Digital and Paper Microfluidic Diagnostic Devices

Design Document

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Summary

The project aims to design two complementary systems for performing biological assays and diagnostic tests. The first system, using digital microfluidics, is reusable and allows for precise fluid control. The second system, paper microfluidics, uses inexpensive materials to provide rapid results.

Introduction

Microfluidics is a field of science and engineering which aims to study and develop systems to manipulate small (µL to nL scale) volumes of fluid. The field was enabled by microfabrication techniques developed for integrated circuit fabrication. These techniques allow researchers to create micrometer-scale channels capable of manipulating fluids. Inkjet printers, most ubiquitous example of microfluidic technology, rely on small chambers of fluid and integrated heating elements to propel droplets of ink onto the paper.

A large part of the rapidly developing field of microfluidics focusses on “lab on a chip” (LOC) applications. LOC systems integrate laboratory functions, like detecting biological compounds, on a single microfluidic device. Traditional microfluidic devices are fabricated using an elastic polymer, PDMS, bonded to a glass slide. The most sophisticated devices may have embedded electrodes for applying electric fields to the fluid flows. Recently, two alternative systems, digital microfluidics and paper microfluidics, have gained attention.

Digital microfluidics involves transporting droplets of fluid in two dimensions over a grid or electrodes using only electric fields. The system works in the following way: first, a droplet is placed on a grid of electrodes, similar to a pixel grid on an LCD display. Then, a voltage is applied to an electrode adjacent to the electrode containing the droplet. A phenomenon called electrowetting causes the droplet to move towards the new electrode. The ability to move discrete droplets in two dimensions is a unique advantage of digital microfluidics. Digital microfluidic (DMF) devices offer a flexible platform for lab on a chip applications.

Paper microfluidics is a method of manipulating fluid flows using the wicking property of paper. Several systems have been developed for creating fluidic channels in paper, with wax printing being the most promising option. Melting the wax through the paper forms a fluid barrier, enabling channels to be created. Paper microfluidics can be used to develop more sophisticated lateral flow assays, or test strips, which are commonly used for point of care testing.

Project definition, goals, and deliverables

Digital

Medical diagnostic tests are currently expensive, time consuming, and prone to human error when performed by lab technicians. Such tests involve numerous pipetting and mixing steps, requiring either expensive pipetting machines or skilled human hands. Digital microfluidic devices have the potential to automate diagnostic tests. All the required fluidic operations; transport, dispensing, merging, and mixing, can be carried out on a DMF device. Unlike traditional microfluidic devices, a DMF device is flexible enough to be used for a variety of different diagnostic tests. DMF devices have the potential to benefit resource-poor hospitals where lower cost fluid handling systems would improve the speed, accuracy, and throughput of diagnostic tests.

To create the digital microfluidics system, we will first replicate the work of other researchers, who recently released open-source plans for an electronic control system with a software interface. The control system allows high voltage signals to be applied to an electrode array. We will design our own electrode arrays and fabricate them using standard photolithography techniques. In the first semester, we will demonstrate a functioning DMF system, and in the second semester we will improve the system to create a robust system capable of performing a biological assay.

Paper

The current market for paper-based low-cost diagnostic devices is in its very early stages. While there are several small groups leading the market, there is still much room for growth. The goal is to, from research done on current technology in the market, create a paper-based diagnostic device capable of being competitive with the current market. To achieve this, we hope to first create a device to test the pH of saliva, and then next semester take the project a step further and modify the device to check other properties, potentially being viscosity and presence and/or concentration of a certain analyte, such as amylase. In addition, we hope to enhance the device by incorporating a downloadable smartphone app capable of reading the results of the saliva tests and providing a user-friendly output.

System level design

System requirements

Digital

The main goal of our project is to make a device that enables manipulation of the droplets on an array of electrodes. In order to make the digital device successfully work as a low cost medical device it will need to be portable, easy to setup, safe, and modular. Over time pieces of the system will experience wear and tear, so parts of it such as the ITO circuit board will need to be replaced without having to purchase an entirely new system. The device uses high voltages (over 50V) which require safety features to prevent users from hurting themselves. The system design is complex and uses multiple components that a user will not be expected to fully understand, but must be able to utilize simply and effectively. Finally a degree of portability will be needed; currently an external power source is required to operate the machine. Users should be able to assemble it in various locations while minimizing required space.

Paper

The functional requirements of our project are that it can run several different tests and display the results colorimetrically for easy interpretation of pH, viscosity and hydration of saliva. Our saliva testing device is a single-use at-home test kit. No power source or electricity needed for the saliva testing device. The saliva testing device requires only a small amount of fluid, usually less than 5 ml.

Our non-functional requirement of this project is that the saliva testing device needs to be relatively cheap. One of the goals of the project is to provide a cheaper alternative to standard high-cost diagnostics. The device must also to be very easy to use. Users who don't have medical training should be able to do the tests at home by following the instructions. Lastly, the entire testing process should be relatively quick, taking less than 30 minutes.

Functional decomposition

Digital

Digital microfluidic (DMF) devices offer a flexible platform for lab-on-a-chip applications. The DMF device can manipulate discrete fluidic droplets on the surface of an array of electrodes coated with a hydrophobic insulator. The device can move discrete droplets in two dimensions, which is a unique advantage of digital microfluidics.

