Worm-on-Chip: fully automated whole-organism platform for screening and identification of toxicity using the nematode *Caenorhabditis elegans*

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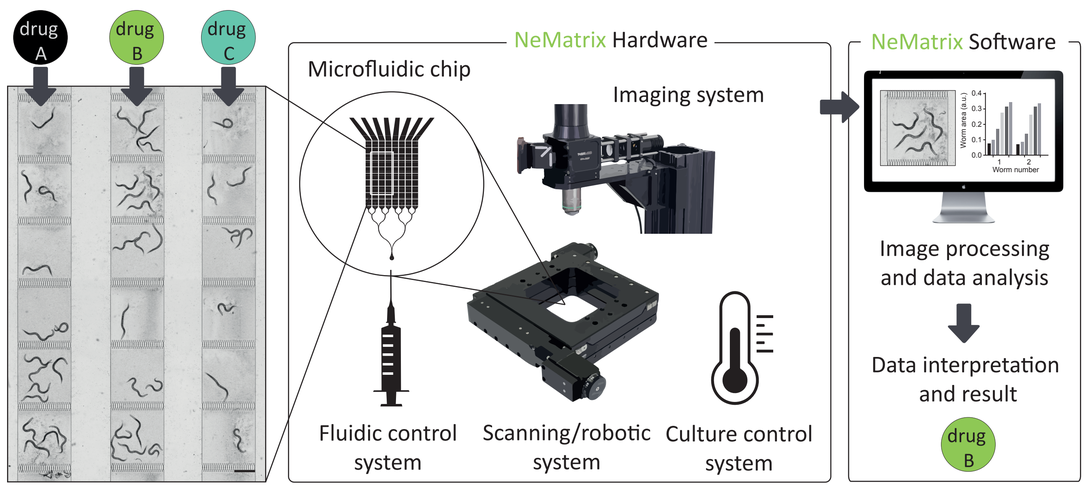
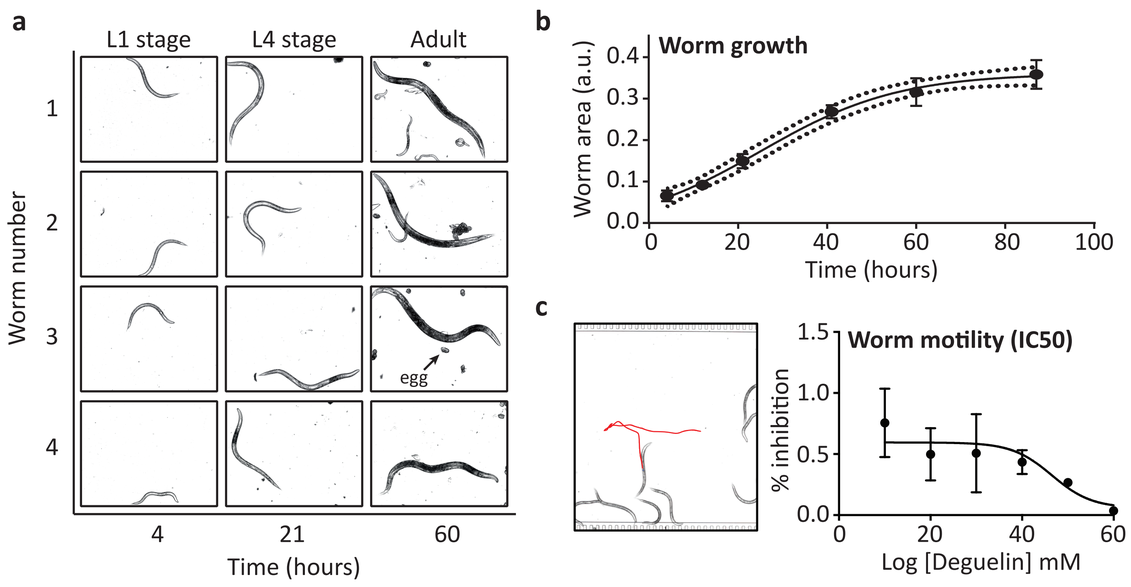
We describe an innovative platform for fully automated handling and observation of *C. elegans*, combined with dedicated software for data collection and analysis. Our microfluidic device allows, for the first time, automated high-content phenotyping of worms at medium/high-throughput, *via* accurate control and real-time monitoring of multiple physiological parameters in the worms. This screening format significantly minimizes the amount of compound needed for each test and could be readily used to identify toxicity mechanisms of substances, through specific phenotypic responses in the worms, within only 3 days. As a pilot study, we validated our platform by screening compounds with well-defined toxicity profiles. By monitoring larval growth, worm fertility and motility over 3 day-experiments, we sucessfully identified distinct patterns related to specific toxicity profiles of the tested compounds. In conclusion, we propose an innovative solution for rapid identification of toxic compounds and their potential mechanism of toxicity, using a biological model that perfectly bridges the gap between *in vitro* and *in vivo* assays.

