

Arizona State University Criteria Checklist for

NATURAL SCIENCES [SQ/SG]

Rationale and Objectives

In a relatively short time in the history of civilized societies, humankind moved from what was essentially an agrarian population into an industrial age, which in recent years has been profoundly shaped by such scientific and technological advances as genetic engineering, the computer, and space exploration. Our history of irrepressible ingenuity makes a compelling case for a future that will be even more profoundly influenced by science and technology. It is imperative that we react expeditiously and effectively to the problems and the promise that these advances create. We must ensure that technological change is directed to the benefit of society and that it will promote human dignity and values. Success in achieving this goal will depend upon the insight and knowledge of political and public opinion leaders, and the scientific enlightenment of educated citizens. To a significant degree, the ability of these individuals to understand the nature of the issues and the alternative courses of action will be determined by the quality of science presented at the nation's institutions of higher learning.

The recommendation of at least one laboratory course that includes a substantial introduction to the fundamental behavior of matter and energy in physical or biological systems derives from a number of considerations. First, all physical and biological phenomena have at their roots the fundamental principles governing the behavior of matter and energy. These principles have been shown over a period of time to be a value in reliably predicting and rationalizing a broad range of phenomena. Unless the lines to these roots are established, our understanding of the broader range of the sciences, and other fields upon which these sciences impinge, will be impaired. Second, because these fundamental principles have been experimentally established beyond reasonable doubt, the essentials of the scientific method can be clearly and coherently revealed by their study. Third, the study of the behavior of matter and energy illustrates the usefulness of mathematics in precisely describing and rationalizing certain physical phenomena, and the expressiveness of mathematical equation.

10/1989

REV: 1/1991, 3/1991, 1/2000, 10/2008

Proposer: Please complete the following sections and attach appropriate documentation.

ASU--[SQ] CRITERIA			
I. - FOR ALL <i>QUANTITATIVE</i> [SQ] NATURAL SCIENCES CORE AREA COURSES, THE FOLLOWING ARE CRITICAL CRITERIA AND MUST BE MET:			
YES	NO		Identify Documentation Submitted
<input checked="" type="checkbox"/>	<input type="checkbox"/>	A. Course emphasizes the mastery of basic scientific principles and concepts.	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	B. Addresses knowledge of scientific method.	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	C. Includes coverage of the methods of scientific inquiry that characterize the particular discipline.	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	D. Addresses potential for uncertainty in scientific inquiry.	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	E. Illustrates the usefulness of mathematics in scientific description and reasoning.	syllabus, lab syllabus, text TOC, cardiovascular and respiratory lab protocols
<input checked="" type="checkbox"/>	<input type="checkbox"/>	F. Includes weekly laboratory and/or field sessions that provide hands-on exposure to scientific phenomena and methodology in the discipline, and enhance the learning of course material.	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	G. Students submit written reports of laboratory experiments for constructive evaluation by the instructor.	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	H. Course is general or introductory in nature, ordinarily at lower-division level; not a course with great depth or specificity.	syllabus, lab syllabus, text TOC
II. - AT LEAST ONE OF THE FOLLOWING ADDITIONAL CRITERIA MUST BE MET WITHIN THE CONTEXT OF THE COURSE:			
<input checked="" type="checkbox"/>	<input type="checkbox"/>	A. Stresses understanding of the nature of basic scientific issues.	syllabus, lab syllabus, text TOC

☒	☐	B. Develops appreciation of the scope and reality of limitations in scientific capabilities.	syllabus, lab syllabus, text TOC, lab protocols
☒	☐	C. Discusses costs (time, human, financial) and risks of scientific inquiry.	syllabus, lab syllabus, text TOC
NOTE: CRITERIA FOR [SG] COURSES BEGIN ON PAGE 4.			

III. - [SQ] COURSES MUST ALSO MEET THESE ADDITIONAL CRITERIA:

YES	NO		Identify Documentation Submitted
<input checked="" type="checkbox"/>	<input type="checkbox"/>	A. Provides a substantial, quantitative introduction to fundamental principles governing behavior of matter and energy, in physical or biological systems.	syllabus, lab syllabus, text TOC
		B. Includes a college-level treatment of some of the following topics (check all that apply below):	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	a. Atomic and molecular structure	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	b. Electrical processes	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	c. Chemical processes	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	d. Elementary thermodynamics	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	e. Electromagnetics	syllabus, lab syllabus, text TOC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	f. Dynamics and mechanics	syllabus, lab syllabus, text TOC
[SQ] REQUIREMENTS CANNOT BE MET BY COURSES:			
<ul style="list-style-type: none"> • Presenting a qualitative survey of a discipline. • Focusing on the impact of science on social, economic, or environmental issues. • Focusing on a specific or limiting but in-depth theme suitable for upper-division majors. 			

Proposer: Please complete the following section and attach appropriate documentation.

ASU--[SG] CRITERIA			
I. - FOR ALL <i>GENERAL</i> [SG] NATURAL SCIENCES CORE AREA COURSES, THE FOLLOWING ARE CRITICAL CRITERIA AND MUST BE MET:			
YES	NO		Identify Documentation Submitted
<input type="checkbox"/>	<input type="checkbox"/>	1. Course emphasizes the mastery of basic scientific principles and concepts.	
<input type="checkbox"/>	<input type="checkbox"/>	2. Addresses knowledge of scientific method.	
<input type="checkbox"/>	<input type="checkbox"/>	3. Includes coverage of the methods of scientific inquiry that characterize the particular discipline.	
<input type="checkbox"/>	<input type="checkbox"/>	4. Addresses potential for uncertainty in scientific inquiry.	
<input type="checkbox"/>	<input type="checkbox"/>	5. Illustrates the usefulness of mathematics in scientific description and reasoning.	
<input type="checkbox"/>	<input type="checkbox"/>	6. Includes weekly laboratory and/or field sessions that provide hands-on exposure to scientific phenomena and methodology in the discipline, and enhance the learning of course material.	
<input type="checkbox"/>	<input type="checkbox"/>	7. Students submit written reports of laboratory experiments for constructive evaluation by the instructor.	
<input type="checkbox"/>	<input type="checkbox"/>	8. Course is general or introductory in nature, ordinarily at lower-division level; not a course with great depth or specificity.	
II. - AT LEAST ONE OF THE ADDITIONAL CRITERIA THAT MUST BE MET WITHIN THE CONTEXT OF THE COURSE:			
<input type="checkbox"/>	<input type="checkbox"/>	A. Stresses understanding of the nature of basic scientific issues.	
<input type="checkbox"/>	<input type="checkbox"/>	B. Develops appreciation of the scope and reality of limitations in scientific capabilities.	
<input type="checkbox"/>	<input type="checkbox"/>	C. Discusses costs (time, human, financial) and risks of scientific inquiry.	

[SG] REQUIREMENTS CANNOT BE MET BY COURSES:

- | | | |
|--|---|--|
| | <ul style="list-style-type: none">• Presenting a qualitative survey of a discipline. | |
| | <ul style="list-style-type: none">• Focusing on the impact of science on social, economic, or environmental issues. | |
| | <ul style="list-style-type: none">• Focusing on a specific or limiting but in-depth theme suitable for upper-division majors. | |

Course Prefix	Number	Title	Designation
BIO	202	Human Anatomy & Physiology II	SQ

Explain in detail which student activities correspond to the **specific** designation criteria. Please use the following organizer to explain how the criteria are being met.

Criteria (from checksheet)	How course meets spirit (contextualize specific examples in next column)	Please provide detailed evidence of how course meets criteria (i.e., where in syllabus)
E. Illustrates the usefulness of mathematics in scientific reasoning and understanding	The course incorporates a number of physiological experiments in which students collect and analyze data. Some data must be used in mathematical equations and students must graph their data	The attached Cardiovascular Physiology Lab Protocol utilizes mathematical equations to transform data that is collected by the students. Students are required to graph their data and write detailed lab reports.
B. Develops appreciation of the scope and reality of limitations in scientific capabilities.	The course incorporates a number of physiological experiments in which students collect and analyze data.	All of the attached lab protocols require students to perform various elements of scientific experimentation. Students must make predictions, form hypotheses, collect and analyze data, and form conclusions. Students will experience the difficulty of designing good experiments with testable hypotheses.

Bio 202: Human Anatomy & Physiology II Cardiovascular Physiology Protocol

I. Lab Goals

1. Continue to develop scientific reasoning skills by:
 - a) Designing & conducting your own experiment
 - b) Analyzing & presenting your data
2. Improve your understanding of cardiovascular physiology concepts
3. Learn how to measure new physiological variables
4. Learn how to conduct & analyze an electrocardiogram

II. Introduction

During exercise skeletal muscles require additional energy in the form of ATP. At sustainable levels of exercise, this increased production of ATP in skeletal muscles is accomplished aerobically. In other words, during sustainable exercise, oxygen is used in the catabolism (breakdown) of fuels to produce ATP. Thus, the cardiovascular system has to deliver more oxygen-rich blood to the muscles for aerobic catabolism to continue during exercise. One mechanism that could be used to meet the muscle demand for oxygenated blood is an increase in cardiac output. Recall that cardiac output (CO) is the volume of blood ejected from the left ventricle to the aorta in 1 minute.

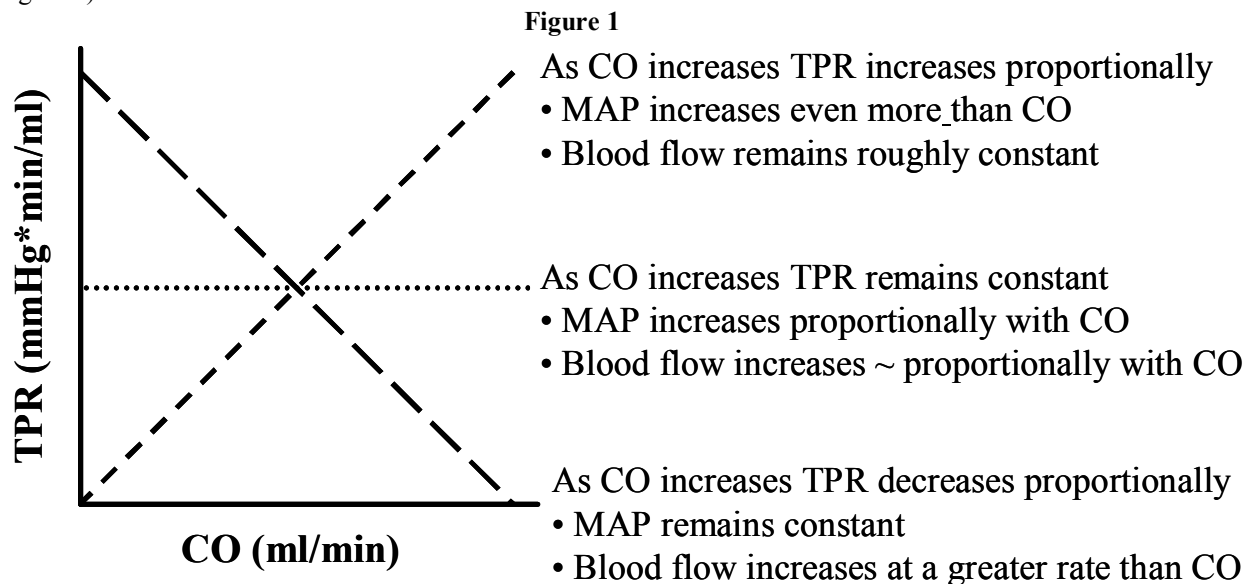
$$\text{Cardiac Output (ml/min)} = \text{Stroke Volume (ml/beat)} \times \text{Heart Rate (beats/min)}$$

Therefore, cardiac output could be increased through increases in either heart rate and/or stroke volume.

However, after the blood leaves the heart it still has to travel through the blood vessels to get to the muscles. The resistance of the vessels along the way will contribute to determining what the actual blood flow is when it reaches the muscle. Unfortunately we can't really measure blood flow (e.g. ml/min) at the muscle arteries, but measuring blood pressure (mmHg) gives us some insight into blood flow, however imperfect (see Figure 1). Recall that blood pressure is the hydrostatic pressure exerted by blood on the walls of a blood vessel. Blood pressure depends upon both the rate of blood flow coming from the heart and the width or resistance of the artery walls. Specifically, mean arterial blood pressure (MAP: the average blood pressure in arteries throughout the cardiac cycle) is a function of both cardiac output and total peripheral resistance (TPR: resistance to blood flow in entire systemic circulation).

$$\text{MAP (mmHg)} = \text{CO (ml/min)} \times \text{TPR (mmHg*min/ml)}$$

Therefore, whether MAP and/or blood flow will increase or not when CO increases depends on TPR (see Figure 1).



III. Scientific Method

In this lab, the scientific method has already been started for you. You will design & carry out an experiment to test the hypothesis below.

1) Puzzling Observation: Yesterday I ran on an elliptical machine in the SRC, and I noticed the machine reported that my heart rate increased while I ran.

Known Fact: I know that exercise also increases stroke volume, and Cardiac Output (ml/min) = Stroke Volume (ml/beat) x Heart Rate (beats/min). So, not only my heart rate, but my whole Cardiac Output (CO) must have increased while I ran.

2) Causal Question: Why did running make my CO increase?

Known Fact: Sustainable exercise increases muscles' demand for oxygen. More oxygen *can in theory* be supplied to muscles by increasing the rate at which oxygen-rich blood flows to the muscles.

3) Hypothesis: Perhaps my CO increased while I ran because during sustainable exercise increasing CO causes blood flow to the muscles to increase.

Known Facts: After CO increases, the blood still has to travel through the vessels to reach the muscles. So, I can't tell if increasing CO increases blood flow to the muscles unless I also look at resistance. I can measure resistance via TPR. However, I can not directly measure blood flow to the muscle, so I'll have to use a measure that should correlate roughly with blood flow (MAP). However MAP is a rough estimate of blood flow so I should look at both TPR and MAP as CO increases in order to determine whether blood flow to the muscles is actually increasing.

4) Experiment: Design your own.

Assumption: I know that during exercise muscles need more oxygen, but I *think* that sustainable exercise, such as we are discussing here, will not cause the cardiovascular system to increase blood flow to the max (i.e. at least above Figure 1 'As CO increases TPR decreases proportionally' line). Yet, *I think* blood flow must increase a good deal (i.e. at least below Figure 1 'As CO increases TPR increases proportionally' line). Thus I think the middle line on Figure 1 (As CO increases TPR remains constant) most likely.

5) Prediction: As CO increases during sustainable exercise:

- a) MAP will increase proportionally with CO
- b) TPR will remain constant

IV. Formulas & Calculations

In this lab you will need to use the calculations below to transform data we can collect (i.e. blood pressure & heart rate) into variables of interest.

A. Calculating MAP

- MAP is the average arterial blood pressure throughout the cardiac cycle, but diastole lasts longer than systole. So MAP is NOT the average of systolic and diastolic blood pressures.

$$\text{MAP (mmHg)} = \text{Diastolic pressure (mmHg)} + 1/3 \text{ Pulse pressure (mmHg)}$$

$$\text{Pulse pressure} = \text{Systolic pressure (mmHg)} - \text{Diastolic pressure (mmHg)}$$

B. Calculating Cardiac Output (CO)

- CO is hard to measure directly (unless we have any volunteers for aortic catheterization). However, remember $\text{CO} = \text{SV} \times \text{HR}$. We can easily measure HR (heart rate) directly, and we can make some reasonable assumptions about SV (stroke volume).
- Assume "**normal**" stroke volume is 70 ml. This will be an underestimate for some and an overestimate for others.
- Assume that "**mild exercise**" increases stroke volume by 10% in untrained individuals, and 20% in trained endurance athletes.
- Assume that "**moderate aerobic exercise**" increases stroke volume by 25% in untrained individuals and 50% in trained endurance athletes.

