TissUUmaps

Release 3.0

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This page hosts the documentation for TissUUmaps 3.2. You can find a pdf version of this documentation here.

For more information on the TissUUmaps project, including video tutorials and demos, visit our website: https://tissuumaps.github.io.

Work in progress!

We are working actively on writing this documentation, more content will be available soon!

CHAPTER

ONE

INTRODUCTION

1.1 About TissUUmaps

TissUUmaps is a free and open source browser-based tool for GPU-accelerated visualization and interactive exploration of tens of millions of datapoints overlaying tissue samples. Users can visualize markers and regions, explore spatial statistics and quantitative analyses of tissue morphology, and assess the quality of decoding in situ transcriptomics data. TissUUmaps provides instant multi-resolution image viewing, can be customized, shared, and also integrated in Jupyter Notebooks. We envision TissUUmaps to contribute to broader dissemination and flexible sharing of large-scale spatial omics data.

Currently, microscopy data can be cumbersome to share: physically transferring the images is often necessary and dedicated software must be installed. Instead, researchers can now share their findings with a simple link to a website running TissUUmaps. The images are loaded in real time, together with annotations, markers, and masks that may also be modified by the user. We also provide tools for quality control and image processing. The software is designed to display and interact with images at multiple resolutions and large numbers of markers, especially data from spatially resolved omics techniques and tissue atlases. TissUUmaps is compatible with many different bioimage informatics tools, and provides new ways to develop insights when exploring and sharing data.

You can access the TissUUmaps project gallery with interactive examples to explore data from in situ sequencing and spatial transcriptomics experiments and view localized quantification of cell and tissue morphology, including links to publications. For seeing examples of TissUUmaps compatibility with other platforms you can access the tutorials page.

1.2 Installation

TissUUmaps is a browser-based tool for fast visualization and exploration of millions of data points overlaying a tissue sample. TissUUmaps can be used as a web service or locally in your computer, and allows users to share regions of interest and local statistics.

1.2.1 Windows installation

1. Download the Windows Installer (`.exe`) from the last release and install it. Note that the installer is not signed yet and may trigger warnings from the browser and from the firewall. You can safely pass these warnings.

1.2.2 MacOS installation

1. Install vips with `brew install vips` (needs homebrew installed https://brew.sh/).
2. Download the macOS installer (`.dmg`) from the last release and install it. Download the `x86_64` file if you have an Intel CPU, or download the `arm64` file if you have an Apple Silicon (M1/M2) CPU.
3. In the installer, drag-and-drop the TissUUmaps bundle to the Applications directory.
4. When the copy is finished, double-click the Applications icon in the installer and right-click + open TissUUmaps from the Applications menu. - A warning should be prompted "macOS cannot verify the developer of TissUUmaps (...)", click open and the program should launch.

1.2.3 Debian / Ubuntu installation

1. Download the Ubuntu installer (`.deb`) from the last release .deb file (20.04 or 22.04 depending on your Ubuntu version)

1.2.4 PIP installation

If you want specific Python packages to be installed with TissUUmaps, or if your no installer is available for your operating system, you will need to install TissUUmaps using `pip`:

1. Install `libvips` for your system: https://www.libvips.org/install.html:

   An easy way to install `libvips` is to use an Anaconda environment with `libvips`:

   ```
   conda create -y -n tissuumaps_env -c conda-forge python=3.9
   conda activate tissuumaps_env
   ```

2. Install dependencies using `conda`:

   ```
   conda install -c conda-forge libvips pyvips openslide-python
   ```

3. Install the TissUUmaps library using `pip`:

   ```
   pip install "TissUUmaps[full]"
   ```

   Note: If the installation fails with PyQt6, you can remove `[full]` from the previous command and run step 5 to start TissUUmaps server.

4. Start the TissUUmaps user interface:

   ```
   tissuumaps
   ```

5. Or start TissUUmaps as a local server:

   ```
   tissuumaps_server path_to_your_images
   ```

   And open http://127.0.0.1:5000/ in your favorite browser.

1.3 Citing TissUUmaps

Please cite our preprint on bioRxiv if using TissUUmaps in your work:

TissUUmaps 3: Interactive visualization and quality assessment of large-scale spatial omics data. *Nicolas Pielawski, Axel Andersson, Christophe Avenel, Andrea Behanova, Eduard Chelebian, Anna Klemm, Fredrik Nysjö, Leslie Solorzano, Carolina Wählby,* bioRxiv 2022.01.28.478131; doi: https://doi.org/10.1101/2022.01.28.478131.

