OXYMICRO

fiber-optic oxygen measurement systems with microsensors

INSTRUCTION MANUAL

PC-controlled one-channel fiber-optic oxygen meter for oxygen microsensors; excitation wavelength of 505 nm; quartz-quartz glass fibers of less than 150 µm diameter connected by ST-fiber connectors. Also available with two 12-bit analog outputs and external trigger input.

022003

World Precision Instruments

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1 Preface

Congratulations!

You have chosen a new innovative technology for measuring oxygen!

The OxyMicro is a compact, easy to transport and PC-controlled fiber-optic oxygen meter. The data evaluation is PC supported.

The OxyMicro was specially developed for very small fiber-optic oxygen sensors. It is based on a novel technology, which creates very stable, internal referenced measured values. This enables a more flexible use of oxygen sensors in many different fields of interest.

Optical oxygen sensors (also called optodes) have important advantages over common Clark type electrodes:

- They are extremely small
- They do not consume oxygen
- Their signal does not depend on the flow rate of the sample

Therefore, they are ideally suited for the examination of very small sample volumes and for measuring oxygen gradients with high spatial resolution in heterogeneous systems. Their small dimensions even allow measurements in living systems.

A set of different microsensors is available to make sure you have the sensor which is ideally suited to your application.

Please feel free to contact our technical support team to find the best solution for your application.

2 Safety Guidelines

PLEASE READ THESE INSTRUCTIONS CAREFULLY BEFORE WORKING WITH THIS INSTRUMENT!

This device has left our works after careful testing of all functions and safety requirements.

The perfect functioning and operational safety of the instrument can only be ensured if the user observes the usual safety precautions as well as the specific safety guidelines stated in these operating guidelines.

- Before connecting the device to the electrical supply network, please ensure that the operating voltage stated on the power supply corresponds to the mains voltage
- The perfect functioning and operational safety of the instrument can only be maintained under the climatic conditions specified in Chapter 10 "Technical Data" in this operating manual.
- If the instrument is moved from cold to warm surroundings, condense may form and interfere with the functioning of the instrument. In this event, wait until the temperature of the instrument reaches room temperature before putting the instrument back into operation.
- Balancing, maintenance and repair work must only be carried out by a suitable qualified technician, trained by us.
- Especially in the case of any damage to current-carrying parts, such as the power supply cable or the power supply itself, the device must be taken out of operation and protected against being put back into operation.
- If there is any reason to assume that the instrument can no longer be employed without a risk, it must be set aside and appropriately marked to prevent further use.
- The safety of the user may be endangered, e. g., if the instrument
    - is visibly damaged;
    - no longer operates as specified;
    - has been stored under adverse conditions for a lengthy period of time;
    - has been damaged in transport
- If you are in doubt, the instrument should be sent back to the manufacturer WPI for repair and maintenance.
- The operator of this measuring instrument must ensure that the following laws and guidelines are observed when using dangerous substances:
    - EEC directives for protective labor legislation;
    - National protective labor legislation;
    - Safety regulations for accident prevention;
    - Safety data-sheets of the chemical manufacturer

The OxyMicrois not protected against water spray;
The OxyMicrois not water proof;
The OxyMicromust not be used under environmental conditions which cause water-condensation in the housing;
The OxyMicrois sealed;
The OxyMicromust not be opened;

We explicitly draw your attention to the fact that any damage of the *manufactural* seal will render of all guarantee warranties invalid.

Any internal operations on the unit must be carried out by personal explicitly authorized by WPI and under antistatic conditions.

Needle-type sensors are housed in extremely sharp syringe needles. Avoid injury by handling the needle carefully. Please pay attention to all safety guidelines for safe handling of sharp needles and syringes. Beware of injuring with the needle as well as with the sensor tip. The glass fiber can break if pricked into the skin and can cause inflammation.

The OxyMicro may only be operated by qualified personal.
This measuring instrument was developed for use in the laboratory. Thus, we must assume that, as a result of their professional training and experience, the operators will know the necessary safety precautions to take when handling chemicals.

Keep the OxyMicro and the equipment such as TEMP100 temperature sensor, power supply and needle type sensors out of the reach of children!

As the manufacturer of the OxyMicro, we only consider ourselves responsible for safety and performance of the device if

- the device is strictly used according to the instruction manual and the safety guidelines
- the electrical installation of the respective room corresponds to the DIN IEC/VDE standards.

The OxyMicro and the microsensors must not be used *in vivo* examinations on humans!

The OxyMicro and the microsensors must not be used for human-diagnostic or therapeutically purposes!

3 Description of the OxyMicroDevice

The **OxyMicro** is a precision, ***temperature compensated***, oxygen meter, designed for fiber-optic oxygen microsensors.

The small outer dimensions, low power consumption and robust box makes it ready for indoor and **outdoor** application.

For data visualisation and storage the instruments have to be connected to a PC computer.

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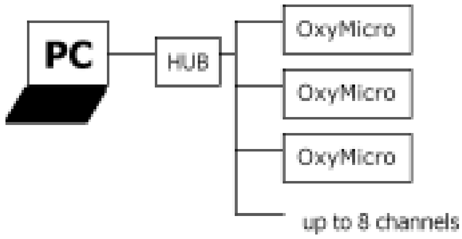
OxyMicro instruments features:

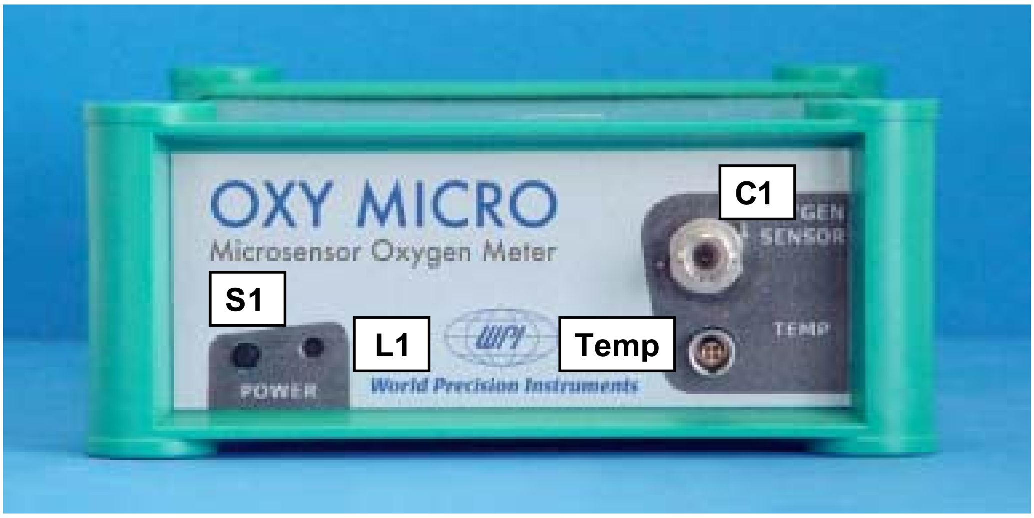
- high precision
- portable (battery power optional)
- analog/digital data output (on request)
- temperature compensation

There also exits the possibility to combine several single OxyMicro oxygen meter to obtain a multichannel system. It allows the user to create a 2, 3, 4 or more channel system.



The OxyMicro oxygen meter contains a dual 12 bit analog output and an external trigger input. The analog output values can be programmed with the PC software (included). The user can choose between oxygen, temperature, amplitude or phase for each channel independently. OxyMicro can be used as a stand-alone instrument when combined with an external data logger.

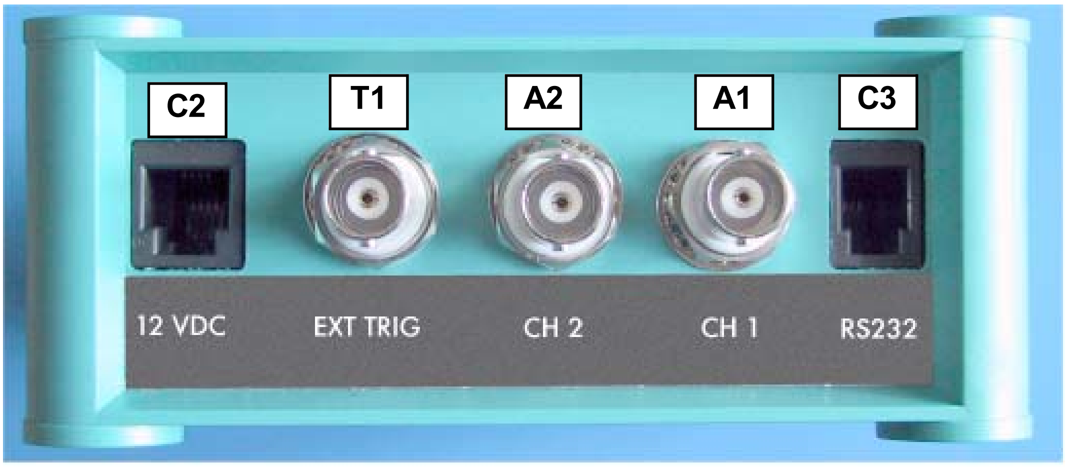
Front Panel



ELEMENT	DESCRIPTION	FUNCTION
S1	ON/OFF switch	Switches the device ON and OFF
C1	ST fiber connector	Connect the fiber-optic oxygen microsensor here.
L1	Control LED	red: instrument off; green: instrument on; orange: stand by;
Temp	Connector for TEMP 100 temperature sensor	Connect the TEMP 100 temperature sensor for temperature compensated measurements here.

Rear Panel of the OxyMicro device

Two standard BNC connectors are added for analog output channels 1 and 2, another one for external trigger input. See figure below.
The electrical specifications of all rear panel connectors are given in technical specification sheet. Please read also the technical notes to avoid mistakes.



ELEMENT	DESCRPTION	FUNCTION
C2	Line adapter for power supply	Connector for 9 - 36 V DC power supply.
C3	RS232 interface (male)	Connect the device with a RS232 data cable to your PC/Notebook here.
A1	Analog out (channel 1)	Connect the device with external devices, e.g. a data logger
A2	Analog out (channel 2)	Connect the device with external devices, e.g. a data logger
T1	External trigger input	Connect the device with external devices, e.g. data logger with a trigger output, pulse generator

Features

- microsensor oxygen meter with temperature compensation
- 2 x 12bit, programmable analog channels, with optical isolation
- measuring range 0 - 500% air saturation
- 9 – 36 V supply voltage (or 220/110V AC adaptor)
- RS 232 interface
- robust metal box

4 Required Basic Equipment

- Oxygen meter OxyMicro*
- Software for OxyMicro*
- PC / Notebook
  (System requirements:
  Windows 95/98/2000/Millenium/NT 4.0; Pentium processor, at least 133 MHz, 16 MB RAM)
- RS 232 Cable *
- Line adapter (110 - 220 V AC, 12 V DC) *
- Temperature sensor TEMP 100*
- Oxygen-sensitive microsensor
  The microsensors are mounted into different types of housings
- Vessels for calibration standard 100 (water-vapor saturated air ∫ 100 % air saturation) and calibration solution 0 (oxygen-free water)
- Laboratory support with clamp, micro-manipulator

*included with the order

5 Fiber-Optic Oxygen Microsensors: Sensors and Housings

5.1 Oxygen-Sensitive Microsensors

5.1.1 Sensor Characteristic

The principle of the sensor operation is based on the quenching of luminescence caused by collision between molecular oxygen and luminescent dye molecules in the excited state. Figure 5.1 shows a typical response curve of an oxygen-sensitive microsensor. In the presence of oxygen the signal - in our case the phase angle Φ - decreases. The phase angle Φ can be related to the oxygen content as shown in Figure 5.2. The theoretical aspects are explained more detailed in the appendix.

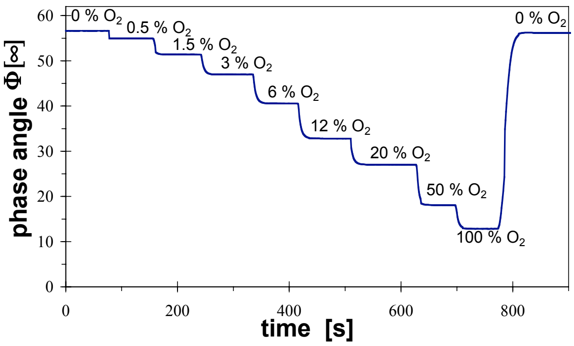


Figure 5.1 Response of an oxygen microsensor toward changes in the oxygen concentration.

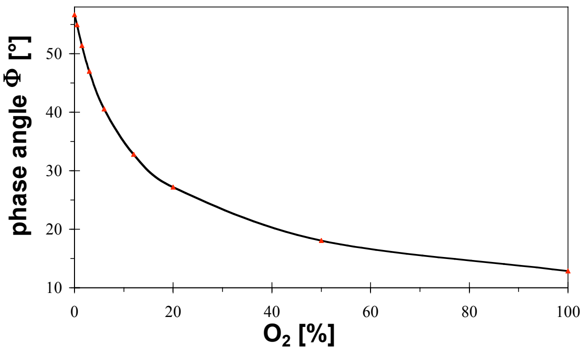


Figure 5.2 Effect of the phase angle of an oxygen microsensor on different oxygen contents

Measuring range

The optimal measuring range of WPI oxygen-sensitive microsensors is from 0 to 50 % pure oxygen (_250 % air-saturation). However, it is also possible to measure oxygen up to 100%. Please contact the WPI service team to get the appropriate software when measuring up to 100 % oxygen.
Table 5.1 displays the measuring range of WPI microsensors in different oxygen units.

Table 5.1 *Measuring range of WPI microsensors.*

oxygen unit	optimal measuring range	maximal measuring range
air-saturation	0 - 250 %	0 - 500 %
oxygen-saturation	0 - 50 %	0 - 100 %
hPa	0 - 500 hPa	0 - 1000 hPa
Torr	0 - 400 Torr	0 - 760 Torr
mg/L (∫ppm)	0 - 22.6 mg/L	0 - 45 mg/L
µmol	0 - 800 µmol	0 - 1.4 mmol

Resolution

The WPI OxyMicro has a phase resolution of better than 0.05°. Since the oxygen calibration plot displays a non-linear behavior, the oxygen resolution is determined for three different partial pressures at 20 °C. The resolution in oxygen is also transformed in different oxygen units.

Table 5.2. *Oxygen resolution at different oxygen contents at 20 °C and 1013 mbar.*

hPa	***% air-sat.***	***mg/L (∫ppm)***
2 ± 0.1 hPa *60* ± 0.26 hPa 200 ± 0.87 hPa 500 ± 3.54 hPa	1 ± 0.05 % air-sat. 30 ± 0.13 % air-sat. 100 ± 0.44 % air-sat. 250 ± 1.8 % air-sat.	0.09 ± 0.005 mg/L 2.72 ± 0.01 mg/L 9.06 ± 0.04 mg/L 22.65 ± 0.17 mg/L
Torr	***% oxygen***	***µmol***
1.55 ± 0.08 Torr 46.7 ± 0.20 Torr 155.5 ± 0.65 Torr 388.8 ± 2.65 Torr	0.21 ± 0.01 % oxygen 6.3 ± 0.03 % oxygen 20.9 ± 0.09 % oxygen 52.4 ± 0.38 % oxygen	2.83 ± 0.14 µmol 85.0 ± 0.3 µmol 283.1 ± 1.3 µmol 798.0 ± 5.2 µmol

Temperature:

Microsensors can be used in the temperature range of -10 to 80 °C. WPI offers a TEMP100 temperature sensor in combination with the OxyMicro to record temperature variations which are compensated using the OxyMicro software (see Chapter 7, ***Calibration of Microsensors*** and Chapter 8, ***Measurement***). In the appendix you will find a detailed description of the ***Temperature Dependent Constants Affecting the Oxygen Content*** (chapter 13.4) and ***Temperature Compensation of the Response of Oxygen Sensors*** (chapter 13.5).

Cross sensitivity:

There exists no cross sensitivity for carbon dioxide (CO_2), hydrogen sulfide (H_2S), ammonia (NH_3), pH, any ionic species like sulfide (S_2^-), sulfate (SO_4^{2-}) or chloride (Cl^-).

The sensors can also be used in methanol- and ethanol-water mixtures as well as in pure methanol and ethanol.

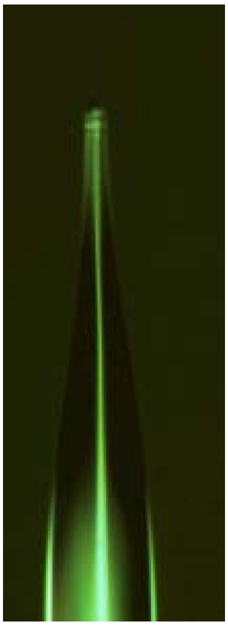
We recommend not to use other organic solvents, such as acetone, chloroform or methylene chloride, which may swell the sensor matrix.

Interferences were found for gaseous sulfur dioxide (SO_2) and gaseous chlorine (Cl_2).

5.1.2 Design of the Sensor Tips

All the sensors mounted in different housings are available with two different glass-fiber tips, (**A**) a < 50 µm tapered tip and (**B**) a 140 µm flat-broken tip.

(**A**) ***Tapered sensor tip***



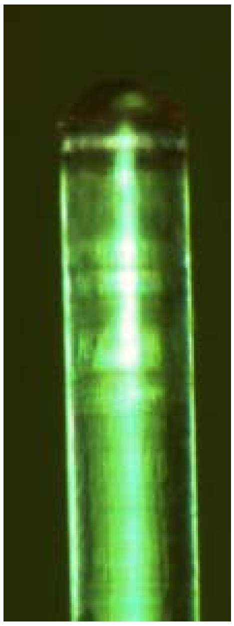
Advantages of microsensors with a tapered tip

- high spatial resolution (<50 µm)
- very fast response times (t_{90} up to 1 s in the liquids and < 0.2 s in the gas phase)

Disadvantages of microsensors with a tapered tip:

- fragile
- display photobleaching

(**B**) ***Flat-broken sensor tip***



Advantages microsensors with a flat-broken tip

- more photostable than tapered ones

➔ long-term stable

- more robust

Disadvantages of microsensors with flat-broken tip

- response times (t_{90}) in the order of 30 s

Response time

Of course, the response time (t_{90}: time for 90% of the total signal change) in water is dependent from the sensor tip size and typical ranges from < 3s (for a sensor tip tapered < 50 µm) to about 30 s for a flat broken 140 µm tip.

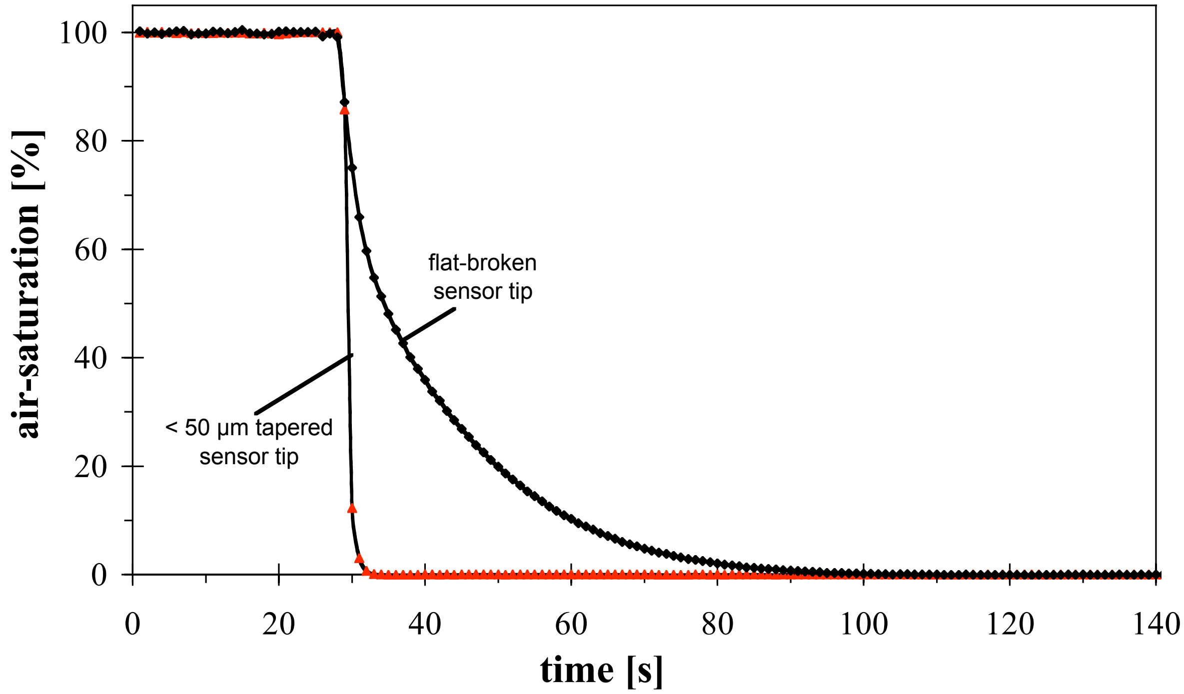


Figure 5.3 *Comparison of the response characteristics of a microsensor with a sensor tip tapered < 50 µm and a microsensor with a flat-broken sensor tip (Δ 140 µm).*

Photo-Decomposition

The oxygen-sensitive material may be subject to photo-decomposition resulting in a signal drift. Photo-decomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light.

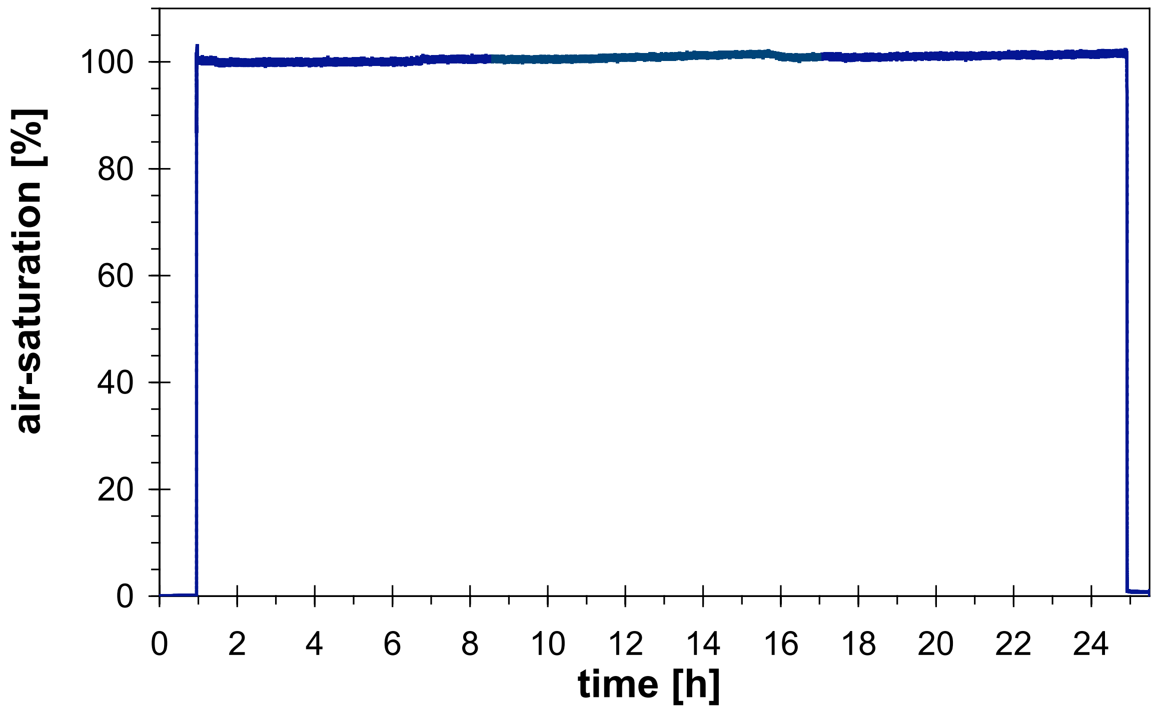


Figure 5.4 *Photostability of a tapered oxygen microsensor.*
Drift in % air-saturation at 100% air-saturation when illuminating the microsensor with a tapered and flat broken sensor tip for 1, 12 and 24 hours in the continuous mode

Mode	Drift per hour (3600 measuring points)	Drift per 12 hours (43200 measuring points)	Drift per 24 hours (86400 measuring points)
tapered sensor tip			
continuous mode (1 s)	< 0.6 % air-saturation	+1 % air-saturation	+1.6 % air-saturation
flat broken sensor tip			
	< 0.5 % air-saturation	< 0.5 % air-saturation	< 0.6 % air-saturation

5.1.3 Optical Isolation

Optical isolated sensor tips are required, if your sample shows intrinsic fluorescence between 600 - 660 nm. Consequently, an optical isolation is recommended measuring in whole blood, urine or chlorophyll containing samples. Using optical isolated sensors exclude the impact of colored samples and ambient light on measurements. Furthermore, an additional optical isolation improves chemical resistance of the sensor membranes.

Optical isolated sensor tips of oxygen microsensors enable measurement in photosynthetically active samples, since stimulation of photosynthesis, due to emission of blue-green light from the fiber tip, is avoided.

WPI offers additional optical isolation for all types of sensor. However, using optical-isolated sensor tips, the tip diameter increases to 60 - 80 µm, whereas tip diameter of non-isolated sensor tips is typically 30 to 40 µm for tapered microsensors. Since the response time of the microsensor is dependent from the diffusion rate of oxygen through the sensor layer, additional optical isolation, of course, increases the response time (t_{90}) to 3-7 seconds while the response time (t_{90}) of non isolated sensors is 1-3 seconds.

5.2 Housings of Oxygen-Sensitive Microsensors

WPI fiber-optic oxygen microsensors are based on 140 µm silica optical fibers. To protect the small glass fiber tip against breaking, suitable housings and tubings around it, depending on the respective application, were designed.

Of course, it is possible to build ***customer-specific*** housings. Please feel free to contact our service team to find the best solution for your application.

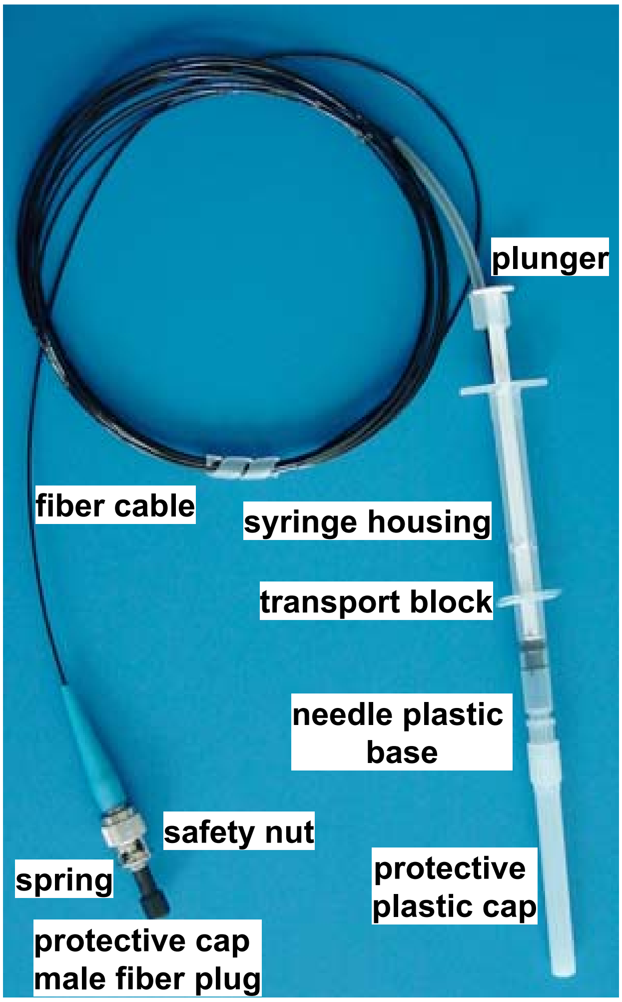
WPI offers the following standard designs:

Needle-type housing oxygen microsensor.

Flow-through cell housing oxygen microsensor.

Implantable oxygen microsensor.

5.2.1 Needle-Type Housing Oxygen Microsensors



WPI offers highly flexible needle-type oxygen microsensors. Needle-type oxygen microsensors are miniaturized optical chemical oxygen sensors designed for all research applications were a small tip size (< 50µm to 140 µm) and fast response time (t_{90} up to 1s) are necessary.

