Patch-Clamp Microchip Interface

By Sdmay18-10 Team Member:
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http://sdmay18-10.sd.ece.iastate.edu/

Team Roles and Responsibilities

Chenhang Xu : Communication Leader

Contact with advisor/client and graduate student project team members

Li Qian : Team Leader

Follow up activities of project and team

Daiyuan Ding : Web Engineer

Maintain website, tester

Ningyuan Zhang: Project Programmer

Draft documents, set up reminder, organize the meetings

Yigao Li : Test Engineer

Test the correctness of setups and debug the content in project results and documents

Introduction

- The patch clamp technique is a laboratory technique in electrophysiology used to study ionic currents in individual living cells, tissue sections, or patches of cell membrane
- This Project is focusing on how to build up a set of electrical environment and circuit to provide operational interface between the PC-ONE and microchip module.
- For our project, we use PC-ONE device to get the voltage graphs when the neuron are excited by the given voltage.

Project Statement

Catch the neuron and get the current plot for it

- Design and build the microchip
- Set up the whole system, which allowed us to do the test on the neuron
- Catch the cell under the microscope
- Collect the test result
- Calculate for the final result

Deliverables

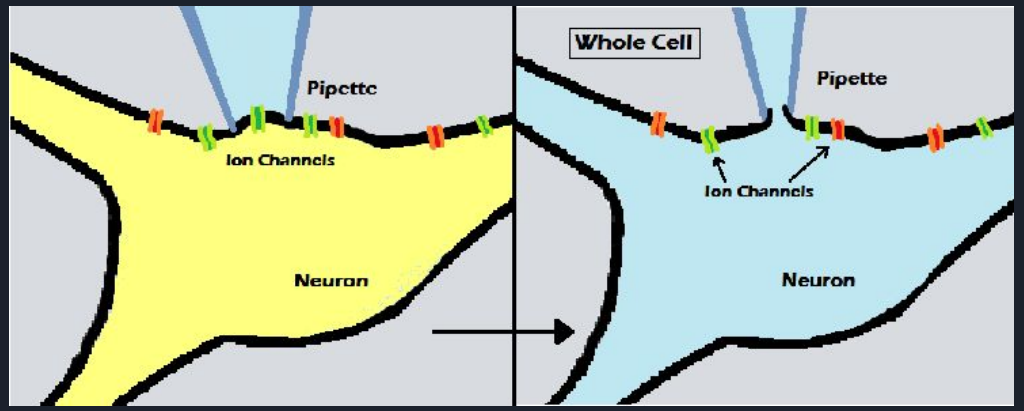
- Our goal is to build up a set of electric environment and circuit to provide interface between the patch-clamp and electron microscope, which is operational to catch neuron and observe the bioelectricity behavior of the neuron.

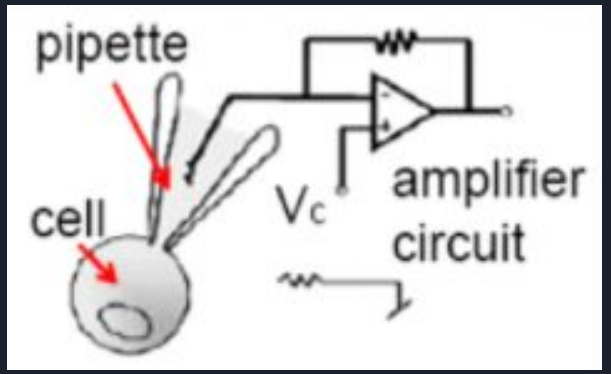
- We are able to measure the ion channel potential of neuron and the action potential of the neuron under external stimulations by using the PC-ONE patch-clamp and microchip module.

Intended Users

- Patch-clamp has wide range of applications in biological field.
- Experimenters who wants to know the ion current on the membrane when we apply different voltages or stimulations.

