Quickstart guide

Cytosplore Viewer

TU Delft

LUMC Leiden University Medical Center

NWO Applied and Engineering Sciences

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Introduction

Cytosplore Viewer is a desktop PC software application for fast and interactive exploration of cell types from single cell sequencing data of the Allen Institute in Seattle. Data displayed in this viewer was downloaded from the Allen Cell Types Data, (© 2015 Allen Institute for Brain Science). The Allen Cell Types Database is available from: http://celltypes.brain-map.org/. Cytosplore Viewer was developed in close collaboration between the Allen Institute for Brain Science, the Division of Image Processing and the Computational Biology Center at Leiden University Medical Center, and the Computer Graphics and Visualization Group at the TU Delft, the Netherlands.

Using Cytosplore Viewer, you can:

- View the cellular hierarchies of the Allen Cell Types Data that were defined from pre-defined gene subsets and cellular taxonomies as published in [6,7].
- Perform interactive a-tSNE computation of the subclasses of cells.
- Explore transcriptome-wide gene expression in combination with metadata of individual cells.
- Perform differential analyses and statistics between manual selections of cells, or between pre-defined clusters throughout the cellular hierarchy.
- Visualize the results of differential analyses.
- Export the results of differential analyses for further analysis in external tools.

Datasets currently include nuclear sequencing data of several areas of the human cortex (for instance MTG [6]). It also includes single cell sequencing data of the mouse cortex and hippocampus, up to the October 2019 data release of the Allen Cell Types Database.

System requirements

The data size and computational demands of interactive GPGPU-tSNE require sufficiently modern hardware to ensure the desired responsiveness in Cytosplore Viewer. We recommend the following:

- Windows 7 or higher (64bits)
- Intel Core i7 with 2 cores or better (preferably generation 6 or higher)
- 8GB of memory or more
- Dedicated Graphics Card (preferably nVidia GTX 960 or better) with at least 2GB of GPU memory

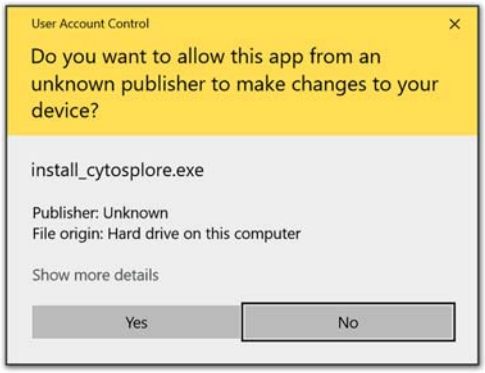
Configurations with lesser specifications may work, but will result in longer wait times for data loading and computing. We have tested Cytosplore Viewer on different configurations, including Windows 7 and Windows 10, intel Core i7 and intel Xeon CPUs as well as different Graphics Cards (nvidia GTX 980, GTX 1080, GTX Titan, AMD Radeon r9 M290, and AMD Radeon Pro 460). It is highly recommended to update the drivers of the graphics card to the latest versions prior to installing Cytosplore Viewer. Remote operation of Cytosplore Viewer through Remote Desktop is not supported.

Installation and updates

Cytosplore Viewer is provided as an installer through viewer.cytosplore.org. Clicking the download button will save the installer to your local disk. Double-click the icon to start the installation.

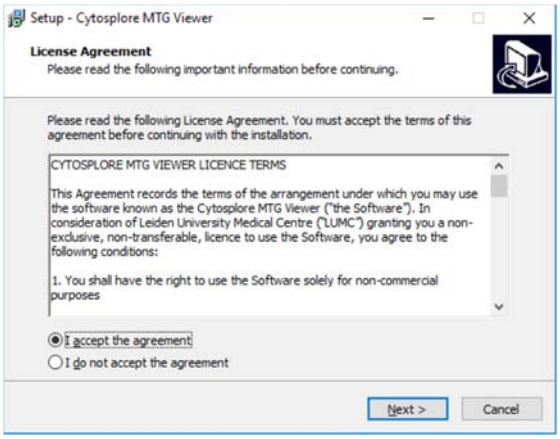
The installer will ask for administrator rights on the computer. This is due to the fact that we include the Microsoft Visual C++ Redistributable Packages. Cytosplore Viewer itself does not need or makes use of these rights. To continue the installation click *Yes*. You will be prompted to enter the administrator password.

Click *Yes*, to continue the installation.

