Patch-Clamp Microchip Testing Circuit Interface

Final Report

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sdmay18-10

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1 Introduction

1.1 Acknowledgement

This final report was supported by project advisor/client Dr. Que Long and his graduated student patch-clamp project team. We thank them for their great assistance in this project, although they may not agree with all of the interpretations of this final report.

1.2 Problem and Project Statement

Patch-clamp is a technology in biological engineering field used to catch cell with tiny clamp and observe the bioelectricity behavior of the cell. This Project is focusing on how to build up a set of electrical environment and circuit to provide operational interface between the PC-ONE patch-clamp and microchip module. With patch-clamp technology, scientist will be able to observe a clear picture about cells, which is neuron, the nerve system cells for this particular project. This could be done by obtaining a voltage square graph of the currents that generated when ions try to enter and leave the membrane of neuron. These behaviors of neurons will let biological engineers to look for many possible cures of some serious nerve diseases that could not be cured right now.

1.3 Operational Enviroment

The whole operational environment should be in the lab provide by the advisor/client. Once operation begins, limited number of electronic devices are allowed to work near the testing equipments to reduce the electronic noise. The headstage and all its related connections should be placed inside the aluminum foil to reduce the room noise. The operations involve with microchip module must be done with proper equipments weared by operators. The operation of Patch-Clamp Microchip Testing Circuit Interface must be done safely under required professional assistance.

1.4 Intended Users and Uses

Patch-clamp technique can not only be used in neuroscience but also a huge variety of physiological questions. Since this technique is still the laboratory technique, so the user of this technique should be experimenters, who want to know the ion current behavior on the surface neuron's membrane when apply different voltage or other stimulation applied to neuron. The most commonly use of this technique in the future is new drug experiment. With the observations from ion current behavior on membrane of neuron, more possible experiments could be done by scientists.

1.5 Assumptions and Limitations

Assumptions:

- All tests can be finished in 5 days(the useful period of neuron split cycle for this project).
- The equipments can work properly in three tests(-20mV PC-ONE build in voltage, empty pipette test and with cell test).
- The data collected can be relatively accurate.
- The calculation can be correct.

Limitations:

- Limit time to collect the data: have to finish the whole test within 5 days include interface setup.
- There is no air bubble actuator for this project. It is very possible that we cannot catch the cell within the living cycle of neuron.
- The possibility of successfully catching neuron is pretty random because of the limitation of the gap between the tip of pipette and neuron.

1.6 Expected End Product and Deliverables

At the end of this project, we are supposed to deliver a fully set up patch-clamp microchip testing interface, which is operational to catch neuron and observe the bioelectricity behavior of the neuron. The goal is to build up a set of electric

environment and connections to provide operational and functional user interface between the PC-ONE patch-clamp and microchip module. At the end of the two semesters we are supposed to measure the ion channel potential of neuron and the action potential of neuron under external stimulations successful by using the PC-ONE patch-clamp and microchip module.

2. Specifications and Analysis

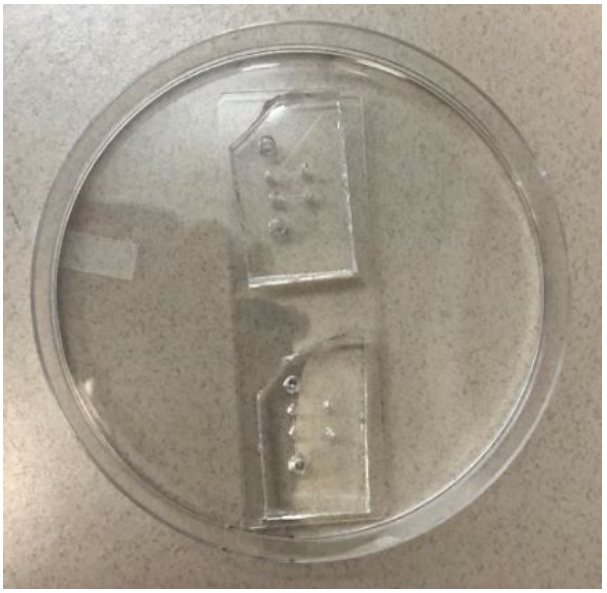
2.1 Design Specifications

Microchip module design specifications:

1. Prepare the silicon wafer for the fabrication process.

graph 2.1.1: Masked Si mold

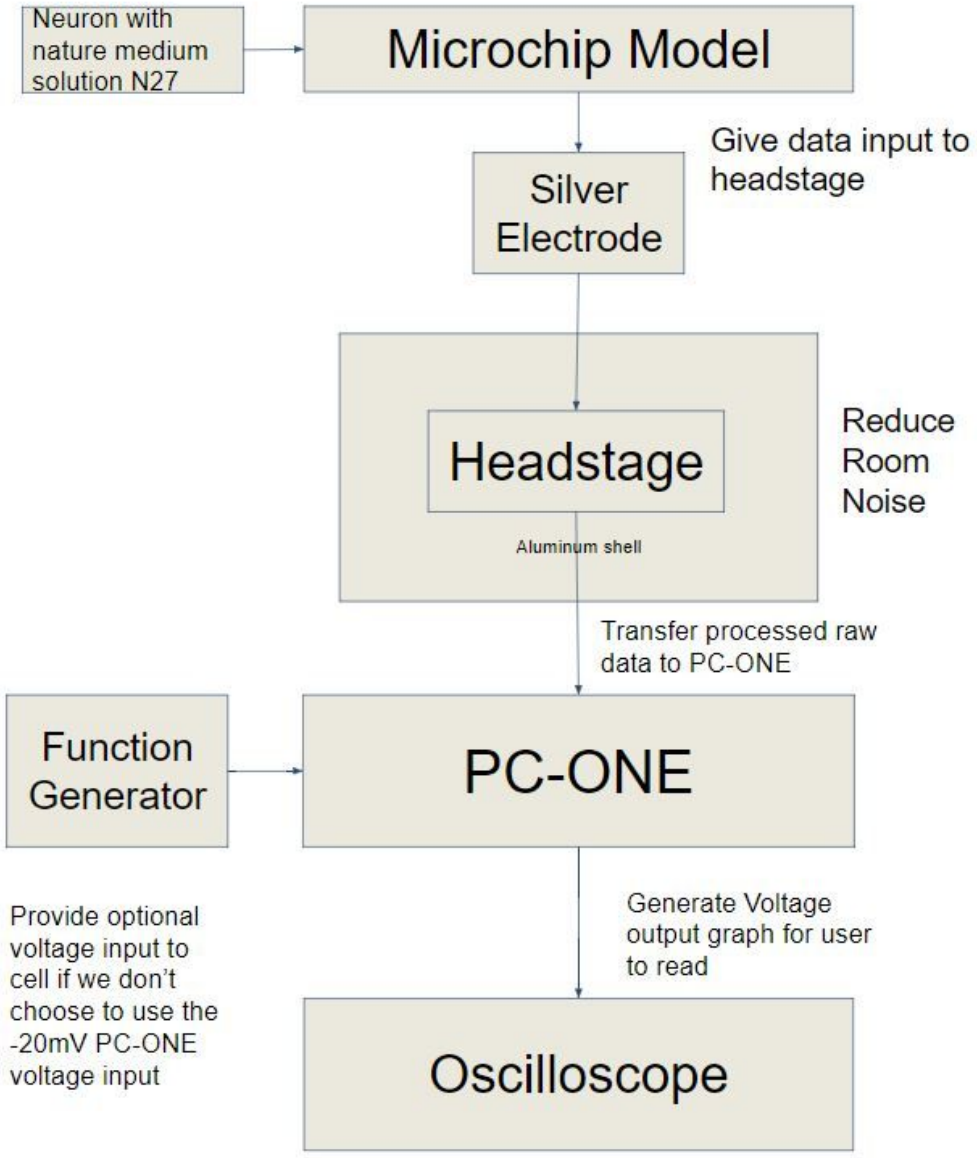
2. 4*4 um mold of the pipette is first pattened on the silicon wafer using RIE process.
3. A mold of the well and cell flow channel are aligned to the pipette mold.
4. PDMS layer is casted onto the mold
5. Bonding the PDMS layer with the glass slide.



