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D1.1: EVOLUTIONARY MODEL DEFINITION AND SPECIFICATIONS

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Abstract	The artificial evolution of nanoparticle-based anti-cancer therapies is a complex problem that reaches multiple scales. To build the evolvable Cancer Simulator we will use a combination of stochastic-based (NanoDoc) and agent-based (PhysiCell)		



	simulators. Where necessary, we will modify and extend existing source code to include cancer stem cells and vasculature modeling capabilities. Nanoparticles will also be modeled at different scales: for representing their mass kinetics we will use stochastic simulators and BioFVM, while for the more fine grained simulations we plan to use molecular dynamics and cellular automata based approaches. Simulated evolution of nano-therapies will be based on novelty search evolutionary algorithms.	
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EXECUTIVE SUMMARY

NP-based drug-delivery systems will be targeted to cancer stem cells (CSC), one of the main reasons for tumour relapse and metastasis. To build the evolvable Cancer Simulator we identified three main domains that should be considered:

1. Tumour - Using existing literature we developed a model of tumour growth which incorporates cancer stem cells, differentiated cells and extracellular matrix.
2. Biological Barriers - To reach the tumour, NPs must overcome a number of biological barriers: Circulation in the bloodstream, extravasation, intratumoral diffusion, cellular penetration. Since these barriers span different temporal and spatial scales, for each of them we will use appropriate modeling approaches.
3. NP composition and properties - We will identify which nanoparticle properties (size, charge, functionalization, material, drug loading) will result in drug delivery systems with desired distribution profiles in the tumour (e.g. ability to accumulate at lethal levels in CSCs).



Since the entire simulation platform will deal with multi-scale systems where both stochastic fluctuations and spatial inhomogeneity plays a crucial part, we will use combination of stochastic (using NanoDoc) and agent-based (using PhysiCell) simulations to better capture randomness in initial conditions and time evolution of parameter values. For simulating intracellular penetration of NPs, we will use molecular modelling approach (LAMMPS or GROMACS software) with collaborators external to this proposal.

NanoDoc has been an online platform for testing novel NP designs since 2013. Using NanoDoc, it is possible to test the influence of NP-based treatment parameters on tumour penetration such as the size of the NPs, their binding affinity, their interaction with other NPs, the dosage of single or multiple species of NPs and the treatment administration time

PhysiCell is an open source, agent-based modeling framework for 2-D and 3-D multicellular simulations. It implements combined approaches: at the individual cell level it operates as agent-based environment, while for diffusing substrates, it is coupled with BioFVM, a multi-substrate diffusion solver that deals with vectors of diffusing substrates.

Where necessary, we will change and extend source code to allow greater flexibility of simulations and to include cancer stem cells and vasculature modeling capabilities.

Due to the extensive search space in nanoparticle design, we plan to implement evolutionary computational algorithms with niching capabilities, for instance Differential Evolution mutation strategies. The evolutionary optimization framework will be developed in Python, will be open source and available on a publicly available software repository. The framework will interface stochastic-based (NanoDoc) and agent-based (PhysiCell) simulators via txt log files or wrapper code.

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LIST OF ABBREVIATIONS

Abbreviation	Description
NP	Nanoparticles
CSC	Cancer stem cells
ECM	Extracellular matrix
IFP	Interstitial fluid pressure
EPR	Enhanced permeability and retention
ODE	Ordinary differential equations
PDE	Partial differential equations
EA	Evolutionary Algorithm



1 INTRODUCTION

1.1 BIOLOGICAL BACKGROUND

1.1.1 TUMOUR GROWTH AND CANCER STEM CELLS (CSC)

Cancer stem cells (CSC) have been increasingly recognized as the main reason for tumour relapse and metastasis (Pattabiraman and Weinberg, 2014). This tumour cell subpopulation is more resistant than differentiated cancer cells to most of the conventional anticancer therapies, antimetabolic agents or radiation. Such aggressiveness is a consequence of the unique CSC phenotype. They have increased capacity to migrate and to invade, are more permissive to DNA damage, and contain high number of multidrug resistant (MDR) channels on the cell membrane, which are able to pump-out chemotherapeutic drugs.

As a result of increased knowledge of CSCs, several models that describe tumour initiation, growth and heterogeneity have been proposed (Relation et al., 2017; Rich et al., 2016). Putting aside their differences, experimental evidence demonstrated:

- existence of CSC subpopulation in solid tumours,
- ability of two-way interconversion between CSC and differentiated tumour cells, and
- the rate of such interconversion is influenced by the state of the cells' immediate environment (niche).

Niches are defined as specialized microenvironments that influence CSC growth, differentiation and dedifferentiation through cell-cell contacts and secreted factors that are predominantly produced by surrounding CSC (Peitzsch et al., 2017; Plaks et al., 2015; Relation et al., 2017).

Extracellular matrix (ECM) is an essential component of the niche (Plaks et al., 2015). Stiffer ECM puts pressure on blood vessels leading to hypoxia which then increases proliferation and self-renewal of CSCs (Mohyeldin et al., 2010; Peitzsch et al., 2017; Relation et al., 2017; Vinogradov and Wei, 2012). Oxygen gradient in microenvironments develops as a function of limited diffusion in rapidly growing tissues (Keith and Simon, 2007).

