Micro Oxymax
Instruction Manual

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1 Hardware

1.1 Introduction

The Micro Oxymax is a multi channel respirometer that is capable of measuring up to 6 gases in up to 80 different gas streams (channels) The system consists of a System Sample Pump, Expansion Interface and an individual gas sensor for each gas that is measured. The System Sample Pump contains a micro processor that controls the system and communicates to the host computer through the RS-232 serial port

System Overview

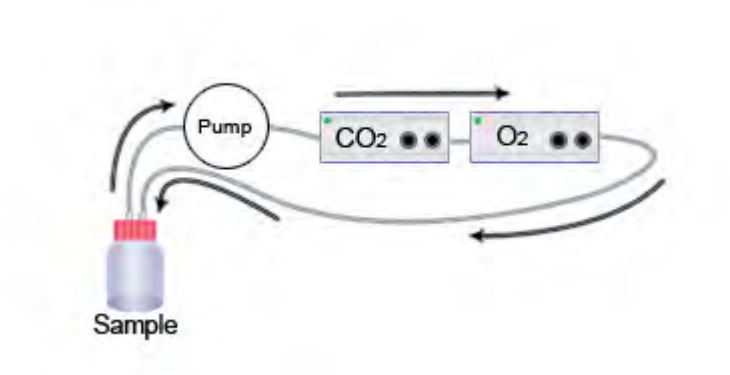
Multiple gas streams are sequentially multiplexed into the system sample pump through the expansion interface(s) The system sample pump directs the sample gas to the sample drier to remove the water vapor, this is needed to make accurate gas concentration measurements. Then the gas is circulated through each individual sensor at a constant flow rate and pressure to prevent errors caused by barometric pressure changes. The model Micro Oxymax employs a host computer for data collection, storage and presentation device. The software supplied with the system allows the user to configure the system for use with a wide variety of gas samples

The following ranges and gases are available

O2	19-21%,0-100$ (user programmable for any range in 0-100%
CO2	0-2000ppm,0-1%,0-3%,10-10% 0-30%,0-100%
CH4	0-1000ppm, 0-1%,0-5%,0-30%,0-100%
H2	0-1000ppm,0-2000ppm
H2S	0-200 ppm
N20	150 ppm
N02	1000 ppm
CO	0-1000 ppm, 0-1%

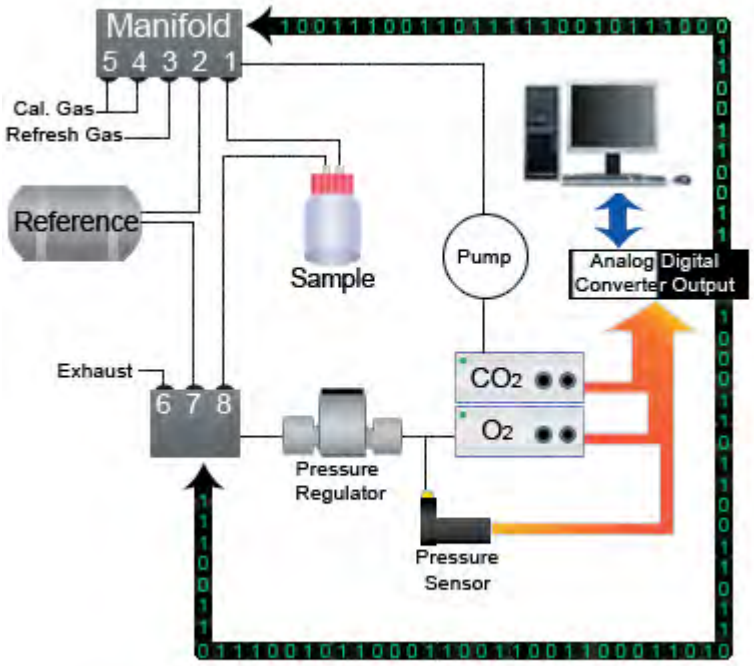
Introduction: The Micro-Oxymax Respirometer measures the production and/or consumption of many gases simultaneously. Data is typically reported as a percentage concentration of each individual gas with respect to the given sample. These percentages are used to calculate other data such as rates and cumulative gas production / consumption. However, the measurement of these simple percentages becomes surprisingly complex when working with such small volumes and concentration changes. The Micro-Oxymax boasts a maximum sensitivity of 0.2µL / hour rate calculation. Automatically, one begins to think about the accuracy of the gas sensors and their ability to accurately measure such small changes in concentration. Though the sensors do play an important role, what really makes the system this sensitive is the "Closed Loop Measurement Method." This can be further enhanced by adding certain options that are specific for the experiment. The following article will describe, in simple terms, how the Closed Loop Measurement Method works, describe the various techniques used by the different gas sensors employed in the Micro-Oxymax System, and break down the optional components for tailoring the system for specific experiments.

The Closed Loop Measurement Method is such a key principle of measurement that we've patented it in the United States, Europe, and Japan. For reference, the patent numbers are 4947339 (US), 0372429 (EUR), and 2117492 (JAP). The 16 page document is full of mathematical equations and descriptions of measurement procedures that can be difficult for the everyday user to fully understand. In short, the Closed Loop Measurement Method is simply a control mechanism to isolate a sample's head space gas, thoroughly mix it, pass it through gas sensors, and return it to the sample chamber.



Simple enough, but the patent also describes the method by which we calculate concentration percentages. Granted, the gas sensors provide us with an immediate value. However, the value we get from the sensors is not exactly representative of the concentration value within the sample chamber. Simply put, the values given by the gas sensors must be back-calculated to get the corresponding concentration of gas inside of the sample chamber. In order to back-calculate from the concentration of gas in the sensors to the concentration in the Sample Chamber, the volumes of each must be measured before hand. But measuring these volumes in the traditional manner (length * height * width) is very difficult, especially considering that the volume of the sample must be accounted for in the Sample Chamber; this would be impossible to measure by hand. The Micro-Oxymax utilizes a pressure sensor; by pressurizing various volumes and comparing the pressure readings of known volumes versus unknown volumes, we can use Boyle's Law (P1V1 = P2V2) to calculate the volumes automatically. But in the effort to avoid all of the math in the patent, it's easiest to follow through the 10 basic steps of the 1st measurement cycle. This includes measuring barometric pressure, sensor volume, sample chamber volume, reference air gas concentrations, purge air gas concentrations, sample head space gas concentrations, and calculations of sensor drift.

Before we get into each individual step, its helpful to know what components lie inside of the system and what they do. Refer to the diagram below for the flow diagram.



Reference Chamber: This is exactly 1L in volume and made of stainless steel. It has two main functions. 1) It is a fixed volume that we use to calculate sensor and sample head space volume using various pressures. 2) It stores a known concentration of air that we use to calculate sensor drift after each measurement cycle.

Analog / Digital Converter: This takes the analog signals from the gas sensors and pressure sensor and converts them to a digital signal. This is needed for 2 reasons. 1) The PC requires digital input. 2) The valves that control air flow through the system receive a digital signal (open or closed).

Manifold and Valves 1 - 8: These simply direct the air flow for each stage of the measurement process.
Pressure Sensor: This measures the amount of pressure passing through the pressure regulator. This is primarily used during the sensor and chamber volume measurements. Recorded pressures are used to calculate volumes using equations derived from Boyle's Law.
Pressure Regulator: This keeps the pressure constant as the gas enters the sensors. Any changes in pressure inside of the sensors would result in erroneous readings.

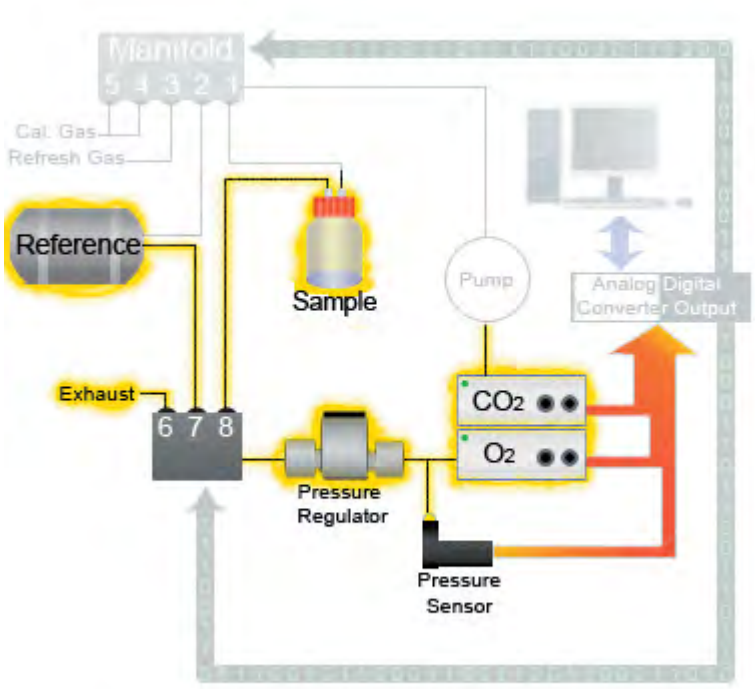
Pump: Simple enough, the Pump pumps air into, out of, and through the system for all the various functions. Its the quiet humming sound heard when the system is up and running.

Outside of the system are the sample chamber and the gas sensors, which are located in their own separate cabinets. The principles of operation of the sensors will be discussed later. The Sample chamber shown in these diagrams is connected directly to the "System Sample Pump." Most Systems have multiple samples with a multiplexer lying between the System Sample Pump and the Sample Chambers, called an "Expansion Interface." The Expansion Interface plays a passive role during these processes. It is comprised of a system of manifolds and valves that act as an intermediary between each individual Sample Chamber and the System Sample Pump and Gas sensors. In other words, the Analog / Digital Converter and Controller tell the Expansion Interface which sample it needs to measure and the Expansion Interface opens the necessary valves and closes the rest. Since its role is passive, it is left out

of the diagrams to more closely relate the System to the sample.

10 Steps of the First Measurement Cycle:
Step 1: The first step is to measure the Barometric Pressure in the room. We open the Reference Chamber, Sample Chamber, and Sensors to the outside environment. The pressure inside the system equalizes with the barometric pressure in the room . The pressure inside the system is recorded. The Sample Chamber and Reference Chamber are opened to the outside since they comprise the bulk of the volume in the closed system.

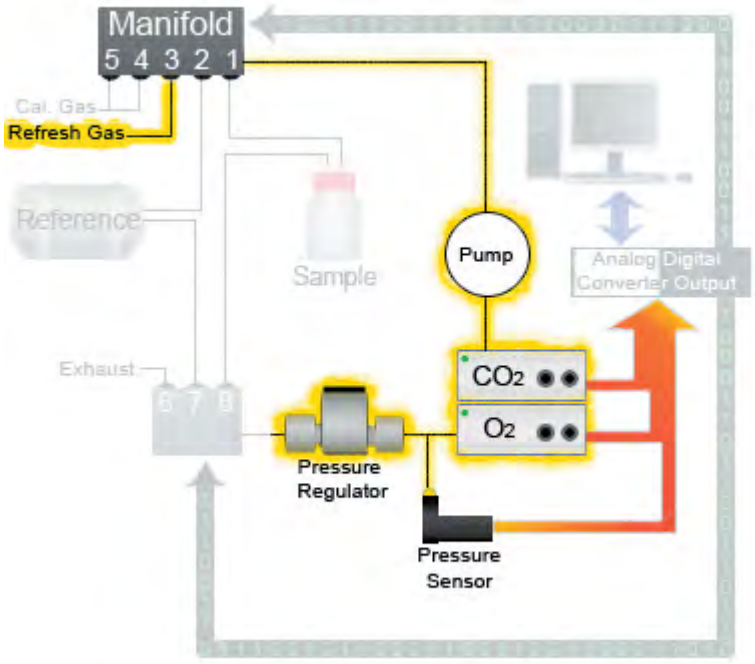
step 1

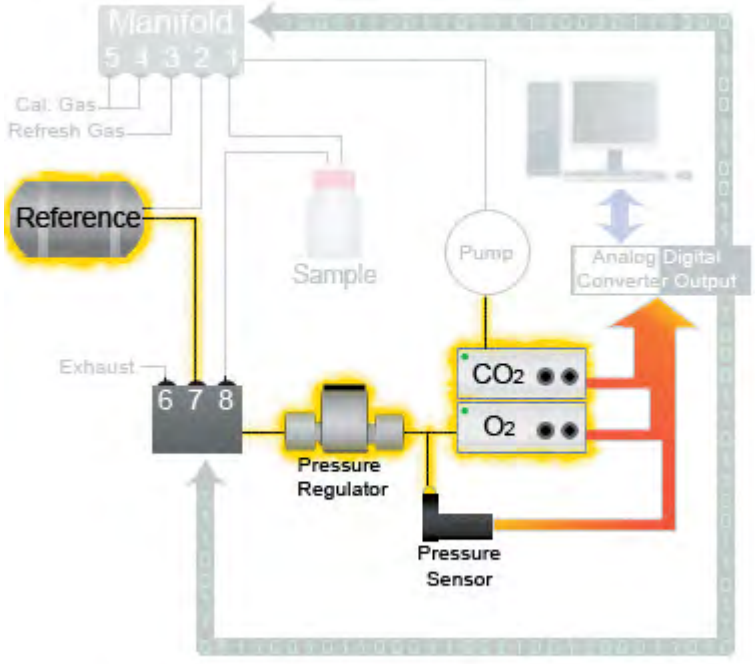


Step 2: Measuring the sensor volume is next. The sensor volume is important to know so that the volume can be accounted for and subtracted when the sample is measured. When the gas analyzers report their values, it includes gas concentrations inside the Sample Chamber plus all of the tubing and the sensors themselves. Since concentration is a percentage of a volume, the sensor and plumbing volumes must be subtracted to yield accurate measurements for the sample.

Measuring the sensor volume is a 3 step process. First, fresh air is used to pressurize the sensors to a value about 50 mmHg above the barometric pressure. Once this is achieved, the valve allowing the flow of fresh air (aka. Refresh Gas) is shut of and everything pauses momentarily to allow the pressure to settle. Typically, the pressure will still rise a little bit after the initial pressurization. Once settled, the pressure is recorded. Since this is the second pressure we've recorded, we'll call this P2 for labeling sake.

Step 2





Next, the pressurized sensors are allowed to equalize with the Reference Chamber . Once this pressure settles, it is recorded. Typically this pressure is only slightly higher than barometric pressure. The original 50+ mmHg pressure difference within the small sensor volume is greatly reduced when equalized with the 1000 ml reference chamber that is at barometric pressure (from Step 1). We'll call this Pressure P3.

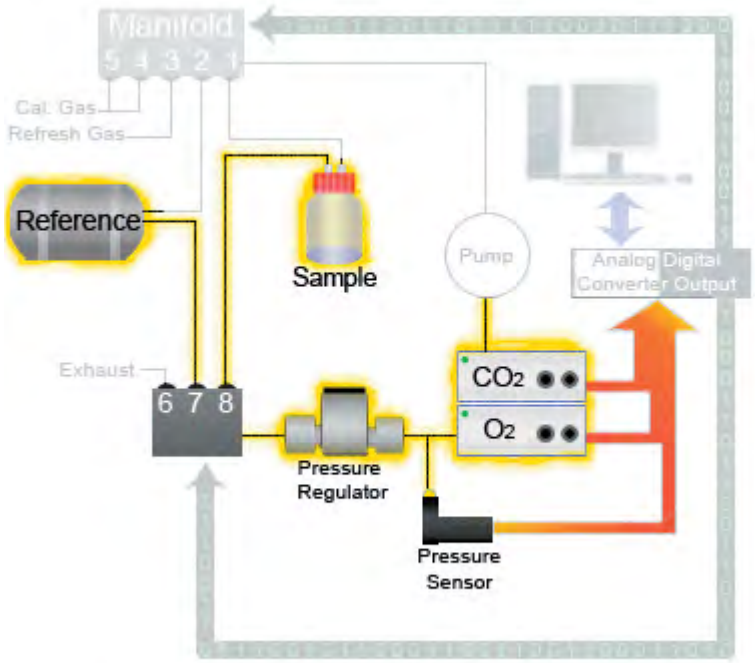
The final step for measuring the Sensor Volume is the calculation. If we compare: A) the pressurized sensors's pressure value (P2), subtract the barometric pressure and then compare it proportionally to B) the equalized pressure with respect to the Reference Chamber (P3) minus the barometric pressure, and use the Reference Chamber volume as a known volume, you can solve for the sensor volume using Boyle's Law. Confused? We subtract barometric pressure from the two pressures we record in this step because the reference chamber is already at barometric pressure. Ideally what we want (in order to use Boyle's Law) is to have a single pressure at Volume 1, then take the same mass of air and distribute it to a Volume 2 which gives a different pressure. We happen to know Volume 2 (its 1 L), and we measure both pressures before and after the volume change, so we 're solving for Volume 1. By subtracting the barometric pressure, effectively what we 're doing is subtracting the mass of air that was already in the Reference Chamber when we mixed them, it cannot be included for the calculation of sensor volume. The actual formula we use, derived from Boyle's Law, is as follows:

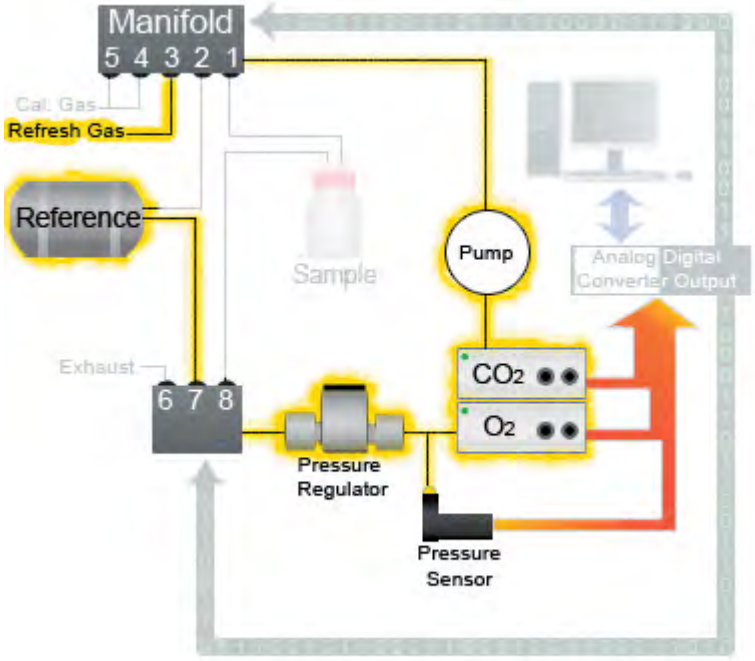
$$Vs=Vr/(((P2-Pa)/(P3-Pa))-1)$$

Where:
Vs=sensor volume
Vr=Reference chamber volume
Pa=Barometric pressure
1=remainder from the cancellation of units

Step 3: Now we measure the Sample Chamber Volume. All we do here is basically repeat Steps 1 and 2. First, we equalize the pressure between the Reference Chamber, Sample Chamber, Sensors and room air until they reach barometric pressure. We close all the valves and then begin pressurizing the system with fresh air again. Except this time, we start with the Reference Chamber and Sensors and bring them to a pressure that is about 50 mmHg above barometric pressure (step 3). We allow the pressure to settle and record our fourth pressure (P4). Once this pressure is recorded, we then allow the pressure to equalize with the Sample Chamber Once settled, we get our fifth pressure (P5).

Step 3





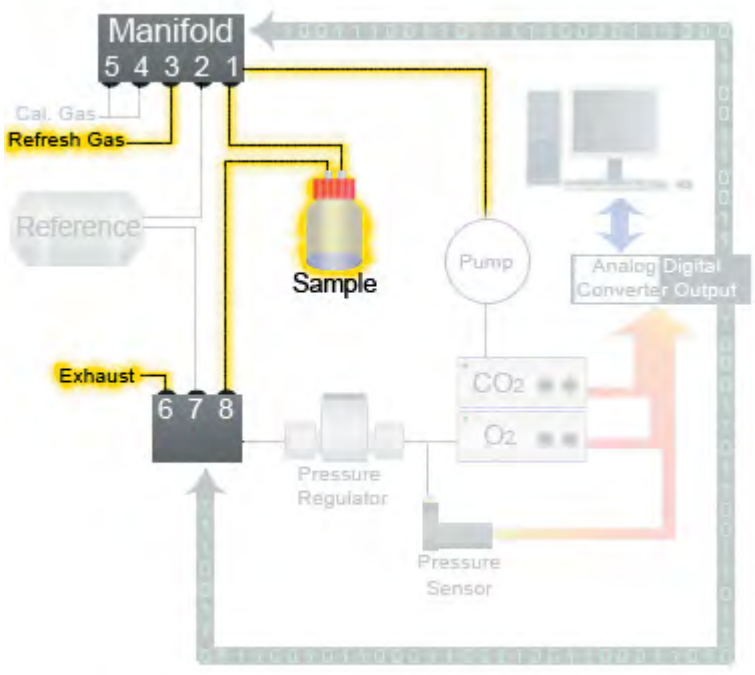
Vt=Vr+Vs/((((P4-Pa)/Ps-Pa))-1)

Where:
Vt=Sample chamber volume
Vs=sensor volume
Vr=Reference chamber volume
Pa=Barometric pressure
1=remainder from the cancellation of units

Now we 're ready to calculate our Sample Chamber Volume. This calculation is only slightly different than the calculation for sensor volume. The difference is that we use the volume of the Reference Chamber plus the Sensor Volume for our known volume (we pressurize both the Reference Chamber and Sensors in Step 3, versus the Sensors only in Step 2). Again, using Boyle's Law, we calculate volume with the following derived formula:

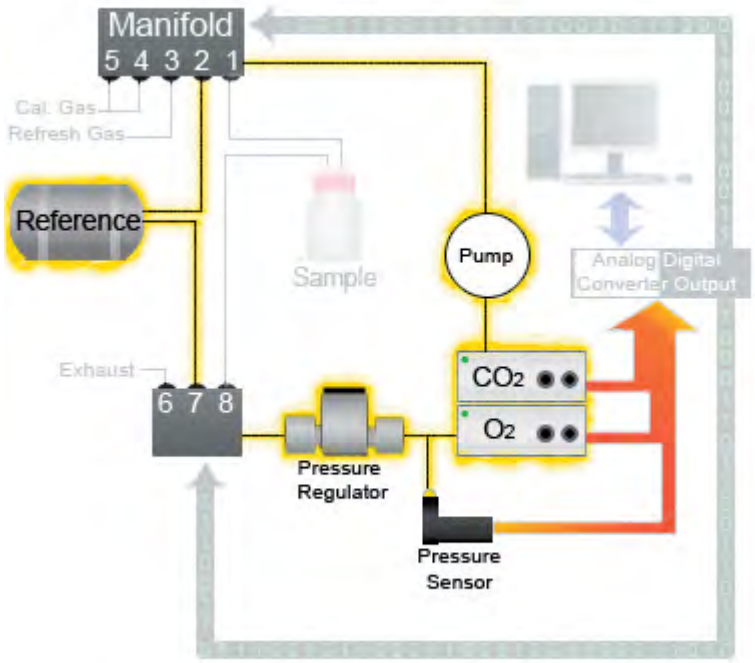
Step 4: Now that we have the volumes measured, we adjust them to STP (Standard Temperature and Pressure), and we refresh the Sample Chambers with fresh air . A custom mixed gas can be used instead of ambient air if desired. This can be in the form of a pre-mixed cylinder or a dynamic gas mixer (like our Pegas 4000MF). For multiple chamber systems, the Sample Chambers are refreshed individually so that the sample air between chambers is not mixed. At this time, the reference chamber is flushed with the same air (fresh air / mixed gas).

Step 4



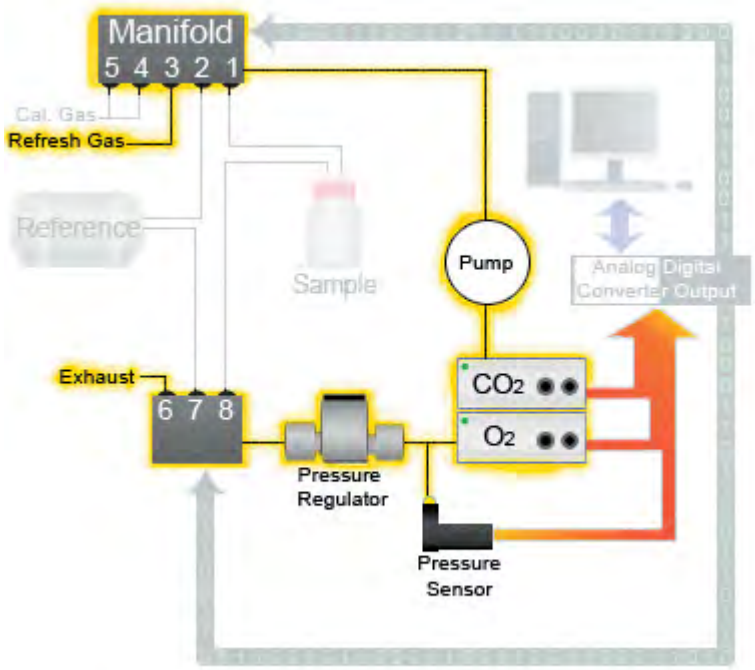
Step 5: Next we measure the concentration of the gases inside of the Reference Chamber. The Reference Chamber is still full of ambient air / mixed gas at this point (from Step 4) so that we have a start point for a reference air measurement. The reason we measure the concentration of the gases inside of the Reference Chamber is to account for sensor drift (discussed later in step 10).

Step 5



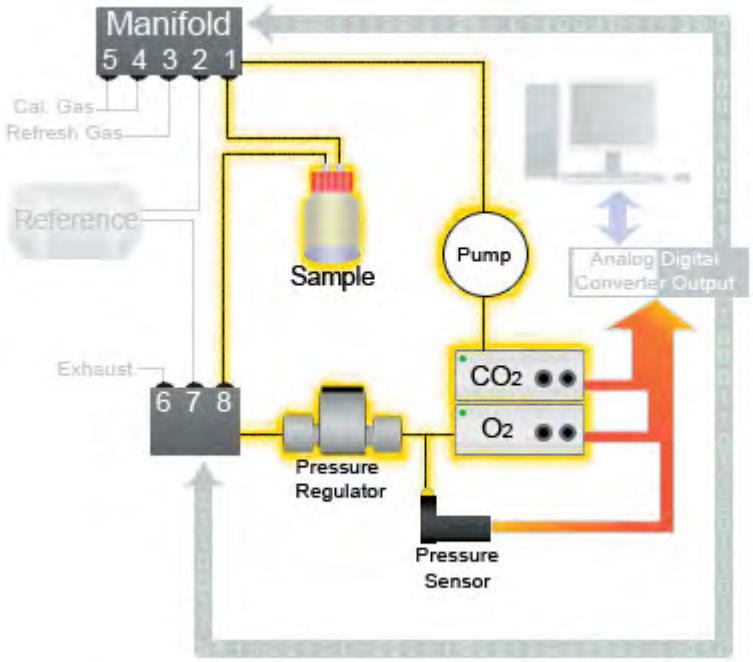
Step 6: Now the purge gas concentrations are measured. This step is rather important. In between the measurement of each sample, the sensors are purged with ambient air to remove the residual gas left in the sensors from the previous sample. But when we measure the next sample, the small amount of gas inside the sensors is mixed with the Sample Chamber gas. So the purge gas is measured by the gas sensors so we know what we've mixed in. We later subtract it from the sample's measurement.

Step 6



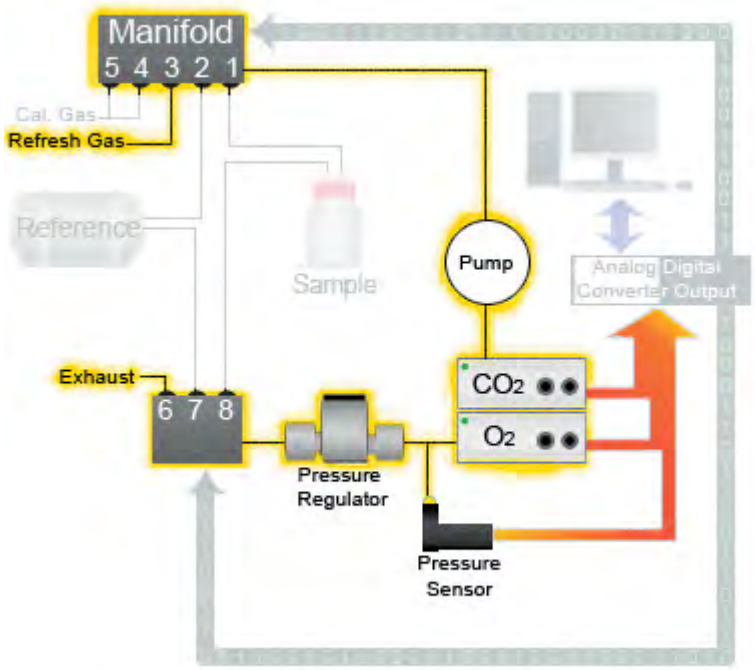
Step 7: We can finally measure our sample! The System circulates the gases from the head space of the Sample Chamber through the sensors then re-circulates them back into the Sample Chamber for a factory set time period of three minutes, and takes a reading from the sensors.

Step 7



Step 8: After we measure the head space of the first sample, the gas sensors are purged to remove any residual sample gas.. This is necessary in order to prevent the previous sample's readings to interfere with the next, and to prevent them from mixing back into the next sample's head space.

Step 8

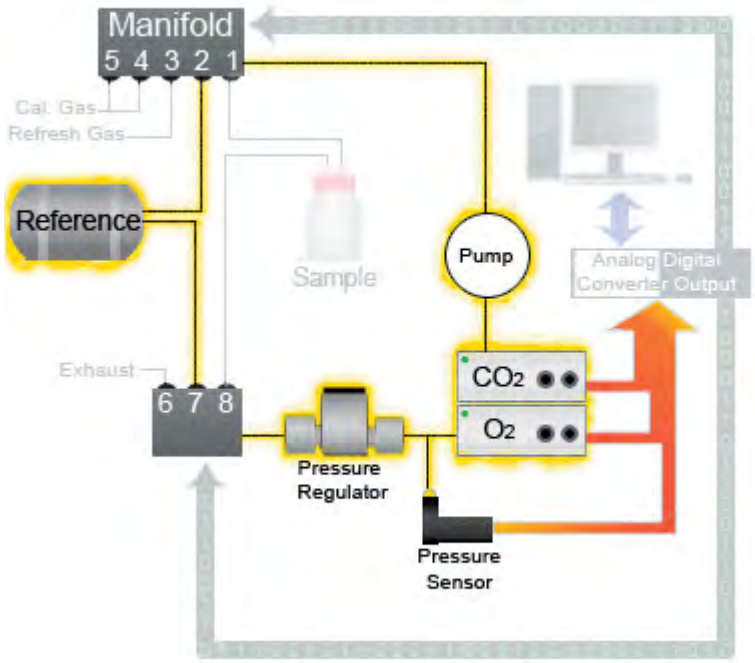


Step 9: After the sensors are purged, we move onto the next sample. We repeat Steps 7 and 8 until all samples have been measured. This gives the first column of data which shows the starting concentrations of each gas, but no rates have been calculated yet. A second data point is needed for this. If "Auto Interval" was selected at the start of the experiment, we immediately go back to the first sample and repeat Steps 7 and 8 again. If the "Auto Interval" was not selected and a time put in its place, the System waits for that time before sampling the first chamber again.

Once two samples have been taking, we compare the difference in gas concentrations, multiply by the volume of head space, and divide by the time of the sample interval

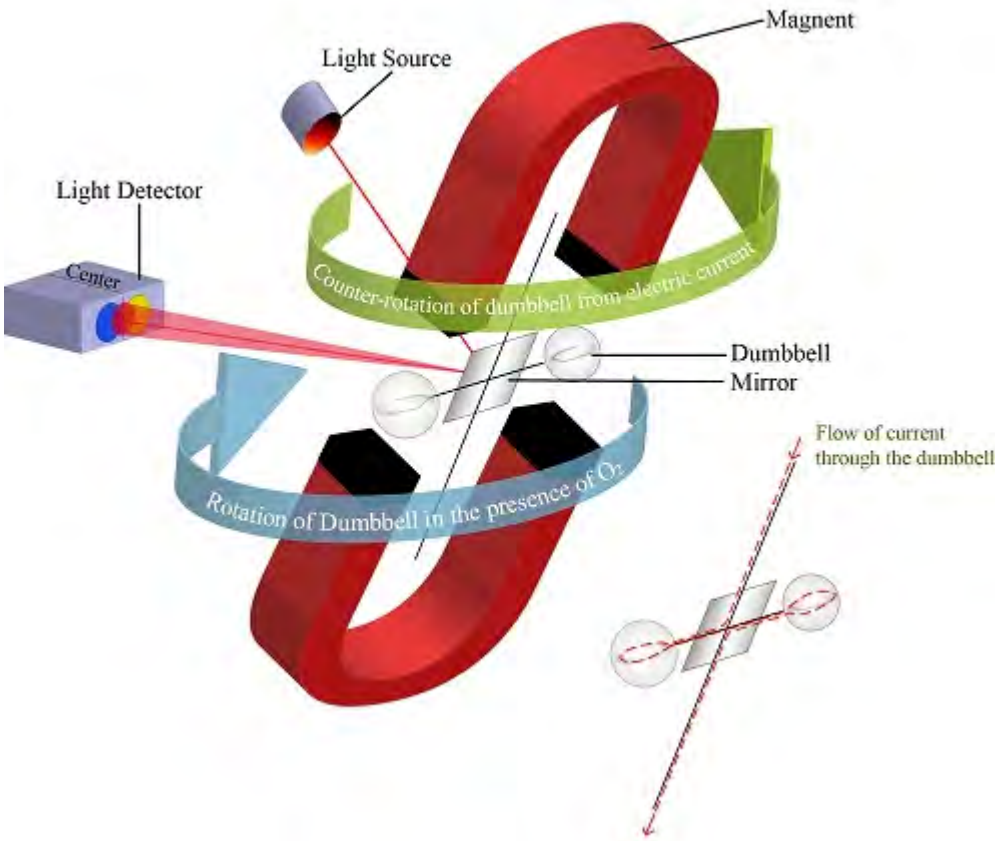
Step 10: Lastly, we measure the Reference Chamber air again We compare these numbers to those recorded in Step 5. The difference in the two values (if any) is termed "Sensor Drift." Over the course of a long experiment, the sensors begin to wander off from their calibration setting. Since calibrating in the middle of an experiment is impossible, we measure a known sample of air at the end of each measurement cycle, and see how it compares to itself over time. The values reported for the samples are adjusted to account for drift step 10 Sensor drift is very slight. From cycle to cycle its not noticeable. But over the span of several days, slight movements in reported values add up and can make a difference by the end of the experiment. Some sensor technologies are more susceptible to drift than others. Electrochemical sensors are the most susceptible. Others, such as Infra Red and Paramagnetic sensors, experience virtually no drift. This is due to the nature of the measurement principles, which we will discuss now.

Step 10

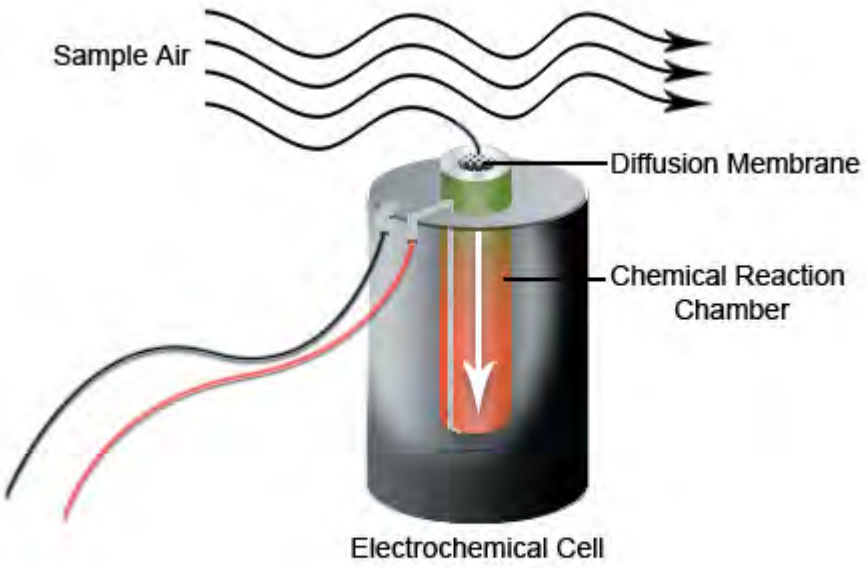


Gas Sensor Technologies: The Micro-Oxymax uses several types of gas sensor technologies to measure the concentrations of different gases. These include Paramagnetism, Electrochemical Fuel Cells, and Non-Dispersive Infrared detection. Following is a brief description of each and their principle of operation.

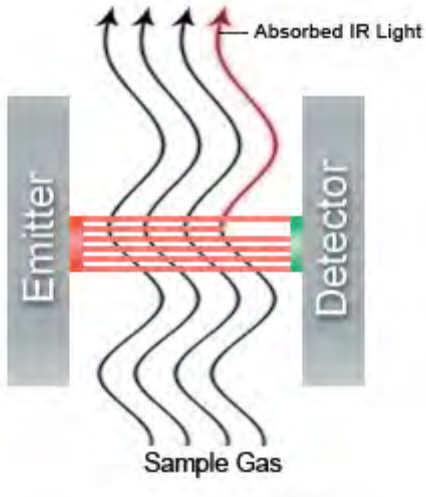
Paramagnetism: This is a physical property of Oxygen that is shared by very few other gases. It exhibits a rather unique behavior within a magnetic field and how it interacts with other objects in that magnetic field. The Paramagnetic Oxygen Sensor consists of a "dumbbell" suspended in a magnetic field by a very fine wire. Sample gas enters the sensor at a very low flow, under 200 ml/minute. As the sample gas passes around the dumbbell, the paramagnetic properties of Oxygen pushes the dumbbell causing it to rotate, which in turn twists the wire suspending it. The rotation of the dumbbell is directly proportional to the concentration of Oxygen present, the more Oxygen, the stronger the rotation. As the dumbbell rotates and the wire is twisted, a small mirror bounces a light source to a detector which tracks the rotation of the dumbbell. A small current is passed through the wire, which brings the dumbbell back to its original position. The more Oxygen present, the more current needed to bring the dumbbell back into position. This current is the signal generated from the sensor.



Electrochemical Fuel Cell: Like the name implies, this sensor is indeed a battery. And just like a battery, it has a finite life span. The gradual "death" of the cell results in sensor drift (which we account for at the end of each measurement cycle). We use it for the detection of Oxygen, Hydrogen, Hydrogen Sulfide, Nitrous Oxide, Carbon Monoxide (2000 ppm), and Sulfur Dioxide. Electrochemical Cells work by chemically reacting to the respective gas present in the sample. The target gas (Oxygen, for example) passes by the cell resulting in a chemical reaction which produces an electric current proportional to the concentration of oxygen in the sample. Electrochemical Fuel Cells consume some of the target gas during the measurement. Therefore, the amount consumed is taken into consideration when making calculations and subsequent measurements.



Non-Dispersive Infrared Detection: This method of measurement is made by simple spectroscopy; emitting a known amount of a specific wavelength of infra-red light through a sample and recording the amount absorbed by the sample. We use this method for the detection of Carbon Dioxide, Methane, and Carbon Monoxide (10%). Each of these compounds absorb a very narrow wavelength range of infra-red light. Each respective sensor emits the corresponding wavelength of IR light, it passes through the sample gas, and a detector records how much of the IR light makes it through. This value is inversely proportional to the concentration of gas in the sample.



Summary: In short, the Closed Loop Measurement Method can be summarized as: Atmospheric air is introduced into reference and sample chambers, whereupon alternating circulation of the gases in the

chambers through gas sensors, a pressure sensor, and a pressure regulator before the gases are returned to their respective chambers. The calculation of gas consumption / production rate by a sample is controlled by a microprocessor which receives signals from the sensors and controls the circulation of gases. Sensor drift is compensated for in the calculation through the use of multiple reference chamber readings and through volume determinations for the reference and sample chambers and the sensors. The gas sensors employed use dependable measurement principles that are applied strategically to accomplish the desired work. The system, being built on a semi-custom basis, can be modified to
handle almost any nature of sample imagined for respirometric measurements.

1.2 Specifications

Power consumption

Micro Oxymax system: 200 Watts
Controller / computer: 200 Watts

Physical dimensions

Sample Pump & Sensors: 13" x 11.5" x 12" (33 x 29 x 30 cm)
Expansion Interface: 13" x 11.5" x 7.5" (33 x 29 x 19 cm)
Controller: 17" x 17" x 7" (43 x 43 x 18 cm)
CO2/CH4/H2S Sensor 13" x 11.5 x 4" (33 x 29 x 10 cm)
Paramagnetic O2 Sensor 13" x 11.5 x 7.5" (33 x 29 x 19 cm)

Weight

System Sample Pump & Sensors: 20 Ibs. (9 Kg)
Paramagnetic O2 Sensor: 15 lbs (6.8 Kg)
Electrochemical O2 Sensor 6 lbs (2.7 Kg)
CO2/CH4/H2S Sensor 6 lbs (2.7 Kg)
Expansion Interface: 12 Ibs. (5.5 Kg)

Gas Flow Rate 100 cc/min 1 - 500 cc/min (500 cc/min standard)

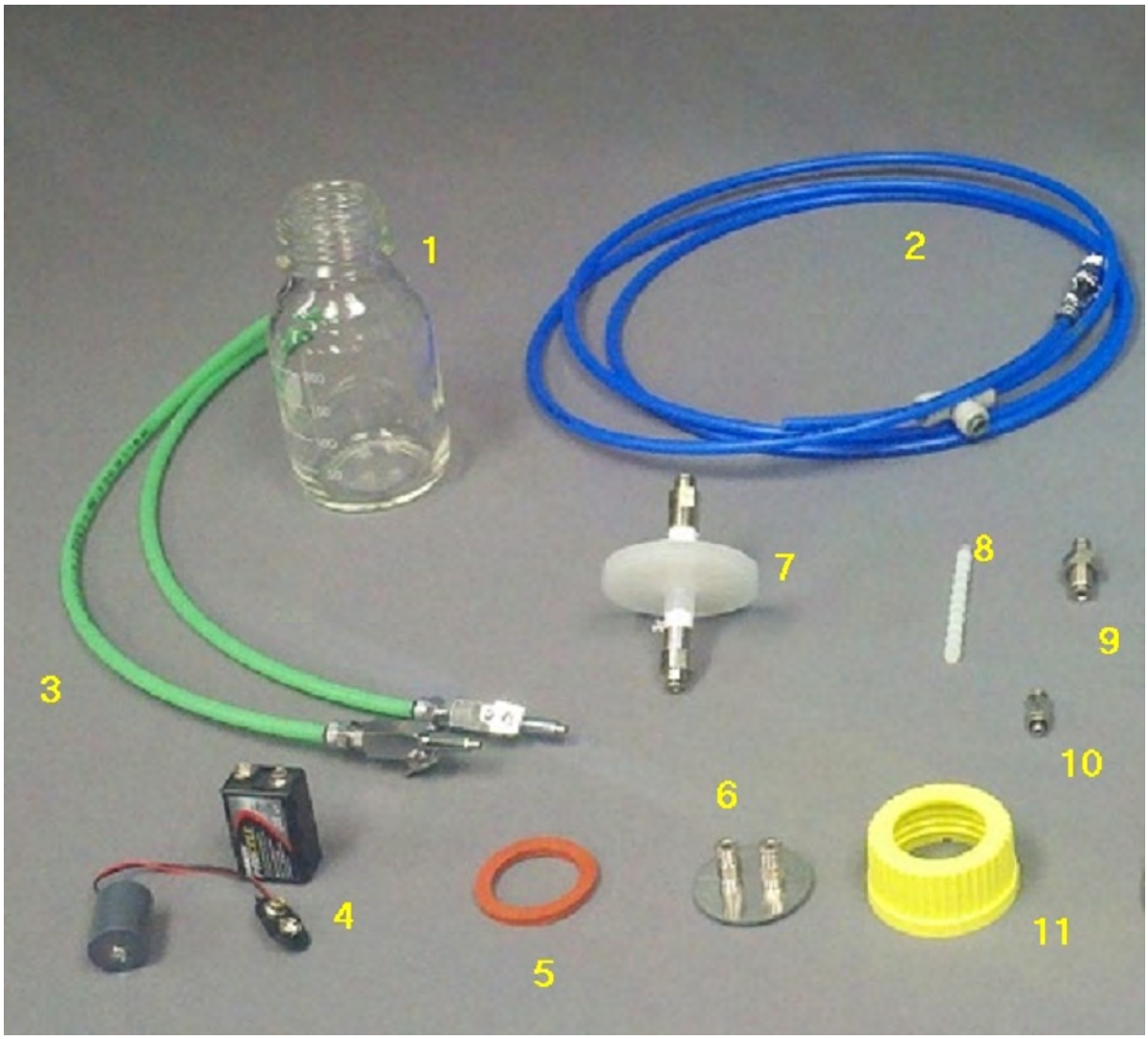
Sensor Ranges

Unless the system is equipped with sensors with non-standard ranges, the range of the oxygen sensor is 19.3% to 21.5%, and the range of the carbon dioxide sensor is 0% to 1.0%. If the oxygen level or the carbon dioxide level goes outside these measurable ranges during the experiment, the consumption and production readings will be incorrect. Normally, this problem can be solved by turning on the automatic refresh. However, in some cases the respiration rate of the sample is so high that not even one sample can be taken before the gas levels cross outside the allowable concentrations. In this case one of the following must be done: decrease the size of the sample, increase the test chamber volume, or shorten the sample interval.

To operate the system with gases other than ambient air (e.g. air with elevated CO2 content or depleted O2 Content) Columbus Instruments can supply the appropriate sensors and system modifications. Contact Columbus Instruments for prices and availability.

