Wmicrotracker®

Installation Guide and User Manual

Data Acquisition System: WMicrotracker ONE
Hardware Version: WMTK09-R01/V1.4-R01
Software Version: WMTK V3.6-API (2021)

This product is protected under international patents, owned by the Argentinian National Research Council CONICET (P20060105084AR, PCT/IB2007/054628, EPO & US patent granted) and licensed to PHYLUMTECH S.A. All rights reserved.

Any partial or total copy is prohibited and will be subject to law penalties. Product provided for use “AS IS”. No modifications allowed without PHYLUMTECH permission. When the client acquires this product, the client understands and accepts these rules.

For research purposes only. Not for human diagnostic use. (©2020). Made in Argentina.

Thank you for acquiring the ONE system. The following document will guide you through the installation process.

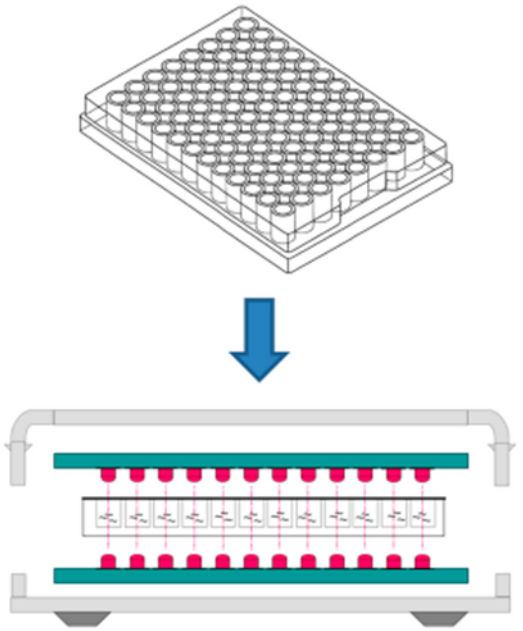
Contents

I. About the ONE

WMicrotracker ONE 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 an Infrared microbeam array of 384 independent sensors and simultaneous channel readouts.



Infrared locomotor tracking system
(Worm Microtracker)

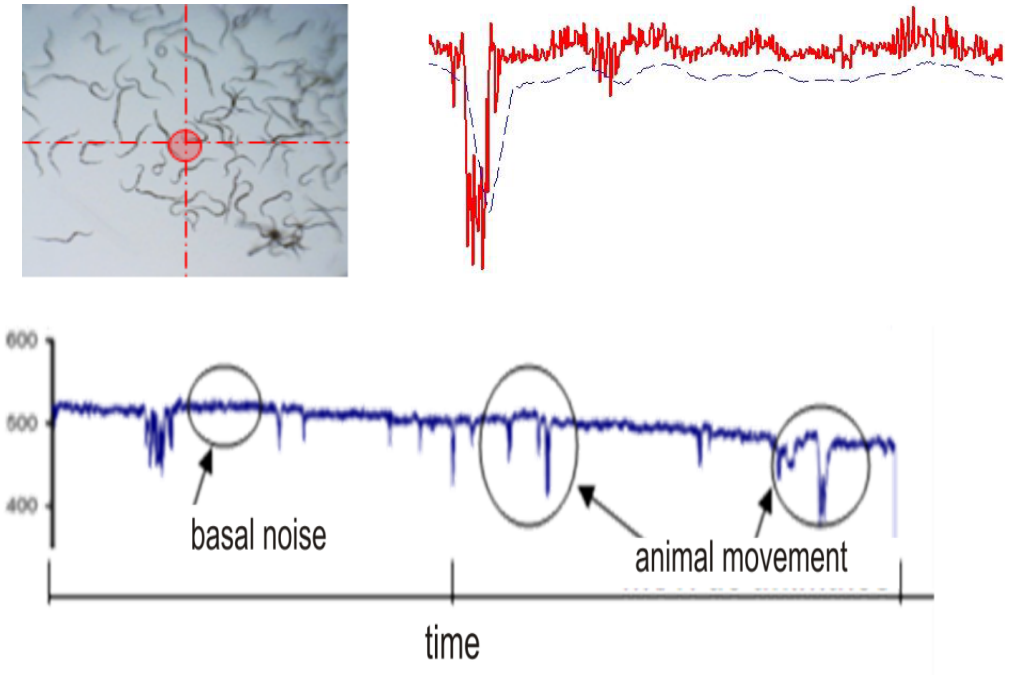
As the animals cross the sensor grid, locomotor activity is detected as interruptions in light beams;

-6 Wells microplate [30-32 microbeams/well] - `W384/W24´plate adapter
-24 Wells microplate [4 microbeams/well] - `W384/W24´plate adapter
-96 Wells Flat bottom [2 microbeams/well] - `W96F´plate adapter
-96 Wells U shape bottom [1 microbeams/well] - `W96U´plate adapter
-384 Wells microplate [1 microbeams/well] - `W384/W24´plate adapter

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.



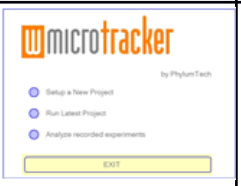
Using this approach is possible to evaluate multiple protocols using liquid culture media. Preferred microplate culture format for ONE is 6well, 24well, 96 well “flat bottom”, 96 well “U bottom” and 384 well “flat bottom”;

Recommended:

- 6-Well (Greiner Bio-One #657160)
- 24-Well (Greiner Bio-One #662160)
- 96-Well “Flat” (Greiner Bio-One #655180)
- 96-Well “U” (Greiner Bio-One #650161 + lid #656161)
- 384-Well “Flat” (Thermo Scientific #95040000 + PS clear lid)

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.		9V DC, 1.5Amp switching Power Source*
	USB-B cable.		Microplate format adapters: -384/24 wells -96 w. flat bottom -96 w. "U" shaped bottom.
			Acquisition Software: available from www.phylumtech.com

* 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 :
  - 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.
  - 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

- 22cm x 28cm x 9.1cm (8.7in x 11.03 in x 3.6 in)

II. Installation & Setup Guide

Software Installation

We recommend periodically referencing the Phylumtech website for software updates.

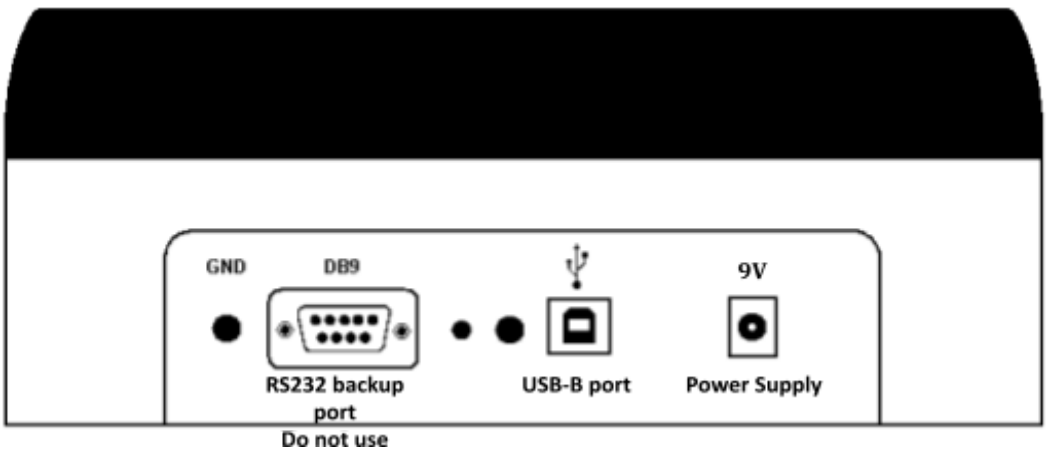
1. You'll find the Software Installation .zip folder for downloading at Software download zone (https://www.phylumtech.com/home/en/support/). To download right-click the link and choose "save link as".
2. Unzip the files and copy WMicrotracker folder to C:\WMicrotracker and follow the instructions detailed 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_vXX.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, plug in the power supply (9VDC Switching Power Supply with 1.5 Amp output) to any regular Power Outlet and the Power Supply`s output cable to the back of your ONE ("9VDC" socket).



2. When you connect the Power Supply to the green light on top of it should turn on and the blue light will flash three times (this is a system check of the microprocessor).