C. Calculating Total Peripheral Resistance (TPR)

- We can not measure TPR directly but, TPR can be calculated from CO & MAP

$$\text{TPR (mmHG*min/ml)} = \text{MAP (mmHG)} / \text{CO (ml/min)}$$

V. Lab Procedures

A. General Information

1. **Safety First.** We will be exercising in this lab, so it is important to control your level of activity so that you do not hurt yourself or others. DO NOT exercise at or near your maximal levels during this lab. Students with medical concerns (e.g. pregnant, high blood pressure, etc...) should restrict or avoid exercise. No student should feel obligated to exercise. The exerciser should always have a safety spotter in case of light-headedness, and if you do exercise vigorously make sure you warm down.
2. **Clothing.** As we will be exercising in lab please make sure you are wearing clothes and shoes that will be comfortable for 10-15 minutes on an exercise bike.

B. Learn To Measure Blood Pressure & Heart Rate (15 minutes)

1. **Blood Pressure Manually.**
 - Practice blowing up & slowly emptying the cuff.
 - a) Turn the silver knob just above the football shaped bulb until it is closed (allows no air out).
 - b) Squeeze the football-shaped bulb repeatedly until the cuff is full.
 - c) Turn the silver knob slowly and with your hand put some pressure on the cuff until the air comes out slowly.
 - Put the EMPTY cuff on a subject.
 - a) Wrap the cuff around the biceps (upper arm) so that the arrow, if there is one, points towards the middle of the anterior side of the elbow joint.
 - b) Place the disc end of the stethoscope on the brachial artery – middle of the anterior side of the elbow. Since the end of the cuff rests here the disc should be approximately half under the cuff
 - c) The subject should be in a seated *stationary* position with their arm almost straight. Movement of the subject will cause errors.
 - Pump up the cuff.
 - a) Put the stethoscope to your ears.
 - b) Place or hold the pressure readout so that you can see it clearly.
 - c) Close the valve with the silver knob and pump the bulb until the pressure is **160-180** mm Hg (**no higher!**). This pressure will stop blood flow to the hand so don't hold cuff at this pressure any longer than necessary. Also, don't record pressure on the same arm more than twice in a half an hour.
 - Determine systolic and diastolic blood pressure.
 - a) Turn the silver knob until air starts to *slowly* flow out of the cuff. You should see the hand on the pressure readout start to slowly come down.
 - b) Note the pressure at which you can first hear the heartbeat. This is the systolic pressure.
 - c) Note the pressure at which you can no longer hear the heartbeat. This is the diastolic pressure.
 - Release the pressure by opening the valve the whole way (rotate the silver knob).
 - Remove the empty cuff from the subject.
2. **Heart Rate Manually**
 - You can not take heart rate manually while the blood pressure cuff is on.
 - Subject should be seated and stationary with palm side of lower arm placed on table.
 - Use your first 2 fingers to find the radial artery. It is on the lateral side of the arm approximately 1 inch superior to the wrist.
 - Use a clock or stopwatch to count the number of beats in 30 seconds.
 - Multiply by 2 and record that number as heart rate (beats/minute)

3. **Heart Rate on LabScribe**
- If you measure blood pressure using the manual cuff you can either take the heart rate manually, as described above, or on LabScribe.
 - To get heart rate from LabScribe set up the equipment, program, and pressure transducer (do not bother with the ECG leads) as described in the ECG section below.
 - Use Marks to Record at least 30 seconds of clean heart rate. I recommend recording a full minute.
 - Then select **View**, then **Analysis**
 - Find the section of data you want to analyze by either using
 - Zoom Out or In using the mountain buttons until you can see the marks you are interested in.
 - Select **View**, then **Marks**, then **Marks** again. A marks dialog box will appear displaying each mark as a row. Select the row of the Mark you wish to go to and click '**Go To Mark**'.
 - **Right click** on the Pulse channel. Select **Scale**, then **AutoScale** to change the y-axis in that channel.
 - Select the '**Double Cursors** button'.
 - At the top left click **Add Function**, then select **General**, then select **Time1**. Repeat the process selecting **Time2**, and **T2-T1** (Time2-Time1). You should now see at the top of each of the 2 channels values for Time1 (the time where the leftmost cursor is located), Time2 (where the 2nd / right cursor is located), T2-T1 (the length of time between the 2 cursors).
 - Move the cursors to select 30 seconds of clean data. The length of data selected can be seen in the T2-T1 column.
 - Count the number of peaks (beats) within your 30 clean seconds and multiply by 2 to get beats/minute.
4. **Blood Pressure & Heart Rate Automatically**
- Practice blowing up & slowly emptying the cuff.
 - a) Hit the button and squeeze the football shaped bulb to make the cuff inflate.
 - b) Hold down the small knob next to the football shaped bulb to release the air.
 - Put the EMPTY cuff on a subject.
 - a) Wrap the cuff around the biceps (upper arm) so that the arrow points towards the middle of the anterior side of the elbow joint (i.e. the brachial artery).
 - b) The subject should be in a seated *stationary* position with their arm almost straight. Movement of the subject will cause errors
 - Pump up the cuff.
 - a) Hit the button on the machine
 - b) Repeatedly squeeze the bulb until the cuff pressure is **160-180 mm Hg (no higher!)**.
 - Determine blood pressures & heart rate.
 - a) The air will slowly leak out of the cuff as the device automatically determines systolic pressure, diastolic pressure, and heart rate.
 - b) When it has finished it will display systolic and diastolic pressure and then heart rate. If the device reports an error immediately use the knob to release the air from the cuff. Make sure your subject did not move and that there were any other factors that caused the error.

C. Learn the Exercise Equipment (15 minutes)

In order to figure out what levels of sustainable aerobic exercise you will want to use in your experiment you will first want to try the exercise. What is mild exercise for one person may be moderate or extreme exercise to another. Also, keep in mind that some students may be restricted in exercise. Exercise bikes will be provided, but you may use other forms of exercise if you wish (e.g. stairs, jumping jacks, etc...) as long as you use them safely. Try the exercise in order to determine what specific levels of speed and duration (and for the bike resistance) produce mild and moderate levels of aerobic exertion in each subject. **For each subject the exercise must be aerobic!!!!** If a

subject exercises too strenuously the muscles will switch to anaerobic metabolism (metabolism that does not use oxygen), and thus the muscles' demand for oxygen will change (i.e. mess up experiment). Note that you will **take measurements (heart rate & blood pressure) as soon as the person finishes exercising, NOT DURING.**

D. Design Your Experiment (10 minutes)

Work with your lab partners to design an experiment that tests the hypothesis above. Make sure fill in all of the blanks on the worksheet including explicitly stating your independent variable, dependent variable(s), and at least 2 controls. Exercise bikes will be provided, but you may use other forms of exercise if you wish (e.g. stairs, jumping jacks, etc...) as long as you use them safely. **Note** that the how strenuous an exercise is (mild or moderate) depends upon the individuals' training.

E. Alternate Predictions (5 minutes)

The prediction given to you in the 'Scientific Method' section above is based on the hypothesis AND an assumption. Is this the only possible prediction based on the given hypothesis? Develop at least one alternate prediction that is based on the given hypothesis. You may make your own alternate assumptions to develop these alternate predictions, or you may not need an assumption.

F. Conduct Your Experiment (75 minutes)

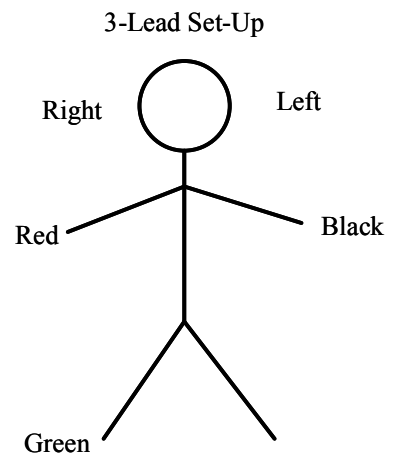
Now that your group has decided what data you want to collect it is time to actually collect the data. REMEMBER that in addition to conducting your experiment you also need to conduct an ECG during lab time today. So, if you are running out of time to perform the experiment two of you may want to break off and conduct the ECG. In order to collect data dividing into two groups of two and rotating through the following positions has proved efficient.

- 1) **Experimental Subject.** This is the person on whom measurements are being taken.
- 2) **Safety Spotter / Pressure & Heart Rate Technician / Secretary:** This person performs all of these jobs at different times.
 - a) Take baseline pressure & heart rate measurements.
 - b) Record the results
 - c) Monitor the experimental subjects exercise (speed & duration). You are responsible for their safety!!!! You may also start on the calculations while the subject is exercising.
 - d) Tell the subject when to stop exercising.
 - e) Immediately take pressure and heart rate measurements.
 - f) Record the results.
 - g) At this point you have to **wait to perform the 2nd level of exercise until the subject has returned to baseline**, so you may want to switch jobs, and continue as above till all measurements are taken.

G. Perform at ECG (15 minutes)

The 2nd component of this lab is for you to learn how to conduct and ECG and successfully identify all of its components. There are two roles at this station are 'ECG subject' and 'LabScribe Recorder'. Each person should rotate though both positions, but you only need to print out 1 ECG recording for your lab write-up.

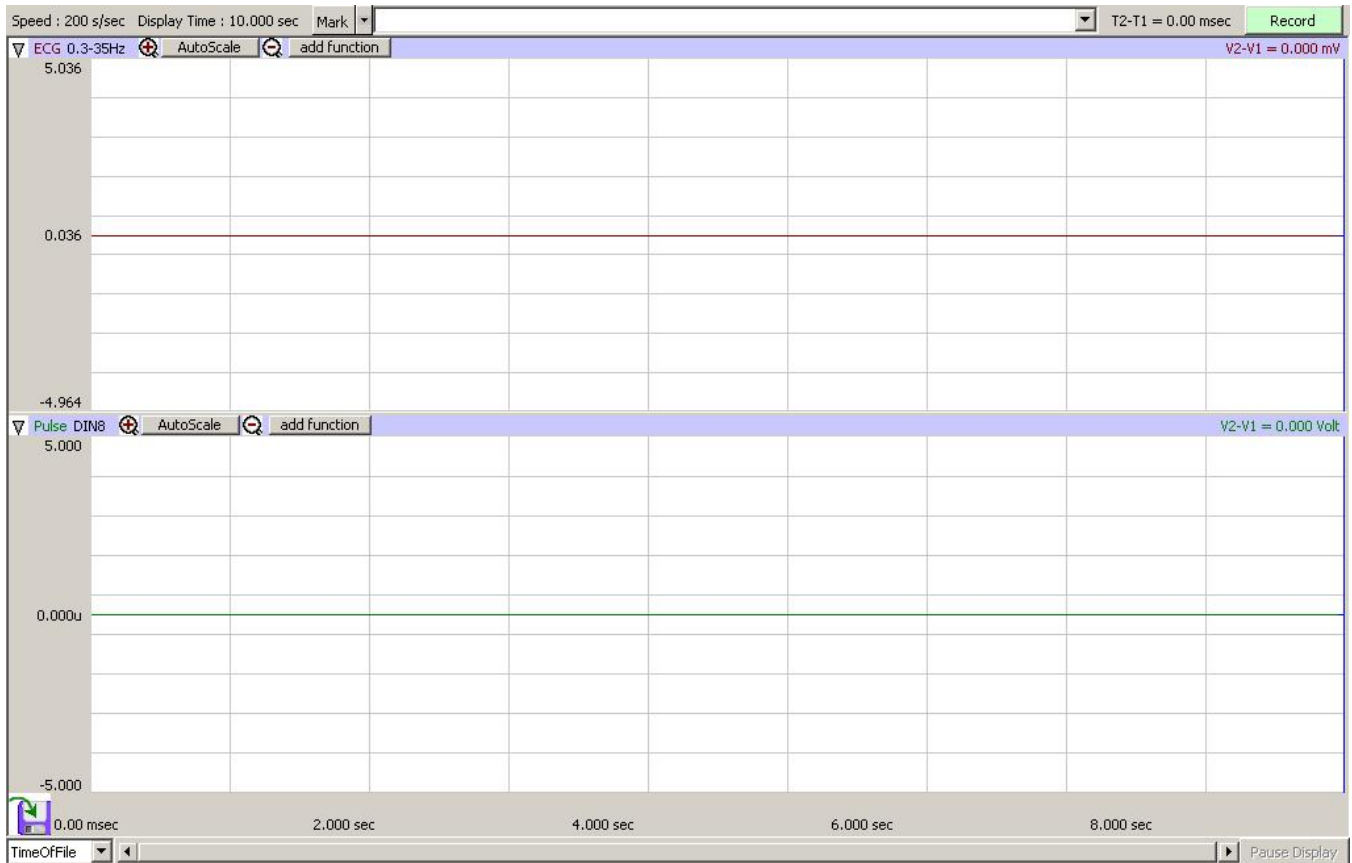
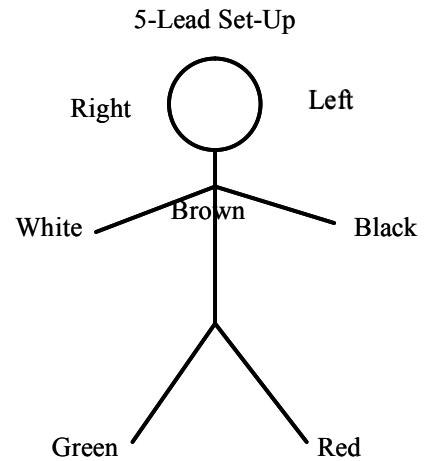
- 1) Check Equipment Set-Up
 - a) The iWorx box is on & connected to the computer via a USB cable.
 - b) The pressure transducer we used to measure heart rate in the previous experiment is plugged into Channel 3 of the iWorx box.
 - c) The ECG leads are connected to channel 1 of the iWorx box.
- 2) Attach the transducers to the experimental subject.
 - a) Make sure the subject is comfortably seated in a stationary position.
 - b) Attach the white pressure transducer that to the pad of the thumb, hand palm side up, just like you did for the previous GSR experiment. Use the Velcro to secure the pressure transducer snugly, but not too tight.



- c) To perform an ECG using 3 leads
- Rub the subjects' wrists & right ankle with alcohol. Alcohol pads will be provided.
 - Attach the **red** electrode to the right wrist, the **black** electrode to the left wrist, and the **green** electrode to the right ankle. *To avoid disturbing screams during electrode removal, pick a relatively hairless spot to place the electrodes.*

- d) To perform an ECG using 5 leads
- Rub the subjects' wrists, ankles, and chest with alcohol.
 - Attach the **white** electrode to the right wrist, the **green** electrode to the right ankle, the **black** electrode to the left wrist, the **red** electrode to the left ankle, and the **brown** electrode to the chest. *To avoid disturbing screams during electrode removal, pick a relatively hairless spot to place the electrodes.*

- 3) Start LabScribe
- a) Make sure the iWorx box is ON
 - b) Double-Click the LabScribe icon.
 - c) Select '**Settings**', then '**Bio 202**', then **ECG-Pulse**. The following screen should appear.

