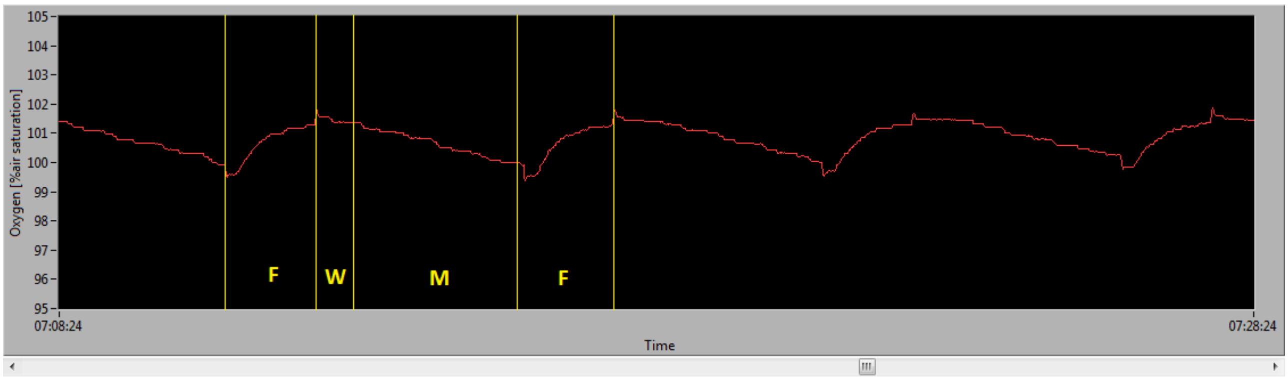
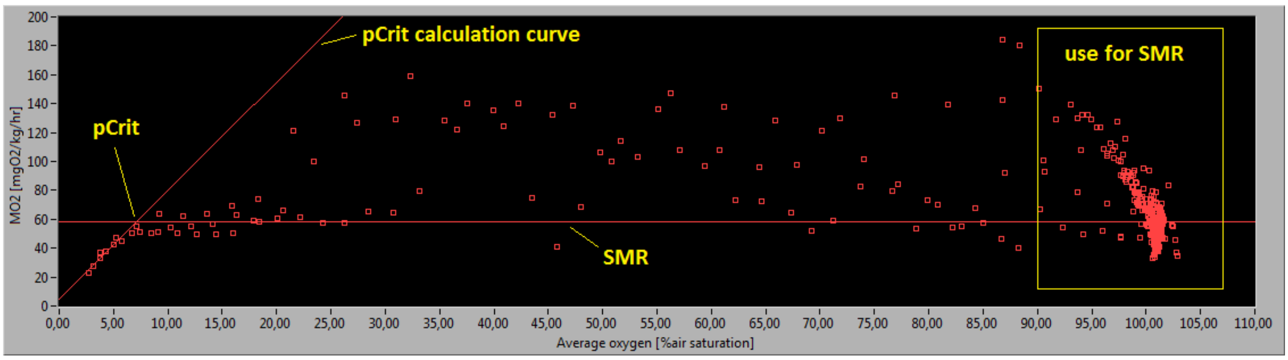
Loligo® Systems

USER MANUAL

AutoResp™

Version 2.3.0





CONTENTS

1. GETTING STARTED

Please follow these few steps below to get started. For more details go to the page numbers listed under each step.

1. Uninstall any previous versions of AutoResp™ software (page 69).

2. Install the new AutoResp™ software on a PC with Windows 10, 8 or 7 (page 31).

   Minimum PC requirements:

CPU	Duo Core 2,4 GHz or similar
RAM	4 GB
USB ports	2-5 (system dependent)
Monitor	1024 x 768

3. Connect the Bluetooth power strip (NETIO) to a grounded wall socket. Connect the USB Bluetooth dongle to the PC (page 22).

4. Set up your Witrox instrument(s) (page 13).

5. For swim tunnel respirometers set up the DAQ-BT instrument(s) (page 17)

6. Mount your chambers with a pump for flushing and a pump for recirculating the chamber using a minimum of tubing (page 10). Remember to thermostate your chamber(s) by placing it submersed in a surrounding (ambient) tank.

7. Connect all flush pumps to RE1 on the Bluetooth power strip (NETIO) using a power strip or adapter cable supplied with the system. Connect all recirculating pumps to RE2.

8. Insert the green hardkey protection (WiBu) dongle (page 44) containing license information.

9. Start the AutoResp™ software (page 45).

   Do not run any other programs at the same time, neither software delivered with Loligo™ instruments, nor software from other vendors. Thus, software delivered with fiber optic instruments and needed for stand alone use, should not be run in parallel with AutoResp™.

10. Set up AutoResp™ to the instruments you have by using the Auto Configure button and chose one of the system (page 4)

11. For reviewing respirometric data or graphs and for post analysis, load raw data files via the File menu in AutoResp™ (page 50).

12. Calculated data is saved in an Excel compatible text file.

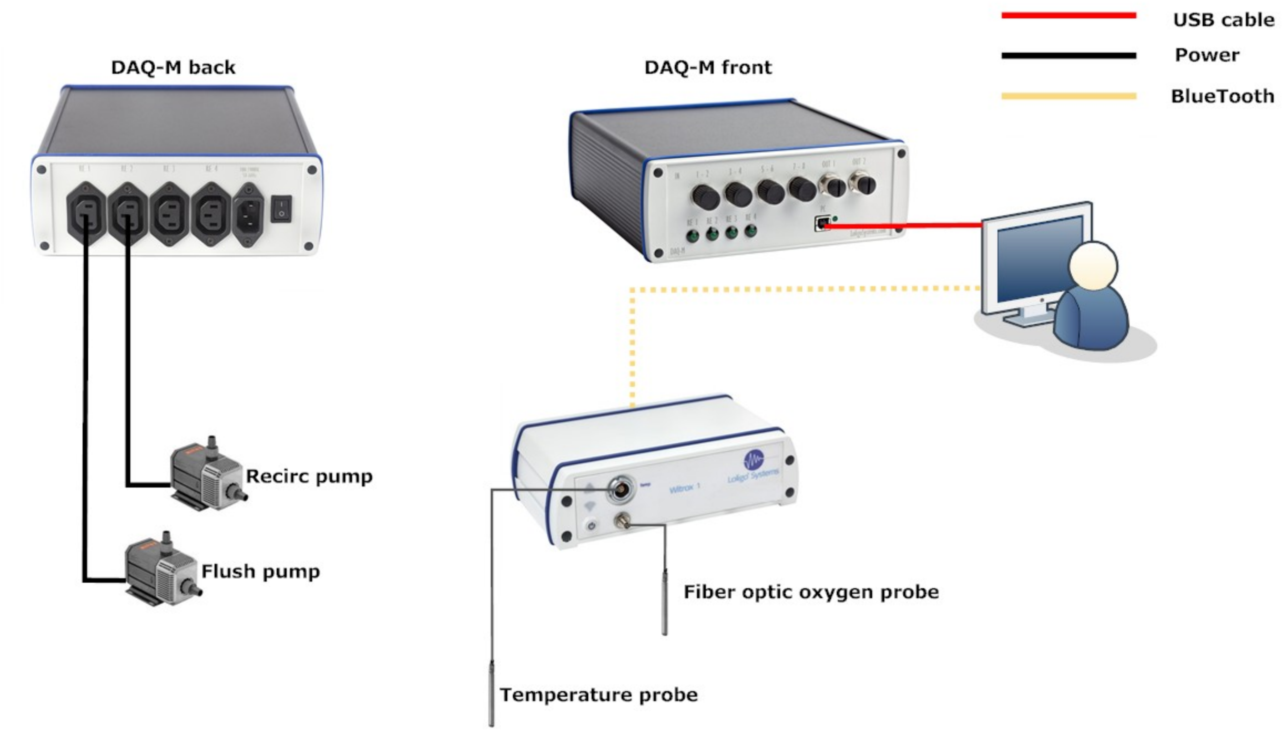
2. PACKAGES (DAQ-PACs)

2.1 DAQ-PAC-WF1

This package for measurements of oxygen consumption rates in a single respirometer chamber includes fiber optic oxygen sensing technology suited for any chamber volume, and equipment for monitoring water temperature. With optional equipment water temperature can also be controlled and/or water O_2 saturation can be monitored or controlled.

- Single chamber
- Any chamber volume
- Water temperature measurements (regulation optional)
- Optional measurements and regulation of ambient water oxygen saturation

NB! Please note that no fiber optic oxygen sensors are included in the package since these should match the application. More about fiber optic sensors can be found on page 13.



2.1.1. *List of parts*

- DAQ-M instrument
    - Power cord
    - Converter piece (230 V/110 V)
    - Device connector (2x)

- AutoResp™
    - USB MEMORY STICK
    - Wibu dongle

- Witrox 1 oxygen instrument for mini sensors
    - AC/DC travel adapter (1.5 m)
    - USB power cable for PC (1.5 m)
    - PT1000 sensor (4W,ClassA, 1.9x40mm, 5 m cable)
    - Plastic suitcase (345x285x122 mm)

2.1.2. *Optional*

For measuring effects of hypoxia/hyperoxia on respiration, we offer an oxygen instrument and an (DO-SET) accessory kit for injection of N_2 or O_2 gas.

For oxygen consumption measurements in two chambers, add a Witrox 1 + sensor, or consider a Witrox 4 + sensors for multiple chambers.

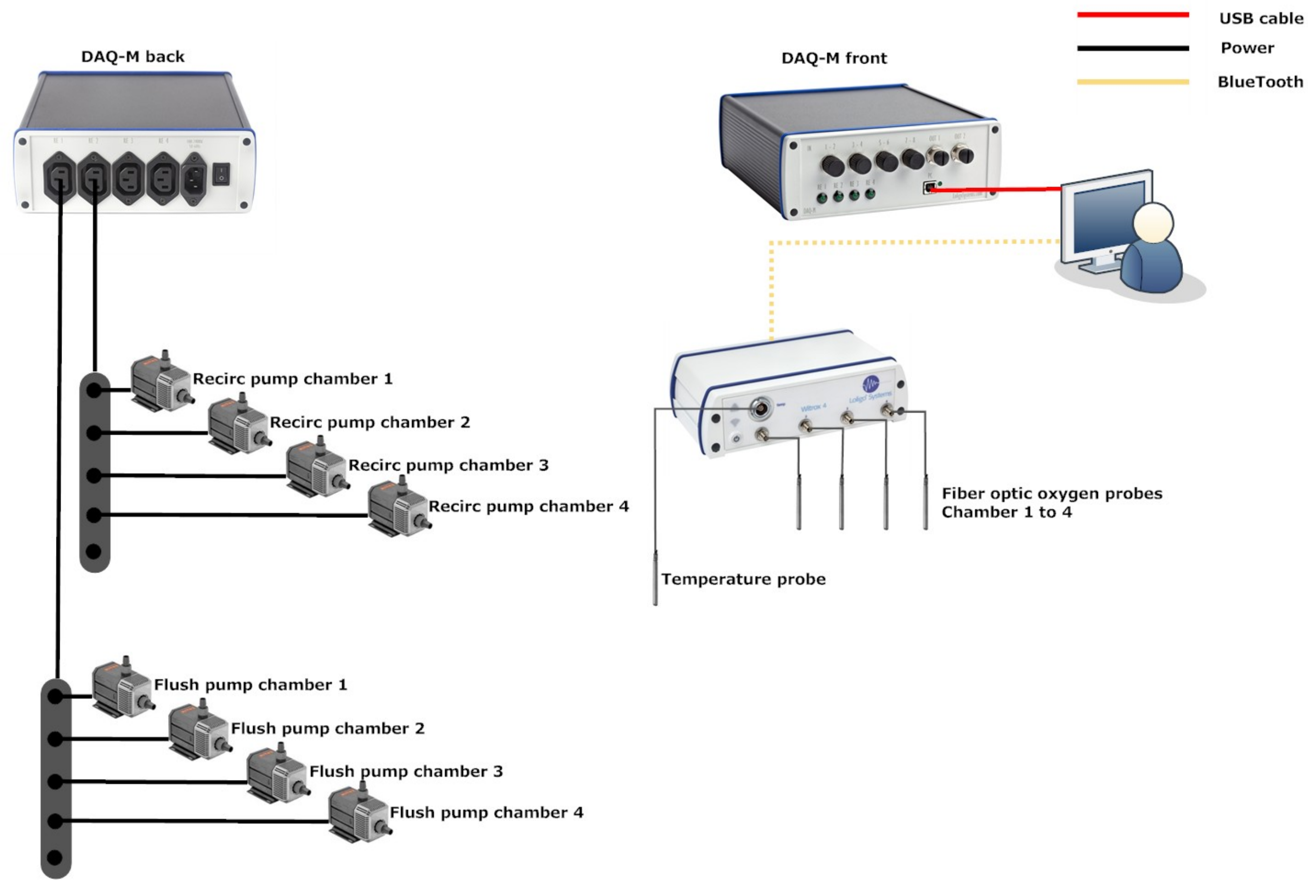
For regulating water temperature, all you need is a TMP-SET accessory kit (#AC10150/#AC10160).

2.2DAQ-PAC-WF4

This package for measurements of oxygen consumption rates in up to four respirometer chambers includes fiber optic oxygen sensing technology suited for any chamber volume, and equipment for monitoring water temperature. With optional equipment water temperature can also be controlled and/or water O_2 saturation can be monitored or controlled.

- 1-4 chambers
- Any chamber volume
- Water temperature measurements (regulation optional)
- Optional measurements and regulation of ambient water oxygen saturation

NB! Please note that no fiber optic oxygen sensors are included in the package since these should match the application. More about fiber optic sensors can be found on page 13.



2.2.1. *List of parts*

- DAQ-M instrument
  - Power cord
  - Converter piece (230 V/110 V)
  - Device connector (2x)

- AutoResp™
  - USB MEMORY STICK
  - Wibu dongle

- Witrox 4 oxygen instrument for mini sensors
  - AC/DC travel adapter (1.5 m)
  - USB power cable for PC (1.5 m)
  - PT1000 sensor (4W,ClassA, 1.9x40mm, 5 m cable)
  - Plastic suitcase (345x285x122 mm)

2.2.2. *Optional*

For measuring effects of hypoxia/hyperoxia on respiration, we offer an oxygen instrument and an (DO-SET) accessory kit for injection of N_2 or O_2 gas.

For oxygen consumption measurements in 5-8 chambers, add a Witrox 4 oxygen instrument + sensors

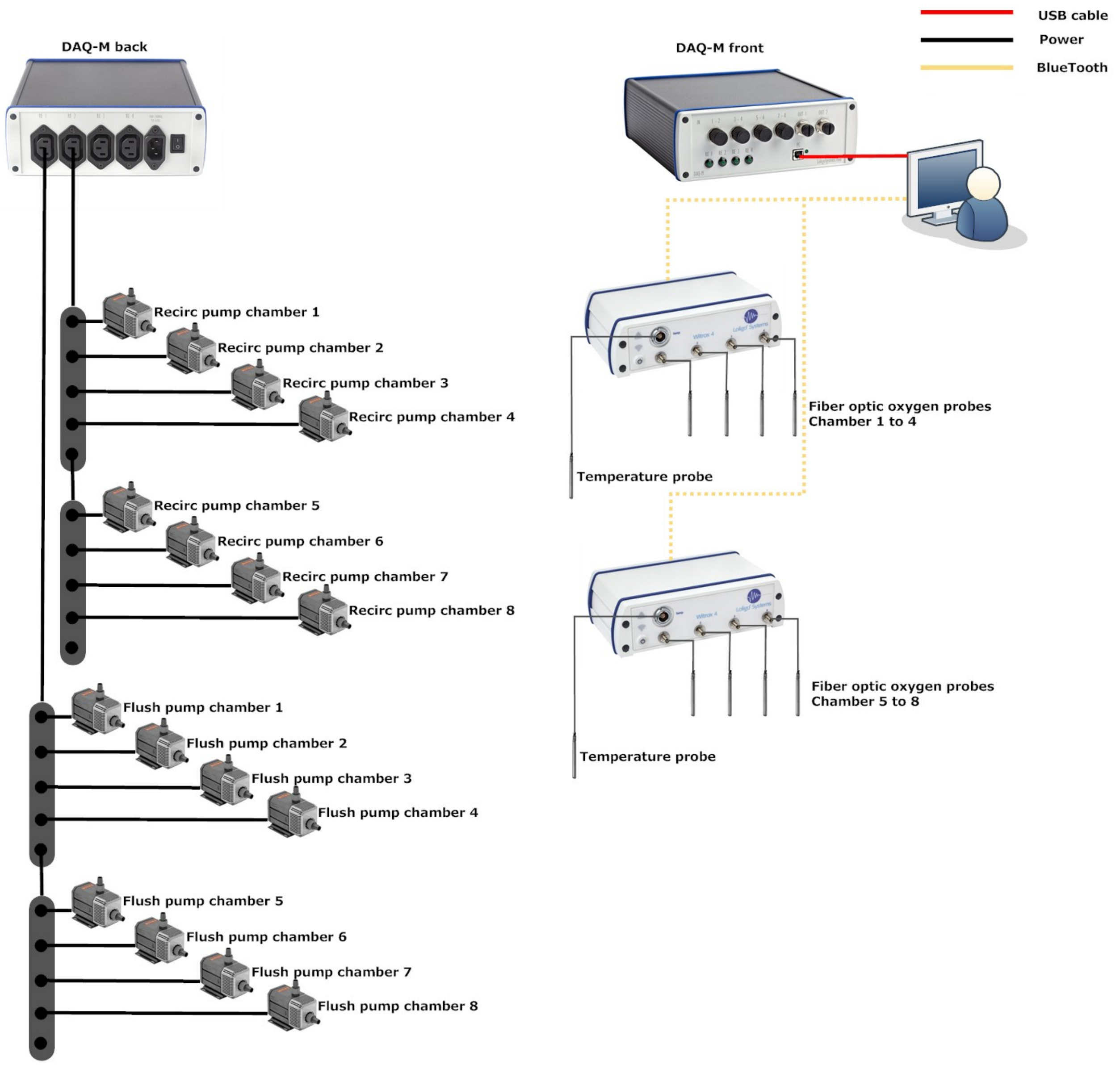
For regulating water temperature, all you need is a TMP-SET accessory kit (#AC10150/#AC10160).

2.3DAQ-PAC-WF8

This package for measurements of oxygen consumption rates in up to eight respirometer chambers includes fiber optic oxygen sensing technology suited for any chamber volume, and equipment for monitoring and regulating water temperature. With optional equipment water temperature can also be controlled and/or water O_2 saturation can be monitored or controlled.

- 1-8 chambers
- Any chamber volume
- Water temperature measurements (regulation optional)
- Optional measurements and regulation of ambient water oxygen saturation

NB! Please note that no fiber optic oxygen sensors are included in the package since these should match the application. More about fiber optic sensors can be found on page 13.



2.3.1. List of parts

- DAQ-M instrument
  - Power cord
  - Converter piece (230 V/110 V)
  - Device connector (2x)

- AutoResp™
  - USB MEMORY STICK
  - Wibu dongle

- Witrox 4 oxygen instrument for mini sensors (2x)
  - AC/DC travel adapter (1.5 m)
  - USB power cable for PC (1.5 m)
  - PT1000 sensor (4W,ClassA, 1.9x40mm, 5 m cable)
  - Plastic suitcase (345x285x122 mm)

2.3.2. Optional

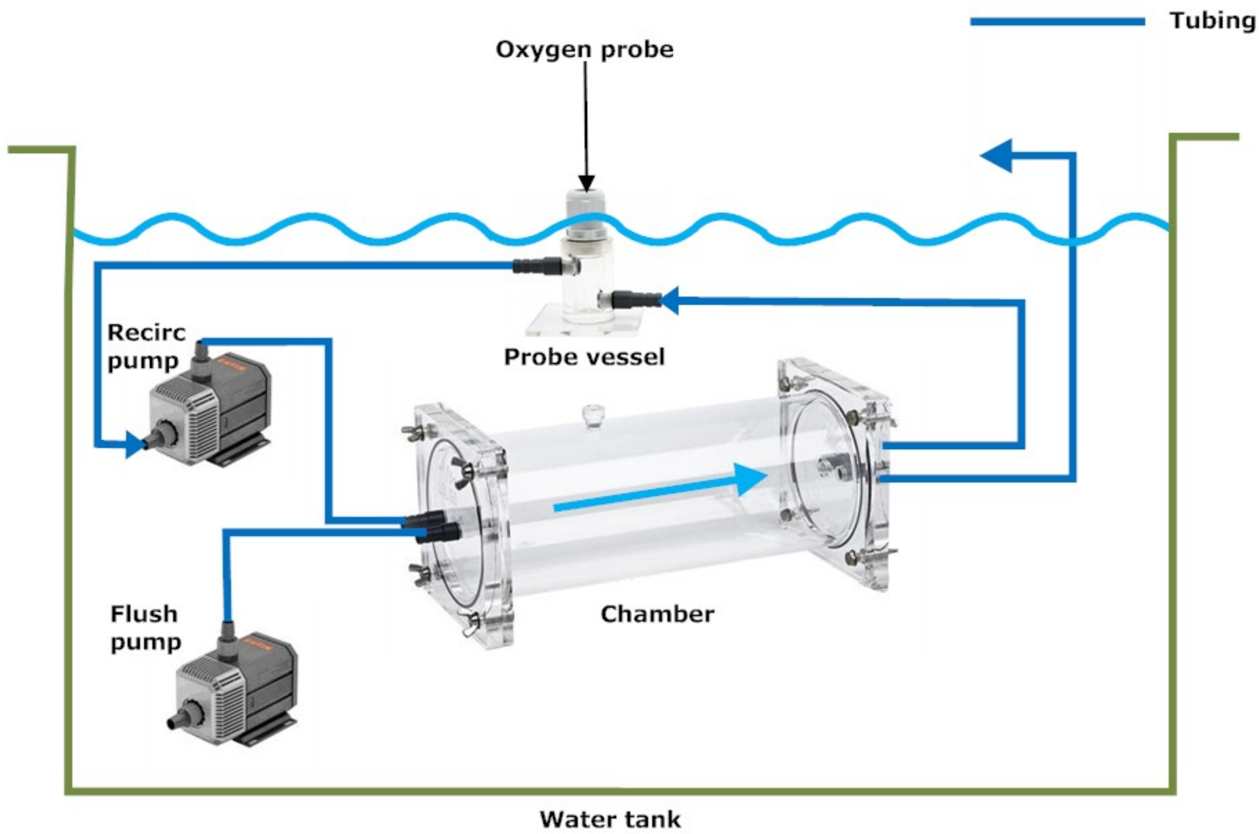
For measuring effects of hypoxia/hyperoxia on respiration, we offer an oxygen instrument and an (DO-SET) accessory kit for injection of N_2 or O_2 gas.

For regulating water temperature, all you need is a TMP-SET accessory kit (#AC10150/#AC10160).

3. CHAMBERS

3.1 Static chambers

When using AutoResp™ with horizontal chambers for determining the metabolic rate of resting/inactive animals, the chamber and pumps must be assembled as indicated in the diagram below.



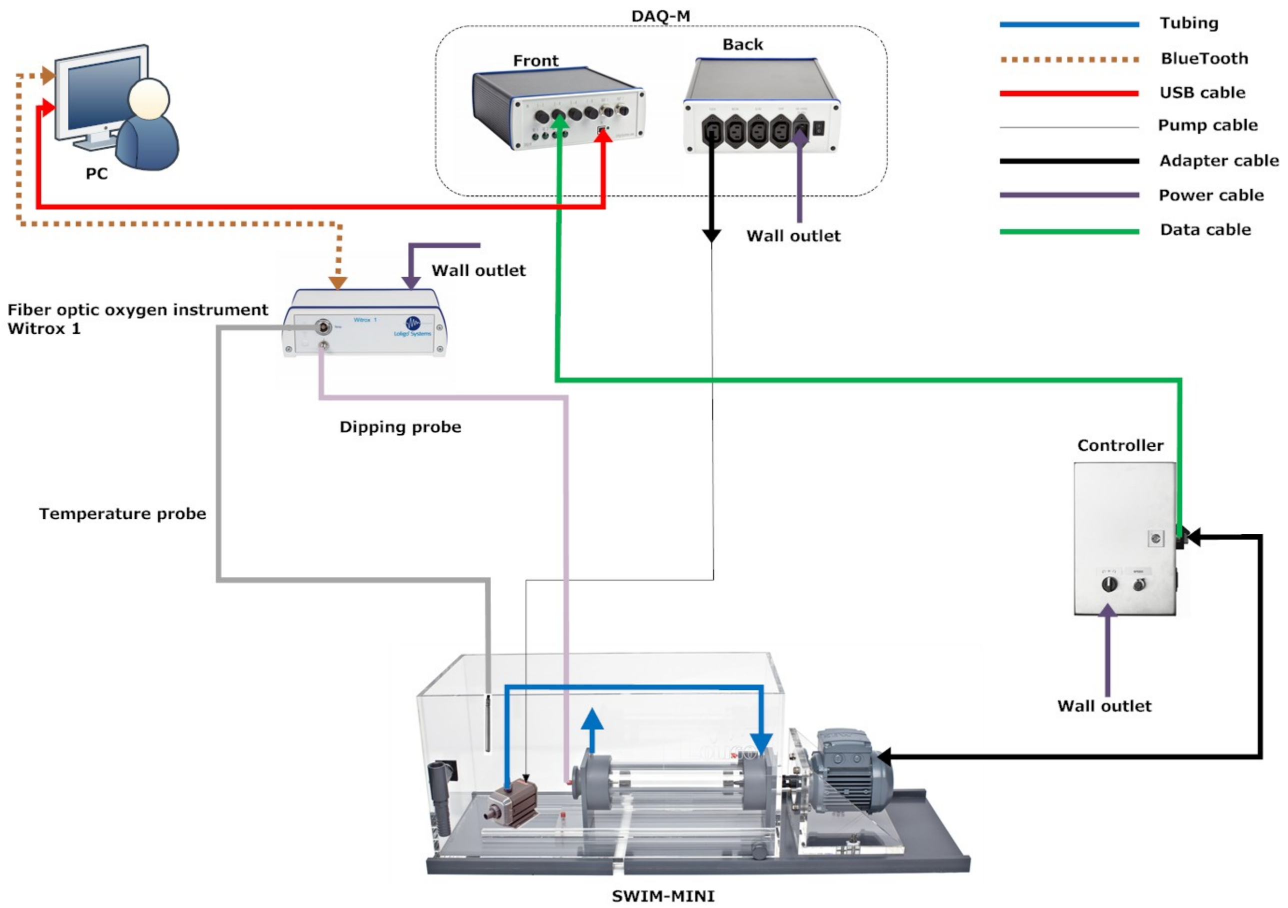
Place the outlet hose just above the water surface for visual check of pump flow and to avoid siphon water entering the respirometer when the flush pump is turned off!

Avoid excess tubing to limit volume, gas diffusion and surfaces for bio film.

For more info on intermittent resp. principle see page 63.

