# Wmicrotracker®

Installation Guide and User Manual
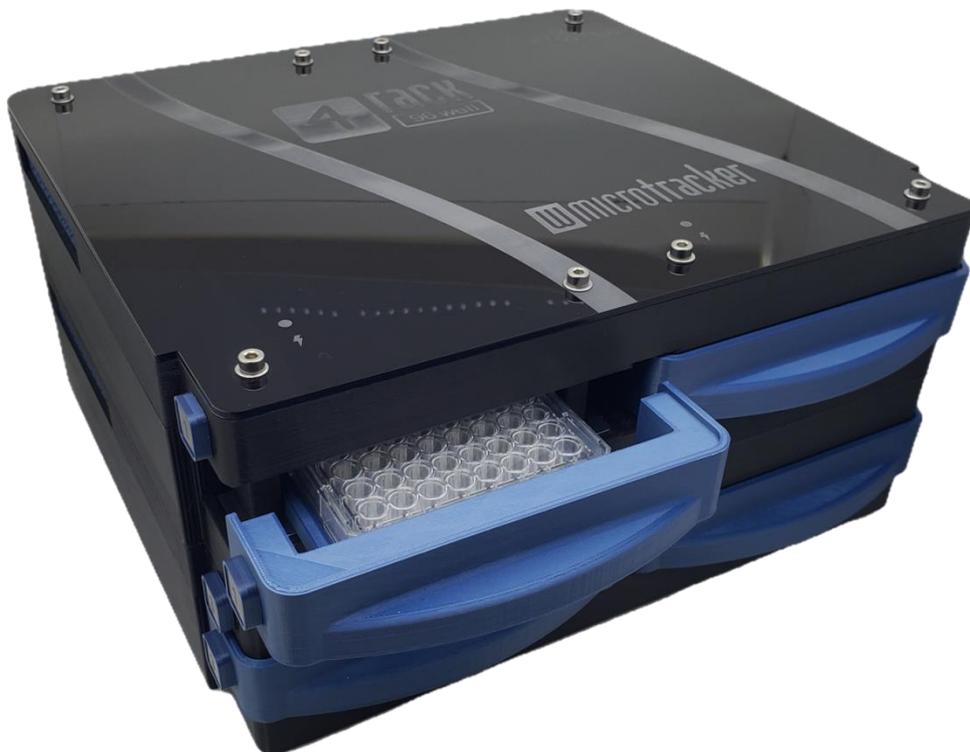
Data Acquisition System: WMicrotracker 4-RACK
Hardware Version: MKT 1.40-RACK
Software Version: V5.1 WMicrotracker (2026)

Thank you for acquiring the WMicrotracker 4-RACK system. The following document will guide you through the installation process.
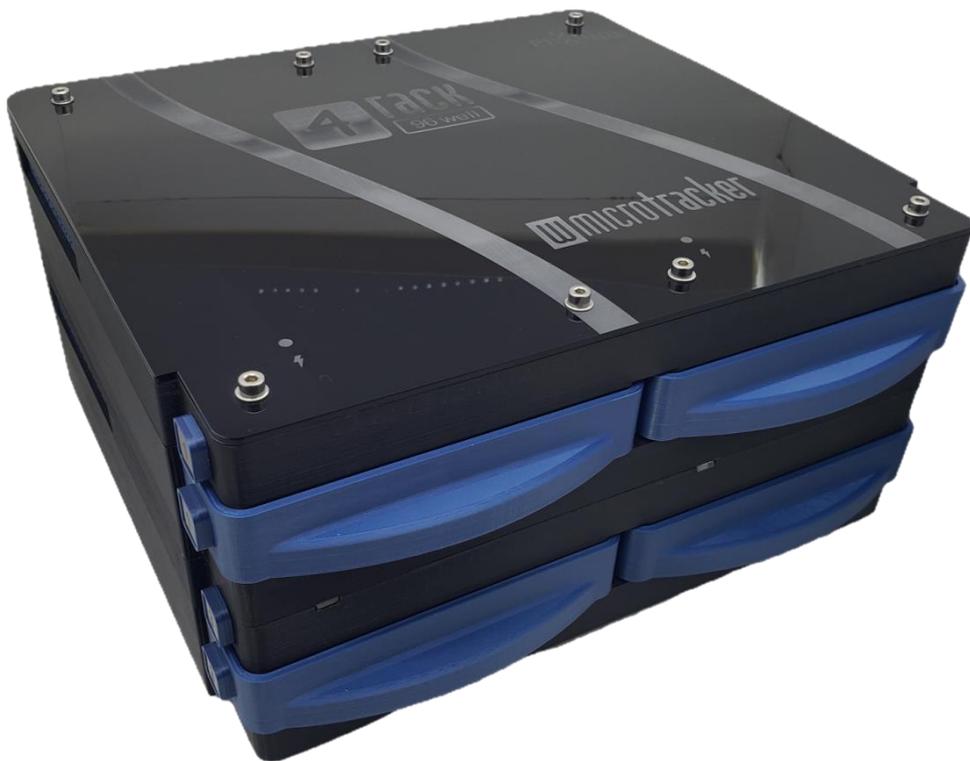
Contents

I. About the 4-RACK

WMicrotracker 4-RACK technology is based on an infrared micro beam system that detects light refraction through the animal body, a methodology originally published by Simonetta SH et al 2007 (https://doi.org/10.1016/j.jneumeth.2006.11.015).

The locomotor activity recording system counts infrared photo-beam interruptions (bins) in a fixed time lapse. Our system senses transient analogical changes in order to detect how individual worms move across a microbeam of infrared light.

The detection is based on four stations of Infrared microbeam array of 96 independent sensors and simultaneous channel readouts.

