Supplementary Material – Short-term memory effects in the phototactic behavior of microalgae

Taha Laroussi,[1] Mojtaba Jarrahi,[2] and Gabriel Amselem[1]

[1] *Laboratoire d'Hydrodynamique (LadHyX), CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, 91120 Palaiseau, France*
[2] *Université Paris-Saclay, CNRS, FAST, 91405 Orsay, France*

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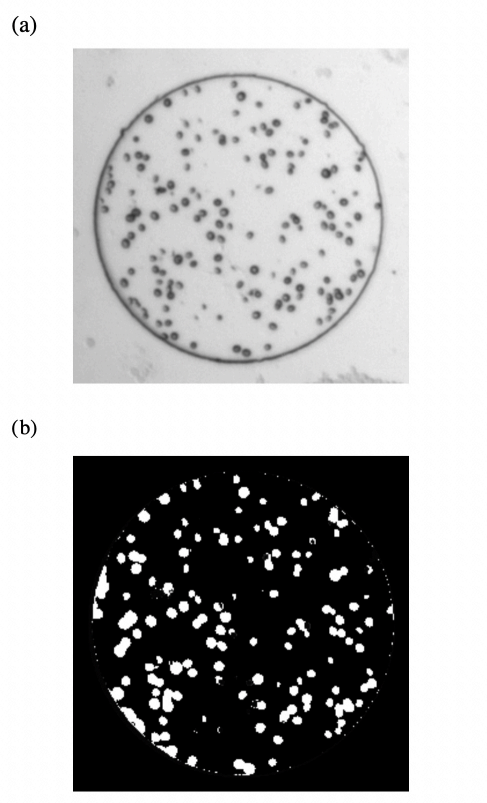
I. MATERIAL AND METHODS

A. Concentration of the algal solution

The algal culture was taken 4 hours after the beginning of the "day", and underwent three centrifugation steps, leading to a solution concentrated in motile algae, and enabling to get rid of low-motility algae, dead algae, and cellular debris. First, 45 mL of the liquid culture were centrifuged at 1057g for 10 minutes. Then, 39 mL of the supernatant were removed to obtain a concentrated pellet of cells. The bottom 6 mL were homogenized and then centrifuged at 73g for 2 minutes. The supernatant, containing the motile cells, was kept and centrifuged again at 285g for 5 minutes to obtain a final solution highly concentrated in motile algae. This solution was then diluted at the desired concentration for the experiments. Before experiments, algae were left to rest in the dark for 60 minutes, allowing the cells that had deflagellated during the centrifugation process to regrow their flagella [1, 2]. It is known that the phototactic response of microalgae may be regulated by its inner biological circadian clock through the day [3]. To ensure the reproducibility of our experiments, experiments systematically started 6 hours after the beginning of the "day".

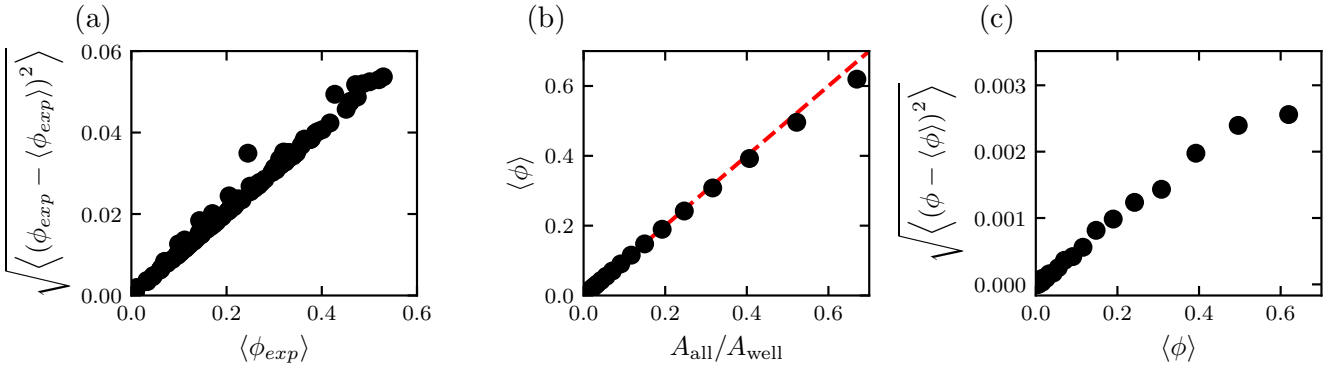


Supp. Fig. 1. Binarization of the experimental images using a threshold on pixel intensity. (a) An experimental image of a well with algae in it. The algae are darker than the background. (b) Thresholding the experimental image leads to a binarized image where algae appear in white in a dark background.

B. Projected area fraction ϕ

The concentration in algae in each well was determined by calculating, in each well, the fraction of area occupied by the algae. At the beginning of an experiment, the algae were not stimulated by any blue light, and swam randomly in their wells. We used Otsu thresholding to binarize the images, see Supp. Fig. 1 and obtain, for each well, the total area occupied by the algae A_p. In each well, this area was renormalized by the well area A_{well}. We then defined the projected area fraction $\phi \equiv A_p/A_{\text{well}}$, which was used as a proxy for the concentration in algae. This was repeated for the first 100 images of each experiments, and used to calculate the mean value and the standard deviation of ϕ. We find that the relative error is of the order of 10%, see Supp. Fig. 2a.

The uncertainty of 10% on ϕ in our experiments results from the imperfect binarization of images, and not from the fact that algae overlap in z. Indeed, at the values of $\phi \leq 0.5$ used in the experiments, there is almost no overlap. This can also be checked by simulating N solid spheres with a radius $R = 8$ μm placed randomly in a cylindrical well of height 32 μm, and calculating their projected area. The obtained projected area is equal to the projected area of N spheres as long as $\phi \leq 0.7$, see Supp. Fig. 2b. The theoretical error on ϕ, determined by calculate the standard deviation of ϕ in 100 identical simulations, is of the order of 0.5%, much smaller than the experimental error, see Supp. Fig. 2c.



