WMicrotracker®

Installation Guide
and User Manual
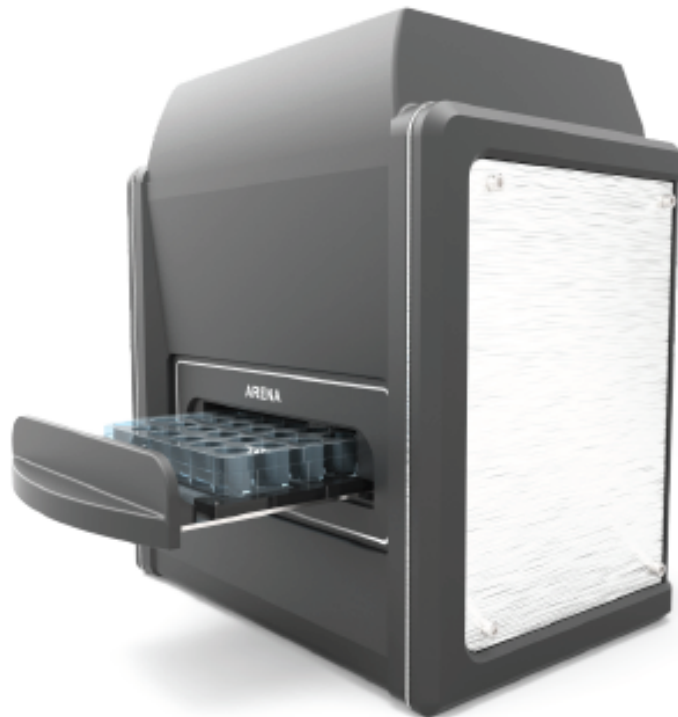
Data Acquisition System: ARENA
Hardware Version: ARENA 100
Software Version: ARENA V1.8 (2021)

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

Contents

I.   About the ARENA

ARENA technology is based on the 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 area composed of more than 20,000 infrared microbeams, each 100µm wide, illuminating the microplate from the bottom side. The light beams are generated by infrared LEDs separated by 0.5mm.

An optical system of 6 video cameras (1M-pixel each) capture the light beams from the top side, and the analog signal is mathematically processed to detect the passage of small animals in the sensing area.
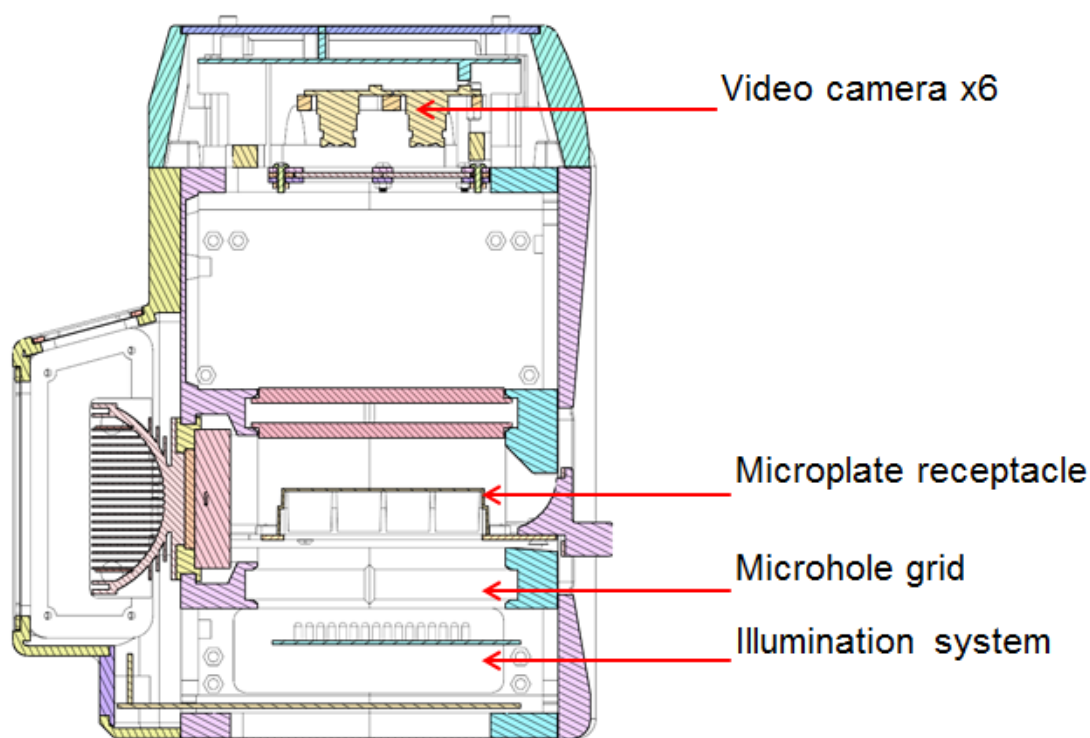
Acquisition algorithms multiplex the microplate image in groups of wells, 1 row of wells per camera at time. The multiplexing mode will take 10 pictures per row, with a maximum rate of 1frame/second, according to each culture format.
This mode allows to optimize the detection of fast moving small animals in any culture medium (liquid or solid).
Detection algorithm acquires the signal of each microbeam and processes it mathematically to detect the light scattering caused by animal movement. When the movement is detected, an activity counter is incremented. The total activity calculated will be the integration of activity events in a time frame defined by the user.

Example of capture of one microbeam in a well:
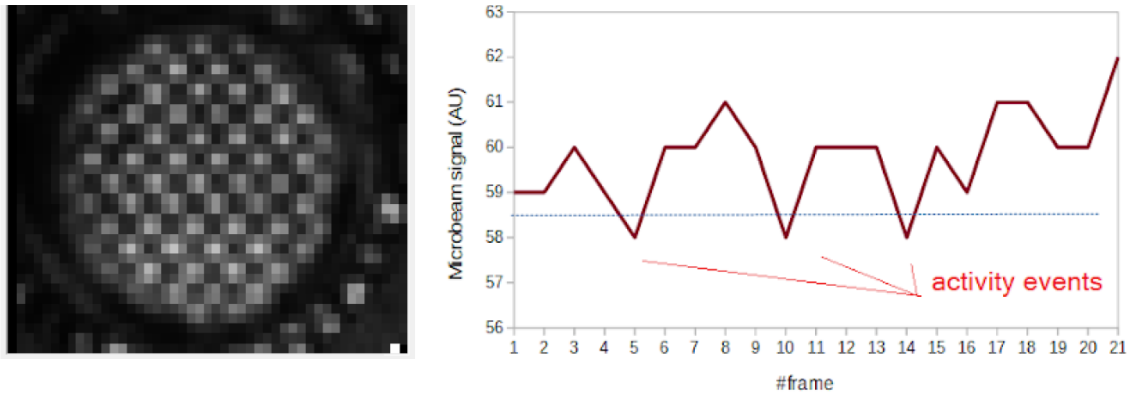
Example of worm population activity detected in 35mm Petri dish with NGM agar:
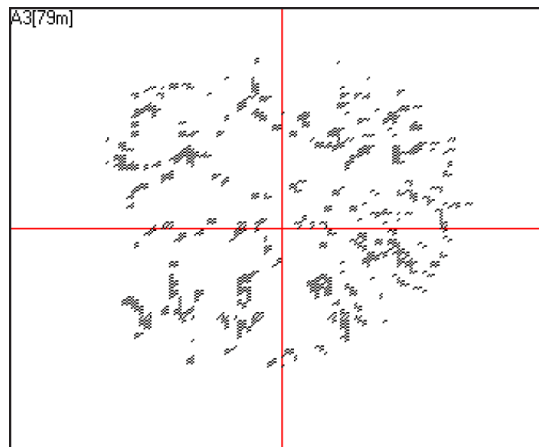
Using this approach it is possible to evaluate multiple protocols using NGM-agar cultured animals, and even liquid culture media. Preferred microplate culture format for ARENA is 6-well, 24-well microplates and 35 mm clear petri dishes;

Recommended:
- 6-Well   (Greiner Bio-One #657160)
- 24-Well   (Greiner Bio-One #662160)
- 35 mm   (Greiner Bio-One #627161)

NOTE: All plate formats must be run with the lid on. It is recommended to seal the plate/ microplate with film (decrease the formation of condensed drops on the lid).

Included Components

	Microplate Reader System.
	USB-B Cable.
	Plastic Adapter for 35 mm Petri Dish.
	12V DC, 6 Amp Switching * Power Source (5.5 x 2.1 mm plug. Center +).

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

Additional Requirements

- IBM PC compatible with the following minimum requirements:
  - Pentium Core i3 processor or above
  - 2Gb of RAM memory
  - 1 USB port 2.0 available
  - Windows 7 32-bit (or higher) operating system
  - At least 200Mb of free HD space
  - Automatic shutdown/sleep/hibernate mode must be disabled.
- Ambient operating temperature of 15ºC to 25ºC with humidity below 50%. This range is for optimal functionality of equipment only; biological samples may have unique temperature requirements.
- Minimize the vibration and dust in your working area.
- Do NOT locate the instrument near a window or bright light.

Product Dimensions

- 30cm x 30cm x 30cm (11.8in x 11.8in x 11.8in)

II.   Installation & Setup Guide

Software Installation

We recommend periodically referencing the Phylumtech website for software updates.

1. You'll find the Software Installation .zip folder for downloading at Software download zone (https://www.phylumtech.com/home/en/support/). To download right-click the link and choose "save link as".
2. Unzip the files and copy WMicrotracker_arena folder to c:\wmicrotracker_arena folder and follow the instructions detailed in the Readme.txt file:
   a. Run USB_Driver Installer before plugging the adapter. Follow the instructions on screen to install the driver.
   b. Plug the equipment to the USB2.0 port.
   c. Run Arena_vXX.exe

Additional comments:

- In case an online "autoupdate" starts (sometimes windows seven tries to find drivers through the internet), select cancel, and choose to "find driver files automatically in the local computer".

