



Pipeline for Corchea fluorescence data acquisition

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Abstract

Synthetic biology uses microfluidic devices to characterize genetic circuits under controlled environments. Microfluidics has not been widespread because is expensive and needs dedicated infrastructure, making access difficult to developing countries like Chile. Previously I developed Corchea, an open-source low-cost paper-based microfluidic device, using 3D print, laser cut and wax patterning. Corchea fits into the Fluopi fluorescence imaging system for time lapse acquisition. In this report I describe a computational pipeline to obtain fluorescence data automatically from images of paper discs with bacteria.

Key words: genetic circuits, microfluidics, Corchea, fluorescence, pipeline



1. Introduction

The ability to perform computation in a living cell will revolutionize biotechnology by improving existing products and enabling new applications (Brophy & Voigt, 2014). Genetic circuits characterization is key to understand transcription and translation processes as well as metabolic and environment interactions. Microfluidic systems has been used to maintain cells under fine controlled environmental conditions to study dynamic genetic circuits like oscillators (Potvin-Trottier, Lord, Vinnicombe, & Paulsson, 2016). The access to microfluidics devices is restricted due to its high cost and the need of dedicated infrastructure (Akyazi, Basabe-Desmonts, & Benito-Lopez, 2018). Paper-based microfluidic devices emerge as a way to overcome these limitations with another set of features (Xia, Si, & Li, 2016). I developed Corchea an Open-Source Low-Cost Paper-Based microfluidic device to culture and communicate bacteria (DOI: 10.13140/RG.2.2.18038.14407). This device use capillarity and negative pressure to generate a constant flow of media through paper. The paper can be patterned with wax to create hydrophobic barriers that constrain liquid flow. I use this feature to create 5 parallel channels of media that to have replicates of the discs on these channels in the same device. Corchea makes accessible features of microfluidic devices like laminar flow and change on the composition of media. Corchea fits into Fluopi to timelapse multi-fluorescent imaging (Nuñez et al., 2017). To obtain fluorescence data from images you usually select the area of interest like cells or paper discs by hand using circular, elliptic or rectangular shapes. The ideal is to automate the process and remove the human from the selection of the area of interest. Human selection is biased and with an algorithm you can increase the number of measurements without an increase of human work. In this report I describe a computational pipeline to obtain fluorescence data automatically from images of paper discs with bacteria.

2. Methodology or experimentation

Cell strains

Two *Escherichia coli* TOP10 where used in this study transformed with plasmids that confers constitutive expression of RFP (cRFP: J23101+B0034+mBeRFP+B0015) and transformed with plasmids that confers homoserine lactone C6 induced expression of GFP (1LU2:



J23101+B0034+C0062+ECK9600, pLux34G: pLux76+B0034+sfGFP+ECK0818). Both described in (Nuñez et al., 2017).

Paper discs culture

A set of 5mm paper discs where placed over solid Luria-Bertani (LB) media with corresponding antibiotic resistance. 5uL of overnight bacterial culture were pipetted on the discs and the petri dish where placed in an incubator at 37 °C for 18 hours.

Corchea setup

Corchea device where ensembled and the source were filled with liquid LB using the corresponding antibiotics. Paper discs with bacteria where placed over Corchea hydrophilic channels. Corchea where placed in Fluopi inside an incubator at 37 °C to acquire fluorescence images every 5 minutes for 1000 time steps or until the experiment where finished.

Induction pulse

Corchea where removed from the incubator and placed into a flow hood, the source where removed and replaced by one with LB with 10^{-7} M HSL C6. After this process Corchea where placed again in the Fluopi inside the incubator at 37 °C for 40 timesteps. After this time Corchea where removed from the incubator and placed into a flow hood, the source where removed and replaced by one with LB. After this process Corchea where placed again in the Fluopi inside the incubator at 37 °C for the rest of the experiment.

3. Results and discussion

Data acquisition is crucial for the usability of a new device. In Corchea I aim to detect the discs with bacteria, calculate its area and extract fluorescence only in this part. Different approaches can be used to segment the disc like circle detection, ellipse detection, convolutional neural network and edge based segmentation. Edge based segmentation was the algorithm with the best results in segmentation quality, detection of all paper discs and computation time.