3. Connect the USB-B cable to the USB COM PORT on your computer and to the back of the ONE device.

4. The hardware is going to be auto-detected at COM Port 1 to 15.

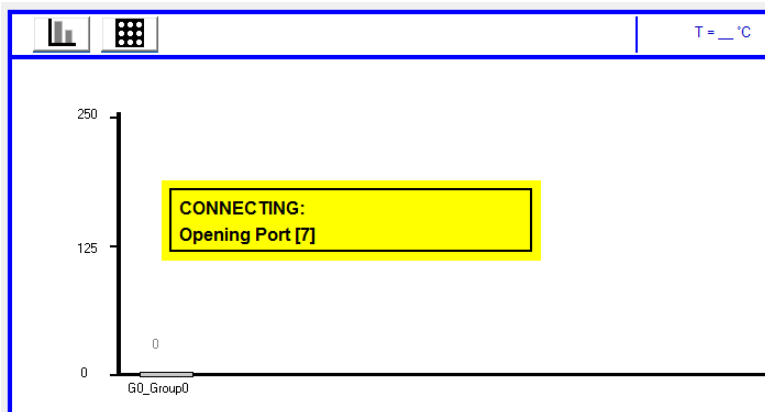
Note:
-See Devices & Printers Window to verify that a new COM Port was detected.
-RS232 backup port is not necessary to connect. It is only maintained for compatibility issues.

III. Software Use

Software Launch

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

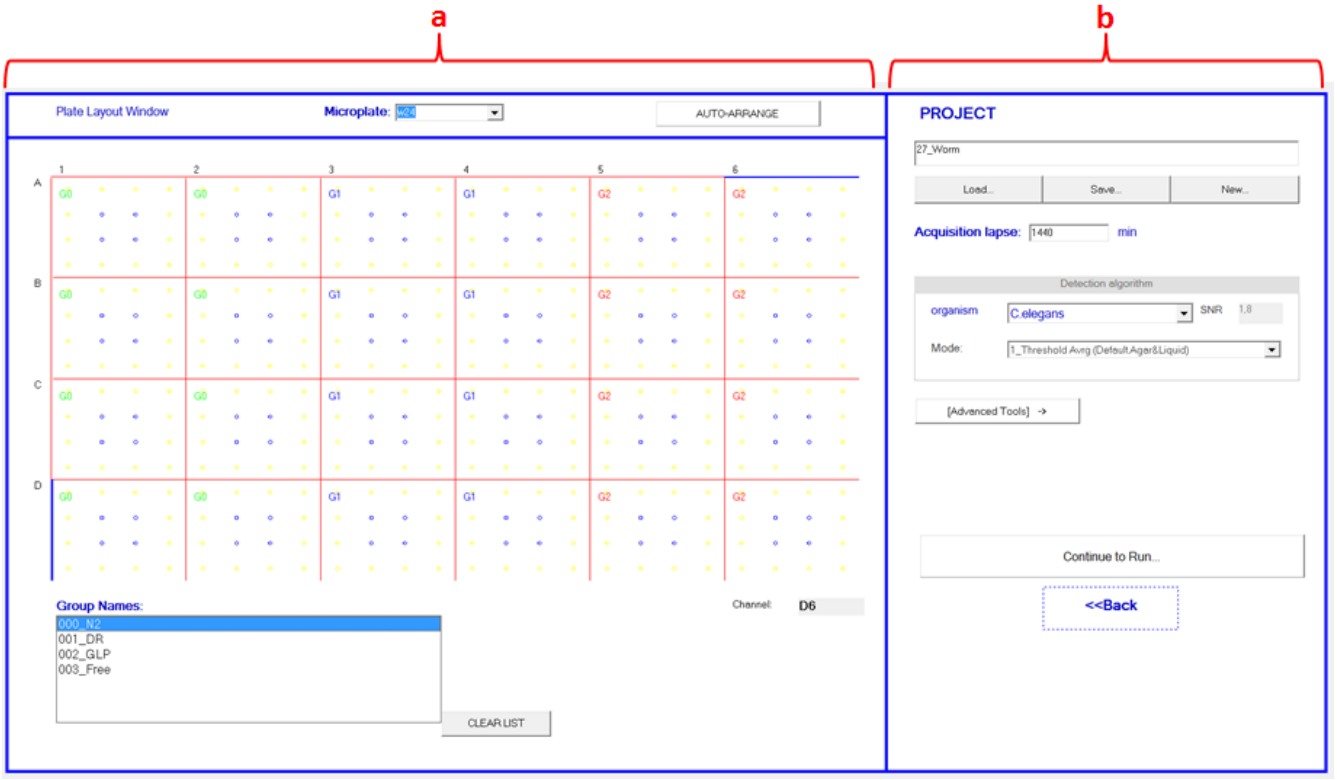
2. Check if the hardware is detected correctly by going to: "Run Latest Project". An auto diagnostic popup yellow window will appear:



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 Setup is shown when you select "Setup a New Project" on the Start window.



a. Plate Layout:

i. Microplate Box:

-Currently the WMTK ONE has been validated to work with 6-well plate, 24-well plate, 96-well "Flat bottom", 96-well "U" bottom and 384-well "Flat bottom" format. To use different microplate formats, be sure to use the correct plate adapter in the device.

-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.
- Options allow for single wells and replicates.

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.

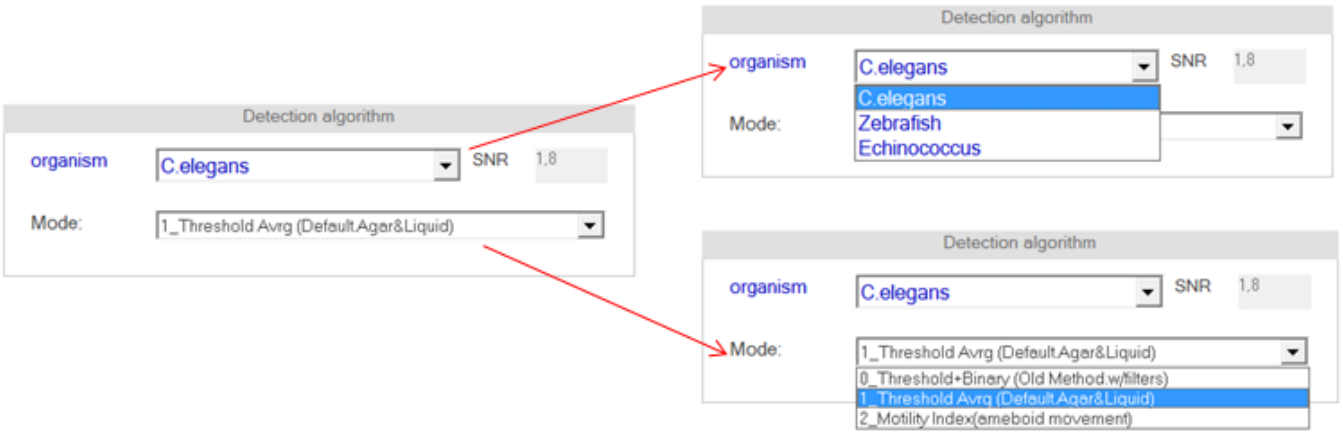
vi. “Detection algorithm” Box:

- 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 “U bottom” microplate.

vii. “Advanced Tools” Button:

- Allows saving raw information from sensors using the “Save real time data” option. If this option is unchecked, it will not allow re-analysis of the data. Unchecked only if it is extremely important to reduce your disk space.
- Option of “temperature control” and “Light control” available only in some hardware versions.

