Spatial Transcriptomics Viewer – User Manual

Version 0.6.2

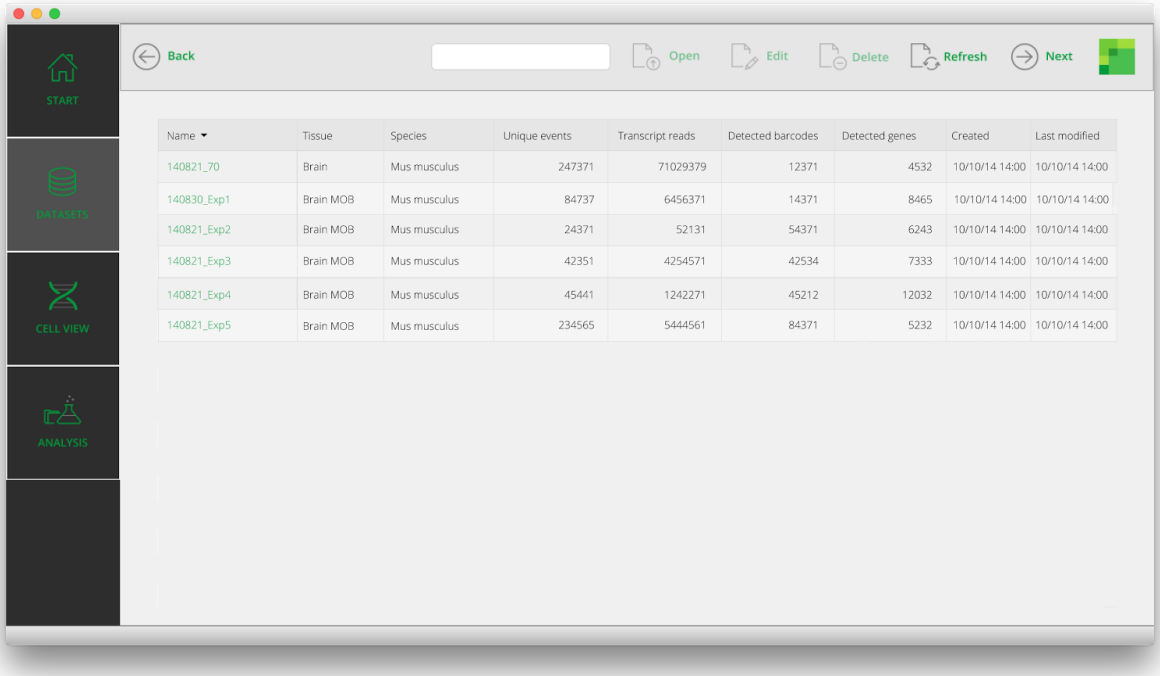
This Manual is old, some of the figures must be updated and some of the options must be updated as well.

The Spatial Transcriptomics Viewer (stVi) is a desktop application that allows users to securely access and visualize spatially distributed gene expression profiles data with their respective tissue image. At the same time it allows users to analyze the data directly in the program or to export it for their own analyses. The application can obtain the data from a secured server (Update the configuration file for this) and/or from local files.

Interface

The application has a main window what we call “cell view” and then it has some extra windows (Datasets and Selections).

Datasets



Dataset view with six different datasets available

The dataset view shows basic information about the dataset associated with the user currently logged in. The primary component of the view, the dataset table, shows key information about each dataset (e.g. name, tissue type, species, etc.). The table will adapt automatically to the content but can also be resized manually if needed.

A search box allows you to filter the dataset list for specific keywords.

To open a specific dataset you have to select it and click on the “Open” button above the table. This will bring you to the Cell View.

The “Edit” option allows you to change name and comments of a dataset.

The “Delete” option deletes a dataset and the selections made in the selected dataset. After deleting a dataset it is no longer accessible by the user.

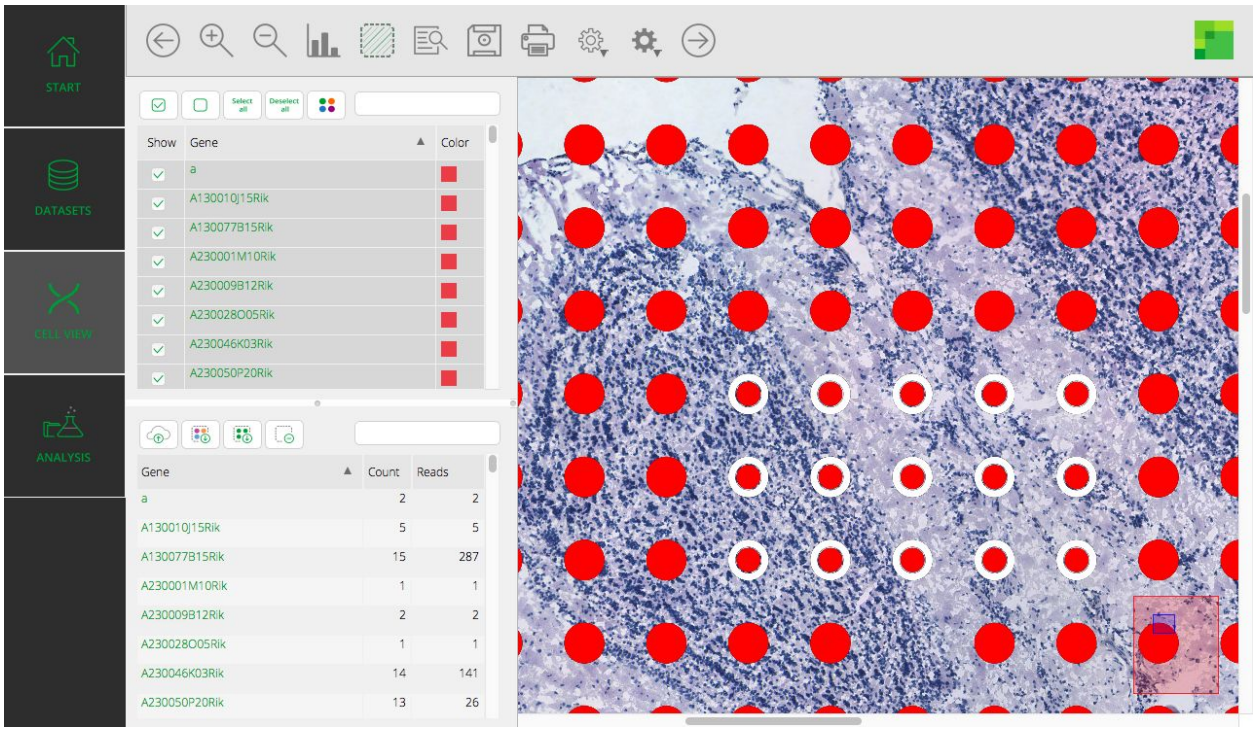
By clicking “Import dataset” the a form will appear where user can import datasets locally (ST data, alignment and images are required).

By clicking the refresh button the list of datasets including all additional information is updated.