The code use os and glob packages to import all the images from a selected folder and sort them to obtain ordered files. A for loop makes a 4 dimensional array of the images, dimension 1 is the number of the image, dimension 2 is the height, dimension 3 is the width and dimension 4 is the



RGB channel, this finish the import of data. To create a mask to analyze discs you need to give to the code 2 inputs: the mask channel, it could be the green or red channel and the mask time, the timestep at which you will create the mask. The code plot the selected image (Figure 1a) to make the mask to show the user the image that it will be using in this process. With this masking image you run the segmentation algorithm, at the end it will plot the segmented masks (Figure 1b). This algorithm is robust and usually you don't need to change anything, but if the don't find the mask that he want, he can modify markers to obtain it. Background is calculated using a circle that the user can modify the number, position and radius, here we use 1 circle of 50 pixels radius to calculate background, and the code plot an image of the background to see if it is correct (Figure 1c). Now that all masks needed were created an empty pandas DataFrame is created with the neat format. A nested for loop iterates over timestep and disc mask to extract the average fluorescence of both channels subtracting the average background. For example to calculate the average red fluorescence of disk 1, the mask of disc 1 and the background circle are multiplied by the red channel image at timestep t and divided by their corresponding area. The result of the background is subtracted from the result of the disc to obtain the average fluorescence of disc 1 in time t, 1. A new row is created with the "Time": t, "Sample": disc 1, "Value": average fluorescence of disc 1 in time t, "Name": "RFP"(because is the red channel). This row is appended to the previously created DataFrame and at the end show you the DataFrame. The code incorporate a seaborn plot to see your data (Figure 1d) and gives you the option to save your data as CSV file to store or share it.

To obtain the fluorescence of constitutive expression (Figure 2a-d) you only need to compute masks one time and the multiply the masks with the image in the corresponding channel. In experiments of induction pulse (Figure e-h), Corchea is moved from his position to load LB with inducer, plugged to acquire images and then moved again to change the media to LB without inducer and plugged again. Everytime Corchea is moved you need to compute masks again or you will obtain information from places without discs. The algorithm is robust enough to allow the user plug and unplug Corchea and keep the experiment going without loss on data quality (Figure 3). A jupyter notebook with the python code is available at <https://github.com/SynBioUC/Corchea>



4. Conclusions

The use of this pipeline makes easy the data analysis from images of Corchea. This feature can encourage the usage of this device for other research groups improving reproducibility and shareability. Open-Source devices should be user friendly to spread the usage of these devices. If more academic labs, city labs, lab spaces and other groups use your device will give you a nice feedback and even improve your designs. In order to work as a community the reproducibility and team work should be our aim. This pipeline makes possible to the user to remove Corchea, change some conditions and plug it again to keep the experiment course which is a nice feature that characterization tool usually lacks.

Acknowledgments

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Glossary

Pipeline: design or execute (a computer or instruction) using the technique of pipelining.

Microfluidics: Study of the behaviour, precise control, and manipulation of fluids that are geometrically constrained to a small scale (typically sub-millimeter) at which capillary penetration governs mass transport.

Open-Source: Software for which the original source code is made freely available and may be redistributed and modified.

Fluorescence: Is the emission of light by a substance that has absorbed light or other electromagnetic radiation.



References

- Akyazi, T., Basabe-Desmonts, L., & Benito-Lopez, F. (2018). Review on microfluidic paper-based analytical devices towards commercialisation. *Analytica Chimica Acta*, 1001, 1–17. <https://doi.org/10.1016/j.aca.2017.11.010>
- Brophy, J. A. N., & Voigt, C. A. (2014). Principles of genetic circuit design. *Nature Methods*. <https://doi.org/10.1038/nmeth.2926>
- Kim, J., Yin, P., & Green, A. A. (2018). Ribocomputing: Cellular Logic Computation Using RNA Devices. *Biochemistry*, 57(6), 883–885. <https://doi.org/10.1021/acs.biochem.7b01072>
- Nuñez, I., Matute, T., Herrera, R., Keymer, J., Marzullo, T., Rudge, T., & Federici, F. (2017). Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering. *PLoS ONE*, 12(11), 1–21. <https://doi.org/10.1371/journal.pone.0187163>
- Potvin-Trottier, L., Lord, N. D., Vinnicombe, G., & Paulsson, J. (2016). Synchronous long-term oscillations in a synthetic gene circuit. *Nature*, 538(7626). <https://doi.org/10.1038/nature19841>
- Xia, Y., Si, J., & Li, Z. (2016). Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review. *Biosensors and Bioelectronics*. <https://doi.org/10.1016/j.bios.2015.10.032>

