Slime-U-Lator (slime mold simulation)
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Students' Protocol

A Quick Background:
Slime mold cells (*Dictyostelium discoideum*) are eukaryotes that live in the soil. Much of their life is spent as solitary cells, eating bacteria. When food sources get low, however, the cells come together, or aggregate, into a mound of cells. The gathered population of cells forms a slug that is phototactic and thermotactic. After the slug travels for a while and reaches the soil surface, 25% of the cells form a cellulose stalk that lifts the remaining cells. The remaining cells then produce spores that germinate later when food may be more abundant. Slime-U-Lator is a mathematical model Excel file that helps students understand the emergent property of slime mold aggregation.

Aggregation:
In order to understand what is happening in the Slime-U-Lator, you must know what happens to real cells during slime mold aggregation. When a cell becomes starved it begins to emit a chemical called cyclic adenosine monophosphate (cAMP) which signals to the other cells the lack of food. The cAMP emanates from the cell like waves of water radiating out from a stone thrown into a pond. When surrounding cells are hit by a cAMP wave, they move towards the higher concentration of cAMP for a minute or so and then release a wave of cAMP on their own to propagate the cAMP wave which in turn attracts more cells. This series of cAMP secretion and cell movement repeats about every 6 minutes under laboratory conditions (Kessin, 2010). However, after the cells move, they become unresponsive to cAMP for a period of time (the refractory period). During the refractory period, starved cells cannot respond to cAMP which prevents the cells from following the cAMP wave away from the emitter cell after the wave has passed them. After the refractory period, cells become responsive to cAMP again and are ready to respond to another cAMP wave. The periodic process of moving followed by resting continues until each cell reaches the aggregated population of cells.

How the program represents the biological action:

Slime-U-Lator is a dynamic and interactive simulation that allows both students and teachers to mathematically model and understand more fully the emergent property of slug formation as a consequence of the interaction between cAMP and Dictyostelium cells. A single emitting cell is in the center of the viewing field (red box), and users can manipulate parameters to alter the wave of emitted cAMP and the surrounding cells' response to the cAMP wave. The red cell in the center will emit cAMP waves as defined by the user. The blue boxes represent Dictyostelium cells which are able to respond to a cAMP wave. Yellow boxes represent moving cells that have already perceived the cAMP wave and black boxes are in their refractory period. Users can alter three parameters:

1) the percentage of the viewing field that contains cells,
2) the time between each cAMP wave,
3) and the duration of the refractory period.

By experimenting with various numbers for each of the three parameters in Slime-U-Lator, users can determine for themselves the rules used by real cells move towards the central cell instead of away from it.

In the Excel file, there is a pair of tandem viewing grids showing two different aspects of the same physical space (Figure 1). The grid on the left shows the Dictyostelium cells with the emitting cell (red) in the center. The right field shows just the cAMP wave. These two grids are interconnected and work with each other. When a cAMP wave starts in the middle of the cAMP grid on the right, and passes over a cell in the left grid, the blue cell changes to yellow and the cell moves if it is receptive to cAMP (*i.e.* not refractory). The cAMP wave has a width of 3 units which allows the wave to incorporate a concentration gradient with three distinct levels of cAMP—low (1), high (3), and medium (2). Thus, depending on the cAMP concentration surrounding a cell that is responsive to cAMP, the cell can move towards the center or away from the center.
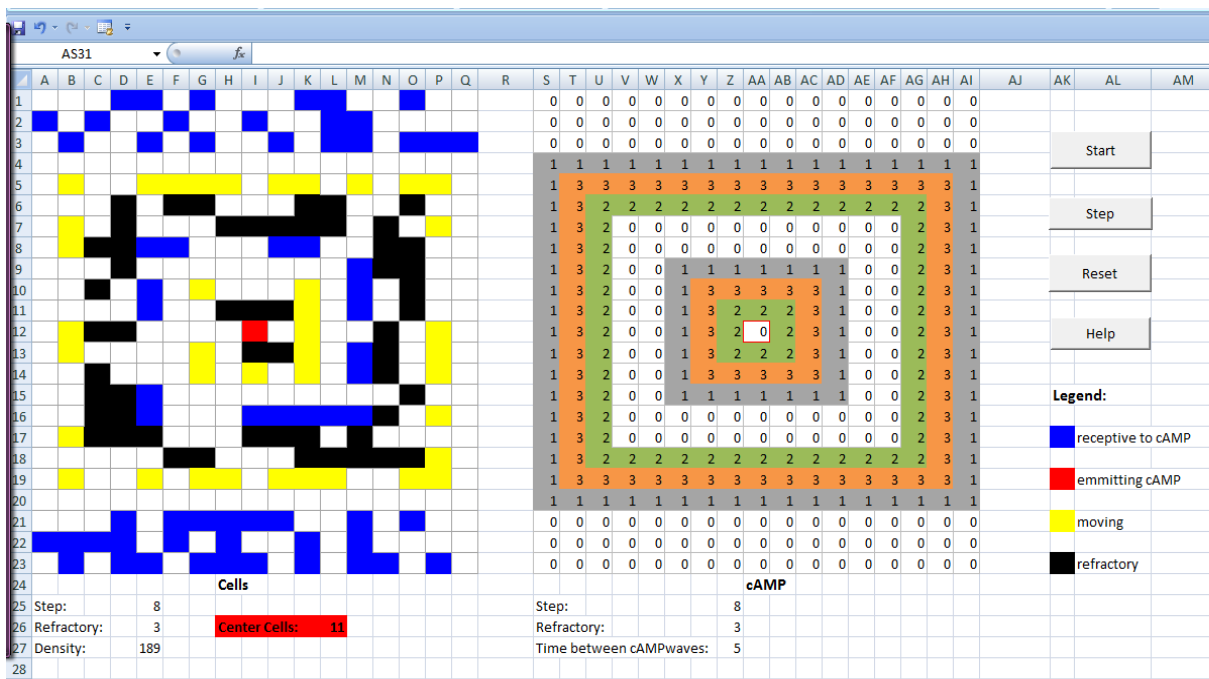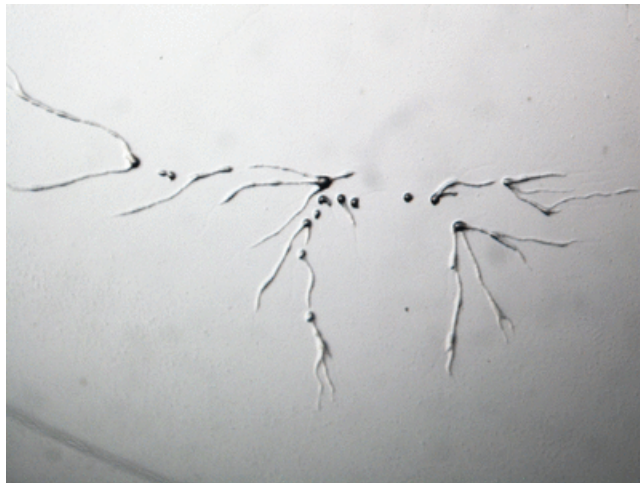

