Supplementary File 2

eVOLVER design, hardware and software

Contents

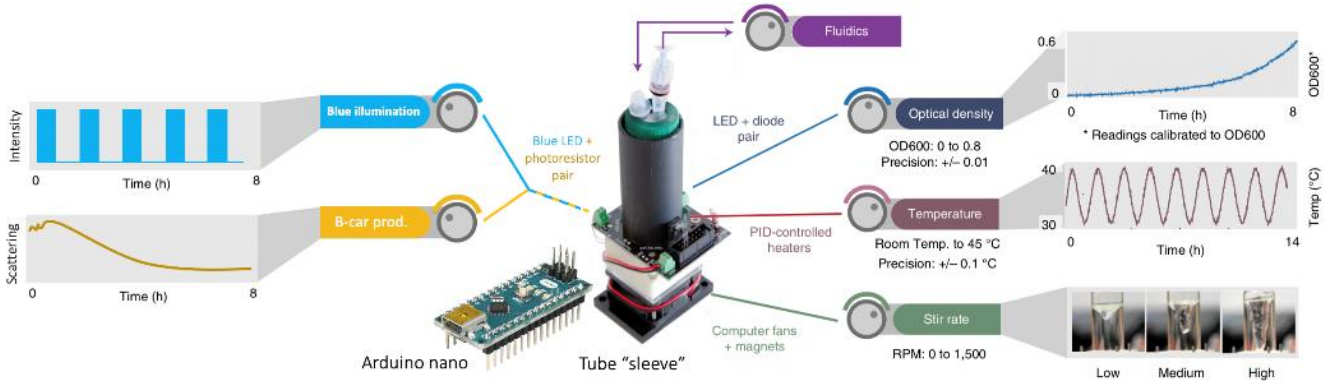

Figure 1. Overview of the eVOLVER sleeve, adapted from Wong 2018[1].

1 Unit development

New platform structure. Instead of sleeves connected to a large motherboard with complex connections, we aimed to develop a simpler (*i.e.*, for someone with limited knowledge of electronics) and more modular system. Thus, each sleeve was connected to a single Arduino and all Arduinos were connected to a centralizing computer. This design may appear less efficient, less integrated and more costly in terms of electronics components; but is easier to start with, and each unit can be used independently of the whole system. Here, we refer to a "unit" as a sleeve and an Arduino nano.

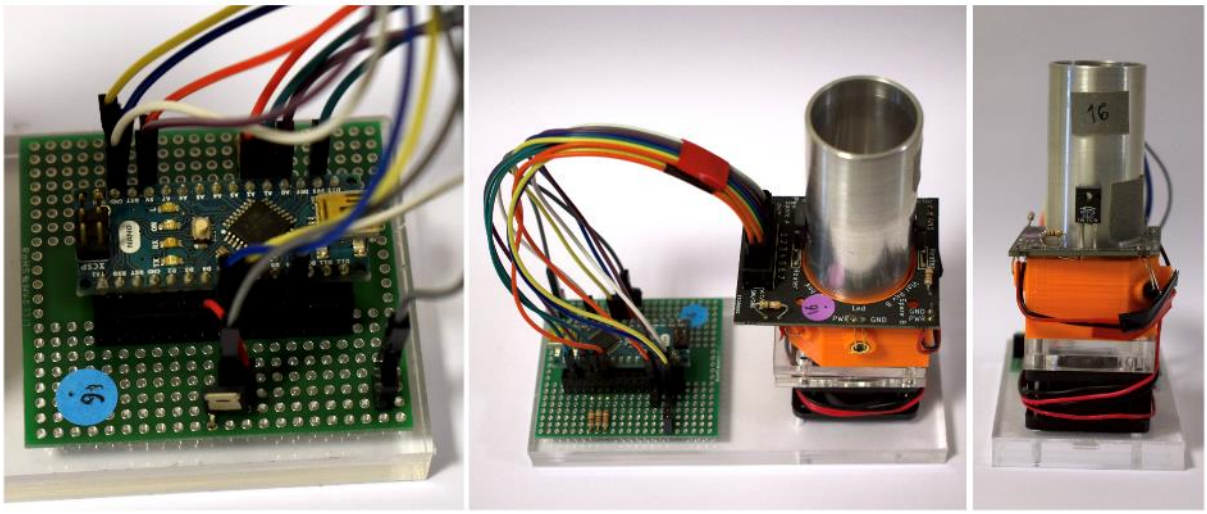

Figure 2. One constructed unit: made of one Arduino board and one sleeve. Each unit can be plugged and unplugged and controlled independently via the software through its Arduino.

With this design, one Arduino controls and monitors all of the parameters for a single sleeve. Although the user could become limited by the capacities of the Arduino (number and types of pins), an Arduino Nano proved sufficient to control one sleeve. PWM pins were allocated to components that require potential intermediate values (rather than 0 or maximum – needed for the blue LEDs and stirring), simple digital pins to the IR LED (needs to be either full on or off), and analog pins used to receive information from the photoresistor (beta-carotene production), IR photodiode (OD) and thermistor (temperature).

Optical Density measurement is performed as indicated in the original eVOLVER paper[1]. The IR LED is connected to a D5 pin and a 39Ω resistor is soldered on the hollow mount board. The IR photodiode was soldered opposite to the current, fed constantly with 3V3, and connected to the A1 analog pin and to the ground with a 1 MΩ resistor. Although the eVOLVER documentation indicates that changing this latter resistor could change/improve the OD detection range, we did not manage to improve the range any further and remained with this original design. The two components are placed at a 135° angle from each other, such that light scattering is measured with this setup.

