Patterns of Collective Bacterial Motion in Microfluidic Devices

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We have studied active and passive forms of pattern formation and synchronized motion in high-density bacterial cell suspensions with phase contrast and epifluorescent videomicroscopy. We have shown that in high-density cultures of non-motile cells sedimentation patterns form. By comparing bacterial and colloidal suspensions we suggest that hydrodynamic interactions between sedimenting bacteria lead to patterns that one may consider a passive form of collective motion. Furthermore, we used microfluidic devices to investigate how solid boundaries influence the motion patterns of flagellated swimming bacteria in high-density cultures. We studied the emergence and dynamics of collective swimming of *Escherichia coli*. Using microfabricated chambers, we were able to control and stabilize the swimming patterns formed. Our results suggest that the physical features of the environment (solid boundaries and geometric constrictions) have strong effects on the swimming behavior and pattern formation of motile bacteria. Such effects may need to be considered when culturing bacteria in microchambers and microreactors.

Key words:
synchronized motion, bacterial motility, collective behavior, microfluidics

Introduction

Interactions between individuals in a group of organisms often lead to the emergence of new patterns of behavior. Such interactions on the most fundamental level can lead to the synchronization of motion velocity and direction of individual organisms. The emerging patterns of collective motion are observable in the living world on a wide range of scale and complexity: from the building blocks of the cytoskeleton of eukaryotic cells,[1] to high-density cultures of microorganisms,[2-4] to schools of fish, and flocks of birds.[5–6] Although bacteria are considered mostly as solitary, single cell organisms, active and passive forms of cell-cell interactions deeply influence bacterial life on the population level. However it is important to note that the term "collective motion" or "collective swimming" don't necessarily imply a social aspect to these phenomena as these expressions are commonly used as synonyms to "correlated motion" in the literature.

Self-propelled microswimmers, as *Escherichia coli* (*E. coli*) in high-density cultures are able to synchronize their swimming speed and direction, and from a physicist's point of view form an active fluid medium with enhanced diffusion rate.[7] This synchronization of active motion can provide several benefits for the whole population (e.g. better nutrient availability or faster biochemical signal propagation due to enhanced diffusion).

Interestingly, pattern formation has also been observed for suspensions of non-active agents.[8] Settling of colloid particles under gravitation is an actively studied field of statistical mechanics, and computational physics,[9-11] although the precise details of equilibrium, and non-equilibrium sedimentation processes are not fully understood. The experiments are mainly focusing on the physical processes of sedimentation of micron-sized acrylate or monodisperse glass particles,[8,9] but the results can be easily applied to living systems, such as blood cells,[12] macromolecules,[13] or on appropriate time scales, as we suggest here, non-swimming bacteria.

Colloid particles in liquid media under the influence of the gravitational field tend to reach an equilibrium state by forming several layers on the bottom of the container. As Royall *et al*. showed[8] by turning the sample container upside down, one can suddenly drive such a system out of equilibrium. In this scenario the lateral distribution of cells changes, however, only transiently. The emergence of finger-like inhomogeneities in the horizontal plane and formation of a well-defined network-like structure of the particles can be observed. Until now this phenomenon has only been studied in artificial particle systems. We observed non-motile bacterial cultures in liquid media acting as the abovementioned system, and showing coherent sedimentation patterns.

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We consider this as synchronization of passive movement as opposed to synchronized swimming of actively moving bacteria.

The collective motion of swimming bacteria in high-density cultures is being extensively studied by several research groups. This phenomenon interests both physicists and biologists. From the physics point of view, such systems may be suitable for testing more general physical models of interacting self-propelled particles. Collective motion is important in bacterial swarming, which enables the fast spreading of colonies. However, the exact biological relevance of swarming is not entirely clear yet. It has been suggested for example that swarming (and the underlying physical and biological processes) may affect the resistance of the colony to environmental stresses.[14]

Active swimmers, like peritrichously flagellated bacteria (such as *E. coli*) can align their swimming direction and harmonize their behavior in high-density suspensions. *E. coli* is a rod-shaped bacterium with 6–10 flagella, driven by molecular motors.[15] The flagella assemble into a bundle and propel the cell if rotating in the same counterclockwise direction. Each flagellum, coupled to its motor, can change its direction of rotation, and secede from the bundle. This disassembly of the flagellar bundle causes the cell to stop and change its swimming direction. With this so-called run-and-tumble motion,[16] cells are able to effectively map their local environment, while essentially perform a random walk.[17] This kind of swimming behavior can significantly change under specific conditions, such as at high cell densities. In high-density suspensions, close range interactions between neighbouring cells overrule the run-and-tumble type motion, and the bacteria align their swimming direction forming fast, fluctuating, constantly changing swimming patterns with emerging vortices, jets, and whirlpools.

