OPERATOR’S MANUAL

THERMOACOUSTIC CELL SORTER

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IMPORTANT SAFETY INSTRUCTIONS

GENERAL WARNINGS

- Care must be taken when handling the syringe
- Gloves must be worn when creating different gels, and using alcohol solvents
- Ensure spin coating and plasma cleaning takes place under a fume hood to reduce the risk of headache or drowsiness
- Do not look directly at the photolithography UV device when it is activated
- Operate the spin coating machine in a dark room
- Handle the Silicon wafers with extreme care when applying the aluminum foil boat
- When using the magnetic particles, use full body protection as the particles are on the nanoscale and can penetrate many surfaces
- When using the magnetic particles it is especially important to wear a mask to make sure that the particles are no inhaled
- Wear gloves at all times when handling PDMS.
- Cured PDMS must be removed from the oven using tongs, as it reaches high temperature
- Make sure when using the magnet that the power source’s current output does not exceed 2 amps
- The frequency of light we are using is potentially harmful to the human eye. Protective eyewear must be worn at all times while the device is activated
- The laser diode is sensitive to static discharge. The operator must wear a conductive bracelet connected to ground when handling the laser diode driver during, before, and after use
- Open containers of water should be kept away from the laser diode driver and all electrical components
- Avoid all bodily contact with the IR QC1 dye
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H-GATE

GLASS WAFER

SILICON WAFER
PDMS CONTAINER

BEAKER
GLASS MIXING ROD

SCALPEL BLADE

WS-400-6NPP-LITE SPIN COATER, HOT PLATE, AND VACUUM PUMP
ULTRAVIOLET EXPOSURE CHAMBER

OVEN AND VACUUM CHAMBER
VACUME PUMP

STANDARD TUBING

PDVF SENSORS
CONNECTORS

LASER DIODE

PROTO BOARD
TEKTRONIX TDS 2024 OSCILLOSCOPE

FUNCTION GENERATOR

NATIONAL INSTRUMENTS PXI CONNECTOR
NATIONAL INSTRUMENTS BNC 2120

FLASH MEMORY CARD

USB CONNECTOR
LABVIEW PROGRAM

LG VX5500
There are a variety of purposes for this project which make it unique. The main goal for the project will be the ability for Dr. Kotha and his team to further their research and fine tune the device for later commercial use. Although this device will be less efficient than currently used cell sorting devices, the much smaller size will allow for implantation inside a human body. The special part about this project is that the future devices based upon the TACS will have a potentially large impact on the human population. Upon successful implantation, this will allow for the filtration of specifically targeted cells. The most likely location for the implantation of this device will be the circulatory system. If implanted in the circulatory system, the device could potentially remove harmful cells and particles from the bloodstream, or be used to administer drugs to targeted cells, rather than allowing diffusion of the drugs into all cells including those which would be unaffected or damaged by the drug. The device could also be used to sequester a sample of specific cells from the blood for further investigation, without having to remove an entire blood sample then separate the cells. If implanted in the lymphatic system, the filtration and isolation of cancerous cells would be the final goal.

The device needs to be constructed in such a way that the proof of concept will enable construction of the device on a scale appropriate for in vivo use. Traditional cell sorting devices use a combination of an optically based data gathering technique, and electromagnets to physically move the cells into different bins. The electromagnetic sorting would not be applicable inside the human body. This necessitates the use of the actuating gates inside the micro-channel, a considerably slower method. Because our sorting rate is now limited by the actuating gates, it allows us to use a less efficient cell identification procedures and data analysis technique based on the thermoacoustic principle.

