UNISENSE

MICRORESPIRATION SYSTEM USER MANUAL

MicroRespiration System User Manual

Version February 2022

MICRORESPIRATION SYSTEM

UNISENSE A/S

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WARRANTY AND LIABILITY

Microsensors are consumable items. The sensors are tested thoroughly before packaging and shipment.

Warranty

N_2O sensors: 60 days from shipment.
Standard Oxygen sensors: 180 days from shipment.
All other sensors excluding special sensors: 90 days from shipment.
If, within the above specified period, the sensor(s) fail to perform according to the specifications, Unisense will replace the sensor(s) free of charge.

Unisense will only replace dysfunctional sensors if they have been tested according to the instructions in the manual upon receipt of the sensor(s). The warranty does not include repair or replacement necessitated by accident, neglect, misuse, unauthorized repair, or modification of the product.
Physical damage to the tip of the sensor is not covered by the warranty.

The MR2-Stirrer Controller and MR2-Rack is covered by a one year warranty. The warranty does not include repair or replacement necessitated by accident, neglect, misuse, unauthorized repair or modification of the product.

Liability

In no event will Unisense be liable for any direct, indirect, consequential or incidental damages, including lost profits, or for any claim by any third party, arising out of the use, the results of use, or the inability to use this product.

Repair or adjustment

Sensors and electrodes cannot be repaired. Other equipment that is not covered by the warranty will, if possible, be repaired

by Unisense with appropriate charges paid by the customer. In case of return of equipment please contact us for a return authorization. For further information please see the document "General Terms of Sale and Delivery of Unisense A/S".

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If you wish to order additional products or if you encounter any problems and need scientific/technical assistance, please do not hesitate to contact our sales and support team. We will respond to your inquiry within one working day.

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Further documentation and support is available at our website www.unisense.com

If you find errors in this manual or have suggestions to improvements, do not hesitate to contact us at sales@unisense.com

OVERVIEW

The MicroRespiration System performs high precision measurements of oxygen concentrations, or any other analytes, and calculates rates of oxygen consumption (e.g. respiration) or production (e.g. photosynthesis) in small, closed systems. Only one sensor is required for measuring in several micro systems concurrently during one experiment. The system can be used to measure in vials with e.g. small aquatic and terrestrial animals, animal tissue, algae cultures, or fish eggs.

The system is based on specially designed respiration chambers and state of the art microsensors, and meets all standard challenges in microrespirometry. The SensorTrace Rate software keeps track of the oxygen measurements in the different chambers and makes fast consumption and production rate calculations. The system can also be used with other electrodes than the oxygen sensor.

This manual gives an overview of the system and operating instructions. Information about the SensorTrace Rate software is found in the SensorTrace Suite manual. Information on how to prepare and calibrate the different sensors is found in the specific sensor manuals.

The MicroRespiration System typically consists of the following main products:

- MicroRespiration Chambers with lids (MR-Chambers). They come with a nylon or glass ring and net (MetNet) to separate fragile samples from the rotating magnets*
  - Various chamber types and sizes are available
- MicroRespiration sensors and electrodes
- Microsensor amplifier (e.g. Opto-F1/Opto-F4 UniAmp, fx-6 UniAmp, fx-3 UniAmp or O_2 UniAmp)
- MicroRespiration Rack (MR2-Rack)
- MicroRespiration Stirrer controller (MR2-Co) and glass coated magnets (different sizes)
- SensorTrace Rate software for datalogging, sensor calibration and rate calculations
- Relevant cables and power supplies

* Rings and MetNet are not compatible with our 400 µL chambers

Properties of the MicroRespiration System

- Only one sensor is required for measuring in several chambers during one experiment
- Sensor guide and chamber racks prevent sensor tip from breaking
- Micro and mini chambers are available in several standard sizes and custom chamber sizes can be provided
- Chambers are effectively sealed with water or oil. The lid construction prevents diffusion into and out of the chamber
- Stirring system prevents oxygen gradients in the chamber
- For fragile samples, magnet and organisms can be separated*
- Easy sterilization of chambers
- Insignificant analyte consumption by the sensor
- High temporal resolution
- Submersion in water bath enables precise temperature control
- Constant pressure (in equilibrium with the outside pressure)

* Rings and MetNet are not compatible with our 400 µL chambers

Software for the MicroRespiration System

SensorTrace Logger and SensorTrace Rate are part of the SensorTrace Suite software package and can be used to log data from microrespiration experiments.

SensorTrace Logger is used for basic data application and comes as freeware. It offers time series datalogging and calibration features. It supports simultaneous logging from multiple channels.

