QUICK GUIDE | Microplate system

1.7 LOLIGO® SYSTEMS

FIRST TIME USE

1 Download the latest version of MicroResp™ from our website: www.loligosystems.com/downloads Follow the instructions on the screen and then restart the PC.

2 Insert the Loligo® license dongle (2) in a USB port on the PC to unlock the full software. MicroResp™ will run in demo mode, if the license dongle is not inserted. Running MicroResp™ in demo mode will enable virtual hardware that simulates data during an experiment (i.e., you cannot use real hardware), but you can still analyze data files.

3 Connect power supply (A) to splitter (B), splitter to PC, and connect the first reader to the splitter (3). Connect additional reader(s) to the previous reader(s).

SETUP

4 **Preparation of sensor spots.** Place the white plastic guide onto the reader and place the microplate glass plate inside the guide (4). For **aquatic use**, hydrate the sensor spots for 30-45 min. using water of the same type and temperature as during trials. For **use in air**, acclimate the reader, guide, and glass plate for 30-45 min. at the trial temperature before use.

5 **Temperature control**

Water bath: Use our optional flow-through water bath (5) to control sample temperature. The water bath sits directly on top of the reader, and only the sealed glass plate (and silicone pad and compression block, if in use) must be submerged in the water bath. Use a refrigerated circulating bath to pass water through the acrylic water bath.

Incubator: Keep only the readers (and guide and glass plate), not the splitter, inside the incubator. Use the flat, black piece on the splitter-to-reader cable for the incubator door.

Climate controlled room: The entire microplate system can be placed in a climate controlled room for sample temperature control.

RUNNING AN EXPERIMENT

6 Start MicroResp™ and click **Experiment** in the main menu to detect the reader(s). Open the **Settings** tab to change setup settings and to configure and perform (or verify) the calibration data (*see step 16*).

Need more help?: Watch the MicroResp™ video tutorial: www.youtube.com/LoligoSystems

7 **Measurement in gas/air.** Experiment > Settings > General. Enable **Is air breather**, and change the humidity value, if needed. MicroResp™ will now assume you are measuring in air (*i.e.*, that you have no water in the glass plate wells).

8 **Number of animals.** Experiment > Settings > Organisms per well (8). If **Set individual** is set to *No*, all wells must have the same number of organisms, and the number is entered in the **Number** field. Change *Set individual* to *Yes* to allow for a custom number of animals per well, and enter the specific number in each of the wells below.

9 **Normalizing data.** Experiment > Settings > Calibration. Next to the **Normalization factor** field, click to normalize oxygen data from all the sensor spots, e.g. to 100 % air.sat. (9). Normalization will set all air sat. values to 100 % (by multiplying with a factor), so that each oxygen curve has the same starting point. If the oxygen sensor calibration is good, normalizing will not change the % air sat. values much (< +/- 10 %), if performed at 100 % air sat.. Normalization should be performed after setting both calibration points, and when there is 100 % air sat. water/air in the wells.

10 **Treatments.** Experiment > Settings > Treatment (10). Add + or remove ✕ a treatment or select one from the list. Change its name and color, if needed. Add the number of blanks you want on your plate. Open the **Treatments** tab and click the **Randomize** button (10.1, **red arrow**) to let MicroResp™ choose the treatment and blank/control assignment for each well. Alternatively, click on each well and select its treatment. Control wells are required for determining background respiration due to bacteria, biofilm, etc.

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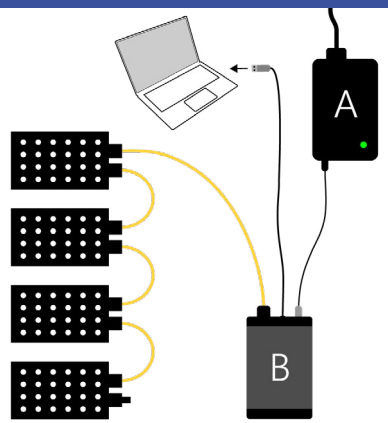

