

Vizardous: Interactive Analysis of Microbial Populations with Single Cell Resolution

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Requirements

- Java Runtime Environment 7 or later.

Starting Vizardous

1. Open a new command line
 - a. Start – Execute – cmd
2. Go to the folder in which the Vizardous-*.jar is located
3. Execute as shown in Figure 1

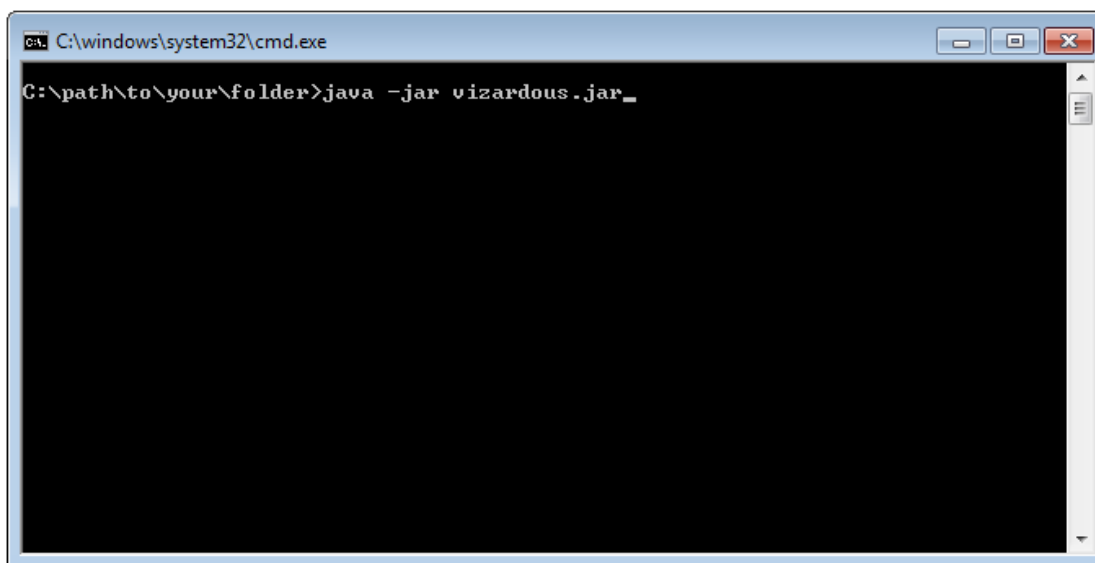


Figure 1. Executing Vizardous from the command line.

Vizardous user interface

After starting Vizardous, the main functions of the software can be accessed through several tabs on the left side (Figure 2). Additionally, many features can be accessed through the menu bar as well as the tool bar. In particular, a connection to an OMERO server can only be established through the *File* menu.

More details on the functions can also be found via the *Help* menu.

