

Data and text mining

Vizardous: interactive analysis of microbial populations with single cell resolution

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Associate Editor: Jonathan Wren

Received on March 3, 2015; revised on July 29, 2015; accepted on August 5, 2015

Abstract

Motivation: Single cell time-lapse microscopy is a powerful method for investigating heterogeneous cell behavior. Advances in microfluidic lab-on-a-chip technologies and live-cell imaging render the parallel observation of the development of individual cells in hundreds of populations possible. While image analysis tools are available for cell detection and tracking, biologists are still confronted with the challenge of exploring and evaluating this data.

Results: We present the software tool *Vizardous* that assists scientists with explorative analysis and interpretation tasks of single cell data in an interactive, configurable and visual way. With *Vizardous*, lineage tree drawings can be augmented with various, time-resolved cellular characteristics. Associated statistical moments bridge the gap between single cell and the population-average level.

Availability and implementation: The software, including documentation and examples, is available as executable Java archive as well as in source form at <https://github.com/modsim/vizardous>.

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Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

Single cell analysis with time-lapse (fluorescence) microscopy has been widely established in the life sciences in recent years. Whereas microarray analysis and flow cytometry yield population level data at a particular point in time, time-lapse live-cell imaging targets the assessment of individual cell parameters with their spatial and temporal (lineage) context. Microfluidic lab-on-chip technologies have enabled the parallel cultivation of hundreds of cells over several generations (Nobs and Maerkl, 2014; Wang *et al.*, 2010). Combined with time-lapse imaging, it has been used, e.g. to study the dynamics of cellular regulation mechanisms and stochasticity of metabolism (Kiviet *et al.*, 2014; Levine *et al.*, 2013; Locke and Elowitz, 2009; Young *et al.*, 2012), to investigate cellular size homeostasis (Campos *et al.*, 2014; Taheri-Araghi *et al.*, 2014) and phenotypic heterogeneity in dependence of stressors as well as the detection of rare events (Balaban *et al.*, 2004; Rosenthal and Elowitz, 2012).

The easy availability of time-resolved data poses new challenges on data analysis by the mere amounts and complexity of acquired information. While several, typically organism-specific, image analysis tools are available for cell detection, feature extraction and tracking tools for the generic task of analyzing and understanding this information are lacking. We developed the software tool *Vizardous* to assist researchers with the following single cell data related tasks: (i) visualize data with single cell resolution in the lineage context, (ii) visually detect emerging structural patterns such as (a)symmetries in subtrees and (iii) assessing joint cellular properties and structural motifs.

2 Description

The single cell community has adopted phylogenies (lineage trees, Fig. 1) to visualize mother–daughter relationships in context of

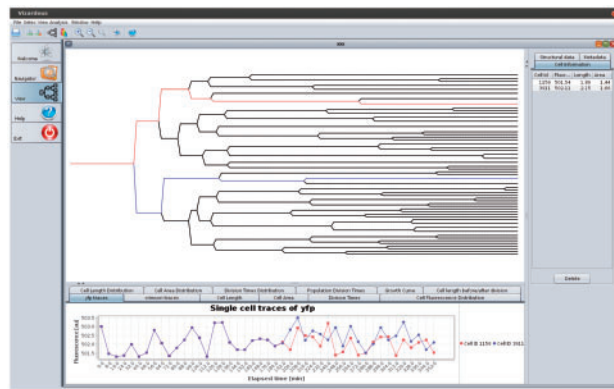


Fig. 1. Lineage tree extracted from an experiment with fluorescence reporters to elucidate the regulation dynamics of key genes. Time-resolved fluorescence traces are shown in the bottom chart for two lineages. Cellular characteristics, i.e. cell area, fluorescence, are shown for selected cells in the table on the left

cellular development and to link time-resolved data to the tree structures. Here, in particular a flexible ‘pattern and outlier’ detection based on spatio-temporal phenotypic observables of individual cells is essential. Because the objectives in single cell analysis are notably different from those in evolutionary biology, conventional software tools for analyzing phylogenies are unsuitable in the single cell domain (see [Supplementary Table S2](#) for a characterization of selected tools).

Vizardous is an open-source software that is designed as out-of-the-box solution to deal with the data from live-cell imaging experiments. The software is implemented using published methods and available libraries wherever appropriate (see [Table S3](#)). Mother-daughter relationships are provided in the standardized phyloXML format ([Han and Zmasek, 2009](#)) and cellular characteristics (e.g. cell length, area, volume, fluorescence reporter signal) as well as population average information in the specially designed document format MetaXML (cf. [Supplement 5](#)). An intuitive user interface enables experimentalists to browse quantitative single cell data in the lineage context. While the major use case of *Vizardous* is the visualization of lineage trees, the underlying information can also be acquired from the data models using an API (cf. [Supplement 4](#)) which allows users with programming experience to script the data analysis tasks directly with Java, Jython ([www.jython.org](#)), or by using the implemented export capabilities. *Vizardous*’ features are summarized in [Supplementary Table S1](#).

3 Vizardous exploration workflow

The most important analysis steps are explained with a representative evaluation workflow using pre-processed time-lapse videos of a growing *Corynebacterium glutamicum* population under transient carbon limitation (for details see [Supplement 1](#)):

Step 1: Importing experimental data. Single cell data are read from phyloXML and MetaXML file pairs containing lineage information and figures about individual cells, respectively. One or more of such file pairs can be imported from a local file system or an external storage location, e.g. OMERO ([Allan et al., 2012](#)).

Step 2: Visualization of lineage trees. Lineage trees are generated and visualized alongside population distributions for a complete experiment or a specific time point (temporal slice). As an indicator of population heterogeneity statistical moments (mean, SD) are calculated for the selection.

Step 3: Highlighting individual cells. When screening for rare events or outliers in populations, the highlighting of single cells in a lineage tree according to cellular properties is especially useful. Two different approaches are implemented:

1. Change the property of a lineage tree element (node or branch) according to a specified cellular property, e.g. nodes are sized according to the cell area.
2. Set a threshold for a cellular property and highlight all cells that exceed the defined value.

Step 4: Selecting cells for inspection. The user has the possibility to interactively select (and deselect) individual cells of interest from the lineage tree. The associated information is visualized in trace charts ([Fig. 1](#)). These charts unlock the contextual interpretation of feature dynamics along the temporal axis therewith bridging the gap between the ancestral relationships of cells and their meta-information.

Step 5: Exporting lineage trees and charts. The lineage trees and single cell traces are fully customizable and exportable to publication ready vector graphics (SVG) or bitmap formats (JPEG, PNG). Additionally, the data underlying single cell traces can be saved to tabular data formats (XLS, CSV) for further processing.

4 Results and conclusions

Vizardous has been used in several studies, e.g. ([Binder et al., 2014](#); [Mustafi et al., 2014](#); [Nanda et al., 2014](#); [Probst et al., 2015](#)), by scientists with different backgrounds to answer a variety of scientific questions. These and further case studies (cf. [Supplementary Data](#)) show that *Vizardous* is a versatile tool that supports researchers in various aspects with the discovery-driven, explorative analysis of single cell experiments. By supporting additional input formats and seamless integration with available bioimage analysis software we anticipate that a range of fields including biological and medical sciences will benefit in the future.

Acknowledgements

We thank Julia Frunzke for giving the motivation for our work, Christina Krämer for constant feedback and experimental data as well as Christian C. Sachs for providing a case study.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft [WI 1705/16-1 to S.H.].

Conflict of Interest: none declared.

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