A Discrete Cell Migration Model [1]

James Nutaro, Kara Kruse, Richard Ward, Elizabeth O'Quinn, Matthew Woerner, Barbara Beckerman
Oak Ridge National Laboratory
Oak Ridge, Tennessee, USA
{**nutarojj;krusekl;wardrc1;oquinnec;woernermm;beckermanbg**}**@ornl.gov**

Stacy Kirkpatrick, Deidra Mountain, Oscar Grandas
University of Tennessee Medical Center
Knoxville, Tennessee, USA
{**SKirkpat; DMountain; OGrandas**} **@mc.utmck.edu**

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Abstract

Migration of vascular smooth muscle cells is a fundamental process in the development of intimal hyperplasia, a precursor to development of cardiovascular disease and a potential response to injury of an arterial wall. Boyden chamber experiments are used to quantify the motion of cell populations in response to a chemoattractant gradient (i.e., cell chemotaxis). We are developing a mathematical model of cell migration within the Boyden chamber, while simultaneously conducting experiments to obtain parameter values for the migration process. In the future, the model and parameters will be used as building blocks for a detailed model of the process that causes intimal hyperplasia. The cell migration model presented in this paper is based on the notion of a cell as a moving sensor that responds to an evolving chemoattractant gradient. We compare the results of our three-dimensional hybrid model with results from a one-dimensional continuum model. Some preliminary experimental data that is being used to refine the model is also presented.

1 INTRODUCTION

This paper introduces an individual cell migration model that is based on the notion of a cell as a moving sensor. In this model, each cell is assumed to move constantly at a fixed speed. The cells live in a solution that contains a chemical attractant, and cells tend to move towards stronger attractant concentrations. This is accomplished by sensing the local concentration gradient.

A relatively simple and very intuitive sensor model is used to reproduce several essential features of observed cell migration paths (see, e.g., (Maheshwari and Lauffenburger 1998; Tranquillo 1990)). The cell sensor apparatus is described by two parameters; sampling frequency and filter gain. A cell can change direction when it acquires a new sample, and it moves in a constant direction between sampling instants. The filter gain amplifies the perceived gradient at the cell location, in effect boosting the gradient signal to noise ratio. The cell always moves in the direction of the perceived gradient.

New locations for individual cells are calculated at the sampling instants. Cell positions in between sampling events can be easily determined by following the cell's velocity vector from its last computed position. The diffusion of the chemoattractant is simulated in tandem with the discrete event migration model using the approach described in (Nutaro, Kuruganti, and Shankar 2007). New concentrations are computed as needed using a variable time step finite difference approximation of the diffusion process. Concentration and gradient values at points not on the spatial finite difference grid are computed with an interpolating polynomial.

The motion model described in this paper shares two important features with the discrete cell migration model described in (Jabbarzadeh and Abrams 2005). The first is an explicit tendency for cells to persist in a particular direction of motion. The second is a gain that amplifies the perceived chemoattractant gradient and helps the cell to stay oriented even when the gradient is very small.

Our proposed model achieves the first effect by assuming constant motion between sensor sampling instants. At each sampling instant, the cell selects a new direction of motion based on the current value of the gradient at the sample location.

In contrast to our approach, the model presented in (Jabbarzadeh and Abrams 2005) uses a cell memory parameter to determine how much of the previous direction is retained when determining a new direction. With their model, direction changes are computed at each step of the chemoattractant

diffusion simulation. Because of this, the time that a single cell persists in one direction is not controlled directly. Rather, the memory parameter ensures a persistence time that is correct on average, but may not reflect a valid cell behavior over short time intervals.

The two models are nearly identical in their use of a gain parameter. However, the gain is described explicitly in our proposed model, and it can be linked directly to more fundamental cellular features through the cell's chemotactic index. The signal gain described in (Jabbarzadeh and Abrams 2005), on the other hand, is specified indirectly by a combination of chemoattract sensitivity and memory terms. Moreover, these model parameters are not linked explicitly to more fundamental cellular processes.

The major deviation of our proposed model from the one presented in (Jabbarzadeh and Abrams 2005) is the simulation protocol. In that model, the description of the migration process is closely intertwined with the time stepping numerical scheme that is used to simulate the diffusing chemoattractant. The chemoattractant diffusion process is solved using an integration time step Δt_{conc}. The chance that a new position is computed for any cell at each integration time step is $\Delta t_{conc}/\Delta t_{cell}$, where Δt_{cell} is the average time between cell motion calculations. When a new position is calculated, the cell is moved a distance that is determined by sampling a normally distributed random variable.