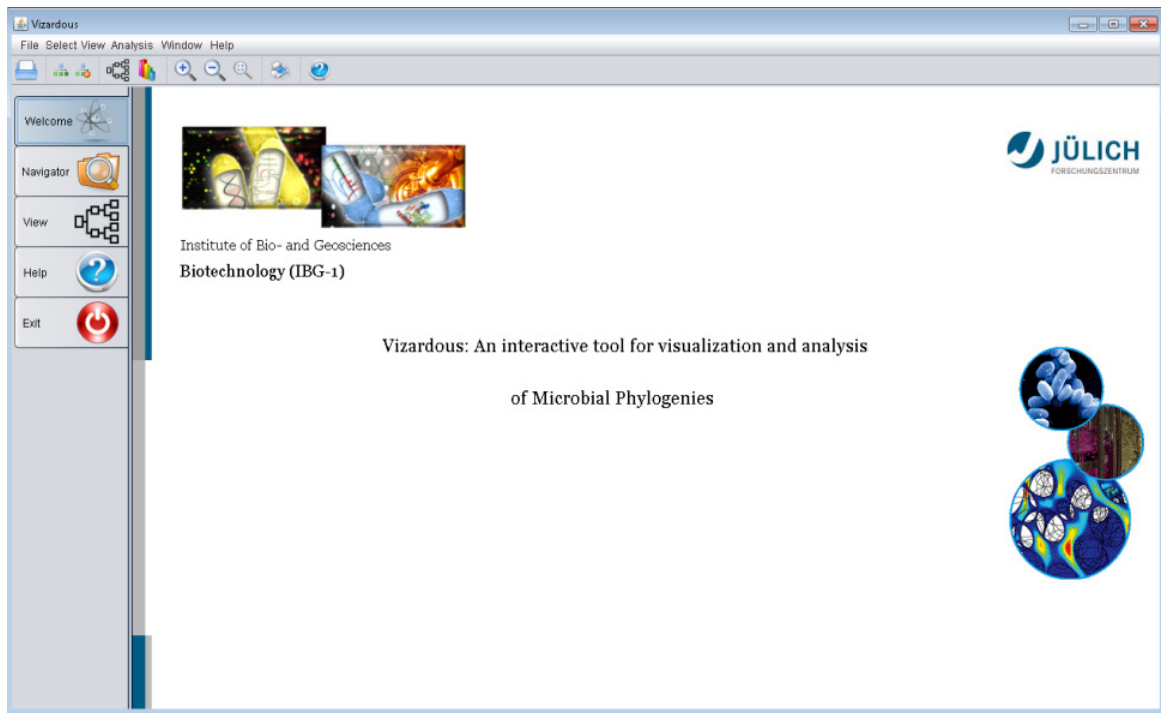


Figure 2. The start screen.

Importing data

Vizardous reads information from phyloXML and MetaXML files which contain the mother-daughter relationships and cellular characteristics, respectively.

Importing from a local file system (see Figure 3)

1. Start Vizardous
2. Select *Navigator* tab
3. Select the PhyloXML, e.g., *nutrient_limitation.xml*
4. Click *Select File 1*
5. Select the MetaXML, e.g., *nutrient_limitation_meta.xml*
6. Click *Select File 2*
7. Add the file pair to the import window
8. Click the *Import* button to start the import process

To import several files, repeat steps 3 to 7 before starting the import process.

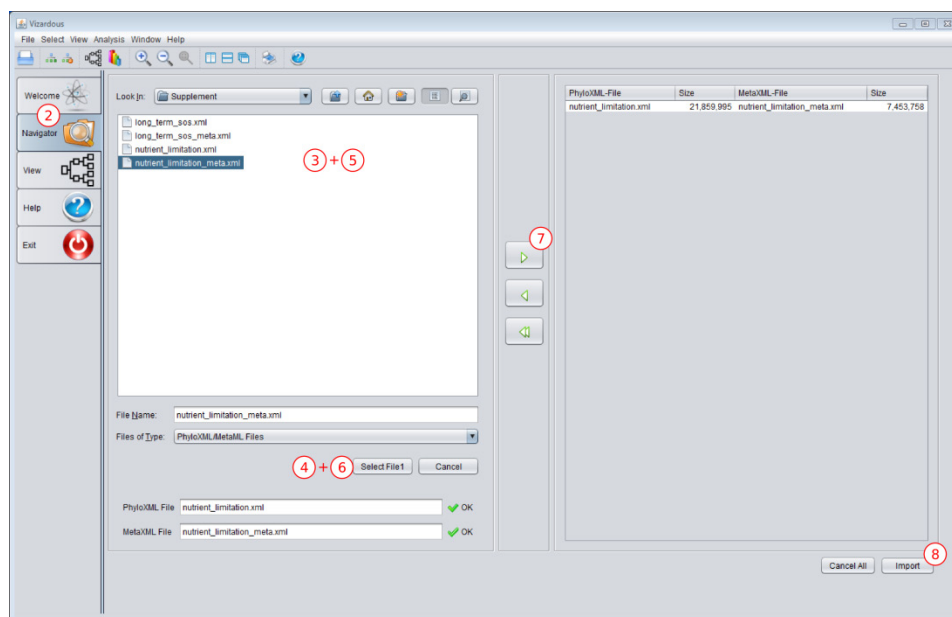


Figure 3. The import window. Numbers denote the steps for importing a phyloXML/metaXML pair.

