

Large-scale flow formation in ensembles of swimming bacteria: Experiment

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We study experimentally self-organization of concentrated ensembles of swimming bacteria *Bacillus Subtilis*. Experiments are performed in a very thin (of the order of 1 bacterium diameter) fluid film spanned between four supporting fibers. Small amplitude electric field is used to adjust dynamically the density of bacteria inside the experimental cell. Our experiments revealed only gradual increase of the large scale flow correlation length with the increase in number density of bacteria, and no sharp transition.

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Problem of collective motion in dense colonies of swimming microorganisms (bacteria, algae, etc) attracted significant attention of experimentalists [1–4] and theorists [6]. One of the most surprising features is the onset of large-scale collective flow driven by flagella of individual bacteria. The experiments in Ref. [1] demonstrated that the correlation length of collective motion exceed the size of individual cell by an order of magnitude. In additions, the collective flow is typically by factor of five faster than the swimming speed of individual bacteria. The collective swimming was found at large enough volume (or number) density of the microorganisms; in dilute suspensions of the bacteria no collective flow was observed and the correlation length was typically of the order of the one bacterium size. Recent works [1, 6] signified the importance of hydrodynamic interaction between swimming bacteria as a primary reason for the onset of collective flow. This phenomenon is in strong disagreement with conventional wisdom arguing the irrelevance of advective motion compared to diffusion due to the smallness of corresponding Peclet number Pe for individual cell. However, once concentrated, the collectively driven hydrodynamics globally outcompetes diffusion.

The onset of coherent motion and nature of the phase transition in groups of locally interacting self-propelled particles (flocks, schools, herds) is also highly debated topic in nonequilibrium statistical mechanics [8–12]. One of the fundamental questions is what is the nature of transition to collective motion and the relation between highly simplified “flocking”-type models based on local interactions between elements [9–12] to the problem of collective swimming where non-local hydrodynamic interactions are obviously important.

In order to address these questions we performed systematic experimental studies of collective bacteria swimming in thin film geometry. To study the transition in detail, we developed a new technique capable of adjustment of the number density of bacteria by small electric field in the course of experiment. It allowed us to perform measurements of the correlation length and the

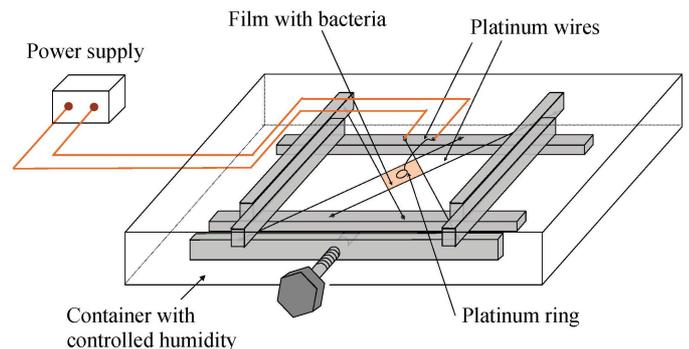


FIG. 1: Schematics of experimental setup. Thin film containing bacteria is spanned between adjustable 2 Pt wires and two glass fibers. Electric current is transmitted between Pt wires and small Pt ring lowered on the film. The cell is placed in a container with controlled humidity.

mean swimming velocity in the range of densities and on the same bacterial colony, thus greatly reducing statistical fluctuations between experimental data due to the physiological differences between colonies. In disagreement with simplified theories [8–12], our results revealed only gradual increase of the correlation length with the increase of the density, and no sharp transition. This observation can be explained as a noise-induced smearing of the phase transition, with the main source of noise due to strong fluctuation in orientation of individual bacteria [2].

The schematics of experiment is shown in Fig. 1. Experiments were conducted on suspensions of strains 1085 and YB886 of *Bacillus Subtilis*, a peritrichously flagellated rod-shaped bacterium, length about $5 \mu\text{m}$ and diameter of the order $1 \mu\text{m}$. Spores stored on agar or sand were used to inoculate nutrient medium. The suspension of grown cells were then washed and centrifuged to separate from water. Our experimental setup is similar to that used in Ref. [2], however a number of important modifications were made. A small drop containing suspension of bacteria was placed between four support-

