Project Title

The Green Mother Machine Reloaded

Report Title

Progress report on the project: The Green Mother Machine Reloaded

Summary

A very short summary or abstract of your project and the main outcomes (200 word max)

In this project we want to build a microfluidic device which allows the observation of *Synechococcus elongatus* PCC 7942, a well-studied cyanobacterium, at the single cell level. We based our design on a well-established device called the mother machine and tailored it to the specific needs of *Synechococcus elongatus.* One of the biggest challenges in adapting the mother machine to *Synechococcus elongatus* is to keep the cells alive and to load the cells into the growth channels. Here we optimize the loading and survival of *Synechococcus elongatus* in the green mother machine by improving the loading protocol and the experimental setup. In addition, we tested various prototypes for the robust media switching between different media.

Report and outcomes

The report should be a short written description of what you accomplished and how, including an evaluation of where things didn't go to plan! Please include links to or copies of any outputs (papers, posters, data, code, hardware designs, photos, blogs, videos) - these do not need to be duplicated in the report. If you are attaching supplementary files, please refer to them in the text or add a list with a brief description at the end e.g. OpenPlantReport.pdf: Formatted version of full project report. FooSequences.fasta: Sequence file for DNA parts ACB123 and DEF345. Refer to the reporting guidelines for more information.

Initially we had less than one cell per channel during the loading process. After modifying the loading protocol by using a combination diffusion and spin loading (using a spin coater https://www.spincoating.com/en/spin-processor-models-substrate-sizes/spin-coater-spin150i-spin-processor-spincoating-machine/77/) almost all channels are fully loaded.
To improve the survival of *Synechococcus elongatus* in the device we reduced the stress levels due to phototoxicity by using a very light sensitive sCMOS camera (Photometrics Prime; https://www.photometrics.com/products/scmos/prime.php). We also tried different designs of the Dial-a-Wave junction (https://dialawave.wikispaces.com/), a clever way of handling the fluids for robust switching. To achieve the switching, we had to upgrade our syringe pumps to two computer controllable pulse free pumps (fig 1).

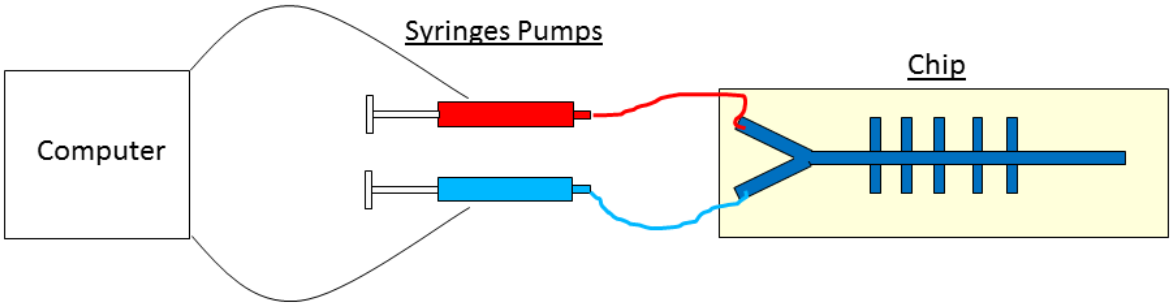

Figure 1. Illustrating Pump setup.

After rigorous tests of different pumps, we ended up going for the KDS legato 200 as it has a good balance between performance and price (http://www.kdscientific.com/legato-200-syringe-pump.html). The switching with the Dial-a Wave junction worked but the growth channels in our initial prototype ended up being too high. Thus the cells could not be trapped in the growth channels. With the additional money we want to get the growth channel height right and investigate different channel designs.

Expenditure

A summary of how you have spent the £4000 budget so far.

We spent the money on two computer controllable pulse-free KDs legato 200 syringe pumps (http://www.kdscientific.com/legato-200-syringe-pump.html). They turned out to be more expensive than expected due to the weak pound. These pumps allow us to do reliable switching between two different media.

This is how we spent the money:

Item	Price per Item	Quantity	Total Amount
KDS Legato Syringe Pump	£ 2351.25	2	£ 4702.50

James Locke has covered the difference (£ 702.50) between our spent amount (£ 4702.50) and the allocated funds (£ 4000.00).

Follow on Plans

A short description of your plans for follow-on work with a breakdown of how you will spend the additional £1000 (if requested) and any remaining funding from the initial £4000. Please include timings as it is expected that all funds will be spent within six months of this report, after which point a brief report from the follow-on activities and return of the remaining funding will be requested.

With the additional £ 1000 we will be covering our clean room costs to optimize the channel heights for our switching device. We will use these additional funds as soon as they have been allocated to us.