Importing from OMERO

File > OMERO opens a connection dialog in which the OMERO server is specified. Once a connection is established, the import window (Figure 3) is presented with the available data on the OMERO server.

Visualizations

Once the import process is finished, the user is presented with the lineage tree(s) (Figure 4), some population average charts, as well as the empty cell information tables (Figure 5).

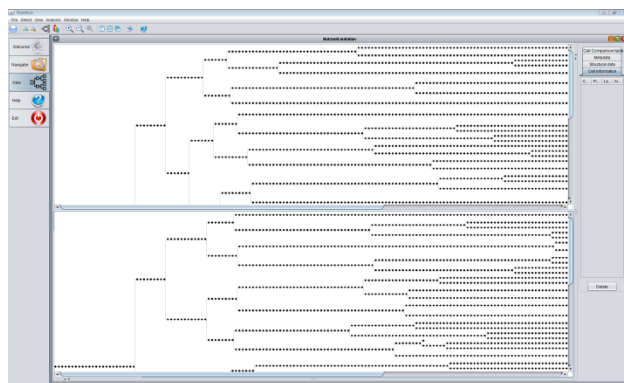


Figure 4. Multiple lineage trees for comparison.

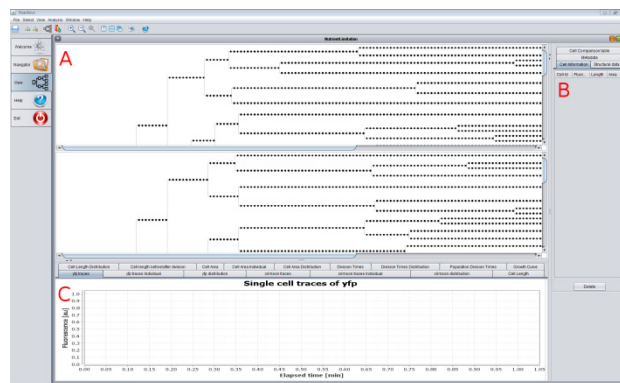


Figure 5. A. Lineage tree view. B. Cell information tables. C. Charts for time-resolved and population average data.

Lineage tree

To get a glimpse of the available cellular information, hover over a cell to show information in a tooltip window. By selecting (clicking) the cell, the information panel opens (Figure 6), showing additional information about predecessors and successors, as well as fluorescence information, etc. The information window is also used to select the cell and its predecessors for plotting in the lower panel of the user interface (*Add to InfoTable*).

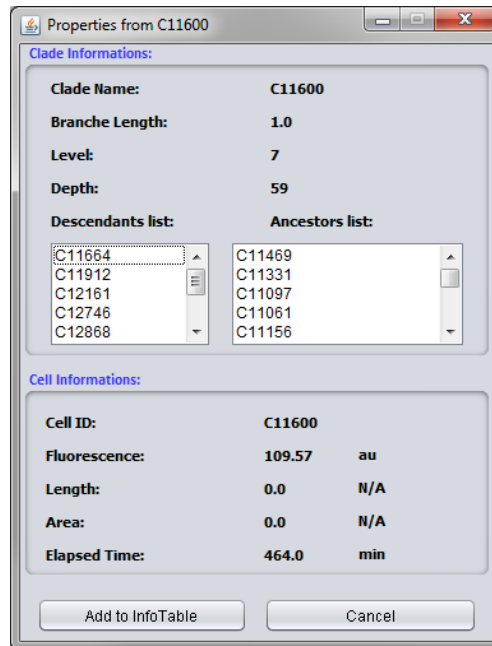


Figure 6. Properties window for a selected cell. Information about the location of the cell within in the tree is provided together with the predecessors and successors. Furthermore, cellular characteristics stored in the MetaXML file are provided.

Charts

Vizardous offers a variety of charts for visualizing time-resolved information for selected cells. Furthermore, population information as well as information about temporal slices can be visualized. Table 1 describes the available plots for single-cell data as well as population data.

