An agent based model capable of simulating a large number of tumour cells (01.09.2017-31.08.2022.)

From 02.06.2018. to 01.06.2020. the project was temporarily suspended while I was on maternity leave with the permission of NKFIH.

Abstract

In silico computations are inevitable in many fields of wet-lab research throughout experimental design, model simulations and data analysis. However, specifically in the field of tumour growth research, the usage of an accompanying computational software is not yet common practice. Here, we introduce LattiCS (Lattice based Cellular Simulator) an *in silico* tool designed to replicate experimental results of *in vitro* and *in vivo* setups. The heterogeneity of tumours requires representing each cell as a separate agent in the simulations. The software design is modular, where individual modules, such as cell cycle, cell-cell adhesion, phenotype transition, cell movement and diffusion of chemicals may be individually switched on or off. To aid clarity and customization to specific needs, the code base is written in Python. While this is a trade-off in terms of computational power, due to the lattice-based design, LattiCS is still capable of simulating hundreds of thousands of tumour cells.

Introduction

Lattice-based Cellular Simulator (LattiCS) is software tool designed to replicate in vitro and in vivo tumour growth experiments. The basic modelling structure consists of a two or three dimensional grid with fixed boundaries. The simulation agents are the different (i.e. malignant) cells, whose behaviour follows a set of rules, they interact with each other and the environment. The environment may include chemical species, whose behaviour is governed by a system of reaction-diffusion equations.

In silico reproduction of *in vitro* colony formation

On the agent-based model simulations I have been working together with Dániel Kiss. During the first nine months of the project, grid-free agent-based simulations were conducted where the mechanical properties of the cells were also taken into account. Specifically, simulations were conducted to model *in vitro* colony formation. In the corresponding cell culture experiments, different cancer cell types were plated and their colony-formation propensity has been observed by taking microscope images during a week-long time-course. From an initial configuration of pre-generated agents, as time progressed, colonies started to form various shapes depending on the model parameters. The simulation process was based on a Monte Carlo rejection sampling method. Our aim was i) to inspect

whether the agent-based model could replicate the experimental observations and ii) to uncover the specific parameter values whose modification could explain the differences between the different cell lines. We found that the produced colony shape features strongly related to only a few parameters of the model. The change of one of the calculated shape parameters obtained *in vitro* and *in silico* are depicted in Fig. 1. The results were presented as a poster at an international conference [4].