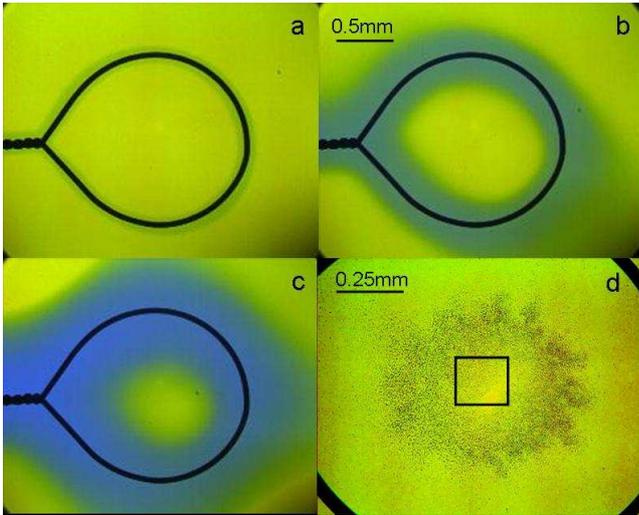


FIG. 2: (a)-(c) Sequence of images illustrating gradual decrease of pH near the electrodes as a result of transmission of electric current. (d) Concentrated bacteria colony (darker part of the image), the square shows the field of view of microscope.

ing movable fibers, two Platinum (Pt) wires (to exclude contamination of the film) and two dielectric glass fibers. The drop was then stretched between the fibers up to the necessary thickness (about 1 micron) by pulling all fibers apart by adjusting the position of the control screw. The film was placed in the container with saturated water vapor pressure to minimize evaporation. In contrast to experiment with *e-Coli* in Ref. [2], no addition surfactant was used to stabilize the fluid film. In our case, the metabolism products secreted by *Bacillus Subtilis* created necessary surface tension and elasticity to sustain the stability of the film in the course of experiment, typically 2-3 min. After that time the film either ruptured, or the “methabolism” products solidified and motion ceased to exist. The images were obtained by high-speed video camera mounted on the high-resolution long focal distant microscope, camera frame rate was 100 frame/sec. The digital image processing was performed by custom-designed software based on Matlab toolboxes. Since we work with the monolayer-thick film of bacteria, our technique allows to identify, count and track all individual cells in the field of view.

In order to adjust the density of bacteria (or filling fraction) in the course of one experiment we used the following technique. Small platinum ring (diameter is approximately 1 mm) was gently lowered on the stretched film containing bacteria, and voltage about 2V was applied between the ring and two Pt wires, thus creating electrolysis. The electrolysis results in change of pH level in the vicinity of electrodes. To monitor the change of pH level, the indicator fluid Bromothymol blue was added to the solution. The evolution of pH level as the function of

time is shown in Fig. 2 a-c. As one sees from the Figure, the blue areas, corresponding to lower pH levels, expands after the transmission of electric current on both sides of the ring electrode due to ion diffusion. The change of pH in turns triggers chemotactic response in bacteria: the bacteria tend to swim away from the electrodes towards the area of more comfortable pH level (about 7.2) in the middle of the ring, see Fig. 2d. Remarkably, this technique stimulated response of living bacteria only; the dead cells remain immobile and were left behind. Thus, with this method we were able to change dynamically the number density of bacteria by the factor of 5 and more. The images were acquired from $230 \times 230 \mu m^2$ small area in the middle of the ring.

Select images of bacterial patterns for different density values ν are shown in Fig. 3 ($0 < \nu < 1$ is measured as a fraction of full surface coverage by bacteria). The bacterial flows velocity field \mathbf{V} was extracted from a number consecutive images by the particles-image velocimetry (PIV) technique, the bacteria themselves were serving the role of tracers. Since we had to work with rather high filling fraction of the film by bacteria, the traditional method of visualization of fluid flow field by passive markers happens to be rather ineffective since small marker particles tend to attach to bacteria. In addition to PIV we extracted from each image the vector field of bacterial orientation $\boldsymbol{\tau}$. To do that we first extracted from the images the director \mathbf{n} by finding the direction of maximal projection of bacteria on a certain direction. Then, assuming that the absolute value of the angle difference between orientation vector $\boldsymbol{\tau}$ and bacteria flow velocity field is smaller $\pi/2$, i.e. $(\boldsymbol{\tau}\mathbf{V}) > 0$ (since bacteria swim in the direction of orientation), one may extend the definition of director \mathbf{n} and to reconstruct uniquely the vector field $\boldsymbol{\tau}$ [13].

