

Polylux

User Manual

Data Visualization and Analysis

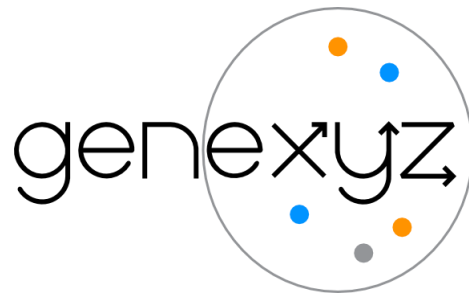


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1. Introduction

The Genexyz technology allows to localize up to 100 transcripts in tissue sections or cultured cells. Data analysis is based on the quantitative and qualitative information provided by the position of single RNA molecules relative to one another and biological structures. The PolyLux tool is at the core of this process, because it allows to overlay single molecule positions onto the same sample imaged with a variety of techniques or staining methods.

2. Installation

Important

- Viewing of molecules and analysis is done using PolyLux which is a plugin for ImageJ2.
- ImageJ is a Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation. ImageJ2 is available with a permissive BSD-2 license.
- 64-Bit Java needs to be installed on your system for ImageJ2 and PolyLux to run. Java can be downloaded and installed at <https://www.java.com/en/download/>

Procedure

1. Download ImageJ2 or copy the provided version to your computer.

ImageJ Download link: <https://imagej.net/Downloads/>

2. Place the "PolyLux_V1.6.1.jar" file into the plugins folder of your ImageJ2 installation.

Current version: Version 1.6.1

3. Features

In this section the main features of the PolyLux plugin are described in detail.

3.1 Menus

There are five menus in the menu bar

1. File Menu
2. Options Menu
3. Regions Menu
4. Coloc Analysis Menu
5. Tools Menu

3.1.1 File Menu

File menu can be used to load or save the files. The different menu items in file menu are described as follows:

Load transcripts

This menu item is used to load the transcripts file into the tool (PolyLux) with different coordinates of the positions of the individual transcript molecules in the image and their names. The file has x, y and z coordinates of the transcript along with the corresponding gene name. These values are separated by tabs to form different columns in the text file. Each row represents one single transcript with coordinates data, gene name and optional q-value score.

Once the file has been selected and successfully loaded, a list of all the gene names in the file is displayed on the list panel on the left side.

Save Settings

This menu item is used for saving all the settings of the tool (PolyLux) in a text file. The text file generated can later be used to load the settings back into the tool. The settings include shape, size and color or the displayed signals defined by row in the PolyLux tool. (Note: Settings are not saved per transcript in the current version of the tool).

Load Settings

This menu item is used for loading the settings file into the tool that was previously generated using the save settings menu function. Once the settings file has been successfully loaded; settings for size, color and shape of the transcripts are restored.

Load Groups

This menu item is used to load the group file into the tool. The file to be loaded is a tab separated text file with gene names in the first column and group membership in the subsequent columns. For example:

1		Group A	Group B	Group C
2	Aldoc	1	0	0
3	Aqp4	1	0	0
4	Ascl1	1	1	0
5	Aurkb	0	1	1
6	Bcl2	0	1	1
7	Bhlhe22	0	0	1

Here we have six genes and three groups. While the digit 1 represents the membership of gene to the group, the digit 0 represents non-membership.

Once the file has been loaded, the group of genes can be selected from the drop down on top of the gene list on the left side of the panel.

Group files can be generated in excel or any text editor.

3.1.2 Options Menu

There are nine menu items in the options menu. These are as follows:

1. Show labels
2. Undo

3. Redo
4. Use default color
5. Reuse default colors
6. Show spot density
7. Define spot density
8. Change number formats
9. Take snapshot of spots

Show labels

This function is used to visualize the spots on the images along with the labels of the corresponding gene names.

Undo

This menu function is used to undo an action and return the plugin software (Polylux) to a previous state.

Redo

This function in the options menu is used to redo or repeat the action that was undone using undo feature.

Use default color

This feature is used to use default colors for the representation of genes from a range of 12 predefined colors. The colors are predefined to be different from each other and easier to be distinguished upon visualization of the spots on the image. Select the genes in the table that you want use default color representations for and then click on this menu item to apply the color settings.

Reuse default colors

This feature resets the available colors for use to the 12 predefined colors.

Show spot density

This feature shows the density of the transcripts corresponding to the genes in the selected area on the image in the “counts” column of the table.

Define spot density

The unit of density in terms of pixels in the image can be defined using this feature to adjust the density value.

Change number formats

The format of the numbers -e.g. signal counts-that are displayed can be set using this feature. For instance, a comma could be used instead of a dot as a fraction separator. The number of decimals can also be adjusted with this setting

Take snapshot of spots

This feature flattens the image with the transcript visualizations into a single image and opens it as a new image. This image may be saved for later viewing. (Note: Flattening the images is essential, images that are saved without flattening will not display Genexyz signals.)