3.2Swim tunnels

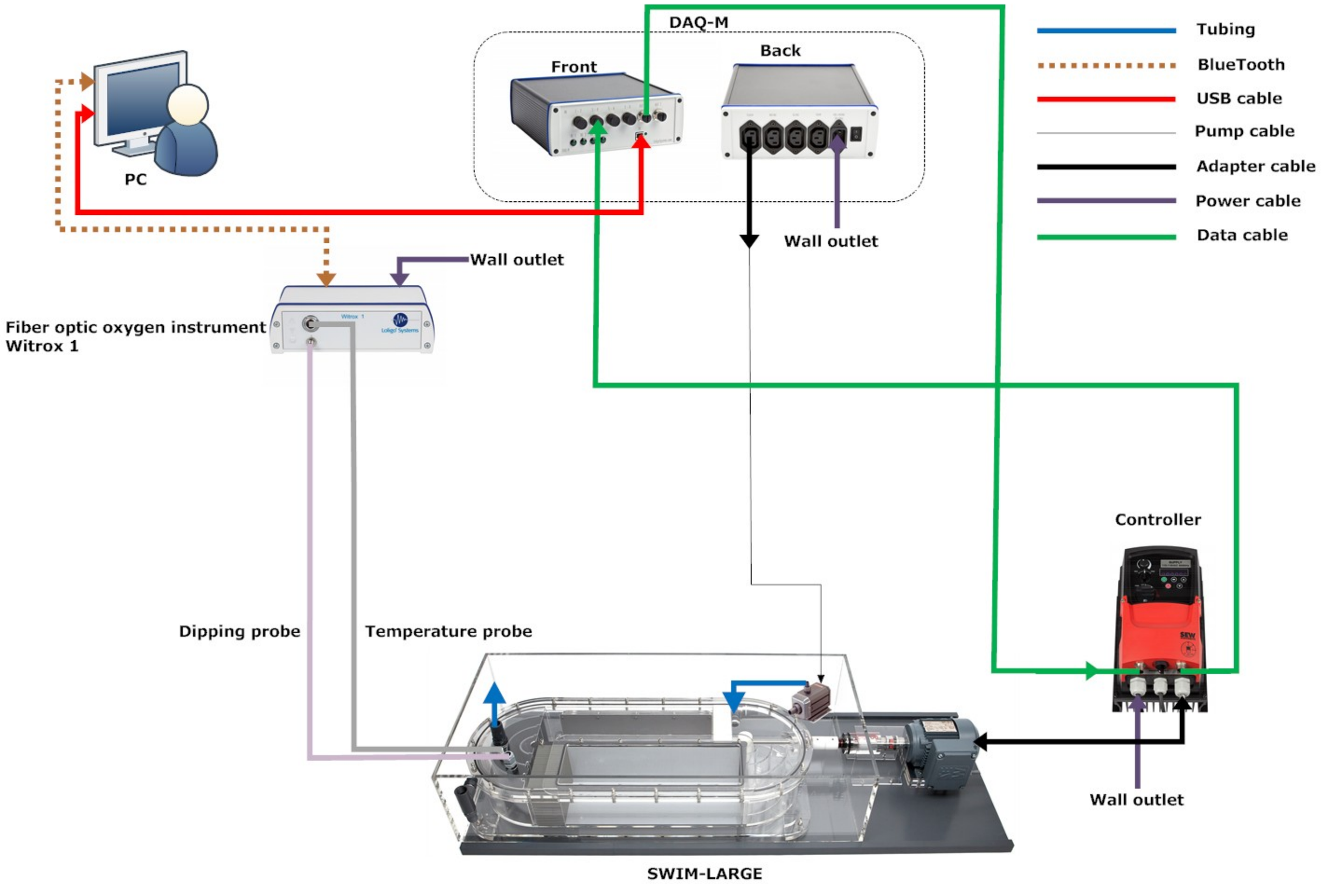
For SWIM-MINI tunnels assembled as indicated in the diagram below.



When using a DAQ-BT instead of a DAQ-M instrument connect the input and output connectors from the swim tunnel motor controller to the DAQ-BT input and output connectors. For the PULSE output please use the long data cable. For the analog input please use the short data cable.

Connect the flush pump to RE1 on the BlueTooth power strip (NETIO) instead of the DAQ-M.

For large swim tunnels assembled as indicated in the diagram below.

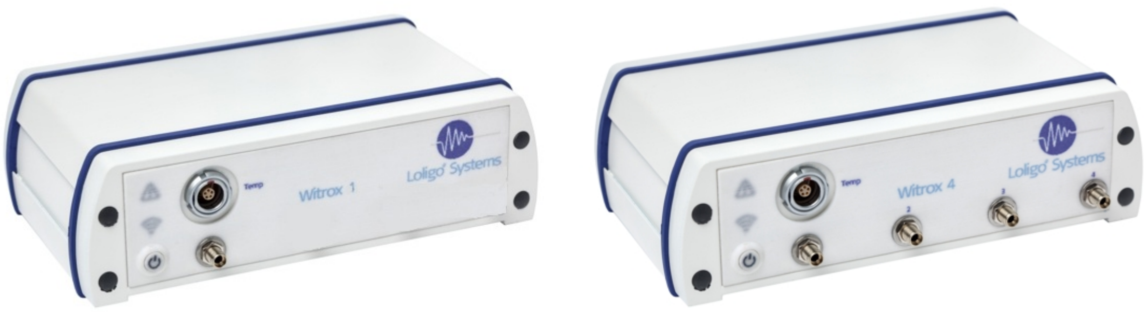


When using a DAQ-BT instead of a DAQ-M instrument connect the input and output connectors from the swim tunnel motor controller to the DAQ-BT input and output connectors.

Connect the flush pump to RE1 on the BlueTooth power strip (NETIO) instead of the DAQ-M.

4. INSTRUMENTS

4.1 Witrox



The Witrox is either a 1 channel (Witrox 1) or 4 channel (Witrox 4) oxygen instrument for measuring dissolved oxygen using fiber optic mini sensors (optodes) and monitoring temperature.

NB! Do not use WitroxView software while running AutoResp™.

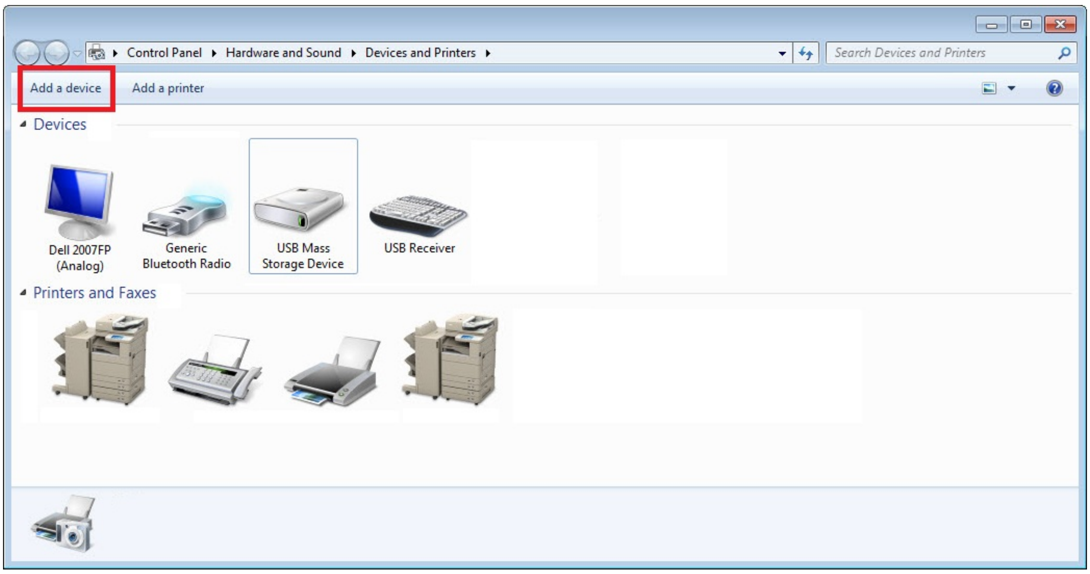
SETUP

Connect the Witrox instrument to the power adapter by using the USB cable on the backside of the instrument.

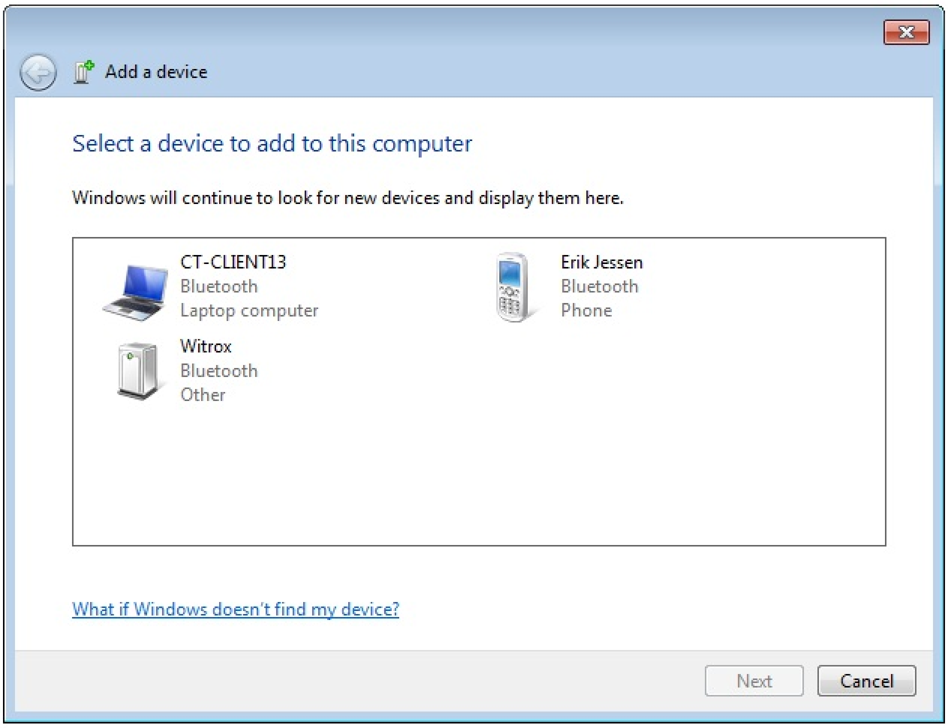
Connect the power adapter to a wall outlet.

Push the Power button, the power LED will turn green. The LINK LED will flash green.

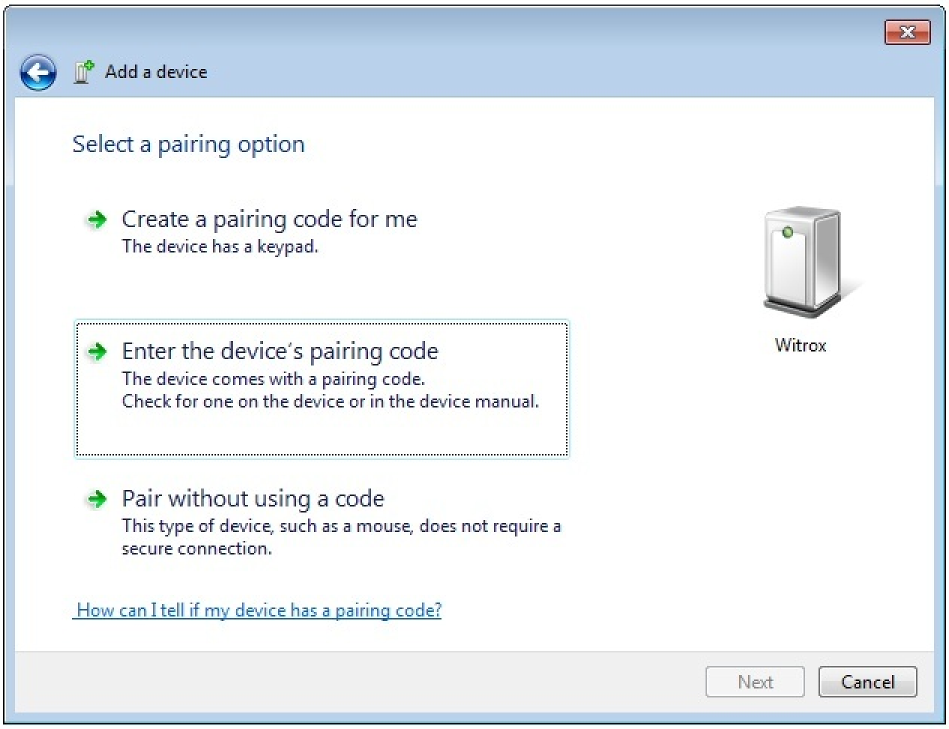
Press the Windows Start button and choose Devices. An overview of the connected devices is listed.



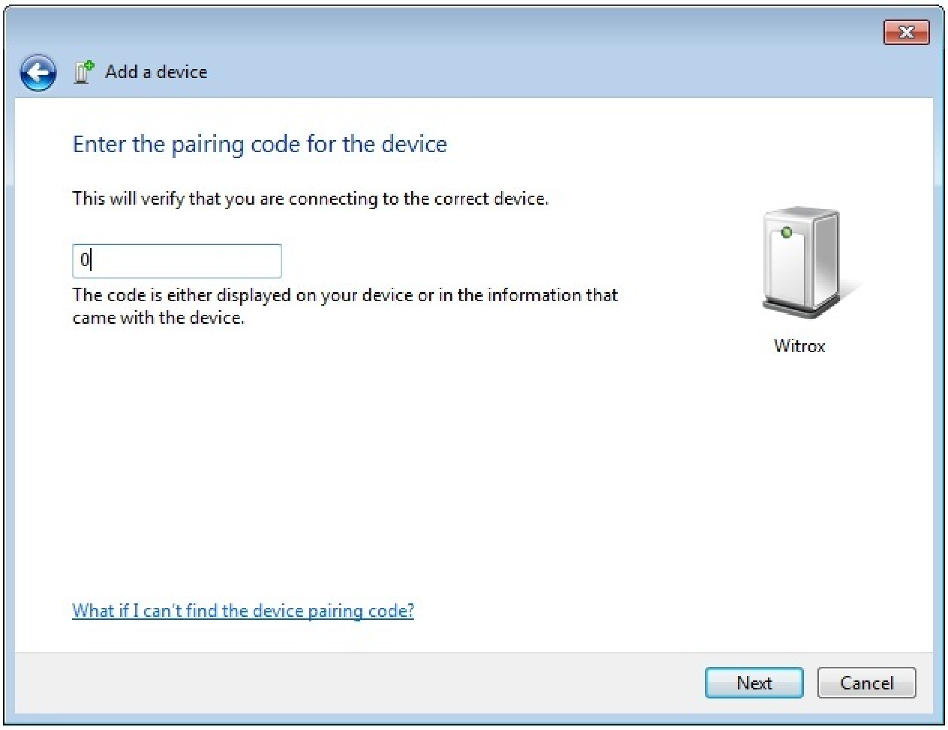
Please click on the “Add a device” button.



Click on the Witrox instrument, then click Next.



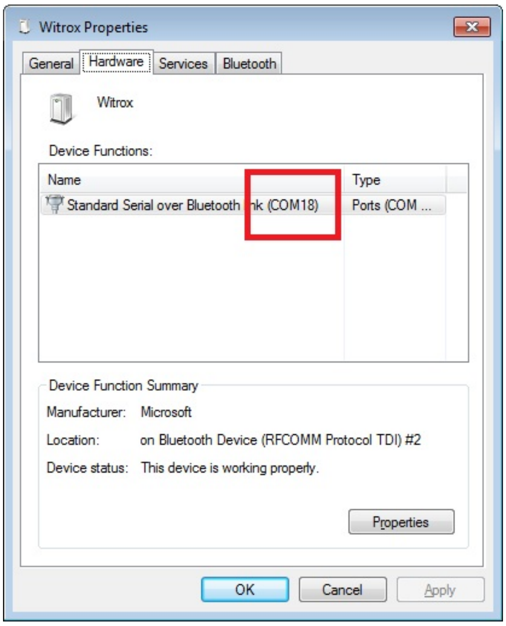
Please choose “Enter the device´s pairing code”, then click Next.

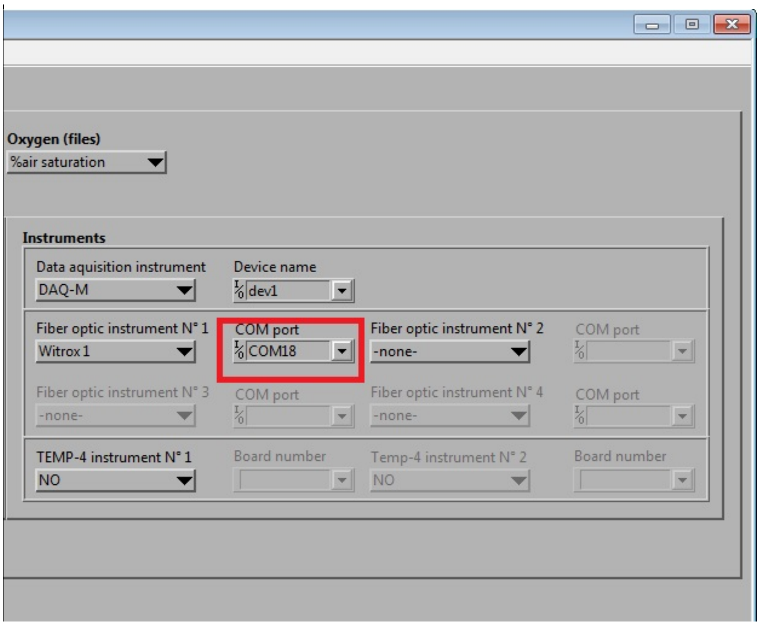


Enter a 0 (zero) in the pairing field, then click Next. The WITROX driver will now be installed.

The instrument is now ready to be used in AutoResp™.

Right click on the device and choose Properties. Check the assigned COM port number. This COM port number must correspond to the COM port number set in AutoResp™.





Connect a fiber optic sensor to the front input (marked *CH1-CH4*). Connect the Pt1000 temperature sensor to the front input marked TEMP.

For more information on fiber optic sensors, see page 13.

4.2DAQ-BT

The DAQ-BT instrument is used for wireless data acquisition and automated control of Loligo® swim tunnels in combination with AutoResp™ software. The wireless Bluetooth communication means that the PC can be placed at a distance from the swim tunnel without data cables that can pick up noise in a lab environment.

Apart from the inputs and outputs needed for controlling the swim tunnel motor (RPM) and acquiring data from it, the DAQ-BT instrument has extra channels for analog data acquisition and TLL control of other devices.

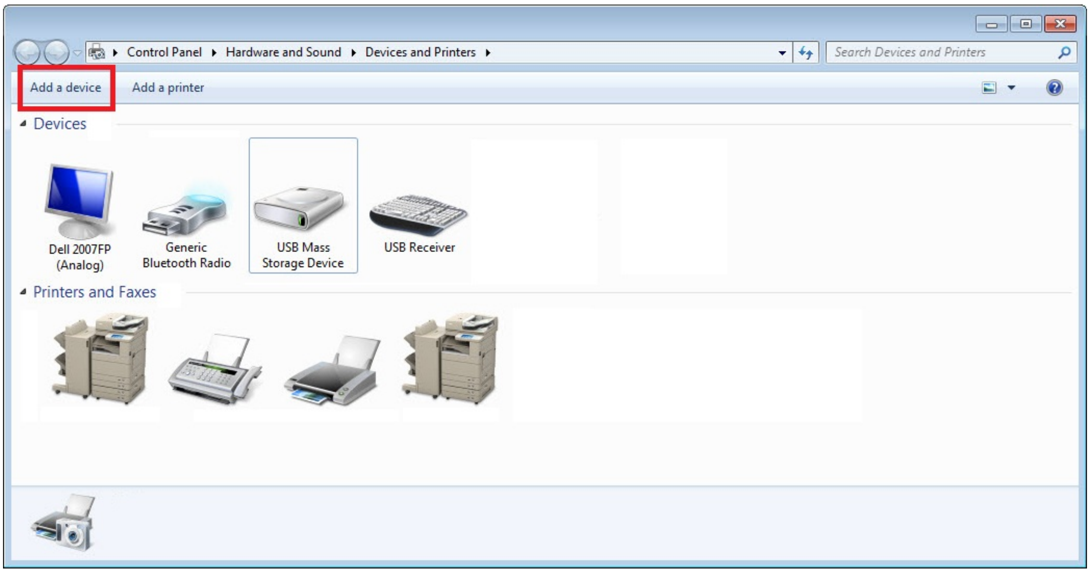
SETUP

Connect the DAQ-BT instrument to the power adapter by using the USB cable on the backside of the instrument.

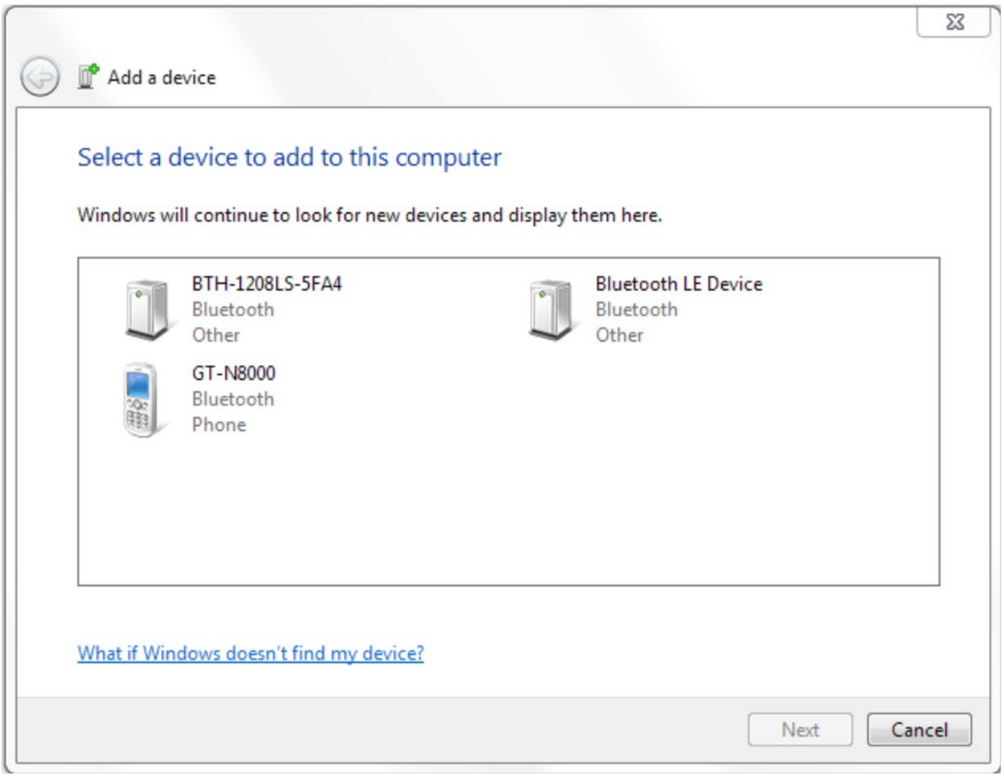
Connect the power adapter to a wall outlet.

Push and hold the Power button for at least 5 seconds until the Power and Status LED flashes green. The pairing mode for the DAQ-BT is now enabled.

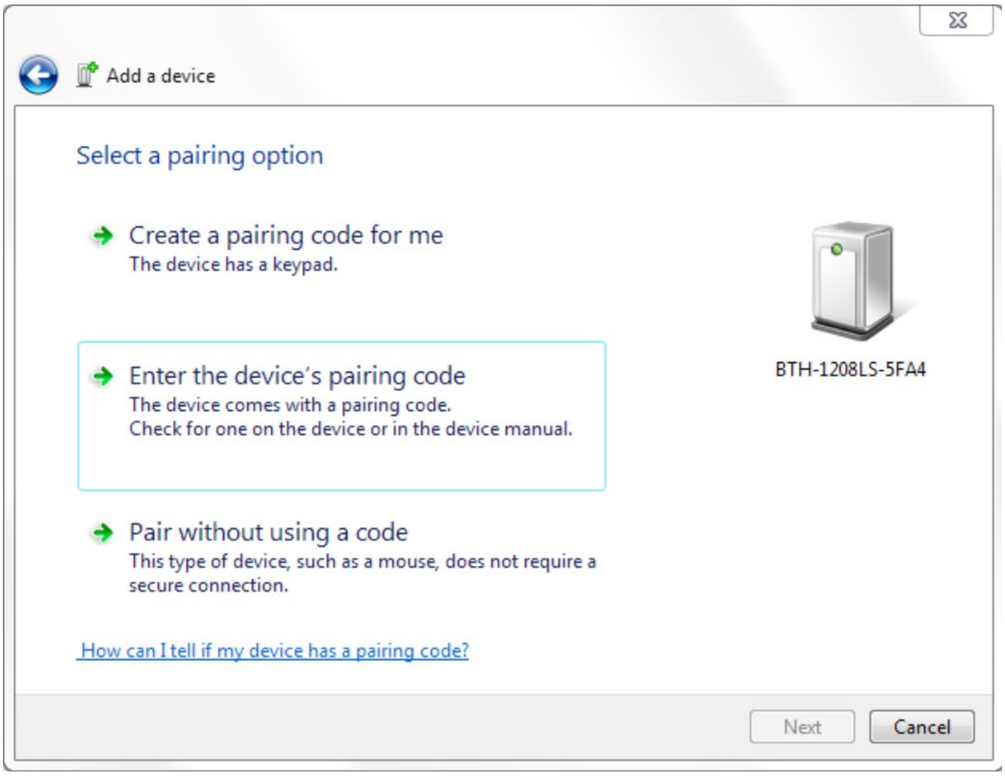
Press the Windows Start button and choose Devices. An overview of the connected devices is listed.



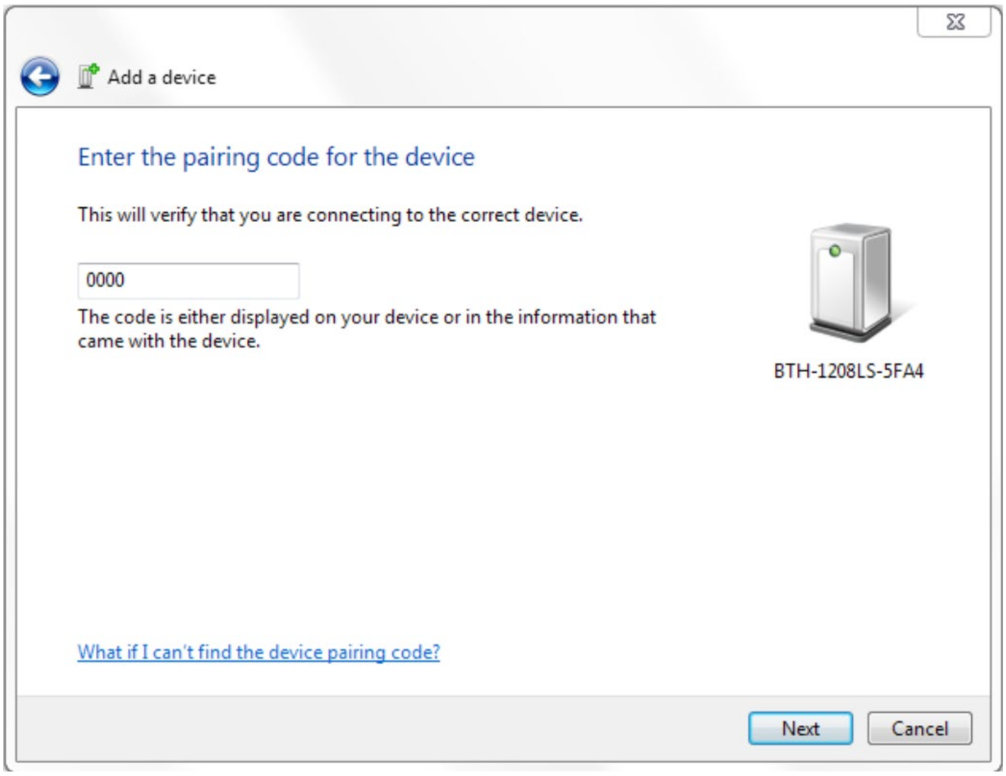
Please click on the “Add a device” button.



