User Manual

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Patents and Patents Pending

Table of Contents

1. MouseOx® Plus Configuration Chart

The fundamental MouseOx® Plus package comes with the MouseOx® Plus control unit and basic software. Features of this system are listed in the following table.

	MouseOx® Plus Standard Features

Software	Parameters:
	  - Arterial Oxygen Saturation
	  - Heart Rate
	  - Breath Rate
	  - Pulse Distention
	  - Breath Distention

	Vital Signs Numeric Summary
	Anesthesia Alarms
Hardware	Analog Pulse Signal Output

In this manual, descriptions of the product will include all of the following accessories.

MouseOx® Plus has a number of accessories available to enhance your ability to make measurements in many different environments and conditions. Details about each of these appear in the following table:

Accessory	Description	Inclusions

Premium Monitoring Software	Added functionality of software:
	  - Vital Sign Real-Time Trends
	  - Scheduling Software
	  - Diagnostic Averaging w/ Recording
	  - File Recording and Event Marking
		- Premium Monitoring Software

Body Temperature Option	System that monitors real-time rectal temperature measurements on subjects	- Body Temp Software
		- Rectal Temp Sensor
		  - Regular w/ 6 ft Cable
		    OR
		  - MRI-Compatible w/
		    20 ft Cable

Conscious Subjects Option*	System that measures vital signs on conscious, unrestrained subjects	- Conscious Subjects Software
Conscious Measurements Enclosure (CME)	Animal holding chambers for conscious measurements – rat and mouse sizes	- Rat or Mouse CME

MRI Compatible Oximeter Sensor	Sensor that measures vital signs in small-animal MRI environments	- MRI-Compatible Sensor w/
		  5 ft of Fiberoptic Cable and
		  15 ft of Electronic Cable

STARR-Link™ Analog Output Module	Device that converts all MouseOx® Plus parameters to analog voltages	- 8-Channel STARR-Link Box - One 6' USB 2.0 Cable
Multiplexer - 16	Device that coordinates MouseOx® Plus measurements on any one of up to 16 conscious subjects, one at a time	- 16-Subject Multiplexer Box - One 6' USB 2.0 Cable - One Patch Cable
Multiplexer - 8	Device that coordinates MouseOx® Plus measurements on any one of up to 8 conscious subjects, one at a time	- 8-Subject Multiplexer Box - One 6' USB 2.0 Cable - One Patch Cable
Multiplexer - 8 w/ Temperature	Device that coordinates MouseOx® Plus measurements on any one of up to of 8 conscious subjects, one at a time, with 8 corresponding ports for temperature sensors	- 8-Subject Multiplexer Box w/ 8 Temperature Ports - One 6' USB 2.0 Cable - One Multiplexer Patch Cable - One Temperature Patch Cable

*Requires Premium Monitoring Software

2. MouseOx® Plus Packaged Parts

The following is a list of parts that are included in your MouseOx® Plus shipping box:
- MouseOx® Plus Control Box
- 12 VAC Power Supply (you will have one or the other of the following)
  - US and Canada……Standard External Plug-in Power Supply
  - International……….Power Transformer and Power Cord to fit your wall receptacle
- 6-foot USB Cable
- CD with MouseOx® Plus Software and Electronic User Manual
- Universal Cable
- MouseOx® Plus User Manual (new customers only)

Under separate cover, you should also receive your choice of the following:
- Selected size of CollarClip™, ThroatClip™, Rat Foot Sensor and/or Thigh Sensor chosen at purchase

Please check to see that you have all of these parts. If you find any part missing, please contact STARR Life Sciences® at 1-866-978-2779, or by e-mail at support@starrlifesciences.com.

3. Computer and Electrical Requirements

The MouseOx® Plus has the following computer hardware requirements:

Processor
PC with Pentium®-class processor (Pentium 1 GHz or higher recommended)

Computer Hardware
CD-ROM drive (optional for software installation, but you can also download from the internet)
VGA or higher resolution monitor (Super VGA recommended)
2.0 USB port

Operating System
Windows® XP, Vista, 7 or newer
MouseOx® is compatible with Apple® computers when using a Windows® emulator

Memory
1 GB RAM
5 MB Hard-Drive Space for program (does not include data files)

Minimum Screen Resolution
1024 by 768 pixels

The MouseOx® Plus has the following power requirements:

Operating Wall Voltage:
100-240 VAC @ 50-60 Hz **(You must use ONLY the STARR Life Sciences power supply sent with your MouseOx® Plus!)**

Device Operating Voltage:
12 VAC

Max Operating Analog Current:
200 mA - MouseOx® Plus
200 mA - Multiplexer™

Max Operating USB Current:
    85 mA - MouseOx® Plus
    40 mA - Multiplexer™
    180 mA – STARR-Link™
    (When using more than one of these devices, you must not operate the computer on batteries.)

4. Warnings, Cautions and Operational Notes

WARNING	Replace control unit if housing is broken or cracked.
WARNING	DO NOT substitute cables or power supply. Use only parts that are packaged with the MouseOx® Plus device.
WARNING	Investigational device only.
WARNING	NOT for use on humans.
WARNING	DO NOT place control box in an environment with oxygen fraction greater than 30%.
WARNING	DO NOT immerse control box in liquids.
WARNING	DO NOT immerse lead ends of sensor cable in liquids.
WARNING	DO NOT directly expose control box to flammable or explosive gases.
WARNING	Replace power supply if cable is frayed.
WARNING	Inspect sensor cables after each use. Replace sensor cables if cable insulation is frayed or wires are exposed.
WARNING	Replace control box if inside is suspected to be wet.
WARNING	DO NOT use STARR Life Sciences® MouseOx® Plus power supply or sensors with any other device.
WARNING	Wipe external surfaces of box, sensors and leads, and sensor clip with light detergent and water after each use on animals.
WARNING	Avoid spilling fluids on control box.
WARNING	Unplug the power cord to the controller when the device is unattended.
WARNING	Use only the power supply shipped with your MouseOx® Plus with this device. Do NOT substitute power supplies.
WARNING	DO NOT place the MouseOx® Plus in a region with high magnetic fields as it contains ferrous material.
CAUTION	The MouseOx® Plus power supply has an AC output. Do not substitute the power supply.
OPERATIONAL NOTE	Do not place any sensor on any cannulated appendage. Doing so could result in a poor signal due to blood flow obstruction.

OPERATIONAL NOTE	If the animal has dark fur, or signals are very small, the sensor location should be shaved.
OPERATIONAL NOTE	The user must have administrative rights to access computer USB ports and access to the software that is loaded onto the computer from the MouseOx® Plus CD.
OPERATIONAL NOTE	Before installing an upgrade or re-installing a current software version, you must first un-install any old versions of MouseOx® Plus software.
OPERATIONAL NOTE	It is recommended that the computer to be used with MouseOx® Plus not be set to go into System Standby or System Hibernate sleep modes.
OPERATIONAL NOTE	The MouseOx® Plus system DOES compensate for dysfunctional hemoglobin.
OPERATIONAL NOTE	It is strongly recommended to use a light blocking cloth or other light-reducing mechanism to shield sensors and prevent interference from ambient lighting.
OPERATIONAL NOTE	Always use only the specified sensor clip for your particular application, paying particular attention to the weight range.
OPERATIONAL NOTE	You must never attempt to open a data file while it is open and being written to by the MouseOx® Plus software.
OPERATIONAL NOTE	When changing a sensor, you must exit the software then restart so that you can select the correct settings for your new sensor!
OPERATIONAL NOTE	MouseOx® Plus sensors are not backwards-compatible with the original MouseOx® system, and original MouseOx® sensors are not forward-compatible with the MouseOx® Plus.
OPERATIONAL NOTE	We recommend that you do not use the rat cage for mice because the spring applying upward force on the entry wire represents a much larger percentage of the weight of a mouse than it does of a rat. This will tend to lift the animal.
OPERATIONAL NOTE	When using the Conscious Measurements Enclosure (CME), DO NOT insert partial pieces of food into the food tray as this may cause them to jam up at the base, restricting the food supply to the animal.
OPERATIONAL NOTE	Always use bedding in the CME since it has the effect of blocking light that can reflect off of the enclosure bottom surface, which can cause distortions in the measurement signals.
OPERATIONAL NOTE	Make sure that your computer's operating system language is English.

Conformance with Regulatory Standards

The MouseOx® Plus, Multiplexer™ and Temperature Sensor have been tested to the Low Voltage Directive (72/23/EEC) and amended directive (93/68/EEC), and EMC Directive (2004/108/EC), based on the following standards: EN 60950-1, EN 55011 and EN 61326-1.

The MouseOx® Plus device was shown to be in compliance with all requirements except for the Electrical Fast Transient/Burst and Surge tests. For these tests, the device tested only to Level C, whereas the directive requires Level B. **The fault, which is the same for both incidences, does not involve a safety**

issue, either to the user or to the subject under test. Because the fault is only a possible, though unlikely, inconvenience to the user, the company has chosen not to address it at this time.

The problem can manifest itself when a power surge or draw on the main power line causes the voltage level inside the MouseOx® Plus to fluctuate such that USB communications with the computer are severed. To solve this, shut down the program, unplug the USB cable on either end, wait about 10 seconds, plug it back in, wait another 10 seconds, then restart the program. If this problem persists, a better solution is to purchase a universal power supply (UPS), which can be obtained from any electronics or computer store. We recommend that you purchase a UPS with a minimum of 450 volt-amps.

5. Physiologic Parameters

The MouseOx® Plus provides the following physiologic measurements:

Oxygen Saturation (S_pO_2)
The MouseOx® Plus provides real-time percent oxygen saturation, in % of functional arterial hemoglobin.

Heart Rate
The MouseOx® Plus provides real-time cardiac pulse rate. Heart rate is given in bpm.

Breath Rate
The MouseOx® Plus provides a real-time breath rate measurement that is updated every few seconds. Note that this parameter is actually derived from respiratory effort, not airflow, and will be present even if the animal is experiencing an obstructive apnea, as long as breathing effort is present. Breath rate is given in breaths per minute or brpm.

Pulse Distention
Pulse distention is a measurement of the change in distention of the arterial blood vessels residing between the sensors pads due to a cardiac output pulse. It is a direct measurement of changes in local blood volume that accompany each cardiac pulse. For a given vascular compliance, pulse distention can also provide a surrogate for pulse pressure. This will be explained in more detail below.

Pulse oximetry measures the oxygen content of **arterial** blood. Blood is identified as being arterial because of its pulsatile nature. This pulsation is identifiable because it causes a cyclic change in the absorption of light energy from the red and infrared LEDs (Light Emitting Diodes) as it passes through the tissue due to the presence of changing quantities of blood that occur with every heart beat. All other non-blood material such as skin, fat and muscle do not change light absorption level with heart rate. Additionally, non-arterial blood (venous or capillary) does not change light absorption level with heart rate either, because pulsation is essentially completely diminished by the termination of the arterioles[1]. Thus, our pulse oximetry measurement is being made across only pulsating blood, which can only be arterial. Because this blood is arterial, it possesses systemic arterial oxygen content, which is what we measure.

Pulse distention is simply a measurement of the change in the effective path length of the light that passes through only the arterial or pulsating blood, and it has true linear distance units of μm. You could picture this by thinking of placing all of the arterial blood residing in the light path between the sensor pads into a cylinder that has a cross-sectional area equal to the cross-sectional area of the column of the light beam passing from the LEDs to the photodiode. If the cylinder had one inlet and one outlet for the blood to enter and exit, then the level of blood in the cylindrical chamber would rise with each cardiac ejection stroke, and lower during each subsequent cardiac filling phase. The change in height of the blood in that cylinder between ejection and filling, or systole and diastole, would then be measured directly as pulse distention.

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[1] AC Guyton and JE Hall, Textbook of Medical Physiology, 9th ed., W.B. Saunders Co., 163, 1996.

Pulse distention can be used to determine if you have sufficient arterial blood to be able to make good oximetry measurements. As a practical example, if you were to place the sensor across your Achilles tendon, which is very poorly perfused, you would not be able to make an oximetry measurement, and the pulse distention would be nearly 0 because there is essentially no arterial blood in the tendon. On the other hand, placing the sensor across a small artery would provide very large signals, indicated by large pulse distention values.

Thus, in order to make an oximetry measurement, there must be sufficient arterial blood in the light path, and because pulse distention quantifies the amount of arterial blood in the light path, it provides a good indicator of the ability to make an oximetry measurement. **The larger the pulse distention value, the more arterial blood will be available to make oximetry, as well as heart rate and breath rate, measurements.**

Pulse distention is only calculated when making anesthetized measurements. During measurements made from conscious subjects, pulse distention is not calculated, but is replaced by Activity, although it is displayed for aiding in proper sensor placement.

Breath Distention
Breath distention is a measurement of the change in distention of the arterial blood vessels residing between the sensor pads due to breathing effort. For a given vascular compliance, we have shown in our lab that breath distention provides a surrogate for intrapleural pressure.

The mechanisms that cause the breath distention signal at the sensor site are the same as those for pulse distention, and are described in detail just above. The reason that we can detect a pulsation at breathing rate however, is due to a cyclical pumping mechanism, just like that produced by the heart, that provides a pulse of blood to the periphery, except at the rate of breathing instead of heart rate.

From our own investigation, we have observed that the breath distention signal is caused by a phenomenon that we term the thoracic pump. In the thoracic pump model, pressure changes in the thoracic cavity that accompany breathing or mechanical ventilation, act directly on the large arteries present there. When intra-thoracic pressure is less than its mean, such as during inhalation, the great arteries become slightly dilated in response, causing blood to fill the new dilated volume, which reduces blood flow to the periphery. Conversely, when intra-thoracic pressure rises above its mean level, as occurs during exhalation, this pressure exerts external force on the great arteries in the thorax, pushing more blood into the periphery. This oscillatory increase and decrease in peripheral blood flow that accompanies breathing effort, and which causes a subsequent oscillatory change in LED light absorption at the sensor site, can be calculated as a distention, as is done with the cardiac blood pulse in Pulse Distention described above.

Note also, that this respiratory blood pumping occurs simultaneously with cardiac blood pumping, and the two are actually super-imposed on one another on the actual light absorption signals that we measure. In the human clinical realm, this respiratory effect, if it is large enough to become visible, is referred to as *pulsus paradoxus*, although one should not also apply the connotation of abnormality commonly associated with that term.

Given this model, the breath distention signal would have some useful applications. Because it is caused by changes in intra-thoracic pressure, it may be considered to be a surrogate for intra-thoracic or intra-pleural pressure. Measurement of intra-pleural or intra-thoracic pressure generally involves some sort of invasive or semi-invasive physiologic intrusion, such as use of an esophageal pressure balloon sensor. Correlation of breath distention with intra-thoracic pressure is not absolute without calibration however, but it can be used for trending.

One specific application of breath distention is that it can provide monitoring of breathing effort put forth by the animal. An example of its utility is when an animal becomes over-anesthetized. As breathing effort is impeded by excessive anesthesia, the animal will start to gasp in order to move air as it becomes hypoxic

and hypercapnic. The increase in effort that accompanies gasping can greatly increase the breath distention, and this phenomenon is particularly notable when it is compared with the pulse distention, which does not change nearly as significantly as does breath distention. This utility of these dual phenomenon is captured in our distention-based anesthesia alarms. Thus, breath and pulse distention may be used to alert you to excessive levels of anesthesia.

Another application is an extension of intra-thoracic pressure measurement when it is used in conjunction with tidal volume or air flow rate. In such a case, breath distention can provide a surrogate or trending parameter for work of breathing, or power of breathing.

Breath distention is only calculated when making anesthetized measurements using only the Foot Clip or Thigh Clip sensors. It is not calculated for any measurements made from the neck of an animal.

As with pulse distention, breath distention is given in true linear distance units of μm.

Body Temperature
When you purchase the MouseOx® Plus Body Temperature Option, the system provides real-time body temperature derived from a rectal probe. Temperature can selectively be displayed in either °C or °F,

Activity
Activity is a binary-valued parameter that indicates when the animal is moving versus sitting still. It provides a value of 1 when the animal is moving, and 0 when it is still. It is provided as part of the MouseOx® Plus Mobile Subjects Option, and appears in place of the Pulse Distention parameter on all output displays.

6. MouseOx® Plus Hardware Description

MouseOx® Plus Control Unit
The MouseOx® Plus device is the core element of a robust measurement system that provides many useful measurements, including rectal temperature, measurement of up to 16 animals from one control box and our new Conscious Subjects Software that allows you to obtain measurements on unrestrained, conscious animals. **The MouseOx® Plus Control Unit is NOT backward compatible with sensors or cables from the original MouseOx® device.**

The connections on your MouseOx® Plus control unit are detailed in the figures below.
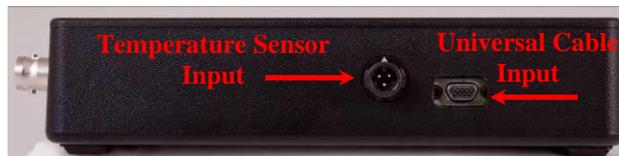
The serial number on your MouseOx® Plus control unit is located on the upper left-hand corner of the label on the bottom of the device. If you call in with questions about your device, you should have this number available.
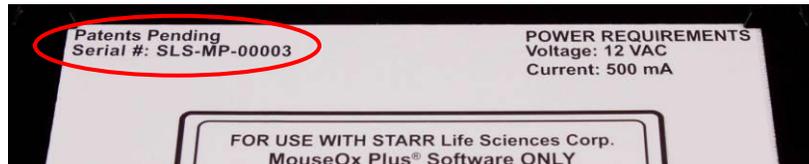
Power Supply
The MouseOx® Plus Control Unit does not have an on-board battery, so all power is provided by the external plug-in power supply. **DO NOT substitute power supplies!!**

For MouseOx® Plus devices sold in the US and Canada, your MouseOx® Plus was shipped with a 120V wall-mount power supply. For MouseOx® Plus devices sold internationally (outside US and Canada), your MouseOx® Plus was shipped with a desktop 120V/230V switchable transformer and the appropriate wall receptacle power cord for your country.

USB Cable
MouseOx® Plus comes standard with a 2.0 USB cable. The USB cable must be connected between the MouseOx® Plus and the computer in order for data to be transferred. The order of connection is inconsequential.

Universal Cable
The Universal Cable, shown below, connects the sensor clip to the MouseOx® Plus control box. The cable assemblies should be connected as shown in the following figures, depending on the application.

