Doctoral School of
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Mathematical models of cell cultures

Doctoral Thesis

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Introduction

With the emergence of personalized therapeutic approaches, computational drug testing, and even artificial tissue engineering modeling of cell cultures have become a prominent area of mathematical and *in silico* biology. Understanding the complex collective behavior emerging from simple phenomena, such as migration, proliferation, or intercellular communication of individual cells is essential to be productive in these fields.

Researchers use a wide variety of mathematical and numerical tools to describe the behavior of populations. A feasible modeling framework should be able to spatial behavior of the population, incorporate actors that are discrete entities, often very few in number. Moreover, the behavior of these actors are driven by a large variety of traits, and we are interested in the dynamics that evolves through the actual interactions between them.

In this thesis we follow the agent based computational simulation approach to investigate the behavior of cell cultures. ABMs, unlike continuum models, regard every particle as an individual that follows a prescribed set of rules. Information about the system can be obtained by analyzing the collective behavior of the agents with statistical methods.

Background

In this thesis, we shall adapt and improve the stochastic simulation algorithm (SSA)[1] introduced by D.T. Gillespie to produce computer-based numerical experiments of chemical reactions. A

[1] A general method for numerically simulating the stochastic time evolution of coupled chemical reactions,
Journal Of Computational Physics 22 (1976), 403–434

key assumption in the SSA is that the chemical species are reacting in a well stirred environment. This may be feasible in chemical systems, but seems to serve poor results for spatially inhomogeneous biological systems.

Gillespie's method was applied by Baker and Simpson to model interacting cells that may move and divide on a lattice[2]. Although the discrete lattice may at first appear to be a crude approximation of the continuous space, it turned out to be extremely practical when the effect of volume exclusion plays important role.

Volume exclusion asserts that two cells cannot occupy the same volume in space, thus cells are physical obstacles to each other.

Methods

In this work we introduced new stochastic simulation models and the corresponding algorithms to efficiently simulate cell cultures on a lattice of 1, 2 or 3 spatial dimensions. We used probabilistic reasoning to show the equivalence of the new algorithm with the former widely used one.

We implemented these algorithms in Python and run *in silico* experiments to explain the behavior of cell populations. We also fitted our models to real *in vitro* experimental data to check the accuracy of the models.

[2]Baker, R. E., and Simpson, M. J. Correcting mean-field approximations for birth-death-movement processes. *Phys. Rev. E* 82 (10 2010), 041905

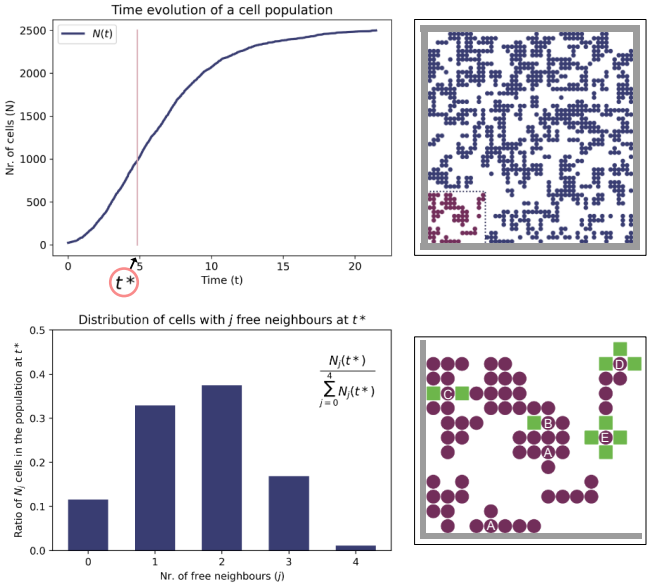

Figure 0.1: **Time evolution of a cell culture.** The *top left figure* shows the time evolution of a proliferating cell population on a lattice with carrying capacity $K = 2500$. In this experiment we chose the proliferation rate to be 1 and we assumed that no other reaction can happen to the cells. The *bottom left figure* shows a snapshot of the ratio of cells with j free neighbors in the population at $t* = 4.8$ (see the formula in the figure). In this state the total number of cells is $N(t*) = 1000$ and very few cells – only 1.1% of the population – have $j = 4$ free adjacent sites. In fact, most of the cells have $j = 2$ neighbors at $t*$. The *top right figure* shows a snapshot of the lattice configuration at $t*$ and the *bottom right figure* is an enlarged part of the highlighted area of the whole lattice. On this latter figure we selected some cells: A, B, C, D, and E having $0 \dots 4$ free adjacent sites, in order. Their corresponding free neighbors are colored green and the border of the lattice is marked in gray. We assume that the cells perceive the boundary as an occupied neighbor – thus both 'A' cells have zero neighbors at $t*$.

