Quad Rat Vitals Monitor

Client: Dr. Alex Converse

Advisor: Dr. Tom Yen

Group Members:

Jack Ho – Team Leader

Nathan Werbeckes – Communicator

Joseph Yuen – BSAC, BWIG

Final Paper for BME 402, Spring 2009

Table of Contents

Abstract

In the course of our client's research, PET imaging of rats are often used. During each scan, four rats are anesthetized and manually kept under anesthesia for the course of a few hours. Because the current system of anesthesia requires manual adjustments, the vitals of each rat must be monitored constantly. Currently our client is using a veterinary pulse oximeter designed for "small animals" (such as dogs, cats, and monkeys). However, since the rat has a relatively much higher heart rate, the machine often displays inconsistent readings, greatly reducing the efficiency of anesthesia delivery. Our novel monitoring device design allows for the record and display of SpO2 levels, heart rates, and body temperatures of four rats simultaneously during PET imaging experiments in order to maintain appropriate anesthesia dosages on each of the four rats independently. Design and construction of the breathing rate and temperature components of the device have been constructed and tested, and are able to simultaneously on four rats, accurately monitor and record rectal temperature (32-42° C) and respiratory rate (around 20 breaths/min) so that the anesthesiologist is able to determine the adequate dosage of isoflourine to keep the rats anesthetized.

Background

A pulse oximeter can be used to determine the SpO2 and heart rate non-invasively. It consists of a pair of red and infrared LEDs, which has wavelength at 660 and 940 nm respectively. These LEDs face a translucent part of the body, such as the earlobe or fingertip, and on the other side there is a photodiode, which produces current depending on how much light reaches the photodiode. Absorption at wavelengths corresponding to red (660 nm) and infrared (940 nm) differs significantly between oxyhemoglobin and its deoxygenated form, therefore from the ratio of the absorption of the red and infrared light the oxy/deoxyhemoglobin ratio can be calculated (Webster, 1997).

After the transmitted red (R) and infrared (IR) signals pass through the measuring site and are received at the photodetector, the R/IR ratio is calculated.

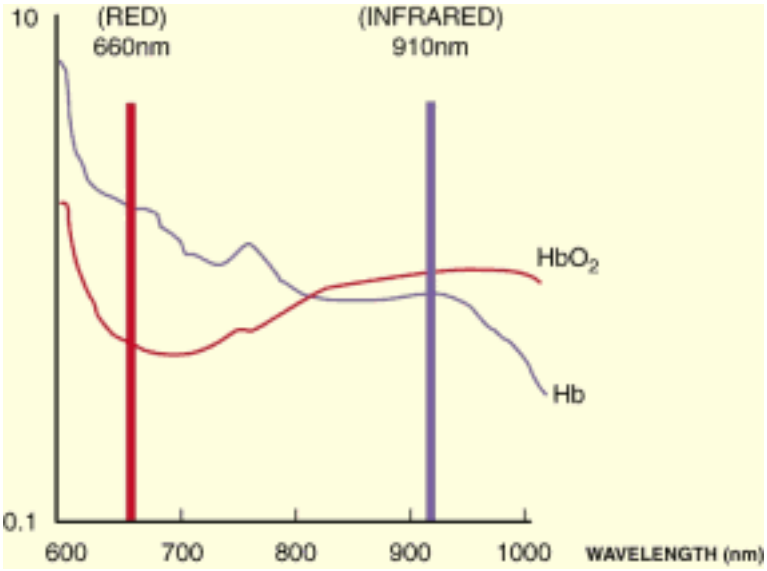


Figure 1. Difference of absorption at different wavelength (http://www.oximetry.org/pulseox/principles.htm)

From Beers law, $I=I_o e^{-\alpha ct}$, where I is the amount of light detected by the photodiode. R/IR ratio = $\ln(I_{MaxRed}/I_{MinRed}) / \ln(I_{MaxInfraRed}/I_{MinInfraRed})$

The R/IR is compared to a "look-up" table (made up of empirical formulas) that convert the ratio to a SpO_2 value. Typically a R/IR ratio of 0.5 equates to approximately

100% SpO_2, a ratio of 1.0 to approximately 82% SpO_2, while a ratio of 2.0 equates to 0% SpO_2 (Webster, 1997).

Companies such as Nonin, Nellcore produce pulse oximeter currently in the market. However, they are mostly for humans. There are also some veterinary oximeters in the market. However they are not suitable for use with mouse. Mouse has an average pulse rate of 300 beat per min, which is more than threefold of what human would have had. Our client is currently using pulse oximeter designed for humans on mice, and the pulse rate shown on the device is always out of the usual range of mice. The reason is due to the algorithm of signal processing as there is less blood passing through the artery with each beat, comparing to human. Currently there is a pulse oximeter specialized for mouse, named MouseOx, which is produced by Starr Life Science, however the cost for this device is estimated to be more than $7,000 USD.

Figure 2. PulseSense™ portable oximeter by Nonin.

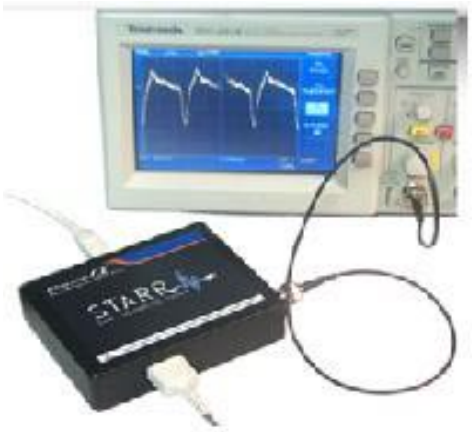


Figure 3. MouseOx™ by Starr Life Sciences.

Heartrate can also be detected because the arterial blood vessels expand and contract with each heartbeat. This is shown on the waveform of the pulse oximeter. By measuring the time distance between two consecutive peaks, we can calculate the pulse rate by 1/time.