Anesthetized Applications
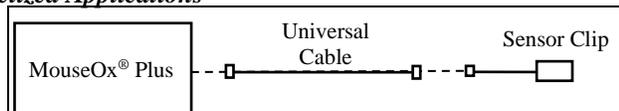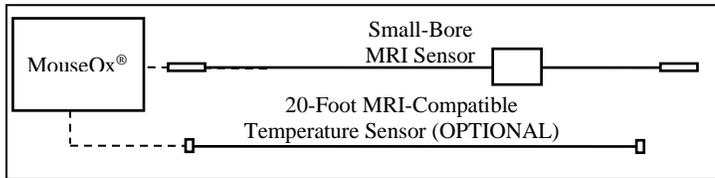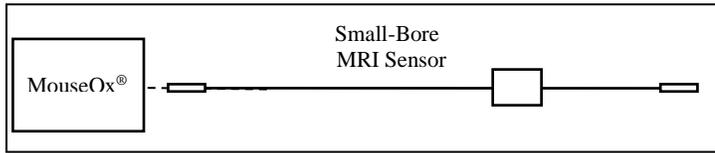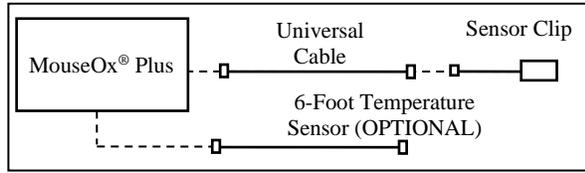
Awake/Mobile Applications
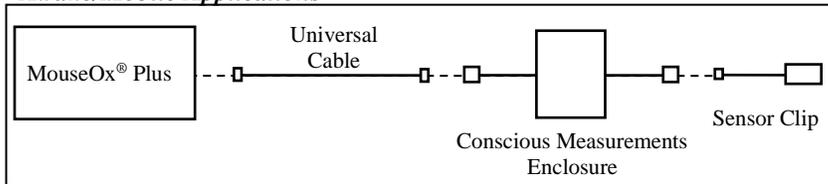
Note: Requires Conscious Subjects Software

MouseOx® Plus Sensors

MouseOx® Plus sensors are designed to provide the highest quality measurements. To assure the highest level of accuracy it is recommended that you replace each sensor after 100 hours of use. Each sensor is shipped with a "Handle Support" in place across the finger tabs to prevent plastic deformation that can accumulate over long periods of time. When not in use always store the sensors with the handle clamp in place.

MouseOx® Plus sensors are visually differentiable from those of the original MouseOx® by virtue of the **orange** or **black** sleeve adjacent to the connector. MouseOx® Plus sensors are not backward compatible

with the original MouseOx® system. Likewise, original MouseOx® sensors are not forward compatible with your MouseOx® Plus.

MouseOx® Plus Wavelengths
The MouseOx® and MouseOx® Plus sensors use wavelengths of 660 nm (red) and 940 nm (infrared).

Calibration of the MouseOx® Plus
Your MouseOx® Plus was calibrated at the factory, and will be fully calibrated when you receive it. Calibration constants are contained in the FLASH memory of the device. If a change in calibration is suspected, please contact STARR Life Sciences® Corp. technical service at 1-866-978-2779, or by e-mail at support@starrlifesciences.com.

Cleaning the MouseOx® Plus
The MouseOx® Plus device and all accompanying cables should be cleaned using only light detergent and water. Additionally, we have a link to an approved gas sterilization vendor.

Temperature Sensor
See section detailing the Temperature Sensors

Multiplexer-16, Multiplexer-8 and Multiplexer-8 w/Temperature
See section detailing the Multiplexer™ models below.

7. Installing the MouseOx® Plus Software

If you are installing an upgrade or are re-installing a current version of the MouseOx® Plus software, you must first un-install any versions of the original MouseOx® or MouseOx® Plus software.

Please note that the software has limitations when Windows® is set to a language other than English. The following steps must be completed to adjust the system language:
In Windows XP:
Go to: Control Panel → Date, Time, Language, and Regional Options → Regional and Language Options Formats tab

- In the drop-down menu labeled "Select an item to match your preferences"
- Select English (United States)

In Windows Vista:
Go to: Control Panel → Regional and Language Options Formats tab

- In the drop-down menu labeled "Under Current Format"
- Select English (United States)

7.1. Un-Installing Old Software

Skip this section if you are installing MouseOx® software for the first time.

1] On your computer, click the "Start" button from the desktop, then click on either "Settings" then "Control Panel," or just "Control Panel." The order depends on which version of Microsoft® Windows® you have.

2] On the Control Panel Menu, double-click on "Add or Remove Programs" or "Add/Remove Programs" or "Uninstall a program (under "Programs)" or "Programs and Features" button.

3] After the list of software has been populated, find "MouseOx Revision X.X," then click on the "Add/Remove" or "Change/Remove" or "Uninstall/Change" button. Ignore any error messages that may arise.

4] Make sure all programs named "MouseOx" are removed from this list, in the event that a previous version was installed more than once without first un-installing.

5] While remaining in the "Add/Remove Programs" section of the "Control Panel," find "InstaCal and Universal Library," then click on the "Add/Remove" or "Change/Remove" or "Uninstall a program (under "Programs)" or "Programs and Features" button.

7.2. Software Installation

1] Be sure that the USB Cable is disconnected from the oximeter control box.

2] Use the STARR Life Sciences software download page or use the optional MouseOx Plus® CD to open the MouseOx Plus® installation menu.
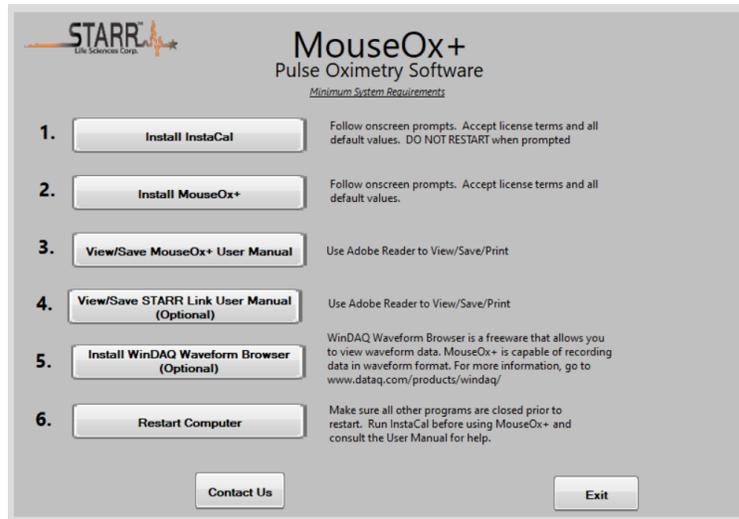
3] Click on Step 1 "Install *Insta*Cal and Universal Library," then do the following:

> **a]** Select "OK" on the WinZip Self-Extractor dialog box.
>
> **b]** On the WinZip Self-Extractor – icalsetup.exe dialog box, select "Setup."
>
> **c]** On the Welcome Screen window, select "Next."
>
> **d]** On the Destination Folder window, accept the default and press "Next." **DO NOT change the default file location.**
>
> **e]** On the Ready to Install Program window, select "Install." At this point, the *Insta*Cal software will load. However, if you have Vista, a Windows Security dialog box may pop up to ask if you want to install this software. This will occur at various stages during the operation. Click

"Install" each time, or click the "Always trust software from Measurement Computing Corporation" check box, then click "Install."

f] On the Install Shield Wizard Completed window, select "Finish."

g] When asked to restart the computer, answer "No."

4] Once you have finished, the MouseOx® Plus installation menu will reappear. Click on Step 2 "Install MouseOx Plus," then do the following:

a] On the MouseOx Plus Revision X.X Setup dialog box (X.X represents the version of software that you are currently loading), select "OK."

b] On the next dialog box, click on the ==computer icon== button to start the download. Although you have the option to change the directory, **DO NOT DO THIS**.

c] On the MouseOx Plus Revision X.X Setup – Choose Program Group dialog box, although you have the option to choose a new program group, **DO NOT DO THIS**. Simply select "Continue" to accept the default group "STARR Life Sciences." After selecting "Continue," the files will be downloaded.

d] Select "OK" at the prompt after the download, and the installation menu will reappear.

5] (OPTIONAL) If you want to download the WinDaq® Waveform Browser, click on Step 3 "Install WinDaq Waveform Browser," then do the following:

a] On the Welcome dialog box, select "OK."

b] On the License Agreement dialog box, select "Accept and Continue."

c] On the Select Destination Directory dialog box, although you have the option to choose a new destination directory, **DO NOT DO THIS**. Simply select "OK" to accept the default directory "C:\WINDAQ." After selecting "OK," the files will be downloaded.

d] On the Select Program Manager Group dialog box, although you have the option to choose a new group name, **DO NOT DO THIS**. Simply select "OK" to accept the default group name "WinDaq."

6] If you want to download or view the MouseOx® Plus and/or Windaq® User Manuals, click on their indicated buttons and follow the simple instructions.

7] After completing the download of any of the optional software, the MouseOx® Plus installation menu will reappear. At this time, all of the software is now loaded and ready to use. You may now close the installation menu.

8] At this point, **you must restart your computer** before you can run any software.

9] After restarting, you will need to complete a check to ensure that proper USB communication with the computer has been established. Please do the following:

a] Reconnect the USB Cable to the oximeter control box.

b] Select "InstaCal" inside the "Measurement Computing" group under the Windows® Start Menu.

c] After about 30 seconds, a message should appear indicating that *Insta*Cal detected a new Plug and Play Board.

d] If the new Plug and Play board message does not appear, then exit *Insta*Cal and restart *Insta*Cal a second time (step 10b).

e] Select "OK" on the message that indicates that the device has been recognized.

f] Close *Insta*Cal.

10] Start the MouseOx® Plus program using the new "MouseOx" icon inside the "STARR Life Sciences" group under the Windows® Start Menu. You are now ready to go.

Multiplexer note: If the Multiplexer is connected and the MouseOx® Plus and Multiplexer are not detected, there may be a problem with the way *Insta*Cal has the products organized. The MouseOx® Plus (USB1408-FS) needs to be set to board number 0. The Multiplexer (USB-1024LS) needs to be set to board number 1.

Close the MouseOx® Plus software and open *Insta*Cal then follow these steps:

Step #1-Make sure all of the USB plugs are connected correctly to the host computer. Open *Insta*Cal and select "Yes" or "Ok" for any questions asked of you. This is just *Insta*Cal connecting with the devices.
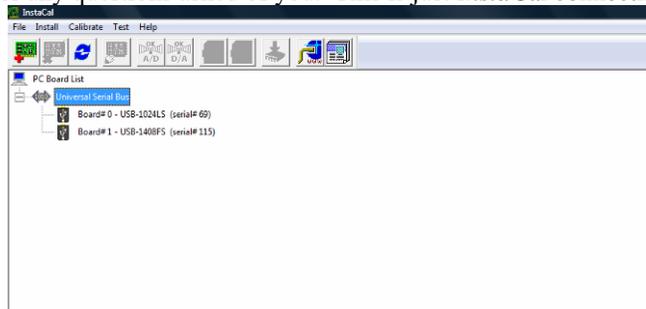
Step #2-Put the devices in the correct order. Some windows versions may require that these devices be in the correct order. USB-1408FS is the DAQ card inside the MouseOx Plus, this card must be board #0. USB-1024LS is the DAQ card inside the Multiplexer, this card must be Board #1.

Use the mouse to right click on the board to display the above drop down menu. Select "Change Board#..."

Organize the board numbers until the USB-1408FS is board number 0 and the USB-1024LS is board number 1.
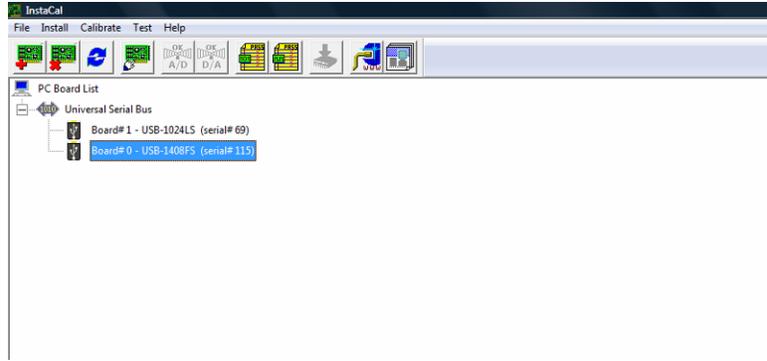
Close *Insta*Cal and run the MouseOx® Plus software. The system should operate as intended.

8. Using Sensor Clips in Anesthetized Applications

Instructions on the use of each type of STARR Life Sciences® sensor is described in this section.

System Configurations

To use your sensors, the components should be connected as illustrated in the following schematic.

Sensor Clips
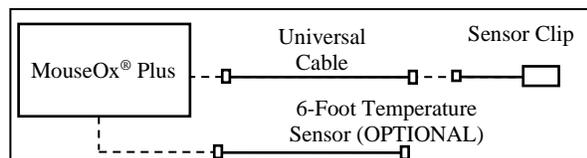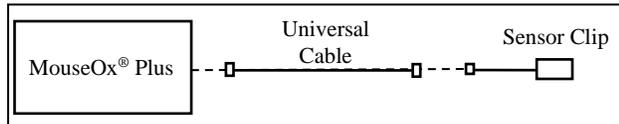
Pulsar™ Small-Bore MRI Sensor
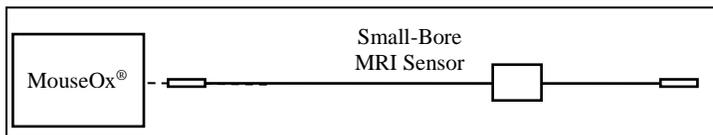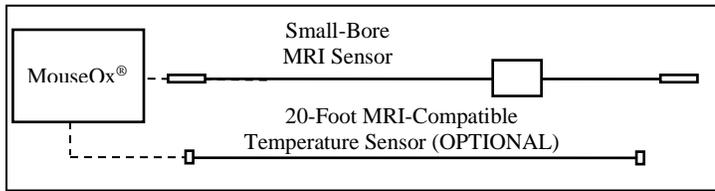
8.1. Sensor Clips

Clip Application and Sizing Chart

Following is the sensor sizing chart. To get **ACCURATE MEASUREMENTS** from your clips, it is imperative that you follow this chart when choosing a sensor for a given animal. Note that the clip size is engraved on the back of each of the clip handles.

Size (Collar or Throat)	Mouse		Rat
	Unshaven	**Shaven**	
XS	1 - 15 gm	1 – 20 gm	N/A
S	> 15 gm	> 20 gm	5 – 30 gm
M	N/A	N/A	31 – 100 gm
L	N/A	N/A	101 – 300 gm
XL	N/A	N/A	301 – 500 gm
2XL	N/A	N/A	> 500 gm
Thigh	< 60 gm		≤ 100 gm
Foot	N/A	N/A	> 100 gm
Paw	>5 gm	>5 gm	N/A

Other Notes

- When changing a sensor type, you must exit the software then restart so that you can select the correct settings for your new sensor!
- MouseOx® Plus sensors are not backwards-compatible with the original MouseOx® system, and original MouseOx® sensors are not forward-compatible with the MouseOx® Plus.

CollarClip™/ThroatClip™ Differentiation

The CollarClip™, which is colored blue, is to be applied from the **back of the neck** of an animal, and can be used in both awake and anesthetized applications. The ThroatClip™ is colored black, it has a much longer wire lead length than that of the CollarClip™, and it is applied from the **front of the neck**. Thus, it is used only in anesthetized applications. **It is important that you use the correct clip for the correct application in order to get good measurements.**

When to Replace Sensor Clips

Slight degradation of absolute accuracy can occur as sensors wear. To ensure the highest level of accuracy it is recommended to replace sensors when any of the following are observed:

1] significant buildup of hair or material covering the clip faces,

2] good signals (data values on the software screen are in color) take an inordinately long time to appear,

3] significant deformation or damage to the sensor that could result in misalignment of optics.

Removing the Clip Handle Support
Before using a sensor clip, you must remove the handle support. To do this, slide the handle support off sideways, NOT over the end, as diagrammed below. **We recommend that you replace the handle support when the clip is not in use.**
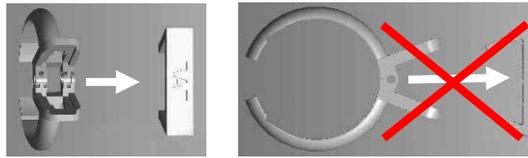
Chemical Hair Removal

The Blank Clips that come with each CollarClip™ or available separately can be used to apply depilatory creams to the sensor site on the animal. This method is preferred over shaving, although not necessary, unless the animal has dark fur. If you do use a depilatory, we suggest that you do so a few hours to a day before you will be conducting tests on your animals.

NOTE: You MUST remove hair from the sensor site of animals with non-white fur.

1] It is recommended that you anesthetize the animal for hair removal.

2] Lay a bead of depilatory cream (e.g., Nair®) around the entire inside of the Blank Clip as shown.

3] Place the clip on the animal in the same location in which testing will be done, and let the clip and depilatory cream remain in place for the duration specified by the manufacturer.

4] Remove the clip, then carefully wash the cream completely off of the animal using swabs and a small water dish. The hair will be removed with the cream when you do this. The depilatory cream must be washed away completely or it will react with the heat of the LED from the sensor. Removing the hair a day or more before using the MouseOx® Plus is strongly advised.

8.1.1. Mouse/Rat Pup ThroatClip™ – Sizes XS and S

Mouse/Rat Pup ThroatClip™ Sizing

Be sure to choose the correct clip for use with a given animal. Use the following chart to pick the correct clip.

Mouse Weight Range (gm)		Clip Size	Rat Pup Weight Range (gm)
Unshaven	Shaven		
1 – 15	1 - 20	**XS**	N/A
>15	>20	**S**	5 - 30

Note that the Clip Size is engraved on the back of the clip handles.

Attaching the ThroatClip™ and Making Measurements

1] Anesthetize the animal, then connect the MouseOx® Plus, Universal Cable and ThroatClip™ sensor.

2] If you are using an animal with non-white fur, you MUST either shave the entire sensor site WELL, or use a depilatory product such as Nair® (See Chemical Hair Removal at the top of the section).

3] With the animal laying in the supine position, push/slide the skin down on either side of the throat, then place the clip over the throat while holding the skin down so that the tines end up BEHIND the ears and in FRONT of the paws. The clip handles should be centered on the throat of the animal. Be careful that the tips of the tines do not catch on the lab surface such that the clip does not close completely.

4] Check that the wiring assembly is connected to the MouseOx® Plus, then start the software. Follow the menu of buttons, choosing those appropriate to your measurement conditions.

5] If signals do not come in quickly, or performance degrades over time, try the following:

- Re-position the sensor   - Shave the animal   - Replace the sensor

8.1.2. Thigh Sensor

Thigh/Rat Foot Clip Sizing

Be sure to choose the correct clip for use with a given animal. Use the following chart to pick the correct clip.

Sensor Type	Mouse Weight Range (gm)	Rat Weight Range (gm)
Thigh	< 60	≤ 100
Rat Foot	N/A	> 100

Attaching the Sensor and Making Measurements

1] Anesthetize the animal, then connect the MouseOx® Plus, Universal Cable and Mouse Thigh sensor.

2] If you are using an animal with non-white fur, you MUST either shave the entire sensor site, or use a depilatory product such as Nair®.

