WormWatcher Activity Experiments

C. elegans activity measurements in multiwell plates

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Synchronize the animals

Start with healthy, well-fed cultures. For bleach synchronization, recover embryos from gravid hermaphrodites using alkaline hypochlorite as described in WormBook Protocol 4. To obtain a synchronized L1 cohort, hatch the recovered embryos without food following the synchronization principle in Protocol 6, then return the larvae to food. If early starvation could affect the endpoint, consider a timed egg lay and use the same method across comparison groups. [1, 2]

Grow to the target stage

Culture the synchronized animals on seeded nematode growth medium (NGM) plates to late L4 or the specified adult age. Escherichia coli OP50 is a common maintenance food. Use the experimental food during growth when your design requires it. A common starting temperature is 20 °C. Verify stage under a stereomicroscope because development depends on strain, temperature, food, and treatment. [1]

Late L4 and adult worms are practical starting stages for WormWatcher observation. Confirm visibility under your own optics. For solid-medium assays, grow synchronized animals on 6 cm culture plates seeded with the same food strain used in the assay. Aim for approximately 1,000–1,500 animals per culture plate. If using floxuridine (FUdR, 5-fluoro-2′-deoxyuridine), transfer at late L4 to suppress progeny. Earlier exposure can interfere with development, and later transfer can carry over eggs or progeny. Define adult age relative to the L4-to-adult molt for adult assays. [3, 4]

Plan the plate layout

Before preparing plates, define the endpoint, medium, animals per well, and recording duration. Map strains, treatments, and matched controls to wells. Distribute comparison groups across plate positions and repeat across independently prepared cohorts. Where worms share a well, the well is the unit for a collective activity measurement. Use replicate wells and independently prepared cohorts to assess variability. For runs long enough for progeny to affect the readout, decide in advance whether to use FUdR or another progeny-control method. Document the dose and timing and check whether the method affects the endpoint in your strains and treatments. Remes et al. provide examples of lifespan assays with and without FUdR. [3–5]

Prepare the multiwell plates

WormWatcher systems can be used with either solid or liquid media in compatible multiwell plates. Choose the medium for the behavior you want to measure, then select a plate and animal density that let you see that behavior clearly.

Selecting a plate format from 6 to 96 wells

Format	Practical considerations
6 or 12 wells	Larger arenas give worms more room to move. Larger food sources are unlikely to need replenishment during a lifespan experience with progeny inhibited.
24 wells	Intermediate well size and throughput. Verify that the plate has open interwell space if using the hydration fill.
48 or 96 wells	Higher throughput with smaller arenas. Better suited to short term liquid media experiments where food supply is not relevant.

Confirm the exact plate and lid fit the holder on your hardware. Pilot well shape, bottom clarity, focus, medium depth, and usable imaging area. Set fill and food volumes for the selected geometry and assay. Scale the suggested volumes to the chosen plate geometry.

Solid media

Use seeded solid medium for crawling and longitudinal activity in a compatible multiwell plate, such as a 6- or 24-well plate. Standard NGM agar is an option [1]. The recipe below uses optically clear gellan gum (Gelrite) and a 24-well plate. A separate hydration fill can be added if the plate has open spaces between the wells. The number of plates per batch depends on the fill volumes and plate format. [4]

Ingredient or stock	In-well gel	Interwell gel
Purified water	200 mL	300 mL
Gelrite	5.0 g	7.5 g
NaCl	0.60 g	0.90 g
Peptone	0.50 g	Omit
Potassium phosphate stock[1]	5.0 mL	7.5 mL
$CaCl_2$ stock, filter-sterilized[2]	200 µL	300 µL
$MgSO_4$ stock, filter-sterilized[2]	200 µL	300 µL
Cholesterol, 5 mg/mL in ethanol	200 µL	300 µL
FUdR, 40 mM stock[3]	1.0 mL	Omit

[1] Prepare stock with 54.15 g KH_2PO_4 and 17.8 g K_2HPO_4 per 500 mL. [2] Prepare calcium and magnesium stocks with 147 g/L $CaCl_2 \cdot 2H_2O$ and 246 g/L $MgSO_4 \cdot 7H_2O$, respectively, and filter-sterilize both. [3] Optional for progeny control. [3, 4]

Equipment for preparing Gelrite plates includes a clean hood, sterile plates and dispensing supplies, a heated aluminum sheet, a hot plate with magnetic stirring, stir bars, a thermometer, 250 mL and 500 mL flasks, and a pipette controller with 25 mL and 50 mL serological pipettes.

1. Disperse the gel. Label the 250 mL flask for the well gel and the 500 mL flask for the interwell fill. Mix each set of dry ingredients and add a stir bar. Stir in about half the purified water, then add the remainder gradually. Stir for at least 10 minutes, preferably an hour. Remake any batch with persistent clumps, which can create imaging artifacts.

2. Pour the interwell fill. If the plate has open space between the wells and the assay calls for a hydration fill, proceed as follows. In the hood, prewarm the sterile plates on an aluminum sheet at about 70 °C if the plates permit it. Set a separate stirring hot plate to about 100 °C to keep the interwell mix fluid. Heat the mixture in a microwave in short intervals until clear, watching for boil-over. Cover with clean foil for transfer to the hood. Add the stocks while stirring briskly without foaming. Immediately dispense enough gel to fill the interwell space (approximately 30 mL for some 24-well plates), starting in the center and dispensing into corners as needed. Keep the lid in place until the pipette is positioned to avoid dripping into the wells.