Summary of the research

We introduced a novel approach to incorporate possible cellular reactions into stochastic models of cell populations. To the best of our knowledge, these reaction types have not been taken into account in lattice-based stochastic simulations of cell cultures. These new types of reactions may be easily applied to complicated systems, enabling the generation of biologically feasible stochastic cell culture simulations.

- We treat the reactions systematically and categorized them according to how they depend on the local environment of the cells.
- We introduced contact-inhibited, contact-promoted, and spontaneous reactions. See Fig. 0.2.

Reaction		In a finite environment...
Contact-inhibited	Movement ($\otimes$) Division ($\otimes$) Cell death Cellular biochemical reaction	the per capita rate decreases as the population grows,
Spontaneous	Cell death Cellular biochemical reaction Non-cellular biochemical reaction	the per capita rate is independent of pop. size,
Contact-promoted	Cell death Cellular biochemical reaction	the per capita rate increases as the population grows.

Figure 0.2: **Common types of reactions that may occur in a cell culture**. Symbol $\otimes$ indicates volume exclusion. The reactions defined in this table are intended to provide a modeling framework for the exact simulation of cell cultures, that can be easily adapted to the problem the reader is considering.

We improved and derived new general purpose cell culture simulation algorithms.

- We significantly extended the lattice based cellular simulation algorithm by Baker and Simpson with the newly

defined reactions. We call this algorithm the Prompt Decision Method (PDM). The PDM algorithm is a classical accept–reject montecarlo simulation algorithm that may be very computationally intensive due to the possible large number of rejections in a crowded environment.

- Then we proposed and derived from first principles our exact method, the Reduced Rate Method (RRM). It is based on the classification of cells according to their free adjacent sites (see Fig. 0.1).
  In this algorithm, every selected reaction is executed, and therefore, the running time and the resource requirements see dramatic reductions.
  From a theoretical viewpoint, this approach enables us to define a state space in which the system is 'well-stirred' in the sense that the probability of a reaction to happen in the next time interval δt does not depend explicitly on the positions of the reactants (cells), it only depends on the number of their free adjacent sites. Thus, with the proper scaling of the reaction rates and some appropriate assumptions, we reduce the problem to Gillespie's SSA formalism

- We proposed the equivalent but simplified version of the RRM algorithm, the marginal Reduced Rate Method (see Fig. 0.3)

- We showed that the PDM algorithm and our RRM algorithm are mathematically equivalent.

- Since the design of the RRM and mRRM algorithm is based on the classification of cells according to their free adjacent sites, including new classes to this method is straightforward. For example, one may include several cell types.

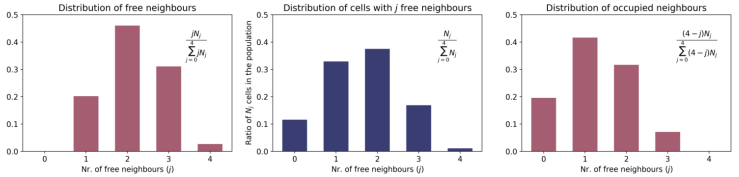

Figure 0.3: **Illustration of the distributions in the marginal RRM algorithm.** The figures correspond to the snapshot of Fig.0.1. Suppose we had all three reaction types in the simulation at state $\underline{X}(t_*)$ of the system. In case the next reaction would be a *spontaneous reaction*, we should choose the class j of the target cell according to the distribution on the middle figure, and then choose a particular target cell from class j at random. This is equivalent of choosing a cell randomly from the total population. It is clear from the figure that we would choose the cell class $j = 2$ cell with the highest probability, as the ratio of cells with two free adjacent sites is the highest in the population. Since there are cells in all five classes, we may choose any class with nonzero probability in a spontaneous reaction. If the next reaction would be a *contact-inhibited reaction*, we should choose the class of the target cell according to the distribution on the left figure. Since $0 \cdot N_0 = 0$, we choose class $j = 0$ with zero probability. Thus cells with zero free neighbors do not participate in contact-inhibited reactions. At this state of the system we would choose a class $j = 2$ cell with the highest probability. If the next reaction would be a *contact-promoted reaction*, we should choose the target cell type from the distribution on the right. Since $(4 - 4) \cdot N_4 = 0$, we choose class $j = 4$ with zero probability – cells with 4 free adjacent sites do not participate in contact-promoted reactions. We would choose class $j = 1$ with the highest probability at this state.

