Electronic Supplementary Information

A microfluidic photobioreactor array demonstrating high-throughput screening for microalgal oil production

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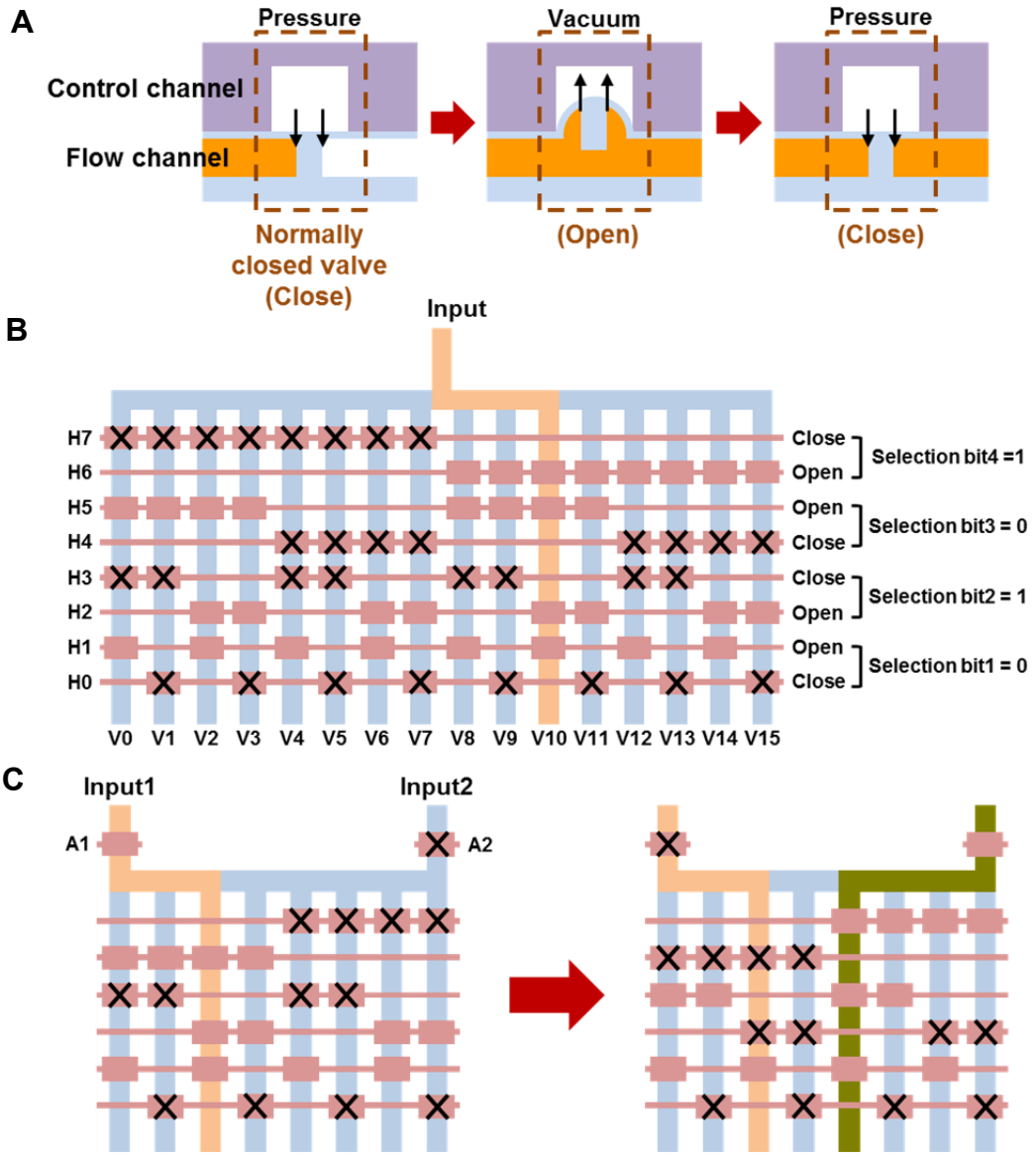
Supplemental Information (SI)

