

Fabrication and Control of Microfluidic Devices for On-chip Gene Synthesis

by

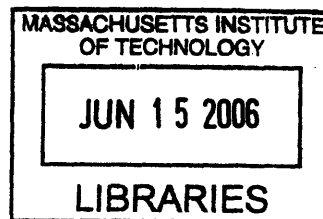
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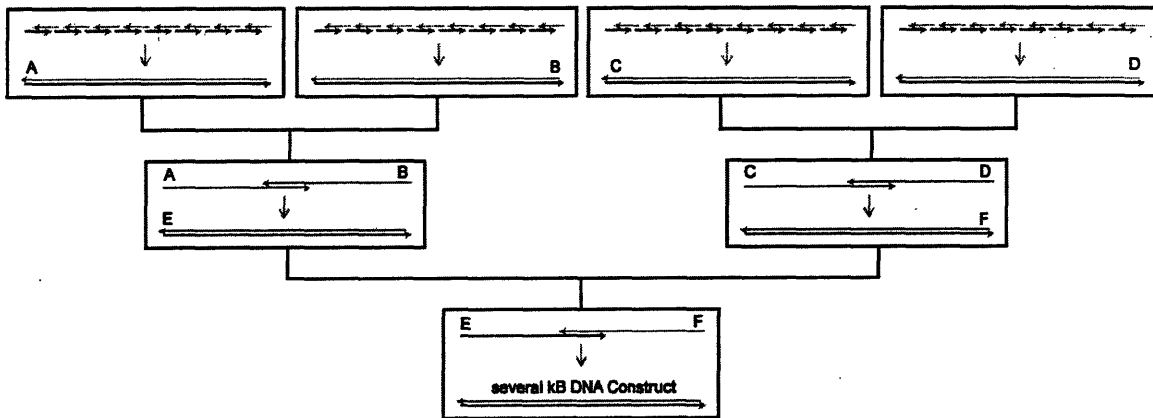
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## Background and Introduction

In biological research today, there is great demand for synthesized biological compounds. The sequencing of the Human Genome has been completed, as well as that of many other organisms. Current work is now shifting towards the production of biological macromolecules. More specifically, this includes gene and genome synthesis from user-defined sequences. A series of A, T, G, and C nucleotides are specified in advance, and then constructed. Because of the error rates in perfectly synthesizing these specific DNA chains, it is more efficient to synthesize smaller oligonucleotide chains (oligos) and then allow them to self-assemble them into a larger oligos. These assembled chains are then brought together to form even longer chains, in a repeating process known as hierarchical assembly.



3-stage hierarchical gene assembly<sup>2</sup>

For example, 25 MegaBase long constructs can be assembled from oligo chains 50 bases long. This process uses Caruthers (phosphoramidite) chemistry to first build small chains one nucleotide at a time, and then assembles the larger structures with enzyme mediated techniques. For example, in the past few years people have

synthesized polio (2003, Wimmer et al., Science) and bacteria (2004, Venter et al., PNAS) chromosomes from chemical bases.

Due to the great length of the target molecules, easily in the tens of thousands, this has been traditionally a long and expensive process. In a field where probabilities and scaling of processes are becoming increasingly important, cost and time are real limiting factors for the advancement of knowledge. A similar parallel in recent history is the dramatic shrinking size and increasing complexity of computer chips. What previously required an entire room to perform the most basic tasks can now be done faster, more cheaply, and using less power on the area of a postage stamp.

A similar analogy can be likened to current developments in microfluidics. Moving biological reactions from test tubes and beakers to microfluidic chambers has a number of advantages. By miniaturizing the reaction volume, the amount of reactants is drastically cut, saving costs. Many more reactions can occur at once as a result, and in a smaller area. Small volume also allows faster and more efficient thermal cycling, commonly used in PCR. All these advantages can be leveraged to create automated systems in which multiple steps of synthesis, assembly, and reaction can occur autonomously on a single chip. This “Lab-on-chip” technology will be the highly integrated fluidic equivalent to the modern integrated circuit.