3] Place the animal either prone or supine on the lab bench surface. Place the Thigh Sensor on the THIGH (not the lower leg!) of the animal by using the half arc shape of the sensor to grasp tissue around the thigh bone so that the sensor protrudes in a perpendicular orientation from the thigh. This will help align the sensor with the large arteries that reside next to the bone. Gently press the clip together on the thigh to improve the surface contact.

NOTE: Do not use this sensor on an area other than the upper thigh.

4] Check that the wiring assembly is connected to the MouseOx® Plus, then start the software. Follow the menu of buttons, choosing those appropriate to your measurement conditions.

5] If signals do not come in quickly, or performance degrades over time, try the following:

- Re-position the sensor   - Shave the animal   - Replace the sensor

NOTE: Shaving is NECESSARY for any animal with non-white fur.

8.1.3. Rat ThroatClip™ – Sizes M, L, XL and 2XL

Rat ThroatClip™ Sizing

Be sure to choose the correct clip for use with a given animal. Use the following chart to pick the correct clip.

Clip Size	Animal Weight Range (gm)
M	31 – 100
L	101 – 300
XL	301 - 500
2XL	500 +

Note that the Clip Size is engraved on the back of the clip handles.

Attaching the ThroatClip™ and Making Measurements

1] Anesthetize the animal, then connect the MouseOx® Plus, Universal Cable and ThroatClip™ sensor.

2] If you are using an animal with non-white fur, you MUST either shave the entire sensor site WELL, or use a depilatory product such as Nair® (See Chemical Hair Removal at the top of the section).

3] With the animal laying in the supine position, push/slide the skin down on either side of the throat, then place the clip over the throat while holding the skin down so that the tines end up BEHIND the ears and in FRONT of the paws. The clip handles should be centered on the throat of the animal. Be careful that the tips of the tines do not catch on the lab surface such that the clip does not close completely.

When applying the clip, to facilitate good contact,

> **1] push the skin down on either side of the neck,**

> **2] push down on the handles toward the neck, then,**

> **3] squeeze the tines together around the animal's neck.**

These steps are VERY important in order to obtain good signals.

4] Check that the wiring assembly is connected to the MouseOx® Plus, then start the software. Follow the menu of buttons, choosing those appropriate to your measurement conditions.

5] If signals do not come in quickly, or performance degrades over time, try the following:

    - Re-position the sensor   - Shave the animal   - Replace the sensor

NOTE: When using the 2XL clip, it is recommended that the animal be shaved regardless of fur color.

8.1.4. Rat Foot Sensor

Thigh/Rat Foot Clip Sizing

Be sure to choose the correct clip for use with a given animal. Use the following chart to pick the correct clip.

Sensor Type	Mouse Weight Range (gm)	Rat Weight Range (gm)
Thigh	< 60	≤ 100
Rat Foot	N/A	> 100

Attaching the Sensor and Making Measurements

1] Anesthetize the animal, then connect the MouseOx® Plus, Universal Cable and Rat Foot sensor.

2] Place the animal either prone or supine on the lab bench surface. Place the Rat Foot sensor on the foot of the animal so that the sensor protrudes directly off the end of the foot past the toes, as shown in the figure below.
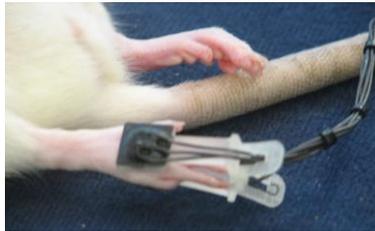
It is also important to observe the following:

- Make sure that the clip is CENTERED on the foot laterally

- Do not place the clip on the base of the foot near the ankle

- Do not place the clip too far toward the toes such that light can pass directly between the sensor pads without passing through tissue.

3] Check that the wiring assembly is connected to the MouseOx® Plus, then start the software. Follow the menu of buttons, choosing those appropriate to your measurement conditions.

4] If signals do not come in quickly, or performance degrades over time, try the following:

    - Re-position the sensor   - Shave the animal   - Replace the sensor

8.1.5. Mouse Paw Sensor

Sizing and Placement
The Mouse Paw Sensor can be used on any mouse(>5 gm) as long as the paw covers the photodiode when the sensor is attached. Center the paw over the photodiode per image below.

Photodiode
(3mm x 3mm)

Sensor Center

Paw Center

Signal Acquisition
1] THE MOUSE MUST BE WARM enough to ensure sufficient blood flow through the paw to ensure a good signal. If necessary warm the mouse enough to ensure a core body temperature of ~37C. If you have trouble getting a reading, try using the other paw.

2] Due to the small size of a mouse paw it will generally take ~30 seconds after you press "Start/Reset" for the MouseOx to perform the optimization process and acquire a signal.

8.2. Pulsar™ Small-Bore MRI Sensor

1] Connect the DB9 Connector (9-pin connector) on the end of the thin black cable to the MouseOx® Plus Control Box. Be sure that the MouseOx® Plus software is not running when you do this.

2] Connect a disposable sensor clip to the fiber-optic cable. **You MUST USE a Mouse Thigh Clip (any mouse or a rat ≤ 100 gm) or a Rat Foot Clip (for rats > 100 gm)**. Place the protrusion on the end of the fiber-optic cable labeled "LED" into the hole on the clip half marked "LED," then do the same for the side marked "PD." Make sure that the fiber-optic cable is oriented so that it aligns over the handle as shown.
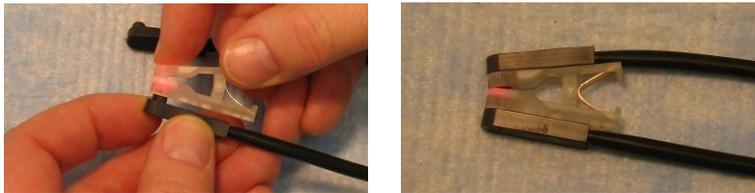
3] Connect the sensor clip to the animal:
A] *Mouse Thigh* – Place the clip on the thigh of a mouse as shown. For non-white fur, you **MUST** shave both locations of the sensor site. On white fur, shaving is not necessary, but will improve signal strength.

B] *Rat Foot* – Place the clip over the toes and locate it so that light shines through the **CENTER** of the foot. Support the clip/cable so that the animal's foot is NOT TWISTED relative to its position before attaching the clip. The clip half marked "PD" should be on the bottom side of the foot.

Mouse Thigh	Rat Foot

4] After locating the clip on the animal, distribute the dual black fiber-optic cable such that it proceeds straight from the animal and that it DOES NOT twist the animal's foot. Try to lay the sensor clip so that both the LED and PD cables are laying on the table.

5] Run the MouseOx® Plus software. To get to the Monitor Subject screen, choose "Anesthetized Measurements" then "Adult Mouse" or the appropriate rat button, then pick the MRI sensor, which is at the bottom of the list.

6] Other recommended guidelines:
- Keep the body (rectal) temperature of the animal above 36°C.

- The fiber-optic cable portion of the sensor is very delicate. Be careful not to bend or coil the fiber-optic cable up too tightly for storage or the small fibers in the cable can become damaged resulting in a reduced quality signal.

- Make sure that the Pulse Distention exceeds 20 μm when operating the system. If the Pulse Distention is less 20 μm, it is recommended that you try to relocate the sensor clip to improve it or **warm the animal**.

- If you are having trouble getting a good signal, try shaving the sensor location if applicable.

- The non-ferrous spring will weaken with multiple uses. An unreasonably low oxygen saturation measurement (a healthy subject with a sat of 88% or less) is a clear sign of an over-used spring. To prevent this, and to promote infection control, replace the clip before each MRI session.

- CAUTION: The converter box contains trace amounts of ferrous material. Keep it away from the magnet bore.

9. MouseOx® Plus Conscious Subjects Option

Instructions on the use of each type of STARR Life Sciences® sensor is described in this section.
In order to use the Conscious Subjects Option, you must have the MouseOx® Plus-Premium software.

System Configuration

To assemble and use your sensors, the components should be connected as illustrated in the following schematic.

Conscious Subjects Application
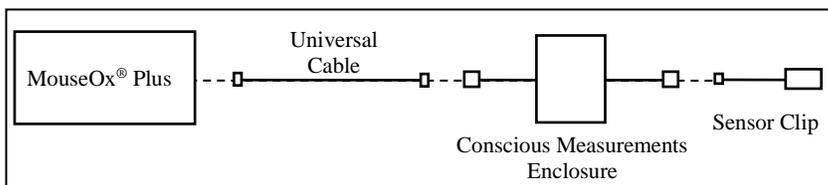
Note: Requires Conscious Subjects Software

9.1. CollarClip™ Sensors

Clip Application and Sizing Chart

Following is the CollarClip™ sizing chart. To get **ACCURATE MEASUREMENTS** from your clips, it is imperative that you follow this chart when choosing a sensor for a given animal. Note that the clip size is engraved on the back of each of the clip handles.

Collar Size	Mouse		Rat
	Unshaven	**Shaven**	
XS	1 - 15 gm	1 – 20 gm	N/A
S	> 15 gm	> 20 gm	5 – 30 gm
M	N/A	N/A	31 – 100 gm
L	N/A	N/A	101 – 300 gm
XL	N/A	N/A	301 – 500 gm
2XL	N/A	N/A	> 500 gm

Other Notes

- When changing a sensor type, you must exit the software then restart so that you can select the correct settings for your new sensor!
- MouseOx® Plus sensors are not backwards-compatible with the original MouseOx® system, and original MouseOx® sensors are not forward-compatible with the MouseOx® Plus.

CollarClip™/ThroatClip™ Differentiation

The CollarClip™, which is colored blue, is to be applied from the **back of the neck** of an animal, and can be used in both awake and anesthetized applications. The ThroatClip™ is colored black, it has a much longer wire lead length than that of the CollarClip™, and it is applied from the **front of the neck**. Thus, it is used only in anesthetized applications. **It is important that you use the correct clip for the correct application in order to get good measurements.**

Removing the Clip Handle Support
Before using a sensor clip, you must remove the handle support. To do this, slide the handle support off sideways, NOT over the end, as diagrammed below. **We recommend that you replace the handle support when the clip is not in use.**
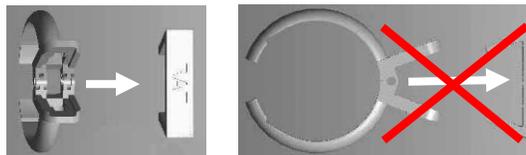
Collar Training

Each CollarClip™ comes with a Blank Clip that matches the size of the sensor clip. It can be used for training an animal to accept the CollarClip™ by placing the clip on the animal for a period of time prior to your experiments. A minimum of 1 hour is recommended, but longer (3-6 hrs.) is preferred.

When to Replace Sensor Clips

Slight degradation of absolute accuracy can occur as sensors wear. To ensure the highest level of accuracy it is recommended to replace sensors when any of the following are observed:

1] significant buildup of hair or material covering the clip faces,

2] good signals (data values on the software screen are in color) take an inordinately long time to appear,

3] significant deformation or damage to the sensor that could result in misalignment of optics.

Chemical Hair Removal

The Blank Clips that come with each CollarClip™, or available separately, can be used to chemically remove hair from the sensor site on the animal. This method is preferred over shaving, although not necessary, unless the animal has dark fur. If you do use a depilatory, we suggest that you do so a few hours to a day before you will be conducting tests on your animals.

NOTE: You MUST remove hair from the sensor site of animals with non-white fur.

1] It is recommended that you anesthetize the animal for hair removal.

2] Lay a bead of depilatory cream (e.g., Nair®) around the entire inside of the Blank Clip as shown.

3] Place the clip on the animal in the same location in which testing will be done, and let the clip and depilatory cream remain in place for the duration specified by the manufacturer.

4] Remove the clip, then carefully wash the cream completely off of the animal using swabs and a small water dish. The hair will be removed with the cream when you do this. The depilatory cream must be washed away completely or it will react with the heat of the LED from the sensor. Removing the hair a day or more before using the MouseOx® Plus is strongly advised.

Neonatal Mice

For very young neonatal mice, it is recommended that the sensor be placed on the animal's head over the ears. We recommend using either an XS ThroatClip™, or a Thigh Clip. Both are shown here.
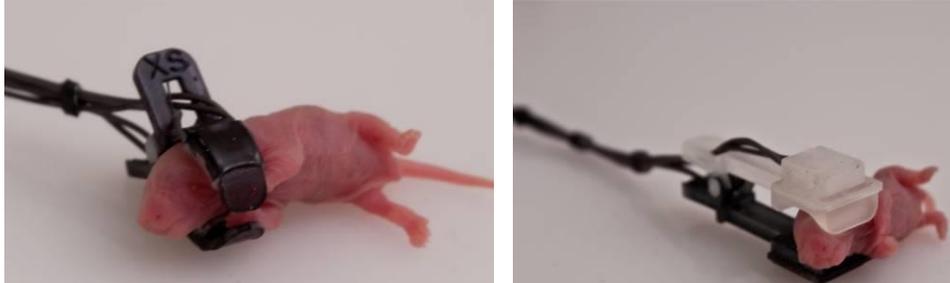
You can also place the clip over front of the head as shown here.

Anesthetized Attachment and Suture Option

The MouseOx® Plus CollarClip™ sensors come equipped with eyelets on the end of the clip arcs that allow you to tie the sensor onto the animal using #5 suture or smaller (the hole is 0.6 mm diameter). This option may be useful for those conducting a long, unattended study. We have found that this attachment approach is more necessary with rats than mice, because it is easier for rats to dislodge the clip.

To attach the sensor using this method, first make sure that the sensor is not connected to the CME. Anesthetize the animal, then retrieve a suture needle from its packet (size 5 or finer). Although we do not recommend a specific

method for attaching the suture to the clip eyelets, we have found that it is helpful to pre-tie the suture onto the clip. By pre-tie, we simply mean that all threading of the suture through both eyelets should be done BEFORE attaching the clip to the animal. This is especially helpful if the sensor location has not been shaved. You will find that it is very difficult to thread the suture once the clip is in place on the animal.

Once the suture is loosely in place, slide the clip with suture over the animal's nose and place the clip in its final location following these rules:

- Place the clip BEHIND the ears and in FRONT of the paws. DO NOT capture the ears under the clip!

- Center the clip handle on the vertebral column of the animal.

- Make sure that the clip is pushed down onto the back of the neck.

- For smaller animals, locate the clip further back away from the head to capture more tissue between the clip tines.

Once the clip is in the proper location, complete the knot to tie the suture down, then cut away any loose ends so that the animal cannot play with them. Once the suturing process is complete, place the animal in the CME, then connect the sensor wire to the connector on the bottom of the commutator.
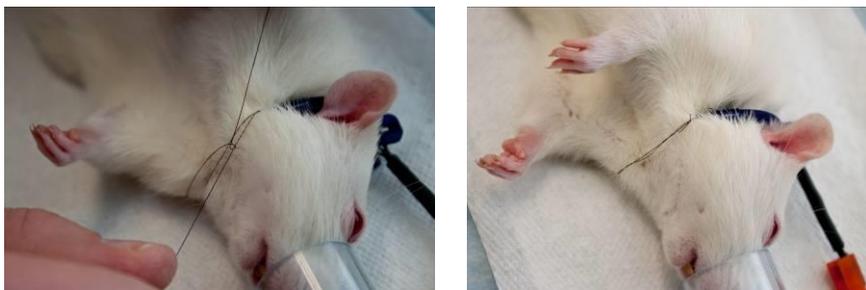
It is important to be sure that you **DO NOT TIE THE SUTURE TOO TIGHT**. The goal of this tie-down approach is not to hold the clip on the animal. This should be left to the spring force in the clip itself. The suture is to be used

merely to prevent the animal from dislodging the clip.

10. Using a Light-Blocking Cloth

A light-blocking cloth is not included with your MouseOx® Plus. If there is the possibility of ambient light effecting the quality of the signal a cloth can be used to reduce the brightness of ambient light that enters the photodiode on your sensor. Excessive ambient light, such as that from surgical lighting or direct sunlight, can reduce the ability of the MouseOx® Plus device to provide consistent and accurate data.

We must also caution users that fluorescent lighting can produce interference that can simulate breathing or cardiac input to the MouseOx® Plus. Such input can disrupt proper operation of the MouseOx® Plus.

Anesthetized Applications
To use a cloth, set up the animal with the clip as described in the previous section. After you have determined that the sensor clip placement is sufficient, place a cloth directly over the sensor clip. If positional adjustments must be made to the sensor clip to improve signal levels, remove the cloth, adjust the sensor position, and then replace the cloth over the sensor. When optimal signals have been achieved, leave the cloth in place over the sensor clip. If signals become poor while you make measurements, remove the cloth and check that the sensor has not moved.

Awake Applications
A cloth cannot be used on an awake, mobile animal. Regardless, we must stress that you reduce the ambient light reaching the sensor. This can be done by reducing the room lighting, or by covering the cage with something to help block out excessive light. Also, if you are using a Conscious Measurements Enclosure, you must use cage liner to prevent light form reflecting off of the bottom surface of the enclosure.

11. Using the MouseOx® Plus Software

11.1. Description of the MouseOx® Plus User Screens

Before describing the MouseOx® Plus software, we note that you can double-click in the title bar at the top of any of the screens to maximize that screen across your display. To return a screen to its original size, you can simply double-click again in the title bar.

You also have the ability to resize all of your screens and windows using the standard Windows® resizing feature. This is done by moving the mouse pointer to any edge of the screen or window until the resizing arrow appears. Once the arrow appears, right-click and drag the edge to the desired shape and size.

11.1.1. MouseOx® Main – Startup Screen

The following is a picture of the MouseOx® Main screen that appears at startup of the software. The Options box on the lower right part of the screen indicates the add-ons or options that you have purchased.
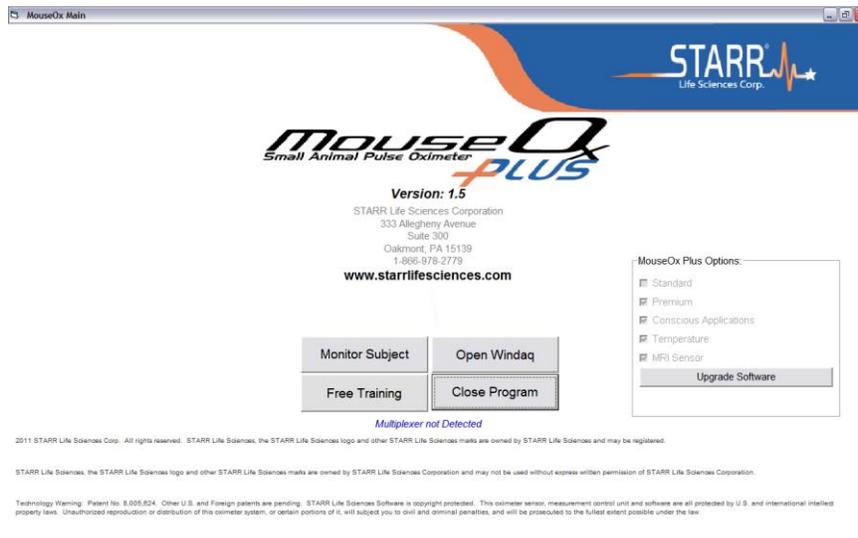
11.1.1.1. Software Product Key

The box entitled MouseOx Plus Options displays the list of options available with MouseOx® Plus (these are Premium Monitoring Software, Body Temperature Option, Conscious Subjects Option and/or MRI Compatible). A check is provided in each box corresponding to those that you have purchased.
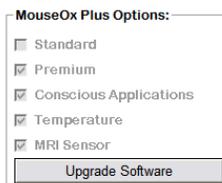
The first time that you operate your MouseOx® Plus, the following screen appears.
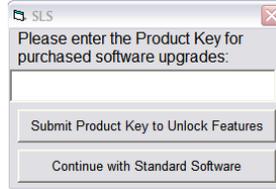
If you have purchased any upgrades, you will have received a Product Key that is associated with the serial number on the bottom of your MouseOx® Plus device. Make sure that the MouseOx® Plus is detected by the computer and the MouseOx® Plus software then enter the Product Key in the box and press the "Submit Product Key to Unlock Features" button. If you have only purchased Standard software, you can press " Continue with Standard Software" or close the box. Note that if you have a new Product Key and you press "Continue with Standard Software," you will only activate the Standard Software and you will need to re-enter your Product Key to open additional software modules.