First, the user will pipette a liquid droplet (such as blood, saliva, or sweat) on top of the Indium Tin Oxide (ITO) glass electrode array (Figure 1), and then setup the sequence of droplet movement by using the software. The droplet(s) will then move in the sequence specified by the user.

Second, the DMF software can communicate with the control board through the Arduino, while a webcam records the real time events on the electrode array. The software can record sequences of electrode activation steps needed to manipulate the discrete droplets to perform an assay. The image of electrode on the computer screen should be matched with the electrodes on ITO glass. Third, the control board will receive commands from the computer, transmit signals to the switching board, and send feedback to the computer. The control board also communicates with the driver boards and each relay switch connects to a single electrode on the DMF device via a custom pogo-pin connector. The board measures the amount of current passing through the device according to the device impedance and amplifier output.

Next, the amplifier converts input signal from the control board to high voltage output. It is connected through a feedback resistor back to the control board to facilitate amplifier-output monitoring. Then, the switching board receives command signals from the control board and transmits a high voltage signal to pogo pin board. It is connected directly to an array of electrodes patterned on Indium Tin Oxide (ITO) glass. The signal is received from the high voltage switching board. Once the voltage is supplied to the electrode, the liquid droplet moves to that electrode.

Using the webcam and software program, a user can see all the electrodes on ITO glass and actuate those by just clicking the mouse on the screen to move the droplet. Since a user can actuate more than 1 electrode, a user can split the droplet in two or cause the droplets to merge.

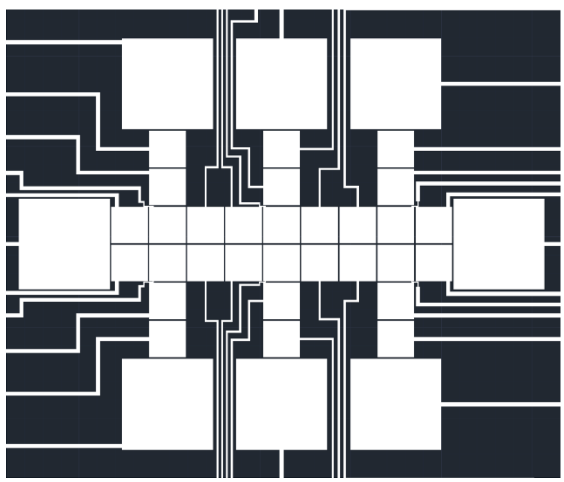

Figure 1. Electrode Array (small squares measure 1mm x 1mm)

Paper

The paper device consists of several steps: saliva application at the source point, wicking of the saliva along the wax-defined channels, and colorimetric output.

The saliva will be applied at a small paper opening at the end of the device. While most of the device will be coated with cold lamination to prevent contamination, this small area provides means of application. Saliva can be applied to this source point by dipping the source point into a saliva "collection cup" which has been filled to a reasonable level by the user, spitting on to the source point, or by holding the source point in the user's mouth.

Once the saliva has been applied, it wicks along the paper through the wax-defined channels. Thinner channels are generally preferred because the reduced flow area requires less saliva per unit distance. Enough saliva must be applied to reach the pH indicator at the end of the channel in the "bulb" shape. Note: the pH indicator is applied in the bulb area and allowed to dry before the cold lamination and application of saliva onto the testing device.

The last step is the colorimetric output. Upon reaching the pH indicator at the end of the channel, the saliva reacts with the indicator, which changes to a color dependent on the pH of the saliva. The resultant color is compared to a standardized reference sheet of colors. The user matches the color with a color on the reference sheet, which shows each color's corresponding pH. Two sets of reference sheets and output regions may be used instead of one. The advantage of two sets is more resolution in the pH range. Also, the indicators tend to be more sensitive at different pH ranges, so two or more indicators would provide better results.

System analysis

Digital

Control- The DMF control board receives commands from the computer, transmits a signal to the switching board, and sends feedback to the computer. The board also measures the amount of current passing through the device according to the device impedance and amplifier output.

Signal switching - The switching board receives command signals from the control board and transmits a high voltage signal to pogo pin board. It has a 40 high voltage relays that allow it to transmit the high voltage signal on 40 channels.

High-Voltage Amplifier - The amplifier converts input signal from the controller board to high voltage output. It connects through a feedback resistor back to the control board to facilitate amplifier-output monitoring.

ITO glass - The Pogo pin board receives signals from the high voltage switching board and transmits them to an array of electrodes patterned on Indium Tin Oxide (ITO) glass.

Video - The webcam will record the real time events on the device's surface electrodes.

Microdrop software – Provides a graphical user interface and communicates with the control board through the Arduino. The software can record sequences of electrode activation steps needed to manipulate the discrete droplets to perform an assay.

