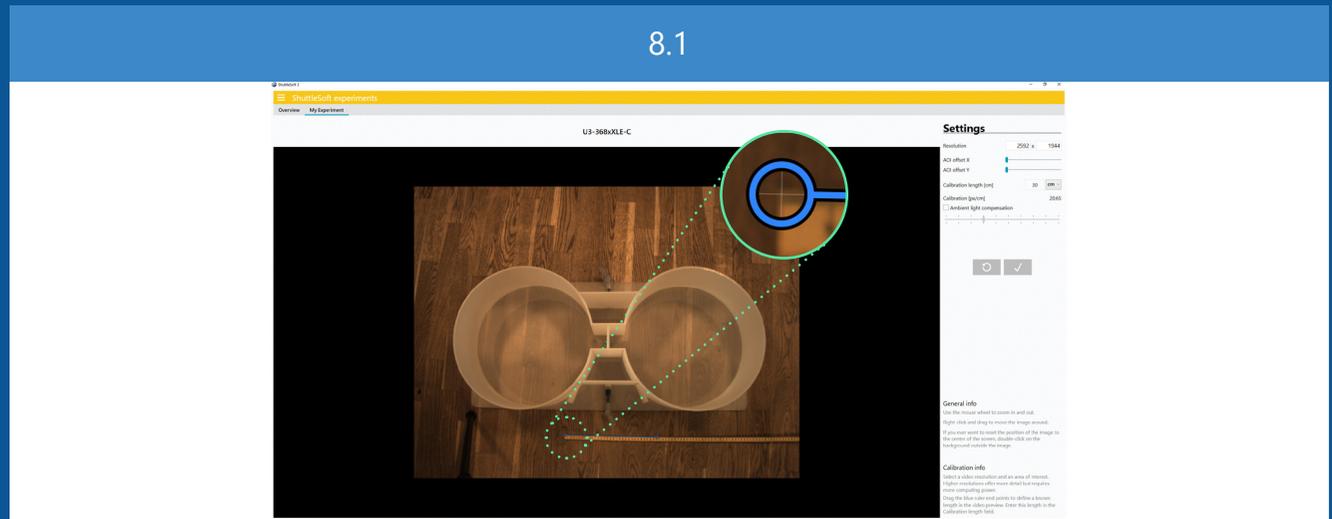
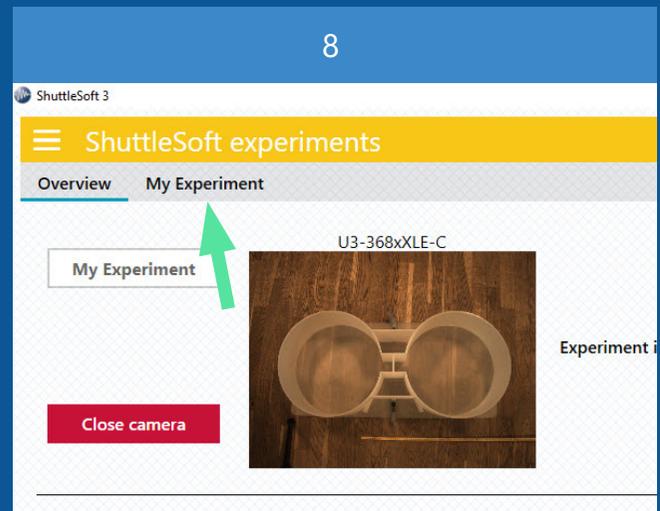
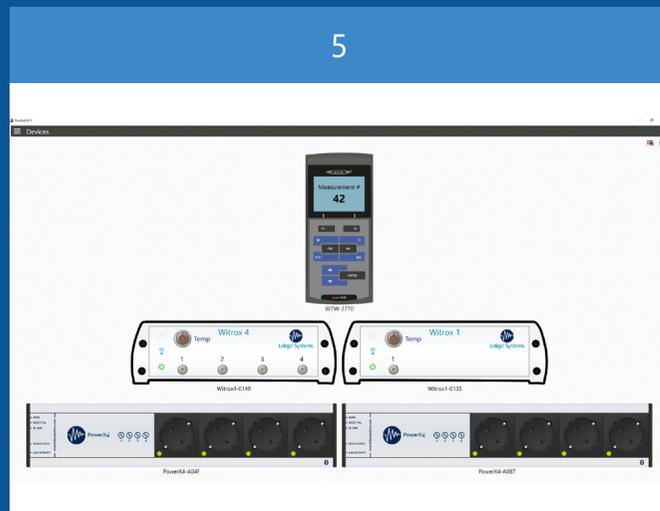
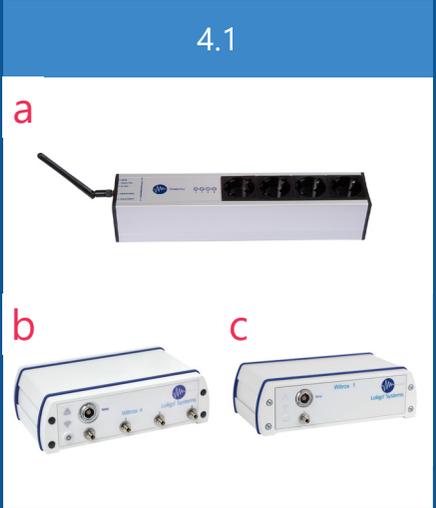