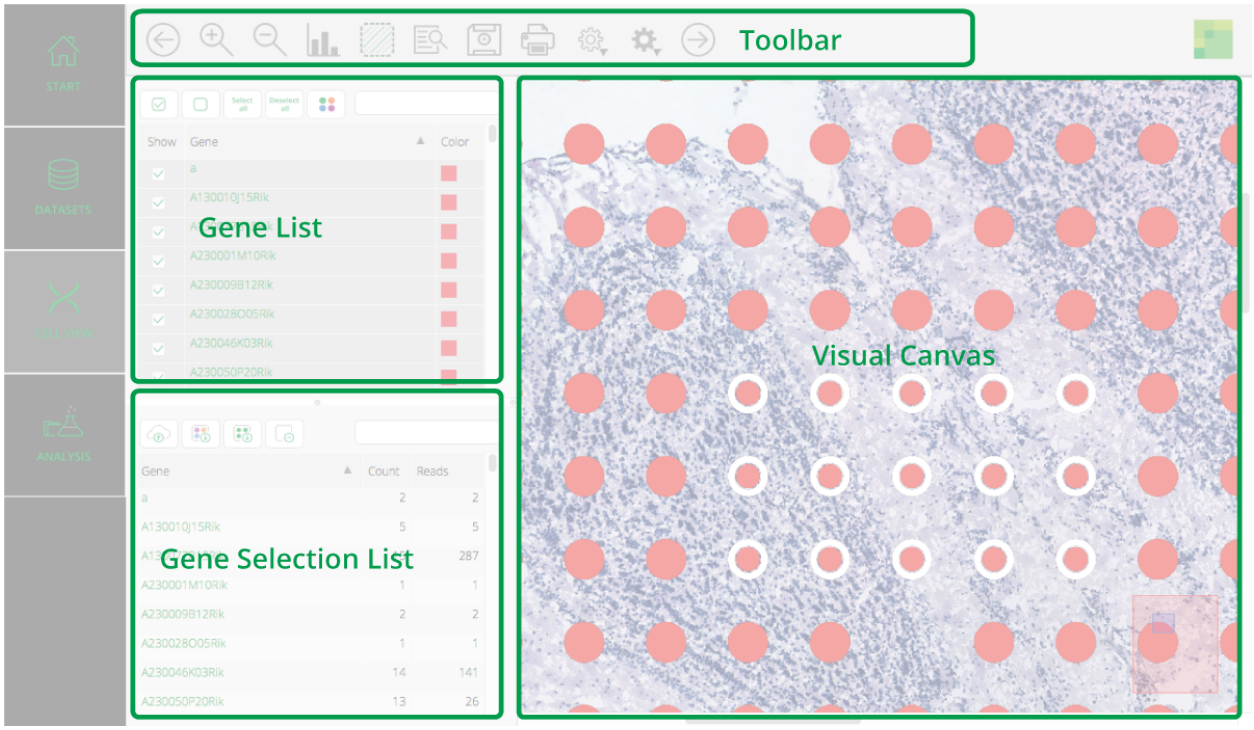
Note:
Each dataset is associated with a substantial amount of data, which will be accessed after a dataset has been selected and opened. As such the transition between the views might require a few moments. It is worth noting that the data is cached, which implies that once it is downloaded, the next time the same dataset will open much quicker. This does not apply if the dataset is updated in the cloud in the meantime.

Cell view

The cell view shows the dataset image and visualized gene activity on the image. It provides various means for customizing the visual aspect, searching and filtering for genes and different options for exporting the current visualization. The main components of this view are the *gene list*, the *gene selection list*, the *visual canvas* and the *toolbar*.



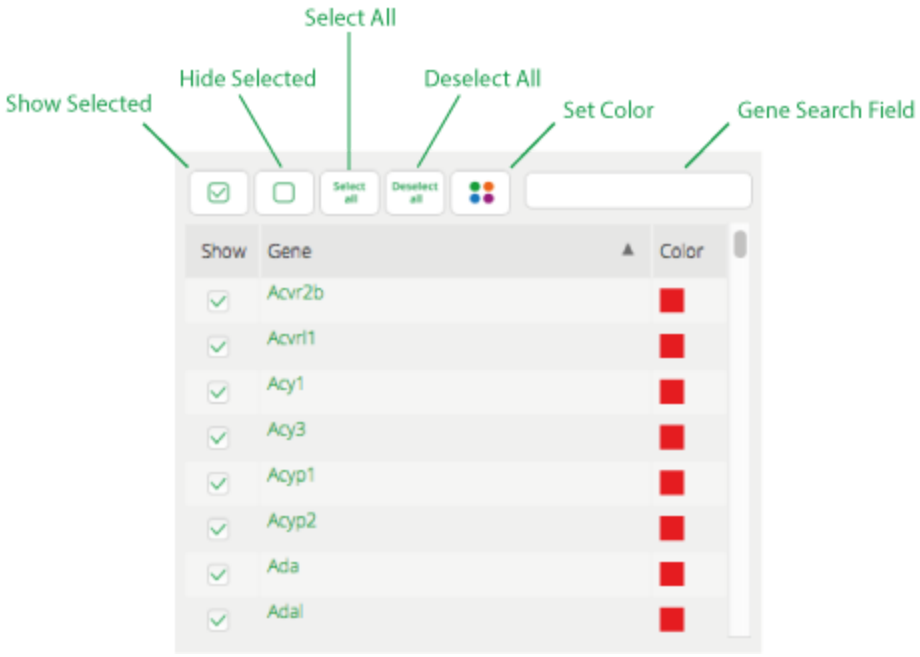
Cell view (normal mode) visualizing the features of all genes in a dataset with a few genes



Cell view (normal mode) visualizing the different parts of the screen

Gene List

The upper table at the left side of the Cell View screen is called “Gene List” and lists all the genes associated with the current dataset. It provides an easy interface to include or exclude the features for specific genes in the visualization as well as it assigns all genes individual colors.



Gene List with toolbar description

Using the “**Show selected**” and “**Hide selected**” buttons shows/hides the genes that are selected in the list in the visual canvas.
For the sake of convenience a “**Select All**” button and the “**Deselect All”** button have been included in order to by performing a one-push action select or deselect all genes at the same time.
In addition a “**Gene search field**” allows the list to be filtered by name. This does not affect the selection but rather provides a shorthand means of showing the genes which match specific name patterns.
The function “**Set color of selected genes**” allows you to choose custom colors for single genes or groups of genes in order to increase clarity.
In the gene selection list clicking on the column headline will sort the list based on the content.
To scroll through the list you can either use the scroll function of the mouse or the scroll bar on the right side of the list.

Selection of Genes

There are two different ways to make a selection of genes. You can select them manually by using the manual “Selection” option or automatically with the “Regular Expression” option which can both be found in the top menu.
A list of all selected genes appears in the “Gene Selection list” which is described below.

Gene Selection List

The lower table at the left side of the Cell View screen is called “**Gene Selection List**”. Here you can see all the genes that have been selected in the visual canvas.
It is important to note that selected genes of the same type are aggregated (reads are added up), even if they originate from a different feature within the selection.

The gene list consists of 4 columns. The content can be sorted based on the content in a column by clicking on the column name field (e.g. “Gene” or “Count”)

