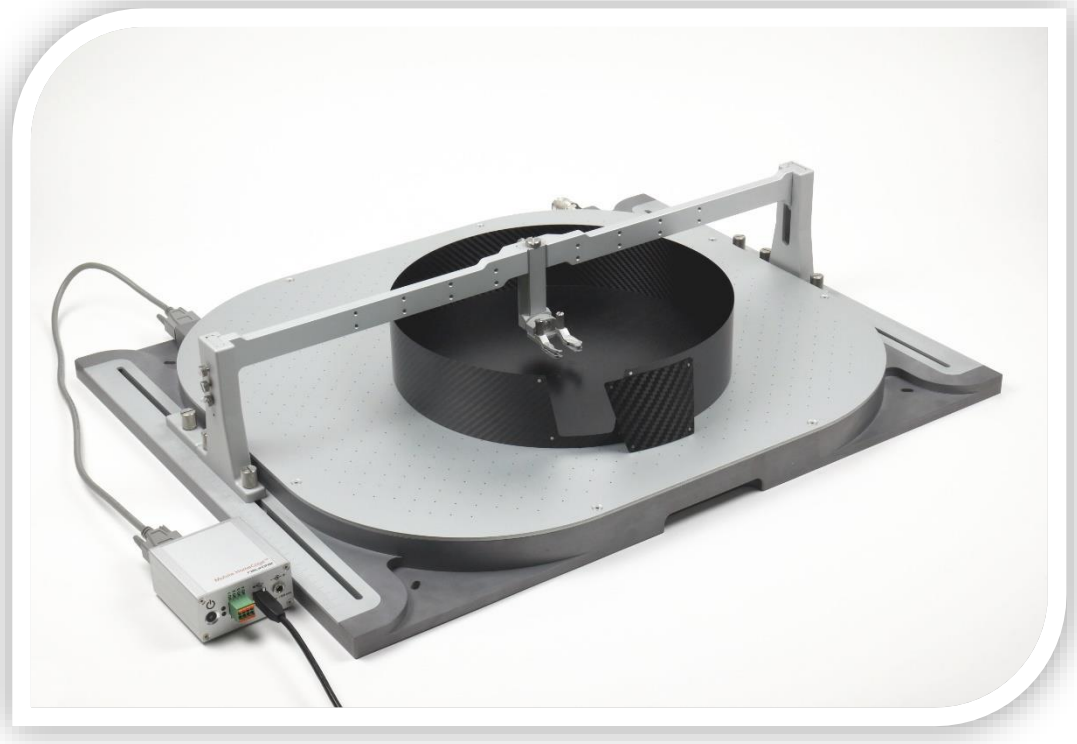
Mobile HomeCage® locomotion tracking software



User Manual 2.7

1. Table of content

2. Installation of Supporting Programs

2.1. ECU Driver Installation

Open folder CP210x_Windows_Drivers located on the USB drive or dowload (free of charge) from:

https://www.silabs.com/documents/public/software/CP210x_Windows_Drivers.zip

Install the drivers in accordance with the local system parameters (please ask system administrator for assistance).

2.2. Tracking Software and National Instruments Programs' Installation

Open folder "MHC_Tracker_Software_v2.2.x.x_full_installer" located on the USB drive (x.x is the subversion of the software). Run "setup.exe" file and follow installation instructions on the screen.

Please note, that installer will update any previously installed versions of the tracking software from v.2.2 branch, but if you have older versions installed (i.e. from 2.1 branch), they will remain unchanged. Both v.2.1 and v.2.2 can be run on the same computer independently.

You may need to reboot the computer when installation is completed.

2.3. NI Drivers' Parameters Set-up (optional)

☛ Connect the tracking device to computer and switch it ON before continuing.

Go to "Start" -> "NI MAX" and set the parameters according to the Figure 1 below:

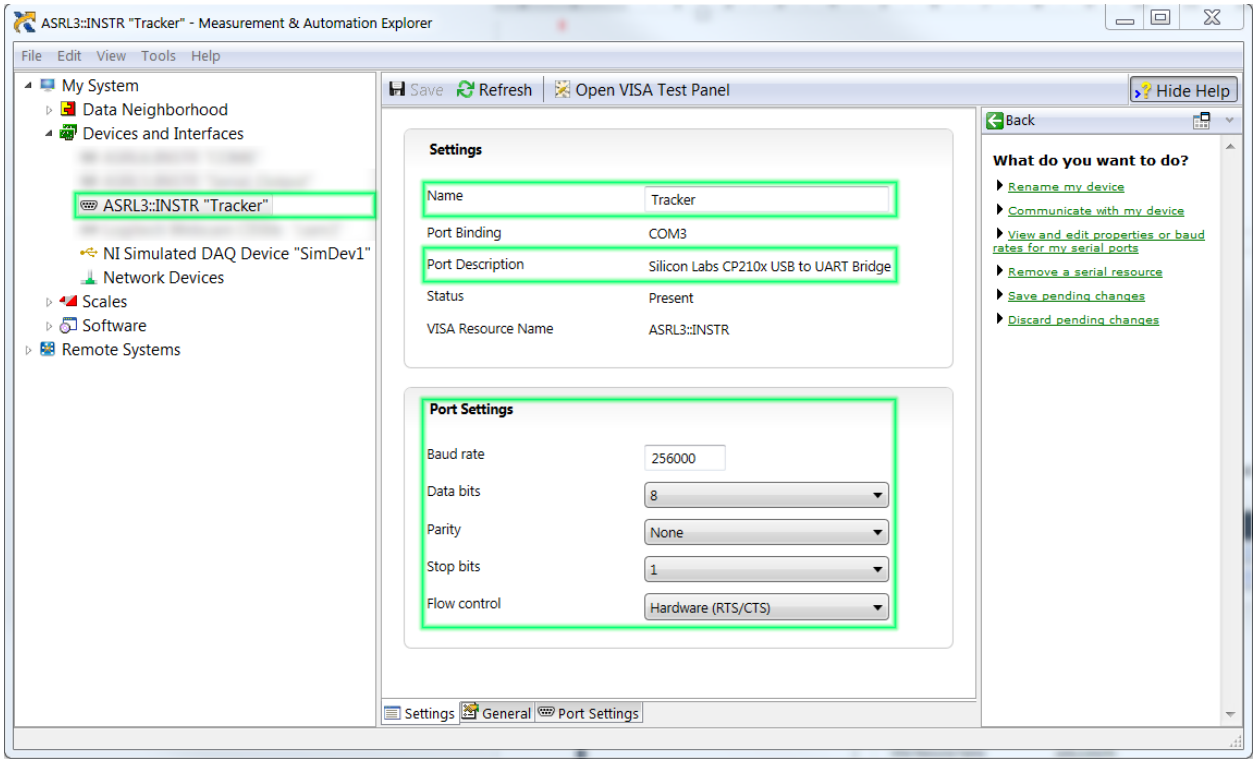


Figure 1. NI MAX Port Settings

2.4. Tracking Software Update

The most recent software version can be downloaded from GitHub:
https://github.com/Neurotar/standard-tracker-application/releases