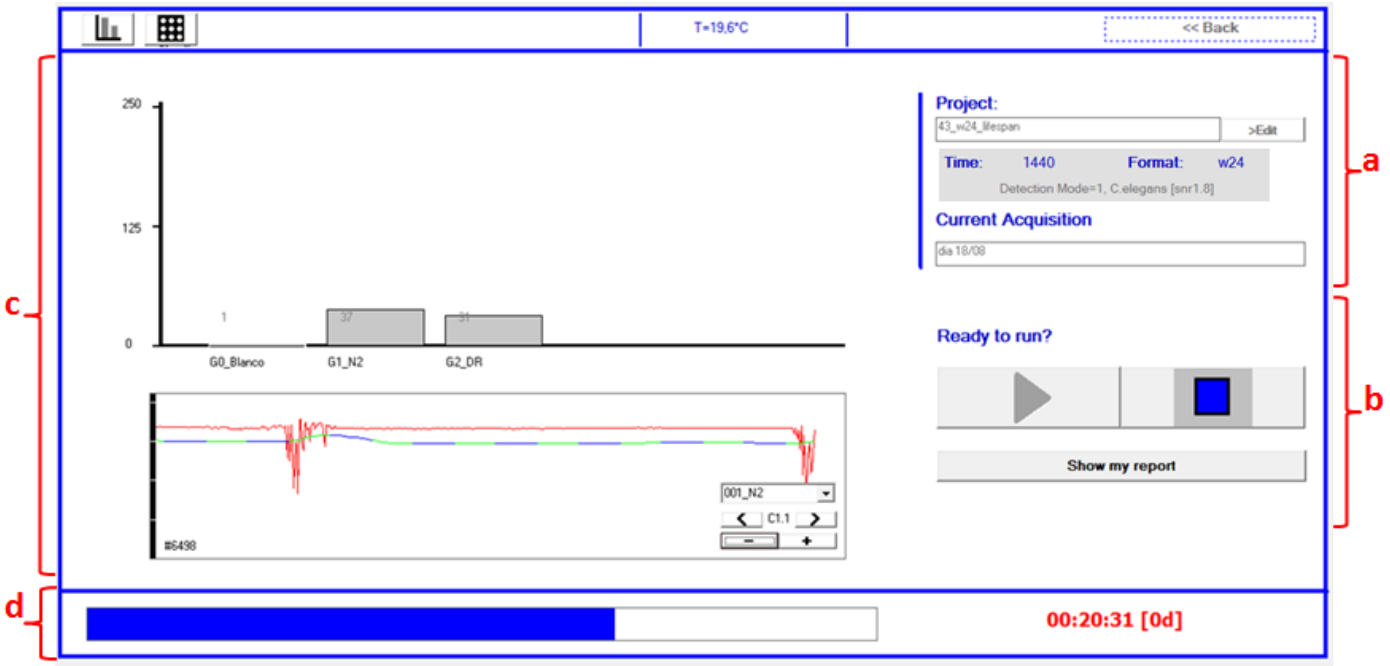
viii. “Continue to Run” Button:

- Press here to continue your experimental run.

ix. “Back” option:

- Returns to the initial screen

2. **The second screen Main 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.

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. Indicates your working room temperature.

iv. “Signal plot” graph on the bottom side:

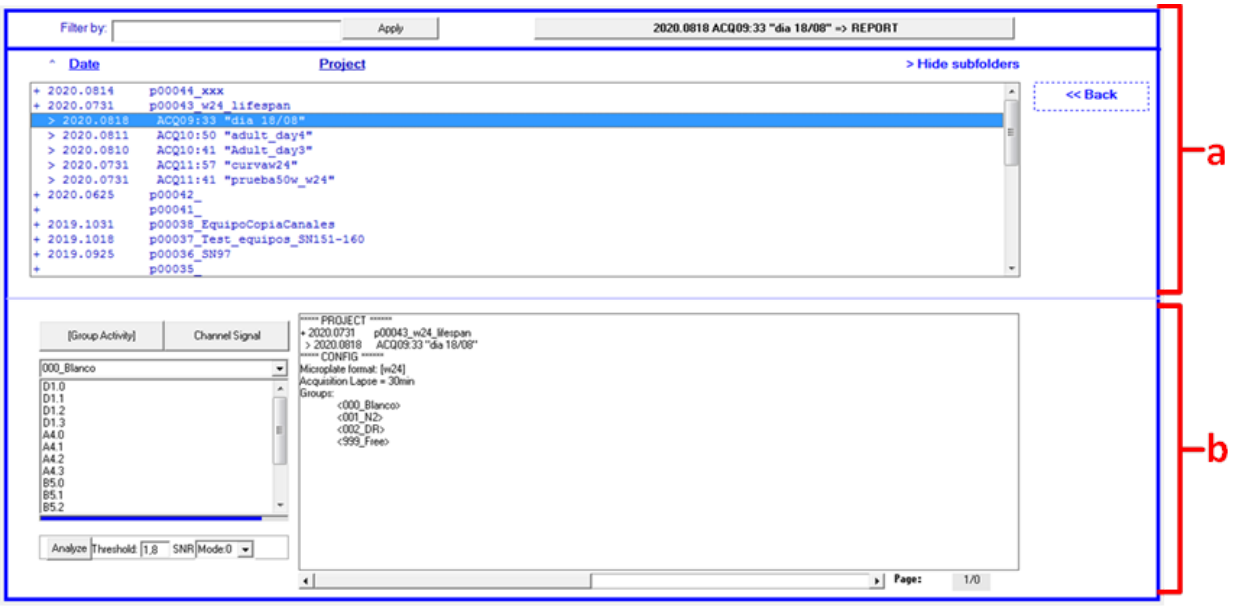
- Displays the individual signal from the sensors. It allows the selection of sensors per well within an experimental group.

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.

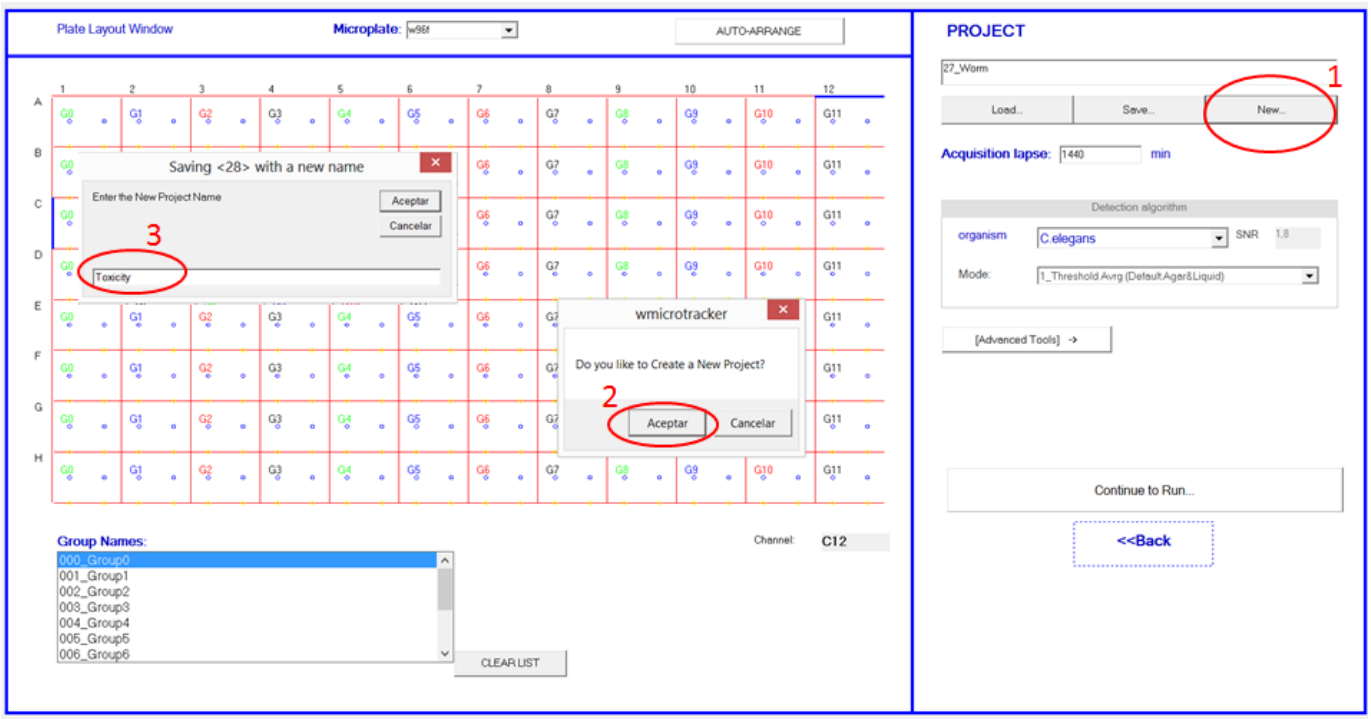
iii. Channel signal button:

-Shows the raw signal of each selected sensor.