## FIRST TIME USE

- Download the latest version of **ShuttleSoft 3** from our website: [loligosystems.com/downloads](http://loligosystems.com/downloads). Follow the installation instructions on the screen and then restart the PC.  
For troubleshooting, please visit our online FAQ: [loligosystems.com/faq](http://loligosystems.com/faq)
- Power on the WTW meter by pressing the **Power** button. Press and hold the **ENTER** button to enter the **Storage & config** menu. Select **System > Interface > Baud rate**, and set the baud rate to 19200. Press the **F1** button until you return to the start screen (3c).
- Connect the green (WiBu) copy protection dongle to a USB port on your PC (3a).  
Connect the **Long range Bluetooth dongle** to a USB port on your PC, and let it initialize (3b). Disable any other Bluetooth radios on your PC.  
Connect the **USB C camera** to a USB C (or >USB 3.0) port on your PC, and let it initialize (4). The LED on the back of the camera will light green, when the camera is ready to use. Keep the spacer ring (green arrow, 4) between the camera house and lens to get correct focus.
- Connect the **PowerX4** power strip to a grounded wall outlet (4.1a).  
Connect the **WTW meter** to a USB port on your PC. Power on the meter.  
*If you are using a Witrox oxygen instrument...* Connect the **Witrox 1/4** to a power source (PC USB or USB adapter) (4.1b/c). Click the red **Power** button. The blue connect icon will now flash blue (for 5 min.) indicating that the Witrox is in pairing mode.
- Open **ShuttleSoft 3 > Devices > Choose Scan for new devices**. When scanning is complete, press and hold the **F2** button on the WTW meter until the **Autom. USB Output menu** appears. Change **Interval rate**, if necessary. Otherwise, choose **Continue** to exit.  
Go back to the main menu, when all connected instruments have been found (5).

## LIVE EXPERIMENTS

- Repeat step 3-4, and open ShuttleSoft 3. Now choose **Use current configuration** and complete step 5.
- The WTW oxygen sensor must be calibrated with the WTW meter. See the WTW meter user manual for more instructions.
- If you are using a Witrox oxygen instrument...* Main menu > **Calibration** > Choose Witrox instrument: Each oxygen sensor must be calibrated before use. Follow the [Quick guide for Witrox Instrument](#) to perform a manual calibration. The temp probe is pre-calibrated.
- Main menu > **Live experiments**: The software will now load available cameras; one for each experiment tab. Close any unwanted cameras. Click on the **Experiment** tab to open the experiment (green arrow, 8).
- Start by performing a **pixel-calibration** to enable correct distance measurements. Drag the blue ruler endpoints to define a known length (e.g., by placing a ruler in the background) on the live preview (8.1). Input the defined length in the **Calibration length** field. Adjust the resolution and area of interest (AOI) to improve performance. Click **Accept settings** to continue.  
**IMPORTANT: Once the camera is in place, the camera must not be moved! Otherwise, a new pixel-calibration is necessary to continue.**
- Video** tab: Shows the live feed from the camera. Any mask, zones or filter can be turned on/off at the bottom of the screen.



