ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Funding of this work was provided by the EU Ideas program (ERC-2008-AdG-23118 and ERC-2012-AdG-320404) and the EU People program (Marie Curie Initial Training Network 'Biomax', Grant No. 264737)

An Automated Microfluidic Platform for Reversible Immobilization and Imaging of *C.Elegans*

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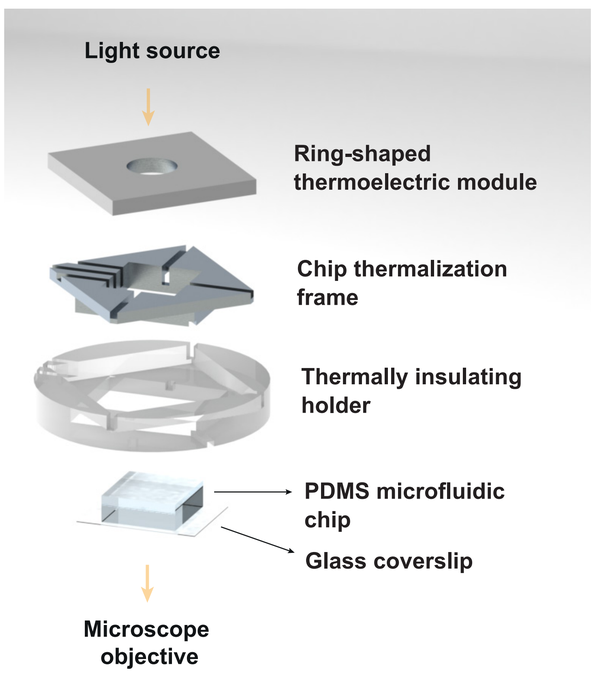
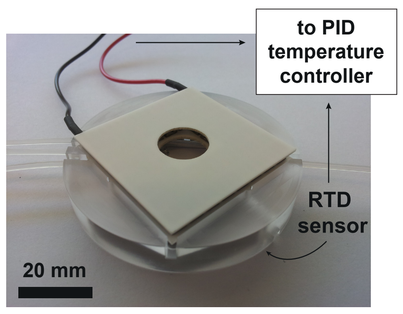
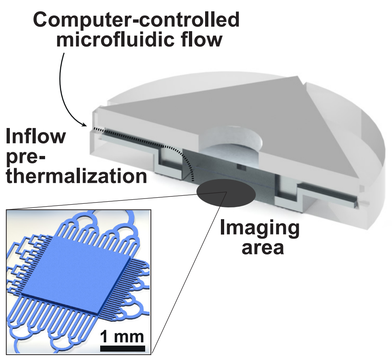
We describe a microfluidic platform for the automated culture, treatment and long-term high-resolution imaging of Caenorhabditis elegans nematodes under normal physiological conditions. Our device features: (i) a microfluidic design tailored for the isolation of L4 larvae from a mixed larval population 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.

Microfluidic device overview







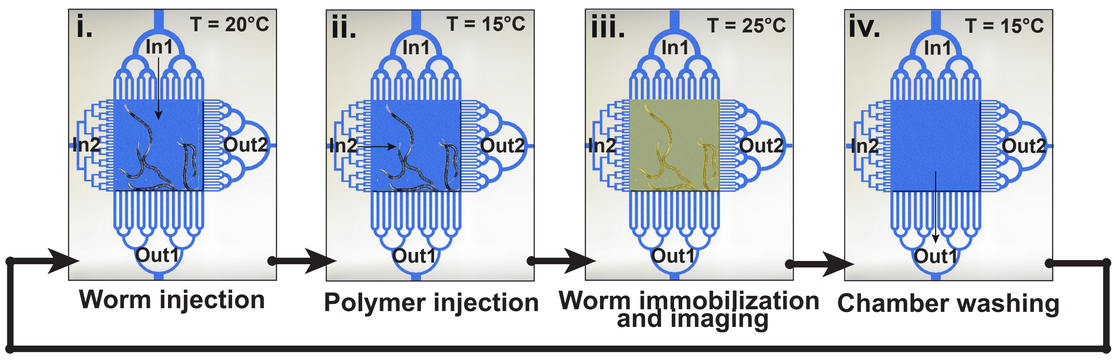
The polydimethylsiloxane (PDMS) microfluidic chip is bonded to a 150 µm-thick glass coverslip, for high-resolution microscopic imaging through oil immersion objectives

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.

The microfluidic inflow is controlled via external automated syringe pumps and pre-thermalized in the microfluidic tubing connected to the chip.

