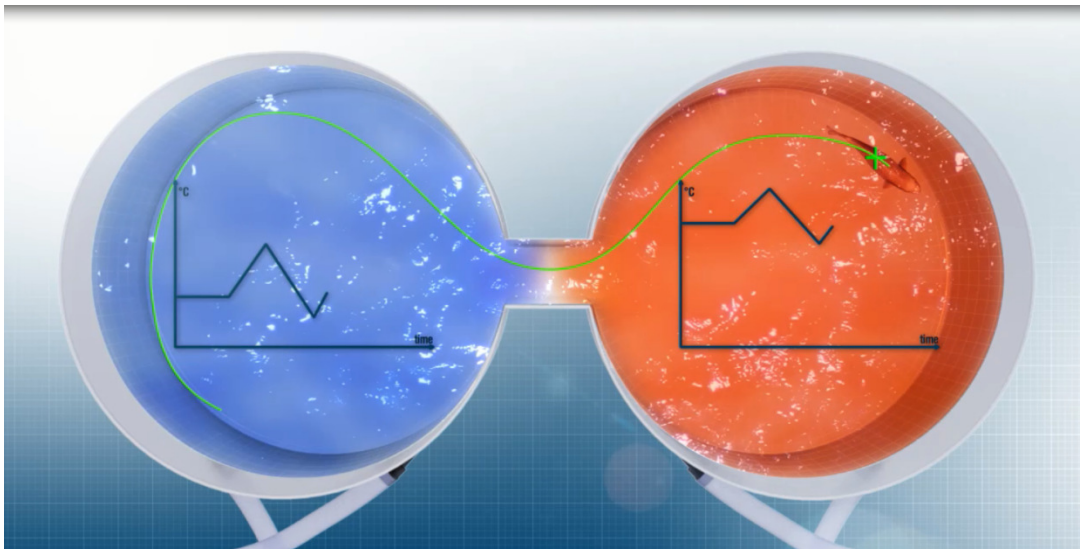
Loligo® Systems

# Shuttle Box System Temperature




# Installation & User Manual

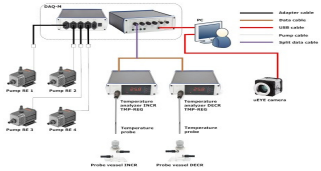
www.loligosystems.com

# Shuttle System Quick Guide

## Follow these steps to quickly start using the system

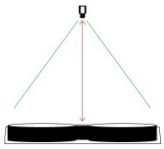
1. Install the ShuttleSoft software and the uEye-camera driver from the Loligo memory stick.
   *See Section 3*

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

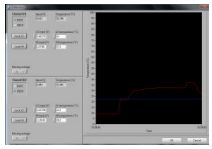


3. Start the ShuttleSoft software.
   *See Section 5.1*

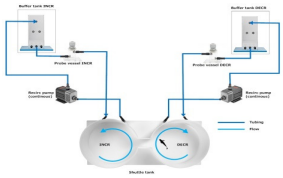
4. Mount the video camera above the shuttle tank. Make sure the image covers the entire tank.
   *See Section 4*



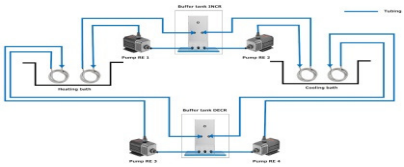
5. Calibrate the output from the temperature analyzers in ShuttleSoft.
   *See Section 5.2.5*



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



7. Place the coils in heating/cooling baths and connect them to the pumps and the buffer tanks.
   *See Figure 3, Section 4.3*



8. Set up an experiment in ShuttleSoft.
   *See Section 5.2*

# Content

# 1. List of parts

- USB memory stick (Loligo®)

- USB hardkey dongle (Wibu)

- LabView Vison runtime license certificate

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

- DAQ-M instrument
  - Adapter cables, qty. 4
  - TMP-SET for temperature control, qty. 2
  - Split data cable, qty. 1

- Temperature analyser (TMP-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 water temperature 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 water temperature to preferred values through behavior. Or you can set up two different (constant) temperature 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 temperature preference in aquatic ectotherms (as well as avoidance behavior), and automated computerized systems have been made for a range of other environmental factors like water turbidity, salinity, oxygen saturation, 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](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***

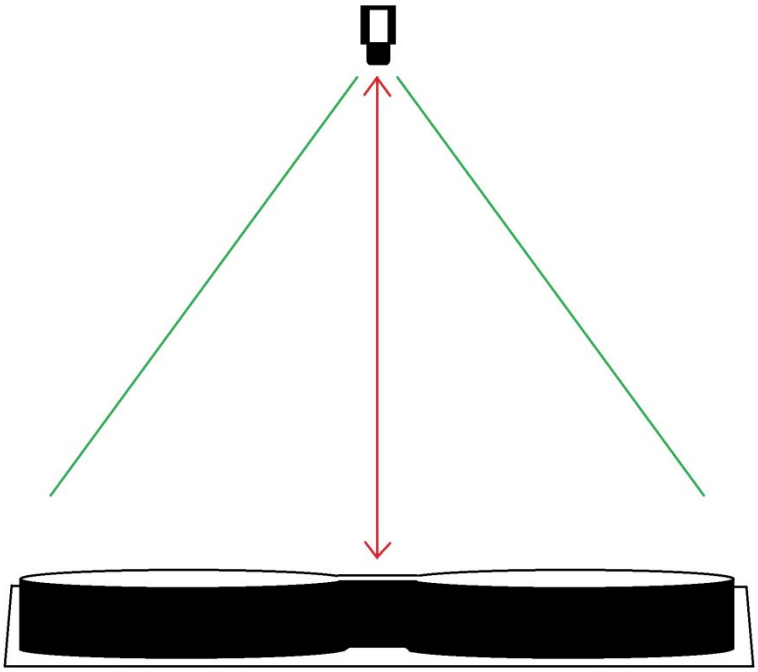
Connect the uEye USB camera to the PC.

- 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 temperature

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 pumps

Connect the DAQ-M instrument to the PC and connect the temperature instruments and temperature control pumps to the DAQ-M instrument as shown in Figure 1 and described in detail below.

