Microscopic Simulation of Growing Bacterial Swarms

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Abstract

Systems of bacteria swimming in a single layer of fluid above a nutrient-rich substrate show exponential growth in area as the bacteria push the boundaries of the fluid outwards. Considering bacteria to be self-propelled particles making up an active matter system, this exponential growth can be explained by a nonlocal pressure driven by growth at the center of the swarm, though no theoretical basis for such a pressure has been found. We simulate bacterial multiplication and interactions in the presence of a movable wall using GPU code in order to investigate the forces on the edges of a bacterial swarm and the density of the bacteria as a function of position and time. We verify through the simulation that the front grows exponentially and find that the local bacterial density near the movable wall grows linearly.

Summary

We examine bacterial interactions as the bacteria swim in a single layer of fluid and grow increasingly quickly in number. Jeckel et al. has found experimentally that, as the bacteria at the edge push the boundaries of the fluid outwards, the area the fluid takes up grows increasingly quickly. Hence, the bacteria at the edge must have knowledge of the growth at the center of the swarm, despite the bacteria not being in perpetual contact with their neighbors. To better investigate the forces at the edges of a bacterial swarm and the density of the bacteria as a function of position and time, we simulate bacterial multiplication and interactions in the presence of a movable wall. Our simulation verifies the exponential growth of the front and finds that the local bacterial density near the movable wall grows linearly.

1 Introduction

Statistical physics was developed in the late 1800s to relate macroscopic and microscopic properties of molecules based on the assumption that different configurations that have the same total energy have the same probability of occurring. It considers systems with a large number N of particles —for example, $N \approx 10^{23}$ —and analyzes the average values of their properties.

For instance, in a gas with $N \gg 1$ particles, microscopic quantities include the positions and momenta of each particle, and macroscopic quantities include the pressure and temperature. Systems can also be considered on a mesoscopic level—more detailed than the macroscopic level's consideration of the entire system and more general than the microscopic level's analysis of each individual particle. An example of a variable defined on a mesoscopic scale is the density ρ in a region, as a function of position and time.

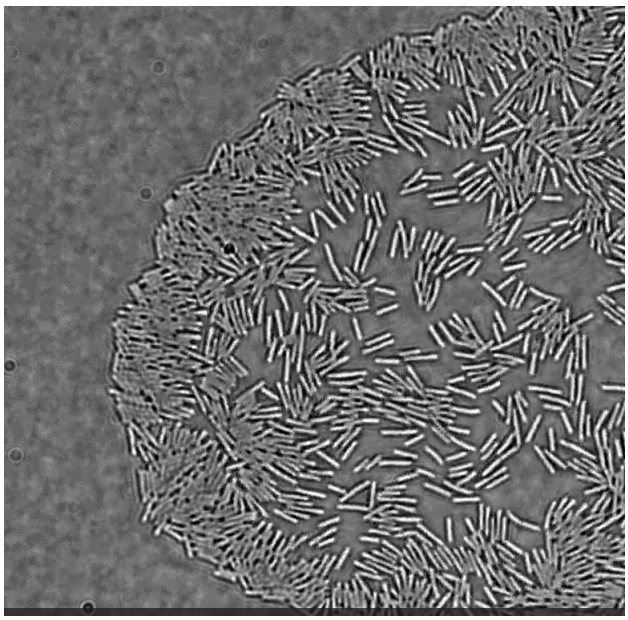
We are interested in active matter systems, a subfield of statistical mechanics which has emerged in the last two decades after Toner and Tu [1] used ideas from condensed matter to model the flocking of birds. Active matter refers to systems composed of units that each have their own source of energy and act relatively independently but show distinctive patterns collectively, such as flocks of birds and schools of fish.

An example of a difference between active matter systems and thermal equilibrium systems is the definition of pressure. In thermal equilibrium systems, pressure is defined as the derivative of free energy with respect to volume, $P = -\frac{\partial F}{\partial V}$, which coincides with the kinetic theory interpretation as the force per unit area caused by particles bumping into walls. However, Solon et al. [2] have shown that general active matter systems do not have equations of state or a consistent definition for free energy. Moreover, the force per unit area on the walls behaves differently for active systems than for systems in equilibrium. For example, the force for active matter depends on the stiffness of the confining walls, while this is not

true for the force for systems in equilibrium.

We build on the bacterial swarming experiments of Jeckel et al. [3], who placed a single bacterium on a nutrient rich agar substrate and allowed the bacterium to grow and multiply. The swarm of bacteria that emerged drew fluid from the substrate via osmosis and swam in a single layer in this fluid. Jeckel et al. captured videos over the course of a few hours as the bacteria expanded from around 10 μm to span 5 cm, resulting in frames such as the one shown in Figure 1.

Figure 1: Video frame of bacteria [3].



The number of bacteria grew exponentially, and Jeckel et al. found experimentally that the area of the swarm also grew approximately exponentially as the bacteria pushed the edge of the fluid outwards. This growth is likely the result of a nonlocal process driven by the growth of bacteria in the interior of the swarm, as the front—that is, the outer boundary of the fluid—would move linearly if the bacteria at the edge were only aware of conditions in their immediate environments.

On the other hand, Trinschek et al. [4] found linear growth in the front for biofilms in

an experiment similar to Jeckel et al.'s, though the former's biofilms were made up of many layers of bacteria in contact with each other, while the latter's bacteria swam in a single layer of fluid. Trinschek et al. used local processes, such as surfactin production and osmosis, to explain the linear growth they found.

To further investigate bacterial growth, we use a GPU computer simulation with bacteria colliding and repelling in 2D and consider the pressure on the confining wall. We verify Jeckel et al.'s observation that the wall is pushed upwards exponentially quickly, while the density near the wall grows linearly.

Understanding how bacterial swarms behave is essential to understanding how bacteria explore and colonize a nutrient-rich environment. It is related to other growth problems such as the growths of biofilms and tumors. Moreover, investigating the microscopic interactions among bacteria contributes to the eventual development of a scientifically grounded mesoscopic model of the bacteria's movement, positions, and orientations. This would further understanding of active matter because previous work in the field has generally kept the number of units constant, while we allow the bacteria to multiply.

2 Previous Work: Hydrodynamic Theory

Equations to describe relations between mesoscopic quantities in statistical physics have been derived, such as Bertin et al.'s [5] use of microscopic particle dynamics to derive Toner and Tu's [1] equations of flocking.

