

INSTALLING AND RUNNING THE SOFTWARE

- Download the latest version of AutoResp™ v3 from our website: loligosystems.com/downloads. Follow the instructions on screen and then restart the PC.
- Insert the Loligo® license dongle (2a) in a USB port on the PC to unlock the full software. AutoResp™ v3 will run in demo mode, if the license dongle is not inserted.
- Insert the recommended **long-range Bluetooth adapter** (2b) in a USB port on the same PC, and let Windows initialize it. Disable any built-in/other Bluetooth radios on your PC.

SETTING UP HARDWARE

Witrox 1 and 4 oxygen instrument

- Insert the power adapter for the Witrox instrument (4) to a wall outlet and the USB cable to the backside socket (alternatively, power the Witrox directly from a USB port).
- Insert the PT1000 temperature sensor to the socket labelled "Temp" on the front of the Witrox instrument.
- Insert fiber optic cables for oxygen sensor(s) to the SMA ports labelled **1-4** on the front of the Witrox instrument.
- Power on the Witrox by pushing the power button. Pairing mode is enabled, when the connection icon flashes blue. The instrument will time out after 300 seconds of inactivity.
- The Witrox is now ready to connect in AutoResp™ v3.

DAQ-BT swim tunnel controller

Power the DAQ-BT (using its DC adapter and USB power cable) from a wall outlet. Press and hold the power button (5) on the front of the DAQ-BT until the POWER and STATUS LED flash green rapidly. Bluetooth pairing mode is now enabled, and the DAQ-BT is ready to connect in AutoResp™ v3. Alternatively, the DAQ-BT can be powered and controlled via USB directly from your PC.

PowerX4 power strip

Power the PowerX4 from a grounded wall outlet. The PowerX4 is now ready to pair via Bluetooth. For wired computer connection, insert an LAN/ethernet cable into the port on the PowerX4 (6.1) and into an ethernet port on your router (or PC) that is connected to the same network that your PC is also connected to. Choose LAN when scanning for devices (step 7).

Connect devices

In AutoResp™ v3 > Main menu > **Devices** > Select **Scan for new devices**. Each device can be renamed by clicking on its device name. For testing purposes, each relay on a PowerX4 can be switched ON/OFF by clicking on the relay. Return to the main menu, when all connected devices have been found.

CALIBRATION

Calibrate oxygen sensors: Main menu > Calibration > Select a Witrox instrument (8, **red arrow**). Now follow the instructions in the video: [AutoResp™ v3 - How to calibrate your oxygen sensor](#) on our YouTube channel.

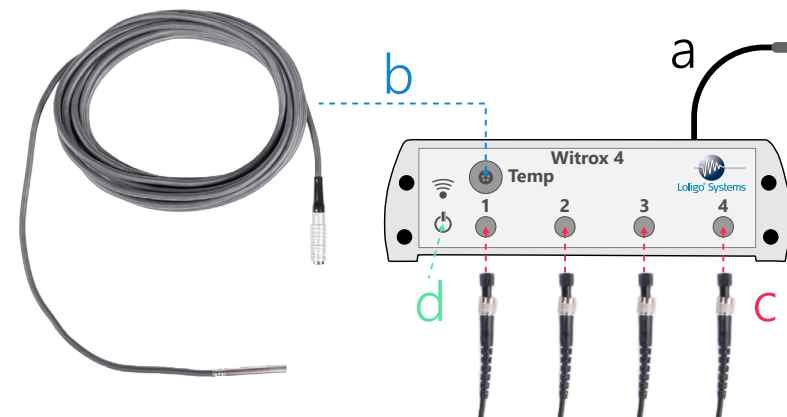
Note that you should calibrate your oxygen sensors in the Loligo® software you intend to use. If you calibrate your oxygen sensors in another Loligo® software, those calibration data will not be transferred into AutoResp™ v3. Use only one Loligo® software at a time.

Calibrate water velocity: Main menu > Calibration > Select a DAQ-BT (8, **blue arrow**). Now follow the instructions in the video: [AutoResp™ v3 - How to calibrate your swim tunnel](#) on our YouTube channel.

