

Supporting Information for

Light-Induced Cell Aggregation of *Euglena gracilis* Towards Economically Feasible Biofuel Production

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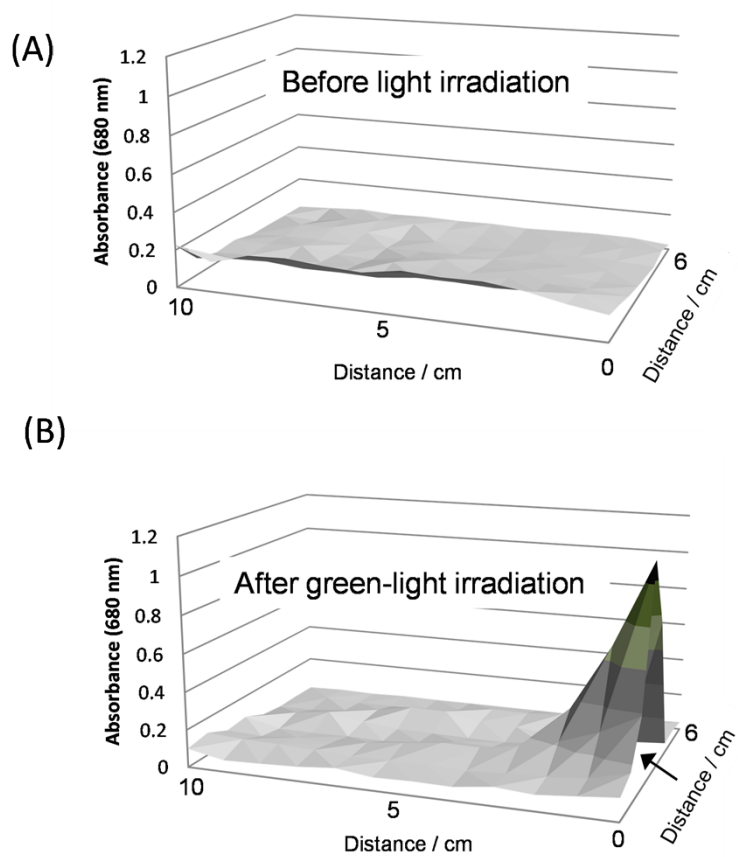


Figure S1. Cell amount in a transparent rectangular petri dish (6 cm x 10 cm) before (A) and after (B) 530-nm light irradiation. The arrow in (B) indicates the position of the light source.

Notes:

To evaluate the cell amount before and after light irradiation, the cell concentration of the cell suspension at each region of the petri dish was measured. This was achieved by conducting the photoresponse measurements in a transparent rectangular petri dish. Because the petri dish was of the same size as the well plate, the cell distribution across the entire petri dish could be measured using a plate reader (Tecan Infinite 200 PRO).

Before light irradiation of the cell suspension, the absorption at 680 nm was approximately 0.2 at all regions of the plate, whereas after irradiation with 530-nm light for 24 hours, the absorption varied depending on the region (Fig. S1). The total cell amount can be represented by the sum of the absorbance in each region. The sums of the absorbance values before and after light irradiation were 7.68 and 9.97 respectively, indicating only minimal cell growth had occurred. Therefore, the photo-induced change of cell distribution is predominantly attributable to cell movement, rather than cell growth.

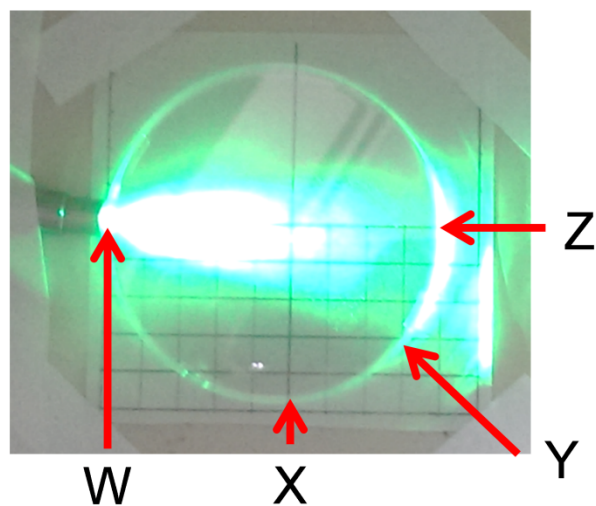


Figure S2. Light intensity at different regions of the petri dish with 530-nm light irradiation of approx. 1.5 mW/cm^2 . W: 1.44 mW/cm^2 , X: $0.11 \text{ }\mu\text{W/cm}^2$, Y: $2.71 \text{ }\mu\text{W/cm}^2$, Z: $76.6 \text{ }\mu\text{W/cm}^2$.

Notes:

We employed radially emitted light generated from a Xe lamp with interference filters and cells were gathered from all regions of the petri dish (Fig.2). However, the light emitted from the optical fiber spreads out in a cone-like shape and consequently, parts of the petri dish are not directly exposed to green light. By measuring the light intensity at different regions of the dish, it was confirmed that cells gathered towards the light source from areas with light intensities as low as $0.1 \text{ }\mu\text{W/cm}^2$.

