Simulation of Multicellular Tumor Spheroids Growth Dynamics

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Introduction

Understanding the fundamental laws driving the tumor development is one of the biggest challenges of contemporary science [1, 2]. Internal dynamics of a tumor reveals itself in a number of phenomena, one of the most obvious ones being the growth. Overtaking its control would have profound impact to therapeutic strategies. Cancer research has developed during the past few decades into a very active scientific field taking on the concepts from many scientific areas, e. g., statistical physics, applied mathematics, and nonlinear science [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. From a certain point of view, the evolution of tumors can be understood as an interplay between the chemical interactions and geometric limitations mutually conditioning each other. Consequently, it is believed that malignancy of a tumor can be inferred exceptionally from the geometric features of its interface with the surrounding tissue [16, 17]. Formation of the growing interface is in a continuum approximation described by a variety of alternative growth models, such as Kardar-Parisi-Zhang [18], molecular beam epitaxy (MBE) [19], or Edwards-Wilkinson equations. At the same time, the growth properties can be classified into universality classes [20], each of them showing specific scaling behavior with corresponding critical exponents. As implies scaling analysis of the 2-dimensional tumor contours [21, 22], the tumor growth dynamics belongs to the MBE universality class characterized by, (1) a linear growth rate (of the radius), (2) the proliferating activity at the outer border, and (3) diffusion at the tumor surface.

In vitro grown tumors usually form 3- (or 2-) dimensional spherical (or close to) aggregations, called multicellular tumor spheroids (MTS) [23]. These provide, allowing strictly controlled nutritional and mechanical conditions, excellent experimental patterns to test the validity of the proposed models of tumor growth [24]. These are usually classified into two groups, (1) continuum, formulated through differential equations, and (2) discrete lattice models, typically represented by cellular automata [25, 26, 27], agent-based [28], and Monte Carlo inspired models [29].

Here we present the inverse geometric approach to the MTS growth simulation, enabling us to evolve an initial MTS by required rate as well as desired fractal dimension of its contour. The method is based on the cellular automata paradigm with the transition rules found by the genetic algorithms.

Simulation and optimization tools

Cellular automata (CA) [30] were originally introduced by John von Neumann as a possible idealization of biological systems. In the simple case they consist of a 2D lattice of cells \( s_{ij}^{(t)} \in \{0, 1\} \), \( i, j = -(L_D/2), -(L_D/2) + 1, \ldots, L_D/2, \) where \( t = 0, \ldots, \tau \) is the time step and \( L_D \) size of the 2D lattice. During the \( \tau \) time steps they evolve obeying the set of local transition rules (CA rules) \( \sigma \), formally written

\[
\begin{align*}
\sigma_{ij}^{(t+1)} &= \sigma(s_{i-1j}^{(t)}, s_{ij}^{(t)}, s_{i+1j}^{(t)}),
\end{align*}
\]

which defines the CA rules \( \sigma \) as the mapping

\[
\sigma: \{0,1\} \times \{0,1\} \times \ldots \times \{0,1\} \rightarrow \{0,1\}.
\]

Any deterministic CA evolution is represented by the corresponding point in \( 2^n \)-dimensional binary space enabling, in principle, immense number of \( 2^n \) possible global behaviors, predestining CA to be the efficient simulation and modeling tool [31]. Inherent nonlinearity of CA models is, however, a double-edged sword. On the one hand, it enables to model a broad variety of behaviors, from trivial to complex, on the other hand it results in difficulty with finding the transition rules generating the desired global behavior. No well-established universal

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technique exists to solve the problem, and, despite sporadic promising applications of genetic algorithms (GA) to solve the task \cite{42,33,31}, one typically implements CA by ad hoc or microscopically reasoned definition of the transition rules.