The first experimental description of organized swimming patterns of bacteria was given about fifteen years ago,[3] when emergence of short-lived jets and whirls was observed in a thin liquid film of *Bacillus subtilis* colonies on agar plates. Mendelson *et al.* used micron-sized beads to probe the fluid flow and characterize the swimming patterns. These observations inspired several subsequent experiments to reveal the fundamental physical and hydrodynamical interactions that govern the synchronization of the swimming behavior of bacteria in high-density cultures.

Several work focused on the diffusivity in such active biological fluids (bacterial suspensions). The enhancement of diffusion has been shown both in quasi 2D (thin films) and 3D (droplet) samples.[18,19]

Other experiments have raised the question how the spatiotemporal correlation of these self-organized swimming patterns depends on the cell density.[20] A gradual increase in spatial correlation length with increasing bacterial density was found.

Self-concentration of bacteria and large-scale coherence of *Bacillus subtilis* cultures were observed in fluid droplets.[4] The authors connected the emergence of increasing concentration of cells and the formation of well-defined patterns with buoyancy effects and chemotaxis towards oxygen-rich regions of the droplet. On the other hand, the collective motion of microorganisms with large-scale spatial coherence emerged due to purely hydrodynamic interactions between the cells and the surrounding fluid. The high cell density emphasized the effect of advective motion in the fluid, compared to diffusion.

Beside the extensive fundamental experimental and theoretical work[21–25] on the physics of the collective swimming behavior in high-density bacterial cultures, "engineering" applications have also started to appear.

A numerical simulation predicted that swimming bacteria are able to rotate microscopic wheels.[26] Experiments proved the simulation and its derived model right.[27] Furthermore, a more efficient rotation effect was seen with synchronously swimming bacteria.[28]

Microfluidic devices were used to study the possible applications of exploiting the motility of swimming *E. coli* cells to drive currents in well-designed microengineered environments.[29] The results foretell that we are on the way towards developing and implementing of microfluidic devices powered by microorganisms.[27,28]

In the last few decades, the advancement of microfluidic technology revolutionized engineering sciences (e.g. chemical engineering and biotechnology), as well as medicine and traditional microbiology. Cell culturing (bacterial and/or eukaryotic),[30-33] and performing experiments in microreactors and microchambers is a rapidly improving, and very promising technology, which provides us with the advantages of a precisely controlled microenvironment for cells. Therefore, it is of great interest to understand how cells move around and distribute in such microstructures.

In this paper, for the first time we describe a passive form of synchronized motion of non-motile cells, where the bacteria (acting as a colloidal suspension) formed gravitational sedimentation patterns due to the physical interactions. On the other hand, we studied the swimming patterns of actively moving high-density bacterial cultures as well. A microfabricated device containing microchambers

with different geometries was used to stabilize, shape and study the fundamental features of the emerging swimming patterns within and outside the microchambers. Our results show the importance of geometry and structure of the physical environment on a microscopic scale in shaping the swimming behavior of bacterial populations.

Materials and methods

The microfluidic device

The microfluidic device was designed using KLayout (klayout.de), an open source layout editor program. The pattern of the device was printed into a chromium mask (JD Photo-Tools Ltd.). We used standard photolithography and soft lithography techniques[34] to make the microfluidic chip. In short, a 40 μm thick SU-8 2050 (MicroChem Corp.) photoresist layer was spin-coated onto a 100 mm diameter silicon wafer. The wafer was then soft baked, exposed to UV-light (Newport New Illumination System, Newport Corp.) through the chromium mask. After a post-exposure bake, the exposed pattern was developed in SU-8 developer (MicroChem Corp.). The wafer with the SU-8 microstructures was used as a master mold for PDMS (polydimethylsiloxane, Sylgard 184, Dow Corning Corp.) casting. In order to prevent sticking of the PDMS, the mold was silanized with (Tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (Gelest Inc.). The PDMS replicates were cured at 90 °C, inlet holes were punched, and the device was bound to glass coverslips after an oxygen plasma treatment.

The microfabricated device contained several microchambers of different size and shape (Fig. 1). Round chambers with radii of 10–50 μm were created. In addition, chambers consisting of two merged circular parts (with radii of 10–50 μm) were fabricated. The microchip also contained larger open areas between the microchambers where unconstrained correlated swimming could be observed.

Cell culturing

Fluorescent *Pseudomonas aeruginosa* PUPa3 cells were used in the sedimentation experiments. Overnight cultures were diluted 1000× in 3 mL LB medium (Sigma-Aldrich Corp.) containing 50 μg mL^{-1} ampicillin in sterile 14 mL polystyrene tubes. The cells were grown at 30 ºC in a shaker incubator shaken at 200 RPM until they reached an optical density of 0.5–0.8 at 600 nm.