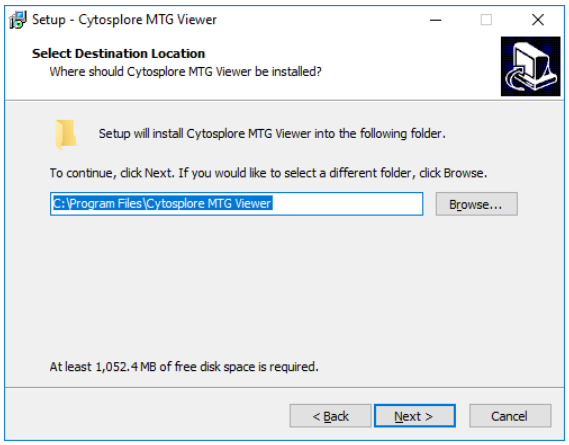


The installer will show the license agreement, that you have to accept. Most importantly it states that the software can be used free of charge.

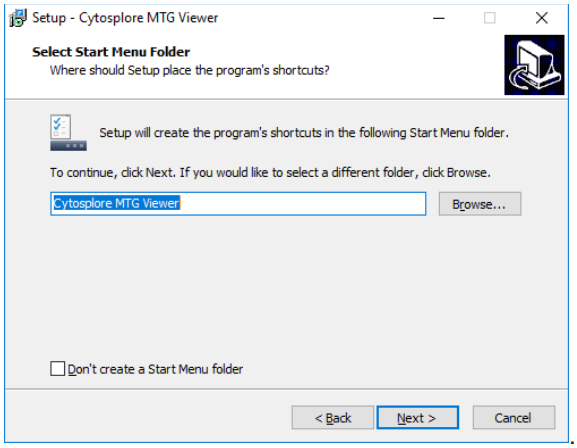
Click *I accept the agreement* and then *Next >*.



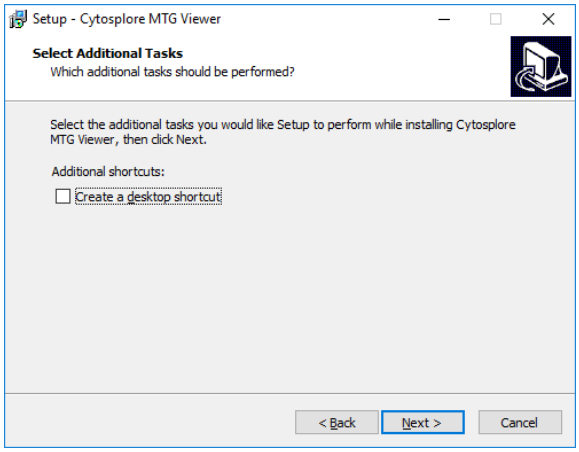
Next you can define the location where Cytosplore Viewer should be installed or simply click *Next* >.



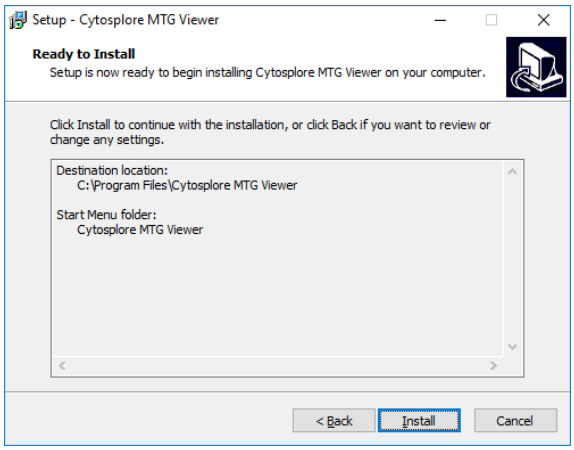
In the next dialog you can define the name of or decide not to create a t Cytosplore Viewer start menu folder. Click *Next* > to continue without changes.



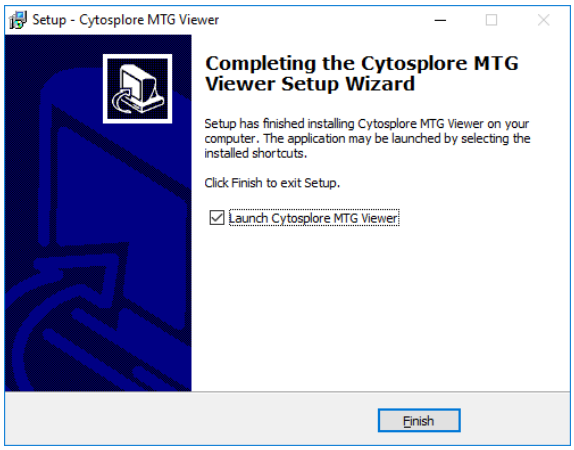
Now you will be asked if you want to create a desktop shortcut. If yes, just click *Next* > to continue.



