ROBOTIC DEVICE FOR FULLY AUTOMATED HIGH-CONTENT SCREENING ON C. ELEGANS AS A NOVEL NAMS PLATFORM FOR EARLY TOXICITY ASSESSMENT

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ABSTRACT

Purpose: Current toxicology testing methods heavily rely on experiments with mammalian models, which is not only expensive but also raises significant ethical concerns. On the other hand, alternative testing methods typically rely on cellular models, which are limited in their ability to predict complex organism-level responses, such as multi-organ crosstalk and metabolic processing of the tested substances. Nematode *Caenorhabditis elegans* constitutes a valuable NAMs model for multiple applications, including predictive toxicology. This microscopic worm gained popularity for its ideal short size, short life cycle, ease of cultivation and propagation, and powerful genetic toolkit. While *C. elegans* has the potential to complement in vitro models to better predict toxic outcomes in mammals, the current experimentation methods lack automation and standardization, limiting their wider use in screenings.

Methods: In response, we developed a microfluidic-based robotic platform that automates the entire process of *C. elegans* culture, treatment, high-content imaging, and phenotypic analysis. The imaging potential of the platform is further extended by a possibility to acquire fluorescent pictures, allowing to benefit from the existing large collection of reporter strains.

Results: As an illustration of the platform capabilities, we evaluated the reproductive and developmental effects of twenty benchmark chemicals, amongst which were bisphenol A, thalidomide, hydroxyurea, paraquat, busulfan, and 5-fluorouracil, using the proposed platform. Synchronized populations of worms were chronically exposed to five doses of test compounds starting from the last larval stage (L4). Time-resolved phenotypic readouts were automatically extracted from the hourly-collected images of the worms, including growth dynamics, sexual maturity, fertility, embryonic viability, progeny accumulation and survival rate. Out of the tested compounds, methotrexate showed the most pronounced embryonic viability adverse effects, while bisphenol A strongly impacted the development of the mothers.

Discussion: We propose an innovative solution for rapid identification of toxic compounds and their mechanism of toxicity, using a model that perfectly bridges the gap between in vitro and in vivo assays. Our technology allows not only the collection of endpoint measurements, but also the monitoring of the dynamics of the biological responses.

MICROFLUIDIC PLATFORM OVERVIEW

Our microfluidic technology allows **large-scale** studies for the parallel characterization of drugs and chemicals 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.

Features

- Patented microfluidic design, relying on passive hydrodynamics
- 16 fluidic lines, enabling tests of 16 independent conditions
- Plug & play chip-to-device connectivity and fluidic operations

Features

- Active culture, treatment and study of 64+ independent conditions
- Programmable acquisitions of BF and fluo images and videos
- Active temperature control in the 10-40°C range

Features

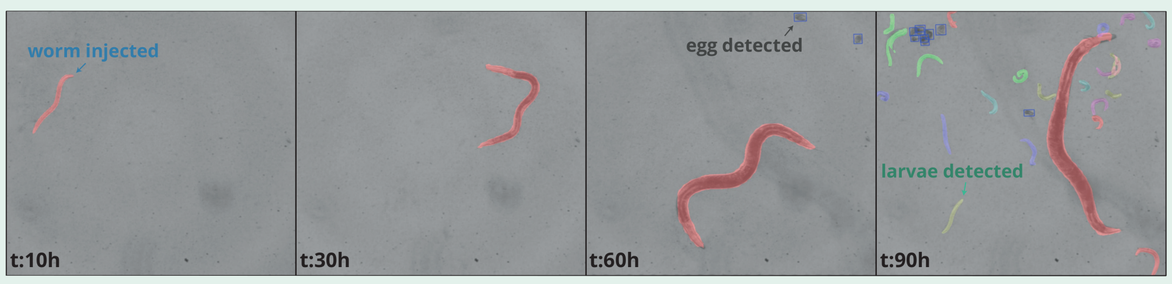
- User-friendly software to design, run and monitor experiments
- Time-resolved/high-content data extraction based on AI
- Integrated statistical analysis /data interpretation algorithms

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 compounds according to the defined treatment plan. The pictures of each microfluidic chamber **are acquired via time-lapse microscopy** at desired frequency.