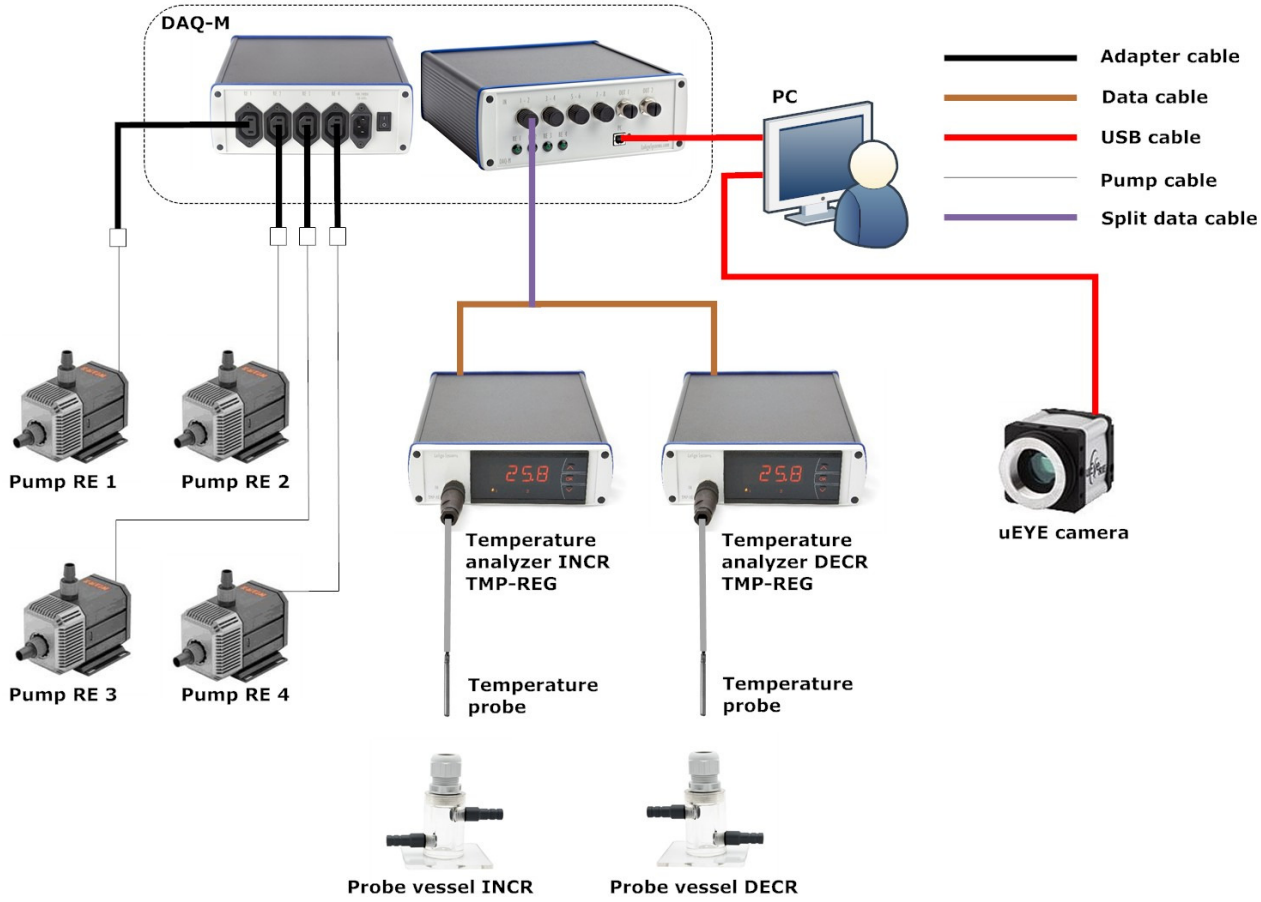


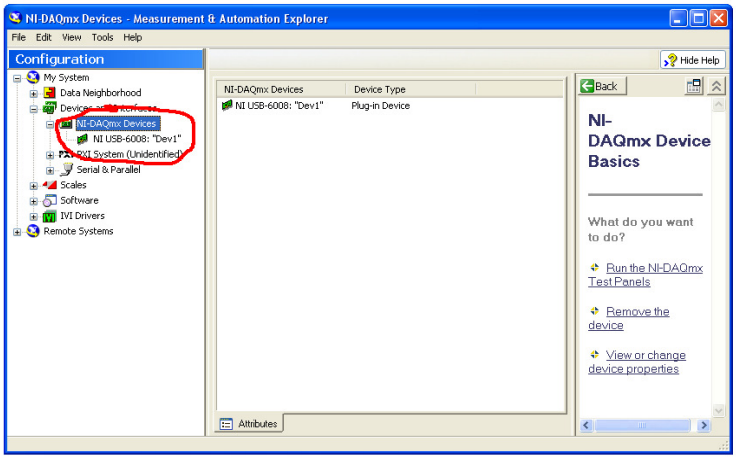

**Figure 1**: How to connect the temperature instruments (TEMP-REG), temperature probes, temperature regulation pumps (RE pumps) and uEye camera for running a ShuttleBox temperature experiment.

### 4.1.1 Connect DAQ-M instrument

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

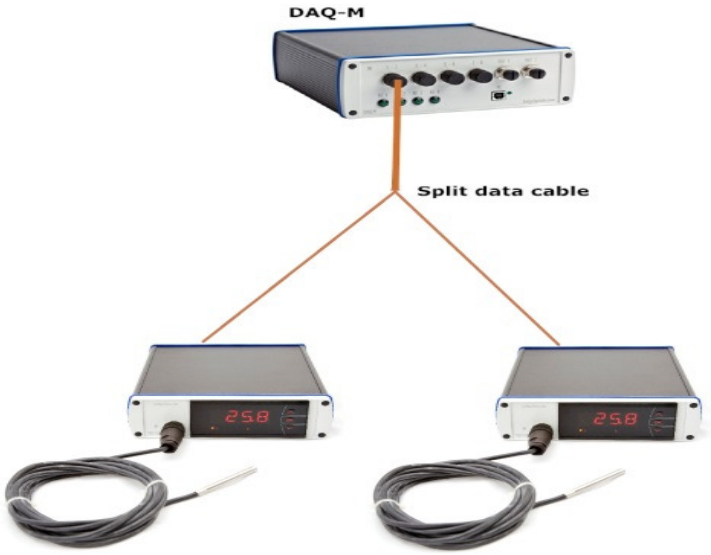
- After a few seconds the software installation will start automatically. Select "Install the software automatically (Recommended)" and follow instruction on the screen.

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 temperature analyzers and probes

Connect the two temperature analyzers (TMP-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 Pt100 temperature probe to the input labeled IN on the TMP-REG instrument front panel.

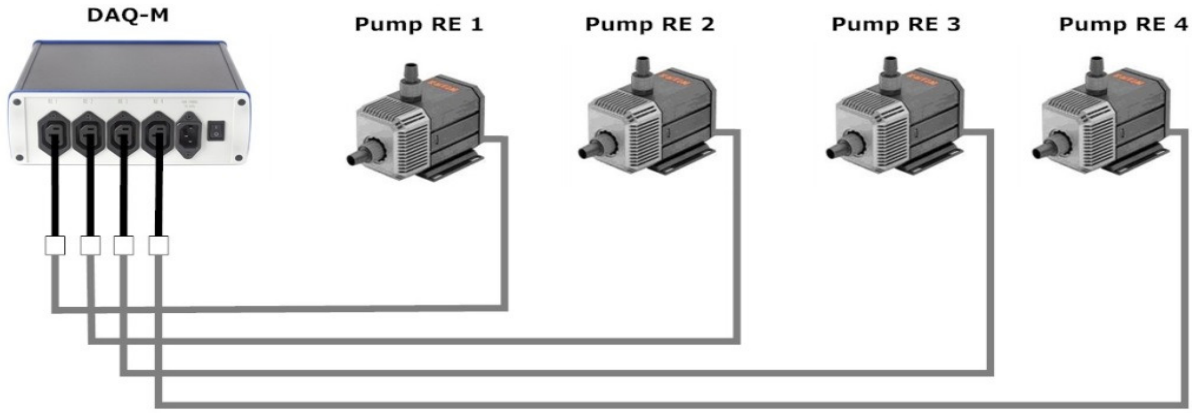
Each temperature probe is placed inside a flow through probe vessel (Figure 2). The probe should only be inserted to the top of the flow-through vessel, to allow free water flow past the probe. Do not push the probe fully into the bottom of the vessel.