LIVE EXPERIMENTS

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**Mask** tab: Use masking tools in settings panel to mask out any unwanted areas by choosing a tool and drag on video preview to create shapes (11). Adjust shapes using anchor points. Objects in blue areas will not be tracked. The mask can be saved via *Save current mask to file*. Click *Accept mask* to apply the current mask.

11

**Zone** tab: Use masking tools in settings panel to create zones by choosing a zone and tool, and then drag on video preview to create shapes (11). Click on the *Zone 1* or *Zone 2* button to activate the zone, and adjust the shape using anchor points. The zones can be saved for later use via the *Save current zones to file* button. Click *Accept zones* to apply the current zones.

12

**Filter** tab: Use filter tools in settings panel to create a filter that will threshold the image into object(s) (red pixels), that you want to track, and irrelevant objects or background (12). Select filter mode A (threshold by color) or B (threshold by background subtraction):

**A. Threshold objects based on color contrast:**

1. Place color-picker on object and click to threshold object (blue arrow, 12).
2. Place color-picker on background and hold SHIFT + click to indicate background.

**B. Threshold objects by subtracting static background pixels:**

1. Static background pixels will be found automatically, and the *Background image* will indicate what the current background is. Adjust *Sampling time* or *Freeze background*, if necessary.

**IMPORTANT:** If the object stands still for a long period of time, the object may be identified as the background. The object will then show up on the *Background image*, and the tracking of the object will fail.

Fine tune the filter selection by adjusting filter strength and object size sliders in the *Settings* panel. Click *Accept filter* to save the filter.

13

**Hardware** tab: Assign available hardware for temperature and/or oxygen control by clicking the "Assign hardware" buttons. The number of available PowerX4 and input channels will show up on the right. A connection overview will show underneath indicating which relays and temperature/oxygen channels that must be connected to zone 1 and 2 (13). Make sure to set these required hardware connections in your physical setup before continuing.

14

**Regulating** tab: Control the regulation of oxygen and temperature levels in each zone. Use the input fields and drop-down menus to specify the regulation to your liking (14). An in-depth description of each parameter is available in our [online FAQ](#). Click *Start regulating* to begin regulating the zones.

*NOTE: The regulation can run independently from the data logging and/or video recording. You can start/stop the regulation, data logging and video recording from the Overview tab.*

Alternatively, regulate the zones from a custom protocol via the *Open protocol* button. To design a custom protocol, see step 18.

15

**Log data** tab: Select a logging file output path and change the *Data sampling rate*, if necessary. Click *Start logging* to begin logging data.

16

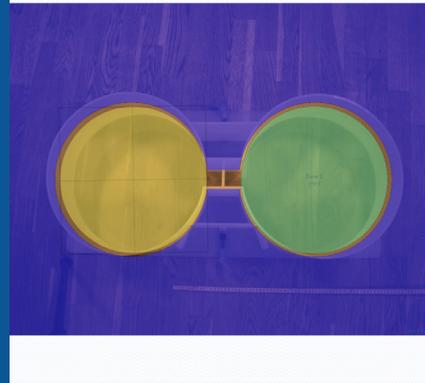
**Record video** tab: If you want to record the experiment, select a video file output path and change the recording settings, if necessary. Click *Start recording* to begin recording video.

**IMPORTANT:** A high frame rate may not be achievable depending on the chosen resolution, current PC performance load, PC specifications etc. Use the *Time-lapse* function and/or the [FAQ Optimizing your PC for data recording guide](#), if necessary.