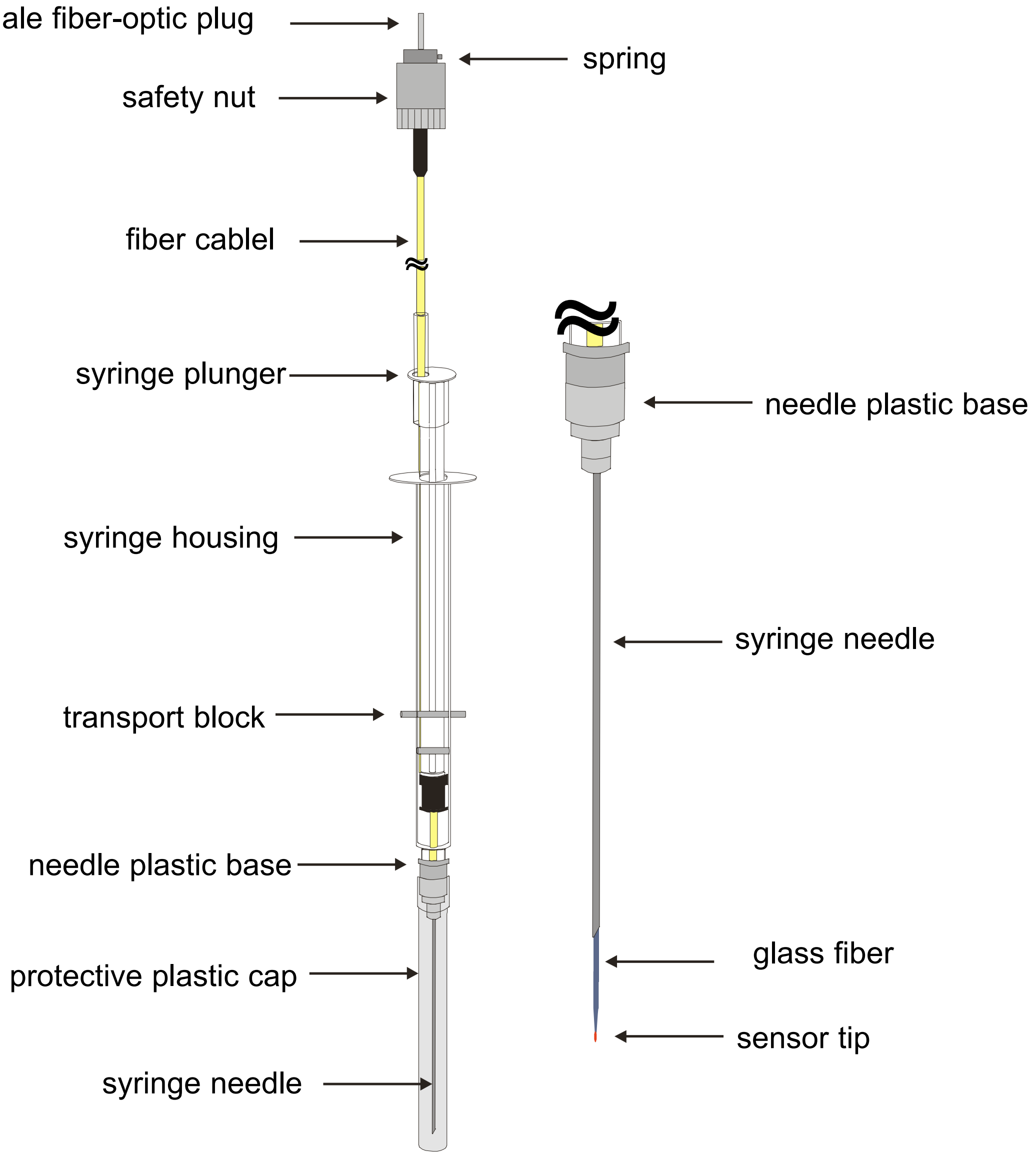
Needle-type oxygen microsensors are ideal for measuring oxygen distribution profiles in sediment and biofilms with a high spatial resolution of less than 50 µm. The glass-fiber with its oxygen-sensitive tip is protected inside a stainless steel needle and can be extended for measurement. If the sensor tip is sheltered inside this needle, it can be easily penetrated through a septum rubber or any other harsh material.

A 1 mL syringe tube made from polypropylene is used as the probe housings.

Features

- high spatial resolution
- penetration probe for insertion into semisolids like sediments or biofilms
- easy to handle and robust
- no breaking of tips during storage, transport or shipment
- sterilizable (H_2O_2, EtOH)
- **not** autoclavable since the syringe is made out of polypropylene (For autoclavable needle-type probe housings please contact our service team)

Schematic drawing of a needle-type housing microsensor



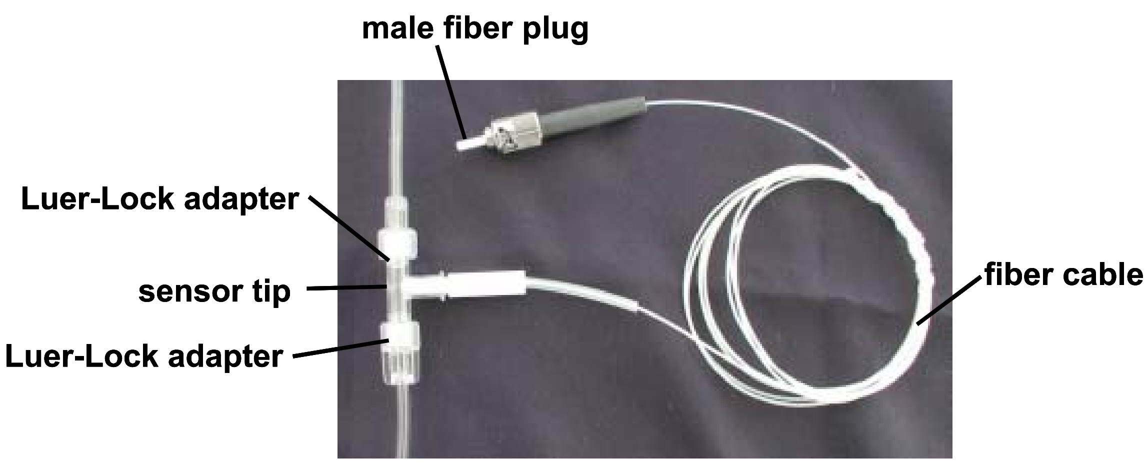
Ordering information

WPI # 501656 MicroTip, needle type housing fiber-optic oxygen sensor.

With this catalog # you will order a microsensor mounted in a needle-type housing, with a glass fiber length of 2.5 m, a sharp-tapered sensor tip of smaller than 50 µm, mounted in a stainless needle of 22 mm length and 0.4 mm diameter.

5.2.2 Flow-Through Cell Housed Oxygen Microsensors

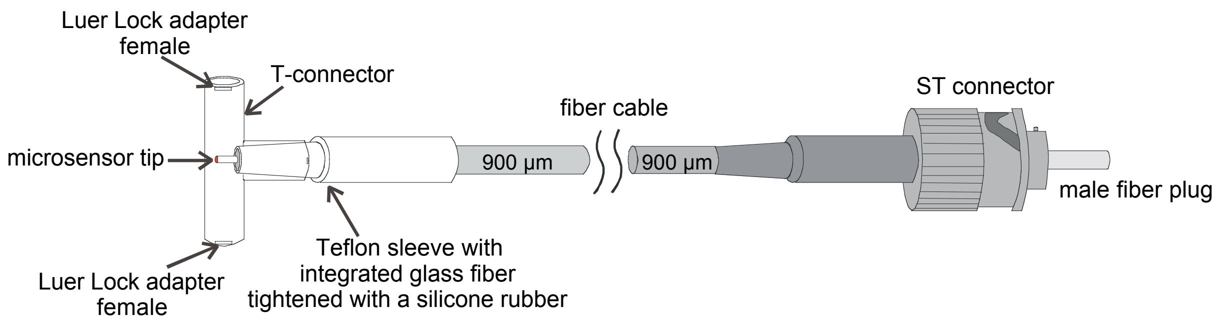
WPI offers miniaturized flow-through-cells with integrated oxygen microsensors. They can be connected via Luer-Lock adapters to tubings. Liquids (water, blood) can be pumped through the cell. Online monitoring in real-time is possible.



Features

- easy to handle and robust
- online monitoring
- sterilizable (autoclave (130 °C, 1.5 atm), EtOH, H_2O_2)

Schematic drawing of flow-through cell housed microsensors

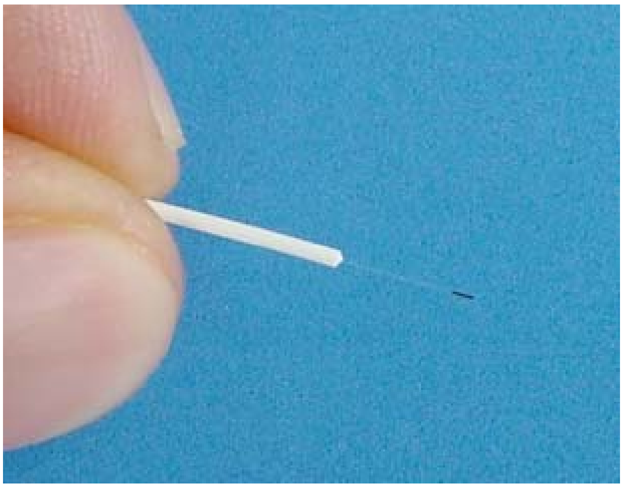


Ordering Information

WPI # 501657 MicroFlow, flow-through housed oxygen microsensor

With this catalog # you will order a flow-through cell with an integrated microsensor, with a glass fiber length of 2.5 m, a sharp-tapered sensor tip of smaller than 50 µm.

5.2.3 Implantable Microsensors



WPI offers highly flexible implantable oxygen microsensors.

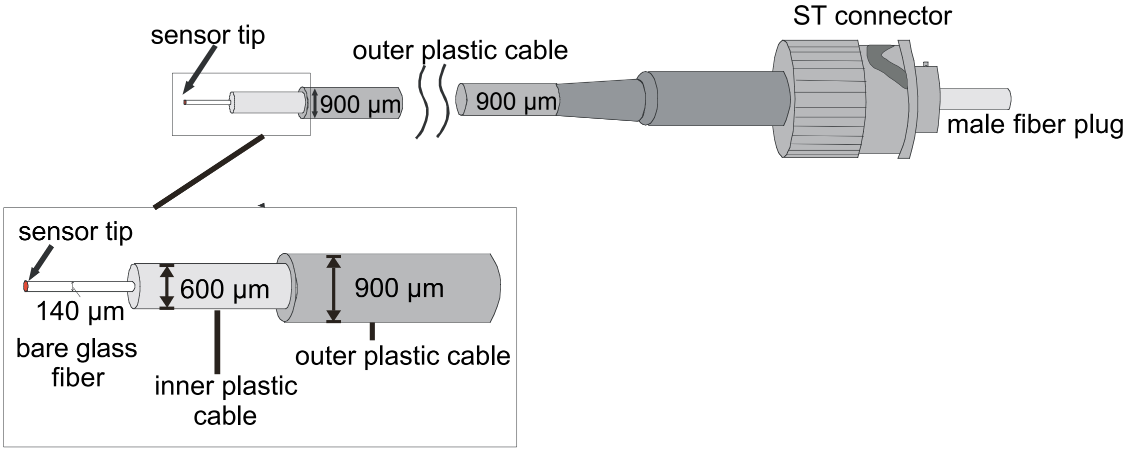
The microsensor tip is not housed in any additional housing. The bare glass-fiber tip can be mounted to your own costum-made housing, home-made steel tubes, costum-made micro respirometer chambers, etc. It can be deployed in soil or implanted into the blood circuits of living animals or the liquid circuits of trees to measure oxygen online and in real-time.

Small outer diameters of 900 or even 600 µm allows insertion into implanted Venflon-tubes.

Features:

- high spatial resolution
- high flexibility
- without any housings (the microsensor is protected with a glass housing during the transport)
- sterilizable (autoclave (130 °C, 1.5 atm), EtOH, H_2O_2)
- implantation into animal blood circuits
- soil implantation
- implantation in customer-made housings

Schematic drawing of implantable microsensors



Ordering Information

WPI# 501658 Microplant, implantable oxygen microsensors

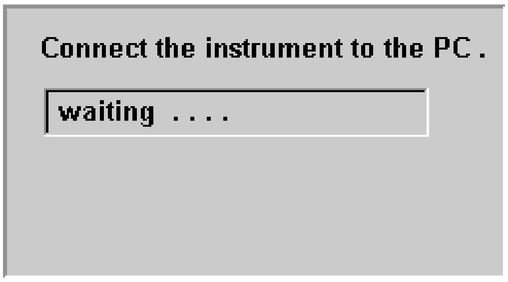
With this catalog # you will order an implantable microsensor mounted in a glass housing. The outer plastic cable with a diameter of 900 µm is 2.5 m long, the inner plastic coating (∆ 600 µm) user specified length in cm and a bare glass fiber length (∆ 140 µm) of 5 mm. a sharp-tapered sensor tip of smaller than 50 µm.

6 Description of OxyMicro Software

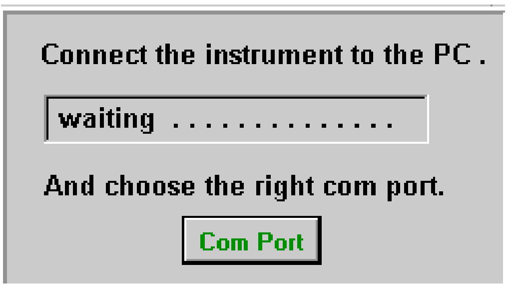
This software is compatible with Windows 95/98/2000/Millenium/NT4.0/XP.

6.1 Software Installation and Starting the Instrument

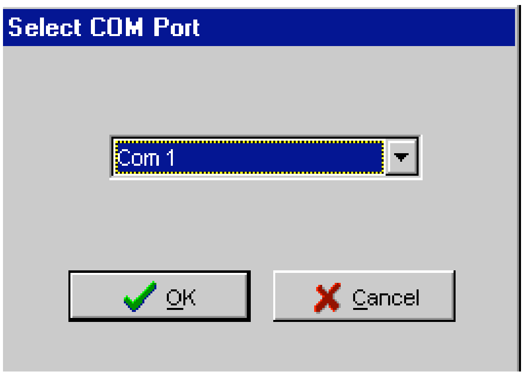
1. Insert the supplied disc/CD into the respective drive. Copy the file oxymicro01.exe onto your hard disk. (for example, create C:\OXYMICRO\oxymicro01.exe). Additionally, you may create a link (icon) on your desktop.
2. Connect the OxyMicro via the supplied serial cable to a serial port of your computer. Tighten the cable with the screws on your computer and on the OxyMicro.
3. Connect the power supply.
4. Please close all other applications as they may interfere with the software. Start the program oxymicro01.exe with a double click. The following information window appears:



5. If the left com port is adjusted this information window disappears within a few seconds. If the wrong com port is adjusted you are asked to set the right com port:



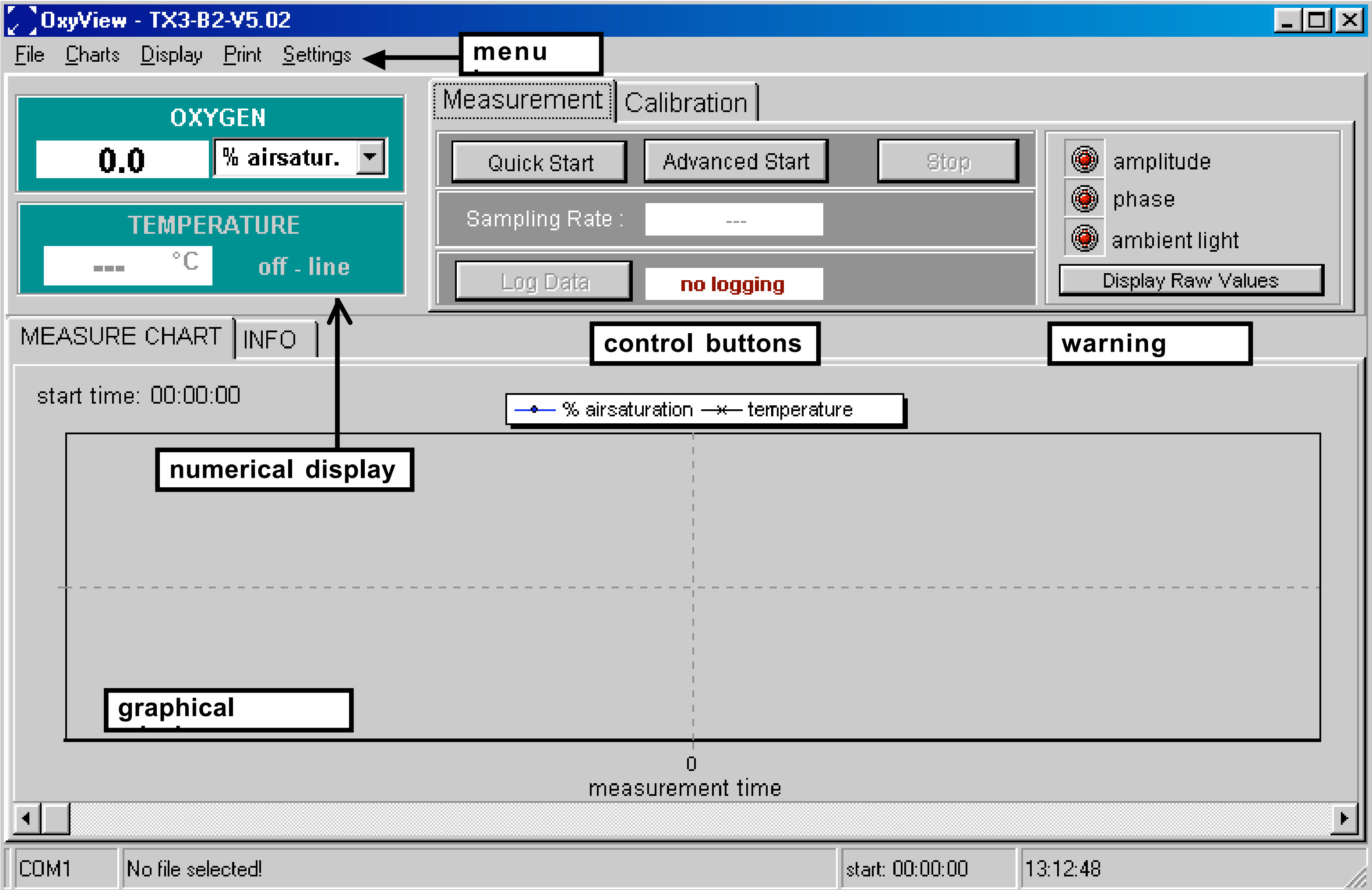
With a right mouse click onto '***com port***' you are able to set the right com port. Please confirm your selection by clicking the '***OK***' button. The information window disappears if the right com port is adjusted.



6.2 Function and Description of the OxyMicroProgram

The window shown below is displayed after starting the software microx.exe:
The program has 4 main sections:

1. Menu bar
2. Graphical window
3. Status bar
4. Control bar, divided into numerical display, control buttons and warning lights



6.2.1 Menu Bar

File	***Charts***	***Display***
→ Exit	→ Oxygen	→ Zoom → AutoScaleY1 → Undo Zoom
	→ Phase	
	→ Amplitude	→ Clear Charts
	→ Temperature	→ Dimensions

Print	***Settings***
→ Charts	→ Com Port
	→ Instrument Info
	→ analog settings
	→ LED Intensity

File

Exit

Closes the program.

Charts

The respective charts of the measurement can be displayed (√) or hidden

Oxygen:
Oxygen content in the chosen unit

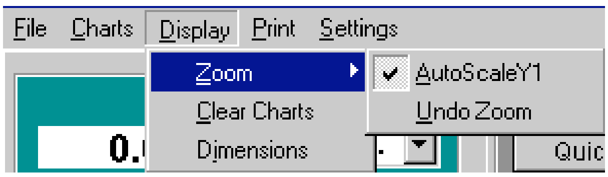
Phase:
Phase angle, the raw data

Amplitude:
The magnitude of the sensor signal

Temperature:
The measured temperature

Display

Zoom:

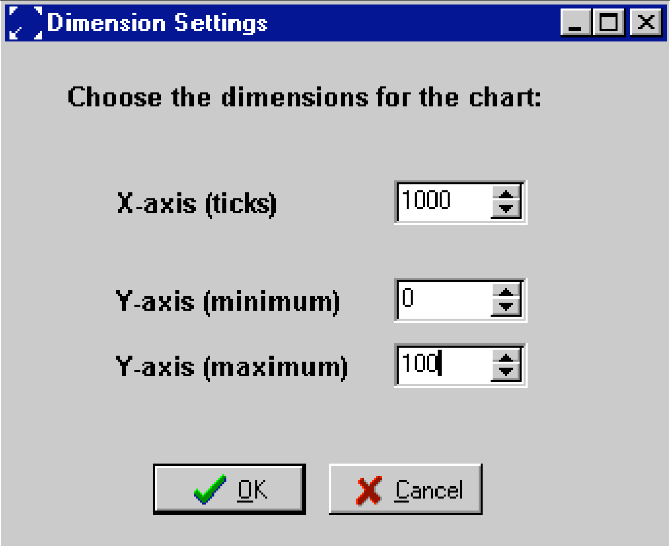


AutoScaleY1 is the default setting. AutoScaleY1 means that the y-axis is scaled automatically.

Undo Zoom: The original display is recovered; see also graphical display

Clear Charts: The graphs shown on the display is cleared.

Dimensions:



You can adjust the number of measurements points on the x-axis shown in the display (maximum number of points are 5000)

Furthermore, you can adjust the minimum and the maximum of the y-axis.

The AutoScaleY1 function is switched off.

Print

Charts: *The charts shown in the display can be printed*

Settings

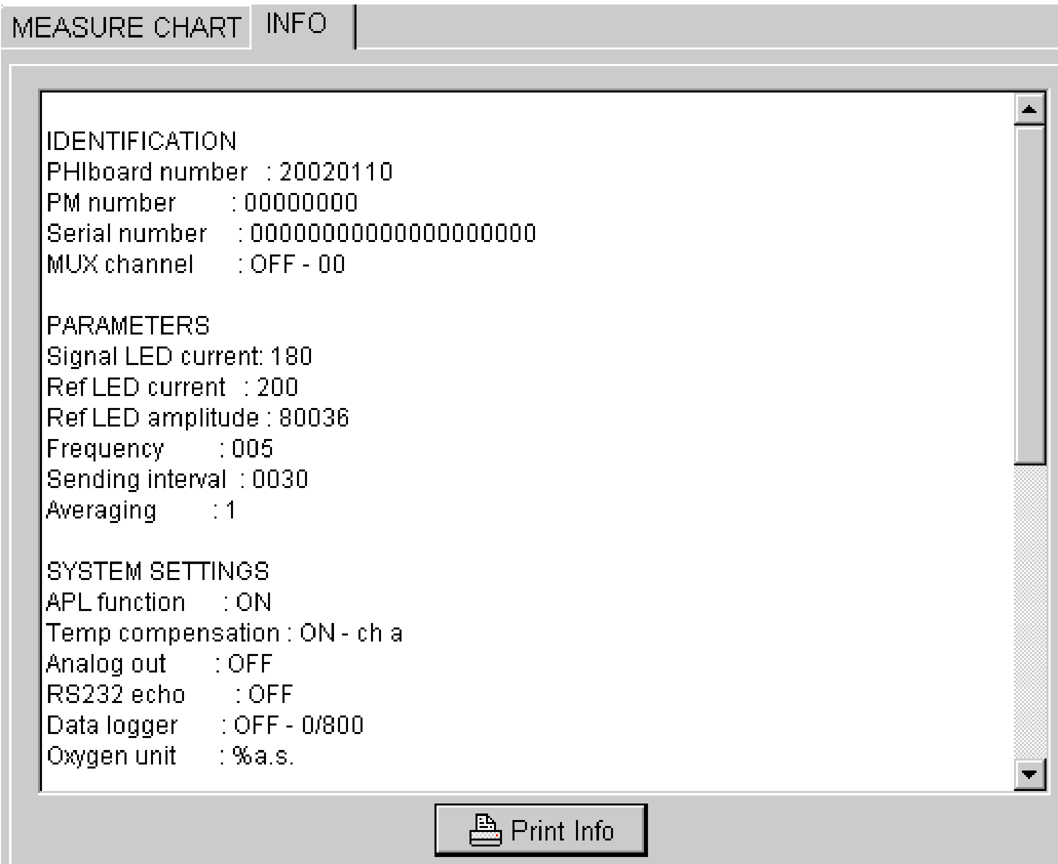
ComPort

The serial comport (com1 – com20) for the serial interface (RS 232) can be chosen in this window. COM 1 is the default setting. If you choose the wrong Com port, the information window '***Connect the instrument to the PC and choose the right com-port***' does not disappear.

Instrument Info:

Here you can find the version of the software and some important settings of the instrument. If you have a problem with the OxyMicro oxygen meter, please contact our service team and have the software and instrument information ready.
To change back to the graphical window click the '***Measure Chart***' button.

Instrument Info



LED-Intensity

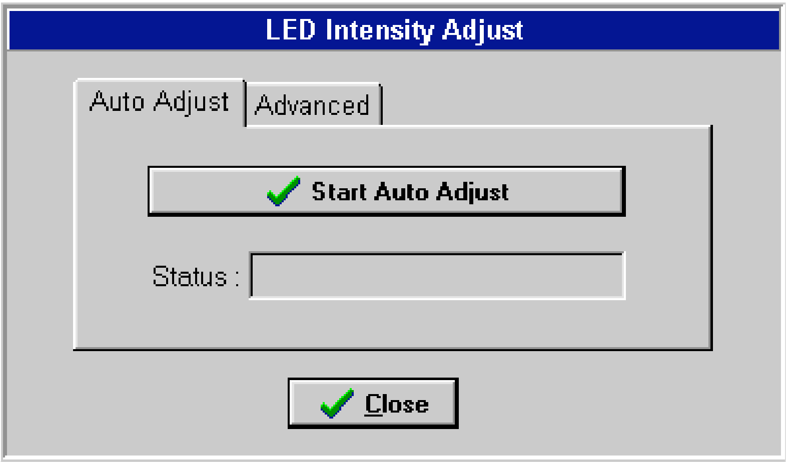
With the current of the LED you can adjust the amount of light illuminating the sensor spot.

You can choose between an '**Auto Adjust**' of the LED where the OxyMicro adjusts the optimal LED current itself, or you can select '**Advanced**' where you can adjust the LED current yourself.

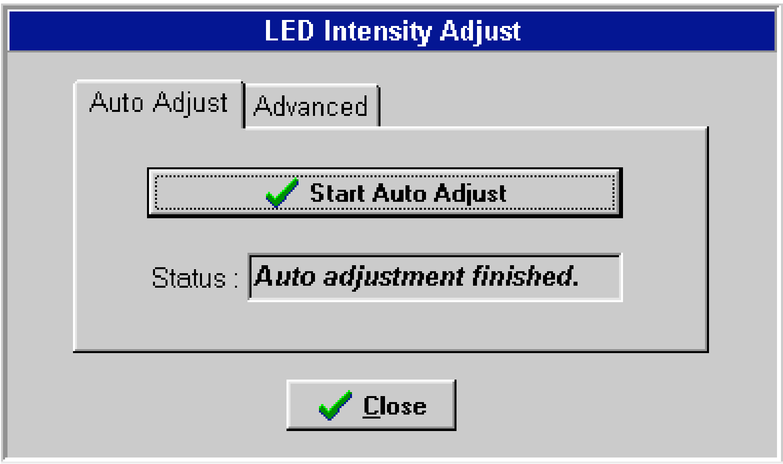
If you increase the LED current, the signal amplitude increases, since a higher light density illuminates the sensor spot.

Auto Adjust:

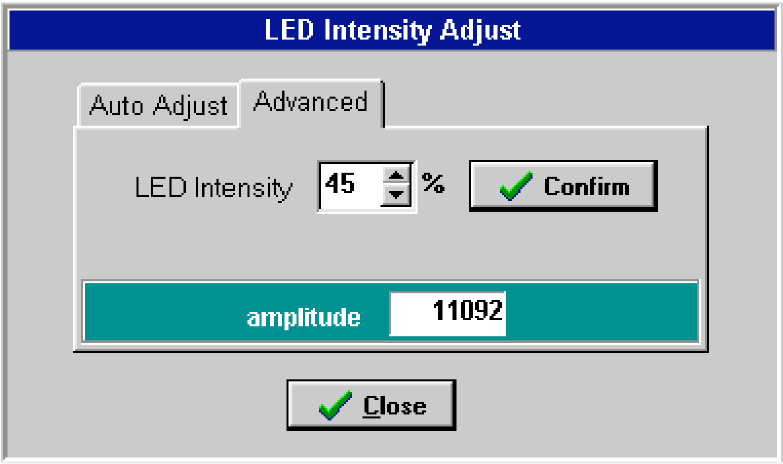
To make the adjustment of the LED intensity automatically, just click the button '**Start Auto Adjust**'. Please check that the oxygen microsensors has been connected to the instrument.



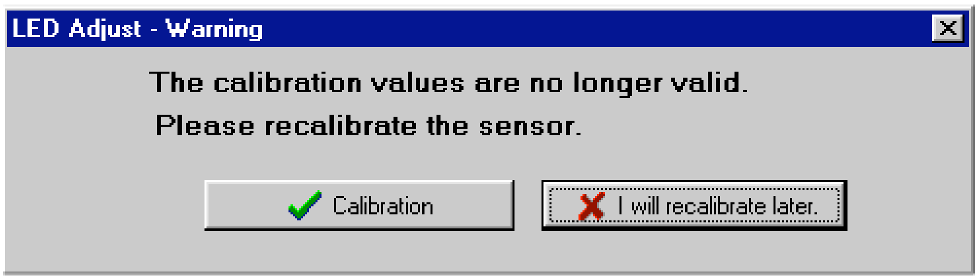
The automatically adjustment of the LED intensity is finished when in the status window the message '**Auto adjustment finished**' appears. Click the '**Close**' button to confirm the settings.



Advanced:
Click the '**Advanced**' button to change the LED current manually. Values between 10 and 100 % are possible. After clicking the 'confirm' button you can see the change of the amplitude in the window below.



Please note, that after changing the LED intensity you should re-calibrate the oxygen microsensor. A warning window points you to re-calibrate the oxygen microsensor.