1.4 Changelog

1.4.1 3.2

- Add annotation tools (in collaboration with Sanofi Digital R&D)
- Move to WebGL rendering for regions
- Refactor completely the region tab to better handle large number of items
- Allow import of regions from multiple GeoJSON files
- Add support for Geobuf (.pbf) region files
- Add drag-and-drop opening of GeoJSON files
- Add histogram button for analyzing individual regions
- Make regions work in collection mode and with multiple image layers
- Add marker outline and fill options in Advanced options
- Increase number of barcodes that can be displayed for markers
- Add validation of .tmap files (via tissuumaps-schema)
- Move standalone installer from Python 3.8 to 3.11
- Move to PySide6 instead of PyQt6
- Add installers for MacOS X (.dmg) and Linux (.deb)
- Update to OpenSeadragon 4.1.0
- Add dzi support (opening as images directly)
- Lots of minor fixes

1.4.2 3.1.1.6

- Add a min-max filter to image layers for better contrast (@glbarlow)
- Fix marker filtering
- Update license from BSD-3 to MIT
- Add collapsible sections in plugins
- Minor fixes

1.4.3 3.1.1.5

- Fix multiple images dropped on empty project
- Change threading to allow higher priority to GUI than to server side
- Add .tmap extension to all saved projects
- Fix pie charts display update and colors
- Add error message if update view fails

1.4.4 3.1.1.4

- Fix bug of modal not hiding properly
- Fix capture with scale of 1
- Fix loading of AnnData files without X matrix
- Add DEFAULT_PROJECT parameter for docker and server

1.4.5 3.1.1.3

- Minor fixes

1.4.6 3.1.1.2

- Make load of HDF5 data parallel
- Add Colormaps and Channel splitting filters

1.4.7 3.1.1

- Move to OpenSeadragon 4.0.0
- Move docker to python-alpine for security reasons
- Add sorting options of markers (applied automatically on AnnData observations)
- Add zOrder parameter for draw order between datasets
- Make Update View button always visible
- Add colorbar canvas to png captures
- Add flip and rotation of markers for each collection item
- Add transformation inputs in Image Layers menu
- Add background color input
- Add gaussian marker shape
- Fix flask deprecated function
- Minor fixes

1.4.8 3.1.0.8

- Fix chunk size for h5 files
- Fix iPhone not loading markers with > 192 collection items

1.4.9 3.1.0.7

- Critical fix for Mac OS (see row_major in WebGL2)

1.4.10 3.1.0.6

- Minor fixes

1.4.11 3.1.0.5

- Add flip and rotation of markers for each collection item
- Add transformation inputs in Image Layers menu
- Fix colormap with NaN values

1.4.12 3.1.0.4

- Fix issue with slow rendering of web-gl using instancing
- Hide colorbar on png captures when needed
- Other minor fixes

1.4.13 3.1.0.3

- Fix h5 autocomplete on iframes
- Fix wrong marker index for color column when more than 100k markers
- Add colorbar canvas to png captures
- Small fixes

1.4.14 3.1.0.2

- Fix crash on layer from a parent layer.
- Change dropdown selection from Chosen to Select2 for faster loading.
- Update docker to Alpine for security reasons.
- Small fixes

1.4.15 3.1.0.1

- Move docker to python-alpine for security reasons
- Add sorting options of markers (applied automatically on AnnData observations)
- Make Update View button always visible
- Minor fixes

1.4.16 3.1

- Adding HDF5 support on the client side
- Adding AnnData support on the server side / GUI
- Adding Network diagram visualization
- Tabs now saved automatically even without buttons
- Adding Plugin helpers in javascript
- Many fixes on the interface
- Move to PyQt6

1.4.17 3.0.10.4

- Fix path issue on json loading from server

1.4.18 3.0.10.3

- Reset all input dropdowns when new data selected

1.4.19 3.0.10.2

- Add scale factor for coordinates of markers

1.4.20 3.0.10.1

- Add optional offset (x, y) and scale properties to tmap.layers

1.4.21 3.0.10

- Add collection mode (to display images next to each other with markers correctly placed)
- IFrame mode (to hide navbar and make menu smaller when TissUUmaps is run inside an iFrame)

1.4.22 3.0.9.6

- Add debug menu when running in debug mode, with debug access in javascript
- Fix linux bugs with Qt displaying all blank
- Fix empty columns in marker csv file