- If MSWindows doesn't find the driver, you can manually search for "mchpcdc.inf" driver file from this folder.

- To check if the USB-driver was properly installed, verify if a new COM Port has been detected in your computer (into Devices & Printers Windows menu) after you connect the equipment.

Hardware Setup

1. After the driver has been installed, plug in the power supply (12VDC Switching Power Supply with 5 Amp output) to any regular Power Outlet and the Power Supply's output cable to the back of your ARENA ("12VDC" socket). The green light will turn ON.

2. When you connect the Power Supply to the equipment the green light at the top should turn on and the yellow light will flash (this is a system check of the microprocessor).
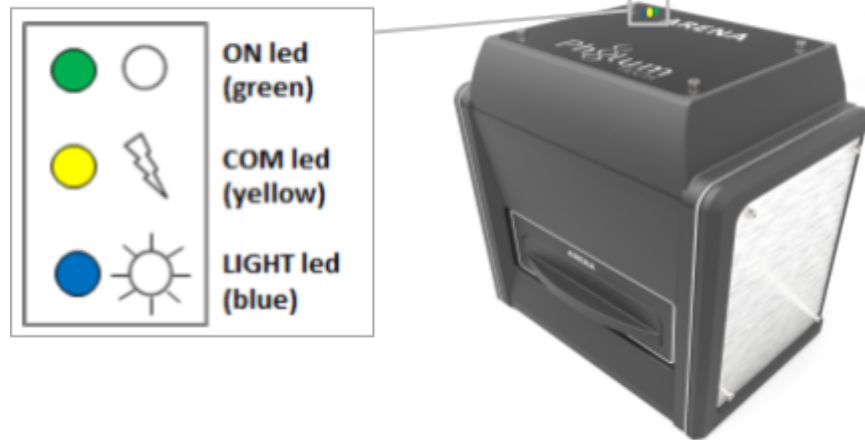
3. Connect the USB-B cable to the USB COM PORT on your computer and to the back of the ARENA. USB2.0 or 3.0 (Blue plastic is recommended).

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

NOTE: See Devices & Printers Window to verify that a new COM Port was detected.

III. Software Use

Software Launch

1. Run the **"Arena_Video"** executable file from the folder you chose during the installation step. The application should start immediately with this window.
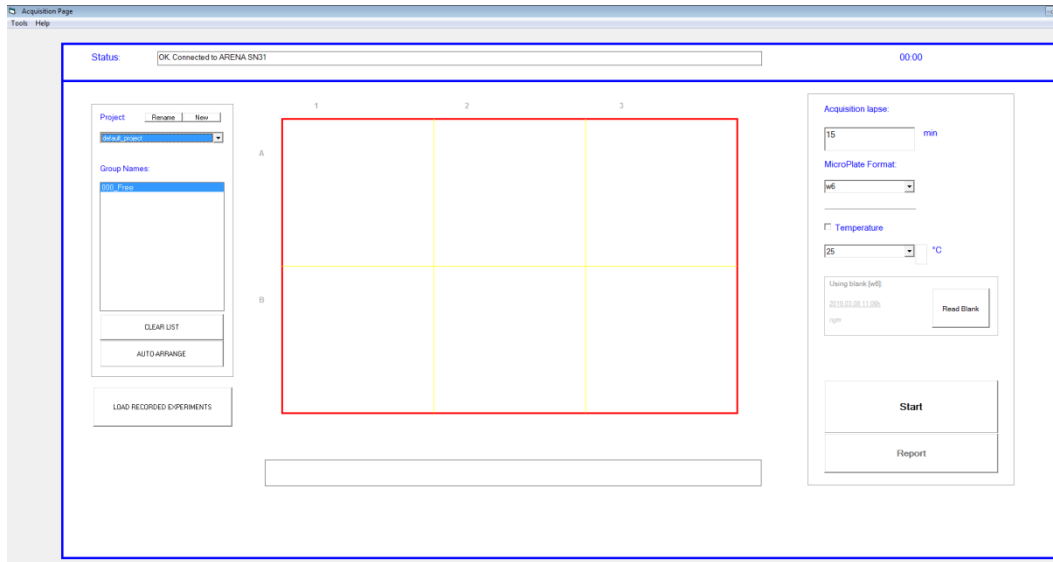
You will be able to define, run and record experiments from this screen, as well as export experiment run data as a report.

Main Screen Components
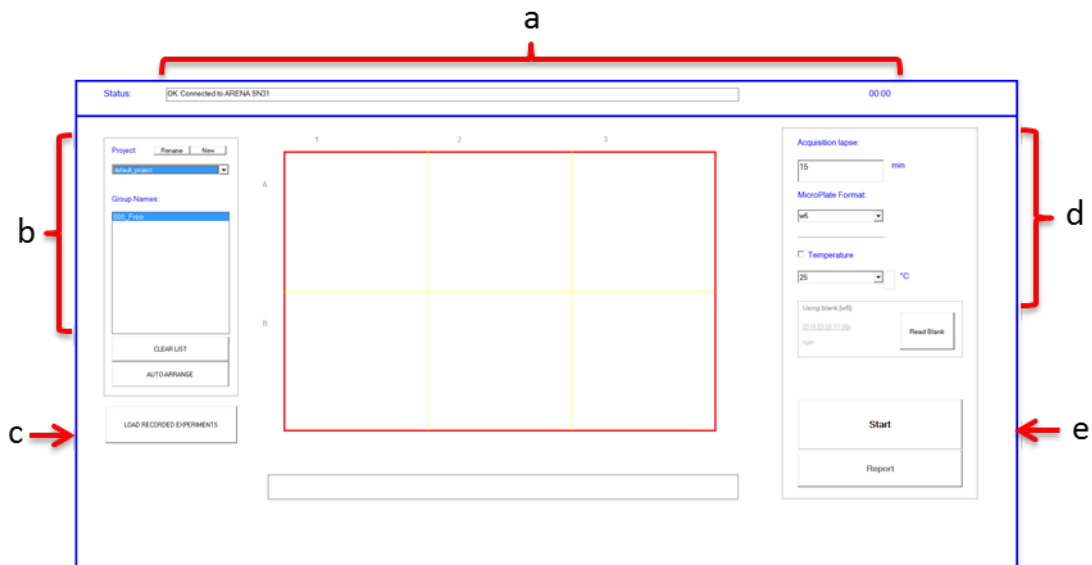
a. Status Bar:
i. Status Bar:
   - Shows progression of auto-calibration during experiment pre-run checks.
   - Shows progression of frames taken per well throughout the run.
ii. Timer:
   - Displays total time of experimental run; this counts up.

b. Project Menu:
i. New Project Creation Button:
   - This will result in a pop-up window.
   - Create and name a new project or new project layout using this window.
ii. Project Layout Dropdown Menu:
   - Upload an existing project layout from the dropdown.
   - Projects are auto-labeled with the date created in addition to the project name you provide.
iii. Group Name for Project Layouts:
   - "Group" refers to an experimental group defined on your plate.
   - "Group" numbering begins with 000_Group0.
   - You can change the Group Name (well name) as desired to best fit your experimental needs (ex: "control sample," "experimental sample A," etc).
iv. Clear List:
   - This will clear all the "Group Name" designations you have set for your plate layout.
v. Auto-Arrange:

- Shows options for arranging the plate layout to read differing experimental set-ups.
- Options allow for single wells and replicates.

c. Load Recorded Experiments:
- Allows you to access data acquired from previous runs, directly from the software main screen.
- A pop-up window will appear, listing projects by date and name, with individual experimental runs within each project.
- To access data from outside the software main screen, simply go to the WMTK ARENA location on your computer, and navigate to the "Reports" folder.

d. Experiment Set-Up Menu:
i. Acquisition Time:
- Set the total run time for the plate reading in minutes (ex: 120 minute run time for a 2 hour plate reading experiment).
- Acquisition time cannot be less than 15 minutes.
ii.   Plate Format:
- Choose your appropriate plate format using this drop-down.
- Currently the WMTK ARENA has been validated to work with 6-well plate and 24-well plate formats, as well as 35mm single plates when used with an adapter (included).
- For use of the WMTK ARENA with 35mm plates, ensure that the 35mm plates fit snugly within the adapter.
- For use of the WMTK ARENA with 35mm plates, choose the "6-well" option in the Plate Format drop-down menu (data acquisition arrangement for the instrument is the same for both the 6-well plate and the 35mm plate setup).
iii.   Temperature:
- Set the experimental run temperature, selecting the Temperature's check-box and using the drop-down menu to choose the desired temperature. Run temperatures can be set between 20ºC and 37ºC.
- The check-box allows you to pre-heat or pre-cool the instrument prior to starting your run.

e. Start Button:
- Press here to start your experimental run!
- The software will run through several automatic calibration steps, and the instrument will begin scanning your plate.

