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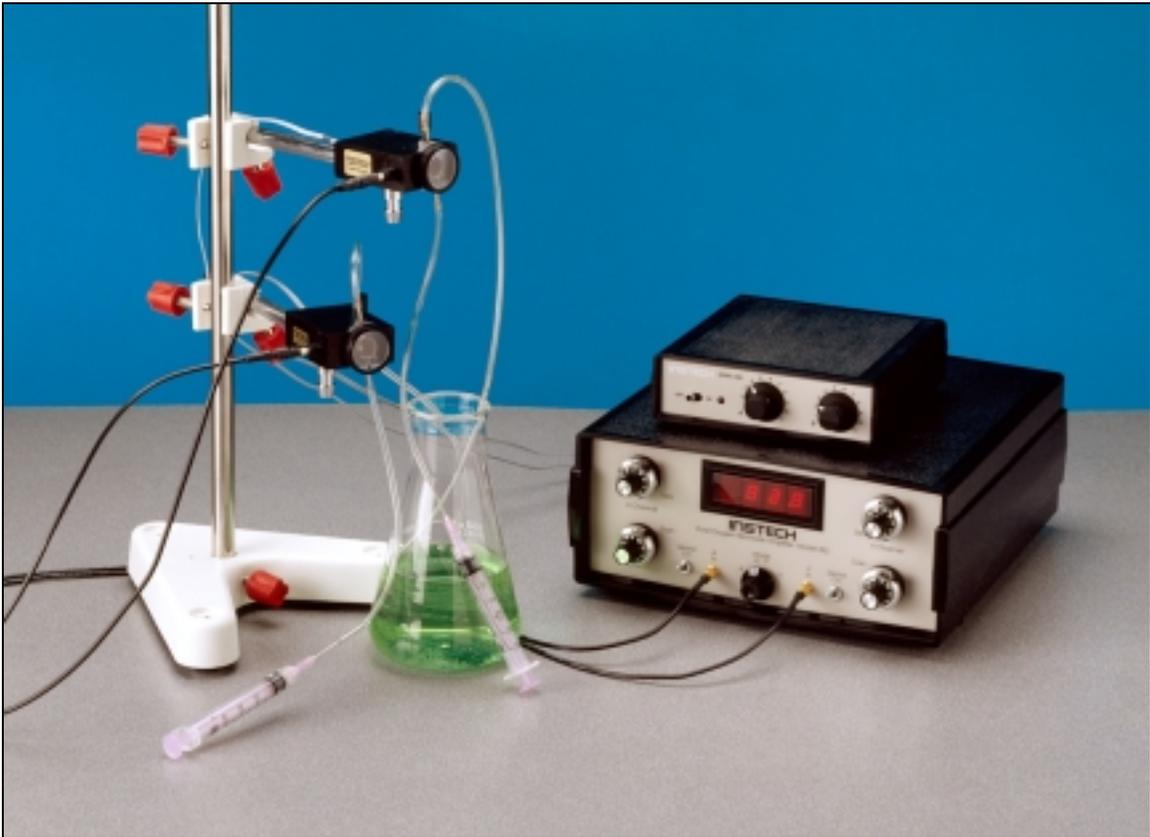
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**INSTECH**

# Dissolved Oxygen Measuring System

System 203 Operation Manual



NOTE: This system is intended for in vitro physiological and biological studies. It is **not** intended for use on humans. Instech Laboratories, Inc. cannot assume liability from improper use of its products.

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# Set-up

Your Dual Chamber Oxygen Measuring System (SYS203) should include the following items:

## 1 Dual Channel Amplifier

- 1.... Amplifier
- 2.... 42” output cables

## 2 Standard Electrodes, each with

- 1.... Standard oxygen electrode with cable
- 1.... Electrode sleeve
- 1.... Electrode nose piece
- 15.. O-rings
- 1.... O-ring installation tool
- 1.... 72 in<sup>2</sup> membrane material
- 1.... 4 in<sup>2</sup> polishing paper
- 1.... 4 in<sup>2</sup> Scotch-Brite®

## 2 Batch/Flow Chambers, each with

- 1.... Chamber block
- 1.... Batch chamber cup with fill port plug
- 1.... Batch chamber window
- 1.... Hamilton syringe fill port plug
- 1.... Vial of silicone grease
- 1.... High flow cell
- 1.... Low flow cell (with white dot)
- 1.... 10” lengths of 1/8” OD Tygon tubing
- 1.... 4” lengths of 1/8” OD Tygon tubing
- 1.... Motor/magnet stirring assembly

## 1 Dual Channel Stirring Controller

- 1.... Dual channel precision speed controller
- 1.... 9 VDC power adapter

## 1 Electrode Chlorider and Accessory Kit

- 1.... Electrode chlorider
- 1.... 9 VDC power adapter
- 15.. Electrode o-rings
- 1.... O-ring installation tool
- 1.... 8 in<sup>2</sup> Scotch-Brite®
- 1.... 72 in<sup>2</sup> polyethylene membrane material
- 1.... 24 in<sup>2</sup> electrode polishing paper

This manual describes the set-up for two basic modes of operation: **batch cell mode**, for measuring dissolved oxygen concentration in a fixed 600µl sample, and **flow cell mode**, for measuring the oxygen concentration in fluid as it passes through the flow cell. For both of these modes, there are five basic steps to the set-up:

