Intracellular Electrometer

Lucas M. Essenburg

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INTRACELLULAR ELECTROMETER

by

Lucas Essenburg

A thesis submitted to the Graduate College in partial fulfillment of the requirements for the degree of Master of Science in Engineering (Electrical) Electrical and Computer Engineering Western Michigan University December 2019

Thesis Committee:

Damon A. Miller, Ph.D., Chair
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John Jellies, Ph.D.
ACKNOWLEDGMENTS

The author thanks Dr. John Jellies for access to his neurobiology laboratory throughout the work of this thesis, and also Thomas Groves for his time and efforts in assisting with experimental data capture for electrometer validation. The author also gratefully acknowledges the support of the Michigan Space Grant Consortium via undergraduate and graduate research fellowships.

Lucas Essenburg
The Western Michigan University Neurobiology Engineering Laboratory conducts research in energy-efficient electrical stimulation of biological neurons. Experimental work requires an intracellular electrometer to inject nanoamp-level currents into a biological neuron and to measure its membrane voltage response. In order to fully understand the generation of such minute currents, an ‘in-house’ electrometer was designed, constructed, and validated. This required exploration of various design trade-offs including precision of generated nanoamp-level currents vs. susceptibility to noise, portability vs. functionality, and cost vs. functionality. The electrometer was successfully used to stimulate and measure responses of neurons from the medicinal leech *Hirudo verbana*. 
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1 Intracellular Neuron Stimulation

Biological cells can be stimulated in a number of ways as shown in the hierarchy of Figure 1.

Extracellular stimulation techniques include stimulation using magnetic fields [4], optical recording of neural function using voltage sensitive dyes under microscopic photodetector arrays [5], and capacitive stimulation [6]. In general, these extracellular techniques are non-invasive and are used in applications such as neuroprosthetics, brain tissue stimulation, and macro-scale stimulation (cultured network of neurons). Extracellular techniques were not explored in-depth since the focus of this thesis is on intracellular methods.

Selecting an intracellular stimulation technique from the three methods shown in Figure 1 depends on objectives of the experiment.
In patch clamp stimulation a small patch of cell membrane is captured within a pipette and the flow of individual channel currents through the cell membrane is recorded. This method was developed by Neher and Sakmann, who were awarded a Nobel Prize in 1991 based on their work in this field [7]. In voltage clamp stimulation a voltage is maintained across a cell membrane and the flow of current through the membrane is measured. The voltage clamp technique was developed in the 1940s by Marmont and Cole, but was used in later work by Hodgkin and Huxley, leading to a Nobel Prize for the two researchers in 1963 [7]. In current clamp stimulation a current is injected into a cell and the membrane voltage response is measured. In 1971 Brennecke and Lindemann developed a system of alternating current injection and voltage measurement using the one single electrode in their development of the current clamp technique [7]. Since then, the applications for current clamp intracellular stimulation have grown widely. The current clamp method is supported by the design presented in this thesis.

1.1 Current Clamp: Techniques and Circuit Models

This section relies heavily on material from [1] that describes the function and operation of instrumentation for single and two electrode current clamp stimulation. The circuit diagrams in Figures 2 - 9 have been adapted and modified from those presented in [1]. These figures provide equivalent circuit diagrams used to analyze intracellular techniques.

Table 1 shows an overview of the different techniques explored in this section. A brief description including drawbacks and advantages is provided. The voltage of interest is the neuron membrane voltage $V_{\text{membrane}}$ and the current of interest is the stimulation current $i_{\text{stim}}$. 

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Technique & Description & Advantages & Drawbacks \\
\hline
Patch clamp & Captures a small patch of membrane & High spatial resolution & Difficult to maintain \\
Voltage clamp & Maintains a fixed voltage & High temporal resolution & Requires a stable voltage source \\
Current clamp & Injects a current & High control over current & Requires a current source \\
\hline
\end{tabular}
\end{table}
Table 1: Overview of current clamp stimulation technique sketches. Based on material in [1].