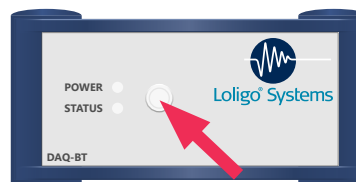
2



4



5



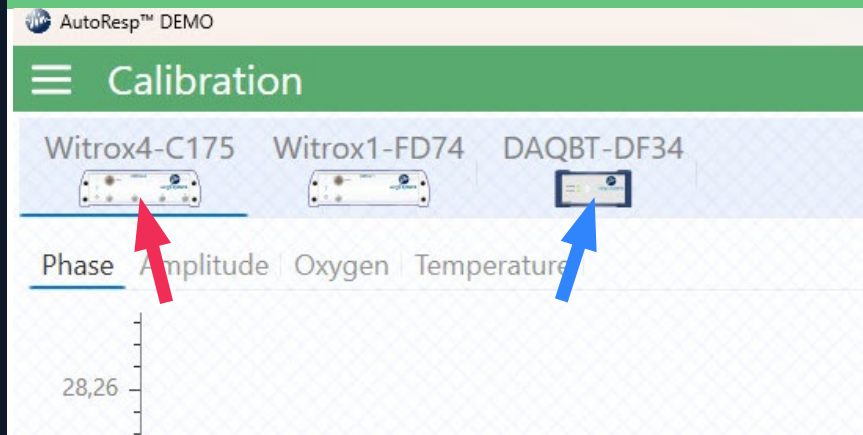
6



6.1



8



SETTING UP YOUR SYSTEM

10

Main menu > **System designer**. In the **Configurator tab** (10), click on the number of resting or swim tunnel chambers you want to set up (10.1). The number of available chambers is based on the hardware devices that you have connected. The available power relays and sensors are shown under **Unassigned hardware**. Now select the number of available O₂ or temperature controls you want to use (10.2) and select whether you want single (up OR down) or double regulation (up AND down).

The chamber and control tabs (11, **red arrow**) show an animated setup illustration, chamber settings, and the hardware that has been automatically assigned. Click on each chamber and control tab to open them.

NOTE: When a DAQ-BT is detected, you can only choose to set up swim tunnel systems. Resting chamber systems can be set up, if no DAQ-BT is detected.

Setup illustration (11): Arrows on tubing show direction of water movement and thus the pumps that are currently ON. Hover the mouse over the elements in the illustration to get more information, and to see which relays or sensor channels they apply to in the hardware overview below.

Settings (11a + 11b): Fields lined with red are required. Once filled out, the red checkmark in the tab will turn green indicating that the chamber has all required information. Change or add values to the remaining fields, if needed. Select between real-time temperature compensation of oxygen data from a specific Witrox instrument, or select *Fixed temperature*, if you have exact control of sample water (e.g., your setup is in a climate-controlled room). The Fixed temperature value is inputted in the Experiment menu.

Hardware list (11c): The connected hardware devices are automatically assigned to each chamber, and the assigned hardware is shown beneath the setup illustration. Mouse-over on relays and sensor channels will show you which pump or sensor they correspond to in the illustration. Helpful tool-tips are applied to most elements upon prolonged mouse-over.

The **Overview tab** (11, **blue arrow**) shows the entered information for all your chambers and controls. Use this tab to check that your entire system is ready to go. If needed, your system setup can be saved as a file in the lower right corner of the overview tab. This is also where you load a system setup file. Exit to the main menu.

NOTE: AutoResp™ v3 will automatically remember the last system setup (including connected devices) that was used. If the same devices are not found during initial scanning (step 7), the system setup in the System designer cannot be used. Enter the Configurator tab to create a new system setup.