Genetic Algorithms (GA) \cite{35} are general-purpose search and optimization techniques based on the analogy with Darwinian evolution of biological species. In this respect, the evolution of a population of individuals is viewed as a search for an optimum (in general sense) genotype. The feedback comes as the interaction of the resulting phenotype with environment. Formalizing the basic evolutionary mechanisms, such as mutations, crossing-over and survival of the fittest, the fundamental theorem of GA was derived (schema theorem) which shows that the evolution is actually driven by multiplying and combining good (quantified by an appropriate objective function), correlations of traits (also called schemata, or hyperplanes). The remarkable feature resulting from the schema theorem is the implicit parallelism stating that by evaluating a (large enough) population of individuals, one, at the same time, obtains the information about the quality of many more correlations of the traits.

Below we present the application of GA optimization to find the CA rules producing the 2D CA evolution by required rate as well as fractal behavior of the contour.

Optimization Problem

In the below numerical studies two competing hypotheses of the rate of the desired tumor mass production have been distinguished, a) broadly accepted exponential growth of the tumor mass:

\[ M^{(t)} = B + \left[\pi(R^{(0)})^{2} - B\right] \exp(Ct), \quad (3) \]

and,

b) the growth of the mass with linearly growing radius \cite{21,22}:

\[ M^{(t)} = \pi(R^{(0)} + At)^{2}, \quad (4) \]

where \( R^{(0)} \) is the initial cluster radius; \( A, B, C \) are constants parameterizing the growth process. The term \( B + \left[\pi(R^{(0)})^{2} - B\right] \) in Eq. (3) was chosen to start from the initial cluster size \( M^{(0)} = \pi(R^{(0)})^{2} \) for any \( B \).

At the beginning, the chain of concentric circles (patterns) with randomly deformed close-to-circular contours \( p_{ij}^{(t)} \in \{0,1\}, \ i,j = -(L_{D}/2), -(L_{D}/2)+1, \ldots, L_{D}/2, \) for \( t = 0, \ldots, \tau \), were generated accordingly to

\[ p_{ij}^{(t)} = \begin{cases} 0, & \text{for } \sqrt{i^2 + j^2} > R^{(t)} + 1, \\ 0, & \text{or 1 drawn with probability } \frac{1}{2} \\ 1, & \text{for } \sqrt{i^2 + j^2} < R^{(t)} - 1. \end{cases} \quad (5) \]

where the increasing tumor radius \( R^{(t)} \) is taken as

\[ R^{(t)} = \sqrt{M^{(t)}/\pi} \quad (6) \]

in the case of exponential tumor mass production Eq. (3), and

\[ R^{(t)} = R^{(0)} + At \quad (7) \]

in the case of linearly growing radius model Eq. (4), respectively.

The optimization task solved by GA was to find the CA transition rules \( \sigma^{*} \) Eq. (2) providing the growth from the initial pattern \( \{s_{ij}^{(0)}\} = \{p_{ij}^{(0)}\}, \ i,j = -(L_{D}/2), \ldots, (L_{D}/2) - 1, L_{D}/2 \) through the sequence of the square lattice configurations \( \{s_{ij}^{(t)}\}, \ t = 1, \ldots, \tau \), generated accordingly to Eq. (1), with the minimum difference from the above ”prescribed” patterns \( \{p_{ij}^{(t)}\} \) in the respective \( t \), quantified by the objective function

\[ f_{1}(\sigma) = \frac{1}{\tau} \sum_{t=1}^{\tau} \left( \frac{\sum_{i,j}^{L_{D}} p_{ij}^{(t)} + \sum_{i,j}^{L_{D}} s_{ij}^{(t)} }{1 + w_{0} \sum_{i,j}^{L_{D}} p_{ij}^{(t)} \delta_{p_{ij}^{(t)} s_{ij}^{(t)}}} \right)^{2}, \quad (8) \]

where \( \delta \) is the standard Kronecker delta symbol, \( w_{0} \) is the weight factor, in our case \( w_{0} = 2 \). The above expression of the objective function Eq. (8) reflects the programming issues. The larger overlap of \( \{s_{ij}^{(t)}\} \) with \( \{p_{ij}^{(t)}\} \) enhances the denominator of Eq. (8), the prefactor \( p_{ij} \delta_{p_{ij} s_{ij}} \) reduces the computational overhead by ignoring the calculation \( \delta_{p_{ij} s_{ij}} \) for \( p_{ij} = 0 \).