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Comments</th>
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<tr>
<td>Figure 2</td>
<td>current clamp - two electrode</td>
<td>Technique for stimulating a cell and measuring membrane voltage response using two separate electrodes. Requires very large current source resistance and large amplitude stimulation voltages.</td>
</tr>
<tr>
<td>Figure 3</td>
<td>current clamp - single electrode</td>
<td>Technique for stimulating a cell and measuring membrane voltage response using a single electrode. Voltage measurement affected by electrode voltage drop. Requires very large current source resistance and large amplitude stimulation voltages.</td>
</tr>
<tr>
<td>Figure 4</td>
<td>current clamp - single electrode with bridge balancing</td>
<td>Technique for stimulating a single electrode with bridge balancing. Undesired voltage drop across electrode is negated using a balancing differential amplifier. Requires very large current source resistance and large amplitude stimulation voltages.</td>
</tr>
<tr>
<td>Figure 5</td>
<td>current clamp - single electrode with floating voltage source</td>
<td>Technique for stimulating a cell and measuring membrane voltage response using a single electrode with floating voltage source. Lower current source resistance can be employed in this technique (compared to passive circuits) to ensure a current source resistance of 100-1000 times larger than electrode resistance. Generally, lower stimulation voltages can be used in active circuits to generate the same stimulation currents due to the lower current source resistances.</td>
</tr>
<tr>
<td>Figure 6</td>
<td>current clamp - stimulation side current monitoring</td>
<td>Technique for measuring stimulation current on instrumentation side of experiment. Bias currents of amplifier skew membrane voltage measurements.</td>
</tr>
<tr>
<td>Figure 7</td>
<td>current clamp - bath side current monitoring</td>
<td>Technique for measuring stimulation current on bath side of experiment. Introduces small series resistance to cell and skews membrane voltage measurements.</td>
</tr>
<tr>
<td>Figure 8</td>
<td>current clamp - virtual ground current monitoring</td>
<td>Technique for measuring stimulation current on the bath side of experiment using differential amplifier with a virtual ground.</td>
</tr>
<tr>
<td>Figure 9</td>
<td>current clamp - 'ideal' setup</td>
<td>'Ideal' current clamp setup using bridge balancing, floating voltage source, and virtual ground current monitor.</td>
</tr>
</tbody>
</table>
Figure 2: Two-electrode current clamp. Adapted from [1] page 40, Figure 1.
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Figure 7: Single electrode current clamp with floating voltage source and bath side current monitoring. Adapted from [1] page 53, Figure 7.
Figure 8: Single electrode current clamp with floating voltage source and virtual ground bath side current monitoring. Adapted from [1] page 55, Figure 8.

\[ i_{\text{stim}} = \left( \frac{V_m + V_s}{R_{\text{c}} - R_{\text{m}}} - V_{\text{in}} \right) \]

as \( A \) increases,

\[ i_{\text{stim}} = \frac{-V_m}{R_{\text{m}}} \]

Note: Measures total current of cells in solution if stimulating more than one cell.

\[ i_{\text{stim}} = \frac{(V_m + V_s) - V_{\text{in}}}{R_{\text{c}}} = \frac{V_s}{R_{\text{c}}} \text{ as before} \]
Figure 9: Single electrode current clamp ‘ideal setup’ with bridge balancing, floating voltage source, and virtual ground bath side current monitoring. Adapted from [1].
1.2 Electrode Capacitance

Adjustable capacitive current control is included in electrometers to offset the varying current through the electrode capacitance; however, this method can increase noise levels due to the commonly-used feedback configuration [7].

Figure 10 shows a circuit that allows manual capacitive adjustment to negate parasitic electrode capacitance [2]. Setting $R_f = R$ yields

$$V_o = 2V_i.$$  \hfill (1)

Then

$$I(s) = -V_i(s)sC_{\text{neutralization}}$$  \hfill (2)

and

$$Z_{\text{in}}(s) = \frac{-1}{sC_{\text{neutralization}}}.$$  \hfill (3)

