MICROFLUIDIC CHANNEL FROM GELATIN USING LASER PRINTER

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ABSTRACT

Microfluidic is the science of manipulating and controlling fluids. Microfluidic deals with very precise fluid control, under small volumes and space. Nowadays, Microfluidics has used a variety of materials such as silicon, glass, polymer, ceramic, etc. All of these are wasted and pollution in our environment. Furthermore, the process of making microfluidic is sophisticated. Therefore, this problem was inspired to make it is simple and environmentally friendly. This microfluidic fabrication uses a laser printer for making the path and gelatin as material for fabricated. The objective of this research is to fabricate microfluidics from gelatin and apply the gelatin microfluidic for rapid *E. coli* detection. The microfluidics channel patterns were formed by using the standard laser printer created the mold template. Moreover, we investigated the applying *E. coli* detection by gelatin microfluidic under the specific catalytic reaction of enzyme. The methodology starts with the design pattern on a computer and printed on a transparent plastic sheet as a template mold for fabricating microfluidic. To determine the ability of gelatin microfluidic detecting *E. coli* as the indicator of bacterial contamination evaluation of the sample, this examination measured the fluorescent synthesis of 4-methyl-umbelliferone (4MU), which is the product of the catalytic reaction of the enzyme, D-glucuronidase (GUD) secreted by *E. coli* and its substrate 4-methyl-umbelliferone-β-D-glucuronide (MUG). The enzyme GUD has been widely used as the biomarker for indicating the amount of *E. coli* in the sample by measuring the detectable fluorescent synthesis product of 4MU, which has the excitation wavelength at 372 nm and emission wavelength at 445 nm. The results indicated that the microfluidic gelatin can be fabricated by using the standard laser printer. The minimize channel that the liquid could be flow through was 0.19 mm height and 1.78 mm width. Furthermore, the gelatin microfluidic could be used efficiently for rapid *E. coli* detection.

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TABLE OF CONTENTS

Page

ABSTRACT iii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF TABLES ix

LIST OF FIGURES x

LIST OF SYMBOLS / ABBREVIATIONS xv

CHAPTER 1 INTRODUCTION 1

1.1 Background and Significance of the research 1

1.2 Objectives 2

1.3 Research hypothesis 2

1.4 Research scope 3

1.5 Research plan 3

CHAPTER 2 RELATED THEORIES 4

2.1 Microfluidic chip 4

2.1.1 Fundamental of microfluidic chip 4

2.2 Microfluidic dynamics 7

2.2.1 Basic information of microfluidic dynamics 7

2.2.2 The Narvier-stokes equations and Reynolds number in microfluidic devices 8

2.3 Other equipment 10

2.3.1 Light microscope 10

2.3.2 Vernier caliper 11

2.4 Gelatin 12

2.4.1 Basic information of gelatin 12

2.4.2 Properties of gelatin 13
2.5 Laser printer 13
2.5.1 Basic information of laser printer 13
2.5.2 Laser printer features 14
2.6 Diffusion 14
2.6.1 Factors that affect diffusion 15
2.7 Light 16
2.8 Ultraviolet light 17
2.9 Fluorescence 18
2.10 Spectrophotometer 19
2.11 Method of Isolation of Microorganisms 21
2.11.1 Streak plate technique 21
2.11.2 Spread plate technique 22
2.12 *Escherichia coli* (*E. coli*) 23
2.12.1 Contaminated of *E. coli* 26
2.12.1.1 Contaminated food of *E. coli* 26
2.12.1.2 Contaminated water of *E. coli* 26
2.12.2 Determination of *E. coli* 27
2.12.3 Substances used for fluorescence determination of *E. coli* 28
CHAPTER 3 METHODOLOGY 30
3.1 Gelatin microfluidic fabrication 30
3.1.1 Galatin preparation 30
3.1.2 Microfluidic fabrication 30
3.2 Channel pattern design methodology 31
3.2.1 Node design 31
3.2.2 Channel pattern design 34

3.2.3 Measurement of channel 36

3.3 Characteristics of gelatin microfluidic channel 37

3.3.1 UV cut on gelatin 37

3.3.2 Diffusion of liquid in gelatin 38

3.4 Reaction of enzyme GUD and substrate MUG, and product 4MU test 38

3.4.1 Fluorescence comparison between 4MU and GUD and MUG reaction 39

3.4.2 Fluorescence comparison between 4MU and GUD and MUG reaction in the gelatin microfluidic 39

3.5 Mixing MUG in gelatin 43

3.5.1 Enzymatic reaction of GUD and MUG under MUG mixed gelatin microfluidic 43

3.5.2 Enzymatic reaction from *E. coli* under MUG with nutrient agar 44

3.5.2.1 Streak plate 44

3.5.2.2 Spread plate 44

3.5.3 Enzymatic reaction from *E. coli* under MUG in gelatin microfluidic channel 44

3.5.3.1 Capillary channel 45

3.5.3.2 Printed 30 times layer channel 46

CHAPTER 4 EXPERIMENTAL RESULTS AND DISCUSSION 48

4.1 Result of node design 48

4.2 Result of channel design 48

4.3 Result of measurement of the channel size 52

4.4 Result of gelatin microfluidic channel characteristics 57

4.4.1 UV cut result 57

4.4.2 Result of diffusion 58

4.5 Reaction of enzyme GUD and substrate MUG, and product 4MU test 61

4.5.1 Result of fluorescence comparison between 4MU and GUD and MUG reaction 61

4.5.2 Result of fluorescence comparison between 4MU and GUD and MUG reaction in the gelatin microfluidic 62

4.6 Result of fluorescence from GUD and MUG mixed in gelatin 66

4.6.1 Enzymatic reaction of fed GUD and MUG mixed gelatin microfluidic 66

4.6.2 Enzymatic reaction from *E. coli* under MUG with nutrient agar 67

4.6.3 Enzymatic reaction from *E. coli* under MUG in gelatin microfluidic 69

4.6.3.1 Capillary channel 69

4.6.3.2 Printed 3 pt and 30 times layer channel 71

CHAPTER 5 CONCLUSION 74

5.1 Conclusion 74

5.2 Suggestion 76

REFERENCES 77

LIST OF TABLES

Table Page

1.1 Show research plan since August 2020 to April 2021 3

4.1 Width and thickness of printed ink on plastic sheet measured by microscope (red) and vernier caliper (black) 54

4.2 Diffusion distance (mm) of liquid color far apart from the edge of the channel measured by vernier caliper and the diffusion rate (mm/hr) at varied times 59

LIST OF FIGURES

Figure Page

2.1 Microfluidic chip 4

2.2 Schematic of Photolithography process for fabricating microfluidic device 5

2.3 Schematic of Soft-lithography process for fabricating microfluidic device 6

2.4 Schematic of fuid flows (a) Laminar flow in a closed pipe; (b) Turbulent flow in a closed pipe 8

2.5 Parts of microscope 10

2.6 Diagram of Vernier caliper 12

2.7 (a) Gelatin powder; (b) Gelatin sheet 13

2.8 Laser printer CANON LBP-6030 14

2.9 Diffusion diagram 15

2.10 Several bands based on the wavelength of light 16

2.11 Ultraviolet spectrum 17

2.12 Fluoresce light with several wavelength 18

2.13 Jablonski energy diagram of absorbance and fluorescence 19

2.14 Spectrophotometer 20

2.15 The diagram shows principle of spectrophotometer 20

2.16 Streak plate technique 22

2.17 Streak plate technique 22

2.18 Example of isolation of microorganism by spread plate technique 23

2.19 Diagram of spread plate technique 23

2.20 Scanning electron micrograph of *Escherchia coli* (*E. coli*) 24

2.21 Pathogenic schema of diarrhoeagenic *E. coli* 26

2.22 Graph of (a) The fluorescence spectra of different *E. coli* concentration samples; (b) The fluorescence intensity versus the bacterial number (red curve), fit line (blue line) 28

2.23 The reaction of GUD and MUG with the systhesis product, 4MU molecule 29

3.1 Diagram of fabrication process 31

3.2 Straight line pattern with the various points of line 32

3.3 Various points of straight line with circular node pattern 32

3.4 Various points of straight with triangular node pattern 33

3.5 The channel of various points of straight-line pattern with circular node on Gelatin 33

3.6 Branches channel with single inlet and open four branches outlet 34

3.7 Four branches channel combine symmatric four branches with one single inlet and outlet 34

3.8 (a) T-shape pattern; (b) Printed 30 times of T-shape pattern 35

3.9 (a) Y-shape pattern; (b) Printed 30 times of Y-shape pattern 35

3.10 (a) Y-shaped with double wave pattern; (b)Printed 30 times of Y-shaped with double wave pattern 35

3.11 Zig-zag pattern 36

3.12 (a) Branch with curve line; (b) Printed 30 times of branch with curve line 36

3.13 (a) Wave line; (b) Printed 30 times of wave line 36

3.14 Measurement of the width and thickness of channel under microscope equipped with Opticam B1 camera program 37

3.15 Experiment set for investigating the diffusion characteristic of gelatin 38

3.16 4MU in straight-line channel of microfluidic 40

3.17 Side view of feeding 0.4ml of 4MU in inlet node of the straight-line channel of microfluidic 40

3.18 Separately fed GUD and MUG in inlet node of straight-line channel of microfluidic 41

3.19 Feeding mixed GUD and MUG in inlet node of straight-line channel of microfluidic 42

3.20 Feeding GUD and MUG in Y-Shape channel of microfluidic 42

3.21 Feeding GUD at the inlet node of the microfluidic channel fabricated from MUG mixed with gelatin 43

3.22 A microfluidic channel fabrication from MUG mixed with gelatin with capillary tube 45

3.23 Side view of fed cultured and liquid cultured residue of *E. coli* at the inlet node of the capillary tube microfluidic channel which fabricated from MUG mixed with gelatin 46

3.24 A microfluidic channel fabrication from MUG mixed with gelatin with printed 30 times layer channel diagram 47
3.25 Side view of fed cultured and liquid cultured residue of *E. coli* at the inlet node of the printed 30 times layer microfluidic channel which fabricated from MUG mixed with gelatin 47
4.1 Water-soluble liquid color able to flow in four branches channel combined symmetric four branches with one single inlet and outlet 49
4.2 The liquid flowed through the 20 times printed layer channel of various width sizes of 0.25 pt to 4 pt 50
4.3 The liquid flowed results of 30 times printed layer channel of various width size of 0.25 pt to 5 pt 50
4.4 Result of flowing of liquid in T-shape pattern 51
4.5 Result of flowing of liquid in Y-shape pattern 51
4.6 Result of flowing of liquid in Y-shape with double wave pattern 51
4.7 Result of flowing of liquid in branch with curve line pattern 52
4.8 Result of flowing of liquid in wave line pattern with (a) Top view; (b) Side view 52
4.9 Edge of gelatin channel under a microscope 40x 53
4.10 The middle region of channel under a microscope 40x 53
4.11 Top view of laser printer ink pigment printed on the transparent plastic sheet used as the gelatin microfluidic template 54
4.12 Side view of laser printer ink on plastic sheet under a microscope 40x 54
4.13 The display of the program of Optika B-292 microscope equipped with a digital camera (Opticam B1) measured the size of laser printer ink 55
4.14 The relation of measured width (mm) of the channel and the number of printed layers 56
4.15 The relation of the thickness of ink printed on the plastic sheet of line points 1 to 5 pt and the number of printed layers 56
4.16 The UV light source exposed to (a) UV cut lens; and (b) Gelatin 57
4.17 The relation of measured transmittance of gelatin and wavelength 58
4.18 Liquid color diffused for 30 minutes (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope 59

4.19 Liquid color diffused for 1.0 hours (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope 59
4.20 Liquid color diffused for 1.5 hours (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope 60
4.21 Liquid color diffused for 2.0 hours (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope 60
4.22 The relation of measured diffusion distance (mm) of liquid color diffused through gelatin far apart from the edge of the channel 61
4.23 (a) 4MU solution in microcentrifuge tube; (b) Fluorescence of 4MU product of enzymatic reaction of GUD and MUG 62
4.24 (a) Fluorescence of 4MU solution in capillary tube; (b) Fluorescence of 4MU solution in microfluidic channel 63
4.25 Fluorescence from the reaction of enzyme GUD and substrate MUG in microfluidic channel 64
4.26 Fluorescence of mixed enzyme GUD and substrate MUG solution fed in microfluidic channel 65
4.27 GUD and MUG in Y-Shape channel pattern 66
4.28 (a) Fluorescence in 30 minutes; (b) Fluorescence in 1.0 hours; (c) Fluorescence in 1.5 hours; (d) Fluorescence in 2.0 hours 67
4.29 *E. coli* culture from streak plate; (a) Without MUG added to nutrient agar; (b) MUG with nutrient agar 68
4.30 *E. coli* culture from spread plate; (a) Without MUG added to nutrient agar; (b) MUG with nutrient agar 68
4.31 Cultured *E. coli* in capillary channel (a) Fluorescence in 2 hours; (b) Fluorescence in 3 hours; (c) Fluorescence in 4 hours; (d) Fluorescence in 5 hours 70
4.32 Liquid cultured residue of *E. coli* in capillary channel (a) Fluorescence in 2 hours; (b) Fluorescence in 3 hours; (c) Fluorescence in 4 hours; (d) Fluorescence in 5 hours 70
4.33 Capillary channel at 3 hours (a) Distilled water; (b) 1700U enzyme GUD; (c) Cultured *E. coli*; (d) Liquid cultured residue 72
4.34 Printed 30 times layer channel at 3 hours (a) Distilled water; (b) 1700U enzyme GUD; (c) Cultured *E. coli*; (d) Liquid cultured residue 72

4.35 Cultured *E. coli* in printed 30 times layer channel 73
4.36 Liquid cultured residue in 30 times printed layer channel 73

LIST OF SYMBOLS / ABBREVIATIONS

Symbols/Abbreviations	**Terms**
PDMS	Polydimethylsiloxane
4MU	4-methylumbelliferone
GUD	β-D-glucuronidase
MUG	4-methylumbelliferyl-β-D-glucuronide
E. coli	*Escherichia coli*
EHC	*Enterohemorrhagic Escherichia coli*
ETEC	*Enterotoxigenic Escherichia coli*
EIEC	*Enteroinvasive Escherichia coli*
EPEC	*Enteropathogenic Escherichia coli*
EAEC	*Enteroaggregative Escherichia coli*
DAEC	*Diffusely adherent Escherichia coli*
UV	Ultraviolet
dpi	Dots-per-inch
CFUs	Colony-forming units

CHAPTER 1
INTRODUCTION

1.1 Background and Significance of the research

Nowadays, the number of patients is increasing and the occurrence of epidemics. In particular, new epidemics such as the Coronavirus disease (COVID-19) is the cause of a large demand for diagnostic equipment based on the biosensor. Which made the researchers realize the importance of developing microfluidics technology for various medical applications, such as microlitre-based measurement of small quantities of substances, the microbe flow test can be used as an experimental model for the study of intravenous blood flow. Microfluidics is a small measuring device for manipulating small volumes of fluids or substances to be measured or a substance used for measuring that are relevant to sensing. Thus, making it fast to measure for disease diagnosis and reduce the cost of the substances used in the measurement. It can also be developed as a portable measuring device for diagnosis due to its small size and lightweight.

Microfluidics is the science of manipulating and controlling fluids which is a technology of fluid manipulation in channels on the micro- and nanometer scale. Academically, it is a subdiscipline of fluid mechanics. This is obtained by applying knowledge from the fluid motion equation of the Navier-Stokes equations that were used in design and fabrication. Wherewith the size of the channel is very small in micron-scale, causes the properties of laminar flow or capillary forces to affect the fluid flow because of the weight of the fluid as the fluid mass has little effect. At present, the microfluidics generation process uses a wide variety of substrate materials (silicon, glass, ceramics, polymers such as PDMS for PolyDimethylSiloxane), all of which pollute the environment. The process of making microfluidics is very complex and the production process has a high price. There are several methods and materials have been applied for microfluidics fabrication. The most common technique for microfluidics fabrication is Photolithography employing Polydimethylsiloxane (PDMS) material. This common technique requires several sophisticated steps, including 1) using a photoresist deposit on a silicon or glass. 2) expose the substrate with ultraviolet (UV) light through the photomask, which has the channel pattern for making the master mold.

3) pour PDMS on this master mold and incubate in the oven. 4) drilling the hole on PDMS for making node. And etching adapted from the miniature circuit design and development industry. The fabrication procedure of microfluidics from PDMS requires specific equipment. Moreover, the PDMS or other usual materials are disposable. Therefore, the microfluidics for a medical device is one of the medical wastes. The Globe Newswire, 2019 reported the global medical waste management market was valued at USD 11.77 billion in 2018 and is expected to reach USD 17.89 billion by the year 2026. This means the medical waste is increasing every day due to the increasing number of patients. Therefore, microfluidics fabrication from green materials is an attractive alternative to reduce medical wastes. In this research, the gelatin was selected to fabricate the microfluidics because it is a natural protein that is abundant and has the property of gel strength. It is feasible for melting and setting, and biodegradable.

1.2 Objectives

1. To fabricate microfluidic from gelatin.
2. To fabricate the gelatin microfluidic by using the standard laser printer creates the channel patterns printed as the template mold.
3. To investigate the optimal channel size and patterns for flowing the fluid in gelatin microfluidic.
4. To apply the gelatin microfluidic with the rapid of *E. coli* detection under the principle of catalytic reaction of enzyme.

1.3 Research hypothesis

1. Gelatin can be used as material for microfluidic fabrication.
2. Printed line pattern from standard laser printer can be used as microfluidic channel mold.
3. Gelatin microfluidic can be used as a device for *E. coli* detection.

1.4 Research scope

This research applied engineering and biochemistry knowledge for the fabricated microfluidic channel from gelatin by laser printer which is a novel method for fabrication microfluidic channel. The gelatin material is used because it is a green material, low cost, and reusable. This gelatin microfluidic can applied to many applications such as determine *E. coli* as the indicator of bacterial contamination evaluation of the sample.

1.5 Research plan

Table 1.1 Show research plan since August 2020 to April 2021

Operation plan	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb	Mar.	Apr.
Study about microfluidic, fluid mechanic, and gelatin.									
Find resolution of printer to fabricate gelatin channel.									
Flowing test under gelatin channel.									
Flowing test with more complex channel pattern.									
Measure channel size									
Investigated characteristics of gelatin channel.									
Fluorescent reaction between GUD and MUG, and 4MU test in laboratory.									
Test fluorescent reaction under gelatin channel.									
Examination enzymatic reaction from *E. coli*									
Examination enzymatic reaction from *E. coli* under gelatin channel.									

CHAPTER 2

RELATED THEORIES

2.1 Microfluidic chip

2.1.1 Fundamental of microfluidic chip

Microfluidics is the science of manipulating and controlling fluids which is a technology of fluid in micro and nanoscale in channels. Microfluidics involves the manipulation of very small volumes of fluids within the tens to hundreds of micrometers channels. Microfluidic channels pattern was fabricated by molded or engraved into a material such as glass, silicon, or polymer (PDMS - PolyDimethylSiloxane). The microchannels incorporated into the microfluidic are connected to the outside by inputs and outputs pierced through the chip. The solution is injected into and removed from the microfluidic chip (Figure 2.1). Microfluidics can devise into 2 systems which are external active systems (through the tubing, syringe adapters, or even simple holes in the chip) and passive systems (through hydrostatic pressure). Fluids are directed, mixed, separated, or manipulated to attain multiplexing, automation, and high-throughput systems. The channel pattern design must be precise to achieve the desired features such as lab-on-a-chip, detection of pathogens, electrophoresis, DNA analysis, etc. [1, 2, 4, 6].

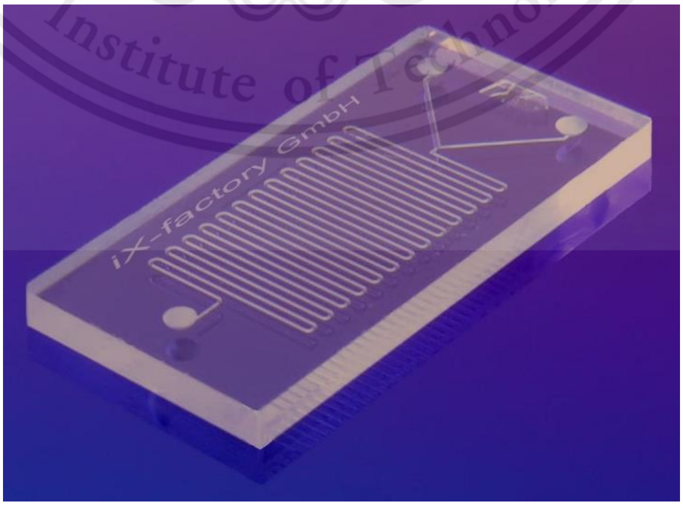


Figure 2.1 Microfluidic chip [3]

The process of fabricated microfluidic chip was shown in Figure 2.2. The fabrication of a microfluidic chip starts with the design of microfluidic channels with design software such as AUTOCAD, Illustrator, LEDIT, and etc. Transferred on a photomask (chrome coated glass plates or plastic films for the most common templates) following the design is made. The microfluidic channel is printed with UV opaque ink (if the substrate is a plastic film) or etched in chromium (if the substrate is a glass plate). Photolithography which a simple technique, usually developed for microfluidic mold. Photolithography is the process that corresponds to the transfer of microchannels patterns from the photomask into real microchannels on a mold. The wide materials used for fabricated the photolithographic production of microfluidic chip was polymer, Polydimethylsiloxane (PDMS) [4, 5].

