Flagellated bacteria can swim across moist surfaces within a thin layer of fluid, a means for surface colonization known as swarming. This fluid spreads with the swarm, but how it does so is unclear. We used micron-sized air bubbles to study the motion of this fluid within swarms of *Escherichia coli*. The bubbles moved diffusively, with drift. Bubbles starting at the swarm edge drifted inward for the first 5 s and then moved outward. Bubbles starting 30 μm from the swarm edge moved inward for the first 20 s, wandered around in place for the next 40 s, and then moved outward. Bubbles starting at 200 or 300 μm from the edge moved outward or wandered around in place, respectively. So the general trend was inward near the outer edge of the swarm and outward farther inside, with flows converging on a region about 100 μm from the swarm edge. We measured cellular metabolic activities with cells expressing a short-lived GFP and cell densities with cells labeled with a membrane fluorescent dye. The fluorescence plots were similar, with peaks about 80 μm from the swarm edge and slopes that mimicked the particle drift rates. These plots suggest that net fluid flow is driven by cell growth. Fluid depth is largest in the multilayered region between approximately 30 and 200 μm from the swarm edge, where fluid agitation is more vigorous. This water reservoir travels with the swarm, fueling its spreading. Inter-cellular communication is not required; cells need only grow.

Field-labeled bacteria can swim across moist surfaces within a thin layer of fluid, a means for surface colonization known as swarming. This fluid spreads with the swarm, but how it does so is unclear. We used micron-sized air bubbles to study the motion of this fluid within swarms of *Escherichia coli*. The bubbles moved diffusively, with drift. Bubbles starting at the swarm edge drifted inward for the first 5 s and then moved outward. Bubbles starting 30 μm from the swarm edge moved inward for the first 20 s, wandered around in place for the next 40 s, and then moved outward. Bubbles starting at 200 or 300 μm from the edge moved outward or wandered around in place, respectively. So the general trend was inward near the outer edge of the swarm and outward farther inside, with flows converging on a region about 100 μm from the swarm edge. We measured cellular metabolic activities with cells expressing a short-lived GFP and cell densities with cells labeled with a membrane fluorescent dye. The fluorescence plots were similar, with peaks about 80 μm from the swarm edge and slopes that mimicked the particle drift rates. These plots suggest that net fluid flow is driven by cell growth. Fluid depth is largest in the multilayered region between approximately 30 and 200 μm from the swarm edge, where fluid agitation is more vigorous. This water reservoir travels with the swarm, fueling its spreading. Inter-cellular communication is not required; cells need only grow.

**Results**

**Fluid Flows in the Interior of *E. coli* Swarms Exhibit Complex Drift.** Microbubbles were formed following the explosive transformation of micron-sized droplets of the water-insoluble surfactant Span 83 that were placed on the agar surface a few centimeters in front of an advancing swarm (17), see Materials and Methods. Some of the bubbles remained stable for hours and were taken up by the advancing swarm. Many of these traveled within the river at the swarm edge; see, for example, the bubble indicated by the black arrow in Fig. 1A. Occasionally, bubbles moved into the swarm; see, for example, the bubble indicated by the white arrow in Fig. 1A. The motion of bubbles with a diameter of 2.1 ± 0.4 μm (mean ± SD, n = 51) were tracked (Materials and Methods and Movie S1), allowing us to map the flow patterns in the interior of the swarm. These bubbles moved freely within the swarm, without sticking to cells or to fluid boundaries.

At the leading edge of an *E. coli* swarm, there is a monolayer of cells spanning a width of 31 ± 4 μm (mean ± SD, n = 12). Just behind this monolayer is a distinct multilayered region with a width that depends upon colony size (Fig. 1A). The multilayered

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**Author contributions:** Y.W. and H.C.B. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper. The authors declare no conflict of interest.

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region exhibits greater fluctuation in brightness compared to other regions of the swarm, as shown in the plot of Fig. 1B, allowing us to determine the boundaries of the multilayered region (see Materials and Methods). Under our experimental conditions, the width of the multilayered region spans 154 ± 27 μm (mean ± SD, n = 12). The boundaries of the multilayered region remain nearly fixed relative to the swarm edge as the swarm expands.