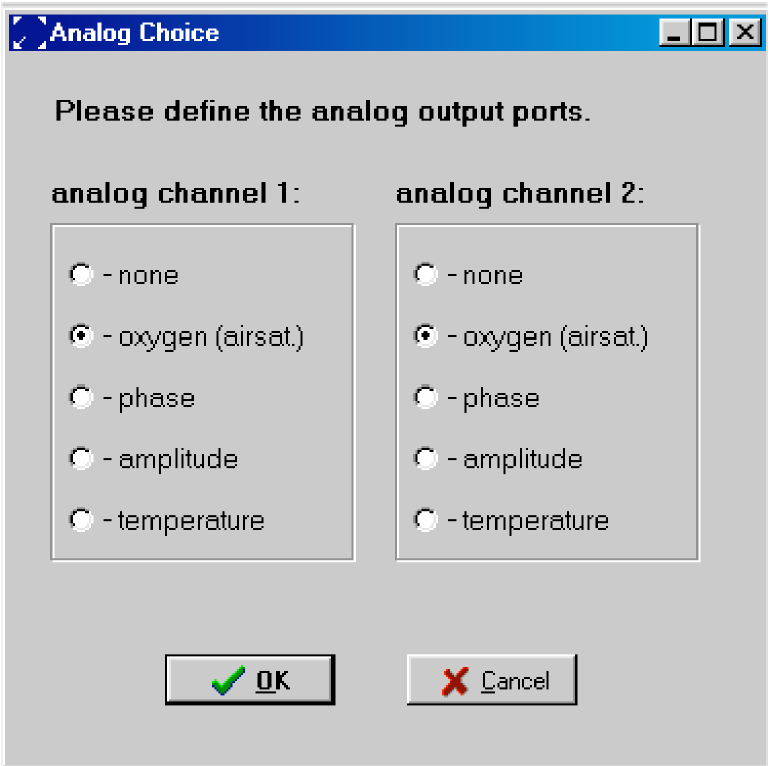
Please note:

By increasing the light intensity you increase the amplitude of the oxygen microsensor. This leads to smoother phase signals. However, increasing the light intensity can increase photobleaching, which decreases the shelf-life of your sensor.

Analogue output
Here you can choose which data are exported via the analog output. The OxyMicrodevice has two analog outputs and one trigger input. The desired data sources (oxygen, temperature, amplitude, phase) can be chosen via the dialog box.

Equivalence coefficient

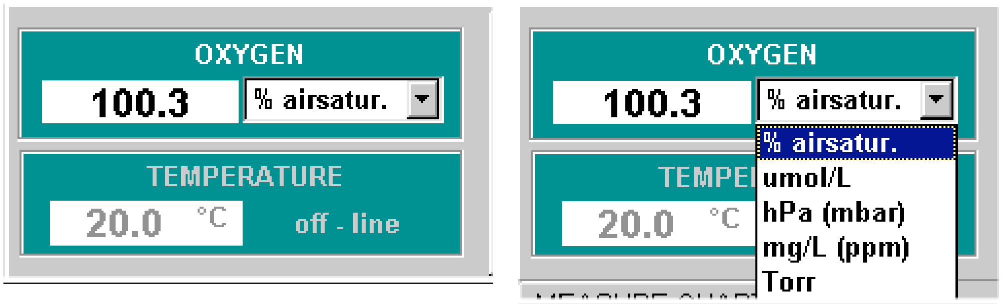
oxygen	1 : 0.1 (e.g. 973mV = 97.3 % air saturation)
temperature	1 : 0.1 (e.g. 208mV = 20.8°C)
amplitude	1 : 20 (e.g. 1110mV = 22200 relative units)
phase	1 : 0.025 (e.g. 1100mV = 27.50°)



Please note:
If you have adjust the desired settings of the analog outputs and want to connect the instrument to a data logger please close the software to store the settings before you disconnect the OxyMicro from the computer.

6.2.2 Control Bar

Numerical display



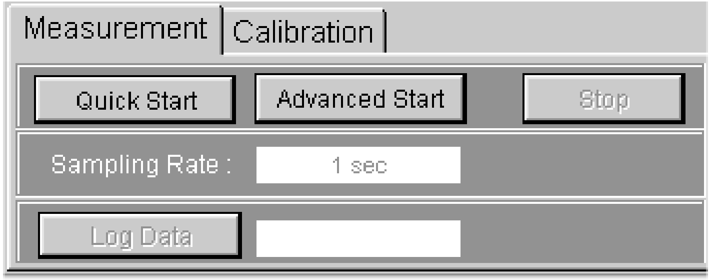
The actual oxygen content in the chosen unit (here % air-saturation) is displayed in the oxygen window. The oxygen unit can be changed by clicking the pull down menu. Tables and formulas for the calculation of different concentration scales are given in the appendix.

Please note:
It is also possible to change the oxygen unit during the measurement.

Temperature measurement:
The actual temperature value of the sample (in the case of temperature compensated measurements) is displayed in the temperature window.
If measurement is performed without temperature compensation, the manual inserted temperature is displayed with the hint that temperature measurement is off–line.

Control buttons:

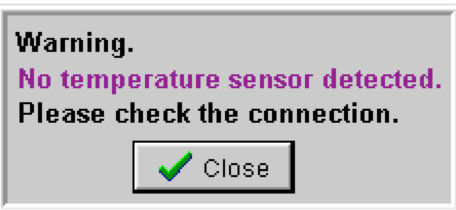
The way to start a measurement is
(A) Calibration of the microsensor with the Calibration Assistant
(B) Start Measurement with Assistant
(C) Log Data



(A) Calibration:
The calibration assistant is opened (see chapter 7, Calibration of Oxygen-Sensitive Microsensors);

(B) Measurement:
The measurement assistant is opened (default setting).

Quick Start:
The measurement is started. The measurement settings are continuous mode which means that each second a new measurement data is recorded. The measurement is temperature compensated i.e. a temperature sensor has to be connected. If no temperature sensor is connected the following warning window appears.



Click the '**Close**' button if you want to continue the measurement without temperature compensation. The temperature is set to 20 °C by the software and temperature compensation of the oxygen content is based on 20 °C.

Connect the temperature sensor if you want to perform a temperature compensated measurement.

If you want to change the measurement stetting click the '**Advanced start**' button.

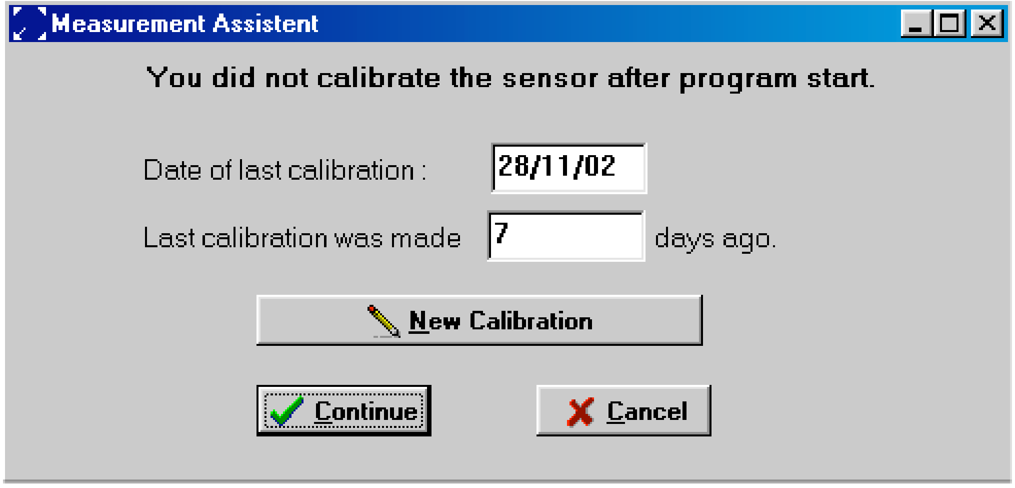
Please note:

The measurement values are not stored. Click the '**Log Data**' button to store the measurement data.

Advanced Start:

In the '***Advanced Start***' mode it is possible to adjust user-defined measurement settings.

If you haven't performed sensor calibration yet the following window appears



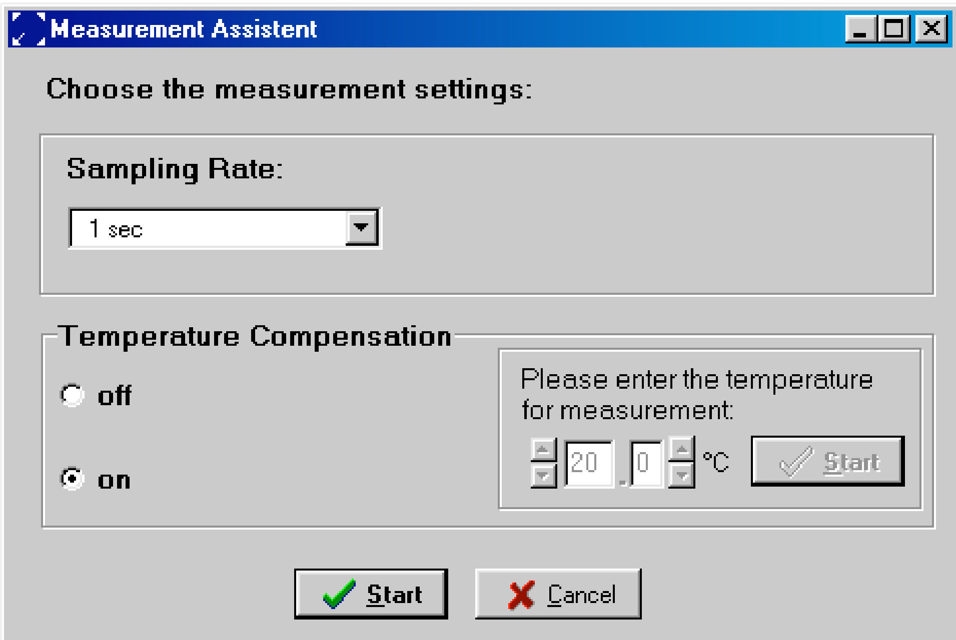
If you want to measure with the last sensor calibration - you can find the '***date of the last calibration***' in the window - click the '***Continue***' button. To obtain reliable results we strongly recommend to perform a sensor calibration before measurement by clicking the '***New calibration***' item when connecting a new sensor.

Follow the instructions given in chapter 7 to calibrate the respective microsensor.

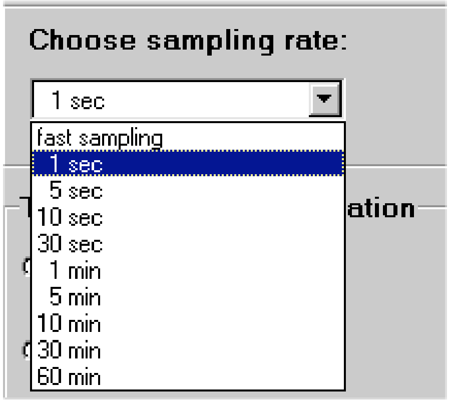
To leave this menu click the '***Cancel***' button.

If you have already performed sensor calibration, the measuring assistant will be opened.

In this window you can choose the measurement settings:



In the ‘**Sampling Rate**’ window you can select the desired measurement mode with a drop-down menu.



By clicking the drop down menu you can choose from ‘***fast sampling***’ (update rate each 250 – 350 ms) to the ‘***60 min***’ mode where each hour a measuring point is recorded.

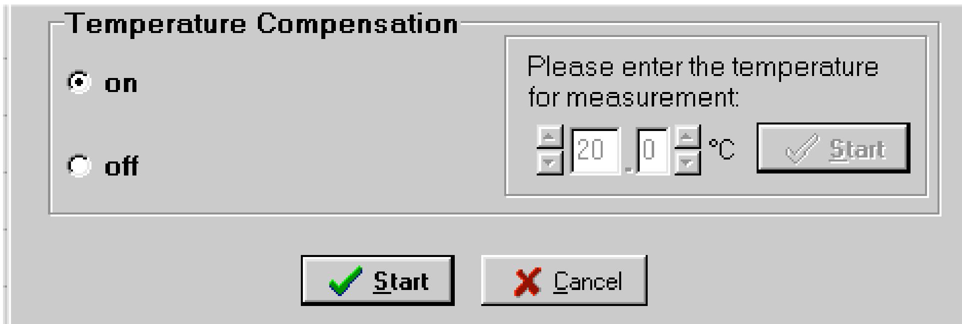
The speed of recording a measurement point in the ‘***fast sampling***’ mode is about 250 ms when no temperature sensor is connected and decreases to about 350 ms when connecting a temperature sensor or activating the analog output channels.

Please note:

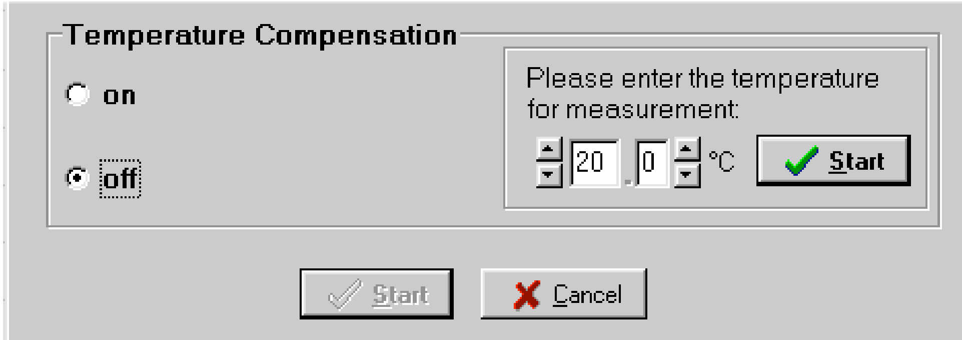
The sensor shelf life can be increased using a slower measuring mode since the effect of photo-bleaching is reduced. The illumination light is switched off between sampling. A further advantage using a high measuring mode is that huge amounts of data for long-time measurement can be avoided.

In the ‘***temperature compensation***’ window you can decide whether you want to measure with or without temperature compensation.

If you want to measure with temperature compensation, click the ’***on***’ button. Please ensure that the temperature sensor TEMP 100 is connected to the OxyMicro, before you click the ‘***Start***’ button to continue. The window where you can enter the temperature manually is disabled.



If you want to measure without temperature compensation, choose the '***off***' button. You will now be requested to enter the temperature of the sample manually. Click the '***Start***' button to start the measurement.

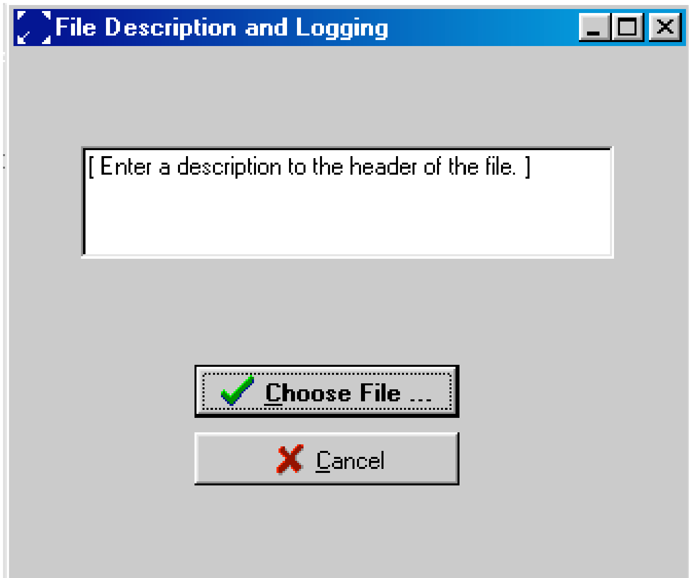


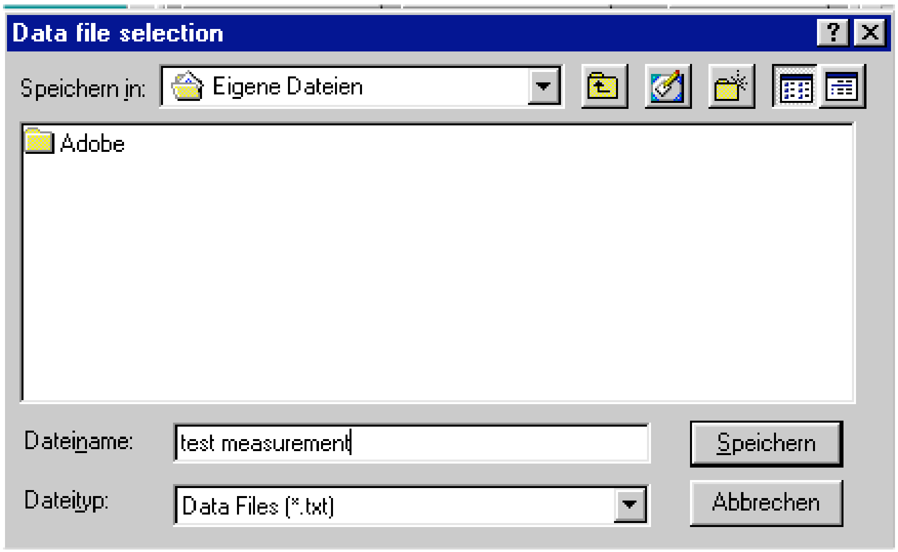
(C) Log Data:

To store the data of your measurement click the '***Log Data***' item. Next to the '***Log Data***' item an information window displays whether the actual measurement is stored to a file (***logging***) or not (***no logging***);

The measurement description which you can enter in the text field '***Enter a description to the header of the file***' is stored in the Ascii File.

By clicking the button '***Choose File***', you can select the location where you want to store the data. Choose as file extension ****.txt***. Click the '***speichern***' button to confirm your settings.





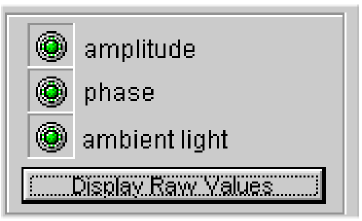
By clicking the '***Stop Log Data***' item you stop data logging which is displayed by the blinking '***no logging***' in the information window next to it.

Stop Measurement

The measurement is ended by a left click on the '***stop***' button in the control bar.

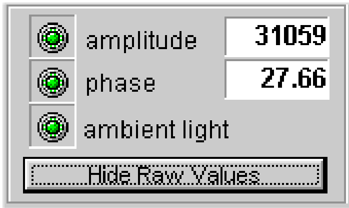
Warning Lights:

At the right bottom of the window you can find the amplitude, phase angle and three warning lights. The warning lights are explained below:



amplitude:	red:	Amplitude is too low, the sensor tip may be damaged or sensor cable may not be connected
	yellow:	Amplitude is critically low, replacement of the sensor is recommended
	green:	amplitude is correct
phase:	red:	phase angle is out of limits
	green:	phase angle is in normal range
ambient light:	red:	background light (e.g. direct sunlight, lamp) is too high. Decrease of false light is recommended
	green:	ratio of sensor signal to false light is acceptable

By clicking the 'Display Raw Values' button, the raw data of phase angle and amplitude are displayed next to the warning lights.



6.2.3 Graphical Window

The respective sensor signal is displayed according to the selection of the 4 control buttons oxygen, phase, amplitude and temperature (menu chart). The oxygen content is displayed in the chosen unit, the temperature in °C. The raw values (the phase angle in degrees and the sensor amplitude in mV) can also be displayed by clicking the button ***'Display Raw values'***.

Zoom Function:

1. Press the left mouse button and drag from left to right to enlarge a certain area of the graphical window. The graphical window displays the selected data points and is not actualized with new data.
2. Press the left mouse button and drag from right to left to recover the original display, or click the '**Undo Zoom**' button in the *display* menu under *zoom*.

6.2.4 Status Bar

sw1: Displays the serial port which is used for communication of the OxyMicro device with the PC
sw2: Displays the file name in which the measurement data are stored. "No storage file selected" is displayed if no file was selected (no data storage).
sw3: Displays the start time of the measurement
sw4: Displays the actual time

6.3 Subsequent Data Handling

In the head of the ASCII file, you find the **description** of your measurement which you have entered by storing the file.

Below you find the '***instrument info***' containing the data of the complete calibration routine and some more important settings of the instrument and firmware.

The '***software info***' below contains the version number of the OxyMicro software, date and time of the performed measurement. If there is a problem with the OxyMicro oxygen meter, please contact our service team and have the software and instrument information ready.

Below, you find the '***measure mode settings***' containing the *dynamic averaging*, and the *measuring mode*.

The following rows, separated by semicolons, list the measuring data. The first two rows contain the **date** and **time**, the third the **log-time** in minutes, the fourth the **oxygen content** in the chosen unit. The raw data - **phase angle** in [°] and the **amplitude** in [mV] - are stored in the fifth and sixth row, respectively. The seventh row contains the **temperature** in °C measured by PT100 temperature sensor. Raw data can be used for user defined recalculations according to the formulas and tables listed in the appendix.

***** DESCRIPTION ***********
test measurement

***** INSTRUMENT INFO *******
***
IDENTIFICATION
PHIboard number : 20020069
PM number : 20020032
Serial number : TX3-AOT-2003-0001---
MUX channel : ON - 01

PARAMETERS
Signal LED current: 160
Ref LED current : 075
Ref LED amplitude : 106091
Frequency : 005
Sending interval : 0001
Averaging : 1

SYSTEM SETTINGS
APL function : ON
Temp compensation : OFF
Analog out : chA o chB o
RS232 echo : ON
Data logger : OFF - 0/800
Oxygen unit : %a.s.

CALIBRATION
Sensor type : 1
0% a.s. phase 1 : 56.00 at 21.0°C amp 000000
100% a.s. phase 2 : 28.07 at 20.0°C amp 021100
Date (ddmmyy) : 060103
Pressure (mBar) : 1013

FIRMWARE
Code ver 1.077 : 12/12/02, 12:50:57
Xilinx built : 20/08/02 (MM/DD/YY)
Reset condition : CONTINUOUS

***** SOFTWARE INFO *********
OxyView - TX3-B2-V5.00 12/2002
© by PreSens
06.01.03
13:12:08

******MEASURE MODE SETTINGS**
Dynamic Aver 1
measure mod 1 sec

start time 12:50:01

date	time/hh:mm:ss	logtime/min	oxygen/% airsatur.	phase/°	amp	temp/°C
06.01.03	13:12:09	0.000	101.510	27.90	20808	20.00
06.01.03	13:12:10	0.017	101.410	27.91	20797	20.00
06.01.03	13:12:11	0.034	101.370	27.92	20785	20.00
06.01.03	13:12:12	0.051	101.960	27.85	20776	20.00
06.01.03	13:12:13	0.069	101.640	27.89	20776	20.00
06.01.03	13:12:14	0.085	101.530	27.90	20761	20.00
06.01.03	13:12:15	0.102	101.710	27.88	20774	20.00
06.01.03	13:12:16	0.119	101.730	27.88	20754	20.00
06.01.03	13:12:17	0.136	101.710	27.88	20747	20.00
06.01.03	13:12:18	0.154	102.080	27.84	20738	20.00
06.01.03	13:12:19	0.170	101.460	27.91	20739	20.00
06.01.03	13:12:20	0.188	101.790	27.87	20736	20.00
06.01.03	13:12:21	0.205	101.760	27.88	20717	20.00
06.01.03	13:12:22	0.222	101.920	27.86	20738	20.00
06.01.03	13:12:23	0.239	101.820	27.87	20718	20.00
06.01.03	13:12:24	0.256	101.990	27.85	20714	20.00
06.01.03	13:12:25	0.273	102.560	27.79	20711	20.00

7 Calibration of Oxygen-Sensitive Microsensors

7.1 Calibration of Needle-Type Oxygen Microsensors

7.1.1 Preparation of the Calibration Standards

Calibration of microsensors is performed using a conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***water-vapor saturated air*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

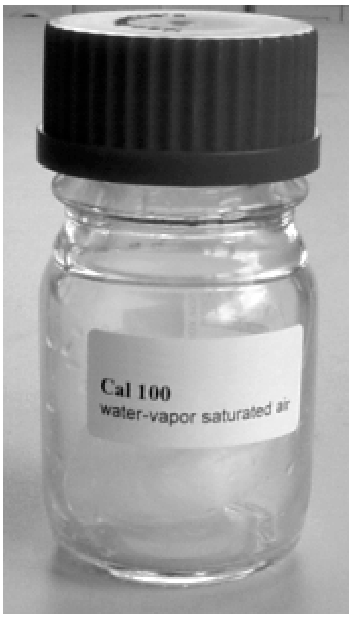
1. Add one gram sodium sulfite (Na_2SO_3) to the vessel and label it '**cal 0**';
2. Dissolve Na_2SO_3 in 100 mL water.
   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination

To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of '**cal 0**' is about 24 hours provided that the vessel has been closed with the screw top.

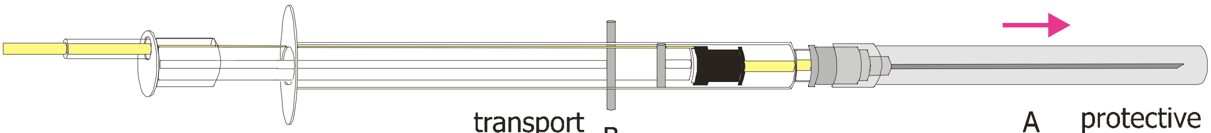
Preparation of calibration standard 100 (water-vapor saturated air)

1. Place wet cotton wool in the vessel with the label '**cal 100**'.
2. Drill two holes for inserting the microsensor and the temperature sensor in the screw top and close the vessel.
3. Wait about 2 minutes to ensure that air is water-vapor saturated

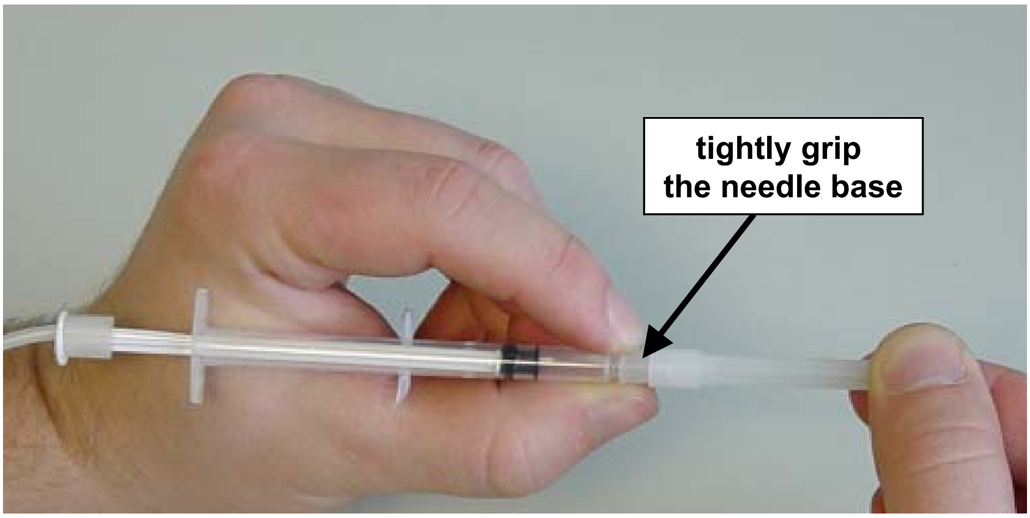


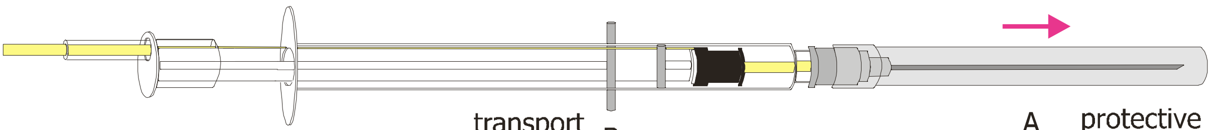
7.1.2 Mounting the Needle-Type Microsensors

1. Remove the microsensor carefully from the protective cover. The needle-type microsensor is housed in 0.4 x 40 mm syringe needle mounted to a 1 mL plastic syringe housing with integrated PUSH & PULL - IN & OUT mechanism. The syringe needle is protected with a protective plastic cap (**A**).



2. Carefully remove the protective plastic cap (**A**) covering the syringe needle.
When doing so, grip the plastic base of the needle tightly. The syringe needle ***must not*** be removed from the syringe housing. Work carefully!



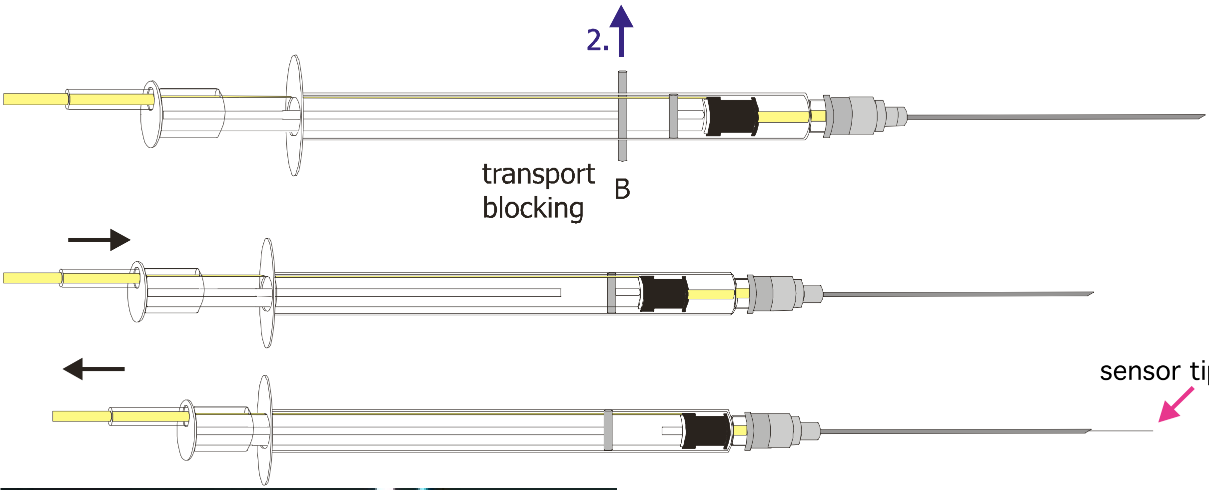


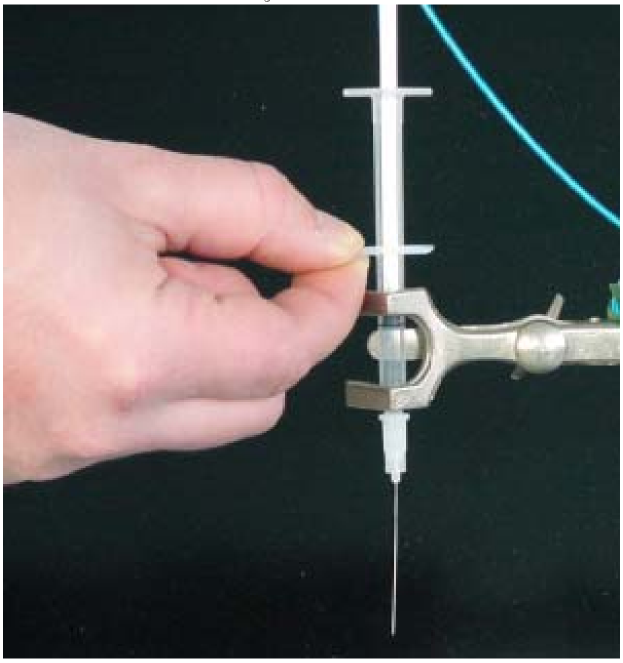
3. Fix the microsensor with a clip to a laboratory support or a similar stable construction.

