

Wmicrotracker®



Installation Guide and User Manual



Data Acquisition System: WMicrotracker® ONE

Hardware Version: WMTK09-R01/V1.4-R01

Software Version: WMTK V5.1-API (2026)

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Thank you for choosing the WMicrotracker® ONE system. The following document provides a comprehensive guide through the installation, setup, and operation of your device.



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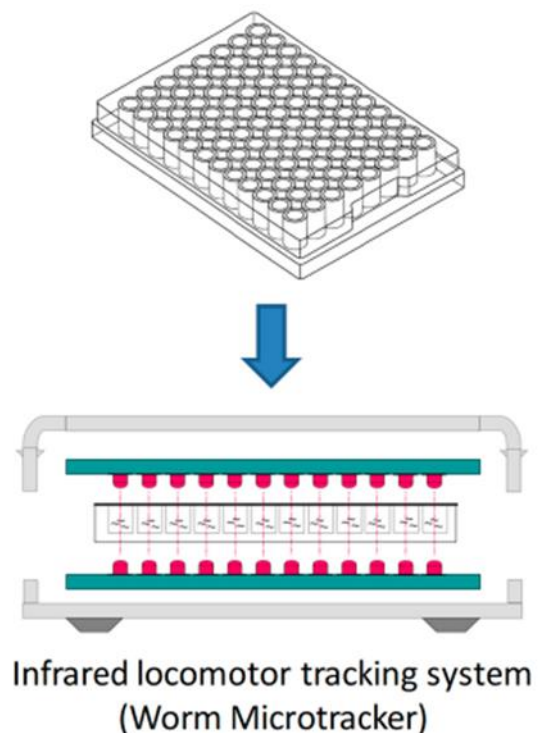
I. About the ONE

WMicrotracker® ONE is a high-sensitivity data acquisition system designed for the automated monitoring of locomotion in small organisms (such as *C. elegans*, other nematodes, zebrafish larvae, etc.) cultured in microplates.

Principle of Operation

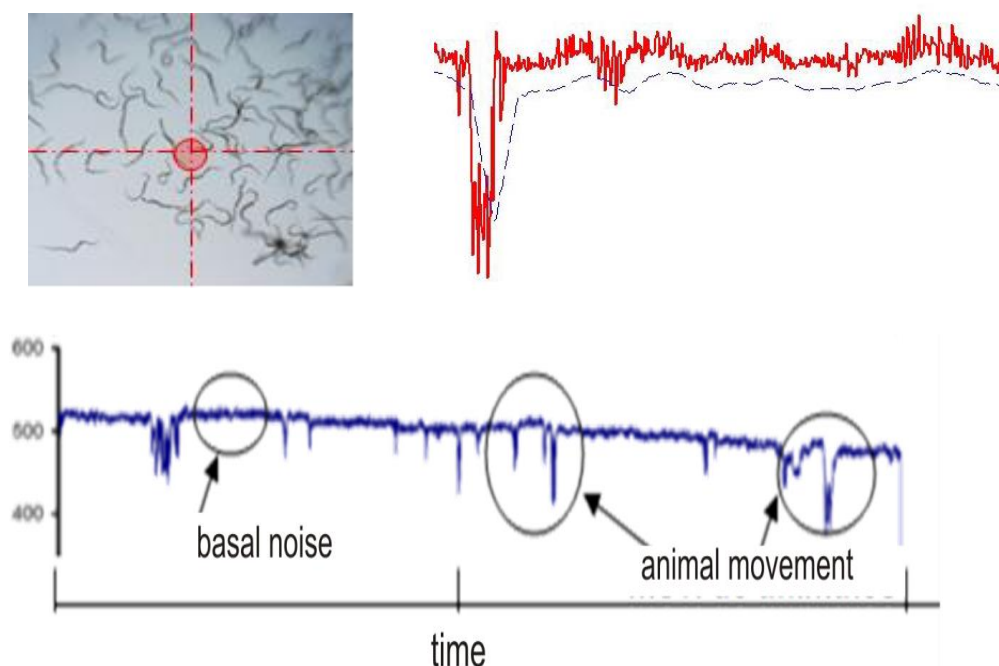
The WMicrotracker ONE technology is powered by a high-precision infrared (IR) micro-beam system designed to detect light refraction through the animal's body. This methodology was originally validated and published by **Simonetta SH et al., 2007** (<https://doi.org/10.1016/j.jneumeth.2006.11.015>).

The system records locomotor activity by counting infrared photo-beam interruptions within fixed time intervals (**bins**). Our technology detects transient analog changes in light intensity as individual organisms move across the micro-beams. This detection is driven by an **array of 384** independent infrared sensors with simultaneous channel readouts by phototransistors, ensuring high-throughput data collection.



As animals move across the sensor grid, activity is registered according to the number of beams assigned per well. A specialized algorithm converts raw signal fluctuations into discrete **Activity Events**. The output from the phototransistors is digitized and transmitted to a PC, where the software analyzes changes in signal amplitude (proportional to light intensity).

The activity counter increments every time the signal falls below a threshold. To ensure data integrity and filter out noise, the threshold line is dynamically smoothed in relation to the raw signal.



Beam Distribution and Plate Compatibility

The system is compatible with several microplate formats using specific adapters to ensure optimal alignment..

The ONE is optimized for 96- and 384-well plates, though it maintains compatibility with 6- and 24-well plates for specific applications or assays with specific organisms. For assays utilizing 6-well or 24-well plates, the use of ARENA is highly recommended.

<i>Microplate Format</i>	<i>Beams per Well</i>	<i>Required Adapter</i>	<i>Recommended Plate Model</i>
6-Well	30–32	W384/W24	Greiner Bio-One #657160
24-Well	4	W384/W24	Greiner Bio-One #662160
96-Well (Flat)	2	W96F	Greiner Bio-One #655180
96-Well (U-Shape)	1	W96U	Greiner Bio-One #650161 (+ lid #656161)
384-Well (Flat)	1	W384/W24	Thermo Scientific #95040000 (+ PS clear lid)

IMPORTANT NOTE: All plate formats must be run with the lid on.

It is highly recommended to seal the microplate with an adhesive film after placing the lid. This significantly decreases the formation of condensation droplets, which can interfere with detection of the infrared beams.

Included Components

Upon receiving your unit, please verify that all the following items are included in the package:

Item	Description
WMicrotracker device	ONE Microplate reader system unit.
Power Supply*	9V DC, 1.5A switching power adapter.
USB Cable	High-speed USB-A to USB-B cable for data connection with the computer.
Plate Adapters	A set of three (3) plate-specific adapters: <ul style="list-style-type: none">• 384/24 well microplate (for 384 and 24-well plates)• 96F well microplate (for 96-well flat bottom plates)• 96U well microplate (for 96-well "U" shape bottom plates)
Software	Installation files available from www.phylumtech.com

** Due to international customs restrictions, the power supply may not be included in shipments to certain countries.*

Additional Requirements

For the system to operate correctly, the following are required:

- **PC Computer:** IBM PC compatible with the following minimum requirements:
 - Pentium II 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.

IMPORTANT NOTE: Ensure the computer is configured to disable "USB Sleep Mode" or "Hibernation" during experiments, as this will interrupt data acquisition.

- **Environmental Conditions:** The equipment should be operated in temperature-controlled environments (between 15°C and 37°C) with humidity below 50%.

Note: The device can be placed inside a biological incubator if the experiment requires it, provided that humidity levels do not lead to internal condensation.

- **Workspace conditions:**
 - *Surface Stability:* Place the device on a stable, level surface. The unit must be kept free from constant vibrations (avoid placing it near centrifuges or heavy machinery).
 - *Environmental Cleanliness:* The workspace should be a clean, dust-free environment to prevent interference with the optical sensors.
 - *Lighting Control:* Do NOT locate the instrument near windows or under direct, bright light sources. The WMicrotracker ONE uses sensitive infrared sensors; external light interference can saturate the detectors and cause measurement errors.

Product Dimensions & Specifications

- **Dimensions:** 22cm x 28cm x 9.1cm (8.7in x 11.03 in x 3.6 in)
- **Technical & Electrical Specifications**

Feature	Specification
Power Source	9V DC, 1.5 ^a
Power Consumption	10W
Detection Technology	Infrared (IR) Phototransistors (x384)
Light Source	880nm Infrared LED Array (x384)
Communication	USB CDC Protocol (115.2 kbps)

II. Installation and Setup Guide

To ensure the system is recognized correctly by your computer, please follow these steps in the order presented.

1. Software Installation

Downloading the Software

1. Visit the **Phylumtech Support Zone** at:
<https://www.phylumtech.com/home/en/support/>
2. Locate the **Software Installation link** corresponding to the device.
3. **Right-click** the download link and select "**Save link as...**" to save the file to your computer.