The last dialog before putting the files in the appropriate locations shows a summary of the previous settings. Click *Install* to prepare the Cytosplore Viewer installation.



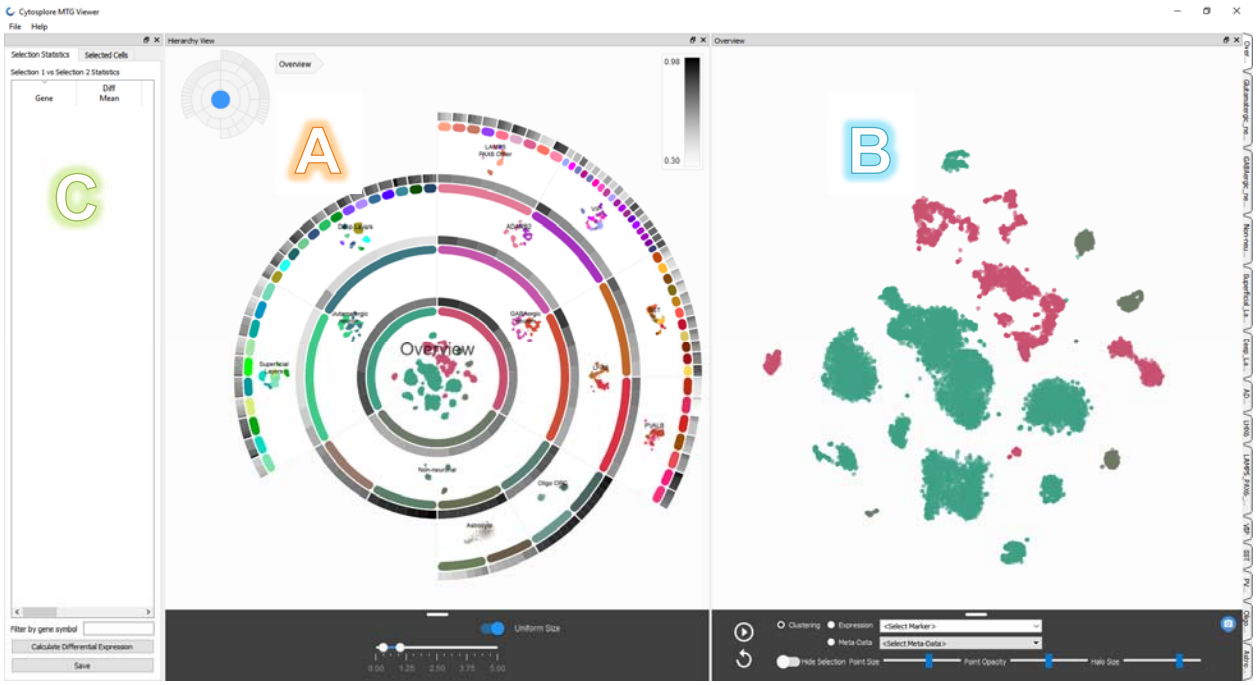
After a successful installation the following dialog will show. You can decide whether to launch Cytosplore Viewer now, and click *Finish* to complete the process.



Cytosplore Viewer includes an automatic updating system. Upon launching, Cytosplore Viewer has the option to automatically check for updates and new versions. You can also choose to manually check for updates through the Help menu.

General overview of the graphical user interface

The graphical user interface of Cytosplore Viewer consists of three main components: A) the Hierarchy Viewer, B) the Map Viewer and C) the Differential Expression Viewer.