3. Software Interface / Operating the Software

3.1. First run

Prior to starting the software, check that all cables are connected, and the tracking device is switched on. During the first run the program will display the Connection error message:

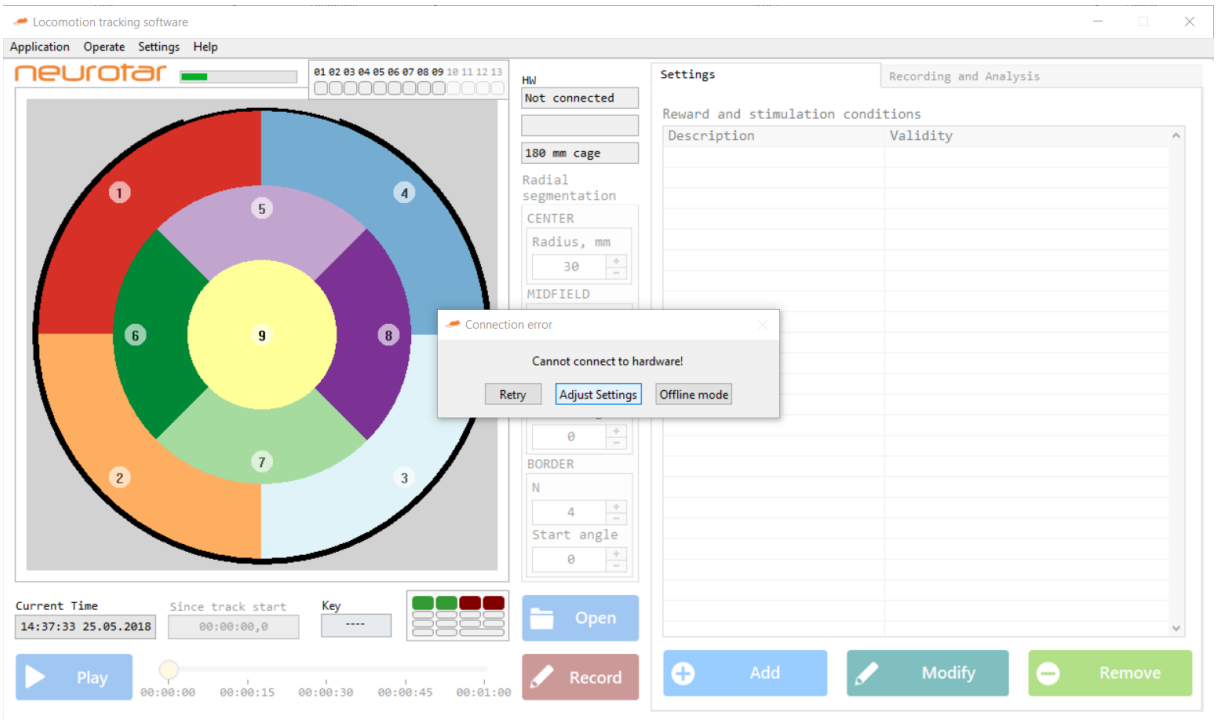


Figure 22. First software run

Press Adjust Settings button. Hardware ports configuration window will appear. Software will try to automatically detect connected hardware. If the row "Silicon Labs CP210x USB to UART Bridge" is not displayed, check that the electronic control unit is connected and switched ON, then press Find HW button. When correct port is selected, press Save Config button.

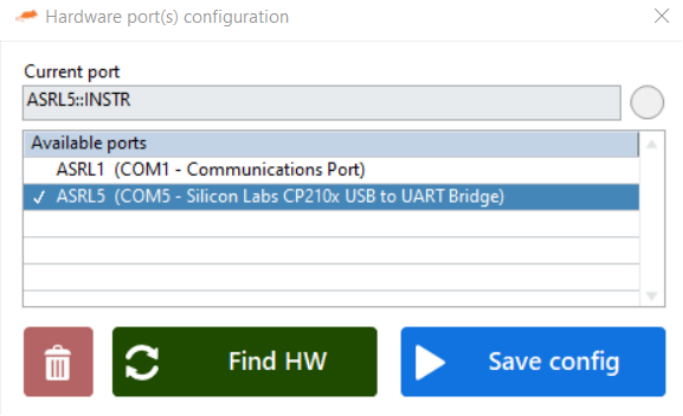


Figure 33. Hardware ports configuration window

☛ Connection error message will appear during normal operation if hardware is not connected or if it is not switched on. In this case, re-connect and switch ON the hardware, then press Retry button. It is also possible to work in the Offline Mode without connecting the hardware (with previously recorded data only).

☛ It is recommended to set-up hardware settings and calibration after the first run. For details refer to section 3.9.

3.2. Main window: layout and Settings tab

The program starts on this window, which displays all settings that can be used during the recording.

