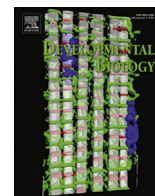




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The Neurosphere Simulator: An educational online tool for modeling neural stem cell behavior and tissue growth



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ABSTRACT

Until very recently, distance education, including digital science labs, served a rather small portion of post-secondary students in the United States and many other countries. This situation has, however, dramatically changed in 2020 in the wake of the COVID-19 pandemic, which forced colleges to rapidly transit from face-to-face instructions to online classes. Here, we report the development of an interactive simulator that is freely available on the web (<http://neurosphere.cos.northeastern.edu/>) for teaching lab classes in developmental biology. This simulator is based on cellular automata models of neural-stem-cell-driven tissue growth in the neurosphere assay. By modifying model parameters, users can explore the role in tissue growth of several developmental mechanisms, such as regulation of mitosis or apoptotic cell death by contact inhibition. Besides providing an instantaneous animation of the simulated development of neurospheres, the Neurosphere Simulator tool offers also the possibility to download data for detailed analysis. The simulator function is complemented by a tutorial that introduces students to computational modeling of developmental processes.

1. Introduction

Over the past two decades, higher education has witnessed a marked increase in online education. According to the [National Center for Education Statistics](#), in fall 2018 there were a total of 19.7 million students enrolled in degree-granting postsecondary institutions in the United States ([National Center for Education Statistics](#), in press). Of them, 6.9 million students (35.3% of the total student population) took some form of distance education course(s).

Despite a steady year-to-year increase since the turn of the century in the number of students who have taken some form of distance education course(s) ([Allen and Seaman, 2015](#)), the statistical data also show that online learning played, until very recently, a rather limited role in higher education teaching, at least in the United States. In fall 2018 only a minority — 3.3 million students (16.6% of the total student population) — were enrolled in exclusively distance education course(s), compared to 12.7 million students (64.7% of the total student population) who did not take any distance education courses ([National Center for Education Statistics](#), in press).

However, the shutdown, in March 2020, of numerous school systems and colleges in the wake of the COVID-19 pandemic, and the rapid transition from face-to-face instructions to online classes, has sparked an

enormous interest among students, parents, and instructors in online learning. This is reflected by an unprecedented surge, coinciding with the campus closures, in search volumes of queries like “remote learning” or “online learning” in the United States (and similarly worldwide) using Google Web Search ([Fig. 1](#)) ([Google Trends, n.d.](#)).

Whereas faculty are faced with the need to rapidly incorporate online components into their teaching, development of their own online courses is usually not feasible, due to the substantial amount of resources required in terms of time, personnel, infrastructure, and funding ([Hollands and Tirthali, 2014](#)). This applies, in particular, to digital science labs that contain, in addition to information presented in form of text, images, and videos, interactive components. Instructors of science labs are, therefore, dependent on (ideally free) digital labs. Such tools have become increasingly popular in recent years as alternatives, or complements, of physical labs ([Huang, 2004](#); [Jones, 2018](#)). Studies in which the acquisition of conceptual knowledge was tested have, overall, not found any significant differences between students that carried out physical experiments and students that ran virtual experiments (for review, see [de Jong et al., 2013](#)).

Here, we describe the development of a web-based interactive simulator that simulates, in the neurosphere assay, tissue growth driven by neural stem cells isolated from the adult central nervous system. This

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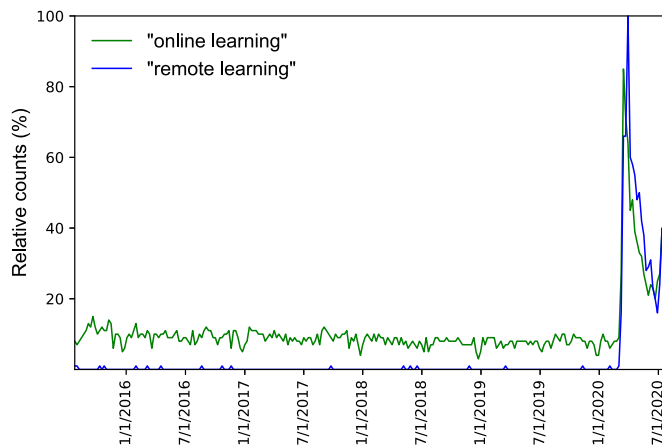


