A ROBOTIC PLATFORM FOR FULLY AUTOMATED AGEING STUDIES IN C. ELEGANS

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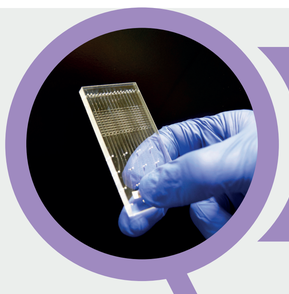
ABSTRACT

C. elegans is a powerful model organism for ageing studies. However, the traditional protocols, which continue to be broadly used, rely on manual handling, making them labor-intensive and time-consuming. Automation of these processes would greatly benefit long-term studies of *C. elegans*. Significant progress has been achieved over the past decade in the techniques to study worm's biology: the introduction of microfluidic approaches for different assay types and the use of machine learning-based algorithms for data processing offer an increase in experimental throughput and a better control of experimental conditions.

We propose here a novel solution for automated ageing studies in *C. elegans*, which involves these new methodologies. Our microfluidic-based robotic platform is capable to fully automate all the key aspects of *C. elegans* experimentation, including worm culture, treatment, imaging, as well as data recording and analysis. The unique characteristics of the platform allow ageing studies on multiple worm populations in parallel that go beyond a simple tracing of the survival curves. We present here a panel of standardized bioassays allowing automated: (1) monitoring of *C. elegans* lifespan, (2) assessment of worm fitness, (3) testing of different stress responses activation.

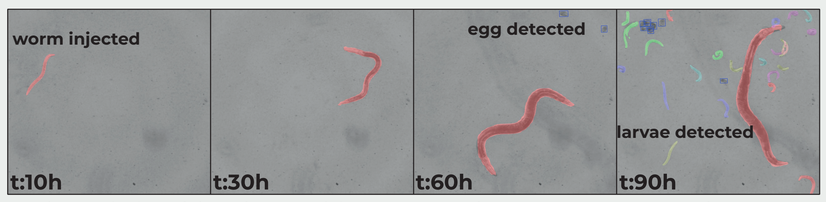
The performance of the assays was corroborated by testing benchmark compounds known to affect *C. elegans* longevity. While the results obtained with our platform were consistent with the results obtained with conventional "manual" methods on plates, the use of microfluidic chips significantly reduced the consumption of test compounds, and fully automated imaging process and data analysis software significantly reduced the number of man-hours required for such study.



Worms are grown inside microfluidic chips. Up to 16 independ biological conditions can be tested per chip.

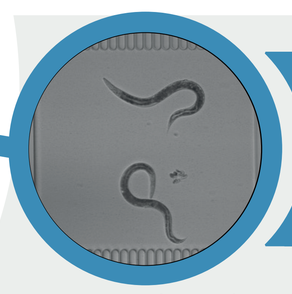
The chips are inserted into the robotic platform, which then ensures a fully-automated worm distribution inside the microfluidic chips and maintains the defined culturing conditions (temperature, food delivery and exposure to test compounds according to the treatment plan defined by the user). It executes time-lapse image and video acquistion for the entire duration of an experiment. Two chip formats are available: depending on the type of assay performed the user can chose to start with a synchronised population of L1 or L4 larvae. The platform can service up to 4 chips in parallel.

The post-processing of the images generated during the experiment is performed by a trained AI software.



AI-based algorithm is capable to recognize worms, eggs and progeny

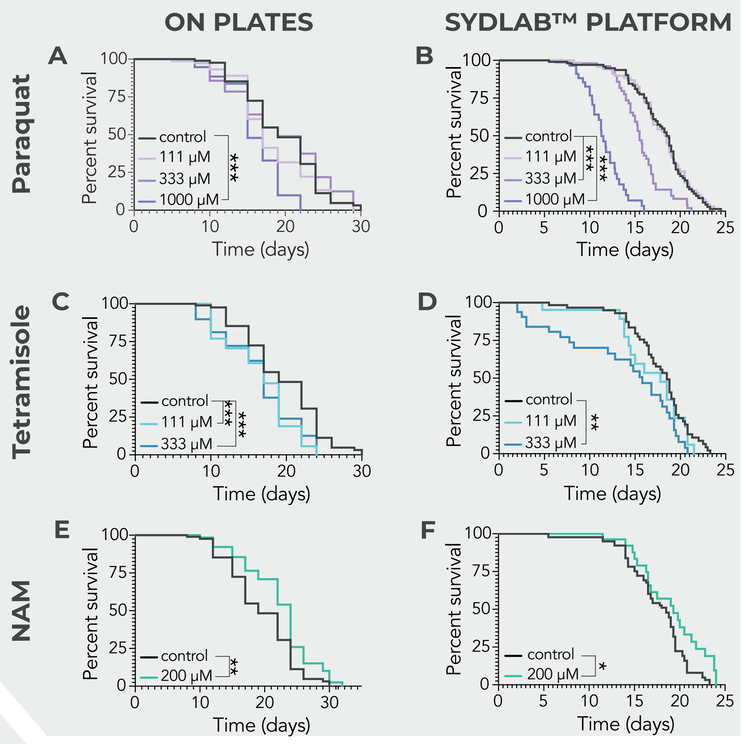
Based on efficient object recognition, the software can extract 20+ features per data point per worm, enabling the monitoring of the growth dynamics of the worm population, assess its fertility and reproductive ability. The software modules not only provide statistical analysis and data interpretation but also enable real-time data visualization.