The use of lateral fluidic connections enables device operation with both upright and inverted microscopes. The whole assembly has the size of a 60 mm diameter well-plate, hence directly fitting standard microscope stages.

Long-term imaging protocol



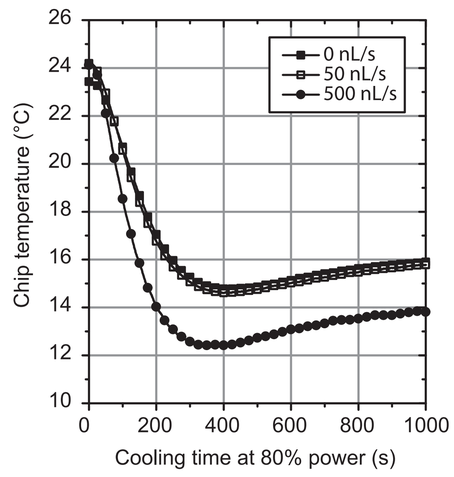
i. Worm injection into the microfluidic chip at a relatively low flow rate (typically 100 nL/s) and synchronization of the population at the L4 larval stage;

ii. Worm culture at a desired temperature (typically 20°C) and feeding by perfusing the chamber with an E.coli suspension;

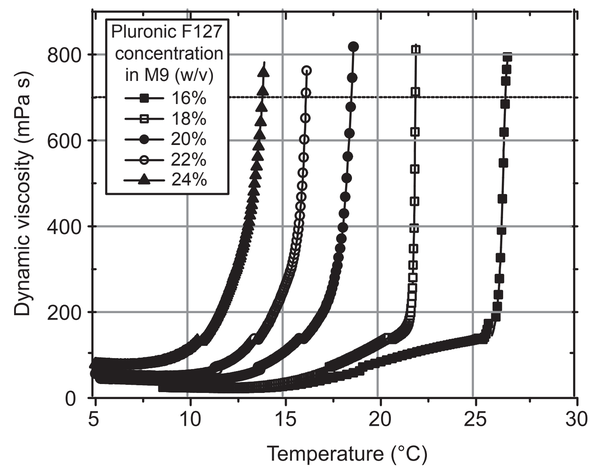
iii. Successive worm immobilization, imaging and release operations. Prior to imaging, the chip temperature is set to 15°C and a liquid solution of 23% Pluronic F127 in M9 buffer is injected into the chamber. For high-resolution imaging, the chip temperature is raised to 25°C, to trigger the gelation 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.

Device characterization



Experimental chip temperature as a function of the device cooling time, for different inflows at the chip inlet

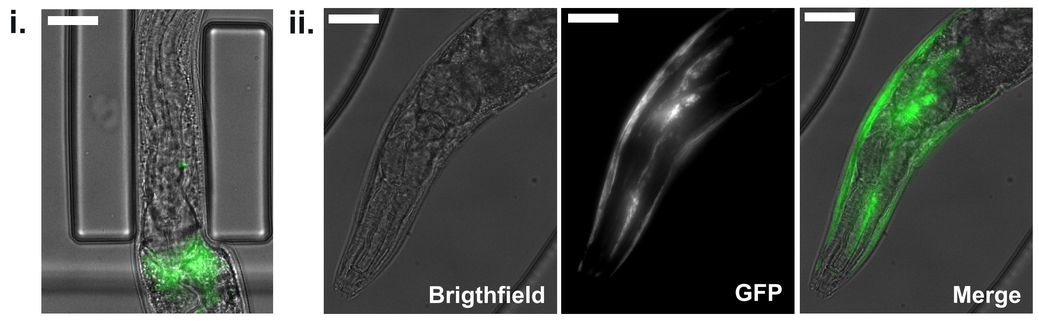


Dynamic viscosity of Pluronic F-127 in M9 buffer as a function of temperature and for different concentrations (measured with a SV-1A tuning fork vibro-viscometer)

Experimental results

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.

Polymer concentration (w/v)	16%	18%	20%	22%	24%
Onset of worm immobilization (°C)	26	22	18.5	16	14



i. 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 µm)

ii. Analysis of Green Fluorescent Protein (GFP) expression in the mitochondria of the worm muscles using pmyo-3::mito-GFP transgenic worms. 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 µm))

Conclusion & Outlook

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.

EMBL conference - Microfluidics 2014

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23 - 25 July 2014

EMBL Advanced Training Centre - Heidelberg - Germany