This could be achieved in the PDM method only in very inconvenient ways, if it is possible at all.

We incorporated realistic cell cycle length to our models.

- We presented a new model and a new algorithm that is able to model the evolution of cell populations with realistic cell cycle length of any distribution.
- Our delay model stratifies the cell cell cycle to two stages: Proliferating (P) and Motile (M). This simple approach

makes it more convenient compared to multistage models[3] where a large number (possibly hundreds) of stages are required to obtain acceptable results.

- We showed that cells may stay in a synchronized state for a long period of time in our modeling method (Fig. 0.4).

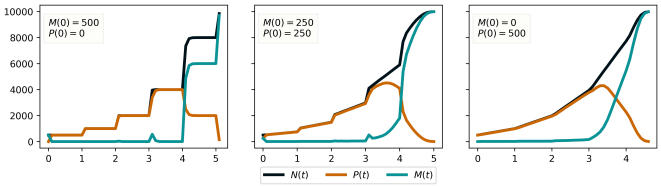

Figure 0.4: Step-wise synchronization of cells may last for a long time, but it is sensitive for the initial conditions. The figures show that the characteristic step-like dynamics may cease to exist if the initial cell population is in the proliferating state and their scheduled division events are scattered uniformly in the interval $[0, \vartheta]$.

- We also fit our model to *in vitro* experimental data (see Fig. 0.5) measured by Vitadello et al.[3].

Then, we put together the tools we have developed to propose the Go or Grow algorithms that may generate accurate dynamics of the behavior of certain tumor cells.

- We proposed four new models and algorithms to overcome the difficulties that cell cycle causes in a spatial population model.

[3] Vittadello, S. T., McCue, S. W., Gunasingh, G., Haass, N. K., and Simpson, M. J. Mathematical models incorporating a multi-stage cell cycle replicate normally-hidden inherent synchronization in cell proliferation. *Journal of The Royal Society Interface* 16, 157 (2019), 20190382

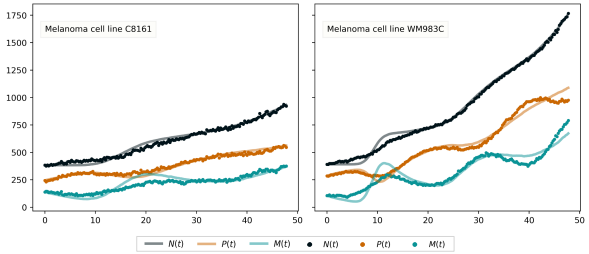

Figure 0.5: Cell cycle model with delay fitted to the experimental data by Vitadello et al[3]. The scatter plot represents the measured data points, the solid lines are the simulation outputs with the fitted parameters.

- Although all of them are based on very different assumptions, they show similar dynamics. Remarkably, the parameter domain for which synchronicity is preserved, is almost the same in all of them (see Fig. 0.6), suggesting that this family of models is robust for this preserving property. Thus, when only synchronicity is concerned, a wrong model selection due to our possibly insufficient knowledge about the system does not seem to elicit problematic behavior.

- We highlighted the possible therapeutic implications of our findings.
  Several antitumor therapies act at a specific stage in the cell cycle. If we manage to include a step to the therapeutic process, in which we syncgronize the cells before the antitumor drug is administered, we may reduce the effective dosis of this substance.

Finally, we introduced the mean field delay differential equation that is obtained from our stochastic cell cycle model.

- We investigated the model with analytical methods and

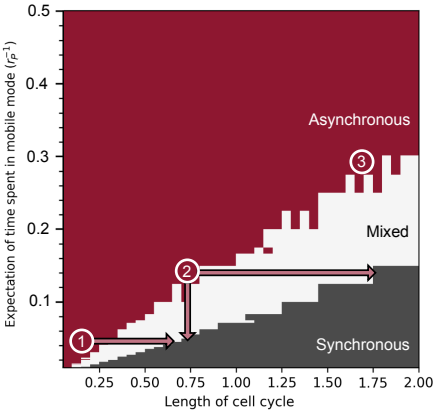

Figure 0.6: The synchronicity landscape may help us to plan tumor therapies which involves manipulation of the cell cycle length or proliferation rate of cells.

compared the model predictions with the predictions of the stochastic model.

Összefoglalás

A személyre szabott terápiás megközelítések, a számítógépes gyógyszerkísérletek, sőt a mesterséges szövet tenyésztés megjelenésével a sejtkultúrák modellezése a matematikai és *in silico* biológia kiemelkedően fontos területévé vált.