1.3 Parts list

1	Drierite column: This connects to the rear of the sample drier
2	Soda lime column: Connects to the N2 port on the rear of the system sample pump. The purpose is to remove CO2 from he air for the zeroing of the CO2 sensor
3	Spare valve: This is a replacement valve for repair of the system sample pump or expansion interface if needed in the future
4	Sensor connection tubing: used for connecting the sensors, system sample pump, and sample drier together
5	USB-serial converter: for connecting the system to computers that do not have a serial port
6	Temperature probe: Connects to the connector labeled temperature probe on the rear of the system sample pump or the first expansion interface. Used to measure the temperature of the sample bottles
7	Connecting cables: Used to connect the sensors to each other and to the system sample pump. Also used to connect the expansion interfaces to each other and the system sample pump



1	Sample chamber (volume from 50 cc-5 liters are available. Also Wide mouth versions are available
2	Calibration connection tubing. This is used to connect the calibration gas cylinder to the calibration port on the system sample pump. It includes a fitting to screw into the pressure regulator on the gas cylinder
3	Drier connection tubing. This connects the drierite column to the rear of the sample drier.
4	O2 standard cell. When the small cylinder is connected to the special zinc air battery this device consumes 10 micro liters/min of O2. It can be placed into the measuring chamber and be used check that the measurements are accurate
5	Flask lid seal, this is used to seal the lid to the glass sample chamber
6	flask lid insert. This goes on top of the sample chamber to allow tubing connections
7	0.2 micron filter. This connects to the IN port on the instrument to protect it from contamination.
8	Replacement plastic ferrules. These are used in the swagelok fittings on the instrument.
9	Replacement expansion interface fitting. This is a spare fitting in case of failure
10	Replacement flask lid fitting. This is a spare fitting in case of failure.
11	Flask lid

1.4 Installation

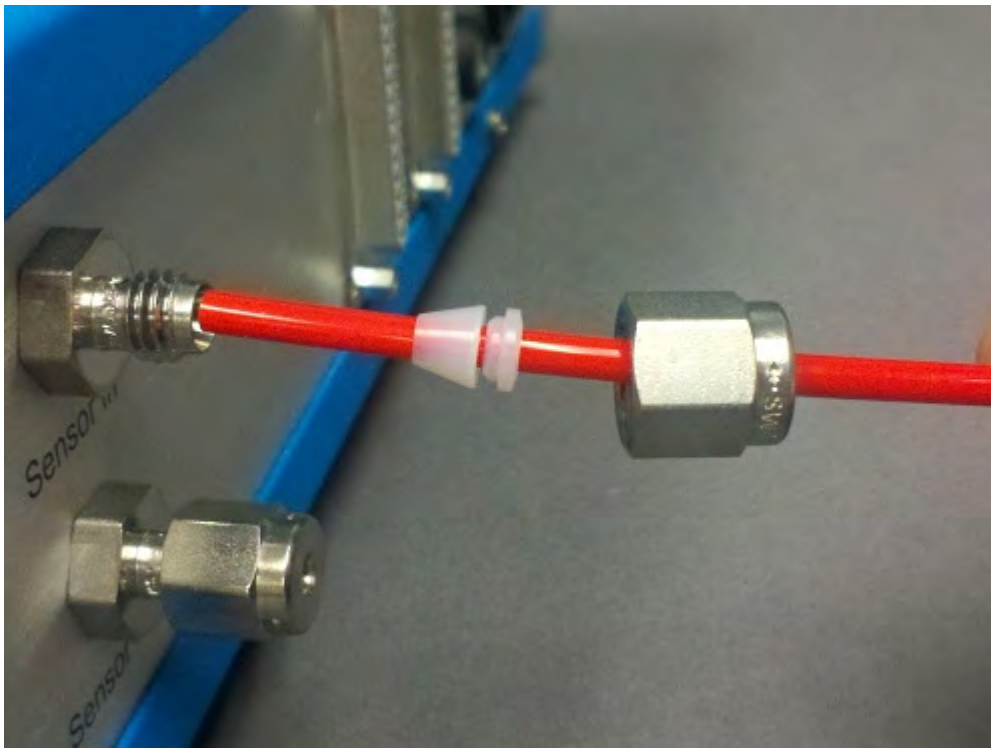
AC Power Filtering and Battery Backup

It is highly recommended to use an AC power filter/surge suppresser with the system to protect all instrumentation. The Micro Oxymax system warranty does not cover damage due to power surges. It is also a good idea to connect the system (including the computer) to an un-interruptable power supply. This will prevent the interruption of experiments and loss of data during brief power outages, and brownouts. Make sure that the un-interruptable power supply delivers a minimum of 500 watts of electrical power.

Tubing Connections

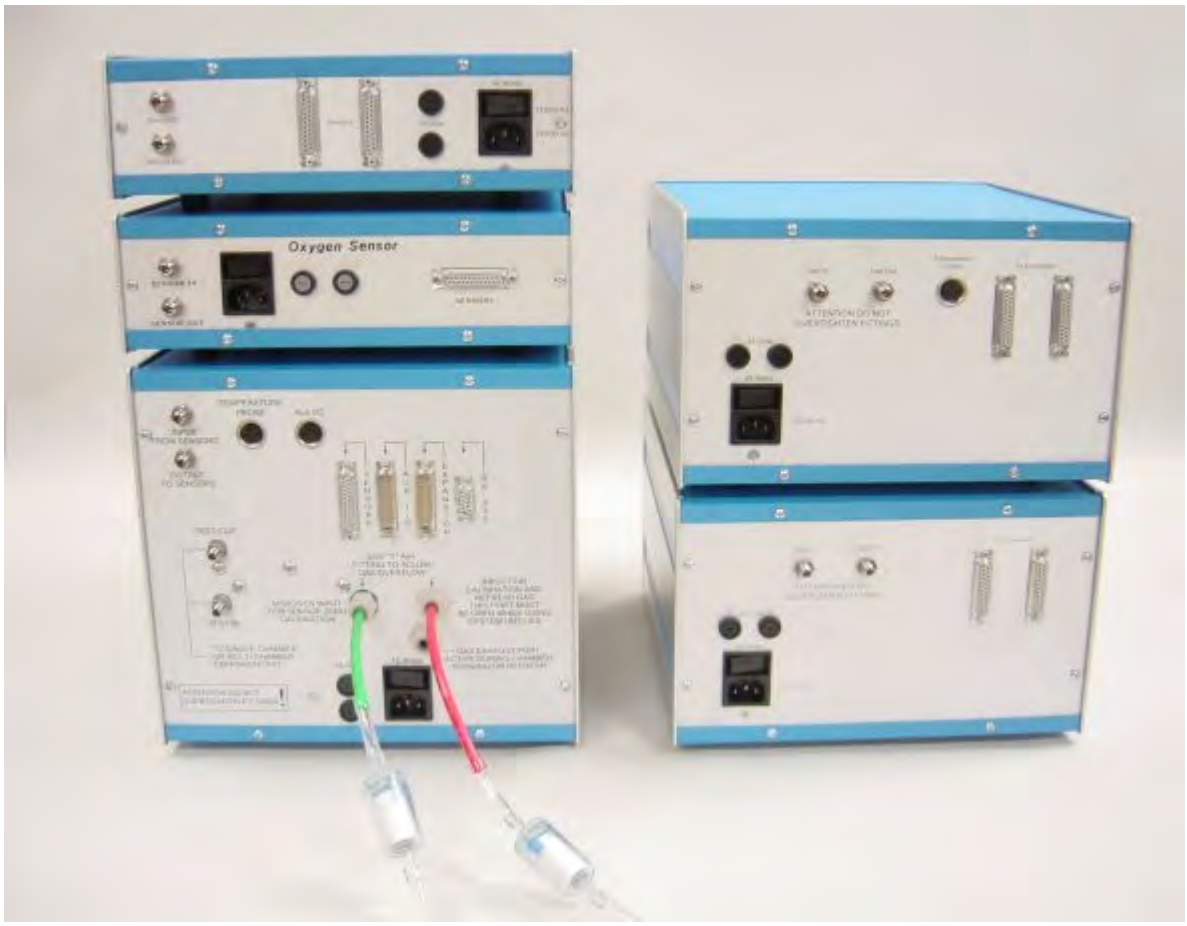
Two types of tubing connection are used on the system. The connections on the back of the sensors, dryers, and System Sample pump employ a metal nut and 2 piece plastic ferrule. The other type of connection is a "quick connect" type fitting and is used on the expansion unit and flask assemblies.

To make a connection using the nut and ferrule, slide the nut onto the end of the tubing and then slide on the two-part ferrule. Allow 0.25 cm of tubing to stick out beyond the end of the ferrule. Screw the nut on to the port finger-tight. Then tighten it another 1 /4 turn using a wrench. **Be careful not to over-tighten these connections. Excessive tightening may result in a pinched tube or damage to the fittings.**



To make a tubing connection using the "quick connect" type fittings, simply press the tubing into the fitting. Tubing should slide approximately 1.75 cm inside the connector and resist dislodging by pulling. To disconnect these fittings, hold the collar around the tubing firmly against the fitting while pulling the tubing. **If excessive leakage occurs with these fittings, most likely the end of the tubing is worn. Cutting a 1 cm piece off the end of the tubing should restore the seal. Be sure to use a sharp knife when cutting the tubing for these fittings to ensure a good seal.** The 1/8" pieces of tubing are supplied in separate bags labeled "Sensor Tubing', "Drier tubing", and "Expansion Interface tubing" (if present). Refer to the following drawing when making the System Sample Pump, Sensor and Expansion Interface tubing connections.

Connect the 2 air filters to the rear of the sample pump as shown below. These protect the instrument against contamination by particles which could damage the instrument.



Connect the large drier filled with Soda Lime (white granules) as shown in the drawing. The bottom fitting (the sealed end) of the large drier connects to the fitting labeled "Nitrogen" on the back of the System Sample cabinet with 1/4" tubing. If the paramagnetic O2 sensor is used there will not be a soda lime column, instead a line going to a tank of offset gas will be connected to the N2 port (see calibration section of the manual)

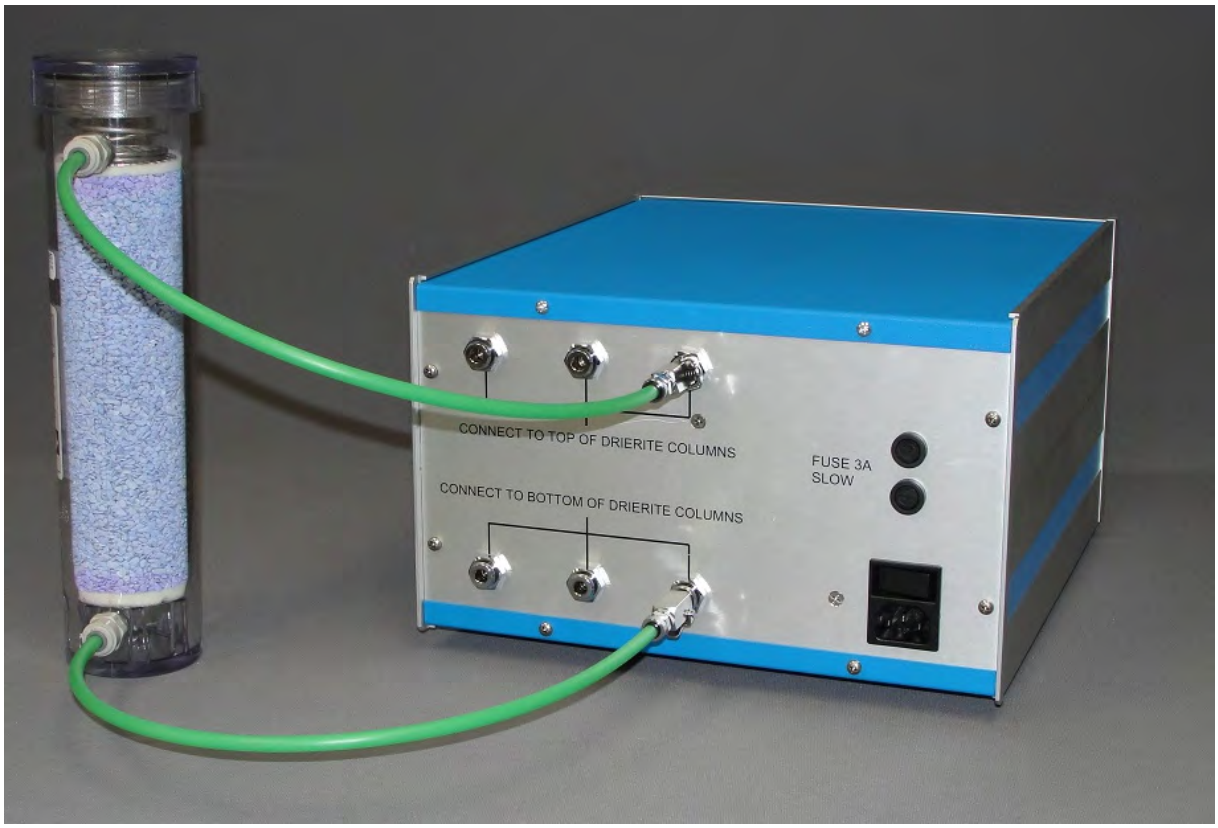
Connection of standard O2/CO2/CH4 sensors

The shortest of the three pieces of tubing labeled "Sensor Tubing" is used to connect the "Sensor Out" fitting on the Carbon Dioxide Sensor to the "Sensor In" fitting on the Oxygen Sensor. The medium-length piece of tubing links the "Sensor Out" fitting of the Oxygen Sensor and the "Sensor In" fitting of the System Sample Pump. If an additional sensor is installed another piece of tubing is used to connect the oxygen sensor's "Sensor Out" fitting to the "Sensor In" fitting on the additional sensor. Finally, the long piece of tubing is used to connect the "Sensor In" fitting of the Carbon Dioxide sensor and the "Sensor Out" fitting of the System Sample Pump.

Sample drier connections

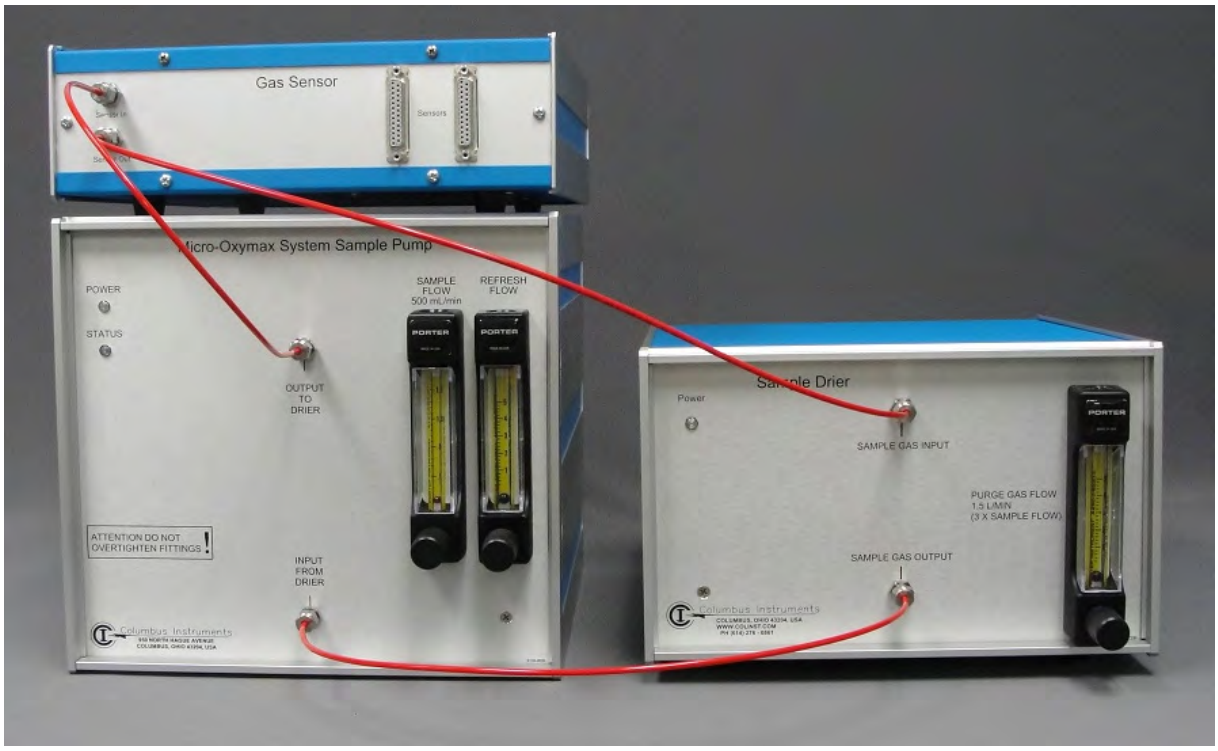
All systems are equipped with a sample drier. The sample drier removes the water vapor for accurate analysis.
Connect as shown with the 1/8" tubing

Connect the drierite columns to the rear of the sample drier as shown in the picture below. There are 2 columns included with the system. They are connected to the additional fittings. Up to 3 columns can be connected at the same time. They operate in parallel so that time between the need to change the columns is increased with each additional column.

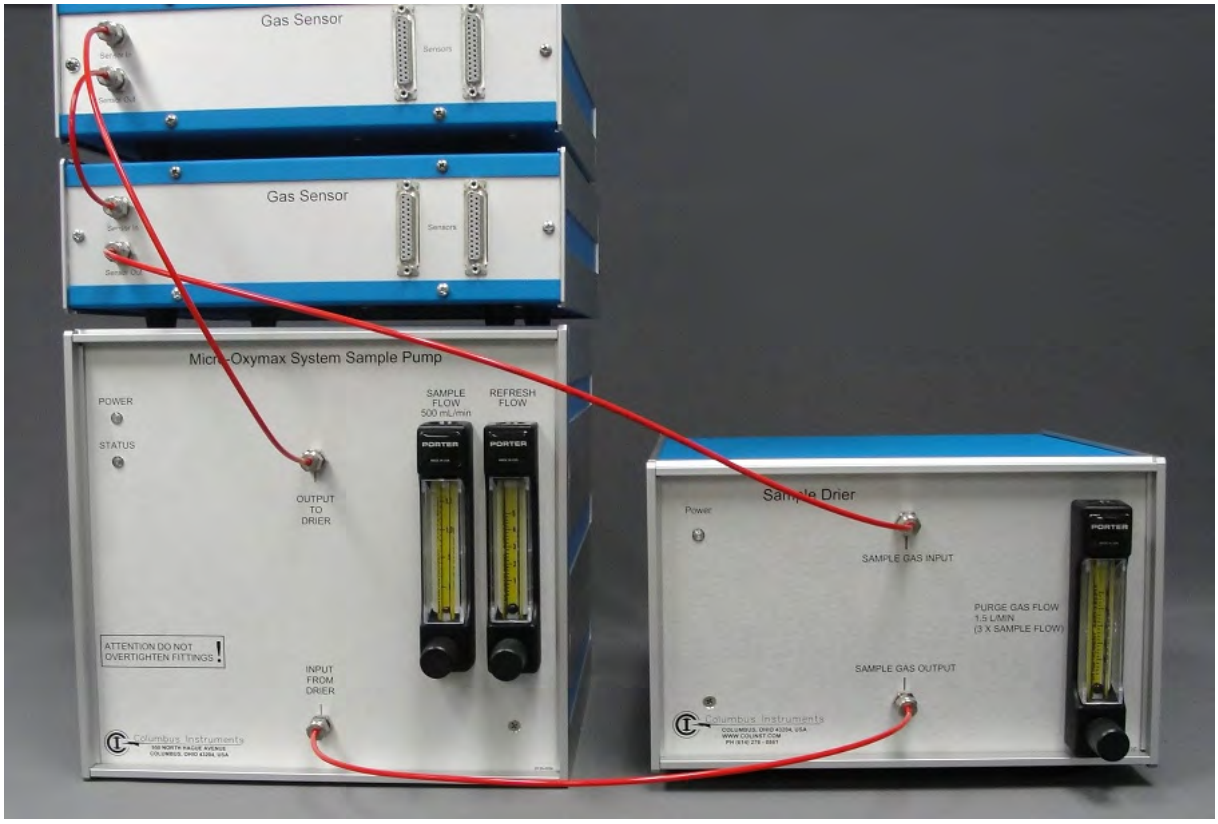


Connection of toxic gas sensors

Some systems are equipped with what are referred to as toxic gas sensors. The H2, H2S, CO, NO2, SO2, NO, NO2 sensors are all toxic gas sensors. They have the special requirement that the sample gas cannot be completely dry. This is because the sensors are electrochemical cells and if the cell dries out it will stop working. Therefore the toxic gas sensors are connected before the sample drier.
The proper connection is shown below.
Connect the connector labeled "output to drier" on the front of the sample pump to the sensor in connector on the toxic gas sensor. The sensor is facing the opposite direction as it normally should. This is for showing the connections clearly.

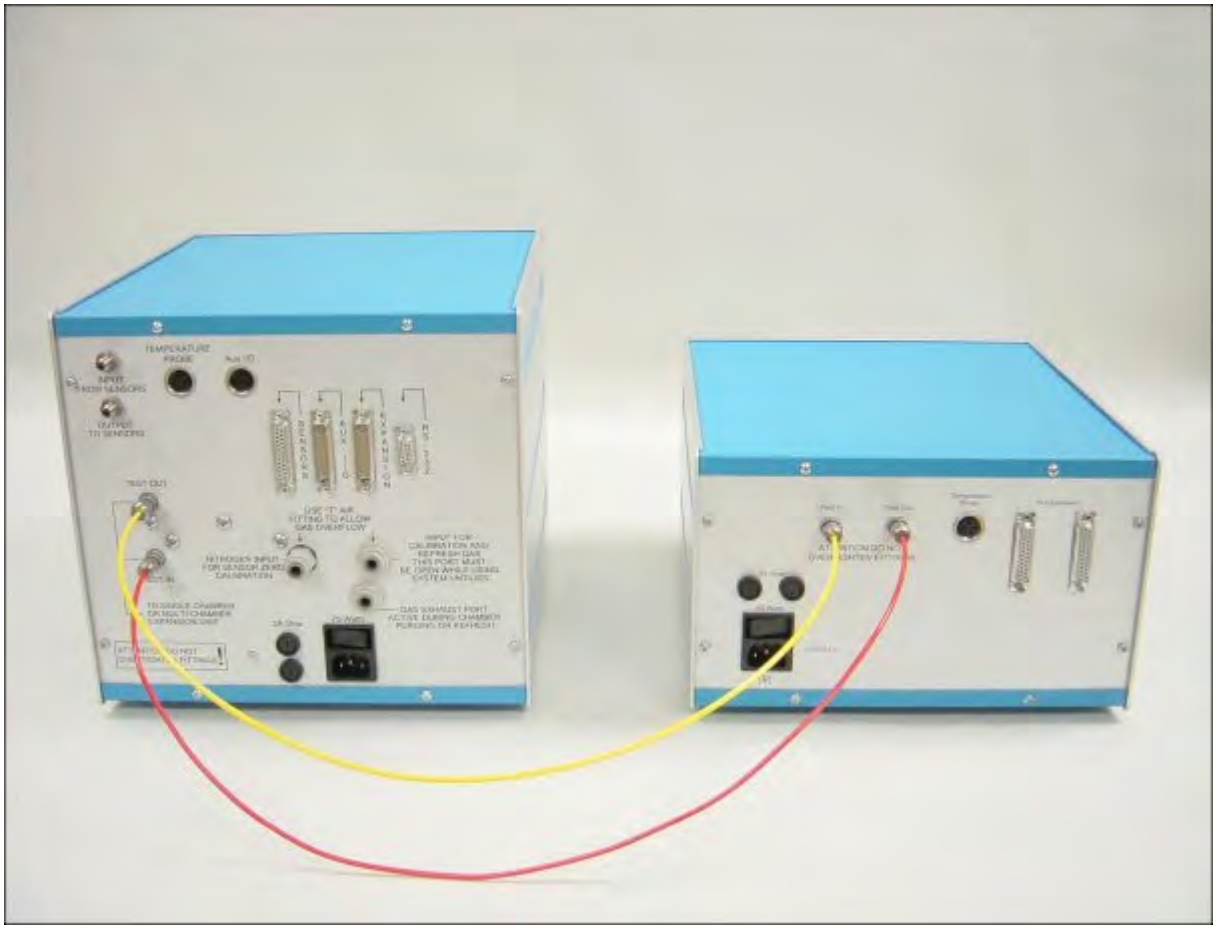


If any additional toxic gas sensors are use they should be connected in series as below

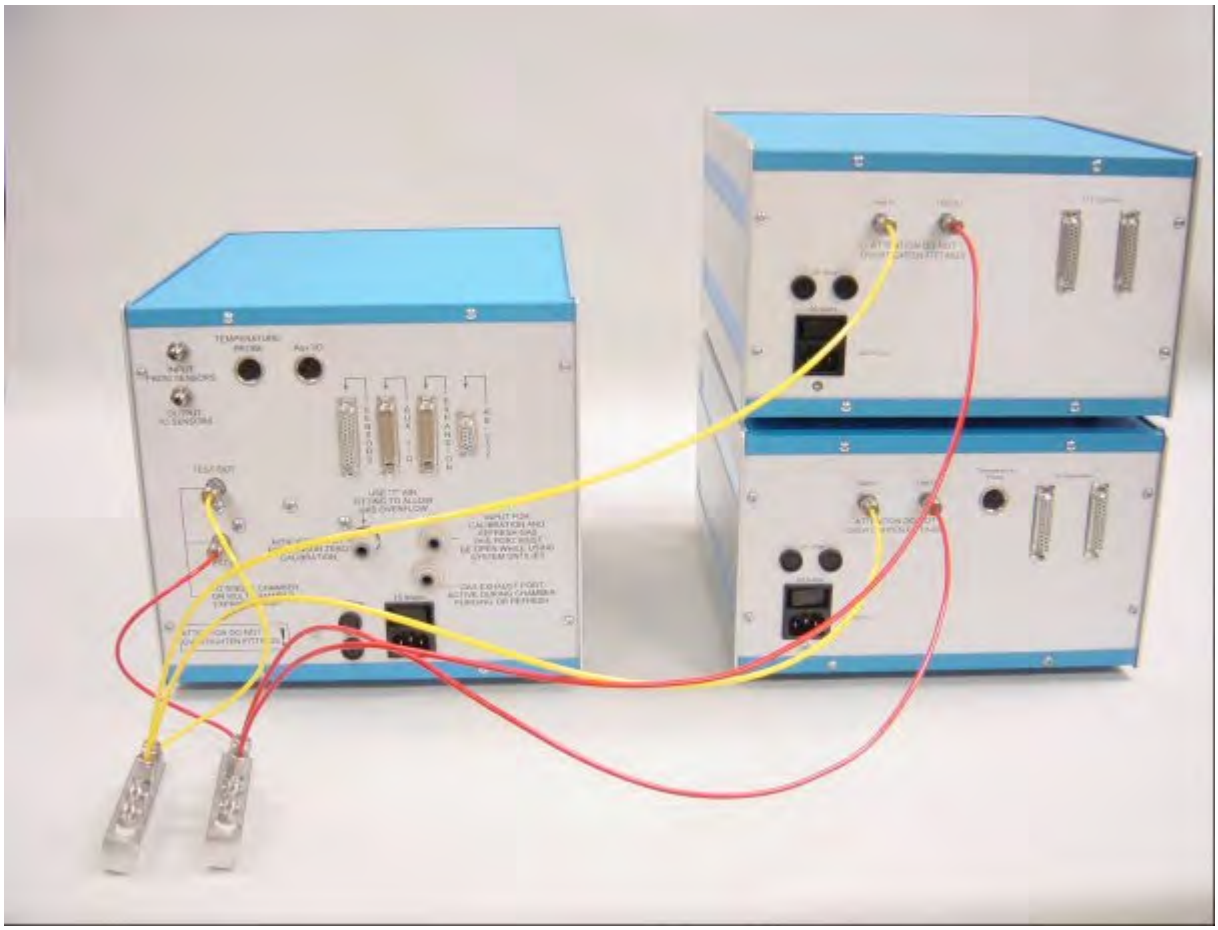


If the system does not include an Expansion Interface (for a single channel system), connect the test chamber directly to the System Sample Pump. Cut two pieces of tubing long enough to reach from the test chamber to the System Sample Pump. These are used to connect the test chamber to the "Test In" and "Test Out" fittings on the back of the System Sample Pump.

If the system includes an Expansion Interface, connect it to the System Sample Pump using the two pieces of tubing labeled "Expansion Interface". Attach one tube from the "TEST OUT" fitting on the System Sample Pump to the "TEST IN" fitting on the Expansion box. Fasten the other tube from the "TEST IN" fitting on the System Sample Pump to the "TEST OUT" fitting on the Expansion Interface.



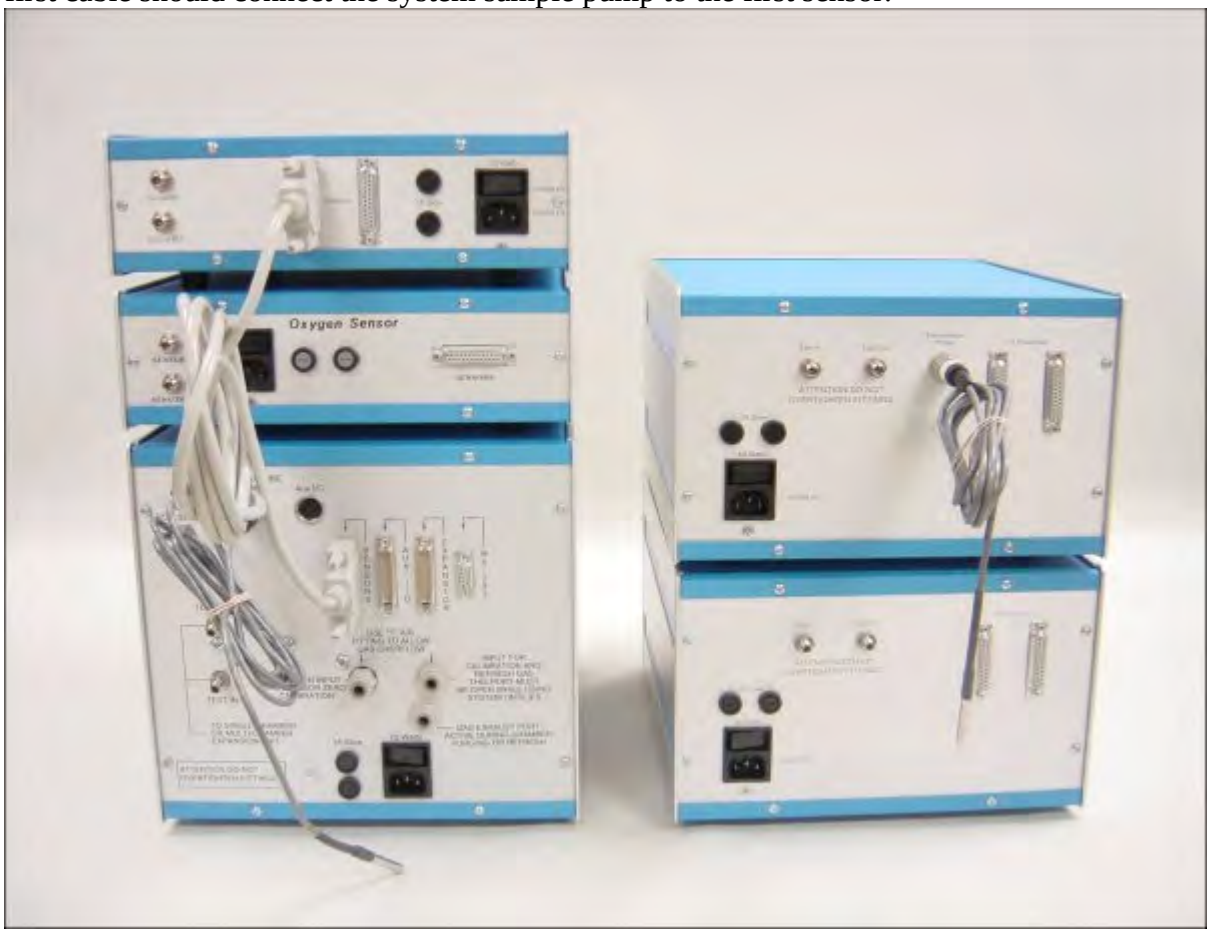
If the system includes more than one Expansion Interface, Two small manifolds will also be included. Connect these manifolds to the system sample pump as shown in the drawing on the next page. Also, connect the tubes from the Expansion Interfaces to the manifolds as shown in the drawing.



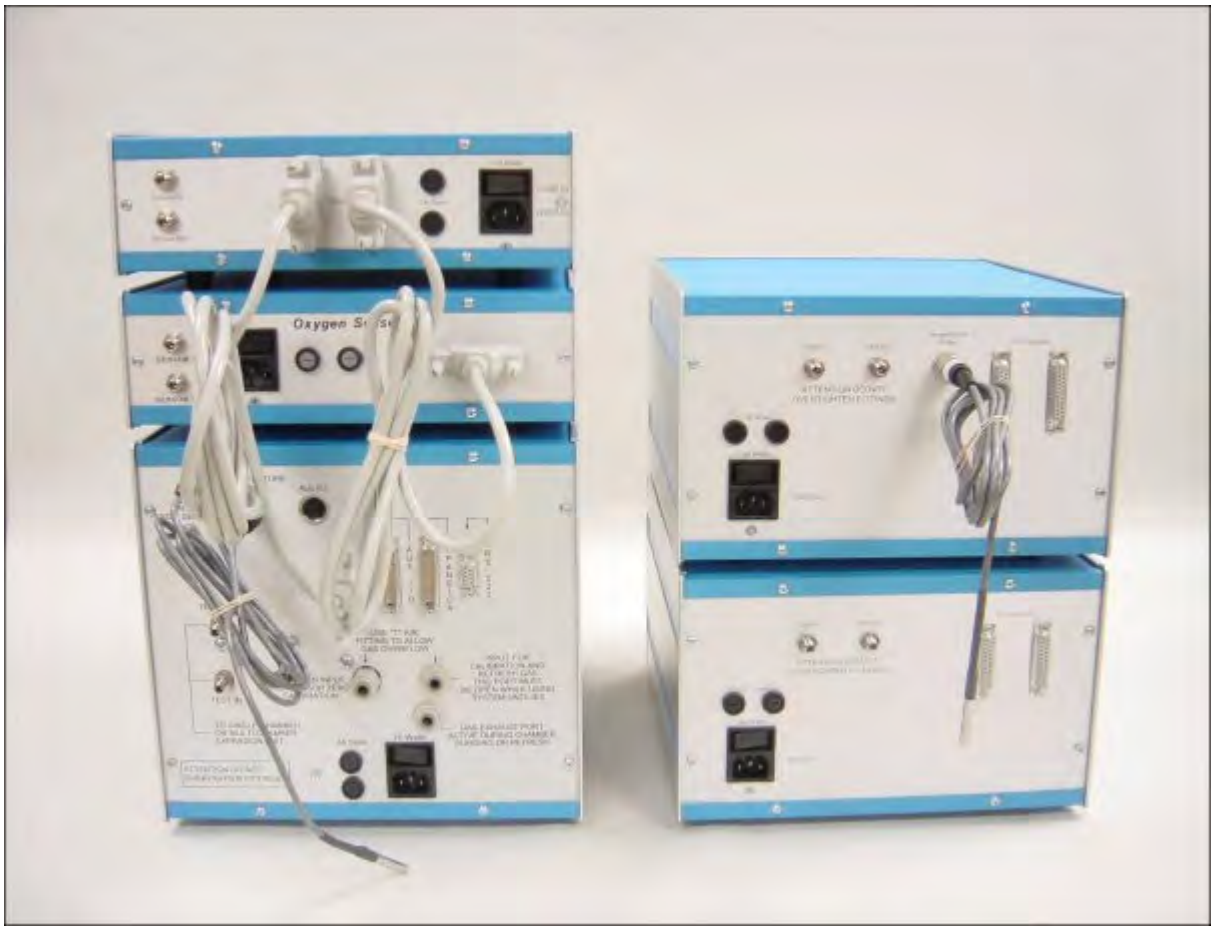
The lengths of these tubes are important. If replacement is necessary, tubes of equal length are preferable. If different lengths of tubing are used, the Expansion Interface volume will change requiring the value in the configuration file to be changed. If the system has more than 10 channels, configuration must be done for each Expansion Interface. To determine the correct volume, connect a short (4") piece of tubing between 'TEST IN" and "TEST OUT' of a selected channel. Next, use the sample head space measurement routine in the system utilities menu to measure the volume of that channel. The measured volume should be 2 ml. Volumes differing from 2 ml will require an adjustment in the calibration file by selecting file-properties from the main menu. The entries at the bottom of the screen titled "Expansion Unit Volumes" contain the settings for each Expansion Interface. (See the Micro Oxymax software instruction manual). Additional replacement tubing and air filters are available from Columbus Instruments.

Cable Connections

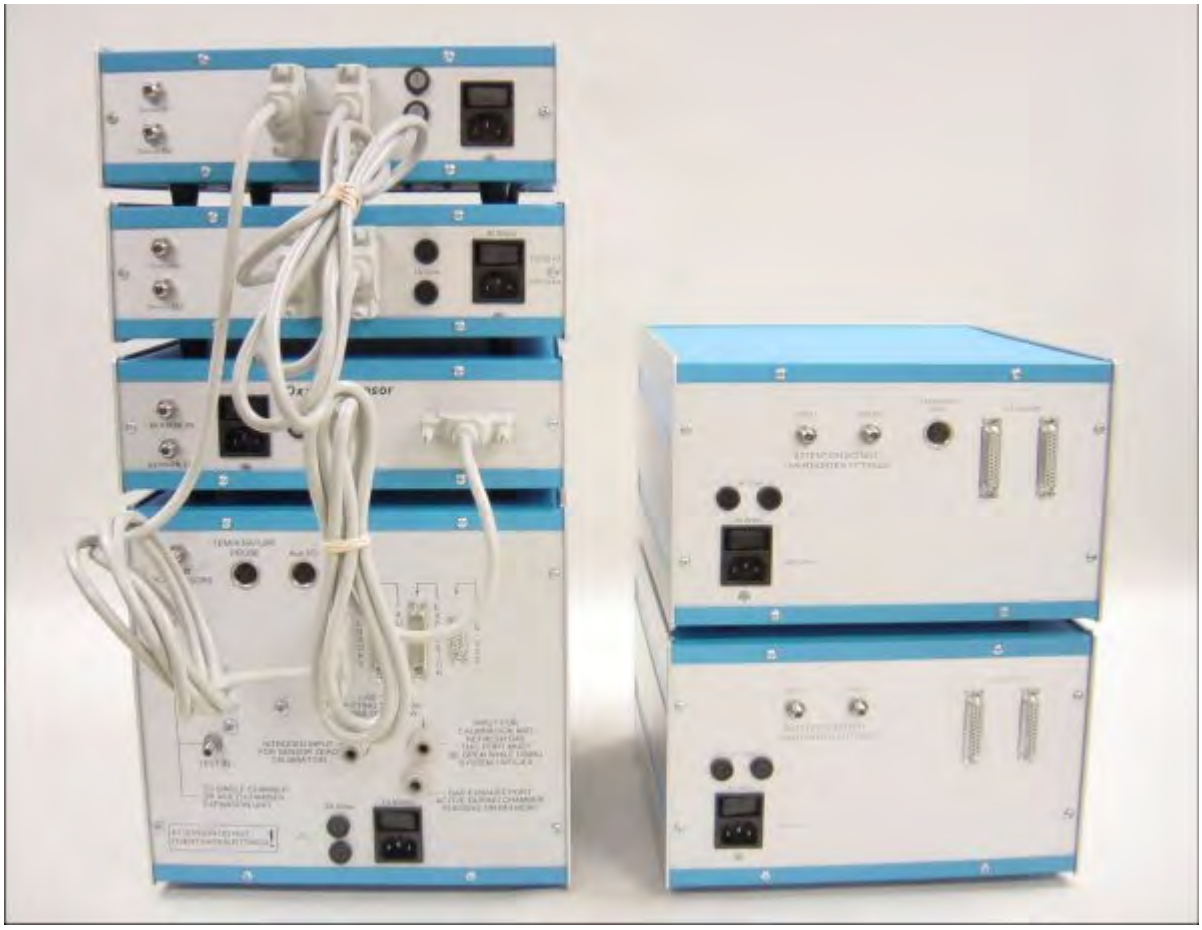
Pictures below depict the proper cable configuration. To connect the cables, find the 25 pin cables with the two male ends. If the system is configured with two sensors then there should be a total of three 25-pin cables. If the system is equipped with additional sensors then there should be an additional cable per sensor. These cables are connected to the connectors labeled "Sensors" which are located on the rear of the sensor and system sample pump cabinets. The first cable should connect the system sample pump to the first sensor.



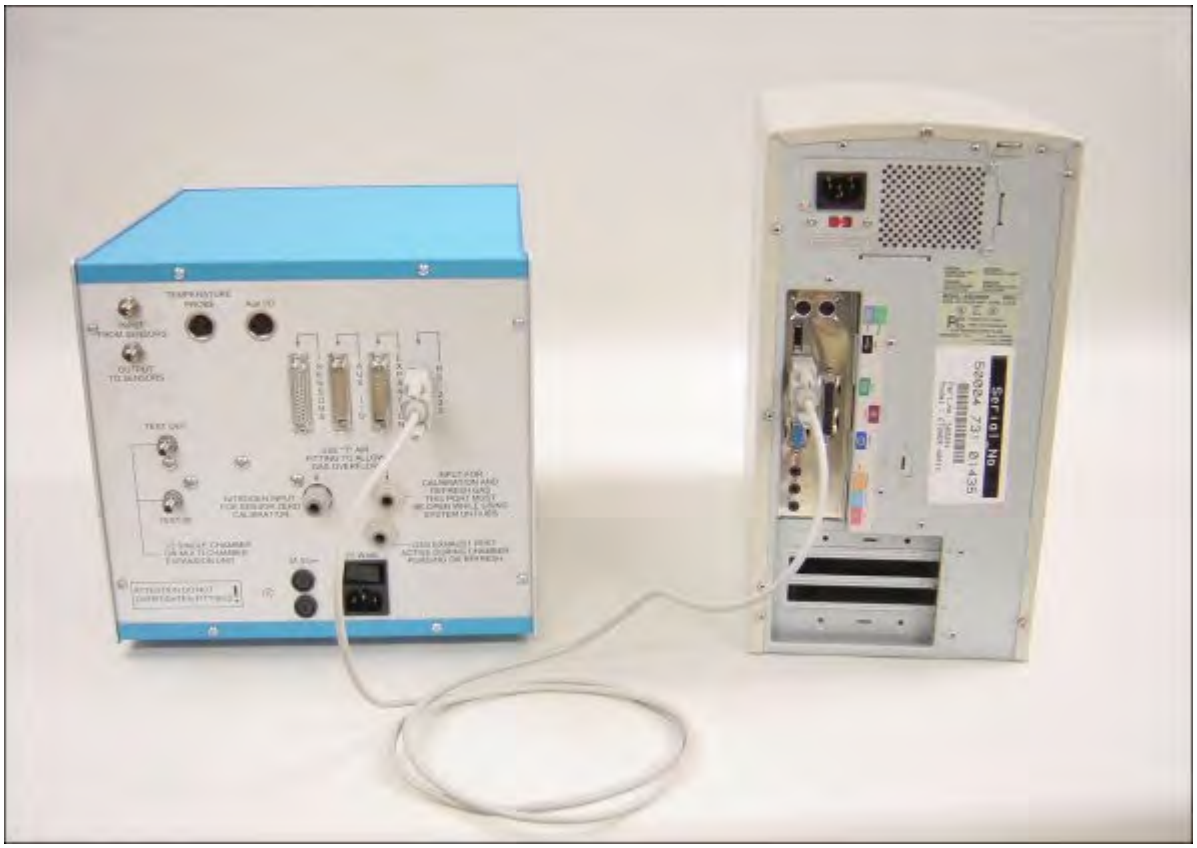
The second cable should connect the first sensor to the second sensor, and so on until all the sensors are connected together.



