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 V1.8
Software Version: SMART V3.3 (2026)

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

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This product is for research purposes only and is not intended for human diagnostic use. Made in Argentina.

Contents

I. Introduction & Overview

1.1 About the WMicrotracker SMARTx8 HD

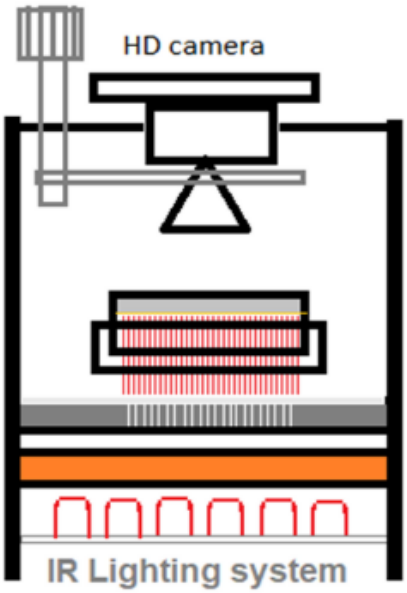
The WMicrotracker SMARTx8 HD is a modular system designed for tracking small organisms in a 35mm Petri dish format. It enables reliable quantification of animal population movement and is compatible with a variety of organisms, including *C. elegans* and related nematodes, zebrafish and Drosophila larvae, and small insects.

Some technical characteristics of the system:

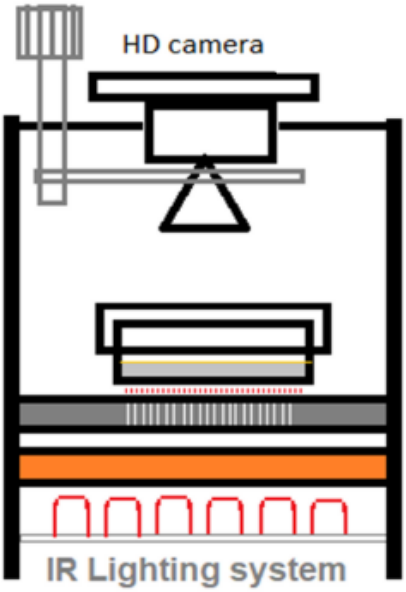
- Non-invasive acquisition
- Undisturbed by bacteria (although very dense or dark bacterial cultures may affect the accuracy of the system).
- Compatible with RNAi and compound screenings.
- Enables the evaluation of multiple protocols using agar-cultured animals, liquid culture media, and air-cultured insects.
- The preferred microplate culture format for the WMicrotracker SMARTx8 HD system is 35 mm clear petri dishes. It is **recommended to use the** 35mm-Greiner Bio-One #627161.
  NOTE: The Petri dish must be run with its lid on, and it is recommended to seal the plate with parafilm.

1.2. Principles of Operation: Dual Acquisition Modes

It has the capability to work in 2 different modes:



Face-down IR Imaging MODE



IR Light scattering MODE

. Multi-Worm Path Tracking (Machine Learning Mode)

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.

. IR Microbeam Light Scattering Mode

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.

1.3. Included System 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

1.4. System Requirements & Laboratory Conditions

1.4.1. PC Recommended Specifications

- BM PC compatible with the following minimum requirements:
- Pentium Core i7 processor or above.
- 1 USB port available.
- MSWindows 10 operating system.
- > 1Gb of free HD space for experiment images storage.

1.4.2. Ambient Operating Conditions

- 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.

1.5. Hardware Dimensions and Manufacturing Specifications

- LWH 21cm x 28cm x 17cm (8.66in x 9in x 8.66in).
- Manufacturing Technology: 3D Printing

II. Installation and Initial Setup

2.1. Software Installation Guidelines.

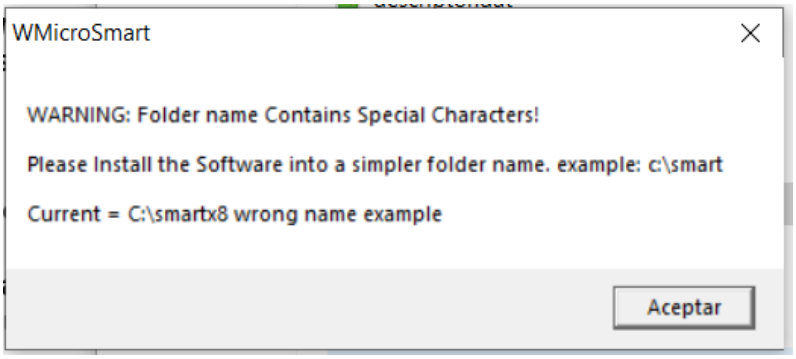
We recommend periodically checking the Phylumtech website for software updates.

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.

Known Troubleshooting Issues:

1. Incorrect Installation Folder Path.

If the software is installed in a folder path that contains special characters (e.g., #, $, @, ñ), an error message will appear upon startup.



While the software will still open, you will not be able to process data or acquire new experiments. To fix this, reinstall or move the software to a directory using only standard alphanumeric characters (e.g., C:\wmicrotracker_smartx8HD).

2. Antivirus or Firewall Blocks

If your antivirus or security software prevents the executable from running, the application will fail to open or process data properly. If you encounter this issue, please ask your IT administrator to manually add an antivirus exception for the software's executable (.exe) files.

2.2. Hardware Connection & Power Sequence.

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 side of your WMicrotracker SMARTx8 HD device.

2. Connect the USB Cable: Connect the USB cable from your computer's USB port to the USB port on the WMicrotracker SMARTx8 HD device. The system will automatically detect the device on COM ports 1 through 15.

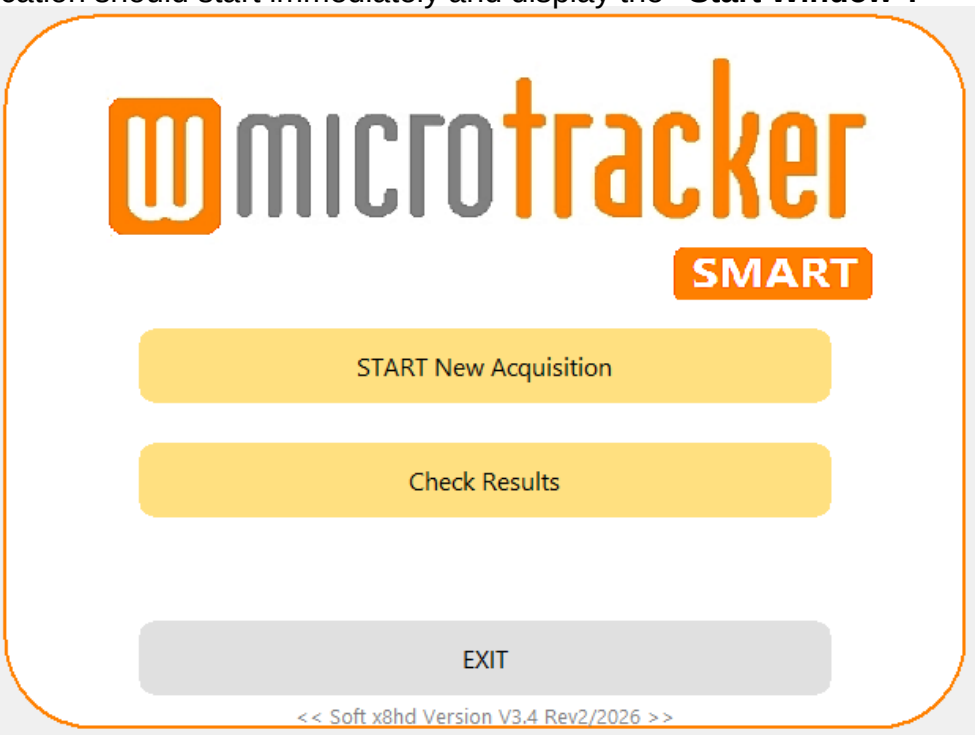
a. Note: You can verify the connection in the Devices and Printers window by checking for a newly assigned COM port.
b. Windows 10 is recommended. Windows 7 is also supported, but requires the latest updates to install the driver properly.

3. Power On the Device: Press the button located on the side of the WMicrotracker SMARTx8 HD 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*.

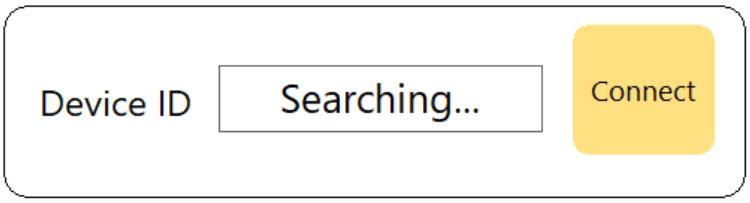
4. Shutdown: **To shut down the device, please use the software controls; otherwise, the memory may be damaged**.The device will automatically shut down after 30 minutes of inactivity. To restart the device, press the button on the side again.

2.3. Software Launch & Initial Connection.

Open the folder where you installed the software and run the **"WMicroSmart"** executable file. The application should start immediately and display the **"Start Window".**



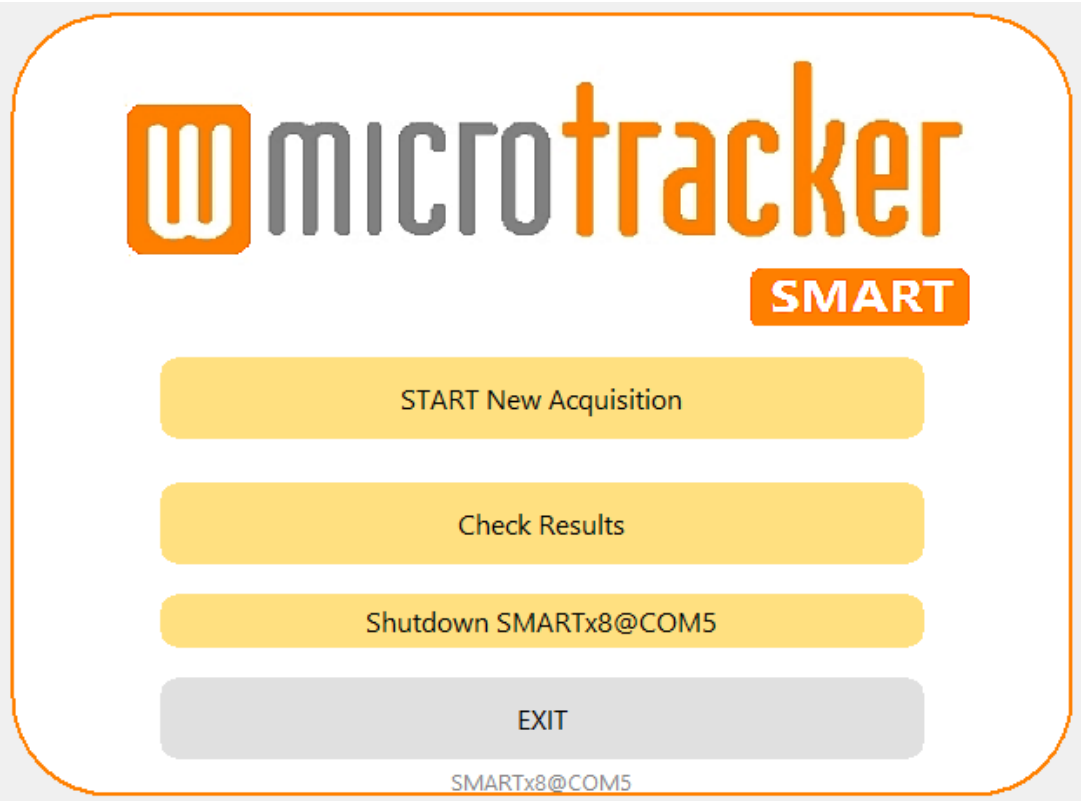
Click on "Start New Acquisition" to connect to your WMicrotracker SMARTx8 HD device. A new window will appear while the software searches for the connected device.



If the device is found on one of the COM ports, please click the Connect button. You will be redirected to the **Data Acquisition Window**.

2.4. Critical Equipment Shutdown Procedure.

After running your experiment, return to the **Start Window**. A new option will now appear to shut down the device.



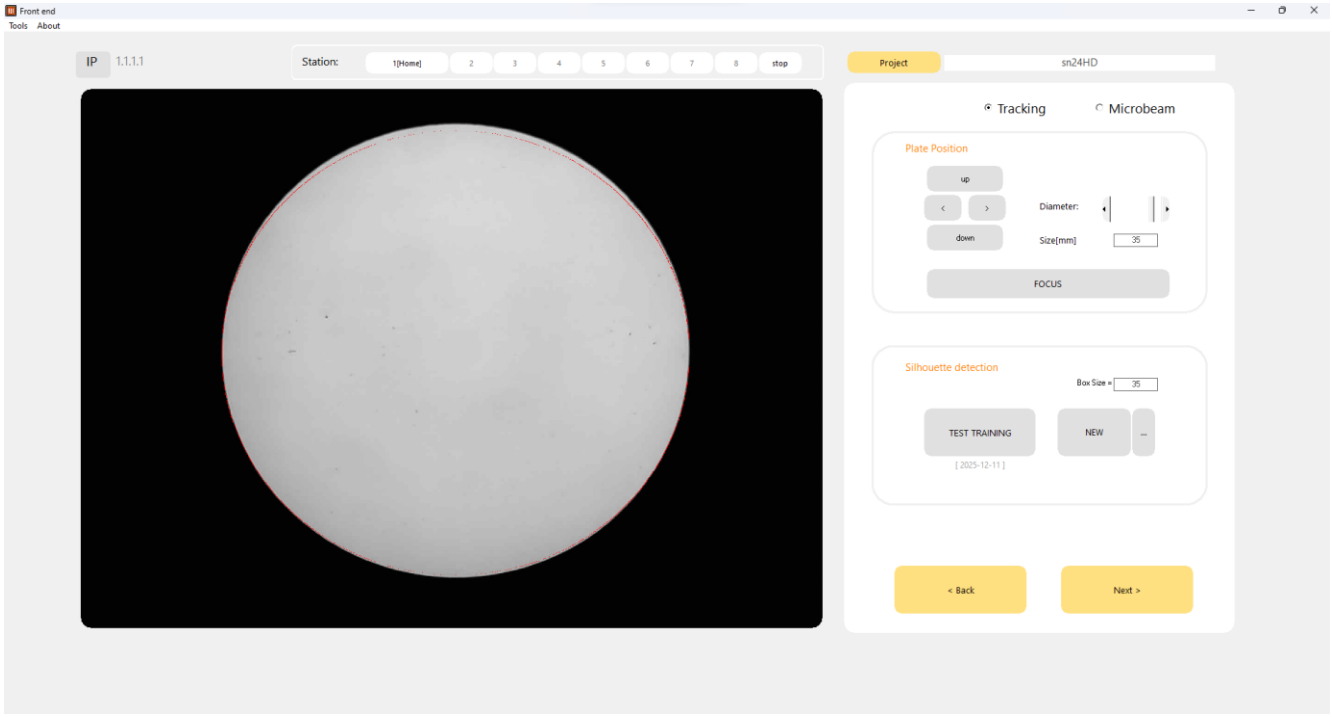
Click the "Shutdown SMARTx8@COM" button. The system will take approximately 20 seconds to turn off completely.

Automatic Timeout: The device will automatically shut down after 30 minutes of inactivity. To initialize the device again, press the physical button on the side and reconnect via the software.

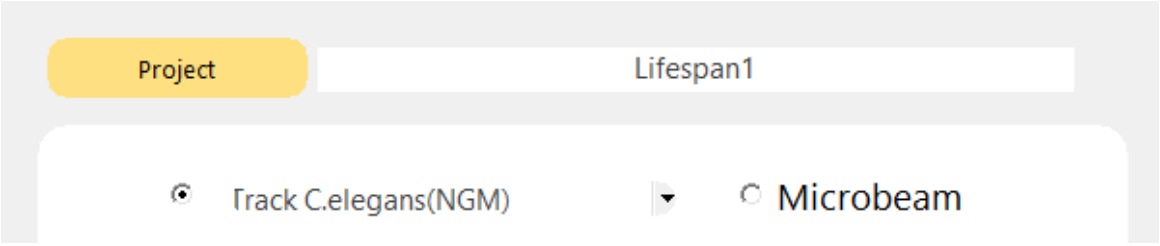
 WARNING: Because the WMicrotracker SMARTx8 HD utilizes an internal Raspberry Pi microcomputer, it **must** be powered off correctly via the software to avoid memory corruption over time.

III. Preparing and Running an Experiment.

Once connected, the Data Acquisition Window will open. Follow the steps below to navigate the screen components and configure your experiment.



3.1 Project Setup & Mode Selection



Project Creation and Management

Use the Project settings to organize your experimental data:

- Create and name a new project folder by entering a unique name directly into the project text field.
- Access or browse previously created projects by clicking the Project button.

Note: The software will automatically generate the project folder inside your main software installation directory. (See Appendix I)

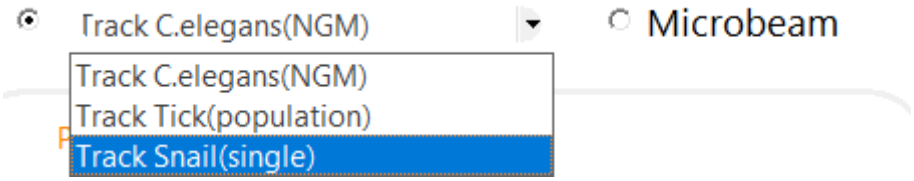
Selecting the Acquisition Mode (Tracking Mode vs. Microbeam Mode)

The system features two primary acquisition modes tailored to different organism sizes, culture media, and experimental designs:

A) Tracking Mode

This mode uses high-resolution imaging combined with machine learning algorithms to identify individual organisms and map their exact trajectories.

Organism *Selection*: The interface features a drop-down menu to select the specific type of organism you wish to track. Based on your selection, the software automatically optimizes and adjusts its detection algorithm parameters.



Depending on your sample type, configure the plate orientation and setup as follows:

- *For C. elegans and similar agar-cultured nematodes:*

The Petri dish must be placed face down (lid down) using the appropriate plate adapter. This setting is specifically compatible with adult-stage C. elegans and similarly sized worms. The system utilizes the optical property of light phase transition (air-worm-agar) to amplify and magnify worm silhouettes when exposed to infrared light. A similar phenomenon has been described in FIM applications. (A Multi-Purpose Worm Tracker Based on FIM | bioRxiv).

- *For small aquatic animals and insects:*

The Petri dish must be placed face up (lid up) using the designated plate adapter for the detection of organisms larger than 1 mm.

B) Microbeam Mode

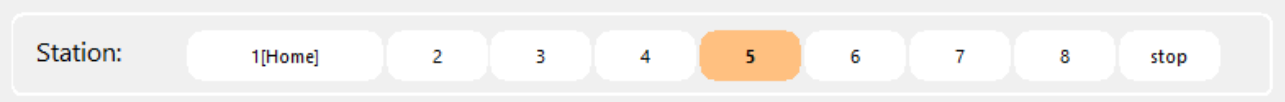
This mode is designed for bulk population locomotor activity quantification without individual particle tracking. It operates using low-resolution video images to calculate overall movement. The system utilizes a specialized plate adapter equipped with a grid of more than 2,000 microholes (100um wide). Activity detection is calculated via a pixel-to-pixel image subtraction algorithm that monitors frame-to-frame fluctuations in light. If the difference between neighboring pixels exceeds a predefined threshold, the system increments an activity accumulator.

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

3.2 Plate Loading, Alignment and Optical Calibration

Plate Loading & Carousel Station Navigation

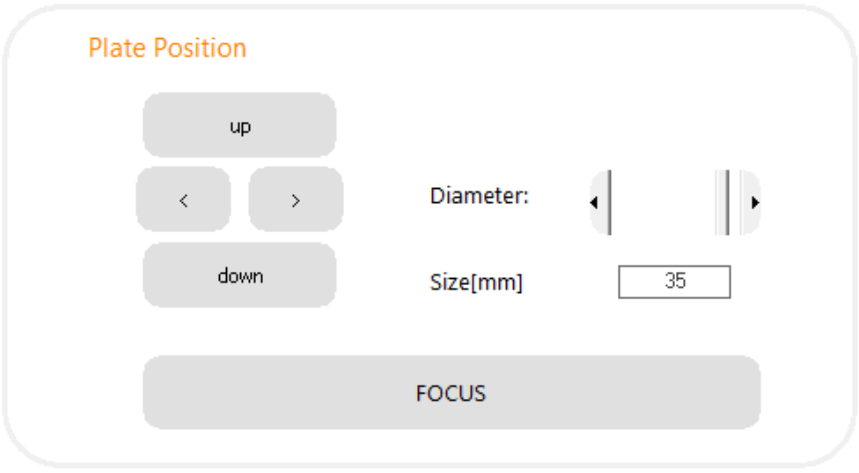
The WMicrotracker SMARTx8 HD is currently validated to work with a 35 mm plate format.



Select your desired plate position by clicking on the corresponding station number (1 through 8) in the software. The device will automatically rotate and align that specific station directly in front of the camera. If you need to manually rotate the carousel by hand, you must click the "Stop" button first to release the motor and allow free movement.

The image displayed in the software panel shows a photo of the plate captured during the last active connection with the device. You can view this plate image in real-time while performing focus adjustments.

Plate Position Alignment and Focus



Position Alignment (Arrow Controls): Use the Up, Down, Right (>), and Left (<) arrows to adjust the position of the red registration circle. Move the circle until it aligns perfectly with the boundaries of your Petri dish.

Diameter: Use this control to increase or decrease the size of the red registration circle. It is critical to align this red circle exactly with the outer edge of the 35 mm base dish.

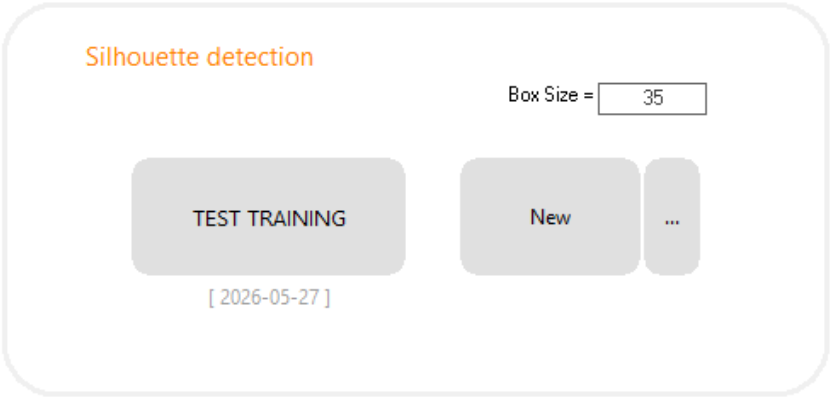
Size (mm): This option allows you to specify the actual physical diameter of the red circle in millimeters (typically 35). The software uses this value as a scale indicator to accurately calculate distance and speed measurements.

Lens Focusing: Click the "**Focus**" button to stream real-time images. While the stream is active, manually adjust the lens focus using the physical focus wheel located at the front of the WMicrotracker SMARTx8 HD device.

The real-time focus stream is programmed to run for 30 seconds. If you need more time to achieve a sharp focus, simply click the "Focus" button again to restart the stream.

3.3 Silhouette Recognition Training (Tracking mode only):

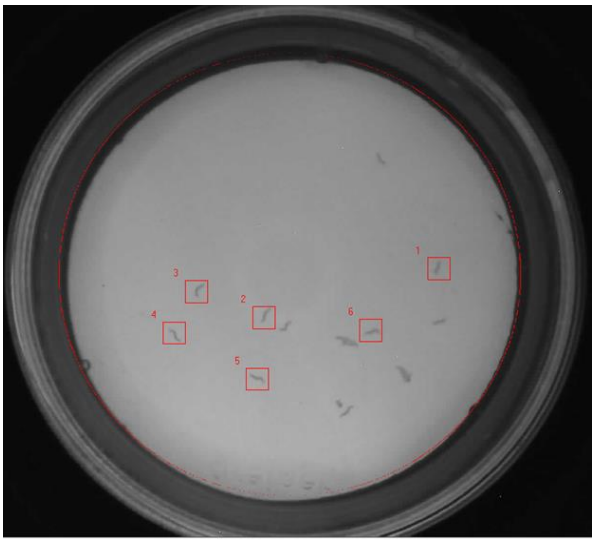
This training step calibrates the machine learning algorithm to recognize your specific organisms.



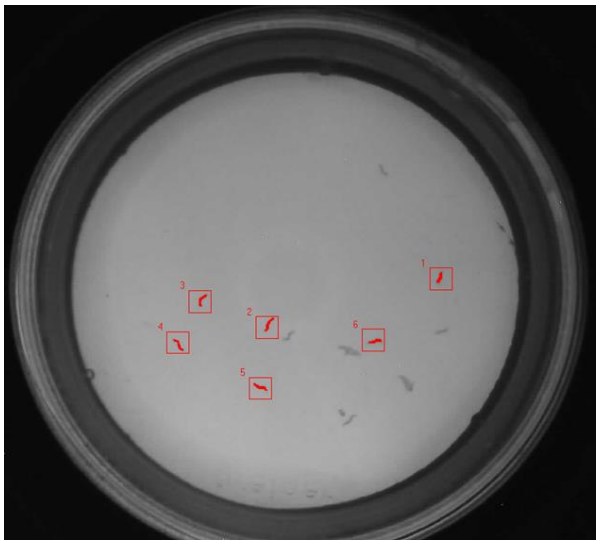
Box Size: Increase or decrease the bounding box size so that the entire microorganism fits completely inside it. The standard box size for C. elegans ranges between 35 to 45 pixels, depending on the developmental stage of the worms.

Training New Silhouettes ("New" Button): Click the "New" button to initialize the training mode.

1. In the plate image, left-click directly on individual organisms to select them. It is recommended to select 5 to 10 representative organisms scattered across different areas of the plate.



2. Click the "Apply" button. The detected silhouettes of the selected animals will turn red to confirm recognition.

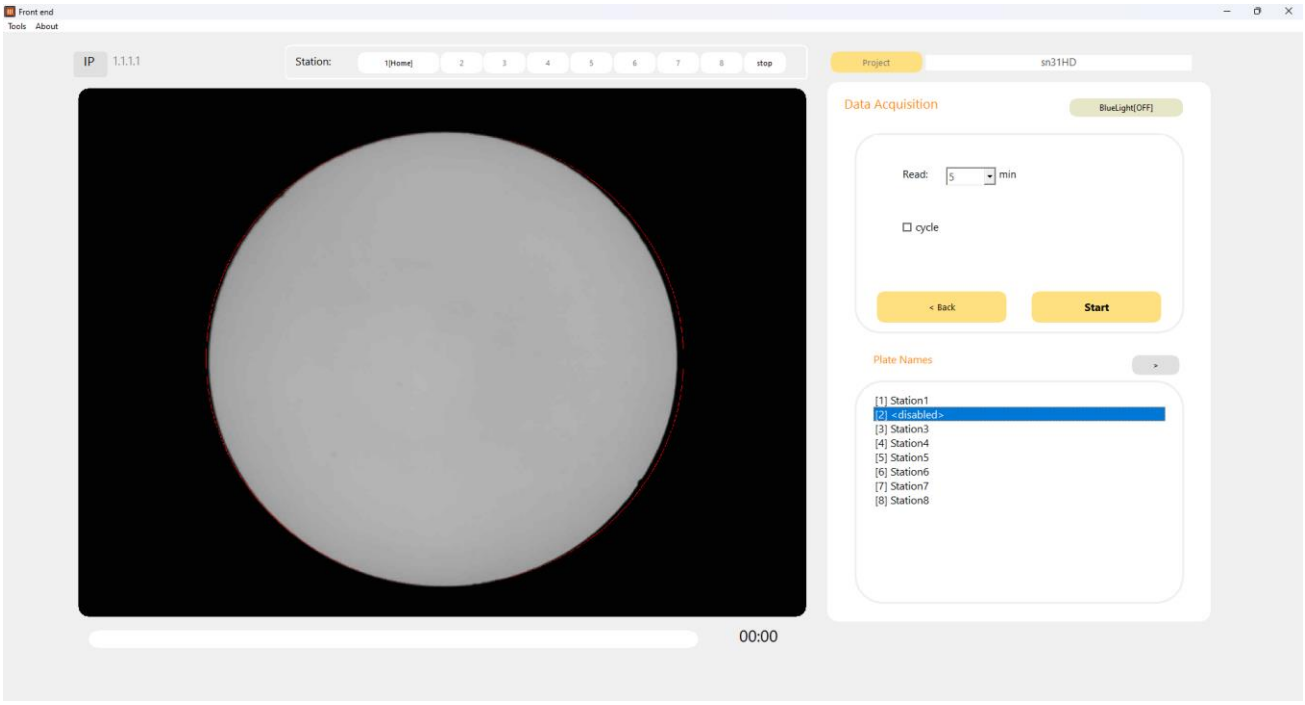


3. Click "OK" to save these training parameters into the system memory.

Click the "Test Training" button to evaluate the accuracy of your newly recorded silhouette recognition pattern. The software will display blue bounding boxes around all particles it successfully recognizes based on your training.

3.4 Run Parameters Configuration

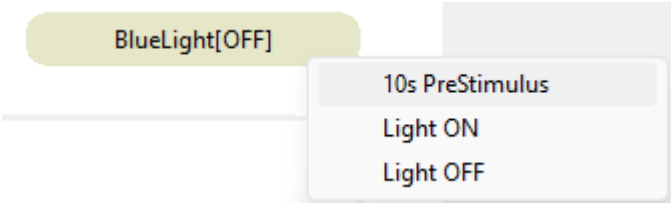
The Image Acquisition screen is automatically displayed once you select the “Next>” button on the initial setup screen.



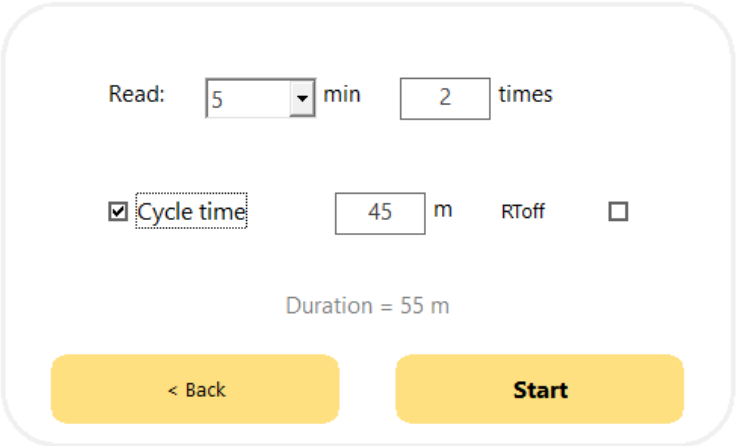
Project Verification: Before starting your acquisition, you still have the option to modify your active project folder or create a new one in the Project text field if needed.

Data Acquisition Settings

- Read (Acquisition Lapse Time): Set the total runtime duration for each individual Petri dish reading between 1 to 5 minutes. The software automatically defaults to a 5-minute lapse time.
- Blue Light & Stimulus Control: Customize the integrated blue light illumination settings prior to starting your data collection:



  - *Light ON:* Select this option to acquire data under active blue light illumination. When enabled, the blue light will automatically turn on at the start of the acquisition and switch off immediately when the run stops.
  - *Pre-Stimulus Option:* Select this option to deliver an automated 10-second blue light pre-stimulus directly before the system begins recording each plate.
- Cycles: To program repetitive measurements, select the 'Cycles' checkbox. The software will prompt you to define:
  - The total number of times you wish to read the same plate.
  - The specific time interval between the start of each consecutive acquisition cycle (Cycle time).



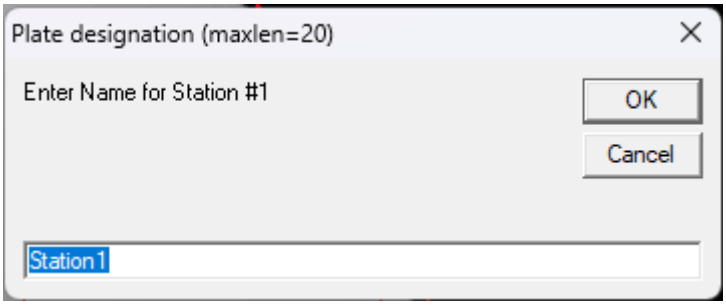
The system will automatically calculate and display the total estimated experiment duration based on these inputs.

RToff: Due to specific resource management traits in MS Windows, performing multiple consecutive acquisitions with real-time image processing can occasionally increase the risk of computer crashes. To mitigate this risk and safeguard your data, it is highly recommended to check "RToff". This disables real-time rendering during the run, allowing you to perform seamless bulk processing once all physical readings are safely completed.

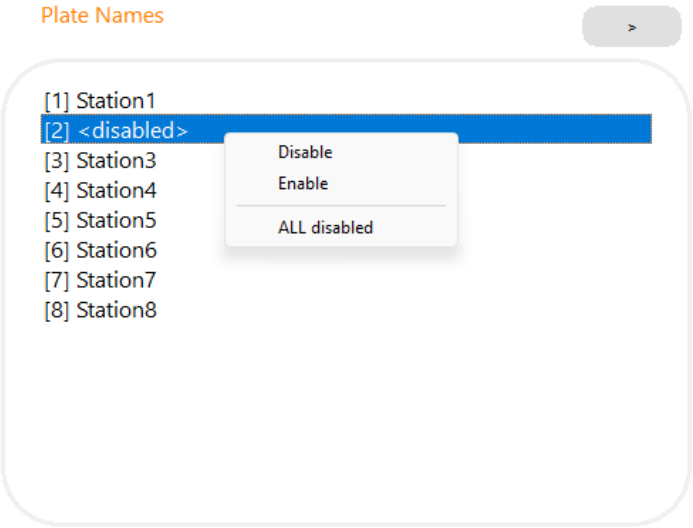
Station Customization

The software allows you to customize the acquisition for the 8 available carousel slots via the Plate Names panel:

- Renaming Stations: Double-click directly on any station name to type a custom label or experimental condition (e.g., Control, Drug treated).



- Enabling / Disabling Slots: Right-click on any station to open a context menu where you can select Enable or Disable.

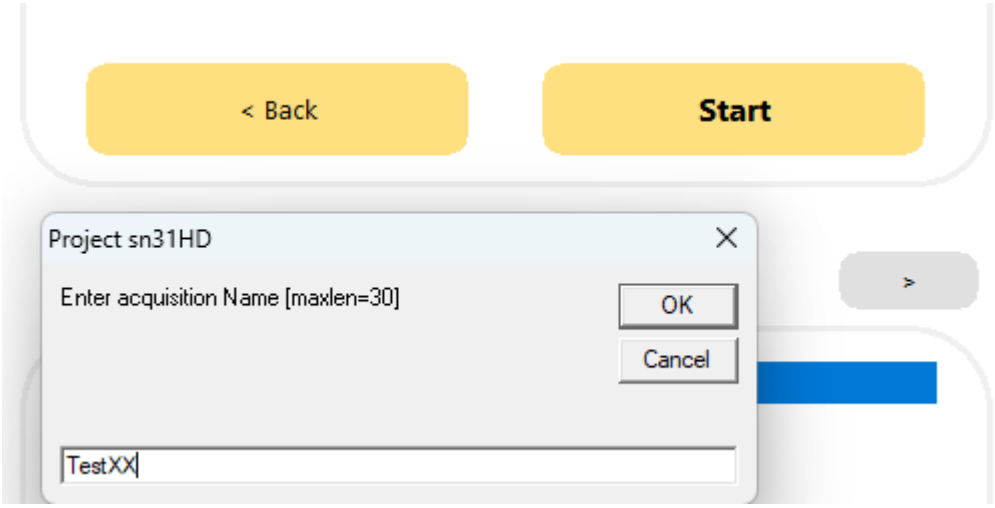


If a carousel station does not contain a plate, change its status to `<disabled>`. This ensures the device automatically skips that slot during the workflow, seamlessly moving on to the next active, enabled plate without losing time.

3.5 Initiating the Run and Monitoring Assay Progression

Starting the Acquisition

Once you have fully configured your experiment settings, click the START button to initiate the data collection process. A pop-up window will automatically appear prompting you to enter a name for your specific run.



It is highly recommended to use a descriptive naming convention to easily identify, filter, and retrieve your data during post-processing and future analysis; since the system automatically appends the current date and time by default, you can focus on entering specific experimental variables to complete the filename (e.g., YYYYMMDD_HHMMSS_Project_Condition)

Real-Time Monitoring

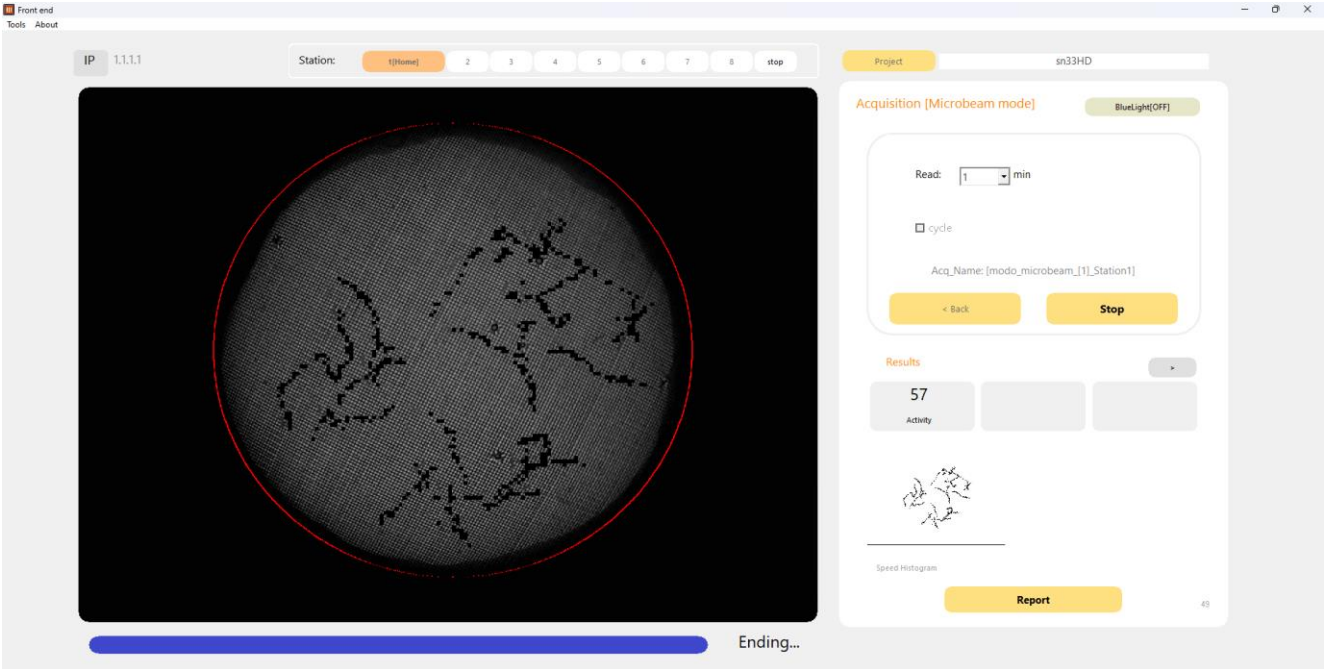
During the acquisition, the software will continuously display the progression of your results alongside live image streams from the active stations.

Depending on the mode selected during initial setup—either Tracking or Microbeam—the data registration process and the specific information displayed in the final report will vary:

- TRACKING Mode:

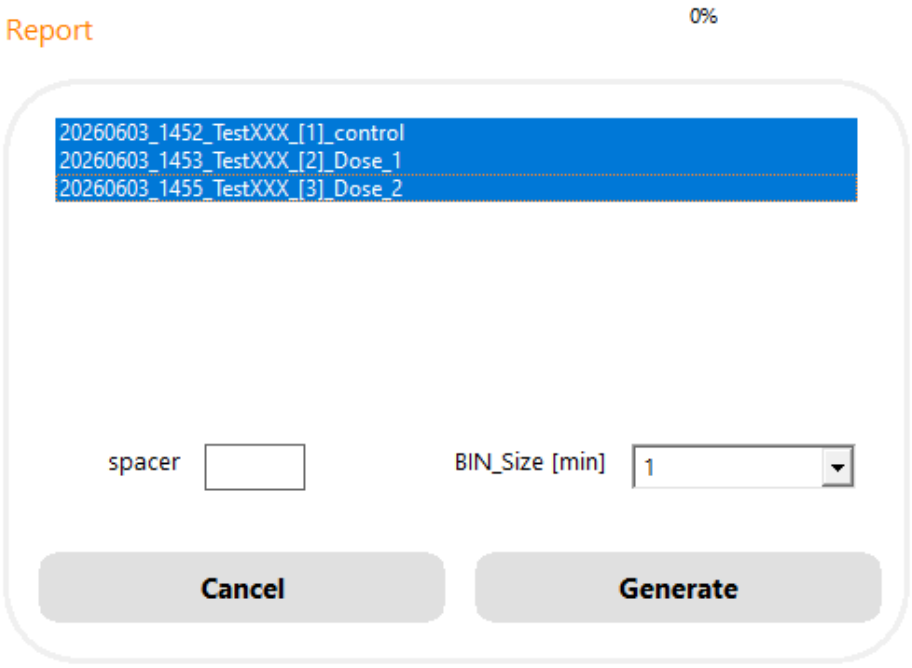
The system captures images at a rate of 1 frame per second (1 fps) and processes each image in real time to automatically detect, isolate, and follow the trajectories of individual organisms within the Petri dish.Real-Time Monitoring

- MICROBEAM Mode:



- *3.6 Report Generation*

Once the acquisition runtime or the programmed cycles are complete, the system will automatically stop recording. To compile your results, select the desired acquisitions from the list and click the **"Generate"** button to export and aggregate the raw data into a structured CSV file. Prior to generation, you can customize the output parameters by selecting the appropriate plate spacer configuration and your desired data binning time.



Note: For a detailed explanation of the output parameters, please refer to Section 4.2 (Report Interpretation).

IV. Results & Data Analysis

4.1 Offline Mode Review (Check Results).

All experiments acquired with the WMicrotracker SMARTx8 HD system are saved into a folder for future access. The images can be recalled to generate a new report or to re-analyze them with a different training To review historical data, navigate to the **"Check Results"** menu located within the main **"Start"** window.

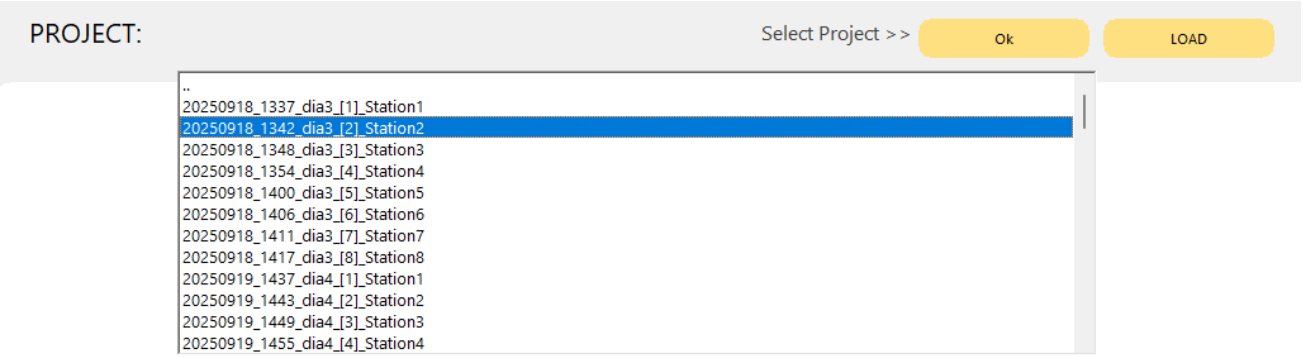
For large-scale studies, you can easily aggregate data from multiple distinct acquisitions within the same project directory by utilizing the **“Joint Report”** function. This tool consolidates separate runs into a single file to streamline your comparative analysis, as explained in detail in the following subsections.

Additionally, if the automated tracking accuracy was suboptimal during a live run, the software provides the flexibility to retrain the system offline. You can apply new silhouette recognition parameters, and select **"Reprocess"** to recalculate the organism trajectories.

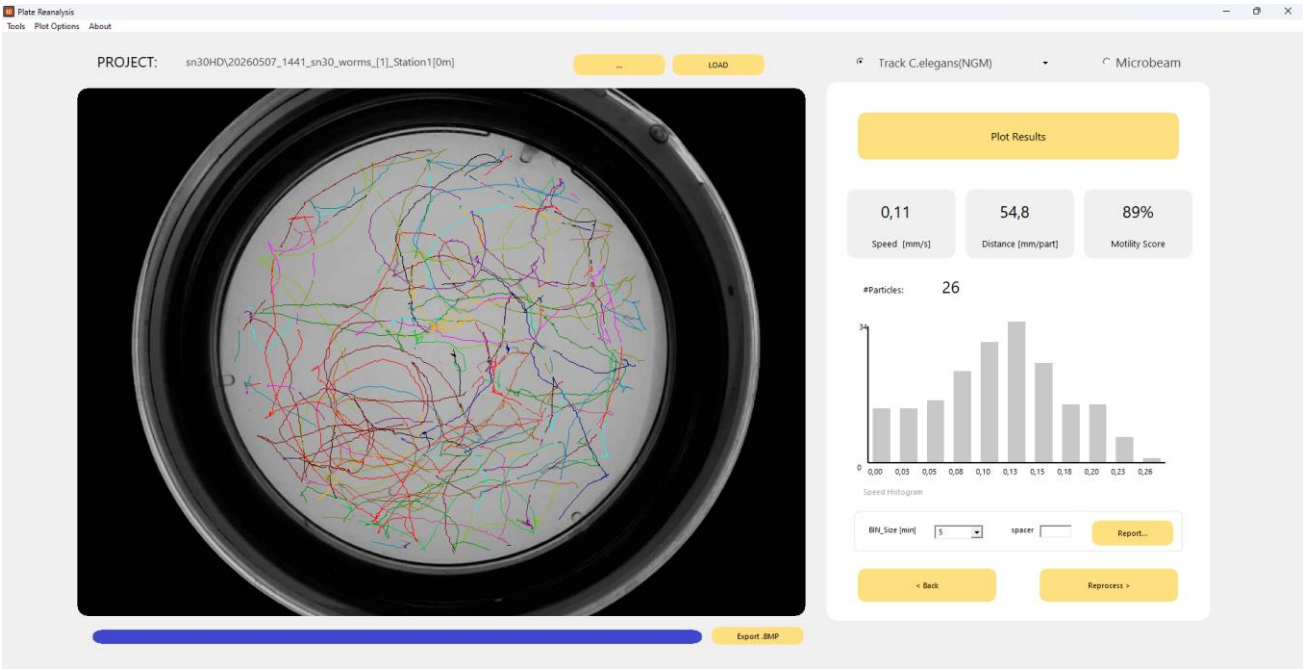
Recalling Saved Data and Plotting Results

Experiment data is easily accessible after an acquisition through the **"Check Results"** menu. To begin, you must select and load the specific acquisition you wish to analyze. All experiments are systematically organized in folders by project name, with individual acquisitions listed chronologically by date. Simply select the desired experiment and click

"Load", or double-click the file to open it.



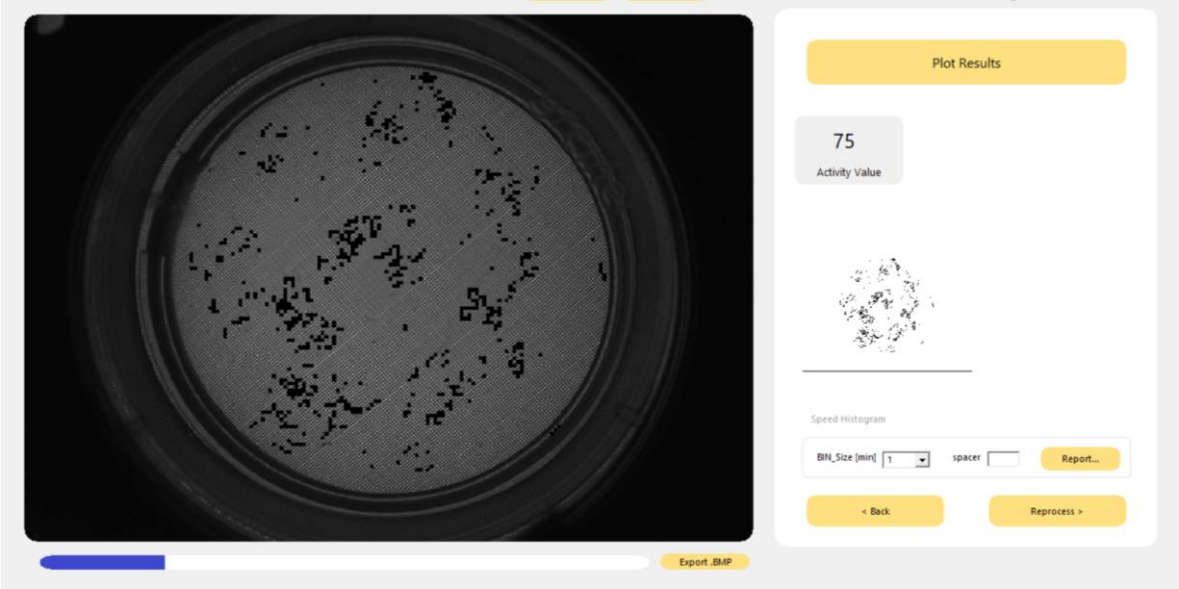
After loading the data, click on **"Plot Results"** to display all the captured images from that acquisition. The visualization in the plate layout area will automatically adapt to your chosen experimental mode. Additionally, you can click on "Export BMP" at any time to save the currently displayed image as a standard .bmp file for external use. In Tracking Mode, the software will display the trajectory paths of the detected particles based on the last saved training configuration.



On the right panel, average population results are presented.

- #Particles detected (average number per frame)
- Particle speed [mm/s]
- Traveled distance [mm/particle]
- Rotation Index
- Speed [mm/s] Histogram

In Microbeam Mode, the system will highlight the exact locations where activity was detected during the run.



To export your data to a datasheet, click on the "Report" button. You can customize the time intervals for your analysis by adjusting the "Bin Size" drop-down menu, which will automatically organize the reported parameters accordingly in the final export. Additionally, ensure you select the appropriate delimiter (such as a comma , or semicolon ;) based on the requirements of your external data analysis software.

4.2 Report Interpretation (report.csv)

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

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	

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 Appendix 1 for an example.

2) **Single particles results:**

A list of results for every single particle detected for at least 10 frames.

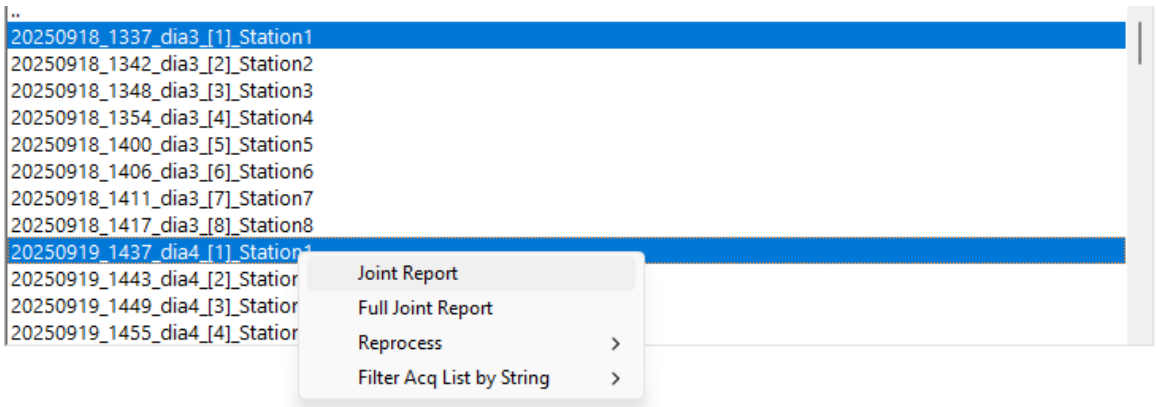
- ID particle: the ID number assigned by order of appearance/recognition.
- Distance: the distance traveled by that particle.
- #frames: the number of frames in which that particle was detected. The device acquires 1 frame per second.
- Speed: the distance traveled by that particle divided by the number 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 Appendix 2 for more information.
Additional data output recording is explained in Appendix 3.

4.3 Generating Consolidated Reports (Joint Report Function)

To generate a consolidated report from multiple acquisitions within the same project, follow these steps:

1. Select the desired acquisitions by holding down the control key and right-clicking on them.
2. Right-click and choose the option to generate either a joint report or a full joint report of all selected acquisitions. The main difference between both types of reports is the quantity of information to include.



Joint *report*:

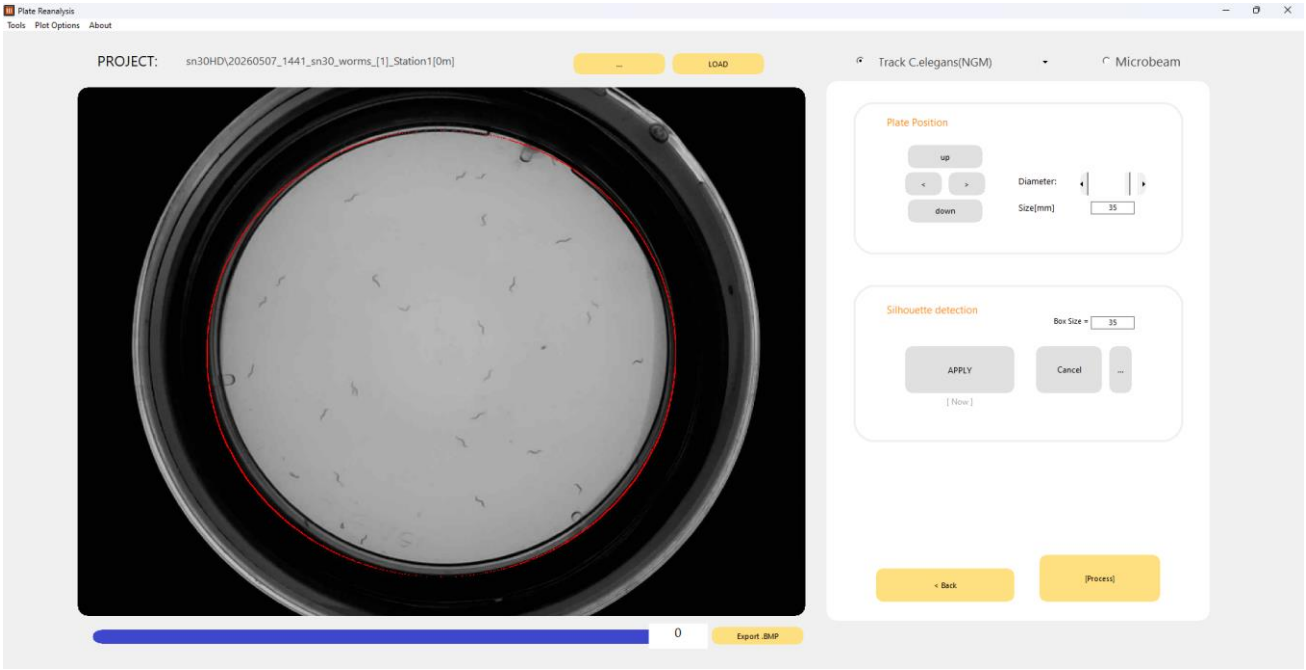
	A	B	C	D	E	F
1	Report File	##########				
2	Folder	C:\PHYLUMTECH\20240214_smartx1\levamisol				
3	Acquisition Lapse[m]	5				
4						
5	Acq_Name	Acq_Date	Acq_Time	#Particles	Average_speed[mm/s]	Motility_Score
6	0A_1	4/4/2022	08:49	36	0,144	0,93
7	OA_2	4/4/2022	09:24	37	0,147	0,97
8	OA_3	4/4/2022	10:00	34	0,142	0,97
9	OA_4	4/4/2022	10:35	32	0,142	0,94

Full joint report:

	A	B	C	D	E	F	G	H
1	Report File	##########						
2	Folder	C:\PHYLUMTECH\20240214_smartx1\levamisol						
3	Acquisition Lapse[m]	5						
4								
5	Acq_Name	Acq_Date	Acq_Time	#Particles	Average_speed[mm/s]	Travelled_Distance[mm/worm]	Motility_Score	Rotation Index
6	0A_1	4/4/2022	08:49	36	0,144	65,23	0,93	6,91
7	OA_2	4/4/2022	09:24	37	0,147	71,74	0,97	6,45
8	OA_3	4/4/2022	10:00	34	0,142	67,04	0,97	6,7
9	OA_4	4/4/2022	10:35	32	0,142	69,43	0,94	6,76

4.4 Offline Image Reprocessing

If silhouette detection requires adjustment post-acquisition, you can retrain the machine learning algorithm to recognize your specific organism. To do this, click the “Reprocess” button, which will direct you to the following screen:



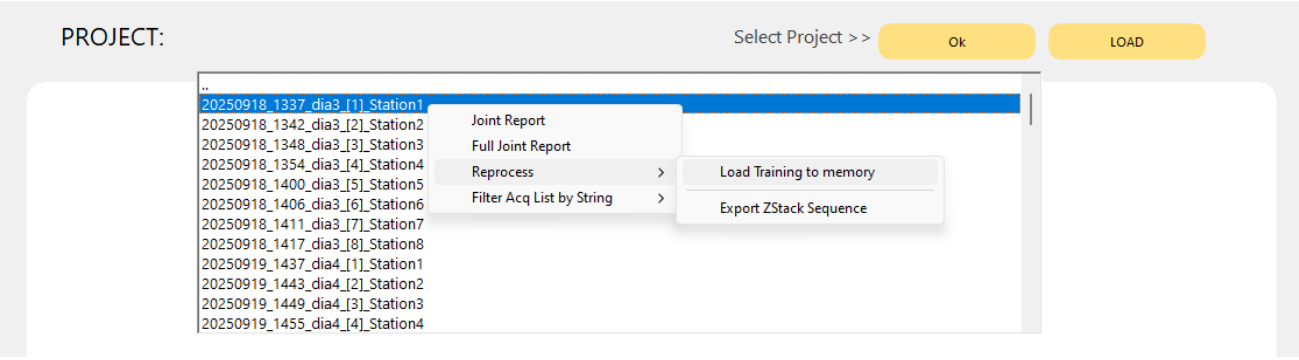
Following the same principles established during the pre-acquisition setup, first adjust the "Box Size" field to ensure the bounding box expands or contracts enough to fit the entire organism inside. Click the "New" button to initialize the training mode, then left-click directly on 5 to 10 representative organisms scattered across different areas of the plate image, taking care to avoid the extreme boundaries. Once your selections are complete, press the "APPLY" button to turn the targeted silhouettes red—confirming system recognition—and click "OK" to save these updated training parameters into the local project memory. Please note that saving this new configuration will permanently overwrite the previous training parameters for this acquisition, as older training data is not retained.

To verify the performance and accuracy of your newly recorded silhouette recognition patterns across the entire sample, click the "Test Training" button. The software will evaluate the frame and overlay blue bounding boxes around every particle it successfully recognizes based on the new criteria. Once you are satisfied with the detection accuracy, press the "Reprocess" button to command the software to recalculate all trajectories and paths across the complete image set. This automated calculation takes approximately one minute to finish. After the reprocessing cycle is complete, you can safely navigate back to the primary report window to visualize the updated path tracking graphics and regenerate your finalized datasheet report.

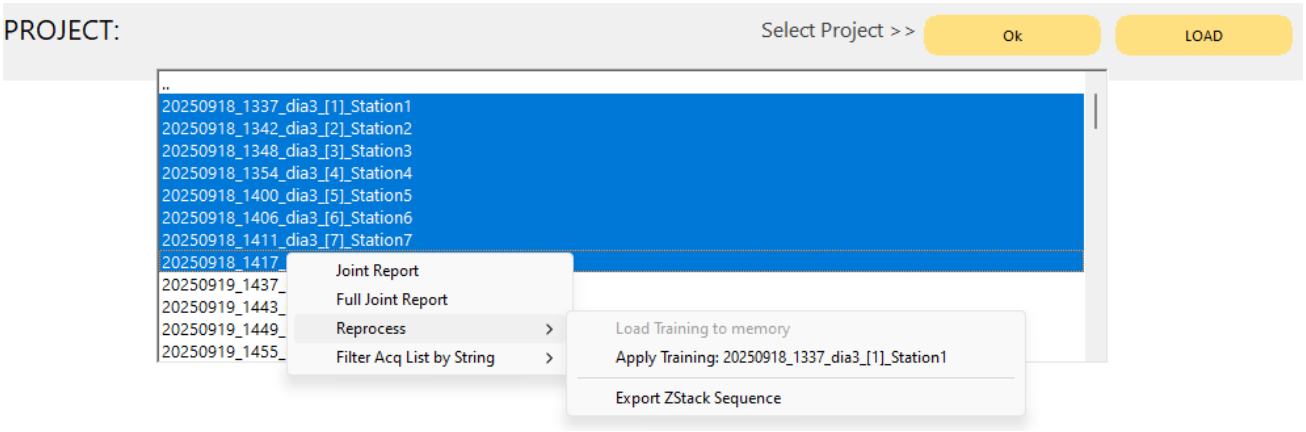
Batch Reprocessing of Multiple Acquisitions

When managing large-scale studies with multiple acquisitions that require adjustments, you can utilize the automated batch reprocessing feature to apply a single set of optimized training parameters across several datasets simultaneously. To begin, open a representative acquisition and perform the offline retraining process as detailed in the previous section. This initial re-analysis serves as the baseline configuration for the rest of your batch.

Once this first acquisition has been successfully reprocessed, right-click on its name within the file directory listing and navigate to “Reprocess/Load Training to memory". This action saves the active parameters you’ve set into the software's temporary cache, making them available for application to other acquisitions.



With the desired parameters loaded into memory, hold down the control key (Ctrl) to select all the remaining acquisitions in the list that require the same update. Right-click the highlighted group, navigate to the **"Reprocess"** menu, and select **"Apply Training"** followed by the specific timestamp label of your saved configuration.



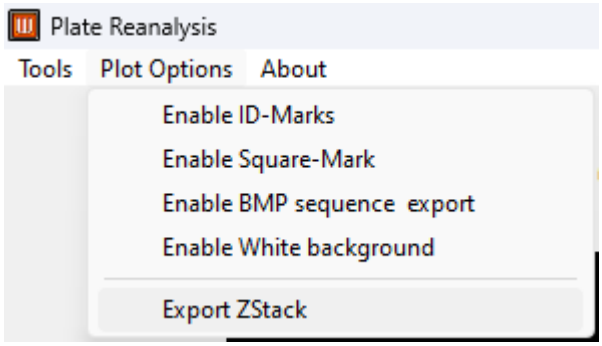
The software will automatically execute the post-acquisition calculations sequentially across all selected runs, eliminating the need for manual parameter input, reducing risk of error, and ensuring absolute consistency throughout your entire project directory.

4.5 Advanced Visualizations

- Export Z-Stacks

A Z-stack is a merging of images in the Z-dimension, which is a common tool for confocal microscopy. However, utilizing Time as the Z-dimension generates a rich, integrated image file that contains the complete trajectory of moving particles in the X-Y plane.

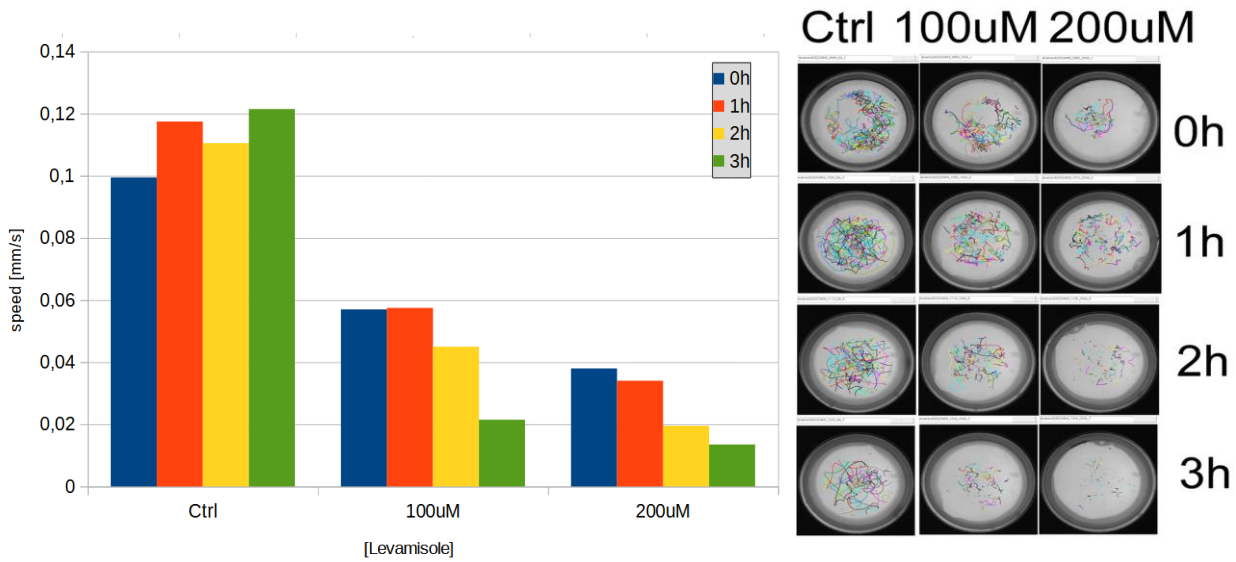
By using this timing Z-stack, you can reconstruct the cumulative movement of worms on NGM agar The software automatically calculates these Z-stacks by using the minimum pixel value of the images for the final merging process. To generate this file, navigate to Plot Options / Export ZStack Sequence in the top menu.



V. Data output Examples: Path Tracking mode

5.1 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:

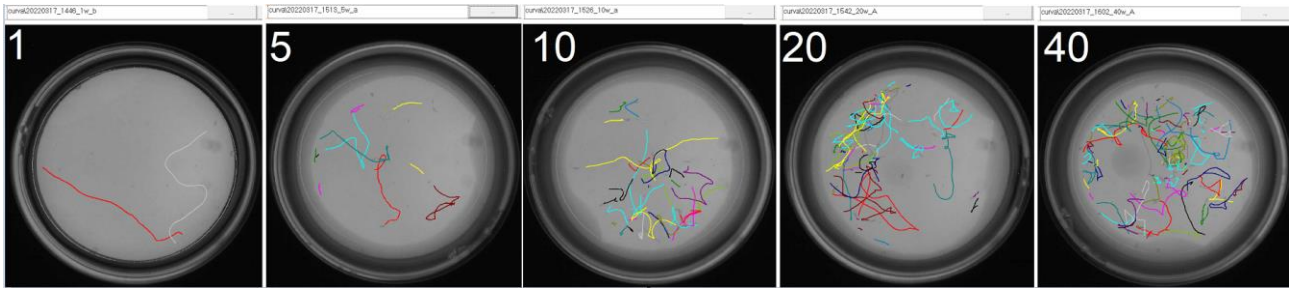
1. Grow synchronized populations of adult day-1 worms in seeding NGM plates (OP50).
2. Remove worms from plates using M9 buffer and transfer them to a sterile 2ml micro tube.
3. Let the worms settle. Decant the supernatant, taking care not to disturb the pellet.
4. Wash the worms by adding 2 ml of M9 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 SMARTx8 HD to record worm activity for 5 minutes. Immediately before the acquisition, stimulate the worms by tapping the plate three times.

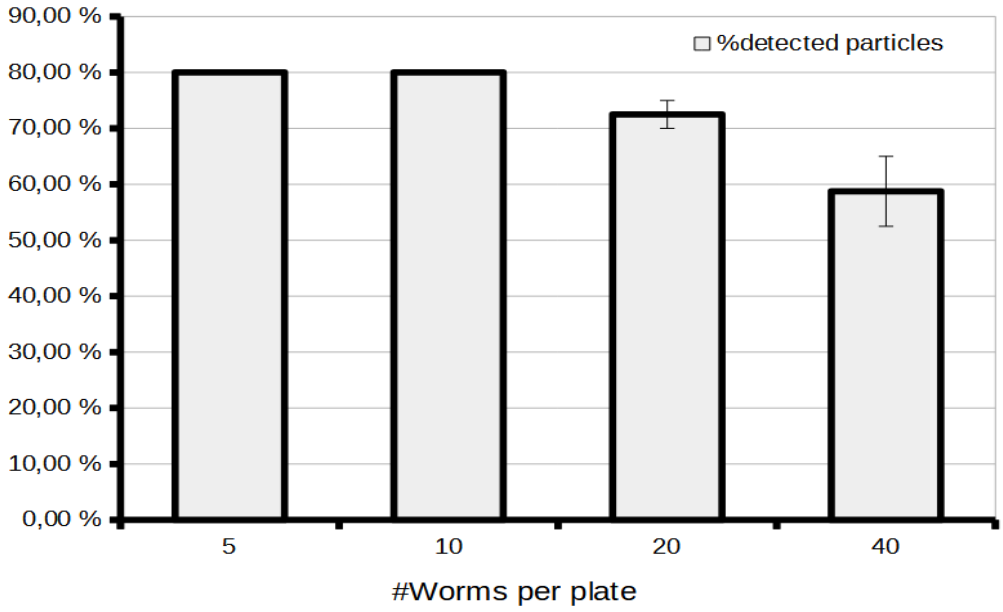
Microplate preparation:

- 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.

5.2 C. elegans detection curve [Optimization of animal number]

The WMicrotracker SMARTx8 HD 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 SMARTx8 HD to register worm activity during a 5-minute interval. Before starting the acquisition, stimulate the worms by tapping the plate three times.

Notes:

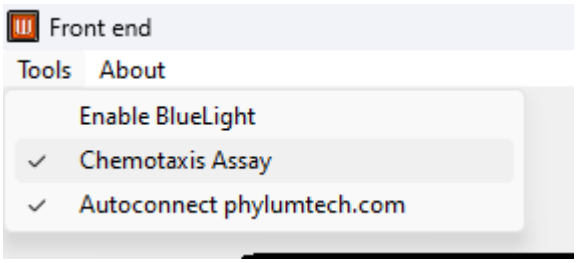
- Before adding the worms to each plate, make a ring of 100 mM copper sulfate around the edge of each plate to prevent worms from crawling out of the agar.
- When adding the worms, be careful not to scratch the agar – worms tend to crawl into any break in the agar surface.

5.3 Chemotaxis Assay.

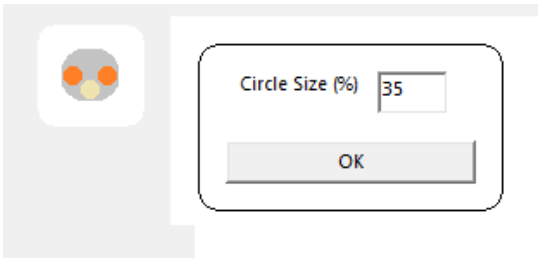
The WMicrotracker SMARTx8 HD includes a specialized Chemotaxis Assay Tool designed to evaluate preferential migration and behavioral responses of worm populations in real-time. By defining custom regions of interest within a 35 mm Petri dish, the system automatically quantifies the spatial distribution and movement of organisms across different sectors of the arena. When the Chemotaxis Assay Tool is enabled, the final software report automatically the Chemotaxis Index. Using programmed multi-cycle measurements, the WMicrotracker SMARTx8 HD enables researchers to effortlessly monitor the kinetics

Protocol and Software Activation:

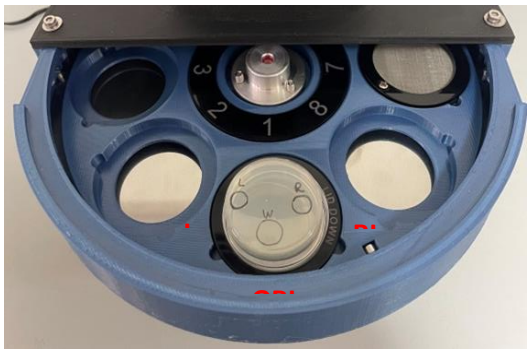
1. Position the red circle at the edge of the bottom of the plate with the menu in the right panel.
2. Bring the worms into focus. We recommend using an extra plate to take the necessary time to properly adjust both focus and illumination.
3. Once the image is in focus, activate the tool via: *Menu → Tool → Chemotaxis Assay*.

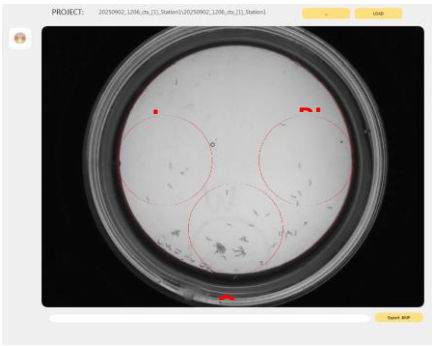


4. A scheme of the section of the plate will appear at the left. Click on it to open a menu where the circle size can be adjusted as a percentage of the total plate area.



5. Place the plates into the carousel.





6. Start the acquisition and set the number of cycles required to monitor the entire chemotaxis process.
7. When the run is complete, generate the report. With the Chemotaxis Assay Tool enabled, the report will display the *fraction of particles detected in each defined area*.

	A	B	C	D	I	J	K	L	M
1	Report File	##########							
2	Folder	G:\20250827_smartx8\20250902_1206_ctx_[1]_Station1							
3	Acquisition L	5							
4	Chemotaxis	Circle_diame	40%						
5									
6	Acq_Name	Acq_Date	Acq_Time	#Particles	**Detections_Left**	**Detections_Right**	**Detections_Origin**	[#AcquiredFrames]	
7	ctx_[1]_Stati	9/2/2025	12:03	17	**0.12**	**0.14**	**0.4**	100%[300/300]	
8	ctx_[1]_Stati	9/2/2025	12:15	22	**0.11**	**0.6**	**0.08**	100%[300/300]	

To estimate how many worms were detected on average in a particular spot, simply multiply: Particles × Detection_fraction

Example:

If #Particles = 17 and Detection_fraction (left area) = 0.12,

Then,

17 × 0.12 = 2 particles (worms) detected on average in the left area.

VI. Appendix

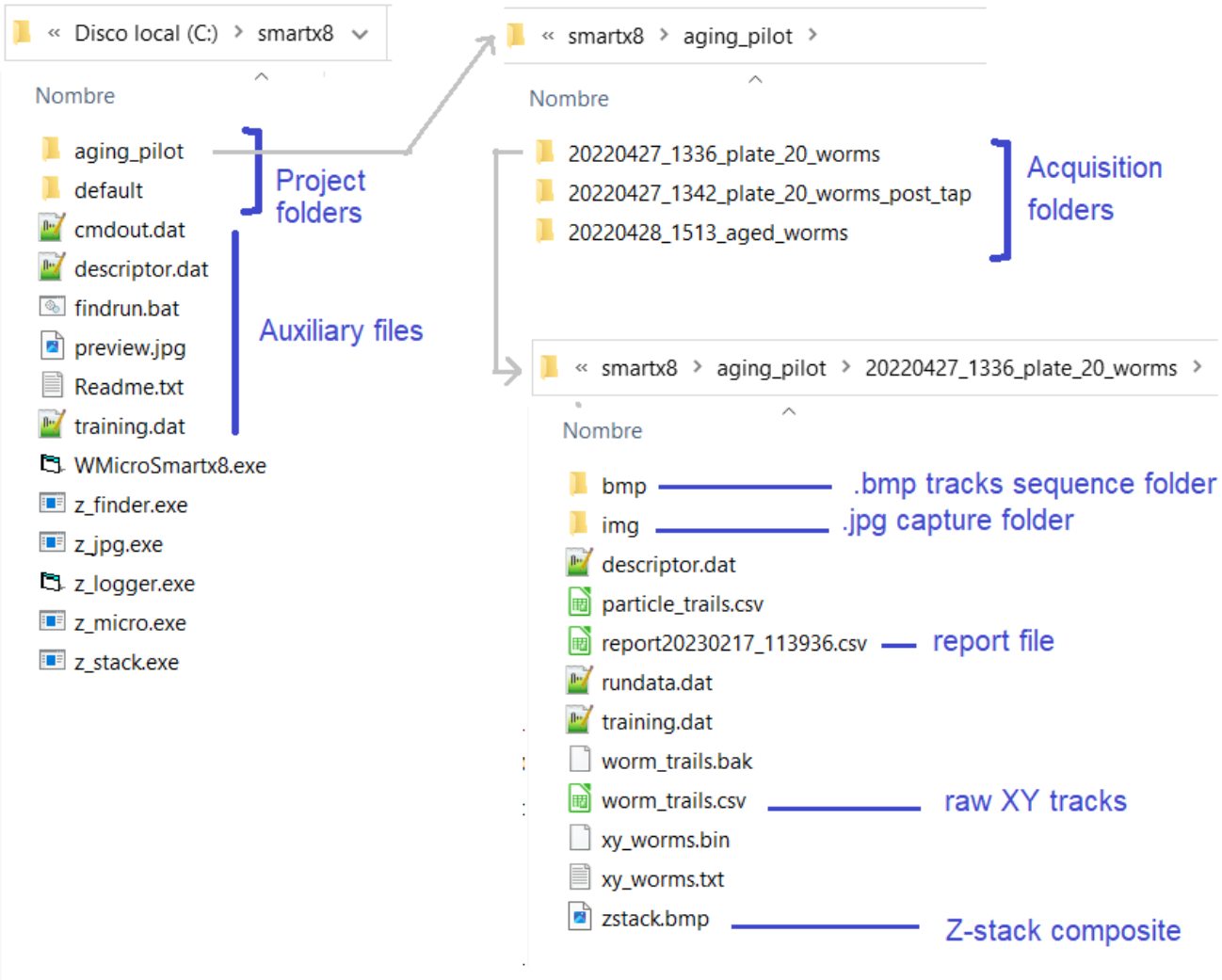
Appendix 1: *Structure of directories.*

Once you start acquiring experiments, you will see that projects are created in the installation directory. Inside each project, you will have the acquisition files sorted by date. All raw data, images, and reports are saved into these acquisition folders.

To access the folder through a network, you should share this folder with your workgroup. To transfer the experiments to another computer for analysis, you should copy the project or acquisition folder. Try keeping the name simple without spaces to be loaded. Acquisition folders need to have the name yyyymmdd_hhmm to be accessible by the software.

Please remember to keep the structure project.acquisition folder in order to allow the software to read and access the data.

Example of data structure:



Appendix 2: *Technical Details on Average Population & Rotation Index*

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

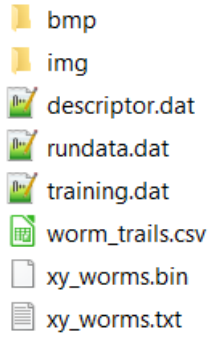
0	0	0	0
0	1	1	1
1	1	0	0
0	0	0	0

3

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

Appendix 3: *Raw Data & File Recording Description*

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



\img folder
contains all images captured during the acquisition lapse. These images can be used to generate a .GIF or .AVI video using ImageJ software

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

Worm_trails.csv

This is the data file containing worm tracks. The data can be processed by users with their 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 i7 processor or above
- 4/8 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 SMARTx8 HD?

The WMicrotracker SMARTx8 HD 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 SMARTx8 HD 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 SMARTx8 HD?

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 SMARTx8 HD?

The temperature in the WMicrotracker SMARTx8 HD 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?

To detect 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"). To detect 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 SMARTx8 HD? 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.

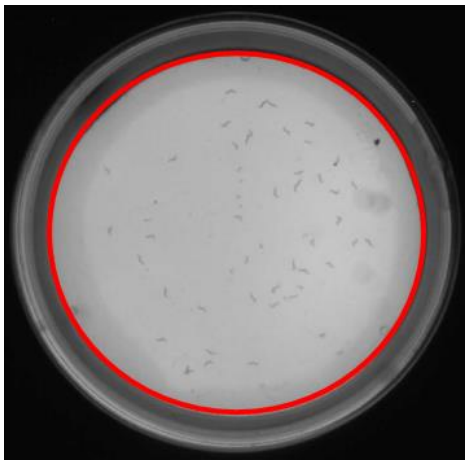
I can't set the focus for the whole area of the plate, what should I pay attention to?
When preparing NGM plates, avoid moving them, as they can create a natural curvature (meniscus), making it difficult to focus the whole area of the plate.

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 (*Section 4.4*)

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.

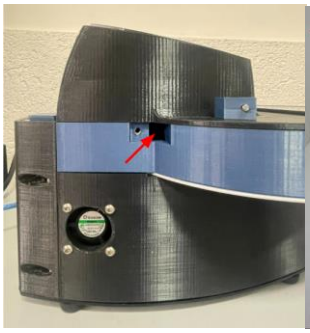
Can I change the measured intervals to be reported?

The WMicrotracker SMARTx8 HD 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 may enter the detection area over time. A compartment located on the rear of the equipment allows access to the detection area for cleaning.

1. Power off the equipment and disconnect it from the power supply.
2. Remove the screw from the cleaning access compartment on the rear of the unit.



3. Carefully open the compartment.
4. Use compressed air to clean the detection area.
5. Close the cleaning compartment, and secure it with the screw.

 Do not use liquids or abrasive materials when cleaning.

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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