We expressly warn you not to handle with microsensors without the support - especially when the sensor tip is extended.

4. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the OxyMicrodevice. The female fiber-plug of the OxyMicrohas a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.

5. The glass fiber with its sensing tip is prevented from slipping using a transport block (**B**). Remove the transport block from the hole in the syringe housing. Now it is possible to retract or extend the glass fiber with its sensor tip by pushing or pulling the plunger. Before pushing out the sensor tip, make sure that you have removed the protective plastic cap and have some space in front of the syringe needle.





WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK

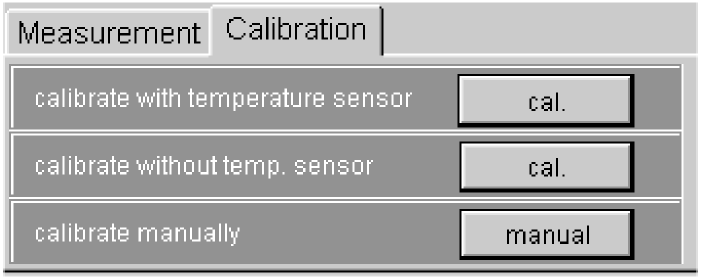
7.1.3 Calibration without Automatic Temperature Compensation

Using the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor TEMP 100 is not connected to the OxyMicro.

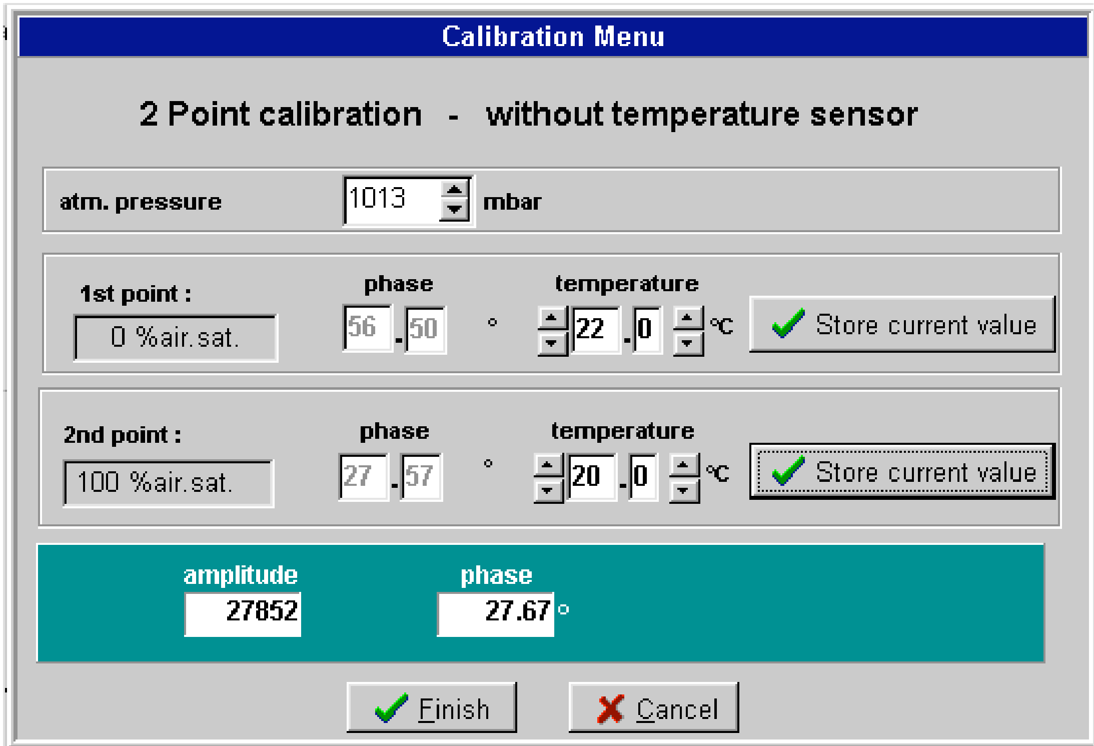
Please note:
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen microsensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the OxyMicro via the RS232 cable to your computer.
2. Switch on the OxyMicro and connect the microsensor as shown in Chapter 7.1.2 '***Mounting the Needle-Type Microsensors***'.
3. Start the OxyMicro software on your computer and click the '***calibration***' menu item.

4. Select the calibration routine: '***calibrate without temp sensor***' and click the '***cal.***' button



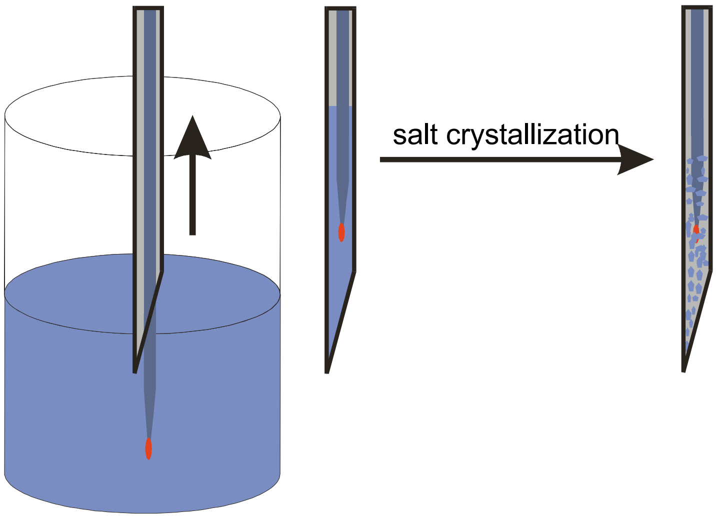
5. Enter the actual **atmospheric pressure** and the **temperature** of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



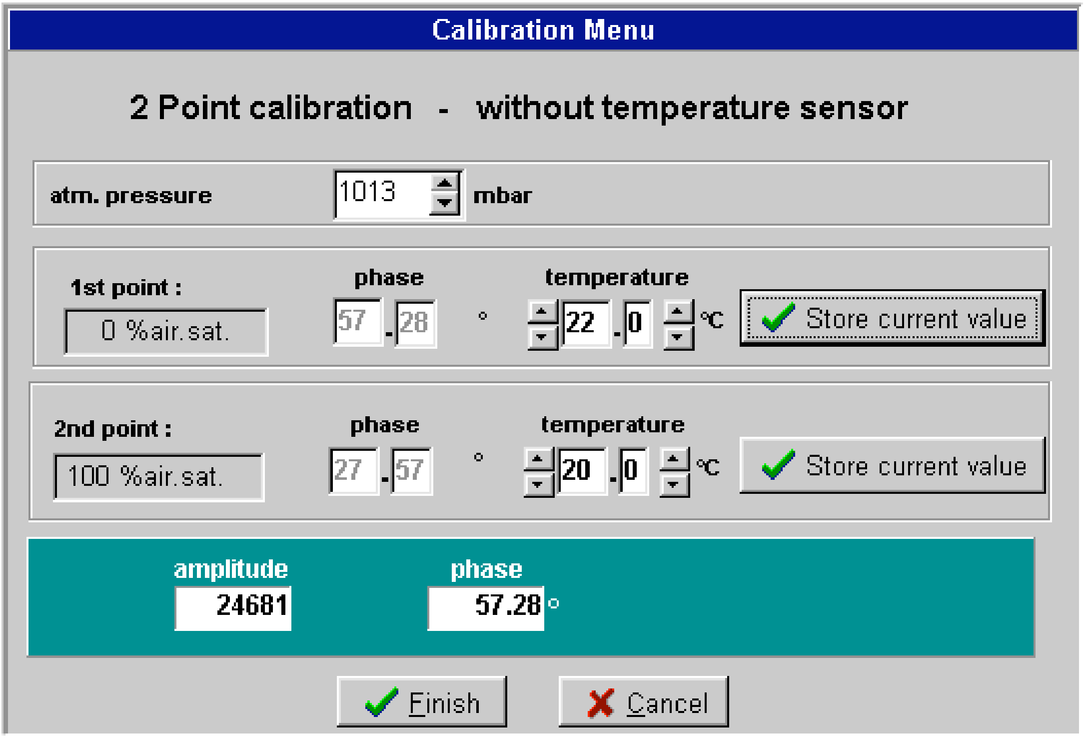
6. Place the vessel with the label '**cal 0**' underneath the microsensor. Please ensure that the glass fiber with its sensor tip is ***not extended***. Locate the syringe needle carefully about 5 mm above the water surface. Slowly press the syringe plunger and extend the glass fiber with its sensor tip from the protective syringe needle.

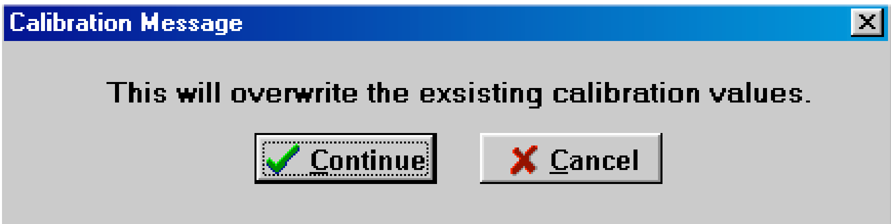
   Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not the protective syringe needle.

If the syringe needle has been dipped into ‘**cal 0**‘ by mistake, please wash the glass fiber and the syringe needle with distilled water to avoid salt crystallization within the syringe needle. Salt crystallization may seal the syringe needle and the glass fiber with its sensor tip will break when extended.



7. Wait about 30 sec. until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the ‘***Store current value***’ button to store the **0% air-sat.** and **temp. at 0%** values.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the ‘***Continue***‘ button to store the new calibration data.





8. Afterwards, wash the glass fiber with its sensor tip with distilled water to clean it from sodium sulfite. Don't retract the sensor tip back into the protective syringe needle. Exchange the calibration solution 0 with an identical vessel filled with distilled water. Make sure not to touch the sensor tip. Dip the sensor tip about 4 mm into the washing solution. Afterwards, retract the glass fiber back into the protective syringe needle without absorbing water.

9. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label '**cal 100**' has to be closed by the screw top containing the two holes.

   *Make sure that the glass fiber is **not** extended!*

   Insert the syringe needle through one of the holes until it is about 1 cm deep inside the vessel.

   Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.

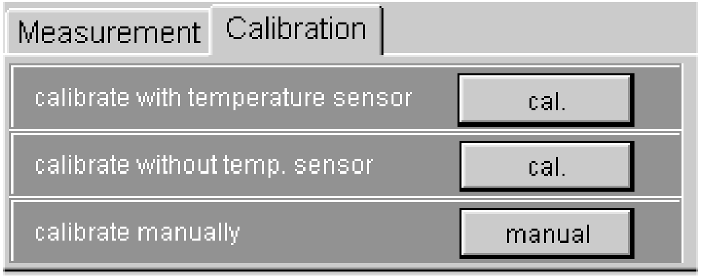
   Extend the sensor tip, wait about 30 s until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '***Store current value***' button to store the **100% air-sat.** and **temp. at 100%** values.
   Again, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

10. Now, calibration is complete. Confirm the calibration values by clicking the '***Finish'*** button.
11. Pull the sensor tip back into its protective syringe needle before removing the microsensor from the calibration vessel.
12. Protect the syringe plunger against slipping out by inserting the transport block back into the syringe housing and don't remove it again until just before measurement.

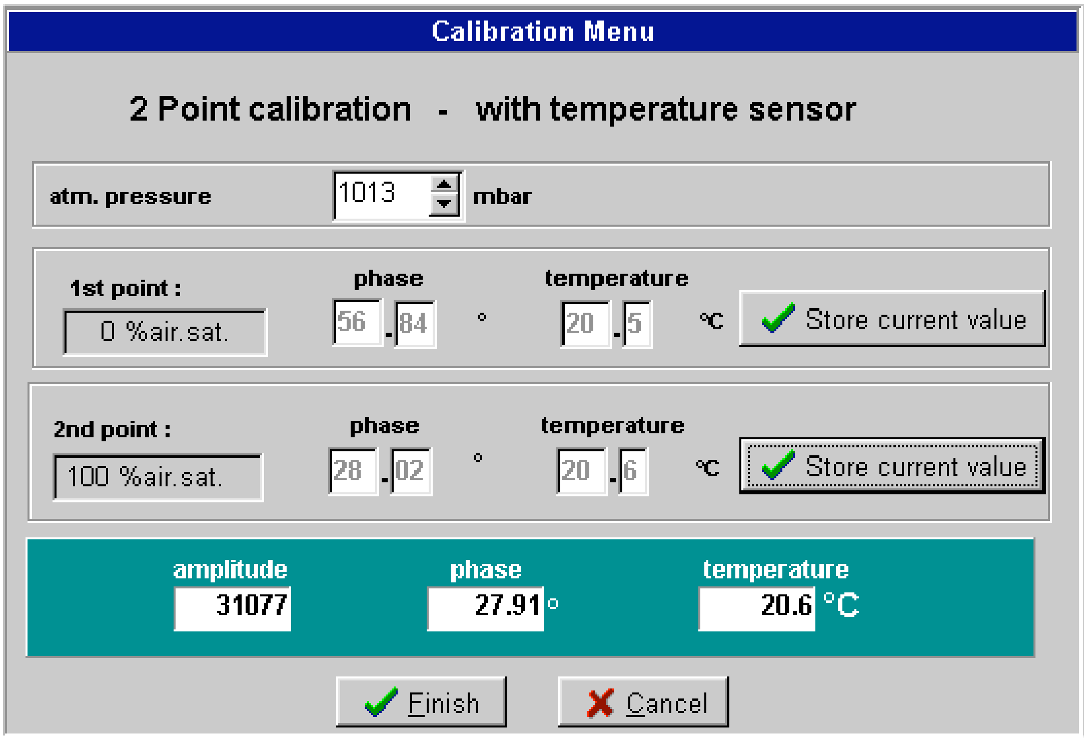
7.1.4 Calibration with Automatic Temperature Compensation

1. Connect the OxyMicro via the RS232 cable to your computer.
2. To perform temperature compensated measurement, connect the temperature sensor TEMP 100 to the 4-pin connector in front of the OxyMicro. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.

3. Switch on the OxyMicro and connect the microsensor as shown in Chapter 7.1.2 '***Mounting the Needle-Type Microsensors***'.
4. Start the OxyMicro software on your computer and click the calibration menu item.
5. Select the calibration routine: '***calibrate with temperature sensor***' by clicking the '***cal.***' button.



6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation in partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

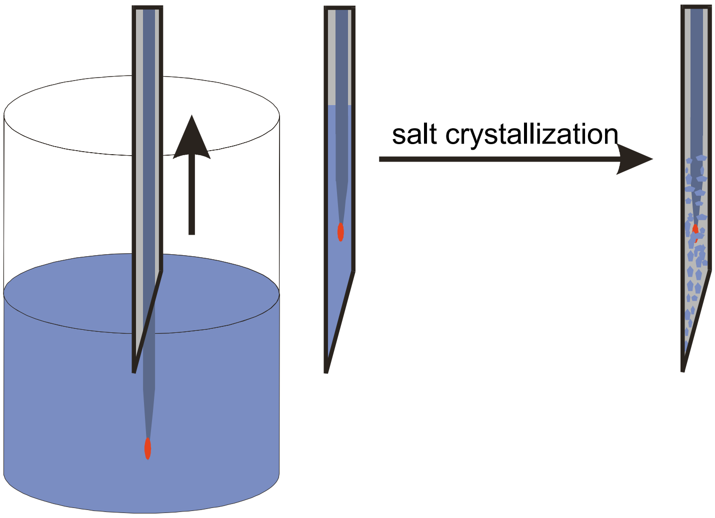


7. Place the vessel with the label '**cal 0**' underneath the microsensor (see picture below). Please ensure that the sensor tip is ***not extended***. Locate the syringe needle carefully about 5 mm above the water surface. Slowly press the syringe plunger and extend the sensor tip from its protective syringe needle.

   Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not its protective syringe needle.

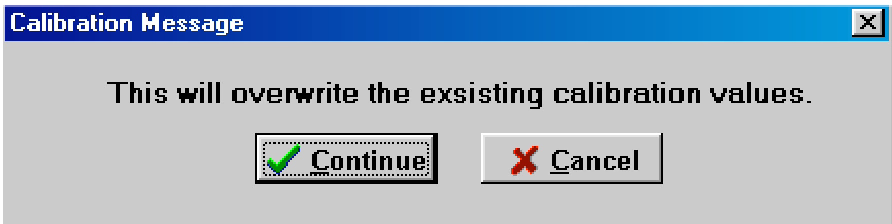
   Ensure that the temperature sensor has been dipped about 1-2 cm into the calibration solution.

   If the needle has been dipped into '**cal 0**' by mistake, please wash it with distilled water to avoid salt crystallization within the syringe needle. Salt crystallization may seal the syringe needle and the glass fiber with its sensor tip will break when extended.



8. Wait about 30 sec. until the phase angle and the temperature value is constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the '***Store current value***' button to store both the '***0% air-sat.***' and the temperature '***temp at 0%***'.

   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



9. Afterwards, wash the sensor tip with distilled water to clean it from sodium sulfite. Don't retract the glass fiber back into the protective syringe needle. Exchange the calibration solution 0 with an identical vessel filled with distilled water. Make sure not to touch the glass fiber. Dip the sensor tip about 4 mm into the washing solution. Afterwards, retract the glass fiber back into the protective syringe needle without absorbing water.

   Also wash the temperature sensor by dipping it into water.

10. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label '**cal 100**' has to be closed with the screw top containing the two holes.

   *Make sure that the glass fiber with is sensor tip is **not** extended!*

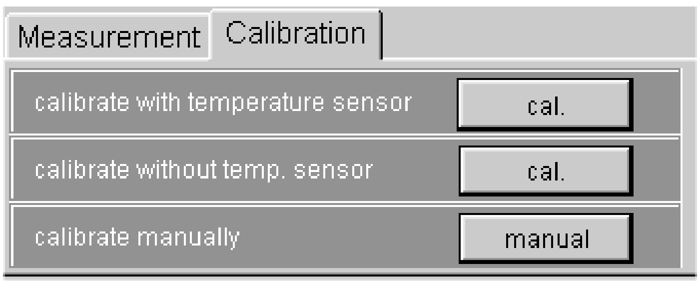
   Insert the syringe needle through one of the holes until it is about 1 cm deep inside the vessel.
   Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.
   Insert the temperature sensor through the other hole and make sure that it doesn't touch the microsensor. Extend the glass fiber with its sensor tip, wait about 30 s until the phase angle and the temperature is constant (the variation of the phase angle and temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '***Store current value***' button to store the '**100% air-sat.**' and **temp. at 100%** values.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

11. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Finish***' button.
12. Pull the glass fiber with its sensor tip back into its protective syringe needle before removing the microsensor from the calibration vessel.
13. Protect the syringe plunger against slipping out by inserting the transport block back into the syringe housing.

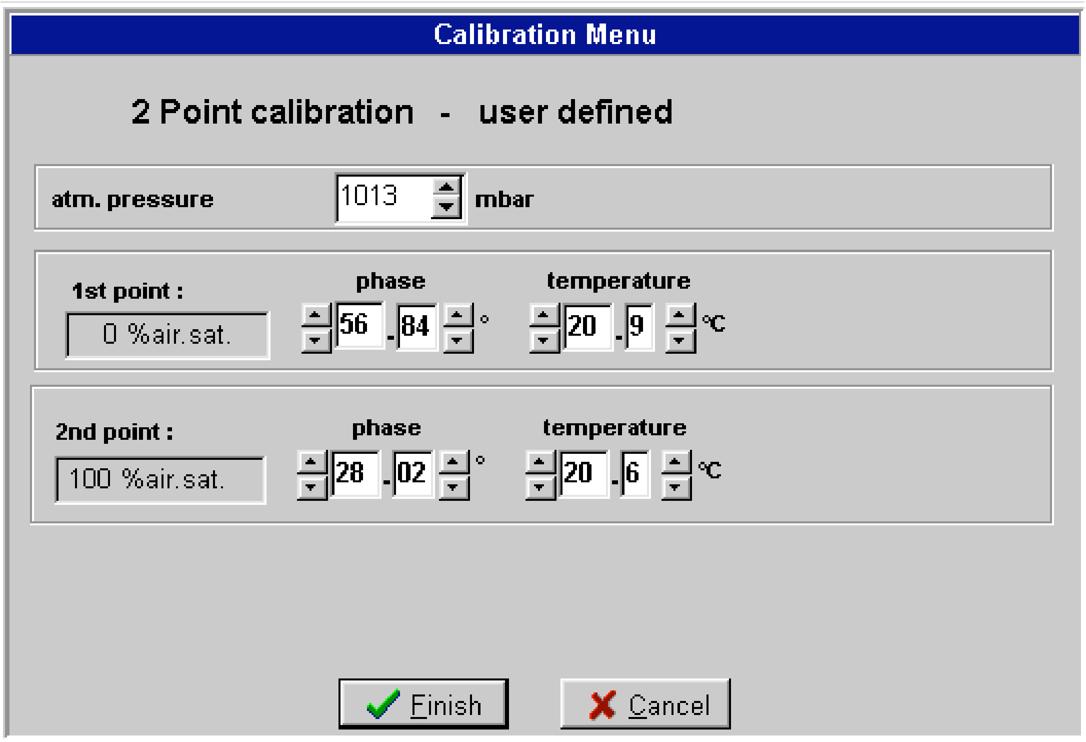
7.1.5 Manual Calibration

A manual calibration should be applied, if you don't want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

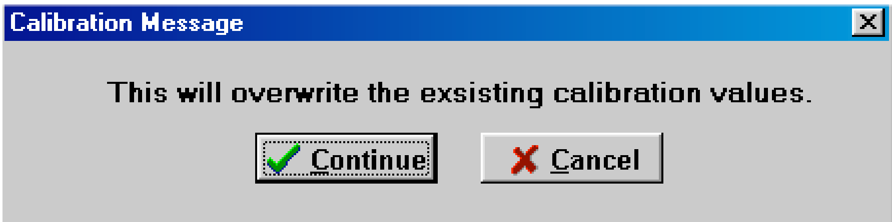
1. Connect the OxyMicro via the RS232 cable to your computer.
2. Switch on the OxyMicro oxygen meter
3. Start the OxyMicro software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' and click the **manual** button



5. Enter the **atmospheric pressure** at which calibration was performed (not the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now, user-defined calibration is complete. Confirm the calibration values by clicking the **Finish** button. A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



7.2 Calibration of Flow-Through Housed Oxygen Microsensors

7.2.1 Preparation of the Calibration Standards

Calibration of microsensors is performed using conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***air-saturated water*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add one gram sodium sulfite(Na_2SO_3) to the vessel and label it '**cal 0**';
2. Dissolve Na_2SO_3 in 100 mL water.
   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination

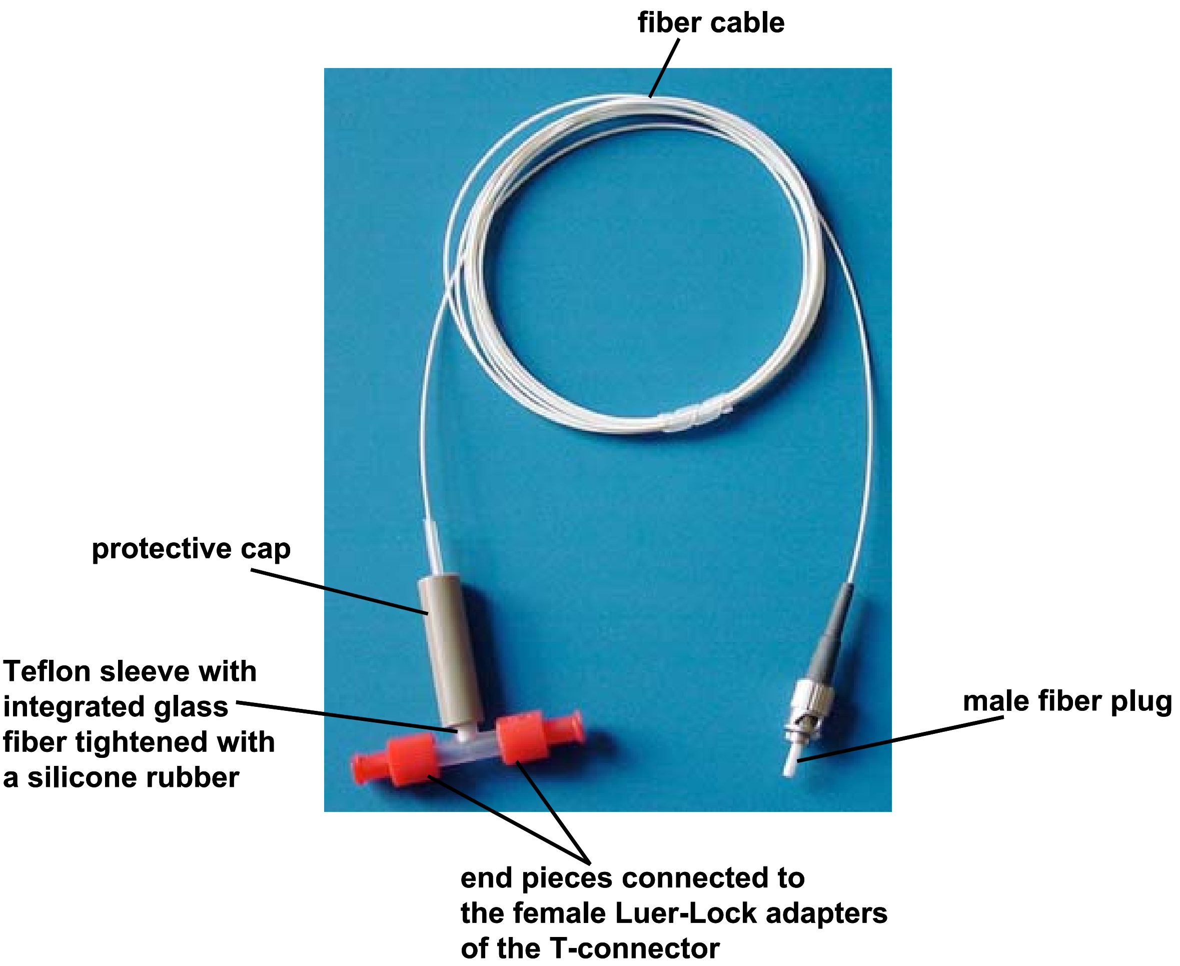
To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of '**cal 0**' is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration solution 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it '**cal 100**'.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch of the air-pump and stir the solution for further 10 minutes to ensure that water is not supersaturated.

7.2.2 Mounting the Flow-Through Housed Oxygen Microsensors

1. Remove the flow-through housed microsensor carefully from the protective cover. The microsensor is integrated in a sleeve consisting of Teflon tightened with a silicone rubber, which is mounted to a T-connector. The sleeve is protected with a plastic cap screwed to the T-connector. The T-connector has two female Luer-Lock adapters for connection with plastic tubings.



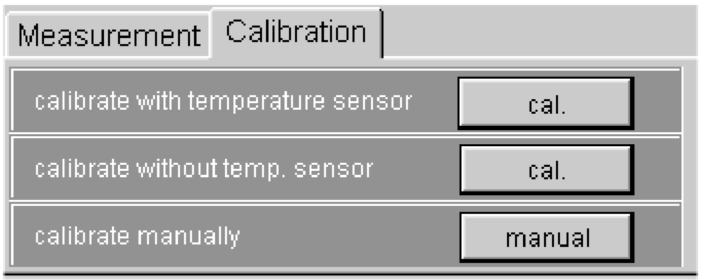
2. Fix the microsensor with a clip to a laboratory support or a similar stable construction.
3. Remove the two red end-pieces from the T-connector and connect the female Luer-Lock adapters with the tubings of your flow-through system.
4. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the OxyMicro device. The female fiber-plug of the OxyMicro has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.

7.2.3 Calibration without Automatic Temperature Compensation

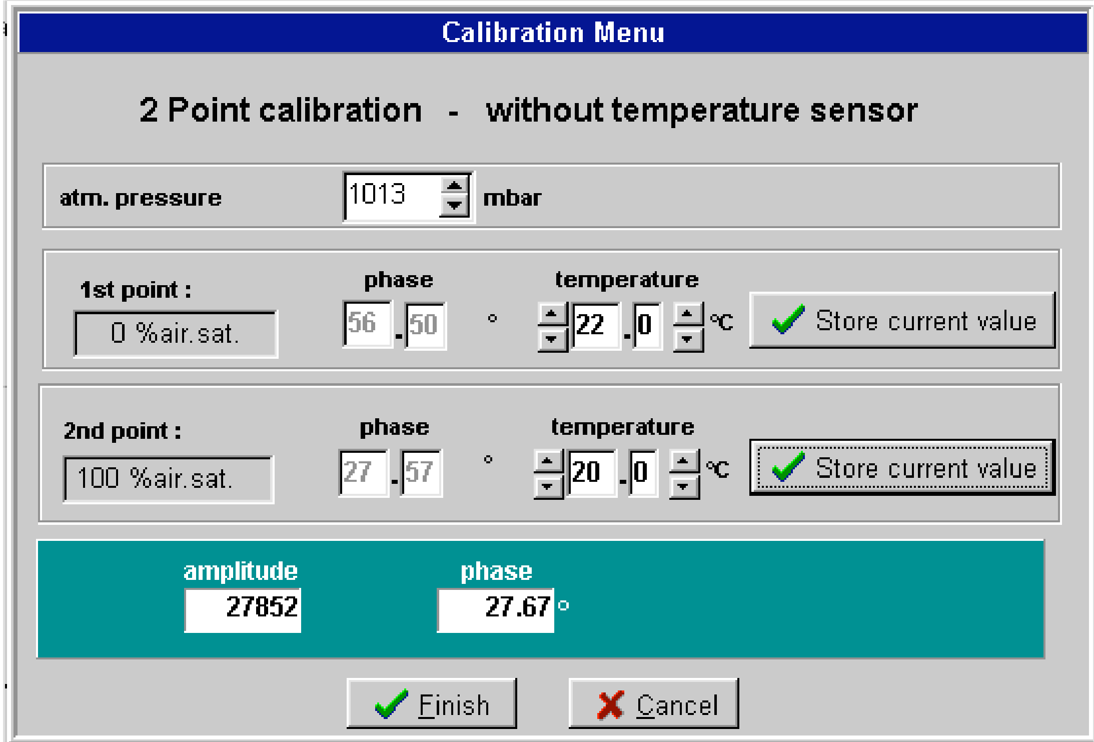
Using the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor TEMP 100 is not connected to the OxyMicro.

