HEAT REGULATED SAMPLE CHAMBER TO MAINTAIN OPTIMAL THERMAL CONDITIONS FOR BIOLOGICAL CELL MICROSCOPY

by

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Heat Regulated Sample Chamber to Maintain Optimal Thermal Conditions for Biological Cell Microscopy

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Abstract: In order to acquire useful and accurate data from cells during biological cell microscopy, a temperature of 37° Celsius is required to maintain viability. To achieve a constant body temperature within a closed microscope slide, a feedback circuit is used as a thermostat. This circuit contains an operational amplifier that compares a constant voltage to the voltage from a thermistor and a transistor that activates a ceramic heating ring depending on the output voltage of the operational amplifier. The heating ring, which heats the slide to 37° Celsius, rests on the glass coverslip. A thermistor is used to monitor the temperature of the sample chamber. As the thermistor heats up, its resistance decreases, thus the voltage applied to the operational amplifier is increased. As the voltage from the thermistor rises above the constant voltage setpoint, it will cause the operational amplifier to decrease the current to the transistor, thereby turning off the heater. Here, a schematic of the circuit and verification of its effectiveness is presented.

INTRODUCTION

Aristotle, a Greek philosopher, was the first scholar to articulate the theory of spontaneous generation, the idea that life can arise from non-living matter.[1] It wasn't until the 19th Century that Louis Pasteur disproved this theory in his famous swan-neck flask experiment.[2] The swan-neck flask was designed to allow oxygen into the sterile environment where there was a pool of broth, but keep out dust. Pasteur observed that the broth showed no signs of life until he broke the neck of the flask, allowing dust and microbes access to the experimental chamber, proving that life arises from other life, not spontaneously.[3] The work of Pasteur led to the development of cell culture techniques. Shortly after Pasteur's experiment, Robert Koch employed the first incubator in his microbiological studies after Alexis Carrel and Montrose Burrows recommended it following their experimentation with cell culture of warm-blooded species.[4]

Historically, incubators have been the primary tool of someone conducting cell culture, allowing for ideal conditions for cell reproduction. Cell culture is required for a number of disciplines, including but not limited to toxicity studies, monoclonal antibody production, human viral vaccine production, artificial tissue engineering, and cell and gene therapy.[5]

Biological Cell Microscopy is a common practice when researching biological systems. To perform accurate and meaningful research, cells must be studied under their most natural conditions to remain viable and functioning. Specifically, human cells require a temperature of approximately 37℃, which is the average body temperature, to remain viable for biological study.

THEORY

Ohm's law is the equation that generally defines analog electronics, allowing for the determination of fundamental characteristics of circuitry based on measurable quantifies. Ohm's law is given in Equation 1 where V is potential difference, I is electrical current, and R is resistance.

$$V = IR \tag{1}$$

Analog Circuitry

Resistance is what causes the reduction in current flow in a circuit and is caused by the electron conducting nature and external heating impurities of a resistive unit. Every material has a finite resistance. Reactance arises from inductive or capacitive elements and cause the alternating current in a circuit to be out of phase with the electromotive force that is producing it. Resistance and reactance contribute to but differs from a circuit's impedance. The impedance of a circuit is the effective resistance of the circuit and is shown in Equation 2 where resistance is added in quadrature with the total reactance of a circuit.

$$Z = \sqrt{R^2 + (X_L - X_C)^2} \tag{2}$$

Z is the impedance of the circuit, R is the resistance, X_L is the reactance due to inductors, and X_c is the capacitive reactance.

It is important to consider input impedance, or a measure of the loads susceptibility to draw current, and output impedance, or the ability for a device to deliver an unrestricted current to a load resistor, when considering impedance bridging. To achieve impedance bridging, ideally the load impedance is much larger than the source impedance, allowing the load to be the limiting factor in the circuit. A component that is very effective in impedance bridging is an operational amplifier. This concept will be revisited in the operation amplifier section of this paper.

Voltage, Current, and Ground

To facilitate the flow of electrons from one position to another, known as an electrical current, a potential difference must exist between the two points. The potential difference, also commonly referred to as a voltage or voltage difference, results in an electromotive force (EMF) which causes the free electrons within a given conductor to drift from one point to another. A positive voltage can be created through a number of methods, including a chemical or mechanical reaction. Batteries use a chemical reaction to create a potential difference while the positive potential created in a wall outlet results from a mechanical process where a steam turbine is turned by steam created by thermal energy due to burning fossil fuels or biomass, nuclear fission, geothermal energy, or solar energy, powering a generator that creates a voltage. Symbols used to represent a power source are given in Figure 1.

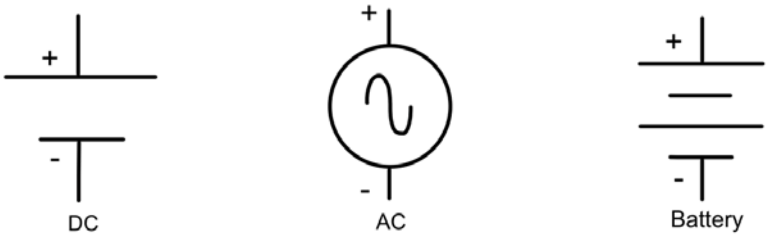

Figure 1. Symbols used to represent a DC voltage source, AC voltage source, and a potential difference produced by a battery.

Ground is what is used to refer to a potential difference of 0 V and is used as a reference point for all voltage measurements. Ground is named so because it refers to the body of the earth, which acts as an electrical sink, but 0 V can typically be obtained by connecting to the ground prong of a wall outlet that is connected to a metal rod in the ground, a cold water pipe, the negative terminal of a battery, or the metal chassis of an instrument. There are several symbols to indicate ground within a circuit, shown in Figure 2, and each symbol represents a different type of ground. In practice these symbols are typically used interchangeably.

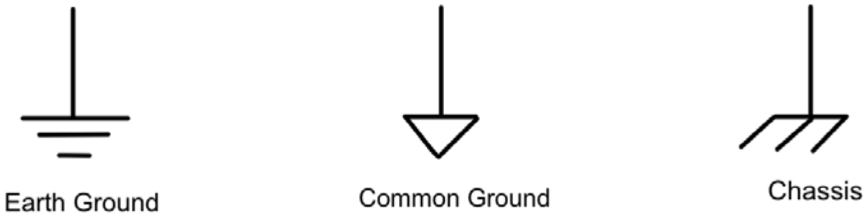

Figure 2. Symbols used to represent ground connections. The symbols are specific to application but distinction between the symbols is rarely made and leads to common interchangeability.

It should be noted that electrons flow from a position of lower potential to higher potential, but Benjamin Franklin established a scientific precedent that conventional current flows from higher voltage to lower voltage by assuming that charge carriers had a positive charge rather than a negative charge. Electronic formulas used in this paper were developed based on Franklin's theory and therefore this incorrect assumption will be utilized when executing mathematical and theoretical explanations of concepts to account for negative sign errors that would arise. Within the circuit described, a Tenma 72-2685 Digital-Control DC Power Supply 30V 3A power supply set to 10 V is used to create a potential difference.

Thermistors

A thermistor is a variable resistor whose resistance changes as temperature varies. All variable resistors, including thermistors and potentiometers use the circuit symbol shown in Figure 3 when being represented in circuit diagrams.

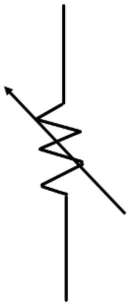

Figure 3. The circuit symbol for variable resistors is presented. This symbol is used throughout this paper to represent both thermistors and potentiometer.