IMPORTANT NOTE: *We recommend periodically referencing the Phylumtech website for software updates.*

Installation Steps

1. **Unzip** the downloaded files.
2. **Copy** the entire WMicrotracker folder directly to the root directory: **C:\WMicrotracker**.
3. Open the folder and follow the sequence below:
 - Run the **USB_Driver Installer** *before* plugging in the device. Follow the on-screen instructions to complete the driver setup.
 - Plug the WMicrotracker ONE into a **USB 2.0/3.0 port** on your computer.
 - Launch the application by running **wmicrotracker_vXX.exe**

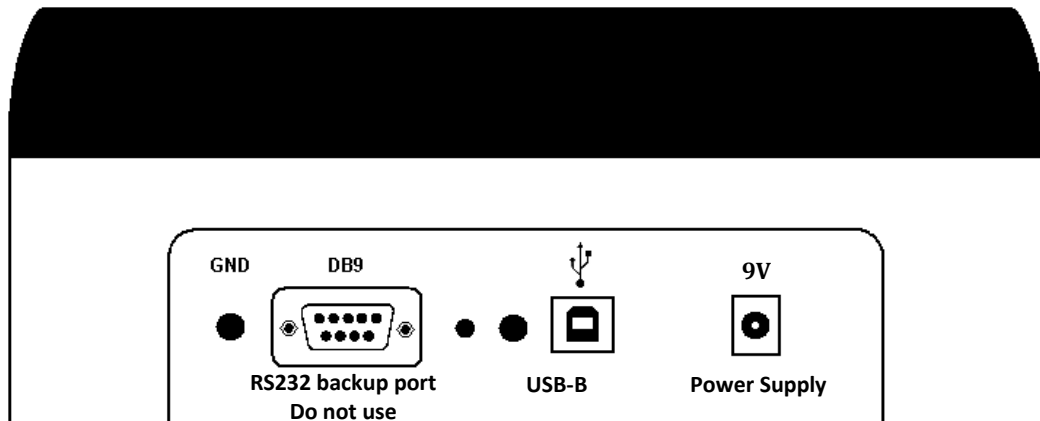
Critical Installation Comments

- If Windows (especially Windows 7) attempts to search for drivers online through "AutoUpdate," click Cancel. Instead, select the option: "*Find driver files automatically on the local computer.*"
- If Windows fails to locate the driver automatically, you can manually point the installer to the folder and search for the mchpcdc.inf file.
- To confirm the driver was installed correctly:
 1. Connect the equipment.
 2. Go to the Devices & Printers menu (or Device Manager) in Windows.
 3. Verify that a new COM Port has been detected and assigned to the device.

2. Hardware Setup

After the software is installed, you can proceed to connect the device:

1. **Power Connection:** Connect the 9V DC power supply to the power jack on the back of the WMicrotracker ONE and plug it into a stable wall outlet.



2. **Connection:** Connect the device to your computer using the provided USB cable. Windows will automatically detect the device and assign it a COM port (typically between COM1 and COM15).

Once connected, observe the LED indicators on the top of the device:



- **Green Light:** Should turn on and remain steady, indicating the device is receiving power.
- **Blue Light:** Will flash three (3) times. This indicates a successful internal system check of the microprocessor.

Notes:

- See Devices & Printers Window to verify that a new COM Port was detected.
- RS232 backup port is not necessary to connect. It is only maintained for compatibility issues.

- Ensure the device is placed on a level surface, free from vibrations and away from direct sunlight or bright light, as specified in the "Workspace Conditions" section.

III. Software Use

To start using the system, navigate to the C:\WMicrotracker folder and double-click the **wmicrotracker_vXX.exe** icon (or use the desktop shortcut if created).

Once the software is launched, the **Start Window** will appear. This is your main dashboard to manage experiments. The interface offers four primary functions:

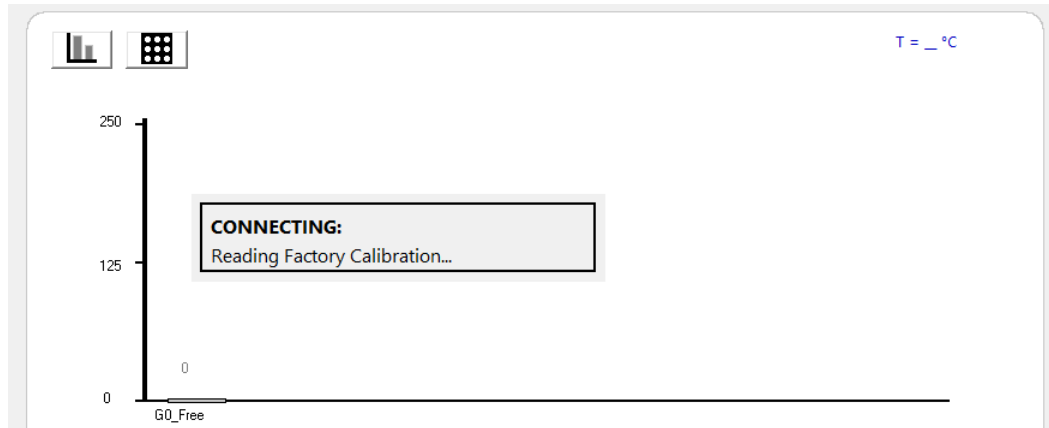
1. **Run Latest Project:** Quickly resume or repeat the most recently configured experiment. This is ideal for routine testing using the same parameters.
2. **Setup a New Project:** Access the configuration menu to define a new experimental design, including plate format, well group assignment, and acquisition duration.
3. **Analyze Recorded Experiments:** Enter the data management area to review previous runs or generate reports for further analysis.
4. **EXIT:** Safely close the application and terminate the connection with the WMicrotracker ONE device.



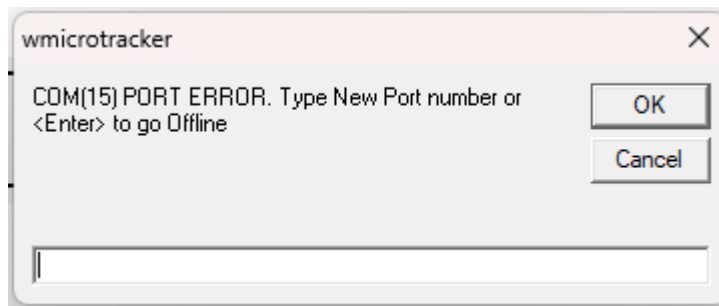
System Diagnostic Check

Before starting a full experiment, verify that the hardware is correctly detected by the computer:

1. Click on "**Run Latest Project**".
2. An **Auto-Diagnostic** popup window will appear:



- **Successful Detection:** If the system is found, the diagnostic window will close automatically, allowing you to proceed.
- **Connection Error:** If there is a problem detecting the hardware, a "COM PORT ERROR" popup will be displayed.

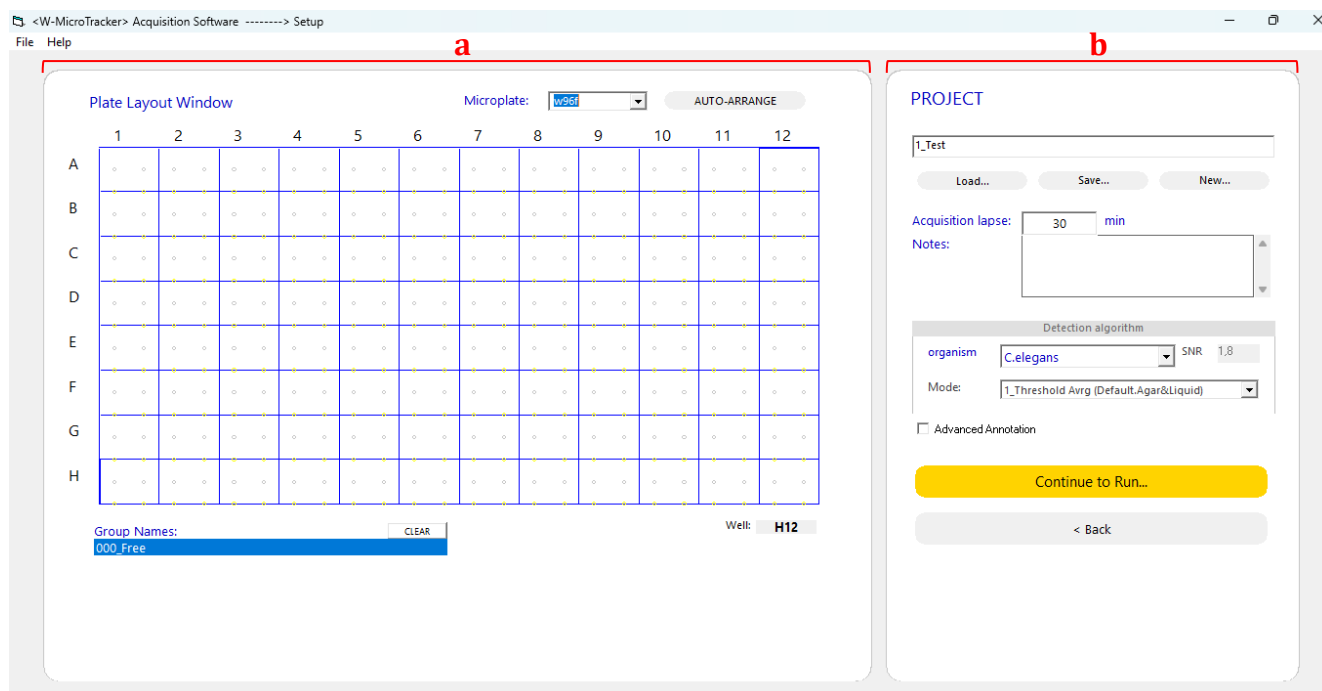


- **Troubleshooting:** In case of a connection error, please ensure the USB cable is securely connected and that the green/blue LED sequence occurred as described in the Hardware Setup section. For further assistance, refer to *Appendix D: Troubleshooting*

Screen Components

1. “Setup a New Project” window

This screen appears after selecting “**Setup a New Project**” from the Start Window. Here, you will define the physical and experimental layout of your microplate and acquisition.



