

# AN AUTOMATED MICROFLUIDIC PLATFORM FOR LONG-TERM HIGH-RESOLUTION IMAGING OF *C. ELEGANS*

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## ABSTRACT

We describe a microfluidic platform for the automated culture, treatment and long-term high-resolution imaging of *Caenorhabditis elegans* nematodes under normal physiological conditions. Our device features: (i) a microfluidic design tailored for the isolation of L4 larvae from a mixed larval population and for successive culture and treatment; (ii) a worm immobilization method, based on the thermo-reversible gelation of a biocompatible polymer inside the microfluidic chip, thereby enabling high-resolution imaging; (iii) an integrated temperature control system, both to ensure viable environmental conditions for *C. elegans* culture and to steer the worm immobilization/release process.

**KEYWORDS:** *C.elegans* immobilization, long-term imaging, Pluronic F-127, thermoreversible gelation

## INTRODUCTION

The nematode *C. elegans* is one of the most employed small model organisms in biology [1, 2]. Because of its small size (a few hundreds of microns), high-magnification imaging is usually needed to extract relevant biological information, while studies on transgenic animals often require the accurate observation of highly localized fluorescent signals inside the worms. Hence, during imaging, animals have usually to be fully immobilized to prevent any movement, which otherwise would result in image distortion. Moreover, to observe the dynamics of biological processes, the same worm has to be immobilized repeatedly in a reversible manner. This should be done under normal physiological conditions in order to ensure minimal impact during the imaging phase. For high-throughput analyses, automation of this process is highly preferable. Several worm immobilization methods have been recently proposed [3], nevertheless, only few of them fulfill all the aforementioned requirements. Here, we propose a novel automated microfluidic platform to solve this issue.

## THEORY AND EXPERIMENTAL

Our system employs the thermoreversible gelation of the triblock copolymer Pluronic F127 for reversible worm immobilization. Unlike previously reported work employing this technique [4], our microfluidic device comprises a simple monolithic polydimethylsiloxane (PDMS) chip, which does not need to be equipped with integrated valves, bonded on a 150  $\mu\text{m}$ -thick glass coverslip and inserted in a custom-made assembly for active control of the temperature inside the device (Fig. 1a,b). The temperature of the microfluidic environment is accurately controlled via a proportional-integral-derivative (PID) controller-regulated thermo-electric module combined with a resistive temperature sensor. This solution offers clear advantages in terms of platform versatility, since it is directly adaptable to any PDMS chip. More complicated microfluidic designs for controlling the chip temperature, e.g. via liquid cooling, may be avoided. Lateral positioning of the microfluidic tubing to the chip enables pre-thermalization of solutions and device operation with both upright and inverted microscopes. The microfluidic flow is controlled via external automated syringe pumps. We characterize the rheology of different concentrations of Pluronic F127 in M9 buffer by measuring the dynamic viscosity of the solutions as a function of both the temperature and polymer concentration (Fig. 1c). Solutions at 23 to 25% (w/v) concentration are found to be best suited for the immobilization of worms.