1.1.2 TRANSPORT BARRIERS FOR NANOPARTICLE DELIVERY

To reach the tumour, NPs must overcome the following barriers, at different scales:

Circulation of the NPs in the vascular system. Here, important factors that should be considered are:

- Development of protein corona around NPs, which leads to clearance from the blood stream;
- Different flow regimes between large vessels and microcapillaries (Fedosov et al., 2014).

Extravasation. Most important factors are:



- Enhanced permeability and retention (EPR) effect, which causes nanoparticles to escape from leaky angiogenic vessels into the high-pressure tumour environment. Note that while EPR can be strong in some tumours, it can be negligible in others and should not be considered a general feature of all tumours [Bjornmalm et al., 2017].
- High interstitial fluid pressure (IFP) can counteract the extravasation of nanoparticles and even drive their diffusion back into the capillary.

Tumour penetration– intratumoral diffusion. Three main factors are:

- High IFP, tumour saturation and a dense extracellular matrix may impede the diffusion of nanoparticles in the tumour (Heldin et al., 2004, Nichols and Bae, 2012; Barua and Mitragotri, 2014).

Cellular penetration. NP targeting ligands, and their interaction with receptors on cells:

- Biological cell membranes separate cell from the environment, act as a protective barrier for the cell and control transport into and out of the cell by triggering specific ligand-receptor interactions. (Lehn, 2013, Oroskar, 2016)

1.2 FUNCTIONALIZED NANOPARTICLES

Our main goal is to functionalize NPs with existing drugs and thus create novel drug delivery systems with altered bioactivity, toxicity and biodistribution. As NPs, we will use gold spheres functionalized with different anti-tumour and/or anti-CSC ligands. Currently most promising candidates are Niclosamide, YM155, Panobinostat, Zileuton and 8-Quinolinol (AKA 8-Hydroxyquinoline). In the model we intend to test ranges of functionalized NPs with variable:

- Size
- Overall charge
- Targeting ligands
- Drugs
- Concentration

Further details are explained in the section 2.5.

1.3 EVOLUTIONARY ALGORITHM APPROACH

Due to the high complexity across different scales, it would be at best impractical, if not impossible to deterministically test all design parameters and strategies. Instead we will use evolutionary algorithms, mainly utilizing a novelty search approach. Details of the approaches will be described in Section 3.4.



2 META-MODEL DEFINITION

In this section we will define approaches, rules, constraints and models we will use for developing eCS.

2.1 MULTI-SCALE APPROACH

Tumour growth, NP influence on tumour and influence of biological barriers on NP distribution described in 1.1.2 can be modeled using various approaches. Most popular and with the longest history are continuous numerical approaches using ODEs and PDEs (ordinary and partial differential equations). Although they can capture global properties, ODEs and PDEs are not very suitable for representing multi-scale systems where both stochastic fluctuations and spatial inhomogeneity plays a crucial part.

Therefore, instead of ODEs and PDEs, we will use combination of stochastic (section 2.2) and agent-based (section 2.3) simulations to better capture randomness in initial conditions and time evolution of parameter values. Stochastic simulations are in the form of a multivariate master equation where spatial domain is discretized into subvolumes. In agent-based simulations individual cells are fundamental units so the entire tumour growth and behavior emerges as a result of local interactions.

2.2 STOCHASTIC SIMULATIONS USING NANODOC

NanoDoc has been an online platform for testing novel NP designs since 2013. Using NanoDoc, it is possible to test the influence of NP-based treatment parameters on tumour penetration such as the size of the NPs, their binding affinity, their interaction with other NPs, the dosage of single or multiple species of NPs and the treatment administration time. It was previously used to crowdsource NP design through gamification, where players were encouraged to experiment with different parameter combinations in order to pass levels in which the tumour environment became increasingly complex.

NanoDoc uses the stochastic simulation compiler (SSC) (Lis et al., 2009) to solve the reaction-diffusion equations that describe NP transport through the tumour environment. SSC is an open-source tool written in C++ for creating exact simulations of biochemical reaction networks. Within an SSC simulation, different spatial regions can be prescribed different reaction-diffusion profiles allowing effective spatial simulations to be carried out. Various improvements and adjustments have been carried out previously to allow for fast computation of simulation solutions (such as through coalescing similar cellular regions) as well as novel NP-behaviours including self-assembly under certain conditions. An example of a typical NanoDoc simulation (presented in its gamified version) is shown in Figure 1.



Figure 1. Example NanoDoc simulation output. The left hand side provides user input for the current gamified version of the software. Changes to the NPs include the treatment time (top bars), number of NP populations (circles under “Nanoparticles”), the size of the NPs (large circle within the editor), the binding affinity and surface functionalisation (outer ring and black line on the large circle within the editor). The right hand side shows the current tumour environment, where the vessel is represented as a red square in the top left corner, healthy cells are light grey ovals and dark grey rectangles (bottom right) are tumour cells. The three cells in the bottom right (star shaped) represent cells killed by the NP treatment.

As the scale of NPs is several orders of magnitude smaller than an individual cell or even a collection of cells (approximately 10nm radius opposed to 10um radius for NPs and cancer cells, respectively), it is not feasible to simultaneously simulate both the entire tumour region as well as the NP drug dosage within the tumour environment. Therefore, NanoDoc provides a framework within which the NPs can be modelled within a tumour subsection of some tens of cells. NanoDocs is able to include different cell populations (such as healthy, cancer stem cells and differentiated cells) through their different binding profiles with certain NPs as well as the various NP parameters (see section 2.5) either individually or in combination.