Regarding bacterial swarming, Jeckel et al. developed the following model for the density of bacteria as a function of position and time, though this equation is grounded in experimental data instead of physics:

$$\frac{\partial \rho}{\partial t} = \alpha \rho + (D_0 + D_g N_e(t)) \nabla^2 \rho. \tag{1}$$

Density is represented by ρ, as usual, and denotes the number of bacteria per unit area of space, while α, D_0, and D_g are constants. The linear term $\alpha\rho$ in Equation (1) conveys the bacteria's exponential growth, as each bacterium splits into two every 20 to 30 minutes. The term involving the Laplacian $\nabla^2\rho$ accounts for diffusion: if an area is more dense than its neighboring regions, the bacteria there will tend to spread out more and decrease the density there. The function of time $N_e(t)$ denotes the population size above a critical density ρ_e:

$$N_e(t) = \int_{\Omega(t)} \mathrm{d}^2x \max\{0, \rho(\vec{x}, t) - \rho_e\},$$

where x represents position, t represents time, and $\Omega(t)$ ranges over the area the bacteria take up at time t.

The term $D_g N_e(t)\nabla^2\rho$ is a nonlocal term that takes into account the configuration of the entire swarm rather than the conditions around each bacterium, and it was added because Jeckel et al. found that the resulting equation better imitated experimental results of bacterial behavior.

To better describe bacterial interactions above a microscopic level, we want further confirmation of the presence of a nonlocal factor at work among the swarm.

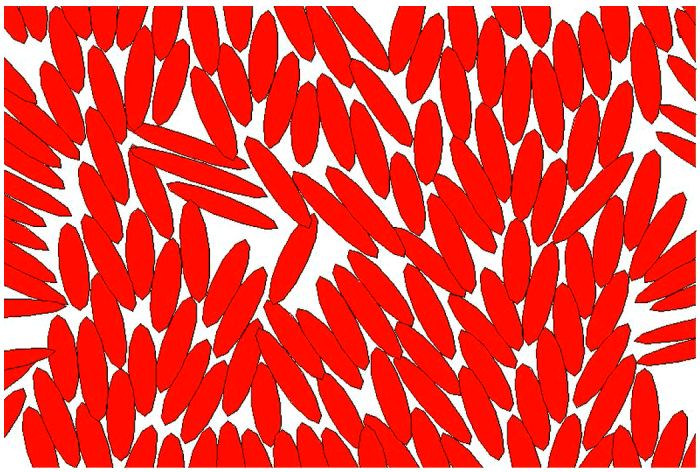
3 Methods

3.1 Using a Graphics Processing Unit

To facilitate investigations into the bacteria's densities and alignments under certain conditions, Jeckel et al. [3] also coded a simulation of the interaction between bacteria, allowing plots of the bacteria's locations as shown in Figure 2 to be made, where each red ellipse represents a bacterium. The code employs periodic boundaries, where bacteria that reach the simulation edges appear again on the opposite side. We modify the code to make the two horizontal sides nonperiodic, to allow the upper wall to move when pushed by bacteria,

and to enable the bacteria to grow and multiply.

Figure 2: Sample plot of bacteria locations, with unitless dimensions, as generated by Jeckel et al.'s initial simulation [3].



The code is designed so that the majority of its calculations run on a graphics processing unit (GPU), which contains hundreds of cores. In contrast, central processing units (CPUs) typically contain fewer than ten cores. The cores of a CPU are fast at doing sequential operations such as loading and unloading data, while those of a GPU are usually slower. However, a GPU's multitude of cores makes it faster at running operations in parallel, making it preferable for doing many calculations independently from each other because it can run each separate thread on a different core.

Molecular dynamics simulations such as Jeckel et al.'s often involve many independent calculations that motivate the usage of a GPU. In Jeckel et al.'s program, the force with which each pair of bacteria act on each other is calculated based on the distance between them. At each timestep, the new position, orientation, and velocity of each bacterium depend only on the system's configuration at the previous timestep and can hence be calculated in parallel from the new properties of the other bacteria.

3.2 Implementing Walls and Bacterial Multiplication

We replace the periodic boundaries of the upper and lower boundaries with walls confining the bacteria by considering the bacteria to experience a further potential—in addition to the potential they possess from the presence of the other bacteria—the farther they move above the upper boundary or below the lower boundary. We consider the potential V from the wall to be quadratic in the distance from the boundary:

$$V(y) = \begin{cases} \frac{\lambda}{2}(y - y_0)^2, & y > y_0 \\ \frac{\lambda}{2}y^2, & y < 0 \\ 0, & \text{otherwise,} \end{cases}$$

where y is the y-coordinate of a point on a bacterium, the upper and lower boundaries are the horizontal lines $y = 0$ and $y = y_0$, respectively, and λ is a constant.

Then, the force from the wall on a point is

$$F = -\nabla V = \begin{cases} -\lambda(y - y_0), & y > y_0 \\ -\lambda y, & y < y_0 \\ 0, & \text{otherwise,} \end{cases}$$

where the negative signs denote that the force acts to push bacteria back within the boundaries. We can approximate the force from the wall as such because we know it starts at 0 at the boundary and is increasing moving farther into the wall. We choose λ to be 0.001 so that the bacteria are pushed back quickly enough that they rarely move completely past the boundary; hence, only a small range of the force as a function of position is relevant, justifying the linear approximation because all continuous functions are approximately linear within a small range.

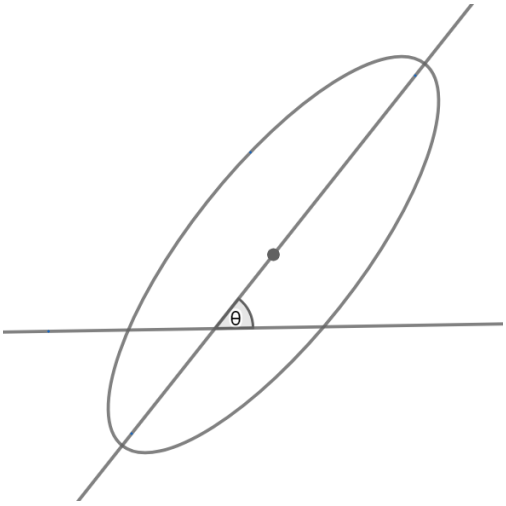


Figure 3: Model of a bacterium as an ellipse.