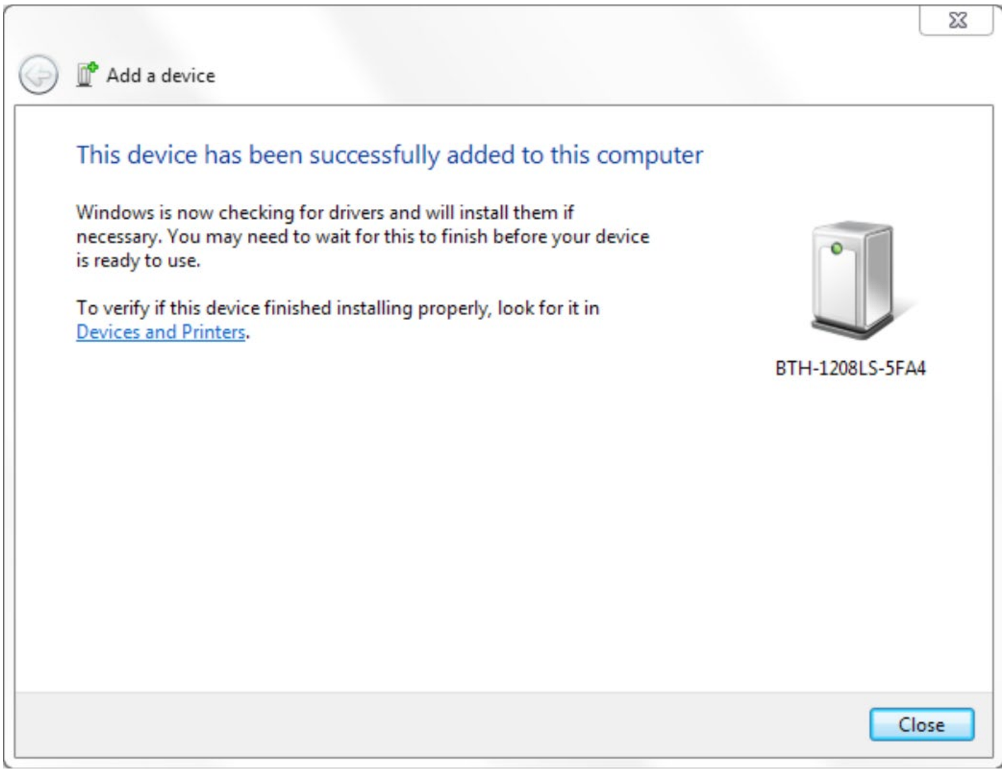
Click on the BTH-1208LS instrument, then click Next.



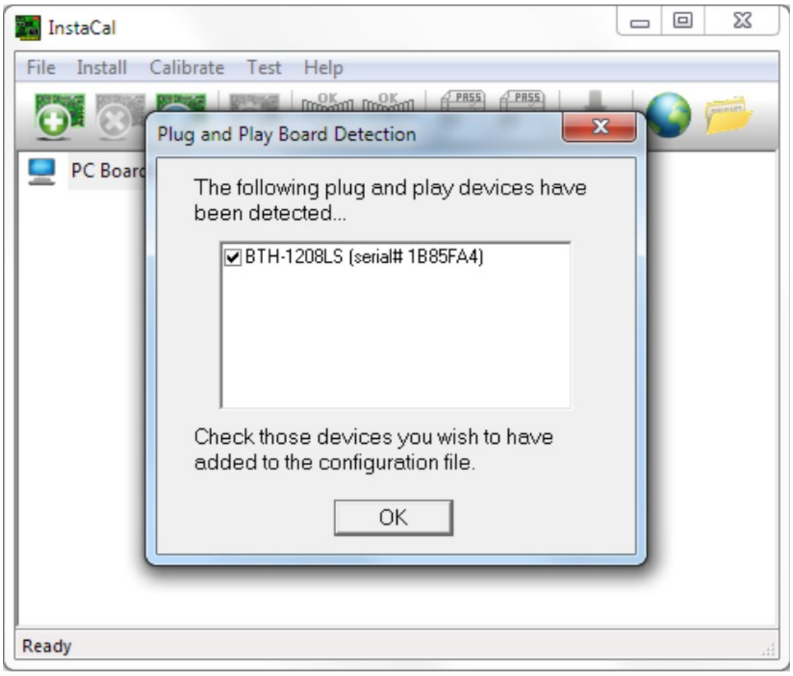
Please choose "Enter the device´s pairing code", then click Next.



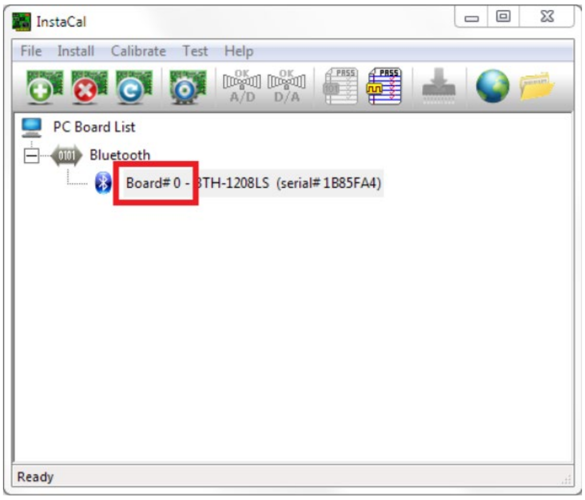
Enter 0000 (four zeros) in the pairing field, then click Next. The DAQ-BT driver will now be installed.

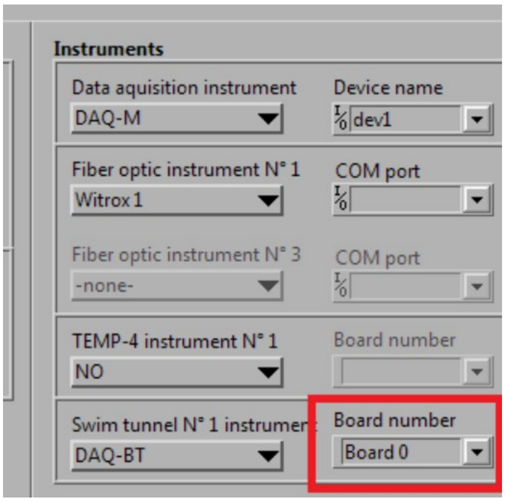


Press the Windows Start button and open InstaCal from all programs/Measurement Computing. InstaCal will detect the DAQ-BT and configure it properly.



The instrument is now ready to be used in AutoResp™. The assigned board number must correspond to the board number set in AutoResp™.





To turn the instrument OFF push and hold the power buttons until both LEDs are turned OFF.

To turn the instrument ON without enabling the pairing mode push and hold the power button for approximately 3 seconds until the power LED turns ON.

When the instrument is communicating with the software the status LED will start flashing.

To measure a Loligo swim tunnel connect a data cable from a swim tunnel controllers output labeled OUT to the DAQ-BT instruments input labeled IN.

To control a Loligo swim tunnel connect a data cable from the DAQ-BT instruments output labeled OUT to a swim tunnel controllers input labeled IN.

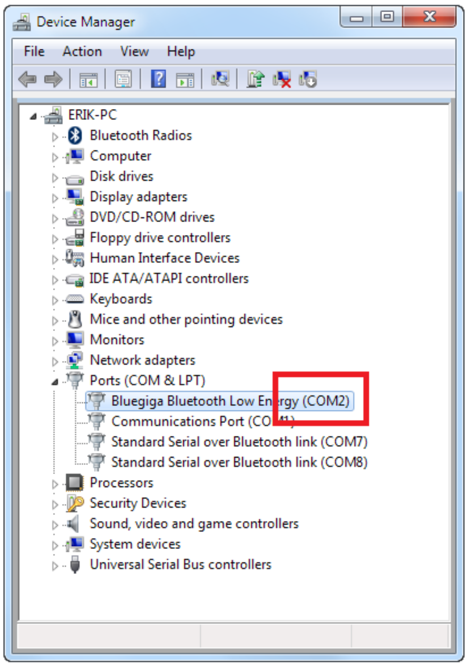
4.3Bluetooth power strip (NETIO)

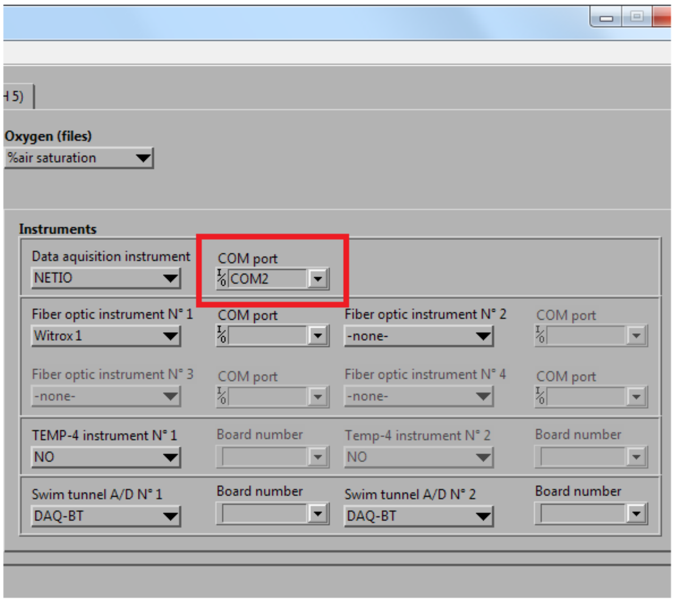
The Bluetooth power strip (NETIO) is essentially a 4-fold power strip for wireless software control of equipment connected to one of the four independent electrical sockets, ***i.e***., turning pumps, valves, stirrers etc. on and off from a distance.

SETUP

Connect the Bluetooth power strip to a wall outlet. Turn ON the power strip with the power button. When the power strip is ready for use the Bluetooth LED will light blue.

Connect the USB Bluetooth dongle to the PC. After the dongle has been found a COM port will be assigned. Please open the device manager and check the COM port. This COM port number must correspond to the COM port number set in AutoResp™.





The Bluetooth power strip is now ready for use in AutoResp™.

Loligo® Systems

4.4 DAQ-M

The DAQ-M instrument for USB is used with AutoResp™ software for automated oxygen consumption measurements in up to eight static chambers or two swim tunnels, and for monitoring and regulating ambient water temperature and oxygen saturation.

SETUP

To power up the instrument connect the power cord to a <u>grounded</u> wall outlet and the socket labeled *100-240 VAC 50-60Hz* on the back side of the instrument. Remember to switch the instrument ON/OFF using the main power switch. Connect the USB cable to the front input (marked *PC*) and to a free USB port on your PC.

First time the DAQ-M instrument is connected to the PC, the drivers will be installed. This might take some time.

All analog outputs from oxygen and temperature instruments, and swim tunnel motors, must be connected to input channels 1-8 on the DAQ-M instrument using either single or split (double) data cables supplied with the devices.

The digital relays are used to control pumps for flushing/recirculating and pumps or valves for ambient water quality control. See page 56.

SPECIFICATIONS

Supply voltage:	100-240 VAC, 50-60 Hz
PC interface:	USB 2.0 (1.1 compatible)
Input channels:	8
Input voltage:	0-10 V
Output channels:	2
Ouput voltage:	0-10 V
Resolution:	14 bit (single-ended)
Signal noise:	62 dB
Frequency:	1000 Hz
Relays:	4
Relay voltage:	100-240VAC, 50-60 Hz
Relay max. power:	2 A per channel (max 6.3 A for all relays)

4.5 TEMP-4

The TEMP-4 is a four channel instrument for monitoring, recording and controlling water temperature with AutoResp™ or TempCTRL software.

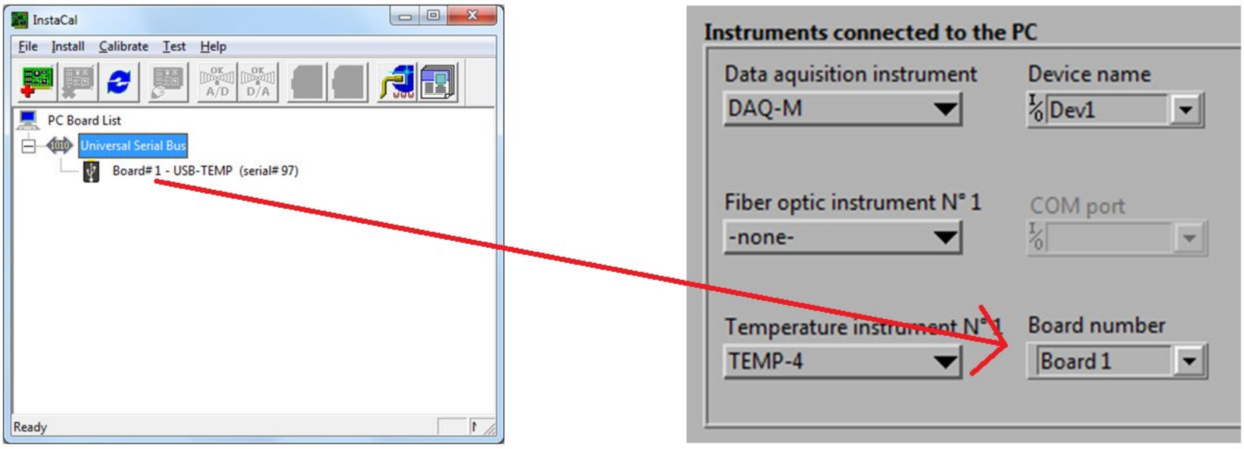
SETUP

To power up the instrument connect the power cord to a <u>grounded</u> wall outlet and the socket labeled 100-240 VAC 50-60Hz on the back side of the instrument. Connect Pt100 temperature probe(s) to the front inputs (marked *IN 1 – IN 4*). Then connect the USB cable to the front input (marked *PC*) and to a free USB port on your PC.

SPECIFICATIONS

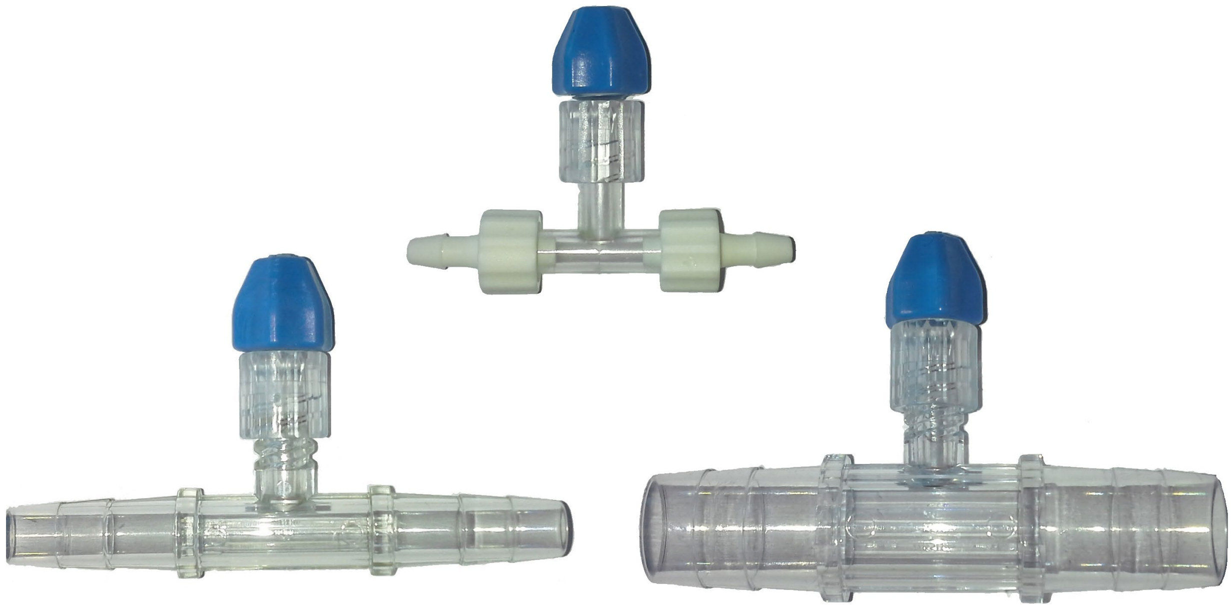
Supply voltage:	100-240 VAC, 50-60 Hz
PC interface:	USB 2.0 (1.1 compatible)
Inputs:	4
Resolution:	24 bit
Signal noise:	60 dB
Frequency:	16 Hz
Relays	4
Relay voltage: :	100-240VAC, 50-60 Hz
Max. relay current:	2 A per channel, 6,3 A all channels

First time the TEMP-4 instrument is connected to the PC, the drivers will be installed. This might take some seconds. Then open InstaCal (this software is installed together with AutoResp™) and check the assigned board number. This board number must correspond to the board number set in AutoResp™.



5. SENSORS

5.1 Flow-through cell mini sensor



The flow-through oxygen mini sensor is for use with 10 - 13 mm tubing, and requires a fiber cable with bare tip for use with Witrox 1 or Witrox 4 instruments. The re-usable (non-disposable) oxygen sensor has excellent long-term stability. Clean it with ethanol or even ETO, recalibrate, and use it again and again.

Features:

Easy to handle
Very robust sensor
Excellent long-term stability
No self-consumption of oxygen
Signal independent on flow velocity
Insensible towards electrical interferences
Measures oxygen in liquids as well as in gas phase

5.2Dipping probe mini sensor

The dipping probe oxygen mini sensor, consists of a polymer optical fiber (POF) with a polished distal tip which is coated with a planar oxygen-sensitive foil. The end of the polymer optical fiber is covered with a high-grade steel tube, to protect both the sensor material and the POF. The cable has an outer diameter of 2.8 mm. The inner diameter of the POF is 2.0 mm. The steel tube has an outer diameter of 4 mm. Usually, the fiber is coated with an optical isolated sensor material in order to exclude ambient light from the fiber tip and to increase chemical resistance especially against oily samples as well as to reduce bio-fouling on the sensor membrane. This type of oxygen sensor has an excellent long-term stability.

Features:
Easy to handle
Usable for process application
Limit of detection: 0.15 % air-saturation, 15 ppb dissolved oxygen
Measuring range: 0 - 100 % oxygen, 0 - 45 mg/L
Very robust sensor with excellent long-term stability
Sterilizable by H_2O_2, ethanol, gamma irradiation, EtO_2
No self-consumption of oxygen
Signal independent on flow velocity
Insensible towards electrical interference and magnetic fields
Dry gases applicable
Robust sensor body

5.3Sensor spot mini sensor

Sensor spots are thin planar oxygen mini sensors immobilized onto either polyester or glass supports. The latter is autoclavable. The sensor spots are glued inside chambers, flasks or disposables with translucent and non-fluorescent walls (e.g. glass, polyester, acrylic etc.). Then oxygen measurements can be done in a non-invasive and non-destructive way from outside and through the wall of your vessel.

Features:

Excelent long-term stability
Easy to handle and robust
Non-invasive and non-destructive measurement from outside through the wall of the flask
Usable for process application
No self-consumption of oxygen
Signal independent on flow velocity
Insensible towards electrical interferences and magnetic fields
Measures oxygen in liquids as well as in gas phase
Excellent mechanical stability and long-term stability
Online monitoring

5.4Temperature sensor Pt1000

The Pt1000 temperature probe is robustly constructed with a stainless steel protection sheath, moulded mini handle with built in stress relief and complete with 5 metre of cable and connector for our Witrox instruments.

The Pt1000 sensor has a tolerance of 1/3 of Class B sensor giving a very high accuracy of +/- 0.15°C.

6. SOFTWARE INSTALLATION

6.1 AutoResp™ installation

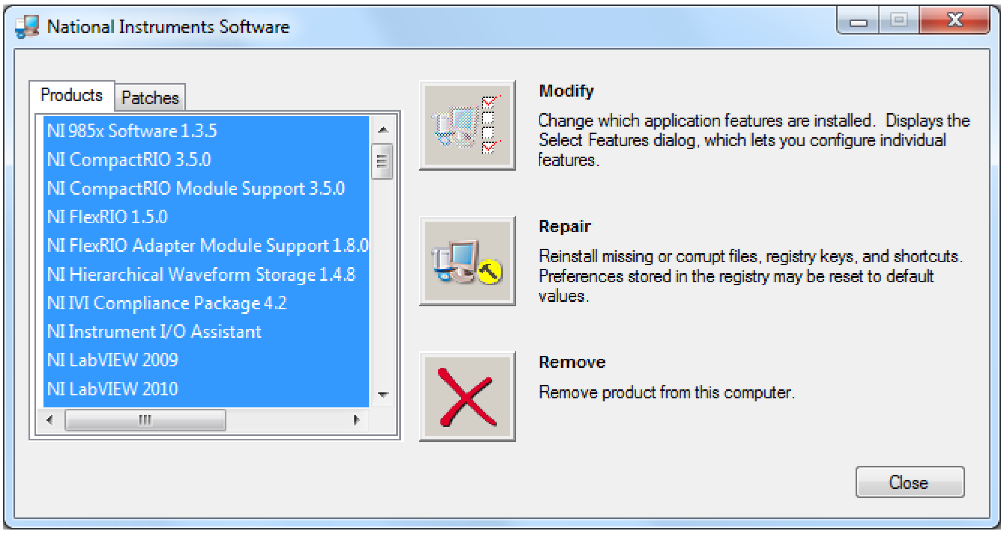
The following steps will explain how to install AutoResp™ and instrument drivers on your PC.

Minimum PC requirements:

CPU	Duo core 2,4 GHz or similar
RAM	4 GB
USB ports	2-5 (system dependent)
Monitor	1024 x 768

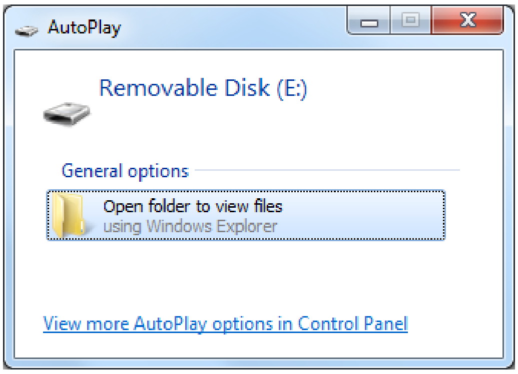
It is important to remove any previous versions of AutoResp™ before starting to install new AutoResp™ software:

1. Click Start→Control Panel
2. Open Programs and Features
3. Double click on National Instruments software
4. Select all packages, and then click on Remove.

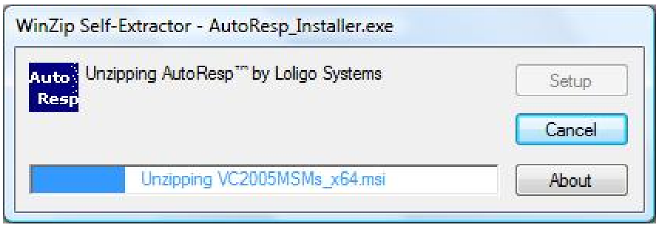


5. You will then be notified that AutoResp™ also will be removed. Click Yes
6. Now wait until all packages are uninstalled. This might take some time.
7. Windows will now ask for a restart.
8. When the computer is restarted, please proceed and install the new AutoResp™ software.

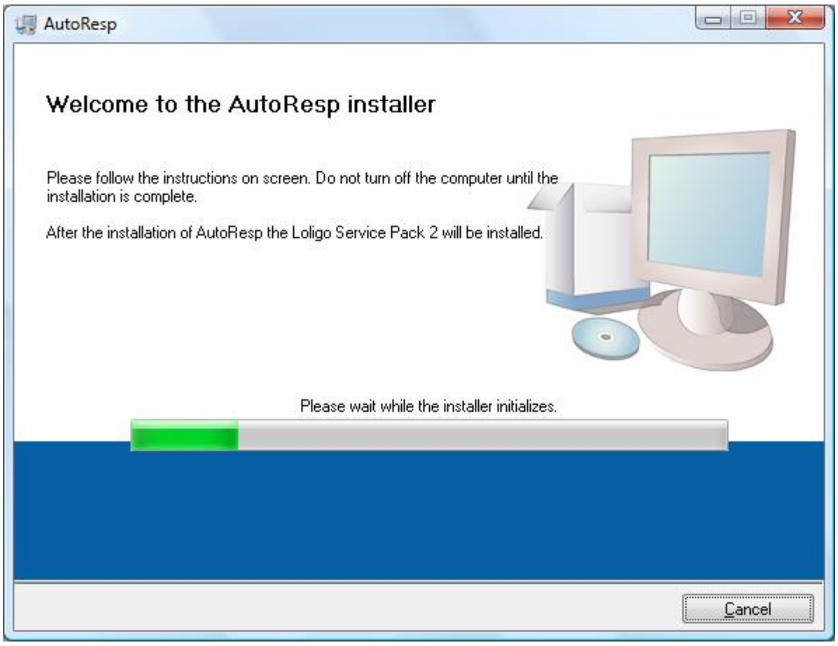
Insert the USB memory stick labelled Loligo and wait until you see the following screen.

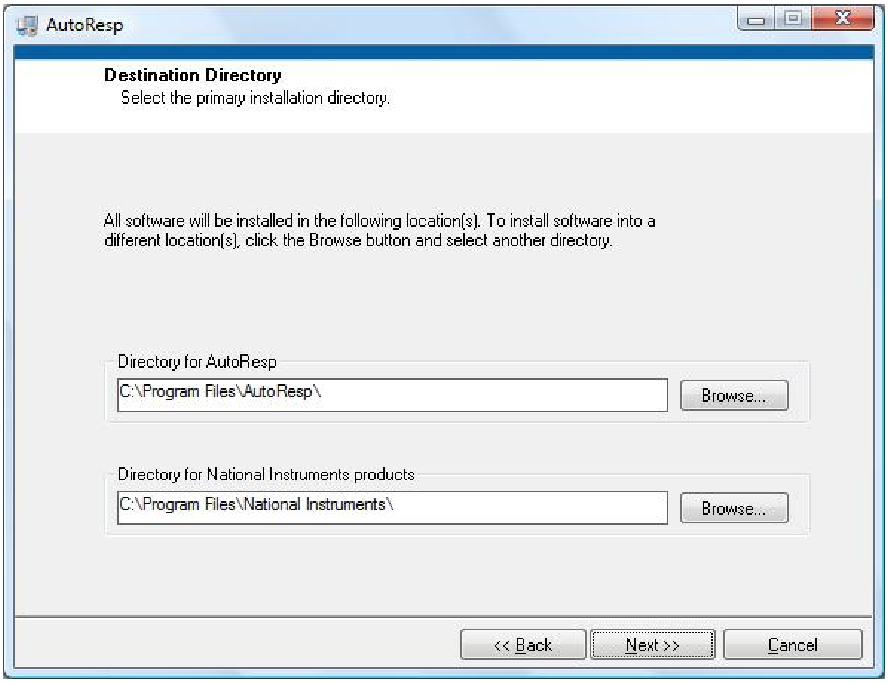


Click Open folder to view files and double click on the icon labelled AutoResp_Installer.exe.