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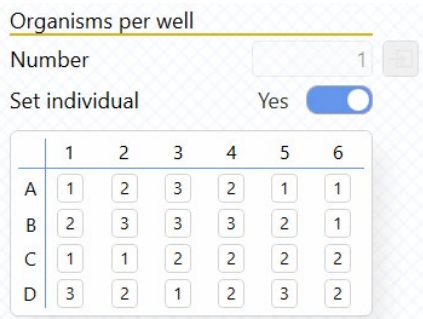

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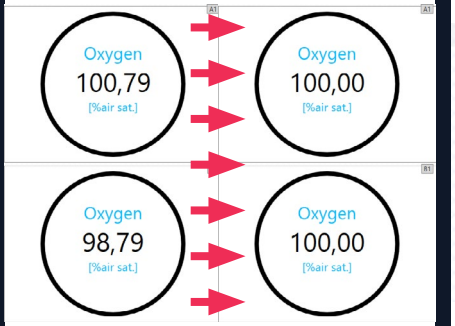

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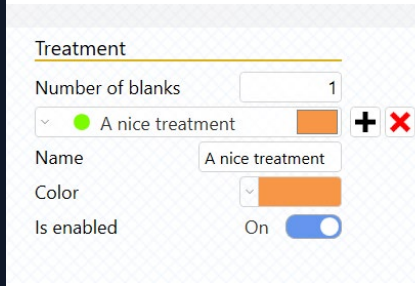

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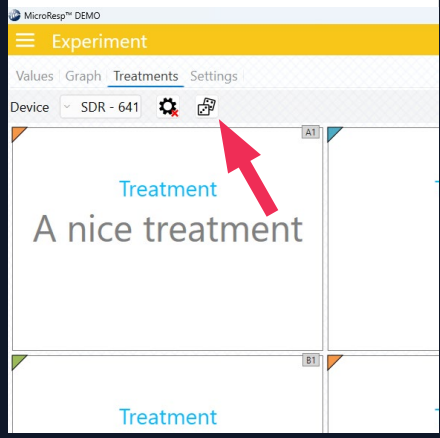

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RUNNING AN EXPERIMENT

11 Fill the wells with water (avoid air bubbles) or air, and add test organisms. *Avoid exposing the microplate system to strong UV light as this can affect oxygen readings, and bleach sensor dye.*

12 To seal the glass plate, line it with a sheet of microplate sealing film (12) or other gas tight foil. If using parafilm, then cover the entire microplate with the silicone pad and place the compression block on top. For experiments with inactive aquatic organisms, place the reader on a shaker-table to agitate the water somewhat.
Need more help?: Watch "Sealing glass microplates" on www.youtube.com/LoligoSystems

13 **Logging data.** Click **START LOG** to create a data file and start logging data from a reader. Data logging must be started seperately for each reader by selecting each reader from the Device-list beneath the tabs.

14 When the experiment is over (or a critical lower oxygen level is reached), click **STOP LOG** for each reader.

ANALYZING AN EXPERIMENT

15 Main menu > Analysis > Load data file. Open the **Settings** tab > **Filters** to adjust the data range for the linear regression analysis used for calculating the slope of the oxygen curve. Data points meeting the criteria will appear **red** in the graphs (15). Settings tab > **General** > Enable **Edit loaded data** to adjust setup parameters for the experiment. Changes will be visible in the MO_2, Slope and Graph tabs. Click to export the analysis as an Excel data file. This data file contains graphs as well as data, including time-stamped MO_2 values.

CALIBRATION, SERVICE & MAINTENANCE

16 To calibrate the oxygen sensor spots, select **Experiment** > **Settings** tab and choose either:

1. Perform a **Manual** (user-defined) calibration (16):
   a. Fill the wells with a mixed air-equilibrated water sample. This can be achieved by purging atmospheric air into sample water, e.g. with an air pump. The temperature of the water sample must be within +/- 1.5 °C of the experiment water temperature, otherwise an additional calibration is needed for higher/lower temperatures.
   b. Wait for the readings to stabilize, and then click **Read current values** (16.1b) to save the current sensor signal as the HIGH calibration value (100 % air saturation).
   c. Then fill the wells with an oxygen free water sample, e.g. by purging nitrogen gas into sample water or by dissolving ~10 grams of Na_2SO_3 in 500 ml of distilled water. The temperature of the water sample must also be within +/- 1.5 °C of the experiment water temperature.
   d. Wait for the reading to stabilize, and then click **Read current values** (16.1d) to save the current sensor signals as the LOW calibration value (0 % air saturation).