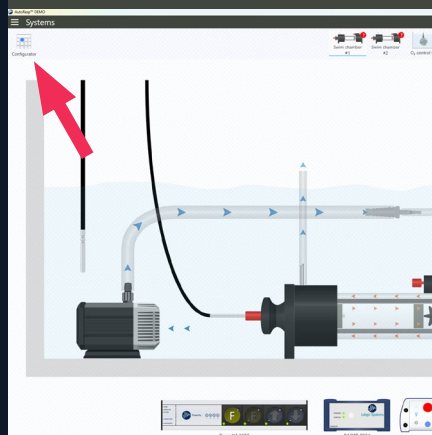
How to manually assign power relays

Hardware is automatically assigned to the relays on the PowerX4 in the System designer menu. Yet, you can manually assign hardware to each power relay, e.g., to control multiple flush or recirculation pumps from a single relay.

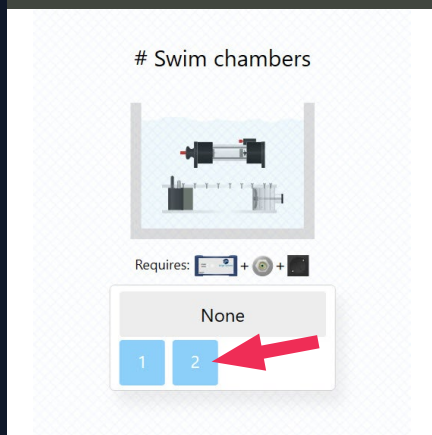
Go to: Main menu > Settings > Enable the **Manual relay configuration** toggle button. In the System designer menu, click on a PowerX4 in the legend panel in a chamber, control or overview tab, and then click on the relay the specific hardware should connect to in the dropdown field.

Watch the [AutoResp™ v3 - How to assign power relays](#) on our YouTube channel for more information.