Paper

Out saliva testing kit uses paper as a substrate. The patient will deliver a sample of saliva, the saliva flows through the paper through the designed pattern. On the paper, we designed the pattern by using the wax. The wax barriers of the design can keep the sample within the boundaries of the pattern so that the saliva can flows through the paper through the designed pattern. With the pattern designed paper, patients will be able to do the test at home. The device will measure the pH and viscosity of the sample. For pH, the tests will provide colorimetric results for visual observation and any difference of color should be discernible by eye directly. We will use the following pH indicators:

- ***Cresol Red***
- ***Bromothymol Blue***
- ***Phenol Red***
- ***Neutral Red***

These indicators have the following properties:

Table 1. pH indicator solutions

Cresol Red	**Cresol Red** (pH indicator) *below pH 7.2* — *above pH 8.8* 7.2 ↔ 8.8
Bromothymol Blue	**Bromothymol Blue** (pH indicator) *below pH 6.0* — *above pH 7.6* 6.0 ↔ 7.6
Phenol Red	**Phenol red** (pH indicator) *below pH 6.8* — *above pH 8.2* 6.8 ↔ 8.2
Neutral Red	**Neutral red** (pH indicator) *below pH 6.8* — *above pH 8.0* 6.8 ↔ 8.0

The devices are very easy to use and the results are relatively easy to read and understand. The viscosity test will give results based on distance that the saliva flows into the test area.

Block diagrams of the concept

Digital

The block diagram in *Figure 2* provides a high-level guide to how the Digital Microfluidic system will run. Everything except the fluids used in the assay are reusable for the system. When an assay has been selected and the system configured, the microcontroller will select the voltage and determine where the droplet moves. The user can easily see the results through the clear ITO and record data. The ITO plate can then be cleaned, dried and reused for future tests.

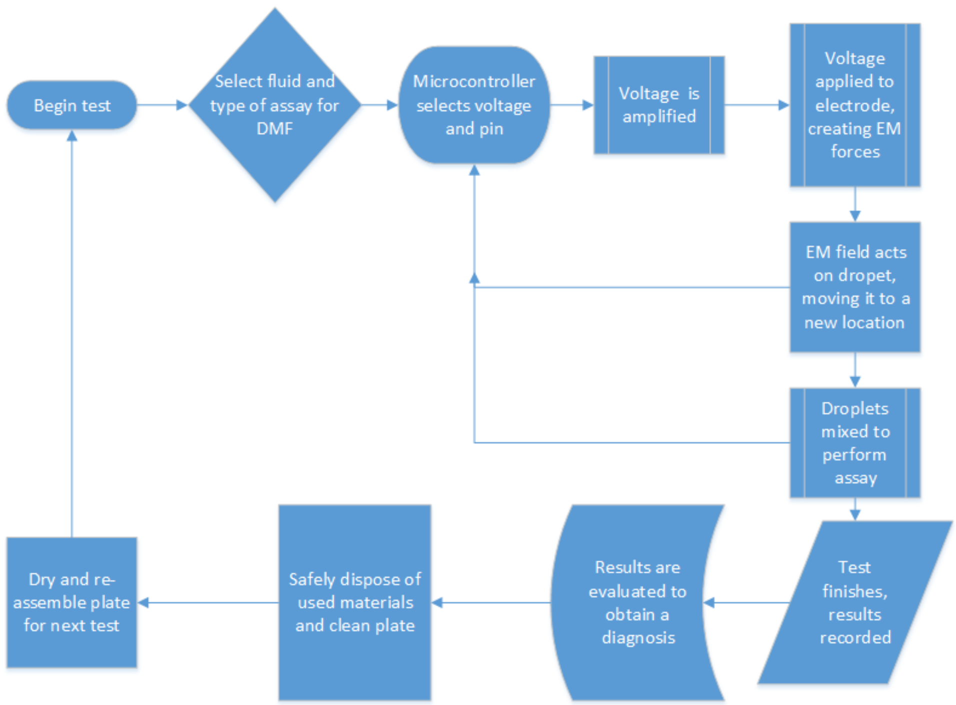

Figure 2. Digital Microfluidics Block Diagram

Paper

The system block diagram is shown in *Figure 3.* This diagram illustrates the overview of our system. The sample is deposited on the paper substrate by the patient. The saliva flows through the paper through the designed pattern. The wax barriers of the design keep the sample within the boundaries of the pattern. The viscosity test will give results based on distance that the saliva flows into the test area. The pH, alcohol, or other analyte tests will provide colorimetric results for visual observation.

