Fractal evaluation of a discrete model for simulation of avascular tumor growth

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Abstract. The paper analyses a number of mathematical models describing the growth and development of nonvascular tumors and proposes a new lattice-based computer model to simulate the tumor growth with nutrient consumption constraints. The modeling solution is able to reproduce the classic three layer structure familiar from multicellular spheroids: cell proliferation, quiescent and necrosis. The accuracy of this model is tested by comparing with a fractal morphometric technique two patterns, one obtained by simulation, the other developed in-vitro. The evaluation of the geometrical complexity is made by measuring the irregularities of the boundary, typical for tumor growth, in a bi-dimensional representation.

Keywords: tumor growth simulation, lattice-based model, fractal morphometry, geometrical complexity

1 Introduction
Tumor growth is a complex process, ultimately dependent on tumor cells proliferating and spreading in host tissues. A very important implication of the spatial and temporal symmetries of tumors is that certain universal quantities can be defined to allow the characterization of the tumor growth dynamics. Modeling and simulation of tumor growth in competition with the immune system is certainly one of the challenging frontiers of applied mathematical which could have a great impact both on the quality of life and development of mathematical sciences. In general, simulations may need the use of dedicated computer devices, to solve systems that include biological variables in the various transport phenomena related to biological systems. The common feature of the above mathematical approach is that the equation model living matter and the ability of cells to organize their dynamics needs to be an essential feature of these mathematical models.
Tumor evolution is a most complex process involving many different phenomena. Understanding the dynamics of cancer growth is one of the great challenges of modern science. Solid tumors develop initially as a single mass of cells. These divide more rapidly than the cells around them because of a proliferative advantage caused by mutation, and a number of genetic pathways responsible for these mutations have been identified over the last decade [1]. Because there are three distinct stages (avascular, vascular, and metastatic) to cancer development, researchers often concentrate their efforts on answering specific questions on each of these stages. The avascular stage of tumor growth is characterized by small tumors, which gain the nutrients and oxygen they need for survival and growth by diffusion from external blood vessels. Since there are no blood vessels within the tumor to supply the mass needed for such volume expansion, this must also enter through the tumor’s periphery. An individual tumor cell has the potential, over successive divisions, to develop into a cluster of tumor cells. Further growth and proliferation lead to the development of an avascular tumor consisting of approximately $10^6$ cells, which feed on oxygen and other nutrients present in the local environment.
Angiogenesis is the process by which tumors induce blood vessels from the host tissue to sprout capillary tips, which migrate towards and ultimately penetrate the tumor, providing it with a circulating blood supply and, therefore, an almost limitless source of nutrients. The vascular growth phase, which follows angiogenesis, is marked by a rapid increase in cell proliferation and is usually accompanied by an increase in the pressure at the centre of the tumor.
This may be sufficient to occlude blood vessels and, thereby, to restrict drug delivery to the tumor. In the earliest stages of development, tumor growth seems to be regulated by direct diffusion of nutrients and wastes from and to surrounding tissue. When a tumor is very small, every cell receives nourishment by simple diffusion and the growth rate is exponential in time. However, this stage cannot be sustained because as a nutrient is consumed its concentration must decrease towards the centre of the tumor. The concentration of a vital nutrient at the centre will fall below a critical level. After the early stages of growth, the avascular spheroids consist structurally of an inner zone of necrotic cells (dead due to lack of nutrients) and an outer zone of living cells. This outer zone can be further divided into a layer largely composed of quiescent cells and a layer largely composed of proliferating cells, although dead cells are found adjacent to both quiescent and proliferating cells. At this stage the spheroids tend to reach a finite size of at most a few millimeters in diameter [2]. However, even at very early stages of their growth, tumors become highly non-homogeneous. Fast growing tumor cells can significantly change their environment leading to formation of gradients of different metabolites, such as oxygen, glucose, growth factors and other nutrients. Changes in metabolite concentration cause in turn the development of microregions occupied by tumor cells of different phenotypes, such as: proliferating, quiescent or necrotic cells. Those subpopulations of cells are characterized by different growth and functional properties as well as by diverse responses to therapeutic factors.