Infrared locomotor tracking system
(Worm Microtracker)
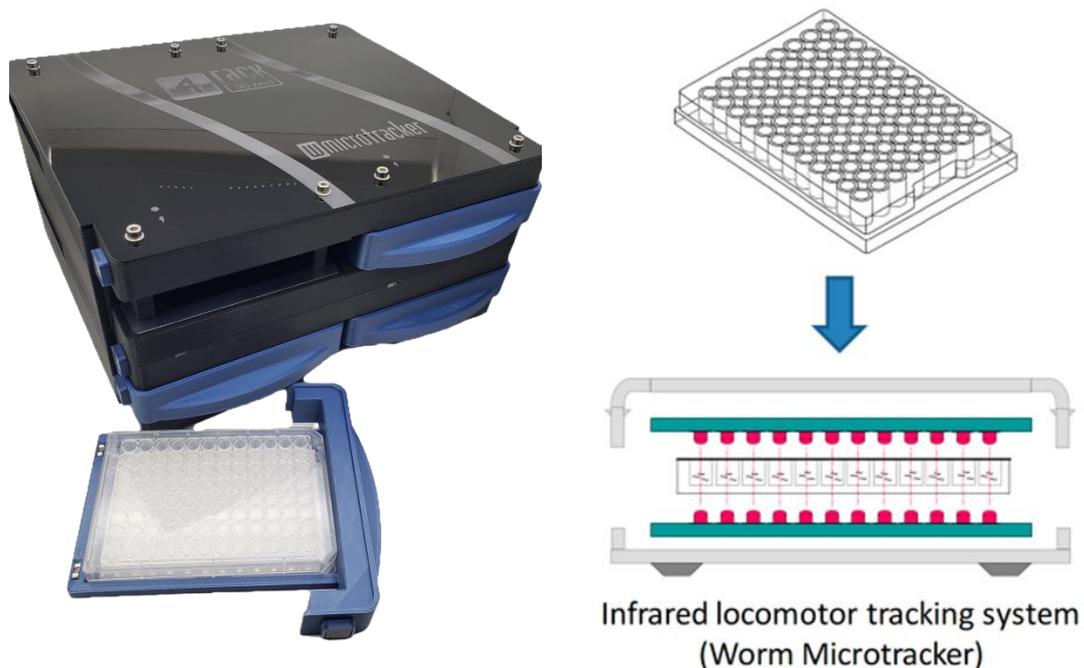
As the animals cross the sensor grid, locomotor activity is detected as interruptions in light beams;

-96 Wells Flat bottom [1 microbeams/well]
-96 Wells U shape bottom [1 microbeams/well]

The 4-RACK version of Wmicrotracker does not require the use of a special adapter for these microplate formats. However, a system customization can be made on request for larval ticks tubes.

-Glass tubes, 8 to 32 per adapter [4-6 microbeams/tube]

A simple algorithm was programmed to convert signal changes into activity events. The output of phototransistors are digitized and recorded in a PC. Digital analysis of signal changes (proportional to light intensity) allowed us to detect the movement of the worm passing through the light beam. A software algorithm calculates the number of activity events per time block;

Example: Mode 1_Threshold Average

Every time the signal falls below threshold the activity counter is incremented. The threshold line will be smoothed in relation to raw signal.
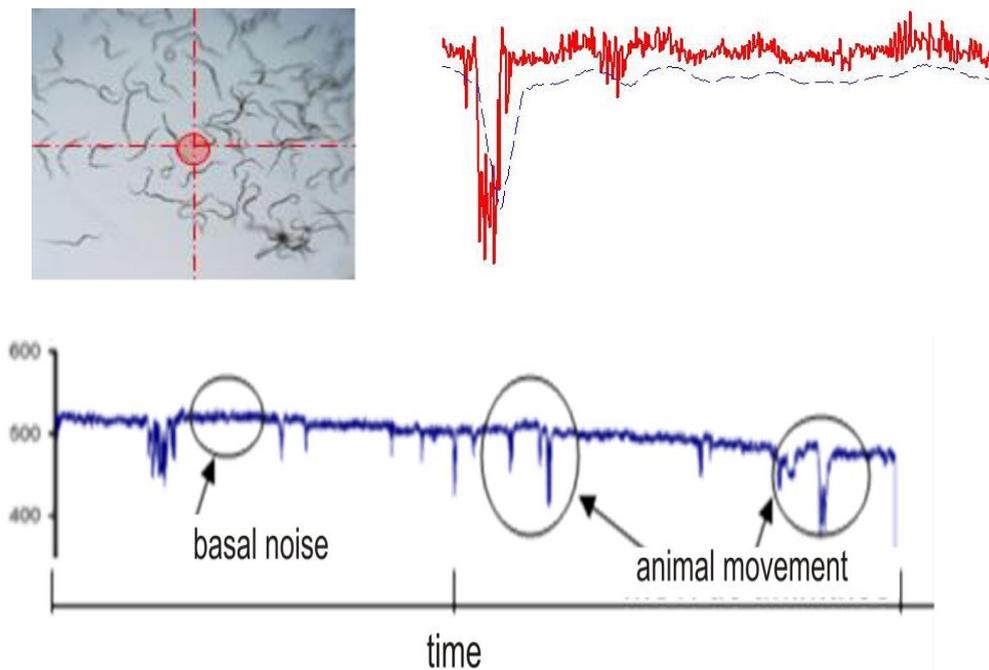
Using this approach is possible to evaluate multiple protocols using liquid culture media. Preferred microplate culture format for 4-RACK is 96 well "flat bottom" and 96 well "U bottom".

Recommended:

- 96-Well "Flat"   (Greiner Bio-One #655180)
- 96-Well "U"   (Greiner Bio-One #650161 + lid #656161)

NOTE: All plate formats must be run with the lid on. It is recommended to seal the plate/ microplate with film (This decreases the formation of condensed drops on the lid).

Included Components

	Microplate reader system. 4-RACK		USB-B/USB-A cable. (*)
	Acquisition Software: available from www.phylumtech.com		5 V DC. 2 Amp. Plug 5.5x2.1 Pin + interno

- Due to customs restrictions, in some countries the shipping might not include the power supply

Additional Requirements

- IBM PC compatible with the following minimum requirements :
  - Core i5 processor or above (>1GHz clock).
  - 512Mb of RAM memory.
  - 1 USB port available.
  - DVD-ROM unit (optional)
  - Windows XP 32bits (or higher) operative system.
  - At least 200Mb of free HD space.
  - Automatic shutdown/sleep/hibernate mode must be disabled.
- Ambient operating temperature of 15ºC to 37ºC with humidity below 50%. This range is for optimal functionality of equipment only; biological samples may have unique temperature requirements.
- Minimize the vibration and dust in your working area.
- Do NOT locate the instrument near a window or bright light.

Product Dimensions

- 29.0 cm x 25.8 cm x 13.8cm (≈ 11.4 × 10.2 × 5.4 in).

II.   Installation & Setup Guide

Software Installation

We recommend periodically referencing the Phylumtech website for software updates.