It contains a semiconductor bead whose electrical conductivity decreases as temperature increases. A negative temperature coefficient (NTC) thermistor is a temperature sensitive resistor that's resistance varies inversely with temperature as shown in the Steinhart-Hart equation in Equation 3.[6]

$$\frac{1}{T} = A + B\ln(R) + C\ln(R)^3 \tag{3}$$

Where T is the absolute temperature of the thermistor, R is the resistance of the thermistor in ohms, and A, B, and C are constants that can be experimentally determined using three difference temperature and resistance measurements and a system of equations. The experimental derivation for the Steinhart-Hart equation can be found in Steinhart and Harts 1968 paper, Calibration curves for thermistors.[7] When conducting the experimental derivation, Steinhart and Hart determined that the curve fit equation for a given thermistor may vary across five different natural logarithm curve fits, each having a given uncertainty in the fit. Using a Thorlabs TH10k 10 kΩ thermistor, the ideal curve fit given by the manufacturer is the variation of the Steinhart-Hart equation, given in Equation 4.

$$(T + 273\ ^\circ\mathrm{C})^{-1} = A + B\ln\left(\frac{R_t}{R_{25^\circ\mathrm{C}}}\right) + C\ln\left(\frac{R_t}{R_{25^\circ\mathrm{C}}}\right)^2 + D\ln\left(\frac{R_t}{R_{25^\circ\mathrm{C}}}\right)^3 \tag{4}$$

Where T the temperature of the thermistor in Kelvin, R_t is the measured resistance of the thermistor in Ω, $R_{25^\circ\mathrm{C}}$ is the resistance of the thermistor at 25 ℃ in Ω and is given to be 10,000 Ω, and A, B, C, and D are constant coefficients determined experimentally by the manufacturer. The coefficients for the Thorlabs TH10k 10 kΩ thermistor are provided in Table 1 below.

Table 1. Coefficients of the Steinhart-Hart Equation for varying resistance ranges for the Thorlabs TH10k 10 kΩ thermistor as provided by Thorlabs.[8]

Resistance Range (Ω)	A $(x\,10^{-3}K^{-1})$	B $(x\,10^{-4}K^{-1})$	C $(x\,10^{-6}K^{-1})$	D $(x\,10^{-8}K^{-1})$
692,600 to 32,770	3.3570420	2.6214848	3.3743283	-6.4957311
32,770 to 3,599	3.3540170	2.5617244	2.1400943	-7.2405219
3,599 to 681.6	3.3530481	2.5420230	1.1431163	-6.9383563
681.6 to 187	3.3536166	2.5377200	0.85433271	-8.7912262

By manipulating Equation 4, an equation for resistance as a function of temperature can be obtained as shown in Equation 5.

$$R_t = R_{25°\mathrm{C}}\,(e^{a+\frac{b}{(T+273°\mathrm{C})}+\frac{c}{(T+273°\mathrm{C})^2}+\frac{d}{(T+273°\mathrm{C})^3}}) \tag{5}$$

The variables a, b, c, and d are constants that were experimentally determined by the manufacturer and are given in Table 2 for various ranges of temperature.

Table 2. Coefficients of the Steinhart-Hart Equation solved to be a function of temperature, for varying temperature ranges for the Thorlabs TH10k 10 kΩ thermistor as provided by Thorlabs.[8]

Temperature Range (°C)	a $(x\,10)$	b $(x\,10^3\ °\mathrm{C})$	c $(x\,10^5\ °\mathrm{C}^2)$	d $(x\,10^7\ °\mathrm{C}^2)$
-50 to -1	-1.6443767	6.1080608	-4.4141671	2.4159818
0 to 49	-1.5470381	5.6022839	-3.7886070	2.4971623
50 to 99	-1.4807463	5.1550854	-2.9717659	2.2904187
100 to 150	-1.4862658	5.2676519	-3.5374848	3.1207901

By examining the second line of Table 2, the coefficients for the temperature range of interest are identified. The Temperature versus Resistance graph for the Thorlabs TH10k 10 kΩ thermistor, using the coefficients for the temperature range of 0°C to 49°C, is provided in Figure 4.

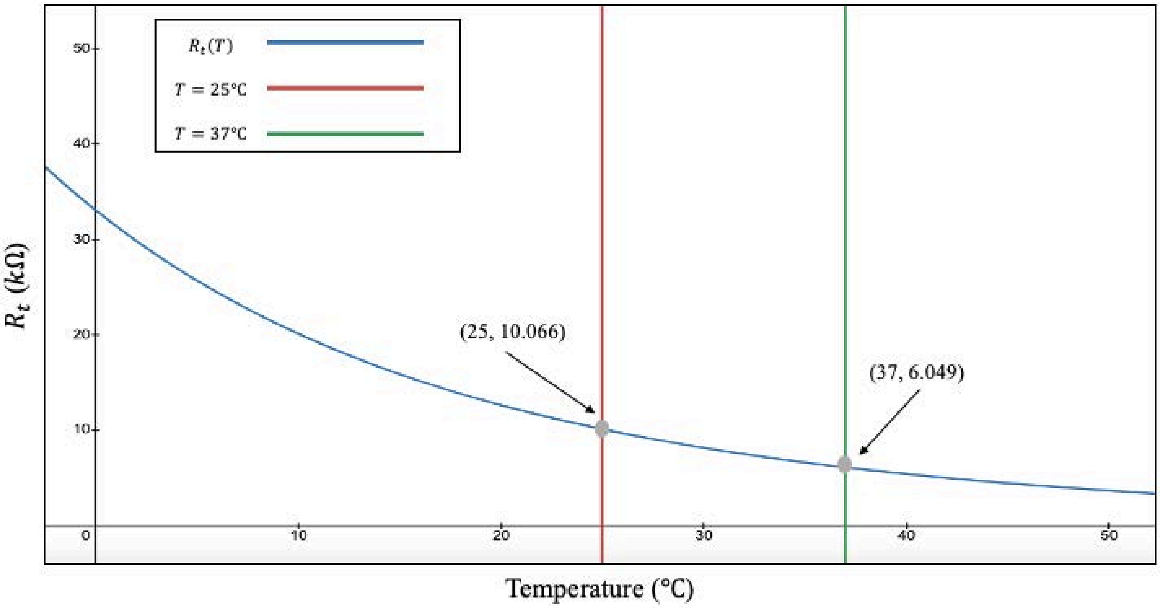

Figure 4. The resistance versus temperature graph of the Thorlabs TH10k 10 kΩ thermistor using coefficients for the temperature range of 0 °C to 49 °C, with lines representing 25 °C and 37 °C superimposed on the plot, created using Desmos graphing software.

When horizontal lines that represent the temperatures at the reference point given by the manufacturer and body temperature are superimposed on the graph of resistance versus temperature, the resistances of the thermistor at those temperatures are graphically determined to be 10.066 kΩ and 6.049 kΩ respectively. Using Equation 1, Ohm's Law, and a current of 0.0003789 Amps it is determined that the voltage drop across the thermistor at body temperature is 2.292 V.

Potentiometers

A potentiometer is a manually variable resistor that allows the potential difference to a load to be adjusted. Figure 5 below shows a diagram of how to wire a potentiometer so that the voltage to the load can be varied as well as a graph that shows the load voltage compared to the potentiometer resistance.

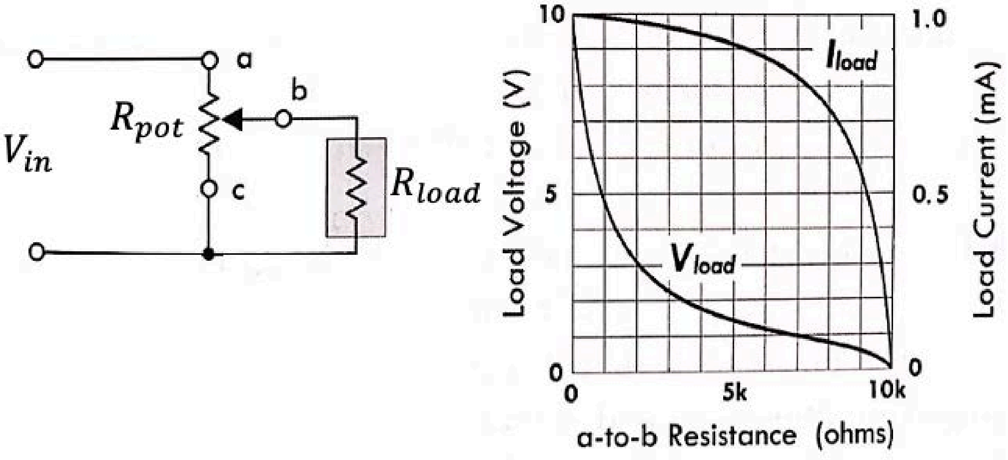

Figure 5. A circuit diagram of a potentiometer configured to vary the voltage to the load resistor and a graph of load voltage and load current compared to potentiometer resistance. This figure comes from Figure 3.58 in Practical Electronics for Inventors.[9]

Rotary potentiometers, made for manual control, contain a knob that is user controlled to turn up to 300° and determines resistance of the potentiometer based on the ratio of turn to the maximum resistance. By turning the taper knob, a sliding wiper element moves along the resistive unit, changing the outputted resistance at the wiper terminal.[10] Referring to Figure 6, the resistance of a potentiometer is determined by its wiper placement in relation to terminals a and c. Terminal b, or the "wiper", slides along the fixed resistor as the knob is turned and varies the resistance outputted at terminal b.