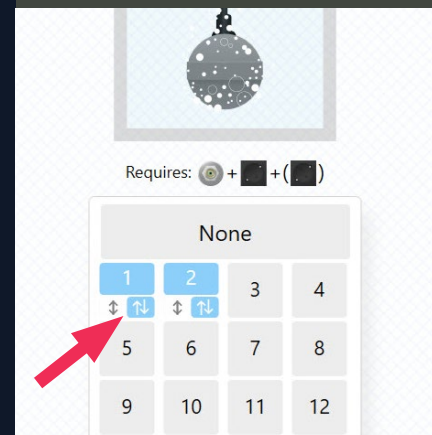
10



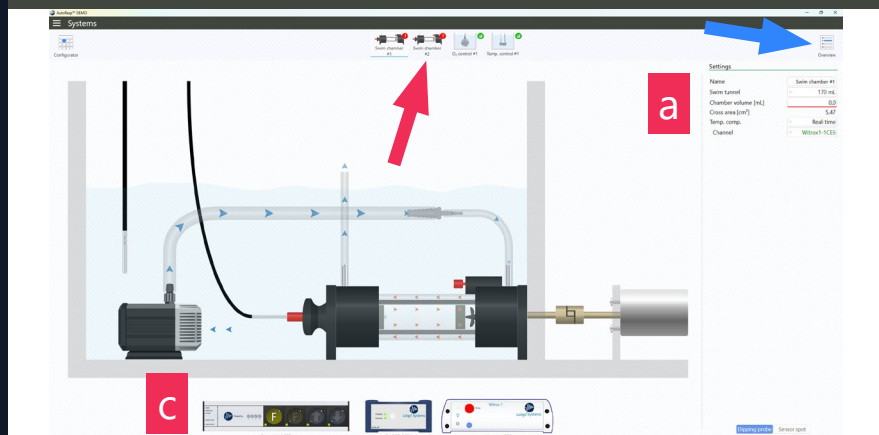
10.1



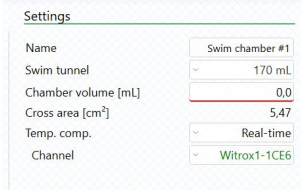
10.2



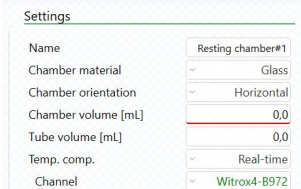
11



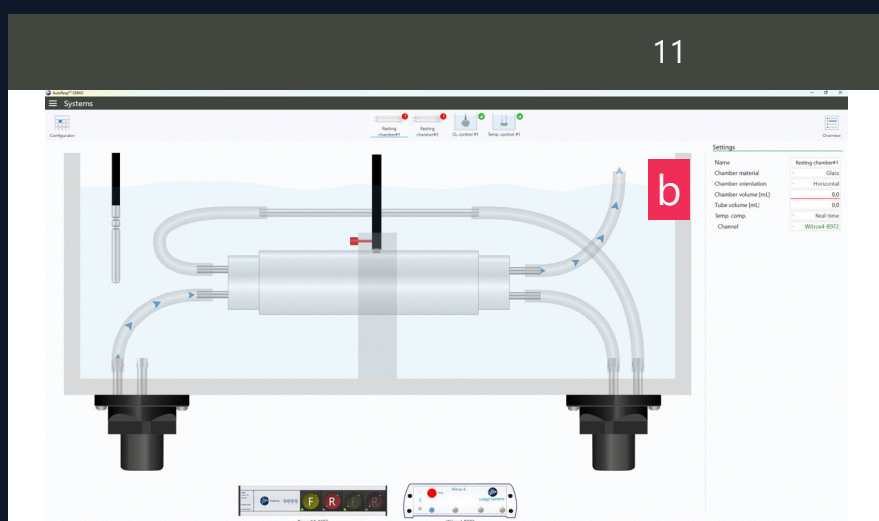
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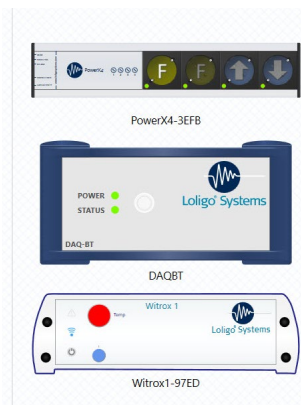
b



11



c



STARTING AN EXPERIMENT

Main menu > **Experiment**. The experiment menu is sectioned into tabs for setting up, monitoring, and controlling your system.

Select your **Project folder** (12, red arrow). This is where all your data files will be saved.

You can start logging data for individual chambers and controls using the ▶ icon in the Setup tab (12.1) and System tab. Use the ▶ icon next to the Project folder field to start/stop all chambers and controls (12, blue arrow). Logging is continuous.

AutoResp™ v3 will save a **.ar3 file** (that you load in the AutoResp™ Analysis menu), a **.calc file** (with calculated experiment data), and a **_raw file** (with raw data) for each chamber. Controls will also be saved as a .ctrl file.

Setup tab: Enter data for each chamber and control in your system before you start your chamber.

- **Animal length** (*swim tunnel only*): Standard or total length of animal.
- **Chamber volume:** Internal volume of chamber only (not water bath).
- **Density:** Density of the animal. If in doubt, use 1.
- **Experiment:** Start and stop individual chambers.
- **Mode:** Select type of respirometry or control mode. Click **i** for mode descriptions.
- **Model:** Shows the resting chamber, swim tunnel model, or control type.
- **Name:** Name of the chamber and control. Can be changed, and appears in the file name.
- **Note:** A note that will be added in the .log and .calc data file.
- **P_{atm}:** Atmospheric pressure. Default 1.013,25 hPa.
- **Ratio:** Chamber volume / Animal volume
- **Resp. volume:** Chamber volume + tube volume - wet weight
- **Salinity:** Salinity of the chamber water.
- **Solid blocking** (*swim tunnel only*): Apply and adjust solid blocking effect, if needed.
- **Temp. comp.:** Type of temperature compensation for Witrox oxygen measurements. If Fixed, the value can be changed here throughout the experiment.
- **Treatment:** Create, edit, and add treatments.
- **Tube volume** (*resting chamber only*): Internal volume of tubing in connection with the chamber.
- **Velocity regulation** (*swim tunnel only*): Select type of water velocity regulation.
- **Wet weight:** Wet weight of the animal.
- **O₂/Temp. regulation:** Select type of O₂ or temperature regulation. Mouse-over the PowerX4 relays to see their functionality.