Since the device was intended to be novel, all the subunits may be classified as “unusual” (see TACS BME 4910 Final Report). The primary novel features of the design include the magnetic gel actuating microfluidic gate, the H-gate design to regulate the flow and sorting of cells, and the use of the thermoacoustic theory to acquire a signal from a cell.
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1 INTRODUCTION

1.1 GENERAL OVERVIEW

The TACS project was designed to find an alternative method for flow cytometry. Our client specified the need for a more cost effective and innovative method for sorting cells. The proposed innovations that were given in the initial project description were researched and refined during the two semesters. This experimental design concept required swift design changes throughout the year as it became evident that some concepts were unable to be implemented into the TACS, while others had the potential for implementation if more research was conducted. This document includes all protocols followed for all methods, including those that produced less than optimal results.

The current iteration of the TACS project is not a real-time cell sorter, rather it is an attempt to prove that each of the parts required to build a real-time cell sorter work. Each component of the device was tested separately.

The electronic component of the device consists of a circuit used to power an infrared laser diode, and PVDF ultrasound sensors. A commercial oscilloscope is used to for data acquisition and a National Instruments PXI 6251 card connected to a BNC 2120 is used to generate a trigger pulse for the laser diode driver and to trigger the oscilloscope. These films are responsible for detecting different ultrasound frequency ranges. The films are attached directly to the first channel of the device. This is the location that the particles get excited and upon relaxation, release an ultrasonic wave. To prove this concept, a channel made out of PDMS and a glass slide was constructed. The different frequency PVDF films were attached to the glass downstream from the excitation laser in order to detect a signal. Infrared dye and an infrared laser diode were used in this process.

Once the particles get excited and the ultrasound is detected, the signal can be saved to a flash card in the oscilloscope and transferred to a computer for further signal analysis.
The flow is directed by the use of an H-Gate. The H-Gate allows the flow of cellular stock to be redirected without having to pass through microgates which could potentially damage them. These gates allow or block flow from two saline containers. The gates will either be active or inactive depending on which direction the cellular flow should go. In order to prove the magnetic gate concept, one was created to test the different flow effects. Also, an H-Gate was constructed in order to try to mimic the flow mechanics that are involved. Although the TACS is not one complete device, many ideas that had the potential to be used in this design were omitted due to feasibility or other reasons. In the absence of these ideas, a few key concepts emerged as the most appropriate means for the device.

1.2 INSTRUCTIONS

1.2.1 PVDF CHANNEL

To test the flow of water or other liquid solutions in a micro channel, the use of a syringe is required. Make sure the syringe has the appropriately sized needle tip and that it is secured to the syringe body. Place the syringe needle in the desired solution and pull back the trigger to uptake the liquid. Take the filled syringe and put the needle inside one of the standard tubing inputs. Make sure to firmly insert the syringe needle into the tube so there is a pressure seal at the end of the tube. This ensures that all of the pressure is directed in the tube rather than leaking at the end of it. Make sure to slowly start pressing the trigger to see the different flow velocity. If there is too much resistance from the channel on the syringe and no flow is starting, see the trouble shooting guide to fix any potential problems. If there is successful flow, make sure to carefully regulate the flow velocity. Once the testing is complete be sure to empty the contents of the syringe in the proper waste receptacle and then fill up the syringe with air to blow out any excess liquid or solution that still remains in the micro channels.
1.2.2 MAGNETIC GATE

To test the magnetic gates a few steps must be followed. First, fill a syringe with the fluid to be injected through the gate. Using a fluid with food coloring can help to identify the rate of flow. Use a syringe and draw the desired fluid into it. Then attach the syringe to one end of the tubing that is attached to the gel.

![Image of syringe and tubing]

The magnet comes with two leads. Connect one of the leads to ground. Connect other lead to the output of a power source. The voltage should be set to 27 volts and the current to 2 amperes.

![Image of magnet and leads]

Put the magnetic gate assembly on top of the magnet where the field is strongest. Begin to force liquid through the gate at a constant rate. Turn the power source on and watch as the flow rate subsides. Turn the power source back off to resume the initial flow rate. To slow the flow rate further, place a magnetic material, such as an iron rod above the magnetic gel channel.
1.2.3 LASER DIODE

To use the laser diode, begin by turning on the National Instruments PXI-1036. Then power up the personal computer. If these tasks are not completed in this order, the computer may not recognize the hardware and the LabVIEW program will not be able to produce a trigger pulse. Ensure that the BNC 2120 board is connected to the NI PXI-6251.