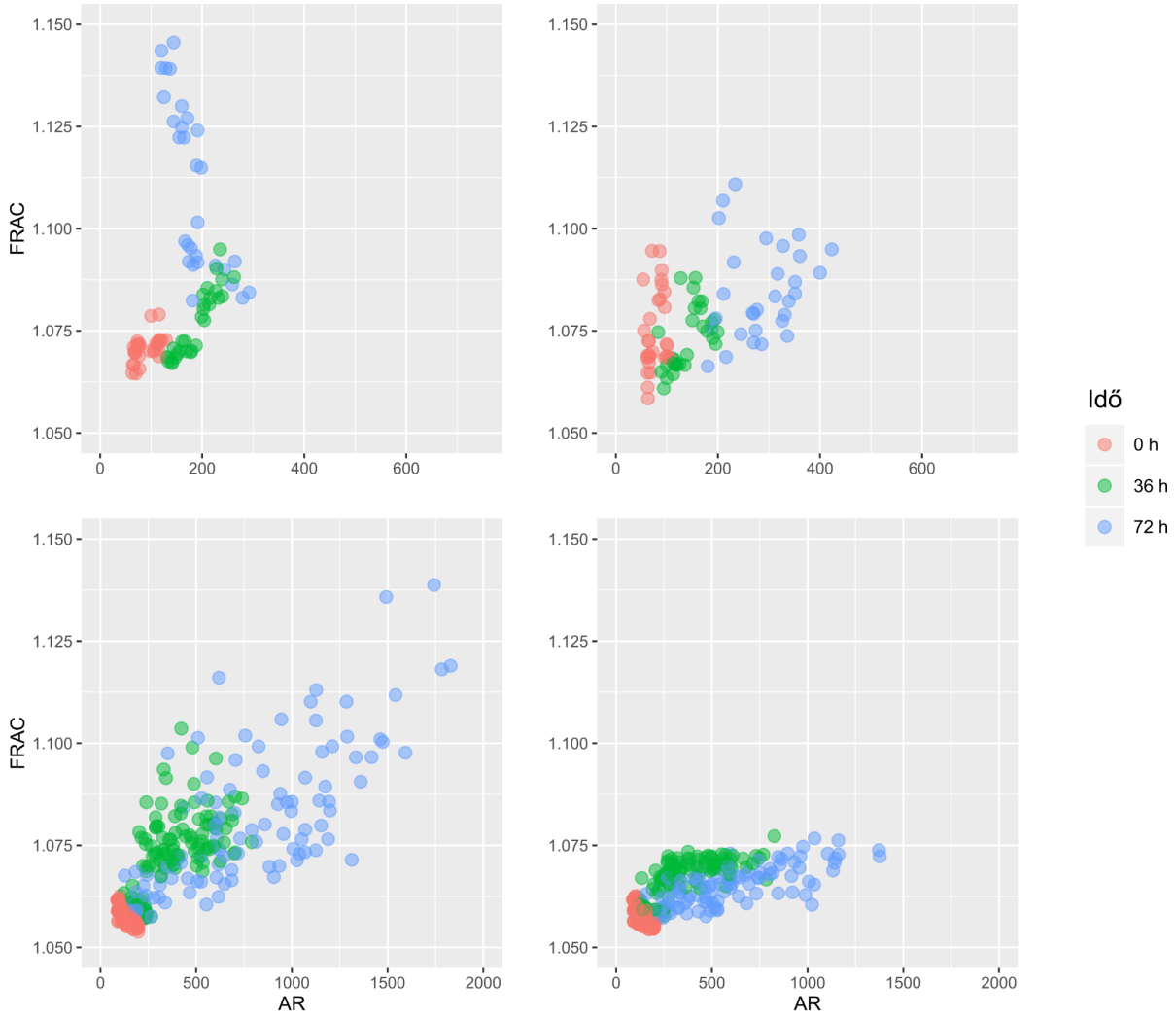

Figure 1. The time course of FRAC (fractal dimension index): an index of the ratio of the circumference and area of the shape of a cell colony. This index is independent of size. Weakly (left) and strongly (right) attaching cells in measurements from *in vitro* experiments (up) and *in silico* simulations (down).

Since the computational time was too long, the simulations were restarted using a different software design by incorporating the LAMMPS Molecular Dynamics Simulator (https://www.lammps.org). This is a simulation tool designed to model chemical particles and optimised for speed. A master student (Zoltán Balázs) is involved in this project, whose role is to develop the code. Now we are working on conducting multiple parallel simulations that will serve as an input for a neural network based parameter estimation. This is a still ongoing side-project, which will continue beyond the OTKA grant.

Tumour growth modelling using a system of ordinary differential equations

Tumour growth can also be modelled using a system of ordinary differential equations. By fitting the mathematical model output to experimental results, parameters that are not accessible by wet-lab experiments could be obtained. An ODE model capturing tumour growth and necrotic volume dynamics, including the drug pharmacodynamics, was formulated to describe a therapy with a chemotherapeutic agent in collaboration with the group of Levente Kovács at Óbuda University. Model results were fitted to in vivo experimental data published earlier by my colleagues (Füredi et al (2017) J. Control. Release vol. 261, pp. 287–296). The results showed that the system of ODEs was suitable for modelling the effect of chemotherapy, however not drug resistance. Incorporating drug resistance into the model is the subject of future work. The work outlined above was published in two conference papers and in a research journal [5-7]. Fitted model parameters were later utilised in the agent-based simulations conducted by LattiCS.

