

Transient state dynamics of *Chlamydomonas reinhardtii* cells during phototaxis

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The directed movement of microorganisms toward light is called phototaxis—a fundamental process shown by many uni- and multicellular photosynthetic microorganisms for their survival and growth. Here, we determine the time-dependent response of a population of model phototactic single-celled algae *Chlamydomonas reinhardtii* to changing light conditions. When the condition changes from dark to light, we show that the response of a dark-adapted isotropic population depends on not just the light intensity but also the cell number density. On the other hand, when conditions change from light to dark, the time dependence of the recovery from an aligned state to an isotropic state is independent of cell density and light intensity. We find that such kinetics emerges from the coupling between flagellar dynamics, cell speed, and incident photon flux. We further show that existing minimal noninteracting models, which robustly describe the steady-state properties of populations of *Chlamydomonas* under given light conditions, capture this time-dependent phenomenon only for dilute suspensions.

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I. INTRODUCTION

Collective behavior is ubiquitous in the microbial world wherein both free-swimming organisms such as *Escherichia coli* [1,2] and *Chlamydomonas reinhardtii* (CR) [3] and surface-associated organisms such as *Myxococcus xanthus* [4], *Paenibacillus vortex* [5], *Bacillus subtilis* [6], and *Proteus mirabilis* [7] self-organize to gain leverage in growth and survival. Interactions of individual cells with their neighbors lead to the emergence of this rich phenomenology [8]. Collective self-organization becomes tunable when the microorganisms undergo taxis, a fundamental stimulus response behavior. For example, bacterial cells sense chemical gradients and collectively move in the direction of the gradient [9–11]. Similarly, a wide variety of motile photosynthetic microorganisms steer themselves to align their swimming direction with the light stimulus direction, a phenomenon called phototaxis [12–15].

While collective effects in bacterial chemotaxis have been extensively studied [16–20], our understanding of collective phototaxis is limited [3,21] and has mainly been studied for single cells or dilute suspension. For example, it is well established that biflagellate CR cells scan their surroundings for light by rotating the cell body about its own axis and using the single eyespot as the detector [13,22–24]. Under light exposure, the beating of the *cis*-flagellum (closer to the eyespot)

is inhibited, whereas the beating of the *trans*-flagellum (away from the eyespot) is enhanced, resulting in aligning of the cell body toward the light source [22,25–31].

Recently, it has been shown that the steady-state collective phototaxis of CR cells is reentrant in nature due to the coupling between cell speed and the light detection mechanism of the cells [32,33]. It is unknown whether similar effects are present for the dynamical measures such as response time of the cells. We, therefore, use high-speed video microscopy to track individual CR cells in a population to determine the growth and decay timescales of orientational order within the suspension in response to switching on and off of the light, respectively, as a function of light intensity and cell concentration. We find that the response time of CR cells has a nontrivial dependence on cell concentration and light intensity when dark-adapted cells are exposed to light (step-up). In contrast, the timescale of loss of orientation of aligned phototactic cells due to switching off (step-down) of light stimulus is independent of the cell concentration.

II. RESULTS

A. Framework for analysis

Figure 1(a) shows a schematic of the experimental setup. The sample chamber is a three-dimensional rectangular chamber made of a glass slide and a coverslip sandwiching two double-sided tapes of thickness 66 μm separated by 5 mm. A blue laser beam at one end of the chamber acts as a phototactic light (see Appendix). We observe the cell suspension in the rectangular chamber (50 mm $\times$ 5 mm $\times$ 66 μm) on a phototactic setup using red light of wavelength 760 nm and above. Cell trajectories are captured over a long time (57.1 s) in 10 $\times$ magnification using a high-speed CMOS camera at 50

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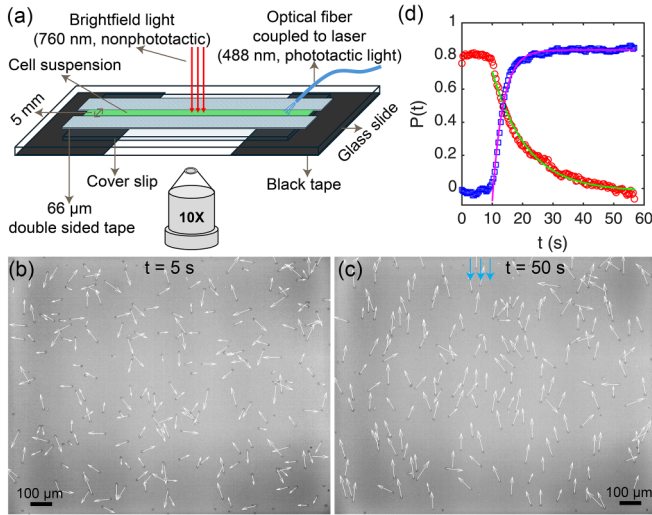