Microfluidic pneumatic binary demultiplexer

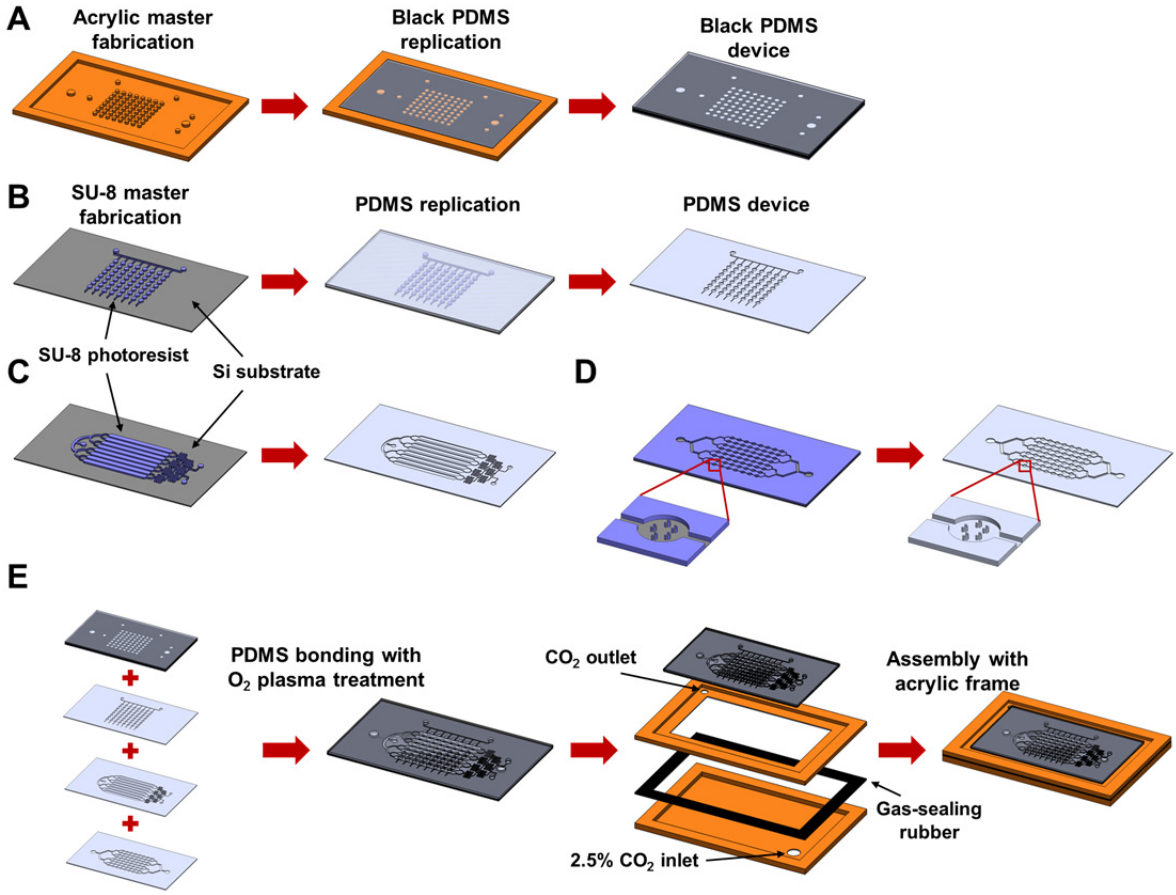
The microfluidic pneumatic binary demultiplexer[1] was composed of two distinct PDMS layers where the control layer containing control lines (H0 – H7 in SI Fig. 1) to actuate microvalve patterns were placed on top of the flow layer comprising of input and output channels to be controlled (V0 – V15 in SI Fig. 1). The microvalve patterns were formed at the junction where the top control lines crossed the bottom flow channels so that the thin membrane between the top and the bottom channels could be deflected by pneumatic actuation. This resulted in opening (negative pressure applied) or closing (positive pressure applied) of the bottom flow channels (SI Fig. 1A).