2.3 AGENT-BASED SIMULATIONS USING PHYSICELL

PhysiCell is an open source, agent-based modeling framework for 2-D and 3-D multicellular simulations (Ghaffarizadeh et al., 2018). It implements combined approaches: at the individual cell level it operates as agent-based environment while for diffusing substrates it is coupled with BioFVM (Ghaffarizadeh et al., 2016), a multi-substrate diffusion solver that deals with vectors of diffusing substrates. BioFVM supports diffusion, decay, cell-based secretions/uptake, and bulk supply/uptake functions.

PhysiCell includes a standard library of sub-models for cell fluid and solid volume changes, cycle progression, apoptosis, necrosis, mechanics, and motility. Since PhysiCell only supports differentiated cancer cells, we will modify its source code to include CSC (see section 3.3) and vasculature growth, based



on the simplified model of tumour growth developed by UNSPF in consultation with VHIR, which incorporates cancer stem cells, differentiated cells and extracellular matrix (Fig. 2).

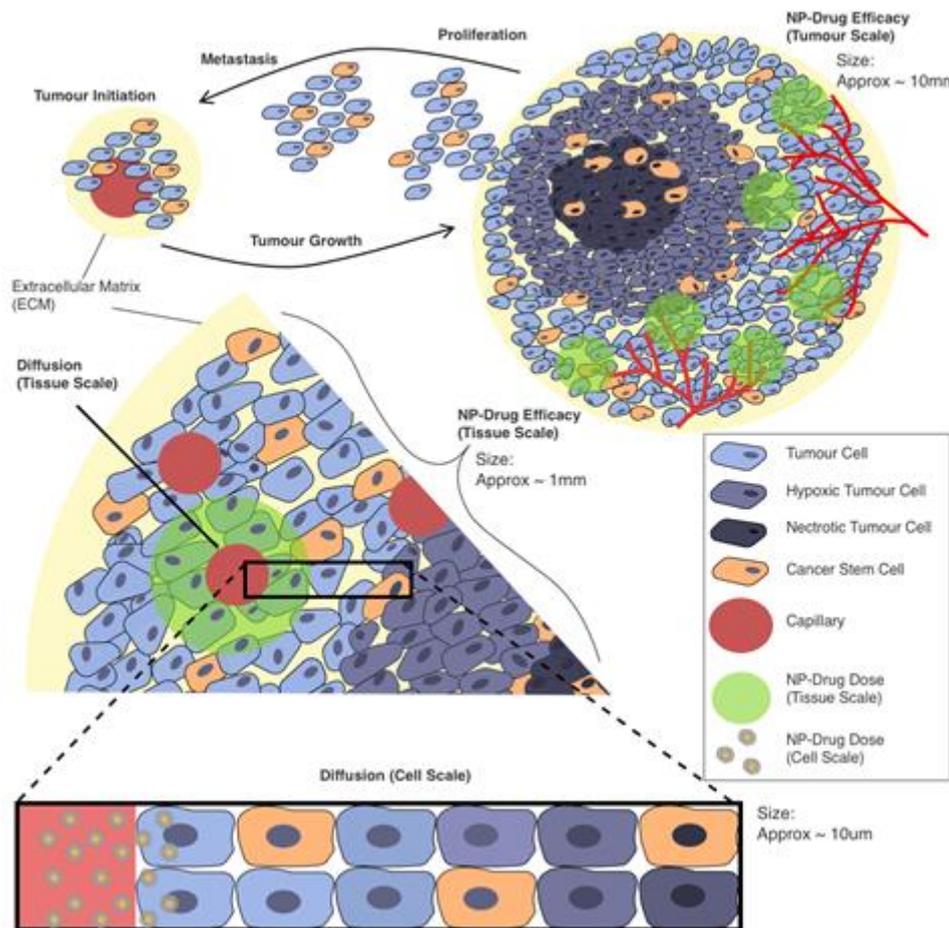


Figure 2. Model of tumour growth across different scales including transportation barriers to NPs. The model includes the initial growth, proliferation, metastasis and secondary tumour initiation and the corresponding NP-diffusion through the tumour at one of these growth stages.

In the model, tumour is initially dormant and relies on the diffusion of oxygen and nutrients from the normal tissue vasculature. Tumour usually cannot grow beyond 1-2 mm in diameter without an adequate blood supply (Helczynska et al., 2003; Vaupel and Harrison, 2004). After that, tumour cells lack nutrients and oxygen which leads to hypoxia, then necrotic cell death (Vaupel and Harrison, 2004) which causes the release of proinflammatory factors (Richards et al., 2011). Under hypoxic conditions, CSC produce factors that stimulate growth and angiogenesis (Keith and Simon, 2007; Pietras, 2011; Vinogradov and Wei, 2012).