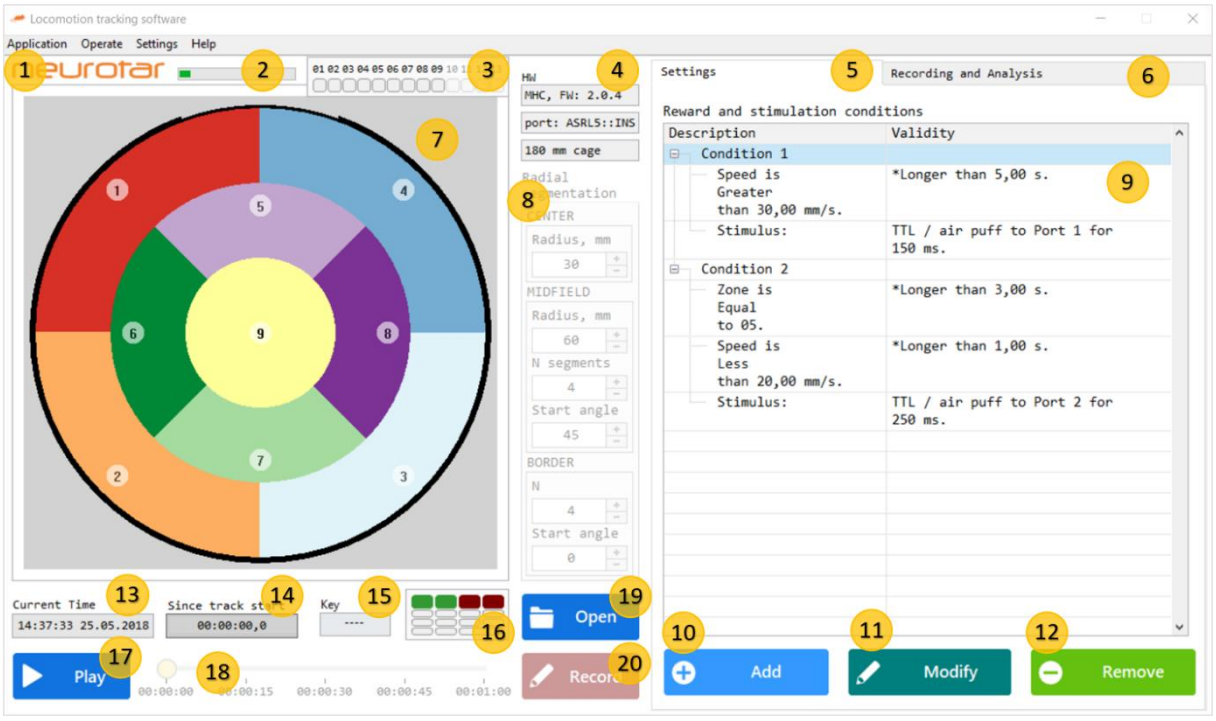


Figure 4. Software Interface: layout

☛ Correct orientation of the magnets inside the cage is essential for the proper functioning of the software in general and for segmentation in particular.

The item numbers below correspond to the circled numbers in Figure 4 (above).

1. **Main menu.** Menu items are listed in the section 3.6.
2. **CPU load indicator**. Shows current CPU load. If the load exceeds 70%, the software automatically reduces the user interface update rate in order to keep other vital activities (such as data recording) running. When the CPU load drops, UI functioning is automatically restored.
3. **Current zone indicator**. Shows the zone, where the mouse is currently located.
4. **Hardware information**. Shows type of the connected device (either MHC or MHC-L), ECU firmware version, port information and diameter of the cage (the latter can be changed in the settings).
5. **Settings tab**. Allows setting the conditioned stimulus delivery.
6. **Recording and Analysis tab**. Shows information about on-line or uploaded data.
7. **Canvas window.** Shows the cage with zones' layout settings.
8. **Cage segmentation block:**

   Only available when Play is active (#17).

   a. **CENTER** defines a zone radius (maximum radius is 5 mm smaller than midfield radius by default).
   b. **MIDFIELD** defines a number of zones (up to 8), a zone radius (maximum radius is 5 mm smaller than the border and 5 mm larger than the center) and a starting angle (0-360).
   c. **BORDER** defines a number of zones (up to 12) and a starting angle (0-360).

   Total number of zones (including the central zone) should not exceed 12.

9. **Reward and stimulation conditions.** Shows the list of currently set conditions.
10. **Add.** Adds a new condition to the list (up to 5). Click on the conditions' list to make this button active. For more details refer to section 3.10.
11. **Modify.** Modifies an existing condition.
12. **Remove.** Removes an existing condition.
13. **Current time.** Shows current timestamp saved alongside the locomotion data.
14. **Since track start.** Shows the track's timestamp saved alongside the locomotion data.
15. **Key.** Shows if one of the four directional keys (Up, Down, Left or Right) is pressed. Keys' status is saved alongside the locomotion data (this functionality is added in response to a specific user request).
16. **I/O status indicator** helps to visualize I/O configuration status of TTL ports (see section 3.3).
17. **Play.** Play button starts a data playback (either live or recorded). When playing live data, it is possible to adjust the settings and see their implementation immediately. Thus, it is possible to test the reward and stimulation conditions before starting an actual experiment.
18. **Timeline.** Shows length of the current track while playing live data or recording. Allows navigating along the recorded data (see section 3.5).
19. **Open.** Opens and loads an existing recording.
20. **Record.** Starts new track recording.

3.3. Main window: I/O configuration and status indicator.

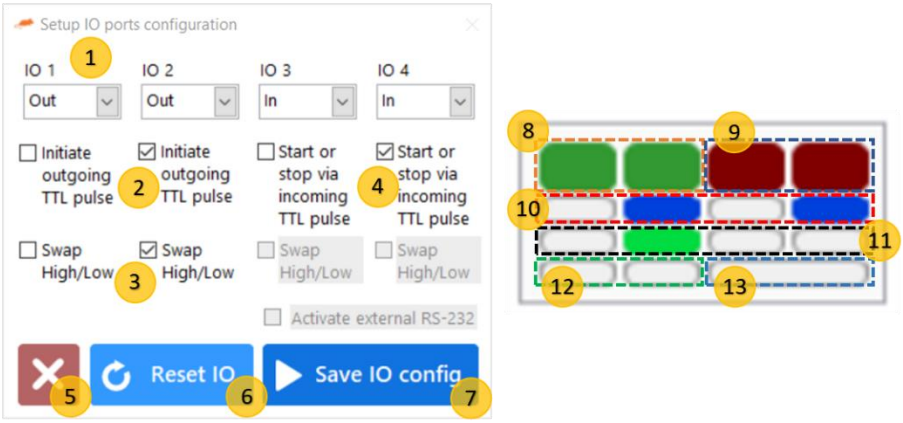


Figure 5. TTL ports I/O configuration window and status indicator

To set up TTL I/O ports configuration go to Menu -> Settings -> I/O Configuration.

The item numbers below correspond to the circled numbers in Figure 5 (above).

1. I/O 1-4 shows the current ports' assignment. Available options: In, Out, Sync (hardware-dependent).
2. When selected, outgoing TTL pulse will be initiated at start and/or end of the recording.

Length of the pulse is 50 ms at the start of the recording and 25 ms at the end of the recording.

3. When selected, TTL output polarity will be swapped, i.e. "low" signal will produce +3.3 V output and "high" signal will produce 0 V output.
4. When selected, incoming TTL pulse will be used to start and/or stop the recording.

