Wmicrotracker®

ɯmicrotracker
SMART

Installation Guide
and User Manual

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

Data Acquisition System: SMART
Hardware Version: SMART V 1.2
Software Version: SMART V1.1 (2022)

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

Contents

I. About the SMART

WMicrotracker SMART is a modular bug tracking system to quantify small organisms behavior in small Petri dish format (35mm Petri dish). It allows reliable quantification of animal population movement. Organism compatibility includes: C.elegans and related nematodes, zebrafish larvae, drosophila larvae, and small insects.

It has the capability to work in 2 different modes:
1- **Face-down IR Imaging:** Allows the Multi Worm Path Tracking using NGM cultures.
2- **IR Light Scattering:** allows the overall quantification of small animal movement in liquid and NGM culture using a big microbeam infrared array.
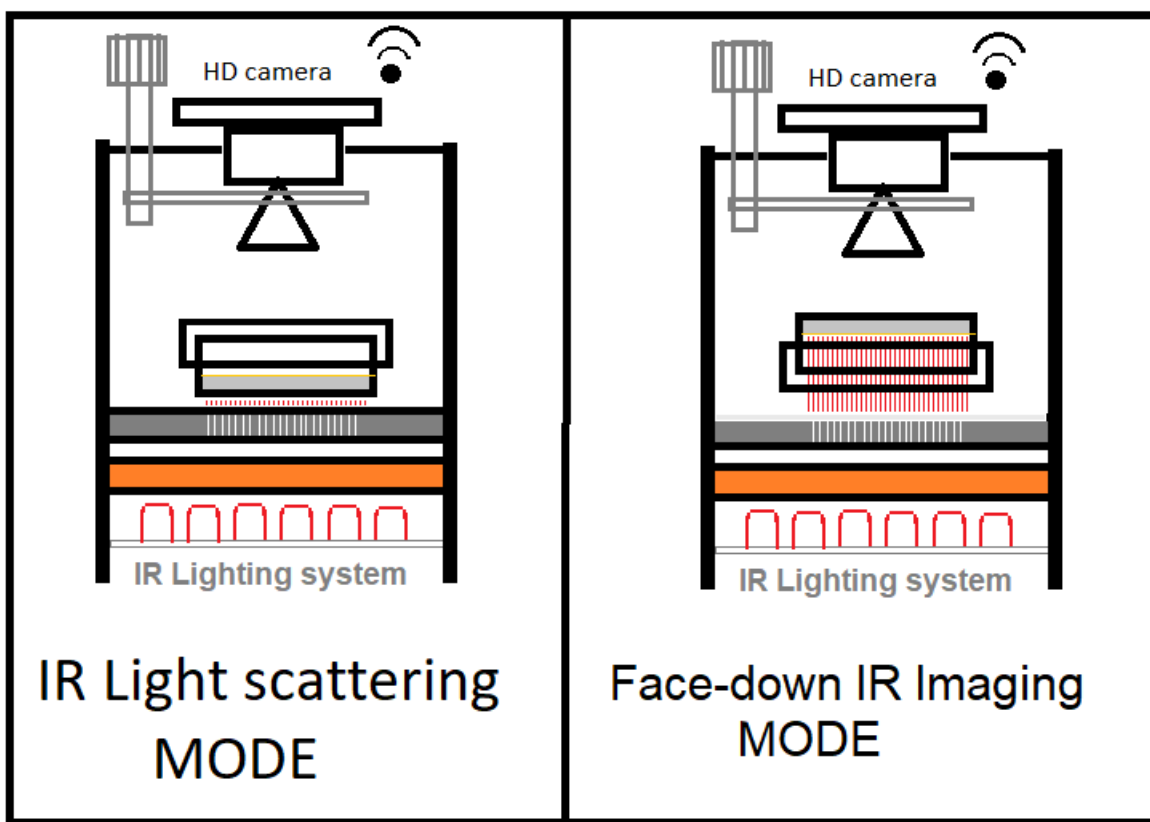
Some technical characteristics:
- non invasive acquisition
- undisturbed by bacteria.
- Compatible with RNAi and compound screenings.
Using this approach it is possible to evaluate multiple protocols using agar cultured animals, liquid culture media and air cultured insects. Preferred microplate culture format for SMART is 35 mm clear petri dishes;

Recommended:
   ● 35 mm   (Greiner Bio-One #627161)
NOTE: Petri plate must be run with the lid on. 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
	Tray petri plate p35
	WIFI Mini Router Access point configured to provide "phylumtech.com" network connection
	5V DC, 1 or 2 Amp_Power Supply MicroUSB* *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 to power WiFi access point.
  - MSWindows 7 (or higher) operating system
  - > 1Gb of free HD space for experiment images storage.
- Ambient operating temperature of 10ºC to 40ºC 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 clear window or bright light.

Product Dimensions and Manufacturing

- LWH 10cm x 10cm x 22cm (3.94in x 3.94in x 8.66in).
- Manufacturing Technology: 3D Printing

II. Data output Examples: Path Tracking mode

C.elegans Levamisole curve [Toxicity/Anthelmintics research]

WMicrotracker SMART is able to get and plot the path tracking of a population of worms, calculating average movement speed in real time. A kinetics plot comparing levamisole concentration treatment (0 to 200uM) is shown below. The plots show the correlation between levamisole concentration and movement speed decrease.
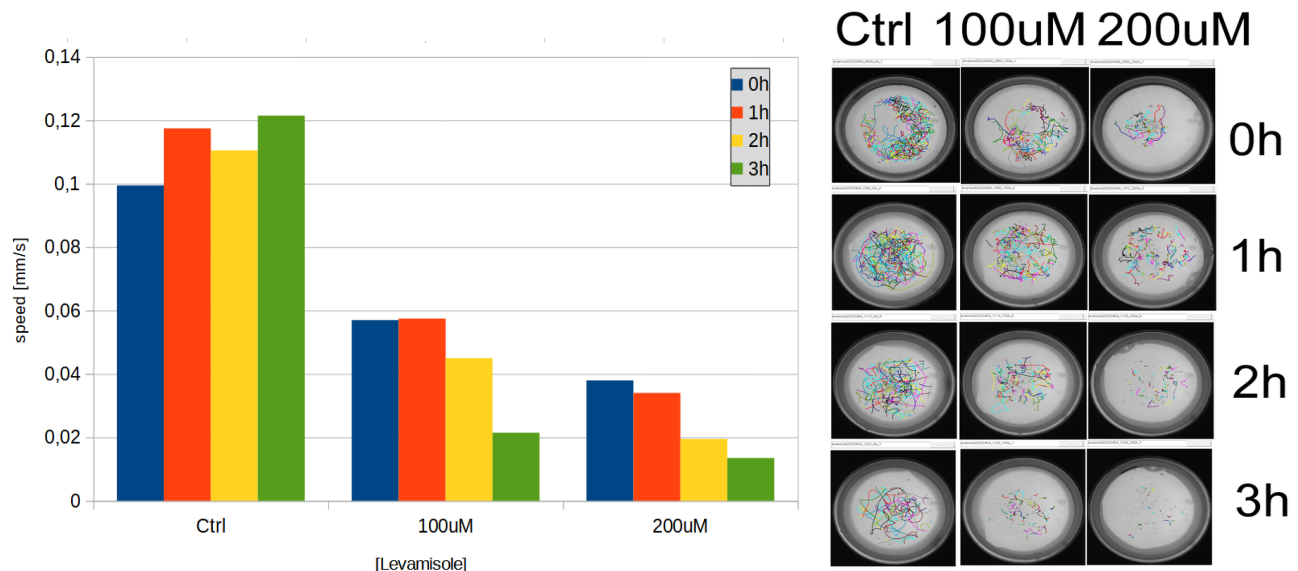
Using a 5 minute acquisition lapse every hour, the WMicrotracker SMART is able to quantify the effect of levamisole paralysis on C.elegans worms.
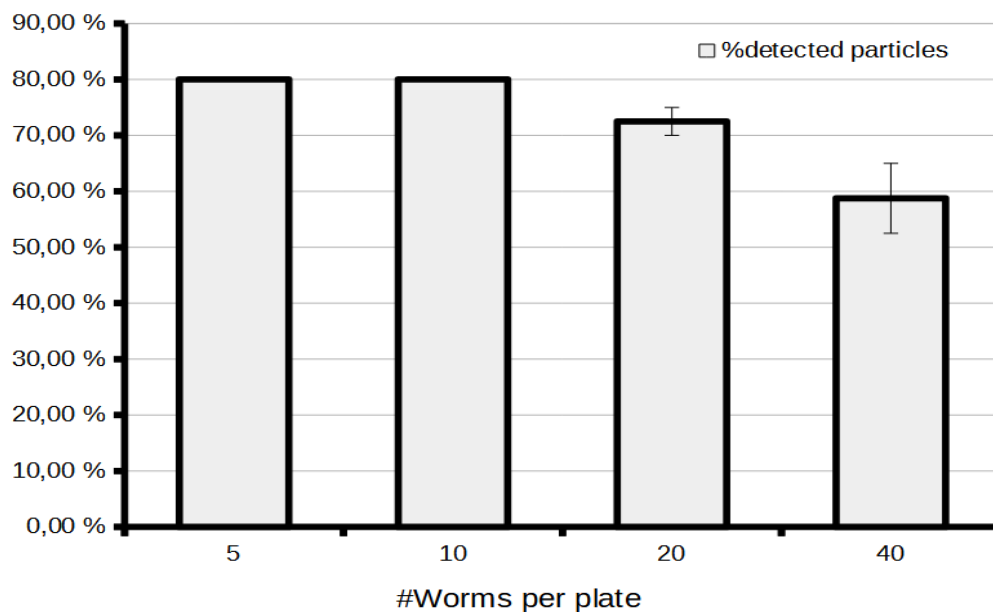
Protocol:
1. Grow synchronized populations of adult day-1 worms in seeding NGM plates (OP50).
2. Remove worms from plates using M9 buffer and transfer them in a sterile 2ml micro tube.
3. Let the worms settle. Decant the supernatant taking care not to disturb the pellet.
4. Perform a wash with 2 ml of M9 buffer. Briefly shake or invert the tube
5. Repeat the decantation step. Throw out 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 to get [25 worms/10 µl]
9. Transfer 10 µl of worm solution to a 35mm plate. Wait until the drop is absorbed.
10. 5 minutes later, register worm activity using WMicrotracker SMART during 5 minutes. Immediately before the acquisition, stimulate the worms by subjecting the plate to mechanical stimulus (tap 3x).

Microplate preparation:
- NGM was 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 assay is performed on plates that had been allowed to template to room temperature prior to 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]

As shown below, the software is able to automatically recognize and follow up to 80% of the particles.

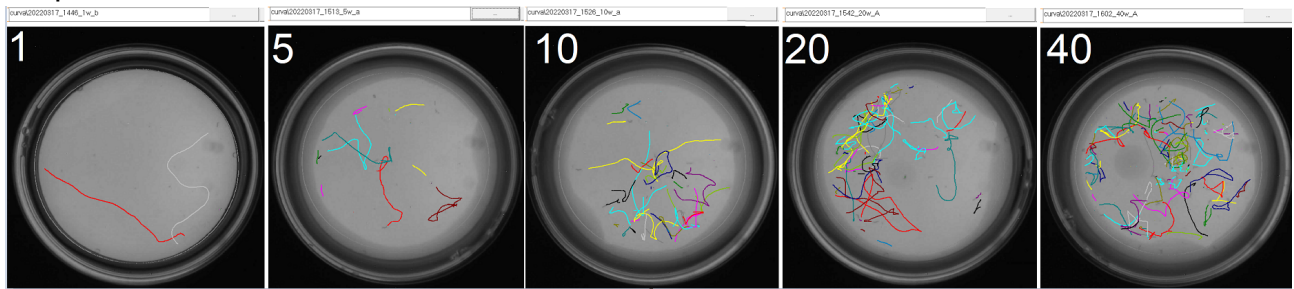| Experiments can be performed using 10 to 30 worms per 35mm Petri dish. |
|---|

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 in a sterile 2ml micro tube.
3. Let the worms settle. Decant the supernatant taking care not to disturb the pellet.
4. Perform a wash with 2 ml of M9 buffer. Briefly shake or invert the tube
5. Repeat the decantation step. Throw out 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 one plate extra with more than 10 worms to train the software. (See Manual Page 12 -   1.d. Silhouette detection).
9. Transfer to 35mm NGM plates without food the different number of worms: 1/5/10/20/40. Check at the magnifying glass the number of worms transferred. Wait until the drop is absorbed.
10. 5 minutes later, register worm activity using WMicrotracker SMART during 5 minutes. Immediately before the acquisition, stimulate the worms by subjecting the plate to mechanical stimulus (tap x3).

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

<u>Software Installation</u>

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_smart folder to c:\wmicrotracker_smart folder and follow the instructions:
   a. Plug the USB-Wifi communication device. It will create a WiFi Network called "phylumtech.com"
   b. Connect your computer to the WiFi network "phylumtech.com" using the password "WMicroSMART"
   c. Run the WMicrotracker_smart_vXX.exe executable file
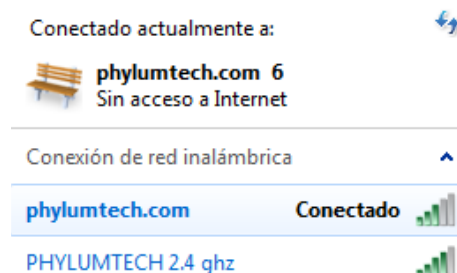
Additional comments:
- Because the WMicrotracker SMART uses a direct WiFi communication protocol, unless you have a wired LAN, the internet will not be available on your PC when you connect to the device.

Hardware Turn on and Setup

1. Plug the USB-Wifi communication device
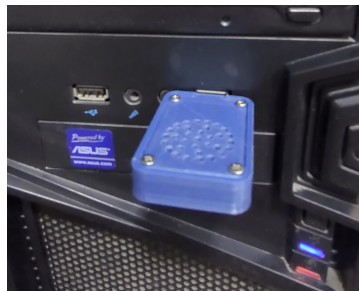
2. Connect your computer to the WiFi network "phylumtech.com" using the password "WMicroSMART".

3. Plug in the power supply (5VDC Switching Power Supply with 1 Amp output) to any regular Power Outlet and the microUSB cable to the back of your SMART. The display will turn ON.

4. The display will turn ON and will show the network configured password while trying to connect to the "phylumtech.com" WiFi network (wait a few seconds).

If after 1 minute the equipment fails to connect, check "phylumtech.com" network availability, and restart the equipment by unplugging it for a few seconds.

5. After successful connection, the display will show the assigned IP (identification number) in the Wi-Fi network. This number is used to link the acquisition software to the device.
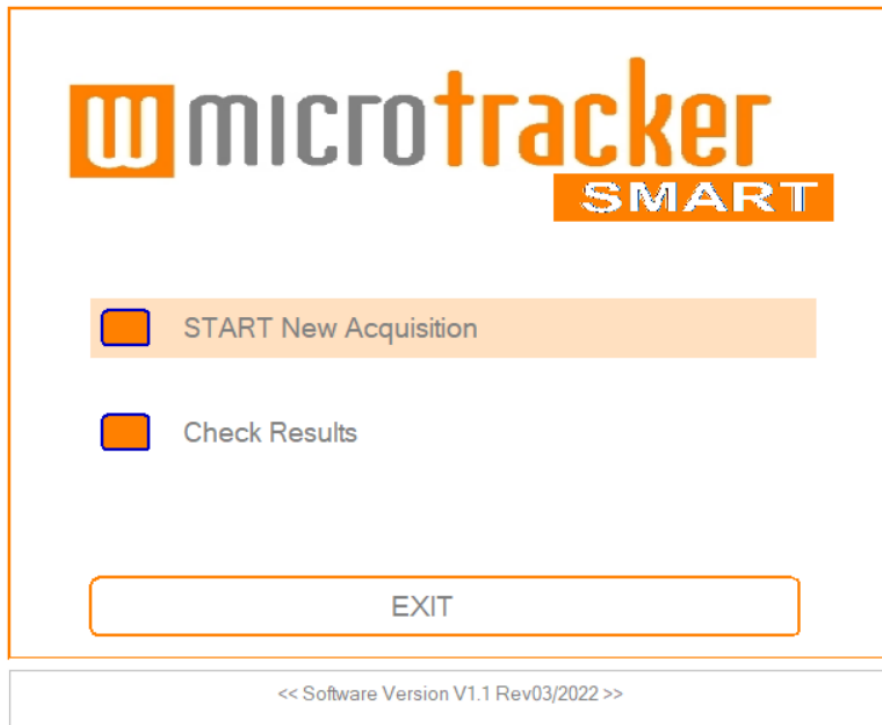

Note: This random number is automatically assigned by the Access Point (WiFi router) at connection time.

IV. Acquisition Software

Software Launch

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

2.   Press Start New acquisition to connect your SMART. The following window will appear:

3.   Complete the Device ID with the IP indicated on the display. For example;

4.   Press the Connect button. If the connection succeeds, you will enter the data acquisition Window

Data acquisition Window: Screen Components and Operation

1. Plate <u>Setup</u> & training is shown when you configure the IP device in the software.

a. Plate Layout:

i.　　Petri plate Box:

-Currently the WMTK SMART has been validated to work with 35 mm format.

The picture is a photo of the plate during the last connection with the device. While setting the focus of the device you will be able to see the plate in real time.

b. MODE Menu:

i.a.　"Tracking Mode" *for C.elegans and similar nematodes cultured on agar:*

- In this mode, the system exploits 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 bigger 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:
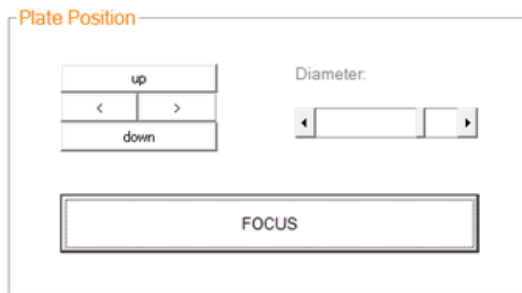
i.   The arrows allow you to move the position of the registration area (red circle) of your petri dish. "Up", "Down", ">" Right, "<" Left.

ii.   "Diameter": Allows you to enlarge or reduce 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.   Focus: Allows you to acquire real time images in order to adjust the lens focus using the focus wheel on the back of the SMART. The time to adjust the focus is set to 20 seconds. If it is not achieved in that time, you can press it again.
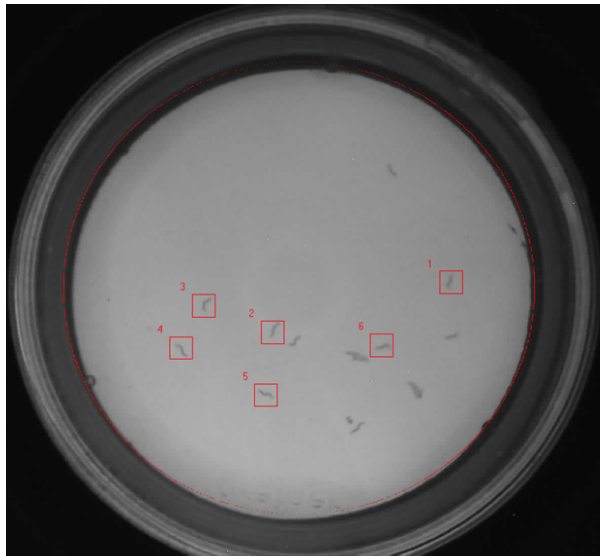
**d. Silhouette detection (only enabled for tracking mode):**
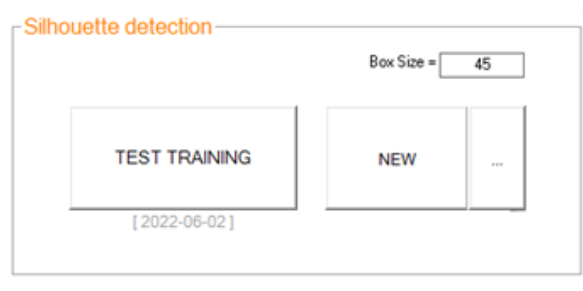
i. "New" button: Allows the software to be trained to recognize silhouettes.To select silhouettes of organisms in the image, just click with the left mouse button on the particle (click 5 to 10 representative organisms). For example;


Box size: increase or decrease the size of the box so that the whole microorganism fits inside. The reference box size for C.elegans is 35 to 45 pixels according to the worm stage.

Then press the "APPLY" button and the shadows of the animals will turn red
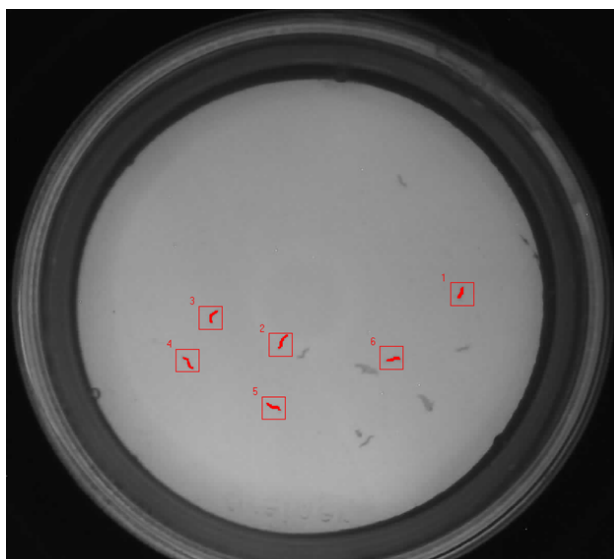
Finally, press "OK" to save the training parameters

ii. "Test training" Allows to check the performance of recorded silhouette recognition patterns.
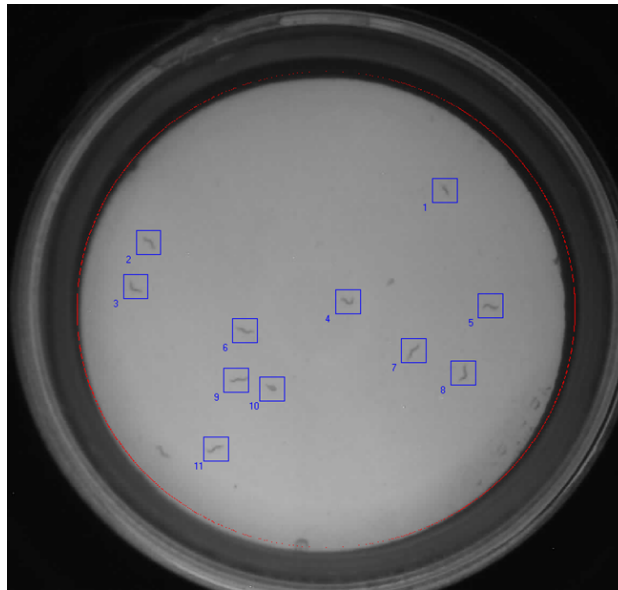
2. Image acquisition screen is shown when you select the "Next>" button on the First screen Setup.

a. Status Bar:
    i.   "Status Bar":

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

b. Data Acquisition:
    i.   "Acquisition" Lapse time:
- Set the total run time for the petri plate reading, in minutes
- Current software version allows 5 minute lapse time by default.

c. Project folder Menu:
    i.   You can create and name a new project folder by typing in the box.
    ii.   You can call a previous project name if you display the box.

    Project folder will be saved inside your software installation folder.

STARTING acquisition:

After configuring the run, press the START button to initiate acquisition.
A pop-up window will appear to enter the acquisition name. Write a name for your experiment in order to recognize future data recalls.
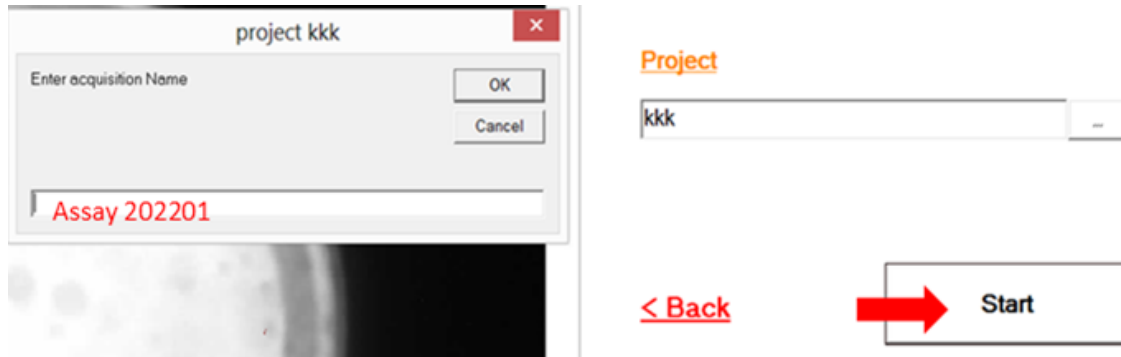

The window will show the results progression, and real time image acquisition.

Depending on the mode initially selected "TRACKING" or "MICROBEAM", the registration mode and the information displayed in the report will be different. Below each of the modes will be detailed individually:

⇨TRACKING Mode

The system will start capturing 1 image per second, processing each image in real time to detect and follow single animals within the plate.
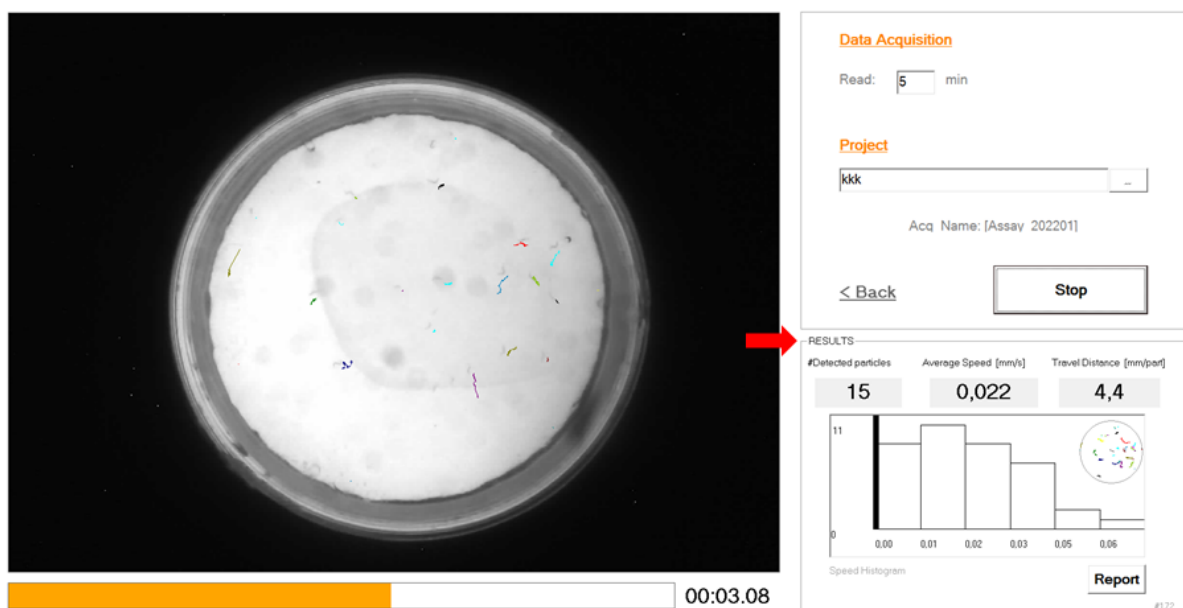
OUTPUT Results/Report

Report file [report.csv]:
- Report file contains quantification results grouped by user defined time-block:

Average population results:
#Particles detected (average number per frame)
Particle speed [mm/s]
Traveled distance [mm/particle]
Rotation Index -> 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.

Single particles results:
A list of results for every single detected particle.

report20220507_1426

Report File
2022/05/07 14:26:20
C:\wmicrotracker_smart\levamisol\20220404_0930_100A_2

Time	#Worms	Average_speed[mm/s]	Travelled_Distance[mm/worm]	Rotation_index
5	36	0,459	33,867	6,1

Particle trails:

| ID_particle	distance	#frames	speed	rotation
0	0,97	29	0,033	5,83
1	1,01	14	0,072	8,21
2	0,86	14	0,061	7,57
3	17,25	197	0,088	6,76
4	4,26	43	0,099	6,51
5	0	9	0	0
6	0	9	0	0
7	4,58	49	0,094	7,53
8	8,51	73	0,117	7,16
9	8,97	71	0,126	7,49
10	1,66	17	0,098	6,12
11	9,85	116	0,085	7,16
12	10,15	97	0,105	8,19

- **Additional data output recording is explained on Annex I**

ANNEX I

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 100kbytes

\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.
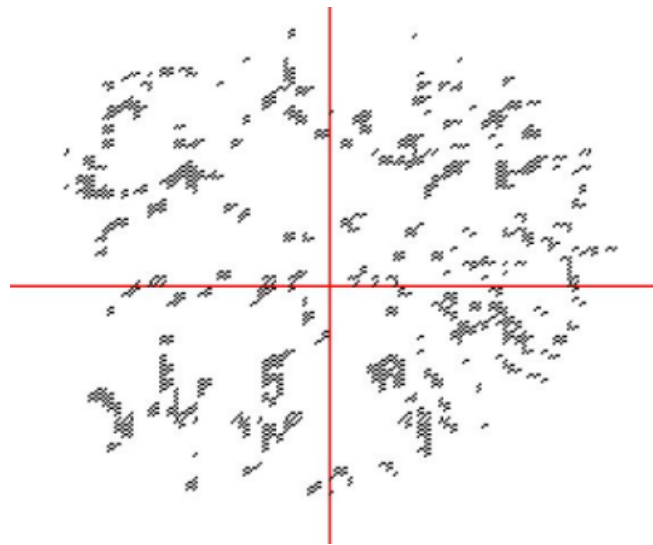
IR Microbeam Ligth Scattering MODE

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.
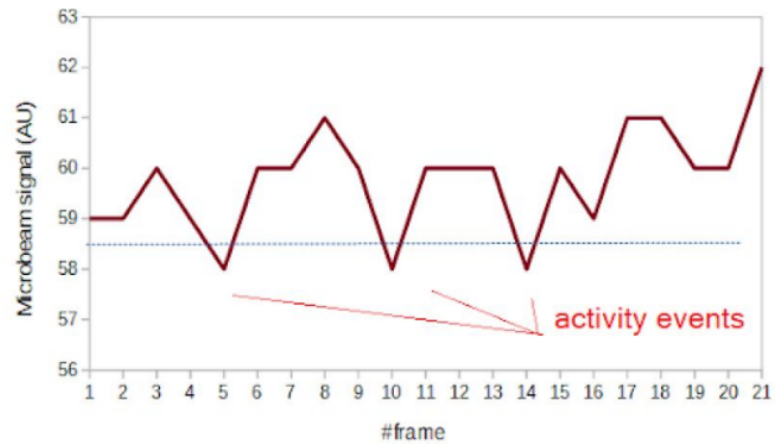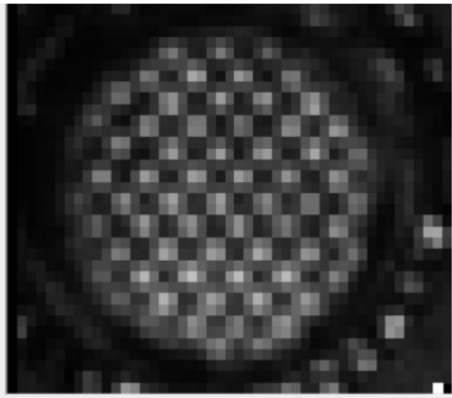
Example of worm population activity detected in 35mm Petri dish with NGM agar:

Using this method is possible to quantify locomotor activity of worm population cultured in liquid or agar medium, and get statistical information of worm population location (useful for chemotaxis experiments)

*for more information and application of this acquisition method data check WMicrotracker ARENA user MANUAL.

For more information contact us
info@phylumtech.com