STREAMLINING AGING STUDIES IN C. ELEGANS BY END-TO-END AUTOMATED HIGH-THROUGHPUT ANALYSIS

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ABSTRACT

High-throughput analysis is a challenge in *C. elegans* research. One example is the conduction of lifespan assays that rely on heavy manual work for a long period of time. In response, we developed a robotic microfluidic-based platform, the SydLab™ One, capable of handling culture, treatment, and imaging of up to 64 worm populations in parallel in a fully automated manner. Combined with AI-based image analysis and data compilation, our platform provides, in record time, large comprehensive datasets spanning all essential features for aging studies.

To illustrate SydLab™ One's robustness, we present a series of lifespan studies. We tested benchmark interventions known to affect *C. elegans* lifespan, including defined conditions for dose-dependent responses to caloric restriction. We also validated the behaviour of 2 classic long-lived mutants (*daf-2(e1370)* and i*sp-1(qm150)*). For all these conditions, the lifespan and healthspan of worms were evaluated throughout automated analysis of short brightfield videos acquired every 6 hours for up to a month. In addition, SydLab™ One also quantified the evolution of key aging features, including egg laying, size, motility and intestinal atrophy. With the large amount of data generated, we are investigating key age-related phenotypes that may serve as potential predictors of aging.

Altogether, SydLab™ One's integrated technologies (microfluidic chips, robotics, optics, and AI-based software analysis pipelines) significantly reduced the bench time while exponentially increased the amount of datapoints obtained for multiple conditions in parallel. Therefore, promoting standardization and reproducibility thanks to end-to-end automation.

MICROFLUIDIC PLATFORM OVERVIEW

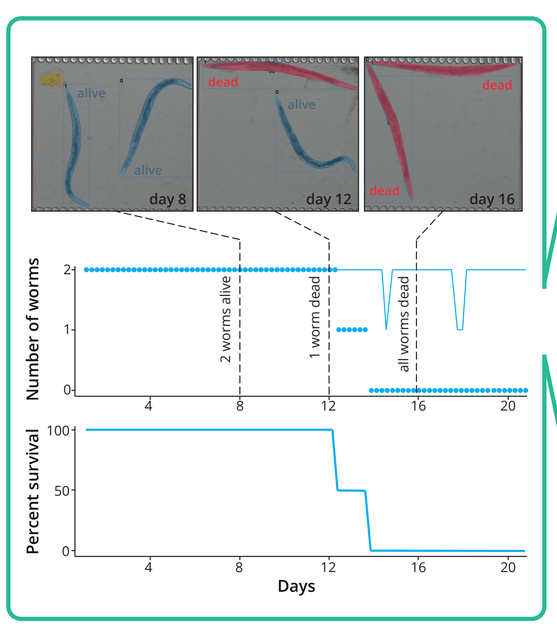
Our microfluidic technology allows **large-scale** studies for the parallel characterization of drugs, chemicals and genetic interventions in *C. elegans*. The SydLab™ One platform provides **fully-automated culture, treatment, imaging and analysis** of the worms over long-term experiments. The high-content information extracted using our image processing and data interpretation algorithms enables detailed multi-phenotypic screening at the whole-organism level.

Worms are **automatically injected into the microfluidic platform** and confined within dedicated chambers of the microfluidic chips. They are then continuously fed with *E. coli* solution and can be exposed to the test interventions according to a defined treatment plan. Pictures or videos of each micro-chamber **are acquired via time-lapse microscopy** at desired frequency.