The biology of tumor microregions has been investigated experimentally using the multicell spheroid model [3]. In experimental setting, the spheroids are usually initiated from aggregates consisting of several cells, but as their size increases, their growth kinetics become similar to those of tumor in vivo, such as micrometastases or pre-vascular primary tumors. The multicell spheroids develop layered structure with a central necrotic core surrounded by quiescent cells and a thin rim of proliferating cells. Steep gradients in oxygen, glucose and other metabolites are also observed in such spheroids.

Mathematical modeling is an ideal approach for teasing apart mechanisms of cancer invasion because it can simultaneously and quantitatively consider interactions between multiple factors. In general, simulations may need the use of dedicated computer devices, to solve systems, which include biological variables in the various transport phenomena related to biological system. The common feature of the above mathematical approach is that the equation model living matter and the ability of cells to organize their dynamics needs to be an essential feature of these mathematical models.

For cancer growth, one of the most aggressive phenomena in biology, numerous mathematical models have recently been investigated. Examples include studies, based on classical reaction-diffusion equations, of the growth of tumor spheroids [4], cancer evolution and its interaction with the immune system [5] and the fundamental problem of tumor angiogenesis [6].

2 Mathematical models for tumor growth

The process of nutrient consumption and diffusion inside tumors has been modeled since the mid 1960s. A consistent review of this area of tumor modeling published over the last few years appears in [7]. However, they all focus on different aspects to those we address. Most models fall into two categories: *continuum mathematical models* that use space averaging and thus consist of partial differential equations and *discrete cell population models* that consider processes on the single cell scale and introduce cell-cell interaction using cellular automata type computational machinery.

2.1. Continuum cell population models

Mathematical models describing continuum cell populations and their development classically consider the interactions between the cell number density and one or more chemical species that provide nutrients influence the cell cycle events of a tumor cell population. Thus these models typically consist of reaction-diffusion equations. One of the best parameterized of these models is due to Casciari et al [8].

2.1.1. Cell population and nutrient consumption model

Tumor cells consume nutrients. Nutrients diffuse into the tumor tissue from the surrounding tissue. Therefore, if the tumor is very large the nutrients cannot reach all parts of the tumor tissue. This leads to decrease in tumor cell proliferation and eventual cell death in regions lacking nutrients. The steady size of the tumor spheroid is reached when the cell proliferation in regions rich in nutrients balances cell death in regions poor in nutrients. Early models of nutrient-limited tumor growth
calculated the nutrient concentration profiles as a function of tumor spheroid radius that was changing due to the rate of cell proliferation [9]. The later models have incorporated differing degrees of complexity for cell movement. For example, cells can be considered to move in either a convective manner or actively in a diffusive manner, or in a diffusive/chemotactic manner. Most models consider tumor cell proliferation and death to be dependent on only one generic nutrient (most often oxygen). The equations describing the distribution of molecular species inside the tumor spheroid are classical transport/mass conservation equations.

### 2.1.2. Tissue mechanics models

Tissue mechanics models consider the mechanical interactions between tumor cells with the aim of answering questions about how the mechanical properties of the tumor and the tissue in which the tumor grows influence tumor growth. Since the late seventies there have been several models that in one way or another have introduced the concept of pressure or force between tumor cells [10], [11]. In addition, there have been a number of papers, which have proposed ways to include the effects of mechanical interaction between tumor cells into the existing mathematical framework of nutrient limited spheroid growth [12].

### 2.2. Discrete cell population models

With the huge advances in biotechnology, large amounts of data on phenomena occurring on a single cell scale are now available. This, combined with in vitro experiments using tumor spheroids, sandwich culture, etc., and high power confocal microscopy that enables tracking of individual cells in space and time, has brought about the possibility of modeling single-cell-scale phenomena and then using the techniques of upscaling to obtain information about the large-scale phenomena of tumor growth. There are several upscaling techniques; the most popular ones are cellular automata [13], lattice Boltzmann methods [14], agent based [15], extended Potts [16] and the stochastic (Markov chain) approach [17]. The difficulty with automatons models is realistically modeling cell motion. The first step in setting up rules for cell motion is to partition the physical space into automaton cells. The simplest partition is to discretise into a regular lattice; rectangular lattices are usually chosen for simplicity. The second modeling decision is whether the lattice is fixed in time or varies as the elements move. It is far simpler to consider a fixed lattice, with each automaton cell corresponding to either a biological cell or vacant site, and cells able to move into a nearby lattice site containing a vacant site. In particular, while the rules of motion for fixed lattices can be formulated simply in terms of cells moving between lattice sites, if the lattice is free to move and the cells can grow.

