UNIVERSITY OF CALIFORNIA, IRVINE

Application of Fused Deposition Modeling and Stereolithography Processes for Fabrication of Point-of-Care Bioassay Platforms

THESIS

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MASTER OF SCIENCE

in Materials Science and Engineering

by

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ABSTRACT OF THE THESIS

Application of Fused Deposition Modeling and Stereolithography Processes for Fabrication of Point-of-Care Bioassay Platforms

By

Yangchung Lee

Master of Science in Material Science and Engineering

University of California, Irvine, 2016

Professor Marc Madou, Chair

This study aspires to apply elastic material to print LOC (Lab-on-the-chip) immunoassay with manufacturing additive techniques. The device is designed for the detection of infectious diseases in the field of developing countries. Applying elastic material can shorten the fabrication time of the device.

The first manufacturing technique we choose is Fused Deposition Modeling (FDM). The reason we choose FDM is because of its low cost and popularity. The elastic material TPU has been chosen to print our LOC immunoassay device. In order to accomplish the final design, different states of prototypes were developed to find the proper settings, geometry, functionality, and to solve emerging difficulties resulted from the characteristics of the elastic material. From our tests, we found leakage in our prototypes; we think this problem is resulted from the resolution limit and characteristics of the FDM technique. After we applied the plastic dips method on the prototypes, the leakage problem improved and the LOC immunoassay functioned properly.

We then employed the SLA technique, which can provide higher resolution and density. We used Formlabs SLA printer with their flexible resin to print our prototypes. The
problem of leakage did improve, but there are two main issues. First, the elasticity of SLA prototypes is not as good as the FDM prototypes. Second, the clog problem caused by the flexible resin increased the difficulty of miniaturization. A further research on utilizing the SLA printer to print the immunoassay with elastic material is needed.

Lastly, an automated Malaria Ab immunoassay device was developed. The device can automatically perform the standard immunoassay steps specified in the Malaria – Ab ELISA manual from IBL international.
1 INTRODUCTION

1.1 Motivation

Microfluidic technology is a promising field. It has the potential to influence areas from chemical synthesis and biological analysis to optics and information technology. The first applications of microfluidic technologies have been in analysis: the ability to use very small quantities of samples and reagents and to perform separations and detections with high resolution and sensitivity. It is low cost, short time for analysis, and small size for the analytical devices. [1, 2]

Conventional methods of producing micro-scale components for MEMS applications such as microfluidic devices can only produce relatively simple geometries and are inefficient for prototype production. Rapid prototyping techniques may be applied to overcome these limitations. The 3D printing system offers great potential and flexibility in creating prototypes of complex 3D structures and parts of any geometry for microfluidic and MEMS devices. It is also easier to test the prototype concept through 3D printing techniques. [2, 3]

However, these techniques need more research to make them more feasible to be applied in the development of microfluidic devices. In order to increase the versatility of 3D printing microfluidic devices, we will use elastic material like TPU and rapid prototyping techniques including FDM and SLA to produce the microfluidic immunoassay devices on demand.
1.2 Background

1.2.1 History of Additive Manufacturing

The concept of 3D printing, also referred to as additive manufacturing, rapid prototyping, was developed by Charles Hull. After graduating from the University of Colorado with a B.S. in engineering physics, Hull started work on fabricating plastic devices from photopolymers in the early 1890s at Ultra Violet Products in California. [22] Because of the lengthy fabrication process and the high probability of design imperfections, Hull was motivated to improve current prototyping methods.

In 1986, Hull obtained the patent for Stereolithography. [23] and went on to acquire countless more patents on the 3D printing technologies. In 1986, he established 3D Systems and developed the .STL file format, which would help transmit electronic files of CAD for the printing of 3D objects. [24]

Hull and 3D Systems continued to develop the first 3D printer termed the “Stereolithography Apparatus” as well as the first commercial 3D printer available to the general public, the SLA-250. With Hull’s work, in addition to the subsequent development of fused deposition modeling (FDM) by Scottj Crump at Stratasys in 1990, 3D printing was ready to revolutionize manufacturing and research. [24] MIT professors Michael Cima and Emanuel Sachs patented the first apparatus termed “3D printer” in 1993 to print plastic, metal, and ceramic parts. [5] Many other companies have developed 3D printers for commercial applications, such as DTM Corporation and Z Corporation, Solidscape and Objet Geometries (which merged with Stratasys). Others include Helisys, Organovo, a company that prints objects from living human tissue, and Ultimaker. 3D printing technology has found applications in the automotive and aerospace industries for printing prototypes of
car and airplane parts and in the consumer goods industry for prototype development for companies.

The applications of 3D printing in private and government defense have been rapidly recognized. For example, applications in gun prototyping and manufacturing processes for the military have already been established. Medical applications of 3D printing date back to the early 2000s with the production of dental implants and prosthetics.[25] Applications in the food industry have also emerged. With regard to research fields, 3D printing has been limited to biomedical applications and engineering, although it shows great potential in the chemical sciences.

### 1.2.2 CAD-file Preparation for Printing

The 3D models of the rapid prototyping originally are generated by a computer aided design (CAD) program such as AutoCAD or SolidWorks. The original design is drafted in a CAD program, where it is then converted to a .STL file. The .STL file format has been accepted as the standard for data transfer between the CAD software and a 3D printer. The .STL file stores the information for each surface of the 3D model in the form of triangulated sections. [4, 5] By increasing the number of triangles that define a surface, more data points exist in the text file to spatially define the part surface. This increases the resolution of the printed device. [5]

The 3D printer interprets the digitally supplied coordinates derived from the .STL file by converting the file into a G-file via slicer software present in the 3D printer. The G-file divides the 3D .STL file into a sequence of two-dimensional horizontal cross sections (25–100 μm, depending on the fabrication technique), [5, 6] which allows the 3D object to
be printed in consecutive layers of the desired material from the base, essentially constructing the model from a series of 2D layers derived from the original CAD file. [5, 7, 8]

1.2.3 Introduction to the Additive Manufacturing Technologies –

FDM and SLA

According to the principles, 3D printing techniques could basically be separated into four main categories.

- **Molten polymer deposition**: Fused Deposition Modeling (FDM), Directed Energy Deposition.
- **Photopolymerization**: Stereolithography (SLA).
- **Sheet Lamination**: Laminated Object Manufacturing (LOM).
- **Granular materials binding**: Selective Laser Sintering (SLS), Selective Laser Melting (SLM), Binder Jetting (BJ)  [9, 10, 12]

In our study, mainly two kinds of additive manufacturing technologies will be employed: one of the techniques is Fused Deposition Modeling (**FDM**), the other is Stereolithography (**SLA**).

The **Fused Deposition Modeling (FDM)** technique is based on Molten Polymer Deposition. The filament of thermoplastic materials is melted and then extruded; after the deposition, the material would cool down and harden again. Then, the structure is formed layer by layer. The filament is usually stored on a spool. Another term for this technique is Fused Filament Fabrication (FFF). [10] A detailed FDM diagram is shown in Figure 1.1.