For ease the temperature signal should be calibrated in ShuttleSoft before adding water to the ShuttleBox system. See Section 5.2.5 for signal calibration.

### 4.1.3 Connecting pumps

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

Make sure pumps are connected in the right order (see Figure 1) to ensure correct temperature 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.
- 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.

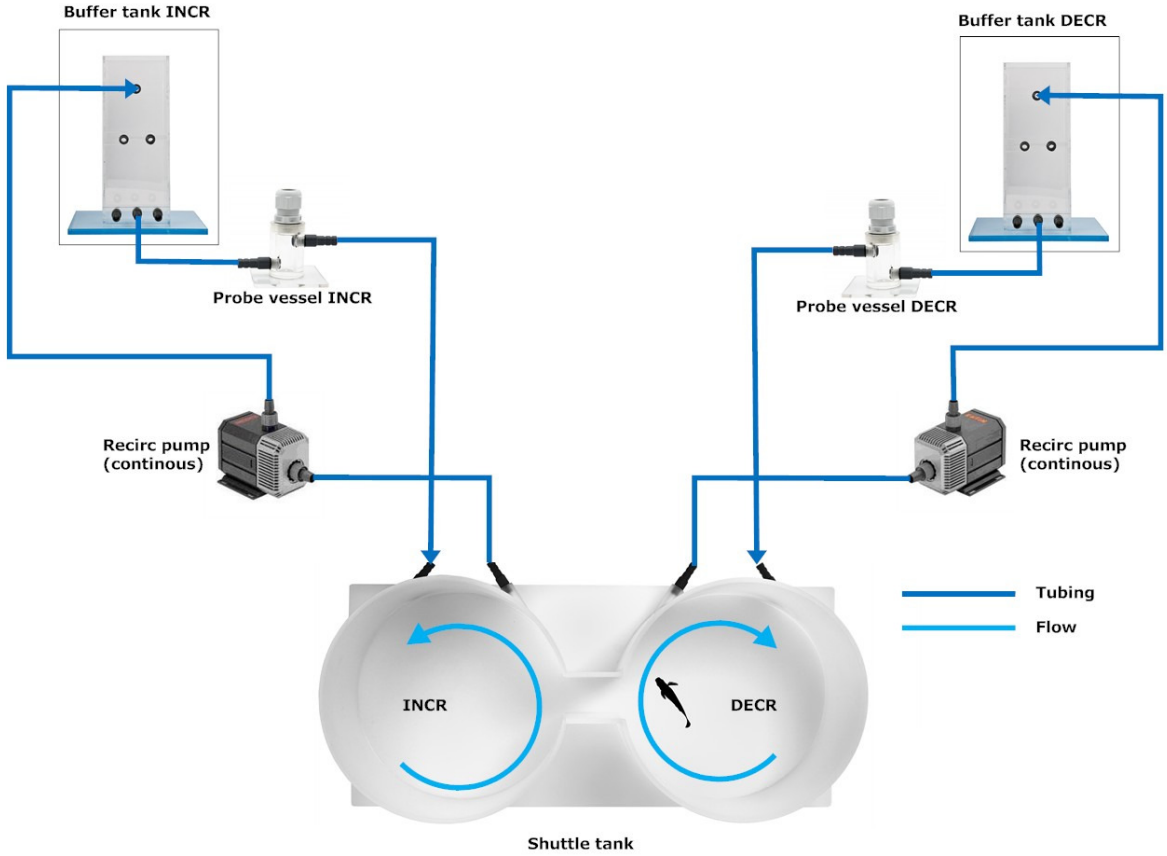
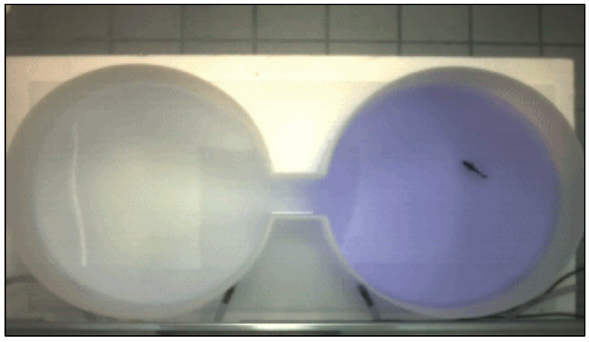



**Figure 2:** How to connect the Shuttle tank with the recirculation pumps, buffer tanks and temperature probe vessels for running a ShuttleBox temperature experiment.

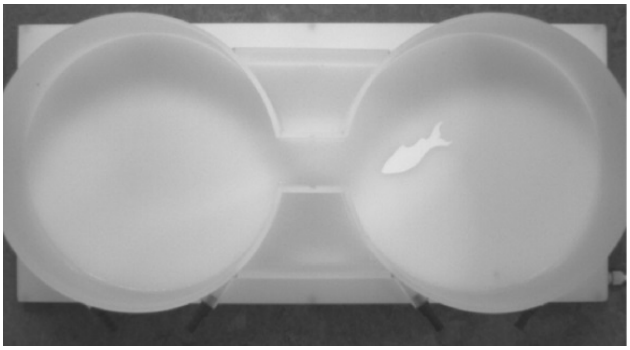
- To avoid mixing between the two sub-compartments, it is important that the water surface level is equal between the two sides, i.e. to avoid water from one side entering the other due to pressure differences (gravity). The separation of flow between the two sub-compartments can be visualized by adding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



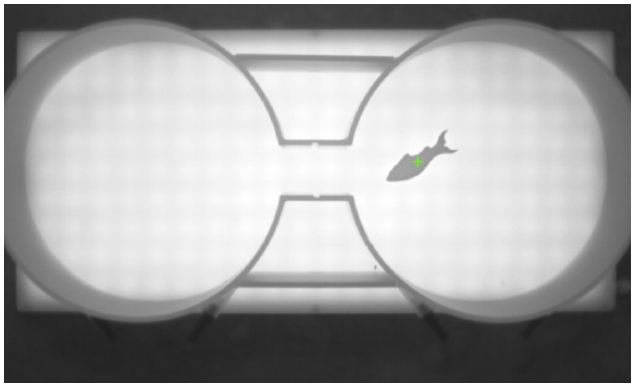
Optional

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 and 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 Cooling and heating baths*

Connect the buffer tanks with the cooling and heating coils and the temperature regulation pumps (RE) as shown in Figure 3. Place the coils in the cooling and heating water baths (not included).

Optional

For extreme temperature ranges insulate tubes and/or buffer tanks (relative to room temperature).

