NEW TECHNOLOGIES: METHODS AND APPLICATIONS

Live from under the lens: exploring microbial motility with dynamic imaging and microfluidics

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Abstract | Motility is one of the most dynamic features of the microbial world. The ability to swim or crawl frequently governs how microorganisms interact with their physical and chemical environments, and underpins a myriad of microbial processes. The ability to resolve temporal dynamics through time-lapse video microscopy and the precise control of the physicochemical microenvironment afforded by microfluidics offer powerful new opportunities to study the many motility adaptations of microorganisms and thereby further our understanding of their ecology. In this Review, we outline recent insights into the motility strategies of microorganisms brought about by these techniques, including the hydrodynamic signature of microorganisms, their locomotion mechanics, chemotaxis, their motility near and on surfaces, swimming in moving fluids and motility in dense microbial suspensions.

Soft lithography
A technique used for fabricating, at the micrometre to nanometre scale, features in elastomeric materials such as polydimethylsiloxane (PDMS).

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Motility underpins many microbial processes, such as behavioural responses of microbial cells to chemical stimuli, the interactions between cells and biotic or abiotic surfaces, and cell–cell interactions in microbial suspensions. Microorganisms have developed numerous motility strategies, typically mediated by flagella or pili, to swim through liquid, and to swarm, glide and twitch on surfaces. The dynamics of microbial motility span a broad range of timescales, from mechanical instabilities in bacterial flagella[1,2] that unfold over milliseconds, to dispersal on surfaces that occurs over many minutes[3].

Live imaging is the most intuitive and often the most robust approach for understanding the dynamic nature of many processes in microbial ecology, with motility as a prime example, and is widely used in macroecology. Advances in experimental technologies and quantitative analysis methods are now offering new dynamic imaging capabilities at the scales relevant to microbial ecology (BOX 1), for processes ranging from single-cell growth[4] to antibiotic resistance[5] and microbial motility[1,2].

Optical microscopy has long been an essential tool for microbiologists, but the ongoing technological developments in digital imaging and image analysis have greatly expanded the opportunities for adding a dynamic dimension to observations. The combination of optical microscopy with these new technologies has revealed hidden mechanisms and has enabled the quantification of microbial processes. Advances in time-lapse video microscopy, achieved by the seamless integration of high-quality optical microscopes with fast and sensitive digital cameras, have enabled the imaging of microbial processes that occur at timescales ranging from milliseconds to days, with exquisite precision and a high signal-to-noise ratio. Dramatic increases in computational resources enable the rapid processing of very large imaging datasets to automatically analyse microbial behaviours and interactions at a wide range of spatial scales, from the nanoscale mechanics of flagella[2] or pili[6] to the microscale tracking of individual surface-attached bacteria[7] that self-organize into millimetre-scale biofilms[3,8].

The full potential of this new level of dynamic imaging is realized when paired with the ability to precisely control the microbial environment using microfluidic technology[9,10] (BOX 2). Modern microfluidics, which is based on the soft lithography of inexpensive, biocompatible, transparent polymers, is becoming an increasingly enabling tool in the field of microbial ecology[11,12]. The reasons for this success are twofold. First, microfluidics enables the creation of environments that greatly facilitate the dynamic, microscale imaging of microbial processes. Second, microfluidics provides unprecedented control over multiple facets of the environment to which a microorganism is exposed, including the chemical environment (for example, generating ephemeral resource

Box 1 | **Dynamic microbial imaging**

Microscopic visualization and image analysis are powerful tools used to study dynamic microbial processes — in particular, microbial motility. Each image acquired from a digital camera is a two-dimensional array of intensity values, with each value being proportional to the number of photons that hit the corresponding camera pixel. For dynamic imaging, a sequence of images (a video) is acquired, ranging approximately from 1 frame per millisecond to 1 frame per hour, as required by the timescale of the process. Below, we provide basic information on the use of image analysis for the quantitative interrogation of microbial processes.

The image of a microorganism can be brighter or darker than the background, depending on the microscopy configuration. A simple image processing technique for a rapid first analysis — for example, in real time during acquisition — is to take for each pixel the maximum (or minimum) intensity value of that pixel over the entire video (see the grey trajectories in the figure, part **a**)[2]. This generates a time-integrated view of the distribution and movement of microorganisms in a population, which is similar to a photograph taken with a long exposure time.

Cell identification

The power of image analysis resides in the automated digital identification of individual microorganisms in each frame of a video. When combined with time-lapse imaging, this provides quantitative information on cell dynamics, including, for example trajectories (panel **a**), propulsion mechanics[2] or growth[81]. The first step often hinges on image segmentation, whereby every group of pixels that satisfies a set of prescribed attributes (for instance, their intensity or intensity gradient exceed a threshold value, or their size and/or shape fall within given ranges) is identified as a microorganism[82] (panels **b**–**c**) or a flagellum[2,83] (panel **d**). From the group of pixels, the position of microorganisms can be calculated (for example, the centroid of the group, which is the average position of all the identified pixels in the shape), as well as their orientation (for example, by fitting an ellipse to the shape) and size.

Cell tracking

Cell trajectories are reconstructed from individual cell positions in each frame through automated tracking algorithms. Tracking routines can be complex, but the fundamental approach is to identify the same microorganism in two consecutive frames (for example, by finding the

nearest neighbour in the previous frame, when the rate of imaging is sufficiently high). Repeating this process across frames, until a microorganism is 'lost' (that is, it swims out of the field of view or focus), yields its trajectory (see the figure, part **a**, yellow lines). This then enables the computation of swimming statistics, including speed, direction and reorientation events such as reversals (see the figure, part **a**, green circles) and flicks (see the figure, part **a**, red squares)[2].

Tracking in three dimensions has also been used to study microbial motility. The operating principle of 3D tracking microscopes is based on automatic motion of the microscope stage in 3D through a feedback control loop that maintains a single microorganism in focus. This technique provided early data that were key to understanding the motility of *Escherichia coli*[84] and was recently used to capture both the orientation and 3D position of individual *Caulobacter crescentus* cells[85]. Powerful recent alternatives to 3D tracking are digital holographic microscopy (DHM)[56] and defocused microscopy[86,87], which can capture the positions of hundreds of microorganisms simultaneously without movement of the microscope stage.

Particle image velocimetry (PIV)

Although cell tracking provides swimming kinematics, it is necessary to track the movement of the fluid to obtain the hydrodynamic signature of a microorganism, which can be achieved using particle image velocimetry (PIV). The most frequently used method is microscale PIV (microPIV), which involves seeding the fluid with small tracer particles (that are often 0.2–1.0 μm in diameter) and imaging their motion as they are transported by the flow. Each frame is subdivided into rectangular boxes, which are as small as possible but large enough to contain several tracer particles. Correlation techniques are then used to determine the mean displacement of the particles in a given box between consecutive frames, yielding the local fluid velocity[88].

Both tracking and PIV algorithms are widely available in commercial and free software packages (for example, ImageJ and MatPIV), making cell tracking and fluid flow measurements in microbial systems broadly accessible. The images in parts **a** and **d** are adapted from REF. 2, Nature Publishing Group. The images in parts **b** and **c** are adapted from REF. 82, National Academy of Sciences.
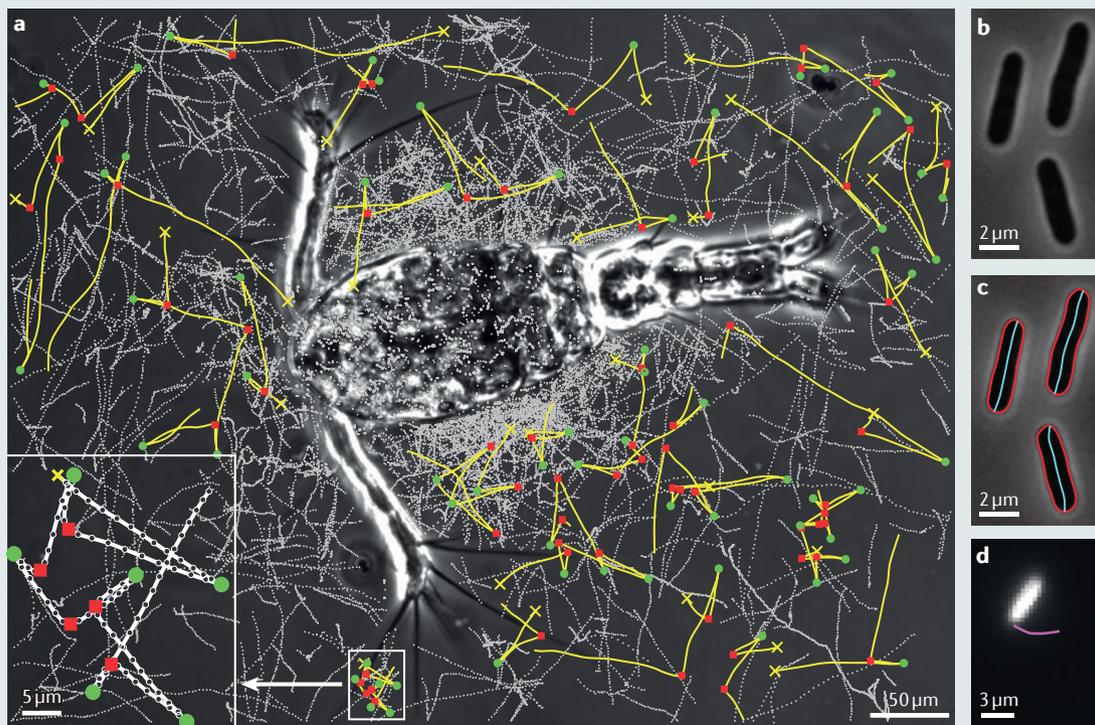
Defocused microscopy
A microscopic imaging technique whereby the distance of a microorganism ('into the plane') from the imaging plane is determined by matching its defocused ring size with a reference stack.

pulses or precise gradients), the physical environment (for example, generating controlled fluid flows and velocity gradients) and the biological environment (for example, controlling the relative positioning of microbial cells). This high-precision spatiotemporal control in conjunction with dynamic imaging is having a major impact on the study of microbial ecology, by enabling the observation of microorganisms in controlled settings that mimic salient features of their natural habitats.