Microbubbles displayed mostly random movement in the interior of the swarm (Movie S1), but when an ensemble of bubble trajectories was averaged, we found net radial displacements (i.e., drifts). The direction of drift was either inward (i.e., toward the center of the swarm) or outward (i.e., toward the edge of the swarm), depending upon the distance from that edge. As soon as bubbles entered the cell monolayer at the edge of the swarm, they drifted inward for approximately 5 s and then outward for approximately 10 s until overtaken by the cells in the multilayered region. Fig. 2A is a plot of the mean radial displacement in the laboratory reference frame of 29 bubbles, shown as a function of time, \( r_x(t) \). The drift speeds, indicated by the initial and final slopes of this curve \( \langle v_x \rangle \), dashed lines were \( -1.1 \pm 0.1 \mu m/s \) and \( 1.3 \pm 0.1 \mu m/s \), respectively. These values were determined by the best linear fits of the \( r_x(t) \) plot. Although the inward drift could be an artifact due to the selection of inward moving bubbles at the beginning of the experiment, the outward drift is most likely generated by the action of rotating flagella of cells stuck at the swarm edge that tends to move fluid from thicker to thinner regions of the swarm (17). The outward drift speed \( (1.3 \pm 0.1 \mu m/s) \) is comparable to the average swarm expansion rate \( (1.7 \pm 0.3 \mu m/s) \).

Once the bubbles reached the multilayered region (Fig. 2B), \( \langle r_x(t) \rangle \) exhibited a complex and unexpected pattern. The bubbles moved inward for approximately 20 s \( \langle v_x \rangle = -1.51 \pm 0.05 \mu m/s \) and then moved outward for approximately 40 s \( \langle v_x \rangle = 0.05 \pm 0.04 \mu m/s \), and then moved outward for the final 40 s of data acquisition \( \langle v_x \rangle = 0.6 \pm 0.1 \mu m/s \). Defining the intersecting points of the linear fits in Fig. 2B as switching points, switching from inward to outward drift occurred at \( t = 20 \pm 1.4 \) s and \( \langle r_x(t) \rangle = -62 \pm 2 \mu m \). Taking account of the distance the swarm has expanded, the switching point corresponds to a distance from the swarm edge of 96 ± 7 μm. At the end of the data acquisition, the bubbles were 208 ± 32 μm from the swarm edge. Thus, the data presented in Fig. 2B cover the entire multilayered region.

To probe the flows beyond the multilayered region, we tracked bubbles starting at distances of 200 and 300 μm from the swarm edge. Beginning at 200 μm, bubbles drifted outward at \( \langle v_x \rangle = 0.34 \pm 0.01 \mu m/s \) (Fig. 2C). Taking account of the distance the swarm has expanded, these bubbles ended up at a distance of 292 ± 23 μm from the swarm edge. Beginning at 300 μm, the bubbles remained driftless, with \( \langle v_x \rangle = -0.01 \pm 0.03 \mu m/s \) (Fig. 2D).
Fig. 2 B–D suggest that the motion of swarm fluid displays outward drift from approximately 170–300 μm from the swarm edge and then remains stationary approximately 300 μm from the swarm edge.

To convince ourselves that these patterns represented motion for the fluid as a whole, and not just for its uppermost layer, we repeated these measurements with a smaller number of polystyrene latex spheres (1.4-μm diameter) or carboxylate-sulfate-modified polystyrene latex spheres (1.0-μm diameter) or hydroxylate polystyrene latex spheres (0.9-μm diameter) (Polysciences, Inc.). The polystyrene latex spheres have larger density than water and tend to sink, so their movement should reflect the motion of the lower portion of the swarm fluid. The spheres behaved in a similar way as microbubbles, drifting inward and then outward in the multilayered region (Movie S2). However, the latex spheres tended to move back and forth between the surface of the agar and the body of the swarm, sticking briefly, wandering freely for a time, and then sticking again; among the three types of latex spheres, the 0.9-μm-diameter hydroxyxlate spheres appeared to have the greatest mobility.