Status tab: Shows the actual status and real-time data for each chamber and control. Swim tunnel motors can be stopped immediately by clicking the **⏹** icon.

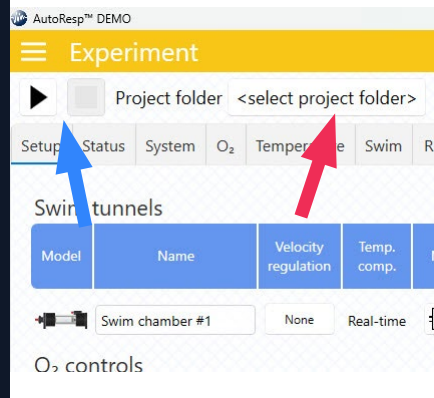
System tab: Shows real-time oxygen and temperature graph (Chart) or pump status (Drawing) for an individual chamber or control. Select other chambers and controls in the bottom tabs (12.3, blue arrow).

- The **Control** panel (12.3, green arrow) allows you to start/stop the chamber, change respirometry mode, set velocity regulation and stop motor (swim tunnel only), and enable the recirculation pump to always be ON (resting chamber only) - similar to the options you find in the Setup tab.
- The **Data** panel (12.3, yellow arrow) shows real-time data values for the selected chamber or control.
- A **Phase-meter** (12.3, red arrow), and info about each of the respirometry phases will appear, when an experiment is running. Click on the Phase-meter to skip to the next phase.
- Assigned hardware, relays, and sensor channels are displayed below the graph.

Data graph tabs: Shows real-time data. The graph layout can be customized (12.3a, red arrow) and exported (12.3a, blue arrow) individually to Excel using the fold-down legend menu.

Control graph tabs: Shows real-time data for the O₂ and temperature regulation. Regulation type can be changed using the **⚙** icon in the legend panel.

