Application Note

Automation of early toxicity profiling using *C. elegans* nematodes on the SydLab System

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Automation of early toxicity profiling using
C. elegans nematodes on the SydLab System

A scientifically validated solution in toxicity assessment

Currently existing biological testing strategies still involve an extensive animal experimentation in vertebrate models, which is expensive and is associated with a number of important ethical concerns and regulatory constraints. The alternative methods to animal testing are typically based on cellular models. The main limitation of these in vitro techniques is that they cannot predict complex responses at the level of an organism, usually involving a multi-organ crosstalk. *Caenorhabditis elegans* (*C. elegans*) has been known for more than 60 years as a powerful model organism in fundamental research, allowing exploration of different facets of aging, development, neurosciences and genomics.

This nematode gained popularity amongst the scientific community due to its small size, short life cycle, ease of cultivation and propagation and a powerful genetic toolkit. Despite their relative simplicity, nematodes present a wide range of behaviors and possess well-defined tissues.
C. elegans offers an excellent ethical alternative to vertebrate animal testing, providing fast and high-throughput results through a whole-organism, validated by 60+ years of scientific research. Toxicity data obtained in worms proved them- selves to be predictive of outcomes in mammals and the LC50 ranking in *C. elegans* matches the LD50 ranking in mouse and rat.

Nagi Bioscience introduces the first Organism-on-Chip technology

Nagi Bioscience's "Organism-on-Chip" technology introduces a paradigm-shift in *C. elegans*-based drug/chemical testing. We completely replaced the traditional manual protocols of *C. elegans* research by standardized operations within the Nagi Chips – disposable microfluidic cartridges – integrated in our fully automated laboratory equipment, the SydLab System.

The SydLab System: An all-in-one platform for in vivo high-content screening

Our microfluidic technology allows large-scale studies for the parallel characterization of multiple drugs and chemicals in different *C. elegans* populations. The SydLab System 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 SydLab Device and confined within dedicated chambers of the Nagi Chips. They are then continuously fed with *E. coli* solution and can be exposed to the test compounds according to the treat- ment plan defined by the user. The pictures of each microfluidic chamber are collected via time-lapse microscopy at desired frequency.

Sydlab System Features

Nagi's Chips
Microfluidic cartridge

SydLab System

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

SydLab System
Benchtop device

➕

Nagi Chip

- ✓ 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.

SydLab Analyzer

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

The Sydlab Analyzer software: Our unique data processing pipeline

Computer vision algorithms, based on state-of-the-art artificial intelligence, are employed to extract multi-phenotypic information from the images and videos generated by the SydLab Device, during the experiment. Data comparison, clustering and statistical analysis tools are then provided to support the user in the data interpretation process, e.g. for the rapid identification and ranking of toxic compounds in the experiment.

✓ ML-based object detection

worm injected
t:10h
t:30h
egg detected
t:60h
larvae detected
t:90h

✓ Objects detected
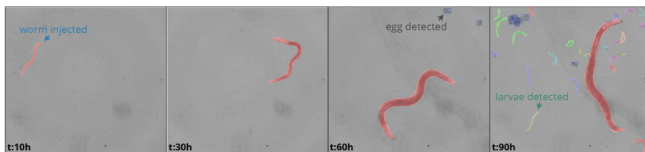
- ✓ Worm (Size)
- ✓ Eggs (number)
- ✓ Progeny (number)

The time-lapse brightfield pictures obtained during the experimental procedure are post-processed by an extensively trained machine learning (ML) software. 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 apparition and number) and progeny production, with a high degree of reproducibility across replicates.

✓ Automated data analysis

✓ Reproducibility
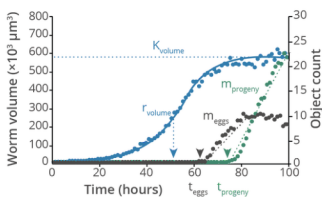
Parameter	Value	Variation	Experiments
Maximum size (K)	570'388 (μm^3)	±6.5%	15 repeats
Growth dynamic (r)	2.24 (days)	±5.6%	15 repeats
Sexual maturity (t_{eggs})	56.44 (hours)	±5.4%	15 repeats

The efficient object recognition allows the ML algorithms to extract the following parameters:

K_{volume} : the maximal volume reached by the worm (a similar maximal value is obtained for the length of the worm),

r_{volume} : the time required to reach 1/2 of the maximal volume (a similar dynamic is obtained for the length of the worm),
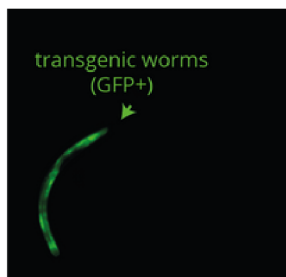
t_{eggs} : the time point when the first egg is detected in each microfluidic chamber,

$t_{progeny}$: the time point when the first larvae is detected in each microfluidic chamber,
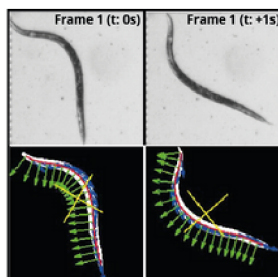
m_{eggs} : the dynamic of egg accumulation,

$m_{progeny}$: the dynamic of progeny accumulation.