Temperature control is achieved via two ceramic-based heaters lying against the aluminum tube. The Arduino is not able to provide enough power to generate a sufficient temperature rise, thus a 12 V power source needs to be brought in; four 12 V power-plugs are used, each feeding the heaters of four eVOLVER units. The power is carried through electric wires running along the main Arduino power source cable. The activation of the heaters is controlled in real-time based on the temperature given by the thermistor (connected to the 5 V and to the A0 analog pin and ground with a 10 KΩ resistor). This PID controller turns on and off the heater when needed. The control is carried out by the Arduino D4 pin via a mosfet transistor as shown in Fig.3.

Stirring occurs via the round magnet glued to the fan placed at the bottom of the sleeve, below the culture glass vial. Note that round magnets should be glued well and centered on the fan, otherwise the unbalance may create disturbances and difficulties in operating the fan under certain conditions. The fan is controlled by the PWM D9 pin. The PWM input values range from 0 to 255 (corresponding to outputs of 0 to 5 V, with a maximum of 40 mA per pin).

Illumination is carried out via a blue LED soldered in the available empty slot on the sleeve mount board and connected to the PWM D11 pin and protected with an 82 Ω resistor. The LED therefore illuminates the culture medium externally from the bottom *side* of the glass vial. To accommodate for this addition, the 3D printed holder was redesigned with the addition of two supplementary holes, one for the LED and one for the photodiode (see after). We take this opportunity to thank the authors of the original paper for anticipating the needs of others in their designs and purposefully leaving empty slots, which truly allowed us to adapt their design. Further improvements for illumination are presented below, with notable 2 additional blue LEDs connected to D7 and D8 (illuminating internally).

A photoresistor was also added to the original design; similarly to the side blue LED, an empty slot was available on the mount board and the holder and aluminum tube were modified accordingly. The photoresistor takes 5 V, inputs a voltage measured by the A7 analog pin and exits via a 1.2 KΩ resistor connected to the ground.

This new electronic design is presented below. Once established, 16 units were constructed.

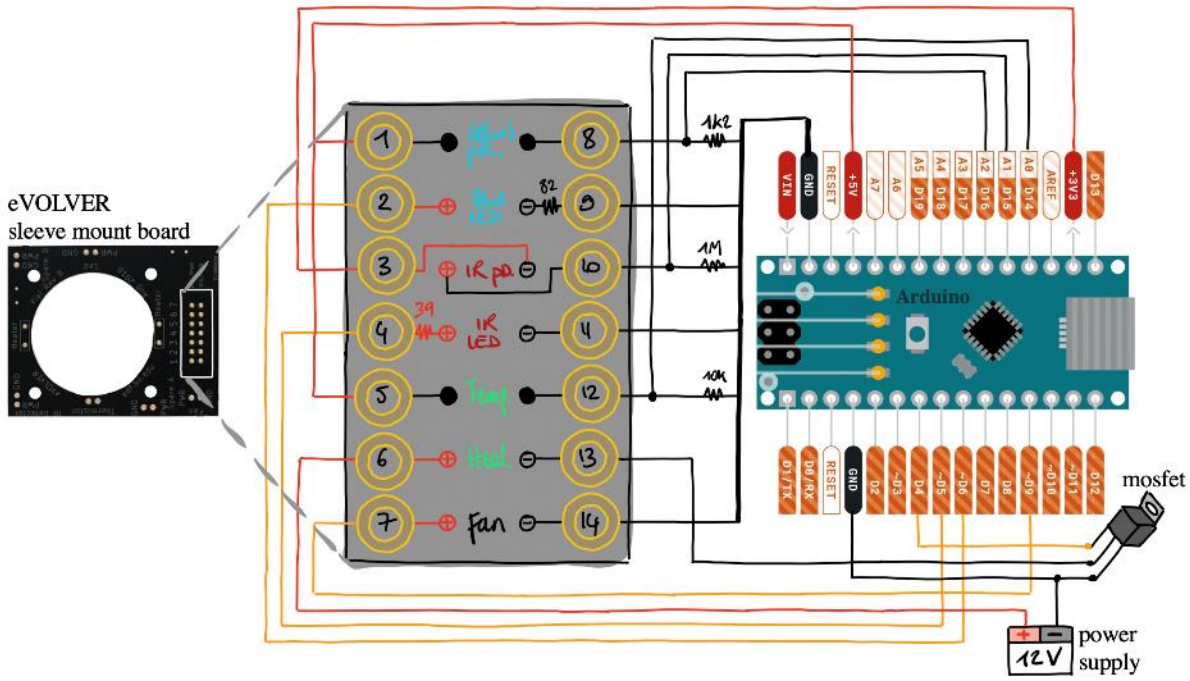

Figure 3. Electronic design to connect the eVOLVER mount printed circuit board (to which electronic components of the sleeve are connected) to the Arduino Nano.

2 Setup

Design. As explained earlier, the platform setup was simplified such that no intermediary electronic boards are needed, except for one Arduino microcontroller per sleeve, making up a single unit. As such, each unit can be directly plugged into the computer via the Arduino USB port. Two USB hubs are used to accommodate 16 units (and a 17th unit used as working prototype), which allows for data exchange with the sleeve via the Arduino and powers the Arduinos requiring 5 V. For the heaters requiring 12 V, four 12 V power supplies are connected to a hub (a simple breadboard) where each unit's two plus and minus wires can be connected and transmit power to the heaters when needed. The two USB hubs are connected to the computer and the code is run using Node-RED software, communicating with every Arduino.

