School of Physics

Masters Project
Computational Physics

Self Organisation in Dictyostelium Discoideum

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Abstract

This project uses a two stage Cellular Automata/Differential Equation model to simulate the behaviour of Dictyostelium Discoideum (Dd) in the early stages of aggregation. The model is used to explain the formation of spiral patterns, the aggregation streams and the increase in speed observed when Dd amoeba travel in groups. It is then used to show that auto-cycling amoeba may not necessary to trigger aggregation, and presents an alternative method.

Declaration

I declare that this project and report are my own work.

Signature: Date:

Supervisor: Dr. Graeme Ackland

Contents

1 Introduction

The aim of this project was to reproduce some of the complex, multicellular behaviour of the slime mould Dictyostelium Discoideum (Dd) using a simple model. Dd has a fascinating life cycle, going through several morphological changes as the individual amoeba work co-operatively when resources are scarce. On an individual scale, the amoeba perform a few basic functions which are relatively simple to incorporate into a simulation. When many of the amoeba are brought together, they organise themselves in a complex way. Understanding how the complex behaviour arises from the basic amoeba functions is the purpose of this project.

The model is based on the work of Prof. Paulien Hogeweg, a member of the Bioinformatics group at Utrecht University in the Netherlands. It consists of a Cellular Automata to describe the individual Dd amoeba and a set of differential equations to simulate the signalling chemical used by Dd amoeba, cyclic adenosine monophosphate (cAMP). A second method for modelling the cAMP dynamics was created by the author and both models were used to investigate the the streaming which occurs during aggregation and the spiral patterns which form.

Dictyostelium Discoideum has been widely studied by many different groups, Toni Collis created a model of the mound formation at the University of Edinburgh where each amoeba was treated as a particle in a molecular dynamics model. Prof. Hogeweg has successfully reproduced the complete life cycle of Dd using a model similar to that presented here with more complex extensions to simulate gravity, cell differentiation and cell stiffening [1]. Palsson and Othmer created a model of Dd where each amoeba was defined as an ellipsoid with a position in space and forces due to chemotaxis or adhesion would deform the shape of the amoeba and translate it's centre of mass. They managed to successfully reproduce the aggregation streams, mound formation and locomotion of the slug [2].

This project does not give an analytical description of Dd, but attempts to develop a deeper understanding of some of the processes by which high level behaviour can arise from simple premises.

2 Background

2.1 Dictyostelium Discoideum

The slime mould Dictyostelium Discoideum is a type of amoeba which lives in top soil and leaf mould. They feed on bacteria and fungi and divide every few hours. When food is plentiful, each amoeba operates individually and is repelled by the smell of its own kind, making them disperse as widely as possible.

If the supply of food is exhausted, this situation changes dramatically and the amoebas start to group together. This process is controlled by a chemical called cyclic adenosine monophosphate (cAMP). It is not fully understood how the signalling process begins, it is possible that the amoeba which has been starving for the longest time switches to an 'auto-cycling' state, where it emits regular pulses of cAMP. It is also possible that all starving amoeba emit small amounts of cAMP which can reproduce the same results and is one of the experiments carried out in this project.

Each pulse of cAMP induces the surrounding amoeba to move towards the source of the pulse and to secrete their own pulse, creating a wave of cAMP. Regular waves of cAMP travel out from each aggregation centre, causing the distant amoeba to travel inwards in 'streams' (Figure 1).

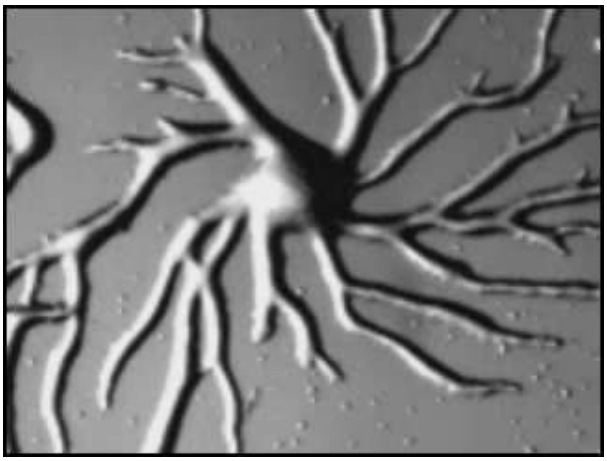

Figure 1: Under the influence of cAMP, Dd amoeba form streams which participate in the formation of a multicellular tissue by chemotaxis. From R. Firtel, UCSD (http://www.dictybase.org/Multimedia/development/Agg.stream2.mov)

As more and more amoeba join the aggregate centre, it forces the central bulk of amoeba up into a mound which continues to grow. When the mound gets too big it topples over and forms a crawling slug like structure, pushed along by waves of cAMP travelling along it's body. The slug is responsive to light and heat (usually it heads for the surface of the soil) and can travel for up to 20 days, at which point it stops moving and the next stage

of development begins.

During the aggregation and slug stages, the amoeba differentiate into pre-spore and pre-stalk types which sort themselves and move to the front and back of the slug respectively. The pre-spore amoeba develop into a spore head which is lifted from the soil by the pre-stalk amoeba, which grow into a long, stiff structure. The final stage in the life cycle is the emission of spores from the spore head, which germinate into new amoeba, while the amoeba in the stalk structure die (Figure 2).

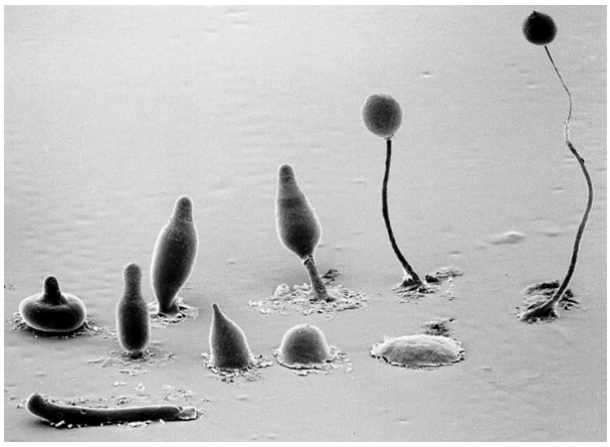

Figure 2: Scanning Electron Micrograph of the Dictyostelium Developmental Stages Copyright, M.J. Grimson & R.L. Blanton, Biological Sciences Electron Microscopy Laboratory, Texas Tech University (http://www.dictybase.org/Multimedia/LarryBlanton/dev.jpg)

Dictyostelium Discoideum makes an excellent candidate to model because the underlying processes performed by each amoeba can be simulated relatively simply. The model does not try to recreate the biochemical reactions which occur inside the cell, rather it attempts to recreate the high level behaviour, and in doing so, gives insight into how simple systems can create complex behaviour.

The three process which have been incorporated into this model are differential adhesion, chemotaxis and cAMP mediated cAMP response.

Differential Adhesion - Once the aggregation phase has begun, the Dd amoeba clump together due to adhesion between their cell membranes. The adhesion between amoeba is necessary for the slug to stay together, and also allows amoeba in a group to travel faster than an individual (explored in one of the experiments in this project). The adhesion plays another not so obvious role, cell sorting. Steinberg [5] suggested that the process of cell sorting could take place if amoeba of different types adhered to each other with different strengths, the Differential Adhesion Hypothesis (DAH). Glazier & Graner [3] created a model of biological cells based on the standard large-Q Potts model which incorporates the DAH, and this model showed that many different types of cell sorting can be recreated using this hypothesis.