Taken together, the drift patterns of bubble motion described in Fig. 2 reveal that only the outer approximately 300-μm-wide rim of the swarm-fluid film spreads. The fluid in the outermost edge of this rim (i.e., in the swarm-cell monolayer) flows outward, directly supporting swarm expansion. Remarkably, the swarm fluid farther inside this rim flows (in the reference frame of the laboratory) toward a region approximately 100 μm from the swarm edge, suggesting that the swarm-fluid film in the multilayered region has a greater depth above the agar. To support these flows, swarm fluid must be constantly supplied from the underlying agar.

Swarm Fluid in the Multilayered Region Is Highly Agitated. Individual microbubbles within swarms diffused with drift, and the diffusivity varied with the distance from the swarm edge. For the bubble trajectories reported in each panel of Fig. 2, we calculated the mean-squared displacement (MSD) corrected for drift, \( \text{MSD}(t) = \frac{1}{N} \sum_i^{N} (\vec{r}(t) - \vec{r}(0))^2 \), where \( N \) is the number of bubble trajectories, \( t \) is trajectory index, \( \vec{r} \) is the displacement of the \( i \)th trajectory, and \( \bar{r}(t) \) is the mean displacement of \( N \) trajectories (i.e., the drift). The diffusion processes of bubbles in different regions within the swarm were then characterized by the effective self-diffusion coefficient \( D_{\text{eff}} \) and the anomalous diffusion exponent \( \alpha \), which are defined in \( \text{MSD}(t) = D_{\text{eff}} t^\alpha \). At short timescales (5 s), microbubbles within the swarm displayed superdiffusion, with \( \alpha > 1 \) (Fig. 3).

The value of \( D_{\text{eff}} \) in the multilayered region (64 ± 2 μm²/s) was about twice as large as in other regions of the swarm (approximately 30 μm²/s), reflecting greater agitation.

The diffusive flows described here are similar to the surface flows observed near “bacterial carpets” (18), but with a higher level of agitation. The 1-μm-diameter beads near bacterial carpets had an effective diffusion coefficient of 19 ± 5 μm²/s, about 40 times larger than expected for such beads in bulk water, but only about one-third as large as found for the 2-μm bubbles in the multilayered region of the swarm. In this region of the swarm, bubbles are immersed in a bath of freely swimming cells, which drift outward with uniform radial speeds (approximately 0.4 μm/s), as determined by particle image velocimetry (Fig. 4).

Surprisingly, cell drifts and fluid drifts do not appear to be correlated. For other reports of enhanced tracer diffusion in concentrated bacterial suspensions, see refs. 19 and 20.

Cell-Density Profiles in the Multilayered Region Correlate with Fluid Drift Patterns. Because cells are mostly water, bulk fluid flow could be driven by metabolic activities associated with cell growth. To verify this idea, we measured metabolic activities, utilizing a short-lived GFP, and cell number, utilizing a membrane-specific fluorescent dye, neither of which appeared to affect swimming (see Materials and Methods). We monitored the fluorescence of swarm cells of E. coli strain MG1655 expressing a short-lived derivative of GFP, called ASV, which degrades with a half-life < 1 h and is rapidly cleared from nongrowing cells, thus reflecting the current rate of biosynthesis and serving as a reporter of cellular metabolic activities (21, 22). ASV is named for the last three amino acids (alanine, serine, valine) of a 13 amino acid C-terminal extension of GFP. The swarms were grown on agar supplemented with the dye FM 4–64, which fluoresces when absorbed by cell membranes (23), thus indicating cell number. The GFP was green and the FM 4–64 was red, so the two could be measured simultaneously using an FITC/Texas red cube, as shown in Fig. S1. The GFP and the FM 4–64 fluorescence plots were similar, suggesting that cells near the swarm edge are metabolically active and have similar growth rates. For E. coli strain HCB1668, the FM 4–64 fluorescence plot shown in Fig. 5 revealed that the cell densities (hence growth activities) were higher in the multilayered region, peaking at a distance of 76 ± 11 μm (mean ± SD, n = 5) from the swarm edge, close to the point where the swarm-fluid flows switch from inward to outward (Fig. 2B). The ratios of the slopes at 30–50 and 150–200 μm from the swarm edge (2.4 ± 0.1) (Fig. 5, dashed lines) coincide with the ratio between the drift speeds in these two regions (2.5 ± 0.4). These results verify this idea, we measured metabolic activities, utilizing a short-lived GFP, and cell number, utilizing a membrane-specific fluorescent dye, neither of which appeared to affect swimming.
support the view that fluid drift rates in the multilayered region are caused by cellular metabolic activities.