IV. Defining and Starting Your Experiment

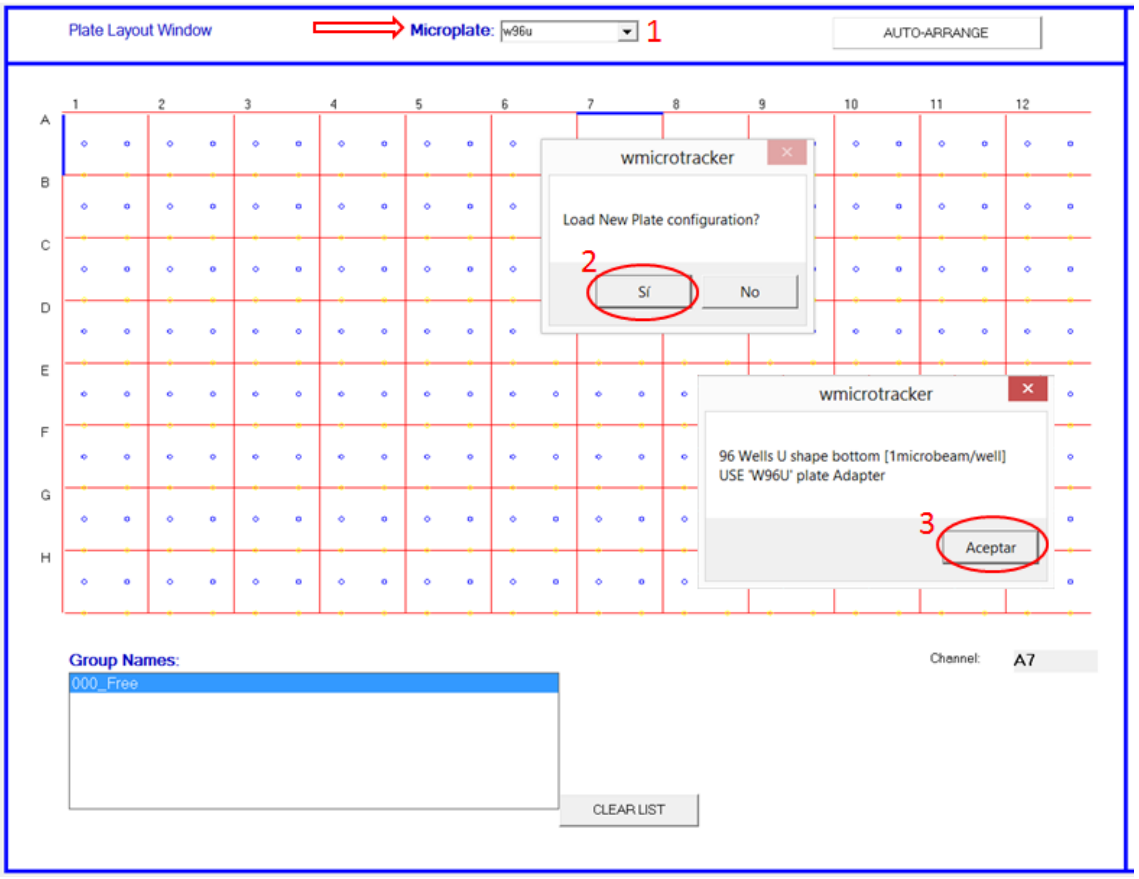
1. In the Setup Window, create a new project by clicking **"New"** on the right-hand project menu (Step 1).

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



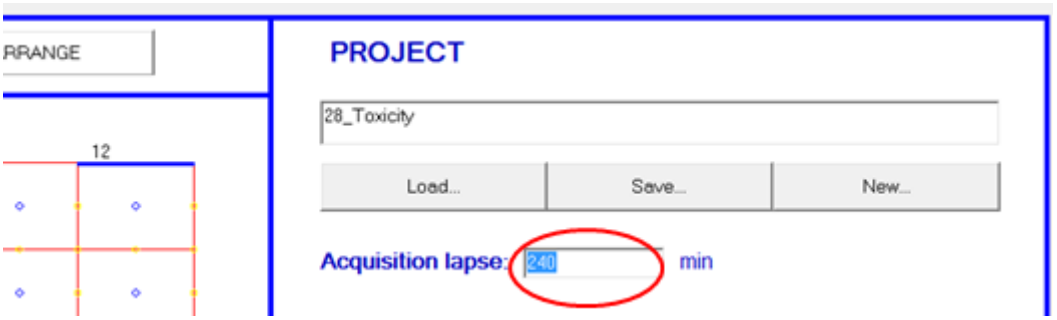
3. Enter the **"MicroPlate Format"** size that you will be using.
   -Select plate format (Step 1)
   -Accept changes (Step 2)
   -Confirm that you are using the correct adapter for your plate format (Step 3)

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.



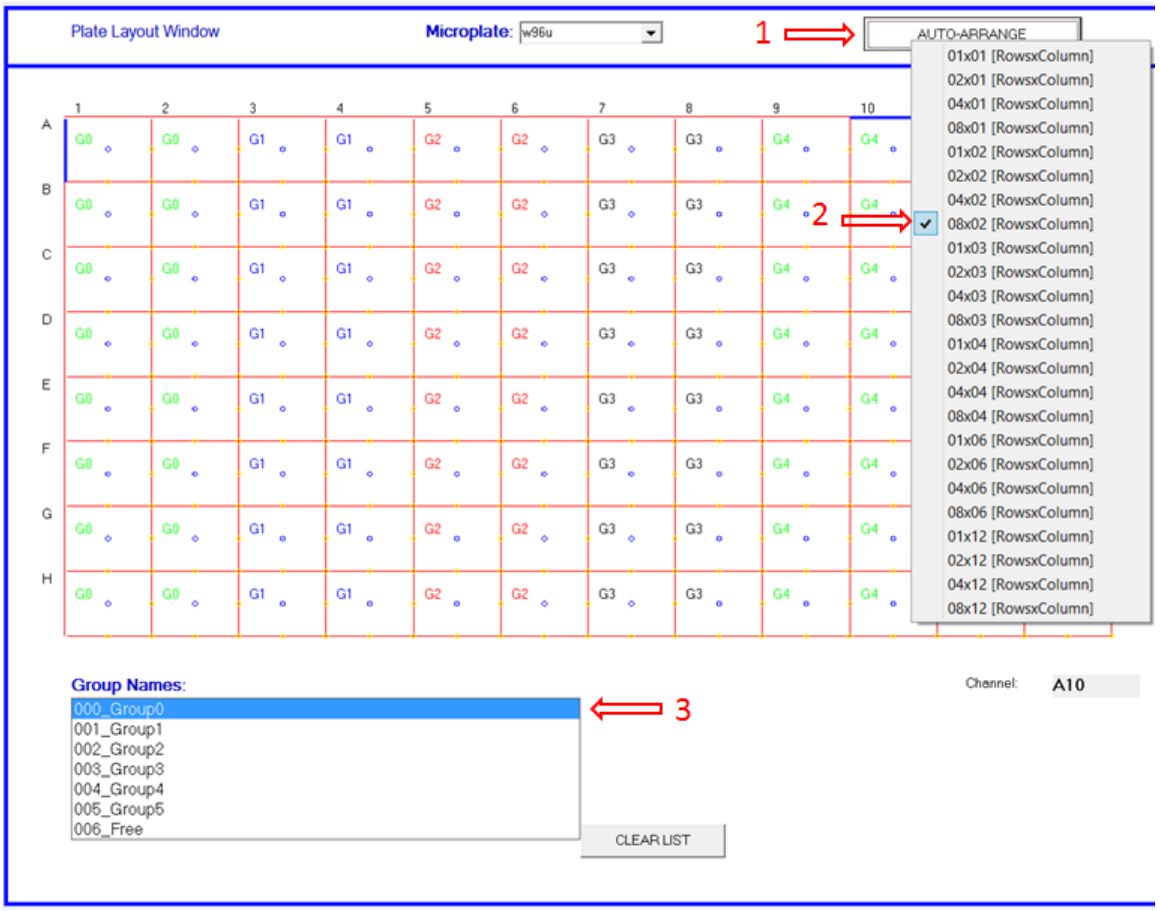
5. Set your plate layout in the **"Group Names"** section; this can be done either manually or by using the **"Auto-Arrange"** feature.

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 (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.