3. Fill the wells. Microwave the well gel until clear, typically 3–5 minutes depending on the microwave. If bubbles persist during stirring, reheat briefly before adding stocks. Cool while stirring to 65–67 °C and hold there. Add phosphate, calcium, magnesium, and cholesterol in order. If using FUdR, add it last at 65–67 °C and protect it from light. This recipe gives approximately 200 µM FUdR in the well gel. Use a 25 mL serological pipette to dispense without bubbles. Use 1 mL per well as a starting volume for 24 wells and adjust for other well sizes. Empty the tip between fills and replace it if gel sets inside. [3, 4]

4. Dry and store. Cool in the hood and dry with lids off for 3–4 hours. Dry the lids separately. Seed once dry, or seal with laboratory film and refrigerate inverted at 4 °C. Wrap FUdR plates in foil to protect them from light. For short assays, omit FUdR if progeny cannot affect the measurement. [3, 4]

5. Seed the bacterial lawns. Prepare LB at 20 g/L for this preparation and autoclave it. Grow the selected strain overnight at 37 °C with shaking. Scale the culture volume to the number of wells, the intended dose per well, and the culture density. Pellet the bacteria using locally established relative centrifugal force (× g) and duration. In the hood, remove the supernatant and resuspend the pellet in a small measured volume of LB by vortexing or pipetting. Record the volume or bacterial density.

Dispense concentrated bacteria in the center of each gel surface. Start with a small drop, such as 50 µL, and adjust the volume to keep the lawn clear of the walls. A 6-well plate is an easier starting point because its wider wells leave more room around a central lawn, more closely resembling a standard C. elegans culture plate. Worms can climb the wall or leave the imaging area if the lawn reaches the edge. Keep plates level and undisturbed while the spots dry, typically 2–4 hours. Use promptly or store sealed, inverted and protected from light at 4 °C for up to 4 days. [1]

Liquid media

Use liquid for swimming endpoints. Select the buffer, food status, volume, and exposure time from the relevant assay. Add equal volumes to matched wells, account for liquid carried in with the worms, and keep the transfer-to-recording interval consistent. Pilot the fill depth and lid strategy for focus, reflections, bubbles, evaporation, and edge-well effects. For extended recordings, also verify that food availability and aeration are suitable for the endpoint.

Many liquid swimming assays use a food-free buffer. Suspended bacteria can cloud the liquid and reduce contrast, making moving worms harder to see or track. If the assay requires bacterial food, verify visibility and tracking at the intended bacterial concentration. [5–7]

Short individual swimming assay. Remes et al. used the Basic Imaging Platform for 10-second videos in S Basal and measured body bends per second for individual animals. See §2.9 for the method. The same paper describes a separate solid-medium activity assay in §2.7. [5]

Extended multiwell swimming assay. Bastien et al. used M9-based liquid in 96-well plates for population activity during compound exposure. Smisko et al. describe 96-well M9 assays for groups and individual worms. Follow the selected paper for its loading, controls, and analysis. Population pixel-change activity and individual body-bend frequency are distinct endpoints. [6, 7]

Load the worms

One animal per well

For an individual readout, transfer one staged worm with a pick or a small pipetted aliquot suited to the medium. Verify the count microscopically. On solid medium, avoid damaging the gel or introducing enough liquid to spread the lawn. In liquid, match final volume across wells and confirm that the animal remains visible through the usable depth and near the well edge.

Multiple animals per well

For collective activity, load a defined number per well and verify counts. For a solid-medium group assay, 30–50 late L4 worms per well is one starting point. Choose a consistent target suited to the plate format, medium, and readout. Check for overlap, crowding, and food depletion. If the readout requires tracking individual animals, verify that the analysis can separate them.

Bulk loading onto solid media

Rinse the staged animals from the 6 cm culture plates with sterile liquid NGM and collect them in a sterile 15 mL tube. Work in batches that can be transferred within 20 minutes to limit time away from food. Pellet using locally verified worm-handling settings, remove most of the supernatant, and resuspend in the remaining liquid. Gently remix between transfers so animals do not settle.

Use a sterile P20 tip to dispense a 1 µL test aliquot under a stereomicroscope. Adjust concentration or transfer volume to load 30–50 worms per well, preferably in less than 5 µL, to avoid spreading the lawn. Let the plates dry in the hood for about 10 minutes, then check the animal count and surface moisture. If needed, add worms or dry for another 10 minutes before rechecking.

Close the plate

To reduce condensation, apply a thin coat of Tween to the inner lid with a lint-free wipe if appropriate for the assay. Let it dry fully and keep it out of the wells. Seal the plate with pre-stretched laboratory film. Cut a strip long enough to wrap the plate, then split it lengthwise, fold each piece lengthwise, then wrap the plate perimeter at least three times and press the film against the sides.

Record the plate with WormWatcher Hardware

For liquid assays, check for bubbles and transfer debris. For either medium, record the plate map, actual starting counts, developmental stage, medium, temperature, food or treatment, and loading time. Inspect representative wells, including edge wells, for clear contrast and focus.

Place the prepared plate in the compatible holder on the WormWatcher Imaging Station, WormWatcher robotic system, or Basic Imaging Platform. Orient it to match the plate map. The settings below apply to a WormWatcher Imaging Station configured for WWRecorder. On the robotic system or Basic Imaging Platform, use the acquisition controls provided with that configuration.

WWRecorder records image sequences on a station configured for its camera, lighting, and stimulation hardware. You can record one sequence, repeat sequences with a pause, or switch imaging or stimulation modes at scheduled times within each sequence.

Set up and verify a trial recording

Power on the station. Connect and power on the camera and imaging station, then open WWRecorder using your usual shortcut or launcher. If asked for a **System name**, enter a recognizable station name.

Frame the plate. Select **DARKFIELD** under **Preview mode**. Position and focus the sample in the separate **Preview** window. Double-click the image to zoom; double-click again to return to the full view. Zoom is available during preview only.

Set trial timing. Under **Acquire settings**, set **Acquisition mode** to **DARKFIELD**, **Record time (s)** to **10**, and **Frames per second** to **4** for a short trial.

Adjust exposure. Expand **Exposure time (ms)** and adjust **Dark field** until the image is clear without washing out bright areas. Press **Enter** after each edit and allow about five seconds for preview settings to refresh. The preview screenshot below shows 75 ms as an example. Set the exposure for your own plate and station.

Disable optional features. Set **Events enable** to **false** and **loop acquisition > enable** to **false** for this trial. Leave **Image post-processing** disabled unless your protocol calls for it.

Choose a destination. Click **Acquire / Stop**. In the save dialog, choose a parent location and type a new, unique folder name for this experiment. Although the dialog resembles a file-save dialog, the name specifies the experiment folder.