After the first time, if you purchase an upgrade, press the Upgrade Software button on the bottom of the MouseOx® Plus Options box.
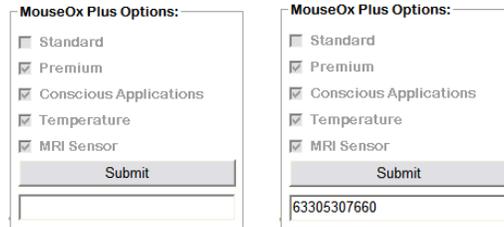
Enter the Product Key in the box, and press Submit. Your MouseOx® Plus Options check boxes will update to reflect your active software, and you can begin using it immediately. In order to avoid problems with the typing in the wrong code, the following error will appear if you type in an invalid product code. This message will also appear if the MouseOx® Plus is not detected by the software. The Product Key is intended to be used with a specific MouseOx® Plus control box and the system must be detected by the MouseOx® Plus software in order for the Product Key to unlock software modules.

If you receive the above error message and you have entered the Product Key correctly do the following steps to ensure that the MouseOx® Plus control box is detected by the software:

1. Close the MouseOx® Plus Software
2. Unplug the USB cable from either the MouseOx® Plus control box or the host computer
3. Wait 10 seconds
4. Open *Insta*Cal (Located in the Windows Start menu → All Programs → Measurement computing group)
5. *Insta*Cal will detect that a MouseOx® Plus is no longer connected to the host computer
6. *Insta*Cal will remove the corrupt driver from the referenced location
7. Close *Insta*Cal
8. Plug the USB cable back in
9. Wait 10 seconds
10. Open *Insta*Cal
11. *Insta*Cal will detect the MouseOx® Plus and install a new driver that is not corrupt for proper USB communications

12. Close *Insta*Cal
13. Restart the MouseOx® Plus software and communications should be re-established

11.1.1.2. Buttons

Monitor Subject
The Monitor Subject button takes you to the screen that controls the recording and display of MouseOx® Plus data received from the test subject. When this button is pressed, a screen appears inquiring whether your subject is anesthetized or awake.
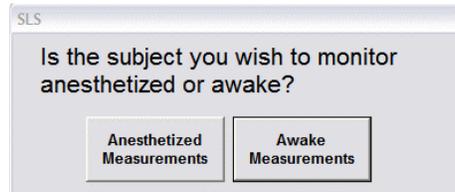
After pressing one or the other of these buttons, you will be taken to a series of screens with self-explanatory buttons that eventually lead to the Monitor Subject screen. Note that if you have not purchased the Conscious Subjects upgrade, the Awake Measurements button will not be active.

Close Program
This button closes the MouseOx® Plus program.

Open Windaq
This button opens the WinDaq® Waveform Browser software from Dataq® Instruments. The WinDaq® Waveform Browser software allows you to perform a variety of post-processing functions on data recorded from the MouseOx® Plus. If you have not downloaded Windaq® from the setup CD, this button should initiate no action.

Free Training
When you click this button, a dialog box opens that describes the free, interactive, web-based training and how to schedule a session. You can also schedule a session by calling (866) 978-2779.

HARDWARE CONNECTION MESSAGE
In the space just below the user-controlled buttons, a message may appear that indicates that the MouseOx® Plus is not connected to the computer or is not recognized by the software. The self-explanatory messages are:
>   *MouseOx® Not Detected*
>   *MouseOx® and Multiplexer Not Detected*
>   *Multiplexer Not Detected*

A Multiplexer™ message will appear if you do not have one.

If you see any of these message and the referenced piece of equipment is plugged into your computer, close down the program, unplug and re-plug the USB cable (make sure that you wait a few seconds before and after re-plugging), then open up the MouseOx® Plus software again.
If the messages still appear, please contact technical service by calling or emailing STARR Life Sciences® at: 1-866-978-2779, or support@starrlifesciences.com.

11.1.2. Monitor Subject – Data Collection Screen

In the following software description, if you have not purchased one or more of the software add-ons, the buttons associated with the functionality of each add-on will be inactive and the associated function will be inaccessible.

Below are pictures of the anesthetized and awake "Monitor Subject" screens.   The Monitor Subject screen uses the same form, but has 2 titles in the upper left corner of the title bar. These are "Monitor Anesthetized Subject" and "Monitor Awake Subject," corresponding to an answer of "Anesthetized" or "Awake" respectively, to the question posed when entering the Monitor Subject Screen. As stated above, if you have not purchased the Conscious Subjects option, the Awake button will be inactive and you cannot reach the screen shown below.

Monitor Awake Subject

Monitor Anesthetized Subject

11.1.2.1. Buttons

Start/Reset
At the beginning of use, the Start/Reset button initiates data collection by the MouseOx® Plus. You will notice that the sensor light comes on when this button is pressed. No data are being saved or recorded if only this button is pressed.

If you start the software by hitting the Start/Reset button before attaching the sensor clip to the animal, or if you detach the sensor clip from the animal after hitting the Start/Reset button, and then apply the sensor to the animal again, it is recommended that you press the Start/Reset button again. This will reset the amplifier gains so that the MouseOx® Plus can hone in on measured values more quickly.

Pause
This button stops data monitoring by the MouseOx® Plus. If Start/Reset is pressed after Pause, the system will continue monitoring from the point at which it was stopped, including resuming at all of the previous control settings and chart data streams. If data files are being recorded, pressing the Pause button will stop the program from writing to the file until Start/Reset is pressed again. Once Start/Reset is pressed again, the program will resume writing to the file at the next available location. However, no marker will be placed into the file to indicate the stoppage location.

Stop
This button stops data monitoring by the MouseOx® Plus. If Start/Reset is pressed after Stop, the system will reset. Control settings will be re-initialized and Data Charts will be cleared. If you are writing to a data file and press the Stop button, you will be prompted to first cease file saving before you can use the Stop button.

Clear Charts
This button clears all of the data charts while data are being collected. It has no effect if pressed while the MouseOx® Plus is either paused or stopped. It does not affect the writing of data to a file.

Subject 1 (select)
Details on the function of this button are given in the section "MouseOx® Plus Multiplexer™."

Cruise Control ON/OFF Toggle
Cruise Control ON should be the normal operating condition for the MouseOx® Plus. When Cruise Control is ON, the MouseOx® Plus controller can optimize all signals at any time. When Cruise Control is OFF, the internal data monitoring electronics lock in the current state to permit continued data monitoring when normally the system would try to optimize gains because of low or high signal levels. This condition permits you to effect some new condition on the animal without the data collection system trying to optimize itself. The benefit is that if the condition is reversed, the system is in the same electronic state in which it started prior to initiation of the new condition.

If you press Start/Reset while the Cruise Control is OFF, the system will not be affected until the Cruise Control is turned back ON, at which point the gains will be reset to their default start values.

The default value at startup is Cruise Control ON.

If you are making measurements on an awake and unrestrained animal that is very active, tuning the Cruise Control OFF can be helpful in getting measurements more often. However, you must let the controller optimize the signals. First of all, let any "9 – Signal Optimization" errors run their course. Next, you want to make sure that you have good signals when the animal is still. You can identify this by looking for clean signals in the Pulse Pleth window when the animal stops. If they are fairly coincident, and either of their amplitude is not too large (near the upper and lower edges of the display), you can turn off the Cruise Control. This will allow the measurements to be made more quickly when the animal stops moving.

Main Menu
This button returns you to the MouseOx® Plus Main screen. If monitoring has been started and not stopped, a message box will prompt you to first stop monitoring. If data are being recorded, a message box will appear to remind you to stop recording data and to stop monitoring.

CSS
This button will open a message box displaying the status of the sensor that is being used. This status indicates the percent of usage that the sensor is expected to have left.

SCH
This button will open the Scheduling Software screen. The Scheduling Software allows the user to have the ability to dictate when the system turns on and off. To open the Scheduling Software simply select the "SCH" button on the "Premium Recording" "Monitor Subject" screen.

Time ON (minutes):
In this text box the user can input the time in minutes that they would like the system to be turned ON for. If a text file is being saved the software will have the actual computer time in the file so the user can use this data to know the time that events happened.

Time OFF (minutes):
In this text box the user can input the time in minutes that they would like the system to be turned OFF for.

Counter (min:sec):
This is simply a count down timer for the next cycle. If the system is ON then this will indicate the amount of time remaining until the system shuts off, if the system is OFF then this counter will indicate the amount of time remaining until the system turns back ON.

AUTO TIMER START
After the time that the user wants the system to cycle ON and OFF for are keyed into the appropriate text boxes, this button will start the cycling process. If the user presses the button again the cycling process will be stopped and the system will stay in the cycle that it is in when the button is pressed.

Timer options:
As of revision 1.5.X there is only one option.

Increment FM with every new ON cycle

Revision 1.5.X and later have the ability to indicate as many file markers as needed. With this option turned on every time the system turns ON with the scheduling software a file marker will be placed in the saved data file.

Summary/Basic Monitoring
This window is actually the Basic Monitoring window and is the only window available with the basic software package. However, if you have purchased the Premium Software Package, this button is used to shift back to the Basic Monitoring screen, which is shown in the figure below. It provides a running summary of the data presented on the primary screen. It has a number of the same buttons described in other parts of this section of the manual. Any button on this screen that activates a function that you have not purchased will be inactive.

Mark Trend File
This button only functions when data are being recorded. It places a marker in the data stream at each file location at the time the button is pressed. It also places a vertical line on the Oxygen Saturation chart at the location at which time the button was pressed. This line follows across the scrolling screen. You are permitted 50 file markers per data file. During data recording, the currently available marker number is displayed in the adjacent box.

Note that markers can also be placed in the file by pressing the keyboard space bar, after the Mark Trend File button has been highlighted once. Also, this button performs the same function whether on the main Monitor Subject screen, or on the Summary screen.

Because the file marker only indicates a location within a file, it is up to you to separately record the significance of any marked event.

Set Alarms
Upon pressing this button, a separate screen titled Alarm Values appears over the top of the monitoring charts. A pictorial example is given in the next figure below.

Parameter Alarms
Note that the alarms are de-activated at startup. You must activate each alarm to get it to function.

You can separately adjust the upper and lower alarm thresholds of each of 5 parameters (Breath Distention does not have its own alarm). All upper and lower threshold alarms can be turned on or off separately by clicking the box adjacent to the chosen Alarm On title. If a given signal passes above its high alarm setting, or if a given signal passes below its low alarm setting, an audible alarm sound will be initiated and the corresponding data chart will change background color. The audible alarm can be shut off by pressing

the Alarm Silent toggle button on the Monitor Subject screen. In addition to the audible and visual alarms, a message will appear in red in place of the pertinent chart title describing the possible cause of the alarm.

All alarms will be automatically de-activated if the pertinent parameter in that alarm is under an active error code. The values are defaulted to the following (Breath Distention does not have an alarm):

> *High Alarms* (all high alarm defaults are also the maximum value for each parameter)
> - Pulse Distention   800 μm
> - Pulse Rate   900 bpm
> - Oxygen Saturation   100 %
> - Breath Rate   450 brpm
> - Body Temperature   43 °C
>
> *Low Alarms*
> - Pulse Distention   10 μm
> - Pulse Rate   120 bpm
> - Oxygen Saturation   80 %
> - Breath Rate   40 brpm
> - Body Temperature   32 °C

The alarm levels can be adjusted by choosing one of the incremental values from the pull-down menu associated with a given alarm, or by typing a new value directly into the pull-down menu box for that alarm.

Anesthesia Alarm Settings
The MouseOx® Plus software provides you with two types of alarms that can be used specifically to monitor depth of anesthesia. These are the Distention Compare Alarm and the Rate Compare Alarm. It should be noted that we do not recommend specific values for these alarms, and that they are available for your use based on your own experience. The reason that we do not make specific recommendations is

because parameter levels may be dependent on items that we cannot control, such as dosage, time after dosage, type of anesthesia, where it is delivered, etc.

NOTE: When monitoring awake subjects, these alarms are deactivated and cannot receive input.

In our alarm configuration, parameter alarms (described above) have precedence over anesthesia alarms. Additionally, an anesthesia alarm will be disabled whenever an error code for a pertinent parameter within the given alarm is active.

Distention Compare Alarm
The Distention Compare Alarm allows you to use the relative percentage of Pulse Distention to Breath Distention as a means of monitoring depth of anesthesia. The value of the alarm limit is literally the percent that Breath Distention is to Pulse Distention, just as it reads on the screen. Because this alarm is a little confusing, an example of each type of alarm is given below.

Example 1 – **Anesthesia Too Deep** Distention Compare Alarm
If the Anesthesia Too Deep alarm is set to 80%, the alarm threshold would be met when the Breath Distention becomes greater than 80% of the Pulse Distention. The idea behind this alarm is that excessive anesthesia levels generally tend to suppress breathing. The response to decreased ventilation is gasping, the result of which, is an increase in breathing effort, which increases Breath Distention relative to Pulse Distention.

Example 2 – **Anesthesia Too Light** Distention Compare Alarm
If the Anesthesia Too Light alarm is set to 20%, then the alarm threshold would be met when the Breath Distention becomes less than 20% of the Pulse Distention.

The alarm settings cannot be set such that there is overlap between the Too Deep and Too Light alarm settings. Additionally, you do not have the ability to type in individual alarm values. You can only choose from the lists provided on the drop-down menus in the combo-boxes. The limits and steps for each of the Distention Compare Alarms are given below.

> Anesthesia Too Deep
>> Range: 70 – 140%
>> Step: 10% increments
>> Default: 100%

> Anesthesia Too Light
>> Range: 10 – 40%
>> Step: 10% increments
>> Default: 20%

Note that this alarm is automatically disabled if the Pulse Distention drops below 25 μm.

Rate Compare Alarm
The Rate Compare Alarm allows you to adjust the ratio of Heart Rate to Breath Rate as another means of monitoring depth of anesthesia. The value of the alarm limit is literally the direct ratio of Heart Rate to Breath Rate, as it reads on the screen. Examples of each type of alarm are given below.

Example 1 – **Anesthesia Too Deep** Rate Compare Alarm
If the Anesthesia Too Deep alarm is set to 7.5, the alarm threshold would be met when the Heart Rate becomes greater than 7.5 times the Breath Rate. The idea behind this alarm is that excessive anesthesia

levels generally tend to suppress breathing, causing the breath rate to drop, usually without a concomitant change in heart rate.

Example 2 – **Anesthesia Too Light** Rate Compare Alarm
If the Anesthesia Too Light alarm is set to 3.5, then the alarm threshold would be met when the Heart Rate becomes less than 3.5 times the Breath Rate, irrespective of which one actually changed.

With this alarm, you can insert your own limit values in the combo boxes provided. However, the system will not permit you to insert values that are overlapping, i.e., the Too Deep alarm limit less than the Too Light alarm limit. You can also choose from the list provided on the drop-down menu in the combo-box. The limits and steps for each of the Rate Compare Alarms are given below.

        Anesthesia Too Deep
            Range: 5.0 – 10.0 bpm/brpm
            Step: 0.5 bpm/brpm increments
            Default: 7.5 bpm/brpm

        Anesthesia Too Light
            Range: 2.5 - 4.5 bpm/brpm
            Step: 0.5 bpm/brpm increments
             Default: 3.5 bpm/brpm

Save Alarm Settings
The Save Alarm Settings button saves all alarm parameters that appear in the current windows so that they will be retained as the functional values any time the MouseOx® Plus is used.

Default Values
The Default Values button resets the alarm parameters to the default values listed in the Parameter Alarms section above.

Set Alarms and Close Window
This button must be pressed in order to set any changed alarm values. This button also closes the Alarm Values screen.

Alarm Silent (toggle)
This button shuts off all audible alarms until the button is pressed again, which restarts the audible alarms. When first pressed, its name changes to "Turn Sound On." Audible alarms occur whenever one of the 4 chart signals crosses either above or below the user-selected threshold. For all signals, alarms will initiate as soon as a new calculated value falls above or below the set threshold.

Under an alarm condition, the monitoring screens will indicate the source by changing the associated parameter chart background to a caution yellow. Additionally, the chart title is replaced by a red label warning of the infraction.

View Temperature Window
Details on the function of this button are given in the section "MouseOx® Plus Body Temperature Option."

View Diagnostic Window
This button opens the Quick Averaging Diagnostics window, which appears in the figures below. The Quick Averaging Diagnostics window allows you to collect an average value for each of the 5 MouseOx® Plus parameters. The length of the average is selectable by grabbing the maroon arrow slider and moving it vertically. You can choose averaging durations from 5 to 30 seconds, in 1 second increments.

Averaging can be done manually, or automatically at constant intervals of your choosing. Automated averages always take precedence over manual averages.