Please note:
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen microsensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the OxyMicro via the RS232 cable to your computer.
2. Switch on the OxyMicro and connect the microsensor as shown in Chapter 7.2.2 '***Mounting the Flow-Through Housed Microsensors***'.
3. Start the OxyMicro software on your computer and click the '***calibration*** ' menu item.
4. Select the calibration routine: '**calibrate without temp. sensor**' and click the '***cal.***' button



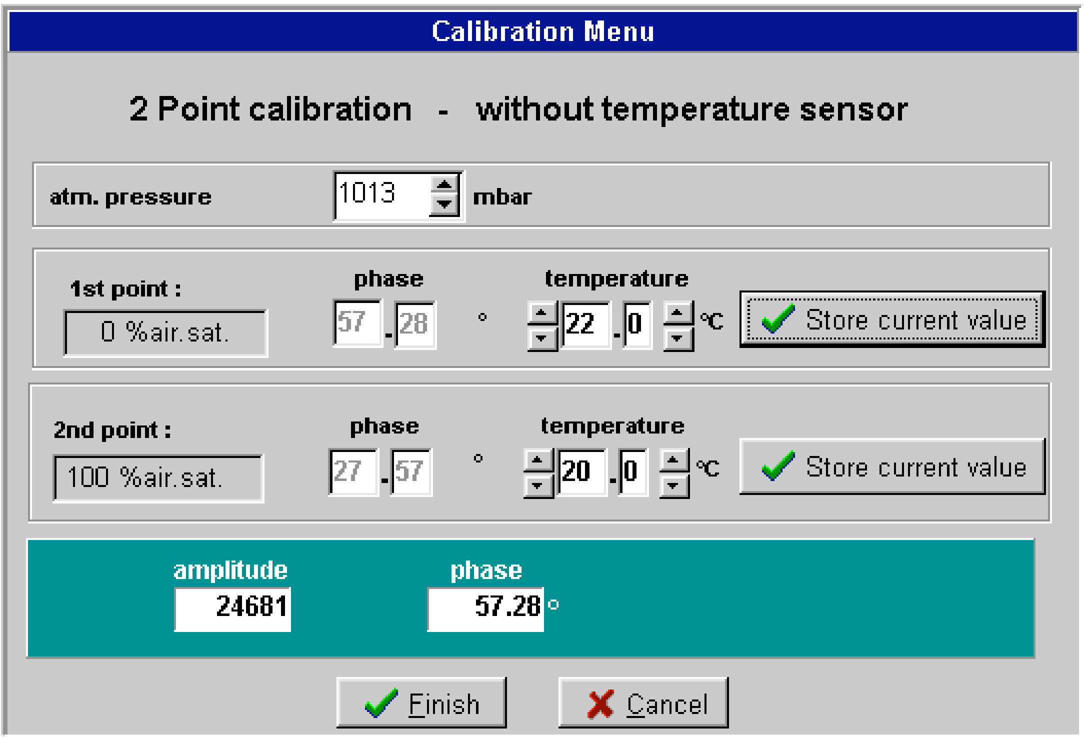
5. Enter the '***actual atmospheric pressure***' and the '***temperature***' of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

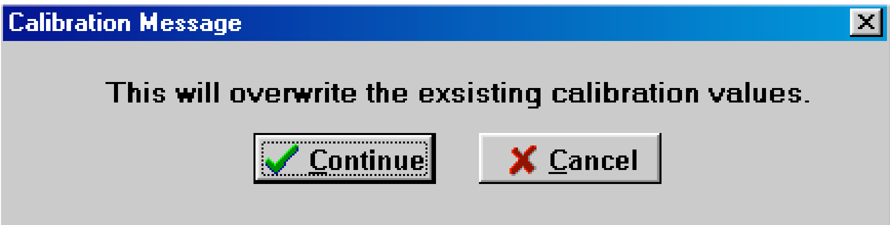


6. Connect one of female Luer-Lock adapters with a plastic tubing which dips into the vessel containing the calibration solution 100, '**cal 100**'. Connect a syringe to the other female Luer-Lock adapter and fill the syringe slowly with calibration solution 100. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.

7. Wait about 30 sec. until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **100% air-sat.** and **temp. at 100%** values. Afterwards, press the calibration solution into the waste.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

8. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, '**cal 0**' and fill the syringe slowly with it. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.
   Wait about 30 s until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '**Store *current value***' button to store the **0% air-sat.** and **temp. at 0%** values. Afterward, press the calibration solution into the waste.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

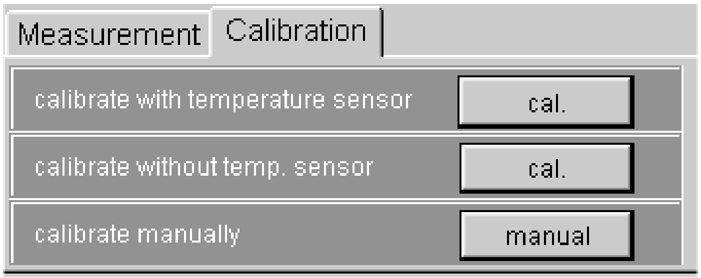




9. Now, calibration is complete. Confirm the calibration values by clicking the '***Finish'*** button.
10. Now you have to wash the glass fiber with its sensor tip with distilled water to clean it from sodium sulfite. Dip the plastic tubing into a vessel containing distilled water and fill the syringe. Press the washing solution to the waste, not back into the vessel. Repeat this washing procedure 3 times.

7.2.4 Calibration with Automatic Temperature Compensation

1. Connect the OxyMicro via the RS232 cable to your computer.
2. To perform temperature compensated measurement, connect the temperature sensor TEMP 100 to the 4-pin connector in front of the OxyMicro .
3. Switch on the OxyMicro and connect the microsensor as shown in Chapter 7.2.2 '***Mounting the Flow-Through Housed Microsensors***'.
4. Start the OxyMicro software on your computer and click the calibration menu item.
5. Select the calibration routine: '***calibrate with temperature sensor***' and click the '***cal.***' button.



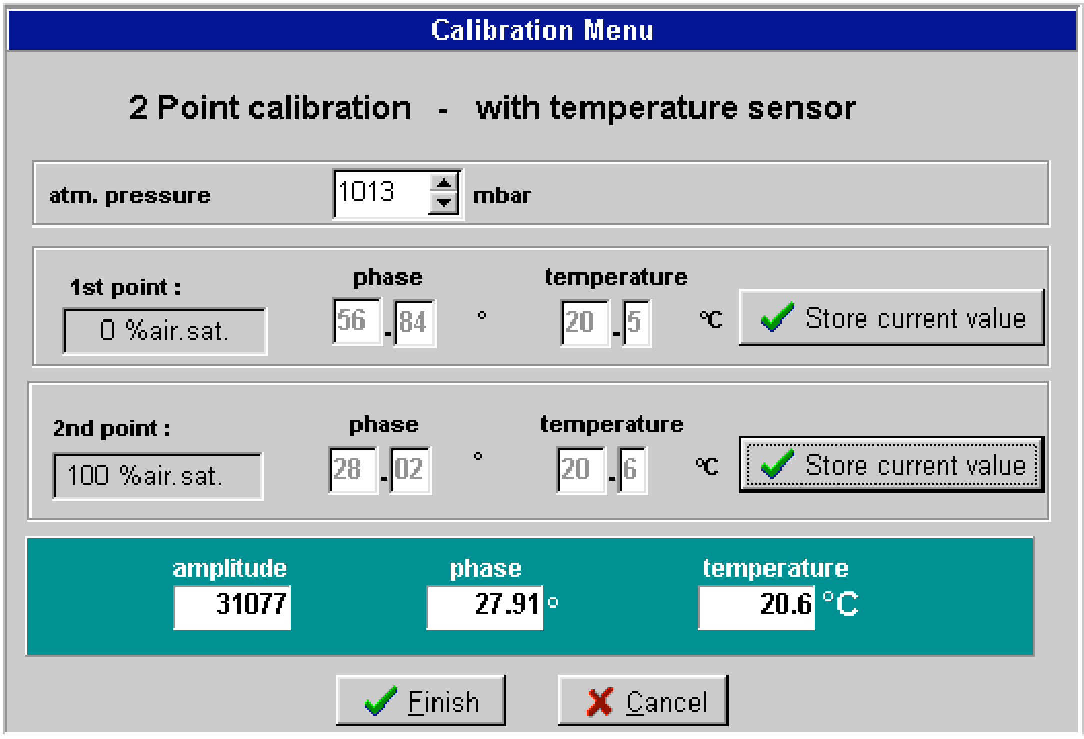
6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.
7. Connect one of female Luer-Lock adapters with a plastic tubing which dips into the vessel containing the calibration solution 100, '**cal 100**'. Connect a syringe to the other female Luer-Lock adapter and fill the syringe slowly with calibration solution 100. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.

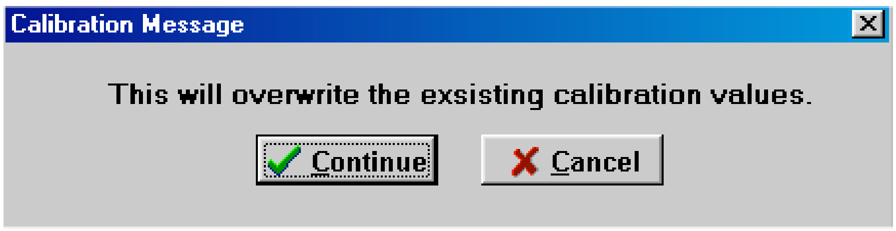
   Ensure that the temperature sensor has been dipped into the calibration solution '**cal 100**' and that there are no temperature differences between the calibration vessel and the flow-through cell.

8. Wait about 30 sec. until the phase angle and the temperature value is constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the '***Store current value***' button to store both the ***100% air-sat.*** and its temperature '***temp at 100%***'. Afterwards, press the calibration solution back the waste.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

9. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, '**cal 0**' and fill the syringe slowly with it. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.
   Ensure that the temperature sensor has been dipped into the calibration solution '**cal 0**'.

10. Wait about 30 s until the phase angle and the temperature is constant (the variation of the phase angle and temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '***Store current value***' button to store the **0% air-sat.** and **temp. at 0%** values. Afterwards, press the calibration solution into the waste.
    Again, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



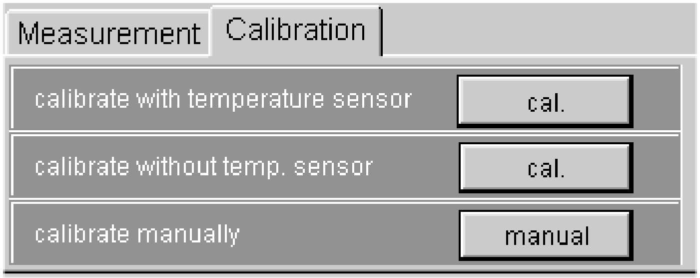


11. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the ‘***Finish’*** button.
12. Now you have to wash the glass fiber with its sensor tip with distilled water to clean it from sodium sulfite. Dip the plastic tubing into a vessel containing distilled water and fill the syringe. Press the washing solution to the waste, not back into the vessel. Please repeat this washing procedure 3 times.
Also wash the temperature sensor by dipping it into water.

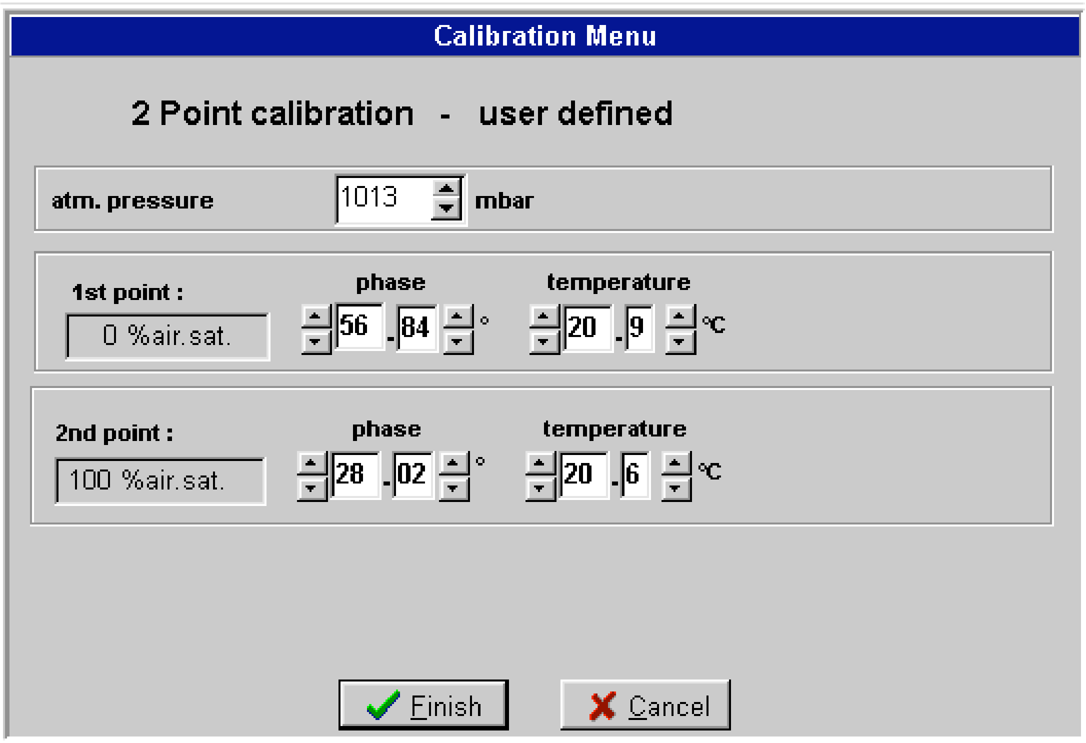
7.2.5 Manual Calibration

A manual calibration should be applied, if you don't want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

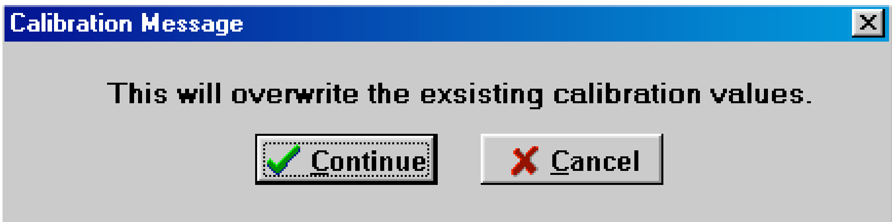
1. Connect the OxyMicro via the RS232 cable to your computer.
2. Switch on the OxyMicro oxygen meter
3. Start the OxyMicro software on your computer and click the **Calibration** menu item.
4. Select the calibration routine ‘**calibrate manually**’ and click the **manual** button



5. Enter the **atmospheric pressure** at which calibration was performed (not the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now, user-defined calibration is complete. Confirm the calibration values by clicking the '**Finish**' button. When doing so, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



7.3 Calibration of Implantable Oxygen Microsensors

7.3.1 Preparation of the Calibration Standards

Calibration of microsensors is performed using conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***water-vapor saturated air*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add one gram sodium sulfite(Na_2SO_3) to the vessel and label it '**cal 0**';
2. Dissolve Na_2SO_3 in 100 mL water.
   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is eliminated (removed) by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water is oxygen-free.

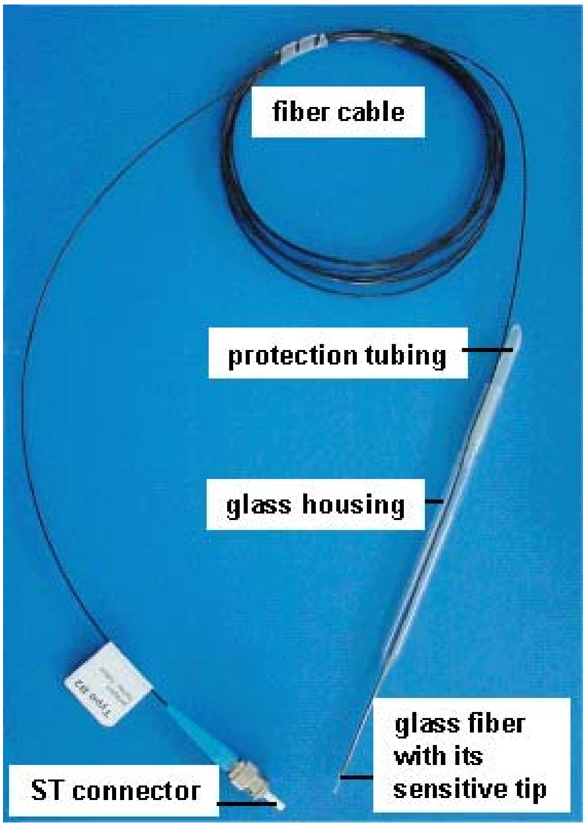
Close the vessel after calibration with a screw top to minimize oxygen contamination
To prepare oxygen-free water you also can use sodium dithionite ($Na_2S_2O_4$). The shelf life of '**cal 0**' is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration standard 100 (water-vapor saturated air)

1. Place wet cotton wool in the vessel and label it '**cal 100**'.
2. Drill two holes for inserting the microsensor and the temperature sensor in the screw top and close the vessel with it.
3. Wait about 2 minutes to ensure that air is water-vapor saturated

7.3.2 Mounting the Implantable Microsensors

1. Remove the microsensor carefully from the protective cover. The microsensor is protected with a glass housing during the transport.
2. Fix the glass housing microsensor with a clip to a laboratory support or a similar stable construction.



We strongly advise you not to handle with microsensors without the support - especially when the sensor tip is extended.

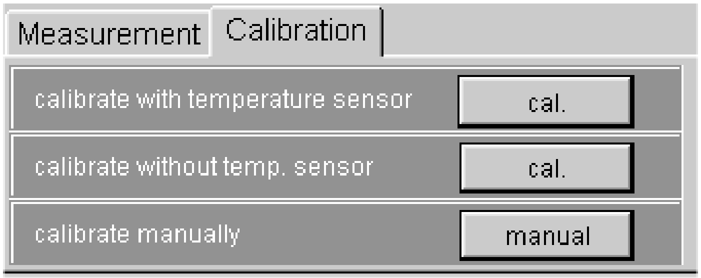
3. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the OxyMicro device. The female fiber-plug of the OxyMicro has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.

7.3.3 Calibration without Automatic Temperature Compensation

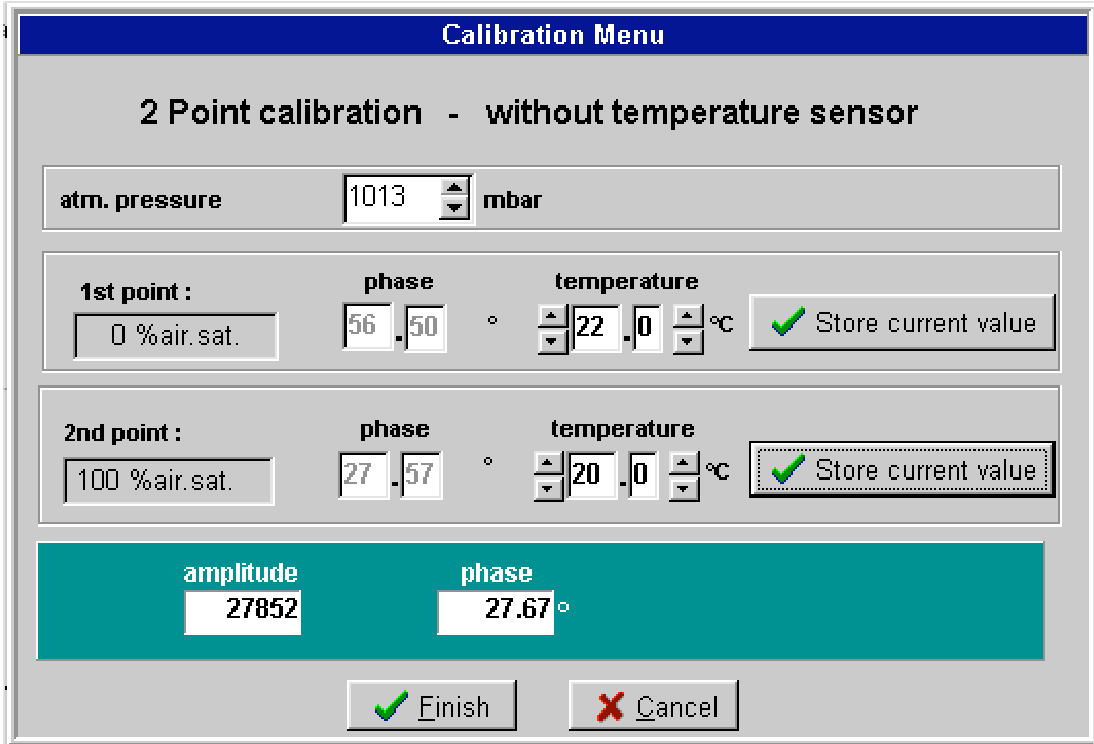
Using the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor TEMP 100 is not connected to the OxyMicro .

Please note:
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen microsensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

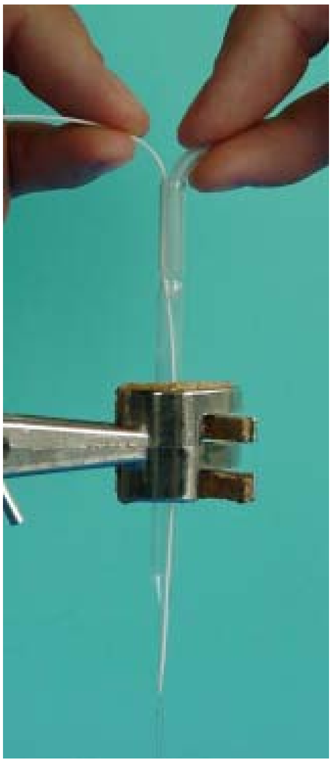
1. Connect the OxyMicro via the RS232 cable to your computer.
2. Switch on the OxyMicro and connect the microsensor as shown in Chapter 7.3.2 '***Mounting the Implantable Microsensors***'.
3. Start the OxyMicro software on your computer and click the '***calibration***' menu item.
4. Select the calibration routine: '***calibrate without temp. sensor***' and click the '***cal.***' button



5. Enter the actual '***atmospheric pressure***' and the '***temperature***' of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



6. Place the vessel with the label '**cal 0**' underneath the microsensor. Please ensure that the glass fiber with its sensor tip is in the protective glass housing. Locate the glass housing carefully about 5 mm above the water surface.
7. The glass fiber with its sensing tip is prevented from slipping using a protection tubing. Slacken the protection tubing from the glass housing, extend the sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing



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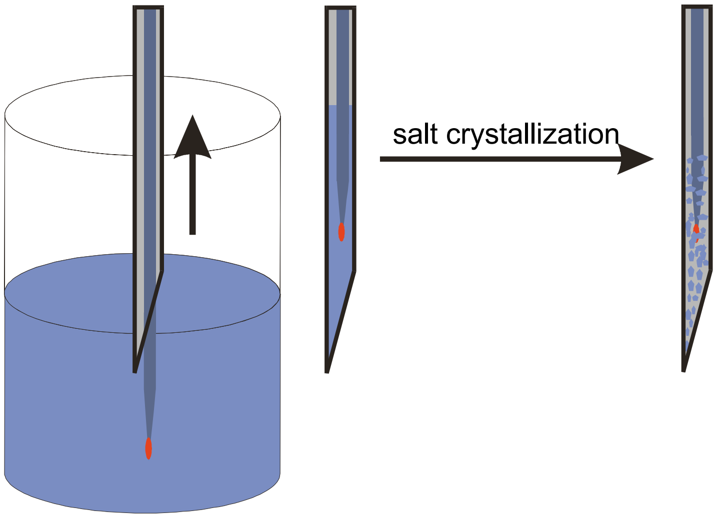
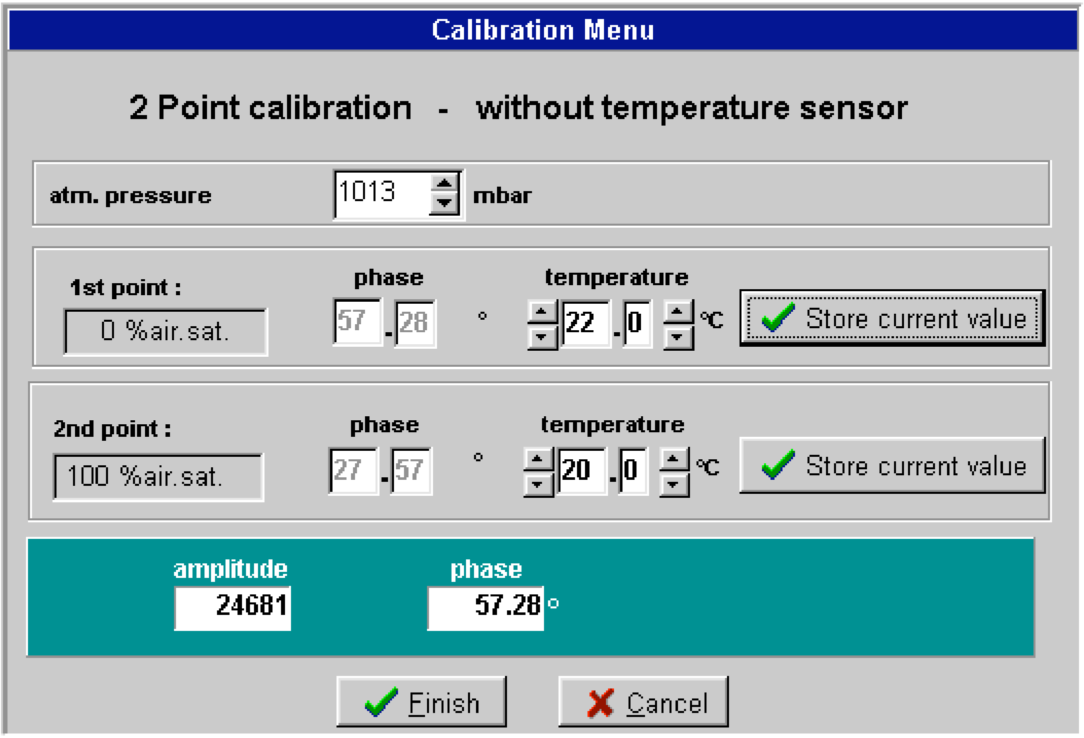
WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK!

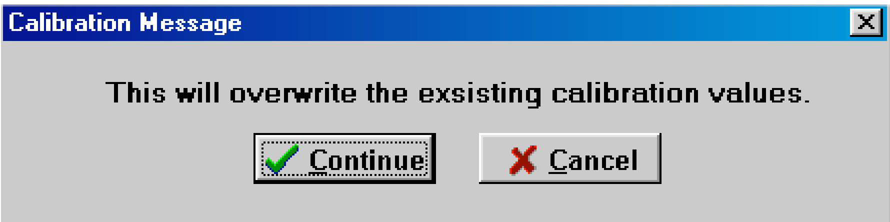
8. Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not the protective glass housing.

If the glass housing has been dipped into '**cal 0**' by mistake, please wash the glass fiber and the glass housing with distilled water to avoid salt crystallization within the housing. Salt crystallization may seal the housing and the glass fiber with its sensor tip will break when extended.



9. Wait about 30 sec. until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **0% air-sat.** and **temp. at 0%** values.
A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.





10. Afterwards, wash the sensor tip with distilled water to clean it from sodium sulfite. Make sure not to touch the sensor tip. Retract the glass fiber back into the protective glass housing without absorbing water.

11. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label '**cal 100**' has to be closed with the screw top containing the two holes.

    *Make sure that the glass fiber is **not** extended!*

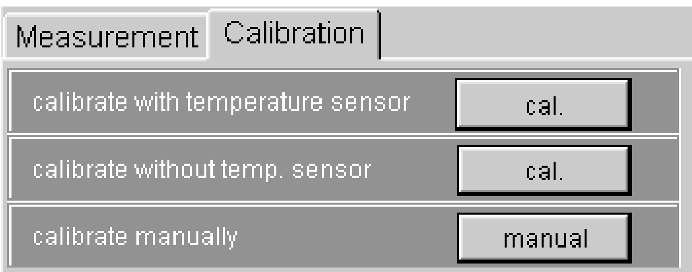
    Insert the glass housing through one of the holes until it is about 1 cm deep inside the vessel.
    Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.
    Slacken the protection tubing from the glass housing, extend the sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing.

    Wait about 30 s until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '***Store current value***' button to store the **100% air-sat.** and **temp. at 100%** values.
    A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

12. Now, calibration is complete. Confirm the calibration values by clicking the '***Finish***' button.
13. Pull the glass fiber with its sensor tip back into its protective glass housing before removing the microsensor from the calibration vessel.