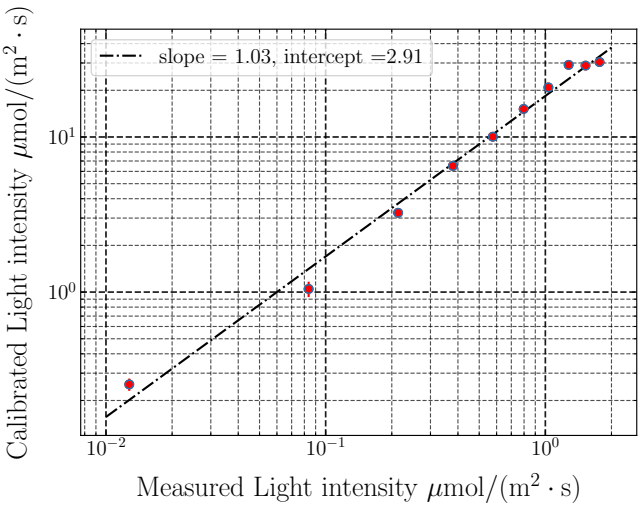
Supp. Fig. 2. (a) The error in the concentration measurement of each experiment is quantified by calculating the standard deviation in the projected area of algae in the first hundred images before the light is turned on. The relative average standard deviation is 10% of the concentration ϕ. Points: experiments. (b) Checking for overlaps: projected area fraction $\phi \equiv A_p/A_{\rm well}$ of N confined spheres which can overlap in the z direction, as a function of the projected area $A_{\rm all}/A_{\rm well} \equiv N\pi R^2/A_{\rm well}$ of N spheres of radius R. The red dashed line has slope 1, showing that overlaps can essentially be neglected for $\phi \leq 0.7$. (c) Standard deviation of the projected area fraction $\phi \equiv A_p/A_{\rm well}$ as a function of the average projected area fraction $\langle\phi\rangle$. The average and standard deviation are calculated over 100 simulations.

C. Determining the flux of photons seen by the microalgae

To determine the flux of photons reaching the microalgae, we proceeded in two steps.

For all experiments, we measured the flux of photons from the blue LED reaching the camera sensor. To do so, we first determined the camera offset value by blocking off all the light to the camera and taking a 16-bit image. The spatial average intensity in grey value of all the pixels in the image was the camera's offset. Then, a 16-bit setup image of the sample was taken at the current experimental condition, with the light of the microscope turned off and the blue LED light on. The camera offset value was then subtracted from each pixel in the setup image. The grey values were first converted to number of electrons by dividing each pixel in the image by the conversion gain of the camera. The electrons were converted to photons by dividing the number of electrons from each pixel by the quantum efficiency (QE) of the sensor at $\lambda = 470$ nm.

Note that the amount of light reaching the camera corresponds to the light scattered by the PDMS. To relate it to the light stimulus experienced by the algae, we measured once the light intensity at the level of the PDMS chip using a light sensor (Adafruit TSL2591), connected to an Arduino. Relating this light intensity to the intensity recorded by the camera provides a calibration curve, enabling to determine the flux of photons reaching the algae. This calibration curve shows that the flux of photons reaching the algae is 20 times higher than the one scattered towards the camera sensor, see Supp. Fig. 3



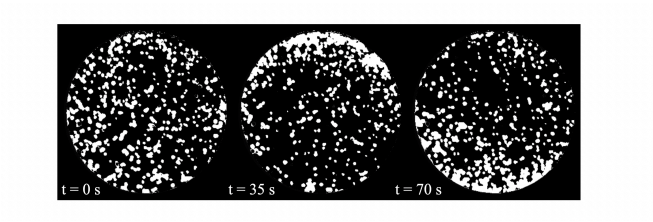
Supp. Fig. 3. Calibration of the light intensity measurement. The light scattered by the PDMS is measured at the level of the camera sensor using the gray values of the recorded images (x-axis). The light intensity at the level of the microwells is measured using a light sensor (y-axis). Both values are proportional, with a coefficient of proportionality ≈ 20.

II. EXPERIMENTAL RESULTS

A. Fraction of algae not responding to light

Not all algae respond to the light stimuli, see the time-lapse in Supp. Fig. 4. In this section we explain how the fraction f of algae responding to light is computed.

For all analyses, including the analysis on the center of



Supp. Fig. 4. Time-lapse of binarized images of *C. reinhardtii* enclosed in a well. The blue light stimulus is turned on at $t = 30$s. The stimulus comes from the upper side of the well. Not all algae react to the stimulus.

mass $z_{\rm cm}$ in the main text, immobile cells were removed by image processing at the beginning of the image analysis. To remove the immobile cells, we thresholded for each experiment the images using Otsu thresholding [4], and took the average of the entire thresholded image sequence. For each pixel in the image, the average gives us the fraction of time that this pixel is "turned on", corresponding to the fraction of time in the image sequence when there is a cell at this position. We say that there is an immobile cell at a location when the pixel is turned on for more than 94 seconds, so more than the stimulus time. All the corresponding pixels are then removed from the individual images. This enables to get rid of truly immobile cells. Note however that it does not remove slowly moving cells, which probably glide on the surface.

The fraction of responding cells was then calculated in two steps, using the binarized images. First, the total area $A_{\rm all}$ occupied by the motile algae was measured at the beginning of the experiments, when algae do not overlap (as shown in Supp. Fig. 2). Then, at all time steps, the largest connected component in the images was identified. The area A_- of all white pixels *not* belonging to this largest connected component was calculated. The fraction f of responding cells was defined as $f = 1 - A_-/A_{\rm all}$. We did not calculate the area of the largest connected component to avoid problems with overlapping cells in this region. The evolution of f as a function of time is shown in Supp. Fig. 5. At the beginning of the experiment, before stimulation, f has no meaning. Once the accumulation has finished, in the last 60s, f is essentially constant, see Supp. Fig. 5. This tends to show that cells with the opposite sign of phototaxis are not counted in our protocol.

The center of mass of responding (resp. non-responding) cells is defined as $z^\star_{\rm cm}$ (resp. $z_{\rm cm,\,not}$). We then have $z_{\rm cm} = z^\star_{\rm cm} f + z_{\rm cm,\,not}(1-f)$, and so $z^\star_{\rm cm} = (z_{\rm cm} - z_{\rm cm,\,not}(1-f))/f$. Results of the renormalization are shown in Supp. Fig. 6. Values of $z^\star_{\rm cm}$ are much closer to ± 1 than values of $z_{\rm cm}$, showing that the main cause of the center of mass not going to ± 1 are the immobile algae.