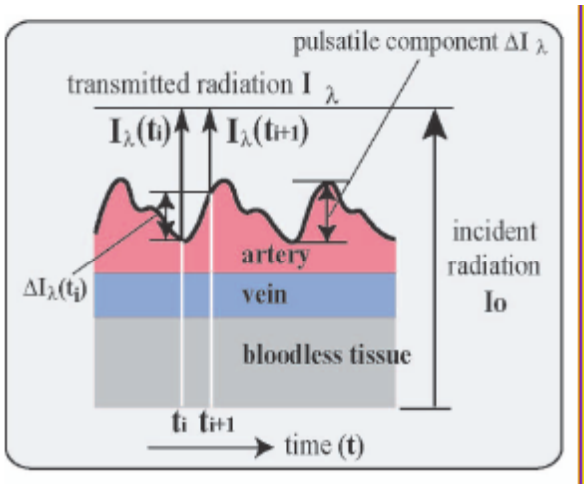


Figure 4. Normal waveform obtained by a pulse oximeter. Heart rate can be determined by the distance between two peaks (Yamakoshi 2006).

Problem Statement

In the course of our client's research, PET imaging of rats are often used. During each scan, four rats are anesthetized and manually kept under anesthesia for the course of a few hours. Because the current system of anesthesia requires manual adjustments, the vitals of each rat must be monitored constantly. Currently our client is using a veterinary pulse oximeter designed for "small animals" (such as dogs, cats, and monkeys). However, since the rat has a relatively much higher heart rate, the machine often displays inconsistent readings, greatly reducing the efficiency of anesthesia delivery. Our novel monitoring device allows for the

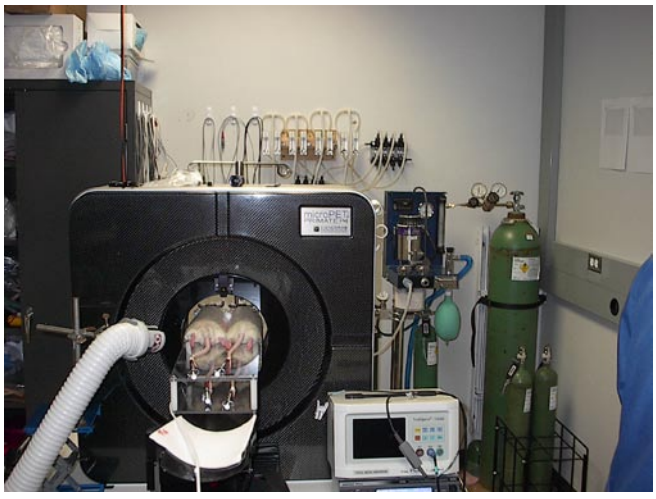


Figure 5. This picture shows the current set up of our client's PET imaging machine. Four rats are placed into the machine, two rats stacked on top of the other two. The pulse oximeter out client is currently using to monitor heart rate and SpO_2 levels is to the right of the PET machine.

record and display of SpO_2 levels, heart rates, and body temperatures of four rats simultaneously during PET imaging experiments in order to maintain appropriate anesthesia dosages on each of the four rats independently. The device will need to be able to, simultaneously on four rats, accurately detect heart rates up to 500 bpm and blood oxygen saturation levels with an accuracy of ±2% so that the anesthesiologist is able to determine the adequate dosage of isoflourine to keep the rats anesthetized. In addition to monitoring the heart rate and SpO_2 levels, our client would also like us to design the device to be able to monitor and record rectal temperature (93-100° F) and respiratory rate (around 20 breaths/min). If the device is able to effectively monitor the rats at the set specifications (up to 500 bpm, ±2% SpO_2, 93-100° F, and detect breathing at around 20 breaths/min), we hope to implement a computer controller to automate the anesthesia delivery system.

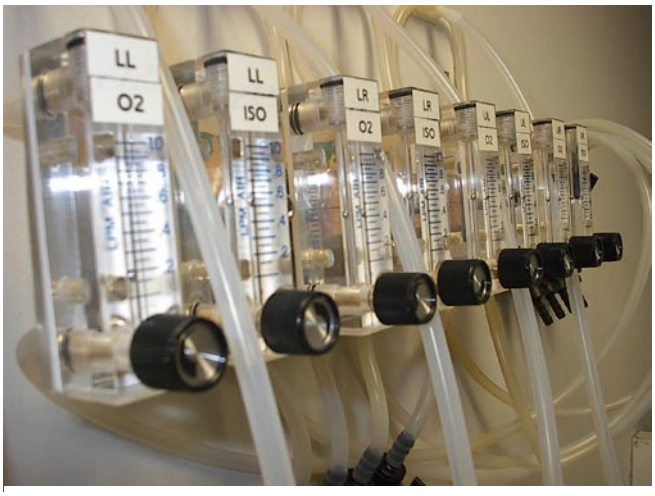


Figure 6. Isoflurane delivery system. Knobs are manually adjusted by an operator to mix appropriate amounts of isoflurane and oxygen in administering the proper concentration of anesthesia.

Last Semester's Idea

An idea we considered was reverse engineering the cheaper parts of the system and replacing the more expensive components. We would begin with a human oximeter sensor. We would adapt this so that it would be able to fit on a rat paw. We would also take apart the cord and find out what signal is being sent in each wire so that we use those voltages as inputs. We would take the voltages and feed them into a data acquisition box,

which would send that data to a LabVIEW program where they would be analyzed and displayed through a LV GUI.

In place of that thousands of dollar box, we will get a simple DaQ and use LabVIEW to process the signals. This cost is well within our budget. The construction is not much more complicated either, but will involve redoing the actual sensor to fit with a rat. This will take a lot more planning and reverse engineering of the output signals. This idea can easily incorporate additional rats or other measurement devices by simply plugging their inputs into our DaQ.

Final Design of Last Semester

LabVIEW

In our revised final design the sensor outputs are fed through the circuits described above and are then recorded using a data acquisition box which sends the analog channels voltage data to LabVIEW. The first step in the LabVIEW coding is to reset the DaQ device and record the initial time in an array. Once the DaQ has been reset it can start recording data. Currently, the data is recorded in two different ways. First, the data is recorded as a waveform and this data is easy to graph and is therefore used to display the information on the front panel. We would like to be able to use this form of data for all the needed calculations, but it is not being stored properly. Right now, the waveform is only holding a single point of data in it. Therefore, it is not possible to see if the data is increasing, decreasing, or if it has just passed a certain threshold. Next semester we hope to find out why the data is being transferred in the way you would expect.

The second way the data is recorded is as a single point. This single point is added to an array of all the previous points. The program then is then told how many seconds to use for a moving average (this is currently manual, but will be internal to the program after some testing for best results). It then adds up all the points in the array that correspond to the designated amount of time and divides by how many points there are. In this way it computes a moving average.