This probabilistic approach to cell motion allows for non-physical behaviors by individual cells. In particular, there is no restriction on the instantaneous speed of a cell. A large sample from the random variable that determines distance can catapult the cell through space. Similarly, it is possible for a cell to move very rapidly by taking a step in space at each simulation step in time. So, while the average speed of a cell, and the average speed of the cell population, looks right, individual cell behaviors can be quite arbitrary.

Our model solves this problem by modeling the connected diffusion/migration processes as a hybrid system. The cell motion is simulated as a discrete event system, with events taking place at the sampling instants. The diffusing chemoattractant can be sampled by a cell at any instant. This is accomplished by encapsulating a suitable numerical scheme within a discrete event process (see (Zeigler, Praehofer, and Kim 2000)).

This formulation of the model has three immediate advantages (Nutaro, Kuruganti, and Shankar 2007);

1. The description of cell behaviors is self-contained; influencing variables are modeled as input to the cell and observable cell properties as output from the cell,

2. The numerical algorithm used to simulate the diffusion process is encapsulated; the process is sampled by individual cells as required, and

3. The model can be easily extended to include dynamic interactions between the individual cells and the chemical environment in which they live.

The simplifications in the sensor model and relative determinism of the cell motion model are aimed at producing a simple, functional model of the migration process. The need for functional, as opposed to mechanistic or phenomenologically detailed, models for the study of biological systems is discussed in (Hartwell, Hopfield, Leibler, and Murray 1999; Pronk, Polstra, Pimentel, and Breit 2007). Our model describes cell migration behaviors in a minimalistic way while preserving two specific features of the system; 1) physical viability of individual trajectories and 2) statistical agreement with continuum migration models. Specifically omitted are observed variability in cell turning frequencies and speeds (see, e.g., (Alt 1980; Rivero, T, Tranquillo, Buettner, and Lauffenburger 1989)).

Our proposed migration model is described in Section 2. In Section 3, our discrete cell migration model is related to a common diffusion-advection type continuum migration model. Simulation experiments conducted with the model are described in Section 4. Section 5 discusses planned model extensions and some initial experimental data that supports it. Section 6 concludes the paper with a discussion of several planned validation experiments and extensions to the model that may be required in that context.

2 DISCRETE CELL MIGRATION MODEL

The model describes the migration of individual cells in the presence of an evolving chemoattractant gradient. The distribution $c(t,\bar{x})$ of the chemoattractant in time and space is described by the diffusion process

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c \qquad (1)$$

with boundary conditions that represent a closed container. Cells will tend to migrate up the chemoattractant gradient ∇c.

The cell trajectory model is built from three basic assumptions. First, a cell's gradient detection mechanism can be modeled by a sensor with sampling frequency f and signal gain G. The cell moves at a constant speed, and it changes direction only when a change in its environment is detected. That is, it can only alter its direction of travel at a rate $1/f$. Between sensor samples, the cell moves in a constant direction.

The migration trajectory of each cell is described by

$$\gamma_n = G\nabla c + \lambda_n \qquad (2)$$

$$p_{n+1} = p_n + \frac{s}{f}\frac{\gamma_n}{\|\gamma_n\|_2} \qquad (3)$$

where p_n is the location of the cell at the nth sampling instant, c is the chemoattractant quantity at the sampling time, λ_n is a

randomly oriented unit vector, s is the cell speed, G is the sensor gain, and f is the sensor sampling frequency. Equation 2 determines the cell's direction of travel γ as a function of the amplified chemoattractant gradient and noise term λ. Equation 3 moves the cell in the selected direction with a speed s.

The parameters for this discrete cell model are summarized in Table 1.

Symbol	Use
D_c	Chemoattractant diffusivity
c	Chemoattractant
p	Position of the cell
f	Cell sampling frequency
s	Cell speed
G	Cell sensor gain
λ	Cell sensor noise direction
n	Sampling instant for the cell, $t = n/f$

Table 1. Discrete migration model parameters and variables.

3 CONTINUUM MODELS

A three dimensional version of the discrete cell migration model can be related to a one dimensional, advection-diffusion type continuum migration model. The continuum model describes cell concentration u in time t and space by

$$\frac{\partial u}{\partial t} = \frac{\partial}{\partial x}\left(\mu\frac{\partial u}{\partial x} - \chi u\frac{\partial c}{\partial x}\right)$$

Here, χ is a the chemotactic coefficient and μ is the random motility (i.e., cell diffusion) coefficient. In what follows, μ and χ are taken to be constant about the values c and $\partial c/\partial x$ that are considered.

