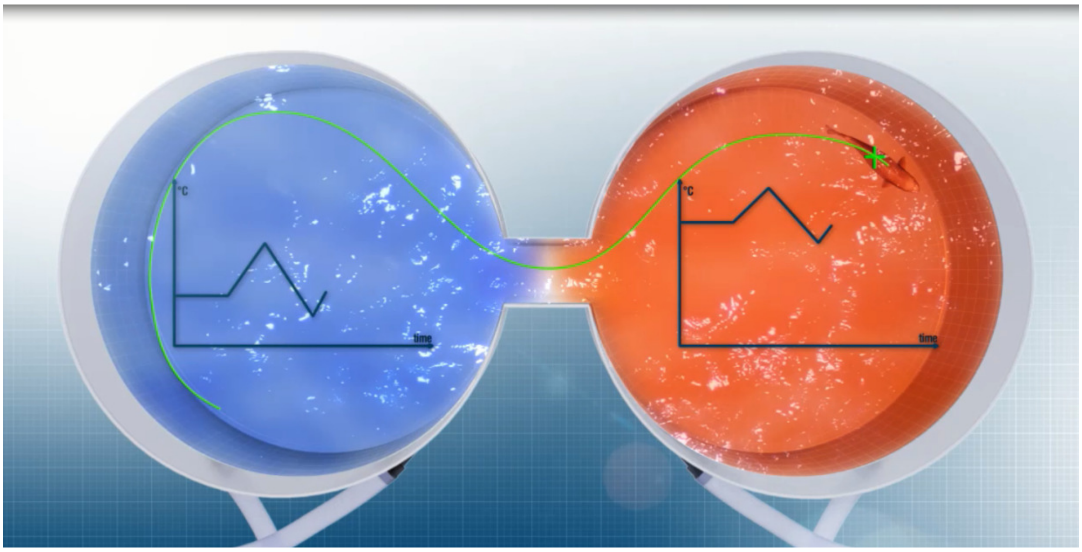
Shuttle Box System
Oxygen



Installation & User Manual

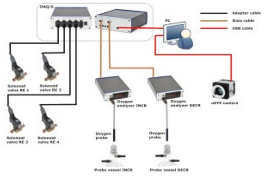
www.loligosystems.com

Shuttle System Quick Guide

Follow these steps to quickly start using the system

1. Install the ShuttleSoft software and the uEye-camera driver from the Loligo memory stick.
   See Section 3

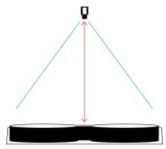
2. Connect the DAQ-M instrument, the two oxygen analyzers and the video camera to the PC.
   See Figure 1, Section 4.1



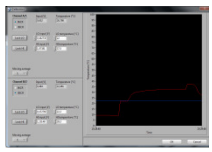
3. Calibrate the oxygen probes.
   See Section 4.3

4. Start the ShuttleSoft software.
   See Section 5.1

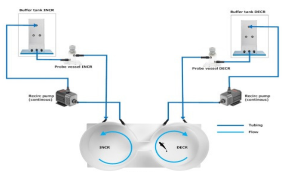
5. Mount the video camera above the shuttle tank. Make sure the image covers the entire tank.
   See Section 4



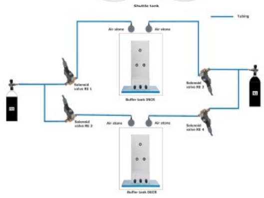
6. Calibrate the output from the two O2 analyzers in ShuttleSoft.
   See Section 5.2.5



7. Connect the shuttle tank, buffer tanks and recirculation pumps and fill with water.
   See Figure 2, Section 4.2



8. Place the air stones in the buffer tanks and connect them to solenoid valves and gas bottles.
   See Figure 3, Section 4.3



9. Set up an experiment in ShuttleSoft.
   See Section 5.2

Content

1. List of parts

- USB memory stick (Loligo®)

- USB hardkey dongle (Wibu)

- LabView Vision runtime license certificate

- uEye USB video camera
  - C-mount lens
  - USB cable
  - Tripod adapter
  - Bracket
  - Software mini CD

- DAQ-M instrument
  - Adapter cables, qty. 4
  - Gas control sets, qty. 4
  - Split data cable, qty. 1

- Oxygen analyser (OXY-REG), qty. 2

- Flow through probe vessel, qty. 2

- User manual

2. General

2.1 Background

The Loligo® shuttle box systems include a test tank for aquatic use that is a modified version of the classic operant conditioning chamber (also known as the Skinner box) used for experimental analysis of behavior, e.g. to study operant conditioning and classical conditioning in animals.

An operant conditioning chamber permits experimenters to study behavior conditioning (training) by teaching a subject animal to perform certain actions (like pressing a lever) in response to specific stimuli, like a light or sound signal. When the subject correctly performs the behavior, a mechanism delivers food or another reward. In some cases, the mechanism delivers a punishment for incorrect or missing responses.

With this apparatus, experimenters perform studies in conditioning and training through reward/punishment mechanisms. Operant chambers have at least one operandum, that can automatically detect the occurrence of a behavioral response or action. Typical operanda for primates and rats are response levers. Despite such a simple configuration (e.g. one operandum and one feeder), it is possible to investigate many psychological phenomena in this way. For this reason operant conditioning chambers have become common in a variety of research disciplines including behavioral pharmacology, and Skinners's Box have been used extensively for behavioral research in primates and rats.

Loligo® shuttle tanks have been developed for aquatic animals like Zebrafish or crustaceans, and the tank design allows for independent control of water quality in two sub compartments. Tank dimensions are made special to accommodate a wide variety of animal species and sizes. Inside the Shuttle tank the animal can freely "shuttle" between two sub compartments with opposite acting controls, e.g. in one tank the dissolved oxygen concentration starts to increase. When the animal enters in the other it starts to decrease.

The computerized Loligo® shuttle systems are equipped with a video camera conditions enabling real-time pc vision software to detect animal locomotion based on contrast. If the animal changes its position from one compartment to the next through locomotion, the computer software (ShuttleSoft) activates/deactivates programmed devices to change environmental conditions inside the tank, e.g. to regulate dissolved oxygen concentration to preferred values through behavior. Or you can set up two different (constant) toxygen concentration levels in the two tank compartments independent of fish behavior for exposure/avoidance/choice tests.

Today a main application of Loligo® shuttle systems is measurements of oxygen preference in aquatic ectotherms (as well as avoidance behavior), and automated computerized systems have been made for a range of other environmental factors like temperature, water turbidity, salinity, pH and pCO_2.

The turnkey systems offered include everything needed for video behavior analysis as well as monitoring and regulating water quality.

For a list of published papers on Loligo® Shuttle boxes, please visit our website: www.loligosystems.com/Support/Published papers

2.2 PC requirements

- Our Shuttle Box systems for preference/avoidance measurements in aquatic organisms, includes ShuttleSoft software for Windows, instrumentation for USB, video equipment and an experimental tank with pumps, tubing and fittings to monitor and control water quality in two independent sub-compartments.

- ShuttleSoft requires a mouse with a scroll button.

- 3 free USB ports are required on your PC:

    - *One for connecting the DAQ instrument.*
    - *One for the digital video camera.*
    - *One for the protection dongle (WIBU) USB hardkey.*

- ***Minimum PC requirements***:

    - *Processor:* ShuttleSoft requires Windows 7 running on a PC with an Intel Pentium IV processor of 2,4 GHz and 2GB RAM or better.

    - *Monitor resolution*: We recommend monitors with a minimum resolution of 1024x768 pixels.

3. Software installation

3.1 ShuttleSoft for Windows

Connect your PC to the internet and insert the USB memory stick labelled Loligo.

Open folder to view files and double click on the icon labelled ShuttleSoft_Installer.exe.

- Install ShuttleSoft by following the instructions on the screen.
- Activate the LabView license by selecting automatic activation. This activation requires an internet connection (during installation only).
- Choose automatic activation and select Next to activate the LabView drivers.
- Enter the serial number found on the LabView Vison runtime license card. Keep this card for future installations and support.
- Enter your contact information to finish the activation.
- Install drivers for the hardkey WIBU dongle.
- In WIBU-KEY SETUP select supported language and continue installation without further changes.

3.2 uEye *USB camera drivers and software*

- Select Next in the self extracting uEye InstallShield Wizard and select unpack and remove, then Next.
- Wait for the files to be unpacked and till the setup screen appears.
- Select Install driver and choose language.
- In Setup Type select Complete.
- Continue installation by following instruction on the screen.
- Continue to install camera drivers.

Restart the Computer

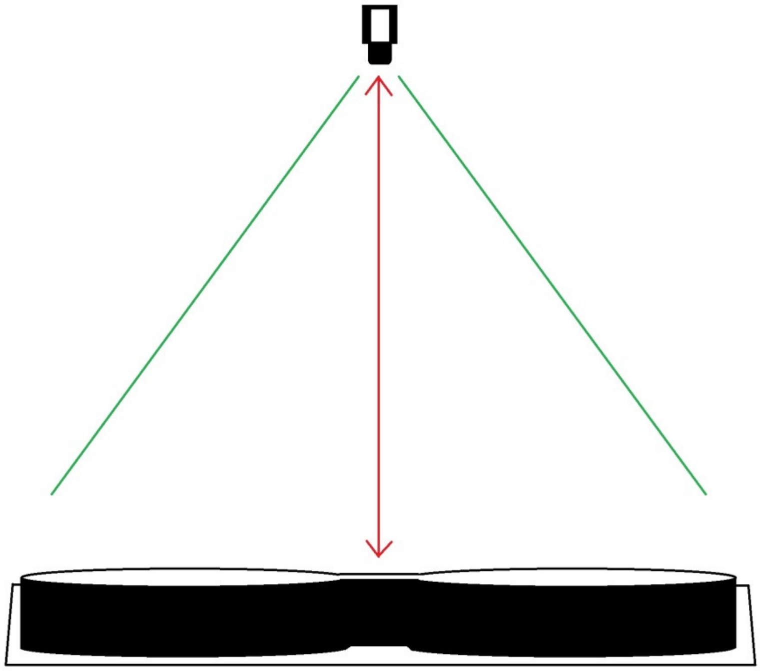
Connect the uEye USB camera to the PC.