1. You'll find the Software Installation .zip folder for downloading at Software download zone (https://www.phylumtech.com/home/en/support/).To download right-click the link and choose "save link as".
2. Unzip the files and copy WMicrotracker_4-RACK folder to C:\WMicrotracker_4-RACK folder and follow the instructions detailed in the Readme.txt file:
   a. Run USB_Driver Installer before plugging the adapter. Follow the instructions on screen to install the driver.
   b. Plug the equipment to the USB2.0 port.
   c. Run wmicrotracker_4-rack.exe

Additional comments:

- In case an online "autoupdate" starts (sometimes windows seven tries to find drivers through the internet), select cancel, and choose to "find driver files automatically in the local computer".

- If MSWindows doesn't find the driver, you can manually search for "mchpcdc.inf" driver file from this folder.

- To check if the USB-driver was properly installed, verify if a new COM Port has been detected in your computer (into Devices & Printers Windows menu) after you connect the equipment.

Hardware Setup

1. After the driver has been installed, connect the USB-B Cable extreme in the 4-RACK device and the USB-A extreme to the USB COM PORT on your computer.

2. When you connect the Power Supply, the green light on top of it should turn on and the blue light will flash (While the system is being checked or during the acquisition).
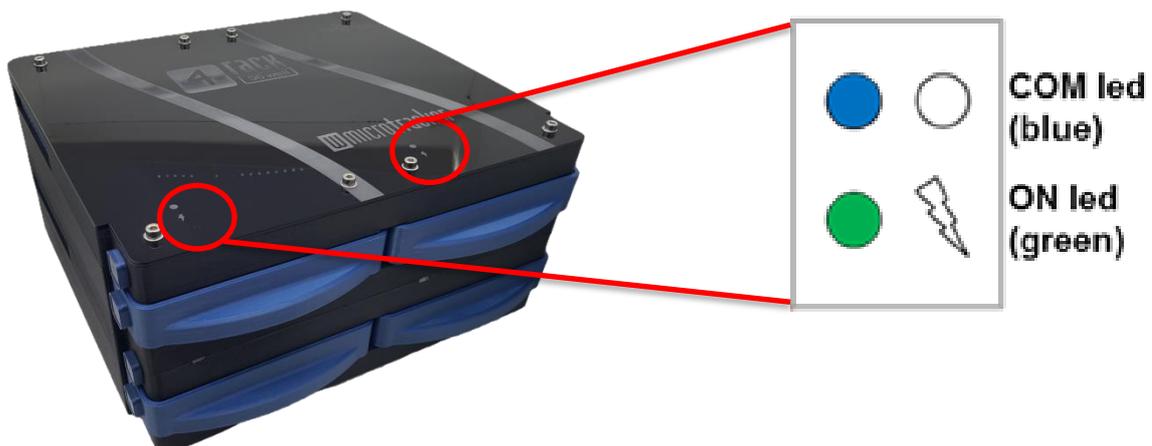
3. The hardware is going to be auto-detected at COM Port 1 to 15.

Note:
-See Devices & Printers Window to verify that a new COM Port was detected.

III. Software Use

Software Launch

1. Run the **"WMicrotracker 4-RACK"** executable file from the folder you chose during the installation step. The application should start immediately with this "**Start Window"**.

2. Check if the hardware is detected correctly by going to: "Run Latest Project". An auto diagnostic popup window will appear:
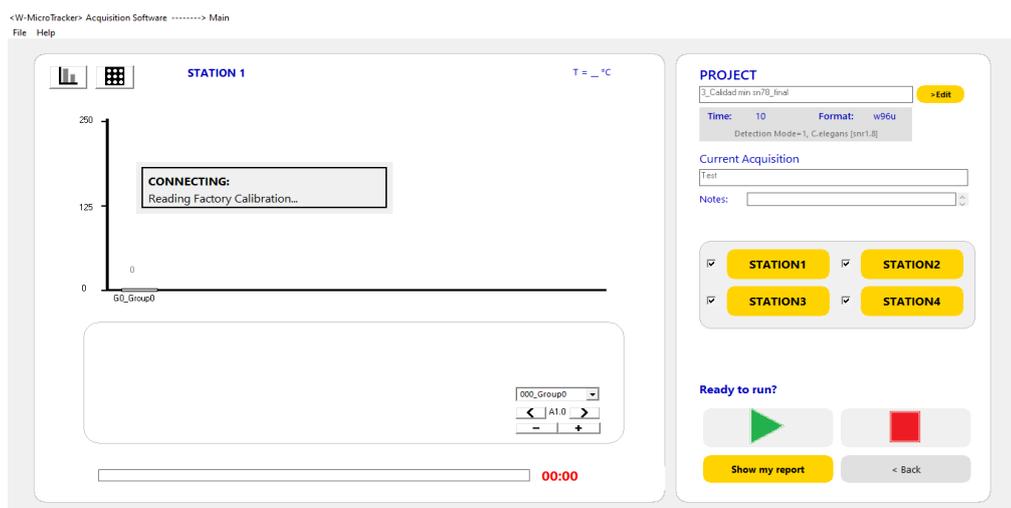
If there is any problem with the detection of the system then a COM PORT ERROR pop-up will be shown (See Appendix D. Troubleshooting).

Screen Components on the Start Window

1. The first screen <u>Setup</u> is shown when you select "Setup a New Project" on the Start window.
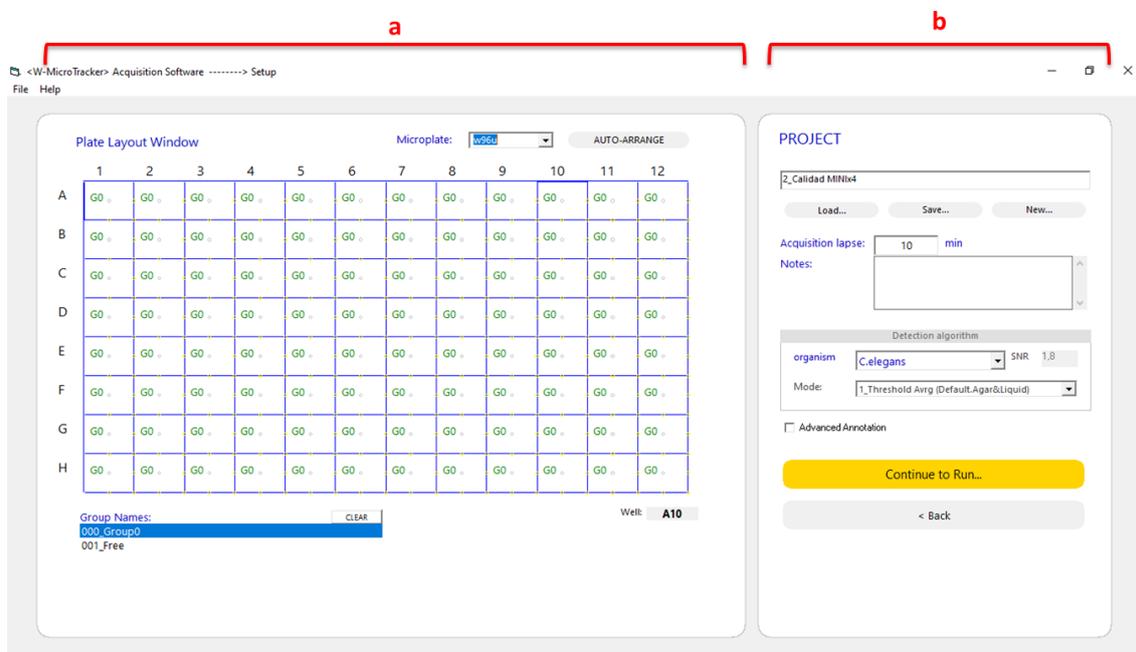
Plate Layout:

a.   Microplate Box:
-Currently the WMTK 4-RACK has been validated to work with 96-well "Flat bottom" and 96-well "U" bottom format. Place the microplate as indicated by the input tray.

-The microplate scheme allows selecting the wells and the distribution of experimental groups.

ii.   *Auto-Arrange Button:*

- Shows options for arranging the plate layout to read differing experimental set-ups.

iii.   *Group Names Box:*

- "Group" refers to an experimental group defined on your plate.

- "Group" numbering begins with 000_Group0.

- You can change the Group Name (well name) as desired to best fit your experimental needs (ex: "control sample," "experimental sample A," etc).

- "Clear list" allows clearing all group name designations you have set for your plate layout.

b.   Project Menu:
i.   *"New" Project Creation Button:*

- This will result in a pop-up window.

- Create and name a new project or new project layout using this window.

ii.   *"Save" Button:*
   - Save the updates values .
iii.   *"Load" Button:*
   - Allows you to select a pre-existed project to load.
iv.   *"Rename" Button:*
- Enter a new name of a pre-existed project
v.   *"Acquisition" Lapse of time:*
- Set the total run time for the microplate reading, in minutes (ex: 120 minute run time for 2 hour plate reading experiments).
- Acquisition time cannot be less than 15 minutes.
vi.   "*Detection algorithm" Box*:
- Allows you to select type of organism:
●   Organisms with sinusoidal movement like C.elegans.
●   Organisms with natatory movement like Zebrafish.
●   Organisms with ameboid movement like Echinococcus.

NOTE: The program will recommend by default a data analysis mode for the selected organism.

- Allows you to select a data analysis mode:
●   DSP Method_0_Threshold+Binary (Old Method.w/filters); Analyze the number of times that small organisms interrupt the beam using a filter of 3-second. Interruptions that occur within a 3-seconds period are computed as a unit of activity, estimating that this is the swimming movement time required to cross the beam.
●   DSP Method_1_Threshold Avrg (Default.Agar&Liquid); Analyze the number of times that small organisms interrupt the beam without a filter of time. Each beam interruption counted as a unit of activity.
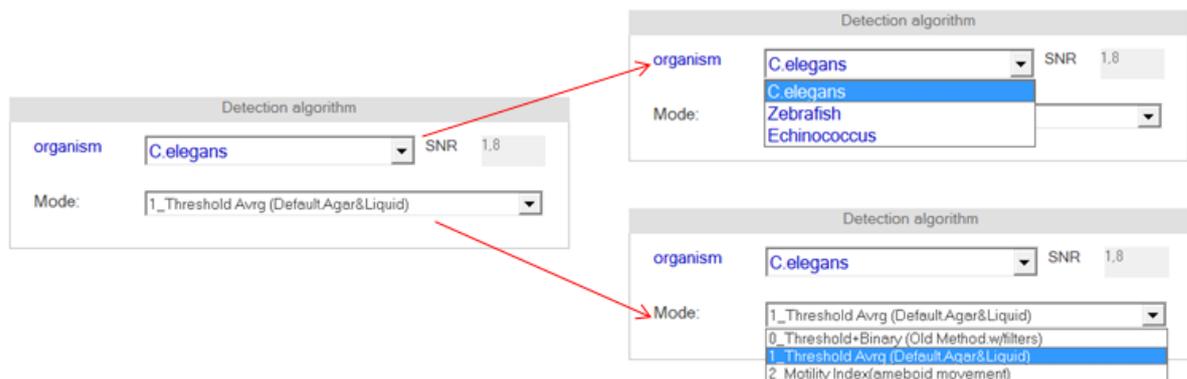
●   DSP Method_2_Motility Index (Ameboid movement); Sensitive detection mode that allows the recording of small fluctuations in organisms with amoeboid behavior. It measures the amplitude of the signal based on patent 4176953_Method and apparatus for measuring the motility of sperm cells (Bartoov et al.). Testing of organisms with amoeboid movement is recommended in the microplate "U bottom".

2. The second screen <u>Main</u> is shown when you select "Run Latest Project" on the Start Window or you press the bottom "continue to Run" in the first screen.

a. Project Window:

i.   "Project" Box:

- Show the name, time and format plate of the current project to run.
- The edit option returns to the Setup Window.

ii.   "Current acquisition" Box:

- You can visualize the name of the current acquisition that you selected.

ii.   "Station" Box:

Select the stations to acquire. Click on the station button to be displayed on the screen

.

b. Run Menu:

i.   Ready to Run?:

- "Start ►"; Starts the data acquisition. The name of the current experiment will be requested for future reference.
- "Stop ■"; Stop the current data acquisition.
- "Show my report" button: Shows the register data of the current acquisition.

c. Real Time Window:

i.   "Bar Plot" a little button of bars on the left top side:

- Displays a bar graph of the real-time activity of the groups selected for the experiment.

ii.   "Heat Map Plot" a little button of array next to the bar plot button:

- Displays a grayscale map according to the presence of activity. Light tones indicate low activity and dark tones indicate more activity.

iii.   "T" Temperature:

-Record the temperature of your test. Indicate your working room temperature.

iv.   "Signal plot" graph on the bottom side:

- Displays the individual signal from the sensors. It allows the selection of sensors per well within an experimental group.

d. Status Bar:

i.   "Status Bar":

-Shows as a blue bar the progress of the run time.