SensorTrace Rate is optimized for the MicroRespiration System. It is used for measuring consumption and production rates of analytes, typically oxygen, based on sensor measurements made in the MicroRespiration chambers. Basic features of the program are:

- Control of settings for experimental start-ups
- Calibration of sensors and optodes
- It keeps track of measurements obtained in different chambers/samples
- Multiple sensors can be used simultaneously
- Calculations based on linear interpolation and regression result in respiration rate data from several chambers using only one sensor
- Oxygen or other analytes consumption and/or production are followed in real time and online

- Data is continuously saved for later data interpretation

After the experiment, all measurements can be exported into Excel or a CSV file for easy data handling and analysis.

GENERAL USE OF MICRORESPIRATION SYSTEM

General applications for the MicroRespiration System include:

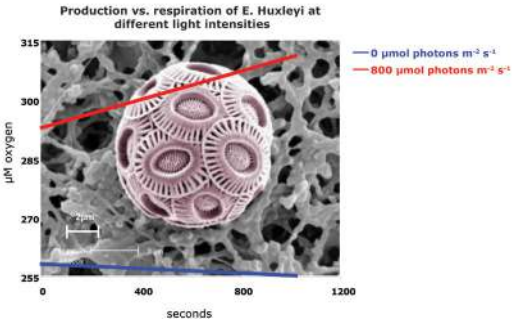
Microrespiration in aqueous or gaseous medium
Measure respiration rates of very small aquatic or terrestrial organisms.

Respiration rates in cultures of bacteria or algae, etc.
The stirring system makes the set-up uniquely suited for measurements in bacteria or algae cultures, where oxygen gradients may occur.

Measurements in fragile samples
The MR-Chambers can be equipped with a metal net to ensure proper stirring while keeping the magnet and the sample separated (not compatible with 400 µL chambers). This facilitates measurements of immobile living organisms vulnerable to the rotating magnet (e.g. eggs, dormant animals, aggregates, tissues, cell cultures etc).

Oxygen production experiments
The MicroRespiration System can be used to measure oxygen production e.g. by an algae culture or photosynthesizing tissues.

Using the MicroRespiration System with other sensors (e.g. enzyme assays)
The MR-Chambers can be used for enzyme assays measuring changes in pH, redox or temperature conditions or by quantifying consumption or production of one of the analytes: H_2, N_2O or H_2S. Please see in the Microsensors section, which sensors are available for use in the MicroRespiration System.



MR-CHAMBERS

The MR-Chambers are specifically designed for measuring respiration of small organisms in either aqueous or gaseous medium using e.g. an oxygen microsensor (OX-MR) or an oxygen microoptode (Opto-MR).

The small and medium MR-Chamber consists of two parts; a chamber and a glass lid with a capillary hole. 40 ml and larger MR-Chambers consist of three parts; a chamber and a lid with a hole for the second lid with the capillary hole.The long and narrow liquid-filled capillary hole effectively prevents diffusion of oxygen into and out of the chamber.

Chamber specification

- Chambers are made of glass and are autoclavable
- Different chamber volumes (small chambers: 400, 500, 750, 1000, 2000, and 4000 µL; medium chambers: 20, 40, 50 mL and large chambers 200 and 400 mL) are available. Customized chambers can be made. Chambers are handmade; therefore the exact volume must be measured for each chamber

- Lid capillary hole dimension is approximately 0.7 mm * 10 mm
- Chambers that fit into the MR2-Rack:
  - 9 mm in diameter chambers: 400, 500, 750 and 1000 µL;
  - 15 mm in diameter chambers: 1 mL, 2 mL and 4 mL
  - 40 mm in diameter chambers: up to 40 ml chamber.
- These chambers can be equipped with a metal net and a glass or nylon ring to ensure proper stirring while keeping the magnet and the sample separated (not compatible with 400 µL chambers).

- Other MR-Chambers available:
  - MR-Ch 0-calibration. With this chamber it is possible to make a 0-calibration regularly without placing the sensor tip directly into ascorbic acid

  - MR-Ch injection: Chamber with injection lid. The lid has an extra capillary hole filled with silicone in the top where it is possible to inject something into the chamber using a syringe with a needle.

- MR-Ch double port: For two sensors (2 x 15 mm in diameter chambers, 2 x 2 mL or 2 x 4 mL). With this chamber it is possible to measure with two different microsensors in the same solution

IMPORTANT

Always pack the glass chamber bottoms and lids separately, both for storage and for transportation. Otherwise, the lid may get stuck and the glass bottom can break

Table 1: MR-Chambers. Custom made chambers are available. All MR-Chambers are handmade and the dimensions are therefore approximate values.