Once a diagnostic average (manual or automatic) is initiated, each parameter window will display the message "Processing…" until the first valid value is entered into the averaging calculation. At this point, each window will display the running average, but in gray text, as shown in the leftward figure below. Once each parameter calculation is completed, it will change from gray to color, as shown in the rightward figure below. Because the system rejects values that are error-coded independently for each parameter, some averaged values may appear in their respective slots before others.
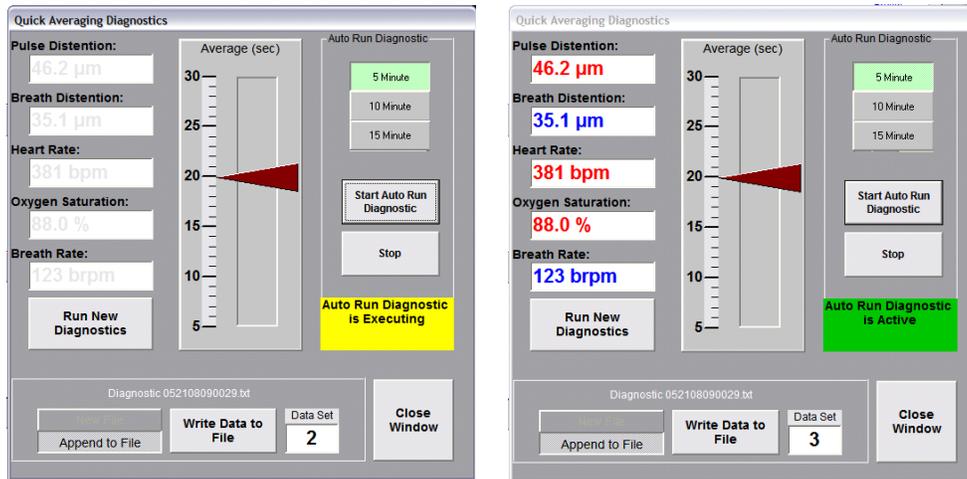
The duration of a given averaging operation for a parameter may be longer than the period set using the slider arrow. The duration corresponding to the position of the slider arrow simply indicates the sum total duration of valid data collected and averaged, irrespective of how long that takes. This is due to the fact that any data subject to an active error code are automatically rejected by the averaging algorithm and are not included in the average. However, all diagnostic runs will time out at a value of 3 times for anesthetized applications (6 times for awake applications) the time selected using the slider arrow, and a value of 0 will be written for any parameters that have not completed an average in that period. In summary, once an average is initiated, the averaging algorithm will simply continue until the full period of non-error code-rejected data are averaged, or one or more uncompleted parameter averages times out.

Run New Diagnostics (Manual Only)
This button will initiate a manual average. If you choose to initiate another average, you can press the Run New Diagnostics button without having to close the window. Also, if you press the Run New Diagnostics button while a previous data averaging is still in progress, you will override that data averaging and initiate a new one. If you press this button while an automatic average is active (not executing), the manual average will proceed, but will be overridden if an auto-run average executes while the manual average is running.

Write Data to File (Manual Only)
This button saves the current manual diagnostic data to a text file of your choice. This text file can easily be read into Microsoft® Excel using the same instructions for reading a Text File into Excel as described in the section below titled "Opening Text Data Files Using Microsoft® Excel."

When you press the Write Data to File button, a standard Windows® file saving dialog box will appear, allowing you to name the file and store it in a location of your choice. A default filename will be provided with the default save location being "c:\mcc\STARR Text Files", and the default name being "Diagnostic mmddyyhhmmss.txt" (month, day, year, hour, minute, second). The data set will be time-stamped at the point at which you press the Write Data to File button. The currently active filename will appear in light text above this button. A sample Excel file with 6 data sets is provided above.

Note that although temperature and animal number do not appear on the Diagnostic Window, they are recorded in each data set, as can be seen in the figure above.

If you press the Write Data to File button before a new diagnostic data collection has been completed, a data set will still be saved, but will contain all zeros. If you press this button while all data are still being averaged, the currently completed data values will be saved, along with zeros for those that are still processing. You cannot write data from the same diagnostic run to a file more than once, so be sure that you have all of your desired parameter calculations completed before saving.

Start Auto Run Diagnostics (Automatic Only)
This button will initiate automatic averaging that will execute at time intervals of your choosing from the palette of duration toggles directly above this button. The available time intervals are 5, 10 and 15 minutes. If you want to change this time interval after having started, without creating a new file, you can press the desired duration toggle, then press Start Auto Run Diagnostics again, which will initiate new automatic averaging at that point.

When you first press the Start Auto Run Diagnostics button, AND you have not opened a file using the Write Data to File button, a standard Windows® file saving dialog box will appear, allowing you to name the file and store it in a location of your choice. A default filename will be provided with the default save location being "c:\mcc\STARR Text Files", and the default name being "Diagnostic mmddyyhhmmss.txt" (month, day, year, hour, minute, second). The data set will be time-stamped at the point at which each auto run is executed. The currently active filename will appear in light text above the Write Data to File button.

In order to initiate saving data to a new file, you must first press the Stop button, then press the New File toggle, then press Start Auto Run Diagnostics again.

Once you press the Start Auto Run Diagnostics button, a yellow banner will appear below the Stop button that says "Auto Run Diagnostic is Executing" (see leftward figure above). Once the data are calculated and stored to the file, the banner will change color and title to green and "Auto Run Diagnostic is Active" (see rightward figure above). If the Auto Run feature has not been initiated, this area will remain gray.

New File/Append to File Toggles
When you first open the Quick Averaging Diagnostics window, the New File toggle will be active. It will remain so until you open a file by pressing either the Write Data to File or Start Auto Run Diagnostics buttons. Once the data set associated with that action is saved, the New File toggle will deactivate and the Append to File toggle will be active. From that point forward, each manual or automatic data set will be

appended to the current file. If you would like to open a new file, simply activate the New File option box, and the same process will repeat.

Data Set Counter
The data set counter will increment each time a data set is saved to a given data file, regardless if it was a manual or automatic execution. If you save to a new file, the counter will be reset to 0.

Close Window
This button closes the Quick Averaging Diagnostics window. It does not affect execution of automatic diagnostics.

Trend Charts
Four Trend Charts appear on the left side of the monitoring screens. These are:
- Pulse and Breath Distention (μm) [Anesthetized Subject] OR Activity [Conscious Subject]
- Heart Rate (bpm)
- Oxygen Saturation (%)
- Breath Rate (brpm)

These charts scroll horizontally from right to left, but each data point represents data collected for **1 heartbeat**, which means that **the points are not necessarily spaced evenly in time**. Each chart holds 1000 heartbeats of information across the screen, but new data simply continue to scroll. In general, heart-related parameters are in red, while breathing-related parameters are in blue.

For all 4 charts, any signal that passes below the alarm threshold (either default or user-set) will cause that chart background color to change from white to yellow, and its title to turn red and display a message. The alarm condition will occur after the first calculated value that falls below the set threshold for that parameter.

The Pulse and Breath Distention chart includes both Pulse and Breath Distention signals. These signals are included on the same graph because they have similar properties, including units (μm). The Pulse Distention plot is in red, and the Breath Distention plot is in blue. Descriptors that detail the color coding appear in opposite upper corners of the plot.

Note that when you use the cursors on a data location at which an error code is active, the error code title will appear for a short period of time directly below the Breath Rate data chart. Also, when an error code is active, we do not adjust the color or appearance of any of the data charts.

Start Recording Data
This button initiates data-writing to a text file or a WinDaq® file. When pressed, a small dialog box with 3 buttons will appear ("Text File," "Windaq File" and "Cancel") that will allow you to choose either format, or cancel a file-saving request. The dialog box will also have 3 option boxes that will allow you to choose the data file saving sample rate (1, 5 or 15 Hz). **Data points are saved in these files at a constant frequency, not by heartbeat, as is the case with data posted to the Monitor Subject chart displays.**
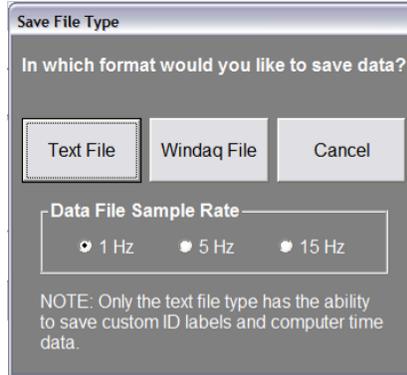
Be sure that you have pressed Start/Reset before recording data, as the software will not indicate that no data are being collected or saved. The reason that no indicator is present is so that you can Pause and Restart data collection without having to stop data recording, which would necessitate the recording of multiple files. If you do Pause and Restart monitoring/collecting, the data will be saved sequentially in the file as if no break had occurred. Note that there will be no indication of this stoppage in the data set. Even the clock time is not universal, but only sequential from the start.

Note also, that if you plan to use Microsoft® Excel to view text files, Excel limits data file size to 64,000 rows. Therefore, you must limit your data files sizes to 64,000 points, or about 70 minutes at 15 Hz, 210 minutes (3.5 hrs) at 5 Hz and 1050 minutes (17.5 hrs) at 1 Hz. However, if you will be using the WinDaq® Waveform Browser, the file size limit will be based on your available computer memory.

Text File
When this button is pressed, a Windows® file saving utility dialog box will appear. This box will provide you with the option to direct the location of the saved file, and to rename it as you wish. The suggested location and file name will appear as in previous software revisions with the default save location being "c:\mcc\STARR Text Files" and the default name being "XTrend mmddyyhhmmss.txt" (month, day, year, hour, minute, second). The character "X" shown at the beginning of the file name will not actually appear, but is a placeholder for the frequency at which the file is going to be saved, and will actually be a value of 1, 5 or 15, depending on that frequency. Information about the file format is given in the section below titled "Opening Text Data Files Using Microsoft® Excel."

WinDaq® File
When this button is pressed, a Windows® file saving utility dialog box will appear as shown below. This box will provide you with the option to direct the location of the saved file, and to rename it as you wish. The suggested location and file name will appear as in previous software revisions with the default save location being "c:\Windaq\" and the default name being "Xwindaq mmddyyhhmmss.wdq" (month, day, year, hour, minute, second). The character "X" shown at the beginning of the file name will not actually appear, but is a placeholder for the frequency at which the file is going to be saved, and will actually be a value of 1, 5 or 15, depending on that frequency. Information about the file format is given in the section below titled "WinDaq® Waveform Browser."
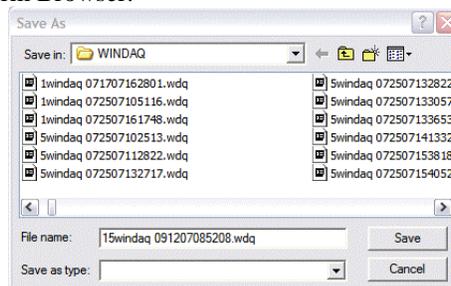
Pulse Pleth

The Pulse Pleth chart in the upper right-hand corner displays the real-time red and infrared (in yellow) signals. The signals scroll right-to-left whenever monitoring is active. This information helps you to determine the quality of the signals and improves your ability to diagnose problems. Generally, a good signal should appear as overlapping sinusoids of both the red and yellow traces.

Error codes that become active during a data monitoring session will be displayed with their numerical designation in the lower center of the Pulse Pleth window in yellow, during the period at which that given code is active. Additionally, the Pulse Pleth chart background will change from black to a translucent yellow-on-black whenever any error code is active, except for "3-Lost Breath Rate." More detail on error codes is given in the next section below.

11.1.2.2. Chart Data

Chart Data displays appear as the leftward column of boxes on the right side of the monitoring screen. In these boxes, we display current numerical values for each parameter, and they are updated continuously (with each heart beat).

When an error code becomes active, the Chart Data displays associated with that error code will change from red and/or blue to gray. Their colors will revert back as soon as the error code disappear. Parametric information for the Chart Data displays is given in the following table.

Parameter	Units	Averaging Information
Time (BIOS clock)	seconds	N/A
Pulse Distention	μm	10 heart beats
Breath Distention	μm	10 breath distention measurements
Heart Rate	bpm	5 heart beats
Oxygen Saturation	%	10 heart beats
Breath Rate	brpm	10 breath cycles
Temperature	°C, °F	no average
Activity	N/A	N/A

11.1.2.3. Cursor Data

The cursor data displays appear in the rightward boxes on the right side of the screen adjacent to the Chart Data Displays. They display the same data as their Chart Data Display counterparts, and appear without values until you click on one of the main charts on the monitoring screen at a given time, or heartbeat, location. Once the chart is clicked, new data from the location of the click will be placed in the Cursor Data boxes and will remain there until the next click at a different location on one of the charts. With each click, all Cursor Data boxes will update to the values at that cursor location.

During monitoring, the cursor position is only visually identifiable for the current heartbeat. However, the data placed in the Cursor Data boxes will be retained until a new cursor position is chosen.

If an error code was active at the location of the cursor, the cursor data associated with that code in these boxes will be displayed in gray rather than in **black**. Additionally, the name of the error code will appear in a box directly below the Data Charts for a short period of time after you locate the cursor.

Motion Sensitivity (active with Conscious Subjects)

The Motion Sensitivity slider is located on the far right-hand side of the Monitor Subject screen.
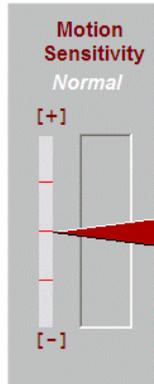
The slider sets the level of sensitivity to motion for the Activity graph. There is no quantitative scale for sensitivity to activity, so this is a feature that you can tailor to your own needs. It should be noted that the size of an animal, or the position of the clip may have some bearing on the natural sensitivity of the system. Therefore, the slider allows you to make adjustments for this as necessary.

In the picture above, the text "Normal" in white italics indicates the level of sensitivity of the slider. The middle position is considered normal. As you raise the slider, there are 2 settings, each with subsequently greater sensitivity to motion. Likewise, as you pull the slider down, the Activity parameter will be flagged with increasingly less sensitivity.

If the motion flag goes off even when the animal appears to be sitting quietly and the sensitivity is set to the lowest value, it is likely due to a low strength cardiac signal. This can be addressed by adjusting the sensor clip to a more optimal position. To do this, make sure of the following:
- Sensor is pushed down on the back of the animal.
- Sensor handles are centered on the vertebral column.

If you are using the MouseOx® Plus in Anesthetized mode, or Awake mode but you have not purchased the Conscious Subjects software, the Motion Sensitivity slider will appear as shown below.

Up - Down Scroll
These buttons, which appear to the left of each main chart, allow you to scroll the vertical axis range up or down. If data are being collected when a given chart appears to be railed (i.e., the Up-Down Scroll has not been adjusted and the trace falls along the top or bottom of the plot), the true data will still be saved.

2X and 1/2X Toggles
Activating the 2X toggle zooms the y-axis span **IN** by a factor of 2. When you de-activate this toggle, the span is doubled back to its original range.

Activating the 1/2X toggle zooms the y-axis span **OUT** by a factor of 2. When you de-activate this toggle, the span is zoomed back by half to its original range.

11.2. Measurement Parameter Rejection Criteria and Error Codes

11.2.1. Parameter Rejection Criteria Specifications

Pulse Pleth Error Messages

When MouseOx® Plus measured parameters are not passing all of our acceptance criteria, one of seven (numbers up to nine) numerical error code messages will be displayed at the bottom of the Pulse Pleth window. These error codes are used to show you which data points for which parameter are valid at any given time during data collection. The numerical designation of any of these codes will be placed in both the text and WinDaq® data files at every temporal location for which the given error message applies. Before describing each of the codes, we must first explain the basic acceptance criteria for each parameter in the MouseOx® Plus.

Heart Rate

In order to calculate the displayed heart rate, an average of 5 consecutive heart rate measurements is used. For the displayed value, we define an erroneous heart rate as occurring after we calculate 5 consecutive individual heart rates that do not meet our internal acceptance criteria. During the period when heart rate is considered not valid by this algorithm, the last value displayed will be retained on the screen (and written to the data file) and the error code "**2-Lost Pulse**" will appear. This code will clear after the passage of 1 heart beat that meets our acceptance criteria.

Breath Rate

Breath rate may appear to have a locked-in value due to its slow update rate, since it is naturally slower than heart rate. It is also derived from a running average of 10 calculated breaths. For the displayed value, we define an erroneous breath rate as occurring after we calculate 10 consecutive breath rates that do not meet our internal acceptance criteria.

During the period when breath rate is considered not valid by this algorithm, the last value displayed will be retained on the screen (and written to the data file), and the error code "**3-Lost Breath Rate**" will appear. This code will clear after the presence of 1 breath that meets our acceptance criteria.

We have an additional complex criterion that activates the breath error code "**3-Lost Breath Rate**" instantaneously. When this criterion triggers the error code, the breath rate value will continue to be updated even though the error code is active and the associated values in the Chart Data displays are grayed out.

Additionally, because breath rate requires even better signal quality than does heart rate, when one of the heart rate error codes occurs, we consider that breath rate is also erroneous, and its value is turned gray while that heart rate error code is active. Thus, breath rate has its own error code, as well as all of the heart rate error codes.

Oxygen Saturation

Although oxygen saturation or S_pO_2 is calculated at every heart beat, highly accurate values of heart rate are not necessary for accurate calculation of S_pO_2. This means that we can still calculate S_pO_2 in the absence of accurate heart rate values (*i.e.,* when a heart rate error code is active). S_pO_2 is calculated based on an average of 10 S_pO_2 calculations that have satisfied the acceptance criteria. If we calculate 10

consecutive values that do not meet the acceptance criteria, the error code "**Lost S$_p$O$_2$**" will appear. This code will clear after the passage of 1 S$_p$O$_2$ value that meets our acceptance criteria.

Pulse Distention
At this time, we do not have an error code specifically for pulse distention. However, because pulse distention requires signal qualities similar to those for measuring heart rate, we have tied pulse distention to the heart rate error codes. Thus, when any of the heart rate error codes are active, the last pulse distention value is turned gray until the error code clears. Additionally, if pulse distention drops below 10.0 μm, we consider the signal to be lost and we display the error code "**6-Lost Signa**l."

Breath Distention
At this time, we do not have an error code specifically for breath distention either. However, because breath distention requires signal qualities similar to those for measuring breath rate, we have tied breath distention errors to the breath rate error codes, which include all of the heart rate error codes as well. Thus, when any of the breath rate error codes are active, the last breath distention value is turned gray until the error code clears.

Body Temperature
There is no error code associated with body temperature.

Activity
There is no error code associated with activity.

We should finally mention that when an error code is active, the parameters associated with that error code will be displayed in gray rather than in their normal colors in the Chart Data displays. Also, a parameter that is subject to any error code will not be included in an average of that parameter when using the Quick Averaging Diagnostics window. The parameters that are affected by each error code are detailed just below.

11.2.2. Error Code Definitions

Following are a list of the error code definitions.

0 – No Errors

2 - Lost Pulse
This error code occurs when 5 consecutive heart rate measurements do not pass the accuracy acceptance criterion (prior to Rev 5.0 software, this error code became active when 20 consecutive heart rate measurements did not pass the accuracy acceptance criterion). This code also applies to breath rate, pulse distention and breath distention, but not to oxygen saturation. This code will be removed when 1 good heart rate measurement is obtained.

3 – Lost Breath Rate
This error code occurs when 10 consecutive breath rate measurements do not pass the accuracy acceptance criterion. If the error code Lost Pulse becomes active while the Lost Breath Rate code is active, the Lost Breath Rate code will be overridden. The Lost Breath Rate code also becomes active if the calculated breath rate falls below 25 brpm. This code will be removed when 1 good breath rate measurement is obtained.

4 - Lost S$_p$O$_2$
This error code occurs when approximately 10 consecutive S$_p$O$_2$ measurements do not pass the accuracy acceptance criteria. This code will be removed when 1 valid S$_p$O$_2$ measurement is obtained.