IV. Defining and Starting Your Experiment

1. Create a new project by clicking **"New"** on the left-hand project menu.
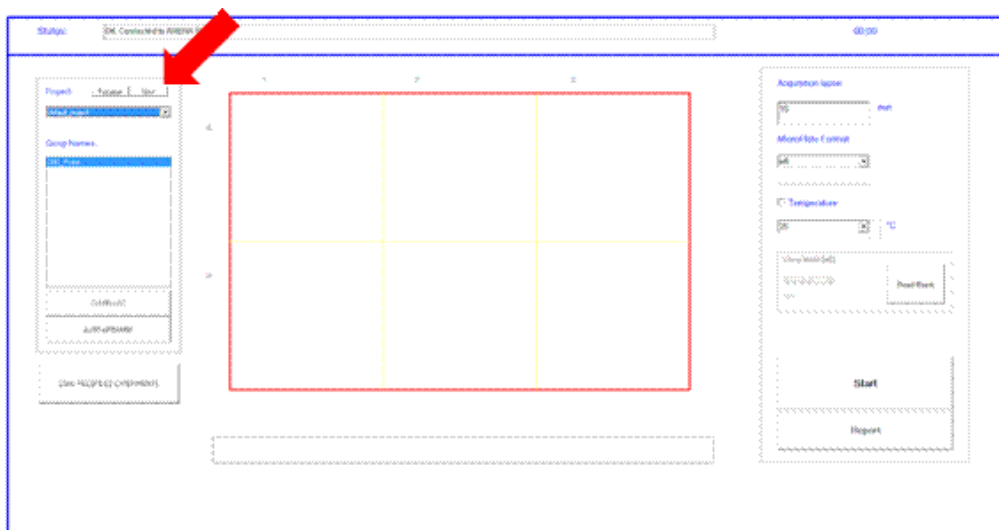
2. A **"New Project Creation"** window will pop up; name your new project in this pop-up window. This is the file name under which you are able to locate your project run reports and acquired data in the WMTK ARENA folder.
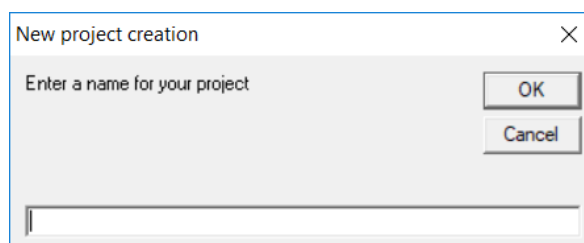
3. Enter the **"MicroPlate Format"** size that you will be using.

NOTE: At this time, we have validated the instrument for use only with 6-well, 24-well and 35mm plate formats.

4. If you are changing to a different plate size, you will be prompted to confirm your selection.
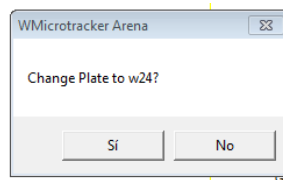
5. Set the **"Acquisition Lapse Time."**

NOTE: The minimum recommended collection time is a 15 min run.

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

NOTE: In 24-well microplate format, the number of technical replicates recommended per group is four wells (average standard deviation < 15% in the activity between homogeneous groups in liquid media).

a. MANUAL Layout
- Highlight the wells that you want to set as a group.
- In the **"Group Names"** section, double-click to label your group; repeat for each group in your experiment.
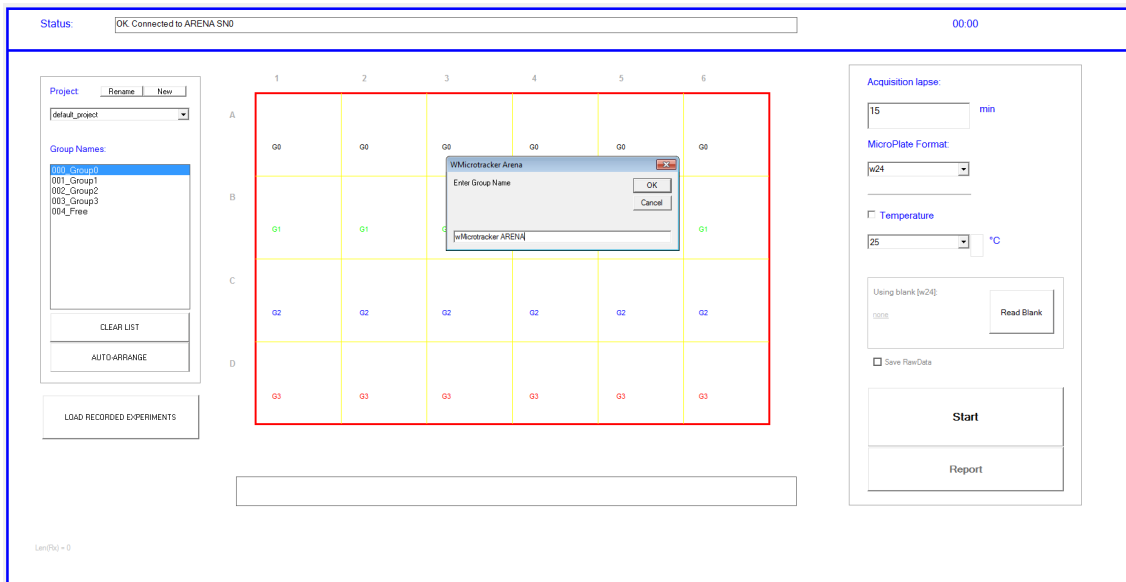
b. AUTO-ARRANGE Layout
- Click **"Auto-Arrange"** to select the number of rows and columns you wish to use; the arrangement options will differ depending on your chosen plate format.
- In the **"Group Names"** section, double-click to label your group; repeat for each group in your experiment.
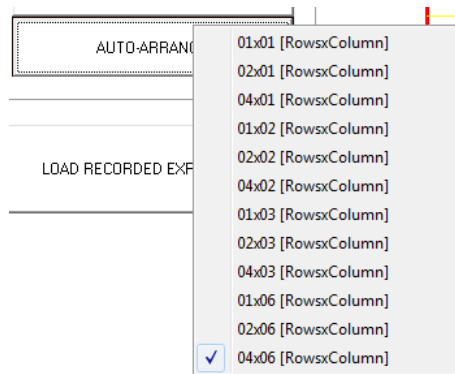
c. CLEAR LIST Button
- This will clear the existing Group Names that you have listed and arranged; no name or arrangement information will be saved.
- You will be prompted to confirm your selection.
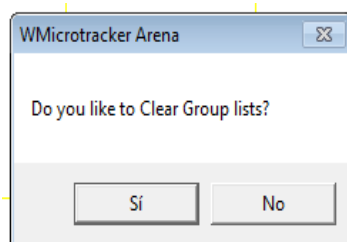
7. Set the option **"Blue light"** if your experiments require it.

In some versions of the equipment you can select the "Enable blue lighting" option from the button "Tools" on the main bar.
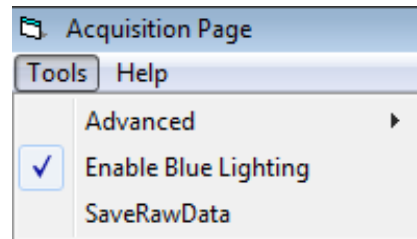
A blue-light menu will appear in the right-hand set-up menu.

-Light Pulse: click on if you want to switch on blue light before the equipment begins to acquire activity.

- Enable: use the check box to enable blue lights cycle during acquisition. Set up the parameters of blue light cycles:

    - ▇ : Set the amount of time (in minutes) that the blue lights stay off in each cycle.

    - ▇ : Set the amount of time (in minutes) that the blue lights stay on in each cycle.

    - Times: number of times that the cycle will repeat.

    - Power: Set the power of Blue light, between 1 (low) and 7 (high).

NOTE: Intensity from 1 to 5 can be used during data acquisition. Intensity 6 to 7 should only be used for stimulus pulses prior to data

acquisition (High power can generate interference/noise during your run).The software will display the next reminder window
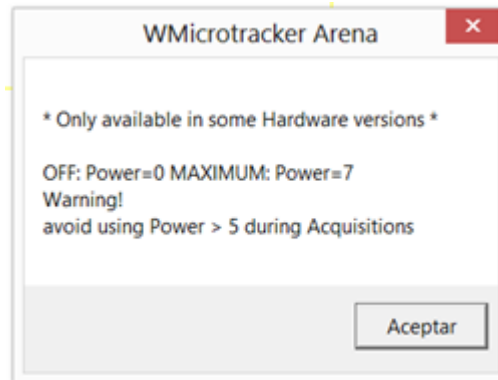
8. Set or change the temperature for your experiment by using the **"Temperature"** check-box and drop-down menu. Instrument reading temperature can currently be set between 20ºC and 37ºC (max low limit = room temperature -5ºC).
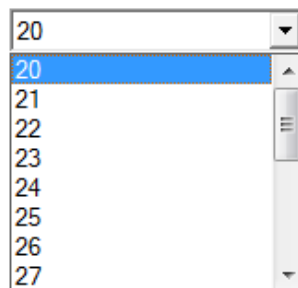
9. After entering the temperature choice and selecting the temperature check box, you will be asked if you want to **"Pre-Heat / Pre-Cool"** the instrument to the temperature you specified.

NOTE: If pre-heating or pre-cooling the instrument, please allow approximately 30 minutes for the temperature to adjust before starting your experiments.
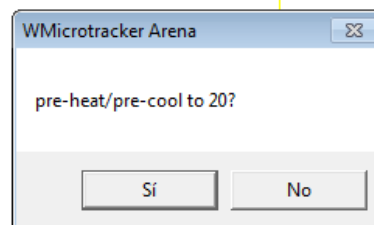
10. You are now ready to load your plate and start your experiment running!

V.   Running Your Experiment

1. After setting experimental parameters, load your plate into the instrument and press **"Start"** to begin your experiment.