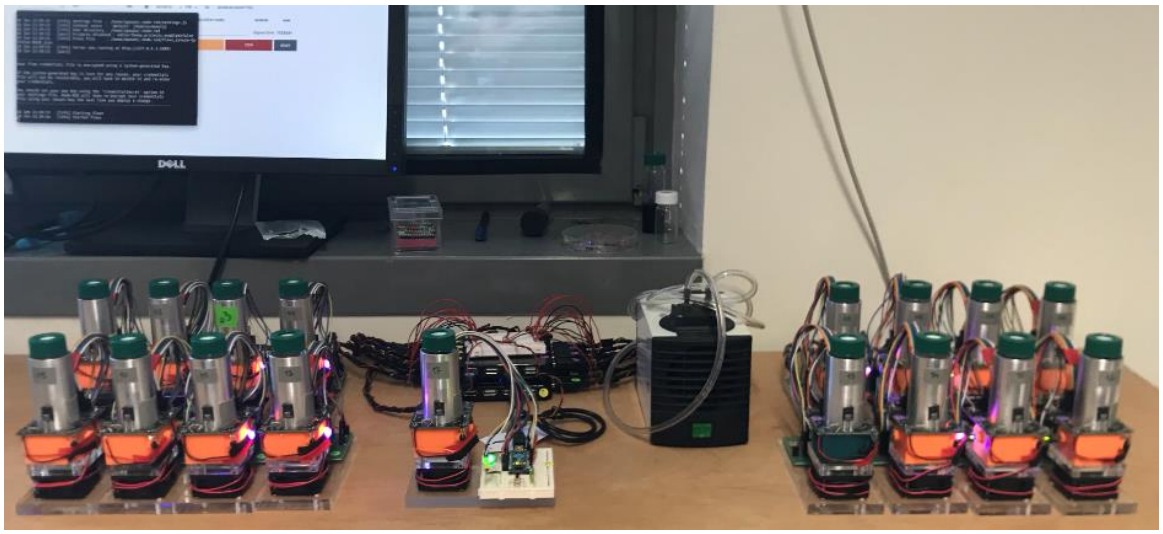

Figure 4. Image of 17 units connected to a computer, with each unit running an experiment with different culture conditions.

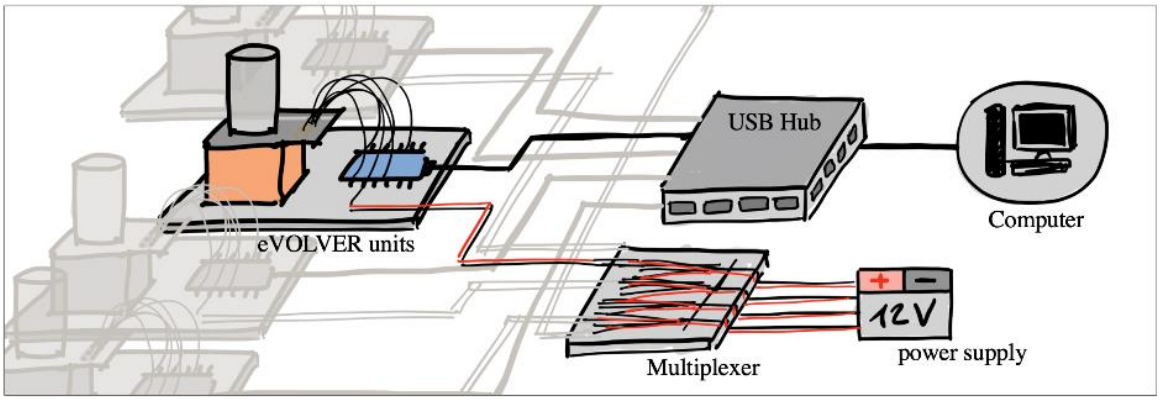

Figure 5. Connections for the platform. Each unit (sleeve + Arduino) is connected to two hubs: a USB hub and a 12 V power input (for the heaters). The USB hub is connected to a computer running Node-RED as the control software.

3 Software

Node-RED was chosen to develop the running code and user interface and to communicate with the Arduinos. This language rests upon the Node.js framework of the JavaScript language. Node-RED is a web-based, flow-based programing language. This visual programing environment makes it easy to program without writing code and to connect nodes together to obtain more and more complex flows. Node-RED can conveniently make different hardware components seamlessly interact together, as well as with diverse online services, via the use of specific nodes transmitting standardized information. Many modules (i.e., packages or plugins) containing many nodes for various applications are available; and new nodes can be easily coded to fit this environment and communicate with other parts of the code. A node can be simply considered either as a function or a device part. A node generally has a

standardized input and output that can be used over and over, in different circumstances, by being connected to other node (like assigning a value to a variable, a counter…). Information will be sent to or received from (typically, a pin of an Arduino) a device part. Although Node-RED was developed by IBM for industry-oriented purposes, it has been recently expanded to include simpler hardware.

For eVOLVER, we mostly use the basic set of nodes to run an experiment, creating loops, editing variables, writing outputs; the `Arduino` module is used to communicate with the eVOLVER units via each Arduino pins, and the `dashboard` module was used to create the user-interface. To allow Node-RED to communicate with an Arduino, the Arduino is loaded with the Firmata template code (available by default in the Arduino IDE) so it can receive instructions from Node-RED and send back information. The code developed is usable by one single unit. For 16 units, the code is duplicated 16 times. To add another unit, the code simply has to be duplicated another time, the Arduino nodes modified with the new Arduino identified, and a few other minor modifications (which can all be carried out in the raw JavaScript code for simplicity). A new unit will automatically be added in the user interface and a new experiment can be easily started. The code is separated into three parts: i. the user interface and setting up the experiment, ii. initialization, iii. the running code for the experiment.

The user interface was created to easily setup a standard experiment, while varying some parameters. OD measurement ("Measure OD") can be setup at regular intervals. Similarly, the blue light can be quickly activated at regular intervals to measure beta-carotene production ("Measure BL"). The third option ("Illuminate BL") allows the setup of a duty cycle and the period for blue illumination of the culture via the side-LED, placed at the bottom of the eVOLVER. In addition to the duty cycle and period, the intensity of the LED can be tuned. Temperature control can be turned on or off and set to a desired value (here, expressed in equivalent to voltage unit (0 to 5 V detected corresponds to 0 to 1023 u.a.), 550 u.a. corresponds to 30°C). The stirring ("fan") can also be set at intermediate values. Another option, "Growing delay", can be set to allow for a non-illumined period before activation of the illumination, i.e., the onset of the illumination pattern (either constant or pulsed). Finally, additional notes can be added and the experiment name can be edited. Additional information is displayed on the user interface (counters, measured values). Once all parameters are set, the experiment can be started and initialization begins. The experiment can be stopped and the user-interface reset at any time.