3. The third screen <u>Report Analysis</u> is shown when you select "Analyzed recorder experiment" on the Start Window.
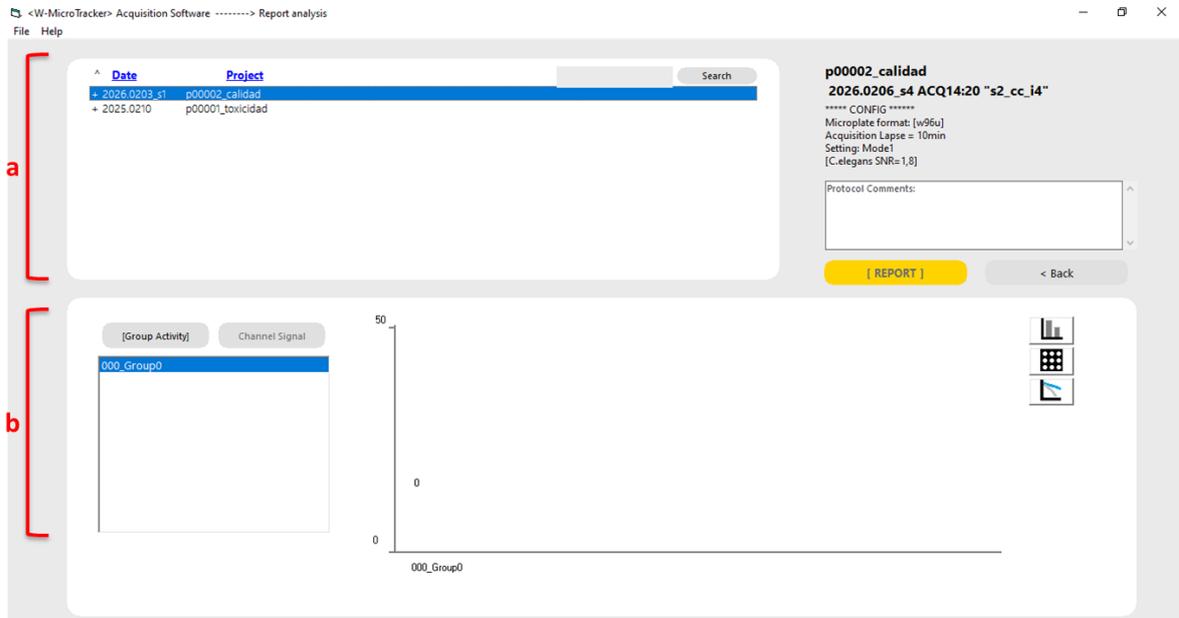
a. Search Window:

i.   "Filter by" Box:

- Project search by word. Press apply to find out your folder.

ii.   "Project" Box:

- You can visualize your project by date and name. The plus symbol allows displaying the sub folders.

iii.   REPORT Bar/Bottom:

- Once the desired project is selected, press this button/bar to access the report options.

b. Offline Window:

i.   Screen Box:

- Shows a summary of the settings of the selected project and the experimental groups.

ii.   Group activity button:

- You can visualize a bar graph with the activity of each experimental group and each selected well.

iii.   Channel signal button:

-Shows the raw signal of each selected sensor.

IV. Defining and Starting Your Experiment

1. In the Setup Window, create a new project by clicking **"New"** on the right-hand project menu (Step 1).

2. A **"New Project Creation"** window will pop up; name your new project in this pop-up window (Step 2 and 3).
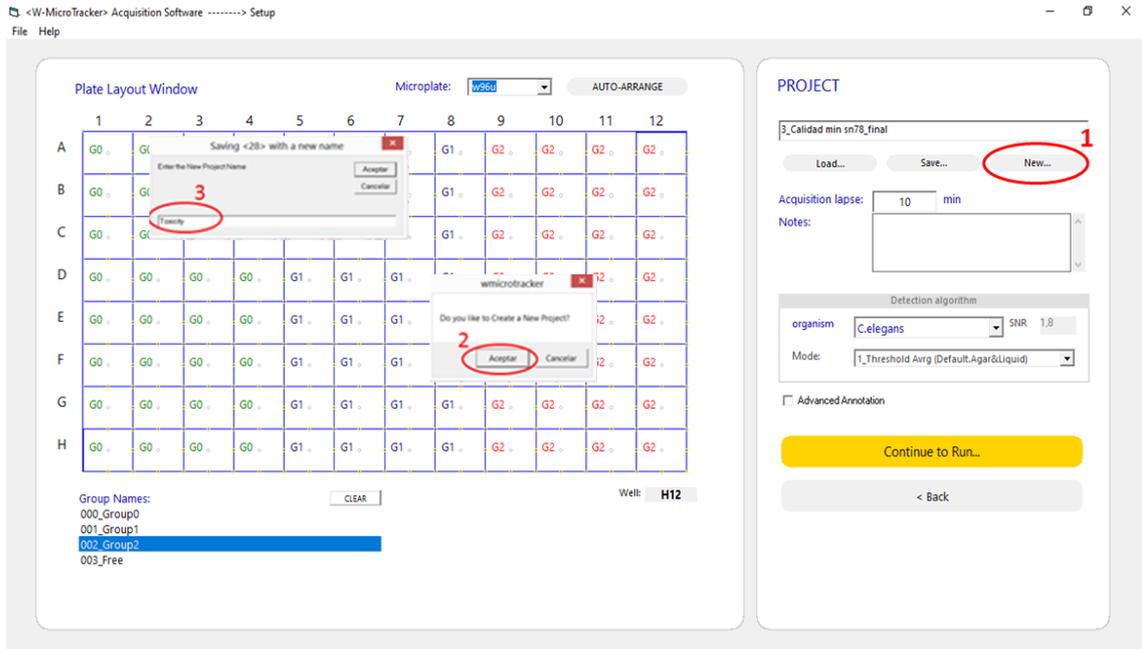
3. Enter the **"MicroPlate Format"** size that you will be using.
   - Select plate format
   - Accept changes

NOTE: At this time, we have validated the instrument for use only with 96-well "Flat" and "U" shape bottom. The MINI version of Wmicrotracker does not require the use of a special adapter for these microplate formats. Just insert the plate on the tray.