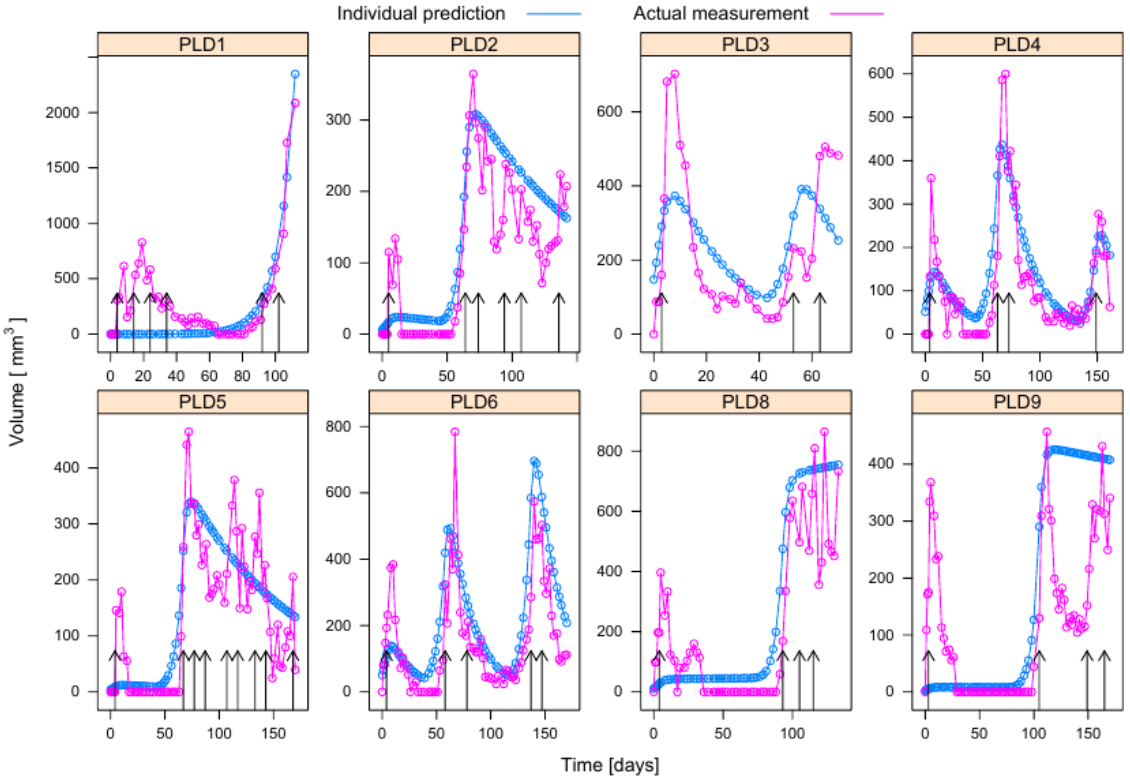

Figure 2. Time course of actual tumour volume changes in mice along with (individual) estimations from the system of ODEs model. Arrows depict addition of 8 mg/kg pegylated liposomal formulation of doxorubicin (PLD).

Existing mathematical approaches to model tumour growth

We have written a review paper about mathematical approaches to model tumour growth and drug response, focusing on personalised treatment and how potential therapy induced resistance should also be taken into account [3]. This paper reviews the main directions in agent-based modelling as well.

LattiCS (Lattice-based Cellular Simulator)

Finally, the results from the first two years were incorporated into the software LattiCS designed to simulate *in vitro* or *in vivo* tumour growth with the aim of contributing to the design and evaluation of wet-lab experiments. To aid customization to specific needs the code-base is written in Python. At the same time in order to optimise computing resources, the LattiCS framework is grid-based, *i.e.* simulated cell agents may only reside on grid points. Simulation results using grid-free or grid-based approaches were compared in a conference paper [2]. We found that the collective behaviour of the agents was similar in both cases, even though there are differences in the individual-level attributes.

Taken together, the framework is capable of simulating hundreds of thousands of cells. The software is freely available to use and modify. We are currently finalising a manuscript [1], where the capabilities of the software are presented through three simulations, each of which reproduces published results obtained by using a different simulation framework. These simulations illustrate as well how 2D and 3D *in vitro* and *in vivo* wet-lab experiments may be reproduced by the software. The examples also highlight the main features of LattiCS, such as *i)* switching different modules on or off for a certain type of run, *ii)* the implementation of cell movement and cell-cell adhesion in the grid-based framework and *iii)* the inclusion of cell cycle within the individual agents.

LattiCS - Wound healing simulation

Walker et al. conducted *in vitro* wound healing experiments along with grid-free in silico simulations (Walker et al. (2004) IEEE transactions on NanoBioscience 3, 153–163.). Using LattiCS, we have reproduced the result by a grid-based software as follows. First, cells were initialised on a 2D computational grid at random mimicking cell seeding in an *in vitro* experiment. After the cells have reached a pre-defined confluence state, a wound was created by deleting cells within a 500μm-wide strip, after which the simulation was continued. During the simulation steps, each cell agent progressed along the cell cycle and were flagged for division at the end of the cell cycle. However, cells could only divide in the case of an empty neighbouring grid. Cell state transition could occur back and forth to G0 state in the case of too many neighbouring cells. Cell movement was modelled by a Monte Carlo approach, modified for a grid based system. Implicitly, cell-cell adhesion was included into the Monte Carlo approach by defining the potential energy between neighbouring cells. In the case of physiological or low calcium levels, the binding between neighbouring cells was strong or weak respectively. Fig. 3 summarises the simulation results. As in the original

in vitro experiments and the grid-free simulations, the grid-based model also predicted a faster wound closure in the case of low Ca^{2+} level.