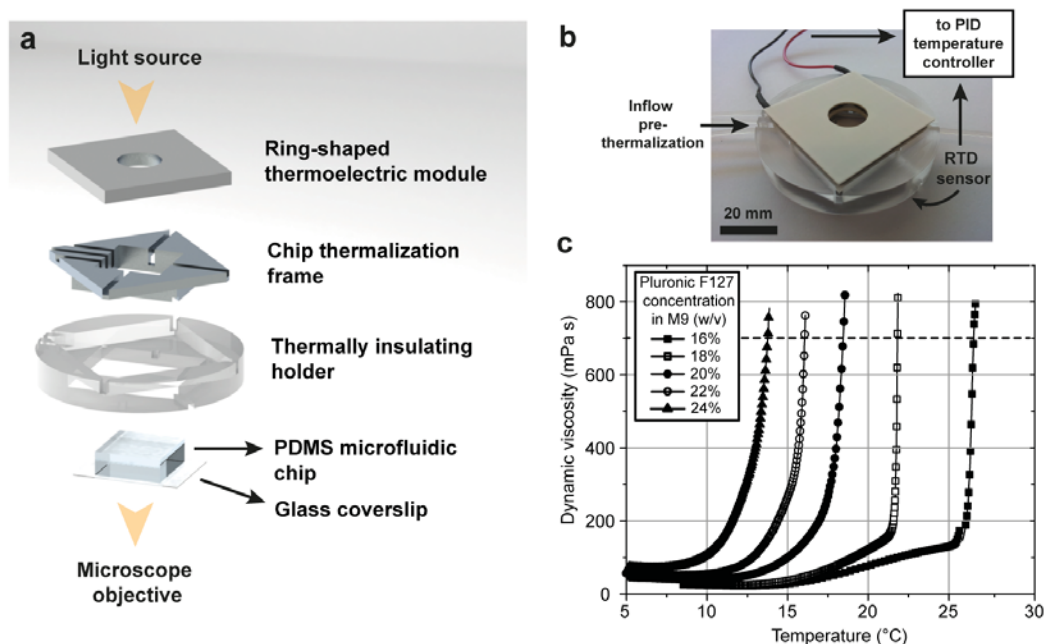


Figure 1. (a) Schematic representation of the constitutive components of our *C.elegans* high-resolution imaging device. A PDMS microfluidic chip is bonded to a 150  $\mu\text{m}$ -thick glass coverslip. The chip is inserted in a metallic “thermalization frame”, which is thermally connected to a thermo-electric module and insulated from the external environment by a custom-made microscope holder. The ring shape of the thermoelectric module ensures efficient light exposure, needed for high-resolution transmission microscopy. (b) Photograph of the device. The whole assembly has the size of a 60 mm diameter well-plate, hence it directly fits standard microscope stages. (c) Dynamic viscosity of Pluronic F-127 in M9 buffer as a function of temperature and for different concentrations (measured with a SV-1A tuning fork vibro-viscometer). All curves show an abrupt increase of the solution viscosity in a narrow temperature range. This onset shifts to lower temperatures for higher Pluronic concentrations.

## RESULTS AND DISCUSSION

Our microfluidic platform allows performing the following automated operations: (i) worm injection into the microfluidic chip at a relatively low flow rate (typically 100 nL/s) and synchronization of the population at the L4 larval stage; (ii) worm culture at a desired temperature (typically 20°C) and feeding by perfusing the chamber with an *E.coli* suspension; (iii) successive worm immobilization, imaging and release operations. Prior to imaging, the chip temperature is set to 15°C and a liquid solution of 23% Pluronic F127 in M9 buffer is injected into the chamber. For high-resolution imaging, the chip temperature is raised to 25°C, to trigger the gelation of the Pluronic solution for worm immobilization; (iv) washing of the chip: after imaging, the chamber temperature is brought back to 15°C, the worms are released and fed again or, if needed, washed out together with the Pluronic solution at a relatively high flow rate (typically 5000 nL/s). Hereafter the process can restart with a new worm population.

By tracking *C.elegans* movements, we empirically define a viscosity of 700 mPa·s as onset temperature which triggers worm immobilization, while dynamic viscosity values above 1000 mPa·s (measurement limit of our instrument) are needed for full immobilization. Both brightfield and fluorescent images of the immobilized worms can be taken at high-magnification. We demonstrate that with our method the expression of specific Green Fluorescent Protein (GFP) reporters in different mutant worms may be analyzed on-chip at a high level of accuracy, both in space and time (Fig. 2).

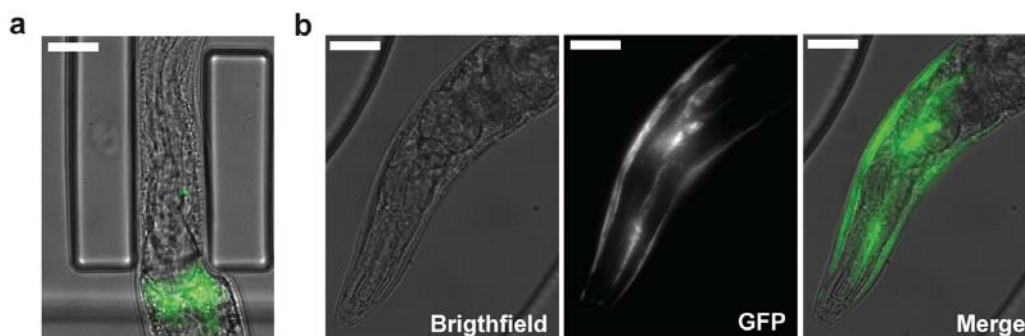


Figure 2. (a) Fluorescent image of a *pges-1::mito::GFP* transgenic worm, showing the intestinal mitochondria, merged to the corresponding brightfield picture for accurate localization of the fluorescent signal (scale bar = 20  $\mu\text{m}$ ) (b) Analysis of GFP expression in the mitochondria of the worm muscles using *pmyo-3::mito-GFP* transgenic worms. Brightfield, GFP and the merged images are shown. The “GFP” picture is obtained by superimposing a z-stack of 10 fluorescent images taken at different focal distances in order to visualize the reporter expression across the whole worm thickness (scale bars = 20  $\mu\text{m}$ )

## CONCLUSION

We developed a compact temperature-controlled microfluidic device for the reliable and reversible immobilization of *C.elegans* nematodes. Its versatility and ease of use makes the proposed microfluidic platform a powerful tool for the design of advanced biological experiments that take advantage of repetitive high-resolution imaging and long-term observation of single *C.elegans* nematodes without affecting the normal physiological conditions of the worm. This goes beyond the possibilities of conventional agar-plate based methods. In particular, we expect interesting applications in the field of drug screening assays or for developmental studies.

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