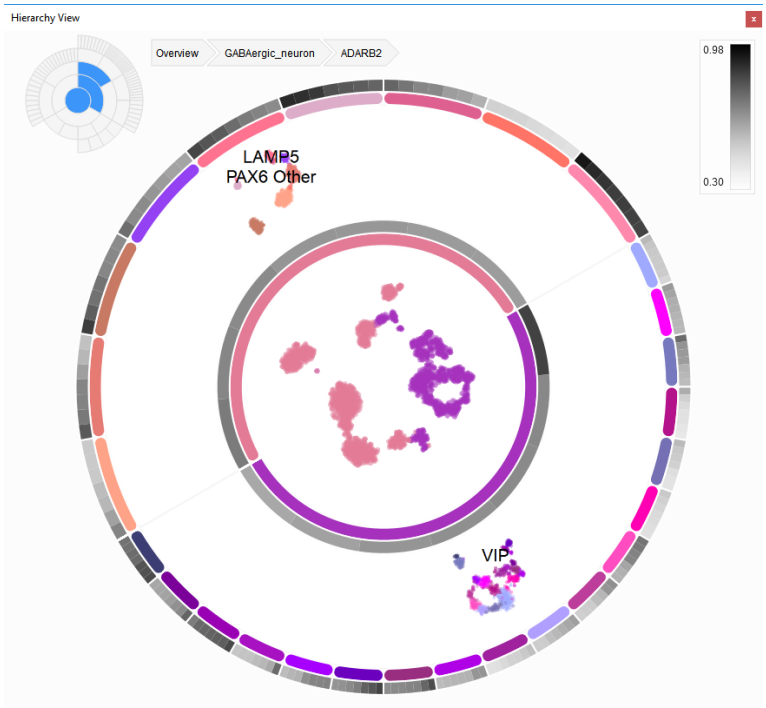
A: Hierarchy viewer: Designed to view a cellular hierarchy of tSNE maps, enabling navigation through submaps in the hierarchy. All tSNE maps are ordered in a ring configuration, and the outer ring segments are the leafs of the tree. The left-top pictogram provides a trail of the map that is being viewed. All maps have been computed using pre-computed gene presets for each submap: i.e. each map is constructed from a different gene set. Maps are pre-loaded upon startup, but can all be dynamically recomputed using the fixed gene presets and inspected. The colored rings represent different cluster aggregations of nuclei throughout the hierarchy. The gray ring panels represent the most distinctive genes expressed within a cluster, compared to the other clusters in the submap. These ring panels are sorted on cluster localization of expression (Beta). The genes accessible through the rings are drawn from the reduced gene presets that were used to construct the maps.

B: Map viewer: Designed to view metadata of each cell on each submap, to manually select sets of cells for differential gene expression analysis, and to display expression of single genes. Gene expression display and differential analysis is performed on the full gene set (in contrast to the gene subsets used to create the tSNE maps). Nuclei can be selected from pre-specified clusters, or by manual drawing.

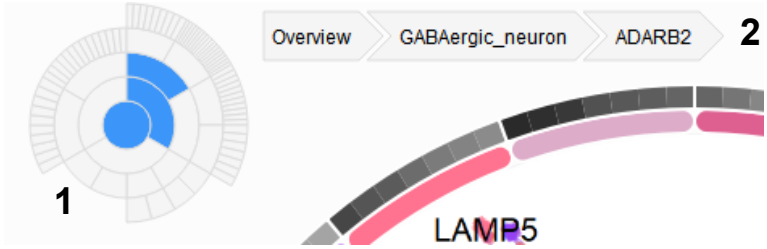
C: Differential Expression viewer: Designed to analyze differential expression between different subselections of nuclei. One selection of nuclei can be contrasted against all other nuclei, or against a secondary selection. A number of differential expression statistics between selections are computed, and genes can be sorted for all these traits. This enables mining the full feature set based on differential features between selections, and all analysis results can be copied to clipboard for export.

Navigating the Hierarchy Viewer

Clicking on a submap in the Hierarchy Viewer activates the submap in the Map Viewer. Upon zooming in on a submap with shift-left click, the submap of interest will be opened in the center of the ring, with its children in the outer rings. This gives individual clusters in the submap more space along the ring and an easier inspection of the cluster specific gene expression in the gray panels.



In the top-left, there are two navigation aids that show where the current map is situated in the hierarchy:



1) A pictogram of the full hierarchy wheel. In blue the current zoom trace is displayed from the overview map to the currently active sub map in the center of the ring. Left-clicking in the small gray panels in the wheel pictogram will zoom into the segment of interest.
2) A “breadcrumb trace” that displays the trace to the overview map (root node of the hierarchy), and the names of the submaps along the trace. Clicking on the trace arrows opens to the corresponding submap.

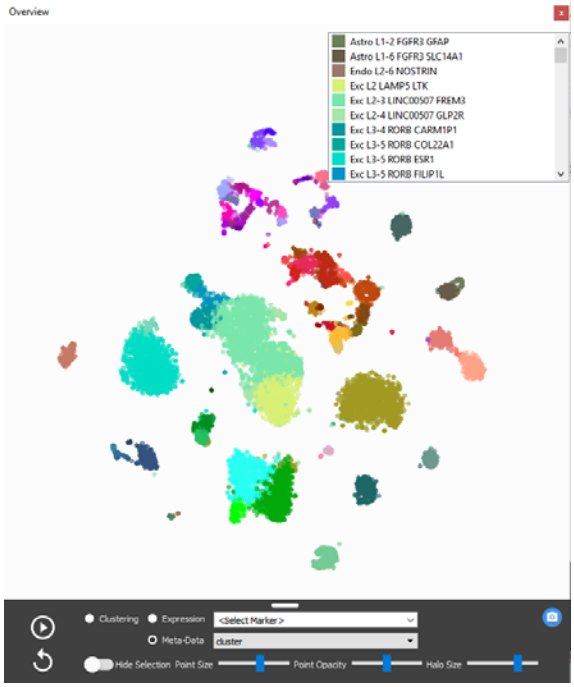
Both these tools can be used to zoom out to the overview level as well.

