QUICK GUIDE | Microplate system

1.2 LOLIGO® SYSTEMS

FIRST TIME USE

1	Insert Loligo USB memory stick into the PC and run the **MicroResp™** installation program. Follow the instructions on the screen and then restart the PC. *Alternatively, download MicroResp™ from the website: www.loligosystems.com/downloads*
2	Connect the green (WiBu) copy protection dongle to a USB port on the PC.
3	Connect power supply (A) to splitter (B), splitter to PC and connect the first reader to the splitter. Connect additional reader(s) to the previous reader(s).
4	When using an incubator, keep the readers in it, not the splitter. Use the flat piece on the cable for the incubator door.

FOR EACH TRIAL

5	Place the white plastic guide on the reader and place the microplate inside it. For aquatic use, hydrate the sensor spots for approximately 30-45 min. using water of the same type and temperature as during trials. *NB. Avoid strong light from above as this can affect oxygen readings.*
6	Start the MicroResp™ program and click **Experiment** in the main menu to detect the reader(s). Click **Settings** to configure and verify the calibration (*see step 14*). *Optional: Watch the MicroResp™ video tutorial: www.loligosystems.com/videos*
7	Click **Normalize** to normalize oxygen data from all the sensor spots, e.g. to 100 % a.s.
8	Click **Treatments** and **Randomize** to let MicroResp™ choose treatments and control wells. The latter is required for determining background respiration due to bacteria, biofilm, etc.
9	Fill the wells with water and test organisms making sure to avoid air bubbles in the wells.
10	To seal all the wells, line the silicone pad with parafilm, PCR film or some gas tight film or foil, then cover the entire microplate with these and place the compression block on top so that the silicone pad will act as a gas-tight gasket. *Optional: For experiments with inactive aquatic organisms, place the reader on a shaker-table to agitate the water somewhat.*

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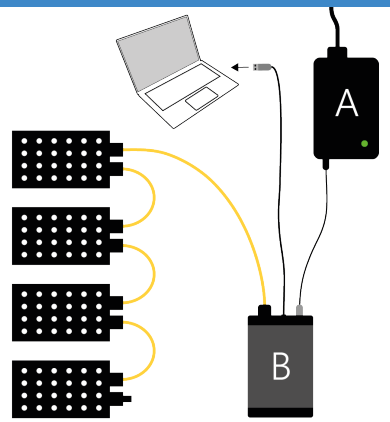

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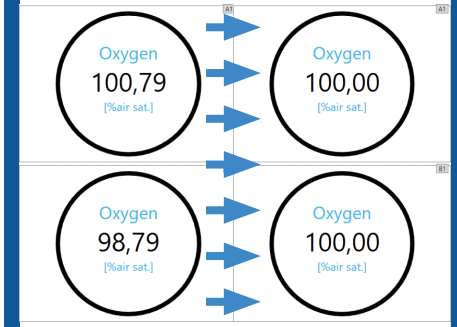

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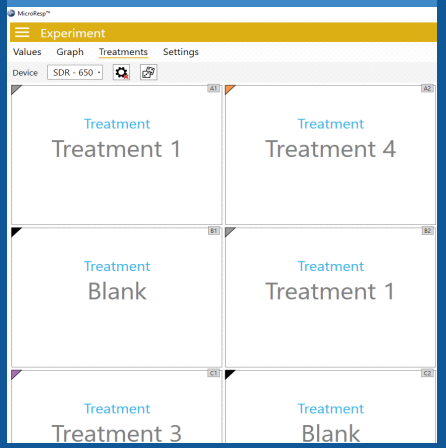

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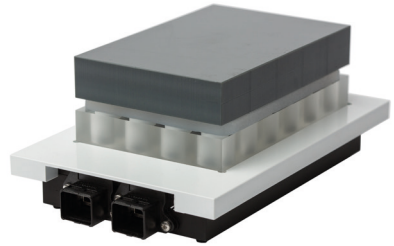

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1.2 LOLIGO® SYSTEMS

FOR EACH TRIAL

11 Click **Start log** to create a data file and start logging data from a reader. *Please note that each data file logs data from one reader only.*

12 When the experiment is over (or a critical lower oxygen level is reached), click **Stop logging**.

13

1. To analyse the data file, click **Analysis** in the main menu and load the data file. Choose **Settings** to set an optional Wait time or Upper/Lower limit 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.
2. Click the **Save Analysis to Excel** icon to create an Excel data file.