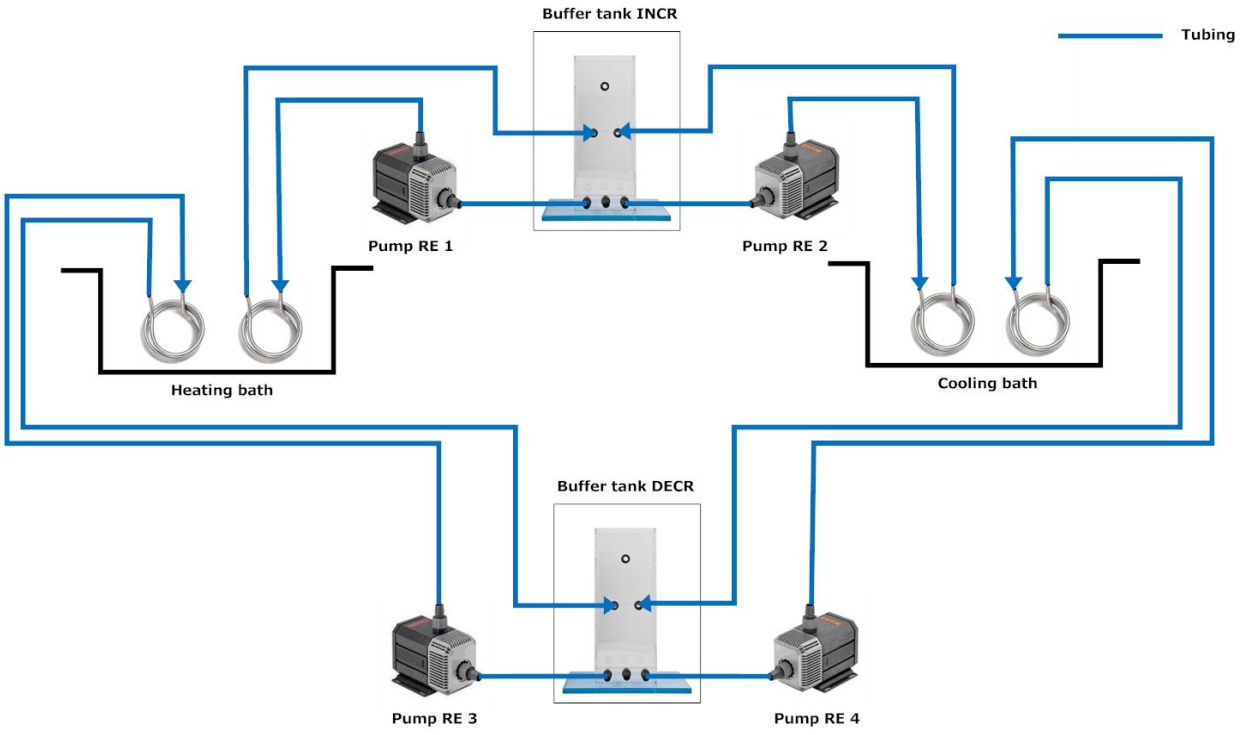



**Figure 3**: How to connect the buffer tanks with the cooling and heating baths (not supplied) for running a ShuttleBox temperature experiment.

# 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 water temperature 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 water temperature.

It is also possible to set up a static experiment during which the water temperature 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 Temperature.

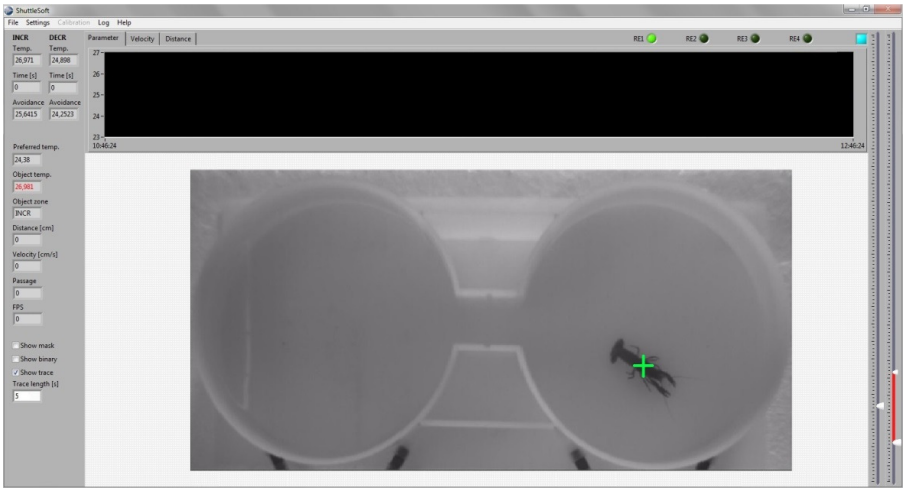
ShuttleSoft will connect automatically to the first uEye camera found.

- 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.

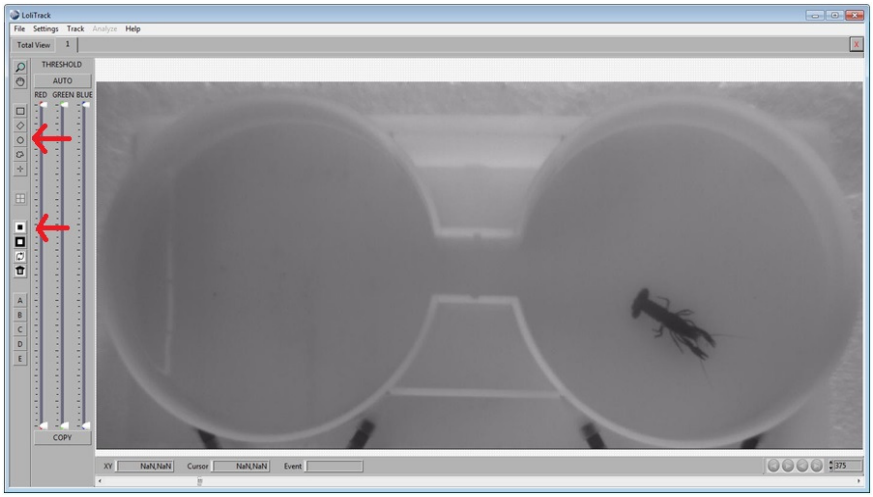
When done select Show Binary again to view unmodified images.