Note that there are two caveats to this analysis: (i) the slowly moving cells can be taken into account in the largest connected component, (ii) the largest connected component always corresponds to accumulated cells, but all accumulated cells are not necessarily in this largest connected component.

B. Estimating the time scales of accumulation

To estimate the time scales of accumulation for positive and negative phototaxis, we calculate the derivative of the center of mass $z_{\rm cm}$ and smooth it using a moving average over 10 s. Then, we define the accumulation time as the moment when the absolute value of the derivative is below a given threshold. This method allows to estimate when the accumulation of algae essentially slows down. We used three different thresholds for three different types of experiments, and verified manually that the accumulation time found automatically indeed corresponds to what we observe by eye. For negative phototaxis, we used a threshold of 0.025. For positive phototaxis, we used a threshold of 0.03 or 0.02, depending on the experiment. Indeed, in some cases, the accumulation shows a first quick response before slowing down, which is evident by looking at the time evolution of $z_{\rm cm}$ by eye.

To obtain the time scale of accumulation for back-and-forth motion, we find the peak in $z_{\rm cm}$, which corresponds to the time scale of positive phototaxis. Then, the time scale of the second accumulation (negative phototaxis) is taken when 90% of the plateau value of $z_{\rm cm}$ is reached. The plateau occurs 30 to 40 seconds after the stimulus is turned on.

C. High concentrations of algae

At too high concentrations, the algae fill the entire well, preventing the algae from swimming towards or away from the light, see Supp. Fig. 7.

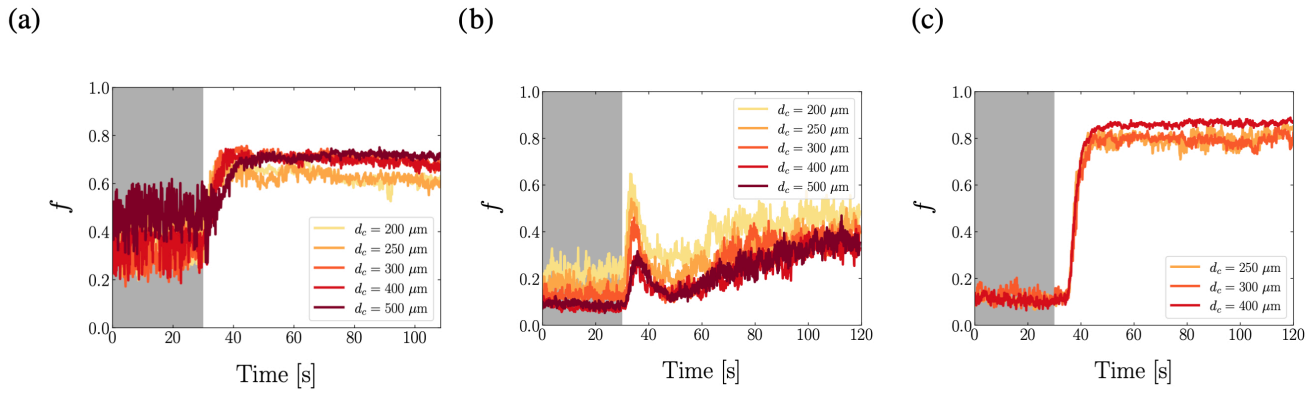
D. Sticking algae

After repeated stimuli, the algae can stick to the glass, see Supp. Fig. 8. This has already been reported, see [5, 6].

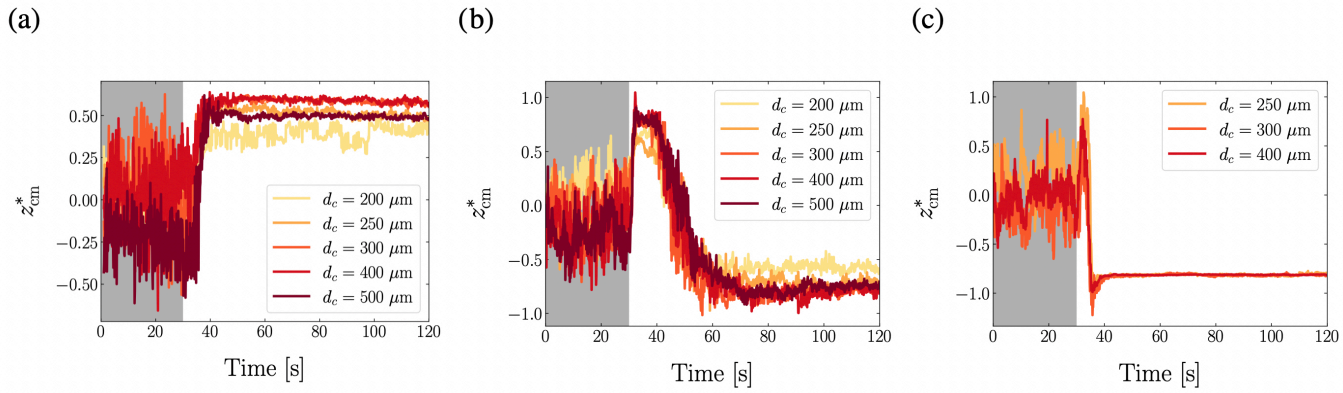
E. Influence of the time between experiments on the change in phototactic behavior

We stimulated populations of algae with a stimulus eliciting as a first response a back-and-forth behavior. Repeating the stimulus at a 10 or 20 minutes interval did not lead to a change in the phototactic response, see Supp. Fig. 9.