Since $C_{eq} = (C_{\text{neutralization}})(C_{\text{electrode}}) = -C_{\text{neutralization}} + C_{\text{electrode}}$, capacitive compensation is achieved. Note that if $R_f = 0$, then $V_o(s) = V_i(s)$ and the magnitude of the complex quantity $Z_{\text{in}}$ is infinite and thus there is no capacitive compensation.
Junction potentials arise from the contact of different solutions within the cell and electrode solution, and the bath and the electrode solution, specifically when solution diffusion of positively and negatively charged particles spread to opposite solutions at different rates, a voltage difference results. This voltage difference results in the measured voltage [7]

\[ v_{\text{Meas}}(t) = v_{\text{Membrane}}(t) + V_{\text{cell}}^{\text{Junction Potential}} + V_{\text{bath}}^{\text{Junction Potential}}. \]  

(4)
These junction potentials are negated in DC offset and bridge compensation circuitry, as will be described in later sections.

Circuit noise is affected by electrode seal quality during intracellular stimulation and recording [7]. Proper insertion of the electrode into the cell membrane involves manipulating the electrode angle of attack and position. Proper electrode shape, sharpness, and taper enable good electrode seal, thus reduced circuit noise.

1.4 Intracellular Electrometers

Electrometer designs have taken into account amplification of output voltages, resistance compensation (bridge balancing), and capacitance compensation [8][9][10], similar to the design contained within this thesis.

1.5 Electrodes Used for Intracellular Stimulation

Electrodes used for intracellular stimulation can be made from a limited list of materials, the most common of which is silver chloride (AgCl). This limited list of materials used for electrodes in intracellular stimulation is due to the chemical properties the materials must exhibit. The materials must allow electron and ion transfer in order to properly interface the instrumentation and the cell. The electrodes used in these experimental settings are assembled by first pulling a glass tube into a sharp electrode using heat and tension. These sharp electrodes are then filled with an electrolyte solution of potassium chloride and the small silver chloride filament is placed within the pipette before impaling. The reaction

\[ Cl^- + Ag \leftrightarrow AgCl + e^- \] (5)

takes place when stimulating the cell [11]. This chemical reaction is reversible, but exhaustible [11].
Platinum electrodes can also be used for intracellular stimulation through the irreversible, inexhaustible chemical reaction [11]

\[ e^- + H_2O \rightarrow OH^- + \frac{1}{2}H_2 \quad \text{or} \quad H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + e^-. \]  

(6)

Several other electrode factors play into effectiveness of stimulation such as fabrication, tip size, tip shape, and polishing of pipettes prior to insertion and stimulation [11].
2 Design

2.1 Specifications

The specifications for the neuron stimulation device were adapted from the World Precision Instruments Duo 773 Electrometer [12] currently used to conduct electrophysiology experiments. Specifically, the specifications of Channel B of the Duo 773 were considered. The electrometer specifications are:

1. Input impedance magnitude of at least $10^{11}$ Ω. (100 GΩ.)
2. Membrane voltage must be amplified by a factor of 10.
3. DC position adjustment of the membrane voltage must be ±300mV.
4. The electrometer must be able to negate an electrode resistance $R_{\text{electrode}}$ within the range of $0 < R < 100\text{MΩ}$ (bridge balance).
5. Electrometer must enable compensation of electrode capacitance $C_{\text{electrode}}$ within the range of $-50 < C < +10\text{pF}$.
6. Current monitoring circuitry must provide a signal of 100mV per nA injected into target cell.
7. A cell ‘tickler’ to mechanically vibrate the target cell must be a variable signal between 0V and 15V amplitude, with a variable frequency between 1kHz and 8kHz variable.

Figure 11 illustrates the quantities that were considered for the specifications as part of a larger electrophysiology rig.
Figure 11: Diagrammatic representation of electrophysiology rig during intracellular experimentation used to develop specifications
2.2 Block Diagram

Figure 12 provides a block diagram of the electrometer that was designed, assembled, and validated. The three main components of the device are the headstage, the main enclosure, and the power supply. The subsystems within each of the three main components are included to illustrate device configuration and user controls.
Figure 12: Electrometer Block Diagram
2.3 Nanoamp Current Source

The foundation of the circuit design is a Linear Technology LTC6081 nA current source circuit as shown in Figure 13. This nanoamp-level current source utilizes (3) individual LTC6081 op-amps to create a nanoamp output current accurate to 10pA [3].

