Supplementary Information

Hsinkai Wang[1] and Ya-Tang Yang[1*]

[1]*Department of Electrical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan, R.O.C.*

This article provides supplementary information to the main text of the manuscript for the details of the experimental setup and additional data.

[*] Correspondence and requests for materials should be addressed to Y. T. Y. (email: ytyang@ee.nthu.edu.tw).

1. Strain preparation and culture

The light sensing *E. coli* has been prepared by transforming competent cell with the plasmid from Addgene pSR43.6r (addgene plasmid number 63197) and psR58.6r (addgene plasmid no 63176). For the experiment with different organic substrates, we have used M9 medium supplemented with different organic substrates. The substrates and their concentration used in this study were glucose (0.20% w/v), sodium pyruvate (0.24% w/v), sodium succinate (0.45% w/v), and sodium acetate (0.27% w/v). All these chemicals were obtained from Sigma Alrich. For the evolutiton experiment under serial dilution transfer, we have used M9 medium supplemented with vitamin B6 (2 μM) and glucose(0.4%,).

2. Calibration of light intensity for the light illumination

Light intensity is controlled via a pulse width–modulated (PWM) signal generated by Arduino control board, which controls red and green LEDs for activation and deactivation of optogenetic circuit. The PWM signals have an 8-bit resolution (256 levels) in duty cycle setting and a full PWM cycle frequency of 980 Hz. Linear relation between the duty cycle and average intensity is found. In our experiment, we fix duty cycle to be 61 % to obtain 0.55 W/m^2 for the data display in Figure 1d and 1e and Figure 2.

3. Calibration of detector for optical density and fluorescence measurement

For calibration of detectors, we dilute an overnight bacterial culture with various dilution ratios to prepare a series of sample in minimal growth medium. The optical density and fluorescence level of each sample is separately measured in the bioreactor and plate reader (Synergy H1hybrid, Biotek). A set of representative calibration curves for optical density and fluorescence are displayed in Figure S1.

4. Cross check with culture under vigorous aeration

To check whether the growth in our bioreactor is in its optimal condition, we grew wild type MG1655 cells in Luria-Bertani(LB) medium and M9 medium with 0.4% glucose at 37 °C. The result of this experiment is shown in supplementary Figure S9. In both medium, the final OD reaches ~0.5 while growth in LB medium shows higher growth rate in the exponential phase. To compare the growth in the photo bioreactor to the growth in the flask, we grew cells of ~50 ml in flask under vigorous aeration at 180 rpm with M9 medium supplemented with 0.4% glucose and the measured end-point optical density at 20 hr reaches ~1.5. Cells in the bioreactor reach lower optical density than in the flask, perhaps due to incomplete oxygenation as a result of the weak magnetic stirring in the culture vessel.