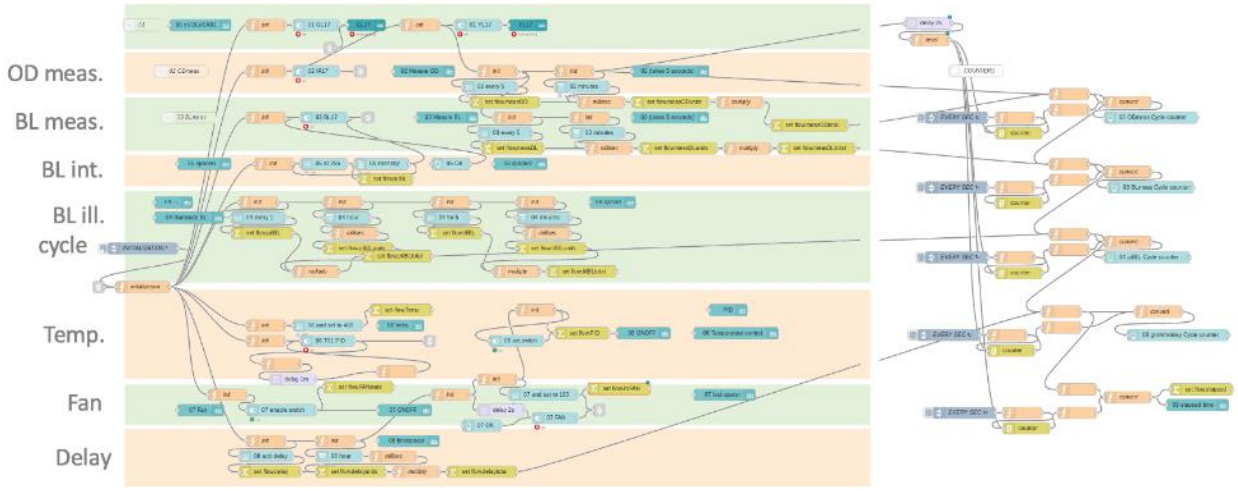

Figure 6. User interface code that allows the user to easily tune their experiment using the platform.

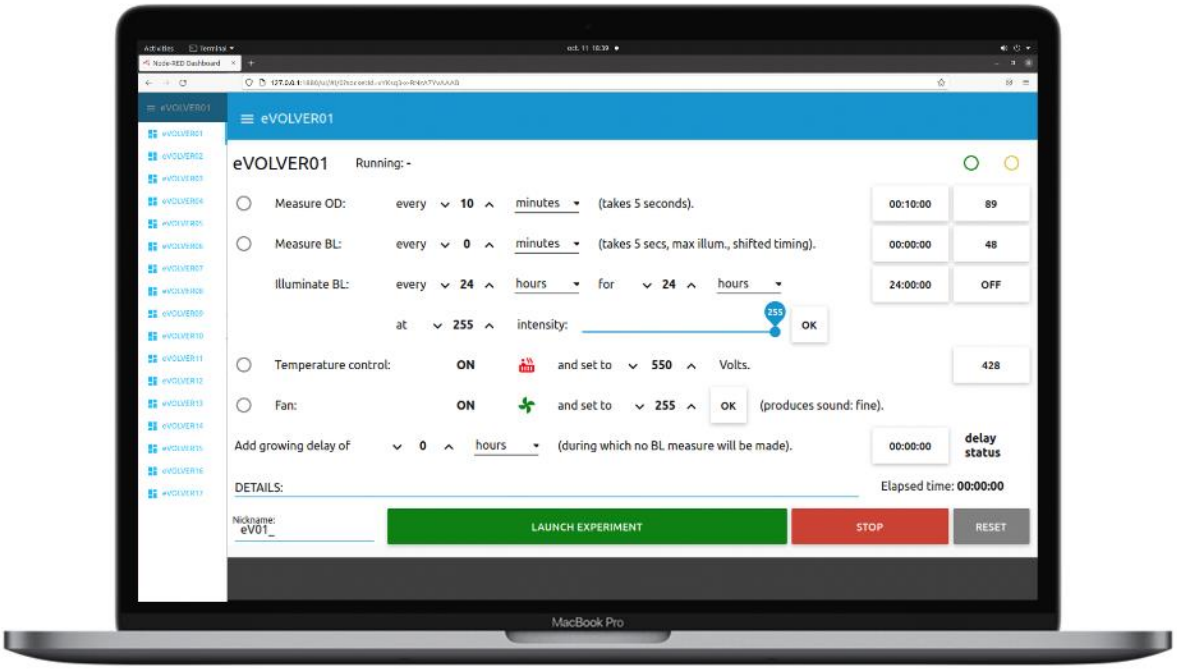

Figure 7. Overview of the user interface made using the Node-RED Dashboard plugin.

Initialization is automatically started upon launching the experiment and mainly consists of setting up the output files that will contain the experimental data. For each eVOLVER experiment launched, a folder will be created and named with the date and hour followed by the name specified in the user interface, if any. Four files are created in this folder: a readme file containing all the experimental variables set up for the experiment as a list and three .csv files containing data from the sensors: the IR photodiode (OD), the photoresistor (blue light absorbance from potential beta-

carotene), and the thermistor (temperature). In each file, the value recorded is followed by a timestamp, to prevent any time inconsistency in the data treatment. Note that, in this design, information is recorded every three seconds: if the IR or blue LED are not on, only a baseline signal is recorded, which will have to be filtered during data processing.

