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TECHNICAL NOTE

SydLab™ One

Unlocking New Frontiers in Research

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SydLab™ One

The Nagi Bioscience Solution - SydLab™ One is a state-of-the-art benchtop automation platform engineered for high-content screening and mid- to large-scale biological testing using *Caenorhabditis elegans* (*C. elegans*).

SydLab™ One integrates advanced and automated microfluidics handling and high-content imaging, providing a complete solution for monitoring biological responses *in vivo* under various experimental conditions. The system automates key steps such as *C. elegans* feeding, compound treatments, imaging and data analysis, ensuring consistent and user-independent results. SydLab™ One 's ability to handle multiple test conditions in parallel and its built-in environmental controls makes it suitable for applications in aging, neurodegeneration, toxicology, drug discovery and genetic research. The platform is designed to streamline workflows in biological laboratories, allowing researchers to generate reliable results with reduced hands-on time.

System Components and Setup

The SydLab™ One system consists of three main components:

The microfluidic chips

The SydLab™ One platform

The Experiment Design and Monitoring software

Each serves a specific function to ensure efficient experimental execution and extraction of comprehensive analysis. Below is a description of each component and its role within the system.

Microfluidic Chips

The patented microfluidic chips are central to SydLab™ One functionality, allowing fine-tuned compound treatments and precise imaging. Each chip contains 16 parallel microfluidic channels, each subdivided into eight chambers. Each SydLab™ One can house up to four chips, enabling testing of up to 64 independent conditions simultaneously.

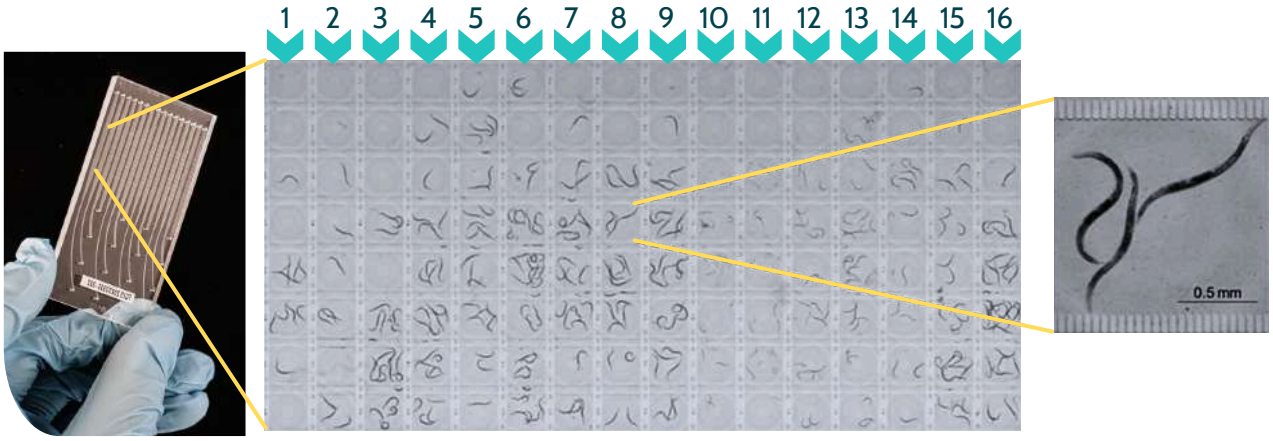


Figure legend: Example of an L4-chip made of transparent polymer (PDMS) and a glass slide for optimal imaging quality. Each microfluidic channel (divided into eight chambers) can be individually loaded with different C. elegans strains, bacterial strains, and compounds providing flexibility in experimental design. The chips are loaded inside the SydLab™ One incubator that guarantee stable temperature control.

The SydLab™ One system offers two types of microfluidic chips (L1 and L4) that cater to different experimental requirements based on the developmental stage of *C. elegans*. Each chip is designed to optimize the automated handling, imaging, and analysis of specific *C. elegans* stages, with unique features that make them suitable for different types of studies. The chambers in the microfluidic chips are interconnected by filters that regulate the movements of *C. elegans* along the same channel. During the *C. elegans* loading process, the system applies pressure to open the filters, allowing *C. elegans* to move between chambers and be distributed across the chambers and along the channel. These filters are engineered with precise dimensions that act as size-based barriers; once the *C. elegans* are loaded, the filters return to their default state, which is sized to retain *C. elegans* of a specific developmental stage within the chambers. This design ensures that, throughout the experiment, *C. elegans* remain confined to their designated chambers, providing a controlled environment for consistent imaging and analysis while preventing the escape or migration of *C. elegans* between chambers.