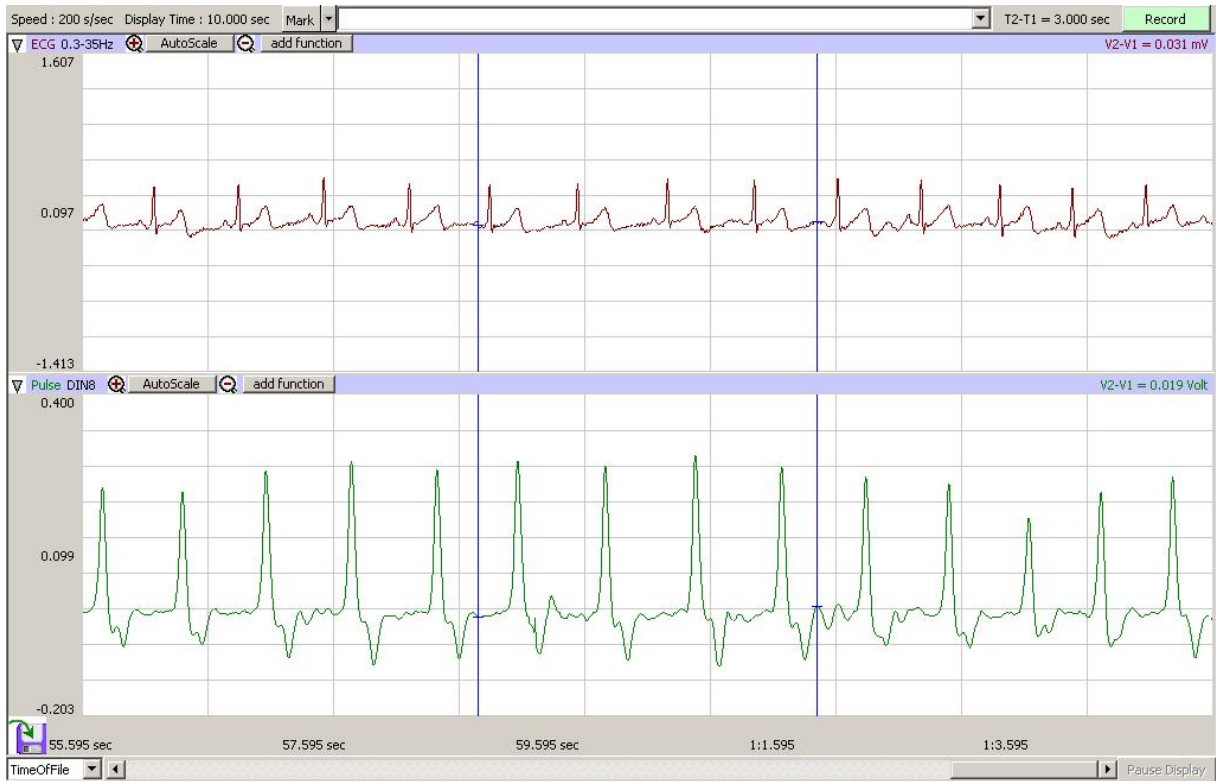


- d) Check the following.
- **Channel 1 = ECG.** This shows the electrical recording from the 3 or 5 electrodes. The ECG electrodes measure electrical events occurring on the surface of your limbs that are triggered by electrical events occurring in the heart. In a good ECG, one can see the P, QRS, and T waves that correspond to atrial depolarization, ventricular depolarization, and ventricular repolarization (hyperpolarization), respectively.

These signals are amplified and displayed on the screen. The range on the left side of this box should read from -5 to +5 Volts

- **Channel 2 = Pulse.** This will show the output from the pressure transducer on the subject's thumb. It will look like a series of peaks. Each peak marks an increase in pressure due to a heart beat (i.e. peak = heart beat). The y-axis is amount of pressure, not beats per minute. The range on the left side of this box should read from -5 to +5.
- **Speed.** In the top left of the screen check that your recording speed is recording 200 events per second.
- **Display Time.** In the top left of the screen you should see that the display time (the length / x-axis of the screen) is set to 10 seconds.

- 4) **Begin Recording**
 - a) Click Record.
 - b) If the QRS spike in the ECG trace is deflected downward, place the cursor in the ECG window, right click and select invert.
 - c) Similarly, if the peaks in the Pulse channel point downwards, place the cursor in the Pulse window, right click and select invert.
 - d) Once you have a clean trace, click on AutoScale in both channels. This should give you a full screen recording as shown below.



- 5) **Record & Save**
 - a) Once the recording appears clean type the subject's name in the text box and click Mark.
 - b) After recording at least 10 seconds of clean data insert another Mark.
 - c) Click Stop.
 - d) Select **File**, then **Save As**, then choose a file location (desktop or flash drive). Type in a file name, so that you will be able to identify it and click **Save**.
- 6) **Print**
 - a) Select **View**, then **Analysis**

- b) Find the section of data you want to print by using any or all of the following techniques.
- Zoom Out or In using the buttons until you can see the marks you are interested in.
 - Select **View**, then **Marks**, then **Marks** again. A marks dialog box will appear displaying each mark as a row. Select the row of the Mark you wish to go to and click '**Go To Mark**'.
- c) **Right click** on the channel you are interested in. Select **Scale**, then **AutoScale** to change the y-axis in that channel.
- d) Press the two cursor button.
- e) Move the cursors to include six complete pulse traces between the mark lines.
- f) Select **File**, then **Print**. You only need one copy for that member to include in their report. Other members will get a printout of their own recording.
- g) Repeat these procedures for each members trace.

VI. Data Analysis & Graphing (remaining time)

Once you have finished recording systolic blood pressure, diastolic blood pressure, and heart rate at baseline, and both levels of exercise, you will need to convert these measurements into our variables of interest (CO, MAP, TPR). Use the excel data sheet to help you calculate and graph these variables. Remember, your hypothesis is about proportional increases, so we are more interested in seeing the % change from baseline instead of the actual values. You will have to decide whether you think it is important to see the data on individuals, or whether a presentation of the mean % change across all subjects is more relevant. $\% \text{ change} = [(\text{measurement} - \text{baseline}) / \text{baseline}] * 100$

Bio 202: Human Anatomy and Physiology II

Endocrine Physiology

I. Goals for this lab

1. To continue to develop scientific reasoning skills.
2. To gain hands-on experience investigating effects of hormones on smooth muscle tissue.
3. To integrate theory about hormones and receptors with visible physiological responses.

Note: You *must study this protocol before coming to lab* because

1. The procedures and equipment set-ups are complex.
2. The smooth muscle tissue is easily damaged by hypoxia (lack of oxygen).
3. Hormones are expensive and can't be wasted.

II. Introduction

Smooth muscle tissue makes up the walls of such organ systems as the digestive tract, reproductive tract, exocrine glands, and the blood vessels. Usually, smooth muscle tissue surrounds an organ with a hollow space or lumen, so that the muscles can exert pressure on the lumen. One of the hallmarks of smooth muscle tissue is the fact that its activity is not regulated by somatic (voluntary) motor neurons. Instead, smooth muscle tissue tends to have its own rhythm. That endogenous rhythm, and the force of contractions, can be increased or decreased by hormones and other factors. In this experiment we will examine the responses of rat uterine and intestinal smooth muscle tissue to two hormones: oxytocin and epinephrine.

Oxytocin is a small peptide hormone synthesized in the hypothalamus, but stored and secreted from the posterior pituitary gland. It stimulates smooth muscle contractions in reproductive organs and thereby plays important roles in childbirth and nursing. Oxytocin does not naturally initiate labor in humans, but does enhance contractions once labor has started by means of a positive feedback loop.

- At the onset of labor, increased stretch of uterine smooth muscle stimulates 'stretch receptors' to send neural signals to the hypothalamus; this stimulates the release of oxytocin from the posterior pituitary gland into the bloodstream.
- Oxytocin then travels through the blood to its major target organ, the uterus. The uterine smooth muscle tissue has ample numbers of oxytocin receptors *at the time of parturition* (child birth). The hormone oxytocin binds to its receptors on the uterine smooth muscle tissue and induces the muscles to increase the strength of their contractions.
- The stronger contractions push the baby further, which increases uterine stretching. The increased stretching causes the stretch receptors to again signal the hypothalamus to release more oxytocin. Thus the positive feedback loop continues until the baby is free of the womb.
- Post-partum, the presence of an infant suckling at the breast is a powerful stimulus for oxytocin release. Sensory receptors in the nipple send neural signals to the hypothalamus, stimulating the release of oxytocin. Now the major target organ for oxytocin is the mammary gland; oxytocin stimulates the smooth muscles surrounding milk ducts to contract, causing "milk let down."
- Several studies report that the sight or sound of an infant, or even the thought of nursing, is sufficient stimulus to induce oxytocin release and subsequent milk let down.
- The brain also has oxytocin receptors. No, oxytocin does not stimulate brain contractions, but it does affect behaviors related to reproduction, childbirth and child rearing. Studies suggest that oxytocin plays stimulates pair-bonding between parent and child (maternal or paternal behavior) and/or between mates (the "cuddling hormone").

The hormone epinephrine also has powerful effects on smooth muscle contractions. Epinephrine is released from the adrenal medulla during stress, and causes changes in the body that are part of the fight or flight response. The release of epinephrine is stimulated by acetylcholine release by neurons that are part of the Sympathetic Division of the Autonomic Nervous System. Epinephrine activates several systems associated with fight or flight (e.g. glucose sparing, increases heart rate, increases blood pressure, stimulates muscle circulation, increases airflow, raises metabolic rate). However, it also acts to depress systems that are deemed nonessential in stressful situations such as digestion (smooth muscle of small intestine) and urine production.

It would be wise to review your notes on smooth muscle structure and function from BIO 201 before this lab.

III. Scientific Method & Experimental Design

- Puzzling Observation A: Roxy was doing a study on women during childbirth and discovered that uterine smooth muscle contractions were positively correlated with the presence of a small peptide in the bloodstream of 100 women. She named the peptide after herself Roxytocin, but the name was changed to Oxytocin.

- **Puzzling Observation B:** Mr. Pine was doing a study on stress. He had 50 subjects attend a class on meditation and another 50 subjects attend a class on meditation that was interrupted by a group of actors posing as hostage takers with guns. He found a substance in the blood of the ‘hostage’ subjects, and that presence of this substance was negatively correlated with small intestine smooth muscle contractions. He named this substance after himself, Pinepine, but it was changed to Epinephrine.
- **Causal Question:** Why does oxytocin affect uterine smooth muscles and epinephrine affects small intestinal smooth muscles?
- **Known Fact:** Only tissue with receptors for a hormone will respond to the presence of a hormone.
- **Hypothesis A (null):** All smooth muscle tissue has receptors for both oxytocin and epinephrine, *and all smooth muscle tissue is affected by those hormones in the same way.* Therefore, oxytocin *positively* affects all smooth muscle tissue and epinephrine *negatively* affects all smooth muscle tissue. **NOTE** – *Italicized* portion is optional. You can include it or take it out. Either is fine, just be specific about which hypothesis you using in the report. Here is a schematic of the above hypothesis.

	Oxytocin	Epinephrine
Uterine smooth muscle	+	-
Small intestine smooth muscle	+	-

- **Hypothesis B (receptor specificity):** Only tissues related to the ‘process’ each hormone is associated with will have receptors for that hormone. So, uterine, but not intestinal, tissue will have receptors for oxytocin, because oxytocin is associated with the process of ‘childbirth’. Similarly, intestinal tissue, but not uterine tissue, will have receptors for epinephrine, because epinephrine is associated with the ‘fight or flight’ process. **NOTE** – This does not say in what way (+ or -) tissue will be affected only that it will be affected by the hormone.

	Oxytocin	Epinephrine
Uterine smooth muscle	+/-	no change
Small intestine smooth muscle	no change	+/-

- **Experiment:** On the worksheet briefly describe your experiment. Your independent and dependent variables are given, but spend a few minutes thinking about controls. Controls are a bit more difficult with this experiment because different groups will be collecting different pieces of data. So the class as a whole will have to agree upon a few things here.

IV. Experimental Set-up

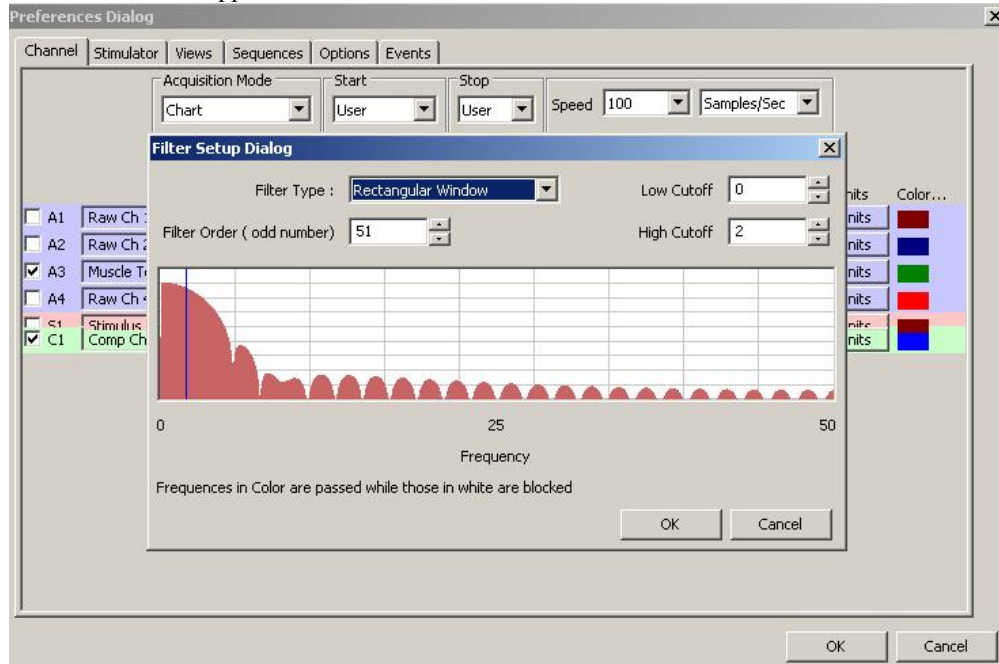
1. **First Things First.** *As soon as you enter the lab check that the water bath is at 37 °C.* If not adjust as necessary, but watch it carefully to avoid overheating.
2. **General Information:**
 - You will work in teams of 4 to collect data.
 - Each team should attempt to get data on how each of the two hormones affects one tissue type. Then if there is time move on to the other tissue type.
 - Since there probably won’t be enough time for each group to perform all 4 treatments we will be sharing data.
 - You must enter your data into the excel data sheet file and ***make sure your TA has a working copy of the file with your data before you can leave class.*** Your TA will compile the class data and post it on the blackboard site for you to use for your lab report.
3. **Personnel Distribution:**
 - **Muscle Caretaker:** The muscle caretaker is responsible for keeping the tissue alive and functioning correctly. Make sure the temperature remains near 37 °C *in the buffer.* To hot or too cold and the tissue will die. Also make sure that the bubbles are not going to fast / bumping the muscle too much, that nothing gets bumped on the table or shifts position.
 - **Lab Scribe Technician:** The lab scribe technician is responsible for running lab scribe. Make sure that everything is calibrated and running correctly *before* you start recording data. Make sure that you put in all of the marks you will need for data analysis. Make sure that you record both baseline and the hormone treatments for 5 minutes each. Remember, you can save your data and analyze it later in the computer lab, LSC 102, if necessary.
 - **Buffer & Hormone Coordinator:** The buffer & hormone coordinator is responsible for both taking notes on the amount of buffer and hormones added and physically adding the correct amounts.
 - **ALL:** You are all responsible for assisting with the setup of the experimental apparatus, ensuring that everything is prepared before you start the experiment, that everything runs smoothly during the experiment, and that everything is cleaned up at the end of the experiment.
4. **Pre-Tissue Setup – Buffer & Water Bath:**

- **Make sure the water bath is within 1-2 degrees of 37 °C.** If you need to make an adjustment with *small* turns of the thermostat. Remember the ultimate goal is to have the buffer in the tube / test chamber at 37 °C, roughly the same temperature it would be at in the rat body.
- Make sure you **have a small dish filled with ~ 250 ml of buffer** (it is clear like water) resting on top of the test tube rack in the water bath. If you do not have enough buffer request some from your TA or ATA.
- Make sure that the **tube / test chamber is secured in the clamp**, and submerged at least half way in the water bath. If not add more warm water to the water bath. You may also need to lower the clamp, but do not let it rest on the water bath itself as this will allow for the transfer of vibrations and messy data.
- Using a pipette, **fill the tube/ test chamber with 35-40 ml of warm buffer** from the small dish. Note down the amount of buffer added on the worksheet. If there is something in the tube empty it into the sink, clean it, and then fill it as described above.
- The **aerator has 2 lines** coming from it. Put one line from the aerator into the small dish of buffer, and one into the test chamber. Make sure that you can control the flow of bubbles to the line you put into the test chamber. There should be either a valve or a clamp that squeezes the air hose to control the flow of bubbles. Use the tape to secure these 2 lines in their position.
- **Secure the aerator** in a position that will lessen the transfer of vibrations from the aerator to the force transducer (i.e lessen messy data). I recommend taping it to the top of the small wall dividing computer cubicles with a pile of paper towels between the aerator and the wall to dampen vibrations.
- **Adjust the amount of bubbles** coming out of each of the line going into the test chamber, using either the valve or the clamp on the air hose, to 5-8 bubbles per second. Too many bubbles in the test chamber will vibrate the muscle and give you inconsistent results. Too few bubbles will deprive the muscle of oxygen and kill the tissue. The flow of bubbles in the small dish filled with buffer can and should be faster than 5-8 per second if possible.
- **Check that you have** a muscle clamp with a heart clamp attached, a heart clamp connected to a piece of string, and both 10 & 30ml syringes with rubber tubing attached to the ends.