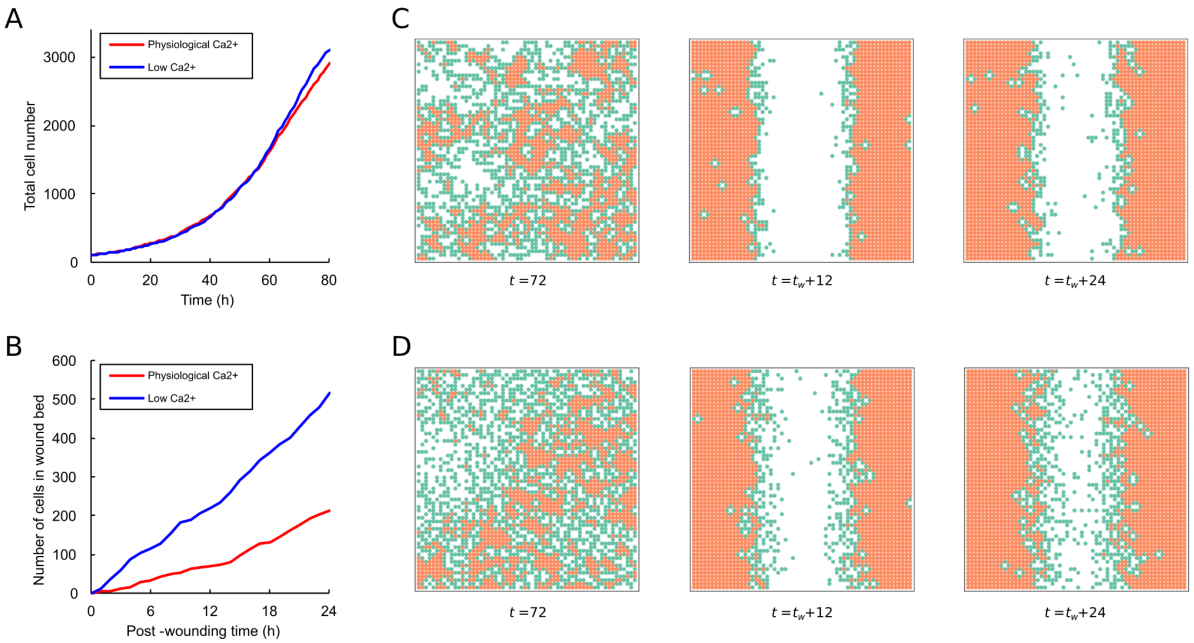

Figure 3. Simulation of epithelial wound healing in monolayers. A: In accordance with Walker et al., the initial growth rate of the population is the same independently of binding affinity. After reaching a specific cell density, a stronger binding affinity (i.e., Ca^{2+} level) prevents cell spreading, which results in a decreased growth rate. B: Higher binding affinity prevents cells from the edge of the wound to migrate inside the wound bed and also elongates cell cycle length, which causes a slower wound closure. C: Representative images taken from a simulation of cells with a high binding affinity (physiological Ca^{2+} level). D: Representative images taken from a simulation of cells without binding affinity (low Ca^{2+} level). Green color means that a cell is proliferative, while salmon-colored cells are in a quiescent state (all of their neighbouring grid points are occupied). Images were taken at 72 hours of simulated time after seeding, then 12 and 24 hours post-wounding, respectively.

LattiCS - Oxygen consumption simulation

This simulation was motivated by the work of Grimes et al. (Grimes et al. (2013), Journal of The Royal Society Interface 11:20131124), where experimental results of tissue hypoxia in 3D tumour spheroids were mathematically modelled using an algebraic equation derived from the diffusion equation. In the LattiCS reproduction, the simulation space consisted of a 3D lattice and each simulation agent represented a tumour cell type. Malignant cells progressed along the cell cycle, and pushed each other apart when they divided. Malignant cells turned into hypoxic cells when the oxygen level fell below a threshold, and hypoxic cells were converted to necrotic cells after a time-delay. On each grid point, the oxygen concentration was also monitored. Oxygen level was constant at the boundary of the simulation space and diffused towards the core of the tumour.