DATA PROCESSING PIPELINE

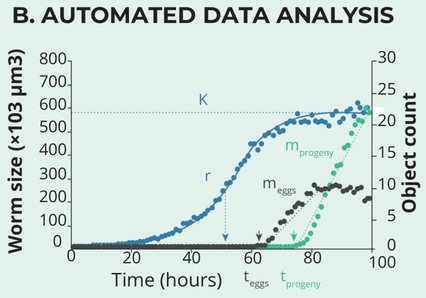
The time-lapse brightfield pictures obtained during the experimental procedure are post-processed by a **machine learning (ML) software (A)**. Through this ML software we monitor the **growth rate** of the worm population within the microfluidic chip over several days, as well as the **fertility** (eggs appearance and number) and **progeny production (B)**, with a high degree of reproducibility accross replicates **(C)**.

A. ML-BASED OBJECTS' DETECTION



The object recognition allows the ML algortihms to extract the following parameters: maximal length (K_{lenght}), time required to reach ½ max length(r_{lenght}), the time point when the first egg is detected (t_{eggs}), the time point when the first larvae is detected ($t_{progeny}$), the speed of egg (m_{eggs}) and progeny accumulation ($m_{progeny}$).

B. AUTOMATED DATA ANALYSIS



C. OBJECTS DETECTED

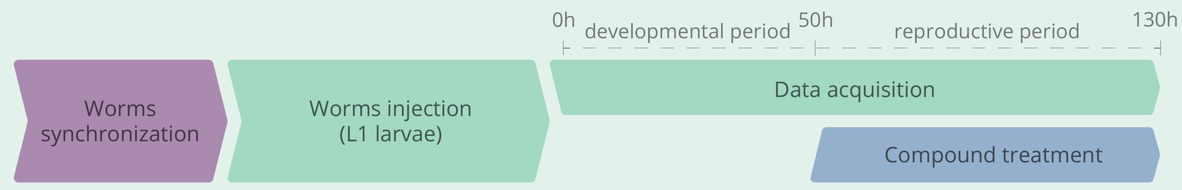
● Worm (size) ● Eggs (number) ● Progeny (number)

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

REPRODUCTIVE TOXICITY ASSAY

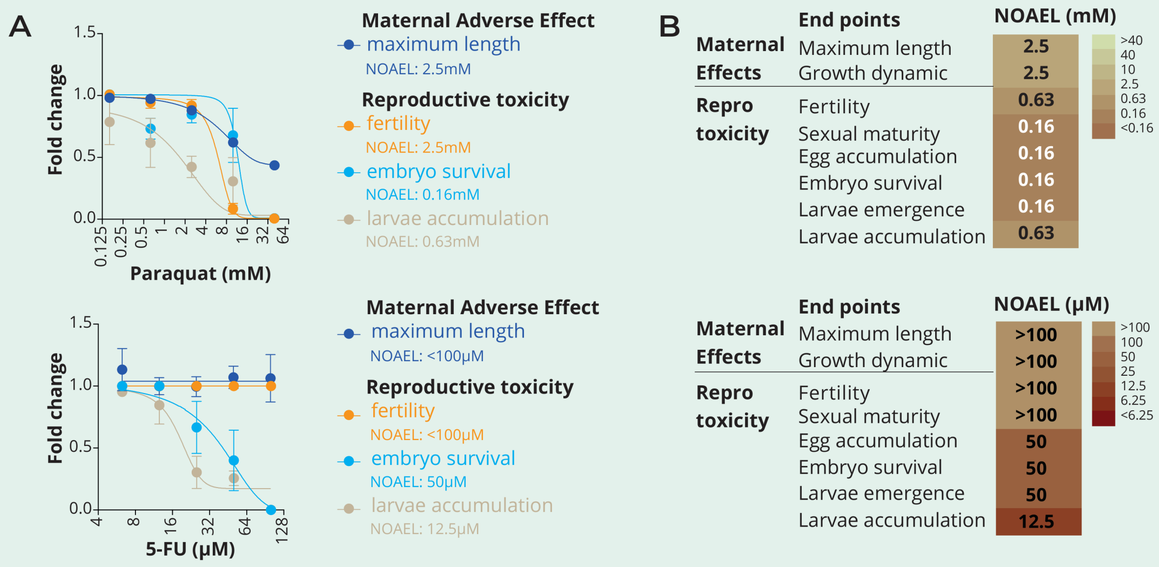
Method description: a synchronized population of *C. elegans* is injected into the microfluidic platform at the first larval stage (L1). Worms are confined within dedicated microfluidic chambers and are continuously fed with an *E. coli* solution.