7.3.4 Calibration with Automatic Temperature Compensation

1. Connect the OxyMicro via the RS232 cable to your computer.
2. To perform temperature compensated measurement, connect the temperature sensor Pt 100 to the 4-pin connector in front of the OxyMicro. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.
3. Switch on the OxyMicro and connect the microsensor as shown in Chapter 7.3.2 '***Mounting the Microsensors***'.
4. Start the OxyMicro software on your computer and click the calibration menu item.
5. Select the calibration routine: '***calibrate with temperature sensor***' and click the '**cal.**' button.



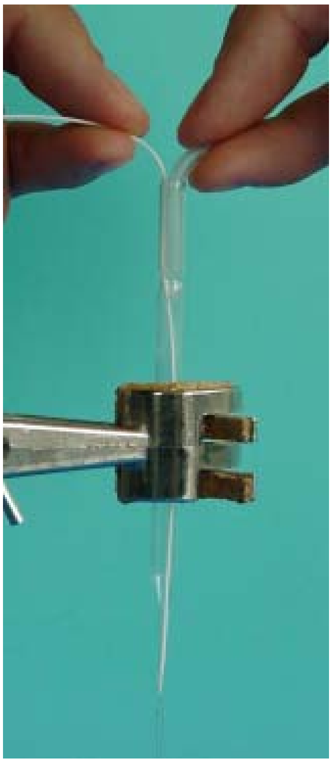
6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual

atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

7. Place the vessel with the label ‘**cal 0**‘ underneath the microsensor. Please ensure that the sensor tip is in the protective glass housing. Locate the glass housing carefully about 5 mm above the water surface.

   Ensure that the temperature sensor has been dipped about 1-2 cm into the calibration solution.

8. The sensing tip is prevented from slipping using a protection tubing. Slacken the protection tubing from the glass housing, extend the sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing.

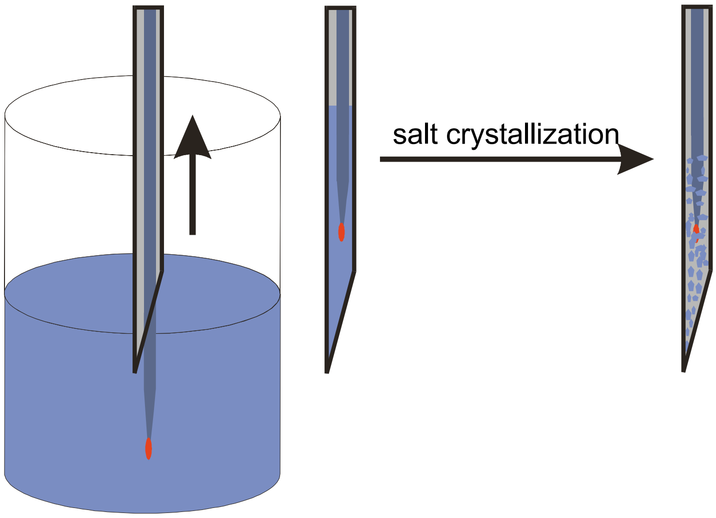


.

WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK!

9. Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not the protective glass housing.

If the glass housing has been dipped into ‘**cal 0**‘ by mistake, please wash the glass fiber and the glass housing with distilled water to avoid salt crystallization within the housing. Salt crystallization may seal the housing and the glass fiber with its sensor tip will break when extended.



10. Wait about 30 sec. until the phase angle and the temperature value is constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the ‘**Store *current value***’ button to store both the ***0% air-sat.*** and its temperature ***temp at 0%***.
    A message window opens and informs you that you will overwrite the existing calibration values. Click the ‘***Continue***‘ button to store the new calibration data.
11. Afterwards, wash the sensor tip with distilled water to clean it from sodium sulfite. Make sure not to touch the sensor tip. Retract the glass fiber back into the protective glass housing without absorbing water.

    Also wash the temperature sensor by dipping it into water.
12. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label ‘**cal 100**‘ has to be closed with the screw top containing the two holes.

*Make sure that the glass fiber is **not** extended!*

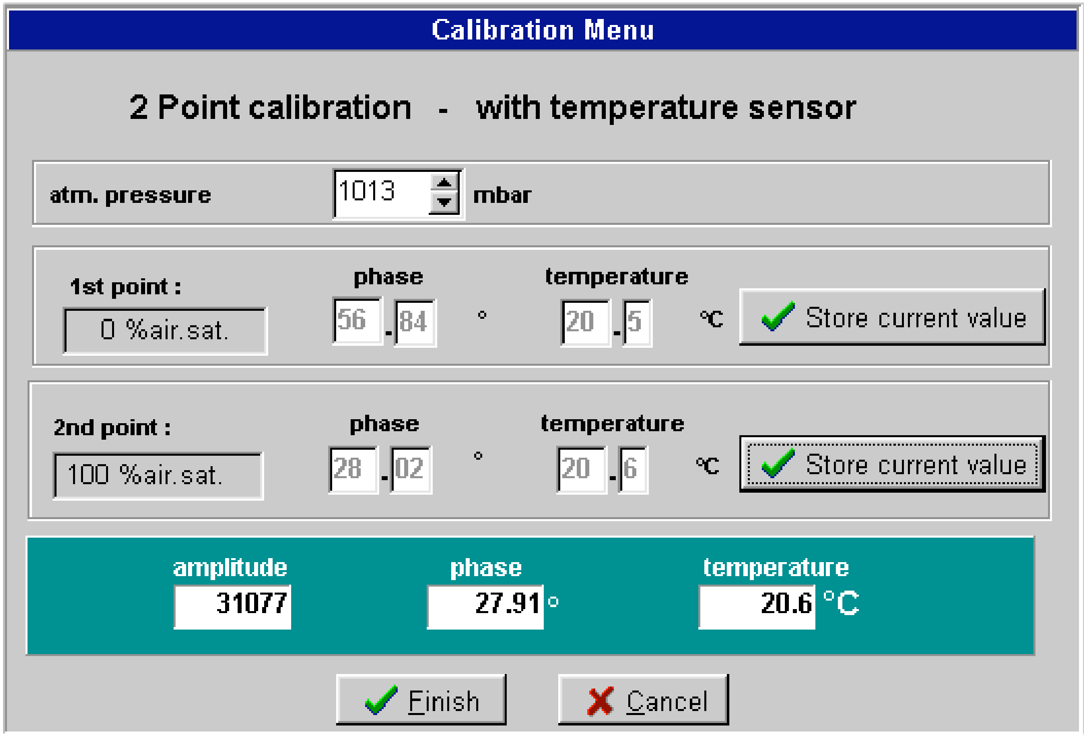
Insert the glass housing through one of the holes until it is about 1 cm deep inside the vessel.

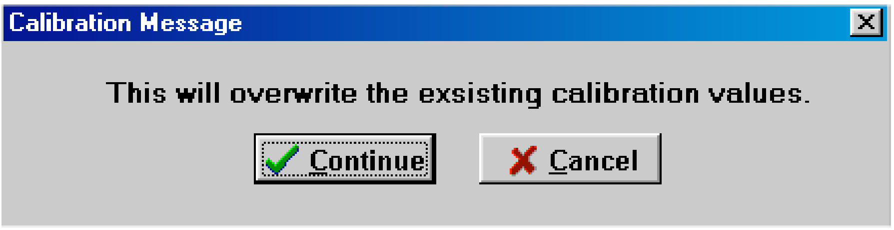
Insert the temperature sensor through the other hole and make sure that it doesn't touch the microsensor.

Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.

Slacken the protection tubing from the glass housing, extend the glass fiber with its sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing.

Wait about 30 s until the phase angle and the temperature is constant (the variation of the phase angle and temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '**Store *current value***' button to store the **100% air-sat.** and **temp. at 100%** values.
A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



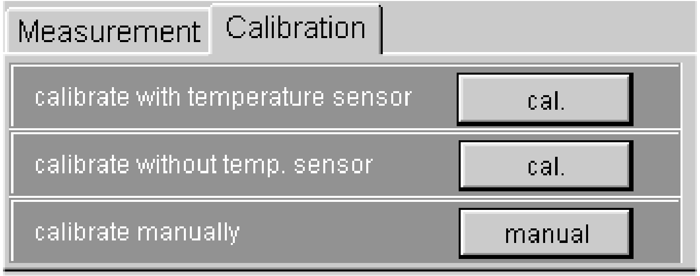


13. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Store'*** button.
14. Pull the glass fiber with its sensor tip back into its protective glass housing before removing the microsensor from the calibration vessel.

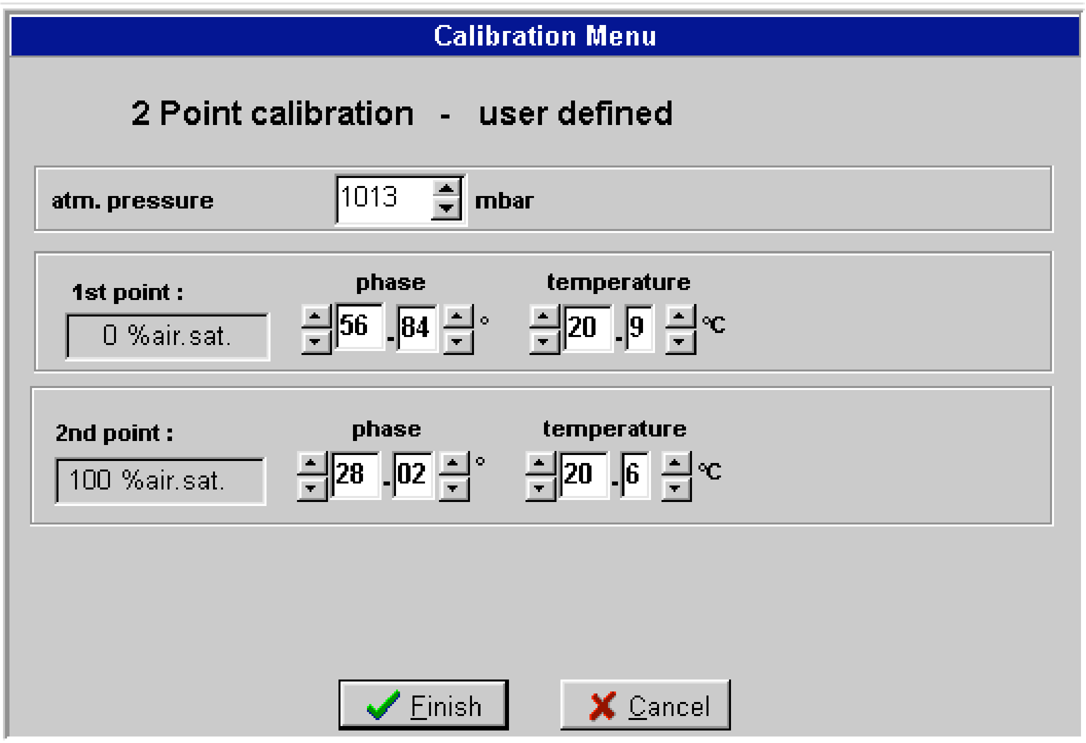
7.3.5 Manual Calibration

A manual calibration should be applied, if you don't want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

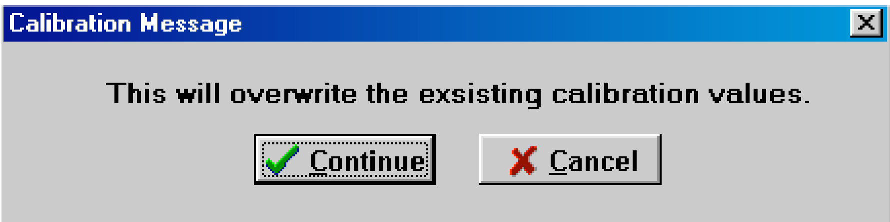
1. Connect the OxyMicro via the RS232 cable to your computer.
2. Switch on the OxyMicro oxygen meter
3. Start the OxyMicro software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' and click the **manual** button



5. Enter the **atmospheric pressure** at which calibration was performed (not the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now, user-defined calibration is complete. Confirm the calibration values by clicking the '**Finish**' button. When doing so, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



8 Measurement

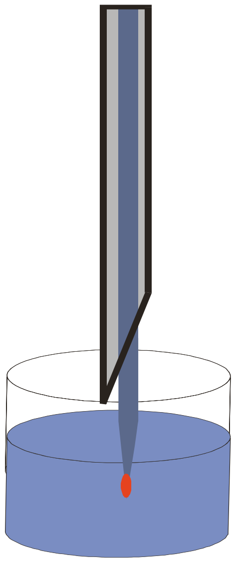
Calibration of the microsensor is recommended before each measurement (see chapter 7 '***Calibration of Microsensors***'). If you don't want to recalibrate the microsensor, you can use the calibration values of your last measurement (see '**User-Defined Calibration**'). Each calibration is only valid for the corresponding microsensor and should be repeated before beginning a new measurement. Especially, after longer measurements (more than 18000 measuring points or 5 h continuous sensor-illumination) the sensor should be re-calibrated. Ensure that the temperature of the sample is known and is constant during measurement, if you do not use temperature compensation. In the case of temperature-compensated measurements, the temperature sensor TEMP 100 should be positioned as close as possible to the microsensor to avoid temperature differences.

8.1 Measurement with Needle-Type Oxygen Microsensors

1. Please carefully read chapter 7.1.2 '***Mounting the Needle-Type Microsensors***' and chapters 7.1.3, 7.1.4 '***Calibration of the Microsensor without / with Automatic Temperature Compensation***' in the manual. There you will find relevant information about the proper handling of microsensors. They are the basic for the following chapter.
2. Connect the OxyMicrovia the RS232 cable to your computer.
3. Connect the temperature sensor TEMP 100 to the 4-pin connector on the front panel of the OxyMicro, to perform temperature-compensated measurement. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.
4. Calibrate the sensor according to chapter 7.1 '***Calibration of Needle-Type Oxygen Microsensors***'. If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose '***User-Defined***' calibration which is described in chapter 7.1.5.
5. Position the microsensor right above your sample. The syringe needle - ***with retracted glass fiber*** – can be punched through a septum or immersed into a tissue. Remove the transport block.

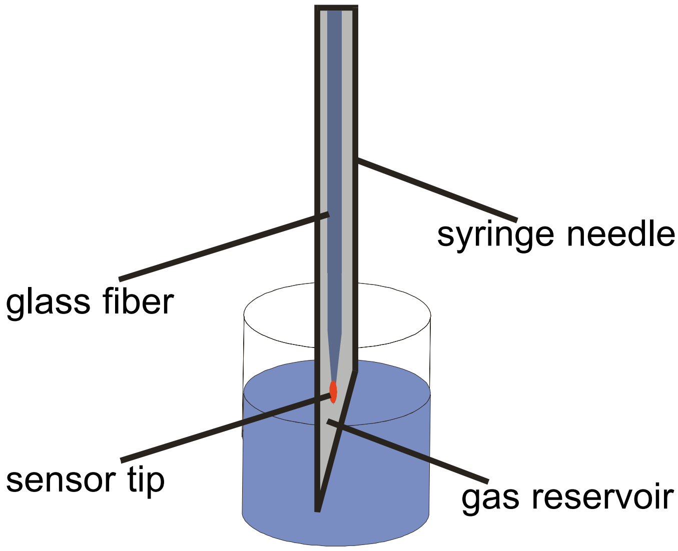
   Extend the glass fiber with its sensor tip from the syringe needle by carefully pressing the syringe plunger. Please take into account that the fine glass fiber with its sensor tip is mechanically quite sensitive! Avoid mechanical stress as far as possible.

6. The sensor tip will only measure accurately if the glass fiber with its sensor tip has been completely extended from the syringe needle. Inside the needle there is an air-reservoir, in which the oxygen content is different to your sample!



right position of the sensor tip!

Sensor tip ***must*** be outside for measurement and calibration!



wrong position of the sensor tip!

There is an air reservoir inside the syringe needle. The oxygen content inside the syringe needle is different to that of the sample, since the gas exchange rate is slow.

7. Please rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Retract the sensor tip into the protective housing and insert the transport block to prevent the syringe plunger from slipping.

8.2 Measurement with Flow-Through Housed Oxygen Microsensors

1. Please carefully read chapter 7.2 "***Calibration of Flow-Through Housed Oxygen Microsensors***" in the manual instructions. There you will find relevant information about the proper handling of microsensors. They are the basic for the following chapter.
2. Connect the OxyMicro via the RS232 cable to your computer.
3. Connect the temperature sensor TEMP100 to the 4-pin connector on the front panel of the OxyMicro and carefully tighten the safety nut, to perform temperature-compensated measurement. Immerse the temperature sensor in your sample and fix it with a laboratory support.
4. Calibrate the sensor according to chapter 7.2 "***Calibration of Flow-Through Housed Oxygen Microsensors***". If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose "***User-Defined***" calibration which is described in chapter 7.2.5.
5. Connect the end-pieces of the T-connector with Luer-Lock tubing or Luer-Lock adapters for tubing and pump your sample through the flow-through cell.
6. Please rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Pump distilled water through the cell until all sample residues are removed.

8.3 Measurement with Implantable Oxygen Microsensors

1. Please carefully read chapter 7.3 '***Calibration of Implantable Oxygen Microsensors***' in the manual instructions. There you will find relevant information about the proper handling of microsensors. They are the basic for the following chapter.
2. Connect the OxyMicro via the RS232 cable to your computer.
3. Connect the temperature sensor TEMP100 to the 4-pin connector on the front panel of the OxyMicro and carefully tighten the safety nut, to perform temperature-compensated measurement. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.
4. Remove the microsensor carefully from the protective cover. The microsensor is protected with a glass housing during the transport. Fix the glass housing microsensor with a clip to a laboratory support or a similar stable construction.
5. Calibrate the sensor according to chapter 7.3 '***Calibration of Implantable Oxygen Microsensors***'. If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose '***User-Defined***' calibration which is described in chapter 7.3.5.
6. The glass fiber with its sensing tip is prevented from slipping from the glass housing using a protection tubing. Remove the fiber cable from the glass housing for implantation. Slacken the protection tubing from the glass housing and carefully extract the glass. Be careful, don't touch the glass housing with the glass fiber tip.

WHEN GLASS-FIBER WITH ITS SENSOR TIP IS EXTRACTED FROM THE PROTECTIVE HOUSING, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK!

7. Be carefully, by implanting the microsensor into your specially designed system. Please contact our service team for custom designed systems.
8. Please rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Retract the sensor tip into the protective housing and insert the transport block to prevent the syringe plunger from slipping.

8.4 Some Advice for Correct Measurement

8.4.1 Signal drifts due to oxygen gradients

Please take into account that the sensor has a high spatial resolution. An oxygen gradient occurs most times in unstirred solutions, which are in contact with ambient air.

In case of needle type sensors, check first if the tip is completely extended from the needle or if air bubbles are on the sensor tip whenever unexpected drifts, gradients or unstable measurement values occur.

In case of flow trough cells, air bubbles located at the sensor tip cause signal drifts.

Critical conditions for bubble formations are, for example, purging with air or other gases and increasing temperature during measurement.

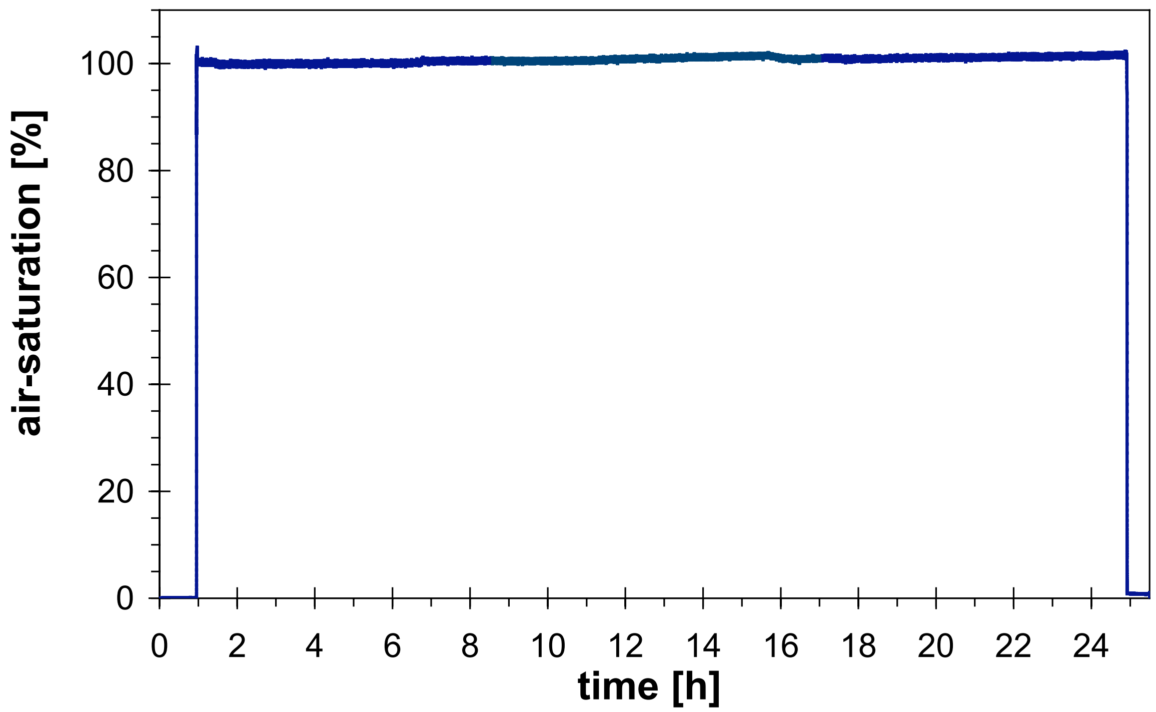
The formation of a bio-film during long-term measurements or the accumulation of other sample components like oil or solid substances may also lead to an oxygen gradient.

8.4.2 Signal drifts due to temperature gradients

A further source of imprecise measurement is insufficient temperature compensation. If you use the temperature compensation, ensure that no temperature gradients exist between the microsensor and the temperature sensors. If you measure without temperature compensation, please bear in mind, that the OxyMicro only measures correctly, if the sample temperature is constant during measurement and the temperature is the same as you typed in at the beginning of the measurement. Please also refer to Chapter 13.5 "Formulas for temperature compensation". If the temperature is measured with a precision of ± 0.2 °C, there is a variation in the measuring value at 100% air-saturation of 100 ± 0.3 % air-saturation. Please choose the measurement with temperature compensation to minimize temperature gradients.

8.4.3 Signal drift due to photo-decomposition

The oxygen-sensitive material may be subject to photo-decomposition resulting in a signal drift. Photo-decomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light. Therefore, the excitation light is minimized.
Continuous illumination of a tapered sensor tip over a period of 24 hours may lead to a phase drift of up to + 1.6 % air-saturation measured at 100% air-saturation at 20°C. However, this effect of photo-decomposition can even be minimized, by changing the measuring mode to the second or minute interval mode. In these modes, the software switches off the excitation light after recording the data point and switches it on after the interval you have chosen.
Please use the interval method whenever it is possible to increase the shelf life of the microsensor.



Photostability of a tapered oxygen microsensor.

Drift in % air-saturation at 100% air-saturation when illuminating the microsensor with a tapered and flat broken sensor tip for 1, 12 and 24 hours in the continuous mode

Mode	Drift per hour	Drift per 12 hours	Drift per 24 hours
tapered sensor tip			
continuous mode (1 s)	± 0.6 % air-saturation	+1 % air-saturation	+1.6 % air-saturation
flat broken sensor tip			
	± 0.5% air-saturation	± 0.5% air-saturation	< 0.6 % air-saturation

8.4.4 Performance proof

If you want to prove the performance during the past measurement, please check the calibration values by inserting the sensor tip in the '**cal 0**' and '**cal 100**' calibration standards when you have finished your measurement. If the device shows 0% air-saturation immersing the sensor tip into the '**cal 0**' solution and 100 % air-saturation measuring the '**cal 100**' standard, the sensor worked perfectly during the whole measurement.

9 General Instructions:

9.1 Warm-Up Time

The warm up time of the electronic and opto-electronic components of the OxyMicro is 5 min. Afterwards stable measuring values are obtained.

9.2 Maintenance

The instrument is maintenance-free.
The housing should be cleaned only with a moist cloth. Avoid any moisture entering the housing! Never use benzene, acetone, alcohol or other organic solvents.
The ST-fiber connector of the microsensor can be cleaned only with lint-free cloth. The sensor tip may be rinsed only with distilled water. Please ensure that no sample residues are inside the syringe needle. If necessary, rinse the glass-fiber with its oxygen-sensitive tip with distilled water.

9.3 Service

Balancing, maintenance and repair work may only be carried out by the manufacturer:

WPI, Inc
Sarasota International Trade Center
175 Sarasota Center Blvd
Sarasota, FL 34240

Phone: 941-371-1003
Fax: 941-377-5428
E-mail: wpi@wpiinc.com
Internet: www.wpiinc.com

Please contact our Technical Support Department should you have any questions. We look forward for helping you and are open for any questions and criticism.

10 Technical Data

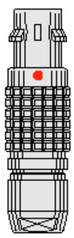
10.1 General Data

MODES	
oxygen	range: 0 - 500 % air-saturation resolution: 30 ± 0.3 % air -aturation 100 ± 0.9 % air-saturation 250 ± 3.6 % air-saturation accuracy: ± 1 % air-saturation
temperature	range: 0 - 50 °C resolution: ± 0.5 °C accuracy: ± 1° C

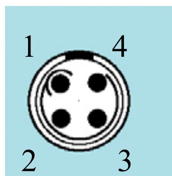
CALIBRATION PROCEDURE	2-point calibration in oxygen free water and humidified air or air-saturated water

OPTICAL OUTPUT / INPUT	
Optical connector	ST compatible, Core/Center 100/140
Channels	1
Wavelength	505 nm

TEMPERATURE SENSOR INPUT

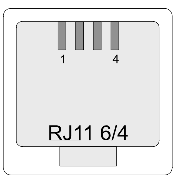


1 ... TEMP100-1
2 ... n.c.
3 ... n.c.
4 ... TEMP100-2

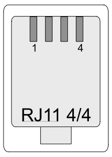
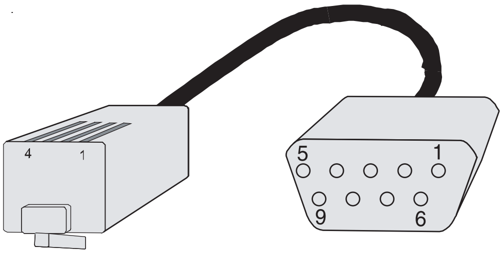


Lemo Connector Size 00	Connector for TEMP100 temperature sensor

DC INPUT	DC-Range : 12 V/1250mA up to 18V/900mA



1 ... GND
2 ... +18 VDC
3 ... GND
4 ... +18 VDC

DIGITAL OUTPUT	
communication protocoll	serial interface RS232 19200 Baud, Databits 8, Stoppbits 1, Parity none, Handshake none
instrument output:  **1 ... TXD** **2 ... RXD** **3 ... n.c.** **4 ... GND**	on RJ11 4/4 plug
Interface cable to PC:  **RJ11 4/4** **1 ... TXD** **2 ... RXD** **3 ... n.c.** **4 ... GND** **D-Sub9 female** **2 ... RXD** **3 ... TXD** **5 ... GND** **1,4,6,7,8,9 ... n.c**	RJ11 4/4 to DSub9:

ENVIRONMENTAL CONDITIONS	
Operating temperature	0 to +50ºC
Storage temperature	-10 to +65ºC
Relative humidity:	up to 95%

OPERATION CONTROL	*LED at the front panel:* red: instrument off green: instrument on orange: stand by

DIMENSIONS	length: 185 mm; width: 110 mm; height: 45 mm; weight: 630 g;

10.2 Analog Output and External Trigger

The OXYMICRO-AOT instrument version is supplied with a dual programmable 12-bit analog output with galvanic isolation and an external trigger input.

- **ANALOG OUTPUT**

GENERAL SPECIFICATION - ANALOG OUTPUT

Channels	2
Connector	BNC
Resolution	12 bit
Output range	0 to 4095mV (±2mV max. error)
Galvanic isolation	500V rms
Shortcut protection	Yes

Programmable to oxygen, temperature, amplitude, phase by software

Equivalence coefficients :

oxygen	1 :: 0.1	(i.e. : 973 mV = 97.3 % air saturation)
temperature	1 :: 0.1	(i.e. : 208 mV = 20.8°C)
amplitude	1 :: 10	(i.e. : 2220 mV = 22200 relative units)
phase	1 :: 0.025	(i.e. : 1100 mV = 27.50°)

Update rate:
The update rate is dependent on the sampling rate of the software. If an external trigger is used, the update rate is equivalent to the trigger pulse rate.

DC SPECIFICATION - ANALOG OUTPUT

Resolution

oxygen	± 2mV ➔ ± 0.2 % air saturation
temperature	± 2mV ➔ ± 0.2°C
amplitude	± 2mV ➔ ± 20 relative units
phase	± 2mV ➔ ± 0.05°)

Accuracy error ± 10mV

- **EXTERNAL TRIGGER INPUT**

GENERAL SPECIFICATION - EXTERNAL TRIGGER INPUT

Channels	1
Connector	BNC
Input voltage range	TTL-compatible / up to 24V
Trigger mode	Low-High-Low (Input must be kept Low for at least 50µs)
Normal state	no current
Isolation	500V rms

Timing Specifications:

Min rise &fall time for trigger	15ns (see TTL-specification)
Max rise &fall time for trigger	2 ms
Min pulse length	3 ms
Min pause length	10 ms
Min periode length	13 ms

10.3 Technical Notes

Power Adapter

OxyMicroshould always be used with the original power adapter (110-220VAC/12VDC). As an alternative power source a battery can be used that meets the DC input voltage given in technical specification. The battery adapter cable is available as an additional accessory.

Analog Outputs

WARNING: The analog outputs are not protected against any input voltage! Any voltage applied to the analog outputs can cause irreversible damage of the circuit.