Conceptual Sketch





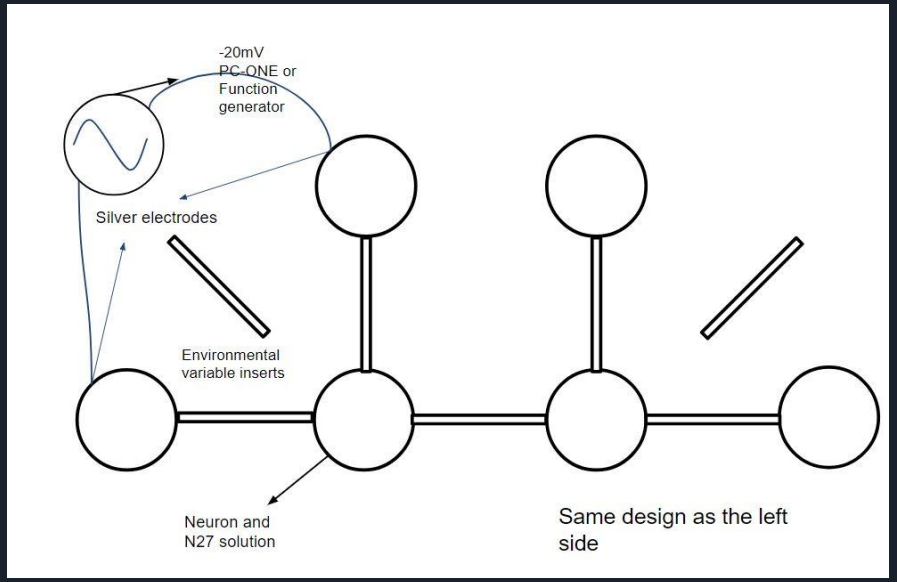
- Whole-cell patch involves recording currents through multiple channels simultaneously, over the membrane of the entire cell.
- The advantage of whole-cell patch clamp recording is that the larger opening at the tip of the patch clamp electrode provides lower resistance and thus better electrical access to the inside of the cell.

