Development of an Open Source Autonomous Imaging Station for Distribution in High Schools, Universities, and Emerging DIY Scientific Communities.

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Who we are

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The idea

The growing availability of prototyping tools such as 3D printers and Laser Cutters (for example in MakerSpaces), combined with the rapid growth of online libraries where scientists can easily share design files (e.g. GitHub and Docubricks) has led to the birth of the "Open Science" movement. In this effort, scientific tools are developed collaboratively, with scientists in labs across the world contributing and modifying designs that are curated publicly online. Recent achievements of the Open Science approach include 3D manipulators, amplifiers, pipettes, PCR machines, gel electrophoresis machines, and Microscopes.

Backyard Brains has contributed to these achievements by developing open technologies for neuroscience and electrophysiology research and teaching, including a smartphone microscope. Synthetic biology has also played a key role in the Open Science movement by promoting the development of open frameworks for engineering biology. iGEM, for instance, has included a hardware track in the competition.

At its most accessible level synthetic biology deals with the engineering of genetic circuits that are implemented in bacteria. Most of the characterization techniques are based on fluorescent measurements obtained from expensive equipment such as fluorimetric plate readers, flow cytometers and fluorescence microscopes. In addition, the study of genetic processes taking place at a larger scale such as in large bacterial populations growing on solid media or whole plants has remained less explored due to the lack of appropriate equipment. Low cost imaging devices for studying - and engineering - these biological processes are still needed. These devices could be useful for engineering and characterizing colony-wide behaviour, morphogenetic processes, microbial biofabrication, distributed computing, microbial consortium engineering, among many others.

Here we propose to develop a standalone tool for imaging and analysing fluorescence in biological samples at a range of scales from individual bacteria, through colonies, plant cells and even whole organisms such as C. elegans. The system will be self-contained and autonomous, including hardware and software for image capture, programmed sequences (e.g. timelapse), and quantitative analysis of samples. We also propose the development of a simple genetic toolkit for the production of fluorescent and pigmented bacteria complementing the device. The entire system, optics, frame, electronics, genetic resources and software will be open source. 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. This project also wishes to highlight the benefits of employing an open framework for academic collaborations that seek to deliver Open Access resources and information. We have formed an industry partnership with the Open Source company Backyard Brains (TM), which has significant experience in creating and distributing open educational and research technology for neuroscience in Latin America and worldwide (backyardbrains.com, backyardbrains.cl).

Implementation

What we will do:

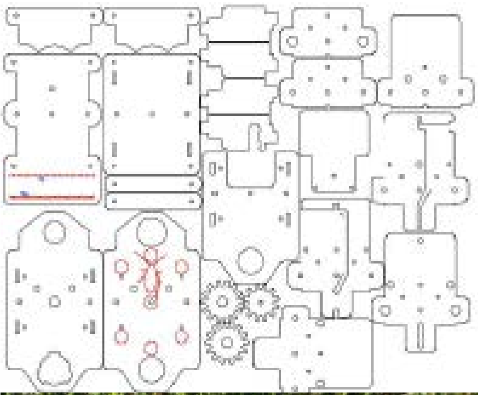
The system will be designed and built in collaboration with scientists from OpenPlant (FF, NP) and Chile (FF, JK and TR) in collaboration with Backyard Brains (TM). We will leverage this experience to develop, test and distribute our system, tailoring it for the needs of Latin American researchers and educators.

Hardware design:

The design will be based on an existing open source product from Backyard Brains (http://www.backyardbrains.cl/products/roachscope) - a smartphone microscope using fixed lenses for imaging at 10X and 100X magnification (Figure 1). This design will be retooled to fit the following specifications:

- Allow range of scales from entire petri dish (90mm) to single bacterium (1-2um).
- Replace smartphone with Raspberry Pi camera for better imaging control and automation.
- Modify casing to incorporate Raspberry Pi and connection ports
- Clamp/holder for petri dishes (30mm, 90mm)
- Clamp/holder for microscope slides
- Fluorescence illumination for sfGFP (485 nm) and other IP-free GFP-like proteins.
- Fluorescence excitation filtering (e.g. 1/8” blue acrylic from acrylite-shop.com)
- Fluorescence emission filtering using cheap commodity materials (e.g. 1/8” amber acrylic from McMaster-Carr)

Figure 1: Open Source Laser Design File, Microscope with Cell Phone placed, and view of microscope in field.