Az egyszerű jelenségekből, például az egyes sejtek migrációjából, proliferációjából vagy sejtek közötti kommunikációjából eredő komplex kollektív viselkedés megértése elengedhetetlen ahhoz, hogy eredményesek legyünk ezeken a területeken.

Ebben a dolgozatban az ágens alapú számítógépes szimulációs megközelítést (ABM - ágens alapú modell) követjük a sejtkultúrák viselkedésének vizsgálatához. Az ABM-ek a kontinuummodellektől eltérően minden modellbeli szereplőt diszkrét entitásnak tekintenek, amelyek egy előírt szabályrendszert követnek. A rendszerről az ágensek kollektív viselkedésének statisztikai módszerekkel történő elemzésével kap-hatunk információt.

A dolgozatban Baker és Simpson sejtek mozgását és osztódását rácson vizsgáló modelljét[4] jelentősen bővítve jutunk el egy olyan modellezési rendszerig (PDM - Azonnali Döntési Módszer), amely új, eddig nem használt reakciók hatását is bevonja a modellbe.

Majd első elvekből származtatunk két ekvivalens modellt (RRM - Redukált Ráta Módszer és mRRM - marginális RRM), melyek lényegesen gyorsabbak a korábbi megközelítéseknél. Megmutatjuk, hogy a PDM és RRM módszerek matematikailag ekvivalensek.

A modelleket továbbfejlesztjük úgy, hogy alkalmasak legyenek arra, hogy a sejtek valós sejtciklus hosszát is figyelembe vegyék.

Részletezzük az eredmények alkalmazhatóságát, sőt kiemeljük azokat a lehetőségeket, melyek egy esetleges terápiás folyamat optimalizálásával csökkenthetik a terápiás gyógszerek mennyiségét.

[4] Baker, R. E., and Simpson, M. J. Correcting mean-field approximations for birth-death-movement processes. *Phys. Rev. E* 82 (10 2010), 041905

Publications of the author

The dissertation is based on the following two published papers

- R. E. Baker, P. Boldog, and G. Röst,
  Convergence of solutions in a mean- field model of go-or-grow type with reservation of sites for proliferation and cell cycle delay;
  Progress in Industrial Mathematics at ECMI 2018 (Cham, 2019), I. Faragó, F. Izsák, and P. L. Simon, Eds., Springer International Publishing, pp. 381–387.

- P. Boldog, N. Bogya, and Z. Vizi,
  Propensity matrix method for age dependent stochastic infectious disease models
  Trends in Biomathematics: Stability and Oscillations in Environmental, Social, and Biological Models, R. P. Mondaini (ed.), Springer, (2021);

and the preprint (submitted to a journal and under review)

- P. Boldog,
  Exact lattice-based stochastic cell culture simulation algorithms incorporating spontaneous and contact-dependent reactions. https://arxiv.org/pdf/2208.04774.pdf

Further publications of the author:

- P. Boldog, T. Tekeli, Z. Vizi, A. Dénes, F. A. Bartha, G. Röst,
  Risk Assessment of Novel Coronavirus COVID-19 Outbreaks Outside China
  Journal of Clinical Medicine, 9, 2, 571 (2020), MDPI,

- G. Röst, F.A. Bartha, N. Bogya, P. Boldog, A. Dénes, T. Ferenci, K.J. Horváth, A. Juhász, C. Nagy, T. Tekeli, Z.

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Early Phase of the COVID-19 Outbreak in Hungary and Post-Lockdown Scenarios, *Viruses.* 2020; 12(7):708.

- P. Boldog, K. Hajdu, M. Magyar, É. Hideg, K. Hernádi, E. Horváth, A. Magrez, K. Nagy, G. Váró, L. Forró and L. Nagy (2013), Carbon nanotubes quench singlet oxygen generated by photosynthetic reaction centers. *Phys. Status Solidi B*, 250: 2539-2543.

- F.A. Bartha, P. Boldog, A. Dénes, T. Tekeli, Z. Vizi, G. Röst, Potential severity, mitigation, and control of Omicron waves depending on pre-existing immunity and immune evasion *Trends in Biomathematics: Stability and Oscillations in Environmental, Social, and Biological Models*, Springer R. P. Mondaini (ed.)

- D. Meszéna, A. Barlay, P. Boldog, D. Cserpán, L. Wittner, I. Ulbert, and Z. Somogyvári, Reconstruction of spatiotemporal membrane potential and resistive current distribution from paired intra- and extracellular recordings *The Journal of Physiology*, Wiley (accepted)