The color panels along the ring represent pre-specified clusters of cells, based on the AIBS clustering and color convention. Hovering over the ring segments shows the cluster name, and clicking on the segment selects the cells in the cluster. The gray panels enable a quick exploration of genes with localized expression in the corresponding cluster. The panels are sorted by locality score Beta, and clicking on a gray panel activates the corresponding submap colored by the expression of that particular gene. Note that the genes in the ring are drawn from the gene subsets that were used to compute the submap. Exploration of the full feature set is possible from the differential expression viewer (see page 7).

Map Viewer

The Map Viewer is designed for interactive exploration of tSNE submaps at the single cell level, with the following features:

- Metadata on each cell can be viewed by scrolling with the mouse wheel while hovering over the Metadata text box. This will bring up a legend box with the metadata items in that particular submap.
- tSNE maps can be recomputed on the fly, and paused during computation for detail inspection and analysis. Note that linear structures during A-tSNE computation with exponential force decay have been shown to suggest differentiation pathways and lineage relations [5].
- Expression of an arbitrary gene can be painted in the map by starting to type the gene name in the expression text box, and selecting the target gene from the drop down menu.
- Hovering over a cell brings up an information panel with the detail metadata on each nucleus.



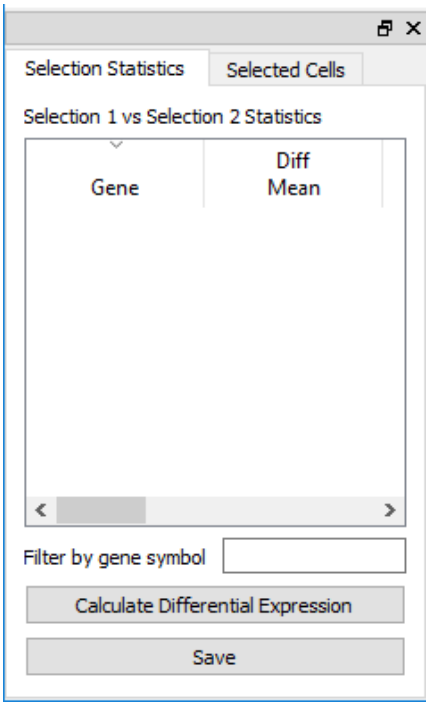
Differential Expression Viewer

The Differential Expression Viewer is designed to mine the full feature set for differential expression between two selections of cells.

There are three methods to select cells / nuclei:

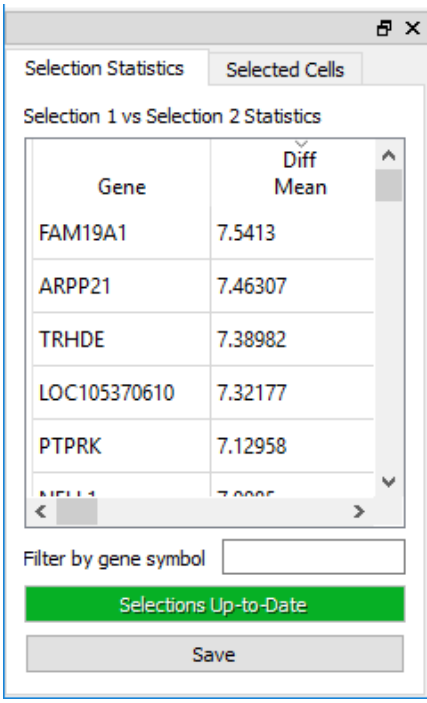
- Manual drawing of cell selections in the Map Viewer (Left-click and drag).
- Clicking on a colored ring segment in the hierarchy viewer selects the cells in that cluster / preset.
- Using the metadata legend in the map viewer: clicking on a metadata item in the legend selects the corresponding cells.

Selections can be emptied by left-clicking in an empty space in the map viewer. By default selections are stored in selection 1. Pressing the left-alt key while performing these actions will fill selection 2.