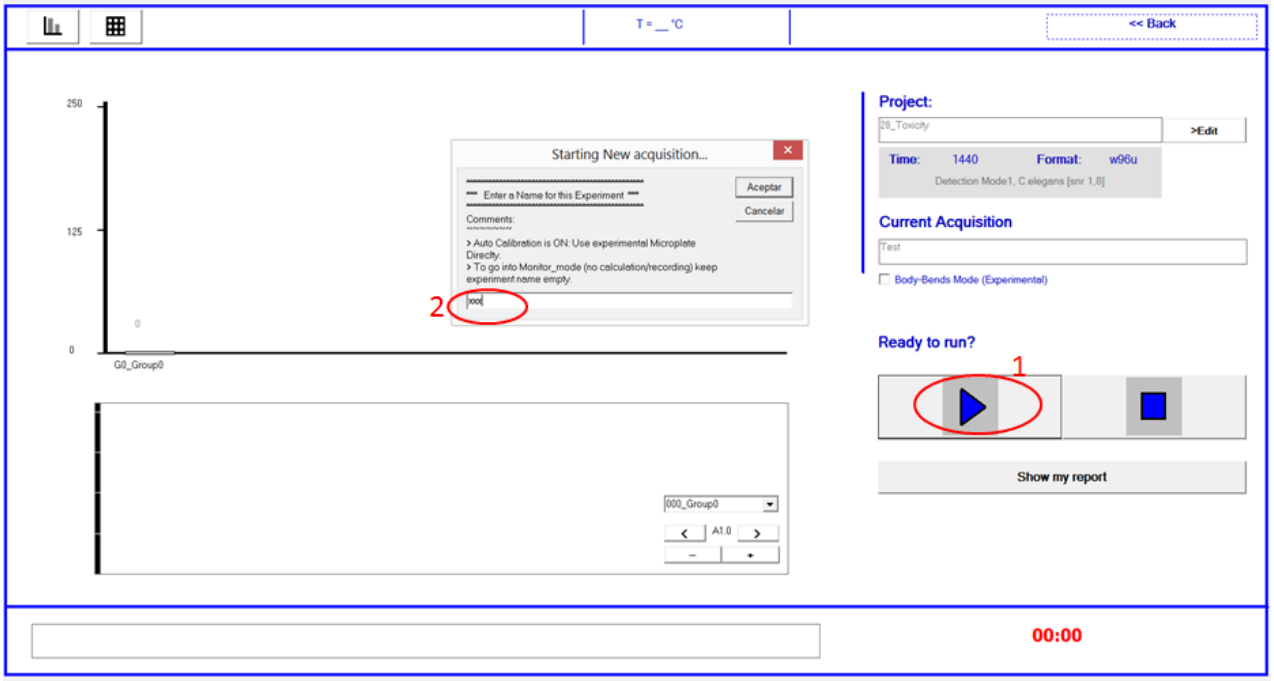
6. You are now ready to load your plate and press the **"Continue to Run"** button.

Start your experiment running!

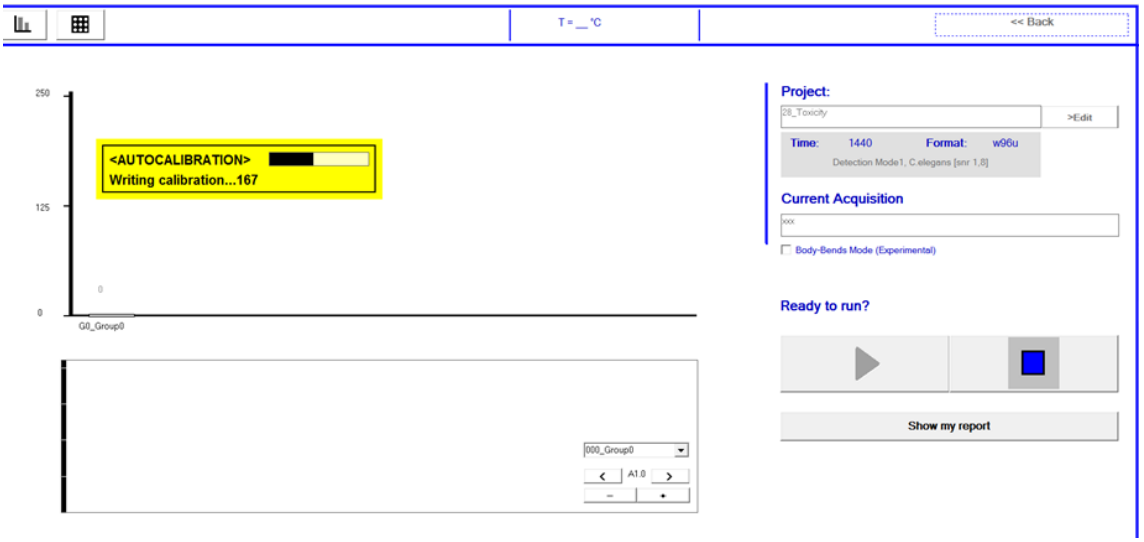
V. Running Your Experiment

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.

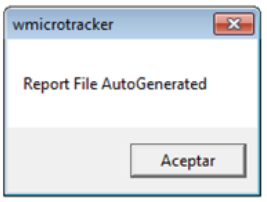
VI. Accessing Experiment Data

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

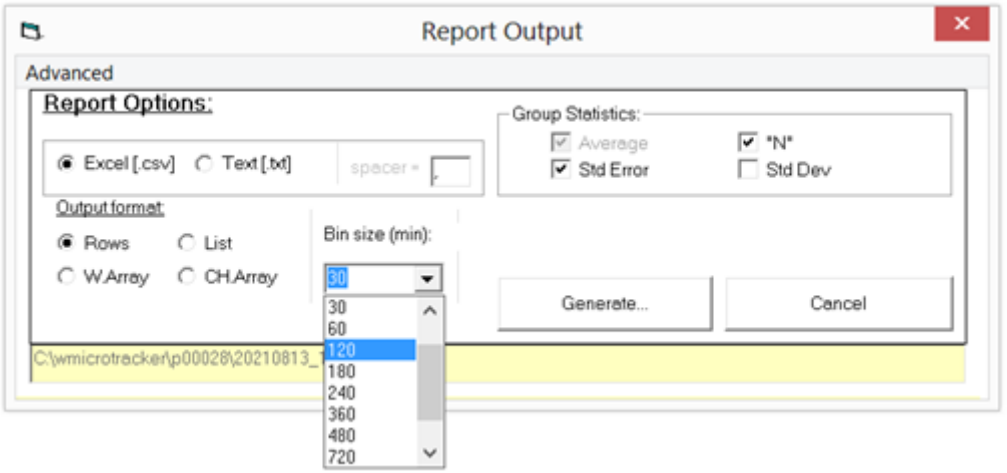
NOTE: Experiment reports are generated as a .CSV file by default; we recommend Excel for quick export and ease of use of the report files.

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.

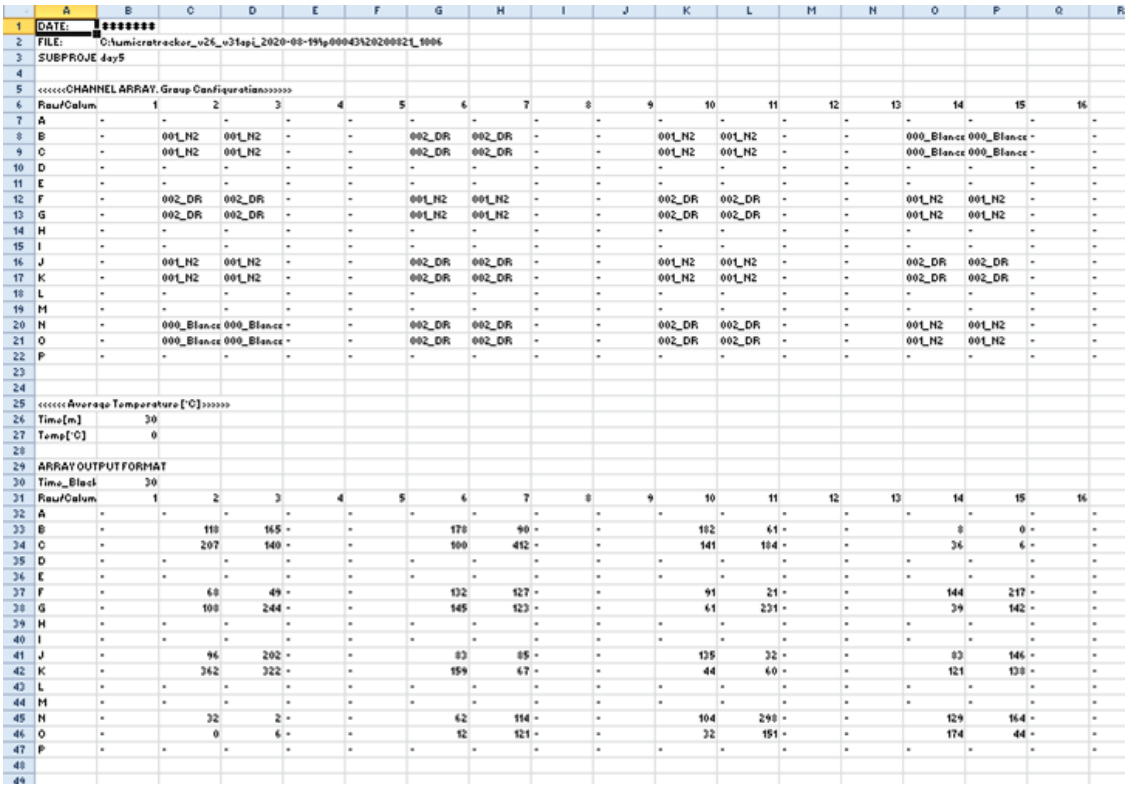
5	<<<<<< Group Activity: Average Activity Counts per Data Interval>>>>>>		
6	Time	Group	Value
7	5	000_Blanco	1
8	10	000_Blanco	2
9	15	000_Blanco	2
10	20	000_Blanco	2
11	25	000_Blanco	2
12	30	000_Blanco	2
13			
14	5	001_N2	38
15	10	001_N2	25
16	15	001_N2	22
17	20	001_N2	16
18	25	001_N2	15
19	30	001_N2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