Figure 13: Nanoamp level current source schematic. Circuit is from [3]

Note that

\[ V_1 = V_{load} + R_{10} \cdot i_{load} \]  \hspace{1cm} (7)

and ignoring \( C_2 \) yields

\[ V_2 = V_1 - V_{load} = R_{10}i_{load}. \]  \hspace{1cm} (8)
Also,
\[ i_{R_3} = i_{C_1} = \frac{V_2 - V_{\text{stim}} \frac{R_2}{R_1 + R_2}}{R_9}. \]  

(9)

Now the transfer function between the load current \( I_{\text{load}}(s) \) and input voltage \( V_{\text{stim}}(s) \) is found. Assuming \( R_3 \) has little effect on the transfer function, then
\[ V_1(s) \approx V_{\text{stim}}(s) \frac{R_2}{R_1 + R_2} - I_{C_1}(s) \left[ \frac{1}{sC_1} \right]. \]  

(10)

Further investigation shows that
\[ V_{\text{stim}}(s) \frac{R_2}{R_1 + R_2} - I_{C_1}(s) \left[ \frac{1}{sC_1} \right] = I_{\text{load}}(s)Z_{\text{load}}(s) + R_{10}I_{\text{load}}(s). \]  

(11)

Expanding this equation yields
\[ V_{\text{stim}}(s) \frac{R_2}{R_1 + R_2} - \frac{R_{10}I_{\text{load}}(s)}{R_9} - V_{\text{stim}}(s) \frac{R_2}{R_1 + R_2} \left[ \frac{1}{sC_1} \right] = I_{\text{load}}(s)Z_{\text{load}}(s) + R_{10}I_{\text{load}}(s). \]  

(12)

After gathering terms
\[ \frac{I_{\text{load}}(s)}{V_{\text{stim}}(s)} = \frac{\frac{R_2}{R_1 + R_2}}{R_{10} \left[ \frac{1}{sC_1 R_9} + 1 + \frac{Z_{\text{load}}(s)}{R_{10}} \right]} = \frac{\frac{1}{R_{10}} \left[ \frac{1}{sC_1 R_9} + 1 + \frac{Z_{\text{load}}(s)}{R_{10}} \right]}{\frac{1}{sC_1 R_9} + 1 + \frac{Z_{\text{load}}(s)}{R_{10}}}. \]  

(13)

and simplifying
\[ \frac{I_{\text{load}}(s)}{V_{\text{stim}}(s)} = \frac{R_9 C_1(s) + 1}{\frac{R_2}{R_{10} R_1 + R_2} \left[ R_9 C_1(s)(1 + \frac{Z_{\text{load}}(s)}{R_{10}}) + 1 \right]}. \]  

(14)
The DC transfer function gain is

\[
\left. \frac{R_9 C_1 s + 1}{R_10 R_2 R_{1+} R_{2}} \right|_{s=0} = \frac{10^{(-10)}}{V} = -200\text{dB} \frac{A}{V}. \quad (15)
\]

If \( V_{\text{stim}} = 10\text{V} \), then \( I_{\text{load}} = 10^{(-9)}\text{A} = 1\text{nA} \), which matches the LTC6081 datasheet specifications.

Figures 14 and 15 show the Bode plot and step response for the transfer function of the nanoamp current source provided by MATLAB®. Figure 16 shows the Bode plot computed with LTspice®. There is a qualitative agreement between the Bode plots at low frequencies.

Figure 14: Nanoamp current source transfer function (16) Bode plot from MATLAB®
Figure 15: Nanoamp current source step response plot from MATLAB®

Figure 16: Nanoamp current source circuit LTspice® Bode plot
2.4 Power Supply

The linear power supply of Figure 17 was designed to provide ±5V for electronics of the electrometer. This power supply utilizes a 30VA transformer paired with common 7805 and 7905 ±5V regulators and large capacitors to provide a low-noise ±5V output for the electrometer electronics. Fuse protection ensures the output current is limited to a modest 500mA level on the ±5V side of the transformer and 250mA on the 120V side. Secondary fuse protection is much smaller than the allowable output current of the transformer, but was selected to be well below the maximum current values of the ±5V regulators.
Figure 17: Linear power supply schematic
2.5 Bridge Balance

Intracellular stimulation requires the ability to ‘balance’ the electrode during cellular stimulation experiments. This design requires nulling of the voltage drop created by the nanoamp-level current flowing through the stimulation electrode, that is between 0 and 100MΩ.