Please note the delay between the incoming TTL pulse and the software response. The delay is approximately equal to a single hardware frame time (i.e. 10 ms, 13 ms or 20 ms, depending on the device model and software settings).

5. Exit without saving the configuration.
6. Reset to default configuration.
7. Save the configuration and exit.
8. Green colour Indicates that the port is used as Out or Sync.
9. Red colour Indicates that the port is used as Input.
10. Indicates whether TTL output port polarity is swapped (off by default, see #3 above).
11. Indicates whether the port is used to produce outgoing TTL or receive incoming TTL pulses during start and/or stop.
12. Indicates whether the output port is used for Sync purposes (IO 1-2 only).
13. Indicates whether the external RS-232 mirror port is activated (not available at the moment).

3.4. Main window: Recording and Analysis tab (live view or recording mode)

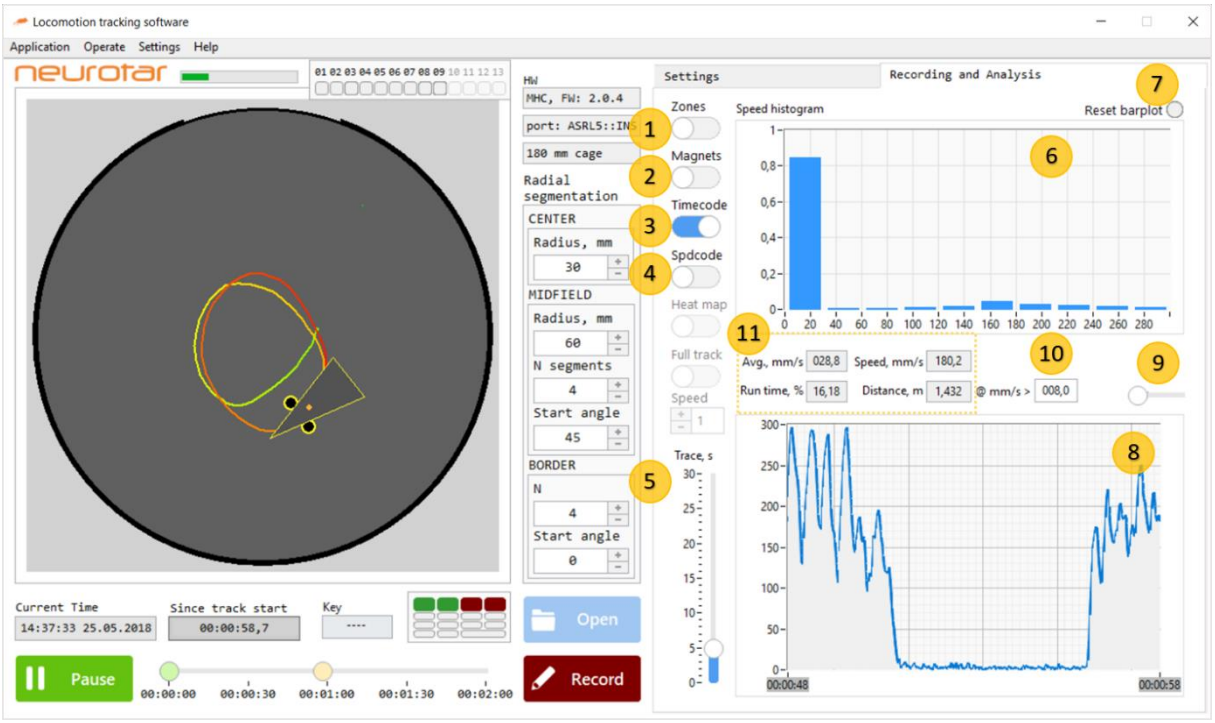


Figure 6. Software Interface: Recording and Analysis tab (live view or recording mode)

The item numbers below correspond to the circled numbers in Figure 6 (above).

1. **Zones.** Enables or disables zones layout overlay.
2. **Magnets.** Enables or disables magnets location overlay (according to the default magnet position).
3. **Timecode.** Enables or disables time color coding of the animal's location trace; cannot be used simultaneously with speed color coding (#4).
4. **Spdcode.** Enables or disables speed color coding of the animal's location trace; cannot be used simultaneously with time color coding (#3).
5. **Trace, s.** Allows setting desired visible trace length in seconds. Default is 5 seconds.
6. **Speed histogram.** Shows distribution of animal's movement speed above the threshold (#10).
7. **Reset barplot.** Resets speed histogram (#6).
8. **Speed plot.** Shows speed history plot over time.
9. Changes the time axis scale of the speed plot (#8).
10. **Speed threshold.** Any speed below or equal to the threshold will be considered as 0 for all calculations (except plot #8).
11. Simple statistics' block displaying speed, average speed, run time percentage and distance travelled.