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	R
1	DATE:	*******																
2	FILE:	O:\umicratracker_v26_v31api_2020-08-19\p00043%20200821_1006																
3	SUBPROJE	day5																
4																		
5	<<<<<<CHANNEL ARRAY. Group Configuration>>>>>>																	
6	Row/Colum		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_Blanco	000_Blanco	-	-
9	C	-	001_N2	001_N2	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-	000_Blanco	000_Blanco	-	-
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_Blanco	000_Blanco	-	-	002_DR	002_DR	-	-	002_DR	002_DR	-	-	001_N2	001_N2	-	-
21	O	-	000_Blanco	000_Blanco	-	-	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_Black	30																
31	Row/Colum		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
32	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	B	-		118	165	-	-		178	90	-	-	182	61	-	-	8	0	-	-
34	C	-		207	140	-	-		100	412	-	-	141	184	-	-	36	6	-	-
35	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	F	-		68	49	-	-		132	127	-	-	91	21	-	-	144	217	-	-
38	G	-		100	244	-	-		145	123	-	-	61	231	-	-	39	142	-	-
39	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	J	-		96	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:

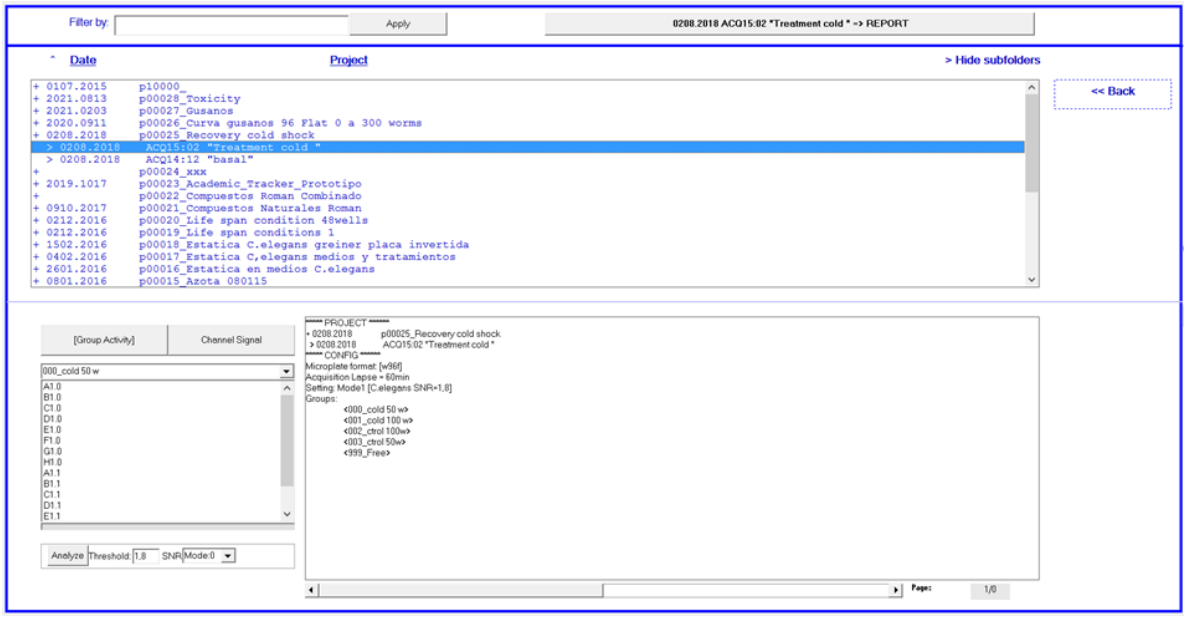
   - Select the desired project name from the list; each project will expand to show the acquisitions (if more than one) that were collected for that project. Select and double-click the appropriate acquisition and press the “REPORT” bar/button located at the top-right of the screen.

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.



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.

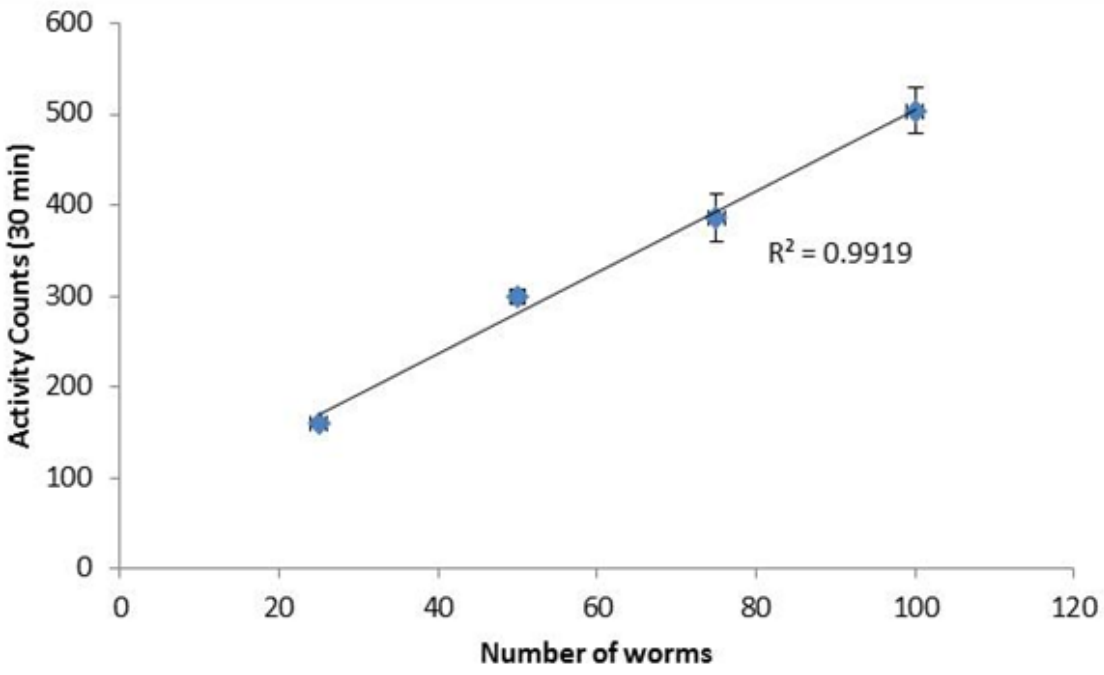
KEY APPLICATIONS:

- 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 (R2=0.99). More than 100 worms are not recommended, a plateau of activity is observed.

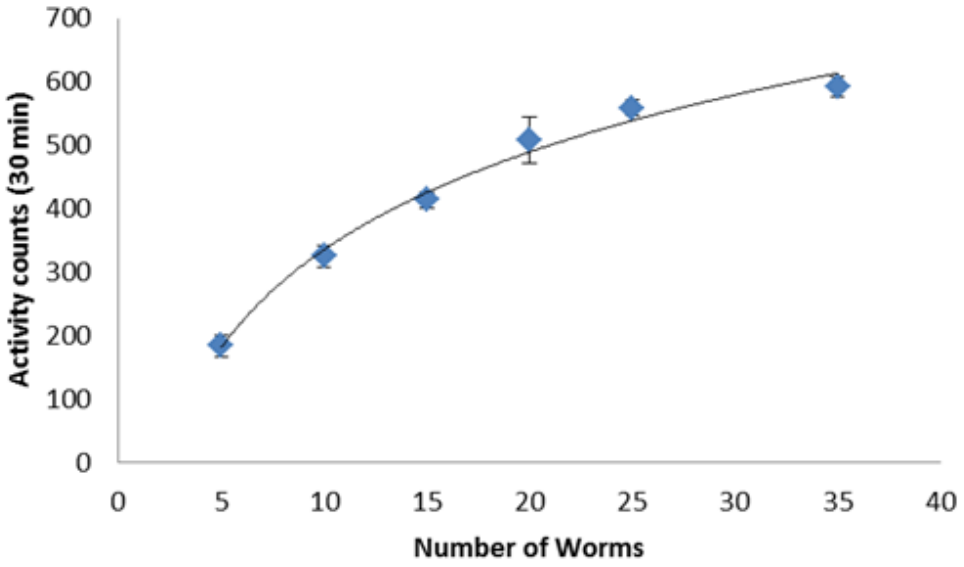
NOTE: Data shown are averages over two independent biological replicates with four technical replicates for each. Error bars represent +/- S.D. Analysis detection Mode 1.