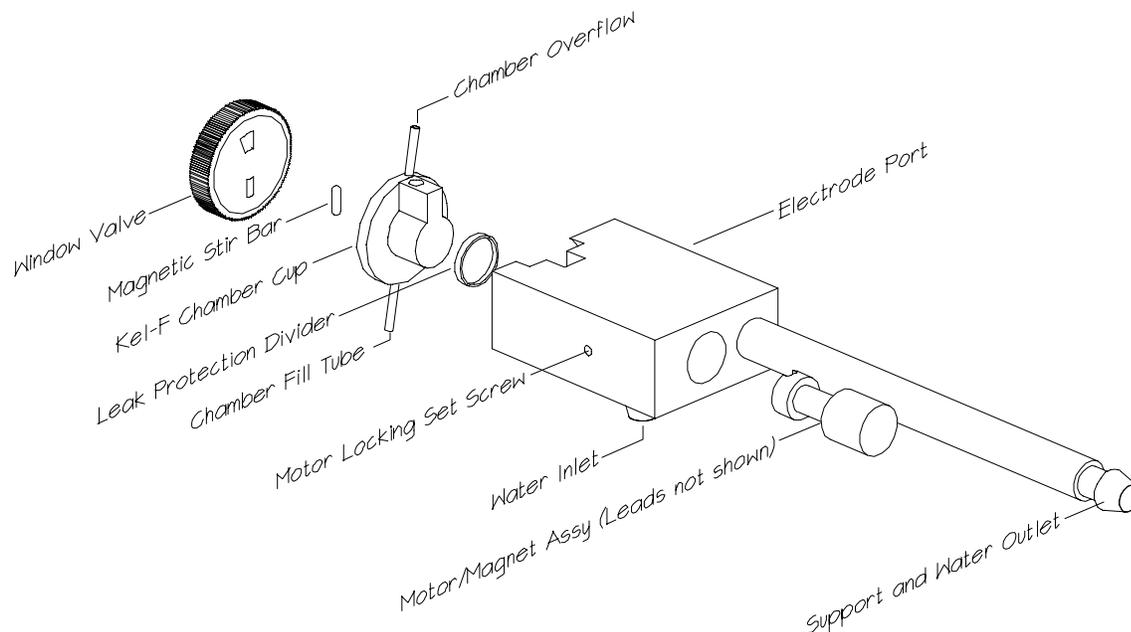
1. Set up the chamber
2. Prepare the electrode
3. Install the electrode and complete the chamber set-up
4. Set up the amplifier
5. Calibrate the amplifier.

For your convenience, this manual describes the set-up of the entire system; you do not need to refer to the smaller manuals included with the electrode or the chamber system.

## Setting Up the Chamber

These initial steps apply to both the batch and flow cell modes:

1. *Mount the chamber to a ring stand or other suitable support.*
2. *Attach a circulating water bath to the inlet and outlet tubes.* The block should be kept at a constant temperature as the oxygen electrode is sensitive to temperature changes. The fittings are designed for 3/8” ID tubing. Be sure to secure the tubing with a hose clamp or cable tie to prevent blow-off.

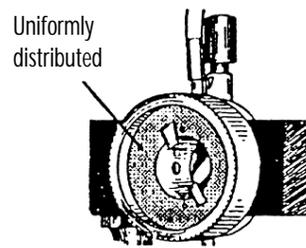
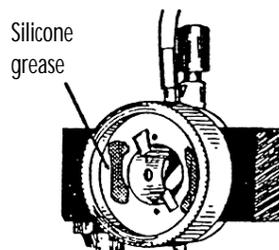


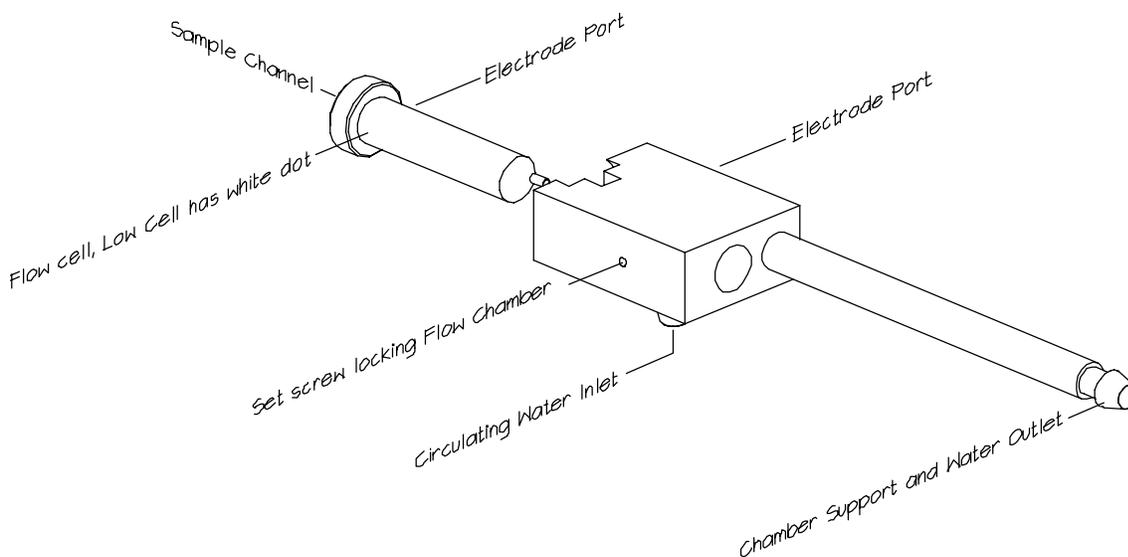
Batch cell chamber assembly

### Setting Up the Batch Cell Chamber

The batch cell mode uses the chamber cup with a magnetic stirring motor mounted behind it. The chamber cup is sealed with the window valve which is held in place by a thin layer of silicone grease.

