Title: Open Source Autonomous Imaging Station

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

We proposed to create a set of tools for scientific research, engineering and teaching biology at the scale of whole colonies and entire plates. First, we designed and built a standalone tool for imaging and analysing fluorescence in biological samples at three different spectra and at a range of scales from individual colonies to whole plates (easily extensible to plant cells and even whole organisms such as *C. elegans* and *Marchantia spp)*. The system is self-contained and autonomous, including hardware and software for image capture, programmed sequences (e.g. timelapse), and quantitative analysis of samples. We also developed a simple genetic toolkit for the production of fluorescent and pigmented bacteria complementing the device. We have optimized genetic construction protocols and adopted fluorescent markers that can be used with 470 nm single excitation. Altogether, our system allows for construction and integral experimentation with hardware, software and genetic instructions from off-the-shelve, open access and distributed resources.

**Project Report**
**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.**

# Introduction

We proposed to create a set of tools for scientific research, engineering and teaching biology at the scale of whole colonies and entire plates. First, we designed and built a standalone tool for imaging and analysing fluorescence in biological samples at three different spectra and at a range of scales from individual colonies to whole plates (easily extensible to plant cells and even whole organisms such as *C. elegans* and *Marchantia spp)* (Fig. 1). The system is self-contained and autonomous, including hardware and software for image capture, programmed sequences (e.g. timelapse), and quantitative analysis of samples.
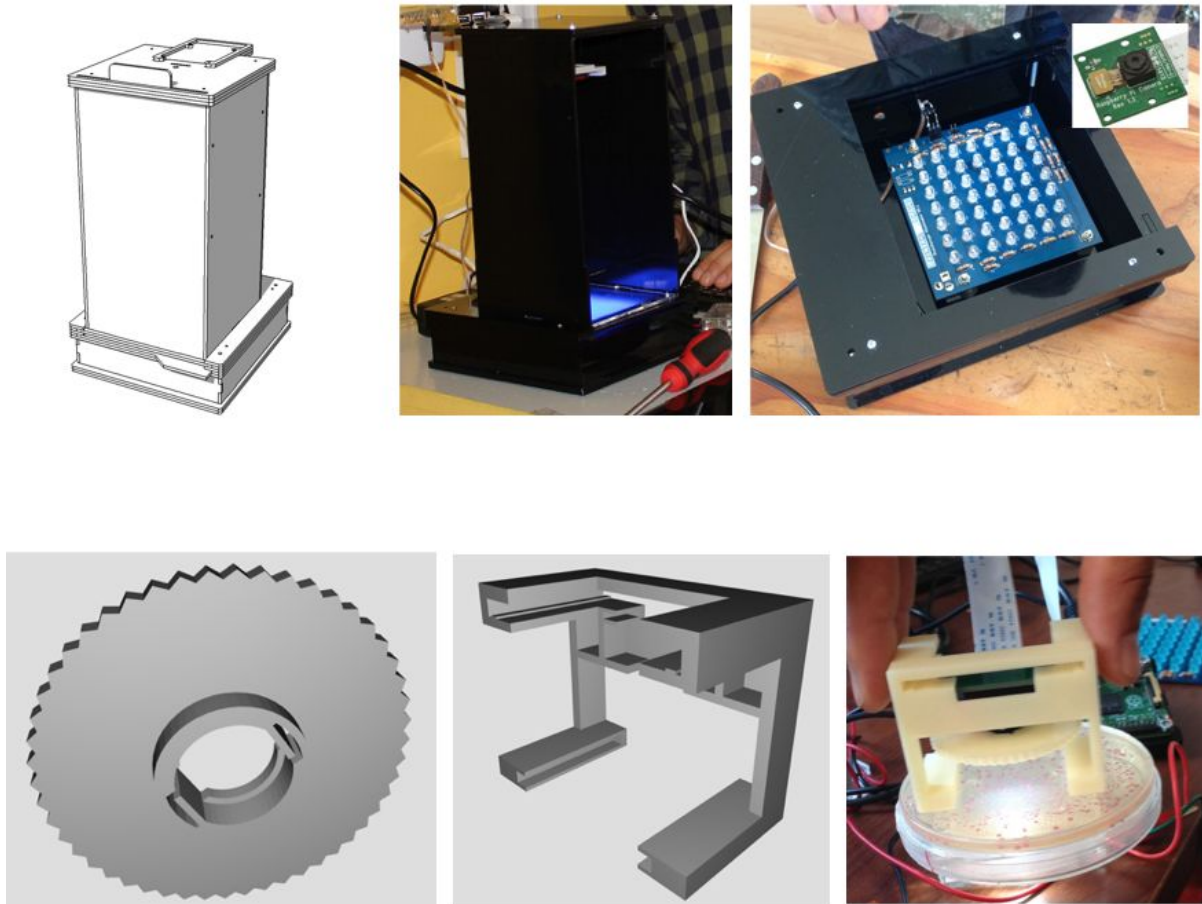
Fig. 1: Imaging station

