

The Green Mother Machine Reloaded

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Proposal Title	The Green Mother Machine Reloaded
The Idea	Cyanobacteria have the ability to exploit sunlight to convert atmospheric CO₂ into carbohydrates, and possess a very simple but robust circadian clock. The relatively fast doubling times of cyanobacteria compared to plants and the simplicity of their circadian clock make cyanobacteria ideal organisms to study the interaction of the circadian clock with other biochemical networks (both endogenous and synthetic). These networks can interact in non-intuitive ways and often the mechanisms of such interactions are only apparent at the single cell level. Considerable efforts to build tools to observe bacteria at the single cell level have focused on other types of bacteria, such as <i>E. coli</i> or <i>B. subtilis</i>. These organisms have been preferred because of their ubiquity as laboratory model organisms and extremely fast growth rates. In this project we want to build a microfluidic device which allows the observation of <i>Synechococcus elongatus</i> PCC 7942, a well-studied cyanobacterium, at the single cell level. A clever microfluidic design was developed in the Jun Lab at the University of California in San Diego for the study of <i>E. coli</i> [1]. In this device the bacteria grow in dead-end channels, which are slightly wider than the diameter of an <i>E. coli</i> cell. Thus the colony grows in a line, rather than randomly across a surface, which makes the segmentation and tracking of cells easier. Moreover, the cell at the bottom of the channel can be followed for very long times. Unfortunately, it is not possible to grow cyanobacteria in this traditional Mother Machine as <i>S. elongatus</i> cells are slightly larger than <i>E. coli</i> and their growth is inhibited when in direct contact with PDMS, the standard polymer used in microfluidic applications. We started this project in 2015 when we received funding from SynBio Fund to build a Mother Machine type of microfluidic device optimized for cyanobacteria. We call this device the Green Mother Machine. With the funds from SynBio Fund we have been able to build a prototype of the Green Mother Machine. To get the cells to grow, we chemically removed the uncured PDMS and passivated the channels. However, the loading of the cells into the channels is still very poor, with, on average, fewer than one cell lineage per field of view. In addition to building a better device with improved loading, we want to extend this project to build a Green Mother Machine that will allow for ultrafast switching of media on the device without generating any backflow. We intend to do this by exploiting the dial-a-wave valve pioneered by the Hasty lab at University of California in San Diego [2]. Backflow when two channels merge is a common problem in microfluidics, and this backflow causes fluctuations in the switching times. A traditional solution to this problem is to use a solenoid valve outside of the chip. These solenoid valves are expensive (£100- £1000), and the switching involves a lag time as the switch is located

outside the chip. However, with the dial-a-wave valve it is possible to switch without any backflow.

Thus, for this follow-up project, we have defined the following two goals:

1. Optimize the geometry of the channels and growth conditions to improve loading and growth in the Green Mother Machine.
2. Implementing the Dial-a-Wave valve into the Green Mother Machine.

[1] P. Wang et al., Robust growth of Escherichia coli. Curr. Biol. 20, 1099–1103 (2010).

[2] M. Ferry et al., Microfluidics for synthetic biology: from design to execution. Methods Enzym. 497, 295-375 (2011)

Who We Are

Christian Schwall (cs687@cam.ac.uk), Background: Physics/ Quantitative Biology
Philipp Braeuninger-Weimer (pab96@cam.ac.uk), Background: Engineering
Bruno Martins (bmc36@cam.ac.uk), Background: Physics/ Quantitative Biology
Arijit Das (akd41@cam.ac.uk), Background: Microbiology
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Antony Hall (anthony.hall@uea.ac.uk), Background: Circadian Clock plant biology

Implementation

I. Optimize the geometry of the channel and growth conditions

Our plan is to change our Green Mother Machine prototype in the following ways:

1. Dead-end funnel shaped channels.
 - a. This channel shape should help getting the cells into the growth channels.
2. Open-ended channels.
 - a. One reason why the cells exhibit sub-optimal growth might be that the media is not exchanged quickly enough in the growth channels. This effect would be reduced when flushing media through two openings in the small growth channels.
3. Larger channels which can host small colonies of up to 20 to 30 cells.
 - a. These micro colonies could be followed for very long times. The biggest advantage of this design change would be that the growth channels could be fabricated with conventional photolithography, which makes the work flow a lot faster and easier.

II. Implementing the Dial-a-Wave valve

While, optimizing the growth channel geometry, we will investigate possibilities to use the Dial-a-Wave valve with the Green Mother Machine.

All this work will be done within the framework of our well established collaboration between Christian Schwall (microfluidics expert), Bruno Martins (cyanobacteria expert), Philipp Braeuninger-Weimer (photolithography expert), and our new collaborators Anthony Hall (imaging expert on circadian clocks), Arijit Das (cyanobacteria expert), Chao Ye (cyanobacteria expert) and Toby Livesey (single cell microscopy expert).

Benefits and outcomes	For this project we will continue our collaboration between the Department of Engineering (Philipp Braeuninger-Weimer) and the Sainsbury Laboratory (Christian Schwall, Bruno Martins, Arijit Das, Chao Ye and Toby Livesey). In addition, we have been able to establish a new collaboration with Anthony Hall, at the Earlham Insitute, who will help us with understanding how best to image the circadian clock in cyanobacteria. To our knowledge there is no working Mother Machine device specifically designed for the cyanobacterium <i>S. elongatus</i>. The direct benefits of this project to the framework of synthetic biology are two-fold: 1. <i>S. elongatus</i> is the model organism of choice for research on the prokaryotic circadian clock and it is a promising host of synthetic circuits for metabolic engineering. Despite its importance, specific single-cell analysis tools have, so far, prioritized other types of bacteria. We believe that, in order to optimally characterise and manipulate the interaction between a cyanobacterium 'chassis' (i.e., a cell) and an exogenous circuit, we must first study systems in a rigorously controlled environment at the single-cell level. Our device will provide such a level of control. On the other hand, while we will design and test our device with cyanobacteria, there is no impediment to it being tweaked and used to grow other bacteria. 2. Microfluidics has emerged as a pivotal technique to study organisms at single-cell or micro-colony levels. However, fabricating the devices can be challenging and this discourages usage by the wider community. It is therefore essential that cross-talks between advanced engineering and fundamental biology are maintained. Our proposal addresses this cross-talk by: i) partnering skills in materials engineering, cell biology and plant biology from two different Cambridge departments and the Earlham Institute in Norwich; ii) accomplishing all steps that link an engineering blue-print to a real everyday experiment in a biology lab. Once we have a working device we will share the CAD files and the established protocols. We will also make epoxy replicas of the working device available, as epoxy replicas are an easy and cheap way of sharing design.
Sponsor for the research and cost centre	Dr. James Locke, The Sainsbury Laboratory, james.locke@slcu.ac.uk , who as agreed to match all funding.
Budget	<u>Budget:</u> We budget our project as follows: Masks to test new channel geometries: £ 1000.00 Mutli-channel pump: £ 2000.00 Consumables: £ 500.00 Cleanroom Access: £500.00 Total: £ 4000.00 Additional support for the project will be provided as follows: Free access to the clean room and electron beam time will be provided by Philipp Braeuninger-Weimer. Dr James Locke has agreed to make his soft lithography lab (plasma cleaner, oven and desiccator) and cyanobacteria strains available for project.