A MICROFLUIDIC PLATFORM FOR AUTOMATED PHENOTYPING DURING FULL LIFESPAN OF *CAENORHABDITIS ELEGANS* AT HIGH-THROUGHPUT

H. B. Atakan[1*], M. Cornaglia[1], L. Mouchiroud[2], J. Auwerx[2] and M.A.M. Gijs[1]

[1]*Laboratory of Microsystems, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*

[2]*Laboratory of Integrative System Physiology, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*

ABSTRACT

We present a multiplexed and automated microfluidic platform for phenotyping of *C. elegans* during their full lifespan and at high-throughput. We used the fluorescent *hsp-6::gfp* strain of worms and performed experiments to observe the effect of four different *E. coli* concentrations supplied for growth. We cultured worms in microfluidic chambers during their lifespan and quantified their motility. Nematodes were also confined at various time points inside tapered constriction channels for imaging to observe the mitochondrial stress, as expressed by fluorescence, and to measure their growth parameters as function of the amount of applied food.

KEYWORDS: *C. elegans*, microfluidics, phenotyping, high-throughput screening

INTRODUCTION

The nematode *C. elegans* is an ideal model organism in biomedical research to study drug screening, aging and longevity due to its short lifespan, hermaphrodite behavior and genetic similarity with humans. However, their small size and high motility pose challenges to the worms' manipulation and imaging. Moreover, in order to obtain significant biological data, worms must be studied at high-throughput. Previous aging, longevity and drug screening platforms had high-throughput but lacked immobilization [1], had good confinement but low-throughput [2], or good confinement at high-throughput but for a single larval stage [3]. To our knowledge, no one has demonstrated nematodes' culture during their full lifespan at high-throughput and involving a method of immobilization for high-resolution imaging.

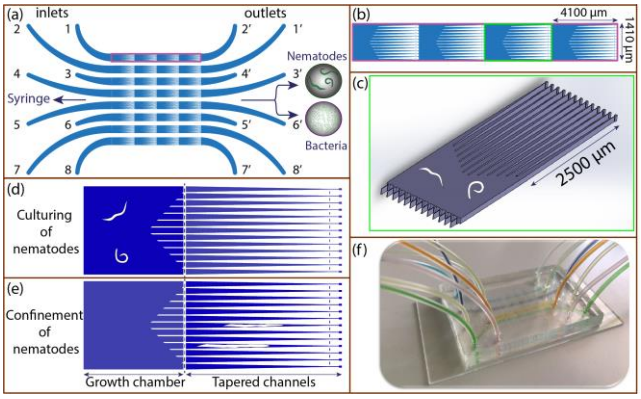

Figure 1: (a) Layout of the PDMS microfluidic chip, consisting of 8 parallel channels, each of which (b) is composed of 4 growth/imaging chambers, the structure of which is detailed in (c). In culture mode (d), worms are fed with a liquid bacteria solution and can freely move in the growth chamber, while videos are automatically recorded every 7 hours for determining worm motility. For imaging (e), a flow from the left is applied every 35 minutes to reversibly immobilize a worm in one of the 13 tapered constrictions with 90 µm-wide entrance. After that, worms are released back to the growth chambers and kept to be fed until the next confinement. (f) Picture of the chip with the microchannels filled with dye solutions.

EXPERIMENTAL

In our design, *C. elegans* are initially distributed among 32 growth chambers, targeting one to three worms per chamber, followed by bacteria feeding from the same inlet (Figure 1). Hereafter our automated Python script is initiated and the experiments are carried out over 5-7 days. Our script automatically controls the flow in the microfluidic channels and triggers image capturing at designated time points. Growth chambers are utilized for the culture of *C. elegans* and extraction of their swimming properties in a multiplexed manner during their full lifespan. Upon a trigger signal from our script to increase the fluidic flow, worms are temporarily confined in the tapered constriction channels, where both brightfield and fluorescence images are captured to quantify development parameters and mitochondria stress, respectively. We also provide an automatic video and image post-processing algorithm for the motility and development studies, respectively.