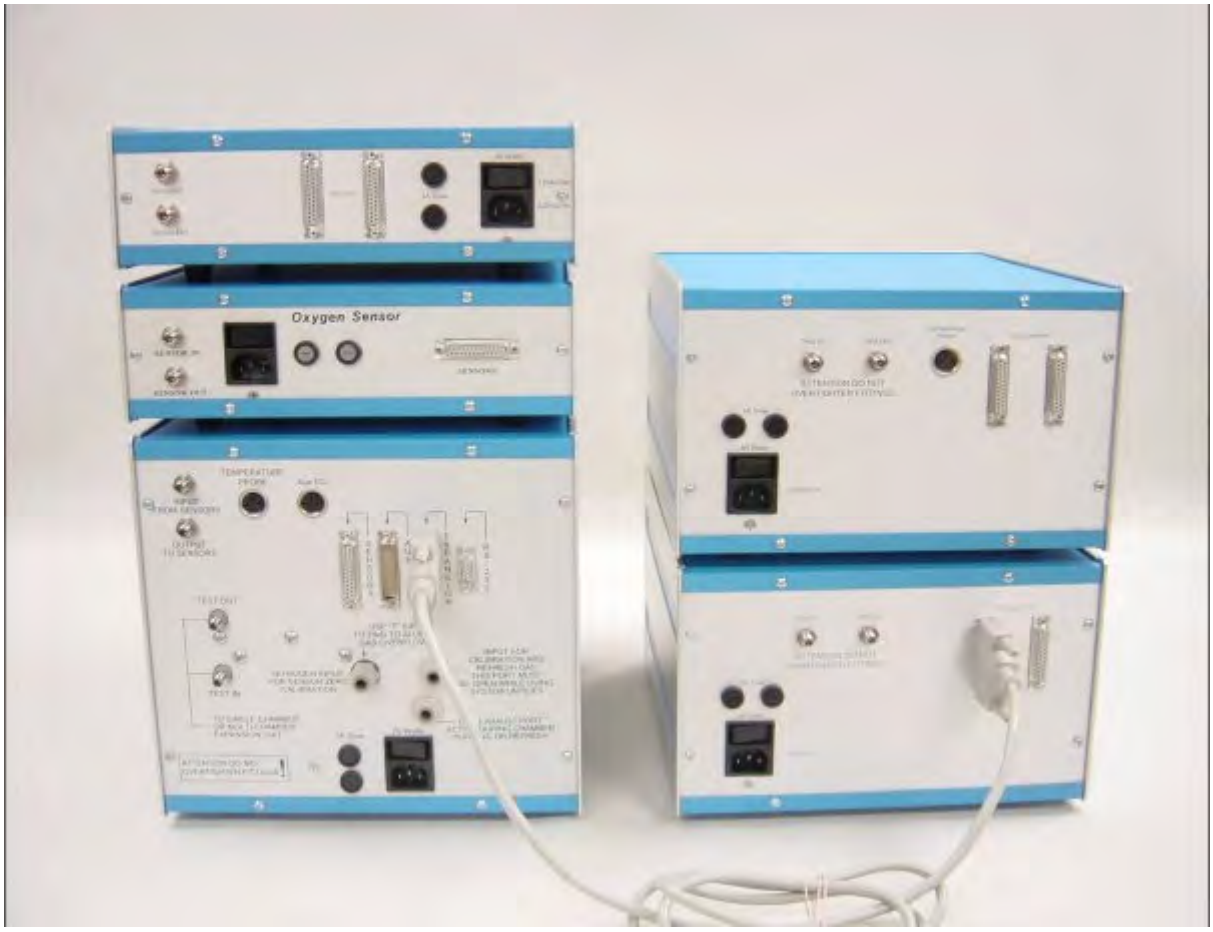
Each additional sensor is connected in series with the 25 pin cables

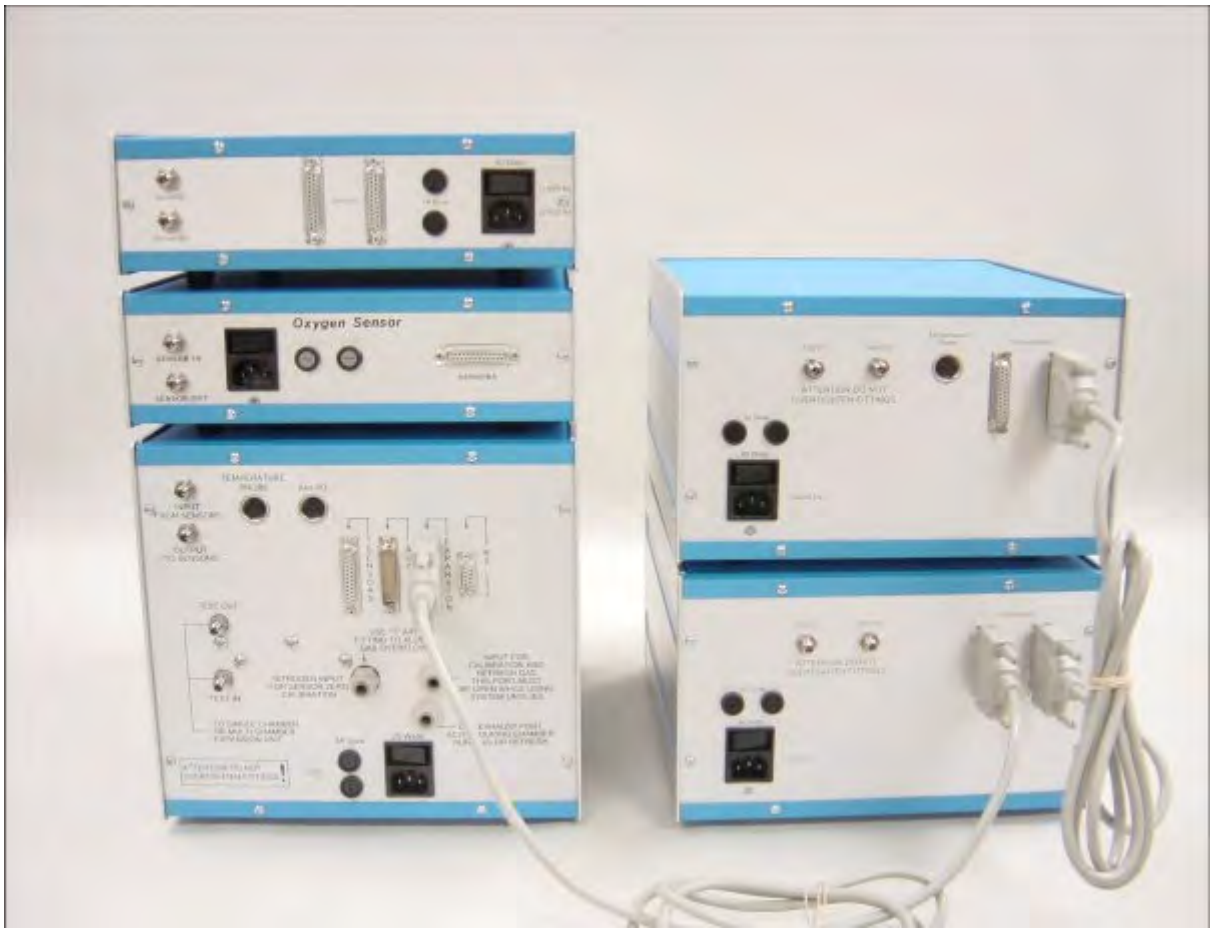


Locate the smaller 9-pin cable with a male and female connectors. This cable is used to connect the system sample pump's serial communication port to the computers serial communication port. The cable is a direct cable connection.



Use the remaining 25 pin cables with male connectors to connect the expansion units to the system sample pump. Like the sensor cables, there will be one cable per expansion unit. The first expansion unit is connected directly to the system sample pump's connector labeled "Expansion Unit". Additional expansion units are connected in series to one another through the additional 25-pin cables.



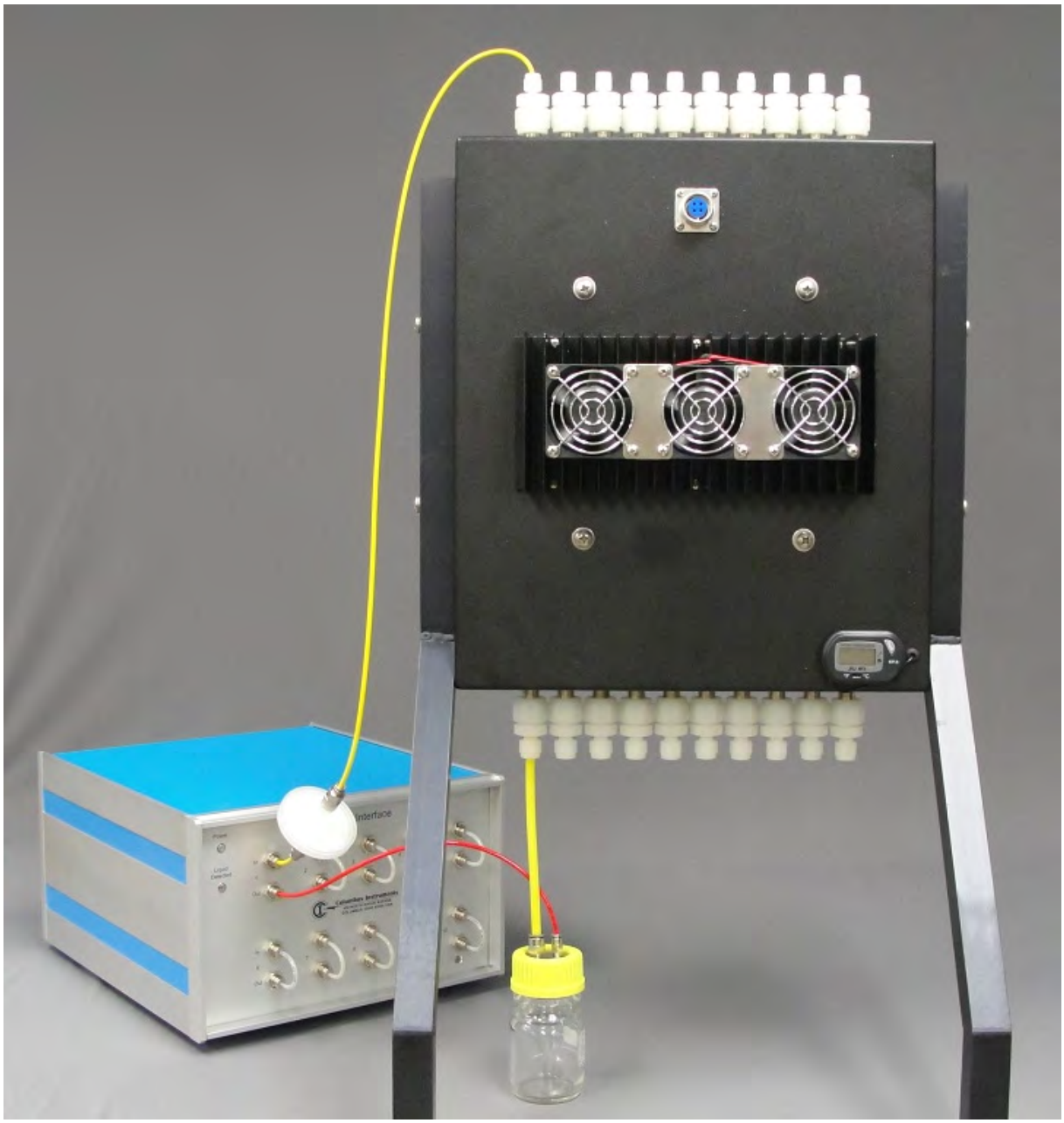


Connect the temperature probe to the "TEMPERATURE PROBE" input on the rear of the System Sample Pump. A second temperature probe, labeled "Expansion", is included with the Expansion Interface and inserts in the "Temperature Probe" connector located on the back panel of the first Expansion Interface.

Connect the AC power cords to the System Sample Pump, gas sensors, Sample Drier, and Expansion Interface(s) if present, and switch the units ON. The power switch is a black rocker type switch located on the same connector that the AC power is connected to the system through.

Condensing Air Drier

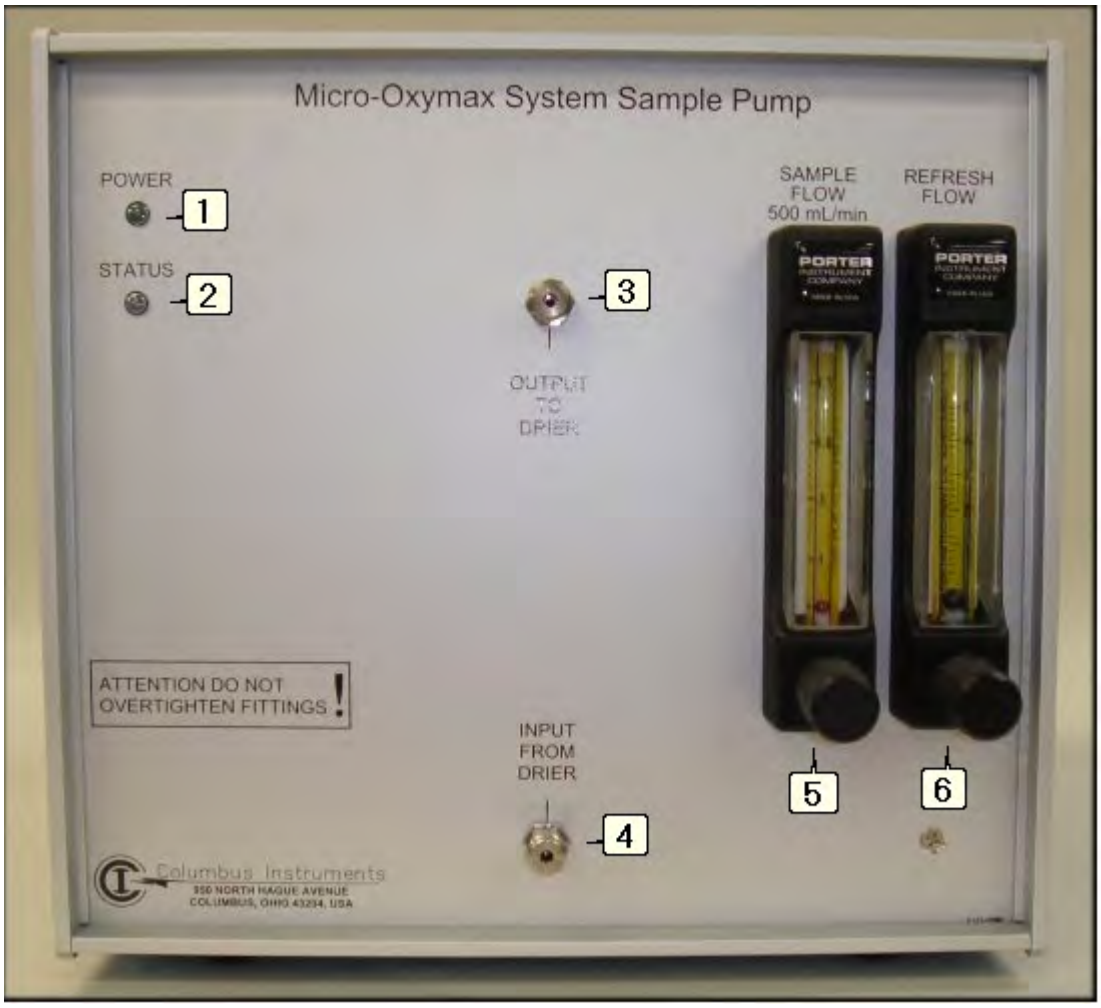
When measuring samples that contain water, the sample tends to have a loss of water when measured over an extended period of time. The condensing air drier was designed to remove the water from the vapor phase of the sample stream and return it to the reactor. The condenser contains 10 tubes that are cooled to 1 degree centigrade. As the sample air passes though the tube the majority of the water in the vapor phase condenses onto the wall of the condenser tubing and drops back into the reactor.



Hardware Controls

System Sample Pump

The flow meter on the left shows the flow rate of the sample stream of air through the sensors. The flow should be set to 0.50 l/min The flow meter on the right shows the flow rate of the refresh air. The flow meter on the right will only indicate airflow during the refresh cycle. The control valve on this flow meter should be normally be adjusted fully counterclockwise. The following is a list of the connections and hardware controls on the front panel of the System Sample Pump.



1. POWER LED. This indicates power is on

2. STATUS LED. This indicates the status of the system sample pump. During normal operation this LED should blink. When the LED does not blink an error has occurred and the system should be reset.

3. DRIER OUTPUT FITTING. This is where the tube connects to the input on the sample drier (or to the input on the toxic gas sensor if the system is equipped with a toxic gas sensor)

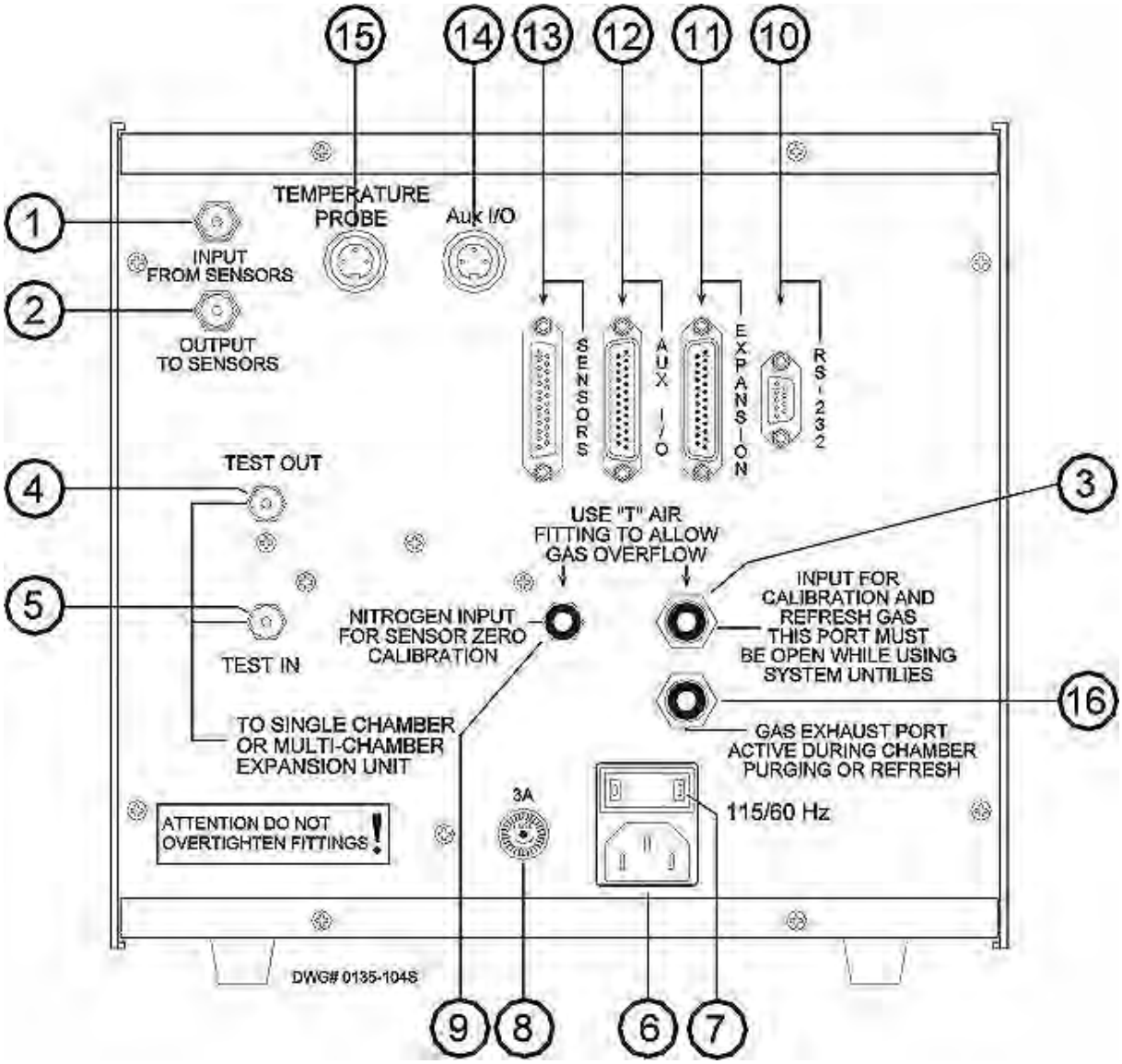
4. DRIER INPUT FITTING. This is where the tube connects to the output on the sample drier.

5. SAMPLE FLOWMETER. This indicates the flow of sample gas through the sensors. The

flow should be adjusted to 0.50 L/min. (500 cc/min)

6. REFRESH FLOWMETER. This indicates the approximate refresh flow. It should indicate a flow of 2 l/min. or greater. The refresh flow only occurs during the refresh cycle.

The following is a list of the connections and hardware controls on the rear panel of the System Sample Pump. The numbers corresponds with the labels in the following diagram.



1. SENSOR IN FITTING. This is connected to the "sensor out" connector on the 02 sensor.

2. SENSOR OUT FITTING. This is connected to the "sensor in" connector on the CO2

sensor.

3. REFRESH/CALIBRATION FITTING. This is where the system draws the gas in for flushing the chambers. Also the mixture gas used for calibration of the sensors is drawn in through this connector. A mixed gas bottle can be connected here for refreshing the chamber and purging the sensors, but the pressure must be regulated to 0.5 psi while the chamber is being refreshed. **If a mixed gas is used, it must be disconnected from this connector during calibration & when using the system utilities and diagnostics**. There should always be a "Balston" air filter connected to this fitting to prevent debris from entering the system

4. TEST OUT FITTING. This is connected to the sample chamber, for single channel systems. For multiple channel systems, this connects to the "Test In" connector on the Expansion Interface.

5. TEST IN FITTING. This is connected to the sample chamber, for single channel systems. For multiple channel systems, this connects to the "test out" connector on the Expansion Interface.

6. AC POWER CONNECTOR. This is connected to the specified voltage.

7. POWER SWITCH. Turns power to the System Sample Pump on and off.

8. FUSE. Use 3.OA fuse for replacement (2.0A for 220 V systems). Use of a fuse with a different rating may result in damage to the instrument

9. NITROGEN FITTING. This is where the system draws in Nitrogen or CO2 free air for calibration of the gas sensors (for "zeroing" CO2 & CH4 sensors). There should always be a "Balston" air filter connected to this fitting.

10. RS-232 CONNECTOR. This is connected to serial communication connector located on the computer.

11. EXPANSION CONNECTOR. This is connected to the Expansion Interface(s) with the 25-pin male to male cable.

12. AUX I/O CONNECTOR. The connector is used for connecting the optional equal flow expansion unit to the system sample pump.

13. SENSORS CONNECTOR. This is connected to the gas sensor(s) with the 25-pin male to female cable.

14. AUX I/O CONNECTOR. This connector is used to connect the optional gas mixer to the system sample pump. The connector supplies a signal to the gas mixer when a mixed gas is used by the system sample pump for refresh or system purging.

15. TEMPERATURE PROBE CONNECTOR. The primary temperature probe plugs into

this port. The temperature probe should be placed in the same environment as the sample chambers.

16. EXHAUST FITTING. This is where the gas flows out from the chambers during the refresh, purging sensors, and cycle. This port can be vented to an exhaust vent hood or outside.

Gas Sensors

The Micro Oxymax can be configured to monitor up to three different gas sensors. Each sensor is preset at the factory to operate with one another. If more than three sensors were purchased there is a chance that two or more sensors are configured to operate at the same Analog address. Please contact Columbus Instruments for details.

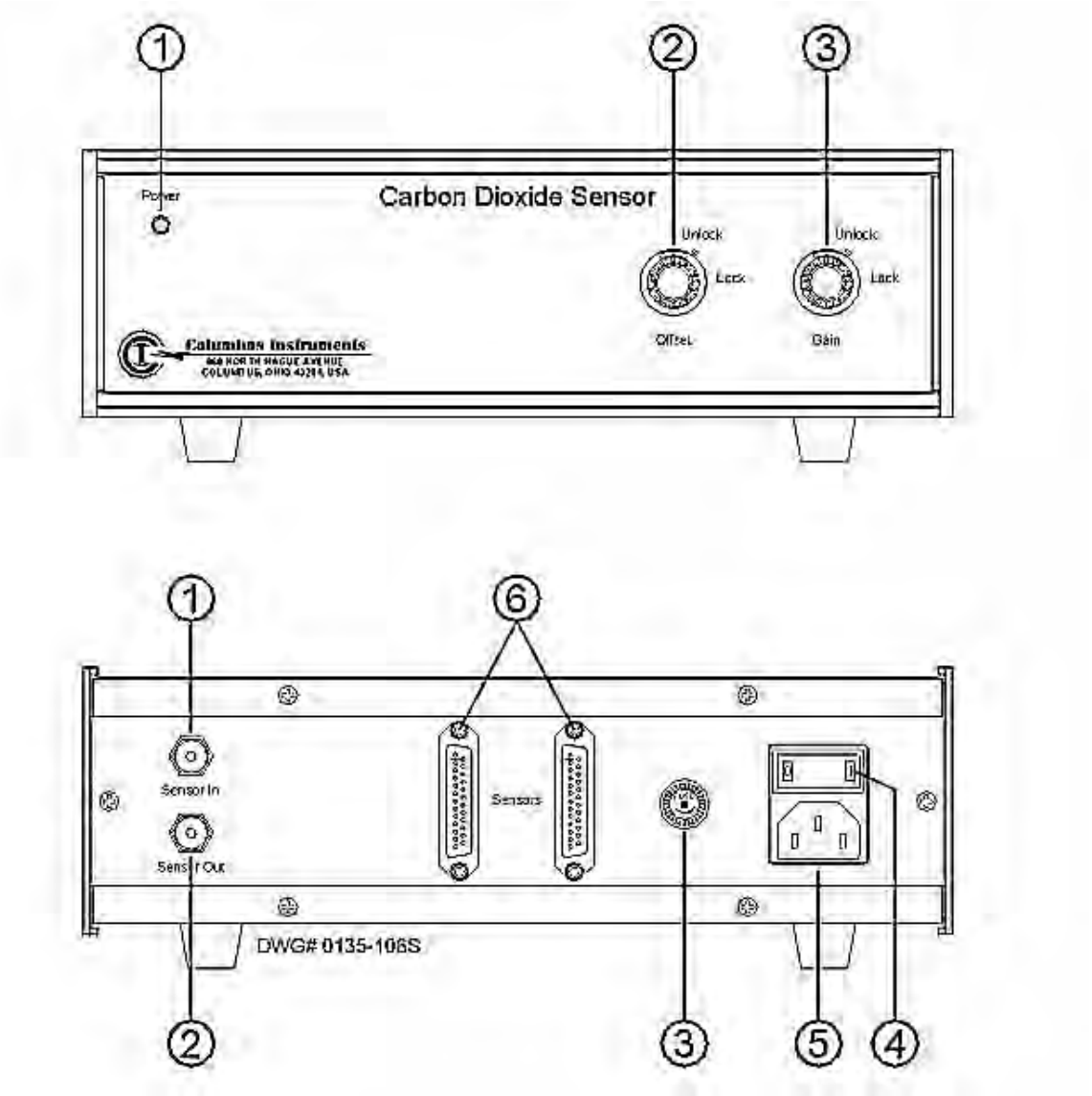
CO2 / CH4 Sensors

The standard CO2 sensor has an operating range of 0 - 1.0 percent. If a higher CO2 concentration is to be measured, an optional 0 - 10 or 0 - 100 percent sensor is available. The standard CH4 sensor operates from 0 - 5 percent. Both sensors require a two-point calibration procedure. The first step is to offset the sensor, which is done with nitrogen gas. The second step is to span the sensor. This is accomplished with a set-point gas that has a concentration that falls in the upper 1/3 of the sensor's range. A more detailed explanation is found in the software manual.

1. POWER LED. Indicates power is on.

2. OFFSET DIAL. Used to adjust the offset or zero point of the sensor during the calibration procedure.

3. GAIN DIAL. Used to adjust the gain or span of the sensor during the calibration procedure.



1. **SENSOR IN FITTING.** Connects to the Sensor Out connector on the System Sample Pump.

2. **SENSOR OUT FITTING.** Connects to the Sensor In connector on the next installed sensor (if equipped) or to the Sensor In connector of the System Sample Pump.

3. **FUSE HOLDER.** Replace only with a 3.0 amp fuse slow blow.

4. **POWER SWITCH.** Turns sensor on and off.

5. **AC POWER CONNECTOR.** Connects to the power mains.

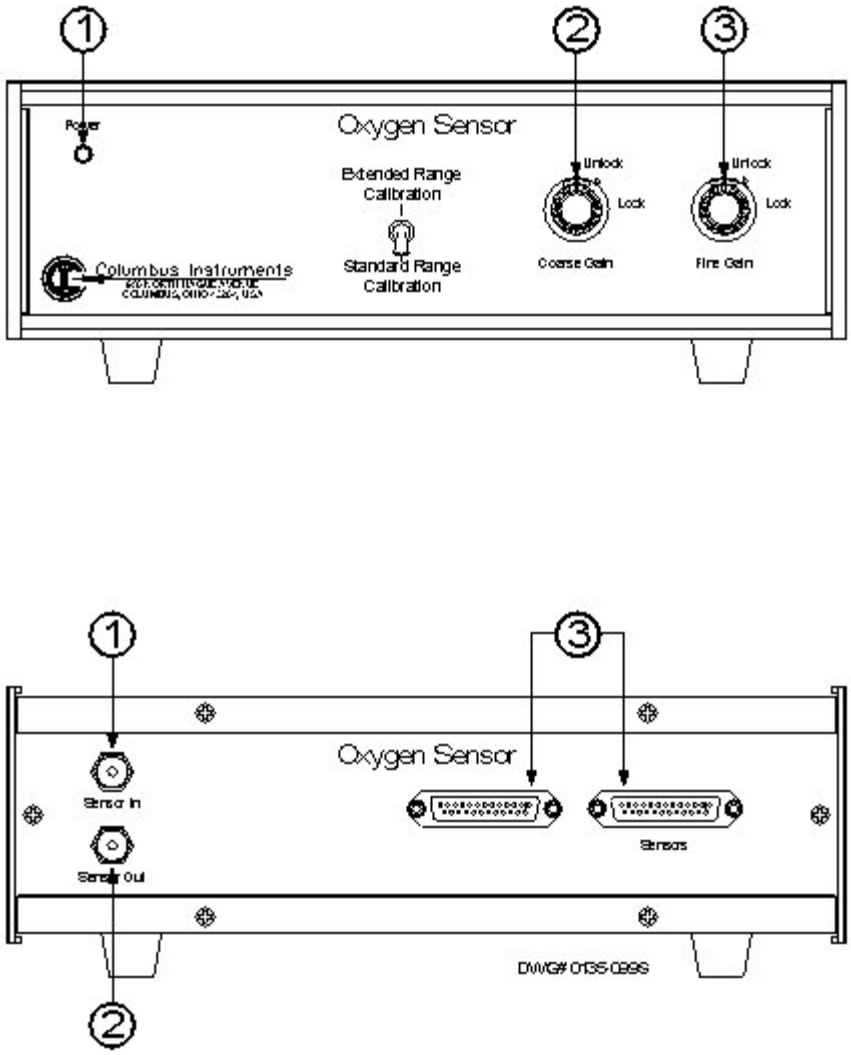
6. **SENSORS CONNECTOR.** Connects the sensor to the other sensors and to the System Sample Pump via the 25 conductor ribbon cable.

Electrochemical Oxygen Sensor
The standard electrochemical oxygen sensor has controls for adjusting the gain of the sensor. There will be a switch on the front panel of oxygen sensors equipped with the dual range option to select the range of operation. **Whenever the range of operation is changed, the sensor must be recalibrated and the software reconfigured for the correct range of operation.** The following is a list of controls and connectors corresponding to the following diagrams.

1. **POWER LED.** Indicates power is on.

2. **COARSE GAIN DIAL.** Used to adjust the gain set-point of the O2 sensor during the calibration procedure.

3. **FINE GAIN DIAL.** Used to fine-tune the gain or span of the O2 sensor during the calibration procedure.



1. SENSOR IN FITTING. This is connected to the "Sensor Out" connector on the CO2 sensor, or to the "Sensor Out" connector on the System Sample Pump for O2 only systems.

2. SENSOR OUT FITTING. This is connected to the "Sensor In" connector on the System Sample Pump.

3. SENSORS CONNECTOR. This connects to the sensor to the other sensors and or to the System Sample Pump via the 25 pin cable.

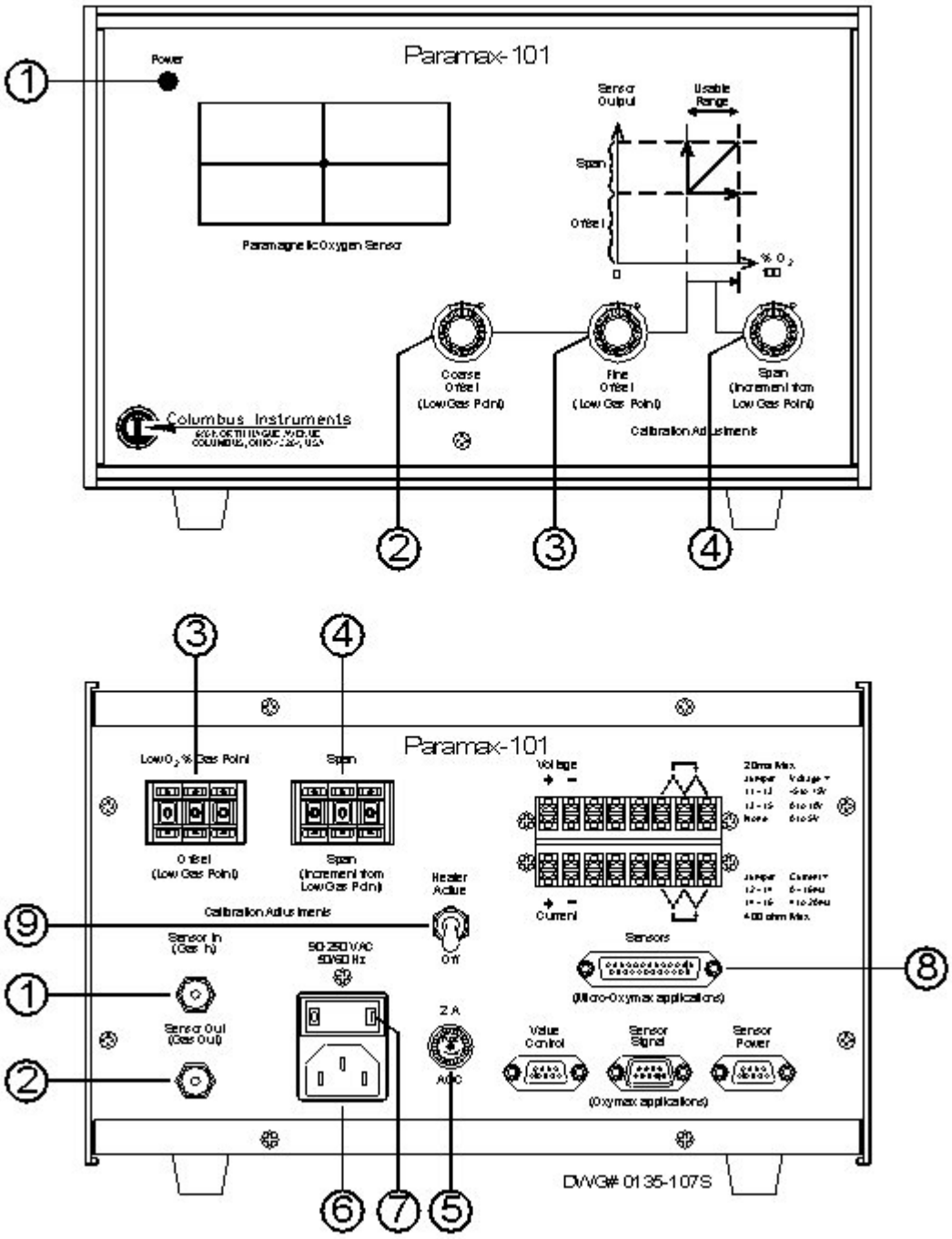
Paramagnetic Oxygen Sensor
The Micro Oxymax system can be equipped with an optional paramagnetic oxygen sensor. The paramagnetic oxygen sensor offers programmable ranges of operation by the controls on the rear panel. The following is a list of controls and connectors that correspond to the appropriate drawings.

1. POWER LED. Indicates power is on.

2. COARSE OFFSET DIAL. Used to adjust the offset set point of the O2 sensor during the calibration procedure.

3. FINE OFFSET DIAL. Used to fine-tune the offset set point of the O2 sensor during the calibration procedure.

4. SPAN DIAL. Used to adjust the span or gain of the O2 sensor during the calibration procedure.



1. SENSOR IN FITTING. This is connected to the "Sensor Out" connector on the CO2 sensor, or to the "Sensor Out" connector on the System Sample Pump for O2 only systems.

2. SENSOR OUT FITTING. This is connected to the "Sensor In" connector on the System Sample Pump.

3. OFFSET PROGRAMMING SWITCH. Selects the offset or lowest concentration of

oxygen to be measured. It is set as ##.# % O2

4. SPAN PROGRAMMING SWITCH. Selects the gain or the difference in oxygen above the offset that is the highest concentration of oxygen to be measured. It is set as ##.# % O2.

5. FUSE HOLDER. Replace with 2.0 amp fuse only.

6. POWER CONNECTOR. Connects to the power mains.

7. POWER SWITCH. This switch used to turn the power on to the unit.

8. SENSOR CONNECTOR. This connects the sensor to the other sensors and to the System Sample Pump via the 25-pin conductor ribbon cable.

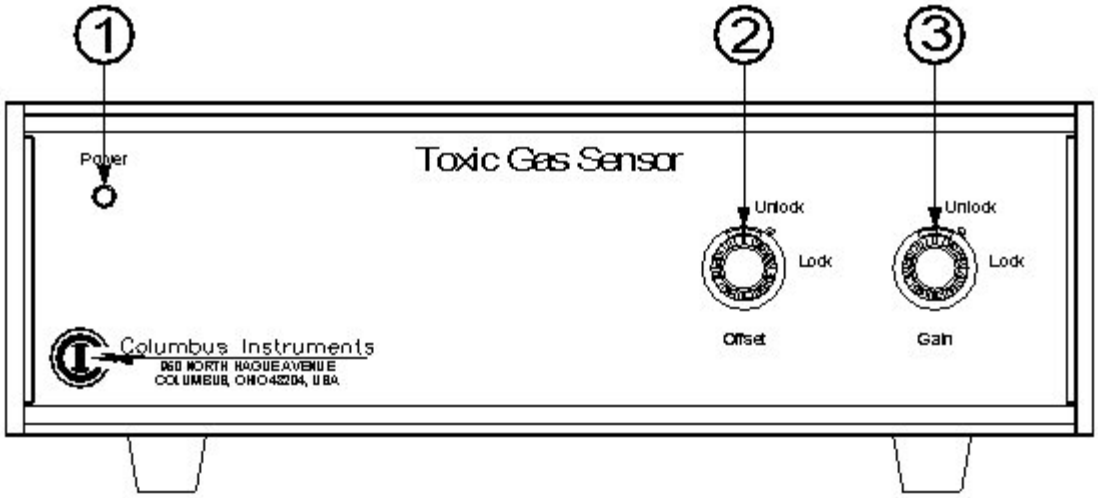
9. HEATER ACTIVE. This switch is used to turn off the heater that heats the Oxygen Sensor. This is normally turned on.

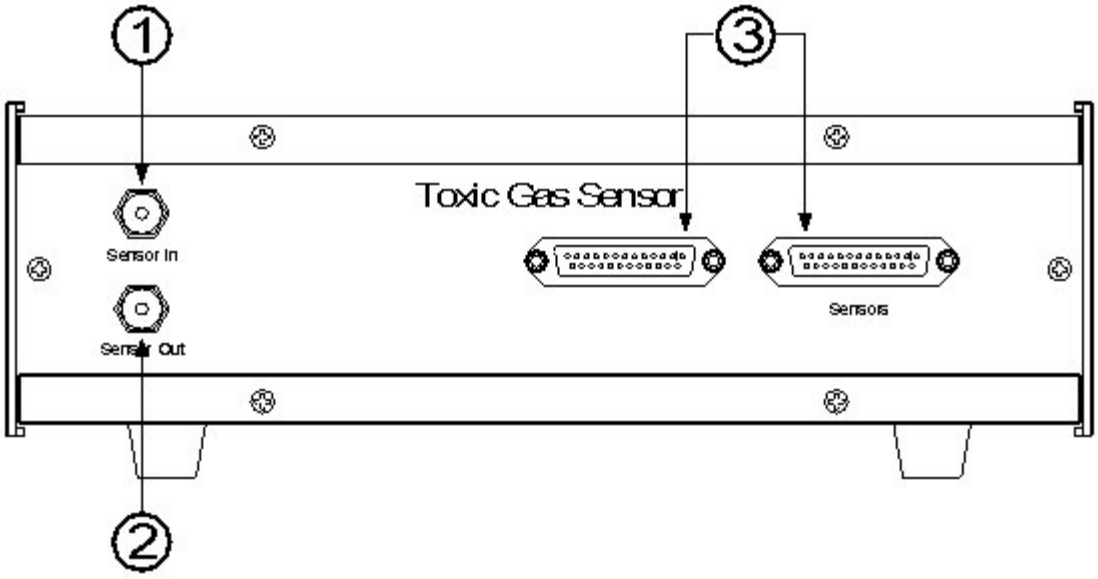
Toxic Gas Sensors (H2, H2S, CO, NO2, SO2, NO, NO2)
These sensors are electro-chemical based and require a two-point gas calibration procedure. The first step is to offset (zero) the sensor, which is done with gas that does not contain specific gas the sensor is designed to measure. The second step is to span the sensor. This is accomplished with a set-point gas that has a concentration that falls in the upper 1/3 of the sensor's range. A more detailed explanation is found in the software manual.

1. POWER LED. Indicates power is on.

2. OFFSET DIAL. Used to adjust the offset or zero point of the sensor during the calibration procedure.

3. GAIN DIAL. Used to adjust the gain or span of the sensor during the calibration procedure.





1. SENSOR IN FITTING. Connects to the Sensor Out connector on the previous sensor.

2. SENSOR OUT FITTING. Connects to the Sensor In connector on the next sensor (if equipped) or to the Sensor In connector of the System Sample Pump.

3. SENSORS CONNECTOR. Connects the sensor to the other sensors and to the System Sample Pump via the 25-pin conductor cable.

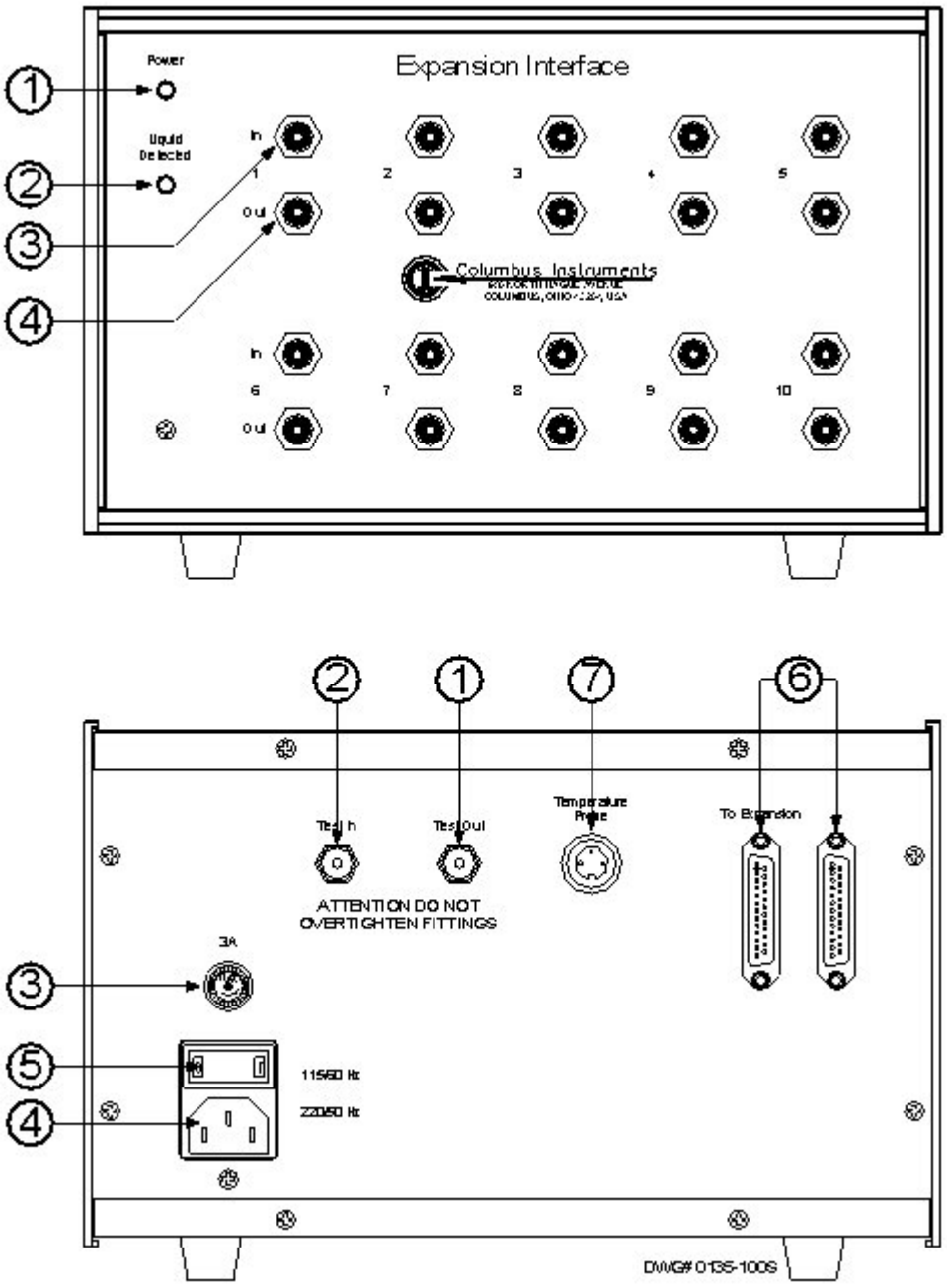
Expansion Interface
System equipped with more than one channel will include one or more Expansion Interface(s). The expansion interface contains valves to select the gas stream from multiple chambers and direct it through the sensors. The following is a list of controls and connectors that corresponds with the following diagram.

1. POWER LED. Indicates power is on.

2. LIQUID DETECTED INDICATOR. This LED lights up when there is liquid in the expansion interface. This circuit measures the conductance between the front panel and the internal valve manifold, and it will not detect distilled water.

3. GAS SAMPLE IN FITTING. This is where the gas from sample chamber (flask) enters the system. Always connect a 0.2 micro PFTE filter to this connector.

4. GAS SAMPLE OUT. Fitting, this is where the gas is returned to the chamber (flask). A 0.2-micron PFTE filter can be used on this port to prevent cross contamination of samples.



1. **TEST OUT FITTING.** This is connected to the "Test In" fitting on the Sample Pump.

2. **TEST IN FITTING.** This connects to the "Test Out" fitting on the System Sample Pump.

3. **FUSE HOLDER.** Replace with 3 amp fuse only.

4. **POWER CONNECTOR.** This connects to the power mains.