2. You will then be prompted to enter a specific name for your experiment run ("Acquisition"); this will be filed under your project name.
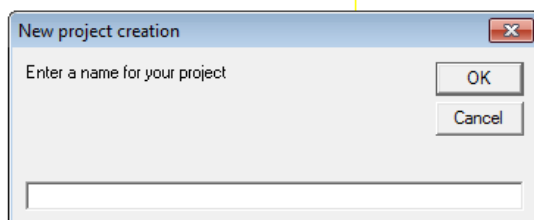
3. After entering the acquisition name, select **"Play"** and the ARENA will automatically begin calibrating the 6 cameras (CAM 0-5) to the unique conditions of your experiment. This process will take a few minutes to complete; calibration progress will be indicated on the main screen.
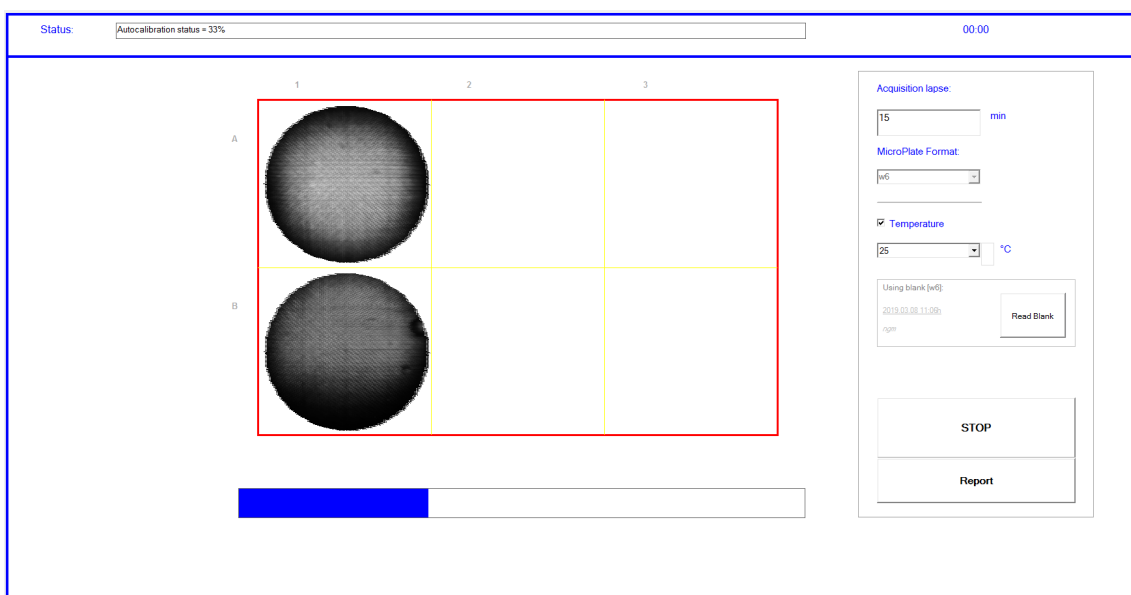
4. When calibration is complete, acquisition of your plate will begin automatically and the ARENA will start collecting data. The clock in the upper right-hand corner will begin to count up and the progress bar will begin to monitor progress as samples are being analyzed.
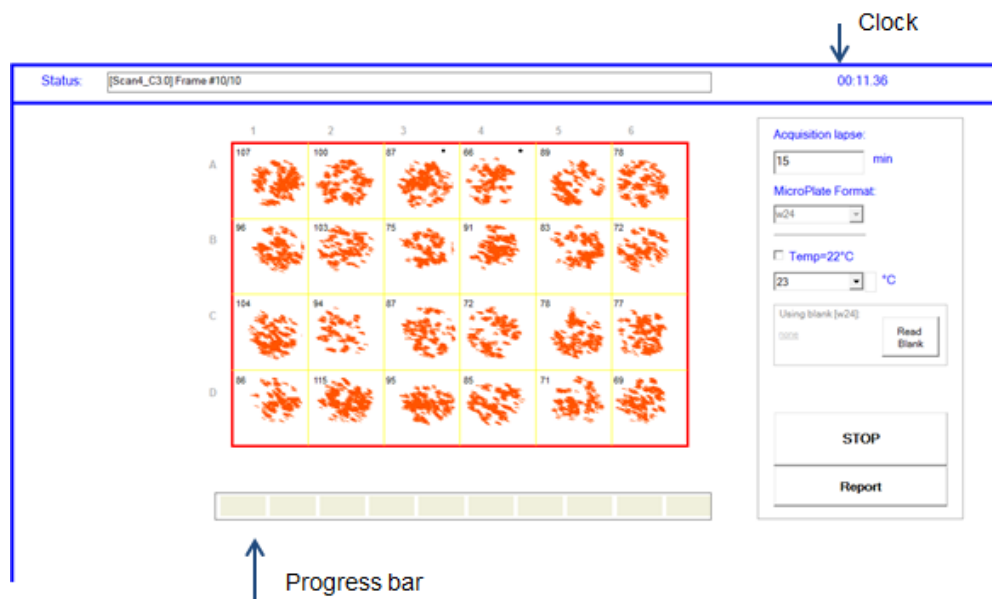
5. During acquisition, the plate layout on the main screen will continuously update with current run information:
   - The top-left corner of each well will indicate total cumulative activity count for that well.
   - The location of activity within a well will be indicated by a population of red marks.
   - The well(s) currently being analyzed will be indicated by a small black dot in the top-right corner of that well.
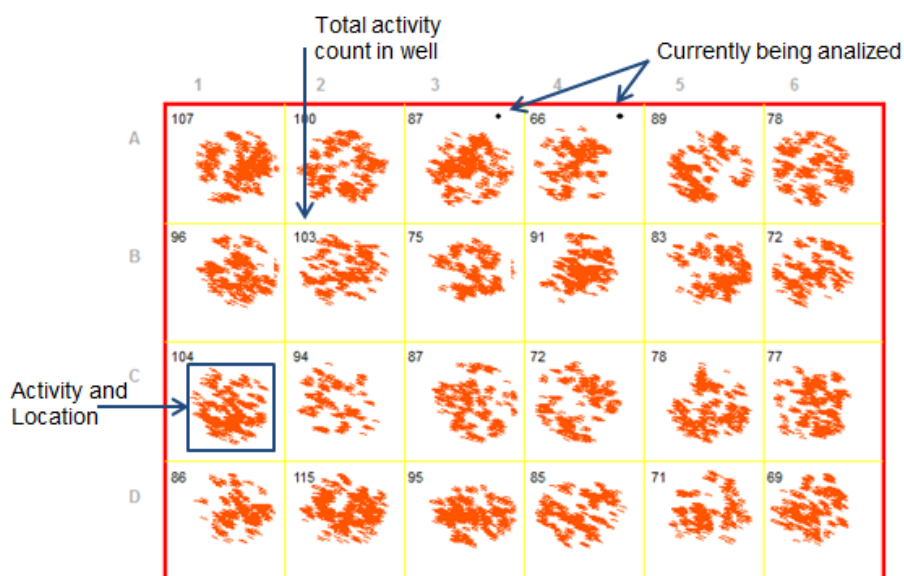
6. When the experimental running has finished, the main screen status bar will show "DONE!". The scan is complete, and the instrument will automatically open up the **"Generate Report"** pop-up window. You can export your data immediately or later by utilizing the **"Report"** button.

VI. Accessing Experiment Data

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

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

Generate an Immediate Report

1. At the end of an experiment run, the following "Generate Report" window will automatically appear:

2. You have several options for data export arrangements: by **"Rows"**, **"Array"** or **"Map Plot"** format.

   i. **Rows** will provide data for each well in an individual sheet row.

7	******GROUP_Average******								
8	Group/Time[	30,0	60,0	90,0	120,0	150,0	180,0	210,0	240,0
9	000_Control	333	225	205	181	200	190	186	186
10	001_100uM Le	249	189	118	90	70	42	43	36
11	002_200uM Le	241	135	52	14	2	1	1	2
12									
13	******STANDARD ERROR******								
14	Group/Time[	30,0	60,0	90,0	120,0	150,0	180,0	210,0	240,0
15	000_Control	41,0	10,0	9,5	11,5	14,0	2,0	12,5	3,0
16	001_100uM Le	11,5	6,0	3,0	8,5	12,5	12,0	8,0	1,0
17	002_200uM Le	7,0	4,0	2,5	5,5	1,0	1,0	0,0	1,5
18									
19	******N******								
20	Group/Time[	30,0	60,0	90,0	120,0	150,0	180,0	210,0	240,0
21	000_Control	2	2	2	2	2	2	2	2
22	001_100uM Le	2	2	2	2	2	2	2	2
23	002_200uM Le	2	2	2	2	2	2	2	2
24									
25	***WELL ACTIVITY***								
26	Well	Group/Time	30,0	60,0	90,0	120,0	150,0	180,0	210,0
27	A1	000_Control	292	215	214	192	214	192	198
28	B1	000_Control	374	235	195	169	186	188	173
29	A2	001_100uM L	237	183	115	81	57	30	35
30	B2	001_100uM L	260	195	121	98	82	54	51
31	A3	002_200uM L	234	131	54	19	1	2	1
32	B3	002_200uM L	248	139	49	8	3	0	1
33									
34									
35	***TEMPERATURE***								
36	Time[m]	0,0	3,4	6,8	10,3	13,7	17,2	20,8	24,2
37	Temperature	23,3	23,4	23,2	23,4	23,5	23,5	23,4	23,4

ii. **Array** will provide data for each well in plate-format layout.