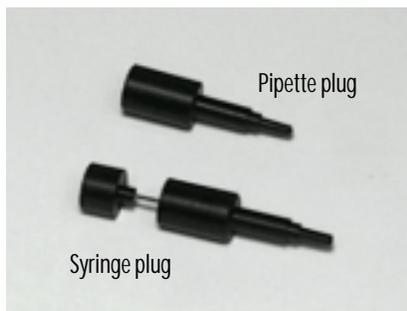
1. *Plug in the speed controller.* Plug the AC adapter into the DC IN jack of the speed controller first, then plug the AC adapter into a wall outlet.
2. *Plug the motor into MOTOR OUT jack of the speed controller.* The motor should run when the speed controller is turned on.
3. Temporarily insert the chamber cup into the chamber block. *Push in the red leak protection divider from the rear of the chamber block* using a pencil until it hits the chamber cup. The opening should face away from the chamber cup.
4. *Insert the motor/magnet assembly into the chamber block* until it hits the red spacer, then pull it back about 1 mm.
5. *Gently tighten the set screw* to hold the motor using the provided allen wrench.
6. *Insert the chamber cup into the front of the chamber block.*
7. *Place the stir bar into the chamber cup.* It should couple to the magnet and rotate freely against the back of the cup when the speed controller is turned on.
8. *Apply a small amount of silicone grease to the flat front surface of the chamber cup.* Avoid the small fill and overflow port holes.
9. *Press the window against the cup and rotate it to distribute the grease uniformly across the face.*





Flow cell chamber assembly

10. Pull off the window valve and clean off excess grease from the inside of cup using a toothpick. Check the port holes as well. Do not clean the layer of grease from the face of the cup – this will form the seal for the window.
11. Clean off all grease from the window valve. An acetone dampened tissue works well.
12. Press the window valve back on to the chamber cup and rotate to distribute the grease across the face.
13. Install the appropriate fill port plug into the top of the chamber. If you plan to use a plastic-tipped micropipette to add fluid to the chamber during your experiment, use the single-piece pipette plug. If you plan to use a microliter syringe, use the two-piece syringe plug.

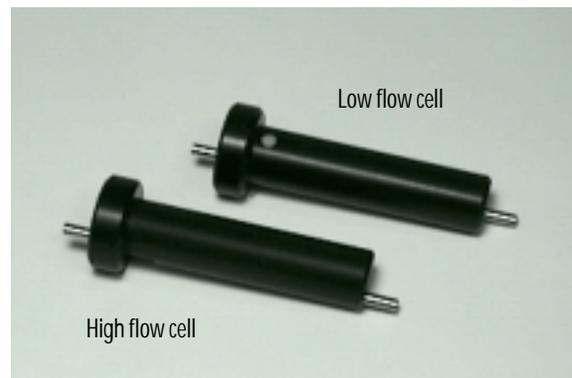


The chamber is now ready for the electrode. When inserted, the electrode will hold the cup

firmly in place. Skip to the section *Preparing the Electrode*.

### Setting Up the Flow Cell Chamber

The flow cell mode uses one of two flow cells provided with your system: the **low-flow cell** for flow rates less than 15 ml/minute, the **high-flow cell** for rates greater than 15 ml/minute. (See Appendix for more details.) The low flow cell has a white dot on it. The various pieces of equipment designed for the batch cell mode (i.e., speed controller, motor, chamber cup, window valve, and red spacer) are not needed and should be set aside.



Most in-line experiments involve measuring the difference in oxygen level at two points in a system, and thus will require that you set up two flow cells.

To set up the flow cell chamber:

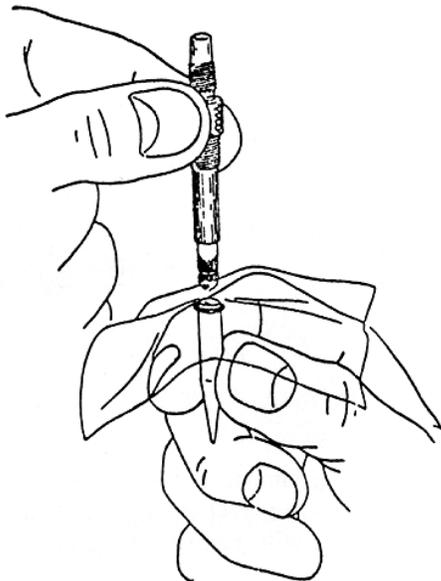
1. *Insert chosen flow cell into the chamber block from the front.* Be sure that batch cell parts have been removed from the chamber block and that the set screw does not protrude into the hole.
2. *Attach inlet and outlet tubes of your experiment to the flow cell.* The system is designed for 1/16" ID tubing, such as Tygon.

The flow cell is now ready for the electrode.

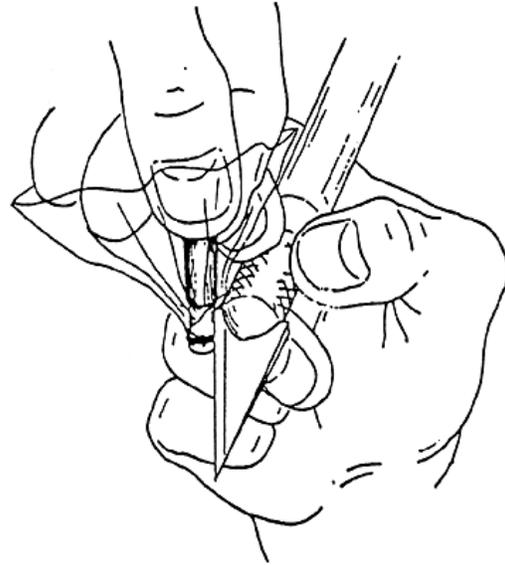
## Preparing the Electrode

Oxygen electrodes need to be prepared with fresh electrolyte and a new membrane before each use.

1. *Prepare fresh KCl solution daily.* The solution should be about 1/2 saturated (add about 15 gms KCl to 100 ml water). Concentration is not critical, purity is.
2. *Remove the old membrane and rinse the electrode tip with water,* if it has been previously used.
3. *Slide the electrode sleeve over the electrode.* Be sure the sleeve is clean, or it may damage the electrode tip.
4. *Roll an o-ring onto tapered end of the installation tool* and position it as close as possible to the flat end by holding the flat end against a tabletop and rolling the o-ring to the bottom.