From this point on the operator must wear a conductive bracelet which is grounded. Remove the ETX 10A laser diode driver from its protective casing and connect the numbered wires to the numerically corresponding ports in Connector B.

Open the file “TACSMain.vi” and click the start button (an arrow that points to the right). Ensure that “External Acquisition” is selected in the radio list. From this point forward, the operator should wear protective eyewear. Switch the switch labeled laser diode.

To be sure that the laser diode is pulsing, the LG VX5500 camera phone is used. Flip the device open and hold the “End” button to power it on. Click the button on the right side of the phone; it has a camera icon on it. Hold the device so that the beam of the laser diode is directed into the camera lens. If the beam of the laser light is not normal to the camera lens, it will not appear on the camera.

![Activated Laser Diode](image)

**Figure 1 - Activated Laser Diode**
If everything is functioning, a bright bluish white spot can be seen in the center of the laser diode. Switching the “Laser Diode” switch in LabVIEW should turn the light off and on.

### 1.2.4 DATA ACQUISITION

To begin data acquisition, the PVDF sensors need to be connected to the circuit board. If they are not already connected to Connector A, connect the numbered leads of the PVDF sensors to the numerically corresponding ports on Connector A. If the aluminum interference probe is going to be used, connect its lead to the numerically corresponding port on Connector A as well. Connector A should already be connected to the circuit board. The white wires are directly grounded whereas the red wires are separated from ground by 10 kΩ resistors.

Turn on the Tektronix TDS 2024 Oscilloscope by pushing the power button on the upper left corner of the device. If they are not already attached, attach voltage probes to channels 1, 2, 3 and ext/5. If the aluminum interference sensor is going to be used, connect a voltage probe to channel 4 as well. Connect the voltage probes of channels 1-3, and 4 if in use, to the marked nodes on the protoboard, and connect their grounds to the ground strip on the protoboard. Connect the ext/5 probe to the marked node on the protoboard.

The oscilloscope should be configured as follows:

- **Trigger Menu**
  - Type: Pulse
  - Source: Ext/5
  - When: >
  - Set Pulse Width: 132ns
  - Polarity: Positive
  - Mode: Normal
  - Coupling: LF Reject

- **Save/Recall Menu**
  - Action: Save All
  - Print Button: Saves All To Files
- Acquire Menu
  - Acquire: Average
  - Averages: 128

- Knobs
  - Volts/Division: Between 2 mV and 50 mV for each channel
  - SEC/DIV: 100 ns
  - Vertical Position: Each channel should be vertically separated so that they can be distinguished from each other
  - Trigger level: 2.52V

The device should appear frozen as long as the TACSMain software program is disabled. As soon as the “Laser Diode” switch is activated, data acquisition should begin.

To attach the PVDF sensors to a specimen, use the soft piece of PDMS. The PDMS should be gently cleaned using alcohol and lint-free Kim wipes to remove any dust particles from the soft surface. Gently pressing the PFDF sensors into the soft gel causes them to adhere to the gel temporarily. Now the PVDF can be pressed against a specimen so that the sensors are sandwiched between the specimen and the gel.
Figure 2 - PVDF Films in Contact with the Dye Specimen Using the Soft PDMS

To save data, a compact flash memory card needs to be inserted into the port labeled “Type 1 Compact Flash” on the rear side of the oscilloscope. A specific folder can be selected as the save location by clicking the “SAVE/RECALL” button then choosing the “Select Folder” button. Pushing the “PRINT” button will cause the raw data as well as a screen capture to be saved to the selected folder.
1.2.5 DATA TRANSFER

To transfer the data from the oscilloscope to the computer, begin by removing the flash memory card from the oscilloscope. Insert the card into the USB adapter, and plug the adapter into the computer. Open the “My Computer” explorer to find the files.