The bridge balance circuit is centered around a differential amplifier (U5), as shown in Figure 18. It amplifies the difference of the output load voltage and a scaled version of the input signal that corresponds to the voltage that would be measured at the output if a load of 100MΩ resistance was installed and the resulting output current was injected into this load. For example, for a 1V input (yielding a 1nA current), the bridge balance circuit amplifies this signal by a factor of 1/10 to yield 100mV. This is the same amplitude that would result if the 1nA electrode current were injected into a 100MΩ resistance. U5 then amplifies the difference between the measured output load voltage and a variable 0-100mV provided by U8 via the bridge balance potentiometer. Once the difference between these signals is negated, the bridge is said to be ‘balanced’. When balanced, the position of the 10-turn potentiometer indicates the approximate electrode resistance measured in megaohms. The maximum 100MΩ value that the bridge balance can negate was selected to be since 100MΩ is the largest electrode resistance reasonably expected for intracellular stimulation of this type.
Figure 18: Bridge balance and DC offset adjustment schematic
2.6 DC Offset Adjustment

Electrophysiology experiments require the ability to offset the measured response of a stimulated cell by several hundred millivolts to allow for proper observation of waveform characteristics and shape. This design allows a ±450mV adjustment, which exceeds the Duo 773 specifications. The design of this DC offset adjustment circuit is based around a differential amplifier with input from a variable DC voltage between ±450mV and the bridge balanced output load voltage. This allows the user to offset the output load voltage by ±450mV through the manipulation of a 1-turn potentiometer during cellular stimulation experiments.

2.7 Cell Tickler

A tickle signal source allows the user to stimulate the cell with a varying voltage that can be used to mechanically vibrate the electrode tip into the cell when the electrode is on the verge of passing through the cell membrane or eliminating possible unwanted cell membrane pieces within the electrode tip.

The Duo 773 provides a variable cell tickle voltage 0-15V variable and 1-8kHz. The designed cell tickler is limited by the power supply voltages of ±5V and provides a variable tickle signal of roughly 0-8V, 1-8kHz variable. The design, shown in Figure 19, is based on a LM555 timer configured to have a constant duty cycle of 50%. The square wave output of the LM555 timer was filtered using a parallel LC circuit. The output amplitude and frequency of this oscillator are controlled by 10-turn potentiometers located within the main enclosure case.
Figure 20 shows an output waveform of the tickle circuit with a frequency of approximately 5kHz. Due to the filtering and the limitations of the supply voltages, the peak-to-peak amplitude is roughly 5.6V.
Figure 20: Tickle signal voltage waveform at approximately 5kHz
2.8 Capacitance Neutralization

Electrode capacitive leakage currents (as discussed in Section 2.2) yield undesired changes to injected current waveforms and measured membrane voltages. To mitigate these capacitive effects, a capacitance neutralization circuit able to negate up to 60pF of capacitance was designed and implemented within the electrometer as shown in Figure 21.

This circuit operates by providing a capacitive current at the node in which the neutralization is desired. This capacitive current can be adjusted via a 10-turn potentiometer to ensure the current is equal and opposite to that of the capacitive current drawn by the electrode impedance. To provide the equal and opposite capacitive current, a non-inverting op-amp configuration provides the desired negative value of capacitance.

![Negative Capacitance](image)

**Figure 21:** Negative capacitance schematic, based on a generic negative impedance converter design (NIC) [2]

The function of this type of negative capacitance circuit was described in detail in Section 2.2.
2.9 Current Monitor

A current monitor circuit (Figure 22) provides verification of the current waveform that is being provided to the cell. This circuit amplifies the voltage drop across $R_{10}$ (10MΩ) in the nanoamp current source circuit to provide a voltage signal of 100mV/1nA.