1.4.23 3.0.9.5

- Add / fix key shortcuts (https://tissuumaps.github.io/TissUUmaps-docs/docs/starting/shortcuts.html)
- Change default GUI port to avoid collisions with server
- Add plugin support to docker server

1.4.24 3.0.9.3

- Go back to webGL 1 for compatibility issue with Safari 14
- Fix missing .tissuumaps folder for recent files

1.4.25 3.0.9.1

- Enable larger markers at high resolutiion (up to 1024x1024px)
- Fix pinch to zoom center
- Add code of conduct
- Clean code and use ci (pre-commit)

1.4.26 3.0.9

- Move to webgl2
- Add Open Recent sub menu in File menu
- Fix path for linux and mac in server mode
- Minor fixes

1.4.27 3.0.8.9

- Make it possible to update to newer version of plugins
- Fully support –debug option in command line
- Add tooltip title for piecharts
- Add documentation https://tissuumaps.github.io/TissUUmaps-docs/
- Fix marker picking when pixel ratio != 1
- Other minor fixes and cleaning

1.4.28 3.0.8.5

- Minor fixes.

1.4.29 3.0.8.4

- Add tiling to viewport capture for higher resolution output
- Increase resolution of markers on high resolution devices
- Fix jumps on pan with mouse gesture (mobile)
- Add fix for bright image canvas on Safari
- Add an option to remove markers' outlines.

1.4.30 3.0.8.3

- Fix png artifact in Firefox, by generating jpg tiles.

1.4.31 3.0.8.2

- Add high resolution capture of viewport, up to 4096x4096 pixels.

1.4.32 3.0.8.1

- Fix multiple dataset alignment when no background image

1.4.33 3.0.8

- Fix black images generated by VIPS
- Fix Linux and Mac open of captures
- Auto save datasets as buttons when saving tmap projects
- Add `mpp` (microns per pixel) option in tmap files, to add scale bar to viewer
- Make region line thickness depend on zoom level
- Add compatibility with JupyterLab
- Add opacity per marker option

1.4.34 3.0.7

- Add menu to load plugins through an update-site

1.4.35 3.0.6

- Fix multiple plugins opening always last plugin
- Move to OpenSeadragon 3.0.0
- Add tooltip format in Advanced Settings
- Add drag and drop to open CSV files and images
- Add “Add layer” button for flask version
- Add viewport capture

1.4.36 3.0.5

- Move csv loading to Papa Parse streaming, to allow better memory management

1.4.37 3.0.4

- Add filtering of markers

1.4.38 3.0

- Add tissuumaps.jupyter module

CHAPTER

TWO

GETTING STARTED

2.1 Images

2.1.1 Supported image formats

TissUUmaps can read whole slide images in any format recognized by the OpenSlide library:

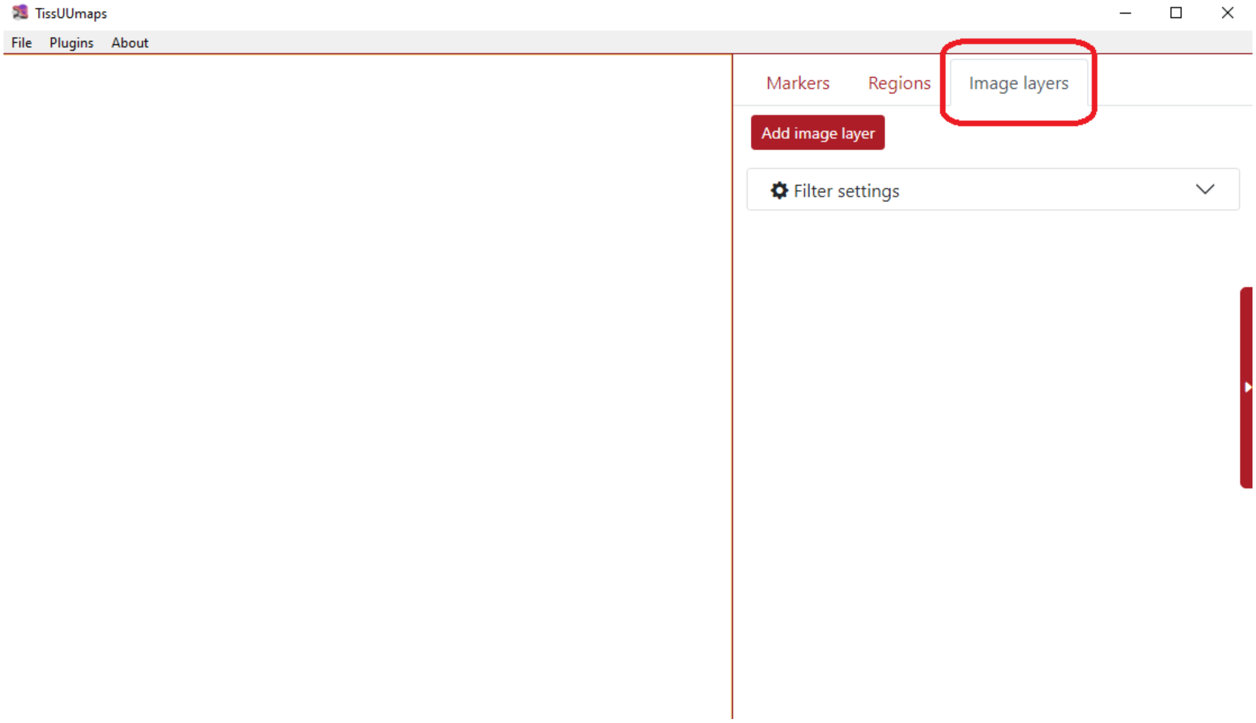
- Aperio (.svs, .tif)
- Hamamatsu (.ndpi, .vms, .vmu)
- Leica (.scn)
- MIRAX (.mrxs)
- Philips (.tiff)
- Sakura (.svslide)
- Trestle (.tif)
- Ventana (.bif, .tif)
- Generic tiled TIFF (.tif)