We also developed a simple genetic toolkit for the production of fluorescent and pigmented bacteria complementing the device. It is worth to note that a series of fluorescent proteins have been screened in order to find those that can be excited with a single illumination source (470 nm LEDs) and detected with the same filter (amber acrylic) (Fig. 2). Being able to excite different probes with a single setup reduced significantly the cost and time of fabrication since mobile parts (e.g filters) are no longer needed.
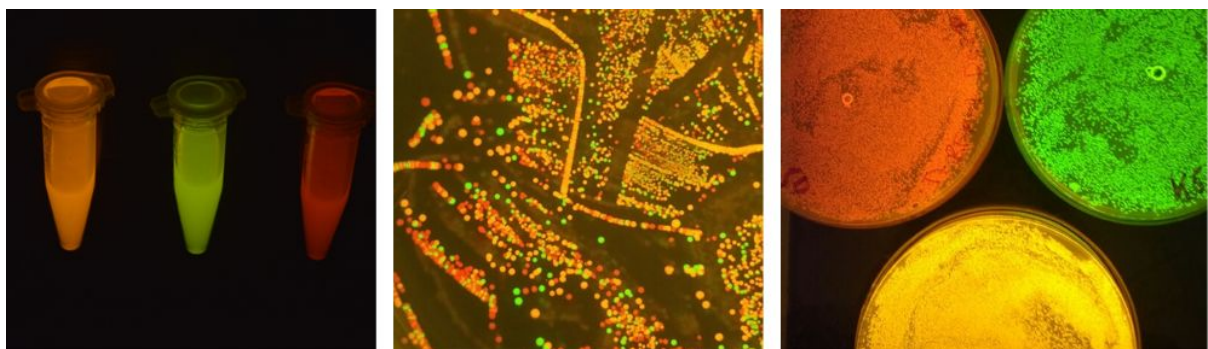


Fig. 2: sfGFP, mBeRFP and CyOFP fluorescent proteins under 470 nm excitation.

We have created a set of vector that combine Golden Gate and Gibson assembly methods for low cost fabrication of multiple genetic functions. These vectors, along with the 470nm-compatible fluorescent  probes, regulatory sequences, response regulators and

genes coding for cell-to-cell signalling components, are compatible with methods resources and methods widely used by the synthetic biology community. They use conventional sequences and type IIs enzymes to easily compile transcriptional units (ie functional units of protein expression) from growing libraries of standardized subcomponents being constantly developed by different labs around the globe (e.g CIDAR MoClo, iGEM MoClo parts and PUC level 0 parts). This approach not only simplifies considerably the protocols for DNA fabrication but also promotes new practices such as co-development and the use of distributed resources. These tools can be therefore maintained, expanded and improved by collaborative communities of distributed around the globe. This means tools developed in one high school or lab are useful and utilizable by another group elsewhere, and vice versa. Of course, this relies fully on being able to share information and resources under open source licenses (eg OMTA).

We also combined this method with Gibson assembly for multigene construction. Once transcriptional units have been assembled by Golden Gate, PCR-based assembly are used for building multigene constructs (Torella et al., 2014). We are optimising the entire protocol to use the fewest number of expensive equipment or replace them with low cost alternatives. For instance, we have adapted bed-heating blankets for growing bacteria overnight and successfully used methods of DNA transformation that doesn´t require refrigeration of cells (Fig. 3).



Fig. 3: low cost setups for growing bacterial plates. Bed-heating device (USD 20).

Open source code has been also developed to run and analyze experiments (https://github.com/SynBioUC/Raspiscope.git), providing a round package for teaching software, hardware and biology in an integrated manner. All together, our tools allow running one week-long workshops that cover a wide range of concepts (Fig. 4).



The entire system, optics, frame, electronics, genetic resources and software are open source. Design files will be distributed in our github and a full documentation will be done on docubricks.

This project also wishes to highlight the benefits of employing an open framework for academic-industry collaborations. We have formed a successful partnership with the Open Source company Backyard Brains (TM), which has vast experience in creating and distributing open educational and research technology for neuroscience in Latin America and worldwide (backyardbrains.com, backyardbrains.cl).

Fig. 4: Workshops in primary schools in Puerto Natales (top) and Valdivia (Bottom), Chilean patagonia.

**Outputs**
1. Library of genetic parts and set of vectors.
2. Github public repository of open source: hardware designs, control software (python), analysis and teaching tools (Jupyter notebooks).
3. Workshops using the developed resources:
    a. Puerto Natales, school, community
    b. Valdivia, Universidad Austral de Chile, students (university and highschool) and community.
4. Draft paper in preparation for submission to an open journal

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

All the invoices are in the financial systems of Plant Sciences Department. All the expenses have been supervised by Jim Haseloff. All the costs of DNA sequencing and lab consumables have been covered by funds in Fernan´s projects.

|  | GBP |
|---|---|
| BackYardBrains * | 2000 |
| waveshare electronics | 160,85 |
| stratagene | 1305,6 |
| waveshare electronics | 561,09 |
| **Total** | **4027,54** |

* These costs covered acrylics, LEDs, and minor electronics as well as laser cutting and 3D printing services. These also covered Roberto Herrera services for design and construction of prototypes as well as PCBs.

**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.**

We believe this robust and affordable package will enable independent, inexpensive experiments and observation for scientists in emerging scientific cultures in Latin America as well as in schools, colleges and universities. We would like to produce a few imaging stations and distribute them to schools, artists and labs along with the accompanying genetic resources.

Our plan is to use the remaining funds to build those devices.