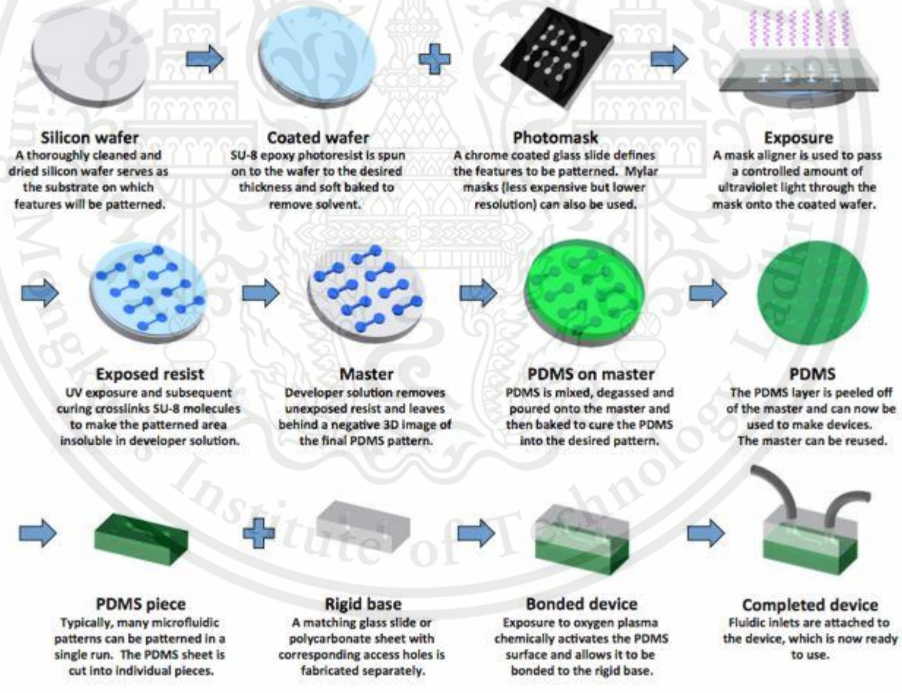


Figure 2.2 Schematic of Photolithography process for fabricating microfluidic device [7]

The fabrication process of photolithography and etching is sophisticated, expensive, and inflexible. Most researchers use a Soft lithography method instead of photolithography later. Soft lithography is a various set of techniques that use a soft elastomer material (PDMS) to transfer patterns to a substrate material. The process consists of building elastomeric microchannels as shown in Figure 2.3. The specific program was used for designed these microchannels and printed onto a high-resolution transparency mask or remodeled them into a conventional chrome mask to produce a master which will serve as a mold for the soft material. Soft lithography has a variety of advantages over other forms of lithography, which can find its low cost and easy fabrication process; its rapidity, its tolerance over a wide variety of materials. In addition, soft lithography allows to the creation of precisely delimited and manageable surface chemistries [8].

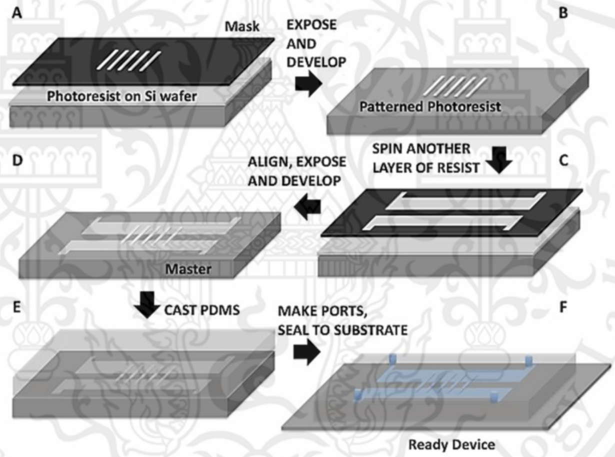


Figure 2.3 Schematic of Soft-lithography process for fabricating microfluidic device [9]

Microfluidic technology has found many applications. In the biomedical field, with laboratories on a chip because it can the integration of many medical tests on a single chip. In cell biology research, because microchannels have the same characteristic size as biological cells. Thus, microfluidic chips allow easy manipulations of single cells and rapid drug changes. In protein crystallization, because microfluidic devices allow the generation on a single chip of a large number of crystallization conditions. And also many other areas such as drug screening, glucose tests, chemical microreactor, electrochemistry, microprocessor cooling, or micro fuel cells [4].

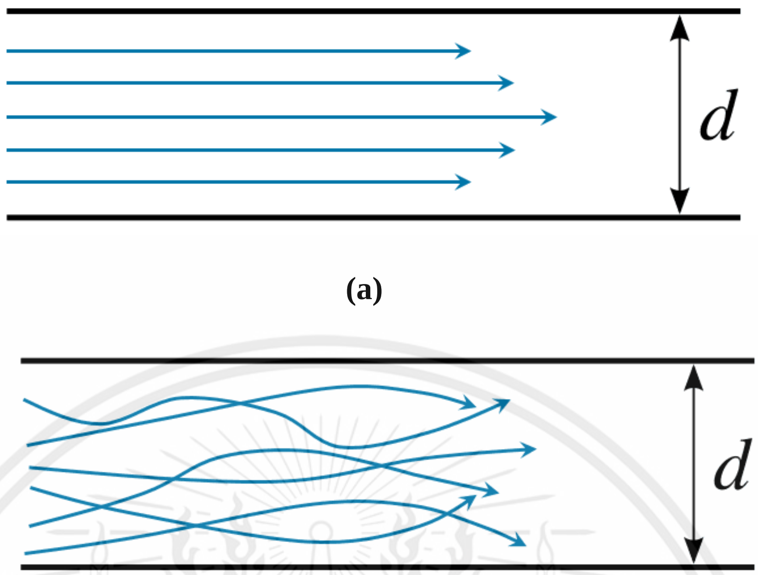
2.2 Microfluidic dynamics

2.2.1 Basic information of microfluidic dynamics

The physics of microfluidics is associated with fluid dynamics in which consider fluid that flow under micrometer scales channel. The fluid flows can be divided into 2 different types which are laminar flows and turbulent flows. Laminar flow occurs when the fluid flows in infinitesimal parallel layers with no disruption between them. In laminar flow, fluids layers slide in parallel with no swirls or currents normal to the flow itself. (Figure 2.4 (a)) The laminar flow is also called streamline flow because it is characterized by non-crossing streamlines. Laminar flow normally occurs under small pipes and low flow velocities [10].

The other type is Turbulent flow. The fluid flows with turbulent in which the fluid undergoes irregular fluctuations, or mixing, in contrast to laminar flow, in which the fluid moves in smooth paths or layers. The speed of fluid under turbulent flow is continuously changed in both magnitude and direction, for example, the flow of wind and river as in Figure 2.4 (b). The air or water swirls and eddies while its overall bulk moves along a specific direction. The general examples are blood flow, and oil transport in pipelines. Turbulent flow also generally occurs at high flow rates with large pipes [10, 22, 23].

Therefore, the fundamental laws of fluid mechanics are concerning microfluidic as well. However, due to the effects like fluid turbulence, gravity can be neglected, the equations describing microfluidic systems are typically considerably simplified versions of the equations of fluid physics. This makes microfluidic an attractive discipline for the study of the properties and dynamics of fluid flow wherever effects, such as diffusion, can be studied at very high resolution [10, 22, 23].



(a)

(b)

Figure 2.4 Schematic of fuid flows (a) Laminar flow in a closed pipe; (b) Turbulent flow in a closed pipe [11]

2.2.2 The Narvier-stokes equations and Reynolds number in microfluidic devices

The motion of fluid under small volumes and space can be using the Navier-Stokes Equations, which are the equations for fluid flow. It can be derived from the principle of Mass, Momentum, and Energy and simplified by using assumptions to specific problems [11, 12].

The velocity field of an incompressible fluid can be derived from the Navier-Stokes equations 2.1 and 2.2

$$\frac{\partial u}{\partial t} + (u \cdot \nabla)u = -\frac{1}{\rho}\nabla p + \varepsilon v \nabla^2 + f \qquad (2.1)$$

$$\nabla \cdot u = 0 \qquad (2.2)$$

where $u[m \cdot s^{-1}]$ is the fluid velocity field

$\rho[kg \cdot m^{-3}]$ is density

$p[Pa]$ is the pressure field

$v[m^2 \cdot s^{-1}]$ is the pressure field

$f[m \cdot s^{-1}]$ is an external acceleration field (due to gravity or electrostatic forces for example)

These equations physically describe the space-time evolution of the velocity field of a given fluid in a given domain. They are respectively obtained from simplified laws of conservation of momentum and mass, with the assumption that the fluid is incompressible (density is constant over space and time) and Newtonian (viscosity is uniform). We set the non-dimensional parameters $\mathbf{\nabla}^* = \mathrm{L}\mathbf{\nabla}$, $\mathbf{u}^* = \mathbf{u}/\mathrm{U}$, $\mathrm{t}^* = \mathrm{t}/(\frac{\mathrm{L}}{\mathrm{U}})$, $\mathrm{p}^* = \mathrm{pL}/(\rho \nu U)$ and $f^* = f/f_0$. If L is the characteristic length of the geometry (also called hydraulic diameter in a microchannel), U the typical velocity of the fluid, and f0 the typical intensity of the acceleration field, each of these non-dimensional parameters are of the same order of magnitude.

Furthermore, Equation 2.3 can be written in the following non-dimensional form 2.4

$$Re(\frac{\partial u^*}{\partial t^*} + (u^* \cdot \nabla^*) = -\nabla^* \mathrm{p}^* + \nabla^{*2} \mathrm{u}^* + \frac{f_0 L}{U^2} \mathrm{f}^* \tag{2.3}$$

with

$$Re = \frac{UL}{v} \tag{2.4}$$

Which Re is called the Reynolds number. This non-dimensional number gives the ratio between inertial and viscous forces. Scaling down in dimension and/or flow velocity tends to decrease this number. It is a powerful indicator of the flow regime. Reynolds' principle, which states that the pressure drop of a flowing fluid is proportional to the flow rate at low velocities. The Reynolds number (Re) is a dimensionless number expressing the ratio between inertial and viscous forces. Therefore, the physical characteristics of the fluid flow in microfluidic channels are the Reynolds number which use to calculate which size of the channel can make a solution flow. From this, we can apply with a capillary force to make blood flow by itself.

The Reynolds number defined as equation 2.5

$$Re = \frac{\rho L v_{avg}}{\mu} \tag{2.5}$$

where ρ is the density of the fluid

L is the characteristic length (or hydraulic diameter)

μ is the dynamic viscosity of the fluid

v is the kinematic viscosity of the fluid.

When Re is close to 1, it is viscous forces dominate and the flow is well-behaved. When Re is very large (ie. greater than 2000), inertial forces begin to have a considerable effect on the profile of the flow and can result in turbulence. In microfluidics, Re is usually less than 100, and often less than 1.0.

If Re is in the range 0 - 2300, the flow condition is laminar flow.

Re is in the range 2301 - 4000, the flow condition is transition region.

Re is more than 4000, the flow condition is turbulent flow.

2.3 Other equipment

2.3.1 Light microscope

A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects and enlarging them [13].

Which all the components are as follows (Figure 2.5)

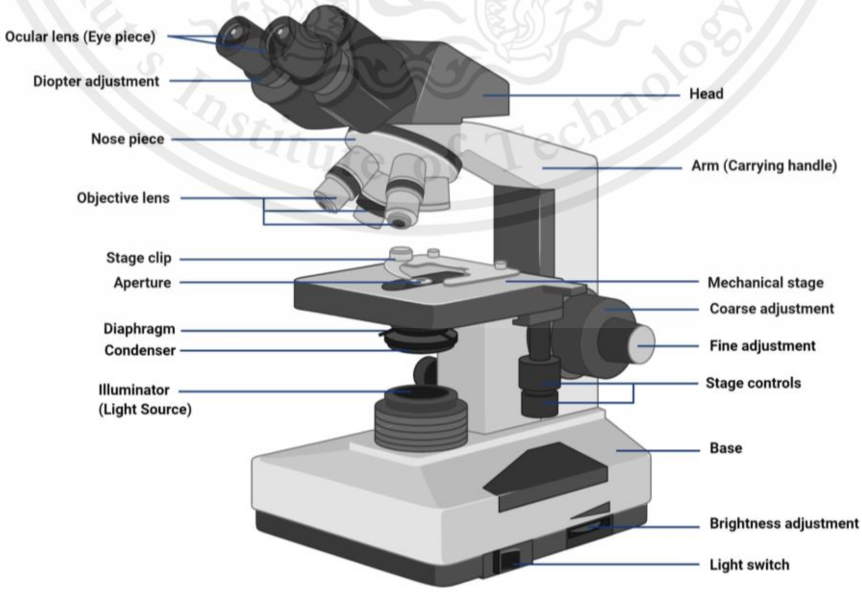


Figure 2.5 Parts of microscope [13]

1. Eyepiece: The lens the viewer appearance through to visualize the specimen. The eyepiece usually used a 10X or 15X power lens.

2. Body tube (Head): Connects the eyepiece with the objective lenses.

3. Arm: Connects the base with the body of the microscope.

4. Coarse adjustment: Adjust the general focus.

5. Fine adjustment: Adjust the fine focus to increase the detail of the specimen.

6. Objective lens: One of the most important parts of a microscope, as the lenses closest to the specimen.

7. A standard microscope has 3, 4, or 5 objective lenses with a range of power from 4X to 100X. The slice, lens, and destroy the specimen could break unless beware the objective lens and touch to the glass slide

8. Specimen or slide: The specimen is the object being examined. Most specimens are placed on glass slides which are thin and flat rectangles.

9. Stage: The flat platform use for placing the glass slide.

10. Stage clips: Metal clips that hold the glass slide.

11. Stage height adjustment (Stage Control): Use the knobs for controlling the stage left and right or up and down.

12. On/off switch: This switch on the base of the microscope control the illuminator off and on.

13. Illumination: The light source for a microscope that uses a low-voltage bulb located at the bottom of the stage.

14 Iris diaphragm: Adjusts the amount of light that reaches the specimen.

15. Condenser: Gathers and focuses light from the illuminator onto the specimen being viewed.

16. Base: Use for supports the microscope and it is where the illuminator is located.

2.3.2 Vernier caliper

A vernier caliper is a device used to measure the dimensions which measure the internal and the external diameters of an object. The components and instruction are as follows; (Figure 2.6) [14].

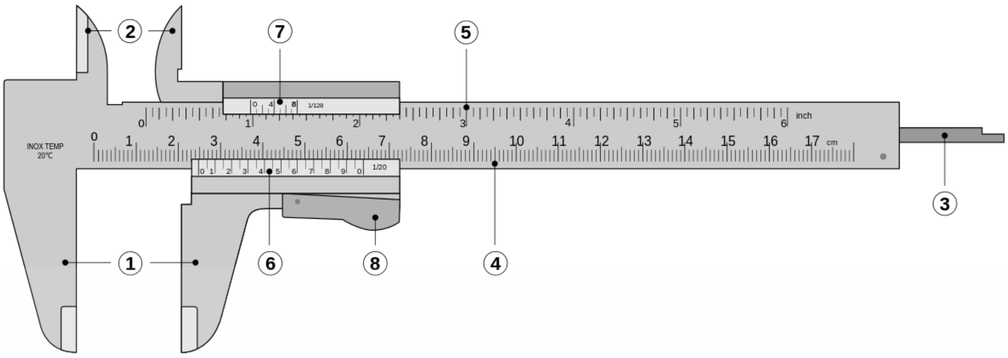


Figure 2.6 Diagram of Vernier caliper [14]

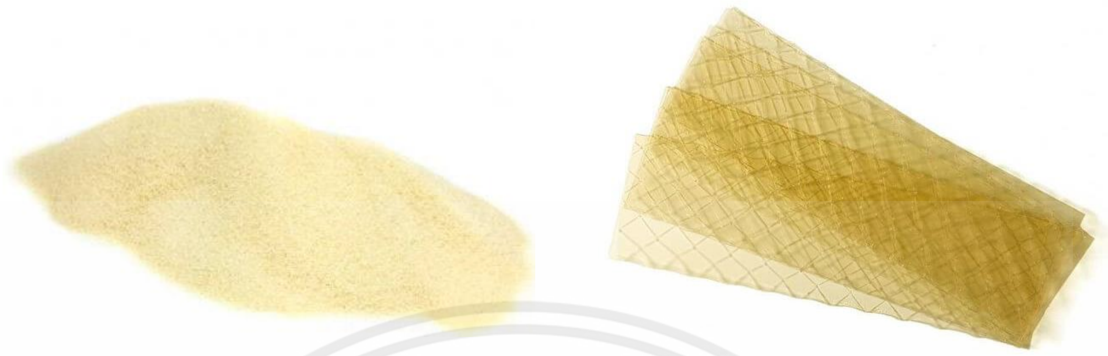
The labeled parts of Vernier caliper are

1. Outside large jaws: used to measure the external diameter or width of an object.
2. Inside small jaws: used to measure the internal diameter of an object.
3. Depth probe/rod: used to measure depths of an object or a hole.
4. Main scale (Metric): scale marked every mm.
5. Main scale (Imperial): scale marked in inches and fractions.
6. Vernier scale (Metric) gives interpolated measurements to 0.1 mm or better.
7. Vernier scale (Imperial) gives interpolated measurements in fractions of an inch.
8. Retainer: used to block movable parts to allow the easy transferring of a measurement.

2.4 Gelatin

2.4.1 Basic information of gelatin

Gelatin is a protein obtained by boiling skin, tendons, ligaments, and/or bones with water. It is brittle when dry and sticky when moist. Gelatin is commonly used as a gelling agent in food, medications, drug and vitamin capsules, photographic films and papers, and cosmetics. Normally, gelatin has 2 forms as powder and sheet (Figure 2.7) [15].



(a) (b)

Figure 2.7 (a) Gelatin powder [16] **; (b) Gelatin sheet** [17]

2.4.2 Properties of gelatin

Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals such as domesticated cattle, chicken, pigs, and fish. During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Its chemical composition is, in many aspects, closely similar to that of its parent collagen [18].

Gelatin is nearly tasteless, odorless, and colorless or slightly yellow in appearance. Most important are its gel strength, setting and melting temperatures, and its viscosity. Gelatin is able to form clear transparent solutions, which gel when cooled and melt again when warmed. It increases viscosity, forms films on surfaces, is able to absorb large quantities of water and functions as a buffer [18].

2.5 Laser printer

2.5.1 Basic information of laser printer

A laser printer is a printer that uses a focused beam of light to print text and images onto paper. The laser beam fires at the surface of a cylindrical drum called a photoreceptor. This drum has an electrical charge typically positive, which is reversed in areas where the laser beam hits it. The laser beam prints the pattern such as text and pictures onto the photoreceptor by reversing the charge in the specific area of the drum. Then coated with toner from a toner cartridge. The positively charged toner clings to areas of the drum that have been negatively charged by the laser. The drum will be given a strong negative charge while the paper was passed through the printer which allows the toner to transfer and stick to the paper. The result is a clean pattern, text and/or picture, written on the paper [19].

Figure 2.8 Laser printer CANON LBP-6030 [20]

2.5.2 Laser printer features

Laser printer resolution: The standard resolution in most laser printers is 600 dots-per-inch (dpi). A high-end production printer should have a resolution of 2400 dpi [21].

2.6 Diffusion

Diffusion is a physical process which concerns the net movement of molecules from a high concentration to a lower concentration region. The materials that could be diffused are solid, liquid, or gas. One of the main characteristics of diffusion is the movement of molecules along the concentration gradient. On the other hand, facilitated diffusion by other molecules does not directly involve high-energy molecules such as adenosine triphosphate (ATP) or guanosine triphosphate (GTP). The rate of diffusion depends on the nature of the interaction between the medium and material [24].

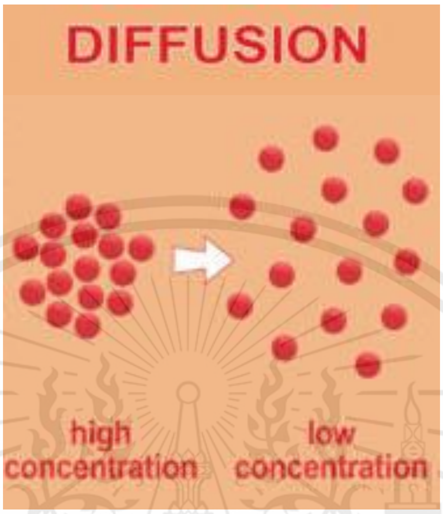


Figure 2.9 Diffusion diagram [25]

2.6.1 Factors that affect diffusion

1) Temperature

In diffusion, molecules are moving with a specific amount of kinetic energy in any system. This does usually not occur directly in characteristic and can appear random. The direction of movement changes as well as the changes of momentum and velocity when the molecules collide with one another. With increase in temperature, the kinetic energy of all particles in the system increases [24].

2) Area of interaction

The area of interaction is affected when two liquid reactants are mixed. The area of interaction can be increased when stirring two chemicals and allows these molecules to diffuse towards each other more quickly. Furthermore, any solute that is broken down into small pieces, which means the area of interaction increases, and stirred into the solvent, the molecules are diffusing better [24].

3) Steepness of the concentration gradient

Since diffusion is powered primarily by the probability of molecules moving away from a region of higher saturation, it immediately follows that when the medium (or solvent) has a very low concentration of the solute, the probability of a molecule diffusing away from the central area is higher [24].

4) Particle size

The diffusion of a small particle will be more rapid than a larger-zed molecule at any temperature. The mass of molecule and surface area are related too. A heavier and larger surface area molecule will diffuse slowly while smaller and lighter will diffuse more quickly [24].

2.7 Light

Light or visible light is one kind of electromagnetic radiation within the portion of the electromagnetic spectrum that can be seen by the human eye. Visible light usually has a wavelength within the range of 400-700 nm which between the infrared (longer wavelength) and the ultraviolet (shorter wavelength. In physics, sometimes the term 'light' refers to electromagnetic radiation of any wavelength, whether visible or not and also gamma rays, X-rays, microwave, and radio waves are light [26, 27].