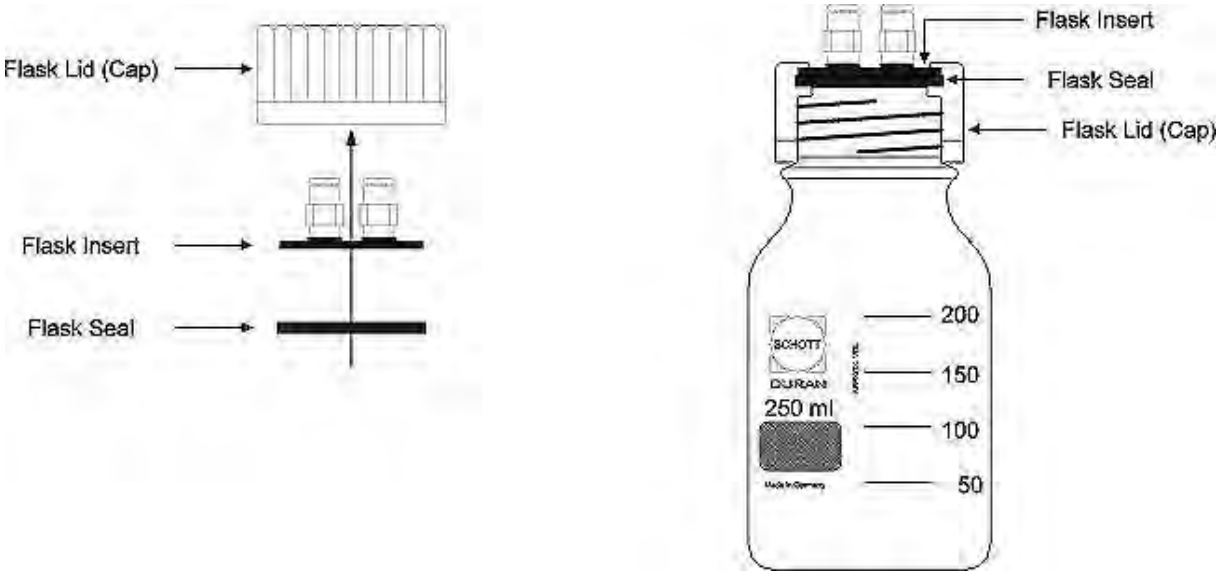
5. **POWER SWITCH.** This is used to turn the power on and off to the Expansion Interface.

5. **EXPANSION CONNECTOR.** This connects to any other expansion interfaces (if present), and to the "EXPANSION" connector on the System Sample Pump.

Sample Chamber Hardware

The nature of the Micro Oxymax measurement process enables the system to be adapted to a wide range of sample vessels. There are only two requirements, the sample chambers must be leak free and the walls of the reactor must be rigid. Sample chamber volumes can range from 50-ml up to 2000-ml when using the automatic sample chamber volume measurement utility. Larger sample chambers can be used when the user calculates the volume manually. Columbus Instruments provides a standard screw top chamber that incorporates two or three port inserts that allow the system to be attached to a condensing air drier. Below is a diagram of the standard sample chamber assembly.

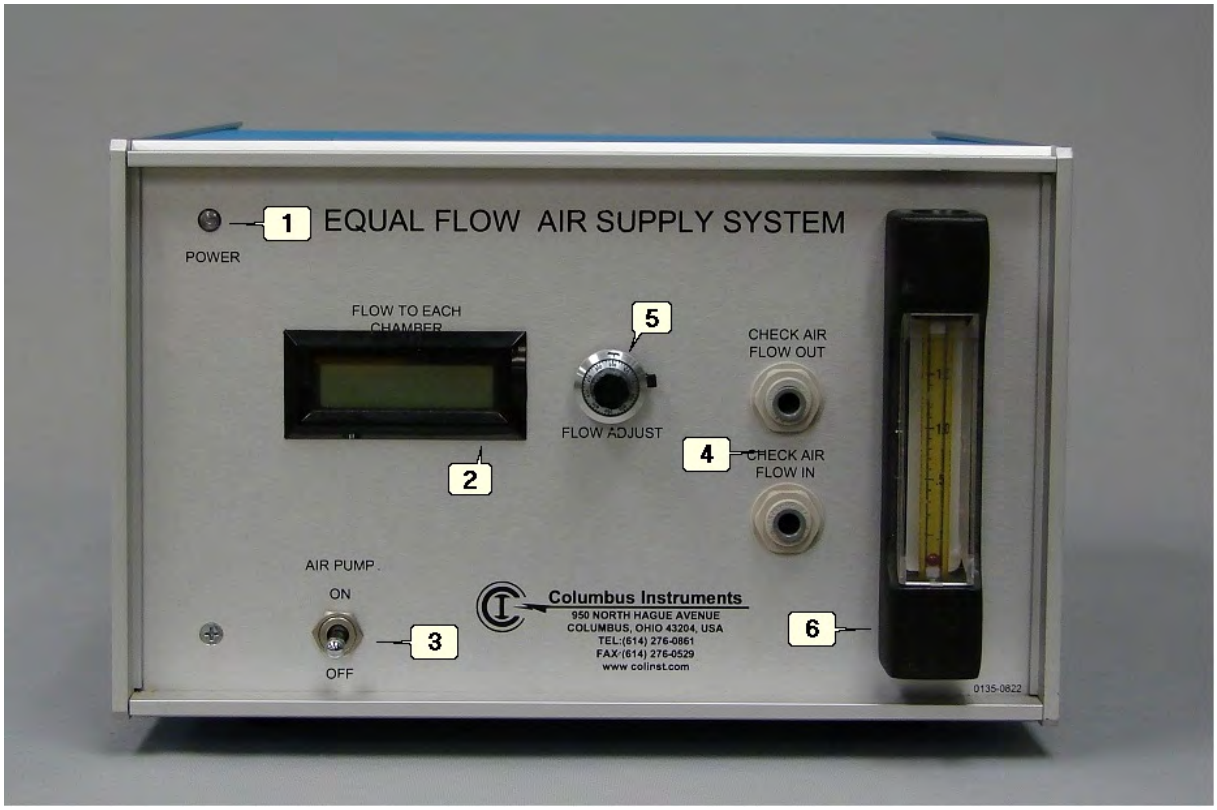
Sample Flask Assembly



1.5 Open flow hardware option

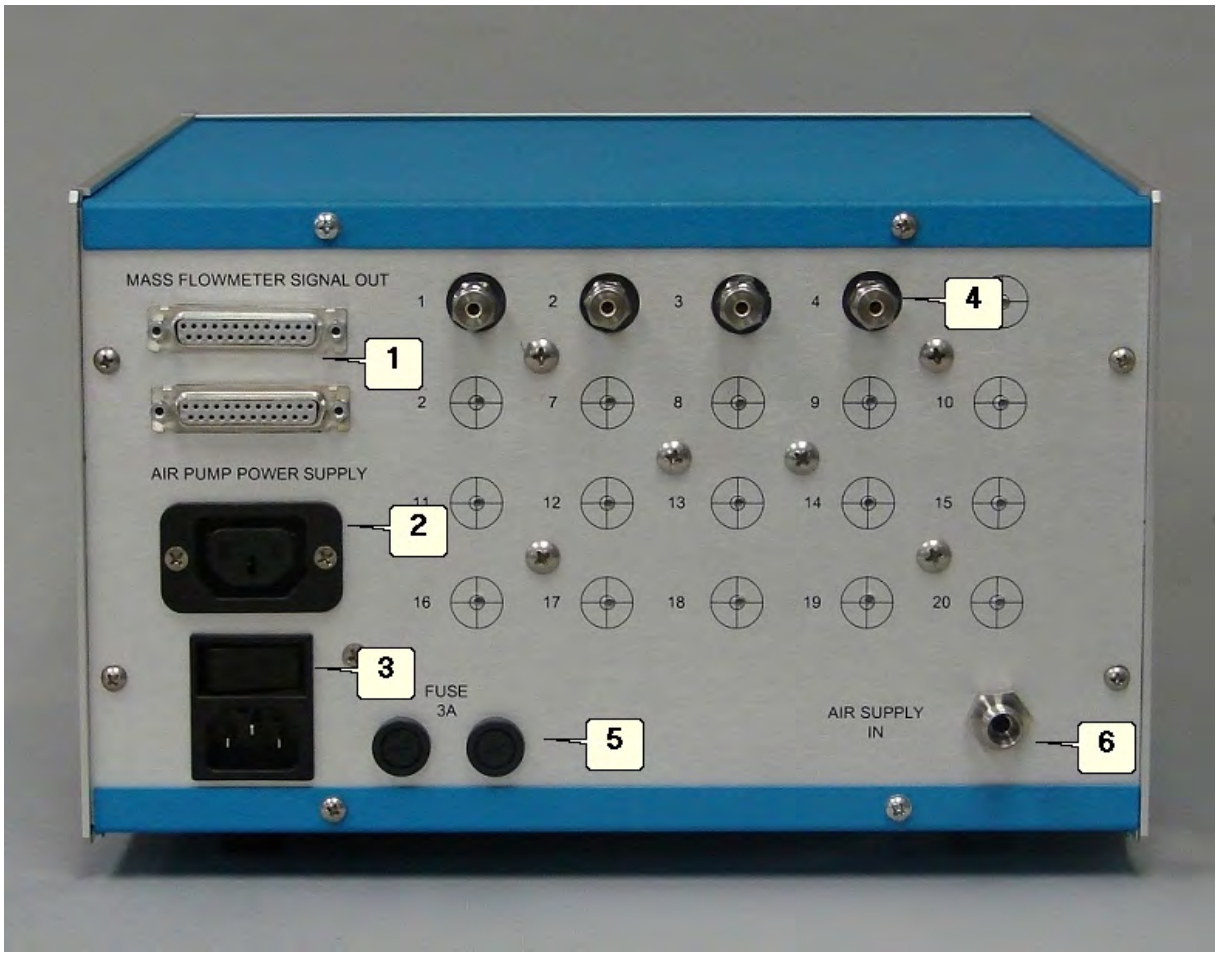
The open flow option for the Micro Oxymax allows the system to operate as an open flow respirometer. This is useful for measuring samples with activity (gas consumption and production) which is too high for the closed mode of operation to measure. These type of experiments are usually for animals or compost. If the samples have too much activity they will make the readings from the gas sensors exceed their normal range in a short time. The open flow option operates by precisely measuring the air flow rate being supplied to the measuring chamber(s) and the system measures the gas concentrations at the input and output of the chamber. From these 3 values the rate of gas production/consumption can be calculated as well as the accumulated amount of gas. There is a front panel control on the equal flow box to adjust the flow rate of fresh air. The air flow rate should be adjusted so that the difference in gas concentration between the input and outlet of the chamber (delta) is at the minimum 10% of the sensors range (for example the standard 1% CO2 sensor has a range of measurement of 0-1% so the minimum delta would be 0.1% but it is better if it is 0.2% or 0.3%. If the delta is too low the fresh air flow rate should be reduced. If the delta is too high the fresh air flow should be increased.

Equal Flow Front Controls



1	Power lamp	Indicates if the AC power is on.
2	Flow display	Shows the flow supplied to each chamber in either ml/min or l/min depending on capacity of air supply
3	Air pump switch	Turns on or off power to the fresh air supply pump
4	Check air flow fittings	The air flow to each channel can be verified by connecting the tubing from a channel to the check air flow in fittings
5	Flow adjust	This control adjusts the fresh air supply to each channel
6	Check air flow meter	This indicates the flow of air going into the check air flow in fitting

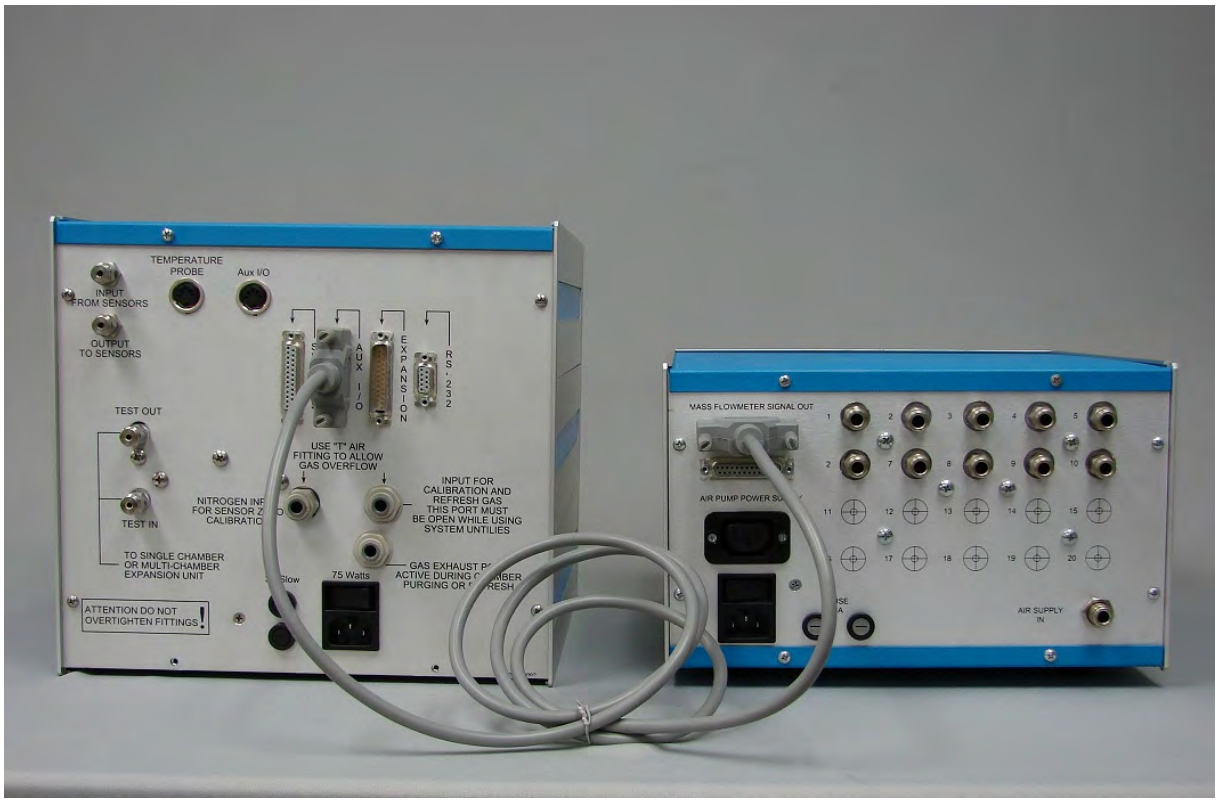
Equal Flow Rear Panel Connections



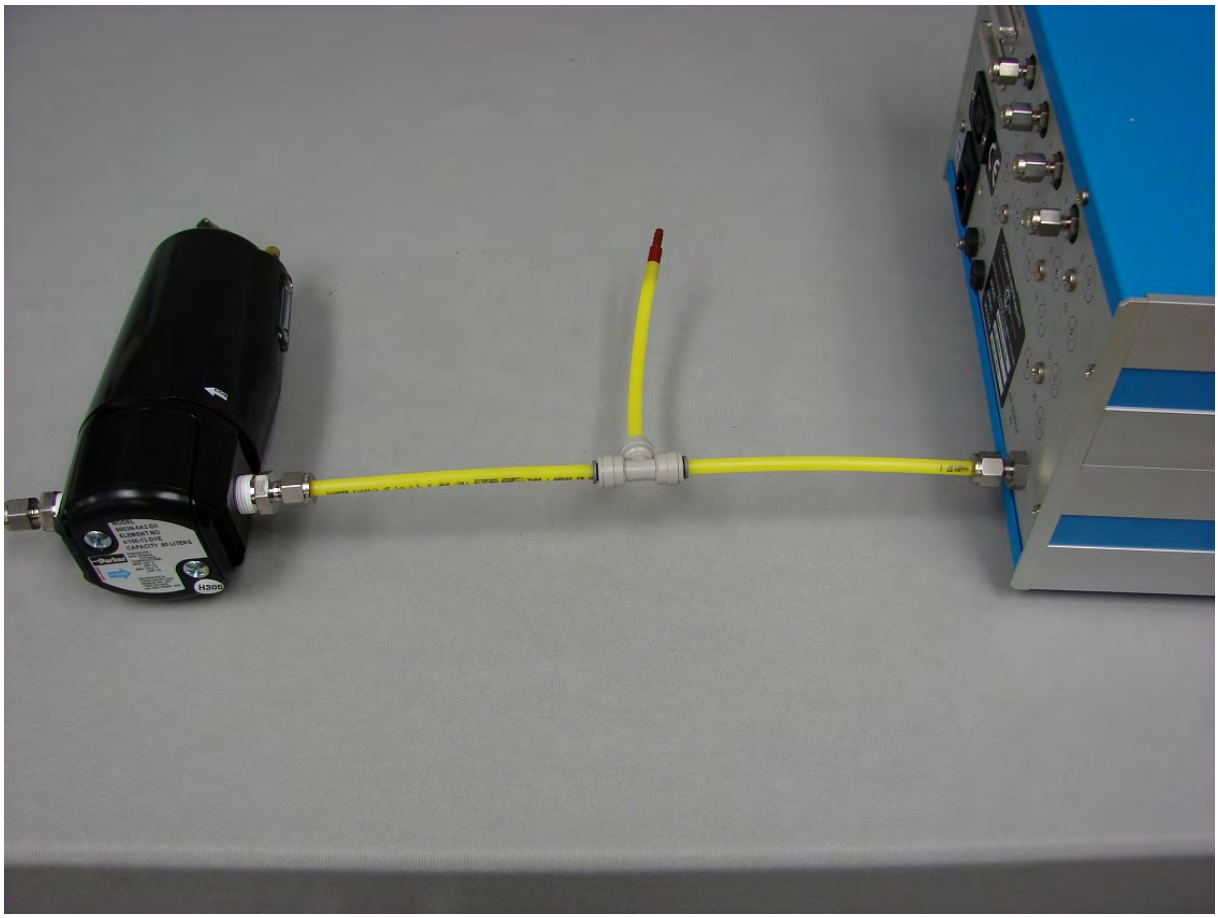
1	Mass flow meter signal out connector	This is where the cable connects from the equal flow box to the system sample pump
2	Air pump power connector	The air supply pump connects here, so it can be turned on and off by the front panel control
3	Power connector / power switch	The AC mains power connects here and the power switch is located here
4	Air outputs	These are the outputs for the fresh air. The flow restrictors connect here
5	Fuse	The fuses for the AC mains are located here and should be replaced with 3 amp slow fuses
6	Air supply in	The tube from the air supply pump connects here

Connecting the equal flow system

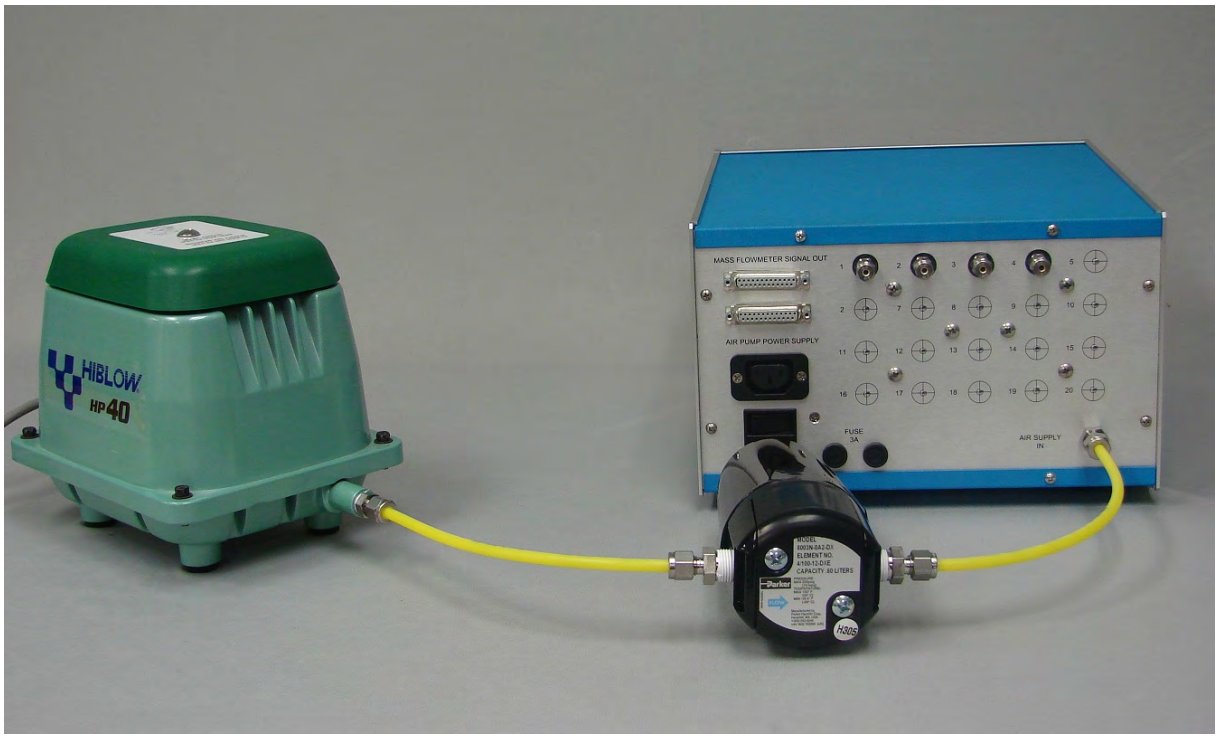
Connect the electric power cable from the fresh air supply pump to the air pump power supply connector on the rear of the equal flow air supply system



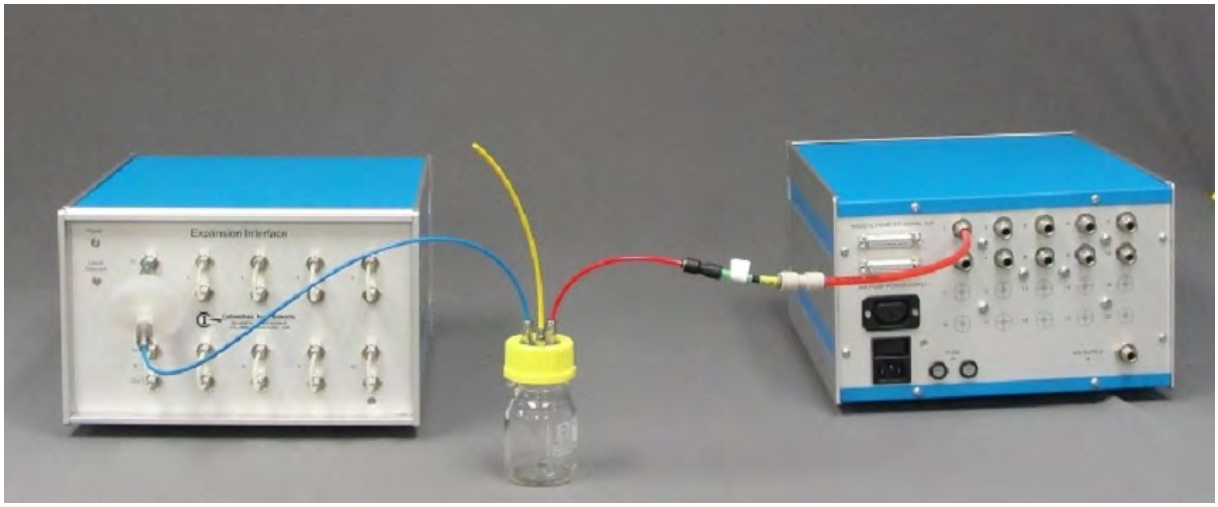
Connect the cable from the Aux I/O connector on the rear of the system sample pump to the mass flow meter out connector on the rear of the equal flow box. If there is more than one equal flow box is used connect the remaining mass flow meter signal out connector to then mass flow out connector of the next equal flow box



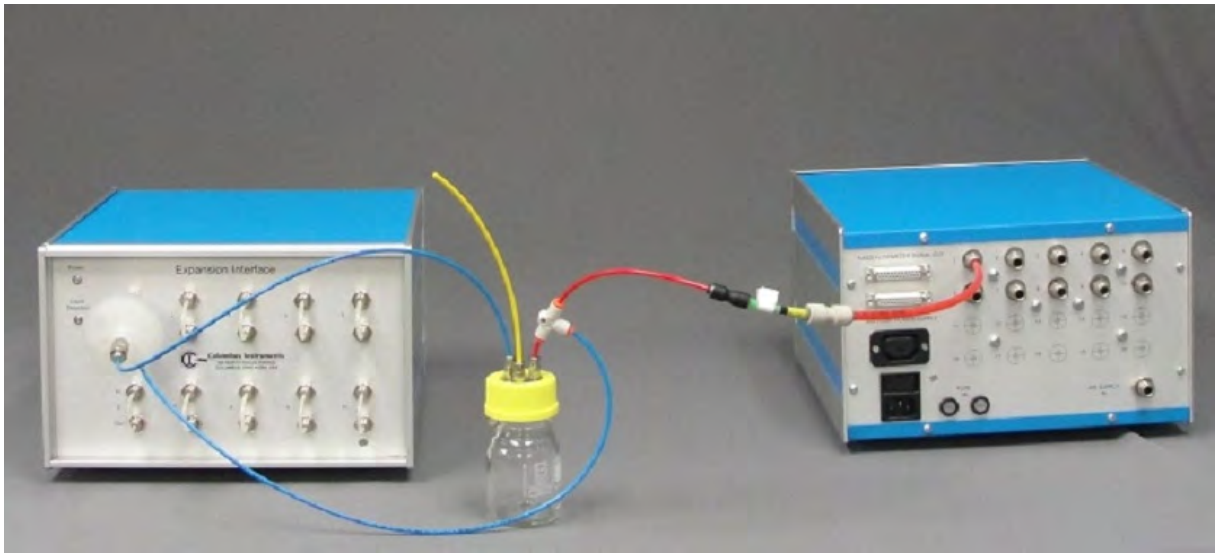
Connect the fresh air pump to the filter then connect the filter to the air supply in connector on the rear of the equal flow box. Note that the arrow on the filter should point towards the equal flow box, or if the filter is marked with in and out the pump must be connected to the in port.
The tubing has been shortened for the purpose of illustration, actual lengths will be longer. Depending on the flow rate used and pump used there may be a pressure relief overflow fitting as shown above, but some systems will not have them as shown below.



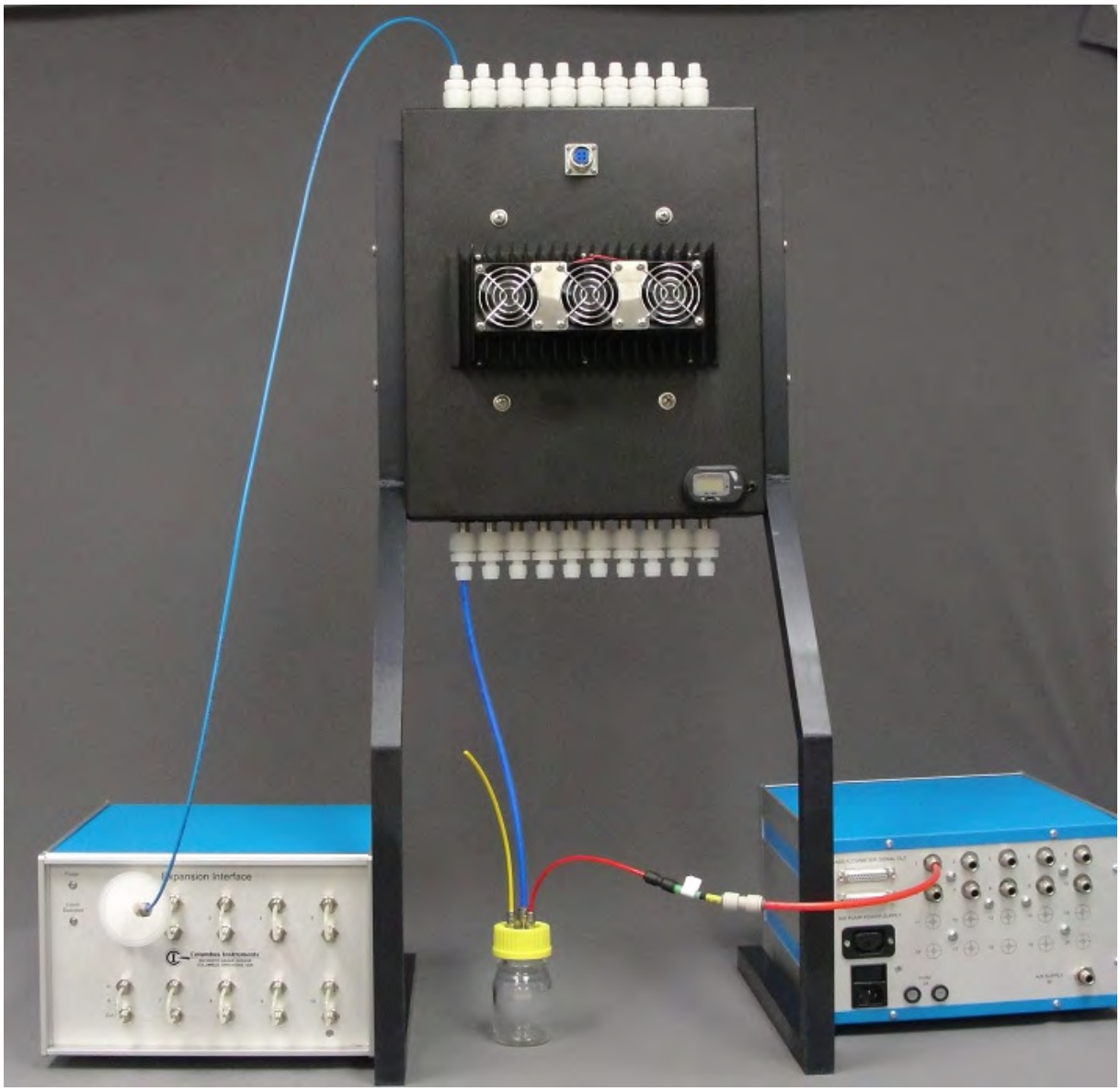
Connect the flow restrictors as shown for all channels. Please note all ports must have the restrictors connected even if all channels are not being used!
Now the sample chamber can be connected. One sample chamber per restrictor.



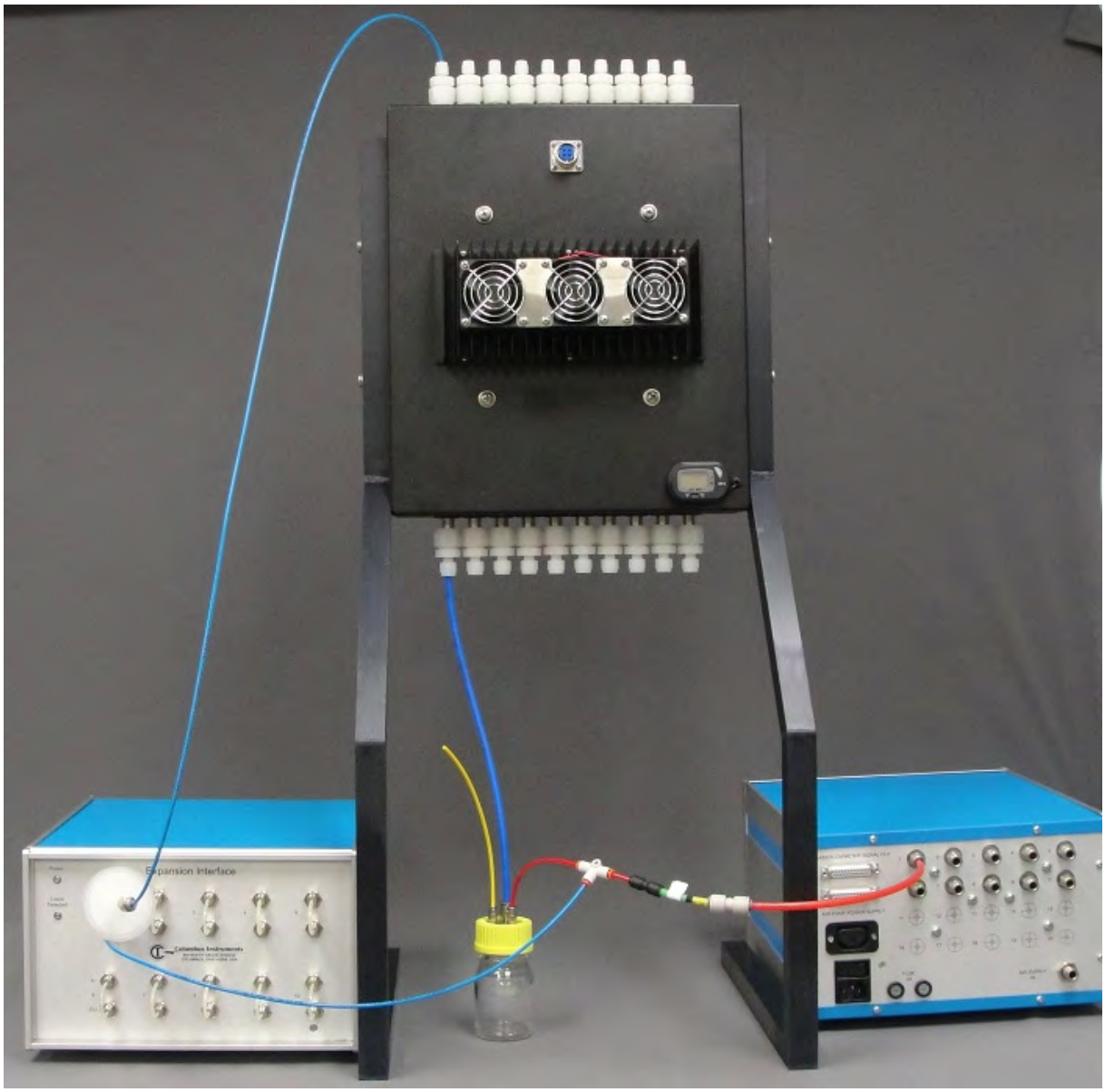
Now connect the tubes from test in port on the expansion interface to the sample chamber. This is the standard connection used when the fresh air supply flow is greater than the sample flow and the out port from the expansion interface is exhausted to the atmosphere and not recirculated to the chamber. Note the yellow tube for the extra overflow air to come out.



This is the configuration where the fresh air flow is less than the ample flow and the sample air is recirculated to the measuring chamber. So there will be a tube connected from the test out port back to the chamber.
Note the yellow tube for the extra overflow air to come out.



if a condensing air drier is used the connection will be slightly different as shown below (the main difference being the additional tube coming from the condenser to the bottle. This is the configuration where the fresh air flow is greater than the sample flow.



The connections for the open flow option with the condenser are shown. This is for the configuration where the fresh air flow is less than the sample flow and the sampled gas is recirculated back to the chamber
Note the yellow tube for the extra overflow air to come out.

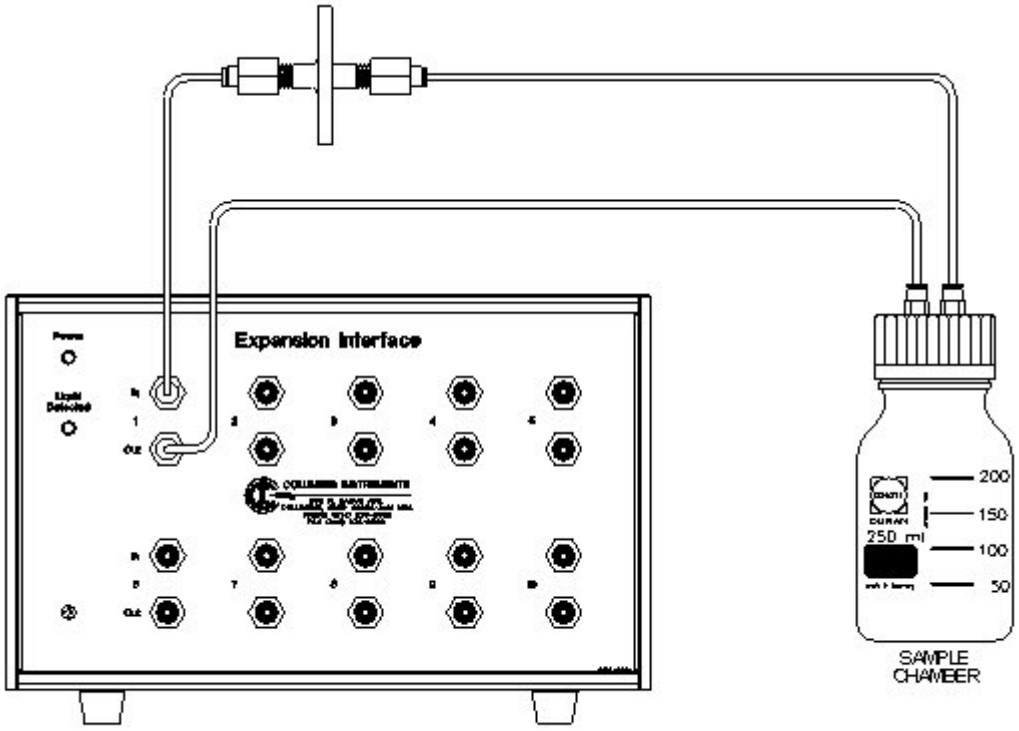
1.6 Operations

Before starting an experiment, the system should warm up for three (3) hours to allow the sensors to come to operating temperature. The system may be left on continuously so that the warm up period can be omitted. What follows is a description of different factors that affect the operation of the system

Preventing Liquid and Bacterial Contamination

If the system includes more than one channel, an Expansion Interface will be included. The

Expansion Interface includes a hardware circuit that detects liquid entering the system. If liquid is detected, the LED on the front panel will turn on, and all the valves in the Expansion Interface will close. To remove any liquid, open the cabinet and drain all contaminants from the clear tubing inside.
For increased protection against liquid entering the system, PFTE hydrophobic membrane filters should be placed in the gas sampling line going to each chamber. The drawing on the following page shows the connection for these filters. These filters are available from Columbus Instruments. An additional advantage of filters is that they prevent contamination of the sensors and expansion units by bacteria as well as cross contamination between the chambers.
Attention: After initial installation of the filters, do not change the orientation of the filter as particles that accumulate on one side of the filter will be sucked into the system when the filters are turned around.

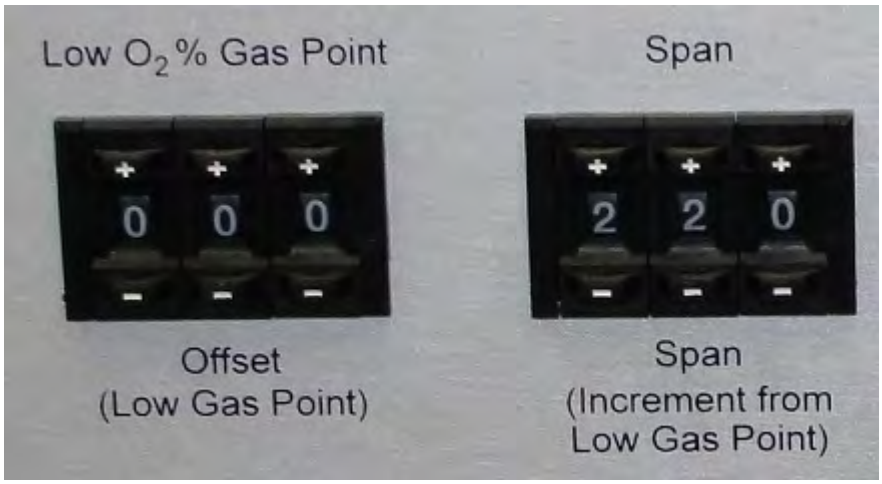


*** NOTE: This circuit detects liquid by measuring conductance, it will not work with distilled water.**

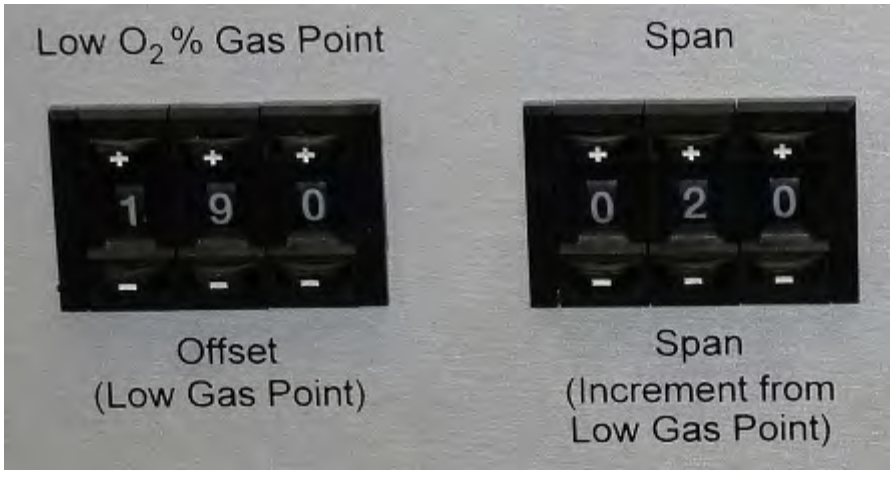
Configuring the paramagnetic O2 sensor.

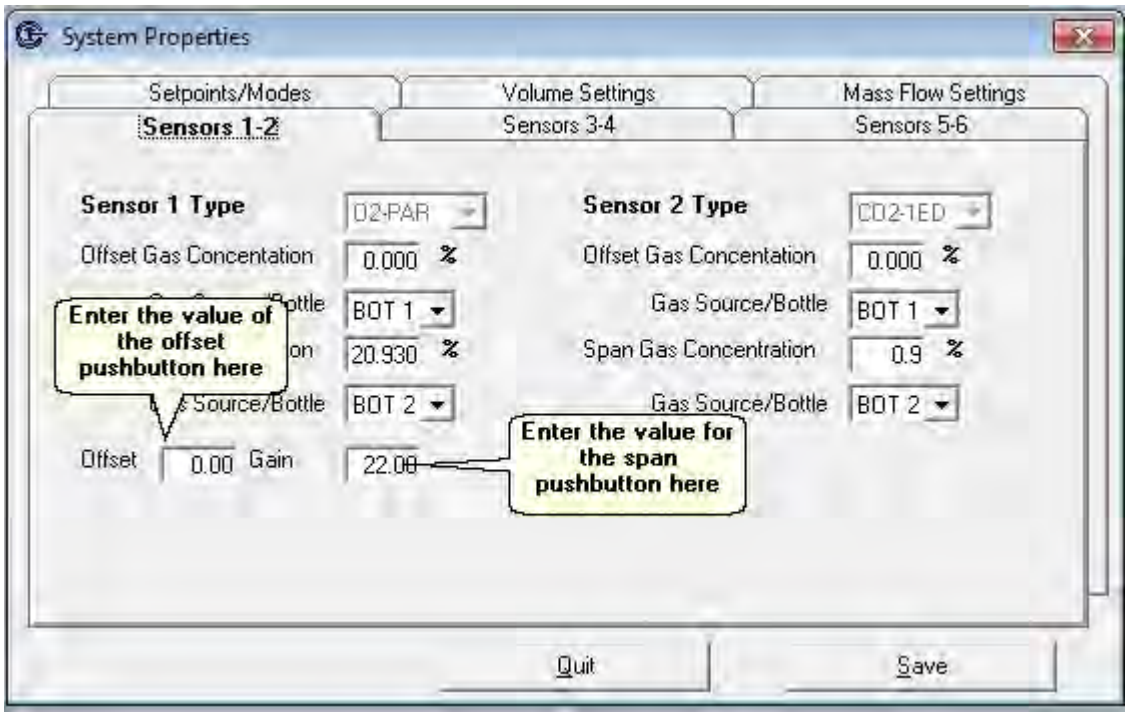
The paramegnetic O2 sensor is unique in the fact that it can be programmed to operate over any range from 0-100%
The offset programming pushbutton selects the lowest concentration of oxygen to be measure. The span programming pushbutton selects the range of oxygen concentration measurement above the offset concentration that the sensor will measure. To help explain a few examples

are shown:
In the example below the range of measurement would be 0-22 % O2. An offset gas with 0 % O2 would be required and a span gas with 90% of the full range would be required. (actually ambient air can be used, it contains 20.93% O2)



In the below example the range of the sensor will be 19 - 21% O2. An offset gas with 19% O2 will be required as well as a span gas with 90% of the full range would be required. (actually ambient air can be used, it contains 20.93% O2)
This example will have a higher sensitivity of measurement because of the smaller range of measurement





Air Flow-Rate Settings

The sample flow should be set to 0.5 l/min. This is set during the calibration procedure. There is typically no flow when the system is idle. The sample flow is adjusted using the metering valve on the "Sample Flow" flow meter on the front of the System Sample Pump. If a test chamber larger than 2 liters is used, the sample flow may not be enough to promote adequate mixing of the air within the chamber. Therefore, it is recommended that a small fan be placed in test chambers larger than 2 liters in volume.

The refresh flow rate should be adjusted with the control knob in the fully counterclockwise position. The only time refresh flow will occur is at the beginning of the experiment, and when a refresh cycle occurs during the course of an experiment.

If the test chamber volume is greater than 10 liters, an external pump may be required to refresh the test chambers in a reasonable amount of time. A special pump and control relay is required for this purpose.