A recent three-dimensional cellular automata model, which does not use a regular lattice, is that of Kansal, *et al.* [18]. The model does not include nutrients or mechanical interaction between cells explicitly, but mimics the effect of both in a phenomenological way. The authors use random fixed lattice, with the space that belongs to a single lattice site consisting of points that are nearer to this site that any other lattice site. In this model, the proliferation is determined by the distance of the cell from the tumor boundary to mimic the effects of nutrient diffusion and consumption; only cell within a certain distance from the boundary can proliferate. Similarly, cells a certain distance from the boundary become necrotic.

Whereas in most cellular automata models each lattice point consists of one or more biological cells, in the Potts model approach [19] each biological cell is made up of several lattice points and the movement of each individual cell is determined by some from of energy minimization. However, the benefit of lattice Boltzman models, Markov chain, etc. approaches originating from statistical mechanics, is in the fact that there is already an existing framework from physics and fluid dynamics that deals with formalizing particle-particle, or, in the case of cancer, cell-cell, interactions.

### 3 Fractal morphometry applied to tumors

Cancer is often characterized as a chaotic, poorly regulated growth. By focusing on the irregularity of tumor growth rather than on a single measure of size such as diameter or volume, fractal geometry is well suited to quantify those morphological characteristics that pathologists have long used in a qualitative sense to describe malignancies – their ragged border with the host tissue and their seemingly random patterns of vascular growth. Herein lays the potential of fractal analysis as a morphometric measure of the irregular structures typical of tumor growth.

Several comprehensive reviews of the use of fractal dimensions in pathology have recently appeared in the literature. There is a growing literature that
shows fractals to be useful measure of the pathologies of the tumor border, cellular/nuclear morphology, and vascular architecture. Fractals show promise as useful measures of these complex structures.

The surface appearance of many malignancies has a typical morphology that corresponds to disease severity: benign tumors are smooth, whereas aggressive malignancies are ‘‘rough’’. There is a correlation between this ‘‘roughness’’ and the tumor’s invasive potential: studies of photomicrographs of tumor surfaces have succeeded in demonstrating self-similarity at different length scales, and have noticed a relationship between the fractal (Hausdorff) dimension of the tumor surface and its invasive potential.

4 Model implementation

The basic principles included in the model are cell proliferation, quiescent and necrosis. Each cell has associated with the velocity, which indicates the direction and the distance the cell will move in one time step. There are nine velocity channels in each lattice site: $V_0 = (0,0)$, $V_1 = (1,-1)$, $V_2 = (0,1)$, $V_3 = (-1,1)$, $V_4 = (-1,0)$, $V_5 = (1,0)$, $V_6 = (-1,1)$, $V_7 = (0,1)$, $V_8 = (1,1)$, were $V_0$ is resting channel and $V_1$, $V_2$, $V_3$, $V_4$, $V_5$, $V_6$, $V_7$ and $V_8$ represent moving to right, up, left, down and diagonals, respectively. In each lattice site, we allow at most one cell (necrotic cells or tumor cells) with each velocity.

Now in each lattice site one of following reactions can occur at each time step:

Quiescent:

\[
\begin{align*}
C_{i,j} &\rightarrow C_{i,j} \\
N_{i,j} &\rightarrow N_{i,j} \quad \text{if and only if } (C_{i,j} \geq 1)
\end{align*}
\]

Proliferation:

\[
\begin{align*}
C_{i,j} &\rightarrow C_{i,j} +1 \\
N_{i,j} &\rightarrow N_{i,j} \quad \text{if and only if } (C_{i,j}+N_{i,j}<5 \text{ and } C_{i,j} \geq 1)
\end{align*}
\]

Necrosis:

\[
\begin{align*}
C_{i,j} &\rightarrow C_{i,j} -1 \\
N_{i,j} &\rightarrow N_{i,j} +1 \quad \text{if and only if } (C_{i,j} \geq 1)
\end{align*}
\]

where $C$ – tumor cells; $N$ – necrotic cells.