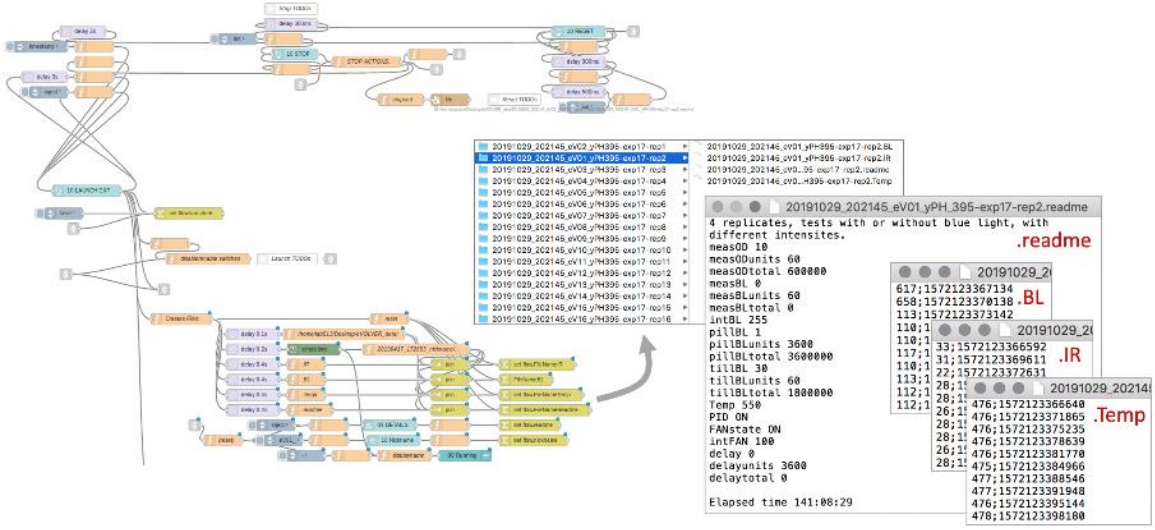

Figure 8. Initialization code that allows an experiment to be launched, stopped and reset and creates output files once an experiment is launched by the user.

The code running the experiment is launched after initialization. First, the stirring and heaters are turned on. Then, the outputs from the sensors via the Arduino are recorded in the appropriate .csv files. The OD measurements start and will restart regularly according to the chosen time. During the OD measurements, the fan is turned off, the blue LED is turned off, and the IR LED turns on for 5 seconds, during which a datapoint is recorded. After these 5 seconds, the IR LED turns off, the fan turns back on, and the blue LED recovers its previous state: ON if it was ON, OFF if it was OFF. Meanwhile, a counter is started if a growth delay was set. If no delay was set, or when time is over, the blue light measurements start as measure cycles identical to the OD measurements. In addition, the chosen pattern of blue light illumination will also start (can be a cycle if the illumination is pulsed, otherwise it is constant illumination).

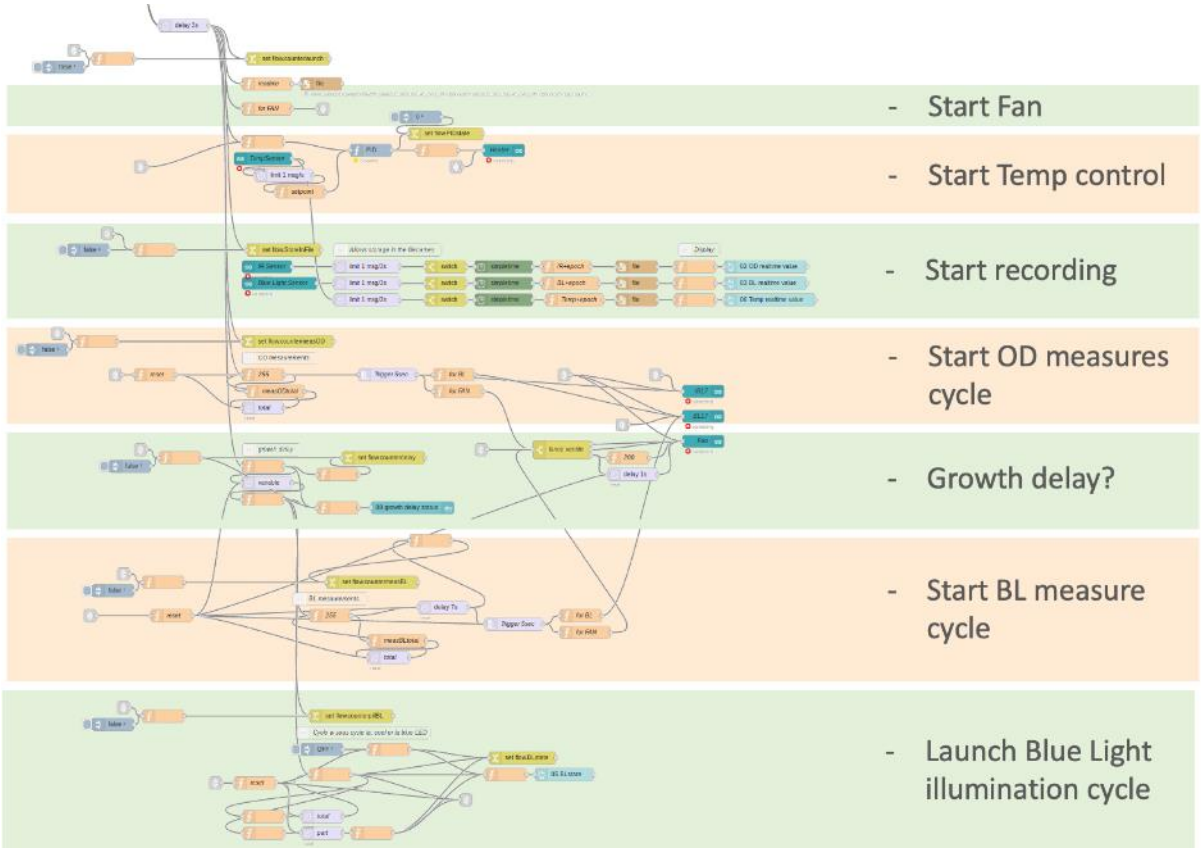

Figure 9. Code running the experiment.