Fig. 1. Search trends in the United States for queries in Google Search using the terms “remote learning” and “online learning” over the period of July 26, 2015 and July 25, 2020. The search volume for each of these terms is normalized to the peak popularity of the “remote learning” query. Note that the start of the surge in queries for both strings coincides with the announcement of the times when U.S. President Donald Trump officially declared a national emergency in wake of the COVID-19 pandemic (March 13, 2020) and when the New York City’s public school system announced that it would close (March 15, 2020).

digital toolbox, titled Neurosphere Simulator, is based on computational models of neurosphere development built as part of our research activities (Sipahi and Zupanc, 2018; Zupanc et al., 2019).

The neurosphere assay is a widely used *in-vitro* system to study the properties of neural stem cells and their progeny. Neurospheres are spherical structures that are formed, under serum-free culture conditions, by proliferation of single neural stem or progenitor cells when stimulated with epidermal growth factor, fibroblast growth factor 2, or both (Reynolds and Weiss, 1992).

Growth factor withdrawal and addition of serum to the culture medium induces neurospheres to differentiate into the three major types of cells in the central nervous system — neurons, astrocytes, and oligodendrocytes. However, since neurospheres are less suitable for analyzing their inner cellular composition, it is customary to study the cellular differentiation of neurosphere cells by transferring them to monolayer culture systems, instead of analyzing this phenomenon directly in the neurosphere assay (for review, see Azari and Reynolds, 2016). Due to this limitation of the neurosphere assay, the focus of the Neurosphere Simulator is on developmental processes other than cellular differentiation. Although differentiation can be simulated with the Neurosphere Simulator, the underlying model does not distinguish between different types of differentiated cells. Instead, terminally differentiated cells of any type are simply subsumed under the term ‘differentiated cells.’

The primary intended users of our Neurosphere Simulator toolbox are undergraduate and graduate students in developmental biology lab courses, lecture classes, or discussion groups. As users can modify a variety of parameters that modulate the development of the simulated neurospheres, a multitude of simulations can be run, making the Neurosphere Simulator also suitable as a research tool — particularly to those who would like to explore how modeling could benefit their work.

Depending on the specific aspects examined through simulations, instructors can design their own lab modules, for example one focusing on normal stem-cell-driven tissue growth, while the other examining the parameters that result in, or suppress, generation and promotion of tumorous tissue growth. In addition to the simulator function, background information is provided in form of a tutorial.

2. Educational objectives of the neurosphere simulator

The Neurosphere Simulator has been developed for enabling students to

- (i) become familiar with an important *in-vitro* system to study neural stem cells and the normal and tumorous development of their progeny;
- (ii) identify key players (e.g., stem cells, progenitor cells, differentiated cells, brain tumor stem cells) and cellular processes (e.g., mitosis, cellular differentiation, apoptosis, phagocytosis) in the development of tissue arising from proliferation of founder stem cells or progenitor cells;
- (iii) explore the effect of various parameters on several developmental events (e.g., of the proliferative potential of stem or progenitor cells on the size of neurospheres);
- (iv) discuss how modeling and simulation can complement experimental studies, thus leading to a deeper theoretical understanding of biological phenomena in general, and of developmental biological concepts in particular.

3. Model development

Our model is a modified version of cellular automata (CA) models built previously (Lehotzky et al., *in press*; Sipahi and Zupanc, 2018; Zupanc et al., 2019). These models are formulated on a 2-dimensional (either square or hexagon) lattice, consisting of distinct lattice sites. Each lattice site is either empty or occupied by an agent (a cell, in our biological context). Using the CA framework, stochastic rules inspired by biological observations were formulated for defining the behavior of these agents, including their interactions with other agents (for a review on how such rules can be formulated in a broader biological context, see Lehotzky and Zupanc, 2019).