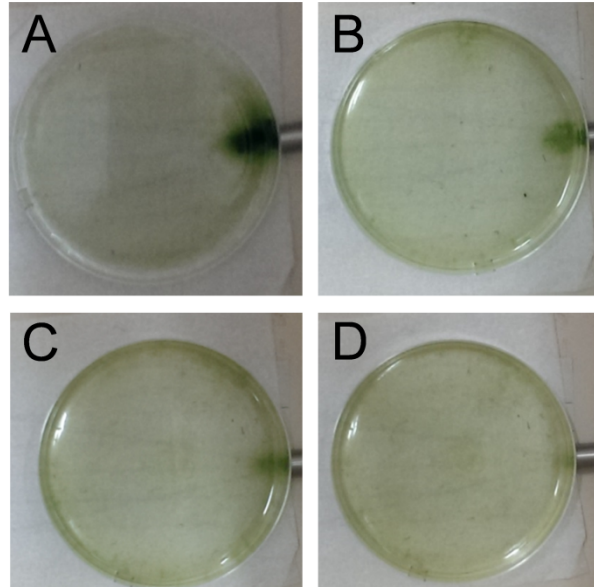


Figure S3. Photo-induced cell aggregation at low light intensities (530 nm). A: $196 \mu\text{W}/\text{cm}^2$, B: $15.7 \mu\text{W}/\text{cm}^2$, C: $5.43 \mu\text{W}/\text{cm}^2$, D: $1.57 \mu\text{W}/\text{cm}^2$. Cells were subjected to 530-nm irradiation for 24 hours prior to the experiment.

Notes:

To confirm the minimum light intensity that *E. gracilis* cells can sense, we irradiated 530-nm light at low intensities. From these results, the minimum light intensity for *E. gracilis* cells to sense green light is implied to be between 1.57 and $5.43 \mu\text{W}/\text{cm}^2$.

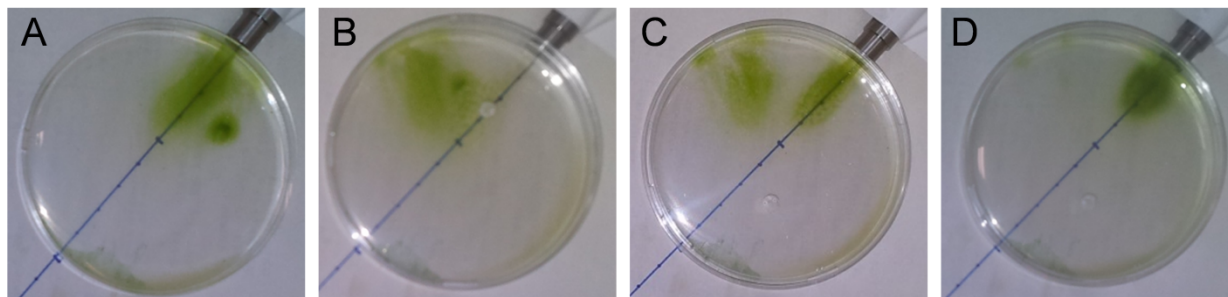


Figure S4. Effects of pre-irradiation on photo-induced cell aggregation. A: 24 hours after green light irradiation. The petri dish was rotated 90 degrees counter clockwise afterwards, and B,C,D correspond to 15 minutes, 2 hours, and 9 hours after the rotation.

Notes:

In order to confirm that the specific time dependence was not due to a photochemical process, we first induced cell aggregation by irradiating 530-nm light for 24 hours (Fig. S4 A), and then rotated the petri dish 90 degrees counter clockwise, so as to move the cells away from the irradiated area. Fig.S4 B, C, and D show the cell distribution 15 minutes, 2 hours, and 9 hours after this rotation. In contrast to the cells without light pre-irradiation, a retreating phase could not be observed, and cell aggregation at the light source was clear in 9 hours.

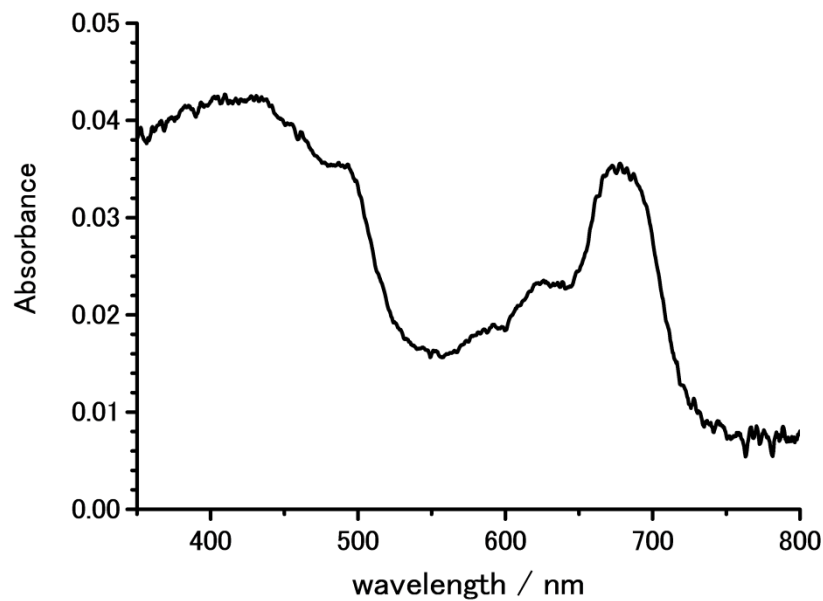


Figure S5. Diffused-transmission UV-Vis absorption spectrum of *E.gracilis* cells in Cramer-Myers medium. The optical length of the cell was 1.0 mm and OD_{680} was 0.4. Pure medium was used as reference (Shimadzu UV-2550, MPC-2200).

Notes:

In this work, we propose of irradiating 530-nm light at the OLI (2.5 mW/cm^2) to induce cell aggregation. When the effective irradiation depth is assumed to be the optical length for the light to decay to approx. $5 \text{ } \mu\text{W/cm}^2$ (Fig. S3), the effective irradiation depth can be estimated as approx. 15 cm from Lambert-Beer's law.