FIG. 1. Experimental setup and the kinetics of phototactic orientation during response and recovery. (a) Schematic of the experimental setup. The sample chamber comprises a glass slide and a coverslip sandwiching two double-sided tapes of thickness $66\ \mu\text{m}$ separated by $5\ \text{mm}$. The resulting rectangular channel is filled with cell suspension, and one end of the channel is illuminated by phototactic light. (b), (c) Representative bright-field image of CR cells under $10\times$ magnification recorded at 50 frames per second at a cell concentration of $1.1 \times 10^7\ \text{cells cm}^{-3}$ and at a light intensity $11.09\ \mu\text{E m}^{-2}\ \text{s}^{-1}$. Dark gray spots are CR cells, and their respective orientation vectors are shown as the white-colored arrow sign. The orientation vector of each cell at the given time t is obtained by adjoining the cell coordinates at times t and $t + 0.08\ \text{s}$. Orientation vectors in the image are rescaled uniformly for visual clarity. Cell orientations in the suspension are random in the dark (b). In contrast, after a few seconds of switching on the light, a large fraction of cells in the suspension reorient toward the light source (c). The blue arrows represent the direction of the light stimulus. (d) Polarization $P(t)$ as a function of time for response (blue squares) and recovery (red circles) at the same cell concentration ($1.1 \times 10^7\ \text{cells cm}^{-3}$) and light intensity ($11.09\ \mu\text{E m}^{-2}\ \text{s}^{-1}$). Initially, cells were in the dark (light), and the light stimulus was switched on (off) at $10\ \text{s}$ for the response (recovery). Solid lines are the corresponding exponential fits [$y = A(1 - \exp(-t/\tau_{\text{res}})) + C$ and $y = A(1 + \exp(-t/\tau_{\text{rec}})) + C$] of the response and recovery polarization from $10\ \text{s}$, resulting in the response time (τ_{res}) and recovery time (τ_{rec}).

frames per second. Two-dimensional coordinates of these trajectories are then used to evaluate the cell's orientation vectors with respect to stimulus light direction as a function of time. The direction of cell motion is completely random in dark conditions and the ensemble is best described as isotropic or disordered [Fig. 1(b)]. When the light is switched on, the ensemble undergoes a transition from an isotropic to an oriented state [Fig. 1(c)]. We capture the movement of the cells as a function of time to observe this transition. Imaging of cells initially kept in the dark is started followed by switching on of light stimulus at $10\ \text{s}$ (from the start of the imaging). The light is kept on until $57.1\ \text{s}$. The ensemble reaches steady state after few seconds wherein a large fraction of cells is reoriented toward the light source. We evaluate polarization $P = \langle \cos(\theta) \rangle$ [Fig. 1(d), blue squares] as a function of time to elucidate this transition. Here, the

angle brackets denotes average over all cells and θ is the angle between the light direction (here negative y axis) and the direction vector of a cell given by joining the cell coordinates at times t and $t + 0.02\ \text{s}$. This measurement is performed for varying light intensity (0.28 – $17.85\ \mu\text{E m}^{-2}\ \text{s}^{-1}$) and cell concentration (3.7×10^5 to $1.1 \times 10^8\ \text{cells cm}^{-3}$) (see Fig. 1 of the Supplemental Material [34]). Figure 1(d), blue squares, shows a representative plot of polarization as a function of time during response. Initially, the isotropic state has nearly zero polarization, $P(t < 10) = 0$. Polarization starts to grow for $t > 10\ \text{s}$ and saturates to a steady-state value close to 0.8 wherein a large fraction of cells move toward the light source. We fit the polarization $P(t)$ to an exponential growth function [$y = A(1 - \exp(-t/\tau_{\text{res}})) + C$], where A and C are constants, to obtain a characteristic timescale of the transition (response time) [Fig. 1(d), magenta line]. The data fit to the exponential growth function reasonably well with $r^2 \geq 0.95$. The time constant τ_{res} (response time) characterizes the time taken by the system to evolve from the isotropic state to the oriented state.

Observing the transition from a disordered state to an oriented state naturally led us to wonder about the transition the other way around, i.e., from an oriented state to a disordered state. Therefore, we also performed experiments wherein the oriented state is achieved by exposing the cells to light stimulus for $50\ \text{s}$, after which we start imaging of cell trajectories. The light stimulus is then switched off at $10\ \text{s}$ from the start of the imaging. A representative plot of polarization during the transition from the ordered state to a disordered state is shown in Fig. 1(d) (red circles). The dynamics of polarization for these recovery experiments at different cell concentrations and light intensities is shown in Fig. S2 of the Supplemental Material [34]. Kinetics of the decay [Fig. 1(d), red circles] of polarization shows that the oriented steady state has $P = 0.8$. The polarization starts to decay from $10\ \text{s}$ and approaches zero in the measured time window. It implies that a large fraction of cells initially oriented toward the light source are now moving in random directions in the absence of the light stimulus. An exponential decay function [$y = A(1 + \exp(-t/\tau_{\text{rec}})) + C$] is fit to the polarization $P(t)$ of the recovery [Fig. 1(d), green line]. Here, the time constant τ_{rec} (recovery time) characterizes the time of transition from an oriented state to an isotropic state.

B. Dependence of response and recovery timescales on conditions

If the dynamics of the transition is controlled solely by single-particle behavior, we should expect that the timescales should be independent of cell density, and its dependence on intensity should be determined by the biochemistry of the underlying process. Surprisingly, response time shows non-trivial dependence on cell density and intensity. The response is fast with an average value of $\approx 2.5\ \text{s}$, regardless of intensity at low cell concentrations [$\leq 1.1 \times 10^7\ \text{cells cm}^{-3}$, blue and green branch in Fig. 2(a)]. In contrast, the response is relatively slower (≈ 4 – $8\ \text{s}$) and nonmonotonically dependent on the light intensity at higher cell concentrations [orange and yellow branch, Fig. 2(a)]. This trend is more evident when response time is plotted as a function of cell concentration