Starting from an initial site within the lattice, each lattice site is updated successively according to these governing stochastic rules. In the Neurosphere Simulator, the initial lattice site always contains a single agent, simulating a founder stem or progenitor cell. During each update, this agent may undergo simulated division, depending on its type and division history. Globally, the resulting generation of new agents leads to simulated tissue growth, mimicking the formation of a neurosphere in the neurosphere assay.

We implemented our model using Python 3.7. Occupied lattice sites and the state of their associated agents are stored in a Python dictionary. State values of agents are successively updated in this dictionary at each time step according to the governing rules. For random number generation and simple algebraic manipulations, the NumPy 1.18 library is used.

4. Web development

The web development was guided by two objectives. First, on the technical side, it was important to prioritize performance, low cost, and the use of modern tools. Second, on the user side, visitors of the website should be able to learn about CA modeling through an interactive tutorial and then run the neurosphere model and visually observe its outcome.

Following common web development practice, the front-end architecture is separated from the back-end architecture, which runs the CA model and returns its output. The application’s front end is built with JavaScript (ES6) and React.js, a library for building user interfaces with a reusable, component-based approach. React.js was also chosen for its performance benefits due to the use of a memory-based, virtual Document Object Model (DOM). Other JavaScript and Cascading Style Sheets (CSS) libraries were used for styling and validation, including Bootstrap,

Formik, and Yup. The interface was developed with Web Content Accessibility Guidelines (WCAG) 2.0 best practices ([Web Content Accessibility Guidelines 2.0, 2008](#)). Accessible code design makes the Neurosphere Simulator tool available to users with auditory, cognitive, visual, and/or motor impairments by ensuring that it is operable through a keyboard interface, clear to read, and compatible with assistive technologies, such as screen readers or screen magnification software.

The application's back end consists of a Python Flask Representational State Transfer Application Programming Interface (REST API). The API endpoints receive parameters as input to the CA model. The model is run with these parameters, and an animation of cell development (on a square or horizontal lattice) is produced. This visualization is generated concurrently while the model runs, using `matplotlib.animate` in Python 3.7. The API response contains time-series data from the model and an HTML5-encoded video of the animation. It is necessary to re-run the model in response to each user request, even if it has already run with those exact parameters, to showcase the stochastic nature of the model. Consequently, this lack of a database or cache system reduces the application's memory requirements.

The codebase is containerized using Docker Compose, a tool that enables us to run the website as a multi-container Docker application. In our configuration, an Nginx reverse proxy routes incoming web traffic to the front-end service, running in a Node.js container, or to the back-end service, running in a Flask/uWsgi container. With this setup, the web server starts with a single command. The server is hosted on an Ubuntu 16.04 virtual machine (VM) partitioned in a Microsoft Azure cloud server.

5. Website content

The Neurosphere Simulator website is available online at <http://neurosphere.cos.northeastern.edu/> (Fig. 2). Besides the Neurosphere Simulator Tool page, the website contains a tutorial, suggestions for teaching lab exercises, and a list of publications relevant to stem cell biology, the neurosphere assay, and modeling of neurosphere development. Each reference is associated with a link to the article on the publisher's website or the PubMed entry of the United States National Library of Medicine at the National Institutes of Health (<https://pubmed.ncbi.nlm.nih.gov/>).

5.1. Tutorial

The Tutorial is divided into three major parts: Modeling and Simulations, Modeling Developmental Processes, and Growing Tissue.

The Modeling and Simulations part provides a brief introduction to CA modeling as a discrete modeling framework on which the Neurosphere Simulator is based. Among the properties of CA that make them particularly well suited for applications in developmental biology is the ability to dissect a given biological system down to the level of individual cells (represented by agents in CA) and to define, through specific rules extracted from biological studies of intercellular interactions, local interactions between these agents.