The pneumatic binary demultiplexer was used to choose one particular channel out of the 16 output channels through which input solution could flow (SI Fig. 1B). Each pair of control channels (4 pairs in total) was connected to a group of microvalves regulating half of the flow channels. Thus, a pair of control channels formed a complementary pair (e.g., H0–H1, H2–H3, H4–H5, and H6–H7), and constituted one selection bit. To open (or select) a single output channel, only one control channel from each complementary valve pair had to be opened (actuated with a negative pressure, "open") while the other was closed (actuated with a positive pressure, "close"). Thus, the open-close states of the two control channels forming a selection bit were always opposite. For instance, if the selection bit was 0, H0 was closed while H1 was open. On the other hand, if the selection bit was 1, H0 was open and H1 was closed. By deciding the state of each selection bit, opening and closing of the 16 output channels could be independently controlled. For example, when selection bit 1, 2, 3, and 4 were in state 0, 1, 0, and 1, input solution could flow through the selected output channel V10 (0101_2 = 10; SI Fig. 1B). Due to the complementary microvalves organized in a binary architecture, 16 output microchannels (N) could be controlled with 8 control microchannels ($2log_2N$).

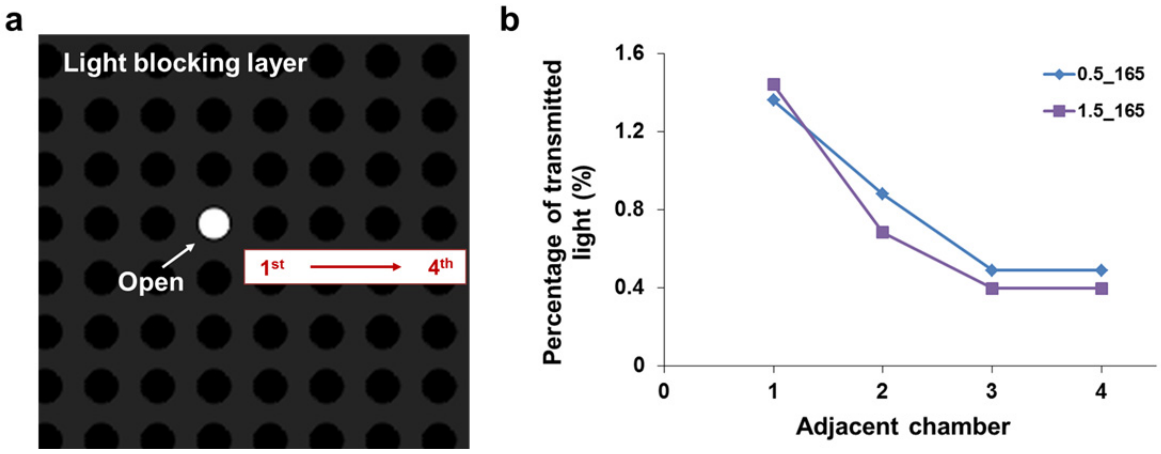
For our developed microalgae photobioreactor array to control the different light-dark cycles, a modified microfluidic pneumatic binary demultiplexer having two inputs (DI water and black dye) instead of a single input as described above was used. The overall working principle in the modified schematic was same, with the only difference being that two additional microvalve structures (A1 and A2 in complementary state) were used to control the two inputs (SI Fig. 1C). Depending on whether A1 was open and A2 was closed, or A1 was closed and A2 was open, either black dye (input 1) or DI water (input 2) could flow into the system. This selected input solution could then flow into one of the 8 output channels selected by the pneumatic binary demultiplexer (SI Fig. 1C).