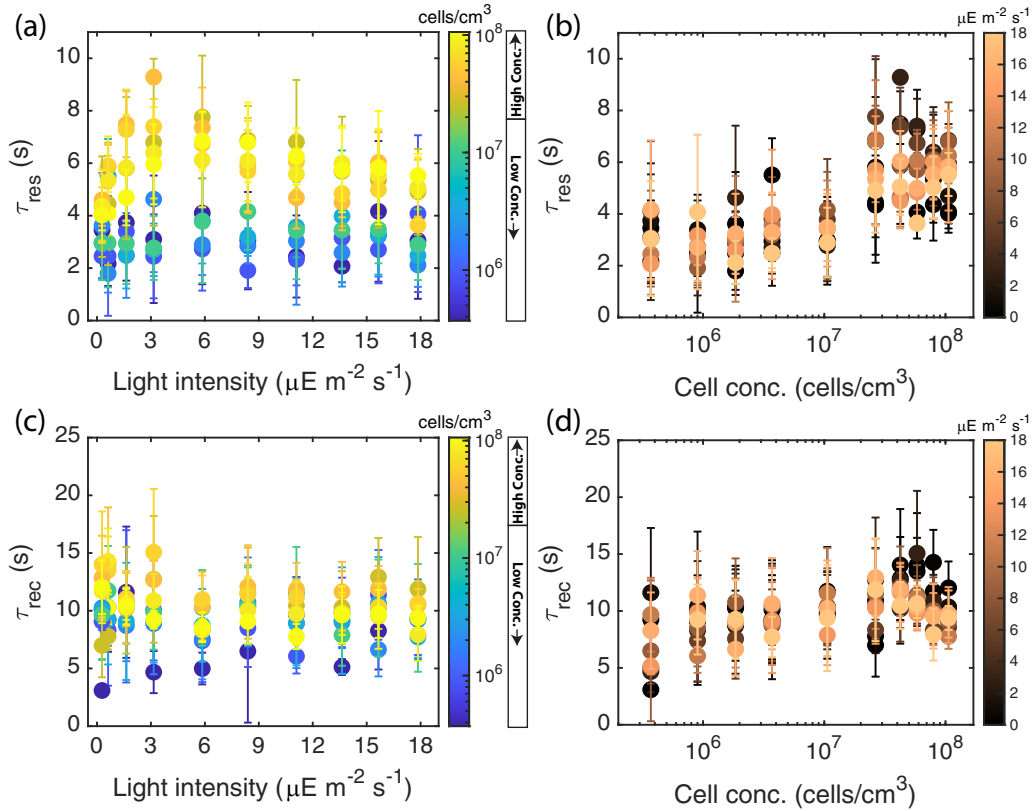


FIG. 2. The dependence of response and recovery time on the cell density and light intensity. (a) Response time (τ_{res}) as a function of light intensity at varying cell densities. At lower cell densities ($\leq 1.1 \times 10^7$ cells cm^{-3} , blue and green branch), the response is faster (≈ 2.5 s) and independent of light intensities. In contrast, at higher cell densities (orange and yellow branch), the response is slower (≈ 4 – 8 s) and follows a nonmonotonic dependence on light intensity. (b) Response time (τ_{res}) with cell concentration at varying light intensities. The plot shows the two distinct regions of the density-dependent response time. (c) Recovery time (τ_{rec}) as a function of light intensity at varying cell densities. (d) Recovery time as a function of cell concentration for varying light intensity. τ_{rec} (≈ 10 s) is independent of light intensity and cell density. The error bars in all the plots correspond to the standard deviation (SD) of respective physical quantities.

[Fig. 2(a)]. The two regions, namely, low cell concentration and high cell concentration, show faster and slower responses, respectively. A recent study [22] on free-swimming single CR cells shows that the timescale of the phototactic turning is ≈ 1 s. This timescale is less than the observed timescale in our case (2.5 s), but lies within the error bar; hence, we assume an agreement with the dilute suspension behavior in our case.

We plot the recovery time τ_{rec} as a function of light intensity for varying cell concentrations [Fig. 2(c)] and as a function of cell concentration for varying light intensities [Fig. 2(d)]. The data show that the recovery time constant is independent of light intensity and cell concentration (for the statistical significance, see Sec. 10 of the Supplemental Material [34]). We note that the recovery time constant is around 10 s.

C. Comparison of experimental and active Brownian particle model of dynamics of phototaxis

In order to uncover the origins of the collective response in the transient state dynamics, we now outline our current understanding of the steady-state properties of a population under constant light conditions. As shown in previous work [33], the orientation of cells with respect to the direction of

light is well described by a von Mises distribution of the form $e^{\kappa \cos(\theta)} / 2\pi I_0(\kappa)$, where $\kappa(\rho, I) = \gamma / D_R$ is a constant that depends on intensity and density, and I_0 is the modified Bessel's function of the first kind. This suggests that the dynamics of the system in the transient state can be captured by a time-dependent Fokker-Planck equation describing the probability density $\psi(\theta, t)$ for the orientations of cells of the form

$$\partial_t \psi(\theta, t) = D_R \partial_\theta^2 \psi(\theta, t) + \gamma \partial_\theta [\sin \theta \psi(\theta, t)], \quad (1)$$

where $\gamma(\rho, I)$ is a torque that depends on density and light intensity, and D_R is a rotational diffusion coefficient that is characteristic of single cells in the absence of light.