The other requirement to the desired growth relates to the geometric properties of the contour/interface. Broadly accepted invariant measure expressing the contour irregularity is the fractal dimension, \( D_{F} \). Using the box-counting method it can be calculated as

\[ D_{F} = \lim_{\epsilon \to 0} \frac{\log N_{B}(\epsilon)}{\log(1/\epsilon)}, \quad (9) \]

where \( N_{B}(\epsilon) \) is the minimum number of boxes of size \( \epsilon \) required to cover the contour. Here, it has been determined as the slope in the log-log plot of \( N_{B}(\epsilon) \) against \( 1/\epsilon \) using the standard linear fit.

To obtain the CA rules generating the cluster with the required fractal dimension \( (\sigma^{*}) \), \( D_{F} \), the objective function Eq. (8) has been multiplied by the factor

\[ f_{2}(\sigma) = 1 + w_{1} (D_{F}^{*} - D_{F})^{2}, \quad (10) \]

where \( D_{F}^{*} \) is the fractal dimension of the cluster kept after the \( \tau \) steps with the CA rules \( \sigma \); the weight \( w_{1} \) was kept 1 in all the presented numerical results.

Finally, the rule-dependent objective function has been written

\[ f(\sigma) = f_{1}(\sigma) f_{2}(\sigma). \quad (11) \]

To sum up, the optimum rule \( \sigma^{*} \) is the subject of GA optimization

\[ \min_{\sigma} f(\sigma) = f(\sigma^{*}). \quad (12) \]
Results and discussion

All the CA runs started from the pattern \( \{ p^{(0)} \} \) Eq. (5), with the radius \( R^{(0)} = 5 \). In all the below GA optimizations, all the CA evolutions ran on the 2-dimensional box of the size \( L_D = 300 \) cells and length of the CA evolution \( \tau = 100 \) (300) steps. The GA search has been applied to find the set of CA rules, \( \sigma^* \), which gives minimum objective function values (Eq. (8), or Eq. (11), respectively). The size of the population was kept constant (1000 individui), the probability of bit-flip mutation 0.001, the crossing over probability 1, and ranking selection scheme applied. The length of the optimization was 3000 generations.

Exponential growth vs. linear radius dilemma

The simplest mathematical models of MTS growth \cite{36} assume exponential increase of the MTS mass during the time Eq. (3). The above assumption is applied mainly because of feasibility of differential and integral calculus, nevertheless revealing, hopefully, some of the characteristics of the real growth. Bru et al. \cite{21, 22} have shown from the morphometric studies that the mean radius of 2D tumors grows linearly. At the same time, they have experimentally shown that the cells proliferation is located near the outer border of cell colonies. In this work the former assumption has been tested and compared with the alternative exponential growth (Figs. 1, 2). For that reason the GA search has been carried out to find the CA transition rules which minimize the criterion Eq. (8) with exponentially growing pattern Eq. (5) during \( \tau = 100 \) steps. In the inset of Fig. 1 one can see that on the interval of the optimization (\( \tau = 100 \)) the CA evolution can be in principle fitted by the exponential Eq. (3) (as it was required) as well as by Eq. (4), corresponding to the growth with linearly increasing radius, both with small systematic error, which can be possibly hidden in the stochasticity of the real biological growth. On the other hand, the extrapolation of the fits beyond the interval of optimization shows obvious divergence of the CA mass production from the exponential fit, meanwhile its deviation from the regime with linearly growing radius stays small (nevertheless systematic). We attribute the latter discrepancy to the fact that the growing interface during the CA evolution is neither smooth, nor of zero thickness, which is true also for real tumors growth. In Fig. 2 we show the comparison of the CA mass production, using the same CA rules as in Fig. 1 fitted by the Eqs. (3) and (4), respectively, on the interval much longer than the interval of minimization (\( \tau = 100 \)). One can see that both the fits are still possible, nevertheless the exponential fit deviates crucially on the interval of optimization (the inset of Fig. 2). The above results show better agreement of the CA mass production with the hypothesis of the MTS growth with linearly growing radius Eq. (4), with a slight implication towards experiments - the growth of close-to-circular MTS is probably slightly faster than proposed by Bru et al. \cite{22}, nevertheless not exponential.