A. Plate Layout

- **Microplate Selection Box:**

The ONE is optimized for 96- and 384-well formats, but remains compatible with 6- and 24-well plates (*See Beam Distribution and Plate Compatibility*).

Important: Verify that the physical Plate Adapter installed in the device matches your software selection to ensure precise data acquisition.

- **Auto-Arrange Button:**

- This feature provides automated templates to quickly organize your plate layout. It includes pre-set configurations for single wells or multiple replicates, optimizing setup time for large-scale assays.

- **Microplate scheme:**

- The interactive microplate scheme allows you to select active wells and manually define the distribution of your experimental groups.

- **Group Names Box:**

- Groups are indexed starting from 000_Group0. You can customize these names to match your experimental conditions (e.g., "Control_Sample", "Drug_Concentration_10uM") by double-clicking on the group name
- Clear List: Use this function to reset all group designations

B. Project Menu:

- **Project Controls:**

- **Load Button:** Allows you to browse and select a previously saved project layout.
- **Save Button:** Saves any changes or updates made to the current project settings.
- **New Project Button:** Opens a pop-up window to create and name a new project layout.
- To **rename** an existing project, simply double-click on the project name field and enter the new title in the pop-up window.

- **Acquisition lapse (Duration):** Set the total run time for the microplate reading in **minutes** (e.g., enter 120 minutes for a 2-hour experiment).

Note: The minimum acquisition time is 15 minutes.

- **Notes:** Use this field to add specific comments or experimental details. These notes will be saved with the project and can be viewed later during data analysis.
- **Detection Algorithm Box:** The system optimizes data processing based on the movement type of your model organism.

Select the organism that you are using and the software will automatically recommend a default Analysis Mode based on your selection of organism.

Analysis Mode (DSP Methods):

- **DSP Method_0 (Threshold + Binary):** Uses a 3-second filter. Any interruptions to the infrared beam within a 3-second window are counted as a single unit of activity. This 3-second filter is the estimated time an organism takes to fully cross the beam.
- **DSP Method_1 (Threshold Average):** The default mode for most Agar and Liquid assays. Every beam interruption is counted as a unit of activity without time filtering.
- **DSP Method_2 (Motility Index):** A high-sensitivity mode designed for small fluctuations in amoeboid behavior. It measures signal amplitude based on patented sperm motility technology (Bartoov et al.). *Recommendation: For amoeboid movement, the use of "U-bottom" microplates is highly recommended for better detection.*

- **Navigation controls:**
 - **Continue to Run:** Press this button to proceed to the run window.
 - **Back:** Returns to Start Window.

2. “Run Project” window

Once you click "Continue to Run", the main execution screen appears. This window allows you to monitor the experiment in real-time.



A. Project Information

- **Project Box:** Displays the project name, total programmed time of acquisition, and microplate format selected. Use the **Edit** option to return to the Setup Window if changes are needed before starting.
- **Current Acquisition Box:** Shows the name of the specific run.

You can view the notes here and modify them or add new comments during the acquisition.

B. Run Menu:

- **Start (▶):** Begins data acquisition. The software will prompt you to enter a name for the current experiment for future data reference.
- **Stop (■):** Terminates the current data acquisition.
- **Show My Report:** Generates a data register/summary of the current acquisition.

C. Real-Time Visualization Window:

- **Visualization tools:** You can toggle between different views using the small icons at the top left:

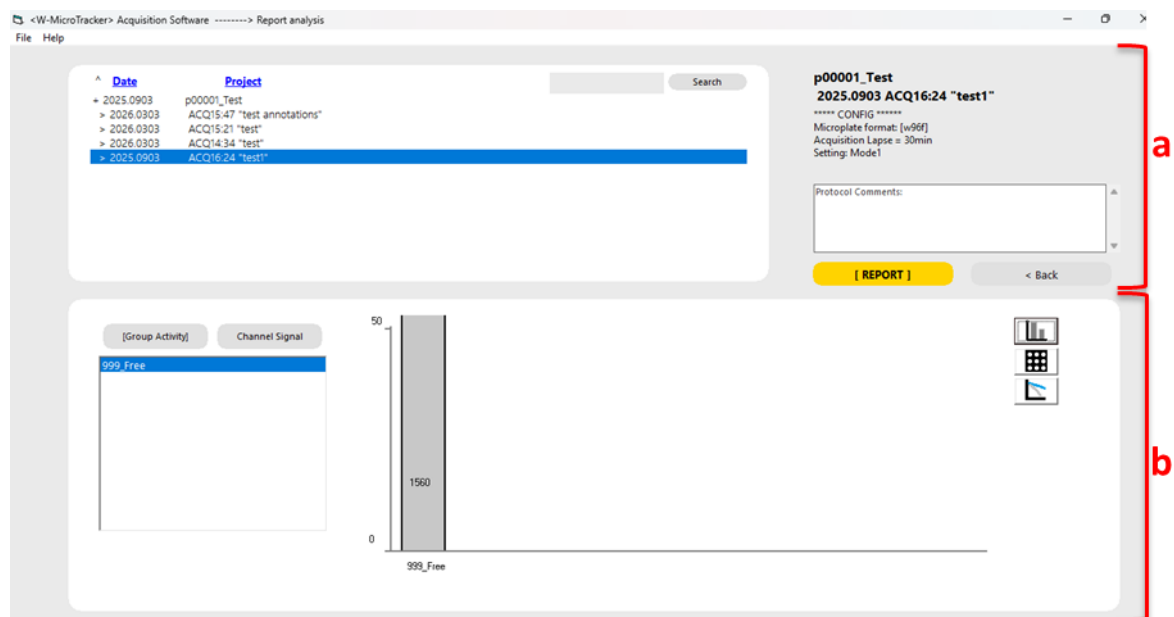
- **Bar Plot (Bar Icon):** Displays a bar graph of real-time activity for the selected experimental groups. Click on the group names or use the navigation arrows within the plot area to change through your defined experimental groups.
- **Heat Map Plot (Grid Icon):** Provides a grayscale visual map of the entire plate. Lighter tones indicate low activity, while darker tones represent higher activity levels.
- **Temperature (T):** Records and displays the ambient temperature of your working environment/room during the test.
- **Signal Plot (Bottom Graph):** Displays the raw individual signals from the sensor selected.

D. Progress Tracking

- **Status Bar:** A blue progress bar at the bottom of the screen indicates the elapsed time of the total programmed run time.

3. “Analyze Recorded Experiments” window

This section is accessed from the **Start Window**. It allows you to review, filter, and export data from previously runs.

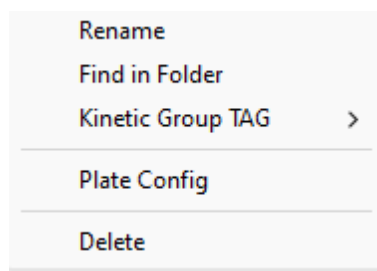


A. Search Window:

Browse the list of available projects, conveniently organized by Date and Name. Click the **plus symbol (+)** to expand subfolders and locate specific acquisition files. Double click in the specific run you wish to visualize.

The **Right Panel** will automatically display a brief summary of the selected experiment's parameters. Review the comments and experimental conditions recorded during the setup and the live run.

Advanced Options (Right-Click Menu): by right-clicking on a selected acquisition in the list, you can access a context menu with the following management tools:



- ✓ **Rename:** Allows you to change the name of the specific acquisition for better identification.
- ✓ **Find in Folder:** Automatically opens the local directory where the acquisition data files are stored on your computer.
- ✓ **Kinetic Group TAG:** Assign a specific "TAG" to related acquisitions. This allows the software to link their datasets, enabling you to visualize Line Plots that combine data from all acquisitions sharing the same TAG.
- ✓ **Plate Config:** Enables to reassign wells to different experimental groups after the run is complete. Once you have finished editing, click "Exit Group Edition" and save your changes to update the analysis.
- ✓ **Delete:** Removes the acquisition from the software's list. The data folder will remain archived even if it is removed from the software view.