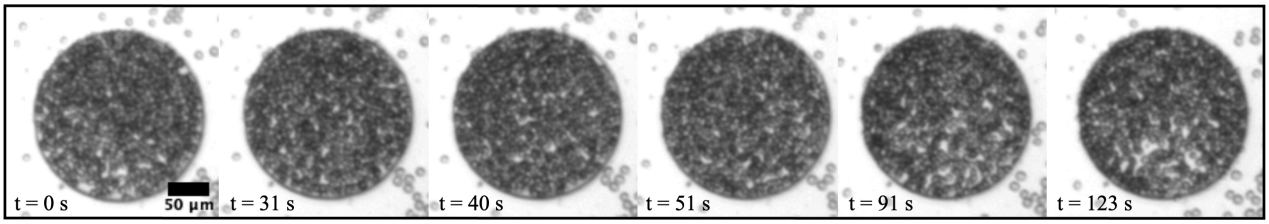
Repeating the stimulus after a 5 minute interval also does not lead to a change in the phototactic sign, see the first two graphs in Supp. Fig. 10. Repeating the stimulus



Supp. Fig. 5. The light stimulus is turned on at $t = 30$ s and the fraction f of algae accumulated at the wall, and therefore that react, is tracked over time. (a) $I = 0.38\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show positive phototaxis. 75% of the algae react to the light stimulus and accumulate at the wall. (b) $I = 2.4\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show first positive phototaxis, then negative phototaxis. At most, 65% of the algae react to the light stimulus and accumulate at the wall during the transient positive regime and 50% during negative phototaxis. (c) $I = 28\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show negative phototaxis. 89% of the algae react to the light stimulus and accumulate at the wall. Each curve is the fraction of accumulated algae averaged over 5 experiments. These are the same experiments as in Fig. 3 of the main article.

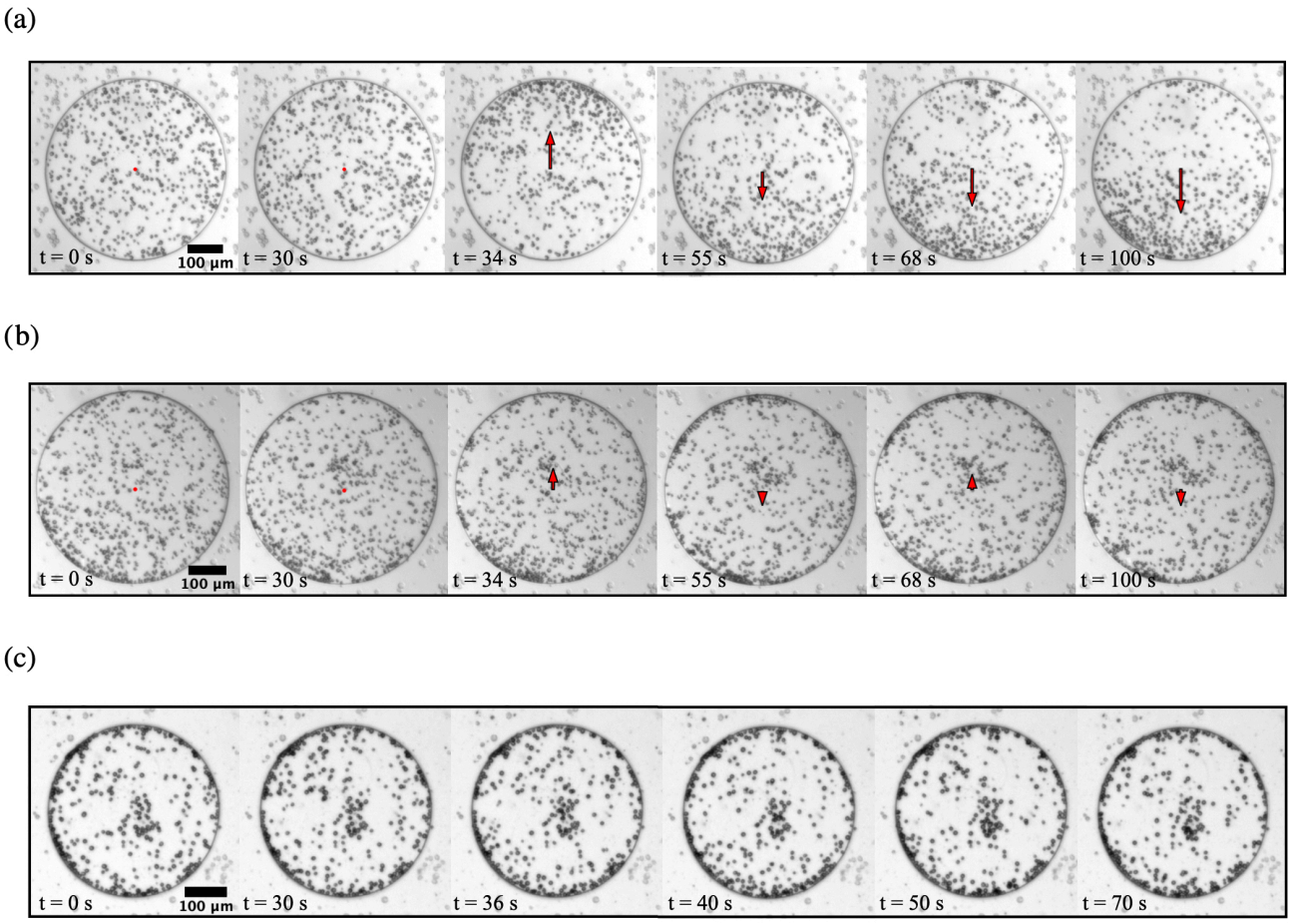


Supp. Fig. 6. The light stimulus is turned on at $t = 30$ s and the corrected position of the center of mass z^*_{cm} is tracked over time. (a) When exposed to an intensity $I = 0.38\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show positive phototaxis. The average projected area fraction of algae in 30 wells is $\phi_{avg} = 0.36$. (b) At intermediate intensities $I = 2.4\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show first positive phototaxis, then negative phototaxis. The average projected area fraction of algae in 30 wells is $\phi_{avg} = 0.28$. (c) At a high intensity $I = 28\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show negative phototaxis. Each curve is an average over 4 to 7 experiments. The average projected area fraction of algae in 17 wells is $\phi_{avg} = 0.11$. These are the same experiments as in Fig. 3 of the main article.



Supp. Fig. 7. *C. reinhardtii* enclosed in a well. The blue light stimulus is turned on at $t = 30$ s. The stimulus comes from the upper side of the wells. The algal population fills the entire well, preventing the algae from migrating towards or away from the light.

after 90 seconds leads to a change from back-and-forth to negative photaxis, see last graph in Supp. Fig. 10.