Watch the recording. Watch for **RECORDING** in the image window. When the sequence finishes, wait for pending images to finish saving and for **PREVIEW** to return.

Verify the saved frames. Open several saved frames and check focus, contrast, well coverage, frame count, and timing before choosing settings for the full experiment.

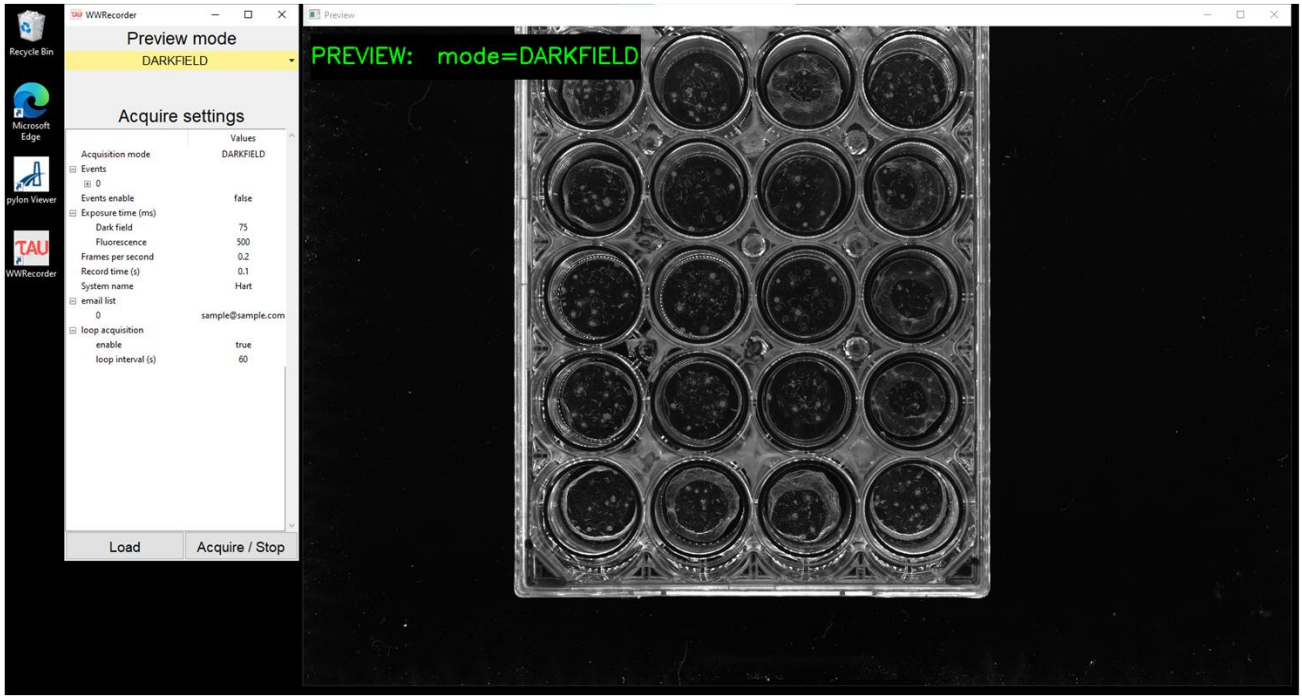


Figure 1. Darkfield preview of a multiwell plate. This screenshot shows a separate low-cadence setup with 75 ms darkfield exposure, 0.2 fps, a 0.1 s requested sequence, and a 60 s pause.

Edit and reuse settings

Expand settings groups using the arrows beside their names. Double-click a value in the **Values** column, type the new value, and press **Enter** to save it. Enter **true** or **false** for enable switches. Press **Escape** while editing to cancel an unfinished edit.

Edits persist automatically; there is no separate Save button. Make experiment changes before starting acquisition. An active recording uses the settings selected when it started.

Load imports a saved settings file, including an earlier experiment's **metadata.json**. The current station's **System name** is retained. Review the imported timing, exposure, events, and hardware selections before acquiring. When importing settings from another station, have the station operator check hardware compatibility first. Restart WWRecorder after changing the camera or LabJack device selection.

Lists such as **Events** and **email list** have additional controls for adding and deleting entries.

- Click the list name in the left column to reveal **Add**. A new entry copies the first entry, so edit every value that needs to differ.
- Click an entry's number in the left column to reveal **Delete**.
- The last entry cannot be deleted. To ignore all scheduled events, use **Events enable = false**.
- **Add** is disabled for an empty list. Load a prepared settings file with an existing entry if you need to populate an empty list.

Choose imaging and stimulation modes

Preview mode controls the live view before and after recording. **Acquisition mode** selects the normal mode used during recording, outside scheduled events. Set both deliberately. Changing the preview selection does not change the acquisition setting.

Enter mode names exactly as shown below when editing **Acquisition mode** or an event's **Mode**.

Mode	Illumination or stimulation	Exposure setting used
NONE	Lights and vibration off; images can still be acquired.	Dark field
DARKFIELD	Red dark-field illumination.	Dark field
FLUO_BLUE	Blue excitation, with red illumination off.	Fluorescence
FLUO_GREEN	Green excitation, with red illumination off.	Fluorescence
STIM_BLUE	Blue stimulation with red dark-field illumination.	Dark field
STIM_GREEN	Green stimulation with red dark-field illumination.	Dark field
STIM_VIB	Vibration with red dark-field illumination.	Dark field

Modes require the corresponding hardware to be connected and configured. A mode name alone does not confirm that the station has that accessory.

Use fluorescence and stimulation preview briefly. Blue preview is a finite pulse. Reaching its configured maximum switches preview to **NONE** and displays a message. Selecting the mode again requests another pulse. Other prolonged stimulation or fluorescence use can also trigger automatic shutdown.

Recording duration and frame rate

Setting	Meaning
Record time (s)	Requested duration of one image sequence, in seconds. With looping enabled, this applies to each sequence, rather than the entire experiment.
Frames per second	Requested image acquisition rate. For example, **4** means one image about every 0.25 seconds; **0.1** means one about every 10 seconds. Use a positive value.