The bacteria are elliptical in shape, as shown in Figure 3, where θ represents the angle between the ellipse's major axis and the horizontal upper wall. Because the averaging of statistical physics allows approximations, we can treat the ellipses as sticks of negligible width, as shown in Figure 4, for the purposes of calculating the total forces and the torques about the center that they experience.

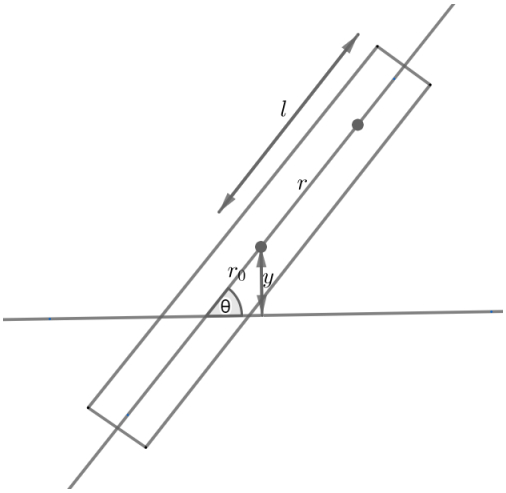


Figure 4: Approximation of a bacterium as a stick with negligible width.

The average force acting on the part of a bacterium past a horizontal boundary is the force acting on the center of this part. For a bacterium completely beyond a boundary that

has length 2ℓ and center of distance y from the nearest horizontal boundary, the total force F is then

$$F = -\lambda y \cdot 2\ell.$$

For a bacterium partly past a boundary forming an angle θ with the horizontal boundary, the total force is

$$F = -\lambda \left(y + \ell \cdot \sin\theta\right) \cdot \left(\frac{y}{\sin\theta} + \ell\right).$$

To calculate the torque about the center from the wall's force, we integrate over all points on the rod approximation. For a bacterium partly past a boundary, let r_0 be $\frac{y}{\sin\theta}$, τ be the torque about the center, and r be the distance from the center for some point on the bacterium. Then

$$\begin{aligned}\tau &= \int_{-r_0}^{\ell} \lambda \cdot \sin\theta \cdot \cos\theta \cdot r \left(r_0 + r\right) \cdot \mathrm{d}r \\ &= \lambda \cdot \sin\theta \cdot \cos\theta \cdot \left[\frac{1}{2} r_0 \cdot \left(\ell^2 - r_0^2\right) + \frac{1}{3}\left(\ell^3 + r_0^3\right)\right].\end{aligned}$$

If a bacterium is completely past the nonperiodic boundary, the torque acting on it about the center is equivalent to that in the $r_0 = \ell$ case, and we get

$$\tau = \frac{2}{3}\lambda \cdot \sin\theta \cdot \cos\theta \cdot \ell^3.$$

Moreover, we allow the top wall to move when pushed against by bacteria. To implement the idea that moving the front requires an energy cost, we make the change in the height of the available space proportional to the total force the bacteria exert on the wall

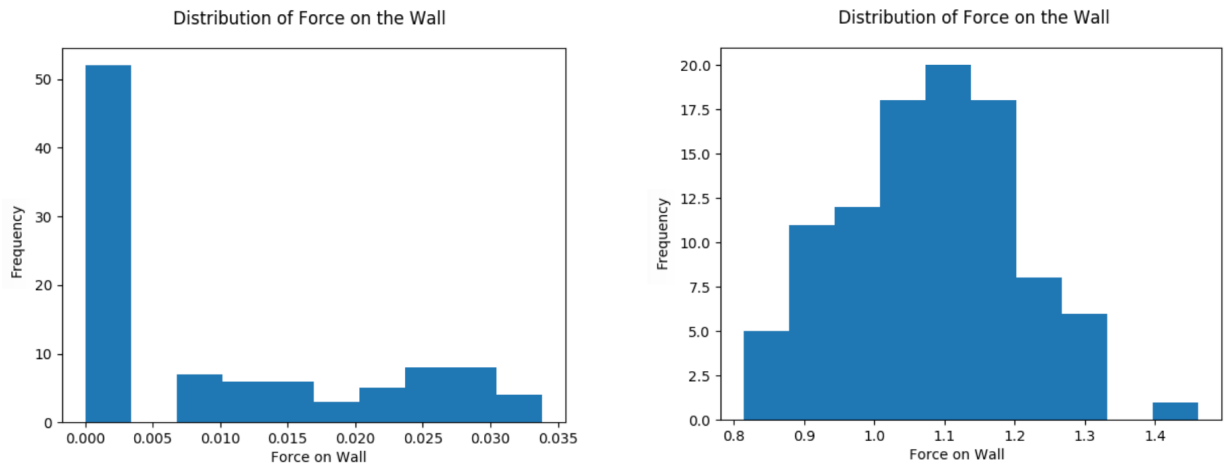
$$\frac{\mathrm{d}y_0}{\mathrm{d}t} = \alpha \cdot F_{\text{total}},$$

where y_0 is the distance between the two horizontal boundaries and α is a constant. Notice that F_{total} is the negative of the sum of the forces the bacteria past the top boundary

experience from this wall. Histograms of the force over many timesteps are shown in Figure 5.

Figure 5: Comparison of force outputs when there are 10 bacteria (left histogram) and 100 bacteria (right histogram). Force is unitless in the simulation.

(a) (b)



Finally, we implement the multiplication of the bacteria by allowing each bacterium to be duplicated with a small probability after every given number of timesteps; in order for the number of bacteria to remain under 200 after 700000 timesteps and hence maintain simulation running times of under one hour, we choose for each bacterium to duplicate with probability 0.007 every 1000 timesteps. Duplicating a bacterium involves adding another bacterium with the same length as the first and position and orientation shifted from those of the first by small randomly generated numbers, so that the two bacteria tend to be barely overlapping.

