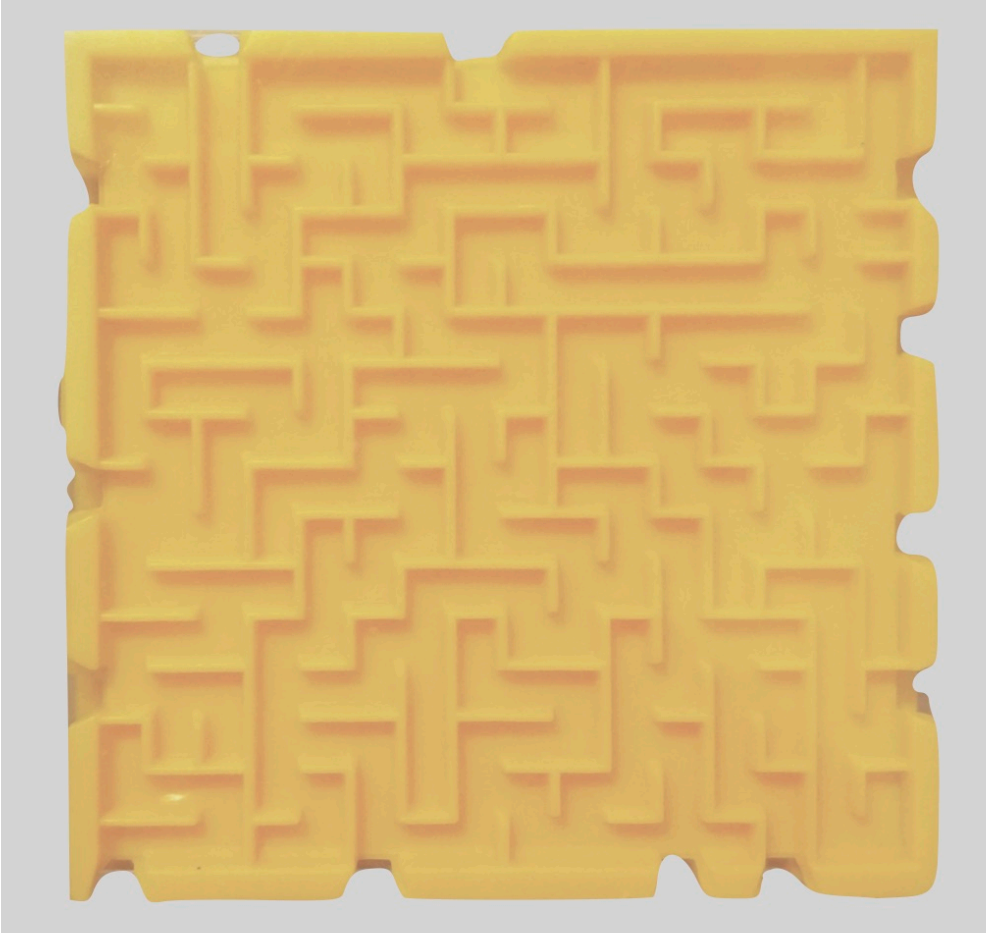


PEARL:

Puzzling BactEriAl Pets with Reinforcement Learning

Varun Kothamachu, Emre Ozer and Tanya Hutter

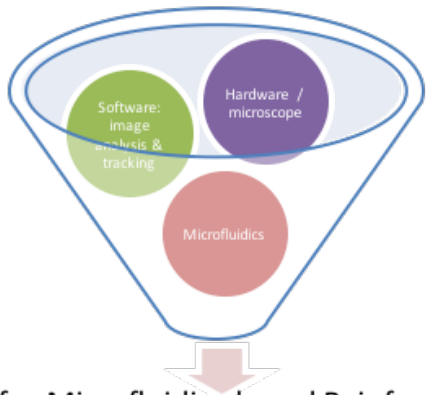
Introduction

Single celled organisms like bacteria use complex signalling pathways to process information about their environment by integrating signals from outside the cell, within and from other members in the colony. Previous examination of decision-making processes and social interactions in bacterial cells have shown that some of the features seen in bacterial cells are like those exhibited by larger, more complex multicellular cells. With this experiment, we attempt to examine the notion of cognition, memory and learning in bacterial colonies [1,2], by training them to navigate and solve maze traversal puzzles.