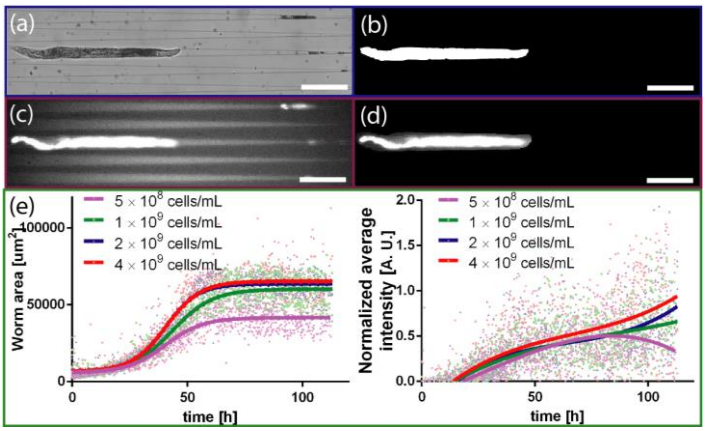

Figure 2: Image processing to quantify worm development parameters and green fluorescent protein expression. (a) During worm immobilization in the tapered constrictions, a raw brightfield image is captured. (b) The image is thresholded and the algorithm is forced to find the largest connected components matching to the worms accommodated in that chamber. (c) In parallel, a raw fluorescence image is also taken. A dilated version of the thresholded image is used as a binary mask that is applied to the fluorescence raw image. (d) The multiplied image is used to quantify the fluorescent intensity inside the worms. (e) Plot of the worm area and normalized fluorescent intensity for different feeding conditions. Data are presented as mean ± S.E.M. based on measurements on N = 18 to 24 worms. Scale bars = 300 µm.

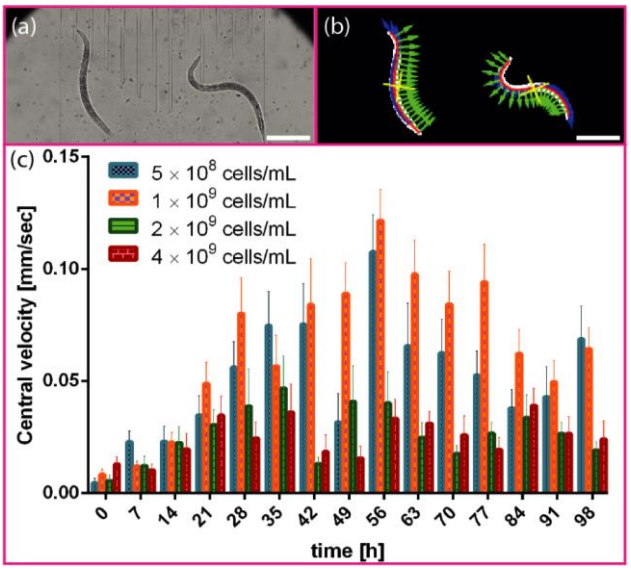

Figure 3: Video processing approach to obtain worms' motility parameters. (a) Screenshot of a video that was captured for 10 seconds at a rate of 5 frames per second. (b) After background removal, an automatic algorithm detects the worms in a growth chamber, after which the worms' positions are fitted with splines enabling to quantify up to 11 swimming parameters per worm. (d) Worm velocity versus time for 4 different bacteria feeding concentrations. Data are presented as mean ± S.E.M. The histogram data are averages based on motility measurements on N = 18 to 24 worms. Scale bars = 300 µm.

RESULTS AND DISCUSSION

In imaging mode, brightfield images were obtained for the worms in the constriction channels, which besides delivering growth parameters like worm area, also were used as masks for the fluorescence image analysis, indicating that more mitochondrial stress develops for more caloric feeding (Figure 2). We recorded regularly short videos of growth chambers, fit splines to the nematodes after an adaptive background removal to extract up to 11 swimming properties and to deduce the effect of *E. coli* concentration on the nematode motility (Figure 3), which is characterized by a decreased motility for more caloric bacterial feeding protocols.

CONCLUSION

Our platform has the ability to address a wide range of questions because of it is high-throughput, automation, full lifespan culturing and the possibility to quantify at the same time both motility and development parameters.

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CONTACT

* H.B. Atakan; phone: +41 21 69 36587; baris.atakan@epfl.ch