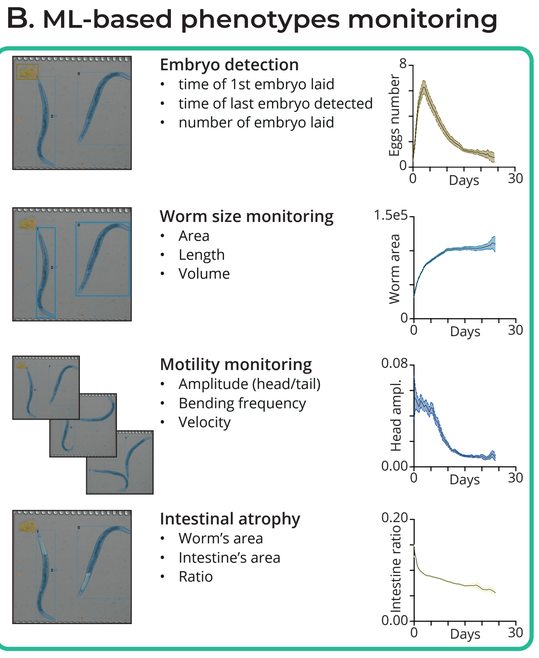
DATA PROCESSING PIPELINE

The time-lapse brightfield videos obtained during the experimental procedure are **post-processed by a machine learning (ML) software** (A). Through this ML software we detect the death of individual worms within the microfluidic chip. Over the course of the lifespan assay, a subsequent analysis pipeline **extracts multiple phenotypes from the alive population** (B), assessing several hallmarks of aging, **including the reproductive span, worm morphology, motility and intestinal atrophy.**

A. ML-based death' detection

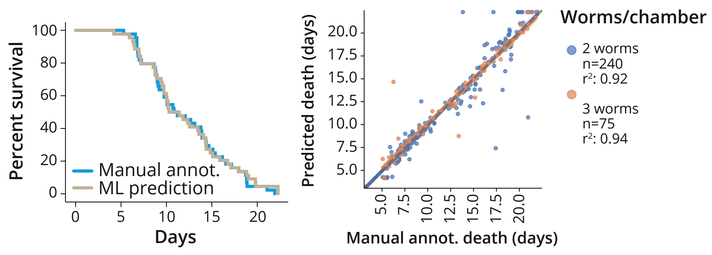


B. ML-based phenotypes monitoring

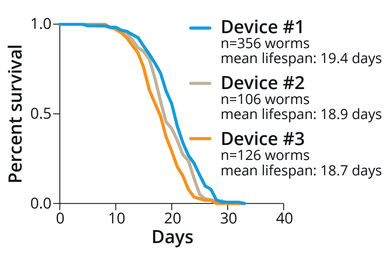


Our ML-based death' detection algorithm shows a **high accuracy for predicting survival propability** of worm's cohorts over the whole range of possible lifespans, matching human annotated performance (C). Lifespan assays performed on SydLab™ One show results aligned with previous works from the literature (median=19.1 days at 20°C) and a **high inter-device reproducibility** (One-way ANOVA: p value=0.399) (D).

C. ML-based death' detection



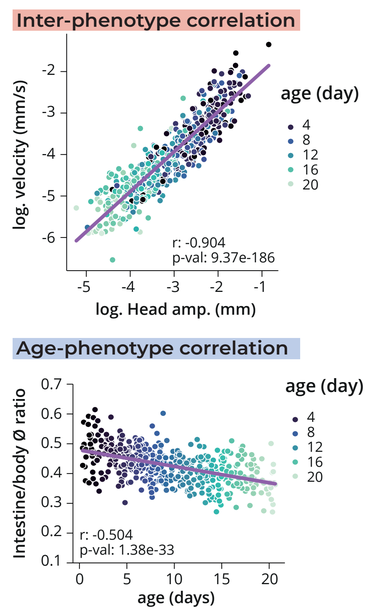
D. Inter-device reproducibility



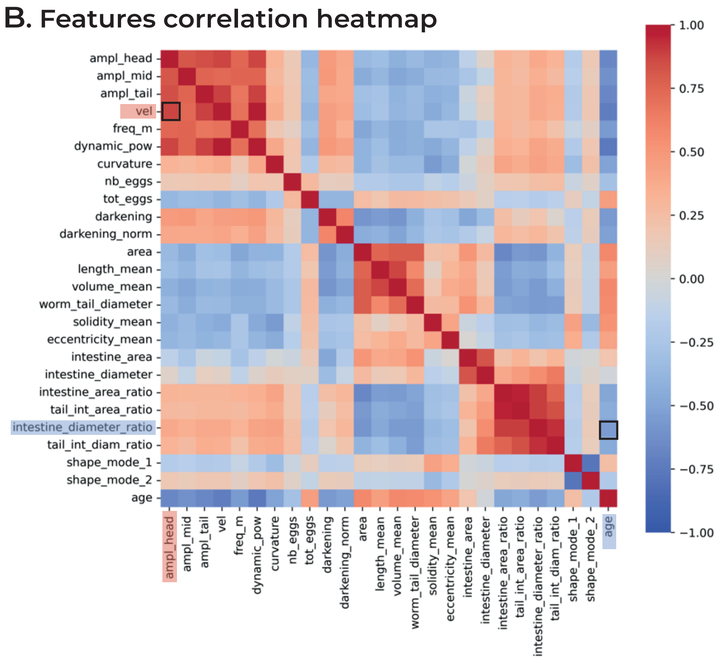
HALLMARKS OF AGING: EXTRACTED PHENOTYPES