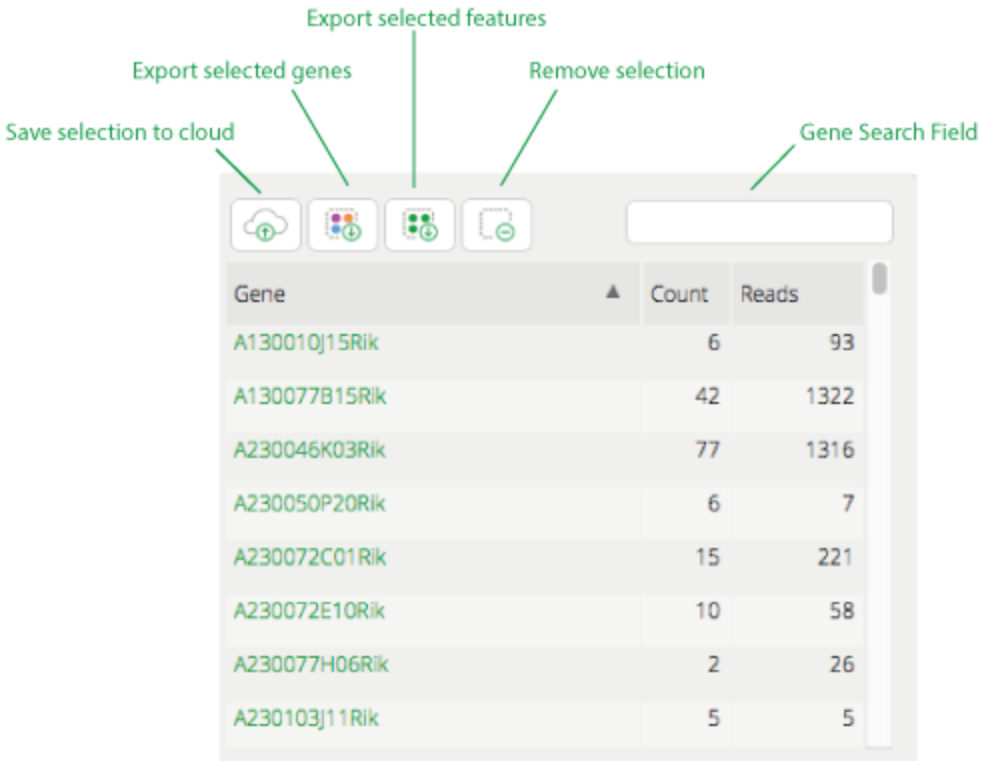
The columns are:

“**Gene**” lists the gene names.

“**Count**” means how many times a gene was present in the selection in different features.

“**Reads**” provides the number of reads that were sequenced for this gene. This number is also averaged like the pixel intensity below.

Observe that in a selection for each gene present in many features its reads will be averaged and the count increased.



Gene Selection List with toolbar description

“**Save selection**” saves the current list of selected genes to the cloud which can be accessed later on.

“**Export selected genes**” saves the current list of selected genes to a file at a location that you choose in the popup window. The selected features can be exported both in a plain text as well as XML format. The exported data includes gene name, reads_count as well as normalized_reads_count (normalized against the current min/max interval).

# gene_name	gene_count	reads_count
Schip1	11	69
Dhx9	3	573
Gsta3	9	569
Gsta4	10	822
Mtap	2	2
Dnah1	1	54
Pdlim1	8	456
Vtn	1	95
Pdlim2	4	283
Pdlim3	3	172
Dnah5	1	25
Cerk	9	530
Pdlim4	1	66
Pdlim5	3	24
Erf	7	290
Erh	10	669
Pdlim7	4	274
Dnah9	1	59
Tha1	1	43
Rundc3a	13	666
Rundc3b	3	121
BC026585	1	7
Tomm40	5	230
Nsdhl	2	51
Abcc10	1	22
Asna1	8	424
Olfr169	1	95
Xcr1	8	16
Cdhr1	12	682

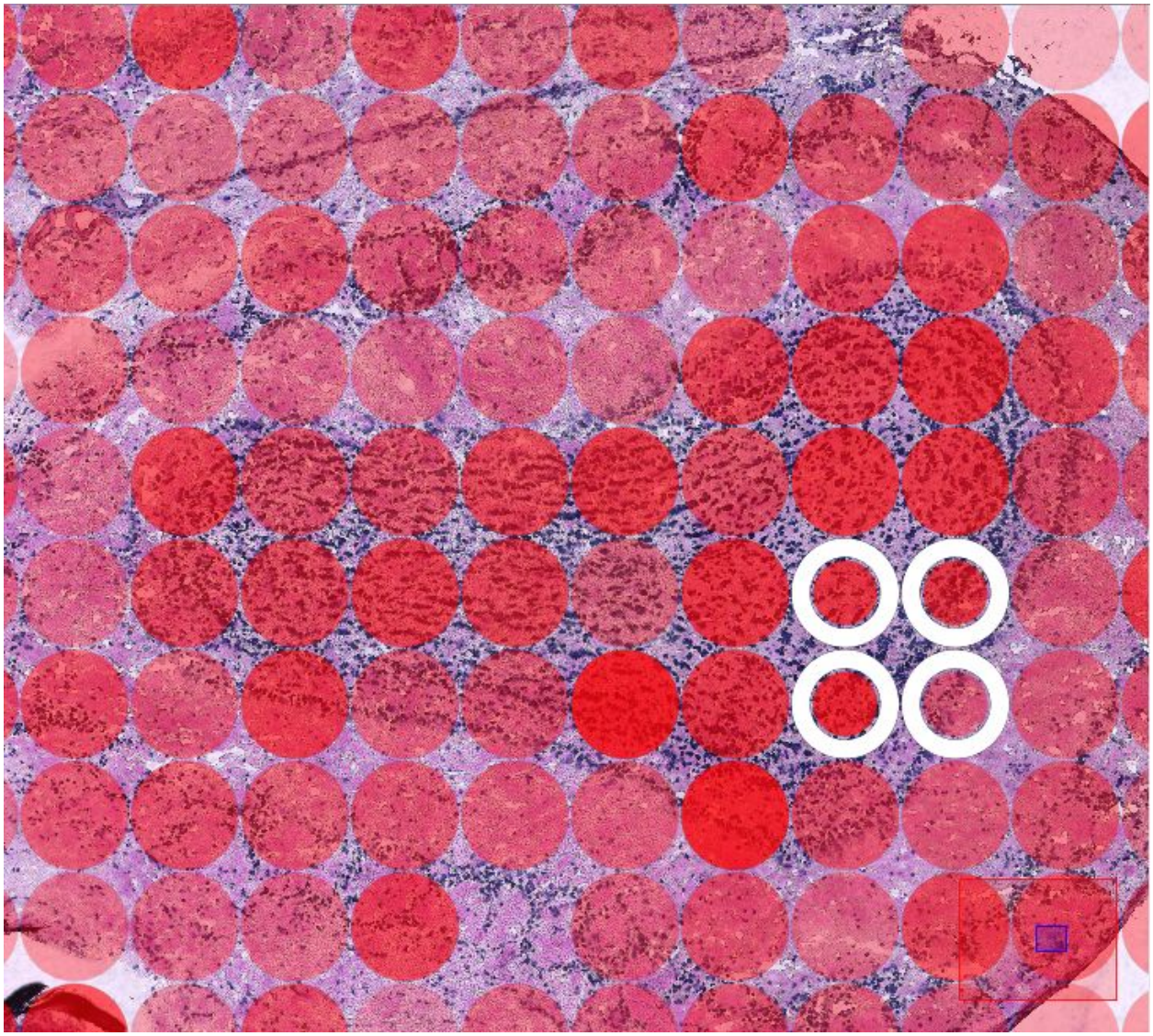
Figure: Sample gene selection exported in plain text format.

“**Export selected features**” saves the current list of selected features to a file at a location that you choose in the popup window. The selected features can be exported in the same way as the selected genes above.

“**Delete selection**” removes the current selection both in the visual canvas and the gene selection list.

“**Gene search field**” allows to filter the list by name. This does not affect the selection but rather provides a shorthand means of showing the genes which match specific name patterns.

Visual Canvas



Screenshot showing the visual canvas in dynamic range mode

The main component of the cell view is the visual canvas. It shows the cell tissue image and highlights the features of the (selected) genes on the image. The image is a high resolution[1] image which support multiple magnification levels. The user can navigate the visual canvas in many different ways.
Each colour circle represents one single feature on the chip. How exactly the information in that makes up the circle is composed differs for the three different visualization modes. More information can be found in the description of the respective visualization mode below.

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Tool Bar

The final component, the tool bar, provides various functions and options to modify or otherwise interact with the current visualization. It includes simple functions for zooming, selecting, exporting as well as more detailed functions for changing the visualization setting of the canvas and the genes.

"Back" option

Clicking the "Back" button closes the dataset and brings you back to the Dataset view.

