

Computer Simulation and Analysis of a Growing Mammalian Cell Colony

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Abstract

All living creatures are made of cells - small membrane-bounded compartments filled with a concentrated aqueous solution of chemicals. This paper deals with simulation and characterization of a growing mammalian cell colony. A colony is modeled as a set of contacting deformable particles. Variation of size, orientation and form of a cell is assumed, although ellipsoidal shape of a cell is used as a basic form. Modeled cells were compared to AHH-1 human lymphocyte culture cells by visual inspection and with the help of the fractal analysis methods.

Keywords: geometric modelling, artificial life, implicit surface, collision detection, packing, human cell colony, fractal dimension.

1. Introduction

The problem of computer simulation for creating artificial life has been widely studied lately. Biology is one of the main sources of complex problems that require computer simulation. This paper deals with simulation of a growing mammalian cell colony. A colony is modelled as a set of contacting deformable particles. A particle can be substituted by a pair of new particles, modelling the process of cell division. From the geometric modelling point of view, this application has identified a complex problem of modelling arbitrarily shaped geometric particles, their collisions and packing. Before a computer-simulated model of a colony can be built, we have to develop algorithms for simulation of dynamic behaviour of particles and communication between them, controlling particles geometry and rendering.

The main goals of the work are:

- To provide better understanding of dynamical characteristics of the cell reproduction process,
- To apply a geometrical approach to modeling “communications” between particles,
- To study cell growth as a property of its behavior rather than as a property of internal physiology,
- To address how can such a simulation be validated.

The remainder of the paper develops as follows. The biological origin of the model is observed in the next Section. Section 3 is devoted to the related works. Section 4 describes our algorithm of colony growth simulation. Section 5 evaluates biological adequateness of the model.

2. Biological origin of the model

All living creatures are made of cells - small membrane-bounded compartments filled with a concentrated aqueous solution of chemicals. In all cells of higher plants and animals, mammals and human, the bulk of hereditary material - DNA - is isolated in a membrane-bound nucleus lying in cytoplasm (Trinkaus 1984). The nucleus is regularly ovoid or spherical and darkly staining, while the surrounding cytoplasm is more lightly staining (Freshney 1987). The high density, size and shape of nucleus are much more conservative than those of cytoplasm because the nucleus contains highly condensed genetic material. The nucleus usually is in the range of 3 to 14 microns in diameter, and most of mammalian cells are in the range of 5 to 50 microns. There is great variation of the appearance, size, and shape of cells, which reflects the different functions of different cell types. There is also dependency on the location of a cell and its neighborhood. The nucleus is usually

located close to the cell centre. Both the nucleus and the entire cell are enveloped by a membrane that is only 7.5 nm thick, and thus is often invisible under the light microscope.

Isolated from organism, cells can be grown *in vitro* on plastic or glass surface in the presence of liquid media containing all necessary ingredients (Freshney 1987, Hay et al. 1992). These are so-called cell cultures. In cell culture, cells are growing exponentially by cell division (duplication) while all necessary nutritional components are available, so that a single cell plated onto plastic surface after a certain number of generations forms a colony of cells. One colony consists of several cells to several thousand cells. Single cell division involves division of the cytoplasm which follows immediately after the division of the nucleus (Alberts et al. 1983). In most cells, the division of nucleus occurs by mitosis to ensure that each of two daughter cells gets equal DNA genetic content identical to that of the parent cell. Normal (non-tumour) cells usually form flat single-level colonies and have a limit of about 40 generations *in vitro* (Hayflick's limit) (Hayflick and Moorhead 1961). Tumour cells have no such limit, and are able to grow without obligatory single-layer growth and form multi-level colonies. Although cells in a colony can perform slow amoebic movements, cell-to-cell adhesion (cell junctions) is quite resistible to mechanical forces tending to separate cells. It was indicated that spaces between cells or between a cell and a surface are narrow, on the order of 15 to 25 nm. Currently there are several thousand cell lines used in research and industry (Hay et al. 1992), but the biology of cell colonies, namely the dynamics of their growth, is not enough investigated.