L1 Microfluidic Chip

The L1 chip is optimized for experiments using synchronized L1 larvae, the first larval stage of *C. elegans*. The L1 chip is dedicated to studying compound toxicity, developmental and reproductive toxicity, stress resistance, drug's efficacy and growth rates in the initial stages of life (until adulthood). Routine experiments with L1 chips span approximately five to eight days, depending on the accumulation rate of the progeny within the chamber.

L4 Microfluidic Chip

The L4 chip is designed for experiments with *C. elegans* at the fourth larval stage, preceding the adult stage, and characterized by a larger body size compared to L1 larvae. With L4 worms confined inside this chip, after image acquisition, the newly generated progeny is flushed away maintaining only the *C. elegans* originally injected at the beginning of the experiment. Experiments on the L4 chip can be conducted from one week up to seven weeks, making it suitable for studies focused on adult physiology, reproductive behavior, healthspan, lifespan, and/or neurodegeneration experiments.

SydLab™ One platform

The SydLab™ One platform houses the core components, including four slots for inserting microfluidic chips, the automated microscope, microfluidic fluidics control, and the incubator. The system's compact footprint allows it to fit easily on a standard laboratory benchtop. The unit requires a stable surface and sufficient clearance for proper ventilation and access to doors.

Figure legend: Power Requirements: 100-240V ±10%, 50/60Hz; consumption varies between 250W and 350W depending on the model. Designed for laboratory environments (0.67m x 0.61m x 0.71m, 100kg) with ambient temperatures between 15-26°C and humidity below 70%. Includes a main power connection and USB 3.0 Type-A port for data transfer. Both connections must meet the system's specified requirements for proper operation.

Imaging System

The imaging system incorporates a microscope with automated focusing and an integrated CMOS camera. It supports both brightfield and dual color fluorescence imaging, with configurations suitable for time-lapse studies and high-content screening. The 10x objective (6x effective magnification) with a numerical aperture of 0.5, provides a balance between resolution and size / depth of field. The camera resolution is 2048 x 2048 pixels, and the image has a final pixel size of 0.9075 µm. The microscope is also optimized for imaging green and red fluorescent light (for example GFP and RFP), using an epifluorescence illumination scheme with excitation wavelength of 470 nm for the green color, and 565 nm for the red color.

Temperature Control

The SydLab™ One includes an integrated incubator that maintains a controlled temperature environment for the *C. elegans*, ranging from 17°C to 37°C (±0.2°C). This temperature range supports various experimental needs, from standard worm cultivation to stress response studies (heat-shock); in fact, the system can increase the temperature by 5°C in approximately 30 minutes and decrease it by the same amount in about 60 minutes.

Internal Trays

The SydLab™ One's fluidic system automatically manages the delivery of compounds, bacteria, and other reagents to the microfluidic chips. For these functions, key components are:

- The reservoir trays holding 64 individual 600 µL tubes allowing for simultaneous testing of 64 conditions.
- The priming tray, tailored for efficient and automated chip priming.
- The cleaning tray facilitating automated cleaning routines.

Flow control

The microfluidic system is fully automated and consists of four independent fluidic control systems, one for each chip. Each fluidic line has flow measurement control through flow sensors and pressure control using pressure sensors. The system uses a combination of these to manage all the fluidic operations that include:

- **Priming:** fill the system with bubble-free worm culture buffer.
- ***C. elegans* loading:** inject and confine the *C. elegans* into the fluidic chip chambers.
- **Feeding and treatment:** steadily feed the *C. elegans* with bacteria and treat them with compounds automatically, for a desired time period and up to their full lifespan duration.
- **Washing:** wash out bacteria and compounds from a channel and/or larvae and/or eggs (for certain applications).
- **Cleaning:** cleaning the device with cleaning agents specifically defined for SydLab™ One device.

