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Incorporating FUCCI technology in discrete random walk models of collective cell spreading

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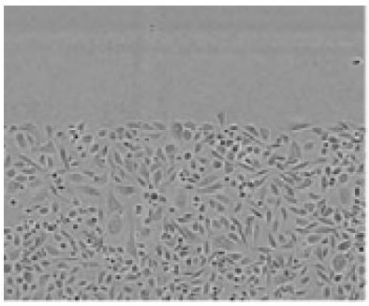
Abstract

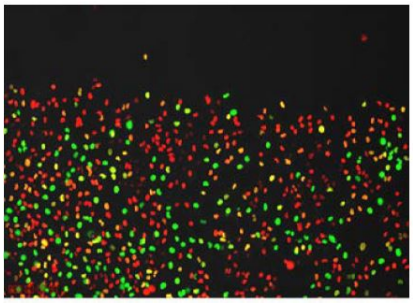
Scratch assay experiments are conducted to observe the behaviour of cancerous cells in wound healing and tumour growth scenarios. FUCCI is a new technology which allows the age of melanoma cells to be observed on a scratch assay as different colours. We developed a lattice-based random walk model that incorporates cell migration, cell-to-cell crowding, and we represented various ages of cells within the cell cycle as a series of interacting sub-populations. Numerical simulations were used to explore how the population-level behaviour depends on the individual-level mechanisms. The Gillespie Stochastic Simulation Algorithm was used to incorporate stochasticity into the random walk model by randomly determining the time between events. To provide more formal insight, we applied averaging arguments to produce a series of new continuum reaction diffusion models that can be used to describe experiments performed with FUCCI. An accurate and easily adaptable model was developed that experimentalists can use to model melanoma cell behaviour.

1 Introduction

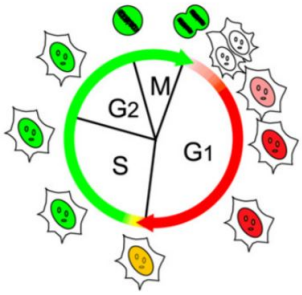
The behaviour of cancerous cells is a widely studied area of biology and has been thoroughly modelled in mathematical biology. Experimentalists observe cell migration and proliferation by conducting a typical scratch assay experiment. Melanoma cells are placed on a petri dish and a scratch is made through the dish to remove some of the cells (Figure 1(a)). Experimentalists then observe over a 5 day period how the cells move to fill the gap and how the number of cells increases due to proliferation. These experiments have the potential to be useful in predicting wound healing and tumour growth.



(a) Typical scratch assay [1]



(b) Scratch assay with FUCCI [2]



(c) Melanoma cell cycle [3]

Figure 1: Experimental scratch assays

There exists several discrete and continuous mathematical models which model melanoma cell migration and proliferation. Discrete models take the form of lattice-based or lattice-free random walks. Baker & Simpson investigate a discrete birth-death-movement process for an initially uniformly seeded lattice [4]. The Fisher-Kolmogorov model is a continuous model which considers cell migration and proliferation in terms of a partial differential equation (PDE). Jin *et. al* studies the diffusion and proliferation parameters required for the Fisher-Kolmogorov model [1].

The fluorescent ubiquitination-based cell cycle indicator (FUCCI) is a new technology that allows experimentalists to monitor cell age, as well as migration and proliferation, on a scratch assay [5]. The stages of the melanoma cell cycle are observed as different colours on the scratch assay as seen in Figure 1(b). Figure 1(c) indicates how melanoma cells transition through the cell cycle. FUCCI highlights cells in the G1 phase as red, the S phase as yellow, and the S/G2/M phases as green.

In this study, we develop a lattice-based model which incorporates cell migration, cell-to-cell crowding and cell phase transitions. To incorporate each phase of the melanoma cell cycle, we consider 3 interacting sub-populations on a hexagonal lattice. We apply averaging arguments over a series of time intervals to produce a series of new continuum reaction, diffusion models that simulate experiments performed with FUCCI. In this report, we explain the development of the lattice-based model and the use of the Gillespie Stochastic Simulation Algorithm (SSA). We define the sub-population interaction rules and the assumptions made in this model. We then investigate the density of red, yellow and green cells over a 5 day period and further investigate the applications of this model.