Report Button: Click this button to access the Reporting Options menu. From this menu, you can configure the specific data you wish to include and generate a report in CSV format of your results, ready for further analysis in external software.

B. Data Visualization & Review

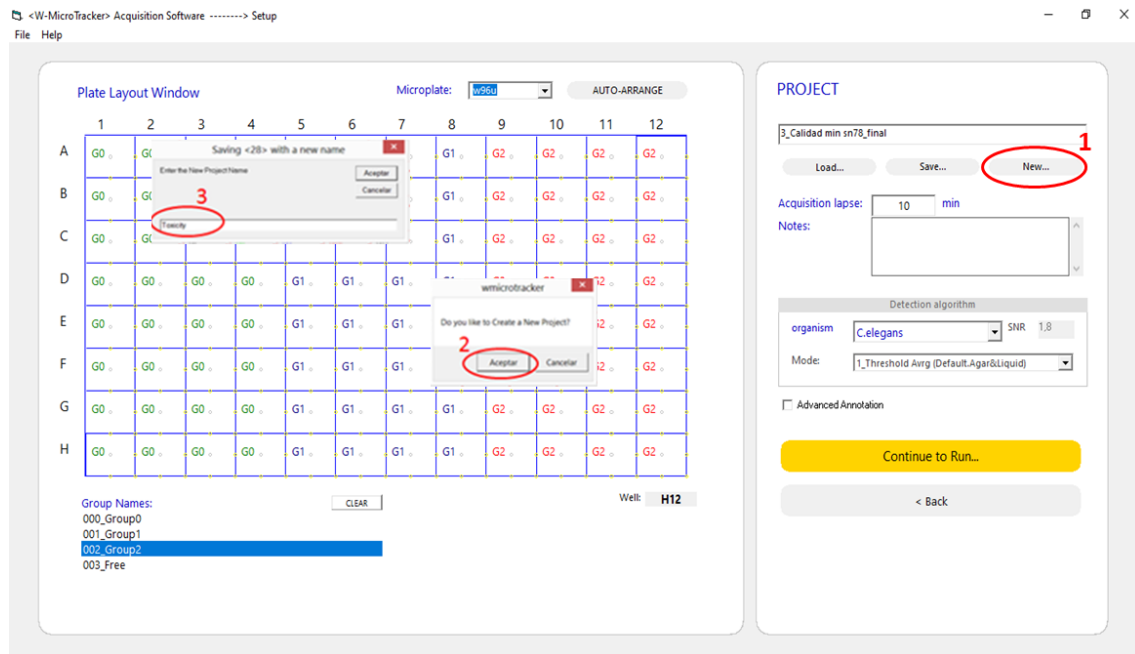
- Group activity button: You can visualize the activity of the different experimental groups. To switch between different data visualization modes of the group activity, use the three icons located on the right side of the panel:

- **Bar Plot:** Displays the accumulated activity during the acquisition for each defined group.
- **Heat Map:** Represents the entire microplate in a grayscale format. Light tones indicate low while dark tones indicate higher activity levels.
- **Line Plot:** Displays a temporal graph showing how activity evolves over time for the different groups.
- **TAG kinetics plots:** enables users to create kinetics plots from datasets of different acquisitions. Previously you have to tag the related acquisition with the same tag. After that, by clicking on the desired TAG in the visualization area, it will show you the kinetic plot.

- Channel signal button: Shows the raw signal of each selected sensor by double click.

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).

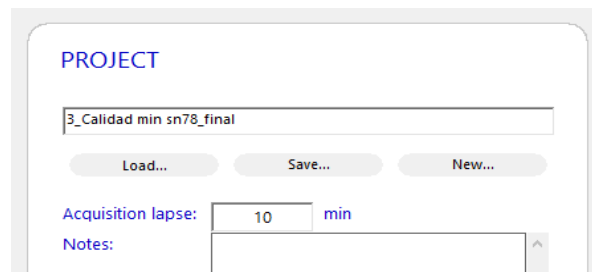


2. A “**New Project Creation**” window will pop up; name your new project in this pop-up window (Step 2 and 3).

3. Enter the “**MicroPlate Format**” size that you will be using.

NOTE: At this time, we have validated the instrument for use only with 6-well, 24-well, 96-well “Flat” and “U” shape bottom, 384-well “Flat” bottom.

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.

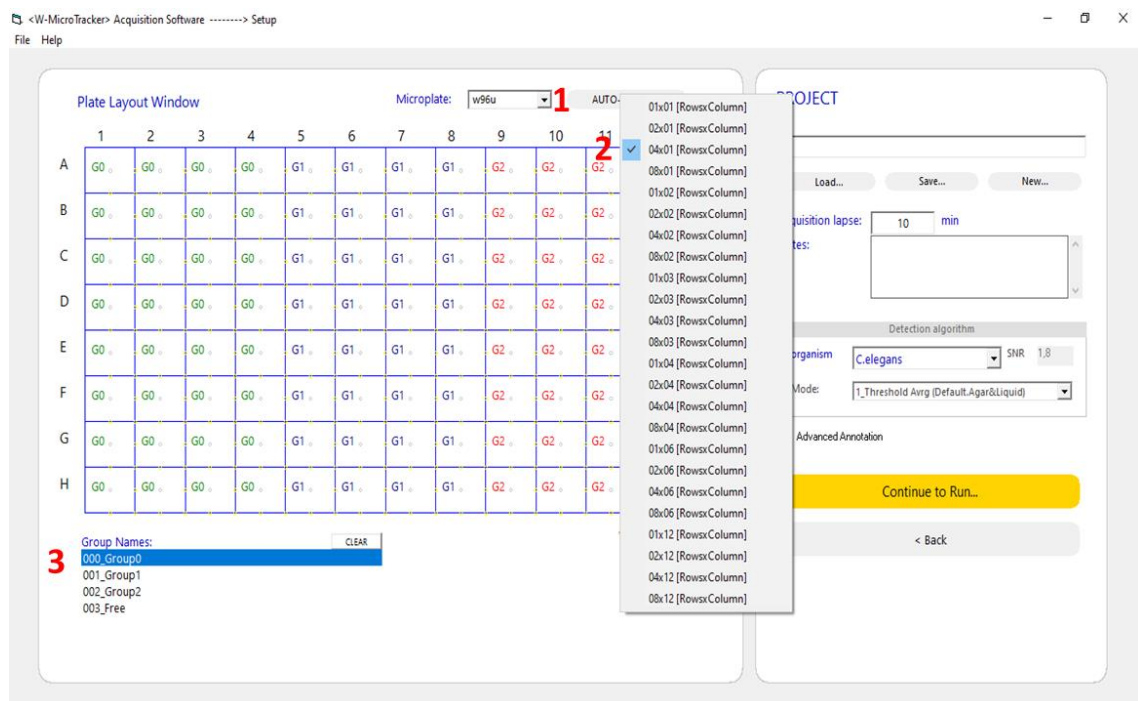
NOTE: In 96-well microplate format, the number of technical replicates recommended per group is four wells (average standard deviation < 15% in the activity between homogeneous groups).

a. MANUAL Layout

- In the “Group Names” section, select a group in the box.
- Double-click in the left mouse button to rename your group.
- 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**” to select the number of rows and columns you wish to use; the arrangement options will differ depending on your chosen plate format.
- In the “**Group Names**” section, double-click to label your group; repeat for each group in your experiment.