As a first step, we consider the recovery behavior within this paradigm. In the absence of light stimulus, the proposed dynamics equation (1) reduces to a rotational diffusion equation. Therefore, it predicts that $\psi(\theta, t) = \sum_{n=0}^{\infty} a_n \cos(n\theta) e^{-n^2 D_R t}$, where a_n 's are determined by the initial condition. In our experiments, the system is well described by the von Mises distribution before the light is switched off. Therefore, the model predicts that polarization $P(t) = [I_1(\kappa) / I_0(\kappa)] e^{-D_R t}$. As noted earlier, $\tau_{\text{rec}} \approx 10$ s is independent of cell concentration and intensity. This is consistent with

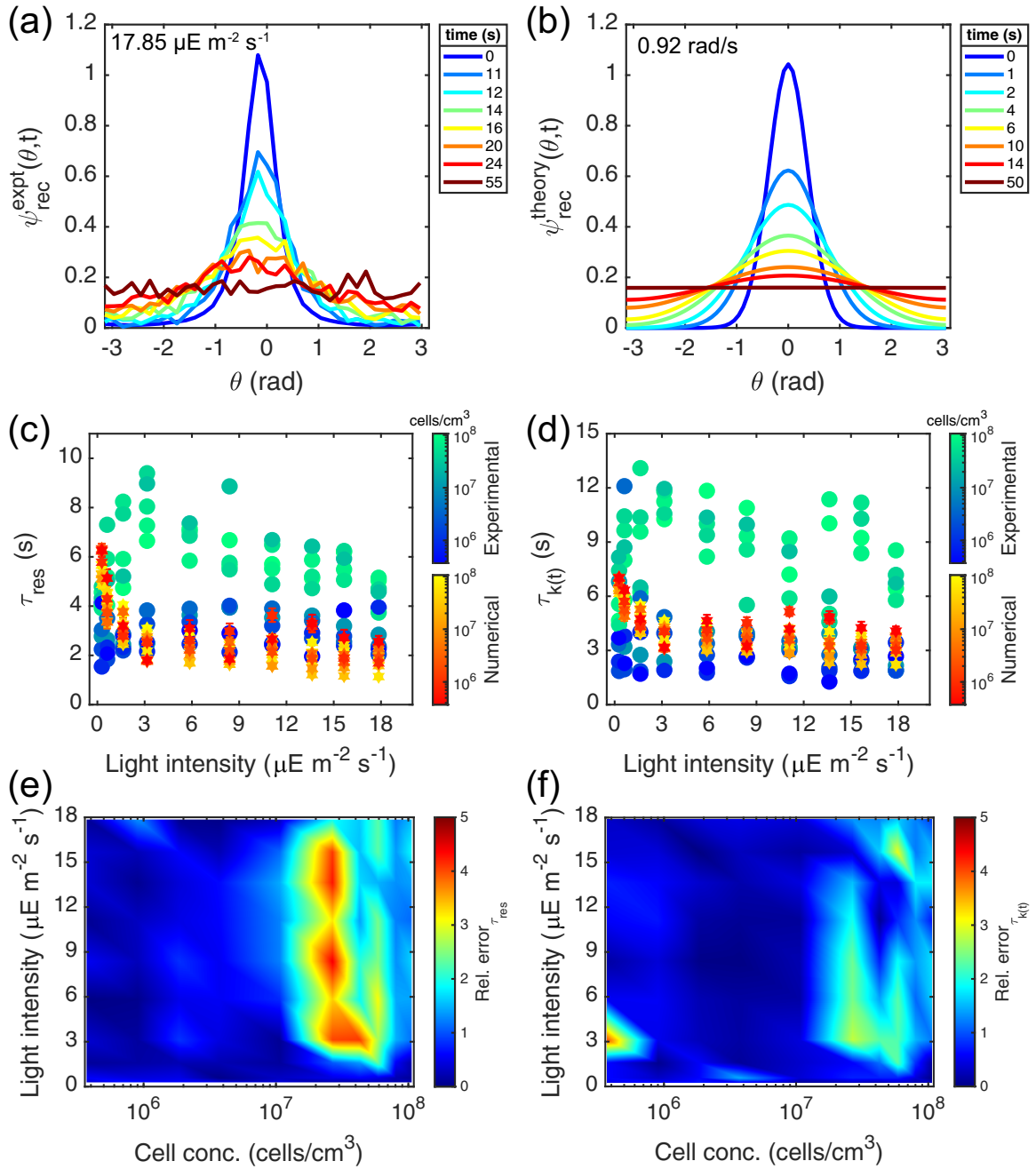


FIG. 3. Deviation of the theoretical model from the experimental observation. (a) A representative plot of the experimental probability distribution during recovery for the cells oriented (in the steady state) under a light intensity of $17.85 \mu\text{E m}^{-2} \text{s}^{-1}$ and at a cell concentration of $2.6 \times 10^7 \text{ cells cm}^{-3}$. The light stimulus is switched off at 10 s. (b) The theoretical probability distribution during recovery corresponding to the torque strength $\gamma = 0.92 \text{ rad s}^{-1}$ obtained experimentally for the light intensity and cell concentration as in panel (a). (c) Experimental (circles) and numerical (hexagrams) τ_{res} as a function of light intensity for varying cell concentration. (d) Experimental and numerical $\tau_{k(t)}$, the time constant obtained from $k(t)$, as a function of light intensity for varying cell concentration. The error bars in panels (c) and (d) for the experimental data are fitting errors that are small and within the symbol, whereas for the numerical data, they are obtained based on the SD of steady-state γ . To compare the experimental results more clearly with theory, we define the relative error as $e = (|\tau_{\text{num}} - \tau_{\text{expt}}|)/\tau_{\text{num}}$ and plot it in panels (e) and (f).

the theoretical paradigm as $1/D_R \approx 8 \text{ s}$ in our experiments (see Fig. S3 of the Supplemental Material [34]). The full probability distribution $\psi(\theta, t)$ from the theory [Eq. (A1)] and the experiments shows excellent qualitative agreement as well [Figs. 3(a) and 3(b)] [35]. We conclude that the recovery process is well captured by the single-cell behavior.