Software design:

Software will be developed using open source libraries and accessible programming languages (e.g. python, python-picam, scikit-image). This will be packaged as an open source module and hosted on github for further iterative development and incorporation into other software projects for our hardware. Further, a simple user interface based on this module will be developed to allow educators to easily use the system for teaching. This will include a graphical user interface for setting up automated timelapses, quantifying fluorescence, and plotting/visualising resulting analyses.

Biological samples for testing and teaching:

We will create a series of simple test samples, protocols and analyses that can also serve as teaching material with further development. We propose to create a protocol and accompanying resources for colony-wide artificial signaling that can be tracked by time-lapse fluorescent microscopy. We aim to develop this genetic material as an easy to use DNA fabrication kit based on Golden Gate and Gibson Assembly. We are currently working in collaboration with a Chilean High School (Colegio Blest Gana) and three undergraduate students of PUC Chile in the development of teaching resources that follow standards for transcriptional unit fabrication (CIDAR MoClo) and multigene assembly (UNSes).

We will explore the development of three types of biological material:
1- a bidirectional signaling system based on quorum sensing molecules already used by the applicants and two fluorescent proteins compatible with the fixed optical filters and lighting (e.g green and long stoke shift orange fluorescent protein).

2- Paenibacillus swarming colonies compatible with transmitted white light setups (video attached). This soil-borne bacteria is non-pathogenic and will allow its use in public spaces.
3- CRISPR/Cas9 regulation of swarming phenotypes for visible output of CRISPR genome Regulation.

We will buy and use IP-free fluorescent and chromogenic proteins from DNA2.0.

Outcomes:

Similarly to other Backyard Brains inventions and open source educational programmes, the outcomes will be:
1. Design and production of the machine, software and bioware.
2. Online publication of the design files and software in various open libraries (NIH 3D Print Exchange, Docubricks, Github, etc.).
3. Online publication and submission of genetic material (e.g. AddGene) and teaching materials.
4. Deployment in universities (Universidad Catolica), high schools (e.g Colegio Blest Gana and Metodista N7-Valdivia), and with various interested hobbyists through the Backyard Brains monthly community workshops.
5. Iterative changes to the hardware, software, bioware and teaching material based on feedback from these early adopters.
6. More rounds of distribution to schools and other users .
7. Formal scientific publication after 6 months -- 1 year of use in the field.

Who will be involved:

The design specification will be generated collaboratively between all team members, who all have marked experience in maximizing cultural impact and distribution of open source science tools and teaching of advanced biological concepts.

Publications include:
Baden T, Chagas AM, Gage GJ, Marzullo TC, Prieto-Godino LL, Euler T. Open Labware – 3D printing your own lab equipment Open Labware – 3D printing your own lab equipment. PloS Biology. Mar 20;13(3)

Marzullo TC and Gage GJ (2012) The SpikerBox: A Low Cost, Open-Source BioAmplifier for Increasing Public Participation in Neuroscience Inquiry. PLoS ONE, 20 March 2012, 7(3).

Design and production will be done by Backyard Brains. Testing will be carried out at Cambridge University (FF) and PUC (JK and TR). Software will be developed at JIC (NP) and PUC (JK and TR). Biological samples for teaching will be designed and assembled at Cambridge University (FF) and PUC (JK and TR).

The academic team has significant experience in fluorescence assays and time-lapse microscopy:

Rudge TJ, Federici F, Steiner PJ, Kan A, Haseloff J, Cell Polarity-Driven Instability Generates Self-Organized, Fractal Patterning of Cell Layers, ACS Synthetic Biology (2013).

Rudge TJ, Federici F, Brown JR, Dalchau N, Phillips A, Ajioka JW, Haseloff J, Characterization of intrinsic properties of promoters, ACS Synthetic Biology (2015).

Federici F., Dupuy L., Laplaze L., Heisler M., and Haseloff J. Integrated genetic and computation methods for in planta cytometry. Nature Methods 9: 483-485 (2012).

http://www.wired.com/2013/10/beautiful-microscopic-art-is-also-world-changing-science/?viewall=true

Van Vliet S, FJH Hol, T Weenink, P Galajda, JE Keymer The Effects of Chemical Interactions and Culture History on the Colonization of Structured Habitats by Competing Bacterial Populations.BMC Microbiology, May 7, 2014 Vol. 14:116 doi:10.1186/1471-2180-14-116. (Highly Accessed paper.)