As one of the most dynamic processes in the microbial world, the study of microbial motility is naturally suited to benefit from these technical advances. In this Review, we use specific examples to describe how dynamic imaging, often combined with microfluidic technology, has helped to further our understanding of microbial motility, from its biophysical underpinnings to its ecological consequences. We first describe the hydrodynamic signature of swimming microorganisms

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Box 2 | Microfluidics for the control of microbial environments

Fabrication of microfluidic devices has now become commonplace at most research institutions, and microfluidic devices are also commercially available, making them widely accessible to microbial ecologists. A range of fabrication methods and materials exist[89]. However, soft lithography[90] that uses the elastomeric polymer polydimethylsiloxane (PDMS) remains the most common owing to several advantages: PDMS is optically transparent, biocompatible, chemically inert, gas-permeable, flexible and inexpensive. Below we highlight key features of microfluidics that enable precise physical and chemical control of microbial microenvironments.

Controlling fluid flow
Microfluidic channels can be used to generate carefully controlled fluid flows[91], thereby mimicking salient features of the physical environment in microbial habitats. Flow inside microfluidic channels is typically laminar. The velocity of flow inside the channel is non-uniform (see the figure; the blue arrows in part **a** indicate flow velocity and direction): it is zero at the top, bottom and sidewalls and maximum at the centre (see the figure, part **a**). For a microfluidic channel with a high aspect ratio cross-section, the velocity profile has the shape of a parabola across the smallest dimension (often the depth of the microchannel; see the figure, part **a**, the blue arrows along the z-direction) and is almost uniform across the widest dimension (see the figure, part **a**, the blue arrows along the y-direction)[92]. Cells in suspension are transported by the flow, whereas surface-attached cells experience flow as a drag force.

The non-uniformity of the velocity profile implies the existence of velocity gradients — or 'shear'— which are strongest at the channel sidewalls, where fluid is at rest. Cells in suspension are continuously rotated by shear[63], whereas surface-attached cells experience shear as a torque that can cause them to bend[8].
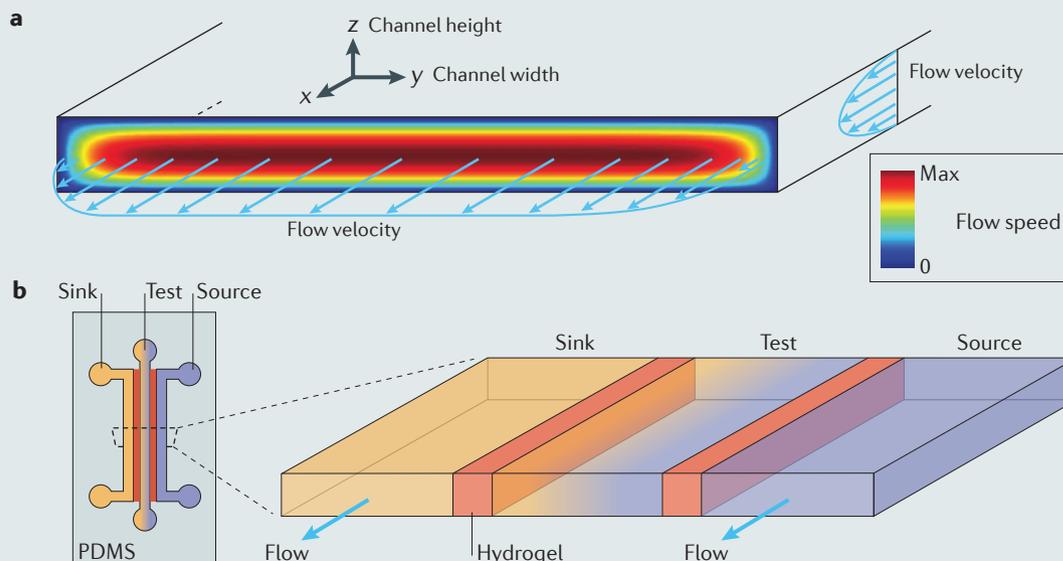
Controlling chemical gradients
Microfluidic devices are ideally suited to create a wide range of chemical gradients (steady or unsteady, linear or nonlinear) of small molecules or
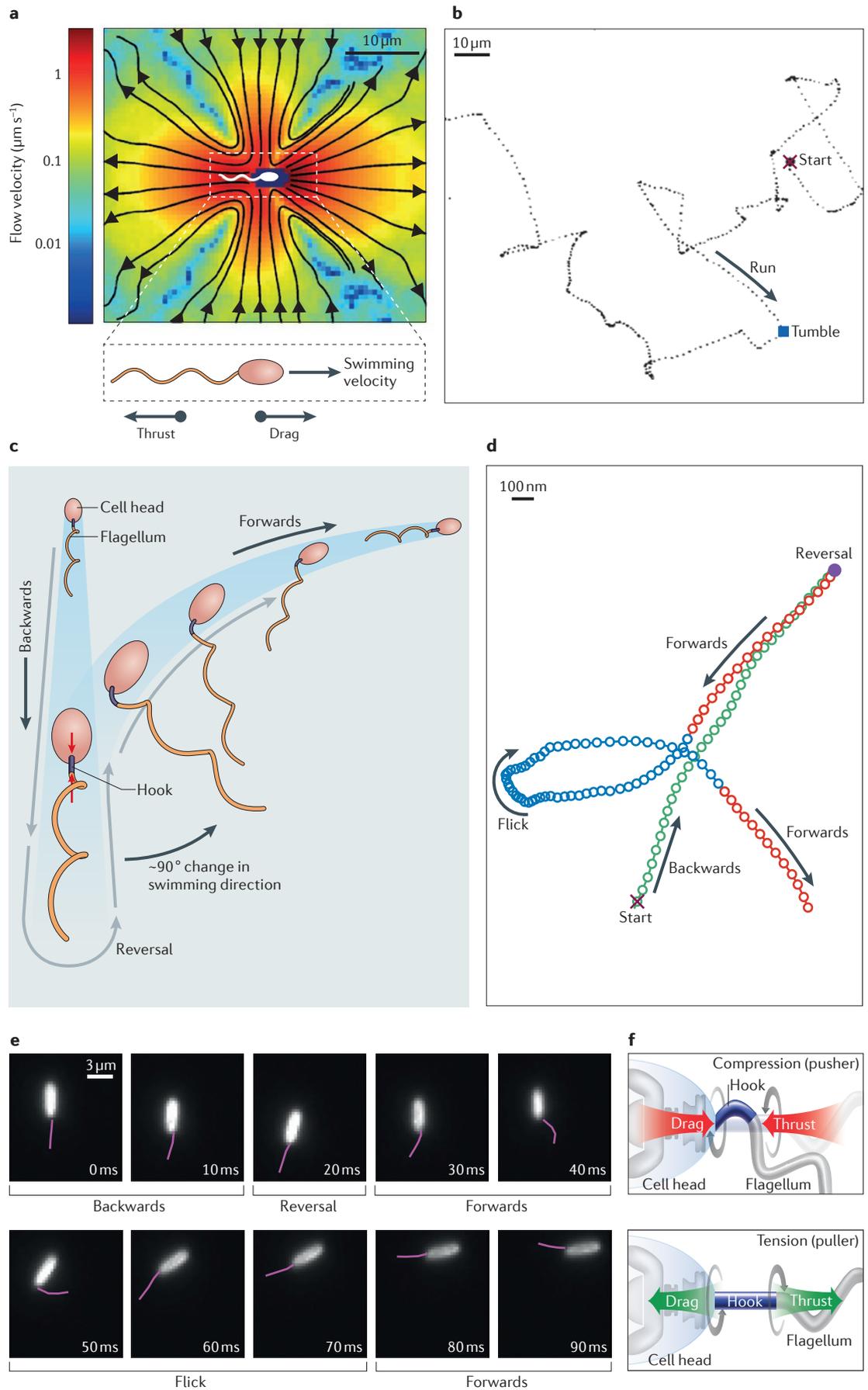
gases at the scale relevant to the chemical ecology of microorganisms[31,93,94]. For example, unsteady chemical gradients designed to mimic ephemeral resource hotspots can be generated by creating a microscale solute band that subsequently spreads by molecular diffusion to become homogeneously distributed.