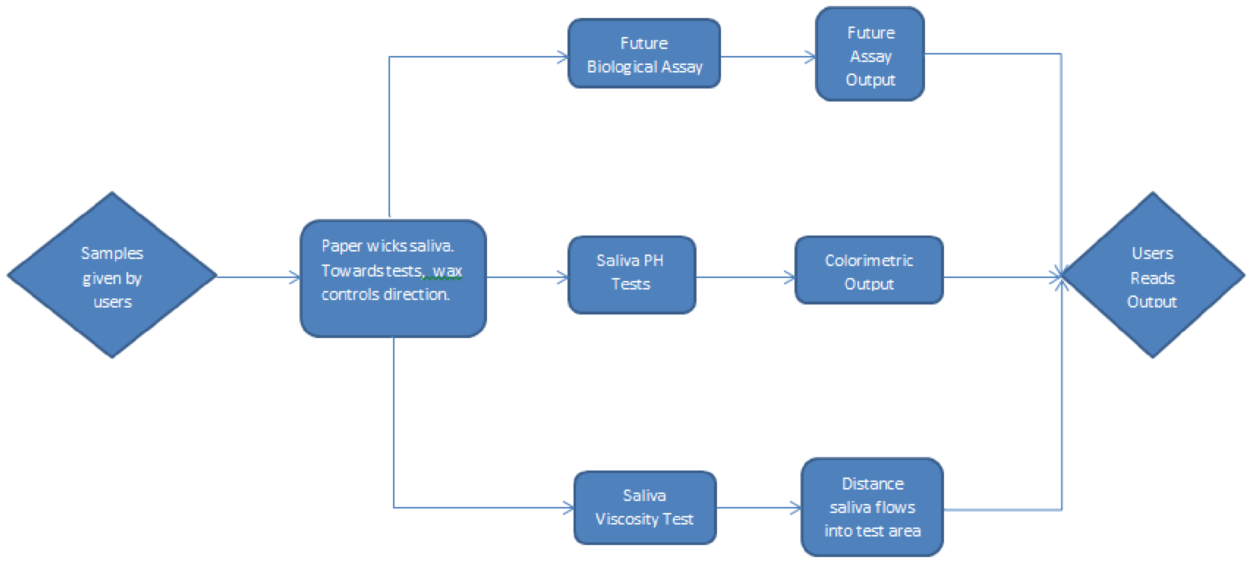

Figure 3. Paper Microfluidics Block Diagram

Detailed description

I/O specifications

Digital

The following subsystems must be configured within the tolerances listed below in order to ensure proper output.

- *Voltage* - The amplified voltage must be between 40 to 100 Volts. These values are defined though the microcontroller output, and then passed to the high-voltage amplifier. If the voltage is too low the droplet will not be able to overcome static friction forces. If voltage is too high, the system could be damaged by dielectric breakdown.
- *Microcontroller pin selection* – The pins must be activated in a logical manner that will begin at the location of the droplet.
- *Reagent* - The reagent selected must not react with Teflon.
- *Cleaning* – The Teflon coating must not be damaged during cleaning.

Paper

The paper fluidic system will include the following parts:

- Saliva collector which will use a special polymer that can collect the saliva without touching the indicator
- Test strips can be dipped into the collected saliva and will have the PH indicator on it, will be able to turn into different colors to indicate the PH of the saliva.
- Smart phone app which will use the rear-camera to take a picture of the test strip and assess the result

Interface specifications

Paper

The user interface will include three parts, the saliva collector, the pH strip with indicator, and the smartphone app with the ability to analyze test-strip color. The collector tube will be designed to be hand held and user friendly. The size of the test strip will also be big enough for user to see the result just by eyes, also the app for the device will be easy and simple to use without instructions.

Hardware/software specifications

Digital

Hardware specifications

Indium Tin Oxide (ITO) glass substrate - All the wires and electrodes are connected. Each wire needs to be connected only one electrode. Also, the glass needs to be coated with ~50nm of Teflon-AF 1600 by spin-coating (1000 rpm, 30s)

Signal - Since the DMF device uses AC signal, a control board generates a 0-1.4 Vrms variable-frequency sine wave.

Amount of voltage - To move the droplets, approximate 100~150V are required. After the signal is generated by a control board, it will be amplified by a high voltage amplifier.

Supply the voltage - Once a signal is amplified, a high voltage switching board will send the signal to an electrode on ITO glass. When the electrode is actuated, it will attract the liquid droplet, and the droplet will move to the actuated electrode.

Software specifications

Using the webcam and Microdrop software, a user can see all the electrodes on ITO glass and actuate those by clicking the mouse on the screen to move the droplet.

- A graphical user interface is written in Python
- GUI – click to activate electrode to attract the droplet
- Record actuation steps and how much voltage is applied
- The image of electrode on the computer screen is matched with the electrodes
- Communicate with the control board through the Arduino

Paper

Background:

The paper allows for the saliva to travel vertically because of the cohesion between molecules in the liquid, or surface tension, and adhesion between the saliva and the paper fibers. Because there are many fibers in close relation to one another, the diameter of flow for the liquid throughout the paper is relatively small. This allows for a high percentage of the saliva molecules to be in contact with the paper, therefore, a large amount of adhesive forces result between the paper and saliva.

Preparation:
Once the design has been printed, the paper is heated until the wax has completely melted through the paper (2 min @ 150°). A piece of contact is applied and pressed firmly onto the back of the design. This is done to fight the liquid's desire to flow outside of the design when applied. At one test location, a small drop of (indicator x) is placed as far away from the pathway as possible to keep as much of the indicator contained within the test area. At the other location, (indicator y) was applied in a similar manner. Once the paper was dry, another piece of contact paper was applied to the front of the paper, leaving only a small area (1-5 mm) exposed for sample application. The paper is now ready for testing.

Software specifications
We are going to make an iOS and android app which will be able to compare image's color taken by the cellphone on the PH test paper. The app will be able to list the potential health problems for the user and give the best advice according to some parameters such as PH, viscosity, hormone of user's salvia.