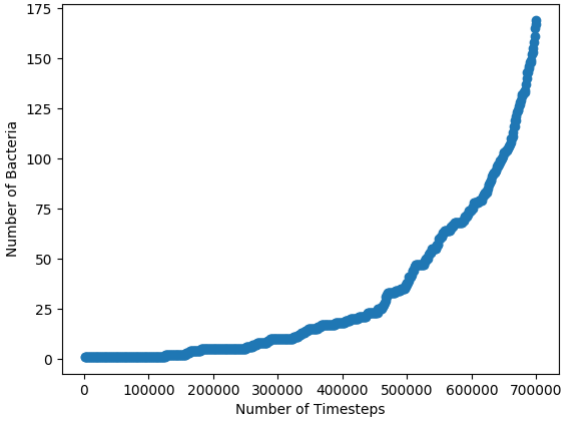
4 Results

As a control, we first run simulations while keeping the upper wall fixed and allowing the bacteria to multiply slowly, with the graphs of number of bacteria and force on the wall over

time shown in Figure 6. We plot the log of both the number of bacteria and the force over time, as shown in Figure 7. Because each bacterium has length around 8 or 9 units along its major axis, we choose both the width and height of the available space to be 40 units so that the periodic vertical boundaries' effects do not affect the interaction between two adjacent bacteria.

Figure 6: Number of bacteria and force on the upper wall with respect to time when the wall is held fixed.

(a)



(b)

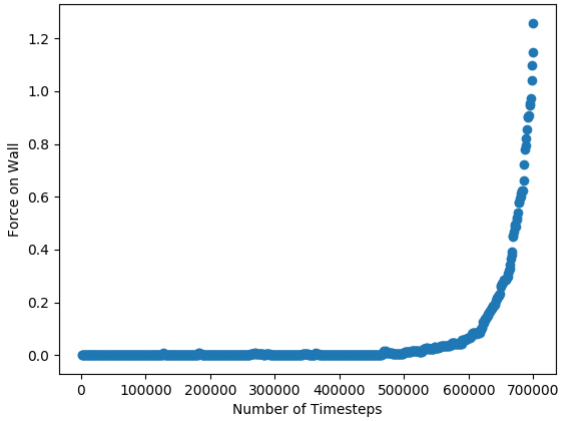
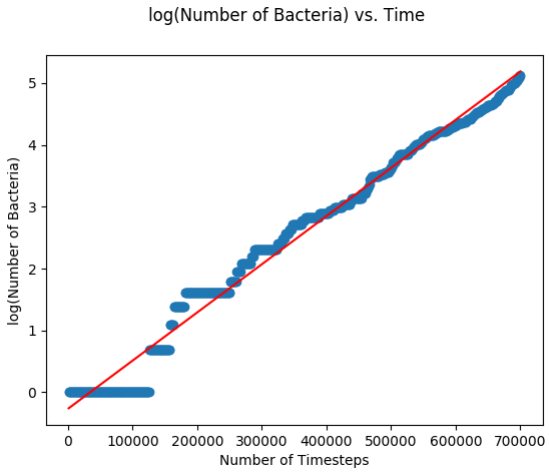
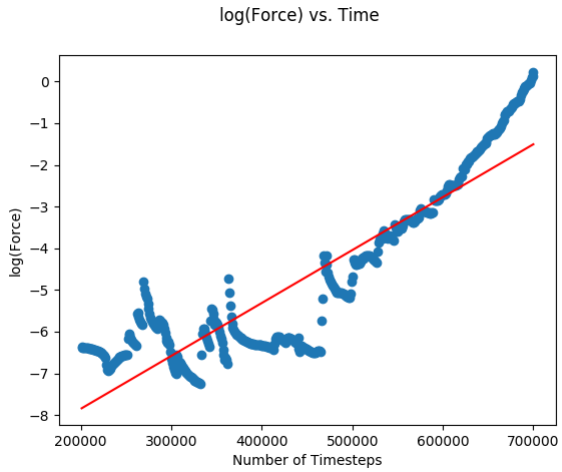


Figure 7: Subfigure (a) is a scatter plot of the log of the number of bacteria over time, including its least squares regression line with $r^2 = 0.9824$. Subfigure (b) is a scatter plot of the log of the force on the wall over time, including its least squares regression line with $r^2 = 0.8060$.

(a)



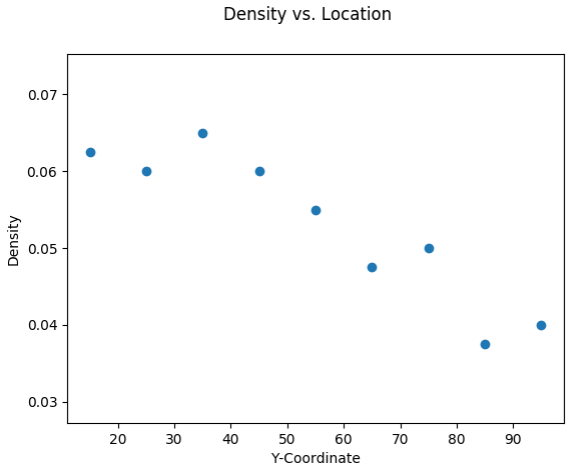
(b)



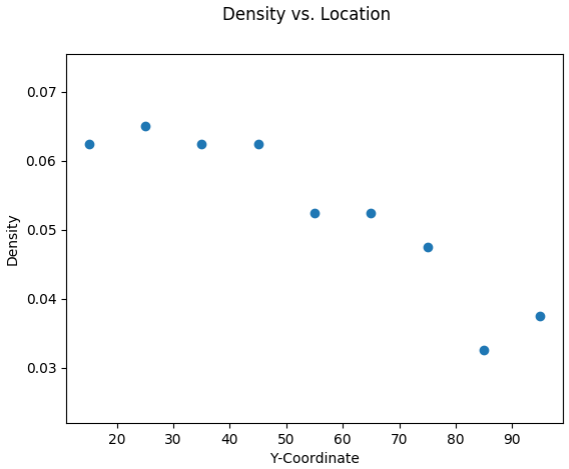
We now allow the upper wall to move, maintaining a region width of 40 units. We choose α, which controls the stiffness of the border and determines how quickly the upper wall moves, to be 0.1 and 1.0. The values were chosen to be not so large that the wall could easily be pushed by one bacterium—causing there to be effectively no upper boundary for the majority of the bacteria—and not so small that the bacteria would build up along the upper wall and cause the density near the wall to grow exponentially. Figure 8 shows the distribution of densities in horizontal strips centered at different y-coordinates. The figures show the distribution we desire from our values of α, as the density is relatively constant but lower nearer the upper wall due to its outwards movement.