c. CLEAR LIST Button

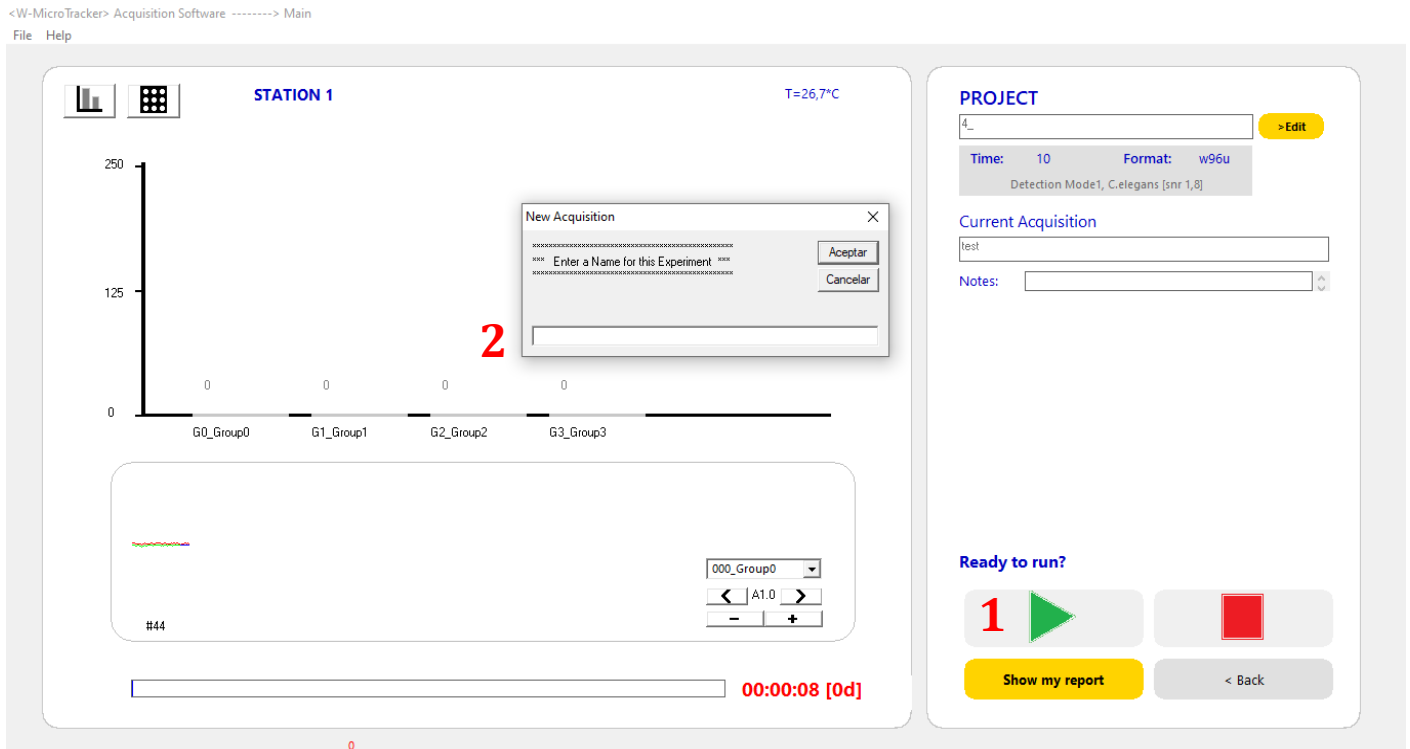
- This will clear “ALL” the existing Group Names that you have listed and arranged; no name or arrangement information will be saved.
- You will be prompted to confirm your selection.

6. You are now ready to load your plate and click on “**Continue to Run**” button.

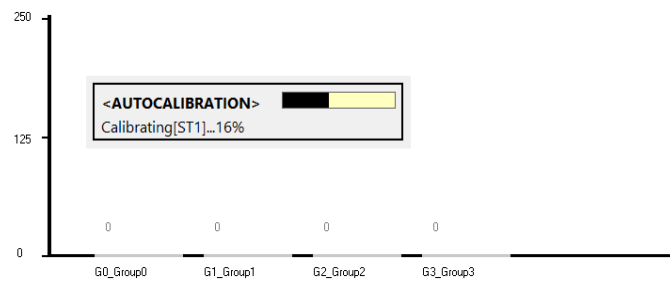
Start your experiment running!

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 384 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 ONE 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 90 seconds the accumulated activity for each group at the TOP plot.

5. When the ONE 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.

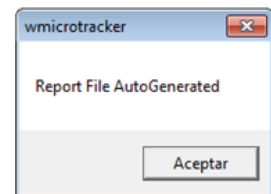
VI. Accessing Experiment Data

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

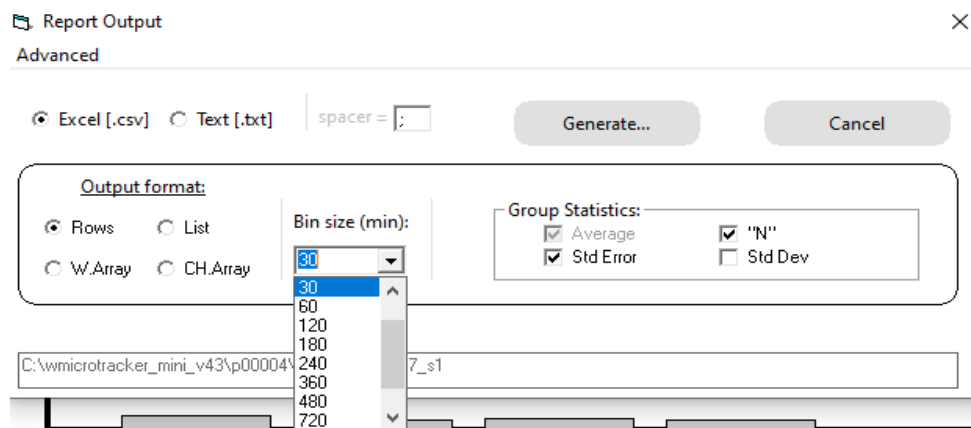
NOTE: Experiment reports are generated as a .CSV file by default; we recommend Excel for quick export and ease of use of the report files.

Generate an Immediate Report

1. At the end of an experiment run, the following “Report file Auto Generated” pop-up window will automatically appear. Then you could press the “**Accept**” button and after that press the button of “**Show my report**” on the screen.



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			

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	R
1	DATE:	*****																
2	FILE:	O:\humitracker_v26_v3\api_2020-08-19\p00043120200821_1006																
3	SUBPROJ	day5																
4																		
5	*****CHANNEL ARRAY Group Configuration*****																	
6	Row/Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
7	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	B	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	000_Blance	000_Blance	-	-
9	C	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	000_Blance	000_Blance	-	-
10	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	F	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-
13	G	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-
14	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	J	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-
17	K	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-
18	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	N	-	000_Blance	000_Blance	-	-	002_DR	002_DR	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-
21	O	-	000_Blance	000_Blance	-	-	002_DR	002_DR	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-
22	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23																		
24																		
25	*****Average Temperature [°C]*****																	
26	Time[m]	30																
27	Temp[°C]	0																
28																		
29	ARRAY OUTPUT FORMAT																	
30	Time_Block	30																
31	Row/Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
32	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	B	-	110	165	-	-	170	90	-	-	102	61	-	-	8	0	-	-
34	C	-	207	140	-	-	100	412	-	-	141	104	-	-	36	6	-	-
35	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	F	-	60	49	-	-	132	127	-	-	91	21	-	-	144	217	-	-
38	G	-	100	244	-	-	145	123	-	-	61	231	-	-	39	142	-	-
39	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	J	-	94	202	-	-	83	85	-	-	135	32	-	-	83	146	-	-
42	K	-	362	322	-	-	159	67	-	-	44	60	-	-	121	138	-	-
43	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	N	-	32	2	-	-	62	114	-	-	104	290	-	-	129	164	-	-
46	O	-	0	6	-	-	12	121	-	-	32	151	-	-	174	44	-	-
47	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48																		
49																		

Export Previous Experiments

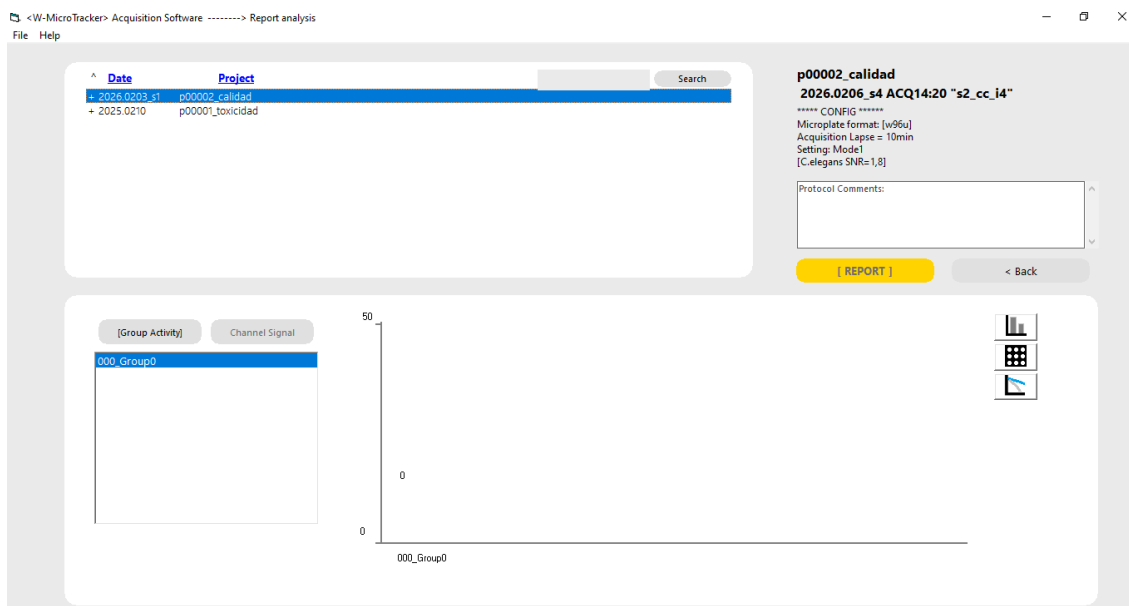
1. You can access your data at any time directly through the software Start Window.

- Press the “**Analyzed recorded experiments**” on the Software Start Window; this will open a “Report analysis” screen with previous experiments:

- Select the desired project name from the list; each project will expand to show the acquisitions collected under that layout. 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 ONE device.

- In the computer location you chose for the software, 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.

