An Example Model of Self-Organized Aggregation and Protective Differentiation of Simple Autonomous Agents

Technical Report UT-CS-04-523

Bruce J. MacLennan*

Department of Computer Science
University of Tennessee, Knoxville
maclennan@cs.utk.edu

April 1, 2004

Abstract

We describe experiments with simple bacteria-like agents that, when distressed, will self-organize into compact colonies with a protective cyst enclosing dormant bacteria; when favorable conditions return, the agents break out of their spore-like clusters and resume normal behavior. The population can be cycled between these two states any number of times. We define a simulated regulatory network governing this self-organizing behavior and explore the effects of its parameters. In particular, self-organization is regulated by the diffusion of three different chemicals, for aggregation, for differentiation of dormant and cyst bacteria, and for synchronization of spore formation. Although intended as an exploration of the principles of self-organization rather than as a model of any particular organism, the mechanisms and behavior are suggestive of quorum testing and biofilm formation by real bacteria.

1 Introduction

An important problem is how bacteria and other simple organisms self-organize to protect themselves from host defenses and other threats. We have taken this problem as a test case to improve our understanding of how simple autonomous agents can perform self-organized aggregation and differentiation of function in order to form a spore-like defensive colony. Although we are not modeling any specific bacterial species, the kinds of behaviors our agents exhibit are similar to the behavior of bacteria in nature [2, 3, 4], and it is plausible that bacteria could be genetically engineered to implement the behavior described in this report.

Specifically, our goal is to design (simulated) bacteria exhibiting the following collective behavior:

1. behave normally, including random locomotion and reproduction, under favorable environmental conditions;
2. under adverse environmental conditions, to aggregate into dense colonies;
3. to differentiate in these colonies between outer "boundary" cells and inner "interior" cells;
4. to have the interior cells enter a dormant state until favorable conditions return;
5. to have the boundary cells die after secreting a protective cyst material (e.g. cellulose), thus forming a spore containing the interior cells;
6. upon return of favorable conditions, to have the dormant bacteria reanimate, break out of the spore, and resume normal activity; and
7. to have the population able to go though this hibernation/reanimation cycle any number of times.

The bacteria can be in several regulatory states depending on the local concentrations of three signaling chemicals, which diffuse through the environment. Before presenting the details of the regulatory network (Sec. 3), we make a few remarks on the use of diffusion in self-organized systems. Readers uninterested in the mathematical analysis may prefer to skip directly to Sec. 3 (p. 7).

2 Diffusion

2.1 Gaussian Smoothing

Diffusion is a valuable tool for controlling and coordinating self-organization; for example, it can be used for massively-parallel unbiased search and communication. The system described in this report makes use of the diffusion of three chemical

compounds (called A, D, and S). To understand their application consider a simple n-dimensional diffusion equation (with conduction coefficient $c > 0$):

$$\frac{\partial \Phi}{\partial t} = c\nabla^2 \Phi, \tag{1}$$

where for simplicity we assume an unbounded domain, $\lim_{\|\mathbf{r}\|\to\infty} \Phi_{\mathbf{r}}(t) = 0$. If the initial condition is given by $\Phi_{\mathbf{r}}(0) = \varphi_{\mathbf{r}}$, then it is easy to show that

$$\Phi_{\mathbf{r}}(t) = G_{2ct}(\mathbf{r}) \otimes \varphi_{\mathbf{r}}, \tag{2}$$

where "$\otimes$" represents convolution and G_v is an n-dimensional Gaussian with variance v:

$$G_v(\mathbf{r}) = (2\pi v)^{-n/2} \exp\left(-\frac{\|\mathbf{r}\|^2}{2v}\right).$$

Thus, as intuitively expected, diffusion causes Gaussian smoothing of the initial field φ, with the standard deviation of the Gaussian kernel, after time t, given by:

$$\sigma_t = \sqrt{2ct}. \tag{3}$$

2.2 Discrete and Continuous Diffusion Parameters

In the simulations described in this report, 2-dimensional diffusion is simulated on a discrete grid in discrete time steps, and so it will be worthwhile to relate the continuous parameters to the discrete. A typical discrete approximation of $\partial\Phi/\partial t = \nabla^2\Phi$ is given by

$$\frac{\Delta \Phi_{x,y}}{\Delta t} = \frac{c}{h^2}\left(-4\Phi_{x,y} + \Phi_{x+h,y} + \Phi_{x-h,y} + \Phi_{x,y+h} + \Phi_{x,y-h}\right), \tag{4}$$

where h is the grid spacing in both dimensions. However, in these simulations, diffusion is modeled by equally distributing a fraction C of the field's value in a patch to the eight adjacent patches on each time step. That is, $\Phi_{x,y}$ is decreased by $C\Phi_{x,y}$ and the eight neighbors $\Phi_{x\pm h,y\pm h}$ are increased by $C\Phi_{x,y}/8$ each. This computation implements the following discrete approximation to the diffusion equation:

$$\Phi'_{x,y} = (1 - C)\Phi_{x,y} + \frac{C}{n}\sum_{\mathbf{r}\in N_{x,y}} \Phi_{\mathbf{r}}, \tag{5}$$

where $N_{x,y}$ represents the set of coordinates of the $n = 8$ neighbors of patch (x, y). Hence, it is computing the discrete approximation:

$$\Delta \Phi_{x,y} = -C\Phi_{x,y} + \frac{C}{n}\sum_{\mathbf{r}\in N_{x,y}} \Phi_{\mathbf{r}}. \tag{6}$$

This may be compared to the ordinary discrete approximation, analogous to Eq. 4, but based on n neighbors:

$$\Delta\Phi_{x,y} = \frac{c}{h^2}\left(-n\Phi_{x,y} + \sum_{\mathbf{r}\in N_{x,y}} \Phi_{\mathbf{r}}\right)\Delta t. \tag{7}$$

Equating Eqs. 6 and 7 relates the parameters of the discrete and continuous diffusions:

$$C = cn\Delta t/h^2. \tag{8}$$

If for convenience we set $h = 1$ grid unit and $\Delta t = 1$ time step (this is just a choice of units), then:

$$C = cn. \tag{9}$$

This is the basis for the continuous and discrete conduction coefficients shown in Table 1 (below, p. 9, in which $n = 8$).

We may compute from Eq. 3 the standard deviation of the Gaussian smoothing after one time step ($\Delta t = 1$) in terms of the discrete parameters:

$$\sigma_{\Delta t=1}/h = \sqrt{2C/n}.$$

This shows the effective width of the averaging, in grid units, at each time step. For $h = 1$ (grid units) and $n = 8$,

$$\sigma_{\Delta t=1} = \sqrt{C}/2 \tag{10}$$

per time step. For example, if $C = 0.5$ (Table 1), $\sigma_{\Delta t=1} \approx 0.35$, and if $C = 0.75$, $\sigma_{\Delta t=1} \approx 0.43$.

2.3 Diffusion as Spatially-Smoothed Time-Averaging

In the context of these experiments, signaling chemicals are assumed to obey a diffusion equation such as this:

$$\frac{\partial\Phi}{\partial t} = c\nabla^2\Phi - d\Phi + \Psi, \tag{11}$$

where c is the diffusion or conduction coefficient, d is a degradation rate, and the source field $\Psi_{x,y}$ represents the rate of secretion by the bacteria at location (x, y). If this rate is the same for the entire population Q, then the source field $\Psi(t)$ is proportional to the local population density field $Q(t)$: $\Psi(t) = sQ(t)$. As we have seen, the diffusion term $c\nabla^2\Phi$ causes spatial Gaussian smoothing with an increasing standard deviation $\sigma_t = \sqrt{2ct}$. Within a region of relatively uniform density $\bar{Q}$, diffusion can be neglected, and Eq. 11 acts like

$$\frac{\mathrm{d}\Phi}{\mathrm{d}t} = -d\Phi + s\bar{Q}. \tag{12}$$

Of course, this has the point attractor $\Phi^* = s\bar{Q}/d$, which defines the basic scale factor s/d for the population density. For example if, as in Table 1, $d_\mathrm{A} = 0.1$ per time step and $s_\mathrm{A} = 10$ quanta of A per time step, and there is an average of one bacterium per patch ($\bar{Q} = 1$), then $A^* = 100$ quanta. More generally, for the values in Table 1, $D^* = 2000\bar{Q}$ quanta and $A^* = 100\bar{Q}$ quanta.

In summary, if $\Psi = sQ$, then Eq. 11 generates a field proportional to the population density field Q, but smoothed by the Gaussian kernel. Although the Gaussian kernel is spreading in time, the Φ field is also decaying in proportion to the degradation rate d. Thus the Φ field represents a moving average, exponentially decreasing into the past, of the smoothed Ψ. That is, in the absence of the source field, it decays $\Phi(t + \Delta t) = e^{-d\Delta t}\Phi(t)$, and the time constant is

$$\tau_\Phi = 1/d. \tag{13}$$

In the simulations, field degradation proceeds in discrete steps, $\Phi' = f\Phi$, where $f \leq 1$ is the degradation factor. To relate the discrete and continuous parameters, observe that $f\Phi(t) = \Phi(t + \Delta t) = e^{-d\Delta t}\Phi(t)$, so

$$d = -\frac{\ln f}{\Delta t}. \tag{14}$$

Setting $\Delta t = 1$ time step gives

$$d = -\ln f. \tag{15}$$

To get the time constant in terms of simulation time steps, substitute Eq. 14 in Eq. 13:

$$\mathrm{T} = \frac{\tau}{\Delta t} = \frac{d^{-1}}{\Delta t} = -\frac{1}{\ln f}.$$

This gives us a way of assessing the recentness of the average reflected in Φ. For example, for $f_\mathrm{A} = 0.5$ (Table 1), we have $\mathrm{T}_\mathrm{A} = 1.44$ time steps.

When we include the source field in the discrete simulation, $\Phi(t+\Delta t) = f\Phi(t)+sQ$, then,

$$\Phi(t + k\Delta t) = f^k\Phi(t) + \frac{1 - f^k}{1 - f}sQ.$$

The asymptotic value is

$$\Phi(t) \to \frac{sQ}{1 - f}.$$

Since the expansion of the Gaussian kernel is checked by field degradation, a useful time-invariant measure of the spatial smoothing effected by Eq. 11 is the standard deviation of the kernel after one time constant, which we call the *smoothing radius* ρ:

$$\rho = \sigma_{\Delta t=\tau_\Phi} = \sqrt{2c\tau_\Phi} = \sqrt{2c/d} = 4\sqrt{-C\ln f}. \tag{16}$$

In many cases it is useful to know the effective *area* over which the Gaussian kernel is averaging, and for this purpose we may use its variance, which is proportional to the area. Hence we may define the *smoothing area* α:

$$\alpha = v_{\Delta t=\tau_\Phi} = 2c/d = -16C\ln f. \tag{17}$$

2.4 Aggregation and Gaussian Sharpening

A typical model of the chemotactic cell aggregation resulting from following an increasing gradient of Φ is the following population density equation [1, pp. 114–116]:

$$\frac{\partial Q}{\partial t} = \nabla \cdot [c(Q)\nabla Q] - \nabla \cdot [\chi(r)Q\nabla\Phi],$$

where $c(Q)$ is a conduction coefficient dependent on the cell density (since movement is impeded by cell clumping), and $\chi(r)$ reflects the desensitization of cells to Φ (r is the fraction of active receptors). The first term represents random motion of the cells, the second represents chemotaxis. Typical expressions for the variable coefficients are

$$c(Q) = \mu_1 + \mu_2 \frac{q_{\text{th}}^4}{q_{\text{th}}^4 + Q^4},$$

where q_{th} is the population density at which clumping begins to occur, and

$$\chi(r) = \chi_0 \frac{r^m}{A^m + r^m},$$

for appropriate constants μ_1, μ_2, χ_0, A, and m [1, pp. 114–115].

Here we will consider a simpler model of aggregation in order to explain the operation of the simulation. Therefore we may take the velocity vector of the bacteria to be proportional to the gradient of an aggregation signal chemical,

$$\mathbf{V} = r\nabla\Phi,$$

where $r > 0$. In fact, in our simulations, the bacteria orient to the gradient but move at constant velocity, so $\mathbf{V} = r\nabla\Phi/\|\nabla\Phi\|$ is a more accurate description. Further, when $\nabla\Phi \approx 0$ the velocity is random, so we should write $\mathbf{V} = r\mathbf{U}/\|\mathbf{U}\|$ where $\mathbf{U} = \nabla\Phi + \epsilon\mathbf{u}$, where $\mathbf{u}$ is a random unit vector and ϵ is sufficiently small, but we will ignore these details.

The number of bacteria leaving an $h \times h$ patch in time Δt is approximately

$$\left(QV_x + \frac{\partial(QV_x)}{\partial x}h\right)h\Delta t - QV_x h\Delta t + \left(QV_y + \frac{\partial(QV_y)}{\partial y}h\right)h\Delta t - QV_y h\Delta t.$$

Therefore, the *flux*, or loss of population density per unit time, is given by the divergence,

$$\frac{\partial QV_x}{\partial x} + \frac{\partial QV_y}{\partial y} = \nabla \cdot Q\mathbf{V}.$$

Since this is the rate of flow of population density *out* of a differential patch, the change of population density due to chemotaxis is

$$\frac{\partial Q}{\partial t} = -\nabla \cdot Q(r\nabla\Phi) = -rs\nabla \cdot Q\nabla Q, \tag{18}$$

if, as usual, $\Phi = sQ$. We may expand Eq. 18 as follows:

$$\frac{\partial Q}{\partial t} = -rs\left(Q\nabla^2 Q + \|\nabla Q\|^2\right).$$

Neglecting for the moment the second term and the factor Q on the divergence, consider the equation $\partial Q/\partial t = -rs\nabla^2 Q$. By replacing t by $-t$, $\partial Q/\partial(-t) = rs\nabla^2 Q$, we can see that this is a *reverse diffusion* equation, and results in *Gaussian sharpening* according to the equation

$$G_{2rs\Delta t}(x, y) \otimes Q_{x,y}(t + \Delta t) = Q_{x,y}(t).$$

Multiplying by Q, as in $\partial Q/\partial t = -rsQ\nabla^2 Q$, only amplifies this effect (since $Q_{x,y} \geq 0$). Therefore, we can say that the effect of chemotaxis is sharpening of the original population distribution. In this way structures (clusters) are created by amplifying nonuniformities in an original, approximately homogeneous distribution.

3 Design of the Simulated Bacteria

Figure 1 depicts a regulatory network that implements the desired behavior. We will explain this network region by region in the following sections to show how it produces the intended self-organizing behavior. (The colors in the figure are correlated, to the extent possible, to the colors used in the simulation screen images beginning in Sec. 4.)

3.1 Normal State

The top-center of the diagram represents a sensor for any ambient condition, which, for concreteness, we have taken to be ambient temperature. It could be any condition that changes effectively instantaneously (i.e., within one time step) throughout the simulation space. If the temperature is greater than a critical value T_{c} then conditions are favorable for the bacteria, and they are in their *Normal* state, indicated by the brown network in the upper right corner, in which they wander randomly and reproduce.[1] The reproduction rate is determined by a parameter p_{R}, which is a bacterium's probability of reproducing in each time step. (Default parameters of the model are collected in Table 1.)

3.2 Distressed State

If the ambient temperature ever falls below T_{c}, then the bacteria enter the *Distressed* state, represented by the red network in the upper-left of Fig. 1. A distressed bacterium will die after $T_{\mathrm{d}} \pm V_{\mathrm{d}}$ (uniformly distributed) time units, unless it returns to

[1] In the Normal state they also "destroy cysts," which is explained in Sec. 3.5 below.

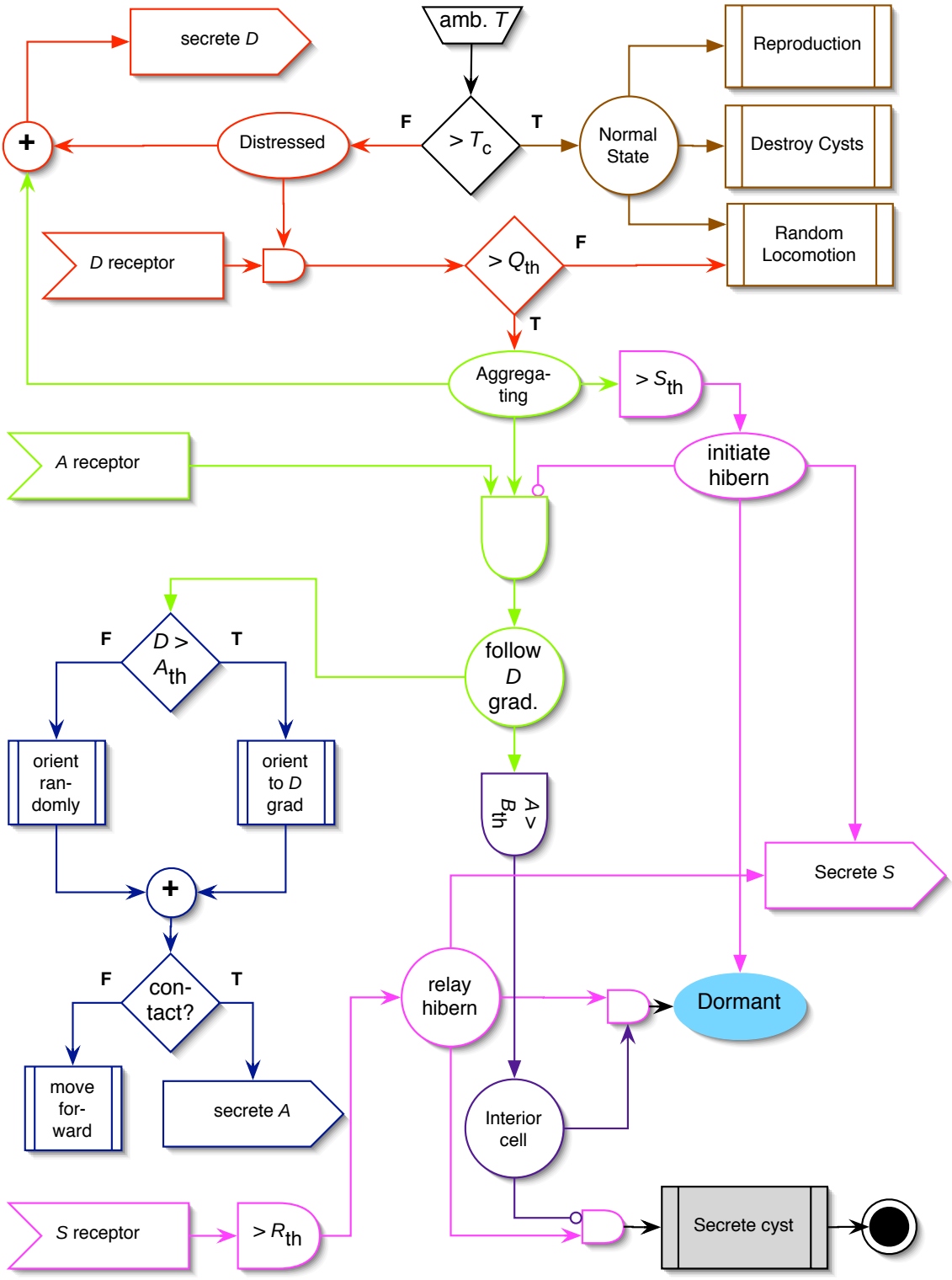


Figure 1: Regulatory network of simulated bacterium. See Sec. 3 for explanation.

Table 1: Default Parameters

Parameter	Default Value	
	Discrete	Continuous
D diffusion	$C_{\mathrm{D}} = 0.5$	$c_{\mathrm{D}} = 0.0625$
D degradation	$f_{\mathrm{D}} = 0.99$	$d_{\mathrm{D}} = 0.01$
D secretion quanta	$K_{\mathrm{D}} = 20$	
D secretion probability	$p_{\mathrm{D}} = 0.2$	
Refractory period	$T_{\mathrm{r}} = 5$	
Quorum threshold	$Q_{\mathrm{th}} = 90$	
Aggregation threshold	$A_{\mathrm{th}} = 70$	
Hibernation signal threshold	$S_{\mathrm{th}} = 300$	
A diffusion	$C_{\mathrm{A}} = 0.5$	$c_{\mathrm{A}} = 0.0625$
A degradation	$f_{\mathrm{A}} = 0.90$	$d_{\mathrm{A}} = 0.1$
A secretion quanta	$K_{\mathrm{A}} = 10$	
Boundary threshold	$B_{\mathrm{th}} = 75$	
S diffusion	$C_{\mathrm{S}} = 0.75$	$c_{\mathrm{S}} = 0.09375$
S degradation	$f_{\mathrm{S}} = 0.5$	$d_{\mathrm{S}} = 0.69$
S secretion quanta	$K_{\mathrm{S}} = 100$	
Relay threshold	$R_{\mathrm{th}} = 1$	
Initial population density	$p_{\mathrm{P}} = 0.1$	
Reproduction rate	$p_{\mathrm{R}} = 0.02$	
Mean distressed lifetime	$T_{\mathrm{d}} = 500$	
Distressed lifetime variability	$V_{\mathrm{d}} = \pm 25$	
Simulation space	$P_{\max} = 45 \times 47$	

Dimensioned quantities are in units of time steps, grid spacing, and quanta of chemical compounds, as appropriate.

the Normal state or enters the Dormant state (Sec. 3.4). A distressed bacterium signals its state by secreting the D compound; in each time step it has a probability p_{D} of secreting K_{D} units of the chemical (which is thus added to that already present). After each such secretion it enters a refractory period for T_{r} time steps, during which it cannot secrete D.

Distressed bacteria are sensitive to the D concentration in their immediate vicinity. If it is less than Q_{th}, the *quorum threshold*, then they continue to wander randomly. However, if $D > Q_{\mathrm{th}}$, then they enter the *Aggregation* state, for there are enough distressed bacteria in the vicinity to attempt to aggregate into "spores."

3.3 Aggregation State

The basic aggregation network is shown in green in Fig. 1. The bacterium continues to secrete D, but by suppressing the refractory period, it effectively increases the rate of D production.[2] Therefore the D field reflects the local population density of aggregating bacteria, which attempt to follow the gradient ∇D toward larger concentrations of D (and thus higher population densities), provided the signal is sufficiently strong. This causes the aggregating bacteria to clump together (see Sec. 2.4 above).

The gradient-following network is shown in dark blue on the left of the lower half of Fig. 1. If $D \leq A_{\mathrm{th}}$, the *aggregation threshold*, then the bacterium orients randomly because the signal is weak, but if $D > A_{\mathrm{th}}$ then the bacterium orients in the direction of increasing concentration. In either case the bacterium attempts to move forward. If it cannot do so, because it is in contact with another bacterium,[3] it secretes K_{A} quanta of the A signal chemical, which thus reflects the density of *clumped* or *aggregated* bacteria.

The concentration of the A substance is used to determine whether bacteria will become dormant (Interior state, $A > B_{\mathrm{th}}$) or will sacrifice themselves by secreting cyst material to protect the others (Boundary state, $A \leq B_{\mathrm{th}}$). (This discrimination is not made until spore formation takes place, but in the simulations an aggregating or clumped bacterium's color indicates the local A concentration — purple for $A > B_{\mathrm{th}}$, green for $A \leq B_{\mathrm{th}}$ — so that we can see how the bacteria are differentiating.)

