded at one time (Borbeau, 2009). This makes the total processing capacity of the WASP around 378 dishes at a time.

In the 1980s, Sasaki has used a conveyer-belt system for his automated laboratory. This system is designed to integrate the laboratory by linking multiple workstations together. Although it is a great application in total laboratory automation, it is not a suitable for a standalone instrument. A preferable alternative would be the use of robotic hand or arms. The use of robotic hand have a good reproducibility of motions and positional repeatability (Boyd, 1996). However, the operation of robotic hand require a well-trained technician to interact with the software and the peripheral interface. Boyd also stated that pharmaceutical laboratories have benefited from the usage of robotic hand not because of the reliability and repeatability of the robots, but also because the laboratories employ competent systems integrators who can produce and maintain working pharmaceutical robotic testing systems.