**Discussion**

We have studied the motion of fluid near the outer edge (the rim) of *E. coli* swarms, using microbubbles as unique flow tracers. The flows exhibit complex drift patterns that differ in different regions of the swarm. The fluid beyond the swarm-edge monolayer in the multilayered region flows inward and outward toward a region approximately 100 μm from the swarm edge. These flows maintain a water reservoir of greater fluid depth extending between approximately 30 and 200 μm from the swarm edge. In this reservoir, fluid flows are more highly agitated.

**The Cause of Fluid Drift.** The flows within the multilayer region appear to be driven by cellular metabolic activities (Fig. 5 and Fig. S1), not by cell motility (Fig. 4). Metabolic activities can affect water activity in at least two ways. One way is by the increase of cell number (cell volume) per unit area of agar surface. This requires process water because cells are approximately 80% water. The other way is by secreting osmolites, as by-products of metabolism, raising the osmolarity of the extracellular medium. A spatial gradient in metabolic activities will then be accompanied by a gradient in osmolality, which will drive fluid up the gradient. The cell-density profile shown in Fig. 5 implies that cells will by a gradient in osmolarity, which will drive fluid up the gradient.

**Fluid Balance Near Swarm Edge.** Because the height profile of a swarm remains constant as the swarm expands, we can derive a fluid balance equation for the fluid film near the edge of the swarm. Denoting the net height of swarm fluid at position *x* and at time *t* as *h(x, t)* (excluding the volume occupied by cells), the change of *h(x, t)* at *x* due to the drift to the right (at the speed of swarm expansion, *v*<sub>f</sub>) of the entire height profile equals the change of fluid volume (area in the 2D representation of Fig. S2) due to the fluid flows within the swarm [*v*<sub>f</sub>(x, t)] plus the change in volume due to the flow from the agar substrate (*v*<sub>a</sub>(x, t)) minus the change in volume due to fluid taken up by cells for volume increase and division. For convenience, we set *t* = 0 and define *h(x, 0) ≡ H(x).* The following equation is obtained (see SI Results):

\[ v_f(x) = rC(x) - v_s \frac{dH(x)}{dx} + \frac{d[v_f(x)H(x)]}{dx}. \]  

Here *r* is the growth rate of cells (chosen as 1/1,200 s<sup>−1</sup>; see SI Results) and *C(x)* is the cell volume per unit area of agar surface. *C(x)* is proportional to the normalized fluorescence intensity from FM 4-64 stained cells measured in Fig. 5. *H(x)* can be inferred from *C(x)*, and *v_f(x)* can be approximated by fitting the measured flow speeds in different regions of the swarm (Fig. 2); see Figs. S3 and S4. With *C(x)*, *H(x)*, and *v_f(x)*, Eq. 1 allows us to calculate *v_a(x)* as a function of the distance from the swarm edge, as shown in Fig. 6A. The result suggests that water is drawn from the agar mostly within approximately 70 μm from the swarm edge, with *v_a* greater than zero and peaking at approximately 0.15 μm/s near approximately 30 μm from the swarm edge (i.e., the boundary between the multilayered region and the swarm edge monolayer). In the region between approximately 70 and 200 μm from the swarm edge, the fluid balance requires that the agar absorbs water from the swarm (*v_a < 0*). This surprising behavior can be understood if the osmolarity in the swarm fluid of this region is smaller than that in the agar underneath.