![Current Monitor Schematic](image)

Figure 22: Current monitor schematic

2.10 Analog Switching

Electronic switching provides operator selection of the signal being applied to the electrode, either the nanoamp-level output current or the tickle signal. Electronic switches were used to provide a fast switch between output signal states to minimize the time an op-amp is pin floating which might lead to op-amp oscillation and/or inadvertent overstimulation of a cell. Figure 23 shows the configuration of the MAX319 switches used. When a particular signal is not being applied to the electrode, it is connected to a resistor to ground. The analog switches are controlled through the same logic signal that is provided via a momentary button housed within the main enclosure of the device shown in Figure 23.
2.11 Assembly and Construction

Figure 24 shows the assembled electrometer.
The electrometer utilizes three interconnected enclosures. The first enclosure houses the power supply which includes the transformer, connections, and all internal electronics mounted on a solderable breadboard. This power supply enclosure connects to the main enclosure and provides ±5V power for the other two enclosures. The other two enclosures, connected to each other by shielded cable soldered in place, house device electronics. Separation of these electronics into two enclosures is required due to the sensitivity of the nanoamp-level current source with respect to proximity of the load. To mitigate op-amp oscillations, the nanoamp-level current source must be as close as possible to the load to minimize capacitance generated by long lengths of cabling from the op-amps to the load. The main enclosure uses the same metallic box as the power supply to act as a Faraday cage around sensitive electronics. A similar, yet smaller enclosure was utilized for the headstage enclosure to allow for headstage mounting onto the existing micromanipulator used to position the electrode. A solderable breadboard system was used to assemble the circuit above a common ground.
plane to minimize capacitance between exposed circuit parts. Due to the extreme sensitivity of the circuit to load capacitance to ground, the pin orientation at the output was modified to further distance the pin from the ground plane to yield even lower output capacitance to ground.

Figures 25, 26, 27, and 28 show assembly details.
Figure 25: Power supply enclosure schematic and connections
Figure 26: Main enclosure schematic and connections
Figure 27: Headstage enclosure schematic and connections
Figure 28: Final device connections diagram
Figures 29, 30, and 31 show photos of the inside of the individual electrometer enclosures.

Figure 29: Photo of electrometer power supply enclosure interior
Figure 30: Photo of electrometer main enclosure interior
Figure 31: Photo of electrometer headstage enclosure interior
3 Cost Analysis

The retail price of a research-quality advanced World Precision Instruments Duo 773 Electrometer is approximately $5300. The functionality of a basic electrometer is encompassed within the designed intracellular electrometer. In total, the device cost is around $200. A detailed bill of materials is provided in Table 2.
### Bill of Materials

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<tr>
<th>Component</th>
<th>DigKey Part #</th>
<th>Price</th>
<th>Quantity</th>
<th>Extended Price</th>
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<td>LTC6081CM388#PBF-ND</td>
<td>$3.56</td>
<td>5</td>
<td>$17.80</td>
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<tr>
<td>MSOP TO DIP-8</td>
<td>PA0026-ND</td>
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<td>5</td>
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<td>10M OHM RESISTOR</td>
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<td>0.033mF CAPACITOR</td>
<td>BC1081CT-ND</td>
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<td>10K OHM 10 TURN POT (TRIMMER)</td>
<td>490-2875-ND</td>
<td>$1.50</td>
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<td>POWER SUPPLY CAPACITOR (ELECTROLYTIC) - 1uF</td>
<td>I189-1662-1-ND</td>
<td>$0.43</td>
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<td>390-13923-1-ND</td>
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<td>100mH INDUCTION</td>
<td>M10077-ND</td>
<td>$1.73</td>
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</table>

Total: $206.44
4 Validation

4.1 Experimental Setup

Experimental validation of the device was conducted at the neurobiology laboratory of Dr. John Jellies. This included individually stimulating both Retzius and P-type cells of a medicinal leech. Stimulation and data capture of the intracellular experiments was supported using a custom LabVIEW™ application developed by the lab. This application provided a voltage signal proportional to the desired stimulation current via a USB-6211 digital to analog interface. This stimulation signal was then converted to a nanoamp-level output current by the electrometer and applied to a particular cell within a cell ganglia that was in a grounded bath.