The usual frame-rate limit is **8 fps**, although the station's configured limit may differ. WWRecorder rejects a request above that limit. Actual timing also depends on exposure, mode changes, camera speed, and storage speed; verify timing from the saved images in a trial recording.

At **0.25 fps or below**, or when **Record time (s) is 1.5 seconds or less**, acquisition uses illumination around frame requests and turns it off between frames outside active events. At other settings, the normal acquisition illumination can remain on between frames.

Set exposure and image appearance

Exposure time

Dark field applies to DARKFIELD, NONE, and all STIM modes. **Fluorescence** applies to both FLUO modes. Exposure is the time the camera collects light for each image; increasing it usually brightens the image but can increase motion blur and limit acquisition speed.

Keep exposure comfortably shorter than the time between frames. At 4 fps, frames are 250 ms apart, so a 500 ms fluorescence exposure cannot sustain that rate. Allow additional time for camera overhead and mode transitions. Switching into fluorescence can introduce an approximately one-second settling delay, so trial short events before relying on them for measurements.

Exposure control is supported for the Basler camera. In **WEBCAM** mode, these exposure settings are not applied; adjust the webcam through its own controls if needed.

Image post-processing

The **Dark field** and **Fluorescence** groups have independent appearance settings. Dark-field processing applies to DARKFIELD images; fluorescence processing applies to FLUO_BLUE and FLUO_GREEN images. These adjustments do not apply to STIM or NONE images.

Option	Effect
enable	**true** applies the group's adjustments; **false** leaves them off.
black level	Pixel values at or below this level become black. Raising it suppresses dim background but can remove faint signal.
white level	Pixel values at or above this level become white. Lowering it brightens the displayed range but can clip bright signal.
gamma	Adjusts midtone brightness. **1.0** leaves midtones unchanged, values above **1.0** brighten them, and positive values below **1.0** darken them.

Use **0 <= black level < white level <= 255** and a positive gamma. The neutral values are black **0**, white **255**, and gamma **1.0**.

Post-processing changes the saved images as well as the preview. Keep it disabled if you need unadjusted pixel intensities, and keep settings consistent when comparing experiments.

Schedule events within a sequence

Set **Events enable** to **true** to activate the **Events** list. Each event temporarily replaces the normal acquisition mode. After the event, acquisition returns to the normal mode. Event timing starts at the beginning of each sequence and repeats with each sequence when looping is enabled.

Event option	Meaning
Mode	Imaging or stimulation mode to use during the event.
Start time (s)	Delay from the beginning of the sequence to the start of the event.
Duration (s)	How long the event lasts. Use a positive duration.

Keep event start times at or above zero and each event's end within **Record time (s)**. FLUO, STIM_BLUE, and STIM_GREEN events longer than **30 seconds** are rejected. A shorter event can still exceed the station's operating limits; passing this check does not guarantee that the event can run uninterrupted.

Avoid overlapping events. Only one event takes priority at a time, with the first active entry in the list taking precedence. Separate events with enough time for mode changes and any required cooling. Events use the sequence's requested frame rate. An event shorter than the interval between frames may occur without a saved image during it.

The settings below record before, during, and after a two-second blue stimulus.

Setting	Value
Acquisition mode	DARKFIELD
Record time (s)	20
Frames per second	4
Events enable	true
Event Mode	STIM_BLUE
Event Start time (s)	5
Event Duration (s)	2

This requests dark-field imaging, blue stimulation from 5 to 7 seconds, then dark-field imaging for the rest of the sequence. Verify the result in a short trial on your station.

To acquire fluorescence instead, use **FLUO_BLUE** or **FLUO_GREEN** and set **Exposure time (ms) > Fluorescence** appropriately. Allow for exposure changes and settling time when choosing event duration.

Repeat sequences with loop acquisition

Option under loop acquisition	Meaning
enable	**false** records one sequence. **true** repeats sequences until stopped or interrupted by an error.
loop interval (s)	Pause after one sequence ends and before the next begins. This is an end-to-start delay, rather than the time between sequence starts. Use a nonnegative value.
split into timepoint folders	**false** saves images together in the experiment folder. **true** puts each sequence in its own dated timepoint folder.

For example, a 10-second recording with a 50-second loop interval starts a new sequence approximately every minute, plus acquisition overhead. Illumination and stimulation are off during the pause. The image window shows **WAITING**, the remaining pause time, and the last acquired image; that image is not a live view during the pause.