FDM was first introduced in 1991 and commercialized by Stratasys in 1996. [5] FDM is
one of the most widely used manufacturing technologies for rapid prototyping today. It is widely used for the building of prototypes and the fabrication of functional parts in engineering plastics because of its simplicity and affordability. It builds complex structures by a simple process of extruding heated filaments through a nozzle onto a platform based on a digital model. FDM is furthermore appreciated by industry, academia, and consumers for its reliability and affordability. [14] A notable advantage of FDM is that it can create objects fabricated from multiple material types by printing and subsequently changing the print material, which enables more user control over device fabrication for experimental use. [5]

The materials are positioned on spools on the back side of the printer. Different materials can be used for the built and the support structures. Driving wheels push the filament to extrude through the nozzles, while liquefiers inside the nozzle head heat up and melt the materials. The printer head extrudes the material layer by layer onto a preheated base and proceeds X-Y-movement of the nozzles. The Z-motion is controlled by the movement of the platform base. The base is a flat plate that can be heated for a better contact between the material and base. [15]

The printouts have usually two layers of material on the surface and are filled with a honeycomb structure generated by the software. The amount of material used for the honeycomb structure can be chosen by changing the settings for the infill in the software.
The **Stereolithography (SLA)** technique belongs in the category of Photopolymerization. Photopolymerization techniques cure a liquid material layer by layer by displaying light on the area of the layer, which is part of the desired shape. After curing one layer the z-stage lowers and a sweeper provides new liquid material on top of the cured material. Then the light hardens the new layer of liquid again. [10] A SLA diagram is shown in Figure 1.2.

Stereolithography (SLA) was the first commercialized 3D printing technique. It was developed by Chuck Hull at 3D Systems in 1987. [11] It uses ultraviolet light from a laser to cure the liquid ultraviolet light sensitive polymer. The main advantage of Stereolithography is its high resolution and smoothness of the models.
Figure 1.2: Functional Principle of Stereolithography; liquid photopolymer hardens by lasercuring the area of the design, the scanning mirror leads the laser beam in the destination area cf. [9]

There are several different approaches to SLA, including direct-laser writing and mask-based writing. The approaches can be separated into a free surface or constrained surface technique depending on the orientation of the laser source. The direct-laser writing technique contains the common components of a movable base, a tank of liquid resin, a UV light beam, and a computer interface. The mask-based writing also contains the movable platform, resin vat, computer, and UV beam as well as a mask that allows for the curing of a single layer at once. [5] Table 1.1 shows a comparison between FDM and SLA technique.
Table 1.1: Comparison between FDM and SLA Techniques

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<td>Name of techniques</td>
<td>Fused Deposition Modeling (FDM)</td>
<td>Stereolithography (SLA)</td>
</tr>
<tr>
<td>Materials</td>
<td>Thermoplastics (eg. ABS, PLA, TPU)</td>
<td>Epoxy, resins acrylate</td>
</tr>
<tr>
<td>Advantages</td>
<td>Higher mechanical and thermal properties than SLA, high degree of freedom in material deposition, very interoperable with automation</td>
<td>High level of details, very smooth surfaces</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Rougher surfaces, lower level of details than SLA, distinct built line between layers</td>
<td>Lower mechanical and thermal properties than SLM an FDM</td>
</tr>
</tbody>
</table>

1.2.4 Advantages of Additive Manufacturing Techniques for the Microfluidic Device

The reasons we choose additive manufacturing techniques to produce our microfluidic device are listed as follows:

1. Additive manufacturing techniques can be applied to overcoming the limitation of conventional methods which are inefficient for prototype production and incapable to produce complex geometries. [2] For example, the FDM system offers great potential and flexibility in creating prototypes of complex 3D structures and parts of any geometry for microfluidic and MEMS devices.

2. When fast concept-to-chip time is a priority, one-step manufacturing techniques are favored over conventional methods. Conventional photolithographic methods for three-dimensional designs need accurate alignment. The ability of the additive manufacturing
techniques to transform digital designs directly into physical models will not only accelerate the fabrication process but also make practical evaluation of different designs faster and easier. [26] Due to the time and cost-effectiveness of additive manufacturing techniques for the production of microfluidic devices, it is straightforward to alter the design of the devices in terms of geometry.

3. An advantage of 3D printed devices is that they can be printed on demand, and the design files could be shared online or as part of the publication process so that technology can be shared between laboratories.

4. As FDM printer are available starting at $500 and a spool of material can be purchased around $15-$70 it is a very affordable technique [10]. This is even more important for microfluidic devices for biomedical purpose. Because of contamination, the devices frequently need to be disposed after the first usage. In addition to the low investment and material costs, the process itself is also very cost-efficient. This is because FDM does not need to be done under protective gas or in cleanrooms. It is also very easy to operate does not need highly trained personnel

1.3 Researches on FDM Microfluidics

In 2001, Hengzi et al published their proceedings for the application of FDM for producing microchannels by using ABS. They utilize the printed device to study the mixing behavior of fluids through the microchannels. They analyzed the minimum diameter of printable channels and their surface roughness. The minimum diameter of a permeable channel was found to be 0.5mm. A problem they found is the leaking due to insufficient bonding between adjacent layers. One solution to this problem may be to smooth out the
surfaces by chemical coating. The roughness of the structures can also be improved by optimizing the control of the FDM equipment and by proper selection of FDM process parameters [2].

In 2012 Phillip J. Kitson et al fabricated FDM printed reactionware devices using Polypropylene (PP). Their function was demonstrated by performing gold nanoparticle synthesis in the microfluidic device. The devices were found to be robust, reliable and due to the use of PP inexpensive and chemically inert. [3]

A research group at the State University of Michigan published an evaluation of 3D printing for Biotechnology in 2014 comparing FDM printed microfluidic devices to devices fabricated with different 3D printing techniques. They found FDM to be a fast 3D printing technology for the fabrication of devices with embedded cannel. [5]

In the biomedical field, because of its leakage problem and biofouling nature, 3D printed ABS devices are not widely used in comparison to materials such as silicones and polyurethanes which offer greater native biocompatibility. In order to improve the impermeability of the 3D printed ABS devices, McCullough et al studied the surface modification of 3D printed ABS devices. They tested different methods to close the spaces between adjacent layers. They used a treatment of the plastic with acetone. In order to increase hydrophicillity of the ABS photo-induced graft polymerization of poly (ethylene glycol) was used. The received devices were characterized for water tightness and microfluidic properties. [20]

A research project proceeded by Stratasys Inc. and a research group at the W.M. Keck Center for 3D Innovation gives an overview over different treatments for FDM printed ABS parts for different pressures. This report analyses cases of failure of ABS parts and presents
a guideline for choosing the right surface treatment for the desired application in order to avoid leakages and catastrophic failure. [21]

There are several main advantages of the FDM printing technique, for printing microfluidic devices are:

- The possibility to print different materials,
- The possibility to print elastomeric materials,
- Low costs.

The possibility of printing different materials derives from the availability of multiple printer heads in some FDM printers. [5] Each printer head can be loaded by a different material and the provided software supplies with the specific extrusion temperatures for the different materials. As the printer heads are attached to each other, only one material is printed at a time. But as soon as one printer head stops extruding, another one can be activated.

The FDM technique also enables to print thermoplastic elastomers. Hence rigid and flexible materials can be combined in one print. This enables to make different features like valves or squeezable chambers for microfluidic devices.

In our research, we use Thermoplastic polyurethane (TPU) on an Airwolf printer. The results prove that thermoplastic elastomers can build good quality designs on FDM printing machines.