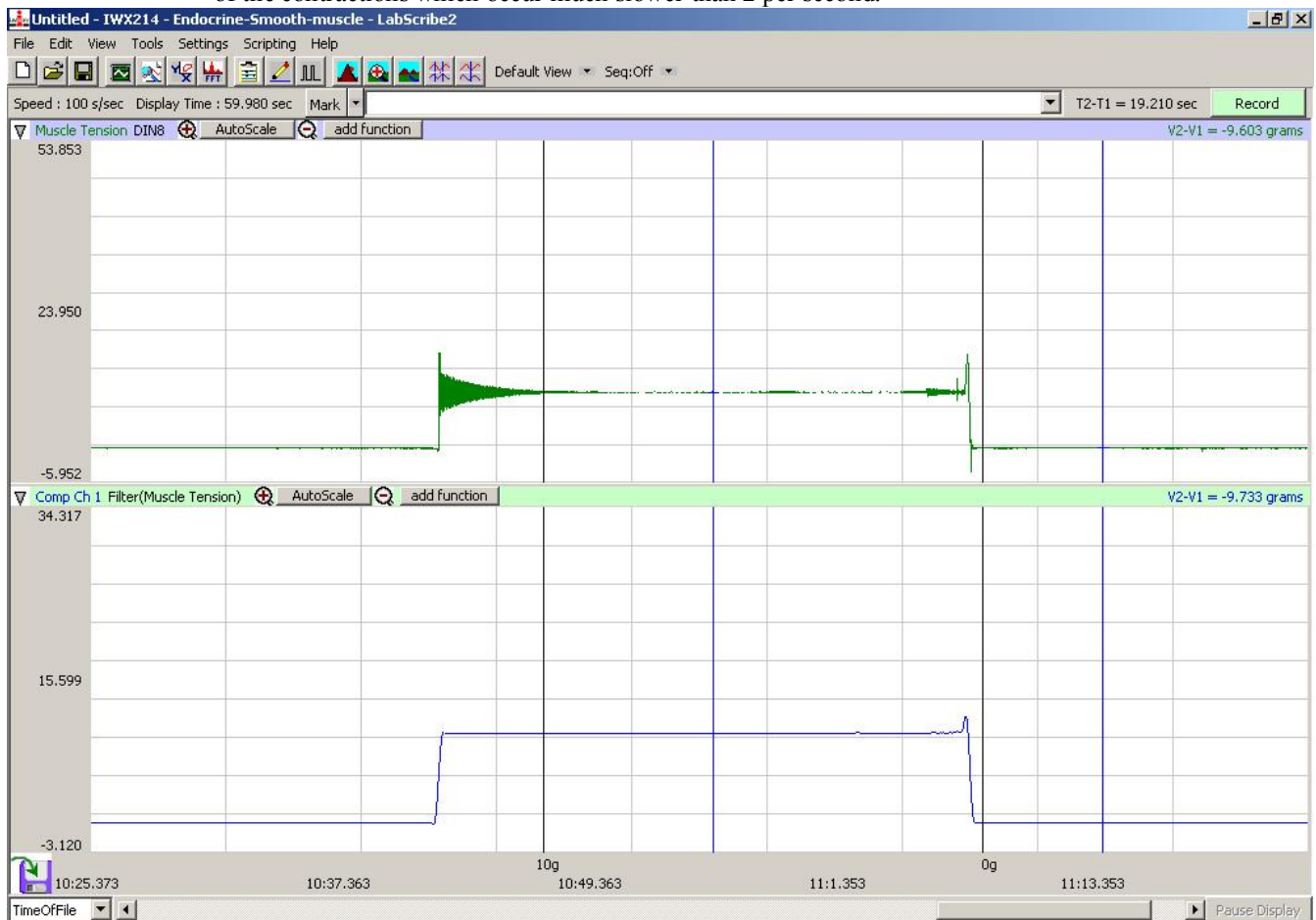
5. Pre-Tissue Setup – Lab Scribe

- Make sure that the **iWorx box** is on (red light is on) and that the force transducer is plugged into channel 3.
- **Double click** on the **Lab Scribe** icon on the computer desktop if it is not already open
- Select **Settings**, then **Bio 202**, then **Endocrine – Smooth muscle**.
- Check that in the top left corner the **Speed** is set to **100** samples per second and the **Display Time** is set to **60** seconds.
- Check that in the top right corner, under Record, the display is reading in **Grams**. If not follow the calibration directions below. *If it is reading grams skip to adding a filter.*
- Calibration Directions:
 - Click **Record**
 - In the Mark box type in **0g** and click **Mark**. Record for at least 10 seconds.
 - In the Mark box type in **1g** and click **Mark** as you add the weight to the top edge of the force transducer.
 - Click **AutoScale**, and if the 1g weight deflected the trace downward right click anywhere in the channel and select Invert
 - Record the 1g weight until the trace is nice and level (~10 seconds)
 - In the Mark box type in **0g** and click **Mark** as you remove the weight
 - In the Mark box type in **20g** and click **Mark** as you add the weight to the top edge of the force transducer
 - Record the 20g weight until the trace is nice and level (~10 seconds). You may need to hit **AutoScale** again to see the trace.
 - Remove the 20g weight and click Stop
 - Click on the **2 cursor** button and move the 1st (left cursor) to an even patch on the 1g recording and the 2nd cursor to an even patch on the 20g recording.
 - Right click** anywhere in the channel, select **Units**, then **Simple**, then a dialog box should appear
 - In the right-hand box next to **Value at Cursor 1** type **1** into the box
 - In the right-hand box next to **Value at Cursor 2** type **20** into the box
 - In the box labeled **Unit Name** type in **Grams**
 - Click **OK**. The unit is now calibrated.
 - To check it, try putting a 10g weight on and seeing what weight it reads.
- Adding a Filter
 - Select **Edit**, then **Preferences**, the Preferences Dialog Box should appear

- ii. In the Muscle Tension row click on the **Add Function** button, then select **Filter**. The following screen should appear

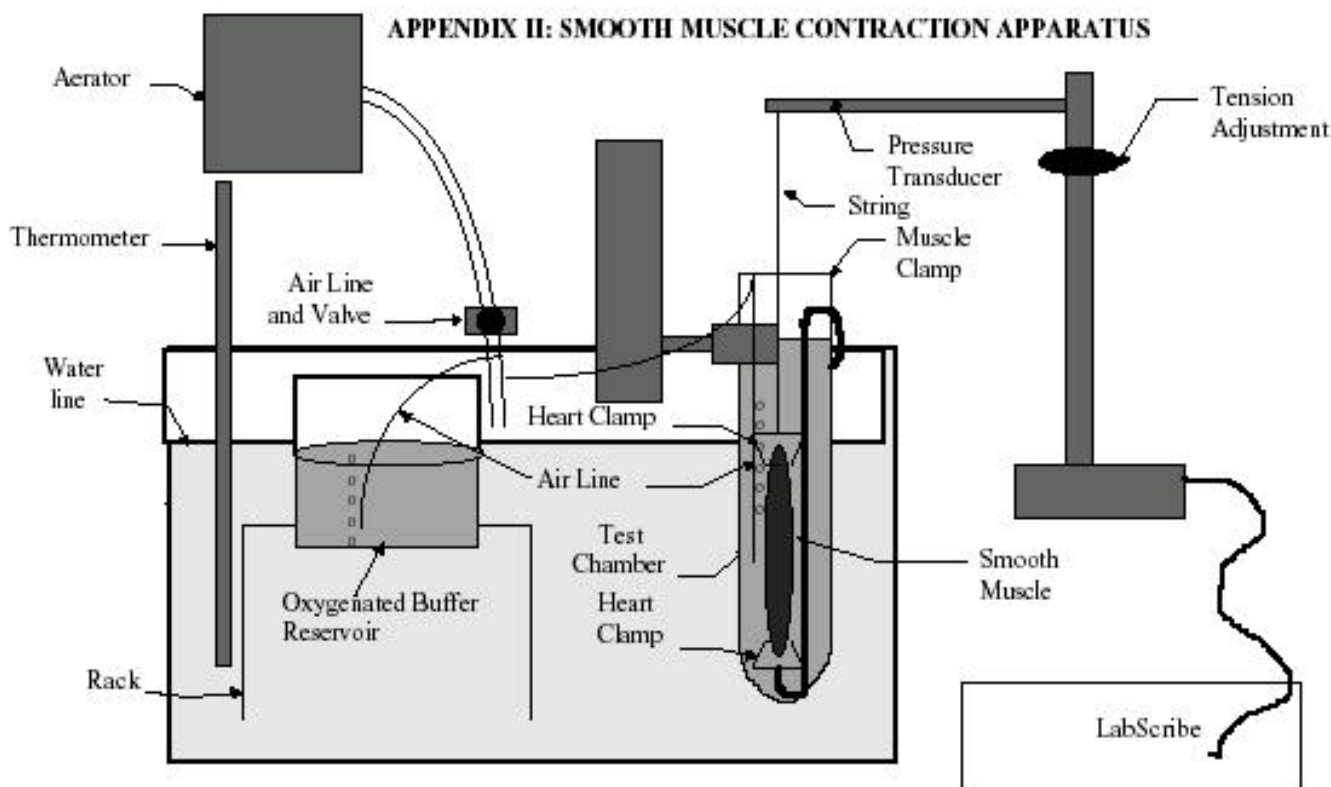


- iii. For Filter Type select **Rectangular Window** and for High Cutoff type in **2**. Click **OK**, and then click **OK** again.
- iv. You should now be seeing 2 channels, such as the image below. The lower filtered channel eliminates vibrations that occur faster than 2 per second (such as the aerator) giving you a cleaner picture of the contractions which occur much slower than 2 per second.



6. Tissue Setup:

- At the start of class the TA will dissect the uterus and smooth intestine from a pregnant female rat and place the tissue in warm aerated physiological buffer.
- Your group will be **assigned a tissue type and a hormone** to start with, so that the class will have data from each treatment even if your group does not have time for all 4 treatments.
- **Get a 0.5 to 1cm long piece of your tissue** type from your TA or ATA and put it immediately in your small dish of warm aerated buffer.
- Get the muscle clamp and **firmly attach the heart clamp on the muscle clamp to one end of the tissue**. Try to do this while *keeping the tissue in the buffer*. Remember, that the tissue has directionality. You must attach the heart clamps so that contractions will bring them closet together.
- **Attach the 2nd heart clamp**, the one with string attached, to the opposite end of the tissue. **DO NOT** stretch the tissue.
- **Place the whole setup (tissue and clamps) into the test chamber** as seen below. You will need to hold the string attached to the muscle loosely so that it does not fall in or stretch the muscle. Attach the muscle clamp to the side of the test chamber while the other end holds the tissue near the bottom of the test chamber.



- **Tape the string** attached to the 2nd heart clamp to the top of the force transducer so that there is GENTLE tension holding the muscle up and down in the tube. **DO NOT OVERSTRETCH** the muscle. You will have a chance to tighten it later if it is a bit loose.
- Make sure the **muscle is covered by buffer**, if not use the pipette to add more and note how much. If your tube is overflowing with buffer remove it.
- **Check the bubbles**, which should still be at 5-8/sec, so that they touch the muscle as little as possible.
- Getting Proper Muscle Tension:
 - To start with the string between the muscle and the force transducer should have a little slack as described above.
 - In LabScribe, click **Record**
 - Carefully raise the height of the force transducer by twisting the adjustment knob on the stand counter-clockwise until the muscle is *slightly* stretched.
 - Make sure that the muscle is not touching the sides of the container.