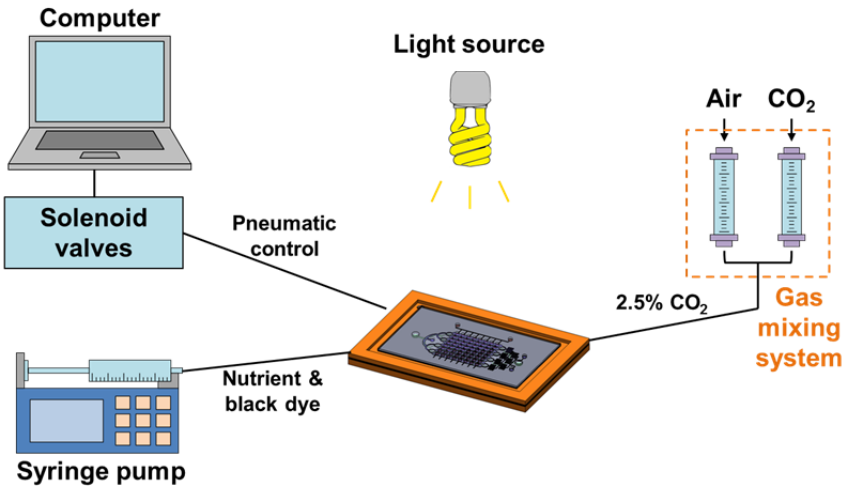
SI Fig. 1. Operation principle of a microfluidic binary demultiplexer. (A) Microvalve composed of a control layer and a flow layer utilized in this demultiplexer. (B) Binary demultiplexer in which 16 output channels were regulated with 8 control microchannels. (C) Modified binary demultiplexer having two inputs and two additional microvalves, which was utilized in the high-throughput microfluidic microalgal photobioreactor array to control different light-dark cycles.



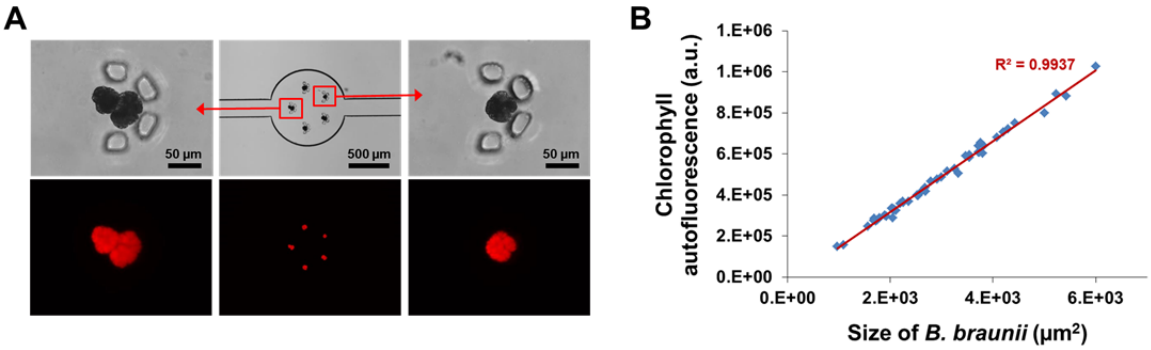
Supplementary Fig. S1. Fabrication process of the microfluidic microalgal photobioreactor array. (A) Light blocking layer. (B) Microfluidic light-dark cycle control layer. (C) Microfluidic light intensity control layer. (D) Microalgae culture layer. (E) Bonding of all PDMS layers using O_2 plasma treatment and assembly into a gas-tight acrylic frame for CO_2-controlled environment.



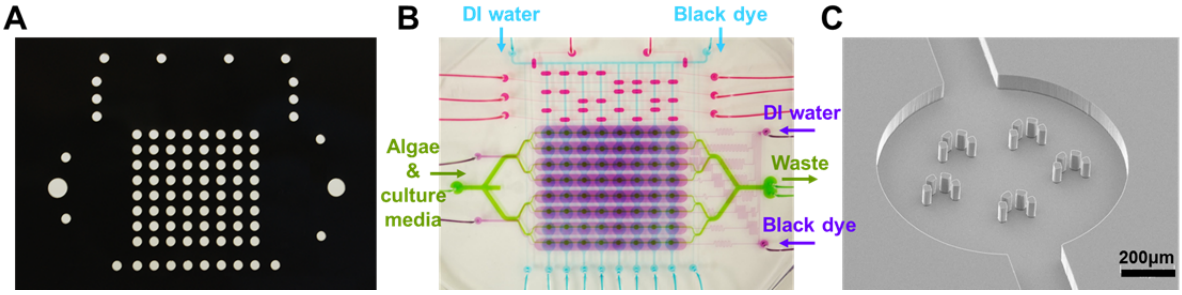
Supplementary Fig. S2. Light interference among neighboring chambers. (A) Schematic showing the setup for this measurement. All chambers in the light blocking layer were blocked except for one chamber (highlighted as "Open"), and the intensities of light underneath this particular chamber as well as adjacent chambers were measured and compared. (B) Comparison of the degree of transmitted light from neighboring chambers by changing the distance from the bottom of the platform used (0.5 and 1.5 in the graph legend indicate 0.5 and 1.5 mm). Number 165 in the graph legend indicates the intensity of incident light, 165 μmol photons·m^{-2}·s^{-1}. Less than 1.5% light transmittance was observed, which is negligible.