Chemotaxis - In the cell membrane of Dd amoeba are receptors which monitor levels of cAMP in the surroundings. Once the concentration of cAMP reaches a threshold level, the amoeba enters an excited state, and moves towards the source of of cAMP. This process is called Chemotaxis and accounts for the aggregation of amoeba into a mound, and subsequently for the locomotion of the slug.

cAMP Mediated cAMP Response - While in the excited state and performing Chemotaxis, the amoeba also emits it's own pulse of cAMP, exciting neighbouring amoeba, which create their own pulse and so on. This is the cAMP Mediated cAMP response. When an amoeba has been in the excited state for some time, the receptors in the cell membrane become desensitised and the amoeba enters a refractory, or resting state. In this state, the amoeba emits a catalyst called phosphodiesterase which breaks down the cAMP, in preparation for the next wave to pass. This combination of a cAMP mediated cAMP response and the excited-refractory states allows the waves to travel outwards from the centre of aggregation, creating the streams (Figure 1).

2.2 The Model

The model consists of two components, a Cellular Automata to describe the amoeba and a set of differential equation to control the cAMP field.

2.2.1 Cellular Automata

This model uses a Cellular Automata (CA) to describe the physical structure and locomotion of the Dd amoeba. Cellular automata are dynamical systems which are discrete in space and time, operate on a uniform, regular lattice - and are characterised by 'local' interactions. They consist of four components, a lattice, a set of states, an interaction neighbourhood and a set of update rules.

The Lattice is a regular pattern of sites[1] each of which can be in one of a finite number of states. Commonly, CA lattices are one or two dimensional, but they can extend into any number of dimensions. The lattice can be of any size, but the size can influence the results of the problem being tackled by introducing finite size effects. There are different ways to deal with the boundary of the lattice; static, mirror and periodic boundary conditions.

Static Boundary Conditions - the sites at the edge of the lattice are held at a fixed value and cannot change.

Mirror Boundary Conditions - any transformation which tries to act on a site outside the boundary is reflected back.

[1]The individual sites in the lattice are normally called 'cells', hence the name Cellular Automata. In this report, the discrete elements in the lattice will be called 'sites' and Dictyostelium Discoideum amoeba will be called 'amoeba'.

Periodic Boundary Conditions - each edge of the lattice is connected to the opposing edge, so if a transformation moves a state off the left boundary, it will re-appear on the right boundary. A one dimensional CA with Periodic Boundary Conditions (PBS) corresponds to a connected loop, a two dimensional CA with PBS corresponds to a torus.

The States are a set of objects which represent the different properties each site can take. Normally the states are just integers, as these are simple and fast to process, but they can be much more complicated.

The Interaction Neighbourhood is the range of neighbours which each site can 'see'. For example, in a simple CA which represents forest fires, each site on the lattice looks at the sites in it's interaction neighbourhood and if any of them are on fire, it sets on fire. Two common 2d neighbourhoods are the Moore neighbourhood (4 nearest neighbours) and the Von Neumann neighbourhood (8 nearest neighbours).

Figure 3: *Left* - The Moore Neighbourhood. *Right* - The Von Neumann Neighbourhood.

The Transition Rules determine how the system evolves. Based on the previous forest fires example, the transition rules for that system could be:

- If you were alive and a neighbour was burning, you are now burning
- If you were burning, you are now ash
- If you were ash and no neighbours were burning, you are now alive

There are different ways to apply the transition rules, simultaneously and stochastically. Simultaneous updating runs through the entire lattice in order and stores the updated values of each site in a second lattice. It then runs through the second lattice and stores the result in the first etc. Stochastic updating picks random sites in the lattice and updates them to the same lattice. The two update algorithms can produce very different results.

2.2.2 cAMP Dynamics

The cAMP dynamics are fairly complex and have been studied in great detail (REFERENCES). Experiments using dark-field illumination have shown that spatial distribution of cAMP throughout the cells forms intricate spiral patterns (Figure 4). Two approaches have been used to attempt to recreate this behaviour in the model:

- The Explicit Emission Model (Section 3.2.1) - This model was created by the author. The cAMP behaviour is passive bar plain diffusion with a removal term to simulate the catalysis of cAMP by phosphodiesterase. It more closely mimics the biological system as the emission of cAMP is controlled by the amoeba themselves.
- The Implicit Emission Model (Section 3.2.2) - This model was used by Hogeweg [4]. The cAMP dynamics are approximated by a partial differential equation which models waves travelling through an excitable medium.

The Implicit model has been used in much of the research by Hogeweg, and reproduces many of the properties of the biological system. The Explicit model also reproduces the spirals, but does so in a different way. By implementing both models, it is possible to compare the two approaches.

Figure 4: Spiral signalling patterns during the aggregation of Dd amoeba. The light and dark bands show up under dark-field illumination and arise from the differences in optical properties between moving and stationary amoeba. The light areas show amoeba which are excited and moving chemotactically, the dark areas show stationary amoeba. The image above is a 5mm by 5mm square. (http://www.uni-magdeburg.de/abp/pics/dictyostelium1.jpg)

3 Model Implementation

The model consists of two components, a Cellular Automata (CA) to describe the amoebas and a differential equation to describe the cAMP dynamics.

3.1 Cellular Automata

3.1.1 Initialisation

The Cellular Automata (CA) is based on the model of Glazier & Graner [3] and consists of a two dimensional square lattice with static boundary conditions. Each amoeba is assigned a unique identification number σ, which is copied onto all sites in the CA lattice corresponding to that amoeba. Any lattice sites which are not part of an amoeba form part of the medium are are assigned the value 0. The ideal number of sites occupied by each amoeba, ν, is called the target volume and is set by the user on initialisation. In many other models, each amoeba is modelled by one site on the lattice. Although that approach allows for a simpler model, it is the use of extended bodies for the amoeba which allows them to squeeze past each other and apply pressure on each other via membrane deformation.

The initialisation algorithm works as follows:

1. Pick a random site on the lattice which is empty (forms part of the medium).
2. Copy the id of the current amoeba into this site and store the coordinates in a list.
3. Pick a random site from the list and look for a empty space in the four nearest neighbours.
4. If current volume $< \nu$ goto step 2, otherwise move on to the next amoeba.
5. If no empty neighbours can be found for any sites in the list, store the current volume of the amoeba and move on to the next.

It distributes amoeba randomly throughout the lattice, ensures that the sites making up each amoeba are connected and allows the amoeba density to be varied from 0.0 to 1.0 without getting caught in a infinite loop. If the required amoeba density is high, not all amoebas will be at the target volume after initialisation as some will be squashed between others. Figure 5 shows a possible state of the lattice after three amoeba have been initialised.

3.1.2 Hamiltonian

The amoebas can have different properties (pre-stalk, pre-spore, auto-cycling) and this is incorporated by assigning each amoeba a type, τ. Each amoeba can only be of one type, but there can be many amoebas of the same type in the simulation. The medium is assigned it's own type.