In the next step, the program checks to see if the voltage has just surpassed this average. The most current value of the array is compared to the moving average. If the current value is above the moving average and the data point directly before the current point is below the moving average the data has just spiked above average. The program then records exactly what time this event occurred at.

Whenever a new time point is added a new bpm is computed. This time the program is told to count back a certain number of pulses—that is currently inputted manually (that will be internalized after more testing)--and takes all of these pulses and measures the time between each one. These data are added together and divided to find the average length of time between pulses. This number is in milliseconds, which is then converted to a bpm with simple arithmetic. This heart rate is then displayed on the front panel.

Circuit Design

Because of the use of light in the absorbance measurement, we need a "light-to-voltage" conversion using current as the input signal. The classes of photodiode amplifiers suitable for pulse oximetry applications are the classical resistor-feedback transimpedance amplifier and the capacitor-feedback switched integrator. This circuit is

also common in use with piezoelectric sensor, which also gives out a small current with respond to stimulus. This is the first stage of our circuit (Figure 7).

In the pulse oximeter case, the light shining on a photodiode produces a small current that runs to the amplifier-summing junction and through the feedback resistor. Given the very large feedback resistor value we used (4.7 MΩ), this circuit is extremely sensitive to changes in light intensity. A small capacitor (390 pF) is used to control gain peaking caused by the diode capacitance.

The second stage of the circuit is a differential amplifier, which is used to filter out the common noise from the input. The third stage is a non-inverting amplifier with a gain of 22 so the output signal can be observed on the oscilloscope.

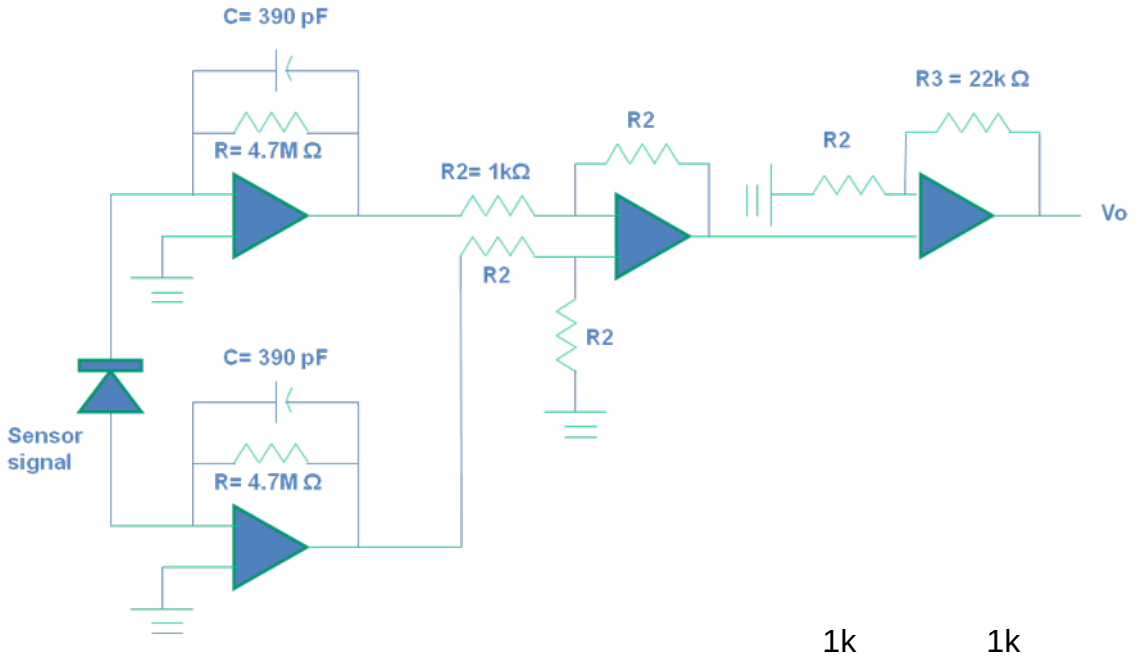


Figure 7. Circuit diagram for the pulse oximeter.

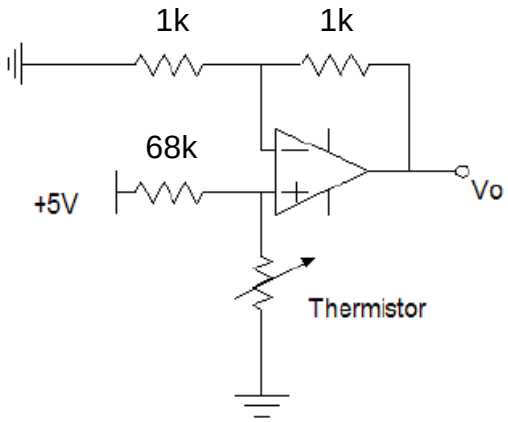


Figure 8. Circuit diagram for the thermistor.

For the thermistor circuit (Figure 8), we used the thermistors from digital human thermometers purchased from Walgreen's (which is what our client currently uses) and use a non-inverting amplifier to get the signal. We place the thermistor at the positive input at the op-amp. When the temperature causes resistance to change, the voltage across the thermistor changes.

Current Semester's Work

Signal imported to LabVIEW

The biggest short coming of last semester was that we were unable to aquire the output signal of our pulse oximeter sensor and import it into LabVIEW. After adjusting numerous settings on both the DAq box and LabVIEW itself, we were finally able to import the signal into LabVIEW and do some signal processing.

Breathing Rate

This semester we designed for the ability of our device to monitor breathing rate. Initially we came up with three different methods on how we could acquire a signal corresponding to breathing rate.

Force Sensing Resistor

Our third design, the design we finally chose, is using a force sensing resistor (Figure 9) to measure the force exerted to the sensor when the rat breaths. In this case, the resistor is placed right beneath the abdomen of the rat. When the abdomen expands, it exerts force to the resistor and the resistance changes. Applying a simple voltage divider would allow us to get the fluctuation in resistance in terms of voltage. From the pattern, we can then calculate the breathing rate. The advantage for this design is a simple circuit and that the resistor is always fixed in place (beneath the abdomen), as well as being relatively cheap.