MR-Chamber (mL)	Chamber diameter (mm)	Fits into MR2-Rack	Height w/o lid (mm)	Height w. lid (mm)
Small				
400 µL	9	x	15	24
500 µL	9	x	19	29
750 µL	9	x	23	34
1000 µL	9	x	27	37
2000 µL	9	x	44	52
1 mL	15	x	19	31
2 mL	15	x	27	38
4 mL	15	x	45	55
Medium				
~ 20 mL	42		53	63
~ 40 mL	40	x	45	59
~ 50 mL	42		73	83
Big				
~ 200 mL	60		105	140
~ 400 mL	60		210	245
Other MR-Chambers				
Double chambers				
2 x 2 mL	15	x	28	38
2 x 4 mL	15	x	45	55
0-cal chamber	15	x	45	53
Lids				
MR-lid	9			
MR-lid	15			
Injection lid	15			
0-cal lid	15			

MR-SENSORS

The microsensors used in the MicroRespiration System are built into aluminum sensor guides with adjustable plastic tips. These guides enable the user to move the sensor between samples with the sensor tip retracted. It also controls the insertion depth of the sensor tip into the sample, protecting it from unnecessary exposure and damage. The sensor guide is fixed between the chamber rack and the lid, the plastic tip of the sensor is placed in the hole at the top of the MR-lid, and the sensor tip is inserted through the capillary hole for measurements.

MR-Sensor types

The most common parameter measured in the microrespiration chambers is oxygen using a microoptode or microsensor, however Unisense offers other sensor types compatible with the system as well.

The full sensor portfolio for the MicroRespiration System includes:

- O_2 - oxygen
- H_2 - hydrogen
- H_2S – hydrogen sulfide
- N_2O - nitrous oxide sensor
- NO - nitric oxide
- Rd - redox
- pH
- Temperature

All microsensors have the following advantages:

- Fast response
- Low sensitivity to stirring
- Insignificant consumption of analyte

For details on the various sensor types please see the specific sensor manuals. These can be found on our website www.unisense.com

Using the sensor guide

MR-Sensors have been specifically developed to minimize the risk of breaking, and easily making successive measurements in different chambers.

The MR-Sensor consists of the sensor and the sensor guide - an aluminum sensor shaft and an adjustable pipette tip. When the sensor is completely retracted, the tip will rest about 2 mm inside the shaft of the guide. When the sensor is extended, the tip should protrude no more than 2 mm below the capillary hole in the lid of the chamber. This should be checked and adjusted by lifting or lowering the pipette tip accordingly prior to measurements. When the plastic tip is placed in the correct position, tighten the locking nut.

The plastic tip is used to adjust the extended sensor tip. Two black rubber o-rings are placed at the end of the aluminum shaft. In the outer position, the black rubber o-ring will prevent the sensor from slipping out of the guide. In the inner position, the rubber ring keeps the sensor in place in the retracted position.

Lowering and fixing the sensor

In the guide shaft there is about 1 mm of slack. As it is not possible to make a sensor completely symmetrical, this slack facilitates a slight movement of the sensor in the shaft allowing it to adjust while it is lowered to prevent it from breaking. If resistance is encountered while lowering the sensor, please allow for this adjustment.

Amplifiers

Sensors and electrodes have to be connected to an amplifier.
Following amplifiers can be used for MR-Sensors:

fx-6 UniAmp

O_2 UniAmp

Opto-F1 and Opto-F4 UniAmp

Field Microsensor Multimeter

MR2-RACK AND GLASS COVERED MAGNETS

MR2-Rack

The MR2-Rack holds both the MR-Sensor/s and MR-Chambers so the sensor can easily be placed into the chamber. The MR2-Rack has magnetic stirring controlled by the MR2-Stirrer Controller. How to use the MR2-Rack as a stirrer is described in the MR2-Stirrer Controller section.

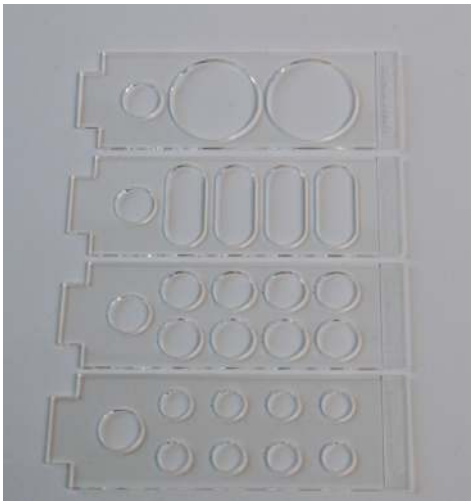
The MR2-Rack holds up to eight chambers (≤ 4 mL) and eight sensors. It comes with up to four different chamber holders:

- 8 x 9 mm chambers (400, 500, 750 and 1000 µL)
- 8 x 15 mm chambers (1 mL, 2 mL, 4 mL)
- 4 x 15 mm double chamber (2 x 2 mL or 2 x 4 mL)
- 2 x 40 mm chambers – only included if needed (custom made up to 40 mL)

All holders also have space for the 0-calibration chamber.

The MR2-Rack is waterproof and can be placed into a water bath.

Avoid water inside the LEMO connection as this can damage the equipment when connected to the MR2-Stirrer Controller.