- After a few seconds the camera software (uEye Cockpit) installation will start automatically. Select Install the software automatically (Recommended) and follow instructions on the screen.
- Mount the lens on the uEye camera.
- The uEye camera is now ready for use with ShuttleSoft.
- Do not use the software uEye Cockpit while using ShuttleSoft. uEye Cockpit is being used when using the camera for other applications such as recording video files or stills.

4. Installing ShuttleBox system for oxygen

The ShuttleBox system is built by numerous components and extends in space depending on the size of the Shuttle tank.

- The video camera must be positioned sufficiently high above the Shuttle tank to obtain a full view of the entire tank.



- For background illumination of the experimental animal in the Shuttle tank an infra-red light panel can be placed under the tank (see section 4.2).
- All data acquisitioning instruments should be placed on a laboratory bench away from water.
- Place the buffer tanks in close vicinity to the Shuttle tank to keep tubes at a minimum length.
- Ensure sufficient space to access all pumps, fittings and sensors.

4.1 Connecting instruments and valves

Connect the DAQ-M instrument to the PC and connect the oxygen analyzers and gas control solenoid valves to the DAQ-M instrument as shown in Figure 1 and described in detail below.

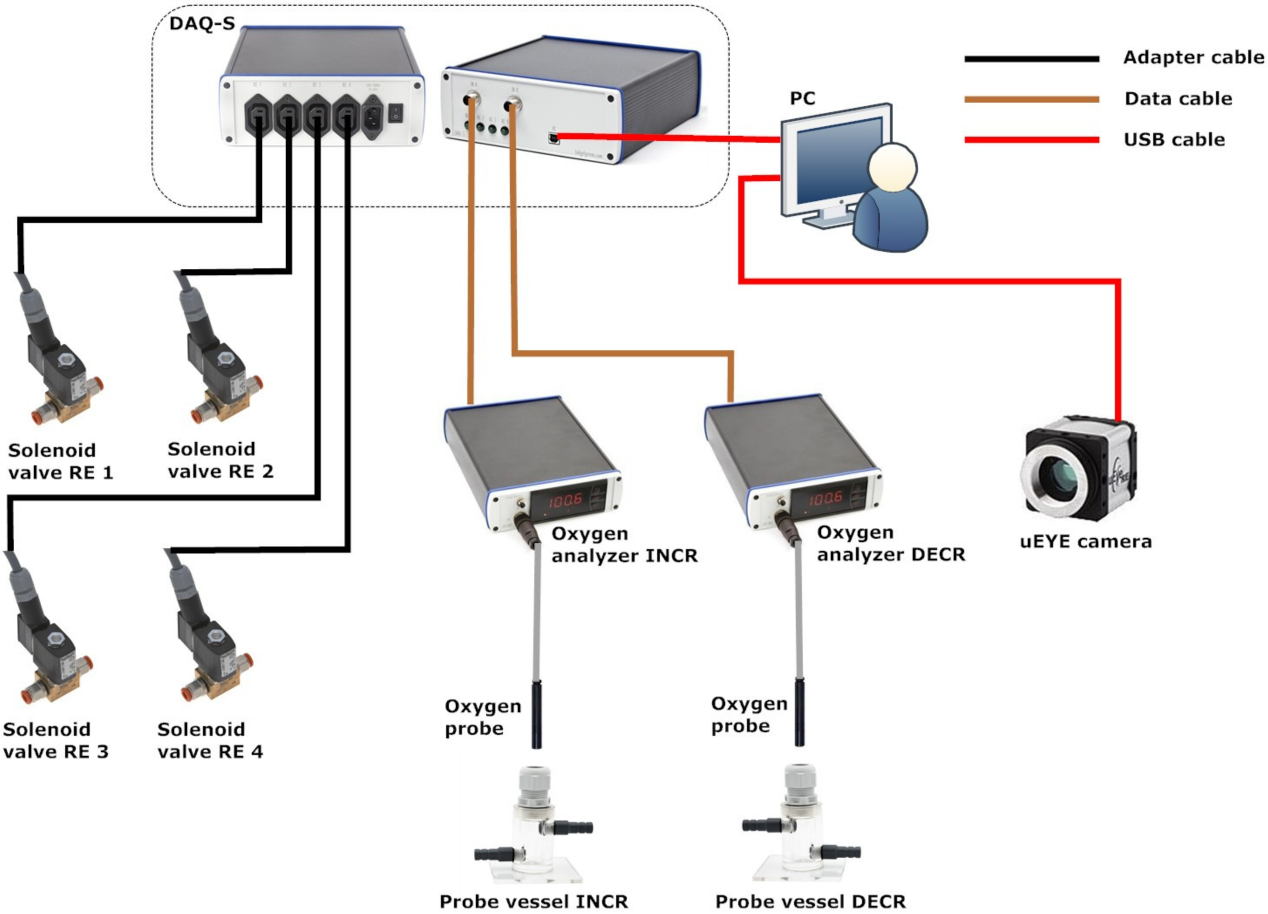


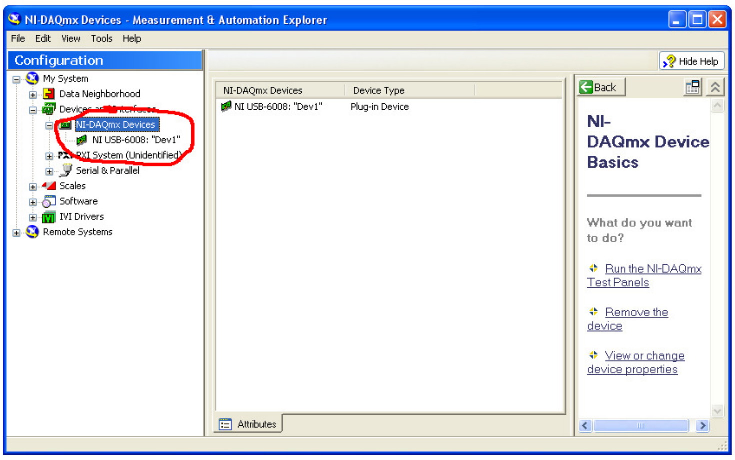
Figure 1: How to connect the oxygen analyzers (OXY-REG), oxygen probes, oxygen regulation valves (RE valves) and uEye camera for running a ShuttleBox oxygen experiment.

4.1.1 Connect DAQ-M instrument

Connect the DAQ-M instrument to the PC using the USB cable.

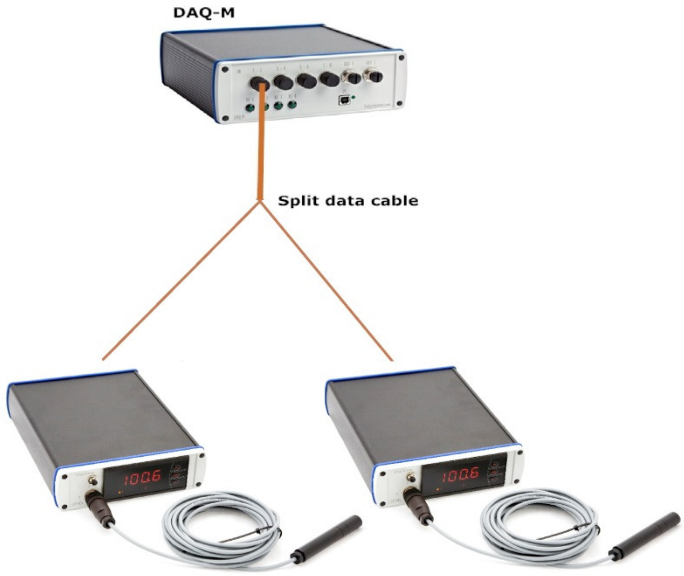
- After a few seconds the software installation will start automatically. Select "Install the software automatically (Recommended)" and follow instruction on the screen.

To check if the installation was successful, open the Measurement and Automation Explorer (MAX) by clicking Start→All Programs→National Instruments→MAX. If the installation was successful, the USB-6008(DAQ-S)/USB-6009(DAQ-M) card is now shown here.



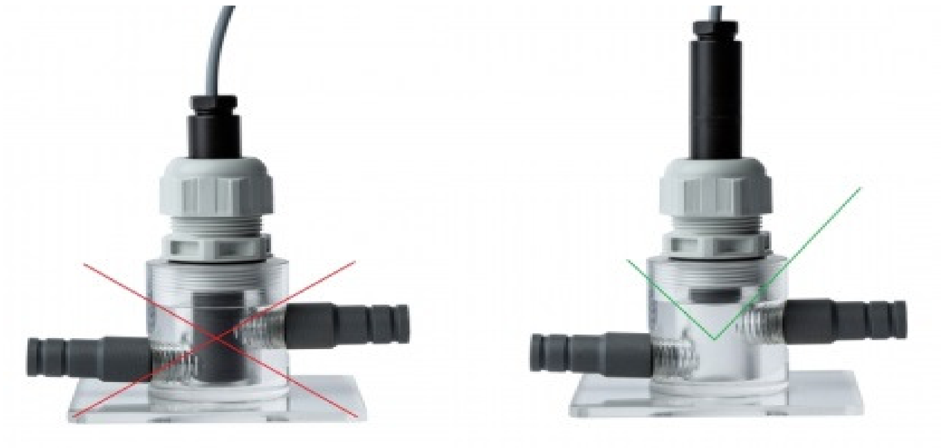
4.1.2 Connecting the oxygen analyzers and probes

Connect the two oxygen analyzers (OXY-REG) to the DAQ-M instrument via a split data cable to the input 1 channel on the front of the DAQ-M instrument.