The differential adhesion hypothesis [5] claims that the interaction between two amoeba involves an adhesion surface energy which varies according to amoeba type. Each boundary between two different amoebas (or an amoeba and the medium) has an associated dimensionless bond energy, which is stored in the adhesion matrix $J_{\tau(\sigma),\tau(\sigma')}$. The Hamiltonian for each site on the lattice is then defined as:

$$H = \sum_{neighbours} J_{\tau(\sigma(x,y)),\tau(\sigma(x',y'))}(1 - \delta_{\sigma(x,y),\sigma(x',y')}) + \lambda(\nu - V(\sigma(x,y)))^2 \qquad (1)$$

Where $\sigma(x, y)$ is the id of the selected site and $\sigma(x', y')$ is the id of the neighbour. The Kronecker Delta ensures that the bond energy between two sites in the same amoeba or between two medium sites is zero. The sum is over the 8 nearest neighbours (Von Neumann neighbourhood). The last term depends on the current volume of the amoeba and is minimised if the current volume is equal to the target volume. λ is a multiplier specifying the strength of the volume constraint and physically corresponds to the elasticity of the cell membrane.

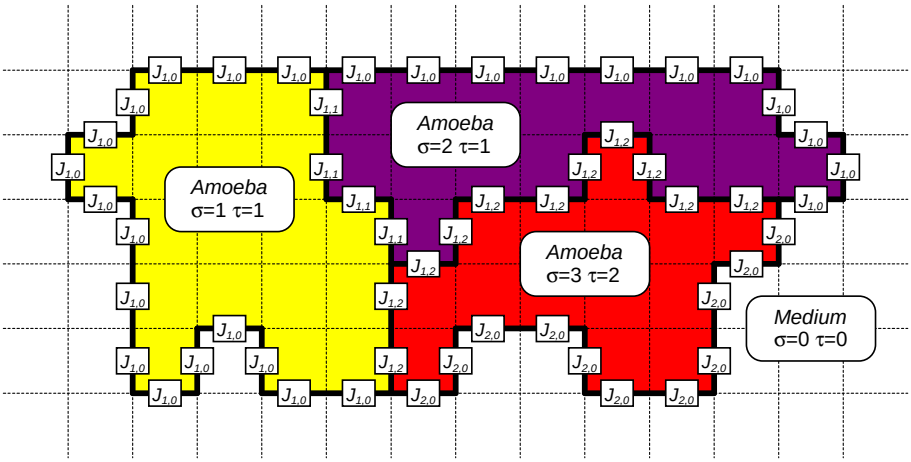

Figure 5: Schematic of 3 amoebas after initialisation. Note that the yellow and purple amoebas are both type 1 and the red amoeba is type 2. For clarity, only the bonds between the 4 nearest neighbours have been shown, in the model 8 nearest neighbours are used.

3.1.3 Iteration Procedure

The time advancement of the Cellular Automata is based on the Metropolis Algorithm. Each step is reversible, so the algorithm satisfies Detailed Balance:

1. Select a site on the lattice at random and calculate its Hamiltonian.
2. Copy the state of one its neighbours onto the selected site and calculate the difference in the Hamiltonian, dE, from before and after the swap.
3. If $dE \leq 0.0$ accept the swap and continue, otherwise accept the swap with probability $e^{-\frac{dE}{T}}$.

In one time-step the number of sites randomly selected is equal to the total number of sites on the lattice, ensuring that on average, each lattice site is selected once per time-step. The constant T controls the probability of accepting a swap when the change in H is positive. It corresponds to the temperature in the Potts Model and in this model controls the mobility of the amoeba.

3.1.4 Cell Sorting due to Differential Adhesion

The Glazier & Graner [3] algorithm described above is capable of reproducing the cell rearrangement of many biological systems. Two examples are shown below, that of the checkerboard and complete cell sorting, both between two cell types light (l) and dark (d). The screen shots were taken from a version of the model implemented by the author.

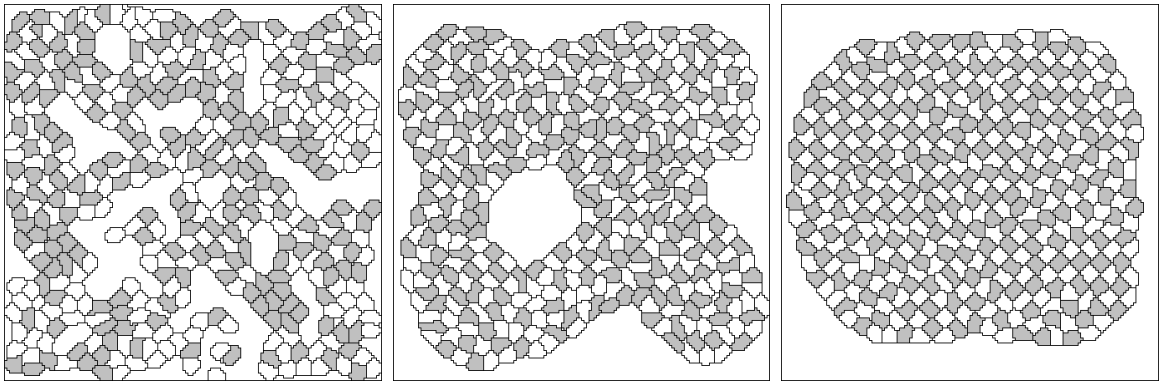

Figure 6: The checkerboard pattern formed by Differential Adhesion in the CA model of Glazier & Graner. On the left, after initialisation. In the centre, after 4,000 iterations. On the right, after 10,000 iterations. The parameters used were: $T = 10, \lambda = 1, J_{ll} = 10, J_{dd} = 8, J_{ld} = 6, J_{lM} = J_{dM} = 12$

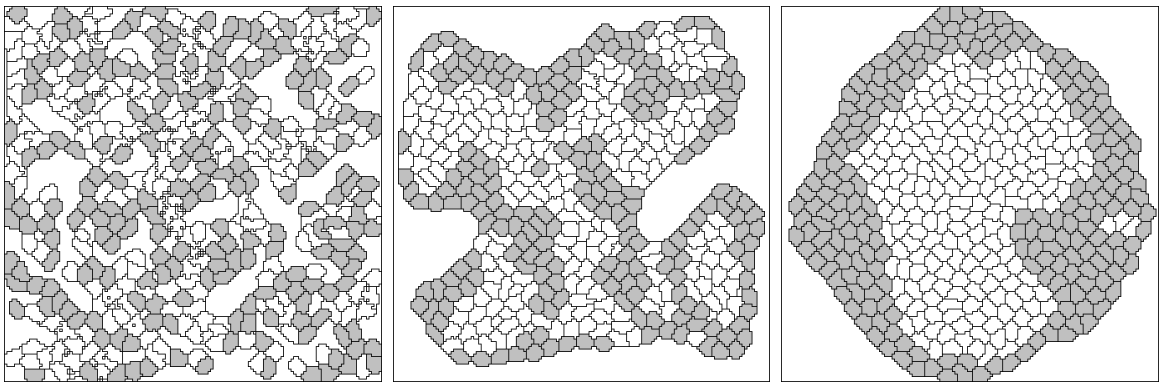

Figure 7: Cell sorting due to Differential Adhesion. On the left, after initialisation. In the centre, after 4,000 iterations. On the right, after 10,000 iterations. The parameters used were: $T = 10, \lambda = 1, J_{ll} = 14, J_{dd} = 2, J_{ld} = 11, J_{lM} = J_{dM} = 16$

3.2 cAMP Field

The cAMP field is comprised of a lattice which is the same size as the Cellular automata, each site on the cAMP field corresponding to one site on the CA. Two different methods have been used to model the cAMP dynamics, the explicit emission model where the amoeba in the CA add cAMP directly to the cAMP field and the implicit emission model where the cAMP dynamics are controlled entirely by a set of differential equations and the amoeba never directly modify the cAMP field.