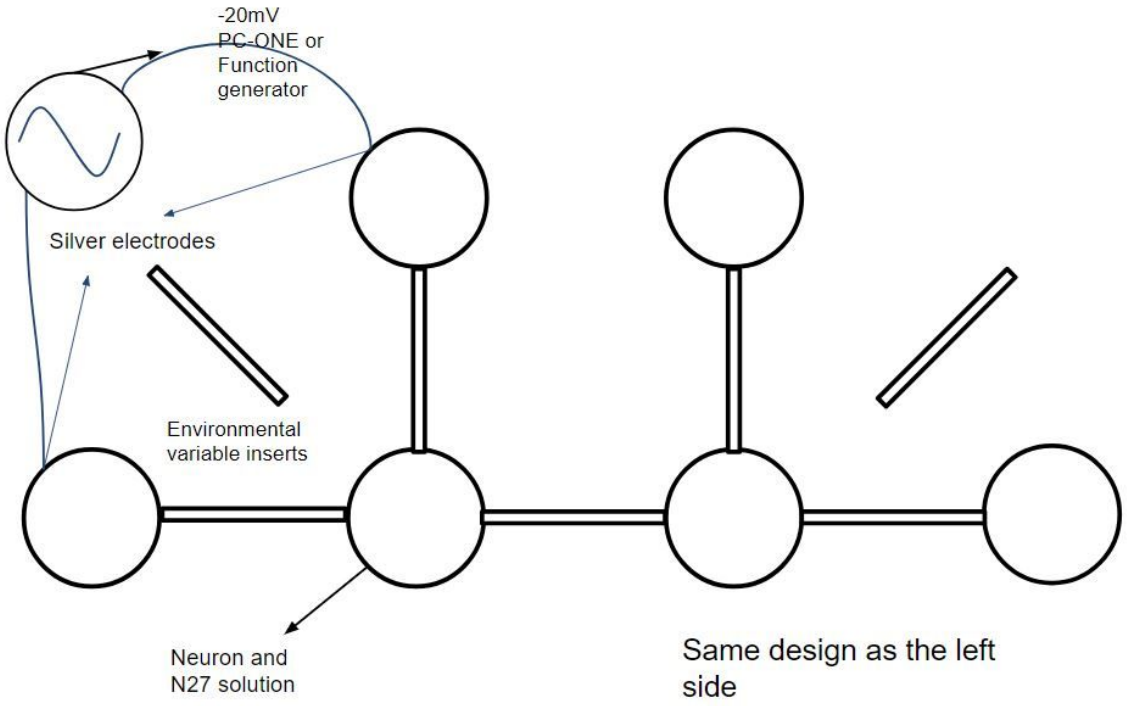
graph 2.1.2: microchip module with glass on button and PDMS on top.

2.2 Proposed Design

The design of the setup and microchip module are shown below as graphs:



graph 2.2.1: Implementation block diagram



graph 2.2.2: Microchip module design diagram

In the setup part, we used the PC-ONE given -20mV voltage or custom function generator provided voltage to give an simulated voltage to neuron in the microchip module. Then, we will connect headstage to PC-ONE, and two heads of it to the electrodes that inserted into the microchip module's micro chambers. After that, we have to set up the proper testing parameters on PC-ONE, which is also the most important step to get the accurate result reading of oscilloscope. The correct parameters will generate voltage reading which will be output through the BNC cable that connected between PC-ONE and oscilloscope. Then, we could get the current graph generated when electrons enter or leave the membrane of neuron through the connected oscilloscope by calculations.

Functional requirements:

- Pipette could catch neuron inside microchip module.
- Voltage could be observed through PC-ONE output to oscilliscope.
- Neuron must be alive during operation.
- Noise should be reduced to an acceptable range.

- Data reading and ralculation should be correct.

Non-functional requirments:

- We need to strictly follow the equipment manual to operate all operations to fulfill the project requirements.
- We have to strictly follow the safety guide of labolarity to make the experiment process safely.
- We have to cite every sources we used during the development, and ensure that the project is totally independent.
- Plan regular meeting with client to make sure the project is meeting the requirement.
- Timely maintain the project to avoid critical bugs.
- Refresh reports and document on time to meet current process.

2.3 Design Analysis

We read the manual and document of patch clamp, tried to set up the patch clamp, and helped the graduate to make the cell module, which is also called microchip. The microchip is a glass made square chip that has three micro chambers to let the pipette and Ag-AgCl electrodes insert in it.

From Whole Cell test, we see that the room noise is a big impact towards the result, So we need to place the headstage into the aluminum foil to reduce the room noise.