RS232 Interface

The unit uses special interface cable. Another cable can cause the unit's malfunction.

Optical Output (ST)

The ST connector is a high precision optical component. Please keep it clean and dry. Always use the rubber cap to close the output when not in use.

10.4 Operation Notes

Oxygen Measurement

To achieve the highest accuracy OxyMicroshould be warmed-up for 5min before starting the measurement. Please see the details of measurement process described in OxyMicromanual.

Temperature Compensation

No other than supplied temperature sensor could be used with the unit. The use of any other temperature sensor can damage the oxygen meter.

11 Trouble Shooting

Error	Cause	Action
Device does not work and LED on the front panel is not lit	Device is not switched on No power supply	Switch on device with ON/OFF switch on the rear panel Connect power supply with device
Device does not work and LED on the front panel is on	No connection to PC	Check connection of the device to your PC (RS 232)
Temperature compensation failed, no temperature measurement possible	TEMP 100 sensor is not connected properly TEMP 100 Sensor is faulty	Check connection, Contact our service
Warning light: Amplitude: red	Microsensor is not connected properly ST-Connector is contaminated Sensor tip is damaged	Check connection of the ST-connector Clean connector with a soft, lint-free cloth rag Replace oxygen sensor; (Send the sensor back to WPI for re-coating service;
Warning light: Phase: red	phase angle out of limits	- check connection of the microsensor - replace microsensor
Warning light: overload: red	too much false light	- reduce false light - use optical-isolated microsensors
no cal	calibration failed Sensor was not in the right calibration standard sulfite solution has aged	- calibrate again - check calibration solutions Immerse Sensor in the proper calibration vessel Prepare fresh sulfite -solution
Strong signal fluctuations	Air bubbles at sensor tip The glass-fiber with its sensor tip is not extended from the syringe needle	Remove air bubbles by carefully tapping Completely extend the sensor

12 Concluding Remarks

Dear customer,

With this manual, we hope to provide you with an introduction to work with the OxyMicro fiber-optic oxygen-meter.

This manual does not claim to be complete. We are endeavored to improve and supplement this Version.

We are looking forward to your critical review and to any suggestions you may have.

You can find updated information at www.wpiinc.com

With best regards,

Your WPI Technical Support Department

13 Appendix

13.1 Basics in Optical Sensing of Oxygen

13.1.1 Dynamic Quenching of Luminescence

The principle of measurement is based on the effect of dynamic luminescence quenching by molecular oxygen. The following scheme explains the principle of dynamic luminescence quenching by oxygen.

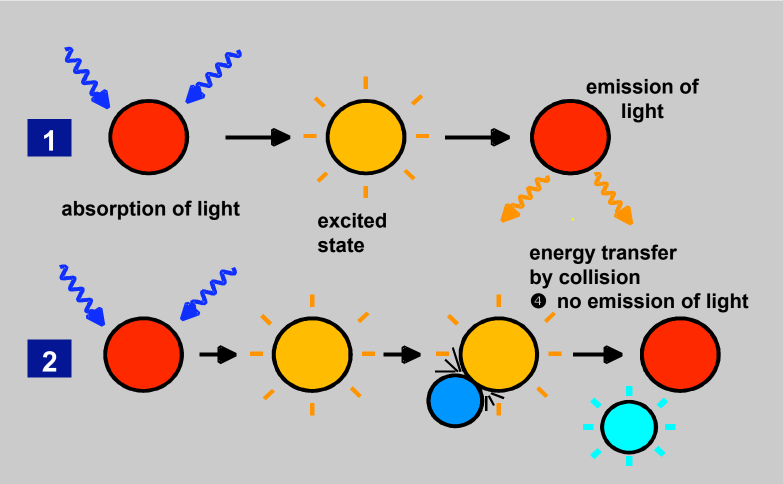


Figure 13.1 *Principle of dynamic quenching of luminescence by molecular oxygen*
(1) Luminescence process in absence of oxygen
(2) Deactivation of the luminescent indicator molecule by molecular oxygen

The collision between the luminophore in its excited state and the quencher (oxygen) results in radiationless deactivation and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable luminescence signal decreases.
A relation exists between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime which is described in the Stern-Volmer-equation (**1**). Here, τ_0 and τ are the luminescence decay times in absence and presence of oxygen (I_0 and I are the respective luminescence intensities), $[O_2]$ the oxygen concentration and K_{SV} the overall quenching constant

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2]$$
$$I = f([O_2])$$
$$\tau = f([O_2]) \qquad (1)$$

I: Luminescence intensity in presence of oxygen

I_0: Luminescence intensity in absence of oxygen

τ: Luminescence decay time in presence of oxygen

τ_0: Luminescence decay time in absence of oxygen

K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)

$[O_2]$: oxygen content

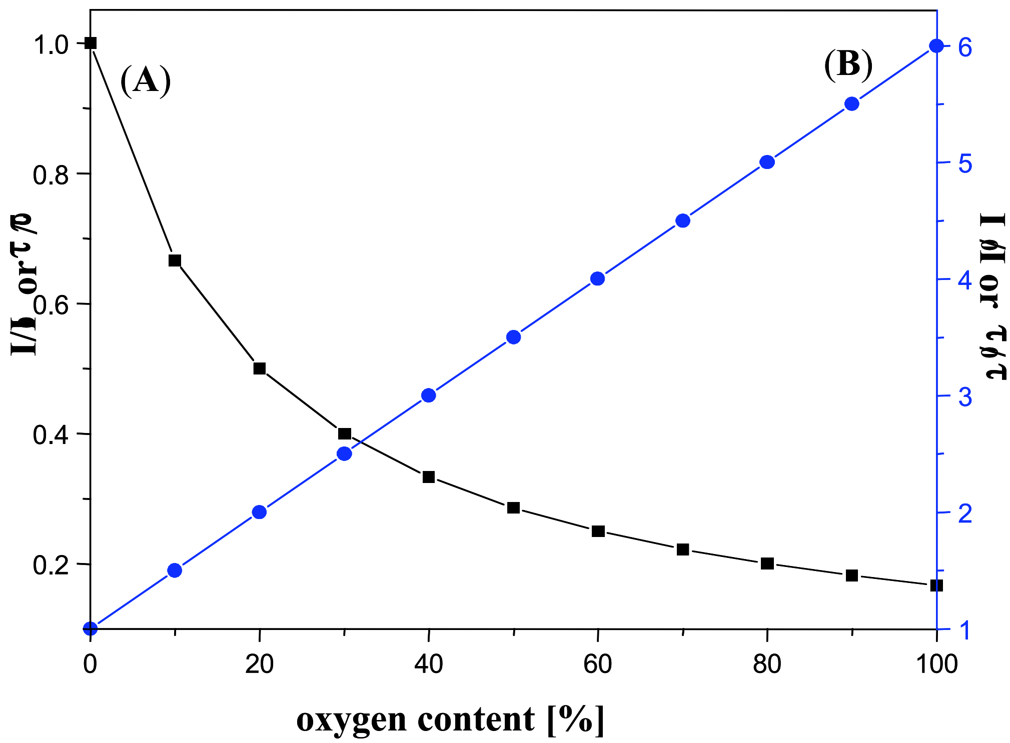


Figure. 13.2 *(**A**) Luminescence decrease in the presence of oxygen. (**B**) Stern-Volmer plot.*

Indicator dyes quenched by oxygen are, for example polycyclic aromatic hydrocarbons, transition metal complexes of Ru(II), Os(II) and Rh(II), and phosphorescent porphyrins containing Pt(II) or Pd(II) as the central atom.

13.1.2 Major Components of Fiber-Optic Microsensors

In optical chemical sensors, the analyte interacts with an indicator and changes its optical properties. The result is either a change in the color (absorbance or spectral distribution) or the luminescence properties (intensity, lifetime, polarisation). Light acts as the carrier of the information.

The major components of a typical fiber optical sensing system are

- a light source to illuminate the sensor (laser, light emitting diode, lamps)
- an optical fiber as signal transducer (plastic or glass fiber)
- a photodetector (photodiode, photomultiplier tube, CCD-array)
- the optical sensor (indicator immobilised in a solid matrix)

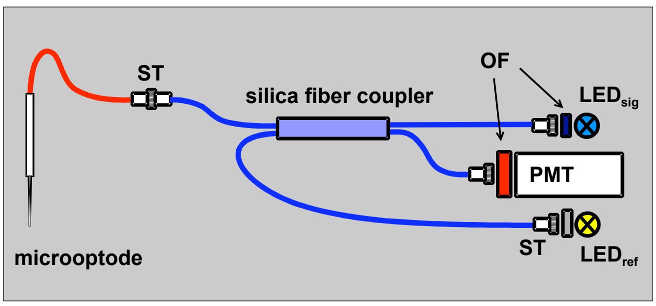


Figure 13.3 Schematic drawing of the optical setup of a measuring system with microsensors (LED: light emitting diodes, PMT: photomultiplier, OF: optical filters, ST: fiber connector)

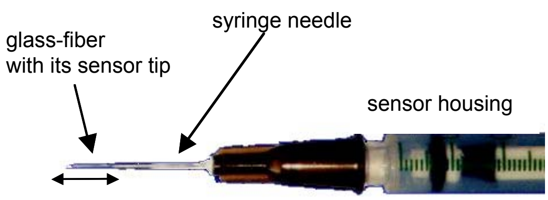


Figure 13.4 *Scheme of a microsensor*

13.1.3 Advantages of Optical Oxygen-Sensitive Microsensors

- no oxygen is consumed during the measurement;
- the signal is independent of changes in flow velocity;
- high spatial (< 50 µm) and temporal resolution (t_{90} < 1 s)
- they are able to measure the oxygen content in dry gases
- they are insensible towards electrical interferences and magnetic fields;
- they are more sensitive than conventional electrodes (up to ppb-range);
- using silica fibers, it is possible to measure in samples while physically separate from the light source and detectors;
- light conducting fibers are able to transport more information than power currents (information can be simultaneously transferred, e.g., intensity of light, spectral distribution, polarisation, information such as decay time or delayed fluorescence);

13.1.4 Luminescence Decay Time

The OXYMICRO measures the luminescence decay time of the immobilised luminophore as the oxygen dependent parameter.

$$\tau = f([O_2]) \qquad (2)$$

The OXYMICROuses the phase-modulation technique to evaluate the luminescence decay time of the indicators. If the luminophore is exited with a sinusoidally intensity modulated light, its decay time causes a time delay in the emitted light signal. In technical terms, this delay is the phase angle between the exiting and emitted signal. This phase angle is shifted as a function of the oxygen concentration. The relation between decay time t and the phase angle F is shown by the following equation:

$$\tau = \frac{\tan\Phi}{2\pi \cdot f_{mod}} \qquad (3a)$$

$$\tan\Phi = 2\pi \cdot f_{mod} \cdot \tau \qquad (3b)$$

$$\tau \int \tan\ \Phi \int \Phi \int f([O_2]) \qquad (3c)$$

τ: luminescence decay time; Φ: phase angle; f_{mod}: modulation frequency

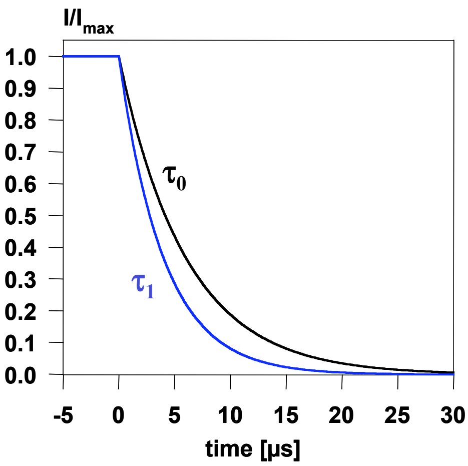


Figure 13.5 *Schematic of the single exponential decay (t_0 > t_1).*

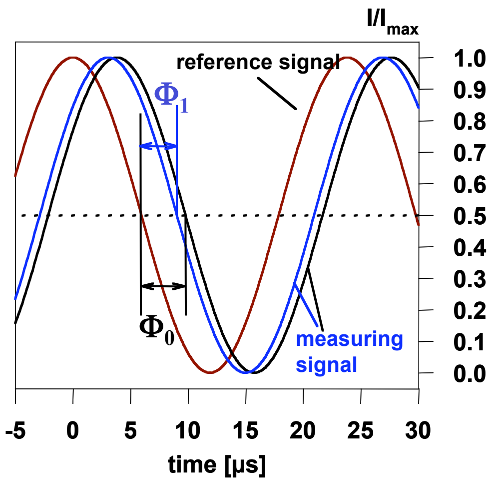


Figure 13.6 *The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state*

The measurement of the luminescence decay time, an intrinsically referenced parameter, has the following advantages compared to the conventional intensity measurement.

- The decay time does not depend on fluctuations in the intensity of the light source and the sensitivity of the detector;
- The decay time is not influenced by signal loss caused by fiber bending or by intensity changes caused by changes in the geometry of the sensor;
- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ Photobleaching and leaching of the indicator dye has less influence on the measuring signal;

The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

13.1.5 Literature

If you want to find out more about this subject, we recommend the following publications.

- Wolfbeis O.S. (Ed.), ***Fiber Optic Chemical Sensors and Biosensors***, Vol. **1**&**2**, CRC, Boca Raton (1991).
- Klimant I., Wolfbeis O.S., ***Oxygen-Sensitive Luminescent Materials Based on Silicone-Soluble Ruthenium Diimine Complexes***, Anal. Chem., **67**, 3160-3166 (1995).
- Klimant I., Kühl M., Glud R.N., Holst G., ***Optical measurement of oxygen and temperature in microscale: strategies and biological applications***, Sensors and Actuators B, **38-39**, 29-37 (1997).
- Holst G., Glud R.N., Kühl M., Klimant I., ***A microoptode array for fine-scale measurement of oxygen distribution***, Sensors and Actuators B, **38-39**, 122-129 (1997).
- Klimant I., Meyer V., Kühl M., ***Fiber-optic oxygen microsensors, a new tool in aquatic biology***, Limnol. Oceanogr., **40**, 1159-1165 (1995).
- Klimant I., Ruckruh F., Liebsch G., Stangelmayer A., Wolfbeis O.S., ***Fast Response Oxygen Microsensors Based on Novel Soluble Ormosil Glasses***, Mikrochim. Acta, **131**, 35-46 (1999).

13.2 Determination of the Oxygen Concentration Using a Modified Stern-Volmer Equation

The Stern-Volmer equation (**4**) displays a linear correlation between $\tan\Phi_0/\tan\Phi$ or τ_0/τ and the oxygen concentration $[O_2]$.

$$\frac{\tan\Phi_0}{\tan\Phi} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2] \qquad (\mathbf{4})$$

Φ_0 phase angle of oxygen-free water
Φ measured phase angle
K_{SV} Stern-Volmer Constant
$[O_2]$ oxygen content in %-air-saturation

The Stern-Volmer plots of all our sensors show a distinct non-linearity in their response behavior which is also observed for many other oxygen sensors described in literature. This non-linear response behavior can be described with a modified Stern-Volmer equation.

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{f_1}{1+K_{SV_1}\cdot[O_2]} + \frac{1-f_1}{1+K_{SV_2}\cdot[O_2]})\right)^{-1} \qquad (5)$$

This model is based on the assumption that the indicator is distributed in the polymer matrix at two different sites and each fraction (f_1, 1-f_1) shows a different quenching constant (K_{SV1}, K_{SV2}).

For practical use this model is not very convenient since it has too many parameters which have to be calibrated. Therefore, two simplified models based on equation 5 can be used.

In the first model, one fraction of the indicator is assumed to be non-quenchable (K_{SV2}=0).

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{f_1}{1+K_{SV}\cdot[O_2]} + (1-f_1)\right)^{-1} \qquad (6)$$

Equation 7 was used to describe the oxygen calibration plot of a microsensor type B2. The correlation coefficient R^2 of this fit was higher than 0.999.

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{0.89}{1+K_{SV}\cdot[O_2]} + 0.11)\right)^{-1} \qquad (7)$$

The oxygen content in %-air-saturation can be calculated according to equation 8.

$$[O_2] = \frac{1-\frac{\tan\Phi}{\tan\Phi_0}}{K_{SV}\cdot\left(\frac{\tan\Phi}{\tan\Phi_0} - 0.11\right)} \qquad (8)$$

A second model, which also is based on equation 5, can be used for describing the oxygen calibration plot. In this model, K_{SV2} is set to be x*K_{SV1}. f_1 was determined to be 0.805 and x was determined to be 1/22.9. The correlation coefficient R^2 of this fit was higher than 0.9999.

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{0.804}{1+K_{SV_1}\cdot[O_2]} + \frac{0.196}{1+\frac{1}{22.9}\cdot K_{SV_1}\cdot[O_2]})\right)^{-1} \qquad (9)$$

The oxygen content in %-air-saturation can be calculated according to equation 10.

$$[O_2] = \frac{-B + \sqrt{B^2 - 4 \cdot A \cdot C}}{2 \cdot A} \quad \textbf{(10)}$$

with the coefficients

$$A = \frac{\tan\Phi}{\tan\Phi_0} \cdot x \cdot K_{SV}^2 \quad \textbf{(10a)}$$

$$B = \frac{\tan\Phi}{\tan\Phi_0} \cdot K_{SV} + \frac{\tan\Phi}{\tan\Phi_0} \cdot x \cdot K_{SV} - f_1 \cdot x \cdot K_{SV} - K_{SV} + f_1 K_{SV} \quad \textbf{(10b)}$$

$$C = \frac{\tan\Phi}{\tan\Phi_0} - 1 \quad \textbf{(10c)}$$

13.3 Oxygen Conversion Formulas

Please note:
These conversion formulas are only valid in aqueous solutions and humidified air. These formulas have to be modified if measurements are performed in organic solvents or solutions with high salinity.

% -Saturation

%-air saturation

Default-Setting of the instrument (see equation 10 in 13.2)

%-oxygen saturation

$$\%O_2 = \% \text{ air-saturation} \cdot \frac{20.95}{100} \quad \textbf{(11)}$$

0.2095: volume content of oxygen in air

ppm in the gaseous phase:

$$\text{ppm}[O_2] = \%\text{air} - \text{saturation} \cdot \frac{20.95}{100} \cdot 10000 \quad \textbf{(12)}$$

$$1\text{ppm} = \frac{1}{1000000} = \frac{1\text{mg}}{1\text{kg}} = \frac{1\mu\text{L}}{1\text{L}} = \frac{1}{10000}\%$$

Partial pressure of oxygen

in hPa

$$p_{O_2}[\text{hPa}] = \left(p_{\text{atm}}[\text{hPa}] - p_W(T)[\text{hPa}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \qquad (13)$$

in mbar

$$p_{O_2}[mbar] = \left(p_{atm}[mbar] - p_W(T)[mbar]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \qquad (14)$$

in Torr

$$p_{O_2}[Torr] = \left[\left(p_{atm}[mbar] - p_W(T)[mbar]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095\right] \cdot 0.75 \qquad (15)$$

Please note:
1 mbar = 1 hPa = 0.750 Torr

Oxygen Concentration

in mg/L

$$c_{O_2}[\text{mg/L}] = \frac{p_{\text{atm}} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \qquad (16)$$

in ppm

$$c_{O_2}[\text{ppm}] = \frac{p_{\text{atm}} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \qquad (17)$$

in µmol/L

$$c_{O_2}[\mu\text{mol/L}] = \left[\frac{p_{\text{atm}} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M}\right] \cdot 31.25 \qquad (18)$$

p_{atm}: actual atmospheric pressure;

p_N: standard pressure (1013 mbar);

0.2095: volume content of oxygen in air;

$p_W(T)$: vapor pressure of water at temperature T given in Kelvin;

$\alpha(T)$: Bunsen absorption coefficient at temperature T;

$M(O_2)$: molecular mass of oxygen (32 g/mol);

V_M: molar volume (22.414 l/mol);

13.4 Temperature Dependent Constants Affecting the Oxygen Content

13.4.1 Water Vapor Pressure

As shown in equation 13 - 18, the water vapor pressure p_w influences the oxygen partial pressure of air-saturated water and water-vapor saturated air.

Oxygen partial pressure in dry air:

$$p(O_2) = p_{atm} \cdot 0.2095 \quad \textbf{(19)}$$

$p(O_2)$: oxygen partial pressure in dry air at a barometric pressure p_{atm}
0.2095: volume content of oxygen in air;

Oxygen partial pressure in air-saturated water and water-vapor saturated air:

$$p(O_2)' = (p_{atm} - p_W(T)) \cdot 0.2095 \quad \textbf{(20)}$$

Water vapor pressure is strongly affected by temperature variations and this, of course, influence the oxygen partial pressure as shown in equation 19.

Table 13.1 Variation of water vapor pressure $p_W(T)$ with temperature.

θ [°C]	0	5	10	15	20	25	30	35	40	50
T [K]	273	278	283	288	293	298	303	308	131	323
$p_W(T)$ [mbar]	6.1	8.7	12.3	17.1	23.3	31.7	42.4	56.3	73.7	123.3

A convenient fitting function is given by the Campbell equation 20

$$p_W(T) = \exp\left(A - \frac{B}{T} - C \cdot \ln T\right) \quad \textbf{(21)}$$

where T is the temperature in Kelvin and A, B and C constants given in Figure 13.7

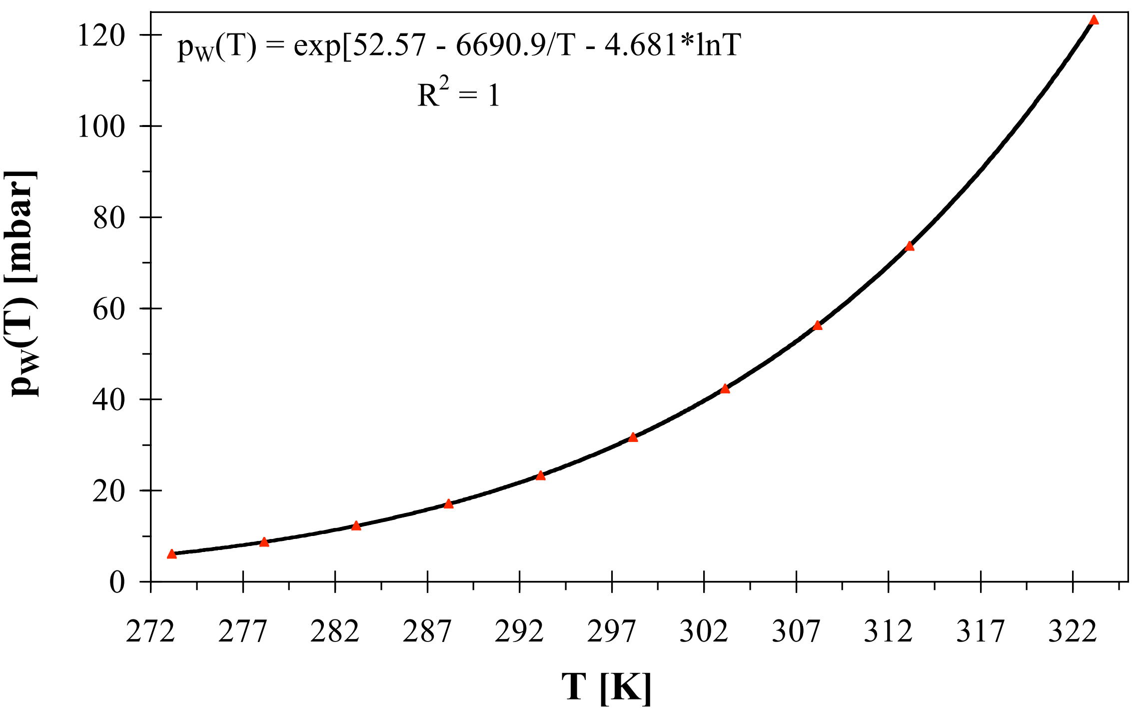


***Figure. 13.7** Variation of water vapor pressure with temperature.*

13.4.2 Bunsen Absorption Coefficient

The solubility of oxygen in water is temperature dependent and can be described using the Bunsen absorption coefficient $\alpha(\theta)$ and the oxygen partial pressure $p(O_2)$ according to equation 22. With increasing temperature, the solubility of oxygen in water decreases.

$$c_S(p,\theta) = \frac{p(O_2)}{p_N}\alpha(\theta) \qquad (22)$$

$c_S(p,\theta)$: temperature dependent solubility of oxygen in water
$p(O_2)$: oxygen partial pressure
p_N: standard pressure (1013 mbar);

Table 13.2 Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature.

θ [°C]	0	5	10	15	20	25	30	35	40	50
$\alpha(\theta)\cdot 10^3$	49.01	42.94	38.11	34.17	31.01	28.43	26.30	24.63	23.16	20.85

The data in Table 13.2 can be described by two forms of equations.

The first form of equation to describe the temperature dependent variation of the Bunsen absorption coefficient $\alpha(\theta)$ is obtained by fitting a general power series to the values in Table 13.2. A fourth degree polynomial fit can be chosen, yielding equation 23

$$10^3\alpha = a + b\cdot\theta + c\cdot\theta^2 + d\cdot\theta^3 + e\cdot\theta^4 \qquad (23)$$

where θ is the temperature in °C and a - e the coefficients calculated by standard curve fitting procedures.

a = 48.998
b = -1.335
c = $2.755 * 10^{-2}$
d = $-3.220 * 10^{-4}$
e = $1.598 * 10^{-6}$

The square of the correlation coefficient is 0.999996.

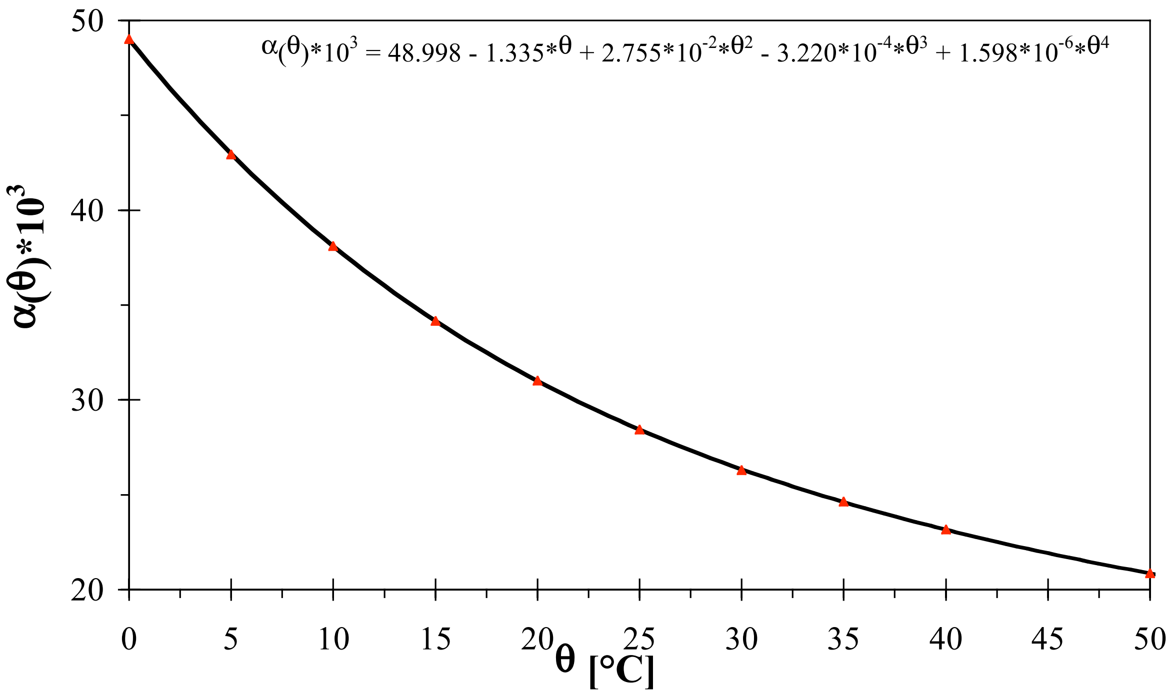


Figure 13.8 Variation of Bunsen absorption coefficient α (θ) with temperature.

The other form of equation to describe the variation of α with temperature can be derived from a thermodynamical correlation and gives an equation of the form

$$\ln 10^3 \alpha = \frac{A}{T} + B \cdot \ln T + C \qquad \textbf{(24)}$$

where A, B and C are constants and T is the temperature in K. For oxygen dissolved in water we find by fitting the equation to the values of α in Table 13.2 that A = 8.553 * 10^3, B = 2.378 * 10, and C = -1.608 * 10^2.

Values of α calculated from eqns. 23 and 24 for the same temperature agree within ±0.5%.

The Bunsen absorption coefficient, however, is not a very practical measure. Values of $\alpha(\theta)$ have therefore to be converted to mg/L, and the method for doing this is best illustrated by an example.

Example: Calculation of the oxygen content ($c_S(p_{atm},\theta)$) in air-saturated water at a temperature θ of 20°C.
Equation 24 allows the solubility of oxygen in air-saturated fresh water to be calculated for any temperature and pressure provided that the values of the Bunsen absorption coefficient $\alpha(T)$ and the vapor pressure $p_W(T)$ at the particular temperature are known. Equation 23 or 23 can be used to obtain α, and p_W can be calculated from equation 21. Table 13.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C.

$$c_S(p_{atm}, \theta) = \frac{p_{atm} - p_W(\theta)}{p_N} \cdot 0.2095 \cdot \alpha(\theta) \cdot \frac{M_{O_2}}{V_M} \qquad \textbf{(25)}$$

In equation 25 p_{atm} is the actual atmospheric pressure corrected for the contribution of the water vapor pressure p_w and related to standard pressure p_N. The corrected pressure is multiplied by 0.2095, the volume content of oxygen in air, by $\alpha(\theta)$ and by the molecular mass of oxygen (M_{O2}) divided by the molar volume V_M.
At a given atmospheric pressure of 1013 mbar ($p_{atm} = p_N$) and a temperature of 20 °C the oxygen content can be calculated according to equation 26.

$$c_S(1013\,\text{mbar}, 20°C) = \frac{1013 - 23.3}{1013} \cdot 0.2095 \cdot 0.031 \cdot \frac{32 \cdot \text{g/mol}}{22.414\,\text{mol/L}} = 0.009\,\text{g/L} = 9.06\,\text{mg/L} \qquad \textbf{(26)}$$

Figure 13.9 shows the temperature-dependent oxygen solubility in air-saturated fresh water and Table 13.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C.