3.5. Main window: Recording and Analysis tab (playback of recorded data mode)

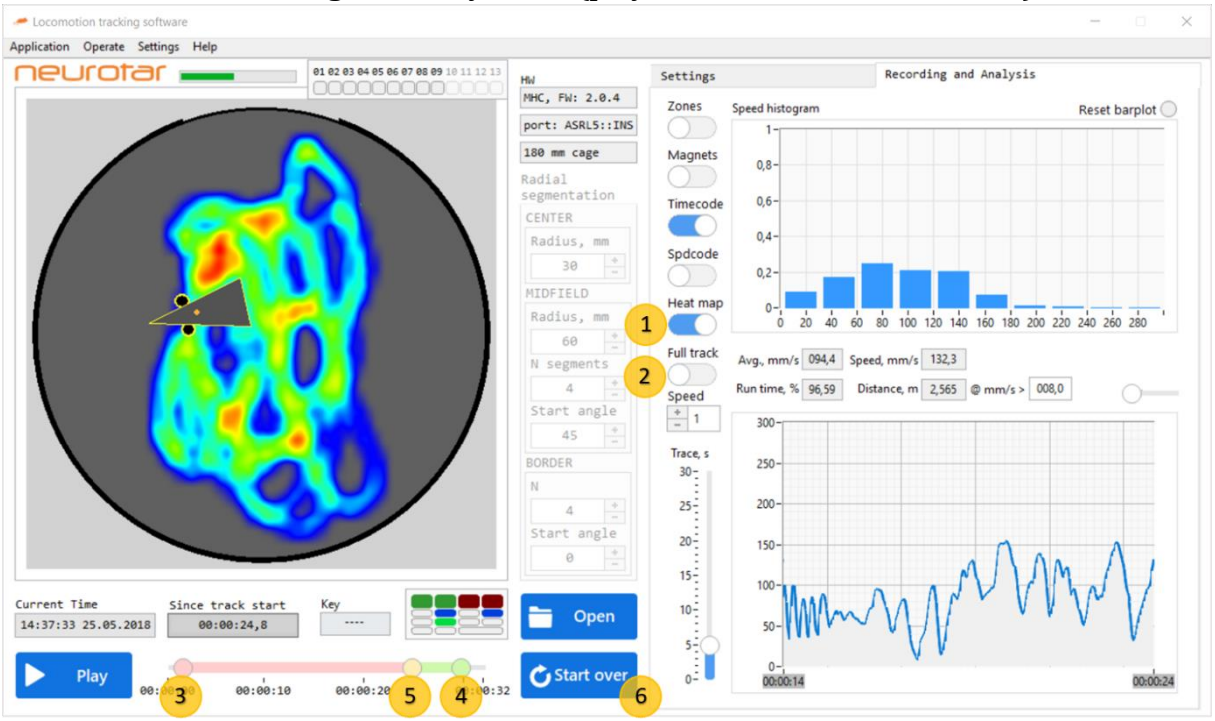


Figure 7. Software Interface: Recording and Analysis tab (playback or recorded data mode)

The item numbers below correspond to the circled numbers in Figure 7 (above).

1. **Heat map.** Enables or disables heat map overlay. Two heat map options are: location preference (shown above) and speed preference. Switching between the options is possible using Timecode and Spdcode switches.

   Heat maps are calculated and converted according to the formula $hm = log_2(x + 1)$ in order to fit the entire dynamic range. Heat maps can be exported as pictures and as a data matrix using Application -> Save density maps menu item.
2. **Full track.** Enables or disables full track overlay. Can be time and speed color coded. Color is assigned to every two second interval according to its average parameter value.
3. **Begin selection knob.** Allows selecting the starting point for the displayed track. Move the knob to the desired position and left click on it to apply the selection.
4. **End selection knob.** Allows selecting the final point for the displayed track. Move the knob to the desired position and left click on it to apply the selection.
5. **Current position knob.** Shows the current playback position.
6. **Start over.** Discards all unsaved changes and returns to the main screen to start a new recording.

3.6. Main window: menu

1. **Application.**
   a. **Save density maps.** Saves maps of location preference and average speed at location (heat maps) as pictures and as a data matrix. Maps are calculated and converted according to the formula $hm = log_2(x + 1)$ in order to fit the entire dynamic range.
   b. **Exit.** Exits application saving all settings.
2. **Operate.** Allows starting and stopping (live) acquisition, starting and stopping the recording and starting over (i.e. discarding all unsaved changes and preparing for a new recording).

3. **Settings.**
    a. **Save Settings.** Saves all settings into configuration file for future use.
    b. **Load Settings.** Loads settings from a previously saved configuration file.
    c. **Configure port(s).** Opens Hardware ports configuration dialog window (see section 7.4.3.1).
    d. **I/O configuration.** Opens TTL I/O configuration dialog window (see section 7.4.3.3).
    e. **Cage and Recording.** Opens Cage and recording settings dialog window (see section 7.4.3.7).
    f. **Dispensing rate calibration.** Opens dispensing rate calibration dialog window (see section 7.4.3.8). Dispensing rate must be set prior to using an (optional) liquid reward hardware.
    g. **Timings calibration.** Performs hardware and software timers' calibration (see section 7.4.3.9). Place the magnetic cage into the device prior to initiating this function.

3.7. Cage and Recording settings

These settings can be accessed via Settings -> Cage and Recording menu item.

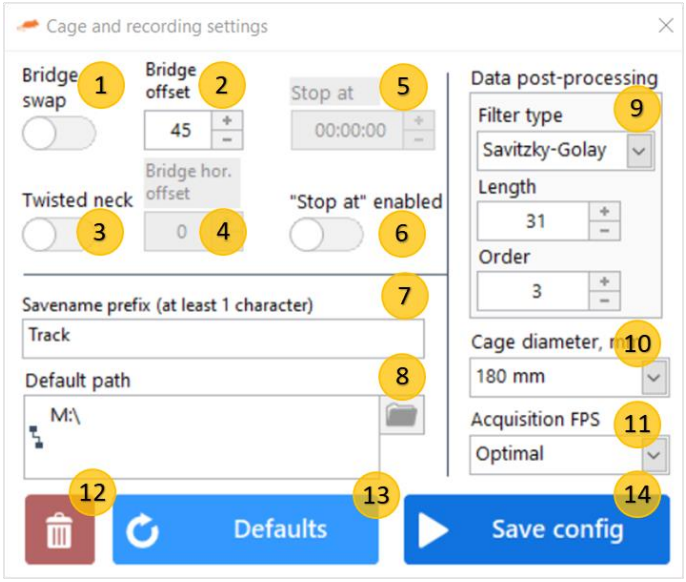
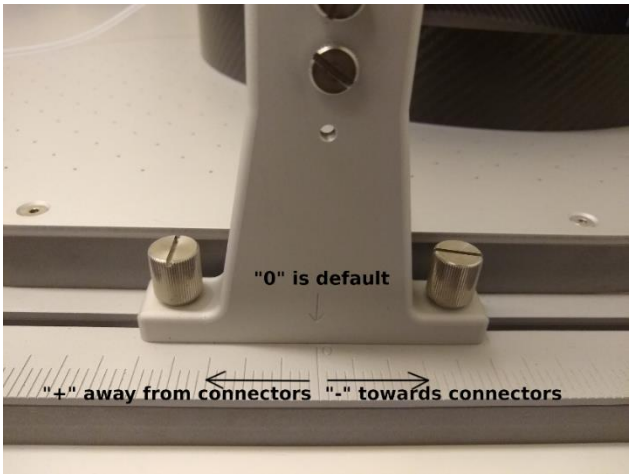


Figure 8. Cage and recording settings dialog.

The item numbers below correspond to the circled numbers in Figure 8 (above).

1. **Bridge swap.** Allows adjusting software functions for bridge orientation which is different from default. Default: mouse facing away from the tracker connector.
2. **Bridge offset.** Allows adjusting software functions if the bridge is positioned off-center (see picture on the left). If playback (live) is active, then this setting is applied instantly. Can be used for fine on-line adjustment.
3. **Twisted neck**. Allows adjusting software functions for using the special *twisted neck*.
4. **Bridge horizontal offset.** Allows adjusting software functions if the bridge is positioned off-center in combination with the *twisted neck*.