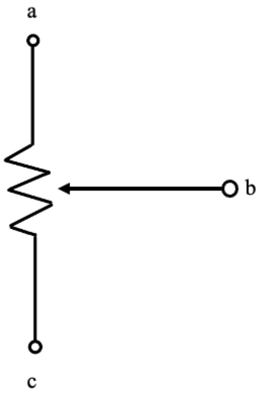

Figure 6. A partial circuit diagram that shows the three terminals of a potentiometer and a visual description of wiper placement versus resistance.

The potentiometer used throughout this paper is a Bourns Inc. Pot 10 Kohm 1W Plastic Linear Potentiometer, which contains a linear taper.[11] The taper rotation determines the wiper position. Because of the linear taper the resistance of the potentiometer has a linear relationship to the rotation of the tapper knob. The resistance of a potentiometer can be determined using Equation 6.

$$R_{pot} = R_{max}\frac{\theta}{300°} \tag{6}$$

Where R_{pot} is the resistance of the potentiometer, R_{max} is the maximum resistance of the potentiometer, and θ is the angel is degrees that the potentiometer knob was turned. For the potentiometer used, R_{max} is 10 $k\Omega$ with uncertainty in the resistances of the potentiometer being $\pm$ 20%. The plot of R_{pot} versus θ is shown in Figure 7, demonstrating the linear relationship between the two variables.

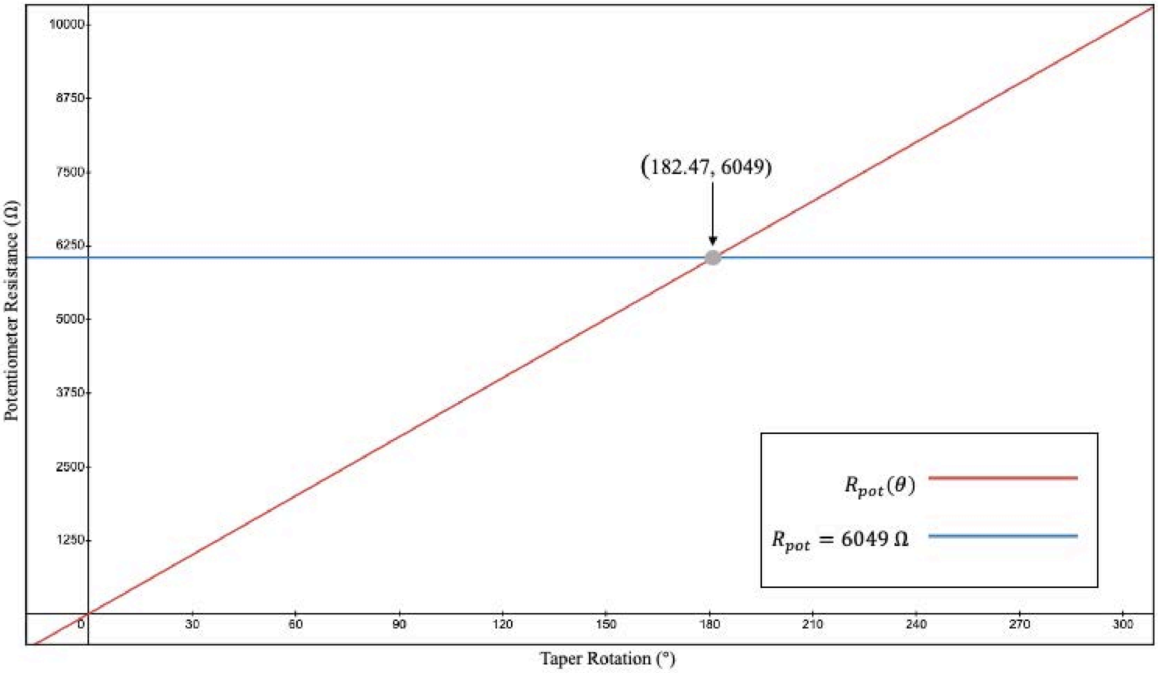

Figure 7. The linear plot of the potentiometer resistance versus the taper rotation for the Bourns Inc. Pot 10 Kohm 1W Plastic Linear Potentiometer, created using Desmos graphing software.[11]

According to Figure 7, when the line for 6049 Ω is superimposed on the plot of $R_{pot}(\theta)$, the taper rotation to achieve the proper set resistance for 37℃ is 182.47°. The resolution of a potentiometer is the smallest change in tapping ratio that can be made by moving the potentiometers wiper, and is given by the manufacturer to be theoretically infinite for this potentiometer.[11] This potentiometer uses conductive plastic as the resistive unit.

Voltage Dividers

Utilizing a voltage divider allows for the reduction of voltage within a circuit in a predictable fashion. A voltage divider is created using two resistors as shown in Figure 8.

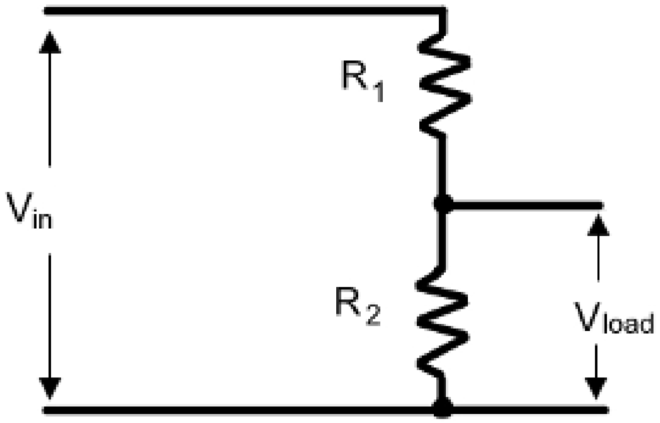

Figure 8. A schematic of a voltage divider, where V_{in} is the potential difference across the two resistors and V_{load} is the potential difference across resistor 2.

The voltage to the load is given by Equation 7.

$$V_{load} = V_{in}\frac{R_2}{R_1+R_2} \tag{7}$$

Where V is the voltage provided by the power supply, and R_1 and R_2 are the resistors referenced in Figure 8. Voltage dividers are employed after both the thermistor and the potentiometer to allow for the limiting of voltage into the operational amplifier.

NPN Transistors

An NPN transistor is a type of bipolar transistor, which is a three-terminal device that acts as an electrically controlled switch.[9] The circuit symbol for an NPN transistor is presented in Figure 9 below.

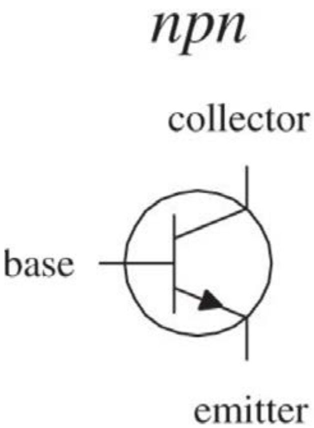

Figure 9. The circuit diagram for an NPN transistor, showing the location of the base, collector, and emitter pins within the orientation of the device. This figure came from figure 4.44 in Practical Electronics for inventors.[9]

Bipolar transistors require a process called "doping" to be manufactured. Doping, or the addition of impurities, allows for semiconductors to become useful in electronics, creating electron depletion and excess regions in p-type and n-type doped semiconductors respectively. An NPN is made by sandwiching a p-type doped semiconductor between two n-type doped semiconductors. A schematic of an NPN transistor, showing labeled doped regions, the depletion zone, and the pins of the transistor is presented in Figure 10.