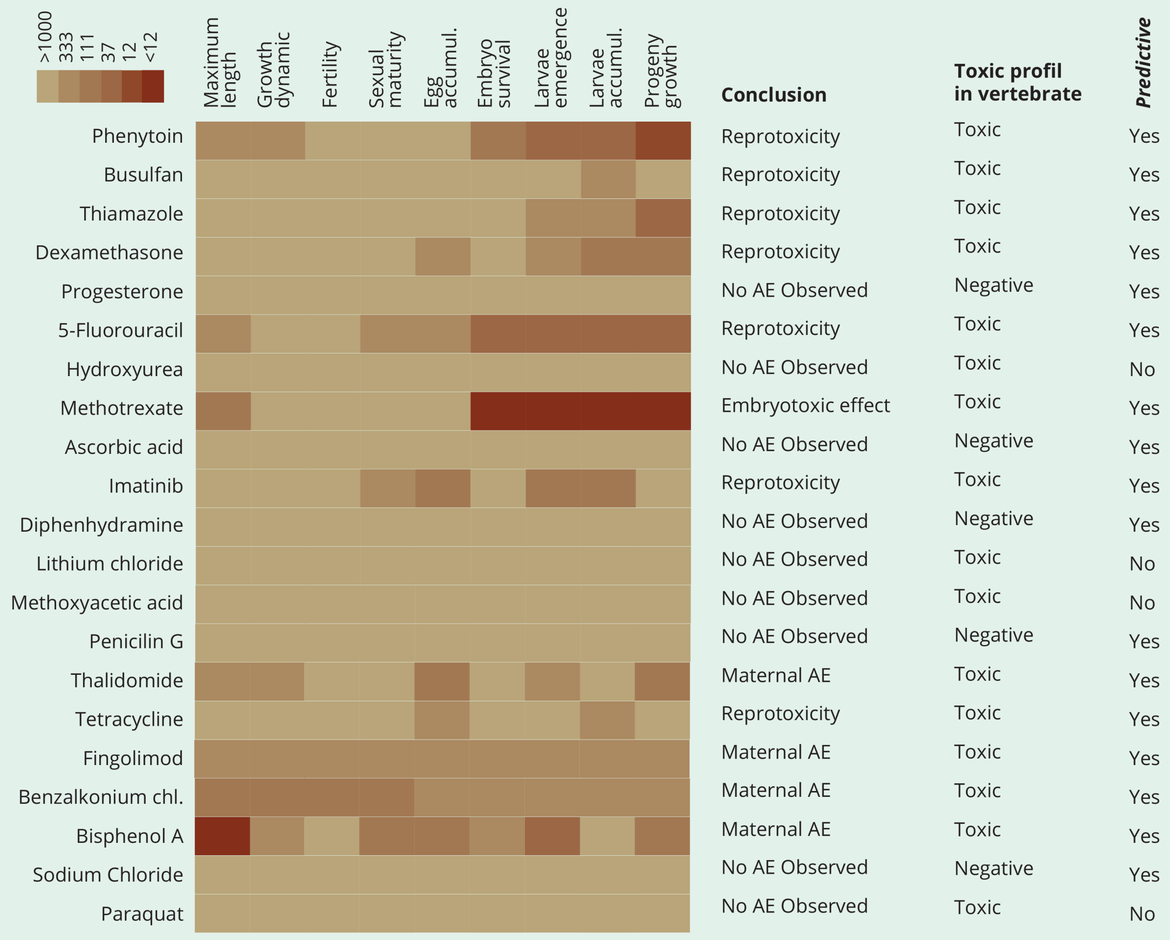
Worms are then chronically exposed to the test compounds starting **from the last larval stage prior to sexual maturity (L4)** for 80 hours (day 3 of adulthood). The protocol was specifically designed to avoid a treatment with the compounds during the developmental phase: the goal is **to evaluate the potential adverse effects on *C. elegans* reproduction only**. The images of each microfluidic chamber are recorded every hour. **Time-resolved phenotypic readouts** are then extracted from the collected images.