Air flow Rate considerations when using the open flow option

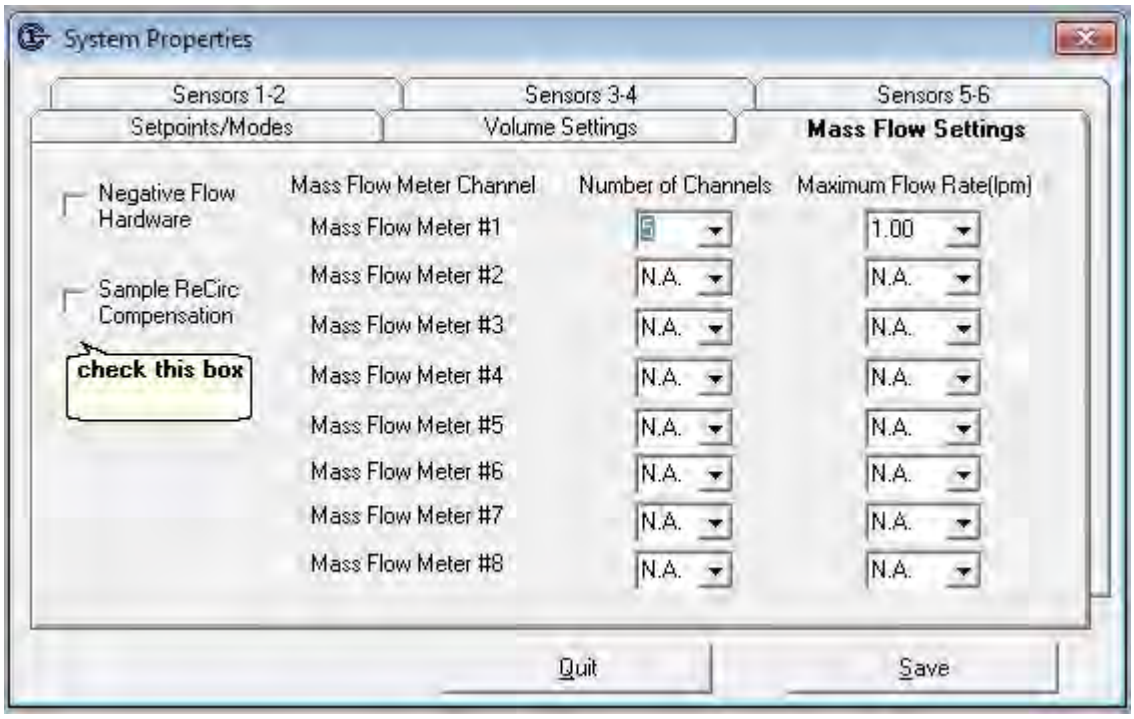
The open flow option measures the difference in gas concentrations going into the chamber and coming out. This is referred to as the delta or change in gas concentration.
The fresh air flow should be set so that the delta O2/CO2 are between approximately .2% and .8%. This is for a system with a standard range sensor (0-1% CO2) The delta can be proportionally higher for systems with higher range sensors (ie for a 3% sensor the delta should be between 0.6% and 2.4% This insures a good signal to noise ration while still keeping the gas concentrations within the sensor's range of measurement.

When the fresh air flow rate is higher than the sample flow rate (500 ml/min or 0.5 l/min) the out line from the expansion interface does not need to be connected to anything the system just samples what it needs and the excess is vented to the atmosphere.

If the fresh air flow rate is close to or less than the sample flow rate, the out port should be connected to the sample bottle to recirculate the sample gas back to the bottle. This is required because if the sample gas is not recirculated back to the chamber, there will be no overflow at the open port of the chamber and atmospheric air will be sucked back into the chamber from the overflow and will cause large errors in the readings. Also it can be desirable to use lower sample flow rates in some situations. The air condensing drier operates more efficiently so higher temperature samples with high moisture content can benefit from this. If the sample flow rate is decreased the time for the gas to circulate through the sensors should be increased proportionally .
For example, if the sample flow rate is decreased from 500 ml/min to 250 ml/min then the sample circulation time must double. See below for which parameters should be channel. Also the decreased sample flow rate is desirable for open flow tests using low fresh air flow rates (below 500 ml/min.

Sample gas recirculation compensation

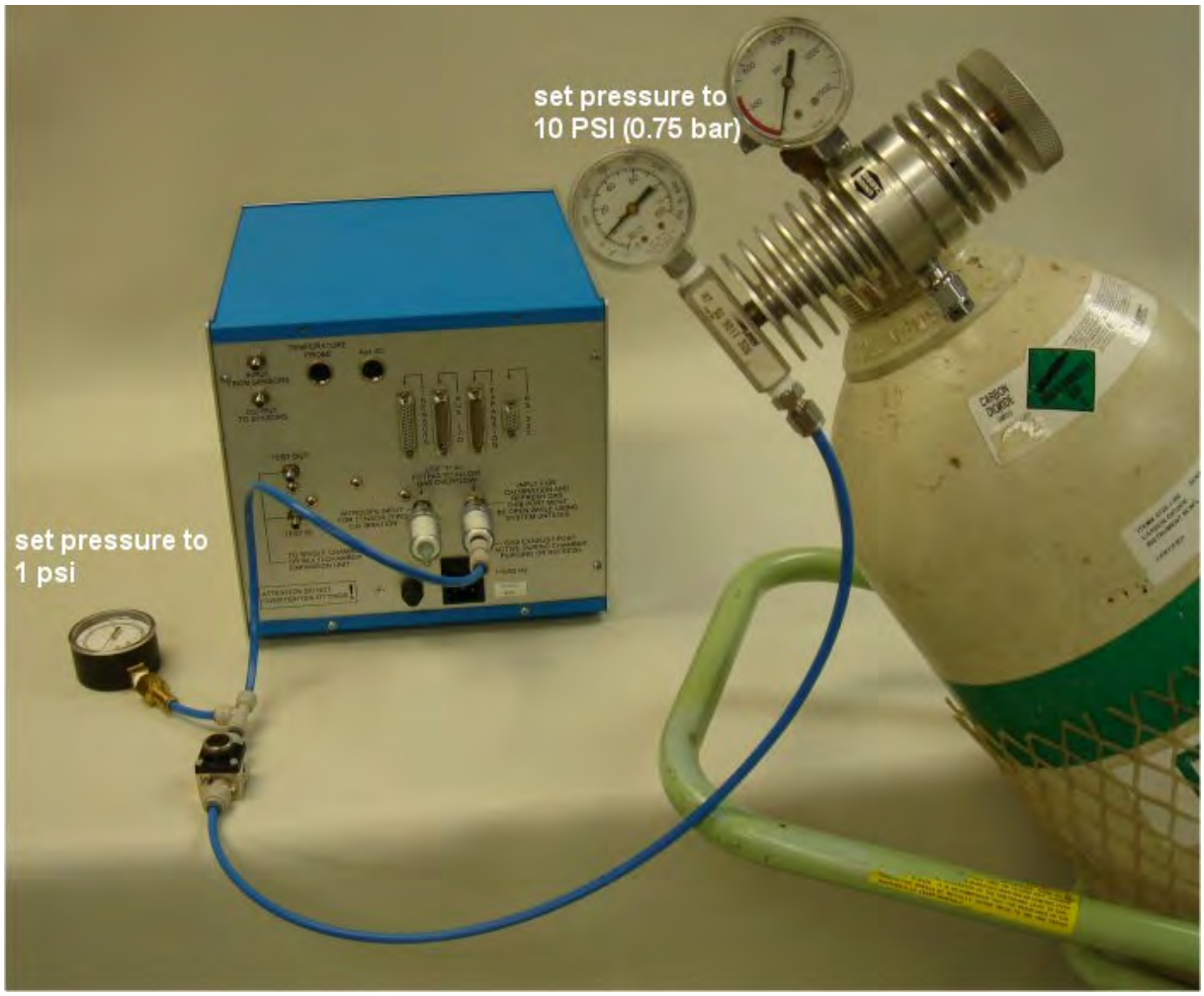
In open flow experiments where the flow rate of the fresh air must be lower than the sample flow rate, it is necessary to recirculate the sampled gas to the chamber so that there will be an overflow of air from the chamber and fresh air will not be drawn into the overflow tube. In this situation a compensation can be applied to the reading to enhance the accuracy and reduce the error caused be recirculating the sampled gas to the chamber. This correction can be enabled by going to file-properties-mass flow settings and checking the Sample Recirc Compensation box.
When this compensation is enabled, the chamber head space volume must be measured with the overflow port blocked, and this head space volume must be entered into the experiment setup. (See experiment setup)



Refreshing from bottled gas

The diagram on the following page shows the connection of the Micro Oxymax for use with a bottled gas. When using a bottled gas for refresh, the pressure at the refresh gas inlet should be 0.5 PSI while the chambers are being refreshed. The pressure will be higher when the system is not refreshing. A special low-pressure regulator is available form Columbus Instruments for this purpose.

Micro Oxymax System Setup
Refresh From Bottled Gas

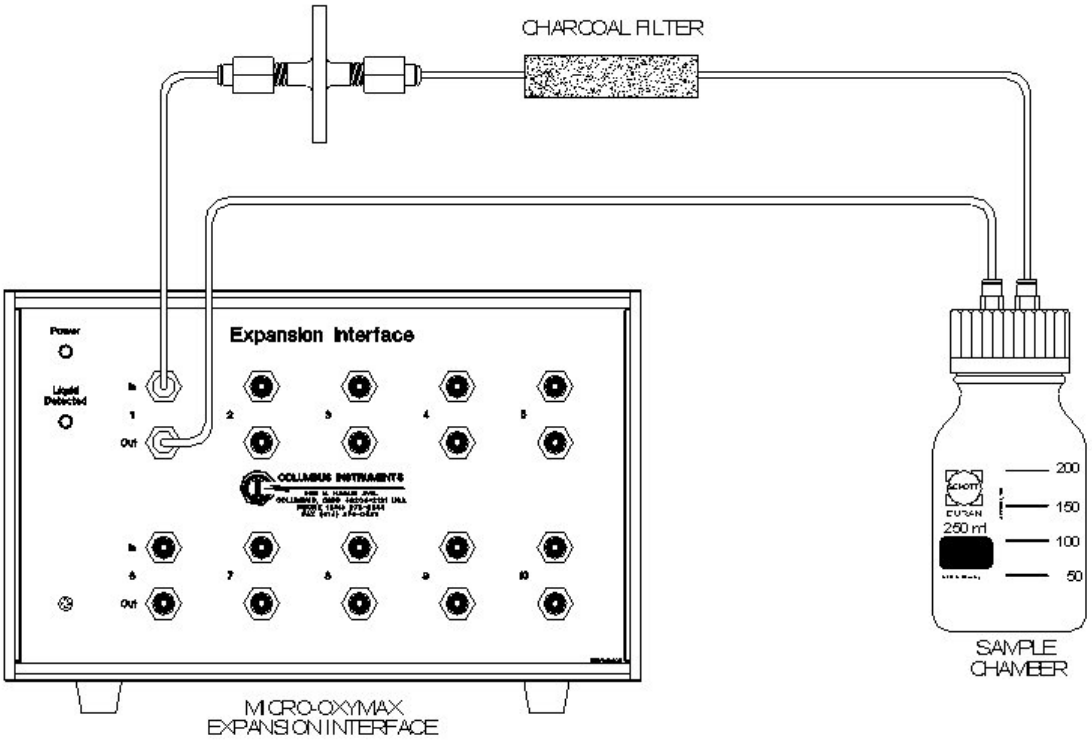


Use with Volatiles

When using the Micro Oxymax with samples that contain volatiles, an activated charcoal trap must be used to remove the volatiles so that the gas sensors are not damaged and the readings are not distorted. The following drawing shows the connection of the charcoal trap to the Micro Oxymax and sample chamber. **Never place charcoal inside the drying column with the magnesium perchlorate!**

Micro Oxymax System Setup

Charcoal Filter Connection

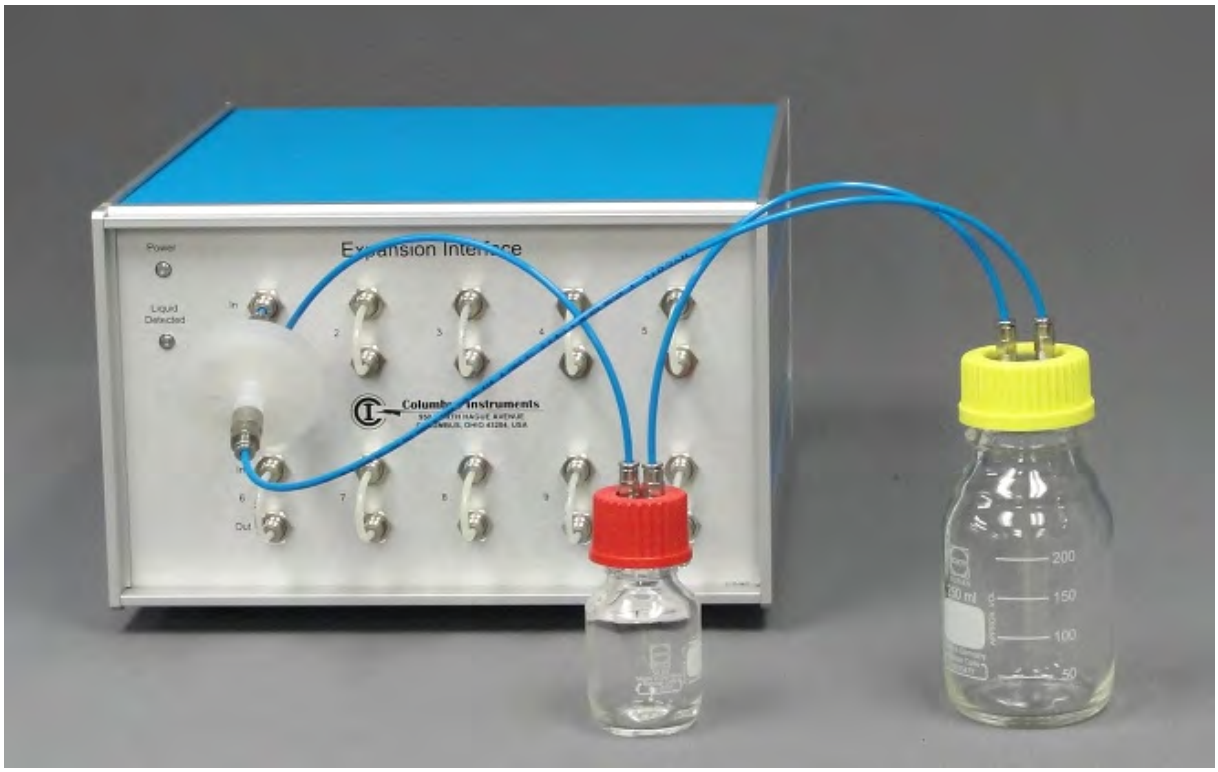


Adding or removing substances from a chamber

In some experiments it is desirable to add or remove materials from a chamber during the experiment. Liquids can be added or removed from the chamber through the septum lid option (available from Columbus Instruments) via a syringe without disturbing the experiment. If the amount of substance being added or removed is less than 5% of the chamber's head space volume, there will be little affect on the O2 and CO2 readings. If an amount larger than 5% is to be added, an equal amount of substance should be withdrawn first to keep the head space constant. If an amount larger than 5% is to be withdrawn, an equal amount of water should be added to keep the head space constant. If the sample chamber must be opened, It must be done either immediately before a refresh cycle, or the experiment must be terminated then restarted after re-closing the chamber using the append option to append the new data to the original file.

Re humidifying air returning to the chamber

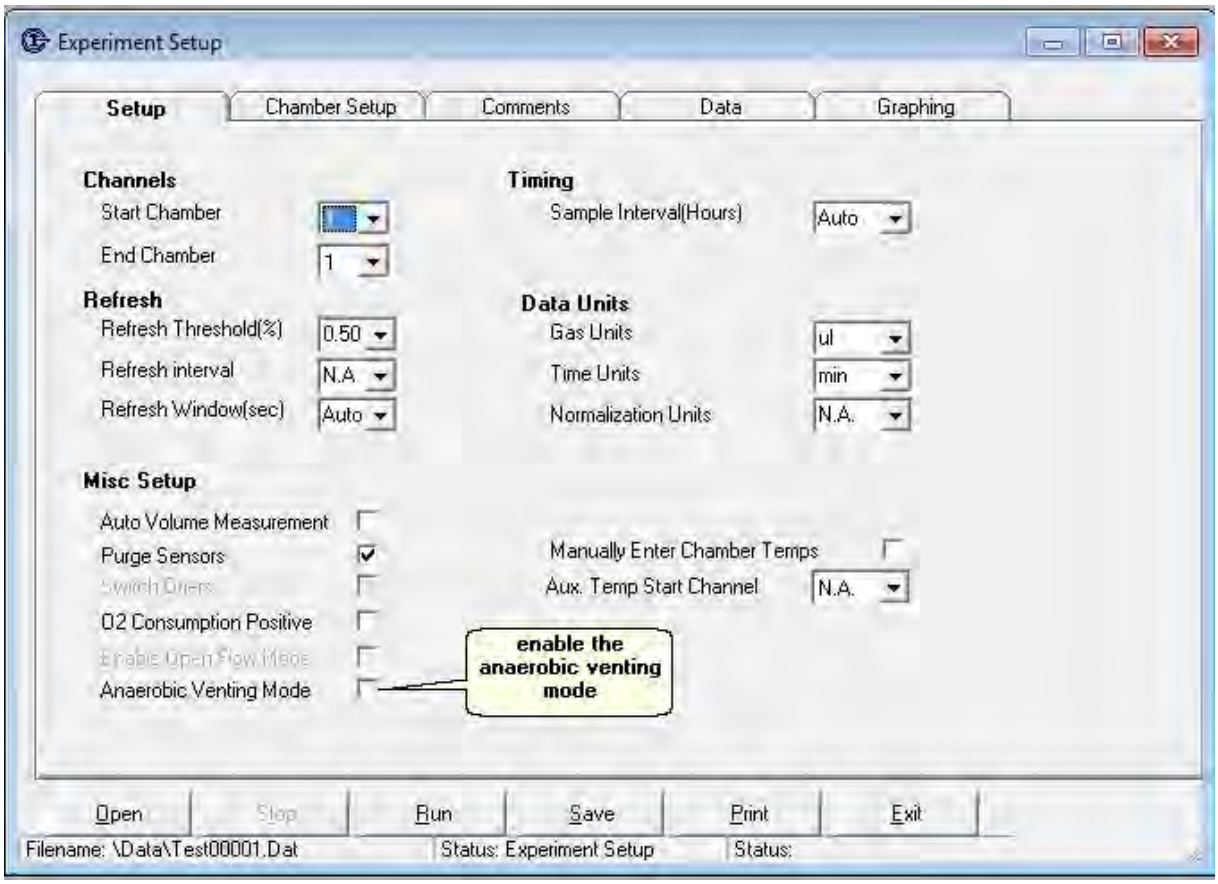
When the air is sampled from the chamber, all of the water vapor is removed. When the air returns to the chamber it is sometimes necessary to re humidify it, To do so, place a small flask in series between the out port of the expansion interface and the sample chamber as shown below:



1.6.1 Anaerobic operation

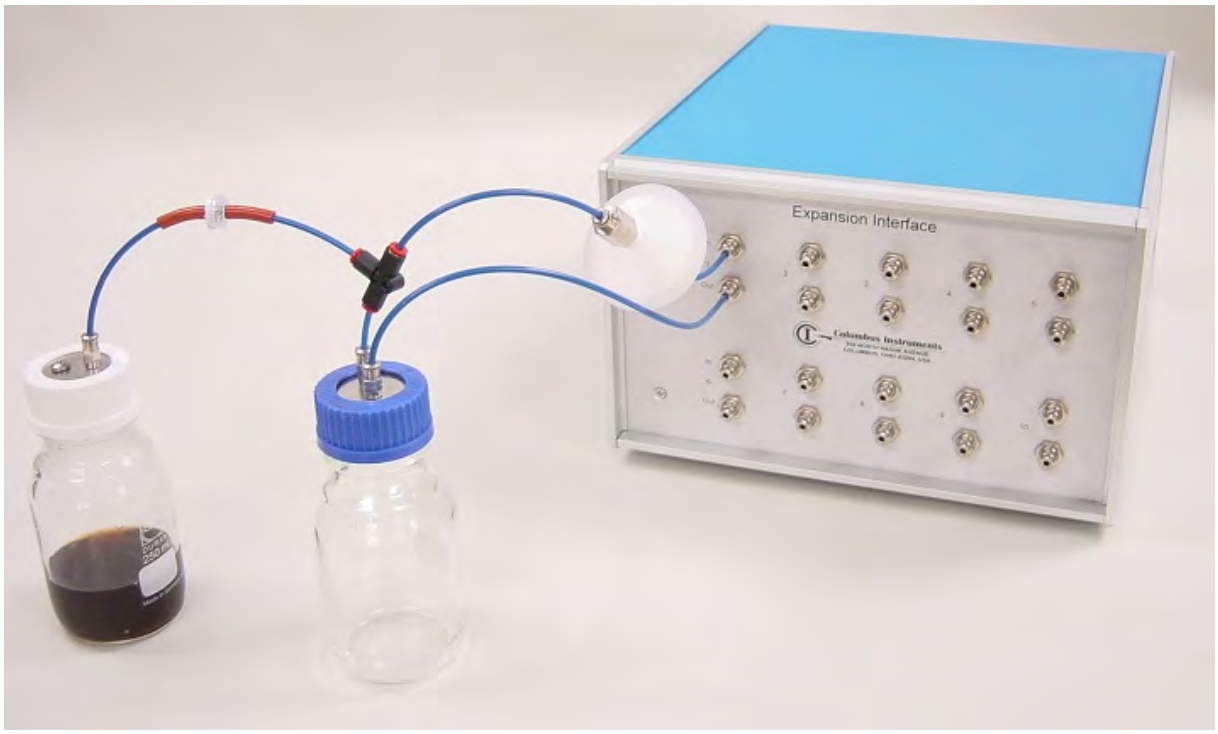
The Micro Oxymax system can be used to measure the production of gases in anaerobic experiments. There are 2 different ways to configure the system to operate anaerobically. The methods will be described below and configuration will be shown.

With either method the check box for anaerobic venting on the experiment setup should be checked. The purpose of the anaerobic venting option is to vent excess pressure to the atmosphere after each measurement of the chamber. In normal aerobic operation of the system, the net pressure in the chamber tends to remain constant because O2 is consumed and CO2 is produced. In an anaerobic experiment gases are produced and none are consumed so the pressure in the chamber goes up over time.

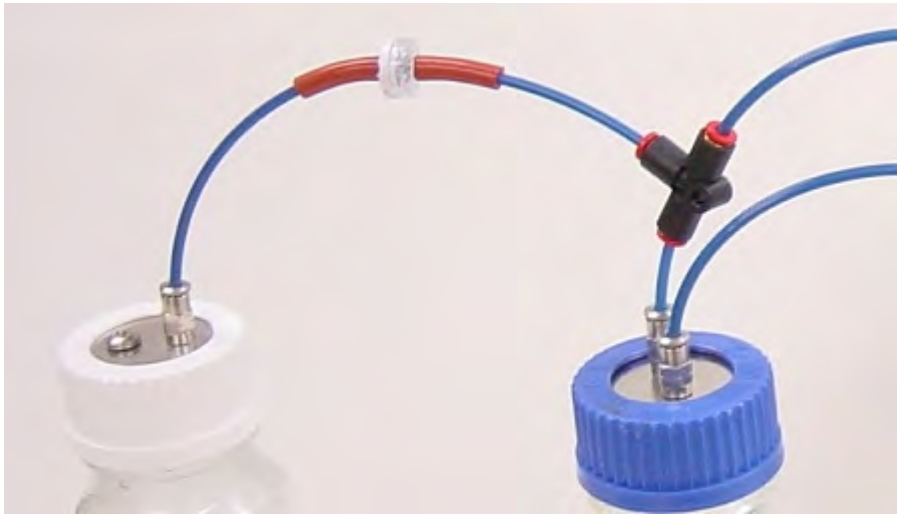


The 2 bottle method

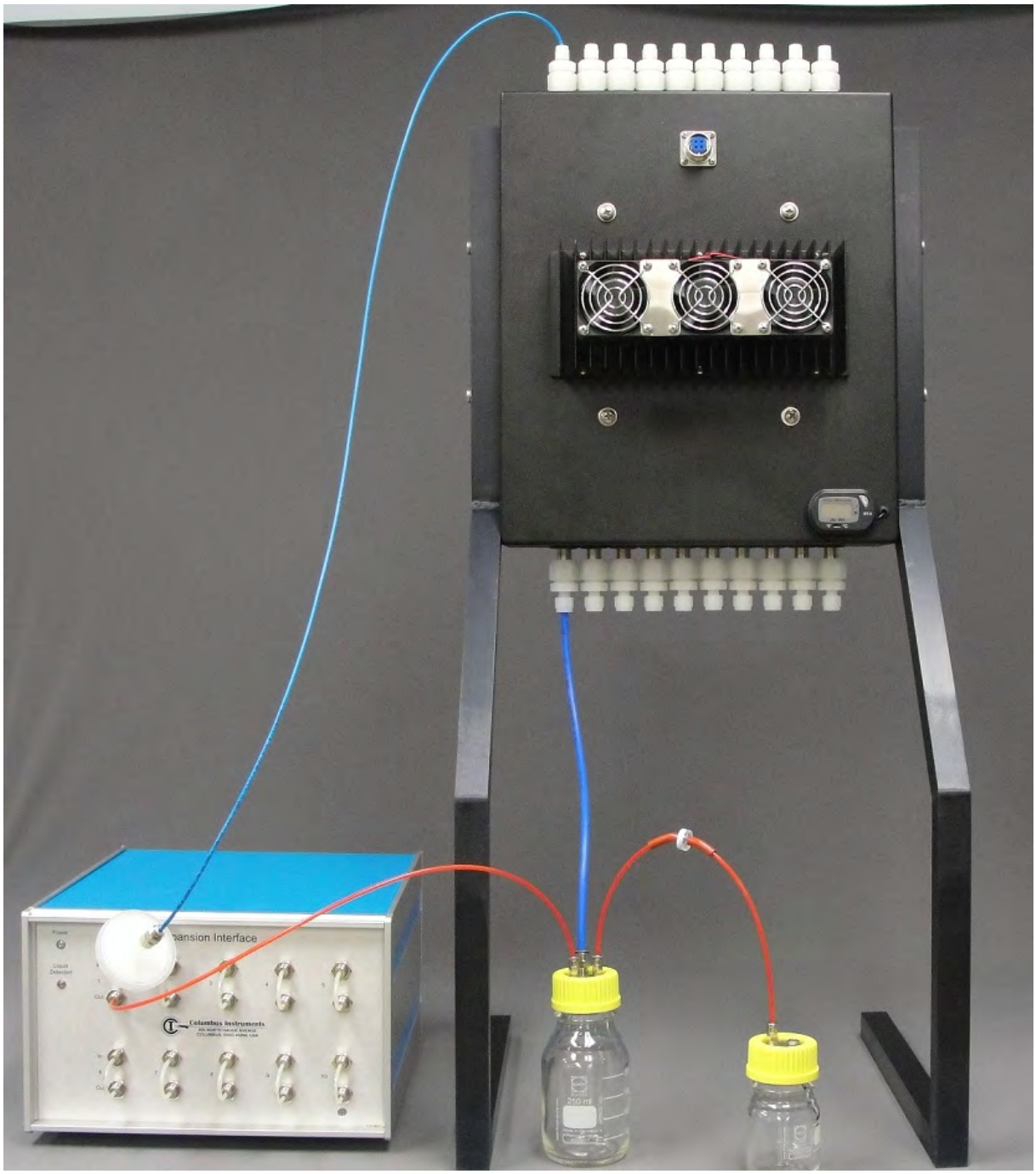
The advantage of the 2 bottle method is that no source of nitrogen needs to be connected to the system to maintain anaerobic conditions. It is called the 2 bottle method because 2 bottles are connected together with a check valve (one way valve) between them. this valve has a very low opening pressure. One bottle is referred to as the reactor bottle and contains the sample to be measured. The other bottle is referred to as the sample bottle. The sample bottle connects to the expansion interface (or in the case of a single channel system to the test in and out ports on the rear of the system sample pump). The reactor bottle is connected to the sample bottle via one way valve. The connection is shown below:



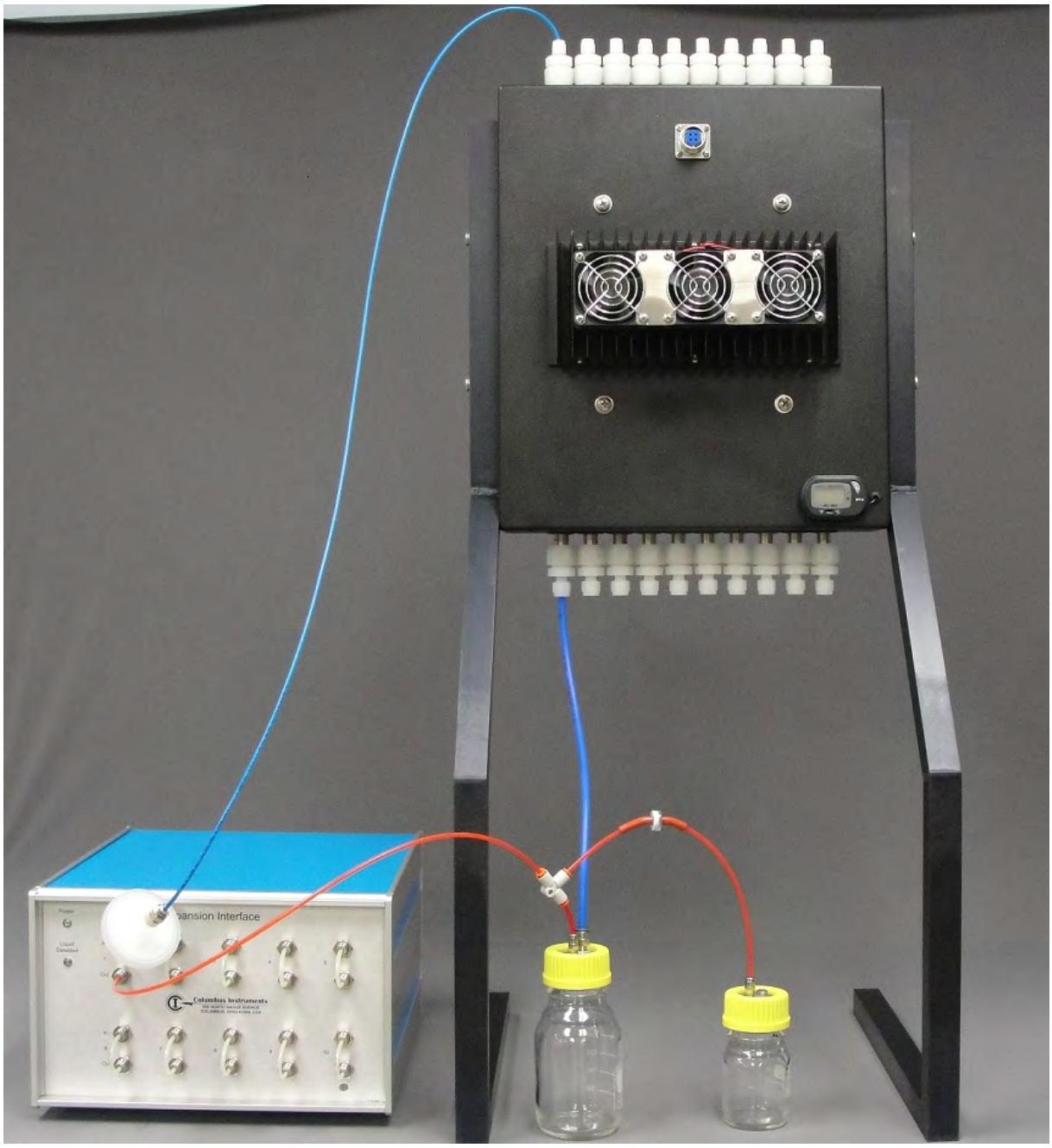
Be sure the clear side of the one way valve points to the sample bottle:



Connection with the condenser with a 3 port flask

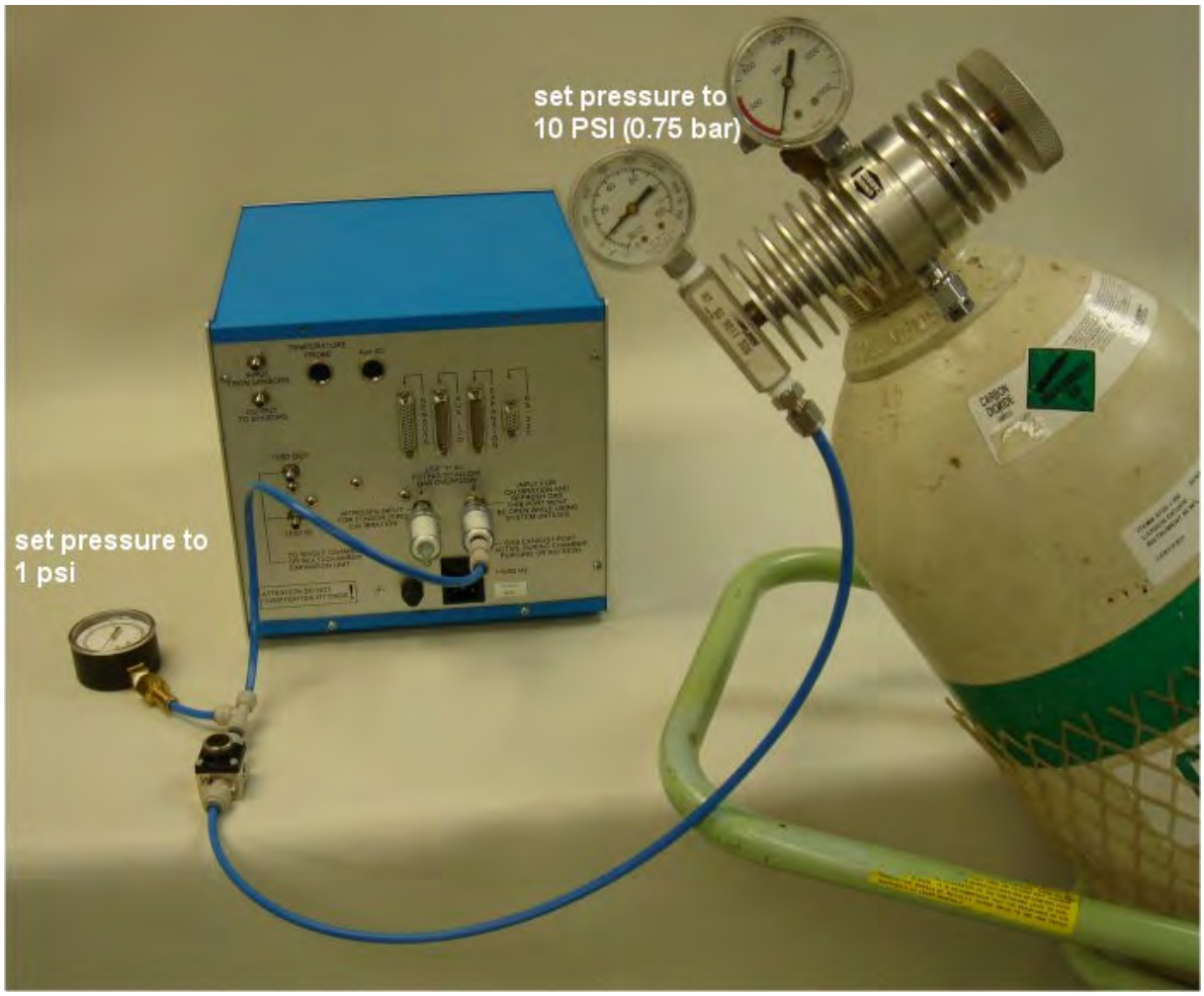


Connection with condenser with 2 port flask



Nitrogen method

With the nitrogen method, a bottle of pure nitrogen is connected to the system sample pump where it brings in the gas used for purging the sensors and refreshing the chamber. This maintains anaerobic conditions in the chamber. The disadvantage is that a considerable amount of nitrogen will be used. The amount of nitrogen can be roughly calculated in the following way: (purging time + sampling time) / 60 * 0.5 * number of channels used = nitrogen used per sample interval in liters.
The nitrogen source must be connected to the system sample pump by a special low pressure regulator sold by Columbus Instruments. The inlet pressure should be set to 1 PSI (.068 bar). The connection is shown below:

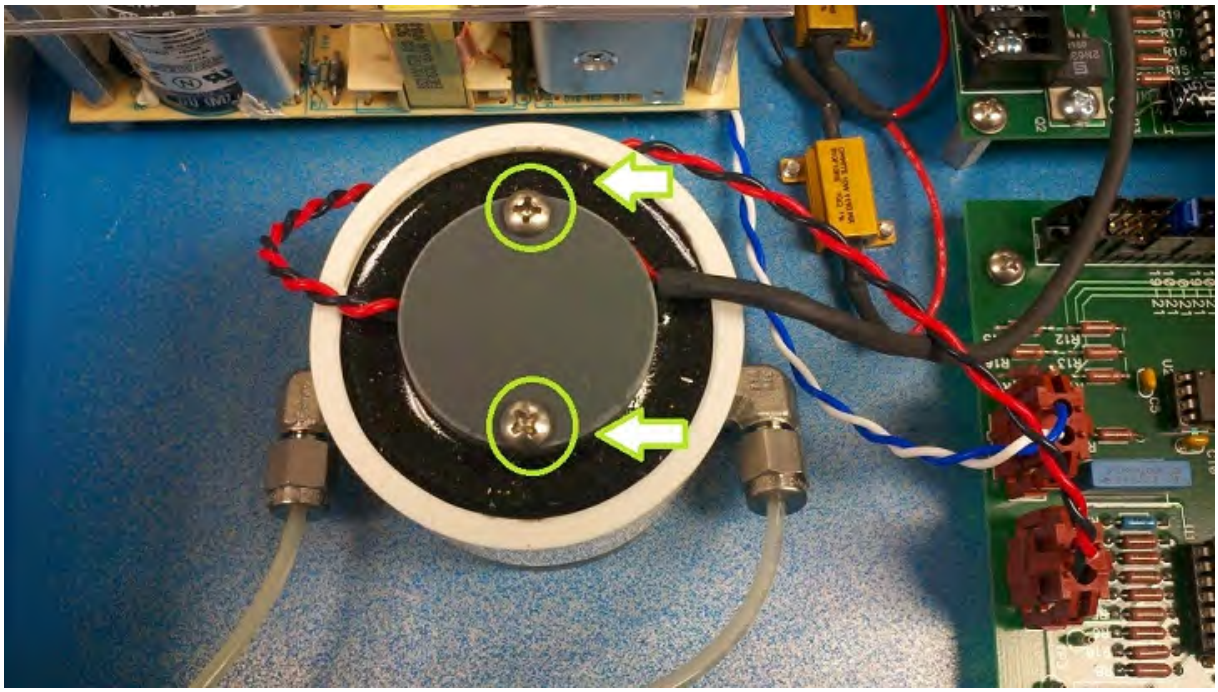


1.7 Maintenance

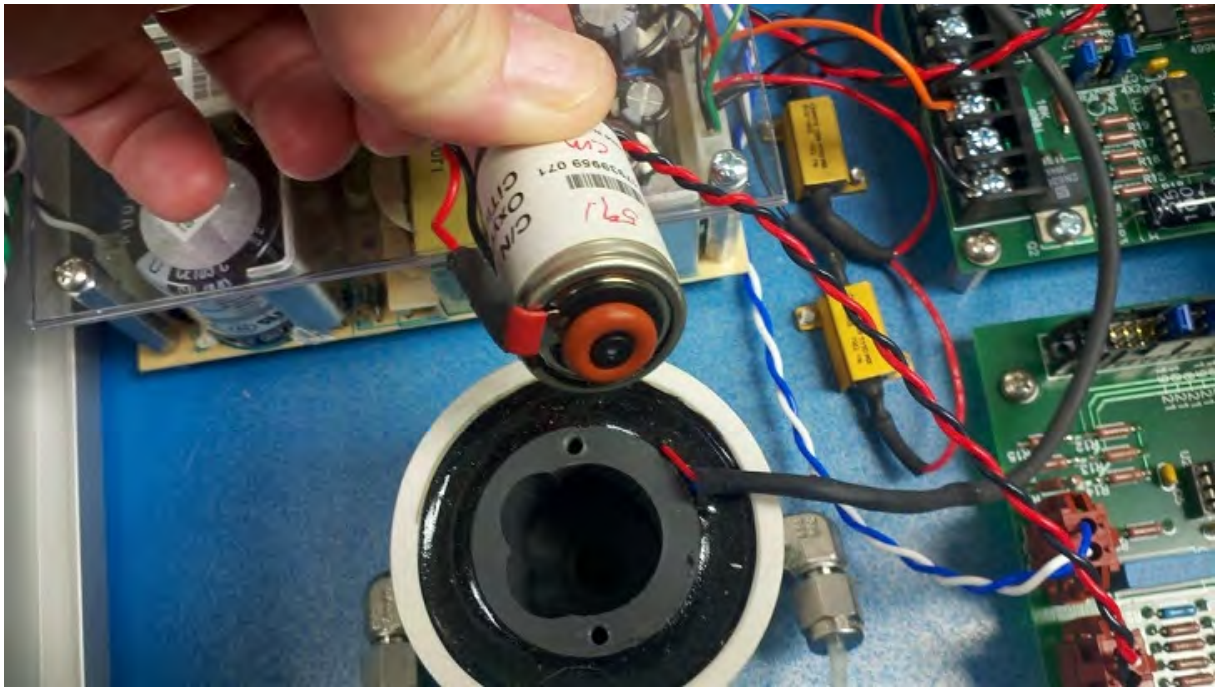
Replacement of the Oxygen Sensor

The Micro Oxymax oxygen sensor is designed to supply about nine months of service. Replacement of the sensor is indicated when the desired calibration Set-point oxygen level can no longer be obtained (ie increasing the gain during calibration has no effect). The drawing on the following page shows the oxygen sensor housing assembly. The following steps outline the procedure for replacement:

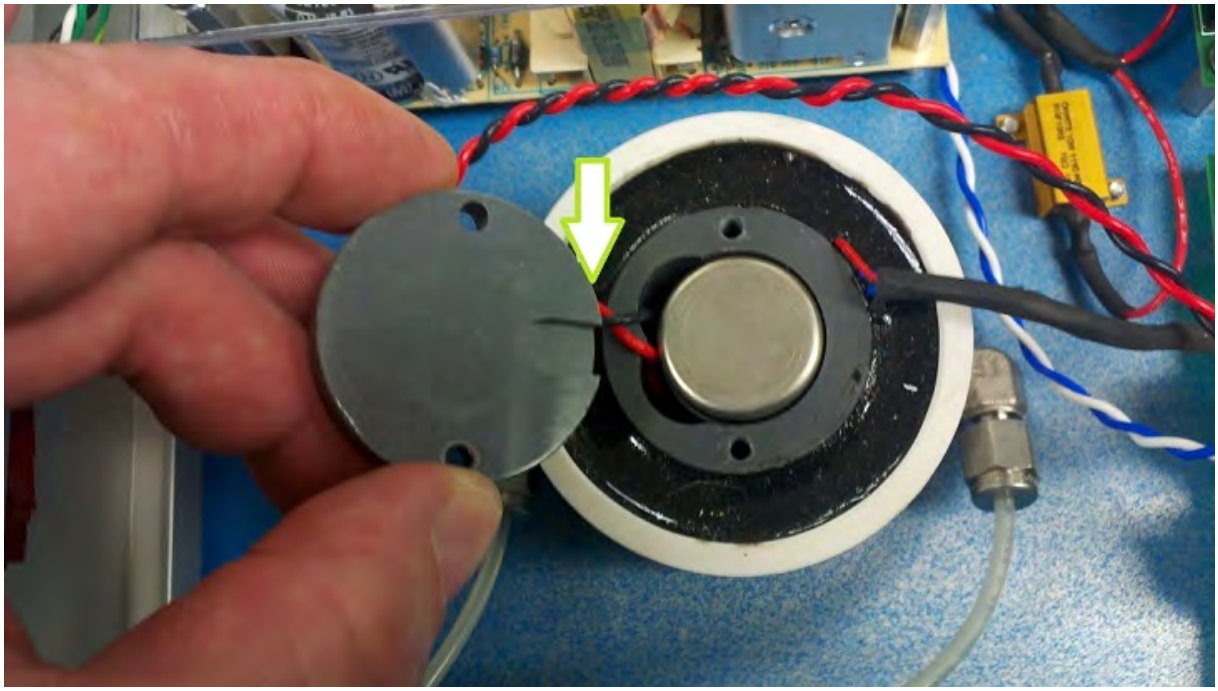
1. Unplug the System Sample Pump and carbon dioxide sensor from the AC power source.
2. Remove the tubing from the "Sensor In" and "Sensor Out" fittings on the rear of the Oxygen Sensor cabinet.
3. Unplug the cable from the Oxygen Sensor cabinet.
4. Place the Oxygen Sensor on a clean workspace and remove the two Philip's screws that fasten the top panel. Remove the top panel.
5. Remove the two screws from the top end of the housing



8. Slide the sensor assembly out and remove the red and black wires from the plug in connector on the circuit board
9. Plug the red and black wires from the new sensor into the green circuit board
10. Slide the assembly back into the housing. Be sure the red silicone O ring is on the nipple on the end of the sensor



11. Replace the cover over the sensor cell making sure the relief slot for the wires is positioned over the place where the wires come out of the housing



12. Replace the top panel and secure it with the two Philip's screws and lock washers.
13. Return the Oxygen Sensor cabinet to its location in the system and reconnect all the cables and hoses that were removed.

Hydrophobic filters

The use of hydrophobic filters on the chamber sampling lines are required to prevent liquid and or particle contamination of the expansion unit. These filters are used to prevent bacteria from entering the expansion unit during the sampling of the chamber head space gas, and will prevent cross contamination of the samples during the return of the measured head space gas. These filters should be replaced once per year Replacement hydrophobic filters can be obtained from Columbus Instruments. The part number for a complete filter assembly is 7395-40B. For just the filter replacement use part number 7395-40A.