3.4 Dormant State

If the local D concentration near a bacterium reaches the threshold S_{th}, then that bacterium triggers the formation of a spore, a process we call hibernation; the network subserving this function is shown in magenta on the lower right and bottom edges of Fig. 1. When a bacterium detects $D > S_{\mathrm{th}}$, it secretes K_{S} quanta of the S signal

[2]The effective rate of D secretion increases from $\hat{p}_{\mathrm{D}} K_{\mathrm{D}}$ to $(\hat{p}_{\mathrm{D}} + p_{\mathrm{D}}) K_{\mathrm{D}}$, where $\hat{p}_{\mathrm{D}}$ is defined by Eq. 19 in Sec. 5.1 on p. 48.

[3]This might be implemented by having the bacteria sense a chemical that is expressed on the surface of all bacteria, and cannot diffuse away.

compound, and enters the *Dormant* state, in which it will remain until the ambient temperature increases above T_c (see Sec. 3.5 below). S is a rapidly diffusing, quickly degrading volatile compound, intended to cause the nearly synchronous hibernation of the entire colony. (Occasionally it may propagate to an adjacent colony.) Whenever a bacterium in the Aggregation state detects $S > R_{th}$ (the relay threshold), it secretes K_S quanta of S, thus continuing the propagation of the hibernation signal.[4] In addition, if $A > B_{th}$, then this bacterium is an Interior cell, so it enters the Dormant state (violet subnet). On the other hand, if $A \leq B_{th}$, the bacterium is a Boundary cell, so it secretes a quantity of protective cyst material and dies. In this way the Boundary cells create a protective layer around the dormant Interior cells, and a spore is formed.

3.5 Reanimation

Whenever the ambient temperature becomes favorable by increasing above T_c, the surviving bacteria (in any state) return to the Normal state controlled by the brown network in the upper-right corner of Fig. 1. Dormant bacteria will attempt random locomotion, but they are confined by the protective cyst. However, as the diagram indicates, in the Normal state bacteria will destroy cyst material (eating or dissolving it) whenever they encounter it. Thus the bacteria will be able to break out of the spore and return to their normal behavior, wandering and reproducing.

4 Typical Behavior

The best way to get an intuitive understanding of the self-organization process is to watch several simulations. To capture some of the dynamics of a moving simulation, this section presents a large number of screen images from a typical run, which is perhaps the best that can be done in a static document. The simulation used the default parameters shown in Table 1.[5]

[4] The function of the relay threshold is to prevent trace quantities of S from triggering premature hibernation.

[5] The simulations were implemented in Java StarLogo 2.0 and run under both Mac OS/X Panther and MS Windows 2000 Professional; the program is about 225 lines of StarLogo code.

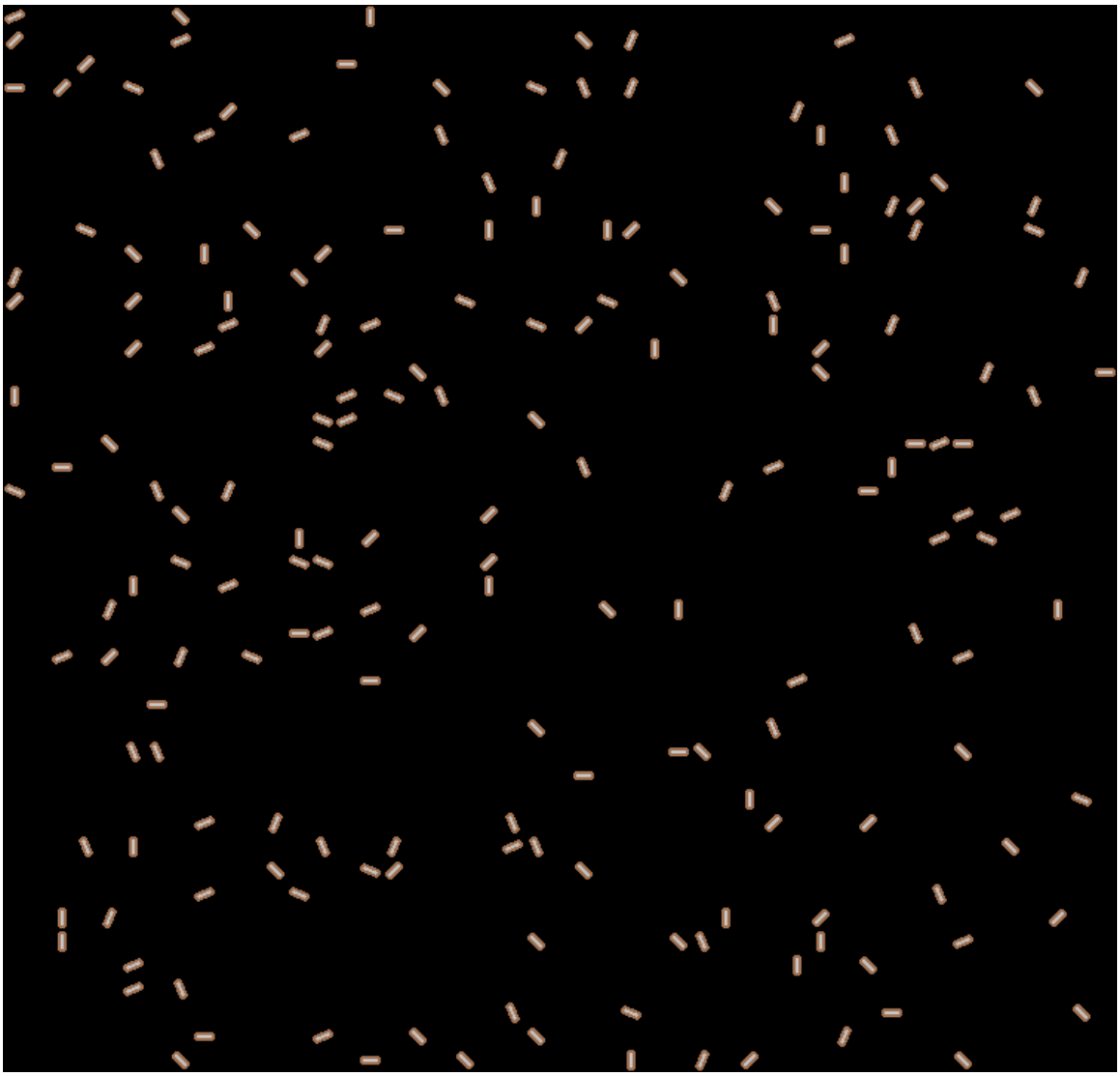


Figure 2: $T = 0$, initial population $P_0 = 190$. Bacteria in the Normal state (indicated by tan color) wander randomly and periodically reproduce. The population is initialized randomly according to the initial population density parameter and the size of the space, $P_0 \approx p_{\mathrm{P}} P_{\max}$.

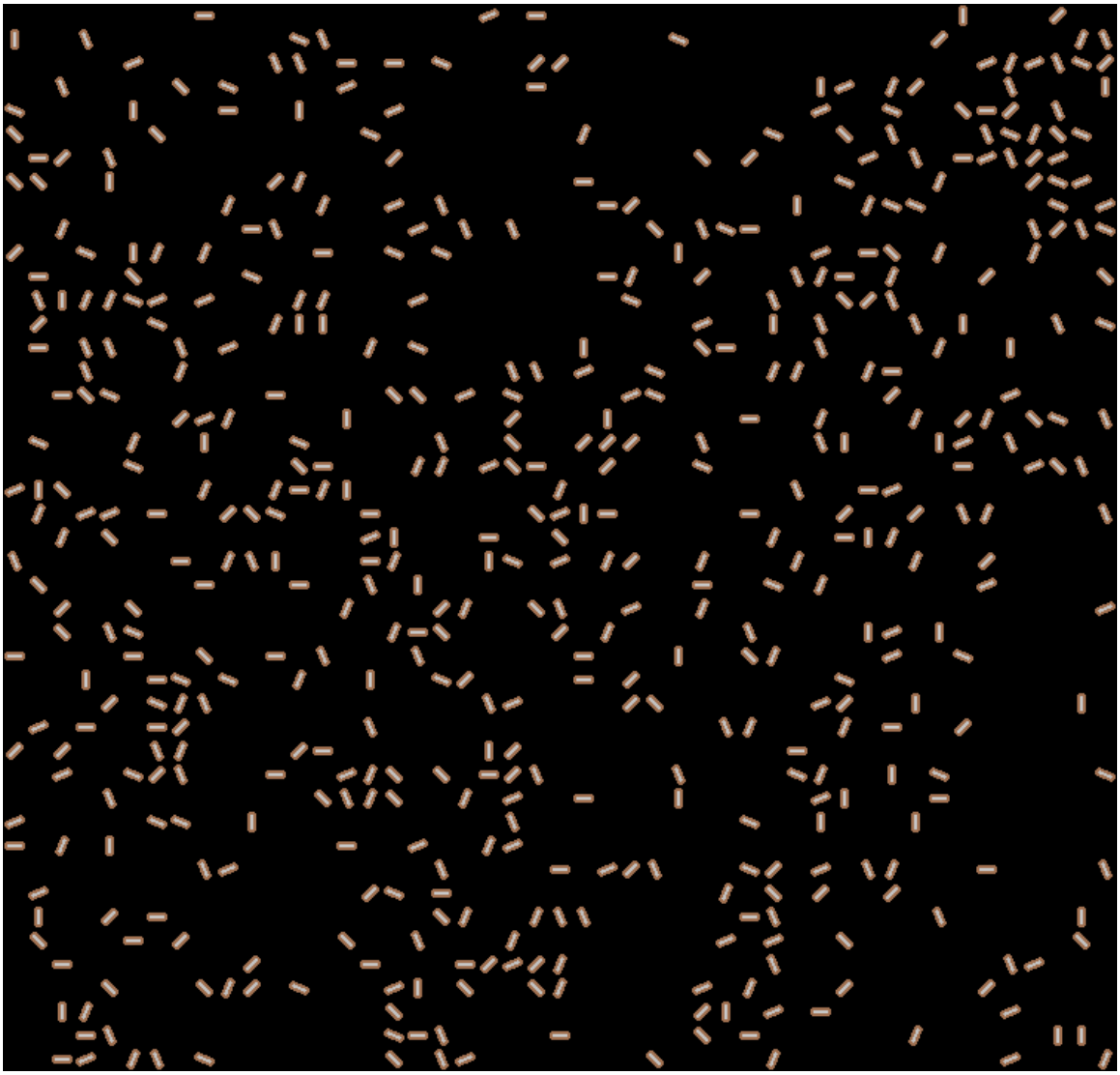


Figure 3: $T = 49$. The population has increased to $P = 539$. At this point the ambient temperature is decreased to less than the critical temperature T_c, creating unfavorable conditions for the bacteria. Bacteria begin signaling distress by secreting D chemical.

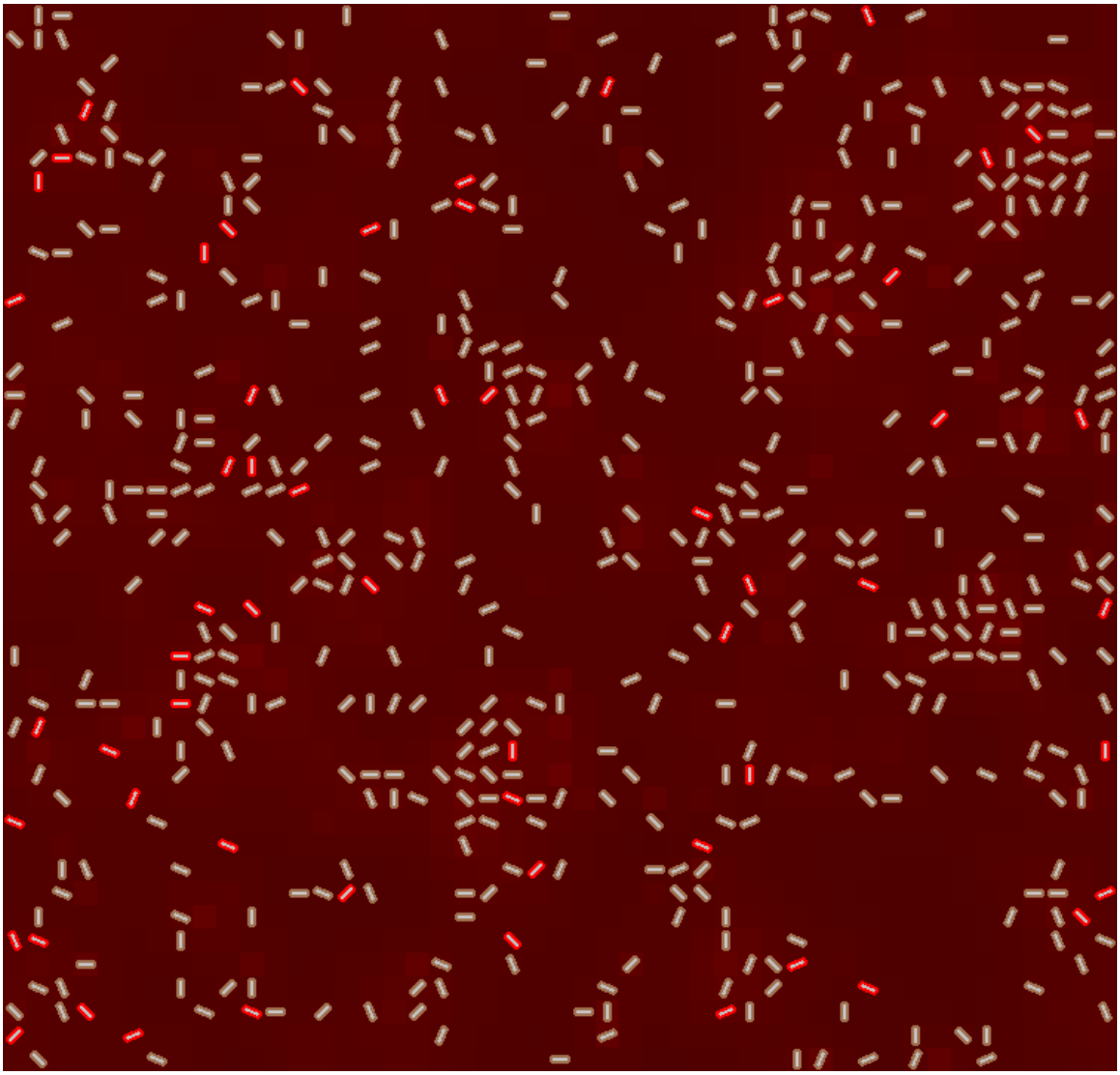


Figure 4: $T = 64$, D field. Bacteria are signaling distress. The bacteria signaling at a given time are shown in red. The background color indicates the strength of the D field (darker is weaker). Some clumping of the bacteria is already apparent.

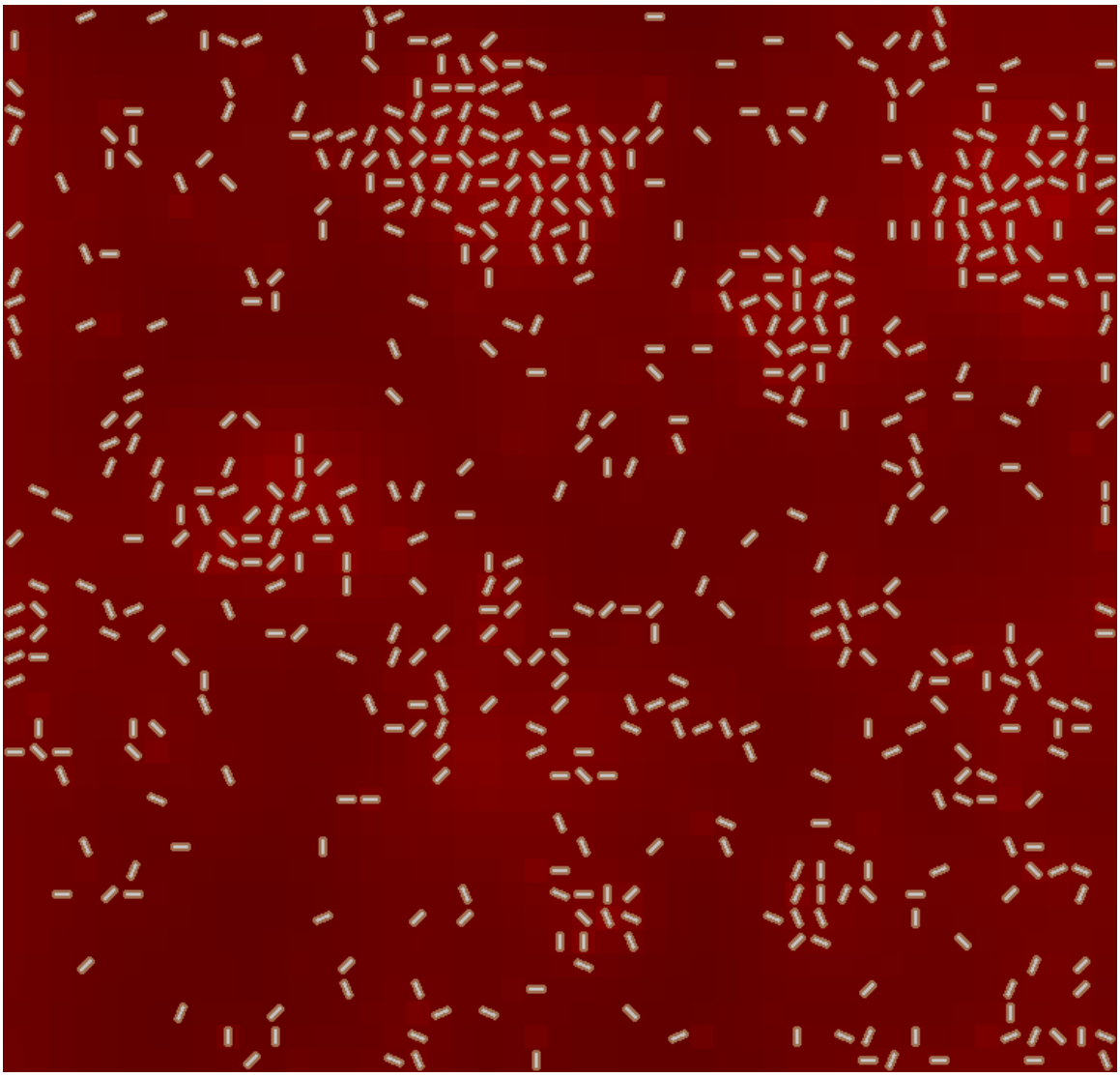


Figure 5: $T = 129$, D field. Aggregation in upper center and right is apparent, which is where the colonies will eventually form.

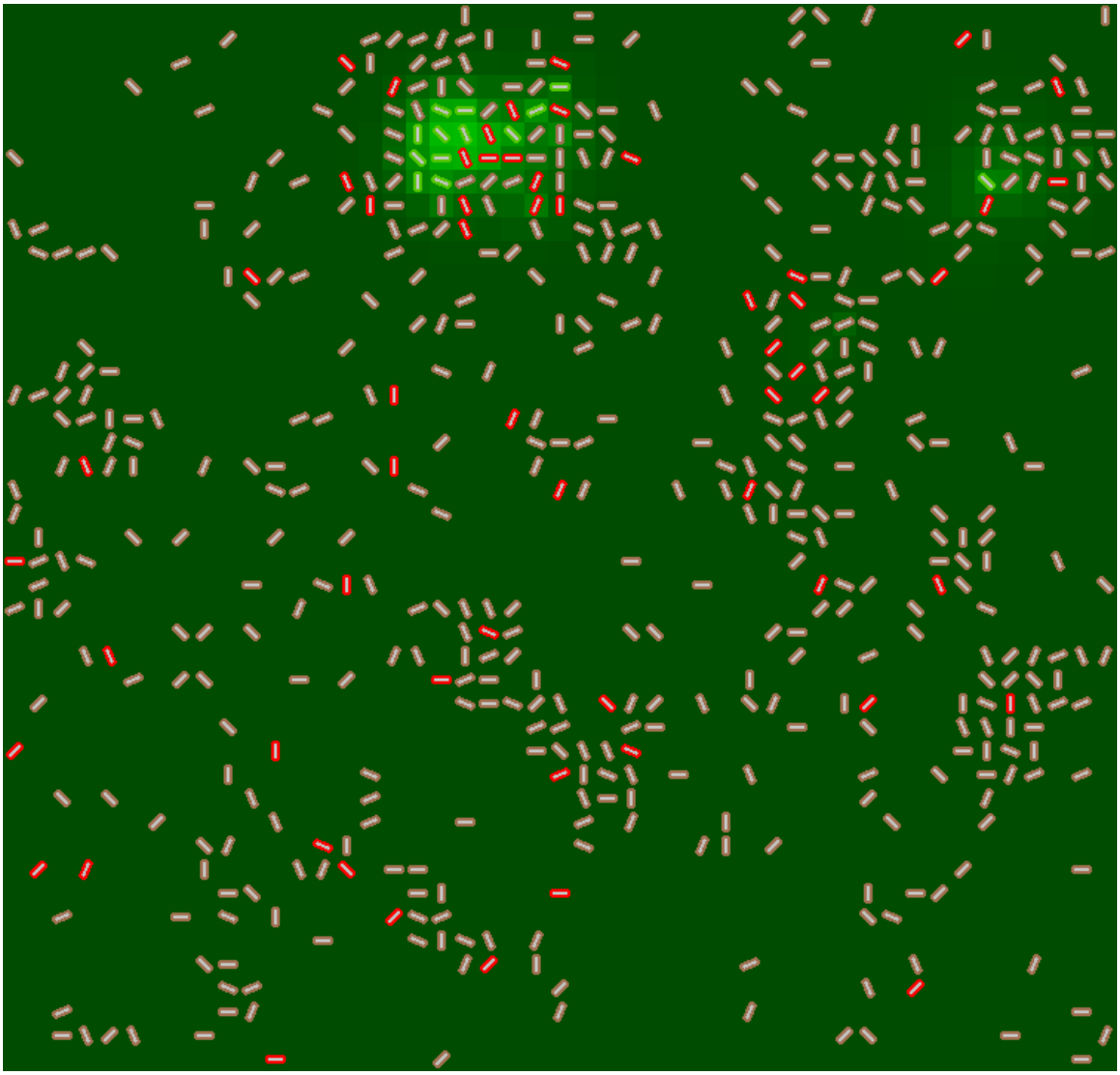


Figure 6: $T = 171$, A field. Aggregating bacteria ($D > A_{\text{th}}$) are shown in green (until $A > B_{\text{th}}$, see Fig. 10); the others (tan) are still wandering randomly. The background color depicts the strength of the A field generated by aggregated (clumped) bacteria (lighter color is stronger field).