Phenotypes extracted in the time-course of the experiment **show strong correlations with aging** (A). **Up to 25 phenotypes can be monitored** for each single individual, allowing to capture complex interactions between phenotypes, and with lifespan (B).

A. Phentoypes correlation

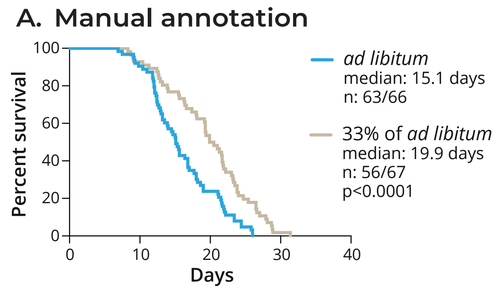


B. Features correlation heatmap

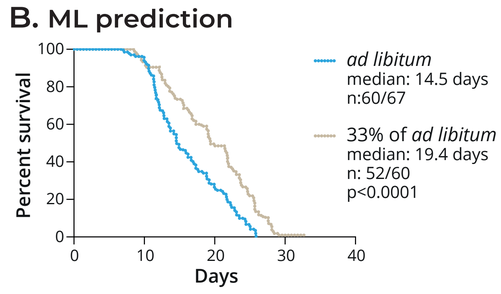


RESULTS

A. Manual annotation

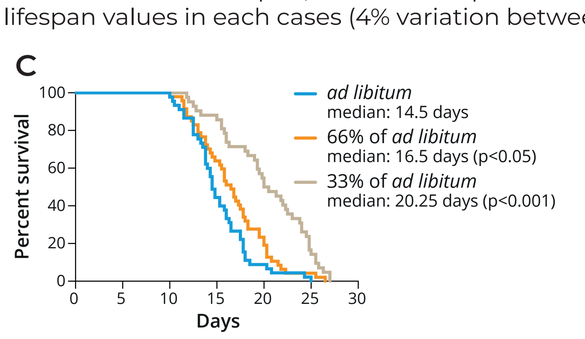


B. ML prediction

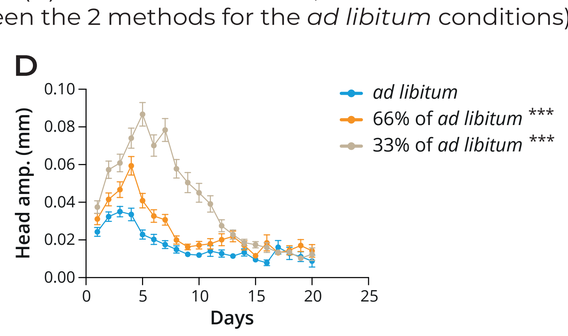


Our algorithm **accurately identifies anti-aging interventions**, as demonstrated by the comparable lifespan extensions observed with food dilution: manual annotations (A) indicates a 32% increase in median lifespan, while the ML prediction (B) shows a 34% increase, with similar median lifespan values in each cases (4% variation between the 2 methods for the *ad libitum* conditions).