6 – Lost Signal

This error code occurs whenever the conditions for both the Lost Pulse and Lost S_pO_2 error codes are valid simultaneously. This error code can also become active if the Pulse Distention drops below 5.0 μm.

7 - Lost Breath Rate & Lost S_pO_2
This error code occurs whenever the conditions for both the Lost Breath Rate and Lost S_pO_2 error codes are valid simultaneously. When using the Conscious Subjects Software, this error also occurs when the Activity is high.

8 – Breathing Artifact
This error code occurs when the MouseOx® Plus detects that breathing, or motion caused by breathing, overrides the heart pulse signal, which makes all other parameters difficult to measure accurately. This code will be removed when cardiac pulse signals become strong enough such that accurate measurements can again be made.

9 – Signal Optimization
This error code becomes active whenever the MouseOx® Plus controller is adjusting system gains. This code will be removed after the refractory period of the given controller change has elapsed.

The table below provides guidance as to the validity of specific parameters for each error code. In this table, each of the error codes is defined for the MouseOx® Plus parameters listed on the right-hand side of the table. For a given error code, the parameter at that location will be either **Good** or **Unsure**.

Good means that the given measurement satisfies all of our acceptance criteria.

Unsure means that the given measurement does not meet our acceptance criteria during the presence of that particular code.

Error Codes	Error Description	Oxygen Saturation	Heart Rate	Breath Rate	Pulse Distention	Breath Distention
0	No Error	**Good**	**Good**	**Good**	**Good**	**Good**
2	**Lost Pulse**	**Good**	**Unsure**	**Unsure**	**Unsure**	**Unsure**
3	**Lost Breath Rate**	**Good**	**Good**	**Unsure**	**Good**	**Unsure**
4	**Lost S_pO_2**	**Unsure**	**Good**	**Good**	**Good**	**Good**
6	**Lost Signal**	**Unsure**	**Unsure**	**Unsure**	**Unsure**	**Unsure**
7	**Lost Breath Rate & Lost S_pO_2**	**Unsure**	**Good**	**Unsure**	**Good**	**Unsure**
8	**Breathing Artifact**	**Unsure**	**Unsure**	**Unsure**	**Unsure**	**Unsure**
9	**Signal Optimization**	**Unsure**	**Unsure**	**Unsure**	**Unsure**	**Unsure**

11.3. File Saving Options and Utilities

11.3.1. Opening Text Data Files Using Microsoft® Excel

MouseOx® Plus text (or trend) data files and Quick Averaging Diagnostics data files can be opened easily in Microsoft® Excel. To do so, open Microsoft® Excel, and click "Open" under the "File" tab, just as you would open any Excel file. A dialogue box titled "Open" will appear. Because MouseOx® Plus text files use a ".txt" extension, you must open the pull-down menu at the bottom left titled "Files of Type," and choose "All Files." This will permit the text files to appear. Simply highlight the desired file and click the

"Open" button. Another dialogue box will appear with the title "Text Import Wizard – Step 1 of 3." When this appears, choose the following steps:
- Choose "Delimited," then select "Next>."
- Check the "Comma" box, then select "Next>."
- Choose "General" as the column data format, then select "Finish."
The data will appear in 11 columns as shown in the figure here.

Note that Microsoft® Excel limits data file size to 64,000 rows. Therefore, you must limit your data files sizes to 64,000 points, or about 70 minutes at 15 Hz, 210 minutes (3.5 hrs) at 5 Hz and 1050 minutes (17.5 hrs) at 1 Hz. This limitation does not exist for the WinDaq® Waveform Browser.

File Marker
The File Marker column will start at 0 and hold the value of the last file marker that was inserted. Thus, the location of the next file marker is at the location of the change in numerical value in this column.

Error Code
The Error Code can have values 0 through 9, excluding 1 and 5. If the value is 0, all data points are valid. A non-zero error code indicates that some or all of the data points may be unreliable at that temporal location. The error code table just above provides guidance as to the validity of specific parameters for each error code value.

We have also added some useful functionality to help you deal with error codes in your Excel files. In the following section, we provide you with equations that you can type into your data file after it has been loaded into Excel, which will allow you to clear cells that have parameters that are associated with an active error code.

11.3.2. Filtering Files in Microsoft® Excel to Blank Out Error Coded Data

So now you have moved your data into Microsoft® Excel, but you still have to figure out how you are going to deal with data points that have associated active error codes. As you know, the error codes provided with your data indicate which data points do not pass our internal acceptance criteria. Thus, we will describe here how you can filter your data so that you can **CLEAR** cells that have an associated error code.

If you load your data into Excel exactly as described in the previous section, you will have the parameters loaded into the Excel columns as shown in the figure above, and as detailed in the following table.

Immediately below this table is the table that provides expressions to be used in Excel to filter your data.

To filter the data for each parameter, type the text of the given formula from the middle column of that table into the associated cell listed in the right column of that table. Note that there are no filters for the Elapsed Time, Custom ID, Subject Number, File Marker, Error Code, Activity, Core Temperature, and Computer Clock parameters.

Parameter Column Location

Parameter	Excel Column
Elapsed Time	A
Custom ID	B
Subject Number	C
File Marker	D
Error Code	E
Oxygen Saturation	F
Heart Rate	G
Breath Rate	H
Pulse Distention	I
Breath Distention	J
Activity	K
Core Temperature	L
CPU Clock	M

Conversion Formulae

Parameter	Filter Function	Formula Cell Location
Oxygen Saturation	=IF(OR(E4=0,E4=2,E4=3),F4,"")	O4
Heart Rate	=IF(OR(E4=0,E4=3,E4=4,E4=7),G4,"")	P4
Pulse Distention	=IF(OR(E4=0,E4=4),I4,"")	Q4
Breath Distention	=IF(OR(E4=0,E4=3,E4=4,E4=7),J4,"")	R4
Breath Rate	=IF(OR(E4=0,E4=4),H4,"")	S4

Instructions for Implementing the Error Code Filtering Formulae:

1] Make sure that the numerical data in your Excel file start in Row 4 and that the last column of data (titled Computer Clock) are in Column M.

2] Type the **EXACT** text of the Filter Function from the middle column of the table immediately above, into the Formula Cell Location listed in the rightmost column of the table above.

3] After the formulas for each parameter are typed in, highlight all of the formulas along that row (Row 4, Columns O through S), then drag them or copy them down to the last row of your data set. The result of this will be that the data will be copied, but for every active error code, the cells for the associated parameter values will be left **blank**.

4] **In order for these filtering functions to work as written, your original data must be in the rows and columns specified above (start at Row 4 and encompass Columns A-M), AND you must place each function in the given Formula Cell Location specified in the table just above!**

11.3.3. WinDaq® Waveform Browser

The WinDaq® Waveform Browser is an advanced data analysis software package from Dataq® Instruments. A copy of this software is included with your MouseOx® software and can be downloaded onto your

computer from the MouseOx® Plus CD. For information regarding the use of the WinDaq® Waveform Browser, Dataq® Instruments provides a very detailed user manual for this software, and we have included a copy of it on your MouseOx® Plus CD under Step 5 of the Installation Menu.
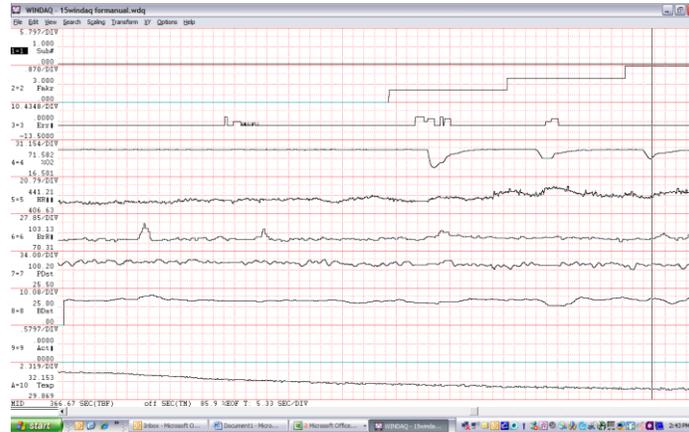
11.3.4. Converting WinDaq® Waveform File Data to Microsoft® Excel

Converting WinDaq® files to be readable by Excel can be done using the following steps:

1] Compress the Data
When you open a WinDaq® file, all of the data channels will be displayed on one screen, but only a small segment of the total duration of the file will be shown. The first step is to compress the data so that all of it will be on the screen. To do this, press the F7 key and select the "Maximum" button on the bottom of the dialog box that appears. When you do this, all channels of all of your data will appear on the screen.
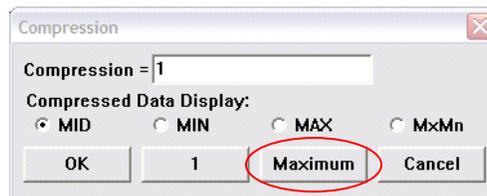
2] Insert WinDaq® Cursors
You must now select the section of data that you are interested in loading into Excel. To do this, you must move the cursor to the beginning of the section of data that you are interested in saving. Once the cursor is in place, press the F4 key. By doing this, you will have set a time marker to "0" at the cursor location that you have chosen. See the figure below.
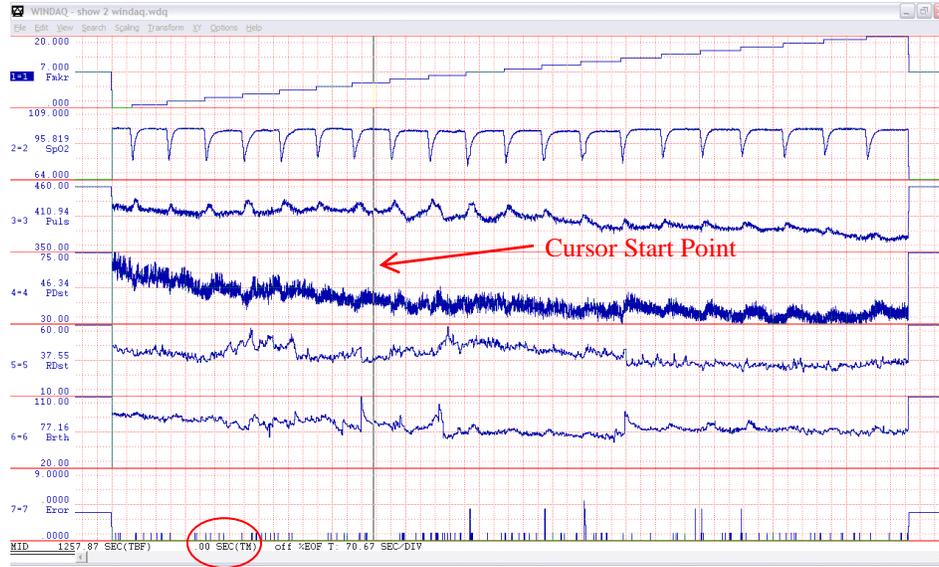
You must now move the cursor to the end of your data section of interest. The data between these two cursor locations can now be saved for use in Excel. Note that a cursor start point mark is placed at the starting location on channel 1, as shown below.
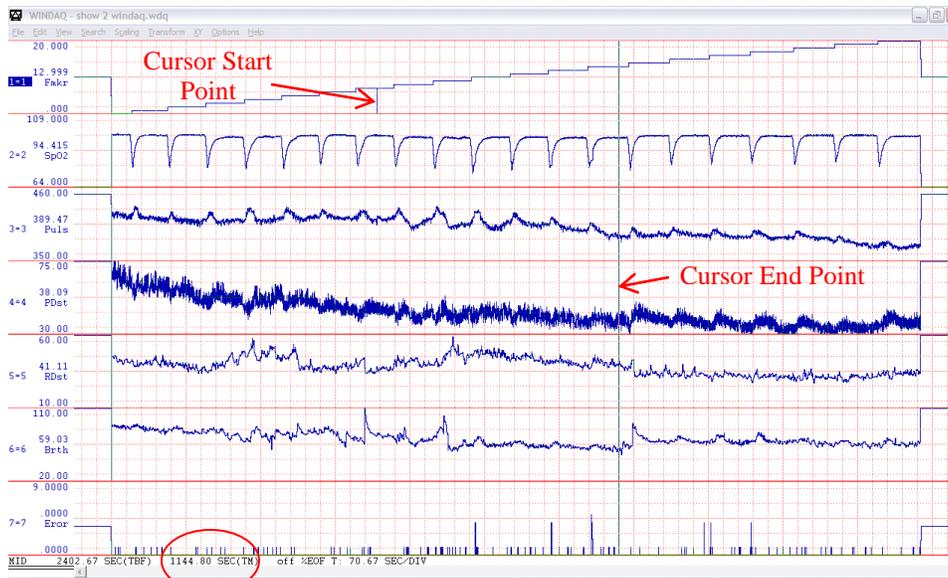
Please note that Excel can only handle about 70 minutes (210 min, 1050 min) worth of your data collected at 15 Hz (5 Hz, 1 Hz), so do not choose a section longer than this. The data box at the bottom of the screen will update the period of the data capture in seconds as you move the second cursor along the data stream. The location of this counter is circled in the picture just above.

3] Uncompress the Data
After your cursors are set, press the F7 key again, and select the "1" button on the bottom of the dialog box that appears. This will uncompress the data to its original span. This step is important since it will allow the data to be saved in the 15, 5 or 1 Hz format in which it was originally captured.
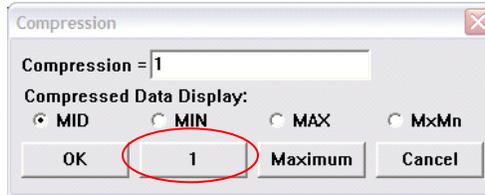
4] Save the Data to the Appropriate File Format
Go to the File drop-down menu and choose "Save As." On the right-hand side of the dialog box that appears, please choose the format "5) Spreadsheet print (CSV)". You should then choose the desired filename and location, then press the "Save" button.
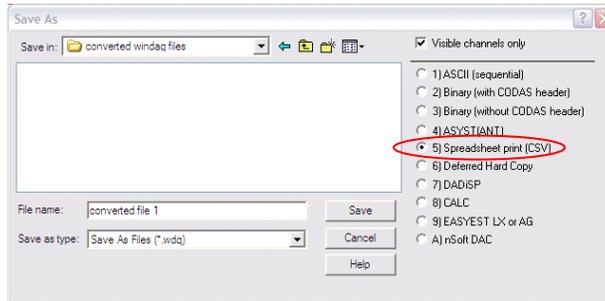
After you press the save button, the following dialog box will appear. We recommend that you just press "OK." However, there are a couple of options that you can choose that will improve the representation of your data once it is in the Excel format. Firstly, if you want to transfer the time-base to your Excel file, you should check the "Relative Time" box. Secondly, if you desire to transfer the channel names that appear on the left side of the Windaq® charts (see Figure in Step 2 above) to your Excel file, you can select "Engineering Units" and all data columns will have the channel names appear as column headers. Once you have done this, you can then press "OK."
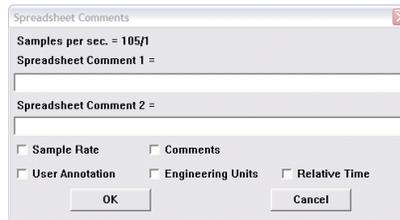
5] Opening the File in Excel
The data are now in a file format similar to a MouseOx® Plus text file. To read them into Excel, follow the instructions given in the section above, "Opening Text Data Files Using Microsoft® Excel."

12. MouseOx® Plus Analog Pulse Signal Output

The MouseOx® Plus Analog Pulse Signal Output (APSO) provides you with the capability of looking at raw infrared light signals before they have been processed with our software algorithms. These signals can be obtained from the BNC connector protruding from the left side of the control unit. You can use a BNC cable to connect the MouseOx® Plus device directly to a measuring device, such as an oscilloscope, or to an alternate measurement device for triggering. **Note that the signal operates on a floating ground, and that you must be sure that the device to which you connect the signal has a floating, differential input**.
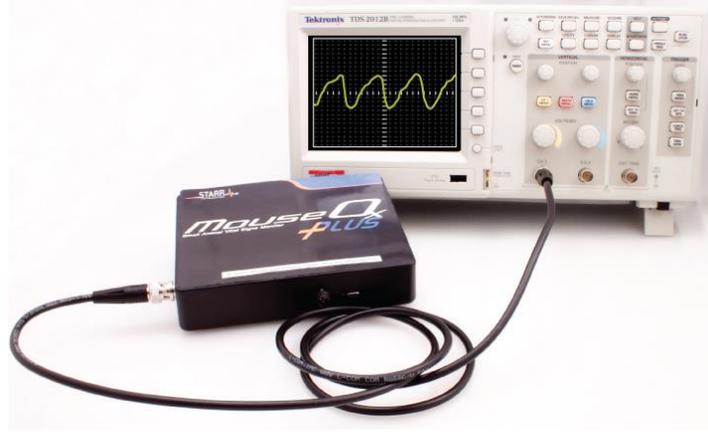
The primary application for the MouseOx® Plus APSO is event triggering. The signal provided is an electronic version of the yellow Pulse Pleth trace that you see on the Monitor Subject screen, which can be used for cardiac triggering. There are some things to be aware of before you use this signal however. The output signal from the APSO spans ± 5 volts, but is usually much smaller. It is AC-coupled, indicating that its mean value resides around 0 volts, so it can easily be amplified externally.

The signal does undergo the analog filtering and amplification that occurs before we digitize it and use it to calculate the oximetry parameters. However, its amplitude is subject to all of the analog amplification methods used by the MouseOx® Plus control system. Thus, it can suddenly increase or decrease in amplitude as the MouseOx® Plus controller makes its adjustments. You can eliminate these changes by turning off the Cruise Control.

The signal is continuous and has no delay, outside of that associated with both low and high-pass analog filtering. This delay is inconsequential for the purposes of cardiac triggering, although we do not know its magnitude specifically.

Additionally, the signals are subject to various forms of noise in our system, which includes motion artifact due to animal motion, variations in amplitude due to changes in signal amplification from the MouseOx® Plus controller as we just described, small variations in amplitude due to breathing artifact on the signal and various characteristics of peripheral microvascular physiology that can make the signal shape vary.