All code is available at **https://github.com/spouze/opto_eVOLVER**

4 Type experiment

For an **experiment**, the culture medium is prepared beforehand and glass vials with lids and rotating magnets are autoclaved and cooled. Precultures are set overnight before starting the experiment; these cultures can be diluted in the morning to obtain an exponentially growing culture at the beginning of the experiment and skip as much of the lag phase as possible. An appropriate amount of culture volume is inoculated to obtain a OD_{600} of 0.05 and is placed in eVOLVER glass vials. Lids are only slightly screwed onto the vials to allow gas exchange to occur. Then, the glass culture vials are placed in the eVOLVER unit sleeves.

A summary of the growth of each culture can be drawn with the three output files. First, one can verify that the **temperature** remained constant at 30 °C (i.e., 550 u.a.) during the entire culture.

Growth is detected by the IR LED and photodiode and plotted here in blue. As explained before, OD is only measured at set intervals, but the signal is read every three seconds, such that a baseline at about 150 u.a. (which corresponds to the photodiode signal without any IR light) can be seen in the

raw data. The measured value only has meaning when the IR light is turned on. The growth pattern is as expected: an exponential phase followed by a stationary phase. However, one should not be misled by the familiar pattern here. The plateau reached here is a technical plateau, as it is the result of the detection range of the coupled IR LED and photodiode. We know that the OD can continue to increase, because the OD signal subsequently decreases. Indeed, the increase in the OD is detected by scattering of more and more IR light to the photodiode as cell number increases, such that the detected value increases as well. Once the detection threshold is reached, the output value remains constant for a few hours. However, at some point, the OD reaches a value where absorption of the IR light takes over the scattering, such that the signal decreases. Therefore, under our conditions, only a small portion of the growth detected can be used to attempt to compute growth rates.

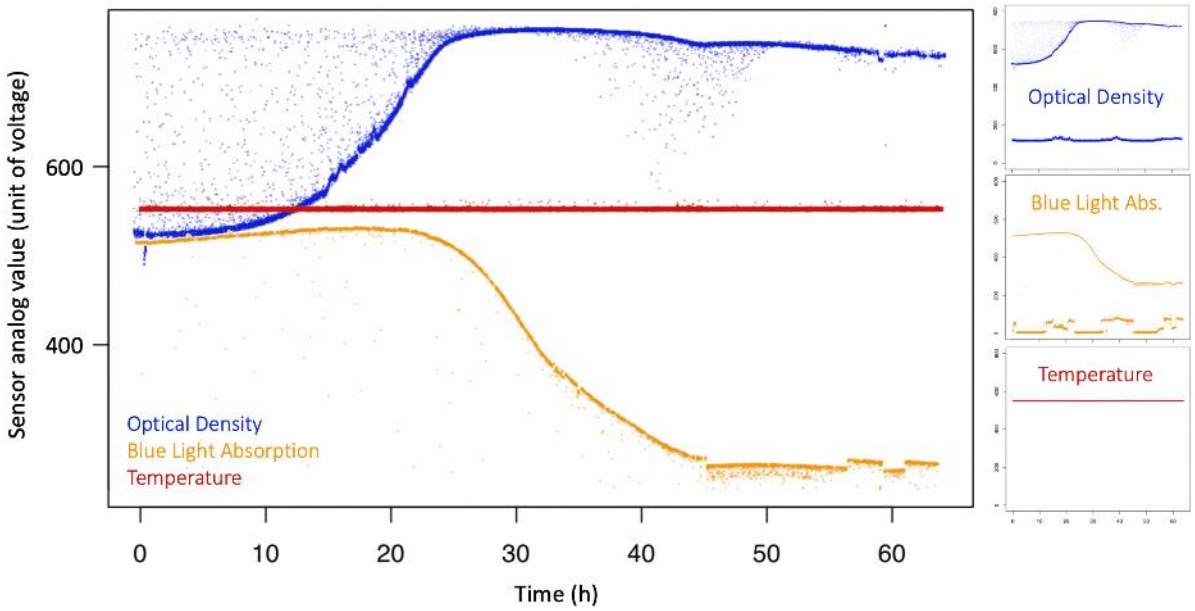

Figure 10. Type experiment (main figure). Superimposition of the Arduino outputs: optical density (growth), blue light absorption (beta-carotene production) and temperature. (right). Raw unfiltered data displaying baseline values.

Beta-carotene detection follows a similar detection pattern but with a different sensitivity. Detection of blue light by the photoresistor is less sensitive to variations in scattering but more sensitive to absorbance. Indeed, during growth, the blue light absorbance value increases only slightly, meaning detection is already close to its maximum value with few cells in the medium – this can be due to the intensity of the light, the wavelength, and/or the sensor. Absorbance only takes over scattering at a certain point, and the photoresistor value decreases accordingly. Even though we used a beta-carotene-producer strain, this is also the case, though to a lesser extent, with a non-producer strain (see Fig.11). In addition, similarly to the OD detection, a baseline is constantly recorded for beta-carotene detection (Fig.10– right middle): we can see strong variations in the baseline (that also appear in the main figure), which correspond to fluctuations in the ambient light in the room where the experiment was carried

out, which impacts light detection in the photoresistor. While the IR photodiode is specialized for IR detection, the photoresistor is more sensitive to a broad range of wavelengths.

Below, we show an example of two different strains grown successively in the same unit. CEN.PK2-1C is a WT strain and yPH_554 is the constitutive beta-carotene producer.