Figure 8: Bacteria densities at different y-coordinates, when $\alpha = 1.0$ in Subfigure (a) and when $\alpha = 0.1$ in Subfigure (b).

(a)



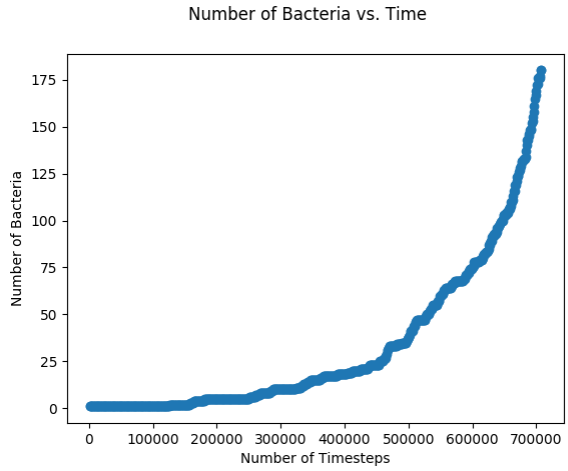
(b)



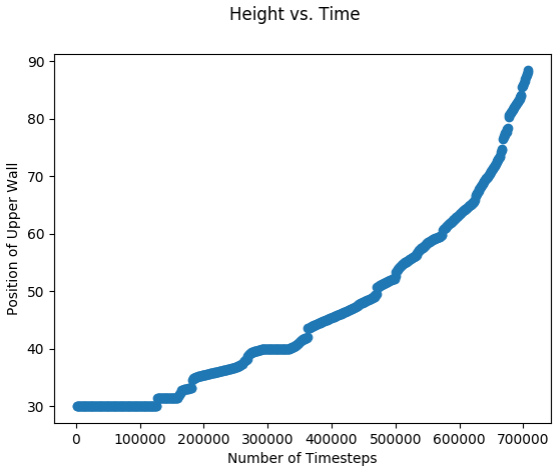
For both values of α, we graph the number of bacteria, the y-coordinate of the upper wall, the force exerted on the wall, and the density in the horizontal strip right below the wall as they vary over time, as shown in Figures 9 and 10. We also plot the logs of the number of bacteria and the height of the available region over time, with $\alpha = 1.0$ shown in Figure 11 and $\alpha = 0.1$ shown in Figure 12.

Figure 9: Shows the changes over time in each of number of bacteria, height of the available region, force on the upper wall, and density near the wall when $\alpha = 1.0$.

(a)



(b)



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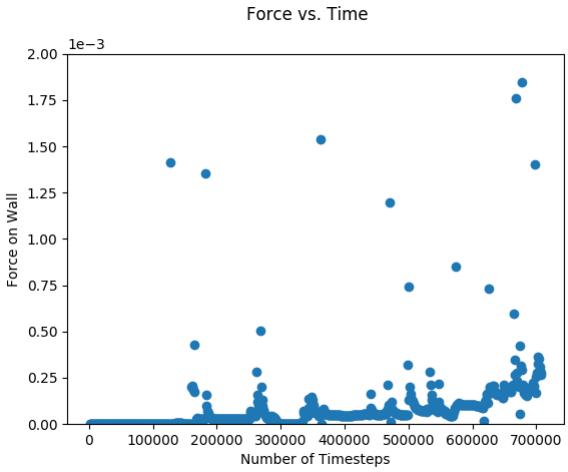

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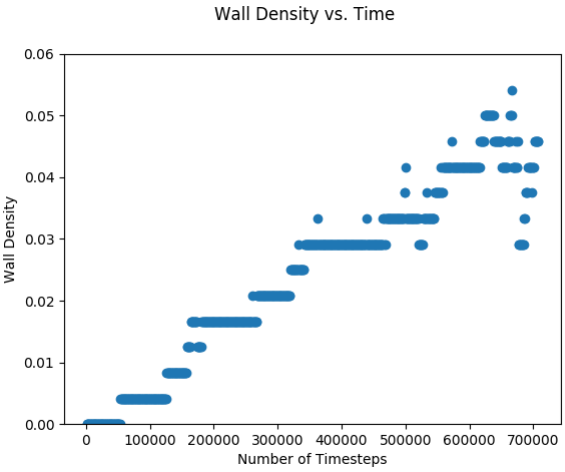
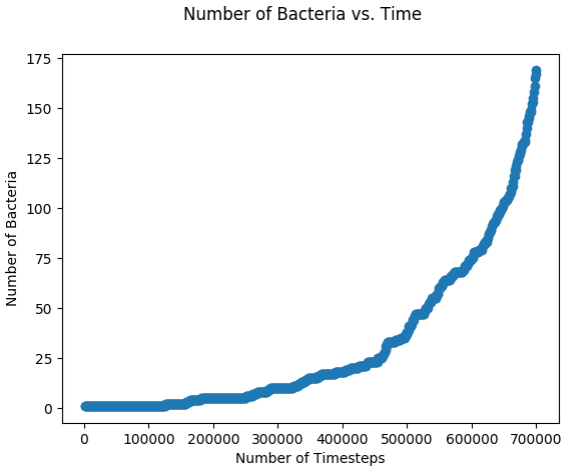
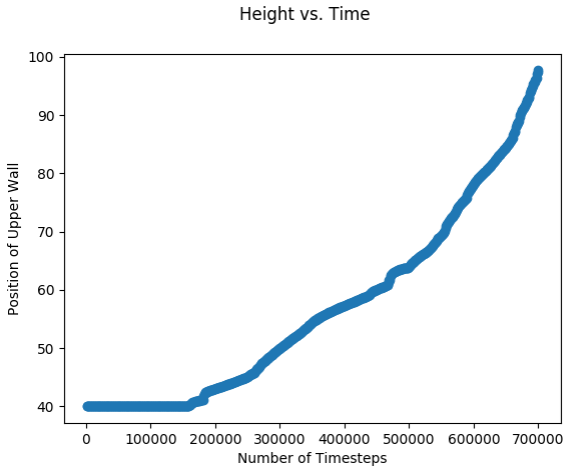


Figure 10: Shows the changes over time in each of number of bacteria, height of the available region, force on the upper wall, and density near the wall when $\alpha = 0.1$.