Microfluidic platform overview

Our microfluidic technology allows generating **large-scale matrices** of *C. elegans* in a microfluidic chip format, suitable for parallel testing of several conditions (e.g. compounds and/or concentrations). The "NeMatrix" platform is conceived to provide **fully automated culture and analysis** of the worms. Dedicated software algorithms process the data and generate unbiased high-content information for each drug screening test, facilitating data interpretation and enabling hypothesis generation.

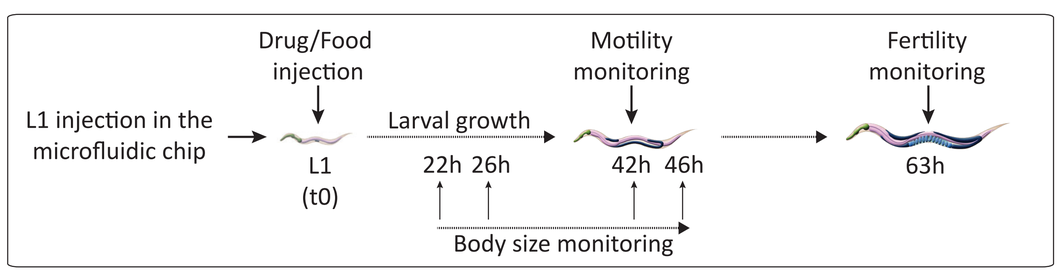


Our microfluidic design is tailored for the **isolation of larvae** at a synchronized developmental stage in separate fluidic chambers and for **automated long-term culture and imaging**. *Via* image-processing on time-lapse brightfield pictures, we monitor the **growth rate** of the worm population within the microfluidic chip over several days (**a-b**), as well as their **fertility** (egg production) and **motility** *via* the MovementTracker software (**c**).



Design of the toxicity testing pipeline

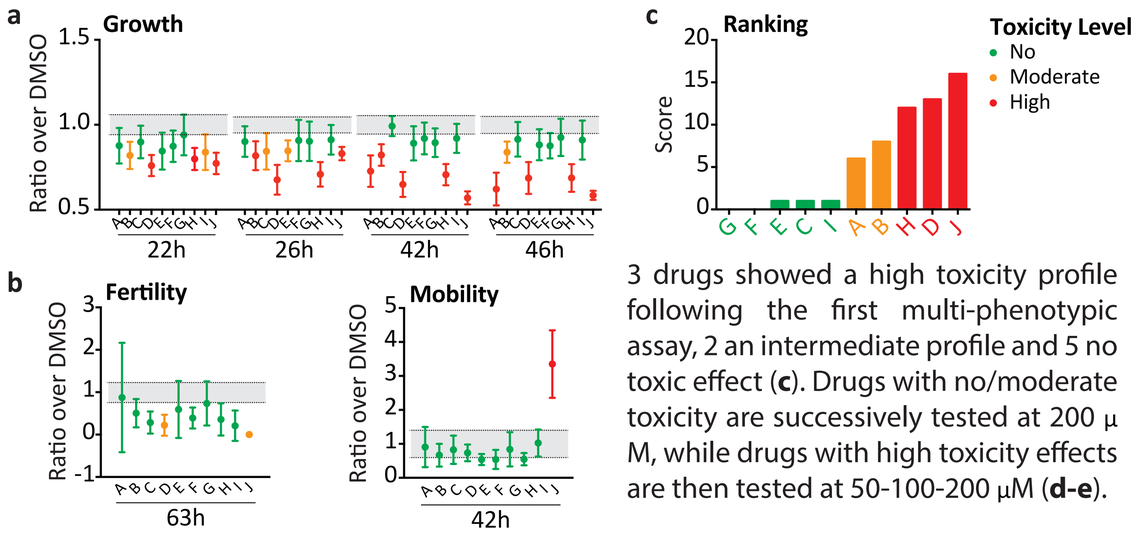
Ten drugs were tested in blind for their toxicity profiles throughout a series of 3 successive assays. N2 wild-type worms were **injected at the L1 larval stage** in the microfluidic device. The drugs were then mixed with maintenance medium containing *E. coli* bacteria as food and the worms were exposed at the final concentration of 100 µM. Drugs were **perfused continuously** during the assays and pictures/videos are recorded at different time frames.