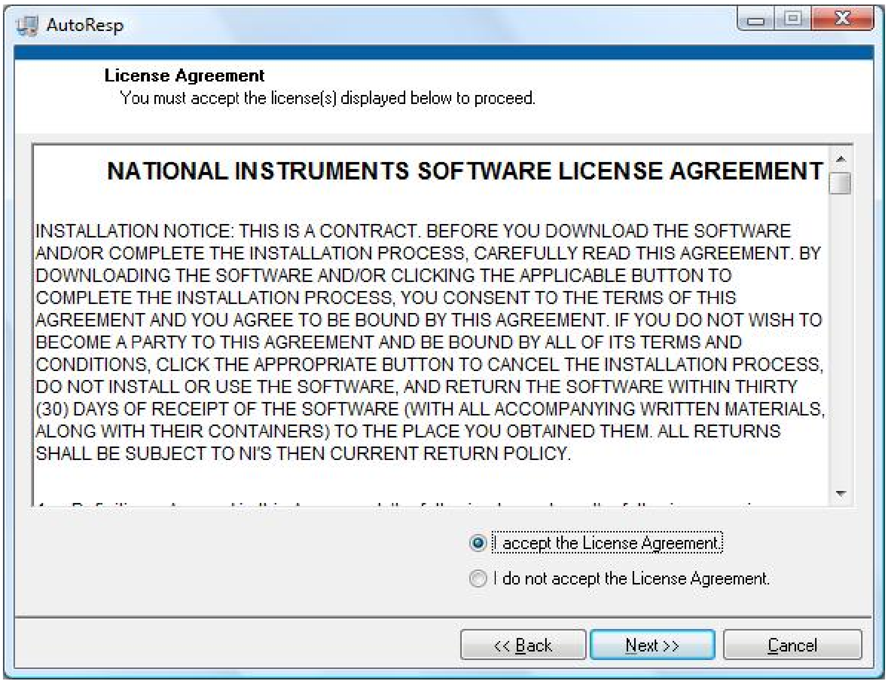


After the file has been unzipped, the Welcome screen will appear.

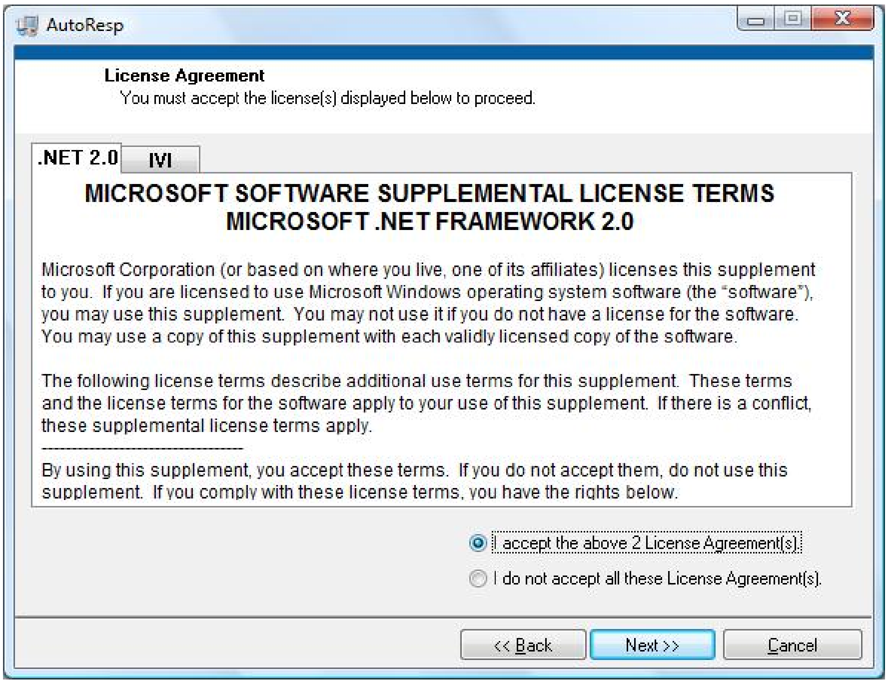




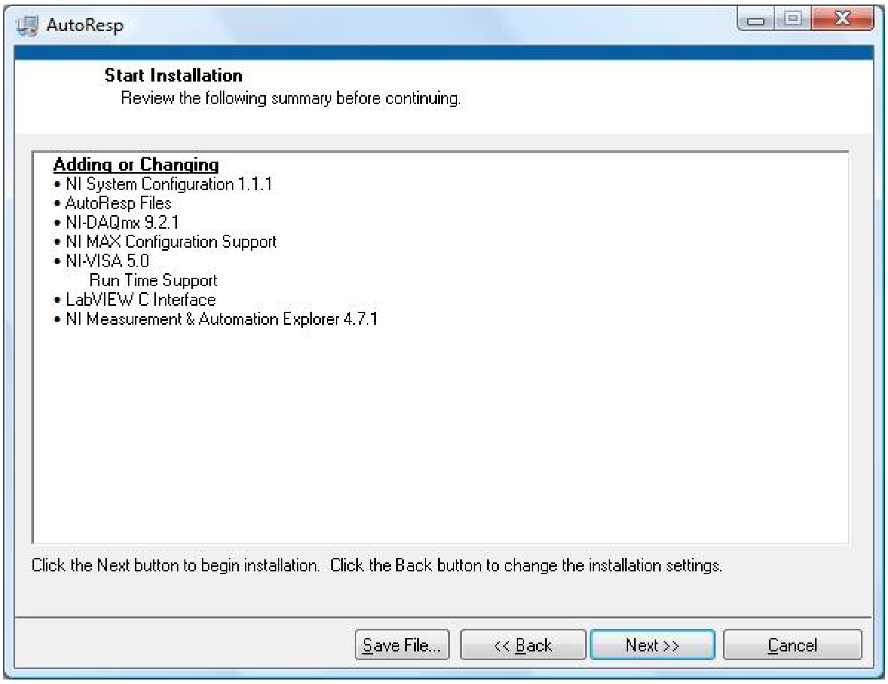
Select destination directory for AutoResp™ and for the drivers and click Next.



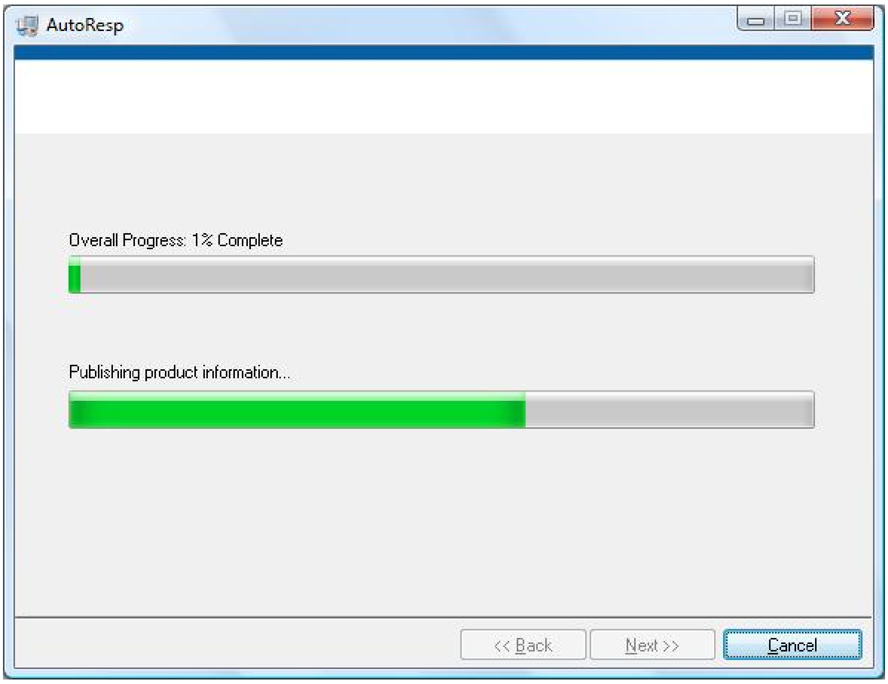
If you accept the License Agreement, please select "I accept the License Agreement. Then click Next.



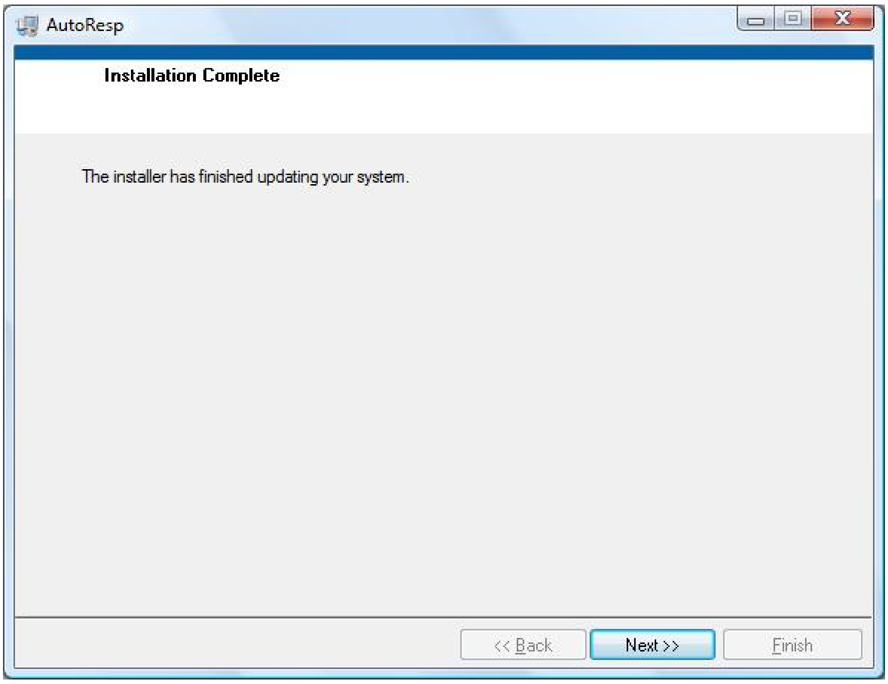
If you accept the License Agreement, please select "I accept the License Agreement(s). Then click Next.



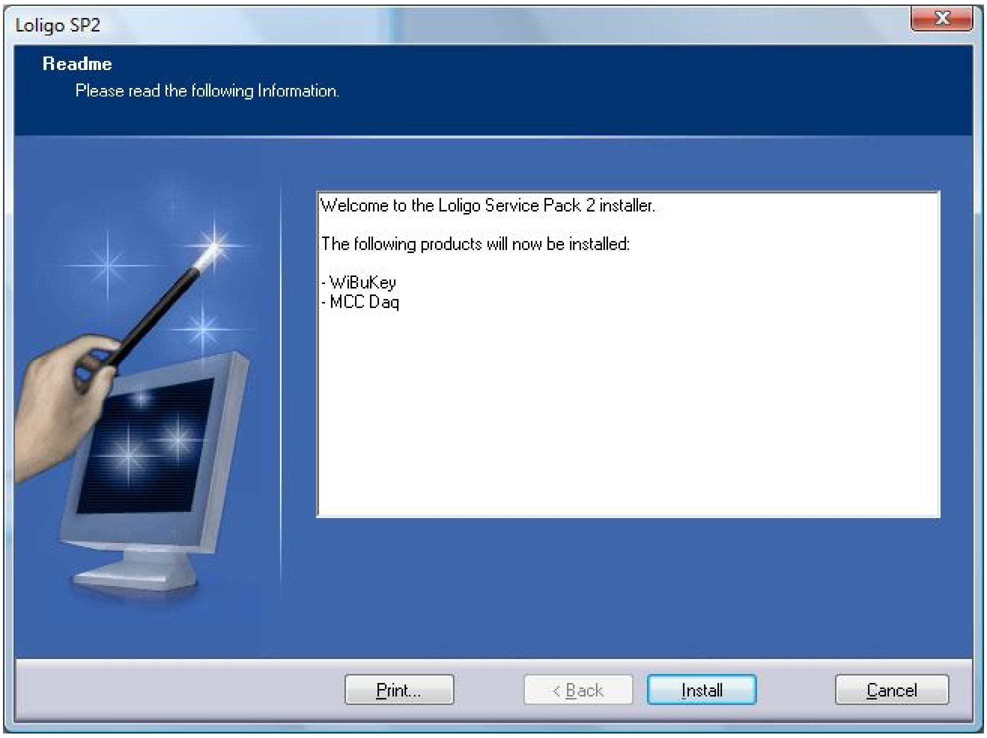
A summary is given of the products to be installed. Click Next.



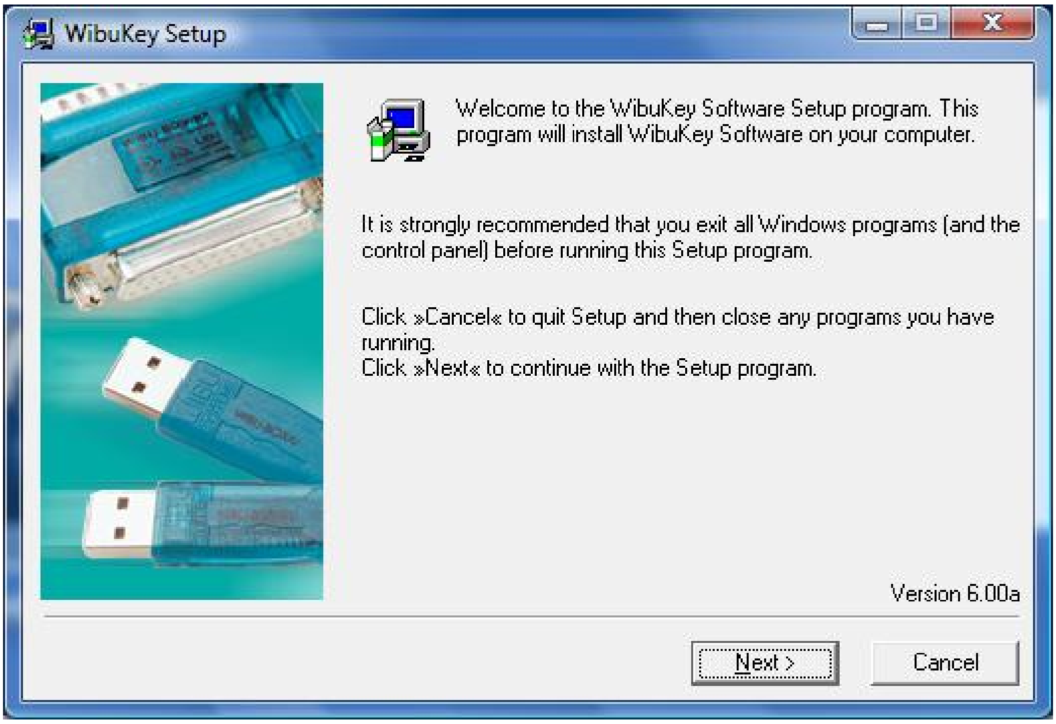
The installation may take a while.



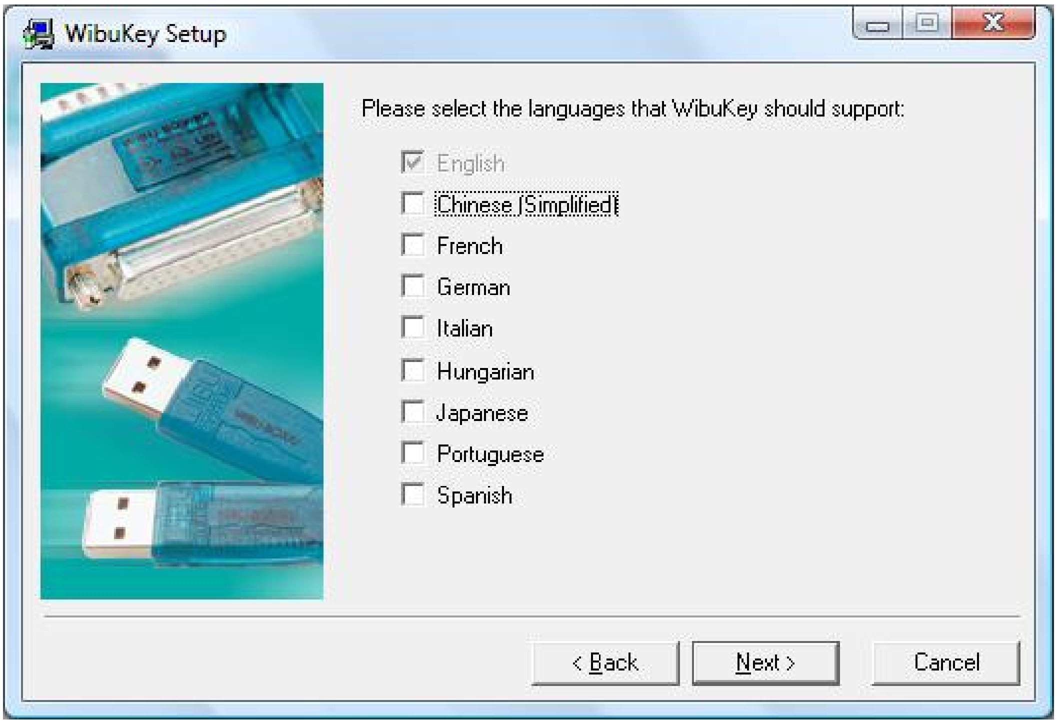
When installation is complete click Next.



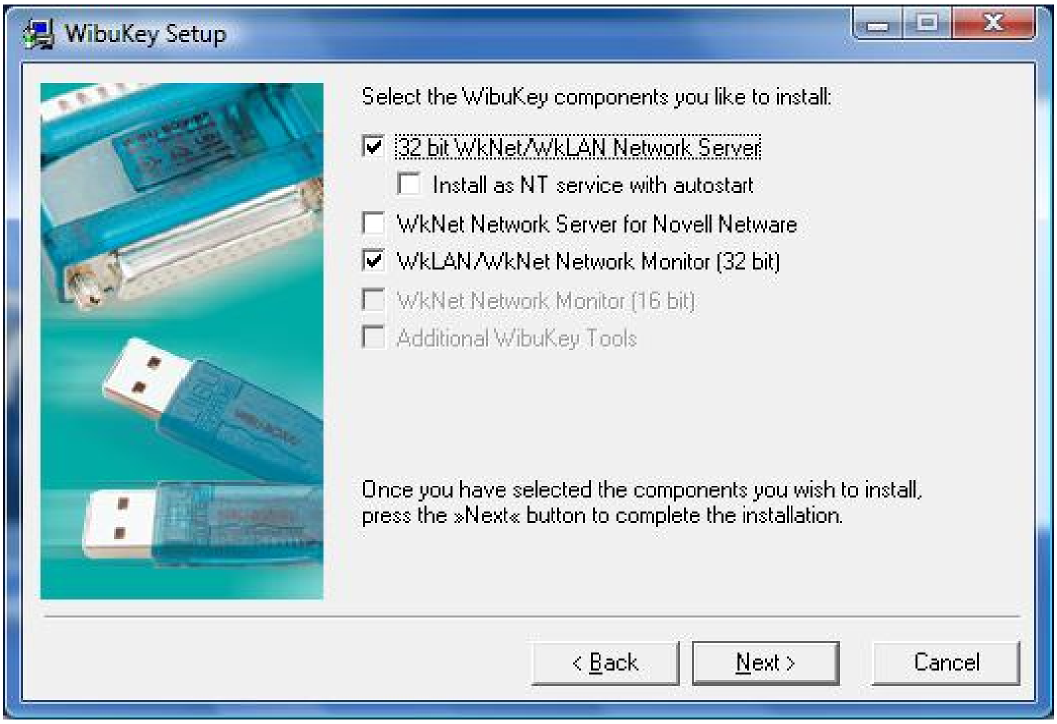
Now to the installation of drivers for the WiBu hardkey protection dongle. Click Install.



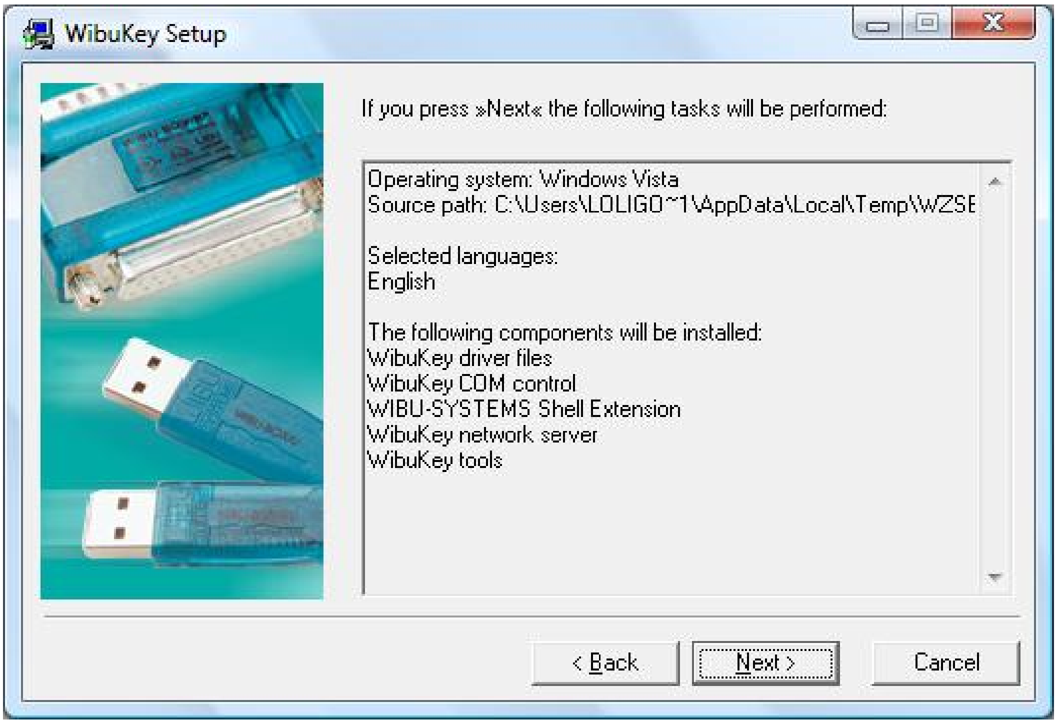
Click Next.



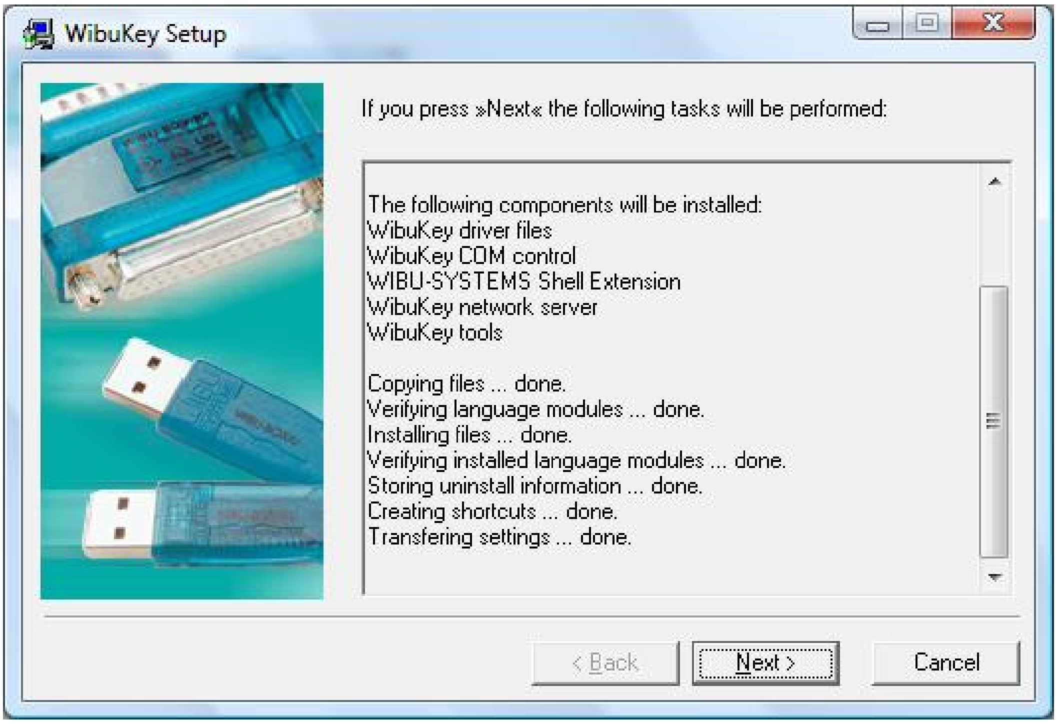
Click Next.



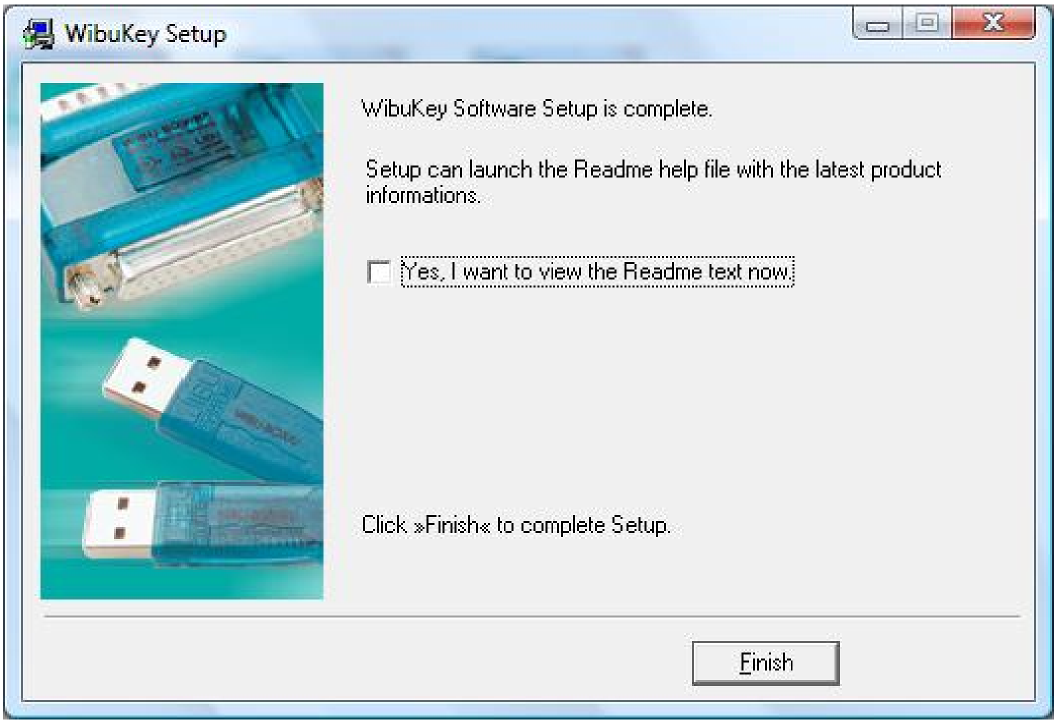
Click Next.