To obtain a one dimensional continuum model from the discrete three dimensional model, we assume that the chemoattractant gradient in the y and z directions is negligible. Denoting vector components with subscripts, and noting that $\partial c/\partial y = \partial c/\partial z = 0$, we can write the cell velocity v_x in the x direction as

$$v_x = \frac{s\left(G\dfrac{\partial c}{\partial x} + \lambda_x\right)}{\sqrt{\left(G\dfrac{\partial c}{\partial x} + \lambda_x\right)^2 + \lambda_y^2 + \lambda_z^2}} \qquad (4)$$

where c is the concentration given by Eqn. 1. Motion of the cell in the x direction is then given by

$$p_{n+1} = p_n + \frac{v_x}{f}$$

Notice that the cell velocity in the x direction will vary even though the cell is moving with constant speed in three dimensions. The maximum speed of the cell in the x direction is

s, with $v_x < s$ indicating random motion in the y and z directions.

The continuum model's chemotactic and random motility coefficients are related to the cell gain, sampling frequency, speed, and spatial dimensionality d of the continuum model by (see (Rivero, T, Tranquillo, Buettner, and Lauffenburger 1989; Maheshwari and Lauffenburger 1998; Alt 1980))

$$\mu = \frac{1}{d}\frac{s^2}{f} \qquad (5)$$

$$\chi = E[v_x] \qquad (6)$$

where $E[v_x]$ is the expected value of v_x.

It is apparent from Eqn. 4 that χ is a function of the gain G and the chemoattractant gradient $\partial c/\partial x$. This observation can be used to relate G and the cell chemotactic index ϕ (denoted CI in some papers). The chemotactic index is related to $E[v_x]$ and s by (see, e.g., (Rivero, T, Tranquillo, Buettner, and Lauffenburger 1989; Farrell, Daniele, and Lauffenburger 1990; Maheshwari and Lauffenburger 1998))

$$E[v_x] = \phi s \qquad (7)$$

Denoting $E[v_x]$ by $E_x(G)$ to emphasize its dependence on the sensor gain and substituting this expression into Eqn. 7 gives

$$\phi = \frac{E_x(G)}{s}$$

Given s and ϕ, the gain G is a real, positive root of

$$\frac{E_x(G)}{s} - \phi = 0 \qquad (8)$$

which can be found numerically.

4 NUMERICAL EXPERIMENTS

The relationship between the discrete and continuum migration models can be illustrated with a simple numerical experiment. For this experiment, the chemoattractant gradient along the x axis is fixed at $\partial c/\partial x = 1.0 \times 10^{-7}$ M/mm. The individual cell parameters are taken to be

$$s = 2.0 \times 10^{-3} \text{ mm/min,}$$
$$f = 3.3 \times 10^{-2} \text{ 1/min,}$$
$$\text{and using } \phi = 0.2 \text{ gives}$$
$$G = 3.0 \times 10^{6}$$

These parameters are representative of a alveolar macrophage's response to the chemoattractant C5a (see (Farrell, Daniele, and Lauffenburger 1990); the approximate values from (Maheshwari and Lauffenburger 1998) are used

in this experiment). The continuum model parameters that correspond to these values are

$$\mu \approx 1.2 \times 10^{-4} \text{ mm}^2/\text{min, and}$$

$$\chi \approx 4.0 \times 10^{-4} \text{ mm}^2/\text{min} \cdot \text{M}$$

Using these parameters, the discrete migration model was exercised in a box with dimensions 7 mm $\times$ 7 mm $\times$ 7 mm. The corresponding continuum experiment took place on a line 7 mm in length. The cells were initially located about the 1 mm mark. The chemoattractant concentration was fixed at $c(t,x) = 1.0 \times 10^{-7}x$.
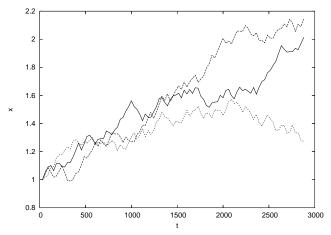

Figure 1. Trajectories produced by the discrete migration model for three cells. Each line traces the motion of a single cell along the x axis.
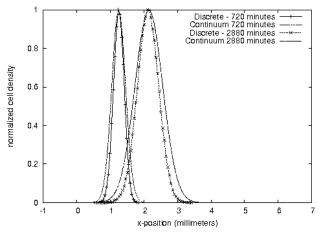

Figure 2. Comparison of cell densities produced by the continuum and discrete migration models.

Figure 1 shows the x position of several cells as a function of time when their trajectories are computed with the discrete migration model. The corresponding cell densities given by

the continuum and discrete models as a function of time and space are shown in Fig. 2. In both figures, the cell densities are normalized. Cell density for the discrete model is computed as the number of cells in an x-band 0.05 mm in width; a total of 100,000 cells were used in the experiment.