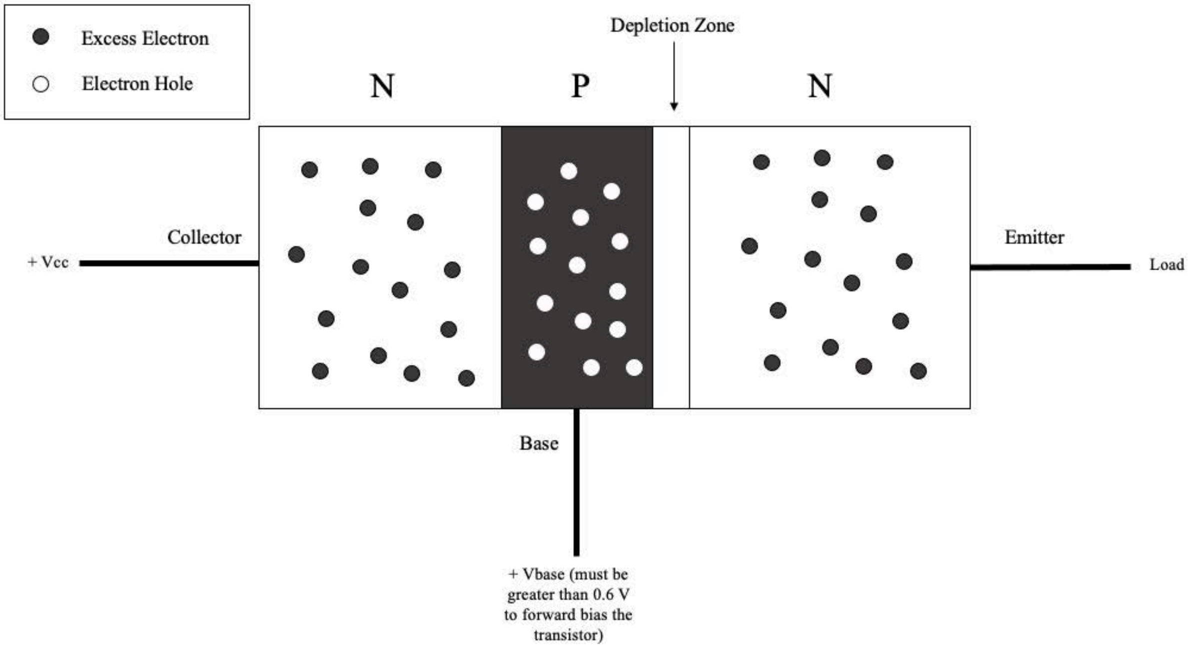

Figure 10. A theoretical schematic of an NPN transistor.

The two p-n junctions created in the transistor cause current to not flow to the emitter pin, unless a strong enough base voltage is applied to forward bias the junction. When the transistor is forward biased, electrons are able to overcome the atomic forces keeping them on the n-side of the junction, causing the depletion zone between the p-n junction to vanish, and causing current to flow from the collector to the emitter.

Operational Amplifiers

An operational amplifier is an integrated device that contains an inverting input, a non-inverting input, two DC power supply leads, an output terminal**,** and leads that allow for fine tuning and acts as a differential amplifier. An ideal operational amplifier has an infinite input impedance to prevent loading down from any signal, an output impedance of zero to prevent limiting of power supplied to the load, an infinite gain to allow for ease of operation, and the input terminals draw no current. Realistically this is never the case, and a real operational amplifier typically has an input impedance in the range of $10^6\ \Omega$ to $10^{12}\ \Omega$, an output impedance in the range of $10\ \Omega$ to $1000\ \Omega$, a gain in the range of 10^4 to 10^6, and the input terminals draw current in the picoamps to nanoamps range. Schematics for both the ideal operational amplifier and real operational amplifier are given in Figure 11.

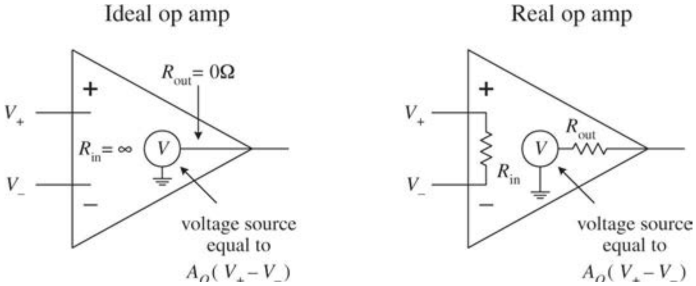

Figure 11. The left shows an ideal operational amplifier while the right shows a more realistic operational amplifier circuit, Figure 8.6 from Practical Electronics for Inventors.[9]

The inner circuitry is more complicated than the diagram shown on the right side of Figure 11, an equivalent circuit that shows the various NPN and PNP transistors, as well as the resistors within a typical low-cost general purpose bipolar operational amplifier is shown in Figure 12.

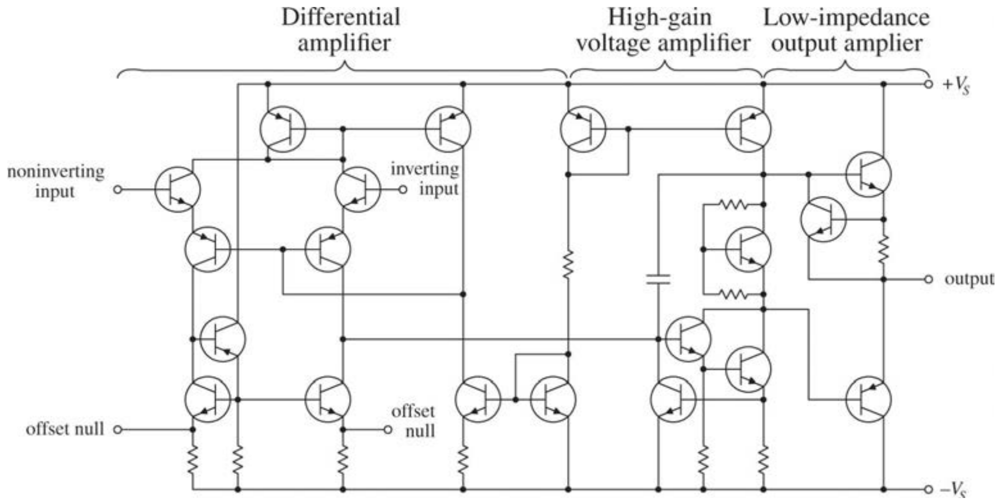

Figure 12. A schematic diagram of a typical low-cost general purpose bipolar operational amplifier, Figure 8.5 from Practical Electronics for Inventors.[9]

Both the circuit diagram symbol and a more realistic chip illustration of an operational amplifier are given in Figure 13.

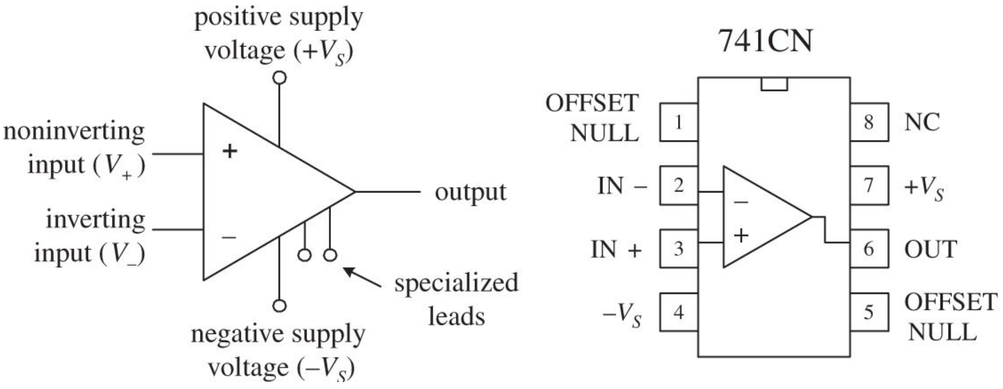

Figure 13. The left shows the circuit symbol for an operational amplifier, with the pins labeled. The right shows a 741CN Operational Amplifier chip pin out. This is figure 8.1 from Practical Electronics for Inventors.[9]

When used as a simple comparator, as in the circuit discussed in this paper, an operational amplifier's function becomes very simple. When the potential coming into the noninverting input is greater than that of the inverting input then the outputted voltage is equal to the positive supply voltage. When the potential at the inverting input is greater than that at the noninverting input, then the voltage at the output terminal is equal to the negative supply voltage.