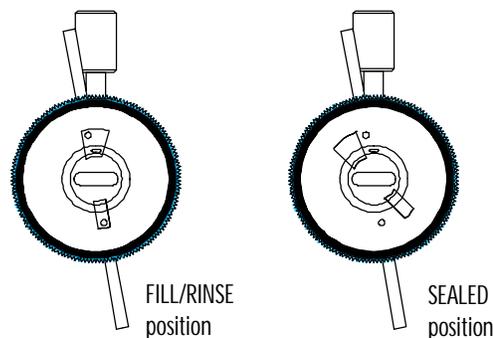
5. *Lay a sheet of membrane material (approximately 1" by 1") on the top of the installation tool.* The best material for membranes is usually common polyethylene sandwich bags. (See **System Theory** for more details.) Be careful not to touch the area of the membrane that you will be using.
6. *Dip the electrode tip into the KCl solution and allow a droplet to form.*
7. *Place this drop on the membrane over the hole in the installation tool.*
8. *Dip the electrode tip into the KCl again.*
9. *Press the tip with the hanging droplet into the depression in the installation tool.*
10. *Roll the o-ring off the tool and into the groove on the electrode to secure the membrane.*
11. *Examine the tip of the electrode; if you see an air bubble, install a new membrane.*
12. *Cut off excess membrane using a sharp knife against the silver body of the electrode.*



The electrode is now ready to be installed into your chamber set-up. If you plan to use the electrode outside the chamber block, *screw the electrode nose piece onto the electrode sleeve.* This will form a seal against the o-ring and prevent solution from leaking behind the membrane.

Install the electrode quickly; if it is exposed to dry air for much more than one minute, the electrolyte will evaporate and you will need to install a new membrane.

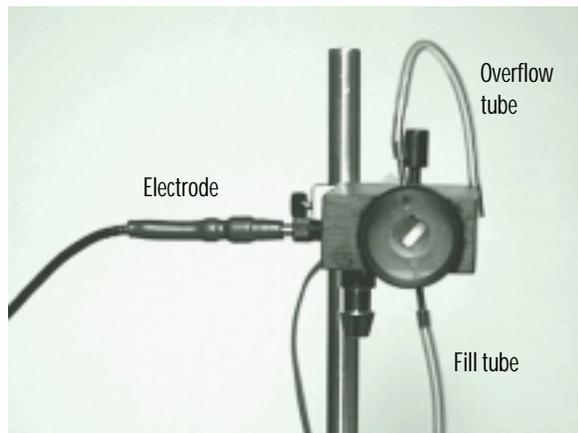
If you are using this electrode for the first time, you may wish to determine the true electrode zero offset before installing the electrode in your system. (Skip to the section *Calibrating the Amplifier - Determining True Zero* and return to *Completing Chamber Set-Up* when finished.



## Completing Chamber Set-up

### Completing Batch Cell Chamber

To complete the batch cell chamber set-up, you need to install the electrode and fill the chamber with your buffer solution.



1. *Align cup and chamber block electrode holes and insert electrode.* Gently screw in the electrode sleeve so that the o-ring forms a seal against the cup. Be careful not to puncture the membrane during this process.
2. *Attach the overflow tube to the top of the chamber.* Be sure that the other end of the tube is above the top of the chamber cup, otherwise it may siphon out the chamber. Place a beaker under the tube to collect the overflow.
3. *Attach the fill tube to the bottom of the chamber.*

1. *Fill the chamber with buffer solution using a syringe inserted into the fill tube.* The window valve must be turned to the FILL/RINSE position with both depressions in the window valve aligned with the

chamber cup holes. You must be careful not to have any air bubbles trapped in the chamber as this will obviously distort your measurements. Positioning the stir bar vertically by joggling the speed controller on and off will help. If you do get an air bubble, suck the solution back into the syringe and try again.

2. *Turn the window valve to the SEALED position.*
3. *Turn on the stirring motor and the circulating water bath.*

Your batch cell chamber is now set up. Skip to the section *Setting Up the Amplifier*.

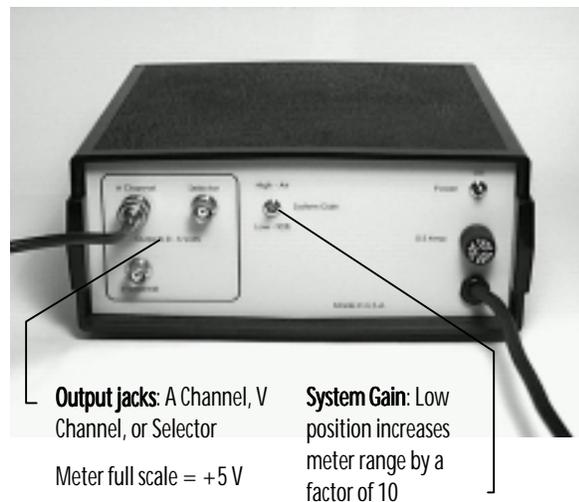
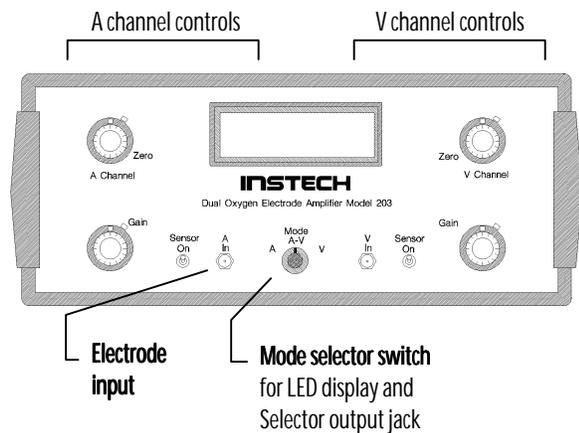
### Completing Flow Cell Chamber

1. *Align flow cell and chamber block holes and insert electrode.* Gently screw in the electrode sleeve so that the o-ring forms a seal against the cell. Be careful not to puncture the membrane during this process.
2. *Fill the flow cell with solution.* Be sure to do this within one minute after preparing the electrode to prevent evaporation of the electrolyte.