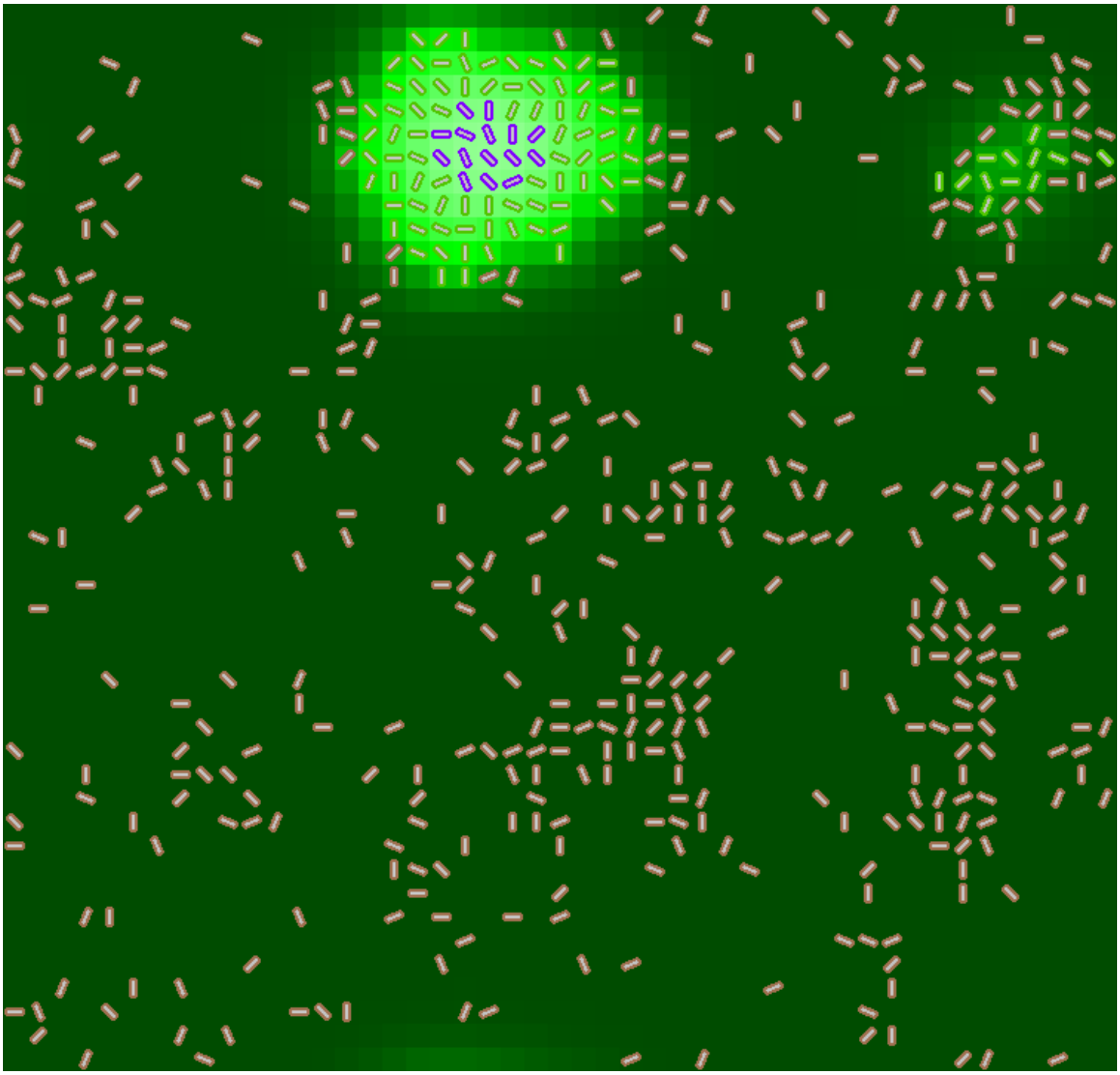


Figure 7: $T = 204$, A field. The size of the main colony has increased sufficiently that some of the bacteria are in the Interior state (indicated by violet color), as a consequence of A concentration passing the threshold B_{th}. The simulation space is a torus (wraps around vertically and horizontally), so some of the A diffusing from the large cluster can be seen on the bottom of the screen.

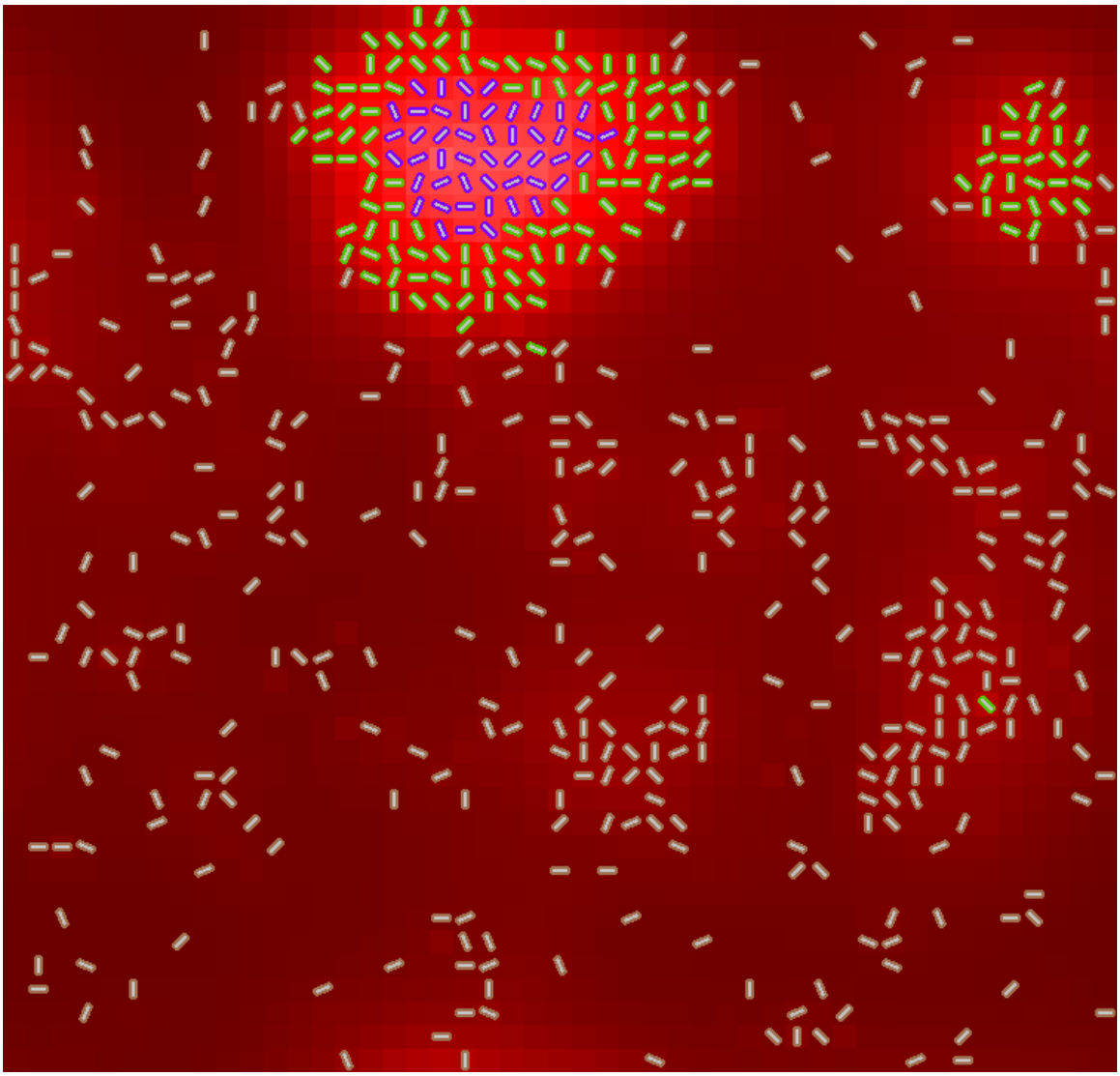


Figure 8: $T = 236$, D field. Continued aggregation in two main colonies; additional clustering is apparent to right of center.

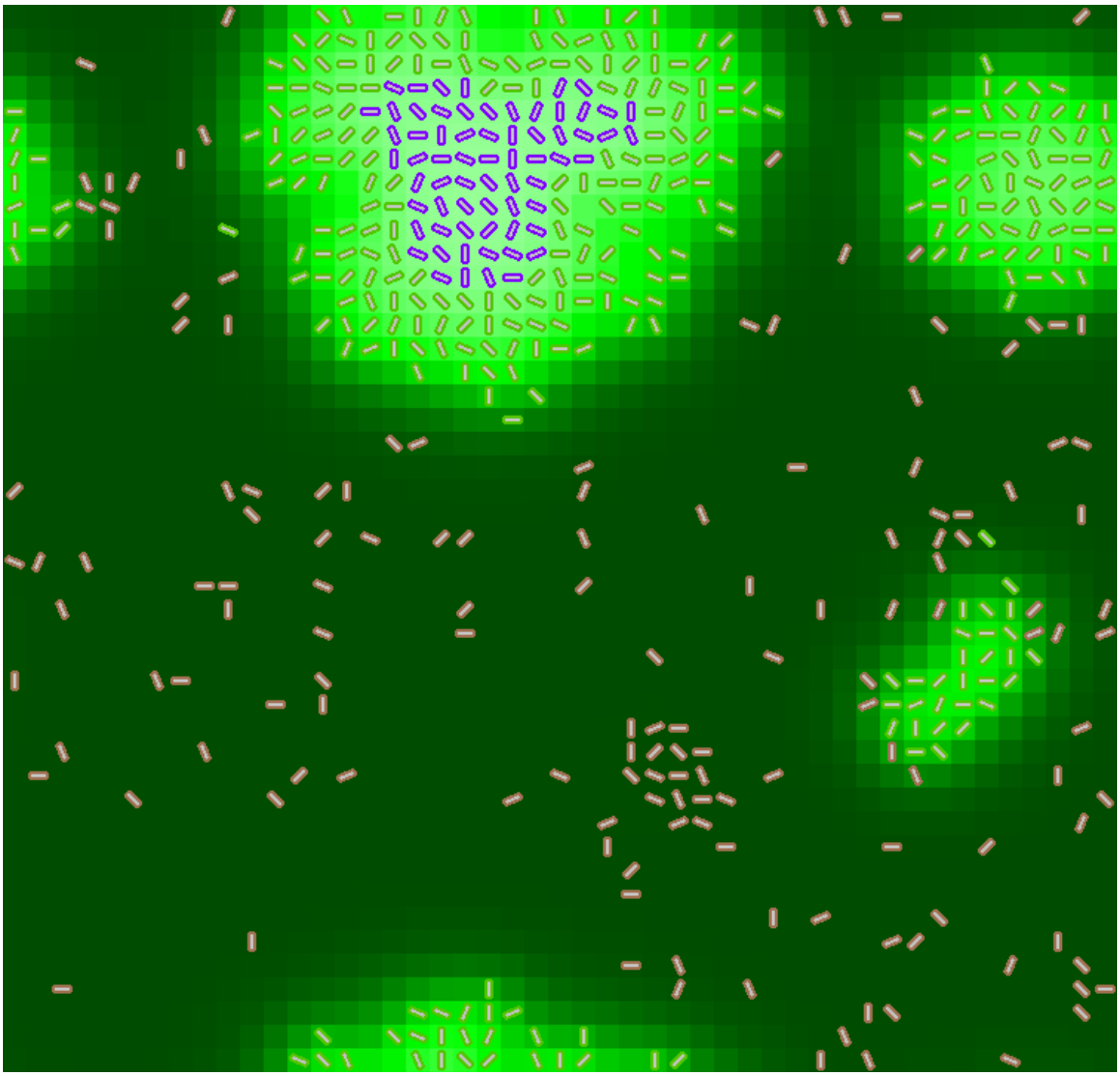


Figure 9: $T = 302$, A field. The largest colony has Interior cells, the smaller ones do not. D concentration is nearing the hibernation threshold S_{th} indicated by the lightest shade of the green background color. Recall that the simulation space is a torus, so there are three clusters, not five.

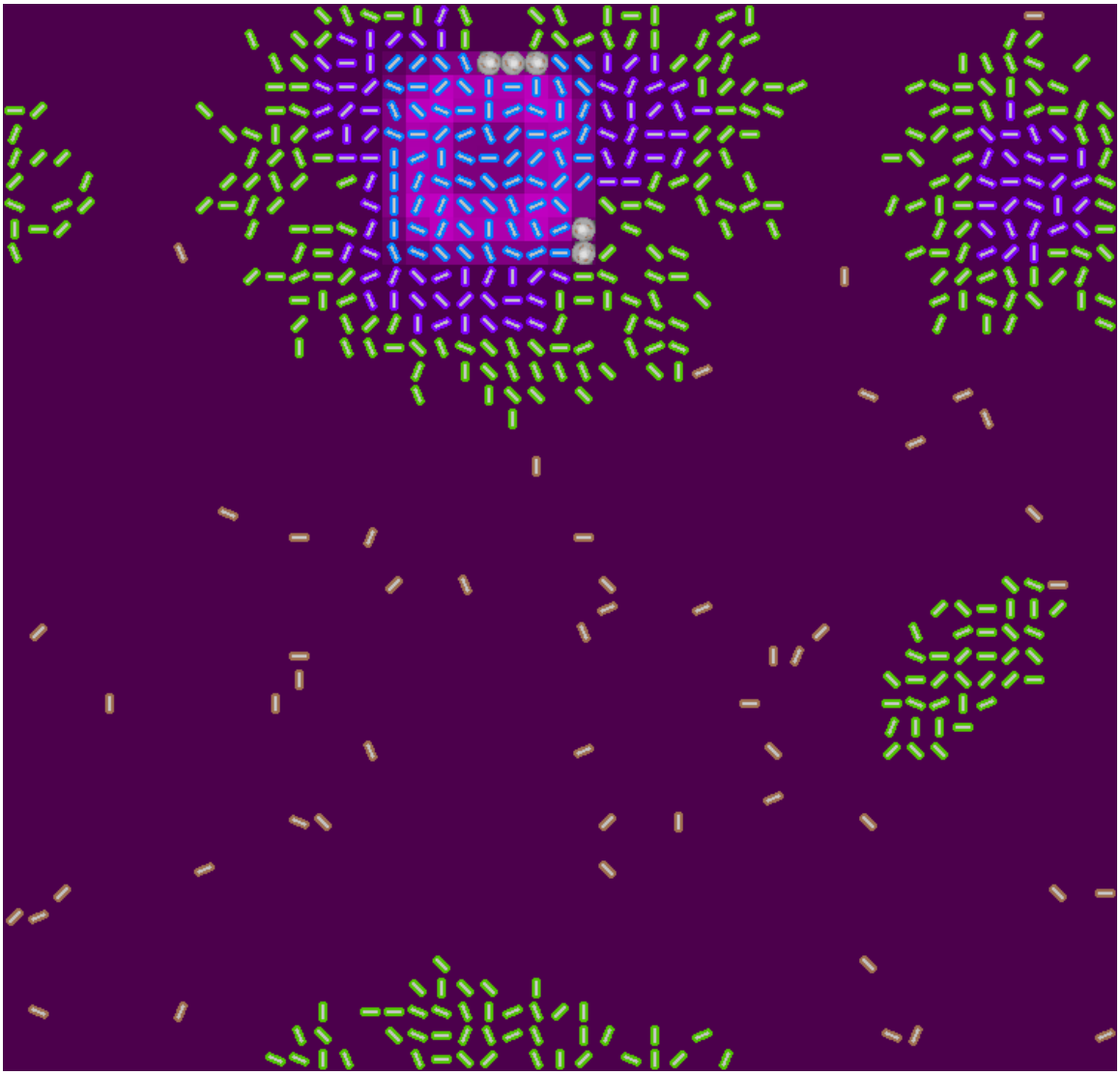


Figure 10: $T = 343$, S field. The D concentration near a bacterium in the largest cluster has reached the hibernation threshold S_{th}. This has caused it to secrete K_{S} quanta of the volatile S compound (indicated by background shades of magenta; lighter color is higher concentration). The bacterium that emitted the S signal has gone into a dormant state (indicated by light blue color). Further, as each bacterium in the Interior state ($A > B_{\text{th}}$) receives the S signal, it relays it by secreting K_{S} quanta of S, and then going into the dormant state. The outwardly propagating wave of S is clearly visible as a lighter shade of magenta. Aggregating bacteria in the Boundary state ($A \leq B_{\text{th}}$, green) also relay the S signal, but after doing so, they secrete protective cyst material (indicated in grey), and then die. (Note that the middle-size colony has Interior cells by this time.)

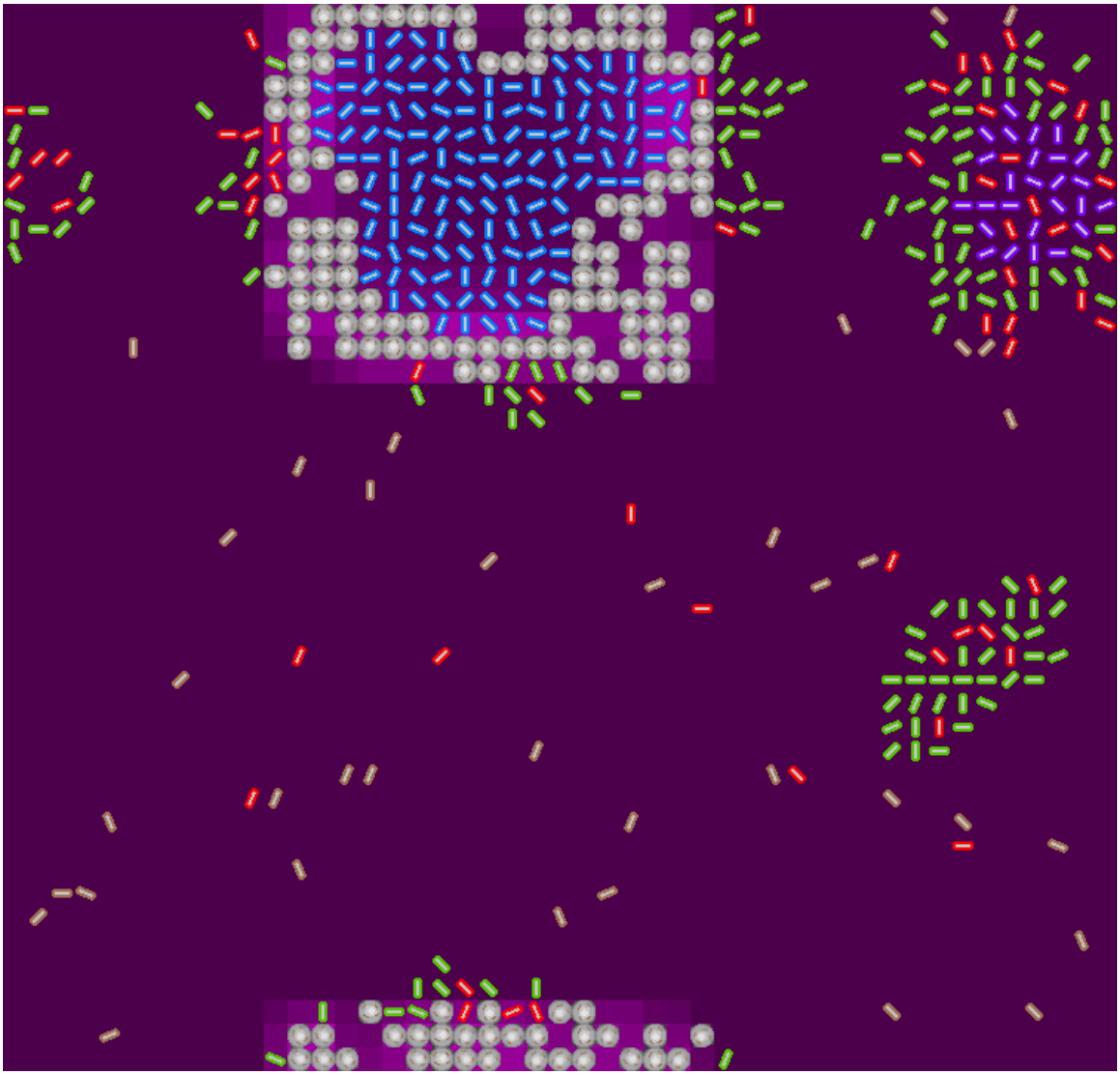


Figure 11: $T = 347$, S field. Continued spread of S signal. Note that bacteria continue to signal distress (red) throughout aggregation, until they become dormant or die.

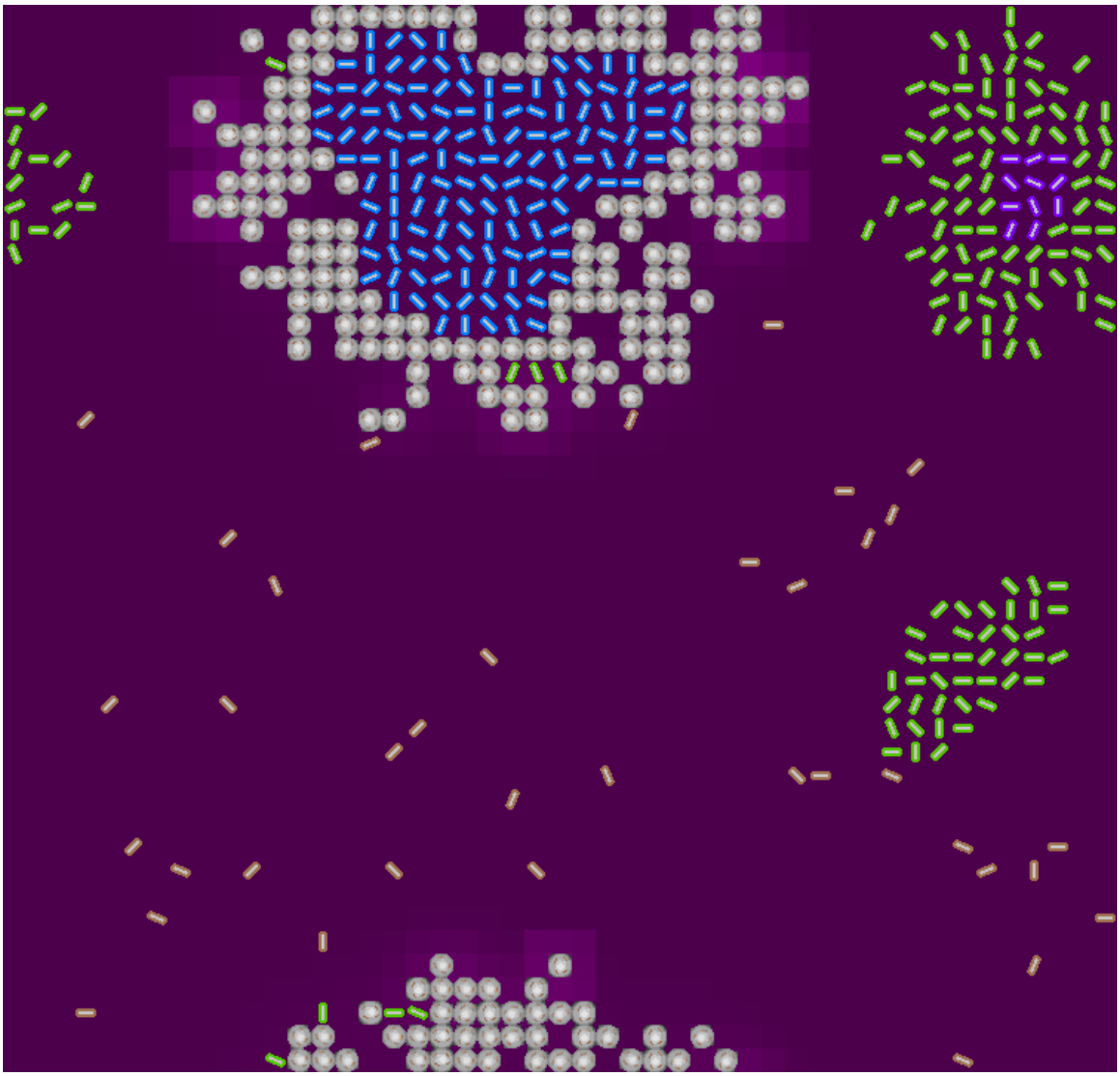


Figure 12: $T = 351$, S field. S chemical has largely dissipated, and formation of first spore is complete. The protective layer is of moderate but irregular thickness.