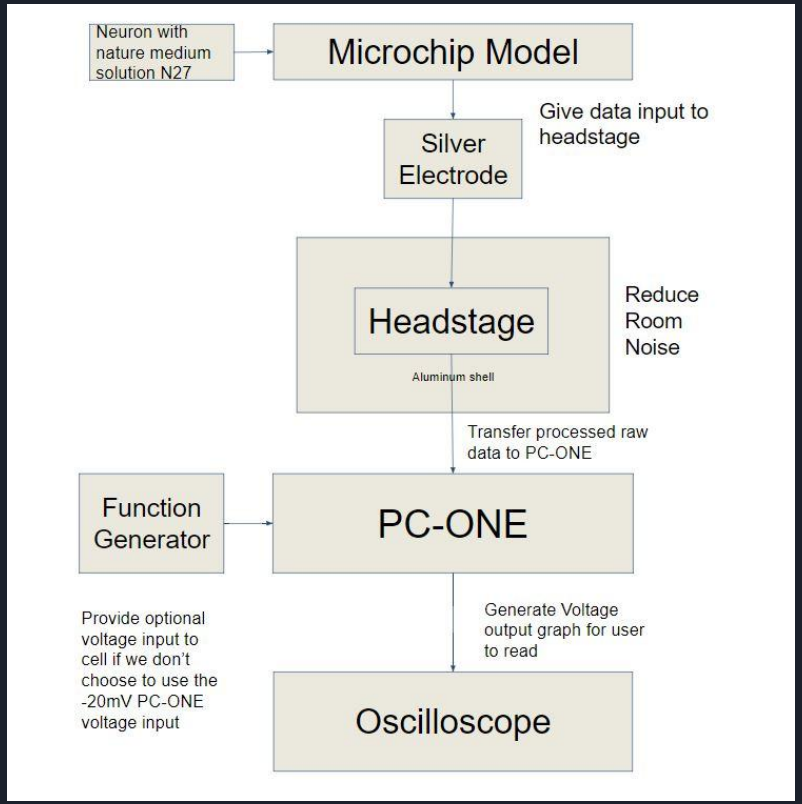
Design

Microchip Module

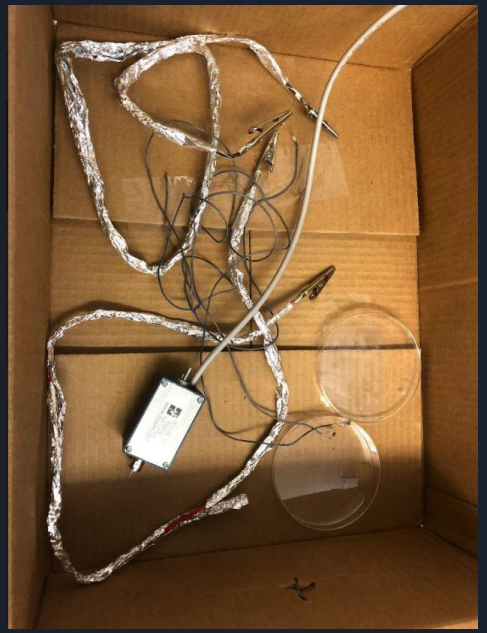
1. Make mold using IRE Process
2. Pour PDMS solution onto the mold
3. Peeling the PDMS
4. Bond the peeled PDMS with glass