Air Supply Filters

The "Balston" Air Filters are used on both the Refresh/Calibration and Nitrogen Ports located on the rear of the system sample pump. When these filters become expired they turn from white to pink. Once the filter is 90 percent pink in color it should be replaced. These filters prevent any debris from entering the Refresh/Calibration and Nitrogen Gas ports. Replacement Balston filters can be obtained from Columbus Instruments. The part number for a complete filter assembly is 7395-78. They should be replaced once per year

Recharging columns with O2 only systems

Systems with the O2 only measurement capability use glass columns filled with soda lime (NaOH) in series with the sampling line. This is because the CO2 must be absorbed from the sampled gas prior to measuring the O2 concentration. The variations in CO2 levels will cause errors in the O2 measurement. In a system equipped with a CO2 sensor, the variations are corrected for in software. The soda lime has an indicating chemical which changes to a violet color when it becomes saturated with CO2. When 75 % of the column turns violet the chemical could be changed for new.

Changing the drying columns on the Sample drier

The large drying columns connected to the rear of the sample drier need to be periodically changed. The chemical inside (drierite) will change from blue to light pink when it is consumed. These should be changed when approximately 75% of the chemical changed to pink. When multiple columns are connected to the sample drier, One can be recharged while leaving the other(s) connected as not to disrupt the experiment.

Changing the drying tube in the sample drier

It is possible for the permeable tube used in the sample drier to stop working after some time. This can be caused by old age or chemical contamination of the permeable tube. Failure of the permeable tube is indicated by the chemical in the **drying columns no longer being consumed as the experiment progresses.**

2 Software

2.1 Software Introduction

System Requirements

The Micro Oxymax Windows software requires a 500Mhz Pentium Computer with 128 megabytes system RAM and 20 megabytes hard drive for a minimum. A free RS232 communication port will also be needed to communicate with the Micro Oxymax hardware. The software is compatible with Windows 98, NT4.0, 2000 or XP operating system. It is also recommended that the system be equipped with a Super VGA monitor capable of screen resolutions 800 X 600 or more.

Installation

The software to operate the Micro Oxymax is supplied on a CD-ROM The software is installed by running the setup file located on the supplied CD-ROM. Once the software has been installed a new program group named "Micro Oxymax" will be added to the Programs

Menu. To run the software simply click on the Micro Oxymax Icon located in the Programs Menu.

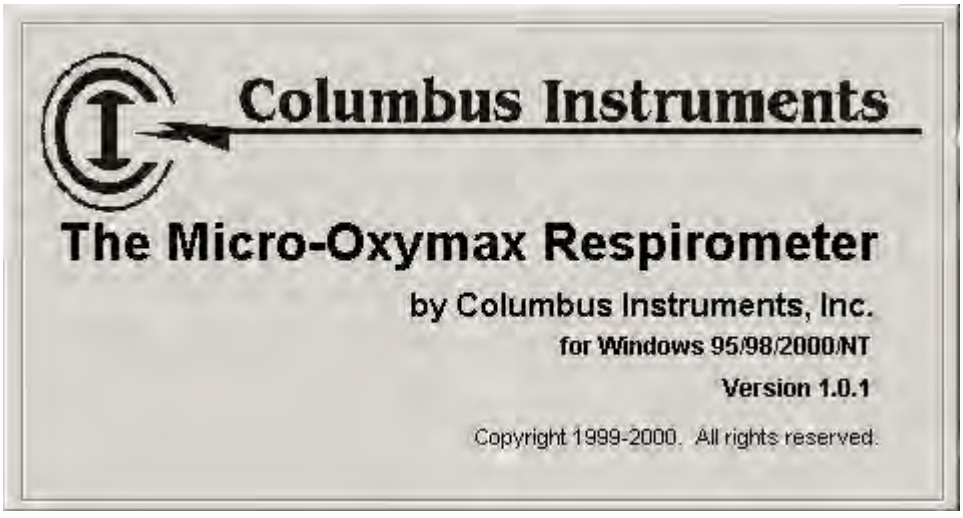
Special notes on installing software:

For Windows 7 & and Vista operating systems
User account controls must be turned off!! failure to do so will result in incorrect installation and improper configuration of the system
This can be done under control panel -> user accounts -> change user account control settings >change the setting to never notify

If the software is installed on a computer with regional settings other than United States, the decimal separator must be changed from , to . under control panel -> regional settings. Failure to do so will result in incorrect configuration of the system and incorrect functioning of the system

Running Software

The Micro Oxymax software can be run by clicking on the Micro Oxymax folder located in the Programs Menu. Once this file has been copied the software will finish loading and the system will search the available RS232 ports for The Micro Oxymax installed hardware. If the system is unable to find the Micro Oxymax hardware the system will notify the user that there is no hardware present and the system will need to be restarted to initialize hardware.

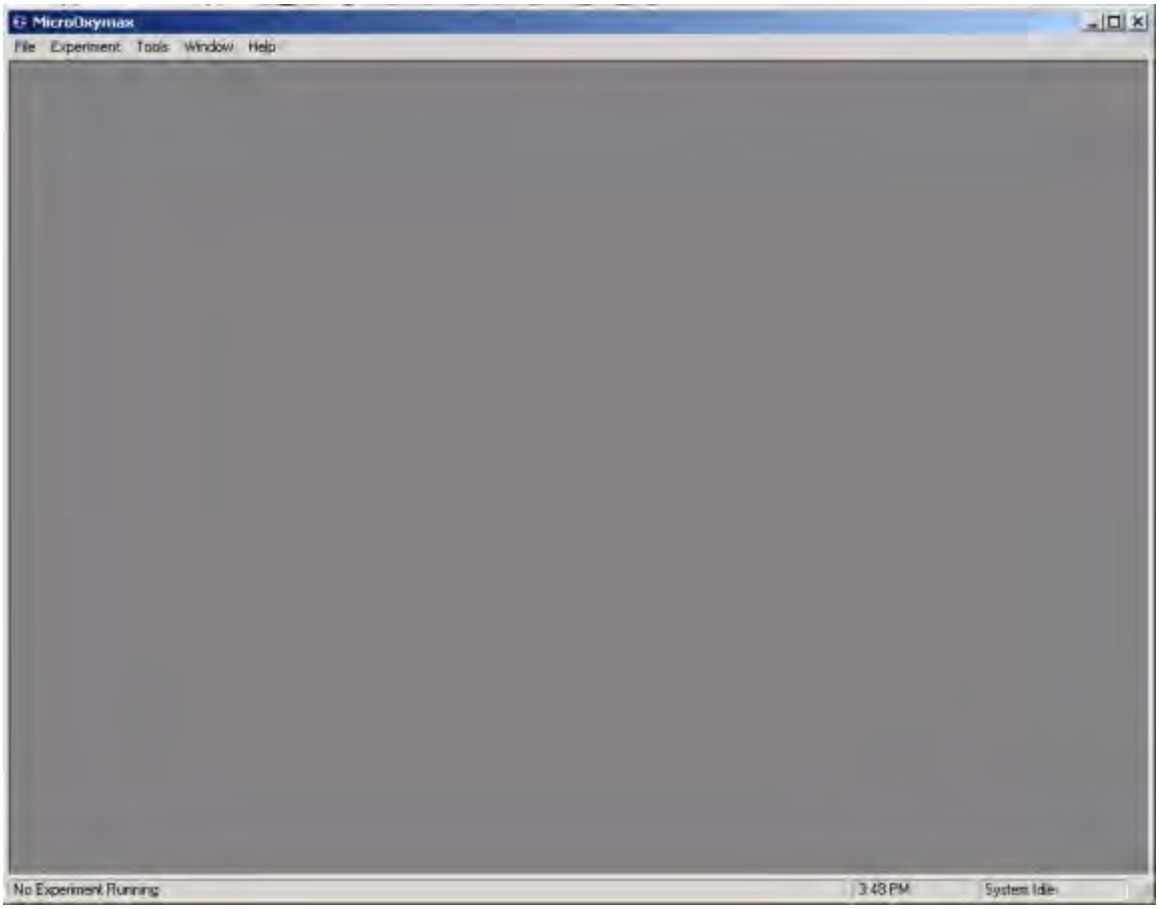


As the Software loads and the Micro Oxymax hardware is initialized this screen will be display. This screen contains the version number of the software

As the software loads the system will automatically search the computers communication ports for the Micro Oxymax hardware. If the hardware is not found then the user will be notified that the Device is not connected. The program will need to be restarted to reestablish the connection with the hardware.

2.2 Main Menus

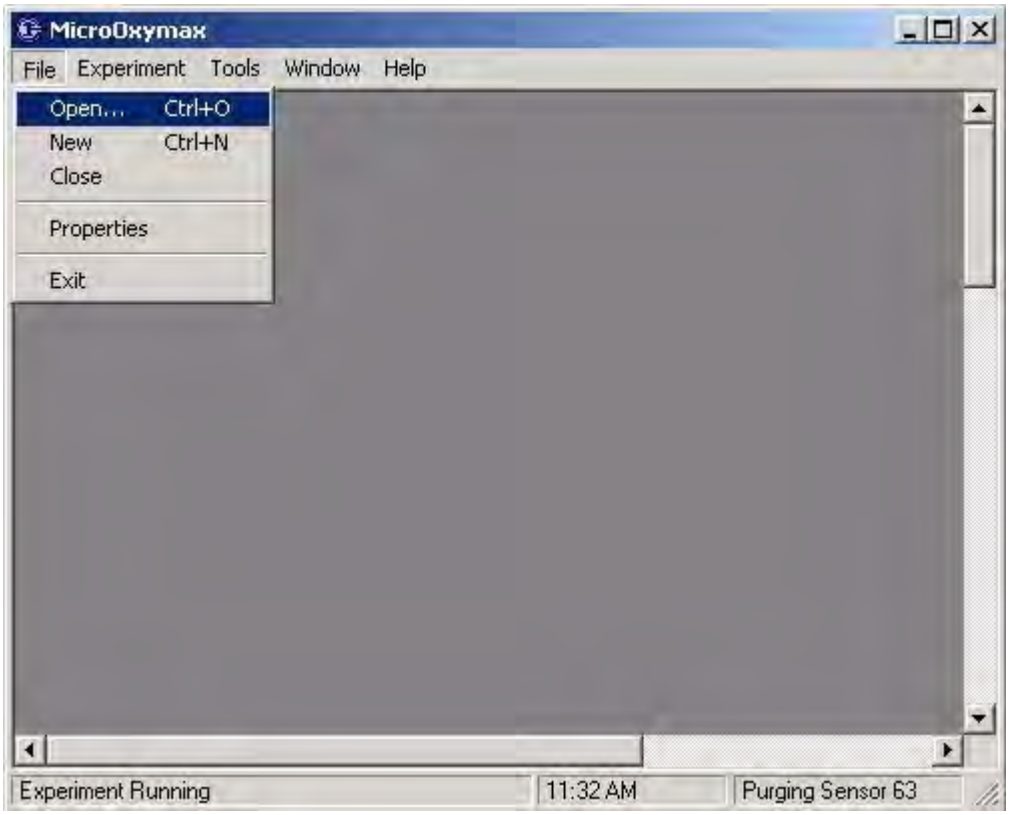
The main menu contains links to setup, view and run experiments, test system volumes and leakages, calibrate gas sensors and flow meters, as well as run diagnostics to test the system integrity.

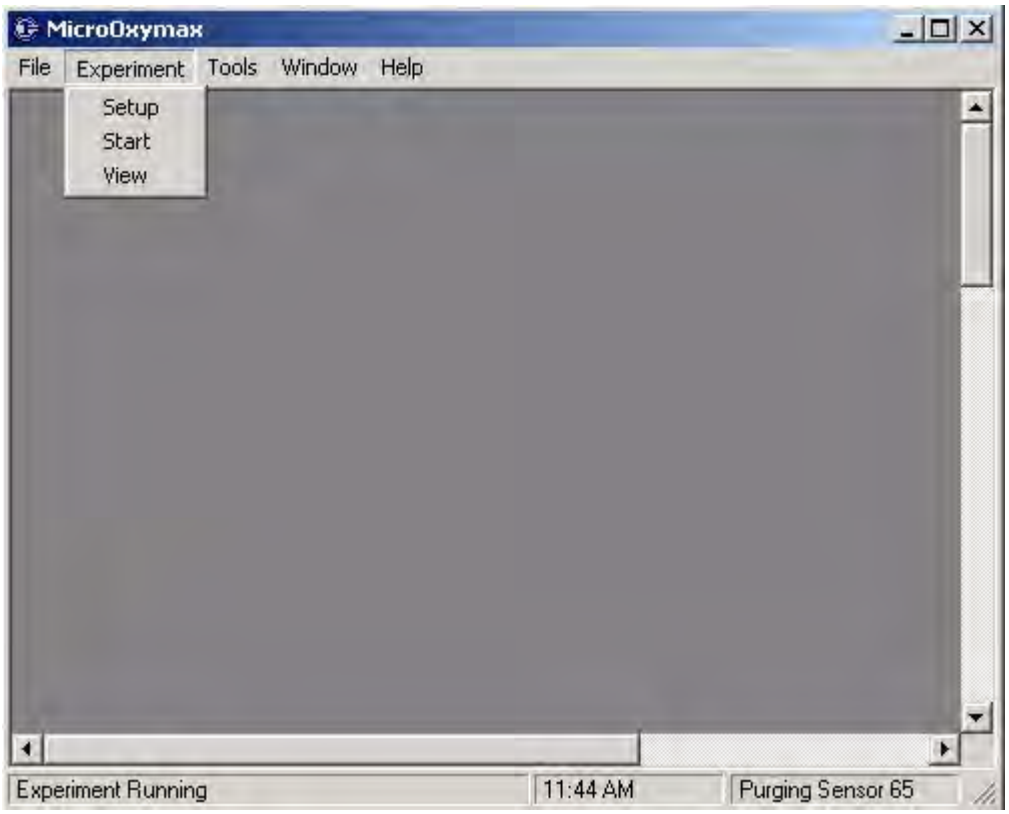


On the Bottom of the main menu a status bar is displayed. This status bar contains the current time, experiment status and status of the current task function being performed (i.e.. reading chamber, purging sensor, measuring volumes).

File Menu

The File menu allows functions to open existing files, configure new experiments, set the System Properties and Exit the Program.



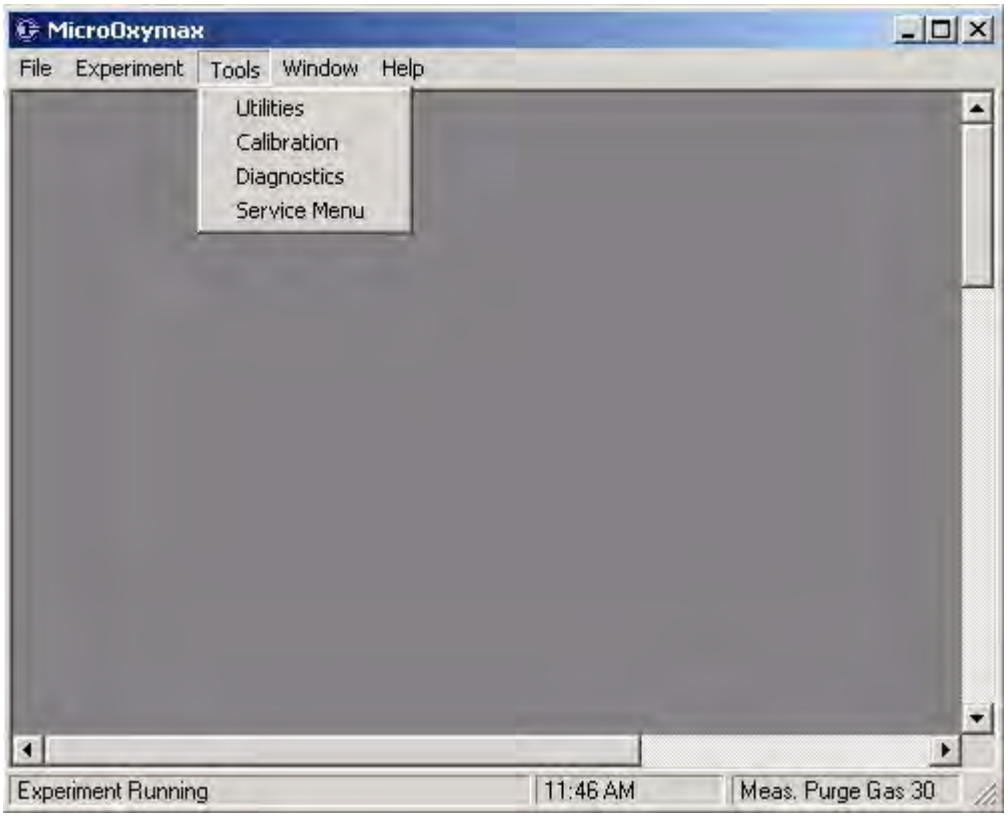


Experiment Menu

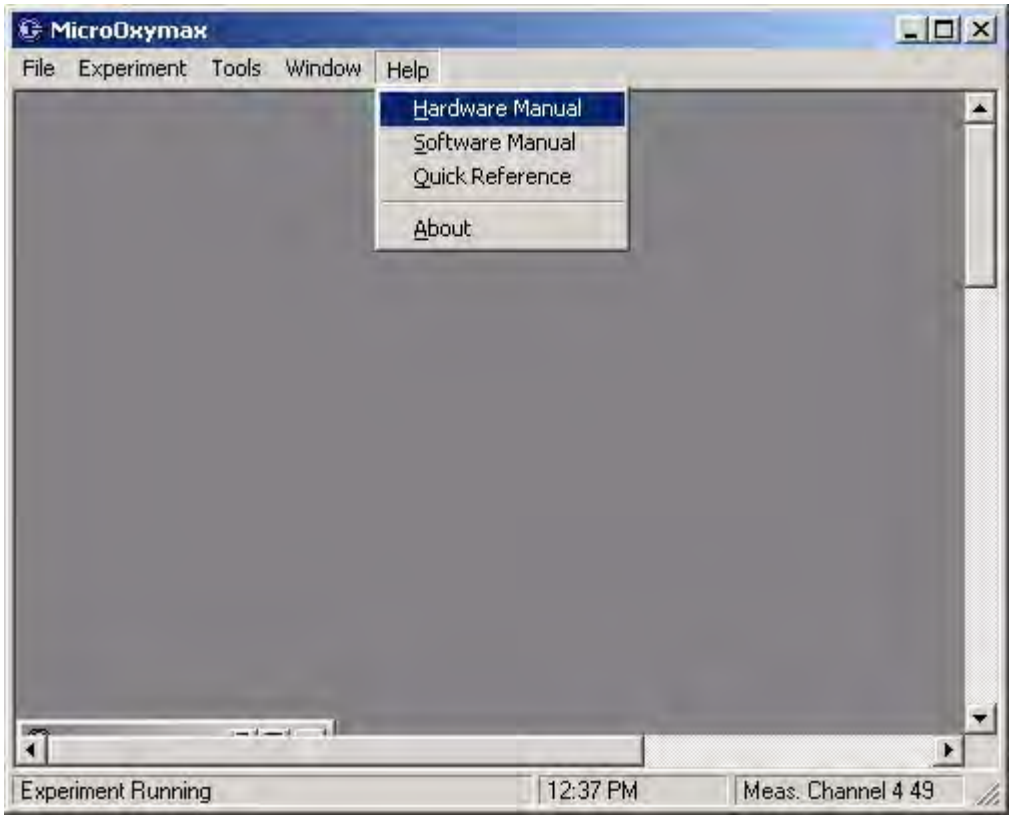
The Experiment menu is used to setup parameters for new experiments. A new experiment can also be started from this menu. Experiments that have been run previously can also be opened and viewed from this menu.

Tools Menu

The Tools menu contains utilities to measure chamber leakage, volumes ad restrictions. Extensive System Diagnostics used to test each component of the system can be run from this menu. Sensor calibration can be performed from this menu as well.



Help Menu
The Help Menu contains links to the Hardware, Software and Quick Reference manuals. This menu also contains a link to the About menu. The about menu contains important Software information such as version number and compatibility issues.



2.3 System Properties

The system properties contain variables that are specific to the Micro Oxymax hardware. Most of these settings are configured at the factory and can not be changed. Some values that pertain to calibration settings and volume settings can be set by the end user. The System Properties Window contain 6 Tabs. The tabs labeled Sensors ... contain information about the gas sensors installed with the system. The tab labeled SetPoints/Modes, contains variables that set the calibration modes, sensor pressures, and calibration bottles installed in the system. The tab labeled Volume Settings contains the volume setup for the entire system including sensors, expansion unit, and various shared volumes. The last tab is labeled Mass Flow Settings. this tab is used to setup the Mass flow meters if the Open flow option is present.

Setpoint**s Tab.** The sensors tab contains information used to configure the sensor present with the system. The Micro Oxymax windows software can support up to 6 sensors.

Sensor Type. Sensor type selects what type of sensor is installed in the system. This setting is configured at the factory when the instrument is purchased.

Offset Gas Concentration. If the sensor requires an offset calibration this setting will be enabled. Normally this setting is 0.00 for all infrared and toxic gas sensors. If the system is configured with an Electo-Chemical Oxygen Sensor the offset gas is not required for calibration of that sensor.

Offset Gas Source/Bottle. This is used to select which calibration gas bottle contains the offset gas. There can be a total of 6 different individual gas bottles. the Gas bottle setup can be found in the Set point/Modes tab.

Span Gas Concentration. The calibration gas is used to span the sensor. This value can be found by the composition analysis tag which is included with the calibration gas. The calibration gas concentration should be between 50 and 75 percent of the sensors range. Contact Columbus Instrument for calibration gas Specifications.

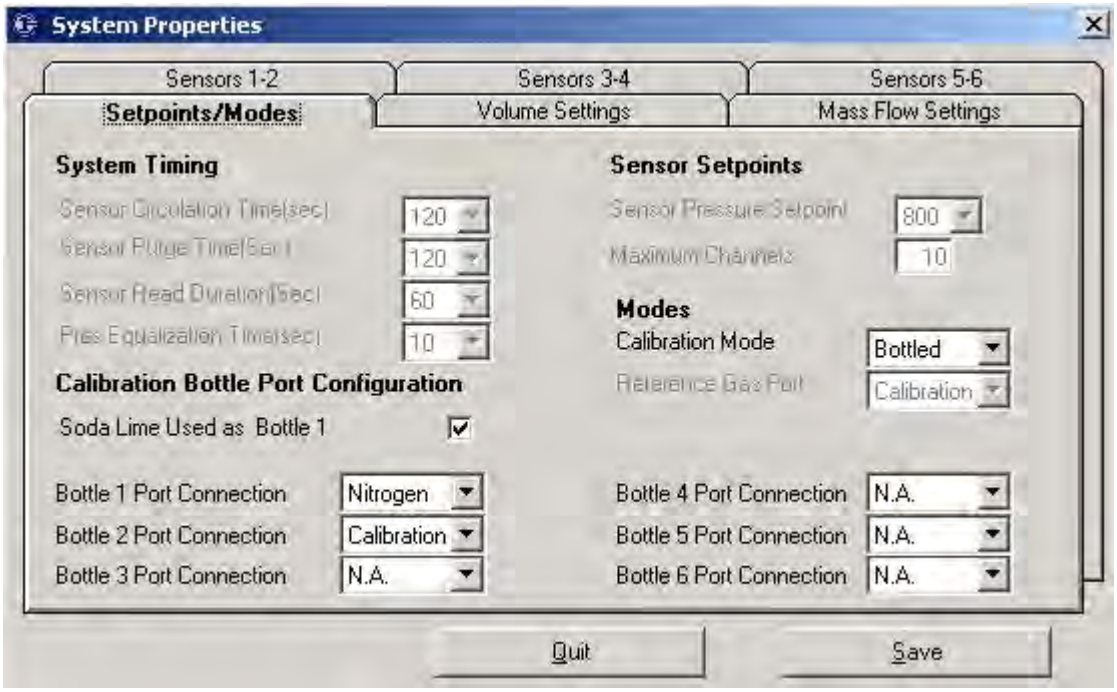
Calibration Gas Source/Bottle. This is used to select which calibration gas bottle contains the calibration gas. There can be a total of 6 different individual gas bottles. The gas bottle setup can be found in the Setpoint/Modes tab.

Sensor Offset. This setting is only used when there is an oxygen sensor installed with the instrument. This setting is factory set and can not be changed by the customer.

Sensor Gain. This setting is only used when there is an oxygen sensor installed with the instrument. This setting is factory set and can not be changed by the customer.

Sensor Cell Consumption. This setting is only used when there is an oxygen sensor installed with the instrument. This setting is factory set and can not be changed by the customer.

SetPoints/Modes
The Setpoints/Modes tab contains information about the systems timing and pressures, calibration modes, as well as calibration bottle setup.



System Timing. These values are used to set the amount of time the system circulates gases during purging, settling and purging of the system. These values are factory set and can not be changed.

Sensor Setpoints. The Sensor Pressure Setpoint is used to set the pressure that is maintained in the sensor during the measurement process. The sensor is pressurized approximately 50 mmhg above the local barometric pressure, to eliminate any pressure influence. The Maximum Channels determines how many channels is available in the hardware. Both of these values are factory set and can not be changed.

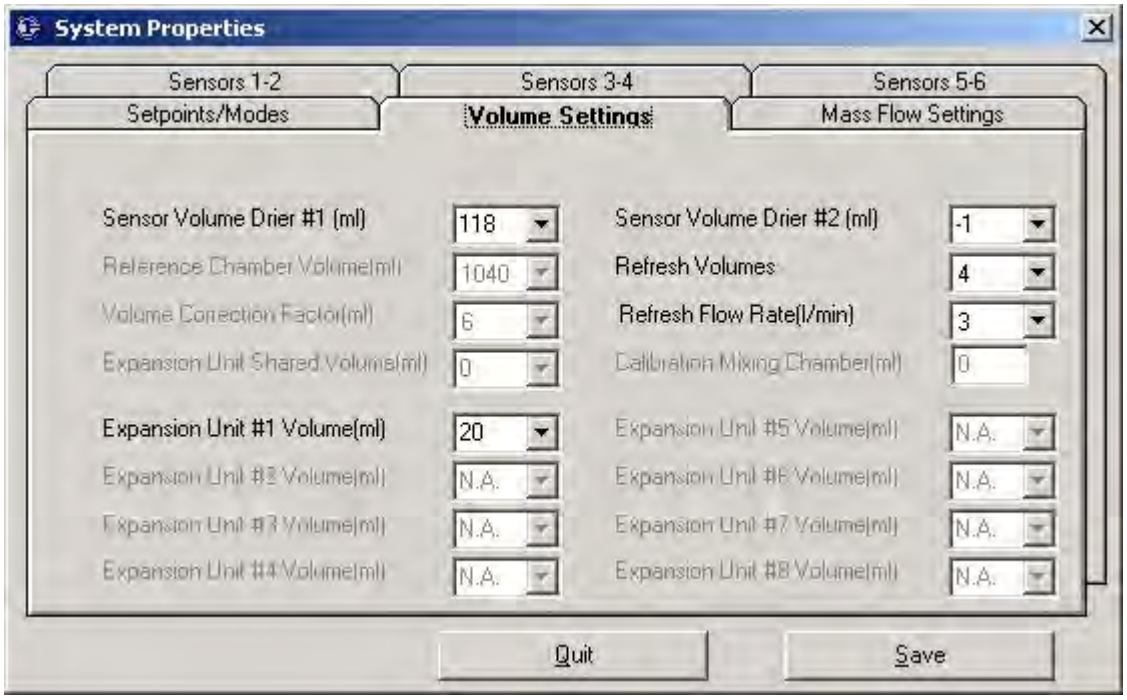
Modes. There are two types of calibration modes available with the Micro Oxymax. The standard calibration is the bottled method. the bottled method requires that a set of calibration bottles standards be purchased. The composition of these bottles depend upon the sensors installed with the system. The alternative calibration method can be used to calibrate CO2 sensors. This method involves mixing your own calibration gas by injecting a small amount of pure CO2 into a Mixing chamber. For a more detailed explanation of this method refer to the Section "Sensor Calibration" found later in this manual.

Calibration Bottle Port Configuration. The section is used to help configure the calibration process.

- Soda Lime Used as Bottle 1. For many of the infrared or toxic gas sensor the offset portion of the calibration procedure can be done with normal room air with the addition of a Soda Lime Column. This column removes CO2 that is in the stream of gas. If the soda lime column is used Calibration Bottle 1 is set to the Soda Lime Column.

- Bottle 1-6 Port Configuration. When the system is configured with 2 or more sensors multiple calibration bottles are needed to calibrate the system. These section enables the user to label which port is used for a specific calibration bottle.

Volume Settings
The Volume Setting tab contains variables that are used to setup the all the volumes associated with the instrument. The accuracy of these volumes are important since they are used in the calculations.



Sensor Volume Drier 1 and Drier 2. These volumes are used to set the internal sensor volume through drier 1 and drier 2. These volumes include all the internal volumes of the sensors, tubing and the drier itself. These volumes are automatically measured when ever the volume measurement utility is performed.

Refresh Volume. The refresh volume determines the amount of refresh air that passes through the chamber during a refresh. The factory setting for this value is 6. This indicates that there is a refresh 6 times the volume flows through the chamber.

Refresh Flow Rate. The Refresh flow rate is an estimate of the amount of refresh flow the system will use during a refresh. This value should be set to 3 lpm.

Reference Chamber Volume. The reference chamber is located inside the system sample pump. This chamber is used in volume measurements. When the system is running an experiment this chamber is also used to determine sensor drift. This volume is factory set and

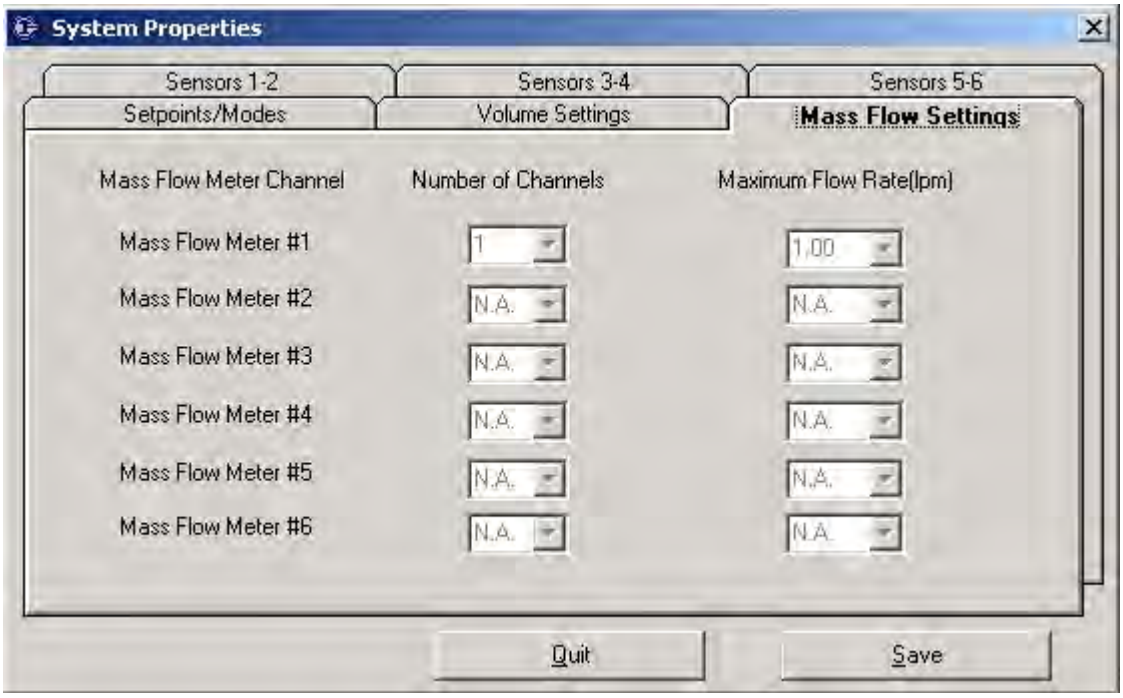
can not be changed.

Volume Correction Factor, This value is associated with the internal sampling pump. This volume is factory set and can not be changed.

Expansion Unit Shared Volume. For systems that contain less than 10 chambers this value is set to 0. For system larger than 10 chambers this value is the volume of the expansion manifold connecting the expansion units to the system sample pump. This value is factory set and can not be changed.

Expansion Unit 1-8 Volume. Each expansion unit has an internal volume associated with the multiplexing manifolds. This volume includes the internal volume for each individual expansion interface as well as the tubing connecting it to the system sample pump. This value is factory set and should not be changed unless the tubing that connects the expansion unit is changed in length.

Mass Flow Settings
If the Micro Oxymax is supplied with the Open Flow High Metabolic option this tab is used to setup the type of mass flow meter supplied with the system



Mass Flow Meter 1-6. The Micro Oxymax is capable of running with 6 different mass flow meters. Each mass flow meter can have one or more channels associated with it. In the case of

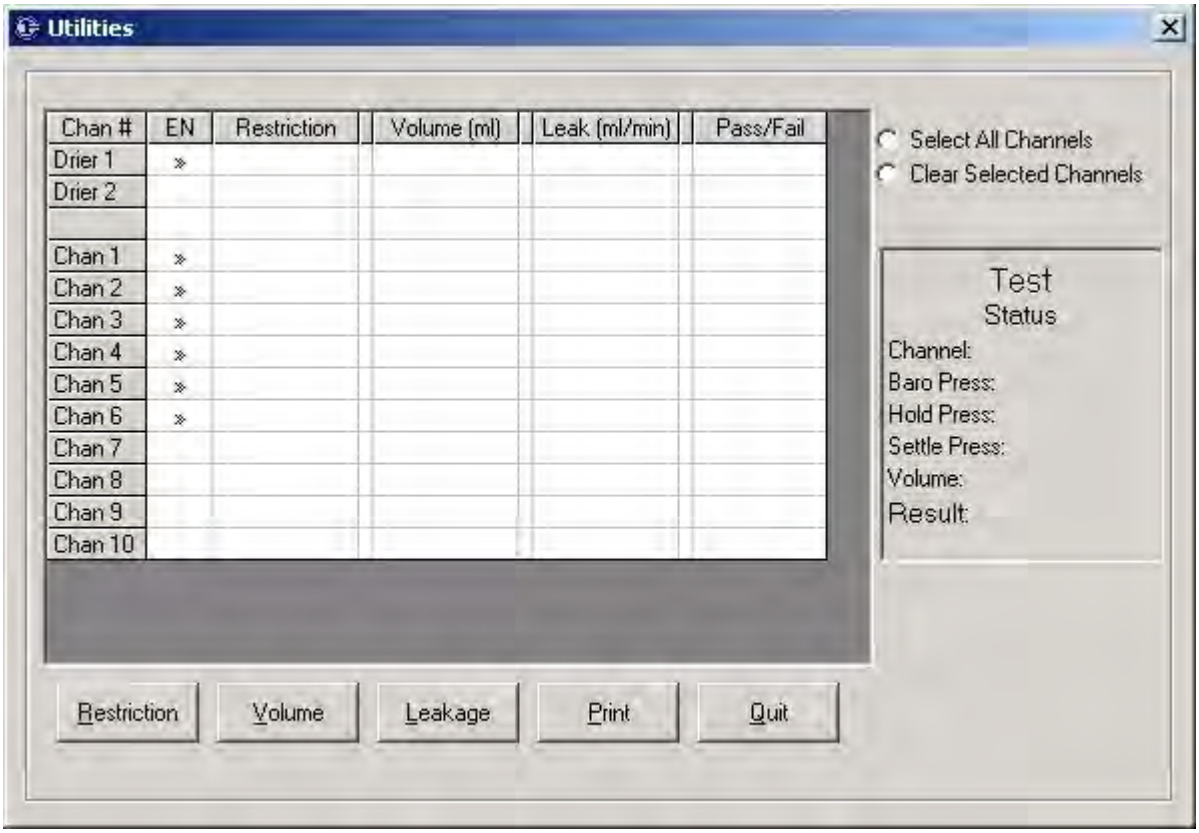
an Equal Flow unit the mass flow meter can have a total of 20 channels. This value is set at the factory and can not be changed.

2.4 System Tools

Once the system is properly configured the hardware will need to be tested to ensure that the system was installed properly. The system tools enables the user to calibrate sensors, measure volumes, leakages and restrictions as well as run an assortment of diagnostics used to test the entire systems functionality.

System Utilities

The Micro Oxymax measures volumetric gas changes in a closed reactor, therefore it is important that the system volumes are measured before each experiment. The system utilities enabled the user to measure sensor and chamber volumes. System leakage is also an important value to measure. To ensure accuracy of the measurements system leakage including the sensor and chambers need to be kept to a minimum. Excess sensor leakage of 0.3 ml/min could cause errors in the readings. Excessive leakage in the chamber is any value greater than 0.50 ml/min.



Volumes Measurement

Sensor Volume Measurement
The Sensor Volume must be known for accurate measurements. New systems only have a single connection to the sample drier and the sensor volume will not change. For older systems with glass columns filled with descant, there two possible sensor measurements: one using Drier #1 and one using Drier #2. If Drier #2 is not used there is no need to measure it. The sensor volume using Drier #1 must always be current. The volume is the total head space of the sensors, the external air drier column, and the tubing connecting the sensors to the System Sample Pump. If the lengths of the tubing connecting the sensors are changed, or the drier is replaced or the desiccant changed, the sensor volume must be re-measured.

Clicking on Volume will begin the volume measurement. Volume measurements should be made after the system has warmed up for at least an hour. When the sensors are measured, the value(s) will be displayed and the system configuration file will be updated with this new information. Repeat the volume measurement two to three times to verify that the readings are consistent (+/- 2 ml). Sensors should always be checked for leakage especially when the displayed volumes are inconsistent or out of acceptable range:

Small Drier (1 " (25 mm) diameter): Acceptable Range: 120- 180 ml
Large Drier (3 " (75 mm) diameter): Acceptable Range: 250 - 320 ml

Chamber Volume Measurement
The Chamber Volume must be known for accurate measurements. The volume is the total head space of the chamber and the tubing connecting the chamber to the Expansion Unit (or System Sample Pump if a single chamber system).
Clicking the Volume Button will begin the Volume Measurement. If an experiment was currently setup or the System Utilities was started from the Experiment Setup window, all the volumes will be measured that are setup in the experiment. The volume is measured to within 2% of the actual volume. This utility cannot be used for measuring volumes greater than 2 liters. Larger chambers must be manually calculated. Volume measurements should be made after the system has warmed up. If a test chamber contains a sample which gives off water vapor, wait 15 minutes after placing the sample in the chamber before making the measurements. If a chamber is placed in a different temperature (e.g. water bath) wait 15 minutes before making measurements.

If the system reports a measured volume which is obviously too high or low:
- A leak may be in the Test Chamber or Sensor.
- A leak may be in the tubing connecting the Test Chamber to the Expansion Unit.
- A Test Chamber is not sufficiently rigid to allow the volume measurement to work correctly.

If the chambers are not measured by the Micro -Oxymax system, the volumes can be manually calculated:
Head space = Empty Chamber Volume - Samples Volume + tubing volume

+ 1
Tubing Volume = 1 ml / foot for 1/8" tubing or 4 ml / foot for 1/4" tubing
Note: do not use tubing lengths longer than 10 feet when using 1/8" tubing.
Empty Chamber Volume can be measured by filling chamber with water and measuring the water. Note: be sure to dry the chamber and/or tubes before placing in an experiment.

Sensor Leakage Measurement

The Sensor Leakage measurement is used to indicate loose tubing and/or drier connections. Excessive leakage in the sensors will reduce the accuracy of measurements. Inconsistent or erratic volume measurements usually indicate sensor leakage. A normal value for the leakage is +/- 0.2 ml/min.

By clicking on the Leakage Button Leakage Measurement will begin. To verify leakage, measurements should be repeated. Sensor leakage is usually due to the drier not being completely closed or loose tubing connections connecting the sensors to the System Sample Pump. The second drier, if used, should also be tested for leakage. The result will be displayed on the screen as a positive or negative number. If the leak is excessive (> 0.2 ml/min for a positive leak or < -0.2 ml/min for a negative leak) the procedure below should be followed to locate it.

Positive Leak:

Leak Located on input side of the Sampling Pump (inside of System Sampling Pump)
Contact Columbus Instruments for assistance if a positive leak is detected.

Negative Leak:

Possible Locations of Leak:
In the Drier on the Front Panel
In one of the Gas Sensors
In the tubing or connections to the Gas Sensors
Output side of the Sampling Pump (inside of System Sampling Pump)

Procedure to find a leak (negative)

1. Replace drier with a piece of tubing
Repeat leak measurement (Check connections & O-ring seals)
If leak is fixed Drier is leaking, check seals
If leak remains goto Setp 2

2. Disconnect sensors from System Sample Pump (Sensor In & Sensor Out) connect a small (50ml) to the sensor in and sensor out connectors.
If leak is fixed then sensors are leaking goto Step 4
If leak remains goto Step 3

3. Leak is inside System Sample Pump.
Remove cover and inspect integrity of internal fittings and tubing.
Goto Step 5

4. Disconnect flask and connect each sensor
to the System Sample Pump one at a time.
if the leak remains then current sensor connected is leaking goto Step 5
if the leak is fixed current sensor is ok, try next sensor.

5. Contact Columbus Instruments

Chamber Leakage Measurement
The Chamber Leakage measurement is used to indicate loose tubing and/or drier connections. Excessive leakage in the Chamber will reduce the accuracy of measurements. Inconsistent or erratic volume measurements usually indicate Chamber leakage. A normal value for the leakage is +/- 0.5 ml/min.

By clicking on the Leakage Button Leakage Measurement will begin. To verify leakage, measurements should be repeated. Chamber leakage is usually due to the flask lid assemblye not being completely closed or loose tubing connections connecting the chamber to the Expansion Unit. The result will be displayed on the screen as a positive or negative number. If the leak is excessive (> 0.5 ml/min for a positive leak or < -0.5 ml/min for a negative leak) the procedure below should be followed to locate it.

Procedure to find a leak (negative)

1. Disconnect expansion unit from System Sample Pump.
Connect a small leak chamber between the "Test In" to "Test Out" ports located on the system sample pump.
If leak is fixed goto step 2
If leak remains then goto step 4

2. Reconnect expansion unit. 'Short Out' "Test In" and "Test Out" ports on front of Expansion Unit with 4-5" tubing loop.
If leak is fixed then leak is in the chameber or tubing connecting the chamber.
If leak remains then leak is inside expansion unit goto Step 4.

3. If leak is approximately the same for each chamber:
leak is probably in a tubing connection either inside the expansion box or between the System Sample pump and the expansion box.
If leak appears on only one of the channels:
then the quick connect fitting on the front of expansion box is probably leaking.
If leak is approximately the same for all chambers but one (chamber n):
then the leak is in the valve in the expansion box at chamber n.

4. See Sensor Leak Procedure - above, if leak is not in sensor: Goto 5.

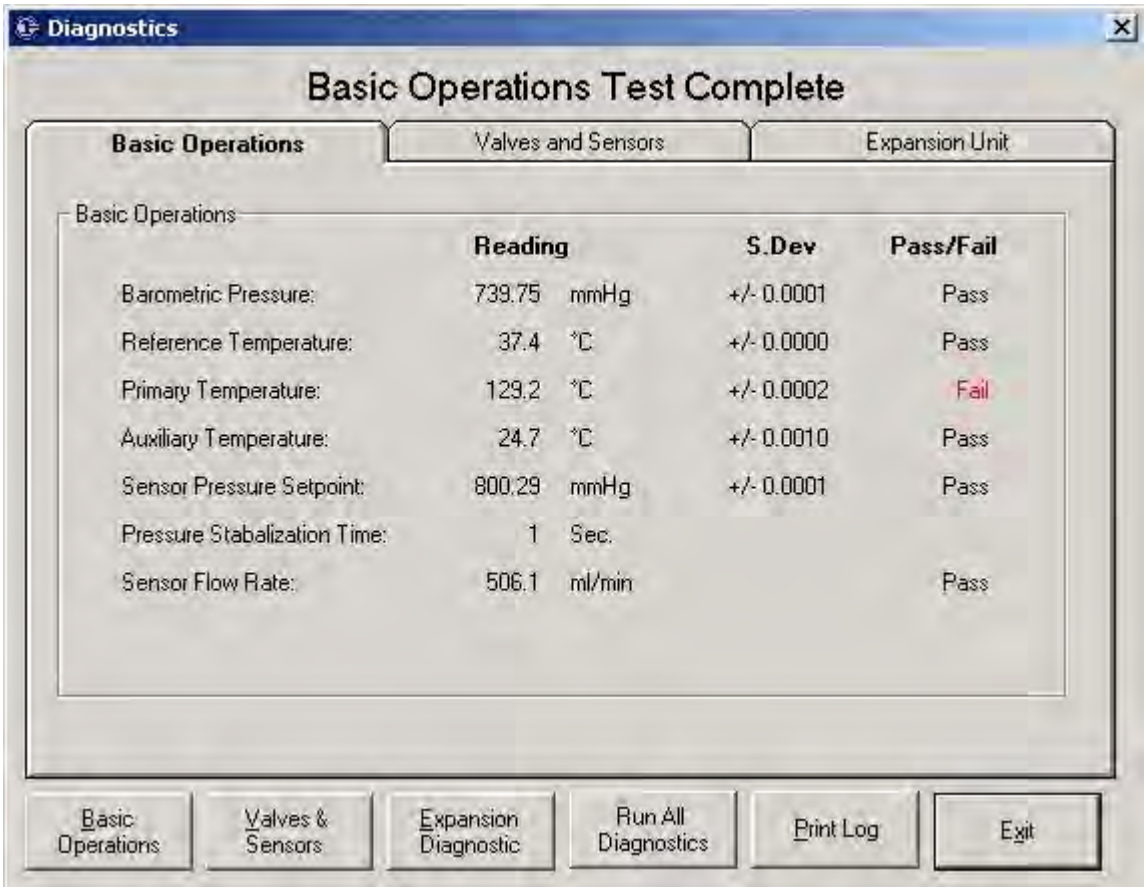
5. Contact Columbus Instruments.

