Fully automated laboratory device for aging studies using the nematode *Caenorhabditis elegans*

David Liaskos[1,2], Elena Katsyuba[1], Laurent Mouchiroud[1], Johan Auwerx[2], and Matteo Cornaglia[1]*

[1] *Nagi Bioscience SA, EPFL Innovation Park, Chemin de la Dent d'Oche 1B, CH-1024 Ecublens, Switzerland;*
[2] *Laboratory of Integrative Systems Physiology, EPFL, CH-1015 Lausanne, Switzerland;*
**email: matteo.cornaglia@nagibio.ch*

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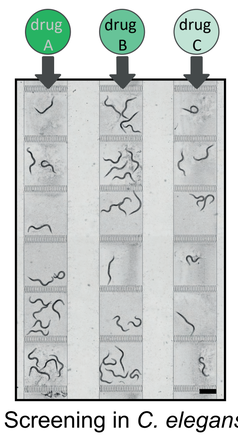
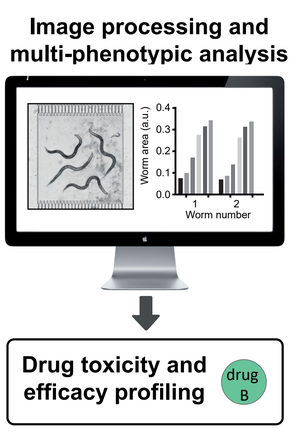
Here we describe an innovative microfluidic platform using the nematode *Caenorhabditis elegans*, as an ideal model organism for the study of aging due to its short lifespan and tractable genetics. Our platform allows full automation of the entire process of culture, treatment, imaging and analysis of *C. elegans*, at unprecedented levels of throughput and standardization. The unique possibilities offered by this microfluidic platform allow the automated execution of customized bioassays for: (i) monitoring *C. elegans* lifespan and healthspan; (ii) identifying developmental phenotypes that can serve as potential predictors of aging; (iii) quantifying the GFP expression of reporter strains. In particular, to test the potential of our lifespan and healthspan bioassay, we expose wild-type worms to different dietary restriction plans and an anti-aging compound. In this study, we show that the platform allows the detection of early phenotypes related to the aging process, such as size and reproductive aging, and we successfully demonstrate the extended lifespan of *C. elegans* as a result of precise dietary restriction plans and treatment with a geroprotective drug. Finally, we employ the *hsp-6:gfp* transgenic strain as a specific reporter for the mitochondrial unfolded protein response (UPR^{mt}). By treating *hsp-6::gfp* worms with the antibiotic doxycycline, we can activate the UPR^{mt} and efficiently quantify its level by measuring the fluorescence intensity of the worms.

Microfluidic platform overview

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

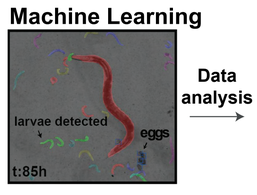


Screening in *C. elegans*



The time-lapse brightfield pictures obtained during the experimental procedure are post-processed by an extensively trained **machine learning (ML) software** (**a**). Through this ML software we monitor the **growth rate** of the worm population within the microfluidic chip over several days, as well as the **fertility** and **progeny production** (**b**), with a high degree of reproducibility accross replicates (**c**). Our technology also allows worm monitoring throughout their **entire lifespan**. Via video acquisition we extract several **motility** parameters (**d**) and detect dead worms if no movement is observed in successive videos (**e**).

a



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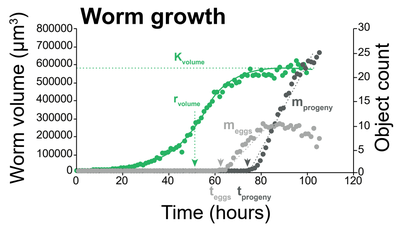


c

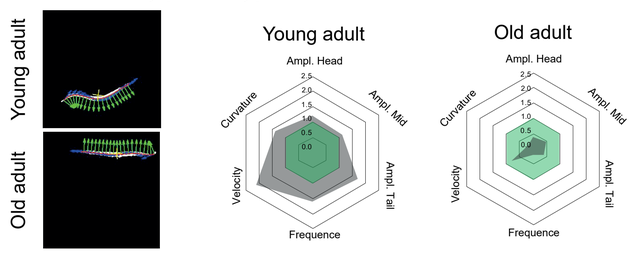
Objects detected

● Worm (size) ● Eggs (number) ● Progeny (number)

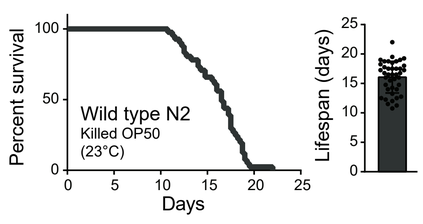
Reproducibility

Parameter	Value	Variation	Experiments
Maximum size (K)	570'388 (µm³)	±6.5%	15 repeats
Growth dynamic (r)	2.24 (days)	±5.6%	15 repeats
Sexual maturity (t_{eggs})	56.44 (hours)	±5.4%	15 repeats