The microchip, we design for our project is lateral cell trapping. The reason for us to design this kind of microchip is we can observe the process of catching cell under the microscope.

The electrode is made by Ag/AgCl and it has the pipette size of 4*4 um and the size for the electrode is 1mm. the resistor for it is about 1M ohms. The fabrication process of this chip is start with a silicon wafer. A 4*4 um mold of the pipette is first patterned and formed in a silicon wafer using IRE process. The diameter for the microchamber on the microchip is 1.5mm.

3 Testing and Implementation

3.1 Interface Specifications

In this project, we need to use PC-ONE patch/whole cell clamp.

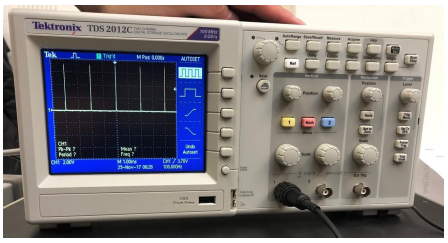
We are going to use PC-ONE Whole Cell Patch-Clamp to get the signal from PC-ONE-10 Headstage and amplify the signal so we could output it to the oscilloscope. The pipette with microchip is also required to connect with headstage so we could use headstage to process the signal from the probe connected with the Silver(Ag-AgCl) electrode that is inserted inside the membrane of the neuron. Neuron will be put into solution and flow through the cell flow located on the microchip. The oscilloscope will catch the output from PC-ONE to generate the voltage graph we are wanting.

3.2 Hardware and software

Hardware used:

The hardware we used in our project are: PC-ONE whole cell patch, whose main job is amplifying the signal generated by headstage; the function generator that provides the custom(other than -20mV) voltage to electrodes; the microchip module that is fabricated for the neuron container and main operational environment; the Ag-AgCl electrodes inserted into the microchip module to provide positive and negative voltage to neuron; Olympus IX73 Electrical Microscope to capture the image of operation;N27 rat neuron cell and its cell pecificated culture medium; micro tube with syringe; and a PC.

graph 3.2.1: PC-ONE Whole Cell Patch-Clamp



graph 3.2.2: Oscilloscope

graph 3.2.3: Function Generator

graph 3.2.4: Micro Chip

Software used:

We use Microsoft Office Excel to collect and calculate the data and results.

We use CellSens Standerd to capture images of neuron in microchip under Olympus IX73 Electrrical Microscope during operation.

3.3 Functional Testing

We used three different variables to complete the tests.

1. Voltage sourse test

   In this test, we applied the general setup of the whole system but replaced the whole microchip component with an open circuit/resistor.

2. Empty pipette test

   In this test, we applied the general setup of the whole system with only inserting the N27 specificated culture medium into the micro chambers and placing electrodes into the culture medium without neuron inside.

3. With cell test

   In this test, we applied all needed materials into the setup to get the final results.

3.4 Non-functional Testing

For non-functional test, We followed every instructions from the equipments' manual and obey every safety guide of lab. We did a lot of researches according to the PC-ONE patch-clamp, include contacting DAGAN, the company produced this device to approach the most possible accurate setting of PC-ONE. All resources we used in our documents, reports and plans are well cited.

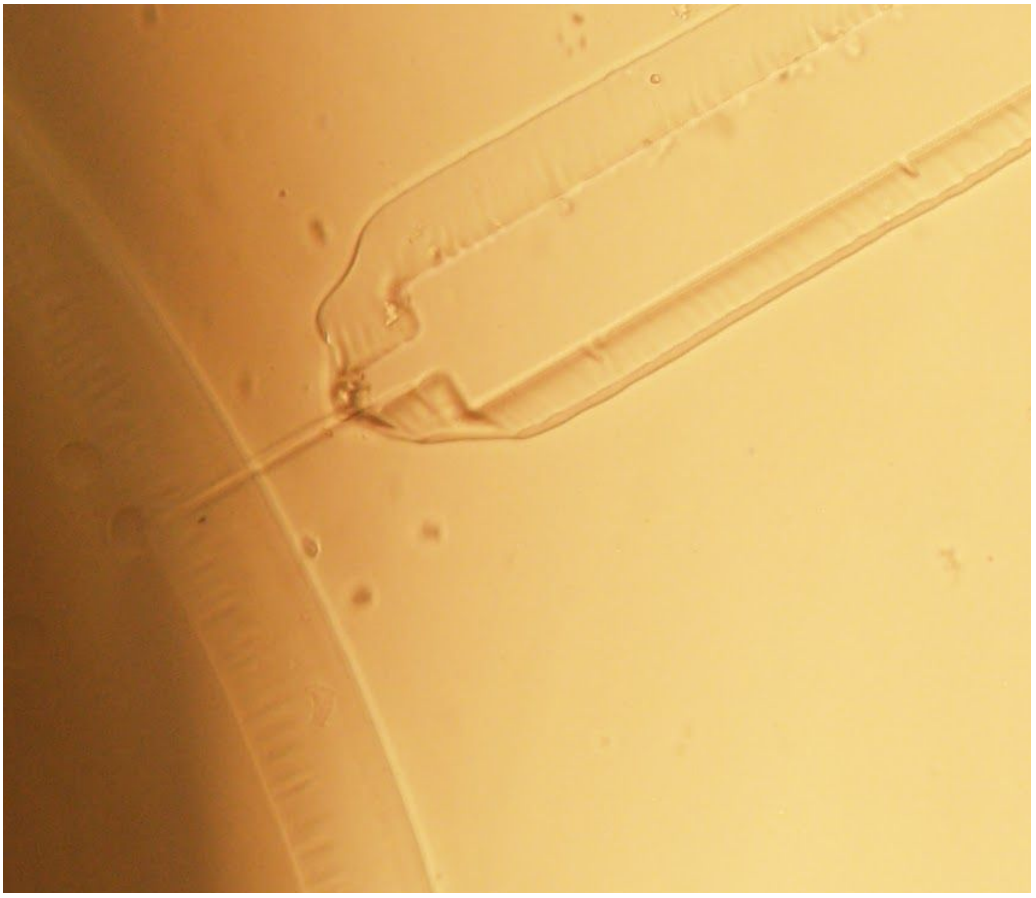
3.5 Challenges

- Neuron are spread into culture medium with radom distance.
- Need to let neuron be closer to the tip of pippette.
- Need to elimate the small gap between the pippette and neuron.
- Neuron division cycle is about 7 days. Need to finish the measurements within 5 days.

