Dynamic optical rectification and delivery of active particles: Supplementary Information

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1 Extracting the bacterial density

Under our experimental conditions, the image pixel intensity variance σ_0^2 , is proportional to the cell density. Since bacteria show up phase dark, one might expect the average intensity to be vary linearly with the cell number density. However, the phase-dark image of each bacterium is surrounded by a rather wide brighter halo. The difference in the average intensity between an image with and without a bacterium is therefore close to zero, and difficult to extract above the background noise. We find, however, that the variance of the image intensity in the dilute limit has no such issue, and increases linearly with the number of bacteria¹.

For different areas of our experimental images, Fig. 1, we show the normalized variance $\sigma_0^2(t)/\sigma_0^2(0)$ as a function of time, Fig. 2). The areas correspond to the centre areas of the static and dynamic patterns (squares of $110 \mu m \times 110 \mu m$), as well as a $21 \mu m$ -thick border of the experimental image, where the bacteria are not illuminated by green light and so do not swim. We analysed an area in the centre of each pattern that is slightly larger than the actual central dark square to ensure that the whole region of interest is included.

The dynamic and static areas behave as discussed in the main text. However, the normalized variance from the border shows a reduction from 1.0 to 0.8 within a few minutes, Fig. 2 (•). The border is populated by non-swimmers, so that we do not expect the cell concentration to change within this time scale. Indeed, visual inspection of the images showed no decrease in the border cell density. Using borders of thickness $14\mu m$ and $7\mu m$ produced exactly the same result. The observed reduction of the image, which was noticeable to direct visual inspection. Figure 3 shows the normalized variance of an image of a constant density of nonswimming bacteria at various degree of defocussing (where z = 0 is where the image is in focus). We find that a drift of about 4 microns is enough to produce the degree of reduction in the normalized variance seen in the border data in Fig. 2.

We correct for this effect by normalising the variance of the patterns by the change in variance of the border at each timepoint. The variance used in the main text then becomes:

$$\sigma^{2}(t) = \sigma_{0}^{2}(t) [\sigma_{\text{Border}}^{2}(t) / \sigma_{\text{Border}}^{2}(0)]^{-1}.$$
 (1)

In any case, this correction to the concentrations is minor and does not affect any substantive points or conclusions set out in the main text.

2 Differential Dynamic Microscopy analysis

The swimming speed of the bacteria under green illumination was quantified with differential dynamic microscopy (DDM), a high-throughput technique used for measuring the motility of swimming microorganisms^{2–4}. Acquiring movies as described in the main text (10x Phase Contrast, 50 frames per second), we calculated the differential image correlation function $g(q, \tau)$, which is the power spectrum of the difference between pairs of images delayed by time τ , with q the spatial frequency defining the length scale of interest $L = 2\pi/q$. For isotropic motion and appropriate imaging conditions^{3,4}, $g(q, \tau)$ is related to the intermediate scattering function $f(q, \tau)$, which is the q^{th} Fourier component of the



Figure 1 Image of the experiment at $t = 400 \, \text{s}$. The variances of the highlighted areas as functions of time are shown in Fig. S2. Other patterns in the image correspond to different speeds and conditions discussed in the main text.

density temporal autocorrelation function, via:

$$g(q,\tau) = \mathscr{A}(q)[1 - f(q,\tau)] + \mathscr{B}(q)$$
⁽²⁾

Here $\mathscr{B}(q)$ relates to experimental setup noise and $\mathscr{A}(q)$ is the signal amplitude. Fitting to a suitable swimming model for *E. coli*, we obtain the average speed, the width of the speed distribution, the fraction of non-motile bacteria and their diffusion coefficient.

DDM allows us to extract bacterial swimming speeds at 1 s intervals¹. By alternately switching the light on and off, Fig. 4, we determined two separate time scales (τ_A and τ_B) with which our cells adapt to the current illumination level (see main text equations (4) and (5) respectively). Fitting the observed time-dependent speed to

$$\bar{v}(t) = ABv_0, \tag{3}$$

$$\dot{A} = (I - A)/\tau_A,\tag{4}$$

$$\dot{B} = (I - B) / \tau_{\rm B},\tag{5}$$

where *I* is the state of illumination (1 for on and 0 for off) (compare main text equations (2), (4) and (5)), we obtained $v_0 = 9.2 \mu \text{m s}^{-1}$ for the speed of the bacteria under steady-state illumination, A(t = 0) = 1, B(t = 0) = 0.926, $\tau_A = 1.6$ s and $\tau_B = 100$ s.