4. Set the **"Acquisition Lapse Time."**

NOTE: The minimum recommended collection time is a 15 min run.
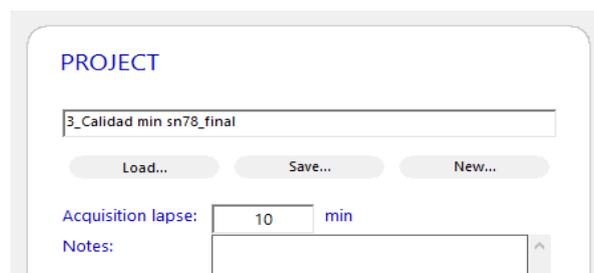
5. Set your plate layout in the **"Group Names"** section; this can be done either manually or by using the **"Auto-Arrange"** feature.

NOTE: In 96-well microplate format, the number of technical replicates recommended per group is six wells (average standard deviation < 15% in the activity between homogeneous groups).

a. MANUAL Layout
    - In the "Group Names" section, select a group in the box (Step 3)
    - Double-click in the left mouse button to rename your group (Step 3)
    - Highlight the wells that you want to set as a group. Click the left mouse button on the microplate plot to add wells. Click the right mouse button to remove wells from the group.
    - Repeat this procedure to each group in your experiment. Always remember to select a new group!

b. AUTO-ARRANGE Layout
    - Click **"Auto-Arrange"** (Step 1) to select the number of rows and columns you wish to use (Step 2); the arrangement options will differ depending on your chosen plate format.
    - In the **"Group Names"** section, double-click to label your group (Step 3); repeat for each group in your experiment.
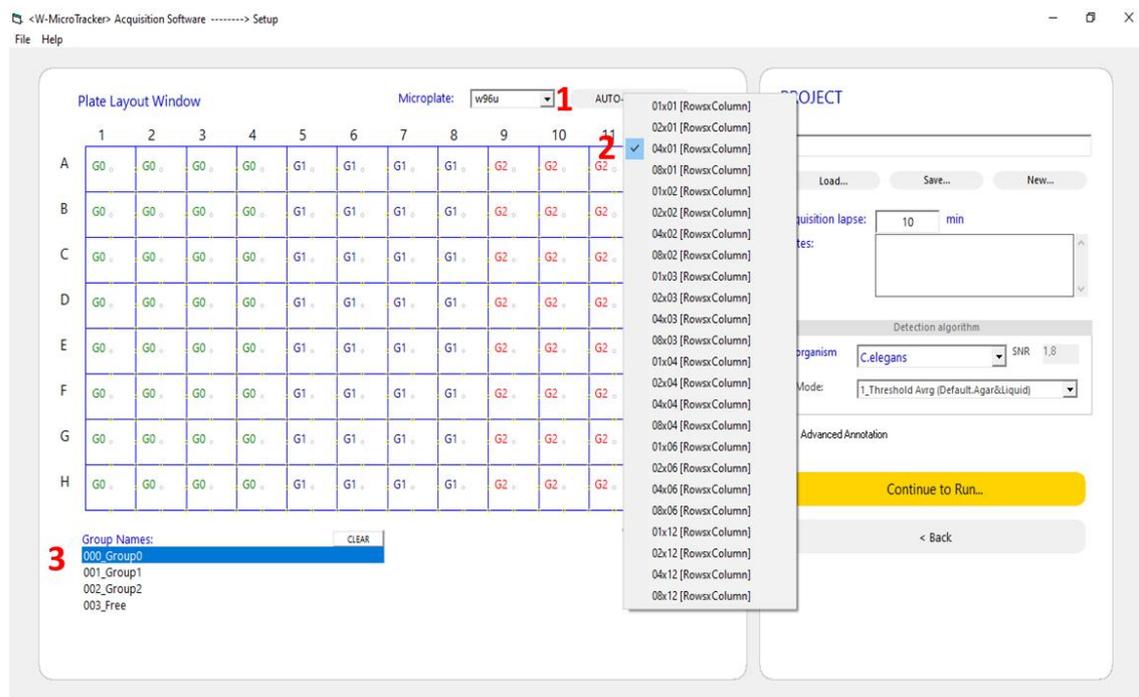
c. CLEAR LIST Button
    - This will clear "ALL" the existing Group Names that you have listed and arranged; no name or arrangement information will be saved.
    - You will be prompted to confirm your selection.

6. Press the **"Continue to Run"** button.

7. You are now ready to load your plate.

Start your experiment running!

V. Running Your Experiment

1. After setting experimental parameters, load your plate into the instrument and press **"Start ►"** to begin your experiment (Step 1).