1.2.6 CELL STAINING AND PREPARATION

MATERIALS

- Licor QC-1 NHS Ester Dye
- 50 mM Potassium Phosphate Buffer at pH 8.5
- Cellular stock in protein-free fluid medium (or specimen particles)

PROCEDURE

Weigh out 100 micrograms of CQ-1 dye
Pipette 100 microliters of potassium phosphate buffer and combine it with the dye in a sterile test tube
Pipette between 50 and 200 microliters of the cellular stock into the test tube
The reaction completes in 2 hours at room temperature, or overnight at 4°C Celsius

NOTES

These volumes should be as small as possible. Hundreds of microliters will suffice.
Once the cells or specimen particles are labeled they can be further diluted so that the microchannels will not get too crowded
It is recommended to use tinted vials to shield the dye from light

1.2.7 CELL SORTING GUIDELINES

To use the TACS for its intended purpose, the soft piece of PDMS should be used to attach the PVDF sensors to the glass slide on the bottom of the microchannel. Stained cells can be taken up using a syringe and injected into the microchannel. Pressure should as low as
possible to ensure slow and steady flow. The laser diode should be positioned so that it aims directly into the channel from the glass side.

![Cell Sorting Configuration](image)

**Figure 4 - Cell Sorting Configuration**

## 2 MAINTENANCE

### 2.1.1 MICRO CHANNELS

The contents of the syringe must be released at a very slow rate in order to ensure that the microchannel is not damaged by the pressure. If the contents of the syringe do not readily empty into the channel, the syringe should be released and the microchannel should be inspected to see if there are any blockages. Blockages cause high pressure resistance and can cause damage to the channel or syringe. High pressure resistance is common in the H-Gate channel. The input channel requires close attention so that too much force is not applied from to the syringe. Too much pressure may cause leaks in the channel or even remove the adhesion effects of the PDMS pattern from the glass. If there are leaks caused by misuse or high levels of pressure then they can be resealed using PDMS. Use aluminum foil and create an enclosed cup
like structure around the channel and glass slide. Pour PDMS either directly on the location of the leak or distribute an even layer throughout the whole slide. When curing the PDMS, a temperature of 60°Celsius is used to avoid melting the beads of glue that are between the tubing and the PDMS.

2.1.2 MAGNETIC GATE

There is little maintenance required for the finished magnetic gate. The PDMS does not readily degrade in air and the bond to the glass is effectively permanent. Thus, the maintenance surrounding the magnetic gate is more concerned with the raw materials. The PDMS and the curing agent should be kept refrigerated after opening. The magnetic particles should be stored in a cool dry area in a sort of closet or pantry. The magnetic particles should be kept contained in a bag inside of a bin to make sure that the particles do not all become airborne immediately after opening.

2.1.3 SYRINGE

When putting the syringe into one of the standard tubing inputs make sure the needle does not puncture the tubing to avoid any potential leaks. Once the syringe is done being used remove the contents into the proper waste receptacle and dispose of the needle. It is not advised to re-sheath the needle once it has been used due to chemical contamination and dulling of the needle.

2.1.4 SPIN COATING MACHINE

After each use of the spin processor make sure to clean up. Any excess SU-8, or other material used, that was spun off of the substrate and into the lid of the spin coater must be cleaned. It is advised that this is done after each use to ensure that multiple layers are not being deposited on each other.
2.1.5 LASER DIODE

When not in use the laser diode should be kept in a protective bag to protect it from static discharge. Before handling the laser diode driver, the operator must wear a conductive bracelet on the right wrist and connect it to electrical ground. This prevents static charge from the operator’s body from damaging the sensitive circuitry. To attach the laser diode driver to the circuit, the operator connects each of the numbered wires on the laser diode to numerically corresponding ports on connector B. The ground bracelet should be worn until the laser diode is disconnected again and returned to its protective case.