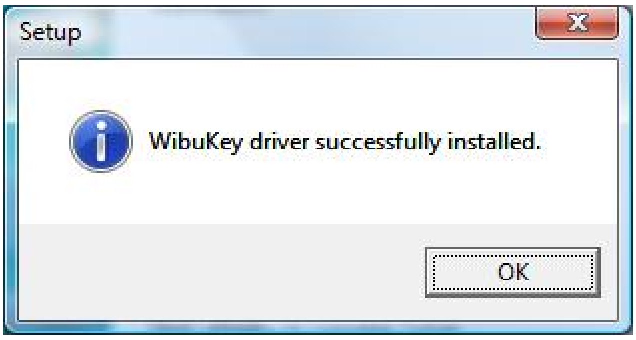
Click Next.



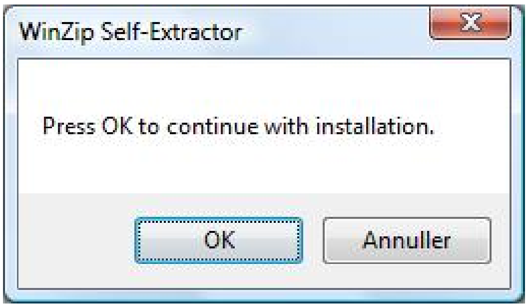
Click Next.



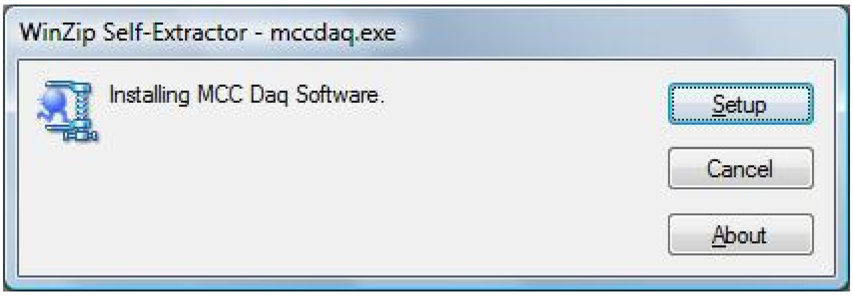
Click Finish.



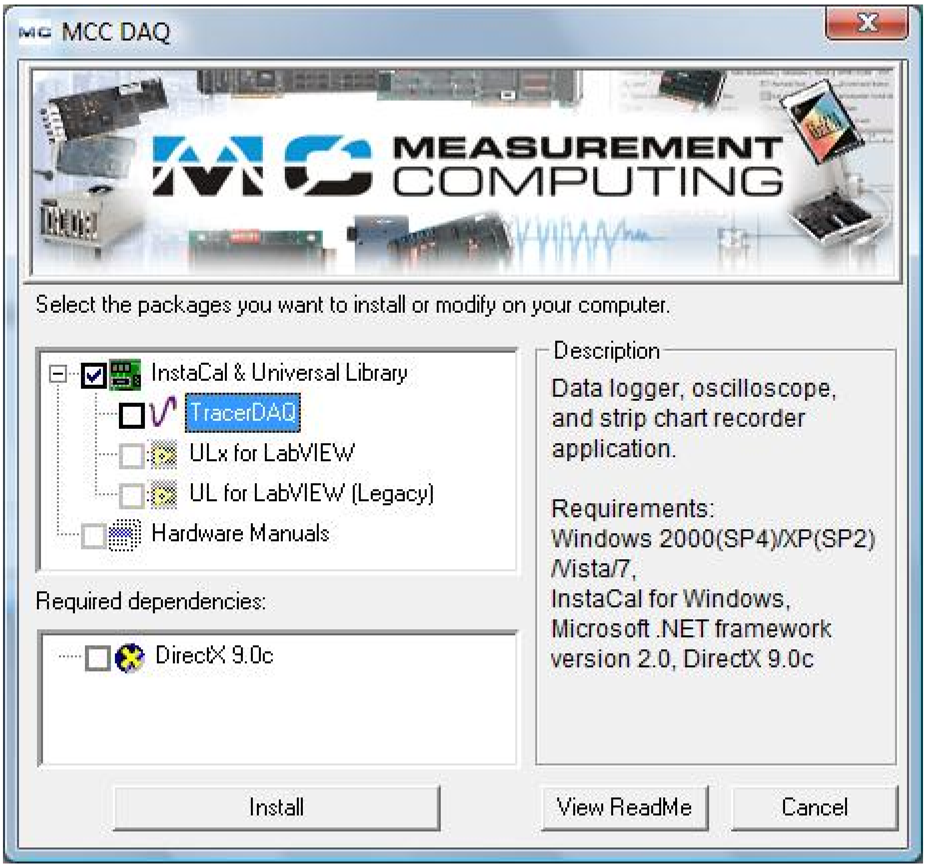
Click OK.



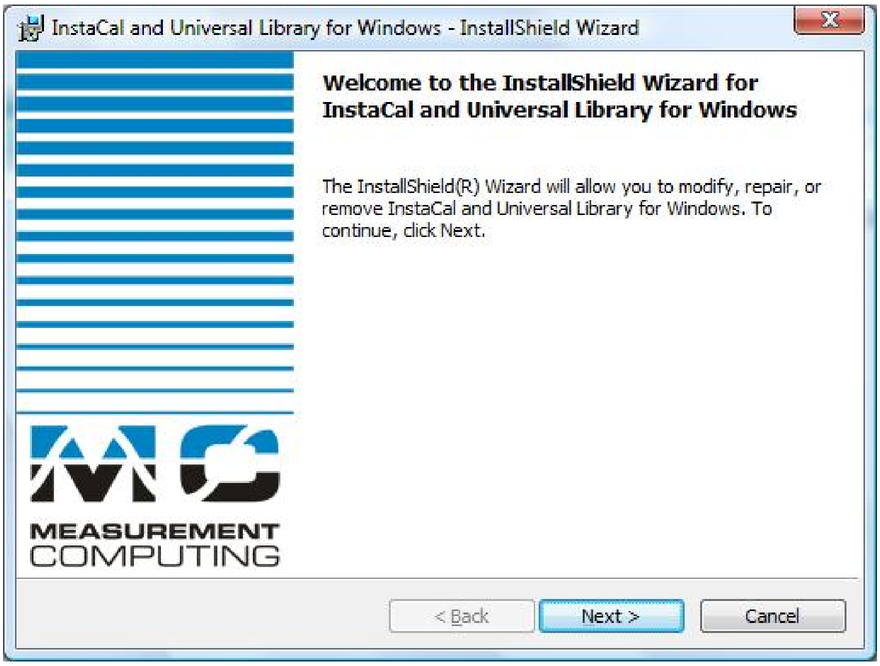
Click OK.



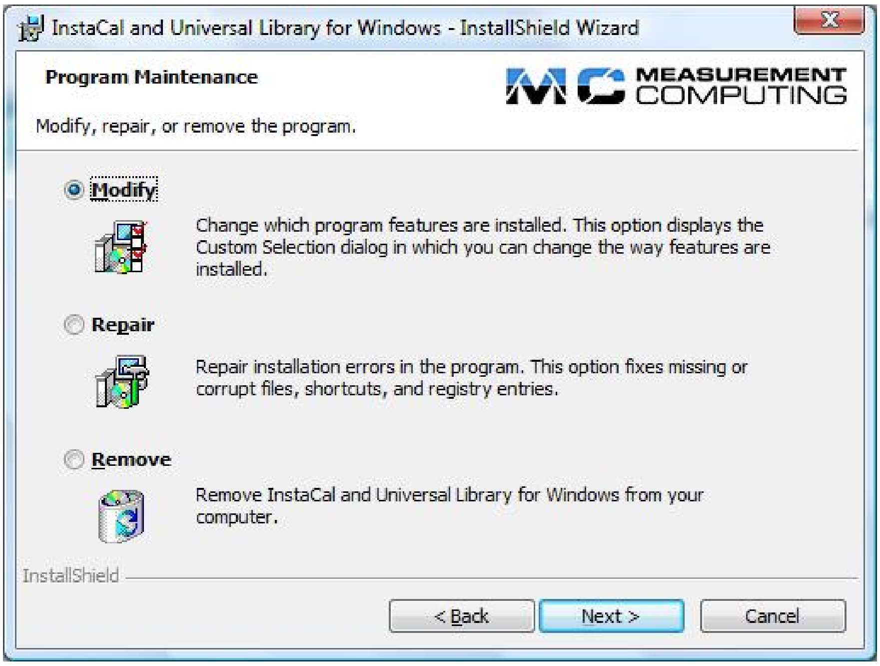
Click Setup to start installing third party (Measurement Computing) device drivers.



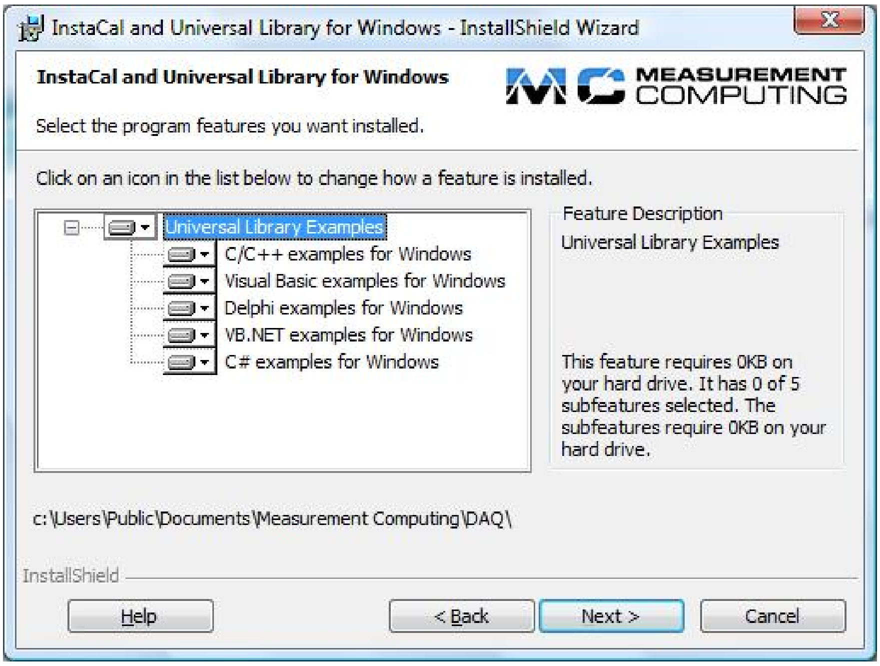
Deselect TracerDAQ, then click Install.



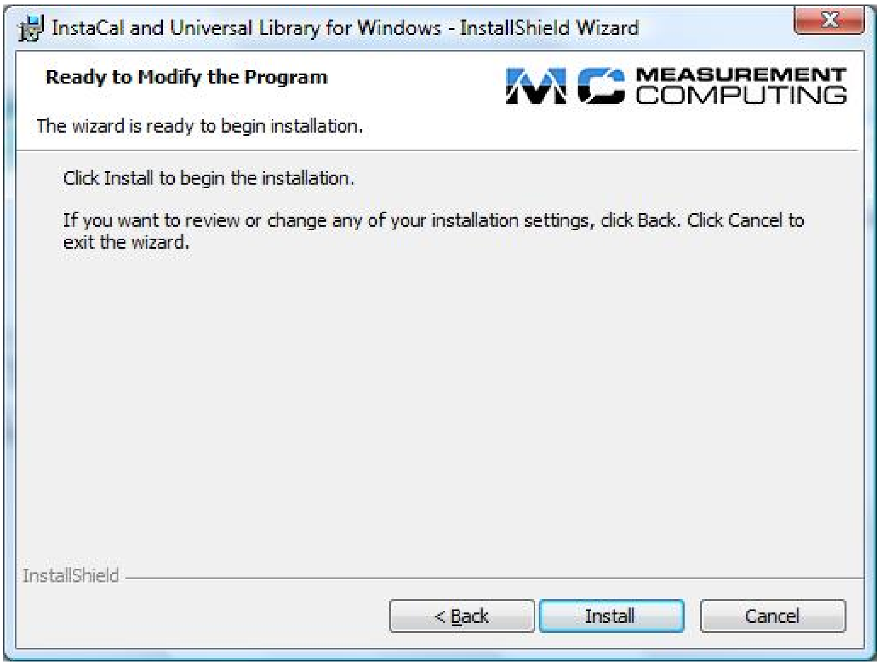
Click Next.



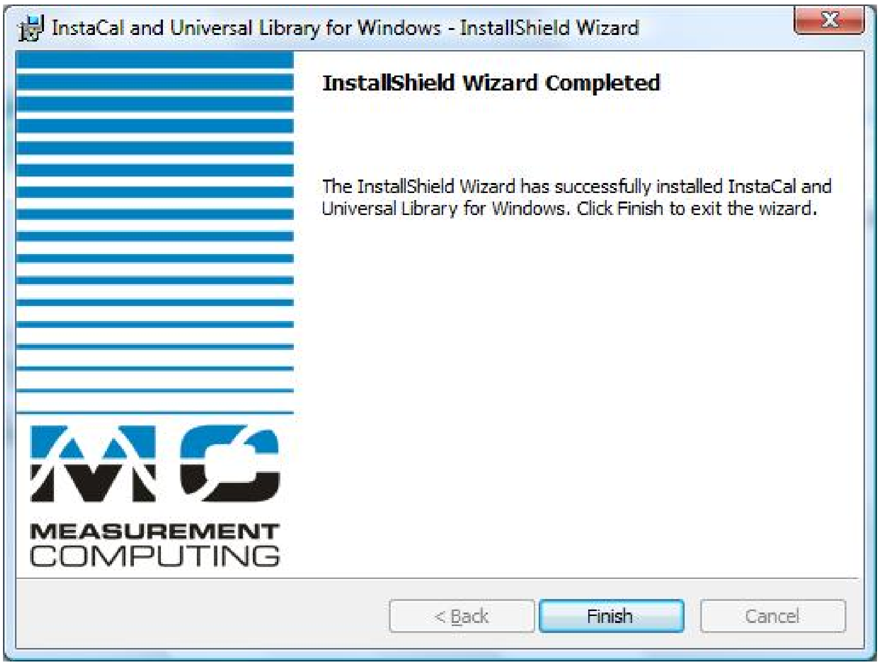
Click Next.



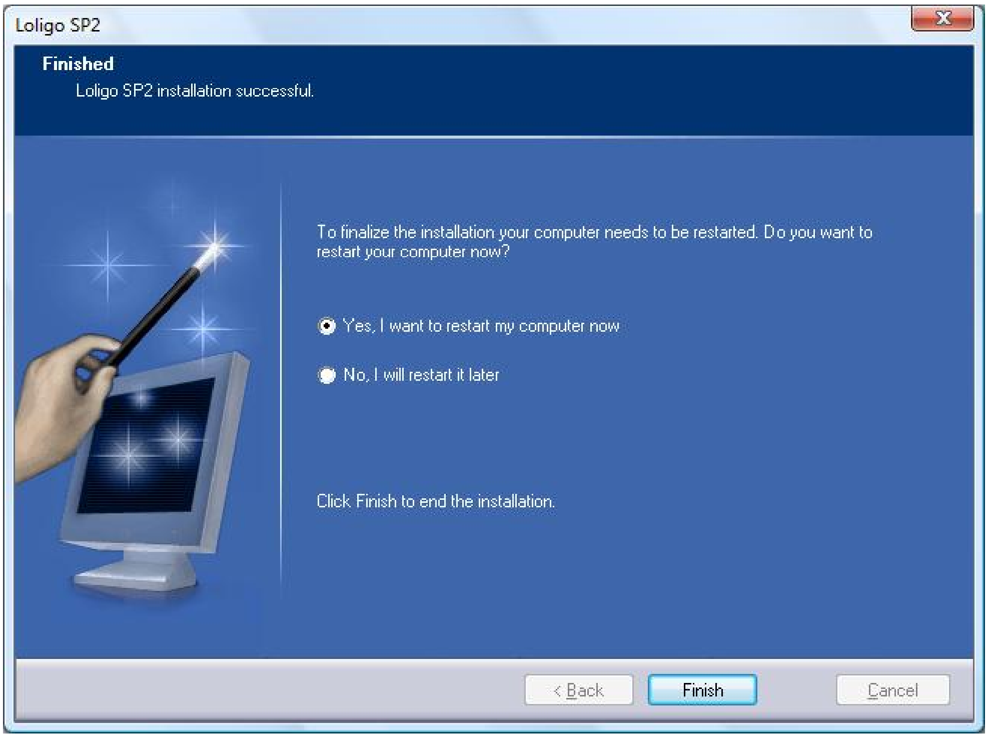
Click Next.



Click Install.



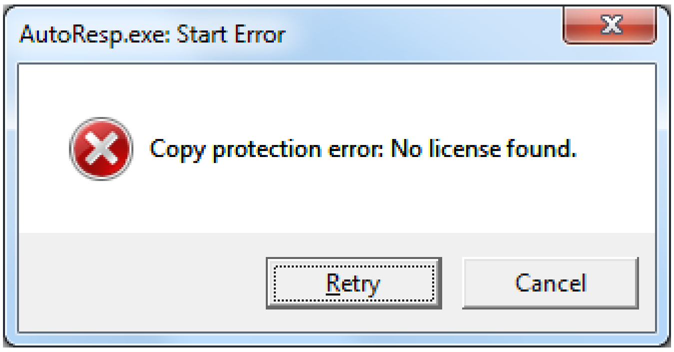
Click Finish.



Choose “Yes, I want to restart my computer now”, then click Finish. After the PC has restarted you have installed all AutoResp™ software and device drivers.

6.2 WiBu software protection

AutoResp™ is protected with an USB hardkey dongle (WiBu), and will only run if a valid dongle is connected to an USB port on the computer. If not, the error message below will appear.



Plug in the WiBu hardkey dongle and wait to let it be recognized by Windows. Only then can AutoResp™ be used.

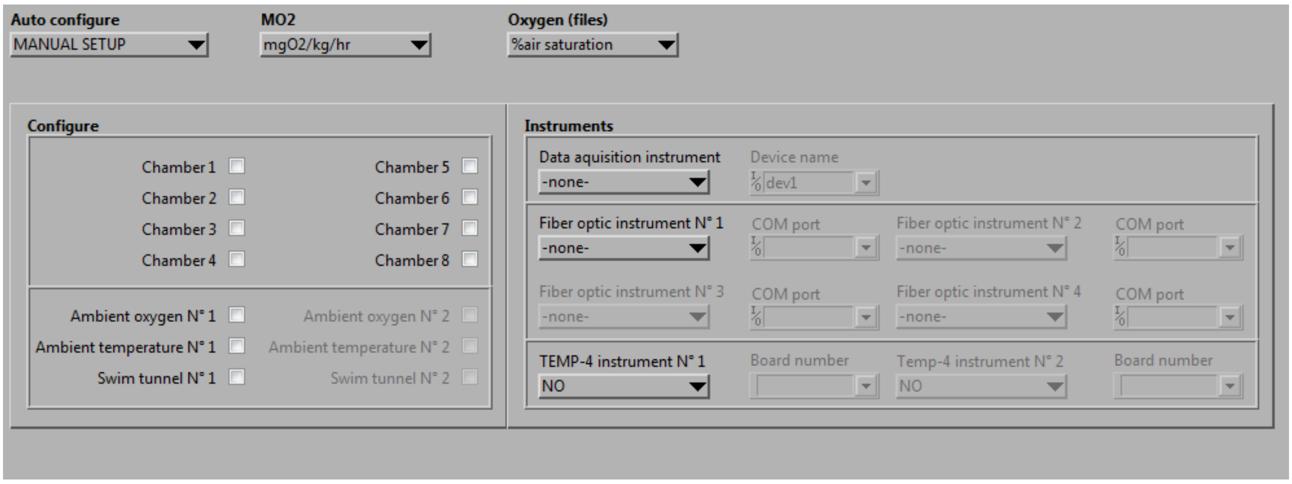
7. USE AUTORESP™

NB! To enjoy all functions in AutoResp™ it is necessary that the PC user has administrator status. (page 71)

Start AutoResp™ from the start menu in Windows. It might take a few seconds to load the program initially. Watch the Windows task bar.

AutoResp™ will start in the screen mode shown below. Here you can connect the different instruments that you have, and configure the input channels on your DAQ instrument. Here you also choose units of measure.

If one of our a DAQ-PACs or systems are used, please use the Auto configure function for a quick setup.



NB! In AutoResp™ users can freely choose between the oxygen units % air saturation, kPa (partial pressure of oxygen) and mgO2/L (oxygen concentration), but this requires a user input for barometric pressure and salinity. Temperature is also required, but can be acquired automatically if connecting a temperature instrument to the system.

1. The digital output from the oxygen instrument must be calibrated in the AutoResp™ software to show correct oxygen values.

2. Measure at 0% air saturation, and press LOCK LO. Since fiber optic oxygen sensors are sensitive to temperature, it is also necessary to enter this value.

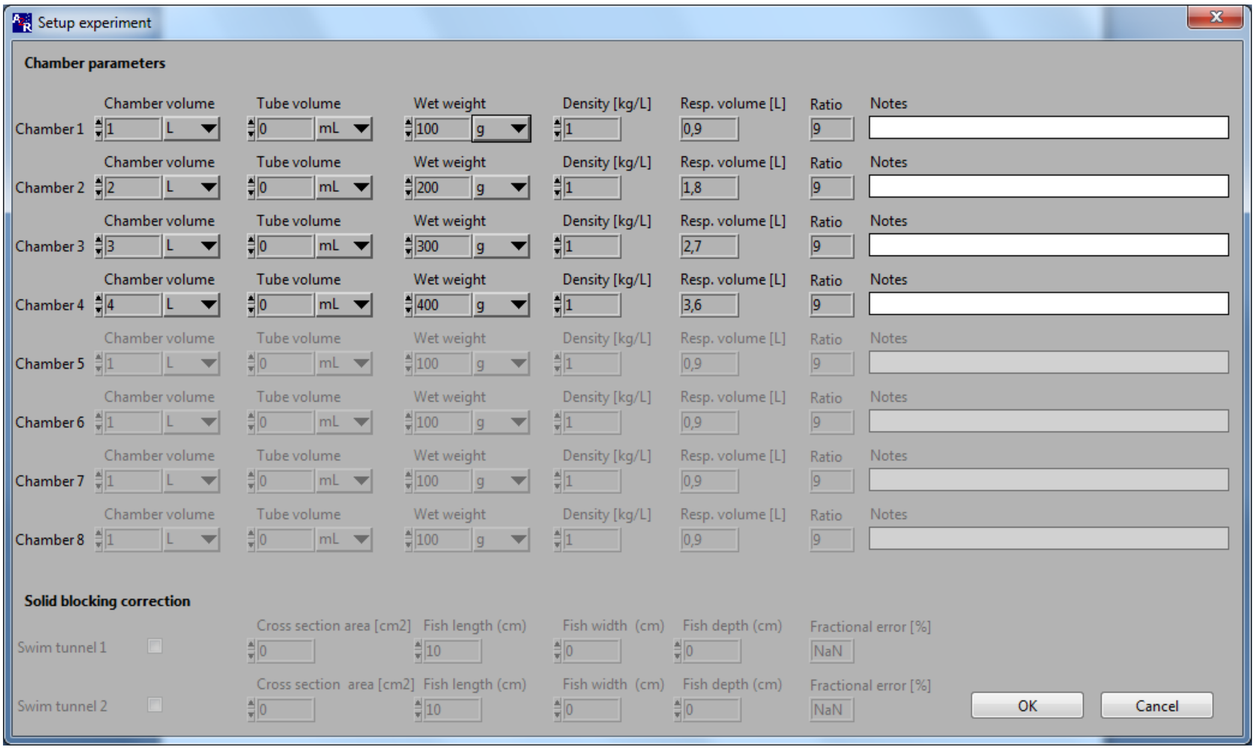
3. Measure at 100% air saturation, and press LOCK HI. Since fiber optic oxygen sensors are sensitive to temperature, it is also necessary to enter this value.

4. Choose Online (Ambient) as temperature input channel. It is very important to temperature compensate the fiber optic oxygen values, since the oxygen values are very temperature dependent.

5. Repeat this step for every oxygen channel.

6. The calibration and configuration is continuously saved to a binary file (AutoResp.conf).

7. Now choose Experiment→Start to start a respirometry experiment.



8. Before the experiment starts a number of parameters must be given in order for AutoResp™ to calculate oxygen consumption rate etc.

9. First enter the chamber volume and choose the correct unit.

10. Enter the volume of tubes in the recirculating loop only and choose the correct unit of measure. In swim tunnels this tube volume should be set to zero (0).