3. Related works

In the following overview we mention the related works of several types: artificial life, non-rigid bodies modelling, collision detection and packing. Artificial life endeavors to synthesize lifelike behaviours within computers. The key parameters for creating artificial life in virtual worlds have been identified by the authors of (Thalmann and Thalmann 1994). They notice that very simple creatures have been discussed and there have been little visual representation of living organisms in previous work. The authors of (Ransom and Matela 1986) mentioned that simulation techniques of cell division processes have their origins in the cellular automata. The authors use graph theory to represent cell division. The cellular automata approach is used to simulate botanical colony growth in (Kunii and Takai 1989). Now, various approaches should be integrated to create truly virtual worlds with autonomous living beings, plants, animals and humans with their own behaviour and real people should be able to enter into these worlds. Paper (Francon and Lienhardt 1994) presents methods of subdivisions or partitions of geometric spaces into cells (vertices, edges, faces, etc.) and basic operations to define sub-object behaviour.

The survey (Gascuel 1994) presents the state of the art in animation of non-rigid bodies. In particular, models based on the physical theory of elasticity in continuous media and discrete mechanical models which integrate discrete mechanical models are observed. This survey demonstrates some optimization techniques to specify constraints on the behaviour of the objects and collision detection and response algorithms. Constructing a virtual world requires the ability to simulate geometric collisions in order to provide collision avoidance, for example, in behavioural procedural modelling (Reynolds 1982). In (Sims 1990) a particle animation system using data parallel computation on the Connection Machine CM-2 is presented. The author emphasizes that particle systems are well suited for highly parallel computation. Lubachevsky and Stillinger (1990) investigated random disk packing of spheres which enlarge in size and offered an effective algorithm for determination of pair collisions. Detecting and eliminating collisions between plant organs while optimizing their packing are presented in (Fowler et al. 1992), whose proposed method arranges plant structures of varying sizes on arbitrary surfaces of revolution. Motion of time-dependent parametric surfaces is simulated in (Von Herten et al. 1990), where the authors state that the collision detection problem is insoluble for arbitrary time-dependent surfaces. To fill the gap in the collision detection problem for simulation of dynamic behaviour of rigid solids with implicitly defined surfaces, a numerical algorithm for collision detection was used in (Savchenko and Pasko 1994).

In (Fleischer et al. 1995) an approach was proposed for generating patterns of geometric elements using a biologically-motivated cellular simulation. The cellular development system forms the basis this work and combines cell-cell interactions, cell-cell adhesion, oriented particles, and surface constraints. The developed system is a powerful method of creating attractive computer graphics models of organic objects. The authors of (Fleischer et al. 1995) noticed that simulations can be slow for some kinds of cell programs, even for the spherical shapes.

Since artificial objects can seem very similar to natural ones, the question, *How can such an object or model be validated?*, appears with some risk to accept similarity as validation. Simulation leaves open the question of how to validate the results and we suppose that simulation has to reproduce experimental data, regularities and features of phenomena. Paper (Green, 1989) presents a good example how it is possible to validate rules and behaviour of complex structure via the help of computing simulation and visualization.

4. Simulation of colony growth

4.1 Particles geometry and intersection

We model living cells by geometric objects called particles. To define a geometric object the function representation is used: both simple and complex objects are represented by inequality $f(x,y,z) \geq 0$, where f is a real continuous function of Cartesian coordinates of a point. The function has negative values for points outside an object, positive values for inside points and zero values for points on the surface. Surfaces defined by equality $f(x,y,z) = 0$ are usually called implicit surfaces. This is very convenient for defining simple surfaces like spheres, ellipsoids, tori and others. For description of more complex form a set of orthogonal functions, for example, can be used to express a defining function as a set of spherical harmonics. Although, the numerical algorithms applied here were designed for arbitrarily shaped objects, in our application we limit ourselves by using a "noisy ellipsoid" as a particle:

$$f(x,y,z) = 1 - (x/a)^2 - (y/b)^2 - (z/c)^2 + \text{noise}(x,y,z),$$

where $\text{noise}(x,y,z)$ is a continuous stochastic function usually called "solid noise" and represented here by Gardner's series (Lewis 1989).

Points belonging to the interpenetration area of two particles can be detected with the help of the interference relation. This relation is defined by the bivalued predicate:

$$S(f_1, f_2) = \begin{cases} 0, & \text{if } f_1(p) \& f_2(p) < 0; \\ 1, & \text{if } f_1(p) \& f_2(p) \geq 0, \end{cases}$$

where f_1 and f_2 are defining functions of two particles; p is a point with coordinates (x,y,z) in an admissible domain P (a rectangular domain such that all points of intersection belong to it); $\&$ is a symbol of set-theoretic intersection. Analytical definitions of set-theoretic operations in the form of so-called R-functions have been studied by Rvachev (1974), see also (Shapiro 1994) for a survey. The simplest type of R-intersection is well-known: $f_1 \& f_2 = \min(f_1, f_2)$.