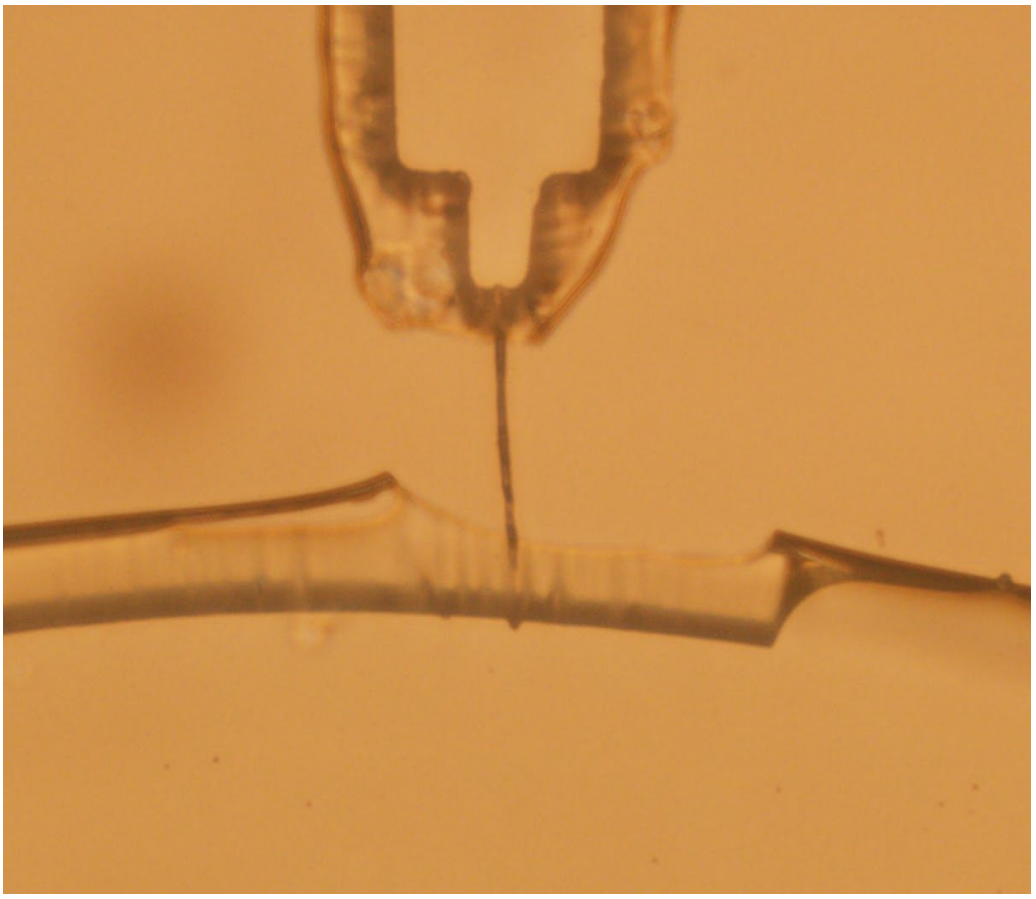
3.6 Technical Approach

- Use PC-ONE equipment to catch the neuron
- Inject proper voltage to the neuron
- Amplify the electron moving by microchip, enlarge the result so that we could obtain in the oscilloscope
- Reduce the electronic noise and keep tracing the neuron moving clearly.

3.7 Results



graph 3.8.1: Pipette catched a neuron successfully



graph 3.8.2: Pipette inner structure under Olympus IX73

Above two images are captured by CellSens Standard and show parts of the inner structure of the microchip module under Olympus IX73 Electrical Microscope. The first image was captured during one operation and the pipette has already catched a neuron successfully.
The second image shows the pipette structure without neuron attached.

Data of Voltage Source:

1	Record Length	2.50E+03		-0.05	0
2	Sample Interval	4.00E-05		-0.04996	0
3	Trigger Point	1.25E+03		-0.04992	0
4				-0.04988	0
5				-0.04984	0.004
6				-0.0498	0
7	Source	CH2		-0.04976	0
8	Vertical Units	V		-0.04972	0
9	Vertical Scale	1.00E-01		-0.04968	0
10	Vertical Offset	0.00E+00		-0.04964	0
11	Horizontal Units	s		-0.0496	0
12	Horizontal Scale	1.00E-02		-0.04956	0
13	Pt Fmt	Y		-0.04952	0
14	Yzero	0.00E+00		-0.04948	0
15	Probe Atten	1.00E+00		-0.04944	0
16	Model Number	TDS2012C		-0.0494	0
17	Serial Number	C041037		-0.04936	0
18	Firmware Versior	FV:v24.26		-0.04932	0.004
19				-0.04928	0
20				-0.04924	0
21				-0.0492	0
22				-0.04916	0
23				-0.04912	0
24				-0.04908	0
25				-0.04904	0
26				-0.049	0
27				-0.04896	0
28				-0.04892	0.004
29				-0.04888	0
30				-0.04884	0
31				-0.0488	0
32				-0.04876	0
33				-0.04872	0
34				-0.04868	0
35				-0.04864	0
36				-0.0486	0
37				-0.04856	0

chart 3.8.1: Data of Voltage Source

Data of Empty Pipetty:

	A	B	C	D	E
1	Record Length	2.50E+03		-0.05	-1.92
2	Sample Interval	4.00E-05		-0.04996	-1.92
3	Trigger Point	1.25E+03		-0.04992	-1.88
4				-0.04988	-1.92
5				-0.04984	-1.92
6				-0.0498	-1.92
7	Source	CH1		-0.04976	-1.92
8	Vertical Units	V		-0.04972	-1.92
9	Vertical Scale	1.00E+00		-0.04968	-1.92
10	Vertical Offset	0.00E+00		-0.04964	-1.92
11	Horizontal Units	s		-0.0496	-1.92
12	Horizontal Scale	1.00E-02		-0.04956	-1.92
13	Pt Fmt	Y		-0.04952	-1.92
14	Yzero	0.00E+00		-0.04948	-1.92
15	Probe Atten	1.00E+00		-0.04944	-1.92
16	Model Number	TDS2012C		-0.0494	-1.92
17	Serial Number	C041037		-0.04936	-1.92
18	Firmware Versior	FV:v24.26		-0.04932	-1.92
19				-0.04928	-1.92
20				-0.04924	-1.92
21				-0.0492	-1.92
22				-0.04916	-1.92
23				-0.04912	-1.92
24				-0.04908	-1.92
25				-0.04904	-1.92
26				-0.049	-1.92
27				-0.04896	-1.92
28				-0.04892	-1.92
29				-0.04888	-1.92
30				-0.04884	-1.92
31				-0.0488	-1.92
32				-0.04876	-1.92
33				-0.04872	-1.92
34				-0.04868	-1.92
35				-0.04864	-1.92
36				-0.0486	-1.92

chart 3.8.2: Data of Empty Pipette

Data of With Cell:

	A	B	C	D	E
1	Record Length	2.50E+03		-0.05	-0.584
2	Sample Interval	4.00E-05		-0.04996	-0.584
3	Trigger Point	1.25E+03		-0.04992	-0.584
4				-0.04988	-0.584
5				-0.04984	-0.584
6				-0.0498	-0.584
7	Source	CH1		-0.04976	-0.584
8	Vertical Units	V		-0.04972	-0.584
9	Vertical Scale	2.00E-01		-0.04968	-0.584
10	Vertical Offset	0.00E+00		-0.04964	-0.584
11	Horizontal Units	s		-0.0496	-0.584
12	Horizontal Scale	1.00E-02		-0.04956	-0.584
13	Pt Fmt	Y		-0.04952	-0.584
14	Yzero	0.00E+00		-0.04948	-0.584
15	Probe Atten	1.00E+00		-0.04944	-0.584
16	Model Number	TDS2012C		-0.0494	-0.584
17	Serial Number	C041037		-0.04936	-0.584
18	Firmware Versior	FV:v24.26		-0.04932	-0.584
19				-0.04928	-0.584
20				-0.04924	-0.584
21				-0.0492	-0.584
22				-0.04916	-0.584
23				-0.04912	-0.584
24				-0.04908	-0.584
25				-0.04904	-0.584
26				-0.049	-0.584
27				-0.04896	-0.584
28				-0.04892	-0.584
29				-0.04888	-0.584
30				-0.04884	-0.584
31				-0.0488	-0.584
32				-0.04876	-0.584
33				-0.04872	-0.584
34				-0.04868	-0.584
35				-0.04864	-0.584
36				-0.0486	-0.584
37				-0.04856	-0.584

chart 3.8.3: Data of Cell

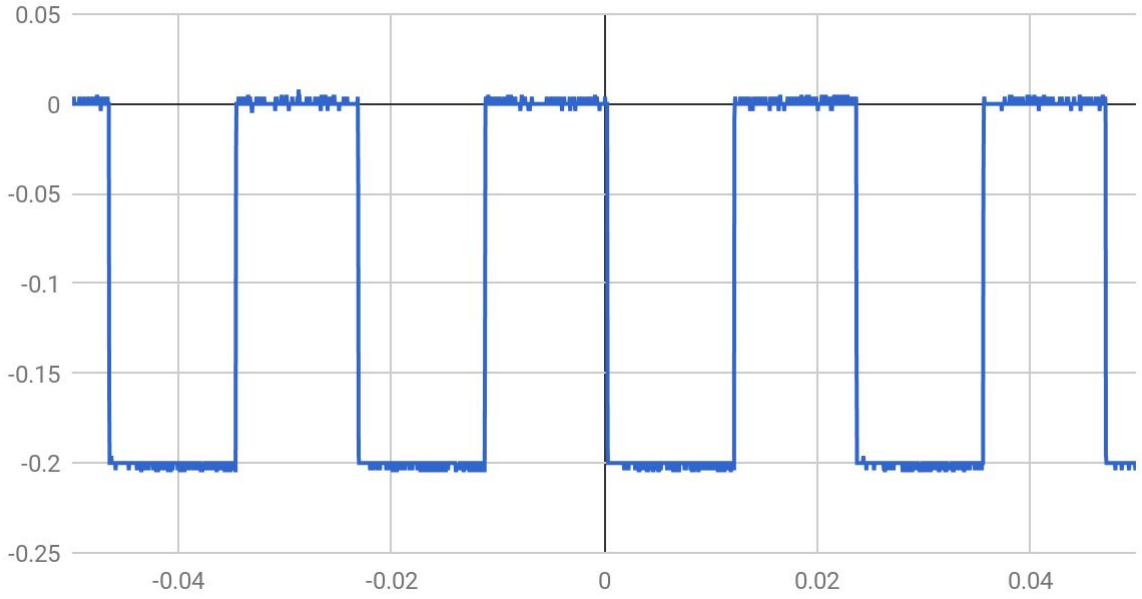
	A	B	C	D	E	F	G
1	Empty Voltage(V)	WithCell Voltage(V)	R_E(Ohm)	R_WithC(Ohm)	R_cell(Ohm)	I(A)	Time
2	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.05
3	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04996
4	-1.88	-0.584	1.06E+06	3.42E+07	3.32E+07	6.03E-10	-0.04992
5	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04988
6	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04984
7	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.0498
8	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04976
9	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04972
10	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04968
11	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04964
12	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.0496
13	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04956
14	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04952
15	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04948
16	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04944
17	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.0494
18	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04936
19	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04932
20	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04928
21	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04924
22	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.0492
23	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04916
24	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04912
25	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04908
26	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04904
27	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.049
28	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04896
29	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04892
30	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04888
31	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04884
32	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.0488
33	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04876
34	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04872
35	-1.92	-0.584	1.04E+06	3.42E+07	3.32E+07	6.02E-10	-0.04868

chart 3.8.4: Final results and calculation of neuron current and other resistance