Sensor Restriction Measurement.
The restriction through the sensor should be periodically measured to test the health of the drier assembly. High sensor restriction is caused by a drier with exhausted desiccant and or a drier filter clog. A restriction greater than 10.0 mmhg is consider excessive. An excessive restriction in the sensor can be fixed by replacing the descant and or replacing the filter located on the bottom of the drier assembly.

Chamber Restriction Measurement.
The restriction through the chamber connections should be measure periodically to prevent excessive pressure buildup during the measurement process. A restriction higher than 45 mmhg is considered excessive. A higher chamber restriction can be caused by a clogged Hydrophobic filter used in the sampling line. The connecting tubing between the expansion unit and the chamber can also cause high restriction if it exceeds 3 meters in length. Also a valve that does not open can cause high restriction.

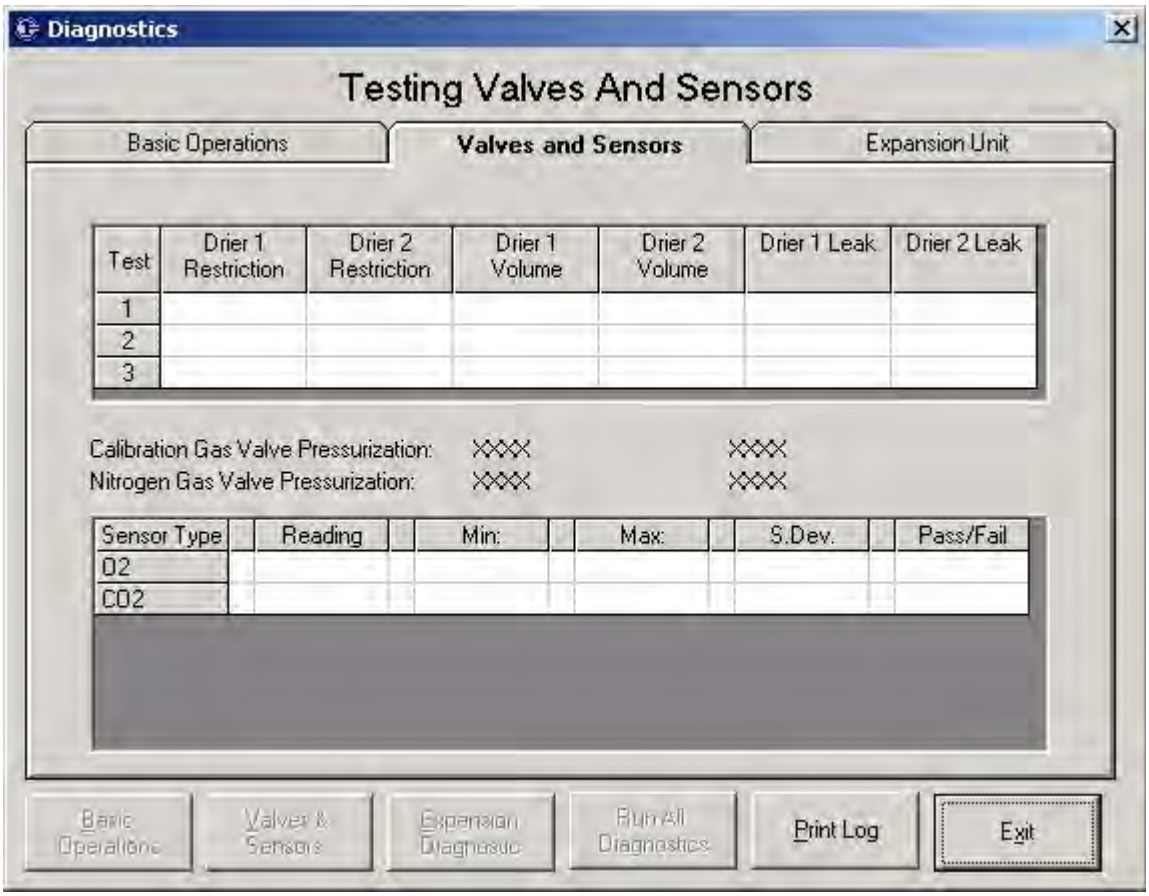
2.5 System Diagnostics

Basic Operations
The Basic Operations Diagnostics tests the general operation of the system temperature and pressure sensors. Each test is followed by a standard deviation and a PASS/FAIL statement. Once the Test has finished the user will be prompted to save the results to the system log which can be later sent to Columbus Instruments for analysis.



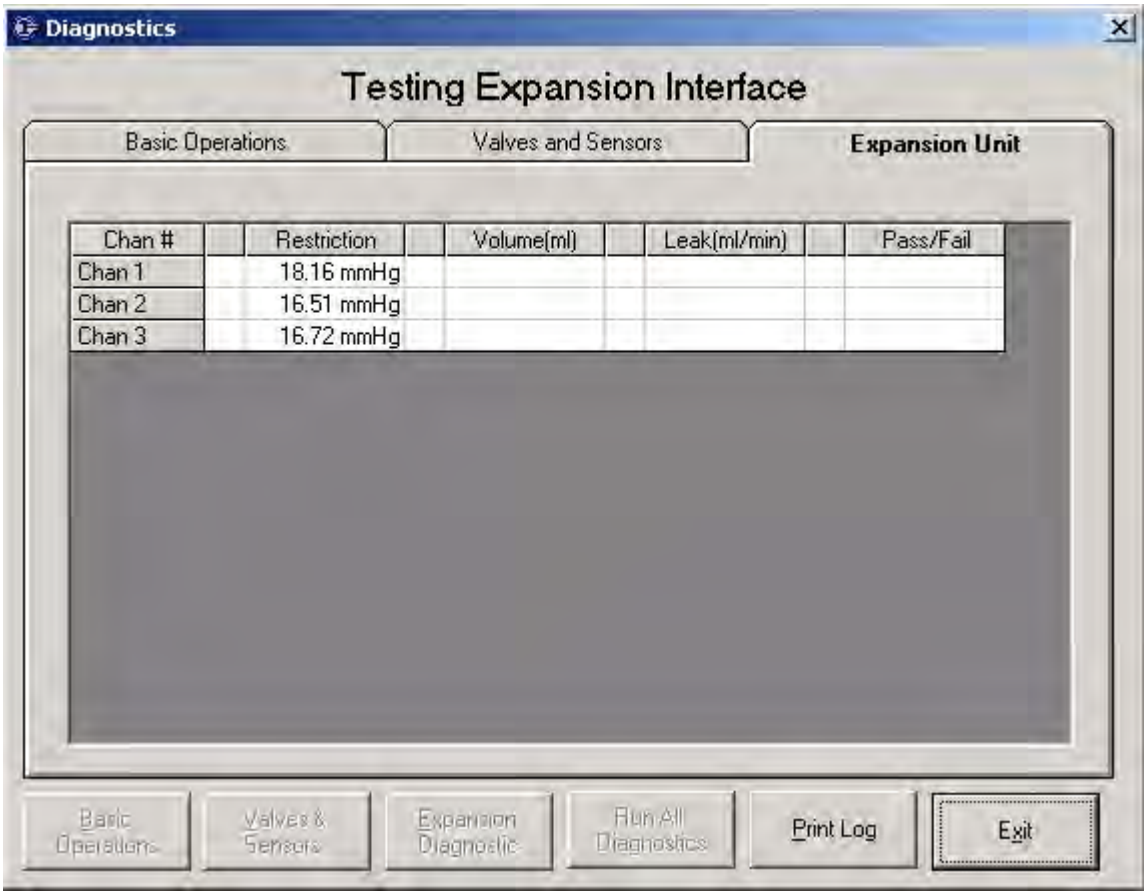
Valves and Sensors

The Valves and Sensor Diagnostics tests the sensors, restriction , leakage and volumes. Once these tests are performed the Calibration and Nitrogen Ports are checked for high restrictions. Finally the test performs a stability test for the gas sensor installed with the system. Each test is followed by a standard deviation and a PASS/FAIL statement. Once the test has finished the user will be prompted to save the results to the system log which can be later sent to Columbus Instruments for analysis.



Expansion Unit

The Expansion Unit Diagnostic tests integrity of each connector and valve in the expansion unit. Each chambers restriction, leakage and volume is measured to ensure proper operation. By replacing the chamber with a short piece of tubing the operation of the expansion unit can be tested.



Run All Diagnostics
By clicking on Run All Diagnostics the system will automatically run each diagnostics test and save the results in the System Log File. This function is helpful when running the diagnostics automatically without user intervention.

Print Log
Once the diagnostics tests have been completed the log file can be printed. When troubleshooting the Micro Oxymax hardware the Log file can be faxed to Columbus Instrument for analysis.

2.6 Calibration

The Micro -Oxymax requires periodic calibration of the gas sensors to insure precise measurements. The calibration procedure consists of the application of a gas of known composition and adjusting controls on the front of the Oxygen, Carbon Dioxide and any other gas sensors to obtain readings that reflect the contents of the calibration gas. It is recommended that the Micro -Oxymax system be calibrated prior to the start of each experiment.

Calibration Gas Specifications

The recommended calibration gas is termed "Primary Standard" by suppliers and is usually the most precise mixture available.
The cylinder of gas must be ordered from a local gas supplier. The supplier will include a certificate of analysis which indicates the actual concentrations of the bottle
All sensors require a 2 point calibration with a zero gas (gas containing none of the gas to be measured) and a span gas that is 80-90% of the full scale range of the sensor. There is an exception, which is the electrochemical O2 sensor which only requires a span calibration. Room air can even be used. All sensors except the electrochemical O2 sensor require an offset or zero calibration. All sensors (except the paramagnetic O2 sensor) can use air passed through a soda lime (calcium hydroxide) which is used to remove the atmospheric CO2 as a zero (offset) calibration

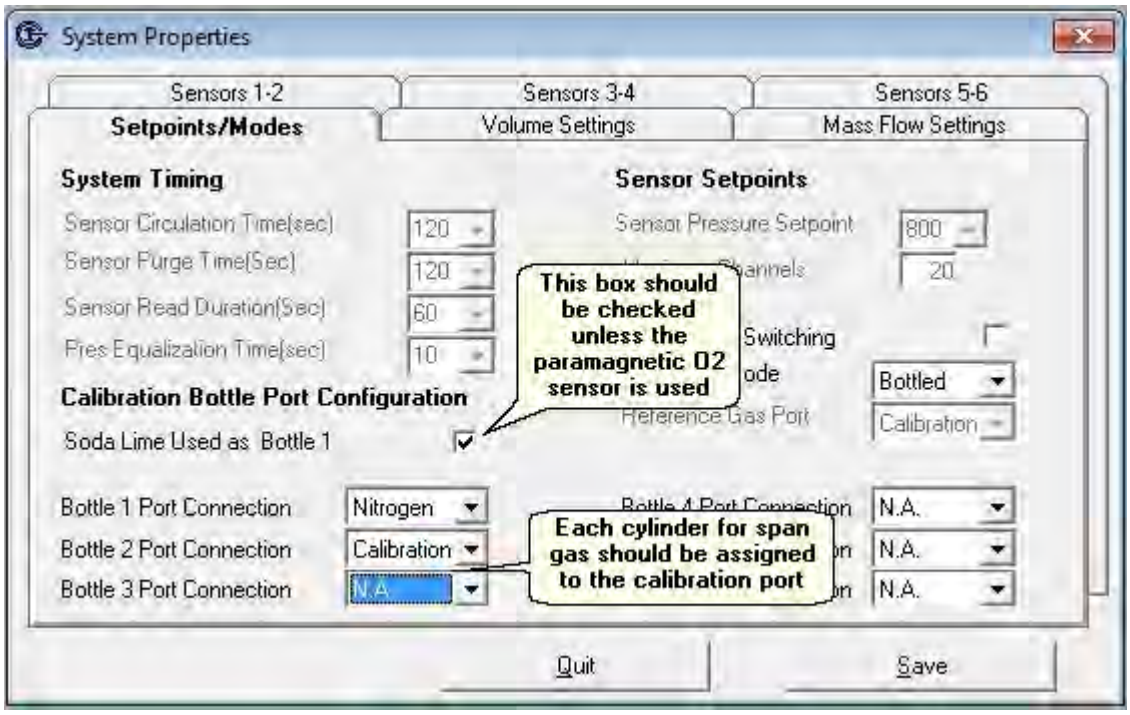
Table of primary standard calibration gas specifications

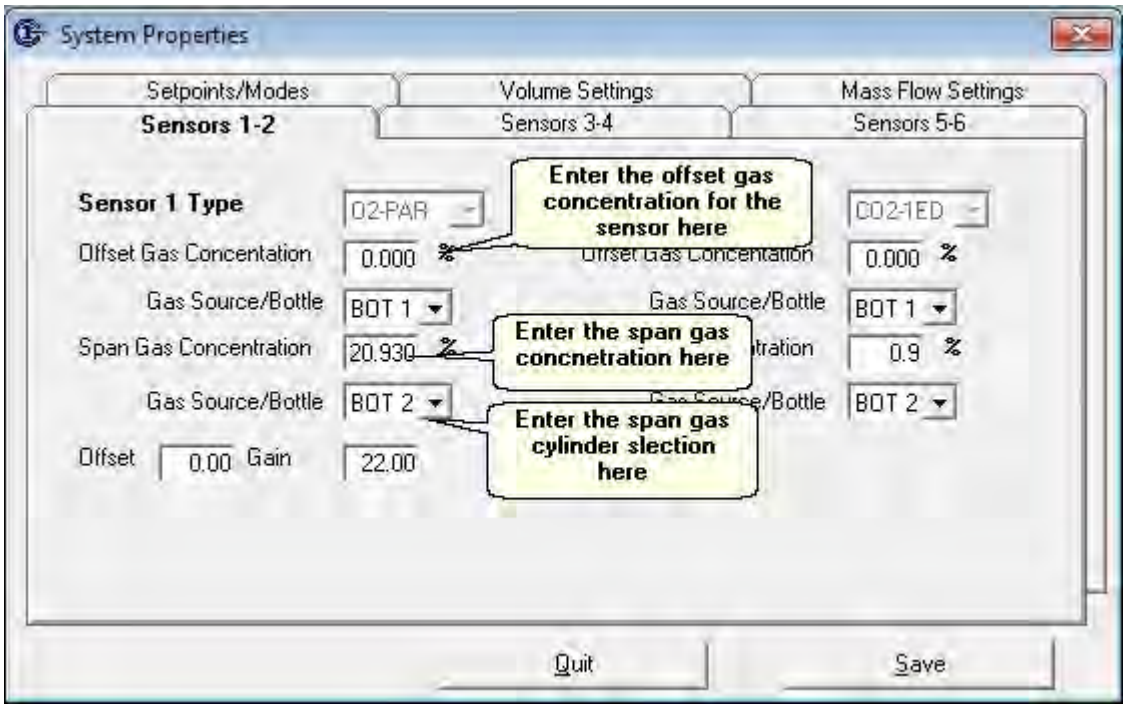
Concentration Range	Preparation tolerance	Certification accuracy
5-50%	+/- 1% of component	+/- 1% of component or +/- 0.02% absolute whichever is smaller
1-5%	+/- 1% of component	same as above
0-1%	+/- 5% of component	same as above

For sensors with a range of 100% gas, a bottle of the pure gas which the sensor measures can be used, but the adjustment on the screen must be 99.99% to ensure accuracy (this is because once the reading goes above 100%, it is not known how much above 100% the sensor is reading.

The value for the analyzed gas concentrations on the certificate of analysis must be used

When the gas cylinder is obtained the values on the certificate for each of the span gas (gases if a mixture containing more that one gas is used) must be entered into the software configuration (under System Properties, and the tab for each sensor).
To tell the system the concentrations of the gases, the configuration of the connection of the cylinder, and use of the soda lime column must be specified as well as the concentration of the span gases. This is accomplished by going to File - Properties menu





Some examples

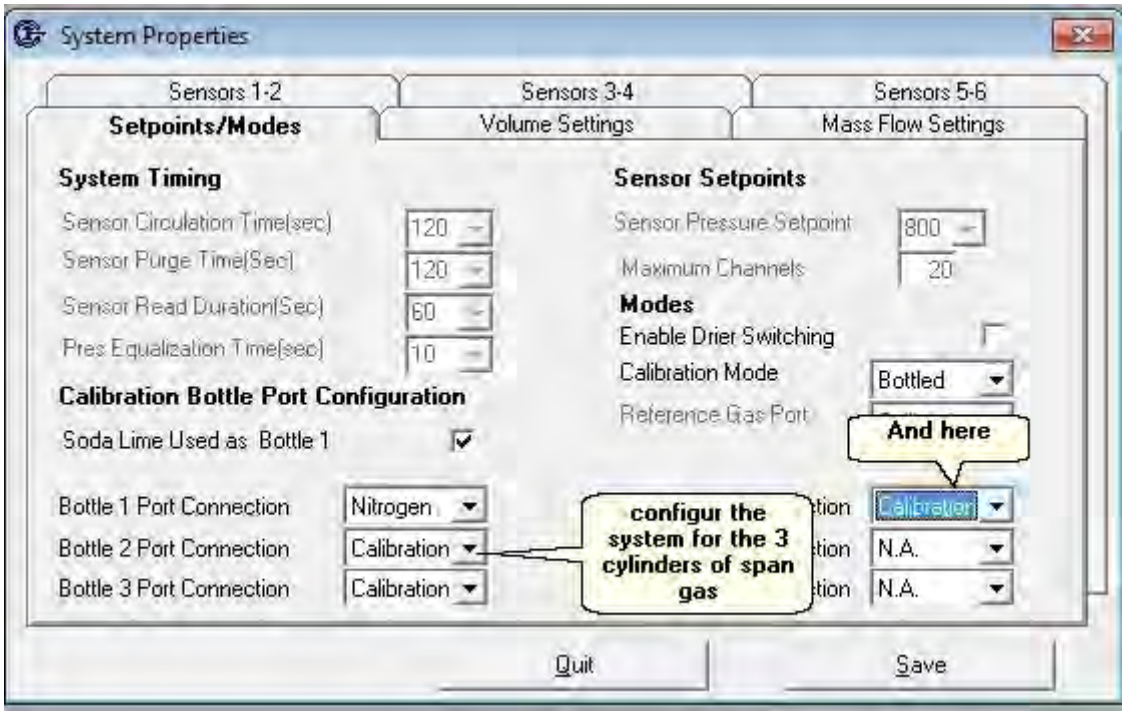
1 Typical system

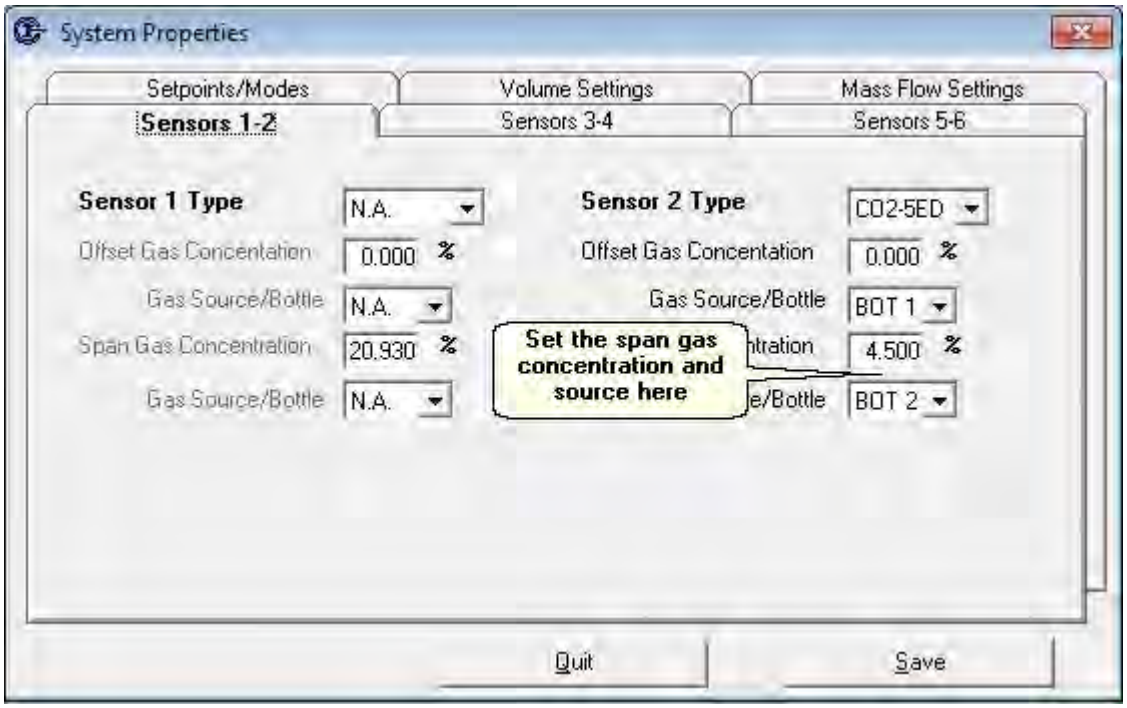
A system equipped with a standard electrochemical O2 sensor and 0-1% CO2 sensor. The

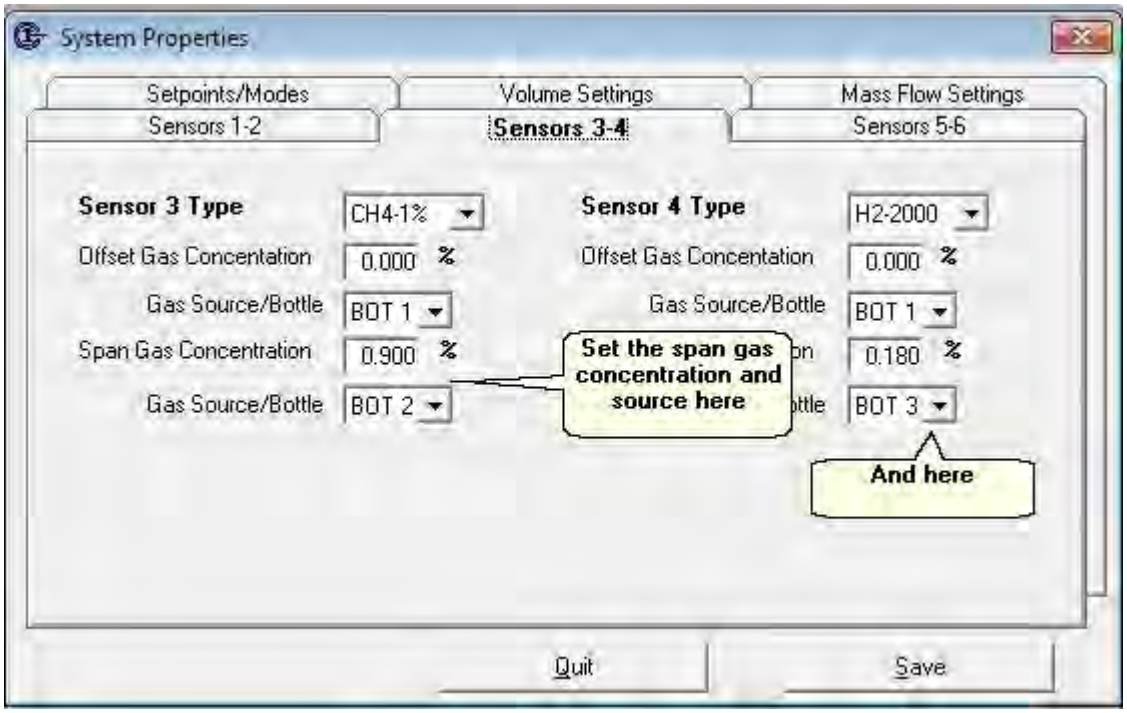
span gas for the CO2 sensor will be 0.9% (90% of the full scale 1%). Atmospheric air will be used for the span calibration. So in this example under sensor 1 - Offset - gas source/bottle is set to n/a because the electrochemical cell does not have a offset. The entry for sensor 1 span gas concentration is set to 20.93 because we are using room air, and the entry for span gas source/bottle will be 1 because the gas should come from the nitrogen port to remove the CO2 to give the correct concentration of O2. For sensor 2 the offset concentration will be 0.000 and the gas source/bottle will be 1, for span gas the concentration the value will be 0.9% (or what ever the value is on the certificate)

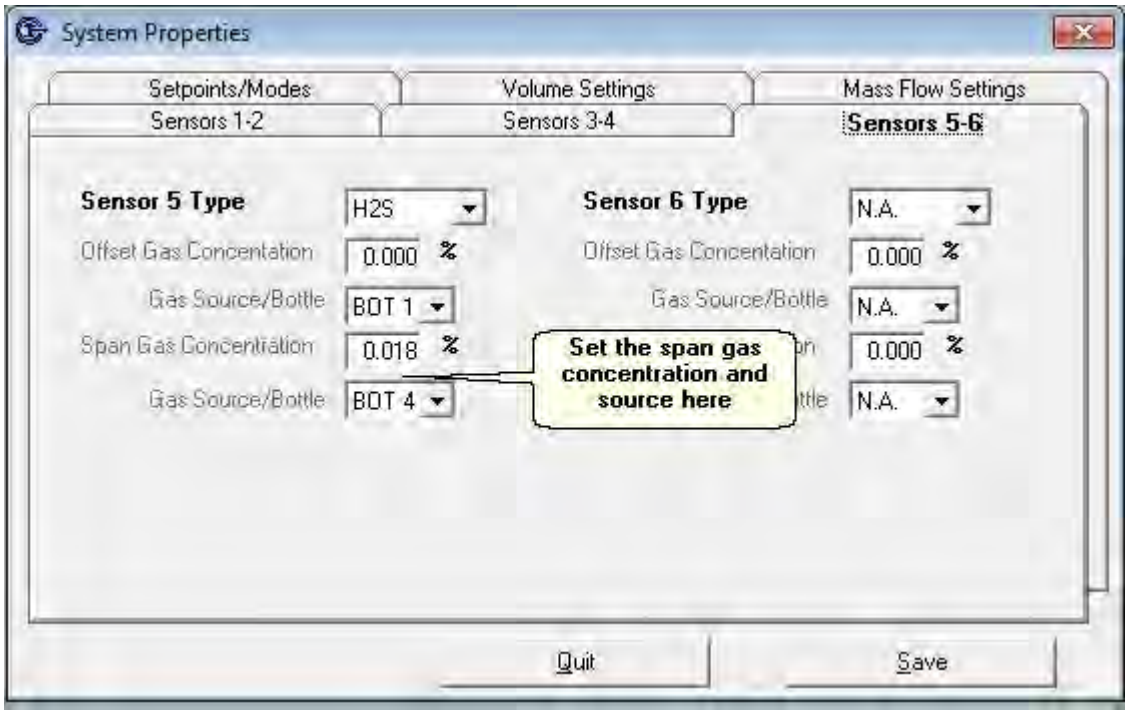
2 More complex system

In this example the system is equipped with a 5 % CO2 sensor, a 1% CH4 sensor, a 2000 ppm H2 sensor, and a 200 ppm H2S sensor. Lets say that the gas was supplied in 3 cylinders one containing 4.5% CO2 + 0.9 CH4, one containing 1800 ppm H2 and a third containing 180 ppm H2S. Below would be the correct configuration:

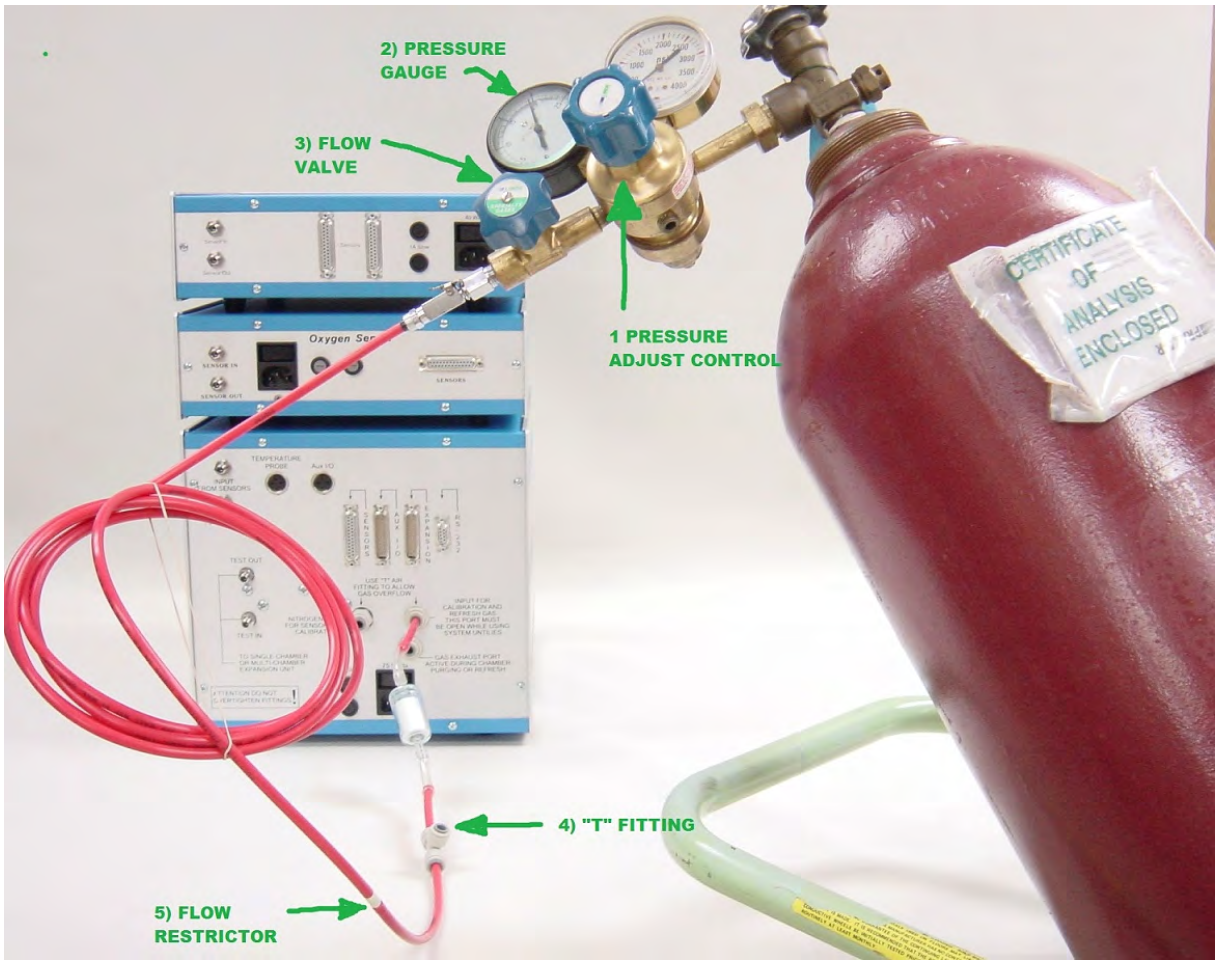








See the picture below for the connection of the span gas(s) cylinder(s)

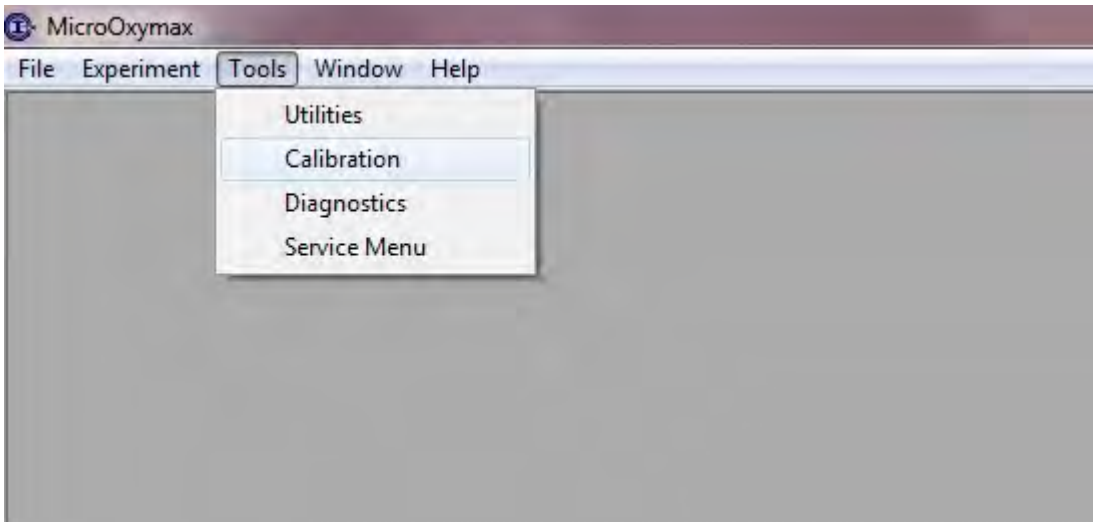


A dual stage pressure regulator must be connected to the cylinder. The pressure control knob (1) should be adjusted for a reading of 5 PSIG on the pressure gauge (2). Some regulators will have a flow control valve (show as number 3 in the picture). Make sure this is opened fully counterclockwise to allow flow of gas.
Use the calibration gas tube included with the system. It will have a restrictor inline with the tube (5 in the picture) to control the flow rate of gas. The tube is connected to the included "T" fitting. The other end of the "T" fitting is connected to the port labeled "Calibration" through the supplied filter.

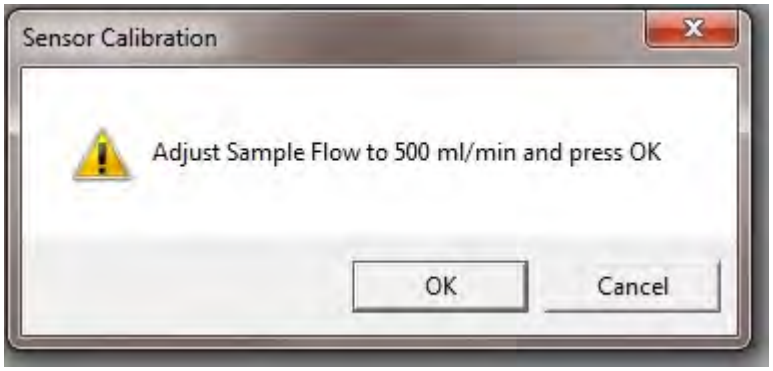
When the system is initially setup a column containing soda lime (white granules) should be connected to the port labeled "Nitrogen" The purpose of the soda lime column is to remove the atmospheric CO2 to provide a "zero" gas to the set the zero (offset) of the CO2 sensor. The connection is shown below: The soda lime column can remain connected all the time.

Please note that if the paramagnetic oxygen sensor is used in the range where the offset if 0, a bottle of nitrogen must be connected to the nitrogen port with a "T" fitting in the same was as the calibration gas cylinder.

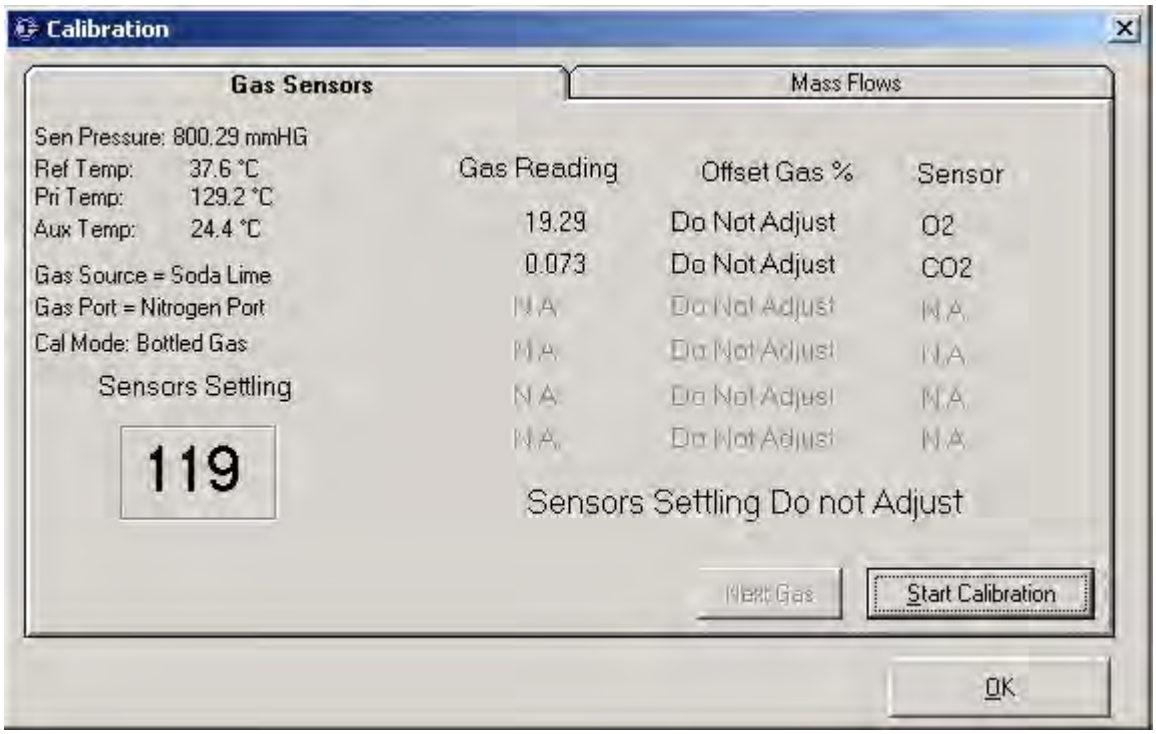
To start the calibration at the main menu of the software go to Tools - Calibration



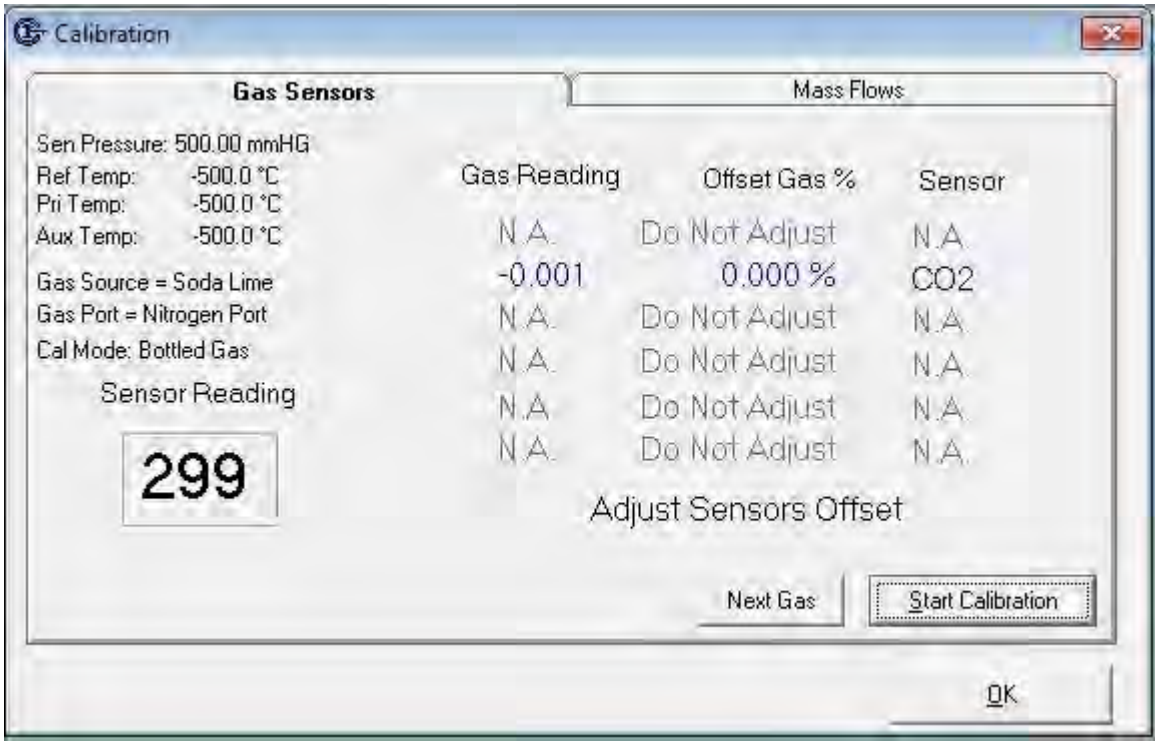
Click on the button labeled "start calibration" in the lower right corner. A window will appear prompting to adjust the sample flow to 0.5 l/min. At this point adjust the knob on the sample flow meter, on the front of the system sample pump for a reading of 0.5 l/min
Then click OK



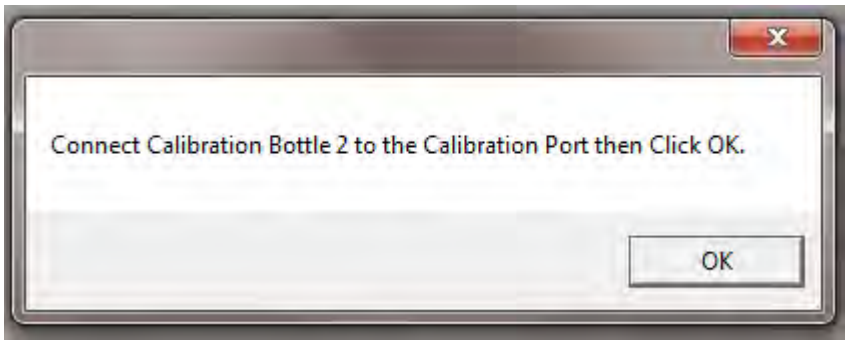
Then a timer will countdown to zero and during this time the gas is just allowed to circulate through the sensors.



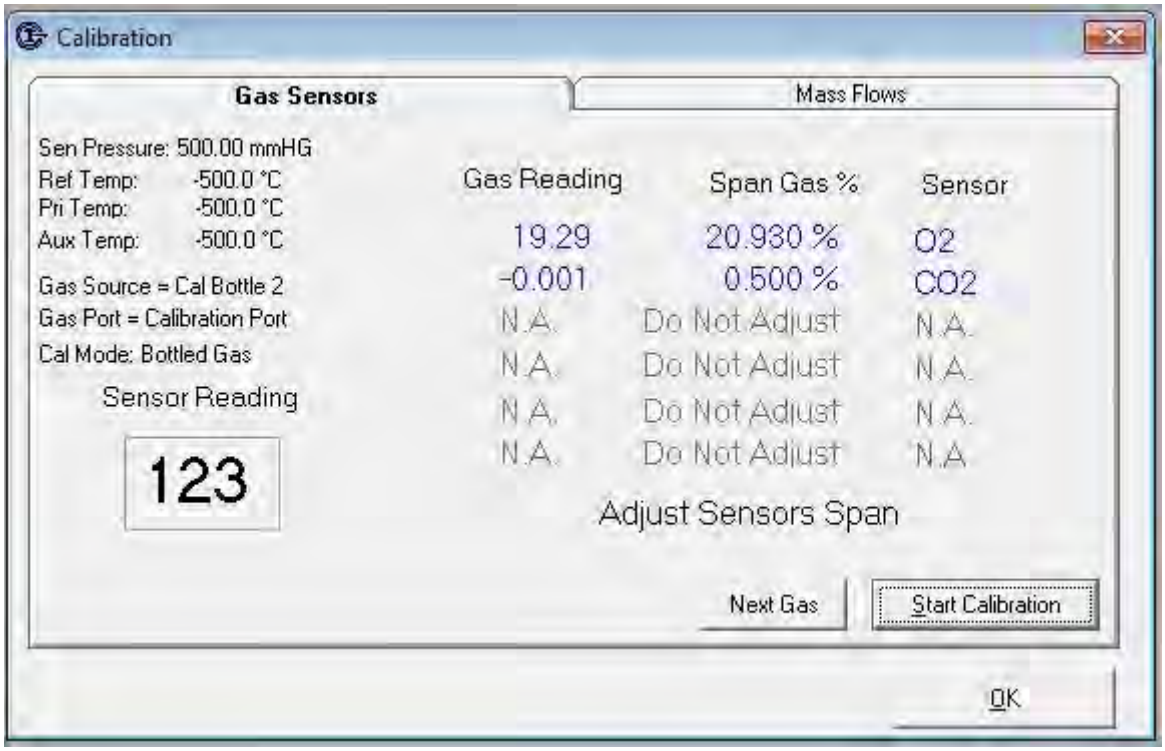
After the timer reaches zero, then the user will be prompted to adjust the zero knob on each sensor for a reading of 0 on the screen. The numbers will turn blue to indicate which sensor(s) should be adjusted at this point. The timer will start increasing after it reaches 0, just ignore this.



After the knob(s) has been adjusted, click on the button labeled "next gas" The software will then prompt you to connect the calibration cylinder to the calibration port. Ensure the bottle is connected as shown previously in this section then click ok.



Again the screen will start counting down to zero and then will prompt to adjust the span on the sensor colored in blue so the values in the gas reading column are equal to the values in the Span gas % column.
Adjust the knobs for the correct reading.