	WELL ACTIVITY						
7	0.3m						
8	ROW/COL	1	2	3	4	5	6
9	A	28	24	23	0	23	0
10	B	17	29	23	34	0	26
11	C	31	23	30	31	25	21
12	D	0	19	31	31	19	27
13							
14	4.0m						
15	ROW/COL	1	2	3	4	5	6
16	A	30	22	16	0	16	0
17	B	17	24	19	28	0	20
18	C	15	15	23	21	20	8
19	D	0	14	16	25	16	19

iii. **Map Plot** will provide data of the activity relative-location for each well, calculating the Chemotaxis Index at each ScanTime. Choose the correct design of the plate for the analysis (2 sides/quadrant) and the test spot location. Then, click on the Process Files button to run the analysis.
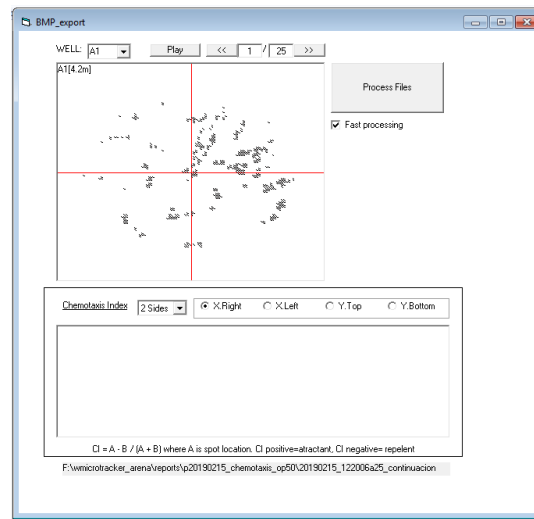
5	Chemotaxis_Index								
6	Well	Group/Time[m]	0.4	3.6	6.5	9.4	12.5	15.5	18.7
7	A1_CiXright	-001_Free	0.2	0.3	0.3	0.3	0.3	0.3	0.3
8	B1_CiXright	-001_Free	-0.8	-0.5	-0.2	0	0.2	0.2	0.2
9	A2_CiXright	-001_Free	0.5	0.9	0.9	1	0.9	0.9	0.9
10	B2_CiXright	-001_Free	-0.8	-0.5	-0.3	-0.1	0	0	0
11	A3_CiXright	-001_Free	-0.3	-0.1	0	0.2	0.3	0.3	0.3
12	B3_CiXright	-001_Free	-0.7	-0.4	-0.2	0.1	0.3	0.3	0.3

14	Activity_map							
15	Time[m]	0.4		3.6		6.5		
16	A1_top	214	595	470	1386	738	2378	
17	A1_bottom	2984	3930	3160	4823	3641	5128	
18								
19	B1_top	2864	307	3160	725	2510	1072	
20	B1_bottom	2809	419	2622	1017	2211	2021	
21								
22	A2_top	0	33	0	86	0	480	
23	A2_bottom	723	2125	187	2454	178	3863	
24								
25	B2_top	1438	158	1460	384	1372	674	
26	B2_bottom	1894	201	2047	797	2062	1326	
27								
28	A3_top	0	5	0	49	8	136	
29	A3_bottom	807	478	926	660	917	809	
30								
31	B3_top	647	140	604	245	575	356	
32	B3_bottom	422	57	504	195	434	371	

3. Export and view the data using different "Bin Size" formats.
    i. Scan Time bin size will output data for each individual scan taken of each well in your plate.
    ii. Minute Bin sized activity (ex: 30, 60 min bins) will transform scanned counts into Time blocked activity and will output that information.

   Important: When the data is exported in Bin Sizes of different # Minutes the software will integrate "scan time" data using one minute resolution timeframe transformation. So, the result will differ from the raw sum of scantime activity.

4. You can also modify the "File" and "Spacer" fields as best suited to your data analysis needs.
5. Apply internal reference. For default it will apply factory internal reference.
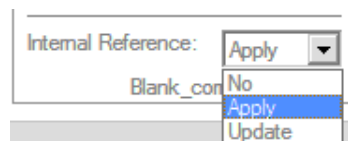
6. Apply Blank Correction. (See Blank measurement in Appendix D).
7. When you are ready to view your data, press **"Generate"**. This will automatically open a new window with your data displayed; data can be analyzed or further saved from this new window, as required.

Export Previous Experiments

1. You can access your data at any time after a completed run directly through the software main screen.
        - Press the **"Load recorded experiments"** button at the bottom right; this will open a **"Previous Experiments"** menu on the main screen:

- Select the desired project name from the list; each project will expand to show the acquisitions (if more than one) that were collected for that project. Select and double-click the appropriate acquisition and the **"Generate Report"** window will appear, allowing you to export data in the format you wish.

2. You can also access your data at any time directly through the **"Reports"** folder that was installed with your software. It will contain all the project data acquired on your ARENA instrument.

- In the computer location you chose for the software, simply navigate to the Reports folder to see all stored project data:

- Select and open the project folder you wish to analyse; this will lead you to files containing activity data and temperature data for the experiment.

- Select and double-click the "_report.csv" file that you wish, this will automatically open a new window with your data displayed; data can be analysed or further saved from this new window, as needed.

Joint Reports

To obtain a unique report of a project with the data of all acquisitions,

1- Select all together the subprojects you want to join and press right-click, click-on Joint Report.
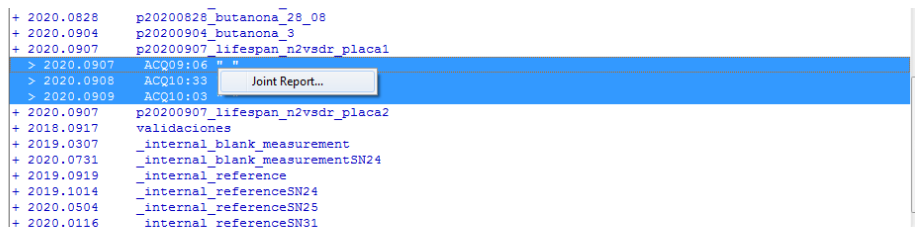
2- You will be asked if you want to remove outliers from the analysis. Activity from one scan is considered as an outlier when the activity obtained from one scan gives plus/minus 3.5 means of 2 scans before and 2 scans after the scan acquisition. Removing outliers is recommended when you expect to get constant activity during each day's acquisition.

3- Then, the window "Generate Joint Report" will appear.

After choosing the reports options, press "**Generate Joint Report**". This will automatically open a new window with your data displayed; data can be analyzed or further saved from this new window, as needed.
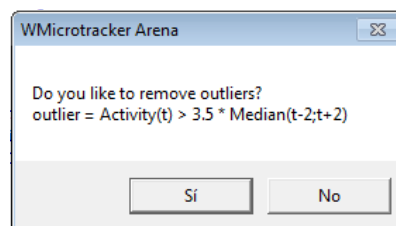
The WMicroTracker Arena provides the capability to measure the locomotor activity of small animals cultured in function of time and space. The system detects the movement of organism populations through the interference caused by them in a large array of infrared light microbeams.

ARENA Quick Start

1. Launch ARENA software from your chosen computer location.

2. Create and name a new project, or load an existing project.
   - If creating a new project, allocate plate layout and name groups.
   - If loading an existing project, double-check plate layout.

3. Set experiment acquisition time
   - NOTE: Minimum acquisition time recommended is a 15 min read.

4. Set experiment well format.
   - NOTE: Only 6-well, 24-well and 35mm plate formats currently validated. All plate formats must be run with the lid on. It is recommended to seal the plate/ microplate with film (This decreases the formation of condensed drops on the lid).

5. Set experiment run temperature (20ºC – 37ºC).
   - Select the temperature check-box and the desired temperature.
   - Allow approximately 30 minutes for temperature adjustment if pre-heating/pre-cooling instrument.

6. Load your experiment plate into the instrument.

7. Click "START" to begin experiment reading.
   - Instrument will automatically run through several calibration steps.
   - Instrument will begin data acquisition immediately following calibration.

8. Upon read completion, the status bar will indicate "DONE!".
   - Generate a report for immediate analysis or access the data later.
   - Remove your experiment plate and exit the software.

KEY APPLICATIONS:
- Toxicity Assay
- Aging and healthspan
- Chemotaxis on agar
- Heatshock stress

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Curve 6W Microplate: Young Adult_N2 (*C.elegans*)_NGM
The system presents a very good linearity of detection between 10 and 300 worms, observing a plateau at more than 300 (R2=0.99).

$R^2 = 0.9911$

(Activity (Counts/60min) vs Number of Worms)
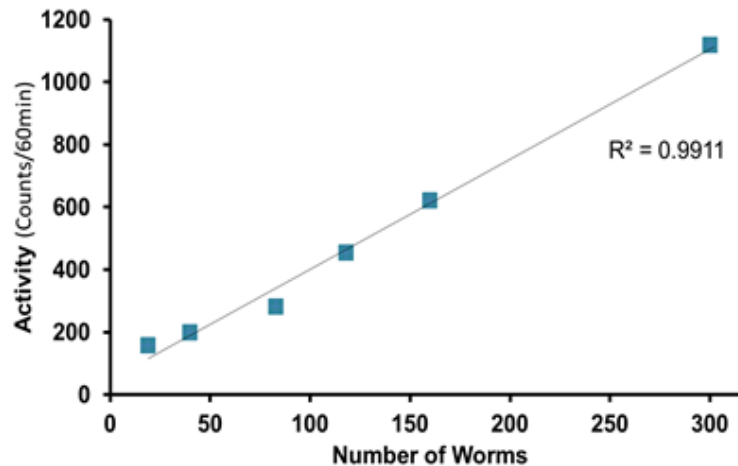
Curve 24W Microplate: Young Adult_N2 (*C.elegans*)_NGM
The system presents very good linearity of detection between 5 and 100 worms (R2=0.96), observing a decrease at more than 100 worms.

$R^2 = 0.9677$

(Activity (Counts/60min) vs Number of worms)

NOTE: These results were obtained using NGM plates, no seeded bacteria. Worms' locomotor activity changes in presence of food.