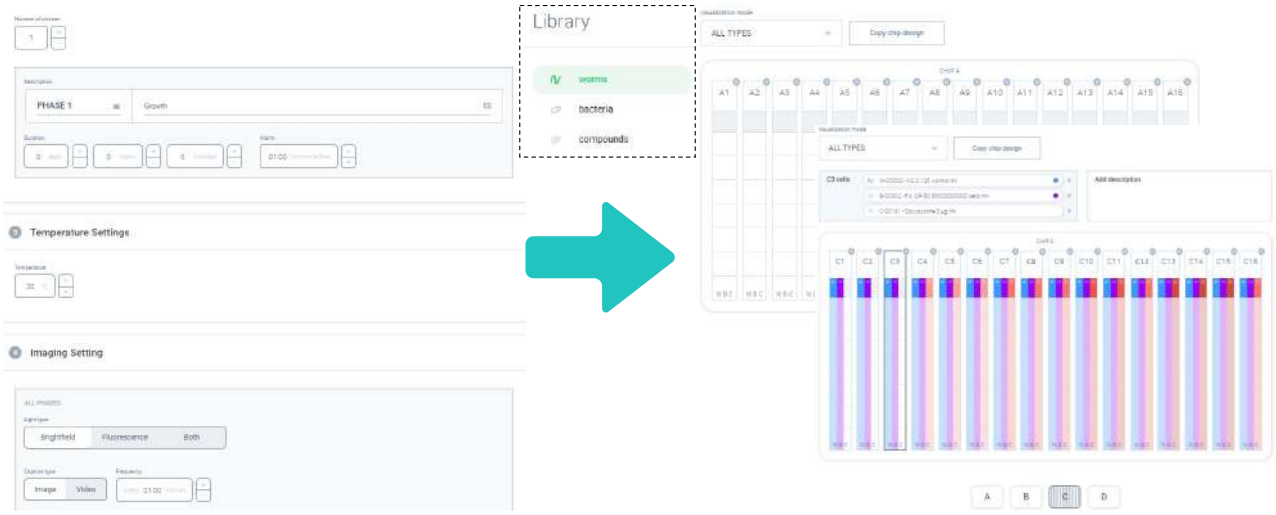
Software

The SydLab™ One platform employs a software guided systematic workflow, encompassing preparation, experiment execution, and data analysis. Each step is designed to maximize reproducibility and minimize user intervention.

Designing the Experiment (Experiment design GUI)

The SydLab™ One software offers a graphical user interface (GUI) for experiment design, allowing for easy configuration of various parameters:

- **Library Management:** The software maintains libraries of *C. elegans* strains, bacterial strains, and chemical compounds. These can be loaded into the experiment design for easy reference.
- **Phases and Conditions:** The experiment can be divided into multiple phases, each characterized by different settings for temperature, imaging, and reagent delivery. This allows for dynamic experimental designs, such as changing treatments at specified time points.
- **Chip and Channel Layout:** Define the specific conditions for each of the 16 to 64 channels across all chips. *C. elegans*, bacteria, and compounds can be assigned to individual channels with precise concentration settings.



Running and Monitoring the Experiment (Monitoring tool)

After finalizing the design, using the "Run" button executes the protocol according to the set phases and parameters. During the run, a real-time monitoring tool provides a view of the images already acquired. Heatmaps and graphs update continuously, allowing for immediate identification of phenotypes.

If necessary, it is possible to pause the experiment to adjust conditions manually. Automatic phase transition is the standard for hands-free operation. If bubbles or contamination are detected in specific channels, those can be manually excluded from the analysis by updating the experimental design.

Data Analysis

The SydLab™ One software automates the collection and initial analysis of data, which includes images, videos, and quantitative metrics for each *C. elegans* and experimental condition.

Segmentation and Image Analysis

During acquisition, in real-time, the SydLab™ One platform uploads the captured images to a secure cloud server, where they are processed and analyzed. SydLab™ One platform utilizes advanced deep learning-based algorithms for segmentation and image analysis, enabling precise quantification of *C. elegans* metrics in each chamber. During the imaging process, brightfield images are used by the segmentation algorithm to distinguish worms, eggs, and larvae from the background. The segmented data are then used to extract quantitative metrics of morphology, mobility and lifespan (see tables of metrics at the end of the document). Fluorescence images are instead used to extract measurement of fluorescence intensity (see tables of metrics at the end of the document). This cloud-based approach ensures that computationally intensive tasks do not overload the local system, while providing access to powerful computational resources.