Supp. Fig. 8. *C. reinhardtii* enclosed in a well. The blue light stimulus is turned on at $t = 30$ s. The stimulus comes from the upper side of the wells. (a) In the first experiment performed at intermediate intensity $I = 1.78\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the algae show first positive phototaxis, then negative phototaxis. The algae are motile. (b) At the beginning of the fourth experiment performed at intermediate intensity $I = 1.78\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ many algae remain accumulated at the walls from earlier experiments. We can see aggregates forming at the center of the well and at the walls during the course of the experiment. (c) The sixth experiment of a series performed at intensity $I = 6.6\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ is displayed. The algae have formed aggregates at the wall and at the center and do not react to the light stimulus anymore.

III. SIMPLIFIED PHOTOTACTIC MODEL

A. Determining the parameters of the simplified phototactic model

The dynamics of the inner biochemical species S in our simplified phototaxis model evolve according to:

$$\frac{ds^\star}{dt} = \gamma I_0 (s_{\text{tot}} - s^\star) - \tau^{-1} s^\star, \tag{1}$$

where γ is the reaction rate at which the inactive species of concentration s is converted into the active species of concentration $s^\star$. The total concentration is conserved and is called $s_{\text{tot}} = s + s^\star$. The transition from inactive to active state depends on the light intensity I_0, while the reverse transition occurs at a constant rate τ^{-1}.

The solution to this equation writes

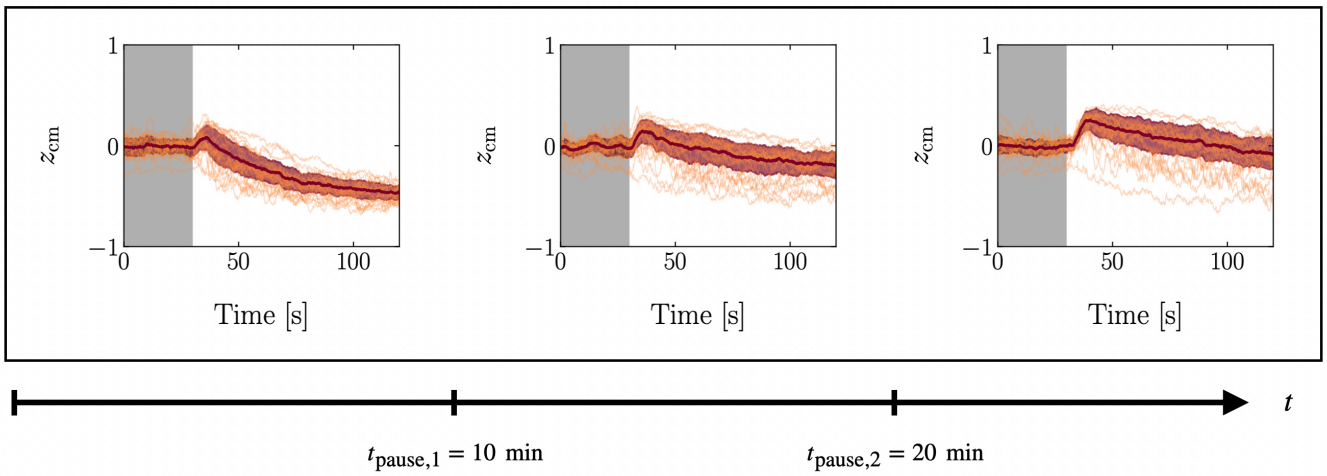
$$s^\star(t) = \frac{\gamma I_0 \tau}{\gamma I_0 \tau + 1} s_{\text{tot}} \left(1 - \exp\left\{-\left[\frac{\gamma I_0 \tau + 1}{\tau}\right] t\right\}\right). \tag{2}$$

We use $s_{\text{tot}} = 1$ for simplicity. To obtain time scales close to the experimental time scales, we choose $\tau = 5$ min.

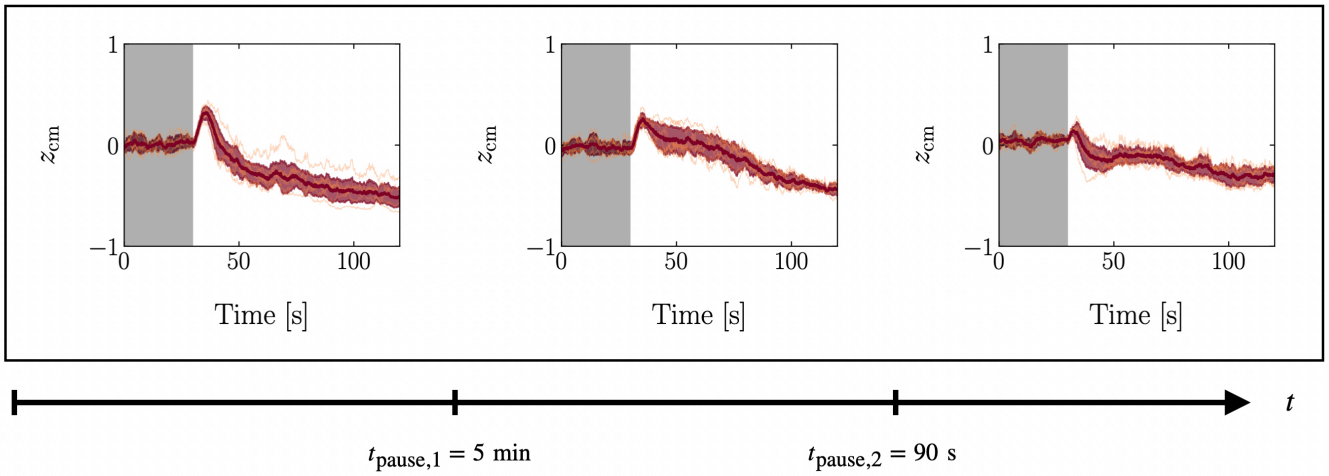
Then, we assume the position z of the alga evolves according to:

$$\frac{dz}{dt} = -\text{sign}(s^\star - s_T) v_0, \tag{3}$$

where s_T is the threshold concentration at which cells transition from positive to negative phototaxis, and v_0 is the characteristic speed of the alga. We know experimentally that $v_0 \approx 100\ \mu\text{m.s}^{-1}$.