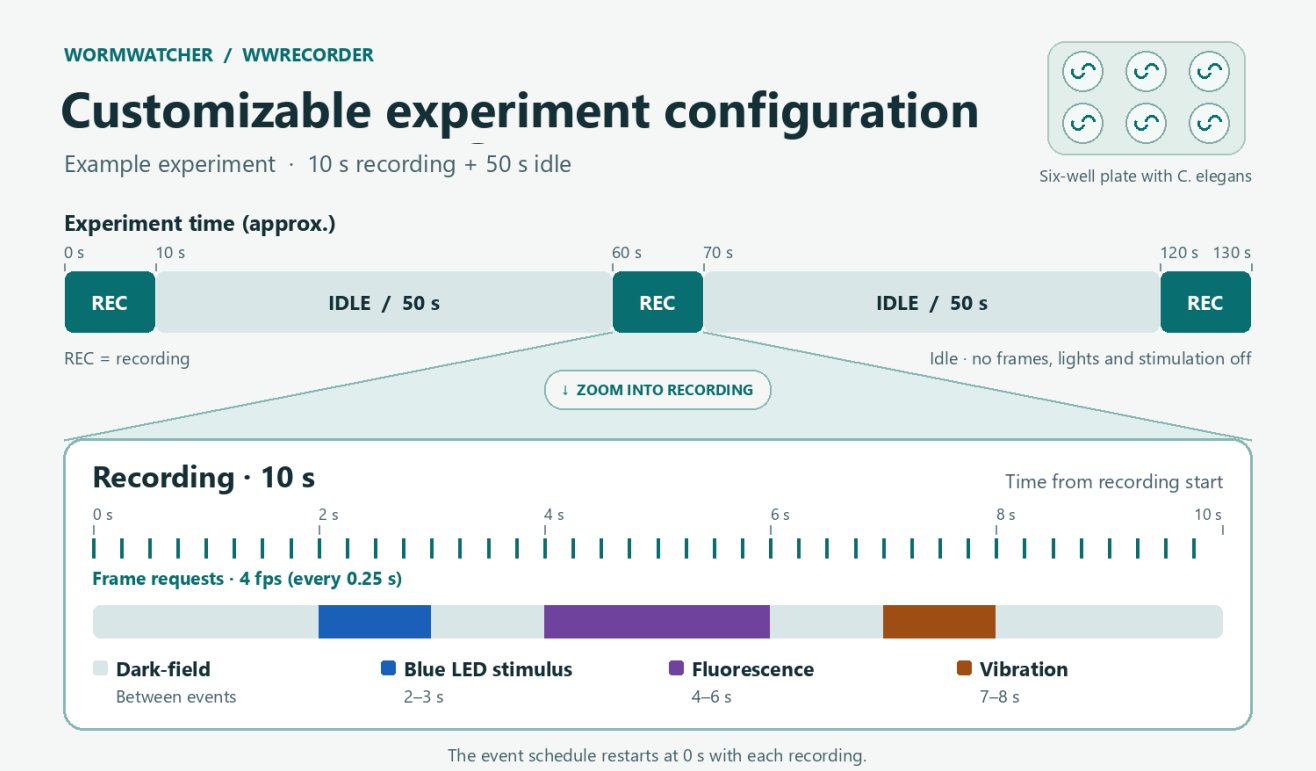


Figure 2. Example timing for repeated recordings and mode changes within a sequence. The diagram is illustrative. Use the event and loop settings above to define your own timing.

There is no loop-count or total-experiment-duration control. Stop the experiment when you have collected enough sequences.

Use timepoint folders only at 1 fps or slower. In this layout, image names have only whole-second timestamps. Multiple images acquired in the same second can overwrite one another. For faster recordings, leave **split into timepoint folders = false**.

Save and monitor an experiment

Always choose a new, empty experiment folder. WWRecorder asks for confirmation if the selected folder contains data; reusing it can overwrite earlier results.

During acquisition, the image window shows the current mode, elapsed/requested sequence time, and **save queue**. The queue counts images still waiting to be written. A persistently growing queue indicates that storage cannot keep up; reduce the frame rate for subsequent runs or use faster storage.

To stop early, click **Acquire / Stop** again, or click the image window and press **Esc**. Esc must be pressed with the image window active. Stopping also ends a loop during its waiting period. Wait for the save queue to drain and preview to return before closing WWRecorder. **ABORTING** can appear while pending images finish saving.

The experiment folder contains the following files.

- **PNG images**. The recorded frames. With the normal layout, names include a series number, frame number, and elapsed time. With timepoint folders, folder and image names contain dates and times.
- **metadata.json**. The settings used when acquisition started. Keep this with the images; it can also be loaded to reuse the setup.

Preview zoom and the status text overlaid on the image window are not included in the saved images. Enabled image post-processing is included.

Check that images are present, open several frames, and confirm brightness and timing before leaving a long experiment unattended.

Station options and status

Item	How to use it
System name	Identifies the imaging station, including in acquisition error alerts. Use a recognizable name.
email list	Recipient addresses for acquisition error alerts. Replace example addresses with real recipients. Delivery requires a working internet connection and notification service; do not treat the absence of email as confirmation that an experiment is running.
Camera device	**BASLER** selects the station camera; **WEBCAM** selects the default webcam. Restart after changing this selection.
LabJack device	**AUTO** uses automatic selection; **U3** or **UD** selects the U3 connection; **T4** or **LJM** selects the T4 connection. Match the installed station and restart after changing this selection.
max STIM or FLUO time (s), if present	Configured maximum on-time used for preview and prolonged-output protection. The fallback is 10 seconds. Have the station operator confirm an appropriate limit for your hardware and protocol.
Safety temperature	Temperature reported by the dedicated safety sensor. **FAULT** indicates a latched safety fault; **INHIBITED** indicates that the safety output is not permitted; **unavailable** means no current usable reading is available.

Blue-output experiments require a successful safety check before the save dialog opens. If WWRecorder reports a failed check, temperature fault, or excessive on-time, stop and resolve the reported cause with the station operator. A mode selection or acquisition request asks WWRecorder to recheck the interlock; it cannot bypass an unresolved fault.

Hardware port assignments, recording performance limits, and debugging options are hidden from the normal experiment settings panel. Have the station operator handle those settings. Confirm that a station used for practice has been returned to real camera, real hardware, and real file-saving operation before collecting experimental data.

Common setup problems

What you see	What to check
Wrong lighting during acquisition	Check **Acquisition mode** and enabled events, independently of **Preview mode**.
Image too dark, too bright, or blurred	Adjust the relevant exposure; check whether post-processing is enabled. Use shorter exposure to reduce motion blur.
Requested frame rate rejected	Reduce **Frames per second** to the station's configured limit or below.
Recording slower than requested	Shorten exposure, reduce frame rate, and allow time for mode changes. Check storage speed if the save queue grows.

What you see	What to check
Events do not occur	Check **Events enable**, exact mode names, event times, and availability of the required hardware.
Event setup rejected	Keep events within the sequence duration and within the event-duration limits.
Fewer images than expected in timepoint folders	Check for rates above 1 fps. Use the normal single-folder layout to prevent whole-second filename collisions.
No images saved	Check the chosen experiment folder and available disk space. Have the operator confirm that file-saving simulation is disabled.
Blue preview switches to NONE	The finite preview pulse may have ended. Read the message before selecting the mode again.
Lights shut off or acquisition stops with an error	Read the reported safety, camera, or storage error and correct its cause before restarting.
Error explicitly says a restart is required	Close and reopen WWRecorder after addressing the reported problem, then run a short trial.

Analyze output images or videos

Many options for analysis

WormWatcher recordings are available in standard image or video formats. Choose an analysis method based on the assay endpoint and whether you need a measure for each well or each animal.

Activity per well. Tau-supplied software quantifies activity in each well. Use this approach when the well is the unit of measurement.