✅ Fluorescence monitoring

Our platform implements both BF and fluorescent microscopy. With the possibility to track GFP signal in a time resolved manner, we can take advantage of the large collection of existing *C. elegans* reporter strains.
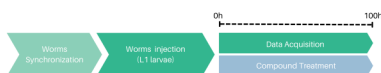
✅ Video acquisition

An option to record videos instead of pictures is also availa- ble. By processing the acquired videos we are capable to extract multiple motility parameters.

Developmental toxicity assay

The assay is designed to explore the potential adverse effects of molecules on the development of *C. elegans*. It can be used as a prediction for teratogenic potency of the test compounds.

Method Description

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

Worms are chronically exposed to the test compounds right after their injection (L1 stage) for 100 hours (corresponding to the full larval development + 2 day of adulthood). The compounds to be tested are mixed with the *E. coli* solution.

Readouts

- ✅ Worm lethality
- ✅ Worm size
- ✅ Growth dynamics
- ✅ Sexual maturity
- ✅ Worm shape

Freeze-dried OP50 *E. coli* are used as a food source for the whole duration of the experiment, preventing the metabolization of the tested molecules by the bacteria.

The images of each microfluidic chamber are recorded every hour. Time-resolved phenotypic readouts are then extracted from the collected images.

Figure 1. Worms growth detection using proprietary ML-based algorithm. Brightfield images of wild-type *C. elegans* (70 hours post-hatching) treated with antibiotic doxycycline starting from the L1 larval stage (right panel) or with vehicle (left panel). Overlay masks for the detection of worms and eggs are automatically generated by SydLab's AI-based computer vision algorithm.
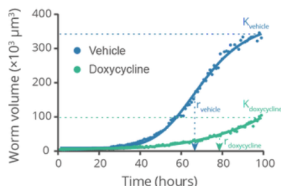
Results Analyzed

Figure 2. Growth dynamics upon Doxycycline treatment

Temporal evolution of the average worm size for wild-type worms treated with the antibiotic dox. starting from the L1 larval stage vs untreated worms (vehicle). The treatment significantly impacts on worm development, size and growth rate.
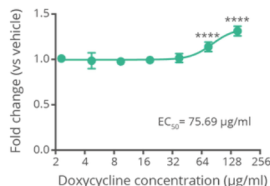

Figure 3. Dose-dependent effect of Doxycycline on the growth rate

Average time to reach half of the maximal size (r) for worms treated with the antibiotic dox. at different doses (data normalized to the value measured for the vehicle). Dox. treatments delay worm development in a dose-dependent manner.

Conclusion

Dox. treatment significantly impacts on worm development, resulting in growth retardation and reduced size.

Reproductive toxicity assay

The assay is designed to explore the potential adverse effects of molecules on the reproductive capacity of *C. elegans*. It can be used as a prediction for fertilization problems/embryotoxic effects.

Method Description

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

Worms are 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 is 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.
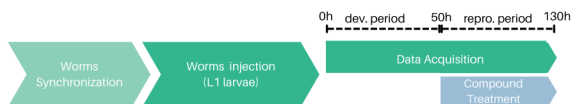
Readouts

- ✓ Worm lethality
- ✓ Worm size
- ✓ Growth dynamics
- ✓ Sexual maturity
- ✓ Worm shape

Maternal effect readouts

- ✓ Fertility
- ✓ Embryonic viability
- ✓ Progeny accumulation

Reprotox readouts
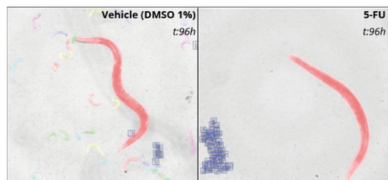
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Figure 1. Eggs and larvae detection using proprietary ML-based algorithm. Brightfield images of wild-type *C. elegans* (96 hours post-hatching) treated with the anticancer drug 5-FU from the L41 larval stage (right panel) or with vehicle (left panel). Overlay masks for the detection of worms and eggs are automatically generated by SydLab's AI-based computer vision algorithm.
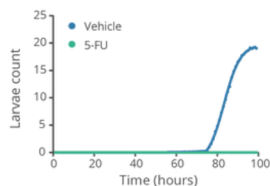
Results Analyzed

Figure 2. Progeny production dynamics upon 5-FU treatment

Temporal evolution of the average number of larvae produced by wild-type *C. elegans* treated with the anticancer drug 5-FU starting from the L4 larval stage vs untreated worms. The drug treatment signifi- cantly impacts the repro- ductive process.
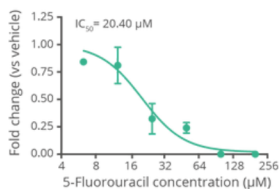

IC_{50} = 20.40 µM

Figure 3. Dose-dependent effect of 5-FU on the reproduction rate

Progeny production rate over the first 24 hours of reproduction for wild-type worms treated with 5-FU at different doses (data normalized to the value measured for the vehicle). 5-FU treatments induce an embryotoxic effect in a dose-dependent manner.