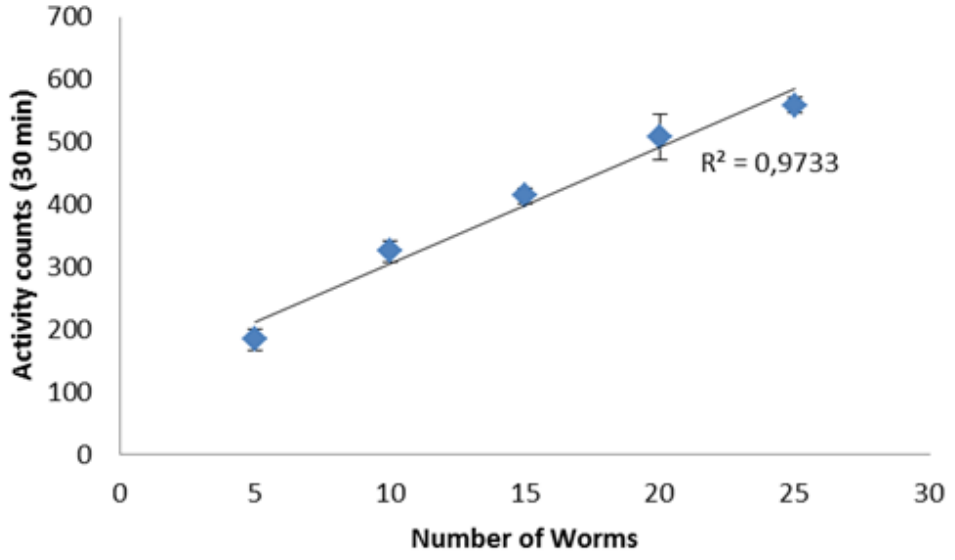


Curve 96W "U" bottom Microplate: Young Adult_N2 (*C.elegans*)_M9 supplemented with BSA 0,05%.

The system presents a very good linearity of detection between 5 and 25 worms, observing a plateau at more than 25 (R2=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.





Appendix C. ONE Applications_Toxicity Test

WHAT YOU NEED:

Young adult worms

96 well "U"-bottom plate with lid.

M9 buffer.

OP50.

Levamisole – Stock solution: 100mM in ddH20.

Synchronized populations of young adult worms.

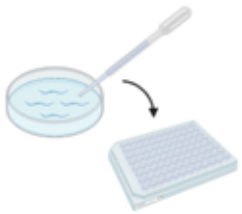
wMicrotracker ONE.

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 µl and adjust the volume to obtain a concentration of [20worms/90µl]. Adjust volume with M9 and add OP50 to 1 mg/ml final concentration.
7. Transfer per well 90µl of the worm solution to a 96 well microplate using multichannel pipette.
8. Add 10µl 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.

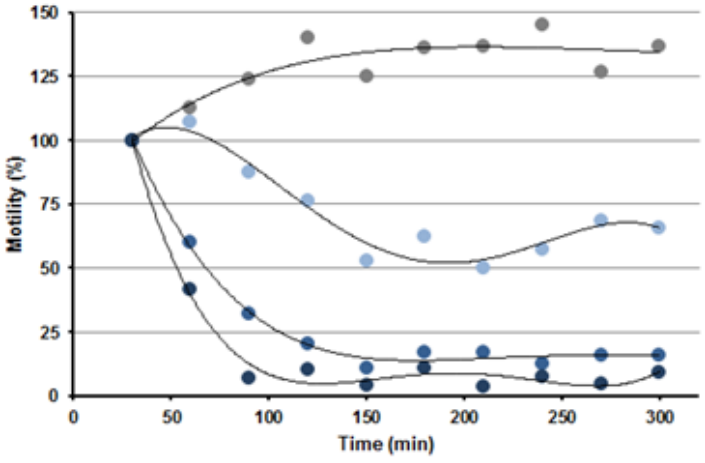


(A) Transfer 20 young adults worms in 90 µl to 96-well "U" bottom plates

(B) Add 10 µl 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

- Control
- Levamisol 5 uM
- Levamisol 10 uM
- Levamisol 100 uM

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 50µM FuDR, 100µg/ml streptomycin and 20µg/ml kanamycin.

Paraquat – Stock solution: 200mM in ddH20.

Vitamin C

Synchronized populations of L4 worms.

wMicrotracker ONE device.

Notes:

- Perform at least three technical replicates and at least two biological replicate.

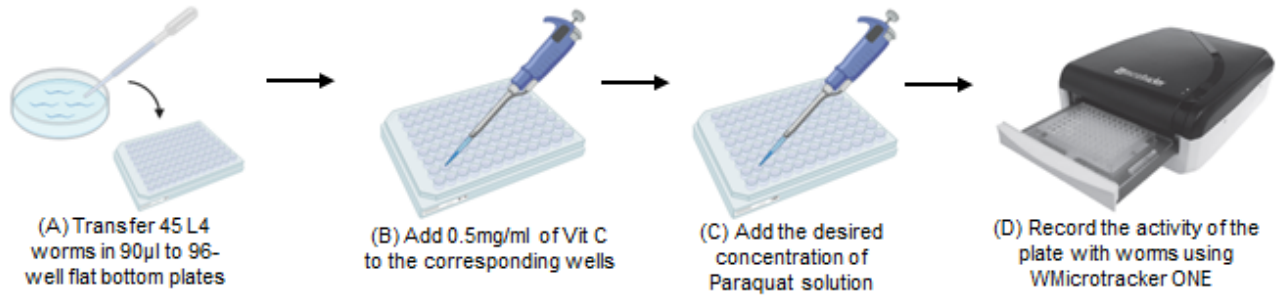
- Before activity measure, stimulate the 96 well plate with worms for 5 second by gently shaking by hand.

- 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 µl and adjust the volume to obtain a concentration of [5 worms / 10 µl].
7. Transfer per well 90 µl 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.



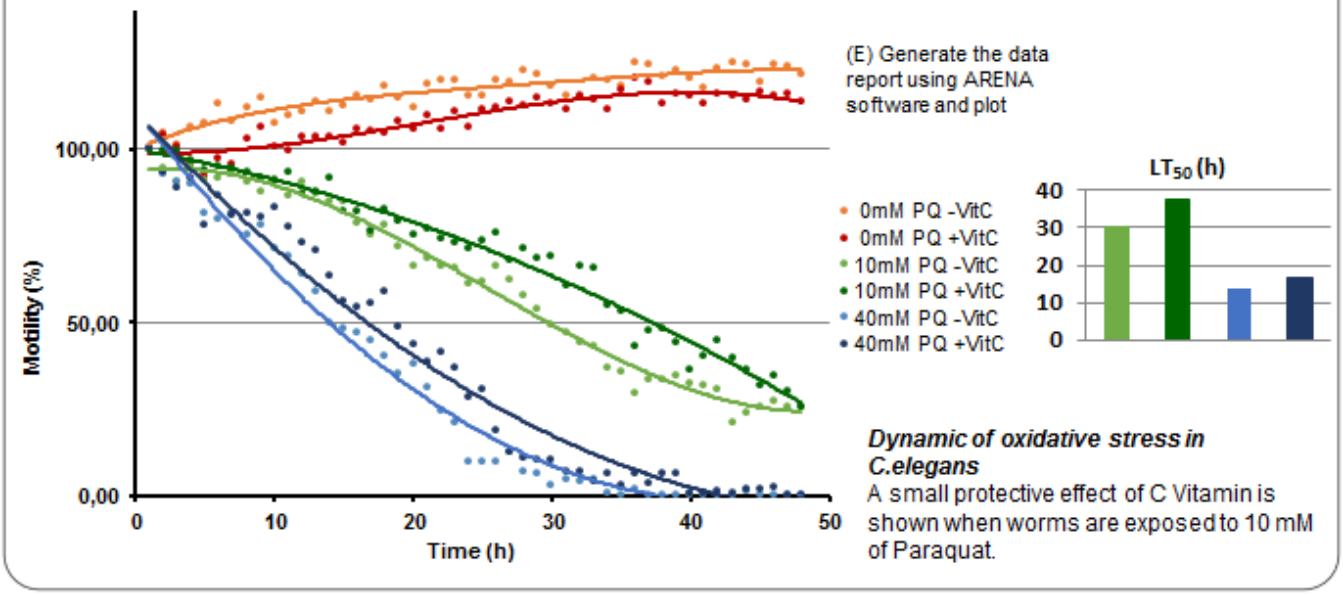
(A) Transfer 45 L4 worms in 90µl to 96-well flat bottom plates

(B) Add 0.5mg/ml of Vit C to the corresponding wells

(C) Add the desired concentration of Paraquat solution

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

(E) Generate the data report using ARENA software and plot



Dynamic of oxidative stress in C.elegans

A small protective effect of C Vitamin is shown when worms are exposed to 10 mM of Paraquat.