Once both selections are set, clicking the Calculate Differential Expression button will compute differential expression statistics over the full feature set and will fill the table. The default behavior for the differential expression viewer is as follows:

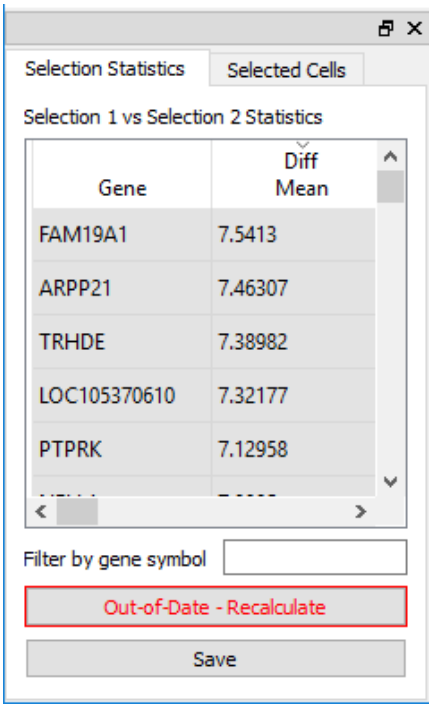
- Differential expression and Beta are only computed if a gene is expressed in at least 5 cells in each selection, and otherwise set to 0.
- P-values for differential expression are computed if a gene is expressed in at least 10 cells in each selection using the Wilcoxon rank-sum test, or otherwise set to 1.
- P-values are corrected for multiple testing through Bonferroni correction.
- Compute times may increase with larger selections.



Once the differential expression computation has finished, the analysis results can be explored as follows:

- ***Gene expression coloring in the map view*** can be performed by clicking on a gene name in the left column. This will display the expression of the selected gene in the Map View
- ***Gene sorting*** can be performed by left-clicking on the sorting variable names at the top of each column. Left clicking again will sort the variable in reverse order. The columns can be reordered by clicking on the column name, and dragging it to a new position in the table.
- ***Gene filtering*** can be performed to reduce the number of listed genes by typing gene symbol characters in the “Filter by gene symbol” field.
- ***Cell selections*** can be inspected, loaded from file or saved through a separate tab “Selected Cells”.
- ***Result export*** can be achieved through the clipboard by right-clicking on the result table, or by pressing the “save” button.

If either of the selections is changed, the results in the table will no longer be up-to-date with the selections: this is indicated in the differential expression viewer by a darker background color for the statistics table and the button “Out-of-Date – Recalculate” will become visible. Note that the previously calculated differential expression results can still be interactively explored.



For an instructional videos highlighting the user interface functionality, see https://vimeo.com/channels/cytosploreviewer

Context-sensitive user interactions in the Hierarchy and Map viewers

Hierarchy viewer
Map selection

- *Leftclick on a submap*: **selects** the map in the map view
- *Shift+ leftclick on a submap*: **zooms in** on the submap. See page 3 for details on further navigation and more detail in the zoomed view

Cluster navigation

- *Hover over a color in the rings*: **displays** the cluster ID
- *Leftclick on a color in the rings:* **selects** the cluster in the current map view in selection 1
- *Alt+ leftclick on a color in the rings:* **selects** the cluster in the current map view in selection 2

Gene selection of cluster-expressed genes

- *Hover over* one of the gray panels in the outer ring segments: shows the gene name of the gene
- *Leftclick* on one of the gray panels in the outer ring segments:
  - switches the map view to the map inside the ring segment
  - paints the corresponding gene expression in the map view