The following numerical algorithm for detection of interpenetration area is proposed:

1. Searching for the admissible domain P is performed through the intersection test of a couple of bounding boxes according to the next scheme: bounding boxes are projected onto three coordinate planes; projection intersections are determined in each plane; a rectangular domain such that all points of intersection belong to it is found. If this admissible domain is empty we have the case when the search for a collision point is of no use;
2. To find intersection points we use Sobol's quasi-random (or *LPT*) points (Sobol 1986). *LPT* points fill n -dimensional space more uniformly than traditional random number generators. Their use can lead at most to an $N^{2/3}$ error term (where N is a number of trials) in Monte Carlo integration applied to estimate an interpenetration area and the position of the centre of mass of a particle.

A particle deformed by contact with its neighbors can be described by as:

$$f_1^{\sim}(p) = f_1(p) - \sum_{j=2}^k f_j(p)$$

where f_1 is the defining function of an initial particle, f_1^{\sim} is the defining function of the deformed particle and f_j ($j = 2, \dots, k$) are defining functions of neighbor particles. Such a description of deformation was proposed in (Gascuel 1993). It has no physical meaning but it is enough to simulate deformations when contacting objects are described by functions of the same type.

4.2 Computer model of a growing cell colony

Mechanical modelling of a growing mammalian cell colony is supposed to provide a better understanding of dynamical characteristics of the cell reproduction process. We believe that it is possible to study cell's life as a property of behaviour rather than as a property of internal cell's physiology. Also, we realize that there is a huge number of factors which may directly cause or just influence cell division and colony growth. Some of these factors have been chosen as important ones for colony growth. The exact numerical data used in the model were based on experimental data obtained from cell biology literature for the "averaged" human or mammalian cell growing in a culture *in vitro* on some kind of surface. In order to study the opportunity of applying geometrical approach to formation of structure, and to estimate time performance of the numerical algorithm, we consider the simplified mechanical model of human cell colony growth. In the model, we take into account contact guidance and inhibition to control a preferred direction of cell motion as major factors. We consider that a living cell can be in two states: active (dividing) or passive (non-dividing). At the stages of colony growth which we take, cell death does not occur. We use the following rules of cell reproduction:

- reproduction of cells is a result of cell division;
- during a single division, a parent cell forms a daughter cell, i.e. a particle is substituted by a pair of new particles;
- the initial number of cells in a colony is one, maximal number is not defined;
- the number of feasible divisions of the initial cell is limited by 40 (Hayflick's limit, see above);
- for cells with open boundaries division occurs every 12 ± 2 hours;
- division does not occur if there is no enough space for both parent and daughter cells;
- cell division is presented by splitting both nucleus and cytoplasm by a plane passing through the nucleus center and a point with minimal distance between the nucleus and a cytoplasm membrane; orientation of the splitting plane is found by a genetic free space search algorithm (see below); a straight line orthogonal to the splitting plane defines the principal splitting direction, which can be modified by the genetic search;
- after division, the cytoplasm is split in two unequal parts and a nucleus in a daughter cell reaches the size of the one of a parental cell;
- within two hours after division a nucleus occupies a position with maximal distance from the cell's faces;
- variation of size, orientation and form of a cell is assumed, although ellipsoidal shape of a cell is used as a basic form;
- "solid noise" is used for attaching irregularity to a cell's shape;
- representation of a cell as a deformable (soft) particle assumes formation of faces between intersecting particles;
- cells that do not intersect with neighbors have random splitting direction.

In the outside physical world there are a lot of initially simple geometric forms that can produce complex forms in accordance with certain rules or principles. In our model, we use the "economy" principle: dividing cells strive to occupy space with the minimal deformation of each other. This principle defines the measure of fitness in free space search. Formulation of the numerical simulation algorithm follows.