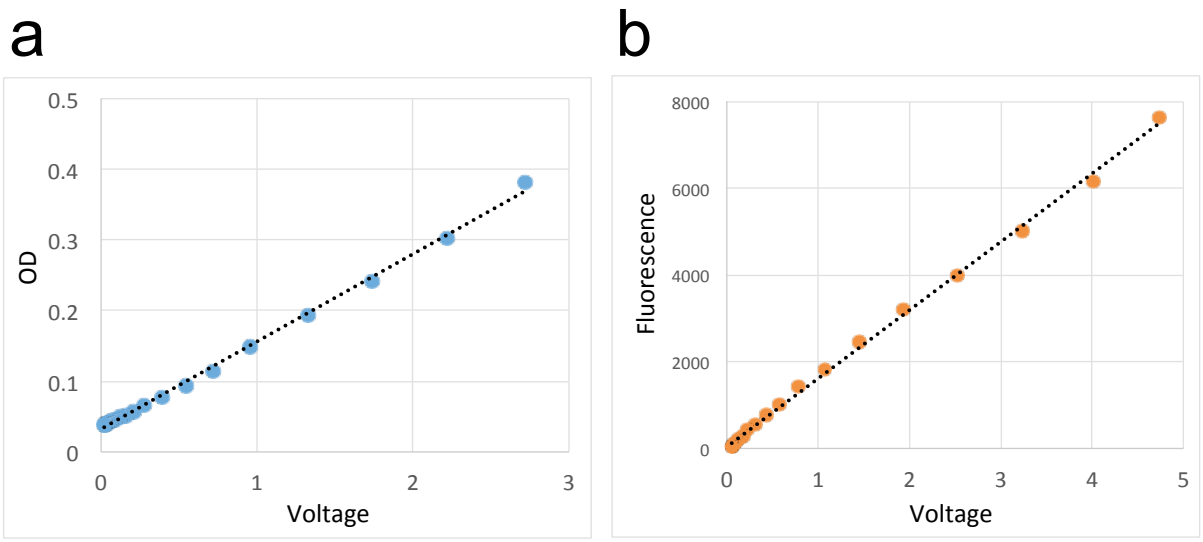

Figure S1. Calibration of the detectors. (a) The voltages (gray circles) created by cultures with known OD values are recorded. A line (black line) is fitted for finding the calibration factor. (b) The voltages (gray circles) created by cultures with known fluorescent intensity are recorded. A line (black line) is fitted for finding the calibration factor.

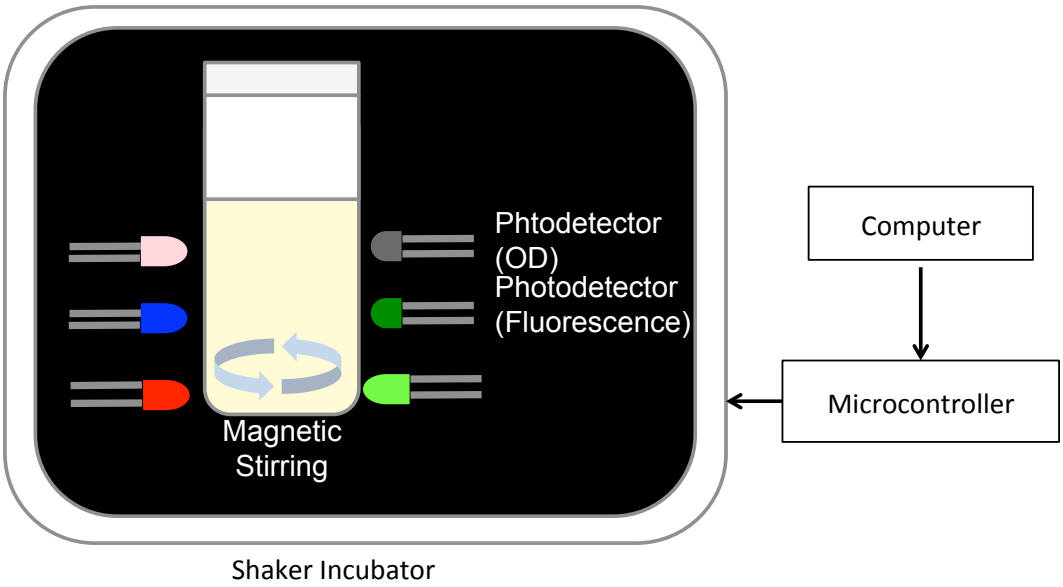

Figure S2. Schematics of the mini photobioreactor. Two LED light sources (Green and Red) are used to activate and deactivate the optogentic circuit. Two LED light sources (Pink and Blue) are used for optical density and fluorescence measurement, respectively. Bacterial cells are stirred continuously to avoid sedimentation. All operations are controlled by an Arduino microcontroller board and a laptop computer.