The use of porous materials (such as hydrogels or membranes) within microfluidic devices enables the generation of steady chemical gradients in flow-free conditions[31]. A prototypical case consists of a 'test' channel containing microorganisms, separated by thin hydrogel walls from a 'source' channel carrying chemoattractant on one side, and a 'sink' channel carrying buffer on the other side (see the figure, part **b**). By diffusing from the source channel through to the sink channel, the chemoattractant establishes a steady uniform gradient in the test channel, enabling the study of microbial behaviour or physiology in a controlled chemical environment over extended timescales. Many variants of this concept exist, with other implementations enabling the creation of multiple, simultaneous chemical gradients (in parallel or in opposition)[37], the generation of temporally alternating gradients[36,48], and the simultaneous quantification of chemotactic migration and surface attachment[48].

Controlling surface chemistry
Surface chemistry can be controlled in microfluidic devices[95] through traditional uniform surface treatments; for example, bovine serum albumin can be used to prevent attachment in chemotaxis studies[96]. In addition, surface chemistry can be spatially controlled through micro-stamping techniques, whereby adjacent areas can be given different surface properties with micrometre resolution, through the use of appropriate stamps[97] — for example, to mimic spatial heterogeneity in natural surfaces for biofilm studies. The image in part **a** is adapted from REF. 92. The image in part **b** is adapted from REF. 48, National Academy of Sciences.

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a

Flow velocity (µm s⁻¹)

10 µm

Swimming velocity

Thrust   Drag

b

10 µm

Start

Run

Tumble

c

Cell head

Flagellum

Forwards

Backwards

Hook

~90° change in
swimming direction

Reversal

d

100 nm

Reversal

Forwards

Flick

Backwards

Forwards

Start

e

3 µm

| 0 ms | 10 ms | 20 ms | 30 ms | 40 ms |

Backwards   Reversal   Forwards

| 50 ms | 60 ms | 70 ms | 80 ms | 90 ms |

Flick   Forwards

Particle image velocimetry
(PIV). A method to measure the
velocity field of a fluid based
on the motion of many small
passive tracer particles.

f

Compression (pusher)

Hook

Drag   Thrust

Cell head   Flagellum

Tension (puller)

Drag   Hook   Thrust

Cell head   Flagellum

◀ Figure 1 | **Microbial flow fields and motility mechanics. a** | The flow field produced by a swimming *Escherichia coli* cell[15]. *E. coli* is a 'pusher', with the flagella at the back pushing the cell head, resulting in fluid moving away from the cell along the swimming direction and towards the cell from the sides (black streamlines). Colours represent magnitude of flow velocity. The arrows for the zoomed-in box show the forces exerted by the bacterium on the fluid. **b** | Planar projection of the 3D trajectory of an *E. coli* cell swimming in a 'run-and-tumble' pattern[84]. Circular markers are cell head positions at 79 ms intervals. **c–f** | Many marine bacteria reorient by the 'flick' motility adaptation, an off-axis deformation of the flagellum that enables certain bacteria with a single flagellum to change their direction of swimming[1,2] (Supplementary information S1 (movie)). **c** | Schematic of the flick, which occurs at the onset of a forward run when the 'hook' is under compression. **d** | A trajectory of the bacterium *Vibrio alginolyticus* swimming in a 'run-reverse-flick' motility pattern. Circular markers represent cell head positions at 1 ms intervals. **e** | The image sequence, captured with high-intensity dark-field microscopy, shows the kinematics of the flagellum (magenta) during a flick[2], for the trajectory shown in panel **d**. The flick occurs ~10 ms after the transition from backward to forward swimming. **f** | The flick results from a buckling instability of the hook, which for a pusher cell is compressed by the drag on the cell head and the propulsion force from the flagellum[2]. For a 'puller' cell the hook is under tension and cannot buckle. The image in part **a** is adapted from REF. 15, National Academy of Sciences. The image in part **b** is adapted from REF. 84, Nature Publishing Group. Figure parts **d–f** are adapted from REF. 2, Nature Publishing Group.

Thermal fluctuations
A source of random noise in a system at equilibrium that induces diffusion of small particles.

Rotational diffusion
For a swimming microorganism, this describes the continuous, random changes in swimming direction owing to thermal fluctuations (passive rotational diffusion) or to intrinsic imperfections (for example, wobbling) in the locomotion system (termed active rotational diffusion).

and the mechanics of microbial locomotion, and then discuss microbial chemotaxis and motility in different environments, specifically near and on surfaces, in flowing fluids and in dense suspensions of cells.

The hydrodynamic signature of microorganisms

The microscale fluid flow that a swimming microorganism produces in its immediate surroundings — that is, its 'hydrodynamic signature' — is an important determinant of the ecology of a microorganism and its motility, particularly how it interacts with its physical and chemical microenvironment. The propulsion of a swimming microorganism inevitably results in the generation of flow in the surrounding fluid[13,14], which is characteristic to the swimming strategy of each microorganism. This flow can affect the transport of chemicals, the physical interaction of a cell with surfaces or with conspecifics, and may act as a hydrodynamic cue, revealing the presence of the microorganism to predators.

Dynamic visualization of the hydrodynamic signature of a microorganism is challenging because of the small length scale of the flow, the fast timescales of the translational and rotational movement of microbial cells, and the influence of Brownian motion resulting from thermal fluctuations. Despite these challenges, the hydrodynamic signature of individual swimming *Escherichia coli* cells has recently been measured with remarkable precision[15] (FIG. 1a) by tracking small 'tracer' particles in the vicinity of the bacterial cells (BOX 1). To illustrate the challenge these measurements pose, >5 billion tracer velocity vectors were necessary to resolve the flow field owing to the importance of Brownian motion at this scale[15].

The resulting hydrodynamic signature confirmed long-standing theoretical predictions for *E. coli* in terms of both the nature and magnitude of the flow[16]. The observed flow field is characteristic of 'pusher' microorganisms — microorganisms that use their flagella to push through a fluid (FIG. 1a). As a result, fluid is pushed away from the cell at the front and the back, and pulled towards the cell from the sides (FIG. 1a). These hydrodynamic signatures can affect the coupled motion of two bacteria in close proximity — for example, by causing their alignment, as the flow field of one microorganism 'attracts' the other and vice-versa. However, the effect of the flow field of one microorganism on neighbouring microorganisms was found to decay sharply with increasing distance from the cell producing the flow. This implies that the effects of the flow field are drowned out beyond a few micrometres from a swimmer by active or passive rotational diffusion and are therefore most pronounced in dense cell suspensions[17–19] and near surfaces[20], as discussed below.

The hydrodynamic signature of a microorganism depends on its locomotion mechanism. Whereas some bacteria are 'pushers', others are 'pullers', with flagella that pull the cell through the fluid. In this case, the flow field will be the same as that observed for *E. coli*, but reversed in every direction (that is, fluid is pulled towards the cell at the front and the back, and pushed away from the cell at the sides). Many bacteria, in particular marine bacteria, alternate approximately every second between pushing and pulling, as evidenced in the 'run-reverse-flick' motility pattern[1,2] (see below), and their hydrodynamic signature will therefore alternate in time between that of a 'pusher' and that of a 'puller'.

The mechanics of microbial motility

In addition to offering insights into how microorganisms interact with their environment during swimming, fast imaging of microorganisms at sub-micrometre resolution (which is required to capture their propulsion appendages, such as flagella) has revealed new motility adaptations in bacteria and fostered the understanding of the underlying mechanics.

Most knowledge of bacterial motility comes from the study of the 'run-and-tumble' swimming pattern of *E. coli*[21] (FIG. 1b), which has four to eight flagella emerging from random points on the cell body. Each flagellum is driven by a rotary motor, which is powered by a proton gradient across the inner membrane of the cell. The resulting rotation of the helical flagella causes them to 'push off' from the surrounding fluid and generate propulsion. Direct visualization of flagella[22] revealed that when all motors spin counterclockwise (as seen from behind), the flagella form a bundle that enables the cell to swim in nearly straight 'runs' at speeds of 10–30 µm s⁻¹. The bundle comes apart when one or more motors switch direction, which occurs approximately every second as part of the natural swimming behaviour of the cell, causing it to reorient in a nearly random direction ('tumble'). The transition between 'running' and 'tumbling' is the key to chemotaxis in *E. coli* and is common among peritrichous bacteria (that is, those with multiple flagella distributed randomly over the cell body)[21], including the pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium and the soil-dwelling *Bacillus subtilis*[23].

The swimming strategies of bacteria living in other habitats can be radically different from that of *E. coli*. It has been estimated that 90% of motile marine bacteria are monotrichous[24] — they have a single flagellum

— and therefore cannot reorient using the tumbling strategy observed with *E. coli*. Instead of tumbling, monotrichous marine bacteria, including *Vibrio algino-lyticus*, *Shewanella putrefaciens*, *Pseudoalteromonas haloplanktis* and *Cobetia marina*, change their swimming direction by reversing the direction of rotation of their single motor[23]. Unlike the 'pusher' bacterium *E. coli*, which always swims forwards with its flagella at the rear, these bacteria alternate between forwards swimming (flagellum pushing the cell head) and backwards swimming (flagellum pulling the cell head) (FIG. 1c).