11. Enter the wet weight of the animal(s) and choose the unit of measure.

12. Then enter the density of the animal(s) for the software to calculate the respirometric volume.

13. The respirometric volume is calculated as:

    Resp. voume [L] = chamber volume + tube volume - volume of organism(s)

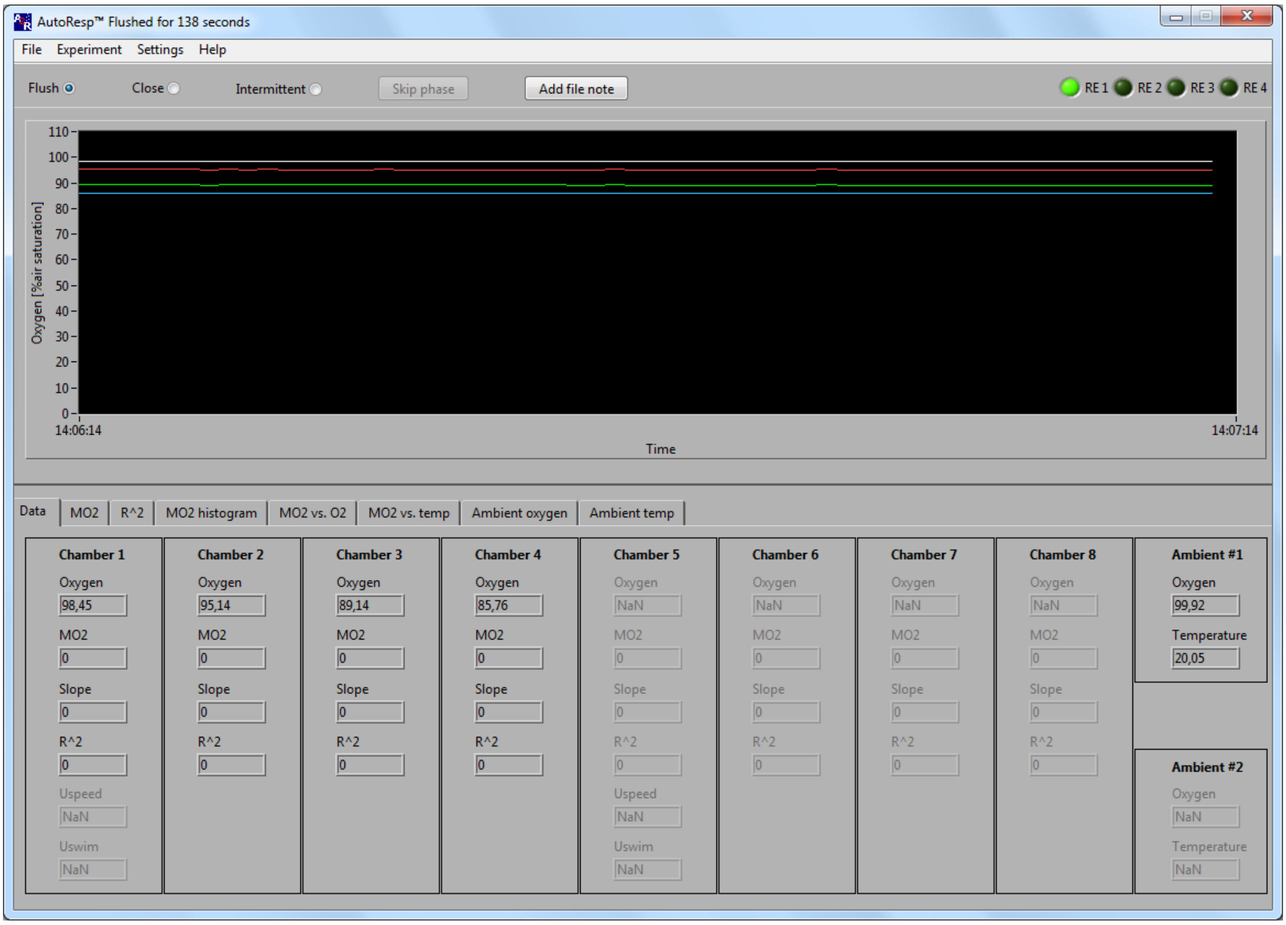
14. The ratio field indicates the ratio between the Resp. volume and the wet weight volume. As rules of thumb, this ratio should be 10-20 for measurements in resting animals, 10-50 for micro respirometry (<<50 mL) and up to 200 for measurements in active animals (swim tunnel respirometry) for reliable MO2 measurements. If experimental temperatures are high the ratio can be higher without compromising the quality of MO” data.

NB! The solid blocking correction fields are only available when running with a swim tunnel, see SWIM TUNNEL RESPIROMETRY, page 52.

15. Now enter correct values for chamber 2.

16. When done, press OK.

17. Now a file dialog pops up, where the user is asked a file path for the (calculated) data file and raw data file. The raw data file will get the same name as the data file but with "_raw" as an extension to the file name.



18. Now an experiment is started, but in a continous flush mode so that no oxygen consumption measurements will take place until the user sets the operation to either "Close" (closed respirometry) or Intermittent (intermittent respirometry).

19. Move the mouse over the relay indicator diodes in the upper right corner. A Help text will pop up and help the user which relay is used as recirc, flush, ambient control etc.

20. The upper screen shows the oxygen values for the chambers. Right clicking on the graph will show a submenu. It is here possible to hide/show chamber graphs, pause graphs, scale axes etc.

NB! Please note, that AutoResp™ will continue collecting data even though graphs are set on pause (Show cursor). While set on pause the x-scrollbar appears and it is possible to go back to the start time of the experiment using the scrollbar.

NB! If using the cursor and having troubles that others graphs values are shown, use the sticky function. The sticky function will attach a cursor to the curve from one chamber specified by the user.

21. The lower graph panel shows the data and graphs for calculated values.

22. The Data tab will show all current values. Some fields are gray, depending on the configuration mode. So in the current example case, chamber 1 and 2 are shown and the ambient temperature only.

23. Because the ambient temperature was activated, an ambient Temp tab is also accessable, showing ambient water temperature in real time or average values over time (right click). Depending on the configuration, different tabs may appear, such as ambient oxygen, Uspeed (swimming speed) etc.

24. In the upper right corner, the kind of experiment currently running can be chosen.

    Flush – This means that the flush is always on. The object will get fresh water constantly, but this also means that no MO2 values are calculated.

    Close – This means the chamber is closed, and every time a measure period is finished a MO2 is calculated. After the measure period is finished a new measure period will begin immediately. If choosing this mode of operation, take care not to leave the set up to avoid severe oxygen depletion inside the chamber due to animal respiration.

NB! The object will remove all oxygen from the chamber after some time, and then the object will **DIE**! Use with care.

    Intermittent – This means the experiment runs in a loop (flush, wait and measure) as explained in the back ground theory chapter (page 62). During each flush period, the flush pump is turned on to flush chambers with ambient water. The wait periods allow time for steady state before measurements start during the measure period.

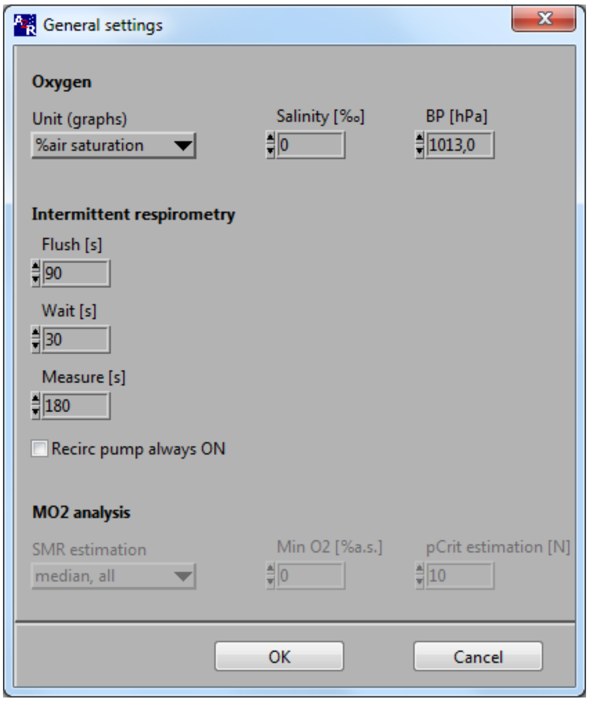
    In Intermittent mode, the skip phase button allow users to jump between these three modes (flush, wait measure) immediately and on-the-fly for special applications or situations, e.g. to start flushing the chamber immediately after reaching Ucrit during a swim trial.

    LOOP

    F1→W1→M1→F2→W2→M2 etc.

    For more information about the Intermittent principle, see page 63.

25. Now choose Settings→General to set the settings for the experiment.



26. In the General settings it is possible to change oxygen units, water salinity or barometric pressure during experiments. It is also possible to change flush, wait and measure times.

    Flush times should be set to allow complete renewal of water inside each chamber. This will restore oxygen values to ambient between each measurement. As a rule of thumb, cylindrical chambers should be flushed with a volume five times the chamber volume for 99% wash-out, but this depends on chamber dimensions.

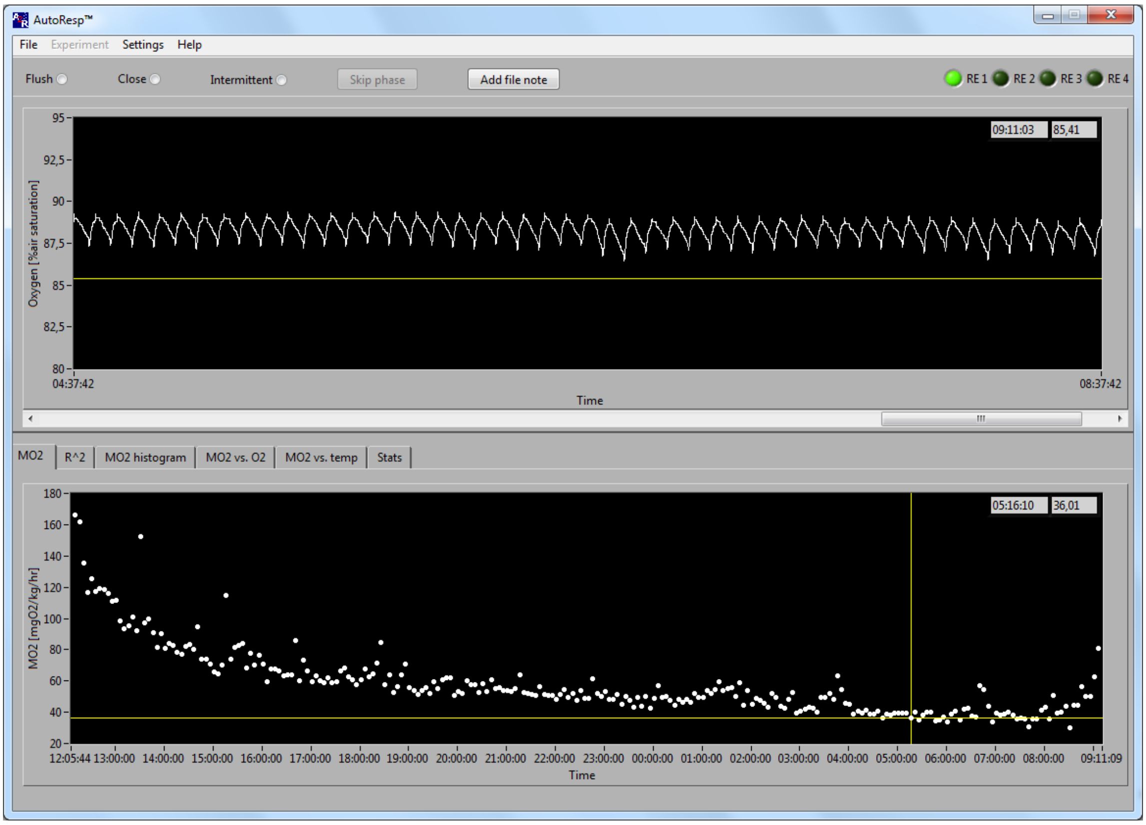
    The measurement times should allow enough data for a reliable determination of the slope of the oxygen curve to be estimated. The slope is determined from a linear regression, and the regression coefficient r^2 express the statistical validity of the calculated slope, e.g. r^2 >0.95 indicates a sound linear relationship (oxygen vs. time), and thus a reliable MO2 value.

    Notice that the recirculating pump can be set to constant activity. This will double the flow during flush periods potentially affecting animal behavior/locomotion, but will provide better oxygen readings if the oxygen probe is placed outside the chamber in a recirculating loop.

27. The MO2 analysis buttons are gray during measurements, but can be used during post analysis when loading saved raw data files form past experiments.

28. Press OK to close the general settings.

29. Now run an experiment for min/hours/days depending on application. Adjust your settings during measurements if needed. Any changes will be written in the raw and data file.



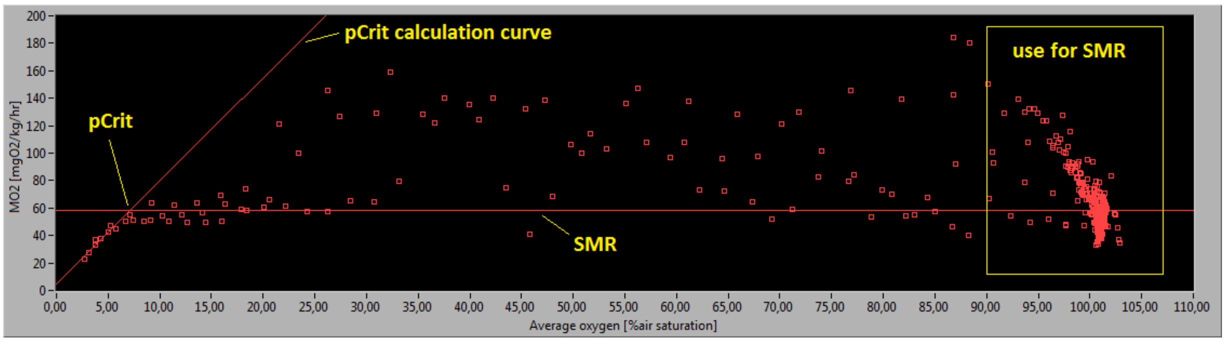
Your graph should look like this. This is a graph for one chamber only.

30. To end the experiment, click Experiment→Stop.

31. Now press File→Load to analyze saved data. The file loading might take a while.

32. When the file is loaded, a new tab labeled Stats are now available for the user. Use this tab for calculations and statistics.

33. The analysis settings are available in Settings→General. Choose SMR estimation method and choose a minimum average oxygen level for estimating standard metabolic rate (SMR) to exclude any hypoxic values. For hypoxia experiments, the critical point for metabolic homeostasis (P_{crit}) can be calculated automatically. Click the button to set the number of low oxygen values that should be part of the oxy-conforme curve when the animal is no longer able to regulate respiration rate independently of ambient water oxygen.



8. SWIM TUNNEL RESPIROMETRY

If you purchased a Loligo™ swim tunnel respirometer to measure oxygen consumption in swimming animals the AutoResp™ software has several features for real-time data acquisition, analysis and control. The AutoResp™ is able to operate one or two swim tunnels at a time.

8.1 Motor output

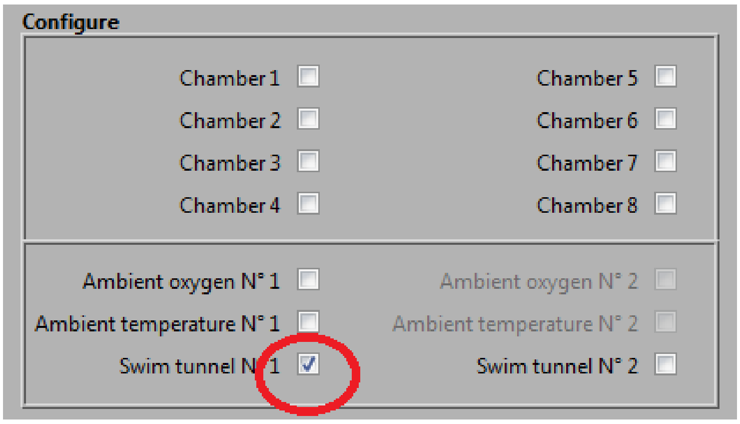
In AutoResp™ it is possible to acquire an analog DC voltage signal or a tacho signal from a Loligo™ swim tunnel motor controller. The tacho signal will give the more precise measurement of water speed (RPM), so we recommend collecting this type of signal if possible.

8.2Motor input

In AutoResp™ it is possible to generate an analog DC voltage signal to control a Loligo™ swim tunnel motor controller.

8.3 AutoResp™ settings

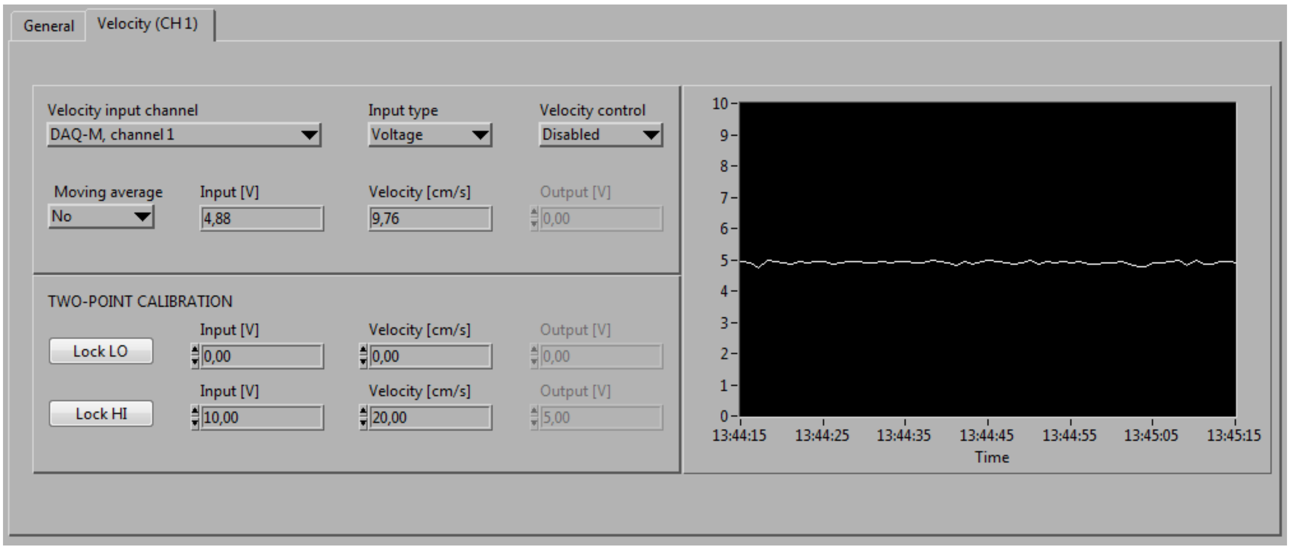
To set up AutoResp™ to collect a swim tunnel motor output signal, the motor must be running. It is possible to measure oxygen consumption rates and swimming speed from up to two swim tunnels simultaneously. Data from swim tunnel N°1 will appear as chamber 1 and data from any second swim tunnel will be saved under chamber 5 in files, graphs etc.



If Swim tunnel N°1 is activated a tab labeled Velocity (CH 1) will appear. If Swim tunnel N°2 is activated another tab labeled Velocity (CH 5) will be active.

AutoResp™ will also be asking if a DAQ-BT is used. If a DAQ-BT is used please enter the board number. No further in or output settings are necessary then.

Click the tab to calibrate the swim tunnel motor output to show correct water and swim speeds in the software.



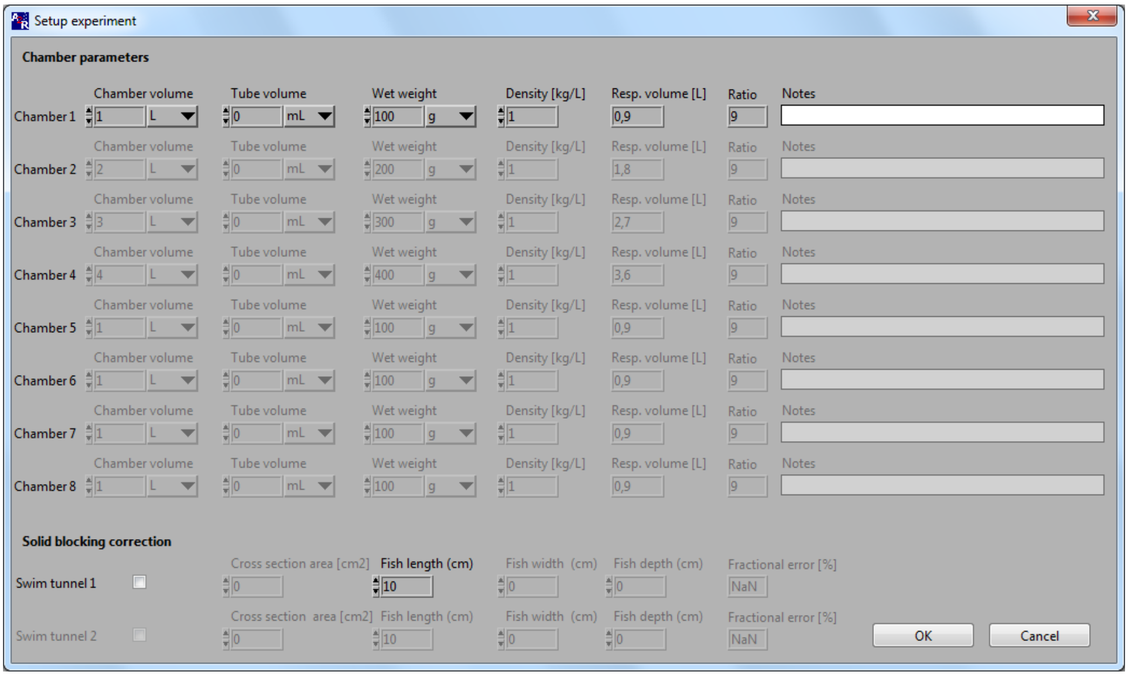
First click on the input channel and choose the input channel. Any of the eight input channels on your DAQ-M instrument can be used. Then choose between a voltage type or tacho type of signal from the motor. If choosing the tacho type of signal, it will take about 10 seconds before the tacho signal starts in the graph.

If AutoResp™ has to control the motor, enable Velocity control. For Velocity (CH 1) the OUT 1 output is used, for Velocity (CH 5) OUT 2 is used. The output [V] can manually be set by entering a value.

Now perform a 2-point calibration at two known velocities and Output [V] values for the software to show correct velocity units (e.g. cm/sec).

E.g. set the LO value to 0,75 V, wait until the Input [V] value gets stable, then press Lock LO, and enter the velocity in the Velocity [cm/s] field.

After the flow calibration, click start experiment. In the setup experiment screen it is now possible to enter the fish length and to choose if solid blocking correction should be used.



Solid blocking correction:

An animal swimming in a (closed) channel obstructs the flow of water, causing the water to run faster past the swimming animal. This results in a fractional error, i.e., a difference in water velocity depending on the size of the animals and the dimensions of the channel.

Formula (Bell & Terhune, 1970):

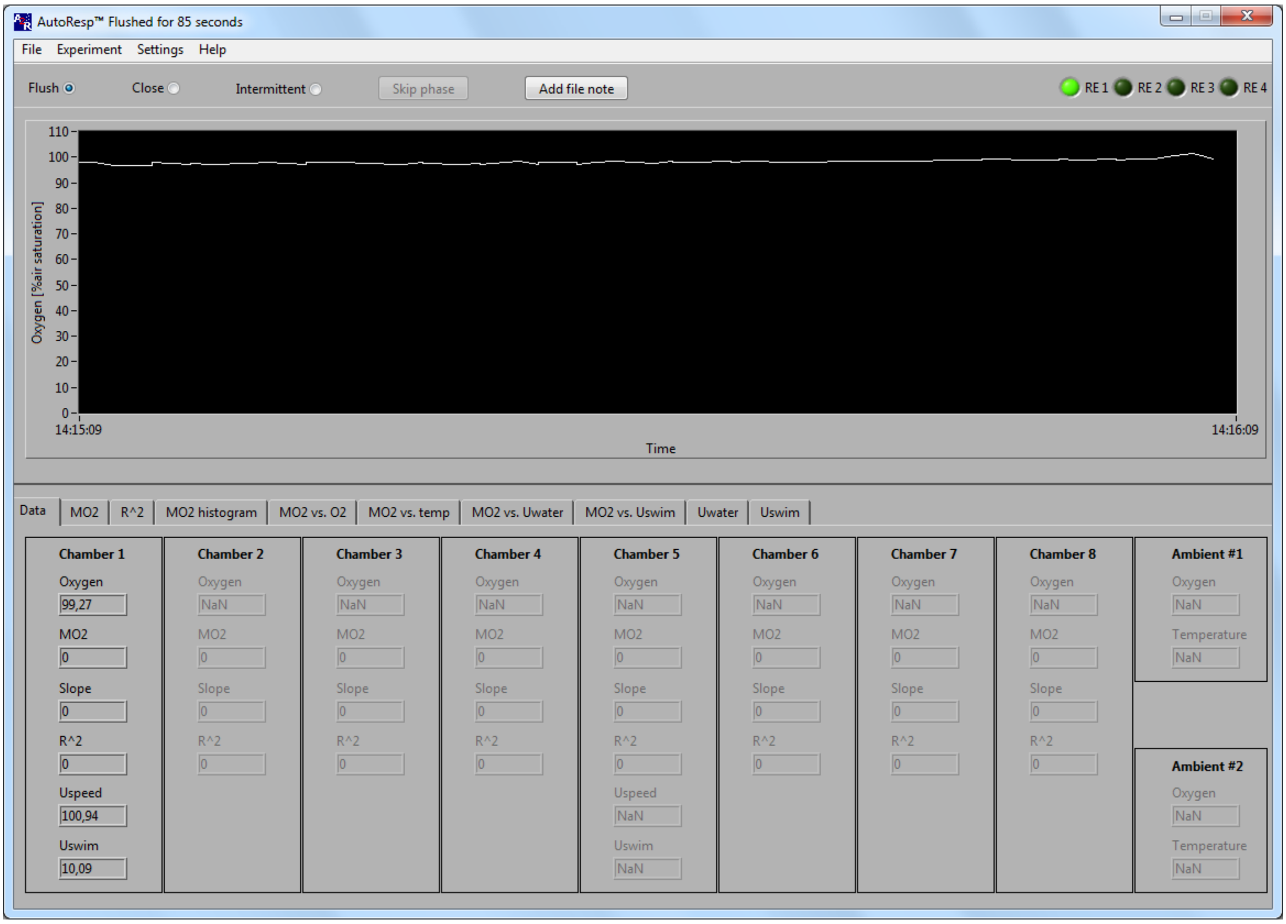
Fractional error = 0.8 • 0.5 (Fish length/fish radius) • (fish square area/cross area)$^{3/2}$

Fish length	: Body length of fish
Fish radius ("Thickness")	: (fish width + fish depth)/4
Fish square area	: π • (fish radius)^2
Cross sectional area	: cross area of swim tunnel working section

The fractional error is used to convert water velocity in cm/s (as measured during flow calibration with no fish in the working section) into corrected relative swimming speed in BL/s during experiments with swimming fish.

<u>Reference</u>

Bell, W.H. & Terhune, L.D.B. (1970). Water tunnel design for fisheries research. Fish.Res.Bd.Can.Tech.Rep. 195, 1-69.



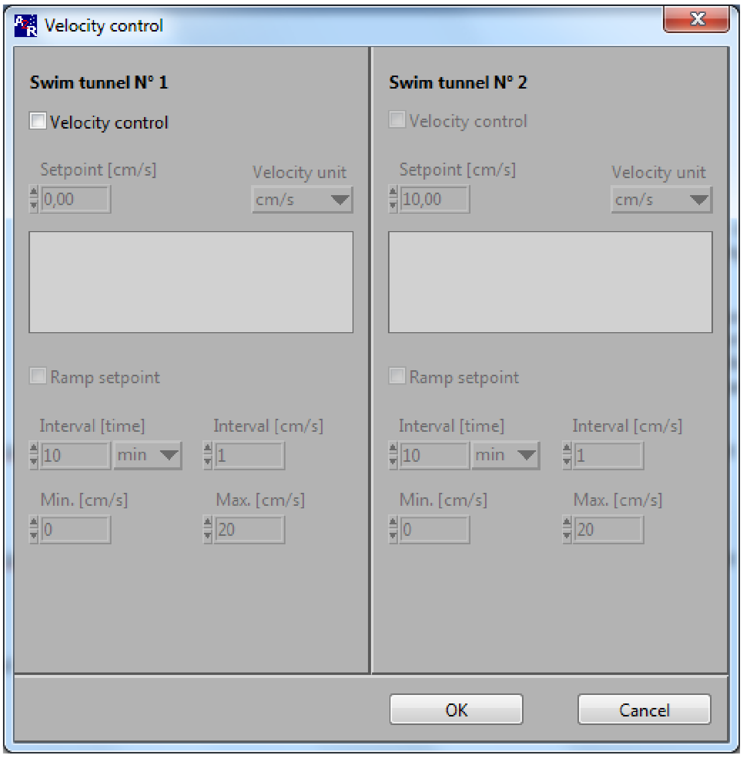
Uwater: This graph plots uncorrected water velocity versus time. By right clicking the graph it is possible to show average values for each measuring period instead of real-time values.