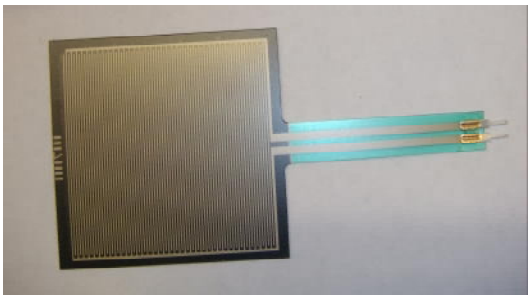


Figure 9. Force Sensing Resistor (FSR)

Once the sensor had been built, we wrote a LabVIEW (LV) program that was designed to calculate the breathing rate. As a first attempt, we took a length of time (10

sec during first testing) and calculated the average voltage during that time frame. Once the LV program knew the average it compared the incoming raw data to that and was alerted when one point was below the average and the data point before was above the average. When this occured the program recorded the time to an array (time was actually taken immediately after the data was first recorded).

To calculate the breaths per minute the program took the last eleven time points and found the total time between those points. Then, that time was divided in ten, so as to represent the average time between breaths over the last ten breath intervals. This time was manipulated to account for the change from milliseconds to minutes and was then displayed to the user.

Heart Rate and SpO_2 with Red and Infrared Waveforms

We need to have two waveforms, one Red and one infrared, to calculate the SpO_2. Usually in a commercial unit, the red LED and IR LED turns on and off alternatively, with timing circuit built in, so we can get two waveforms and find the ratio between the peak and the baseline and then compare it to a table and get the SpO_2 value. However, originally, we only got one waveform, and that was not sufficient to find SpO_2. As we have been solely using positive DC as our LED source, we guessed that if reversed the polarity and a negative DC source was sent in, we could turn on the infrared LED. It had been tested that when a negative DC signal was feed to the LED, we still got a very nice and clean signal just as when we fed in a positive signal, only a difference in magnitude, which proved our assumption was right.

To find SpO_2, we need to determine when the red LED is on and measure it. Alternatively, determine when IR is on and measure that, and take corresponding readings. That is why the signal polarity frequency needs to be carefully considered. But since our output is still from one channel (having only one photodiode), we have to split it into two sets of data, correspond to the polarity of the input signal. This might be done

in LabVIEW. The timing pattern was suggested 480 Hz in Prof. Webster's book (Webster, 1997).

The heart rate of human is around 1 Hz. Assuming that the heart rate is at 1 Hz and the timing pattern is 2Hz. Then for every second, the first 0.5 sec is under RED and second 0.5 sec is under IR. What this means is that the RED data we get is always the first half part of the heartbeat, and IR LED always shines during the second half. Since the highest value of the waveform only appears in the first part and the lowest value only appears during the 2nd part, we never get the appropriate Rhigh / Rlow ratio or IRhigh/ IR low ratio. So a high timing pattern might be the key to get an accurate result.

Rats have a heart rate of around 300-400bpm. Which means the frequency is around 6-7 Hz. Assume it is 6 Hz, which was 6 times higher than human. If the timing pattern is already 480 Hz for human, then we believe that for a rat, the pattern should be much higher in order to capture the right data point. That might be the reason our client is getting a fluctuating and unrealistic low SPO2 value, because the rate is not high enough to capture the correct data point.

Due to the complexity of being able to acquire both waveforms and process them to get the blood oxygen saturation along with the timeframe of this project, the priority has been set to complete the other components of our device to be fully functioning before we move onto acquiring the SpO_2 levels.

Testing

Temperature

The design of the thermistor was tested using a thermometer as a reference. Based on the specification (accurately measuring a temperature range between 32-42 °C), the thermistor and the thermometer were placed in water. The water was then heated to above 45°C and slowly cooled to 30°F. During this process, an analog voltage signal was recorded with measurements from the thermometer. This procedure was repeated twice to

ensure accuracy of the thermistor. Data was plotted measuring the analog voltage output versus temperature (Figure 10). A linear function was chosen to calculate the relationship between output voltage and temperature because the thermistor displayed linear performance through this temperature region. The first trial showed an R square value of 0.9983, while the second trial yielded an R square value of 0.9938. Both R square values are quite high, and repetition of the procedure further improved the R square value (showing increased skill in performing this procedure).

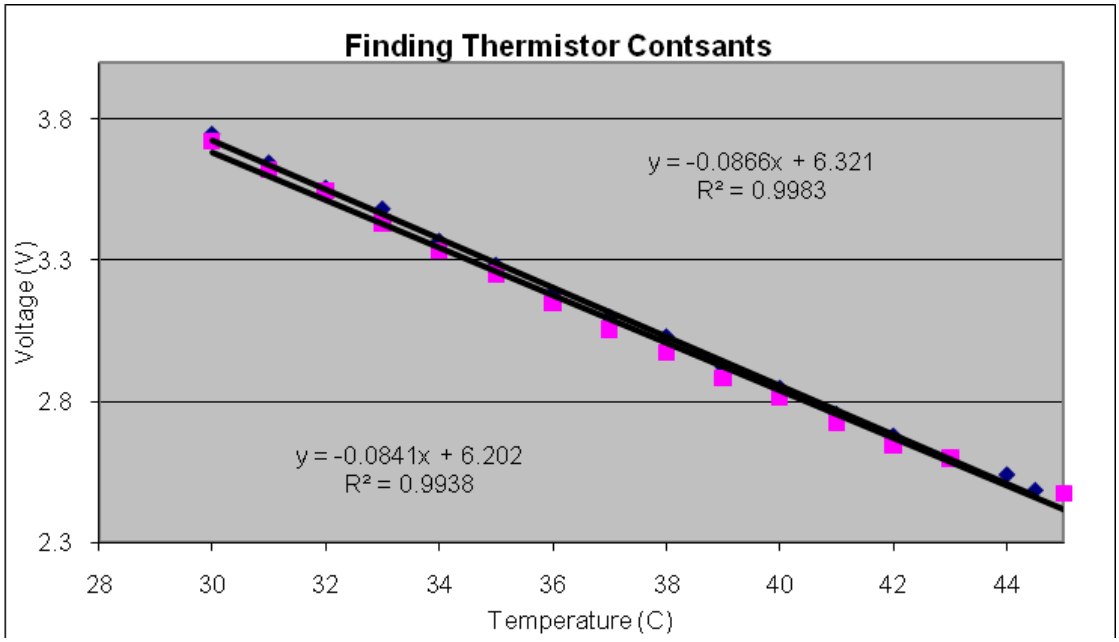


Figure 10. Voltage vs. Temperature. After testing of the thermistor, we found that within our design specification range (~32-42 ˚C) the correlation between temperature and output voltage was very near linear with a R^2 greater than 0.99.