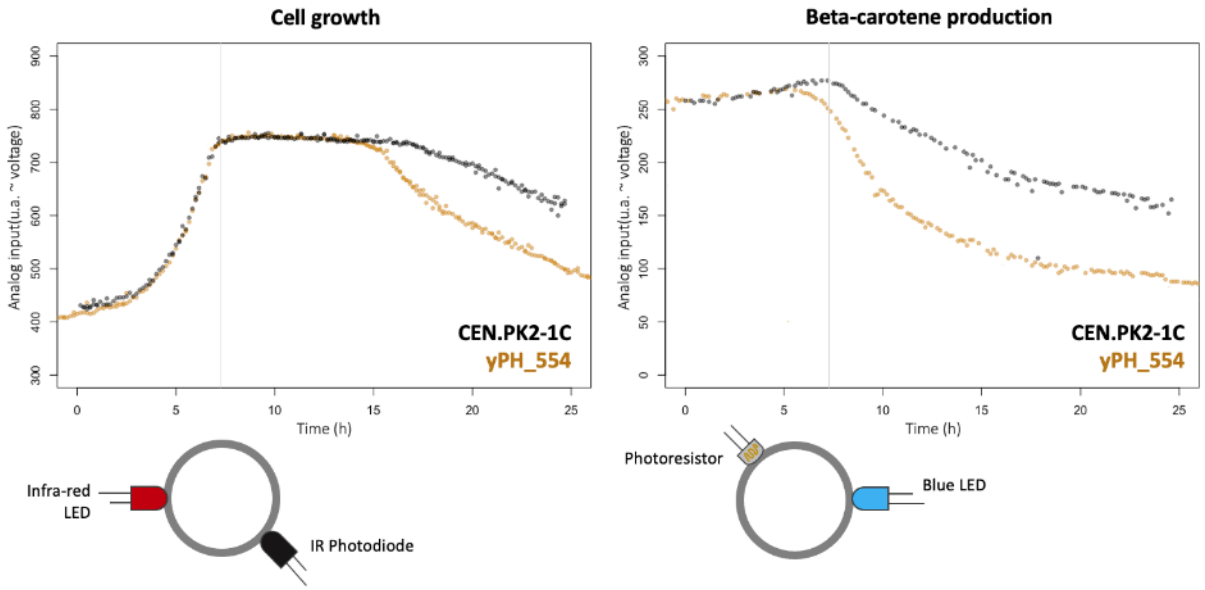

Figure 11. Comparison of eVOLVER outputs. (Left) Cell growth can be measured using the IR LED and photodiode pair. The angle between the two electronic components allows quantification of light, first via light scattering (from 0 to ~7 h, the higher the number of cells, the greater the light scattering and the higher the signal), then absorbance takes over, reducing the signal. (Right) Beta-carotene production can be observed in the eVOLVER using the side blue-LED used to activate the optogenetic system. Here, using the photoresistor, mostly absorbance is considered, and a reduction in the light received leads to a lower signal. With the beta-carotene producer strain, as beta-carotene specifically absorbs blue light, more light is absorbed, leading to an even lower signal. The two strains were tested in the same eVOLVER unit, at different times. In the scheme, the central hole represents the glass vial as viewed from above.

5 Calibrations

Beyond illumination control and automated cultures, one motivation to build eVOLVER was to measure the growth rate in an experiment, and possibly, in real time.

We first assessed reproducibility: we ran the same experiment in all units several times. We found that reproducibility appeared good between experiment in the same eVOLVER unit, but variations between units remained: the detection range for growth measurement varies from unit to unit, likely due to the position (distance, angle of the component) of the IR photoresistor relative to the IR LED, both soldered and placed by hand in the 3D-printed holder. These results did not appear precise enough

to determine a growth rate over the whole experiment, not to mention determining it in real-time during the experiment. It appears that the resolution of the measured growth rate may not be high enough to detect, for instance, a 10% drop in fitness (going from 90 min to 100 min for yeast). Moreover, these inconsistencies remained in repeated experiments and with several trials, we did not manage to obtain sufficiently reproducible results, in contrast to the original eVOLVER paper[1].

One could question the fact that this may be a biological issue, that some units allow for better growth than others, but the low intra-unit repeatability and our various trials to calibrate the units showed that this is likely not the case. We think this failure is more likely to be due to the hand-made manner used to build the platform, which created variability in the measurements, and the fact the components could slightly move over time because they are not completely glued to the holder. However, there is a trade-off between modularity, repairability and the stability of the system; and this trade-off was not resolved here. After many trials, we decided to set this issue aside, using growth curves as an indicator of proper growth.

Other parameters. For beta-carotene measurements, similar problems were to be expected and no calibration was performed; beta-carotene production was quantified using another protocol at the end of the experiments (see paper Methods). The temperature detected by the thermistor was found to remain consistent across eVOLVER units (and thermistors are known not to vary much between components); thus, temperature was not calibrated further. Stirring was also not specifically calibrated. For each experiment, all replicates were performed in different units in the same run, such that these parameters are averaged in any case.

All in all, we used eVOLVER in batches, as it allows us to manipulate the volumes during an experiment and for the controllability over the stirring rate and, especially, over the illumination. Moreover, its throughput and ease of programing makes eVOLVER ideal to test many culture parameters.

6 Illumination

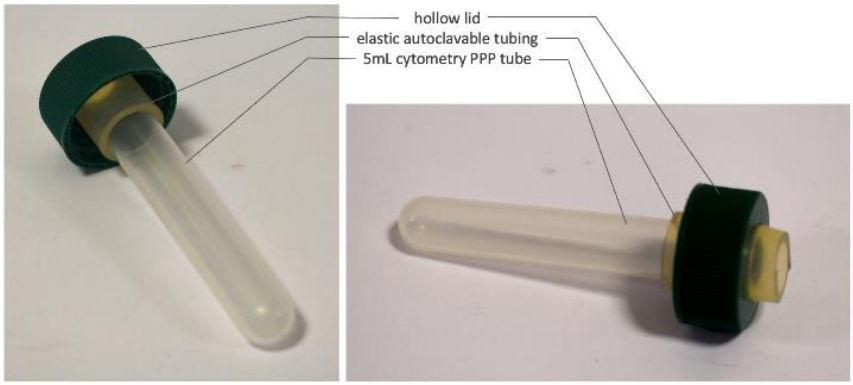

Figure 12. Custom lid for internal culture illumination.