Fluorescently labeled *E. coli* cells of the W3110 strain (carrying a *lac* promoter inserted together with a GFP encoding gene) were used in the microchip experiments. We used the same culturing tech-
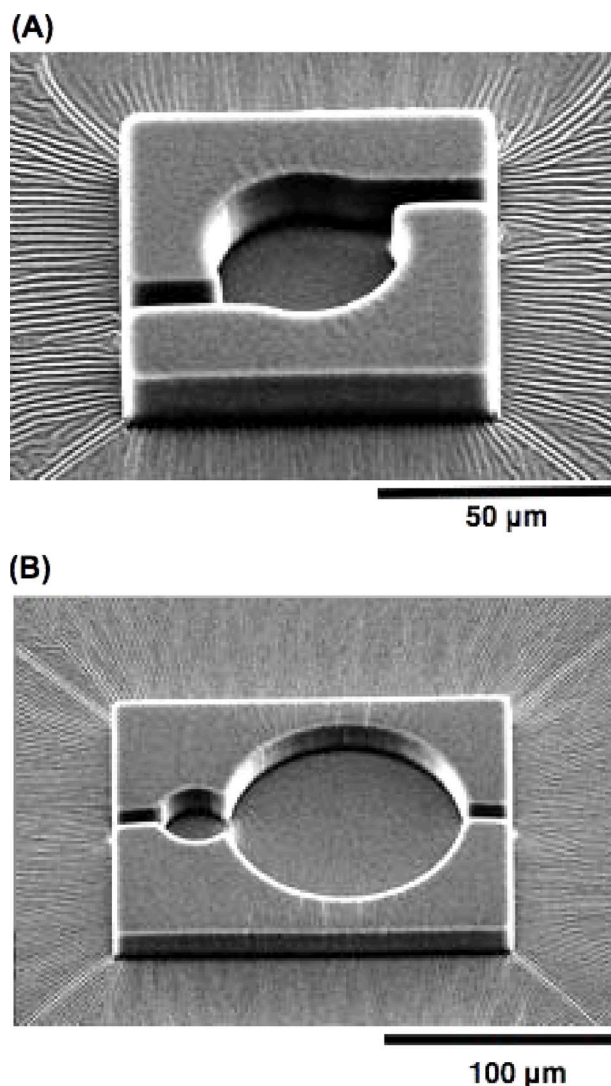
(A)

(B)

Fig. 1 – *Scanning Electron Micrographs of individual microchambers of the microfluidic device*

niques as in the case of the *Pseudomonas aeruginosa* strain, except 1 mM of IPTG (isopropyl β-D-1-thiogalactopyranoside, Sigma-Aldrich Corp.) was added to the medium instead of antibiotics.

Microscope sample preparation

For the sedimentation experiments 2 μL of *P. aeruginosa* PUPa3 cultures were pipetted to form a droplet on a cleaned microscope slide. The sample was sealed with a cleaned coverslip. Two-sided sticky tape was used as a spacer between the microscope slide and the coverslip to maintain the consistency of the droplet and prevent evaporation.

Microfluidic samples were prepared as follows: before inoculating the microchip 1 mL of the *E. coli* culture was centrifuged for 10 minutes at 2700 g with a desktop centrifuge (Wise Inc.). The pellet was resuspended in 10 μL LB medium leading to a 100 times increase in the concentration of bacteria

(yielding cell densities in the order of 10^{10} cells mL^{-1}). With this high-density suspension, the microchambers were filled through the inlet holes, and the chip was sealed with a microscope slide using fast curing PDMS (World Precision Instruments Inc.).

To prepare bulk samples of high-density *E. coli* suspensions, the culture was concentrated as described above. Droplets on microscope slides were prepared as for the sedimentation experiments.

Imaging

A Nikon Eclipse Ti-E epifluorescent microscope (Nikon Inc.) was used during the experiments, equipped with 10×, and 40× Plan Fluor phase contrast objectives, a fluorescence filter set for GFP (Chroma Inc.), a Proscan II motorized microscope stage (Prior Scientific Ltd.), and a Lumen 200Pro metal arc lamp (Prior Scientific Ltd.) as a fluorescence excitation source. Snapshots and video recordings were taken with a Rolera em-c2 digital EM-CCD camera (QImaging Corp.). All the hardware was controlled by the Nikon NIS Elements AR microscopy software (Nikon Inc.). During video recording (time lapse videos), the frame rate was 2 frames per minute for the sedimentation experiments, and 60 frames per second for the high-density bacterial suspensions. We used phase contrast microscopy for recording the sedimentation patterns and fluorescence imaging for the microchip experiments.

Data analysis

Particle Image Velocimetry (PIV) techniques were used to analyse the fluctuating, dynamic patterns in bulk cultures, as well as the stabilized vortices in the microchambers. The data analysis was performed in MATLAB 2013a (Mathworks Inc.) using the PIVlab tool (pivlab.blogspot.com), an open-source software for MATLAB.