Let the 3D domain, initial position and geometry of a cell which can be divided be given. We want to study formation of the shape of a cell colony as a function of time. Global state $S(t)$ of the system at time t is the vector: $(s_1(t), s_2(t), \dots, s_N(t))$, where N is a number of cells in a colony, $s_i(t) = (r_i, r_{-c_i}, A_i, B_i, C_i, W_{i1}, W_{i2}, W_{i3}, T_i)$ is the state of i -th cell; r_i is the centre position vector of a cell; r_{-c_i} is the centre position vector of the nucleus; A_i, B_i, C_i are semiaxes of the ellipsoid; W_{i1}, W_{i2}, W_{i3} are angular velocities of rotation for computing orientation of the cell; and T_i is time of last splitting of the given cell. We use discrete-event approach to advance the state of the system. The discrete-event approach means changing of the state in particular time moment when event (division) takes place. We use a global time step as a minimal time interval for advancement from event to event. At each time step we define free space, new positions and shapes of parent and daughter cells and calculate positions of nuclei that define splitting direction at the next splitting step. A nucleus is moving in a cell after splitting during two hours. Since this time is less than splitting time, we recalculate the position of each nucleus only once after splitting. We calculate the position of a nucleus and possible direction of splitting according to the following scheme:

- Modelling interpenetration area. As it was mentioned above, if particles i and j interact, we can define interpenetration area by $f_{ij} = f_i \& f_j$. Volume V_{ij} of this area is estimated with Monte Carlo integration. It is used to evaluate a fitness function in free space search and packing.
- Computing centre of mass. In order to calculate new position of a nucleus we use Monte Carlo integration. In this case volume of a particle deformed by its neighbours is calculated.
- Search for a point with minimal distance between new centre of mass and the cell's cytoplasmic membrane. We select an LPT point which does not belong to the deformed particle and has minimal distance from the centre of mass. A plane passing through this point and the centre of mass defines the splitting direction.

If a cell can reproduce, it has to define space for a daughter cell. This search for free space looks like communication with the cell's neighborhood. A cell is trying to find free space by pushing all neighbouring cells. Since a colony is continuous, only deformations of cells are allowed in our model. We simulate deformation by interpenetration of cells with a preassigned measure of tolerance. To search for free space, new positions and shapes of parent and daughter cells, we apply a genetic algorithm (GA).

GAs can be attractive in applications work for several reasons (Goldberg 1994):

- GAs can solve sophisticated problems quickly and reliably;
- GAs are easy to interface to models;
- GAs are extensible.

There are many variations of genetic algorithms. A GA is a mathematical search technique based on the principles of natural selection and genetic recombination. The simple genetic algorithm developed by Holland et al. (1986) begins with a set of random structures as possible solutions to the given problem. The structures are evaluated using a fitness weighted random selection scheme, and by applying genetic operators such as mutation and crossover to them. The resulting structures are then evaluated, the new structures with higher fitness join to the population to replace those old ones whose fitness measures are lower. Therefore, the structures with high strength tend to survive and those with a low strength to die out. The process is repeated until a satisfying structure (an optimal solution to the given problem) is obtained. In the genetic algorithm, binary strings represent possible candidate solutions. Each string position takes a value of 1 or 0. In this context, a string is composed of information encoding for modelling parameters $r_i, A_i, B_i, C_i, W_{i1}, W_{i2}, W_{i3}$. These parameters for a new cell are represented by a bit string, from which they can be decoded into real values. A fitness function in GA represents the environment for all individual strings. It provides a measurement of how good a given solution is. Usually, the probability of reproduction during a given cycle is proportional to the fitness of the individual string. The effect of this is that individuals with higher fitness have a higher expected number of offspring than individuals with lower fitness and have a greater chance to survive. The fitness function in our simulation has the following form:

$$Fitness = (V_i - \sum_{j=1}^k V_{ij}) / V_i$$

where, V_i is volume of a new cell, V_{ij} is volume of interpenetration area of the new cell with j -th neighbouring cell. The fitness function has a non-negative real number value in the range of [0,1]. For a new cell to be generated at time t , the GA is used to search for an optimal solution which has a highest fitness at that time.

Conceptually the algorithm consists of two steps: 1) a predictor which provides computation of a daughter cell and its fitness; 2) a corrector (works only if predictor was unable to generate a daughter cell with "good" fitness) which provides new generation calculations applying slight derivations of the cell obtained from predictor, new fitness computation for each generation, best cell selection between those generations, new center of a nucleus computation for the selected cell. Parallelization of the predictor step is a way to increase performance of the algorithm (Savchenko et al. 1995).