In the following pictures, we see sample signals from the APSO on an oscilloscope in three different stages of time compression. Note that the signal is not completely repetitive in shape or amplitude as is the case with a pure sine wave. The reason for this is that the signal represents the received infrared light from the photodiode that has not been digitized and undergone the filtering used to optimize the signals to allow measurements to be made from them by the MouseOx® Plus.
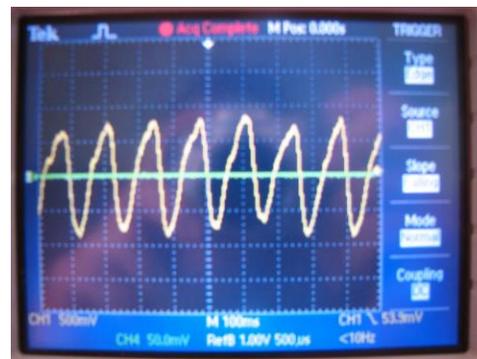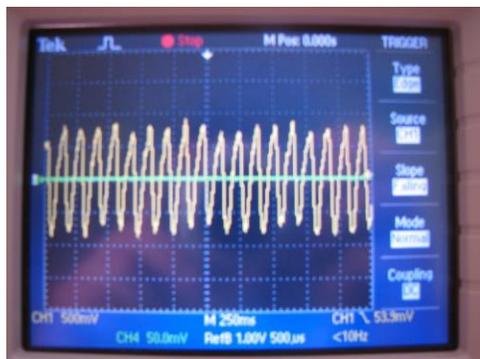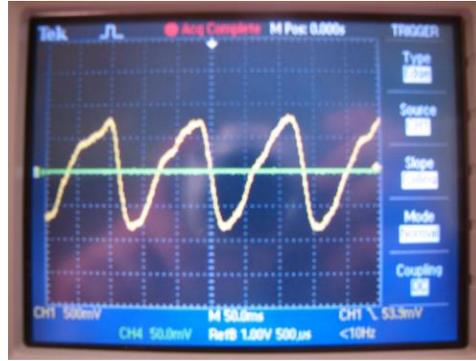
If you observe the figure directly above, you can see that there is a fast downstroke followed by a slow upstroke. The fast downstroke represents increased peripheral blood flow associated with cardiac ejection, or systole. The slow upstroke is associated with diastole. It is our observation that the most repeatable (jitter-free) portion of the signal is the vertical middle of the downstroke, which represents some part in the middle of the cardiac ejection phase. We cannot say exactly what portion this is, since we do not know what delays are associated with blood flow to the periphery. Regardless, it might be best to set the trigger at this point, and add any desired delay externally, if possible.

Recommendations for use as a trigger:

1] Once you get good signals as indicated by the MouseOx® Plus software, turn off the Cruise Control.

2] Use the middle portion of the downstroke as a trigger point, then delay from that location if necessary.

3] Make sure that the animal is anesthetized or restrained sufficiently to prevent motion from deleteriously affecting the signal. However, you do not want to over-anesthetize the animal such that it starts to gasp, as this will cause the signal to "jump" at each inhalation.

4] Make sure that the device into which you are plugging the APSO signal has a high input impedance and that it is non-grounded (*i.e.*, has a floating differential input).

13. MouseOx® Plus Body Temperature Option (ACCESSORY)

13.1. General Information

The MouseOx® Plus Temperature Option accessory allows you to monitor rectal temperature of an animal during any time that other MouseOx® Plus measurements are being made. The MouseOx® Plus Temperature Option comes with the following:
- Body Temperature Software
- 1 Rectal Temperature Sensor, which can be either of the following:
  Regular Temperature Sensor w/ 6' Cable

  MRI-Compatible Temperature Sensor w/ 20' Cable

Some of the characteristics and capabilities of the system include the following:

- The Body Temperature Option works with all MouseOx® Plus software packages.

- Body temperature can be measured and recorded in both Celsius and Fahrenheit scales. The output is user-selectable. Default is Celsius.

- The temperature measurement range is 15°C to 50°C (60°F to 120°F).

- Both types of temperature sensor plug directly into the same port on the MouseOx® Plus control unit.

- Temperature sensors do not have an intentionally limited life.

- The MouseOx® Plus temperature sensors use a very tiny sensor in order to minimize potential trauma to the animal.

- The same sensor can be used on both mice and rats.

- Temperature measurement is functional even if you are operating your MouseOx® Plus on Awake Animals.

- Temperature measurement can be made when using the Multiplexer™, even though the MouseOx® Plus system can only accommodate 1 temperature sensor.

13.2. Using the Temperature Sensors

Body Temperature Option Software
If you purchased the Body Temperature Option with your MouseOx® Plus system, the software is already included.

If you purchased the Body Temperature Option as an add-on to a previously purchased MouseOx® Plus, you must first load the software upgrade. This can be done following the instructions given in an earlier section on software loading.

Measuring Temperature
Connect the temperature sensor to the temperature port on the MouseOx® Plus control unit.

The measurement end of the probe can be used as a rectal thermometer, but can also be used in other locations. Once the sensor has been placed, you are ready to make measurements. Refer to the Software Description section below for further details.

Cleaning the Temperature Sensor
The end of the temperature sensor should be swabbed with alcohol after each use.

13.3. Software Description

The Temperature window can be accessed from the View Temperature Window button in the bottom right-hand corner of the Monitor Subject screen.
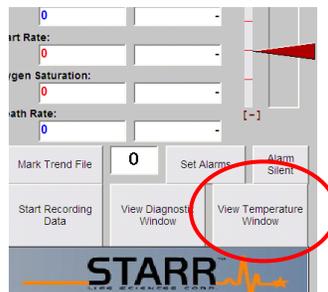
View Temperature Window
The View Temperature Window button brings up the following screen.
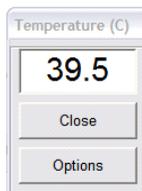
The current temperature is displayed in the upper window. Units are displayed in °C or °F. If no sensor is attached, the box will display "na" and zeroes will be written to an open active data file.   If a sensor is connected and the Temperature Window is not displayed, the data will still be recorded to an open active data file.

Close
The Close button closes the window in both the short and long-form windows.

Options
The Options button expands the temperature window to the longer form shown here.
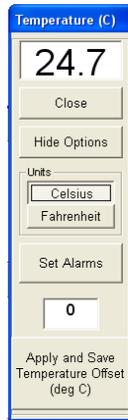
Hide Options
The Hide Options button returns you to the short form temperature window.

Celsius/Fahrenheit
The Celsius/Fahrenheit toggle flips between temperature scales in the display window. The values for the currently displayed temperature scale are saved in both the Windaq® and text data files. If you press this toggle while data are being saved, the saved numerical values will immediately change to the new setting. Note that the units saved in the title row of a data file will remain those active when the data file recording was initiated. The default temperature scale is Celsius.

Set Alarms
The Set Alarms button performs the same function as the Set Alarms button on the Monitor Subject screen. The functionality of this screen is described in great detail in the Using the MouseOx® Plus Software section above. Note that there is a specific alarm setting for temperature.

14. MouseOx® Plus Multiplexer™ (ACCESSORY)

14.1. General Information

The MouseOx® Plus Multiplexer™ is series of 3 accessories that allow you to conduct measurements on up to 16 animals, one at a time, using **one** MouseOx® Plus control unit. One version provides MouseOx® Plus and temperature measurements for up to 8 animals. The Multiplexer™ is shipped with a Patch Cable and a 2.0 USB cable.

Some of the characteristics and capabilities of the system include the following:

- You can monitor all MouseOx® Plus parameters on any one of up to 16 animals. The particular animal whose parameters are active is user-selectable, or you can scroll through each automatically.

- The system connects separately to the same computer used by the MouseOx® Plus, via a second USB cable.

- The Multiplexer™ can be configured to cycle through and record data from each animal connected automatically.

- Custom ID tags allow for user input tags to be correlated with the subject number. This data will show up in a saved text file. Custom ID tags can be used with both manual or auto multiplexing applications.

- When used with the APSO, the APSO output will change to that of the active channel.

14.2. Using the Multiplexer™

Multiplexer™ Software
If you purchased the Multiplexer™ with your MouseOx® Plus system, the correct software and hardware is included. However, the MouseOx® Plus system must detect the presence of a Multiplexer™ in order for that software to become active. This will happen when you plug in the USB cable.

System Connections
The Multiplexer™ comes with a Patch Cable, a 2.0 USB cable and an external power supply. The Patch Cable, shown here, connects the Multiplexer™ to the MouseOx® Plus, while the USB cable provides an independent connection to the same computer into which the MouseOx® Plus is plugged.

There are 3 types of Multiplexer™:
Multiplexer™ - 16
Multiplexer™ - 8

Multiplexer™ - 8 w/Temperature

The Multiplexer™-16 has 16 separate input channels with 8 in the front and 8 in the back. The Multiplexer™-8 provides the same 8 channels as the Multiplexer™-16, and the back plate is empty.

The channels are numbered numerically from 1 to 16. All references to the channels in the software or recorded files use these numbers for identification. **It is up to you to track the association between the test subject and the number of the channel.**

Multiplexer™-16 and 8	
Multiplexer™-16 Backplate	
Multiplexer™-8 Backplate	
Multiplexer™ - 8 w/Temperature Backplate	
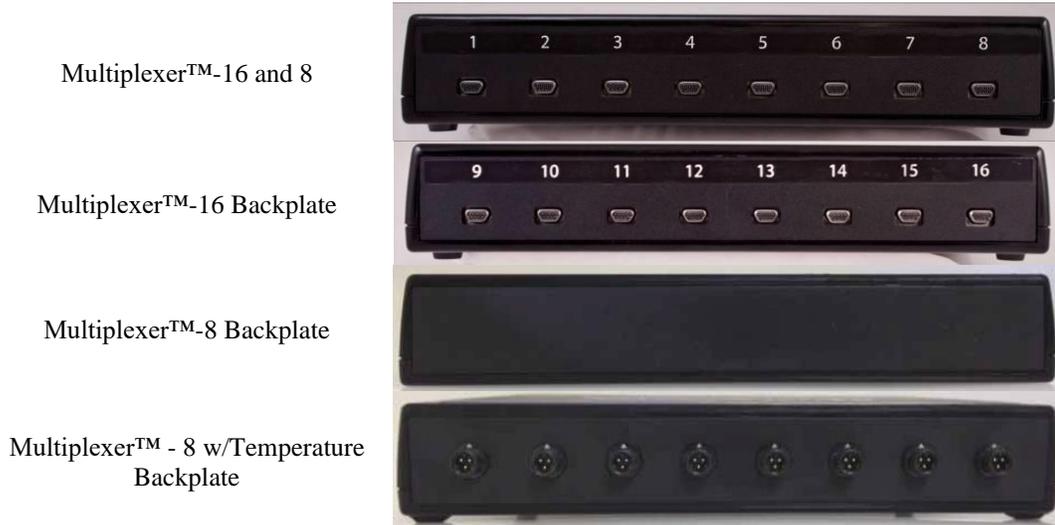
On the Multiplexer™ w/ Temperature, the backplate has a series of 8 connectors that accept STARR Life Sciences® temperature probes. Each temperature port corresponds to the numbered input channel on the opposite side of the device. Also, as with the regular input channels, the only active temperature channels are those that correspond to the active input channels.

When using the Multiplexer™, you are not required to use the channels in order, or even use adjacent channels. For instance, if you are using 2 channels, you can choose 4 and 11, and the system will work as well and as easily as if you used channels 1 and 2. If you try to use a channel that does not have a sensor plugged in, you will get the message "Sensor Not Detected."

The cabling connectors on the Multiplexer™ are shown in the next images.

Wall Power Input
Patch Cable Input
USB Input

(Multiplexer™ -8 w/Temperature)

Patch Cable Input
Wall Power Input
Temperature Patch Cable Input
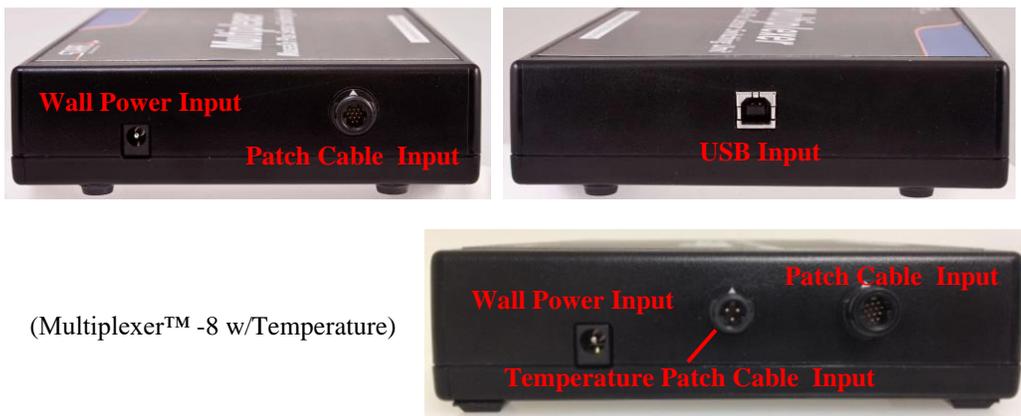
To set up the system, connect the Patch Cable into the mating port on the Multiplexer™, then connect the other end of the Patch Cable into the Universal Cable Input on the MouseOx® Plus. The two systems can be stacked to reduce their overall footprint on the lab bench. The Multiplexer™ - 8 w/Temperature has an

additional patch cable that must be connected between the Multiplexer™ Temperature Patch Cable Input and the MouseOx® Plus temperature port (the same port into which a single temperature cable is plugged in the absence of the Multiplexer™).

Next, connect the USB cable between the Multiplexer™ and the computer, then connect the power supply to the port on the Multiplexer™. The order of connection is inconsequential. Finally, connect the different Universal Cables into the channels of your choice.

Cleaning the Multiplexer™
The MouseOx® Plus device and all accompanying cables should be cleaned using only light detergent and water.

Product Serial Number
Please note that as with the MouseOx® Plus, the serial number for the Multiplexer™ resides on the upper left-hand corner of the bottom label.   Please have this number ready if you call STARR Life Sciences® for technical assistance.
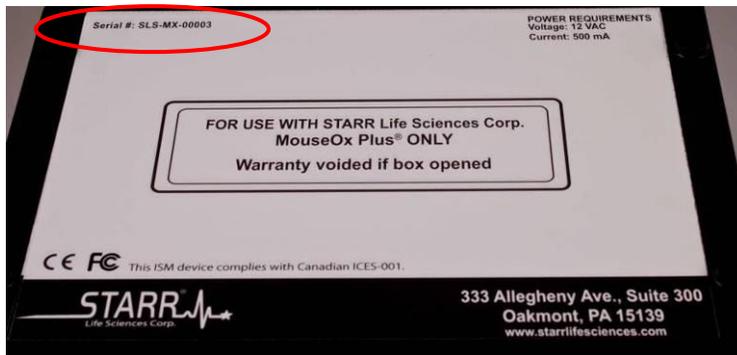
14.3. Multiplexer™ Software Description

The Multiplexer™ can be operated such that data collection is manual or automated. To operate in either mode, you must select the Multiplexer Menu button at the top of the Monitor Subject screen just below the Start/Reset button.
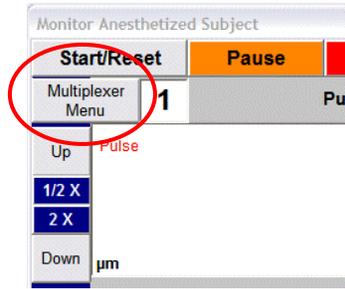
14.3.1. Manual Multiplexer™ Operation

When you press the Multiplexer Menu button, the Manual Multiplexer screen appears.
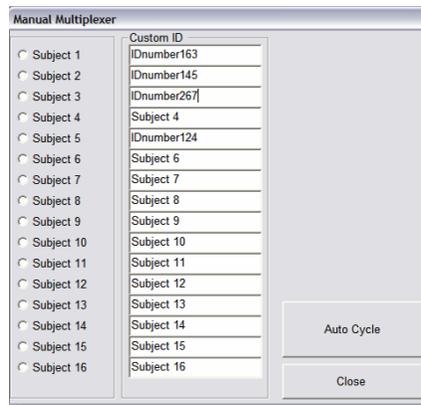
To operate the Multiplexer™ manually, you can change the active subject by clicking on the subject number of interest. Once you do this, the window will close and the active subject number will be changed on the window adjacent to the Multiplexer Menu button on the Monitor Subject screen. When you first do this, you must press Start/Reset to initiate data collection. From that point, all of the parameter values on the screen will be derived from the animal on the active channel. At any time you can key in custom ID tags in the corresponding text boxes next to the subject number. This parameter is sampled for the saved text file, just like any other parameter, so it is best to have this information all set up before you start saving a text file.

Some additional useful information includes the following:

- The MouseOx® Plus uses Channel 1 as the default channel. You can of course, use any channels that you would like, but the system will automatically poll Channel 1 in the absence of your input.

- The MouseOx® Plus software stores all of the electronic gain settings for the currently active channel before it moves to the next channel so that when it returns to this channel, it can pick up where it left off.

- Once a channel is selected, all displayed parameter values are calculated for the animal on that channel where the active subject number again is displayed in the window next to the Multiplexer Menu button.

- If you are saving a data file, all of the parameters saved will correspond to the animal that is associated with currently active subject number. Additionally, in the data file, there is a Subject # column that tells you which animal the associated data on that row correspond to. When the subject number is changed, the data written to the file will immediately switch to that from the new animal.

- If you try to use a channel that does not have a sensor plugged in, you will get the message "Sensor Not Detected."

Auto Cycle
The Auto Cycle button opens the Auto Cycle Multiplexer window (see next section).

Close
The Close button closes the Manual Multiplexer window.

14.3.2. Automated Multiplexer™ Operation

A description of the software associated with automated operation is given here. When you press the Auto Cycle button on the Manual Multiplexer window, the following Auto Cycle Multiplexer window appears. At any time you can key in custom ID tags in the corresponding text boxes next to the subject number. This parameter is sampled for the saved text file, just like any other parameter, so it is best to have this information all set up before you start saving a text file.
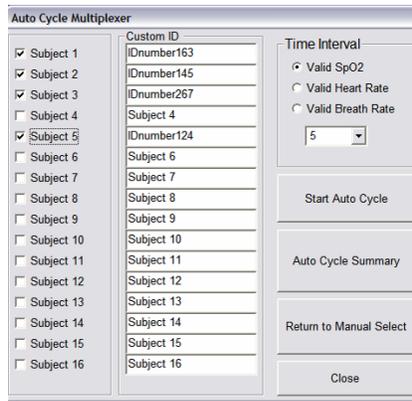
When this window appears, none of the subject numbers on the left side of the window will be selected. You must select the channels you want to utilize, but each must have an active sensor attached. You can edit or update this channel selection menu at any time.

Time Interval
During automated operation, the Multiplexer™ software will roll through each of the selected active channels based on your desired criterion. You must first set the contingent parameter that will drive switching of the subject number. These parameters, which appear immediately under Time Interval on the Auto Cycle Multiplexer window, are S_pO_2, Heart Rate and Breath Rate. You can select the contingent parameter by clicking on the option box next to the associated parameter. The default parameter is S_pO_2.

Below the contingent parameter list is a box in which you can set the duration (in seconds) over which valid data for the contingent parameter will be collected before the system switches to the next active subject number. The system operates such that the clock only counts valid data (data with no error codes for the contingent parameter) cumulatively. Thus, if an error code for the contingent parameter you choose is active for 6 seconds during a selected duration of 10 seconds, the active channel will switch after a total of 16 seconds since at that point, 10 seconds of valid data have been collected.