TAG Kinetics Plots

The **Kinetic Group TAG** is an advanced tool for organizing data when an experiment is divided into several acquisitions. With the TAG, the software recognizes that different acquisitions belong to the same experiment, allowing you to visualize the datasets together.

1. Linking Data Sessions (Grouping)

To link different data sessions:

1. **Select** the acquisitions from the list that are part of the same experiment.
2. **Right-click** on them and select **Kinetic Group TAG**.
3. **Assign** the same name or label (TAG) to all of them. Once assigned, the software will recognize these acquisitions as a continuous sequence or a related set.

2. Visualizing Unified Graphs

To view the unified plot:

1. In the visualization area, select the TAG you created.
2. The system will generate a single kinetic plot that combines the datasets from the different acquisitions.

Appendix A. ONE Quick Start Sheet



The WMicrotracker One measures overall locomotor activity and viability of your worms such as *C. elegans* and parasitic nematodes cultured in liquid media and in multi-well plates. The system detects the movement of organism populations through the interference caused by them in a large array of infrared light microbeams.

ONE Quick Start Sheet

1. Launch ONE software from your chosen computer location.
2. Create and name a new project, or load an existing project.
 - If creating a new project, choose plate layout and assign name groups.
 - If loading an existing project, double-check plate layout.
3. Set experiment well format.
 - *NOTE: Only 6-well plate, 24-well plate, 96-well "Flat bottom", 96-well "U" bottom and 384-well "Flat bottom" microplate formats are currently validated. 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).*
4. Set experiment acquisition time.
 - *NOTE: Minimum acquisition time recommended is a 15 min read.*
5. Set your plate layout "Groups".
 - *NOTE: In 96-well microplate format, the number of technical replicates recommended per group is four wells (average standard deviation < 15% in the activity between homogeneous groups).*
6. Load your experiment plate into the instrument.
 - *NOTE: For use of the different microplate format, please ensure to use the correct plate adapter into the device for your microplate format.*
7. Click "START ►" to begin experiment reading.
 - Instrument will automatically run through several calibration steps.
 - Instrument will begin data acquisition immediately following calibration.
8. Upon read completion, the status is completed in blue color and the software shows a pop-up window "Report file Auto Generated".
 - Generate a report for immediate analysis or access the data later.
 - Remove your experiment plate and exit the software.

KEY APPLICATIONS:

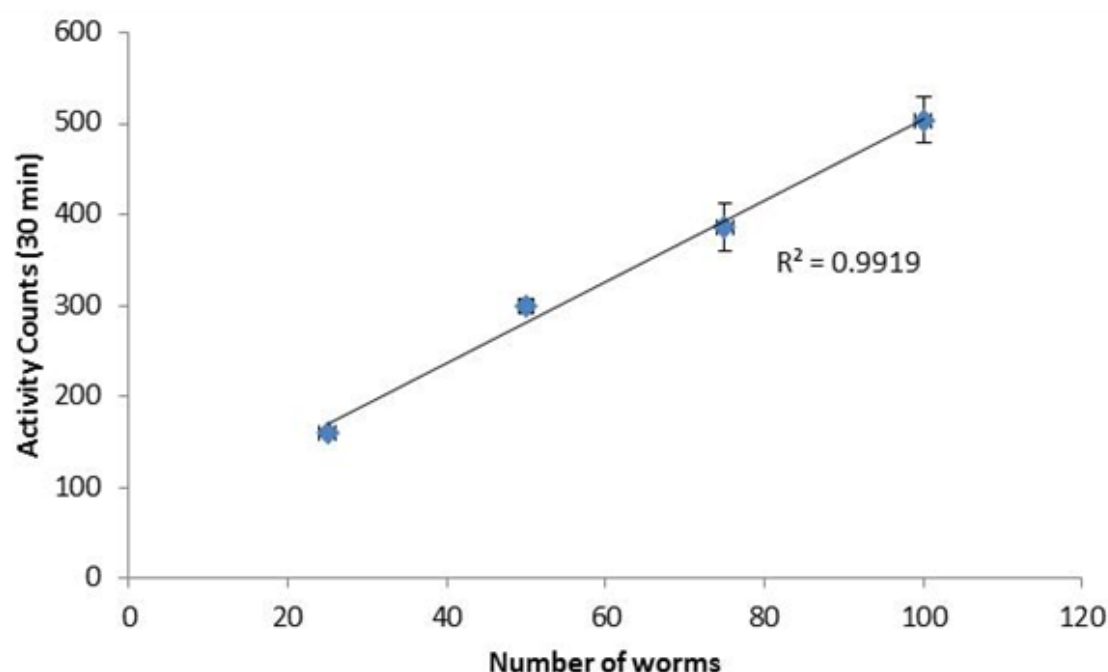
- Toxicity Assay
- Oxidative Stress
- Pathogenesis
- Ecotoxicity



Curve 96W “F” bottom Microplate: Young Adult_N2 (*C.elegans*)_M9 supplemented with BSA 0,05%.

The system presents a very good linearity of detection between 25 and 100 worms ($R^2=0.99$). More than 100 worms are not recommended, a plateau of activity is observed.

NOTE: Data shown are averages over two independent biological replicates with four technical replicates for each. Error bars represent +/- S.D. Analysis detection Mode 1.



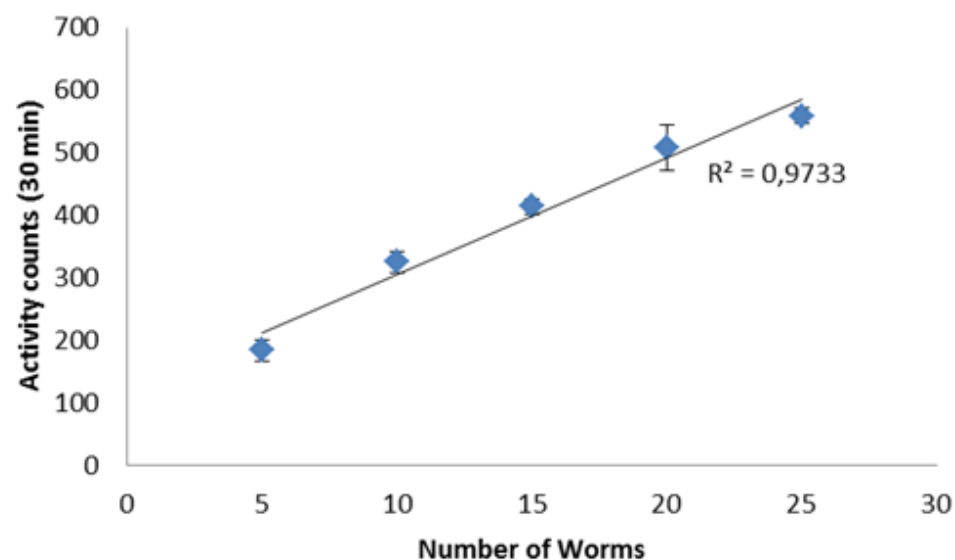
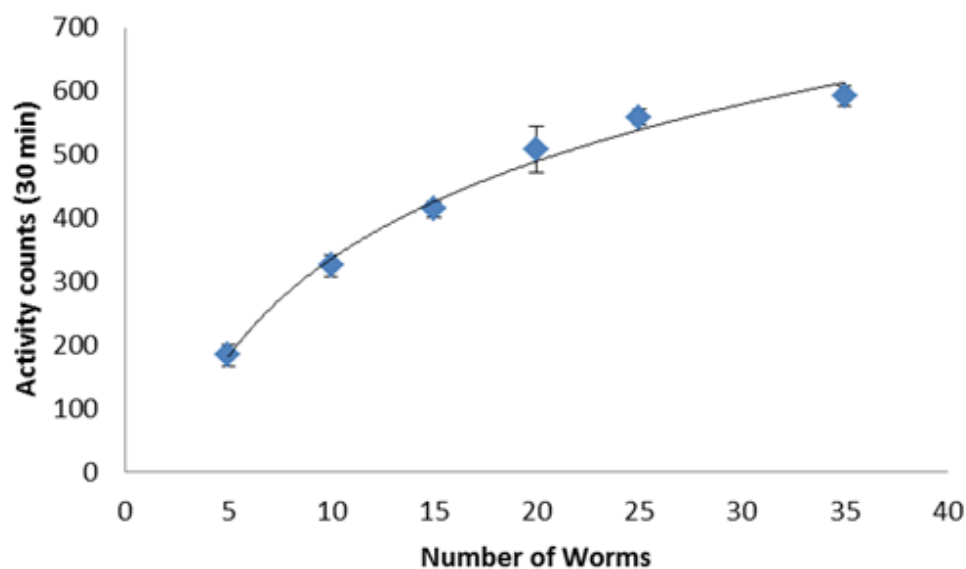
Appendix B. ONE Linearity & Reproducibility



Curve 96W “U” bottom Microplate: Young Adult_N2 (*C.elegans*)_M9 supplemented with BSA 0,05%.

The system presents a very good linearity of detection between 5 and 25 worms, observing a plateau at more than 25 ($R^2=0.97$).