(a)



(b)



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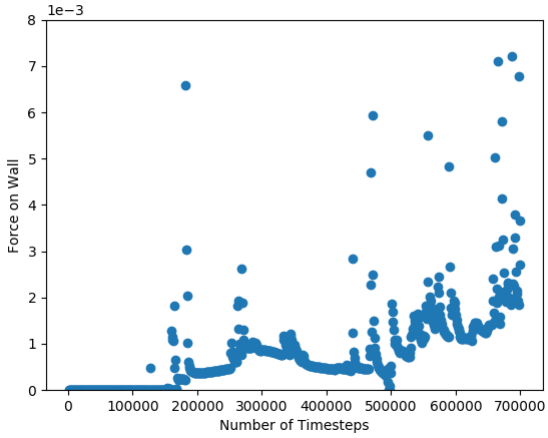

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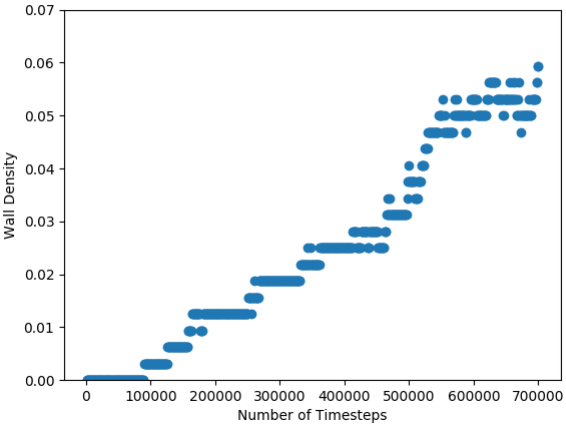


Figure 11: Subfigure (a) is a scatter plot of the log of the number of bacteria over time when $\alpha = 1.0$, including its least squares regression line with $r^2 = 0.9830$. Subfigure (b) is a scatter plot of the log of the height on the wall over time when $\alpha = 1.0$, including its least squares regression line with $r^2 = 0.9743$.

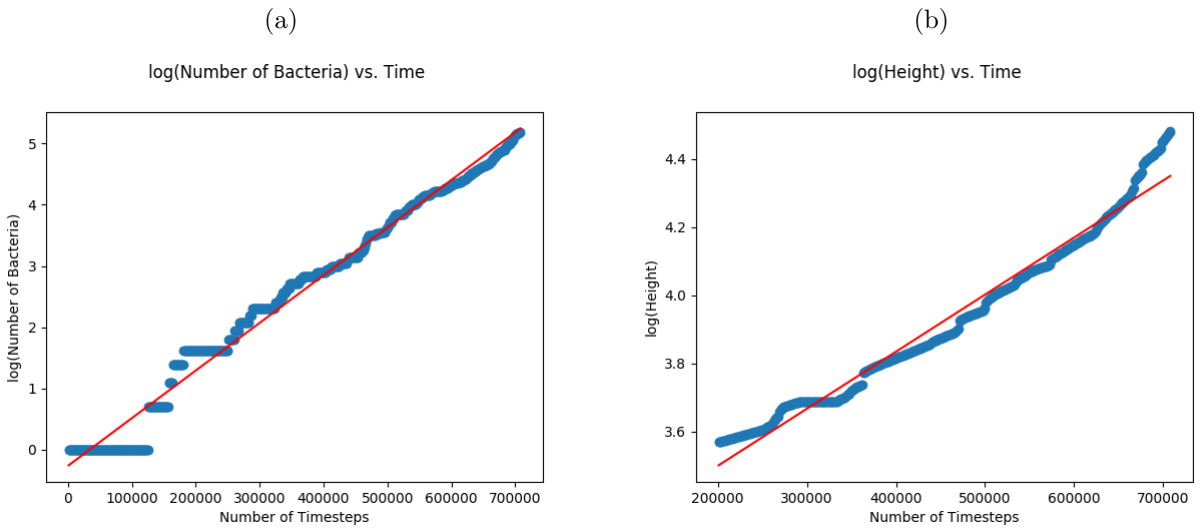
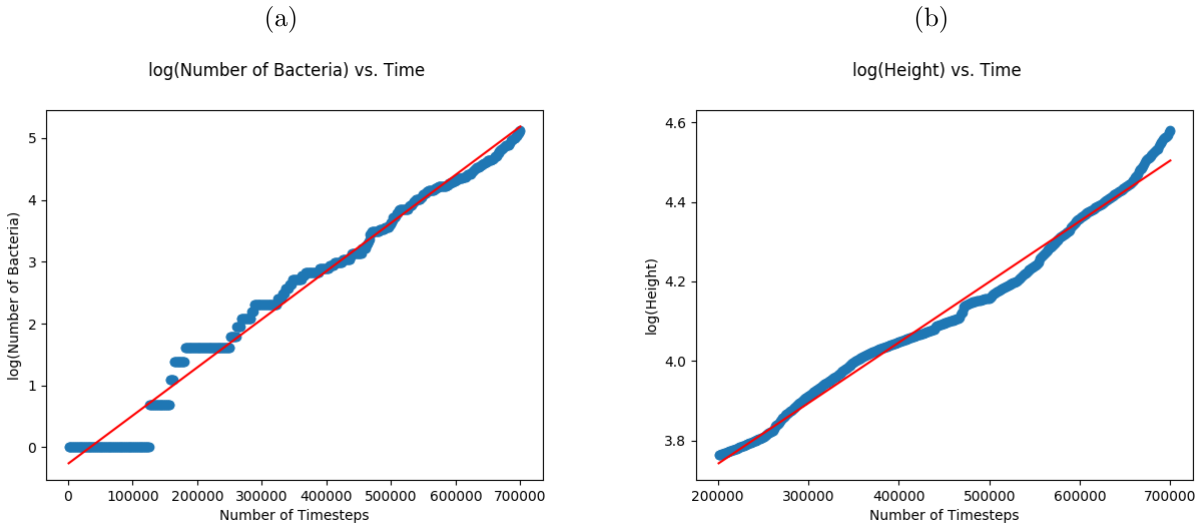


Figure 12: Subfigure (a) is a scatter plot of the log of the number of bacteria over time when $\alpha = 0.1$, including its least squares regression line with $r^2 = 0.9824$. Subfigure (b) is a scatter plot of the log of the height on the wall over time when $\alpha = 0.1$, including its least squares regression line with $r^2 = 0.9873$.