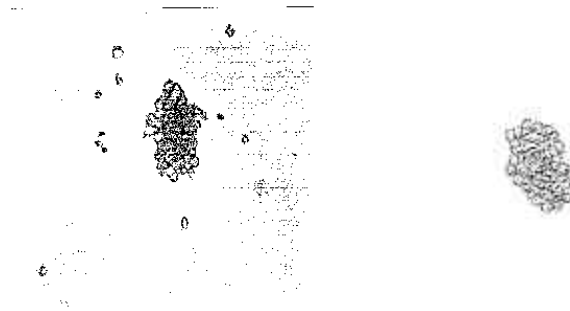


Figure 1. Images of AHH-1 human lymphocyte culture cells (left) and modelled cells (right) after contours extraction.

5. Evaluation of biological adequateness of the model and characterization of surface topography

Modelled cells (Fig 1, right) were compared to AHH-1 human lymphocyte culture cells (Fig. 1, left). Visually, modelled cells are a little different from real cells. They are not transparent, darkly coloured, and have an intensive dark shadow. Under microscope, cells are transparent and have no shadow, because they are illuminated from the bottom not from the side. From the cell biologist's point of view, the model has several definite advantages. Cell shape and size, form of colony and its dynamics are very similar to those of real cells. Modelled cells are different in their size, expressing the variability of forms which is a common feature of biological objects (Freshney 1987). A cell is spread on the surface, and neither its border nor cover are even. It is visible, that in the centre of colony cells are smaller than those on the colony edge. Central cells seem to be restricted in space. It is important, that cells which are standing separately from the visual colony border are significantly different in their sizes and shapes. Cells in colony are able to perform amoebic movements, which lets them stand almost separately from the colony, especially in young colonies. These movements cause some very realistic open spaces in young colonies. Also, cells in the division process, form characteristic doubling cells with a straight border between them. Colonies do not form secondary levels of cells, but instead the rate of cell division goes from the exponential (logarithmic) phase to contact inhibition and density limitation of growth, very characteristic for non-transformed (non-tumour, normal) cells (Hay et al. 1992, Trinkaus 1984). As in a real colony, there is always a mixture of dividing and resting cells. Even dividing cells are different in their sizes. The average cell size in exponentially growing population is decreasing. The size of a colony as well as the number of cells in it is of course growing. But what is important, the colony is changing its shape while growing, and is not at all round. The colony itself is flat (normal cells) but has non-even border. All these characteristics are often observed in the literature (Freshney 1987, Trinkaus 1984, James et al. 1994) and in our experiments with AHH-1 cells.

We clearly understand that human vision or estimation can not be sufficient or complete reason to draw conclusions about model correctness. The main question about the biological adequateness of the model: *Can a relevant statement be made by visual inspection?*, is left open. The same question arises about the use of computers. Digitized images represent foreground and background pixels with some noise, brightness and color. They require careful correction for preserving the details of a studied phenomena. Nevertheless, we suppose that some measurement parameters, for instance, the fractal dimension correlate with the properties of the real-world phenomena. Fractal dimension also seems correlated with human visual response to simulated properties. We use the fractal dimension as a characteristic of objects in a general manner. Different types of real or simulated cells can have significantly different fractal dimensions. We suppose that such differences may allow us to detect the correlation between the real colony and simulated one.

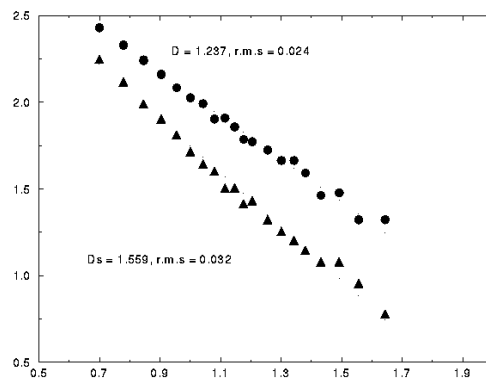


Figure 2. Box counting results for the images shown in Fig. 1. The plot of $\log N(r)$ vs. $\log r$ is linear

There are a number of numerical methods that can be applied in practice to calculate the fractal dimension and to indicate its existence (Vicsek 1992, Peitgen et al. 1992). The box counting method and the information dimension method were implemented. Calculated results (Fig. 2) of the box counting method can be approximated by a straight line with error limits from 0.02 to 0.03. In the box counting method, the number of non-empty squares $N(r)$ is calculated, where r defines the image spacing. The second method gives slightl different but qualitatively similar results.

We would stress that the analysis shows fractal scaling and exhibits some hidden features so often found in the real world. To make a reliable conclusion, in future we have to achieve visual similarity in the model to real cells, making them transparent with visible nucleus. Appropriate image processing of experimental and simulated data should be carefully done to avoid bias in the density histograms.

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