Connect the MINI-DO oxygen probe to the input labeled IN on the OXY-REG instrument front panel.

Each MINI-DO O_2 probe is placed inside a flow through probe vessel (Figure 2). The probe should only be inserted to the top of the flow-through vessel, to allow free water flow past the probe. Do not push the probe fully into the bottom of the vessel.



For ease the oxygen signal should be calibrated in OXY–REG (Section 4.1.3) as well as in ShuttleSoft (Section 5.2.5) before adding water to the ShuttleBox system.

4.1.3 Calibrating oxygen probes in OXY-REG

The galvanic type oxygen probe is stable and quite rugged, but like all other oxygen sensors it needs to be calibrated regularly in order to adjust for signal drift.

Perform a two-point calibration.

- Place the oxygen sensor in an air equilibrated water sample. Air equilibrated water can be prepared by purging atmospheric air into the sample water, e.g. with an air pump and air stone.
- Wait for the reading to stabilize, and then press the **OK** button until the display reads CA.HI. Use the **Λ** or **V** button to toggle to YES, and press the **OK** button to accept the current probe signal for the high calibration value.
- Press the **OK** button until the display reads DI.HI. and the **Λ** or **V** button to adjust the high calibration value to 100 (% air saturation). Press the **OK** button to accept the value.
- Then press the OK button several times, until the display reads "----" to finish the high calibration.

- Perform a zero calibration procedure by short-circuiting the signal wires using the 0-cal button on the front of the OXY-REG instrument. Press and hold this button for approximately 20 seconds to allow the signal to stabilize and then accept by pressing the OK button at the same time.

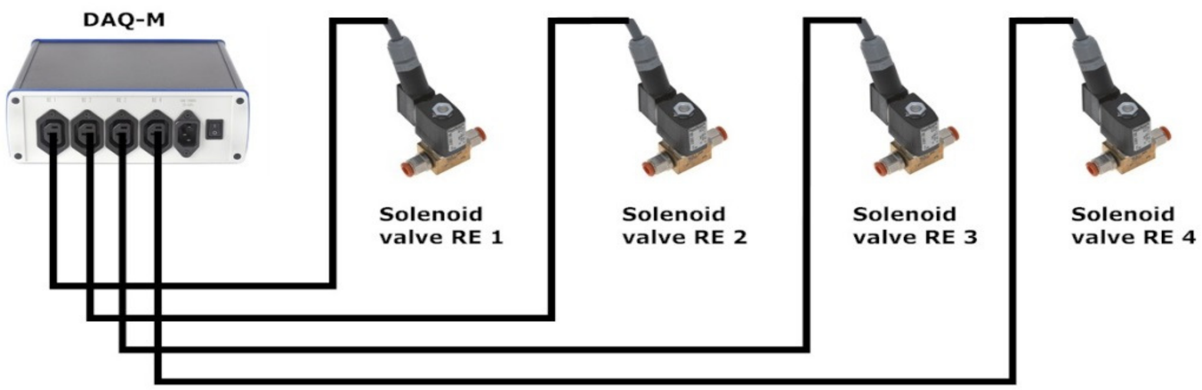
  Alternatively, make a zero calibration by placing the tip of the probe in an oxygen free water sample, press the **OK** button until the display reads CA.LO. Use the **Λ** or **V** button to toggle to YES, and press the **OK** button to accept the current probe signal for the low calibration value. Press the **OK** button until the display reads DI.LO. and the **Λ** or **V** button to adjust the low calibration value to 0 (% air saturation). Press the **OK** button to accept the value. Finally press the **OK** button several times, until the display reads "----" to finish the zero calibration.

Please refer to the oxygen analyser (OXY-REG) user manual for further instructions on how to calibrate and service the MINI-DO probes.

4.1.4 Connecting valves

Connect the four gas regulating solenoid valves to the four relays (RE) on the back side of the DAQ-M instrument using the 4 adapter cables.

Make sure valves are connected in the right order (see Figure 1) to ensure correct oxygen regulation.



4.2 Tube connections

Connect the Shuttle tank to the recirculation pumps, probe vessels and buffer tanks as shown in Figure 2.

- Make sure the Shuttle tank rests on a completely level and firm surface.
- Place buffer tanks in close vicinity to the shuttle tank to keep tubing length to a minimum. It is generally recommend placing the buffer tanks at the same level as the Shuttle tank.
- If a longer diffusion pathway is desired when bubbling with O2 or N2, lower the buffer tanks to obtain a higher water column.
- Ensure sufficient room to access all pumps, fitting and sensors.
- Make sure to check if the direction of flow is correct.
- For optimum separation of flows, it is very important to that both sub-compartments are fed with exactly equal flow rates. We recommend flow rates should of 1-5 cm/sec. To adjust the flow rate use tube clamps on the hose going from each pump, and/or raise/lower the position of the buffer tanks feeding the Shuttle box tank.

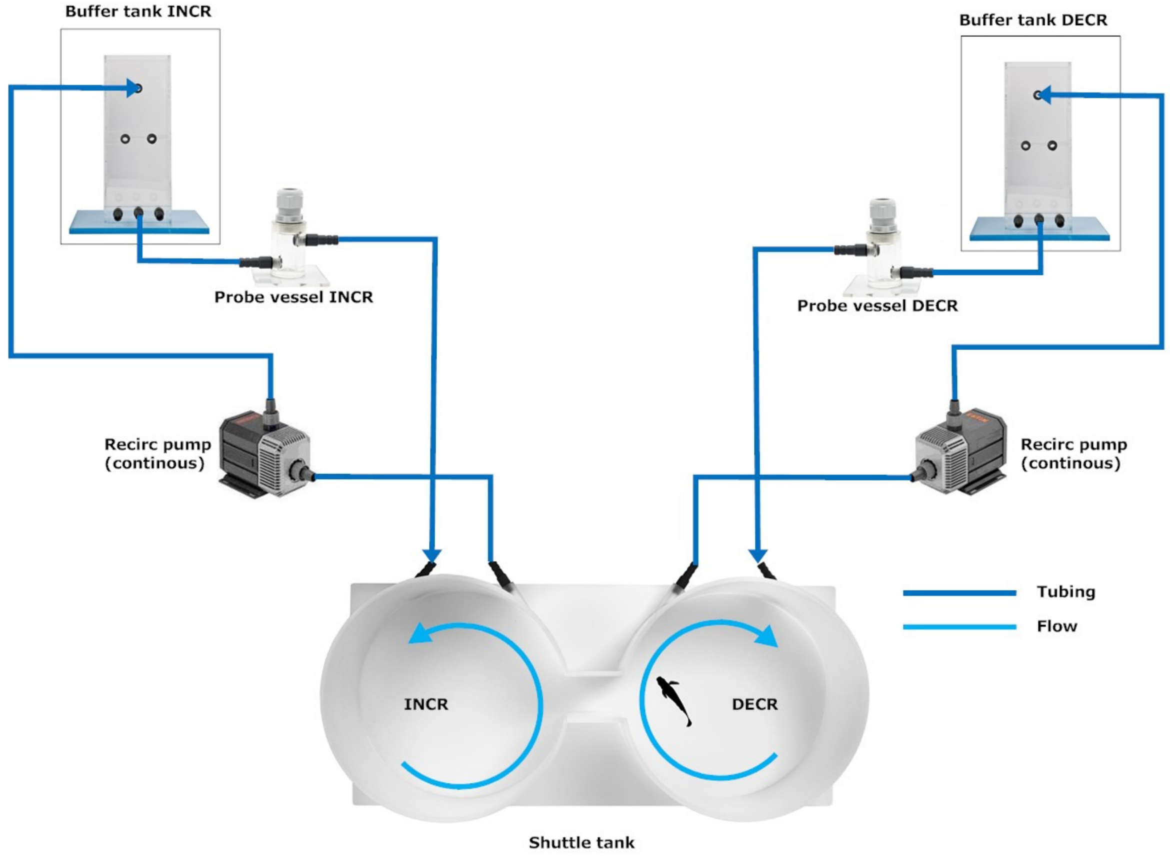
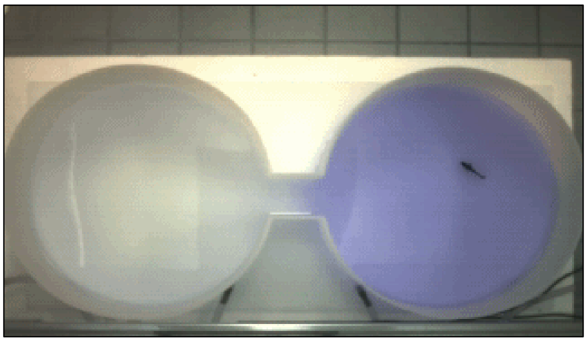


Figure 2: How to connect the Shuttle tank with the recirculation pumps, buffer tanks and oxygen probe vessels for running a ShuttleBox oxygen experiment.

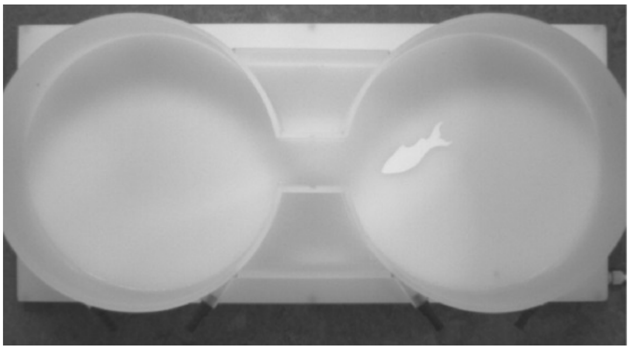
- To avoid mixing between the two sub-compartments, it is important that the water surface level is equal between the two sides, i.e. to avoid water from one side entering the other due to pressure differences (gravity). The separation of flow between the two sub-compartments can be visualized by adding a colored dye (e.g. red wine or food coloring) to one sub-compartment and follow it over time.