2.1.6 PVDF FILMS

PVDF is sensitive to high temperatures. Exposure to temperatures greater than 80° Celsius causes irreversible damage to the PVDF sensors. The device should not be stored near a heater, oven or other device that generates high temperatures. PVDF films should be stored lying flat without any force pressing on them. If the films are bent or undergo stress, they can deform and their effectiveness at recording ultrasound signals will be reduced.

2.1.7 INFRARED DYE

The Licor QC-1 NHS Ester Dye must be protected from light and heat when stored. The dye should be kept frozen at -20° Celsius. The dye should be contained inside a light shielding bag to ensure that the organic molecules are not prematurely degraded.
3 TECHNICAL DESCRIPTION

3.1 GEL PREPARATION

3.1.1 PROTOCOL FOR THE PREPARATION OF THE N-ISOPROPYLACRYLAMIDE (NIPA) GEL.

MATERIALS AND REAGENTS

- 50 ml Beaker
- 40% Acrylamide solution
- 2% Bis-acrylamide solution
- 1 M Hepes solution
- 50 mM Hepes solution
- Temed
- Water
- Magnetic particles
- 1x PBS solution
- 10% Ammonium persulfate (freshly prepared)
- Nitrogen gas (optional)

PROCEDURE

1) Mix 7.5 ml of acrylamide, 2 ml of bis-acrylamide, and 0.4 ml of 1M hepes with 30 ml of water.
2) Add desired amount of magnetic particles
3) De-gas mixture in vacuum chamber for 20 minutes to dispel any oxygen bubbles.
4) Add 20 ul of Temed (cross-linker).
5) Add ammonium persulfate (initiator).
6) Immediately mix solution with sonicator and pour onto mold.
7) Cover mold with paper towel and leak nitrogen gas over setting gel. (optional)
8) Leave for 30 minutes
9) Flood assembly and wash gel with 50 mM hepes solution

**NOTES**

1) It is most important to add the initiator last to the mixture.
2) If the nitrogen gas flow is too high, then incomplete polymerization may occur.
### Materials and Reagents

- 50 ml beaker
- Argarose powder
- Heating plate
- Magnetic particles
- Water

### Procedure

1. Add 0.3 g of Argarose to 30 ml of water to create a 1% solution.
2. Heat on hot plate while stirring until all of the Argarose is dissolved.
3. As the liquid cools to near room temperature, add desired amount of magnetic particles and mix thoroughly.
4. Pour liquid onto mold and let cool for 30 min.

### Notes

1. If the Argarose solution gets too hot the solution may spill over the top.
2. The magnetic particles may be difficult to immerse in a uniform fashion.
3.1.3 PROTOCOL FOR THE PREPARATION OF POLYDIMETHYLSILOXANE (PDMS) GEL (PROTOTYPE)

MATERIALS AND REAGENTS

- Slygard 184 elastomer kit
- Ethanol
- Curing oven
- Vacuum desiccator
- Mold of imprint shape (approximately 10 cm x 10 cm)
- 100 ml beaker

PROCEDURE

1) Add 40g of monomer to 1 g of curing agent (Both included in elastomer kit)
2) Mix thoroughly for 5 minutes
3) De-gas mixture in vacuum desiccator for until all bubbles are gone (approx. 30 min).
4) Pour mixture of over mold and de-gas again for 30 min.
5) Cure in oven at 80 ºC for 45 minutes
6) Peel gel from mold
7) Use ethanol to clean beaker

NOTES

1) The curing times and temperatures can be varied. The gel will polymerize at room temperature in 48 hours
2) When pouring mold, do so slowly to avoid introducing more air bubbles.
3.2 MICROCHANNEL FABRICATION

3.2.1 PROTOCOL FOR THE FABRICATION OF SU-8 CHANNEL MOLDS

MATERIALS AND EQUIPMENT

- Spin coating processer with chuck
- Vacuum Pump
- Nitrogen gas container
- SU-8
- SU-8 Developer
- Isopropyl alcohol
- Silicon wafer or glass substrate
- Photolithography machine
- Transparency sheet
- De-ionized water
- Hot plate
- Timer or stop watch
- Dark room