**Proposed Model of Swarm Expansion.** To summarize, we suggest a model for *E. coli* swarm expansion shown in Fig. 6B. As cellular metabolic activities in the multilayer region draw water from the surroundings, a water reservoir is maintained near the swarm edge (Fig. 6B, solid black profile). In the reference frame of the laboratory, the fluid in the inner half of the water reservoir (between the inner edge of the water reservoir and the profile peak) flows outward, decreasing the fluid depth in this region; the fluid in the outer half of the water reservoir (between the outer edge of the water reservoir and the profile peak) flows inward, with water supplied from the underlying agar, increasing the fluid depth in
E. coli liquefied swarm agar before pipetting at the concentrations used in liquid and MG1655-ASV) and arabinose (for large Plexiglas box. Drops of diluted cell culture (2 \times 15–30 \mu L) were added to the plates and stored at room temperature. Before use, it was melted in a microwave concentrators, HCB1668 and MG1655-ASV swarmed at similar rates and ex-agar in 1% Bacto peptone, 0.3% beef extract, and 0.5% NaCl. At these agar large swarms grew to a diameter of approximately 5 cm.

If the surface were not wettable, water would tend to accumulate in droplets rather than flow outward. In some cases, surfactants are required. For example, a mutant strain of Bacillus subtilis defective in surfactinbiosynthesis (the sfA mutant) cannot swarm on Difco agar; however, externally supplied surfactin restored its swarming capability (12). Strains of E. coli K12 fail to swarm on Difco agar because they are missing the lipopolysaccharide O-antigen, but they do swarm on Eiken agar, which is more wet-table (15). Cell surfaces need to be wettable, as well, so that cells can move into the advancing fluid film. There is a mutant of Salmonella missing a surface component, FhE, that cannot swarm on Difco agar; an externally supplied nonionic surfactant (Tween 80) restored its swarming capability (24).

This is a minimal model of swarm expansion, requiring only cell growth, functional flagella, and wettable surfaces. Only the rim of the swarm, which extends about 300 \mu m from the swarm edge, is involved in the expansion of swarm fluid. We expect that this model applies to the swarming of flagellated bacteria in general, as a simple but effective means for colonizing surfaces. Regulatory mechanisms involving intercellular communication are not required.

Materials and Methods

Bacterial Strains. The strain used for studies of fluid motion was E. coli HCB1668 (FIC S353C), an AW405 derivative that swarms well, developed for flagellar visualization (8). The strain used for studies of cell growth and cell density was E. coli MG1655-ASV (a gift from Kim Lewis, Northeastern Univer- sity, Boston, MA), which expresses a short-lived derivative of GFP (ASV) under control of the ribosomal rrnB1 promoter, developed for studies of cell growth (21, 22). Single-colony isolates were grown overnight in LB medium (1% Bacto tryptone, 0.5% yeast extract, and 0.5% NaCl, pH 7.5) at 30 °C to stationary phase. For E. coli HCB1668, kanamycin (50 \mu g/mL), chlorampheni-col (34 \mu g/mL), and arabinose (0.5%) were added to the growth medium. For E. coli MG1655-ASV, ampicillin (50 \mu g/mL) and chloramphenicol (34 \mu g/mL) were added to the growth medium. These cultures were diluted 10^{-5} to provide cells for inoculation of swarm plates.