Heating Element

The Thorlabs HT10KR 10 W Metal Ceramic Ring Heater is used as a heating element within the circuit discussed throughout this paper. This heating element acts as the load for the circuit, with a resistor value of 50 Ω. The heating element contains thermistor leads that were not employed. The CAD rendering of the heater is shown in Figure 14 below.

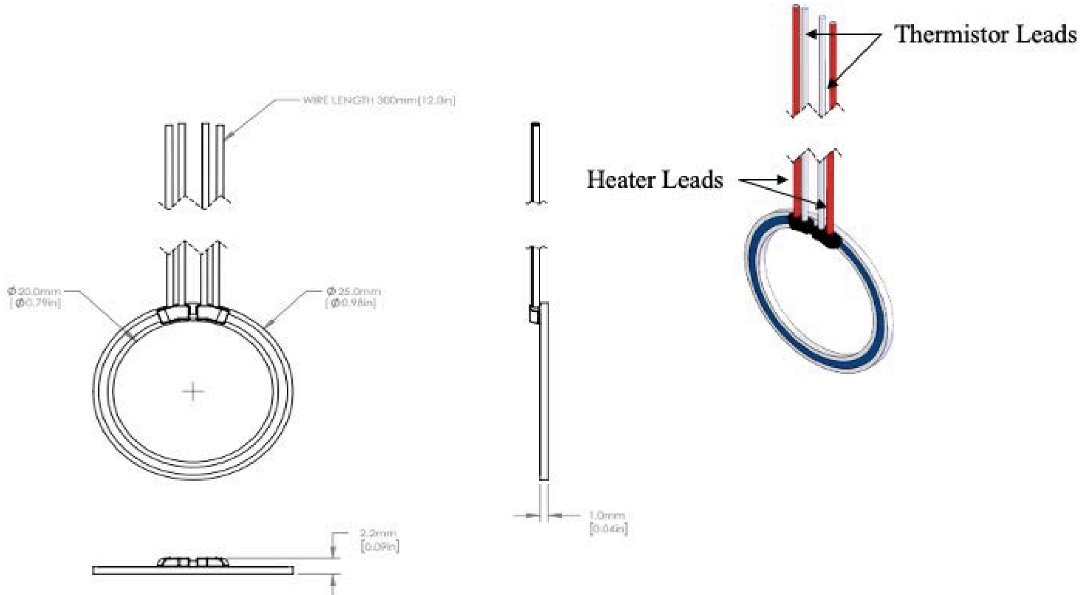

Figure 14. CAD drawing of the Thorlabs 10 W Metal Ceramic Ring Heater with heater and thermistor leads labeled, as provided by Thorlabs.[12]

Thermodynamics

Specific heat capacity (SHC) is defined as the amount of heat required to raise the temperature of some mass of a substance by some temperature.[13] Equation 8 mathematically defines SHC capacity in terms of thermal energy, mass, and change in temperature.

$$c = \frac{Q}{m\,\Delta T} \tag{8}$$

Where c is the specific heat capacity, Q is the thermal energy, m is the mass of the sample in kilograms, and ΔT is the change in absolute temperature. The specific heat capacity of pure water is 4184 $J/kg\ K$.[14] By manipulating Equation 8, solving for temperature change, Figure 15 is produced and shows a plot of temperature change versus thermal energy required to make that change for 0.00225 kg of pure water, the sample size used for data acquisition in this paper.

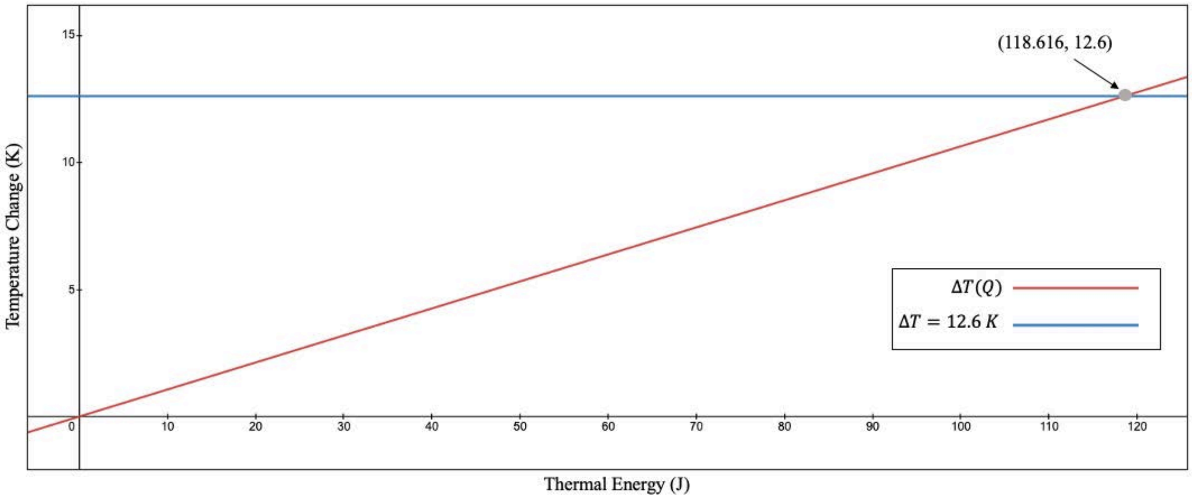

Figure 15. The plot of temperature change of a sample versus thermal energy required for 0.00225 kg of distilled and deionized water, created using Desmos graphing software.

This demonstrates the energy required to change the temperature of 0.00225 kilograms of water by some amount. As seen on the plot, to change the temperature of the water sample by 12.6 K, which is equal to 12.6 ℃, requires 118.616 Joules of thermal energy.

EXPERIMENTAL METHODS

A feedback circuit is used to maintain thermal conditions within the sample chamber. The feedback element in the circuit is the thermistor. A circuit diagram for the feedback circuit is presented in Figure 16.

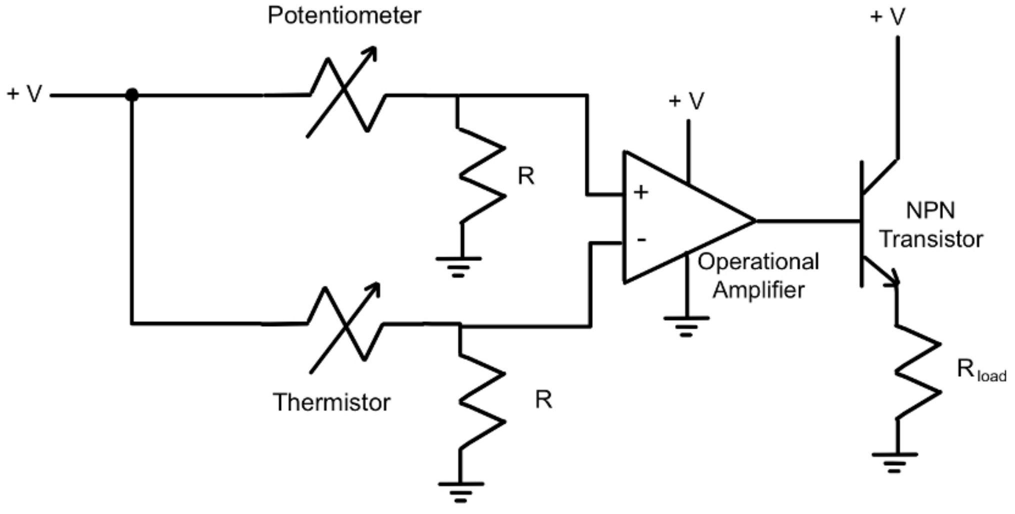

Figure 16. The circuit diagram of the feedback circuit, showing all components labeled.

