STSE: Spatio-Temporal Simulation Environment Dedicated to Biology

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Abstract

Background: Recently, the availability of high-resolution microscopy together with the advancements in the development of biomarkers used as reporters of biomolecular interactions increased the importance of imaging methods in molecular cell biology. These techniques enable the analysis of spatial cell properties and open up many new areas of research in the study of spatial, complex and dynamic cellular system. One of the crucial challenges for the study of such systems is the design of a well stuctured and optimized workflow to provide a systematic and efficient hypothesis verification. Computer Science can ease this task by providing software that facilitates the handling, analysis, and evaluation of biological data to the benefit of experimenters and modelers.

Results: The Spatio-Temporal Simulation Environment (STSE) is a set of *open-source* tools provided to simplify the execution of spatio-temporal simulations in discrete structures based on the microscopy images. The framework contains modules to *digitize, represent, analyze*, and *mathematically model* spatial distributions of chemical species. Digitization is performed using GUI tools allowing for user-friendly editing based on the Voronoi concept. Additionally, it is possible to automatically acquire spatial information from the images based on pixel luminosity (e.g. corresponding to molecular levels from microscopy images).

Here, we describe the details of the STSE implementation and workflow. We demonstrate it's usefulness for the example of a signaling cascade leading to formation of a morphological gradient of Fus3 inside of the mating yeast cell *Saccharomyces cerevisiae*.

Conclusions: STSE is an efficient and powerful novel platform, designed for computational handling and evaluation of microscopic images. It allows for an uninterrupted workflow including digitization, representation, analysis, and mathematical modeling. By providing the means to relate the simulation to the image data it allows for systematic, image driven model validation or rejection. STSE can be scripted and extended using the Python language. STSE is freely available either as a stand-alone version or included in the linux live distribution Systems Biology Operational Software (SB.OS) and can be downloaded from http://www.stse-software.org/. The Python source code as well as a comprehensive user manual and video tutorials are also offered to the research community.

Keywords systems biology, image analysis, simulation, discrete representation, digitization, quality control, complex system analysis

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Background

With the availability of high-resolution microscopy and high-throughput technologies in molecular biology the amount of high quality cellular images is exploding. This leads to a significant increase in the demand for scientific image analysis software being able to use the full capacity of these advancements.
The state-of-the-art way of presenting, assessing and evaluating experimental images qualitatively is being increasingly replaced by computational data evaluation. Quantification of e.g. light intensities coming from fluorescent protein (FP) expression in different cellular compartments can be ascertained in a spatially resolved manner and enables us to mathematically verify the current understanding of biological systems. Unambiguous and reproducible computational extraction increases the quality and exchangeability of information for subsequent automatic processing steps such as digitization, representation, analysis, and modeling. A variety of image processing-, analysis- or modeling-packages easing these tasks already exist, either commercial or open source. Recently, several eminent reviews have been published which outline the most common methods and tools addressing biological image processing, analysis and modeling (see [1–3]). Cell segmentation and property extraction, for example, are well established and can be realized by dedicated software such as CellProfiler [4], Cell-ID [5] or generic image processing platforms like Labview (National Instruments, Austin, USA) or Imaris (Bitplane, Zurich, Switzerland). A widely used and freely

available tool is ImageJ [6], which comprises standard segmentation algorithms as well as surface or profile plots. Also freely available are additional packages for R like EBImage [7], which can be used for the segmentation and analysis steps. When it comes to spatial (stochastic) modeling and/or the simulation of reaction-diffusion systems, tools like MesoRD, Matlab, MCell or VCell may be eminently suited for this purpose [8].

All of these tools offer excellent solutions for the specific problems they were designed to solve. However, there is still a lack of the possibility to perform a contiguous and intuitive workflow, starting with almost raw data images and ending with a running mathematical model, allowing us to directly compare the simulation results with biological data.