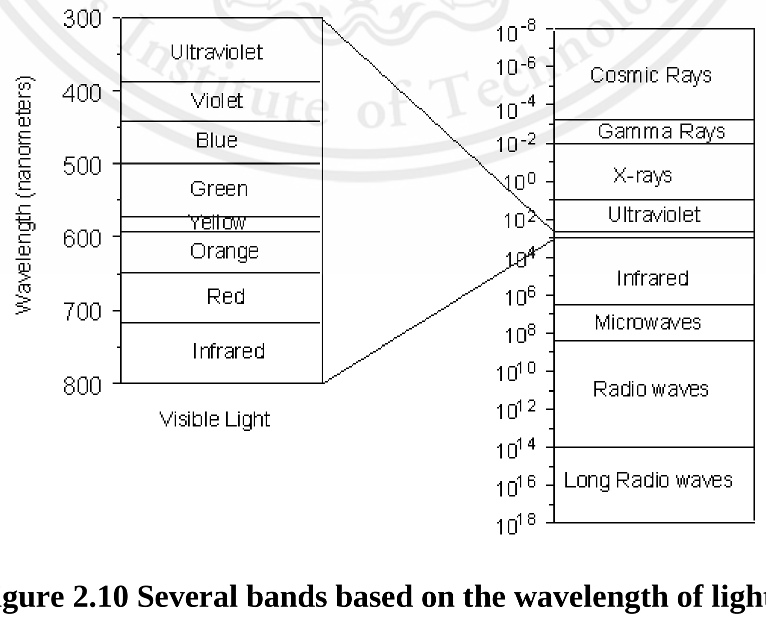


Figure 2.10 Several bands based on the wavelength of light [27]

2.8 Ultraviolet light

Ultraviolet (UV) is a form of electromagnetic radiation. The ultraviolet region covers the wavelength between 10 nm to 400 nm which is X-ray to visible light. The Sun is a natural source of the full spectrum of ultraviolet radiation. It can be produced by artificial sources, such as electric arcs and specialized lights. UV radiation is considered ionizing radiation because its photons have higher energies to ionize atoms. The resulting affects the chemical properties of the atoms and causes chemical reactions to form or break chemical bonds. This can be useful for chemical processing that widely uses to excite the fluorescence. UV radiation is commonly classified into three sub-bands which are UVA, UVB, and UVC, corresponding to the different wavelengths [28, 29].

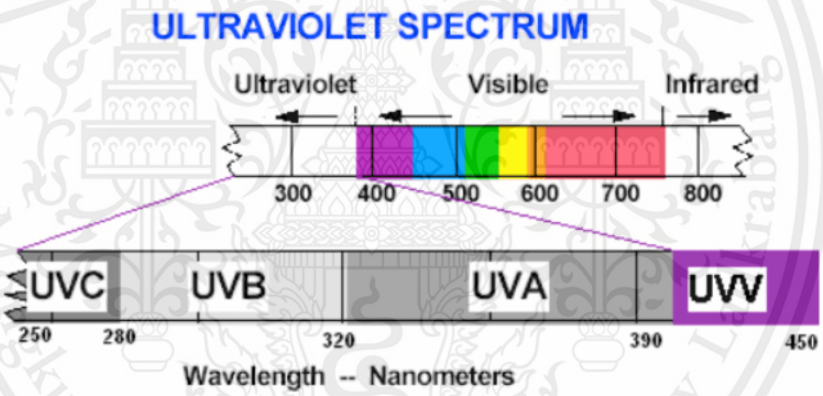


Figure 2.11 Ultraviolet spectrum [30]

UV radiation can be further classified as UVA, UVB and UVC respectively, corresponding to their different wavelengths. UV radiation reaching the earth's surface consists of UVA and UVB only, as all UVC is absorbed by the atmosphere [29, 31].

1. UVA, or near UV (315–400 nm)
2. UVB, or middle UV (280–315 nm)
3. UVC, or far UV (180–280 nm)

2.9 Fluorescence

Fluorescence is the emission of electromagnetic radiation from luminescence substances or molecules in a gas, liquid, or solid chemical system which usually emit visible light. The initial excitation of luminescence molecule is either caused by a physical absorption of light, mechanical as friction, or chemical mechanism. Fluorescence was generated through the excitation of a molecule by absorbed light (photon) at a particular wavelength from the lowest vibrational energy level of the ground state to a higher vibrational level in the excited state. At this state, molecules are unstable and release energy to the lowest energy level as vibrational relaxation of the excited state. The released energy from the excited state to the ground state as the emission of a longer wavelength photon produces the fluorescence spectrum [32].

The emission of fluorescence is emitted in different colors than the absorbed light because different colors of light correspond to different energies. Many animals, plants, and minerals also have luminescence substances or molecules that cause them to fluoresce in different colors. Mostly, absorption of ultraviolet radiation as ultraviolet or blue light is used to excite the fluorescence. Different organisms or materials absorb ultraviolet light and emit other color light such as green, yellow, orange, or red light. For this reason, they are commonly used to locate and identify organic materials and components of complex biomolecular assemblies [32].

Figure 2.12 Fluoresce light with several wavelength [33]

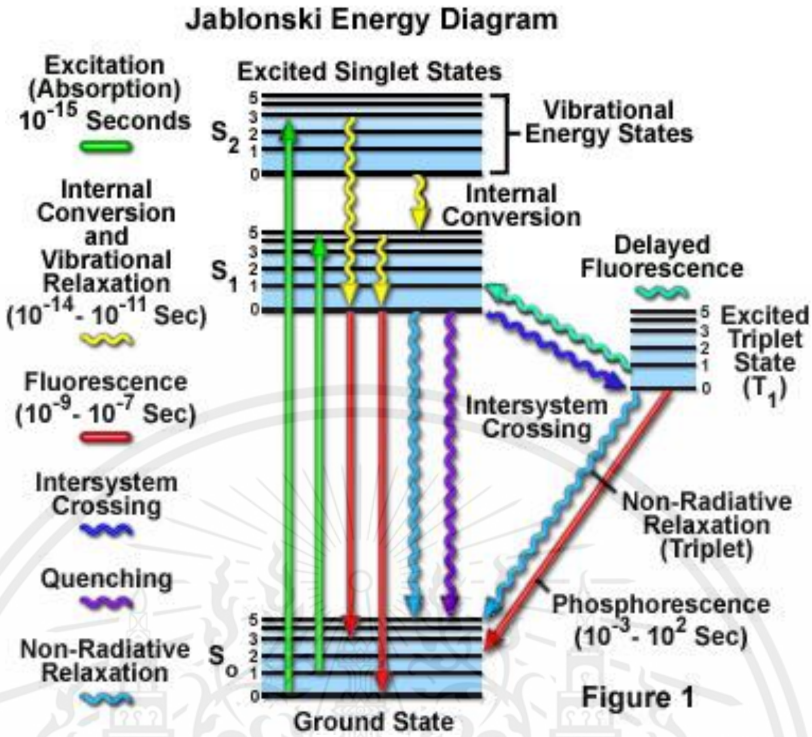


Figure 2.13 Jablonski energy diagram of absorbance and fluorescence [34]

2.10 Spectrophotometer

A spectrophotometer is an instrument that is mostly used for measuring the amount of intensity of light absorbed by a sample. Spectrophotometry is widely used for quantitative analysis in various fields and several variations of applications. It measures the concentration of a chemical substance by measuring the amount of intensity of light (photon) that is absorbed or transmitted after a light beam passes through the sample solution in a cuvette. The intensity of light detected as a function of wavelength that depends on the range of wavelength of light source, classified by electromagnetic radiation spectrum into two types of UV-visible and IR spectrophotometer. The general structure of spectrophotometers consists of two devices which are a spectrometer and a photometer. This is a scientific instrument that is used to disperse, produce, and measure light, and a photometer used to measure the intensity of light. The basic components of spectrophotometer instrumentation include a light source, a collimator (lens), a monochromator (prism or grating), a wavelength selector (slit), a cuvette for sample solution, a photoelectric detector, and a display device [35].

Figure 2.14 Spectrophotometer [36]

The mechanism of a spectrophotometer is that the lamp provides the light source. A collimator transmits the light beam (photons), it strikes the diffraction greeting, which passes through a prism and separates the light into its component wavelengths (spectrum). The grating is rotated that only a desired range of wavelength of light reaches the exit slit. Then the light passes through and interacts with the solution of a sample in a cuvette. From this point, the detector detects the number of photons to measures the transmittance and absorbance of the sample. Absorbance is a measurement of light that is absorbed by the sample. Then, the detector sends the signal of light being transmitted through the sample to a galvanometer and converts this information as a graph and then on the display device [35].

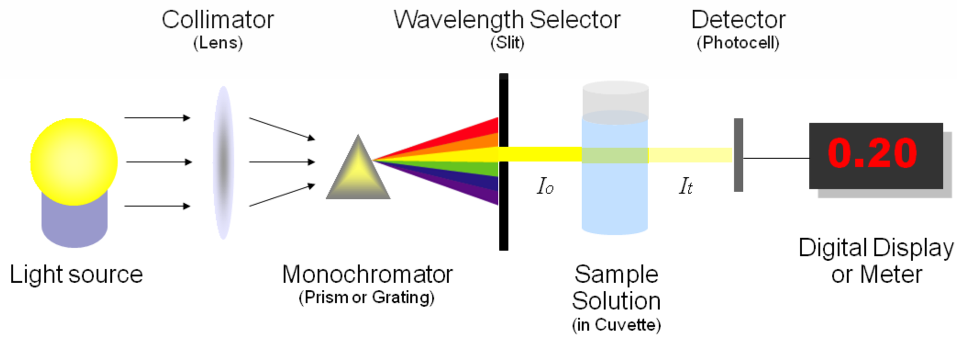


Figure 2.15 The diagram shows principle of spectrophotometer [35]

2.11 Method of Isolation of Microorganisms

Isolation of Microorganisms is a technique to separate and identify strain from a natural environment. Purification is an important and fundamental technique in microbiology, due to in nature and environments include many types of microbes that are mixed with several other forms of life. Therefore, to study the microorganism, the isolation is necessary step for purifying the interested microbial. Depending on the specific characteristic of microbial on culture medium such as the density and viability, metabolism conditions, morphology on solid agar medium and from the biochemical reaction test, the desired microorganism can be identified and isolated. There are several techniques for the isolation of microorganisms. This research specify only 2 techniques which are streak plate and spread plate techniques [37].

2.11.1 Streak plate technique

The streak plate technique is simple and rapid isolation of organisms. This technique involves the dilution of organisms which the density of organisms is decreased when it is streaked normally four quadrants on solid medium. The bacterial cells are separated into some individual cells which are diluted by streaking in third sector or fourth quadrants on an agar plate. This organism grow into a number of cell colonies. This technique uses to identify or estimate the number of the organism in colony-forming units (CFUs). The streaking was done by using an inoculation loop. The inoculation loop was sterilized by passing it through a flame. When the loop was cool, dipped it into the inoculum of the sample. The inoculation loop was then streaked over the surface of an agar plate [38].

Figure 2.16 Streak plate technique [38]

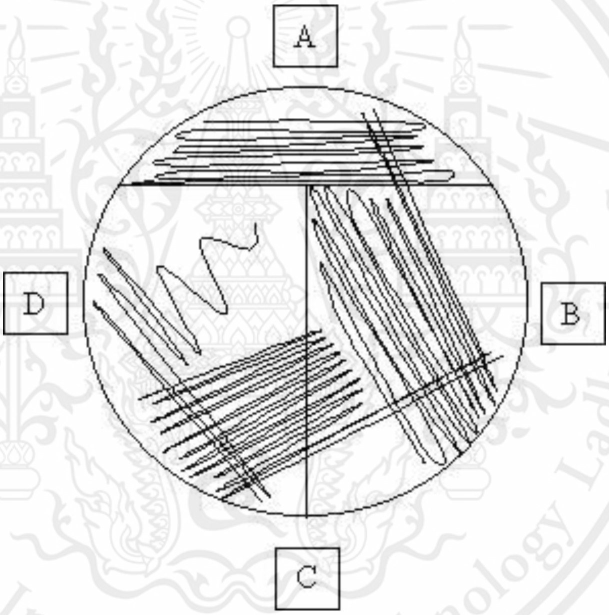


Figure 2.17 Streak plate technique [39]

2.11.2 Spread plate technique

The spread plate technique is easy to isolate and quantify microorganisms in mixed culture in a liquid sample of bacteria. This technique applies the specimen suspended in a solution over a plate which makes bacteria are diluted and evenly distributed from each other to develop the colonies. Consequently, we able to isolate the number of bacterial colonies on the plate. Spread plate technique starts with making a dilution series from the sample. Drop the appropriate desired dilution series onto the surface of an agar plate. Using the sterilized L-shaped glass spreader to spread the sample evenly over the surface of the agar [40].

Figure 2.18 Example of isolation of microorganism by spread plate technique [41]

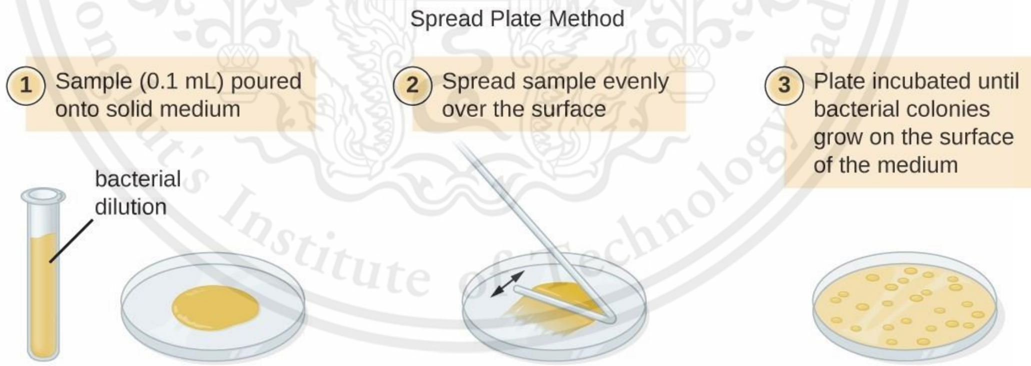


Figure 2.19 Diagram of spread plate technique [42]

2.12 *Escherichia coli* (*E. coli*)

Escherichia coli (*E. coli*) is a gram-negative bacilli and rod-shaped bacterium. These bacteria are commonly found in the intestines of people and animals. Most strains of *E. coli* are an important part of a healthy human intestinal tract and harmless to a healthy human. On the other hand, some *E. coli* strains are pathogenic that can produce strong toxins, cause various common illnesses or severe food-borne diseases, either inside and outside of the intestinal tract. For example, it can cause diarrhea, urinary tract infection, respiratory disease, bloodstream infection, and other illnesses. The types of *E. coli* that can cause illness can be transmitted to others through the consumption of contaminated food and water, such as raw or undercooked meat products, milk, and vegetables, or through contact with animals or persons [43, 51, 52].

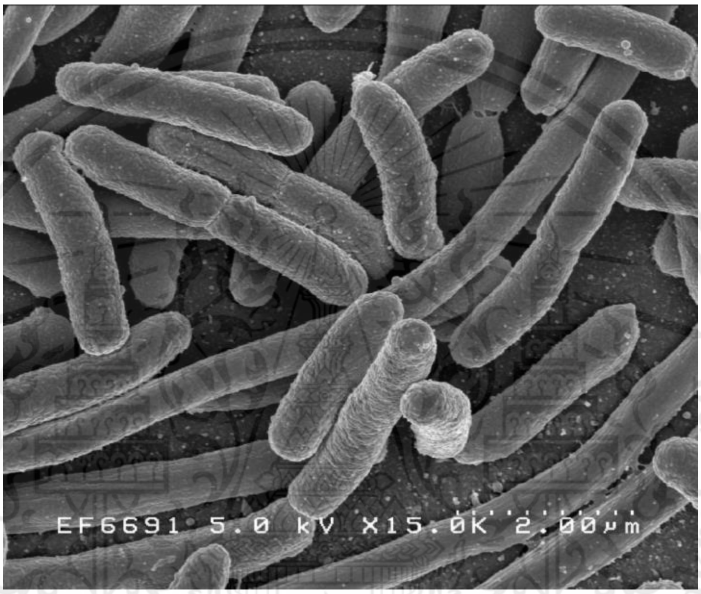


Figure 2.20 Scanning electron micrograph of *Escherchia coli* (*E. coli*) [44]

E. coli consists of various groups of bacteria. Pathogenic *E. coli* strains are categorized into six pathotypes which are: [43]

1. *Enterohemorrhagic E. coli* (EHEC) pathotype is strain O157:H7. This pathotype produces a toxin called Shiga toxin that can elicit an intense inflammatory response. The toxin causes bloody diarrhea and no fever. EHEC can cause hemolytic-uremic syndrome (HUS) and sudden kidney failure. EHEC found in various raw meats, unpasteurized milk, juice, and some vegetables [45, 50].

2. *Enterotoxigenic E. coli* (ETEC), produces enterotoxin, a heat-stable toxin (ST) and a heat-labile toxin (LT), which stimulate the lining of the intestines causing them to secrete excessive fluid, thus producing diarrhea. ETEC is transmitted to hosts by contaminated food or water from animal or human feces [46].

3. *Enteroinvasive E. coli* (EIEC), is pathogenic that is commonly found in the intestines. They do not produce toxins but use adhesin proteins instead. Adhesin proteins bind and enter through an intestinal cell leading to inflammation and ulceration of the mucosa. Furthermore, they use mechanical cell destruction to damage the intestinal wall which is a pathogenic mechanism similar to Shigella. The infected patient has the symptoms of bacillary dysentery [47].

4. *Enteropathogenic E. coli* (EPEC), are diarrheal pathogens of young children. It uses an adhesin known as intimin to bind host intestinal cells. This pathotype has virulence factors similar to Shigella. EPEC is normally found in raw meat and contaminated water [48].

5. *Enteroaggregative E. coli* (EAEC), involves the aggregation of and adherence of the bacteria to the intestinal mucosa, where they elaborate enterotoxins and cytotoxins that damage host cells and induce inflammation that results in diarrhea [49].

6. *Diffusely adherent E. coli* (DAEC), are divided into two classes, which expressing the afimbrial adhesins (Afa)/Drori antigen (Dr) adhesins in urinary tract infections. This pathotype causes infantile diarrhea that expresses an adhesin involved in diffuse adherence. These strains are involved in diarrhea in children and not involved in diarrhea in adults [53].

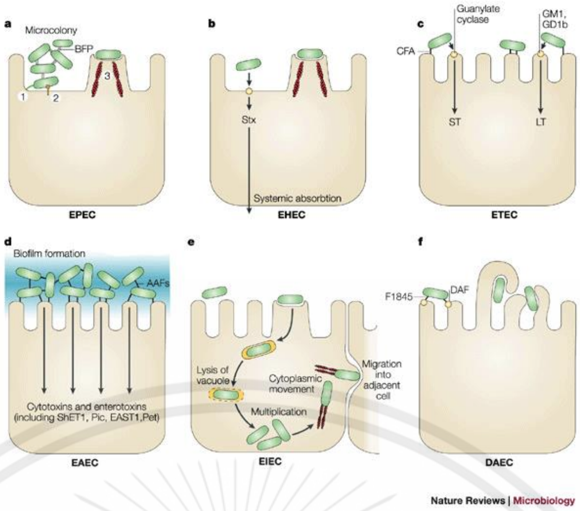


Figure 2.21 Pathogenic schema of diarrhoeagenic *E. coli* [54]

2.12.1 Contaminated of *E. coli*

2.12.1.1 Contaminated food of *E. coli*

Eating contaminated food of *E. coli* is one of the most ways to get an infection and cause a foodborne illness. Most of the *E. coli* infection from eating is found from meat products of many different animals. Unclean transformation processed of meat can cause bacterial contamination of the *E. coli* where it comes from the animal's intestines and transfers into the meat. In addition, substandard packaging and eating undercooked or raw meat can increase the risk of contamination; Fresh produce, fresh vegetables, and fruit can be contaminated by *E. coli* because *E. coli* live in water and soil that used for growing vegetables and fruit. Vegetables are particularly vulnerable to contamination of *E. coli*. Unpasteurized milk which is not heated to unpasteurized and kill bacteria also can be contaminated *E. coli* from cows' breasts or milking equipment [54].

2.12.1.2 Contaminated water of *E. coli*

Drinking is one of the most common ways to get contamination of *E. coli* since water resources can be contaminated by human and animal stool which pollute soil and water. This water is used in agriculture and causing deep contamination of *E.*

coli. Water resources in rural areas are the most likely to be contaminated so the water quality is under inspection in many areas to prevent contamination of *E. coli* [54].

2.12.2 Determination of *E. coli*

Detection and quantification of *E. coli* that are contaminated in food and water are essential to help reduce the risk of developing the severe disease. There are many methods of examination. The most standard method of examination is used a variety of culture media to isolate microorganisms. After isolation, the suspected biochemical culture must be identified using several laboratory tests. The procedure is highly sensitive. But it is laborious, and it typically takes several days for obtaining results [55].

Alternatively, several methods of detection are based on *E. coli* characteristics, such as the fluorogenic test as detecting the activity of glucuronidase. The β-D-glucuronidase (GUD) enzyme, which separates the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) for fluorogenic radical release is widely used in microbiological analysis. The β-D-glucuronidase (GUD) enzyme, produced by *E. coli* 94 - 96% strains, is classified as a biomarker capable of quantifying *E. coli*. The β-D-glucuronidase (GUD) is useful to identify the amount of *E. coli* in the sample by measuring the detectable fluorescent synthesis product of 4MU, which has the excitation wavelength at 365 nm. This examination of *E. coli* provides close results when compared with the normal method. This method can reduce the steps and complexity of examination. It is easier to perform and requires less time detection when compared to normal procedures [55].

