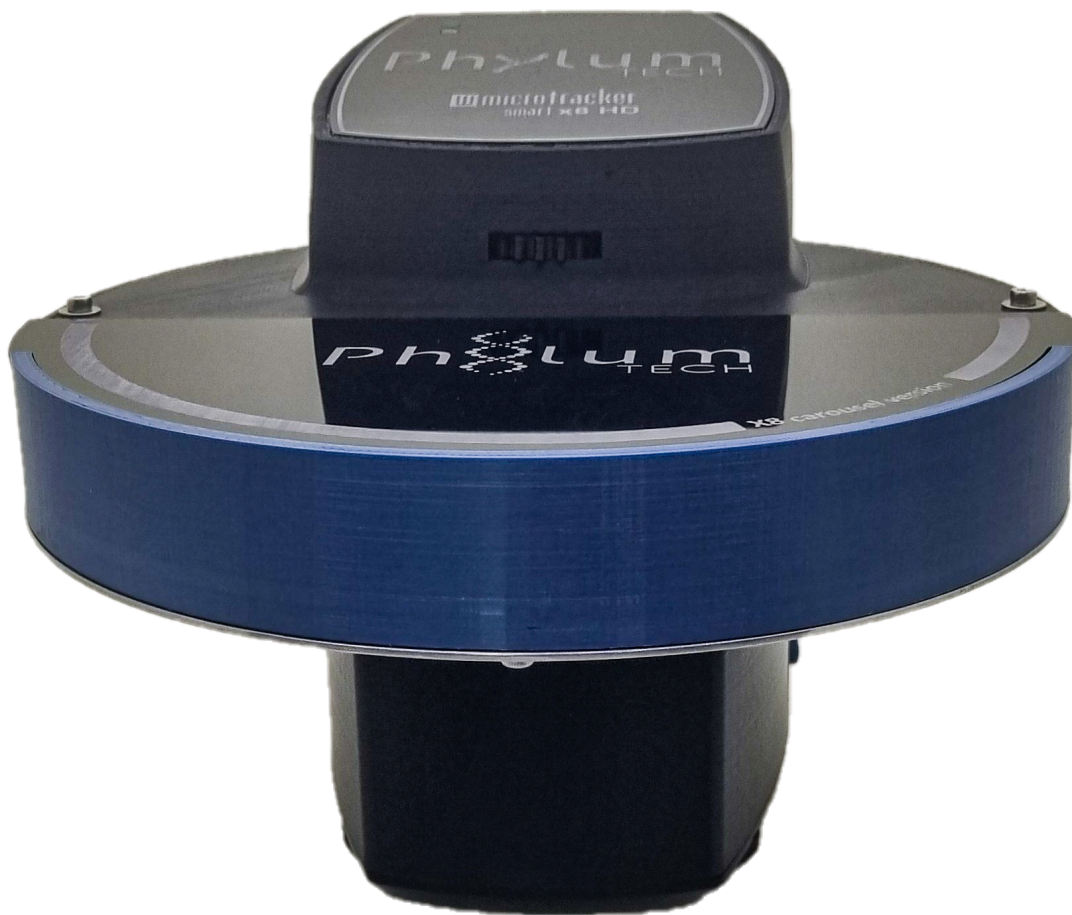# ɰ microtracker
smart x8 HD

Installation Guide
and User Manual

Dual Mode Infrared Imaging system with x8 carousel
Multi-Worm Path Tracking (Machine Learning Algorithm)
+ Infrared Light scattering detection

Data Acquisition System: WMicrotracker SMARTx8 HD
Hardware Version: SMARTx8 V2.0
Software Version: SMART V2.5 (2025)

Thank you for acquiring the SMARTx8 system. The following document will guide you through the installation process.

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Contents

I.   About the SMART

The WMicrotracker SMART is a modular system designed for tracking small organisms in a 35mm Petri dish format. It enables reliable quantification of animal population movement and is compatible with a variety of organisms, including *C. elegans* and related nematodes, zebrafish and Drosophila larvae, and small insects.

It has the capability to work in 2 different modes:

1- **Face-down IR Imaging:** This mode enables Multi Worm Path Tracking using NGM cultures. It relies on the optical phenomenon of Silhouette Amplification by Infrared Refraction, where infrared light waves refract at the worm-agar interface, generating an amplified image captured by a high-resolution camera system. The digital processing of the image is carried out using software specially designed for real-time data acquisition

2- **IR Light Scattering:** This mode enables the quantification of the behavior of multiple organisms larger than 0.1 mm using solid, liquid, or air media cultures. It allows for the definition of different areas of activity on the plate, which is useful in chemotaxis experiments. The patented method detects movement through the scattering of light caused by a grid of infrared microbeams.

Face-down IR Imaging
MODE

IR Light scattering
MODE
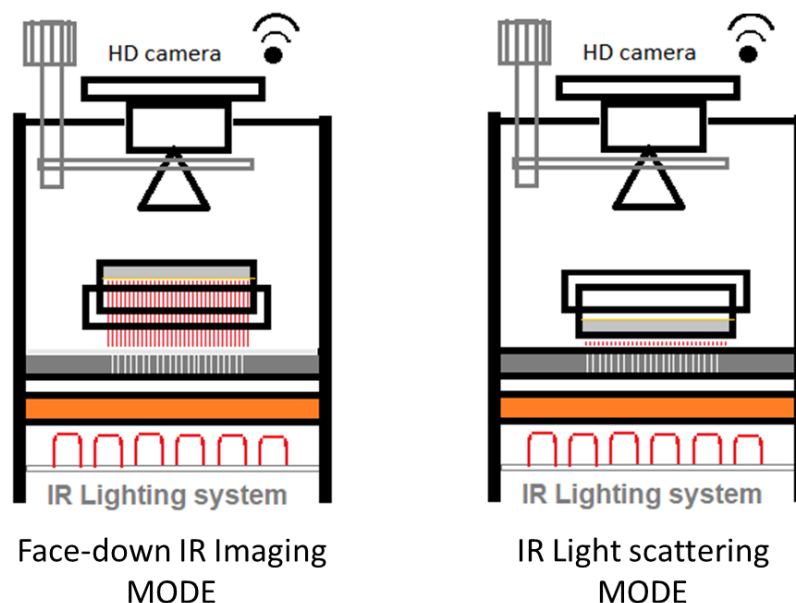
Some technical characteristics of the system:
- Non invasive acquisition
- Undisturbed by bacteria (although very dense or dark bacterial cultures may affect the accuracy of the system).
- Compatible with RNAi and compound screenings.
- Enables the evaluation of multiple protocols using agar-cultured animals, liquid culture media, and air-cultured insects.
- The preferred microplate culture format for the SMART system is 35 mm clear petri dishes. It is **recommended to use the** 35mm-Greiner Bio-One #627161.
  NOTE: Petri plate must be run with the lid on and it is recommended to seal the plate with film.

Included Components

	Wmicrotracker SMART acquisition hardware
	Plate mode adapter: **Microbeam grid side-up** **Tracking mode side-up** **Tracking mode side-down**
	mini USB A Cable
	Power supply 9V 2.5 Amp, plug 5,5 x 2,1, center positive *In some countries might not include it due to customs restrictions

Additional Requirements

- IBM PC compatible with the following minimum requirements:
  - Pentium Core i3 processor or above
  - 2Gb of RAM memory
  - 1 USB port available
  - MSWindows 7 (or higher) operating system
  - > 1Gb of free HD space for experiment images storage.
- The system's optimal functionality requires an ambient operating temperature of 10°C to 40°C with humidity below 50%, but biological samples may have unique temperature requirements.
- Minimize vibration and dust in your working area.
- Avoid locating the instrument near a clear window or bright light.

Product Dimensions and Manufacturing

- LWH 22cm x 24cm x 22cm (8.66in x 9in x 8.66in).
- Manufacturing Technology: 3D Printing

II. Data output Examples: Path Tracking mode

C.elegans Levamisole curve [Toxicity/Anthelmintics research]

WMicrotracker SMART is capable of tracking the movement of a population of C. elegans and plotting their path, while calculating the average movement speed in real-time. This feature is useful for toxicity and anthelmintics research.