The Modeling Developmental Processes part of the Tutorial shows how agents and their rule-governed interactions can be used to simulate some fundamental processes in cell development, such as mitotic division of stem and progenitor cells, cellular differentiation, apoptosis, phagocytosis, and transformation of a neural progenitor cell into a brain tumor stem cell. This part also demonstrates how the effect of contact inhibition on cell proliferation and cell death can be translated into a mathematical algorithm in the model.

The Growing Tissue part provides a preview of the capability of the Neurosphere Simulator. Using the default settings of this tool, it shows how neurosphere growth can be simulated by activation of a single

neural stem (founder) cell, and how the simulated two-dimensional structure compares with the three-dimensional tissue structures that grow in the neurosphere assay.

5.2. Neurosphere Simulator Tool

The Neurosphere Simulator Tool (Fig. 3) is designed to make the running of simulations as simple as possible while providing users with a significant amount of flexibility in the selection of model parameters. First-time users are recommended to take a digital tour of the functions of the input boxes on the Tool's dashboard.

For the initialization of a simulation, the user can use the default settings of the Neurosphere Simulator or modify the following parameters:

- Lattice type: square or hexagon.
- Lattice size: small (total square lattice: 100; total hexagon lattice: 90), medium (total square lattice: 900; total hexagon lattice: 780), or large (total square lattice: 2500; total hexagon lattice: 2150).
- Type of founder cell: active stem cell or progenitor cell.

We offer the option to select each of these two cell types as a founder cell because studies have suggested that a minor portion of biological neurospheres are derived from *bona fide* neural stem cells, whereas the majority appears to originate from progenitor cells (Deleyrolle et al., 2008; Narayanan et al., 2012).

- Minimum probability of mitosis: The probability of mitosis of a stem cell, progenitor cell, or brain tumor stem cell when one lattice site is vacant in the von Neumann neighborhood. On a two-dimensional square lattice, the von Neumann neighborhood is composed of the four squares immediately adjacent to the central agent.

The regulation of mitosis by local cell density ('contact inhibition') is consistent with early observations of the regulatory effect of cellular interactions on the growth of fibroblasts and amnion cells (Eagle and Levine, 1967), and, more recently, with the response of epithelial model tissue to experimental manipulation of the mechanical forces applied to these cells (Streichan et al., 2014).

- Maximum probability of mitosis: The probability of mitosis of a stem cell, progenitor cell, or brain tumor stem cell when all lattice sites are vacant in the von Neumann neighborhood.
- Minimum probability of cell death: The probability that a daughter cell undergoes cell death upon completion of mitosis when all lattice sites are vacant in the von Neumann neighborhood.

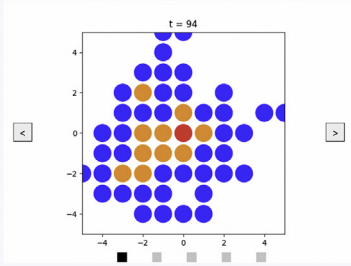
The occurrence of cell death in one of the two daughter cells after mitotic division, and the regulation of this phenomenon by local cell density, has been suggested by a variety of studies (for reviews, see Eisenhoffer and Rosenblatt, 2013; Kuan et al., 2000; Sommer and Rao, 2002).

- Maximum probability of cell death: The probability that a daughter cell undergoes cell death upon completion of mitosis when one lattice site is vacant in the von Neumann neighborhood.
- Quiescence time: The number of iteration steps during which a stem cell cannot be activated to undergo mitosis.
- Probability of stem cell activation: The probability that a stem cell is activated to undergo mitosis.
- Proliferative potential: The number of mitotic divisions performed by a progenitor cell before it terminally differentiates.