Simulations and modeling

Digital

The main goal of our project is to make a device that enables manipulation of the droplets on an array of electrodes. The basic operations required in a droplet-based lab on a chip include:

- Droplet merging - merging multiple droplets into one
- Droplet separation - separating one droplet into smaller ones
- Droplet transfer - migrating droplets from one location to another

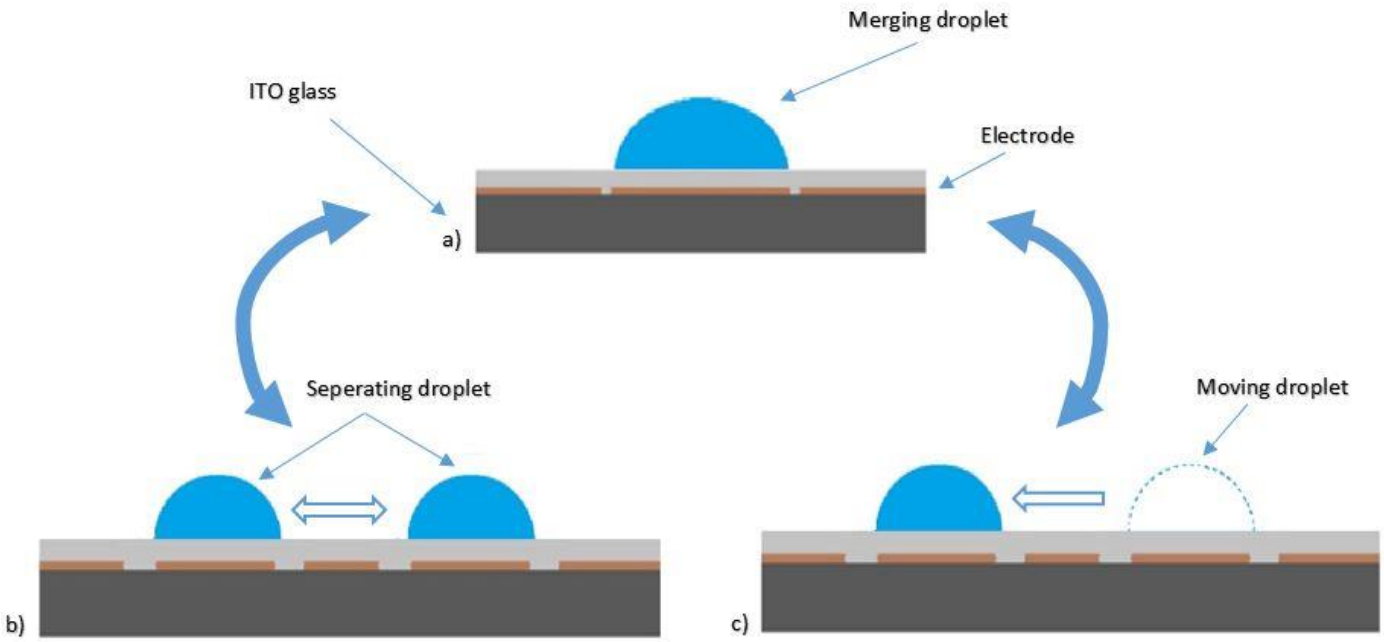

Figure 4. Droplet manipulation operations a) Merging multiple droplets into one; b) Separating a droplet into smaller droplets; c) Moving droplets from one location to another

Paper

In the simulation part, first we are going to collect the saliva using the saliva collector, then dip the saliva on the test strip, and the PH indicator will change the color of. Finally, user can use the smart phone app to take a picture of the PH strip and the app will analyze the result by comparing the result with the default color of the PH strip, the flash light will make sure the light condition stays the same every time.

Implementations issues challenges

Digital

Advantages of DMF devices include reduced the consumption of reagent volumes and faster analysis time. To realize these advantages, a user needs to supply the correct amount of voltage. Too small amount of voltage supply will cause slow droplet motion. Unnecessarily large voltages do not provide higher activation speeds and may damage the electrode array. Therefore, finding the precise amount of the voltage to move a droplet is very important.

Paper

One challenge with the paper is finding the right amount of indicator to add to the device. As described above, the indicator is applied and allowed to dry prior to the saliva being applied. The amount of indicator added affects the distance down the channel that the indicator solution travels. The hope is to find a precise amount and apply exactly that amount each time. This will ensure that the distance the solution travels remains constant each time.

Testing, procedures and specifications

Digital

Move droplet

Procedure:

- Connect the DMF system, high voltage amplifier, and the ITO slides.
- Place a droplet of water on a single electrode to test.
- Select an adjacent electrode from the controls and apply voltage

Data interpretation: If the droplet moves the test is a success.

Merge and mix Droplet

Procedure:

- Place two samples at different locations on the board
- Continue moving one of the droplets until it reaches the electrode with the second sample
- After droplets merge together, move the merged droplets to 6 center electrodes to allow for mixing.

Data interpretation: Based on color or other assay it can be determined if the mixed droplet is now a homogeneous mixture.

Paper

pH Testing:

Insert the strips into the testing cap. Have the patient deposit saliva into the cup so that there is an appropriate amount of saliva for testing. Place the slotted topper on the cup and wait 15 seconds. Remove the topper from the saliva and observe the color change in the test area of the paper. 15 seconds should allot enough time for the saliva to enter our test area, without flooding the area or causing too much dilution of color. Observe the colors and compare with the table below.