Keymer JE, Galajda P, Muldoon C, Austin R Bacterial metapopulations in nanofabricated landscapes. PNAS November 14, 2006 Vol 103(46) 17290-17295. (Commented in Nature Research highlights, Nature 444:126-127 and also in Nature materials nanozone news, Novembre 2006).

Hol FJH, MJ Voges, C Dekker, JE Keymer Nutrient-responsive regulation determines biodiversity in a colicin-mediated bacterial community.BMC Biology, August 27, 2014, 12:68.

As well as open source software development, including:
Rudge TJ, Steiner PJ, Phillips A, Haseloff J, Computational Modelling of Synthetic Microbial Biofilms, ACS Synthetic Biology (2012). [github.com/haselofflab/cellmodeller] https://github.com/NeilPearson

Benefits and Outcomes

Describe how your project fits the remit of the Synthetic Biology OpenPlant Funds and the judging criteria, including details of any new interdisciplinary interactions in the University.
This collaboration will result in the invention, publication, and distribution of an open source portable tool for doing automated analysis of whole plant and bacterial colonies. It will form an important addition to the growing open science movement, and is a unique juxtaposition of scientists affiliated to prestigious universities (FF, JK; TR) that give scientific credibility to the effort, a founder of a science hardware company that has distributed gear to over 70 countries (TM), coding expertise to build the software necessary (TR, NP), and scientists embedded in emerging DIY scientific communities and novel educational programs in schools (TR, JK, FF, and TM). As such, this team is uniquely positioned, with the support of this grant, to make an invention actually used and distributed in the international science Community.

The project by definition is interdisciplinary, with collaborations between engineers, biologists, computer programmers, and inventors, working both in advanced and developing nations, with experience teaching from public high school classes up to advanced graduate level courses. The success of this project will lead to further strengthening of the ties between Cambridge and the emerging scientific culture in the Southern Cone of South America.

The project also seeks to demonstrate the potential of using open frameworks to share and collaborate between industry and academia.

Finally, this project will deliver devices and lectures in various schools across Chile and Argentina, including two ancestral ethnic communities: Williche school at Manquemapu (Chilean Patagonia) and Agroescuela Jocoli (Lavale Argentina).

Budget

Provide costings for your proposal (up to £4000) and indicate if you have access to any additional funding to meet your aims.

First £4000
Hardware design and fabrication: £2350
Backyard Brains estimate 1 month of design and fabrication work, costing approximately £2111, costed as follows:

- £1236 - Contract designer for 1 month
- £618 - Use of machines and production space (lab)
- £256 - Materials for prototyping
- Total: £2110

After design work is completed, each unit should cost around GBP £35 (without Raspberry Pi or camera). Thus, there will be 2100 GBP design fee for Backyard Brains, with the agreement that 20 microscopes that distributed at cost afterwards. After 20 microscopes are distributed, the microscopes will then be sold at market value (typically 3x markup of cost) for the first year.

- 2x Raspberry Pi + Camera = £75
- 2x Micro SD card (8gb + Noobs) = £15
- 2 HDMI monitors = £100 (To complete an imaging station for Manquemapu and Jocoli
- schools)
- 2 USB-integrated Mouse + Keyboard systems = £50 (To complete an imaging station for
- Manquemapu and Jocoli schools)
- Total: £240

Bioware design and fabrication: £1650
We estimate £1000 cost in buying IP-free genes encoding for fluorescent and chromogenic proteins from DNA2.0 and £650 in oligos and DNA sequencing.

Final £1000
Raspberry Pi + Camera for 20x units = 20x (£24.50 + £11.99) = £729.80
Micro SD card (8gb + Noobs) for 20x units = 20x (£6.00) = £120
2 HDMI monitors = £100 (To complete imaging stations for two schools or colleges)
2 USB-integrated Mouse + Keyboard systems = £50 (To complete imaging stations for two schools or colleges)
Total: £999.80

Video of Paenibacillus. Time steps were taken every ten seconds.