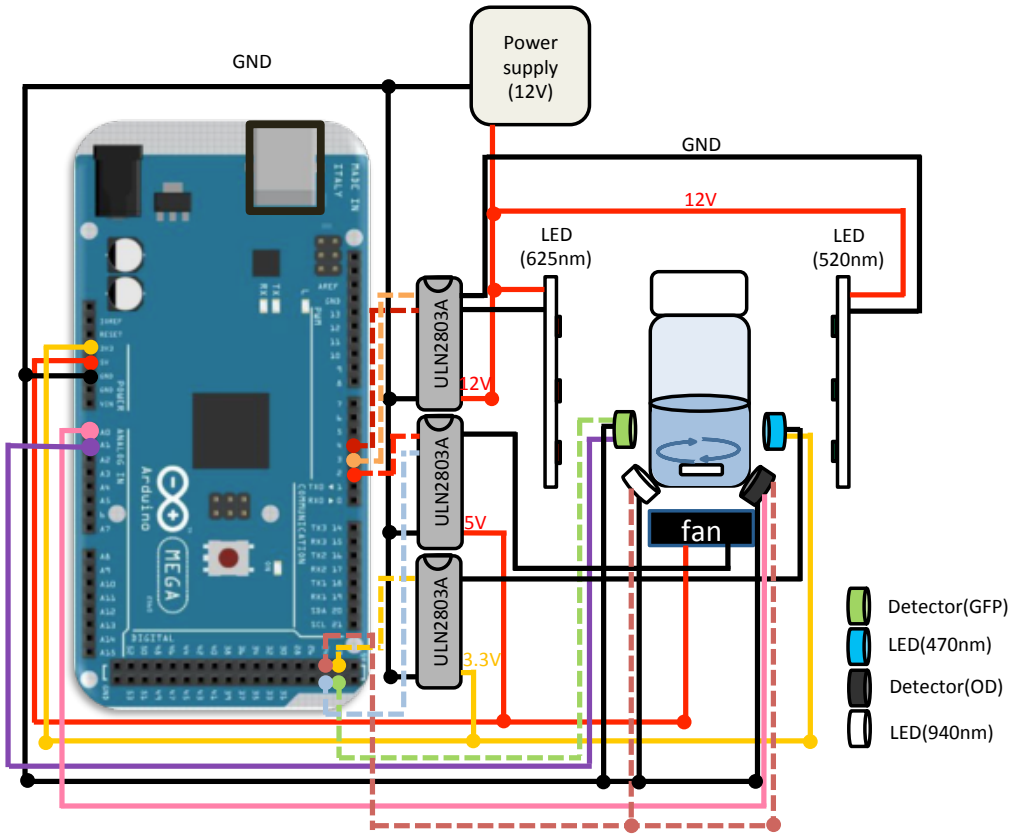

Figure S3. Schematics of the detailed wiring diagram. The diagram shows the detailed wiring through Arduino microcontroller board. The fan with magnet attached is used for stirring. All the LED sources and the fan is powered by a Darlington pair chip UN2803.

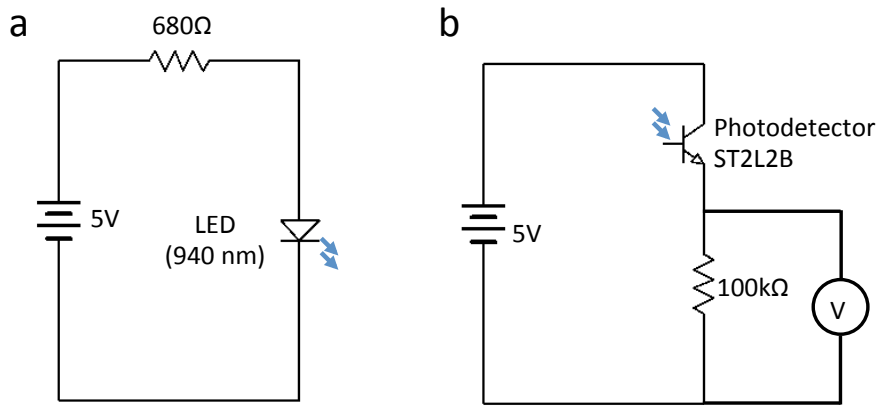

Figure S4. Schematics of circuit diagram for optical density. (a) Biasing circuit for LED of wavelength 940 nm. (b) Biasing circuit for photodetector.