Swarm Plates. Swarm agar was 0.6% (HCB1668) or 0.5% (MG1655-ASV) Eiken agar in 1% Bacto peptone, 0.3% beef extract, and 0.5% NaCl. At these agar concentrations, HCB1668 and MG1655-ASV swarmed at similar rates and exhibited similar morphologies near the swarm edge. The agar was autoclaved and stored at room temperature. Before use, it was melted in a microwave oven, cooled to approximately 60 °C, and pipetted in 25-ML aliquots into 150 \times 15-mm polystyrene petri plates (8). Antibiotics (for E. coli HCB1668 and MG1655-ASV) and arabinose (for E. coli HCB1668) were added to the liquefied swarm agar before pipetting at the concentrations used in liquid cultures. For surface cell-density measurements with E. coli HCB1668 or MG1655-ASV, the dye FM 4-64 (Invitrogen-Molecular Probes) was dissolved in deionized water and added to the liquefied swarm agar before pipetting at a final concentration of 1 \mu g/mL. The agar plates were swirled gently to ensure complete wetting, and then cooled for 30 min without a lid inside a large Plexiglas box. Drops of diluted cell culture (2 \mu L, described above) were inoculated at a distance of 2–3 cm from the edges of the plates, and the plates were dried for another 30 min without a lid, covered, and incubated overnight at 30 °C and approximately 100% relative humidity, until the swarm grew to a diameter of approximately 5 cm.

Microbubble Fabrication. Suspensions of the surfactant Span 83 (Sorbitan ses- quiolate, S3386; Sigma–Aldrich) were prepared in water at a wt/vt ratio of 0.03–0.04%, following the procedures described previously (17). When viewed with a phase-contrast microscope, the suspension appeared full of refractile droplets with diameters ranging from a fraction of a micrometer to a few micrometers. A 0.5-\mu L drop of this suspension was placed 3–4 cm in front of the E. coli HCB1668 swarms. As water in the drop was absorbed by the agar, Span 83 droplets transformed into arrays of micron-sized bubbles (17). Some of these bubbles were stable enough to be engulfed by the advancing swarms.

Phase Contrast and Epifluorescence Imaging. The motion of microbubbles in the interior of swarms was observed in phase contrast with a 10x objective and a 1x relay lens mounted on a Nikon Optiphot2 upright microscope maintained at 30 °C. Recordings were made with a CCD camera at 30 frames per second (model KPC-658BH; KT&C) and a digital tape recorder (model GV-D1000; Sony). The video sequences were transferred to a PC as “avi” files and uncompressed using the free software VirtualDub (Avery Lee) for further analysis. The epifluorescence of cells expressing GFP (ASV) and/or stained by FM 4–64 was observed with the same objective and relay lens, by illuminating the swarm with a mercury arc lamp via a FITC/Texas red cube (S1006; Chroma Technology Corp., excitation 497/20 and 570/30 nm; emission 530/40 and 625/60 nm). The fluorescence was recorded with a Nikon D80 digital camera with 5 s or exposure times in red, green, and blue (RGB) color mode, utilizing the D80 Camera Control Pro 2 installed in a PC. Fluorescence from GFP (ASV) and FM 4–64 was recorded in the green and red channels of the RGB images, respectively.

Image Analysis. Microbubbles were tracked in the phase-contrast video sequences either automatically with a program based on an open-source package (see http://www.rowland.harvard.edu/labs/bacteria/index_software. html) (16), or manually using the MTrack plugin (Erik Meijering, http:// www.imagescience.org/meijering/software/mtrackj/) developed for ImageJ (http://rsbweb.nih.gov/ij/). The sizes of microbubbles were determined by doing Gaussian fits to the light-intensity profiles of lines crossing bubble centers plotted in ImageJ. The width of Gaussian fits (2\sigma) was taken as the diameter of bubbles (17).

The boundaries of the multilayered region of swarms were determined by the brightness fluctuation across the swarm, defined as the normalized standard deviation of pixel values in the transverse direction as a function of the distance from the edge of the swarm. The brightness fluctuation is averaged over a given number of successive video frames and then normalized by the average standard deviation of pixel values at the outer boundary of the multilayered region (defined below). The brightness fluctuation provides a measure of the swarm porosity. Just behind the swarm-edge mono-layer, this function exhibits a local maximum, whose position was taken as the position of the outer boundary of the multilayered region (Fig. 1B, at distance approximately 30 \mu m). This function also exhibits a ramp near the inner boundary of the multilayered region, the midpoint of which was taken as the position of the inner boundary of the multilayered region (Fig. 1B, at distance approximately 170 \mu m).