17

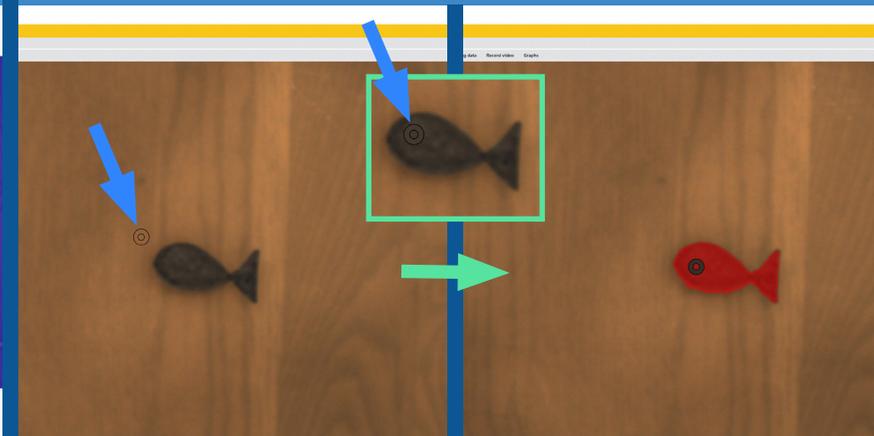
**Graphs** tab: Shows a selection of data for the current experiment. Each individual graph can be customized (green arrow) and exported to Excel (blue arrow) (17). Stop logging to change logging settings and to reset graph data. The complete dataset for the experiment can be analyzed by loading in the experiment log file in the *Data analysis* menu (see step 26).

11



13

12



14

17

PROTOCOL DESIGNER

18

Create custom protocols for regulating oxygen and temperature levels in a live experiment (from step 6). The first protocol row will be highlighted in **blue** which means its settings can be changed in the *Settings panel* (18). Add a new protocol row via the *Add row* button. This will create a row beneath with similar settings as row 1. Click on and highlight row 2 to change its settings, if necessary. Add (or remove) additional rows until you are happy with the result.

Click *Save protocol* to save the current protocol. Then load the saved protocol file during a live experiment.

Alternatively, load a protocol via the *Load protocol* button to change its settings.

VIDEO FILE TRACKING

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Main menu > **Video file tracking**: Start by loading the video you want to track. Now perform a *pixel-calibration* as described in step 8. Change the settings in the *Settings panel*, if necessary. Click *Accept settings* to continue.

20

**Video** tab: Shows the loaded video. Any mask, zones or filter can be turned on/off at the bottom of the screen.

21

**Mask** tab: Use masking tools in settings panel to mask out any unwanted areas by choosing a tool and drag on video preview to create shapes. Adjust shapes using anchor points. Objects in blue areas will not be tracked. The mask can be saved via *Save current mask to file*. Click *Accept mask* to apply the current mask.

22

**Zone** tab: Use masking tools in settings panel to create zones by choosing a zone and tool, and then drag on video preview to create shapes. Click on the Zone 1 or 2 button to activate the zone, and adjust the shape using anchor points. The zones can be saved for later use via *Save current zones to file*. Click *Accept zones* to apply the current zones.

23

**Filter** tab: Use filter tools in settings panel to create a filter that will threshold the image into object(s) (red pixels), that you want to track, and irrelevant objects or background. Select filter mode A (threshold by color) or B (threshold by background subtraction):

**A. Threshold objects based on color contrast:**

1. Place color-picker on object and click to threshold object.
2. Place color-picker on background and hold SHIFT + click to indicate background.

**B. Threshold objects by subtracting static background pixels:**

1. Static background pixels will be found automatically, and the *Background image* will indicate what the current background is. Adjust *Background brightness*, if necessary.

**IMPORTANT:** If the object stands still for a long period of time, the object may be identified as the background. The object will then show up on the *Background image*, and the tracking of the object will fail.

Fine tune the filter selection by adjusting filter strength and object size sliders in the *Settings panel*. Click *Accept filter* to save the filter.