2 Mathematical Model

We develop a discrete random walk model for collective cell spreading which incorporates FUCCI technology on a hexagonal lattice. The physical scratch assay is modelled by a hexagonal lattice and cells are represented as agents. The hexagonal lattice is structurally defined in Figure 2 below where an agent can move to any vacant neigbouring lattice site. Individual agents, belonging to different sub-populations, interact on this lattice in terms of a random walk to simulate melanoma cell migration, proliferation and cell phase transitions.

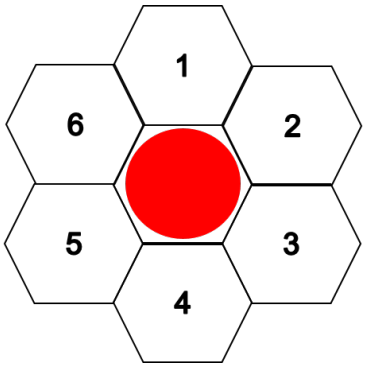


Figure 2: Lattice-based model

Agents participate in migration or transition events. We use the SSA to determine which event occurs and the time between events. Discrete time steps are used to update the system from $t = 0$ until $t = T$. At $t = 0$, the lattice is initialised with a set amount of agents in a specified location. The initial agent pack can be initialised randomly or based on percentages of each sub-population. Agents can either be placed in the middle of the lattice or on the left hand boundary of the lattice. The length and density of the initial agent pack is

specified along with the boundary conditions. When the initial agent pack is placed in the middle of the lattice, we implement a periodic boundary condition. Otherwise, a no flux boundary condition is used when the agent pack is placed on the left hand boundary.

We base the approach to determine which event occurred in a discrete time interval, $[t, t+\tau]$, on the work of Baker and Simpson [4]. The total propensity function, a_0, is defined as the sum of the individual propensity functions for N migration events and M transition events:

$$a_0 = \sum_{k=1}^{N} a_k Q_k + \sum_{k=1}^{M} b_k Q_k \tag{1}$$

where Q_k is the number of agents in the k^{th} sub-population, and a_k and b_k are the rates of migration and transition for the k^{th} migration and transition event respectively. We sample the time between events i and $i+1$ as $\tau = (1/a_0)\log(1/u_1)$ where $u_1 \sim U[0,1]$. We then determine which event occurred in $[t, t+\tau]$ according to $R = a_0 u_2$ where $u_2 \sim U[0,1]$. The position of R in the interval $[0, a_0]$ determines which migration or transition event occurs. After the selected event is attempted, we let $t \to t+\tau$ and repeat the process outlined above until $t \geq T$.

2.1 Sub-population interaction rules

Interaction rules between the different sub-populations are defined based on the phases of cells in the melanoma cell cycle. Each sub-population can participate in migration events where a selected agent attempts to move to a vacant neighbouring lattice site. Once an agent from a particular sub-population is selected, a potential neighbouring migration site is chosen. If the migration site is vacant, the agent is removed from the original site and placed in the chosen migration site. However, if the potential migration site is occupied, the migration event is aborted. This incorporates the effects of cell-to-cell crowding into the model.

Similarly, agents from each sub-population can transition into another sub-population. When agents participate in transition events, they remain stationary. According to Figure 1(c) above, red agents can transition into yellow agents, and yellow agents can transition into green agents. The number of agents in each sub-population is updated appropriately after each event. When a green agents transitions into a red agent, a red daughter cell is placed in a vacant, neigbouring proliferation lattice site. If the chosen proliferation site is occupied, the transition event is aborted. Similarly, the sub-population counts are updated after this event such that the number of red agents in increased by 2 and the number of green agents is decreased by 1.

2.2 Variables

Variables such as the lattice length, initial agent pack length and total time of simulation are defined. Table 1 below outlines the variables required for the model.