Your flow-cell chamber is now set up.

## Setting Up the Amplifier

1. Plug in the amplifier power cord.
2. Attach the electrode cable to the jack on the front panel of the amplifier. (A In or V In depending on which channel you are connecting.)
3. Attach a chart recorder or other data acquisition device to the appropriate output jack on the back of the amplifier. The output on the Selector jack depends on the setting of the Mode switch on the front panel (A, V or AV).



## Calibrating the Amplifier

Finally, you should calibrate the amplifier to convert the nanoamp electrode current into the desired reading on the meter, e.g. mmHg of oxygen. This is done by adjusting the **Zero** control so that the amplifier reads zero when the electrode is turned off (or for a more accurate reading, when the electrode is exposed to an oxygen depleted solution), then by adjusting the **Gain** so that the meter reads the true ambient  $pO_2$  with the electrode on.

### Quick Zero Calibration

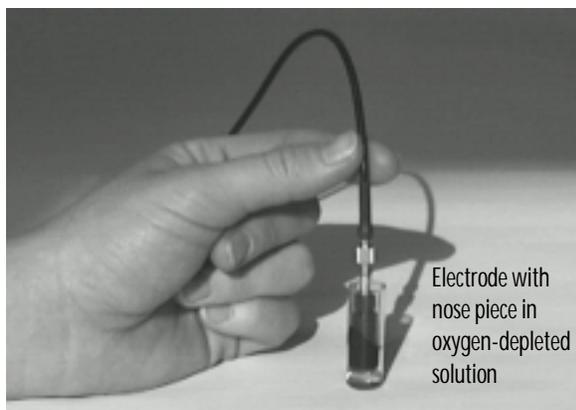
This calibration approximates a zero oxygen level by turning off the electrode sensor. In practice, a healthy electrode will read 1 to 10 mmHg even when the oxygen level is zero due to minor imperfections. If this accuracy is important to your experiment, skip to the section *Determining True Zero*.

1. Turn on the amplifier.
2. Set the **Gain** of the channel you are working with so that the number on the knob reads approximately 2 (a fairly high setting).
3. Turn the electrode sensor switch off.
4. Turn the **Zero** knob so that the LED meter reads zero. Lock the knob in that position with the lever on the side of the knob.
5. Turn the **Gain** back down so that the knob reads about 1.
6. Turn the electrode sensor switch back on.

Skip to the section *Setting Amplifier Gain*.

## Determining True Zero

A healthy electrode will deliver a small current, equivalent to 1 to 10 mmHg, even when the oxygen concentration is zero. Complete this procedure to determine this offset so that it may be subtracted electronically. Unless you suspect a problem, you do not need to repeat this procedure each time you use the electrode.



1. Remove the electrode from the chamber block. It should still be connected to the amplifier.
2. Screw black nose piece onto electrode to seal membrane. Do not immerse the electrode into any solutions outside the chamber without this sleeve in place.
3. Immerse the tip into a vial with a small amount of distilled water and allow the system to stabilize.
4. Adjust the amplifier Zero so that the meter reads 0 with the electrode sensor switch turned off.
5. Turn on the electrode and set the amplifier Gain so that the LED meter reads about 150 (the approximate ambient  $pO_2$  of water in mmHg).
6. In another vial, dissolve about 25 mg of sodium dithionite into 1 ml of water. Sodium dithionite chemically removes dissolved oxygen from the solution.

7. Place the electrode tip into the sodium dithionite solution. The meter reading should immediately fall.
8. Check the LED meter after the reading is stable for one minute. If it reads less than 10 mmHg, the electrode is in good working condition. If the meter reads greater than 10 mmHg, add small amounts of sodium dithionite until no further drop is observed. If the meter still reads greater than 10 mmHg, refer to the **Troubleshooting** section of this manual.
9. Adjust the amplifier Zero so that the meter reads 0 and lock the knob in that position.
10. Rinse the electrode tip well with distilled water, remove the nose piece, rinse again, and return it to the chamber block.

You have now calibrated the amplifier to offset the small signal the electrode delivers when the  $O_2$  level is zero.

## Setting Amplifier Gain

1. Allow the system to stabilize. The oxygen chamber should contain your buffer solution. The temperature should be held constant with the circulating water bath. After a few minutes the meter reading should become constant.
2. Calculate the ambient  $pO_2$  for your solution. You can refer to the charts in the appendix, or use the Intake oxygen data acquisition software to make these calculations for you.
3. Adjust the Gain so that the meter reads the correct ambient  $pO_2$ . If you are working with 95% oxygen solutions (i.e., a  $pO_2$  of about 700 mmHg) you will need to set the System Gain switch on the rear panel of the amplifier to the low position.

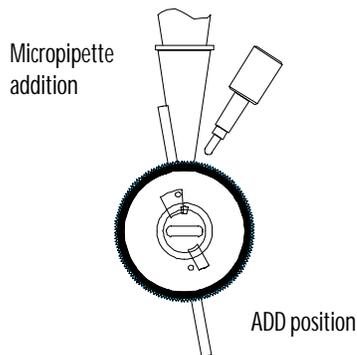
The system is now completely set up and calibrated. You may begin your experiment.

# Operation

This section describes the basics of operating the system in the batch cell and flow cell modes.

## Batch Cell Experiments

Once the system has been set up and has stabilized, a typical experiment begins with the addition of cells or drugs to the chamber.