5. **Stop at.** Sets maximum length of the recording (if set to 10 minutes, all recordings will be exactly 10 minutes long unless stopped manually).
6. **"Stop at" enabled.** Enables and disables "Stop at" function.
7. **Savename prefix.** Allows adding a prefix to saved tracks' names. The value must be at least one character long.
8. **Default path.** Path where tracks are saved by default.
9. **Data post-processing.** Defines data filtering parameters for offline data post-processing. *Function under development*.
10. **Cage diameter.** Must correspond to the diameter of the floating cage used in the Mobile HomeCage set-up.
11. **Acquisition FPS.** Sets device's sampling rate.
    a. **Max.** Maximal sampling rate is 100 fps.
    b. **Optimal.** Optimal sampling rate, helps to achieve the the lowest possible sampling jitter (100 fps for the Mobile HomeCage Large).
    c. **Eco.** Relatively slow sampling, but 20 ms sample intervals are useful for some applications.
12. Exit without saving changes.
13. **Defaults.** Load default values.
14. **Save config.** Save configuration and exit.

3.8. Dispensing rate calibration

These settings can be accessed via Settings -> Dispensing rate calibration menu item.

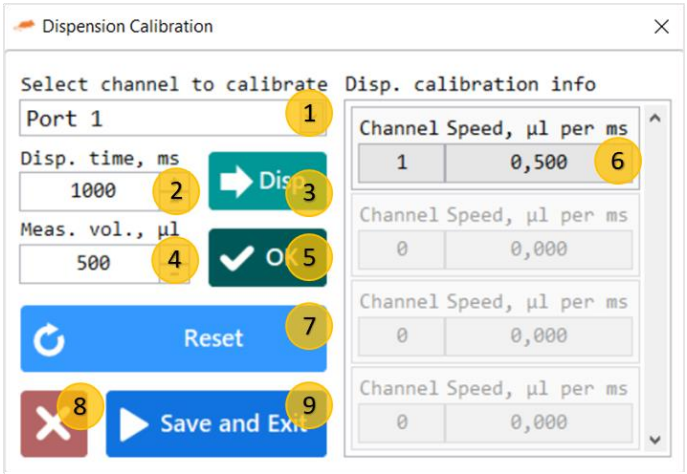


Figure 9. Dispensing rate calibration dialog window.

The item numbers below correspond to the circled numbers in Figure 9 (above).

1. **Select channel to calibrate.** Allows selecting active output channel used to control liquid dispensing device.
2. **Disp. time, ms.** Defines length of dispensing, in milliseconds. Default setting is 1000 ms.
3. **Disp.** button. Performs dispensing.
4. **Meas. vol., µl.** Measured amount of the dispensed liquid should be entered here (in microliters, must be measured by user).
5. **OK.** Applies measured calibration information to selected channel.
6. **Disp. calibration info.** Measured calibration information for each calibrated channel.
7. **Reset.** Resets values to defaults.
8. Exit without saving changes.
9. **Save and Exit.** Save changes and exit.

3.9. Timings' calibration dialog

Calibration can be accessed via Settings -> Timings calibration menu item; the calibration is performed automatically. We recommend calibrating timings prior to operating a new device. Magnetic cage must be placed in the Mobile HomeCage device prior to performing the calibration.

Figure 4. Timings' calibration information.

The item numbers below correspond to the circled numbers in Figure 10 (above).

1. **Frame delta time, ms.** Shows the plot of theconsecuitive frames' intervals in milliseconds.
2. **Hardware.** Shows plot legend for the hardware timer (running on ECU).
3. **Software.** Shows plot legend for the software timer (computer's clock).
4. Shows average hardware frame time in milliseconds.
5. Standard deviation for the hardware timer for the test sample (2000 samples).
6. Shows average software frame time in milliseconds.
7. Standard deviation for the software timer for the test sample (2000 samples).
8. **True FPS.** Shows true sampling rate in frames per second.
9. Short calibration report.
10. **OK.** Save calibration information and exit.