Note that the fitted saturation speed here is different from that in the main text because of the higher illumination used in these measurements. We assume that $\tau_{A,B}$ are not dependent on the saturation speed, which is justified *a posteriori* by the fact that these values of $\tau_{A,B}$ are found to reproduce the data in the main text using our 2D simulations. The independence of the short time, τ_A from the starting intensity has also been explicitly validated in previous experiments¹.

Physically, the short τ_A is determined by the charge/discharge time of the equivalent membrane capacitor and resistor, while the longer τ_B is determined by the time to reassemble/dissassemble



Figure 2 Normalized variance as a function of time for three different areas in the experimental images corresponding to the dynamic and static areas in Fig. 1 of the main text, as well as the image border area where bacteria are not illuminated.



Figure 3 Normalized variance of a non-swimming bacteria sample (optical density \approx 5) as a function of *z*, the focal distance from the bottom coverslip.

stator units for the rotary flagella motors (see 1 and references therein).

3 Rotational Diffusion through tracking

To extract the rotational diffusion coefficient of our bacteria during our experiments, we tracked the swimming *E. coli* and calculated their velocity autocorrelation function. We focussed on a single static light pattern, where the bacteria in the lit region became dilute enough to track. The original images required some pre-processing however, to exclude non-swimmers from the tracking. A moving 2 s average is subtracted from each image before a centre-of-mass tracking algorithm is implemented, Fig. 5 (a, b, c). The tracks from a total of 30 movies of 20 s each at 50 frames per second are then analysed. Using only those tracks in the lit area of the pattern of 3 s duration or longer, we calculated the velocity autocorrelation functions and fit the average with a single exponential decay

$$\langle v(0)v(t)\rangle = V^2 \exp(-D_{\mathbf{R}}t).$$
(6)



Figure 4 The response of the bacterial swimming speed to on-off cycles of illumination (white background = on, grey background = off). Filled circles = data; line = fit to equations (3)-(5).

Here V is the tracked bacteria speed ($V \approx 3.3 \mu \text{m} \text{s}^{-1}$ in the annulus) and the rotational diffusion coefficient was found to be $D_{\text{R}} = 0.05 \pm 0.01 \text{s}^{-1}$, see SI Fig.5d. Corroborating the tracking measurments, DDM of the bacteria in the annulus produced $v_{annulus} = 2.8 \pm 0.3 \mu \text{m} \text{s}^{-1}$.

4 1-Dimensional Theory

We here calculate the mean speeds of left and right going bacteria in a moving, 1D, periodic light field of infinite extent. These calculations are equivalent to those in ref.⁵. In this model, bacteria swim either right (+ve x-direction) or left, at a speed v in the light, and v' = 0 in the dark. They undergo temporally uncorrelated tumbles with a rate k, with equal probability into either direction. A pattern of light and dark bands is applied (the light field), which moves at speed u to the right. In a frame moving with this light field, there are periodic boundary conditions at x = 0 and $x = \Lambda$, and the region $0 \le x < \alpha \Lambda$ is light, with $\alpha \Lambda \le x < \Lambda$ being dark. The Fokker-Planck equation for this system is

$$\frac{\partial f_{\pm}(x)}{\partial t} = -\left[\pm v(x) - u\right] \frac{\partial f_{\pm}(x)}{\partial x} + \frac{k}{2} \left[f_{\mp}(x) - f_{\pm}(x) \right], \tag{7}$$

where the position dependent speed V(x) is v or 0 in the light and dark respectively. The first term accounts for self-propulsion in either direction, and the second term for tumbling. Note that tumbling may result in no change of direction, which accounts for the factor of 1/2.

Eq. (7) applies at all points apart from x = 0 and $x = \alpha \Lambda$, where there are specific boundary conditions. For u > v, the boundary condition at each boundary consists in simply equating the fluxes

$$j_{\pm} = [\pm V(x) - u] f_{\pm}(x), \qquad (8)$$

on either side of the boundary. For u < v, the situation is rather more involved. Right-going bacteria which reach the light-dark boundary at $x = \alpha \Lambda$ become trapped there, moving at speed u, until they tumble away. This means there is a δ -function concentration F_+ of right-going bacteria at this boundary. The amplitude F_+ of this peak couples to the left-and-right fluxes via modified boundary conditions:

$$j_{\pm}(x \to \alpha \Lambda|_B) - j_{\pm}(x \to \alpha \Lambda|_D) = \pm \frac{k}{2} F_+, \qquad (9)$$