"Next" option

Clicking the "Next" button closes the dataset and brings you to the Analysis view (described below).

Zoom

The high definition image can easily be navigated by zooming in and out. This is supported through explicit buttons on the toolbar as well as through the standard approaches utilizing mouse and keyboard inputs.

Zooming is done by mouse scrolling or using the "Zoom in" or "Zoom out" option in the top menu. Moving the picture is done by holding the left mouse key pressed in and moving the mouse around or with the arrow keys on the keyboard.

The thumbnail view in the lower right corner is called "MiniMap" and provides an easy means of navigation as it will adapt to the level of magnification allowing easy navigation through scrollbars. Navigation to a chosen point can be done by clicking on that point in the "MiniMap", whereas the red square shows the total picture and the blue square the current selection. This allows you to see where the current view is located in relation to the whole picture. Clicking on a certain area within in "MiniMap" with cursor keys will bring you to the respective point on the canvas.

Selection

The main means of interacting with the features/genes is done through selection. It is important to mention that all genes present in a feature will be included in the selection regardless whether they are shown in the canvas or not. The cell view includes two means in which this can be done:

- Manual Selection
- Regular Expression Selection

Manual Selection

The primary mode of selection is the manual selection which allows the user to select specific features by highlighting a rectangular area that encompasses them. This mode reacts to key modifiers and supports in addition to normal selection both inclusive and exclusive selection. It is important to mention that the selection of a feature automatically includes all genes present in this feature.

Selection Mode	Modifier	Description
Normal	None	Only the features inside the selection area are selected. Any previous selection is discarded.
Inclusive	Shift	Previously selected features will not be discarded allowing incremental selection where features inside the selection area are simply appended to the current selection.
Exclusive	Shift+Ctrl (Shift+ CMD in MAC)	Previously selected features are not discarded but rather than including new features, the features in the current selection area are excluded from the selection.

Table: Rubberband selection modes.

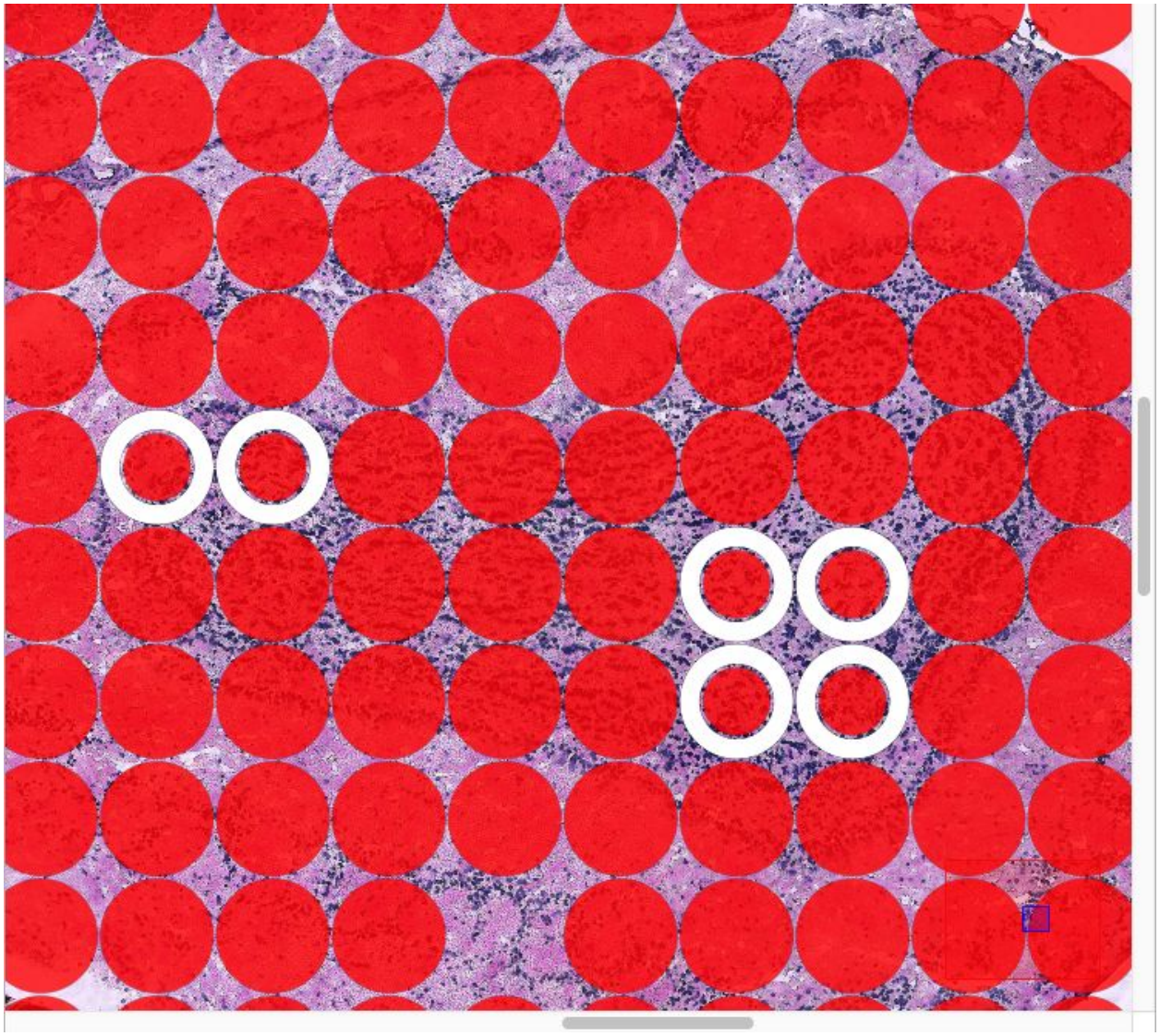


Figure: Gene set of selected genes after having used normal selection, inclusive selection and exclusive selection in that order.

Regular Expression Selection

To simplify the selection of specific genes, the cell view provides a means of selecting features by matching their genes' names against a specific string pattern, a regular expression[2]. This gives the user a powerful tool for customized selections at the possible expense of complexity. In addition the selection dialog provides two options; to include ambiguous genes and to make the regular expression case sensitive.

[2] The regular expression syntax is modelled on the Perl regexp language and further details can be found on the Qt-Project's documentation page: http://qt-project.org/doc/qt-5/qregexp.html

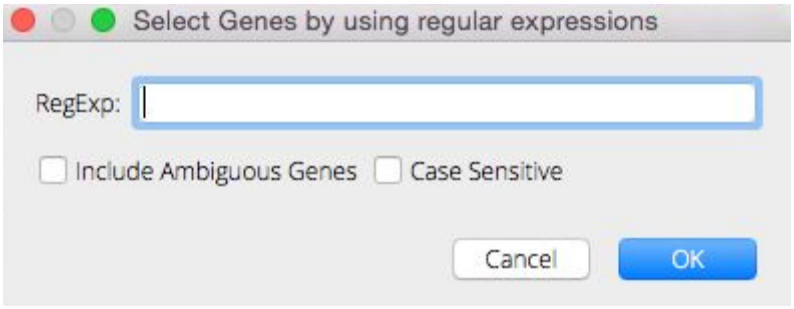


Figure: The selection by regular expression dialog.

By default the regular expression selection excludes ambiguous genes and ignores case differences.

NOTE : Once the user wants a selection to be created and moved to the Selections windows the user must click “Export selection”.

Export Canvas

“**Save Canvas**” exports the canvas as an image file (Currently the supported image formats are JPEG, PNG and BMP).

“**Print Canvas**” button opens a print menu where you can specify your printing preferences.