   or

2. Select **Pre-defined** under **Calibration** (16.2) and select the batch calibration number found on the black plastic bag that the microplate glass plate came in (18).
   IMPORTANT: *The pre-defined calibration should only be used for preliminary trials.*

17 To clean the microplate, use ethanol (<70 % v/v), bleach (<3% H_2O_2), or mild detergent, and rinse with demi water. Then dry (17). If using ethanol to sterilize the spots/wells, then dry the plate thoroughly, at least 2 days at 50-60 °C in an oven, to ensure that all residues have left the sensor dye matrix.

It is recommended to perform a manual calibration after cleaning/sterilizing.

18 Store glass plates in the non-translucent black plastic bag between trials, and avoid exposing the sensor spots to UV light as it will bleach the oxygen sensitive dye causing signal drift (18).

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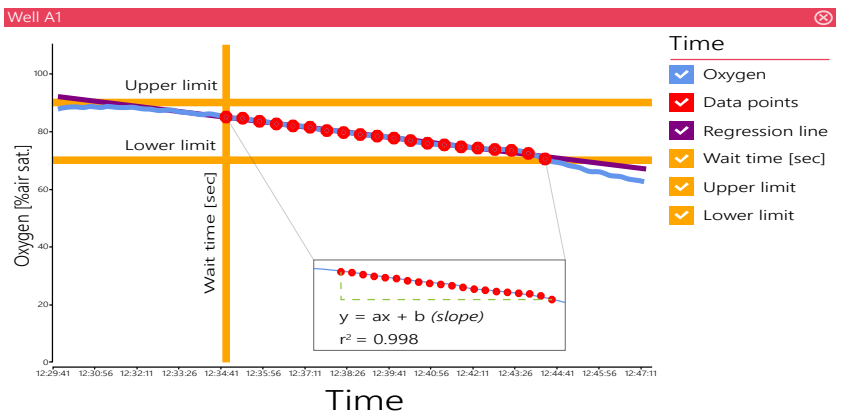

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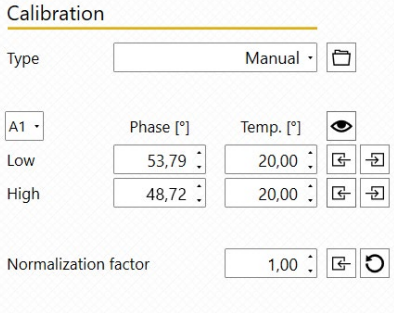

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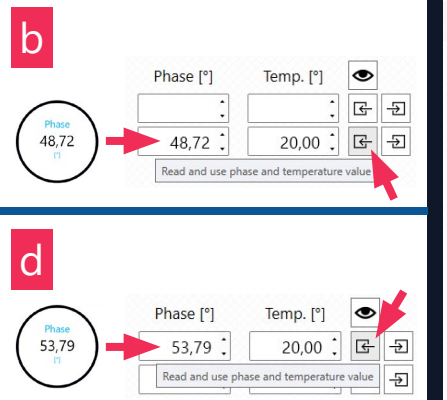

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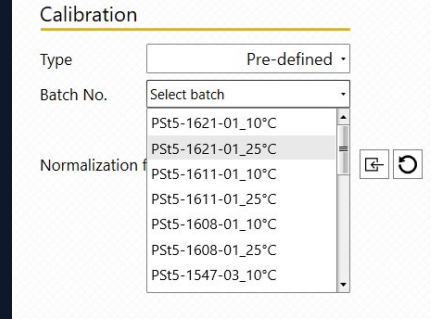

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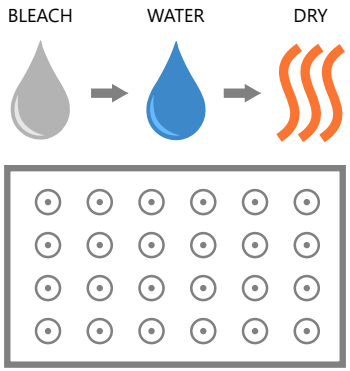

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