Results and discussion

Sedimentation patterns of non-motile bacteria

It is known that hydrodynamic interactions between self-propelled bacteria considerably influence their swimming behavior. It may seem surprising at first that we observed emerging pattern formation in non-motile bacterial suspensions too.

We have prepared droplet samples from *P. aeruginosa* cultures on microscope slides. The droplets were enclosed to prevent evaporation and drying. The sample was left on the bench for 4 days. During this time, the culture in the droplet went into stationary phase and cells completely stopped

swimming. Also, all the bacteria sank down to the bottom of the droplet forming an even layer of dense cell suspension on the glass slide. We drove our system out of this equilibrium state by suddenly turning the sample upside down. When looking at the sample with a microscope, we saw the slow appearance of a mesh-like pattern on the initially homogeneous image of the cell suspension (Fig. 2). The emerging network-like structure started to form immediately, a faint pattern could be observed by the time we placed the sample on the microscope. The pattern reached maximum contrast in 5 minutes and disappeared completely in 30 minutes. From the images, it can be seen that this mesh-like motif corresponds to the spatial distribution of cells with higher cell densities along the lines of the pattern. This was confirmed by repeating the experiment with fluorescent cells (data not shown), where the fluorescence intensity directly correlates with the cell density. Furthermore, it is interesting to note that the pattern was stationary throughout the experiment, only the contrast of the structure changed with time. Our observations resemble those of sedimenting colloidal systems.[8] It is possible that the
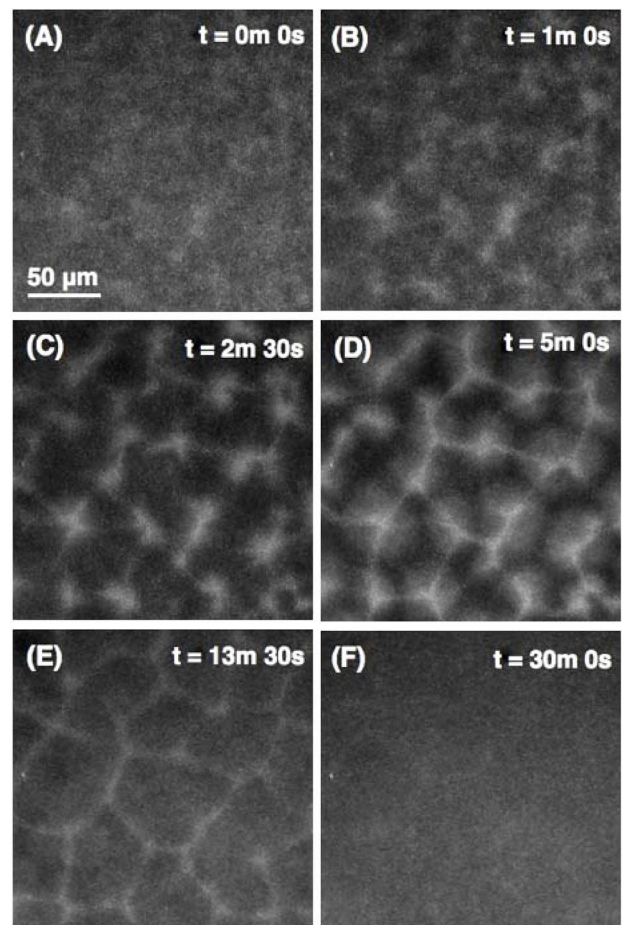

F i g . 2 – *Sequence of microscopy images showing the formation of sedimentation patterns of non-motile P. aeruginosa bacteria*
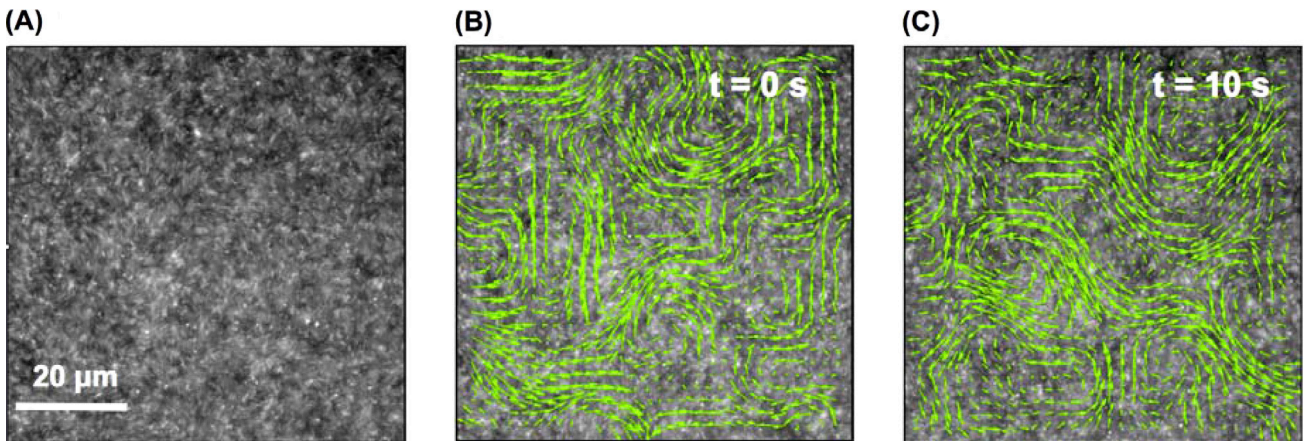
(A)   **(B)**   **(C)**