The noise evident in the graphs of force over time leads us to find a smoother model to

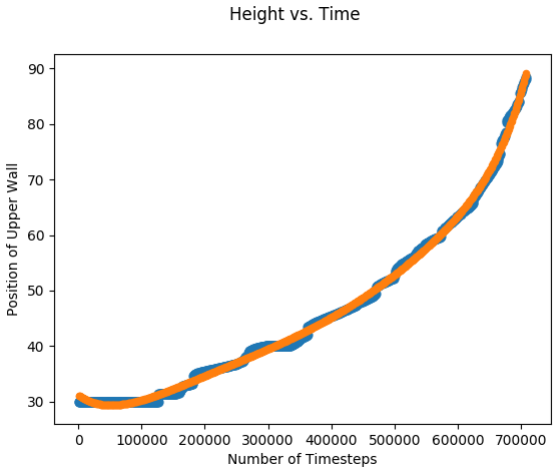
approximate the force in order to better understand its trends when there are thousands of bacteria. Because

$$\frac{\mathrm{d}y_0}{\mathrm{d}t} = \alpha \cdot F_{\text{total}},$$

we use Python to project the graph of y_0 vs time onto a smooth curve of an exponential function, take the derivative, and divide by α to get the graphs of force vs. time as shown in Figures 13 and 14.

Figure 13: In Subfigure (a), the smooth orange curve approximates the height of the available region with respect to time for $\alpha = 1.0$, with the original data points shown in blue. Subfigure (b) shows the approximation of force on the wall as a function of time, found by taking the derivative of the curve in Subfigure (a).

(a)



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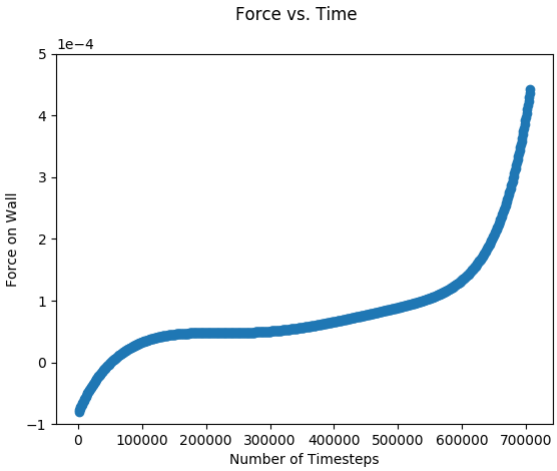
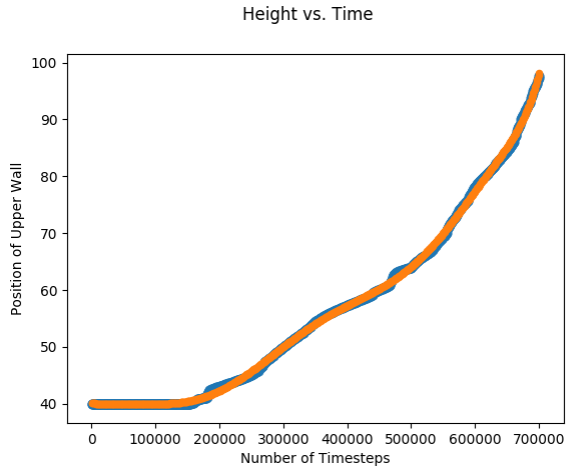


Figure 14: The smooth orange curve in Subfigure (a) approximates height with respect to time for $\alpha = 0.1$. Subfigure (b) approximates the force on the wall as a function of time.

(a)



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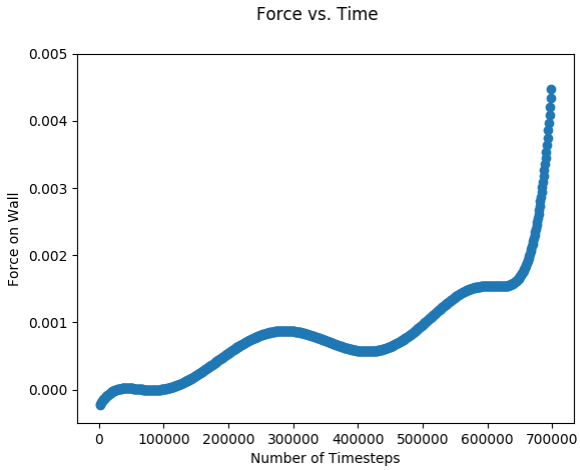
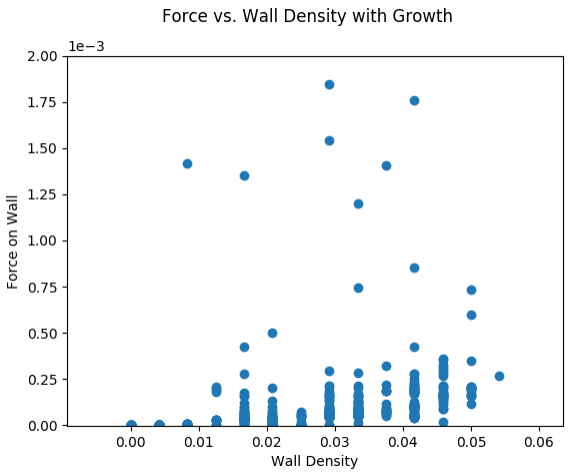


Furthermore, we compare the forces on the upper wall when the bacteria multiply and when they do not. We graph the force against bacterial density to compare the forces when the density is the same in both cases, and we test $\alpha = 1.0$ in Figure 15 and $\alpha = 0.1$ in Figure 16.

Figure 15: Subfigure 15a graphs force against wall density at various timesteps when bacterial multiplication is allowed and $\alpha = 1.0$. Subfigure 15b shows the average forces on the wall at various final densities bacterial multiplication does not occur and $\alpha = 1.0$.