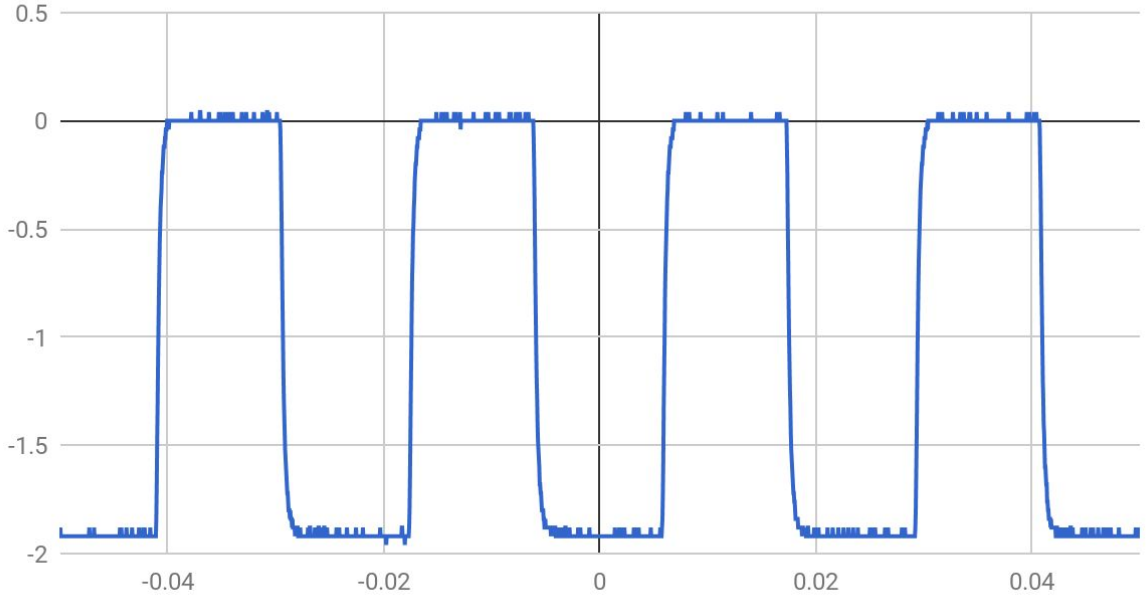
The first three charts of above are generated for three single tests in different setups, data source is the oscilloscope. The D column is the voltage and unit is V, and the E column is time and unit is second.

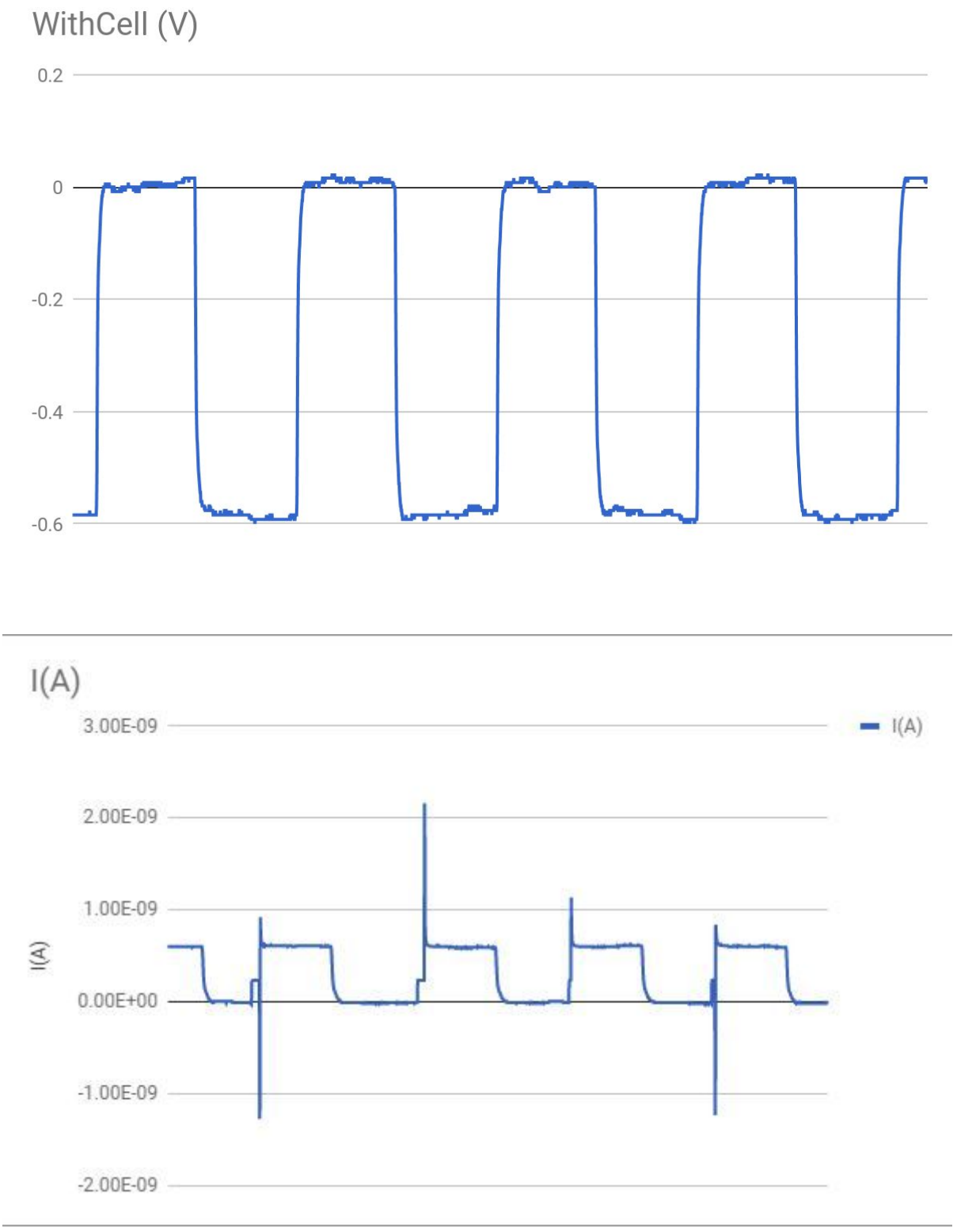
The fourth chart shows the results of the final calculated current value on column F, unit A.

Voltage Source (V)



Empty Pipette (V)





graph 3.8.3: Results Waveforms

First three of the above waveforms are generated from the charts above for three single tests in different setups. The vertical reading is the voltage and unit is V, and the horizontal reading is time and unit is second.
And the fourth waveform is the Time(s) vs Current(A) of cell.

The Gain of Empty Pipette: (a)(b) = 0.1 mV/pA
The Gain of WithCell: (a)(b) = 1 mV/pA

Resistance of Empty pipette = (-20*10^(-3))/((X/0.1)*10^(-9)), unit is Ohm, X is data from chart 3.8.2 column E

Resistance of neuron = (-20*10^(-3))/((X/1)*10^(-9)), unit is Ohm, X is data from chart 3.8.2 column E

Current of cell = -20mV / Resistance of neuron

4 Closing Material

4.1 Conclusion

In this two semester project, we built up a set of electric environment and circuit to provide operational interface between the patch-clamp and electron microscope. According the graphs we got from neurons, we can find the current change when voltage applied to the membrane of neurons and ions try to enter or leave the membrane of neurons. We measure the ion channel potential of neuron and the action potential of neuron under external stimulations successful by using the PC-ONE patch-clamp and microchip module.