3.2.1 cAMP Dynamics in the Explicit Model

In this model cAMP is added directly to the cAMP field by the amoeba when they are in the excited state, and the time advancement is controlled by the diffusion equation with a term to account for the breakdown of cAMP by phosphodiesterase.

$$\frac{dc(x,y)}{dt} = D\nabla^2 c(x,y) - \gamma c(x,y) \tag{2}$$

D is the diffusion constant and is assumed to be space invariant. $c(x,y)$ is the concentration of cAMP at the position defined by the coordinates (x,y). γ is a constant over the whole system and represents the concentration of phosphodiesterase.

In order to represent this equation on a lattice it was converted from a continuous into a discrete function, and then solved using the explicit Euler method as follows.

The discrete form of equation (2) in two dimensions is:

$$\frac{c^1_{i,j} - c^0_{i,j}}{dt} = D\left(\frac{c^0_{i-1,j} - 2c^0_{i,j} + c^0_{i+1,j}}{dx^2} + \frac{c^0_{i,j-1} - 2c^0_{i,j} + c^0_{i,j+1}}{dy^2}\right) - \gamma c^0_{i,j}$$

Where c^0 is the concentration of cAMP in the current time step and c^1 is the concentration in the next time step. The continuous variables x and y have been replaced by the indices i and j. Setting $dx = dy$ and rearranging gives:

$$c^1_{i,j} = c^0_{i,j} + (dt)\left\{D\left(\frac{c^0_{i-1,j} + c^0_{i+1,j} + c^0_{i,j-1} + c^0_{i,j+1} - 4c^0_{i,j}}{dx^2}\right) - \gamma c^0_{i,j}\right\} \tag{3}$$

The five terms inside the inner bracket correspond to the flow of cAMP into the current site from the neighbouring sites (the four positive terms), and the flow out of the current site into it's neighbours (the negative term).

The cAMP field is updated simultaneously, the state of the cAMP field in the next time step, c^1, is calculated from the current state, c^0, and stored in a new array. One complete iteration of the cAMP field takes place for each iteration of the Cellular Automata.

3.2.2 cAMP Dynamics in the Implicit Model

The Implicit model approximates the cAMP field as an excitable medium, using the Fitzhugh-Nagumo (FHN) equations with piecewise linear "Pushchino kinetics" [8] to control the dynamics. A full analysis of this model was considered outside the scope of this project and has not been included. The parameters for the equations have been used as Hogeweg used in modelling Dd [4]. The second variable in these equations, r, is called the refractory index and controls how desensitised the amoeba's cAMP receptors are.

$$\frac{\partial c}{\partial t} = D\nabla^2 c - f(c) - r$$

$$\frac{\partial r}{\partial t} = \varepsilon(c)(kc - r)$$

Where

$$f(c) = \begin{cases} C_1 c & \text{if } c < c_1 \\ a - C_2 c & \text{if } c_1 < c < c_2 \\ C_3(c-1) & \text{if } c_2 < c \end{cases}$$

and

$$\varepsilon(c) = \begin{cases} \varepsilon_1 & \text{if } c < c_1 \\ \varepsilon_2 & \text{if } c_1 < c < c_2 \\ \varepsilon_3 & \text{if } c_2 < c \end{cases}$$

In the Implicit model the length time each amoeba spends in the Excited and Refractory states is controlled by the values of c, the concentration of cAMP and r, the refractory index. An amoeba will enter the excited state if the value of c in any of it's sites is above the threshold value, c_t. After prolonged exposure to high concentrations of cAMP, the receptors in the amoebas cell membrane become desensitised and the amoeba enters the refractory state, in the model this occurs when $r > r_t$. Figure 8 shows the behaviour of c and r as functions of time along with the threshold values used in this model.

The FHN equations are solved on all sites which form part of any amoeba, and equation (3) is solved on the medium sites to simulate the diffusion of cAMP into the medium. The iteration procedure is the same for the Implicit model as for the Explicit model, except that another field has to be used to store the values of r at each point in the lattice.

3.3 Amoeba Behaviour

3.3.1 States

Once the signalling process has begun and the amoeba have started aggregating, they operate in three different states Ready, Excited and Refractory.

Ready The amoeba is stationary and ready to be excited before the cAMP wave.

Excited The amoeba is moving up the strongest local cAMP gradient and emitting it's own source of cAMP. This state lasts for 100 seconds.

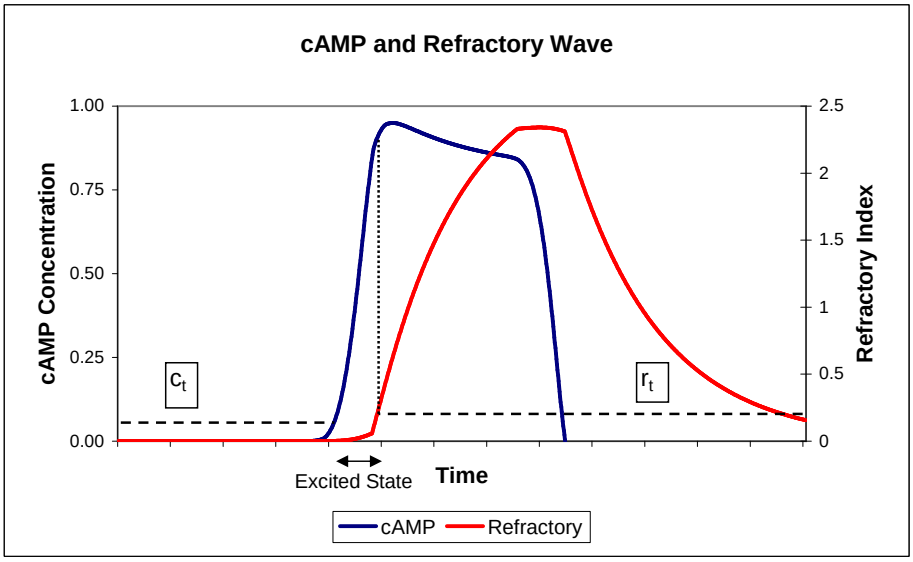

Figure 8: Chart showing the value of c and r at a fixed point in the field as a wave passes. The values of the parameters used in the equations were $C_1 = 20, C_2 = 3, C_3 = 15, a = 0.15, c_1 = 0.0065, c_2 = 0.841, D = 1.0, \varepsilon_1 = 0.5, \varepsilon_2 = 0.0589, \varepsilon_3 = 0.5, k = 3.5$, Time Step = 0.01, Space Step = 0.37

Refractory The amoeba is stationary and unresponsive to cAMP. This state lasts for 120 - 420 seconds.

In the two different cAMP dynamics models, the transition rules between states have been implemented differently. In the Explicit model, the length of each state is defined in the code and corresponds to a number of time steps. An amoeba will enter the excited state if it detects an above threshold concentration of cAMP. In this state the amoeba will add cAMP to the cAMP field whenever it is selected by the iteration algorithm, and will perform chemotaxis (3.3.2). It will remain in the excited state for the predetermined amount of time, then switch to the Refractory state. This state will then last for a predetermined length of time, at which point the amoeba returns to the Ready state (Figure 9).