Supp. Fig. 9. Memory effects on the phototactic response of algae. The light stimulus is turned on at $t = 30$ s and the position of the center of mass z_{cm} is tracked over time. Three consecutive experiments are performed with varying rest times, where the light is turned off in between. There is a 10 min pause between Exp. 1 and Exp. 2, then a 20 min pause between Exp. 2 and Exp. 3. At intermediate intensities $I = 1.3\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the algae show the same phototactic behaviors in each experiment. The center of mass is averaged over 24 experiments.



Supp. Fig. 10. Memory effects on the phototactic response of algae. The light stimulus is turned on at $t = 30$ s and the position of the center of mass z_{cm} is tracked over time. Three consecutive experiments are performed with varying rest times, where the light is turned off in between. There is a 5 min pause between Exp. 1 and Exp. 2, then a 90 s pause between Exp. 2 and Exp. 3. At intermediate intensities $I = 2.4\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the algae the same phototactic behavior in the first two experiments, there is a back-and-forth motion. In the last experiment, the algae switch to negative phototaxis after a short 90 s break. The center of mass is averaged over 6 experiments.

To obtain the phase diagram of the phototactic behavior as a function of γI_0 and s_T shown in the main text of the article, we defined a positive phototactic behavior when $s^\star(t) < s_T$ for $0 \leq t \leq 90$ s, and negative phototactic behavior when $s^\star(t)$ crosses the threshold s_T at one point t such that $0 \leq t \leq = 10$ s. In between, $s^\star(t)$ crosses the threshold s_T at a time 10 $rms \leq t \leq 90;$ s, and this defines a back-and-forth behavior.

Two parameters now need to be determined: γ and s_T. To estimate γ, we use the fact that, at $I_0 = 0.02\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the algae stop responding. We assume this corresponds to less than one molecule of activated S per second [7], leading to $\gamma = 2\times10^{-3}\ \text{m}^2\cdot\mu\text{mol}^{-1}$. Then, we also know that at $I_0 = 2\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the algae exhibit a back-and-forth behavior. This determines $s_T = 0.1$, according to the phase diagram in the main text.

B. Concentration of $s^\star$ for different stimuli

The sign of phototaxis is given in the model by comparing the concentration $s^\star$ of activated chemical, to a threshold value s_T. The evolution of $s^\star$ with time for repeated stimuli of different intensities is shown in Supp. Fig. 11.

C. Response of the phototactic model to consecutive stimuli

We simulated the application of two consecutive, identical stimuli of intensity I_0 and duration 90 seconds, spaced by a time t_{pause}. We can then draw the phase diagram showing when the algae change behavior between the two stimuli. This phase diagram is shown in Supp. Fig. 12. In the diagram, regions filled with a unique color show when the behavior does not change between the two stimuli. Yellow: positive phototaxis. Red: back-and-forth behavior. Blue: negative phototaxis. Regions filled with hatched lines indicate a change in behavior between the two stimuli. Yellow and red hatches: the algae exhibit positive phototaxis in the first stimulus and back-and-forth in the second stimulus. Red and blue hatches: the algae exhibit back-and-forth motion in the first stimulus and negative phototaxis in the second stimulus.

D. Multiple changes in phototactic behavior

It is possible to switch from back-and-forth to negative phototaxis, and from positive phototaxis to back-and-forth in successive experiments separated by a short 90 s break, see Fig. 13.

E. Incorporating the inner biochemistry into the model of Arrieta et al. [8]

It is possible to incorporate the dynamics of $s^\star$ into another model of phototaxis, described in Arrieta et al. [8]. There, the authors report that *C. reinhardtii* describe loops around gaussian light sources, before escaping. They show that this behavior cannot be reproduced by a simple phototaxis model where the sign of phototaxis changes at a threshold intensity I_c. Arrieta et al. assume the position of a cell $\boldsymbol{x}(t)$ and its direction $\boldsymbol{p}(t)$ evolve according to:

$$\dot{\boldsymbol{x}}(t) = v_s \boldsymbol{p}(t) \qquad \text{and} \qquad \dot{\boldsymbol{p}}(t) = \boldsymbol{\omega} \times \boldsymbol{p}(t), \quad (4)$$

where v_s is the speed of a cell and $\boldsymbol{\omega}$ is its angular speed. The angular speed is supposed to be proportional to the local gradient in light intensity ∇I: $\boldsymbol{\omega} = \alpha \boldsymbol{p}(t) \times \nabla I$, where α is the phototactic parameter. In a simple assumption, $\alpha = 1$ (resp. -1) when the local light intensity is below (resp. above) a threshold I_c. This leads to the trochoid-like trajectory shown as a dotted black line in Supp. Fig. 14. We now incorporate our model of the dynamics of $s^\star$ into the phototactic parameter, and assume $\alpha = 1$ (resp. -1) when $s^\star \leq s_T$ (resp. $s^\star > s_T$). For a given set of parameters, this leads to the blue trajectory in Supp. Fig. 14: the algae loops around the light and escapes. The escape is due to the memory: due to a too long exposure to intense light, *C. reinhardtii* becomes negatively phototactic during a time $\approx \tau$. After this time, it has swam far away from the source, and does not feel the gradient of light anymore so does not come back towards the source.