Specifications for MR2-Rack

- Thermo-stable plastic: PMMA and POM
- Temperature: -20 °C to 100 °C
- Sensor holder: Up to 8 sensors.
- Chamber holders: up to four different holders
- Cable: 1.5 m cable and LEMO connection to MR2–Stirrer Controller
- Dimensions: 146 mm (5,7 in) x 108 mm (4,3 in) x 160 mm (6,3 in) (W x D x H)
- Weight: 680 g (1,8 lb)

Glass covered magnets

For the MicroRespiration System it is recommended to use glass coated magnets and not teflon magnets, as the latter can act as oxygen sinks and introduce artifacts to the measurements. The glass covered magnets fits the following chamber sizes: 9, 15 and 40 mm in diameter. The magnets should be handled with care because of the fragile glass around them.

MR2-STIRRER CONTROLLER

The MR2-Rack for the MicroRespiration System is used to stir the samples to ensure homogeneity and avoid build-up of gradients in the MR-Chambers.

The MR2-Rack is connected to the MR2-Stirrer Controller by a 1.5 m long cable. Each magnet can be controlled individually, and the MR2-Rack can run either 8 small magnets or two large magnets as indicated by the black bars at the bottom of the MR2-Rack.
For measuring in fragile samples, a small glass or nylon ring and a piece of stainless steel metal net (MetNet) is provided with the set-up. This is used to separate the samples from the magnet.

WARNING:
Please use only glass magnets in the MicroRespiration System. Teflon magnets will act as oxygen sinks and destroy measurements

MR2-Stirrer Controller

Operating the MR2-Stirrer Controller

Connect the MR2-Rack to the MR2-Stirrer Controller and power it. From the initial window the MENU can be selected, which will lead directly to the stirrer mode previously used. The first time the MR2-Stirrer Controller is used, selecting MENU will lead to Stirrer Mode. To shift between the four stirrer modes select MODE and use the left/right arrows on the keypad.

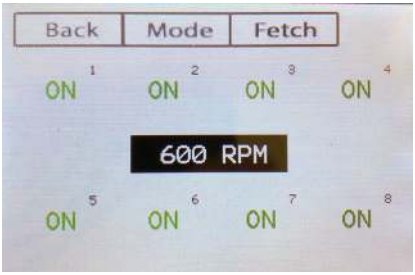
Using the menu system:

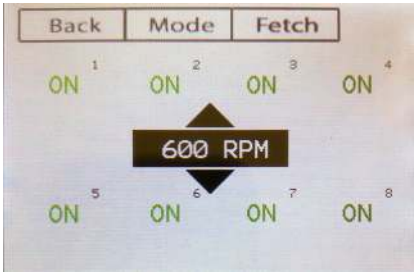
There are three main selections:

- Menu: Opens the control of stirrer speeds and modes (see details below)
- Fetch: Slows stirring down and speeds up gradually over 5 seconds to "catch" the magnets. The range for the MR2-Stirrer Controller is 200-1600 rpm
- About: Shows the versions of the system

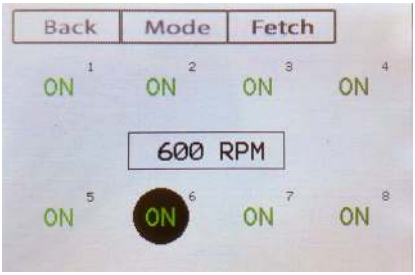
Stirrer modes:
To change stirrer mode and stirrer speed select MENU using the arrow keys and press ENTER. To change between stirrer modes, highlight MODE in the menu bar and press ENTER. Use the left and right arrow keys to shift between the four modes. Press ENTER to select the mode currently displayed.

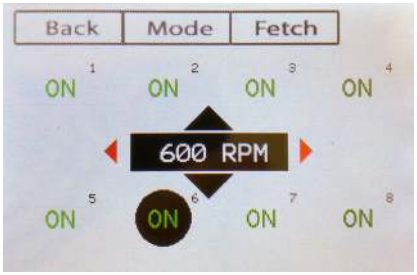
Stirrer mode 1: There are 8 stirrers available and all have the same stirring speed. These are marked with the small circles on the base plate and correspond to the sensor positions 1 – 8 on the top plate of the stirrer rack.
Change stirrer speed: Select the current stirring speed in the center of the display with the down arrow. Press ENTER and change the stirring speed with up and down arrows. Press ENTER to confirm.



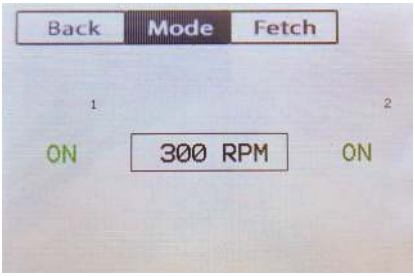


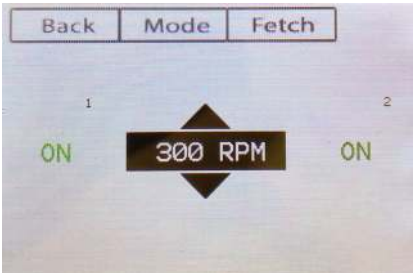
Stirrer mode 2: There are 8 stirrers available. The stirrer speed is set independently for each stirrer spot which may also be turned on or off individually.
Change stirrer speed: Select a specific stirrer spot with the arrow keys and press ENTER.