By inducing angiogenesis, CSCs promote tumour progression through increased nutrient delivery (Relation et al., 2017). Tumour cells exploit their microenvironment by releasing cytokines and growth factors which stimulate neovascular proliferation (Weis and Cheres, 2011). Although the induction of angiogenesis may



initially provide the tumour with more oxygen and nutrients, vasculature in solid tumour is anarchic, unregulated and leaky. Combined with increased diffusion distances between the nutritive blood vessels and the tumour cell, this again leads to hypoxic conditions (Keith and Simon, 2007; Vaupel and Harrison, 2004; Weis and Cheres, 2011). Within the hypoxic region, tumour cells acquire a less mature, dedifferentiated phenotype (Helczynska et al., 2003).

2.4 MODELING VASCULAR FLOW AND EXTRAVASATION

To model vascular flow we will mainly rely on discrete approaches. In contrast to continuous approach whose main disadvantage is the lack of information about the structure of vascular network, using e.g. cellular automata (Topa, 2008) we can produce vascular networks of desired topologies.

In our model, the basic set of the control parameters that will govern tumor induced vascular growth is:

- PAF(pro-angiogenic factors) gradient coefficient.
- Oxygen gradient coefficient.
- PAF threshold that activates vascular growth.

The growth of all vessels is apical. Direction of vessel growth at each spatial subspace will be determined as biased random walk following PAF gradient. Significant proportion of tumour vasculature is composed of dysfunctional vessels which do not transport blood properly. This aspect will be incorporated in the model and all dysfunctional vessels will be removed gradually.

For modeling vasculature we will use following values (Caballero et al., 2017, Nakamura et al., 2016, Nehoff et al., 2014):

	Healthy capillary	Tumor capillary
Diameter (µm)	5–25	15-50
Length (µm)	100 - 1000	100 - 1000
Blood flow (mL g ⁻¹ min ⁻¹)	0.5–1	0–1 (highly heterogeneous)
Blood Pressure (mBar)	20	20 ± 5
Shear stress (dyne per cm ²)	4	1–10 (highly heterogeneous)
IFP mm Hg	0	10 – 40 (tumor tissue)*
Fenestration size range	-	50 to 4,700 nm**

Table 1. Parameters that will be used for modeling vasculature growth

2.5 NANOPARTICLE PARAMETER SPACE AND RANGES THAT WILL BE SIMULATED

Modelling and verifying the NPs action on the tumour will be done for strictly defined ranges of candidate constructs. We will be focusing on small gold Nanoparticles (GNPs) presenting metal core diameters of 5.5 nm due to their relatively easy crossing the cellular membrane and multiple possible mechanisms involved. If needed both simulated and validation experiments may include the diameter size ranges of 12-14 nm and 30 nm. The size range is chosen in accordance with the existing literature on effective design of nanocarriers for tumour targeting (Danhier et al., 2010; Wilhelm et al., 2016).

While the intrinsic and primary feature of NPs must include the full water solubility and stability the functionalization and background ligand selection will be directed to ensure this entry criterion. The NPs' functionalization facilitating solubility will be combined with the ligands enabling low level of nonspecific binding to cellular structures. To achieve both features we plan to utilise two possible strategies:

- Introduction of PEG-ylated moieties within ligand structures raring 3-6 ethylene glycol units;
- Formation of stabilizing NPs corona with ionized distal groups;

The NPs stabilized by ionized groups enables further insight and study of net surface charge influence (zeta potential value) on specific behaviour and activity towards tumour cells. We will model the range of NPs exhibiting positive, neutral and negative zeta potentials. On the empirical level the GNPs surface charge will be measured by Zeta potential measurements and isoelectric point (pKI) titration experiments.

Both eCS and evaluations will cover behaviour scenarios in a range of concentrations of GNPs. To achieve this, the empirical solutions will need precise concentration measurements and serial dilutions curves characteristics. Upon complete gold nanoparticle (GNP) synthesis and functionalization their concentration will be determined by UV-VIS spectroscopy due to fact that GNP absorb light in the ultraviolet range proportionally to concentration, accordingly to Lambert-Beer's law. During the EvoNano project, the traditional concentration of GNPs will be also measured using Multi Angle Dynamic Light Scattering (MADLS).

Chemical properties of proposed GNP constructs will be tracked from the ligand synthesis and characterisation to the final functionalisation corona content analysis by high resolution H-NMR spectroscopy.

GNPs will be labelled fluorescently to enable easy detection, as well as evaluate the microfluidic devices for in vitro anticancer treatment scenarios. We envisage that microfluidic devices testing and in vitro/ in vivo tracking of NPs within the cellular cancer models will need the fluorophore selection both exhibiting measurable signal generation and low energy activation to limit the artifacts and additional detrimental effects on living cells.



2.6. MOLECULAR-SCALE EVENTS

To address the issue of cellular penetration outlined in section 1.1.2, we will use molecular dynamics tools to model interactions between nanoparticles and cellular membrane. We plan to use LAMMPS or GROMACS software. Planned simulations will be performed in the second half of the project after we obtain first experimental verification results which will be used to identify shortcomings of the first round of suggested drug delivery systems.

3 META-MODEL IMPLEMENTATION SPECIFICATIONS

3.1 OVERVIEW OF NANODOC SOURCE CODE STRUCTURE

NanoDoc interfaces with the stochastic simulation compiler (SSC) to run the NP-simulations. SSC is an open-source tool for solving exact stochastic equations using an improved version of the Gillespie algorithm. Both the NanoDoc interface and SSC are written in C++. NanoDoc runs on Linux but requires old versions of both the C++ compiler and OS system (Ubuntu 12.04 and GCC 4.4). Both can be loaded and run using virtual containers (such as Docker).