Here, parameters of the agent-based model were obtained by fitting the size of the tumour spheroid and hypoxic region to the experimental data points. The final parameter set reproduced the original experimental results as shown in Fig. 4.

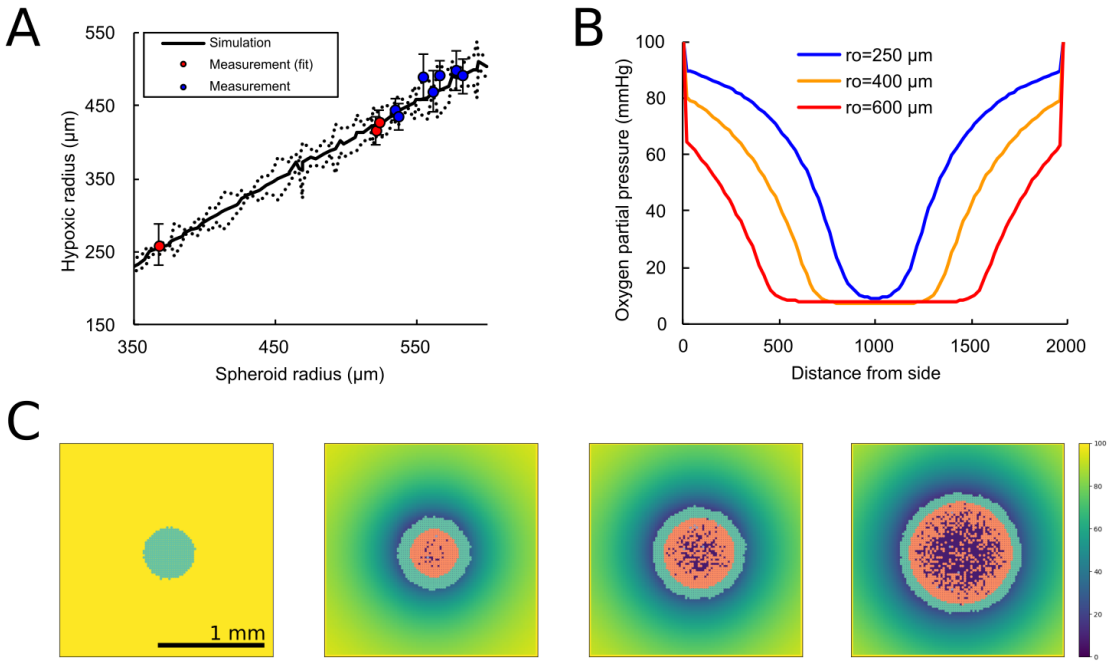

Figure 4. Simulation of development of hypoxia in a 3D multicellular tumour spheroid. A: The radius of the hypoxic inner core correlates to the outer radius of the whole spheroid. Circular markers show *in vitro* measurement data presented by Grimes et al (Grimes et al. (2013), Journal of The Royal Society Interface 11:20131124), the solid black line corresponds to a simulation result (dotted lines shows standard deviations). To fit our agent-based model to the *in vitro* experiment, we only used the first three data points (marked by red). The prediction of the hypoxic core radius of larger spheroids made by the simulation is in agreement with the real observations (blue markers). B: Oxygen concentrations along a selected line parallel to the side of the simulation box through the middle of the spheroid. Oxygen is depleted in the inner regions as the radius of the spheroid increases. C: Representative images rendered from the simulation at different spheroid sizes.

LattiCS - *In vivo* tumour growth simulation

The previously published system of ODEs [5] resulted in a set of fitted parameters that could reproduce *in vivo* tumour growth. These parameters were directly used in the LattiCS simulation (with rescaling if necessary). Additionally, agent-based simulation specific parameters were added, such as cell movement rate or how many cells may be pushed apart in the case of malignant cell division. Simulations were run as a 2D slice of the whole tumour: cells densely packed within a circle were allowed to progress along the cell cycle, divide at the end of the cell cycle or turn into necrotic cells with a drug concentration dependent rate. Cells were only allowed to divide if they could push the neighbouring cells

apart to create room for the daughter cell. Necrotic cells vanished from the system after some time simulating cell wash-out. Drug concentration was homogeneous in space. The concentration increased as a result of drug addition and the rate of drug decay was calculated based on a Michaelis-Menten kinetics, such as in the system of ODEs. Of course, the effect of inhomogeneous drug concentration is a matter of interest, however at present our main interest was to show the capabilities of LattiCS. We plan to investigate further the effect of a spatially inhomogeneous setup in the case of the tumour growth model.
Fig. 5. depicts that using the parameters from the system of ODEs resulted in a good fit in the case of the agent-based simulation as well.