Start Auto Cycle
This buttons initiates the Auto Cycle process starting with the first subject number that you selected. If you press this button any time after initiation, the system will go back to the first active subject number.

Auto Cycle Summary

This button opens the Auto Cycle Summary screen shown below.
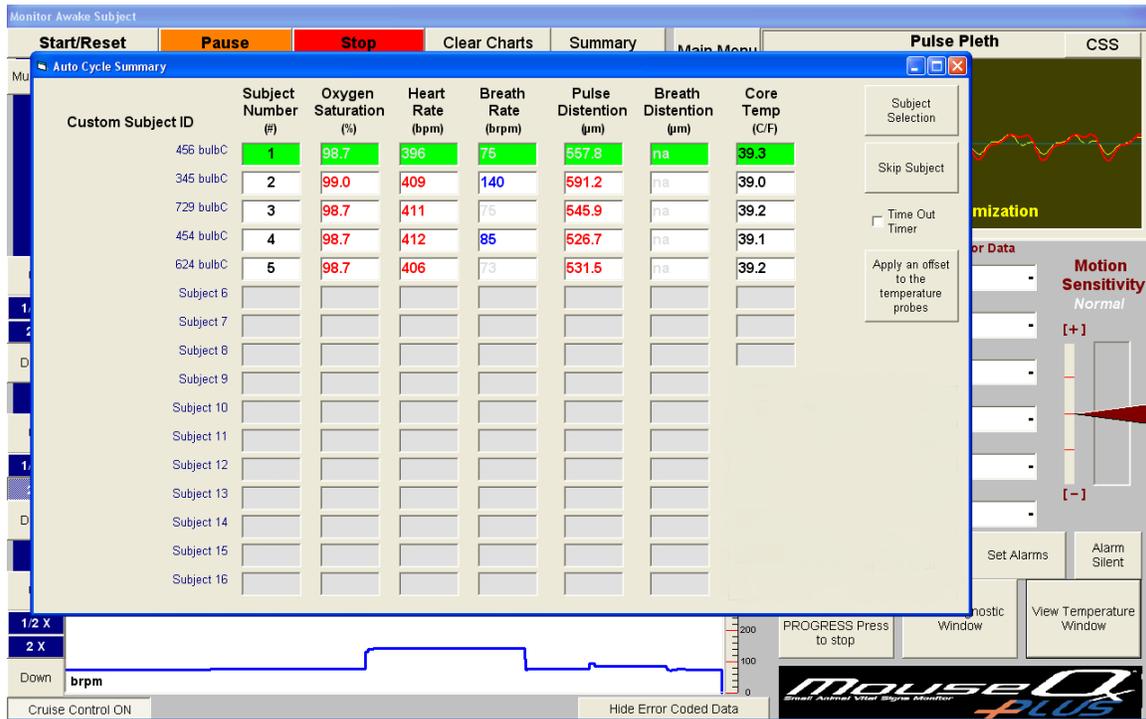
In the figure shown here, there are spaces for all 16 possible subjects. However, windows not selected will be grayed out and all active windows will appear white. Furthermore, the boxes associated with the currently updating subject will appear bright green. Some other properties of this table include:

- When data are being collected, the parameter values will appear gray until valid, non-error-coded data is calculated, at which point, they will appear in their appropriate color.

- When the subject number switches, the parameter values for the new active subject are returned to 0 in each box, and the new calculations will update as described just above.

- The MouseOx® Plus software stores all of the electronic gain settings for the currently active channel before it moves to the next channel, so that when it returns to this channel, it can pick up where it left off.

- Once a channel is selected, all displayed parameter values are calculated for the animal on that channel where the active subject number again is displayed in the window next to the Multiplexer Menu button on the Monitor Subject screen.

- If you are saving a data file, all of the parameters will automatically be saved for the animal with the currently active subject number.

- If you try to use a channel that does not have a sensor plugged in, you will get the message "Sensor Not Detected."

- This window is sizeable.

Subject Selection
This button will reopen the Auto Cycle Multiplexer window so that you can make changes to the list of active subject channels.

Skip Subject

When you press this button, the Auto Cycle processor skips to the next active subject for that cycle. On the next cycle, the skipped subject will be re-interrogated. If you want to skip a subject continually, you will need to de-select it from the list by pressing the Subject Selection button.

Time Out Timer
When this checkbox is highlighted, the time out function for each channel will be active. This means that the active channel will automatically time out after approximately the longer of 30 seconds or 3x the collection period that you have selected in the original Time Interval box. If you de-select this box, the active channel will grind until it obtains sufficient data. This box is defaulted in the de-select position.

Apply an Offset to the Temperature Probes
Upon pressing this button, an additional column appears on this screen as shown below.
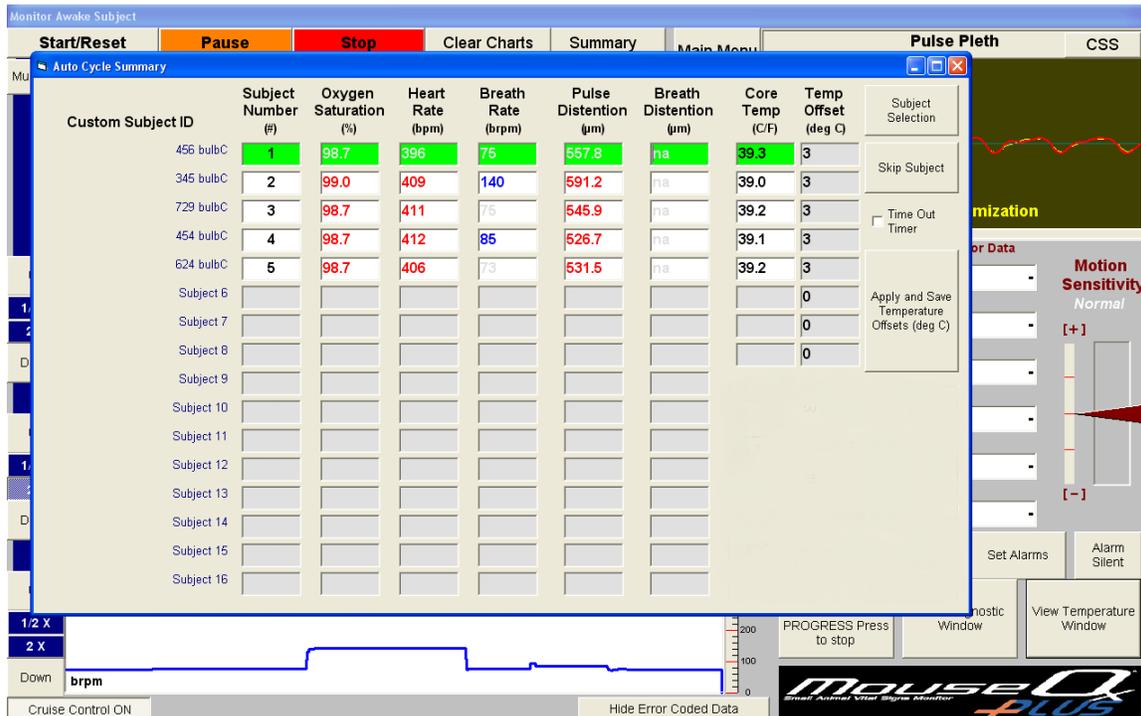
Separate values can be entered into any of the slots under the Temp Offset heading. Each slot corresponds to the subject number to the left. The functionality of this offset is that the displayed value in the Core Temp column will have the corresponding value under Temp Offset added to it. This functionality allows you to make adjustments for any individual sensors that may be evidently in error. After pressing this button, its title changes to Apply and Save Temperature Offsets.

Apply and Save Temperature Offsets
When pressed, this button saves the offset values and applies them to the displayed temperatures.

Return to Manual Select
This button returns you to the Manual Multiplexer window and **it stops the Auto Cycle process**.

Close
This button closes the Auto Cycle menu and returns you directly to the Monitor Subject screen. If the Auto Cycle function is left operating, the number window under the Start/Reset button and adjacent to the Multiplexer Menu button will continue to update the active subject number as the Auto Cycle process continues.

15. Effect of Computer Sleep Modes on the MouseOx® Plus

Screen Saver
If the MouseOx® Plus software is running or collecting data or writing to a data file, and the computer screen saver activates, there will be no effect on any of the operations. As is true any time the computer goes into screen saver mode, you need to move the mouse or touch a key to return the active screen.

System Standby
If the MouseOx® Plus software is open, but data are not being collected, and the computer goes into System Standby, you must close and reopen the program to re-establish USB communications. We also recommend that you Restart the computer before restarting the MouseOx® Plus software. If the MouseOx® Plus software is open and data are being collected, the computer will not go into System Standby. **We recommend that the dedicated MouseOx® Plus computer have the System Standby sleep mode deactivated.**

System Hibernate
If the MouseOx® Plus software is open, but data are not being collected, and the computer goes into System Hibernate, you must close and reopen the program to re-establish USB communications. We also recommend that you Restart the computer before restarting the MouseOx® Plus software. If the MouseOx® Plus software is open and data are being collected, the computer will not go into System Hibernate. **We recommend that the dedicated MouseOx® Plus computer have the System Hibernate sleep mode deactivated.**

16. Troubleshooting

16.1. Questions and Answers

As a starting point, make sure that you have properly followed all of the directions in your User Manual for installing your software and hardware. Please note that the MouseOx® Plus device will not appear to function until you have started data collection by hitting the "Start/Reset" button on the "Monitor Subject" screen in the software.

Q. I had a MouseOx® setup in the lab and everything was working. After some period of time, the software just locked up. What happened?

A. It is likely that USB communication has been lost with the computer. Due to the constraints of Windows®, it is necessary that you close all the way out of MouseOx® Plus, pull the USB cable from either the computer or MouseOx® Plus device, wait about 10 seconds, then plug it back in. After the USB cable is re-inserted, restart the MouseOx® Plus software.

If this problem persists, a better solution is to purchase a universal power supply (UPS), which can be obtained from any electronics or computer store. We recommend that you purchase a UPS with a minimum of 450 volt-amps.

Q. Why do I lose my signal when I put ground shielding around the BNC cable connected to the MouseOx® with APSO (analog pulse signal output)?

A. The ground plane on the APSO is floating. The exposed metal on a BNC connector is attached directly to one of the conductors in the wire. When you apply noise shielding that touches this exposed metal, you drive the signal to ground, causing its loss. The APSO connector must be attached to a floating, differential input.

Q. What other things can I check if signals are poor?

A. You should check on the following if signals are poor:

General

- If you have detached the sensor while the sensors lights are illuminated, you should press the "Start/Reset" button. This will reset the amplifier gains and allow the MouseOx® Plus device to converge on the correct values faster. Note that this will not interrupt data recording.

- Be aware that LED light reflecting off of shiny surfaces can sometimes make signal processing difficult for the MouseOx® Plus. We have observed this from materials as seemingly innocuous as Formica. We recommend that animals be placed on a non-reflective material that hides a reflective lab bench surface.

- A light-blocking cloth should be used in all anesthetized applications if the room is extremely bright from either artificial lights (such as surgical lights) or direct sunlight. Fluorescent lighting in particular, is more problematic than other types of artificial lighting or direct sunlight.

- Did you deliver too much anesthesia? Very high anesthesia levels can lower the breath rate (BR must be ≥ 25 brpm), and even sometimes the heart rate (HR must be > 90 bpm), outside of the valid ranges of the MouseOx®. You can use the anesthesia alarms to help you to assess this, or you can simply note if the animal is gasping, a sure sign of over-anesthesia.

- Dark fur can impede transmission of light. Make sure that you shave the sensor sight if the animal has dark fur. Note as well, that animals with dark fur may also have highly pigmented skin, which can also impede light transmission. If this is the case, it may be necessary to find a thinner section of perfused tissue, or a section of tissue that is less pigmented.

- Even if the subject has light-colored hair, signal quality can always be improved by shaving the sensor site.

Neck Measurements

- For freely moving animals, make sure that the clip is located in FRONT of the paws and BEHIND the ears. Be sure that the clip does not capture either of the ears in any manner. It also helps to push the clip down on the neck of the animal, then to squeeze the tines together.

- Note that the Conscious Measurements Enclosure provides a shiny surface, which can cause reflections that affect the integrity of the light signals used for measurements in the MouseOx® Plus. We recommend that you use some bedding in the enclosure to provide a light-block for the bottom surface of the enclosure.

Thigh/Foot Measurements

- Because pulse distention provides a direct measurement of signal strength, low values of pulse distention (<5.0 μm) indicate a need to reposition the sensor if the weak signal is due to poor access to arterial blood. Note that it is also possible that the animal actually does have a low pulse distention, which may be due to a number of factors such as low fluid volume, low cardiac contractility, low body temperature that causes poor peripheral perfusion, etc.

- Keep the subject warm. If the appendage on which the clip is placed feels cool to the touch, the subject probably needs to be warmed. We suggest a drug store heating pad set on "Low."

- If signals are poor after trying all of the above, move the sensor clip to the other foot or thigh, keeping in mind that it should not be placed on an appendage that is cannulated.

- [*For mice only*] The non-LED side of the sensor should be on the side of the thigh that is not directly exposed to room lighting. Make sure that the sensor clip is covering muscle tissue, and not just skin, since skin is poorly perfused.

- [*For mice only*] Try moving the sensor inward on the thigh, toward the pelvis. Perfusion generally improves proximally.

- [*For rats only*] The non-LED side of the sensor should go on the bottom of the foot (the pad) and the sensor should run lengthwise with the foot, not across it. Both sensor pads should always be COMPLETELY covered/filled by perfused tissue. Thus, the clip should be placed on the center of the foot from side to side.

- [*For rats only*] If good signals cannot be obtained from the foot, try the sensor on the calf or thigh. Remember, if the subject has dark fur, the sensor site will need to be shaved.

16.2. Items Related to *Insta*Cal™

The MouseOx® Plus device contains embedded calibration software and hardware from Measurement Computing Corp. These embedded components are controlled by the *Insta*Cal™ software, which you have loaded into the computer as part of the MouseOx® Plus software. There are some requirements given by Measurement Computing related to use of this software. These are:

1] Windows® Updates

The *Insta*Cal™ program that is included with your MouseOx® software requires that you have conducted all of the most recent Windows® Updates. There are a few ways to get to the Windows® Update to check that you have these. These include:

- Click the "Start" button. For the older Windows® Start button format, there will simply be a button titled "Windows Update" at the top of the menu that appears when you have clicked the "Start" button. Click on that "Windows Update" button and follow the instructions. Select the "Express" option once you are at the Microsoft® website. **OR**

- Click the "Start" button. For the newer Windows® Start button format, click on "All Programs." At the top of the menu that appears, click on "Windows Update," and follow the instructions. Select the "Express" option once you are at the Microsoft® website.

[*For Windows® XP only*] Make sure that the Windows® XP Hotfix KB822603 has been installed on any machine with Windows® XP. This patch fixes an error associated with operation of the USB ports.

2] USB Cable Usage

Do not disconnect the USB cable from either the computer or control unit end while the software is running. Doing so will sever communications between the MouseOx® Plus and the computer, which may cause loss of data.

If the LED on the sensor is illuminated then goes dark, the computer has lost communication with the control box. In order to re-establish communications, you must first exit the MouseOx® Plus software by pressing "Main Menu" from the Monitor Subject screen, then press "Close Program" from the Main Menu. After closing the program, disconnect the USB cable, wait about 10 seconds, then reconnect it and wait another 10 seconds. This should reset communications. At this point, the software can be rerun, and the sensor LED should illuminate.

16.3. System Resource Usage

In some cases, your computer may be running other programs simultaneously with the MouseOx® Plus software, which may use up system resources significantly. System resources are simply defined as computer RAM. This can cause the MouseOx® Plus system to sporadically skip groups of data because calculations for a current set of data are not completed before the next set of data are collected. This problem is not obvious because it does not cause a loss of communication between the MouseOx® Plus and the computer. The solution to this problem is to minimize usage of the memory from other applications by closing them whenever you use the MouseOx® Plus.

Windows® does provide a method for assessing what resources are available, but we have found that these indicators are inconsistent and do not provide a clear means for identifying what you have the capacity to actually do at any given time. Thus, we will simply recommend that you close any applications that are not needed when using the MouseOx® Plus.

17. MouseOx® Parameter Specifications

17.1. Oxygen Saturation (%)

Measurement Range (pulse rate 90 to 900 bpm)
0 to 100% Arterial Blood Oxygen Saturation

Measurement Resolution (pulse rate 210 to 900 bpm)
Resolution ≤ 1.5%, across entire range

Measurement Response Time
S_pO_2 is reported to the user after each heartbeat. Every 0.72 seconds the screen is refreshed to display all of the S_pO_2 values measured since the previous screen refresh.

17.2. Heart Rate (bpm)

Measurement Range
90 to 900 bpm (beats per minute)

Measurement Resolution (heart rate 210 to 900 bpm)

Rate (bpm)   Resolution (bpm)
210   2.4
300   4.9
400   8.7
500   13.5
600   19.4
700   26.2
800   34.0
900   42.9

Measurement Response Time
Heart rate is reported to the user after each heartbeat. Every 0.72 seconds the screen is refreshed to display all of the heart rate values measured since the previous screen refresh.

17.3. Pulse Distention (µm)

Measurement Range (pulse rate 90 to 900 bpm)
0 to 800 µm

Measurement Resolution
≤ 2.4% of measurement

Measurement Response Time
Pulse distention is reported to the user after each heartbeat. Every 0.72 seconds the screen is refreshed to display all of the pulse distention values measured since the previous screen refresh.

17.4. Respiratory Rate (brpm)

Measurement Range (pulse rate 90 to 900 bpm)
25 to 450 brpm (breaths per minute)

Measurement Resolution
Rate (brpm)   Resolution (brpm)
25   < 0.1
100   0.6
200   2.2
300   4.9
400   8.7

Measurement Response Time
Respiratory rate is reported to the user every 1.7 sec. The value reported is the result of a moving average of the rate of breathing for the 10 previous breath rate measurements. Note that the screen still updates at the rate used for the other parameters (0.72 seconds), but new breath rate data are presented at the lower rate.

17.5. Breath Distention (μm)

Measurement Range (pulse rate 90 to 900 bpm)
0 to 800 μm

Measurement Resolution
≤ 2.4% of measurement

Measurement Response Time
Breath distention is reported to the user as it can be validly calculated. It cannot update any faster than breath rate.

17.6. Body Temperature (°C or °F)

Measurement Range
15 to 50°C

Measurement Resolution
±0.1 °C or °F

Measurement Response Time
Body temperature is updated every 0.72 seconds.

18. Contacting STARR Life Sciences® for Technical Support

STARR Life Sciences® values its customers and is dedicated to providing quality products and solutions to meet your needs. For technical support, please call or email STARR Life Sciences® at: 1-866-978-2779, or support@starrlifesciences.com.

19. Intellectual Property Protecting the MouseOx® Plus

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