Northeastern Neurobiology [Home](#) [Tutorial](#) [Neurosphere Simulator](#) [Lab Exercises](#) [Publications](#) [People](#)

Welcome to the Neurosphere Simulator Toolbox

Laboratory of Neurobiology at Northeastern University



As part of a research project funded by the [National Science Foundation](#), the Laboratory of Neurobiology at Northeastern University has built computational models that simulate stem-cell-driven tissue growth *in vitro* and *in vivo*. Based on these models, this website provides the Neurosphere Simulator as a free online tool. This simulator has been designed primarily for educational purposes, but it offers many features that allow users to also explore its suitability for their research.

LEARN MORE

If you are new to cellular automata modeling and its application to developmental neurobiology, and would like to learn more, click here.

[Tutorial](#)

NEUROSPHERE SIMULATOR

If you like to go directly to the Neurosphere Simulator, click here.

[Simulator](#)

RESEARCH

If you are interested in the research that led to the development of the Neurosphere Simulator, click here.

[Research](#)

Fig. 2. Home page of the Neurosphere Simulator Toolbox.

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Neurosphere Simulator Tool

If this is your first time using the Neurosphere Simulator, click here: [Take a tour!](#)

<p>Lattice Type</p> <p><input type="text" value="Square Lattice"/></p>	<p>Lattice Size</p> <p><input type="text" value="Medium"/></p>	<p>Founder Cell Type</p> <p><input type="text" value="Active stem cell"/></p>	<p>Pa</p> <p><input type="text" value="1"/></p> <p>Probability of stem cell activation</p>
<p>Quiescence Time</p> <p><input type="text" value="1"/></p> <p>Number of time steps after mitosis during which a quiescent stem cell cannot be activated</p>	<p>Proliferative Potential</p> <p><input type="text" value="7"/></p> <p>Number of divisions after which a progenitor cell differentiates</p>	<p>Clearance Time</p> <p><input type="text" value="5"/></p> <p>Number of time steps after which a dead cell is cleared from its lattice site</p>	<p>Min Pm</p> <p><input type="text" value="0.5"/></p> <p>Minimum probability of mitosis</p>
<p>Max Pm</p> <p><input type="text" value="1"/></p> <p>Maximum probability of mitosis</p>	<p>Min Pd</p> <p><input type="text" value="0.05"/></p> <p>Minimum probability of daughter cell death</p>	<p>Max Pd</p> <p><input type="text" value="0.5"/></p> <p>Maximum probability of daughter cell death</p>	<p>Transformation to Brain Tumor Stem Cell (BTSC)</p> <p><input type="checkbox"/> Allow BTSC Transformation</p>

[Reset default parameters](#)

[Run Model](#)

This site was built by the Laboratory of Neurobiology at Northeastern University.
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Fig. 3. Dashboard of the Neurosphere Simulator Tool page.

- Clearance time: Number of iteration steps after which a dead cell is cleared through phagocytosis from its lattice site, thereby emptying the site for a new cell.
- BTSC transformation: Enables transformation of a randomly selected progenitor cell into a brain tumor stem cell (BTSC).
- Time steps until transformation: Iteration number at which a randomly selected progenitor cell is transformed into a BTSC. The iteration number is an indicator of the age of the simulated neurosphere tissue.