Particle image velocimetry (PIV) was performed using the open-source package MatPIV 1.6.1 written by J. Kristian Sveen (http://folk.uio.no/jks/ matpiv/). For each pair of consecutive images, the interrogation-window size started at 32.9 \times 32.9 \mu m and ended at 4.1 \times 4.1 \mu m after eight iterations. The grid size of the resulting velocity field was 2.05 \times 2.05 \mu m. The average radial speed of cells at a certain distance from the swarm edge was then calculated by averaging the radial component of all the velocity vectors in the velocity field at that particular distance from the swarm edge.

Fluorescence images were analyzed with MatLab (The MathWorks). After making background corrections, the image data extracted from the green and red channels corresponds to the epifluorescence signal from GFP (ASV) and FM 4–64 stained cells, respectively. The peak location of a fluores- cence intensity profile was determined as the maximum position of the best polynomial fit to the profile.

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**Supporting Information**

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**SI Results**

**Fluid Balance Near Swarm Edge.** Denoting the net height of swarm fluid at position \( x \) and at time \( t \) as \( h(x, t) \) (in the laboratory frame), the change of \( h(x, t) \) at \( x \) due to the drift to the right (at the speed of swarm expansion, \( v_r \)) of the entire height profile equals the change of fluid volume (area in the 2D representation of Fig. S2) due to the fluid flows within the swarm \( \{v_f(x, t)\} \) plus the change of volume due to the flow from the agar substrate \( \{v_o(x, t)\} \) minus the change in volume due to the fluid taken up by cells for volume increase and division.

The net change of fluid volume in the region \( (x - dx, x + dx) \) during \( dt \) is proportional to

\[
\frac{d}{dt}(x, t) \cdot 2dx = -v_f(x + dx, t) \cdot h(x + dx, t) \cdot dt + v_f(x, t) \cdot dt + v_o(x, t) \cdot 2dx \cdot dt - rC(x) \cdot 2dx \cdot dt
\]

\[
= -\frac{\partial}{\partial x} [v_f(x, t)h(x, t)] \cdot 2dx dt + v_o(x, t) \cdot 2dx dt - rC(x) \cdot 2dx dt.
\]

Here \( r \) is the growth rate of cells and \( C(x) \) is the cell volume per unit area of agar surface. This formula can be rewritten as

\[
\frac{\partial h(x, t)}{\partial t} = -\frac{\partial}{\partial x} [v_f(x, t)h(x, t)] + v_o(x, t) - rC(x).
\]

Let \( H(x) \) be the height profile of swarm fluid at \( t = 0 \); i.e., \( H(x) = h(x, 0) \). Because \( h(x, t) = H(x-v_r t) \), at \( t = 0 \) we have

\[
\frac{\partial h(x, t)}{\partial t} = -v_r \frac{dH(x)}{dx};
\]

\[
\frac{\partial h(x, t)}{\partial x} = \frac{dH(x)}{dx}.
\]

At \( t = 0 \), combining Eqs. S1–S3, we obtain Eq. 1 in the main text:

\[
v_r(x) = rC(x) - v_r \frac{dH(x)}{dx} + \frac{d[v_f(x)H(x)]}{dx}.
\]