After several tests on rats, our device showed the ability to effectively monitor the temperatures of all four rats simultaneously, as well as store the data digitally on the computer operating LabVIEW.

Breathing Rate

During testing with the LV program described in the design section, the program worked well only for exceptionally clean data (Fig. 11). Some data looked clean, but because the average favored the baseline it would occasionally be set off by noise (Fig. 12). It was clear that a better method of peak detection would be needed to calculate breaths per minute for average and not just exceptionally good data. Our first idea was to use the same block of previous data, and simply find the minimum and maximum data points. This method favors outliers and so puts the comparison line towards the middle of the waveform and can easily detect breaths for good (Fig. 11) and average (Fig. 12) data.

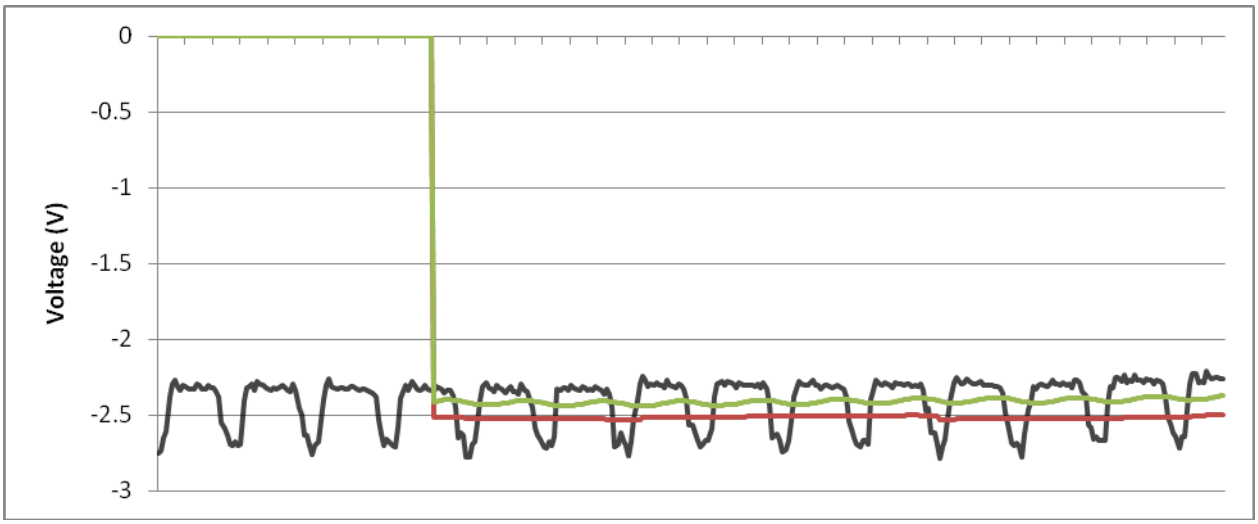


Figure 11. This is exceptionally clean data we acquired after some practice at placing the FSR under the rats. The green line shows the average from a specified amount of time and the red line is the min/max from that same time period. Both of these methods work well for this graph.

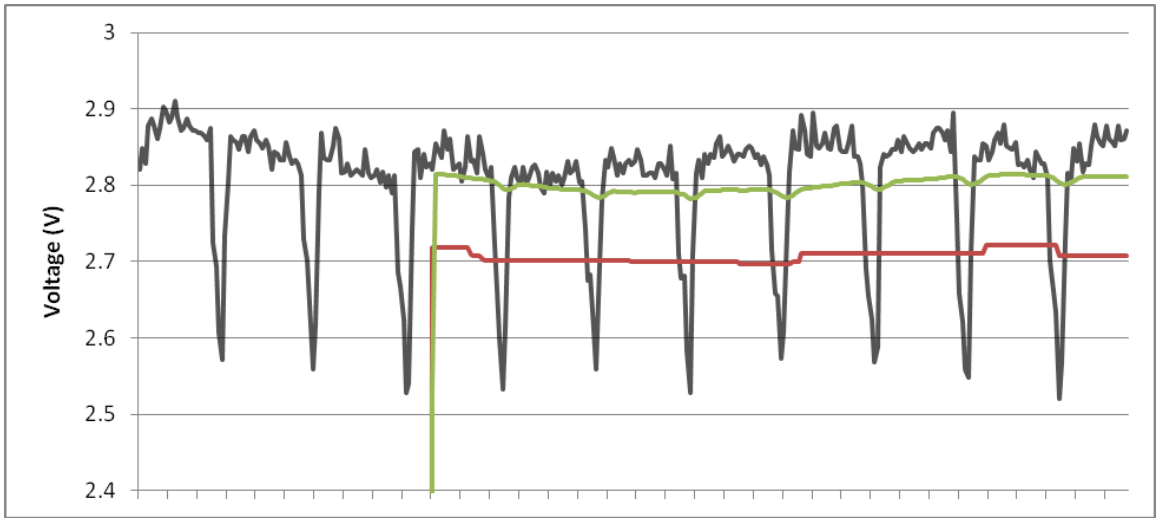


Figure 12. This is some average looking data from our testing. The green line shows a long moving average. That method for breath detection favors the baseline and so some of the noise on the baseline sets off the computer that a breath is taking place. The red line shows a min/max average of the data, this proves to be a much more effective method of breath detection on this data.

However, neither method works for bad, noisy data. Figure 13 shows our first attempt at trying to place our device under a rat and get the breathing rate. If the researcher knows where to place the FSR they should never get data as messy as this, but you shouldn't have to be familiar with our device to be able to use it the first time. Our method to detect breaths mathematically in this mess was to first calculate a shorter moving average which works as a filters to smooth out the noise and react to large peaks. This moving average was compared to another average that takes ten times the data points, this second average works as a DC offset. Figure 14 displays the raw data in the background with the DC offset average v. the filtering average. Using this method will greatly improve our devices ability to detect peaks in even the noisiest of waveforms.