d

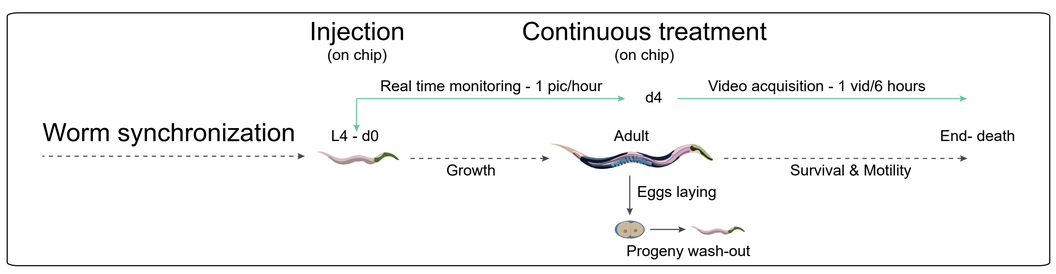


e



Design of the aging bioassay

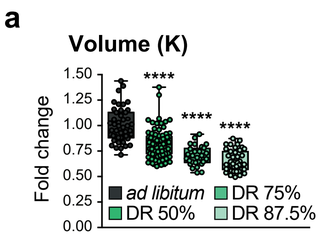
Synchronized populations of **L4 larvae are injected** into the microfluidic chips (d0). Following the L4s injection into the device, each worm population is **continuously exposed** to a well-defined liquid environment, including the compounds to be tested and *E. coli* bacteria as the food source. The growth and reproduction of *C. elegans* are monitored during the first 4 days of adulthood by acquiring brightfield **images every hour**. After the first 4 days, **videos are taken every 6 hours** to analyze worm motility and to identify if a worm is dead or not. Here, we score a worm as dead if no movement is observed for 3+ consecutive videos. The experiment stops when all worms are dead.



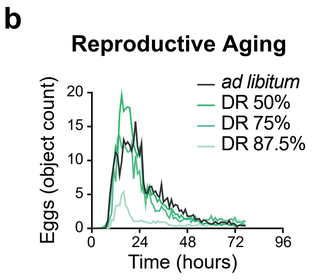
Phenotypic profiles of longevity

The aging bioassay was employed against precise **dietary restriction** (DR) plans. Early phenotypic readouts revealed the significant effect of DR on worm size (**a**) and the progressive decrease in reproductive capacity as DR levels increased (**b**). **Survival analysis** showed that DR reduces the death rate of worms (**c**). Similar to the literature, survival followed a Gaussian distribution around the optimal diet (~50%).

a



b



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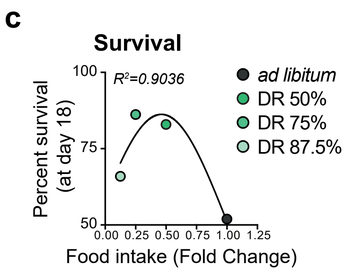
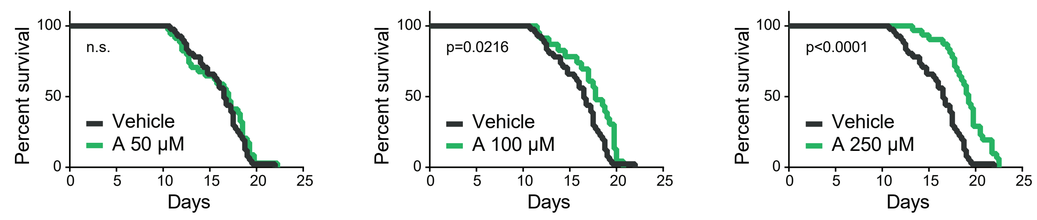


A key feature of our device for aging assays is the ability to rigorously follow individual worms **over their entire lifespan**, and this simultaneously for different conditions. Here we exposed WT worms to an **anti-aging compound** (A) at 3 different concentrations and compared them to the vehicle population. The effect of compound A on the survival of the worm populations was calculated with Kaplan-Meier curves and log-rank (Mantel-Cox) test (**d**). Our results showed a **dose-dependent extension of the mean lifespan** of the worms, with a ~20% increase for the highest dose (250µM).

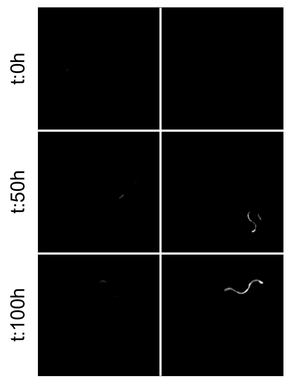
d



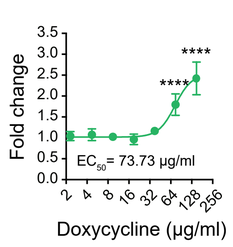
Fluorescence intensity quantification

To exploit the advantage of *C. elegans* in facilitating green fluorescent protein (GFP) visualization, we employed the *hsp-6::gfp* strain in our microfluidic platform as a specific reporter for the mitochondrial unfolded protein response (UPR^{mt}) (**a**). **Quantification of fluorescence intensity** allowed the identification of a **dose-dependent induction of UPRmt** upon treatment with the antibiotic doxycycline (**b**), which is known to promote longevity and healthy aging in *C. elegans*.

a



b



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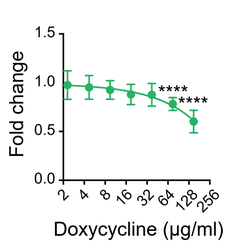
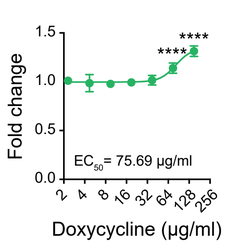


d



Our approach allowed simultaneous monitoring of maximum worm size (**c**) and growth dynamics (**d**).

Conclusion & Outlook

We presented a novel microfluidic platform for *C. elegans* research and demonstrated its potential for conducting aging studies. Our technology offers unprecedented levels of control and automation in long-term *C. elegans* experiments. Dedicated ML software allows monitoring of a variety of worm phenotypes, including body size, fertility, reproduction, motility and survival. We believe this platform represents the first "all-in-one" *C. elegans* microfluidic laboratory and promotes more efficient characterization in the early stages of the anti-aging drug discovery pipeline.