The circuit uses a 10 V power supply, branching off into paths that lead into a potentiometer and a thermistor. The potentiometer and thermistor used are the Bourns Inc. Pot 10 Kohm 1W Plastic Linear Potentiometer and the ThorLabs TH10k 10 kΩ Thermistor respectively. A 20 kΩ voltage divider follows both these, dividing voltage leading into the inputs of the operational amplifier (op amp), which acts as a simple comparator. The operational amplifier used in this circuit is the Texas Instruments LM358P Dual Standard Operational Amplifier. The potentiometer lead goes into the non-inverting input of the operational amplifier, while the thermistor lead is connected to the inverting input. The potentiometer acts to manually set the voltage that the operational amplifier is comparing the voltage from the thermistor to. By adjusting the resistance of the potentiometer, the voltage leading into the operational amplifier changes, as shown by Equation 1. When the voltage from the potentiometer is higher than the voltage from the thermistor, the operational amplifier outputs a positive voltage. An NPN transistor acts as a switch in the circuit, when a positive voltage is incident on the base pin, the transistor outputs a positive voltage to the load. The transistor used is the DigiKey TIP120G NPN Transistor and the load is the Thorlabs HT10KR 10 W Metal Ceramic Ring Heater. The heater then turns on, heating the sample chamber which contains the thermistor inside of it. As the thermistor heats, it's resistance decreases causing the voltage drop across the resistor and therefore increasing the voltage into the inverting input of the operational amplifier. Once the resistance of the thermistor is low enough that the voltage drop across it is greater than that of the potentiometer, the voltage at the inverting input of the operational amplifier is greater than at the non-inverting input and the op amp outputs a ground signal, causing the transistor to not conduct and therefore turning off the heating element. The circuit then continuously oscillates between a heating and non-heating state, keeping the sample chamber at 37.0 ± 0.6 ℃.

To analyze the operation of this circuit temperature, voltage across the potentiometer, and voltage across the thermistor were monitored. The experimental set up for data acquisition is presented in Figure 17 below.

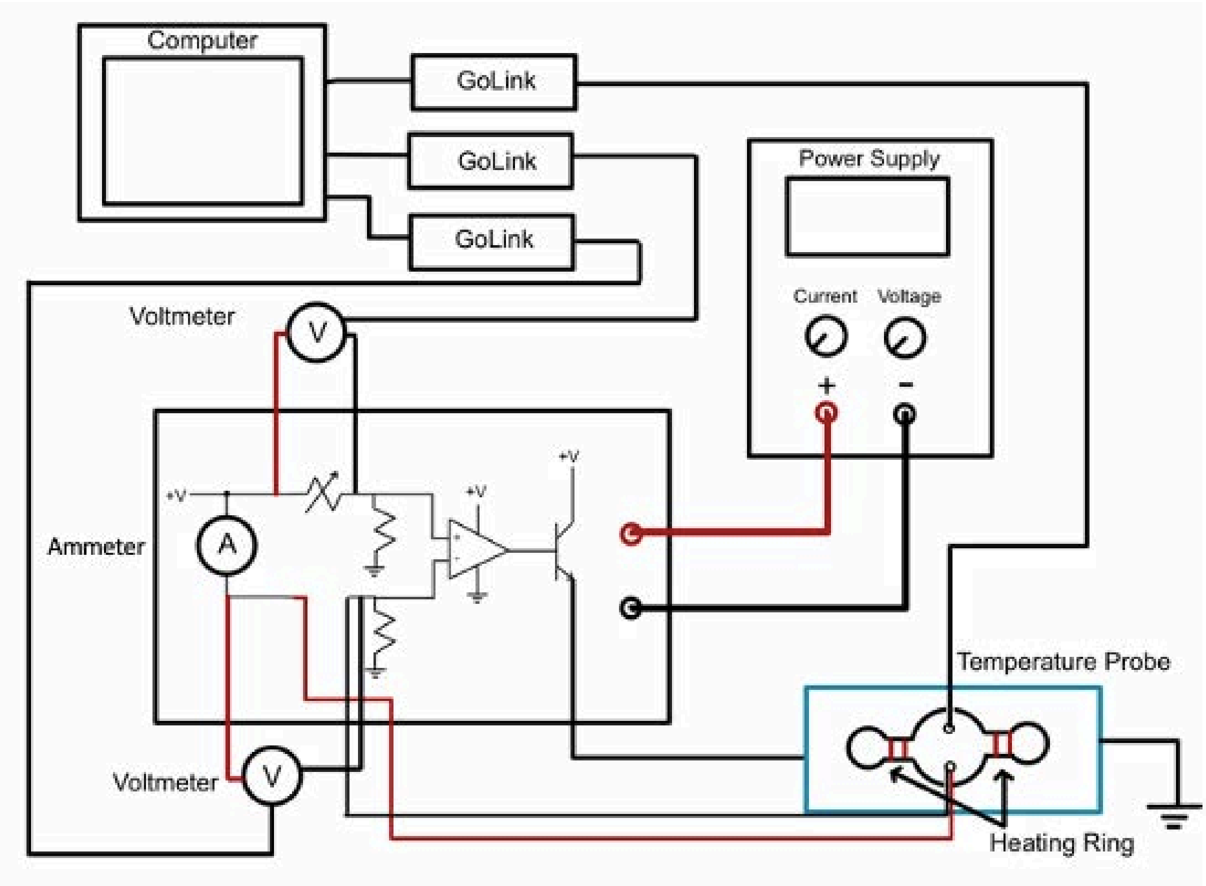

Figure 17. The experimental setup for data acquisition.

To acquire data, a temperature probe is inserted into one side of the sample chamber using a custom 3D printed slide spacer with two channels leading into the sample region. The spacer was designed using the Autodesk Fusion 360 software and printed using a Lulzbot Taz6 3D Printer. The CAD rendering of the slide spacer is presented in Figure 18.

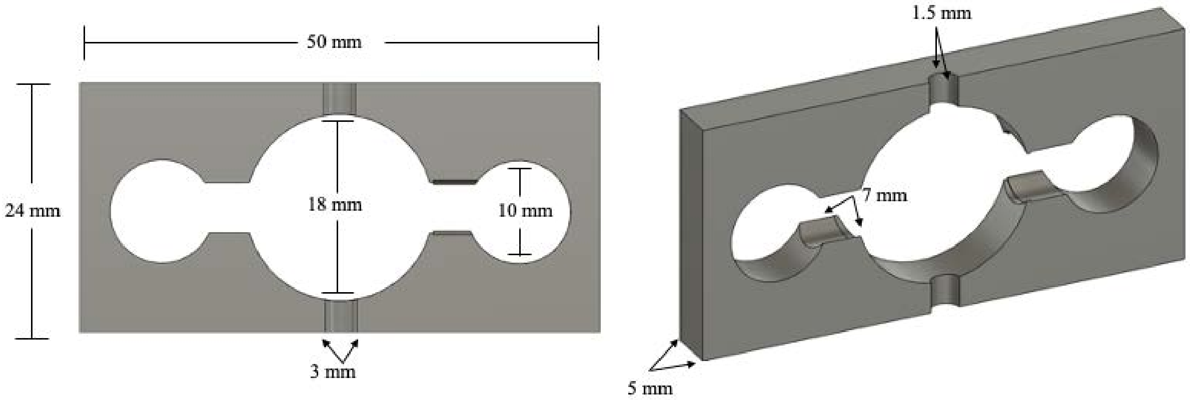

Figure 18. The CAD design of the slide spacer used for data acquisition, designed using Autodesk Fusion 360 and printed using a Lulzbot Taz6 3D printer.

The second channel allowed for the thermistor to be inserted into the sample chamber, allowing the changing temperature of the sample to be monitored by the feedback circuit. The slide spacer is insulated using Dow Corning High-Vacuum Grease and placed in-between two glass microscope slides. The sample chamber then rests atop the heating ring. This is shown in Figure 19.