Another elastic material that was printed by a research group at the University of Seoul around Kyu-Jin Cho printed a Material called E20 with a FDM printer in order to receive soft elastic material. E20 is a polyester-based elastomer used for the fabrication flexible components. [22]
1.4 Researches on SLA Microfluidics

Many researchers think Polydimethylsiloxane (PDMS) is an ideal material for microfluidic devices; PDMS is inexpensive, optically clear, biocompatible, and easy to be molded. However, Anthony k. Au et al point out some disadvantages of PDMS; it is difficult to automate and fabrication process is slow, which means it is not easy to producing large numbers of copies of a device per day for the commercialization process. Although high through-put plastic molding like injection molding exists, the cost of the molds and equipment can be very high, which could be an obstacle for commercialization. [30]

They think Stereolithography (SLA) is an ideal technique that can solve the problem encountered by soft lithography. SLA is more convenient, faster, more cost efficient. It also allows for producing 3D architectures that are not possible with PDMS molding. In their paper, they evaluate the resolution of Stereolithography and find out the main causes of resolution loss is the optical clarity of the devices. They think this technique has key advantages for commercialization:

1) The designer does not need to order expensive molds or set up a company for launching production of his or her devices

2) The customer can order just one print without minimum quantities through mail-order services

In another research, Toshimitsu et al used Stereolithography technique to fabricate a microfluidic device which can be used for preparing monodisperse functional particles. They introduced several different techniques that have been used for the microfluidic devices. In the paper, they mention that capillary microfluidic devices can be fabricated at low cost, but it is troublesome to set the positions and sizes of the capillaries precisely.
Other technique like the soft lithography can more easily control the positions and sizes of channels in the Polydimethylsiloxane (PDMS) device through the design of mask patterns; this technique also has the potential to be used for mass production. However, it is difficult to fabricate a device with complex channels in three dimensions by soft lithography.

Therefore, Toshimitsu et al tried to use Stereolithography (SLA) to fabricate the microfluidic device, because it can build the structure with complex flow channels in three dimensions. Besides that, the device can be directly fabricated from CAD files, fine-tuning of the channels can be performed easily and efficient. [31]

1.5 Objective of Thesis

The objective of this thesis is to utilize elastic material and apply different additive manufacturing techniques to improve our design of microfluidic device for the Malaria-Ab Immunoassay. In our lab, we have used Acrylonitrile Butadiene Styrene (ABS) to produce our embedded microfluidic device for the Malaria-Ab Immunoassay. The prototype and the design are shown in Figure 1.3.

![Figure 1.3: The ABS prototype for the Malaria-Ab Immunoassay](image)
Because ABS is inelastic, we have to use other elastic material, silicone, to make the pump structure by molding. If we can print the whole immunoassay device with elastic material, we can shorten and simplify the fabrication process of the immunoassay device.

To achieve this goal, we will try to use the FDM technique to print our immunoassay devices with the elastic material Thermoplastic polyurethane TPU. To make a comparison, we will also try to apply a different technique SLA to print our immunoassay device. The SLA technique can provide higher resolution than FDM, which can possibly help achieve smaller width of the channels and help decrease the size of the structure. Due to the characteristics of the SLA technique, the layers are highly connected to each other without building holes and density of the prototype could also be highly improved.

In the last part of the thesis, we will design an automated Lab-on-Chip immunoassay device which incorporates our SLA prototype and the automated mechanism. The automated device can perform the standard immunoassay steps specified in the Malaria – Ab ELISA manual from IBL international.
2 MALARIA – AB IMMUNOASSAY

Our design of immunoassay devices is based on the process of the Malaria-Ab ELISA Immunoassay from IBL International. This kit was chosen as it is commercially available, contains break-apart well strips, and is with $153 per kit in the lower price range [27].

Content and Function

This section will give an overview of the content of the kit and the different steps for testing against Malaria. The detailed preparation and procedure steps can be found in the Instructions to Use for Malaria-Ab ELISA, provided by IBL International [28].

The reagents supplied in the kit are:

- 12 break-apart 8-well snap-off strips coated with recombinant Plasmodium antigens (P. falciparum, P. vivax) in aluminum foil.
- 1 bottle Sample Diluent containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2.
- 1 bottle stop solution containing 15 ml Sulfuric acid, 0.2 mol/l.
- 1 bottle washing solution containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2).
- 1 bottle Malaria conjugate containing 20 ml of peroxidase labeled antibody to human IgG and IgM.
- 1 bottle TMB Substrate Solution containing 15 ml 3,3′,5,5′-tetramethylbenzidine (TMB).
- 1 bottle Malaria positive control containing 2 ml.
- 1 bottle Malaria Cut-off Control containing 3 ml.
- 1 bottle Malaria Negative Control containing 2 ml. [20]

For the preparation of the Malaria test, blood samples have to be diluted as well as the washing solution.
The different steps as recommended for processing the Malaria-Ab ELISA are:

1. Dispensing 100µL controls and diluted samples into the wells (leave A1 blank).
2. Covering the wells with foil.
3. Incubating the wells for 1h +/- 5min at 37°C.
4. Removing the foil, aspirating the content, washing the wells (5* 350µL) >5s soak time, with no overflow; removing fluid by tissue paper.
5. Dispensing 100µL Malaria Conjugate in all wells except A1, covering the wells with foil.
6. Incubating the wells at RT for 30min, with no direct sunlight.
7. Removing the foil, aspirating the content, washing the wells (5* 350µL) >5s soak time, with no overflow; removing fluid by tissue paper.
8. Dispensing 100µL TMB Substrate Solution in all wells.
9. Incubating for exact 15min RT, in the dark.
10. Dispensing 100µL Stop Solution in all wells, in the same order and rate like the TMB Substrate Solution.
11. Measuring the absorbance of the specimen at 450/620nm within 30min after dispensing the Stop Solution. [28]

**Steps to implement**

Our goal is to design a lab-on-chip immunoassay device that can implement the steps 1-11. While the interpretation of the results will not be focused on in this thesis, the readout of the color change and hence the readout of the results will be implemented.
3 FDM IMMUNOASSAY PROTOTYPES - DESIGN FOR ADDITIVE MANUFACTURING

![Flashforge 3D Printer (FDM)](image1)

**Figure 3.1**: Flashforge 3D Printer (FDM)

Not every FDM 3D printer can print elastic material. For example, Flashforge 3D printer shown in Figure 3.1 can be used to print ABS prototypes, but it is not an ideal printer to print prototypes with elastic material. The filament of elastic material will be dragged around the cogwheel, which drives the filament through the printer heads, and clogged the printer head. Due to the blockage of the cogwheel, the extrusion will stop. Therefore, we decide to choose Airwolf AW3D HD2x FDM printer which is a more ideal printer to print elastic material. The machine is shown in Figure 3.2.

![Airwolf AW3D HD2x 3D Printer (FDM)](image2)

**Figure 3.2**: Airwolf AW3D HD2x 3D Printer (FDM)
3.1 Printing TPU with Airwolf AW3D HD2x (FDM) Printer

The elastic material we used for our FDM prototypes was Thermoplastic polyurethane TPU. Before we started printing our TPU prototypes, we had to adjust the settings of the printer to optimize the result of our printing. Because of different characteristics of the materials, the settings of the machine for TPU were different from ABS.