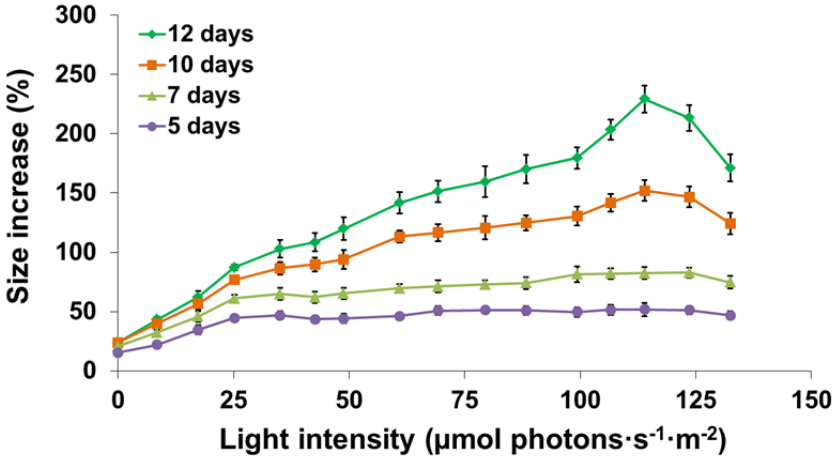
Supplementary Fig. S3. Experimental setup. Air containing 2.5% CO_2 was generated by mixing atmospheric air and 99.9% CO_2 in the ratio of 40 to 1 by controlling each gas flow with compact shielded flowmeters (VWR). This mixed gas was then sterilized through a filter, and flowed into the acrylic culture frame, where CO_2 could diffuse into the microalgae culture compartments through the exposed thin PDMS layer. A 14-W compact fluorescent light bulb (65 K), which could provide different incident intensities of light depending on the distances from the microalgae culture platform, was used. Nutrients were continuously supplied by a syringe pump (1 μl/min, Chemyx Inc.), which introduced fresh culture media into the platform and flushed any waste products out of the platform. The flow of DI water and black dye to produce different light intensities and different light-dark cycles were also controlled with syringe pumps, where different flow rates were used for intensity control (5 μl/min : 0.8 μl/min = DI water : black dye) and light cycle control (1.5 μl/min for both solutions). All control lines in the microfluidic pneumatic binary demultiplexer to regulate the light-dark cycles were operated automatically by an array of solenoid valves and a programmable Labview™ interface.



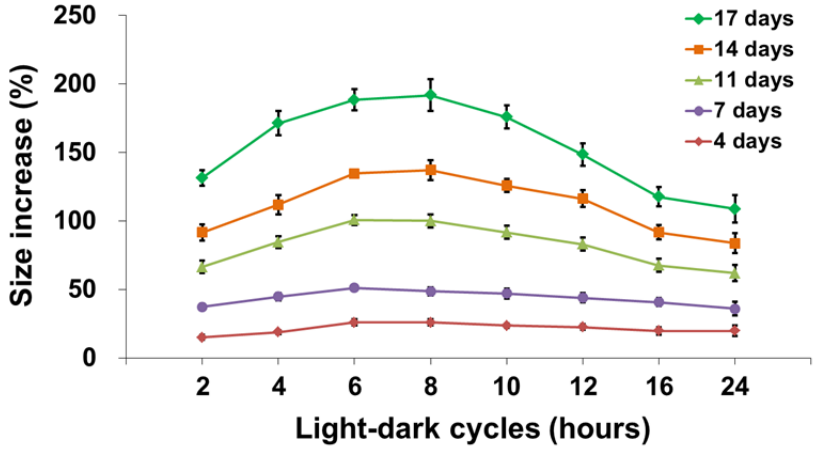
Supplementary Fig. S4. Correlation between size and chlorophyll autofluorescence of *B. braunii* colonies. (A) Chlorophyll autofluorescence and bright field images of captured *B. braunii* colonies inside the platform. (B) Strong linear correlation (R^2=0.9937) between *B. braunii* size and intensity sum of its corresponding chlorophyll autofluorescence, which also indicates strong linear relationship between size and biomass.