Design

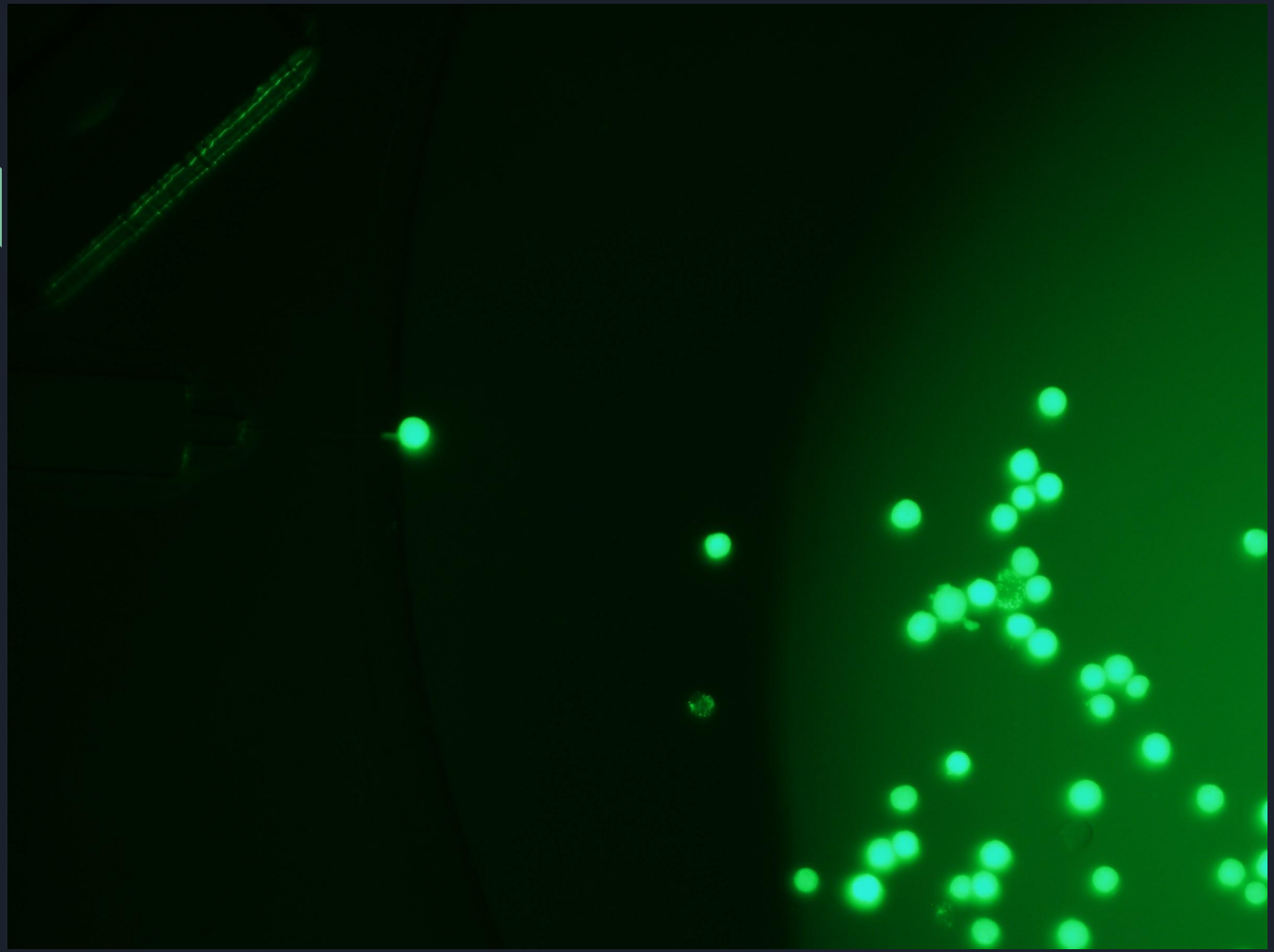

-20mV
PC-ONE or
Function
generator
Silver electrodes
Environmental
variable inserts
Neuron and
N27 solution
Same design as the left
side


Neuron with
nature medium
solution N27
Microchip Model
Give data input to
headstage
Silver
Electrode
Headstage
Aluminum shell
Reduce
Room
Noise
Transfer processed raw
data to PC-ONE
Function
Generator
PC-ONE
Provide optional
voltage input to
cell if we don't
choose to use the
-20mV PC-ONE
voltage input
Generate Voltage
output graph for user
to read
Oscilloscope

Design

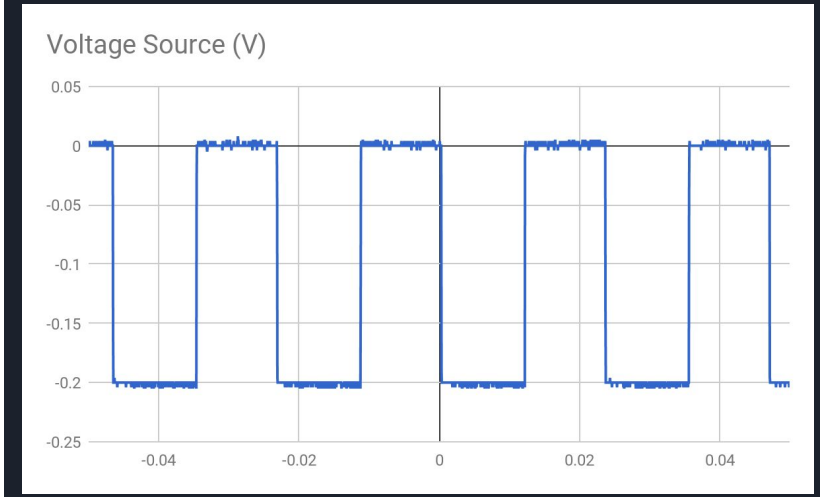


To reduce the room noise while recording samples, we used a carton box covered with aluminum foil.
All of the connections towards headstage will be put in the carton box to avoid noise.