CALIBRATION, SERVICE & MAINTENANCE

14 To calibrate the oxygen sensor spots, choose **Settings** within **Experiment** and choose either:

1. Perform a **Manual** (user-defined) calibration (14.1):
   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.
   b. Wait for the readings to stabilize, and then click **Read current values** (14.1b) 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.
   d. Wait for the reading to stabilize, and then click **Read current values** (14.1d) to save the current sensor signals as the LOW calibration value (0 % air saturation).

   or

2. **Predefined** under **Calibration** (14.2) and select the batch calibration number found on the black plastic bag the microplate came in (16). *IMPORTANT: The predefined calibration should only be used for preliminary trials.*

15 To clean the microplate, use bleach and rinse with demi water. Then dry.

To sterilize, use ethanol (max. 10 % v/v). 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.

16 Store microplates 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.

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CRITICAL LOWER OXYGEN LIMIT

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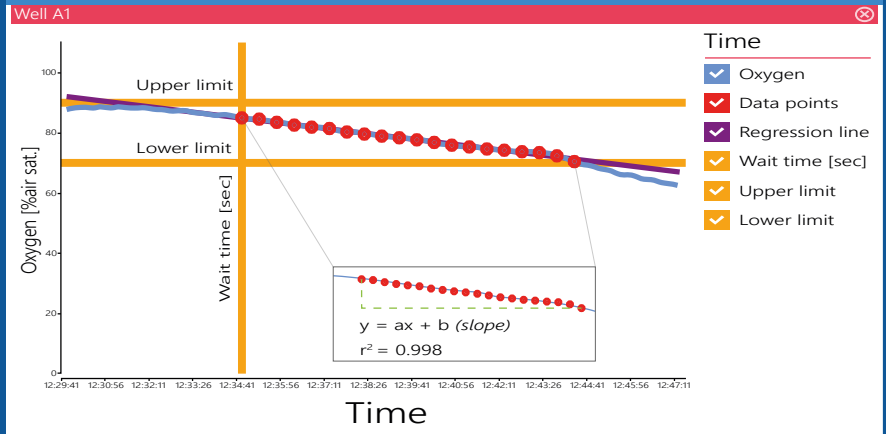

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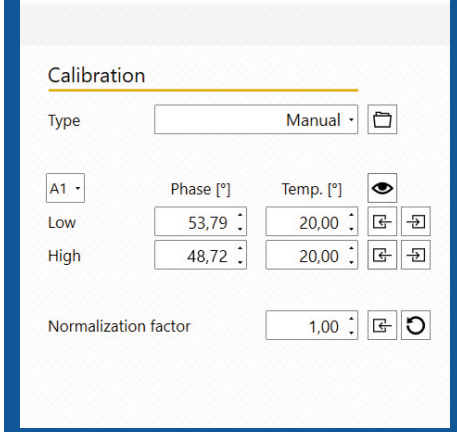

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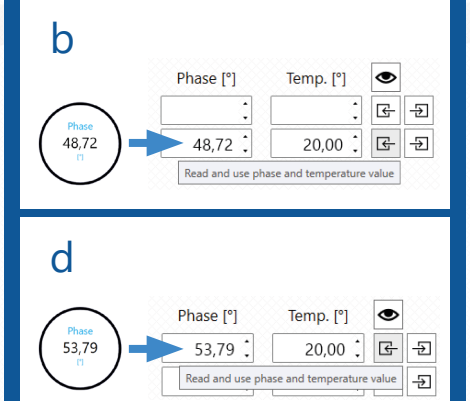

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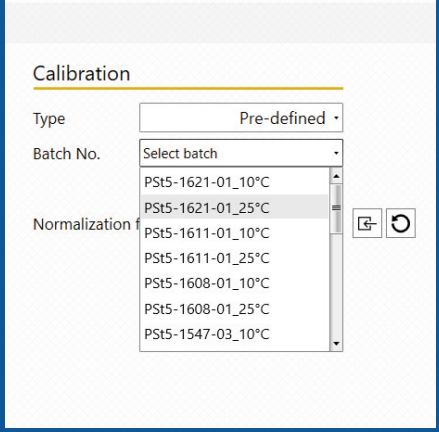

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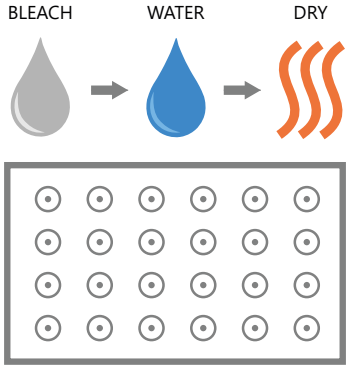

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