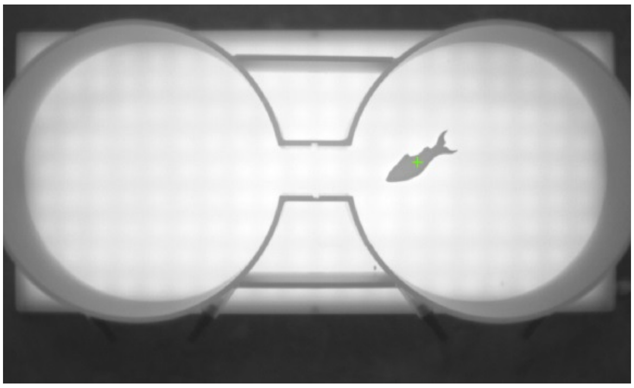
<u>Optional</u>

For background illumination of the experimental animal in the Shuttle tank an infra-red light panel can be placed under the tank. Animals will appear pitch black against a bright white background. With light from above reflections from the water surface can disturb tracking affect animal behavior.

Shuttle tank without IR background lightning.



Shuttle tank with IR background lightning.



It is **HIGHLY RECOMMENDED** using infrared background lighting, when

- working with organisms that change color depending on environment.
- lighting from above result in light reflections from the water surface.
- daylight is an issue, i.e. if changing light regime influence animal behavior.

4.3 O_2 and N_2 for gas concentration regulation

Connect the O_2 and N_2 gas bottles (not supplied) with the solenoid valves (RE) and air stones using gas tight tubing as shown in Figure 3. Place the air stones in the bottom of the buffer tanks.

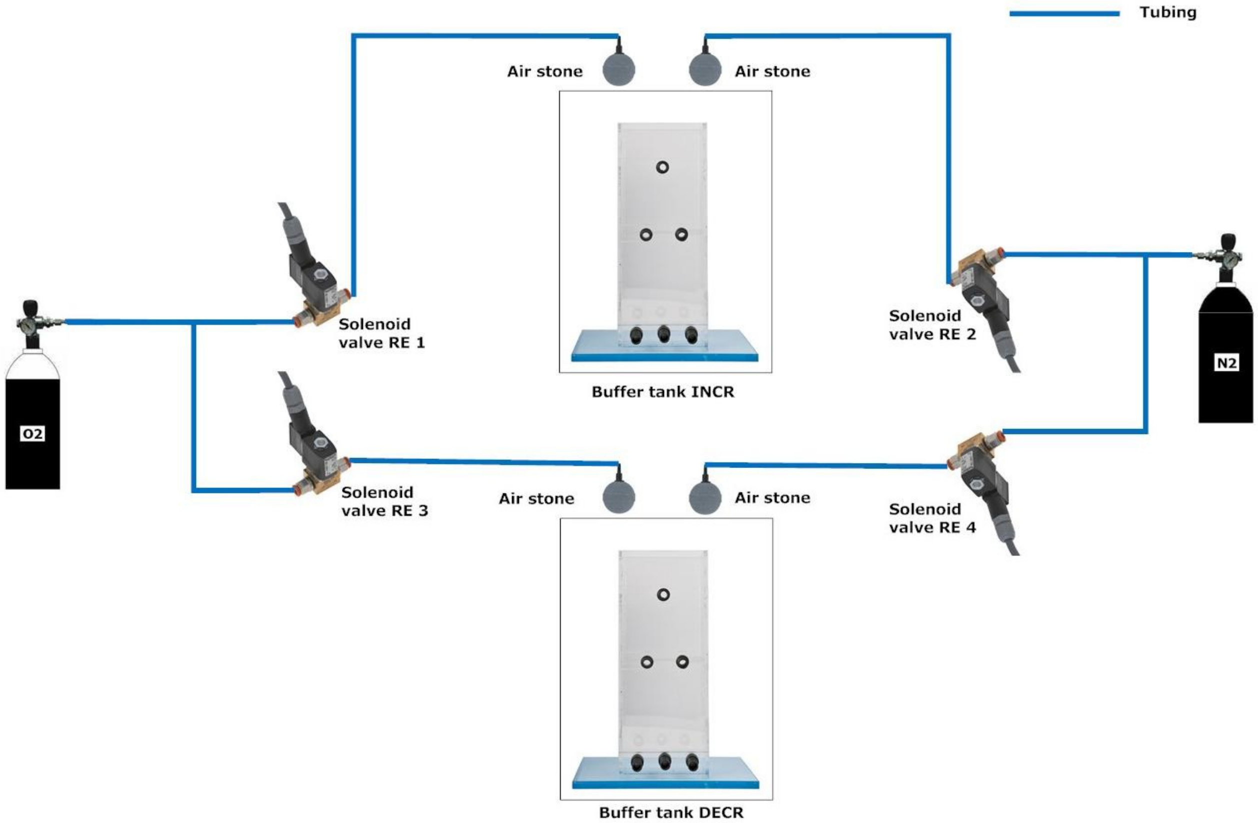


Figure 3: How to connect the buffer tanks with the O_2 and N_2 gas bottles (not supplied) for running a ShuttleBox oxygen experiment.

- Instead of using O_2 supplied in a compressed gas bottle, the oxygen concentration can be controlled using compressed laboratory air or an air pump. The response of the system will depend on the air supply.
- The buffer tank comes with six fittings for tube connections. Only four are used for the Shuttle Box System for oxygen. To close off the remaining two connect them with a short piece of tubing.

5. Using ShuttleSoft

5.1 Start up

The ShuttleSoft software tracks the position of the experimental animal on the principle of contrast between the tank and the animal. It determines in real time whether the animal is in one or the other sub-compartment of the experimental ShuttleBox tank. The software then activates/deactivates solenoid valves or pumps connected to digital relays on the DAQ instrument to control the dissolved oxygen concentration in the two sub-compartments in a dynamic way depending on animal real-time position, e.g. it is the behaviour of the animal that determines the dissolved oxygen concentration.

It is also possible to set up a static experiment during which the dissolved oxygen concentration in the two sub-compartments are kept at fixed levels defined by the user. The software will save all input data and calculated values to an Excel compatible .cvs file.

Log onto the PC as administrator.

- Select Start → ShuttleSoft →right-hand click properties → Compatibility → Select 'Run this program as administrator'
- ShuttleSoft should always be run under administrator rights, by enabling this privilege level, all settings from the last experiment will be saved automatically.

Connect the USB hardkey dongle with the ShuttleSoft Licence and Start ShuttleSoft.

- If the USB hardkey dongle is not connected to the PC, an error dialog box pops up. Connect the USB hardkey dongle to your PC and wait for the device to be recognized, then click Retry.

Choose control variable Oxygen.

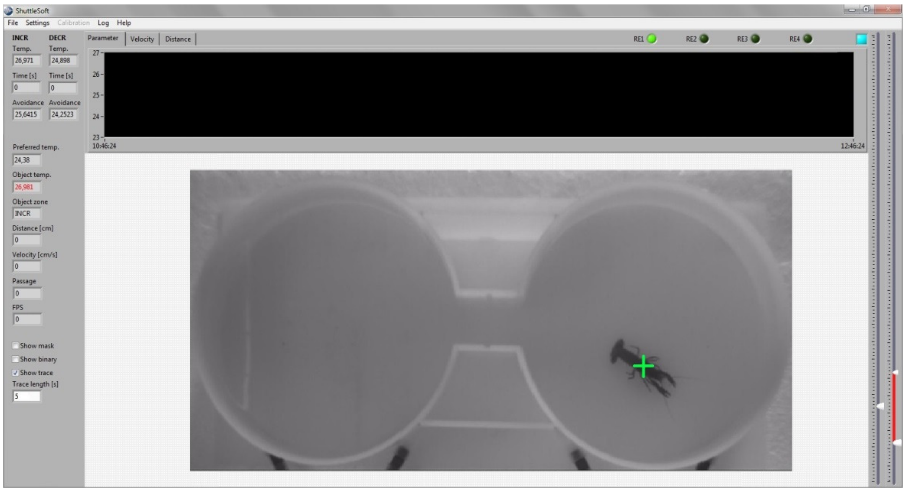
ShuttleSoft will connect automatically to the first uEye camera found.

- If no uEye camera is found, ShuttleSoft will detect all USB cameras connected to the PC and ask the user which camera should be used. Choose uEye.
- If no USB camera is found connected to the PC an error message will appear. Connect the uEye camera to the PC.

ShuttleSoft now looks for a DAQ-M instrument connected to the PC and is named dev1.

- If ShuttleSoft is unable to locate the DAQ-M instrument connected to the PC please see Section 8. TroubleShooting.

When ShuttleSoft has located the DAQ-M instrument, the initialization is done and images from the camera will now be shown on the screen.



5.2 Start an experiment

Place your experimental animal in the shuttle tank to acclimate for an appropriate time.

Adjust the camera height and angle to maximize shuttle tank image (see section 4). Adjust camera settings (frame rate) in uEye Cockpit.

Start Shuttlesoft.

5.2.1 Threshold values

Select Show Binary.

Use the vertical threshold bars to find the animal inside the Shuttle Box.

Start by choosing a narrow range (short distance between the 2 bars) and then use the right hand side slider to scan the image for your animal.

If necessary use the filter options found in Settings→Filter to remove irrelevant pixels in the picture.