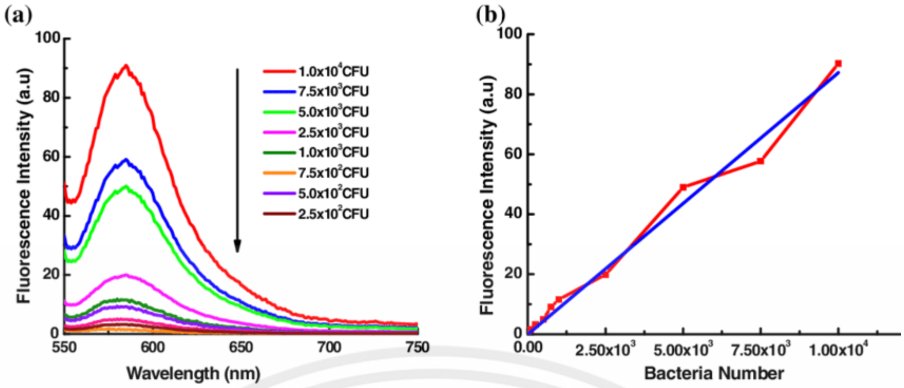


Figure 2.22 Graph of (a) The fluorescence spectra of different *E. coli* concentration samples; (b) The fluorescence intensity versus the bacterial number (red curve), fit line (blue line) [60]

From Figure 2.22, Fluorescence value derived from 4MU which is the product will vary according to the Enzyme activity of GUD. The higher the GUD content is the higher the *E. coli*. Since GUD is an enzyme secreted by *E. coli*, it is classified as a biomarker that can be used to quantify *E. coli*. Based on the results of Wildeboer et al., 2010, it was found that the enzyme activity of GUD secreted by *E. coli* was directly proportional to the *E. coli* content from the colony count of *E. coli* on the culture medium. If MUG was used directly to test *E. coli*, the fluorescence linear correlation was obtained. Similarly, it can be concluded that the 4MU solution concentration or the fluorescence intensity can indicate the amount of *E. Coli* [56].

2.12.3 Substances used for fluorescence determination of *E. coli*

β-D-glucuronidase (GUD) enzyme, produced by *E. coli* 94 - 96% strains. It is an acidic enzyme to accelerate the degradation of Glucuronides from 4-methylumbelliferyl-β-D-glucuronidase (MUG). MUG is hydrolyzed rapidly by the action of GUD enzyme to release fluorescent 4-methylumbelliferone (4MU), which shows blue fluorescence when irradiated with long-wave UV light (366 nm light). 4MU can be detected with a fluorometer. The concentration of 4MU is proportional to the fluorescence intensity. Therefore, it can be used in the quantitative detection of *E. coli* [58, 59].

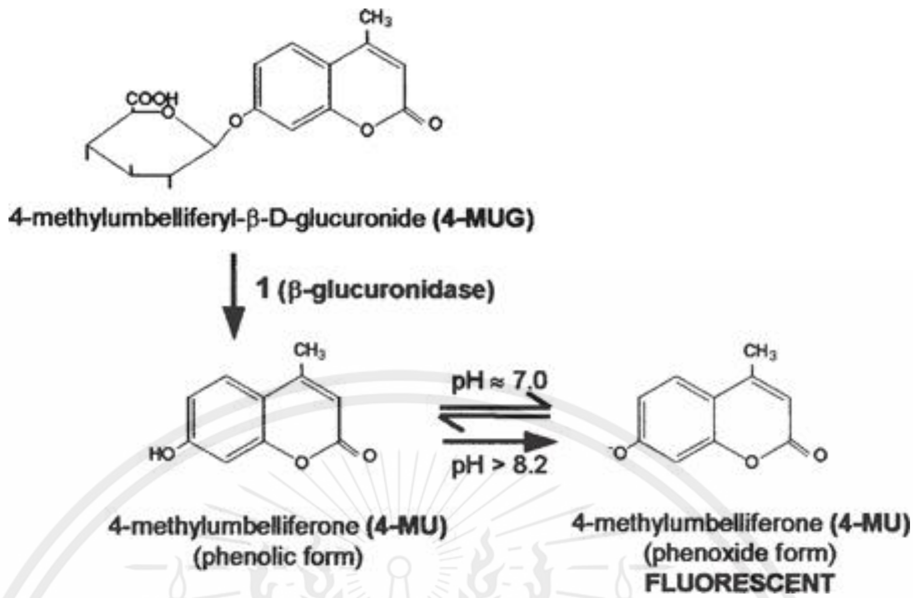


Figure 2.23 The reaction of GUD and MUG with the systhesis product, 4MU molecule [57]

CHAPTER 3

METHODOLOGY

This chapter describes the fabrication method of microfluidic from gelatin. The channel pattern was created and made by laser printer. The fluid flowing test experiments were performed for determining the optimal channel and pattern and size of channel. The application of gelatin microfluidic was performed for detecting *E. coli*, which is used as the indicator of bacterial contamination evaluation. The enzyme β-D-glucuronidase (GUD) is a biomarker for indicating *E. coli* in sample. This experiment was done by detecting the fluorescent product 4-methylumbelliferone (4MU) of enzymatic reaction of β-D-glucuronidase (GUD) and substrate 4-methyl-umbelliferone-β-D-glucuronide (MUG).

3.1 Gelatin microfluidic fabrication

3.1.1 Galatin preparation

In this project, the microfluidic was made by gelatin, the channel featured were designed and printed on transparent plastic for making template mold. The gelatin aqueous was prepared by dissolve gelatin powder in heated distilled water at gelatin: water of 1g:3ml and stirred well until gelatin powder was completely dissolved. Then pour this warmed gelatin aqueous on a channel patterns printed on template transparent plastic sheet. Let the gelatin setting by cooled it down in the refrigerator or leave at room temperature.

3.1.2 Microfluidic fabrication

The overall methodology of the gelatin microfluidic channel starts with a pattern designed on the computer software and printed out the designed pattern on a transparent plastic sheet. This printed channel patterns on transparent plastic sheet was the template for molding the microfluidic. Dissolve gelatin powder in warm water and mixed it well. Pour this warmed gelatin aqueous solution on a printed template plastic sheet. Let it cool down in a refrigerator. The final step was removed the printed template plastic sheet from gelatin and obtained gelatin microfluidic was placed on glass side and then ready to use. The process of gelatin microfluidic fabrication was shown in Figure 3.1.

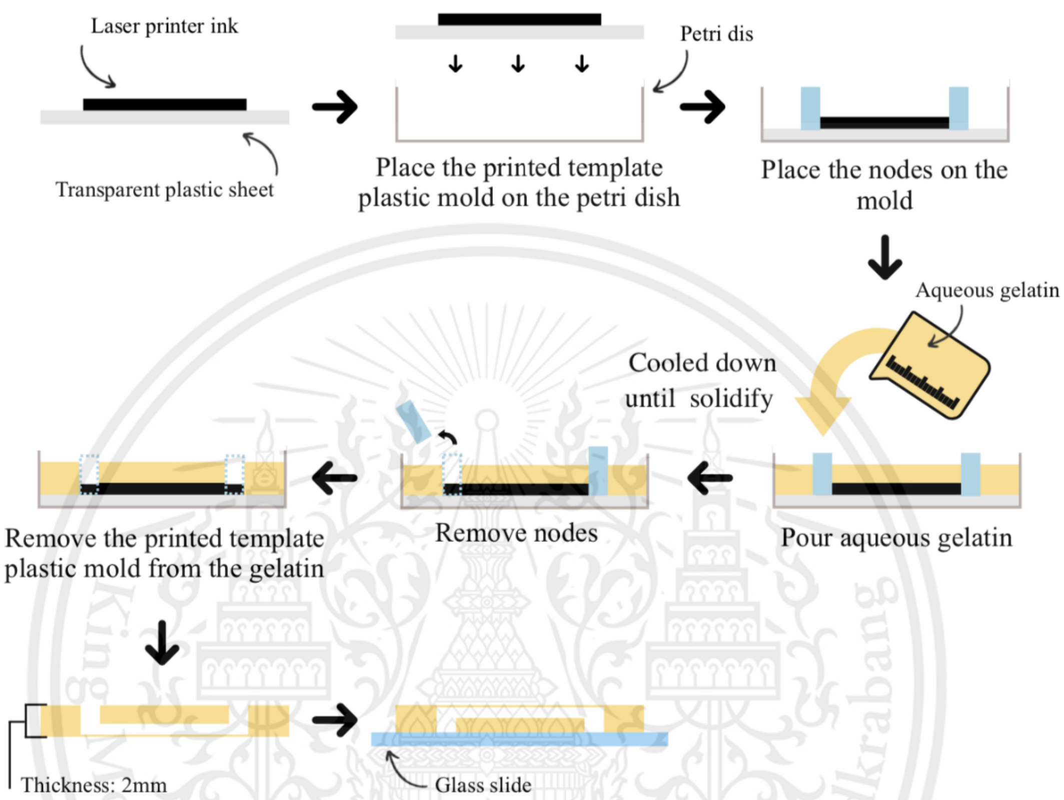


Figure 3.1 Diagram of fabrication process

3.2 Channel pattern design methodology

3.2.1 Node design

Firstly, to determine the optimal channel features, we decided to vary the size of line width for making the channel from 0.01 pt to 1 pt by which 1 pt is equal to 0.353 mm of printed one time layer as shown in Figure 3.2. The circular node as a normal microfluidic node was created at the ends of line (Figure 3.3). This designed pattern was printed out by using laser printer CANON LBP6030 on a transparency plastic sheet. Secondly, to investigate the effect of node shape, we decided to modify the circular node to the triangular node. The width of the channel was varied from 1 pt to 6 pt increased by 0.5 pt (Figure 3.4). The triangular node has a higher pressure than the circular node. From the principle of fluid dynamics, the pressure and velocity of fluid increase when the cross-sectional area of entering flowing fluid decreases.

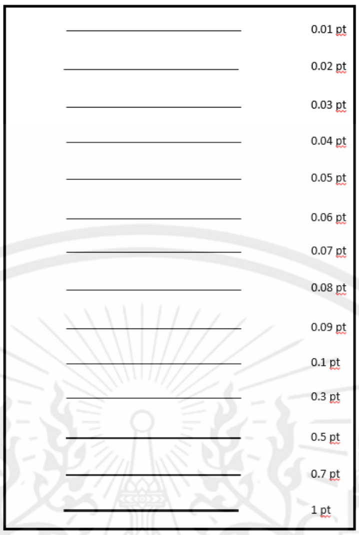


Figure 3.2 Straight line pattern with the various points of line

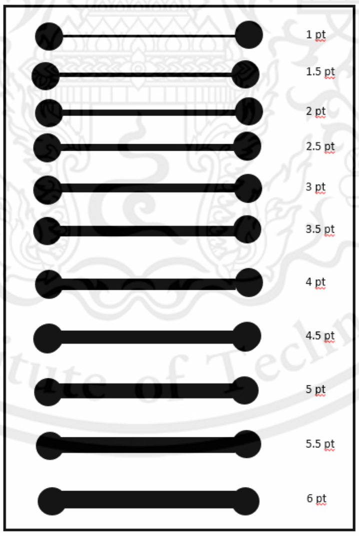


Figure 3.3 Various points of straight line with circular node pattern

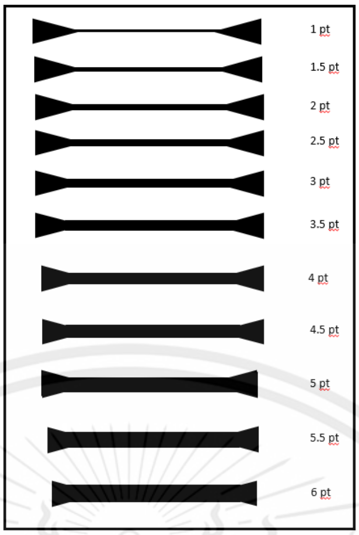


Figure 3.4 Various points of straight with triangular node pattern

Figure 3.5 The channel of various points of straight-line pattern with circular node on Gelatin

3.2.2 Channel pattern design

Several channel patterns were investigated. The straight line was designed first and then the branch channel pattern with 5 pt width and five times printed layer was designed. This branching pattern was designed in 2 types of a single inlet with four open branches outlet at the end as shown in Figure 3.6 and combined the symmetric four branches with a single outlet as shown in Figure 3.7. The first branching pattern, a single inlet with four open branches outlet, was designed to decrease pressure in the channel.

Figure 3.6 Branches channel with single inlet and open four branches outlet

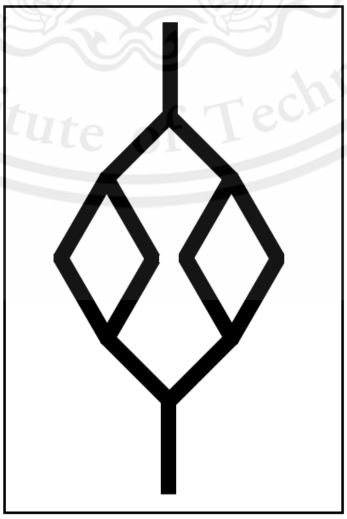


Figure 3.7 Four branches channel combine symmatric four branches with one single inlet and outlet

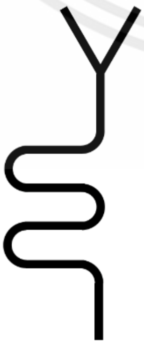
Furthermore, the other channel patterns were designed, including T-shape and Y-shape, Y with double wave pattern, zig-zag pattern, branches with curve line, and wave lines as shown in Figure 3.8, 3.9, 3.10, 3.11, 3.12, and 3.13 respectively. All of these patterns were a common widely used in microfluidic.

(a) **(b)**

Figure 3.8 (a) T-shape pattern; (b) Printed 30 times of T-shape pattern

(a) **(b)**

Figure 3.9 (a) Y-shape pattern; (b) Printed 30 times of Y-shape pattern



(a) **(b)**

Figure 3.10 (a) Y-shaped with double wave pattern; (b) Printed 30 times of Y-shaped with double wave pattern

Figure 3.11 Zig-zag pattern

(a) (b)

Figure 3.12 (a) Branch with curve line; (b) Printed 30 times of branch with curve line

(a) (b)

Figure 3.13 (a) Wave line; (b) Printed 30 times of wave line

3.2.3 Measurement of channel

The size of channel has been varied by the width and the printed times with 1 to 5 pt and 1, 5, 10, 15, 20, and 30 times printed, respectively. A vernier caliper and software equipped microscope were employed to measure the width and thickness of laser printer ink on a transparent plastic sheet. The Figure 3.14 showed the laser printer ink measured width and thickness under microscope with equipped software. When the size of channel was over to observe the entire channel under the microscope, so a vernier caliper has been used instead. The microscope used in this experiment was an

Optika B-292 with eyepiece x10 and objective lens x10 equipped with a digital camera (Opticam B1).

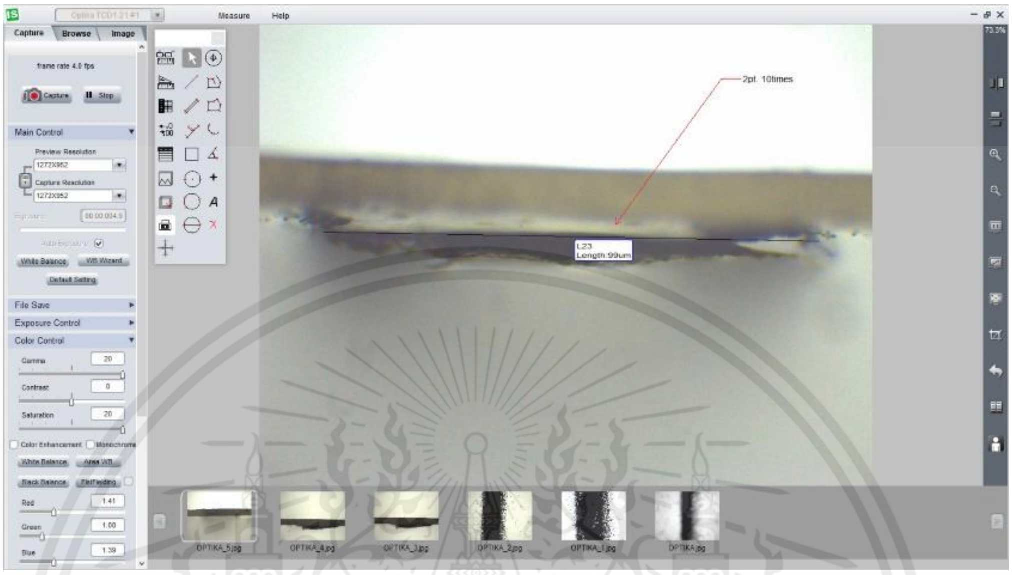


Figure 3.14 Measurement of the width and thickness of channel under microscope equipped with Opticam B1 camera program

3.3 Characteristics of gelatin microfluidic channel

3.3.1 UV cut on gelatin

The *E. coli* can be detected from fluorescent product under the Ultraviolet light source. The UV light source had to exposed on our gelatin microfluidic and it must have some background light reflect out that affected to the fluorescent result. Consequently, we investigated the gelatin UV cut property by compare the result of UV cut property between using UV cut lens and gelatin. Furthermore, the transmittance of gelatin was measured by a spectrophotometer with Metaspec program and ran at the wavelength of 200 – 700 nm. The gelatin in 2 mm thickness was placed in cuvettes slot. In the spectrophotometer, the light source exposes through the gelatin with varies wavelengths. The result of transmittance against the wavelength was graphically plotted and shown on display.

3.3.2 Diffusion of liquid in gelatin

We investigated the diffusion property of liquid in gelatin by fed liquid color solution through the channel as shown in Figure 3.15 and left for 30 minutes to 2 hours. Then observe the diffusion of liquid color in gelatin under microscope.

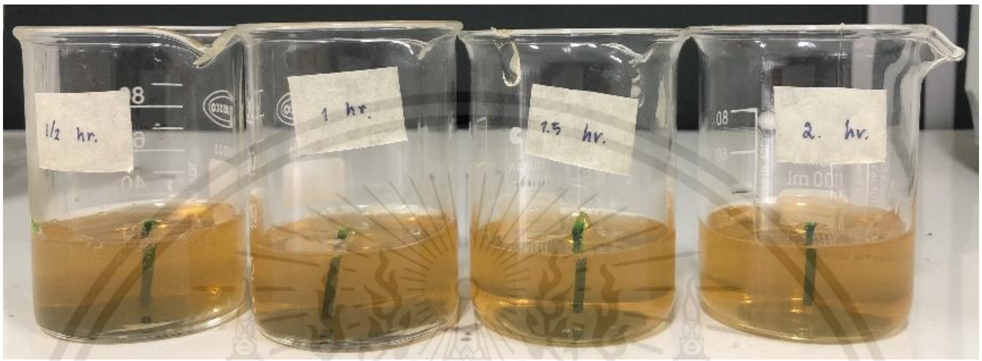


Figure 3.15 Experiment set for investigating the diffusion characteristic of gelatin

3.4 Reaction of enzyme GUD and substrate MUG, and product 4MU test

This experiment studied enzyme interactions between β-D-glucuronidase (GUD) which is secreted by *E. coli* as a biomarker, and a 4-methylumbelliferyl-β-D-glucuronide (MUG) was a substrate. The hydrolysis of MUG substrate produces 4-methylumbelliferone (4MU), which has an excitation wavelength at 365 nm with a fluorescence emission wavelength at 445 nm. The measured fluorescence intensity of 4MU would be proportional directly to the amount of GUD, which indicates the amount of *E. coli* the in sample.

Phosphate buffer solution was prepared by dissolving monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4) according to equation 3.1. The phosphate buffer was used for prepared substrate 4-methylumbelliferyl-β-D-glucuronide (MUG).

$$pH = pK_a + \log\left(\frac{salt}{acid}\right) \tag{3.1}$$

Adjust the pH of prepared buffer solution to 6.8 under pH meter. If the pH was over 6.8, added phosphoric acid (H_3PO_4) to decrease the pH. On the hand, added sodium hydroxide (NaOH) to increase the pH if it was lower than 6.8.

The enzymatic reactions of β-D-glucuronidase (GUD) and the 4-methylumbelliferyl-β-D-glucuronide (MUG) substrate were performed in 2 cases which were in a microcentrifuge tube and using gelatin microfluidic.

3.4.1 Fluorescence comparison between 4MU and GUD and MUG reaction

In this experiment, we used the 4MU, GUD, and MUG that had prepared before for indicating the availability of enzyme GUD and substrate MUG solutions by observed the detectable emitted fluorescent synthesis product, 4MU. Therefore, we examined the enzyme and substrate reaction in the microcentrifuge tube which divided into 3 tubes;

1. Distilled water as a negative control tube.
2. 4MU solution (500 µM) as a positive control tube.
3. Enzymatic reaction from mixed 1700 U of GUD with 500 mM of MUG.

Then placed these microcentrifuge tubes in dark box equipped light source for cut other background light and observed while excited by UV light source wavelength 365 nm. If enzyme GUD mixed with substrate MUG shows the fluorescent emitted as same as 4MU solution, we can ensure that the enzyme-substrate complex of GUD and MUG based on 4-methylumbelliferone (4MU) can be used for the *Escherichia coli* detection.

3.4.2 Fluorescence comparison between 4MU and GUD and MUG reaction in the gelatin microfluidic

To examine the microfluidic channel from gelatin using the laser printer able to use for detecting fluorescent synthesis to detect *E. coli* as the indicator of bacterial contamination evaluation of the sample. The experiment was carried out by flowing the liquid solution under the straight-line channel of 1.78 mm (width) x 0.19 mm (height) and Y-shape channel.

1. Straight-line channel

1.1 4MU

To indicate the availability of channel for flowing liquid by observing the detectable released fluorescent synthesis under gelatin microfluidic channel. This experiment was carried out by used an insulin needle fed 0.4 ml of 4MU (500 μM) solution at the inlet node of the straight-line channel pattern (Figure. 3.17 and 3.18). Placed microfluidic in dark box equipped light source for cut other background light and excited by UV light source wavelength 365 nm for fluorescent synthesis product, 4MU.