The STSE platform intends to close the gaps between *various* tools or software-packages for image assessment that are in majority specifically designed for separate steps and analysis questions. It offers the advantage of stratifying the interaction with different data-structures and minimizes thus the loss of time and information during the export and conversion processes. STSE comprises modules for digitizing and representing microscopy data, enables data analysis as well as manipulation, and can be used for mathematical modeling and simulation of spatial distributions of chemical species. It is a powerful, multifaceted tool for interdisciplinary work which significantly simplifies data handling by addressing many aspects of image analysis and evaluation. It offers abundant state-of-the-art features and an easy-to-use graphical user interface (GUI) but is also flexible and extensible for customizing via the Python script language.

Implementation

STSE is a platform that simplifies the preparation and realization of spatio-temporal simulations, preferably based on microscopy images. The tools are written in Python and have a modular design which allows the modeler to extend their functionality according to custom needs. The default STSE workflow can be summarized as follows (see: Figure):

1. Preprocessing of microscopic images for the studied object.
2. Definition of a discrete representation of the images.
3. Automatic integration of the information from images into the discrete representation.
4. Analyzing the digitized data.

5. Formulating a model: defining interactions between regions of interest and molecules of interest.

6. Running a model: previously digitized images are used as initial conditions for the evaluation of simulation results.

A detailed description of a use case, including all individual steps with examples as well as comparative studies with some of the above mentioned state-of-the-art tools is provided in the Supplementary Materials (text01.pdf). The webpage of the project contains additional examples, video tutorials, access to a discussion group and other helpful information sources. In the following, we give a concise overview of the basic methods used in STSE:

Spatial Segmentation and Digitization

By the process of digitization we mean the generation of a digital data structure, allowing for efficient analysis, representation and modeling. The classical approach is to decompose the microscopy image into physiologically distinguishable compartments (e.g. nucleus, cytoplasm, etc.) which is called image segmentation [9, 10]. Usually, image segmentation results in a data structure linking the compartments with pixels. STSE differs from this approach by introducing an abstract, intermediate layer composed of so-called subcompartments. To generate this layer, each compartment is divided into subcompartments which have the geometry of polygons and are organized in such a way that they fill the entire compartment and do not overlap with each other. The default geometry is automatically composed of equilateral hexagons. The purpose of introducing this abstract layer is to allow for adjusting the digitization precision separately for different compartments, which is useful when it comes to analysis and modeling. To edit the geometry of subcompartments a Voronoi 2D tessellation is used [11]. By moving the subcompartment center (corresponding to the Voronoi vertex), the user specifies implicitly the geometry of each subcompartment. Since these subcompartments share edges, the representation resembles a polygonal mesh (PM).

Each subcompartment has an individual geometry as well as other user-customizable properties such as cellular compartment affiliation, concentrations of specific substances, etc. The graphical user interface (GUI) allows for user-friendly inspecting and editing of these properties. Additionally, due to the software implementation design, it is possible to extend the GUI editor by adding custom editing actions as well as to script the GUI with Python.

With STSE it is possible to acquire spatial luminosity information from microscopic images, which can

correspond e.g. to the inhomogeneous distribution of tagged molecules within the cell. This process is performed on indexed color images (e.g. FP microscopic images). This is an important feature, since it allows to compare the simulation results with observed data. All tasks related with image digitization are covered in the Supplementary Material (text01.pdf, Section: Digitization).

Representation and Analysis

Image representation is performed by the conversion of the Voronoi 2D mesh to a design called "WalledTissue2D", which is an internal STSE data structure. This design involves less constraints and thus allows for more latitude in defining polygonal geometries (e.g. including non-convex ones) as well as physiological information. It is realized by storing the polygon corner coordinates explicitly in the data structure instead of computing them using the Voronoi algorithm. The geometry may be freely altered by a convenient API which exhibits also a 3D surface mesh representation and additionally supports two important operations: removal and division of subcompartments. This allows for simulating structures changing in time, which has been, for instance, successfully used in the dynamic modeling of meristem growth [12].