As an example, a kinetics plot comparing the effect of different levamisole concentrations (ranging from 0 to 200 µM) on the movement speed of C. elegans is shown below. The plots demonstrate a correlation between the concentration of levamisole and a decrease in movement speed.
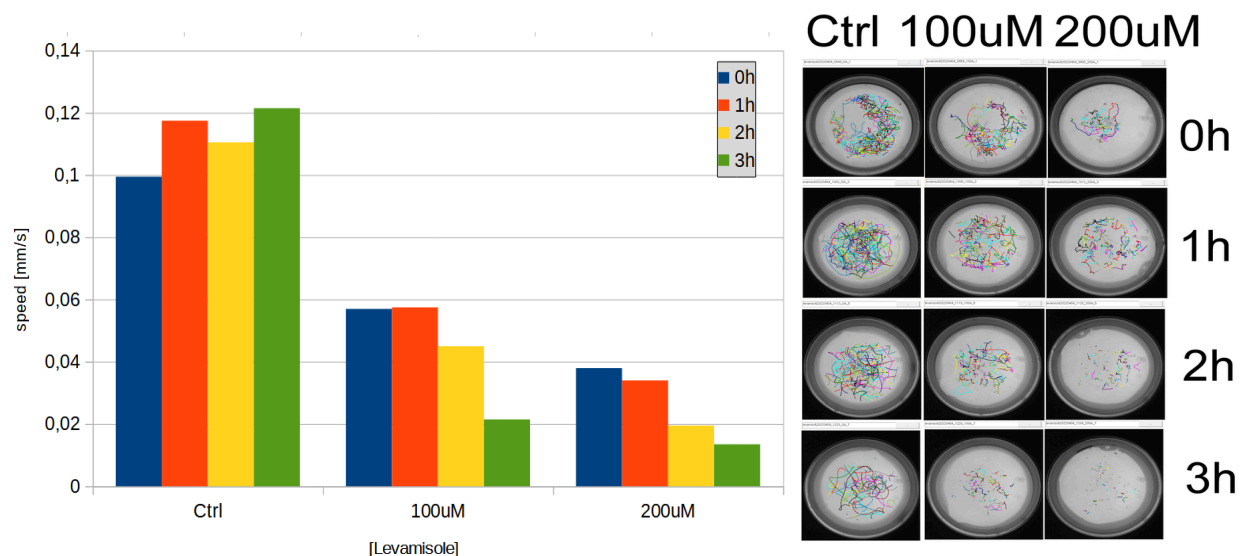
Using a 5 minute acquisition lapse every hour, the WMicrotracker SMART is able to quantify the effect of levamisole paralysis on C.elegans worms.

Protocol:
1. Grow synchronized populations of adult day-1 worms in seeding NGM plates (OP50).
2. Remove worms from plates using M9 buffer and transfer them to a sterile 2ml micro tube.
3. Let the worms settle. Decant the supernatant, taking care not to disturb the pellet.
4. Wash the worms by adding 2 ml of M9 buffer. Briefly, shake or invert the tube
5. Repeat the decantation step and discard the supernatant.
6. Add 2 ml of M9 buffer.
7. Count the number of worms in 10 µl in triplicate and calculate the average.
8. Prepare a suspension of 25 worms/10 µl.
9. Transfer 10 µl of the worm solution to a 35mm plate and wait until the drop is absorbed.
10. After 5 minutes, use WMicrotracker SMART to record worm activity for 5 minutes. Immediately before the acquisition, stimulate the worms by tapping the plate three times.

Microplate preparation:
- NGM is prepared following standard procedure.
- Levamisole is added to the 55°C NGM agar solution immediately after the addition of the salts and cholesterol.
- Levamisole plates were not seeded with OP50.
- The plates are allowed to equilibrate to room temperature before the start of the experiment.

- Before adding the worms to each plate, make a ring of 100 mM copper sulfate around the edge of each plate to prevent worms from crawling out of the agar.
- When adding the worms, be careful not to scratch the agar – worms tend to crawl into any break in the agar surface.

C.elegans detection curve [Optimization of animal number]

The WMicrotracker SMART software can automatically recognize and track up to 80% of the particles, as shown in the detection curve below.
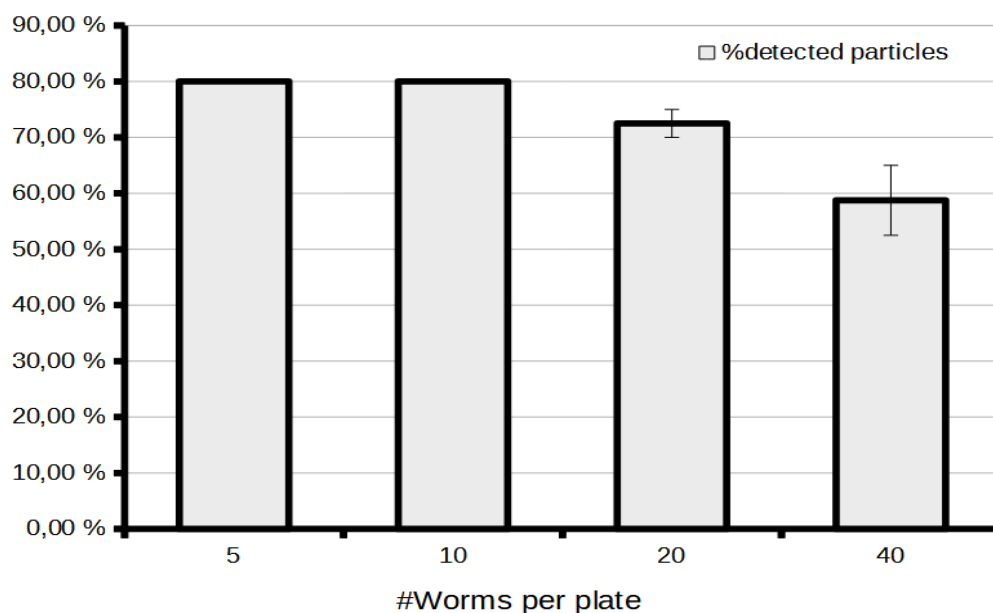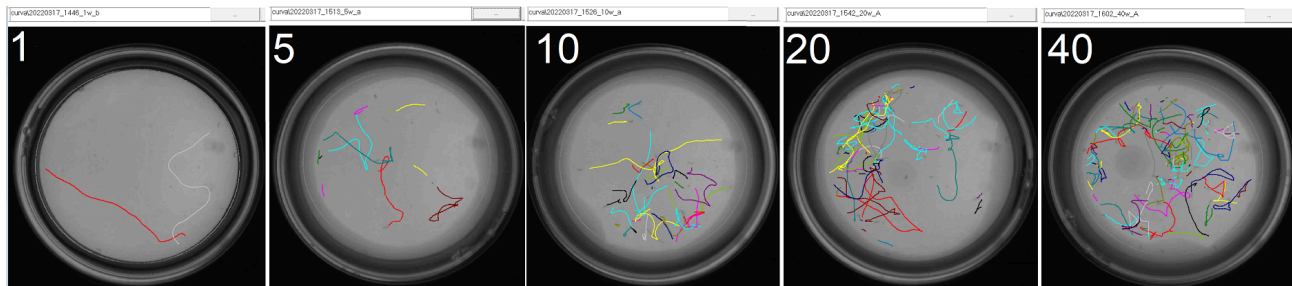
Experiments can be performed using 10 to 30 worms per 35mm Petri dish.

Protocol:
1. Grow synchronized populations of adult day-1 worms in seeding NGM plates (OP50).
2. Remove worms from plates using M9 buffer and transfer them to a sterile 2ml micro tube.
3. Let the worms settle. Decant the supernatant, taking care not to disturb the pellet.
4. Wash the worms by adding 2 ml of M9 buffer. Briefly shake or invert the tube
5. Repeat the decantation step and discard the supernatant.
6. Add 2 ml of M9 buffer.
7. Count the number of worms in 10 µl in triplicate and calculate the average.
8. Prepare an extra with more than 10 worms to train the software. (See Manual Page 12 - 1.d. Silhouette detection).
9. Transfer different numbers of worms (1/5/10/20/40) to 35mm NGM plates without food.

Check the number of worms transferred using a magnifying glass. Wait until the drop is absorbed.

10. Five minutes later, use the WMicrotracker SMART to register worm activity during a 5-minute interval. Before starting the acquisition, stimulate the worms by tapping the plate three times.

Notes:

- Before adding the worms to each plate, make a ring of 100 mM copper sulfate around the edge of each plate to prevent worms from crawling out of the agar.
- When adding the worms, be careful not to scratch the agar – worms tend to crawl into any break in the agar surface.

III. Installation & Setup Guide

Software Installation

We recommend periodically checking the Phylumtech website for software updates.

1. To download the Software Installation .zip folder, go to the Software download zone (https://www.phylumtech.com/home/en/support/), right-click the link, and choose "save link as".
2. Unzip the files and copy the WMicrotracker_smartx8HD folder directly to c:\wmicrotracker_smartx8HD. (Avoid copying the folder to the desktop or into a folder with a very long name.)
   The software WMicroSmartx8HD.exe is ready to run.

Hardware Turn ON/OFF and Setup

1. Connect the Power Supply: Plug the 9VDC switching power supply (2.5 Amp output) into a standard power outlet, and connect the cable plug to the back of your SMART device.

2. Connect the USB Cable: Connect the USB cable from your computer's USB port to the USB port on the SMART device. The system will automatically detect the device on COM ports 1 through 15.

   a. Note: You can verify the connection in the Devices and Printers window by checking for a newly assigned COM port.
   b. Windows 10 is recommended. Windows 7 is also supported, but requires the latest updates to install the driver properly.

3. Power On the Device: Press the button located on the side of the SMART device. A green light will turn on.

   Wait approximately 30 seconds for the internal chip to initialize. When the device is ready to connect to the computer, a yellow light will flash.

4. Shutdown: To shut down the device, please use the software controls.The device will automatically shut down after 30 minutes of inactivity. To restart the device, press the button on the side again.
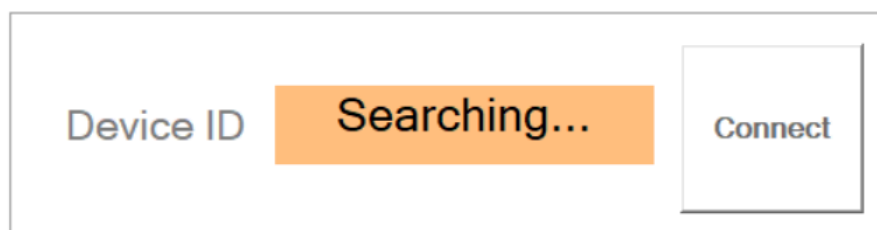
IV. Acquisition Software

Software Launch

1. Open the folder where you installed the software and run the **"WMicroSmart"** executable file. The application should start immediately and display the **"Start Window".**
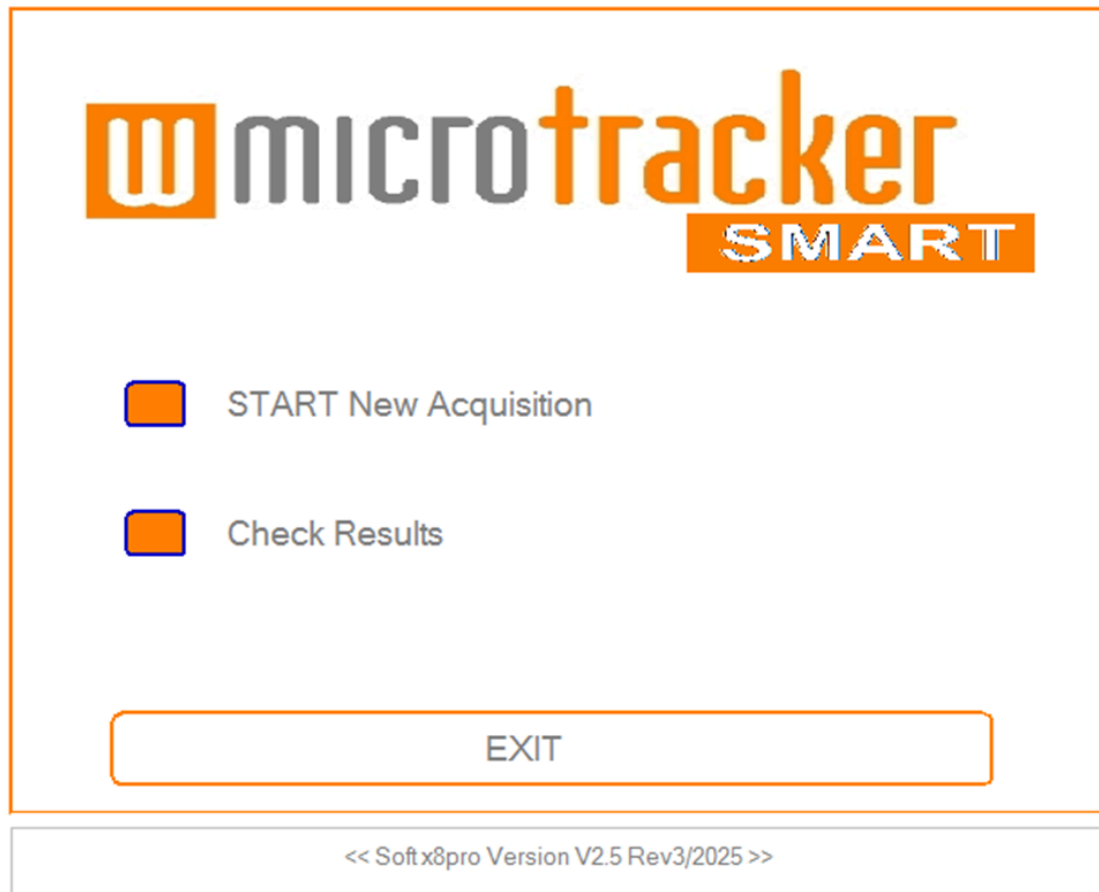
2. Click on "Start New Acquisition" to connect to your SMART device.
A new window will appear while the software searches for the connected device.

If the device is found on one of the COM ports, you will be redirected to the **Data Acquisition Window**.

3. Shutdown:

To turn down the device you have to press the shutdown button, it will take 20 seconds to turn off the device.

The device will automatically shutdown after 30 minutes of inactivity. To initialize the device again, press the button at the side of the device and please go back to the initial window and connect again.
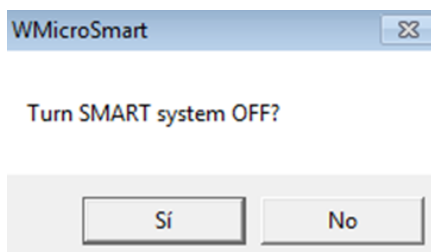
WARNING:
Because the SMARTx8HD uses a Raspberry Pi microcomputer inside, it requires to power off correctly by software to avoid memory corruption over time. Just force the disconnection of the plug power of the device if there is a specific requirement to do that.

Data acquisition Window: Screen Components and Operation

1. Plate Setup & training

a. Station:

You can select your desired plate position by clicking on each number. The device will automatically rotate and align that station in front of the camera.
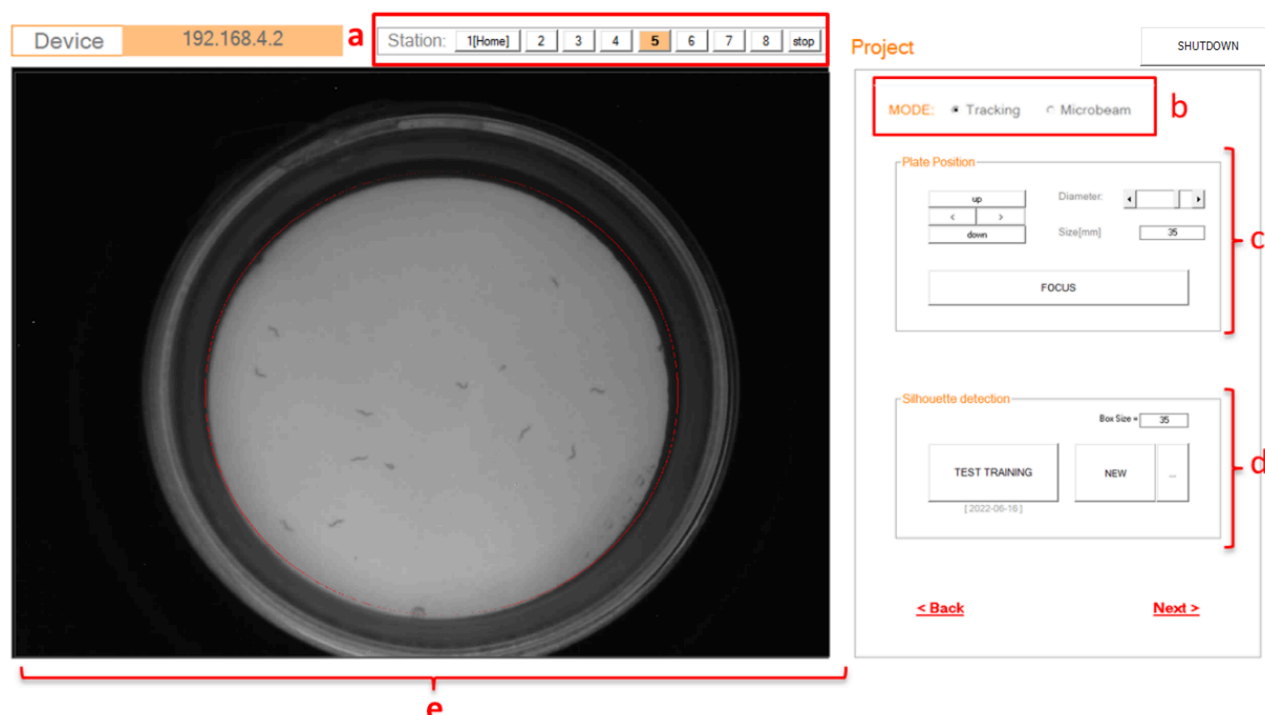
If you wish to manually rotate the carousel, please click on the "stop" button beforehand to allow free movement.

b. MODE Menu:

i.a. "Tracking Mode" for C.elegans and similar nematodes cultured on agar:
- In this mode, the system utilizes the optical property of light phase transition (air-worm-agar) to magnify worm silhouettes when exposed to infrared light. A similar phenomena has been described in FIM applications (A Multi-Purpose Worm Tracker Based on FIM | bioRxiv). High resolution images are processed using machine learning algorithms to identify single worms and to track their trajectories.
- The petri dish must be placed face down (with the lid down and the appropriate plate adapter) for detection of worms cultured on NGM. It is only compatible with adult stage C.elegans and similar sized worms.

*i.b. "Tracking Mode" for small aquatic animals and insects:*
- The petri dish must be placed face up (with the lid up and the appropriate plate adapter) for detection of organisms higher than 1mm.

ii. "Microbeam" Mode:

The microgrid plate adapter possesses a grid composed of more than 2.000 microholes, 100um wide. Activity detection for this mode is based on determining frame-to-frame microbeam lights fluctuation by using a pixel-to-pixel image subtraction algorithm. If the pixel neighbor difference is larger than a threshold, then an activity accumulator is incremented. This calculation mode uses low resolution video images with no single worm detection.

-The petri dish must be placed face up (with the lid up) with the appropriate microgrid plate adapter.

c. Focus and Plate position:
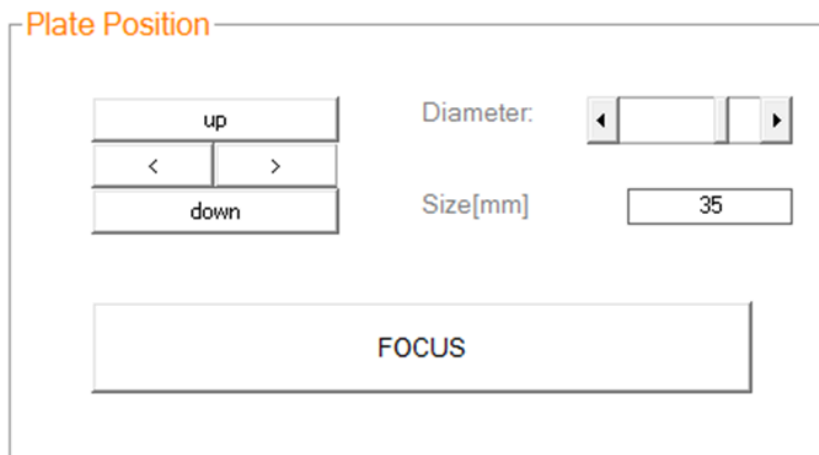
i. The arrows allow you to adjust the position of the registration area (red circle) of your petri dish. Use the "Up", "Down", ">" Right, and "<" Left arrows to move the red circle to the desired location.

ii. "Diameter": Use this option to increase or decrease the size of the registration area (red circle) of your petri dish. It is important to place the red circle on the edge of the 35mm base dish.

iii. "Size (mm)": This option allows you to specify the actual diameter of the red circle in millimeters. It serves as a scale indicator to calculate distances accurately.

iv. Focus: This option allows you to capture real-time images to adjust the lens focus using the focus wheel at the back of the SMART device. The time designated for adjusting the focus is set to 30 seconds. If the focus is not achieved within that time, you can press the focus button again.

FOCUS WHEEL
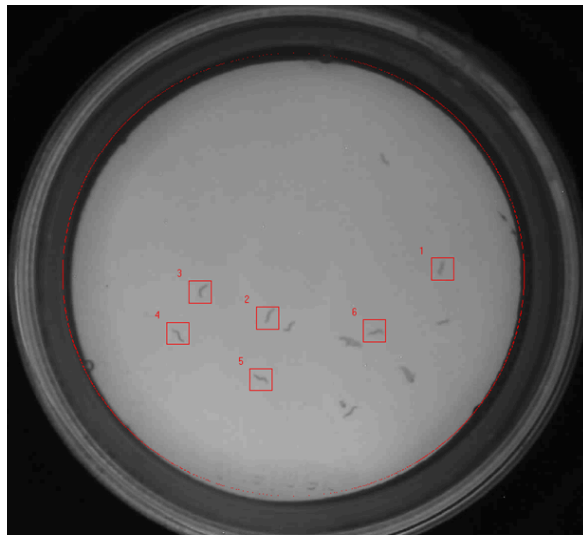
d. Silhouette detection (only enabled for tracking mode):
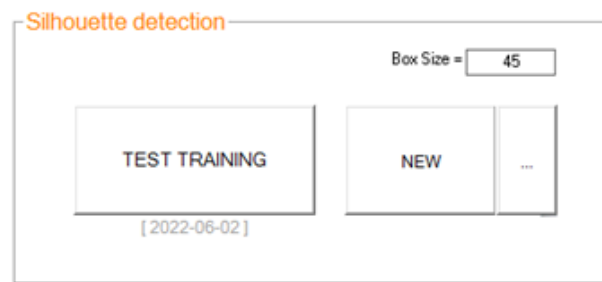
i. "New" button: Allows the software to be trained to recognize silhouettes.To select silhouettes of organisms in the image, click on the particle with the left mouse button (select 5 to 10 representative organisms). For example;

Box size: Increase or decrease the box size so that the entire microorganism fits inside. The reference box size for C. elegans is 35 to 45 pixels depending on the worm stage.
Then, press the "APPLY" button, and the shadows of the animals will turn red.
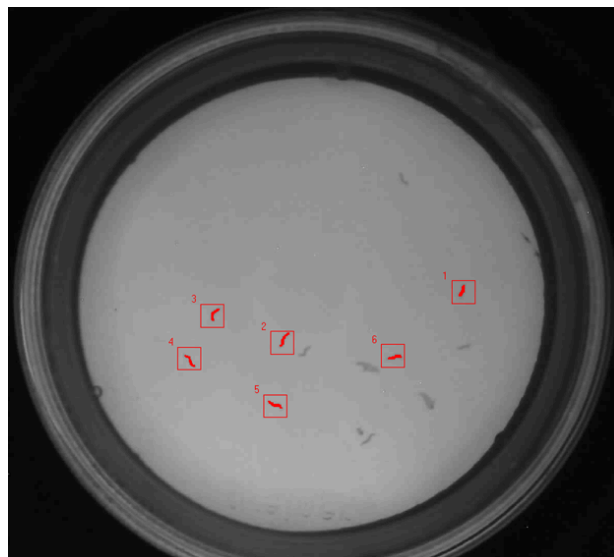
Finally, press "OK" to save the training parameters

ii.   "Test training" Allows you to check the performance of recorded silhouette recognition patterns.
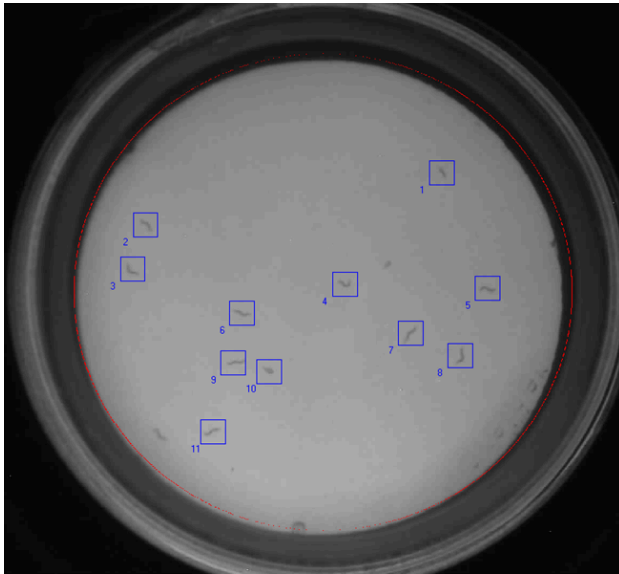
e. Plate Layout:

i.   Petri plate Box:

The WMicrotracker SMART is currently validated to work with 35 mm format. The picture displayed is a photo of the plate taken during the last connection with the device. You can view the plate in real-time while adjusting the focus of the device.

2. Image acquisition screen is shown when you select the "Next>" button on the First screen Setup.

a. Status Bar:
   i. "Status Bar":
   -   Displays the progress of the runtime as a yellow bar.

b. Project folder Menu:
   i. You can create and name a new project folder by typing in the box.
   ii. You can access a previous project name by displaying the box.

The project folder will be saved inside your software installation folder.

c. Data Acquisition:
   i.   **"Read":** use this button to set the acquisition lapse time:
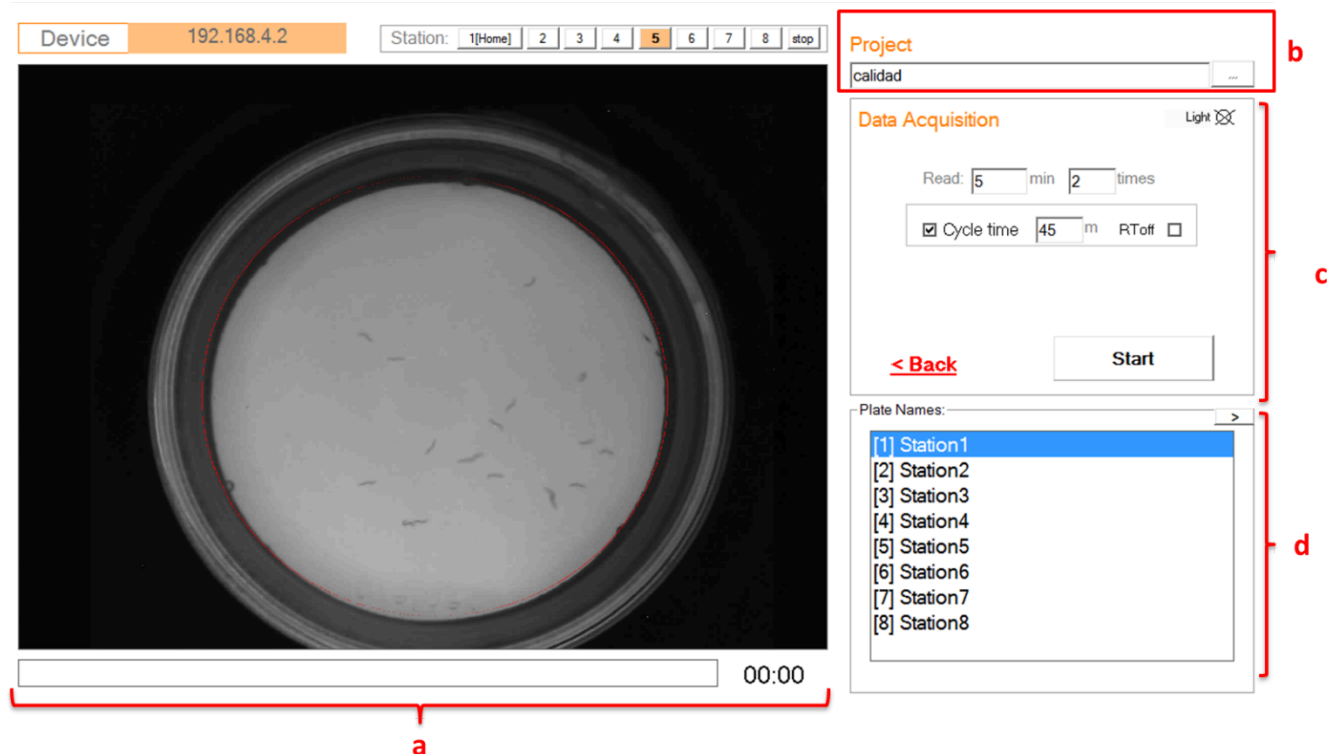   - Set the total runtime for the petri plate reading, in minutes
   - The current software version has a default lapse time of 5 minutes.
   ii. **"Light Stimulus":** if you previously enabled blue light (Tools/Enable Blue light), you can select whether to acquire data in presence of blue light or not. If you select this option, blue light will turn on when the acquisition starts and turn off automatically when it stops. NOTE: Not all the devices have blue light incorporated.
   iii. **Cycles:** to utilize ac to utilize acquisition cycles, select the 'Cycles' option. This will prompt you to specify the number of times you wish to read the same plate and the time interval between the beginnings of each acquisition cycle (Cycle time).The total cycle duration is estimated based on these settings.
   RToff: Due to certain characteristics of MS Windows, conducting multiple acquisitions and processing images in real-time can lead to more frequent computer crashes. To mitigate this risk, we recommend performing acquisitions without real-time processing

(selecting "RToff") and conducting bulk processing once the computer has completed the readings.

*Latest SMARTx8 HD version also reports the current temperature

d. Plate Names:

      i.   Double-click on each station to rename it.

      ii.   Right-click on any station to open a menu where you can choose to enable or disable it for acquisition.
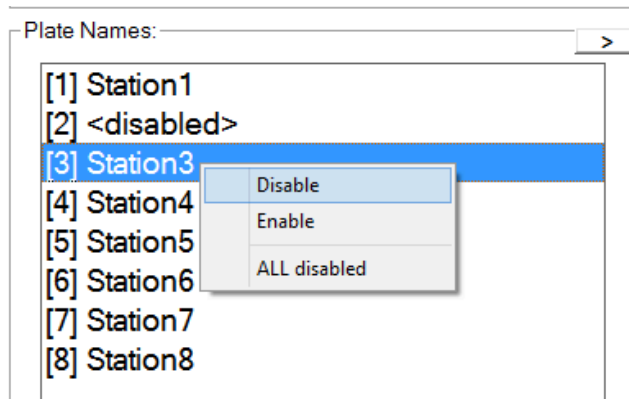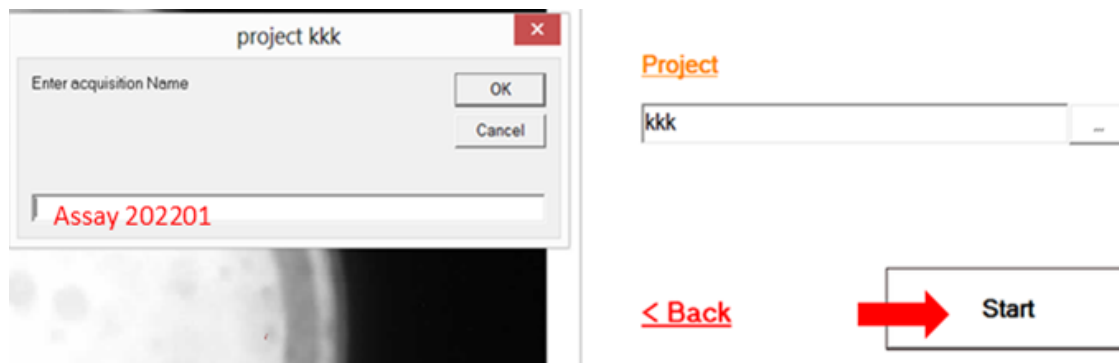
If a station is without a plate, disabling it ensures the device won't acquire data from it, moving on to the next enabled station automatically.

STARTING acquisition:

Once you have configured the acquisition settings, click on the START button to begin the acquisition process.

A pop-up window will appear asking you to enter a name for your experiment. It is recommended to choose a name that will help you identify your data in the future.

During the acquisition, the software will display the progression of the results and real-time images.

Depending on the mode you selected initially, either "TRACKING" or "MICROBEAM," the registration mode and information displayed in the report will vary.

⇨TRACKING Mode

The system will start capturing 1 image per second and processing each image in real time to detect and follow individual organisms within the petri dish.

OUTPUT Results/Report

Experiment report files can be accessed easily either immediately after an acquisition or at a later time from the check results menu.

Report file [report.csv]:
- Report file contains quantification results grouped by user defined time-block. It will first show the average population results, followed by the single particle results at the bottom:

Report File					
28/06/2022 13:38					
F:\wmicrotracker_SMART\para_validar\20220628_1317_5W					
Analysis area[mm]	35				
Time	#Particles	Average_speed[mm/s]	Travelled_Distance[mm/part]	Motility_Score	Rotation_index
5	5	0.149	38.31	0.82	6.21
Particle trails:					
#block0					
#id	distance	#frames	speed	rotation	
0	6.82	32	0.213	5.47	
1	24.73	296	0.084	7.06	
2	6.93	107	0.065	7.67	
3	13.67	71	0.193	5.49	
4	26.54	134	0.198	6.34	
6	0	263	0	2	
7	51.97	234	0.222	8.76	
8	29.9	175	0.171	7.55	
10	30.96	159	0.195	5.56	

1) **Average population results:**
   - #Particles: the average number of particles detected per frame
   - Particle speed [mm/s]: the average speed of all the detailed particles.
   - Traveled distance [mm/particle]: the sum of the distance traveled by all the detected particles divided by average number of particles detected per frame.
   - Motility score: the fraction of detection events corresponding to moving particles (distance>1mm). The range is between 0-1.
   - Rotation Index: the average of the rotation index of all the detailed particles.

   See Annex 1 for an example.

2) **Single particles results:**
   A list of results for every single particle detected for at least 10 frames.
   - ID particle: the ID number assigned by order of appearance/recognition.
   - Distance: the distance traveled by that particle.
   - #frames: the number of frames in which that particle was detected. The device acquires 1 frame per second.
   - Speed: the distance traveled by that particle divided by the number of frames (1 frame per second) in which that particle was detected.
   - Rotation: an index representing the change in rotation of animal body shapes. It is useful for lifespan experiments or animals that stay in place doing small movements, but not long trails. (Range:0-16).
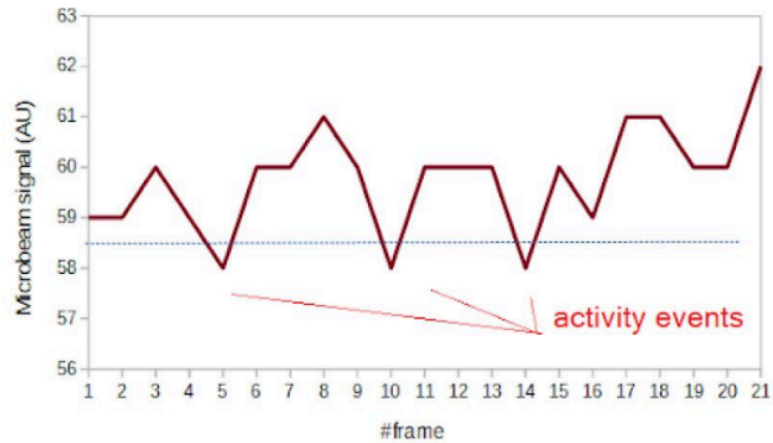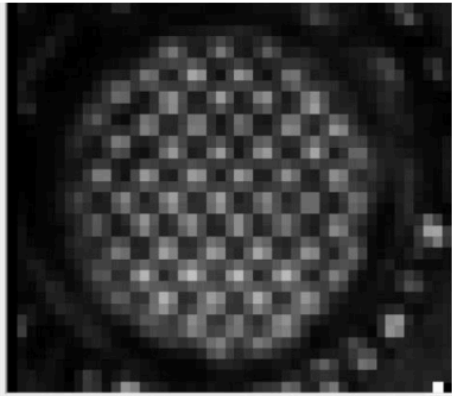
   See Annex 1 for more information.
   Additional data output recording is explained on Annex 2

IR Microbeam Ligth Scattering MODE

Example of capture of microbeams:
Activity detection for this mode is based on determining frame to frame microbeam lights fluctuation by using a pixel to pixel image subtraction algorithm.

Example of worm population activity detected in 35mm Petri dish with NGM agar:
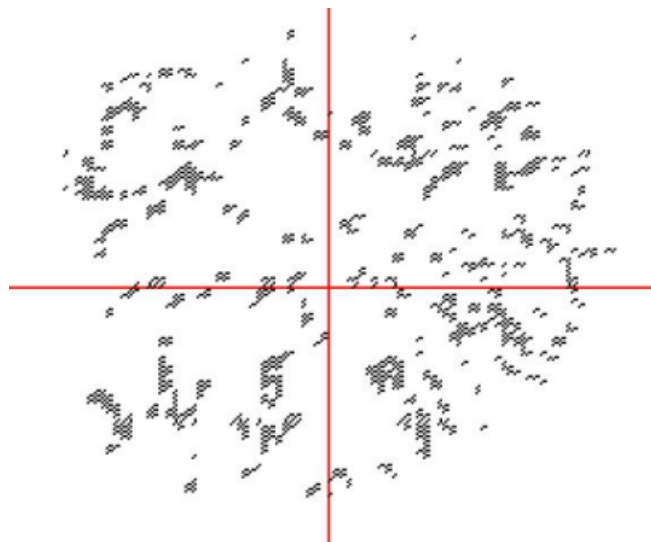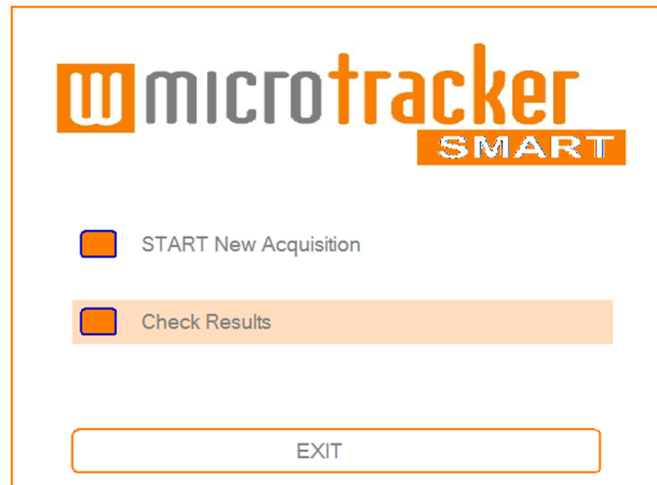
Using this method it is possible to quantify locomotor activity of worm population cultured in liquid or agar medium. This acquisition mode also provides statistical information about the location of the worm population, making it useful for chemotaxis experiments.
*for more information and application of this acquisition method data check WMicrotracker ARENA user MANUAL.

MENU <CHECK RESULTS>

To access data from previous acquisitions, navigate to the "Check Results" menu in the "Start" window. From there, you can view your results, generate reports, and even reprocess your experiment by training the software to recognize silhouettes again.

Plate Reanalysis: Screen components and Operation.
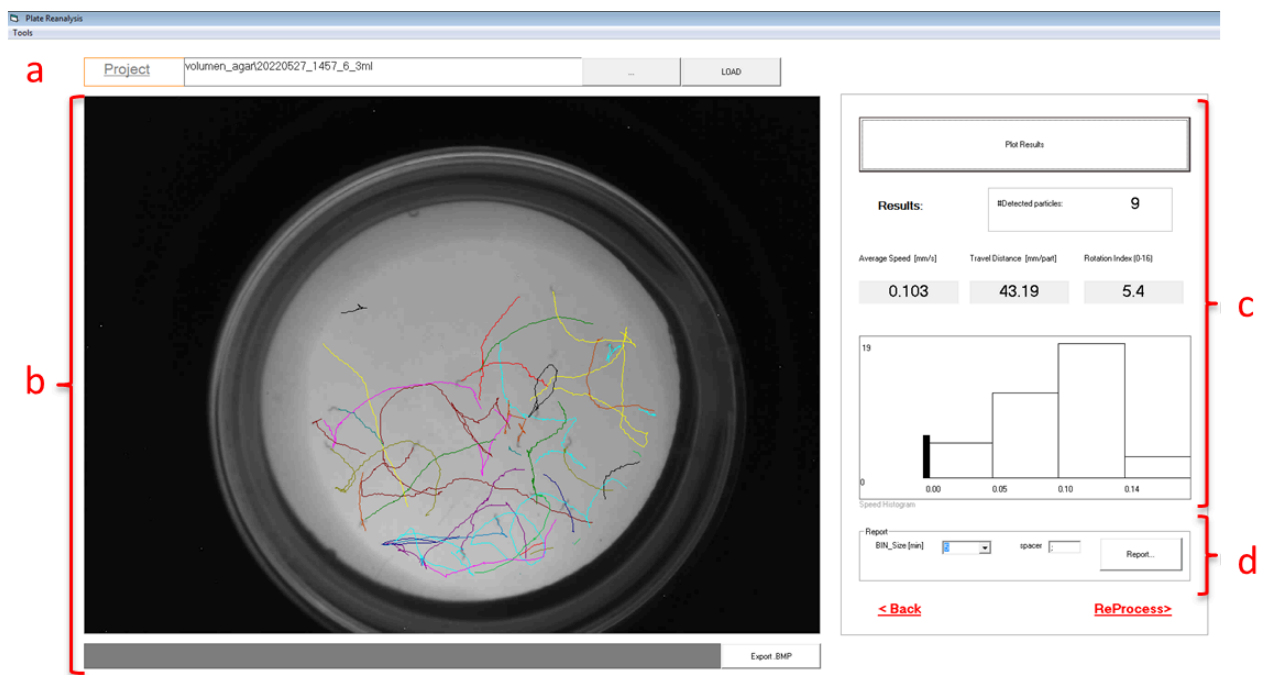
Procedure sequence:
1- Select Project & acquisition
2- Plot Results
3- Export Report
4- Re-Process data if you like to re analyze worm paths using a new training.

Button Description:
a. **Project:**
It shows the name of the project-acquisition that is loaded.
Click on the button ((…)_) to expand the directory where you can search for the experiment of interest. The experiments are organized in folders by project name, and inside each one are all the acquisitions listed by date. Select and double-click to load the desired experiment.

b. **Petri dish Image:**
Display the initial photo of the acquisition, and during "plot results" shows the path of the particles detected.
- Click on "Export BMP" to export the picture of the path of the particles as an image.

c. **Results:**
- Plot results: click on this button to load all the images of the acquisition. The paths of the particles detected according to the last saved training will appear in the plate layout area.
- Results: displays the average population results:
    #Particles detected (average number per frame)
    Particle speed [mm/s]
    Traveled distance [mm/particle]
    Rotation Index
    Speed [mm/s] Histogram

d. **Report:**
- BIN_size (min): choose the time-block for the calculation of the parameters.
- Spacer: select the best "spacer" for your data analysis needs.
- Report: Click on this button to generate a .csv file with the average population and single particle results.

e. **Reprocess:** click on this button to redirect to a window where you can change the plate position or re-train the software for silhouette detection. The menu/options are like the ones in the acquisition window. Press the "Reprocess Images" button to apply changes. The new training will overwrite the last one.

Top Menu: Tools:
- Enable ID-Marks: show the ID particle of each pathway.
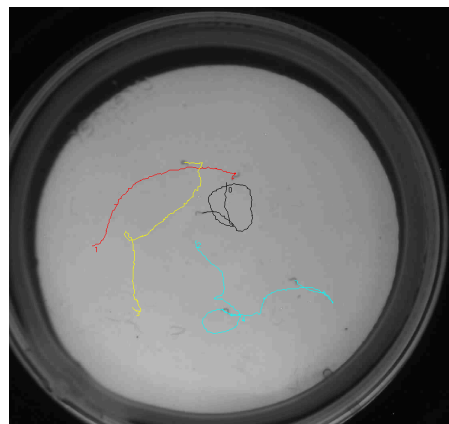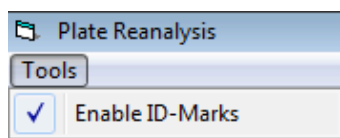
ANNEX 1

Details on report average population results:
- #Particles detected (average number per frame).
- Average speed [mm/s].
- Travelled distance [mm/particle].
- Motility score.
- Rotation Index.

Calculation of Average population results:

Report File					
28/06/2022 13:38					
F:\wmicrotracker_SMART\para_validar\20220628_1317_5W					
Analysis area[mm]	35				
Time	#Particles	Average_speed[mm/s]	Travelled_Distance[mm/part]	Motility_Score	Rotation_index
5	5	0.149	38.31	0.82	6.21
Particle trails:					
#block0					
#id	distance	#frames	speed	rotation	
0	6.82	32	0.213	5.47	
1	24.73	296	0.084	7.06	
2	6.93	107	0.065	7.67	
3	13.67	71	0.193	5.49	
4	26.54	134	0.198	6.34	
6	0	263	0	2	
7	51.97	234	0.222	8.76	
8	29.9	175	0.171	7.55	
10	30.96	159	0.195	5.56	

Travelled distance
= Σ distance / #Particles → =
191.52/5 = 38.31

Average speed
= 0.149

Rotatio index →
average rotation =
6.21

Particle trails:		
#block0		
#id	distance	#frames
0	6.82	32
1	24.73	296
2	6.93	107
3	13.67	71
4	26.54	134
6	0	263
7	51.97	234
8	29.9	175
10	30.96	159

Fraction of Detection Events per particle

0.022
0.201
0.073
0.048
0.091
0.179
0.159
0.119
0.108

→ **#Frames / Total Detection Events**
→ 32/1471 = 0.022

Total Detection Events = 1471

Motility Score
→ Σ fraction of detection events with distance >1

Calculation of Rotation index:

For each detected worm, its silhouette is converted into a binary matrix with a resolution of 4 pixels by 4 pixels, where a value of 1 indicates the presence of the organism. The software then calculates the bitwise exclusive OR (XOR) between the binary matrices of successive frames. The average of the XOR values for all worms corresponds to the reported Rotation Index, which quantifies the amount of rotational movement of the worms over time.