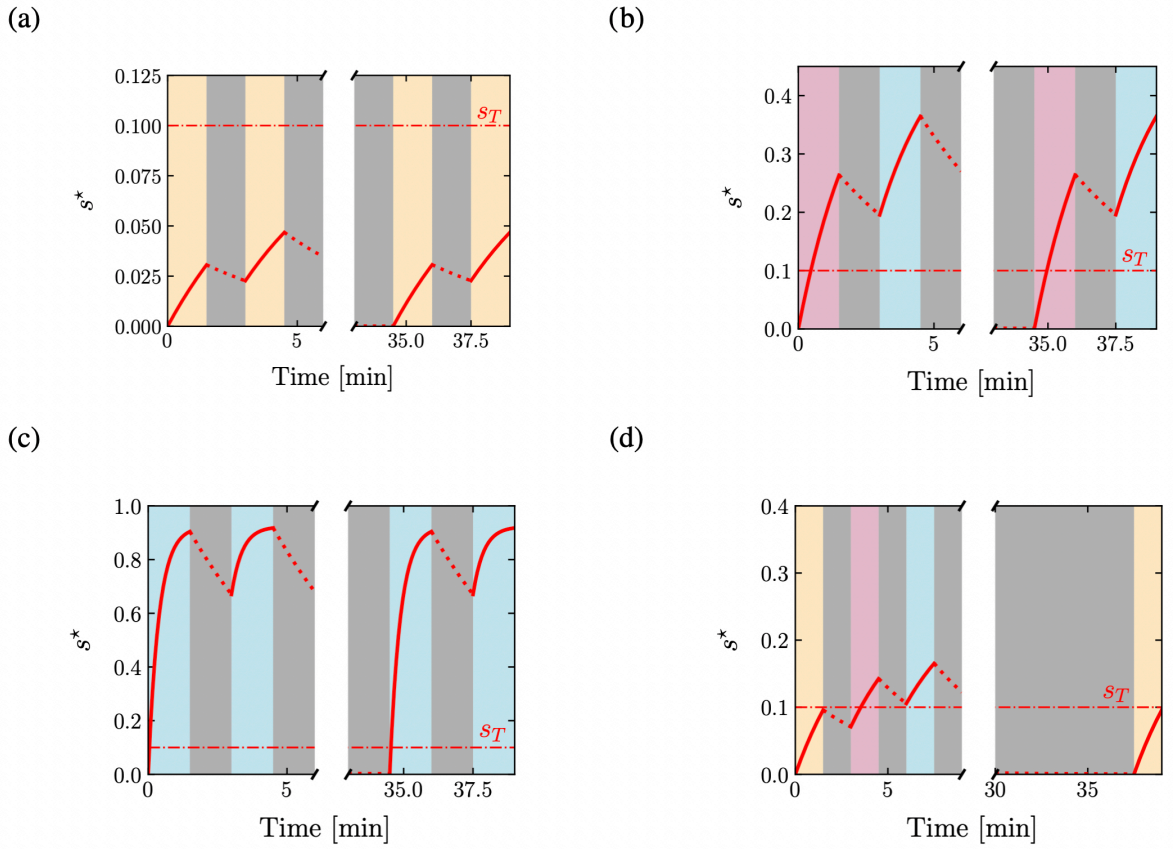
We choose parameters similar to those used by Arrieta et al. in our simulations: a gaussian source of peak intensity $I_0 = 260\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, with a standard deviation $\sigma_I = 700\ \mu$m. In the simple switch model, we simulate a change in phototactic sign at $I_c = I_0/2$, so that the algae exhibit positive phototaxis ($\alpha = 1$) for $I \leq I_0/2$, and negative phototaxis otherwise. Algae are made to start at position $(x_0, y_0) = (500, 500)\ \mu$m, at an angle of 200 degrees. The speed of the algae is $v_s = 50\ \mu$m/s. We obtain loops for $\gamma = 10^{-5}\ \text{m}^2 \cdot \mu\text{mol}^{-1}$ and $s_T = 0.1$. This is a very different value of γ from that used in our model. Yet, note that the algae in the experiments of Arrieta et al. were exposed to light for more than 10 minutes before being observed. It is likely that this induces adaptation, corresponding to a larger value of s_T than that of our model, where cells were kept in the dark before the experiments. Taking another value of s_T will affect the value of γ for which loops are observed.

F. Limits of the model

The model is extremely simple. It does not reproduce some very rare cases we observed, where the algae go back-and-forth twice in the well, see Supp. Fig. 15. Such a behavior could potentially be recovered by introducing another time scale in the model, responsible for adaptation of the algae, which would lead to a change in time of the threshold s_T.

[1] Rosenbaum JL, Moulder JE, Ringo DL. Flagellar elongation and shortening in Chlamydomonas: the use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. The Journal of cell biology. 1969;41(2):600-19.

[2] Lefebvre PA, Nordstrom SA, Moulder JE, Rosenbaum JL. Flagellar elongation and shortening in Chlamydomonas. IV. Effects of flagellar detachment, regeneration, and re-



Supp. Fig. 11. Simulation results for four consecutive experiments performed at a constant light intensity I_0, with different rest times in between. The concentration of active molecules s^* is tracked over time. Each experiment lasts for 90 s. Rest times are shown in shaded gray areas. ($t_{\text{pause},1} = 90$ s, $t_{\text{pause},2} = 30$ min and $t_{\text{pause},3} = 90$ s for (a), (b) and (c)). (a) When exposed to a low light intensity $I_0 = 0.2\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the alga always displays positive phototaxis. (b) At intermediate intensities $I_0 = 2\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the alga varies its phototactic behavior between each experiment. In the first experiment, the alga shows a back-and-forth motion. In the second, the alga shows only negative phototaxis. In the third experiment, the alga displays a back-and-forth motion again. Finally, in the last experiment, the behavior is negative phototaxis. (c) At a high intensity $I_0 = 20\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the alga always shows negative phototaxis. (d) At low to intermediate intensities $I_0 = 0.65\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and with adjusted pause times ($t_{\text{pause},1} = 90$ s, $t_{\text{pause},2} = 90$ s and $t_{\text{pause},3} = 30$ min) it is possible to successively go through positive phototaxis in the first experiment, back-and-forth motion in the second, negative phototaxis in the third and then revert back to positive phototaxis in the last experiment. Other simulation parameters: $s_T = 0.1$, $s_{\text{tot}} = 1$, $v_0 = 100\ \mu\text{m} \cdot \text{s}^{-1}$, $\tau = 300$ s and $\gamma = 0.002\ \text{m}^2 \cdot \mu\text{mol}^{-1}$.

sorption on the induction of flagellar protein synthesis. The Journal of cell biology. 1978;78(1):8-27.