Fig. 3 – *(A) Fluorescence microscopy image of a high-density E. coli suspension. (B, C) Fluorescence images of the same suspension with the calculated velocity vector field overlayed. In 10 seconds, the patterns of motion changed largely, showing the dynamic nature of these structures.*

same microscopic processes and mechanisms lay behind the settling of the bacterial samples, and a plausible scenario of the phenomenon observed may be outlined: sedimented bacteria form a thin, dense layer on the glass surface. When the sample is flipped, cells start sedimenting due to gravity and move in the fluid medium. Hydrodynamic and mechanical interactions emerge between the cells due to the flows induced in the medium. These interactions result in a correlated settling motion of entire groups of bacteria and formation of heterogeneities in the spatial distribution of cells. The hydrodynamic interactions disappear when cells settle, and Brownian motion completely washes away the patterns. Although the mechanisms described for sedimenting colloid particles[8] can explain our observations on bacteria we do not exclude other (biological) processes playing a role here.

Such a sedimentation phenomenon has not been described before for bacterial cells. We consider this process as a form of correlated passive motion, but it is distinct from other types of correlated bacterial motions (such as synchronized swimming) that rely of active motility. However, it is likely that hydrodynamic and mechanical interactions play an important role in both active and passive correlated motions.

Bacterial "turbulence" in bulk cultures and stabilized whirlpools in microchambers

In the following experiments, we studied high-density cultures of *E. coli* bacteria in bulk cultures, as well as in microfabricated chambers in order to determine the role that geometrical constraints and solid boundaries play on the swimming behavior of bacteria. Due to the short-range interactions between the cells, the originally random motion of the individual bacteria becomes a well-synchronized, dynamic swimming in dense cultures.

Because of the alignment of swimming speed and direction between adjacent cells, jets, whirlpools, and vortices emerge. In the case of cells swimming in large open volumes unconstrained by boundaries (at least on microscopic scales), swimming patterns of jets and vortices form and disappear, creating constantly fluctuating dynamic swimming patterns in space and time (Fig. 3).

We used fluorescent videomicroscopy together with Particle Image Velocimetry (PIV) to record, identify, and phenomenologically characterize and describe the emerging patterns of synchronized motion in our bulk culture experiments. We calculated the velocity vector fields, which show that the characteristic size of the large-scale coherent patterns is about 10–30 microns, significantly larger than the size of single bacterial cells (Fig. 3B). The lifespan of the constantly changing vortices and whirlpools was a couple of seconds (compare Figs. 3B and C), indicating the strongly fluctuating, turbulent-like characteristics of the bacterial bath.

To probe the effects of solid boundaries and geometric constrictions on the collective swimming motion and the dynamic patterns, we used *E. coli* bacteria in microfluidic devices.

We loaded the microfabricated device with high-density cell suspension. In two minutes during which we mounted the sample on the microscope, correlated swimming patterns had formed and were observable in the microdevice. These patterns outside of the microchambers were similar to those seen in microdroplets before (such as in Fig. 3). We saw continuously changing dynamic structures of jets and vortices. However, inside the round chambers, we observed stable, regular, circular motion of all the bacteria (data not shown). We have seen no preference in rotation direction of these vortices: however, a few times we experienced a change in directionality in smaller (radius < 30 μm) round

chambers. More data would be necessary for a statistical analysis of this phenomenon. Apart from these rare turnaround events, the vortices persisted in the chambers throughout the experiment (up to about 2 hours).