The difference in the width of the discrete and continuous cell density profiles is due to the relatively small cell population in the discrete migration model. In particular, the largest distance traveled by any of the 100,000 cells is about 2.4 mm, corresponding to an end position of about 3.4 mm at 2880 minutes. However, if 1,000,000 cells are used, the maximum distance traveled by any one cell is about 2.6 mm, corresponding to an end position of 3.6 mm. Similarly, with only 10,000 cells, the distance traveled shrinks to about 2.2 mm.

To actually observe values near the theoretical maximum travel distance of 5.76 mm would require a tremendous number of cells, which is infeasible without correspondingly tremendous computing resources. The continuum model, however, with its assumption of a massive population, predicts a density profile that includes measurable numbers of the rare travel distances, and hence the apparent discrepancy in the density profiles.

Table 2 summarizes the difference in the cell density profiles calculated with the continuum and discrete models. As the number of cells increases, the difference between the two models shrinks. This supports our hypothesis that the discrete model converges on the continuum model as the cell count becomes large.

Number of cells	Total Difference	Average Difference
10^6	4.68	0.0538
10^5	4.87	0.0560
10^4	5.39	0.0620
10^3	7.87	0.0905

Table 2. Total and average difference between the discrete and continuum models when computed with data points at 0.05 mm intervals.

5   CELL ATTACHMENT

It is our intention to validate the migration model with a series of Boyden chamber experiments. The Boyden chamber experiment requires placing a solution with suspended cells above a disc of material through which they will migrate. The chemoattractant diffuses from the bottom well, through the disk, and into the cell solution. This sets up a concentration gradient that motivates the cells to move through the barrier. The experimental setup is sketched in Fig. 3.

A cell can not begin to migrate until it has attached itself to the disc. Because the cells are initially suspended in solution, each cell will require some time to settle and attach before
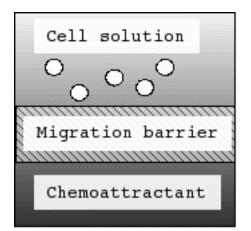
Figure 3. The basic elements of a Boyden chamber experiment.

it begins to move of its own accord. This process is not accounted for in the model as described above, which assumes instead that cells begin migrating immediately. The observable effect of this deficiency on the model will be less cell diffusivity and greater cell speed with respect to the experimental data.

We have begun to conduct a series of attachment experiments to quantify this effect. The experimental data will be used to construct a function that models the cell attachment time. Individual cells will use this function to select statistically valid attachment times.

Figure 4 shows the mean of the results obtained in two replicates of attachment experiments conducted for rat aortic smooth muscle cells. These experiments were carried out using subcultures of rat aortic smooth muscle cells (RASMC) derived from a male rat donor and obtained from Dominion Pharmakine, S.L. (Spain).

The cells were cultured in 2 T-75 cm^2 culture plates (Corning, Inc.; Corning, NY) in DMEM (Gibco, Invitrogen Corp.; Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 10 μg/ml gentamicin, and 0.25 μg/ml amphotericin B, (Cascade Biologics, Inc; Portland, OR), and incubated at 37°C in 5% CO$_2$. After cells reached confluency, one flask was changed to DMEM media with 0.1% bovine serum albumin (BSA) while the other flask remained in DMEM media with 10% FBS. Both sets of cells were incubated for an additional 18 hours. The confluent cultures were subsequently lifted with a treatment of trypsin/EDTA (0.05% / 0.53mM in HBSS, Gibco) and seeded into a six-well culture plate (9.4 cm^2 per well) (Sarstedt, Inc.; Newton, NC) for a total of 3 wells with DMEM+10% FBS and 3 wells with DMEM+0.1%BSA. The plate was incubated at 37°C in 5% CO$_2$.

At 30 minutes the plate was observed under a microscope and the percentage of cells attached to the plate surface was noted. This observation was repeated at 1, 1.5, 2, 3, 4, and 6 hours. The data shown come from two independent experiments. Also shown is the best fit of this data to an S-Curve function in the form

$$\frac{at^c}{1+bt^c}\,.$$

For the experiments using 0.1% BSA, the best-fit parameters, as computed with Mathematica's NonlinearFit algorithm, are

$$a = 25.6297\,,$$
$$b = 0.260103\,, \text{ and}$$
$$c = 4.43234\,.$$

For the experiments using 10% FBS, these parameters are

$$a = 18.3788\,,$$
$$b = 0.186569\,, \text{ and}$$
$$c = 4.8316\,.$$

The data and the results of the above empirical model are shown in Fig. 4.
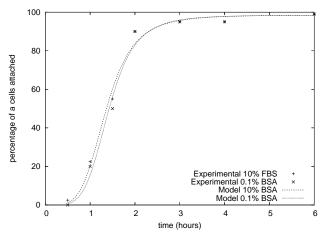

Figure 4. Attachment percentage as a function of time for rat aortic smooth muscle cells using two different media conditions.