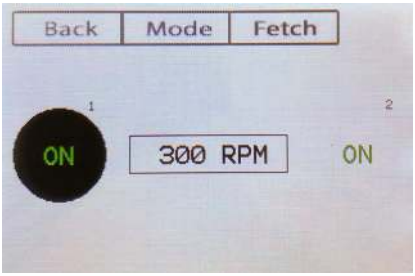


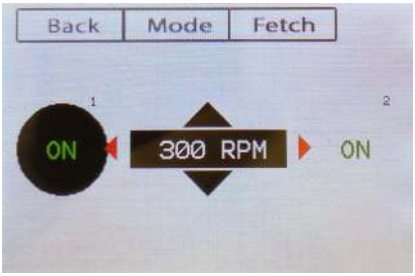
Stirrer Mode 3: There are 2 large stirrer zones available and both have the same stirring speed. The zones are marked by the two large circles on the base plate corresponding to the sensor positions marked A and B on the top plate of the stirrer rack.
Change stirrer speed: Select the current stirring speed in the center of the display with the down arrow. Press ENTER and change the stirring speed with up and down arrows. Press ENTER to confirm.





Stirrer Mode 4: There are 2 large stirrer zones available. The stirrer speed is set independently for each stirrer spot which may also be turned on or off individually.
Change stirrer speed: Select a specific stirrer spot with the arrow keys and press ENTER. Change the stirrer speed using the up/down arrow keys and turn the stirrer on and off using the left/right arrow keys.





Specifications for the MR2-Stirrer Controller

- Mains: 90-264 VAC, 47-63 Hz
- Working temperature: 0°C to 50°C
- Dimensions: 11 cm (4.3 in) x 12.5 cm (4.9 in) x 5 cm (2.0 in) (W x D x H)
- Weight: 674 g (1.48 lbs)

SENSORTRACE RATE SOFTWARE

SensorTrace Rate is a program that can calculate consumption and production rates of analytes, typically oxygen, using the MicroRespiration System. Basic features of the program are:

- Control of settings for experimental start-ups
- It keeps track of measurements obtained in different chambers/samples
- Multiple sensors can be used simultaneously
- Calculations based on linear interpolation and regression results in respiration rate data from several chambers using only one sensor
- Oxygen or other analytes consumption and/or production are followed in real time and online
- Data is continuously saved for later data interpretation
- After the experiment, all measurements can be exported into Excel or a CSV file for easy additional data handling and analysis

The detailed description of the software and how to use it to calibrate a microsensor and make rate experiments can be found in the SensorTrace Suite manual.

Minimum system requirements

- 2 GHz PC
- Windows 7 or newer
- 200 MB free hard disk space
- Ports to connect Unisense instruments
- 4 GB RAM
- Screen resolution 1280 x 800
- Unisense amplifier: e.g. Opto-F1/Opto-F4 UniAmp, fx-6 UniAmp, fx-3 UniAmp or O_2 UniAmp

SETTING UP THE SYSTEM

Successful respiration measurements using the MicroRespiration System are dependent on a correct set-up and proper sealing of the chamber.
The chamber should always be set up in an MR2-Rack allowing for controlled insertion of the MR-Sensor and should always be used under well-defined and stable experimental temperatures, preferably a water bath.

Preparing the samples

1. Always make sure that the chamber and all additional elements are clean. The presence of bacteria/contaminants can seriously affect the measurements. NB! Do not use ethanol on the rack. Please see the Storage and Maintenance section for details.
2. If stirring is required, place the glass covered magnet in the chamber
3. If measuring in a fragile sample, place the glass ring and the MetNet in the chamber (not compatible with 400 µL chambers). See also the Fragile sample section
4. Weigh the chamber, stirring equipment and lid
5. Add the sample to the chamber, making sure that it is completely filled and that no air bubbles get trapped (focus particularly on the MetNet). Close the chamber with the MR-lid. Surplus water will spill through the capillary hole.
6. Weigh the chamber again and calculate the exact volume of the chamber. Enter this value in the software

7. Place the chamber in the rack, making sure all chambers are completely submerged in the water. If measuring in air make sure the chambers are sealed with 0.5% agar, see below.
8. Repeat for all samples

WARNING

If the chambers are cleaned with ethanol or HCl, please be aware that these are potential hazardous agents to your sample

NOTE:

Depending on the type of sample, it may be an advantage to partly fill the chamber before adding the glass ring, magnet, and MetNet. This makes it easier to avoid bubbles forming underneath the net. The chamber is then filled up completely before the lid is put in place.