Configuration of Genes

The cell view provides additional gene related options in a dropdown menu symbolized by the blue gear-wheel. These are primarily related to the visual aspects of each feature as well as the visualization mode of the cell view. It is important to note that the options that modify feature aspects apply these changes to all the features. As such any individual changes (such as color) would be overwritten.

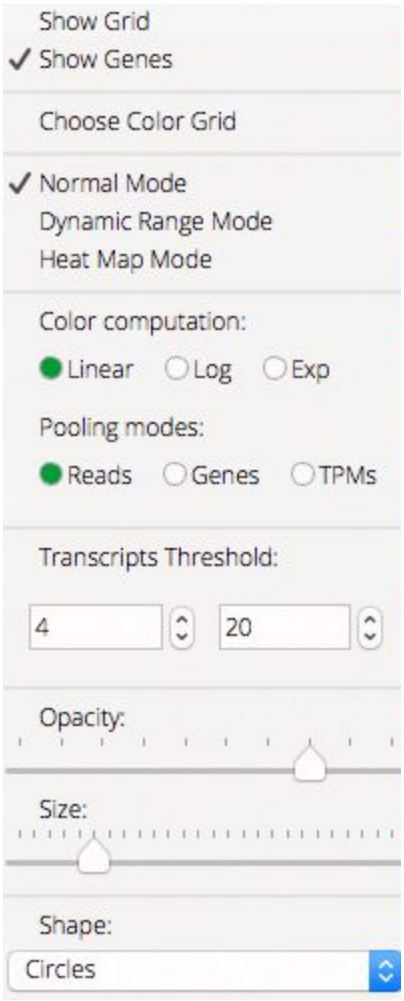


Figure: Gene options menu

"**Show Grid**" displays the original array and its border simplified to 5x5 features per square.

"**Show Genes**" shows/hides all genes that are currently be shown

"**Choose Color Grid**" opens a menu that allows you to select a color for the grid. For that you have different options such as picking a color directly from the screen, or enter RGB values, Hue/Sat/Val values. Colors can be saved by adding them to the custom colors.

Gene display modes

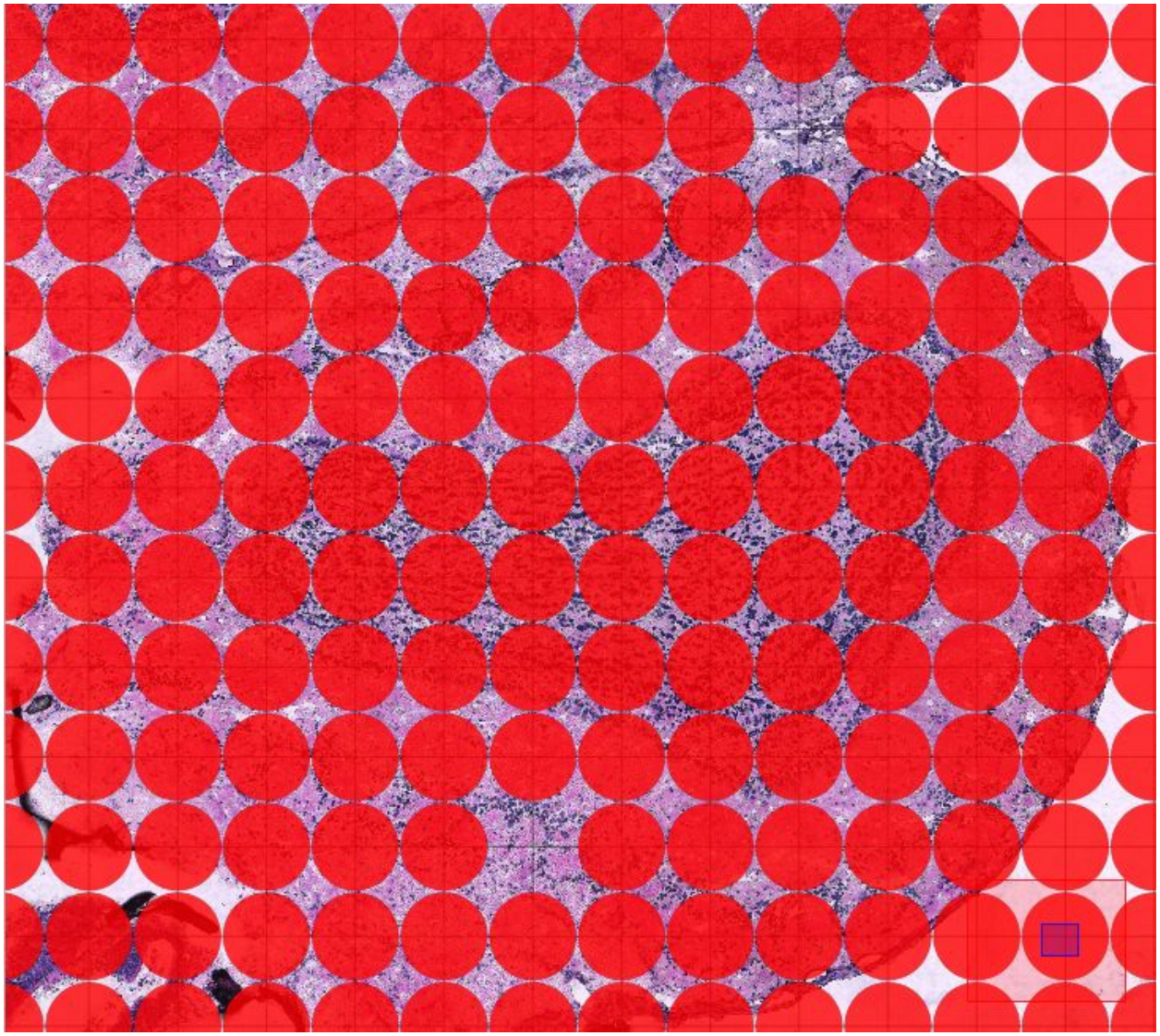
Genes can be visualized in three different modes:

- Normal Mode
- Dynamic Range Mode

- Heatmap Mode

Normal Mode

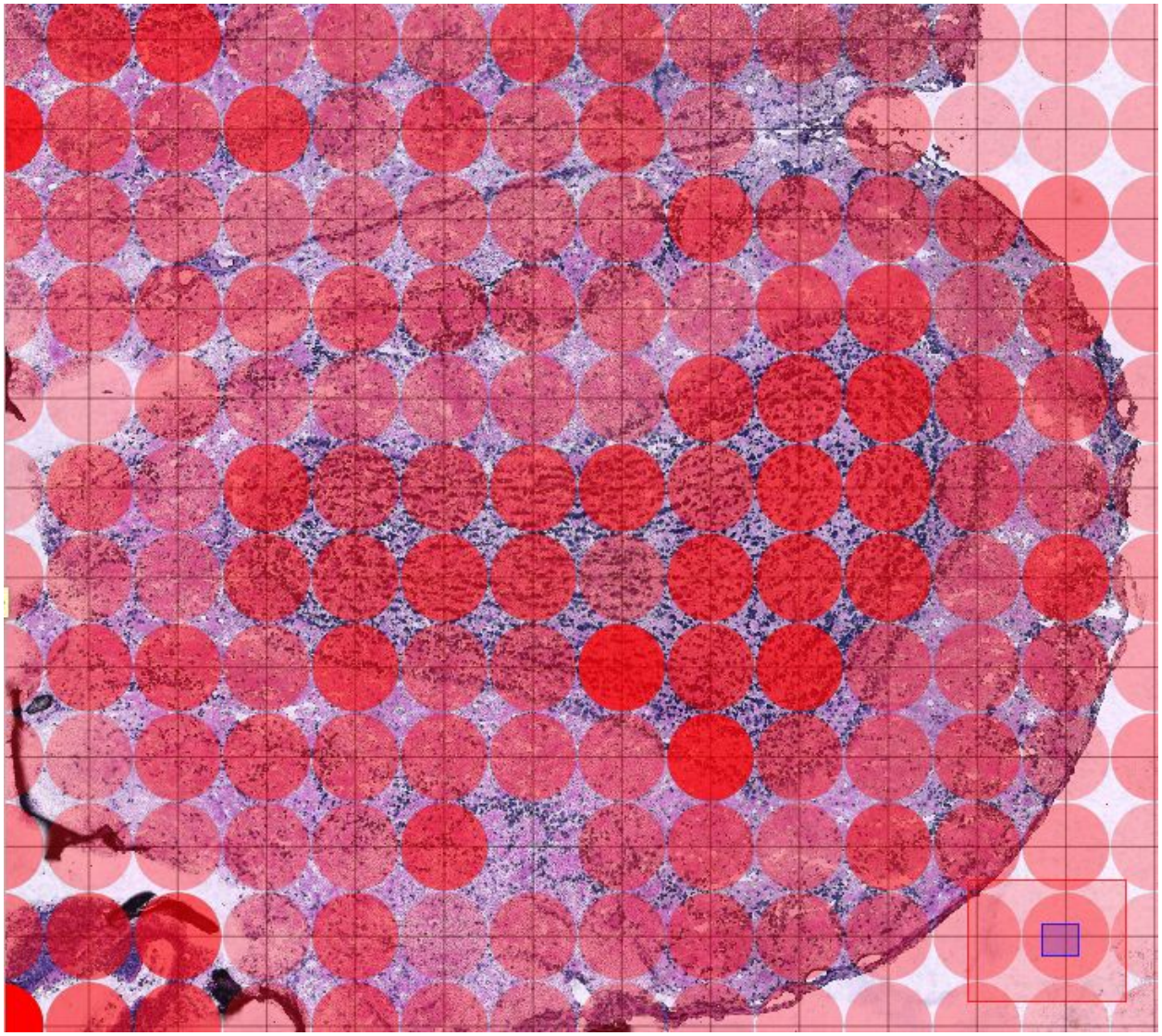
In the normal mode the selected genes in each feature are treated independently. When multiple genes are selected in a feature, the final color of the feature will be a mix of the colors of the selected genes. In normal mode the threshold(upper-lower) treats the genes in each feature separately, therefore the threshold can trim away some of the selected genes in a feature or all. In these cases the final color of the feature will be adjusted accordingly.



Genes shown in Normal Mode

Dynamic Range Mode

In the dynamic range mode, the selected genes in a feature are treated as one unit. More precisely, features are treated as single units whose level of expression is determined by the combination of all the genes selected in the feature. The final expression is normalized and the alpha channel (intensity) of the feature is adjusted according to the normalized final expression of the feature. This way, users can get an idea of the over-all expression level of a feature in relation to other features. The color of the feature will be computed the same way as the normal model (combining the colors of the selected features).
The threshold (upper and lower) in dynamic range mode affects features as a whole and not individually by gene like the normal mode. When the threshold is used, the intensity for all the features is re-computed as the normalization factor might have changed.

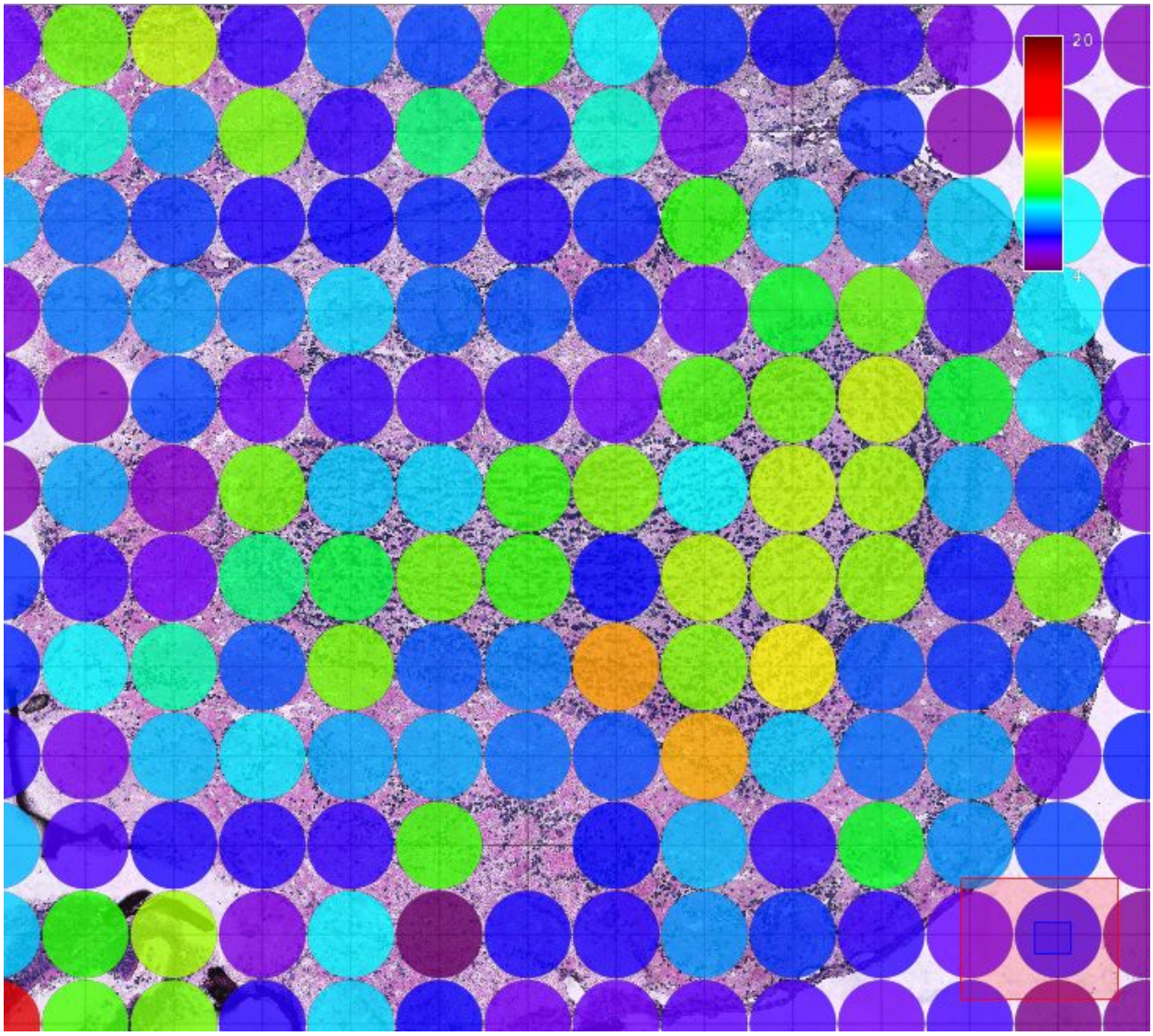


Genes shown in Dynamic Range Mode

Heatmap Mode

The heat-mode uses the same approach as the dynamic range mode but instead of adjusting the intensity, a new color is computed for the feature representing the over-all level of expression. This way, users can see more clearly the differences on the over-all expression for visible features.
The heatmap uses the visual spectrum for color space where the low frequencies (red) represents a low hit-count and high frequencies (blue) a high hit-count. The visual spectrum, minimum and maximum hit-count as well as the current threshold value (see below) is visualized in the heatmap legend in the upper right corner.

The threshold works the same way as in the dynamic range, once features are trimmed away with the threshold, the color range will be recomputed and thus all the colors of the features will be recomputed as well.



Genes shown in Heat Map Mode

“**Transcript Threshold**” fades out features whose expression level is outside of the specified threshold borders (below the lower and above the upper specified limit).