Table 1. Overview of the available charts.

Name	Type	Description
Fluorescence Traces	Lineplot	Time-resolved fluorescence reporter intensities for selected cells
Cell Length	Lineplot	Development of cell length over time for selected cells
Cell Area	Lineplot	Development of cell area over time for selected cells
Division Times	Lineplot	Division times for each generation of the selected cells

Cell Fluorescence Distribution	Histogram	Distribution of fluorescence reporter intensities, plotted for each population individually
Cell Length Distribution	Histogram	Distribution of cell lengths, plotted for each population individually
Cell Area Distribution	Histogram	Distribution of cell areas, plotted for each population individually
Division Times Distribution	Histogram	Distribution of division times, plotted for each population individually
Population Division Times	Boxplot	Boxplot of combined division times per generation
Population Growth Curve	Lineplot	Growth curves, plotted for each population individually
Cell Length Before/After Division	Histogram	Distribution of cell lengths before a division and after a division event for comparison

Single-cell information

Figure 7 shows the fluorescence traces (in this case YFP) for three previously selected cells.

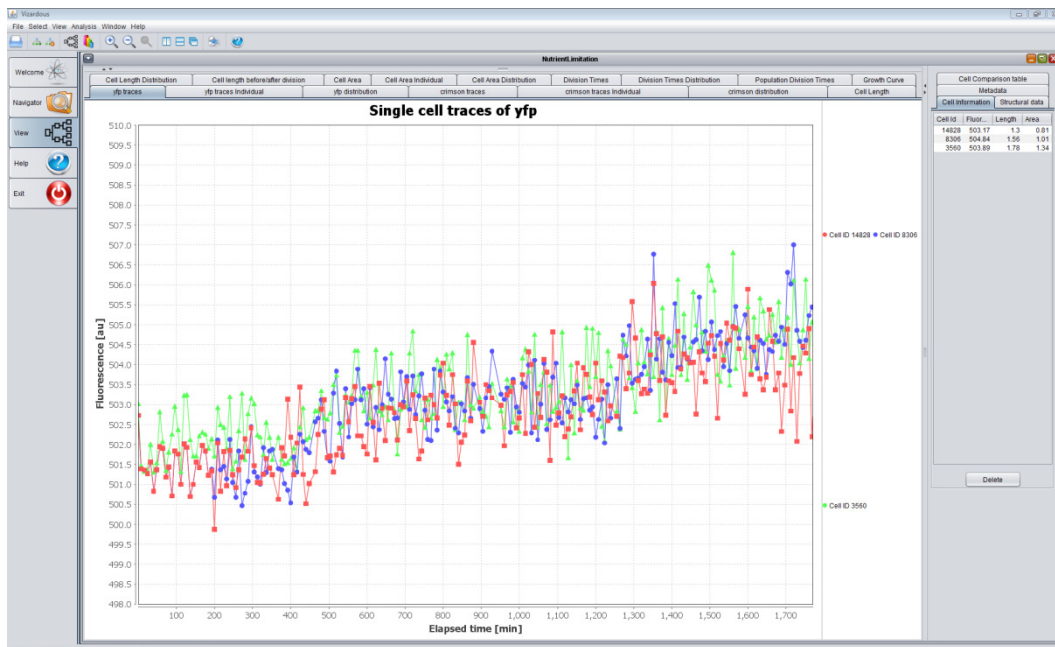


Figure 7. Fluorescence traces for three selected cells (not shown) for the whole experiment.

Selecting cells

1. Select a cell by clicking
 2. Click *Add to Info table* to mark the cell for the generation of time-resolved charts
- or
1. Select multiple cells with the selection rectangle

2. Right click one of the cells
3. Click *Add to Info table* to mark the cell for the generation of time-resolved charts

Once selected, the cells will be colored in the lineage tree and their traces are shown in the charts in the same color (Figure 8).