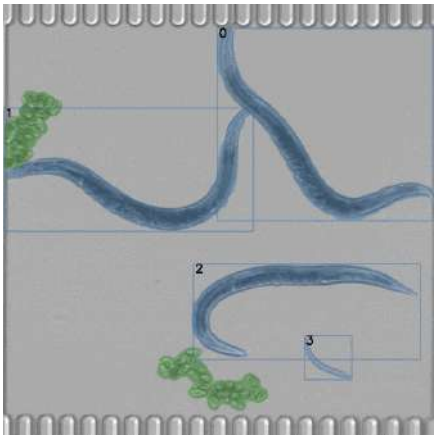


Figure description: The AI-based segmentation algorithm precisely identifies *C. elegans* (overlayed to the respective blue mask) and eggs (overlayed to the respective green mask) in each chamber and at every time point.

These metrics are used for assessing compound effects, toxicological responses, and genetic phenotypes, making the SydLab™ One a comprehensive tool for quantitative high-content screening.

Data Output

At the end of each experiment, SydLab™ One provides a comprehensive data package comprising raw and processed measurements across multiple phenotypic dimensions. Data are available as excel files organized by readout.

- **For an L4 experiment:** growth, motility, survival, reproduction, and fluorescence.
- **For an L1 experiment:** growth, egg, larvae, and fluorescence.

Each module includes raw data at every time point and reports aggregating metrics either per channel or per experimental condition over time.

- **For L4 experiments**, key metrics include worm length, area, speed, body bend frequency, lifespan, egg counts, and fluorescence intensity, among others.
- **For L1 experiments**, key metrics include: worm length, worm area, egg counts and larvae counts among others.

Graphical outputs are also provided as interactive HTML and static PDF reports, showing daily trends per condition. Statistical indicators such as standard deviation and sample size (worms or chambers) are included for every aggregated value. This structured and high-resolution data output enables both granular analyses of individual phenotypes and high-level comparisons across experimental conditions, streamlining interpretation and facilitating reproducibility in *C. elegans*-based studies.

Biological System Validation (Bio-benchmarking)

The SydLab™ One platform has undergone extensive validation through a series of experiments. This validation process involved benchmarking lifespan assays, reproducing known effects of dietary restriction and genetic mutations.

Lifespan Assays

The main goal of a lifespan assay is to measure *C. elegans* longevity. Using SydLab™ One, we analyzed the lifespan of wild type (N2) controls across 18 experiments lasting 22–35 days (A). In line with reported averages using traditional methods[1], *C. elegans* housed in microfluidic chips at 20°C, fed with an optimized freeze-dried OP50 diet, showed a mean lifespan of 18.05 days (95% CI: 17.78–18.33) and a median lifespan of 18.4 days. We then compared median lifespans from SydLab™ One with those from manual plate experiments using HT115 or live OP50 diets (B). As expected, the lifespan was similar between SydLab™ One and plate experiments with HT115, but longer than with live OP50, as inactivated *E. coli* diets extended lifespan compared to live bacteria in both solid and liquid cultures[2–4]. Lifespan assays on plates are labor-intensive, particularly for detecting dead worms. To automate this, we developed a machine learning (ML) algorithm trained on brightfield (BF) videos taken every 6 hours. The algorithm accurately detected deaths, with Pearson coefficients of 0.90–0.94 (C). Motility, a key healthspan indicator, is known to decline with age[5]. As predicted, automated analysis showed a gradual motility decrease over time, particularly during the first 10 days, as demonstrated by reduced head movement amplitude (D). These results highlight SydLab™ One's ability to reliably capture motility loss during aging effectively.