Interesting patterns of swimming motion were observed in double chambers consisting of two merged circular parts (Fig. 4). Two adjacent vortices had formed in these chambers with clockwise and counterclockwise rotating motion, i.e. with opposite vorticity. Although these vortices were stable, the participating cells were replaced gradually in time: we saw bacteria swimming in both directions in the channels leading into the chambers. Through these channels, bacteria were constantly entering and leaving the chambers. Cells entering into the microchamber were compelled to join the stable high-velocity vortex. We also observed cells moving from one side of the channel to the other, i.e. moving between the vortices. These migration events, however, (similar to the cell trafficking into and out of the chamber) did not happen en masse, so the vortex structures were not affected.
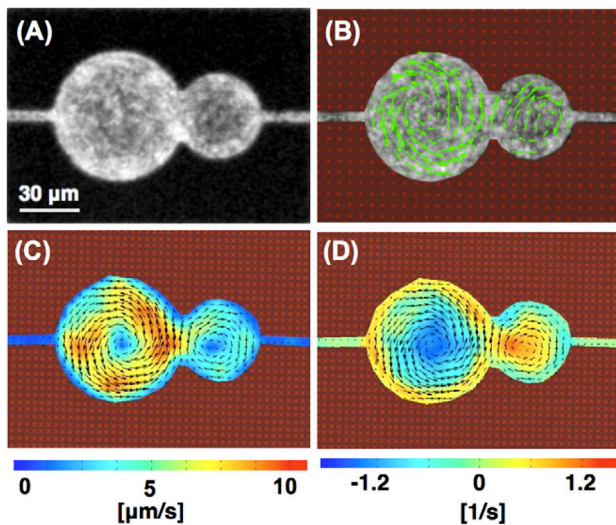

Fig. 4 – *(A) Fluorescence microscopy image of a coupled microchamber filled with a dense culture of bacteria. (B) The velocity field of the bacterial suspension inside the chamber. (C) Time-averaged velocity distribution inside the chamber (averaging was done over a 10 s period). The velocity of the cells is lower near the solid edges of the chamber, due to the direct contact with the wall. (D) The vorticity of the emerging swimming pattern in the microchamber. The formed two vortices have opposite vorticity.*

Based on the abovementioned observation, we can describe the underlying mechanisms that lead to these stable patterns. It has been shown before that swimming bacteria move along solid walls and surfaces after colliding with them.[35,36] Similarly, cells in our round chambers swim around near the chamber boundaries in a circular fashion. Hydrodynamic

and mechanical interactions between swimming cells in dense cultures make the propagation of local effects possible. While these interactions lead to jets and vortices in free suspensions, they also result in synchronized motion within the chambers. However, the constant presence of the orienting effect of the round walls stabilizes these patterns in space and time.

After the PIV (Particle Image Velocimetry) analysis of the images, the velocity vector fields may be reconstructed within and outside of the chambers. These vector fields carry some interesting information. The color-coded velocity magnitudes within a double chamber are shown in Fig. 4C. The maximum average velocity is achieved in a ring-like structure within the chamber. Both at the center of the circular parts of the chambers and at the perimeter (i.e. in the vicinity of walls), swimming speed was reduced. The first seems to be a wall effect, which may be caused by a friction-like mechanism. Moreover, the velocity was higher in the larger side compared to the smaller side of the chamber. It is possible that the sharper turn and the associated more drastic change in swimming direction required in the chamber part with smaller radius of curvature has such an impact on the swimming velocity. A similar effect may cause the lower velocities in the center of the vortices.

Based on the velocity vector fields, we calculated the vorticity (the curl of the vector field) of the emerging whirlpools in the microchambers (Fig. 4D). In the double chambers, the two rotating swimming patterns had opposite directions, which are shown by the opposite sign of vorticity inside the left and right side of the chamber. As we described before, the two counter-rotating vortices were in constant exchange of bacteria. We observed swimming cells seceding from one vortex and joining another. This feature of the coupled vortices did not change with the ratio of the diameters of the two round coupled chamber parts.

We compared the characteristic features of the synchronized motion of high-density bacterial suspensions in the round microchamber and in bulk cultures, to determine the influence of solid boundaries on the swimming behavior. Our microfluidic device contained enough space between the chambers to consider the motion of the cells unrestricted in these open areas. The patterns observed here were indeed similar to those in bulk samples (microdroplets). This offered the possibility to compare the swimming of the bacteria in constrained and unconstrained conditions in the same sample (with the same density and condition of cells) within the same experiment. We calculated the average velocity direction in two 12 µm × 12 µm areas from the velocity vector fields constructed by PIV. One of

the test areas was in the maximum velocity region within a chamber with 60 μm radius. The other area considered was outside the chamber at a 60 μm distance from any walls where dynamic swimming patterns were observed as in bulk. We found that swimming direction is quite constant in the chamber while it is very dynamic and fluctuating outside it (Fig. 5). The stabilizing effect of the geometric constraint results in the suppression of both the fast and slow fluctuations and irregularities.
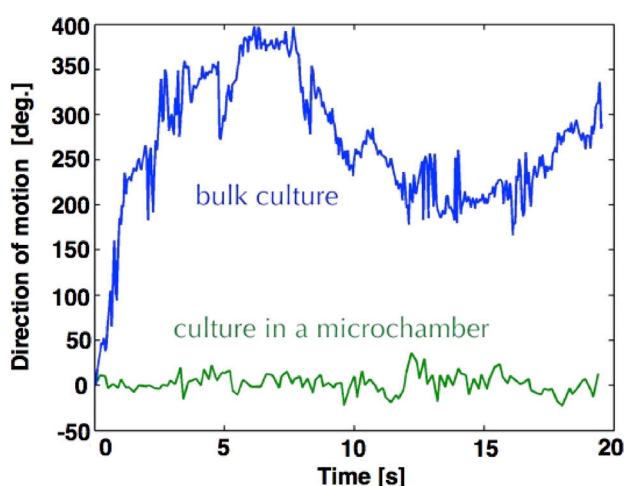