Fragile sample

If you are measuring in a sample that may be at risk of being damaged by the magnet, such as an egg or small animal, the MetNet is used (not compatible with 400 µL chambers). The net is placed and removed with tweezers.

Note that due to the unavoidable uncertainty of sensor and guide tip length, the tip of the sensor may protrude further than 2 mm below the hole in the lid. Depending on the sample type, this can interfere with the sample if the glass or nylon ring together with the MetNet and magnet are used. If possible, Unisense recommends that larger chambers (min. 750 µL) are used for such samples. Alternatively, please check how far the sensor tip protrudes into the chamber and adjust the plastic tip before adding the glass or nylon ring and the MetNet.

Leakage Test

1. Fill the chamber with sterilized oxygen sub-saturated water, preferably at a concentration of oxygen in the interval 0-50 % of atmospheric saturation (by flushing with N2)
2. Close the chamber
3. Position the measuring OX-MR or OP-MR sensor in the middle of the chamber and leave it there

4. Follow the sensor signal and the temperature for several hours/ over night

Sealing the chambers

Proper sealing of the chambers is extremely important. If the chamber is exposed to air, capillary forces will pull up water. If measuring in a water bath, make sure that the rack and all chambers are completely submerged in it. When measuring in the gas phase, seal the lids by adding 0.5 % sterile agar solution into the capillary tube, without air bubbles, using a syringe with a small needle (Verboven et al 2001). Close the chamber with the agar filled lid and place it into a water bath so that the lid is covered with water.

If the chamber is sealed properly, the oxygen tension remain unchanged at a sub-saturated level (between 0 and 100 % saturation, depending on the N_2 flushing time) provided that the temperature is constant. Increasing oxygen tension indicates leakage. Decreasing oxygen tension indicates oxygen consumption by e.g. bacteria or other contaminants.

Use of injection lid

Turn the injection lid upside down and fill the extra capillary hole for injection with the sample using a syringe with a needle. Insert the needle from the bottom of the lid (see picture). When you feel resistance from the silicone don't push the needle further in but fill the hole with the sample. Then close the chamber with the injection lid.

To inject a substance during measurements, use a needle with an outer diameter of 0.4 mm or less (e.g. BD Precisionglide® syringe needle, gauge 30, L 1 inch).
Pierce through the silicone from the top, insert the needle into the chamber and inject the substance.

Please note that the color of the liquid in this photo is just for a clearer demonstration.

Set-up

Prepare a water bath and adjust it to the desired temperature. The water level in the water bath should be high enough to fully cover the chambers including lid, when placed into the MR2-rack, with water.

1. Connect the microsensor to the amplifier and place it near the MR2-Rack
2. Connect the MR2-Rack to the MR2-Stirrer Controller
3. Place the MR2-Rack with the chambers in a thermostated water bath making sure it cannot tip over. As mentioned above, the MR-Chambers should be completely covered with water as this prevents oxygen to diffuse into and out of the chambers. Place a glass covered magnet in each chamber. If possible place a temperature sensor in the water bath near the MR2-Rack.
4. Connect the MR2-Stirrer Controller to the plug and turn it on. Select appropriate mode (for directions, please see the MR2-Stirrer Controller section).
5. Open SensorTrace Rate (or the SensorTrace Logger freeware). Make sure the sensor(s) are recognized by the program in the Settings tab and add number of chambers and their individual volume.
6. Start the experiment

NOTE

In order to avoid noise in the measurements, it may be necessary to ground the set-up by connecting an extra cable to the meter in one end and leaving the other end in the water bath. Use the provided blue cable. (Only for microsensor)

Calibration of oxygen sensor

Both the sensor signal and the respiration rates are affected by temperature. Unisense therefore strongly recommends that the equipment is calibrated under well-defined and stable experimental temperatures e.g. by submerging both experimental samples and calibration samples in a thermostated water bath. As oxygen microsensors respond linearly to changes in oxygen concentrations, a two-point calibration e.g. zero oxygen and full air saturation, is sufficient.

Zero calibration

An anoxic solution can be prepared in one or several ways. Below we describe two methods recommended by Unisense:

1. Prepare a solution of sodium ascorbate and NaOH, both for final concentrations of 0.1 M (2 g sodium ascorbate in 100 ml of 0.1 M NaOH). This zero calibration solution can be stored in a closed container for 1-2 weeks. Expose only the sensor and the plastic tip to the ascorbic acid solution. If ascorbic acid by accident enters the aluminum guide, the guide has to be rinsed carefully with water to avoid ascorbic acid contamination in the sample. See also the Cleaning the sensor between samples section.

2. Vigorous bubbling with oxygen free inert gas (e.g. N_2). It is important to apply vigorous bubbling over a time period (> 5 minutes) sufficient to ensure that all oxygen has been removed. Furthermore, it is important to prevent that the water has any contact with oxygen during bubbling, as oxygen will otherwise be contiuously reintroduced to the water. In practice this means that the headspace above the water must be closed except for a hole slightly larger than the sensor shaft. This effectively prevents ambient air

from entering the vessel. We recommend our calibration chamber (CAL 300) for this.
Place the sensor in the anoxic solution and the point to the software calibration.