If the doubling time of cells is about 20 min, then the growth rate \( r \) equals 1/1,200 s\(^{-1}\). The cell volume per unit area of agar surface \( C(x) \) is proportional to the normalized fluorescent intensity from 4–64 stained cells \([S(x)], \text{see Fig. 5 in main text}\); so \( C(x) = c_1 S(x) \), where \( c_1 \) is a constant. \( H(x) \) can be inferred from \( C(x) \) and from the swarm brightness fluctuation [measure by the normalized standard deviation of pixel values in phase-contrast images in the transverse direction as a function of distance from the swarm edge, denoted as \( P(x) \); see Fig. 1B and main text]. The fluctuation of swarm brightness seen in phase-contrast images is caused by the partial-overlapping of cells, which occurs more often when each cell has more space in which to swim around, so we can assume that \( P(x) \) is inversely proportional to the volume density of cells immersed in the swarm fluid. Denoting the height of the swarm (including cells and swarm fluid) as \( H'(x) \), we have \( S(x)/H'(x) \sim P(x)^{-1} \), or \( H'(x) = c_2 P(x) S(x) \), where \( c_2 \) is a constant. So the net height profile of swarm fluid is \( H(x) = H'(x) - C(x) = c_2 P(x) S(x) - c_1 S(x) \), as plotted in Fig. S3 (green curve). Here \( c_1 \) and \( c_2 \) are parameters chosen as 1.0 and 3.0 μm, so that \( C(x) \approx 0.5 \mu m \) and \( H(x) \approx 0.5 \mu m \) at \( x = -300 \mu m \). These values correspond to a swarm monolayer with a surface packing ratio of approximately 50%; that is, half of the space of the swarm monolayer is filled by cells, and the other half is filled by fluid. This choice of packing ratio is slightly larger than that measured by Darnton et al. (1), who observed faster expansion rates of swarms on agar of lower concentration. Also plotted in Fig. S3 are the expected total volume of swarm fluid and cells \([H'(x), \text{red curve}] \) and the net volume occupied by cells \([C(x)], \text{black curve}\). The best fit of \( H(x) \) is used for further calculations with Eq. S4. On the other hand, \( v_r(x) \) can be approximated as a polynomial fit of the measured flow speeds at different regions of the swarm (Fig. S4).

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Fig. S2. Fluid balance in a 2D representation of the swarm fluid, where $v_o(x)$ is the flow from agar into the swarm, and $rC(x)$ is the flow of fluid into the cells during volume increase and division.

Fig. S3. Height profiles of the swarm. The expected height profiles of the swarm cells (black), of the entire swarm (including the cells and the swarm fluid) (red), and of the swarm fluid only (green) are calculated as described in the supporting text. Note that these are effective-height profiles; cells and fluid in swarms are mixed together, not separated in layers.

Fig. S4. Approximation of the radial flow speed of swarm fluid ($v_f$) as a function of distance from the swarm edge. The $v_f$ is first represented as a step function (black) adopting the flow speeds measured from Fig. 2 of the main text: $v_f = 0.3 \mu m/s$ ($-300$ to $-200 \mu m$); $v_f = 0.6 \mu m/s$ ($-200$ to $-150 \mu m$); $v_f = 0.1 \mu m/s$ ($-150$ to $-100 \mu m$); $v_f = -1.5 \mu m/s$ ($-100$ to $-30 \mu m$); $v_f = 1.5 \mu m/s$ ($-30$ to $0 \mu m$). Then a fourth-order polynomial fit (red) to this step function is taken as the approximation of $v_f$ used in Eq. S4 for the calculation of $v_o$.

Movie S1 Microbubbles moving at the swarm edge and within the swarm. This phase-contrast movie (made at 30 frames per second) is associated with the image of Fig. 1. The movie is played in real time at three frames per second (with the real elapsed time indicated by the stamp in the movie control bar)—i.e., at 10-frame intervals—to reduce the file size. The swarm expands from left to right at $1.7 \mu m/s$, and microbubbles appear as bright spots. At $17$ s, a bubble with a diameter of $2.0 \mu m$ enters the swarm edge monolayer, and its trajectory is followed by the red trace. The image field of the movie is $220 \times 330 \mu m$.

Movie S1 (MOV)
**Movie S2** Polystyrene latex spheres (1.4-μm diameter) moving at the swarm edge and within the swarm. This phase-contrast movie is played in real time at three frames per second (with the real elapsed time indicated by the stamp in the movie control bar). The swarm expands from left to right at 2.1 μm/s, and microspheres appear as bright spots. At 15 s, a sphere in the swarm edge monolayer is moved by the swarm, and its trajectory is followed by the red trace. At 39 s, upon entering the multilayered region, the sphere drifts inward and then outward. The image field of the movie is 247 × 370 μm.

**Movie S2 (MOV)**