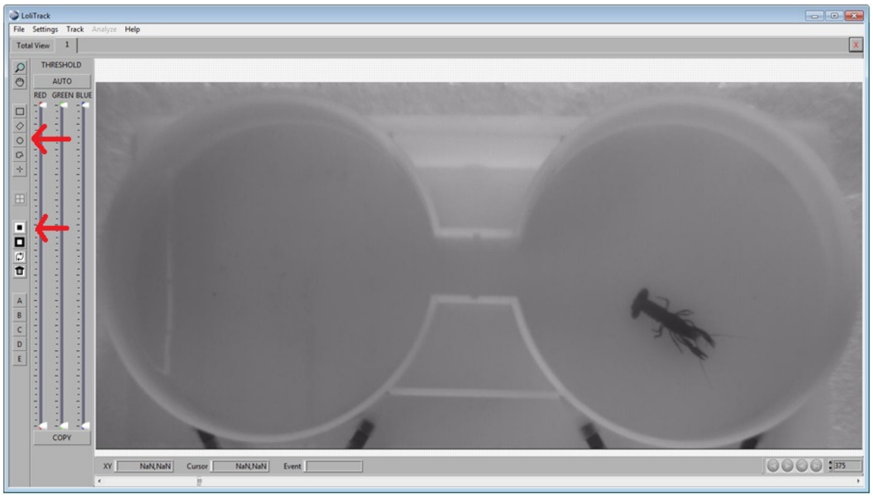
When done, select Show Binary again to view unmodified images.

To zoom place the mouse over the picture and use the scroll button to zoom.

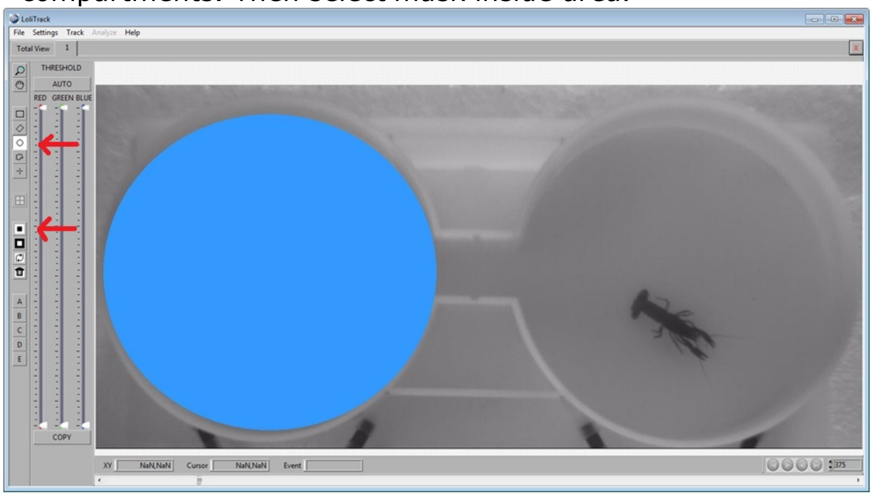
5.2.2 Creating a mask

To avoid tracking any moving objects outside the ShuttleBox system draw a Mask excluding areas outside the experimental Shuttle tank for analysis.

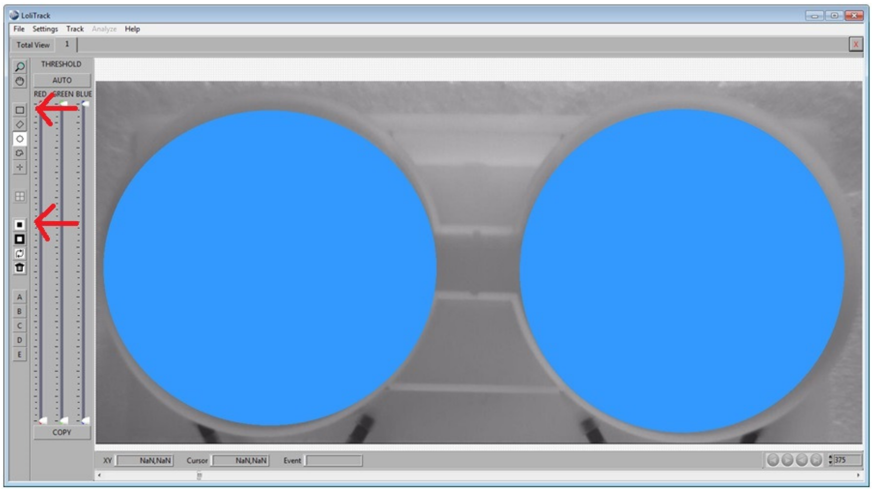
Select Settings→Mask



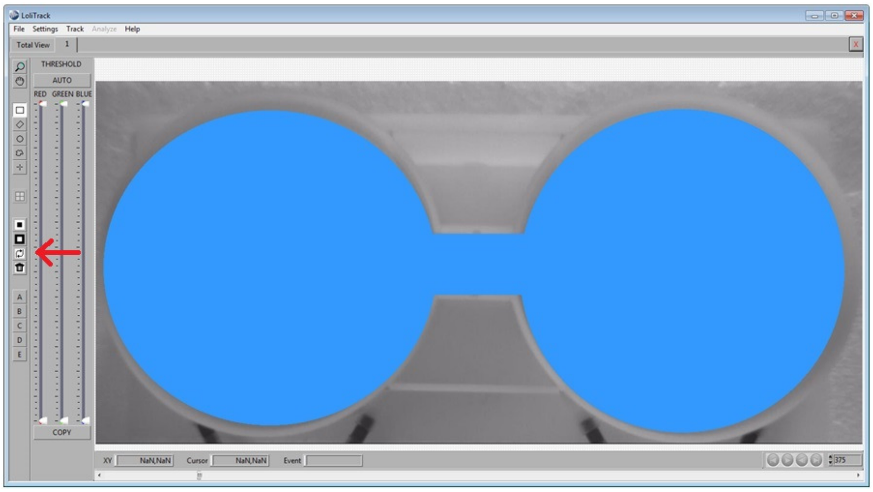
1. Use the circle button to draw a circle in one of the sub-compartments. Then select *mask inside area.*



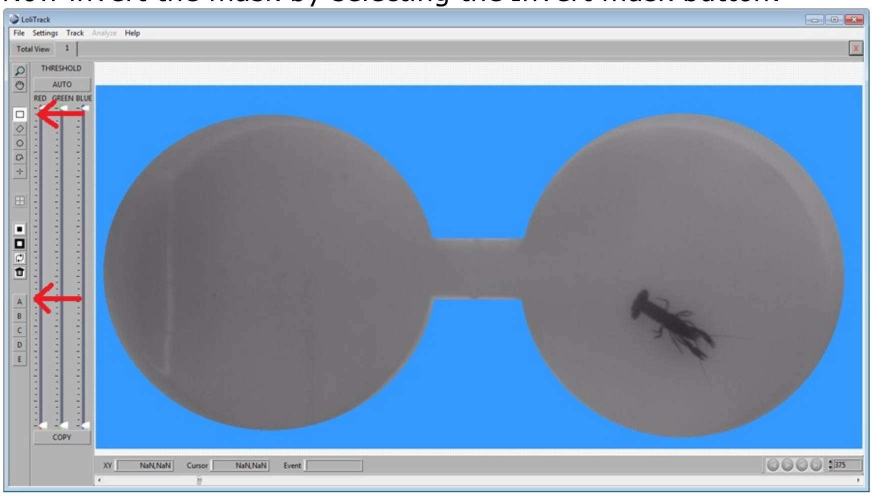
2. Use the circle button to draw a circle in the other sub-compartment. Then select *mask inside area.*



3. Use the rectangular button to draw a rectangle between the two compartments. Then select *mask inside area.*



4. Now invert the mask by selecting the *Invert mask button*.



Created masks can be saved or loaded as bitmap files for later use.

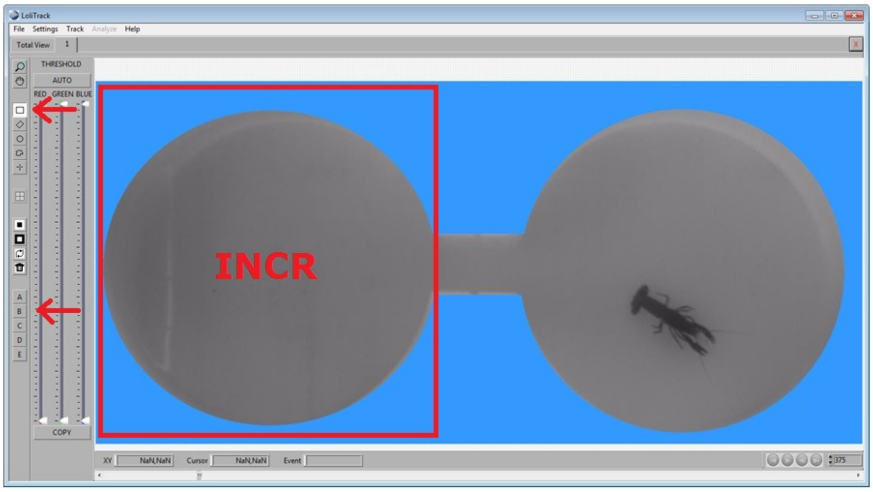
When maximizing the dialog box, the image will scale. When moving the mouse over the camera, it is possible to zoom/unzoom the image via the scroll button on the mouse.

5.2.3 Experimental zones

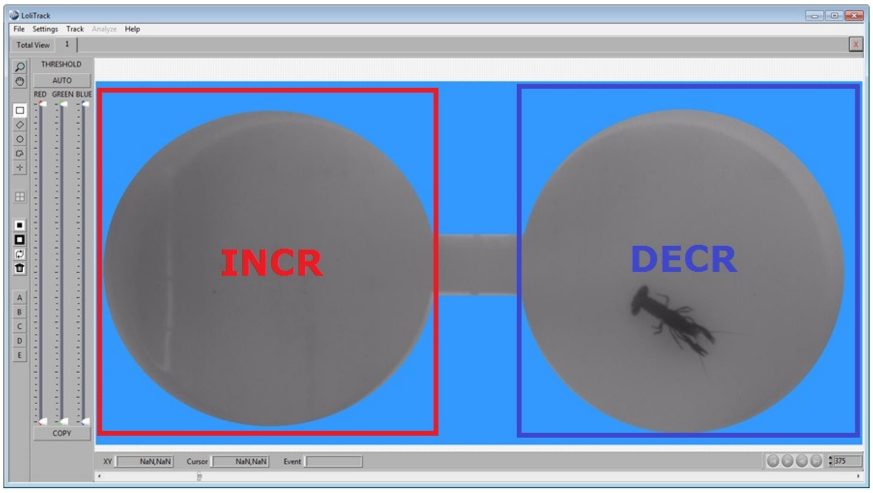
To define in which sub-compartment of the Shuttle tank the dissolved oxygen concentration (DO) will increase and decrease, respectively, experimental zones must be defined.