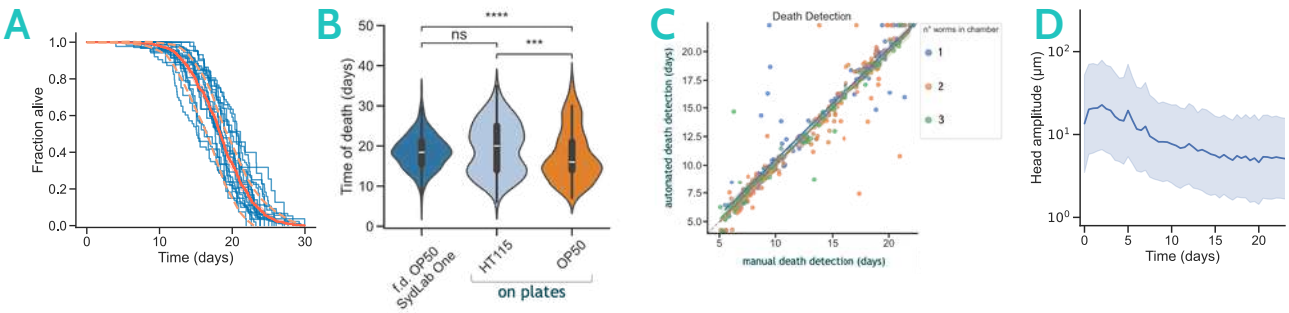


Figure description: (A) Survival curves for N2 worms generated from 18 experiments (blue curves) performed with SydLab™ One. The orange line represents the mean fraction of worms alive, the shaded orange area around the mean displays the 95% confidence interval (CI) of the mean survival and the dashed orange lines provide the standard deviation. For each experiment, the number of worms analyzed is at least 27. Median lifespan = 18.4 days. (B) Violin plots showing the distribution of the time of death of N2 worms during lifespan experiments performed in SydLab™ One with freeze-dried OP50 as a source of food and compared to on plates experiments with HT115 or live OP50 bacteria. Mann-Whitney U tests with Bonferroni correction performed. (C) Correlation between the automated death detection and the manual death detection depending on the number of worms per chamber. Analysis performed on BF movies acquired every 6 hours during 1 experiment in which N2 worms were exposed to different conditions. N, R^2, RMSE (Root Mean Square Error) and MAE (Mean Absolute Error) for chambers containing 1 worm; 2 worms and 3 worms respectively: 174, 0.9, 1.63, 0.37; 239, 0.91, 1.42, 0.33 and 75, 0.94, 1.25, 0.29. (D) Evolution of the head amplitude overtime as an example of motility feature extracted from BF videos during the 18 experiments. Data averaged for N2 worms in chambers containing maximum 3 worms and binned by 12h for 18 experiments. In this figure and below ns: p>0.05; ***: p<0.001; ****: p<0.0001, violin plots illustrate the distribution of data points with boxplots containing 50% of values (interquartile range, IQR), the median time of death (central line) and the whiskers show the range of values within 1.5*IQR.

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2. Garigan, D. et al. Genetic Analysis of Tissue Aging in *Caenorhabditis elegans*: A Role for Heat-Shock Factor and Bacterial Proliferation. Genetics 161, 1101–1112 (2002).
3. Stuhr, N. L. & Curran, S. P. Bacterial diets differentially alter lifespan and healthspan trajectories in *C. elegans*. Commun Biol 3, 653 (2020).
4. Win, M. T. T. et al. Validated Liquid Culture Monitoring System for Lifespan Extension of *Caenorhabditis elegans* through Genetic and Dietary Manipulations. Aging Dis 4, 178–85 (2013).
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Genetic mutants

Numerous genes influence *C. elegans* lifespan (reviewed in Uno & Nishida, 2016). To further validate SydLab™ One, we replicated phenotypes of well-known lifespan mutants: the long-lived *isp-1(qm150)*[1] and *daf-2(e1370)*[2], and the short-lived *daf-16(mu86)* mutants[3–4]. As expected, *isp-1(qm150)* and *daf-2(e1370)* showed significant lifespan extensions (+209.7% and +52.6%, respectively), while *daf-16(mu86)* had a -13.3% reduction in lifespan (A). This aligns with earlier findings, though *daf-16* longevity varies across alleles, highlighting the need for sufficient outcrossing with controls[2–3–5]. We also measured physiological traits and healthspan markers (B-D). *Daf-16* mutants had normal development, egg production, and motility[4]. *Daf-2* mutants were smaller, laid fewer eggs, and were less active at the restrictive temperatures[6]. *Isp-1* mutants were thinner and less active but produced a normal number of eggs[7]. These results confirm the known phenotypes of these genetic backgrounds.