The LabVIEW™ application was used to continuously capture, display, and store waveforms generated by the bridge output and the current monitor output through the USB-6211 at a sampling frequency of 44.1kHz. Figure 32 shows the experimental setup.

Figure 32: Electrophysiology rig used to validate the intracellular electrometer
4.2 Results

4.2.1 Retzius Cell Stimulation and Measurement

The bridge output and current monitor waveforms that were captured during the stimulation of a Retzius cell is shown in Figure 33. The bridge output waveform displays an action potential generated by the cell during stimulation.

![Bridge Output Waveform](image1)

![Current Monitor Waveform](image2)

Figure 33: Bridge output (a) and current monitor (b) waveform captured during Retzius Cell stimulation
4.2.2 P-Cell Stimulation and Measurement

The current monitor and bridge output waveforms that were captured during the stimulation of a P-cell at two different stimulation current values are shown in Figures 34 and 35. The output waveforms show that at a lower stimulus peak current level of 0.75nA the cell did not produce an action potential, but when stimulated by a 1.35nA pulse, an action potential was generated by the cell.

![Bridge Output x10](image)

![Current Monitor](image)

Figure 34: Bridge output (a) and current monitor (b) waveform captured during lower input P-Cell stimulation
Figure 35: Bridge output (a) and current monitor (b) waveform captured during higher input P-Cell stimulation

4.3 Review of Specifications

Table 3 assesses achievement of the desired specifications.
<table>
<thead>
<tr>
<th>Specification</th>
<th>Achievement</th>
<th>Comments</th>
</tr>
</thead>
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<tr>
<td>Input impedance magnitude of at least $10^4 \Omega$.</td>
<td>N/A</td>
<td>This specification was not designed for.</td>
</tr>
<tr>
<td>Shunt capacitance compensation to negate electrode capacitance $C_{\text{electrode}}$ shall be $\pm 10$ to $-50 \mu F$.</td>
<td>✗</td>
<td>To minimize the possibility of excess capacitance at the output causing oscillation, a $60 \mu F$ capacitance neutralization was implemented. No positive shunt capacitance can be added.</td>
</tr>
<tr>
<td>DC offset adjustment of the measured membrane voltage shall be $\pm 300 \text{mV}$.</td>
<td>✔</td>
<td>Designed DC offset adjustment allows for $\pm 450 \text{mV}$ adjustment, exceeding design specification.</td>
</tr>
<tr>
<td>Cell 'tickler' to mechanically vibrate cell shall be a variable signal between 0V and 15 amplitude, with a variable frequency between 1kHz and 8kHz variable.</td>
<td>✗</td>
<td>Due to the power supply voltage of the device being 10V rail-to-rail, the amplitude of the tickle signal was limited to 10V peak-to-peak, and settles to about 8V after filtering.</td>
</tr>
<tr>
<td>Bridge balance ability of the device shall be able to negate an electrode resistance, $R_{\text{electrode}}$ within the range of 0 to $100 \text{MO}$.</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Current monitoring circuitry shall provide a signal of $100 \text{mV}$ per nA injected into a cell.</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Measured membrane voltage shall be output from the device with a x10 amplification.</td>
<td>✔</td>
<td></td>
</tr>
</tbody>
</table>
5 Conclusions

The electrometer successfully stimulated multiple biological neurons with nanoamp-level currents and enabled measurement of neuron responses. The device exhibits functionality similar to that of the Duo 773 such as current monitoring, bridge balancing, DC offset adjustment, capacitive neutralization, and a cell tickler. In contrast to the Duo 773, this electrometer has only one output stage and lacks the ability to change amplification factor of measurements, as well as other robust design features that allow a user to conduct research-level intracellular stimulation experiments.

The cost savings offered by this design provides accessibility to users who have the need for a basic electrometer. Main drawbacks of the device that was assembled include:

- lack of adjustable low pass filter;
- limited range of tickle signal amplitude and frequency; and
- adjustability of current monitor signal.

Significant work is required to adapt this to a consumer-ready product.
References