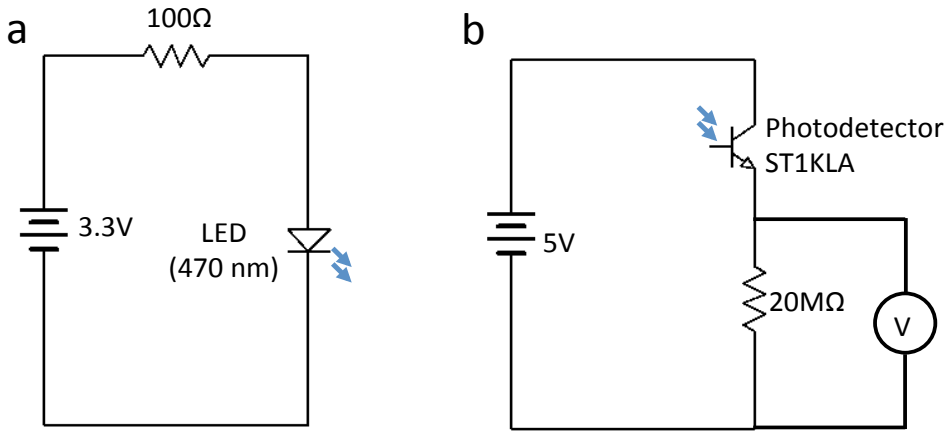

Figure S4. Schematics of circuit diagram for optical density. (a) Biasing circuit for LED of wavelength 470 nm. (b) Biasing circuit for photodetector.

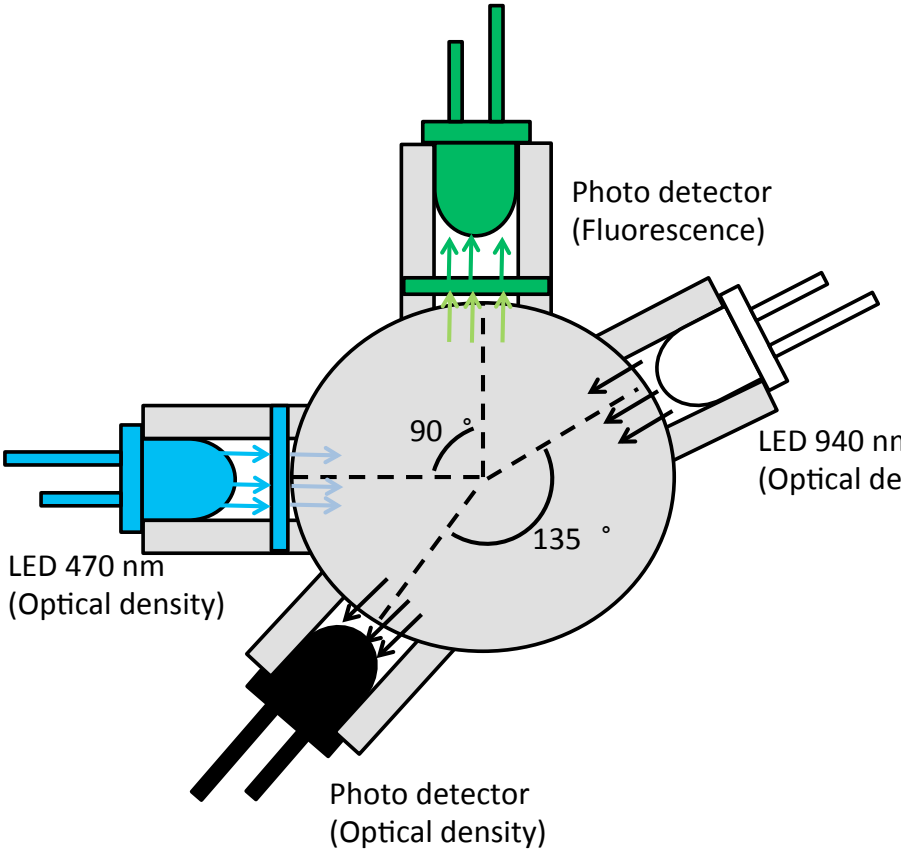

Figure S6. Top view of the tube holder and LED sources for optical density and fluorescence measurement.