F i g . 5 – *Temporal changes in the direction of motion averaged over 12 μm × 12 μm areas inside and outside a microchamber. The swimming direction is steady inside the chamber and rapidly changing by slow and fast fluctuations in bulk cultures.*

Conclusions

In this work we observed and analyzed the correlated motion patterns of actively swimming and passively moving (sedimenting, i.e. externally driven, non-swimming) bacteria.

We observed non-swimming bacterial cells settling from a thin dense layer on the top of a droplet in aqueous media behaving as sedimenting colloid particles. Due to the physical and hydrodynamic interactions between the cells, a heterogeneous, network-like cell density structure appeared in the droplet during the sedimentation process. Due to the physical interactions between the cells, their motion during settling aligned, resulting in the emerging transient patterns. We observed these sedimentation structures with bacterial cells for the first time. Due to the similarities of the underlying hydrodynamic and mechanical driving mechanisms, we consider this phenomenon as a realization of correlated motion of passively moving agents.

In addition, we also studied the correlated motion of actively swimming bacteria. In high-density bulk cultures, microswimmers like *E. coli* bacteria

align their swimming direction and speed, forming a dense active media. Whirlpools, vortices, and jets emerge, translate and disappear on a timescale of seconds, showing fluctuating, dynamic features of this bacterial bath. We described these dynamic patterns phenomenologically.

Using microfluidic devices we showed how geometric constraints and solid boundaries alter the patterns of collectively swimming cells. We were able to stabilize and shape the emerging vortices inside the microchambers.

Recent trends point toward using live bacteria in microchambers and microreactors for scientific research or biotechnological applications. Our results show that the structure of such microdevices may have a deep impact on the motility, distribution and dynamics of bacterial cultures.

References

1. *Schaller, V., Weber, C., Semmrich, C., Frey, E., Bausch, A. R.*, Nature **467** (2010) 73.
   doi: dx.doi.org/10.1038/nature09312

2. *Kessler, J. O., Wojciechowski, M. F.*, Collective behavior and dynamics of swimming bacteria, in Shapiro, J. A. and Dworkin, M. (Ed.) Bacteria as Multicellular Organisms, Oxford University Press, New York, 1997, pp. 417–450.

3. *Mendelson, N. H., Bourque, A., Wilkening, K., Anderson, K. R., Watkins, J. C.*, J. Bacteriol. **181** (1999) 600.

4. *Dombrowski, C., Cisneros, L., Chatkaew, S., Goldstein, R. E., Kessler, J. O.*, Phys. Rev. Lett. **93** (2004) 098103.
   doi: dx.doi.org/10.1103/PhysRevLett.93.098103

5. *Couzin, I. D., Krause, J., James, R., Ruxton, G. D., Franks, N. R.*, J. Theor. Biol. **218** (2002) 1.
   doi: dx.doi.org/10.1006/jtbi.2002.3065

6. *Vicsek, T., Zafeiris, A.*, Phys. Rep. **517** (2012) 71.
   doi: dx.doi.org/10.1016/j.physrep.2012.03.004

7. *Sokolov, A., Goldstein, R., Feldchtein, F., Aranson, I.*, Phys. Rev. E **80** (2009) 031903.
   doi: dx.doi.org/10.1103/PhysRevE.80.031903

8. *Royall, C. P., Dzubiella, J., Schmidt, M., van Blaaderen, A.*, Phys. Rev. Lett. **98** (2007) 188304.
   doi: dx.doi.org/10.1103/PhysRevLett.98.188304

9. *Segrè, P. N., Liu, F., Umbanhowar, P., Weitz, D. A.*, Nature **409** (2001) 594.
   doi: dx.doi.org/10.1038/35054518

10. *Tee, S.-Y., Mucha, P. J., Cipelletti, L., Manley, S., Brenner, M. P., Segrè, P. N., Weitz, D. A.*, Phys. Rev. Lett. **89** (2002) 054501.
    doi: dx.doi.org/10.1103/PhysRevLett.89.054501

11. *Padding, J. T., Louis, A. A.*, Phys. Rev. E., Statistical, non-linear, and soft matter physics **77** (2008) 011402.

12. *Böyum, A.*, Scand. J. Clin. Lab. Invest Suppl. **97** (1968) 77.