2. You will then be prompted to enter a specific name for your experiment run ("Acquisition"); this will be filed under your project name (Step 2).
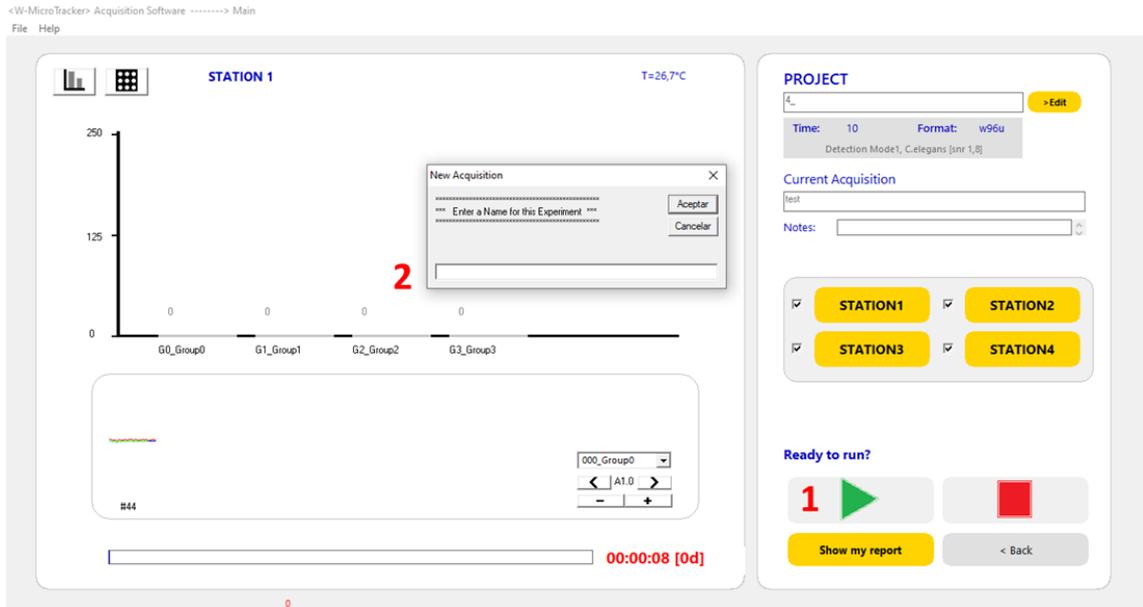
3. The software will automatically calibrate the 96 sensors into the system to recognize the worm movement. This calibration will take about 1 minute for the first time, and a few seconds in the future.
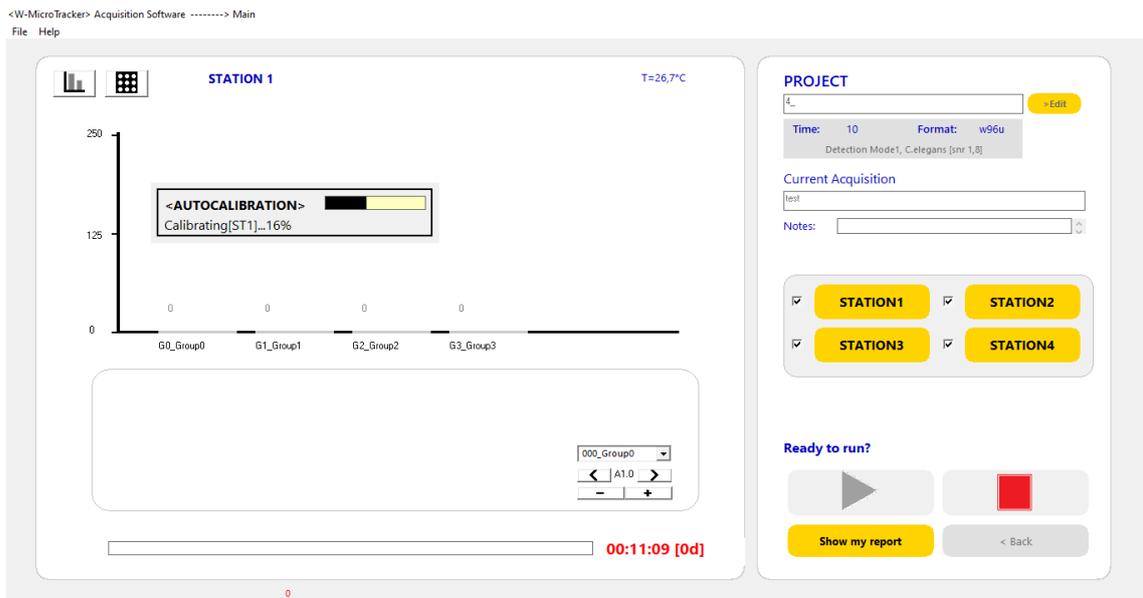
4. When calibration is complete, acquisition of your plate will begin automatically and the 4-RACK will start collecting data. The Status Bar will begin to count up and the progress bar will begin to monitor progress as samples are being analyzed. You will see after 3 minutes the accumulated activity for each group at the TOP plot.

5. When the 4-RACK instrument has finished the experimental run, the status bar is completed. The software shows the next pop-up window "Report file Auto Generated". You can export your data immediately by utilizing the **"Show my Report"** button or export later by utilizing the **"Analyze recorder experiments"** option on the Start Window.

VI. Accessing Experiment Data

Experiment report files can be quickly and easily accessed either immediately after an experiment run, or at any later time.

NOTE: Experiment reports are generated as a .CSV file by default; we recommend Excel for quick export and ease of use of the report files.

Generate an Immediate Report

1. At the end of an experiment run, a pop-up window titled "Report File Auto Generated" will appear automatically. Click the "Accept" button to confirm the report generation. Then, click "Show my report" to display the report on the screen.
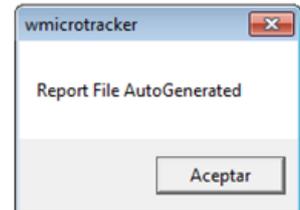
The generated report will correspond to the station that is selected at the moment the **"Generate Report"** button is clicked.

2. You have several options for data export arrangements:
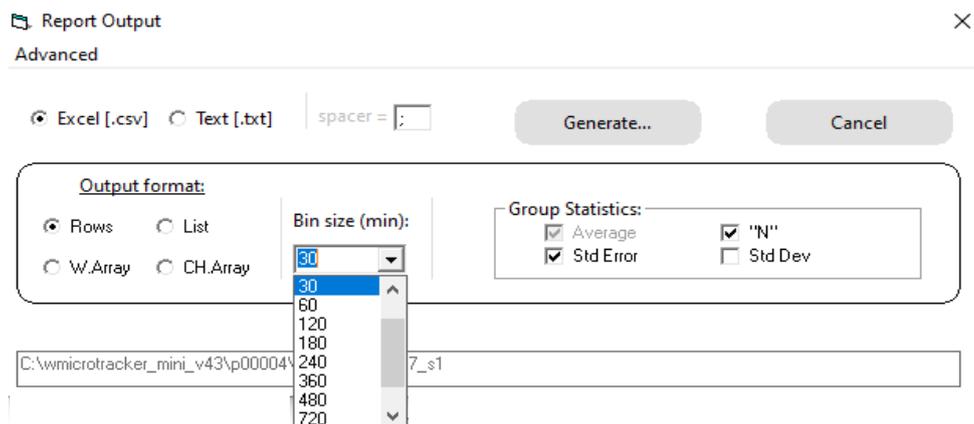
- Export and view the data using different "Bin Size" formats.

> i. Data can be grouped in fixed time-blocks in order to evaluate the kinetics of behavior.

> ii. Alternate bin sizes (ex: 30min bins, 60min bins) will group or "bin" all scans for a run taken in that time, and output only that information.

- You can also modify the "File" and "Spacer" fields as best suited to your data analysis needs.

- You can set the Group Statistics options including Average, Std Error, "N" (Number of replicates) and Std Deviation.

- Export and view the data report by **"Rows"**, **"List"**, **"W.Array"** or **"CH.Assay"** format.

    i. **Rows** show the kinetics of each group arranged in rows.

5	<<<<<< Group Activity: Average Activity Counts per Data Interval>>>>>>					
6	Group/Time[m]	5	10	15	20	25	30
7	000_Blanco	1	2	2	2	2	2
8	001_N2	38	25	22	16	15	13
9	002_DR	27	23	19	13	15	13
10							

    ii. **List** shows the kinetics of each group arranged in a list format.