Figure 3.16 4MU in straight-line channel of microfluidic

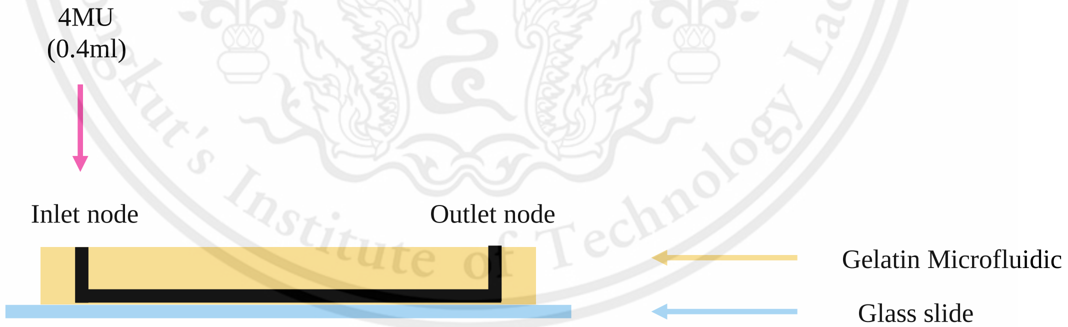


Figure 3.17 Side view of feeding 0.4ml of 4MU in inlet node of the straight-line channel of microfluidic

1.2 The enzymatic reaction of GUD and MUG

1.2.1 GUD and MUG was separately fed in inlet node

To ensure that the enzymatic reaction was achieved under straight-line channel of gelatin microfluidic, the experiment was done by used insulin needle separately fed 0.2 ml of 1700 U of GUD and a 0.02 ml of 500 µM of MUG at the inlet node of the channel (Figure 3.18). And left for 10 minutes of reaction time at room temperature. Then placed it in the dark box equipped light source to observe the generated of fluorescence.

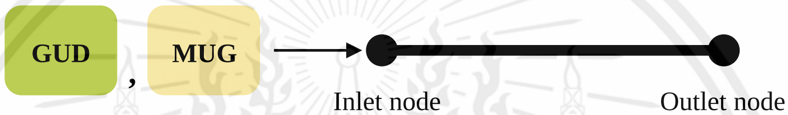


Figure 3.18 Separately fed GUD and MUG in inlet node of straight-line channel of microfluidic

1.2.2 Mixed GUD and MUG fed in inlet node

Mixed 1700 U of GUD and 500 µM of MUG before feeding in the channel was a positive control of enzyme reaction of GUD and MUG under straight-line of gelatin microfluidic. To examine whether the enzymatic reaction was achieved the fluorescent synthesis product of 4MU under the channel, the experiment was compared with separately fed GUD and MUD. This experiment was carried out by mixed 0.2 ml of 1700 U of GUD with 0.2 ml of 500 µM of MUG into a microcentrifuge tube. And left for 10 minutes of reaction time at room temperature. Then insulin needle was used to feed 0.4 ml of mixed GUD and MUG at the inlet node of the channel (Figure. 3.19). Placed it in the dark box equipped light source to observed fluorescent synthesis under the gelatin microfluidic and compared with separately fed enzyme GUD and substrate MUG.

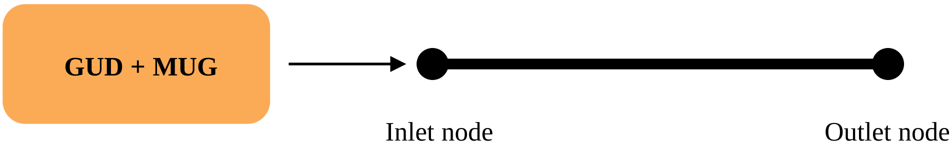


Figure 3.19 Feeding mixed GUD and MUG in inlet node of straight-line channel of microfluidic

2.2 Y-Shape channel

Most microfluidic applications require mixing liquid solution occurs in a short distance of the channel. Y-shape channel is one of the alternatives that liquid solution could be mixed within a short distance. Y-shape channel has configurations of two inlets and one outlet, two fluid streams could be convergence and mixed where the branches met and subsequently flow was extracted at the end of the Y-branch. This experiment used the Y-shape channel. The insulin needle was used to feed 0.2 ml of 1700 U of GUD at the inlet A node and 0.2 ml of 500 µM of MUG at inlet B nodes of the microfluidic channel as shown in Figure 3.21. Then, left for the reaction ran for 10 minutes at room temperature. And then placed it in the dark box equipped light source to observe the generated of fluorescence and compared with mixed GUD and MUG fed in inlet node which is a positive control. To confirm GUD and MUG was mixed in main channel where the branches met.

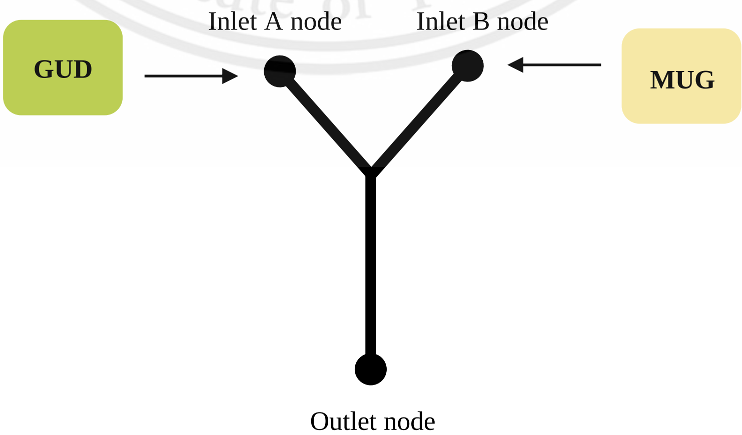


Figure 3.20 Feeding GUD and MUG in Y-Shape channel of microfluidic

3.5 Mixing MUG in gelatin

3.5.1 Enzymatic reaction of GUD and MUG under MUG mixed gelatin microfluidic

Mixing MUG in gelatin was used to determine the diffusion property of gelatin applied to make microfluidic. Mixed solution in gelatin could be decrease MUG concentration. In this experiment, MUG was mixed with gelatin and GUD was fed in the channel. This fed GUD was diffused and bind to its substrate MUG, which the enzyme-substrate complex was occurred and further produces fluorescent 4MU as the product of reaction. A microfluidic channel was fabricated from MUG mixed with gelatin and used a capillary tube as a mold making a channel of microfluidic. Due to capillary tube is larger than printed 30 times layer channel and , therefore, GUD was fed in larger volume.

The gelatin solution was prepared by dissolved gelatin powder in warm distilled water at the ratio of gelatin: distilled water of 1:3 and stirred well until gelatin turns into aqueous. Mixed 500µM of MUG in gelatin aqueous. Pour warm gelatin aqueous on capillary tube placed on the Petri plate as and cooled down in refrigerator or leave at room temperature. Fed 0.3 ml of GUD at the inlet node of the channel (Figure 3.21) and placed it in the dark box equipped light source to excite the UV light to observe the emission of fluorescence.

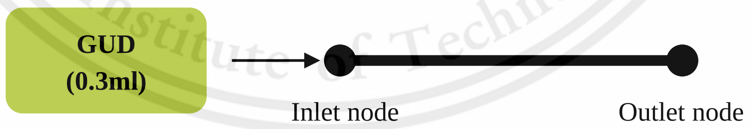


Figure 3.21 Feeding GUD at the inlet node of the microfluidic channel fabricated from MUG mixed with gelatin

3.5.2 Enzymatic reaction from *E. coli* under MUG with nutrient agar

To confirm that *E. coli* secrets extracellular GUD and the enzymatic reaction of GUD and MUG could be achieved. The cultivation of *E. coli* on nutrient agar mixed with MUG substrate was done in this experiment. We prepared the solid medium plate for cultivating *E. coli* by mixing the sterilized nutrient agar (0.5% Peptone, 0.3% Yeast extract, 1.5% NaCl, and 1.5% Agar) with Millipore filtered 100 ml of 125µM of MUG. The final concentration of MUG in nutrient agar was 62.5 µM. The liquid nutrient agar mixed MUG was poured into the petri dish and wait for the liquid turns to solid. Once *E. coli* was cultivated on this nutrient mixed MUG agar plate by streaking and spreading techniques. The colony of *E. coli* on this plate was observed under the UV light source.

3.5.2.1 Streak plate

The streaking was done by using an inoculating loop. The inoculating loop was first sterilized by passing it through a flame. When the loop was cool, dipped it into inoculum of *E. coli*. The inoculation loop was then dragged across the surface of the nutrient mixed MUG plate and incubate at 37°C for 24 hours.

3.5.2.2 Spread plate

Made a dilution series from a sample cultivated *E. coli*. Pipette out 0.2 ml of the appropriate desired dilution series onto the center of the surface of an agar plate. Using the sterilized glass spreader, spread the liquid cultivated *E. coli* evenly over the surface of agar. Incubate the plate at 37°C for 24 hours.

3.5.3 Enzymatic reaction from *E. coli* under MUG in gelatin microfluidic channel

We studied under two features of forming channel, using capillary tube and printed channel template mold by a laser printer. We cultivated *E. coli* in nutrient broth by dipping sterilized inoculating loop into inoculum *E. coli* and inoculated into nutrient broth. Incubated at 150 rpm 30°C for 24 hours, to encourage bacterial growth. The samples of this experiment include:

- Distilled water as a negative control
- 1700U enzyme GUD as a positive control
- Cultured *E. coli* which is cultivate *E. coli* in nutrition broth

- Liquid cultured residue which was separated by centrifuge at 10,000 rpm for 30 minutes of cultivated *E. coli* in nutrient broth.

3.5.3.1 Capillary channel

A microfluidic was fabricated from MUG mixed with gelatin and used a capillary tube to make a channel of microfluidic. The gelatin solution was prepared by gelatin powder dissolved in warm distilled water at gelatin: distilled water of 1:3 and stirred until gelatin turns into aqueous. Mixed 500µM of MUG in gelatin aqueous. Pour warm gelatin aqueous on capillary tube placed on the Petri plate and cooled down in refrigerator or left at room temperature. Fed 0.3 ml of the sample which are distilled water, 1700U of GUD, cultured *E. coli* and liquid cultured residue at the inlet node of the microfluidic channel and placed it in the dark box equipped light source to excite the UV light. Take a video time-lapse for 5 hours to observed fluorescent synthesis under the gelatin microfluidic. The process diagram was shown in Figure 3.22 and 3.23.

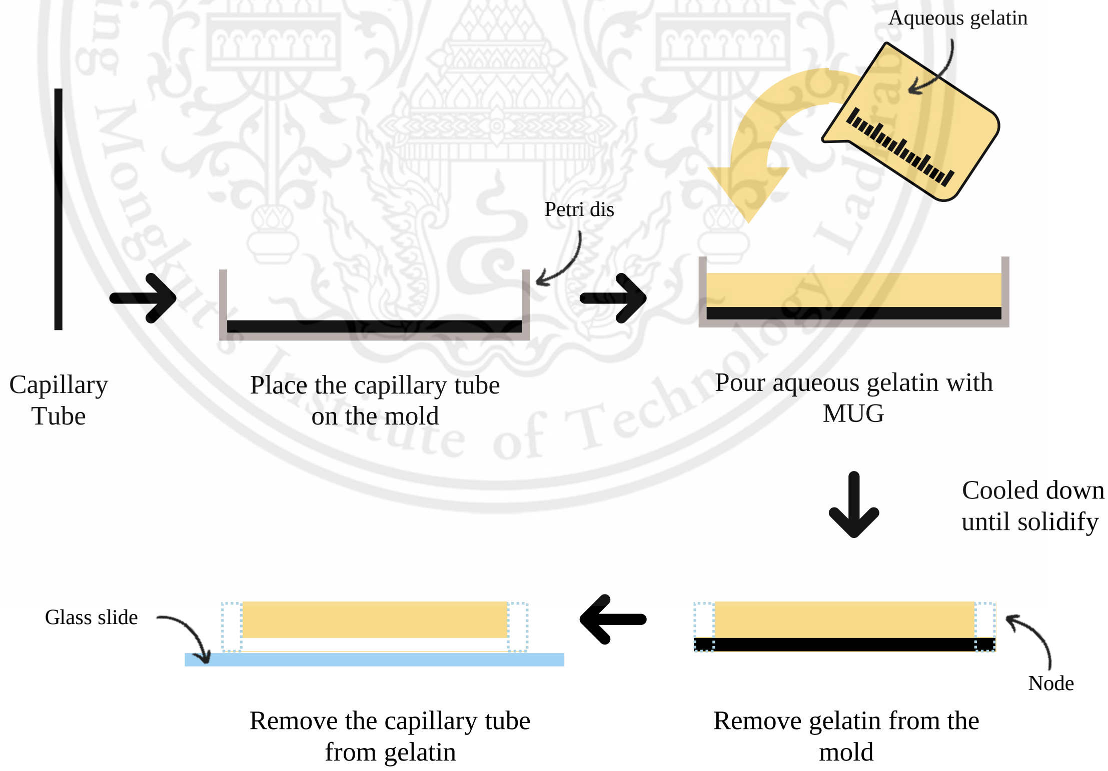


Figure 3.22 A microfluidic channel fabrication from MUG mixed with gelatin with capillary tube

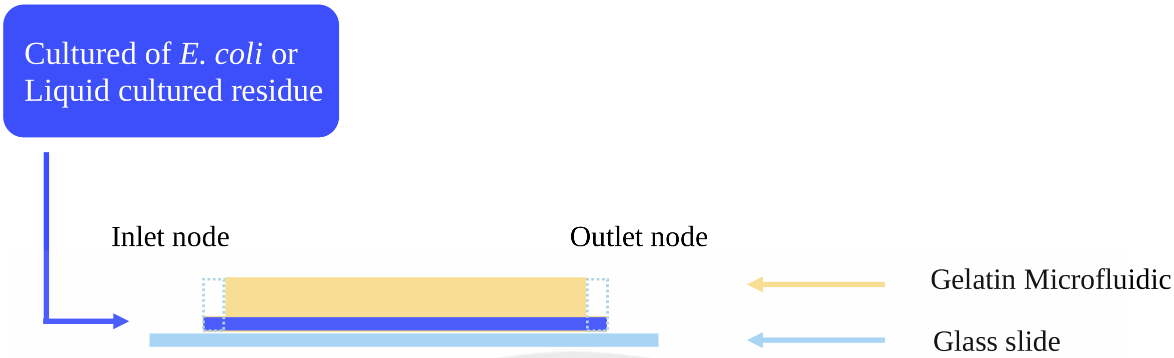


Figure 3.23 Side view of fed cultured and liquid cultured residue of *E. coli* at the inlet node of the capillary tube microfluidic channel which fabricated from MUG mixed with gelatin

3.5.3.2 Printed 30 times layer channel

A microfluidic was fabricated from MUG mixed with gelatin and used a printed 30 times layer of straight-line pattern to make a channel of microfluidic. The gelatin solution was prepared by gelatin powder dissolved in warm distilled water at gelatin: distilled water of 1:3 and stirred until gelatin turns into aqueous. Mixed 500 µM of MUG in gelatin aqueous. Pour warm gelatin aqueous on capillary tube placed on the petri plate and cooled down in refrigerator or left at room temperature. Fed 0.3 ml of the sample which is distilled water, 1700 U of GUD, cultured *E. coli*, and liquid cultured residue at the inlet node of the microfluidic channel and placed it in the dark box equipped light source to excite the UV light. Take a video time-lapse for 4 hours to observed and compare fluorescent synthesis under the gelatin microfluidic with a capillary channel of microfluidic. The process diagram was shown in Figure 3.24 and 3.25.

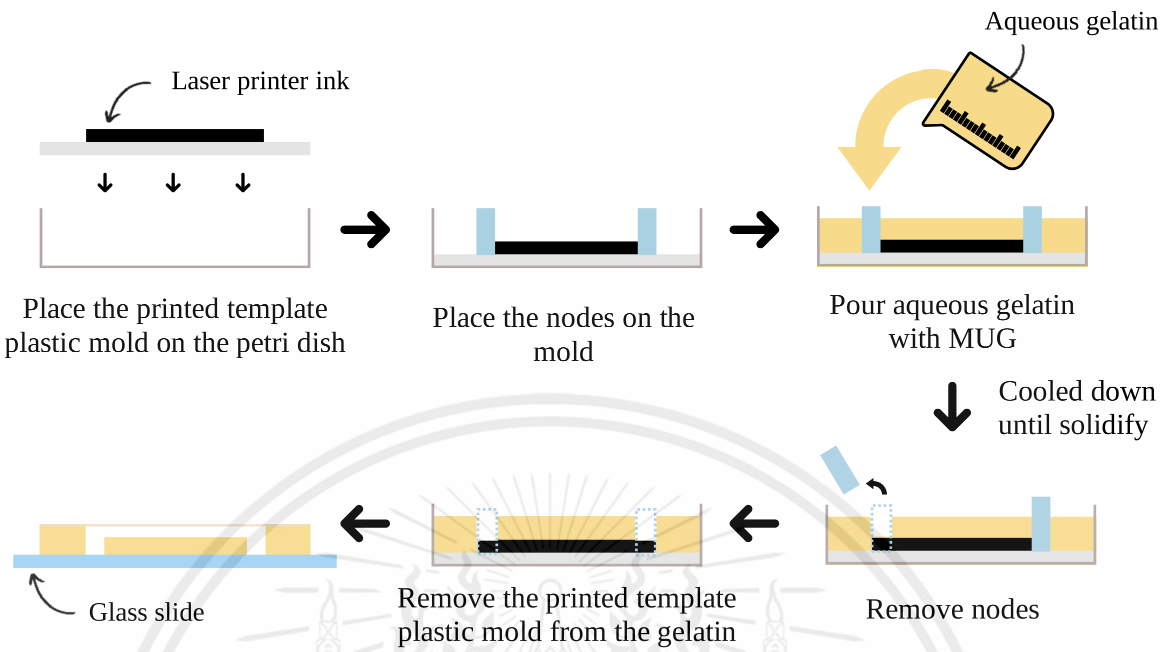


Figure 3.24 A microfluidic channel fabrication from MUG mixed with gelatin with printed 30 times layer channel diagram

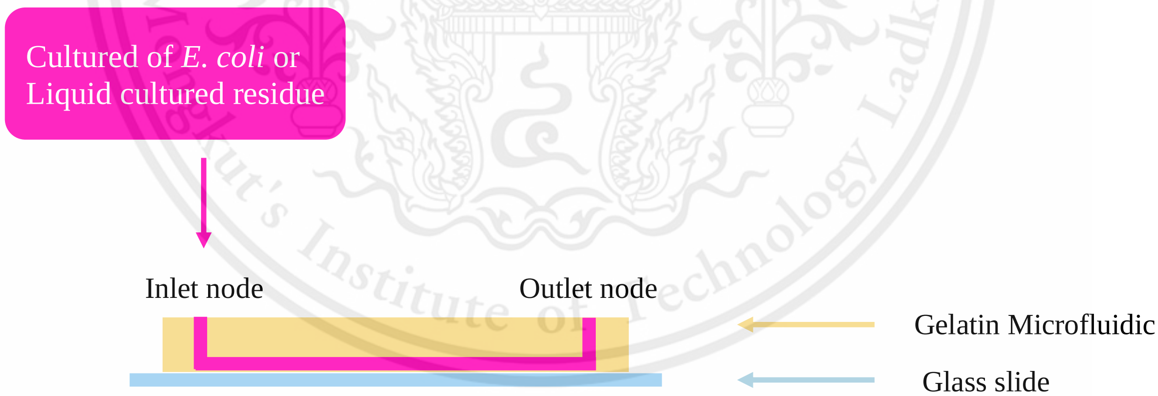


Figure 3.25 Side view of fed cultured and liquid cultured residue of *E. coli* at the inlet node of the printed 30 times layer microfluidic channel which fabricated from MUG mixed with gelatin

CHAPTER 4

EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Result of node design

We investigated the effect of node shape to the flow of fluid in channel. Firstly, circular and triangular nodes were created at the ends of the straight line with varied the width of the channel from 0.01 pt to 1 pt by the height of 1-time printed layer. The result from fed water-soluble liquid color at the inlet node showed that the liquid color was unable to flow throughout from the inlet node to the outlet node. The cause of liquid color unbale to flow was the size of 1-time printed layer channel was probably too small and requires higher pressure to force the water flow through the channel. Therefore, we increased the size of channel by printed at 1 pt varied to 6 pt and 5 times printed layers with the triangle and circular nodes. The results showed that the liquid color was unable to flow throughout whether circular or triangle nodes, which indicated that the node shape was not affected the liquid flow. The significant cause of liquid could flow was the size of channel, which was insufficient in both width of 0.01 pt to 1 pt and thickness of 1-time printed layer and the liquid required higher pressure to enter and further flow through the channel.

4.2 Result of channel design

According to the node design that the liquid could not flow through the channel, the design of the channel was changed from a straight line to 4 branches to reduce the pressure in the channel. Due to our suspect that the size of the channel printed by 1 layer was too small, we increased the height of the channel by printed out 5 times layer on the transparent plastic sheet as a template mold. The first branch design was 4 branches with 4 open outlets and fed the liquid watercolor from one inlet. The result showed that the watercolor could not flow through the channel. The second design was a single inlet and outlet node with symmetric 4 branches combined. The result of the second design was the watercolor could flow through a short distance as shown in Figure 4.1. The reason for this problem was the same as the circular node design with

5 times printed layer, which the channel size was not too small. Consequently, the size of this channel requires a higher width and height and the pressure required to force the fluid flow through the channel was not enough due to the channel was an open microfluidic. So, the channel should be entire closed on the gelatin microfluidic by placing it on the glass slide resulted in higher pressure to enforce liquid to flow along the channel.

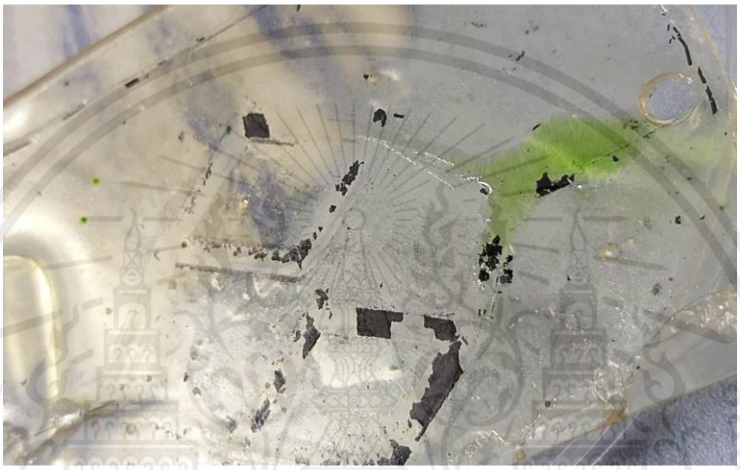


Figure 4.1 Water-soluble liquid color able to flow in four branches channel combined symmetric four branches with one single inlet and outlet

The flowing test of liquid through the different sizes of the channel was examined. The straight-line channel size was varied with the height of 1, 5, 10, 15, 20, and 30 times printed layer and the width of 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 pt which 1pt equal to 0.353mm. The flowing liquid results indicated that the height of the channel less than 20 times printed layers with the width less than 2 pt was insufficient to flow the liquid throughout the channel from the inlet to outlet as shown in Figure 4.2 of 20 times printed layer and Figure 4.3 of 30 times printed layer varied with the width of 0.25 pt to 5 pt. The result shown in Figure 4.3 indicated that the liquid could flow throughout the channel from the inlet node to the outlet node of the width at least 3 pt with 30 times printed layer. Therefore, the minimum size of the channel in which the liquid-based water could flow from the inlet through the outlet node was 30 times printed layer height and 3 pt width as compared in Figures 4.2 and 4.3.