Figure 1: Image of the cell grid (left) and the cAMP grid (right).

How to use the program:

Before beginning, make sure you have enables the macros in Excel. When you launch Slime-U-Lator, there should be a message box asking you to enable the macros for this file. However, if you are not prompted, you will need to enable your macros manually. To do that, go to File then Excel Options. Click on Trust Center and then open Trust Center Settings. Go to the

Macro settings and enable all macros. Please note that this process will differ depending on which version of Excel you own. It is also a good idea to turn your macros off when you are finished using Slime-U-Lator. Alternatively, you can quit Excel and launch Excel by double clicking on Slime-U-Lator which will ask you if you want macros enabled or not – chose to enable macros at that time.

To begin Slime-U-Lator, press the "Start" button. This will open a set of 3 input boxes. Type a numerical integer into each box and press "Ok." To see the results of your parameters, press the "Step" button repeatedly. After the simulation is completed (meaning all of the cells have converged on the center red cell, or the remaining cells are oscillating between the outer two rings), press the "Reset" button before pressing the "Start" button again. Reset clears the two grids and changes your parameters to "0". You can press the "Reset" button at any point during a simulation in order to try new parameters.

Figure 2: Image of streaming Dictyostelium cells. See an animation of real Dictyostelium cells online.

Good things to know:
When using the Slime-U-Lator, it is important to remember a few things that can help you attain the most informative results. As with any good mathematical model, there are ranges within which the model will work best and generate the most biologically accurate results.

Figure 3: Input box 1 for number of steps between cAMP waves.

The first input box (Figure 3) determines the number of steps between the start of each cAMP wave in the cAMP grid. Because you have to press the "Step" button to see your results, it's a good idea to keep this number fairly small so you don't have to press the "Step" button 1,000 times before seeing any change. A good range for the cAMP waves is between 3-25 steps but feel free to