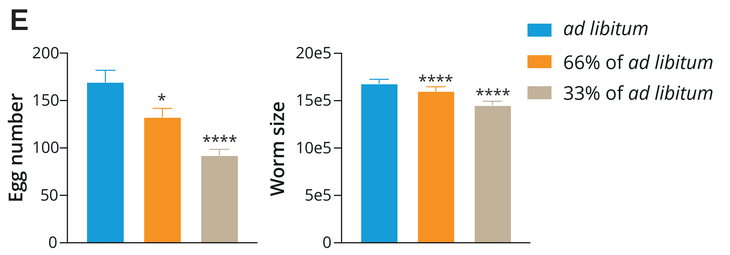
C



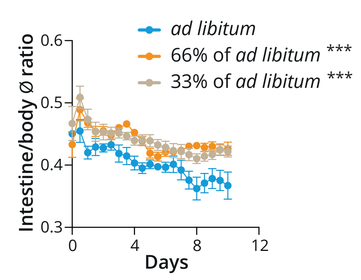
D



E

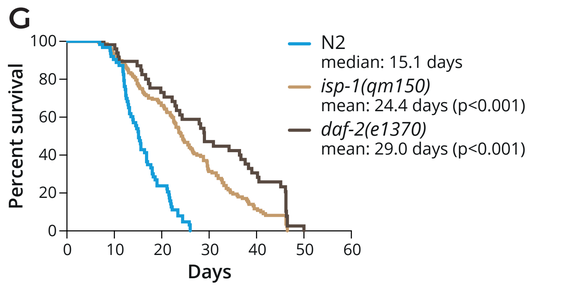


F

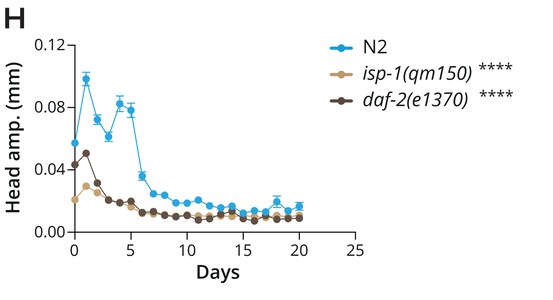


Food dilution (Fd) interventions show lifespan extensions in our microfluidic system (C), consistent with previous literature (66% of *ad libitum*: +10% / 33% of *ad libitum*:+39%). Phenotypic extraction reveals that both Fd intervations significantly improve motility (D), while negatively affecting reproduction and worm's size (E). Additionally, Fd significantly maintains intestine integrity during aging, as indicated by a higher intestine diameter to worm body diameter ratio (F).

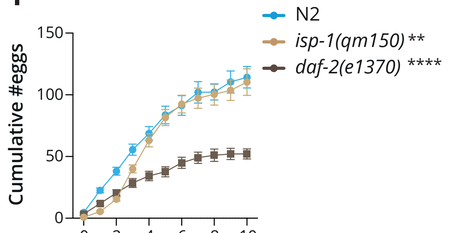
G



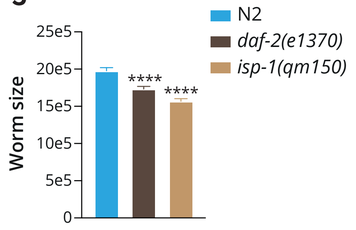
H



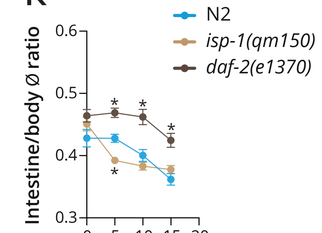
I



J



K



Classic long-lived mutants, namely *daf-2(e1370)* and *isp-1(qm150)*, exhibit previously reported lifespan extension in our platform, with +92% and +61% increases in median lifespan, respectively (G). Unlike Fd interventions, both mutants maintain a low motility profil during aging (H). Although both mutants live significantly longer compared to wild-type N2, they show distinct phenotypic features: *isp-1* shows a delay in egg laying and a non-significant improvement in intestinal atrophy, while *daf-2* mutants produce significantly fewer eggs but maintain a good intestinal integrity (I-K).

CONCLUSION AND OUTLOOK

We presented the SydLab™ One platform, an **all-in-one solution that automates the study of aging in *C. elegans***, using machine learning to detect death and analyze age-related phenotypes like motility and intestinal integrity. The platform provides **high accuracy and reproducibility**, making it ideal for large-scale, multi-phenotypic aging studies. Our results show food dilution and classic long-lived mutants significantly extend lifespan, aligning with previous scientific research. Future perspectives include expanding the platform's capabilities ***to screen a wider range of genetic and pharmacological interventions***, and integrating advanced algorithm and techniques to further unravel the complexities of aging at the cellular and molecular levels.