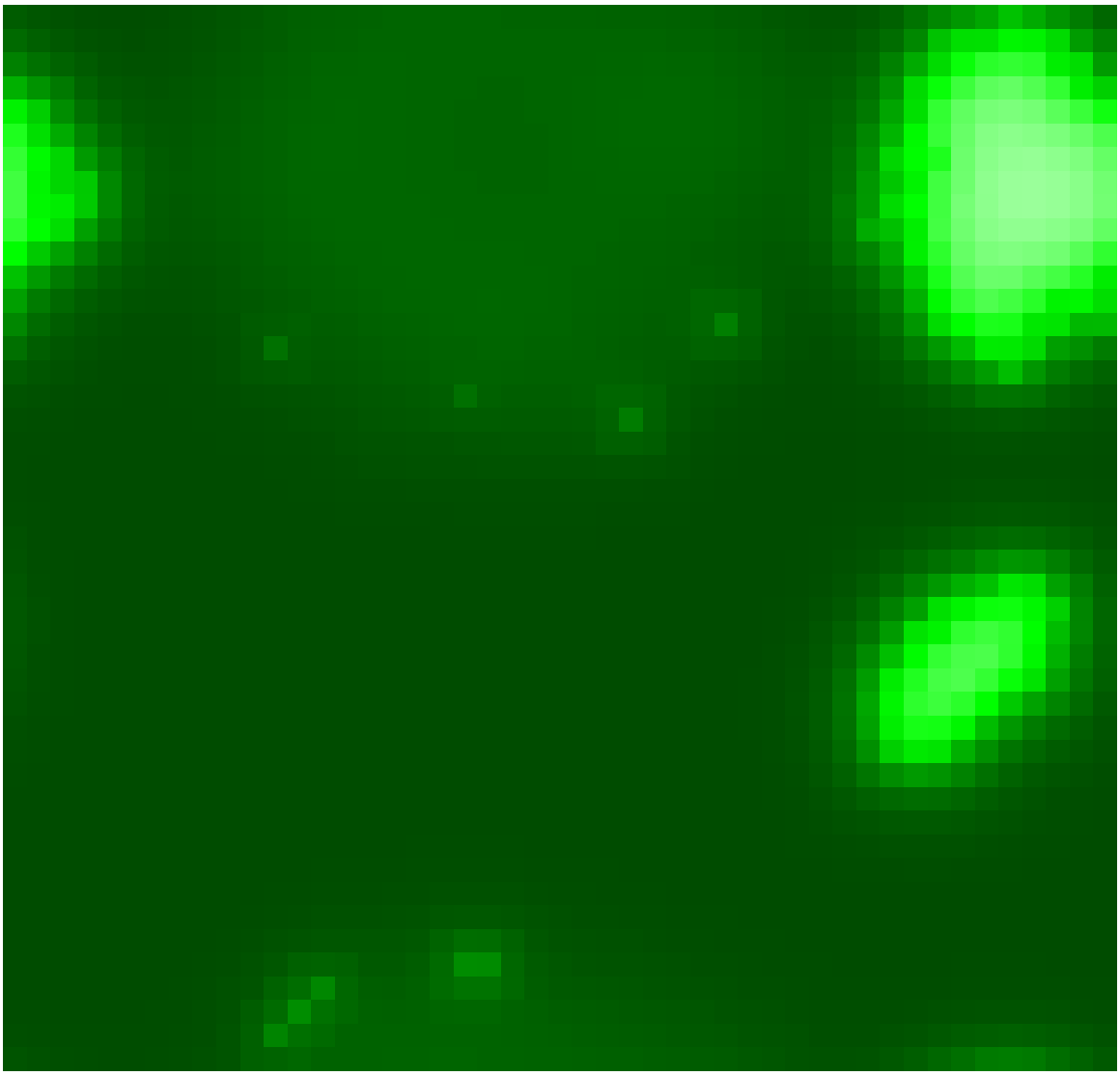


Figure 13: $T = 365$, A field (bacteria omitted). Since the largest colony is in the dormant state, it is no longer secreting the A signal, and so we can see that the A concentration has decreased in its vicinity.

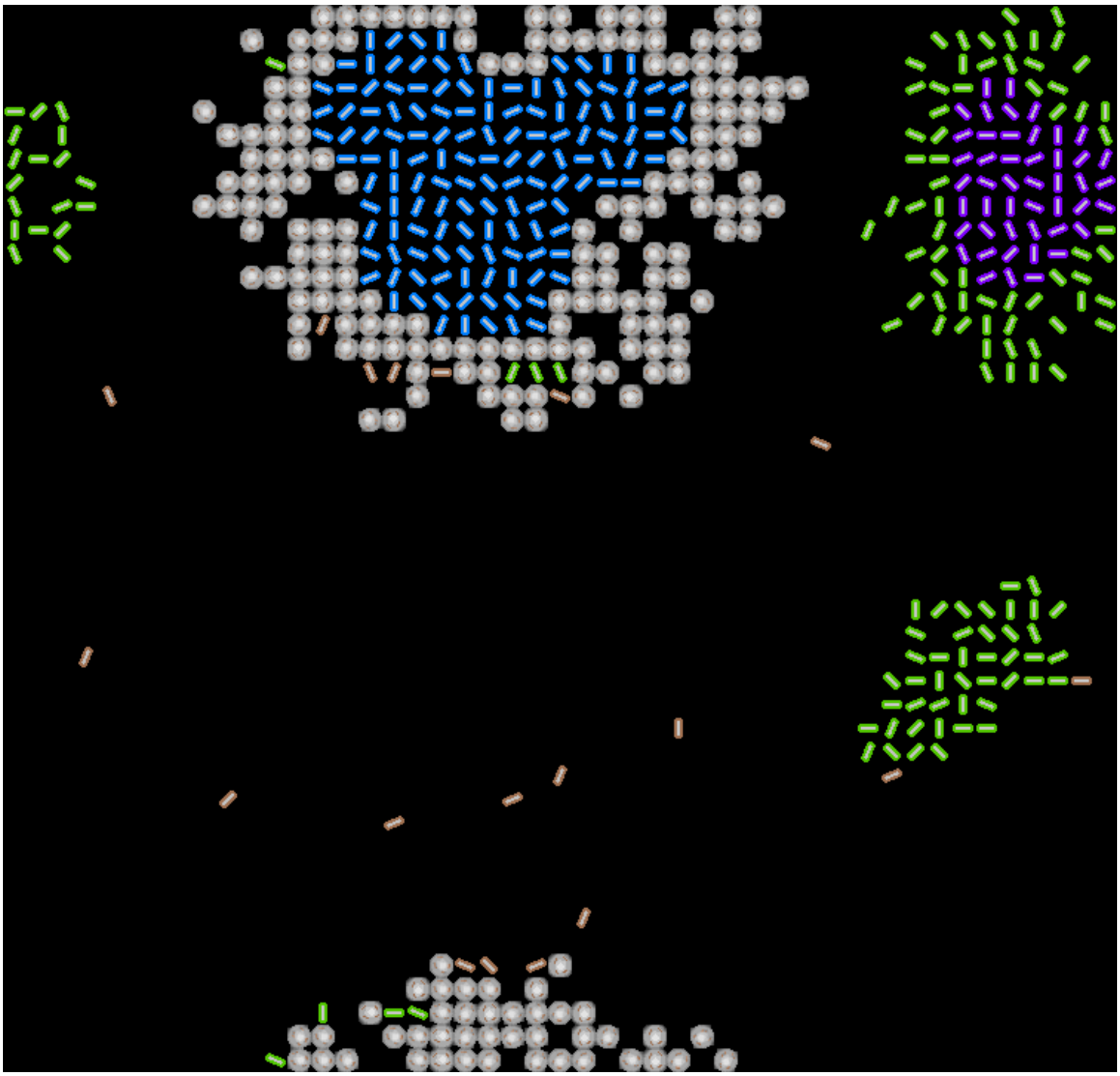


Figure 14: $T = 462$. The two smaller colonies have not reached the critical mass necessary to form spores.

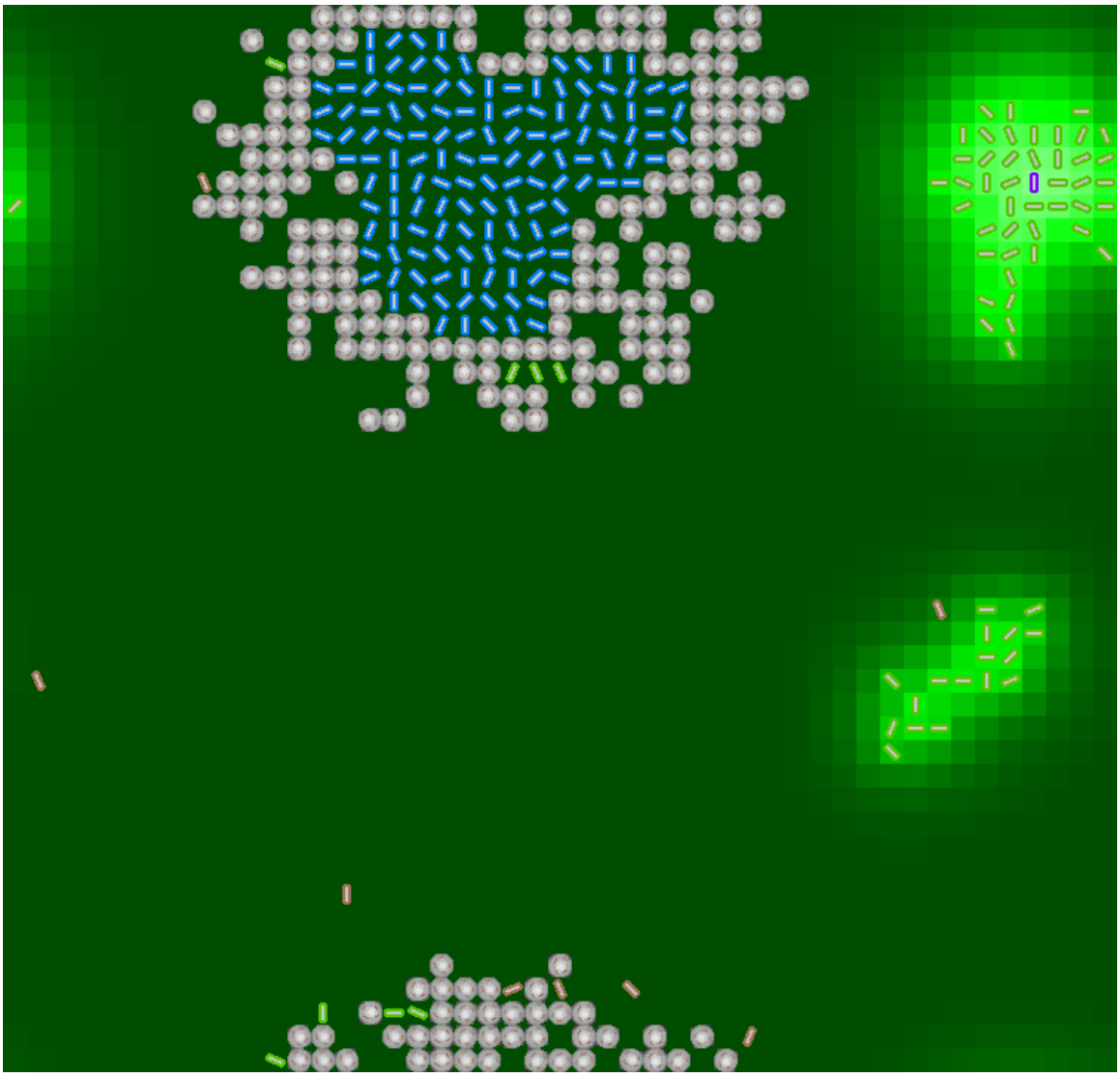


Figure 15: $T = 548$, A field. The two smaller clusters are dying out. In this simulation the lifetime of distressed bacteria was 500 ± 25 time steps, and since the population was stressed at $T = 50$, we expect stressed bacteria to die during $525 < T < 575$.

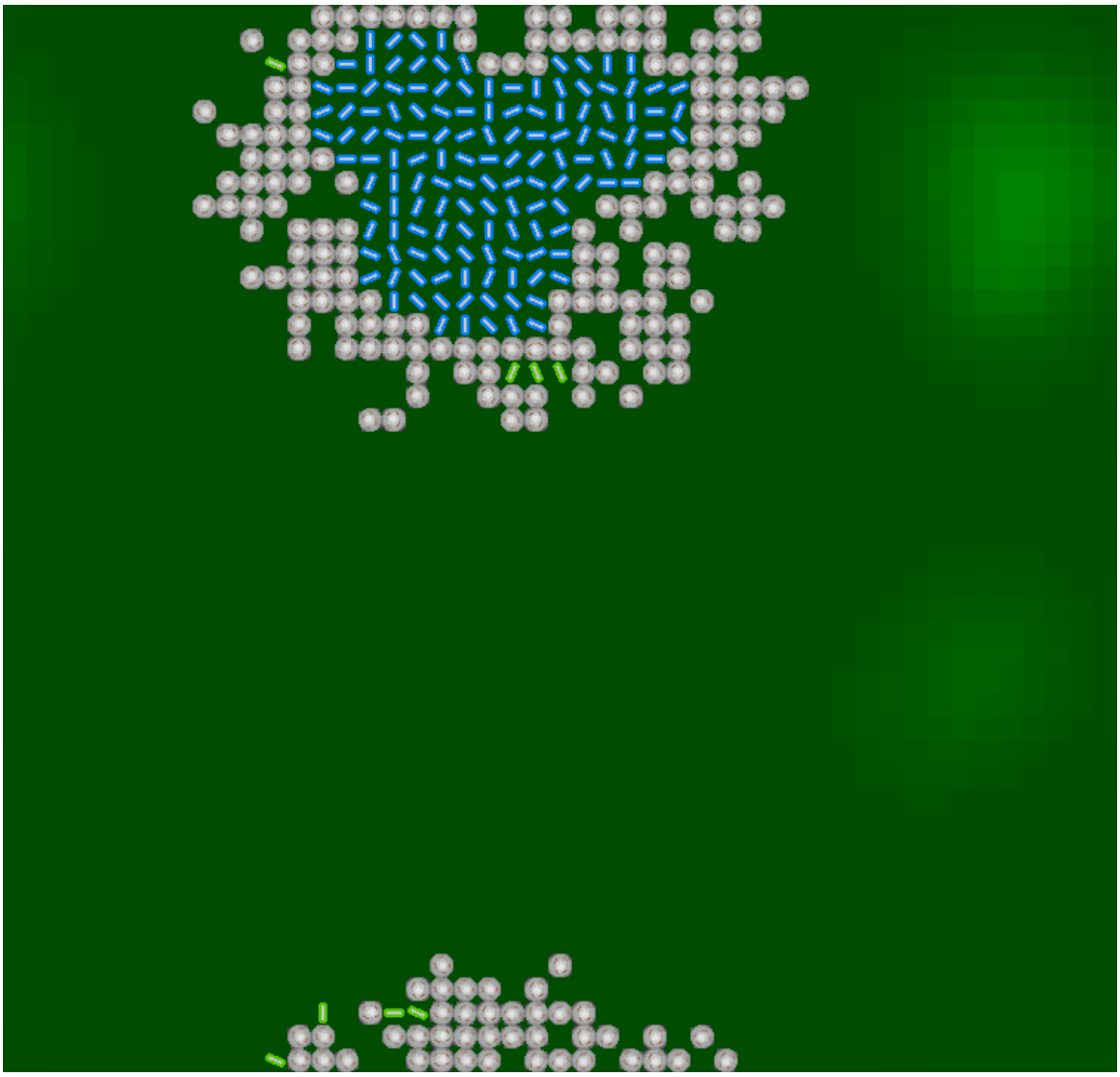


Figure 16: $T = 572$, A field. The smaller colonies have died (except 8 individuals); only the spore remains. Final population $P_{\mathrm{f}} = 136$, protected in the interior of the spore.

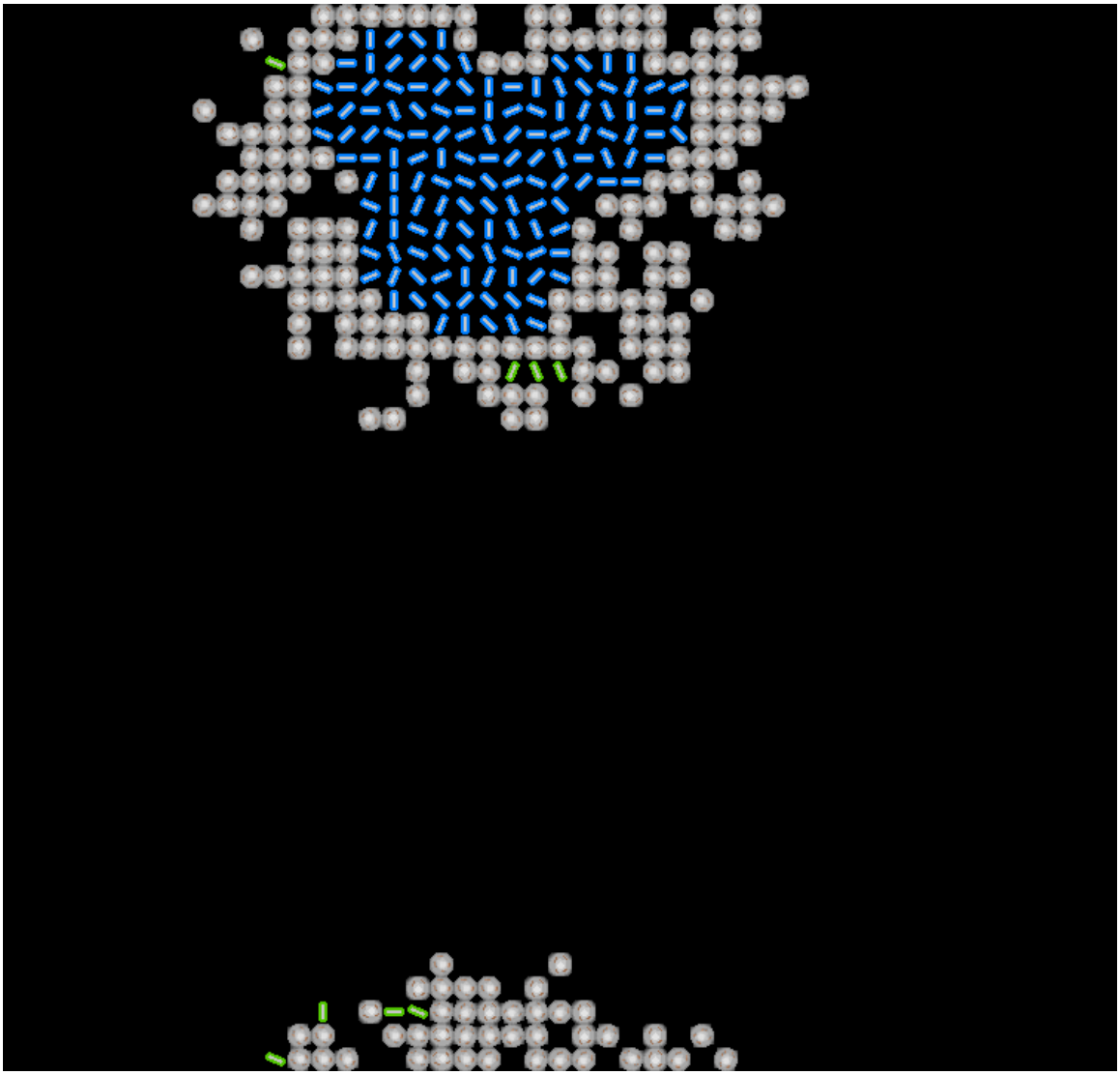


Figure 17: $T = 805, P = 136$. Surviving spore, protecting 136 dormant bacteria, just before favorable conditions restored (temperature above T_c).

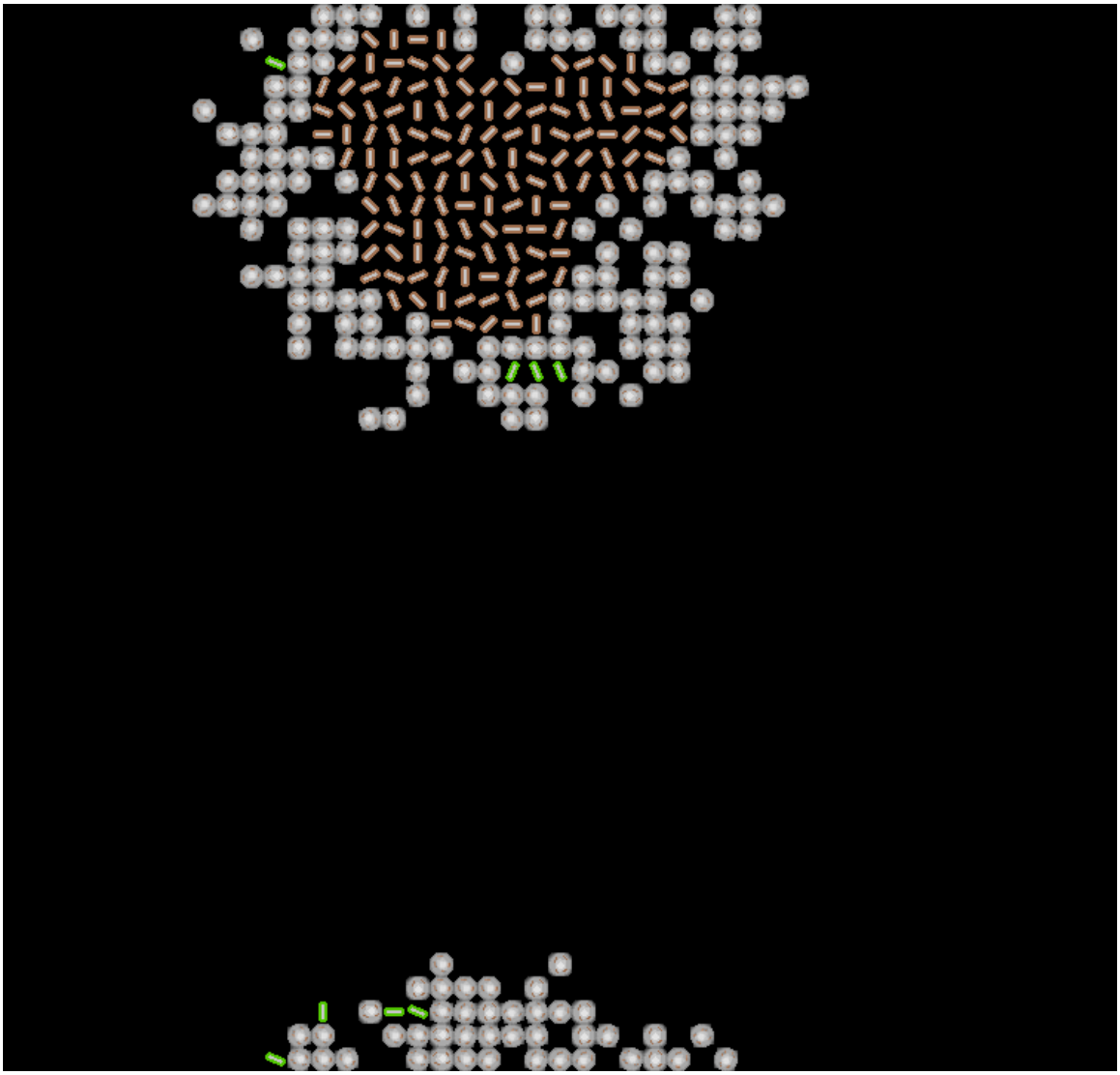


Figure 18: $T = 806$. The temperature has been raised above the critical temperature T_c, and, given these favorable conditions, the dormant bacteria have reanimated (tan color). The bacteria have returned to their Normal state, which includes random locomotion and reproduction, but also destroying the (grey) cysts when they encounter them. In this way they begin to break out of their spore.