Improving illumination came out important for optogenetics (see paper). Although the original design of the eVOLVER sleeve contains an empty slot (which the paper actually suggested for optogenetics), illumination from the side of the sleeve with a resistor limiting the intensity was shown to be limiting in terms of activation (see paper). Since the eVOLVER is surrounded by an aluminum tube and providing more illumination from the outside would require an entire redesign and reassembly, we sought to illuminate the cultures from the inside. One possibility was to take advantage of the hollow lid to place LEDs inside the culture. To prevent contact with the medium and protect the LED from getting wet, different systems were tested using basic lab equipment. The requirements were simple: place the LED sufficiently inside the culture, protect it with autoclavable material, and make sure that the junctions keep everything airtight and preserve sterility. After a few tests, we settled with a customized lid in which a cytometry tube (5 mL Polypropylene Round-Bottom Tubes from BD Falcon REF 352063) is placed inside the hole of the lid via an autoclavable, cut piece of tubing that is just large enough to keep the junction airtight. Therefore, the new lid can be autoclaved, just like any other type of lab equipment.

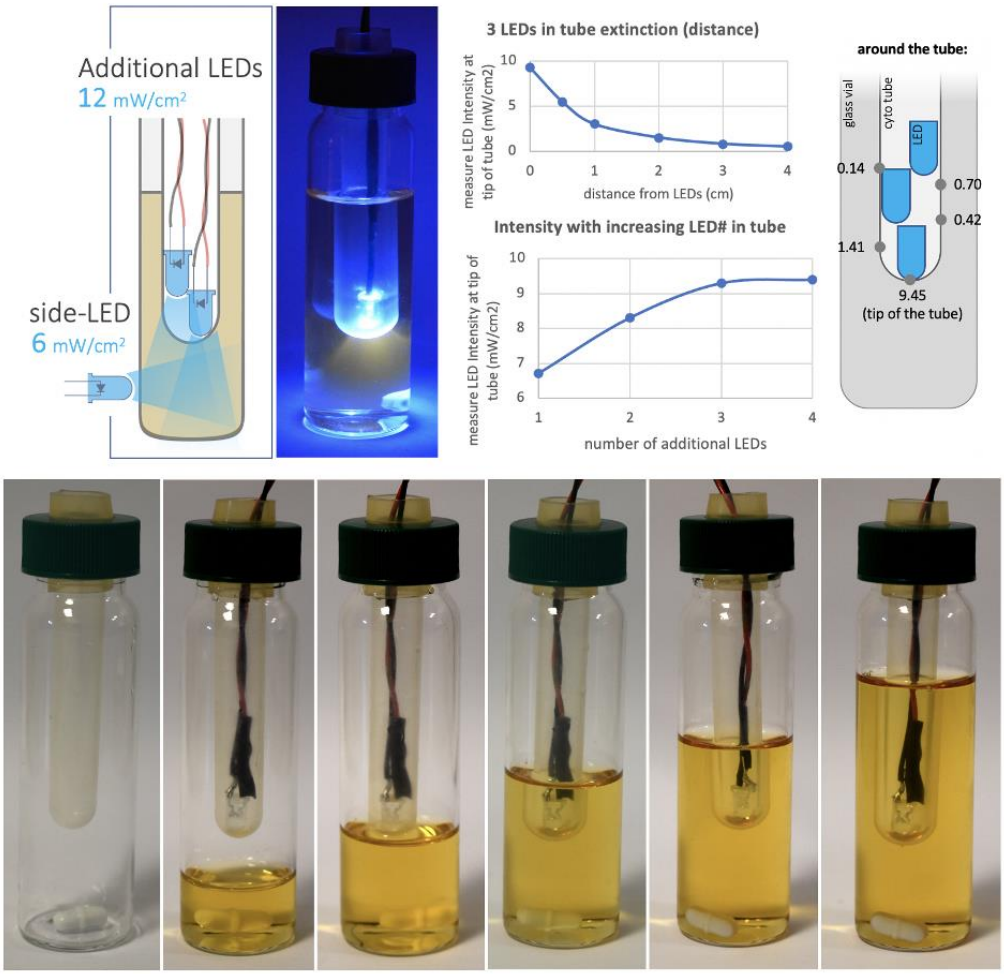

Figure 13. (Top) From left to right: illumination map in the eVOLVER sleeve, 25 mL culture with the LED turned on, measured LED intensities using a power meter, and scheme of intensities measured around the tube containing the LEDs. (Bottom) From left to right: 5, 10, 15, 20 and 25 mL of culture medium.

Technicalities. This illumination arrangement has also certain constraints, such that it mostly illuminates the culture below the additional LEDs, given how the LED is positioned, i.e., only a certain portion of the volume. As such, the volume of the culture has an impact on the cell-to-cell illumination/per-cell amount of light. We used only "naked" LEDs (with no extra piece of plastic or other equipment) to improve the dissipation of light (*i.e.*, no light deflectors, or kinds of lampshades that could help to homogenize the illumination). We also measured the light intensity that was received through the tube and on the sides to confirm that the light beams were mostly oriented downwards. We also measured the light intensity emitted with one to four additional LEDs placed in the tube. While each additional LED can independently emit 12 mW/cm^2, we found that the maximum (measured through the plastic of the tube) was reached with three additional LEDs at 9.45 mW/cm^2.

CITED LITERATURE

1. Wong BG, Mancuso CP, Kiriakov S, Bashor CJ, Khalil AS. Precise , automated control of conditions for high- throughput growth of yeast and bacteria with eVOLVER. *Nat Publ Gr*. 2018;36(April). doi:10.1038/nbt.4151