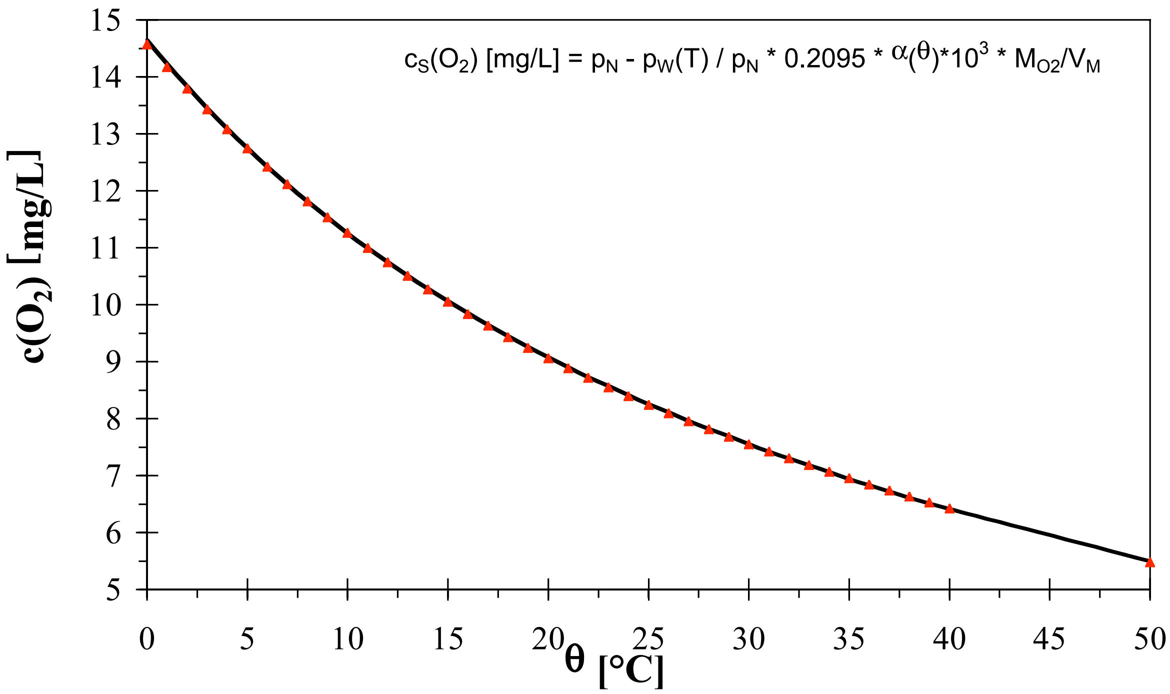


Figure 13.9 *Dependence of the oxygen solubility in air-saturated fresh water on temperature*

Table 13.3 Oxygen solubility in air-saturated fresh water [mg/L].

T [°C]	c_S(T)	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
0	**14.**	64	60	55	51	47	43	39	35	31	27	23
1		23	19	15	10	06	03	99	95	91	87	83
2	**13.**	83	79	75	71	68	64	60	56	52	49	45
3		45	41	38	34	30	27	23	20	16	12	09
4		09	05	02	98	95	92	88	85	81	78	75
5	**12.**	75	71	68	65	61	58	55	52	48	45	42
6		42	39	36	32	29	26	23	20	17	14	11
7		11	08	05	02	99	96	93	90	87	84	81
8	**11.**	81	78	75	72	69	67	64	61	58	55	53
9		53	50	47	44	42	39	36	33	31	28	25
10		25	23	20	18	15	12	10	07	05	02	99
11	**10.**	99	97	94	92	89	87	84	82	79	77	75
12		75	72	70	67	65	63	60	58	55	53	51
13		51	48	46	44	41	39	37	35	32	30	28
14		28	26	23	21	19	17	15	12	10	08	06
15		06	04	02	99	97	95	93	91	89	87	85
16	**9.**	85	83	81	70	76	74	72	70	68	66	64
17		64	62	60	58	56	54	53	51	49	47	45
18		45	43	41	39	37	35	33	31	30	28	26
19		26	24	22	20	19	17	15	13	11	09	08
20		08	06	04	02	01	99	97	95	94	92	90
21	**8.**	90	88	87	85	83	82	80	78	76	75	73
22		73	71	70	68	66	65	63	62	60	58	57
23		57	55	53	52	50	49	47	46	44	42	41
24		41	39	38	36	35	33	32	30	28	27	25
25		25	24	22	21	19	18	16	15	14	12	11
26		11	09	08	06	05	03	02	00	99	98	96
27	**7.**	96	95	93	92	90	89	88	86	85	83	82
28		82	81	79	78	77	75	74	73	71	70	69
29		69	67	66	65	63	62	61	59	58	57	55
30		55	54	53	51	50	49	48	46	45	44	42
31		42	41	40	39	37	36	35	34	32	31	30
32		30	29	28	26	25	24	23	21	20	19	18
33		18	17	15	14	13	12	11	09	08	07	06
34		06	05	04	02	01	00	99	98	97	96	94
35	**6.**	94	93	92	91	90	89	88	87	85	84	83
36		83	82	81	80	79	78	77	75	74	73	72
37		72	71	70	69	68	67	66	65	64	63	61
38		61	60	59	58	57	56	55	54	53	52	51
39		51	50	49	48	47	46	45	44	43	42	41
40		41	40	39	38	37	36	35	34	33	32	31

Example:: c_S(**14.3°C**) = **10.21 mg/L**

13.4.3 Dependence on the Salt Concentration

Table 13.4 gives values of the concentration of dissolved oxygen at several temperatures in solutions with various chloride concentrations. Increasing the salt concentration, there is a decrease in the oxygen solubility. This behavior is characteristic for the solubility of many nonelectrolytes - it is the phenomenon known as the ***salting-out effect***.

Instead of chlorinity [Cl⁻] - the amount of chloride in parts per thousand - which was used as a measure of the amount of salt in water, the term salinity is often used. If salinity is preferred as a measure of salt concentration, then the conversion from g/L can be readily made using equation 27.

$$S = 1.805[Cl^-] + 0.03 \qquad (\mathbf{27})$$

where S is the salinity in [‰] or [g/1000g]

Table 13.4 Solubility of oxygen in water as a function of temperature and salt concentration (Total pressure = 760 torr)

[Cl⁻] (g/1000g)	0	4	8	12	16	20
T [°C]	***Oxygen solubility [mg/L]***					
0	14.5	13.9	13.3	12.6	12.0	11.3
10	11.3	10.8	10.4	9.9	9.5	9.0
20	9.1	8.8	8.5	8.1	7.8	7.4
30	7.5	7.3	7.0	6.7	6.4	6.1

The effect of increasing the salt concentration on the vapor pressure is neglectible small as shown in Table 13.5.

Table 13.5. Variation of solution vapor pressure (p_W) with salt concentration

[Cl⁻] (g/1000g)	0	9	18	26
T [°C]	***Vapor pressure of solution (torr)***			
0	4.6	4.5	4.4	4.4
10	9.2	9.1	8.9	8.8
20	17.5	17.3	17.0	16.7
30	31.8	31.4	30.9	30.4

The dependence of oxygen solubility on salt concentration can also be obtained from equation 21 except that now values calculated from either equation 28 or 29 have to be used.

$$10^3 \cdot \alpha = a + b \cdot \theta + c \cdot \theta^2 + d \cdot \theta^3 + e \cdot \theta^4 - [Cl^-] \cdot (p + q \cdot \theta + r \cdot \theta^2 + s \cdot \theta^3 + t \cdot \theta^4) \qquad (\mathbf{28})$$

where θ is the temperature in °C, a - e are the coefficients used in equation 23 and p - t are new constants given in Table 13.6. The values of these new constants, obtained by fitting the polynomial to experimental data in the ranges 0 ≤ θ ≤ 30°C and 0 ≤ [Cl⁻] ≤ 20‰. To obtain an oxygen solubility from the Bunsen absorption coefficient the same procedure as described previously is used.

An alternative equation to compensate the Bunsen absorption coefficient by the salt concentration displays equation 29.

$$10^3 \cdot \alpha = \exp\left[\left(A + \frac{B}{T} + C \cdot \ln T + D \cdot T\right) - [Cl^-]\left(P + \frac{Q}{T} + R \cdot \ln T + S \cdot T\right)\right] \qquad (\mathbf{29})$$

where T is the temperature in Kelvin, and A - D and P - S are the coefficients also given in Table 13.6. They are based on measurements for 273.1 ≤ T ≤ 308.18 K and 0 ≤ [Cl⁻] ≤ 30‰

and is therefore more extensive than equation **28**. Both equations give values of $10^3\alpha$ which agree to better than ±1%.

Table 13.6 Values of the coefficients in equations **26** and **27**.

Eqn. 26	a	$4.900 * 10$	p	$5.516 * 10^{-1}$
	b	-1.335	q	$-1.759 * 10^{-2}$
	c	$2.759 * 10^{-2}$	r	$2.253 * 10^{-4}$
	d	$-3.235 * 10^{-4}$	s	$-2.654 * 10^{-7}$
	e	$1.614 * 10^{-2}$	t	$5.362 * 10^{-8}$
Eqn. 27	A	-7.424	P	$-1.288 * 10^{-1}$
	B	$4.417 * 10^{3}$	Q	$5.344 * 10$
	C	-2.927	R	$-4.442 * 10^{-2}$
	D	$4.238 * 10^{-2}$	S	$7.145 * 10^{-4}$

Seawater has a salinity typically of 35‰ (35g / 1000g) or a chloride content of about 19‰, and therefore, falls within the scope of both equations.

13.5 Temperature Compensation of the Response of Oxygen Sensors

Temperature affects the luminescence decay time as well as the luminescence intensity of the indicator dye. The collisional frequency of the oxygen molecules with the indicator dye reflecting the diffusion coefficient of oxygen is also affected.
A typical oxygen response characteristic at different temperatures is shown in Figure 13.10. The phase angle Φ is a function of the oxygen content ($\Phi = \tan\Phi = f(O_2)$) and decreases with increasing the oxygen content.

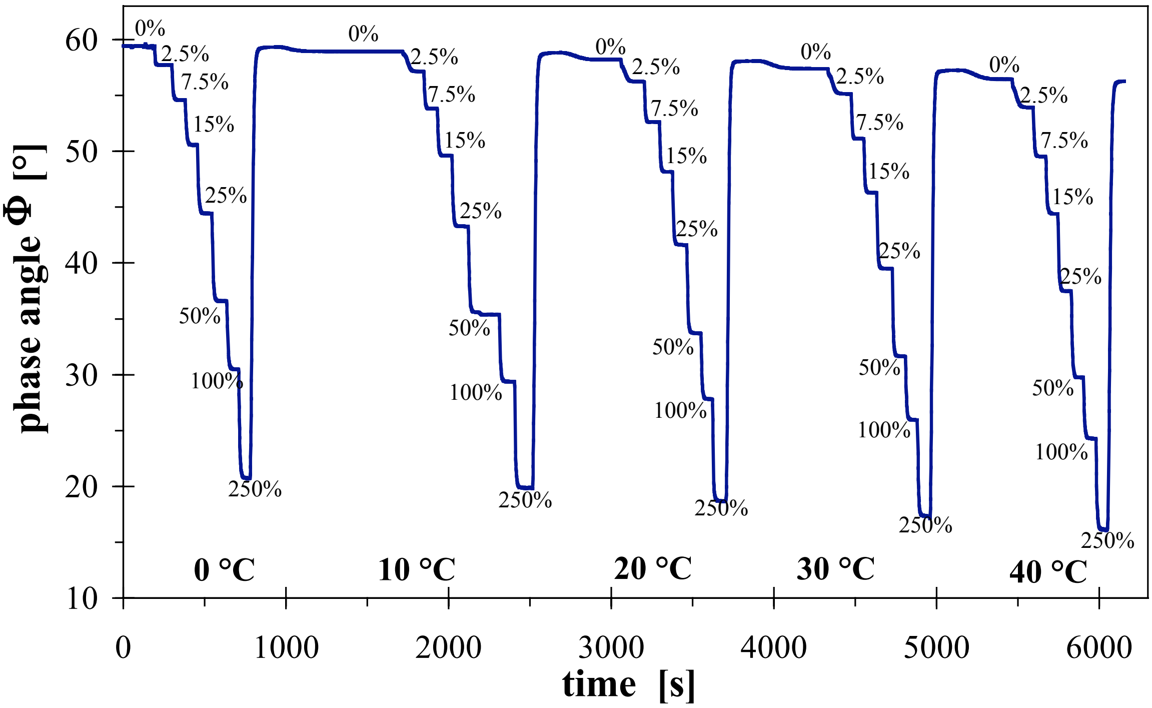


Figure 13.10 *Oxygen response characteristics at different temperatures.*

Figure 13.11 displays the oxygen-dependence of the phase angle at different temperatures and Figure 13.12 the respective Stern-Volmer plots. These two figures and Table 13.7 displays, that both the phase angle in absence of oxygen, Φ_0, and the Stern-Volmer constant, K_{SV}, are temperature dependent. Φ_0 decreases with increasing temperature, while KSV increases with increasing temperature.

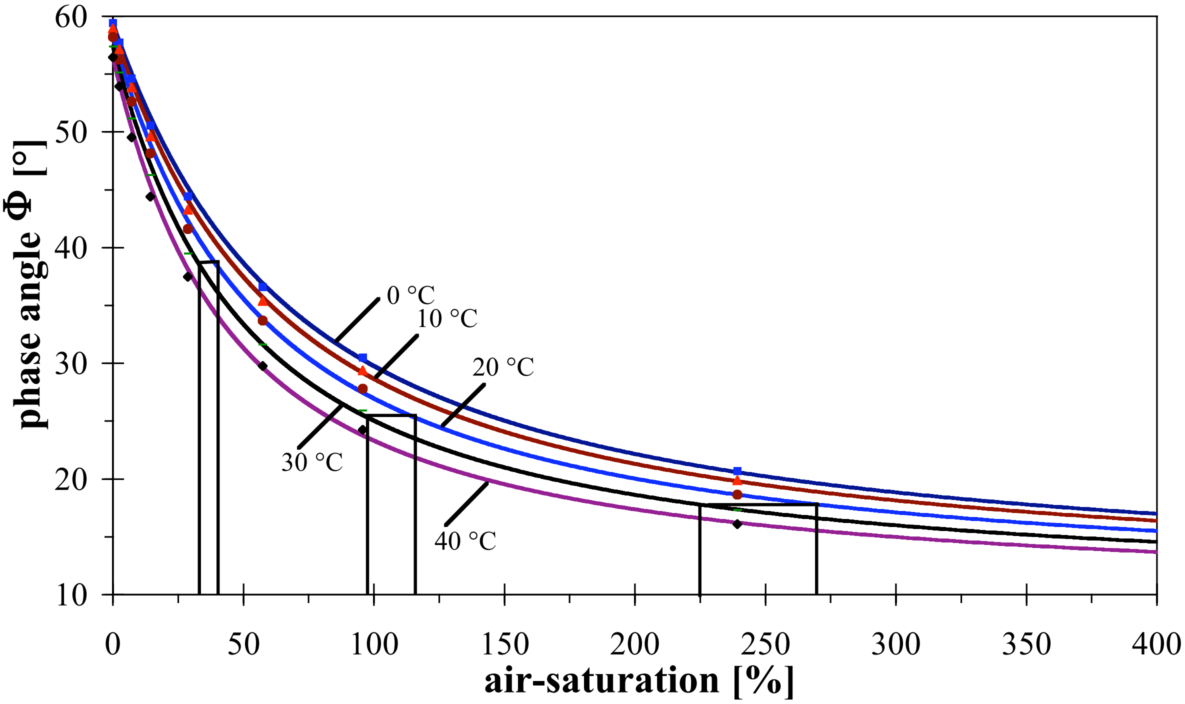


Figure 13.11 *Effect of the temperature on the phase angle at different oxygen contents given in % air-saturation.*

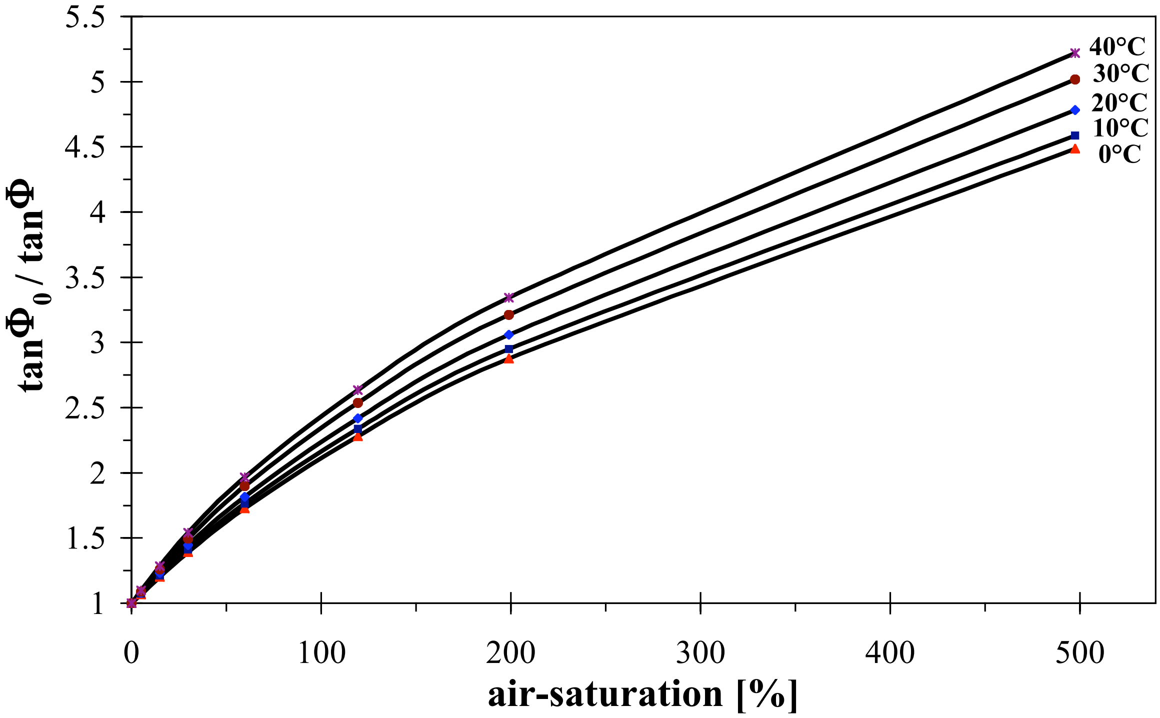


Figure 13.12 *Effect of the temperature on the Stern-Volmer constant.*

Table 13.7 Effect of the temperature on the phase angle in the absence of oxygen (Φ_0) and the Stern-Volmer constant K_{SV}.

θ [°C]	***3.5***	***10***	***20***	***30***	***40***
Φ_0 [°]	59.43	58.94	58.2	57.4	56.44
K_{SV}^a [% air-sat]$^{-1}$	0.03678	0.03929	0.04257	0.04698	0.05085

[a]: The Stern-Volmer constant is determined via equation 9.

Figure 13.13 displays the temperature dependence of Φ_0 and K_{SV}. From Table 13.7 and Figure 13.13 a decreases in Φ_0 of about 0.08° can be calculated by increasing the temperature by 1 K. On the other hand, the Stern-Volmer constant (K_{SV}) increases about $3.8 \cdot 10^{-4}$ [% air-sat.]$^{-1}$ by increasing the temperature by 1 K.

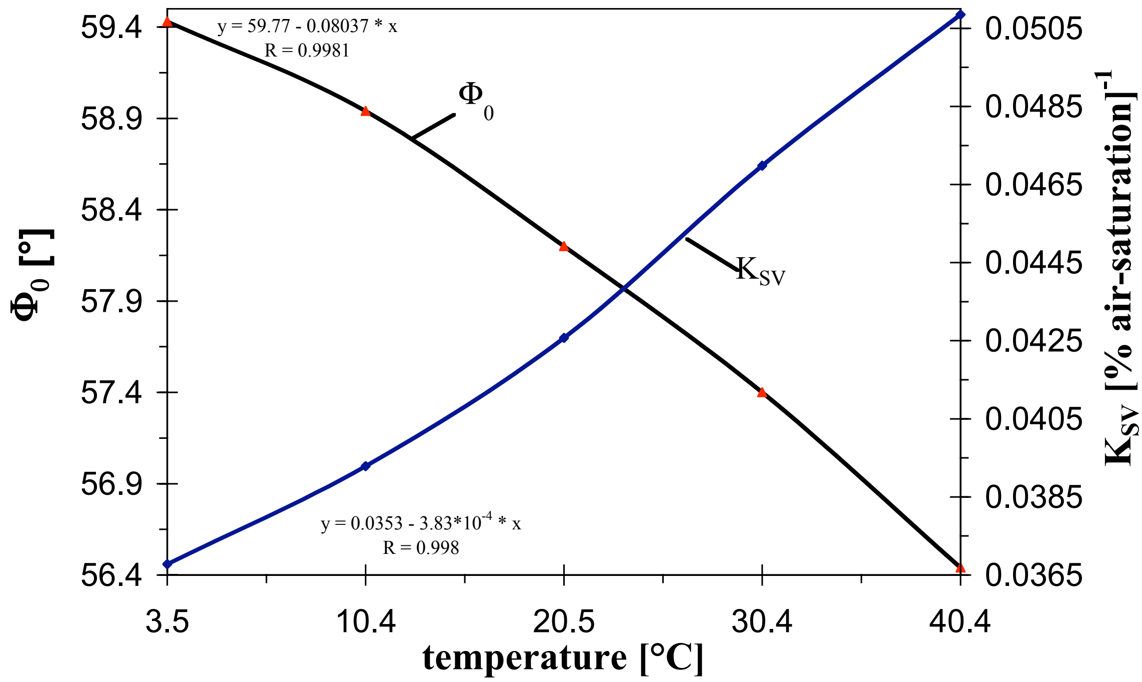


***Figure 13.13** Effect of the temperature on Φ_0 and the Stern-Volmer consant (K_{SV}).*

Consequently, variations in the temperature causes variations in the measuring value at a constant oxygen content (see Figure 13.11). Table 13.8 displays the deviation of the measuring value from the real oxygen content depending on temperature and the oxygen content.

Tabelle 13.8 Variation of the measured oxygen content (Δ % air-saturation) at a constant oxygen content and variations in temperature by ±1K.

% air-saturation	30 %	100 %	250 %
Δ%air-saturation /K	± 0.56 %	± 1.72 %	± 4.68 %

Example:

If the temperature is measured with a precision of ± 0.2 °C, there is a variation in the measuring value at 100% air-saturation of 100 ± 0.3 % air-saturation.

Temperature-compensated oxygen measurements

On our web-page — **www.wpi.com** — you find an excel sheet, tempTypB2.xls, which can calculates the oxygen content in different units after insertion of the calibration values Φ_0, Φ_{100}, the measuring value Φ_m and the respective temperatures T_0, T_{100}, and T_m.
In this sheet, the temperature dependent parameter of the sensor, Φ_0 and K_{SV}, as well as the temperature-dependent solubility of oxygen in fresh water are considered.
Please note:
This sheet in only valid measuring in fresh-water. The effect of salinity on the solubility of oxygen in aqueous samples is not compensated.

program for temperature compensation of oxygen measurements
with WPI oxygen microoptode type B

enter your values here		
input of $\Phi_{0,T0}$	58.94	phase angle of cal 0
input of $\Phi_{100,T100}$	25.95	phase angle of cal 100 (water-vapor saturated air)
input of $\Phi_{m,Tm}$	33.7	measured phase angle
input of T_0	10	temperature of cal 0
input of T_{100}	30	temperatur of cal100
input of T_m	20	temperature at measurement
air pressure p_{atm}	1013	air pressure

Results of calculation + compensation	
[%] air saturation	58.9%
[%] O_2	12.3%
pO_2 [hPa]	122.2hPa
pO_2 [Torr]	91.6Torr
cO_2 [mg/L]	5.34mg/L
cO_2 [ppm]	5.34ppm
cO_2 [µmol/L]	166.80µmol/L

Internal calculated parameter (DO NOT CHANGE !!)	
tan($\Phi_{0,T100}$)	1.559611301
tan($\Phi_{0,Tm}$)	1.608838292
tan($\Phi_{100,T100}$)	0.486652791
tan($\Phi_{m,Tm}$)	0.666917096
$K_{SV,T100}$	0.044378654
$K_{SV,Tm}$	0.040578654
A	136.2596794
B	9.455146335
C	-0.687965334
a	2.98071E-05
b	0.008177656
c	-0.585466669

Figure. 13.14 *Excel Sheet calculating temperature-compensated oxygen contents.*

In this sheet, the evaluation is performed using equation 9

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{0.804}{1 + K_{SV_1} \cdot [O_2]} + \frac{0.196}{1 + \frac{1}{22.9} \cdot K_{SV_1} \cdot [O_2]}) \right)^{-1} \qquad (9)$$

The following definitions are used.

$$\tan\Phi_{100,T_{100}} = \tan\left(\Phi_{100,T_{100}} \cdot \frac{\pi}{180}\right)$$

$$\tan\Phi_{0,T_{100}} = \tan\left([\Phi_{0,T_0} + (-0.08037 \cdot (T_{100} - T_0))] \cdot \frac{\pi}{180}\right)$$

$$\tan\Phi_{m,T_m} = \tan\left(\Phi_{m,T_m} \cdot \frac{\pi}{180}\right)$$

$$\tan\Phi_{0,T_m} = \tan\left([\Phi_{0,T_0} + (-0.08037 \cdot (T_m - T_0))] \cdot \frac{\pi}{180}\right)$$

$$(K_{SV})_{1/2} = \frac{-B \pm \sqrt{B^2 - 4 \cdot A \cdot C}}{2 \cdot A}$$

$$A = \frac{\tan\Phi}{\tan\Phi_0} \cdot x \cdot [O_2]^2$$

$$B = \frac{\tan\Phi}{\tan\Phi_0} \cdot [O_2] + \frac{\tan\Phi}{\tan\Phi_0} \cdot x \cdot [O_2] - f_1 \cdot x \cdot [O_2] - [O_2] + f_1[O_2]$$

$$C = \frac{\tan\Phi}{\tan\Phi_0} - 1$$

$$K_{SV,T_m} = K_{SV,T_{100}} + [3.83 \cdot 10^{-4} \cdot (T_m - T_{100})]$$

$$[O_2]_{1/2} = \frac{-b \pm \sqrt{b^2 - 4 \cdot a \cdot c}}{2 \cdot a}$$

$$a = \frac{\tan\Phi}{\tan\Phi_0} \cdot x \cdot K_{SV}^2$$

$$b = \frac{\tan\Phi}{\tan\Phi_0} \cdot K_{SV} + \frac{\tan\Phi}{\tan\Phi_0} \cdot x \cdot K_{SV} - f_1 \cdot x \cdot K_{SV} - K_{SV} + f_1 K_{SV}$$

$$c = \frac{\tan\Phi}{\tan\Phi_0} - 1$$

T_{100}	temperature in air-saturated water or water-vapor saturated air (‘**cal 100**‘)
T_0	temperature of calibration solution 0 (‘**cal 0**‘)
T_m	temperature at measurement
$\Phi_{100,T_{100}}$	phase angle of ‘**cal 100**‘ at temperature T_{100}
Φ_{0,T_0}	phase angle of ‘**cal 0**‘ at temperature T_0
$\Phi_{0,T_{100}}$	phase angle of ‘**cal 0**‘ at temperature T_{100}
Φ_{m,T_m}	measured phase angle at temperature T_m
$K_{SV,T_{100}}$	Stern-Volmer consant at T_{100}
K_{SV,T_m}	Stern-Volmer consant at T_m

Warranty

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

• Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.

• WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all items returned.

• Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.

• Goods returned for repair must be reasonably clean and free of hazardous materials.

• A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.

• Equipment which was built as a special order cannot be returned.

• Always refer to the RMA# when contacting WPI to obtain a status of your returned item.

• For any other issues regarding a claim or return, please contact the RMA department.

Warning: This equipment is not designed or intended for use on humans.

World Precision Instruments, Inc.

International Trade Center, 175 Sarasota Center Blvd., Sarasota FL 34240-9258

Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK: Astonbury Farm Business Centre • Aston, Stevenage, Hertfordshire SG2 7EG • Tel: 01438-880025 • Fax: 01438-880026 • E-mail: wpiuk@wpi-europe.com

Germany: Liegnitzer Str. 15, D-10999 Berlin • Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com

WORLD PRECISION INSTRUMENTS, INC.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA
Telephone: (941) 371-1003 Fax: (941) 377-5428
e-mail wpi@wpiinc.com

DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

as the distributor of the apparatus listed, declare under sole responsibility that the products:

Title: OXY-MINI & OXY-MICRO Fiber Optic Oxygen Meters

to which this declaration relates are in conformity with the following standards or other normative documents:

Safety: EN 60950: 2001-12

EMC: EN 55022: 2001-11 Class A
EN 61000-3-2: 2001-12
EN 61000-3-3: 2002-05
EN 61000-4-2: 2001-12 through EN 61000-4-6: 2001-12 inclusive
EN 61000-4-8: 2001-12
EN 61000-4-11: 2001-12
EN 61000-6-2: 2001-12

and therefore conform with the protection requirements of Council Directive 89/336/EEC relating to Electromagnetic Compatibility and Council Directive 73/23/EEC relating to Safety Requirements, as well as Council Directive R&TTE 1999/5/EC.

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Mr. Cliff Bredenberg
President
World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA

Mr. Glen Carlquist
Production Manager
World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA