Mucrotracker

ARENA

Fast startup guide

www.wmicrotracker.com

Data acquisition system WMicrotracker ARENA Hardware version: WMTK ARENA 18 / V1.0-R01 Software version: WMTK V1.6 (2020)

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Thank you for acquiring our wMicrotracker ARENA system. The following document should guide you through the installation process.

Packing List

The system includes the following accessories:

 Microplate reader system 	• USB-B cable
 12V DC, 5Amp switching Power Source (5.5mm-2.1mm 	 Plastic adapter for 35mm Petri dish
 Acquisition Software: available from www.phylumtech.com 	

* Due to customs restrictions, in some countries the shipping might not include the power supply and could be delivered separately.

Installation and setup require the following items:

- 12V DC switching power supply with 5 Amps output.
- USB-B connection wire (Provided).
- IBM PC compatible with the following minimum requirements :
 - Pentium Core i3 processor or above
 - 2 Gb of RAM memory.
 - ☑ 1 USB 2.0 port available.
 - 2 Windows 7 32bits (or higher) operative system.
 - At least 200Mb of free HD space.
- Workbench space required for equipment installation: 30cmx30cmx30cm.
- Ambient free of vibrations and dust.
- Ambient Optimal temperature range: 15 to 25°C*. Preferably low humidity (<50%).

* This range is for equipment optimal functionality only; biological samples might have unique temperature requirements.

Technology Description

WMicrotracker technology is based on detection of worm movement through infrared light scattering, a methodology originally published by Simonetta SH *et al* 2007 (DOI: 10.1016/j.jneumeth.2006.11.015).

This system possesses a sensing arena composed of more than 20.000 infrared microbeams, 100um wide, illuminating the microplate from the bottom side. The light beams generated by infrared leds are separated 0.7mm one each other.

An optical system of 6 video cameras (1Mpixel each) capture the light beams from the top side and the analog signal is mathematically processed to detect the passage of the small animals in the sensing arena.



Our acquisition mode will multiplex the microplate in groups of wells, 1 row of wells per camera at time. The multiplexing mode will take 10 pictures per row, with a maximum rate of 1frame/second, according to each microplate format. This mode allows to optimize the detection of fast moving small animals in any culture medium (liquid or solid).

The software will acquire the signal of each microbeam and will process it

mathematically to detect small animals light scattering. If movement is detected, an activity counter will be incremented.

The total calculated activity will be the integration of activity events in a time frame defined by the user.







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



Using this approach is possible to evaluate multiple protocols using NGM cultured animals, and even liquid. Preferred microplate culture format for ARENA is 6well, 24well microplates and 35mm Petri dishes.

Experimental protocol guidelines can be accessed at www.phylumtech.com

INSTALLATION & SETUP GUIDE:

Step One, Software Installation:

- a) Install the "USB driver" in the computer you plan to use for the data acquisition of your wMicrotracker system. You'll find the driver at <u>www.phylumtech.com</u> →Support page → "USB Direct connection".
- Note: If you like to check USB-driver was properly installed, verify if a new COM Port has been detected in your computer (into Devices&Printers Windows menu) after you connect WMicrotracker equipment.
- b) Install the wMicrotracker ARENA Acquisition Software: Download the files from <u>www.phylumtech.com</u> →Support page → Version ARENA
- Unzip the files into a temporary folder and follow the instructions detailed in the file Readme.txt. In Brief:
 - o you will need to copy <u>\worm_arena</u>\ folder to <u>c:\worm_arena</u>
 - o after installing the usb driver, just execute c:\worm_arena\arena_video.exe file

c) If available, copy the /config files to your installation folder. These files will configure your specific equipment to factory calibrated values.

Step Two, Hardware Setup

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a) Connect USB-B cable to the USB COMM PORT at the back of equipment and the USB end to any free USB port on your computer. USB2.0 or 3.0 (blue plastic colour) are recommended.

b) Plug in your Power Supply to any regular Power Outlet and the Power Supply's output cable to the back of your WMicrotracker Arena (5.5mm-2.1mm socket).

Step Three, Running Acquisition Software [V1.6]

Run the "Arena_video.exe" executable file from the folder you choose at the installation step.
 If everything went well the application should start immediately with this window:



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

FIRST TIME connection will automatically search and download Calibrations files from ARENA internal memory.

At Phylumtech we generate calibration files for each system during Quality check. You will receive these customized factory files saved into your equipment, or a file link at time of purchase.

*Just for reference, calibration procedure is described at the appendix.

Running acquisitions and generating report files

Once you have installed setup files for the first time, you will be ready to start acquiring data.

To proceed, setup the following parameters:

1) Acquisition period.

Acq lapse:

60	min
----	-----

2) Microplate format

MicroPlate Format:



3) Incubation temperature



check the box to turn on temperature control.

4) Set saving folder and technical replicate groups *optional

To select the saving folder or to create a new one, check the button or scroll the Project menu.

Project:	New
default_project	-

To assign technical replicates and experimental treatment names, Select the "Group" menu and click on the wells to assign. left click to apply, right click to remove.



Double click Group name to rename.

*Your configuration will be saved for your next experiment.

5) Press START to run the acquisition



The system will ask you to write an experiment acquisition name (useful to remember when you recall an old experiment). Write it and press play to start.

default_project	
Acquisition: <td< th=""><th>></th></td<>	>
Cancel	PLAY

The system will start a short period of autocalibration (2 to 4 minutes), acquiring microplate images to homogenize lighting. After this action, processing of the locomotor activity will start automatically.

Generation of report files

At the end of the experiment (or at any time when the experiment is running) it is possible to generate a report file.

Pressing "Report" button a menu like this will appear:

• Rows	C Array	y C MapPlot
Binsize ScanTime	▼ min.	File .csv Spacer : Internal Reference: Apply Rlank correction

Select appropriated setup for your customization and press "Generate"

MSExcel or the associated .csv software will be open showing your results.

Step Five, Recalling old experiments

In order to recall old experiment press

LOAD RECORDED EXPERIMENTS.

A folder menu will be show on main windows,

X > Hide subfolders

_			
-	+ 2018.0802	backup_projects_withoutname	~
ŀ	+ 2018.0829	default_project	
	+ 2018.0806	validaciones pclab	
ſ	> 2018.0830	ACQ13:55 "1 gusano en fudr placa35mm al-2-b1-2"	
	> 2018.0830	ACQ15:27 "1 adulto x well - placa de 6 wells NGM"	
	> 2018.0830	ACQ16:25 "placa 6 wells 50-25-1 worm"	
	> 2018.0904	ACQ16:23 "al-4 single worm agar 35mm"	
	> 2018.0906	ACQ14:42 "Test curva 75,50,25,12,6,0,75,50,25,12,6,0 w96f"	
	> 2018.0906	ACQ15:20 "Curva w6 agar. A1=100, B1=75,a2=50,b2=25,a3=12,b3=6"	
	> 2018.0906	ACQ16:05 "Misma placa w6 al reves: curva_w6_agar_al_100_b1_75	£ –
	> 2018.0910	ACQ13:55 "al,2,3 l0gus. bl,2,3 5gus agar 6well plate"	
	> 2018.0910	ACQ15:34 "al23 5gus, bl23 l0gus agar w6"	
	> 2018.0910	ACQ16:56 "al23 l0gus, bl23 20gus agar 6w plate"	
	> 2018.0912	ACQ14:24 "curva A123 100gus, B123 75gus. well 6 agar"	
			×

explore the folders to find the desired experiment and double click to load to memory and generate report files again.

Joint Report:

If you want to join different acquisitions (for example a multiple day lifespan experiment), select it by pressing CTRL + Left click. After selection, press Right Click and choose "Joint Report".

> 2018.0917	ACQ14:29 "Health span 35 min"
> 2018.0918	ACQ14:58 "Health Span día 4 adulto en FudR"
> 2018.0919	ACQ13:49 "Health Span 5 dias adulto en FudR"
> 2018.0919	ACQ13:54 "Health Span Dia 5 adulto en FudR"
> 2018.0920	ACQ13:13 "Healthspan Dia 6 NCM con FudD#
> 2018.0920	ACQ14:02 "healthspan_dia_6_n Joint Report gar para arriba'
> 2018.0920	ACO16:15 "Placa 24 wells agar num worms A-20 B-40 C-60 D-80"

APPENDIX 1: Advanced Guidelines

Factory settings Calibration procedure

1) Background Light Calibration:

In order to train the system, background lights need to be read at first time of use. Perform the following steps:

a. Select the menu "Advance/Lighting Calibration/Background Light" and follow the instructions presented by the software.

Acquisition Page

Well Format Calibration	>	
Lighting Calibration	>	Background light
Temperature Calibration	>	'
Factory Tools	>	
	Well Format Calibration Lighting Calibration Temperature Calibration Factory Tools	Well Format CalibrationLighting CalibrationTemperature CalibrationFactory Tools

b. To remove the microplate will be required

The lighting calibration procedure will take from 5 to 10 minutes to run. Setup results are going to be saved for future experiments.

2) Well Position Calibration:

In order to calibrate the sensing area for each camera, an appropriate microplate format needs to be placed inside the equipment. Use an empty plate to calibrate positions.

a. Select microplate format to use, and place the empty plate inside WMicrotracker MicroPlate Format:



*NOTE: For 35mm Petri dishes use the provided plastic adapter and select "w6" format

b. Select the menu "Advance/Well format Calibration Tool/Calibrate"

5. Acquisition Page

Tools Help		
Advanced >	Well Format Calibration	> Calibrate position
	Lighting Calibration	>
	Temperature Calibration	> -
	Factory Tools	> _

c. A windows showing the capture of the microplate portion will appear



Move the columns and rows for each camera in order to accommodate the sensing area for each well. The red line is recommended to be located in the area between wells as shown in the picture.



Repeat the procedure for all the 6 cameras, saving each camera at time, and close the menu. This setup is going to be saved for all the experiments you run using the selected microplate format.

3) Temperature Calibration

The system already came with sensing temperature calibrated in-factory. If there is any issue with temperature correlation, there is a tool to calibrate the temperature sensor inside the system. Contact our tech department to configure this setup.

4) Blank measurement

Due to high sensitivity of sensors, the system could detect small amounts of background noise activity. If you prefer to remove this background activity use the Blank measure tool. In brief, software will ask you to measure an empty plate (only with culture medium) and the system will save into memory the background values in order to subtract it to future acquisitions.

If you have any comment or problem just contact us to info@phylumtech.com.

Software and system updates available at <u>www.phylumtech.com</u>