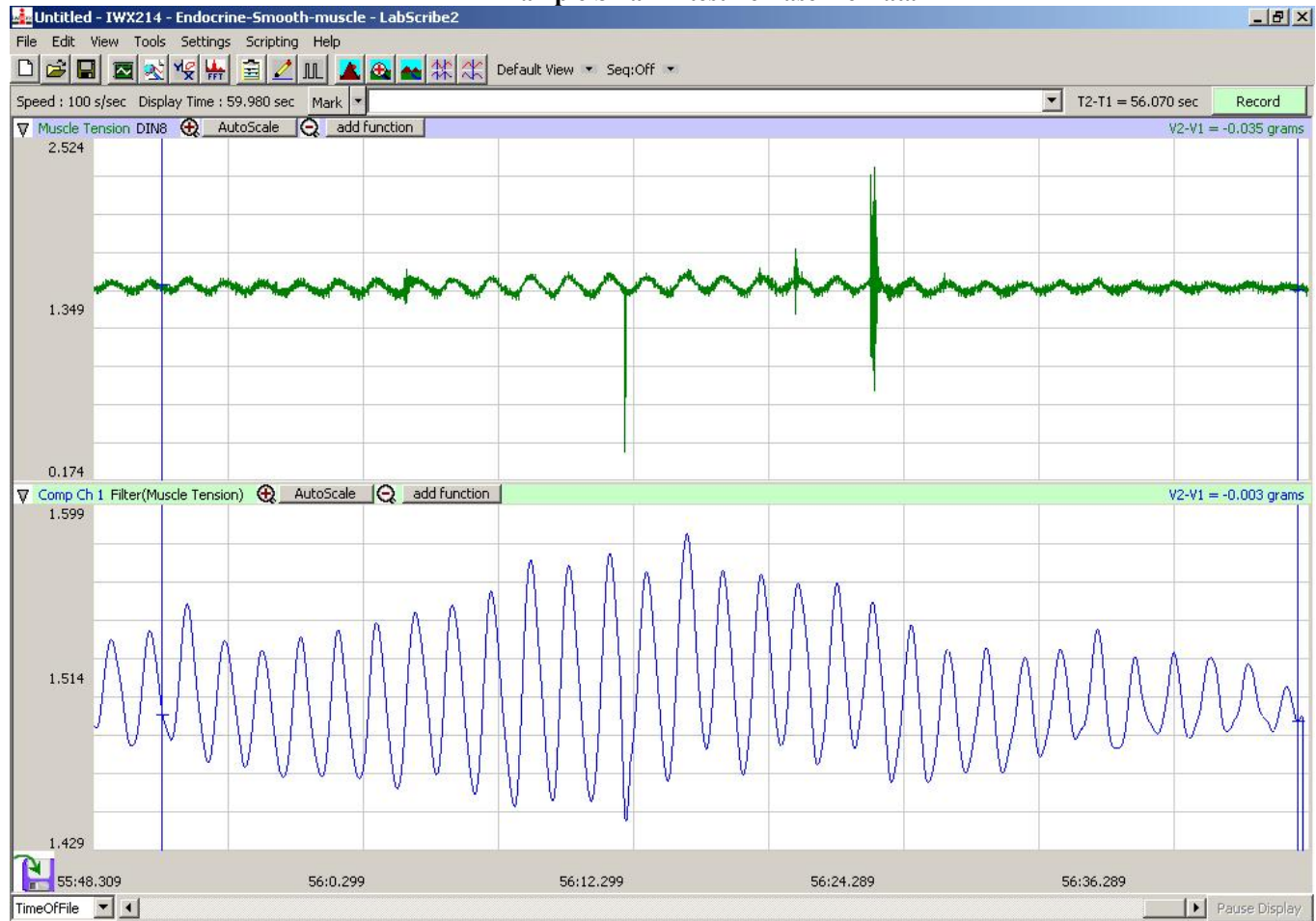
- v. Look for contractions on LabScribe and if you do not see any after 1-2 minutes carefully raise the force transducer a little higher.
- Let the **muscle equilibrate for 5-10 minutes** before recording baseline measurements

V. Performing Experiment

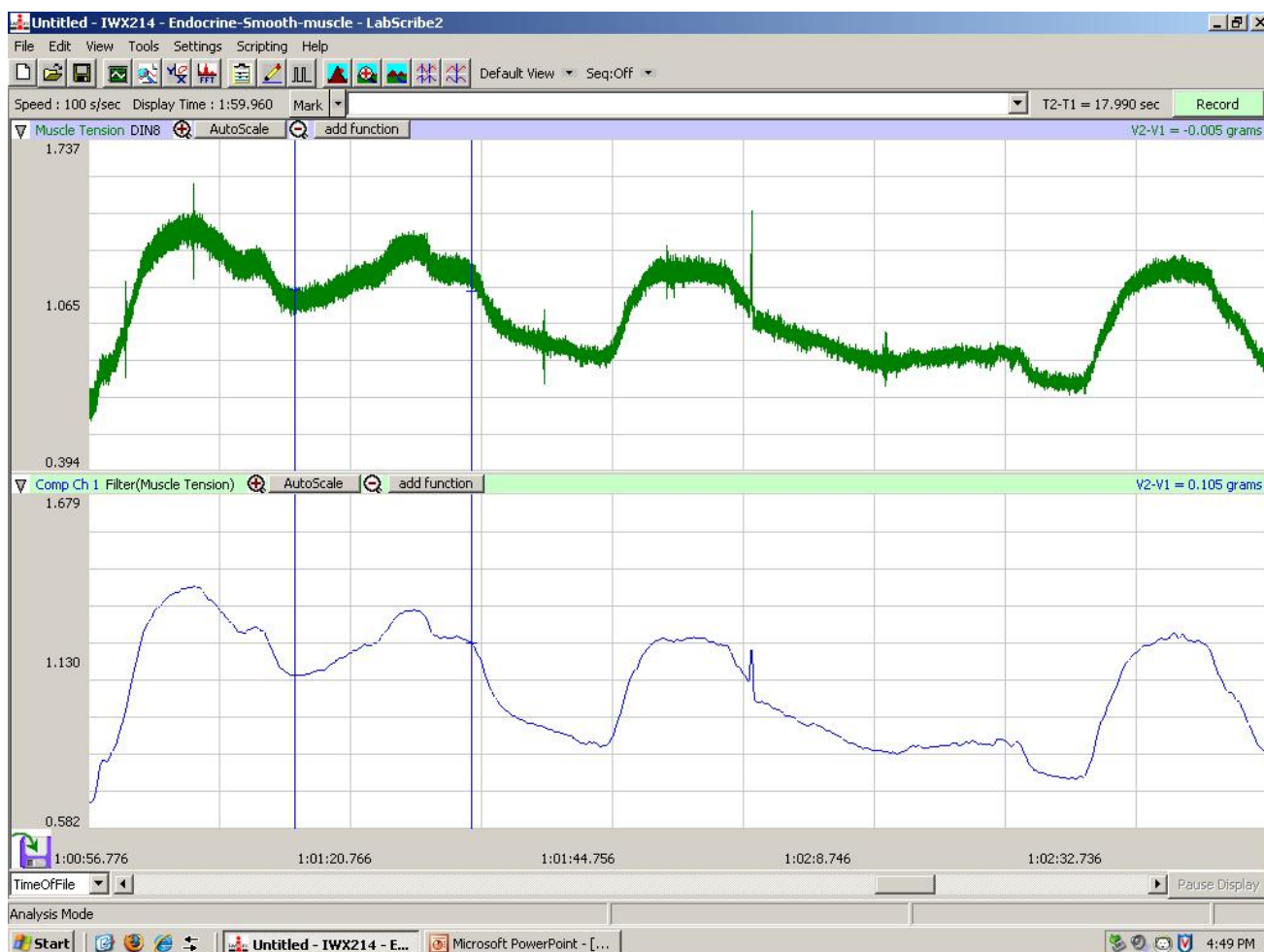
1) Baseline

- After the muscle has equilibrated you may need to re-adjust the tension. Then record at least **5 minutes** of clean baseline data.

Example Small Intestine Baseline Data



Example Uterine Baseline Data



2) Hormone Addition

- Oxytocin stock solution concentration is **5.0 μg oxytocin/ml** buffer.
- Epinephrine stock solution concentration is **20 μg epinephrine/ml** buffer.
- We will be testing the effect of **10-fold dilutions** of the hormones today.
- How do you make a 10-fold dilution of a stock solution of marbles? If you have a stock solution of 8 marbles in 1 L of buffer, add enough buffer (9 L) to your solution so you will have 8 marbles in 10 L of buffer.
- The easiest way to dilute the stock solution 10-fold is by removing one tenth of the volume of physiological buffer from the test chamber and replacing it with the same volume of stock solution.
- For example, you have 40 ml of buffer in your test chamber. You remove 4 ml of buffer and add 4 ml of 5.0 μg oxytocin/ml buffer. That gives you (5.0 μg oxytocin/ml * 4.0 ml = 20) 20 μg oxytocin in a total of 40 ml of buffer or (20 μg oxytocin/ 40ml buffer=0.5) 0.5 μg oxytocin/ ml buffer. That is a 10-fold dilution of the 5.0 μg oxytocin /ml buffer stock.
- Similarly, 40ml buffer minus 4ml buffer, plus 4 ml of 20 μg epinephrine/ml = 80 μg epinephrine in a total of 40 ml or a 2.0 μg epinephrine/ml buffer solution.
- **Remove 1/10th of the buffer** from the total test chamber volume.
- **Add the hormone to the test chamber**, DO NOT pour directly on the tissue.
- Make sure you insert a **Mark** in LabScribe when you add the hormone.
- Record for **5 minutes** after you added the hormone.

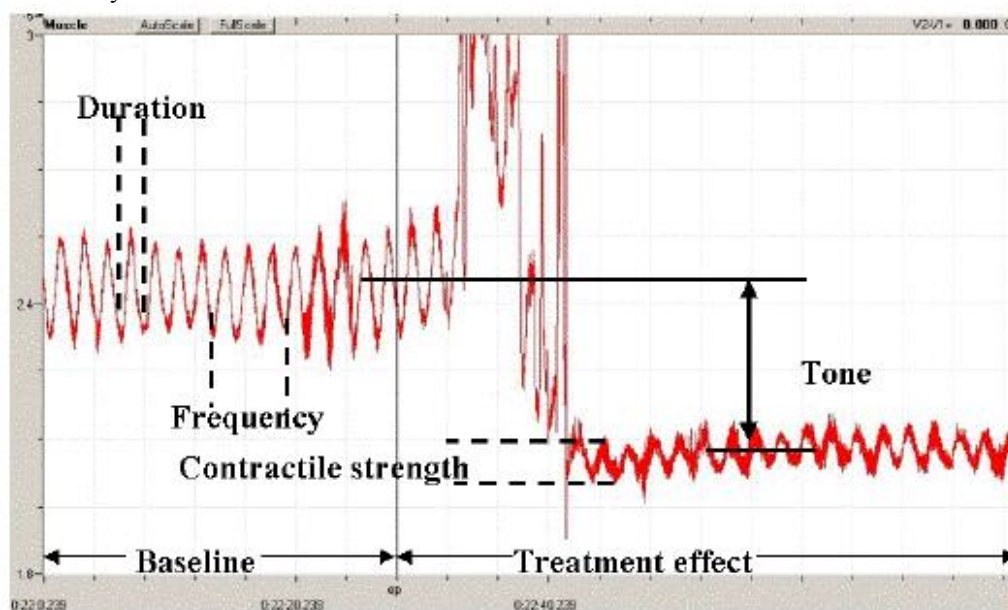
3) Preparing for 2nd Hormone

- In order to use the same already set up tissue for the 2nd hormone you must first use the syringe to **remove all of the buffer** from the test chamber.
- **Immediately add a set amount (35-40ml) of clean warm aerated buffer** from the small dish to the test chamber using a clean syringe.
- **Dump old buffer that included hormone down sink** and clean the syringe with ample water.
- **Record new baseline data for 5 minutes**

- Then **add the 2nd hormone** as detailed above and record for another 5 minutes.
- 4) Once you have completed baseline and hormone treatments for both hormones on one tissue type ask your TA for the other tissue type are repeat the procedure.
 - 5) Dispose of all animal tissue in the marked bag which should be in the sink or the refrigerator. **DO NOT** let rat parts clog the sink drain.
 - 6) Once you have completed all of your recordings make sure that everything is clean and put back where you found it.

VI. Getting Data from LabScribe

- 1) Select **View**, then **Analysis**
- 2) Find the section of the recording you want to analyze by using one or more of the following techniques
 - **Zoom Out** or **Zoom In** using the buttons until you can see the marks you are interested in.
 - If Zoom In does not show all of the section you want to see and Zoom Out makes it too small then click on the **2 cursors** button and move the cursors to select the area you are interested in. Then click on the button that looks like a mountain with a target sign on it.
 - Select **View**, then **Marks**, then **Marks** again. A marks dialog box will appear displaying each mark as a row. Select the row of the Mark you wish to go to and click '**Go To Mark**'. You can also unselect any Marks you do not wish to see in this dialog box.
 - Once you have the area along the X-axis that you want to see on-screen you will probably need to fix the Y-axis. **Right click** on the channel you are interested in (for us Comp Channel 1 Filter). Select **Scale**, then **AutoScale**.
- 3) For each baseline & hormone treatment you will need to measure all 4 of the dependent variables as follows and record the data on your worksheet or excel data sheet.



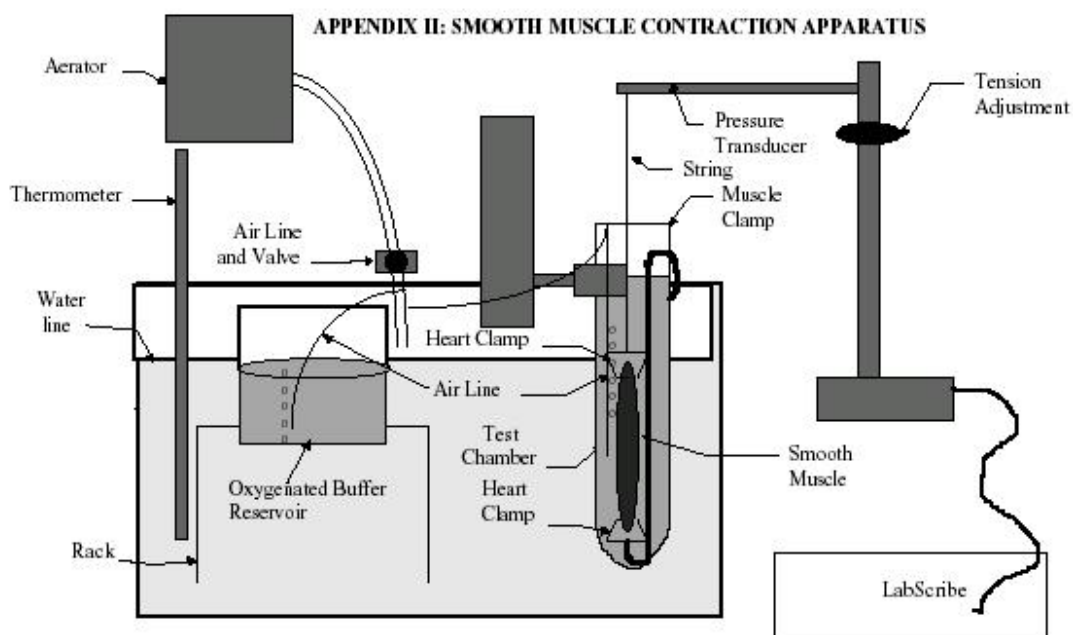
- 4) Click on the **2 cursor** button.
- 5) If there are any functions listed at the top of the channels **right click** and **Delete** them
- 6) Measuring the Frequency of Contractions (#/min)
 - Move the 2 cursors so that they encompass 5 clean peaks
 - Click **Add Function**, then **General**, then **T2-T1**
 - Write down both the number of contractions between the 2 cursors and the T2-T1 value (number of seconds it took for the contractions) from the **LOWER FILTERED CHANNEL**
 - If you do not have 5 contractions use a lower number of contractions and record it.
- 7) Measuring Tone (g)
 - Move the 2 cursors so that they encompass 5 clean peaks
 - Click **Add Function**, then **General**, then **Mean**
 - Write down both the number of contractions between the 2 cursors and the Mean value (average g) from the **LOWER FILTERED CHANNEL**
 - If you do not have 5 contractions use a lower number of contractions and record it.

8) Measuring Contraction Duration (s)

- Move the 2 cursors so that they encompass **1** clean peak
- Write down the T2-T1 value (duration of the contraction in seconds) from the LOWER FILTERED CHANNEL
- **NOTE** before you move on it is easiest to measure contraction duration & strength on the same peak and then move.
- Move the cursors to the next clean peak and repeat the measurement and recording.
- Measure contraction duration for 5 peaks

9) Measuring Contractile Strength (g)

- Move the 2 cursors so that they encompass **1** clean peak from valley to valley.
- Click **Add Function**, then **General**, then **Max-Min**
- Write down the Max-Min value (strength of contraction in g) from the LOWER FILTERED CHANNEL
- Move the cursors to the next clean peak and repeat the measurement and recording.
- Measure 5 peaks



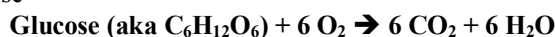
Bio 202: Human Anatomy and Physiology II Respiratory Physiology Protocol

I. Goals for this lab

- A. To increase your understanding of how the respiratory system meets the body's need for O₂ delivery and CO₂ elimination.
- B. To learn how to:
 1. Measure Tidal Volume and Breathing Rate using the LabScribe spirometer
 2. Calculate Minute Volume
 3. Measure Inspired & Expired O₂ and CO₂ levels
 4. Calculate O₂ consumption rates and CO₂ production rates
- C. To gain more experience with the scientific method, experimental design, making predictions, critical analysis of results, and interpretation of your results.