PROCEDURE

1) Clean the Silicon wafer or glass substrate using isopropyl alcohol
2) Let the clean substrate dry either by allowing enough time to air dry or place in an oven at 60 °C for 5 minutes.
3) Place the substrate on the spin coater chuck
4) Open the Nitrogen gas container to 60 psi
5) Turn on the vacuum pump
6) Turn on the chuck vacuum pump on the spin coating display so the display reads a value of V greater than 20.

7) Close the spin coating lid and run the program to ensure the substrate is centered on the chuck.

8) Open the lid, turn off the vacuum, adjust the substrate, turn the chuck back, close the lid, and run the spin coater. Repeat this step until the substrate is centered on the chuck.

9) Once the substrate is centered on the spin coater chuck, apply the desired amount of SU-8 on top of the substrate.

10) Close the lid and run on program E (500 rpm for 10 seconds). After program E is completed run program F (1500 rpm for 30 seconds).

11) Open the spin coater lid to ensure an even distribution of SU-8. If the distribution is not as desired then apply more SU-8 and repeat the process.

12) Take the evenly distributed SU-8 substrate and place on a hot plate at 95 ºC for 15 minutes.

13) Take the substrate off the hot plate and place the desired transparency (smooth side down) pattern on top of the SU-8. Put into the photolithography machine and expose to UV light for 0.6 – 1.0 minutes. (0.6 minutes is typical)

14) Removed the substrate and transparency from the photolithography device, remove the transparency from the top of the substrate and then place the substrate back onto the hot plate for 2 minutes.

15) Remove the substrate from the hot plate and place into SU-8 developer for 5 minutes making sure to shake for part of the time in the solution.

16) Remove the substrate from the developer and wash with de-ionized water.

17) After the rinsing is complete bake the substrate for one more minute.
1) The amount of SU-8 put on the substrate prior to the actual spin processing determines the thickness of the eventual SU-8 layer.
3.2.2 PROTOCOL FOR FABRICATING POLYSTYRENE MOLDS

MATERIALS

- Printable polystyrene sheets
- 600 dpi or higher resolution printer
- Computer
- Oven

PROCEDURES

1) Design the microchannels using CAD or other powerful image editing tool. Microchannels should be 3 X wider than the desired width.
2) Print the designs to scale using the polystyrene sheets in the printer.
3) Cut the Sheets into their desired shapes
4) Preheat the oven to 110° Celsius.
5) Bake the Polystyrene sheets for 10 minutes. While baking, the sheets will curl and shrink. Care should be taken so that they do not stick to each other or themselves. If they do get stuck, remove them from each other using tweezers and continue baking.
6) After shrinking is complete, place the sheets on a flat surface and allow to cool for 30 minutes.

NOTES

1) The higher the resolution setting on the printer, the greater the height of the resulting channel will be.
3.2.3 PROTOCOL FOR THE PLASMA TREATMENT OF GLASS SLIDES TO PDMS CHANNEL MOLDS

MATERIALS AND EQUIPMENT

- Isopropyl alcohol
- Methanol
- Glass slides
- Desired PDMS channel mold
- Plasma cleaner machine
- Cotton swabs
- Oven
- Colored dyes