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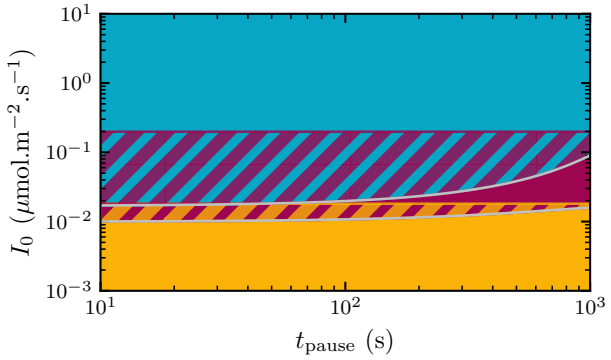
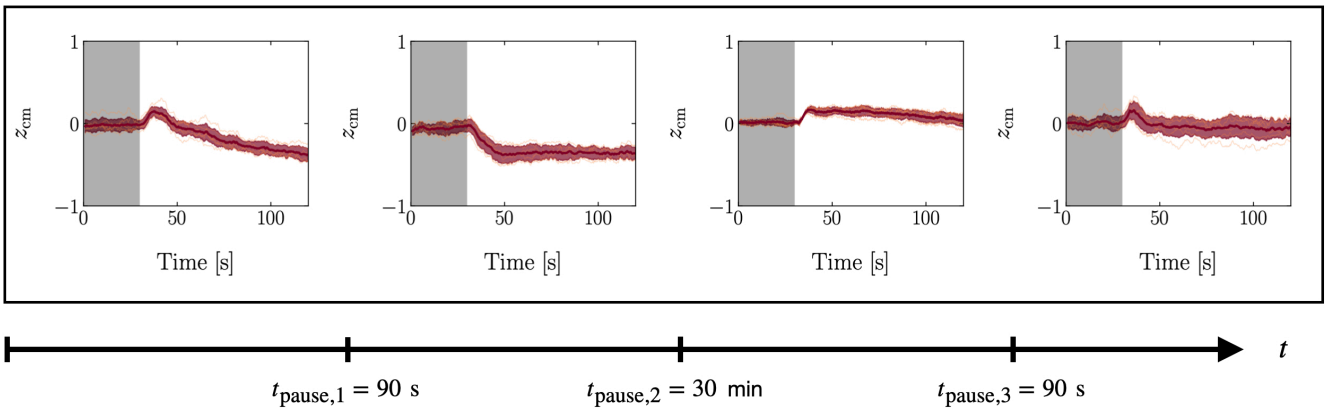
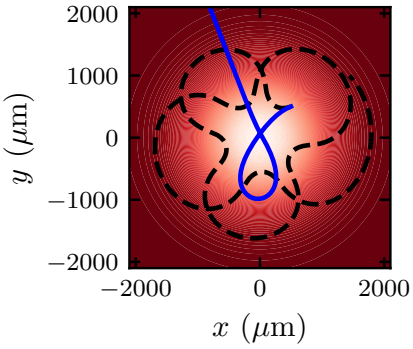


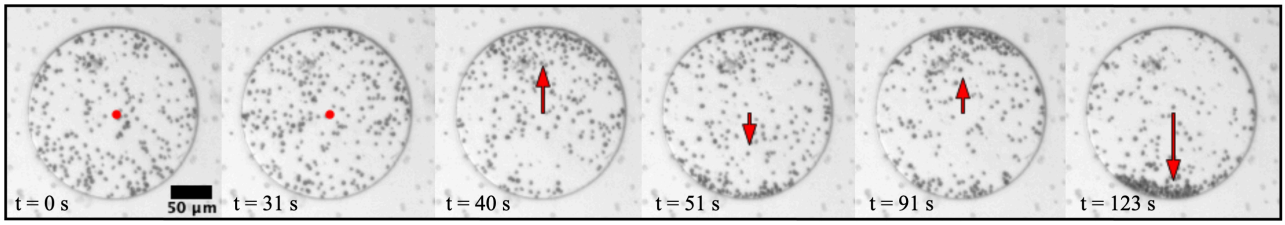
Supp. Fig. 12. Phase diagram of the phototactic behavior after two consecutive stimuli at the same intensity. Yellow: positive phototaxis. Red: Back-and-forth behavior. Blue: Negative phototaxis. Hatched regions indicate where the behavior changes between the first and second stimulus. Yellow and red hatch: positive phototaxis during the first stimulus, back-and-forth during the second stimulus. Blue and red hatch: back-and-forth during the first stimulus, negative phototaxis during the second stimulus. Simulation parameters: $\gamma = 2 \times 10^{-3}$ m$^2 \cdot \mu$mol^{-1}, $s_T = 0.1$, $\tau = 300$ s.



Supp. Fig. 13. Memory effects on the phototactic response of algae. After a pause of 90 seconds, it is possible to switch from back-and-forth behavior to negative phototaxis (Exp. 1 and 2), or from positive phototaxis to back-and-forth (Exp. 3 and 4). The light stimulus is turned on at $t = 30$ s and the position of the center of mass z_{cm} is tracked over time. Four consecutive experiments are performed with varying rest times, where the light is turned off in between. There is a 90 s pause between Exp. 1 and Exp. 2, then a 30 min pause between Exp. 2 and Exp. 3 and finally a 90 s pause between Exp. 3 and Exp. 4. At intermediate intensities $I = 0.46$ μmol $\cdot$ m$^{-2} \cdot$ s^{-1}, the algae show different phototactic behaviors in each experiment. The behavior switches from positive phototaxis to back-and-forth behavior between the third and fourth experiments. The center of mass is averaged over 6 experiments.



Supp. Fig. 14. Incorporating memory in the model of Arrieta et al. [8]. Black dotted line: simulated trajectory of a cell that exhibits positive phototaxis at light intensities $I < I_c$, and negative phototaxis otherwise. The shape of the trajectory is not the shape observed in experiments. Blue line: simulated trajectory of a cell whose phototactic behavior depends on the concentration $s^\star$ of an inner biochemical species, with a characteristic deactivation time $\tau = 300$ s. The cell makes a loop and then escapes.



Supp. Fig. 15. *C. reinhardtii* enclosed in a well. A blue light stimulus of intensity $I = 2.0\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ is turned on at $t = 30$ s. The stimulus comes from the upper side of the wells. In response to the stimulus, the algae go back-and-forth twice in the well.