Although reversals change the orientation of a cell, they do not change its swimming direction, and would by themselves result in swimming primarily back-and-forth along the same direction. The ability of these cells to change swimming direction in the absence of multiple flagella was therefore difficult to explain, until the observation of a new motility adaptation — the 'flick'[1] — which is prevalent among marine bacteria[1,2]. The flick is a large, off-axis deformation of the flagellum that results in a 90° (on average) reorientation in swimming direction (FIG. 1c-e). High-speed imaging of the 20 nm-thick flagellum of *V. alginolyticus* showed that the flick occurs approximately 10 ms after the onset of a forward run[2] (FIG. 1e; [Supplementary information S1](#) (movie)). At that instant, the drag force on the cell head and the propulsion force from the flagellum exert a compressive force on the ~100-nm-long 'hook' (which connects the flagellar filament with its motor inside the cell) that is sufficient to make the hook buckle and causes the flagellum to deform off-axis (FIG. 1f). Although the flick is entirely mechanically driven[2], it seems likely that microbial cells control the onset of this buckling process, as evidenced by the dependence of the forward and backward swimming intervals on the sensing of chemical stimuli during chemotaxis[1]. This observation demonstrates that flexibility can be important in the functionality of bacterial flagella[2], even though the flagellar filament itself is essentially rigid.

There is strong evidence that the run-reverse-flick motility strategy is used by other cultured bacterial isolates (*P. haloplanktis*[1] and *Vibrio coralliilyticus*[2]) and widely among swimming bacteria from natural coastal communities[2]. The flick enables cells to effectively reorient with only one flagellum, saving on the cost of assembling and operating multiple flagella in the often nutrient-scarce ocean. This minimalistic solution for reorienting may enhance the chemotactic performance of bacterial cells[1,23,25,26], although the optimality of any bacterial search strategy depends on the often poorly characterized structure of the resource landscape and remains an important area for future research. Methodologically, the discovery of the flick and of its mechanism exemplifies the power of dynamic imaging at extreme length- and time-scales in helping to understand the exquisite motility adaptations of microorganisms.

Swimming with information: chemotaxis

Many microbial survival strategies are based on sensing chemical gradients in the environment and biasing motility towards favourable conditions through a process called chemotaxis[27]. Chemotaxis is dependent on the ability of microbial cells to sense chemical concentrations using transmembrane chemoreceptors, to process this information through intracellular signal transduction systems, and finally to bias motility towards an environment with better conditions[28]. The bacterial chemotactic network is one of the most extensively studied biological sensory systems, and microbial chemotaxis has fundamental roles in a broad range of processes[29,30], including nutrient consumption and cycling, pathogenesis and surface colonization.

The ability to create chemical gradients at the spatial and temporal scales relevant to microorganisms, which is offered by microfluidics (BOX 2), combined with the possibility of tracking hundreds of individual cells by dynamic imaging (BOX 1), has provided a new tool to study chemotaxis[31]. This has furthered our understanding of both the principles of chemotaxis in model organisms such as *E. coli*, and the ecological implications of chemotaxis under realistic environmental conditions, in particular for marine bacteria.

Chemotaxis in **E. coli**. Careful gradient control has provided new insights into the rescaling response of chemotaxis in *E. coli*. In steady gradients, created within a microfluidic device, the chemotactic response of *E. coli* to the amino acids α-methyl-DL-aspartate and L-serine did not change significantly over a wide range of concentrations and gradients[32]. *E. coli* is able to maintain its chemotactic performance by biasing its swimming not in response to the concentration gradient *per se,* but in response to the gradient normalized by the mean concentration of the amino acids. This property is called logarithmic sensing[32] (because the response is to the gradient of the logarithm of concentration): it ensures a response-rescaling such that the cell retains high sensitivity under a broad range of environmental conditions, similarly to what occurs in human vision and hearing[33,34].

Direct imaging of chemotactic migration in microfluidic devices together with Förster resonance energy transfer (FRET) measurements of intracellular signalling showed that the chemotactic response of *E. coli* has an even stronger rescaling property — fold-change detection (FCD)[35]. In this case, the full time-course of the chemotactic response remains unchanged if the concentration of the chemical eliciting chemotaxis is rescaled by a constant factor. Therefore, cells respond to the time history (that is, shape) of the chemical signal, irrespective of its absolute intensity. In other words, a cell exhibiting FCD will have the same chemotactic response to two time histories of the concentration of a given chemical that differ only in their amplitude. The observation of FCD illustrates the remarkable ability of bacteria to follow chemical cues by chemotaxis under a broad range of conditions, which may be an adaptation to the diversity of chemical conditions that bacterial cells encounter in the environment.

Microfluidic approaches are well suited to studying microbial responses to chemical landscapes that begin to mimic the complexity of natural environments, where

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Buckling
A sudden sideways failure of a structure subjected to compressive load.

Logarithmic sensing
A sensing property in which cells respond to the relative gradient in a stimulus, $\nabla C/C$, in which C is the magnitude and ∇C is the gradient magnitude of the stimulus.

Förster resonance energy transfer
(FRET). A mechanism quantifying energy transfer between two light-sensitive molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon. In chemotactic transduction studies of *Escherichia coli*, FRET is used to measure the level of the chemotaxis signalling molecule phospho-CheY (CheY^p) that controls flagellar reversals.

microorganisms often experience temporal fluctuations in chemical concentration. Imaging the chemotactic response of an *E. coli* population to gradients of either the metabolizable attractant L-aspartate or its non-metabolizable analogue α-methyl-DL-aspartate, the directions of which were periodically switched, revealed that the bacterial cells were able to track the gradient when the switch in direction was not too rapid (that is, >200 s). This ability was related to the time requirement for adaptation[36], demonstrating that the internal adaptation rate, which is controlled by the chemoreceptor methylation level, sets a fundamental biophysical limit on the frequency of environmental fluctuations that *E. coli* can track by chemotaxis.

Moreover, microfluidic approaches have offered insights into microbial responses to multiple chemical gradients, which are another common feature of natural environments. For example, exposing *E. coli* cells to simultaneous, opposing gradients of two different amino acids (α-methyl-DL-aspartate and L-serine) in a microfluidic device showed that the relative preference of the cells for each amino acid depended on the relative number of chemotactic receptors for that amino acid (Tar for aspartate and Tsr for serine)[37]. The receptor expression level was in turn determined by the density of bacterial cells in the population[37], a quorum effect that has largely been neglected in chemotaxis studies, but might be an important feature in realistic environments. This study of bacterial decision making under multiple chemical cues opens the door to the investigation of chemotaxis in more complex chemical landscapes with microfluidics.

Chemotaxis in marine bacteria. Recent research has demonstrated the prevalence and ecological importance of chemotaxis among marine bacteria[29,30]. Chemotaxis enables bacteria in the ocean to exploit the many resource hotspots and organic matter gradients that characterize marine microenvironments, originating from phytoplankton exudation and lysis, sloppy feeding and excretions by larger organisms, and sinking particles.

Imaging has revealed the ability of natural communities of marine bacteria to chemotactically cluster around particles and in the organic-matter rich 'phycosphere' surrounding stressed or dying phytoplankton cells[29,38,39] (FIG. 2a; Supplementary information S2 (movie)). Imaging coupled with cell tracking has shown the remarkable ability of marine bacteria to track phytoplankton: in one case, the marine bacterium *P. haloplanktis* made up to 12 correct consecutive turns in chasing the swimming phytoplankton *Pavlova lutheri*[40], probably by tracking its chemical wake by chemotaxis. These close spatial associations between primary producers and bacterial consumers can provide a fitness advantage to motile bacteria and potentially shape the timescale and modes of transformation of dissolved organic matter (DOM) in the ocean.

Microfluidics has provided a platform for the controlled production of microscale hotspots and the detailed study of the microbial response to them[29,30]. Chemotaxis of marine bacteria in response to ephemeral,

Chemokinesis
The modulation of swimming speed in response to changes in the concentration of a chemical.

microscale DOM pulses has been studied with a microfluidic injector (microinjector) device, in which a precisely controlled band of DOM that is similar in size to microscale hotspots in the ocean (~300 μm) is generated and then freely diffuses (FIG. 2b). Imaging bacteria responding to the band revealed that *P. haloplanktis* exhibits a chemotactic response to these ephemeral DOM pulses that considerably exceeds the fastest chemotactic responses known for *E. coli*, resulting in an order of magnitude enhancement in the nutrient exposure by the fastest cells[41].

Subsequent applications of the microinjector broadened this finding by demonstrating strong chemotactic responses by marine bacteria towards a range of chemical compounds, including DOM exuded from the highly abundant cyanobacteria *Synechococcus* and *Prochlorococcus*[42], the exudates of the harmful algal-bloom-producing phytoplankton *Heterosigma akashiwo*[43] and the sulfur compound dimethylsulfoniopropionate (DMSP). The microbial breakdown of DMSP can produce the climatically active compound dimethylsulfide, the release of which into the atmosphere can affect cloud formation[44]. These microfluidic observations demonstrate and quantify the pervasive behavioural responses of marine bacteria to chemical cues and provide the basis for new ecological frameworks of marine microorganisms and their biogeochemical roles that explicitly take into account microbial motility in the context of microscale resource heterogeneity.