NOTE: Data shown are averages over two independent biological replicates with five technical replicates for each. Error bars represent +/- S.D. Analysis detection Mode 1.



WHAT YOU NEED:

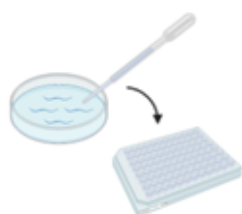
Young adult worms
96 well "U"-bottom plate with lid.
M9 buffer.
OP50.
Levamisole – Stock solution: 100mM in ddH₂O.
Synchronized populations of young adult worms.
wMicrotracker ONE.

Notes:

- Perform at least three technical replicates and at least two biological replicates.
- Before activity measure, stimulate the 96 well plate with worms for 5 second by gently shaking by hand.
- A basal record can be made before carrying out the treatment. This value can be used to relativize the data after treatment.
- If dissolving the drugs in DMSO the final concentrations of DMSO should not exceed 1%.

PROTOCOL:

1. Grow synchronized populations of young adult worms in seeding NGM plates (OP50).
2. Collect worms from plates using M9 buffer and transfer them in a sterile 15 ml tube.
3. Let the worms settle. Remove the supernatant taking care not to disturb the pellet.
4. Perform a wash with 5 ml of M9 buffer. Briefly shake or invert the tube. Repeat step 3.
5. Add 3 ml of M9 buffer.
6. Count the number of worms in a volume of 10 μ l and adjust the volume to obtain a concentration of [20worms/90 μ l]. Adjust volume with M9 and add OP50 to 1 mg/ml final concentration.
7. Transfer per well 90 μ l of the worm solution to a 96 well microplate using multichannel pipette.
8. Add 10 μ l of a 10X concentrated solution of chemicals to test and gently shake microplate by hand. Include a control without compounds.
9. Register worm activity using WMicrotracker ONE.
10. Generate the data report using WMicrotracker One software and plot using MSEXcel.



(A) Transfer 20 young adults worms in 90 μ l to 96-well "U" bottom plates

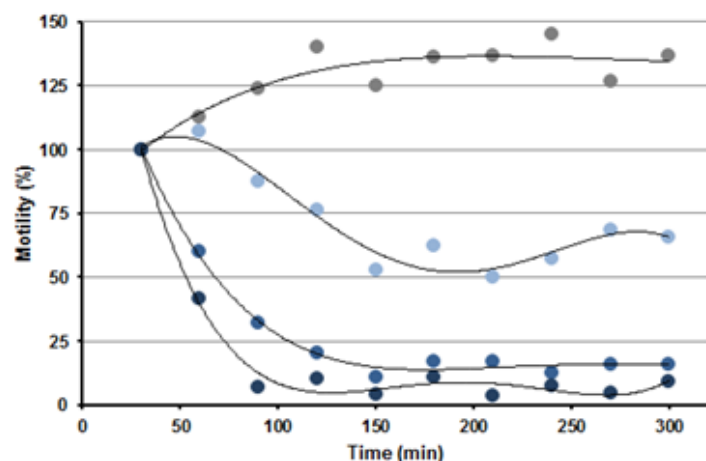


(B) Add 10 μ l of a 10X concentrated solution of chemicals



(C) Record the activity of the plate with worms using wMicroTracker ONE

RESULTS



(D) Generate the data report using ONE software and plot

Kinetic of paralysis of *C.elegans* using levamisole and in W96

In these experiments we can observe the kinetic and dose response to Levamisole. In less than one hour a quantitative dose response effect is obtained.

Appendix C. ONE Applications_Oxidative Stres



WHAT YOU NEED:

96 well flat-bottom plate with lid.

M9 buffer.

3PY medium supplemented with 50 μ M FuDR, 100 μ g/ml streptomycin and 20 μ g/ml kanamycin.

Paraquat – Stock solution: 200mM in ddH₂O.

Vitamin C

Synchronized populations of L4 worms.

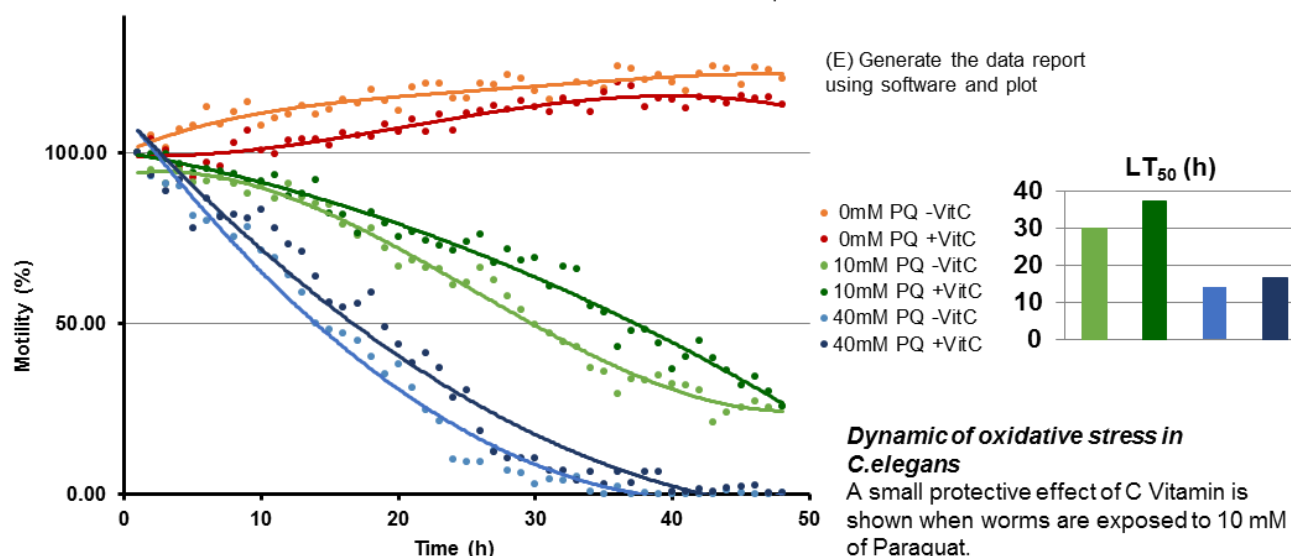
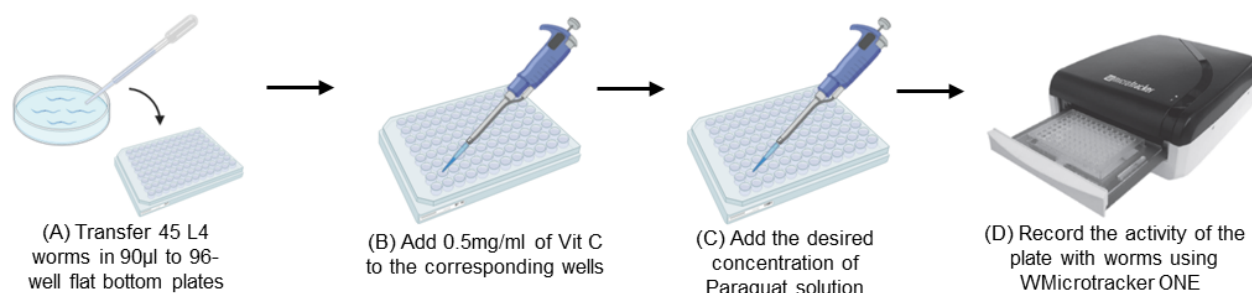
wMicrotracker ONE device.

Notes:

- Perform at least three technical replicates and at least two biological replicate.
- Before activity measure, stimulate the 96 well plate with worms for 5 second by gently shaking by hand.
- A basal record can be made before carrying out the treatment. This value can be used to relativize the data after treatment.
- If dissolving the drugs in DMSO the final concentrations of DMSO should not exceed 1%.

PROTOCOL:

1. Grow synchronized populations of L4 N2 worms in seeding NGM plates (OP50).
2. Collect worms from plates using M9 buffer and transfer them in a sterile 15 ml tube.
3. Let the worms settle. Remove the supernatant taking care not to disturb the pellet.
4. Perform a wash with 5 ml of M9 buffer. Briefly shake or invert the tube. Repeat step 3.
5. Add 3 ml of nutrient medium.
6. Count the number of worms in a volume of 10 μ l and adjust the volume to obtain a concentration of [5 worms / 10 μ l].
7. Transfer per well 90 μ l of the worm solution to a 96 well microplate using multichannel pipette.
8. Add 0.5mg/ml of Vit C to the corresponding wells.
9. Add the desired concentration of Paraquat solution and seal the plate.
10. Register worm activity using wMicrotracker ONE.
11. Generate the data report using WMicrotracker One software and plot using MSEXcel.



WHAT YOU NEED:

96 well flat-bottom plate with lid.

M9 buffer.

3PY medium supplemented with 50 μ M FuDR, streptomycin 100 μ g/ml and kanamycin sulfate 20 μ g/ml.

Bacterial culture supernatant.