By introducing a colony of E. coli cells inside a maze implemented as a microchannel on a microfluidics chip, we will test if cells from the colony can navigate their way out of the maze. Using different maze patterns, food gradients, and employing a strain selection approach to advance successful bacterial colonies to more complex maze patterns, we hope to ask important questions on the ability of these cells to learn from their experience in the maze, and test if these colonies can exhibit a capacity for predicting changes in their environment, beyond just sensing and responding to them. This could potentially lead to interesting real world applications like developing bacterial computers to solve computationally challenging problems [3].

Aim of the Study

The overall aim of this project is to examine the behaviour of chemotactic bacterial cells within a microfluidic maze environment. In doing so, we will attempt to understand the ability of these cells to leverage their chemotaxis machinery and navigate the constraints of their micro environment to solve a maze. To test this, the project will combine several disciplines: microscope hardware, image analysis, cell culture and microfluidics.



Imaging Platform for Microfluidics-based Reinforced Learning with Motile Bacterial Cells

Our **first goal** is to solve challenges associated with the experimental setup needed to achieve monitoring of bacteria in microchannels. *E. coli* cells are very small (~2µm) and

require high magnification. In parallel to working on the experimental setup, our **second goal** is to develop a test chip to begin basic work on the experimental design.

Current Status

Microscope/imaging setup

As we want to measure the movement of cells over a large field of view and capture how and where these cells move over several millimetres, we started working on a programmable motorised stage to capture multiple high resolution images which we plan to use with open source tools like ImageJ for further image analysis. After several iterations, we developed an early prototype microfluidic chip (with the maze puzzle) holding enclosure and an external support frame within which the chip holding enclosure will be placed.

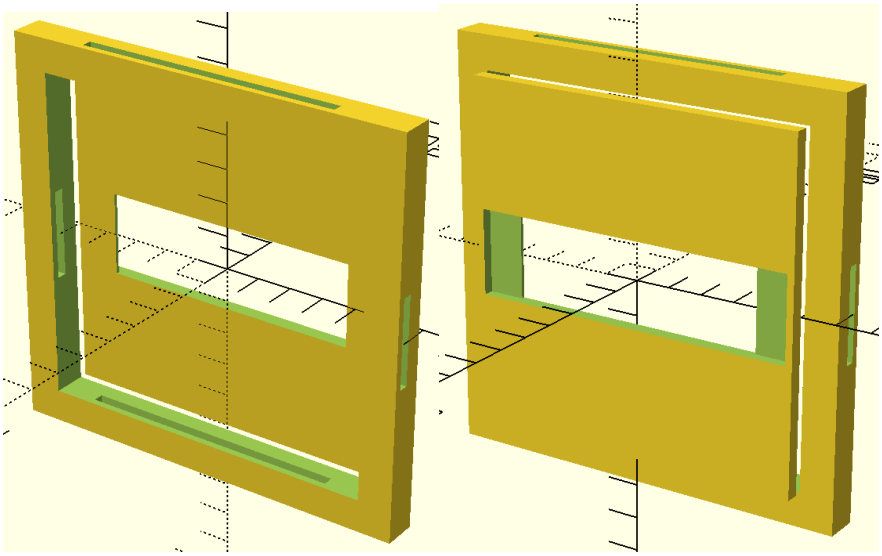


Figure 1: Early prototype designs for a stage holding the microfluidic chip and slits for setting up a light source for imaging.

The design for the outer support enclosure holding the base chip holder, will be further developed to have an X-Y movement track which would allow the movement of the microfluidic chip holding enclosure. Once this is implemented, in the next steps, we plan to use servo motors and micro-controller boards (Arduino based controllers) for precise X-Y movement. The aim of this design will be to allow the resulting programmable stage to work with any existing microscope. The initial designs have been developed using OpenSCAD [5].