Chemotaxis can also favour the association between marine bacterial pathogens and their animal hosts. In a further application of the microinjector device, the coral pathogen *V. coralliilyticus* was observed to undergo chemotaxis with striking speed and directionality towards a layer of the mucus collected from its coral host, *Pocillopora damicornis*[45] (FIG. 2c). This response was driven primarily by DMSP present in the mucus. Tracking instantaneous cell responses showed that, in addition to chemotaxis, *V. coralliilyticus* enhanced its response to mucus by chemokinesis[45]. Chemokinesis has been reported in other marine isolates, including *P. haloplanktis*[46], *S. putrefaciens* and *C. marina*, as well as in enriched assemblages of marine bacteria[47]. Its observation in *V. coralliilyticus*[45] illustrates a potential implication of bacterial chemokinesis in the disease of marine animals. Analogous experiments with mucus from heat-stressed corals revealed that higher temperatures further favour the pathogen, by causing a greater release of DMSP by the coral and consequently enhancing the chemotactic response of *V. coralliilyticus*.

In aquatic environments, an important driver of chemotaxis is the opportunity to encounter and exploit particles that are often rich in organic matter. Successful chemotaxis towards the particle surface can be ensued by significantly different behaviours, even among closely related bacteria. This was demonstrated in a recent microfluidic study, in which attachment and chemotaxis were assayed in two recently speciated, sympatric populations of the marine bacterium *Vibrio cyclitrophicus* to provide insights into how they coexist in nature[48]. By creating microenvironments mimicking both the particle

surface and the chemical gradient near it, this study revealed that one population specialized in colonizing particles by attachment and biofilm formation, whereas the other specialized in dispersing among particles by chemotaxis to take advantage of the occurrence of fresh particles (FIG. 2d). These phenotypic differences were paralleled by corresponding genotypic differences related to surface attachment and biofilm formation. This is the first microbial example of a 'competition–dispersal' trade-off, which was developed and traditionally applied in macroecology, and demonstrates how differences in spatial behaviour can drive coexistence in the environment. These findings also highlight how microfluidic approaches can be used to mimic and study the effects of fundamental features of microbial habitats.
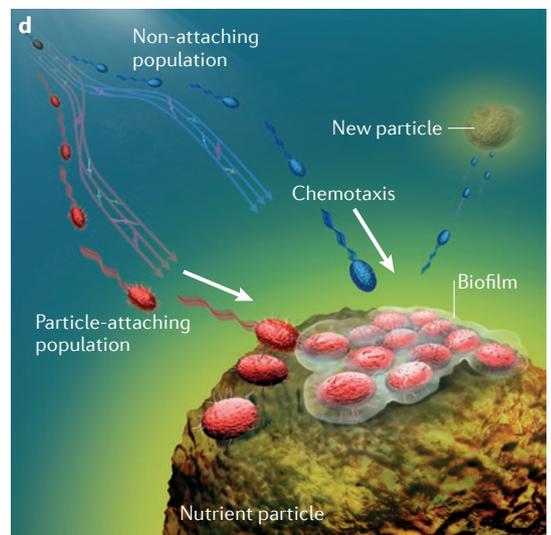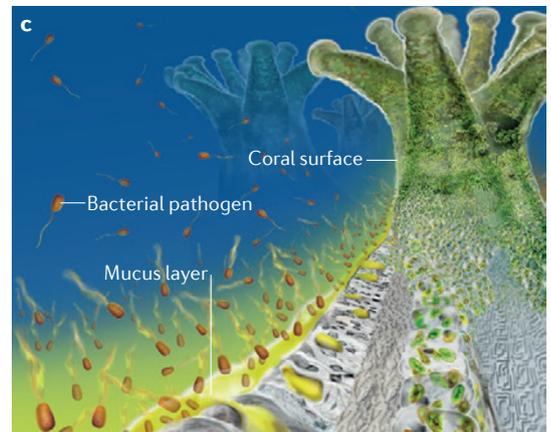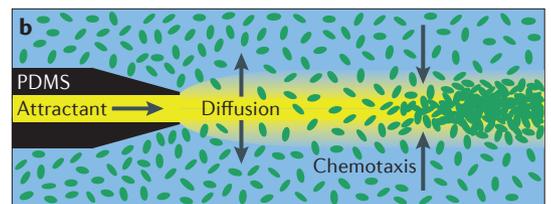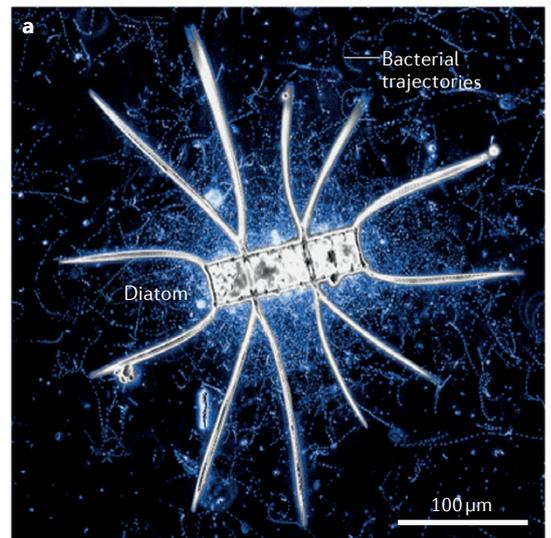
Environmental effects on motility

Beyond chemical cues in the environment, motility is often affected by the physical properties of the environment. The presence of a surface is a prime example[49] and considerably alters the motility of microorganisms. In the vicinity of a surface, hydrodynamic forces can trap swimming microbial cells or bias their trajectories[20], whereas on the surface itself flagella-mediated swimming turns into pili-mediated twitching[6,7]. A second physical property of most microbial environments is fluid flow[11]; rarely are fluids at rest, and their motion can exert forces on swimming microorganisms, redirect them, and trap them in specific regions. Finally, the presence of nearby cells, as observed in dense microbial suspensions, also alters the physical environment of a swimmer, creating patterns of collective motion[17–19] that may significantly affect chemical and biological transport. Imaging techniques and microfluidic technology have provided new insights into how microbial motility is affected by these physical elements of the environment, frequently by tracking individual cells.

Microbial motility near and on surfaces. Surfaces are ubiquitous in microbial habitats[49] and present opportunities for microorganisms to form biofilms, whereby cells attach to a surface by encasing themselves within a self-secreted matrix of extracellular polymeric substances. This offers them increased resistance to antibiotic insults and mechanical stress[50–52]. The principles underlying the onset of biofilm formation have largely remained elusive, in part because of the complexity of the physical interactions between cells and the substrate, and here they are discussed specifically in relation to motility.

The initial steps in the colonization of a surface consist of the approach and attachment of cells, which is frequently followed by dispersal of cells on the surface by a type of motility known as twitching[53]. Tracking individual bacteria near and on surfaces has revealed a range of surface motility adaptations and has yielded new insights into the initial stages of biofilm formation[49,54].

Microorganisms swimming near surfaces have two characteristic features that influence the rate of attachment and, ultimately, biofilm formation. First, they often become effectively trapped near the surface[20], even when

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Twitching
Crawling motion of bacteria on surfaces by means of pili.

a

Bacterial trajectories

Diatom

100 μm

b

PDMS
Attractant
Diffusion
Chemotaxis

c

Coral surface
Bacterial pathogen
Mucus layer

d

Non-attaching population

New particle

Chemotaxis

Biofilm

Particle-attaching population

Nutrient particle

◄ Figure 2 | **Microbial chemotaxis.** **a** | Snapshot of the phycosphere, the organic-matter-rich microzone surrounding individual phytoplankton cells (see also Supplementary information S2 (movie)). The maximum intensity projection image shows trajectories of natural marine bacteria (blue) strongly accumulating around a lysing *Chaetoceros* diatom by chemotaxis[29]. **b** | Schematic of the microfluidic 'microinjector' used to study microbial behavioural responses, particularly chemotaxis, to ephemeral resource hotspots[41–45]. The resource is ephemeral because it is initially in the form of a band of attractant that rapidly diffuses outwards. Bacterial locations and trajectories can be captured by time-lapse imaging. **c** | Bacterial pathogens can detect their coral hosts by chemotaxis. Motility is prevalent among putative coral pathogens, and microfluidic experiments showed that *Vibrio coralliilyticus* exhibits a strong chemotactic response towards coral mucus (yellow shading), which diffuses from the coral surface[45]. This response is likely to be a mechanism used by the pathogen to locate its coral host and is exacerbated under warming conditions[45]. **d** | Model for the coexistence of two closely related populations of marine bacteria based on trade-offs in their spatial behaviours[48]. Dynamic imaging in microfluidic devices showed that both populations of *Vibrio cyclitrophicus* use chemotaxis to migrate towards particles, but only one population attaches and forms biofilms on particles (red cells). The other population (blue cells) remains near the particle and retains the flexibility of rapidly migrating to new, more nutrient-rich particles. This represents the first microbial example of a competition–dispersal trade-off. PDMS, polydimethylsiloxane. The image in part **c** is adapted from REF. 45, Nature Publishing Group.

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they are several body lengths away from the surface. This trapping is caused by the interaction between the flow field generated by a microorganism swimming in a 'pusher' configuration (FIG. 1a) and the surface, which produces a force that attracts the swimmer to the surface. This occurs both in *E. coli*[20], which is exclusively a 'pusher', and in *V. alginolyticus*[55], which alternates between being a 'pusher' and a 'puller'. In the case of *E. coli*, this leads to a bias in the swimming direction of the cells so that the cells swim preferentially parallel to the surface[20]. The reorientation produced by the random tumbles intrinsic in the run-and-tumble swimming pattern of *E. coli* may, in principle, free the cell from the attractive force of the surface and cause its escape, but recent observations made by digital holographic microscopy (BOX 1) revealed that the tumbles themselves may be impaired by the surface[56]. It was found that the tumbling frequency was reduced by approximately 50% within 20 μm of the surface, compared with motility away from the surface. This is likely due to a reduction in the hydrodynamic force responsible for the unbundling of flagella — a process necessary for tumbling[56]. This finding suggests that the flow field and run-and-tumble motility pattern of *E. coli* are either not ideal for escaping surfaces, or alternatively, that they are well suited to ensure that *E. coli* cells remain near surfaces, potentially to enhance attachment and biofilm formation rates or benefit from surface-derived nutrients.