Uswim: This graph plots swimming speed versus time. The unit of measure for Uswim is body lengths per second (BL/s). When solid blocking correction is activated, corrected swim speed in BL/s will be shown. By right clicking the graph it is possible to show average values for each measuring period instead of real-time values..

MO2 vs. Uwater: This graph plots the MO2 value for each measuring period against average Uwater.

MO2 vs. Uswim: This graph plots the MO2 value for each measuring period against average Uswim.

To control the velocity enter the menu Settings→Velocity control.



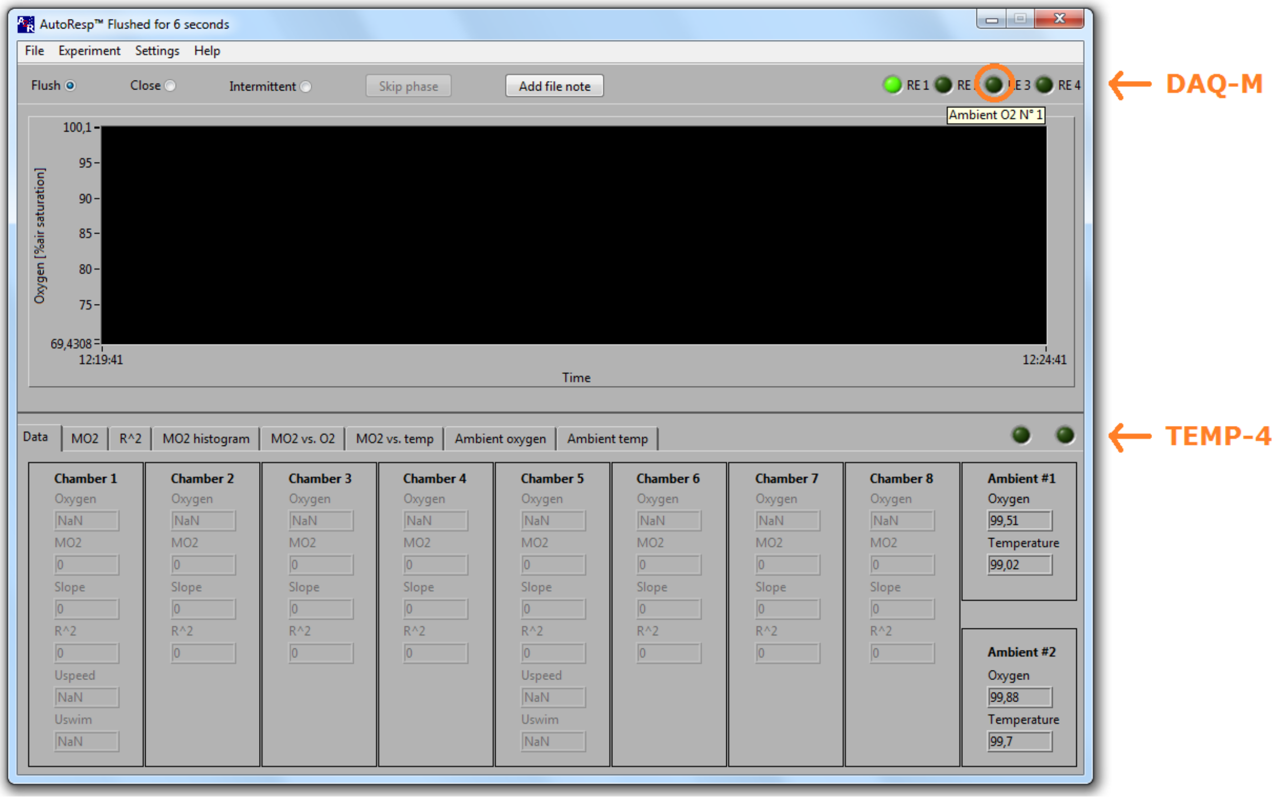
Enable the Velocity control, and enter the setpoint value. A ramp function allow users to let the software change set points up or down (use negative step value). When activating the ramp function AutoResp™ will automatically increase/decrease the setpoint by a given interval and after a given time interval. The duration of the time interval can be set in minutes or in number of loops, During a loop, set points are ramped (changed) at the onset of a flush period.

9. AMBIENT WATER QUALITY

Two relays are available for ambient water quality control (e.g. temperature or oxygen), but if using a TEMP-4 instrument also two extra relays will be available for temperature control only (see table below).

Ambient/Instrument	DAQ-M/NETIO	TEMP-4
Oxygen #1	RE 3	
Oxygen #2	RE 4	
Temperature #1	RE 4	RE 1
Temperature #2	RE 3	RE 2

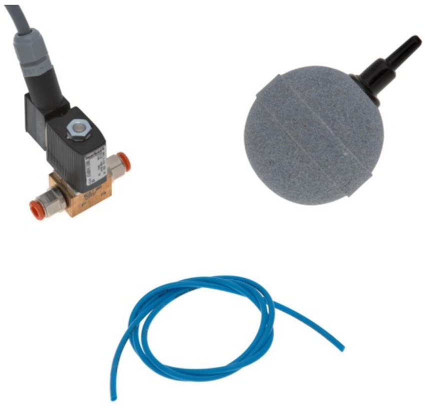
For further help on what instrument relays to use for the different devices, try to place the mouse over any of the four LEDs in the upper right corner in AutoResp®. This will give you a help text depending on the system configuration (see below).



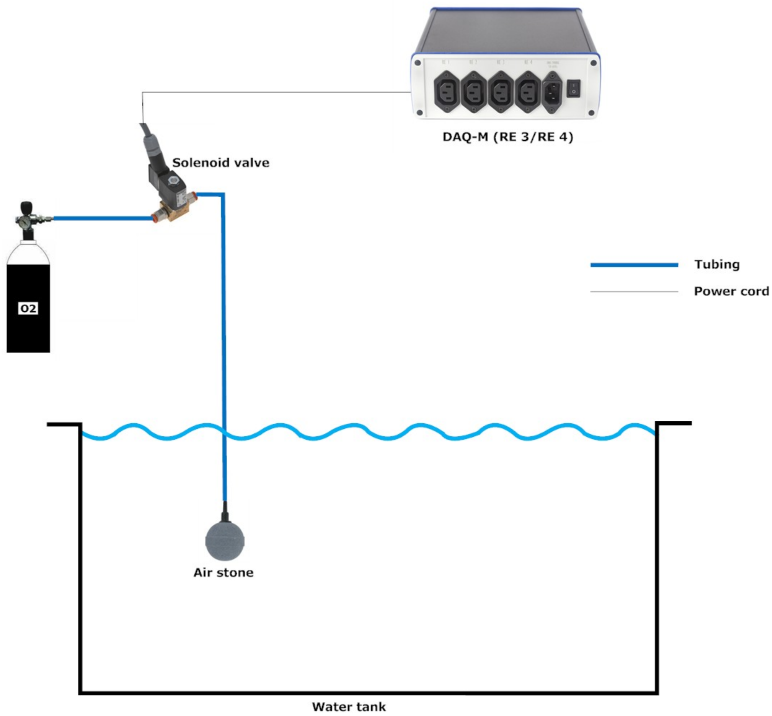
9.1Oxygen saturation

To control the oxygen saturation in the ambient water used for flushing respirometer chambers, a DO-SET is needed. This DO-SET can either be connected to the DAQ-M instrument for software control and monitoring of ambient oxygen levels or an OXY-REG instrument for standalone operation. The DO-SET includes:

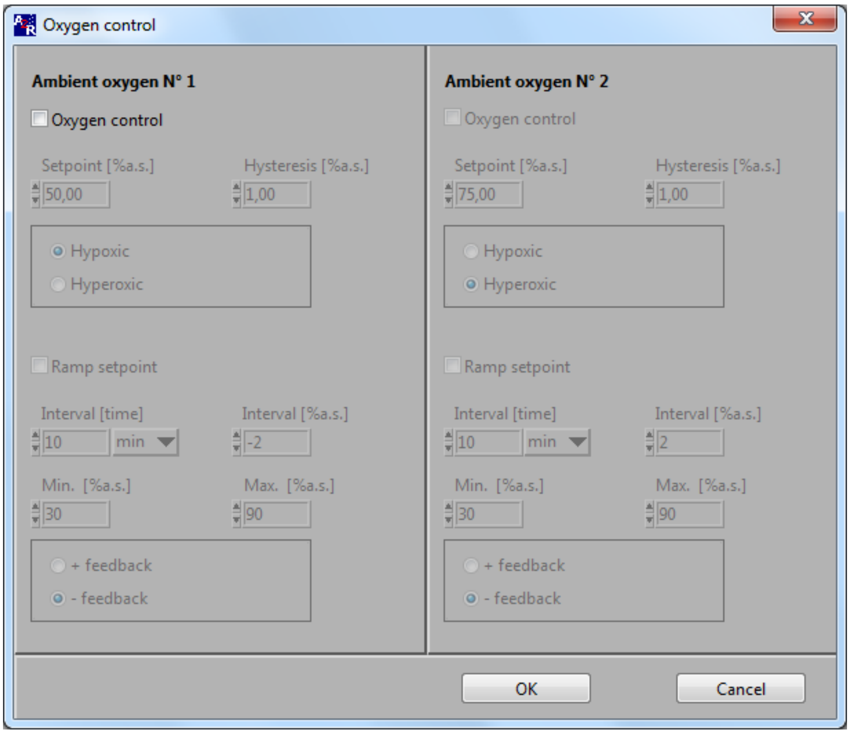
- Solenoid valve
- Air stone
- Tubing



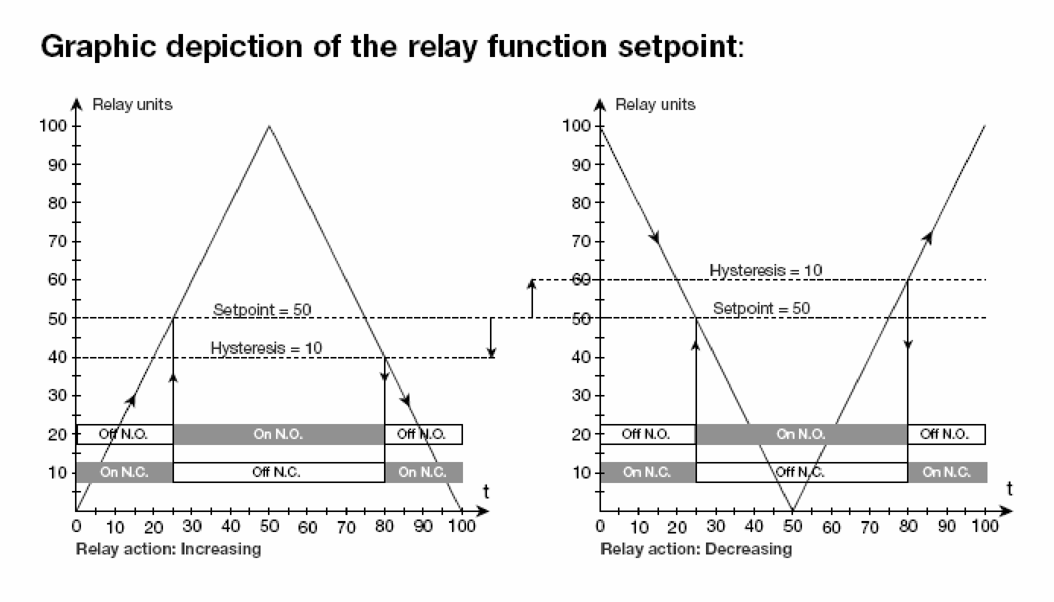
To use the DO-SET assemble the system shown in the figure below. The power cord is connected to the DAQ-M or OXY-REG.



For using a DO-SET with AutoResp™, connect the solenoid directly to one of the DAQ-M/NETIO relays. Now activate ambient oxygen N°1 in the software (see below). After the calibration and experiment settings is finished, the ambient oxygen control settings can be found under Settings→Oxygen control.



Activate oxygen control to start controlling the ambient oxygen saturation. Now set the setpoint and hysteresis and if the you want hypoxic (inject nitrogen gas) or hyperoxic (inject clean oxygen gas) control. Hypoxic control will keep ambient oxygen saturation below normoxic levels to a set point value choosen by the user and visa versa. This set point value can be altered by the user at any point in time.



A ramp function allow users to let the software change set points up or down (use negative step value) in a step-wise way for both temperature or oxygen control. When activating the ramp function AutoResp™ will automatically increase/decrease the setpoint by a given interval and after a given time interval. The duration of the time interval can be set in minutes or in number of loops, e.g. change the set point for ambient oxygen by 10% air sat. after each third measuring loop. During a loop, set points are ramped (changed) at the onset of a flush period.

The user can choose between ramping setpoints with or without feedback. Feedback means that setpoints are not ramped (changed) until the measured value has reached the setpoint value. This to avoid problems with large volumes of water and/or large steps of increment/decrement in values, in which case it can take considerable time to change water temperature or oxygen saturation.
Finally, the user can set minimum and maximum setpoint values to avoid severe changes or harmfull conditions when using the automated ramp functions.

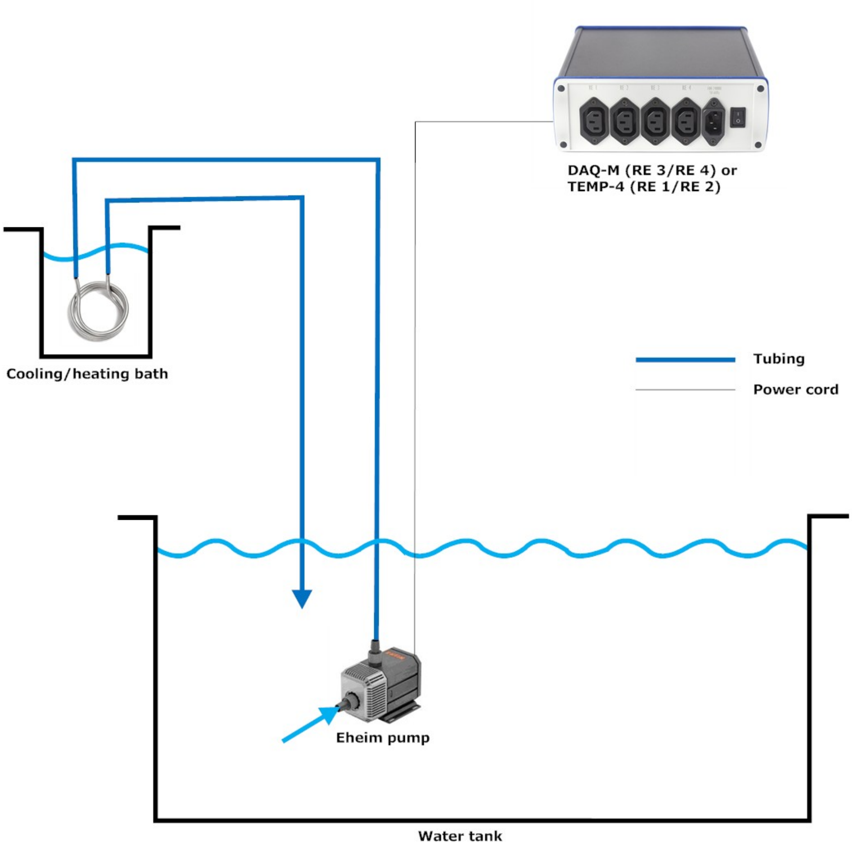
NB! Please note, if changing oxygen units, the ramp function settings should be adjusted accordingly by the user.

9.2 Temperature

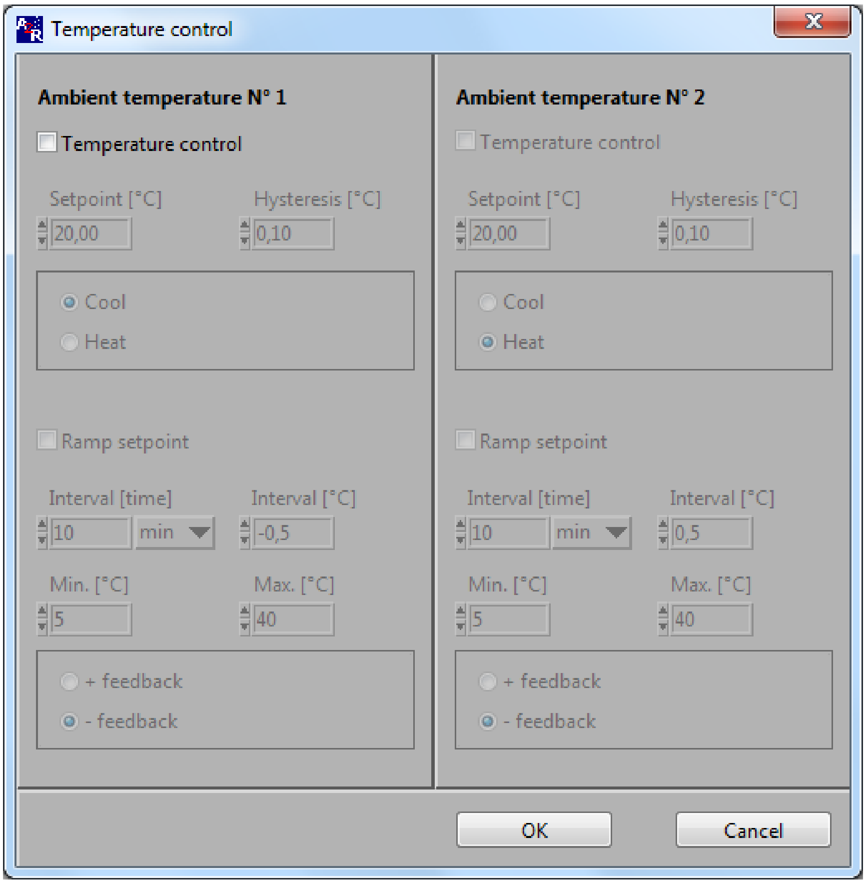
To control the temperature of the ambient water used for flushing and thermostating respirometer chambers, a TMP-SET is needed. This TMP-SET can either be connected to the DAQ-M instrument for software control and monitoring of ambient oxygen levels or an TMP-REG instrument for stand alone operation. The TMP-SET includes:

- Stainless coil
- Pump
- Tubing

To use the TMP-SET, assemble the system as shown in the figure below. The power cord for the pump is connected to a DAQ-M or TMP-REG relay using a power strip or adapter cable supplied with the system.



For using a TMP-SET with AutoResp™, connect the pump to one of the DAQ-M/NETIO relays as explained above. Now activate ambient temperature N°1 in the software (see below). After the calibration and experiment settings is finished, the ambient temperature control settings can be found under Settings→Temperature control.



Activate temperature control to start controlling the ambient temperature. Now set the setpoint and hysteresis and how the relay has to work. The control can either keep the ambient temperature above the setpoint (Heat) or below the setpoint (Cool). If heating the bath in which the stainless coil is submerged must be filled with heated water. If cooling the bath must be filled with chilled water.

For help using the ramp function, see explanation under ambient oxygen control above.

10. BACKGROUND THEORY

10.1 Measuring metabolic rate

Metabolic rate is one of the most important physiological variables for comparing performance and adaptations of different organisms, and it is a measure of the total energy consumption over time. However, it is only possible to measure the amount of energy consumed for metabolism by one of four indirect methods:

1. Energy contents of food minus energy content of waste products
2. Oxygen consumed or CO_2 excreted (respirometry)
3. Heat production (calorimetry)
4. Metabolic water produced (isotopic techniques)

Of these oxygen consumption (MO_2) is the easiest to measure in water, and MO_2 is closely linked to the heat production no matter what organic substrates are burned (in average 20 kJ per litre O_2).
Anaerobic metabolism cannot be determined through MO_2 measurements, causing misleading results in some organisms.

10.2 What is oxygen used for?

All organisms use energy at a rate that can be determined and expressed in different ways. The energy is used for maintaining homeostasis, and for vital and energy consuming processes like osmo regulation, acid-base balance, protein synthesis etc.

The metabolism of higher animal cells (eukaryotes) is primarily based on aerobic respiration requiring oxygen in order to generate energy (ATP).

Simplified reaction:

$C_6H_{12}O_6\ (aq) + 6O_2\ (g) \rightarrow 6CO_2\ (g) + 6H_2O\ (l)$

$\Delta G = -2880$ kJ per mole of $C_6H_{12}O_6$

Oxygen is used in the reaction, thus measured oxygen consumption rate of an organism mirrors the aerobic metabolism.

Oxygen molecules are used as the “terminal electron acceptor” in the respiratory chain during oxidation of organic molecules. The product of this process is energy in the form of ATP (Adenosine Triphosphate), by substrate-level phosphorylation, NADH and FADH2.

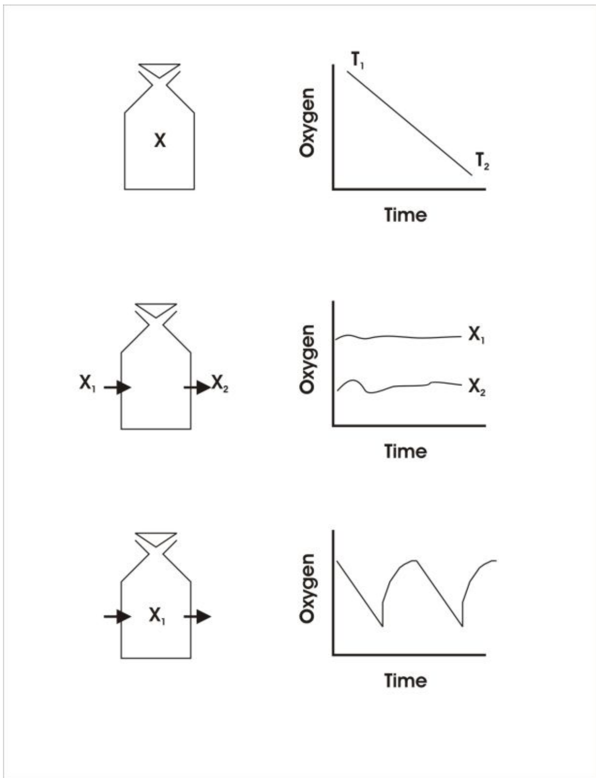
10.3 Intermittent respirometry

The measuring principle behind **AutoResp™** is so-called intermittent respirometry, one of three widespread methods for oxygen consumption measurements (respirometry) in aquatic breathers:

1. Closed respirometry (or constant volume respirometry)

2. Flow-through respirometry (or open respirometry)

3. Intermittent respirometry (or stop-flow respirometry)



10.3.1. Closed respirometry

Measurements in a sealed chamber of known volume, ie. a closed respirometer. The oxygen content of the water is measured initially (t0), then the respirometer is closed and at the end of the experiment (t1) the oxygen content is measured again.

Knowing the body weight of the animal, the respirometer volume and the oxygen content of the water at time t0 and t1 the mass specific oxygen consumption rate can be calculated as follows:

$$VO_2 = \left([O_2]_{t_0} - [O_2]_{t1}\right)\cdot\frac{V}{t}\cdot\frac{1}{BW}$$

VO_2 = oxygen consumption rate (mg O_2/kg/hour)

$[O_2]t_0$ = oxygen concentration at time t_0 (mg O_2/liter)

$[O_2]t_1$ = oxygen concentration at time t_1 (mg O_2/liter)

V = respirometer volume minus volume of experimental animal (liter)

$t = t_1 - t_0$ (hour)

BW = body weight of experimental animal (kg)

An advantage of this method is its simplicity. A disadvantage is that the measurements are never made at a constant oxygen level, due to the continuous use of oxygen by the animal inside the closed chamber. This might cause problems when interpreting data, since animal respiration often changes with ambient oxygen partial pressure.

Furthermore, metabolites from the experimental animal (e.g. CO_2) accumulate in the water, thus limiting the duration of measurements. The limited time for measurements prevents the experimental animal to recover from initial handling stress that often increase animal respiration significantly and for several hours, thus overestimating oxygen consumption rates.

10.3.2. *Flow-through respirometry*

This is a more sophisticated method for oxygen consumption measurements. The experimental animal is placed in a flow-through chamber, with a known flow rate. Oxygen content is measured in both the inflowing and outflowing water, and oxygen consumption rate can then be calculated as:

$$VO_2 = \left([O_2]_{in} - [O_2]_{out}\right) \cdot \frac{F}{BW}$$

VO_2 = oxygen consumption rate (mg O_2/kg/hour)

F = water flow rate (l/hour)

$[O_2]$in = oxygen content in water inflow (mg O_2/liter)

$[O_2]$out = oxygen content in water outflow (mg O_2/liter)

BW = body weight of experimental animal (kg)

The advantages of this method are several:

1) the duration of the experiment is in principle unlimited

2) no accumulation of CO2 and other metabolites

3) its possible to measure at a constant oxygen level

4) by controlling the quality of the inflowing water its possible to measure metabolism at different desired levels of oxygen, salinity etc.

However, this method bring along one significant disadvantage: in order to determine oxygen consumption by open respirometry it is crucial that the system is in steady state. This means that the oxygen content of the in flowing and out flowing water, AND the oxygen consumption of the animal have to be constant.

If the oxygen consumption of the animal for some reason changes during the experiment, steady state will not exist for a while. Not until the system is in steady state again will the above formula give the correct oxygen consumption rate. The duration of the time lag depends on the relationship between chamber volume, wash-out and flow rate. Thus, open respirometry measurements have poor time resolution and are not suitable for determination of oxygen consumption on organisms with a highly variable respiration, e.g. like fish that can triple their respiration rate or more within a few minutes.

10.3.3. *Intermittent respirometry*

AutoResp™ is based on the principle of intermittent respirometry aiming at combining the best of both of the above methods 1) closed and 2) flow-through respirometry.

The experimental animal is placed in a sealed chamber with ports for recirculating the (closed) volume of water inside the chamber during measurements to avoid gradients (mixing) and to maintain adequate flow past oxygen probes with self-consumption of oxygen.

A second set of ports are used to flush the water inside the respirometer chamber intermittently with water from the ambient tank (temperature bath) in which the chamber is submerged to avoid severe oxygen depletion inside the chamber. Hence the name *intermittent respirometry*.

A computer actuated (flush) pump connected to the latter ports is turned on and off intermittently to flush the chamber, When the flush pump is turned off the systems operates like closed respirometry.

When the pc controlled flush pump turns on, it pumps ambient water into the respirometer chamber thus bringing the oxygen content to the level of the surrounding ambient water. In this way, problems with accumulating metabolites and severe changes in oxygen level due to animal respiration are avoided.

As with open (or flow-through) respirometry, the duration of the experiment is in principle unlimited.

However, the most important advantage is the great time resolution of this method. Oxygen consumption rates of animals can be determined for every 5-10 minutes over periods of hours or days, making automated systems extremely well suited for uncovering short and long term variations in respiration.

In summary, AutoResp™ systems for automated oxygen consumption measurements have been developed for prolonged respirometry experiments in a controlled laboratory environment.