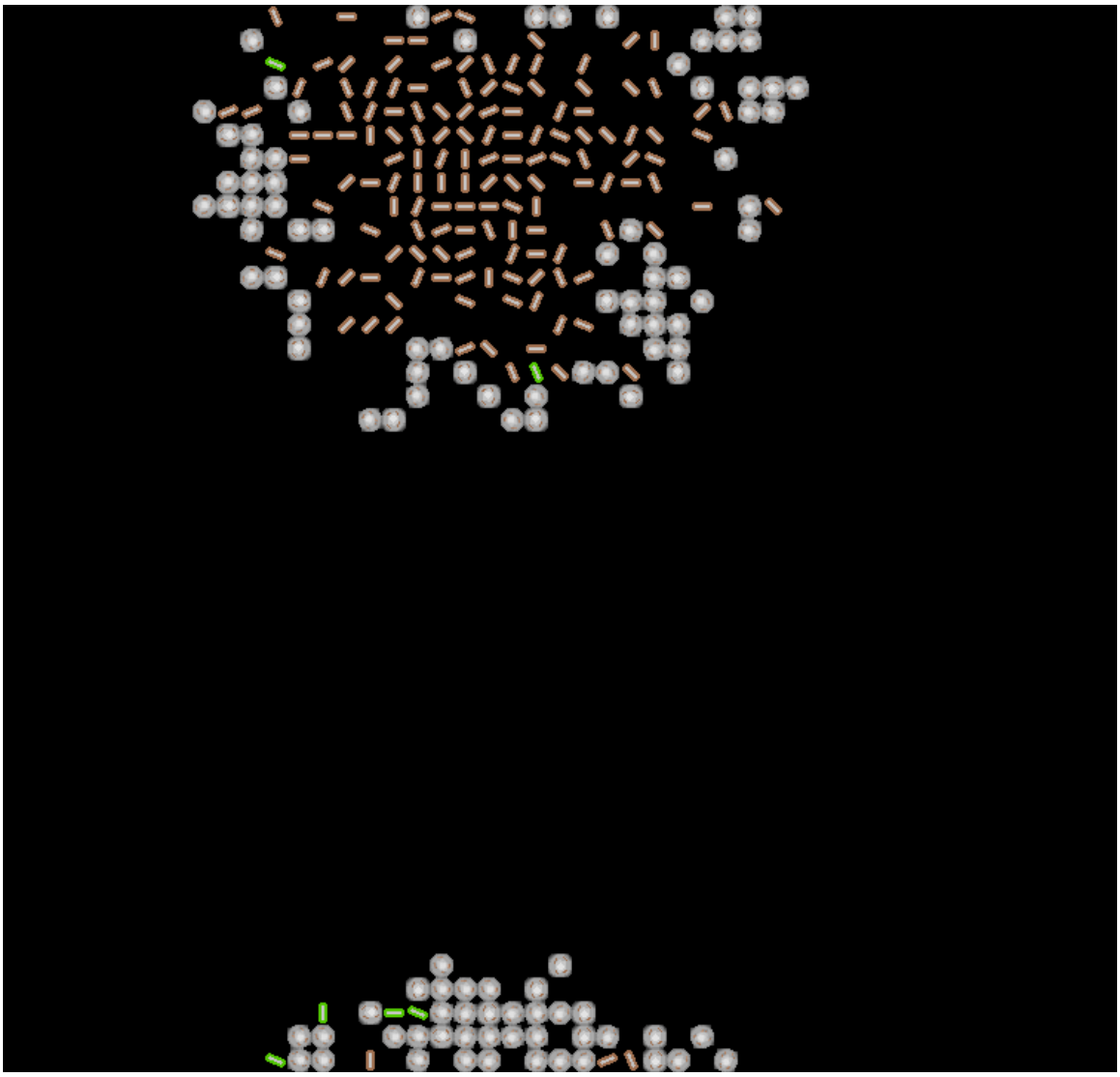


Figure 19: $T = 811, P = 148$. Reanimated bacteria continue to break out of their spore. Population has increased to 148.

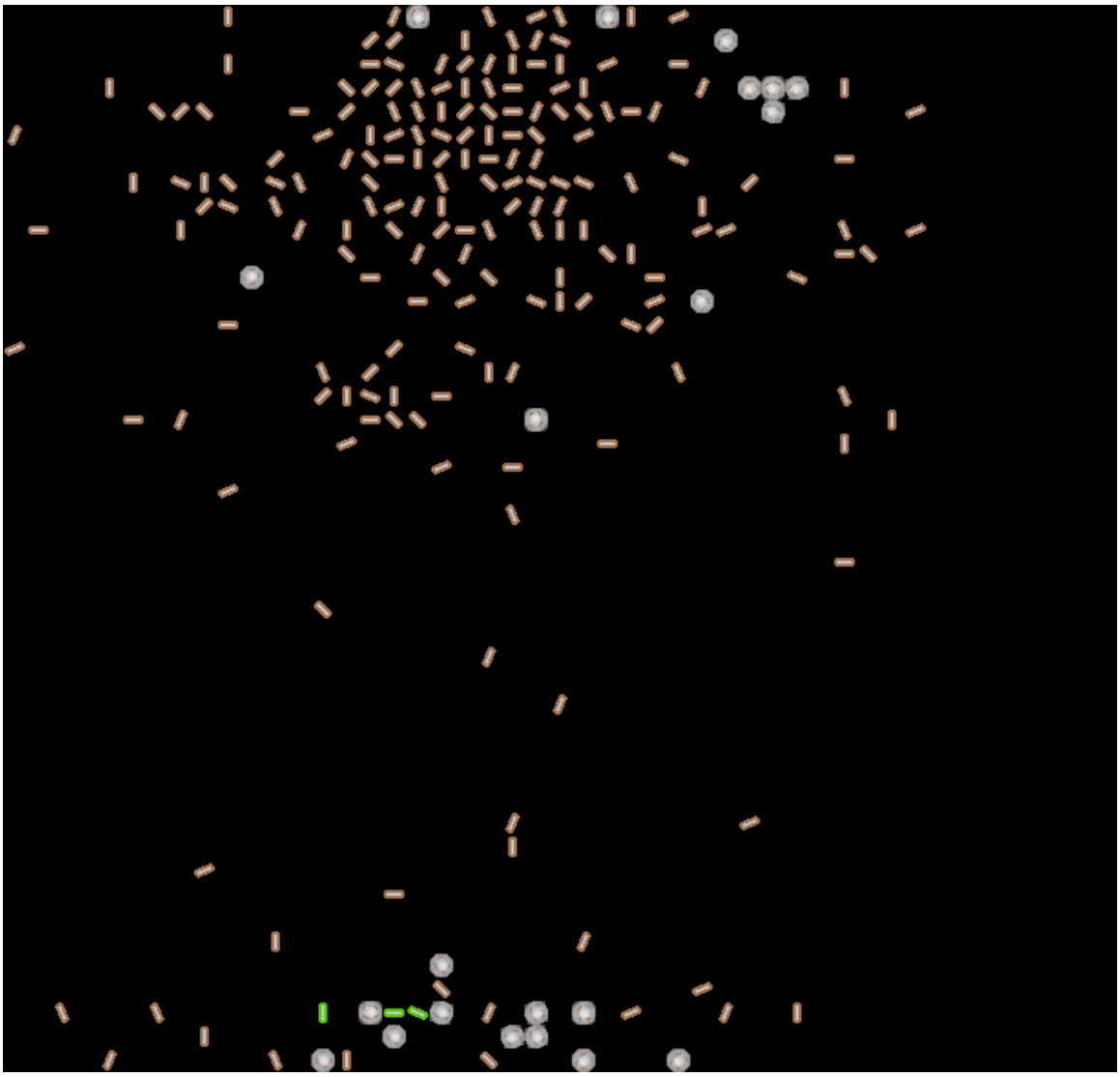


Figure 20: $T = 826, P = 205$. Bacteria have largely destroyed the cysts.

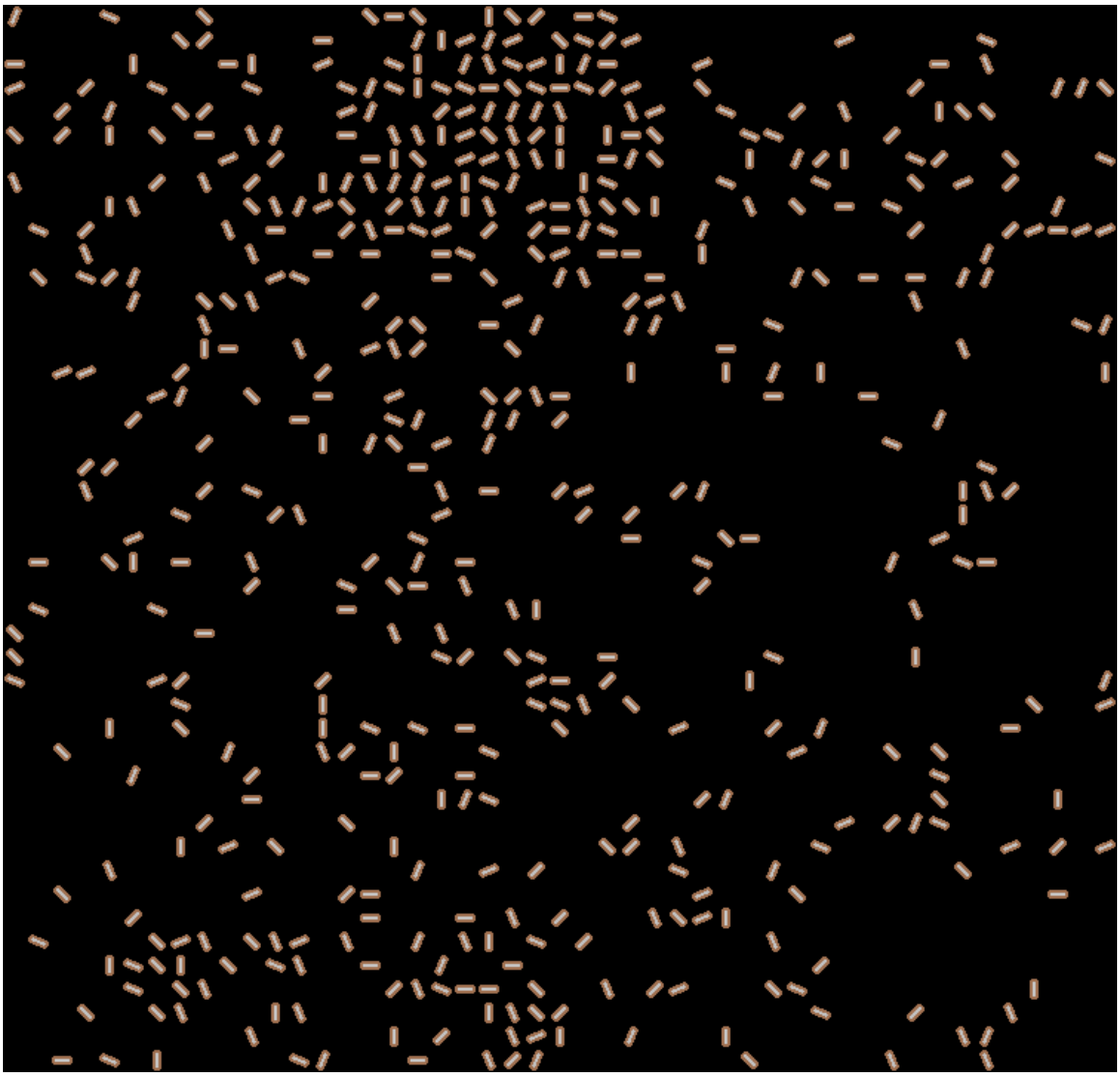


Figure 21: $T = 874, P = 538$. The population has recovered to its level before it was stressed. Although the bacteria wander randomly, their density remains higher in the region of the spore.

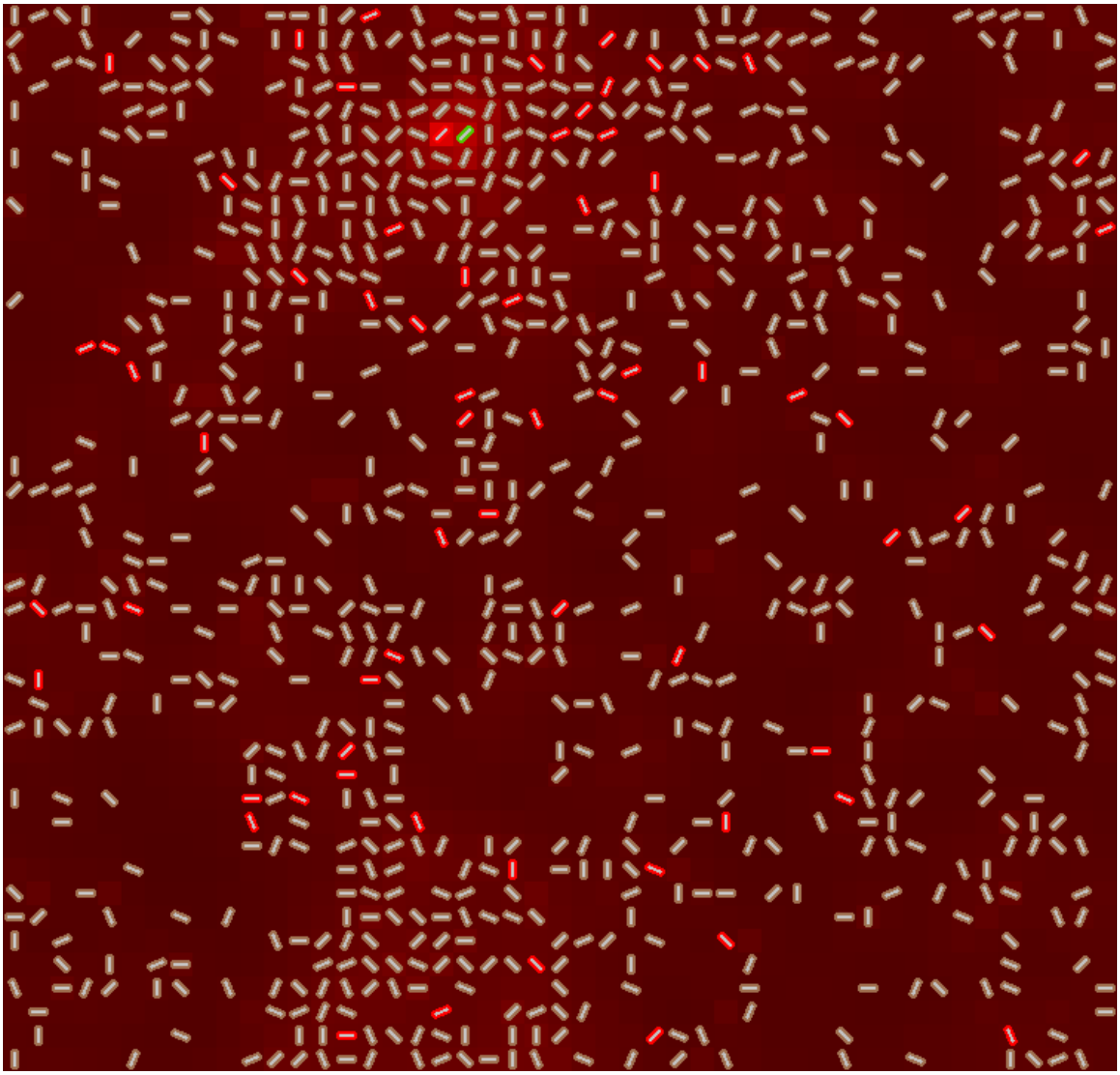


Figure 22: $T = 911, P = 891$, D field. To demonstrate that the system an be recycled repeatedly between favorable and unfavorable conditions, the temperature has been decreased below T_c again. Distressed bacteria are emitting D signal and already beginning to clump, due to the high population density ($P = 891$).

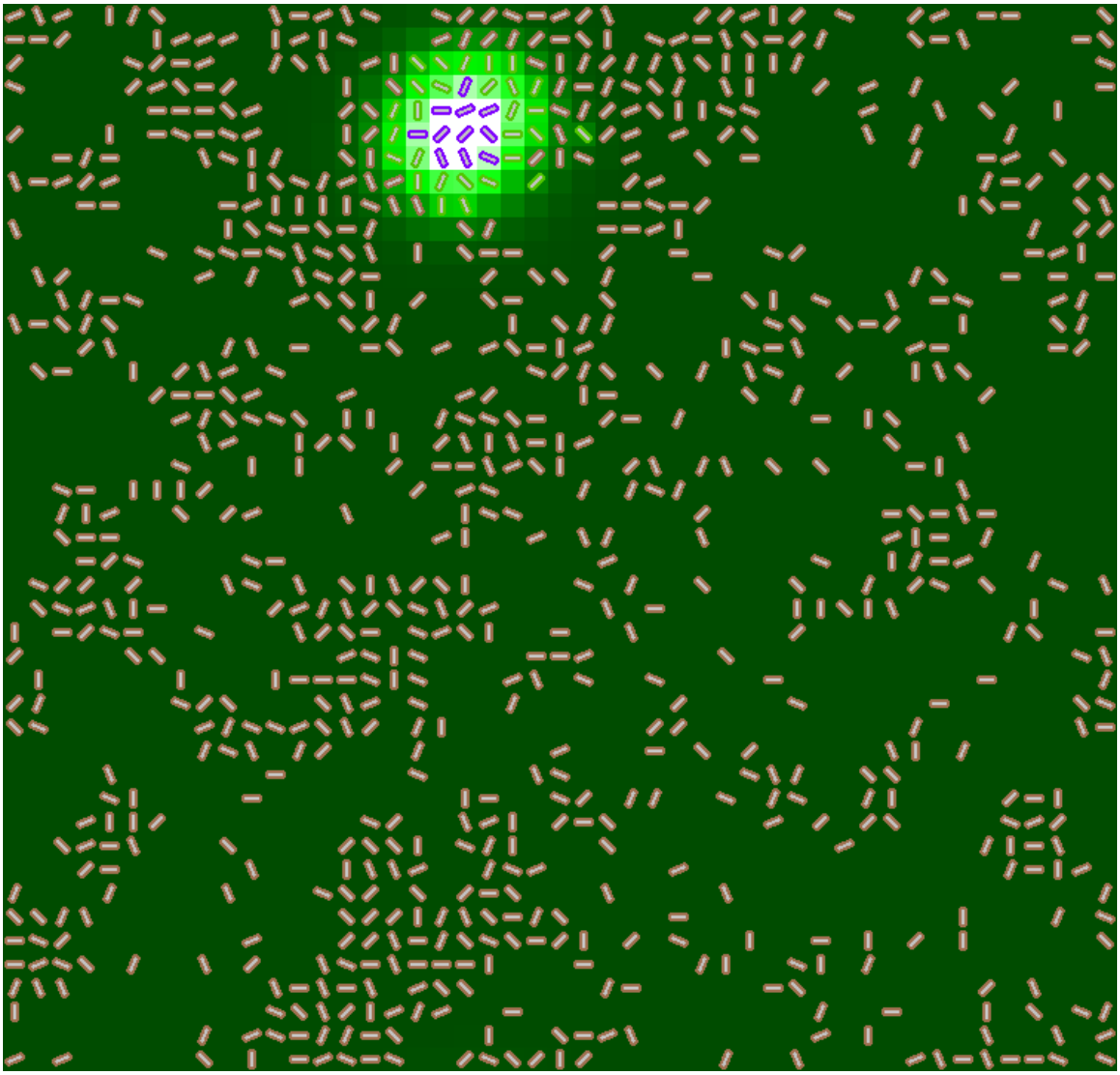


Figure 23: $T = 930$, A field. A cluster with Interior cells is already forming in the vicinity of the old spore (since that is where there is the highest density of bacteria).

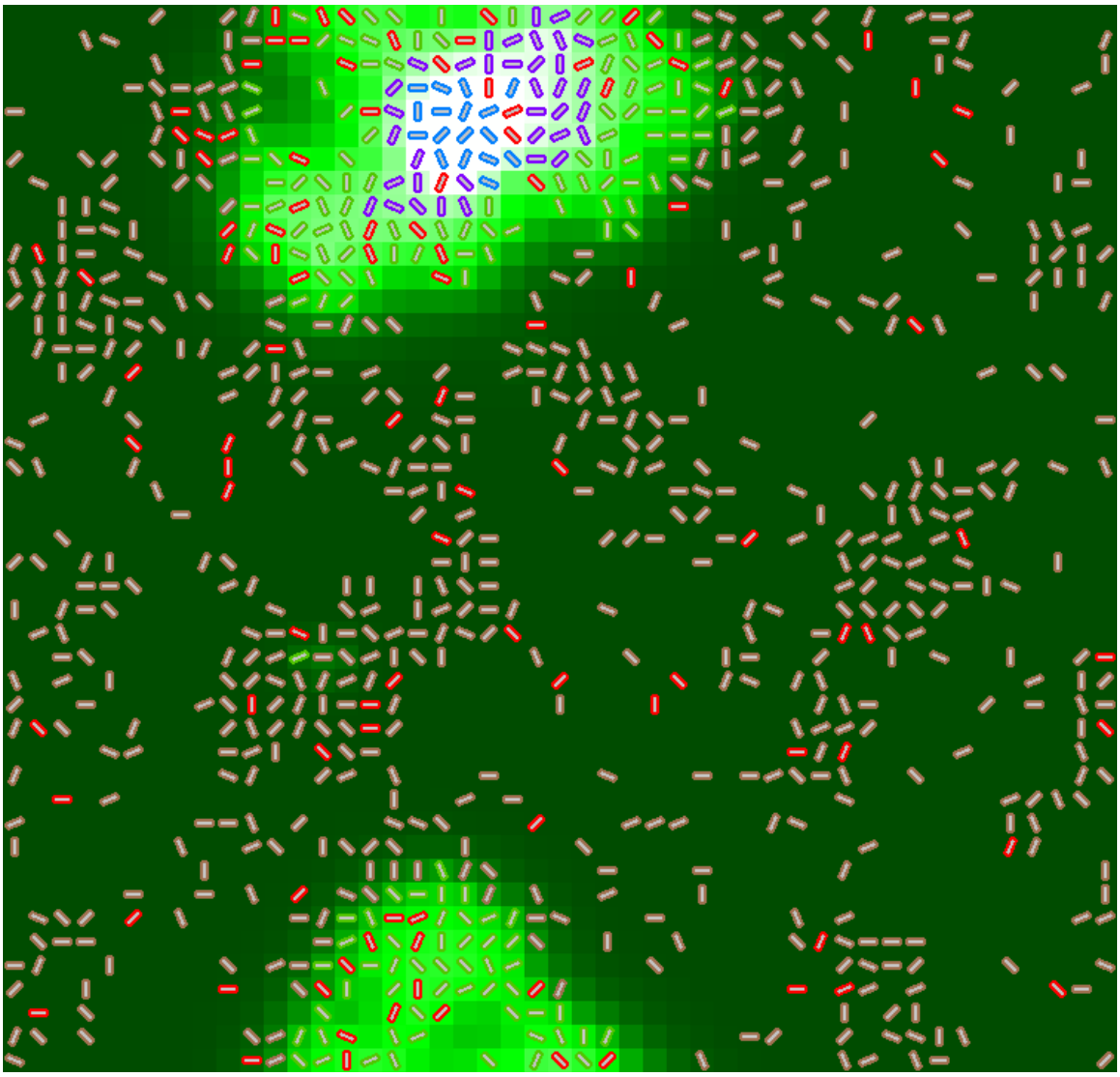


Figure 24: $T = 969$, A field. The colony is beginning to become dormant (light blue).