Select Settings→ Zones

1. Drawing a closed figure (e.g. circle or square) over one of the Shuttle tanks sub-compartments. Decide if the closed figure should be used as INCR (increasing DO concentration) or DECR (decreasing DO concentration) zone by selecting either the I-button or the D-button.



2. Draw a second closed figure over the other sub-compartment and choose this as the alternate zone.



The example above shows what a typical mask and zones could look like running a ShuttleSoft experiment.

Any areas outside the two zones will be referred to as OFF zone.

We recommend leaving a narrow space between the two user-defined zones corresponding to the short channel connecting the sub-compartments. This avoids errors or misinterpretations associated with animals taking positions in the connecting channel for prolonged periods of time.

When maximizing the dialog box, the image will scale.

When moving the mouse over the camera image, it is possible to zoom/unzoom the image via the scroll button on the mouse.

Zones can be saved and loaded as graphics (.bmp file) for later use.

5.2.4 Pixel calibration

In order to obtain behavioural data in metric units, e.g. distance moved in meter or cm a calibration has to be done.

Select Calibration→Pixel

Use the mouse to draw a line along an axis of a known distance in the image, e.g. place a ruler in the experimental tank. Then enter the distance and unit for the line. The pixel-to-meter ratio is now calculated.

5.2.5 Sensor calibration

In order to convert the analog inputs from the oxygen instrument to a relevant unit (% air saturation) a sensor calibration in ShuttleSoft has to be done.

Select Calibration→Oxygen

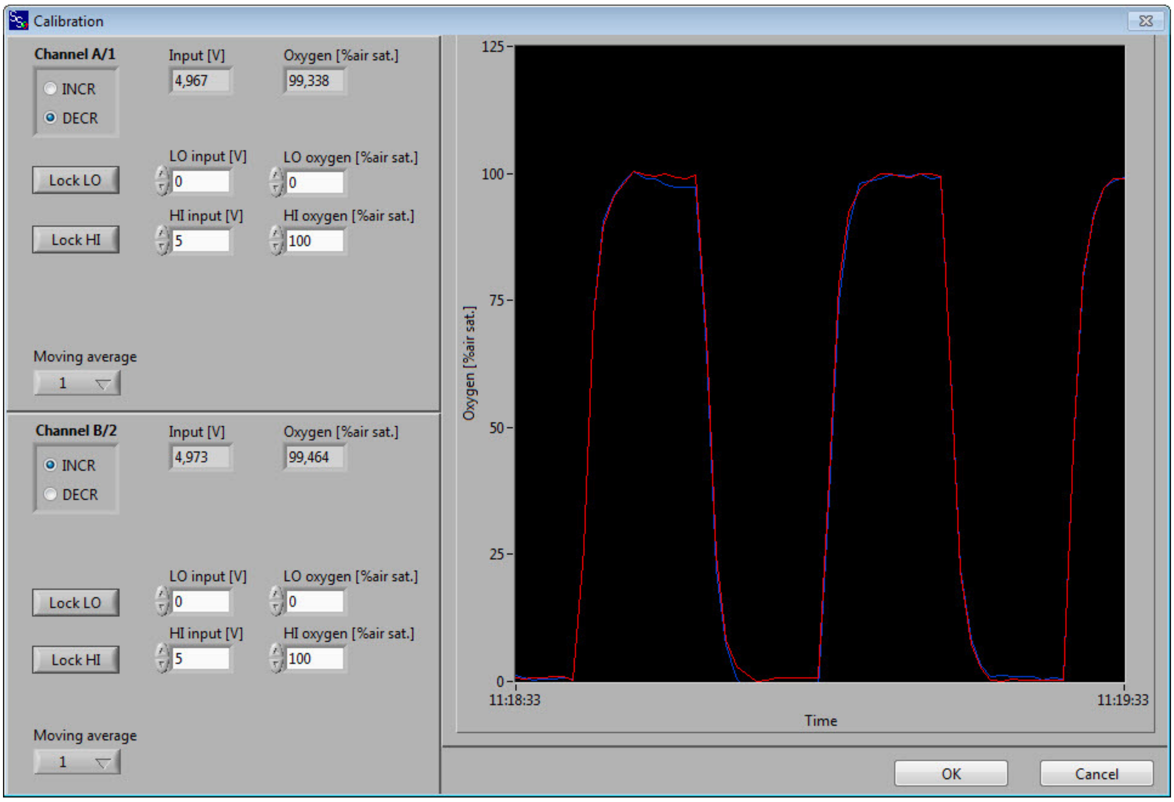
Perform a two-point calibration.

Select if input IN A (or 1) should be used for monitoring oxygen in the (red) INCR zone or the (blue) DECR zone. Do the same for IN B (or 2).

- Place the oxygen sensor in an air equilibrated water sample, wait till the registered oxygen stabilizes and click 'LOCK HI' (100 % air saturation). Air equilibrated water can be prepared by purging atmospheric air into the sample water, e.g. with an air pump and air stone.
- Press the 0-cal button on the OXY-REG instrument to short-circuit the signal displaying 0 on the instrument. Click 'LOCK LO' (0% air saturation) to save the corresponding voltage. Alternatively, place the oxygen sensor of the INCR/DECR zone in an oxygen

free water sample, wait till the registered value stabilizes and click 'LOCK LO' (0% air saturation).

- Replace the oxygen sensor into the probe vessel of the appropriate zone (i.e. INCR or DECR). It should now read the dissolved oxygen concentration of the assigned zone of the Shuttle box.
- Repeat aforementioned steps to perform a two-point calibration of the senor of the remaining zone (i.e. INCR or DECR).



Now ShuttleSoft will show the dissolved oxygen (DO) concentration in the zone (or sub-compartment) in which the animal is present. During times when the animal is in OFF zone, an average between the DO concentration in the INCR and DECR zones will be calculated.

5.2.6 Set-points

Select Setting →Experiment

Choose between a dynamic or static regulation of the water quality.

Static Experiments:

The dissolved oxygen concentration in the two sub-compartments is kept at the chosen levels, e.g. for choice/avoidance experiments.

ShuttleSoft will regulate the dissolved oxygen concentration around the set-points and hysteresis selected for each of the two zones. For example if values 80 % air saturation and 1% are selected for the HI zone, ShuttleSoft will start deoxygenating (bubbling with N_2) the water when the dissolved oxygen concentration rises above 81% air saturation in the INCR zone and will stop N_2 bubbling as soon as the dissolved oxygen concentration reaches 80% air saturation. When the dissolved oxygen concentration drops below 79 % air saturation ShuttleSoft will start oxygenating the water (bubbling with O_2) in the INCR zone.

Enter the HI zone setpoint and LO zone setpoint values and the values for hysteresis.

Dynamic Experiments:

Oxygen concentration in the two sub-compartments will depend on the behaviour of the experimental animal. When in one (INCR) compartment the dissolved oxygen concentration will increase, and when the animal then shuttle to the other compartment, it will decrease, e.g. behavioural oxygen regulation. The dissolved oxygen concentration difference between the two compartments is kept constant (Delta (Δ) value) and the animal's position will determine the dissolved oxygen concentration.

Enter the HI zone setpoint and LO zone setpoint values and the values for hysteresis when running a Static Experiment.

OR

Enter Δ-value and the value for hysteresis when running a Dynamic Experiment.

OPTIONS:

- It is also possible to enter a maximum and minimum dissolved oxygen concentration, e.g. it is the oxygenating and deoxygenating capacity that sets the maximum rate obtainable. If the INCR value is greater than the maximum value ShuttleSoft will not increase the INCR value by triggering RE 1 relay. The same goes for the DECR value, minimum value and RE 4.
- It is also possible to enable and enter a system maximum rate. If enabled ShuttleSoft will calculate the rate of the system by which the INCR and DECR zones increase/decrease and regulate if the rate exceeds the entered max rate.