We mainly tested three different settings that would affect the quality of our printing:

1. Extrusion temperature

   The first approach to improve the extrusion of the filament is the optimization of the extrusion temperature. Even if the filament is only heated at the nozzle, it is assumed that the temperature of the filament before entering the nozzle is highly influenced by the extrusion temperature. As a lower temperature lead to a higher viscosity, a lower temperature could help to prevent the filament from being stuck before entering the nozzle. [32]

2. Extrusion speed

   The extrusion speed is the length of the filament which is extruded through the nozzle per time unit. A lower extrusion speed could lead to a more uniform temperature distribution, while a higher extrusion speed would prevent the filament in the printer head from heating up. However, if the extrusion speed is too high, the extruded filament will start accumulate in certain areas, which would lower the quality and resolution of the printing.

3. Travel speed

   The travel speed is the way in X-Y-direction, which the printer head travels per time unit. This setting will affect the quality of printing. If the nozzle head moves too fast,
the filament of material will not deposit properly on the surface. This setting is correlated with extrusion speed.

**Extrusion Temperature Test**

The extrusion temperature tested were 225°C to 240°C in 5°C steps. At 225°C the filament did not melt enough to get a good attachment to the base. The printer head just trailed the extruded filament behind. When the temperature reached 240°C, the melted filament will keep flowing out of the nozzle; it became hard to control the extrusion of the material. Hence the best temperature setting concerning the material was found to be between 230°C and 235°C.

**Travel Speed Test**

First, we tested how different travel speed would affect the results of the printing. The test samples are shown in Figure 3.3. The sample is a 2.5cm x 2.5cm square with the thickness of 1.5mm. On the top of the sample, there are two structures: one is a T-shape structure and the other is a channel. The inner diameter of the channel is 1.5mm and the outer diameter is 3mm. From this test sample, we can check how different settings affect the channel structure and sharp angle of the sidewall.

The travel speed for each sample from left to right is 15, 20, and 30 mm/s and all other settings remain the same. The results show that the printing quality is best when the printing speed is 15 mm/s, which has fewer holes on the surface than the others. On the other hand, the surface quality is the worst when the printing speed is 30 mm/s. These samples indicate that printing speed has great influence on the quality of the prototype.
Travel Speed and Extrusion Rate Test

After the test of the travel speed, in order to make a more clear comparison, we tested on how printing speed and extrusion rate would affect the results of the printing. In figure 3.4, from left to right, the extrusion rate is 100%, 115%, 130%. From top to bottom, the travel speed is 13mm/s, 15mm/s, and 17mm/s. From the picture, we can see that when we increase the extrusion rate, the material will start to accumulate in some spots, which will decrease the resolution.
After the tests, the settings we chose for our TPU prototypes are listed in the Table 3.1.

<table>
<thead>
<tr>
<th>Travel speed</th>
<th>13 mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrusion speed</td>
<td>100%</td>
</tr>
<tr>
<td>Extrusion temperature</td>
<td>230°C</td>
</tr>
<tr>
<td>Base temperature</td>
<td>Not heating</td>
</tr>
</tbody>
</table>

Table 3.1 Main Settings for the Airwolf 3D Printer

3.2 The Difference between ABS and TPU Printing

When printing ABS, we need to heat up the base of the FDM printer in order to help the prototype stick to the platform better or the prototype might peel off from the platform. For TPU prototypes, we don’t need to heat up the base because of its elastic characteristics. If we heat up the platform, it will be hard to remove TPU prototypes from the platform after the printing is finished and the heating tape on the platform could be tear off.

Second, the process of printing TPU is not as stable as ABS. One of the reasons is the elastic characteristics of TPU. From the previous introduction to FDM, we know that the filament is being pushed by the cogwheels and extruded through the nozzle. If the filament is too flexible, it will increase the difficulty for the machine to send the filament through the nozzle because the cogwheels will not be able to apply enough force on the filament to send it through the nozzle.

A failure TPU sample is shown in Figure 3.5. The middle part of the sample is continuous and even, but the left area is not printed well.
3.3 Design for TPU Prototypes

From the previous tests, we found out what were the optimized settings for our TPU prototype. Our next step was to design and print a microfluidic prototype with TPU.

The idea was to employ elastic domes on top of enclosed chambers. By pushing the domes down, the fluid in these chambers was forced to flow through the channels into the next chambers. One main reason we used elastic material for our prototypes was that we did not need to build the pumps and channels separately.

First, we designed a simple prototype including a pump chamber, a microfluidic channel, and a holder which was for the immunoassay well. The design is shown in Figure 3.6. Then the first TPU prototype was printed and shown in Figure 3.7.
In this prototype, there is a channel connecting the pump and the holder. By pressing the pump, the fluid in the pump can flow through the channel and reach the holder.

### 3.4 Minimize Surface Defects on the Prototype

From the picture, we can see many defects on the surface of the prototype. This is because the machine has two nozzle heads and the other nozzle head not being used scratches on the prototype while printing. This was probably because the platform was not completely horizontal or the two nozzle heads were not parallel, so we calibrated the platform and the nozzle heads to fix this problem.

Beside this problem, we also found out many holes on top of the pump structure. We guessed that it was because the top surface was too flat and there was no support inside the pump. Because of the characteristics of FDM, we need to add supports under a large suspended area, or the area will have defects or collapse.

The first solution to this problem was to print a dome-shaped structure instead of a flat one. A dome-shaped structure could help each layer support the next layer better and could avoid defects on the top. The reason we did not want to use support inside the pump
was that the support would increase the stiffness of the pump and make it hard to press.

The second solution was to decrease the circumference of the pump, which could reduce the top area and decrease the necessity of support for the top.

The third solution was to increase infill density of the pump, which can probably give more support to the top. However, there was a tradeoff for the third solution. Increasing the infill density could increase the stiffness of the pump and make it hard to press.

**Changing the Circumference of the Pump**

First, we tried to decrease the circumference of the pump. The result is shown in Figure 3.8. The first prototype is on the right and the second prototype is on the left. We reduced the thickness of the pump size from 2cm to 1.6cm. We could see the condition improve, but there were still some holes remaining on the top.

![Figure 3.8: Comparison between the Prototypes with Different Circumference; Right: the first prototype with thickness of the pump equal to 2.0cm, Left: the second prototype with thickness of the pump equal to 1.6cm](image)

**Increasing Fill Density**

Next, we tried to increase the fill density of our prototype form 20% to 30%. The “Fill Density” is the amount of infill the printer will use when filling the internal space of your
object. The Cura software allows the user to put in any value from 0% to 100% for the “fill density”. 0% is hollow and 100% is full solid. Just 5% to 10% infill is enough for many objects, however a higher fill density will make your product stronger.

We tested on how the fill density affected the surface quality on the top of the pump. The result is shown in Figure 3.9. We can see that the prototype with 30% fill density has fewer holes on the top than the one with 20%. It is probably because the in-filled material provides better support to the top surface. However, we have to notice that when we increase the fill density, we also increase the stiffness of our prototype.

![Figure 3.9: Comparison between the Prototypes with Different Fill Density; right: the prototype with 20% fill density, left: the prototype with 30% fill density](image)

**Changing the Geometry of the Design**

We then tried to improve the top surface of the pump by changing the geometry. We increased the thickness of the wall around the pump from 1mm to 1.5mm and the thickness on the top by 3mm. The result is shown in Figure 3.10. Comparing to the previous prototypes, we can see that the surface quality does improve; there is no visible defect on the surface. Figure 3.11 shows another more complex TPU prototype we have printed; there are two metering chambers connected to the pump by two separate channels.
Before we continued to print more complex structures, we checked if these prototypes had leakage problem. In our leakage test, the prototypes functioned well. Therefore, our next step was to print a more complex TPU prototype.

### 3.5 Design of TPU Prototypes for Malaria-Ab Immunoassay

#### 3.5.1 The ABS Prototype for Malaria-Ab Immunoassay

Our lab had designed an ABS prototype for the Malaria-Ab Immunoassay. The
prototype included four chambers for the reagents below:

- Washing.
- Malaria Conjugate.
- TMB Substrate.
- Stop Solution.

These were connected to the metering chambers, which distributed the reagents to the detection chambers containing the wells with the antigen. The metering chamber was vented on both ends to allow complete filling with the fluids. The design is shown in Figure 3.12. Because ABS material was inelastic, the elastic domes were made from silicone and glued on the top of four round chambers with Sil-Poxy (Silicone Rubber Adhesive). The silicon pumps were made from molding. The main drawback of this design is that we cannot make the whole device in a single step.

![Figure 3.12: The Design of the ABS Prototype](image)

**3.5.2 First Generation of TPU Prototypes for Malaria-Ab Immunoassay**

For the TPU prototype, because the material is elastic, we can design a prototype that can be printed in one single step without the need to integrate elastic silicone pumps. The
The prototype contained four pumps, which had the same function as the ones in our ABS prototype. Instead of having seven metering chambers, we cut the number of chambers to two to simplify the design. Each pump was connected to the metering chambers with separate channels.

In order to test the function of the prototype, we used needle to penetrate the pump and injected colored water. We then pressed the pump to actuate the fluid to flow through the channels to the metering chambers. We checked if the fluid could go through the channels and if there was any leakage on the structure.

In our test, water could successfully reach the metering chambers. However, we found that the prototype had leakage problem. Leakage occurred mostly in the area of the channels and the edge of the structure. We came up with several approaches to fix this problem.

**Surface Treatment**

Our first attempt to improve the leakage problem was to deal with the surface of the prints. For ABS prototypes, acetone can be used to make the rough surface smoother,
because the acetone dissolves the ABS and fills up the space between the filaments. However, after our test, we found that acetone could not interact with TPU, so this method did not work.

Another surface treatment was to coat the prototype with wax. The goal is to coat the prototype with wax which closes smaller holes and fills up bigger leakages. However, we found that when we coated the prototype with wax, it was hard to coat the channels inside the prototype evenly. Even worse, the wax could clog the channels.

Eventually, we decided to use the third method, plastic dips. We applied melted plastics on the surface of our prototype to seal the leakage of the prototype. In Figure 3.13, you can see the surface of the prototype is rough, which is resulted from our application of plastics on it.

**Geometrical Optimization**

One other approach to improve leakage problem is to change the geometry of the prototype. We simplified the design by reducing two metering chambers to one. The result is shown in the left picture of Figure 3.14. Each pump is connected to one metering chamber by one single channel. To test this new design, we printed the prototype with ABS first, because printing ABS prototypes was more reliable and easy. The white one is our first ABS prototype for the new design. We add supports underneath the channels and the whole structure is printed pretty well. One disadvantage of this design was that the fluid inside the metering chamber could flow back to the pumps and cause contamination. Therefore, we increased the height of our metering chamber and the angle of the channels, which could help prevent the liquid in the metering chamber flowing back to the pumps. The second prototype is shown in the right picture of Figure 3.14.
In order to check the leakage, we injected the colored water and let colored water flow through the channels to the metering chamber. The result in Figure 3.15 shows that the prototype could handle the leakage problem well.

3.5.3 Second Generation of TPU Prototypes for Malaria-Ab Immunoassay

Our next step was to print the TPU prototype with this new design. Figure 3.16 shows the result. The prototype was successfully printed and we applied plastic dips on the surface of the prototype. The surface of the prototype was fuzzy. In the process of printing, because of the characteristics of TPU, the material would keep flowing out of the nozzle
while the nozzle head was moving, which resulted in the fuzzy surface.

Figure 3.16: Second Generation of the TPU Prototype for the Immunoassay

3.6 Discussion on FDM Immunoassay Devices

In this chapter, we successfully used elastic material TPU to print our immunoassay device by applying the FDM technique. Like what we mention earlier, the reason we choose the FDM technique is because of its low cost and popularity. If we can apply this technique to produce the Malaria-Ab Immunoassay device, the device can be available for the detection of infectious diseases in the field of developing countries.

From our tests, we find some limitation of FDM techniques. The resolution of FDM is not as high as some other 3D printing techniques like SLA. This limitation will result in some problems. First, the size of the prototype can’t decrease. Second, this limitation could cause leakage problem when the geometry is too complex. Figure 3.17 shows the picture of our ABS prototype. You can see apparent layer steps on the surface. The resolution of the prototype in z direction is decided by the thickness of one single layer. When the design becomes complex, the leakage will occur easily in the areas between two adjacent layers.
Because of the limitation of the FDM technique, we decide to apply the SLA technique to print our immunoassay devices. Compared to FDM, SLA has higher resolution, which could be beneficial for miniaturization. Besides that, because of the characteristics of the technique, the layers of SLA prototypes could be highly connected to each other without building holes and density of the prototype could also be highly improved, which might help solve the leaking problem.
4 SLA IMMUNOASSAY PROTOTYPES

4.1 Introduction to Form 1+ 3D Printer

The SLA machine we used for our research is Form 1+ 3D printer in Rapidtech. The setup is shown in the Figure 4.1. The company provided different resins for different purposes. For our purpose, we chose flexible resin. [33]

![Figure 4.1: The Setup of the Form lab 1+ SLA Printer](image)

It was not hard to operate this 3D printer. We adjusted the settings by the software and then sent our STL file through USB to the printer. There was only one button on the machine which was used for starting or stopping the printing process. The prototype would be attached to the platform when the printing was finished like Figure 4.2.

![Figure 4.2: Finished Sample on the Platform](image)
After the printing is finished, there will be lots of resin remaining on the prototype. To clean the remaining resin, we need to put the printed prototype in rinse buckets filled with isopropyl alcohol (IPA); IPA will dissolve the remaining resin on the prototype.

The Problem Encountered with Form 1+ 3D Printer

Usually, the result of the printing would attach to the platform, but when we printed our prototype, the prototype failed to attach on the platform. We tried to adjust the settings of the machine to fix this problem.

First, we increased the thickness of supporting base. Supporting pillars and prototypes were created on top of the supporting base. A thicker base could help the part stick better to the platform.

Second, we used ‘fine tuning’ function provided in the software. This setting changes the “zero height” between the platform and the bottom of the resin tank. In our case, to fix the problem of attachment of the parts, we needed to decrease the zero height between the platform and the bottom of the resin tank.

After the adjustments, we found that the first method does not work, but the second one worked. We then adjusted the zero height between the build platform and the bottom of the resin tank from 0 to 0.5 mm.

4.2 SLA Sample Test

The first prototype we printed was a simple structure like the one we printed with the FDM printer. It included a single channel in the middle and a T-shape structure on the edge. The sample is shown in Figure 4.3. We can tell from the picture that SLA prototype had
much higher resolution and smoother surface than the FDM prototype; it also provided higher density than FDM. The base underneath the sample was the support which helped the sample attach to the machine’s platform better. When the printing was finished, we needed to cut the sample off from the supporting pillars.

![Image](image1.jpg)

**Figure 4.3.** The SLA Test Sample

Even though SLA could provide better resolution and smoothness of surface than FDM, there was one disadvantage of SLA. Because the whole process of printing was in the resin, when we printed a channel or enclosed structures like pumps, the resin would be stuck inside. Due to its high viscosity, the resin was not easy to be cleaned from the channel or enclosed structures.

### 4.3 Test on SLA channel’s width and length

We would like to know what the smallest width of the channel could be achieved by this printer. We printed three samples with different length and each of the samples had five channels with different width. Figure 4.4 shows the samples and table 4.1 shows the result from the test.
After the printing was finished, all samples were left in the IPA solution for 30 minutes to clean the remaining resin on the samples. From the table 3, we found that all the channels in the 5cm-long sample were clogged but when the length of the channel decreased, some channels with larger width could be cleaned. From this test, we found that the length of the channel was also an important factor that could affect the smallest width of the channel achieved by the printer; increasing the length of the channel would increase the difficulty of cleaning up the channel.
4.4 Methods to Clean the SLA Channel

We would like to experiment on how we can improve the width and length of channels that could be printed by this machine.

The first method we tried is to extend the period of time in the IPA solution for the SLA sample. After the printing was finished, in order to clean the remaining resin on the surface of the prototype, we needed to leave the printed prototype in the IPA solution for about 30 minutes. We tested if increasing the period of time in the solution could help clean the channels better.

Instead of leaving the samples in the solution for 30 minutes, we left them in the IPA solution for 6 hours. Figure 4.5 and Table 4.2 shows the result of the test. Extending the period of time in the IPA solution did help clean the channel. We could see that the 5cm long channel with the width larger than 1.75mm is cleaned after this process. We also tried extending the period of time from 6 hours to one day. The clog problem did not improve much.

One thing to notice was that the IPA solution could deteriorate the SLA sample if we left the sample in the solution for a long period of time. After leaving the prototype in the IPA solution for one day, some tiny cracks appeared on the surface of the sample.
Figure 4.5 : Test Samples for the Smallest Width of the Channel (IPA)

<table>
<thead>
<tr>
<th>Length</th>
<th>Diameter</th>
<th>0.5mm</th>
<th>0.75mm</th>
<th>1 mm</th>
<th>1.25mm</th>
<th>1.5mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5cm</td>
<td>x</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>3.0cm</td>
<td>x</td>
<td>x</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>5.0cm</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
</tbody>
</table>

Table 4.2 : The Result of the Test on Channels’ Width (IPA); o - the channel is cleaned, x – the channel is clogged

We also tried to use the syringe to help clean the channels. In the Figure 4.6, the left picture shows the sample before we applied the syringe to suck the resin out; the right one shows the sample after we applied the syringe to clean the channels. Table 4.3 shows the result of the test.
Figure 4.6: Comparison between the Samples for the Channel's Width (Syringes); right - the sample before we applied the syringe, left - the sample after we applied the syringe.

<table>
<thead>
<tr>
<th>Diameter</th>
<th>0.5mm</th>
<th>0.75mm</th>
<th>1 mm</th>
<th>1.25mm</th>
<th>1.5mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before applying the syringe</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>o</td>
</tr>
<tr>
<td>After applying the syringe</td>
<td>x</td>
<td>x</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
</tbody>
</table>

Table 4.3: The result of the Test on Channels’ Width (Syringe); o - the channel was cleaned, x - the channel was clogged.

**Discussion on Methods for Cleaning Channels**

For the straight channel, the easiest way to clean the channel might be using the wire to push through the channel. However, the wire can’t be used to clean the tortuous channel. The elastic characteristics of the prototype will also increase the difficulty to push the wire through the channel.

Another method is to use IPA solution to help dissolve remaining resin inside channels. From our test, leaving the printed prototype in the IPA longer did help clean the channel. However, if we left the prototype in the solution too long, the material of the SLA prototype would deteriorate and the prototype would become fragile. If the prototype just included channels, the deterioration probably would not have tremendous effect on it. However, if
the prototype included some mechanical parts like pumps, the deterioration would make the parts easy to break.

Applying the syringe will also be an ideal way to clean the channel. This method can be applied to cleaning tortuous channels and will not result in any negative effects on the prototype. However, the viscosity of the resin increases the difficulty of applying this method for small width channels. To clean small width channels, you need to use the needle with small diameter. The resin will then be stuck in the needle due to high viscosity.

**The Comparison between the Machine’s Resolution Settings: 0.1 mm and 0.05 mm**

One other factor that can affect the performance of the machine on printing channels is the machine’s resolution setting. For this machine, there are two resolution settings: 0.1mm and 0.05mm. The Figure 4.7 and Table 4.4 show the results with two different resolution settings. We can see that the smallest width of the channel achieved with the resolution setting of 0.05mm was 0.75mm; on the other hand, the smallest channel achieved with the 0.1 mm resolution setting was 1mm. Therefore, even though all our channels’ width was larger than 0.1mm, the resolution setting could still affect the printing results.

![Figure 4.7: Comparison of the Samples with Different Resolution Settings; left: the sample with 0.05mm resolution setting, right: the sample with 0.1mm resolution setting](image)
Table 4.4: The result of the test on channels’ width with different resolution setting; o - the channel is cleaned, x - the channel is clogged

<table>
<thead>
<tr>
<th>Diameter Settings</th>
<th>0.5mm</th>
<th>0.75mm</th>
<th>1 mm</th>
<th>1.25mm</th>
<th>1.5mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1mm</td>
<td>x</td>
<td>x</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>0.05mm</td>
<td>x</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
</tbody>
</table>

4.5 Discussion on SLA Prototypes

After all the channel tests, we printed a more complex prototype; the structure was the same as the TPU prototype shown in Figure 3.6. The result is shown in figure 4.8.

Clean the Resin in the Pump

After the print was finished, the pump was filled with resin. We tried to squeeze out the resin through the embedded channel. However, because of the high viscosity of the resin and the small width of the channel, the resin could not go out through the channel. If we applied too much pressure on the pump, the resin would break the pump and flow out.
through the cracks. Therefore, if we want to print a pump structure by the SLA printer, we might need to enlarge the width of the channel, which can help the resin come out more easily, or we can use syringe to suck out the resin inside the pump.

**Elasticity of the SLA Prototype**

One of our goals in the thesis is to print an immunoassay device with elastic material. That is why we choose flexible resin for our SLA prototype. From our tests, we find that the performance elasticity of the SLA prototype is not very good. The deformation of the pump could result in cracks on the structure. However, the same amount of deformation on the TPU prototype did not result in any cracks. A broken SLA prototype is shown in figure 4.9. After we press the SLA pump for several times, the crack on the pump became larger and one side of the pump collapsed. If we want to use the SLA printer to print our immunoassay device, we have to take the characteristics of the flexible resin into consideration.

![Elasticity Performance of the SLA Prototype](image)

**Figure 4.9 Elasticity Performance of the SLA Prototype; the pumps breaks after deformation**

Even though the SLA technique can provide higher resolution and density than FDM, the performance of elasticity of the SLA prototype is not as good as FDM. Besides that, high viscosity of the resin also increases the difficulty of producing the immunoassay device.
5 AUTOMATED MALARIA AB IMMUNOASSAY DEVICE

In the last part of the thesis, we would try to incorporate automated mechanism and an SLA prototype into one automated Malaria-Ab immunoassay device. In order to prove the concept, we used syringes to substitute pumps for the actuation of the fluid, because syringes were more reliable than 3D printed pumps. The syringes were driven by the servo motors and continuous motors which were controlled by the Arduino Uno board.

5.1 Electrical Parts in the Device

Arduino Uno Microcontroller

The Arduino Uno board shown in Figure 5.1 is based on the ATMega328, which has 14 digital input/output pins (six of them can be used as PWM (Pulse-Width Modulation) outputs, six analog inputs, a 16MHz ceramic resonator, a USB connection, a power jack, an ICPS header, and a reset button. The Arduino Uno operates with 5V and requires an Input Voltage between 6-20V (recommended: 7-12V). The six PWM outputs will be important for the control of the servomotors; the easiest way to program PWM on the ATMega328 is to use its PWM outputs. [34] We used the software, Arduino 1.6.8, to program the board.

Servomotor

Servomotors work on PWM (Pulse width modulation) principle, which means its angle
of rotation is controlled by the duration of applied pulse to its Control PIN. Basically the servomotor is made up of DC motor which is controlled by a variable resistor (potentiometer) and some gears. Usually, the range of rotation of the servo motor is 180 degrees, but there are also continuous rotation servomotors which have no limitation of the angle.

**Automated Mechanism for the Syringes**

In our design, we needed total 4 syringes; each of them was for Malaria Conjugate, TMB Substrate Solution, Stop Solution and waste respectively. Because the required volume for Malaria Conjugate, TMB Substrate Solution, and Stop Solution was less than 100 uL, we could use simple mechanism to drive the syringes containing these solutions. The mechanism was shown in Figure 5.2; we used one small servomotor to push each syringe. Because the required volume for the waste was much higher than the other three solutions (the required volume for the waste is about 2000uL), we used 3D printed gear rack shown in Figure 5.2 to drive the syringe, which could provide more space for the actuation of the syringe. Because the rotation range of the servo motor was only 180 degrees, we used continuous rotation servomotor for our gear rack design.

![Figure 5.2: Automated Mechanism for the Syringes; left – Driving mechanism for the syringes of the three solutions, right – Gear rack for the syringe of the waste](image-url)
Automated Mechanism for Washing

For the mechanism of washing, we employed the SLA prototype which included one pump and one channel. Because the stiffness of the pump was too high, the pump could not be pressed down by the regular servomotor. We searched on the internet and found out HS-5645MG Digital High Torque Servo Motor, which could provide enough force to push down the pump. The mechanism is shown in Figure 4.12. When the servomotor rotated, the stick attached to the motor could press down the pump.

![Automated Mechanism for Washing](image)

Figure 5.3: Automated Mechanism for Washing

5.2 The Setup for the Automated Immunoassay Device

The complete setup is shown in Figure 5.4. One syringe for the waste was driven by the gear rack mechanism and each of the other three syringes was driven by one small servomotor directly. All the servomotors were driven by one Arduino board except the continuous servomotor, which was driven by another Arduino board. The reason we used two Arduino boards was that the programming for the regular servomotor and the continuous servomotor was different, so we decided to use two boards to drive the motors, which would make programming easier. The programing code is listed in the appendix.

The whole procedure of this automated Malaria Ab immunoassay device followed the
The procedure is as follows:

1. The well containing 100 µL the sample or control is positioned in the device. (6 in Figure 4.13) We then incubate the well for 1 hour at room temperature.

2. Wash the well three times with 300 µL Washing Solution. The high torque servomotor in the washing mechanism will press the pump three times and the Washing Solution inside the pump will flow into the well. (1 in Figure 4.13) Every time the Washing Solution flows into the well, the gear rack mechanism will actuate the syringe of waste to clean the solution in the well. (2 in Figure 4.13)

3. Send 100 µL Malaria Conjugate into the well by actuating the syringe of Malaria Conjugate. (3 in Figure 4.13) Then wait for 30 minutes.

4. Repeat step 2.

5. Send 100 µL TMB Substrate Solution into the well by actuating the syringe of TMB Substrate Solution. (4 in Figure 4.13) Then wait for 15 minutes.

6. Send 100 µL Stop Solution into the well by actuating the syringe of Stop Solution. (5 in Figure 4.13)

7. If the result is positive, the color of the solution in the well will change; on the other hand, if the result is negative, the color will remain the same like what is shown in Figure 5.5. We can also use the cell phone to detect the color change.
Figure 5.4: The Setup of the Automated Malaria Ab Immunoassay Device; 1 - washing mechanism (high torque server motor and the pump containing Washing Solution), 2 - gear rack mechanism for the syringe of waste, 3 - actuation mechanism for the syringe of Malaria Conjugate, 4 - actuation mechanism for the syringe of TMB Substrate Solution, 5 - actuation mechanism for the syringe of Stop Solution, 6 – the well containing the sample or control

Figure 5.5 : Color change of the samples; top - the samples before adding Stop Solution, bottom – the samples after adding Stop Solution
Discussion on the Automated Immunoassay Device

The device could successfully perform the specified steps, but we found some issues about this device. The first issue was the SLA pump. We had mentioned earlier that the elasticity of the printed SLA prototype was not very good. After being pressed down several times, the pump started cracking. The cracking resulted in leaking and deteriorated the function of the pump. We used tapes to help seal the pump, which did help, but a new design or new material of the pump might be better to fix this problem. For example, like all the other syringe mechanisms, we can use a syringe to substitute the wash pump, which will make the wash mechanism more reliable, and we don't have to worry about the elasticity performance of the material.

Another issue is about cleaning the waste. We used small diameter plastic tubes connected to the syringes to send the solution to the well and clean the waste from the well. There was no problem to send the solution into the well, but when we needed to clean the well, we had to make sure the well was cleaned completely by the tube. Otherwise, this issue would affect the accuracy of the result.
6 CONCLUSION

In order to develop a LOC immunoassay with elastic material that could be produced on demand, the rapid technologies of 3D printing were introduced; Fused Deposition Modeling and Stereolithography were chosen for the manufacturing of different prototypes.

FDM prototypes were first developed to prove the possibility of using elastic material TPU to build enclosed channels, then to test embedded fluidics and their actuation, and finally to be used for immunoassays. We successfully printed a LOC immunoassay device with the elastic material TPU, which shortened the fabrication time of the device because it eliminated the necessity of integrating the elastic parts made from other techniques into our device. One of limitation for the FDM LOC immunoassay is leakage. Because of the characteristics of FDM techniques, the fluid is easy to leak out through the gaps between the layers. We used plastic dips method to help seal the leakage, which worked pretty well. Another limitation is resolution. Compared to other 3D printing techniques, FDM is more affordable and popular; however, it can only print stuff with relatively low resolution, which means it can’t be applied to printing small scale LOC devices.

After we successfully printed the LOC immunoassay device with the elastic material TPU by applying the FDM technique, we tried to apply the SLA technique to printing our device. Because of the characteristics of the SLA technique, we thought it probably can help improve the density of the device and enabled miniaturization. We used the flexible resin bought from the Formlabs company to print our SLA prototypes. Due to the characteristics of the technique, the leakage problem highly improved compared to the FDM technique. However, one issue is that the elasticity of the SLA prototypes is not as good as that of FDM prototypes. When we pressed down a SLA pump structure, the deformation could result in
cracking on the pump, which would result in leakage and deteriorate the function of the pump. Another issue is caused by the flexible resin. When we printed the enclosed structures like pumps or embedded channels, the resin would be stuck inside. In order to clean the channel, the diameter of the channel could not be too small, which would increase the difficulty for the miniaturization of the device.

In the last part of the thesis, we successfully built an automated immunoassay device, which could automatically perform the immunoassay steps listed in the IBL Malaria Ab ELISA manual. [27]

**Future Prospects**

One of the future prospects is that we can use transparent TPU to print our LOC immunoassay. Transparent materials can be used to demonstrate the functionality of the device. Besides that, we can check if there is any leakage happening in the device more easily. Another advantage is that, to detect the color change of the results, we need sufficient light and the device made from the transparent material is beneficial to the readout of the result. Lastly, like what we mention earlier, transparent TPU can be printed by the FDM printer, which is popular and inexpensive.

Another future prospect of the immunoassay device is miniaturizing the design. By shrinking down the size and the volume of the reagents of the disposable part, multiple goals can be reached. Different physical principles will predominate. Hence capillary force will be more dominant and gravity in proportion is less important. This could lead to a better control of the flow, as fluids would not only run through the channels and mix up, but stay at the point where they are being pushed by the pumping force, because of the capillary force. Different surfaces could be used to obtain capillary depression or attraction
and to control the flow. Also the small device can increase its portability and decrease the energy consumption of the push. Also smaller reagents will be sufficient, which will make the assay cheaper. In conclusion, miniaturizing the LOC-Immunoassay could allow to rapidly improving control of the fluids, portability, energy consumption, amount of reagents necessary and duration of the test.

Lastly, another prospect is finding an alternative elastic material for the SLA immunoassay device. In the thesis, we use Formlabs flexible resin for our SLA prototypes and the elasticity is not as good as our FDM prototypes made from TPU. However, one significant advantage of the SLA technique is that it can provide high density for the immunoassay device, which could help prevent leakage of the device. Besides that, the high resolution provided by the SLA technique is also beneficial for miniaturization of the device. If we can find some other elastic material that has high elasticity, the SLA technique will be ideal for the 3D printed immunoassay device.
REFERENCES


usersguide/#directedenergy, 12-032014


[28] IBL International GmbH, Malaria-Ab ELISA. Enzyme Immunoassay for the qualitative determination of antibodies against Plasmodium in human serum or plasma, 01-20-2012

[30] Anthony K.Au; Wonjae Lee; and Albert Folch, Lab Chip, 2014, 14, 1294,
[31] Toshimitsu Kanai; Kanako Ohtani; Masafumi Fukuyama; Toru Katakura; and Masatoshi Hayakawa, Polymer Journal (2011) 43, 987–990
[34] Servo motor basics, URL: http://circuitdigest.com/article/servo-motor-basics
APPENDIX A : The Code for Actuation of Servomotors

#include <Servo.h>

Servo myservo1; // create servo object to control a servo
Servo myservo2;
Servo myservo3;
Servo myservo4;
int pos1 = 10;
int pos2 = 90;
int pos3 = 10;
int pos  = 50;

void setup() {
    myservo1.attach(8);
    myservo2.attach(9);
    myservo3.attach(7);
    myservo4.attach(5);
}

void loop() {

delay(8000);

for (pos = 50; pos <=130; pos += 1) {
    // in steps of 1 degree
    myservo4.write(pos);
    delay(15);
}

for (pos = 130; pos >= 50; pos -= 1) {
    myservo4.write(pos);
}
delay(15);
}
delay(3000);
for (pos = 50; pos <= 130; pos += 1) {
    // in steps of 1 degree
    myservo4.write(pos);
delay(15);
}
for (pos = 130; pos >= 50; pos -= 1) {
    myservo4.write(pos);
delay(15);
}
delay(3000);
for (pos = 50; pos <= 130; pos += 1) {
    // in steps of 1 degree
    myservo4.write(pos);
delay(15);
}
for (pos = 130; pos >= 50; pos -= 1) {
    myservo4.write(pos);
delay(15);
}
delay(5000);

for (pos1 = 10; pos1 <= 120; pos1 += 1) {
    // in steps of 1 degree
    myservo1.write(pos1);
delay(15);
}
for (pos1 = 120; pos1 >= 10; pos1 -= 1) {
    myservo1.write(pos1);
}
delay(15);
}

delay(9000);

for (pos = 50; pos <= 130; pos += 1) {
    // in steps of 1 degree
    myservo4.write(pos);
    delay(15);
}
for (pos = 130; pos >= 50; pos -= 1) {
    myservo4.write(pos);
    delay(15);
}
delay(3000);
for (pos = 50; pos <= 130; pos += 1) {
    // in steps of 1 degree
    myservo4.write(pos);
    delay(15);
}
for (pos = 130; pos >= 50; pos -= 1) {
    myservo4.write(pos);
    delay(15);
}
delay(3000);
for (pos = 50; pos <= 130; pos += 1) {
    // in steps of 1 degree
    myservo4.write(pos);
    delay(15);
}
for (pos = 130; pos >= 50; pos -= 1) {
myservo4.write(pos);
delay(15);
}
delay(5000);
for (pos2 = 90; pos2 >=0; pos2 -= 1) {
    myservo2.write(pos2);
delay(15);
}
for (pos2 = 0; pos2 <= 90; pos2 += 1) {
    // in steps of 1 degree
    myservo2.write(pos2);
delay(15);
}
delay(9000);

for (pos3 = 10; pos3 <= 90; pos3 += 1) {
    // in steps of 1 degree
    myservo3.write(pos3);
delay(15);
}
for (pos3 = 90; pos3 >= 10; pos3 -= 1) {
    myservo3.write(pos3);
delay(15);
}
APPENDIX B: The Code for Actuation of the Continuous Rotation Servomotor

```
#include <Servo.h>

Servo myservo; // create servo object to control a servo

void setup() {
    myservo.attach(5);
}

void loop() {

    unsigned long startTime = millis();

    while(millis() - startTime < 10000UL){
        myservo.write(96.8);
        myservo.write(97);
    }
    myservo.write(95);
    delay(1800);

    while(millis() - startTime < 15500UL){
        myservo.write(96.8);
        myservo.write(97);
    }
    myservo.write(95);
    delay(1800);

    while(millis() - startTime < 21000UL){
        myservo.write(96.8);
    }
```

myservo.write(97);
}
myservo.write(95);
delay(1800);

while(millis() - startTime < 40500UL){
    myservo.write(96.8);
    myservo.write(97);
}
myservo.write(95);
delay(1800);

while(millis() - startTime < 46500UL){
    myservo.write(96.8);
    myservo.write(97);
}
myservo.write(95);
delay(1800);

while(millis() - startTime < 52000UL){
    myservo.write(96.8);
    myservo.write(97);
}
myservo.write(95);
delay(1800);

while(millis() - startTime < 71000UL){
    myservo.write(96.8);
    myservo.write(97);
}
}