Tables and Figures

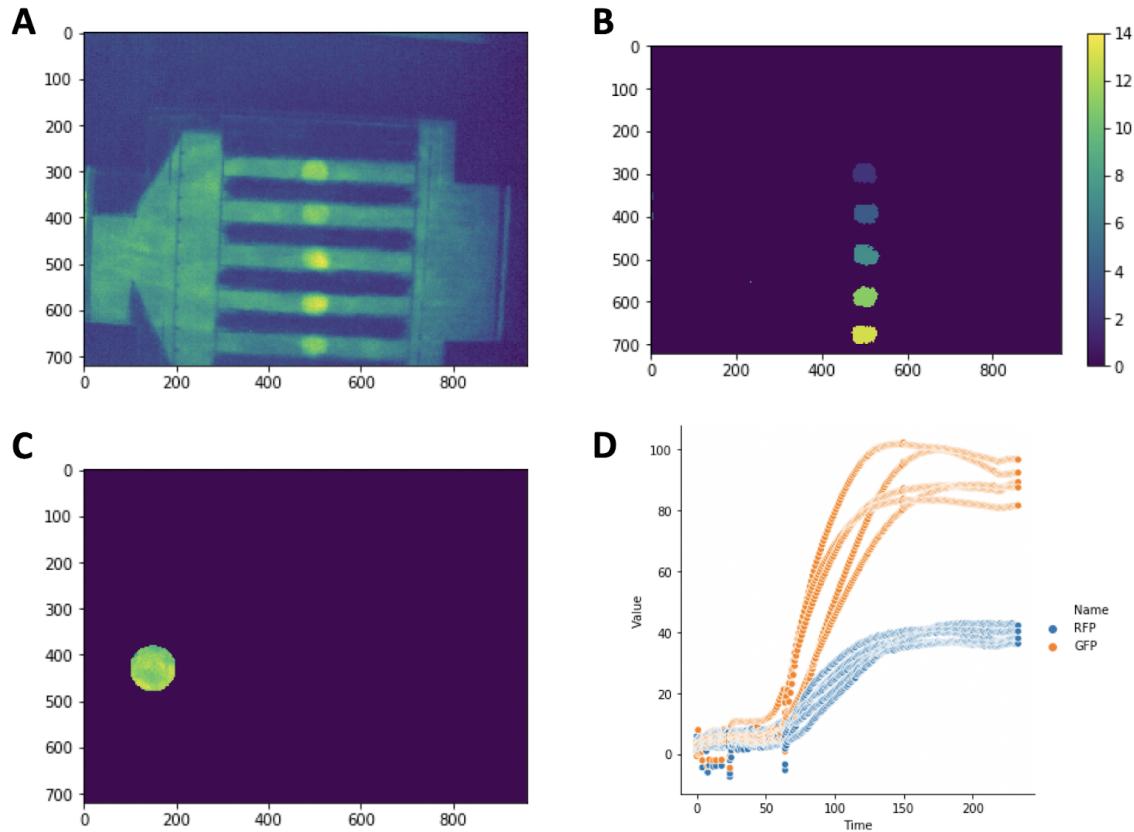


Figure 1. Data acquisition from image timelapse pipeline. a, Green channel of captured image. b, Masks made using edge based segmentation. c, Background measurement on a selected circle. d, Plot of the data obtained using this automated pipeline.