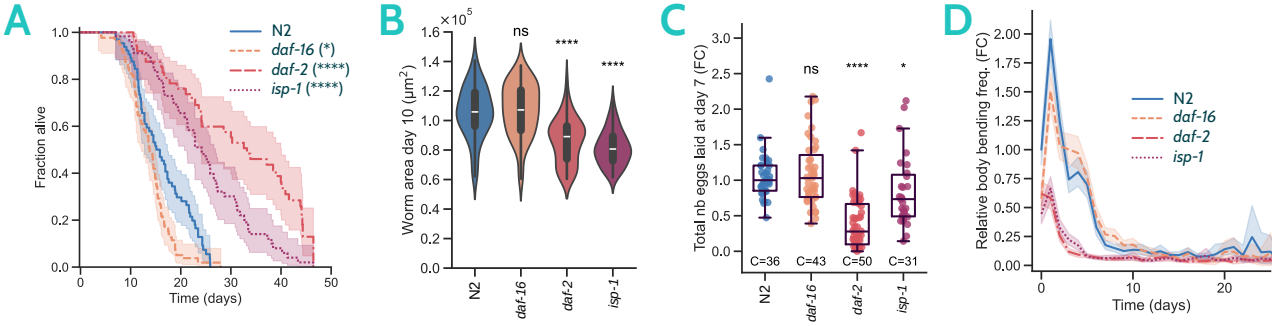

Figure description: (A) Survival curves of classical aging mutants from a lifespan experiment ran over 50 days with the SydLab™ One platform. For N2 *ad libitum* and for the mutant strains *daf-16, daf-2 and isp-1*, the median survival time (days) and the N worms analyzed are respectively: 15.8 and 62; 13.8 and 81, 33.2 and 96; 24.2 and 59. Log-rank tests with Bonferroni correction against the *ad lib.* Condition. (B) Violin plots showing the distribution of *C. elegans*' area measured on day 10. Mann-Whitney tests were performed to compare mutants to N2s. (C) Total number of eggs detected per *C. elegans* at day 7 and normalized to N2s. C=n reports the number of micro-chambers observed. Statistical significance relative to the control condition defined using Mann-Whitney tests with Bonferroni correction. (D) Body bending frequency during lifespan relative to N2 C. elegans' motility at day 0. The mean body bending frequency binned per day (colored lines) and the 95%CI of the mean (bootstrap, n=1000) (shaded areas around the lines) are shown. In panel C here and below, box plots contain 50% of values (interquartile range, IQR), the central line is the median and the whiskers show the range of values within 1.5*IQR.

References

1. Feng, J., Bussière, F. & Hekimi, S. Mitochondrial Electron Transport Is a Key Determinant of Life Span in *Caenorhabditis elegans*. Dev Cell 1, 633–644 (2001).
2. Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. Nature 366, 461–464 (1993).
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Dietary Interventions

Caloric restriction (CR), or dietary restriction, is a proven method to extend lifespan across species, including *C. elegans*[1]. Standard CR studies in *C. elegans* require labor-intensive plate preparations and frequent *C. elegans* transfers to prevent starvation[2]. Mutant strains or genetic knockdowns have also been used[3], but their specificity remains questionable. Typically, food-restricted *C. elegans* live longer, are smaller, lay fewer eggs, and show increased motility, with phenotypes varying by the degree of food deprivation. Using SydLab™ One, we streamlined CR experiments while replicating key phenotypes (A–D). Serial dilutions of freeze-dried OP50 were injected into microfluidic chips, providing precise control of food availability. As expected, CR extended lifespan, with a +68% median survival increase at 16% ad libitum food and +35% at 33% (A). No effect was observed at 66%. CR-treated *C. elegans* were smaller and laid about 50% fewer eggs at 16% (B and C). They also exhibited greater and sustained motility compared to fully fed *C. elegans* (D). These results demonstrate that SydLab™ One simplifies CR experiments, delivering detailed data for lifespan and healthspan studies with minimal effort.