Now, we turn our attention to the response behavior of the population of cells. In order to characterize the transient state dynamics, we begin by considering the polarization $P(t) = \langle \cos \theta(t) \rangle$. Both the experimental data and the numerical solution of the theory have a time-dependent polarization that is well fit by an exponential growth function characterized by

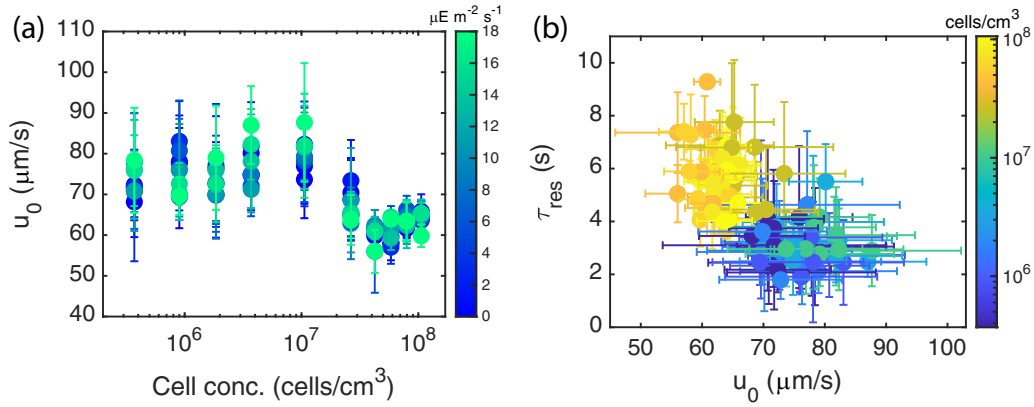


FIG. 4. Relationship between response time and initial cell speed in dark. (a) Initial cell speed (just before the introduction of light stimulus whose intensity value is given on the color scale) in dark u_0 as a function of cell concentration. The initial cell speed in the dark is high at low cell concentration, whereas it is low at high cell concentration. The error bars in the plot correspond to the SD of u_0 . (b) Response time as a function of initial cell speed in the dark for varying cell concentration and light intensity clearly depicts that the slow-moving cells respond slower and fast-moving cells respond faster to the light stimulus. This relationship is also evident by the direct comparison of initial cell speed in the dark as a function of cell concentration in panel (a) with response time as a function of cell concentration in Fig. 2(b).

a single time constant τ_{res} . Figure 3(c) compares the model to the experimental data. It shows that the experimental data have a nontrivial concentration dependence in its transient state dynamics that the theoretical model does not capture. So, even though the theory is robustly validated in the orientationally aligned steady state and its transformation to the disordered state in the dark, there are additional effects present in the experimental data when the dark-adapted cells are responding to a light stimulus.

To investigate the origin of this additional collective response of the cells to the stimuli, we carry out the following quantitative analyses. First, both the experimental data and the numerical solution of the time-dependent Fokker-Planck equation are well fit by a local normal solution of the form $\psi(\theta, t) \propto e^{\kappa(t) \cos(\theta)}$, with $\kappa(0) = 0$ corresponding to the isotropic state in dark and $\kappa(\infty) = \kappa = \gamma/D_R$ the coefficient of the von Mises distribution in steady state (see Fig. S4 of the Supplemental Material, which shows that this is indeed a good fit [34]). Further, $k(t) = \kappa(t)/\kappa_0$ (where κ_0 is the normalization factor; see Fig. S5 of the Supplemental Material [34]) is well fit by an exponential growth function $1 - \exp(-t/\tau_{k(t)})$ with a characteristic time $\tau_{k(t)}$. Figure 3(d) compares this time constant from both experiments and theory. We note that the experimental data show distinct behaviors at low and high concentrations of cells, while the theory does not. To compare the experimental results more clearly with those of the model, we define a relative error, $(|\tau_{\text{num}} - \tau_{\text{expt}}|)/\tau_{\text{num}}$. The relative error in τ_{res} and $\tau_{k(t)}$ is shown in Figs. 3(e) and 3(f), respectively. The error analysis in Figs. 3(e) and 3(f) elucidates that the noninteracting Fokker-Planck model captures the experimental observation in two conditions: (1) at low cell concentration, irrespective of the light intensity, and (2) at high cell concentration and low light intensity. The high cell concentration behavior of the experimental observation, except at low light intensity, shows a significant difference from the model prediction. So, for the experimental data, even though well fit by the local normal form that would follow from a Fokker-Planck description, the effective torque

experienced by the cells has additional density dependence that is not captured in the steady-state distributions. In addition, the Supplemental Material presents a more in-depth analysis, indicating that the experimental data fundamentally contradict the eigendecomposition of the Fokker-Planck operator [34].

D. Interplay of transient state speed and photon flux

Our experimental observations suggest that light stimuli affect cell speed. The cell speed corresponding to polarization data shown in Fig. S6 of the Supplemental Material [34] reveals four stages: (1) constant initial dark speed (before 10 s), (2) decrease in speed the moment cells sense the presence of light (at 10 s), (3) increase in speed during the transition period (10–30 s), and (4) increase in cell speed during the time when polarization shows steady-state behavior (30–57 s). A sharp decrease in speed at 10 s shows that cells momentarily slow down when they see the light. After that, the speed recovers and exceeds even the baseline initial dark speed during the transition when the cells orient themselves toward the light direction. These findings that cells change speed in response to light stimulus lead us to explore whether the initial dark speed has any relationship with the measured response time.