5.2.7 Data Logging and data handling

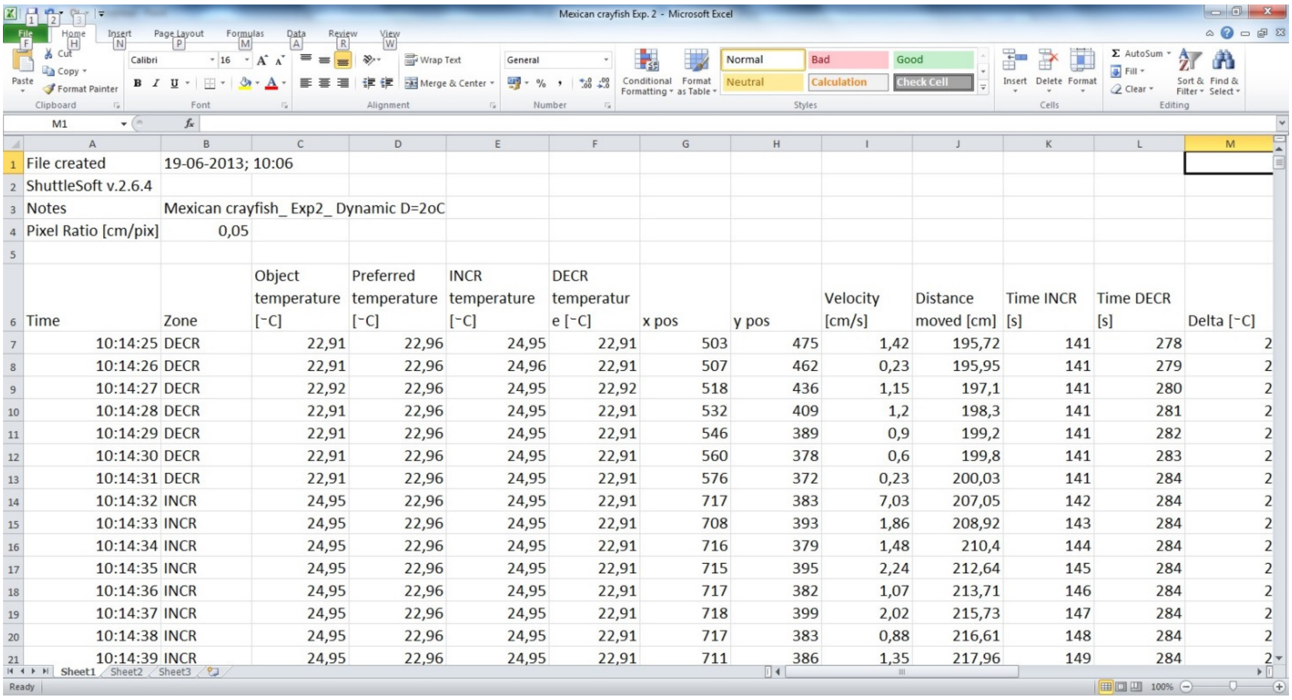
Select Log→Start logging data.

- Enter notes (optional) about the experiment in the notes field.
- Choose a destination for the data file. Select OK and data logging starts.

To stop logging, click Log→Stop

On exit the software will close the camera input and save all settings and then close ShuttleSoft.

Data is saved to a .dat file, which can be imported directly into Excel.



File created	19-06-2013; 10:06											
ShuttleSoft v.2.6.4												
Notes	Mexican crayfish_ Exp2_ Dynamic D=2oC											
Pixel Ratio [cm/pix]	0,05											
Time	Zone	Object temperature [-C]	Preferred temperature [-C]	INCR temperature [-C]	DECR temperatur e [-C]	x pos	y pos	Velocity [cm/s]	Distance moved [cm]	Time INCR [s]	Time DECR [s]	Delta [-C]
10:14:25	DECR	22,91	22,96	24,95	22,91	503	475	1,42	195,72	141	278	2
10:14:26	DECR	22,91	22,96	24,96	22,91	507	462	0,23	195,95	141	279	2
10:14:27	DECR	22,92	22,96	24,95	22,92	518	436	1,15	197,1	141	280	2
10:14:28	DECR	22,91	22,96	24,95	22,91	532	409	1,2	198,3	141	281	2
10:14:29	DECR	22,91	22,96	24,95	22,91	546	389	0,9	199,2	141	282	2
10:14:30	DECR	22,91	22,96	24,95	22,91	560	378	0,6	199,8	141	283	2
10:14:31	DECR	22,91	22,96	24,95	22,91	576	372	0,23	200,03	141	284	2
10:14:32	INCR	24,95	22,96	24,95	22,91	717	383	7,03	207,05	142	284	2
10:14:33	INCR	24,95	22,96	24,95	22,91	708	393	1,86	208,92	143	284	2
10:14:34	INCR	24,95	22,96	24,95	22,91	716	379	1,48	210,4	144	284	2
10:14:35	INCR	24,95	22,96	24,95	22,91	715	395	2,24	212,64	145	284	2
10:14:36	INCR	24,95	22,96	24,95	22,91	717	382	1,07	213,71	146	284	2
10:14:37	INCR	24,95	22,96	24,95	22,91	718	399	2,02	215,73	147	284	2
10:14:38	INCR	24,95	22,96	24,95	22,91	717	383	0,88	216,61	148	284	2
10:14:39	INCR	24,95	22,96	24,95	22,91	711	386	1,35	217,96	149	284	2

Example of a ShuttleSoft Excel data file from a Shuttle Box Systems for Temperature experiment.

6. Additional ShuttleSoft menus and screen views

6.1 Screen views

When running a ShuttleBox oxygen experiment, the development in dissolved oxygen concentration in the two sub-compartments as it develops over time is shown in the **Parameter** view (default pane one).

The dissolved oxygen concentration in the INCR compartment is shown in red and the dissolved oxygen concentration in the DECR is shown in blue. The dissolved oxygen concentration in the compartment where the experimental animal is present is shown in yellow.

The left side of the view always show the O_2 concentration in each zone, the time spend in each zone and the avoidance oxygen cnncetration in each zone.

- The value for the avoidance parameter is defined as the value of the measured parameter (O_2) when the experimental organism leaves the zone. The value shown is the value of the parameter when the organism last left the zone. The average avoidance value is logged in the data file.

Also shown is Preferred oxygen. This is the median dissolved oxygen concentration of the water where the experimental organism was located throughout the experiment.

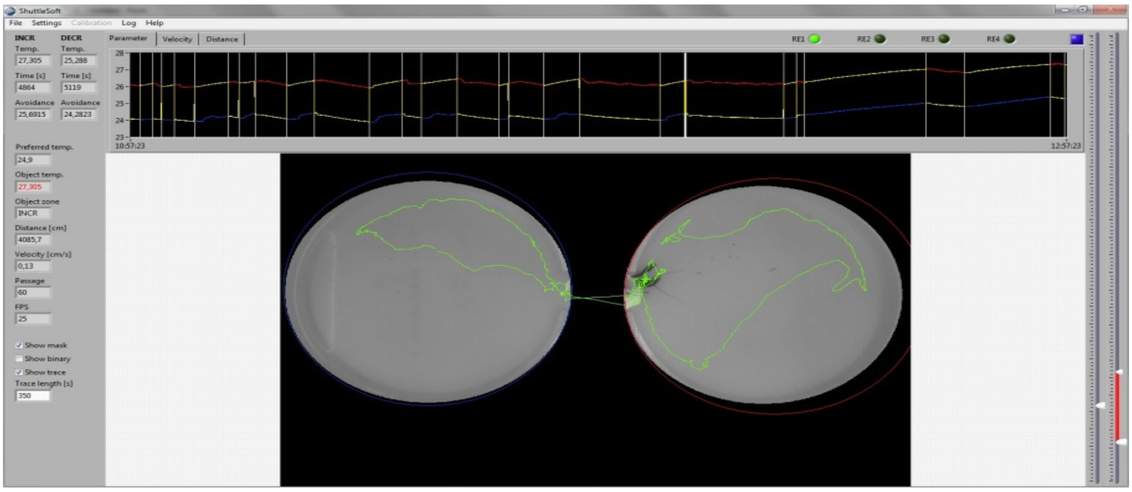
The parameter value for the water where the experimental animal is located at a given time in shown in Object oxygen, and below is shown in which zone (INCR or DECR) the organism is currently positioned.

In the **Velocity** view (second pane) the velocity of the experimental organism is depicted graphically.

In the **Distance** View (third pane) the accumulated distance travelled by the experimental organism over the course of the experiment is depicted graphically.

- To change the viewed period of time point PC cursor on graph view, right click on mouse and select Scale X. Enter time period in minutes (1-120min).
- To change viewed dissolved oxygen concentration/velocity/distance scale point PC cursor on graph view, right click on mouse and select Autoscale Y, or double-click on the highest and lowest value on the Y-axis and enter new maximum and minimum values to be shown.

To show the trace of the path travelled by the experimental animal over a defined period of time (trace length) select Show trace.



Example of trace from a Shuttle Box Systems for Temperature experiment

6.2 Camera settings

Select Settings→Camera

Opens a dialog box where users may set the video settings and resolution of the camera. Please note, that a change in the resolution requires a restart of ShuttleSoft.

6.3 Filters

Select Settings →Filter

This menu will open a dialog box where user may set the filter options.

- *Fill* object is an image processing operation that fills objects that are only partly tracked. Note, that objects in contact with the image border will not be filled. Use Fill Objects if lighting is uneven, enhancing the objects contrast to the background.

- Reject Borders is an image processing operation that eliminates objects that are in contact with the border of an image.

- Erosion is an image processing operation that removes pixels on object boundaries. Using a 3×3 set of coordinate points (a so called structuring element) each pixel is compared to its neighbours, removing any pixel that does not match its neighbouring (foreground or background, respectively) pixels. The erosion procedure is used to remove pixel noise or irrelevant pixels in the picture. If not removed pixel noise may interfere with the tracking of the experimental animal.

6.4 Help Menu

Select Help →About

This menu will open a dialog box, displaying the version number of the ShuttleSoft software and contact information for Loligo® Systems.