1. *Turn the window valve to the ADD position.* This opens the top overflow port, but keeps the bottom port closed.
2. *Remove the fill port plug from the top of the chamber and inject cells or drugs.* If you are using a plastic tipped micropipette, remove the entire single-piece pipette plug. If you are using a microliter syringe, use the two-piece syringe plug and remove only the top part. Excess fluid should drain out through the overflow tube. Be careful not to inject air bubbles into the chamber with the pipette.
3. *Replace the plug and turn the window valve to the SEALED position* to close off the chamber from the atmosphere (see figures on page 7).

4. *When your oxygen measurement is complete, open the window valve to the FILL/RINSE position and flush out the chamber with buffer solution from the syringe.* You are now ready to take another measurement.

## Flow Cell Experiments

Typical flow cell experiments involve measuring the oxygen consumption of some perfused tissue or device by measuring the difference in oxygen level of a flowing solution immediately before and after the object.

Set up your experiment so that the two flow cells are as close as possible to the experimental preparation. Keep all parts of the system at a constant temperature.

To calibrate the amplifier, insert a length of tubing to bypass the preparation, directly connecting the two flow cells. Adjust both electrodes to the  $pO_2$  of the flowing solution. The difference should be zero. The flow rate of the solution during calibration should be the same as the flow rate during the experiment. Reconnect the experimental preparation, and set the amplifier to A-V to measure the difference between the two flow cells.

A varying flow rate across the electrode surface may vary the reading by several percent, particularly at lower flow rates. Check the graph the appendix to determine if this will be a problem for your experiment.

# Maintenance

## Electrode Maintenance

With proper care, your oxygen electrode should last for many years. You will need to rechloride the tip periodically, as the Ag-AgCl layer will become contaminated or wear off under normal use.

### *Storage and Cleaning*

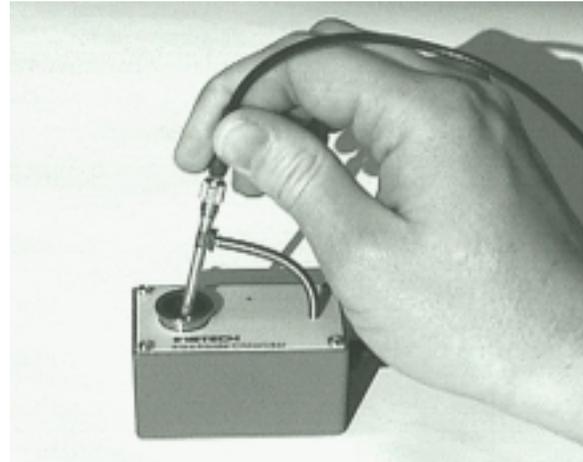
Store the electrode with its membrane and o-ring in place. The electrolyte will evaporate, but the membrane will protect the electrode tip. When you are ready to use the electrode again, remove the old membrane, rinse, and install a new one.

You should only rinse the electrode tip with water. When working with the electrode, be very careful not to get any solutions near the connectors. Salty connectors cannot be cleaned and must be replaced.

### *Rechloriding*

This procedure replaces the Ag-AgCl layer on the tip of the electrode. This layer is critical to the electrode operation, and will wear off under normal use. Excessively high or drifting signals are signs that you should rechloride the electrode.

1. *Remove the old chloride layer with the gray pad of Scotch-Brite® included in the accessory kit. The tip should become a bright silver. Avoid touching the platinum cathode at the center of the tip. Rinse the tip with water when you are finished.*
2. *Plug in the Chlorider and fill the clean cup with fresh KCl solution.*
3. *Clip in the electrode.*
4. *Repeatedly dip the electrode tip into the Chlorider cup until it becomes a uniform dull gray (this usually takes about 1 minute). Avoid touching the electrode to the sides of the cup. If the coating is not uniform, the tip probably was not clean enough; you should remove this layer and start over.*



With a new membrane, your electrode will be ready to use.

### *Tip Renovation*

If the electrode tip becomes contaminated with protein or if the epoxy around the glass seal swells, complete this procedure. You should not have to renovate the tip frequently.

1. *Place the fine gray polishing paper (provided in the accessory kit) on a flat surface and put a few drops of water on the paper.*
2. *Holding the electrode perpendicular to the paper, rub the tip until a fresh layer of platinum has been exposed at the center. Usually this takes only 3-5 passes.*
3. *Rechloride the electrode.*

## Chamber Maintenance

It is not normally necessary to clean the chamber cup or the flow cell after each use; simply flushing the cell with your buffer solution should suffice. Occasionally, you should dismantle the chamber set-up and clean the parts with alcohol or another disinfectant to prevent bacteria buildup. If bacteria exist in your system, you will probably notice their oxygen consumption during your experiment.

# Appendix A: Troubleshooting

Symptom	Potential problem	Treatment
Signal drifts and is excessively high	Membrane perforated	Remove electrode and check for holes in the membrane. If present, clean and rechloride electrode.
	Salt bridge in cable or electrode connectors	Remove electrode from chamber, remove membrane and dry electrode tip. $pO_2$ level should read zero; if not, replace electrode and cable.
	Chloride layer contaminated	Clean and rechloride electrode.
Signal wanders at typical levels	System temperature not sufficiently stable	Use well controlled circulating water bath. Remember that the temperature coefficient of the electrode is approximately 4%/°C.
	Air bubble on or under membrane	Dislodge external bubble or replace membrane.
	Solution not stirred sufficiently (batch cell mode)	Turn on or increase speed of stirring motor.
	Varying flow rate of solution across electrode tip (flow cell mode)	Use low flow cell or modify experiment to stabilize flow rate.
	Chloride layer contaminated	Clean and rechloride electrode.
	Foreign material on platinum cathode surface	Renovate electrode tip.
	Hydrostatic pressure changes effecting membrane stability	No known remedy.
Signal drifts downward steadily, but returns to normal levels with refilling of chamber	Chamber contaminated with bacteria	Disassemble and clean all chamber parts. Alcohol or Clorox will usually kill any growths.
Zero $pO_2$ level (as established by <b>Determining True Zero</b> procedure) is greater than 7% of ambient	Not enough sodium dithionite	Add more dithionite in small steps to titrate down to a minimum reading. Be careful not to use excessive amounts of dithionite, as it will damage the chloride layer.
	Chloride layer contaminated	Clean and rechloride electrode.
	Defective glass to platinum seal	Replace electrode.
Transient spikes during small additions to chamber	Temperature of injected solution different from system temperature	Equilibrate injectate to system temperature
	Heat of dissolution due to addition of a non-aqueous solution (e.g. alcohol or DMSO)	Signal not valid during transient; may be removed mathematically.
Electrode does not respond when sensor turned on	Air or insufficient electrolyte under membrane	Visually inspect electrode tip. Reinstall membrane if required.
	Electrode or cable leads broken	Remove electrode membrane and dip tip into deionized water with sensor turned on. The amplifier should register a very large reading. If not, the leads are probably broken; replace the electrode and cable.
	Amplifier faulty	Test the suspect amplifier channel with another electrode, if available. Call for technical assistance.