1
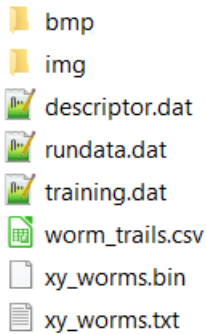
0	0	0	0
0	0	0	1
0	1	1	1
1	1	1	0

2

0	0	0	0
0	1	1	1
1	1	0	0
0	0	0	0

3

0	0	0	0
0	0	1	1
1	1	1	1
0	0	0	0

ANNEX 2

File recording description:

Additional files:
The following raw data files will be located into project acquisition folder:

📁 bmp
📁 img
📗 descriptor.dat
📗 rundata.dat
📗 training.dat
📊 worm_trails.csv
📄 xy_worms.bin
📄 xy_worms.txt
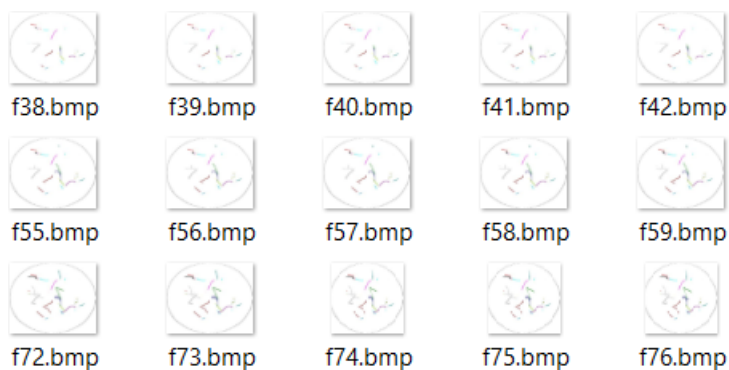
\img folder
contains all images captured during the acquisition lapse. This images can be used to generate a .GIF or .AVI video using ImageJ software

⬛ c192_168_0_201f0.jpg

⬛ c192_168_0_201f1.jpg

⬛ c192_168_0_201f2.jpg

⬛ c192_168_0_201f3.jpg

⬛ c192_168_0_201f4.jpg

Images are enumerated by System_ID + Frame Timing (in seconds).
Each image file size is about 50 to 100 kbytes

\bmp folder
it contains the exported images for incremental worm trails

| f38.bmp | f39.bmp | f40.bmp | f41.bmp | f42.bmp |

| f55.bmp | f56.bmp | f57.bmp | f58.bmp | f59.bmp |

| f72.bmp | f73.bmp | f74.bmp | f75.bmp | f76.bmp |

Worm_trails.csv
This is the data file containing worm tracks. The data can be processed by the user with his own algorithm to determine additional outputs (such as particle vector direction, distribution of particles within plate, etc). Data is structured in the following way:

Particle ID	FRAME	X	Y	#PIXELS
0	0	576	385	74
0	3	575	383	55
0	4	564	380	61
0	5	568	383	66
0	6	569	383	66
0	7	575	384	64
0	8	576	381	57
0	9	570	378	56
0	10	568	372	66
0	11	568	373	57
0	12	566	373	70
0	13	566	373	50
0	14	567	372	44
0	15	567	372	63
0	16	567	372	62
0	17	565	372	60

*Additional information includes **worm shape**

rundata.dat
It contains acquisition configuration (system_ID, folder, project date, acquisition lapse)

training.dat
It contains the parameters used by machine learning algorithms

descriptor.dat
It contains a sample of box frames used for training

xy_worms.txt and xy_worms.bin
It contains information (ascii or binary) about each particle detected on each frame. This data is used by the software to build Worm trails.

FAQ

What are the computer requirements for using the WMicrotracker SMART?

To use the WMicrotracker SMART, you will need an IBM PC compatible computer with the following minimum requirements:

- Pentium Core i3 processor or above
- 2 GB of RAM memory
- 1 available USB port t
- MS Windows 7 (or higher) operating system
- At least 1 GB of free hard drive space.

What brands of plates can I use with the WMicrotracker SMART?

The WMicrotracker SMART was designed to fit 35mm plates from Greiner Bio-One (GBO). Plates from other brands may not fit in the proper plate adapters.

Is the WMicrotracker SMART compatible with all worm strains?

The system is compatible with most worm strains, including wild nematode isolates and parasitic nematodes. Non-good swimming strains, such as unc mutants, can also be detected.

Can I measure movement in *C. elegans* larvae?

Yes, you can measure movement in populations of larvae as early as L3.

How many worms should I put in each plate to get accurate measurements?

It is recommended to place no more than 20/30 worms per plate. If the worms are too close, the software will not be able to identify each individual worm accurately.

Is it possible to measure individual worms?

Yes, it is possible to measure individual worms. We recommend using another plate with at least 5-10 worms to pre-train the software before conducting the experiment.

How long can I keep and measure my worms in the WMicrotracker SMART?

Currently, the software allows you to measure only at intervals of 5 minutes. For experiments spanning a longer time period, multiple measurements of 5 minutes can be programmed to be taken at different times.

Can I control/change the temperature of my samples with the WMicrotracker SMART?

The temperature in the WMicrotracker SMART cannot be preset and will depend on the room temperature. However, it is very compact and can easily fit in your incubator if necessary.

How do I know which plate adapter to use for my experiments?

If you're detecting worms cultured on NGM, use the petri dish with the lid downwards on the appropriate plate adapter ("lid down"). For small aquatic animals and insects larger than 1mm, use the petri dish with the lid upwards on the appropriate plate adapter ("lid up"). If you're working with organisms smaller than 1mm, use the adapter with the grid (IR Microbeam Light Scattering MODE).

Can I use NGM with a lawn of bacteria?

Yes, you can use NGM with a bacteria lawn. However, verify that your bacteria lawn is not too dense, as this can prevent recognition of the worm's silhouette.

Is it important to manually adjust the lens focus of the WMicrotracker SMART? Is it necessary to change the focus between plates?

It is essential to adjust the lens focus for your worm layer during the initial setup. If you use the same volume of media on different plates, it will not be necessary to change the focus between plates of the same experimental set.

I can't set the focus for the whole area of the plate, what should I pay attention to?

When preparing NGM plates, avoid moving them, as they can create a natural curvature (meniscus), making it difficult to focus the whole area of the plate.

What should I use to train the software for the recognition of the silhouette of the worms?

Use a plate with at least 5-10 worms, selecting worms in different areas of the plates. Avoid selecting worms that are on the edge of the plate.

Could I re-entrain after the acquisition?

Yes, you can re-entrain the software after the acquisition.

Should I stimulate the plate before measurement?

For reproducible results it is recommended to stimulate each plate just before each measurement. You could stimulate mechanically (tap-tap) or using blue light.

Where should I place the red line of the circle?

The red circle line serves as a relative caliper for distance, telling the software how much that diameter is in reality. For instance, if the red circle line is on the edge of the 35mm baseplate, you need to input into the software that the actual measurement is 35mm.
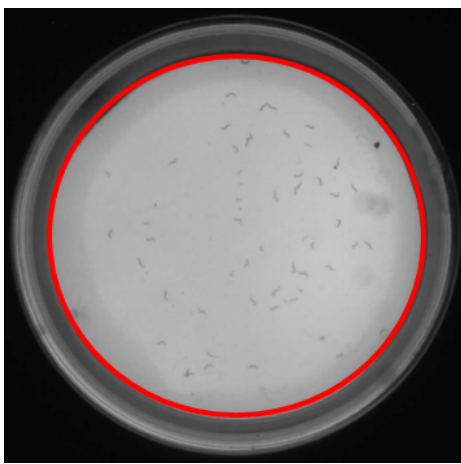
Photograph of a plate with the adapter lid down – Location of the red line.

How can I avoid worms crawling out of the agar?

For short experiments, you can use Cu as a repellent. You can either use a ring of copper or pipet 75ul of 100mM CuSO4 solution in the edge of the plate before placing the worms.

Can I change the measured intervals to be reported?

The WMicrotracker SMART is constantly detecting and recording movement. When you export your data to an excel sheet, you can change the "Bin Size" drop down menu to the desired time interval. The exported data sheet will then show you the average activity count at the intervals you specified.

I see some spots in the detection area, how can I clean them?
Particles and dust can enter the detection area. Use a microfiber cloth to clean the tray compartment.

Why do I observe in the report a list of particles (particle trials ID) higher than the number of worms I put?

When the software stops detecting a worm's silhouette for 10 seconds, that particle's path ends, and the software will assign a new particle ID to that worm when it detects it again.

For more information contact us
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