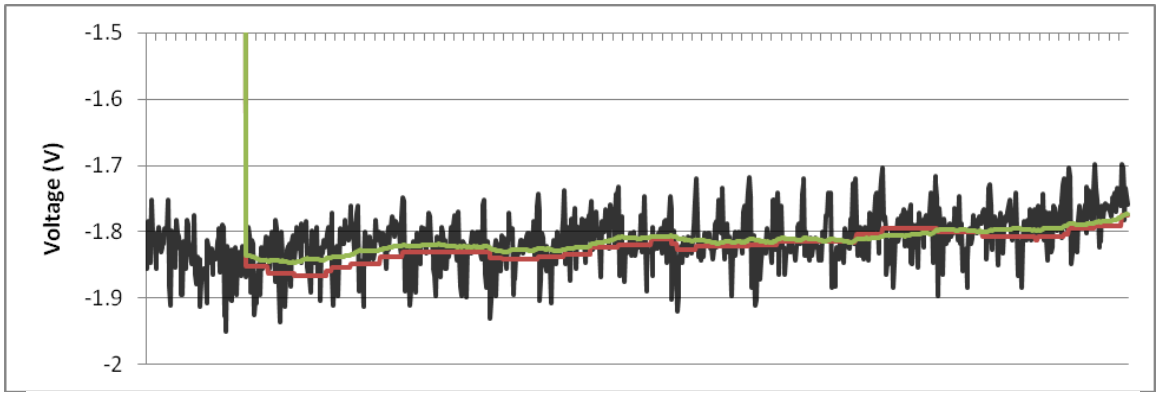


Figure 13. The first data we took using the FSR. The sensor was placed in a very bad spot and was being handled at the same time. Both the long average and the min/max comparison lines would not effectively measure the breathing rate.

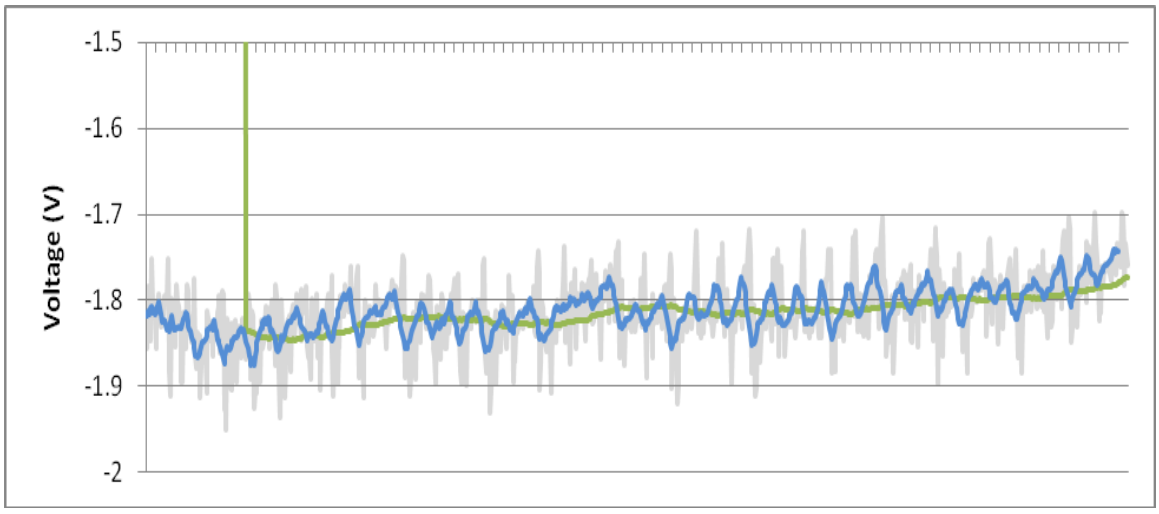


Figure 14. The raw data from a very noisy and misplaced trial is displayed in the background. The blue line is the short average (filtering average) and the green line is the long average (offset). Comparing the blue and green lines will get a reasonably accurate breaths per minute with even the worst data.

Pulse Oximeter

The modified pulse oximeter with the circuit discussed in the final design demonstrated a clear output analog signal (Figure 15). Each heart beat created two peaks (first a larger peak followed by a smaller peak), and the signal was successfully compared by placing existing pulse oximeter on the same finger on the opposite hand. While we were able to obtain a signal from a human, we were unable to obtain any workable signal

from a rat. Through testing we discovered that the signal was too small in comparison to the noise. The small signal is due to there being much less blood being pumped through paw of the rat than compared to the blood pumped through the finger of a human during each pulse. Since the signal is so much smaller, the signal to noise ratio increases dramatically. We tried to modify the pulse ox circuit to try to get a better signal. The anode from the probe is forced to ground and then the signal from the cathode is fed to a series of circuit. The first stage is a voltage follower to ensure the signal is not affected by additional resistance. The second stage is a non inverting amplifier, with varying gain using a potentiometer. The third stage is filtering. We chose a low pass four pole Butterworth filter with a cutoff frequency of 20 Hz. Corner frequency of 10 Hz, 15 Hz were tested but the amplitude of the waveform is lower and it is more harder to distinguish the pulse (See Appendix for reference to filtering data). Even though the signal is filtered, there still is a lot of noise that makes a pulse hard to distinguish. The signal is analyzed with frequency analyzer and there is a peak at 60 Hz even after filtering, signifying that most of the signal is still noise. So despite developing filters using active and cascading low-pass filters, we were

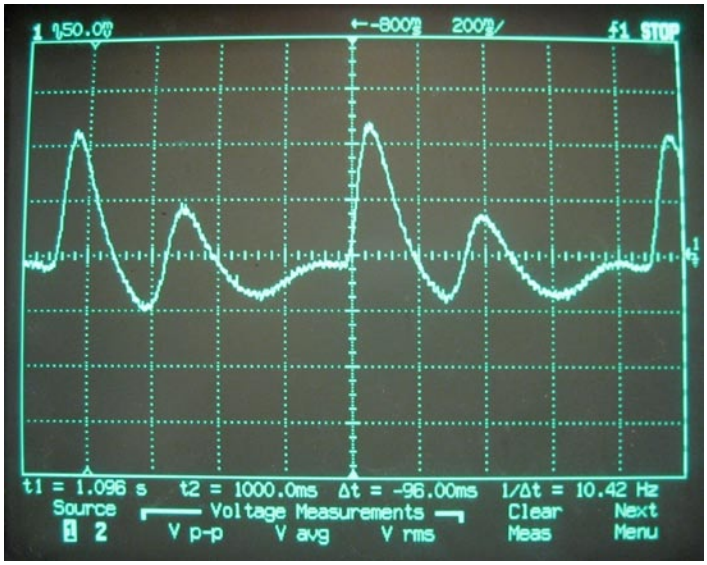


Figure 15. Oscilloscope screen shot. Shows the clean signal obtained from our pulse oximeter circuit. We believe the two peaks are red and infrared transmission of each pulse.

still unable to filter out enough of the noise in order to obtain a workable signal. In consequence, software/digital filtering may be the solution to this problem.