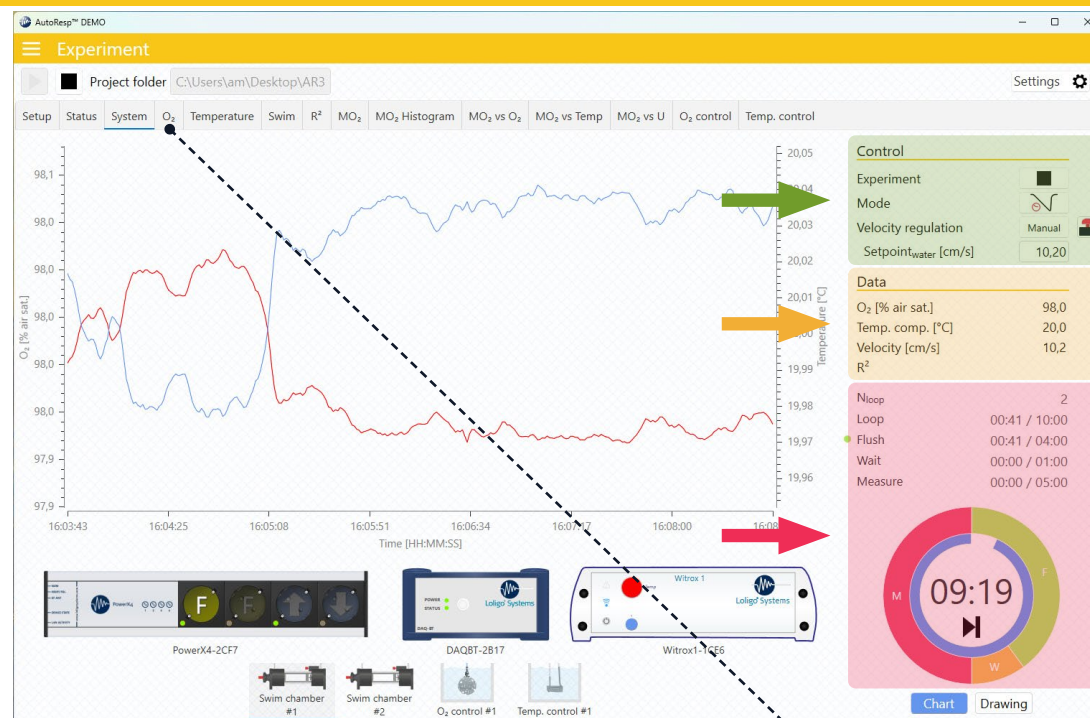
12



12.1



12.3



Time 22:09:06

⌵ ⌴

Swim chamber #1
 Velocity
 Setpoint 11,00

12

ANALYZING YOUR EXPERIMENT

The Analysis section can be used without restrictions while running AutoResp™ v3 in demo mode. This means that you can collect data on the computer the Keylok dongle is connected to, and analyze this data on another computer without a Keylok dongle inserted.

Main menu > **Analysis** > Click the **Load** button (13, **green arrow**) and select the experiment .log files you want to analyze from your Project folder.

The **Project** tab shows an overview of calculated data for all your selected files. The **Status** field (13, **red arrow**) indicates if the .log file has been edited or has finished unexpectedly (red = checksum error). The gear icon next to the Status field allows you to manually set the data range or set the EPOC range for that dataset. Data outside this range will not be included in the analysis. The Project folder location is displayed above the overview list (13, **blue arrow**).

The data range can also be set using the gear icon (13.1, **green arrow**) in the legend in many of the data graphs. You can also set a global data range using the **Settings** pop-up menu (13.1, **red arrow**) or by dragging the global data range handlers (13.1, **blue arrows**). The global data range handlers must be made visible in the **Chart settings** pop-up menu (13.1, **yellow arrow**).

In the **data graphs**, you can toggle to show or hide the graph curves for the data files, and up to several parameters, by clicking on the colored checkmark squares in the legend. Each data graph is also customizable using the legend menu. Some features, like confidence intervals and function equations (14), are only visible when a single data file is checked in the legend.

Further visualization options can be found in the Chart settings pop-up menu. Each option in this menu is explained in more details using the **i** icon or in our [online FAQ](#).

The Settings pop-up menu enables you to select between several algorithm methods, and adjust their settings, for your SMR, MMR, P_{crit} , COT, U_{opt} and EPOC parameters.

You can also enable **allometric scaling** and **temperature normalization of oxygen consumption rate** ($Temp_{norm}$) in the Settings menu. See details in our [online FAQ](#).

The different parameters can be visualized in these data graph tabs:

- MO₂ vs O₂**
 - SMR FAQ: [How to find the Standard Metabolic Rate \(SMR\)](#)
 - MMR FAQ: [How to find the Maximum Metabolic Rate \(MMR\)](#)
 - P_{crit} FAQ: [How to find the critical lower oxygen level \(Pcrit\)](#)

- MO₂ vs Time**
 - EPOC FAQ: [How to find the Excessive Post-exercise Oxygen \(EPOC\)](#)

- COT**
 - U_{opt} FAQ: [How to find the Optimal Swim Speed \(Uopt\)](#)
 - COT_{opt} FAQ: [How to find the Cost Of Transport \(COT\) and COTopt](#)

Refer to the user manual, [AutoResp™ v3 - Algorithms summary](#), for a detailed explanation of each parameter.

Next to the Settings button, you will find a **Sample / Treatment** toggle button. When **Sample** is selected, each data file will be shown individually in each data graph, and calculations like MO₂, SMR, MMR, etc. apply to the individual data files (16). When **Treatment** is selected, the data files are grouped into their respective treatments (16.1), indicated in the Project tab, and calculations are now based on all the data files per treatment.

On some data graphs, the **Average** values in the legend (16.2) are based on all files in the Project tab independent of having Sample or Treatment selected.

Exporting data. The data graph can be exported to Excel using the legend drop-down menu (16, **red arrow**). Any graph customization or grouping (Sample vs Treatment) applied will be exported as well.