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

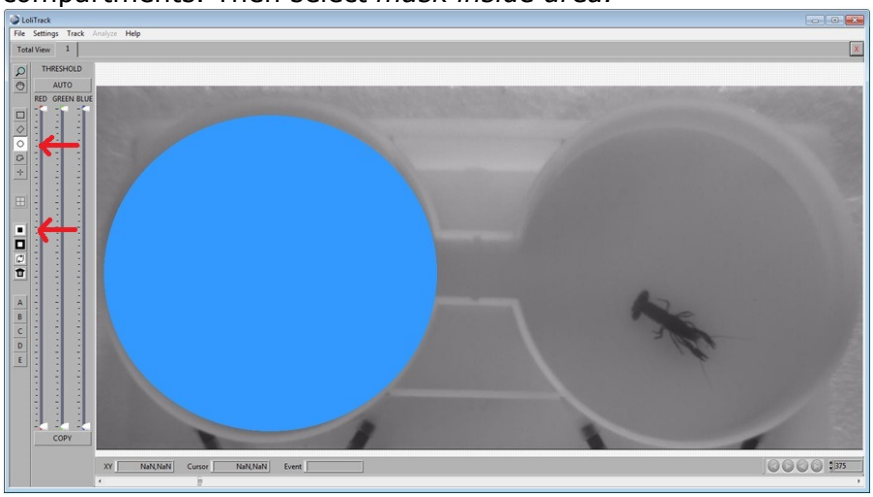
### 5.2.2 Creating a mask

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

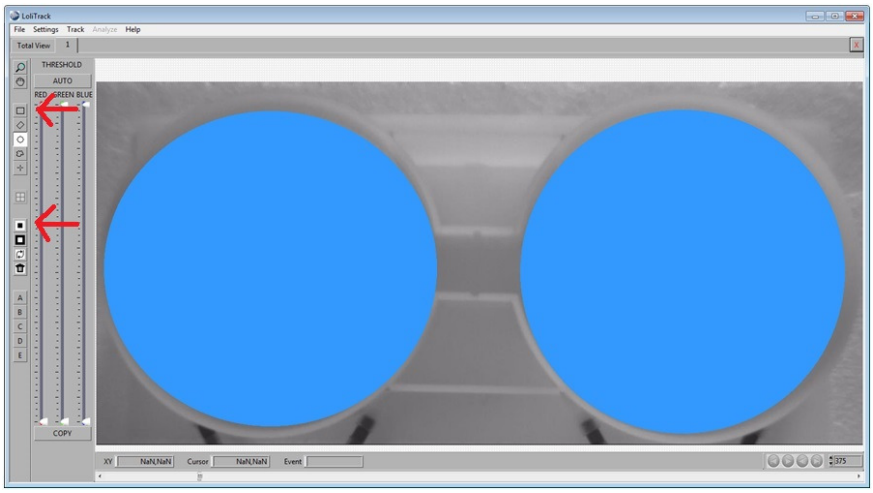
Select Settings→Mask



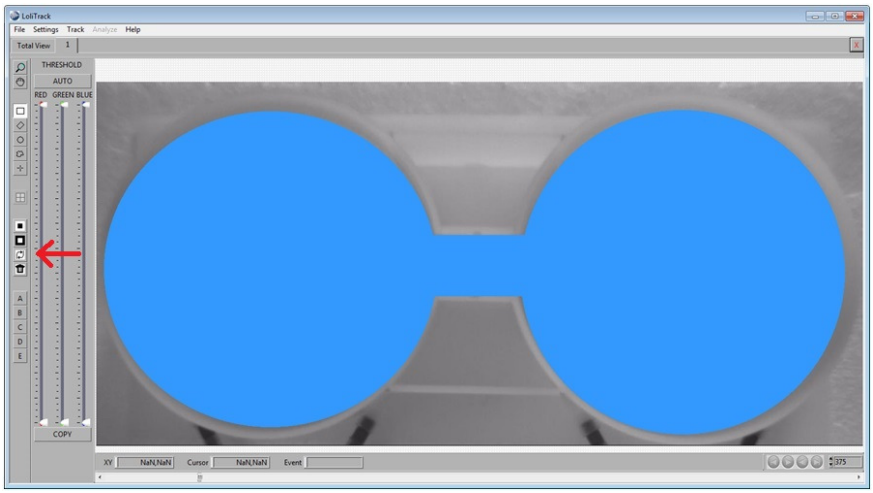
1. Use the circle button to draw a circle in one of the sub-compartments. Then select *mask inside area.*



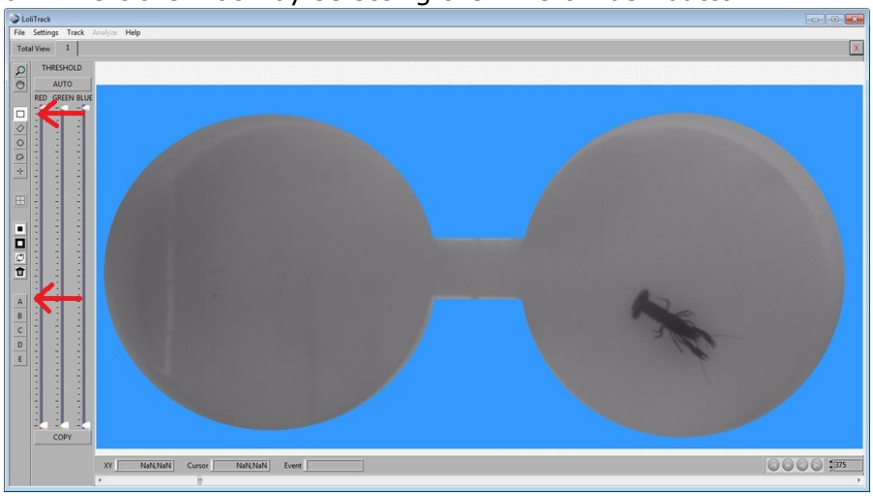
2. Use the circle button to draw a circle in the other sub-compartment. Then select *mask inside area.*



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.

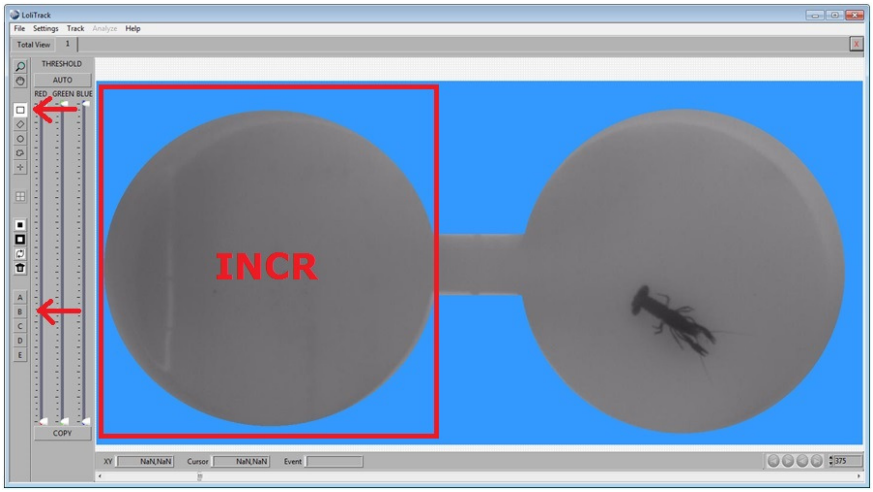
When maximizing the dialog box, the image will scale. When moving the mouse over the camera, it is possible to zoom/unzoom the image via the scroll button on the mouse.