A second characteristic feature of microorganisms swimming near surfaces is that they often swim in circular trajectories (for example, *E. coli* cells swim clockwise when viewed from within the fluid). This phenomenon arises from the reaction forces from the surface acting on the cell head and flagellar bundle of the swimmer, which rotate in opposite directions[57]. The two reaction forces create a torque that makes the cell curve and swim in a circular trajectory (FIG. 3a). Circular trajectories are therefore another feature of microbial cells trapped near solid surfaces. Furthermore, these processes highlight the importance of the hydrodynamic signature of

microorganisms, with different propulsion strategies resulting in different forces and interactions between microorganisms and surfaces[20].

Trapping near a surface caused by flagella-based motility results in a greater reservoir of microbial cells that can encounter the surface and thus probably enhances the rate of attachment and biofilm formation. The actual contact with the surface can be highly dependent on the behaviour of a second type of appendage — pili — in scanning the surface and adhering to it. For example, *Vibrio cholerae* cells swimming near a surface use their mannose-sensitive haemagglutinin pili (MSHA pili) to mechanically scan the physical properties of the surface before attachment[58]. Rotation of the cell body during propulsion causes periodic contact of the MSHA pili with the surface. Depending on the magnitude of the frictional forces between pili and the surface, the cells exhibit either 'roaming' motion, which is characterized by meandering trajectories with low frictional interaction, or 'orbiting' motion, which is characterized by high-curvature trajectories with strong pili–surface interactions. The distinction between these two modes is important in determining surface colonization, because only orbiting cells can attach irreversibly and form microcolonies, which are the precursors of biofilms[58].

The different appendages also offer microorganisms the ability to use distinct surface-motility and chemical-tracking strategies after landing on surfaces to transition from the planktonic state to the surface-associated state and ultimately initiate biofilm formation. For example, *Pseudomonas aeruginosa* cells can repeatedly attach and detach their type IV pili to either 'walk' on a surface in a vertical orientation (FIG. 3b) or crawl on a surface in a horizontal orientation[7,59] (FIG. 3c). Walking *P. aeruginosa* cells exhibit a higher instantaneous velocity (approximately 70 nm s⁻¹) than crawling cells (approximately 40 nm s⁻¹), but their trajectories on the surface are diffusive, enabling rapid local exploration of the surface. By contrast, crawling trajectories are straighter, enabling more effective large-scale exploration. *P. aeruginosa* cells reversibly transition between these two modes of motility[7].

Especially during crawling, the pili of *P. aeruginosa* mediate two distinct actions (Supplementary information S3 (movie)). Multiple pili can pull a cell at a constant velocity, or the 'slingshot' release of a single pilus can transiently propel the cell 20 times faster while also causing it to rotate[6] (FIG. 3d). The 100-ms-long slingshot motion may assist in propulsion through viscoelastic fluids, such as the polymeric matrix of biofilms, the viscosity of which is reduced at the large velocity gradients produced by this rapid action[6].

The crawling patterns of microbial cells on a surface can directly affect biofilm formation. Tracking crawling *P. aeruginosa* cells and quantifying their visit frequency to each location on the surface revealed the mechanism by which cells begin to build biofilms[3]. After landing on a clean surface, *P. aeruginosa* cells begin to deposit a trail of the exopolysaccharide Psl as they crawl on the surface over many hours. This chemical trail influences the motility of bacteria that subsequently encounter the

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Digital holographic microscopy
A microscopic imaging technique where the position of an object 'into the plane' is encoded by the interference fringes it creates by diffracting light and can be reconstructed in post-processing to yield 3D information.

Torque
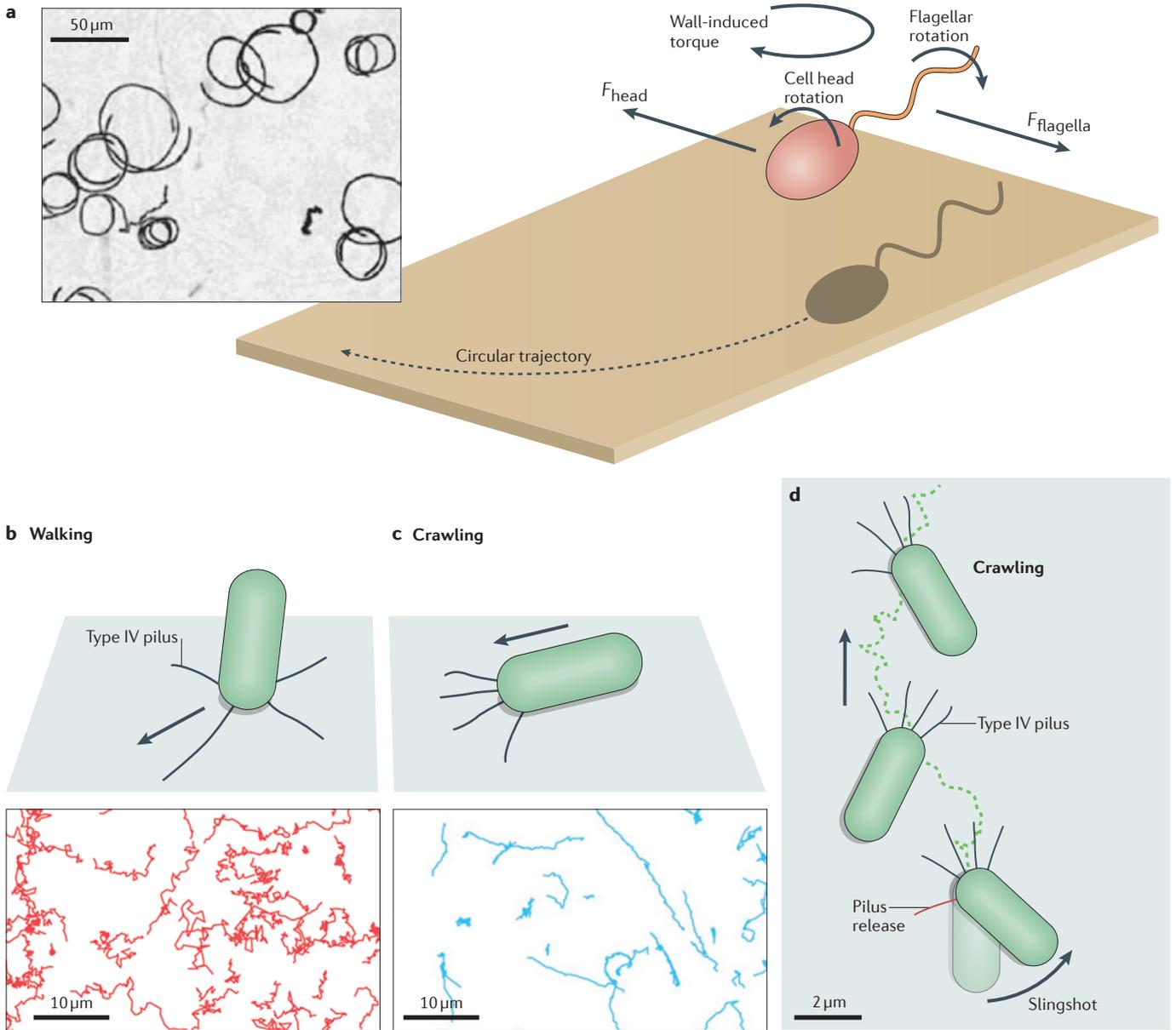The moment of the forces that act on an object, which quantifies their tendency to rotate the object.

Mannose-sensitive haemagglutinin pili
(MSHA pili). One of three type IV pili, which play an important part in biofilm formation.

Type IV pili
Thin, hair-like appendages present on the surface of many bacteria, involved in adherence to and motility on substrates.

Figure 3 | Microbial interactions with surfaces. a | Bacteria near a surface often swim in circles[57]. The rotation of the flagellar bundle near the surface induces a net reaction force on the flagellar bundle ($F_{flagella}$) from the surface; the cell head counter-rotates and experiences a force in the opposite direction (F_{head}). Circular swimming results from the torque induced by these two forces[57,98]. The inset shows observed trajectories of a smooth swimming (non-tumbling) mutant of *Escherichia coli* (HCB437) that also lacks most genes associated with chemotaxis [57]. **b–d |** Live imaging of cells on a surface revealed distinct modes of surface motility in *Pseudomonas aeruginosa*[7,59] (see also Supplementary information S3 (movie)). Cells can either stand up on the surface and 'walk' (part **b**) or lie on the surface and 'crawl' (part **c**). Walking results in jagged trajectories that are better for local exploration, whereas crawling has high directional persistence and enables bacterial cells to more effectively cover distance[7]. Simultaneous pulling of multiple type IV pili that results in steady crawling motility (part **c**) is interrupted by the rapid (100 ms), 'slingshot' release of a single pilus that causes an impulsive forward motion coupled with a change in direction[6] (part **d**). The inset image in part **a** is republished with permission of Elsevier, from Swimming in circles: motion of bacteria near solid boundaries, Lauga, E., DiLuzio, W. R., Whitesides, G. M. & Stone, H. A. **90**, 2, 2006; permission conveyed through Copyright Clearance Center, Inc. The images in parts **b** and **c** are adapted from Gibiansky, M. L. *et al.* Bacteria use type IV pili to walk upright and detach from surfaces. *Science* **330**, 197 (2010). Reprinted with permission from AAAS. The image in part **d** is adapted with permission from REF. 6, National Academy of Sciences.

trail, creating a positive-feedback loop where regions of high Psl concentration result in more Psl deposition, and thereby become nucleation points for microcolony formation[3].