NanoDoc typically models the interactions of dosage of NPs with tens to hundreds of cells. The parameter information for individual cells and NP species are contained with a JSON file that includes the following information:

- 1) Number of individual cells including blood vessels, healthy cells and tumour cells.
- 2) Properties of each cell including the required dosage of NPs to kill the cell, their binding profile and their location within the tumour environment.
- 3) Number of NP species.
- 4) Properties of each NP species including size, binding profile, surface functionalisation, number of NPs within the treatment and the duration of the treatment.

3.2 OVERVIEW OF CHANGES IN NANODOC SOURCE CODE WE PLAN TO IMPLEMENT

NanoDoc has been previously designed for use as an online game that crowdsources NP designs. This has led the software that solves the stochastic simulators to become outdated and no longer compatible with recent OS versions. SSC is also not currently designed for the high throughput computation (HTC) required of large scale parameter estimation and EA optimisation. As a result, we will change the stochastic simulator to one which has greater flexibility, up to date version support and HTC capabilities.

We will use the Stochastic Engine for Pathway Simulation (STEPS) (E. Wils, E. de Schutter, 2009) which allows for exact simulation of reaction-diffusion networks in arbitrary complex 3D geometries. The benefits of using STEPS is three-fold:



- 1) Up to date version support: STEPS is a set of python modules with core computational routines written in C++. It has been tested on Windows, OSX and Linux and has a straightforward installation procedure.
- 2) STEPS has been extended to offer HTC functionality. The most recent publication highlights a parallel simulation with over 2,000 processes which resulted in a 3,600 factor speedup when compared with serial stochastic simulation algorithms. This will allow for both high throughput parameter testing or larger tumour environment to be included in the simulations.
- 3) Previously, NanoDoc assumed that diffusion occurs spontaneously with an appropriate rate between well-mixed cellular compartments. STEPS allows for explicit modelling of diffusion through the extracellular matrix as well as surface functionalisation on the cellular regions using 3D tetrahedral meshes. This is expected to improve accuracy as well as being more readily combined with simulation output from PhysiCell such as the existences of a stem cell niche.

3.3 OVERVIEW OF PHYSICELL SOURCE CODE STRUCTURE

PhysiCell is a cross-platform C++ framework tested on OSX, Linux, and Windows. Recently PhysiCell has been extended with EMEWS to run large scale parallelized simulations (Ozik et al., 2018). It was created by extending the Basic_Agent class in BioFVM (Ghaffarizadeh et al., 2016). BioFVM is an open-source diffusion transport solver. It divides the simulation domain into a collection of non-intersecting voxels: volumetric pixels. Each voxel has a unique integer index: this is its unique address, for quickly accessing its information. BioFVM adds one or more diffusible substrates to this microenvironment. Each substrate has a diffusion coefficient and decay rate. For each voxel, a vector of chemical substrates (densities), and a vector of gradients (one gradient vector for each substrate) are stored.

Each Cell in PhysiCell is an extension of BioFVM's Basic_Agent class. As such, it has access to all the parent class's member data and functions. In PhysiCell, Cells have the following major parts (for details see PhysiCell User Manual, downloadable at <http://physicell.mathcancer.org>):

1. String type name: The name of the type of cell.
2. Custom Cell Data: Custom data attached the cell, which may differ from other cells.
3. Cell Parameters: A set of standardized parameters
4. Cell Functions: A collection of functions used for updating the cell phenotype, including custom functions.
5. Cell State: A small set of standard state variables.



6. Phenotype: A hierarchical organization of the cell's properties.

Each agent inherits the following member data and functions from Basic_Agent class:

1. int ID is a unique integer identifier for the cell. No other cell now or in the future will have the same ID.
2. int index is the cell's current index in `std::vector<Basic_Agent*>`
3. int type is a user-specified (default 0) integer code to classify the cell's type.
4. int get current voxel index(void) returns the cell's positional index in the BioFVM Microenvironment.
5. `std::vector<double>& nearest density vector(void)` allows the user to directly access (i.e., sample or modify) the vector of substrates at the cell's position.
6. `std::vector<double>& nearest gradient(int substrate index)` returns the gradient of the substrate with index `substrate_index`, at the cell's current position.
7. `std::vector<gradient>& nearest gradient vector(void)` returns a vector of all the substrate gradients at the cell's position.

3.4 OVERVIEW OF CHANGES IN PHYSICELL SOURCE CODE WE PLAN TO IMPLEMENT

1. New sample project “cancer_stem_cells” with the same elements as all other PhysiCell integrated projects:

- config (PhysiCell_settings.xml) folder, custom_modules(cancer_stem_cells.cpp, cancer_stem_cells.h) folder and scripts(empty.txt) folder
- Makefile and main-cancer_stem_cells.cpp

All mentioned files will be customized and new files/folders will be created so they follow physiological outline described in section 2.3.

2. Change framework:

- Add options to customize cell division in files PhysiCell_cell.h and PhysiCell_cell.cpp under the method `void divide(void)`.
- Add vasculature module by creating new code and files Groups.h, Point.hpp, Capillary.hpp. These files will be located in the core folder. Optionally we can move vasculature module into cancer_stem_cell part of the PhysiCell.