Initial cell speed in the dark is the mean speed of cells when cells are dark adapted just before the introduction of light stimulus to the cell suspension. Initial cell speed in the dark as a function of cell concentration [Fig. 4(a)] reveals that cell speed is higher at lower cell concentration, and vice versa. Comparing this cell concentration-dependent initial speed with the cell concentration-dependent phototactic response [Fig. 4(b)] manifests that slow-moving cells respond slower and fast-moving cells respond faster to the light stimulus. The close connection between the initial state of the system, namely, the initial dark speed, and the concentration dependence of the response time indicates that perhaps there could be a similar relationship between the cell speed during the transition state [10–30 s in Fig. 1(d)] and response time.

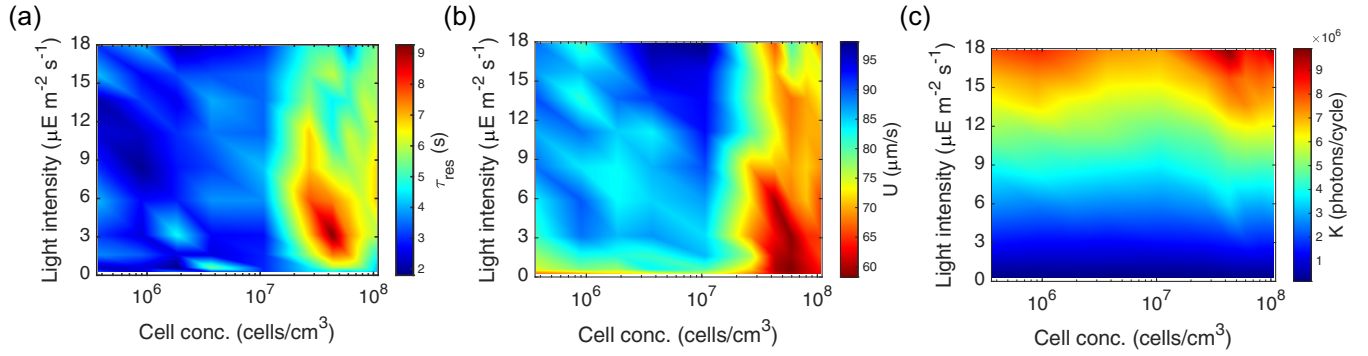


FIG. 5. Effect of transition state dynamics on the response time. (a) Heat map representing response time. Response time at low cell concentration is faster irrespective of intensity, whereas at high cell concentration, response time is relatively slower and depends on light intensity. (b) Heat map representing $U = (1/20\text{ s}) \int_{10\text{ s}}^{30\text{ s}} u dt$. The average linear speed of the cell during the transition (10–30 s), U , shows high speed at low cell concentration irrespective of light intensity. At high cell concentrations, the speed is slower and depends on light intensity. The heat map of U shows direct correspondence to the heat map of τ_{res} , except at the lowest light intensity. (c) Heat map representing $K = (1/20\text{ s}) \int_{10\text{ s}}^{30\text{ s}} F dt$, where F is photons per cycle. The average number of photons per cycle during the transition period, K , primarily depends on the light stimulus intensity and remains nearly constant with cell concentration. However, at a given light intensity, the average number of photons per cycle is higher in the high-concentration regime due to lower cell speed. The average number of photons per cycle in the transition period alters the swim speed, eventually affecting the response time, especially at high cell concentrations.

The monotonic increase in speed during transition depends on light intensity and cell concentration (see Fig. S6 of the Supplemental Material [34]). We compute the average cell speed during the transition period, $U = (1/20\text{ s}) \int_{10\text{ s}}^{30\text{ s}} u dt$, where u is the linear speed. The quantity U is shown in Fig. 5(b) as a heat map plot in cell concentration and light intensity plane. We find that U is higher and independent of light intensity at low cell concentrations. In contrast, U is lower at high cell concentrations and has a nonmonotonic dependence on light intensity. Remarkably, U shows a direct correspondence to the heat map of the response time τ_{res} in Fig. 5(a). This correspondence rather explains our observed phenomenology in a succinct way. This can be understood by the mechanism of how cells turn toward the light. The cell turns toward the light by making the beating of the *trans*-flagellum (farthest from the eyespot) stronger relative to the *cis*-flagellum (closest to the eyespot) [25,26]. Under light exposure, the enhancement in the beating of the *trans*-flagellum of the faster cell will differ from the slower one as the global beat frequency of the cell ν_b will be a determining factor. If ν_b is higher, the enhancement in the beating of the *trans*-flagellum will be stronger compared to that in the slower cell. The speed u of the cell depends on the beat frequency ν_b with $u \propto \nu_b$. Hence, higher speed corresponds to the higher ν_b and stronger enhancement in the beating of the *trans*-flagellum, turning the cell faster toward the light. The slower cell will have lower ν_b and weaker enhancement in the *trans*-flagellum, causing it to turn slowly.

At a more microscopic level, the intensity dependence of response time must be connected to the average number of photons reaching the cells. Therefore, we quantify the photon flux per cell by evaluating the cumulative number of photons falling on the eyespot per rotation of the cell body about its axis, $F = \alpha(2\pi/\omega) \times N_p$, where N_p is the number of photons falling on the eyespot per second (photon flux to the eyespot) and ω is the angular speed. The factor α accounts for the light-dark modulation and varies from 1/2 to 1 as the ensemble

reorients from dark to light (see Sec. 7 of the Supplemental Material [34]). ω is given by $u \times 2\pi/p$, where u is the linear speed and $p = 40\text{ }\mu\text{m}$ is the pitch of the helical path of the swimming cell, which is independent of cell concentration and light intensity (see Fig. S7 and Sec. 8 of the Supplemental Material [34]). The inverse proportionality of photon flux per cell and angular speed reveals that slower cells will receive a larger number of photons per cycle, and vice versa. Again, to quantify the average number of photons reaching the eyespot per cycle in the transition regime, we evaluate $K = (1/20\text{ s}) \int_{10\text{ s}}^{30\text{ s}} F dt$. The heat maps of K [Fig. 5(c)] and τ_{res} [Fig. 5(a)] show that at low concentrations the response time of the cells has the least value achievable by isolated cells and therefore is independent of the average number of photons falling on the cell eyespot per cycle in the transition period. The response time of the cells at high cell concentrations decreases with the increase in the average number of photons falling on the cell eyespot per cycle in the transition period. This is consistent with how sensory apparatus in biology shows signal-dependent response. That is, the larger the input stimuli, the larger is the transducer (sensor) output.