Synchronized populations of L4 worms.

wMicrotracker ONE device.

PROTOCOL:

1. Obtain synchronized populations of adult GLP-4 worms grown in NGM + OP50 at 25°C.
2. Collect worms from plates using M9 buffer and transfer them in a sterile 15 ml tube.
3. Let the worms settle. Remove the supernatant taking care not to disturb the pellet.
4. Perform a wash with 5 ml of M9 buffer. Briefly

shake or invert the tube. Repeat step 3.

5. Add 3 ml of nutrient medium

6. Collect the number of worms in a volume of 10 μ l and adjust the volume to obtain a concentration of [5 worms / 10 μ l].

7. Transfer per well 90 μ l of the worm solution to a 96 well microplate using multichannel pipette.

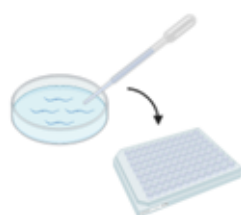
Optional: Let worms to rest for 30 min and measure basal activity using WMicrotracker.

8. Add 10 μ l of a bacterial culture supernatant to test and gently shake microplate by hand.

10. Record the activity of the plate with worms using WMicrotracker.

11. Generate the data report using WMicrotracker One software and plot using MSExcel.

Note: Perform at least three technical replicates and at least two biological replicate.



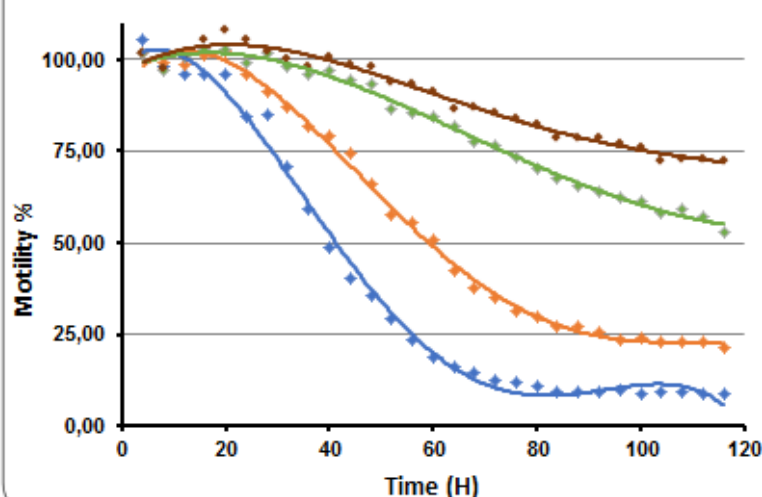
(A) Transfer 45 young adults worms in 90 μ l to 96-well flat bottom plates



(B) Add 10 μ l of a 10X concentrated solution of chemicals



(C) Record the activity of the plate with worms using WMicrotracker ONE



- ◆ CHA0 (dil 1/2)
- ◆ CHA0 (dil 1/10)
- ◆ CHA0 (dil 1/100)
- ◆ Control

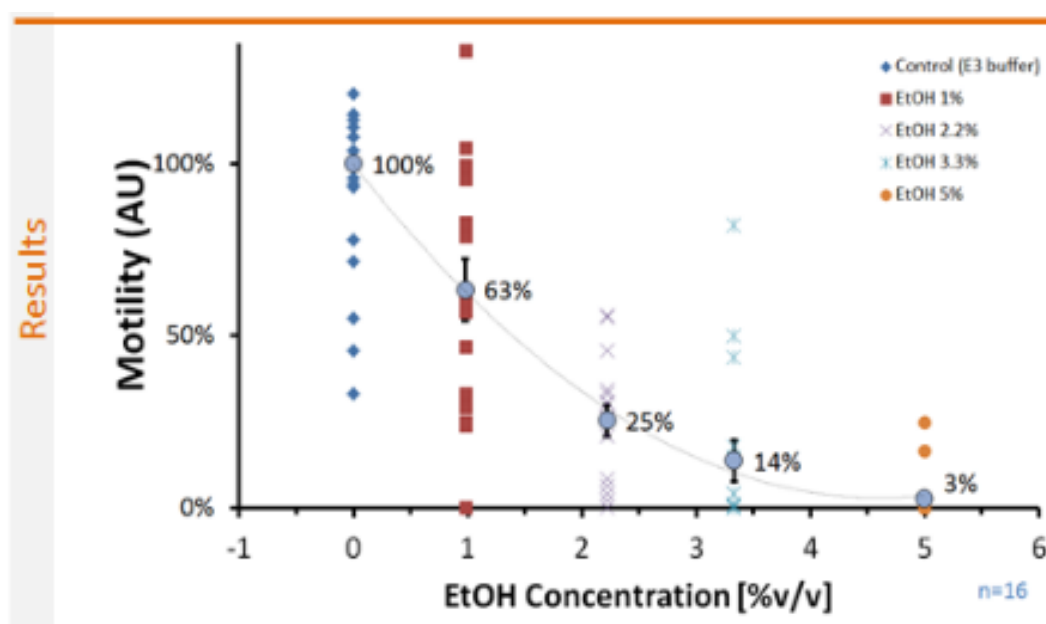
Kinetics of killing using bacterial supernatant of *Pseudomonas Fluorescens* CHA0

In this experiment we can observe long-term kinetic and dose response effect using dilutions of bacterial supernatant of *Pseudomonas* CHA0. Paralytic killing is reported to depend on bacterial hydrogen cyanide production

Using the **WMicrotracker One** system is possible to study Fish toxicity. Global swimming behavior could be a simple readout for toxicity, easy to scale-up in automated experiments. This approach is potentially applicable for fast ecotoxicity assays and whole-organism high-throughput compound screening. Below we present an example of toxicity with “Ethanol” in a Zebrafish model.

Brief Methodology:

(1) Collect fertilized eggs of Zebrafish (*Danio Rerio*) in a petri dish with E3 medium. (2) Transfer one to three 48-hpf non-hatched zebrafish embryos to each well of a 96-well plate. (3) Get a final volume of 200ul of E3 medium. (4) Incubate 48 hs at 28°C to allow hatching. (5) Add 22ul of a 10x concentrated solution of chemicals to test. Use four technical replicates. (6) Record the activity of the plate using WMicrotracker One for a period of 15 minutes. (7) Generate the data report using WMicrotracker One software and plot using MSExcel.



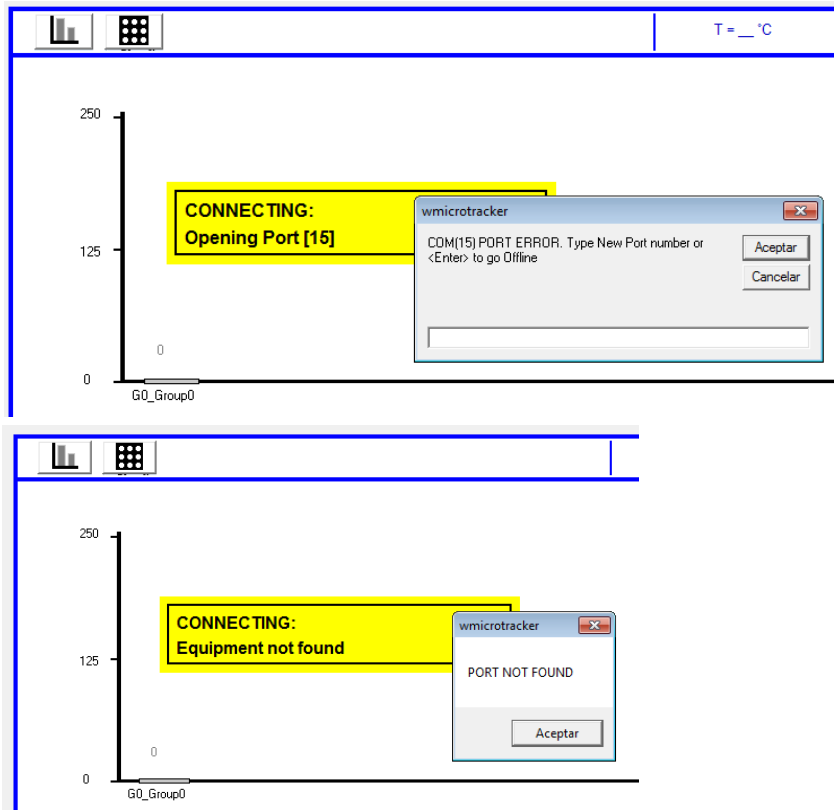
Effect of ethanol in Zebrafish Larvae

In this experiment we can observe the effect of EtOH on zebrafish larvae behavior after 48h of drug exposure. The plot shows the average activity and the natural variability of response between animals (n = 16 per treatment).

Appendix D. Troubleshooting and additional information

Hardware/Software Troubleshooting

If your system is not detecting the new COM PORT, a pop-up window will be shown:



Please check the following:

- a. Test proper system power source.
- b. Ask your software administrator if you are able to install new USB Drivers or change COM Port numbers.

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

**Software and system updates available at
www.phylumtech.com**