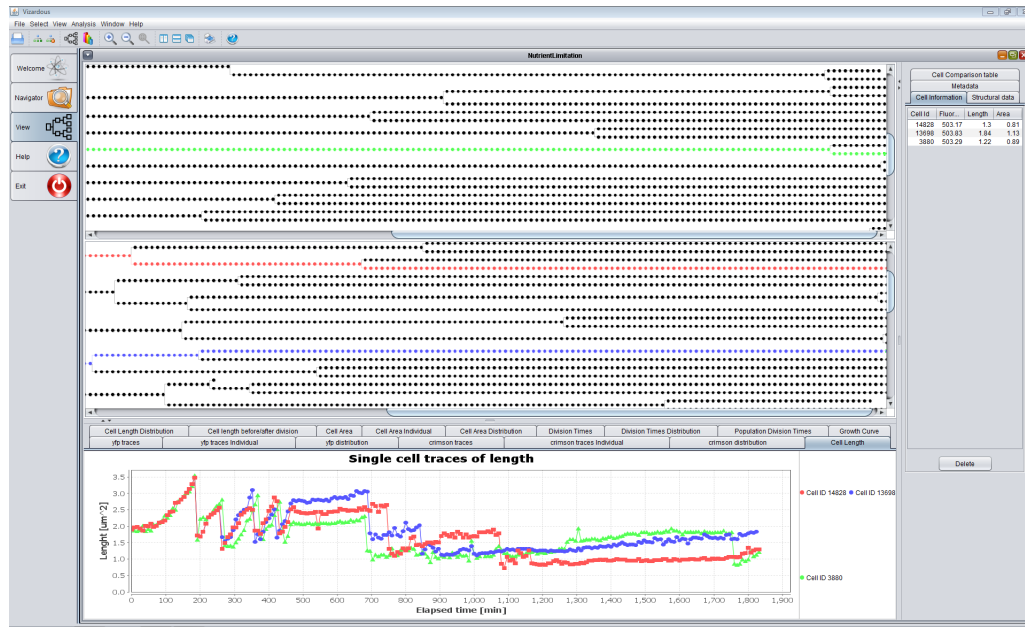


Figure 8. Selected cells are colored in the lineage tree. Length traces are shown for the selected cells in the chart.

Highlighting cells

Vizardous offers the possibility to visualize cells according to a selected characteristic. Figure 9 shows the possible characteristics. Cell sizes can be mapped to fluorescence intensities, cell length, or area. Additionally, it is also possible to highlight cells that exceed a given threshold of, e.g. fluorescence intensities.

Figure 10 shows the resulting lineage tree visualization. Cells of interest can subsequently be selected for further analysis (cf. Selecting cells).

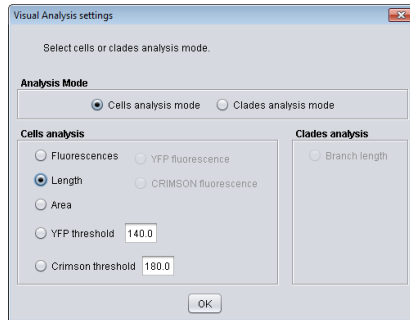


Figure 9. Settings for the highlighting of specific cells.

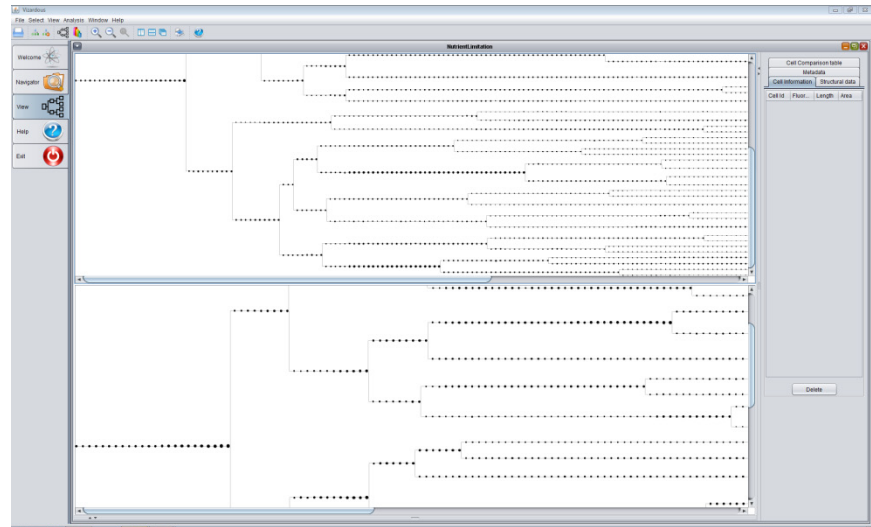


Figure 10. Lineage tree visualization when the node size is proportional to the cell area.