6 CONCLUSIONS

In this paper, we have introduced a model that describes the migration of individual cells in the presence of a chemoattractant gradient. Our model is distinct from earlier, individually based cell migration models in three important ways (see, e.g., (Jabbarzadeh and Abrams 2005; Chaplain, McDougall, and Anderson 2006)):

1. The proposed model admits only feasible migration paths for individual cells. This is apparent in the explicit limit on cell speed, and the absence of long migration

trajectories for the small population simulation in Section 4. This contrasts sharply with the model described in (Jabbarzadeh and Abrams 2005), where the simulation protocol acts to control just the average speed of a cell, but places no limit on its instantaneous speed.

2. The model parameters are directly related to measurable cell attributes. The cell signal gain parameter in particular can be calculated if the cell speed and chemotactic index are known.

3. The simulation can be readily implemented using available hybrid system simulation techniques. This permits the cell migration model and numerical algorithm used to simulate continuous sub-processes to be described separately. In contrast to this, these two aspects are closely connected in the models described by (Jabbarzadeh and Abrams 2005; Chaplain, McDougall, and Anderson 2006).

We are planning Boyden chamber experiments that will provide data for validating the migration model. The experimental data will show cell density as a function of time on the chemoattractant side of the migration barrier. This data will be compared with density profiles, like those shown in Fig. 2, computed with the migration model.

As was noted in Section 5, the cell attachment process is expected to have a measurable impact on the experimental outcome. Because of this, we plan to add this process to our migration model prior to conducting the validation study. As part of the validation process, the we will determine the sensitivity of the model sensitivity to the attachment process. Notably, cell attachment is absent from continuum models that have been validated against Boyden chamber experiments. To our knowledge, Boyden chamber based validation studies of individual cell models have not been previously attempted.

REFERENCES

Alt, W. (1980). Biased Random Walk Models for Chemotaxis and Related Diffusion Approximations. *Journal of Mathematical Biology 9*, 147–177.

Chaplain, M., S. McDougall, and A. Anderson (2006, August). Mathematical Modeling of Tumor-Induced Angiogenesis. *Annual Review of Biomedical Engineering 8*, 233–257.

Farrell, B. E., R. P. Daniele, and D. A. Lauffenburger (1990). Quantitative Relationships Between Single-Cell and Cell-Population Model Parameters for Chemosensory Migration Responses of Alveolar Macrophages to C5a. *Cell Motility and the Cytoskeleton 16*, 279–293.

Hartwell, L. H., J. J. Hopfield, S. Leibler, and A. W. Murray (1999, December). From Molecular to Modular Cell Biology. *Nature 402*, C47–C52.

Jabbarzadeh, E. and C. F. Abrams (2005, July). Chemotaxis and Random Motility in Unsteady Chemoattractant Fields: A Computational Study. *Journal of Theoretical Biology 235*(2), 221–232.

Maheshwari, G. and D. A. Lauffenburger (1998, December). Deconstructing (and Reconstructing) Cell Migration. *Microscopy Research and Technique 43*(5), 358–368.

Nutaro, J., T. Kuruganti, and M. Shankar (2007, March). Seamless Simulation of Hybrid Systems with Discrete Event Software Packages. In *Proceedings of the 40th Annual Simulation Symposium*, Norfolk, Virginia, pp. 81–87.

Pronk, T. E., S. Polstra, A. D. Pimentel, and T. M. Breit (2007, March). Evaluating the Design of Biological Cells Using a Computer Workbench. In *Proceedings of the 40th Annual Simulation Symposium*, Norfolk, Virginia, pp. 88–98.

Rivero, M. A., R. T, Tranquillo, H. M. Buettner, and D. A. Lauffenburger (1989). Transport Models for Chemotactic Cell Populations Based on Individual Cell Behavior. *Chemical Engineering Science 44*(12), 2881–2897.

Tranquillo, R. T. (1990). Theories and Models of Gradient Perception. In J. Armitage and J. Lackie (Eds.), *Biology of the Chemotactic Response*, pp. 33–75. Cambridge University Press.

Zeigler, B. P., H. Praehofer, and T. G. Kim (2000). *Theory of Modeling and Simulation, 2nd Edition*. Academic Press.