II. Introduction

Muscles require more O₂ during sustainable aerobic exercise. Oxygen is necessary in order to produce ATP. ATP is necessary because it is the energy source that muscles use in order to contract and perform work. The equation by which fuel and O₂ are used to generate ATP can be as seen below. From this equation we can see that one molecule of CO₂ is produced for every molecule of O₂ that is consumed when glucose is the fuel. The use of other fuels (e.g. lipids or proteins) produces different ratios of O₂ consumption to CO₂ production. However, whatever the fuel, there is always increased demand for O₂ during *aerobic* exercise



A few weeks ago we learned that the cardiovascular system responds to muscles' increased O₂ consumption and CO₂ production during aerobic exercise by increasing the rate of blood flow to the muscles. The cardiovascular system increases the rate of blood flow to the muscles by increasing Cardiac Output (ml of blood pumped from left ventricle to aorta per minute) and decreasing Total Peripheral Resistance experienced by the blood while traveling to the muscles (i.e. by increasing vessel diameter / vasodilation). However, increasing the rate of blood flow to the muscles will only provide them with more O₂ and rid them of excess CO₂ if there is more O₂ available in the lungs for the blood to pick up.

How does the respiratory system adjust during exercise in order to meet the muscles' increased demand for O₂ to consume and CO₂ to eliminate? This is the question we will be trying to answer during lab today. We know that muscles' need more O₂ during exercise, for the O₂ consumption rate (the volume of O₂ consumed by the body per min) increases during maximal endurance exercise. In fact it can increase 10 -20 times in an untrained individual and up to 30 times in a trained athlete. So how does the respiratory system supply the O₂? Some hypotheses to answer this question seem obvious (e.g. what happens to your breathing when you work out?). Others might require a little more thought about the nature of gas exchange and its dependence on partial pressure differences.

III. Equations & Definitions

Minute Volume (MV) (L/min) = Tidal Volume (L/breath) x Breathing Rate (breath/min)

Minute Volume (MV) (L/min) = total volume of air inhaled and exhaled each minute

Tidal Volume (V_T) (L/breath) = the volume of air inhaled or exhaled in one breath

Breathing Rate (BR) (breath/min) = the number of breaths taken in one minute

Alveolar Ventilation Rate (AVR) (L/min) = Breathing Rate (breath/min) x [Tidal Volume - Dead Air] (L/breath)

Dead Air (L/breath) = the volume of air that fills the conducting pathways (e.g. bronchi and bronchioles) of respiratory system and therefore is not available for gas exchange.

For the purposes of our experiment, **assume Dead Air = 0.15 L OR**, if you feel comfortable with it, **1ml per pound** (e.g. a 200 pound person would have 200 ml / 0.2 L of dead air).

O₂ Consumption Rate (L O₂/min) = Minute Volume (L air/min) x [Inspired O₂ Fraction – Expired O₂ Fraction] (O₂/air Ratio – no units)

Inspired O₂ Fraction (O₂/air Ratio) = you can think of this as L of O₂ in 1L of room / inspired air.

Inspired O₂ Fraction = percent of O₂ in inspired air / 100

= partial pressure O₂ in inspired air (mmHg) / atmospheric pressure (mmHg)

Expired O₂ Fraction (O₂/air Ratio) = you can think of this as the L of O₂ in 1L of expired air.

Expired O₂ Fraction = percent of O₂ in expired air / 100

= partial pressure O₂ in expired air (mmHg) / atmospheric pressure (mmHg)

CO₂ Production Rate (L CO₂/min) = Minute Volume (L air /min) x [Expired CO₂ Fraction – Inspired CO₂ Fraction]
(CO₂/air Ratio – no units)

Expired CO₂ Fraction (CO₂/air Ratio) = you can think of this as the L of CO₂ in 1L of expired air

Expired CO₂ Fraction = percent of CO₂ in expired air /100

= partial pressure CO₂ in expired air (mmHg) /atmospheric pressure (mmHg)

Inspired CO₂ Fraction (O₂/air Ratio) = you can think of this as the L of CO₂ in 1L of inspired air.

Inspired CO₂ Fraction = percent of CO₂ in inspired air / 100

= partial pressure CO₂ in inspired air (mmHg) /atmospheric pressure (mmHg)

NOTE CO₂ Production equation is the opposite (exp – insp) of O₂ Consumption (insp-exp)

IV. Hypothesis Generation & Experimental Design

A. General Idea: Today you will work in a team in order to:

- 1) Develop **TWO hypotheses** about how the respiratory system responds to exercise.
- 2) Design **ONE controlled experiment** to test both hypotheses using the equipment provided.
At this time you may want to make preliminary observations.
- 3) Make predictions about your results based on your hypothesis.
- 4) Carry out your experiment;
- 5) Critically analyze your results.
- 6) Draw conclusions based on the analysis of your results.

B. Safety: As in the cardiovascular lab, students who should restrict vigorous activity for any reason, including a history of respiratory or cardiovascular problems, should not exercise in the lab. *It is the responsibility of the group to ensure that no student exercises beyond what is comfortable; keep it aerobic. A designated safety spotter will position him/her self adjacent to an exercising subject and be alert to the subject's condition. The safety spotter is also responsible for seeing that the subject warms down after exercise.*

C. Developing Hypotheses: How does the respiratory system adjust during exercise in order to meet the muscles' increased demand for O₂ to consume and CO₂ to eliminate? To start answering this question start simple. Do you breathe differently when you exercise? Given your experience with breathing during exercise, develop a hypothesis that will lead to predictions about these 4 dependent variables for hypothesis A (Tidal Volume, Breathing Rate, Minute Volume, and Alveolar Ventilation Rate).

What do you think happens to the *air* you breathe during exercise? You are breathing in air from the environment with a relatively constant composition (~21% O₂, 1% CO₂). But does the air you breathe out remain constant in composition or change? How might your expired air change in composition as you exercise and why might it change in those ways? Consider these issues in order to develop a hypothesis that will lead to predictions about O₂ Consumption Rate and CO₂ Production Rate.

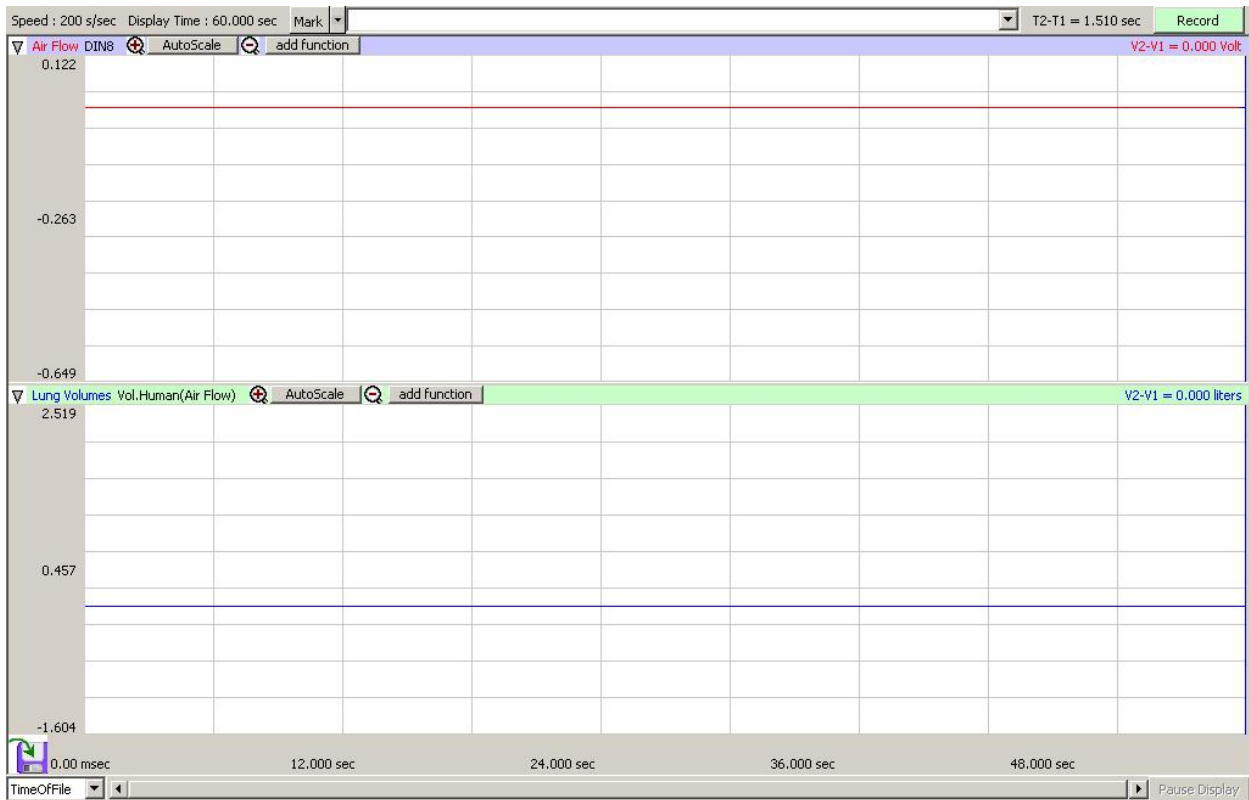
If you are having trouble with your hypotheses, sometimes it is helpful to work backwards from your predictions or to make preliminary observations. **REMEMBER your hypothesis should be about the idea – NOT the dependent variable.** You will not mention your dependent variable in your hypothesis; it goes in the prediction section. It is important to note that *your hypothesis should only apply to aerobic exercise. Respiratory adjustments to non-sustainable exercise are different because of the release of lactic acid from muscles into the bloodstream. Therefore, be sure the subject is within his or her range of sustainable exercise. Displays of athletic prowess by subjects only may provide bad data.*

D. Designing an Experiment:

- Independent Variable = the thing we change in order to see how dependent variables respond = amount of exercise
- Levels of the Independent Variable = same as for cardiovascular physiology lab (light and moderate with a baseline measure for reference).
- Dependent Variables: Tidal Volume, Breathing Rate, Minute Volume, Alveolar Ventilation Rate, O₂ Consumption Rate, and CO₂ Production Rate,
- Controls: THINK about what it is important to keep constant for this experiment. What things other than the independent variable might affect the dependent variables?
- **Preliminary Observations:** You will be measuring Tidal Volume and Breathing Rate using the LabScribe spirometry setup. You will measure Gas Fractions using the O₂ and CO₂ analyzers on the side of the room. Note that there is only 1 analyzer for the entire class, so **ORGANIZE** your data collection to **be as efficient as possible**. No one should be standing around doing nothing. Do not spend a lot of time collecting preliminary data, but make sure that everyone feels comfortable working the equipment before you start your experiment

V. Laboratory Equipment, Instructions, & Techniques

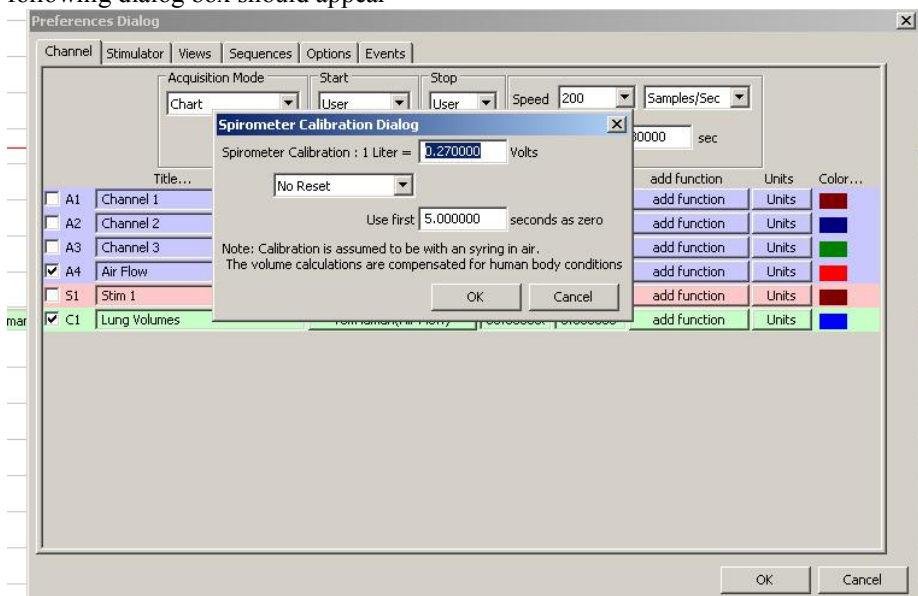
- C. **Setup/Calibration of LabScribe:** The iWorx spirometry lab should be set up for you before you enter the lab. If this is the case proceed to the 'iWorx Spirometer Use' section. If not,
1. Make sure the iWorx box is turned on.
 2. Start LabScribe by double clicking the icon on the desktop.
 - a. When the program opens, select the **settings** menu, then select **Bio202**, then select **Exercise – Respiration**. The following screen should appear (see below).
 - b. You should see a screen with 2 channels.
 - Channel 1 should be called Air Flow.
 - Channel 2 should be called Lung Volumes. This is the only channel we are interested in. We will use this channel to measure both breathing rate and tidal volume.
 - The speed should be set to 200 samples per second and the display time should be 30 seconds.



3. Before you begin collecting data, *you must calibrate the spirometer* (i.e. convert the electrical output of the spirometer into meaningful units of volume). To do this:
 - a. Select **Edit**, then select **Preferences**. The following dialog box should appear.