Fractal behavior of the contour

Figs. 3 and 4 show the efficiency of the above approach to simulate the MTS growth by any required rate (Eq. (5) with \( R^{(t)} \) coming from Eq. (7)), and, at the same time, desired fractal dimensions of the contour, \( D_F \). Here, two GA optimizations have been performed to find the CA

\[ \text{FIG. 1: Comparison of the CA mass production using the CA transition rules obtained by the minimization of Eq. (8)} \]

\[ \text{during } \tau = 100 \text{ steps against the exponential growth Eq. (3)} \]

\[ \text{(dashed line) and the growth with linearly increasing radius Eq. (4)} \]

\[ \text{(solid line). The extrapolation of both the fits beyond the interval of minimization is displayed. The inset shows coincidence of the fits on the interval of the minimization.} \]

\[ \text{FIG. 2: Comparison of the CA mass production using the same CA transition rules as in Fig. 1. Here, the CA mass production has been fitted by both the fits far beyond the interval of optimization. The inset shows the comparison of the fits on the interval of optimization.} \]
rules generating the mass production minimizing both
the criteria Eq. (8) and Eq. (10) in the multiplicative
form Eq. (11) during \( \tau = 300 \) steps and reaching the
desired fractal dimensions \( D_{F}^{300} = 1.35 \) (Fig. 3), and 1.1
(Fig. 4), respectively. The obtained CA rules provide
the growth that fits well to the required rate as well as
desired \( D_{F} \). Fig. 5 shows the final size of the CA clus-

![Fig. 3](image)

**FIG. 3:** The CA mass production using the CA rules obtained
by GA minimization of the Eq. (11) with the patterns Eq. (9)
substitute by Eq. (8) with \( R^{(0)} = 5 \) and velocity constant
\( A = 0.4 \), and the desired fractal dimension \( D_{F}^{300} = 1.35 \) (here
presented CA run gives \( D_{F}^{300} = 1.34 \)). The smooth circles

![Fig. 4](image)

**FIG. 4:** The same as in Fig. 3 requiring the fractal dimension
\( D_{F}^{300} = 1.1 \) (here presented CA run gives \( D_{F}^{300} = 1.09 \)).

ters using the CA rules obtained by the GA minimization
of Eq. (11) requiring the growth accordingly to Eq. (4)
during the \( \tau = 100 \) steps within a broad range of the
velocity constants, \( A \in < 0.1, 0.5 > \), as well as the frac-
tal dimensions, \( D_{F}^{100} \in < 1.0, 1.35 > \). Fig. 6 shows the

![Fig. 5](image)

**FIG. 5:** Resulting contours after \( \tau = 100 \) steps using the CA
rules found by the GA for the respective pairs of the desired
parameters \( A, D_{F} \). The smooth circles correspond to the
growth accordingly to Eq. (4) for the respective \( A \). The real
values of \( D_{F}^{100} \) that correspond to the above CA clusters after
\( \tau = 100 \) steps are depicted in Fig. 6.

![Fig. 6](image)

**FIG. 6:** The real fractal dimensions \( D_{F}^{100} \) using the CA rules
found by GA optimization for the respective pairs of \( A, D_{F} \)
(Fig. 5). Dotted plane shows respective desired fractal dimen-
sions, \( D_{F} \).

The above results demonstrate that a specific fractal behavior
of the growing interface (Fig. 5) out of the broad range
\( (D_{F} \in < 1.05, 1.35 >) \) consistent with the morphometric
results [21] can be intentionally generated by here pre-
sented GA optimization approach. Moreover, the growth
rate can still be kept at desired value. Beyond these lim-
its unwanted artifacts emerge.
Scaling behavior

Bru et al. [21, 22] used the scaling analysis to characterize the geometric features of the contours of a few tens growing tumors and cell colonies. Here we outline the scaling behavior of the contour of the growing CA cluster resulting from our approach.