Microbial motility in moving fluids. Microorganisms often live in dynamic fluid environments[60]. For example, water creeps through soil and the bodies of animals and plants, and creates currents and turbulence in streams,

a | Jeffery orbit

b | Flow profile

Swimming trajectories

c | Cell distribution
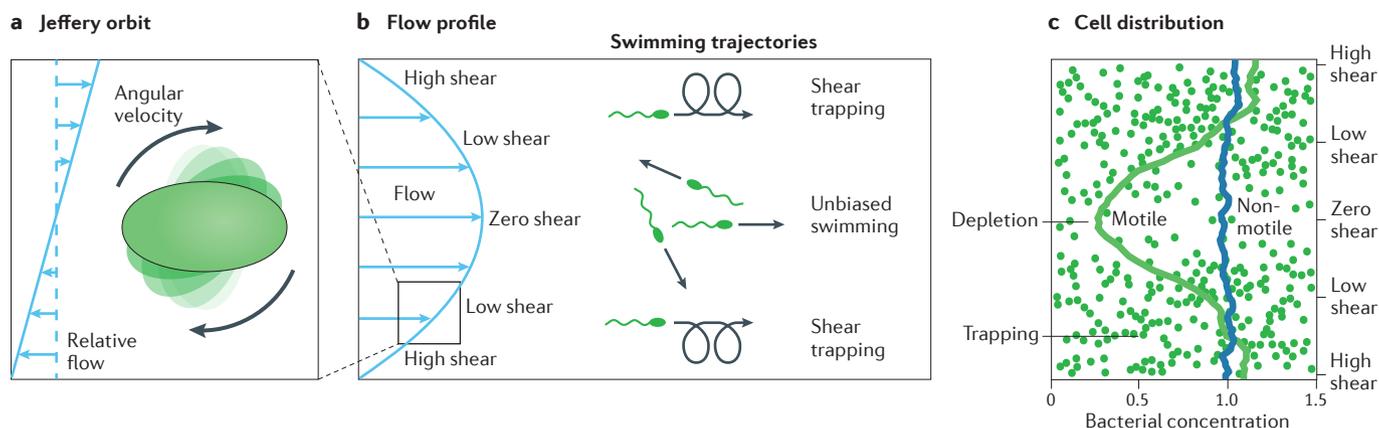
Figure 4 | **Microbial motility in moving fluids. a** | Elongated particles or microorganisms exposed to fluid velocity gradients ('shear') undergo periodic rotations, or 'Jeffery orbits'[13]. The angular velocity varies with orientation relative to the flow, being faster when the cell is oriented transverse to the flow and slower when it is aligned with the flow. Jeffery orbits can considerably affect the transport of microorganisms in the environment. **b,c** | Fluid flow biases the motility of swimming bacteria (see also Supplementary information S4 (movie)). Like many natural flows, the parabolic flow profile in a microfluidic channel has non-uniform shear. Bacteria are free to swim in all directions equally in the central, low-shear region, but preferentially align and become trapped in high-shear regions near the sides[63] (part **b**). Therefore, motile bacteria are depleted in regions of low shear and accumulate in regions of high shear, resulting in strong spatial heterogeneity in the bacterial distribution[63] (part **c**). This 'shear-trapping' increases surface attachment and hinders chemotaxis[63]. Part **c** is adapted from REF. 63, Nature Publishing Group.

lakes and oceans. However, tools to study and quantify the effects of fluid flow on microbial processes — in particular, microbial motility — have been lacking. In the past few years, microfluidics coupled with dynamic imaging has provided an ideal approach to study flow–microorganism interactions, through the precise tracking of microorganisms (BOX 1) in precisely controlled flows (BOX 2). This approach has already revealed important consequences of the coupling between flow and motility, including the formation of strong spatial heterogeneity in the distribution of cells[61–63] as well as biases in the direction of microbial migration[64–67].

In a fluid flow, non-motile microorganisms travel faithfully with the flow, their small size precluding any deviations from fluid streamlines[13]. However, inevitable fluid velocity gradients (known as 'shear'; BOX 2) exert torques that result in a periodic rotation of microbial cells, called a 'Jeffery orbit' (FIG. 4a). If the microorganism is motile, this rotation affects its swimming direction and thus where it ends up in the flow[63]. Many bacteria are highly elongated, particularly if they possess flagella. For example, the hydrodynamic aspect ratio (that is, the length to width ratio) of *B. subtilis*[63] is ~10. Owing to this elongation, the rotation rate in the Jeffery orbit is faster when the cell is oriented transverse to the flow and slower when it is aligned with the flow (FIG. 4a,b). Consequently, in the presence of fluid velocity gradients, elongated microorganisms spend more time aligned with the flow (Supplementary information S4 (movie)), and their ability to migrate across streamlines is hampered.

Fast imaging of *B. subtilis* cells swimming in a laminar flow revealed that Jeffery orbits can strongly alter the spatial distribution of motile bacteria (FIG. 4c). Shear varies linearly across a microfluidic device, being high

near the sidewalls, where fluid velocity is lowest, and low in the centre, where fluid velocity is highest (but mostly uniform) (FIG. 4b). Bacteria are free to swim in all directions equally near the centre of the channel, where shear is low, but, as a result of Jeffery orbits, become trapped and accumulate near the sidewalls of the channel, where shear is high (FIG. 4b; Supplementary information S4 (movie)). This effect caused a >70% depletion of cells from the centre of the channel (FIG. 4c), which occurred very rapidly, over only a few seconds. Strong heterogeneity in the population distribution was observed for the tumbling, wild-type *B. subtilis* and a non-tumbling mutant, as well as for the monotrichous *P. aeruginosa*[63]. This strong redistribution in the positions of microbial cells originates from the competition between the shear-induced alignment of the bacteria and the random reorientations owing to active tumbling and passive rotational diffusion.

Additional microfluidic observations revealed that this 'shear-trapping' can have direct consequences on major microbial phenotypes, including chemotaxis and surface attachment[63]. Given that essentially all motile microorganisms with flagella are highly elongated and that in most flows in nature the shear is spatially non-uniform, shear-trapping is expected to apply to a broad range of bacteria.

Beyond free-swimming cells, live imaging has revealed that fluid flow can have important and counter-intuitive consequences on bacteria moving on surfaces. Microfluidic experiments have shown that *E. coli*[64,65] swimming near a surface (FIG. 5a), as well as *Xylella fastidiosa*[68] and *P. aeruginosa*[66] twitching on a surface, migrate upstream (FIG. 5b) in the presence of flow. This upstream migration results from the shear at the surface, which exerts a torque on the bacterial cells that reorients

Jeffery orbit
Periodic rotational trajectory of an elongated particle (in this case, a microorganism) in a fluid velocity gradient, in which the angular speed varies with orientation relative to the flow.

Laminar flow
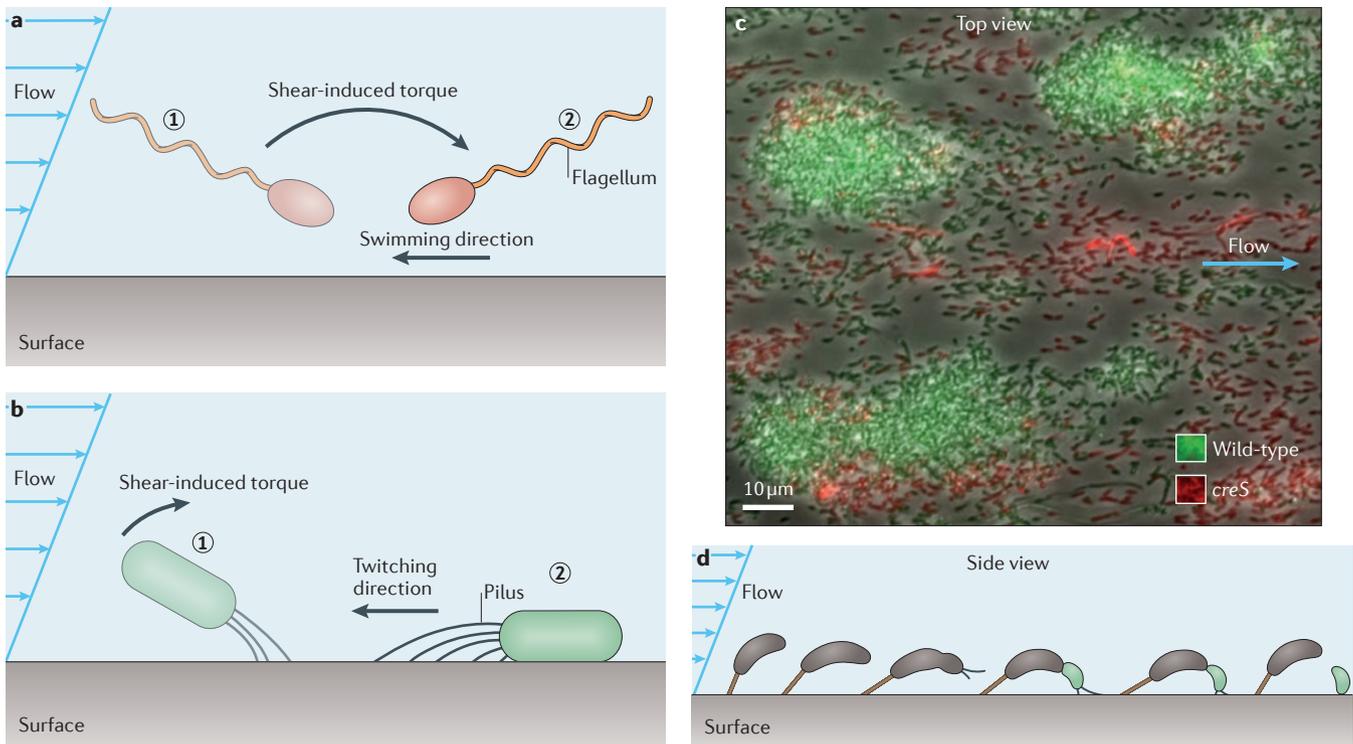Fluid motion devoid of turbulence and typically occurring as a smooth, orderly flow.