In order to address the formation of tumor microregions, we present a two-dimensional time-dependent mathematical model in which every tumor cell is treated as an individual entity characterized by its own geometry and individually controlled cell processes. This model allows one to follow fate of each individual cell and to investigate how changes occurring in individual cells can influence behavior of the whole tumor tissue. For simplicity, we introduce in our model only one external metabolic factor and take explicitly into account only the effect of nutrient consumption on cell growth and metabolism.

5 Simulation results

We have developed a computer model based on random growth to simulate the geometrical complexity of a tumor. The model was implemented using Java Development Toolkit.

The simulations were performed on a 100 x 100 square lattice with central site initially defined to contain one cancerous cell. The size of the lattice is chosen sufficiently large such that the boundaries do not influence the tumor growth within the considered time interval. The simulation has been greatly simplified by neglecting some effects such as: interaction of healthy cells with cancerous cells, the effect of nutrients concentrations and limited volume space for tumor and it seems the addition of these effects is not problematic in this simulation (fig. 1).

![Fig. 1. Spatial development of tumor microregions containing proliferating cells (black), necrotic cells (dark grey), quiescent cells (light grey).](image-url)
Our model estimated fast expansion of tumor cells during the first third of the whole period and significant reduction in tumor growth after developing necrotic cell area. Finally, the tumor enters into a phase of growth saturation. The initial fast tumor growth follows from the fact, the almost all tumor cells proliferated actively. The percentage of proliferating tumor cells is equal to 100% during this time, except of the scattered single points that reflect short periods of time when the newly created daughter cells did not yet enter in the new cell cycle. A subpopulation of quiescent cells becomes more noticeable at the time when the first necrotic cells arise. After subpopulation of necrotic cells arises the tumor growth is characterized by a fast exponential expansion. The percentage of proliferating cells starts to decrease with the increasing of quiescent and necrotic cells.

To measure the fractal dimension and roughness of the tumor boundary we select only the cells at the boundary of the tumors. We define boundary cells as those that have at least one normal neighbor. $D_f$ was calculated using a box-counting algorithm [20]. See the figure 2 where the fractal dimensions of the patterns are plotted against the total number of time steps.

![Figure 2](image)

**Fig. 2.** Plot of the fractal dimensions of the tumor boundary as a function of time steps

Finally, we shall compare the simulated patterns with an *in vitro* model of tumor growth [21] for validation our computational model.

![Figure 3](image)

**Fig. 3.** *In vitro* growth model versus bi-dimensional simulation.

In fig. 3 we show two sections of tumor growth: left panel – *in vitro* model and right panel – simulated patterns. This image is very similar to the patterns exhibited in our simulation. At the beginning, we assume that similarities between these *in vitro* growth model and simulated patterns suggest that some of the functional properties of cancer cells are similar to those built into our model.

### 6 Conclusion

We see the role of mathematical modeling in cancer biology as twofold. Models can help our intuition, provide a framework for thinking about the problem, and make predictions. If a model is well parameterized then these predictions can be quantitative predictions can be significant.

Including mathematical modeling and computer simulations can speed up the process, provide insight into the mechanisms that control tumor evolution and growth, and, hence, suggest directions for new therapies. The theoretical predictions generated from the models and simulations can help optimizing the experimental protocol by identifying for the most promising candidates for further clinical investigation. The ease with parameters can be manipulated in a computer simulation can be identifying new experimental programmes and optimal tumor therapy schedules.

Therefore, we consider that at the moment a comparison between the computer simulations and the real cancerous growths is a first stage of further studies.

**ACKNOWLEDGEMENTS**

This work was partially supported by the Romanian Ministry of Education and Research under Grants No. 61031 /2007 and No. 1032/2007.

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