“**Opacity**” regulates the opacity of all shown feature representations simultaneously

“**Size**” changes the size of all feature representations at the same time

“**Shape**” drop down menu defines the symbol of feature representation. Possible choices are circles, crosses and squares.

Configuration of Canvas

Configuration of Canvas menu

“Show Minimap” makes the Mini Map appear/disappear.

“Show legend” makes the legend describing the different colors in the heatmap mode appear/disappear.

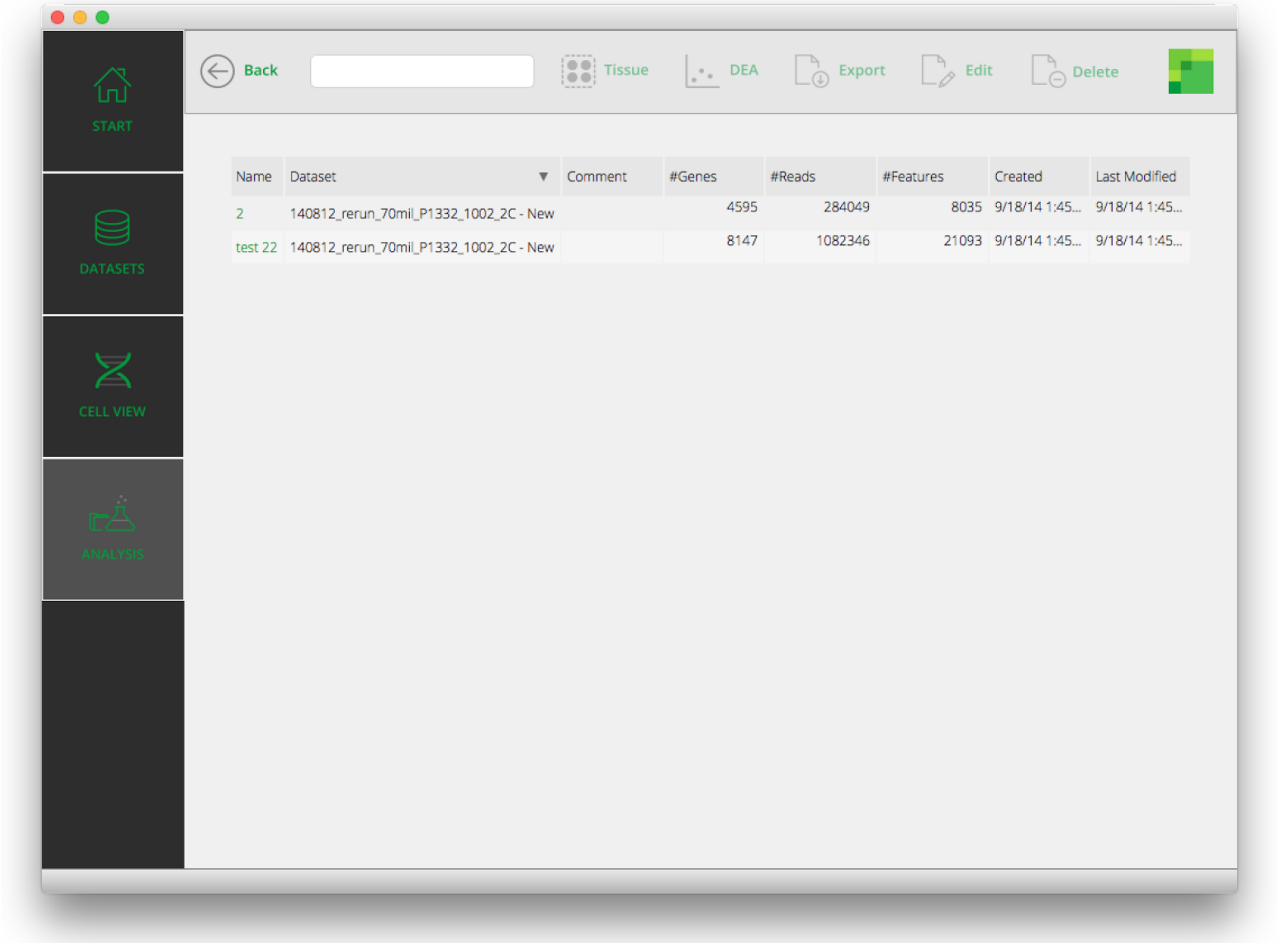
The 4 “Legend ...” options allow you to change the localization of the legend to one of the corners.

The “Minimap …” options allow you to place the Mini Map in one of the corners.

“Show cell tissue” makes the cell tissue image appear/disappear.

The “Brightness” slider regulates the brightness of the cell tissue image.

Selections



Analysis view

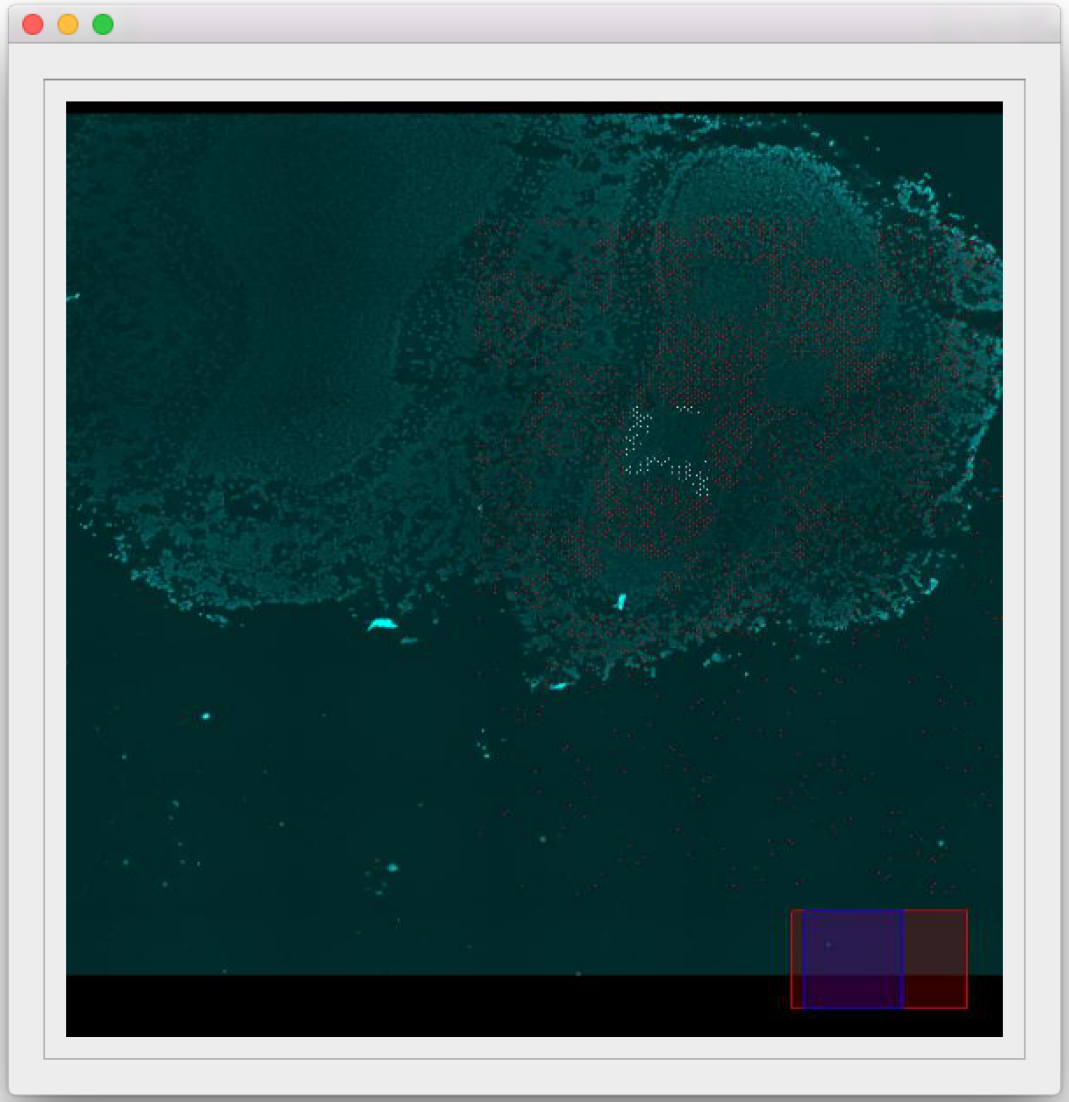
The Selections view allows you to analyze/visualize selections of genes that were previously created in the cell view. By clicking in the row of one gene selection it is selected/deselected.