Cross-correlation between the vector fields $\boldsymbol{\tau}$ and \mathbf{V} in the regime of well-developed large-scale flow (Fig. 2b) over entire span of the image produced a rather puzzling result: the correlation coefficient was smaller than 8%! However, this seeming contradiction to the fact that the bacteria swim in the direction of their orientation can be resolved as follows. In the well-developed chaotic flow the bacteria are advected by the fluid velocity field \mathbf{v}_f created by *all bacteria*. Since each bacterium swims in the direction of its orientation only in the *local frame* moving with fluid velocity \mathbf{v}_f , one should not expect strong global correlation between \mathbf{V} and $\boldsymbol{\tau}$ as long as fluid velocity field is chaotic. In contrast, there is significant correlation between maximal values of these fields since more oriented patches of bacteria create faster flow. In order to demonstrate that we characterized the alignment between these two fields by the coefficient C defined as

$$C = \frac{\langle \cos \phi \rangle - 2/\pi}{1 - 2/\pi} \quad (1)$$

where ϕ is the angle between \mathbf{V} and $\boldsymbol{\tau}$ (note that $-\pi/2 <$

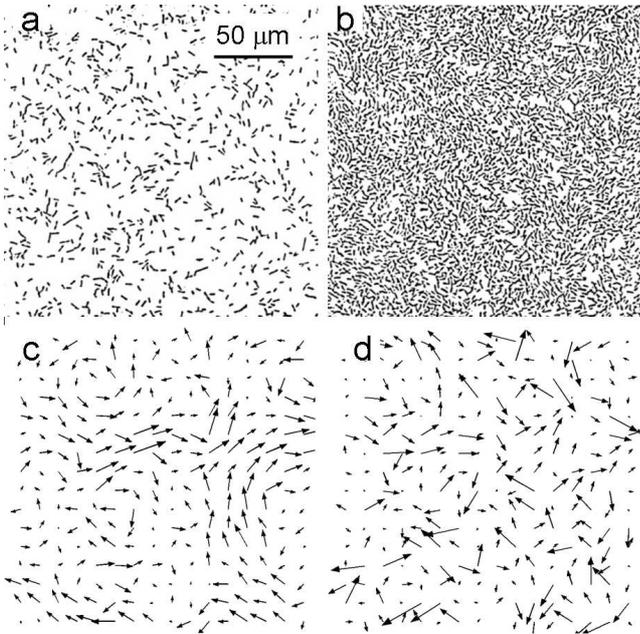


FIG. 3: Bacteria patterns for low density $\nu = 0.14$, no collective swimming (a); high density $\nu = 0.47$, well-developed chaotic large-scale flows (b). Vector fields for velocity \mathbf{V} (c) and orientation $\boldsymbol{\tau}$ (d) for the configuration shown in image (b). See also movies 1 and 2 in [15]

$\phi < \pi/2$ due to our choice of the direction of $\boldsymbol{\tau}$). If the directions between $\mathbf{V}, \boldsymbol{\tau}$ were random, then the value of $\langle \cos \phi \rangle = 2/\pi$ and, $C = 0$. For the perfectly aligned fields $C = 1$. To amplify the contributions from large-amplitude regions of fields $\boldsymbol{\tau}, \mathbf{V}$ we used the following method: in the averaging procedure we excluded the points of both fields whose amplitudes were below certain variable thresholds $V_s = k\langle |\mathbf{V}| \rangle, \tau_s = k\langle |\boldsymbol{\tau}| \rangle$, where parameter k measures the fraction of mean value of each field. The alignment coefficient C vs k is shown in Fig. 4. As one sees from the Figure, the alignment indeed decreases with the density ν due to the effect of large-scale flow discussed above (for low density bacteria swim exactly in the direction of orientation, and $C \rightarrow 1$). With the increase in k the coefficient C indeed increases, supporting the statement that more aligned regions are also moving faster.

We performed systematic measurements of the collective flow properties in the range bacterial number density ν . The density was adjusted dynamically in the course of experiment using the technique described above, see the Inset to Fig. 5. The following quantities were extracted from experimental data: typical bacterial velocity $\bar{V} = \sqrt{\langle \mathbf{V}^2 \rangle - \langle \mathbf{V} \rangle^2}$, and radial velocity correlation functions $K(r)$, defines as (θ is a polar angle)

$$K(r) = \int_0^{2\pi} d\theta [\langle \mathbf{V}(\mathbf{r}') \mathbf{V}(\mathbf{r} + \mathbf{r}') \rangle - \langle \mathbf{V}(\mathbf{r}') \rangle^2] \quad (2)$$

The correlation length was extracted from $K(r)$ by expo-

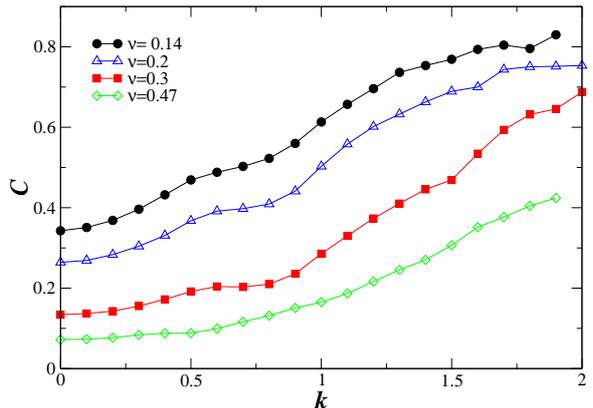


FIG. 4: C vs k for different values of density.