Most of the changes will be located in the cancer_stem_cells, inside custom_modules and config folders and inside main-cancer_stem_cells.cpp file.



3. If necessary we will modify BioFVM to change properties of voxels and micro-environment. It will be done by modifying files BioFVM_basic_agent.h and BioFVM_basic_agent.cpp.

4. To modify rules of cellular interactions we will change PhysiCell core package files that define these properties, such as: PhysiCell_cell.h, PhysiCell_cell.cpp, PhysiCell_cell_container.h, PhysiCell_cell_container.cpp.

3.5 OVERVIEW OF EVOLUTIONARY APPROACH WE PLAN TO IMPLEMENT

Given the very large high-dimensional search spaces and relatively minuscule evaluation budgets resulting from extremely expensive computational cancer simulations, it may not be possible to perform global optimisation using a conventional evolutionary algorithms (EAs).

Thus, the implementation of non-greedy EA optimisation techniques will be targeted, that will favour the exploration of the search space without hindering the exploitation. Exploration refers to the determination of areas in the search space where viable/optimum solutions are found, whereas exploitation refers to the greedy shift towards one optimum solution. One paradigm of these techniques is novelty search methods.

Novelty search is a non-objective search methodology that alleviates the problem induced by searches based on ambitious objectives, namely leading to dead-end local optima. Despite the fact that novelty search is unaware of the fundamental goal of the optimisation process, it managed to perform more efficiently than objective-based search in some cases.

Due to the fact that most of the real-world optimization problems demonstrate highly multimodal landscapes, we expect that the optimization of designing NPs is not an exception. Consequently, we plan to implement evolutionary computational algorithms with niching capabilities, for instance Differential Evolution mutation strategies.

The evolutionary optimization framework will be developed in Python, will be open source and available on a publicly available software repository. The framework will communicate with the combination of stochastic-based (NanoDoc) and agent-based (PhysiCell) simulators through manipulation of files (i.e. .txt, .xml and .mat files).

3.6 IMPLEMENTATION CONSTRAINTS

Nanodoc currently uses SSC which has significant restrictions in both version support and in terms of parallelisation. As explained in the section 3.2 NanoDoc will instead be linked to the Stochastic Engine for Pathway Simulation (STEPS). STEPS is open-source under the terms of the GNU General Public License as published by the Free Software Foundation, either version 2 of the License. The STEPS simulator is also able to be integrated within a high throughput computing framework (Chen and De Schutter, 2017) using an MPI protocol for CPU clusters. It is compatible with Windows, Linux and OSX. It is built as a Python module with computation performed using C++ code.



PhysiCell is open source, licensed under the (3-Clause) BSD License (<https://opensource.org/licenses/BSD-3-Clause>) which means that redistribution and use of its source code and binary forms are mostly allowed.

PhysiCell is GPL v2 and v3 compatible, and suitable for commercial use in many circumstances. It uses cross-platform compatible C++ (tested on OSX, Linux, and Windows), without need for a package manager system or any other complex installation, and it has been parallelized using OpenMP.

To build a high throughput computing (HTC) framework, PhysiCell has been recently integrated with an extreme-scale model exploration platform (EMEWS) (Ozik et al., 2018).

3.7 PERFORMANCE REQUIREMENTS

During development, algorithms will be tested using workstations and computers available at UNSPF, UWE Bristol and UNIVBRIS. Small scale simulations will be run using the same hardware capabilities.

For running large-scale simulations, EVO-NANO has a direct and free access to the following heterogeneous HPC platforms maintained by the Finnish IT center for science:

- Sisu Cray XC40 Supercomputer, (1688 compute nodes, each with two Intel Xeon E5-2690v3 (Haswell microarchitecture), totaling in 40512 cores, peak performance of 1688 TFlop/s). An example of Sisu computing node is presented in the figure 3.

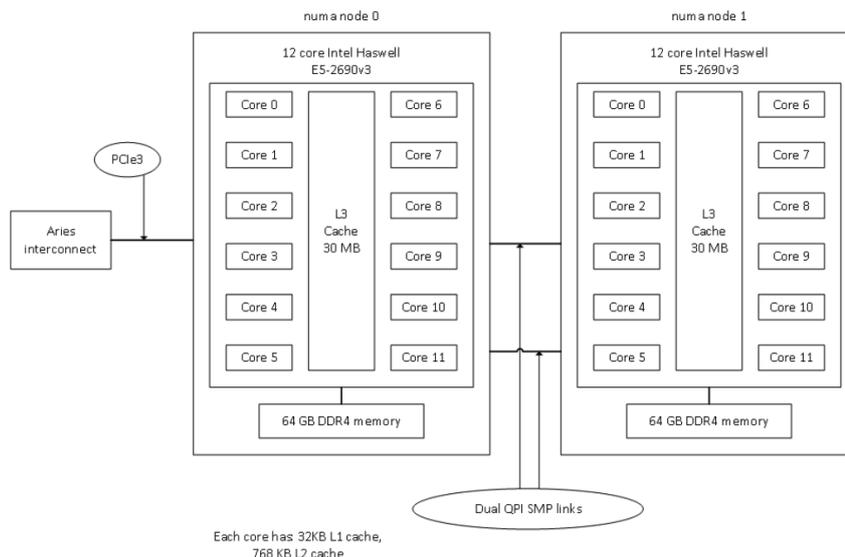


Figure 3. An example of Sisu computing node.