The 0-calibration chamber is a 4 ml glass chamber with a glass lid that has a silicone tube attached to it. This tube is sealed in the end with silicone and is thus only open to the environment through the capillary hole in the lid.
Fill the chamber with the ascorbic acid solution or another antioxidant. The chamber must be completely filled and contain no air bubbles. With a long, slender syringe, fill the silicone tube (through the capillary hole in the lid) with clean, sterile water, again ensuring that no air bubbles are present. Place the lid in the chamber and place the chamber in the MR2-Rack in the water bath making sure the lid is submerged completely. After a few hours, all oxygen in the tube is consumed by the oxidant (2+ hours, depending on the antioxidant).

0-calibration chamber

Atmospherically saturated calibration

The 100 % air saturated calibration is done either externally or internally. In the external calibration you can e.g. use our calibration chamber (CAL 300) to obtain the oxygen concentration at air saturation and it is assumed that the calibration temperature and the sample temperature is the same during the entire measuring period. The internal calibration is used when the calibration should reflect temperature changes during incubation. In this case the 100 % air saturated calibration sample is filled into a MR-chamber and placed in the MR2- Rack in the same water bath as the test sample chamber. However, if the temperature is changing significantly (e.g. 5 °C vs. 25 °C), this will create air bubbles in the calibration sample. In this case we recommend that an external calibration camber (e.g. CAL300) is used instead.

On the next page, the preparation of the external and internal 100% air saturation sample is explained.

External calibration

1. Place the CAL300 calibration chamber in a water bath and leave it to reach the same temperature as the samples in the MR2-Rack
2. Activate the pump and check the temperature
3. Lock the retracted position with the o-ring
4. Take off the plastic tip and place the sensor in the calibration chamber. The bottom rubber o-ring should be placed to ensure that the sensor is not lowered too far into the CAL300 calibration chamber

IMPORTANT

If you are using a pH-MR microelectrode, do the first calibration in the protective tube and then carefully move the sensor from this tube into the provided blue guide.

Internal calibration

1. Prepare a sample of sterile water
2. Oxygenate the water and ensure it reaches the same temperature as your other samples (preferably by placing it in the same water bath)
3. Fill a sterile MR-Chamber with the water and seal it with the lid. It is important that this is done at the same temperature as the other samples
4. In the Calibration window of the Rate software, enter the correct values for temperature and salinity of the sample. The program then calculates the saturated concentration
5. Repeat this process regularly during sample incubation

IMPORTANT

If stirring is used in the test samples, it is important to also use stirring in the atm. saturated calibration chamber. This is due to the stirring sensitivity of the sensors, which can cause a small error in the signal (2%)

This way, small temperature changes are accounted for in the calibrations.

Measurements

After calibrating the sensor the measurements can start:

1. Place the MR-Sensor in the lid of the first sample
2. Lower the sensor into the sample
3. Wait for a stable signal and process as described in the SensorTrace Rate section of the SensorTrace Suite manual
4. Between measurements, the sensor tip can be rinsed with clean water, alternatively with alcohol or HCl as described in the Cleaning the sensor between samples section below

Closely monitor the temperature at all time. Even small temperature changes (0.5 °C) will affect the measurements significantly and recalibration is necessary.

Cleaning the sensor between samples

Between measurements, the sensor tip can be rinsed with clean water. Alternatively, it can be cleaned with alcohol (70 %) or HCl (0.1 M). As potential carry-over will harm the sample it is important to rinse the sensor properly with water.

STORAGE & MAINTENANCE

It is important that the chambers and lids are stored dry and separate to prevent them from getting stuck together.

Chambers can be cleaned gently with soap, water and a small brush.

The chamber, lid, MetNet and glass coated magnets can be autoclaved or cleaned/disinfected by submersion in 70 % ethanol and/or 0.1 M HCl for 15-30 min.

The electrode tip is cleaned with ethanol (70 %) or HCl (0.1 M) and subsequently rinsed thoroughly with clean water.
The MR2-Rack must be cleaned with soap and water and dried thoroughly. As it is made of plastic (PMMA and POM), please do not use ethanol for cleaning.