Swimming frequency and other established endpoints. Freely available *C. elegans* analysis software can be used for established assays. For example, Remes et al. measured individual swimming frequency as body bends per second [5, §2.9].

Application-specific analysis. Use custom software when the assay calls for a specialized measurement or workflow. Tau's most recent development in this area uses deep neural networks to recognize worms in customer-generated images.

Activity analysis in well plates

Use WormWatcher Analyzer (WWAnalyzer) to measure movement within each imaging session and track activity by well throughout a multiwell plate experiment. Step 1 calculates measurements from image differences; Step 2 combines them into activity curves and a spreadsheet for each plate. Where several animals share a well, interpret the result as collective activity rather than individual-animal tracking.

Prepare the experiment folder

Keep each plate in its own folder under the experiment parent folder. Each plate folder should contain one subfolder per imaging session, with that session's images inside. Open WWAnalyzer once, allow it to load, and select the experiment parent directory using Change path.

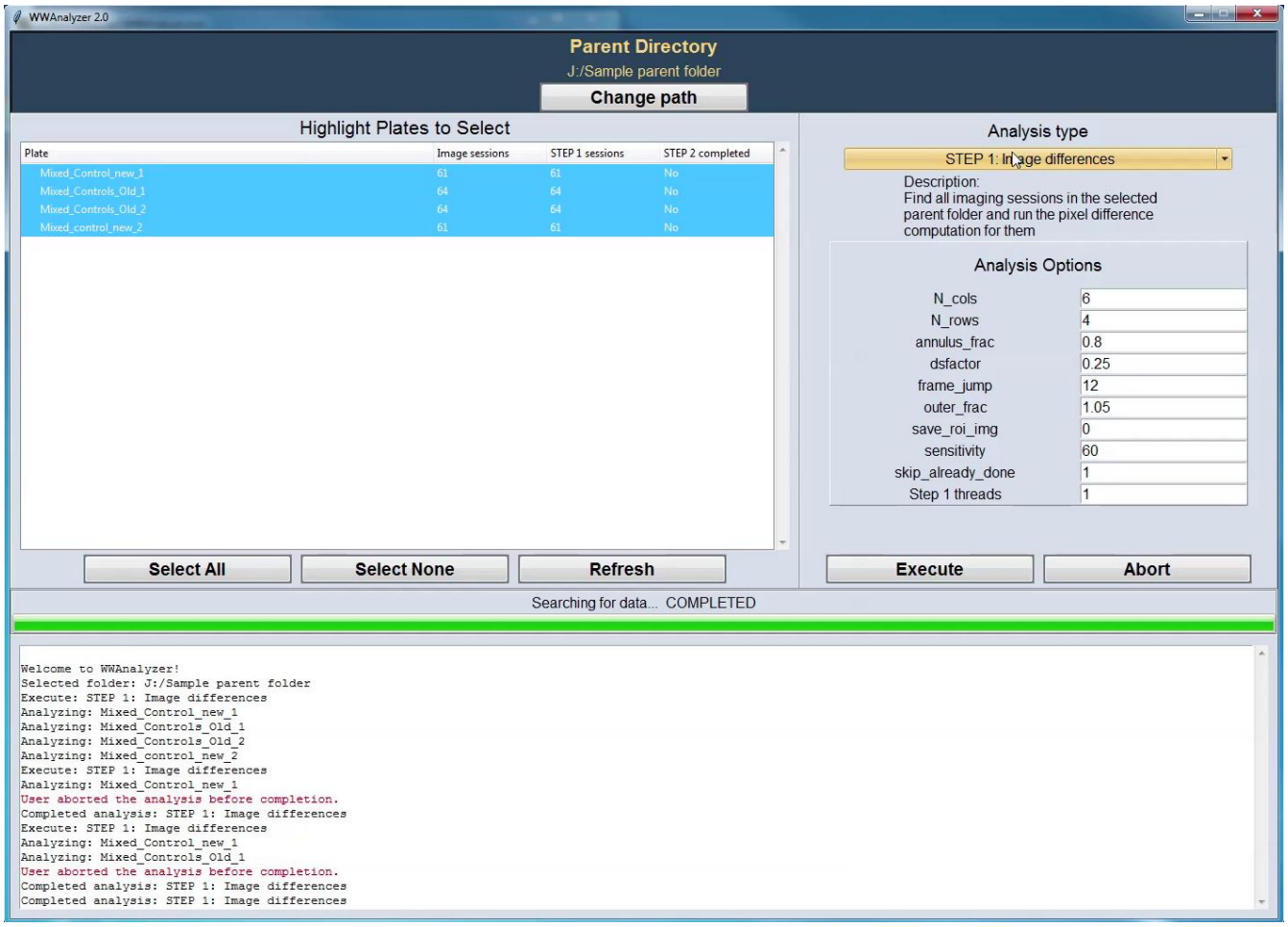
Measure activity within sessions

Under Analysis Type, select Step 1 Image Differences. Match the rows and columns to the plate geometry: the 24-well plate shown has 4 rows and 6 columns (N_rows = 4; N_cols = 6 in the interface shown).

Frame Jump (frame_jump) sets the separation between the images being subtracted. For images acquired every 5 seconds, a jump of 12 compares images 60 seconds apart. Set this interval deliberately and use a consistent analysis setting across comparison plates.

Skip Already Done (skip_already_done) = 1 retains successfully analyzed sessions and processes outstanding sessions; set it to 0 to regenerate results for the selected plates. Some WormWatcher releases distributed in 2020 or later perform Step 1 during acquisition, so check for existing results before reprocessing.

Highlight the plates to process, or click Select All, then click Execute. Each plate opens a progress window. Processing is complete when its progress bar fills and the log reports Completed Analysis. To stop within a plate, focus its progress window and press Esc; the main window's Abort button stops after the current plate.