24

**Tracking settings** tab: Start by inputting *Start time of recording*, *Custom value* settings and tracking settings, if necessary (24).

**Custom values:** Two custom values (or parameters) can be specified for each zone, e.g., the concentration of added compounds in the zones at the time of recording. Input the values and unit/comment, and click *Update custom settings* to apply. The custom settings can be applied to the entire timeline or to an interval via hold SHIFT + drag on timeline.

Click the *Track* button to track the entire timeline or a selected interval.

Protocol designer

Row number	Time span [minutes]	High zone	Temp mode	Temp high	Temp low
1	10	2	Static	15,00	25,00
2	10	1	Dynamic		
3	10	Randomize	Dynamic		
4	10	Invert	Dynamic		
5	1	As before	Dynamic		
6	1	As before	None		

VIDEO FILE TRACKING

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**Graphs** tab: Shows tracking data for the tracked video file. Each individual graph can be customized and exported to Excel. Click *Save data* to export the complete dataset as an Excel file. The data file can be analyzed in ShuttleSoft 3 by loading in the log file in the *Data analysis* menu (see step 26).

DATA ANALYSIS

The **Data analysis** menu enables you to analyze a single or multiple data files from one or more experiments. Click the *Load* button to import a data log file or simply drag and drop the files into the file area at the bottom of the screen (26). Only ShuttleSoft 3 data log files can be imported here, and only files from either live experiments or tracked video files can be analyzed at a time.

A detailed description of each data parameter can be found in our [online FAQ](#).

**View settings:** Change which data parameters that are shown in the data file list. The *Settings* column applies to the *Project settings* tab and the *Data* column applies to the *Project data* tab and graphs.

**Data settings:** Change the logging settings for all experiments in the data file list. You can also enable *Include graphs in Excel export*.

**Project settings** tab: Shows project settings related data for one or multiple experiments.

**Project data** tab: Shows an overview of all available data parameters for one or multiple experiments. Click the *sum icon* (blue arrow, 26.1) to view additional statistics for the given data column. Use the scroll bar at the bottom of the screen to view all parameters.

**Temperature** tab: Shows every temperature related dataset for one or multiple experiments. Each graph can be customized and exported to Excel. Use the scroll bar to the right to view all graphs.

**Oxygen** tab: Shows every oxygen related dataset for one or multiple experiments. Each graph can be customized and exported to Excel. Use the scroll bar to the right to view all graphs.

**Zones** tab: Shows every zone related dataset for one or multiple experiments. Each graph can be customized and exported to Excel. Use the scroll bar to the right to view all graphs.

**Custom value 1/2** tab: Shows every custom value 1/2 related dataset for one or multiple experiments. Each graph can be customized and exported to Excel. *NOTE: This tab is only available when analyzing data from the Video file tracking menu.*

The **Export** button (green arrow, 26.1) exports the compiled dataset (and graphs) for every experiment in the data file list to a single Excel file.

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ShuttleSoft 3

**Data analysis**

Load View settings Data settings Export

Project settings Project data Temperature Oxygen Zones

Filename	Date	Start time	Duration	Decay value, k	P
X Experiment 1.log	September 15, 2021	13:32:44	00:05:03	1	3
X Experiment 2.log	September 16, 2021	14:45:03	01:32:12	1	3
X Experiment 3.log	September 17, 2021	15:01:17	01:30:27	1	3
X Experiment 4.log	September 18, 2021	14:50:32	01:31:48	1	3

26.1

ShuttleSoft 3

**Data analysis**

Load View settings Data settings Export

Project settings Project data Temperature Oxygen Zones

Filename	Total distance moved $\Sigma$	Distance moved (high zone) $\Sigma$
X Experiment 1.log	0,69 cm	0,32 cm
X Experiment 2.log	0,53 cm	0,15 cm
X Experiment 3.log	0,45 cm	0,21 cm
X Experiment 4.log	0,73 cm	0,12 cm