To summarize, the automated measuring procedure runs in three phases:

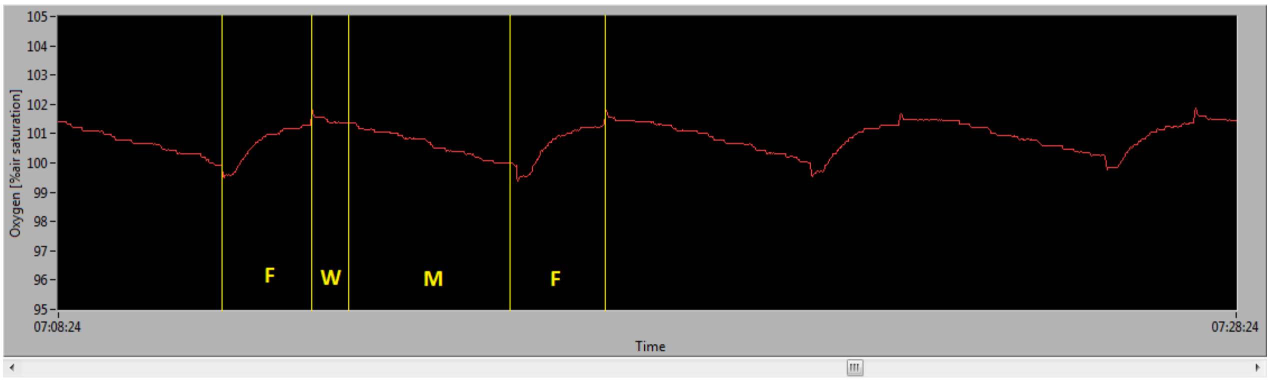
1. Measuring period (M)

2. Flush period (F)

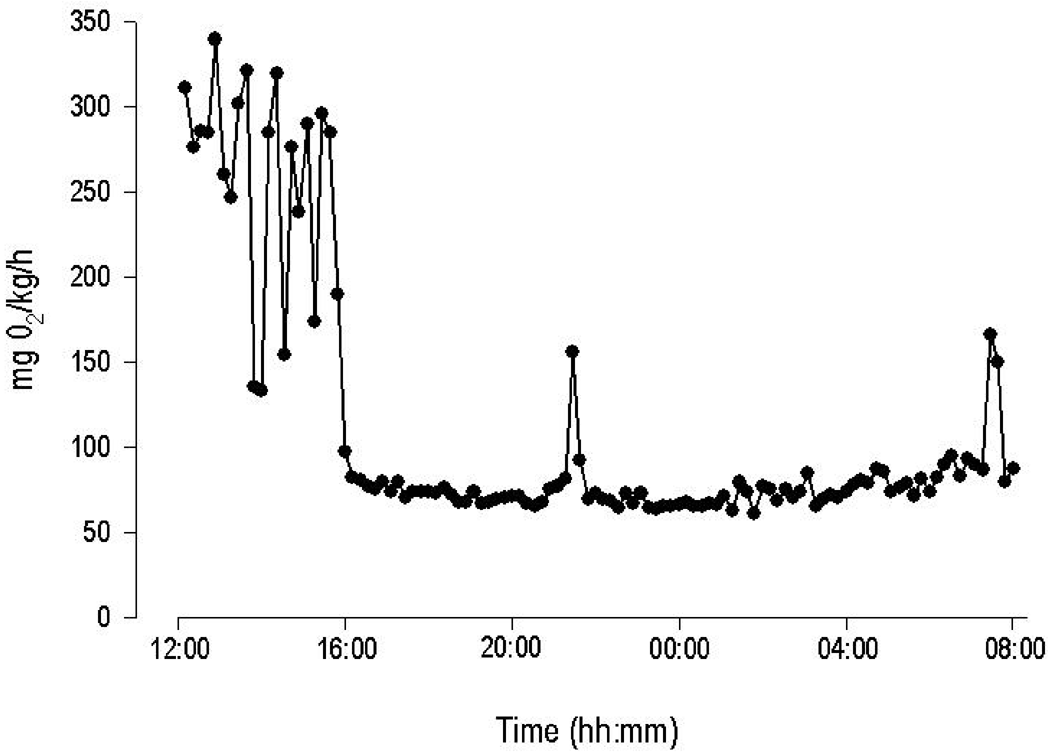
3. Wait period (W)

In the Measuring period (M) the flush pump is off, and the chamber is closed. Fish respiration rate is calculated from the decline in oxygen. During this time the recirculation pump is active to mix the water inside the respirometer and to ensure proper flow past the oxygen sensor.

The measuring period is followed by a Flush period (F) where the flush pump is active pumping water from the ambient temperature bath and into the respirometer. During this period the recirculation pump is inactive and the oxygen curve will raise to approach the level of the ambient water.



Finally, the flush pump stops and the loop ends with a short Wait period (W) before starting a new measuring period. This waiting period is necessary to account for a lag in the system response resulting in a non-linear oxygen curve. During the Wait period the recirculation pump is active.



Examples of raw MO_2 data can be seen from the above diagram. Standard metabolic rate of juvenile Rainbow Trout was determined in a static respirometer chamber and with an automated respirometry system from Loligo™ Systems during approximately 24 hours.

Initial high oxygen consumption rates due to handling stress, were followed by a gradual decline to lower and more stable values indicating standard metabolic rate for the specimen. Notice the high temporal resolution (10 min) of the system revealing sudden changes in MO_2.

10.4 Dissolved oxygen

The oxygen capacitance coefficient ß in water is only 1/30 of that in atmospheric air, depending on barometric pressure. The concentration of oxygen in air-equilibrated water depends on water temperature and salinity. Even more importantly, the diffusion velocities of oxygen molecules are 10.000 times lower than in air. Thus oxygen is scarce and fluctuates due to environmental changes in pressure, temperature and salinity.

$$[O_2] = pO_2 \cdot \beta$$

$[O_2]$ = concentration of oxygen in water (mgO_2/l)

pO_2 = partial pressure of oxygen in water (kPa)

β = capacitance coefficient of oxygen in water (mg O_2/l/kPa)

Atmospheric air contains c.21% oxygen, e.g. 210 mlO_2/l and in general terrestrial animals is rarely challenged by any significant changes in oxygen availability.

In comparison one litre of "saturated" water in equilibrium with atmospheric air, contains only c.10 mlO_2/l, and oxygen solubility will decrease with increasing water temperature and salinity and *visa versa*.

Thus, many aquatic organisms experiences significant pertubations in oxygen levels in their natural environment, and not only due to anthropogenic effects (eutrofication). In environments with high biological activity, algae and plants can add high amounts of oxygen to the water during daytime photosynthesis, whereas oxygen in the water is consumed during night by the respiration of the same organisms and bacteria in some case causing severe hypoxia.

<u>Formulas</u>

$$[O_2] = PwO_2 \cdot \beta$$

Where

PwO2 = oxygen partial pressure in water (kPa)

ß = oxygen solubility in water ($mgO_2/l/kPa$)

See appendix for ß -values in relation to temperature and salinity.

From barometric pressure (BP) and vapor pressure (pH_2O) the partial pressure of oxygen in fully saturated water can be calculated as:

$$pO_2 = (BP - pH_2O) \cdot 0{,}2094$$

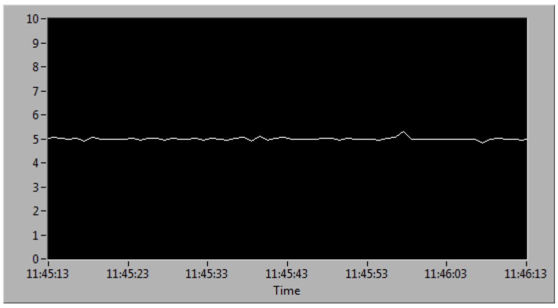
where 0,2094 is the fraction of oxygen in the atmosphere at sea level.

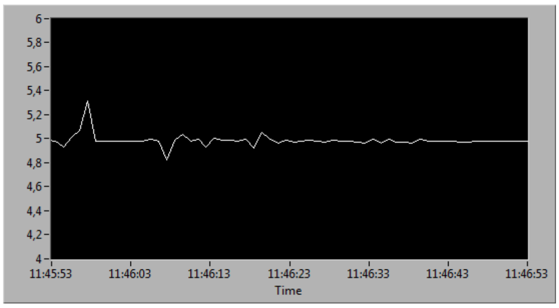
See appendix for pO_2- and pH_2O-values in relation to barometric pressure and temperature

11. TROUBLESHOOTING

11.1 Change Y-scale units

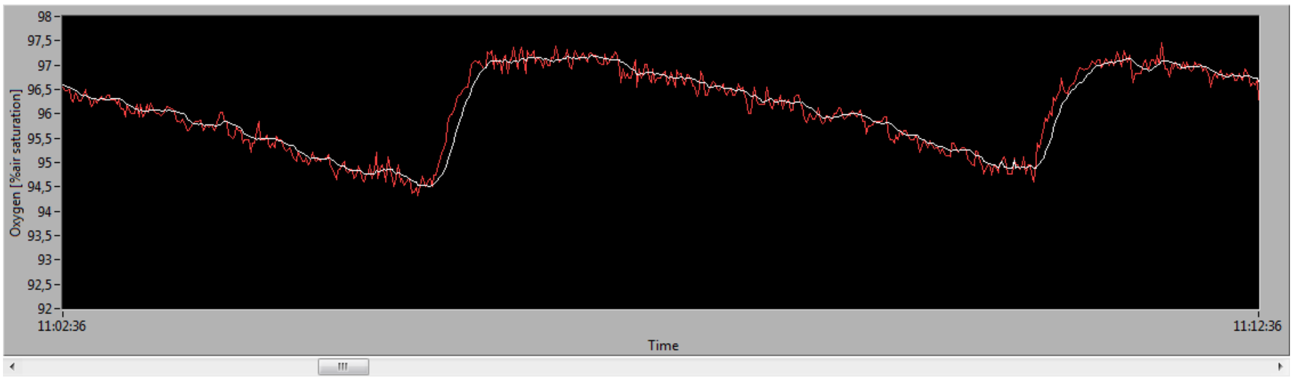
To change the scaling of graph y-axes, double click the upper or lower value of the Y-scale and type a new value using the keyboard numerics.





11.2 Noise

If a noise problem on input signals exist, users can choose a moving average function to smoothen signals.

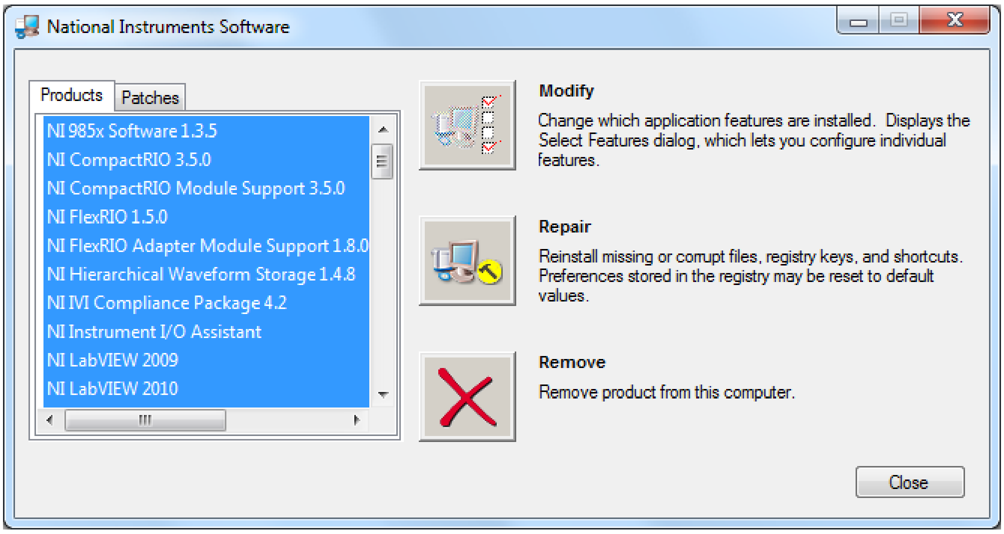


Example. Graph showing raw sensor signal (red curve) and the same data with a moving average of 10 points (white curve).

11.3 Previous versions

It is important to remove any previous versions before installing the new AutoResp™ software:

9. Click Start→Control Panel
10. Open Programs and Features
11. Double click on National Instruments software
12. Select all packages, and then click on Remove.



13. You will then be notified that AutoResp™ also will be removed. Click Yes
14. Now wait until all packages are uninstalled. This might take some time.
15. Windows will now ask for a restart.
16. When the computer is restarted, proceed to install the new AutoResp™ software.

11.4 2-point calibration

If using an oxygen meter with an analog output connected to a data acquisition instrument (e.g. DAQ-M/-S/-1/-4), start by calibrating the sensor against two known standard solutions. This is often done by equilibrating a stirred water sample with atmospheric air to reach 100% air saturation. Use an air pump to bubble the water sample and allow enough time for full equilibration. Then place your oxygen sensor in the sample and wait for the sensor signal to stabilize. Then follow the operating instructions for the meter to set the measured (HI) value to 100%. Repeat this procedure for a zero oxygen solution, setting the measured (LO) value to 0%. Bubble with nitrogen gas or use a sodium sulphite solution to remove all oxygen from the sample.

Now the oxygen meter will convert the oxygen sensor signal into % air saturation and the correct values is displayed on the screen. Alternatively the engineering unit could be changed to mmHg or kPa.

If the oxygen meter has an analog output, this can be connected to an A/D (analog-to-digital) instrument, e.g. DAQ-M/-S/-1/-4 instrument, for PC data acquisition. A typical analog output signal is a 0-5V DC voltage over the entire measuring range, e.g. when the oxygen meter screen reads 0% oxygen saturation the analog output is 0 VDC, and when the meter reads 100% the analog output is approximately 2.5 VDC if the measuring range is 0-200% air saturation.

To convert the 0-5VDC signal into engineering units (e.g. % air sat.) in AutoResp™, a second two-point calibration is necessary, e.g. to convert the output signal from the instrument into % air saturation or another unit of measure.

Place your sensor into a low solution, wait until the voltage stabilizes, then click LOCK LO and read the value on the oxygen meter and write the value in the corresponding LOCK LO oxygen field. Now the voltage is calibrated against an oxygen saturation.

Do the same for a high solution, but press LOCK HI.

When done, the output from the instrument is calibrated in AutoResp™.

11.5 Run AutoResp™ always as admin

1. Right click on the AutoResp™ icon.
2. Choose properties.
3. Go to compatibility.
4. Enable "Run this program as an administrator"
5. Click OK.
6. Now open AutoResp™ by double clicking the icon.
7. Choose Yes, to confirm that you want to start the software as admin.

Next time you want to open AutoResp™ as admin only do step 6 and 7.

12. INDEX

13. APPENDIX

Table 1. Table of oxygen saturation in water

Temperature (deg C)	Air saturated water (ppm or $mgO_2\ l^{-1}$)	Atmospheric pressure (mmHg)
10	11.3	157.3
11	11.1	157.1
12	10.8	156.9
13	10.6	156.7
14	10.4	156.5
15	10.2	156.3
16	10.0	156.0
17	9.7	155.8
18	9.5	155.6
19	9.4	155.4
20	9.2	155.2
21	9.0	154.9
22	8.8	154.7
23	8.7	154.4
24	8.5	154.1
25	8.4	153.8
26	8.2	153.5
27	8.1	153.2
28	7.9	152.8
29	7.8	152.5
30	7.6	152.2
35	7.1	150.0
40	6.6	148.0
45	6.1	145.5

Table 2. Partial pressure of oxygen (pO2) at different barometric pressures and temperatures

Partial pressure of oxygen (pO2) at different barometric pressures and temperatures = ((Pbp-Pvap)*.2094)																
Temperature (deg C)	0	2	4	6	8	10	12	14	16	18	20	25	30	35	37	40
Pvap (kPa)	0,61	0,71	0,81	0,93	1,07	1,23	1,40	1,60	1,82	2,06	2,34	3,17	4,24	5,62	6,28	7,38
Pbp (kPa)																
97,32	20,256	20,237	20,215	20,191	20,163	20,132	20,097	20,058	20,013	19,964	19,909	19,742	19,526	19,248	19,117	18,895
97,59	20,312	20,292	20,271	20,246	20,218	20,187	20,152	20,113	20,068	20,019	19,963	19,796	19,579	19,301	19,169	18,947
97,85	20,367	20,348	20,326	20,302	20,274	20,242	20,207	20,167	20,123	20,073	20,018	19,850	19,633	19,353	19,221	18,999
98,12	20,423	20,403	20,382	20,357	20,329	20,298	20,262	20,222	20,178	20,128	20,072	19,904	19,686	19,406	19,274	19,050
98,39	20,478	20,459	20,437	20,412	20,384	20,353	20,317	20,277	20,233	20,183	20,127	19,958	19,739	19,459	19,326	19,102
98,65	20,534	20,514	20,492	20,467	20,439	20,408	20,372	20,332	20,288	20,237	20,182	20,012	19,793	19,512	19,378	19,154
98,92	20,589	20,570	20,548	20,523	20,495	20,463	20,427	20,387	20,342	20,292	20,236	20,066	19,846	19,564	19,431	19,206
99,19	20,645	20,625	20,603	20,578	20,550	20,518	20,482	20,442	20,397	20,347	20,291	20,121	19,900	19,617	19,483	19,257
99,45	20,700	20,681	20,658	20,633	20,605	20,573	20,537	20,497	20,452	20,402	20,345	20,175	19,953	19,670	19,536	19,309
99,72	20,756	20,736	20,714	20,689	20,660	20,628	20,593	20,552	20,507	20,456	20,400	20,229	20,007	19,722	19,588	19,361
99,99	20,811	20,791	20,769	20,744	20,716	20,684	20,648	20,607	20,562	20,511	20,454	20,283	20,060	19,775	19,640	19,413
100,25	20,866	20,847	20,825	20,799	20,771	20,739	20,703	20,662	20,617	20,566	20,509	20,337	20,114	19,828	19,693	19,464
100,52	20,922	20,902	20,880	20,855	20,826	20,794	20,758	20,717	20,671	20,620	20,563	20,391	20,167	19,881	19,745	19,516
100,79	20,977	20,958	20,935	20,910	20,881	20,849	20,813	20,772	20,726	20,675	20,618	20,445	20,221	19,933	19,797	19,568
101,05	21,033	21,013	20,991	20,965	20,937	20,904	20,868	20,827	20,781	20,730	20,672	20,499	20,274	19,986	19,850	19,620
101,32	21,088	21,069	21,046	21,021	20,992	20,959	20,923	20,882	20,836	20,784	20,727	20,553	20,328	20,039	19,902	19,672
101,59	21,144	21,124	21,102	21,076	21,047	21,015	20,978	20,937	20,891	20,839	20,782	20,607	20,381	20,092	19,955	19,723
101,85	21,199	21,180	21,157	21,131	21,102	21,070	21,033	20,992	20,946	20,894	20,836	20,661	20,435	20,144	20,007	19,775
102,12	21,255	21,235	21,212	21,187	21,158	21,125	21,088	21,047	21,000	20,948	20,891	20,715	20,488	20,197	20,059	19,827
102,39	21,310	21,290	21,268	21,242	21,213	21,180	21,143	21,102	21,055	21,003	20,945	20,770	20,542	20,250	20,112	19,879
102,65	21,366	21,346	21,323	21,297	21,268	21,235	21,198	21,157	21,110	21,058	21,000	20,824	20,595	20,303	20,164	19,930
102,92	21,421	21,401	21,378	21,353	21,323	21,290	21,253	21,212	21,165	21,113	21,054	20,878	20,649	20,355	20,216	19,982
103,19	21,477	21,457	21,434	21,408	21,379	21,346	21,308	21,267	21,220	21,167	21,109	20,932	20,702	20,408	20,269	20,034
103,45	21,532	21,512	21,489	21,463	21,434	21,401	21,363	21,321	21,275	21,222	21,163	20,986	20,756	20,461	20,321	20,086

Table 3. Oxygen solubility in mg O2/liter/kPa at different temperatures and salinities

Oxygen solubility in mg O2/liter/kPa at different temperatures and salinities								From Green & Carrit (1967). J. Mar. Biol. 25; 140-147.					1 kPa = 7,501 mmHg						
Salinity (o/oo)	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Temperature (deg C)																			
0	0,6976	0,6878	0,6781	0,6685	0,6591	0,6498	0,6406	0,6316	0,6227	0,6139	0,6053	0,5967	0,5883	0,5800	0,5719	0,5638	0,5559	0,5480	0,5403
1	0,6788	0,6694	0,6600	0,6509	0,6418	0,6329	0,6241	0,6154	0,6068	0,5984	0,5900	0,5818	0,5737	0,5657	0,5579	0,5501	0,5424	0,5349	0,5274
2	0,6608	0,6517	0,6428	0,6339	0,6252	0,6166	0,6082	0,5998	0,5916	0,5834	0,5754	0,5675	0,5597	0,5520	0,5444	0,5369	0,5295	0,5223	0,5151
3	0,6436	0,6349	0,6263	0,6178	0,6094	0,6011	0,5929	0,5849	0,5769	0,5691	0,5614	0,5537	0,5462	0,5388	0,5315	0,5243	0,5171	0,5101	0,5032
4	0,6272	0,6188	0,6104	0,6022	0,5942	0,5862	0,5783	0,5706	0,5629	0,5553	0,5479	0,5405	0,5333	0,5261	0,5190	0,5121	0,5052	0,4984	0,4917
5	0,6114	0,6033	0,5953	0,5874	0,5796	0,5719	0,5643	0,5568	0,5494	0,5421	0,5349	0,5278	0,5208	0,5139	0,5071	0,5004	0,4937	0,4872	0,4807
6	0,5963	0,5885	0,5808	0,5731	0,5656	0,5582	0,5508	0,5436	0,5365	0,5294	0,5225	0,5156	0,5089	0,5022	0,4956	0,4891	0,4827	0,4763	0,4701
7	0,5818	0,5743	0,5668	0,5595	0,5522	0,5450	0,5379	0,5310	0,5241	0,5172	0,5105	0,5039	0,4974	0,4909	0,4845	0,4782	0,4720	0,4659	0,4598
8	0,5680	0,5607	0,5535	0,5463	0,5393	0,5324	0,5255	0,5188	0,5121	0,5055	0,4990	0,4926	0,4863	0,4800	0,4739	0,4678	0,4618	0,4558	0,4500
9	0,5547	0,5476	0,5406	0,5338	0,5270	0,5203	0,5136	0,5071	0,5006	0,4943	0,4880	0,4818	0,4756	0,4696	0,4636	0,4577	0,4519	0,4461	0,4405
10	0,5419	0,5351	0,5283	0,5217	0,5151	0,5086	0,5022	0,4959	0,4896	0,4834	0,4774	0,4713	0,4654	0,4595	0,4537	0,4480	0,4424	0,4368	0,4313
11	0,5297	0,5231	0,5165	0,5101	0,5037	0,4974	0,4912	0,4851	0,4790	0,4730	0,4671	0,4613	0,4555	0,4498	0,4442	0,4387	0,4332	0,4278	0,4224
12	0,5179	0,5115	0,5052	0,4989	0,4928	0,4867	0,4806	0,4747	0,4688	0,4630	0,4573	0,4516	0,4460	0,4405	0,4351	0,4297	0,4244	0,4191	0,4139
13	0,5067	0,5005	0,4943	0,4882	0,4823	0,4763	0,4705	0,4647	0,4590	0,4534	0,4478	0,4423	0,4369	0,4315	0,4262	0,4210	0,4158	0,4107	0,4057
14	0,4959	0,4898	0,4839	0,4780	0,4721	0,4664	0,4607	0,4551	0,4496	0,4441	0,4387	0,4333	0,4281	0,4229	0,4177	0,4126	0,4076	0,4026	0,3977
15	0,4855	0,4796	0,4738	0,4681	0,4624	0,4568	0,4513	0,4459	0,4405	0,4352	0,4299	0,4247	0,4196	0,4145	0,4095	0,4046	0,3997	0,3948	0,3901
16	0,4755	0,4698	0,4641	0,4586	0,4531	0,4476	0,4423	0,4370	0,4317	0,4266	0,4214	0,4164	0,4114	0,4065	0,4016	0,3968	0,3920	0,3873	0,3827
17	0,4659	0,4603	0,4549	0,4494	0,4441	0,4388	0,4336	0,4284	0,4233	0,4183	0,4133	0,4084	0,4035	0,3987	0,3940	0,3893	0,3846	0,3800	0,3755
18	0,4567	0,4513	0,4459	0,4407	0,4354	0,4303	0,4252	0,4202	0,4152	0,4103	0,4054	0,4006	0,3959	0,3912	0,3866	0,3820	0,3775	0,3730	0,3686
19	0,4478	0,4426	0,4374	0,4322	0,4271	0,4221	0,4171	0,4122	0,4074	0,4026	0,3979	0,3932	0,3886	0,3840	0,3795	0,3750	0,3706	0,3662	0,3619
20	0,4393	0,4342	0,4291	0,4241	0,4191	0,4142	0,4094	0,4046	0,3999	0,3952	0,3906	0,3860	0,3815	0,3770	0,3726	0,3683	0,3640	0,3597	0,3555
21	0,4311	0,4261	0,4212	0,4163	0,4114	0,4066	0,4019	0,3972	0,3926	0,3880	0,3835	0,3791	0,3747	0,3703	0,3660	0,3617	0,3575	0,3534	0,3493
22	0,4233	0,4184	0,4135	0,4087	0,4040	0,3993	0,3947	0,3901	0,3856	0,3812	0,3767	0,3724	0,3681	0,3638	0,3596	0,3554	0,3513	0,3473	0,3432
23	0,4157	0,4109	0,4062	0,4015	0,3969	0,3923	0,3878	0,3833	0,3789	0,3745	0,3702	0,3659	0,3617	0,3575	0,3534	0,3494	0,3453	0,3413	0,3374
24	0,4084	0,4037	0,3991	0,3945	0,3900	0,3855	0,3811	0,3767	0,3724	0,3681	0,3639	0,3597	0,3556	0,3515	0,3475	0,3435	0,3395	0,3356	0,3318
25	0,4014	0,3968	0,3923	0,3878	0,3833	0,3790	0,3746	0,3703	0,3661	0,3619	0,3578	0,3537	0,3497	0,3457	0,3417	0,3378	0,3339	0,3301	0,3263
26	0,3947	0,3902	0,3857	0,3813	0,3770	0,3727	0,3684	0,3642	0,3601	0,3560	0,3519	0,3479	0,3439	0,3400	0,3361	0,3323	0,3285	0,3248	0,3211
27	0,3882	0,3838	0,3794	0,3751	0,3708	0,3666	0,3624	0,3583	0,3542	0,3502	0,3462	0,3423	0,3384	0,3346	0,3308	0,3270	0,3233	0,3196	0,3160
28	0,3819	0,3776	0,3733	0,3691	0,3649	0,3608	0,3567	0,3526	0,3486	0,3447	0,3408	0,3369	0,3331	0,3293	0,3256	0,3219	0,3182	0,3146	0,3110
29	0,3759	0,3717	0,3674	0,3633	0,3592	0,3551	0,3511	0,3471	0,3432	0,3393	0,3355	0,3317	0,3279	0,3242	0,3205	0,3169	0,3133	0,3098	0,3063
30	0,3701	0,3659	0,3618	0,3577	0,3537	0,3497	0,3457	0,3418	0,3380	0,3341	0,3304	0,3266	0,3229	0,3193	0,3157	0,3121	0,3086	0,3051	0,3017