### **Goal and Scope of the Project**

The overall goal of this project was to build a microfluidic system that is capable of performing on-chip hierarchical gene assembly. Successful completion of this project consists of two components. One is designing and creating the microfluidic devices

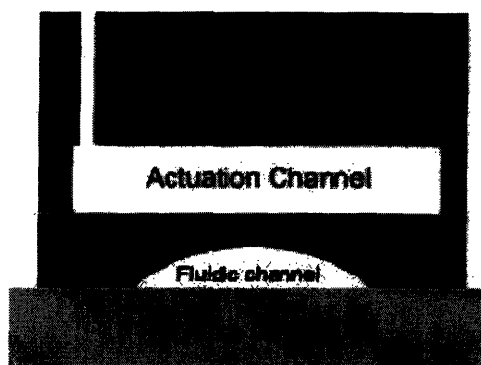
which are capable of running these processes. The second is creating an electronic hardware setup, with corresponding software, that would enable the control of these reactions through a graphical computer interface.

### Microfluidic Devices



The microfluidic devices that I worked with are made using multi-layered PDMS on glass slides. These microfluidic devices work by creating many interconnected channels and reaction pools in the soft PDMS elastomer. Fluid containing select reactants enters specific input locations through metal pins connected to tubing. The flow of fluid is controlled by valves which lie in an actuation layer above the fluid layer.

These valves are controlled by air pressure, and depend on the low Young's Modulus of Elasticity of the PDMS material to deform enough to close each flow channel. Through the opening and closing of these valves the flow of liquid can be controlled. Mixing is stimulated by peristaltic actuation of three valves in series.



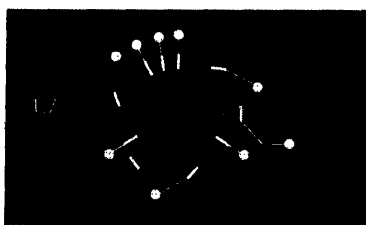
## Device Fabrication

### *Fabrication Overview*

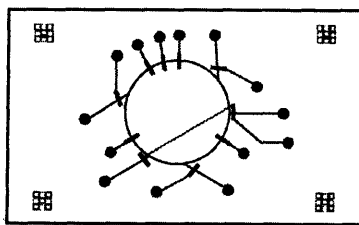
One major aspect of this project was learning how to make these microfluidic devices. The general process is similar to that used in soft lithography. First, masks are designed for both the fluid layer and the actuation layer, and printed onto a transparency. A clean silicon wafer is spin-coated with photoresist, then UV-exposed using the mask. After development, PDMS is poured onto the master mask and cured. Once peeled off, the PDMS layers are bonded together, then bonded onto the glass slide. Each of the steps just described includes a number of challenges and considerations.

### *Mask Design*

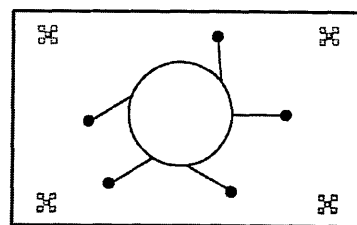
The masks are designed in vector format using Adobe Illustrator, in order to achieve the necessary resolution while fitting onto a glass slide of 24mm x 50 mm. First the flow-channel layer is designed, keeping in mind that the size of each channel and pool will affect the eventual volume, and thus concentration, of oligos cleaved from a microarray chip. Reagent entrance and exit pins are generally located on the sides of the device, but must keep some distance from the edge to facilitate pin insertion.



Actuation Layer



Complete Mask



Flow Layer

Following this step, the actuation layer is created as a separate Group in Illustrator. The width of the valve areas cannot exceed a maximum range without risking

collapse of the fluid layer below. In addition, a line bridging two distant valves can cross over a flow line without risking actuation, but only if the bridge line is less than half the width of the flow line beneath it.

Alignment markers are placed around the edges of the two layers to facilitate proper alignment after fabrication. Because the greater thickness of the actuation layer will cause some shrinkage during the curing process, the actuation mask is scaled up by an additional 1.7% to compensate. After the layers are split up, the actuation layer is inverted in software to create a dark-field mask, while the flow layer remains a clear-field mask.

When sending the mask out for production, the actuation layer masks and flow layer masks are collected on separate wafer-sized disks. Each disk holds six masks of standard slide dimensions. When arranging the masks onto the disk, the actuation masks are tightly packed for efficient cutting of the PDMS, while the flow layer masks are spaced out so that the final devices can be easily separated. This will be further elaborated in the PDMS fabrication section.

### *Wafer Master Fabrication*

The processes for creating the silicon master molds are similar for the two layers, but there are important differences. The flow layer uses AZP4620, a positive resist. Combined with the clear-field flow mask this creates the pattern of the thin flow channels sticking out of the wafer. Moisture can have a significant negative impact on the resist process, so the wafers are first dehydrated for 1 hour at 200°C. The wafers are then coated with HMDS to promote adhesion of the AZP resist, which is spun on at

1500 rpm for 30 seconds. This results in structures approximately 13  $\mu\text{m}$  tall, which create the desired height of the eventual flow layer channels. The wafer is then pre-baked at 90°C for 60 minutes. It is very important to have tight temperature regulation at this step, while avoiding exposure to light which can prematurely expose the resist. Exposure is then performed for 20 seconds at 50% power, and the wafer is developed. During developing it is important to completely remove resist from the non-channel area, but not overdevelop, which can strip off the resist pattern entirely. It helps to vary the mixing motions over this period of about 2.5 minutes. A post-bake at 135°C helps to reflow the resist, rounding out the channels and making them easier to close with the actuation valves.

The process for creating the actuation layer master is similar, but with a few important differences. This wafer uses SU-8 50, a negative resist that is much more viscous, combined with the dark-field mask, to create the actuation layer which is 70-80  $\mu\text{m}$  tall. The process begins also with a dehydration step for 5 minutes, which is not as critical as for the AZP resist. The spin coating is done in two stages- first at 500 rpm to distribute the viscous SU-8 over the surface, then for 30 seconds at 1000 rpm to achieve the desired height. Pre-baking is done for 1 minute at 65°C then for 10 minutes at 95°C. To prevent overheating, the UV exposure is performed in 8 increments of 5 second steps, for a total of 40 seconds of exposure. After cooling, it is developed for 10 minutes, longer than the AZP due to the greater thickness. A brief second rinse with fresh developer solution ensures a clean rinse. At this point, both silicon wafer masters are ready for use.

### *PDMS Fabrication*

PDMS is chosen as the material for the microfluidic devices for several reasons. It is biologically inert, making it compatible for the reactions and thermal cycling which will occur inside. The low elastic modulus facilitates valve actuation. It is also relatively quick and cheap to fabricate with.

The PDMS fabrication process begins with thorough mixing of the polymer with hardening agent in a ratio of 20:1 for the flow layer (10.5 grams), and 5:1 for the actuation layer (75 grams). After the mixtures are de-gased in a vacuum chamber, the wafer masters are placed into a foil dish to contain the PDMS which is then poured on. During this entire process, it is imperative to keep dust off of the PDMS surfaces. Both wafers and the slides are heated in the convection oven for 45 minutes at 80°C. After this step, the thicker PDMS actuation layer is ready to be cut with a razorblade, and holes are poked through the actuation holes. The resulting PDMS rectangles are aligned onto the flow layer wafer where the devices have been spaced apart to allow room for cutting, and baked together to permanently fuse the two layers. These packages are then cut off the flow master wafer, and the flow holes are poked through. At this point, the microfluidic device only needs to be mounted. For mounting on the devices I constructed, a small leftover amount of 20:1 PDMS is spin coated onto glass slides at 2500 rpm for 40 seconds, and pre-baked also for 45 minutes. The device is then placed onto these coated slides and baked overnight for final curing. In the cases where oligos are cleaved from microarrays, the device would be placed onto the microarray slide rather than the coated glass slide. The actuation masters can generally be reused because



the thicker PDMS actuation layer comes off the master wafer cleanly; the thinner flow channel PDMS sticks to the flow master, rendering it only good for one-time use.

### *PDMS Adhesion to Microarrays*

A portion of my thesis work was investigating different types of adhesives which create a stronger adhesion between the PDMS device and the microarray. This is an important requirement, because devices which are bonded weakly may separate or leak internally when pressurized. The step of bonding these two surfaces presents a number of challenges. Methods such as plasma functionalization which typically are effective for sticking PDMS to plain glass slides are not sufficient for the chemically treated microarray surface.

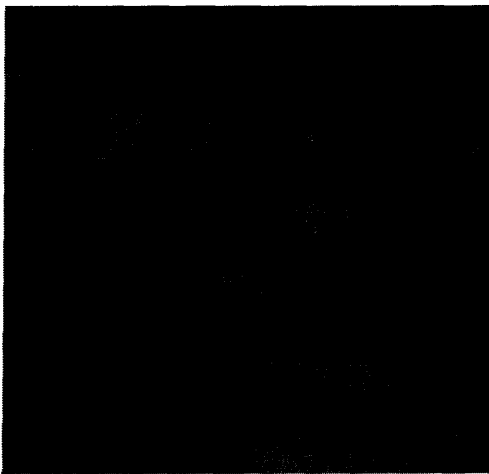
We used the “Stamp and Stick” technique to spin adhesive onto a glass slide, stamp the PDMS device into the resulting adhesive film, and then place the device onto a microarray. One critical factor is the thickness of the adhesive used, which can close off the flow channels if comparable to the channel height of 10  $\mu\text{m}$ . After all necessary curing processes, the adhesion strength was quantified by measuring the pressure level at which the device delaminates from the glass slide or microarray.

A number of adhesives were considered and tested, including epoxies, UV-curable epoxies, urethane, isocyanate, and cyanoacrylate. Of most tested adhesives, viscosity was a major limiting factor. This prevented a sufficiently thin film from being spun, even at speeds of 6000 rpm. Only two adhesives could spin sufficiently thin as to not seal off the flow channels when tested. One was SU-8 2000, typically used as a photoresist, it acts also as a UV-curing epoxy. The other was cyanoacrylate. Both of

these adhesives are still being evaluated for bonding strength, and biological compatibility.

### *Flow-channel Post Spacing*

Cleaving oligos from the surface of a microarray requires a wide reservoir area. The channel height must also be very thin, about 10  $\mu\text{m}$ , in order to maximize the cleaved oligo concentration. Because of these two factors and the soft nature of PDMS, the flow layer can tend to collapse in larger areas, preventing fluid flow through that zone.



To prevent this problem, posts are built into the flow mask to act as columns that hold the layer up. However, each post location covers up oligos below it, wasting that space. As a result, it is desirable to determine the minimum density of posts which can still prevent collapsing of the channel.

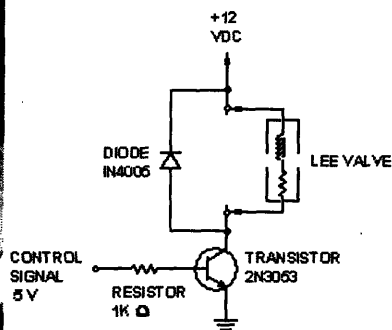
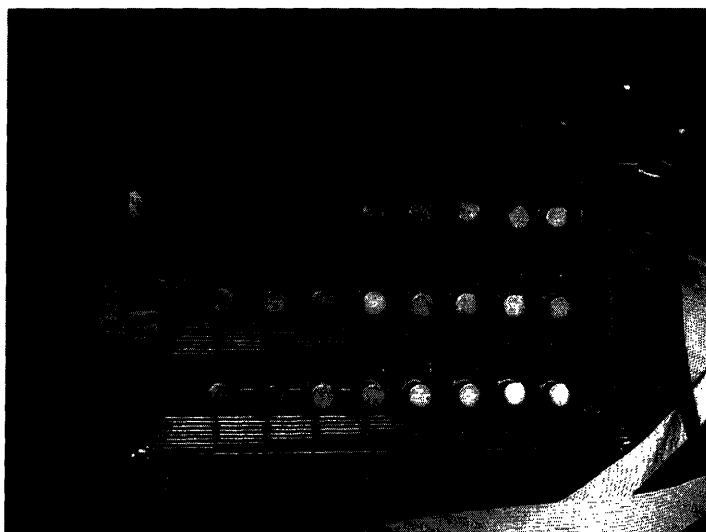
As a small part of my work, I created a set of masks containing wide channels with post spacing varying in a grid pattern from 0.05 mm to 0.625 mm. These masks will be used to create a range of test devices to find the maximum post spacing for a range of different channel heights.

## Control System Overview

### *Hardware System*

An entirely different side of my work was using software and electronics to control these microfluidic devices. Previously, the method for using these devices was entirely hand operated. Pressure valves would be turned on and off by hand to control the actuation valves, and fluids would be moved by pre-loading tubes and then re-connecting them to the hand-operated pressure. There was no good way of performing in-device mixing.

The automated system that I set up allows for computer control of valve actuation in software, as well as automated cycling of mixing patterns. The overall system comprises a National Instruments 6533 DAQ card, which connects to a signal amplifier PCB. The PCB board connects to electronic valves, which are supplied with pressure lines and regulate the passing of this pressure into tubes which run into the microfluidic device.



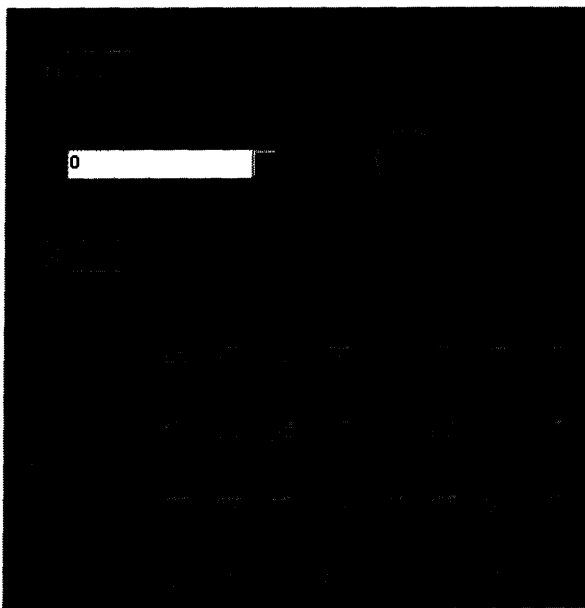
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The board layout was created by Mats Cooper in the Thorsen Group at MIT. It contains an array of 32 simple signal amplifier circuits, basically applying the logic values

from the DAQ card to transistor gates, which each amplifies power across a 12 volt source. My minor additions to the PCB board include adding a power switch and LED indicator light, and changing the valve connectors to be removable instead of permanently soldered on. Standard ribbon cable connects to the current-controlled pressure valve. The tubing extending from these valves enter the microfluidic device through metal pins identically to the manual configuration.

### *Software System*

The software interface runs off of National Instruments (NI) DAQ and Labview 7.1. On the lowest level, the software simply tells the DAQ card when to switch the logic for each of the 32 output lines to High or Low. This can be done in ways that range from very simple to very complex.



Left is a screenshot of the most basic software, supplied to us by graduate student Mayank Kumar, which allows one to manually switch each of the outputs on and off. This is essentially an electronic streamlining of the manual control process, but does not enable any new capabilities. A second version of the software supplied by

Mayank, shown below, includes four sets of mixers which can be controlled in pairs, at frequencies up to 25 Hz. There are six additional individually actuated valves in this software implementation.

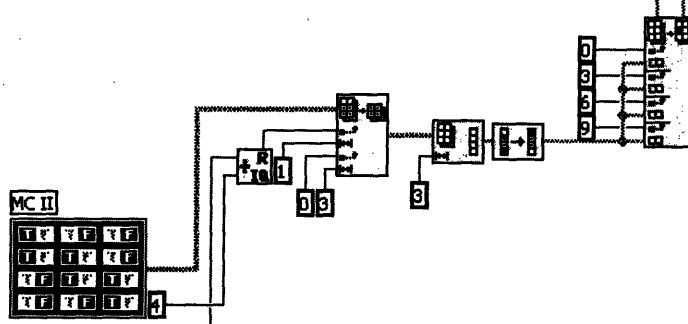


## Software Improvements

### *Peristaltic Mixing Patterns*

During mixing tests using the old software, we noticed that the mixing was very slow and ineffective. This was attributed partially to the mixing pattern previously implemented, which was this 4-stage pattern (where X indicates a closed valve, and O indicates an open valve):

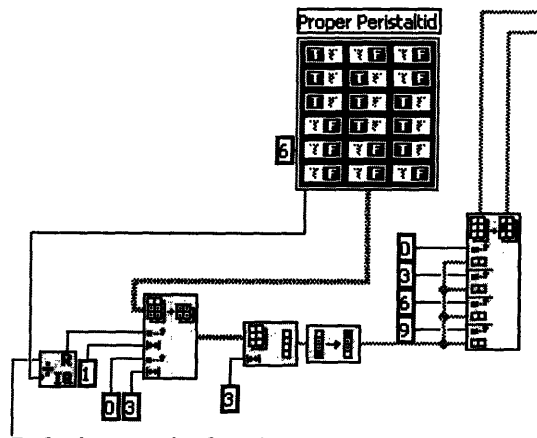
XOO  
 XXO  
 OXX  
 OOX



Labview code for the old 4-cycle mixer

Literature indicated that more effective peristaltic mixing pattern is this 6-stage cycle:

XOO  
 XXO  
 XXX  
 OXX  
 OOX  
 OOO

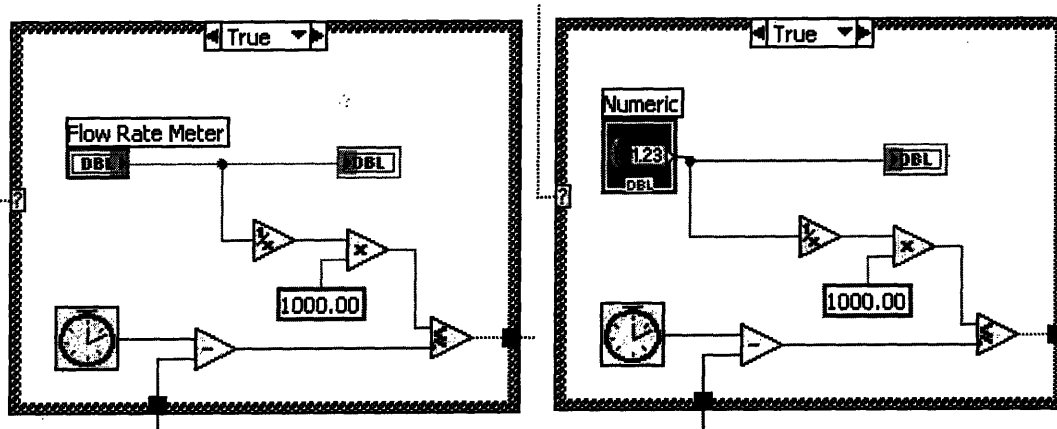


Labview code for the new 6-cycle mixer

I discovered that the mixing patterns are dictated by cycling through an array of binary values, the code for which is shown above. I created a new array with the desired pattern and it resulted in the corresponding mixing pattern. This pattern was confirmed to mix more effectively in tests.

### *Increased Maximum Frequency*

Another strategy for increasing the mixing speed was to increase the maximum frequency allowed by the software for cycling of the mixing valves. This goal was achieved by replacing the adjustable dial which ranged from 0 to 100 with a numeric input which allowed the entry of arbitrarily high frequencies. The maximum valve cycling frequency is now limited by the hardware setup rather than software.



Old low-frequency input

New input for any frequency

### *Control over All Valves*

The previous software which controlled each valve separately did not allow for automated mixing patterns. The previous mixing software only included support for 6 individual valve controls. I added the capability for controlling all 26 of the individual