Drugs with **high toxicity** profiles were tested for **dose/response** (50-100-200 µM)
Drugs featuring **no toxicity** were tested at **higher** concentration (200 µM)

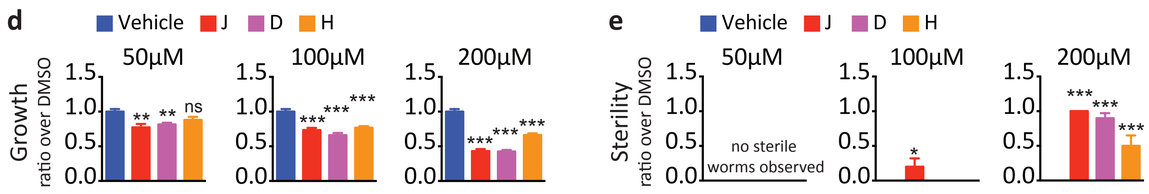
Prototypical profiles of toxicity

All drugs (A to J) were first tested at a single concentration (100 µM) (**a-b**). Toxicity levels (No/Moderate/High) are estimated from the *p* values calculated with respect to the DMSO control. No: $p>0.05$; Moderate: $0.05<p<0.01$; High: $p<0.01$. For each paramaters, a 0 value is assigned to the "no" toxicity condition, 1 to "moderate" toxicity, 3 to "high" toxicity, and summed for an overall ranking (**c**).



3 drugs showed a high toxicity profile following the first multi-phenotypic assay, 2 an intermediate profile and 5 no toxic effect (**c**). Drugs with no/moderate toxicity are successively tested at 200 µM, while drugs with high toxicity effects are then tested at 50-100-200 µM (**d-e**).

The second round of tests allows further distinguishing drugs with respect to to their toxiciy effect on worms: 3 drugs are ranked as highly toxic, 3 as moderately toxic and 4 do not show toxicity effects on worms.



Final toxicity ranking

Our approach allowed identifying 6 toxic compounds among the 10 drugs tested. The unblinding showed a good success rate: only **1 false positive** (Metformin) and **2 false negatives** (Phenformin and Paracetamol).

Conclusion & Outlook

We developed a miniaturized device and a complete platform designed for fully automated analyses of *C. elegans* nematodes. This microfluidic-based technology offers unprecedented control over the worm culture conditions, ensuring robustness and repeatability. At the same time it allows real-time monitoring of a range of worm phenotypes, including body size, fertility and motion that are relevant for early toxicity identification. We believe that this platform will represent a first-of-its-kind example of technological tools allowing rapid identification of toxic compounds. The next step is to map worm phenotypic patterns to specific toxicity profiles to allow the detection of toxicities early on during development using an *in vivo* model applicable for medium-throughput screening.

Eurotox 2017 - 53rd Congress of the European Societies of Toxicology

10 -13 September 2017

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