Symptom	Potential problem	Treatment
Response to oxygen good, but signal level has gradually increased over a period of weeks	Platinum cathode surface may have become roughened, increasing the active surface area and therefore the current	Renovate electrode tip.
Response to oxygen good, but signal level has gradually decreased over a period of weeks	Platinum cathode has become coated or poisoned	Renovate electrode tip.

## Appendix B: System Theory

This dissolved oxygen measuring system is designed around a style of polarographic electrode developed by Dr. Leland Clark.

When a potential of about 0.7V is applied between the anode and the cathode, dissolved gaseous oxygen is reduced at the platinum cathode. This produces a current and consumes the oxygen in the immediate vicinity of the exposed platinum cathode. Oxygen in the sample volume diffuses through the membrane to the oxygen-poor region between the membrane and the electrode. When a steady-state is reached (which usually happens in less than 4 seconds), the electrode current is proportional to the rate of arrival of oxygen molecules at the cathode, which is in turn proportional to the concentration of oxygen outside the membrane.

The oxygen amplifier does two things: (1) it generates the electrode biasing potential and (2) it measures the electrode current, usually only a few nanoamps, and converts it to a voltage.

The electrode is quite sensitive to temperature changes as temperature affects the speed of the chemical reaction, the ease of diffusion across the membrane and the ambient oxygen concentration of the sample solution. Electrode readings will increase by about 4% for a temperature increase of 1°C.

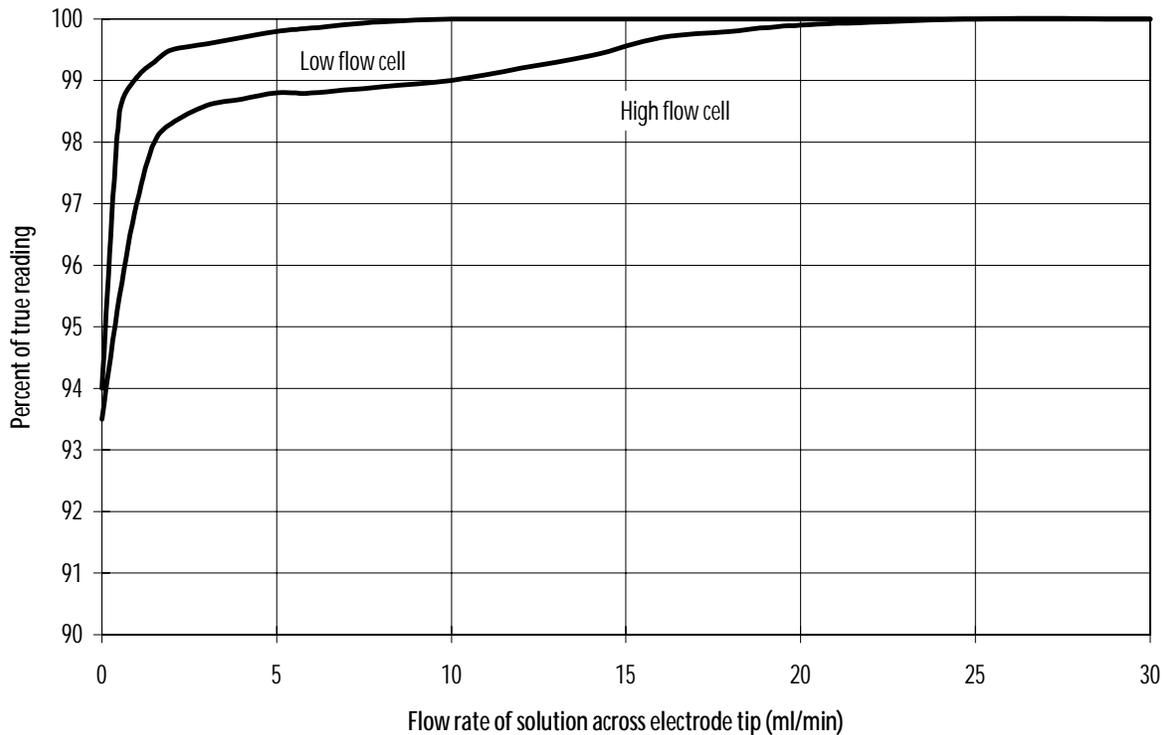
In order to make an accurate measurement of oxygen levels in the entire sample volume, the oxygen around the outside of the membrane must be replaced. If the sample is not adequately stirred when working in batch cell mode, or if the flow rate is too low when working in flow cell mode, a concentration gradient will be set up outside the membrane and the rate of arrival of oxygen at the electrode will decrease. Slight fluid velocity changes will produce a “motion artifact”.