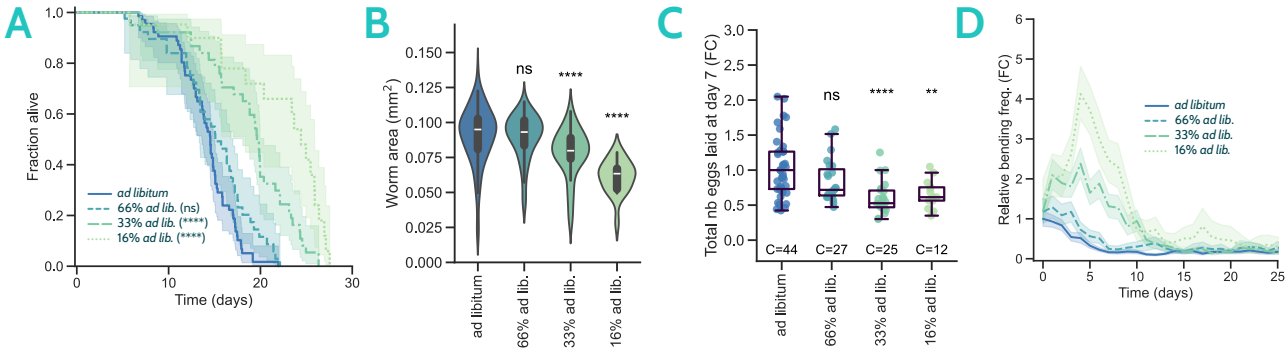

Figure description: (A) Survival curves generated upon *ad libitum* feeding and caloric restriction conditions on N2 worms. For *ad libitum* (*ad lib*), 66% *ad lib*, 33% *ad lib* and 16% *ad lib*, the median survival time (days) and the N worms analyzed are respectively: 14.5 and 75; 15.1 and 42; 19.6 and 42, 24.4 and 21. Log-rank tests with Bonferroni correction were performed compared to *ad lib*. (B) Violin plots for *C. elegans*' area measured at day 10 for different food diets. Statistical significance relative to the control condition defined using Mann-Whitney tests with Bonferroni correction. (C) Total number of eggs detected per *C. elegans* at day 7 and normalized to N2s. C=n reports the number of micro-chambers analyzed. Mann-Whitney tests with Bonferroni correction were performed against the *ad lib*. (D) Body bending frequency overtime and relative to N2 *C. elegans*' motility at day 0. The mean body bending frequency binned per day (colored lines) and the 95%CI of the mean (bootstrap, n=1000) (shaded areas around the lines) are shown.

References

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Conclusions

SydLab™ One offers a powerful and flexible solution for studying aging, neurodegeneration, toxicology, drug discovery, and genetic research in *C. elegans*, with features that streamline and enhance the research process. Its ability to handle a high number of test conditions in parallel makes it ideal for mid- to large-scale studies in compound testing (chemical and RNAi). The fully automated workflow, from imaging to cloud-based analysis, ensures consistent and reliable results, eliminating user bias and reducing variability. Moreover, by automating labor-intensive tasks, SydLab™ One frees researchers from the burdens of manual operations, allowing them to focus on interpreting results and advancing their scientific goals. This combination of throughput, automation, and precision positions SydLab™ One as an invaluable tool for scientific research.

Metrics table, L1 experiments *(e.g. for DART or for compound profiling studies)*

Injected worms		
Injected_worms_Length	Length of worms*	µm
Injected_worms_Area	Area of worms*	µm2
Injected_worms_Straight	Fraction of time worms spend in straight or slight curves shape (paralyzed/dead/molting)	
Injected_worms_Active1	Fraction of time worms spend in semi-circles and gentle curves shape (active worms)	
Injected_worms_Active2	Fraction of time worms spend in S-shapes, sharp bends, complex curves (active worms)	
Injected_worms_Coiled	Fraction of time worms spend in circles, ellipses, highly convoluted shapes (toxic environment)	
Eggs		
Egg_emergence	Time at which the first egg is detected provided by step or absolute time in hours	Step number and hours
Egg_accumulation	Slope of the linear fit applied on egg counts starting from the time of egg emergence	-
Egg_laying	Number of eggs laid per worm within the first 24h starting from time of egg emergence	N eggs per worms per day
Egg_counts**	Number of eggs detected, normalized to the number of worms	N
Larvae		
Larvae_emergence	Time at which the first larvae is detected provided by step or absolute time in hours	Step number and hours
Larvae_accumulation	Slope of the linear fit applied on larvae counts starting from the time of larvae emergence until the end of the experiment	-
Larvae_accumulation_24h	Slope of the linear fit applied on larvae counts starting from the time of larvae emergence on a 24h window	-
Larvae_counts**	Number of larvae detected, normalized to the number of worms	N
Developmental_speed	Larvae speed of development calculated as follows: Larvae_max_area minus Larvae_min_area / first day of larvae detection	μm^2/hour