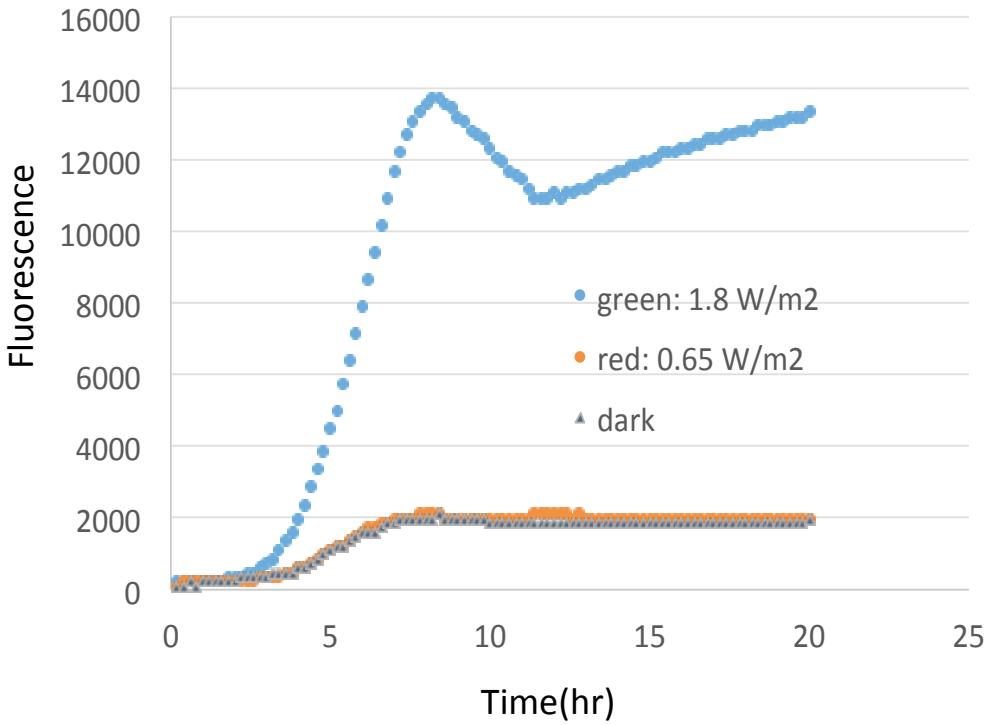

Figure S7. Fluorescence data under green light, red light, and dark condition. A representative data of total gene expression under green light, red light and dark condition is displayed here. The intensity for green and red light is 1.8 W/m^2 and 0.65 W/m^2. The total gene expression shows no appreciable difference between red light and dark condition. The glucose (0.4% w/v) is used as carbon source in minimal medium.