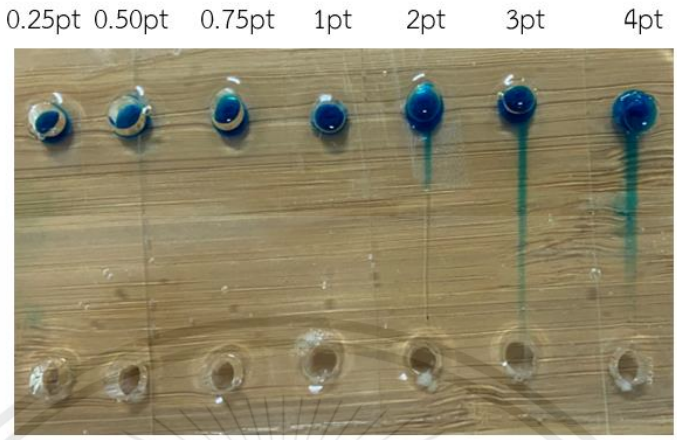


Figure 4.2 The liquid flowed through the 20 times printed layer channel of various width sizes of 0.25 pt to 4 pt

Figure 4.3 The liquid flowed results of 30 times printed layer channel of various width size of 0.25 pt to 5 pt

After we known the optimal size of the channel that liquid could flow, we designed the pattern that is more complex and common widely used in the microfluidic pattern. We tested the flow of the liquid in different channel patterns to evaluate the capability and usability of this gelatin microfluidic. These variety patterns were printed at the optimal size of channel at 30 times printed layer and 3 pt. The result was shown in Figures 4.4 to 4.8 for T-shape, Y-shape and Y-shape with double wave, branch with curve line and wave line, respectively. There results indicated that the liquid could flow throughout the channel from the inlet node toward the outlet node of all designed patterns. Therefore, the gelatin microfluid has the capability and usability as well as using a conventional method of Photolithography with the materials of PDMS.

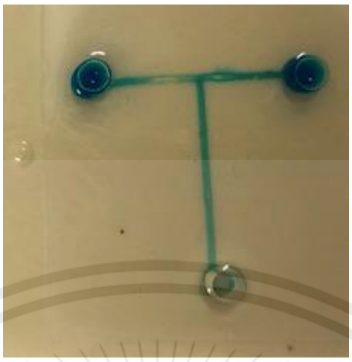


Figure 4.4 Result of flowing of liquid in T-shape pattern

Figure 4.5 Result of flowing of liquid in Y-shape pattern

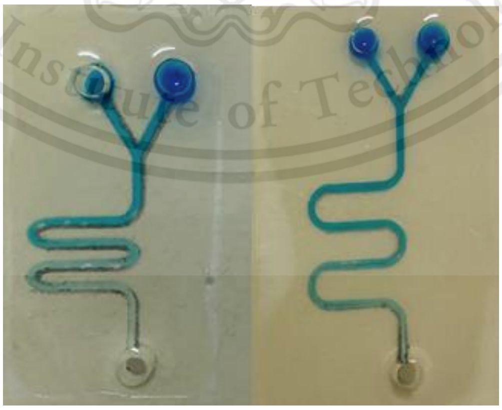


Figure 4.6 Result of flowing of liquid in Y-shape with double wave pattern

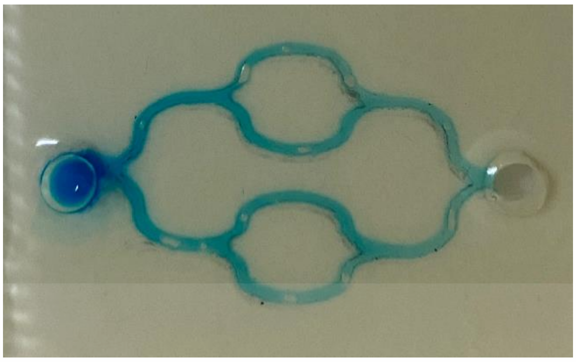


Figure 4.7 Result of flowing of liquid in branch with curve line pattern

(a)

(b)

Figure 4.8 Result of flowing of liquid in wave line pattern with (a) top view; (b) side view

4.3 Result of measurement of the channel size

We measured the size of channel by using the microscope equipped software camera and vernier caliper. The result in Figures 4.9 and 4.10 showed the shape and feature of channel, which is rough along the edge of channel. Even though our channel has a rough surface that different from the conventional microfluidic channel, this is a point of advantage because the rough surface could induce the turbulent flow of fluid in channel, which help the fluids (more than one type of solution are flowed) mix well resulted in increasing the reaction rate while the solutions are flowing through the channel.

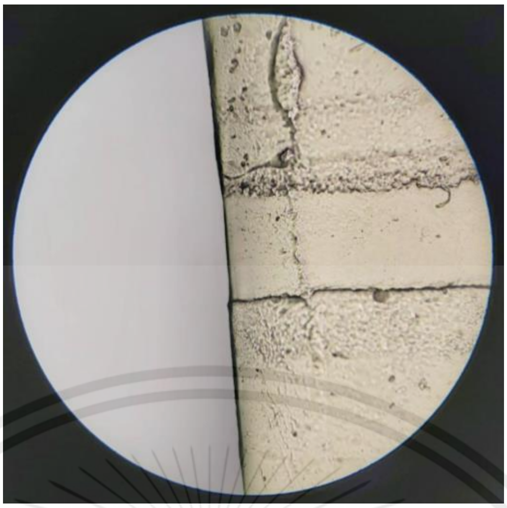


Figure 4.9 Edge of gelatin channel under a microscope 40x

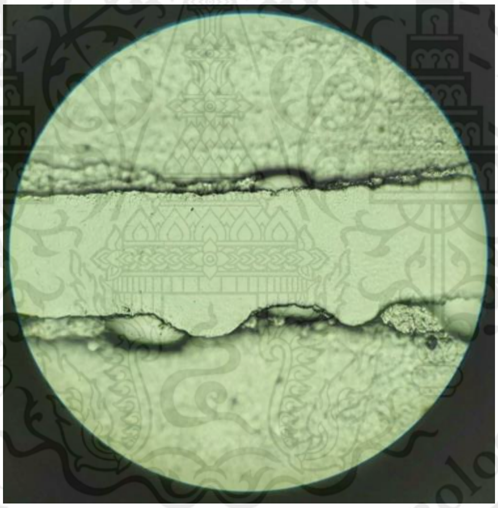


Figure 4.10 The middle region of channel under a microscope 40x

The thickness and the width of laser printer ink on a transparent plastic sheet related to the size of the channel were measured. Figure 4.11 was showed the top view of laser printer ink observed under the microscope. Figure 4.12 was showed the side view of laser printer ink on a transparent plastic sheet under microscope, which indicated that the height of ink was increased due to the number of printed layers increased. The width of 1, 2, 3, 4, and 5 pt with the printed layers of 1, 5, 10, 15, 20, and 30 times, were measured by vernier caliper and microscope with the software of the equipped camera (Figure 4.13). The measured results were shown in Table 4.1.

Table 4.1 Width and thickness of printed ink on plastic sheet measured by microscope (red) and vernier caliper (black)

Printed \ Line	1 times (mm)		5 times (mm)		10 times (mm)		15 times (mm)		20 times (mm)		30 times (mm)	
	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height
1 pt	0.37	0	0.44	0.03	0.66	0.02	0.81	0.07	0.93	0.13	1.02	0.19
2 pt	0.67	0	0.75	0.03	0.91	0.06	0.98	0.07	1.17	0.13	1.42	0.19
3 pt	0.95	0	0.99	0.03	1.16	0.07	1.29	0.1	1.51	0.13	1.78	0.19
4 pt	0	1.40	0.04	1.64	1.64	0.07	1.80	0.1	1.86	0.13	2.05	0.19
5 pt	0	1.83	0.04	1.94	1.94	0.07	2.08	0.1	2.25	0.13	2.44	0.19

Figure 4.11 Top view of laser printer ink pigment printed on the transparent plastic sheet used as the gelatin microfluidic template

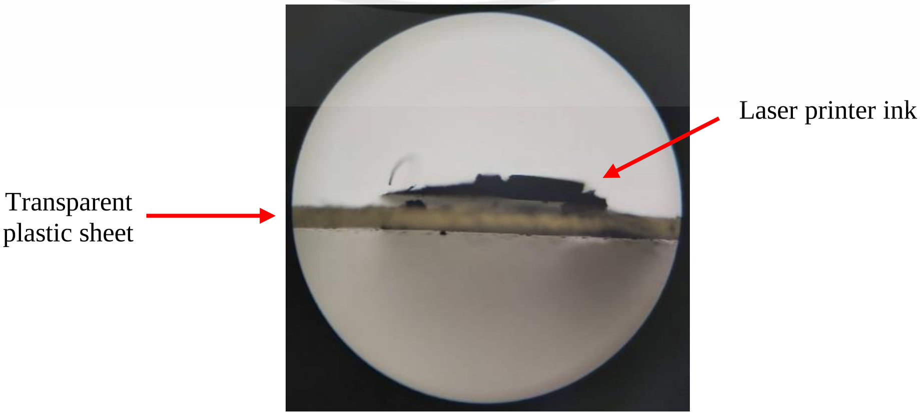


Figure 4.12 Side view of laser printer ink on plastic sheet under a microscope 40x

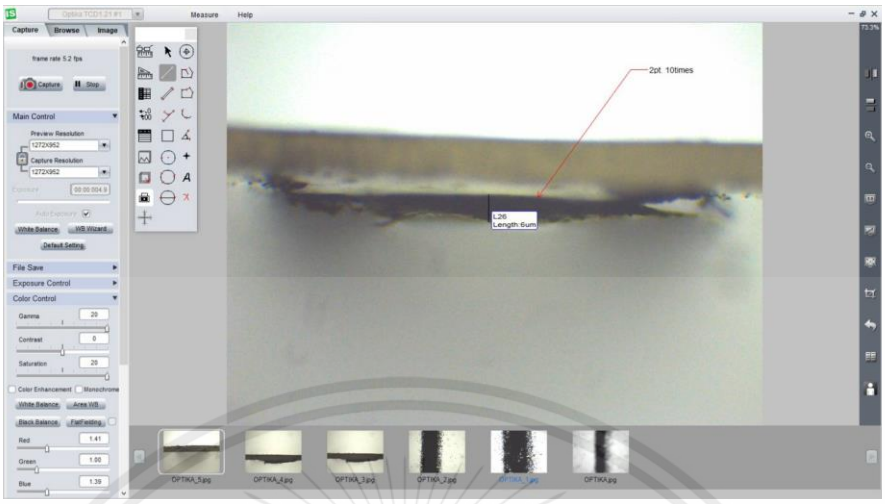


Figure 4.13 The display of the program of Optika B-292 microscope equipped with a digital camera (Opticam B1) measured the size of laser printer ink

These data were graphically plotted as showed in Figure 4.14 and Figure 4.15. The size (mm) of width and thickness of printed ink on a plastic sheet of the channel increased with increasing line size (pt) and the number of printed layers. From these results, the channel of 3 pt width and 30 times printed layer corresponded to 1.78 mm (width) and 0.19 mm (height) was the optimal channel for flowing the liquid water-soluble throughout various patterns of the channel (Figure 4.4 to 4.8 and Figure 4.14 and 4.15). According to this data, the thickness of printed line at fixed width of 3pt was increased with the number of printed layers as shown in Figure 4.15. However, the width of printed ink at the same point (pt) of line was increased unexpectedly with increased the number of printed layers because of the error of the laser printer that the motor maybe unstable, resulted in some staggering causes the ink of each layers were not precisely printed at the same position.

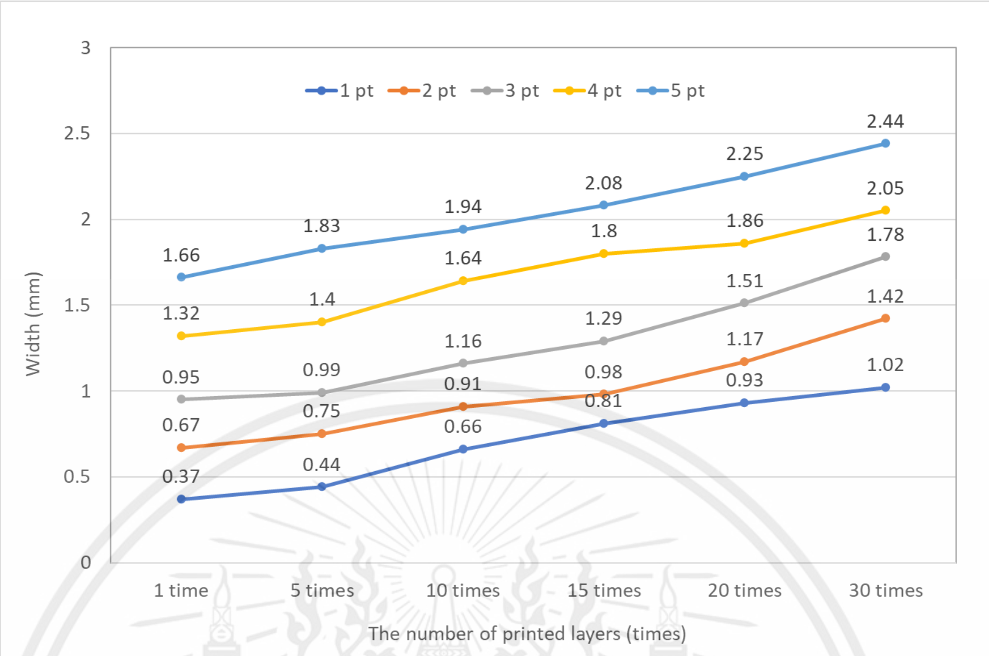


Figure 4.14 The relation of measured width (mm) of the channel and the number of printed layers

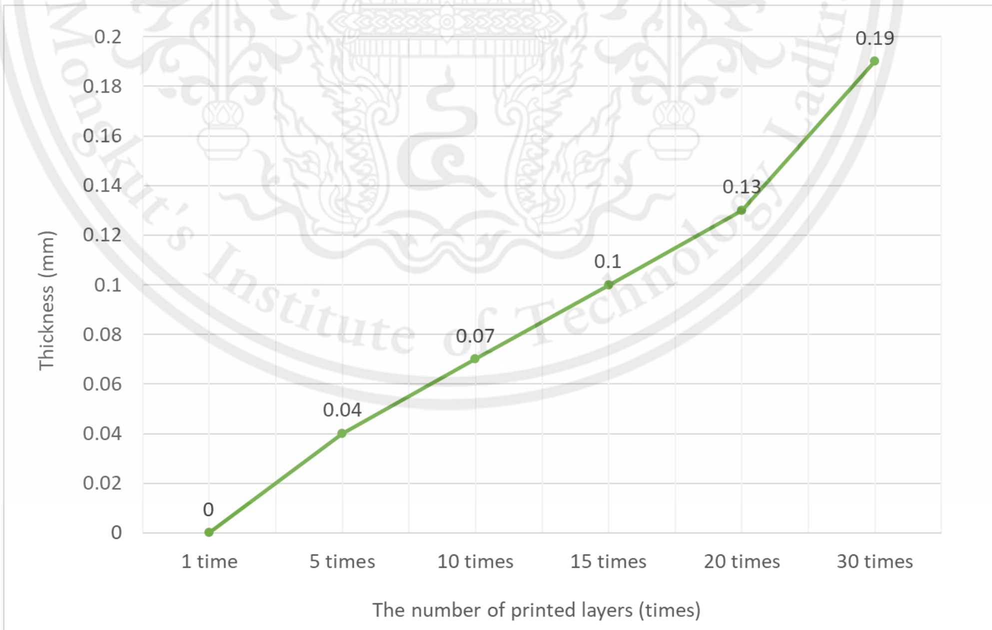
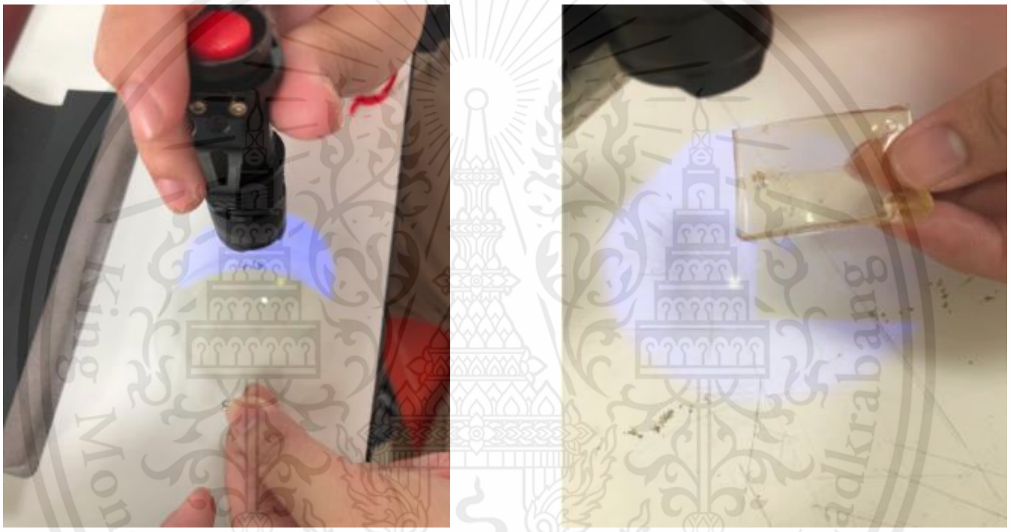


Figure 4.15 The relation of the thickness of ink printed on the plastic sheet of line points 1 to 5 pt and the number of printed layers

4.4 Result of gelatin microfluidic channel characteristics

4.4.1 UV cut result

The gelatin UV cut property was investigated by compared with UV cut lens and measured the transmittance wavelength by spectrophotometer. The result was shown in Figure 4.16. When the UV light at wavelength 365 nm was exposed to the gelatin and UV cut lens, there is no UV light pass through these objects, which indicated that the gelatin was an effective UV cut material as well as the UV cut lens.



(a) **(b)**

Figure 4.16 The UV light source exposed to (a) UV cut lens; and (b) Gelatin

Then the transmittance of 2 mm thickness gelatin was measured by spectrophotometer. This gelatin was placed in cuvette slot of spectrophotometer and ran at the wavelength of 200 – 700 nm. The transmittance data was graphically plotted and shown on display as in Figure 4.17. According to this data, the gelatin was able to cut wavelengths under 290 nm while UV light source has a wavelength between 180 - 400 nm.

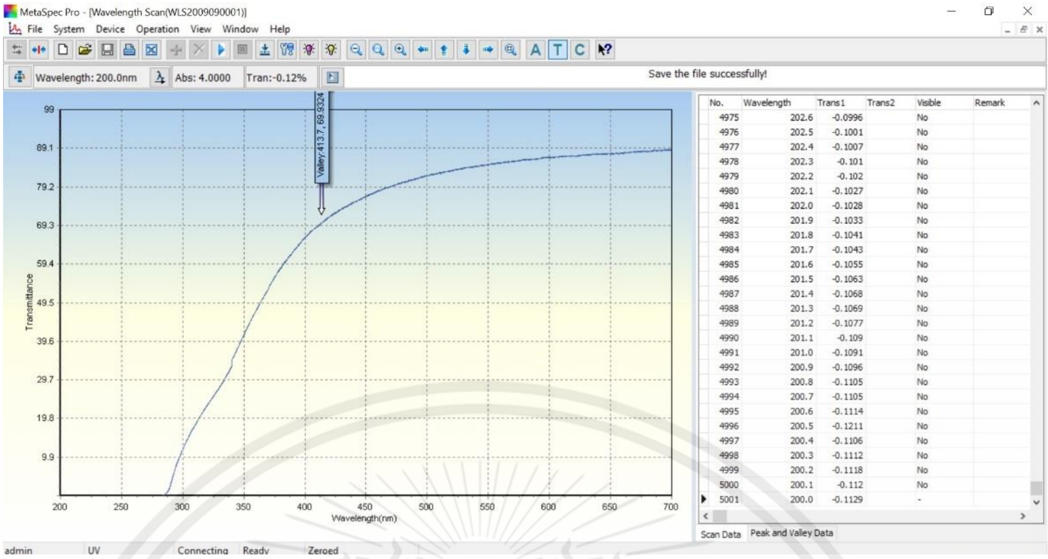


Figure 4.17 The relation of measured transmittance of gelatin and wavelength

4.4.2 Result of diffusion

To investigate the diffusion property of the gelatin microfluidic, the experiment was examined by feeding liquid color through the straight-line capillary tube channel and observed under a microscope at 0.5, 1.0, 1.5, and 2.0 hours. The distance of the liquid color diffused in gelatin was measured by vernier caliper from the edge of channel to the end of liquid color pigment. The measured results were shown in Table 4.2. The molecule of pigments in liquid color solution travel through gelatin resulted in the different color layers of green and yellow were observed. This result under microscope indicated that the liquid could diffuse in gelatin and the cross-section of the gelatin channel were shown in Figure 4.18 to 4.21.

According to these data, we calculated the diffusion rate of liquid color travel through gelatin from the edge of the channel. From Table 4.2, the diffusion distance was increased through gelatin as leaving time increase. However, the diffusion rate decreased due to the amount of liquid gradually decreased with increasing time.