Conclusion

For the final integration of all the sensing components, a printed circuit board was purchased to condense the circuitry and minimize the size of the device (see Appendix for schematic and picture). Four thermistor circuits and four FSR circuits were constructed and connected to the DAq box, in which the signal was then sent to LabVIEW on the laptop computer.

A completed fully functioning prototype for temperature and breathing rate has been tested on the rats and the results show that the device is capable of accurately monitoring the vitals. A GUI has been made that also incorporates heart rate and plethysmograph display for future completion of the pulse ox sensor (Figure 17).

While we were unable to finish the development of the heart rate monitor, our device allows our client to now monitor the temperatures of all four rats without having to probe the rats one by one, as well as store the recorded data digitally. Furthermore, it allows him to monitor the breathing rats of the rats, something that he has never been able to do before.

Another team has expressed interest in finishing the development of the heart rate monitor as well as further developing it into a fully functioning pulse oximeter over the coming summer. Apart from finishing the monitoring capabilities of the device, we hope that in the future our device may be integrated with a computer program that would be capable of automating the anesthesia system as well, removing the need for constant human observation.

If there are further inquiries or comments, please contact us at *RatMonitor@gmail.com*.

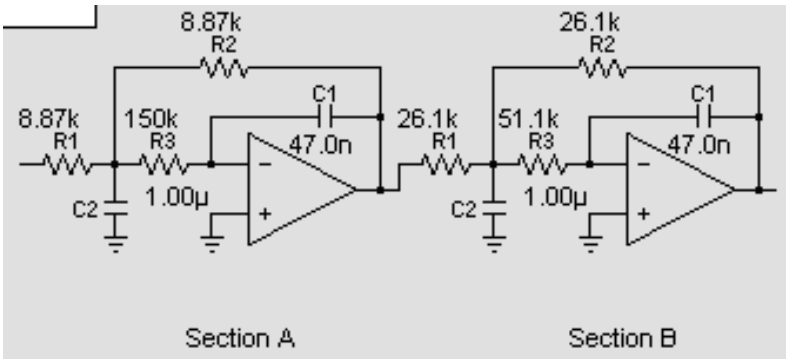
Figure 17. Design of user interface. Graphs are used to display the plethysmographic waveforms of each rat. Heart rate, breathing rate, and temperature will be shown numerically underneath. Warning lights will also be placed underneath alerting the user if a rat has fallen into dangerous conditions.

Acknowledgement

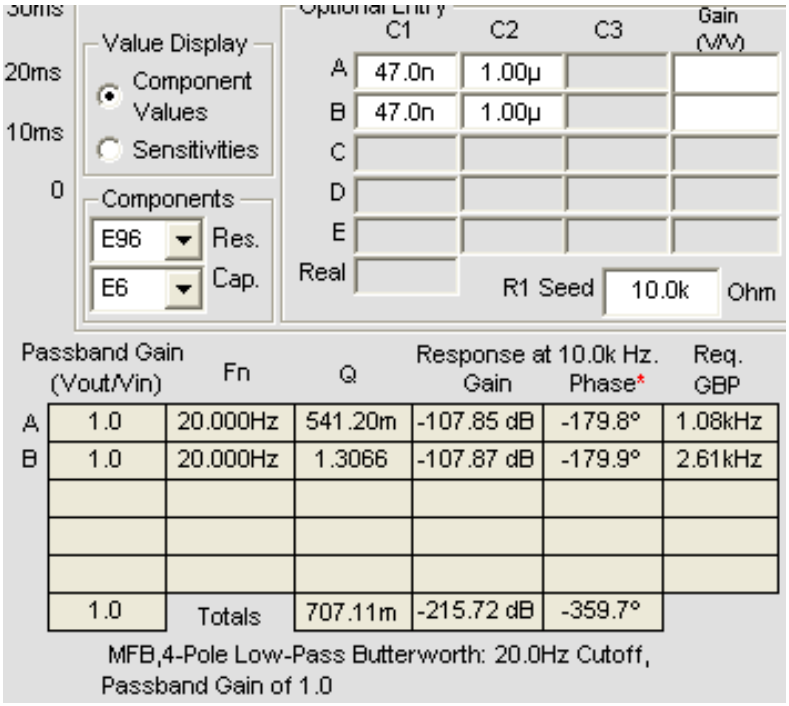
Thank you to all of those who contributed, especially Professor Thomas Yen, PhD and Client Alex Converse, PhD for their support and assistance; Amit Nimunkar, Jon Baran, and Chris Esser for their help in circuit design; Liz Ahlers and Jeff Moirano with their assistance and set-up of rat testing; and we would also like to thank Sharing

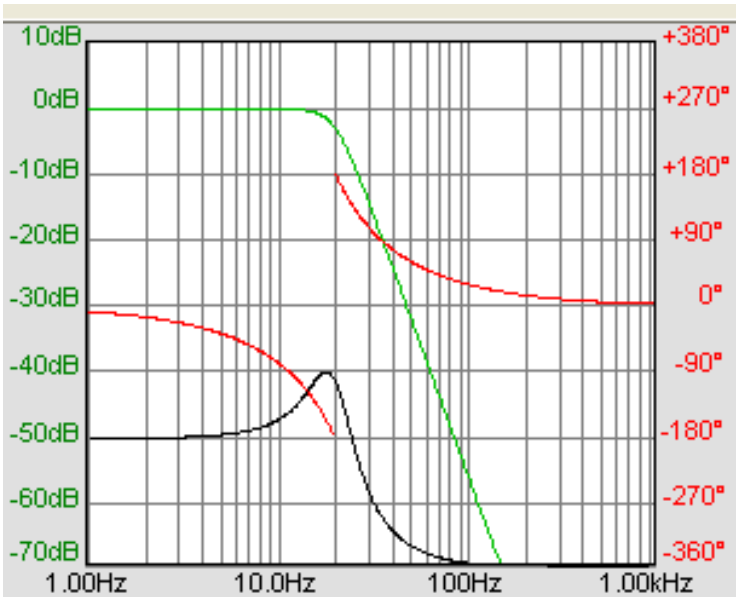
Resources Worldwide for providing us with sensors and equipment, without them this project would not have been possible.