(a)



(b)

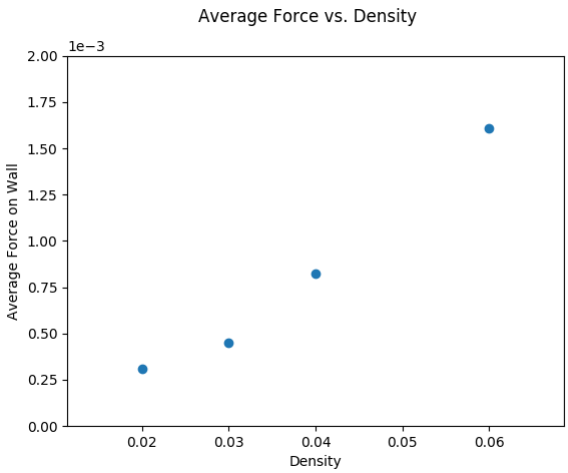
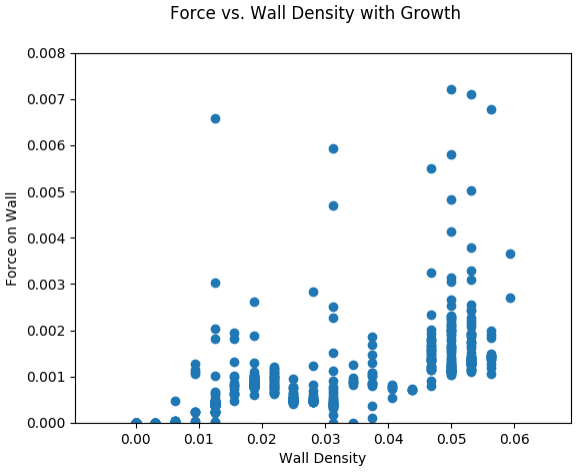
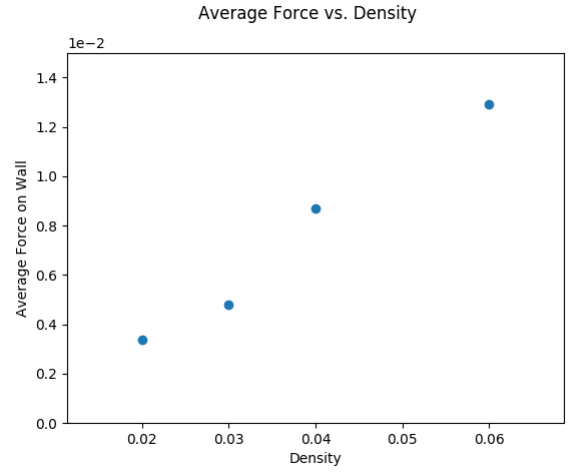


Figure 16: Subfigure 16a graphs force against wall density at various timesteps when bacterial multiplication is allowed and $\alpha = 0.1$. Subfigure 16b shows the average forces on the wall at various final densities when bacterial multiplication does not occur and $\alpha = 0.1$.

(a)



(b)



5 Discussion

When the upper wall is fixed, the r^2 values of the least squares regression lines in Subfigures 7a and 7b are relatively close to 1, meaning that both the number of bacteria and the force on the upper wall increase approximately exponentially with time. Because the area of the available region is constant, the bacterial density in the region is directly proportional to the number of bacteria, meaning that the density also increases exponentially.

We now consider the simulations with mobile walls, described by Figures 9, 10, 13, and 14. The r^2 values of the least squares regression lines in Subfigures 11b and 12b are both close to 1, so for both $\alpha = 1.0$ and 0.1, the y-coordinate of the upper wall increases exponentially, meaning that the area of the region available to the bacteria does too. This result supports Jeckel et al.'s findings that the front is pushed exponentially quickly. As expected, the number of bacteria increases exponentially, as seen through the r^2 values of the least squares regression lines in Subfigures 11a and 12a.

The force on the wall increases superlinearly with respect to time. Interestingly, the wall density increases approximately linearly, instead of the superlinear increase it displayed when the wall was stationary. The approximately linear relations between density and force displayed in Subfigures 15b and 16b suggest that if the force were a function of solely local density, a linear increase in wall density should result in a linear increase in the force. Linear increases in wall density resulting in superlinear increases in force, as described in Figures 9 and 10, indicate that either the force is sensitive on the density—which is unlikely on dimensional grounds—or that the force is unable to be described as a function of the local density and instead depends on other, nonlocal factors.

Nonlocal factors affecting the force on the wall would result in the forces when the bacteria are allowed to multiply being larger than the forces when the number of bacteria was constant and the density of the two were the same. This would be reflected by the average

forces in Subfigure 15a being larger than the forces in Subfigure 15b and the average forces in Subfigure 16a being larger than the forces in Subfigure 16b for the same values of density. However, the figures show that neither of these is true—that, in fact, the forces in Subfigure 15b and Subfigure 16b are larger than the average forces in Subfigure 15a and Subfigure 16a, respectively. We are unsure what could be causing this discrepancy, though we have tried with no avail to adjust for both the wall possibly being too flexible by testing $\alpha = 0.01$ and the lower wall's presence's effect by increasing the available region's final height.

6 Future Work

Because the number of calculations necessary to account for the interaction between every pair of bacteria grows proportionally to the square of the number of bacteria, our simulation is limited by the length of time it would take to model hundreds of thousands of bacteria, as is the case in real swarms. In the future, systems containing more bacteria can be modeled with more computational power.

Our simulation can also be improved by implementing growth in each bacterium and splitting a bacterium into two when it reaches a critical length. The self-diffusion by which bacteria spontaneously change direction can also be implemented by adding randomness to the bacteria's orientations at each timestep.

Ultimately, an equation to relate mesoscopic quantities such as density and pressure for bacterial swarms—similar to Bertin et al.'s [5] for self-propelled particles without growth—should be pursued. Such a model would be useful in further investigations such as that into the case where nutrients are not equally distributed.

If a nonlocal term for force can be derived theoretically, its basis should also be investigated. A possible explanation of the force increasing superlinearly while the density increases linearly is that the bacteria become oriented so that their major axes are vertical, leading to

a greater force on the wall than if more of them had their major axes horizontal. Force can also be transmitted nonlocally through bacteria being temporarily in contact.

7 Conclusion

We modified a computer program that simulated the interactions among a swarm of bacteria: we implemented walls in the y-direction by adding a linear force beyond the boundaries, allowed the upper wall to move in accordance with the total force the bacteria exert on it, and added the capacity for bacterial multiplication. We found that the wall moves exponentially, verifying Jeckel et al.'s observation in a real-life swarm that the front grows exponentially. The density's linear growth suggests that the force on the wall may be affected by a nonlocal pressure originating from the swarm as a whole.

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