Variable Name	Symbol	Value
Lattice length	L	100 μm
Agent pack length	L_0	20 μm
Lattice spacing	Δ	1 μm
Simulation time	T	120 hours
Initial agent density	-	0.99
Number of simulations	-	200
Percentage Red	-	33 %
Percentage Yellow	-	33 %
Percentage Green	-	33 %

Table 1: Table of simulation variables

We define the migration and transition rates of each sub-population based on the work of Jin *et.al* [1]. Refer to Table 2 below.

Phase	Agent colour	Population size (No. agents)	Migration rates (h^{-1})	Transition rates (h^{-1})
G1	Red	Q_r	$P_r = 1.25$	$K_{ry} = 0.1$
S	Yellow	Q_y	$P_y = 1.25$	$K_{yg} = 0.1$
S/G2/M	Green	Q_g	$P_g = 1.25$	$K_{gr} = 0.1$

Table 2: Table of migration and transition rates

From the parameters defined in Table 2, we can state the total propensity function defined in Equation 1 as:

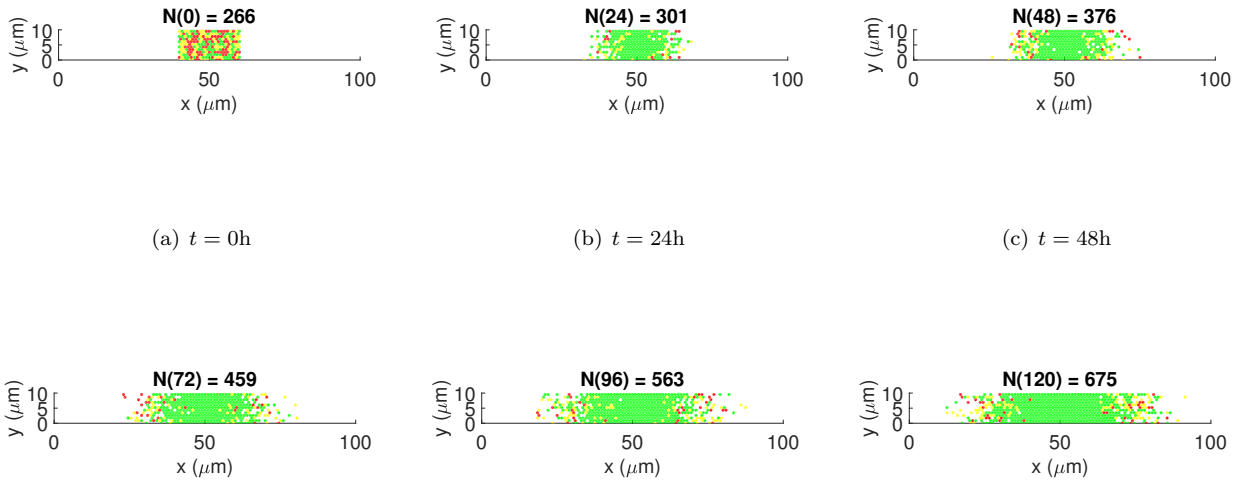
$$a_0 = (P_r + K_{ry})\,Q_r + (P_y + K_{yg})\,Q_y + (P_g + K_{gr})\,Q_g \tag{2}$$

2.3 Assumptions

There are several assumptions made throughout this model. By using a hexagonal lattice, we assume that the shape of a melanoma cell and its preferred direction of movement. Additionally, by implementing periodic boundary conditions, we assume an infinite domain such that the agents never touch the boundaries of the lattice. As death is not included in this model, we assume an infinite, growth cell cycle. A lattice-free model that includes a death event may model the biological process captured by FUCCI more accurately.

3 Results

The above parameters in Table 1 and 2 were used to simulate melanoma cell migration, proliferation and phase transitions over a 120 hour period. Figure 3 illustrates agent migration, proliferation and phase transitions on the lattice at 24 hour intervals.