Appendix

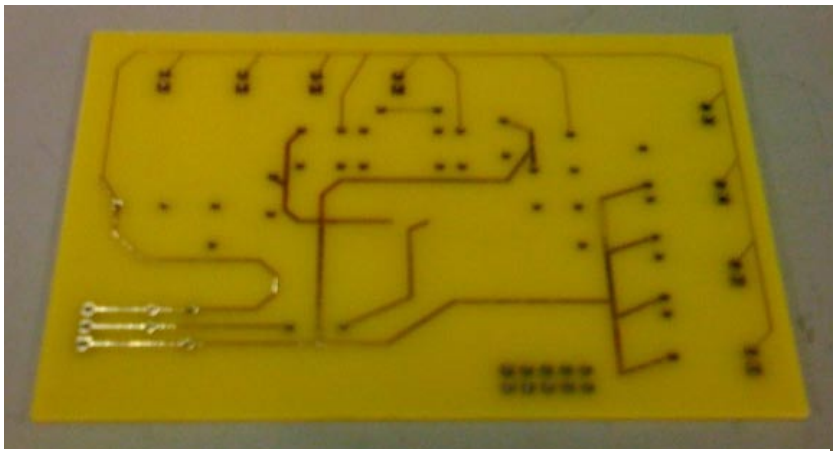


Circuit layout of the 4 pole low-pass filter (stage 3) and corresponding values

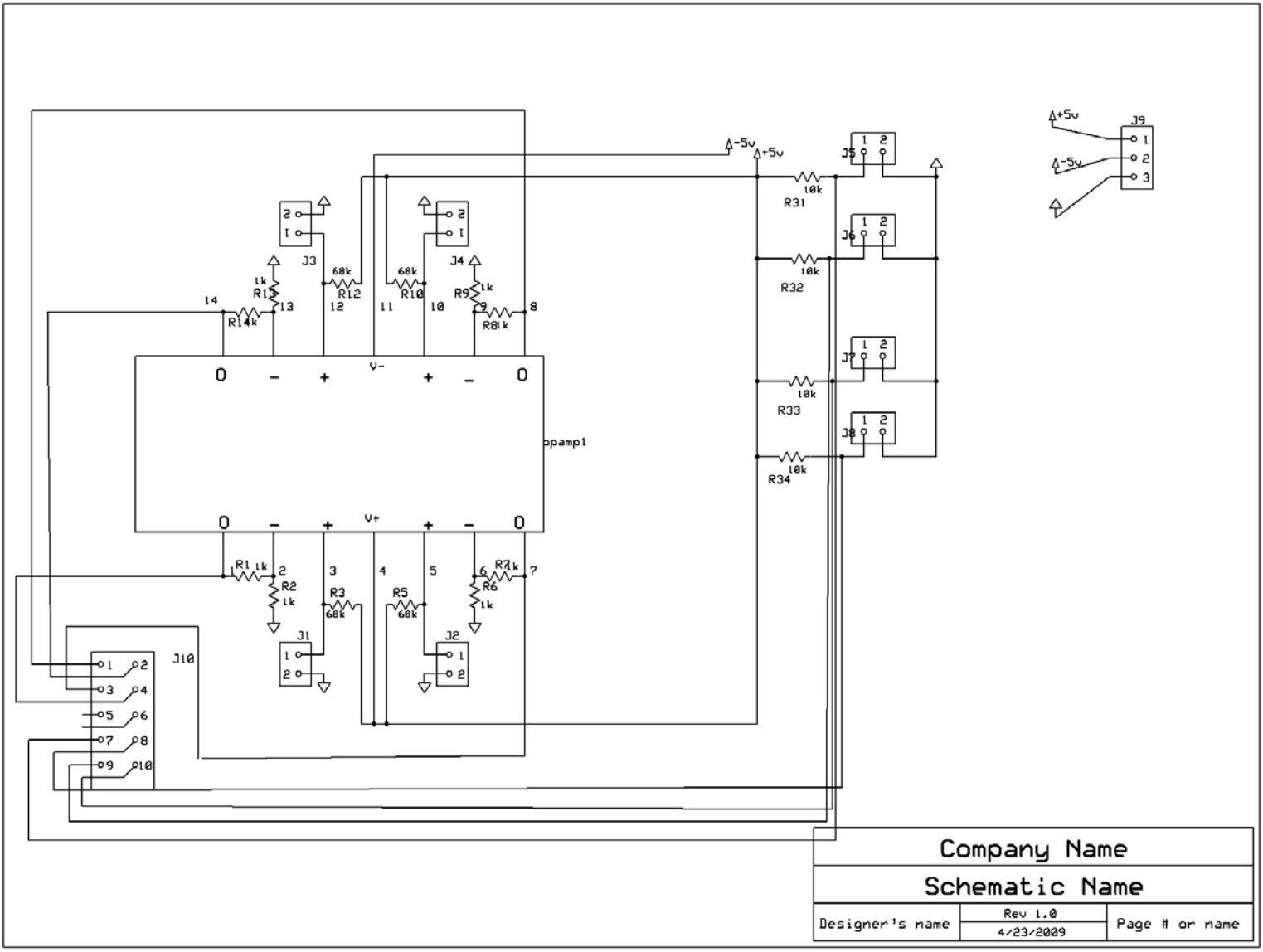




Amplitude response (green)
Phase response (red) of the filter



Actual printed circuit board.



Printed circuit board schematic for the PCB board containing both thermometer and FSR circuit

References

Ford D., D. Nachreiner, R. Thomas (2005), “Design of a Pulse Oximeter for Use in Mice”.

K. Yamakoshi and Y. Yamakoshi (2006). Pulse glucometry: a new approach for noninvasive blood glucose measurement using instantaneous differential near-infrared spectrophotometry. *Biomed. Opt*., Vol. **11**, 054028 ; DOI:10.1117/1.2360919

Starr Life Sciences (2008). *MouseOx*. From http://starrlifesciences.com.

Webster, J. G. (1997), Design of Pulse Oximeters. IOP Publishing Ltd.