Figure 5 | **Upstream motility and downstream bending in flowing fluids. a,b** | Fluid flow causes upstream swimming of *Escherichia coli* [64,65] near a surface (part **a**) and upstream twitching of *Pseudomonas aeruginosa* [66] on a surface (part **b**). Numbers (1 and 2) denote a sequence of events. The torque induced by the shear at the surface orients bacterial cells to point upstream in both cases. For twitching cells, the periodic extension and retraction of pili pulls the cell upstream [66]. Upstream motility can affect transport of bacteria in biomedical settings, including the urinary tract, catheters or blood vessels. **c,d** | Time-lapse imaging in a microchannel revealed the effect of the curved shape of *Caulobacter crescentus* in surface colonization under flow [8]. Curved wild-type cells (green) colonize the surface more successfully than straight cells (red) possessing a mutation in the gene encoding the cytoskeletal protein crescentin (*creS*) (part **c**), because fluid flow more effectively bends the dividing cell (part **d**, grey) towards the surface, conferring a higher surface-attachment probability to the daughter cell [8] (part **d**, green). The image in part **c** is adapted from REF. 8, Nature Publishing Group.

them to point upstream. Therefore, even fast flows cannot be assumed to simply 'wash out' bacteria; this has potential implications for infection processes in the urinary tract, catheters or blood vessels, where upstream migration may cause bacterial transport into unexpected regions of the flow system.

In the presence of fluid flow, the shape of the cell body can be an important phenotype for surface colonization [8] (FIG. 5c,d). In microfluidic experiments in which *Caulobacter crescentus* was attached to a surface by a polar holdfast and a stalk, shear was found to cause the bacteria to bend towards the surface, in the direction of the flow (FIG. 5d). During cell division, the natural curvature of the crescent-shaped *C. crescentus* aids in orienting the adhesive pili at the pole of the daughter cell towards the surface. This is because the concave-side-down configuration is stable in flow whereas the concave-side-up configuration is not. This mechanism promotes the surface attachment of the daughter cell and enhances surface colonization, compared with straight cells expressing a mutated form of the gene encoding the cytoskeletal protein crescentin (*creS*) (FIG. 5c).

These observations represent only the beginning of our understanding of the interactions between fluid flow and motility, and further work is needed to determine how these interactions ultimately affect processes such as chemotaxis, surface adhesion and biofilm formation.

Motility in groups. Microorganisms in certain habitats, including the human gut [69], live in dense suspensions, where cell concentrations can exceed 10^{10} ml^{-1} and interaction with conspecifics becomes predominant. Over the past decade, the physics of dense microbial suspensions has been studied extensively, exploiting the ability of microscale devices to control, confine and visualize dense bacterial suspensions. For motile cells, observations have revealed striking, turbulent-like collective motions of the dense bacterial suspensions [14]. These collective motions take the form of vortices and jets with dimensions ($\sim$50–100 µm) and speeds (50–200 µm s^{-1}) that are considerably greater [70,71] than those of individual bacteria ($\sim$2 µm; 10–50 µm s^{-1}).

These collective behaviours derive from the physical interactions between densely packed cells [17–19], although it remains unclear whether they are caused by hydrodynamic interactions or simple physical contact [72]. According to the former explanation, the flow field produced by an individual bacterium (see above) may

Biofilm streamers
Conglomerates of cells and cell-secreted polymeric substances (exopolysaccharide) that are attached by one end to a surface and otherwise suspended in the flow. These biofilm structures exist in topographically complex environments exposed to fluid flow.

be responsible for the 'bacterial turbulence', whereas according to the latter it is only when microorganisms come in contact that they mechanically affect each other.

Regardless of their origins, these collective motions can enhance the dispersion of bacteria and accelerate the mixing of nutrients and oxygen[73], and may afford bacteria collective resistance to antibiotics[74]. Although the full ecological implications of this physical process remain unexplored, we propose that it holds promise as a framework for microbial dispersion, chemical transport and physical interactions for microbial environments that are characterized by densely packed bacterial populations. This includes environments such as the human gut[69], where motility at the individual-cell scale may affect environmental conditions and, ultimately, population dynamics at the habitat scale.

Outlook
The combination of dynamic imaging and the precise environmental control afforded by microfluidics represents a uniquely powerful approach to capture the fundamental nature of many microbial processes, which are often dynamic, unfold at the level of single cells and depend intimately on the chemical and physical microenvironment. The work reviewed here demonstrates that systems integrating these technologies can offer important new insights into how and why microorganisms move.

An important area awaiting investigation relates to the optimality of microbial motility strategies and how these correspond to environmental conditions, such as the granularity of the resource landscape or the magnitude of hydrodynamic forces. The links between morphology (having one versus multiple flagella), locomotion mechanics (flicking of a single flagellum versus unbundling of many flagella) and motility pattern (run-reverse-flick versus run-and-tumble), as well as their effect on chemotactic performance, are all rich areas of investigation that will require both experimental observations and modelling[25,26] to unearth the ecological underpinning and consequences of different motility adaptations. In addition to its intrinsic importance, this area could have biomimetic applications — for example, inspiring the design of minimalistic robots[75] that use the simplest motility strategies[76] but can be deployed in large numbers[77].

Studies of microbial interactions with the physical environment, primarily surfaces[49] and fluid flow[60], have led to the discovery of biases in microbial migration caused by surface-induced or hydrodynamic forces, which in turn affect the transport of bacteria and the initial surface colonization, ultimately leading to the formation of biofilms[63] and biofilm streamers[78]. These physical interactions represent important features of natural, technological and clinical environments[79], but

have received less attention than chemical or biological interactions. Understanding the microscale mechanisms underpinning these interactions holds promise to provide new insights into ways to tackle ubiquitous biofouling problems.

In some environments, such as the human gut, conditions may be so crowded that current models of free-swimming motility are unlikely to apply. Instead, surface interactions as well as cell–cell interactions are likely to be prevalent. Recent observations indicate that these interactions can produce rich dynamics[17–19]. Understanding the ecological implications of these novel dynamics under realistic conditions will require not only information on the physical and chemical gut microenvironment but also the ability to increasingly mimic such conditions (including, for instance, oxygen gradients) *in vitro*. In this respect, microfluidic approaches are ideally suited to create gut-on-a-chip models of the microbial gut environment and to reveal the role of motility in dense, confined habitats.

A broad range of other microbial processes stand to benefit from dynamic imaging and microfluidics (and in some cases has already begun to do so), because these approaches afford a temporally and spatially explicit understanding of processes that are to date frequently studied in a temporally frozen and/or spatially uniform context. Single-cell dynamic imaging can be meaningfully extended to other important microbial groups —including, for example, archaea and viruses — to observe the interaction between different microbial trophic levels, from predator–prey interactions at the level of the dynamics of individual predation events, to the establishment of symbiotic cell consortia[80], to chemotactic aggregations of microorganisms around larger organisms. Microfluidics provides unprecedented opportunities to mimic increasingly realistic features of complex natural environments such as temporal fluctuations and spatial heterogeneity[11]. Precise control of microenvironmental conditions can be particularly useful for understanding the spatial ecology of microorganisms, and also for creating experimental arenas to study optimal foraging theory and microbial competition for heterogeneous resources.

Imaging — and, in particular, dynamic imaging — appeals uniquely to our understanding of microbial motility in particular, and microbial ecology in general, because it enables the direct visualization of processes for which we have poor intuition, and the mechanisms of which may otherwise be difficult to decipher. The combination of powerful imaging approaches and the growing field of microfluidics and microtechnology has much to contribute to our understanding of the physical ecology of microorganisms, because of the unique capability of controlling and seeing the world of microorganisms, at the scale of microorganisms.

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