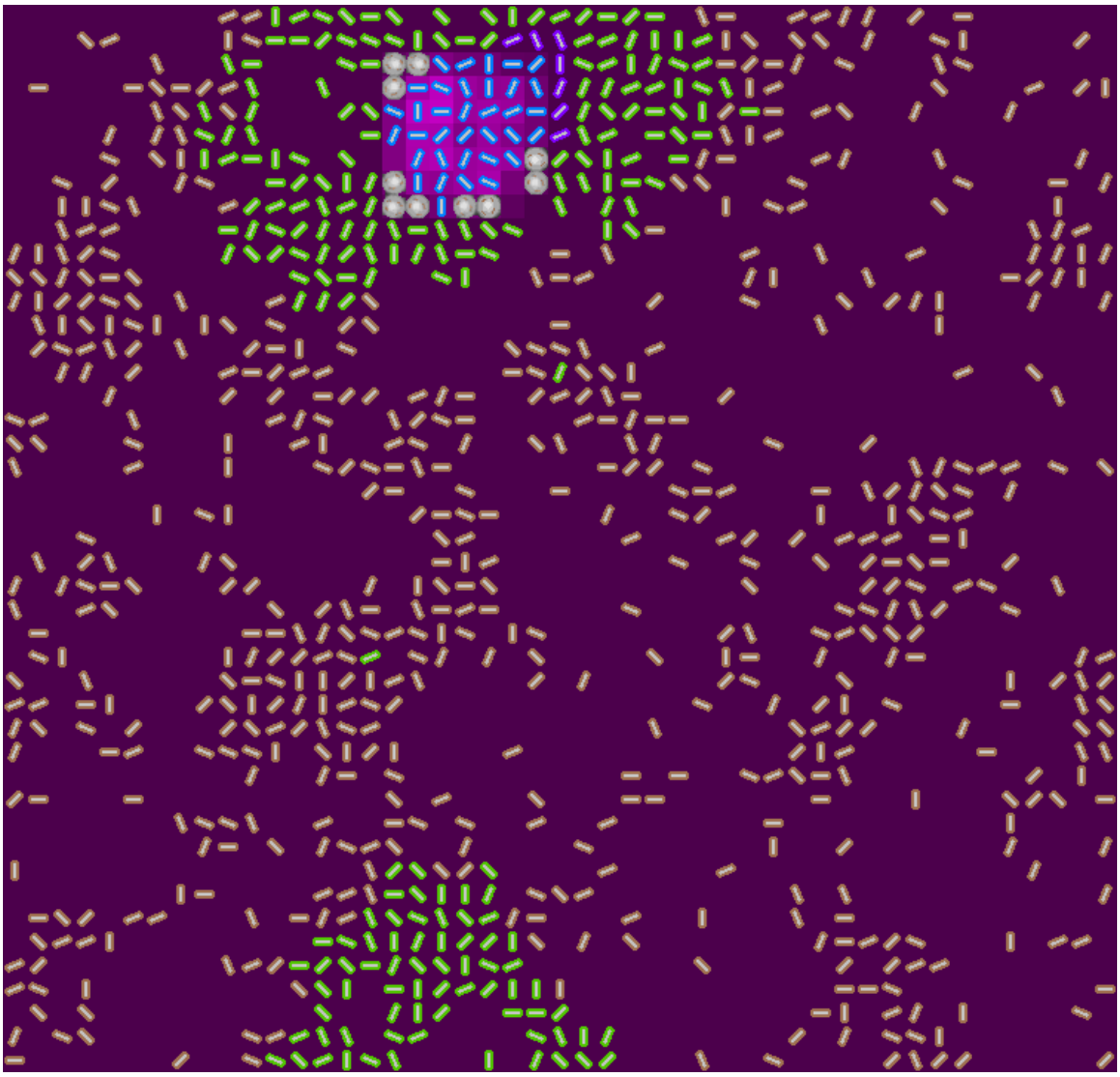


Figure 25: $T = 970$, S field. Spreading wave of S signal.

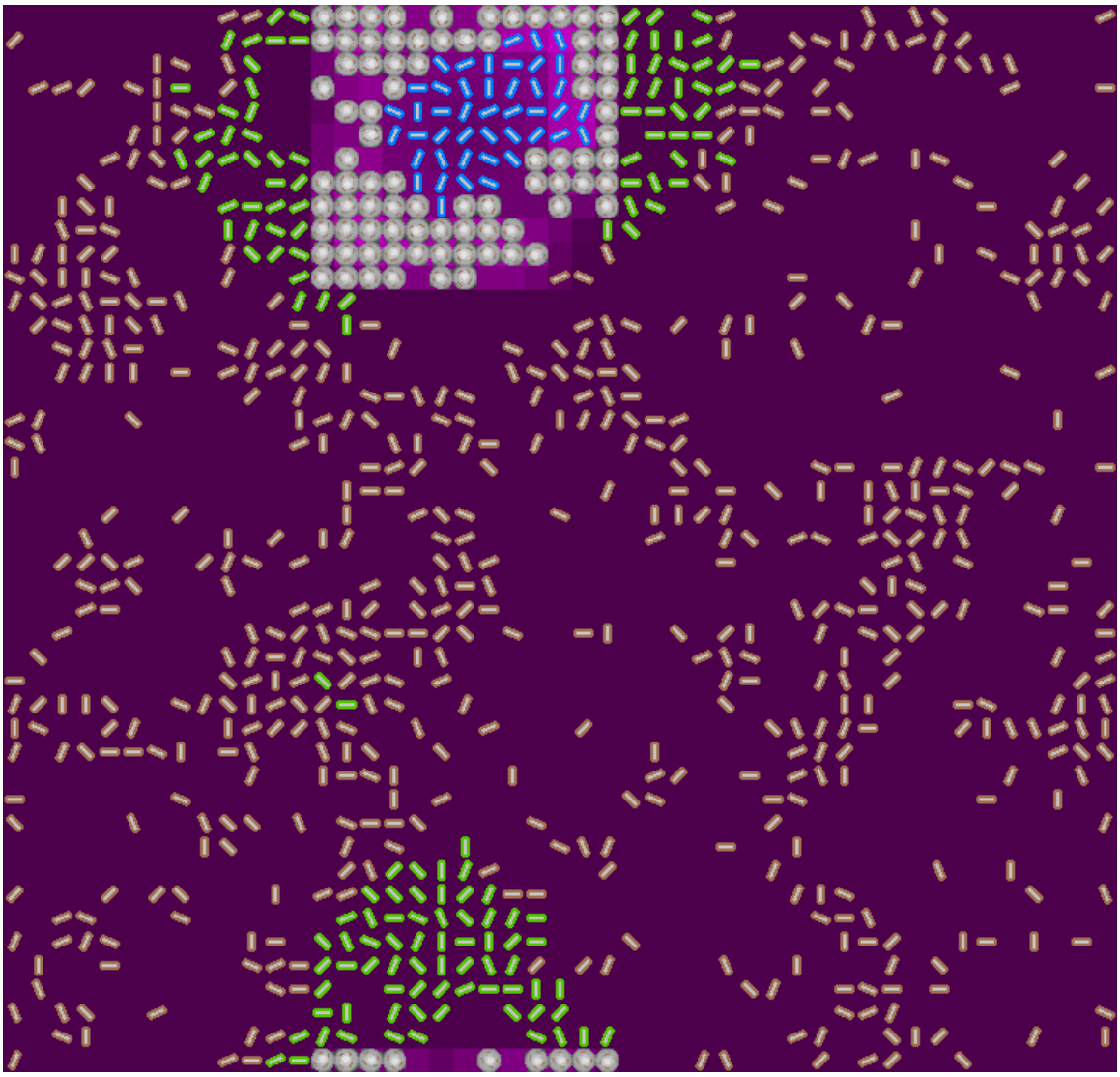


Figure 26: $T = 973$, S field.

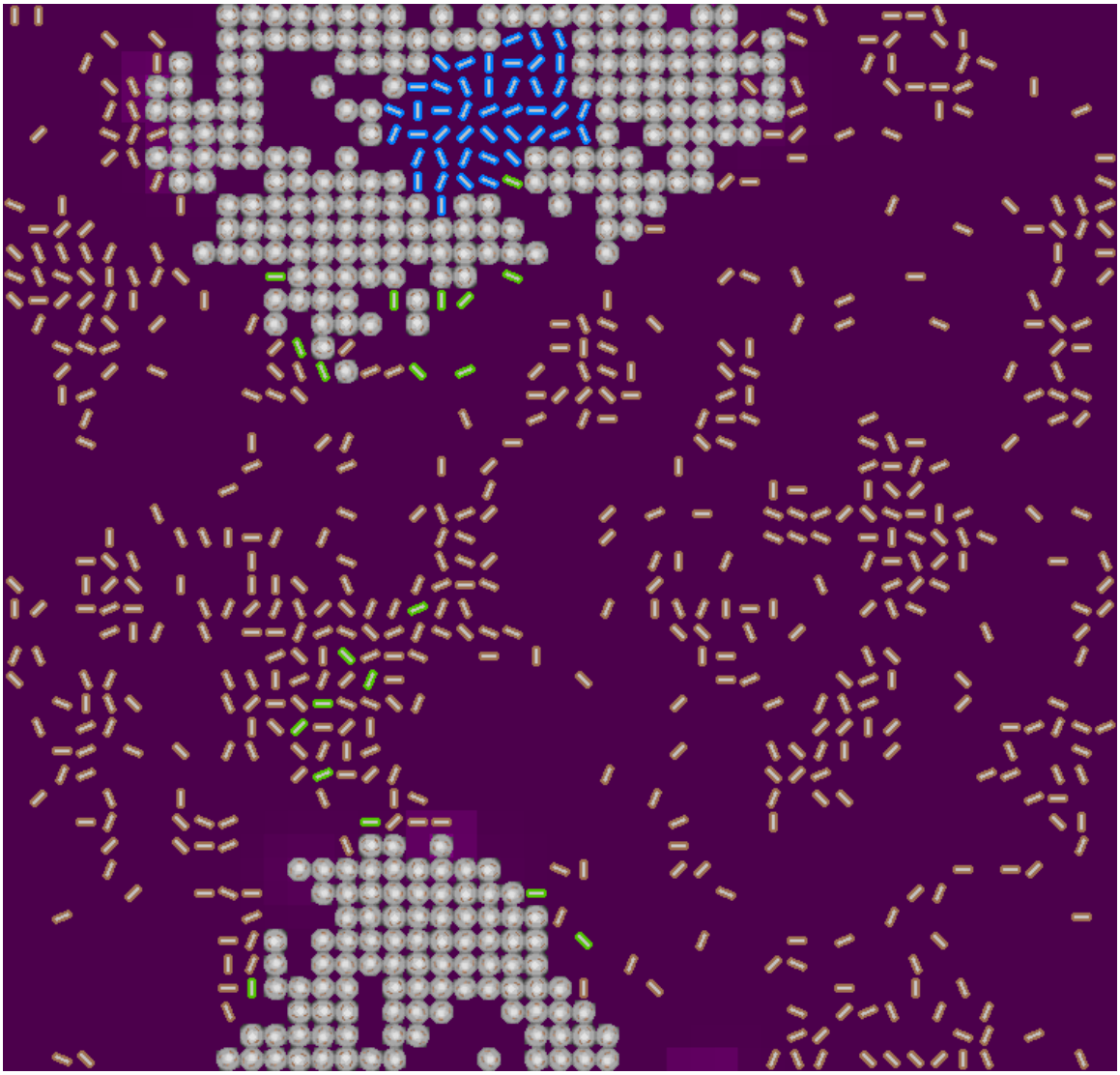


Figure 27: $T = 985$, S field. The spore is complete. Hibernation does not spread further than the aggregating cells (green); the tan cells are still wandering randomly.

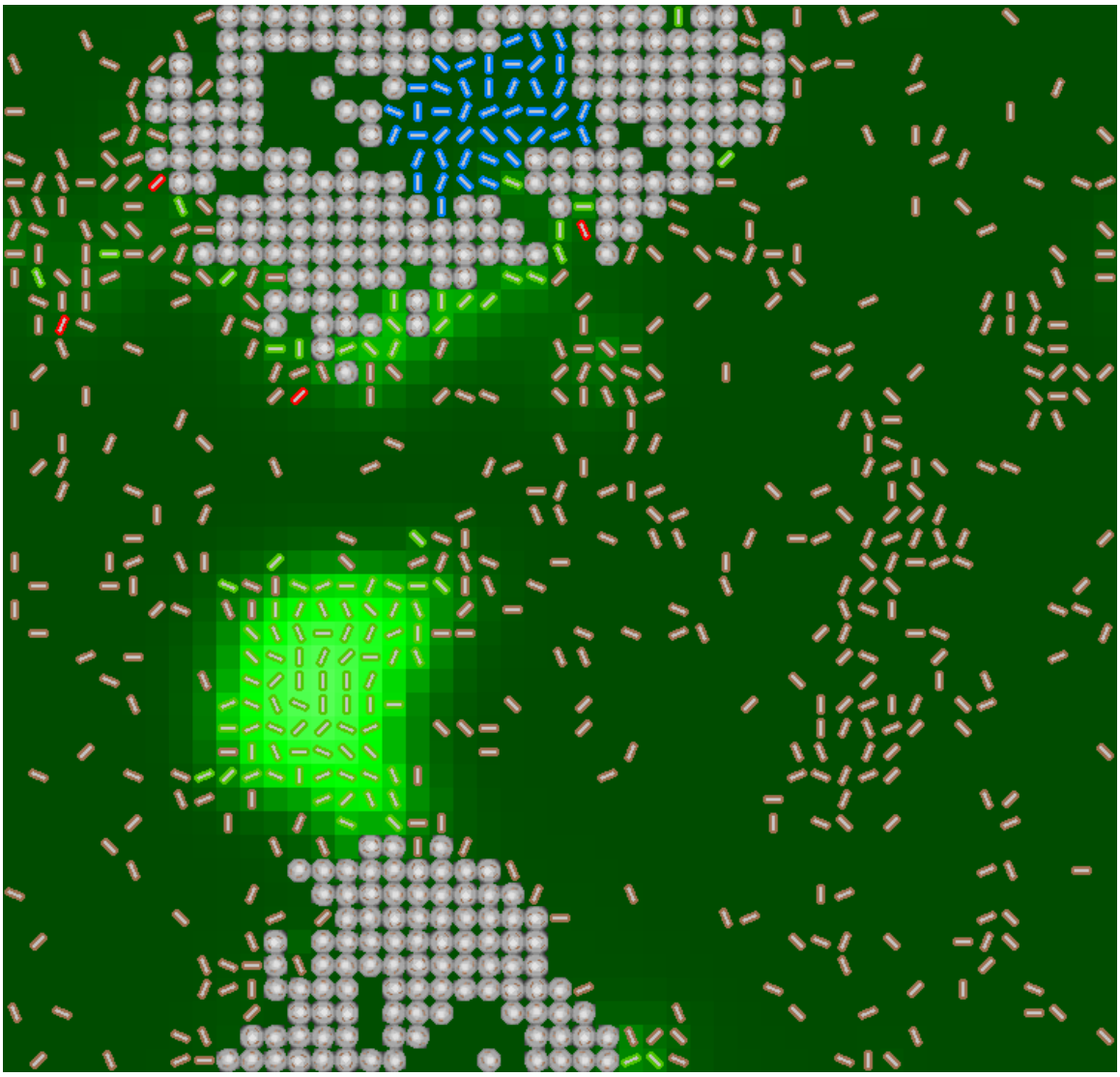


Figure 28: $T = 1010$, A field. A second colony is forming.

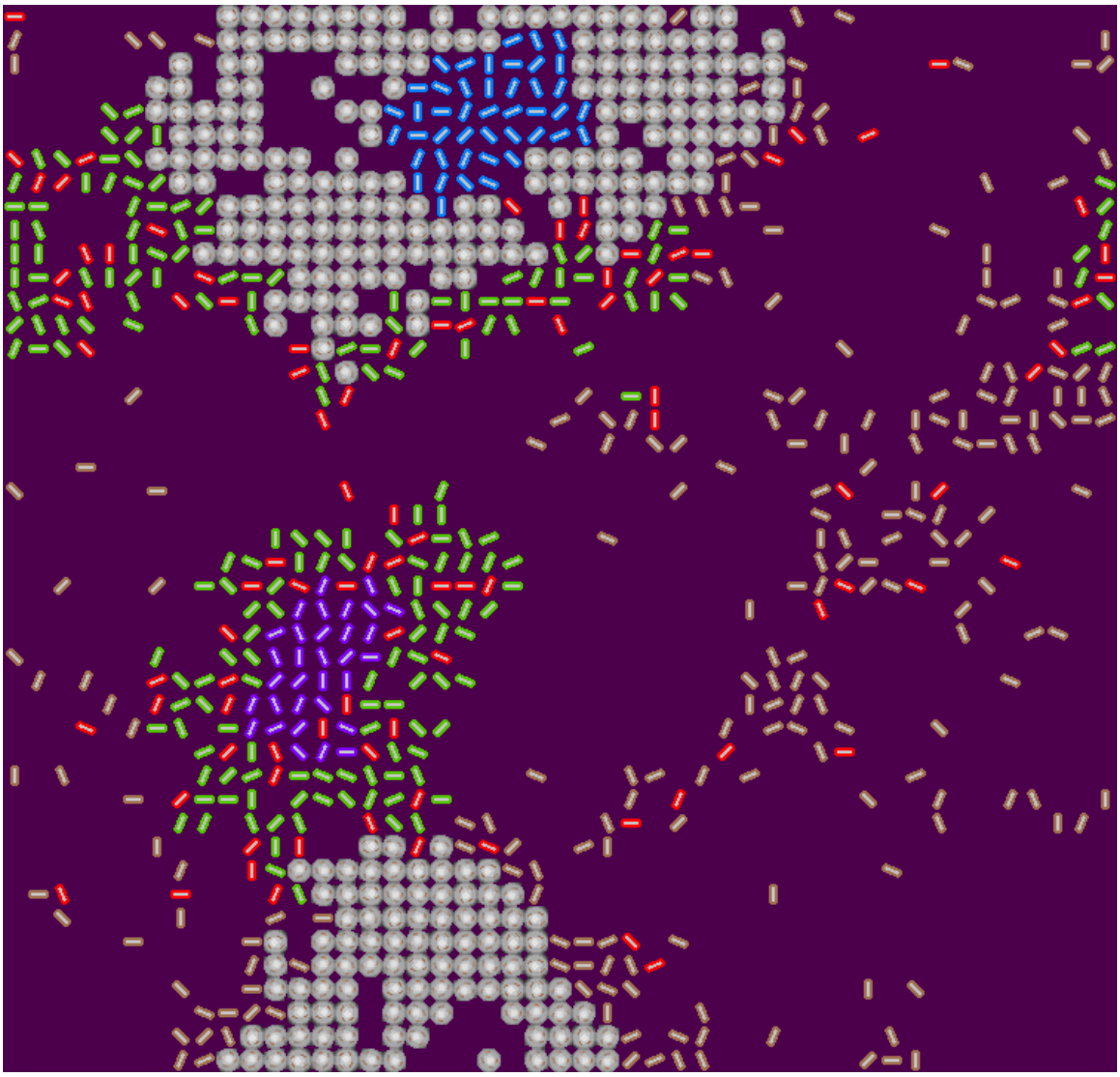


Figure 29: $T = 1054$, S field. The new colony has Interior cells.

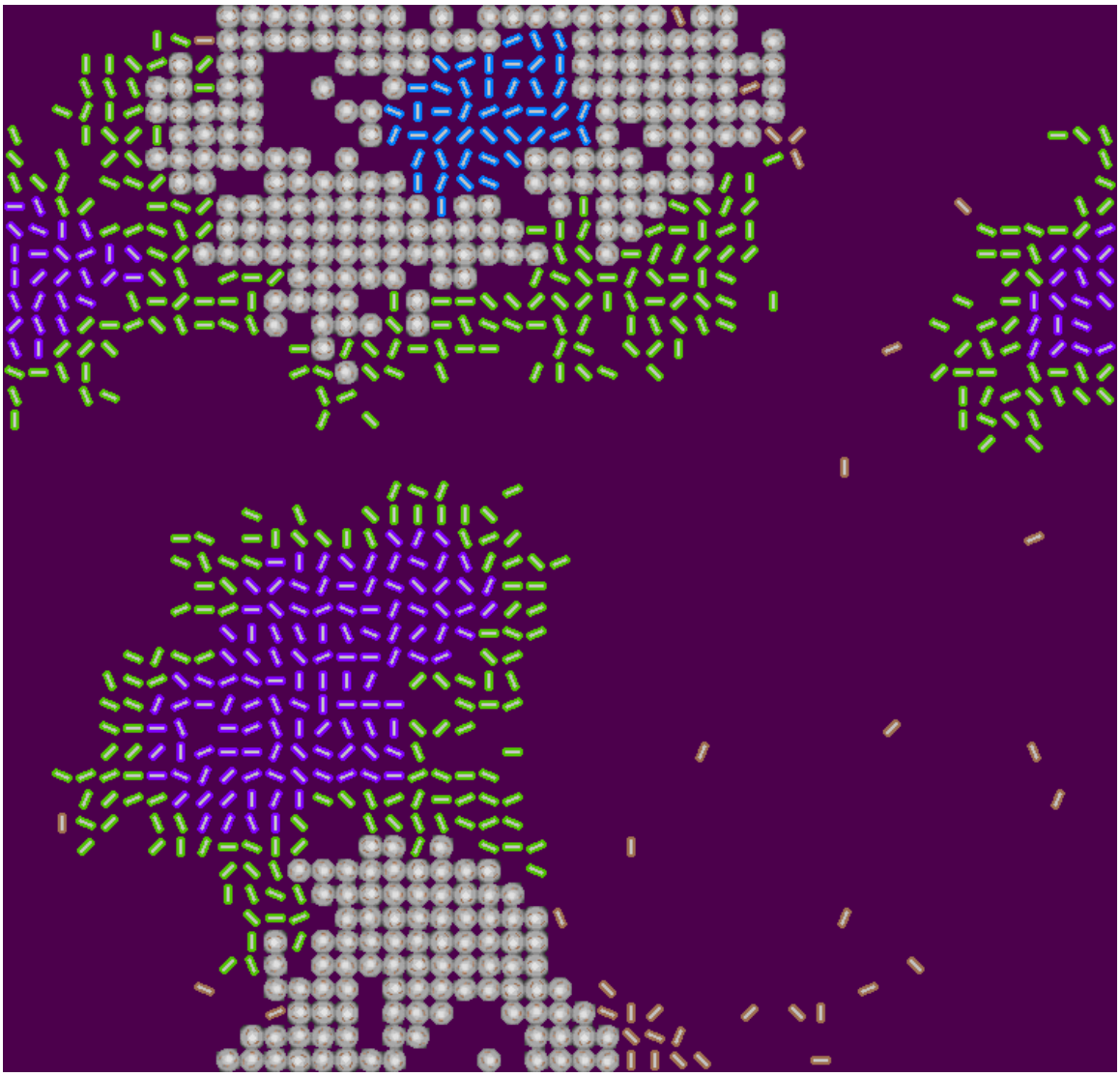


Figure 30: $T = 1155$, S field. A third colony, adjoining the first, has formed and has Interior cells. The secondary colonies are quite large, and very few bacteria are left wandering.