The type of material used as a membrane also critically affects the performance of the system. We have found that 0.001” to 0.0009” thick polyethylene, which is readily available from normal sandwich bags, works best. Membranes that are more oxygen permeable, such as TFE Teflon or silicone, may be required for experiments at low temperatures or when a response rate faster than 4 seconds is desired. However, these materials will cause a greater motion artifact because the concentration gradient extends further into the bulk solution. TFE Teflon will also stretch and thin over time, causing the initial amplifier calibration to become incorrect. FEP Teflon usually works well, but is too stiff to wrap around the small electrode tip. Saran is not sufficiently permeable to oxygen to be useful as a membrane.

## Appendix C: Choosing Between High and Low Flow Cells

When using the flow cells at low flow rates, an oxygen concentration gradient may be set up outside the membrane (see *System Theory*) which will cause the electrode to read lower than it otherwise would. Other than a slight loss in signal strength, this effect should not be a problem so long as the amplifier is properly calibrated and the flow rate does not vary. Use

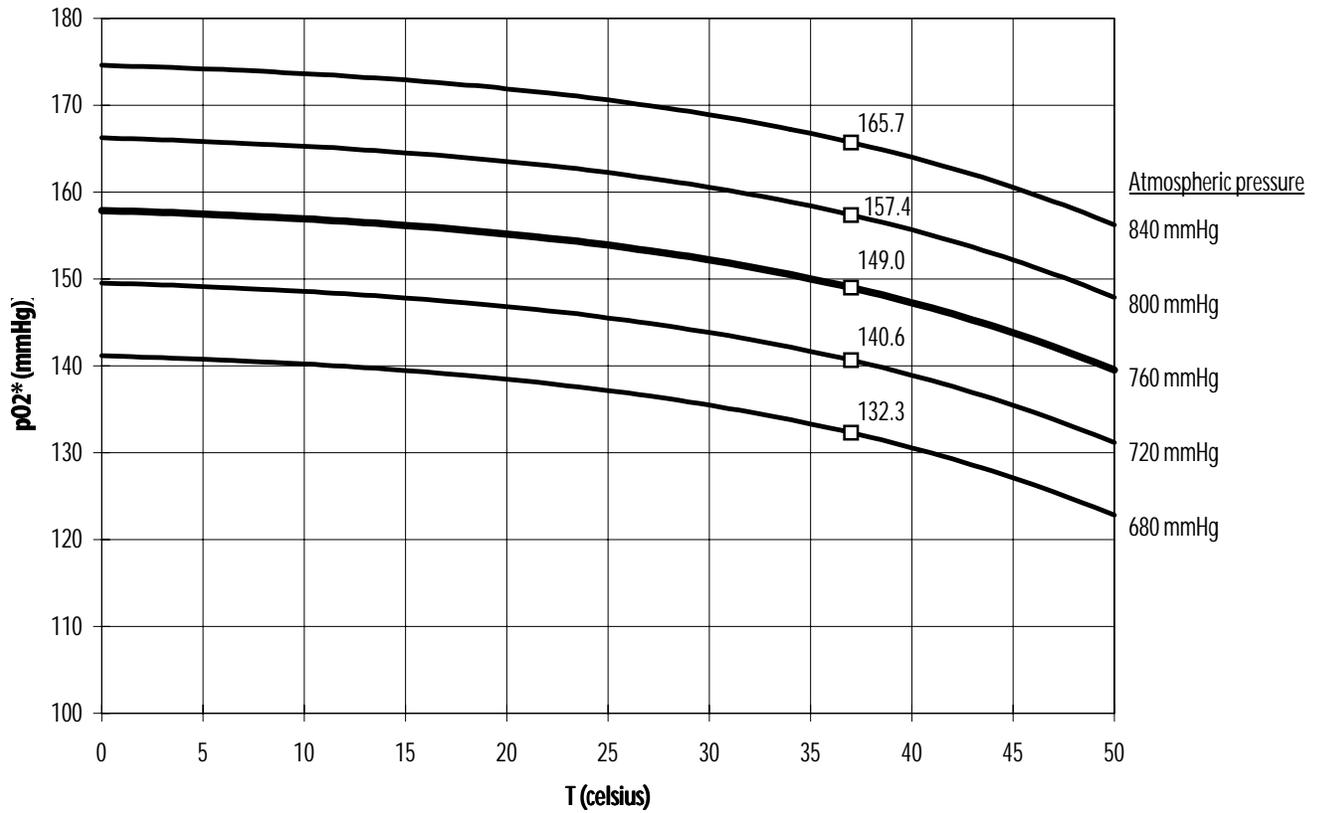
the following chart to understand whether this will affect your experiment, and to choose the best flow cell for your set-up. Typically you will want to use the low flow cell unless your flow rates are greater than 15 ml/minute and the back pressure that the low flow cell generates will bother your experiment.



# Appendix D: Ambient pO<sub>2</sub> vs. Temperature

Use the following chart to help calibrate the amplifier when using buffer solutions equilibrated

with air. When using 95% oxygen solutions, multiply these values by 95/20.9.



\* pO<sub>2</sub> = .209 x (atmospheric pressure – vapor pressure of water)

Source: CRC Handbook (for tables of vapor pressure of water vs. temperature).

## Appendix E: Specifications

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### Electrode (model 125/05)

• Length	1.635	inches
• Body diameter	0.125	inches
• Typical current at ambient pO <sub>2</sub>	18	nanoamps
• Time constant, polyethylene membrane (0-63%)	<4	seconds
• Mating connector (Amphenol part no.)	27-7	

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### Chamber (model SYS600)

• Batch cell cup volume — with stir bar	600	μl (approx.)
• Tube required for water bath connection	3/8" OD	Tygon
• Tubing required for overflow and fill ports	1/8" OD x 1/16" ID	Tygon

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### Amplifier (model 203)

• Size (WxLxH)	10.5x8.75x4.5	Inches
• Weight	3.5	lbs.
• Power requirements — standard model	120	volts AC
	50/60	Hz
• Power requirements — European model	220	volts AC
	50/60	Hz
• Rear panel analog output voltages	0 to 5	volts

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