4.2 References

[1]: IEEE Code of Ethics,
https://www.ieee.org/about/corporate/governance/p7-8.html

[2]: Y Zhao*1, S Inayat*1, D A Dikin*2, J H Singer*3, R S Ruoff*4, and J B Troy*1 , Patch clamp technique: review of the current state of the art and potential contributions from nanoengineering, 08 June 2009, DOI: 10.1243/17403499JNN149.
1: Department of Biomedical Engineering, Northwestern University, Evanston, Illinois, USA.
2: Department of Mechanical Engineering and Department of Physics and Astronomy, Northwestern University, Evanston, Illinois, USA.
3: Department of Ophthalmology and Department of Physiology, Northwestern University, Chicago, Illinois, USA.
4: Department of Mechanical Engineering and Texas Materials Institute, University of Texas, Austin, Texas, USA.

[3]: Zhongcheng Gong, Krithika Nagarajan, Siva Penmetsal, David Millsl, and Long Quel, A patch-clamp device with integrated actuators for cell selection and positioning, Institute for Micromanufacturing, Louisiana Tech University, USA, School of Biological Science, Louisiana Tech University, USA.

[4]: Dagan Corporation, PC-ONE Patch/Whole Cell Clamp Operating Manual Ver. 1.1, www.DAGAN.com.

[5]By Winter20jb - I illusrtated this diagram on my computer, CC BY-SA 3.0, https://en.wikipedia.org/w/index.php?curid=44365802

Appendix I: Operation Menual

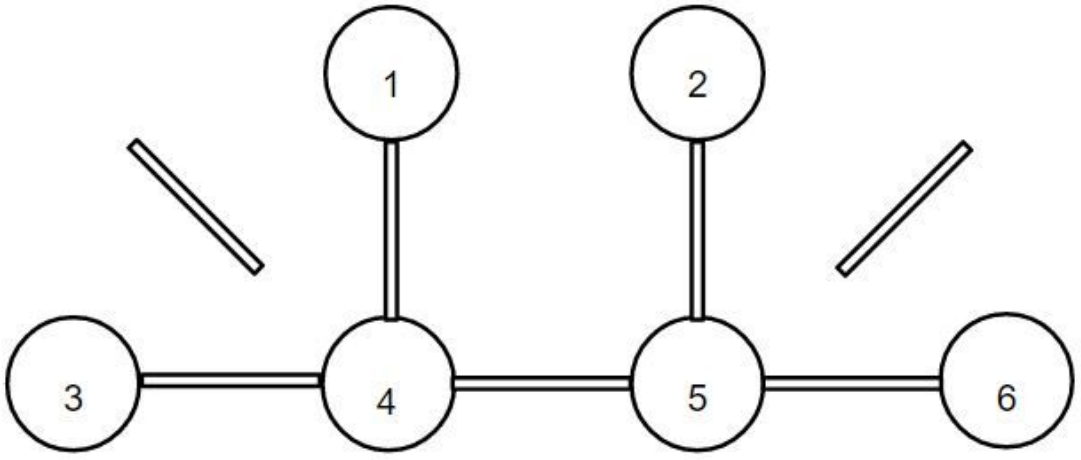
Step 1. Connect oscilloscope input port with PC-ONE FILTERED Im port located on the front panel by BNC-to-BNC cable, this allows us to obtain the voltage square graph of neuron.

Step 2. Connect PC-ONE-10 Headstage to PC-ONE HEADSTAGE connector located on the back of the device.

Step 3. Connect two probe-to-probe cables to the two sides of PC-ONE-10 Headstage.

Step 4. Connect two Ag-AgCl electrodes to another side of the previous probe-to-probe wires.

Step 5. Prepare the Microchip module:



graph A1.1: Microchip Module Preparation

1. Insert the N27 culture medium into the #4/#5 well/micro chamber of microchip.
2. Insert the N27 culture medium into the #1/#2 and the #3/#6 micro chambers.

3. Wait the N27 culture medium to fill up all the grooves between micro chambers and the pipette at the end of the #1-#4/#2-#5 groove.
4. Place N27 rat neuron cell into the #4/5 well. Make sure there is culture medium filled up.

Step 6. Apply positive side to the #3/#6 electrode and negative side to the #1/#2 electrode.

Step 7. Place the headstage and its connections inside the box covered with aluminum foil to reduce the possible room noise and take the wires through holes on the walls of box.

graph A1.2: Headstage in the aluminum foil covered box

Step 8. Setup the peremeters on PC-ONE:

1. HEADSTAGE turned to WHOLE CELL 10M.
2. JUNCTION ZERO set to OFF.
3. COMMAND section: Vhold set to OFF, Vtest set to -20mV, COMD. IN set to OFF
4. COMPENSATION set all three switches to OFF at this moment.
5. MONITOR set to Im at this moment.
6. Im OUTPUT PROCESSING: set GAIN(b) to 10, 3 POLE LOW PASS BESSLE FILTER to 10kHz.
7. MODE set to Vclamp mode.

8. OUTPUT connect the oscilliscope to FILTERED Im (a)(b)mV/pA

Step 9. Turn on the devices, make sure the Olympus IX73 is connected to PC and PC has the CellSens Standard installed.

Step 10. Place the microchip module under the Olympus IX37 to observe the status of catching action and open the CellSens Standard on PC to get the image.

1. Insert the tube connected with syringe to micro chamber #1/#2.
2. Pull the syringe to suckup a neuron while watching this action under Olympus IX37 on the PC's monitor with CellSens Standard.
3. Once the suckup action is confirmed by iamage, pull out the tube and place the Ag-AgCl electrode into the the #1/#2 and the #3/#6 micro chambers.

Step 11. Oscilloscope should now get readings from PC-ONE.

Step 12. Wait until the voltage push neuron and pipette catch the neuron successfully. This could be observed by Olympus IX73 Electrical Microscope and display the images by CellSens Standard on the PC's screen.

Step 13. Collect data from oscilloscope.