- Taito HP Apollo 6000 XL230a/SL230s Supercluster (407 Apollo 6000 XL230a G9 server blades, each with two twelve core Intel Haswell E5-2690v3 CPUs, for a total of 9768 cores, peak performance of 600 TFlop/s)



- Taito Accelerators: Bull B715 GPU cluster (36 nodes with dual Nvidia Kepler K40 GPGPUs and 44 nodes with dual Intel Xeon Phi 7120X coprocessors). The Taito architecture is presented in the Figure 4.

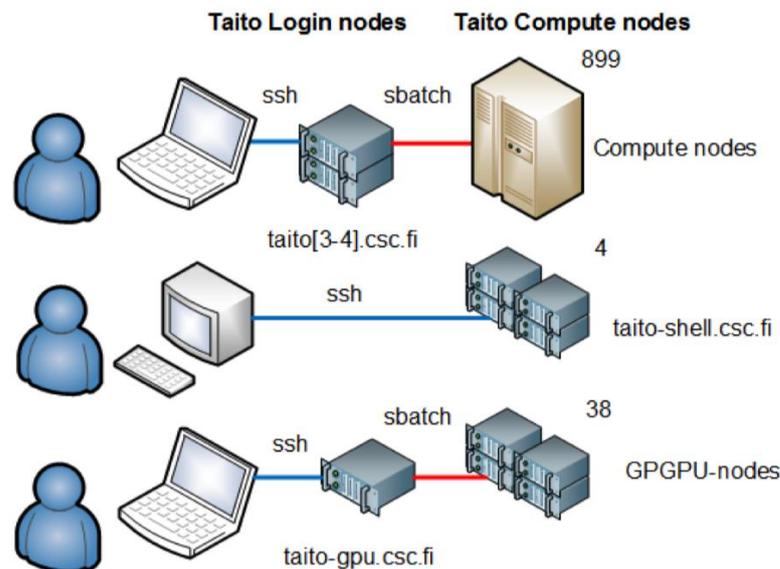


Figure 4. Depiction of the Taito architecture.

- Finnish Grid Infrastructure (51 TFlop/s in CPU capacity and 03 TFlop/s is GPGPU capacity), with a local 96 CPUs cluster owned by AAU

We have already benchmarked preliminaries simulations of PhysiCell with relevant complex examples on our local HPC platform. The benchmarked running time for respective number of simulated cells is as follows.

- 2-D simulation of the “biorobots” example, showing a synthetic multicellular cargo delivery system.
 - total number of cells: 513
 - total runtime: 3 minutes, and 13.048 seconds
- 2-D simulations of the “biorobots” adapted for use as a cancer treatment, where cargo cells detach and secrete a therapeutic once reaching hypoxic tissues.
 - total number of cells: 1593
 - total runtime: 14 minutes, and 14.3346 seconds
- 2-D simulation of a tumor whose heterogeneous oncoprotein expression drives proliferation and selection.
 - total number of cells: 2171
 - total runtime: 1 hours, 13 minutes, and 2.98002 seconds



- 3-D simulation of immune cells attacking a tumor with heterogeneous proliferation and immunogenicity.
 - total number of cells: 111470
 - total runtime: 1 days, 21 hours, 27 minutes, and 47.6682 seconds

The computational platforms have proven to be efficient based on the results of these runs. The main potential foreseeable challenge could be the integration of different simulation tools that are planned to be developed within the framework of this project.

4 DEVIATIONS FROM THE WORKPLAN

None

5 CONCLUSIONS

All planned activities are performed on time and without unexpected obstacles. As a part of preparing to implement the evolvable Cancer Simulator we:

- Built the suitable model of cancer growth based on available published data;
- Identified main sets of model parameters;
- Identified programming approaches we will use when modeling different tumour and NP scales, and
- Outlined source code changes in the existing open-source packages necessary for implementing the Simulator.

We also ran some preliminary tests that demonstrated feasibility of our approach.

6 REFERENCES

- Barua S, Mitragotri S (2014) Challenges associated with penetration of nanoparticles across cell and tissue barriers: A review of current status and future prospects. *Nano Today*, vol. 9, no. 2, pp. 223–243.
- Caballero D, Blackburn SM, de Pablo M, Samitier J, Albertazzi L (2017) Tumour-vessel-on-a-chip models for drug delivery. *Lab Chip* 17(22):3760-3771.
- Chen W, De Schutter E (2017) Parallel STEPS: Large Scale Stochastic Spatial Reaction-Diffusion Simulation with High Performance Computers, *Front. Neuroinform.*, vol. 11(13), pp. 1–15.
- Danhier F, Feron O, Pr at V (2010) To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of controlled release*,148(2), 135-146.
- Fedosov DA, Noguchi H, Gompper G (2014) Multiscale modeling of blood flow: from single cells to blood rheology. *Biomech Model Mechanobiol.* 2014 Apr;13(2):239-58.
- Ghaffarizadeh A, Friedman SH, Macklin P (2016) BioFVM: an efficient parallelized diffusive transport solver for 3-D biological simulations, *Bioinformatics* 32(8):1256-8.