III. DISCUSSION

To summarize, we have presented a systematic quantitative measurement of the kinetics of the transition from an isotropic to an oriented state and vice versa during the phototaxis of a population of CR cells. We find that response is fast, irrespective of light intensity at low cell concentration. In contrast, the response is slow and depends nonmonotonically on the light intensity at high cell concentration. The transition from an oriented state to the isotropic state shows a characteristic time independent of light intensity and cell concentration and has a value close to the inverse of the rotational diffusion coefficient.

The theoretical model developed here considers cells as noninteracting active Brownian particles and successfully

captures the behavior of dilute suspension of CR cells. However, predictions of the model disagree with experimental measurements at high cell concentrations for the case of the response of dark-adapted cells to light stimulus. This implies that at high cell concentrations, intercell interactions become significant. Future studies are required to identify the nature of interactions in the system at high cell concentrations.

We have identified that the linear speed of the cell is tightly coupled with response time. The initial dark speed of cells affects the cell concentration-dependent response. As the initial dark speed is independent of light intensity, it does not correspond with the intensity-dependent response time at high cell concentrations. Instead, the intensity-dependent cell speed during the transition period elucidates the variation of response time with light intensity at high cell concentrations. We note that the underlying mechanism for the reduction in swim speed at higher densities remains unknown. Hydrodynamic effects [36,37], steric interactions [38–41], or changes in metabolic activity [42] may decrease cell speed as density increases.

Previously, we investigated the steady-state phototactic response of CR cells [33]. Here, we extend our study to explore the transient response kinetics of cells during light-on (step-up) and light-off (step-down). We emphasize that the current study reproduces the reentrant nature of phototactic efficiency in the steady state shown in our previous work (see Fig. S8 and Sec. 9 of the Supplemental Material [34]). Although the mechanism of higher efficiency in the steady state is linked to the decrease in steady-state speed (higher photon flux), the transition state dynamics reveals that the slow cell (higher photon flux) will respond slowly, and vice versa.

Recent few studies have reported short-term memory effects and acclimation in the phototactic response of microalgae [43,44]. These higher-order effects were found to be dominant when the cells were dark adapted for a very short period (90 s) between consecutive rounds of switching on of the light stimuli. Our study used a dark adaption time of 360 s between successive response experiments. We also find that the cell speed in the dark after this adaptation is nearly independent of the previous stimulus exposure and similar to the cell speed in the unexposed (to the light stimulus) sample [Fig. 4(a)]. Hence, we believe that our adaptation time is sufficient to relax the sample to its initial starting state. However, it is unclear whether the memory effects played any role in our study.

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We have not used AI-assisted technologies in creating this article.

This work did not require ethical approval from a human subject or animal welfare committee.

We declare we have no competing interests.

DATA AVAILABILITY

The data that support the findings of this article are openly available [45].

APPENDIX: MATERIALS AND METHODS

1. Culture conditions for growing CR cells

Chlamydomonas reinhardtii (wild-type strain, CC1690) cells were grown in TAP medium at 25 °C under cool white (6500 K) fluorescent lighting of intensity of $30 \mu\text{E m}^{-2} \text{s}^{-1}$ in an orbital shaker (137 rpm). Cells were kept under 12 h/12 h light/dark cycle to obtain synchronized cultures. They were harvested for the experiment after 48 h (second day) of inoculation in the middle of the exponential phase. The consistency of harvesting the cell at this particular time has been maintained for reproducibility.

2. Preparation of glass chambers for microscopy observations

Glass slides (75 mm $\times$ 25 mm $\times$ 1.35 mm) and coverslips (50 mm $\times$ 22 mm $\times$ 0.13 mm) were cleaned thoroughly with hot 1% soap solution (Hellmanex, Helma Analytics), ethanol, and 100 mM potassium hydroxide in subsequent steps. Further, cleaned coverslips and glass slides were coated with polyacrylamide brush to prevent adhesion between flagella and the glass surfaces [46]. A rectangular three-dimensional chamber was made with a coated glass slide and a coverslip using double-sided tapes of 66 μm (3M Scotch) as a spacer. Two double-sided tapes (of length slightly larger than the coverslip) separated by 5 mm were sandwiched between the glass slide and the coverslip. Cell suspension (with native media) was injected in the experimental chamber (50 mm $\times$ 5 mm $\times$ 66 μm) using the pipette, and the two openings (inlet and outlet) of the chamber were sealed with UV glue (Norland Optical Adhesive) (by treating it under UV for 20 s) to avoid convective flow in the chamber. Finally, black tapes ($\sim$ 17.5 mm wide; Achem Wonder) were placed from both ends of the chamber till 2.5 cm in the longitudinal direction toward the coverslip side. Thus, on the inverted microscope, black tape at the bottom of the chamber blocks the light stimulus passing through the other side of the transparent chamber and prevents backscattered light from any surfaces that could interfere with the light stimulus spot.