Larvae		
Larvae_area	Area of larvae*	μm^2
Larvae_Straight	Fraction of time that larvae spend in straight or slight curves shape (paralyzed/dead/molting)	
Larvae_Active1	Fraction of time that larvae spend in semi-circles and gentle curves shape (active worms)	
Larvae_Active2	Fraction of time that larvae spend in S-shapes, sharp bends, complex curves (active worms)	
Larvae_Coiled	Fraction of time that larvae spend in circles, ellipses, highly convoluted shapes (toxic environment)	

* Based on the number of pixels that compose the detection mask. A scaling step is applied to convert pixels to microns using a pixel size of 0.45 µm (as the images are binned to 1024*1024 pixels).

** Egg count and larvae count are generated for a time window defined as larvae_emergence * 1.4. Beyond this time window, the chambers become too crowded with eggs, and larvae to allow for accurate object detection.

All measurements are provided for a given time (step and/or absolute time) as averaged data, per channel or per condition, along with the sample size (N) used for averaging, as well as the corresponding standard deviation (SD). A raw data file is also available, containing all extractable metrics listed below, recorded at each time step and at the individual worm level.

Additional metrics extracted from L1 experiments, and available without aggregation:

- Worms_volume,
- Worms_perimeter,
- Worms_solidity,
- Worms_convex_area,
- Worms_eccentricity,
- Worm_equivalent_diameter,
- Worm_extent,
- Worms_minor_axis_length,
- Worms_major_axis_length,
- Worms_orientation,
- Larvae_perimeter,
- Larvae_solidity, larvae_convex_area,
- Larvae_eccentricity,
- Larvae_equivalent_diameter,
- Larvae_minor_axis_length,
- Larvae_major_axis_length,
- Larvae_orientation.

Metrics table, L4 experiments *(e.g. for Healthspan and Lifespan studies)*

Morphometrics		
Length	Length of worms*	µm
Area	Area of worms*	µm2
Eggs		
Egg_emergence	Time at which the first egg is detected	hours
Egg_counts	Number of eggs detected, normalized to the number of worms	N
Motility		
Head	Amplitude of the head measured as the max distance to the straight trajectory (longitudinal axis) [1]	mm
Mid	Amplitude of the middle of the body as the max distance to the straight trajectory (longitudinal axis) [1]	mm
Tail	Amplitude of the tail measured as the max distance to the straight trajectory (longitudinal axis) [1]	mm
Frequency	Body bends frequency	Hz
Speed	Speed of worm displacement in chambers	mm/sec
Curvature	Averaged worm curvature	mm
Survival		
Death_events	Timing of death events	hours
Average lifespan	Average time of death	hours
Lifespan 25% quantile	Time at which 25% population has died	hours
Median lifespan	Median time of death	hours
Lifespan 75% quantile	Time at which 75% population has died	hours

* based on the number of pixels that compose the detection mask. A scaling step is applied to convert pixels to microns using a pixel size of 0.45 µm (as the images are binned to 1024*1024 pixels).

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All measurements are provided as averaged data for a given time, either per channel or per condition, along with the sample size (N) used for averaging and the corresponding standard deviation (SD). A raw data file is also available, containing all the previously described metrics, recorded from each frame of the acquired brightfield (BF) videos at the individual worm level.

Additional metrics extracted from L4 experiments, and available without aggregation:

- Worms_volume,
- Worms_perimeter,
- Worms_solidity,
- Worms_convex_area,
- Worms_eccentricity,
- Worm_equivalent_diameter,
- Worm_extent,
- Worms_minor_axis_length,
- Worms_major_axis_length,
- Worms_orientation.

Metrics table, Fluorescence (available for both green and red colors and both L1 and L4 experiments)

Fluorescence		
Total_Intensity	Mean grey levels of pixels within the object detection masks	0-4095
Tot_Intensity_minus_background	Total_Intensity minus Background_Level	0-4095
Background_Level	Mean grey levels of the 50% least bright pixels within the chamber	0-4095
Pixel_Saturation_Ratio	Number of saturated pixels divided by the total number of pixels of the image	-

Fluorescence quantifications are provided as average data aggregated per step or per day and per channel or per condition for each color (Green/Red). A raw data file is also available, containing all the previously described metrics measured for every chambers at every time points. Two additional metrics, intensity_max and intensity_min are provided in this file.

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