3.10. Reward and stimulus conditions settings dialog

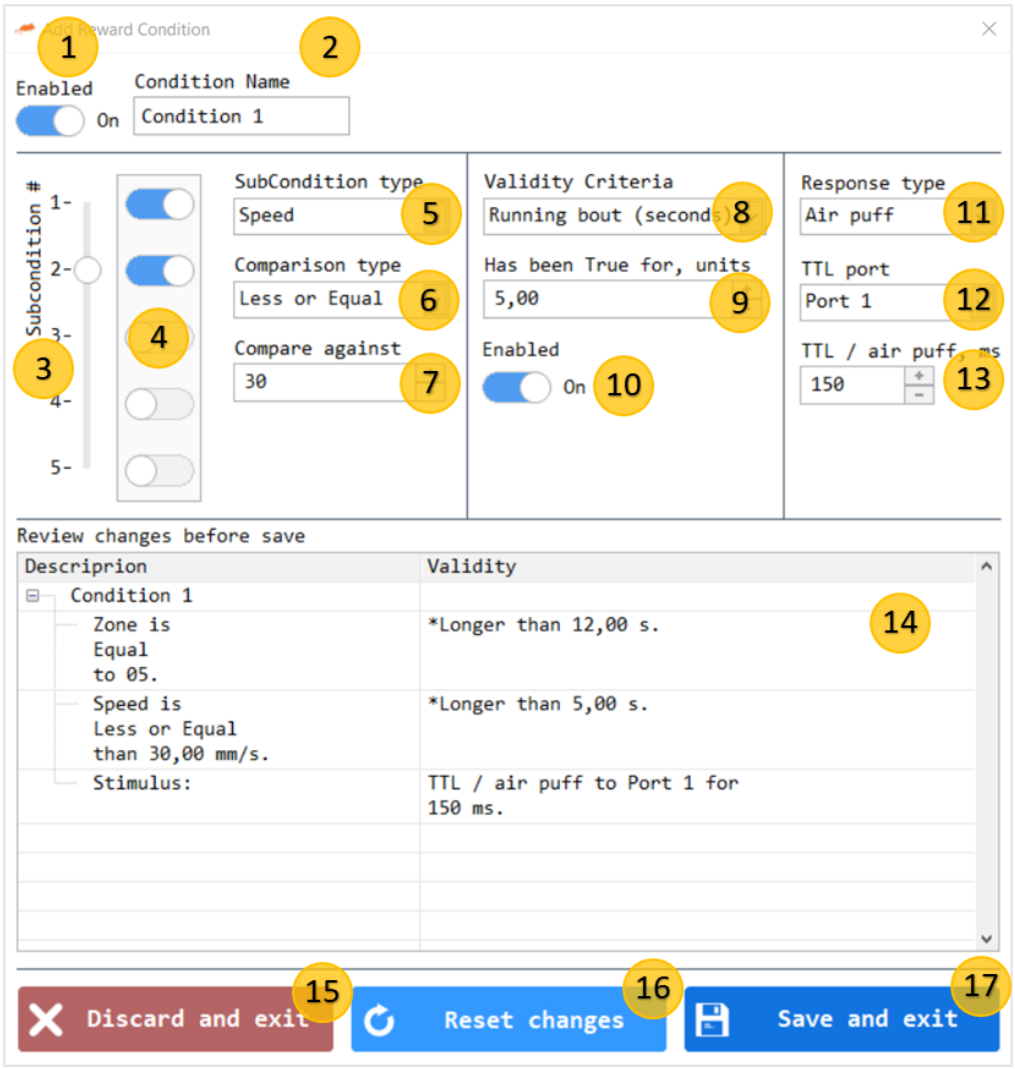


Figure 11. Add/Modify reward condition dialog.

In this dialog user can set up a Reward/Stimulus condition terms.

Definitions:

1. **Condition** – a set of the rules (or subconditions). Whenever all subconditions are met, a desired response is triggered (air puff, TTL output, reward delivery).
2. **Subcondition** – a single rule. For example, animal speed or animal's location.
3. **Validity criterion** defines a set of circumstances under which subcondition is met, e.g. subcondition "enters zone 5" is considered met if the animal remains in zone 5 for 12 seconds after entering (validity criterion).

The max. number of conditions per experiment is five. The max. number of subconditions per each condition is also five. See below for a more detailed description of the parameters. The item numbers below correspond to the circled numbers in Figure 11 (above).

1. **Enabled.** Enables or disables the current condition.
2. **Condition name.** Short description of the condition.
3. **Subcondition #.** Selects a subcondition for editing.
4. Enables or disables specific subcondition.

5. **Subcondition type.** Allows selecting a type of subcondition (e.g. speed, zone and angle to wall).
6. **Comparison type.** Defines a type of the numerical comparison (e.g. equal, greater than, less than.)
7. **Compare against.** Defines numerical reference values.
8. **Validity criterion.** Defines a set of circumstances under which the specific subcondition is met. (Currently only running bout in seconds is supported.)
9. **Has been true for** (followed by a value). Number of seconds, during which the criterion must be valid.
10. Enables or disables validity check for current subcondition.
11. **Response type.** Defines a type of output if the condition is True. Possible values are None, Air puff (the same is used to produce regular TTL output) and Liquid reward (requires liquid reward calibration prior to activation).
12. **TTL port**. Specifies the port for signal output.
13. Defines length of the TTL pulse, or Air puff length in milliseconds, or amount of liquid to dispense in microliters.
14. Summary of the condition parameters.
15. Discard and exit without saving any changes.
16. Reset changes to their original values.
17. Save and exit.

When condition is met, it generates only one outcome (e.g. TTL signal) until it is reset (i.e. until it becomes False).

3.11. Data saving and format description

The software saves the raw data in a TDMS file format in the default folder (see section 3.7) and updates the file every 4 seconds to prevent potential data loss. When recording ends, a new folder marked with the time of recording is created in the application folder. The *.tdms file can be opened with Excel and contains the following data from the recorded session:

1. **Track (root)** tab. Contains technical information about recorded file.
2. **Raw_sensor_data** tab. Contains the magnet's raw and unprocessed Cartesian coordinates (**X1_raw, Y1_raw, X2_raw, Y2_raw**) in the sensor board's coordinate system.
3. **Data** tab. Contains all data with immediate processing where applicable.
    a. **HW_timestamp.** Indicates a hardware timer value from the ECU's own time source.
    b. **Frame_HW_time.** Frame time in milliseconds (from hardware timer).
    c. **SW_timestamp.** Software timestamp (number of seconds between 1st Jan 1904 (year zero) and the moment of the frame's acquisition.
    d. **R** is a linear distance between the mouse and the center of the cage (for explanation of the coordinates axes see Figure 21 below).
    e. **phi** is an angle of the mouse relative to the cage in polar coordinates.
    f. **alpha** is an angle between the mouse's longitudinal axis ("mouse's tail to mouse's nose") and the cage's Y axis ("center of the cage to middle of the door").
    g. **X** is a X coordinate of the mouse relative to the cage center (with filters applied, if selected).
    h. **Y** is a Y coordinate of the mouse relative to the cage center (with filters applied, if selected).
    i. **theta and beta.** Parameters used for debugging.
    j. **w** is the absolute of the angle between the "mouse tail – mouse nose" axis and the tangent of the wall at the point where the mouse axis crosses the wall.

k. **Speed.** Indicates mouse's current speed in mm/s.
l. **Zone.** Number of the zone, in which the mouse is currently located.
m. **TTL_inputs.** Indicates TTL input port's activity status.
n. **TTL_outputs.** Indicates TTL output port's activity status.
o. **Key.** Indicates whether one of the directional keys (Up, Down, Left or Right) is pressed.

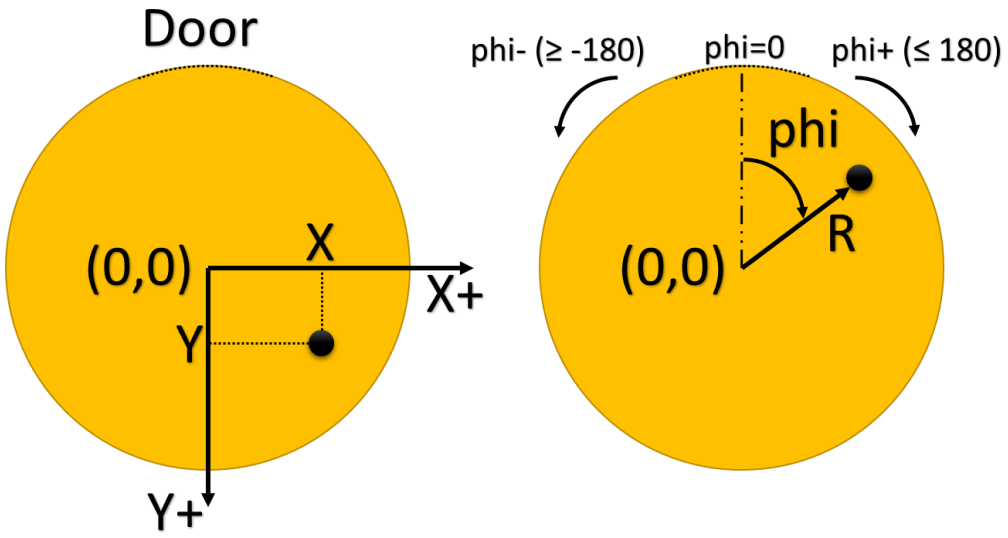


Figure 12. Cartesian and polar coordinates in the Mobile HomeCage.