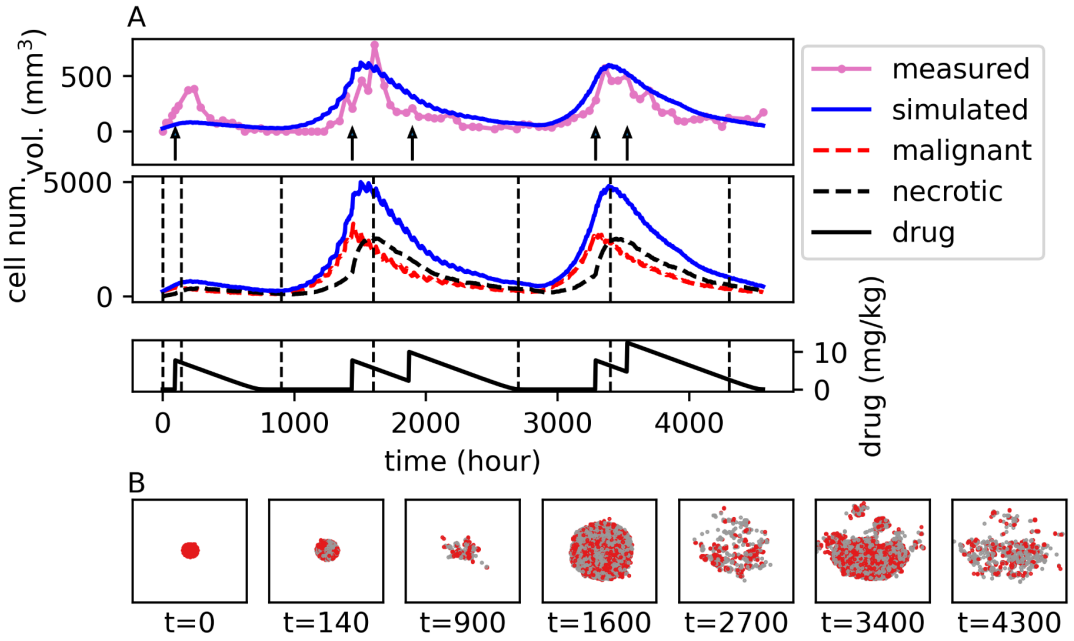

Figure 5. Tumour growth simulation. A. Top panel: measured tumour volume of the ‘PLD6’ mouse along with the scaled simulated tumour volume. Middle panel: number of simulated tumour cells comprising the malignant and necrotic cells. Bottom panel: simulated drug concentration. Horizontal lines depict simulated time points, where the agents were visualised. B. Simulated cell agents at various simulated time points.

LattiCS - performance

The performance of LattiCS was demonstrated by running the selected examples on different numbers of computing cores. Computational time was not measured in the case of the wound healing simulation, as the simulation time for each real-life hour took only a couple of seconds. Fig. 6. demonstrates that simulating hundreds of thousands of cells is feasible, the computation time increases linearly with the number of cells and the total time decreases as more computational cores are used.