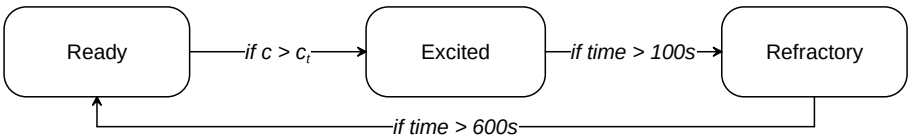

Figure 9: The state cycle in the Explicit model. The times given in the figure refer to experimental data.

In the Implicit emission model, the state of each amoeba is controlled by the FHN equations. Figure 8 shows the values of c and r as a wave passes with the cAMP threshold and refractory index thresholds marked on. If an amoeba in the Ready state detects a concentration of cAMP above c_t it switches to the Excited state. It will remain in this

state until the value of the refractive index on any of it's sites grows bigger than r_t, at which point it switches to the Refractory state. This has been drawn on Figure 8. The thresholds for c and r have been set so that the amoeba will be excited (and moving chemotactically) while the cAMP wavefront passes over it, and refractory for the flat top and trailing edge of the cAMP wave. The amoeba will return to the Ready state when the Refractory level has dropped below r_t (Figure 10).

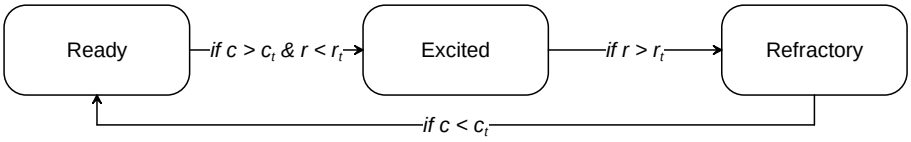

Figure 10: The state cycle in the Implicit model. The length of the Excited state depends on the values of c and r and can be seen on Figure 8.

The auto-cycling amoeba do not go through the same state cycle. They emit a pulse of cAMP once every 600 seconds and do not respond to cAMP in any way.

3.3.2 Chemotaxis

When in the Excited state, the amoeba respond in two ways; by emitting cAMP (cAMP Mediated cAMP response) and by moving up the local spatial gradient of cAMP (chemotaxis). The chemotaxis is incorporated into the model by modifying the energy difference, dE when iterating a site belonging to an amoeba in the excited state.

$$\Delta H' = \Delta H + \mu(c_{automaton} - c_{neighbour}) \tag{4}$$

When equation (4) is active, is has the effect of making an amoeba more likely to move to a site of higher cAMP concentration and less likely to move to a site of lower cAMP concentration. The parameter μ enables the user to control how strongly the amoeba will respond to a spatial cAMP gradient.

4 Experiments & Results

Dictyostelium Discoideum has been the focus of great study for many years and much is known about all the stages of it's life cycle. Hogeweg et al. have been able to reproduce many of the properties of Dd cycle, from aggregation, mound formation, slug locomotion and morphogenesis into spore and stalk structure using a similar but more advanced model than the version presented here. The experiments performed in this project aim to develop a greater understanding of some of the finer aspects of the aggregation phase of Dictyostelium Discoideum.

- The first experiment will demonstrate how the model reproduces the spiral patterns observed as the amoeba begin to aggregate (Figure 4), and attempt to explain how the spirals form.
- The second experiment will look at how the streams form (Figure 1).
- The third will try to explain why the amoeba which usually distance themselves from each other as far as possible cluster together when food is scarce.
- The final experiment shows that the triggering mechanism for the start of aggregation does not necessarily have to depend on a small number of 'special' auto-cycling amoeba.

4.1 Formation of Spirals during aggregation

4.1.1 Experimental Set-up

The first experiment used both the Explicit model with parameters given in Tables 1, 2 and 3 and the Implicit model with parameters given in Tables 1, 2, 4 and the FHN parameters given in the caption of Figure 8. One of the 540 amoeba was made a pacesetter (red), of the rest 20% were pre-stalk (green) and 80% pre-spore (yellow). The colours in brackets refer to the screen-shots (Figure 11), which were taken at regular intervals during the simulation. One time step corresponds to about 0.3 seconds and one site in the lattice to $4\mu m^2$.

4.1.2 Comparison of the Explicit and Implicit Models

Both models recreated the spiral patterns with the given parameters. The implicit model produces smoother spirals because it is governed by a set of equations which operate on a site by site basis, whereas the smallest object which can emit cAMP in the Explicit model is one amoeba. Both models assume the intra-cellular cAMP dynamics are instantaneous, that is, when the state one site in an amoeba changes, the state of the whole amoeba is updated. However, the Implicit model uses the FHN equations to control the states, so a variation of r over one amoeba can be observed, whether the state is updated depends on which sites in that amoeba are selected by the iteration algorithm.

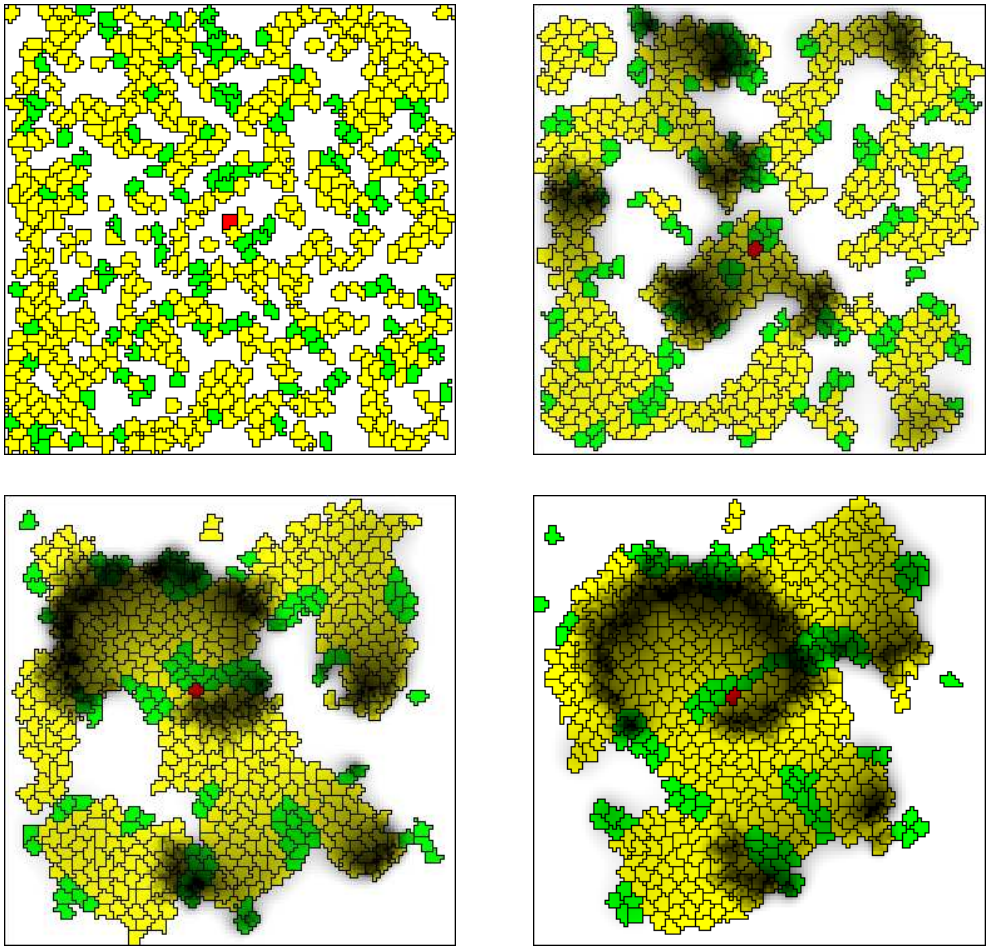

Figure 11: The formation of spiral waves of cAMP in the Explicit emission model. The red amoeba is auto-cycling, green are pre-stalk and yellow are pre-spore. The images show that the different amoeba types sort themselves into groups during aggregation. (a) After Initialisation, (b) 500 iterations, (c) 1,000 iterations, (d) 3,000 iterations.