Table 2. pH Testing

Indicator X Test	Indicator Y Test	Result
Low	Low	pH<5.5
Low	High	5.5<pH<6.5
High	High	pH>6.5

If both indicators show that the pH is below their range, then the solution has a pH of below 5.5 and should be considered a major warning to the patient and they should see a doctor/dentist soon. If indicator X shows a pH below its range, and indicator Y shows a pH above its range, then the saliva has a pH between 5.5 and 6.5. This shows the patient that there may be a problem developing and they should seek the advice at their convenience, or at their next scheduled appointment. If both tests show the pH is above their ranges, then the pH is more than 6.5 and in the normal and healthy region.

Other documents

CAD mechanical, electronic

CAD tools help us efficiently handle the complex design of the digital microfluidics device's integrated circuit boards. Using AutoCAD, we will design an enclosure to contain the three circuit boards, the control board, the switching board, and the high-voltage amplifier. A portable webcam and DMF device on top of the box. A PC/laptop can connect with the DMF enclosure through USB.

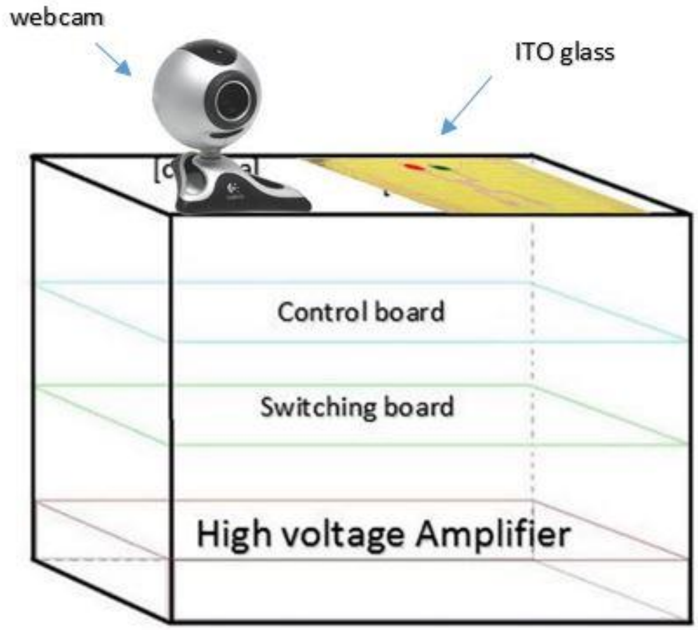

Figure 5. The DMF box contains control board, switching board, and voltage amplifier. There are webcam and ITO glass put on top of the DMF box.

PCB issues

The printed circuit board (PCB) designs we have for the digital microfluidic system are separated into three main boards, the control board, the switching board, and the high-voltage amplifier.

The control board will be the main controller that has unity gain buffers, feedback resistor selection, square wave generator, frequency divider, square or sine wave selector, low pass filter, digital potential meters and the most important one – Arduino Mega 2560.

The switching board will manipulate the discrete electrode according to the signal it receives from control board. It mainly has a 40 channel GPI0- PCA9505DGG, 5 sets of octal buffers and each of the octal buffers distributes signal to 8 relays. So, there are total of 5 octal buffers 74AC540 and 40 Relay – AQW216. The octal buffer is responsible for temporarily storing data and moving data from one place to another.

Our goal for this senior design is to modify the schematic circuits into our own design and simplify the design while maintaining the same functionality.

Software/firmware design documents

Digital

The software program will be designed to enable a user to see all the electrodes on the screen and actuate each electrode by clicking the mouse on the image of ITO glass. The image of electrode needs to be exactly matched with the electrodes on ITO glass. Once the electrode is actuated automatically, it attracts the liquid droplet. The software program records all the actuation steps and how much voltage is supplied.

Paper

Saliva collection device design:

In paper microfluidics, our test device will be at a user's home. The devices should be portable, handheld, light small, easy and safe to test. The design includes two parts, one part is the saliva collector, the other part will be the polymer indicator, which is supposed to show different colors and indicate different health condition for user. So we are designing a device which can collect and test the concentration of pH and viscosity in the saliva. Our saliva collection devices includes a hand hold plastic "stick" and on the other half of the stick is the absorbent polymer, which will collect saliva from a collection tube where we can spit our saliva into.

Schematics

The following schematics were released by a Dr. Aaron Wheeler's research group as part of an open-source digital-microfluidics control system. We are following these schematics to validate their functionality and develop a functioning prototype.