IMPORTANT
Always pack the glass chamber bottoms and lids separately, both for storage and for transport. Otherwise, the lid may get stuck and the glass bottom can break

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Problem	Air bubbles form inside the chamber
Possible cause 1	Leakage
Solution	Repeat the sealing procedure, see leakage test below
Possible cause 2	Extensive oxygen production by algae cultures
Solution	Prepare less dense algae cultures
Possible cause 3	Temperature fluctuations cause a change in the oxygen solubility of the solution
Solution	Repeat the sample preparation making sure that the temperature is kept steady by using a temperature-regulated water bath.
Problem	The glass stopper is stuck
Solution	Moisten the chamber stopper interface and remove the lid gently with tweezers or tongs.
Problem	The oxygen gradient builds up in the chamber
Possible cause	Insufficient stirring conditions
Solution	Turn on the stirring system
Problem	Significant oxygen consumption during a blind test

Possible cause	Contamination by oxygen consuming micro organisms
Solution	Clean/disinfect chamber, stopper and inserts. Use sterilized water/medium
Problem	Sensor signal drift
Possible cause 1	Leakage
Solution	Repeat the sealing procedure, see leakage test section
Possible cause 2	Sensor signal drift
Solution	Calibrate sensor before and after a leakage test
Problem	The sensor tip protrudes too far into the chamber and interferes with the sample or it does not protrude far enough.
Possible cause	The pipette is placed too high or too low on the sensor shaft.
Solution	Adjust the pipette and check the height again.
Problem	"Noisy" measurements
Possible cause 1	Physical vibrations from other appliances on the table are causing movements of the sensor resulting in instability of experimental set-up and disturbances in the measurements.
Solution	Remove all unrelated appliances from surfaces in contact with the experimental set-up and ensure completely stable conditions for the chamber rack and amplifier.

Possible cause 2	Electric noise in the system
Solution	1) Check that the system is properly grounded. Use the blue cable provided. 2) Change the logging frequency in the Rate software. You will need to experiment which settings give a good signal.
Problem	Message "Invalid board number…" appears when Rate is started.
Possible cause	The program does not recognize the analog-to-digital converter.
Solution	Open the program InstaCal which was installed automatically along with Rate. It is located in a program group called Measurement Computing. Open the program and change the board number to 0. Close the program and restart Rate. If you are using the OXY-Meter for other programs find the program InstaCal which was installed automatically along with Rate. It is located in a program group called Measurement Computing. Open the program and check the board number of the A/D-converter unit. Now go to the Advanced Window accessed from the Set-up window when starting Rate. Change the DAQ board number to match.
Problem	You are asked to install the program Instacal
Solution	Click "Always trust..." and OK

If you encounter other problems and need scientific/technical assistance, please contact sales@unisense.com for online support (we will answer you within one workday)

APPENDIX: EQUILIBRIUM O_2 CONCENTRATIONS

Detailed tables are installed with the RATE software and also available on our web page.
At 20 °C and 1 atm.: 1 µmol O_2/l = 0.032 mg O_2/l = 0.024 ml O_2
Table 1. Equilibrium concentrations of oxygen (µmol O_2/litre) at ambient partial pressure of 0.21 atm. in water as a function of temperature and salinity.

‰ /°C	0.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0
0.0	456.6	398.9	352.6	314.9	283.9	257.9	235.9	217.0	200.4
2.0	450.4	393.6	348.1	311.1	280.6	255.0	233.3	214.7	198.3
4.0	444.2	388.5	343.7	307.3	277.3	252.1	230.8	212.4	196.3
6.0	438.1	383.3	339.4	303.6	274.0	249.3	228.3	210.2	194.3
8.0	432.1	378.3	335.1	299.9	270.8	246.5	225.8	207.9	192.3
10.0	426.1	373.3	330.8	296.2	267.6	243.7	223.3	205.7	190.3
12.0	420.3	368.4	326.7	292.6	264.5	240.9	220.9	203.6	188.4
14.0	414.5	363.5	322.5	289.1	261.4	238.2	218.5	201.4	186.5
16.0	408.8	358.7	318.4	285.5	258.3	235.5	216.1	199.3	184.6
18.0	403.2	354.0	314.4	282.1	255.3	232.8	213.7	197.2	182.7
20.0	397.7	349.3	310.4	278.6	252.3	230.2	211.4	195.1	180.8
22.0	392.2	344.7	306.5	275.2	249.3	227.6	209.1	193.0	179.0
24.0	386.8	340.2	302.6	271.9	246.4	225.0	206.8	191.0	177.1
26.0	381.5	335.7	298.7	268.5	243.5	222.5	204.5	189.0	175.3
28.0	376.2	331.2	294.9	265.3	240.6	219.9	202.3	187.0	173.5
30.0	371.0	326.9	291.2	262.0	237.8	217.4	200.1	185.0	171.7
32.0	365.9	322.5	287.5	258.8	235.0	215.0	197.9	183.0	170.0
34.0	3609	3183	2839	2557	2322	2125	1957	1811	1682
36.0	355.9	314.1	280.3	252.5	229.5	210.1	193.6	179.2	166.5
38.0	351.0	309.9	276.7	249.5	226.8	207.7	191.4	177.3	164.8
40.0	346.2	305.8	273.2	246.4	224.1	205.4	189.3	175.4	163.1
42.0	341.4	301.8	269.4	243.4	221.5	203.1	187.3	173.6	161.5

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