The analysis is effected via Python scripts and provides a comprehensive and differentiated overview of topological, geometrical and physiological information. This might be data on the surface, neighbours and distances of the polygons or physiological properties such as quantification of molecules or spatial concentration gradients in the different cellular compartments.

As shown in the Supplementary Material (text01.pdf, Section: Representation and Analysis), the routines provided by STSE allow for visualizing and inspecting compartment properties and can be used for computing different properties and for further analysis of the mesh. All structural information can be exported and saved for persistence and dissemination.

Modeling

The digitized data stored in "WalledTissue2D" can be *directly* used to perform spatial modeling (e.g. as initial conditions or evaluation). The mechanistic model of a studied process needs to be formally stated using a set of ordinary differential equations (ODEs) describing the interplay of different actors (e.g. chemical molecules) and the different cellular compartments with specified kinetic rules on diffusion, chemical reactions, transport, etc. To perform such simulations, the external ODE solver library from SciPy is used [13]. The example of constructing and running a model with STSE is presented in the

Supplementary Material (text01.pdf, Section: Modeling).

Results

In this work we demonstrate how to apply the STSE software to the example of the intensively studied yeast pheromone MAP kinase cascade (see: Figure and Supplementary Material, text01.pdf, Part I). We focus in particular on the distribution of the double-phosphorylated Fus3 ($Fus3^{PP}$) from a shmooing yeast cell [14].

To present how STSE can be used effectively we demonstrate its usage by means of analyzing and characterizing selected aspects of the $Fus3^{PP}$ gradient in a shmooing yeast cell. For this purpose we demonstrate how to:

- Quantify the ratio of the average cytoplasm/nucleus expression of $Fus3^{PP}$ based on fluorescence signal intensity acquired from microscopy images (text01.pdf, Section: Digitization),
- Show gradient curves for $Fus3^{PP}$ along the x-axis of the cell data image and around the nucleus (text01.pdf, Section: Representation and Analysis),
- Simulate the process of $Fus3^{PP}$ diffusion in the cytoplasm to determine the underlying conditions that lead to the qualitative values captured in the image (text01.pdf, Section: Modeling).

We evaluate the results of the simulations and discuss whether the appearance of a $Fus3^{PP}$ gradient throughout the cell can be explained by simple diffusion and how to define which conditions and model parameters are the most plausible and allow to reproduce the experimental observations with the smallest error.

We also compare STSE modules with a selection of other available software tools which allow to perform each of the workflow substeps separately i.e. digitization, representation, analysis or modeling (Supplementary Material, text01.pdf, Part II).

Further versions of the STSE should include additional simulation strategies (e.g. stochastical, agent-based) as well as the opportunity to import SBML files. For the latter, a prior establishment of a standard for spatial modeling would be required.

Conclusion

STSE is an efficient and powerful novel platform, designed for computational handling and evaluation of microscopic images. It allows for an uninterrupted workflow including digitization, representation, analysis,

and mathematical modeling. It conceptually differs from selection-based tools by operating on abstract subcompartments rather than on pixels directly. This approach results in the simplification of further processing and it allows for inhomogenous precision in different cellular compartments.

Availability and requirements

Project name: STSE

Project home page: http://stse-software.org

Operating system(s): Linux (availability on other systems depends on 3rd party libraries)

Programming language: Python

Other requirements: Openalea (http://openalea.gforge.inria.fr/), Mayavi2 (http://code.enthought.com/projects/mayavi/), Qhull(http://www.qhull.org/), NetworkX (http://networkx.lanl.gov/). It is also possible to use the software directly from a live DVD Linux distribution, SB.OS (http://www.sbos.eu/), which comes with a comprehensive list of other systems biology software.

License: GNU GPL

Authors contributions

SS is the main developer of STSE. MF, SG, SS, EK wrote the article. MF, SG, SS applied the STSE workflow to the example described in the Results and performed comparative studies of STSE and other state-of-the-art tools.