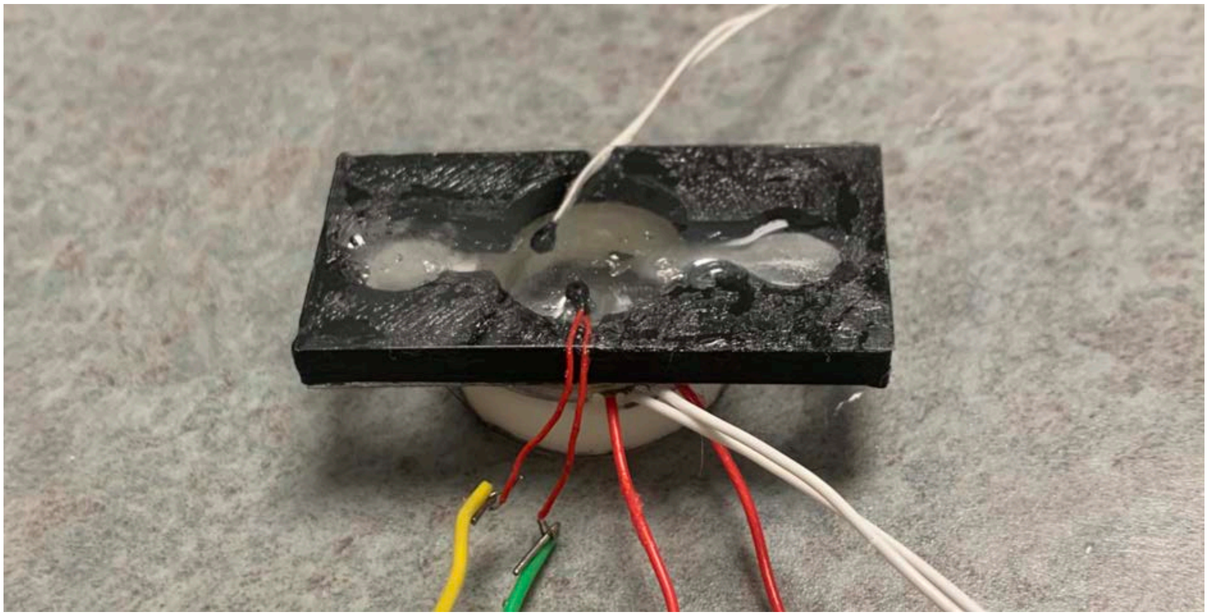

Figure 19. A picture of the sample chamber, including the 3D printed slide spacer, heating ring, thermistor, and temperature probe.

A Tenma 72-2685 Digital-Control DC Power Supply is used to provide a potential difference of 10 V and DC current of 0.147 Amps to the circuit. Two Vernier Software Differential Voltage Probes are used to monitor the voltage drop across the potentiometer and thermistor and a Vernier Surface Temperature Sensor is used to measure the temperature within the sample chamber. All three of these sensors require a Vernier GoLink USB Adapter to connect to a computer and interface with the Vernier Logger Pro 3 software that was used for data acquisition. Using the Logger Pro software, data was able to be collected and graphed in real time, measuring a data point every 1.59 seconds for 1000 seconds for the first data set and 0.5 seconds for 1800 seconds for the second data set. To measure the current through the thermistor, a B&K Precision 2831E 4 ½ Digital Multimeter was used as an ammeter to measure DC current, and a software provided by the manufacturer allowed for the multimeter to interface with a computer and record data electronically at a rate of 0.63 samples per second. The sample used for data acquisition was 0.00225 kg of pure distilled and deionized water produced by a Milli-Q Millipore system.

DATA ANALYSIS AND RESULTS

To verify that the performance of the thermistor matches the specifications provided by the manufacturer for the temperature range of interest, current and potential difference measurements were taken every 1.59 seconds at varying temperatures over the course of 1000 seconds. By manipulating Equation 1, Equation 9 can be obtained and allows for the calculation of resistance values, R, based on measured values of current, I, and voltage, V.

$$R = \frac{V}{I} \tag{9}$$

Using this formula, values for the resistance of the thermistor at difference temperature measurements is acquired. These values were plotted using Python, along with the accepted resistance as a function of temperature equation provided by Thorlabs. This function, with the coefficients that correspond to the temperature range in question, is provided in Equation 10.

$$R_t(T) = 10\ k\Omega\ (e^{(-1.5470381\ x\ 10) + \frac{(5.6022839\ x\ 10^3)}{(T+273℃)} + \frac{(-3.7886070\ x\ 10^5)}{(T+273℃)^2} + \frac{(2.4971623\ x\ 10^7)}{(T+273℃)^3}}) \tag{10}$$

Where the resistance of the thermistor is given in kiloohms. Figure 20 is a superposition of the measured data points and the accepted function for the thermistor resistance versus temperature and was produced using the MatPlot Library in Python.

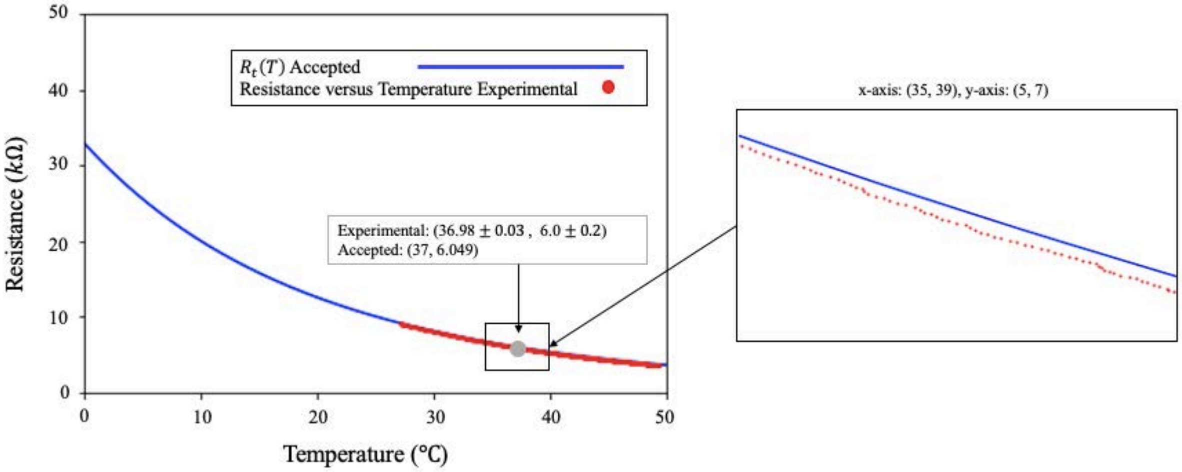

Figure 20. The accepted function for resistance versus temperature for Thorlabs TH10k 10 kΩ thermistor in the temperature range of 0 °C to 50 °C superimposed on the plot of the measured resistance versus temperature data points for a pure water sample of mass 0.00225 kg.

Based on Figure 20, it can be observed that the measured resistance near body temperature is $6.0 \pm 0.2\ k\Omega$. When comparing this value to the accepted value of 6.049 $k\Omega$, provided by Thorlabs, the percent error is determined to be 2%. By examining a scaled in version of the graph, it can be seen that there is a slight discrepancy between the plotted points and the accepted function. Because of the low percent error between the two values of resistance at body temperature, it can be concluded that the Thorlabs TH10k 10 $k\Omega$ thermistor is functioning properly.

To analyze the functionality of the circuit and its effectiveness at keeping the sample at 37 °C, measurements of both the voltage drops across the thermistor and potentiometer, and temperature within the sample chamber were taken every 0.5 seconds for 1800 seconds. Figure 21 is a plot of temperature versus time and shows two regions.