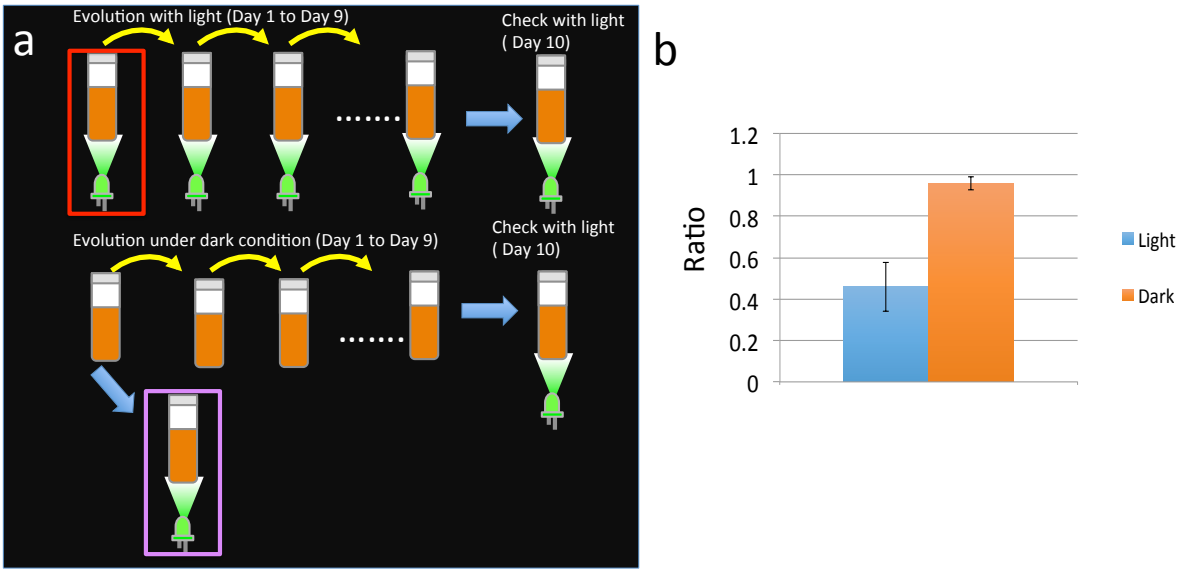

Figure S8. Comparison with evolution under dark condition. (a) The evolution run under dark condition is used to compare the effect due to light illumination. On day 10, both samples are illuminate again to measure gene expression by fluorescence. Red and purple rectangles are used to mark the reference for light illumination and dark condition. (b) The ratios of gene expression on Day 10 to those in reference samples are displayed. The gene expression for evolution under dark condition shows only ~4% degradation. Five replicate and three replicate are used to obtain the error bar for evolution under light and dark condition, respectively.

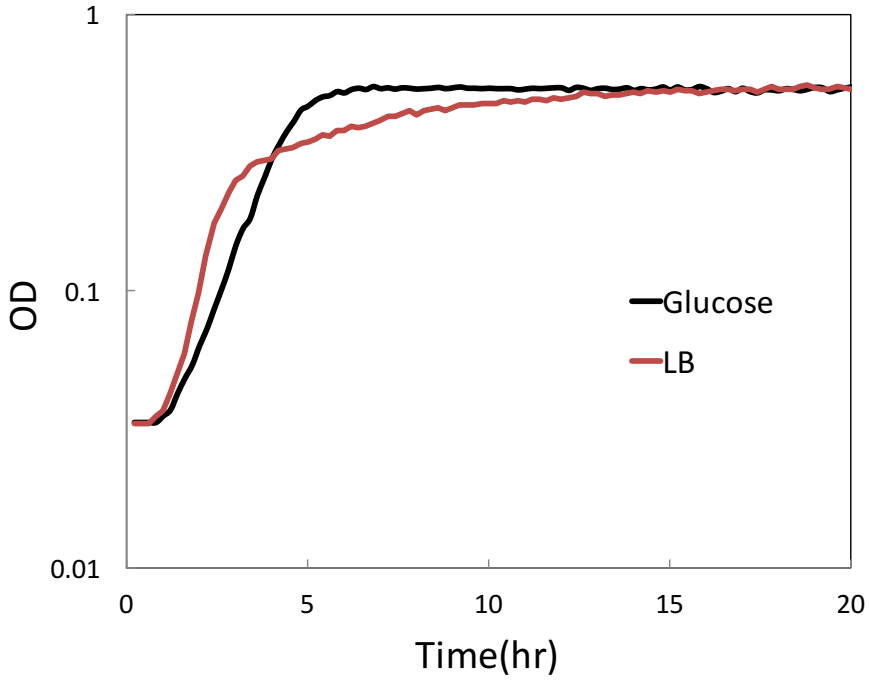

Figure S9. Growth curve of wild type cell. The growth curves of wild type cells in LB and M9 medium with 0.4% glucose are displayed.

Substrate	Growth rate w/o light (hr^{-1})	Growth rate with light(hr^{-1})
Glucose	0.67	0.47
Pyruvate	0.61	0.44
Succinate	0.27	0.28
Acetate	0.233	0.244

Table S1. Growth rate under various organic substrates as carbon and energy source in minimal medium. Growth rates are obtained by fitting the OD versus time data in Figure 1(b).