Conclusion

5-FU treatment specifically affects the worm reproduction, inducing strong embryotoxicity.

Motility Assay

The assay is designed to evaluate the effects of test compounds on *C. elegans* behavior and motility and provide indications about neurotoxicity, muscular toxicity or premature aging process.

Method Description

For this assay, short video sequences are recorded every 1 to 6 hours for each microfluidic chamber. Time-resolved readouts are then extracted from the acquired videos for a diversified characterization of the worms' motility and behavior.

Readouts

- ⊘ Bending frequency
- ⊘ Velocity
- ⊘ Amplitude of the head
- ⊘ Amplitude of the mid body
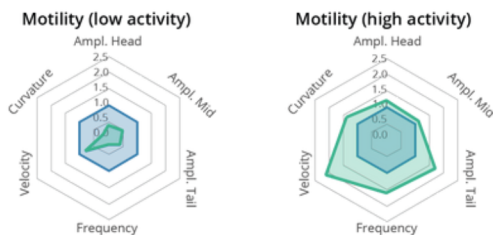- ⊘ Amplitude of the tail
- ⊘ Curvature

Figure 1. Characterization of the worm's motility and behavior. Representative multi-phenotypic fingerprint plot comparing a control worm and a more (left) or less (right) active worm at day 1 of adulthood, which includes readouts of body bends frequency, velocity, curvature and amplitude of the movement at the head, tail and middle of the body. This high-content motion analysis reveals very subtle changes in *C. elegans* movements, beyond average motility measurements.

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Mode-of-Action

The assay is designed to establish which commonly known stress defense pathways are activated by the test compounds in *C. elegans*.

Method Description

For this assay we take advantage of the existing *C. elegans* reporter strains for a variety of key molecular stress pathways, as well as the capacity of our device to perform fluorescent imaging.

Readouts

- ✓ Oxidative stress
- ✓ ER stress
- ✓ Mito stress
- ✓ Cytosolic stress
- ✓ Hypoxic stress

*non-exhaustive list

Mitochondrial stress reporter (*hsp-6::gfp*)
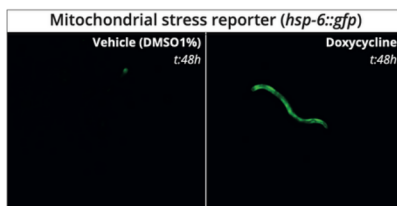
Vehicle (DMSO1%) t:48h | Doxycycline t:48h

Figure 1. Fluorescence detection using proprietary ML-based algorithm. Activation of the mitochondrial stress respsonse in *hsp-6::gfp C. elegans* treated with the antibiotic doxycycline starting from the L1 larval stage (right) vs untreated worms (left), as observed via fluorescence imaging by the SydLab platform.
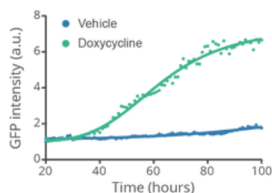
Results Analyzed

Figure 2. Dynamics and level of activation of the *hsp-6::gfp* reporter upon doxycycline treatment

Temporal evolution of the average fluo intensity measured for *hsp-6::gfp* worms treated with the antibiotic dox. vs untreated worms (vehicle). Increased expression of the *hsp-6::gfp* reporter reveals the activation of the mitochondrial stress induced by the drug treatment.
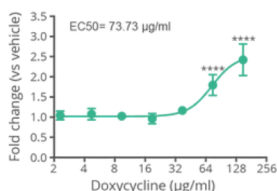

Figure 3. Dose-dependent effect of doxycycline on the mitochondri- al stress patway activation

Average peak fluo intensity for *hsp-6::gfp* worms treated with the antibiotic doxycycline at different doses.
Dox. treatment activates the mitochondri- al stress response in a dose-dependent manner.

Conclusion

Nagi Bioscience proudly introduces the first Organism-on-Chip technology, allowing "*in vivo* testing at the *in vitro* scale", and bridging the gap between cell-based and vertebrate animal models. Our innovative microfluidic platform is designed for fully automated and standardized analyses of *C.elegans* nematodes, a alternative model organism validated by six decades of scientific research. The technology developped by Nagi Bioscience 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 and motility that are relevant for early identification of different toxicity endpoints. This platform represents the first all-in-one *C. elegans* microfluidic laboratory device, allowing rapid identification of toxic compounds in the early stages of the drug/chemical discovery pipeline.

Reference posters available upon request:

Mouchiroud et al., EUROTOX 2017 - 53rd Congress of the European Societies of Toxicology
Mouchiroud et al., Swiss 3Rs Day - 60 years of Replacement, Reduction and Refinement of Animal Experimentations
Mouchiroud et al., SOT 2020 - 59th Annual Meeting
Mouchiroud et al., EUROTOX 2021 - 56th Congress of the European Societies of Toxicology

Interested in using SydLab platform for your research?
Get in touch with us

Our scientific team provides innovative safety and efficacy testing services based on the use of Nagi's Organism-on-Chip technology and tailored to your research needs.

Contact our experts to start designing your project with us today.

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