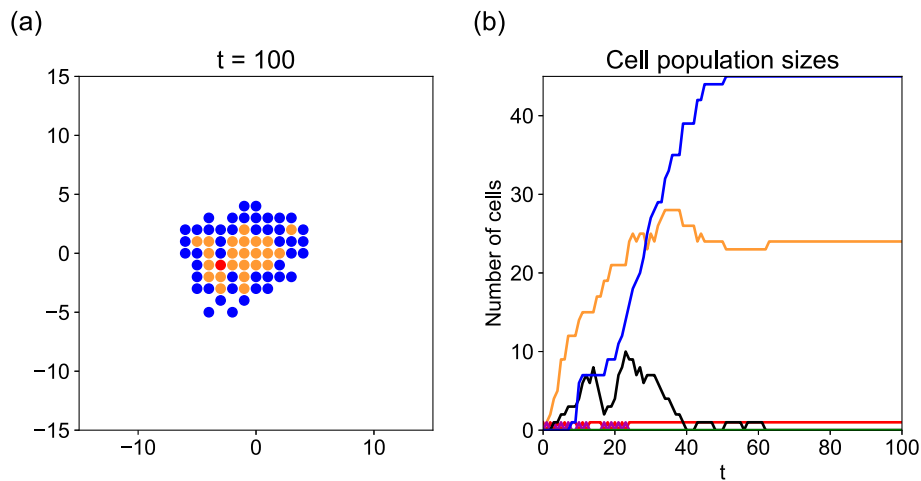


Fig. 4. Simulated neurosphere at iteration step 100 (a). Development of its cellular populations (b). The structure was grown using the default settings of the Neurosphere Simulator. Red, active stem cell; orange, progenitor cells; blue, differentiated cells; black, dead cells; green, BTSCs (not present).

The hypothesis that brain tumors have their origin in the transformation of adult neural stem cells and their progenitors into BTSCs is grounded on the argument that the former two cell types possess already the activated cellular and molecular machinery required for mitotic division, and might, therefore, require less mutations than needed for malignant transformation of a differentiated cell into a tumor cell (Sanai et al., 2005).

The Neurosphere Simulator will run the model for 100 iteration steps while generating an animation of the growing neurosphere and a plot of the development of its cellular population over time (Fig. 4). For performing a custom-designed detailed analysis of each simulation, users can download a data file in .csv format. This data file, titled ‘MQD_Data’, provides information about the position and associated state values of all cells (agents) at each time (iteration) step. For each cell, the data are formatted as: (horizontal position, vertical position): M, Q, D, where the values of M, Q, and D define the state of the cell (for details, see Table 1).

6. Teaching lab exercises

The possibility to modify the parameters of the Neurosphere Simulator enables instructors to design a multitude of teaching lab exercises. In the following, we describe three. We recommend consulting the papers by Sipahi and Zupanc (2018) and Zupanc et al. (2019) for background information and interpretation of the results.

6.1. Effect of founder cell type on neurosphere growth

Biological neurospheres exhibit an enormous heterogeneity in size, even when derived through passaging from the same parental neurosphere and cultured under identical conditions. It has been postulated that size differences within a given neurosphere population are related to the identity of the founder cell, with *bona fide* stem cells giving rise to

larger neurospheres than progenitor cells with more restricted proliferative potential (Louis et al., 2008). However, this hypothesis has been questioned by other investigators (Pastrana et al., 2011).

Hypothesis: In this lab exercise the student will test the hypothesis that stem cells as founder cells give rise to larger neurospheres than do progenitor cells as founder cells.

Approach: Run two sets of simulations: one in which an active stem cell is used as a founder cell, and another in which a progenitor cell is used as a founder cell. In all simulations, set the proliferative potential of the progenitor cells to 7 and select the large animation lattice size. For all the other parameters, use the default settings of the simulator.

Tasks: Run 10 simulations for each of the two sets and calculate the mean size of neurospheres by counting the number of ‘live’ cells (= stem cell, progenitor cells, and differentiated cells) at iteration step = 100. For the cell count, use the MQD data file.

Provide snapshots at iteration step = 100 of simulated neurospheres grown under the two conditions. Based on the visual inspection of these simulated neurospheres, what might limit the growth of the neurospheres, even though the stem cell has unlimited proliferative potential, and the proliferative potential of the progenitor cells may not be exhausted?

6.2. Effect of proliferative potential of progenitor cells on neurosphere growth

Progenitor cells arise from stem cells and undergo a few mitotic divisions before they terminally differentiate. This mitotic activity of the progenitor cells plays an important role in tissue growth.

Hypothesis: In this lab exercise, the student will test the hypothesis that the proliferative potential of progenitor cells correlates positively with the size of neurospheres.

Approach: Select the large animation lattice size and an active stem cell as a founder cell. Modify the proliferative potential from 1-10. For all

Table 1

State variables and their associated values for different cell types.