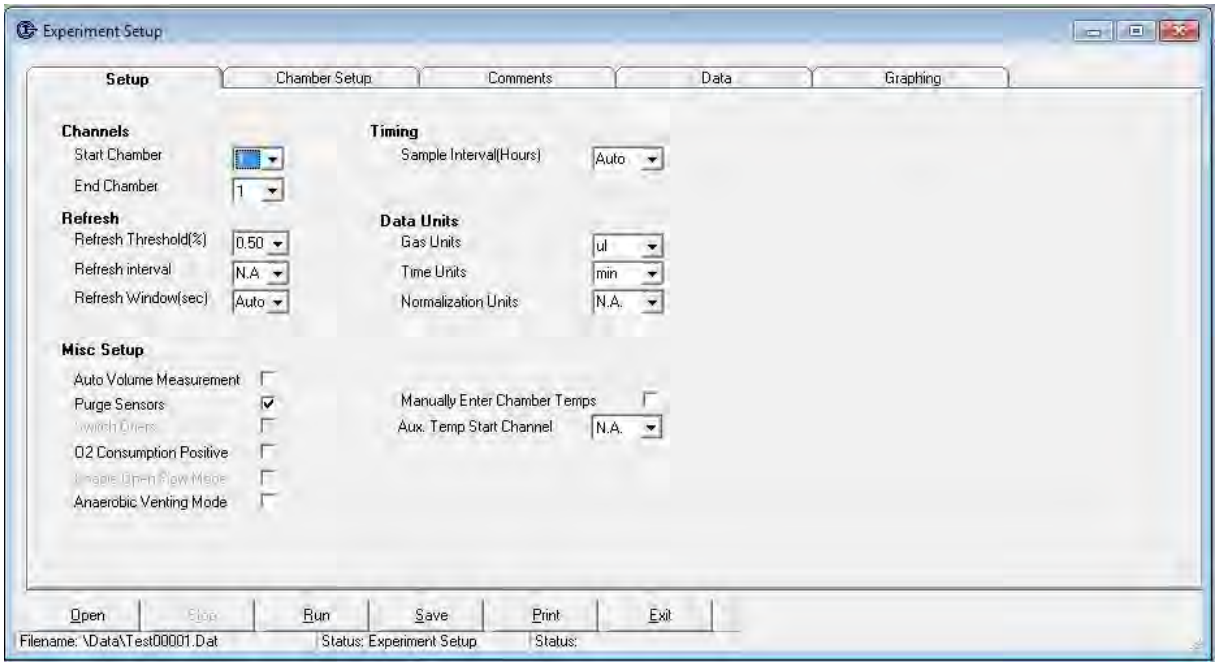


Press next gas and repeat the previous 2 step again for each cylinder (gas bottle) until all gas sensors have been calibrated. After the last cylinder the software will prompt you to turn off the calibration gas bottle.
Calibration is now complete

2.7 Experiment

The Experiment Menu enables the user to setup a new experiment to be run, and view previously run experiments. Only one experiment can be run with the current version of windows. Three previously ran experiment can be viewed at once.

Experiment Setup
To start a new experiment a few variables must be set as well as chamber volumes must be measured before an experiment can be started. When Experiment Setup is selected the user will be prompted to use the default template for the experiment setup. If the user selects the default template all necessary parameters will be set to the factory default. If the user selects not to use the default template for the experiment setup the user can select a previously run experiment.



Start Chamber and End Chamber. Any contiguous group of chambers can be selected for an experiment. The Start chamber is the smallest numbered chamber, the End chamber is the largest numbered chamber. The experiment will read from the Start chamber through the End chamber inclusive. An experiment with only one chamber has the End chamber number = Start chamber number.

Sample Interval. The sample interval is defined as the time period between measurements and is also known as the sample rate. The minimum interval time for a test is based on several variables: There is an "Auto" Function which will at the beginning of the experiment calculate the minimum time required to measure the selected chambers. The minimum time can be calculated as follows.

- With Purging Enabled

- Minimum Interval Time = (# Chambers used) x (6 minutes + Refresh Time) + 9 minutes
- With Purging Disabled
- Minimum Interval Time = (# Chambers used) x (3 minutes + Refresh Time) + 6 minutes

The use of a longer sample interval permits a more sensitive measurement, so one must make a trade-off between frequency of results and sensitivity of measurements. Sample Interval time should be selected based on the amount and type of activity in the subject chamber, and required sensitivity.

Duration of experiment. This is used to limit the length of the experiment. The default value of 0000 will allow the experiment to continue until manually terminated. To set the length of an experiment, enter the duration time between in hours.

Refresh Threshold and Intervals. The measuring chamber may require periodic refreshing since the respiratory activity of the sample can result in a fluctuation of gas - particularly O2 or CO2 levels in the measuring chamber. Refreshing circumvents this problem by replacing the air in the chamber. Fresh air is drawn in from the back port marked "Calibration and Refresh Gas" and circulated through the sensors. Refreshing may be triggered in one of two ways. First it may be triggered after a user selected number of sample intervals have passed. For example, if the sample interval is 15 minutes and the refresh interval is once every 8 sample intervals, refreshing will occur once every two hours. Second, it may be triggered when the gas levels crosses outside user- selected concentration thresholds. The thresholds are specified relative to the ambient gas levels observed at the last sensor purge or stabilization. The user defines a range around the this gas level, for example, +/-0.5%. So that if the gas concentration level rises 0.5% above its starting level or falls 0.5% below its starting level in any of the test chambers, a refresh cycle is triggered. The third way a chamber is triggered into a refresh is not user selectable, When the sensors exceed the normal operating range. To activate the refresh cycle by the number of samples, enter a number between 2 and 99 on the "Refresh Interval" entry. This value will specify the frequency of refreshing in terms of the number of samples taken before the system is refreshed. 0 disables this periodic refresh. To activate the refresh cycle when the sample exceeds a predetermined threshold, enter a number between 0.01 % and 9.99% on the "Refresh Threshold" entry. Otherwise, enter 0.00 to disable.
The experiment printout shows when a refresh cycle has occurred by printing the letter 'R' in the Status column of the data. This means that a refresh occurred immediately after the printed interval.

Refresh Window. When a refresh occurs due to any of the three reasons described above, the time spent to refresh is based on a number of variables: the size of the chamber, the number of volumes required to refresh, flow of the refresh pump and the time it takes to equalize the pressure afterwards.
The Refresh Window is used by the scheduler to calculate the maximum time to refresh a chamber. The Refresh Window must be long enough to refresh the largest chamber in the

experiment. If maximum speed is required, the Refresh Window can be set to 0 - but unpredictable results may occur if a refresh is required. There is an Auto Function which sets this window automatically based on the large chamber head space in the experiment and the refresh flow rate. The automatic calculation should be always used

Misc. Settings

Auto Volume Measurement. Once an experiment is ready to run, the head space above the sample in the chamber must be known. This head space measurement must either be made using one of several ways: previously measured using the System Utilities, known chamber volume + tubing volume - known liquid volume, or automatically measured in the experiment. Automatic measurement may not be desired if: the measurements are already known and entered at edit-time or the time taken to measure the experiment's chamber head space prohibits a successful scheduling of the experiment. Note: measuring the chamber head space before an experiment starts can help detect leakage's. Enter 'Y' to automatically measure the chamber head space. The default, 'N' disables this feature.

Purge Sensors. This setting is turned on throughout system operation. The purpose of purging the sensors is to eliminate the phenomenon of crosstalk between adjacent measuring channels. Crosstalk may be a problem if samples with high respiration rates are measured simultaneously with samples of low respiration rates and is especially troublesome when measuring liquid samples. Purging the sensors between measurements increases the minimum time required to make a measurement from 3 minutes per chamber to 6 minutes per chamber. A limitation to purging the sensors between measurements is that experiment results can tend to have a greater amount of variability if the sensors are purged with room air which varies unpredictably in composition when there is activity in the room. To eliminate this variability, outside or bottled air can be used to purge the sensors. This is done by running a 1/4" 0.D. plastic tube from the outside atmosphere to the back of the System Sample Pump. Connect the end of the tube to the port labeled refresh. A bottle of mixed gas may also be connected to the refresh port provided the pressure is regulated to less than 1 psi. The purging sensors option should be used with liquid samples due to the errors caused by transferred CO2 dissolving into solution. Another advantage of purging the sensors is that the small amount of fresh air in the sensors mixes with the chamber head space, and keeps the gas concentrations from going out of the sensors range, thereby reducing the need for chamber refreshing.

Switching Dryers. This parameter is for older Micro Oxymax systems that used two drier columns. When activated (i.e. setting the parameter to 'Y'), the drier used by the System Sample Pump will be alternated between drier #1 and drier #2. This switching occurs as the experiment becomes active to sample the chambers. Switching dryers requires more time during the interval to clear out trapped gases. Drier #1 is used as the default and will always be used when this selection is set to 'N'. If a long duration experiment is anticipated, drier switching allows the user to replace the inactive drier without disrupting the running experiment. Drier #1 is active when the chambers are initially read at the beginning of the experiment, Drier #2 is then activated when the chambers are read the next time (just before the 1st sample is displayed).
With the new sample drier the glass drying columns have been eliminated and also the feature to switch between 2 driers is no longer used

O2 Consumption Displayed As Positive. By default the oxygen consumption is displayed as a negative number (loss of Oxygen). If this is enabled oxygen consumption is represented as a positive number. In the Micro Oxymax all production of gases is shown as a positive number and the consumption of gases is shown as a negative number.

Enabled Open Flow Mode. If Open Flow High Metabolic hardware is present this will allow the user to set up chambers are open flow. The user can have both open and closed flow chambers running in the same experiment. A chamber is determined open flow if its flow meter is set to a number larger than Zero in the Chamber setup section.

Anaerobic venting mode. Selecting the Anaerobic venting mode causes the system to vent excess pressure in the chamber to the atmosphere after each measurement. In the normal aerobic mode of operation there is little pressure change in the chamber. In the anaerobic mode of operation there is a constant pressure increase in the reactor so the venting keeps the pressure from becoming to high in the chamber and causing problems with the measurement

Manually enter chamber temps. If more than 2 different temperatures are going to be used, the temperature of each channel can be typed into the chamber setup screen if this option is enabled

Starting Chamber for Aux Temperature probe. If an expansion interface is being used, a second temperature probed is included. This variable specifies which test chambers use the second temperature probe reading. The second temperature probe is sometimes useful if two groups of samples are being tested at different temperatures. To enable this capability, connect the group #1 test chambers to Start chamber through N-1 on the expansion interface, and connect the group #2 test chambers to channels N through the last channel. The channel number "N", which is the first channel that uses the Ax temperature probe, is entered on this line. If the second temperature probe is not being used, enter "0" on this line. The temperature readings from the first temperature probe (on the System Sample Pump cabinet) will appear on printout assigned to channels Start chamber through N. The temperature readings from the second temperature probe will appear on the printout assigned to channels N through the last channel.

Data Presentation

Gas Measurement Units. The oxygen consumption and carbon dioxide and other gas production rates can be calculated in various, user specified measurement units. The quantity of a gas can be measured in micro liters (ul), milliliters (ml), milligrams (mg), micrograms (ug) or micro moles (uM). Enter a number between 1 and 5 to select the measurement units.

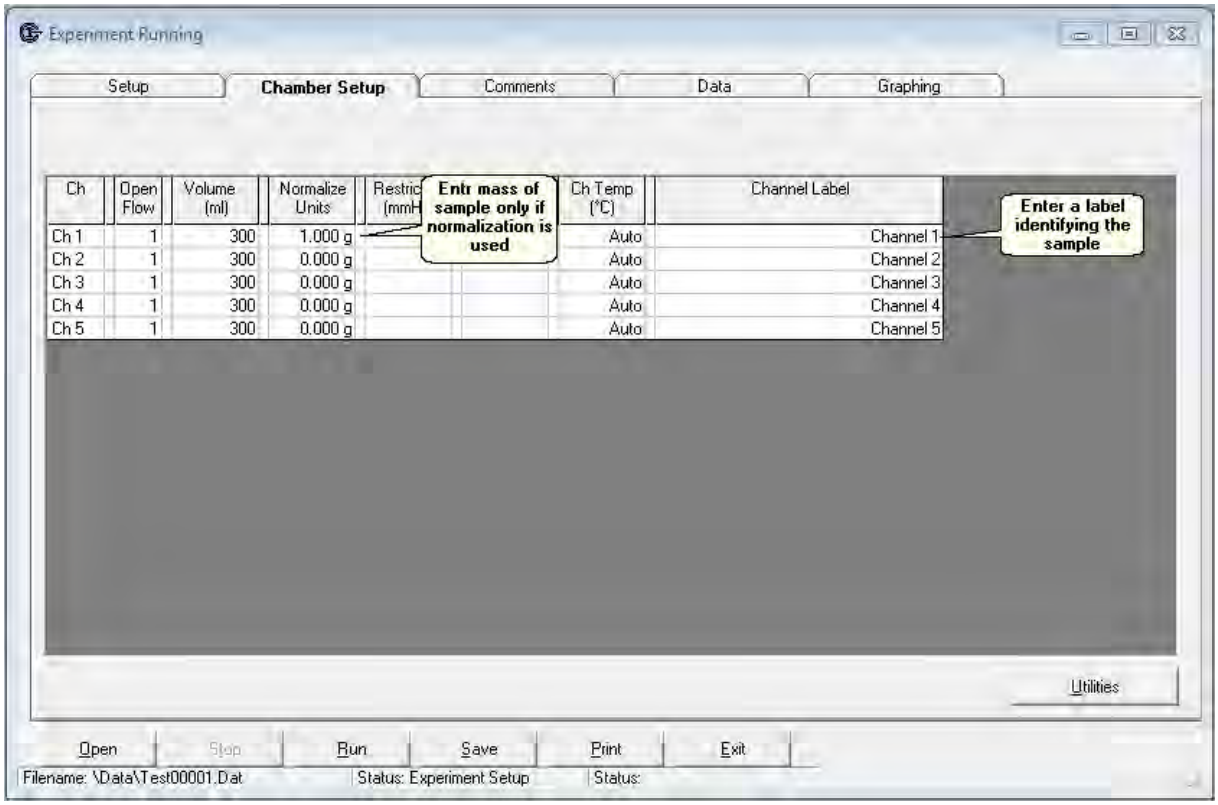
Time Measurement Units. The consumption or production rate can be measured on a per minute or a per hour basis. To specify a time unit of minutes or hours, enter a 1 or 2 respectively.

Normalization Units. By correcting for differences in sample size, the program can normalize the results. This allows the oxygen consumption and carbon dioxide and other gas

productions of a sample to be calculated on a per unit mass or a per unit volume of sample. To activate this feature, select the units in which the samples are to be normalized and enter the Sample Size on the Chamber Setup Page.

Chamber Setup

The chamber setup tab contains information relevant to the selected chambers in the experiment. Chamber volumes, leakage's, restrictions, normalization size and chamber labels. The system Utilities can be run from this tab in order to measure chamber volume, leakage and restrictions.



Open Flow. This parameter will only be displayed if the system is equipped with the open flow option. The default mode of the chamber being sampled is in a Closed Circuit system - 0 (N.A.) indicates this mode. if the open flow option is installed the number 1 is usually used to enable the option on specified channels. Some systems will have more than one flow controller (equal flow box) and each box will be numbered. For example a 20 channel system could include 2 - 10 channel equal flow boxes so channels 1-10 would be configured as open flow 1 and channels 11-20 would be configured as open flow 2.

Chamber Volume. Enter the head space volumes of all active test chambers in milliliters. The test chamber head space only includes the air space in the chamber, and does not include the volume of any sample. The chamber head space also includes the volume of the tubing

connecting the chamber to the Expansion Interface. If the volume of the test chamber head space is unknown, the automatic volume measurement capability may be used to measure it or it may be measured separately by clicking on the Utilities Button.

Chamber Leakage. The chamber leakage is not needed to perform calculations during an experiment, however it is good practice to ensure that there is not a leak present in the chamber before the experiment runs. This data will be saved with the data file when the experiment begins.

Chamber Restriction. The chamber restrictions is not needed to perform calculations during an experiment, however it is good practice to ensure that the chamber restriction is within specifications before the experiment runs. This data will be saved with the data file when the experiment begins.

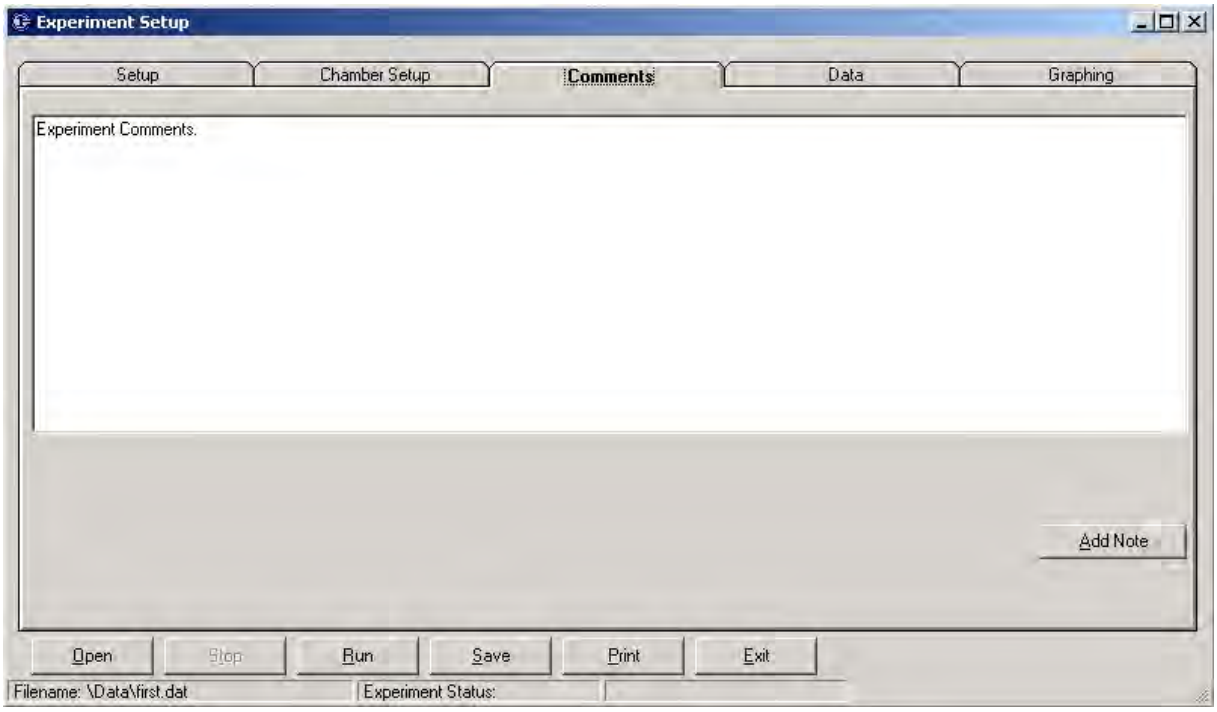
Normalization Size. Activating the Normalize Results feature requires that each sample size be entered in the second column. The size of the sample is measured in the units specified on the first page of the experiment parameters. The normalized oxygen consumption rate and carbon dioxide production rate measured for a particular sample are calculated by dividing the total consumption and production readings by the size of the sample.

CH temp.

Chamber Label or Comment. Enter a short description of sample to help in documenting the experiment. This data is saved with the experiment data when the experiment begins.

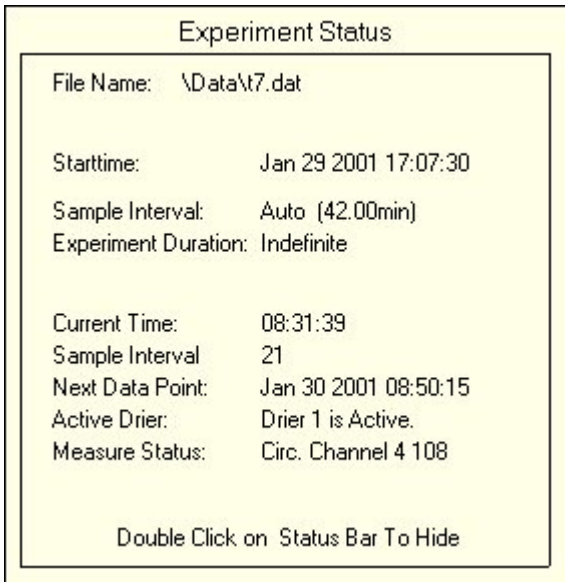
Chamber Comments

The third tab of the Experiment Setup Menu allows the user to enter text to comment the experiment. This page allows the user to type in text to document the experiment.



Experiment Data
Experiment data can be viewed while the experiment is running or after that data has been collected. The Data is presented in both statistical and graphical forms. In the statistical data window the data is scrolled down the screen as the experiment progress. There are scroll bars located on the right of the data grid allowing the user to scroll through the data. If the data is viewed while an experiment is running the Experiment Status Window will be present.

Experiment Status Window
The Experiment Status Window is displayed while an experiment is running. This window can be hidden by double clicking on status bar located on the bottom of the Experiment Window. The Status Window contains the data file name and path, the time the experiment started, the sample interval, experiment duration, sample interval, time until next data point, current active drier and measuring status.



Statistical Data
The statistical data tab displays the data collected since the beginning of the experiment.

Int Column. The interval is displayed in this column. As the experiment progresses the intervals will increase throughout the duration of the experiment.

Ch Column. The chamber number associated with the data is displayed in this column.

Time Column. This column displays the time stamp when the data is collected. This time can be presented in hours since the beginning of the experiment or it can be represented in date format. The format of this column is changed by clicking on the time column.

Ch_Temp. The column displays the temperature of the chamber during that measurement cycle.

RQ(RER). This column displays the Respiration Exchange Ratio. This is the Delta CO2 divided by the Delta O2. This value is helpful when measuring the metabolic rate of small animals.

Gas %. This column is used to display the actual gas concentration in the chamber during the measurement cycle. This value can not be used to manually calculate the rate. The actual rate is calculated by a modified gas concentration corrected for Sensor mixing and drift.

Gas Rate. This column displays the rate of gas production or consumption. A consumption of gas is presented as a negative rate, while a production of gas is displayed as a positive number.

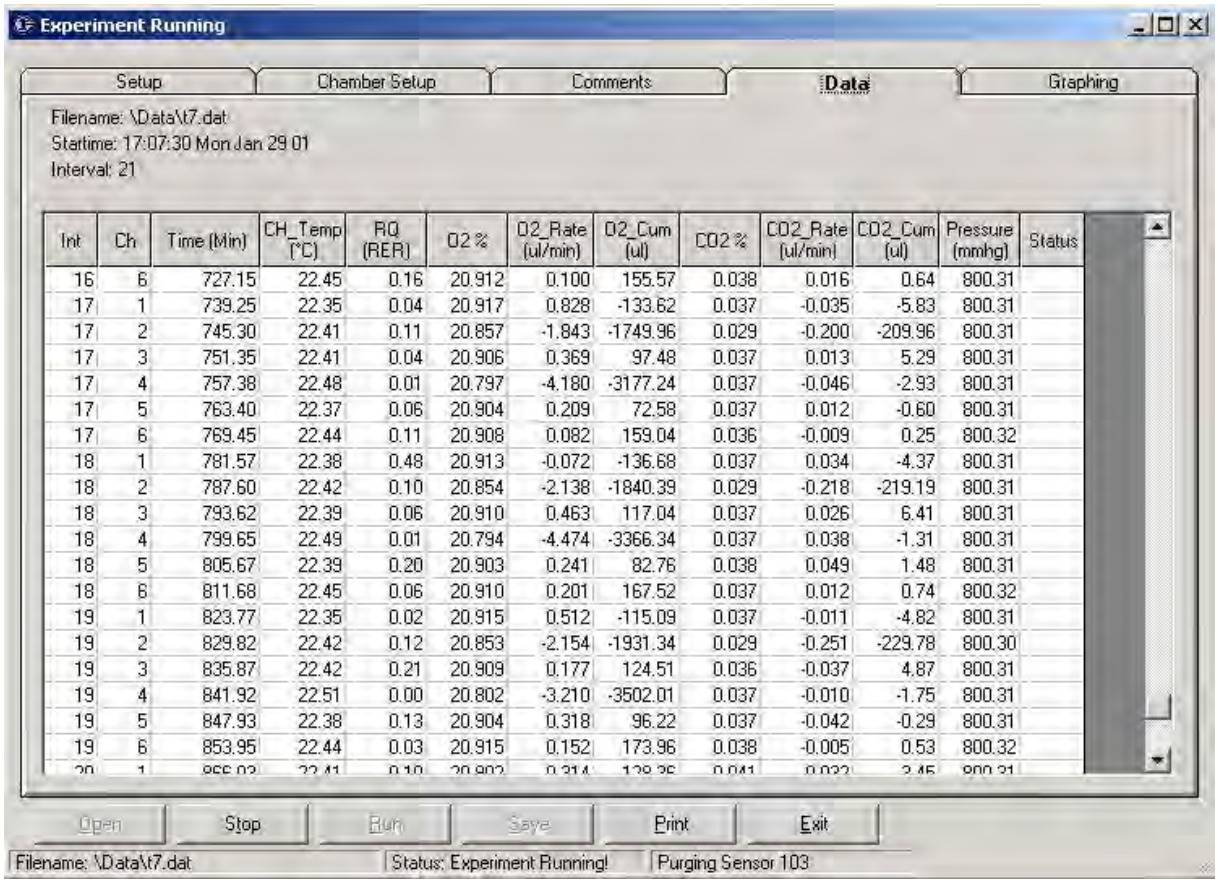
Gas Accumulation. This column displays the total amount of gas change since the beginning of the experiment. This value is not an average but a running total of the gas production or

consumption.

Status. This column is used to indicate various conditions in the operation of the system that occurred, or if an error occurred during that measurement cycle.

Here is a description of each status indicators:

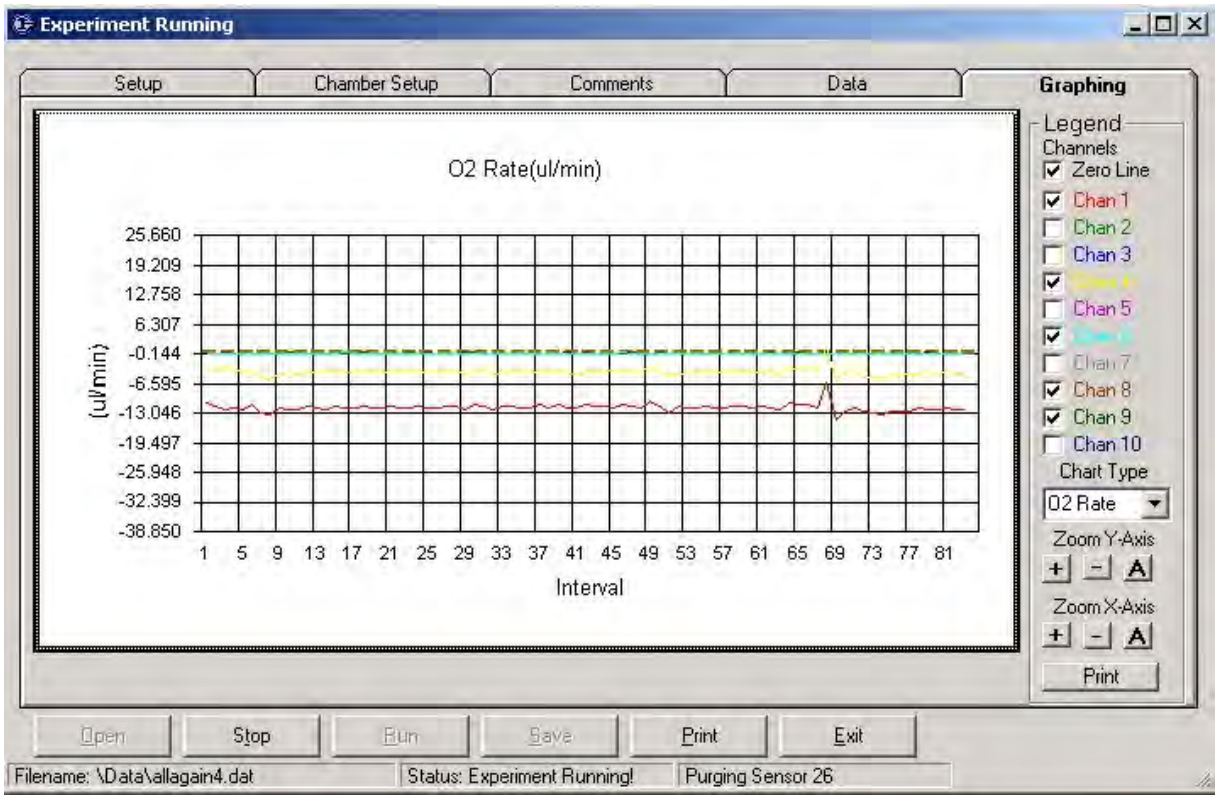
R :A refresh occurred in the chamber
CP + : The pressure in the chamber is elevated above the normal controllable range and cannot be regulated.
CP - : The pressure in the chamber is lower than the normal controllable range and cannot be regulated.
PP + : The pressure in the sensors is elevated above the normal controllable range and cannot be regulated. This indicates an error condition in the sample pump.
PP - : The pressure in the sensors is below above the normal controllable range and cannot be regulated. This indicates an error condition in the sample pump
CO2 + : The level of CO2 is above the normal measuring range of the sensors.
O2 - : The level of O2 is below the normal measuring range of the sensors
(The same status indicators exist for any gas sensor in the system)



Int	Ch	Time (Min)	CH_Temp (°C)	RQ (RER)	O2 %	O2_Rate (ul/min)	O2_Cum (ul)	CO2 %	CO2_Rate (ul/min)	CO2_Cum (ul)	Pressure (mmhg)	Status
16	6	727.15	22.45	0.16	20.912	0.100	155.57	0.038	0.016	0.64	800.31	
17	1	739.25	22.35	0.04	20.917	0.828	-133.62	0.037	-0.035	-5.83	800.31	
17	2	745.30	22.41	0.11	20.857	-1.843	-1749.96	0.029	-0.200	-209.96	800.31	
17	3	751.35	22.41	0.04	20.906	0.369	97.48	0.037	0.013	5.29	800.31	
17	4	757.38	22.48	0.01	20.797	-4.180	-3177.24	0.037	-0.046	-2.93	800.31	
17	5	763.40	22.37	0.06	20.904	0.209	72.58	0.037	0.012	-0.60	800.31	
17	6	769.45	22.44	0.11	20.908	0.082	159.04	0.036	-0.009	0.25	800.32	
18	1	781.57	22.38	0.48	20.913	-0.072	-136.68	0.037	0.034	-4.37	800.31	
18	2	787.60	22.42	0.10	20.854	-2.138	-1840.39	0.029	-0.218	-219.19	800.31	
18	3	793.62	22.39	0.06	20.910	0.463	117.04	0.037	0.026	6.41	800.31	
18	4	799.65	22.49	0.01	20.794	-4.474	-3366.34	0.037	0.038	-1.31	800.31	
18	5	805.67	22.39	0.20	20.903	0.241	82.76	0.038	0.049	1.48	800.31	
18	6	811.68	22.45	0.06	20.910	0.201	167.52	0.037	0.012	0.74	800.32	
19	1	823.77	22.35	0.02	20.915	0.512	-115.09	0.037	-0.011	-4.82	800.31	
19	2	829.82	22.42	0.12	20.853	-2.154	-1931.34	0.029	-0.251	-229.78	800.30	
19	3	835.87	22.42	0.21	20.909	0.177	124.51	0.036	-0.037	4.87	800.31	
19	4	841.92	22.51	0.00	20.802	-3.210	-3502.01	0.037	-0.010	-1.75	800.31	
19	5	847.93	22.38	0.13	20.904	0.318	96.22	0.037	-0.042	-0.29	800.31	
19	6	853.95	22.44	0.03	20.915	0.152	173.96	0.038	-0.005	0.53	800.32	
20	1	866.03	22.41	0.10	20.802	0.314	139.36	0.041	0.032	3.45	800.31	

Graphical Data

The data can be graphed while the experiment data is collected, or while viewing an older experiment. A total of 10 chambers can be viewed at one time in this section. Also the type of data graphed can be selected to be graphed.



Channel Selection. When graphing experiment data, a total of ten chambers can be display at one time. If an experiment contains more than ten chambers any chamber can be displayed at one time, however the total chamber displayed at one time is limited to ten. A Zero line can be displayed to show the zero line on the graph. The Zero line is displayed as a black dashed line.

Chart Type. This list box selects the type of data to be graphed in the graph window. Temperature, Gas %, Rate and Accumulation can be viewed.

Y-axis Controls. The scale of the Y-axis can be adjusted with these controls. The "A" Button is an auto function and will center the graph on the Y-axis. The "+" and "-" buttons will increase or decrease the Y-axis scale.

X-axis Controls. The scale of the X-axis can be adjusted with these controls. The "A" Button is an auto function and will center the graph on the X-axis. The "+" and "-" buttons will increase or decrease the X-axis scale.

2.8 Interpreting Data

The following sections describe how the data is presented and some common errors encountered in running experiments and interpreting data.

Presentation of Data
Data from the Micro -Oxymax is presented in two forms: rate and cumulative. Both forms are useful in different situations. Rate data indicates how fast the gas is being consumed or produced, and can be given per minute or per hour. This information is useful in toxicity tests where the activity level of the organisms is needed. Cumulative data is the total amount of gas (i.e. ul, mg) that has been produced or consumed since the beginning of the experiment. This includes any CO2 produced or O2 consumed during a refresh. The cumulative data may be more useful for Biodegradation type experiments, where the total amount of CO2 produced must be known.

Data File Format
The text shows a small sample of a data file. The file is an ASCII text file and can be easily imported to various other software packages. Some programs may require the user to specify how the data is separated or delimited. The file type is comma delimited Text. Some programs, such as later versions of Excel by Microsoft.
Example of Data File with three data points:

```
[Experiment Data]
Int,Ch,Time,Ch Temp,RQ,O2%,O2 Rate,O2 Accum,CO2%,CO2 Rate,CO2 Accum,Pressure,Status
,,(min),(°C),(RER),%,(ul/min),(ul),%,(ul/min),(ul),(mmhg),
0,1,18.33,0,26.37,21.0081,0.000,0.000,0.0890,0.000,0.000,802.17,R
0,2,24.38,0,26.37,21.0400,0.000,0.000,0.0929,0.000,0.000,802.19,R
0,3,30.43,0,26.37,20.9745,0.000,0.000,0.0861,0.000,0.000,802.18,R
1,1,84.87,9.351955E-02,25.62,20.8705,-12.176,-810.130,0.0679,-1.139,-75.763,802.18,
1,2,90.90,0.6764174,25.61,21.0273,-0.160,-10.625,0.0879,0.108,7.187,802.18,
1,3,96.92,0.1009655,25.46,20.8471,-12.297,-817.539,0.0632,-1.242,-82.543,802.18,
```

CO2 release after Refresh
When running liquid samples, or samples with a high moisture content, CO2 may be released just after refresh. This is indicated by jumps or spikes in the CO2 rate data. This phenomenon is caused by CO2 building up in the head space during the course of an experiment. The CO2 dissolved in solution is in equilibrium with the head space CO2 concentration. After a refresh occurs, the CO2 concentration in the head space decreases considerably. The CO2 dissolved in solution is then released into the head space reaching equilibrium, and showing a jump in CO2 production rate. This variability in the CO2 rate can be seen in the graph on the next page (R indicates where a refresh occurred). The cumulative data for CO2 will be correct and should be used.

Crosstalk between channels.
The software corrects the consumption & production readings for the exchange of gas between chambers. However, due to the high solubility of CO2 in water, the sensor purging

option should be used with liquid or very moist samples. If there is a high amount of respiration on one channel and a low amount on the next channel, the transferred CO2 will dissolve into the liquid on the following channel and the system will indicate CO2 consumption. This is not a problem with the O2 measurement because of the low solubility of O2 in water.

Negative CO2 Rates
When running liquid samples during the beginning of an experiment, data for CO2 rate may give negative readings indicating CO2 consumption. This is caused by using moist samples (i.e. soil or solutions) with very small amounts of dissolved CO2. In the beginning of the experiment, the CO2 from the head space dissolves into the solution to reach equilibrium. This causes some disappearance of CO2 in the head space, resulting in negative CO2 data. This can be avoided by purging the liquid sample with air, or the gas being used for refresh for 15 minutes before the start of the experiment. This will allow the dissolved CO2 to be in equilibrium with the head space CO2 at the start of the experiment.

3 Quick Reference

3.1 System Setup Hints

Power Requirements
The Micro Oxymax System requires a very stable AC power source. Fluctuations in the AC line voltage or frequency can be reflected in the data collected. It is recommended that the System not be powered by an AC circuit that also supplies power to devices that require a large amount of power to operate. These types of instruments include, heating/cooling Water Baths, Circulators, Centrifuges, Environmental Chambers, Shaking Tables and Refrigeration devices. Please contact Columbus Instruments for additional information. If a suitable AC power line is not available an uninterruptible stabilized power supply can be used to provide power to the Micro Oxymax system. When Selecting an uninterruptible power supply consider a device that provides continuous AC Since wave output that has a output rating or 500 to 1000 voltamps. Continuous AC sine wave uninterruptible power supplies are available through Columbus Instruments.

Activated Charcoal Filter Trap
An activated Charcoal filter trap is recommended when measuring samples that contain or produce volatile organics. The trap is used to catch all organics that volatize from the sample during the gas sampling process to prevent damaging the system and mixing with the chemical dryer. CAUTION! If the chemical dryer incorporates Magnesium Perchlorate extreme caution must be used when measure sample with organics. A mixture or magnesium perchlorate and organic solvents make an explosive mixture. Never mix activated charcoal and magnesium perchlorate in the sample dryer column. When replacing the activated charcoal use 4-6 mesh size. If a mesh size smaller than 4-6 a high restriction can result from the filter trap being consumed. Activated charcoal filter traps are available at Columbus Instruments. The part number is 7395-93.

3.2 Sample Reactor

Sample Reactor Size

Due to the nature of the gas measurements in the head space of the sample reactors the Micro Oxymax can operate with sample reactors ranging in size from 20ml to 5 liters. The gas measurement's sensitivity is a function of head space size of the sample reactor, the smaller the head space volume the higher the sensitivity. The inverse is observed when measuring samples that have a high metabolic activity. Samples that have a high metabolic activity require a larger sample reactor to ensure that there is an adequate amount gas reserve available in the head space during the measurement cycle. For most samples a 250 - 500 ml sample reactor will be sufficient.

Sample Preparation

The Micro Oxymax requires a few items in regards to sample preparation. All gas measurements are done in the head space of the reactor therefore the head space of the reactor must be measured and recorded in the experiment setup. The Micro Oxymax has the ability to measure chamber head space volumes up to 2000ml. For volumes larger than 2000ml the user will have to manually calculate the head space volumes. Another item to consider when using liquid sample is the equilibrium between the gas dissolved in the solution and head space area. It is recommended that when measuring liquid samples a stirring mechanism be used to aid in gas equilibrium between the sample solution and head space gas. Care must be taken to prevent any of the solution from entering the sample gas tubing, therefore splashing is not acceptable in the reactor. Hydrophobic Filters should be used on the gas IN ports on the expansion interface. These filters help prevent any of the sample solution from entering the system in the even that a sample reactor tips over.

Sample Reactor Maintenance

Care must be taken when assembling the sample reactor. The reactor bottle, lid and seal assembly must be kept clean of dirt and sample material to ensure a proper seal. The standard lid assembly contains fittings that connect to the sample tubing through a "Quick Connect" type fitting. The fitting itself contains a small O-ring that rests against the tubing as it is inserted into the fitting. As the tubing is inserted into the fitting over time a small ridge will develop where the fitting holds the tubing in place. This ridge will need to be removed by cutting approximately 0.25 inch off the end of the tubing. On the opposite end of the tubing where it connects into the expansion unit a similar ridge can develop over time. That ridge will need to be removed as well.

Sample Reactor Cleaning

The sample reactor assembly will need to be cleaned from time to time. The complete sample reactor assembly can be dissembled and autoclaved if desired. The Nylon Tubing that connects the sample reactor to the expansion unit can be autoclaved as well.

3.3 Running an Experiment

Experiment Checklist
When setting up an experiment there is a few things that will need to be done to ensure that the data that the instrument collects is valid. The following is a list of steps that need to be done to ensure accurate readings.

System Warmed up for 2 hours
This will ensure accurate results when performing the system diagnostics, calibration and volume measurement procedures.

Experiment Parameters Setup
The experiment parameters can be set. In these parameters the number of sample reactors that are going to be measured is set as well as the interval each chamber is measured. For samples that have a high metabolic rate the sample interval should be kept as short as possible. For samples that do not have a high metabolic rate the sample interval can be lengthen to increase the system sensitivity. For samples that vary in their metabolic activity or for moist soil or liquid sample sensor purging should be enabled to prevent gas cross-over. Gas cross-over occurs when a chamber is measured that is very active then a chamber that is not very active is measured. Gas from the active chamber is carried over to the next chamber and can show up in the results. The Reactor Refresh Threshold Parameter should be set. Typically this value is set to 0.5 percent. This indicates that the sample reactor will be refreshed when any of the gases in the reactor's head space changes more than 0.5 percent. The rest of the experiment parameters deal with the units the data will be collected in. (M, (g, mg, (l, can be selected for the gas units and minutes or hours can be selected as the time units. Once the parameters are set the file should be saved.

System Diagnostic Ran
The Micro Oxymax contains diagnostic procedures that will check the function of each section of the instrument for proper operation. The system Diagnostics include the Basic Operations, Valves and Sensor, and the Expansion Unit Diagnostics. If an Error or failure occurs details for that error as well as hints to fix the errors can be obtained by printing out the report. A print out of these test will requested when calling Columbus Instruments for service. If the system is using a gas bottle as its refresh air source the gas bottle must be disconnect from the systems Refresh/Calibration and Nitrogen Ports when running the system diagnostics to prevent false errors and system failures.

Gas Sensor Calibration
The gas sensor installed in the Micro Oxymax require periodic offset and span calibration. Columbus Instruments recommends that the gas sensor before each experiment.

Sample Preparation
The sample can now be prepared for the experiment. If a liquid sample is going to be measured, a stirring or shaking mechanism should be used to aid in the dissolved gasses in the liquid reaching equilibrium with the gasses in the head space. Care should be taken to ensure

that there is no chance that the liquid sample does not splash into the sample tubing ports. Ensure that hydrophobic filters are used on the sample lines that is connected between the sample reactor and the IN port located on the expansion unit. Liquid samples should be purged with room air prior to the experiment to ensure that the sample's dissolved gasses are saturated. If the sample are going to be run at an elevated temperature higher than 40 degrees centigrade the reactor volumes and leakages should be measured before the sample reactors are placed in the elevated temperature.

System Volume and Leak Measurements

Once the samples are prepared and attached to the system the sensor and sample reactor volumes will need to be measured and updated in the experiment parameter file previously setup. After the volumes are measured the system leakages can be measured as a double check that everything is connected properly. An Excessive leakages is a leak that is larger than +/- 0.3ml/min.

Refreshing from a Gas Bottle

If the experiment is going to be refreshed from a compressed gas bottle the gas bottle can be attached to the system at this point. Note that the pressure regulator on the gas bottle must be set to 5 PSIG (250mmHg, 33.3KPa) and connected to the Refresh/Calibration Port located on the rear of the system sample pump. If the sensors are going to be purged with the same source a special 1 PSIG(.068 bar) pressure regulator will be needed. Refer to the operations section for proper setup.

Start Experiment

Now that all the preparation has been completed the experiment can be started. As the data is collected it is stored in the local hard drive. A printed copy or disk copy can be obtained by pressing "P" while viewing the experiment. Real time graphics are also available from this menu by pressing "G".

3.4 System Troubleshooting

Positive Leakage

An excessive positive leak is a leak value that is higher than +0.5 ml/min. The origination of this type of leak is on the input side of the sampling pump and can only occur on the inside of the system sample pump. The main causes of this type of leak is from a valve that does not seat properly, usually caused from debris entering the system. The valves that should be checked are the Nitrogen Gas Valve, Refresh, Calibration Gas Valve and the Test In Valve. All of these valves are located on the inside of the system sample pump on the rear panel. To locate each valve simply follow the labeled port on the rear of the system sample pump.

Negative Leakage

An excessive negative leak is a leak value that is higher than -0.5 ml/min. This type of leak is common with a leak point that is outside the system sample pump. This can include a leaking dryer, loose sensor tubing, or leaking chamber itself. The first step is to determine where the leak point is. A leak in the sensor circuit of the instrument will show the same leak in the

sample reactor, therefore it is recommended that the sensor leakage be measure first.

Troubleshooting Sensor Leakage
Once it has been determined that there is a sensor leak in the system, the next step is to isolate when the leakage is occurring. The most common point of sensor leakage is in the Sample Dryers located on the front of the system sample pump. To check the dryers for leakage simply remove the dryer and short the Dryer "In" and "Out" port with a short piece of tubing and remeasure the sensor leakage. If the leakage the tests less than -0.5 then the leakage is in the dryer that has been remover from the system. (Dryer leakage originates from debris getting on the sealing O-ring of the dryer. Cleaning this seal should eliminate the dryer leakage.) If the Sensor leakage remains larger than -0.5 ml/min then the sensor leakage is in another portion of the system. The next step is to Isolate the leakage to either the sensors themselves, or to the system sample pump. A small flask (100ml) that it leak free can be substituted for each sensor and the sensor leakage can be rechecked. When the leakage drops below -0.5 ml/min, the sensor that has being replaced with the small flask is the cause of the leak. If after substituting all the sensors with a small flask the leakage still remains then the leakage is located inside the system sample pump and will need to be returned to Columbus Instrument's Service Department for repairs.

Troubleshooting Chamber Leakage
Once the leakage has been isolated to the sample chamber, the next step is to isolate the leakage from the Expansion Unit to the tubing and sample reactor. This is done by placing a short piece of tubing between the In and Out port located on the front of the Expansion Unit. The chamber volume will need to be remeasure first before the leakage is measured to give accurate leak results. If the leakage valve is below -0.5 ml/min with the In and Out ports shorted with the short piece of tubing then the leak is located in either the tubing, hydrophobic filter assembly, or the sample reactor itself. To isolate the leakage from the hydrophobic filters the filter assembly can be removed and the chamber leakage test rerun. If the leak goes away then the leakage is in the filter that was removed. If the leakage remains then the next step is to check the sample chamber. In most cases the sample chamber's seal will be dirty or not seated properly. Remove the chamber lid and check the seal. If the seal is seated properly and is clean, then it is possible that the chamber leakage is caused by the chamber tubing's connection to the quick connect type fittings located on both the Sample chamber and expansion unit. A solution of soap and water can be applied to these fittings during the leak measurement test. A leaking fitting will produce bubbles indicating the leak. The leak in the fitting can be caused by worn tubing, or the fitting itself my need to be replaced. If the tubing end is worn a small 0.25 inch piece of tubing can be cut from the end and the tube can be reused.

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