Channel	Title...	Mode/Function	Y Max	Y Min	add function	Units	Color...
<input type="checkbox"/>	A1 Channel 1	Off	5.000000	-5.000000	add function	Units	Red
<input type="checkbox"/>	A2 Channel 2	Off	5.000000	-5.000000	add function	Units	Blue
<input type="checkbox"/>	A3 Channel 3	Off	4.956522	-5.043478	add function	Units	Green
<input checked="" type="checkbox"/>	A4 Air Flow	DIN8	5.000000	-5.000000	add function	Units	Red
<input type="checkbox"/>	S1 Stim 1	Off	5.000000	-5.000000	add function	Units	Red
<input checked="" type="checkbox"/>	C1 Lung Volumes	Vol.Human(Air Flow)	5.000000	0.000000	add function	Units	Blue

- b. Left click on **Vol.Human(AirFlow)**, select **Spirometry**, then select **Volume – Human Body**. The following dialog box should appear



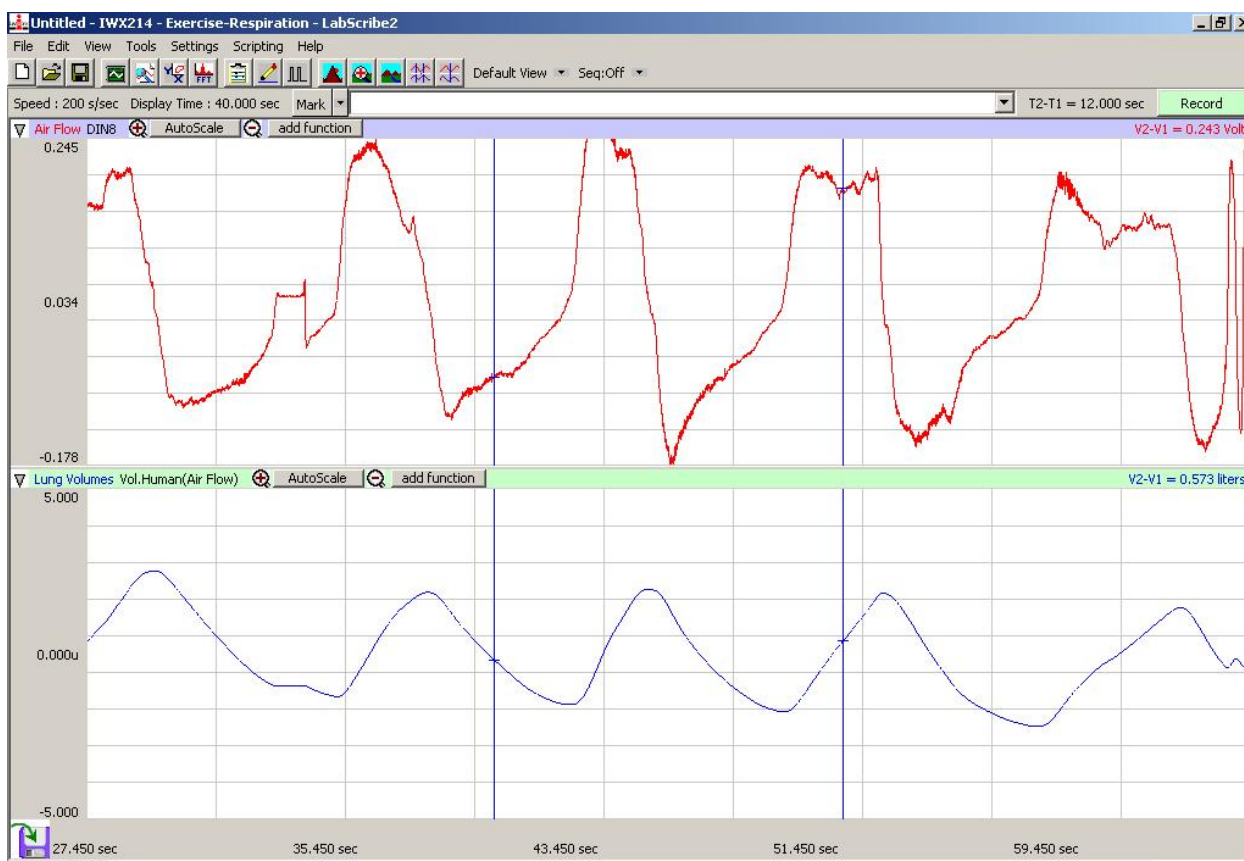
- c. In the Spirometer Calibration Dialog box where there is a place to type in 1 Liter= ___ Volts, type if the number of volts given on the back of the black box attached to Channel 4 on the iWorx box. Make sure the lower box says use the first 5 seconds as zero.
- d. Hit **OK** in the Spirometer Calibration Dialog box. Then click **OK** in the Preferences Dialog box.

B. Setup the Spirometer:

- 1) Get a new clean **bacterial filter** (round plastic thing) and attach it to either end of the spirometer (white round thing). You will use the same bacterial filter for your entire group today, so don't throw it out till you are done with all of your measurements.
- 2) Each individual should get their own **mouthpiece** (cardboard cylinder) and attach that to the bacterial filter. Each individual should try to use the same mouthpiece for the entire experiment today, so keep track of it when you aren't the one blowing into the spirometer anymore.
- 3) The subject should apply a **noseclip** to prevent air from entering or leaving the nose as the subject is breathing into the spirometer. Air that passes through the nose is not included in the volume measurements and will cause errors in these values
- 4) The subject should hold the spirometer so that the **air outlets** on the flow head are always in the **upright position**. This will help to avoid problems with condensation developing in the airflow tubes. **Do not hold the spirometer in your hand**; the heat of your hand will alter the volumes recorded.
- 5) Before the subject starts to breathe, hit the **Start button** and **wait 10 seconds** with the spirometer flow head absolutely still and **NOT near the mouth**. This is absolutely necessary for the spirometer to use room air to establish a baseline. The reading may drift slightly, that is ok.
- 6) Then the subject should place their lips around the **outside** of the opening of the mouthpiece to reduce turbulence. Turbulence in the flow head will produce a noisy signal.
- 7) Then ask the subject to breathe normally. Subjects usually do a better job of breathing normally if they do **not look at the computer monitor**.
- 8) Make sure that the **deflection goes up when the subject inhales**. If not, attach the bacterial filter to the other side of the spirometer.
- 9) Once everything seems to be working enter a Mark (usually subject name, start or stop, and level). Remember that you must already be recording to enter a Mark. Make sure you **get at least 1 minute** of stable breathing before allowing the subject to stop.
- 10) The **AutoScale** function is very useful for giving you optimal size signals for your experiment. However, there is often drift in the absolute scale causing the recording to move off screen. It is suggested to full scale recordings while taking measurements.

C. LabScribe Data Analysis

- 1) Once you start collecting data it should look something like the figure below. When you have recorded at least a full minute you can start to analyze your data.



- 2) Select **View** menu, then **Analysis**
- 3) If there are any functions already showing in this window left click on them and then select **Delete**.
- 4) Left click on **add function**, select **General**, then **T2-T1**. This gives you a column that displays the time difference in seconds between where the 2 blue cursors are located.
- 5) Left click on **add function**, select **General**, then **Max-Min**. This gives you a column that displays the difference between the highest and lowest points between the two cursors.
- 6) If you are not seeing 2 blue lines/cursors press the **double cursor** button.
- 7) Zoom out (the button with 2 mountains) and use the **AutoScale** (anywhere in the lung volume channel **right click**, select **Scale**, then select **AutoScale**) in order to view your complete 1 minute recording of one subject at one level.
- 8) **To measure Breathing Rate** move the two cursors till they enclose 60 seconds of clean recording (i.e. until $T2-T1=60$). Count the number of peaks between the 2 cursors (in 60 seconds) and record that on the data sheet. If you do not have 60 seconds you may use 30 seconds and multiply by 2 in order to record breath/min on the worksheet.
- 9) **To measure Tidal Volume** move the 2 cursors so that the first one is at the highest point of a peak and the 2nd cursor is at highest point of the next peak. You may want to zoom in and / or use **AutoScale** again in order to see the peaks more clearly. Record the **Max-Min** value when the cursor is on the 2 peaks. Make sure you are recording the **Max-Min Lung Volume**, NOT the **Max-Min Air Flow**. Move the cursors to 2 different peaks and record a 2nd and then a 3rd Tidal Volume.
- 10) **Repeat these steps** for each subject at each level.

D. Expired Gasses Data Collection

The oxygen analyzer contains a zirconium crystal in an electric circuit. When the crystal is heated to 750°C within the “sensor cell,” it absorbs O_2 and its electrical resistance is changed in proportion to the amount of O_2 present in the gas sample. We obtain a readout expressed as percentage O_2 in the sample. The CO_2 analyzer works a bit differently. CO_2 absorbs energy in the infrared range, which is why it is a greenhouse gas. The CO_2 analyzer passes an infrared beam through the gas and records how much energy is absorbed.

Procedure:

- 1) Obtain an air sample bag.

- 2) Push down on the white clip till it pinches closed the tube coming from the bag.
- 3) Remove the stopper and squeeze all of the air out of the bag. *It is important that this is a sample of only the subject's exhaled air and is not contaminated by room air or the last subject.* Once the bag is completely empty replace the stopper.
- 4) Attach your cardboard mouthpiece to a bacterial filter and when you are ready to exhale put these in the place of the stopper.
- 5) To collect a sample, exhale into the sample bag, inhale room air, and exhale into the sample bag again until it is about 1/3rd full. **Do not inhale from the sample bag!** Stopper the bag between exhalations and when you have finished collecting a sample.
- 6) When the bag is 1/3rd full carry it over to the gas analyzers IMMEDIATELY.
- 7) When not in use the O₂ analyzer should be reading the percent O₂ of room or **inspired** air (very near to 21%). **Record this reading as Inspired Percent O₂.** Note that the room air does not really change so this value will be the same for all subjects at all levels.
- 8) Similarly, while not in use the CO₂ analyzer should be reporting a CO₂ number that represents the amount of CO₂ in room or inspired air. Record this reading as the Inspired CO₂ number. This will also be the same for all subjects at all levels.
- 9) Attach the tube coming off of the air sample bag to the tube connecting to the air analyzers.
- 10) Open the tube connecting to the bag by releasing the white clip.
- 11) *Gently* Squeeze the bag until the numbers on the displays stabilize. This will push air first through the CO₂ analyzer and then through the O₂ analyzer.
- 12) When the numbers stabilize record your **Expired Percent O₂** and your **Expired CO₂ Number**.
- 13) Remove the bag from the analyzers and prepare it to be used by the next subject.

Bio 202: Human Anatomy and Physiology

Lab 1: Scientific Reasoning Protocol

I. Lab Goals

Your objectives for this lab are to:

- 1) Gain an appreciation for how science is done and develop your critical reasoning skills by developing your own hypotheses and designing your own experiment.
- 2) Learn how to use LabScribe, a sophisticated computerized system for digitizing and analyzing output from physiological transducers that you will use throughout the semester.

II. Introduction

We all know what it means to be stressed – or do we? Physiologists recognize a ‘stress response’ as: 1) the activation of the sympathetic division of the autonomic nervous system, and 2) the release of stress hormones (glucocorticoids) from the adrenal glands. Sensations associated with a stress response include increased heart rate and increased sweating. Such responses may help us to get out of a jam (i.e. fight or flight), or they may just make us feel uncomfortable. Either way, these responses to stressful situations are measurable, and thus useful for this lab.

In this lab you will assess each others’ ‘stress response’ by measuring heart rate and sweat using the iWorx box and LabScribe software. The LabScribe software allows you to record, in digital and graphical form, the output from a variety of physiological transducers. A transducer converts a physiological event (for example, a heart beat) into an electrical signal (change in voltage) readable by the instrument. The LabScribe software is surprisingly user-friendly allowing the user to record, real time, changes in physiological variables (like heart rate) and to produce graphical presentations of the data.

In this lab, the LabScribe program receives input from two external transducers. The first transducer measures voltage differences between your fingers. When someone sweats, there is an increase in electrical current flow along the surface of your skin that is reflected in an increase in voltage (Galvanic Skin Response, GSR). The second transducer measures the "finger-tip pulse" that is used to measure heart rate. With each heartbeat, blood pulses to the tip of your fingers, causing a slight expansion; this piezoelectric transducer can detect the pressure in the fingertip with each heartbeat. *NOTE: Don't confuse this pressure transducer with an electrocardiogram, which records electrical events.*

EVERYONE IN LAB IS EXPECTED TO LEARN HOW TO USE LabScribe!

III. Lab Procedure

A. Learn How to use the Equipment & Explore (30 minutes)

Your first job is to learn how to use the LabScribe software and the transducers (see How to Use Equipment section below). To learn the equipment, rotate through the four positions below so that you are comfortable working with ALL of the equipment BEFORE you start the experiment. *It is especially important to learn to use LabScribe* during this lab period before we move on to more challenging experiments where there will be no time for learning the system. Your 2nd goal during this exploration period is to find a puzzling observation that you find interesting on which to base your scientific method & experiments during the lab.

- 1) **LabScribe Technician:** This person will sit at the computer and be responsible for: running the software, making sure that the recordings are in range and all channels are operating, and marking significant events.
- 2) **Transducer Technician:** This person will be responsible for applying the transducers correctly to the Experimental Subject and working with the LabScribe Technician to test that transducers are reading correctly before the experiment. If your heart rate is 25 or 225 beats per minute, something is wrong and you need help!
- 3) **Group Scribe:** This person will be responsible for correctly recording all of the data the group collects and making sure all lab partners leave the lab with ALL of the data. Also record comments on other pertinent information (e.g., subject was giggling uncontrollably, so data are unreliable).
- 4) **Experimental Subject:** This person will do what the experiment requires.

B. Hypothesize (10 minutes)

Now that you have some sense of what things affect heart rate and GSR, work with your team to develop a simple hypothesis to test during lab today. Use something that you noticed during your exploration as a ‘puzzling observation’ and work from there to form a causal question and a hypothesis. REMEMBER, your hypothesis should be about the idea (stress) not what you are actually measuring (heart rate & GSR). Also, your hypothesis does not have to be right!!!!!! You can receive full credit on your Lab Write-up with a hypothesis that your data does not support. To develop a hypothesis, use your imagination, but please don't offend your experimental subjects or anyone else in the lab! TAs will

remove anyone displaying inappropriate behavior. You are NOT allowed whatsoever to use any kind or type of physical stress.

Example Hypothesis: Being asked to do math in one's head is stressful

C. Design Experiment & Make Predictions (20 minutes)

Design an experiment that tests your hypothesis. Things you should consider in designing your experiment include:

- 1) Test your hypothesis as directly as possible. Does the prediction grow logically from the hypothesis? Will the data you collect using this experimental design allow you to determine if the predictions are met?
- 2) Controls. Have you controlled in some way for all the extraneous / confounding variables that you think could influence heart rate and GSR?
- 3) Appropriate Length. Can the experiment be done in the time allotted? On the other hand don't try to leave as soon as possible and end up collecting so little data that you can't draw conclusions from your results (see Term List section on sample size & replication).

Example Experiment: We will attach GSR and pulse pickup transducers and wait for subject to provide stable values for heart rate and skin conductance. We will then record baseline GSR and heart rate for the subject. Then the subject will be given one level of math problem (easy or difficult) and we will again record GSR and heart rate. After waiting for the subject to return to baseline we will then give them the other level of math problem.

Example Prediction: Heart rate and skin conductance will increase as difficulty level of math problems increases.

D. Conduct Experiment (60 minutes)

Carry out your experiment being sure to rotate positions so that everyone becomes comfortable with all of the equipment. Be sure to mark relevant time points in the LabScribe recording with clear comments. Try to keep testing conditions uniform for each subject (control those extraneous variables!!). Collect data from each member of the group in order to increase your sample size and, therefore, your confidence in your data. When you have finished make sure that every lab member has all of the lab data to take home and use for their write-up.

E. Data Analysis & Presentation (Remaining Time)

Enter the data into a graphing program such as excel and look at it. Which data are the most significant? Would it be better to present raw numbers, or convert the numbers to percent change? Is the best approach to look at mean values for the group, or values for each individual? We suggest you look at your data in more than one way. Then decide on the most effective way to present the data. Some example tables & graphs are in the excel data sheet. Team members may decide how to present data together, but remember the write-ups must be done individually.

IV. How To Use Equipment

A. Setting up the Hardware

The hardware should be already set up for you. However, it is your responsibility to make sure that the equipment is set up properly and that you are able to record the output from each device. The equipment used today will consist of GSR electrodes, a GSR meter box, a pressure transducer and the iWorx box that is in turn connected to the computer. Check the following:

- 1) The iWorx box is connected to the computer via a USB cable.
- 2) The GSR electrodes are plugged into the GSR meter box.
- 3) The GSR meter box is connected to Channel 1 on the front of the iWorx box. GSR determination is based on the fact that the difference in voltage between two recording electrodes increases when the current flows increases. This happens, for example, when a person develops sweaty hands – salt water is a good conductor. To record GSR attach the two electrodes to two different fingers of one of the subject's hands. The electrodes should be snug, but not too tight in order to work correctly.
- 4) The white pressure transducer that we will use to measure heart rate should be plugged into Channel 3 on the front of the iWorx box. To record heart rate attach the pressure transducer

firmly to the palm side of the thumb (either thumb pad or joint) using the Velcro wrap. In order to get a good recording, the transducer should fit snugly but should not be so tight as to prevent blood circulation in the fingertip.