4.1.3 Spiral Formation

When the simulation begins, the amoeba immediately clump together due to adhesion. Once the auto-cycling amoeba has started emitting, the amoeba start to form streams and aggregate together. In these simulations, the spirals form after the aggregation has begun, but before the amoeba have closed all the gaps. The inhomogeneities in amoeba density could be the cause of the spirals. This can be seen in the Explicit model, Figure 11(b), the waves of cAMP starting at the auto-cycling amoeba travel south-west in the image, down one of the streams. In (c) this stream has joined back up with the aggregate, forming a closed loop which the cAMP waves travel around. Eventually, chemotaxis causes the amoeba to close the gap and the spiral is formed, which sustains itself, without the need for the auto-cycling amoeba. A similar situation can be seen in the Implicit model, Figure 19(b), in the south-west of the image a cAMP wave is travelling round a closed loop which has formed a spiral by (c). Simulations were performed on both models with an amoeba density $\rho = 1.0$, and no spirals were generated as no closed loops could be formed.

Parameter	Symbol	Value
Linear Model Size	l	120
Number of Amoeba	n	540
Target Volume	ν	16
Amoeba density	ρ	0.6
Mobility	T	2.0
Membrane Elasticity	λ	1.0
Chemotaxis Constant	μ	30.0

Table 1: Cellular Automata Parameters

$J_{\tau,\tau}$	M	a	k	p
M	0	2	2	2
a	2	3	4	4
k	2	4	3	4
p	2	4	4	4

Table 2: Adhesion bond energies. M = Medium, a = autocycling, k = pre-stalk, p = prespore

Parameter	Symbol	Value
Diffusion Constant	D	0.1
cAMP Excitation Threshold	c_t	0.2
cAMP Decay Constant	γ	0.04

Table 3: cAMP Field Parameters for the Explicit Model

Parameter	Symbol	Value
cAMP Excitation Threshold	c_t	0.1
Refractive Index Threshold	r_t	0.2
cAMP Decay Constant	γ	0.04

Table 4: cAMP Field Parameters for the Implicit Model

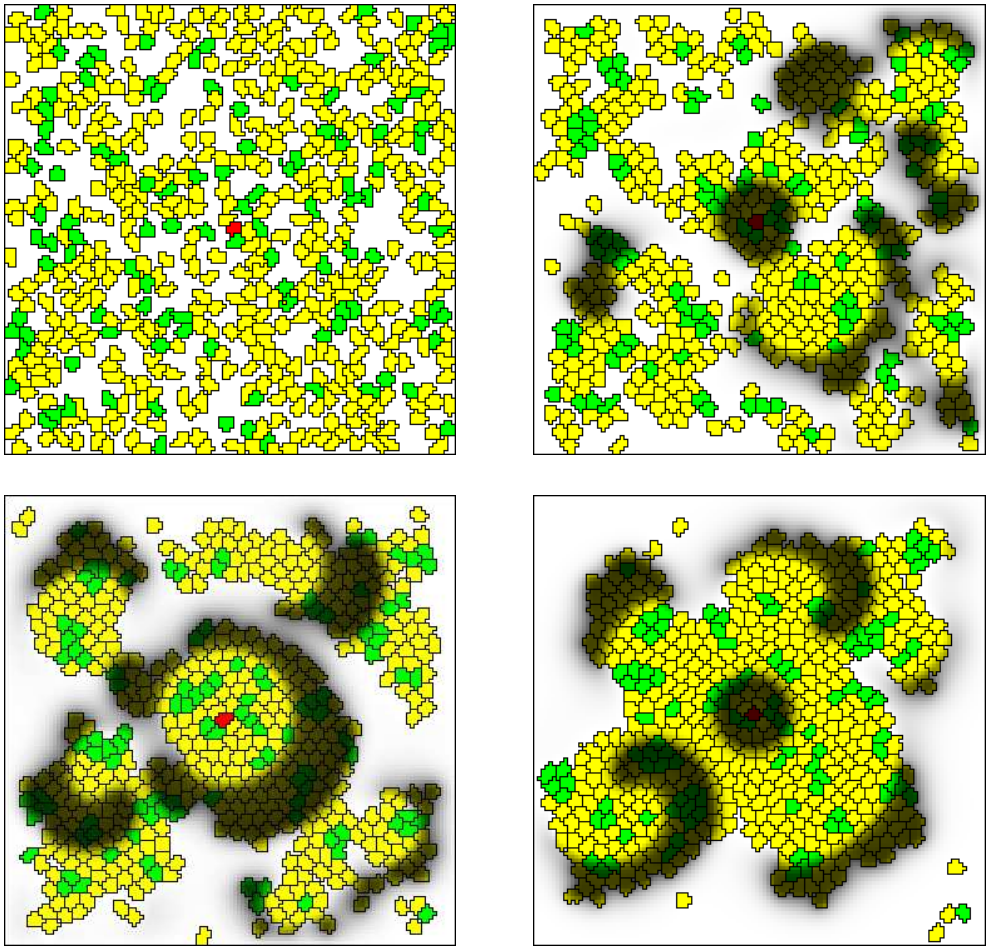

Figure 12: The formation of spiral waves of cAMP in the Implicit emission model. (a) After Initialisation, (b) 500 iterations, (c) 1,000 iterations, (d) 3,000 iterations.

4.2 How do Dd amoeba form streams?

The formation of streams during aggregation is an interesting property of Dictyostelium Discoideum amoeba. Figure 13 shows an enlarged section of a cAMP wave travelling down a stream in the Implicit model. As the wave propagates along the stream, it travels faster down the centre as the amoeba density is higher in the centre than at the edges [9]. This creates a curvature in the wavefront, causing the chemotactically moving amoeba to push towards the centre of the stream (blue arrows), as each wave passes. Diffusion from the edges of the stream excites nearby amoeba which see the strongest gradient of cAMP towards the stream and join. The overall effect is that waves of cAMP make small groups of amoeba form small streams, which attract more amoeba, and the small streams join together to create bigger streams which flow towards the aggregation centre. A further observation is that if the centre of aggregation is a self sustaining spiral, the streams spiral into the aggregation centre, which appears to rotate. This can be seen in Figure 11, where the spiral is rotating in a clockwise direction and the aggregation centre is rotating in the anti-clockwise direction. Figure 19 shows aggregation which has been controlled by the circular waves of cAMP spreading from the auto-cycling amoeba, and

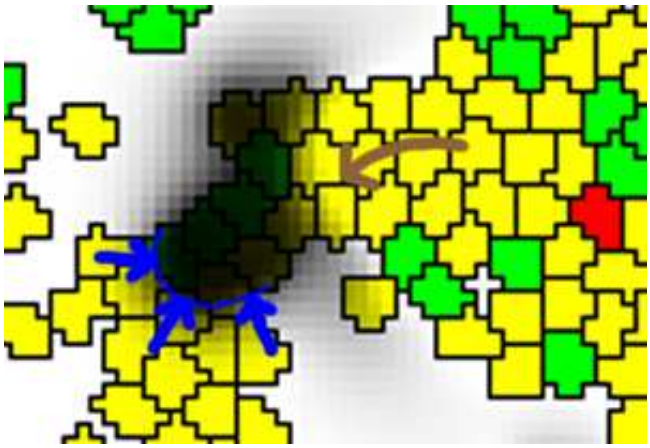

Figure 13: An enlarged screenshot section showing a cAMP wave travelling down a stream. The brown arrow shows the direction of the cAMP wave, the blue curve shows the wavefront and the blue arrows show the direction of chemotaxis.

in this experiment, the aggregation centre does not appear to be rotating.