4. **Pp_Data** tab. Contains the same data as **Data** tab, but post-processed with zero-phase filters where applicable. Timestamps are aligned with the beginning of the recording.

In addition to saving a .tdms file, the software saves a screenshot of the zones' layout, heat maps and the recording settings.

4. Use of Terminal Emulator

It is possible to use the tracker without the tracker software with any terminal emulator (tracker was tested with the Tera Term emulator). To use the tracker with terminal emulator, set the parameters as follows:

Baud rate	256 kbit/s
Data	8 bit
Parity	None
Stop	1 bit
Flow control	RTS/CTS/Hardware

In this mode the data are transmitted in ASCII by default, which may decrease the frame rate (compared to binary mode). Binary mode can be activated in terminal emulator, but the resulting data are not human-readable. Using the binary mode without the tracker software is feasible with a third-party software, but not with a terminal emulator.

4.1. Binary format description

4.1.1. For the "no external TTL recording" mode (PORTS=0):

1. Totally 28 bytes per measurement.
2. 2 bytes start symbol, 0xFF, 0xFF.
3. 4 bytes time code, a 32 bit integer in milliseconds passed since the device is ON.
4. 2 bytes delta time, a 16 bit integer in milliseconds.
5. 2 bytes channel indicator, 0xFE followed by ASCII char A, B, C or D (0x41 - 0x 44). Used only during firmware development.
6. 4 bytes X coordinate of the first magnet, a FP32 little-endian data (i.e. "0x175DB440" = 5.636364). Unit of the measure is 21 mm for the Mobile HomeCage Large and 25 mm for the Standard version, i.e. in order to get real millimeters you need to multiply coordinate by 21 or 25.
7. 4 bytes Y coordinate of the first magnet, a FP32 little-endian data.
8. 4 bytes X coordinate of the second magnet, a FP32 little-endian data.
9. 4 bytes Y coordinate of the second magnet, a FP32 little-endian data.
10. 2 bytes end packet symbol, 0xFE, 0xFE.

4.1.2. For the "with external TTL recording" mode (PORTS=1):

1. Totally 32 bytes per measurement.
2. 2 bytes start symbol, 0xFF, 0xFF.
3. 4 bytes time code, a 32 bit integer in milliseconds passed since the device is ON.
4. 2 bytes delta time, a 16 bit integer in milliseconds.
5. 2 bytes channel indicator, 0xFE followed by ASCII char A, B, C or D (0x41 - 0x 44). Used only during firmware development.
6. 4 bytes X coordinate of the first magnet, a FP32 little-endian data.
7. 4 bytes Y coordinate of the first magnet, a FP32 little-endian data.
8. 4 bytes X coordinate of the second magnet, a FP32 little-endian data.
9. 4 bytes Y coordinate of the second magnet, a FP32 little-endian data.
10. 2 bytes TTL input block start symbol, 0xFE, 0x50.
11. 2 bytes for TTL I/O status (bool AND with ports I/O configuration to separate inputs and outputs).
12. 2 bytes end packet symbol, 0xFE, 0xFE.

4.2. Operation Using the Command Texts

Switching on the power sets the device in an idle mode. In this mode, all commands are entered as ASCII text, and pressing "Enter" after a command starts the command's execution. The commands are shown in Table 3 below:

Table 1. Command set

Command	Function	Notes
M	Perform one measurement	Activates the manual measurement regime. Data output is determined by the selected mode (matrix output mode or coordinate output mode)
A	Auto-measurement	Starts the automatic measurement regime
T	Terminate auto-measurement	Terminates the automatic measurement regime
V	Show firmware version information	Displays the version number of the current firmware
B	Binary format ON	Data are transferred in binary format (coordinate mode only)
D	Binary format OFF	Data are transferred in the default ASCII format
P	Poll for current status	Shows status, output mode, FPS (frames-per-second) rate and several other settings
FPS= [number]	Set target frame rate	Sets the number of measurements/frames per second (FPS)
REBOOT	Reboot the firmware	Mainly used for testing, can be used to return to a known state
R	Reset the timer	Resets the firmware internal timer to zero
LEDS= [number]	Activate / deactivate LEDs	Turns OFF the LEDs if value set to 0, turns ON the LEDs if value set to 1 (default). Note: the Power LED is not affected.
PORTS= [number]	Activate / deactivate external I/O pin reading	When set at 0, no I/O pin data are sent (default); when set at 1, the state of I/O pins is transmitted for every frame
DIR=[number]	Set I/O port direction	I/O pin 1 is set as the lowest bit, I/O pin 2 is set as the second lowest bit, range 0-15, default "1100"=12=0x0C, NOTE: no space!

		Also, for values 10 - 15 Hex notation A - F can be used.
O[number]	Set output pin values	I/O pin 1 is set as the lowest bit, I/O pin 2 is set as the second lowest bit, range 0-15, default 0, NOTE: no space! Also, for values 10 - 15 Hex notation A - F can be used. In the automatic measurement mode A-F notation **should** be used. NOTE: no space between "O" and the number!
SYNC=[number]	Activate automatic sync pulse generation	Uses I/O pins as sync signals to external devices, 1 uses I/O 1 as toggle, 2 uses I/O 2 as pulse, 3 uses both at the same time. User has to set I/O as output. Command SYNC overrides command O.
EXT= [number]	Activate External USART	External USART transmits a copy of the data stream, when set to 1

All of the above commands (except T) will be processed upon pressing ENTER

Note that only two commands (O and T) are usable when in automatic measurement mode

This list is provided for the firmware version 2.0.4+

For stable operation with the current version of firmware/hardware, limit the measurement rate to 80 FPS for the standard *Mobile HomeCage®* and 100 FPS for the *Mobile HomeCage Large®*.