Phylum TECH

wMicroTracker©

arena

WHAT YOU NEED:
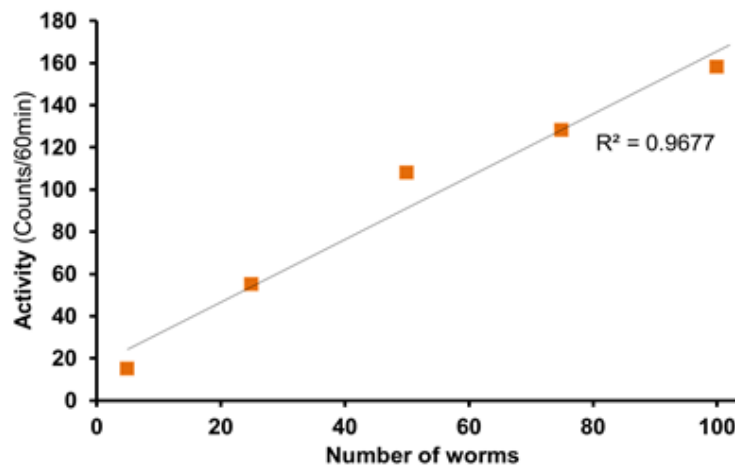
6 well plate with lid.

NGM.

M9 buffer.

Levamisole – Stock solution: 100 mM in ddH20.

Synchronized populations of young adult worms.

wMicrotracker Arena device.

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
- Plates can be used within two weeks after prepared when stored in a container at 4 °C.
- The assay is performed on plates that had been allowed to template to room temperature prior to the start of the experiment.

PROTOCOL:

1. Grow synchronized populations of adult worms in seeding NGM plates (OP50).

2. Collect worms from plates using M9 buffer and transfer them in a sterile 15 ml tube.

3. Let the worms settle. Remove the supernatant taking care not to disturb the pellet.

4. Perform a wash with 5 ml of M9 buffer. Briefly shake or invert the tube. Repeat step 3.

5. Add 3 ml of M9 buffer.

6. Count number of worms in 10 μl in triplicate and calculate the average.

7. Prepare a suspension to get [35 worms/10 μl]

8. Transfer 10 μl of worm solution to 6-well plate.

10. Register worm activity using wMicroTracker ARENA and generate the data report using WMicrotracker software.

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

METHODS
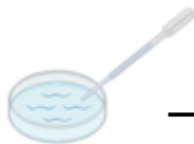
(A) Rinse synchronized worms from the plates using buffer solution.

(B) Count the number and adjust the volume to obtain a concentration of [35 worms /10 μl].

(C) Transfer 10 μl of the worm solution to a 6 well plate containing NGM+levamisole without bacteria.

(D) Record the activity of the plate with worms using wMicroTracker ARENA for 4 h

RESULTS

- Control
- 100μM Levamisole
- 200μM Levamisole
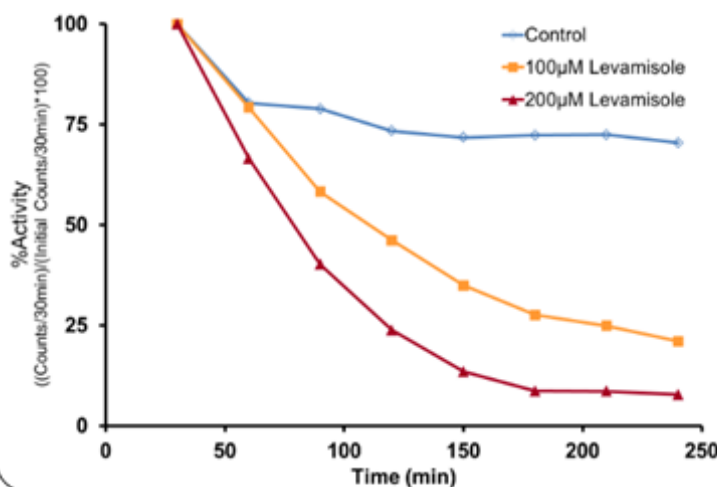
(E) Generate the data report using ARENA software and plot

Dose-inhibition response curve of levamisole on N2 locomotor activity. The effect of different concentration of levamisole (0μM (control),100μM and 200μM) was evaluated. Thirty-five young adult N2 were transferred per well in a 6-well plate. The locomotor activity was recorded at 23°C during 4 hours. Data represents the mean of four independent experiments conducted in duplicate.

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WHAT YOU NEED:

24 well plate with lid.

S complete medium .

Streptomycin, stock 100 mg/ml.

Kanamycin, stock 100 mg/ml.

Amphotericin B, stock 250 µg/ml.

OP50 Feeding bacteria in S-complete medium, stock 100 mg/ml.

Synchronized populations of L4 worms.

wMicrotracker Arena device.

Notes:

Sterile conditions are need by using flow cabinet or flame during manipulation of the worms.

Perform at least four technical replicates and at least two biological replicates.

To prevent starvation of worms, feeding bacteria need to be added. You might have to add bacteria in day 3, 7, 12, 19, 26.
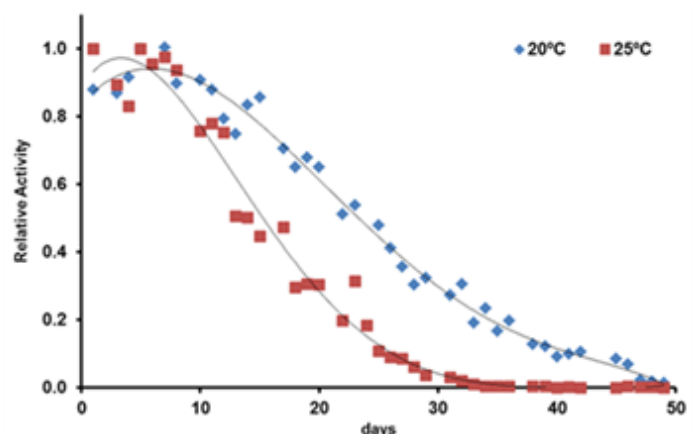
- After recording the activity of the worms using wMicrotracker ARENA, a fast inspection of the plate is recommended to assess if bacteria need to be added.
- Remove the sealer from the micro-plate maintaining sterile conditions.
- Add 20 µL of 10 mg/ml bacterial suspension in each well and seal the plate again.
- Shake the culture gently by hand.
- Return it into the incubator.

DAY 0_Transfer L4 worms to 24-well plate

1. Collect Synchronized L4 worms by washing the NGM agar plates with S Complete medium.

2. Adjust the final volume so that worm concentration in the suspension is approximately 100 worms/ml.

3. Add streptomycin (to a final concentration 100 µg/mL), kanamycin (20 µg/mL) and the antifungal drug Amphotericin B (0,1 µg/mL) to avoid contamination.

4. Add an appropriate volume of feeding bacteria in S-complete media to a final concentration of 3 mg/mL.

5. Add FUdR to a final concentration of 50 µM.

6. Mix the worm suspension. Transfer 500 µL of the suspension in each well of a 24-well plate (final: 50 worms/well).

7. Seal the plate to avoid contamination.

8. Put the plate in the incubator at the desired temperature.

DAY 1 to 50_Measuring locomotor activity

1. Before setting the plate into the wMicrotracker ARENA, make sure there is no condensed water on the plate lid. If necessary, dry the plate lid maintaining sterile conditions.

2. Shake the microplate of worms gently by hand or stimulate it using high intensity blue light to induce animal movement.

3. Record the activity of the plate with worms using wMicrotracker ARENA for 30 min at the same temperature of the incubator.

4. Generate the data report using the software.

METHODS

DAY 0

DAY 1 to 50

Transfer 50 L4 worms in 500 µl to 24-well plate

Register worm activity using wMicroTracker ARENA
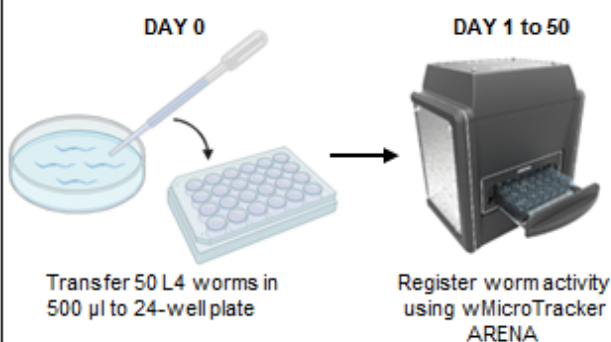
RESULTS

Locomotion-based healthspan assay

Locomotion activity of N2 was evaluated during 50 days. On day 0, L4 larvae stage N2 worms were transferred to S Complete Medium with 50 µM FudR in a 24-well plate. The worms were allowed to grow at 20°C or 25°C. Locomotor activity was recorded at 20°C/25°C during 30 min.

Data represents the mean activity recorded each day (n=9 per group, 2 independent experiments)

PhXlum
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Appendix C. ARENA Application_Chemotaxis

ODORANT TEST SOLUTION – 2SIDES

WHAT YOU NEED:

35mm plates with chemotaxis (CTX) media.

CTX buffer.

Test solution/Control solution.

Synchronized populations of well-fed adult worms in seeding NGM plates (OP50).

wMicroTracker Arena device.

Notes:

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

Do not add azide to the test/control spot.

When adding the worms or the test/control solution, be careful not to scratch the agar with the pipette – worms tend to crawl into any break in the agar surface.

Acquired images will be available to generate .gif animated videos (See Report-Option Map Plot).

PROTOCOL

1. Obtain synchronized populations of well-fed adult-day1 worms in seeding NGM plates (OP50).

2. Collect the worms from the plates using CTX buffer solution. Wash 3 times with CTX buffer (Let the worms settle and remove the supernatant).

3. Count the number of worms in a volume of 10 µl and adjust the volume to obtain a concentration of [50 worms / 10 µl].

4. Transfer 10 µl of the worm solution to left side of a 35 mm Petri dish.

5. At the right sight put 1 µl of the odorant test solution on the agar (0.05%, 1% and 100% Benzaldehyde diluted in EtOH).

6. Seal each plate with parafilm.

7. Record the activity of the plate with worms using wMicroTracker ARENA for 2h at 23ºC.

8. Generate the data report using the MapPlot Software algorithm – 2 sides CI calculator. Plot using MSExcel.

METHODS

(A) Rinse synchronized worms from the plates using CTX buffer

(B) Count the number and adjust the volume to obtain a concentration of [50 worms /10 µl].