4.3 What is the advantage of clumping together when food runs out?

When the amoebas resource supply becomes scarce, they need to move to an area with more resources as quickly as they can in order to survive. By grouping together, amoeba travel more quickly than if they move independently. Bonner showed by experiment that this happens in the migrating slug [6].

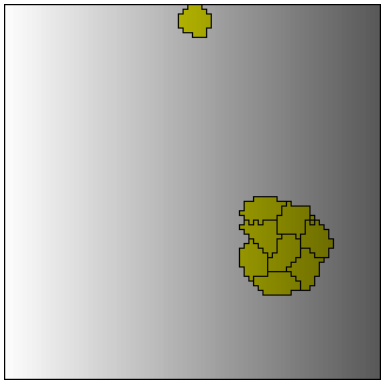

Figure 14: A synthetic experiment showing the movement of a group of amoeba compared to an individual. A static cAMP gradient has been set up smoothly changing from a low concentration on the left to a high concentration on the right.

Figure 14 shows a synthetic experiment to determine how the velocity of a group of amoebas changes with the number of amoeba in the group. The figure shows a cluster of

eight amoebas and a single amoeba chemotactically moving up a static cAMP gradient. The cluster is moves significantly faster than the individual.

In order to measure this behaviour, it was necessary to calculate the speed of a group of amoebas. Each iteration, the centre of mass of the group was calculated. The number of iterations for the centre of mass to move between two fixed points on the grid was then measured and used to calculate the speed of the group. Measurements were taken 5 times and averaged, the results are shown in Figure 15.

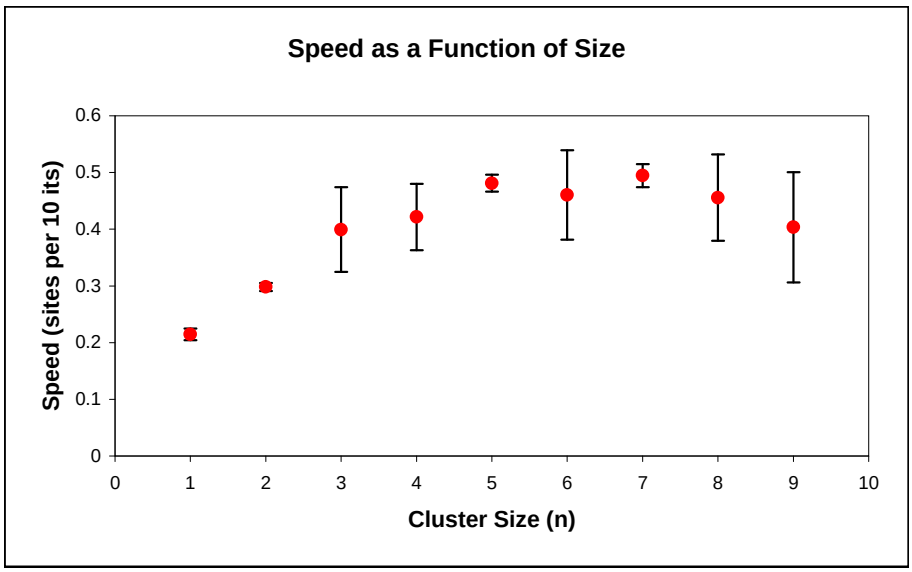

Figure 15: How the speed of a group of amoebas changes with the number in the group. Results were averaged over five runs and the standard deviation displayed in the figure.

Figure 15 shows that, in this experiment, an individual amoeba travels less than half the speed of a larger group, and that for a large n, the speed tends to a constant, which is in accordance with observed experimental results[2] [7].

There are several reasons why amoeba move faster in a group, the most dominant effect caused by the adhesion between cell membranes. If an amoeba moves independently, it can travel a short distance during it's excited period. If this amoeba is surrounded by other amoebas, it gets pulled along by the amoeba moving before it and pushed by the amoeba moving after it, travelling further for each wave of cAMP. A simple representation of this is shown in Figure 16, where the amoeba are connected by springs, which represent the elasticity of their cell membranes.

This experiment used a static cAMP field to demonstrate the how the pushing and pulling motion and adhesion allow amoeba to travel faster in a group. Other effects arise when waves of cAMP are used. Figure 18 shows the motion of a group of amoeba when a wave of cAMP is passed over them. With no cAMP present, they round up into a circular shape to minimise the boundary in contact with the medium. As a wave of cAMP passes through, the group elongates as the amoeba at the front move up the wave front. This

[2]The size of group which reaches saturation velocity in experimental studies is much larger than the groups here as this is an artificial experiment, but the behaviour is the same.

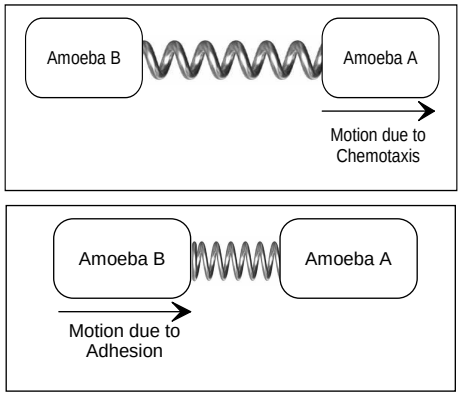

Figure 16: A very simple representation of how surface adhesion allows Dd amoeba to move faster in a group. The top image shows Amoeba A moving chemotatically, stretching the spring which connects it to Amoeba B. The bottom image shows the spring contracting and pulling Amoeba B forwards once Amoeba A has anchored.

elongation makes the amoeba at the rear of the group move forward in an attempt to minimise the surface once again.

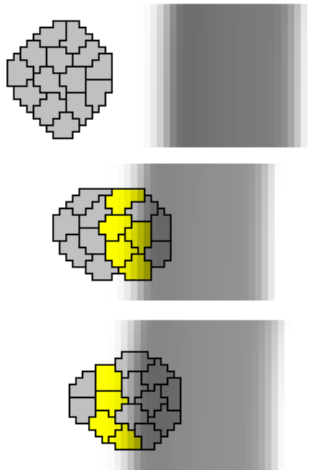

Figure 17: Three screen shots showing a wave of cAMP passing through a group of amoeba. The grey amoeba are Refractory and the yellow are Excited.

4.4 Spiral formation without auto-cycling cells

The final experiment in the project concerns the initial triggering mechanism which starts the aggregation phase. The aim of this experiment was to determine whether the spirals could be generated without using auto-cycling amoeba.

The experiment involved removing the auto-cycling amoeba from the simulation and adding small bursts of cAMP at random sites containing amoeba each iteration.