Map viewer
Viewing

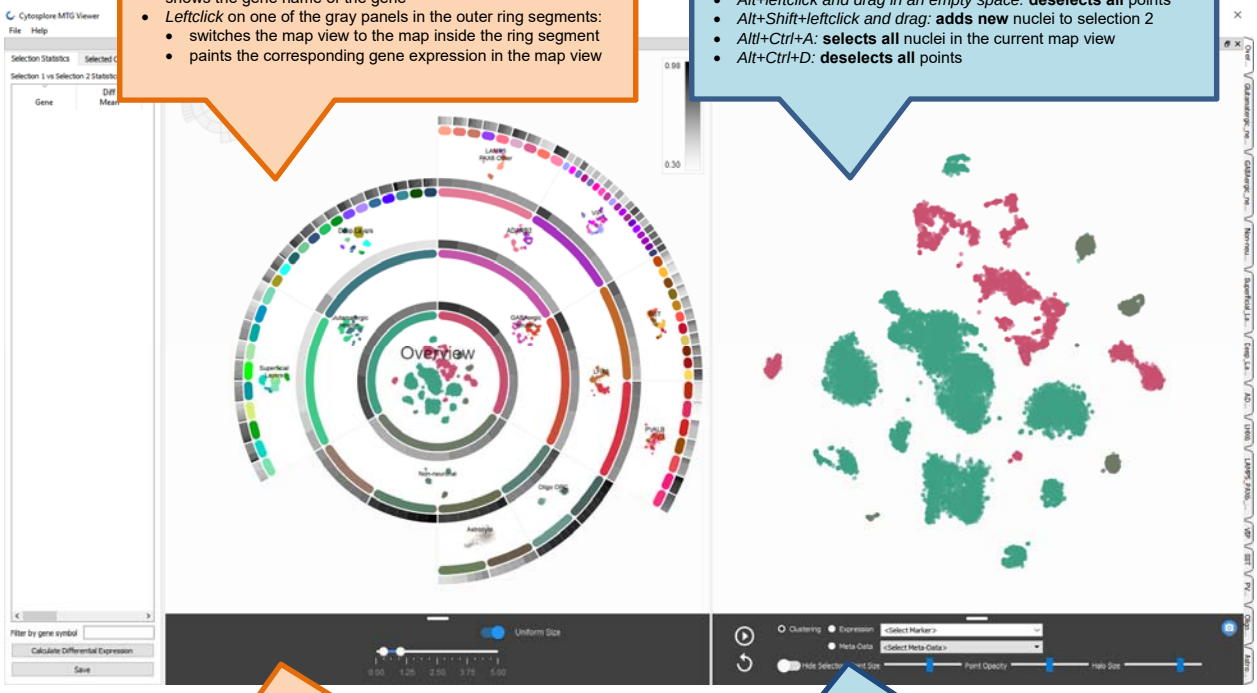
- *Hover over a cell:* displays a widget with metadata on each nucleus under the arrow
- *Left-click on color bar:* enables selection of different expression color maps

Fill selection 1 (highlighted with blue halo)

- *Leftclick+drag*: **selects** nuclei within the dragged square
- *Shift+leftclick and drag*: **adds new** nuclei to selection 1
- *Ctrl+A:* **selects all** nuclei In the current map view
- *Leftclick+drag* in an empty space: **deselects all** points
- *Ctrl+D:* **deselects all** points

Fill selection 2 (highlighted with orange halo)

- *Alt+leftclick and drag:* **selects** nuclei within the dragged square
- *Alt+leftclick and drag in an empty space:* **deselects all** points
- *Alt+Shift+leftclick and drag:* **adds new** nuclei to selection 2
- *Altl+Ctrl+A:* **selects all** nuclei in the current map view
- *Alt+Ctrl+D:* **deselects all** points



Dark gray area

- GUI element dock that can be hidden with the small horizontal stripe at the top center

Uniform size switch (only active in zoomed-out view)

- *Switched on:* all cluster segments have the same arc length within a subsegment.
- *Switched off:* cluster segments are scaled according to the relative proportion of nuclei in the cluster.

Gene panel intensity slider

- Manual adjustment of the intensity scale for the gene panels.

Dark gray area

- GUI element dock that can be hidden with the small horizontal stripe at the top center.

Color Viewing switches

- Enable switching between viewing cluster colors, expression levels, and metadata. To select a gene in the expression mode: start typing the gene name, and select by mouse click the gene of interest that appears in autocomplete.
- *Middle mouse wheel scrolling* can be used to rapidly scroll through the drop-down items (genes or metadata)

Sliders

- Set different size and transparency properties

Hide selections

- Hides the halos of selected points

Play, Pause and Recompute buttons:

- Start, pause or recompute the A- tSNE computation. When pausing the map evolution, all exploration facilities are active.