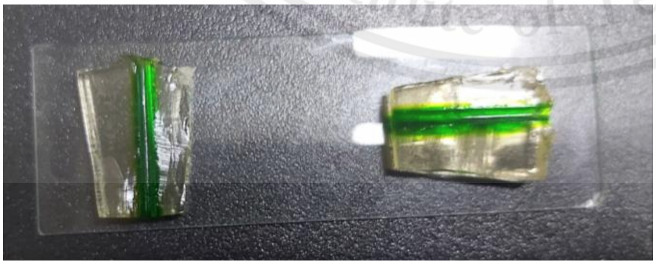
Table 4.2 Diffusion distance (mm) of liquid color far apart from the edge of the channel measured by vernier caliper and the diffusion rate (mm/hr) at varied times

Time (hr)	Diffusion distance (mm)	Diffusion rate (mm/hr)
0.5	1.0	4.0
1.0	1.5	3.0
1.5	2.5	3.3
2.0	3.0	3.0

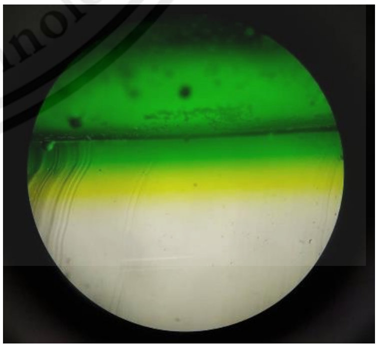
(a)

(b)

Figure 4.18 Liquid color diffused for 30 minutes (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope

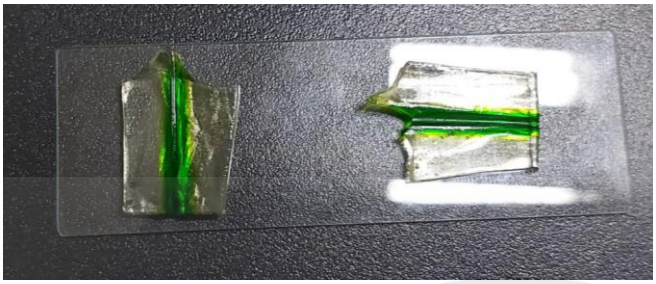


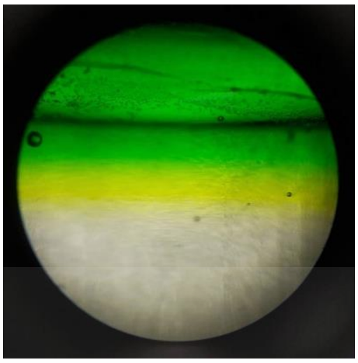
(a)



(b)

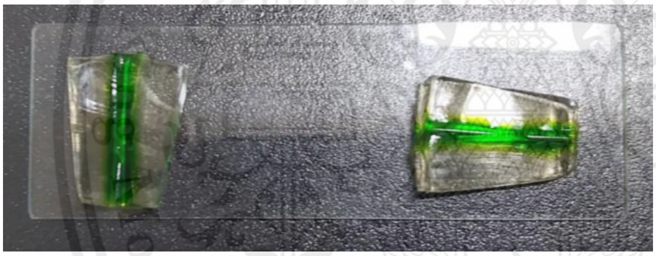
Figure 4.19 Liquid color diffused for 1.0 hours (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope

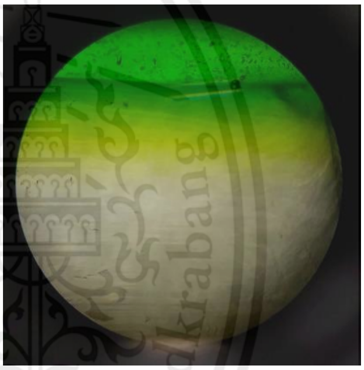




(a) (b)

Figure 4.20 Liquid color diffused for 1.5 hours (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope





(a) (b)

Figure 4.21 Liquid color diffused for 2.0 hours (a) Cross section of gelatin channel; (b) Cross section of gelatin channel observed under a microscope

The data in Table 4.2 was graphically plotted as showed in Figure 4.22. The diffusion distance (mm) of liquid color increased with increasing time (hour) left the liquid in channel.

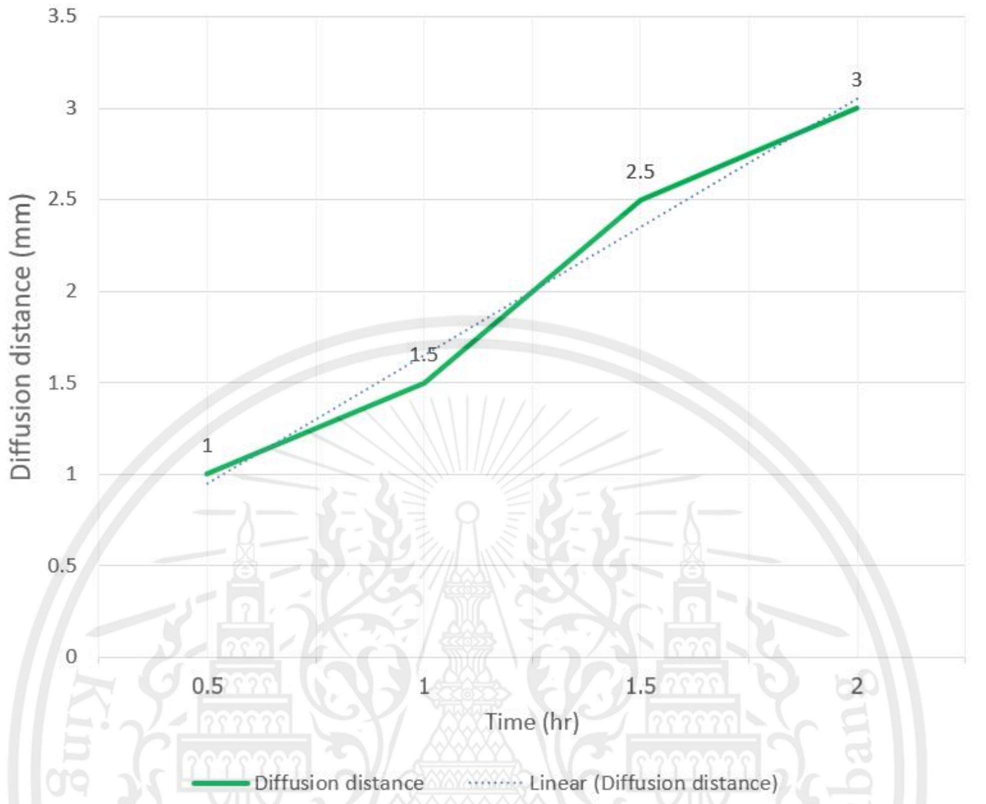
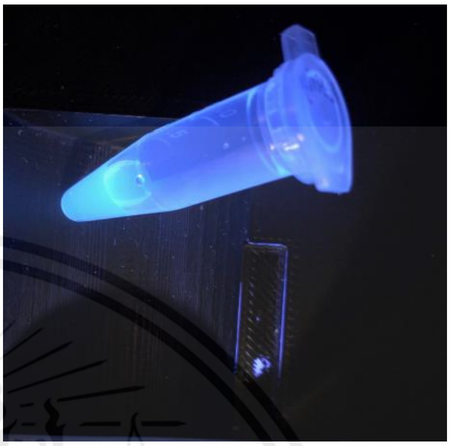


Figure 4.22 The relation of measured diffusion distance (mm) of liquid color diffused through gelatin far apart from the edge of the channel

4.5 Reaction of enzyme GUD and substrate MUG, and product 4MU test

4.5.1 Result of fluorescence comparison between 4MU and GUD and MUG reaction

We compared the fluorescence of 4MU solution and the enzymatic reaction product, 4MU from the reaction of GUD with MUG. To prove that the enzyme-substrate complex of GUD and MUD takes place and give the product of reaction as fluorescent 4MU, the reaction was performed primarily in a microcentrifuge tube. Then these microcentrifuge tubes were placed in the dark box equipped light source. The fluorescent 4MU was excited at wavelength of 365 nm as appear in fluorescence as shown in Figure 4.23. This result confirms that the enzyme-substrate complex of GUD and MUG could occur and the fluorescent 4MU was produced as observed under the UV light.



(a) **(b)**

Figure 4.23 (a) 4MU solution in microcentrifuge tube; (b) Fluorescence of 4MU product of enzymatic reaction of GUD and MUG

4.5.2 Result of fluorescence comparison between 4MU and GUD and MUG reaction in the gelatin microfluidic

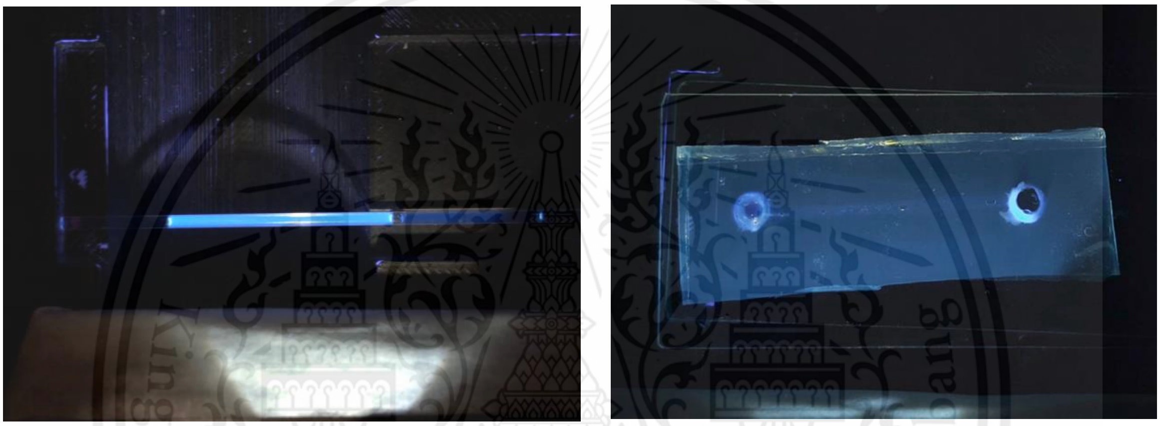
1. Result of the Straight-line channel

1.1 4MU

The 4MU solution was injected into an inlet node of microfluidic and placed in the dark box equipped light source to excite the UV light. The fluorescent 4MU was excited at wavelength of 365 nm as appear in fluorescence as shown in Figure 4.24. This result confirms that the microfluidic channel from gelatin by using the laser printer is available for flowing liquid.

4MU solution in a microfluidic channel, the blue fluorescence was noticeably lighter than the 4MU in the microcentrifuge tube and capillary tube when observed by the naked eye, as compared in Figure 4.24 (a) and (b). The blue light intensity from 4MU in a microcentrifuge tube and capillary tube are clearly visible. While in Figure 4.24 (b), the blue light intensity from a 4MU solution in microfluidics channel was less apparent even though both samples had the same 4MU solution concentration.

Due to the characteristic of gelatin that can partially cut UV light, the gelatin microfluidic is able to cuts background light and reduces scattering and causing the fluorescence light emitted to have fewer chromatic aberrations than normal. Another factor that differentiates the fluorescence emitted is the concentration and volume of the substance which fed into the channel. Because the blue light intensity of 4MU emitted varies with the volume and concentration of 4MU. Accordingly, gelatin microfluidic can be used for fluorescent 4MU detection.



(a) **(b)**

Figure 4.24 (a) Fluorescence of 4MU solution in capillary tube; (b) Fluorescence of 4MU solution in microfluidic channel

1.2 The enzymatic reaction of GUD and MUG

1.2.1 GUD and MUG was separately fed in inlet node

The enzymatic reaction of GUD and MUG was taken place in a microfluidic channel. Enzyme GUD and substrate MUG solution were separately injected into an inlet node of microfluidic channel and left for 10 minutes of reaction time. The sample was then placed in the dark box equipped light source to excite the UV light. There was the fluorescence light emission presented under the straight-line channel as shown in Figure 4.25. This result indicated that the enzyme GUD and substrate MUG solutions were flowed toward and then mixed. The enzyme-substrate complex of GUD and MUG could occur within the channel.

The fluorescence result of GUD and MUG reaction tested in a microfluidic channel was the same as 4MU solution in a microfluidic channel. However, the blue fluorescence from the enzymatic reaction of GUD and MUG in a microfluidic channel was noticeably lighter than in a microcentrifuge tube as shown in Figures 4.23 and 4.25. Due to the characteristic of gelatin that can partially cut UV light and the fluorescence was certainly presented on gelatin, the gelatin microfluidic can be used for fluorescent 4MU detection.

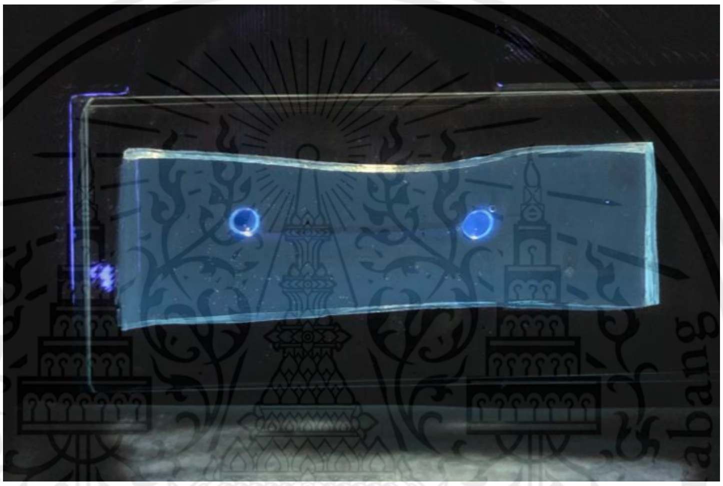


Figure 4.25 Fluorescence from the reaction of enzyme GUD and substrate MUG in microfluidic channel

1.2.2 Mixed GUD and MUG fed in single inlet node

Mixed GUD and MUG before feeding was a positive control to investigate the fluorescent production of reaction under microfluidic channel. Mixed GUD and MUG solution was injected into an inlet node of microfluidic channel and left for 10 minutes of reaction time. The sample was then placed in the dark box equipped light source to excite the UV light. The fluorescence light emission presented under the straight-line channel had a blue fluorescence intensity same as performed in 4MU and separately fed GUD and MUG channel when observed with the naked eye. The result was shown in Figure 4.25 and 4.26. Therefore, the appeared fluorescence on these channels confirms that the gelatin microfluidic can be used for fluorescent 4MU detection.

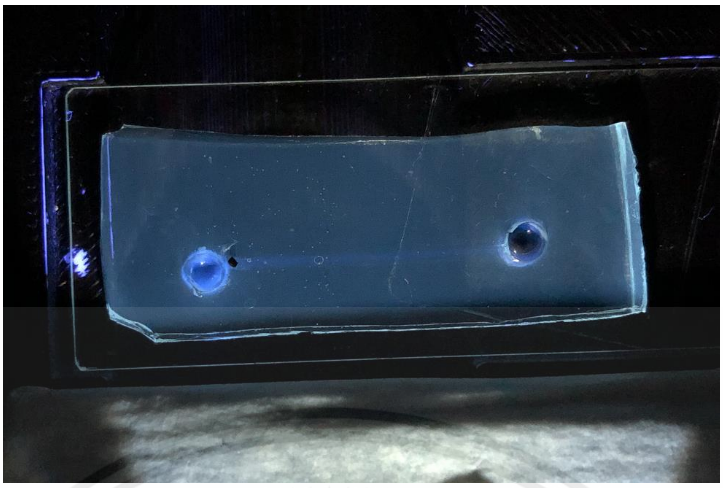


Figure 4.26 Fluorescence of mixed enzyme GUD and substrate MUG solution fed in microfluidic channel

2. Result of the Y-Shape channel

We applied another gelatin microfluidic channel pattern. The Y-shape pattern was used to examine the capability of gelatin microfluidic. The enzyme GUD and substrate MUG were injected into each branch of the Y-branch. The enzyme GUD entered inlet A and substrate MUG entered inlet B nodes of the microfluidic channel as shown in Figure 4.27. The fluorescence was appeared unexpectedly throughout the channel, which indicated that the enzyme GUD and substrate MUG solutions were flowed convergence and mixed together. However, fluid mixed should occur in the channel where the branches met. Because the node was an open node, the pressure while feeding the solution was insufficient to resist the flow from the other side of the channel. Therefore, the solution was leaked and mixed with another solution of the other side of the channel resulted in the fluorescent occurred throughout the Y-shape channel instead of at expected mix position on channel.

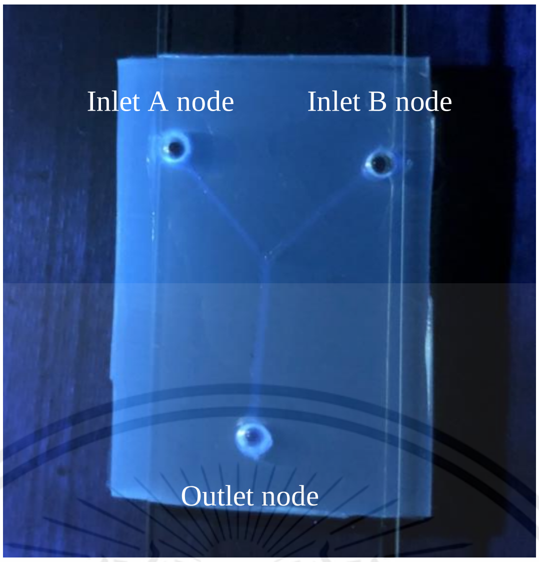
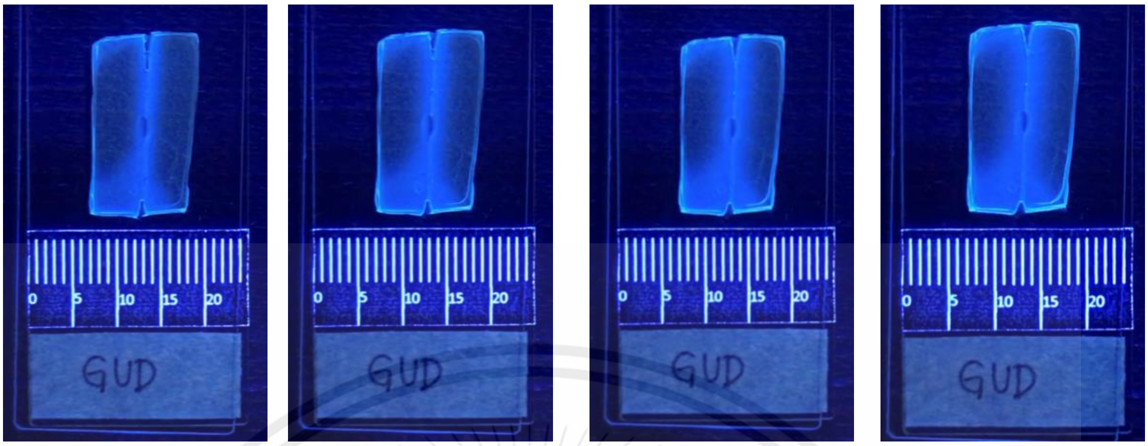


Figure 4.27 GUD and MUG in Y-Shape channel pattern

4.6 Result of fluorescence from GUD and MUG mixed in gelatin

4.6.1 Enzymatic reaction of fed GUD and MUG mixed gelatin microfluidic

The diffusion characteristic of the gelatin microfluidic channel showed that the liquid-based water can diffuse through gelatin. Therefore, we decided to test the hypothesis of GUD can diffuse in gelatin. In this experiment, MUG was mixed with aqueous gelatin and used a capillary tube to make a channel of microfluidic. Then 0.3 ml of 1700 U GUD was fed to the inlet node of the microfluidic single straight-line channel and using a UV flashlight to stimulates the emission of blue fluorescence. The reaction was continually run for 2 hours. The result was observed every 30 minutes as shown in Figure 4.28. This result indicated that GUD can diffuse from the channel area to the gelatin as the fluorescent 4MU was gradually produced and spread out of the channel to gelatin. Furthermore, the appeared fluorescence of 30 minutes reaction indicated that the enzymatic reaction was obviously taken place. Therefore, the reaction time of 30 minutes is probably sufficient for measuring the product 4MU.