"**Back**" brings you back to the Cell View

The Search box allows you to filter the list of created gene selection according to for specific keywords in their name.

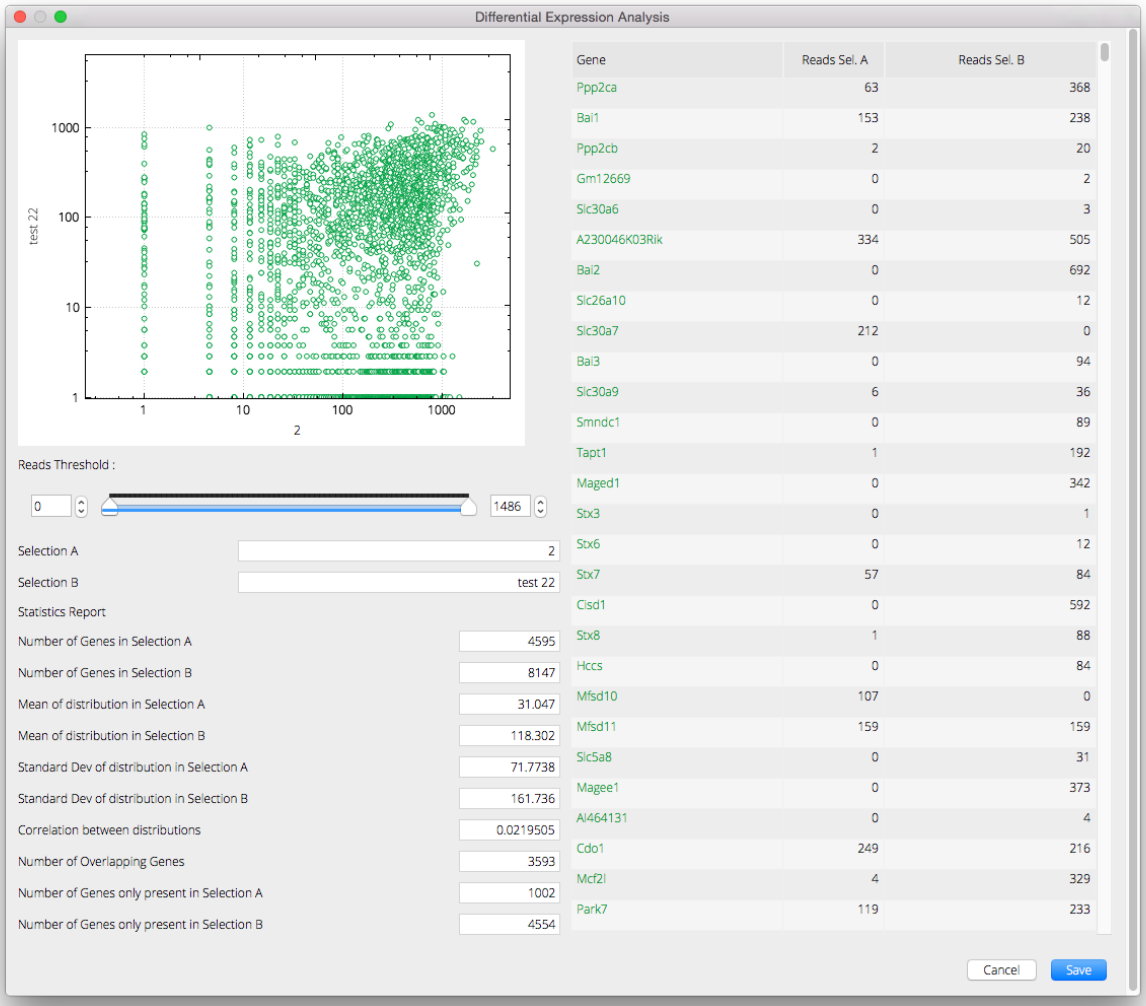
"Tissue"

This function shows a screenshot of the selection in the visual canvas. An example can be seen below.



DEA

When you select two gene selections in the list, you can do a different expression analysis (DEA). Clicking on the DEA symbol automatically performs the analysis and the result appears in a popup window.



DEA result window

On the top left corner you see a scatter plot of the DEA. Underneath There are specifications of the DEA and resulting values. On the right side there is a table with a list of all selected genes and their number of reads in each of the two selected areas.
The scatter plot of the DEA can be saved as .PNG by clicking on the “Save” button”.
The “**Cancel**” button closes the DEA result window.

“**Export**” saves a gene selection as text file. This text file includes four columns, namely: gene name, gene count, reads count and pixel intensity

# gene_name	gene_count	reads_count
Slc3a2	5	9
Hist2h2aa2	1	210
Phc2	2	2
Phc3	1	25
N4bp2l1	1	2
Ppp6r1	1	1
Ppt1	2	4
Ppp6r3	3	3
Tcta	1	32
Col6a2	1	15
Macf1	3	3
4931429L15Rik	1	1
Sgms1	2	7
Tigd3	3	11
Unc50	1	11
Psmg4	1	106
Wiz	1	1
Gm6682	1	1
Sema4b	1	1
1810014B01Rik	2	2
Sema4c	1	100
Dync1li1	3	3
Dync1li2	1	29
S100a1	2	2
Nptxr	1	30
S100a5	4	297
1110002L01Rik	1	1
Trap1	1	230
Gpr68	3	3

Textfile layout of exported gene selection

The “**Edit**” function opens a popup window that allows you to change the name of a gene selection and to add comments to it.

“**Delete**” deletes a selection. Afterwards the selection can not be accessed any more.

Known issues

As the application is still in the early alpha phase it may contain functional as well as visual discrepancies and issues. Some of these are known but were out of the scope of this release.

Below follows a list detailing each known issue, the afflicted platform as well as a brief description of its effect:

- General
    - Speed and memory issues
- Mac OS X
    -
- Linux
    - Some GUI related artifacts
- Windows
    - Some GUI related artifacts

In progress

This is a beta version with limited funcionalities.
Below follows a list of the main features and issues current in development, as well as a brief description detailing the intended change/update:

- Dataset View
    - Allow to copy paste dataset names
    - Highlight the currently opened datasets
    - Make the Import dataset option easier to use
- Cell/Main View
    - Extra customization, selection and visualization options.
    - New selection mode (lazo, circular, automatic segmentation)
    - Speed optimizations
    - More advanced gene selection/visualization options
- Selection view
    - Improvements in the DEA algorithms
    - New Analysis/visualization options
    - Allow to import selections
    - Allow to visualize selections with a chosen color
    - Allow to do dimensionality reduction
- General
    - Better error handling and logging of events
    - Improve graphical layout
    - Add options to main menu bar (global settings, view navigation, etc..)
    - Make components in cell view “docked” so they can be moved around.
    - Store settings automatically among sessions.

Contact

Please report bugs, feedback, questions and errors to:
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