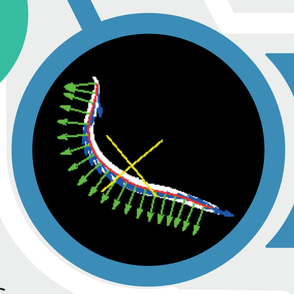
Lifespan assay requires a synchronised population of L4 larvae. The survival data is sampled every 6 hours.

We tested 3 benchmark compounds at indicated concentrations using our Sydlab platform (B, D, F). The treatment was started at the L4 stage. In parallel, the same conditions were reproduced on agar plates (A, C, E), in a traditional way.

As expected, paraquat and tetramisole negatively impacted on *C. elegans* lifespan (A-D), while nicotinamide (NAM) led to a significant extension of worm's longevity (E, F).

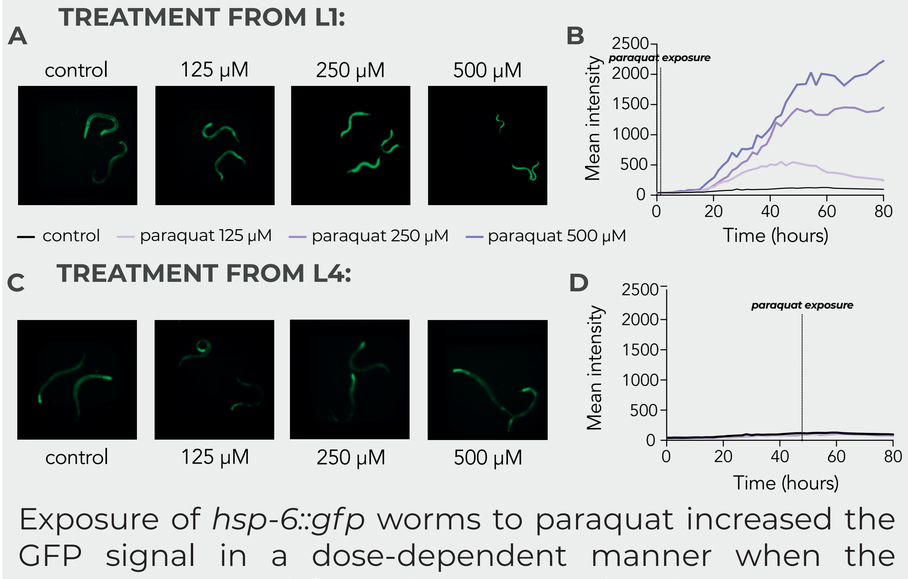


Molecular mechanisms of ageing can be explored with the fluorescent imaging.



The fitness of the worms is assessed based on video recording. Multiple motility parameters are extracted during the post-processing.

The platform offers the possibility to perform fluorescent imaging, thereby enabling the utilisation of a vast collection of *C. elegans* reporter strains. For instance, *hsp-6::gfp* reporter strain is widely used to monitor activation of the mitochondrial unfolded protein response (UPRmt). Our platform allows not only to conduct endpoint measurements, but to evaluate the dynamics of the UPRmt activation.



Exposure of *hsp-6::gfp* worms to paraquat increased the GFP signal in a dose-dependent manner when the treatment started from the L1 stage (A, B). However, no UPRmt activation was detected when exposure to paraquat started from the L4 stage (C, D).

The high-content motion analysis offered by our software can reveal very subtle changes in nematode movements, going beyond average motility measurements. The extracted motility parameters encompass the bending frequency, velocity, and amplitude of the head, tail and mid-body.

The effects of the 3 benchmark compounds were further evaluated on *C. elegans* motility. Interestingly, while both paraquat and tetramisole showed detrimental effects on worm's survival, their effects on worm motility differed. Tetramisole severely reduced all the motility parameters at both tested concentrations (D-F). Paraquat, in its turn, did not exhibit any pronounced effect on motility when averaged over the entire lifespan of the worms (A). However, at 333 µM and 1000 µM doses, paraquat led to overactivity in young worms (B). On the other hand, in the aged population, 1000 µM of paraquat led to a important decrease in motility (C).

NAM did not show a notable impact on worms motility (G-I), but still alleviated the age-related paralysis, as reflected by the amplitudes of the head, tail and mid-body in old worms (I).

Altogether, these examples highlight the importance of multi-factorial characterisation in ageing studies.