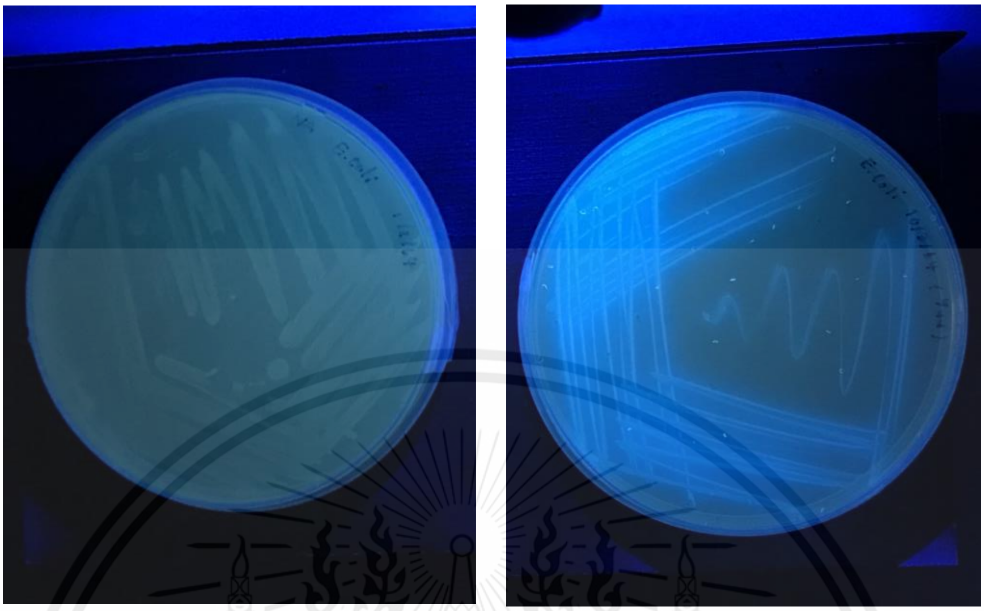


(a) (b) (c) (d)

Figure 4.28 (a) Fluorescence in 30 minutes; (b) Fluorescence in 1.0 hours; (c) Fluorescence in 1.5 hours; (d) Fluorescence in 2.0 hours

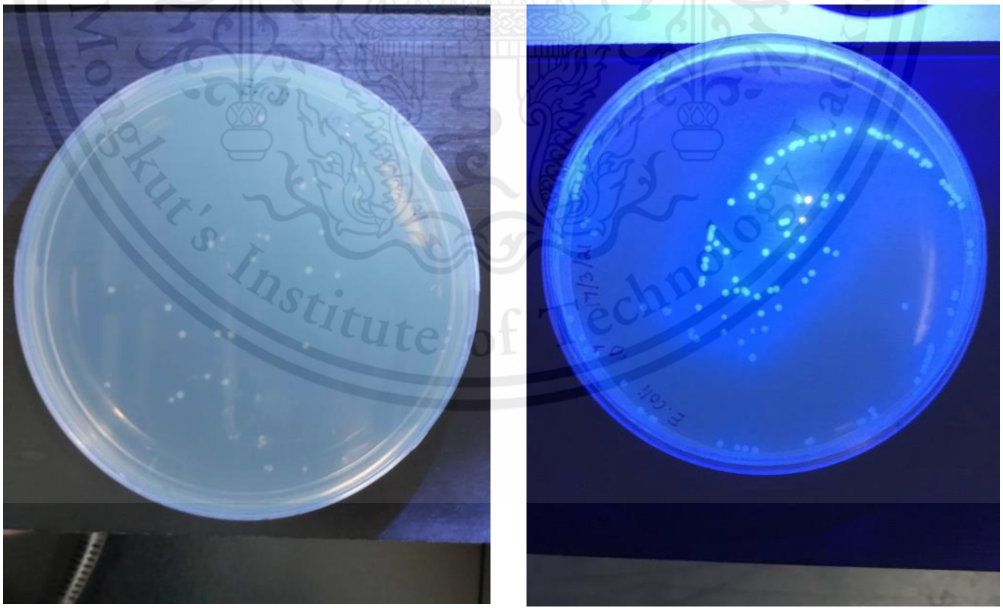
4.6.2 Enzymatic reaction from *E. coli* under MUG with nutrient agar

To confirm that *E. coli* secrets extracellular GUD and the enzymatic reaction of GUD and MUG could be achieved. The cultivation of *E. coli* on nutrient agar mixed MUG substrate was done in streaking and spreading techniques. The colony of *E. coli* was observed under the UV light source. The nutrient agar mixed MUG plate was occurred the fluorescence emission around the colony of *E. coli* as shown in Figure 4.29 (b) and 4.30 (b). On other hand, the fluorescence was not occurred around the colony of *E. coli* on nutrient agar without MUG plate as shown in Figure 4.28 (a) and Figure 4.29 (a). These results confirm the *E. coli* secreted enzyme GUD which can be used as a biomarker for *E. coli* detection by detecting fluorescent synthesis product of 4MU from the enzymatic reaction of GUD and MUG.



(a) (b)

Figure 4.29 *E. coli* culture from streak plate; (a) Without MUG added to nutrient agar; (b) MUG with nutrient agar



(a) (b)

Figure 4.30 *E. coli* culture from spread plate; (a) Without MUG added to nutrient agar; (b) MUG with nutrient agar

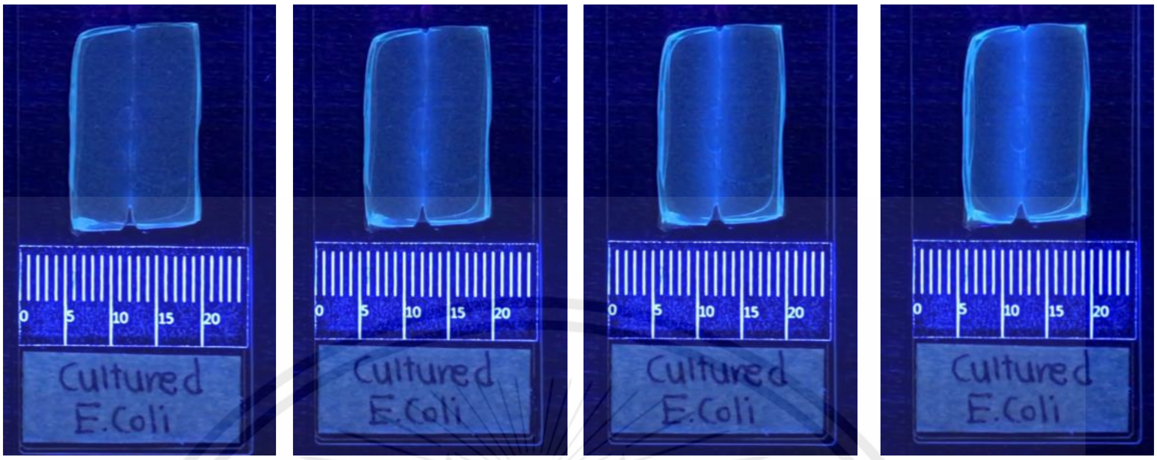
4.6.3 Enzymatic reaction from *E. coli* under MUG in gelatin microfluidic

Due to Figure 4.28 showed that GUD can diffuse from channel to gelatin as the fluorescent 4MU was gradually occur and spread out of the channel to gelatin. This experiment purposed to demonstrated that the enzymatic reaction was obviously take place. Therefore, we decided to test the hypothesis of enzyme GUD secreted by *E. coli* in liquid cultured could produce the blue fluorescence of 4MU as same as using 1700 U of GUD in buffer solution as a positive control of experiment. In this experiment, a microfluidic was fabricated from MUG mixed with gelatin under two features of forming a channel, using capillary tube and printed channel template mold by a laser printer. Then 0.3 ml of samples was fed to the inlet node of the microfluidic channel and using a UV light to stimulates the emission of blue fluorescence.

4.6.3.1 Capillary channel

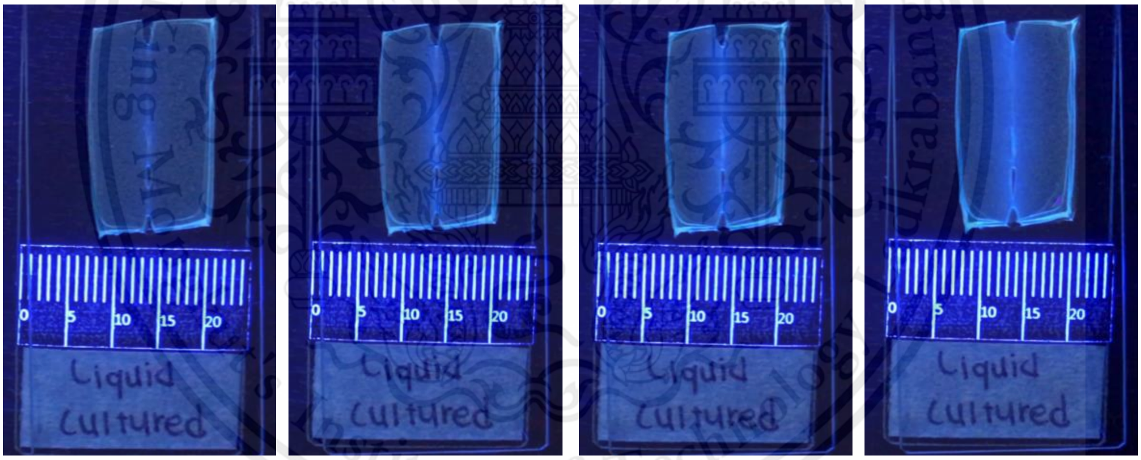
Firstly, we decided to use a capillary tube to make a channel of microfluidic. Because a capillary tube is larger than a printed 30 times layer mold, it can contain more volume inside the channel. In addition, to ensure that the enzymatic reaction of GUD secreted by *E. coli* and MUG in gelatin could be achieved, 0.3 ml of the samples which were distilled water, 1700 U enzyme GUD, cultured *E. coli*, and liquid cultured residue were fed at the inlet node of the microfluidic channel and placed in the dark box equipped light source to excite the UV light. A video time-lapse was recorded for 5 hr.

The results showed that fed cultured *E. coli* and liquid cultured residue presented the fluorescence light emission as same as 1700 U enzyme GUD which is a positive control as shown in Figure 4.28. The fluorescence was occurred at 2 hours and the fluorescence was gradually produced and spread out of the channel to gelatin increasing with time as shown in Figure 4.31 and Figure 4.32. This result confirms that enzyme GUD secreted by *E. coli* can diffuse from the channel to the gelatin and the enzyme-substrate complex was occurred as same as GUD solution. Consequently, the gelatin microfluidic from capillary channel is available for detecting *E. coli*.



(a) (b) (c) (d)

Figure 4.31 Cultured *E. coli* in capillary channel (a) Fluorescence in 2 hours; (b) Fluorescence in 3 hours; (c) Fluorescence in 4 hours; (d) Fluorescence in 5 hours



(a) (b) (c) (d)

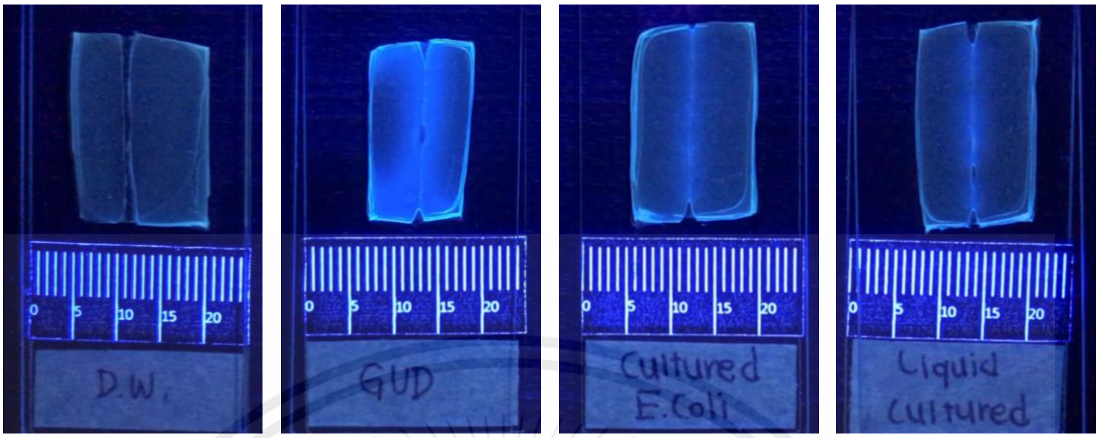
Figure 4.32 Liquid cultured residue of *E. coli* in capillary channel (a) Fluorescence in 2 hours; (b) Fluorescence in 3 hours; (c) Fluorescence in 4 hours; (d) Fluorescence in 5 hours

4.6.3.2 Printed 3 pt and 30 times layer channel

A microfluidic was fabricated from MUG mixed with gelatin and used a printed 30 times layer with 3 pt of straight-line pattern to make a channel of microfluidic. To examine the enzymatic reaction of GUD secreted by *E. coli* and MUG could be occurred in gelatin whether printed 3 pt and 30 times layer channel microfluidic. We fed 0.2 ml of samples including distilled water, 1700 U enzyme GUD, cultured *E. coli* and liquid cultured residue at the inlet node of the microfluidic channel and placed it in the dark box equipped light source to excite the UV light. Take a video time-lapse for 5 hr.

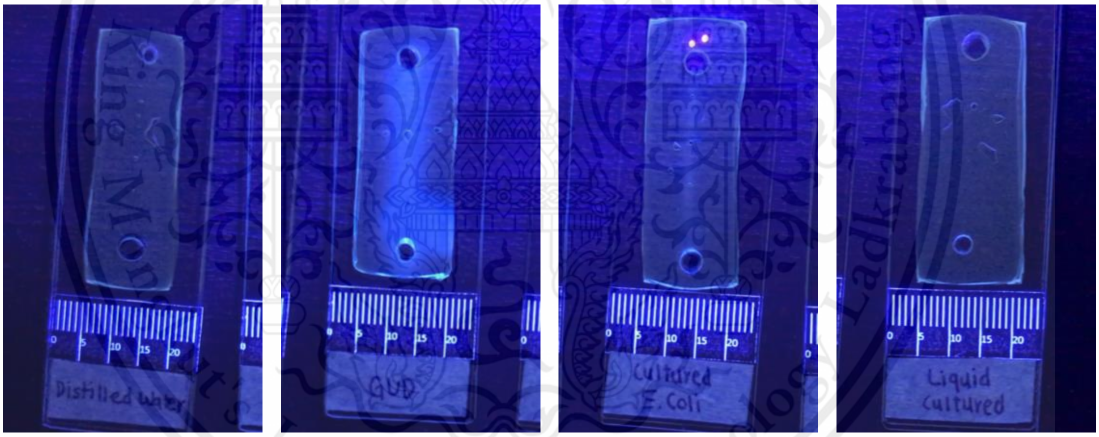
From Figure 4.33 and Figure 4.34, the comparison results of samples of two kinds of forming channels, capillary tube and printed channel at the 3-hour mark which is the appropriate time to compare the results because fluorescence started to occur at this point. The result showed that: (1) distilled water had no fluorescence presented; (2) 1700U enzyme GUD appeared fluorescence in both forming channels. The fluorescent product 4MU was occurred and spread out of the channel to gelatin in the nearby distance; (3) cultured *E. coli* presented the fluorescence light under the printed layer channel but the intensity in the capillary channel was greater than a printed 30 times layer channel. In the capillary channel, the fluorescent was beginning to emit at 2 hours while 30 times printed layer channel began at 2.5 hours. This means the enzymatic reaction in the capillary channel occurred faster than printed 30 times layer channel; and (4) liquid cultured residue, the fluorescence was beginning to emit at 2 hours while 30 times printed layer channel occurred slower than 2 hours as shown in Figure 4.36. These unexpected results probably due to the cultured *E. coli* and liquid cultured residue solutions injected at printed 30 times layer channel was being stored for more than a mouth, but the capillary channel used a newly prepared fresh cultured *E. coli*. Therefore, the metabolism, DNA, protein conformation, and enzyme activity had changed probably resulted in lower intensity of fluorescent product of 4MU presented under printed 30 times layer channel.

Nevertheless, a printed 30-time layer channel showed the fluorescence light emission under the channel, it was gradually produced and spread out of the channel to gelatin. This result indicated that the enzyme GUD secreted by *E. coli* diffused in gelatin and combined with substrate MUG and further release the product 4MU. Therefore, the gelatin microfluidic is available for *E. coli* detection in the sample.



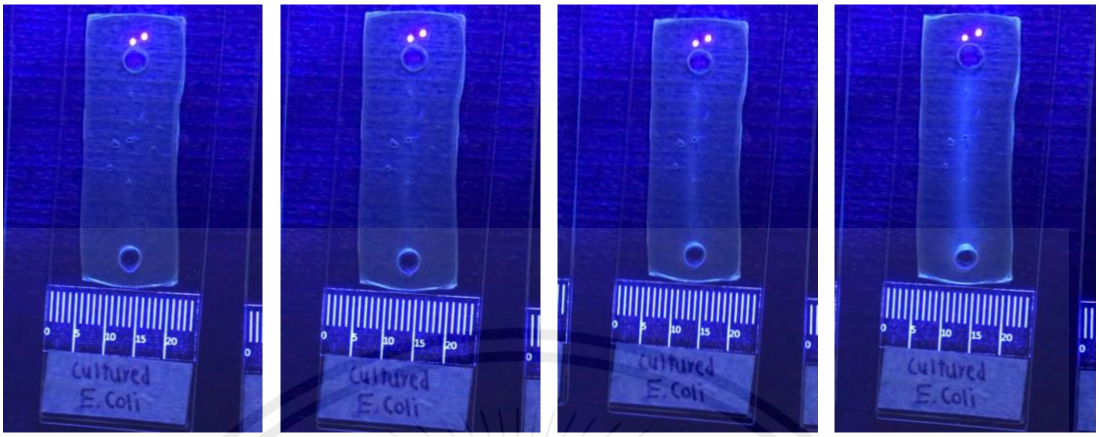
(a) (b) (c) (d)

Figure 4.33 Capillary channel at 3 hours (a) Distilled water; (b) 1700U enzyme GUD; (c) Cultured *E. coli*; (d) Liquid cultured residue



(a) (b) (c) (d)

Figure 4.34 Printed 30 times layer channel at 3 hours (a) Distilled water; (b) 1700U enzyme GUD; (c) Cultured *E. coli*; (d) Liquid cultured residue



(a) (b) (c) (d)

Figure 4.35 Cultured *E. coli* in printed 30 times layer channel

(a) Fluorescence in 2 hours; (b) Fluorescence in 2.30 hours; (c) Fluorescence in 3 hours; (d) Fluorescence in 4 hours

(a) (b) (c) (d)

Figure 4.36 Liquid cultured residue in 30 times printed layer channel

(a) Fluorescence in 2 hours; (b) Fluorescence in 2.5 hours; (c) Fluorescence in 3 hours; (d) Fluorescence in 4 hours

CHAPTER 5
CONCLUSION

5.1 Conclusion

According to, microfluidic fabrication is ordinarily sophisticated, and materials generate waste and pollution in our environment. Therefore, our project was to fabricate a microfluidic channel from gelatin which is a green material, abundant, cheap, environmentally friendly, and reusable. Also, do the microfluidics channel patterning by using the standard laser printer.

To fabricated microfluidic channels, the procedure starts with designed channel pattern on the computer and printed it out on a transparent plastic sheet. The gelatin was prepared by dissolved gelatin powder in heated distilled water at gelatin: water of 1:3. Then pour this warmed gelatin aqueous on a printed template transparent plastic sheet and cooled down for gelatin setting in the refrigerator or leave at room temperature and ready to use.

From the designed node of the microfluidic channel, a circular and triangular node was designed first with the straight-line pattern. The result from fed liquid watercolor indicated that the design of the node does not affect the flow of fluid into channel. The cause of solution could not flow came from the height of channel was not enough which come from the layer of printed ink. Also, in the channel of branches design, a solution could not flow from inlet node to outlet node. Several channel patterns which more complex and commonly used in the microfluidic devices were investigated. Furthermore, the other channel patterns were designed, including T-shape and Y-shape, Y with double wave pattern, branches with curve line, and wave lines.

The gelatin channel formed was confirmed by using the microscope. The result showed that the gelatin channel has a rough surface which is an advantage for increase the reaction rate. The thickness and the width of laser printer ink on a transparent plastic sheet related to the size of the gelatin channel were measured by vernier caliper and microscope with the software of the equipped camera. The size (mm) of width and thickness of printed ink on a plastic sheet of the channel increased with increasing line size (pt) and the number of printed layers. From these results can confirm again that the channel of 3 pt width and 30 times printed layer corresponded to 1.78 mm (width) and 0.19 mm (height) was the effective channel for flowing the water-soluble liquid throughout various patterns of the channel.

The gelatin characteristics were investigated by performing the experiment of UV cut and diffusion. To conclude, the gelatin was able to cut the UV at wavelength under 290 nm which was effective for applied with *E. coli* detection under enzymatic reaction for releasing fluorescent product oof 4MU. The gelatin cut all background of UV light source while detecting *E. coli*. The gelatin also has a diffusion property which a diffusion rate was 0.67 mm/hr. Consequently, the enzyme GUD secreted by *E. coli* was able to diffuse into gelatin.

To examine the microfluidic channel from gelatin using the laser printer applies to varieties of applications. The experiment was applied to *E. coli* detection as the indicator of bacterial contamination evaluation of the sample. This examination measured the fluorescent synthesis of 4-methyl-umbelliferone (4MU), which is the product of the catalytic reaction of the enzyme, D-glucuronidase (GUD) secreted by *E. coli* and its substrate 4-methyl-umbelliferone-β- D-glucuronide (MUG). The enzyme GUD has been widely used as the biomarker for *E. coli* detection in the sample by fluorescent synthesis product of 4MU, which has the excitation wavelength at 366 nm. The experiment was carried out by flowing the liquid solution under the straight-line and Y- shape channel. The enzymatic reaction of GUD and its substrate MUG was used to indicate the availability of channel for flowing liquid by observing the detectable released fluorescent synthesis product of 4MU.

The result was the fluorescence light emission presented under the straight-line channel (Figure 4.25 and 4.26) and Y-shape channel (Figure 4.27). This result indicated that the microfluidic channel from gelatin by using the laser printer is available for efficiently flowing liquid, the enzyme GUD and substrate MUG were flowed toward and then mixed together. The enzymatic reaction proceeded within the channel. This result confirms that the microfluidic channel from gelatin by using the laser printer is available for *E. coli* detection for indicating the bacterial contamination of the sample.

Accordingly, the diffusion property is one of characteristics of gelatin that can be applied to make gelatin microfluidic. A microfluidic was fabricated from MUG mixed with gelatin under two features of forming a channel, using capillary tube and printed channel template mold by a laser printer. To investigate whether enzymatic reaction could be occurred under the channel by used *E. coli* samples which are cultured *E. coli* and liquid cultured residue. The results showed that both capillary tube and a printed 30-time layer channel appeared to have fluorescence, the fluorescent 4MU was gradually produced and spread out of the channel to gelatin. This can be ensured that the enzymatic reaction of GUD secreted by *E. coli* and substrate MUG in gelatin can occur under the gelatin.

This research proves that the gelatin microfluidic fabrication by laser printer is an effective technique compared to the conventional microfluidic fabrication because it can indeed contain the liquid in channel and liquid can flow throughout in various patterns of the channel. Therefore, this gelatin microfluidic by using laser printer can be further effective alternative technique to common sophisticated technique of microfluidic fabrication. Furthermore, we are investigating many applications of this gelatin microfluidic, especially for medical applications.

5.2 Suggestion

1. Find the relation of the amount of *E. coli* colony with the intensity of fluorescent emitted.
2. Practically examine with the water resources, vegetable, or meats

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