PROCEDURE

1) Puncture the PDMS with the correct sized holes and the appropriate locations
2) Clean both sides of the PDMS mold with isopropyl alcohol
3) Clean both sides of the glass slide or other glass substrate with methanol
4) Place the PDMS mold in an oven at 60 ºC for 5 minutes to dry the excess alcohol
5) Place the cleaned PDMS mold with the channel facing up (flat side down) on a holder glass slide.
6) Remove any air bubbles to ensure that there is no exposed PDMS that makes contact with the holder slide. Especially remove any air contact around the edges of the mold.
7) Place the cleaned glass substrate (the piece of glass the actual channels will be stuck to) in the plasma cleaner
8) Place the PDMS mold and glass holder slide together into the plasma cleaner
9) Turn the plasma cleaner on
10) Put the lid on the plasma clean so the valve is pointing to the right
11) Turn the valve 90 degrees to the right so the arrow is pointing down and turn the vacuum pump on.
12) Leave the pump on for 30 seconds
13) Turn the valve 90 degrees further clockwise so the valve arrow is now pointing to the left.
14) Turn the RF pulses on high for 30 seconds (vacuum pump remains on)
15) After 30 seconds, turn the valve counter clockwise so the arrow is pointing down, turn the RF pulses off, and turn the pump off.
16) Turn the valve again counter clockwise so the arrow is facing right to release the air
17) Remove the vacuum lid from the plasma cleaner and remove the PDMS/glass holder assembly and the plasma cleaned glass slide.
18) Place the glass slide on the channel side of the PDMS mold at the proper location. Make sure the location is correct because the adhesiveness of the plasma cleaned surfaces is very strong and peeling off the mold to relocate the mold is difficult.
19) Remove the glass holder slide from the flat side of the PDMS mold
20) Place the glass side and PDMS channel mold on a flat surface and place a small amount of weight to ensure the bonding process is complete
21) Test the channels by using food coloring or other dyes.

NOTES

1) Punch holes in the PDMS mold prior to cleaning. Make sure the channel or desired pattern is facing the individual so particles do not get deflected into the channel.
2) Steps 18 and 19 must be performed very quickly because the time window for the plasma cleaning affect lasts approximately one minute. The holder slide may also become bonded to the PDMS if exposed to air not removed from step 6.
### MATERIALS AND EQUIPMENT

- Properly plasma treated glass slide with the PDMS microchannel patterns
- Hot glue gun
- PDMS (10 mL) See ‘Protocol for the Preparation of Polydimethylsiloxane (PDMS) Gel (Prototype)’ to make
- Oven
- Vacuum oven
- Aluminum Foil
- Standard tubing
- Razor blade
- Ruler

### PROCEDURE

1. Use a ruler and the razor blade to cut the standard tubing into a specific length.
2. Plug the hot glue gun in and let it heat up to use.
3. Apply hot glue approximately 2 mm from the end of the tube circularly around the tube.
4. Wait 30 seconds for the glue to slightly cool.
5. Insert the 2 mm exposed end into the puncture.
6. Repeat steps 3-5 until each puncture has an inserted standard tubing end.
7. Let the hot glue dry for five minutes.
8. Make an aluminum foil boat around the channel slide.
9. Using aluminum foil, wrap all of the inserted tubes up to be held back (almost like a hair tie) place the wrapped tubes and place them on a ledge of the foil boat.
10) Prepare, or used prepared PDMS and pour into the aluminum foil boat

11) Bake for 3 hours at 60 °C

NOTES

1) It is important to make sure the glue is dried prior to pouring PDMS on top of the channel pattern.

2) It is also important to make sure there is no liquid present in the channels or tubing when baking the finished product.

3) The length from the end of the tube may vary depending on the thickness of the PDMS microchannelmold.
The steady state voltage for every node in this circuit is 0 as everything is grounded or connected to ground by a ten kΩ resistor, with only a transient voltage source from the PVDF films.
3.3.2 SPECIMEN EXCITATION

![Excitation Circuit Diagram]