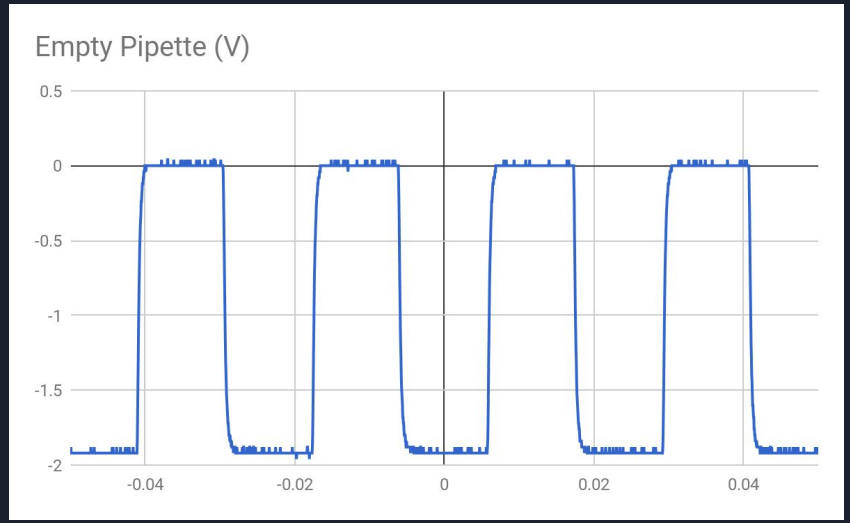
Testing Results(With Voltage Source)

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



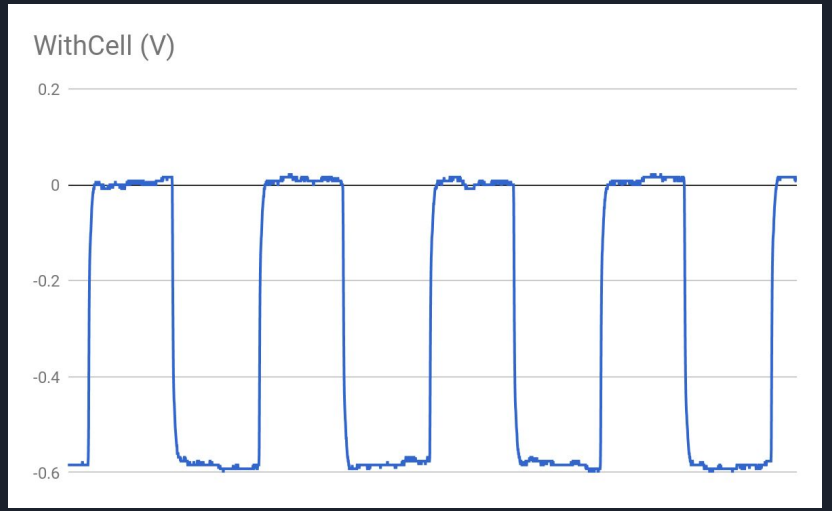
Testing Results(With Empty Pipette)

	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



Testing Results(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 Versio	FV:v24.26		-0.04932	-0.584



Calculation

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

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

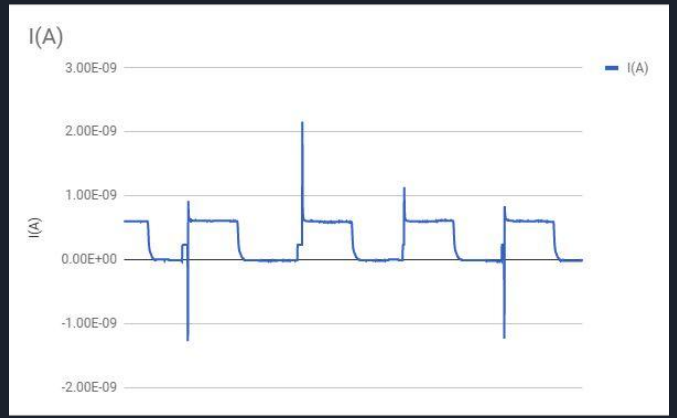
Resistance of Empty pipette = (-20*10^(-3))/((X/0.1)*10^(-9)), unit is Ohm, X is time

Resistance of neuron = (-20*10^(-3))/((X/1)*10^(-9)), unit is Ohm, X is time

Current of cell = -20mV / Resistance of neuron

Testing Results (Resistance and Current)

	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



Challenges

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

Conclusion

In this two-semester project

- Built up a set of electric environment and circuit to provide operational interface between the patch-clamp and electron microscope.
- 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.
- According the data and graphs we got from neurons, we find out the way of current change when voltage applied to the membrane of neurons.

Reference

[1]: 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.

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

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