### 5.2.3 Experimental zones

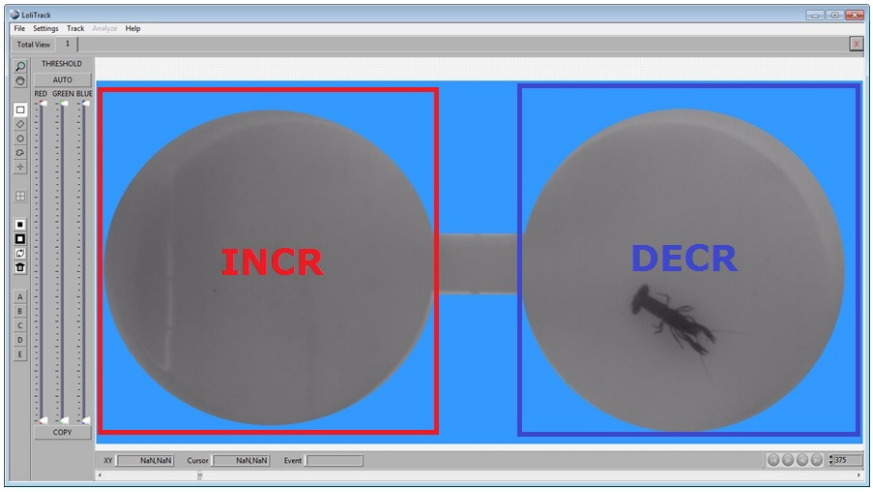
To define in which sub-compartment of the Shuttle tank the temperature will increase and decrease, respectively, experimental zones must be defined.

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 temperature) or DECR (decreasing temperature) 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 temperature instrument to degrees celcius (°C) a sensor calibration has to be done.

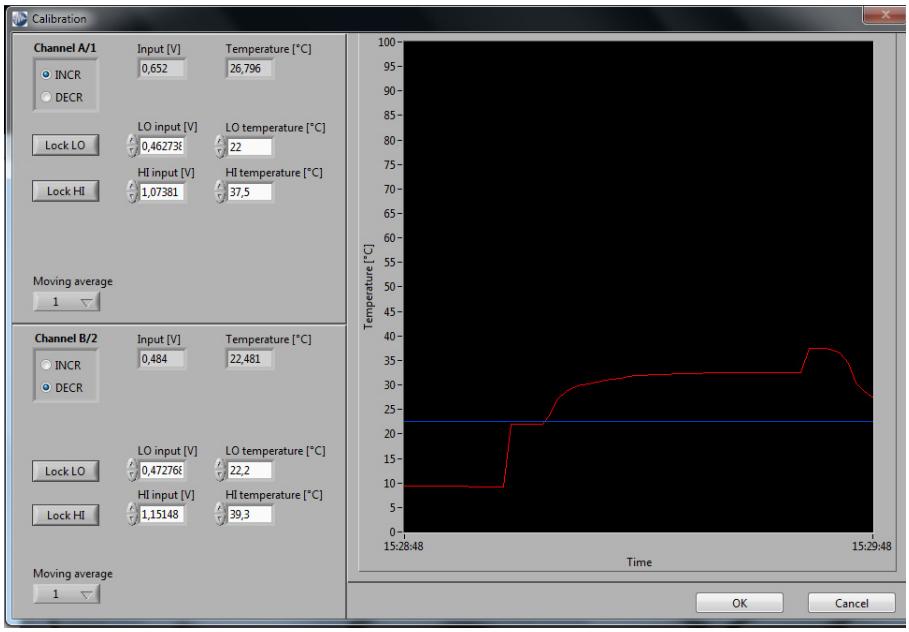
Select Calibration→Temperature

Perform a two-point calibration.

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

- Place the temperature sensor of the INCR/DECR zone in a liquid of low temperature, wait till the registered temperature stabilises and click 'LOCK LO' (in the example below, 22 °C).
- Remove the temperature sensor and place it in liquid of comparatively high temperature, wait till the registered temperature stabilises and click 'LOCK HI' (in the example below, 37.5 °C).

- Replace the temperature sensor into the probe vessel of the appropriate zone (i.e. INCR or DECR), it should now read the temperature of the assigned zone of the Shuttle box (in thie example belwo, 26.796 °C).
- 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 temperature 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 temperature in the INCR and DECR zones will be calculated.

Please refer to the temperature analyser (TMP-REG) user manual for further instructions on how to calibrate or service temperature probes.

## 5.2.6 Set-points

Select Setting →Experiment

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

Static Experiments:

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

ShuttleSoft will regulate the temperature around the setpoints and hysteresis selected for each of the two zones. For example if values 20°C and 0.2°C are selected for the HI zone, ShuttleSoft will start cooling when the temperature rises above 20.2°C in the INCR

zone and will stop cooling as soon as temperature reaches 20°C. When temperature drops below 19.8°C ShuttleSoft will start heating in the INCR zone.

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

Dynamic Experiments:

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

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 value for the parameters, e.g. it is the cooling and heating capacity of your chiller and heater that sets the max 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 estimate the gradual change in the core temperature when water temperature changes. For the calculation of the body temperature Newton´s law of cooling is used.

$$T_b = T_a + (T_i - T_a) \cdot e^{-kt}, where$$

$T_b$ *is body temperature*

$T_a$ *is ambient temperatue*

$T_i$ *is initial temperature*

$t$ *is time* (min)

$k$ *is the rate of change of core temperature* ($\min^{-1}$)

k is dependent of fish size and species (Stevens, E. Don and Sutterlin, A.M. 1976. Heat transfer between fish and ambient water. J. Exp. Biol. 65: 131-145)

- 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

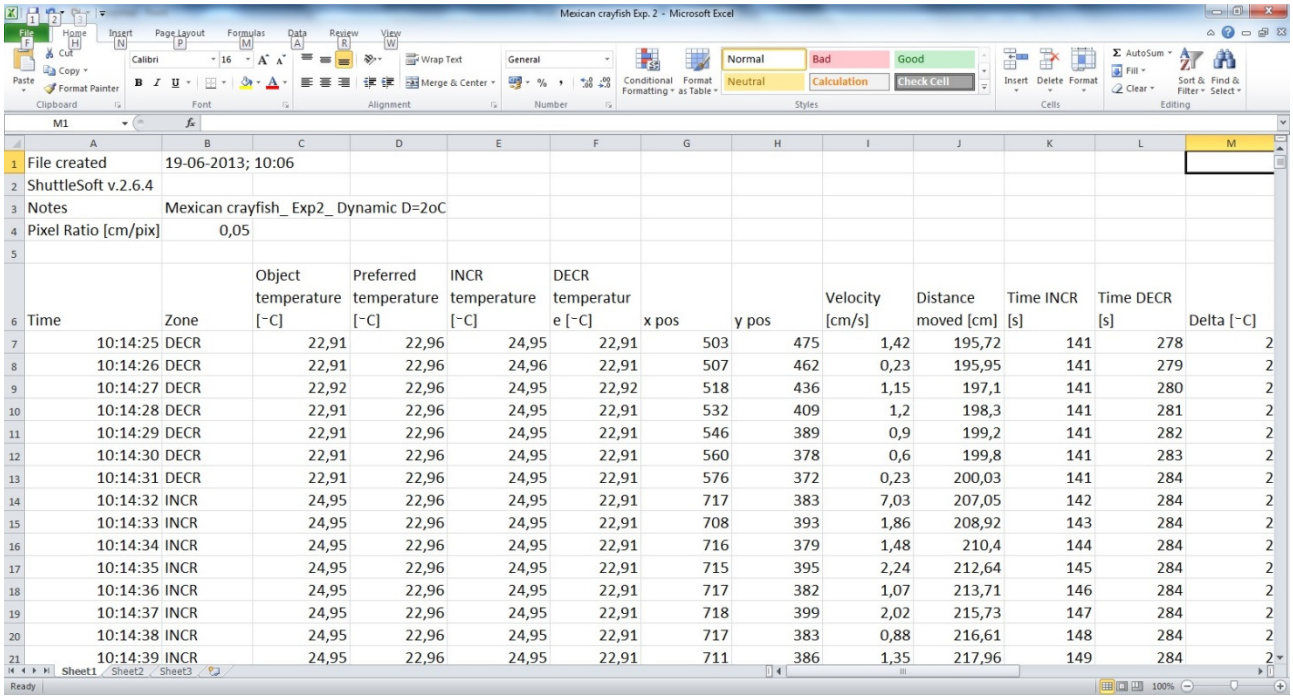
Select Log→Start logging data.

