Title of Project

Puzzle-solving Bacterial Pet: Imaging Platform for Microfluidics-based Reinforced Learning with Motile Bacterial Cells

Primary Contact for the Team

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Team

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Contribution Statement

The initial anticipated contributions are: **Tanya Hutter** will contribute towards building the microfluidics platform for the conditioning hub; **Pahini Pandya** will contribute towards the experimental wet lab work needed for growing the bacteria; **Emre Ozer** will contribute towards designing the microfluidics maze and HW/SW system architecture, and **Varun Kothamachu** will co-ordinate the project and contribute towards electronic microcontrollers needed. All members will be involved in overall experiment design, image analysis algorithm and analysing data generated.

Summary

This proposal is for developing a programmable staging mount, and an imaging platform for a microfluidics-based reinforced learning hub for motile bacterial cells. By developing a maze traversal challenge, we aim to create different scenarios for chemotactic bacterial colonies to employ their decision-making machinery and navigate their way out of the maze. By isolating successful colonies and progressing them further to solve more complex mazes, we aim to identify strains of bacteria that are good at solving maze traversing puzzles. The potential learning from this could lead to an understanding of cognition, memory and learning in bacterial colonies. In addition, we aim to understand a bacterial colony's ability to learn from its experience of solving maze traversal puzzles by measuring complexity level of each of the maze designs.

Proposal

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 cells [1,2], by training chemotactic bacterial cells 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].

Project Implementation Plan

(1) Imaging System:

We need to demonstrate that we can image bacteria using a low-cost microscope/camera. The bacteria will be placed in a microfluidic well [4]. The challenge will be imaging small bacteria with a broad field of view. Microscope normally focuses on a very small area, which will not be enough to see bacteria move long distances. If we use low magnification, then we will not be able to 'see' individual bacteria as they are relatively very small. To see E. coli we will need magnification of 400-1000x according to this video. At a magnification of 400x the field of view is only 450 microns and at 1000x magnification, field of view is 180 microns [7]. Therefore, it is very challenging to

see bacteria move long distances. If we use low magnification, then we will not be able to 'see' individual bacteria as they are relatively very small. To see E. coli we will need magnification of 400-1000x according to this video. At a magnification of 400x the field of view is only 450 microns and at 1000x magnification, field of view is 180 microns [7]. Therefore, it is very challenging to track bacterial movements that are longer than 400 microns.

The team at Stanford University [4] used larger cells, which is significantly easier. Our aim here will be to find a way of measuring small bacteria traveling large relatively distances, possibly this could be achieved by quickly scanning a large area and 'pasting' the images together. We could re-engineer the WaterScope [8] for this. We will also consider using smartphone's camera similar to this paper [9].

(2) Image Analysis Algorithm: Develop an image analysis algorithm that is capable of tracking individual bacteria.

(3) Tracking Bacteria: Show that when food is introduced in one corner of the microfluidic chamber, the bacteria chooses to go in that direction. Demonstrate that our algorithm can track in real time the movement of bacteria and plot individual trajectories.

(4) Teaching Bacteria: Introduce a feeding sequence (at different places in the microfluidic well) and then see if bacteria can predict the next feeding point.

(5) Project Output: Produce detailed technical documentation, release blueprints of the imaging platform and microfluidics maze designs, and disseminate the results online.

Components and Budget

Components to build the imaging system that has a motor to move the camera/microfluidic channel for scanning, including 3D printing of components. In addition to this, we intend to procure some E. coli cells from New England BioLabs and DAPI.

The projected costs are shown in the following table:

Component	Cost (£)
Electrical/Electronic components	100
Microfluidics (Mask + Lithography)	500
3D printing	300
Lab consumables	200
TOTAL	**1000**

References

[1] Chemotaxis: How bacteria use memory
[2] Bacterial computing: a form of natural computing and its applications
[3] The cognitive cell: bacterial behaviour reconsidered
[4] LudusScope: Accessible Interactive Smartphone Microscopy for Life-Science Education
[5] A 3-D printable open source platform for fluorescence microscopy, optogenetics and accurate temperature control.
[6] Globally optimal stitching of tiled 3D microscopic image acquisitions
[7] https://www.microscopeworld.com/t-magnification.aspx
[8] http://www.waterscope.org/3d-printing/
[9] Quantitative Imaging with a Mobile Phone Microscope, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4019540.