5	<<<<<< Group Activity: Average Activity Counts per Data Interval>>>>>>		
6	Time	Group	Value
7	5	000_Blanco	1
8	10	000_Blanco	2
9	15	000_Blanco	2
10	20	000_Blanco	2
11	25	000_Blanco	2
12	30	000_Blanco	2
13			
14	5	001_N2	38
15	10	001_N2	25
16	15	001_N2	22
17	20	001_N2	16
18	25	001_N2	15
19	30	001_N2	13
20			
21	5	002_DR	27
22	10	002_DR	23
23	15	002_DR	19
24	20	002_DR	13

report_1

    iii. **W. Array** will provide data for each well in a plate-format layout.

5	<<<<<<WELL ARRAY. Group Configuration>>>>>>					
6	Row/Column	1	2	3	4	5	6
7	A	001_N2	002_DR	001_N2	000_Blanco	002_DR	000_Blanco
8	B	002_DR	001_N2	002_DR	001_N2	000_Blanco	001_N2
9	C	001_N2	002_DR	001_N2	002_DR	001_N2	002_DR
10	D	000_Blanco	002_DR	002_DR	001_N2	002_DR	001_N2
11							
17	<<<<<<WELL ARRAY. Well Activity>>>>>>					
18	Time_Block[r	5					
19	Row/Column	1	2	3	4	5	6
20	A	34	46	36	1	23	0
21	B	28	44	17	54	2	28
22	C	49	24	22	32	26	20
23	D	2	16	36	42	25	43

iv. CH.Assay will provide data for each sensor/channel in a plate-format layout.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	DATE:	########															
2	FILE:	C:\lumicratracker_v26_v31api_2020-08-19\p00043\20200821_1006															
3	SUBPROJE day5																
5	<<<<<<CHANNEL ARRAY. Group Configuration>>>>>>																

CHANNEL ARRAY. Group Configuration

Row/Colum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	000_Blance	000_Blance	-
C	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	000_Blance	000_Blance	-
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-
G	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-
H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-
K	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-
L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	-	000_Blance	000_Blance	-	-	002_DR	002_DR	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-
O	-	000_Blance	000_Blance	-	-	002_DR	002_DR	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-
P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<<<<<< Average Temperature [°C] >>>>>>

Time[m]	30
Temp[°C]	0

ARRAY OUTPUT FORMAT

Time_Block 30

Row/Colum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	118	165	-	-	178	90	-	-	102	61	-	-	8	0	-
C	-	207	140	-	-	100	412	-	-	141	184	-	-	36	6	-
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	-	68	49	-	-	132	127	-	-	91	21	-	-	144	217	-
G	-	108	244	-	-	145	123	-	-	61	231	-	-	39	142	-
H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	-	96	202	-	-	83	85	-	-	135	32	-	-	83	146	-
K	-	362	322	-	-	159	67	-	-	44	60	-	-	121	138	-
L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	-	32	2	-	-	62	114	-	-	104	298	-	-	129	164	-
O	-	0	6	-	-	12	121	-	-	32	151	-	-	174	44	-
P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Export Previous Experiments

1. You can access your data at any time directly through the software Start Window.

   - Press the **"Analyzed recorded experiments"** on the Software Start Window; this will open a "Report analysis" screen with previous experiments:
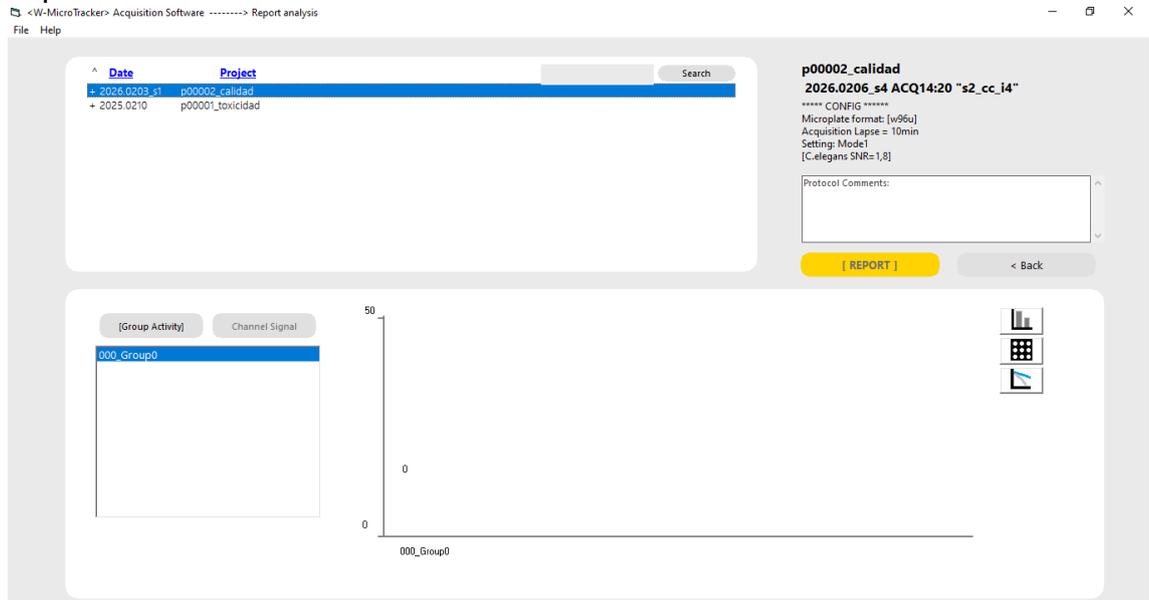
   - Select the desired project from the list. Click on the project name to expand it and view the available acquisitions (if more than one exists). For each acquisition, four reports are provided, corresponding to the reports of each station (_s1, _s2, _s3, _s4).

   - Select and double-click the appropriate acquisition and press the "REPORT" bar/button located at the top-right of the screen.

2. You can also access your data at any time directly through the **"Reports"** folder that was installed with your software. It will contain all the project data acquired on your 4-RACK device.

   - In the computer location you chose for the software folder, simply navigate to the Reports folder to see all stored project data:

   - Select and open the project folder you wish to analyze; this will lead you to files containing activity data and temperature data for the experiment.

   - Select and double-click the "_report.csv" file that you wish, this will automatically open a new window with your data displayed; data can be analyzed or further saved from this new window, as needed.

For technical support, please contact us at info@phylumtech.com

Software and system updates available at www.phylumtech.com