High-Voltage Switching Board

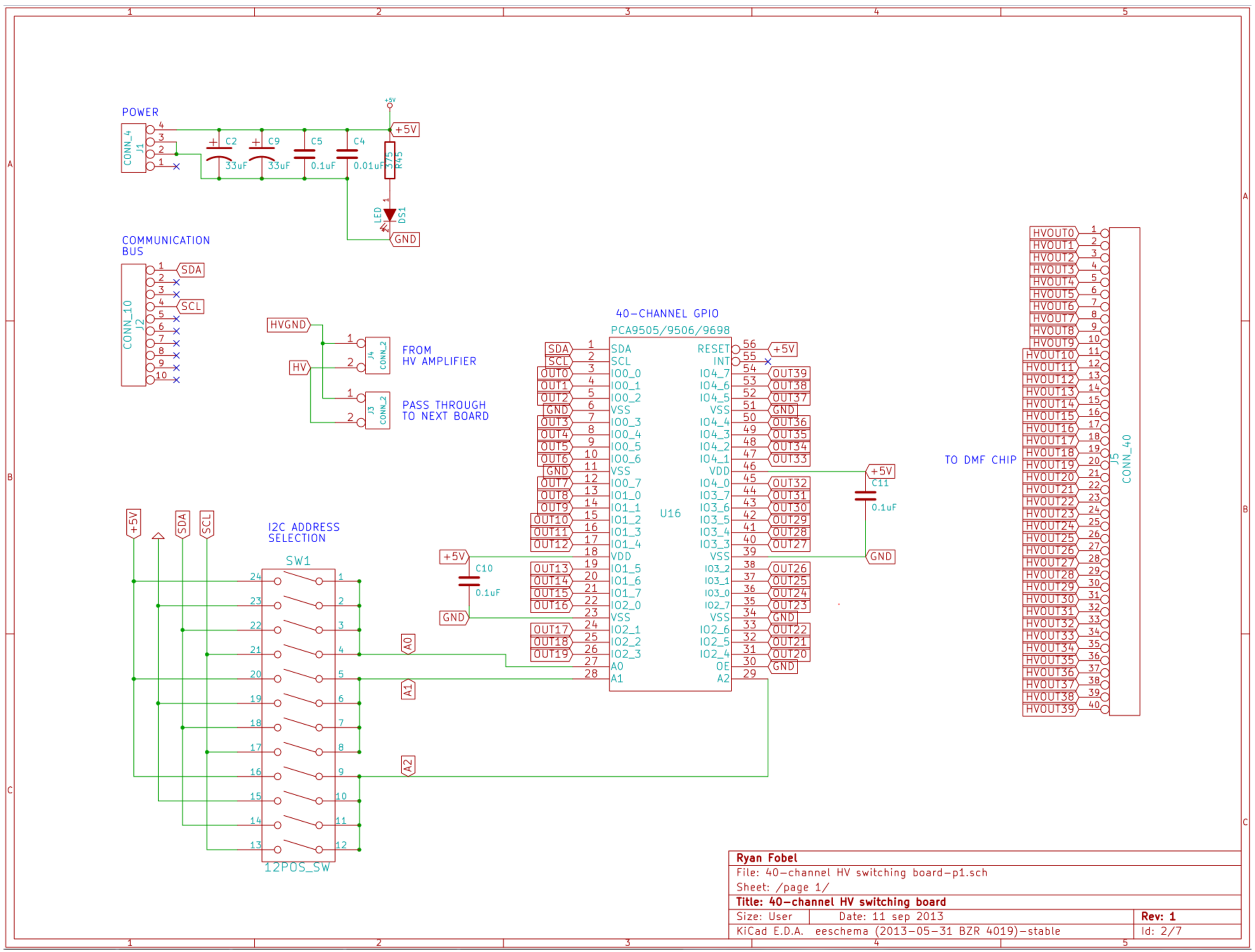

Figure 6. High-voltage switching board

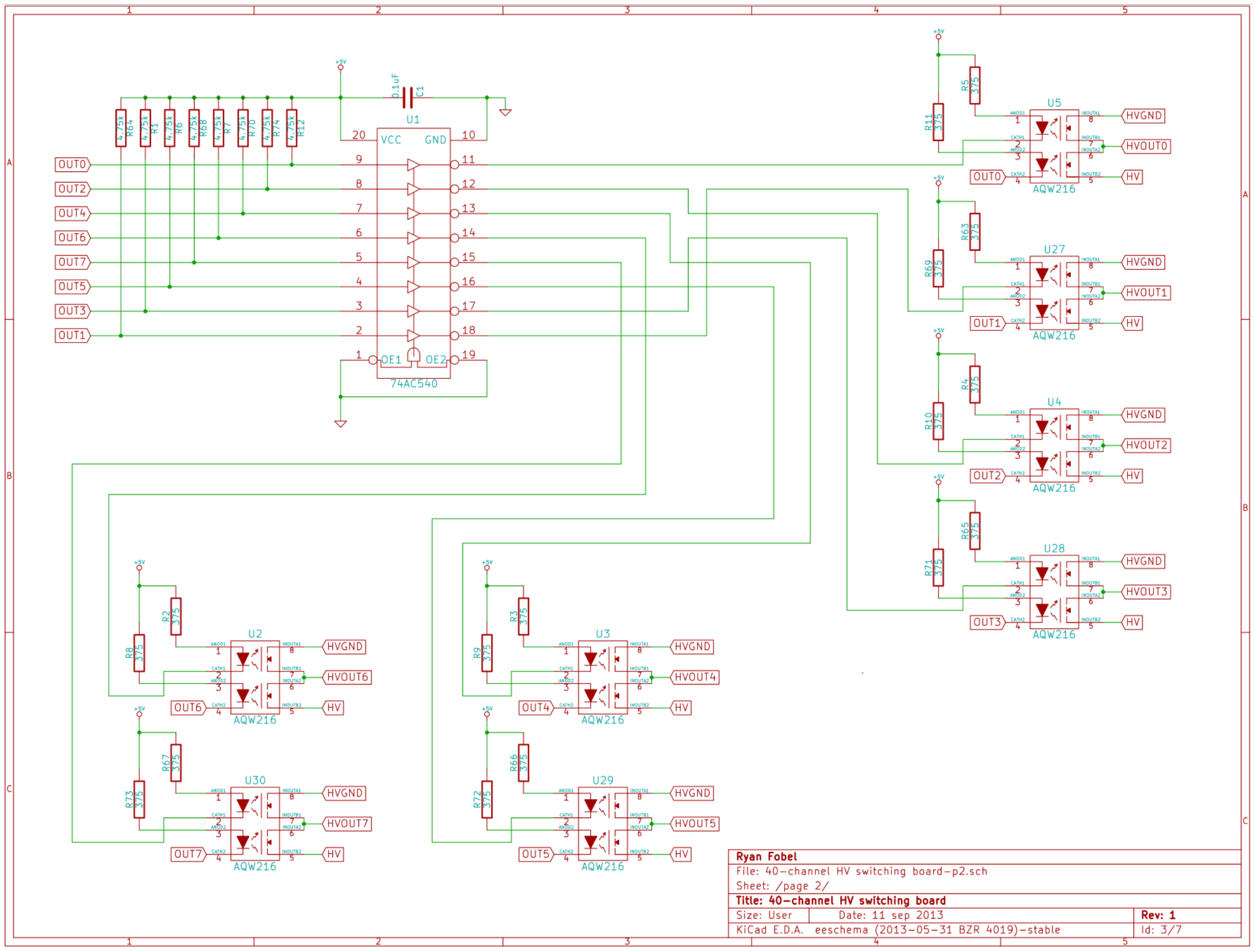