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Figures

Figure 1 - STSE workflow

A detailed description is included in the text. The example image used in the figure is analyzed in the Supplementary Materials, text01.pdf.

References

1. Ljosa V, Carpenter AE: **Introduction to the Quantitative Analysis of Two-Dimensional Fluorescence Microscopy Images for Cell-Based Screening**. *PLoS Comput Biol* 2009, **5**(12):e1000603.
2. Peng H: **Bioimage informatics: a new area of engineering biology**. *Bioinformatics* 2008, **24**(17):1827–1836, [http://bioinformatics.oxfordjournals.org/cgi/content/abstract/24/17/1827].
3. Meijering E, van Cappellen G: *Quantitative biological image analysis*. Imaging Cellular and Molecular Biological Function, Springer Berlin 2007.
4. Lamprecht M, Sabatini D, Carpenter A: **CellProfiler: free, versatile software for automated biological image analysis**. *Biotechniques* 2007, **42**:71–75.
5. Gordon A, Colman-Lerner A, Chin TE, Benjamin KR, Yu RC, Brent R: **Single-cell quantification of molecules and rates using open-source microscope-based cytometry.** *Nat Methods* 2007, **4**(2):175–181, [http://dx.doi.org/10.1038/nmeth1008].
6. Abramoff M, Magalhaes P, Ram S: **Image processing with ImageJ**. *Biophotonics International* 2004, **11**:36–42.
7. Pau G, Fuchs F, Sklyar O, Boutros M, Huber W: **EBImage–an R package for image processing with applications to cellular phenotypes.** *Bioinformatics* 2010, **26**(7):979–981, [http://dx.doi.org/10.1093/bioinformatics/btq046].
8. Resat H, Petzold L, Pettigrew MF: **Kinetic modeling of biological systems.** *Methods Mol Biol* 2009, **541**:311–335, [http://dx.doi.org/10.1007/978-1-59745-243-4_14].
9. Hamilton N: **Quantification and its applications in fluorescent microscopy imaging.** *Traffic* 2009, **10**(8):951–961, [http://dx.doi.org/10.1111/j.1600-0854.2009.00938.x].
10. Wollman R, Stuurman N: **High throughput microscopy: from raw images to discoveries.** *J Cell Sci* 2007, **120**(Pt 21):3715–3722, [http://dx.doi.org/10.1242/jcs.013623].
11. Klein R: *Concrete and Abstract Voronoi Diagrams*, *Volume 200 of* Lecture Notes in Computer Science. Springer-Verlag 1989. [ISBN 3540520554].
12. Stoma S, Lucas M, Chopard J, Schaedel M, Traas J, Godin C: **Flux-Based Transport Enhancement as a Plausible Unifying Mechanism for Auxin Transport in Meristem Development**. *PLoS Comput Biol* 2008, **4**(10):e1000207+.
13. Jones E, Oliphant T, Peterson P: **SciPy: Open source scientific tools for Python**. *http://www.scipy.org/* 2001, [http://www.scipy.org/Citing_SciPy].
14. Maeder CI, Hink MA, Kinkhabwala A, Mayr R, Bastiaens PIH, Knop M: **Spatial regulation of Fus3 MAP kinase activity through a reaction-diffusion mechanism in yeast pheromone signalling**. *Nature Cell Biology* 2007, **9**:1319–1326.

Additional Files

text01.pdf — Supplementary Material

Detailed description of a use case, including all individual steps of the STSE workflow with examples as well as comparative studies with state-of-the-art tools.

fus3_diffusion1.avi — Supplementary animation

Animation showing the dynamics of the exemplary system described in the Supplementary Material, text01.pdf

fus3_diffusion2.avi — Supplementary animation

Animation showing the dynamics of the exemplary system described in the Supplementary Material, text01.pdf