A rough interface is usually characterized by the fluctuations of the height \( h(x, t) \) around its mean value \( \overline{h} \), the global interface width
\[
W(L, t) = \left( \overline{[h(x, t) - \overline{h}]^2} \right)^{1/2},
\]
the overbar is the average over all \( x \), \( L \) is an Euclidean size and the brackets mean average over different realizations. In general, the growth processes are expected to follow the Family-Vicsek ansatz [38],
\[
W(L, t) = t^\beta f(L/\xi(t)),
\]
with the asymptotic behavior of the scaling function
\[
f(u) = \begin{cases} 
  u^\alpha & \text{if } u \ll 1 \\
  \text{const} & \text{if } u \gg 1 
\end{cases},
\]
where \( \alpha \) is the roughness exponent and characterizes the stationary regime in which the height-height correlation length \( \xi(t) \sim t^{1/z} \) (\( z \) is so called dynamic exponent) has reached a value larger than \( L \). The ratio \( \beta = \alpha/z \) is called the growth exponent and characterizes the short-time behavior of the interface.

To adapt the scaling ansatz to the close-to-circular growing CA cluster we identify the constant Euclidean size \( L \) with the time-dependent mean radius \( \overline{r} = \overline{r}(t) = (\sum_{k=1}^{N_C(t)} r_k(t))/N_C(t) \), \( r_k(t) \) being the distance of the \( k \)-th contour point from the center and \( N_C(t) \) the number of contour points in \( t \). Subsequently, we rewrite Eq. 13 into
\[
W_C(t) = \left( \overline{[r_k(t) - \overline{r}]^2} \right)^{1/2},
\]
the overbar being the average over all the contour points in \( t \) and the brackets mean average over different realizations of contours reaching the mean radius \( \overline{r} \) in \( t \), with the scaling ansatz Eq. 14 applied
\[
W_C(t) = t^\beta f(\overline{r}/\xi(t)).
\]
Numerical investigation of the \( W_C(t) \) (Fig. 7) reveals the region with the power law behavior. We identify the respective slope in the log-log plot with the growth exponent \( \beta = 0.723 \) (fitted in the region \( 150 \leq t \leq 700 \)).

To draw more complete scenario of the growing CA cluster scaling behavior, the deeper investigation of site-site correlation functions in both radial and poloidal directions is needed. This type of studies will follow.

![Scaling behavior of \( W_C(t) \). The region with power law behavior can be identified, corresponding to the slope 0.723.](slope_0.723.png)

**Conclusion**

In the paper we have presented the approach to the modeling of multicellular tumor spheroids by required growth rate and fractal dimension. The technique is based on the combination of the CA modeling with the transition rules searched by the GA. Here demonstrated results show the feasibility of the approach in this specific case. Based on the similarity of the geometric properties of the CA evolution and the tumor contours (such as locality of the interaction/communication, deformed contour and nonzero thickness of the proliferating layer) we have reasoned that the MTS mass production is slightly faster than corresponding to linearly growing radius [22]. At the same time our results imply that the often used Gompertz curve of the tumor mass progression comes as a higher level phenomena related to the nutrition, space restrictions, etc. We believe that our approach could be implemented as the backbone into the more sophisticated models of tumor growth encompassing the above higher-level mechanisms. Computationally efficient on the fly scaling analysis during the CA evolution would enable to bias the GA optimization towards the MTS growth with desired scaling properties of the contour; nevertheless its efficient realization is the subject of our ongoing research. Successful implementation of scaling analysis into the optimization process could significantly contribute to the discussion on the scaling behavior of the real tumors [22, 39, 40].

In our opinion the above presented optimization approach to the modeling of growing clusters by required rate and surface properties can find applications in many different fields, such as molecular science, surface design or bioinformatics.
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