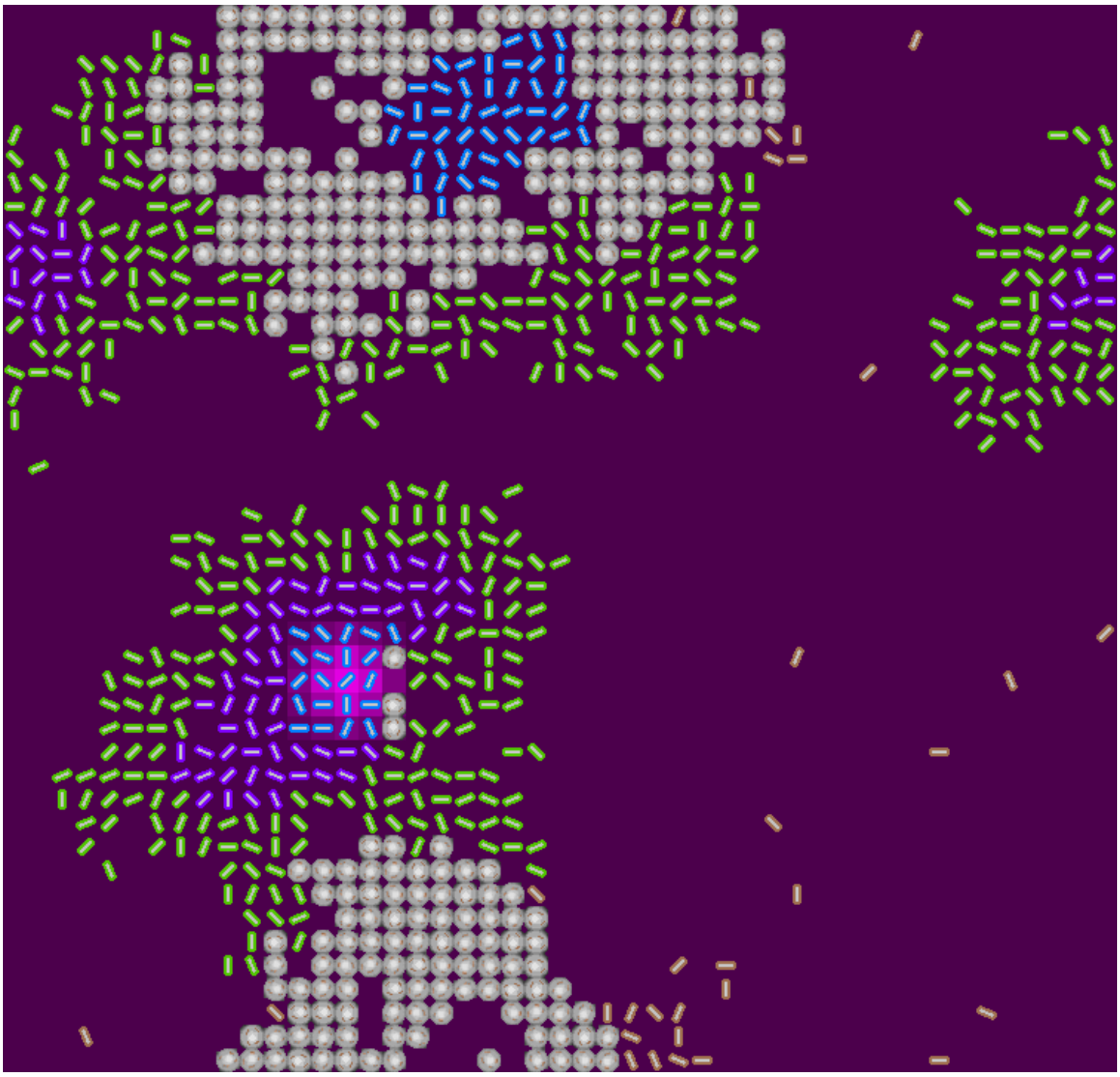


Figure 31: $T = 1161$, S field. The second colony begins to hibernate.

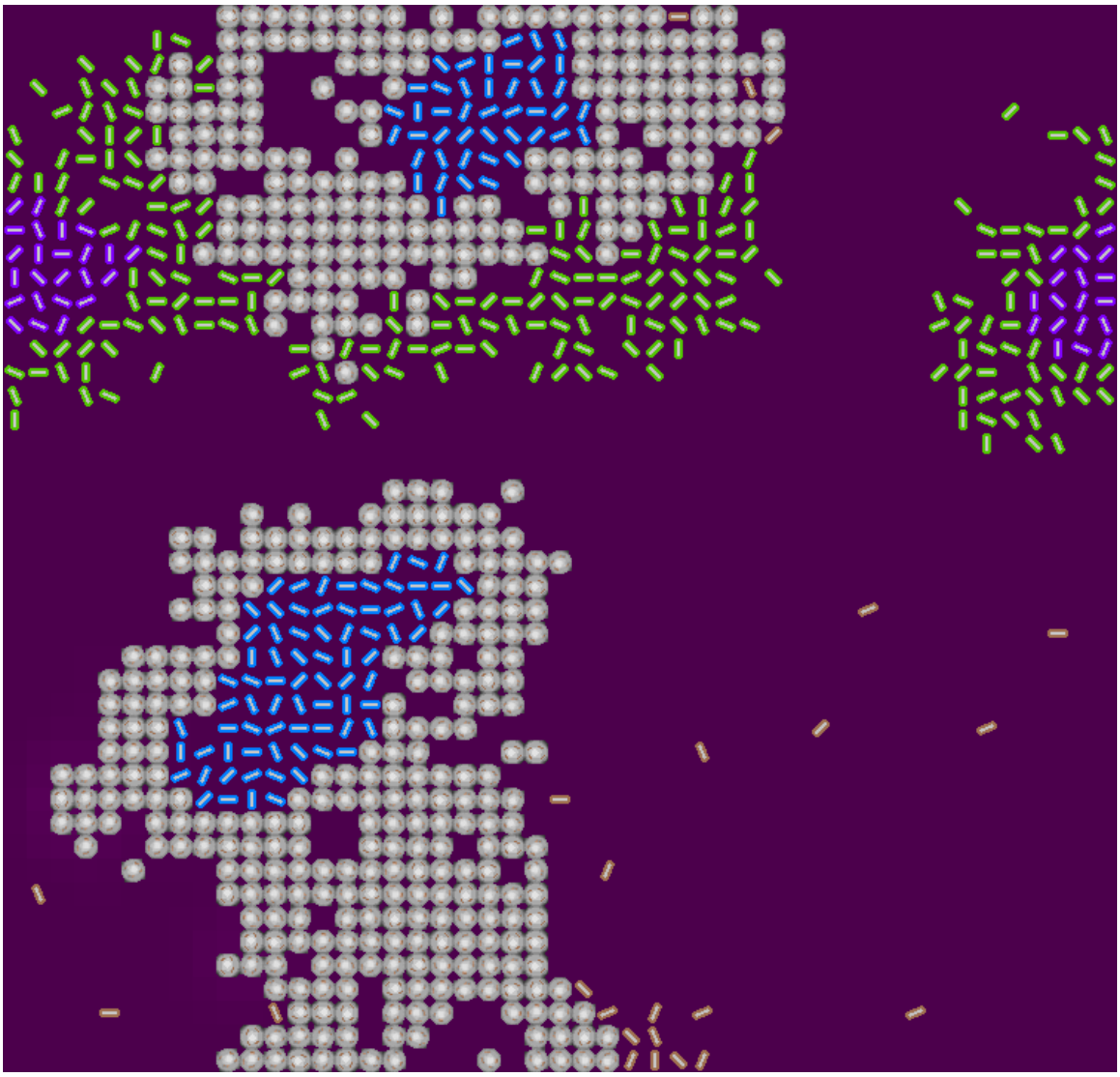


Figure 32: $T = 1173$, S field. A second spore has formed.

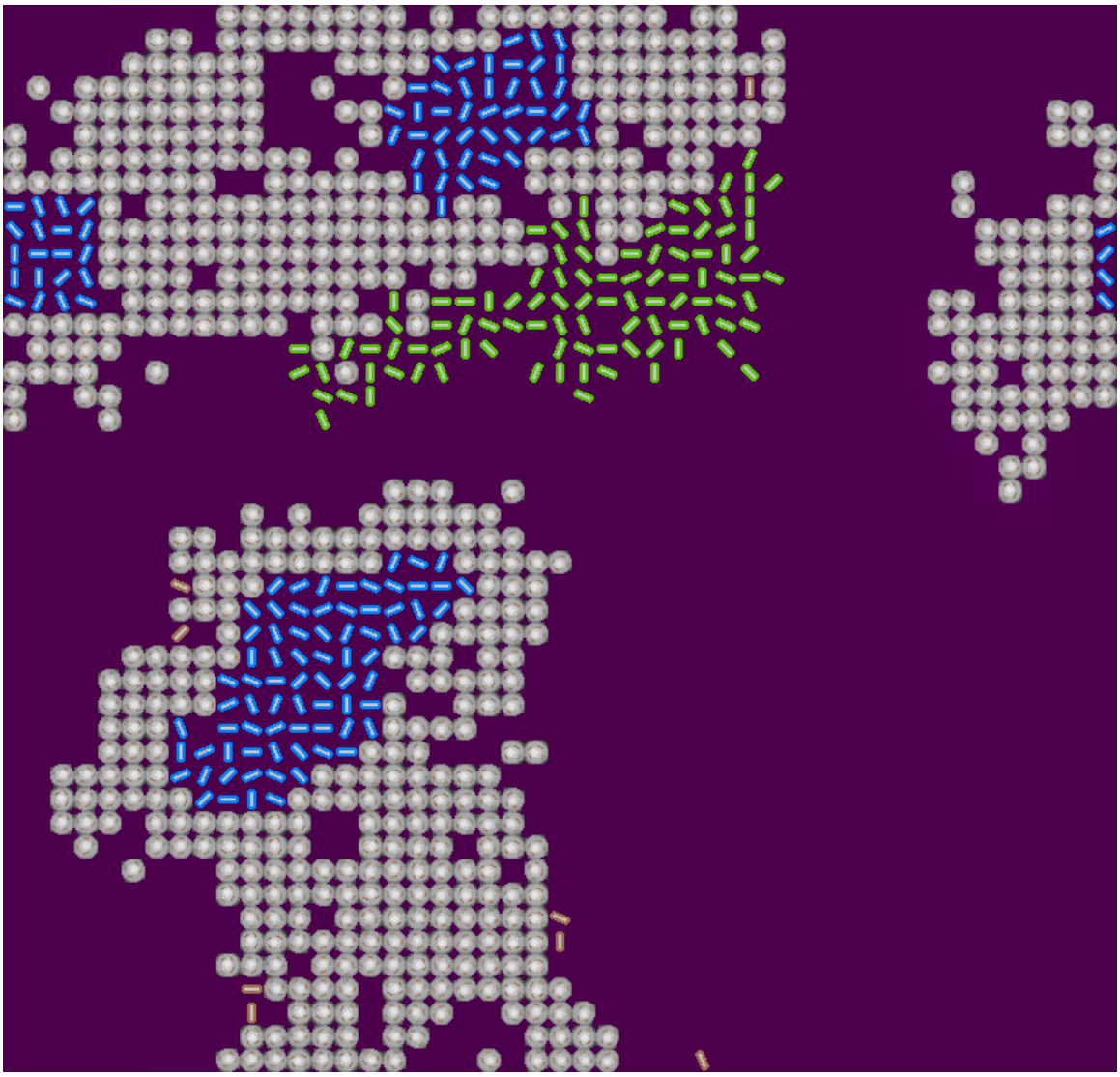


Figure 33: $T = 1317$, S field. A third spore has formed.

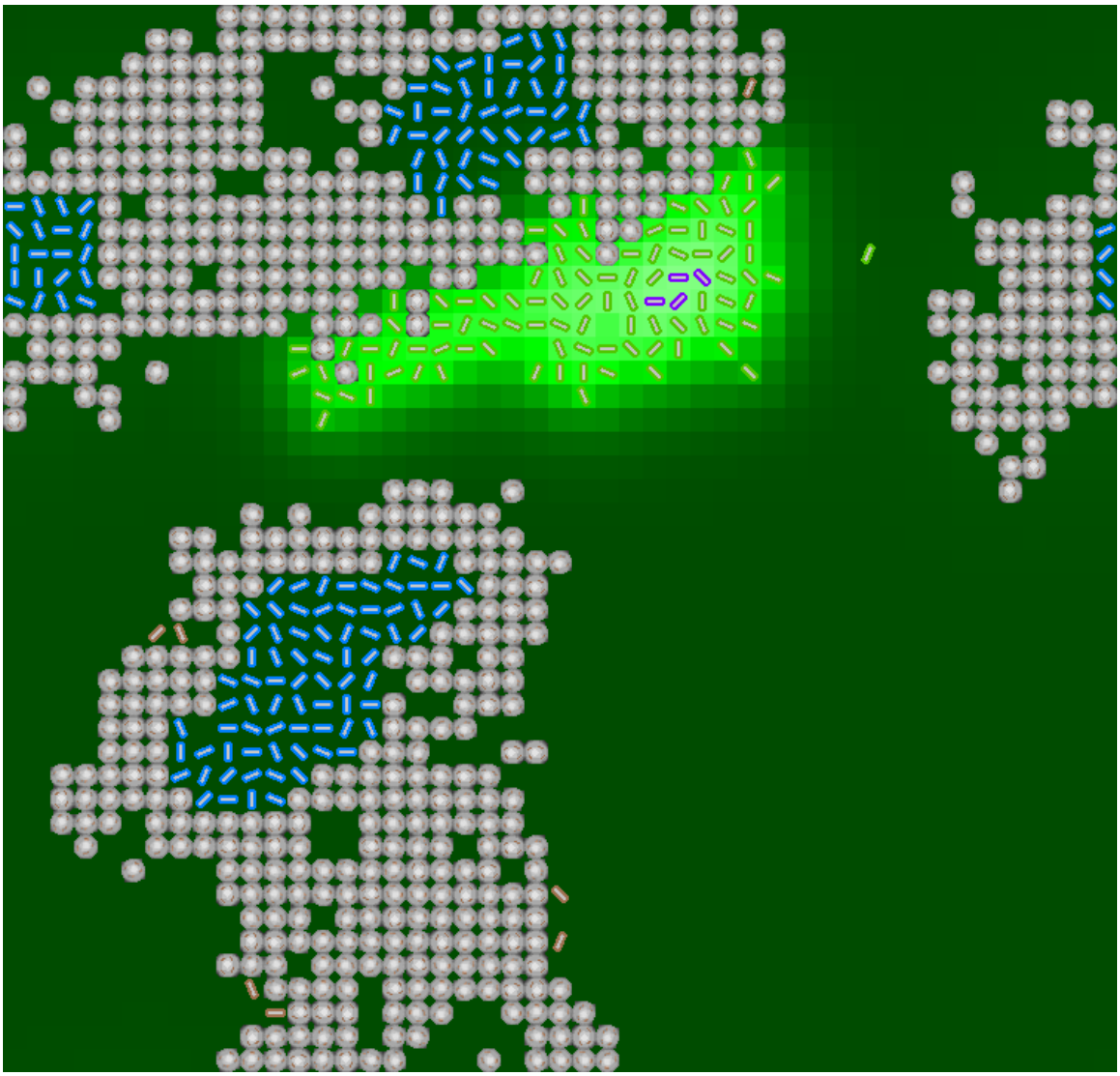


Figure 34: $T = 1337$, A field. A fourth colony has sufficient density to have Interior cells, but not yet the critical mass to hibernate.

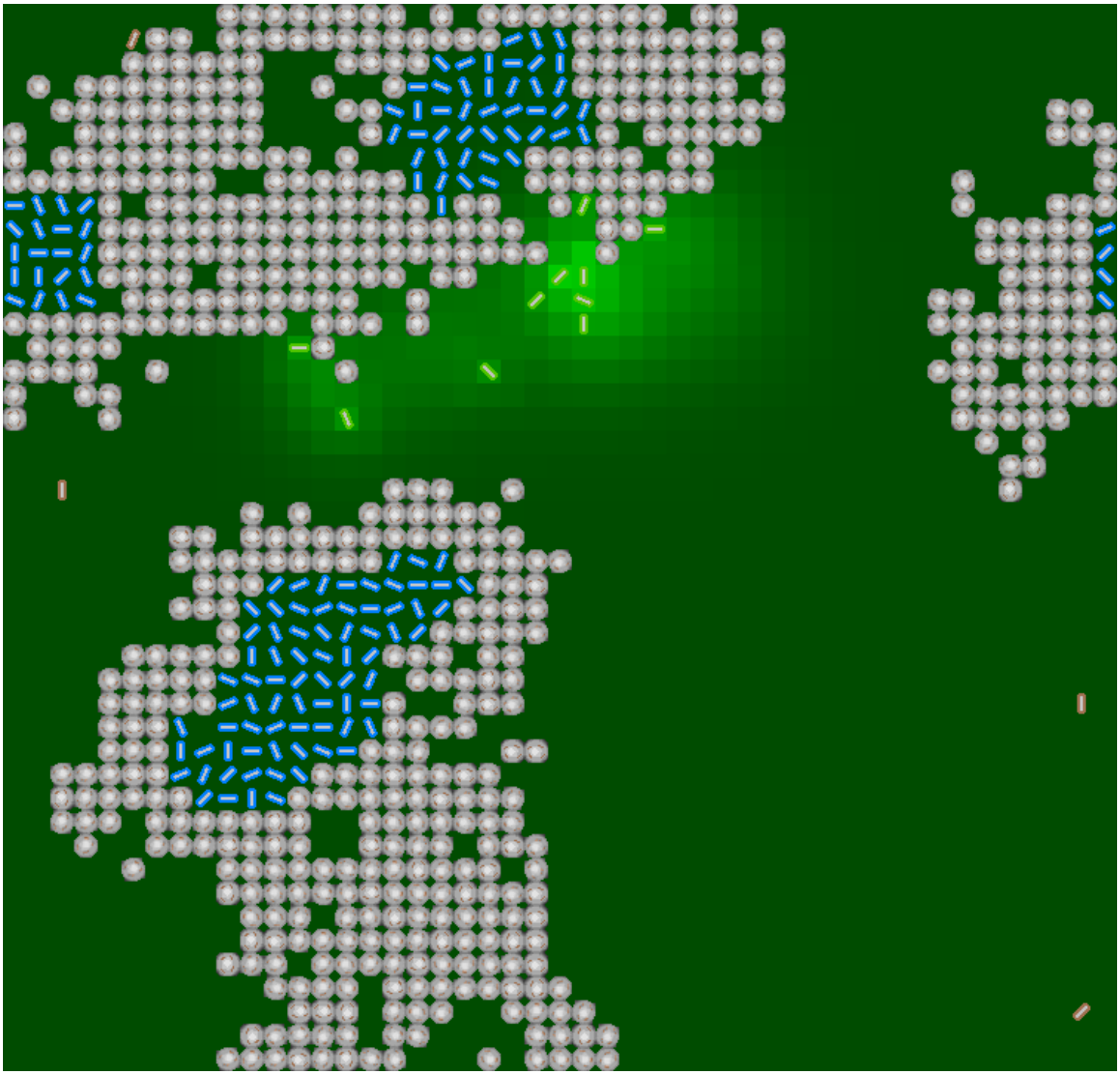


Figure 35: $T = 1406$, A field. Death of remaining, non-dormant bacteria ($1384 < T < 1436$).

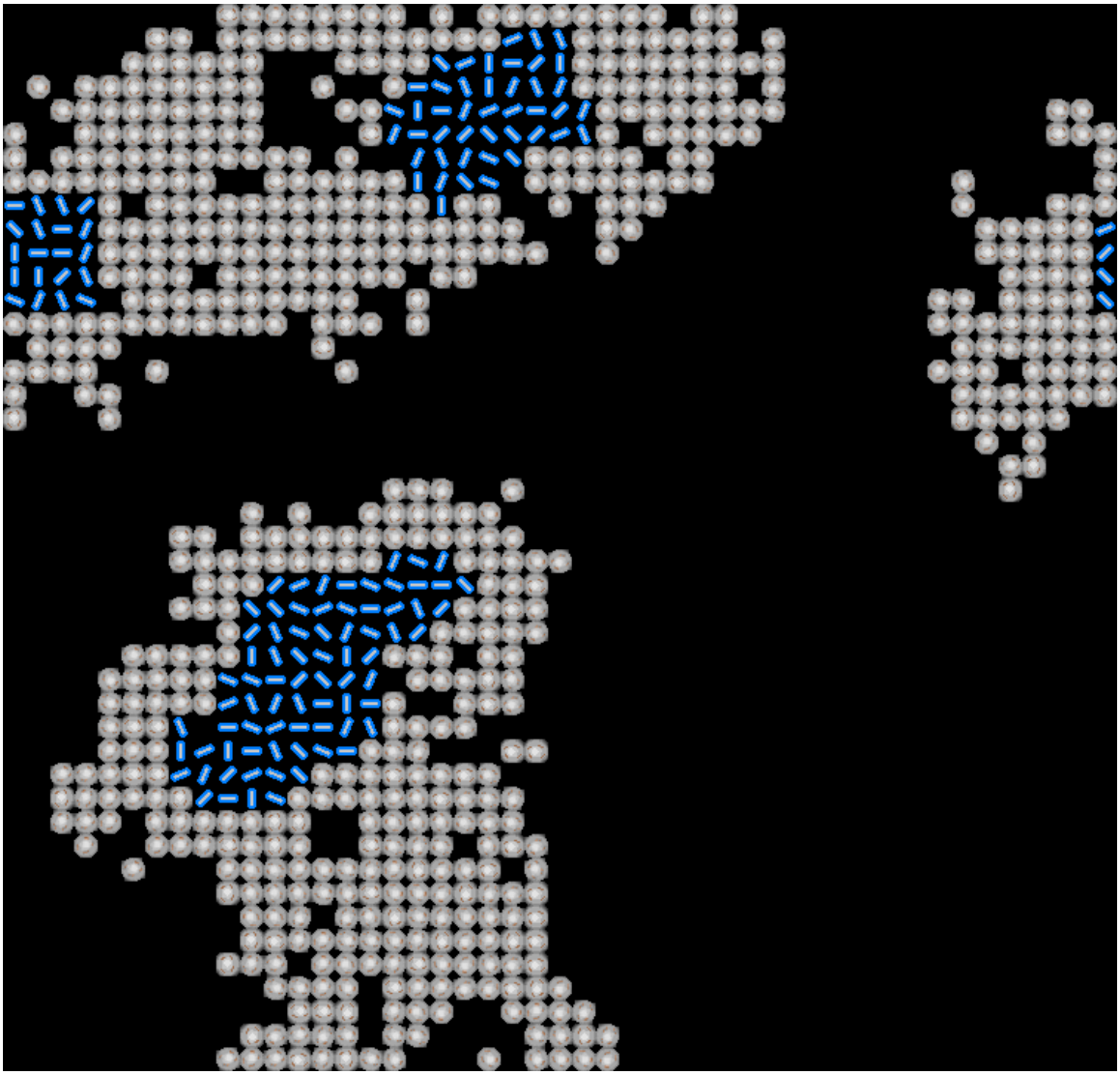


Figure 36: $T = 1434, P = 164$. Three spores protect 164 dormant bacteria until favorable conditions return.