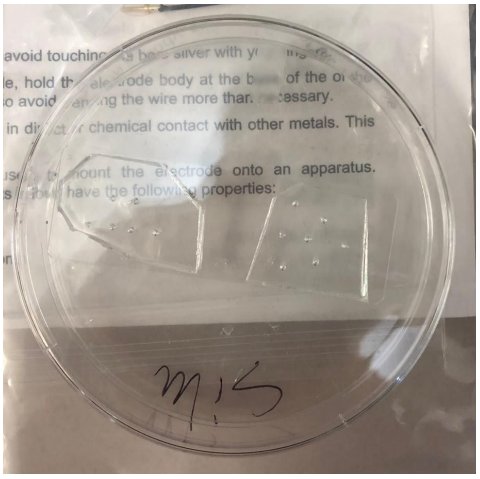
Step 14. If the reading on the oscilloscope is showing white noise(spike) at the end of the result square waves, turn on the CAPACITANCE switch on PC-ONE and adjust the 4 knobs to eliminate them.

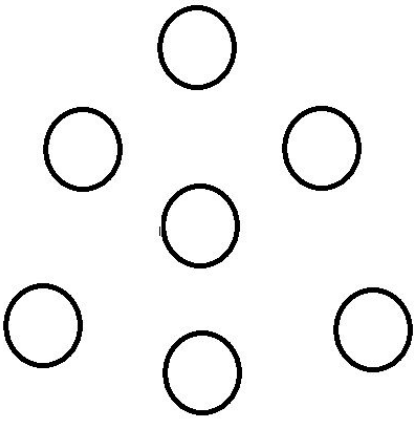
Step 15. If the reading on the oscilloscope is showing that the result square waves are not on the 0 mark of the screen, turn on the JUNCTION ZERO switch on PC-ONE and adjust the MANUAL knob to reset the reading to the middle.

Step 16. After get the expect results, turn off the devices and recycle the microchip module.

Appendix II: Alternative/Other Initial Versions of The Design

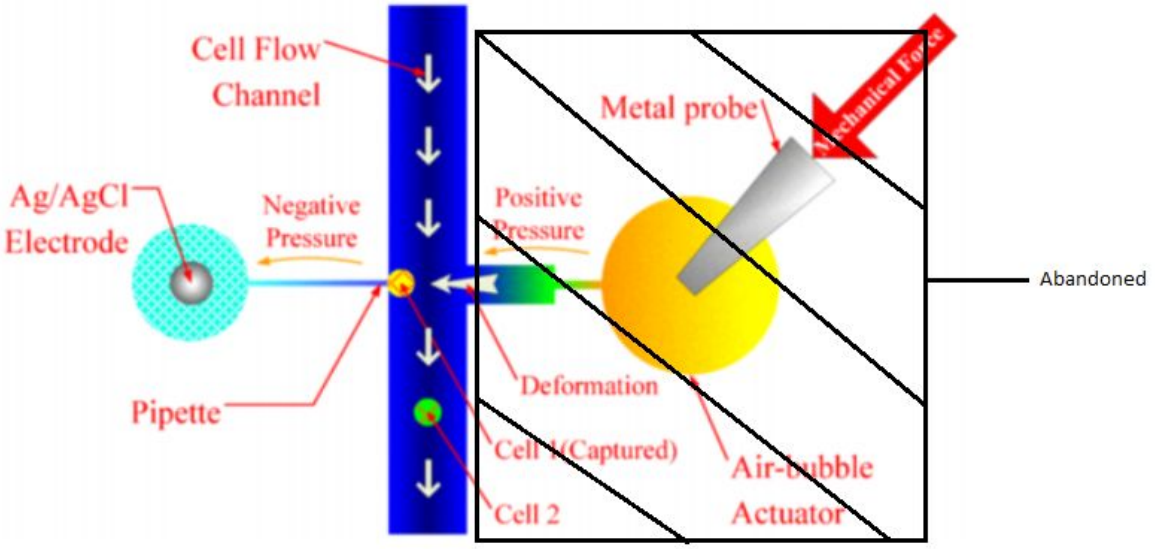
1.The first version of microchip is abandoned . It is not suitable because the electron and pipette have to use the same spot.





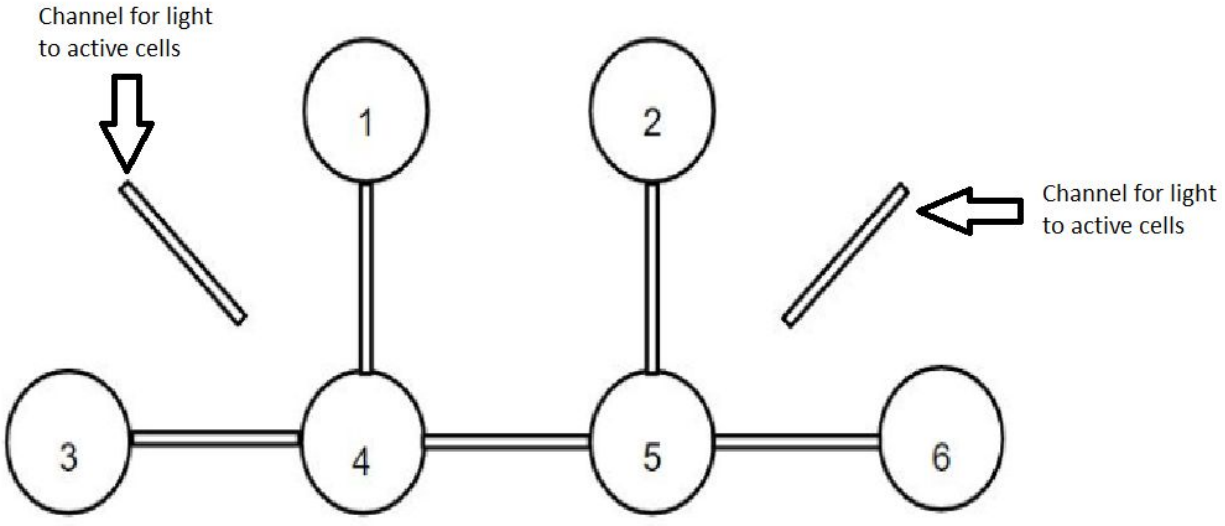
graph A2.1: Failed Microchip Design graph A2.2: Fialed design structure

2. We were planning to use Air-bubble actuator to push the cells to the pipette. However, the method is denied because of high cost. The method we are using is waiting for the cells randomly move to the pipette. Waiting time is between 5 mins to 2hours.



graph A2.3: Not applied design thereo

3. The microchip designed includes two channels for the light to active cells. It' reserved for future research. We don't need them for now.



graph A2.4: Microchip Environment stimulation channel abandoned