7. Terminology and settings

Table 1: Glossary table of terms

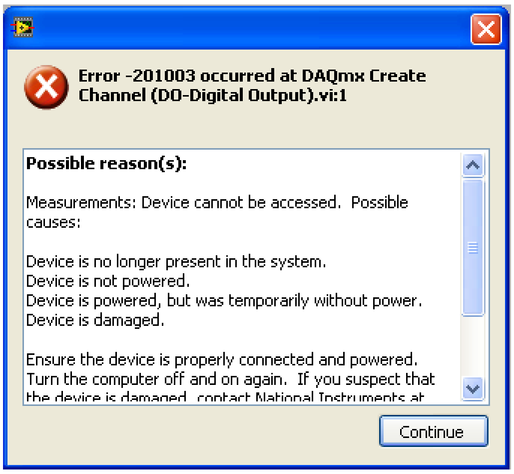
Terms	Definition
INCR O2	This field indicates the current O2 saturation for the INCR zone.
DECR O2	This field indicates the current O2 saturation for the DECR zone.
Time INCR [s]	This field indicates how long the object has stayed the INCR zone. This value will be set to 0, when user starts logging.
Time DECR [s]	This field indicates how long the object has stayed the DECR zone. This value will be set to 0, when user starts logging.
Avoidance Upper	This field calculates the upper avoidance value. Every time the object leaves the INCR zone, the value is stored. The Avoidance Upper value is the mean value of all stored values. All values will be deleted, when user starts logging.
Avoidance Lower	This field calculates the lower avoidance value. Every time the object leaves the DECR zone, the value is stored. The Avoidance Lower value is the mean value of all stored values. All values will be deleted, when user starts logging.
Object zone	This field indicates whether the object is in INCR, DECR or OFF zone.
Object O2	This field indicates the current O2 saturation/ that the animal is exposed to.
Distance	In this field the distance moved by the object is calculated. The unit of measure can be set via the menu for pixel to meter calibration. This value will be set to 0, when user starts logging.
Velocity	In this field the velocity of the object is calculated once per second. Please note that the object has to move at least a half pixel length from frame to

	frame to be obtained as a movement.
Object mean O2	In this field the mean value of the object O2 is calculated. This value will be set to 0, when user starts logging. During dynamic experiments this value will approach the true preference value of the animal.
FPS	Frames per second. This field shows the frame rate of the camera. If frame rate should be low, decrease the resolution in the camera settings menu or use a faster PC.
Threshold bars	Use the bars to set the threshold used to find the binary picture. On the left bar it is possible to set the range of the threshold values. The right bar is to move both sliders. *Example*: The two sliders on the left are set to 40 and 70 and the red indication is between them. Every pixel that has a value between 40 to 70 will be coloured red and all other pixels will be coloured black. The slider on the right bar will stay on 55. Now the user moves the slider to 110. The red indication on the left bar will now be between 95 and 125.
Show Mask	Use this button to toggle between unmodified video images and masked images.
Show Binary	Use this button to toggle between unmodified video images and threshold images.
Show Trace	Use this button to enable/disable a trailing trace from being shown behind the moving animal and set the duration of the trailing trace in seconds.
RE1	This green diode indicator gives the relay status for relay 1. On relay 1 the oxygen valve for the INCR zone must be connected.
RE2	This green diode indicator gives the relay status for relay 2. On relay 2 the nitrogen valve for the INCR zone must be connected.

RE3	This green diode indicator gives the relay status for relay 3. On relay 3 the oxygen valve for the DECR zone must be connected.
RE4	This green diode indicator gives the relay status for relay 4. On relay 4 the oxygen valve for the DECR zone must be connected.
Log	This blue diode will blink while logging. When moving the mouse over the diode, the actual saving path will be shown.
Image	Here the video images from the camera are shown. When maximizing ShuttleSoft on your PC monitor, the image will scale accordingly. Move your cursor over the image and zoom/unzoom by using the scroll button on your mouse.
Parameter / Velocity / Distance graph	On these graphs the values vs. Time are shown, e.g. reading from the inputs IN A (or 1) and IN B (or 2). The y-scale can be changed by double clicking on the scale and entering a new number. Right clicking will open a menu, where x-scale can be changed etc.

8. TroubleShooting

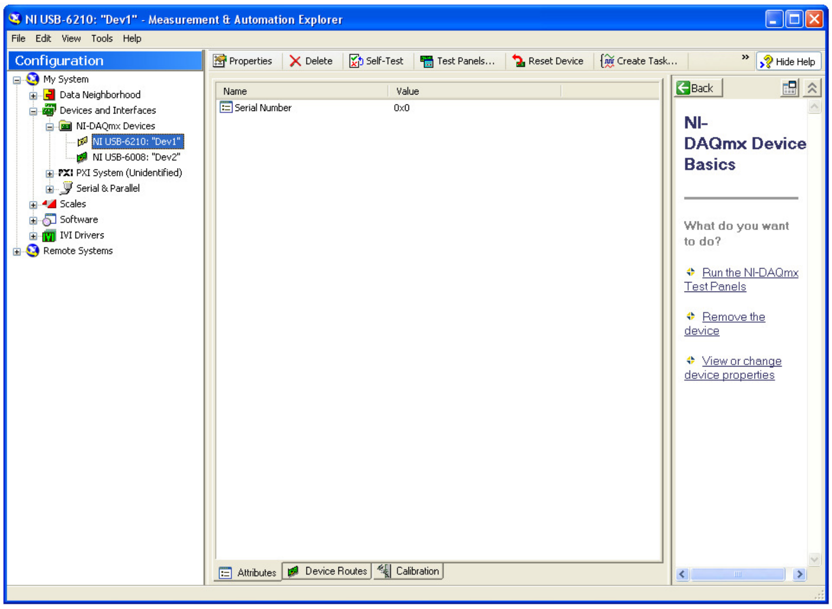
8.1 DAQ connection failure



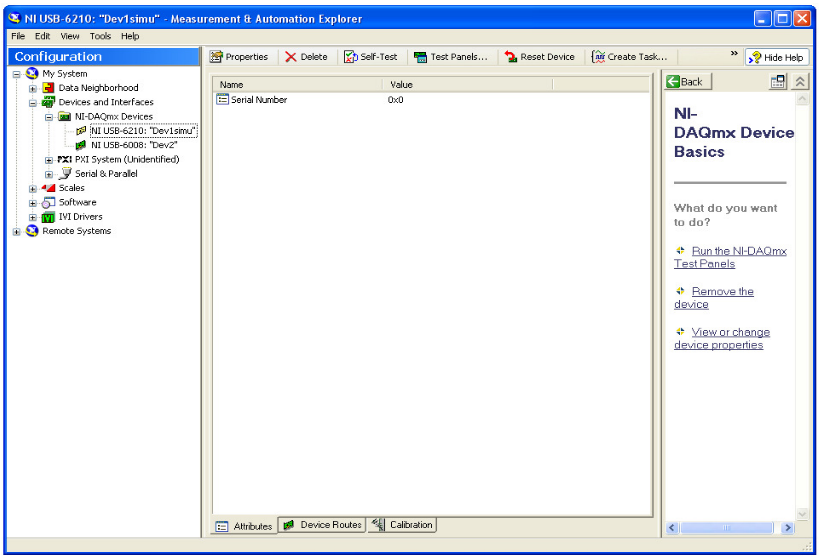
The error shown in the screen above occurs, when the NI-USB 6008/6009 DAQ card is not properly connected to the PC. This error can occur in 2 ways.

8.1.1 Name is not "dev1"

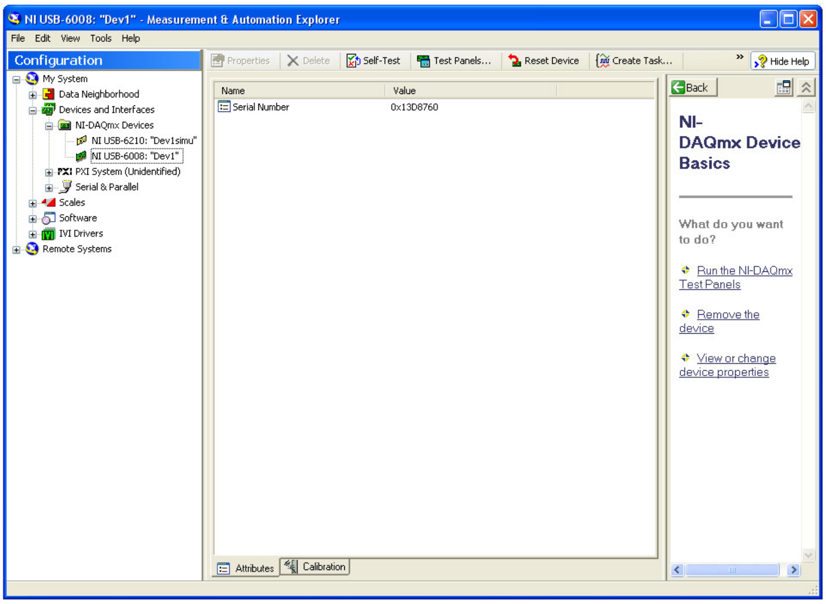
To change the device name, open the NI program called Measurement & Automation (look for desktop icon). The following example on **Fejl! Henvisningskilde ikke fundet.** shows a simulated device named as "dev1". The NI-USB 6008 DAQ card is registered as "dev2"



Right click with mouse on the “dev1”, and choose rename, or press F2. Change the name to something different than “dev1”. In this example the name is changed to “dev1simu”.



Now change the device name of the NI USB-6008 DAQ card to “dev1”. The DAQ-S instrument should now work properly with ShuttleSoft.



8.1.2 Another USB device was improperly removed

If an USB device, like a USB memory stick, is not properly removed, while ShuttleSoft is running, there can be a connection problem with the DAQ-S instrument. To solve this problem, close ShuttleSoft. Then disconnect the USB cable between the PC and the DAQ-S instrument. Wait 30 seconds. Then connect the cable and wait. When the green diode on the front side of the DAQ-S instrument is flashing, the connection is re-established. ShuttleSoft can now be opened again.

8.2 Relays are unresponsive

Make sure the DAQ-S instrument is connected with a power cable, and the Power button is ON.

8.3 uEye camera connection failure

Make sure that no other program is using the uEye camera while using it in ShuttleSoft. Only one software program at the time can acquire data from the camera.

8.4 uEye camera settings

To change camera settings on the uEye camera, open uEye DEMO and set the settings. Then click File→Save parameters→to file.

Save the file and name it uEye_Loligo.ini. Browse to the folder where ShuttleSoft is installed. Backup the old file uEye_Loligo.ini by renaming it to e.g. uEye_Loligo_backup.ini. Now copy the uEye_Loligo.ini file to the folder and start ShuttleSoft. The settings are now loaded.