Figure 5 Extraction of the rotational diffusion coefficient of the bacteria (D_R). (a) The original phase-contrast image (b) The image after subtraction of a 2s moving average (c) Superposition of the bacterial tracks over 2 s (d) The experimental velocity autocorrelation function (solid line), showing a fitted single exponential decay and a fit (dashed line), giving a $D_{\rm R} = 0.05 \pm 0.01 \, {\rm s}^{-1}$.

which accounts for the entry of right-going bacteria into the boundary from both sides, and the transformation of trapped rightgoing into free left-going bacteria by tumbling. Here, the arguments in brackets indicate the limit is to be taken approaching the boundary at $\alpha \Lambda$ either from the bright (B) or dark (D) side. At the other boundary we have $f_+(0) = 0$, since no right-going bacteria can reach this boundary (they move too slowly in the dark, and too quickly in the light), whereas there is the usual balance of fluxes for the left-going bacteria here.

A final condition is to fix the integrated densities of left and rightgoing bacteria to 1 (or some other arbitrary value) i.e.,

$$1 = F_{+} + \int_{0}^{\Lambda} f_{+}(x) dx = \int_{0}^{\Lambda} f_{-}(x) dx.$$
 (10)

Setting Eq. (7) equal to zero then enables a steady-state solution for the bacterial density profiles $f_{\pm}(x)$ to be found, after some algebra; these are piecewise sums of exponentials. From these density profiles, the mean speeds of rightgoing $\langle v_+ \rangle$ and leftgoing $\langle v_- \rangle$ bacteria are obtained by integrating the speed over the whole domain in the lab frame

$$\langle v_+ \rangle = uF_+ + v \int_0^{\alpha \Lambda} f_+(x) \mathrm{d}x,$$
 (11)

$$\langle v_{-} \rangle = v \int_{0}^{\alpha \Lambda} f_{-}(x) \mathrm{d}x.$$
 (12)

The total mean speed $\langle v \rangle = (\langle v_+ \rangle - \langle v_- \rangle)/2$ is the quantity calculated in ref.⁵. The form of the mean speeds for some typical parameter values are shown in Fig. 6.

Defining dimensionless speeds by an overbar, $\langle \bar{v}_+ \rangle = \langle v_+ \rangle / u$ etc., these calculations yield

$$\langle \bar{v}_{\pm} \rangle = \frac{W_{\pm} + \alpha \kappa \gamma^{-1}}{W + \kappa},$$
 (13)



Figure 6 The mean speed of right and left going bacteria as a function of γ (changing v) for the same parameter space as in the main text (fig. 4a) with $\alpha = 0.5$ and $k = 0.1 \text{ s}^{-1}$.

where we define the parameters W_{\pm} and W as

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$$W_{+} = \begin{cases} 2(1 - e^{-Q'}) - \gamma^{-3}(1 + 2\gamma)(1 - \gamma)^{2}(1 - e^{Q}), & u < v, \\ \frac{2\gamma^{-3}\sinh(Q'/2)\sinh(Q/2)}{\sinh(Q'/2 + Q/2)}(1 + \gamma), & u \ge v. \end{cases}$$
(14)

$$W_{-} = \begin{cases} -\gamma^{-3}(1-\gamma)^{2}(1-e^{Q}), & u < v, \\ \frac{2\gamma^{-3}\sinh(Q'/2)\sinh(Q/2)}{\sinh(Q'/2+Q/2)}(1-\gamma), & u \ge v. \end{cases}$$
(15)

$$W = \begin{cases} (1 - e^{-Q'}) - (\gamma^{-1} - 1)^2 (1 - e^Q), & u < v, \\ \frac{2\gamma^{-2} \sinh(Q'/2) \sinh(Q/2)}{\sinh(Q'/2 + Q/2)}, & u \ge v, \end{cases}$$
(16)

with $Q = \kappa \alpha / (1 - \gamma^{-2})$ and $Q' = \kappa (1 - \alpha)$, where $\gamma = u/v$ and $\kappa =$ $k\Lambda/u$.

For the net speed we obtain (as in Ref.⁵, but generalised to an arbitrary light-dark fraction and then calculated for zero swimming in the dark)

$$\langle \bar{v} \rangle = \frac{W}{W + \kappa} \,. \tag{17}$$

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Notes and references

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