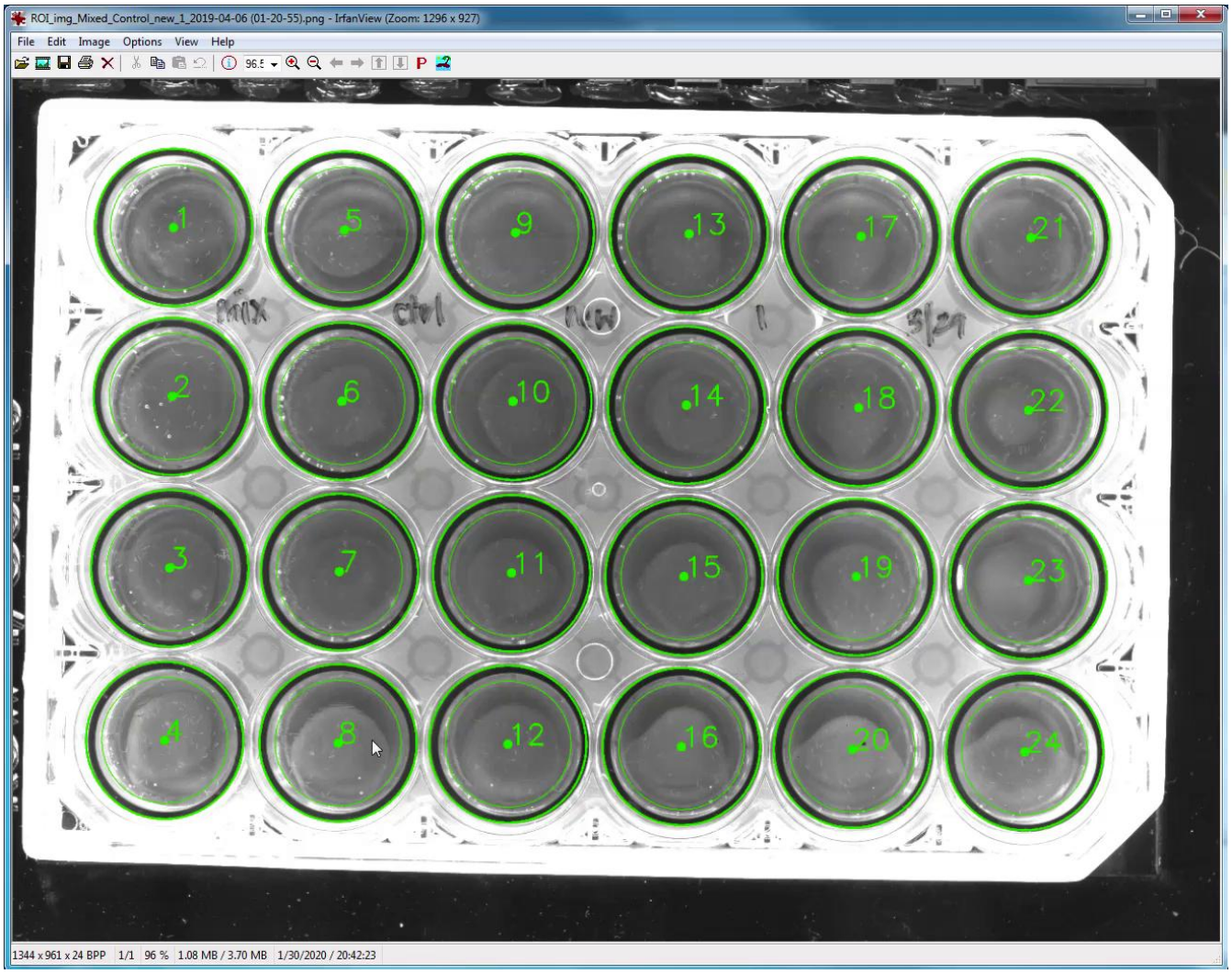
WWAnalyzer interface. Select the experiment parent directory, analysis step, plate geometry and frame jump, then highlight the plates and click Execute. The screenshot shows a 4 × 6 plate setup.

Verify session results and detected wells

Review red log entries and identify the affected plate and session. Sessions may be skipped when they contain too few images or the wells cannot be identified. For detection failures, check source images for obstructions or a plate extending outside the field of view. Consult the detailed WormWatcher analysis log and include it when requesting support.

To check segmentation, select a plate, set Save ROI Image (save_roi_img) to 1 and, if it has already been analyzed, set Skip Already Done to 0. Click Execute. In _analysis under the experiment parent folder, open the matching plate folder and its ROI Images output. Check that every region-of-interest (ROI) outline aligns with its well. The 24-well example shows circular outlines; other plate formats may use different ROI shapes.

After checking, return Save ROI Image to 0 and Skip Already Done to 1 for routine processing. Saved ROI images add files to the analysis folder. Session Data contains one CSV per analyzed session with ROI numbers, coordinates, activity and other measurements.