experiment (3 is the minimum acceptable value since the wave is 3 cells wide). The average run of a good simulation will take about 60 steps, so you do have to press the "Step" button multiple times. However, changing the number of steps does allow you to examine the patterns that emerge during the process.
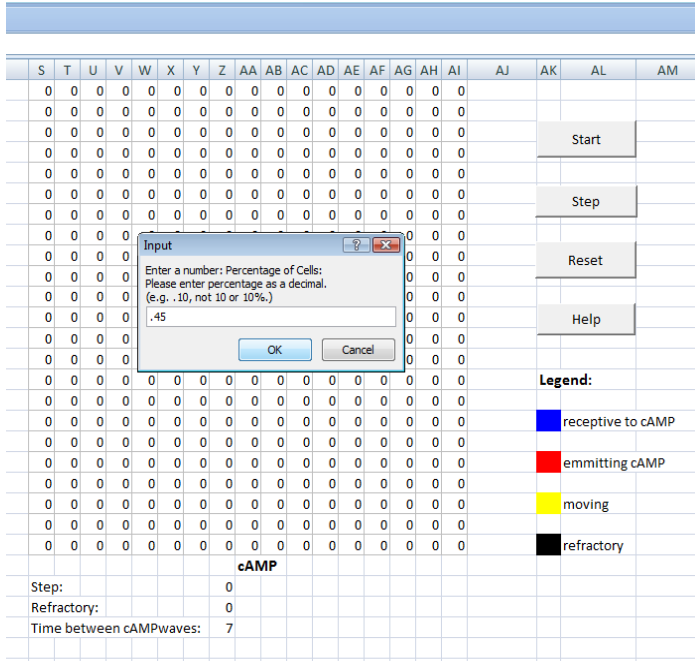
The second input box (Figure 4) determines the density of the cells in terms of the percentage of cells that are colored, out of a total of 391 squares. Users are able to enter a percentage (in terms of decimals) and the program calculates the approximate density of the cell grid. The maximum percentage for density is 100% (entered in as 1).

Figure 4: Input box 2 for cell density.

The third input box (Figure 5) determines how many time steps each cell stays refractory before returning to its cAMP-responsive state. As with the first input box, you don't want this number to be too large because then you will have to wait a long time before the cells can move again.
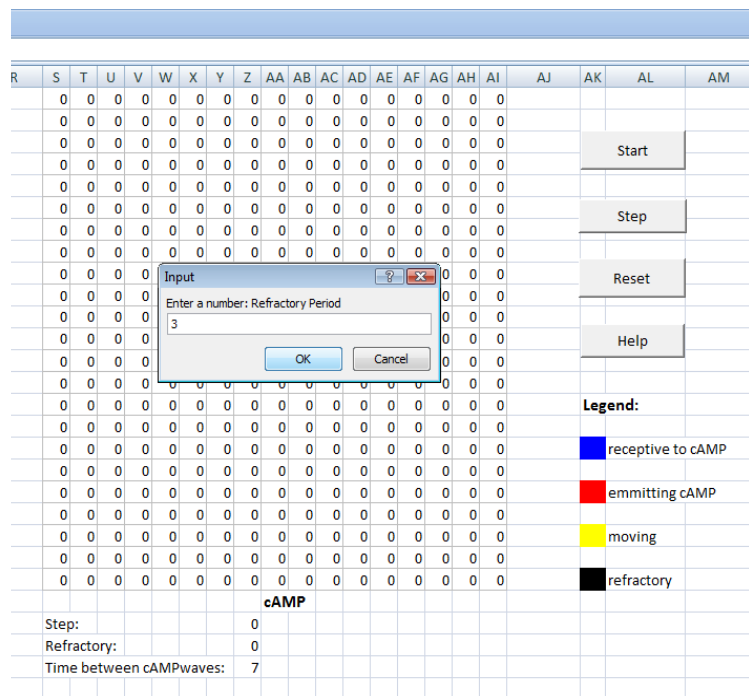
Figure 5: Input box 3 for duration of refractory period.

When the cells converge towards the emitter cell, sometimes they merge on top of each other causing the "cell counter" (seen below the cell grid on the left) to be slightly lower than the original number of cells. Therefore, do not worry if the "cell counter" finishes with fewer cells than your original cell population.

If the cells are oscillating between the outer two rings, the simulation has reached its end. Restart Slime-U-Lator and choose different numbers. When cells appear to move away from the center, it is likely the parameters have caused the cells to chase the cAMP waves away from the emitter cell, instead of towards the center. Perhaps your refractory period was too long, or the time between your cAMP waves was too short.

The emergent property of slime mold slug formation is one of nature's great surprises and the precise timing is the consequence of natural selection and evolution. The Slime-U-Lator should be used as an educational tool, so the main goal is to have fun, try different things, and explore. What happens when the refractory period is higher than the cAMP time? What happens when it's lower? Does density have an effect on how long the simulation runs? Do you see any cell migration patterns? Do you get the same pattern each time you run the simulation with identical input values? What if the refractory period is set to 0? Can you to make the simulation be the most efficient (i.e. make the simulation finish in the least amount of steps)? The only way to find out is to experiment with Slime-U-Lator.

References

Kessin, Richard H., and Jakob Franke. *Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity*. Cambridge: Cambridge UP, 2010. Print.

       Kessin's book provides background information on both the Dictyostelium life cycle and previous Dictyostelium research.

Pilat, Marcin L. "Simplified Models of *Dictyostelium discoideum* Aggregation and Slug Migration." Web. <http://www.pilat.org/docs/605project.pdf>.

       This source discusses various ways of modeling Dictyostelium aggregation and slug movement.

Tyler, Tim. "Texture Garden - Java Fractal Drainage." Web. <http://fd.alife.co.uk/dla/>.

       This model shows one type of model for demonstrating aggregation. It was a starting point in the research for the Slime-U-Lator.