Figure 7. High-Voltage Switching board

Control Board

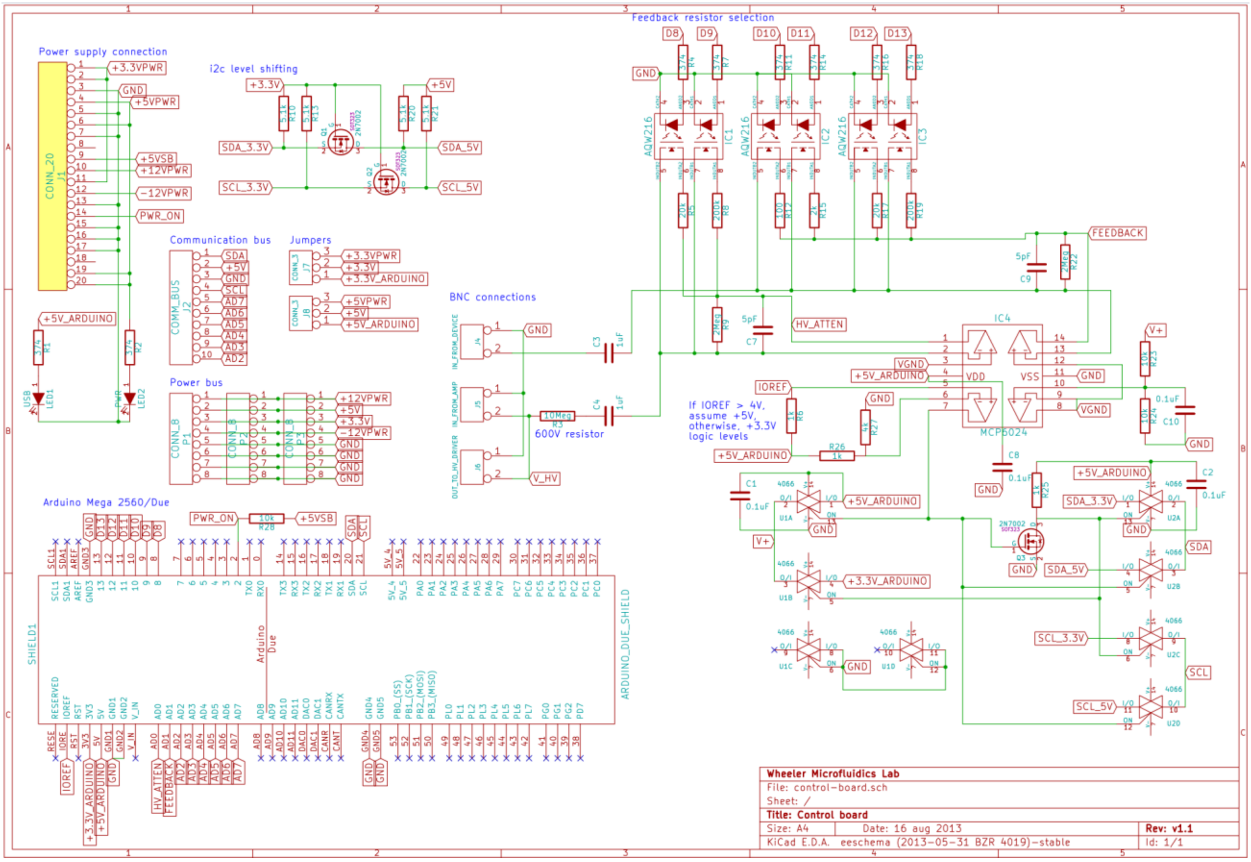

Figure 8. Control Board