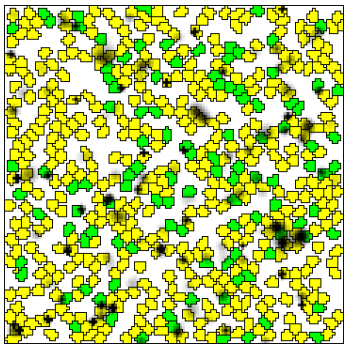

Figure 18: Screenshot of the amoeba with the addition of random noise. Note that the noise has been artificially enhanced for printing purposes. All parameters are the same as those in Figure 19.

The physical reasoning is that when the amoeba begin to starve, they all emit small amounts of cAMP spontaneously, with no special triggering amoeba to start the process.

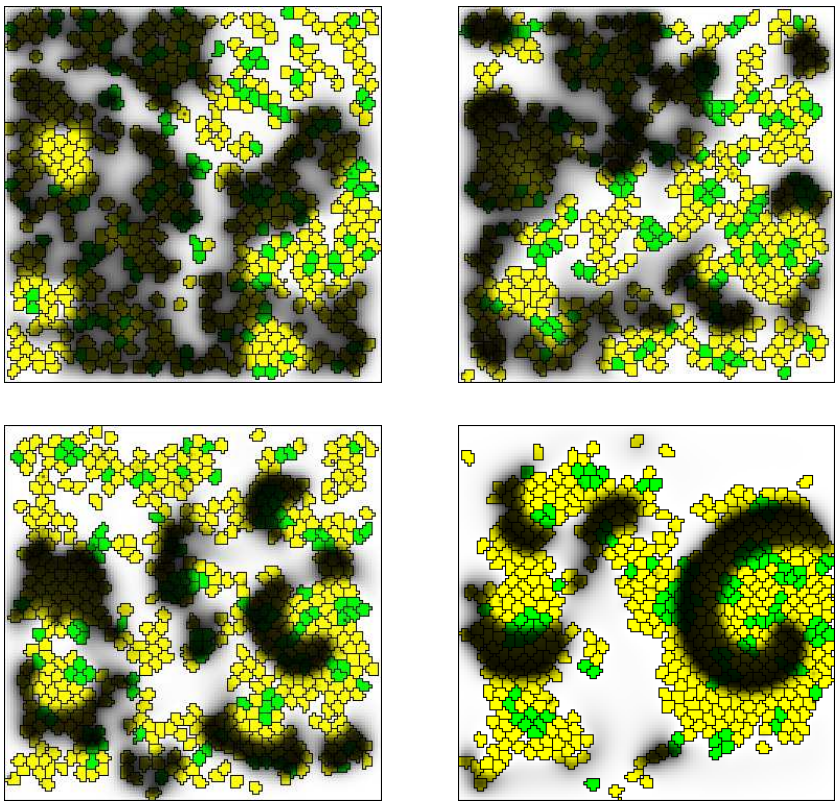

Figure 19: The formation of spiral waves of cAMP due to the addition of small amounts of cAMP at random locations.(a) After Initialisation, (b) 500 iterations, (c) 1,000 iterations, (d) 3,000 iterations.

The results show that initially the noise creates disorder among the amoebas, but after a small amount of time, if a cAMP waves travels around a closed loop, a self sustaining spiral is formed which then controls the aggregation process.

5 Conclusion

The aim of this project was to show that a model constructed from a set of relatively simply rules could reproduce complex behaviour using Dictyostelium Discoideum as the biological system to study. The majority of the model was based on the work of Dr. Paulien Hogeweg, who was contacted during the project and helped solve some of the problems encountered during implementation. Two different ways of modelling the cAMP dynamics were implemented, one based on Dr. Hogewegs work, and one created by the author, both of which produced realistic results.

The results obtained using the model show a strong similarity to the real Dictyostelium Discoideum, reproducing the streaming behaviour and the spiral patterns during aggregation. The model was then used to examine how the streams form and why the amoeba move faster when in a group than travelling individually. Finally, a result which has not been previously seen, the model was used to show that auto-cycling amoeba are not necessary to trigger the aggregation process.

Initially it was hoped that the model could be extended to three dimensions and that the formation of the mound and possibly the slug could be reproduced. Although the model was extended to three dimensions, further work is required for the amoeba to form the mound. In future work it would be interesting to study how the mound forms and what causes it to topple over.

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6 Appendices

How to use the Model

At present, not all of the model parameters have been implemented in a Graphical User Interface, therefore it is necessary to go into the code to change some settings. The model is contained in three files:

- DictyPDE.java - This contains the core simulation class. The code has been commented and all variables defined clearly. It also has a main method for debugging purposes.
- DictyPDEGUI.java - This contains the visualisation and GUI classes. It has a small control frame which allows the user to change some variables while the simulation is running. The left hand panel displays the state of the Cellular Automata and the right hand panel shows the cAMP field.
- MersenneTwisterFast.java - This contains the random number generator used in the model.

To run the code, copy all files into the working directory, compile them with
`javac DictyPDEGUI.java`
and run it with
`java DictyPDEGUI`.

Source Code

Richard Hanes

MPhys Project Personal Statement 2006

I decided on my project title from a list of options supplied by my Supervisor. I decided to take on a project whose aim was to reproduce some aspects of single celled organisms, and to try and find interesting behaviour from this model. This behaviour was very general to start with and did not specify the type of organism, only a few basic properties, movement/growth, nutrition, division and death.

I started writing the code for this project a few weeks into the start of the first term using the project aim sheet supplied by my supervisor. In hindsight, I did not do enough background reading at this point, and so wrote a model which incorporated the basic properties from scratch. The model did recreate movement/growth, nutrition (from a diffusive field) and death, and I had started on the division algorithm when I presented the model at one of the informal NANIA meetings just before Christmas. Questions from colleagues at the presentation highlighted the fact that, although the model was interesting, it had no real direction and the use of extended bodies seemed to be an over complication.

After discussion with my supervisor, I went home for christmas and studied the work of Prof. Hogeweg in more detail. She had created a model of the slime mould Dictyostelium discoideum (Dd) which included movement, adhesion and chemical signalling (cAMP in a diffusive field). After more discussion with my supervisor, I decided to start from scratch and so I started to write a model to reproduce the work of Dr Hogeweg, in the hope that something she had not analysed in detail could be the unique part of my project. Analysis of her work found two possible experiments which I could perform which she had not done. Firstly, the triggering mechanism for the start of the aggregation phase always started with 'Auto-Cycling' amoeba in all the literature on Dd. These 'Special' amoeba somehow knew when to start emitting and this did not seem physical. Secondly, as the amoeba aggregate they form a mound due to pressure from more amoeba joining the aggregate. An analysis of how the mound forms could also give some new results for my project, it would however, require the project to function in three dimensions.

During second term I reviewed articles on Dd, summarised in the Background section of the report. I managed to implement the Cellular Automata part of Hogewegs model, and used a different, simpler approach to model the cAMP signaling (Explicit Model). After unsuccessfully recreating the spirals, I implemented the FHN equations (Implicit Model) Hogeweg used to model the cAMP field, although I have read through a derivation of these equations, I have not analysed them myself and simply used them as described by Hogeweg. I attempted to convert the simulation to three dimensions but ran out of time, so concentrated on the two dimensional model. I tweaked the Explict model and managed to reproduce experimental results with it. I then used both models to explore some of the co-operative behaviour of Dd and managed to trigger the aggregation without auto-cycling amoeba, a result which neither me or my supervisor have seen in any literature.