- 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.



| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | File created | 19-06-2013; 10:06 | | | | | | | | | | | |
| 2 | ShuttleSoft v.2.6.4 | | | | | | | | | | | | |
| 3 | Notes | Mexican crayfish_ Exp2_ Dynamic D=2oC | | | | | | | | | | | |
| 4 | Pixel Ratio [cm/pix] | 0,05 | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | |
| 6 | Time | Zone | Object temperature [°C] | Preferred temperature [°C] | INCR temperature [°C] | DECR temperature [°C] | x pos | y pos | Velocity [cm/s] | Distance moved [cm] | Time INCR [s] | Time DECR [s] | Delta [°C] |
| 7 | 10:14:25 | DECR | 22,91 | 22,96 | 24,95 | 22,91 | 503 | 475 | 1,42 | 195,72 | 141 | 278 | 2 |
| 8 | 10:14:26 | DECR | 22,91 | 22,96 | 24,96 | 22,91 | 507 | 462 | 0,23 | 195,95 | 141 | 279 | 2 |
| 9 | 10:14:27 | DECR | 22,92 | 22,96 | 24,95 | 22,92 | 518 | 436 | 1,15 | 197,1 | 141 | 280 | 2 |
| 10 | 10:14:28 | DECR | 22,91 | 22,96 | 24,95 | 22,91 | 532 | 409 | 1,2 | 198,3 | 141 | 281 | 2 |
| 11 | 10:14:29 | DECR | 22,91 | 22,96 | 24,95 | 22,91 | 546 | 389 | 0,9 | 199,2 | 141 | 282 | 2 |
| 12 | 10:14:30 | DECR | 22,91 | 22,96 | 24,95 | 22,91 | 560 | 378 | 0,6 | 199,8 | 141 | 283 | 2 |
| 13 | 10:14:31 | DECR | 22,91 | 22,96 | 24,95 | 22,91 | 576 | 372 | 0,23 | 200,03 | 141 | 284 | 2 |
| 14 | 10:14:32 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 717 | 383 | 7,03 | 207,05 | 142 | 284 | 2 |
| 15 | 10:14:33 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 708 | 393 | 1,86 | 208,92 | 143 | 284 | 2 |
| 16 | 10:14:34 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 716 | 379 | 1,48 | 210,4 | 144 | 284 | 2 |
| 17 | 10:14:35 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 715 | 395 | 2,24 | 212,64 | 145 | 284 | 2 |
| 18 | 10:14:36 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 717 | 382 | 1,07 | 213,71 | 146 | 284 | 2 |
| 19 | 10:14:37 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 718 | 399 | 2,02 | 215,73 | 147 | 284 | 2 |
| 20 | 10:14:38 | INCR | 24,95 | 22,96 | 24,95 | 22,91 | 717 | 383 | 0,88 | 216,61 | 148 | 284 | 2 |
| 21 | 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 temperature experiment, the development in temperature in the two sub-compartments as it develops over time is shown in the **Parameter** view (default pane one).

The temperature in the INCR compartment is shown in red and the temperature in the DECR is shown I blue. The temperature in the compartment where the experimental animal is present is shown in yellow.

The left side of the view always show the temperature in each zone, the time spend in each zone and the avoidance temperature in each zone.

- The value for the avoidance parameter is defined as the value of the measured parameter (temperature) 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 temperature. This is the median temperature of the water where the experimental organism was located throughout the experiment.

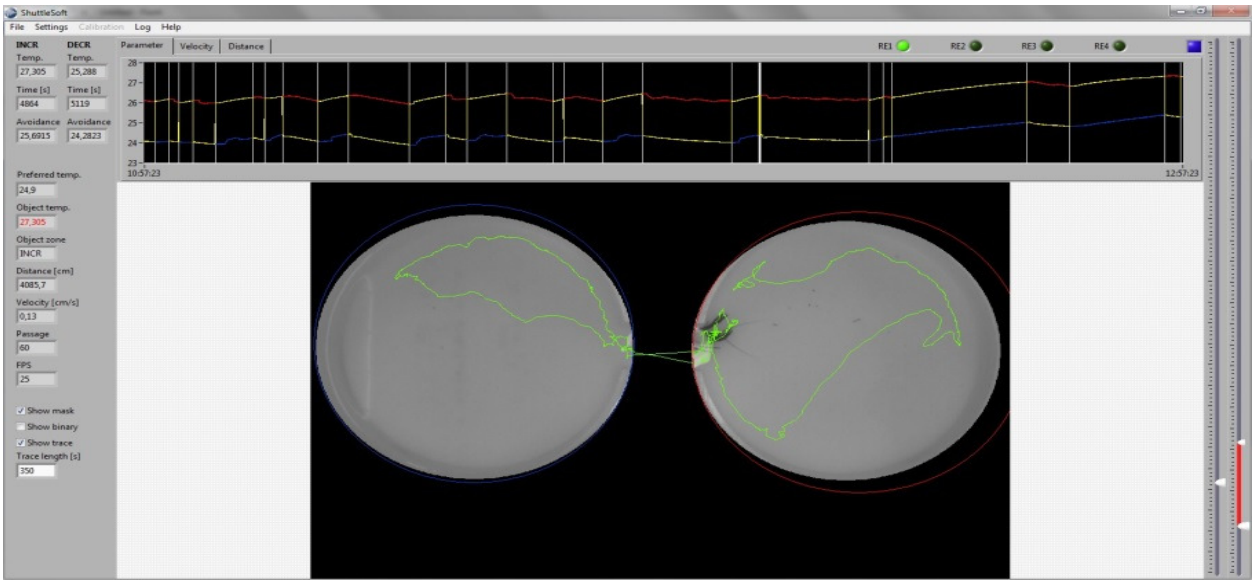
The parameter value for the water where the experimental animal is located at a given time in shown in Object temperature, and below is shown in which zone (INCR or DECR) the organism is currently positioned.

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 temperature/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.