13. *Wang, Q., Tolley, D. H., LeFebre, D. A., Lee, M. L.*, Anal. Bioanal. Chem. **373** (2002) 125.
doi: dx.doi.org/10.1007/s00216-002-1286-y

14. *Kim, W., Killam, T., Sood, V., Surette, M. G.*, J. Bacteriol. **185** (2003) 3111.
doi: dx.doi.org/10.1128/JB.185.10.3111-3117.2003

15. *Berg, H. C., Anderson, R. A.*, Nature **245** (1973) 380.
doi: dx.doi.org/10.1038/245380a0

16. *Berg, H. C.*, E. coli in Motion, Springer-Verlag, New York, 2003.

17. *Berg, H. C.*, Random Walks in Biology, Princeton University Press, Princeton, 1993.

18. *Wu, X. L., Libchaber, A.*, Phys. Rev. Lett. **84** (2000) 3017.
doi: dx.doi.org/10.1103/PhysRevLett.84.3017

19. *Wu, M., Roberts, J. W., Kim, S., Koch, D. L., Delisa, M. P.*, Appl. Environ. Microb. **72** (2006) 4987.
doi: dx.doi.org/10.1128/AEM.00158-06

20. *Sokolov, A., Aranson, I., Kessler, J., Goldstein, R.*, Phys. Rev. Lett. **98** (2007) 158102.
doi: dx.doi.org/10.1103/PhysRevLett.98.158102

21. *Grégoire, G., Chaté, H.*, Phys. Rev. Lett. **92** (2004) 025702.
doi: dx.doi.org/10.1103/PhysRevLett.92.025702

22. *Hernandez-Ortiz, J. P., Stoltz, C. G., Graham, M. D.*, Phys. Rev. Lett. **95** (2005) 204501.
doi: dx.doi.org/10.1103/PhysRevLett.95.204501

23. *Aranson, I., Sokolov, A., Kessler, J., Goldstein, R.*, Phys. Rev. E **75** (2007) 040901.
doi: dx.doi.org/10.1103/PhysRevE.75.040901

24. *Baskaran, A., Marchetti, M. C.*, P. Natl. Acad. Sci. USA **106** (2009) 15567.
doi: dx.doi.org/10.1073/pnas.0906586106

25. *Dunkel, J., Heidenreich, S., Drescher, K., Wensink, H. H., Bär, M., Goldstein, R. E.*, Phys. Rev. Lett. **110** (2013) 228102.
doi: dx.doi.org/10.1103/PhysRevLett.110.228102

26. *Angelani, L., Di Leonardo, R., Ruocco, G.*, Phys. Rev. Lett. **102** (2009) 048104.
doi: dx.doi.org/10.1103/PhysRevLett.102.048104

27. *Di Leonardo, R., Angelani, L., Dell'Arciprete, D., Ruocco, G., Iebba, V., Schippa, S., Conte, M. P., Mecarini, F., De Angelis, F., Di Fabrizio, E.*, P. Natl. Acad. Sci. USA **107** (2010) 9541.
doi: dx.doi.org/10.1073/pnas.0910426107

28. *Sokolov, A., Apodaca, M. M., Grzybowski, B. A., Aranson, I. S.*, P. Natl. Acad. Sci. USA **107** (2010) 969.
doi: dx.doi.org/10.1073/pnas.0913015107

29. *Kaehr, B., Shear, J. B.*, Lab Chip **9** (2009) 2632.
doi: dx.doi.org/10.1039/b908119d

30. *Tourovskaia, A., Figueroa-Masot, X., Folch, A.*, Lab Chip **5** (2005) 14.
doi: dx.doi.org/10.1039/b405719h

31. *Keymer, J. E., Galajda, P., Muldoon, C., Park, S., Austin, R. H.*, P. Natl. Acad. Sci. USA **103** (2006) 17290.
doi: dx.doi.org/10.1073/pnas.0607971103

32. *Keymer, J. E., Galajda, P., Lambert, G., Liao, D., Austin, R. H.*, P. Natl. Acad. Sci. USA **105** (2008) 20269.
doi: dx.doi.org/10.1073/pnas.0810792105

33. *Sun, P., Liu, Y., Sha, J., Zhang, Z., Tu, Q., Chen, P., Wang, J.*, Biosens. Bioelectron. **26** (2011) 1993.
doi: dx.doi.org/10.1016/j.bios.2010.08.062

34. *Whitesides, G. M., Ostuni, E., Takayama, S., Jiang X., Ingeber, D. E.*, Annu. Rev. Biomed. Eng. **3** (2001) 335.
doi: dx.doi.org/10.1146/annurev.bioeng.3.1.335

35. *Galajda, P., Keymer, J., Chaikin, P., Austin, R.*, J. Bacteriol. **189** (2007) 8704.
doi: dx.doi.org/10.1128/JB.01033-07

36. *Li, G., Tang, J. X.*, Phys. Rev. Lett. **103** (2009) 078101.
doi: dx.doi.org/10.1103/PhysRevLett.103.078101