C. elegans development, perturbed in space and time using microfluidics

M2 Internship proposal

Location: Institut Pierre-Gilles de Gennes (IPGG)/ Institut Curie, 75005, Paris
Team/Lab: Quantitative Developmental Biology & IPGG Technology platform
Theme: Nano and Microfabrication
Duration: 6 months
Desired starting date: no later than January 2023

Project & Internship proposal

In this project, we address the question of how multicellular system robustly executes their function in a broad range of environmental temperatures. Our approach is to challenge a small developing multicellular organism (nematode worm, called *C. elegans*) with a **very steep linear temperature gradient** (Fig. 1) in a microfluidic system and follow its development through live-microscopy. By following fluorescent reporters of developmental gene expression as well as skin stem cell divisions, we will investigate whether compensatory mechanisms can counteract the effects of this extremely unnatural environmental condition.

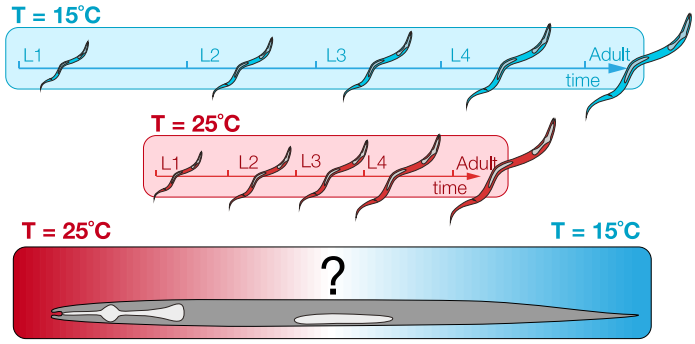

Figure 1. Graphical illustration of the project idea. The C. elegans larva robustly develops through the four larval stages (L1-L4), almost twice as fast at 25˚C compared to 15˚C. The goal of the project is to uncover compensatory mechanisms that enable this adaptivity of development, by challenging *C. elegans* with a linear temperature gradient and observing gene expression dynamics and cell division timings in the hypodermis before, during, and after perturbation.

To obtain this steep temperature gradient, we take inspiration from a study by *Selva, Jullien et al. (JMM, 2009)* and overlay a micro-patterned array of transparent resistors on a microfluidics device that immobilizes the worm along its anteroposterior axis (Fig. 2). All experiments will be performed at the platform at Institut Pierre-Gilles de Gennes (IPGG), taking advantage of its well-equipped cleanroom.

The final aim of the project is to develop a mathematical model that will be helpful to conceptualize our experimental observations and to understand the nature of coupling between the cells enabling tissue synchronization. Testable predictions will be extracted from the model for new types of spatiotemporal perturbations, which will then be experimentally verified with the setup.

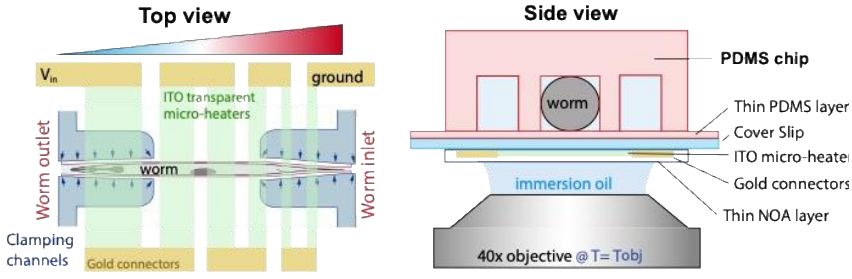

Figure 2. Schematics of the proposed microfluidics device, trapping *C. elegans* larvae inside a channel in a temperature gradient. (A) Bottom view (B) Side view (not to scale). Stable animal confinement is obtained with a channel whose width can be tuned by pressurizing neighboring "clamping channels". The gradient of temperature is obtained through a pattern of transparent ITO micro-heaters (light green), etched on the coverslip surface. Width of the microheaters and input voltage determine the shape and amplitude of the temperature gradient. The imaging objective temperature is set through water cooling.

This project will expose the quantitative laws and limits of adaptability of development in one of the most prominent model organisms for Biology. The involvement of the highly conserved genes on all levels of the genetic regulation of *C. elegans* development suggests that evolutionary conserved general principles of cellular coordination will be revealed by this project. At the same time, the technological innovations that will be developed are likely to find applications in similar studies especially the ones involving other model organisms.

Goals of the internship:

When the future intern will be joining the team, we will already be able to run experiments with the temperature gradient along the worms. Thanks to the interdisciplinarity of the project, this internship could match with different expectations depending on the student profile:

- Working on COMSOL to simulate the expected temperature profile that depends on many parameters (ITO thickness, resistance, applied voltage, temperature of the objective…etc)
- Optimizing the parameters, the fabrication process and the setup to control as much as possible the temperature gradient that the worms are facing
- Developing a new resistor pattern to obtain a step temperature instead of a linear temperature gradient
- Developing a new microfluidic device to image *C. elegans* throughout its entire post-embryonic development
- Perform spinning-disc confocal live-microscopy to track developmental gene expressions and skin stem cell divisions with and without the temperature gradient
- Starting the mathematical model

Working on different parts is obviously feasible and we would be happy to adapt the proposal with your expectations. If you need additional information, please do not hesitate to contact eliot.schlang@curie.fr.

References:

About the linear temperature gradient:

B. Selva, J. Marchalot, and M. C. Jullien, "An optimized resistor pattern for temperature gradient control in microfluidics," J. Micromechanics Microengineering, vol. 19, no. 6, 2009, doi: 10.1088/0960-1317/19/6/065002.

About the microfluidic chip to immobilize C. elegans larvae along its anteroposterior axis:

S. Berger, S. Spiri, A. deMello, and A. Hajnal, "Microfluidic-based imaging of complete Caenorhabditis elegans larval development," Dev., vol. 148, no. 18, 2021, doi: 10.1242/DEV.199674.

About oscillations of gene expressions and skin stem cell divisions during larval stages:

G. J. Hendriks, D. Gaidatzis, F. Aeschimann, and H. Großhans, "Extensive Oscillatory Gene Expression during C.elegans Larval Development," Mol. Cell, vol. 53, no. 3, pp. 380–392, 2014, doi: 10.1016/j.molcel.2013.12.013.

Gritti, N., Kienle, S., Filina, O. et al., « Long-term time-lapse microscopy of C. elegans post-embryonic development" Nat Commun 7, 12500, 2016, doi:10.1038/ncomms12500

About the mathematical model:

B. Novák and J. J. Tyson, "Design principles of biochemical oscillators," Nat. Rev. Mol. Cell Biol., vol. 9, no. 12, pp. 981–991, 2008, doi: 10.1038/nrm2530.