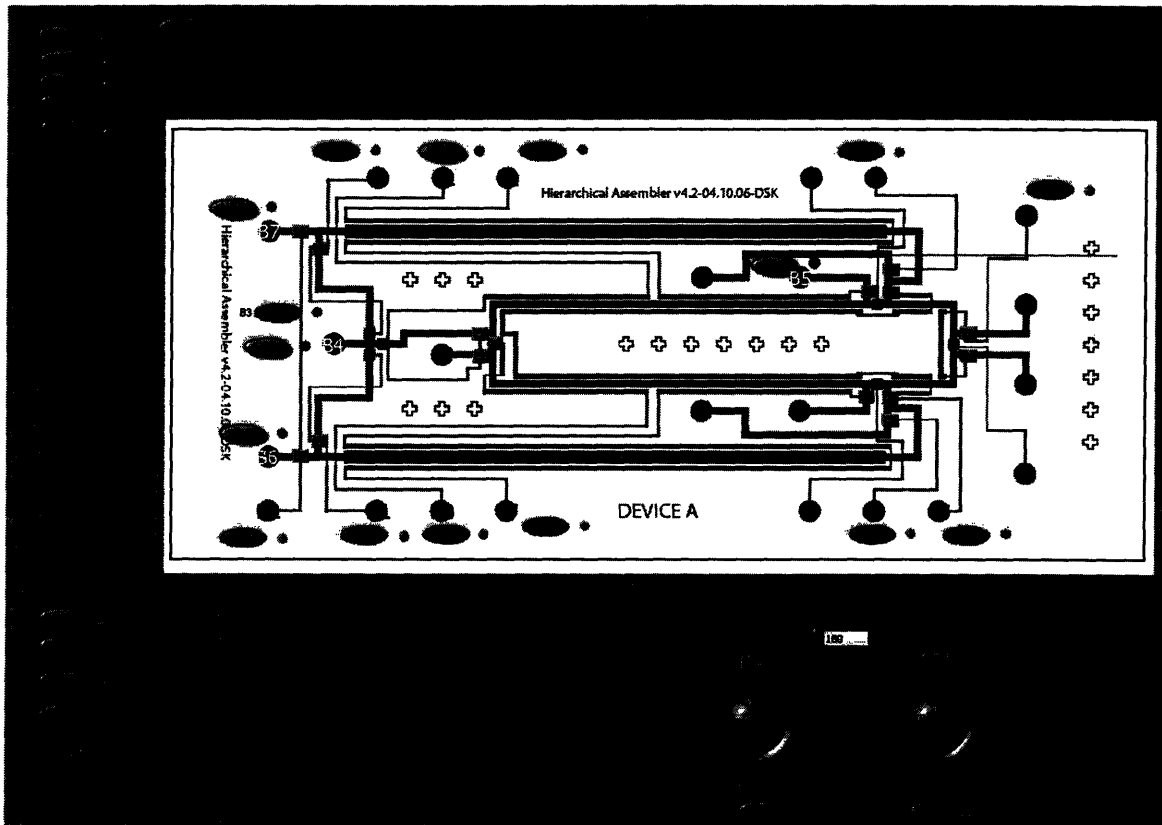
### *Reconfigurable Template*

The final resulting software version has all the added features combined together into one version.





The GUI is arranged into a template format that is easily visually reconfigurable into a layout which matches the device it is controlling for intuitive use. Below is a picture of this template after being arranged to control the latest device from Dave Kong.



### Future Work, and Acknowledgements

I plan to continue this work into the summer and following school term. Some ongoing projects include further investigation into the best adhesive for bonding to microarrays. This will likely encompass a few different types of microarrays including Agilent chips and some from other University collaborators. The post spacing project will include testing of the maximum post interval for different channel heights and other important conditions. Finally, the automation work will continue onto higher-level automation, so that a number of processes may run without human interaction in

specified sequence and timing. This ideally would incorporate communication with the thermocycler as well.

I would like to deeply thank Dave Kong for his guidance through all of these projects. This includes, but is not limited to, teaching me the entire fabrication process, guiding me through the adhesion and post spacing tests, facilitating the collaboration from which the PCB layout and basic software was obtained, and teaching me the basic biological processes which are the end goal. I would like to thank the entire Molecular Machines Group, for their support and excellent suggestions. I would like to thank Lu Chen for her very significant role in the adhesion experiments. I would like to thank Mayank Kumar for supplying the PCB layout and initial software. Finally, I would like to thank Professor Joe Jacobson for being my advisor for this Thesis and Advanced Undergraduate Project.

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