(C) Transfer 10 µl of the worm solution to the left side (w). At the right side put 1 µl of the odorant solution (T)

(D) Record the activity of the plate with worms using wMicroTracker ARENA for 2 h

RESULTS

- 100% Benzaldehvde
- 1% Benzaldehvde
- 0.05% Benzaldehvde
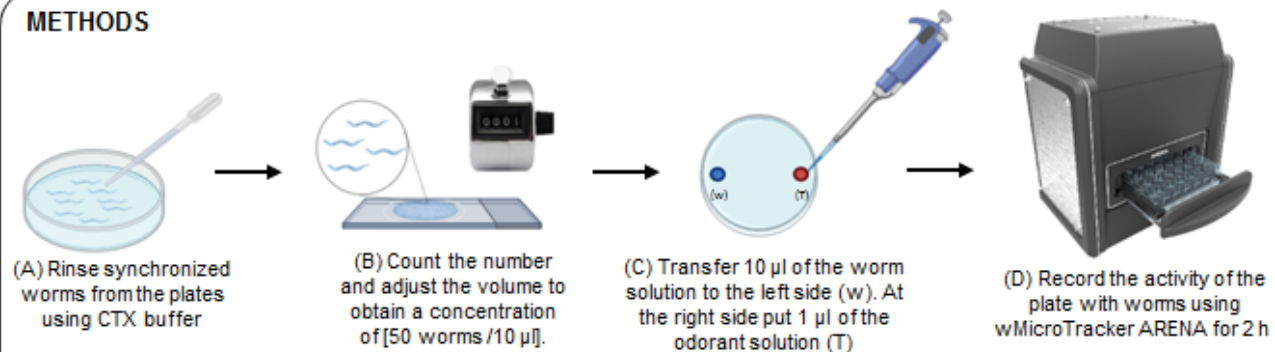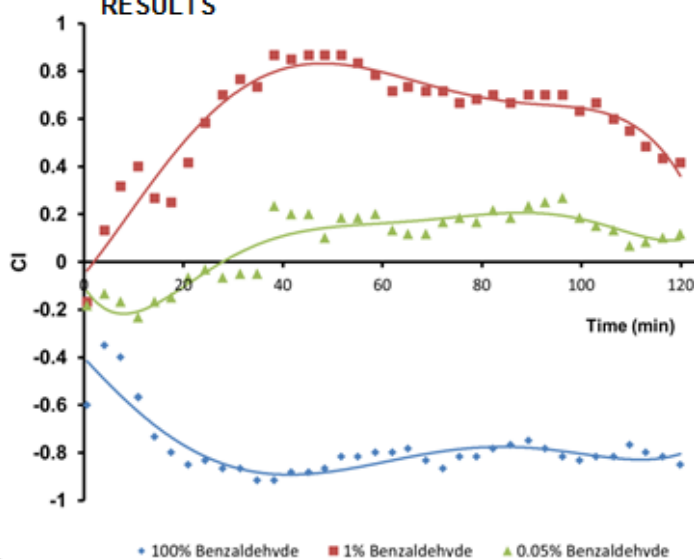
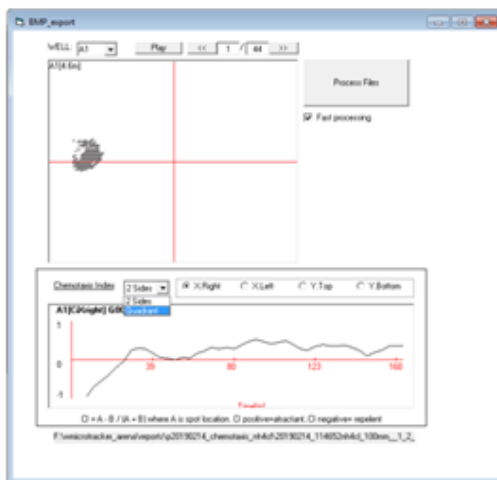(E) Generate the data report using ARENA software and plot

A different chemotactic response is observed when worms are tested against benzaldehyde 0.05%, 1% (attractive) or 100% (repellent). Fifty young-adults N2 worms were transfer on the left side of a 35-mm plate, on the opposite side was spotted the odorant diluted at each concentration. The locomotor activity was evaluated at 23°C during 2 hours. Three independent experiments were performed in duplicate (Data represents the mean).

REPORT – Option MapPlot

• To generate the report of the chemotaxis assay choose MapPlot option

Report	○ Rows	○ Array	● MapPlot	×

BinSize
ScanTime ▼ min.

File | .csv ▼
Spacer | ▼
blank_correction ☐
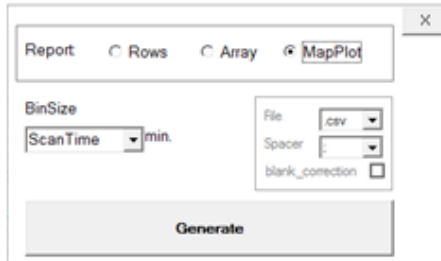
Generate

• The following window will appear

_Choose the correct design of the plate for the analysis (2 sides/quadrant) and the test spot location.

_Click on Process Files button to run the analysis

• A file with the data will be generated

• You can plot CI vs. time

• You can obtain a .gif

After generating a MapPlot report, images are saved in:
wmicrotracker_arena\reports\proyect_name\assay_name\3scans_images

Open Image J
File \ Import \ Image Sequence \ choose the images-assay's folder
Image \ Type \ 8-bit
File \ Save as \ Gif

Sequence Options

Number of images: 180
Starting Image: 1
Increment: 1
Scale Images: 100 %
File name contains: A1 well

(enclose regex in parens)

☐ Convert to RGB
☑ Sort names numerically
☐ Use virtual stack

341 x 277 x 30 (10.8MB)

OK | Cancel | Help

Interpreting the Chemotaxis Index (CI)
CI = (# activity in test spots - # activity in control spots) / Total # activity in the well.
A +1.0 CI score indicates maximal attraction towards the target and represents 100% of the worms arriving to the spots containing the chemical target. An index of -1.0 is evidence of maximal repulsion.

Phylum TECH

wMicroTracker®
arena

WHAT YOU NEED:

6 well plate with lid.
NGM.
M9 buffer.
Synchronized populations of worms in seeding NGM plates (OP50): L3 and 3-day adults.
wMicroTracker Arena device.

Notes:

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

When adding the worms, be careful not to scratch the agar – worms tend to crawl into any break in the agar surface.

PROTOCOL

1. Obtain synchronized populations of L3 and 3-day adults in seeding NGM plates (OP50) at 22°C.

2. Collect worms from plates using M9 buffer and transfer them in a sterile 15 ml tube.

3. Let the worms settle. Remove the supernatant taking care not to disturb the pellet.

4. Perform a wash with 5 ml of M9 buffer. Briefly shake or invert the tube. Repeat step 3.

5. Add 3 ml of M9 buffer.

6. Count number of worms in 10 µl in triplicate and calculate the average. Prepare a suspension to get [35 worms/10 µl].

7. Transfer 10 ul to each well of a 6 well plate containing NGM. Seal the plate with parafilm.

8. Register worm activity using wMicroTracker ARENA for 8 hours:

   a. Set the temperature at 22°C to record basal activity. Aqcuire at least 30 min

   b. For heat shock, high up the temperature to 37°C during 90 min.

   c. Then return temperature to 22°C.

9. Generate the data report. Plot using MSExcel.
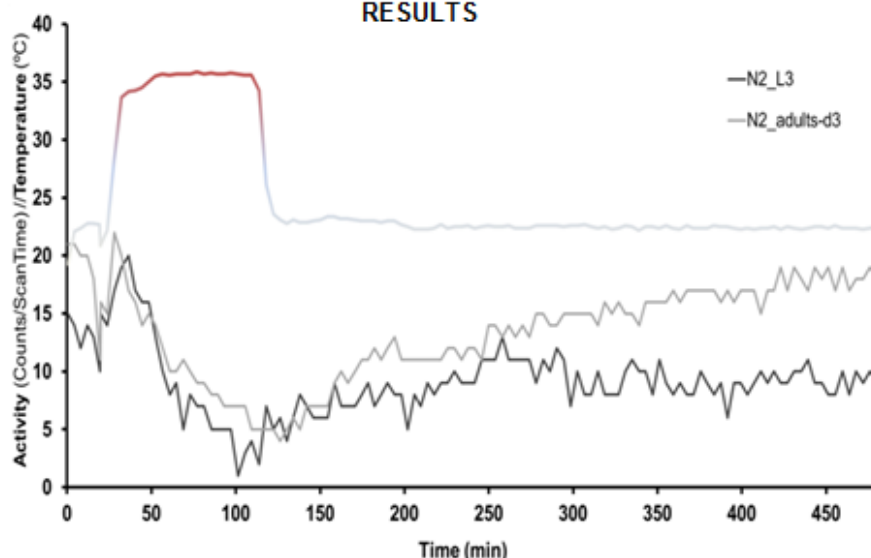
METHODS
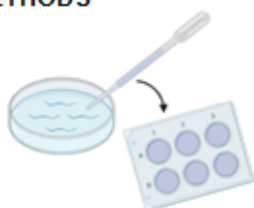
Transfer 35 worms/well to a NGM 6-well plate

Register worm activity using wMicroTracker ARENA

High up the temperature for the heatshock in the wMTK ARENA

RESULTS

— N2_L3
— N2_adults-d3

Differences in locomotor activity following heat shock stress when comparing N2 worms in two life stages. Fifty L3 or adults d3 N2 worms were transferred per well in a NGM 6-well plate. The locomotor activity was evaluated 30 min at 22°C, 90 min at 37°C (heat-shock) and 360 min at 22°C. Data represents the locomotor activity recorded at each time by 3 replicates per condition.
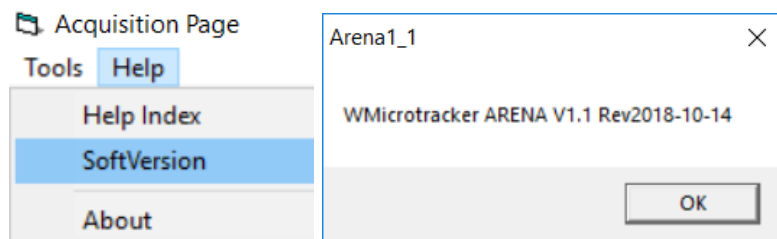
Phylum TECH

1. **Hardware/Software Troubleshooting**

   If your system is not detecting the new COM PORT, please check the following:

          a. Test proper system power source.

          b. Ask your software administrator if you are able to install new USB Drivers or change COM Port numbers.