## 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 neighbors, removing any pixel that does not match its neighboring (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

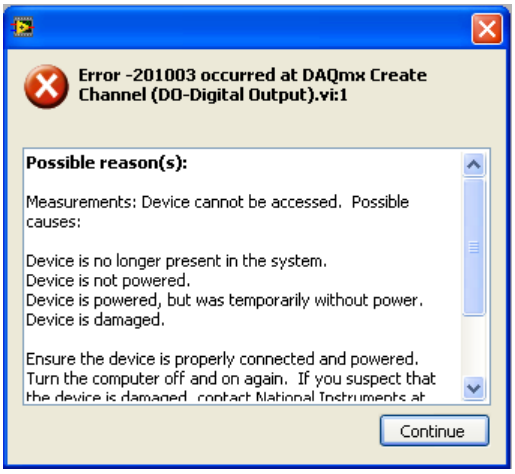
| Terms | Definition |
|---|---|
| **INCR O2 / INCR temp. / INCR sal. / INCR turb.** | This field indicates the current O2 saturation/temperature/salinity for the INCR zone. |
| **DECR O2 / DECR temp. / DECR sal. / DECR turb.** | This field indicates the current O2 saturation/temperature/salinity for 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 / Object temp. / Object sal. / Object turb.** | This field indicates the current O2 saturation/temperature/salinity 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 / temperature/ salinity / turbidity** | In this field the mean value of the object O2/Object temp./Object sal./Object turb. 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.<br>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.<br><br>*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 thresholded 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 heating pump for the INCR zone must be connected. |
| **RE2** | This green diode indicator gives the relay status for relay 2. On relay 2 the cooling pump for the INCR zone must be connected. |

| | |
|---|---|
| **RE3** | This green diode indicator gives the relay status for relay 3. On relay 3 the heating pump for the DECR zone must be connected. |
| **RE4** | This green diode indicator gives the relay status for relay 4. On relay 4 the cooling pump 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

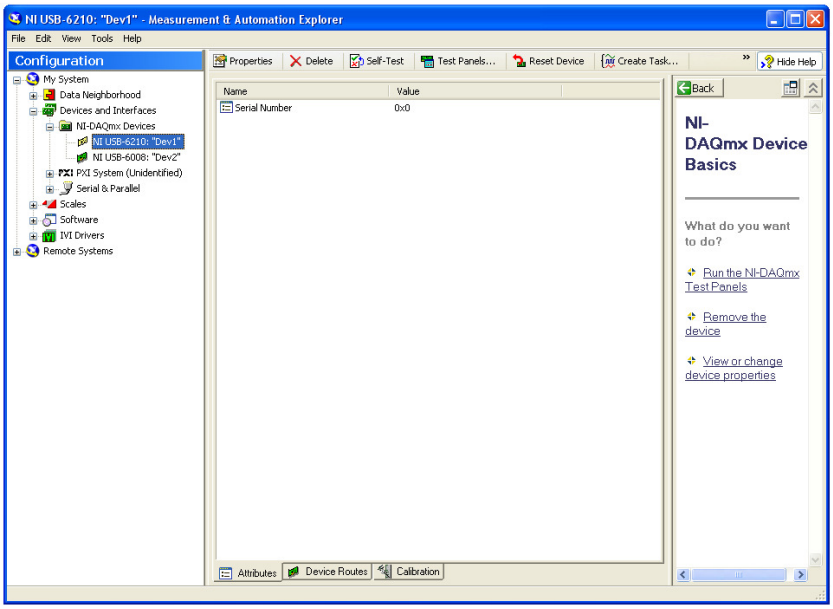
## 8.1 DAQ connection failure



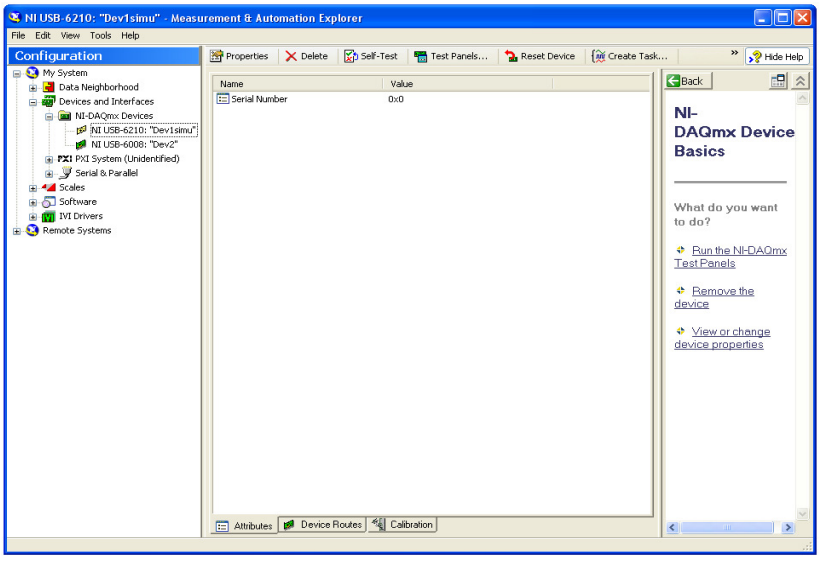

The error shown in the screen above occurs, when the NI-USB 6008/6009 DAQ card is not properly connected to the PC. This error can occur in 2 ways.

### 8.1.1 Name is not "dev1"

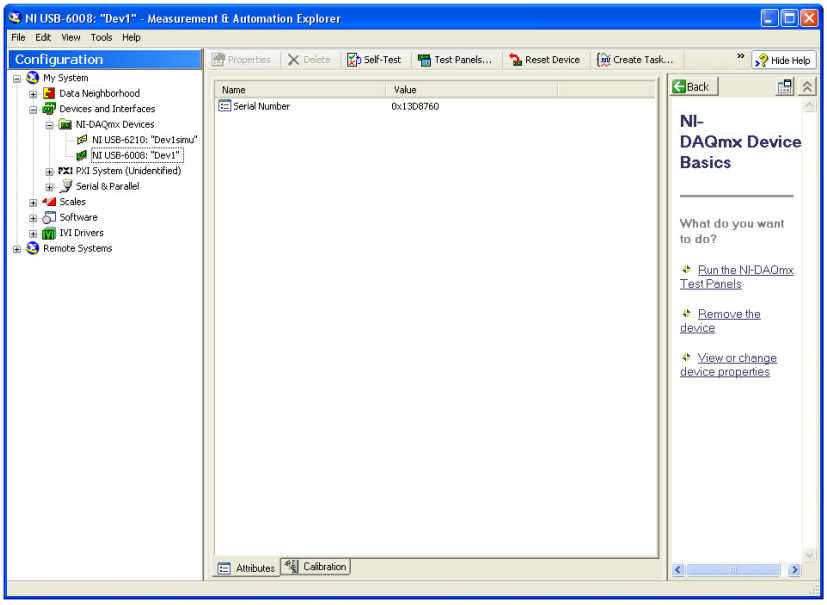
To change the device name, open the NI program called Measurement & Automation (look for desktop icon). The following example on **Fejl! Henvisningskilde ikke fundet.** shows a simulated device named as "dev1". The NI-USB 6008 DAQ card is registered as "dev2"




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.