Maternal effects	**Adverse effects if $p<0.05$ and if:**	**Conclusion:**
Maxium size (K value)	< 1.0 - CTLneg sd (threshold ≈0.90)	Smaller adult worms
Growth dynamic (r value)	<1.0 - 2x CTLneg sd (threshold≈0.85) or >1.0 + 2x CTLneg sd (threshold≈1.15)	Developmental arrest / Slower development
Reproductive toxicity		
Fertility (% eggs detected)	<100%	Lower fertility
Sexual maturity (t eggs)	> 1.0 + CTLneg sd (threshold≈1.05)	Delay in sexual maturity
Egg accumulation (m eggs)	<1.0 - 0.5x CTLneg sd (threshold≈0.71)	Lower egg laying
Embryo survival (% eggs hatched)	<100%	Higher embryotoxicity
Larvae emergence (t progeny)	> 1.0 + CTLneg sd (threshold≈1.05)	Longer egg maturation
Larvae accumulation (m progeny)	<1.0 - 0.5x CTLneg sd (threshold≈0.70)	Lower egg viability
Progeny growth (min size/dev)	<1.0 - CTLneg sd (threshold≈0.90)	Smaller progeny

RESULTS



Worms were treated on the platform with the herbicide **Paraquat** (**A-B**, top row) and the cytotoxic chemotherapy medication **5-fluorouracil** (**A-B**, bottom row). This test set was employed to validate our Reprotox protocol with molecules known to induce different toxic effects in *C. elegans*. Curves depicted on graphics (**A**) show the respective dose-effect on development (max. length) and reproductive capacity (fertility, embryo survival and larvae accumulation). No-Observed-Adverse-Effect Level (NOAEL) were computed for both Paraquat and 5-FU and represented for all the end points (**B**).
Conclusion: Paraquat induced **major maternal AE** at high doses (NOAEL: 2.5mM) and significant reprotoxic effect at lower doses (NOAEL: 0.16mM). As reported, 5-FU at the doses tested has no maternal AE but a **strong reprotoxic impact** at mid doses (NOAEL: 50µM).



21 benchmark chemicals known to induce or not reprotoxic effects were tested in blind on the microfluidic platform. 5 concentrations for each chemical (1mM, 333µM, 111µM, 37µM and 12µM) were tested and compared to the neg. control (DMSO 1%). Three technical repeats were executed, each chemicals were tested twice. NOAEL was determined and their toxicity profiles described.
Conclusion: After unblinding, 17 out of 21 were predicted correctly (according to the ECHA database), providing **a balanced accuracy of 87.5% (Sensitivity: 75% / Specifity: 100%)**.

CONCLUSION AND OUTLOOK

We presented a novel microfluidic platform designed for fully automated analyses of *C. elegans* nematodes. This study highlighted the strong advantages of our innovative approach, including (1) a good reproducibility and accurate results thanks to standardized protocols, (2) an automated and dynamic dosing of chemicals with low liquid consumption and (3) multi-phenotypic readouts in real time. Our approach allowed identifying 12 toxic/reprotoxic chemicals among the 16 tested (Sensitivity: 75%). Furthermore, all the negative chemicals were correctly identified (5 negatives among the 5 tested), providing an overall balanced accuracy of 87.5%. In conclusion, we believe this platform represents the first "all-in-one" *C. elegans* microfluidic laboratory allowing rapid identification of toxic compounds in the early stages of the drug/chemical discovery pipeline.

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