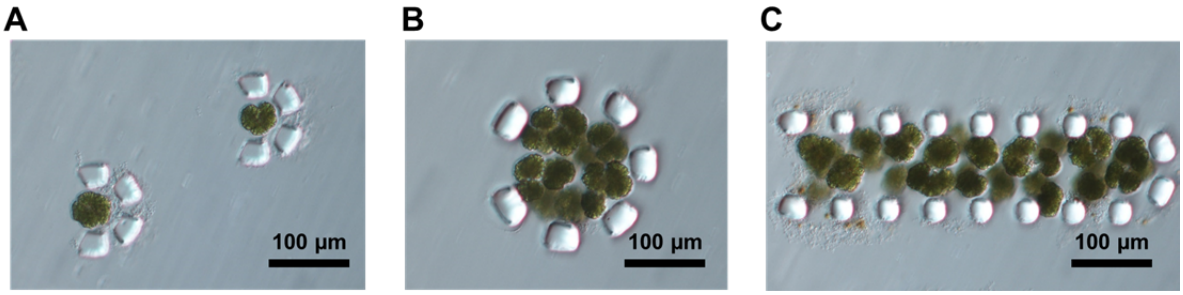
Supplementary Fig. S5. Microfabricated high-throughput microfluidic microalgal photobioreactor array. (A) Light blocking layer. (B) Fully assembled system. Light-dark cycle control layer (cyan: cycle control channels, pink: pneumatic binary demultiplexer) + light intensity control layer (purple) + microalgae culture layer (green) stacked on top of each other. (C) SEM image of a single culture chamber with five *B. braunii* colony trapping sites.



Supplementary Fig. S6. *B. braunii* growth under 16 different light intensities using the developed microfluidic platform. Average size increase of *B. braunii* at days 5, 7, 10, and 12 ($n = 18$) under 16 different light intensities with a 12-hour light-dark cycle were analyzed. Data shown are mean ± standard error.



Supplementary Figure S7. ***B.*** *braunii* growth under 8 different light-dark cycles using the developed microfluidic platform. Average size increase of *B. braunii* at days 4, 7, 11, 14 and 17 (*n = 15*) under 8 different light-dark cycles with a light intensity of 120 μmol photons·m^{-2}·s^{-1} were analyzed. Data shown are mean ± standard error.

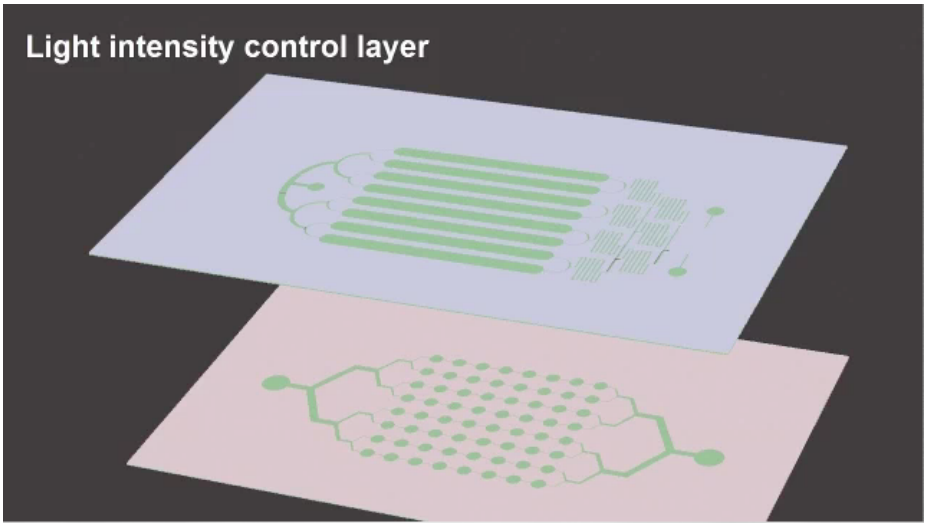


Supplementary Fig. S8. Different designs of algal colony trapping sites. (A) Single-colony trapping design consisting of smaller opening (52 µm). Multiple-colony trapping designs having (B) a large circular structure and (C) a long U-shape structure.