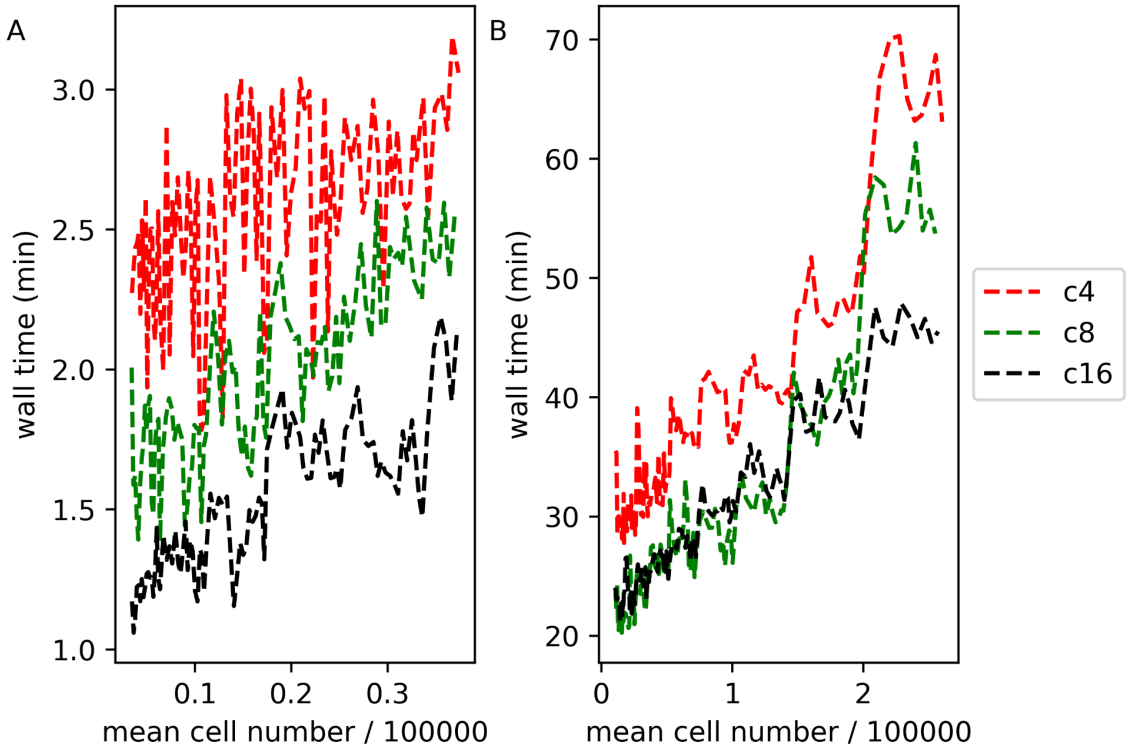

Figure 6. Computing time when using different numbers of cores (4, 8 or 16). Computational time as a function of mean cell numbers. A. one hour of simulated growth in the 'Oxygen consumption simulation'. B. four hours of simulated growth in the '*In vivo* tumour growth simulation'.

Overall, the goal of the OTKA project, *i.e.* simulating a high number of tumour cells was accomplished.

Presentation of the data (new publications in the final year highlighted).

[1] D. Kiss, Á. Pintér, A. Füredi, G. Szakács, A. Lovrics. LattiCS: A lattice-based agent-centred software package specifically tailored to simulate *in vitro* and *in vivo* tumour growth experiments. In the process of writing the manuscript, expected to be submitted within a month.

[2] D. Kiss, A. Lovrics. On-lattice Approximation of a Grid-free Individual-based Model of Growing Cell Populations. In In Proceedings of the 10th Jubilee IEEE International Conference on Computational Cybernetics and Cyber-Medical Systems, 2022.

[3] D. Kiss, A. Lovrics, G. Szakács, A. Füredi, L. Kovács, D. A. Drexler Mathematical and computational methods to model chemotherapy-induced resistance. Magyar Onkológia 65 (2), p 167-175, 2021

[4] D. Kiss, G. Kertész, M. Jaskó, S Szénási , A Lovrics, Z Vámossy. Evaluation of Colony Formation Dataset of Simulated Cell Cultures. Poster presented at the The 11th International Conference on Applied Informatics conference on 29–31 January 2020.

[5] D. A. Drexler, T. Ferenci, A. Lovrics, L. Kovács. Modeling of tumor growth incorporating the effect of pegylated liposomal doxorubicin. In In Proceedings of the IEEE 23rd International Conference on Intelligent Engineering Systems, pages 369–374, 2019.

[6] D. A. Drexler, T. Ferenci, A. Lovrics, L. Kovács. Comparison of michaelis-menten kinetics modeling alternatives in cancer chemotherapy modeling. In In Proceedings of the IEEE 13th International Symposium on Applied Computational Intelligence and Informatics, pages 27–32, 2019.

[7] D. A. Drexler, T. Ferenci, A. Lovrics, L. Kovács. Tumor dynamics modeling based on formal reaction kinetics. ACTA POLYTECHNICA HUNGARICA 16 : 10 pp. 31-44, 2019

Budapest, 09.30.2022. Lovrics Anna