Test chip design

Figure 3 (A, B) show the design of our first test chip. With this, we aim to obtain basic measurements on optimal media composition, and experimental conditions required for setting up imaging. This information will be necessary for defining specifications for implementing mazes on a microfluidic chip.

For initial testing, we decided to use a simple geometry to demonstrate the concept. A channel was mechanically machined on a Perspex piece. The dimensions of the piece were made same as standard microscope slide. The depth of the channel is roughly 100 µm, the total length is 20 mm, channel width 1 mm and the diameter of the wells is 2.5 mm. In this arrangement, E Coli can be introduced in the middle well, then food and repellent on the other two wells on the sides. With this design, *E. coli*, attractants and repellents can be introduced in different combination of wells and the cells moving in the channels imaged[1].

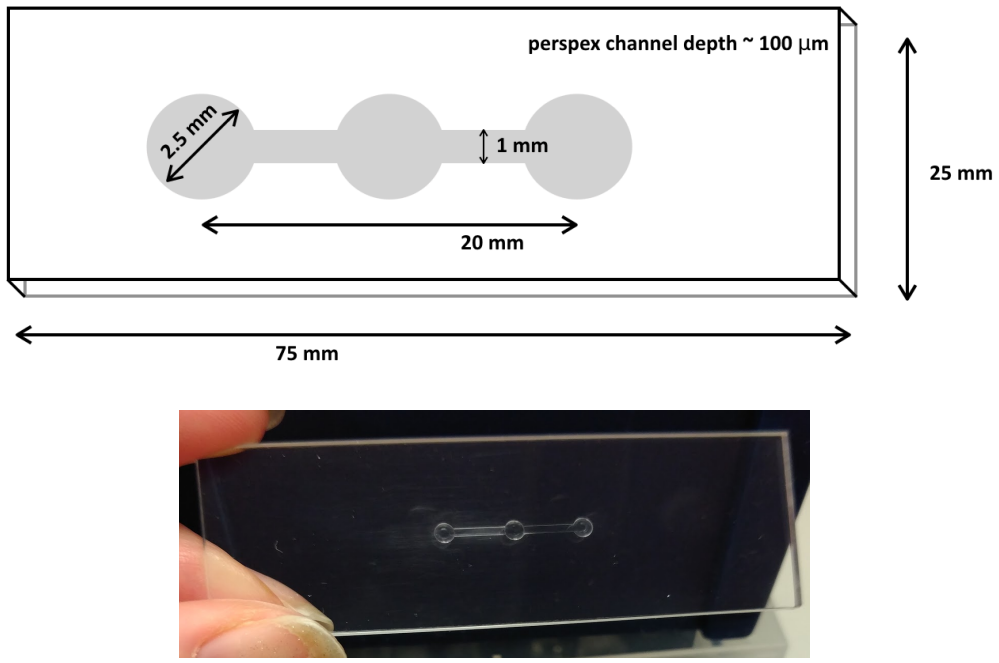


Figure 2: A) Shows a schematic representation of our first test chip (B).

Next Steps

We plan to use E coli AB1157 due to its known usage in motility studies. Alpha methyl aspartate, which is a non-metabolisable analogue of the strong attractant aspartate can be used as the attractant. We will introduce these cells into the test chip and derive our initial set of measurements. These will be necessary for the next steps which are

1. Improving the design of the chip holder enclosure, building the light enclosure and the X-Y movement track.
2. Developing the electronic micro-controller integration and iterating programmable stage design to include precise XY movement.
3. Developing theory for defining maze difficulty and understanding complexity level of maze designs for characterizing bacterial colonies.
4. Using theory developed for maze characterisation to build more complex chip designs.

[1] For longer measurements, and to minimise water evaporation, microscope cover slips can be put on top of the microfluidic chip.

Supplementary files

Design files for the chip holding base plate and its surrounding support mount can be found in the supplementary folder.

Acknowledgements

We thank SRI for the BioMaker challenge grant and support throughout the challenge. We thank Pahini Pandya for her discussions and suggestions.

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