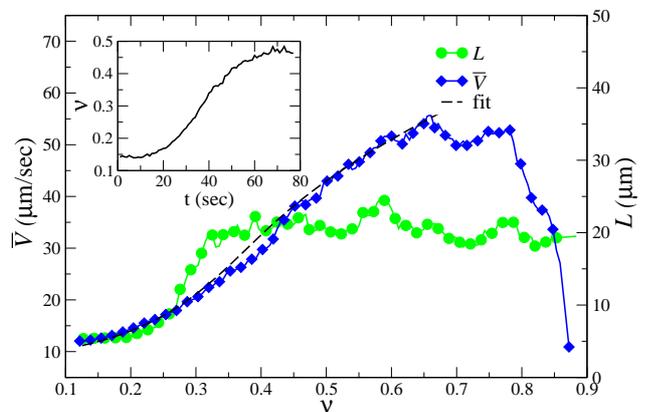


FIG. 5: Typical velocity \bar{V} (diamonds) and velocity correlation length L (circles) as the function of bacterial number density ν . Dashed line shows fit of \bar{V} to solution to Eq. (3). Inset: typical dependence of density ν vs time in the course of experiment.

nential fit $K(r) \sim \exp(-x/L) + const$. We also measured the orientation correlation length, which happens to be roughly 1/3 of L (not shown in the Figure). The results are comprised in Fig. 5. While the shape of correlation functions $K(r)$ and the value of correlation length L are in agreement with previous measurements in Ref. [1], our results present much clearer picture of the phenomenon because they are not contaminated by boundary effects and oxygen concentration gradients. Moreover, over measurements are performed in the film of constant thickness, whereas in Ref. [1, 4] the film thickness variations were significant.

As one sees from Fig. 5, no sharp transition occurs

with the increase of the density. Instead, we observed only gradual (although steep) increase of the velocity \bar{V} and the correlation length L values, overall changes in these quantities were about factor of five. For even higher density ($\nu \rightarrow 1$, i.e. close 90% surface coverage by bacteria) we notices complete termination of motion and formation of biofilm, and, consequently, spores.

Our results are in surprising disagreement with early simplified theories of collective motion in the system of self-propelled particles [9–11] and others. We interpret our observation as smearing of the phase transition by strong noise due to spontaneous orientation fluctuations of individual bacteria. In the following theoretical paper Ref. [7] we develop mathematical model of collective swimming. In the absence of noise the model indeed exhibits second-order phase transition, and the divergence of correlation length L at the critical point. However, for the strong enough noise, the transition smears, and only gradual (although rather steep) increase of correlation length L with density ν was observed, in qualitative agreement with experiment. To support this observation we compared our experimental data for \bar{V} vs ν with those obtained from the normal form equation for generic noisy second order phase transition

$$\partial_t V = (\nu - \nu_{cr})V - V^3 + \zeta(t) \quad (3)$$

where ζ is Gaussian white noise with the intensity D , and ν_{cr} is critical density. In the absence of noise one obtains mean-field result $\bar{V} \sim \sqrt{\nu - \nu_{cr}}$. With the noise one obtains smearing of the transition point ν_{cr} . The fit of solution to Eq. (3) is consistent with the experimental data, see Fig. 5 (the analytical dependence \bar{V} vs ϵ in form of hypergeometric functions is not show).

In conclusion, we presented experimental studies of collective bacteria swimming in thin fluid film. Our results are essentially two-dimensional. In contrast to early work [1], where bacteria accumulated in the vicinity of liquid/air contact line of the sessile drop, the boundary effects play no significant role in our studies. Our results provide a strong evidence for the pure hydrodynamic origin of collective swimming, rather than chemotactic mechanisms of pattern formation [14]. Swimming

of the bacteria in the direction of their orientation is a primary reason for the onset of large-scale chaotic flows. In addition, our technique for concentration and separation of bacteria by electric field may find interesting future applications for bioanalysis and miniature medical diagnostic devices. We thank Michael Graham, Frank Jülicher, Karsten Kruse, Hugues Chate, and Eberhard Bodenschatz for useful discussions. This work was supported by the U.S. DOE, grants W-31-109-ENG-38.

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