Table 2: Effects of Quorum Threshold (Q_{th})

Q_{th}	P_0	Time of First Spore Formation*	Final	
			Spores	P_{f}
0	547	260	1	60
0	536		0	0
10	514		0	0
20	559		0	0
30	542		0	0
35	540	400	1	33
40	556	268	2	117
50	523	367	1	47
60	521	394	1	44
70	516	314	2	78
80	513	420	2	113
90	525	340	1	174
95	528	360	2	162
100	540	361	1	162
109	556		0	0
111	522		0	0
120	539		0	0
150	515		0	0

* Time of first spore formation is estimated by eye.

---

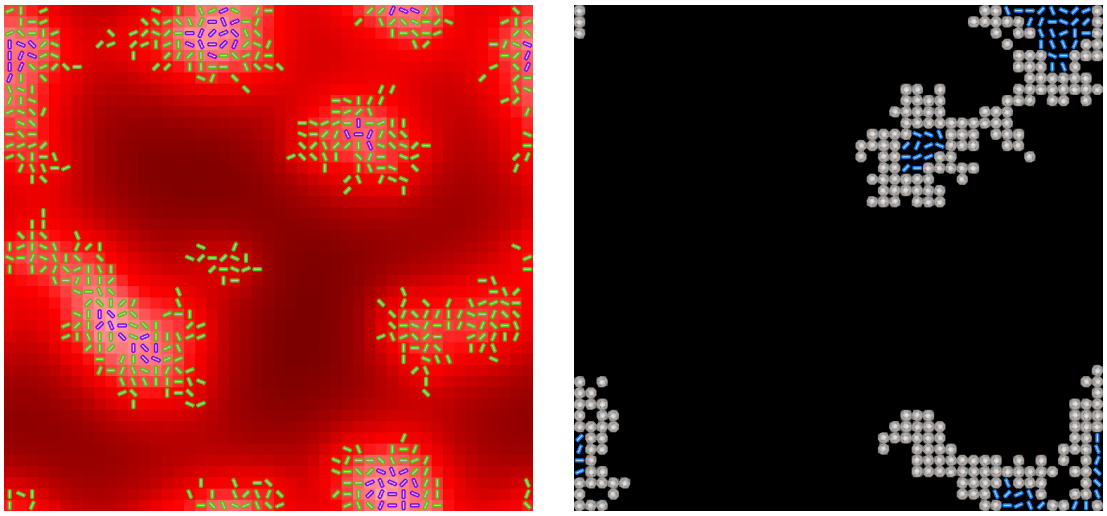
5 Effects of Parameters

5.1 Quorum Threshold (Q_{th})

Table 2 shows a series of experiments in which Q_{th} was systematically varied. This is just one series, and statistically valid conclusions would require many more runs, since there is considerable variability between runs, but it does show the general effect of Q_{th}.

In order to estimate the time to first spore formation, in these experiments the initial population density was set to $p_{\text{P}} = 0.25$, so as to obtain an initial population $P_0 > 500$, and the reproduction rate was set to $p_{\text{R}} = 0$; also the initial ambient temperature was below T_{c}, so distressed behavior began immediately. Except for these and Q_{th}, all other parameters were default (Table 1).

For low values of Q_{th} (30 and below), we generally see small clusters forming, and extinction occurs because none of the clusters reach S_{th} within the lifetime of distressed bacteria (Fig. 37A). However, exceptions occur (e.g., the first row of the



(A) (B)

Figure 37: (A) $Q_{\rm th} = 0, P_0 = 536, T = 290$, D field. With low quorum threshold, bacteria typically aggregate prematurely and do not form clusters of sufficient size to hibernate. The population was insufficiently aggregated and extinction occurred. (B) $Q_{\rm th} = 0, P_0 = 547$. In this case an irregular spore of 60 bacteria formed.

table, $P_0 = 547$). Since at these low $Q_{\rm th}$ values the bacteria aggregate immediately, they tend to form irregularly-shaped spores when they do succeed in hibernating (Fig. 37B).

With higher $Q_{\rm th}$ values, we get fewer clusters, and the larger ones hibernate. We also get larger, better formed spores (Fig. 38). Presumably this is because the bacteria wander randomly for a longer period of time, and thus anneal better. Above $Q_{\rm th} = 100$, extinction occurs, because the bacteria continue wandering with minimal aggregation (Fig. 39).

For moderate values of $Q_{\rm th}$ (35–80), we seem to get 1 or 2 spores, each of size 30–60. For larger $Q_{\rm th}$ (but < 100), we get one large spore about three times this size.

We may estimate $s_{\rm D}$, the rate of D secretion for non-aggregating distressed bacteria as follows. In Sec. 3.2 we explained that in each time step a non-aggregating distressed bacterium has a probability $p_{\rm D}$ of emitting $K_{\rm D}$ quanta of D and then becoming refractory for the next $T_{\rm r}$ timesteps. Therefore, in $T_{\rm r} + 1$ time steps the probability of emission is

$$p_{\rm D} + (1 - p_{\rm D})p_{\rm D} + \cdots + (1 - p_{\rm D})^{T_{\rm r}} p_{\rm D} = 1 - (1 - p_{\rm D})^{T_{\rm r}+1}.$$

Therefore the average rate of emissions is

$$\hat{p}_{\rm D} = \frac{1 - (1 - p_{\rm D})^{T_{\rm r}+1}}{T_{\rm r} + 1}. \tag{19}$$

(A) (B)

Figure 38: (A) $Q_{\text{th}} = 90, P_0 = 525$. (B) $Q_{\text{th}} = 100, P_0 = 540$. Both figures show well-formed spores resulting from high Q_{th} values.

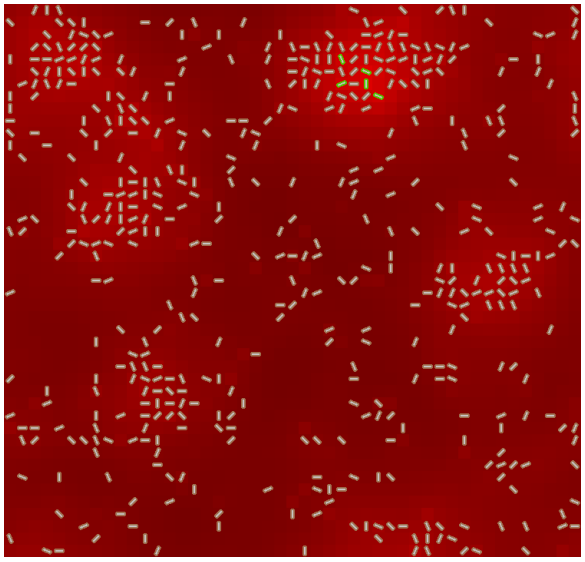


Figure 39: $Q_{\text{th}} = 111, T = 479$, D field. The figure shows the population at the beginning of extinction. At this high Q_{th} value, the bacteria remain wandering (tan color) and do not aggregate into clusters.

Table 3: Effects of Distressed Population Density (q_{d})

P_{d}	Distressed Pop. Density (q_{d})	Time of First Spore Formation*	Final	
			Spores	P_{f}
209	0.10		0	0
243	0.11		0	0
312	0.15		0	0
409	0.19	311	1	135
413	0.20	325	1	136
464	0.22	396	3	118
470	0.22		0	0
524	0.25	260	2	186
527	0.25		0	0
529	0.25	300	1	104
613	0.29	325	2	129
638	0.30	260	4	131
860	0.41	217	6†	234
1081	0.51	214	9†	414
1265	0.60	158	8†	486

* Time of first spore formation is estimated by eye.
† Since spores are connected, this is a count of Interior regions.

For $p_{\mathrm{D}} = 0.2$, $T_{\mathrm{r}} = 5$, and $K_{\mathrm{D}} = 20$ (Table 1), we expect $\hat{p}_{\mathrm{D}} = 0.123$ emissions per time step, and $s_{\mathrm{D}} = 0.123 K_{\mathrm{D}} = 2.46$ quanta of D per time step. For a uniform population density of Q_0, the asymptotic D concentration is

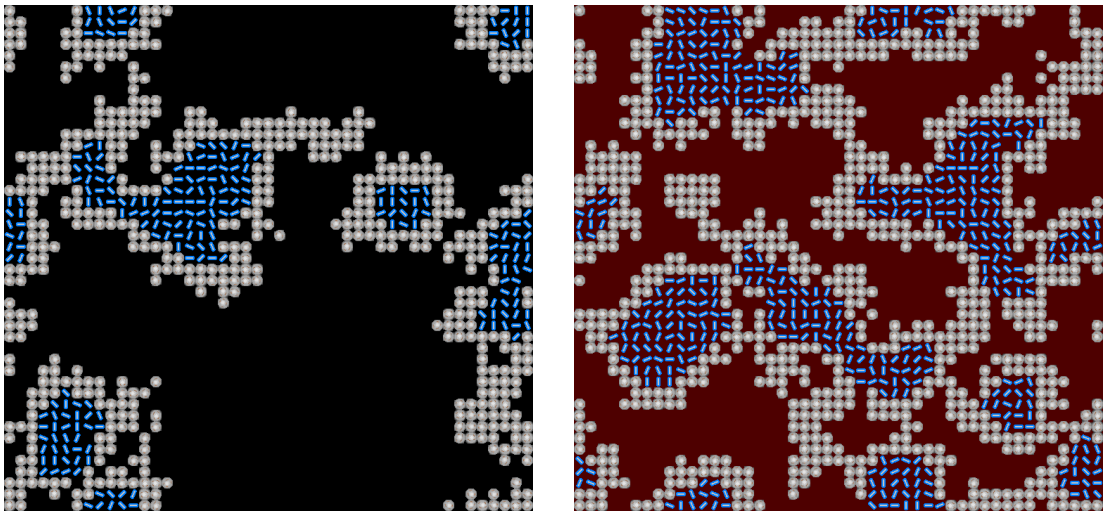
$$D^* = \frac{1}{1 - C_{\mathrm{D}}} s_{\mathrm{D}} Q_0 \approx 61.5$$

quanta of D for $C_{\mathrm{D}} = 0.99$ and $Q_0 = 0.25$. If $Q_{\mathrm{th}} \gg D^*$, it is unlikely the quorum threshold will be reached, but it can occur in regions of locally high population density, which will then, through positive feedback, push the entire population above the quorum threshold.

The time to first hibernation appears to be independent of Q_{th}, but in these experiments was always after $T = 260$, generally after $T = 340$.

5.2 Distressed Population Density (q_{d})

Table 3 displays the results of a series of experiments investigating the effects of the population density of distressed bacteria. They were obtained by initializing the population randomly with a certain density p_{P}, setting the reproduction rate to 0,



(A) (B)

Figure 40: (A) $P_{\mathrm{d}} = 860, q_{\mathrm{d}} = 0.41$. Spores are close together and may be interconnected. (B) $P_{\mathrm{d}} = 1265, q_{\mathrm{d}} = 0.60$, D field. Spores, including Interiors, are interconnected and cannot be easily separated. Both figures illustrate relatively high population densities.

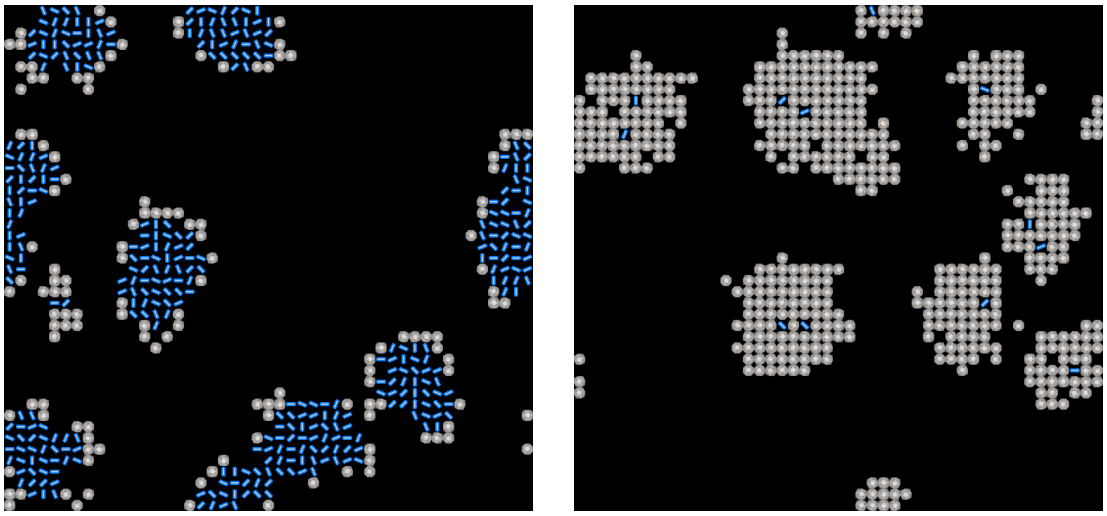
and then initially stressing the population, so that the distressed population was equal to the initial population ($P_{\mathrm{d}} = P_0$). Average distressed population density was calculated as a fraction of the maximum population, $q_{\mathrm{d}} = P_{\mathrm{d}}/P_{\max}$. The simulations were then run until all bacteria were either dormant or dead. Except $Q_{\mathrm{th}} = 60$, other parameters were default (Table 1).

As in the previous experiments, we did not make sufficient runs to draw statistically valid conclusions, but some informal observations can be made. For low population densities (say, $q_{\mathrm{d}} \leq 0.15$) little or no aggregation takes place, and therefore extinction eventually occurs. As we did in Sec. 5.1, we may calculate the asymptotic D concentration for a uniformly distributed distressed population:

$$D^* = \frac{1}{1 - C_{\mathrm{D}}} s_{\mathrm{D}} Q_0 \approx 246 q_{\mathrm{d}}$$

quanta of D for the default parameters. We expect no aggregation if this is much less than the quorum threshold, $246 q_{\mathrm{d}} \ll Q_{\mathrm{th}}$, that is, $q_{\mathrm{d}} \ll 0.24$ (for $Q_{\mathrm{th}} = 60$). However, this is pessimistic, since random fluctuations in the population density may exceed the quorum threshold and allow aggregation to begin.

For moderate densities ($0.15 < q_{\mathrm{d}} < 0.30$) 1 to 3 spores usually form. In the exceptional cases when extinction occurs (such as the $P_{\mathrm{d}} = 470, 527$ runs in Table 3), we observed 5 or 6 clusters forming; extinction occurred because none of them reached critical mass for hibernation. Both have population densities near the critical value $Q_{\mathrm{th}}/246 \approx 0.24$.



(A) (B)

Figure 41: (A) $B_{\text{th}} = 50$, $P_{\text{f}} = 364$. The spores have little or no cyst covering. (At lower B_{th} values there may be no cyst at all, but many more bacteria are dormant — sometimes the entire initial population.) (B) $B_{\text{th}} = 90$, $P_{\text{f}} = 12$. Virtually all the bacteria have formed cyst cells; very few are dormant in the interior. (In both of these cases $Q_{\text{th}} = 60$, but other parameters are as in Table 1.)

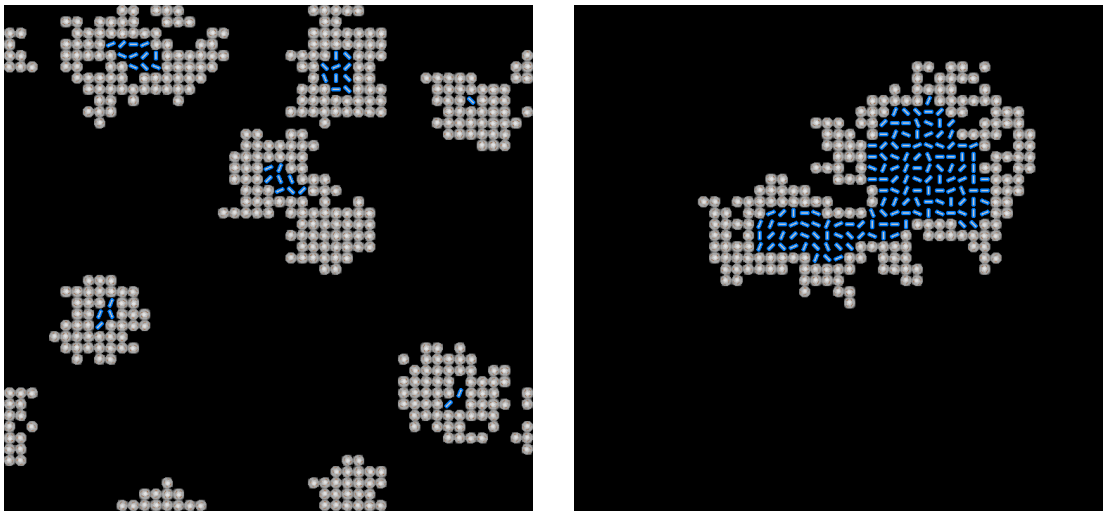
At relatively high densities ($q_{\text{d}} > 0.3$), the population reaches critical mass quickly. Since aggregation has not proceeded very far, the clusters are loose and interconnected; therefore, when the first spore forms, the hibernation signal tends to spread to all the other clusters, causing them to hibernate too. As a result, we may get a large interconnected mass of cysts with multiple interior regions (Fig. 40). In these cases (marked "†" in Table 3) we have counted Interior regions, since the spores are not distinctly separated.

Although the surviving dormant population seems to be independent of initial density for moderate values ($0.20 \leq q_{\text{d}} \leq 0.30$), it is distinctly higher for large values ($q_{\text{d}} \geq 0.40$).

In the following subsections we present some additional observations (not statistically tested). (The aggregation threshold A_{th} didn't seem to have much effect.)

5.3 Boundary Threshold (B_{th})

The diameter of the interiors of spores varies inversely with B_{th}, and boundary thickness seems to vary directly. This makes sense, since a lower B_{th} causes more A-values to be classified as Interior, versus Boundary. There seems to be a tendency with low B_{th} values to get more aggregating clusters with Interior regions, which makes sense, since it is easier for an aggregator to be in the Interior state. See Fig. 41 for typical spores resulting from small and large B_{th} values.



(A) (B)

Figure 42: (A) $C_{\mathrm{D}} = 10$. Eight spores. (B) $C_{\mathrm{D}} = 90$. One large, sprawling spore. (In both of these cases $Q_{\mathrm{th}} = 60$, but other parameters are as in Table 1.)

5.4 D Diffusion Conduction Coefficient (C_{D})

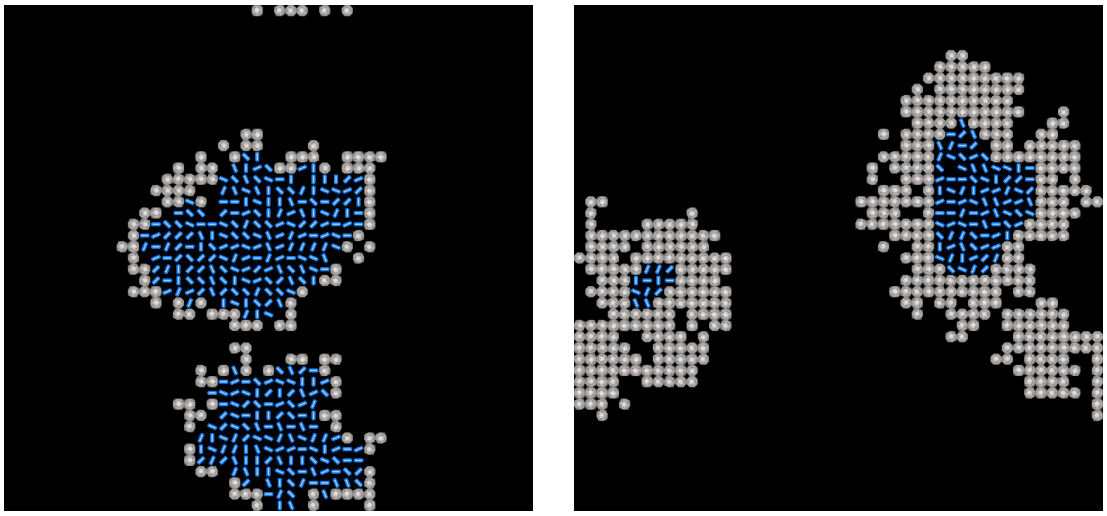
Higher C_{D} leads to larger spores, and thus a smaller number of spores; it also leads to larger interiors, but seems to have little effect on boundary thickness. This makes sense, since more rapid diffusion causes spatial averaging (Gaussian smoothing) over a larger area (Sec. 2.1), and so the interaction lengths are increased. See Fig. 42 for typical spores resulting from small and large C_{D} values.

Eq. 17 (p. 5) shows that the area of the spores should vary directly with the ratio of C_{D} values, and Eq. 16 shows their diameters vary with the square root of this ratio. Therefore we would expect the spores resulting from $C_{\mathrm{D}} = 90$ should be about three times the diameter of those from $C_{\mathrm{D}} = 10$, and this appears to be the case in Fig. 42.

5.5 A Diffusion Conduction Coefficient (C_{A})

Higher C_{A} seems to lead to smaller interiors and somewhat thicker boundaries. This makes sense, since the variance of the Gaussian smoothing kernel is directly proportional to the conduction coefficient (Sec. 2.1). A is secreted only by bacteria in a clumped state, so the source field is exactly proportional to the clumped population. With a higher C_{A} there is more smoothing, and therefore the Interior region is constricted. See Fig. 43 for typical colonies resulting from small and large C_{A} values.

We observed interior diameters of 4 to 20 grid units; if these were real bacteria, 1–10 microns in size, then the interiors might average 60μ. Overall (including the cysts), the simulated spores have diameters of 10–25 grid units, and so corresponding real spores might average 85μ in diameter.



(A) (B)

Figure 43: (A) $C_{\mathrm{A}} = 10$, $P_{\mathrm{f}} = 313$. Thin or incomplete protective cysts. (B) $C_{\mathrm{A}} = 90$, $P_{\mathrm{f}} = 100$. Thick protective cysts.

6 Conclusions

We have demonstrated one means by which simple, bacteria-like autonomous agents can self-organize into protective, spore-like colonies when subjected to unfavorable ambient conditions. The self-organization is governed by the independent diffusion of three chemicals: (1) D, which is secreted by distressed bacteria and guides their aggregation into clusters, (2) A, which is secreted by clumped bacteria and enables their differentiation into dormant interior cells and protective cyst cells, and (3) S, which by its rapid diffusion synchronizes the formation of the spore. When favorable conditions return, the dormant bacteria reanimate, escape from their spore, and resume normal behavior; the population can cycle between the dormant and active states any number of times. A series of experiments demonstrated that the effects of the simulation parameters can be understood in terms of Gaussian smoothing, effected by diffusion, and sharpening, effected by chemotaxis.

References

[1] Camazine, S., Deneubourg, J.-L., Franks, N. R., Sneyd, J., Theraulaz, G., & Bonabeau, E. (2001). *Self-Organization in Biological Systems*, Princeton: Princeton University Press.

[2] Dunny, G. M., & Winans, S. C., eds. (1999). *Cell-Cell Signaling in Bacteria*, Washington, D.C.: ASM Press.

[3] Poindexter, J. S., & Leadbetter, E. R., eds. (1989). *Bacteria in Nature, Vol. 3: Structure, Physiology, and Genetic Adaptability*, New York & London: Plenum Press.

[4] Singleton, P. (1999). *Bacteria in Biology, Biotechnology and Medicine*, fifth ed. Chichester: Wiley.