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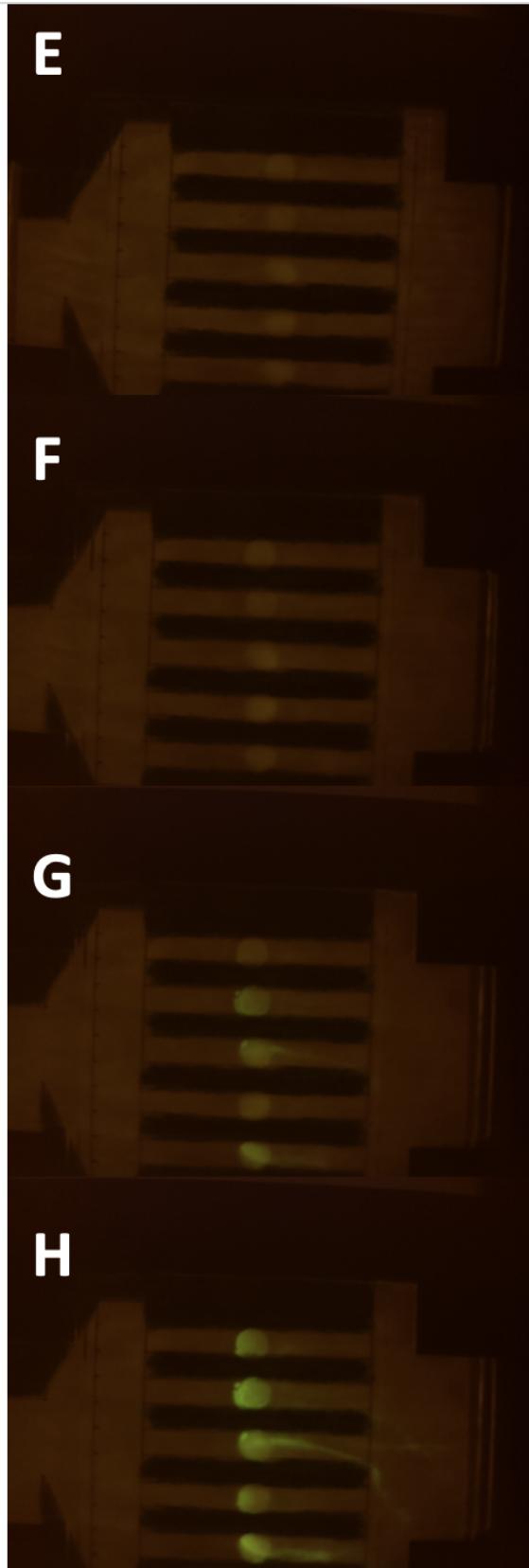
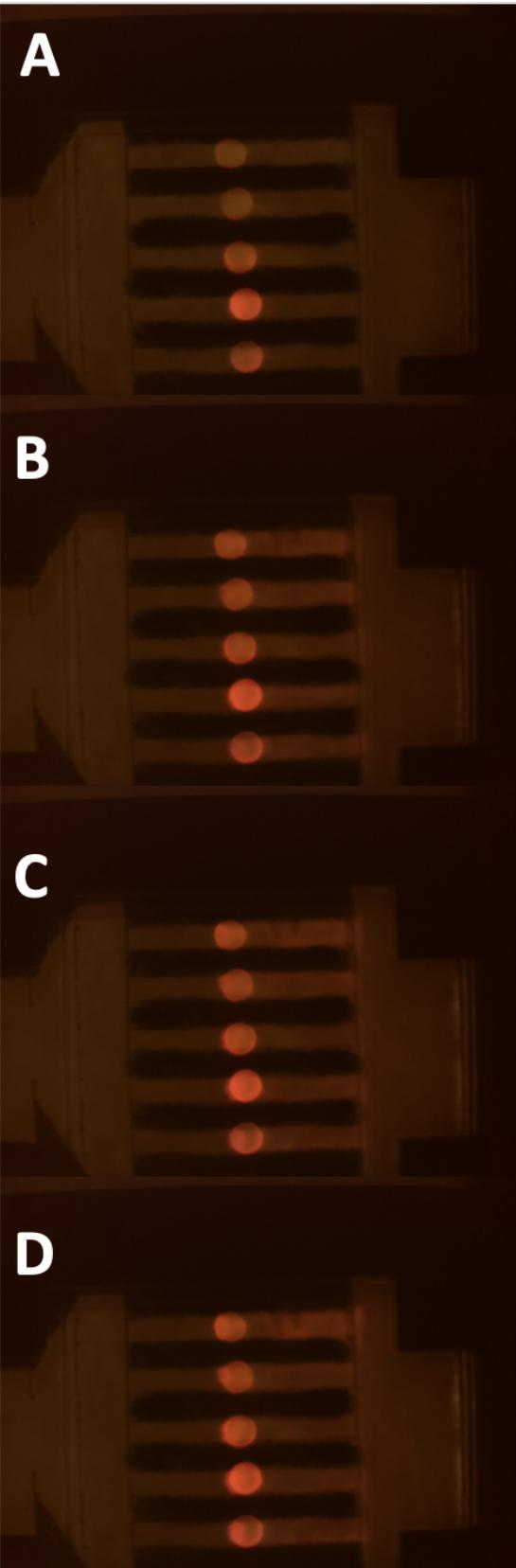