Save a snapshot image of the current view

- Press the camera icon in the right upper corner

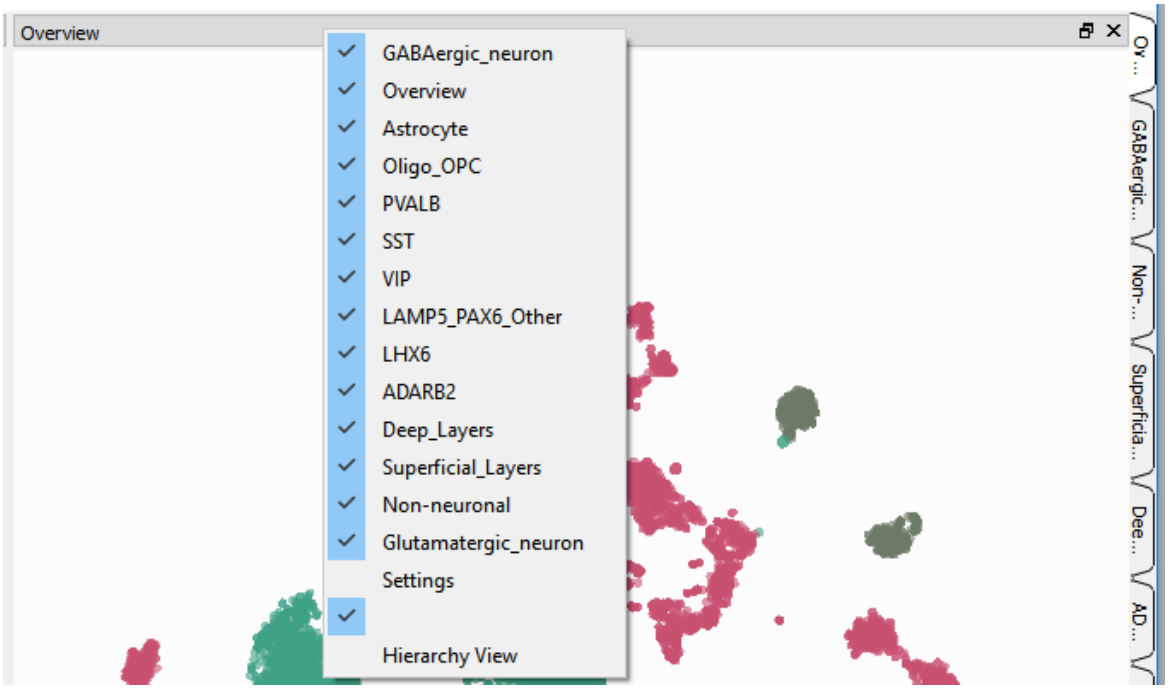
Tab panels on the right border of the map view

- Enables switching between maps without the hierarchy view

General functions to organize views

The default organization of the different views can be customized by clicking and dragging on the top bar of each view. The organization of the windows can be cusomized as follows:

- **Move a view within the application window:** drag and drop within the application window will reorganize the other views. Dropping the view in the middle of another view will add it as a tab to the target view.
- **Resize a view:** each of the views can be resized by moving the mouse over the boundary of the view: arrows appear, and the border can be dragged to resize the view.
- **Dock a view outside of the application:** drag and drop to a location outside of the application window will float the view as a separate window, while maintaining the interactive links to all other views. This can be convenient for instance on a PC with two screens: views can be docked to a second screen.
- **Close a view:** a view can be closed by clicking the X in the right upper corner of each view.
- **Retrieve a closed view:** each view can be retrieved by right-clicking on the top bar of a view: this will bing up a list of views that can be toggled on or off. See below for an example.



Personal Settings

Personal default settings for t-SNE (such as the number of iterations) and visualization options (e.g. for the scatter plots) of Cytosplore Viewer can be customized through the Cytosplore.ini file located in the program folder. Documention regarding the settings can be found in the ini file.

Acknowledgements

Cytosplore Viewer was developed in close collaboration between the Allen Institute for Brain Science in Seattle, the Division of Image Processing and the Computational Biology Center at Leiden University Medical Center, and the Computer Graphics and Visualization Group at the TU Delft, the Netherlands. Cytosplore Viewer development was supported by the NWO-AES grants 12720 (VanPIRE, PI Vilanova) and 12721 (Genes in Space, PI Lelieveldt). Cytosplore Viewer is derived from Cytosplore, a framework for the interactive exploration of single cell mass cytometry data [1-5,8]. Data displayed in this viewer was downloaded from the Allen Cell Types Data, (© 2015 Allen Institute for Brain Science), as reported in [6, 7]. The Allen Cell Types Database is available from: http://celltypes.brain-map.org/.

The main contributors to Cytosplore Viewer development are:

- Trygve Bakken, Jeremy Miller, Rebecca Hodge, Bosiljka Tasic, Zizhen Yao, Lucas Gray, Hongkui Zheng, Mike Hawrylycz, Ed Lein (Allen Institute)
- Jeroen Eggermont (LUMC, Lead Developer)
- Thomas Höllt, Ahmed Mahfouz (LUMC-TU Delft, Visual Analytics and Computational Biology)
- Anna Vilanova (TU Delft, Visual Analytics)
- Boudewijn Lelieveldt (LUMC-TU Delft: PI)

Cytosplore Viewer makes use of the following open-source software:

- QT
- HDF5
- WinSparkle
- FLANN - Fast Library for Approximate Nearest Neighbors
- D3 - Data Driven Documents
- d3-context-menu
- d3-scale-chromatic
- Material Design Lite
- noUiSliser
- wNumb.js

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