- Ghaffarizadeh A, Heiland R, Friedman SH, Mumenthaler SM, Macklin P (2018) PhysiCell: An open source physics-based cell simulator for 3-D multicellular systems. *PLoS Comput Biol* 14(2): e1005991.
- Helczynska K, Kronblad A, Jögi A, Nilsson E, Beckman S, Landberg G, Pålman S (2003) Hypoxia promotes a dedifferentiated phenotype in ductal breast carcinoma in situ. *Cancer Res.*, 63(7), pp. 1441–1444.
- Heldin C-H, Rubin K, Pietras K, Ostman A (2004) High interstitial fluid pressure an obstacle in cancer therapy, *Nat. Rev. Cancer*, 4 (10) (2004), pp. 806-813
- Keith B, Simon MC (2007) Hypoxia-Inducible Factors, Stem Cells, and Cancer. *Cell*, vol. 129, no. 3, pp. 465–472.
- Lehn RC (2013) Effect of Particle Diameter and Surface Composition on the Spontaneous Fusion of Monolayer-Protected Gold Nanoparticles with Lipid Bilayers. *NANO Letters* 13(9), 4060-7.
- Lis M, Artyomov MN, Devadas S, Chakraborty AK (2009) Efficient stochastic simulation of reaction-diffusion processes via direct compilation, *Bioinformatics*, vol. 25, no. 17, pp. 2289–2291.
- Mohyeldin A, Garzón-Muvdi T, Quiñones-Hinojosa A (2010) Oxygen in stem cell biology: A critical component of the stem cell niche. *Cell Stem Cell*, vol. 7, no. 2, pp. 150–161.
- Nakamura Y, Mochida A, Choyke PL, Kobayashi H (2016). Nanodrug delivery: is the enhanced permeability and retention effect sufficient for curing cancer?. *Bioconjugate chemistry*, 27(10), 2225-2238.
- Nehoff H, Parayath NN, Domanovitch L, Taurin S, Greish K. (2014) Nanomedicine for drug targeting: strategies beyond the enhanced permeability and retention effect. *Int J Nanomed.* 9, 2539–2555.
- Nichols J, Bae Y (2012) Odyssey of a cancer nanoparticle: From injection site to site of action. *Nano Today*, 7, 606-618.
- Oroskar PA (2016) Simulated Permeation and Characterization of PEGylated Gold Nanoparticles in a Lipid Bilayer System. *Langmuir* 32(30), 7541-55.
- Ozik J, Collier N, Wozniak J, Macal C, Cockrell C, Friedman S, Ghaffarizadeh A, Heiland R, Macklin P (2018) High-throughput cancer hypothesis testing with an integrated PhysiCell-EMEWS workflow. *BMC Bioinformatics* 19:483
- Pattabiraman DR, Weinberg RA (2014) Tackling the cancer stem cells — what challenges do they pose? *Nat Rev Drug Discov.* 13:497–512. <http://doi.org/10.1038/nrd4253>
- Peitzsch C, Tyutyunnykova A, Pantel K, Dubrovskaya A (2017) Cancer stem cells: The root of tumour recurrence and metastases. *Semin. Cancer Biol.*, vol. 44, pp. 10–24.
- Pietras A (2011) *Cancer Stem Cells in Tumor Heterogeneity*, vol. 112. Elsevier Inc.
- Plaks V, Kong N, Werb Z (2015) The cancer stem cell niche: How essential is the niche in regulating stemness of tumour cells? *Cell Stem Cell*, vol. 16, no. 3, pp. 225–238.
- Relation T, Dominici M, Horwitz EM (2017) Concise Review: An (Im)Penetrable Shield: How the Tumour Microenvironment Protects Cancer Stem Cells. *Stem Cells*, vol. 35, no. 5, pp. 1123–1130.
- Rich JN (2016) Cancer stem cells: Understanding tumour hierarchy and heterogeneity. *Med. (United States)*, vol. 95, no. 1, pp. S2–S7.
- Richards CH, Mohammed Z, Qayyum T, Horgan PG, McMillan DC (2011) The prognostic value of histological tumor necrosis in solid organ malignant disease: a systematic review. *Futur. Oncol.*, vol. 7, pp. 1223–1235.
- Topa P (2008) Dynamically Reorganising Vascular Networks Modelled Using Cellular Automata Approach. In: Umeo H., Morishita S., Nishinari K., Komatsuzaki T., Bandini S. (eds) *Cellular Automata. ACRI 2008. Lecture Notes in Computer Science*, vol 5191. Springer, Berlin, Heidelberg
- Vaupel P, Harrison L (2004) Tumor Hypoxia: Causative Factors, Compensatory Mechanisms, and Cellular Response. *Oncologist*, 9, Suppl 5:4-9.



Vinogradov S, Wei X (2012) Cancer stem cells and drug resistance: The potential of nanomedicine. *Nanomedicine*, vol. 7, no. 4, pp. 597–615.

Weis SM, Cheres DA (2011) Tumor angiogenesis: Molecular pathways and therapeutic targets. *Nat. Med.*, vol. 17, no. 11, pp. 1359–1370.

Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, Chan WC (2016) Analysis of nanoparticle delivery to tumours. *Nature reviews materials*, 1(5), 16014.

Wils S, De Schutter E (2009) STEPS: Modeling and simulating complex reaction-diffusion systems with Python. *Frontiers in Neuroinformatics* 3: 15.