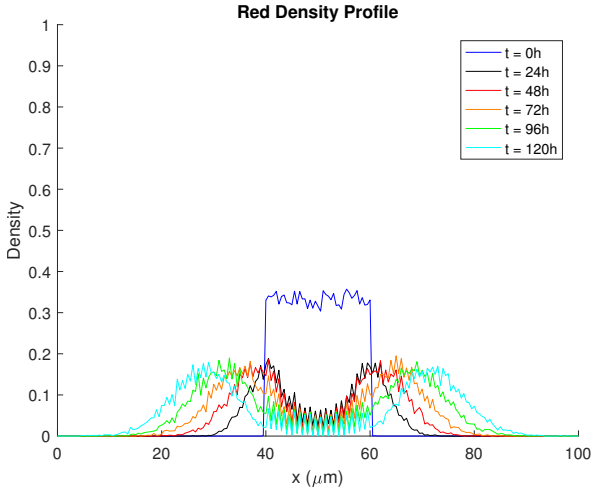


(a) $t = 0$h (b) $t = 24$h (c) $t = 48$h

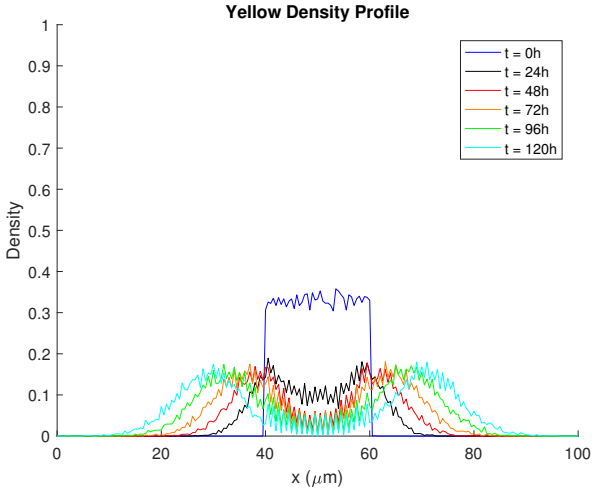
(d) $t = 72$h (e) $t = 96$h (f) $t = 120$h

Figure 3: Lattice snapshots over 120 hours at 24 hour intervals

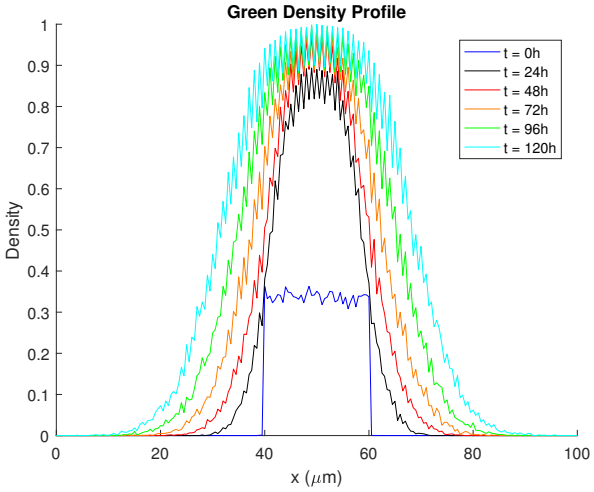
To analyse the density of red, yellow and green agents on the lattice, cell density profiles were constructed. As the initial lattice was densely packed from top to bottom, no net movement of agents in the y direction occurred. Hence, the density of agents with respect to the x position on the lattice was considered. The density of red, yellow and green agents on the lattice was averaged over 200 simulations to determine the overall behaviour of each cell phase. Figures 4(a)-(c) show the cell density profile for red, yellow and green agents respectively, and Figure 4(d) illustrates the total cell density profile.