3.1.3 Regions Menu

The regions menu has the following features:

1. Open Roi manager
2. Export selections to svg
3. Export data of selections
4. Rename selected regions
5. Export point clouds
6. Export for 3D rendering
7. Append cluster no to rois
8. Selects rois with substring

Open Roi manager

This feature opens the roi manager of imagej which can be used to work with selections and regions of interest.

Export selections to svg

Using this feature, the selected regions in the roi manager are exported in an svg file format. SVG is an open -extensible markup language- based vector image format by the world wide web consortium. The exported file may be imported in a different imaging software for example.

Export data of selections

This feature is used to export the data of the selected regions of interest in the roi manager. The counts/density (depending on the “show spot density” setting) of all the genes in the selected regions are exported in a comma separated file format. The first column represents the gene names while there are additional columns for each roi respectively.

Rename selected regions

The selected regions of interest in the roi manager can be renamed using this feature. An integer number starting from 0 is appended at the end of the name and increases with each additional selected region.

Export point clouds

All the transcripts that are present in the selected regions of interest in the roi manager can be exported in a file using this feature. Two text files are generated for each region. One file has the original coordinates whereas the other file has translated coordinates with reference to the bounding rectangle of the selected region of interest. All these files are saved in one zip file at the selected location.

Export for 3D rendering

If you intend to render the transcripts with their colors in a third party 3-dimensional rendering software (e.g. Blender), you have the choice to export the transcripts in the

selected regions of interest along with their rgb color in text files. There is also an option to assign a factor to x, y and z coordinates respectively before the export is completed.

Append cluster no to rois

This feature comes handy when you want to append a cluster number to the names of regions of interest in the roi manager. You can choose a tab separated file name with roi names in first column and cluster number in the second column. The file is read and the cluster number is appended to all the regions in the roi manager which are also present in the file. This is an easy strategy for assigning additional information to rois (e.g. which cell type they correspond to, based on secondary analysis results).

Select rois with substring

If there is a need to select all the rois in the roi manager based on a cluster number, for instance or based on a substring, this feature is used. You can type a substring in the field and all the rois which have that substring in their names will be selected.

3.1.4 Coloc Analysis Menu

This menu is used for the colocalization analysis (this is not a mature feature yes, please contact us for additional information). There are following items in the menu:

1. Settings
2. Run analysis

Settings

There are four parameters in the settings dialog which are used to determine the colocalization algorithm: xy-stepsize, z-stepsize, search radius and distance penalty.

xy-stepsize: This parameter determines the step size between x and y coordinate. The default value given is 1.0 units for this field.

z-stepsize: This parameter determines the step size between z coordinates. The default value is set to 3.5 units in the field.

search radius: This is the radius of the region used in the algorithmic calculation of the colocalization of genes. The default value is set to 50.0 units in the field.

distance penalty: A penalty score is used in the algorithm based on the distance of a transcript from the other gene transcripts within the search radius. A greater distance has higher penalty. The default value is set to 4.0 in this field.

Run analysis

Clicking on this menu triggers the colocalization analysis on the selected region in the image.

A heatmap of the colocalization of genes is generated in a new window. The map represents the colocalization between each pair of gene transcripts in the selected region with an associated color for visual representation as well as values.

show dendogram: This option generates a dendogram of the genes based on the colocalization. The closeness and association of different genes can be seen visually in the form of a tree.

save analysis: The analysis done on a certain region in the image can be saved to computer for later use using save analysis option in the colocalization window.

3.1.5 Tools Menu

The tools menu has the following items:

1. Show density map
2. Set density radius

Show density map

Clicking on this menu item triggers the display of density map of the selected region on the image. The density map is drawn algorithmically and the "Fire" LUT of the imagej is applied to it before the density map image is displayed.

Set density radius

Density radius unit in micrometers can be set using this menu item. The range of values are from 0.5 to 7.0 micrometers and the default value is set to 3.45 micrometers.

3.2 Gene List

This is the left part of the window. When a transcript file is loaded initially, this section is populated with all the gene names present in the file. These genes can then be selected and moved into the table in the middle of the window for visualization and analysis.

3.3 Table

At the center of the window is a table of genes with five columns number, color, show, name and count. Each row in the table represents a single gene with the associated color and a checkbox indicating if transcripts corresponding to it should be visualized on the image. The count column represents the number of all the transcripts of this gene in the selected region on the image.

3.4 Settings

On the right side of the window is the settings panel where the following two kinds of settings can be adjusted:

1. General Settings
2. Specific Settings

General Settings

As the name indicates, these are the settings which are not specific to any genes. The upper and lower limit to z coordinate slices can be set here or the z coordinate can be completely

ignored by checking the “ignore z” checkbox. The stroke-width can also be defined in the units of pixels which is the boundary used in visualization of a spot. Furthermore, the “filled” checkbox may be checked or unchecked to have the spots visualized by filling a color or just having the outer boundary.

Specific Settings

These are the settings which are specific to the individual genes in the table. First one or more genes from the table need to be selected and the specific settings can be used to adjust values for these particularly selected genes in the table. We may assign different properties to the genes in the table using these settings which may come handy when visualizing the data and performing an analysis.