Supplementary Table S1. Light intensity measured through black-dye-filled light intensity control channel in the microfluidic platform and corresponding light transmittance rate.

Light-to-platform distance (cm)	Black dye concentration (%)								
	0 (DI water)	0.3	0.5	1	1.5	2	2.5	3.5	4
	Light intensity (μmol photons·m^{-2}·s^{-1})								
4.5	295.40	269.23	252.12	217.31	185.65	163.82	142.05	111.69	97.93
5.6	261.40	238.68	222.86	198.93	172.92	151.98	128.46	103.49	88.76
7.7	175.78	163.50	155.67	132.87	114.87	101.07	85.71	68.44	58.12
9.7	132.44	123.27	114.14	97.68	87.24	77.51	63.35	51.22	44.35
12.7	80.21	73.90	69.54	60.14	52.44	47.25	38.91	30.98	27.01
25	37.01	34.12	33.27	29.06	24.70	21.63	18.03	14.34	12.40
	Transmittance rate (%)								
4.5	100.00	91.14	85.35	73.56	62.85	55.46	48.09	37.81	33.15
5.6	100.00	91.31	85.26	76.10	66.15	58.14	49.14	39.59	33.96
7.7	100.00	93.01	88.56	75.59	65.35	57.50	48.76	38.94	33.06
9.7	100.00	93.08	86.19	73.76	65.87	58.52	47.83	38.67	33.49
12.7	100.00	92.14	86.70	74.98	65.38	58.90	48.52	38.63	33.68
25	100.00	92.20	89.90	78.52	66.75	58.44	48.72	38.75	33.50
Average	100.00	92.15	86.99	75.42	65.39	57.83	48.51	38.73	33.47
Standard deviation	0.00	0.82	1.86	1.82	1.35	1.25	0.48	0.57	0.33

Light-to-platform distance (cm)	Black dye concentration (%)									
	5	6.5	8.5	10	15	20	40	60	80	100
	Light intensity (μmol photons·m^{-2}·s^{-1})									
4.5	77.36	52.23	34.96	22.72	7.43	3.98	0.26	0.00	0.00	0.00
5.6	67.54	46.54	28.71	21.46	8.00	3.97	1.04	0.75	0.00	0.00
7.7	45.69	32.48	18.93	13.89	5.58	3.03	1.03	0.54	0.15	0.00
9.7	34.10	23.00	14.30	9.88	4.42	2.12	0.47	0.00	0.00	0.00
12.7	20.51	14.00	9.20	6.70	2.78	1.27	0.28	0.16	0.12	0.00
25	9.42	6.63	4.21	2.84	1.14	0.52	0.09	0.05	0.00	0.00
	Transmittance rate (%)									
4.5	26.19	17.68	11.84	7.69	2.52	1.35	0.09	0.00	0.00	0
5.6	25.84	17.80	10.98	8.21	3.06	1.52	0.40	0.29	0.00	0
7.7	25.99	18.48	10.77	7.90	3.17	1.73	0.58	0.31	0.08	0
9.7	25.75	17.37	10.80	7.46	3.34	1.60	0.36	0.00	0.00	0
12.7	25.57	17.46	11.47	8.36	3.46	1.58	0.35	0.20	0.15	0
25	25.45	17.90	11.38	7.67	3.07	1.41	0.26	0.13	0.00	0
Average	25.80	17.78	11.21	7.88	3.10	1.53	0.34	0.15	0.04	0.00
Standard deviation	0.27	0.40	0.43	0.34	0.33	0.14	0.16	0.13	0.06	0.00



Supplementary Video S1. Operation principle of the high-throughput microfluidic microalgae cultivation platform

Supplementary References

1. T. Thorsen, S. J. Maerkl and S. R. Quake, *Science*, 2002, **298**, 580-584.