The steady state voltage at all the numbered nodes is grounded except for node 8 which has a steady state voltage of 5 volts. Node 9 is the trigger signal and varies with time. When activated the signal is a square pulse, with a magnitude of 5 volts, and a pulse width of 150 ns.
### TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uneven SU-8 layer</td>
<td>1. Not enough SU-8</td>
<td>1. Apply more SU-8</td>
</tr>
<tr>
<td></td>
<td>2. Substrate did not spin long enough</td>
<td>2. Re-run program E, if problem persists then run program F</td>
</tr>
<tr>
<td>Spin coater will not start</td>
<td>1. Vacuum is not on</td>
<td>1. Turn vacuum on, make sure the vacuum is displayed on the spin coating display with V &gt; 20</td>
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<tr>
<td></td>
<td>2. Lid is not closed</td>
<td>2. Close the lid</td>
</tr>
<tr>
<td>No microchannel flow</td>
<td>1. Channel is blocked</td>
<td>1. Try inducing flow from another input tube to see if flow goes through the ‘blocked’ channel to remove any debris</td>
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<tr>
<td></td>
<td>2. Standard tubing blocked</td>
<td>2. See step 1. If problem persists then remove the tubing from the PDMS channel mold and replace it</td>
</tr>
<tr>
<td></td>
<td>3. Hole in the tubing</td>
<td>3. Make sure proper syringe etiquette is being practiced to minimize this occurrence. Remove the tubing from PDMS and replace it</td>
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<tr>
<td></td>
<td>4. PDMS pattern is peeling of the glass and is leaking</td>
<td>4. Remove tubing and re-plasma treat the exposed surface</td>
</tr>
<tr>
<td>Issue</td>
<td>Possible Causes</td>
<td>Solutions</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
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<tr>
<td>PDMS will not cure</td>
<td>1. Not enough curing agent was added</td>
<td>1. Make sure to mix the curing agent in the correct weight ratio and stir thoroughly.</td>
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<tr>
<td></td>
<td>2. Oven was not set to the correct temperature</td>
<td>2. Put a thermometer inside the oven to ensure the set temperature is equal to the actual temperature.</td>
</tr>
<tr>
<td>PDMS will not bond to glass during Plasma Treatment</td>
<td>1. The glass or PDMS may be dirty</td>
<td>1. Make sure the proper cleaning procedures were followed.</td>
</tr>
<tr>
<td></td>
<td>2. The RF switch was not turned on</td>
<td>2. Make sure the RF switch was turned on.</td>
</tr>
<tr>
<td></td>
<td>3. The PDMS or glass was not treated for long enough</td>
<td>3. Make sure that the contents were treated for the full time specified.</td>
</tr>
<tr>
<td>Magnet will not create field</td>
<td>1. The current on the power source may be not turned up high enough and could be creating a short in the circuit</td>
<td>1. Ensure that the current is turned high enough as not to short the electrical loop to the magnet.</td>
</tr>
<tr>
<td>Laser cannot be detected on the cameraphone</td>
<td>1. The beam may not be aimed correctly into the camera’s lens</td>
<td>1. Try to move the camera around to get the lens normal to the expected beam from the laser diode. If necessary place the camera lens very close to the laser diode.</td>
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<tr>
<td></td>
<td>2. No trigger signal is being produced at port CTR 0</td>
<td>2. This usually means that the computer did not recognize the NI hardware when it started up. Restart the computer with the NI hardware turned on.</td>
</tr>
<tr>
<td></td>
<td>3. A wire has come loose.</td>
<td>3. Verify that all connections from the BMI *** to the protoboard are firmly attached, and verify that all connections to Connector A are firmly attached.</td>
</tr>
<tr>
<td>The oscilloscope graphs appear frozen, the oscilloscope is not triggering</td>
<td>1. The probe is not attached to the circuit correctly</td>
<td>1. Verify that the probe on the EXT TRIG port is connected to the oscilloscope and completely twisted in. Verify that the tip of the probe is making contact with the protoboard node where the trigger signal is connected.</td>
</tr>
<tr>
<td></td>
<td>2. The configuration is wrong</td>
<td>2. Verify that the configuration options for the trigger menu match those listed in section 1.2.4.</td>
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<tr>
<td></td>
<td>3. The probe connected to the EXT TRIG port is defective</td>
<td>3. Remove the probe and test its resistance. If the resistance is more than a few Ohms, discard and replace the probe.</td>
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</tbody>
</table>