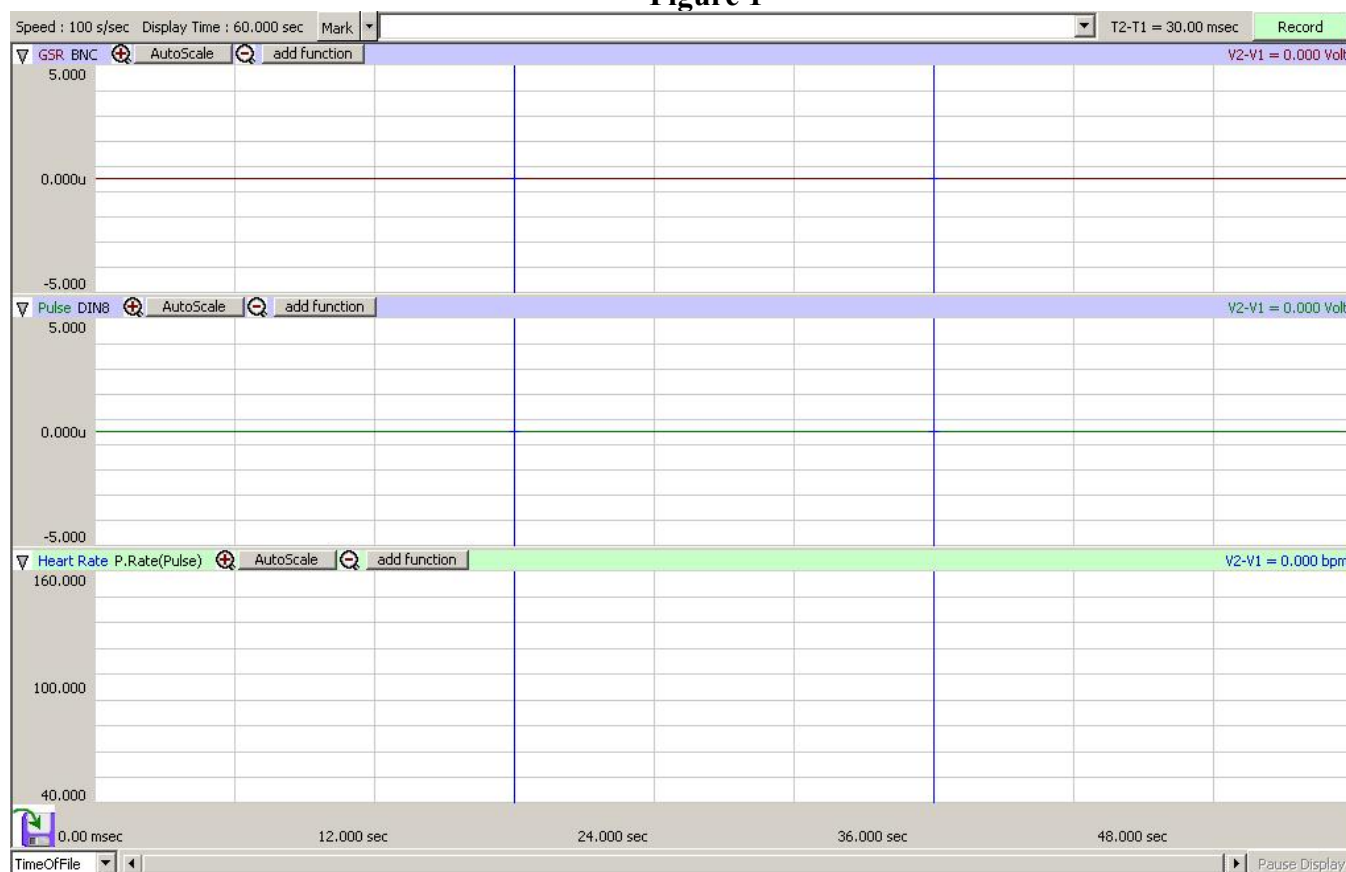
- NOTE. You should attach the GSR and pressure transducer to the same hand of the subject so that the subject only has to keep one hand still.

B. Setting Up LabScribe

The computer & iWorx box should be on and ready to go, but if not follow these steps. The order is critical!!

- Turn on the iWorx box. The switch is on the back of the box. A red light on the front of the box should light up.
- Turn on the GSR meter box. A red light should come on.
- Double Click on the LabScribe icon located on the desktop in order to open the program. You should see something similar to Figure 1.
- If not, select '**Settings**', then '**Bio 202**', then **GSR-Pulse**.
- If that option is not available select **File**, then select **Open**, then tell the computer to 'Look In' the '**Data(D:)**' drive. In the D drive look in the folder '**iWorx**'. In the folder iWorx open the folder labeled '**data**'. In the folder data, double click on the file '**GSR.iwxdata**'

Figure 1



From this screen you can see that you are recording on 3 channels & other features.

- Channel 1 = GSR.** This shows the difference in conductance between the subject's 2 fingers in units of Volts.
 - The range on the left side of this box should read from -5 to $+5$ Volts
- Channel 2 = Pulse.** This will show the output from the pressure transducer on the subject's thumb. It will look like a series of peaks. Each peak marks an increase in pressure due to a heart beat (i.e. peak = heart beat). The y-axis is amount of pressure, not beats per minute.

- The range on the left side of this box should read from -5 to $+5$
- **Channel 3 = Heart Rate.** This channel basically counts the converts how often the peaks occur in Channel 2 (remember peak = beat) into beats per minute. Thus this channel does not display any new information that is not in channel 2. It displays the same information in a format that is easier to understand.
 - The range on the left side of the box should read from 40 to 160 beats/minute
- **Speed.** In the top left of the screen check that your recording speed is recording 100 events per second.
- **Display Time.** In the top left of the screen you should see that the display time (the length / x-axis of the screen) is set to 60 seconds. To change the display time at any time use the following buttons:
 - **Zoom In.** This button at the left-middle top of the screen *looks like a single mountain*. If you click this button the amount of time displayed on the screen will be halved.
 - **Zoom Out.** This button at the left-middle top of the screen *looks like two mountains*. If you click this button the amount of time displayed on the screen will be doubled.
- **Mark box.** In the middle of the top of the screen there is an empty text box with a button labeled **Mark** to the left of it. While LabScribe is recording you can type something into the box (e.g. Janie baseline) and when you hit Mark a line will appear on the screen marking that time with the text at the bottom of the screen. NOTE that *marks can only be added while you are recording* – not after you hit Stop or before you hit Record.
- **Double Cursors button.** On the right of the Zoom Out button is the double cursors button which looks like a recording with 2 blue lines running vertically through it. You will want to use this button when we get to analyzing results in order to select a portion of the recording. Try hitting this button and moving the cursors by clicking on them and holding down the button as you move them.
- **AutoScale.** In the upper left corner of each channel is an AutoScale button. This button changes the Y axis of its channel to match the highest and lowest reading currently on the screen. If the subject moves and there is a large disturbance on the screen, you would want to wait till it moved off the screen and then hit AutoScale to see more the recording more clearly. You can also change the scale and use AutoScale when analyzing data. So, don't worry if something is a bit offscreen while you are recording. The data is still there you can just change the scale when you analyze the data to find it.

C. Practice Recording Data

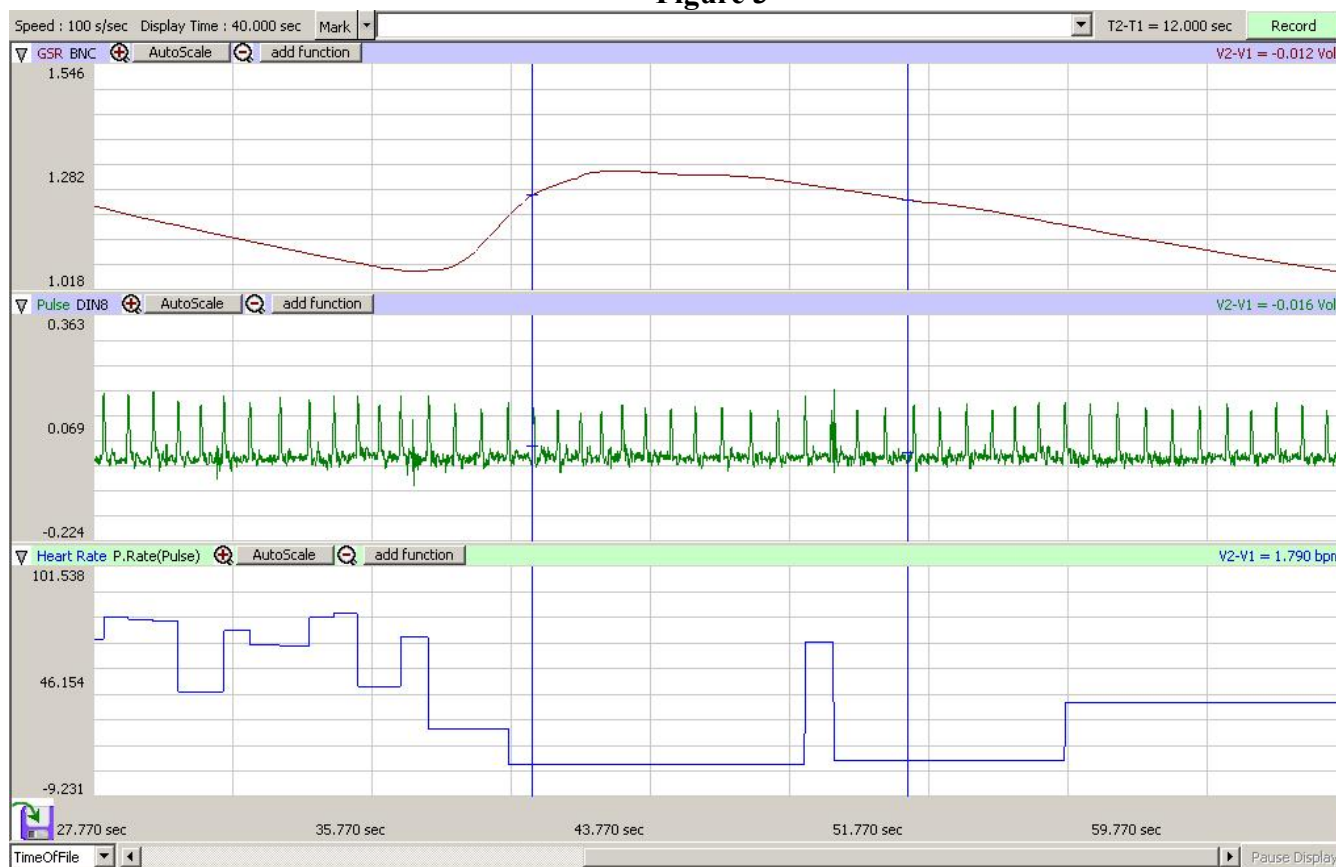
- 1) Connect the GSR and pressure transducer to the subject's hand. The transducers will not work correctly if they are too tight or too loose, so take a few tries to put them on correctly. *The subject should be relaxed and very still with hand resting on the lab bench palm up. If you are the subject, don't watch the screen!* Movement and watching the screen can change your pulse & GSR.
- 2) Click **Record** to begin recording. If you are not seeing any movement in any of the channels on your screen (i.e. they appear flat) hit the **AutoScale** button. Your initial recording may look similar to Figure 2 and require some adjustments to get good recordings of the events and meaningful data.

Figure 2



- Channel 1 Adjustments.
 - Make sure the leads are neither too tight nor too loose.
 - If that does not work try slowly turning one of the knobs on the GSR meter box.
 - Channel 2 & 3 Adjustment.
 - **MAKE SURE the peaks in Channel 2 point upwards!!!** (not like in figure 2). If not, right click while the cursor is in the channel 2 area and select **Invert**.
 - Again, make sure the pressure transducer is neither too tight nor too loose.
 - If all else fails, change hands.
- 3) To stop recording click **Stop**. *When you click Record the next time it will add onto the recording you have already, not record over it. However, if you close LabScribe and then re-open again you will lose any data already recorded.*
 - 4) **Practice clean measurements.** *You will need at least 30 seconds of clear recording for each level* (baseline, level 1 and level 2). Practice remaining completely still for 2 minutes. See how moving even your other hand or other parts of your body affects the recordings. Clear good measurements should look like Figure 3. Note that GSR may look very different.
 - 5) **Practice adding marks** while recording and see whether you think you should hit the mark button before you ask a question or while you ask it.
 - 6) **Practice different jobs.** Make sure everyone knows how to put on the equipment (GSR leads 7 pressure transducer) and how to work LabScribe.
 - 7) **Practice questions you think are stressful.** Try asking your lab partners questions or things that you think could be stressful and interesting enough to base today's lab on.
 - 8) **Practice returning to baseline.** See how long it takes after a stressful question for measurements to return to baseline. *Usually people record for 2 minutes at each level during the experiment* so that they have at least 30 seconds clean after the stimulus / treatment was applied and time to return to baseline before the next stimulus / treatment.

Figure 3



V. Converting LabScribe Recordings to Numbers

Your data set will consist of a long recording with Marks placed at relevant points along the recording. But how will you extract quantitative information from this recording?

- 1) Select **View**, then **Analysis**
- 2) Find the section of data you want to analyze by using any or all of the following techniques.
 - Zoom Out or In using the buttons until you can see the marks you are interested in.
 - Select **View**, then **Marks**, then **Marks** again. A marks dialog box will appear displaying each mark as a row. Select the row of the Mark you wish to go to and click 'Go To Mark'.
 - **Right click** on the channel you are interested in. Select **Scale**, then **AutoScale** to change the y-axis in that channel.
- 3) Select the '**Double Cursors** button'.
- 4) Go to the '**Add Function**' button (top left of your screen). Click **Add Function** then select **General**, then select **Time1**. Repeat the process selecting **Time2**, **T2-T1** (Time2-Time1), and **Mean**. You should now see at the top of each of the 3 channels values for Time1 (the time where the leftmost cursor is located), Time2 (where the 2nd / right cursor is located), T2-T1 (the length of time between the 2 cursors), and the Mean of the values between the 2 cursors.
- 5) Move the cursors to select 30 seconds of clean data. The length of data selected can be seen in the T2-T1 column. See Figure 4.
- 6) GSR. With 30 seconds of clean data selected, record the mean value for GSR between those cursors on your in-class worksheet.
- 7) Heart Rate / Pulse. There are 2 ways to find beats/minute from the data collected.
 - Take the mean from 30 clean seconds of Channel 3.
 - If channel 3 is in any way acting weird, or you want to double check it, count the number of peaks (beats) within your 30 clean seconds and multiply by 2 to get beats/minute.
- 8) Repeat the steps above until you have analyzed all of your data you have collected so far. You may either stop and

analyze data after each subject or wait and do it all at the end.

- 9) To get back to recording data select **View**, then **Main Window**.
- 10) If you do not have time to analyze all of the data during class you may save it and finish analyzing it in the computer lab in LSC 102. To save your data
 - Select **File**, then **Save As**, then choose a file location (desktop is easiest). Type in a file name, so that you will be able to identify it and click **Save**.
 - You may then email yourselves the file or transfer it to a flash drive.
- 11) When you finish collecting data, turn off the GSR meter box.

Figure 4