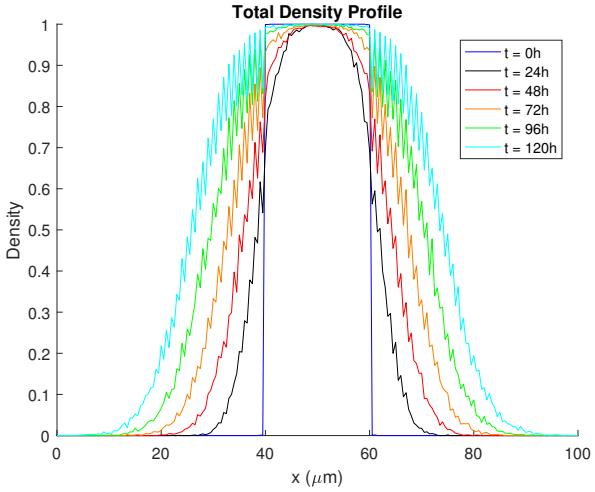
(a) Red cell density profile



(b) Yellow cell density profile



(c) Green cell density profile



(d) Total cell density profile

Figure 4: Density profile for each sub-population and the total population

4 Discussion

A lattice-based random walk model, which describes melanoma cell migration, proliferation and phase transitions has been developed. To determine the overall cell behaviour in each cell phase, we consider the lattice snapshots (Figure 3) and the cell density profiles constructed above (Figure 4). Figure 3(a) illustrates that the same amount of red, yellow and green agents were initially placed in the middle of the lattice. This is similar to the scratch assay shown in Figure 1(b) above, however a scratch is made on both sides of the agent pack. This allows cell migration to be observed in two directions.

Figure 3(b) demonstrates that after 24 hours, the majority of agents in the middle of the lattice have transitioned into green agents. However, the green agents have not been able to transition back into red agents due to space constraints. It is also evident that proliferation has occurred as the number of agents has increased from

266 to 301 after 24 hours. After 120 hours, cell migration, proliferation and cell phase transitions are observed with the number of agents increasing to 675.

It is evident from the dark blue line on Figures 4(a)-(c) that the initial density of red, yellow and green agents placed on the lattice was 0.33. Figures 4(a) and (b) illustrate that after 24 hours, the density of red and yellow agents in the middle of the lattice has significantly decreased. It is also observed that after 120 hours, the red and yellow agents have spread out toward the boundaries with a density of approximately 0.2. This corresponds to behaviour seen in Figure 3 above.

Figure 4(c) demonstrates that after 24 hours, the density of green agents in the middle of the lattice has significantly increased due to cell phase transitions. It is also evident that the density of green agents on the lattice continues to increase throughout the duration of the simulation and the agents migrate toward the boundary. Figure 4(d) illustrates that the initial agent density of the lattice was 0.99 and that over 120 hours, the cells migrate toward the boundary and the total occupancy of the lattice increases, indicating that proliferation has occurred.

There are several advantages and disadvantages of this model. The use of the discrete stochastic simulation means that lattice snapshots can be observed at certain time intervals, i.e. every 24 hours. Additionally, the migration and transition parameters can easily be varied to observe the effects of a biased random walk. The initial placement of agents on the lattice can be varied to a more scattered form. This flexibility allows multiple scenarios of wound healing and tumour growth to be modelled. Furthermore, once this model is validated and calibrated with experimental results, it can be used to simulate and predict melanoma cell behaviour without conducting experiments. If the FUCCI technology advances such that more cell phases can be observed, this model can easily be adapted by including more sub-populations. A death event could be included into the model to account for the carrying capacity of the cell population.

While the advantages of this model are numerous, this simulation is computationally demanding as averaging arguments need to be applied over multiple simulations. PDEs could be used to model FUCCI and are relatively quick to solve numerically. While the lattice-based model is relatively accurate, cell movement is limited by the structure of the lattice. A lattice-free model would better approximate the unbiased movement of cells on the scratch assay. However, would be harder to include the effects of cell-to-cell crowding into a lattice-free model.

5 Conclusion

Overall, the lattice-based random walk model that was developed is an accurate, yet simple and easily adaptable tool, that experimentalists can use to model melanoma cell behaviour. The use of 3 interacting sub-populations on a hexagonal lattice easily incorporated melanoma cell phase transitions into a random walk model of cell migration and proliferation. The SSA included stochasticity into the model by randomly determining which

event occurred and the time between events. The effects of cell-to-cell crowding was included in the model to accurately model cell migration and proliferation on a scratch assay. This lattice-based, random walk model of collective cell spreading can be adapted to model multiple wound healing and tumour growth scenarios.

References

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