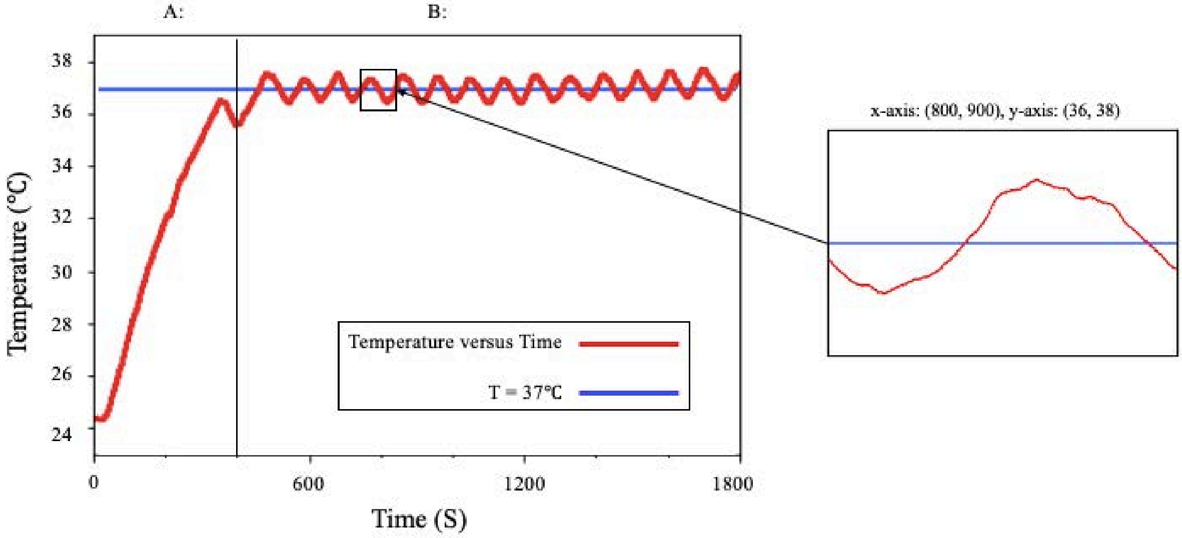

Figure 21. A plot of temperature versus time for a pure water sample of mass 0.00225 kg with a line representing body temperature. Region A, the heating zone, is from 0 seconds to 349 seconds. Region B, the set temperature zone, is from 349.5 seconds to 1800 seconds.

Region A is the heating zone, the ring heater is continuously on, heating the sample from 0 seconds to 349 seconds. Region B is where the set resistance was changed to allow the temperature to vary around a higher temperature. In region B, the temperature continuously varies around 37 °C, with a minimum temperature of 36.55

$\pm$ 0.03 °C, a maximum temperature of 37.58 $\pm$ 0.03 °C, and an average temperate of 37.06 $\pm$ 0.03 °C in a single temperature cycle from 800 to 900 seconds. Based on this data, it can be concluded that the circuit is effective at keeping the sample at a temperature of 37 °C with slight variations of about 0.6 °C, above and below body temperature.

To properly evaluate the circuit design, it is also important to examine the potential differences across both the potentiometer and the thermistor in the same time intervals and regions as in Figure 21. The voltage drops across the two variable resistors versus time is given in Figure 22 below.

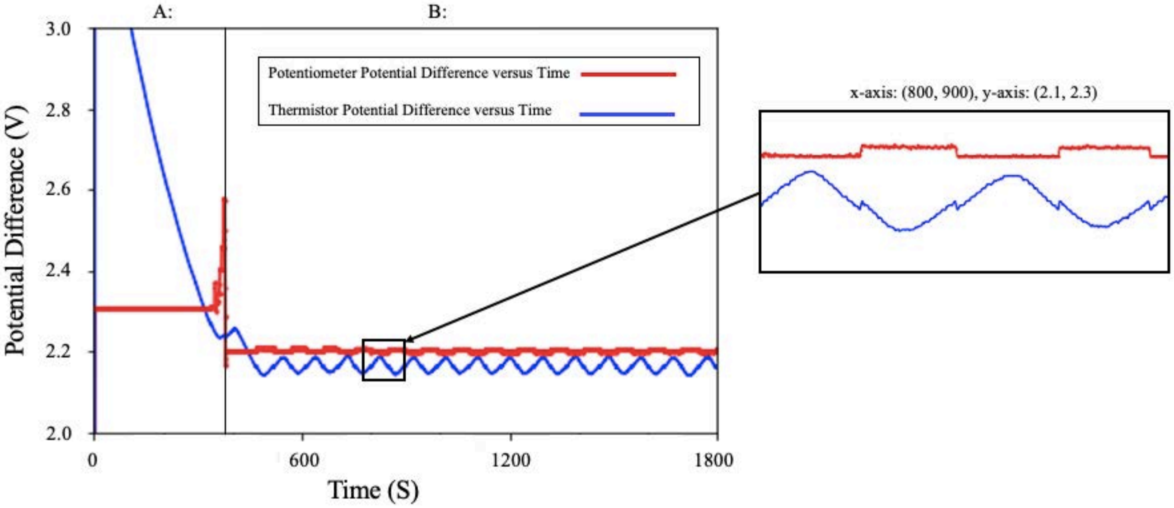

Figure 22. Potential Difference versus Time for the Thorlabs TH10k 10 $k\Omega$ thermistor and the Bourns Inc. Pot 10 Kohm 1W Plastic Linear Potentiometer as temperature is changing. A sample of pure water with mass 0.00225 kg was used.

In region A of Figure 22, it is observed that the potential difference across the potentiometer remains constant until second 349, when the user adjusted the potentiometer taper so that the set resistance would decrease. In region B, the new set resistance forces a lower potential difference across the potentiometer therefore the sample continues to heat and the voltage drop across the thermistor continues to drop. Once the temperature set by the potentiometer is met, the thermistor's potential difference mirrors the temperature changes of the sample. By examining a scaled in version of the plot, it can be seen that there is a 0.02 V threshold on the inputs for the operational amplifier to switch output values, therefore when the voltage drop across the thermistor comes within this threshold, the heater ring will switch from on to off, then once this threshold is exceeded, the ring will turn back on.

DISCUSSION AND FUTURE WORKS

Using graphical and numerical analysis, the effectiveness of the feedback circuit described in this paper to maintain optimal thermal conditions within a sample chamber was evaluated. The sample varied between approximately 36.4 °C and 37.6 °C, maintaining an acceptable temperature range to support long term human cell viability. To verify proper calibration of the circuit, the temperature versus resistance graph of the Thorlabs TH10k 10 $k\Omega$ thermistor was compared to the Steinhart-Hart equation with coefficients provided by the manufacturer. The predicted resistance of the thermistor at body temperature fell within 100 Ω of the experimental value, with a percent error of 1.55% for the measured value.

Throughout the experiment, several improvements were made to allow for higher ease of data acquisition and more accurate measurements of various quantities. Using a 3D printed slide spacer improved the temperature measurements, allowing for more accurate temperature readings to be taken within the sample due to the enclosed nature of the sample that was created due to the custom channels created by printing a plastic slide spacer instead of using a pre-cut silicon spacer. Uncertainty in the temperature reading was also minimized from one data set to another by using a more accurate temperature probe that only took temperature readings within the sample chamber, rather than an 8 inch metal probe that took temperature measurements long the entirety of the probe, allowing for interference from ambient temperature in the room. When measuring current flowing through the thermistor, the original sensor used had a low resolution compared to the current being measured, causing widely inaccurate measurements to be taken. By using the more precise B&K Precision 2831E 4 ½ Digital Multimeter, the current measurement was able to be recorded with far higher accuracy, allowing for more accurate values of thermistor resistance to be calculated.

Several improvements can be made to this apparatus in the future to make it more user-friendly, compact, and precise. To eliminate the trial-error process of identifying the proper potentiometer setting that will result in the ideal temperature range, a digital potentiometer with a 6000 $k\Omega$ setting can replace the analog potentiometer currently in the circuit. This would allow the user to select this setting when using the apparatus in a microscope, rather than requiring a previous calibration of the circuit to identify the correct taper setting. The other improvement that can be made is to lower the space allocated to housing the circuit by rewiring the circuit on a smaller breadboard, or by sending the circuit off to be machined into a small chip. These improvements were unable to be made due to material and time constraints during this project but would be beneficial to implement in the future. The consistently lower resistance measurements of the thermistor at any given temperature can be attributed to the lag of the thermistor's resistance change for a temperature change, resistance measurements would be more accurate if any temperature could be held for a longer time before taking the measurement.

CONCLUSION

The calibration of the Thorlabs TH10k 10 $k\Omega$ thermistor was confirmed to a percent error of 1.55% and the effectiveness of a thermistor based feedback circuit to maintain a temperature of 37 ℃ for a sample of mass 0.00225 kg of pure water was evaluated and confirmed. The improvements made by the author were discussed, as well as future improvements that can be made to optimize the apparatus designed in this paper. These improvements included a 3D printed slide spacer to insulate the sample, a more accurate temperature probe, and the use of a higher resolution ammeter. Future users may consider automating the circuit through the use of a digital potentiometer and consolidating the circuit onto a smaller sized breadboard or chip. This circuit provided ample circuitry, troubleshooting, and data acquisition experience to the author and was a comprehensive and effective thesis project.

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