3. Tuning the cell concentration and its measurement

Low cell concentration for the experiment was achieved by diluting the culture with the native media obtained by centrifuging another aliquot of the same culture at 300 rcf for 3 min. On the other hand, high cell concentration was obtained by centrifuging the culture at 300 rcf for 3 min and resuspending it in the appropriate volume of native media.

The cell concentration was measured precisely in the middle of the x , y , and z dimensions (50 mm $\times$ 5 mm $\times$ 66 μm) of the rectangular chamber [which also corresponds to our field of view (FOV)]. The total number of cells in the FOV of dimension 1280 pixel $\times$ 1024 pixel was obtained using image analysis in MATLAB. The depth of focus (10 μm) of the objective (10 $\times$) is considered the effective height of the chamber in which cells were imaged. The cell concentration was determined by dividing the total number of cells by the image area times the depth of focus.

4. Imaging procedure

The sample was placed on the inverted microscope (Olympus IX73) under red light illumination (760 nm and above). The algae suspension inside the chamber was kept in the dark under dim red light illumination for approximately 40 min for dark adaptation before the experiment. Cell trajectories were imaged using red light and a high-speed CMOS camera (PCO 1200hs) in a bright-field setup under $10\times$ (Olympus, PlanC N, $10\times/0.25$) magnification at 50 frames per second. Each sample was introduced to only three to four light intensities during the measurement. The cells were dark adapted for 6 min between consecutive rounds of switching on of the light stimuli. Fresh samples were used for further light intensities to maintain the required cell concentration in the FOV and to avoid adaptation to higher light intensities. Thus, for a single sample at fixed cell concentration, the measurements were performed for two to three light intensities in increasing order, and then a fresh sample with the same cell concentration was used for the subsequent two to three light intensities and so on till the highest light intensity. In a similar fashion, experiments were repeated over multiple days at the same cell concentration for varying light intensity to achieve robust statistics. Consequently, we get multiple trials (repeats) at a single intensity and cell concentration.

5. Light stimulus source

A blue laser (CW diode laser, Necsel, PD-LD Inc.) of wavelength 488 nm was used as a stimulus for the algae suspension. The laser was coupled to an optical fiber through a plano-convex lens. The guided light through the optical fiber (LMA-GDF-15/130, Nufern) is illuminated at one end of the chamber at an angle of $\approx 30^\circ$ from the horizontal plane (x - y) of the chamber to act as a stimulus source. Due to this, there is a light gradient across the channel to which the cells respond. The light intensity in the field of view was calibrated and measured using the method described earlier [33].

The laser light beam (through the electronic shutter) and the camera were controlled by LabVIEW using a data acquisition device (National Instruments). The light stimulus was switched on exactly at 10 s after the imaging started for the response measurement. In the case of recovery, cells were under light for 50 s to reach a steady state and then imaging was started, followed by turning off the light stimulus at 10 s.

6. Particle tracking

2856 grayscale 16-bit image sequences were acquired at 50 fps for each movie. A $10\times$ magnification provides a large depth of focus, which enables us to record two-dimensional

(2D) projection of cell trajectories lasting typically 40 s. Particle tracking was performed in MATLAB using custom-written image processing codes and a standard particle-tracking algorithm [47]. In order to get robust statistics, 10^3 – 10^4 trajectories were analyzed at a given cell concentration and light intensity.

7. Measurement of rotational diffusion coefficient

Rotational diffusion coefficient D_R was obtained from the two-dimensional cell trajectories in the dark at different cell concentrations using the equation of 2D orientational diffusion: $\langle [\Delta\theta(\Delta t)]^2 \rangle = 2D_R \Delta t$. $\langle [\Delta\theta]^2 \rangle$ is the mean square angular deviation (MSAD) defined as $\langle [\theta(t + \Delta t) - \theta(t)]^2 \rangle$ as a function of delay time Δt , where $\langle \dots \rangle$ designates a time average; $\theta(t) \in [0, 2\pi]$ is the angle between a fixed reference vector (here negative y axis) and the direction vector at time t obtained by adjoining the position coordinates at times t and $t + 0.02$ s. The angular deviation was unwrapped ($\Delta\theta \in [0, \pi]$) prior to the calculation of MSAD.

8. Calculation about the theory of recovery

The initial condition of the diffusion equation is the von Mises distribution $e^{\kappa \cos \theta} / 2\pi I_0(\kappa)$ fitted by the steady state in response. Here, κ is the fitting parameter as a function of cells' density and light intensity, and I_0 denotes the modified Bessel function of the first kind of order 0. The analytical solution for the diffusion equation is expressed by the infinite series:

$$\psi(\theta, t) = \frac{1}{2\pi} + \frac{1}{\pi} \sum_{n=1}^{\infty} \frac{I_n(\kappa)}{I_0(\kappa)} \cos(n\theta) e^{-n^2 D_R t}. \quad (A1)$$

Since the higher order decreases significantly faster, we retained the first ten terms here for the numerical values as the remaining terms are negligible. The polarization, as the first moment, could be derived immediately by orthogonality:

$$P(t) = \frac{I_1(\kappa)}{I_0(\kappa)} e^{-D_R t}, \quad (A2)$$

with the timescale $\tau_{\text{rec}} = 1/D_R$.

9. Numerical solution of Fokker-Planck equation

The numerical analysis of Eq. (1) is conducted using MATHEMATICA, a computational software system that provides a comprehensive capabilities for mathematical and symbolic computations [48]. The numerical solution is obtained by the NDSolveValue function, utilizing the default solver with the maximum step size of 0.01. Fitting procedures for von Mises distribution, exponential decay, and others are performed by the NonlinearModelFit function.

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