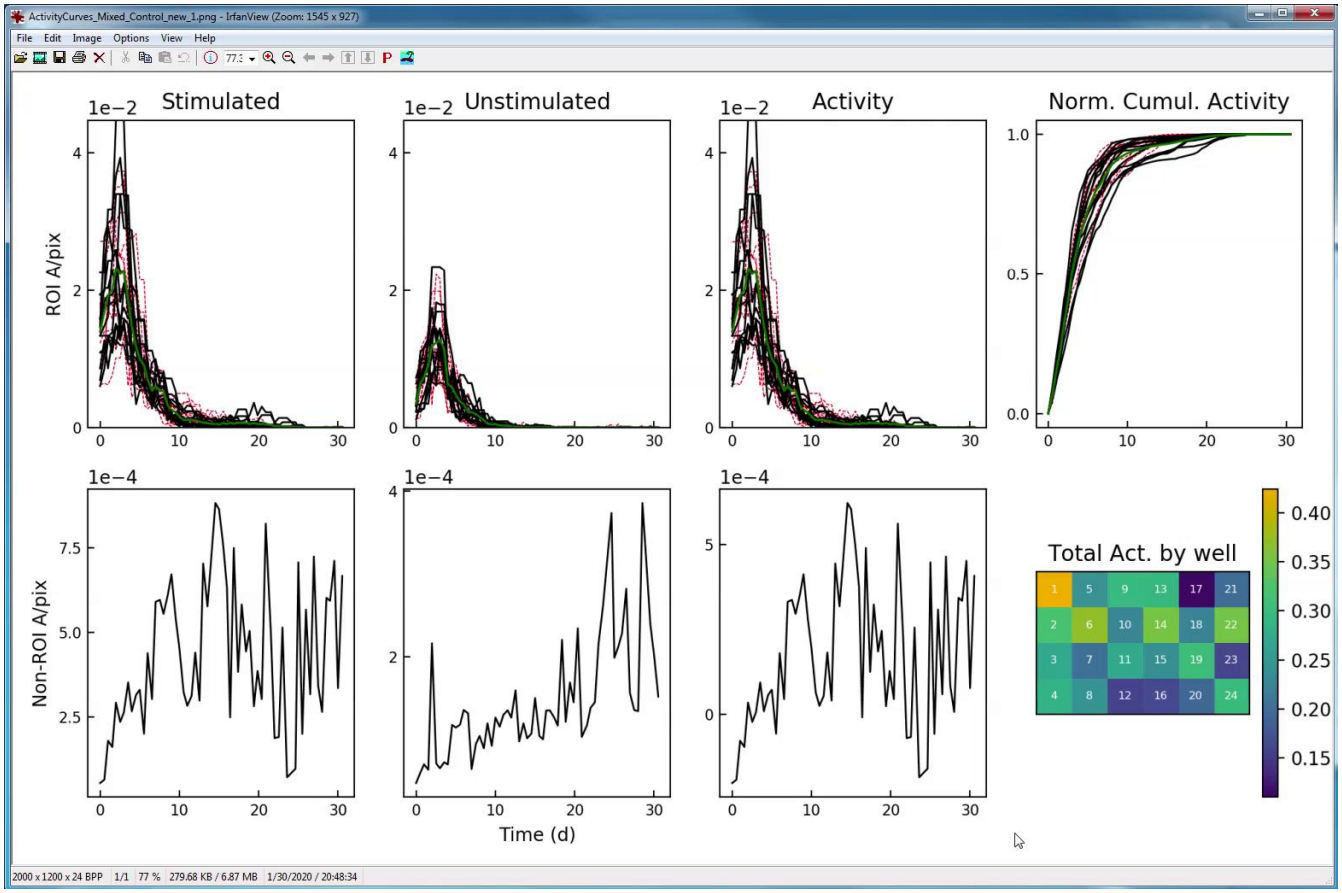


Automatic segmentation of a 24-well plate. Check all 24 outlines against the well boundaries before using the measurements. Numbering runs down each column before moving right: the first column is ROIs 1–4, then 5–8, 9–12, 13–16, 17–20 and 21–24. ROI 17 is the top well in column 5. Match these numbers to the experimental plate map and spreadsheet columns.

Generate activity curves

With the same experiment parent folder selected, choose Step 2 Activity Over Time under Analysis Type. Highlight the plates and click Execute; the tutorial shows no additional settings for this step. Wait for the progress bar to finish. Abort stops processing between plates.

In _analysis, open the matching plate folder. Review the Activity Curves image first, then open the corresponding spreadsheet for numerical values. The _analysis folder can be copied separately, but retain the source images for troubleshooting and reanalysis.



Example activity outputs. Use the per-well curves and plate heat map to identify differences and potential outliers, then verify the numerical values and corresponding source images. These example outputs illustrate the analysis display, not acceptance criteria for a new experiment.

Read the activity plots

Stimulated and unstimulated activity. Stimulated activity measures movement after blue-light stimulation and is zero when stimulation was not used. Unstimulated activity measures movement before stimulation, or during the imaging session in an experiment without a stimulus.

Noise-floor-subtracted activity. In stimulated experiments, this is the stimulated signal minus an estimated background. The software typically estimates the floor from late unstimulated sessions, when little worm movement is expected. For experiments without stimulation, the activity curve uses the unstimulated signal.

Normalized cumulative activity. This expresses accumulated movement as a fraction of its maximum and is used to locate thresholds such as T97 and T99. It describes accumulated activity, not the fraction of animals alive at each time point.

Activity outside the ROIs and total activity heat map. The outside-ROI trace measures movement outside the wells; compare spikes with source images to check for recording artifacts such as a bumped plate. The heat map summarizes total movement by well. Inspect unusually low-activity wells in the source images, because a nearly empty well can also give a low total.

Interpret the activity spreadsheet

Find the desired ROI column and read its values against the time points in each measurement section. Confirm that ROI numbers and Group name assignments match the plate map before comparing groups.

ROI cumulative activity tracks accumulated movement. For stimulated data, use the noise-floor-subtracted cumulative series; for unstimulated data, look for the cumulative non-subtracted series or its equivalent. ROI noise floor subtracted activity reports corrected movement for individual sessions and helps show changes over time.

Stimulated and unstimulated activity fields contain the underlying inside- and outside-well signals. Compare these when investigating spikes. N-worm movements counts movements roughly the size of a worm within a session and should be treated as an exploratory measure. Bulk movements flags large changes within wells; inspect the associated images before excluding data.

Worm fraction estimates worm abundance in plate wells from images, including stationary worms. Consider normalizing raw activity by this measure when comparing wells with different worm abundance, and document the normalization used. Group name identifies the group assigned to each well.

Review artifacts before comparing wells

Compare an outlying activity point with the outside-ROI trace, bulk movement values and source images for that session. A moving plate, light flicker or moving droplet can produce a large signal unrelated to worm activity. If the evidence supports an artifact, exclude the affected point from downstream analysis and record the reason; do not remove a point solely because it differs from the expected result.

Use cumulative-activity thresholds cautiously

In the spreadsheet's T levels section, each row identifies a percentage threshold and the value gives the time when the well's cumulative activity reaches that percentage of its maximum. For example, T99 is the time at 99% of cumulative activity, not a direct count showing 99% mortality.

T97 or T99 may optionally be used as a correlate of lifespan or healthspan in multiwell plate experiments, subject to the customer's own verification. Collective activity monitoring provides an approach to studying aging from movement measurements (Fouad et al., bioRxiv preprint [4]). These thresholds are not direct measurements of survival or healthspan: the customer should verify the chosen threshold against independent measurements of the intended endpoint under their own experimental conditions before using it for comparisons or conclusions.

References

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