Appendix C. ONE Applications_Pathogenesis

WHAT YOU NEED:

96 well flat-bottom plate with lid.

M9 buffer.

3PY medium supplemented with 50µM FuDR, streptomycin 100µg/ml and kanamycin sulfate 20µg/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 25ºC.

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 µl and adjust the volume to obtain a concentration of [5 worms / 10 µl].

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

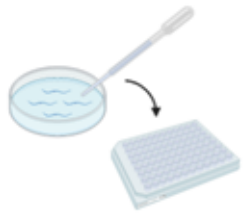
Optional: Let worms to rest for 30 min and measure basal activity using WMicrotracker.

8. Add 10 µl 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.

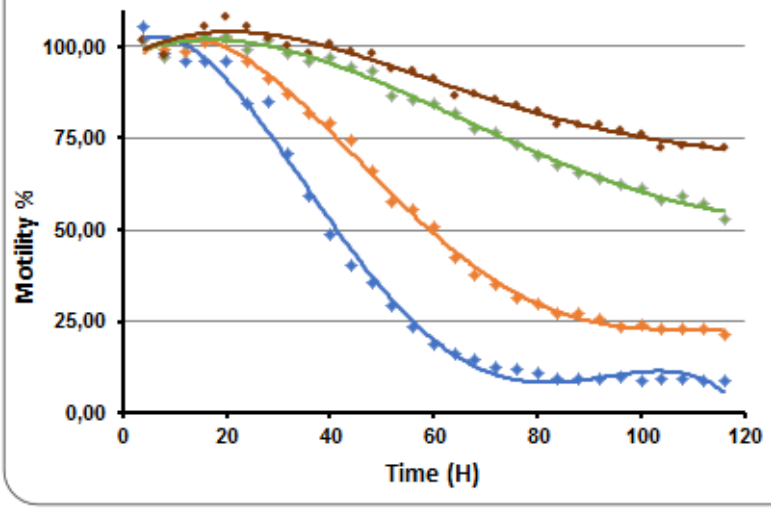
Note: Perform at least three technical replicates and at least two biological replicate.



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

(B) Add 10 µl of a 10X concentrated solution of chemicals

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



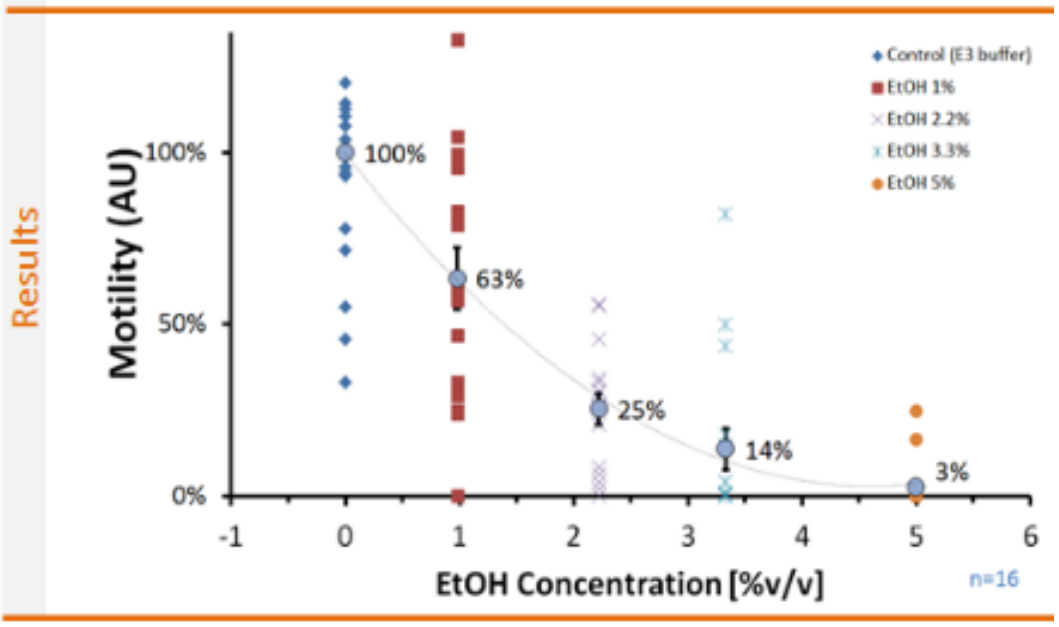
Kinetics of killing using bacterial supernatant of Pseudomonas Fluorescens CHA0

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 28ºC 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.



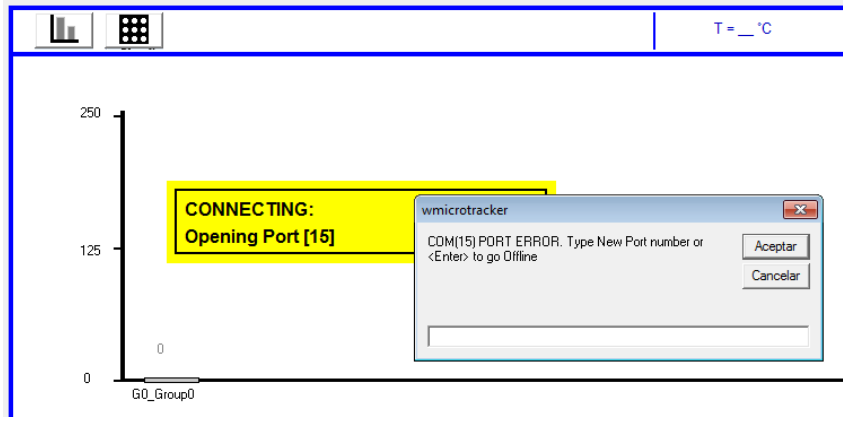
Effect of ethanol in Zebrafish Larvae

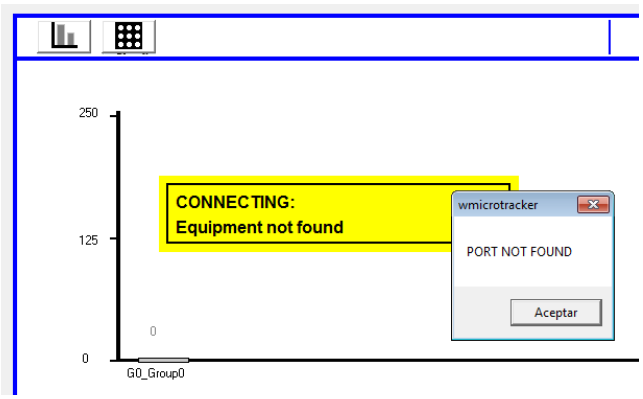
In this experiment we can observe the effect of EtOH on zebrafish larvae behavior after 48h of drug exposure. The plot shows the average activity and the natural variability of response between animals (n = 16 per treatment).

Appendix D. Troubleshooting and additional information

1. 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.

Product Electrical Specifications

WMicrotracker®	ONE
Power Source	9VDC 1.5Amp
Power Consumption	10W
Sensing Detection Technology	IR Phototransistors x384
Lighting Technology	880nm Infrared Led Array x384
Communication Protocol	USB CDC driver. 115.2 KBps
Recommended Ambient Working Conditions	15 - 25ºC, Low Humidity

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

Software and system updates available at www.phylumtech.com