Sorting trees

To sort trees according to meta information, right click in the lineage viewer and select *Tree sort*. It is possible to sort *leaves* (no sorting of other cell types than leaves), *branches* (only looks at the children after a division) and arithmetic sorting, which sorts branches according to the arithmetic mean of their children.

Population information

Distributions of meta information and growth curves (cf. Table 1) are available without selecting individual cells.

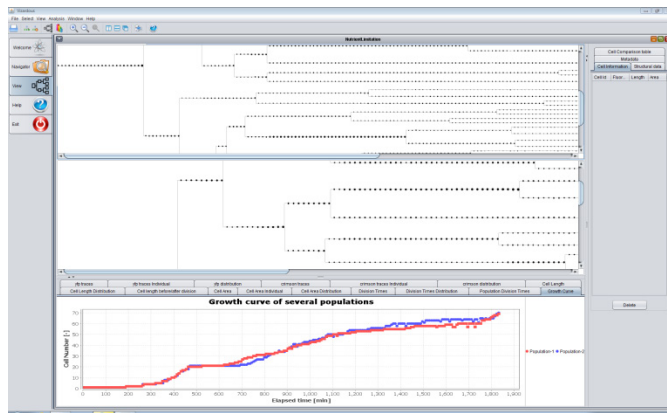


Figure 11. Two lineage trees in connection with their growth curves.

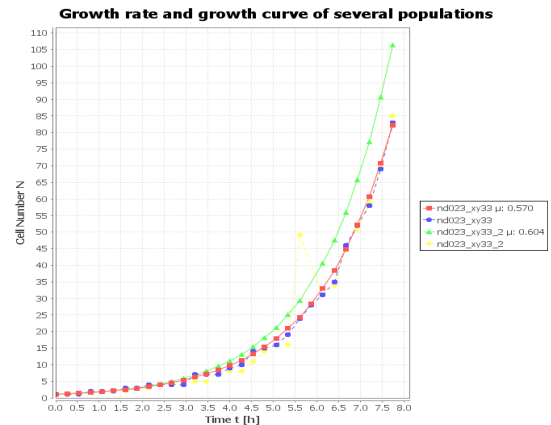


Figure 12. Growth curves (experimentally determined for different dataset) together with exponential fits to obtain the growth rate per population.

Exporting

Lineage trees as well as generated plots can be exported to vector graphics formats (SVG) and bitmap formats (JPEG, PNG). Additionally, raw data can be exported (XLS, CSV) for further processing with external tools.

Exporting lineage trees

Executing a right click on the background of a lineage tree shows a multipurpose pop-up. One of the functionalities is the export of the selected lineage tree via "Export phyloTree as ..." from the popup (Figure 13).

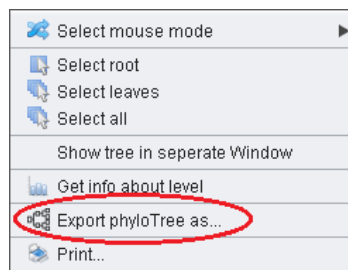


Figure 13. Popup window for exporting the lineage tree visualization.

Exporting charts

Exporting of charts is possible via the context menu (right click) of the charts as shown in Figure 14. This popup also enables the export of the raw data underlying the chart via "Dump data to file".

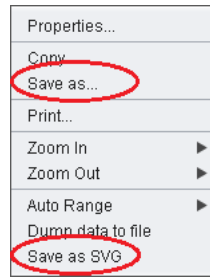


Figure 14. Popup window for exporting charts.