Figure 2. Raw images input of the pipeline. Images of mBeRFP constitutive expression obtained at: a, timestep 5. b, timestep 50. c, timestep 75. d, timestep 100. Images of sfGFP induced expression at: e, timestep 5. f, timestep 50. g, timestep 75. h, timestep 100. Inducer pulse from timestep 26 until 66.

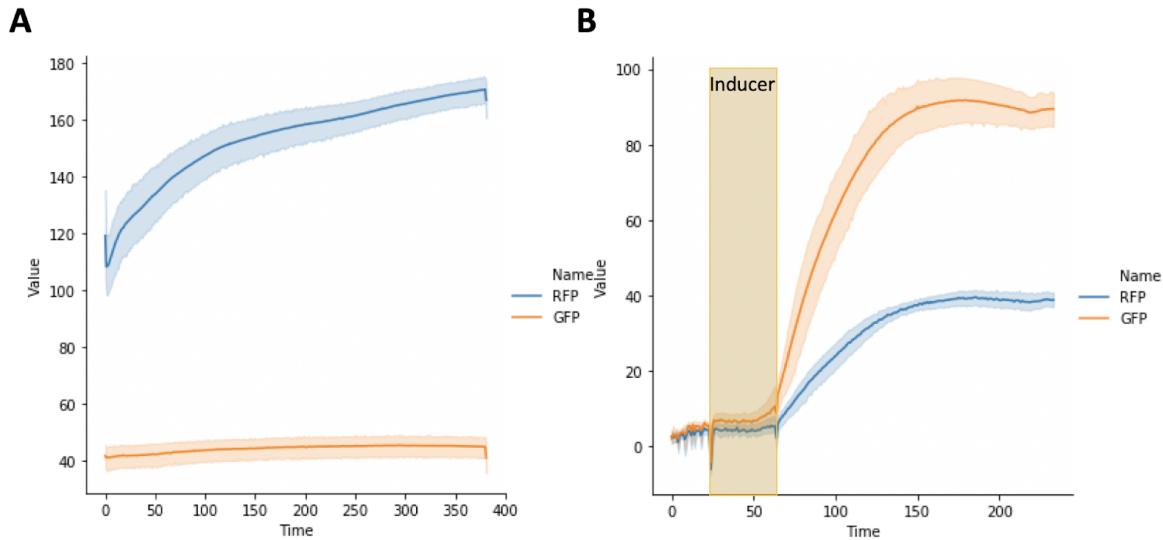


Figure 3. Final data plot. a, Plot of data acquired with the pipeline of images from constitutive expression, Value is the calculated average fluorescence in arbitrary units and Time are timesteps of 5 minutes. Data is from all the discs in 3 Corcheas. b, Plot of data acquired with the pipeline from induced expression of sfGFP, Value is the calculated average fluorescence in arbitrary units and Time are timesteps of 5 minutes. In yellow the window of time of the pulse of LB with HSL C6 at 10^{-7} Molar from timestep 26 until timestep 66. Data is from all discs of 1 Corchea.