2. **Additional Information**

   a. You can find the ARENA software version located in the **"Help"** tab at the top of the software main screen.

   b. Additional calibration tools for the ARENA can be found under the "Tools" tab of the software main screen.

   > **IMPORTANT NOTE!**
   > The system is already calibrated in-factory.
   > Only use the calibration option in case the Phylumtech assistant has recommended it.
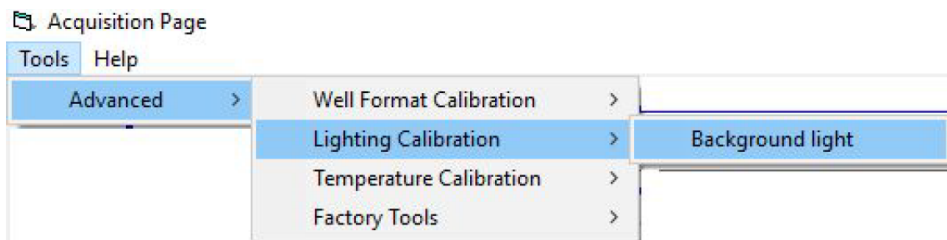
Factory settings Calibration procedure;

> **IMPORTANT NOTE!**
> The system is already calibrated in-factory.
> Only use the calibration option in case the Phylumtech assistant has recommended it.

● *Background Light Calibration:*

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

b. It will be required to remove the microplate. The lighting calibration procedure will take from 5 to 10 minutes to run. Setup results are going to be saved for future experiments.

● *Well Position Calibration:*

a. 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. Select microplate format to use, and place the empty plate inside WMicrotracker ARENA.
NOTE: For 35mm Petri dishes use the provided plastic adapter and select "w6" format.
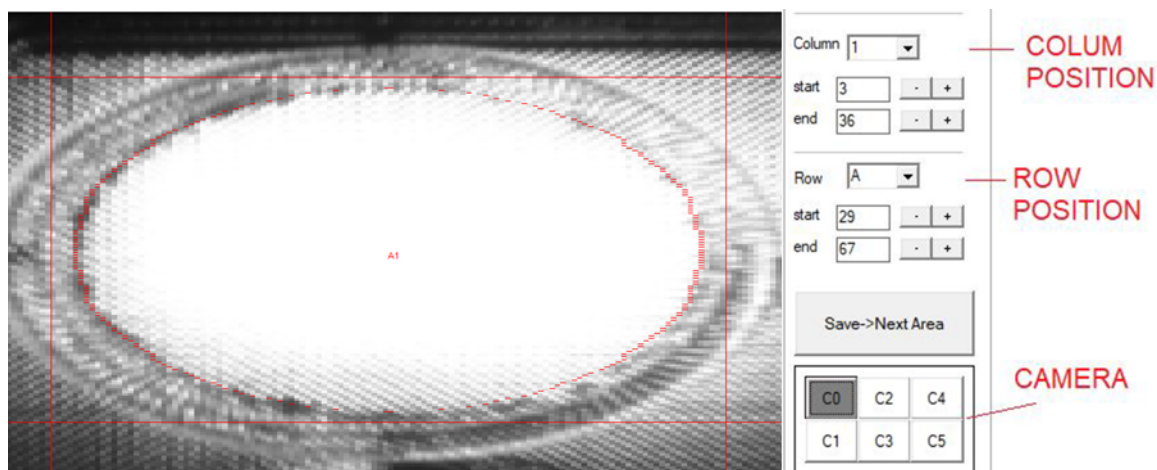

b. Select the menu "Advanced/Well format Calibration Tool/Calibrate"
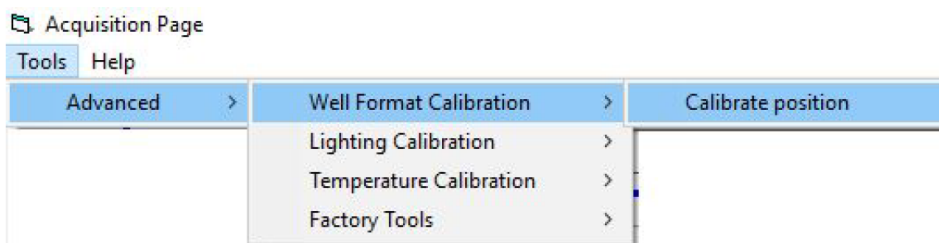
c. A windows showing the capture of the microplate portion will appear

Move the columns and rows for each camera in order to set the sensing area for each well. The red line is recommended to be located in the area near the bottom of the wells. 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. The updated config files will be saved in the PC where you make the well position calibration. If the equipment is going to be used with another PC you will have to copy the folder with the config files to the new PC.

- *Temperature Calibration:*

NOTE: 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.

- *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.

In the "Generate Report" window select Blank correction.

Each experiment report folder contains the following files:

- **_report.csv -** consolidated report, resumes all the information of the acquisition.
- **Adjusted activity.txt -** contains the accumulated activity adjusted by internal factor correction.
  In each row it's detailed the:
  - Time [m] at which the scan finishes.
  - Well scanned.
  - Accumulated activity of the well-adjusted by internal factor correction.
- **Blank_w6.dat - c**ontains the values of the read blank.
- **Chgroup.dat -** contains the group's name and the assignment of the group to each well. In each row it's detailed the:
  - Well.
  - Group number.
  - Group name.
- **Current_activity.txt –** contains raw activity files without internal factor correction applied.
  In each row it's detailed the:
  - Time[m] at which the scan finishes.
  - Well scanned.
  - Raw accumulated activity.
- **current_temperature.txt –** contains the temperature registered at each time.
- **factor_correction.txt** – contains the correction factor.
- **lastread.csv –** contains values of the last scan per camera. To measure the activity, each camera scans' acquire ten sequential pictures.
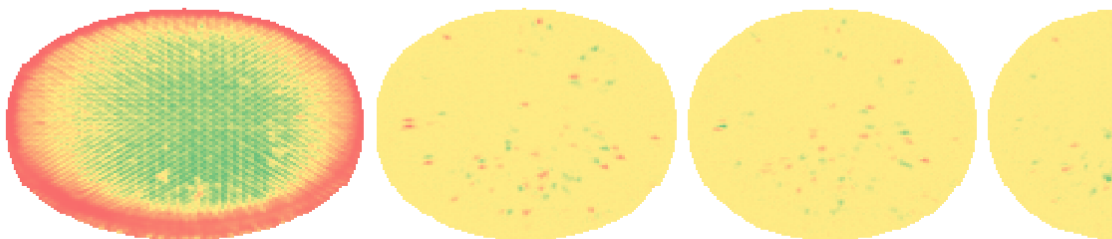
On the first ring it's registered the values for each pixel in the first picture of the last scan of the acquisition. On the following rings it is shown the difference between the pixels of that picture (n) and the picture before (n-1).
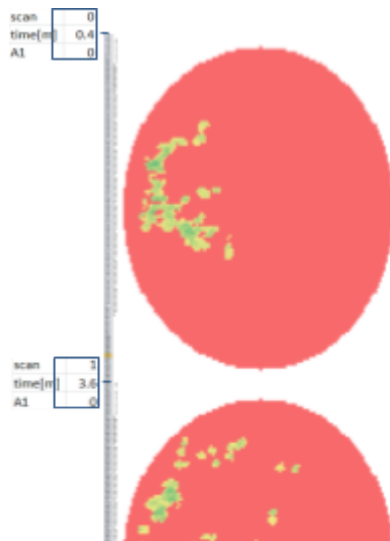Note: the rings are 90º left rotated.

- **Light –** contains the information about the use of blue light during the acquisition.

In each row it's detailed the:
#scan
time[m]
light power[0=off; 1=on]
- **serial.dat** – contains the serial number of the device.
- **subpj.dat** – contains the information about the acquisition
    - Name of the acquisition/subproject.
    - Acquisition time set.
    - Plate format set.
- **wellactivity.csv** – contains the activity calculated for each scan of a camera.

Note: the pictures are 90° left rotated.

Additional Notes:

- Camera numbering order:

0	2	4
1	3	5

- To better visualize the files lastread.csv and wellactivity.csv apply conditional format to each set of data.

Product Electrical Specifications

WMicrotracker®	ARENA
Power Source	12VDC 6Amp
Power Consumption	without Temperature Control:6W with Temperature Control: 70W
Sensing Detection Technology	TTL CMOS camera 1Megapixel x6
Lighting Technology	880nm Infrared Led Array
Blue Light	Royal Blue Leds 3W x12
Communication Protocol	USB CDC Driver. 1Mbps
Cooling / Heating Technology	Peltier
Temperature Regulation Range	20 - 37ºC
Recommended Ambient Working Conditions	15 - 25ºC, Low Humidity

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

Software and system updates available at
http://www.phylumtech.com