State variable	Associated values					
	Active stem cell	Quiescent stem cell	Progenitor cell	Differentiated cell	Dead cell	BTSC
M	1	2	1	5	3	4
Q	Number of divisions = (Q-1)/2	Number of divisions = (Q-1)/2	Number of divisions since asymmetric division of stem cell = (Q-2)/2	0	0	Number of divisions since transformation from progenitor cell = (Q-2)/2
D	Number of time steps since last division occurred	Number of time steps since last division occurred	0	0	Number of time steps since death via apoptosis occurred	0

Table 2

Hours spent by personnel to develop the Neurosphere Simulator website.

Type of personnel	Hours
Principal Investigator	210
Postdoctoral Research Associate	210
Web Developer 1	352
Web Developer 2	352
Total	1124

other parameters, use the default setting of the Neurosphere Simulator.

Tasks: Run 10 simulations for each proliferative potential of the progenitor cells. Plot the mean number of ‘live’ cells as a function of the proliferative potential. Do the results support the hypothesis?

6.3. Tumorigenesis by transformation of progenitor cells into brain tumor stem cells

It has been proposed that brain tumors are caused by transformation of adult neural stem or progenitor cells into BTSCs (Sanai et al., 2005). Based on this hypothesis, we will examine how the cellular micro-environment can modulate the development of tumorous tissue.

Hypothesis: In this lab exercise, we will test the specific hypothesis that encapsulation of BTSCs by neighboring non-BTSC cells can suppress tumor growth.

Approach: Select the large animation lattice size and an active stem cell as a founder cell. Use the following parameter settings: Proliferative potential of progenitors: 6; Clearance time: 10; Minimum probability of mitosis: 0; Maximum probability of mitosis: 1; Minimum probability of cell death: 0.05; Maximum probability of cell death: 0.5; Transformation to BTSC: yes; Time steps until transformation: 10. For the other parameters, use the default settings of the Neurosphere Simulator.

Task: Run 50 simulations with these parameter settings. Plot the frequency distribution of the size of these 50 simulated neurospheres, as assessed by determining the number of ‘live’ cells for each neurosphere. What is notable about the distribution? Does each neurosphere develop into tumorous tissue? Inspect the animated cellular structure of the different types of neurospheres. What role does encapsulation of BTSCs play for the promotion of tumorous growth?

7. Cost analysis

The development of online courses and lab tools consumes considerable institutional resources, particularly in terms of personnel expenditures (Hollands and Tirthali, 2014). To help other faculty and institutions making rational decisions on pursuing similar projects, we present here an estimate of the production costs of the Neurosphere Simulator website. These costs exclude the costs for the generation of previous research versions of the simulator (Sipahi and Zupanc, 2018; Zupanc et al., 2019) but include the costs for translation of the MATLAB codes (on which these research models are based) into Python code that was used for web development.

For our cost analysis, we estimated the hours spent by each of the four team members on the project (Table 2). In the case of the two Web Developers, the number of hours is based on detailed time logs. In the case of the Principal Investigator and the Postdoctoral Research Associate, the number of hours spent were estimated retrospectively at the time of completion of the project. To protect the privacy of the individuals involved and to allow for better comparison across different institutions and geographic regions, instead of actual salaries we used the 2018-19 academic year national average salary of a full professor at a Carnegie Tier-1 university and the amount of an NIH stipend for a postdoctoral fellow with 3 years of experience in 2019. For the salaries of the Web Developers, we used the 2019 median pay according to the U.S. Bureau of

Labor Statistics. In each case, we assumed fringe benefits at a rate of 25%. We assumed that the combined personnel costs (including fringe benefits) represent 75% of total costs, as is typical for delivery of educational interventions (Levin and McEwan, 2001). The remaining 25% are assumed to amount for facilities, equipment, and institutional support.

According to our estimate, we spent a total of 1124 h on the project. The salary expenditures (including fringe benefits) amounted to \$68,352. Thus, the total estimated costs for production of the website, including costs for personnel, facilities, equipment, and institutional support, amounted to \$91,136.

Acknowledgments

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