TissUUmaps will automatically convert any other format into a pyramidal tiff (in a temporary `.tissuumaps` folder created in the original image folder) using vips.

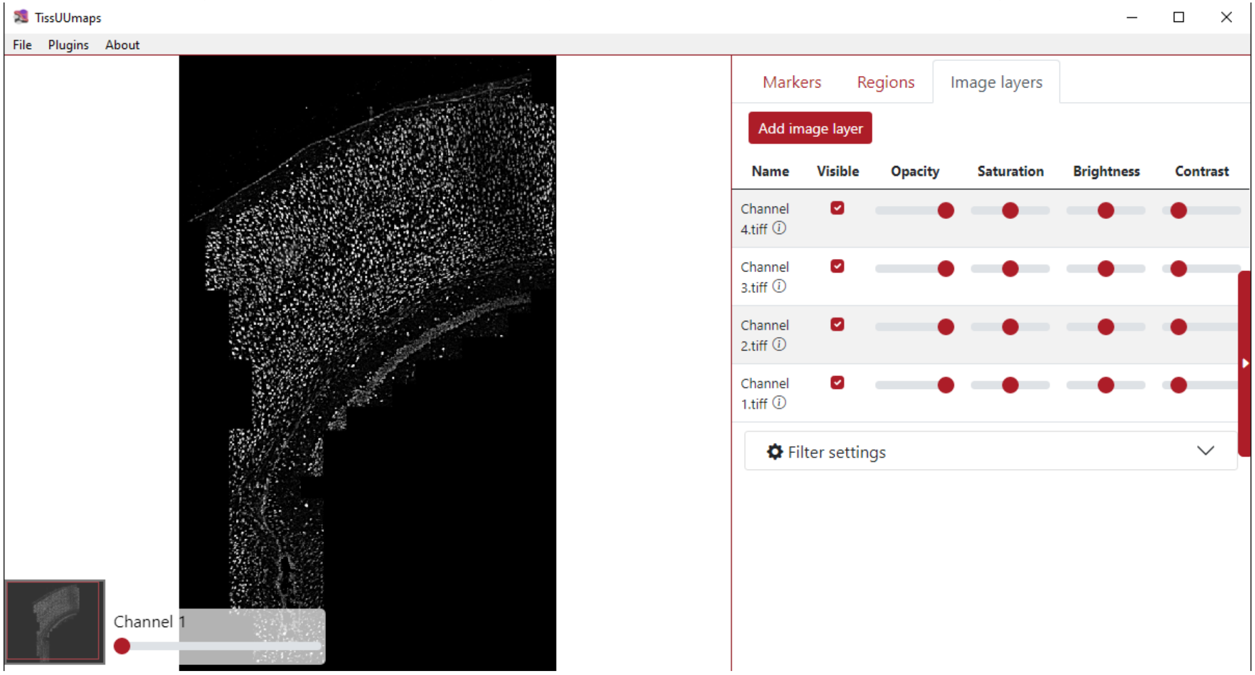
If your image fails to open, try converting it to `tif` format using an external tool.

2.1.2 Load images

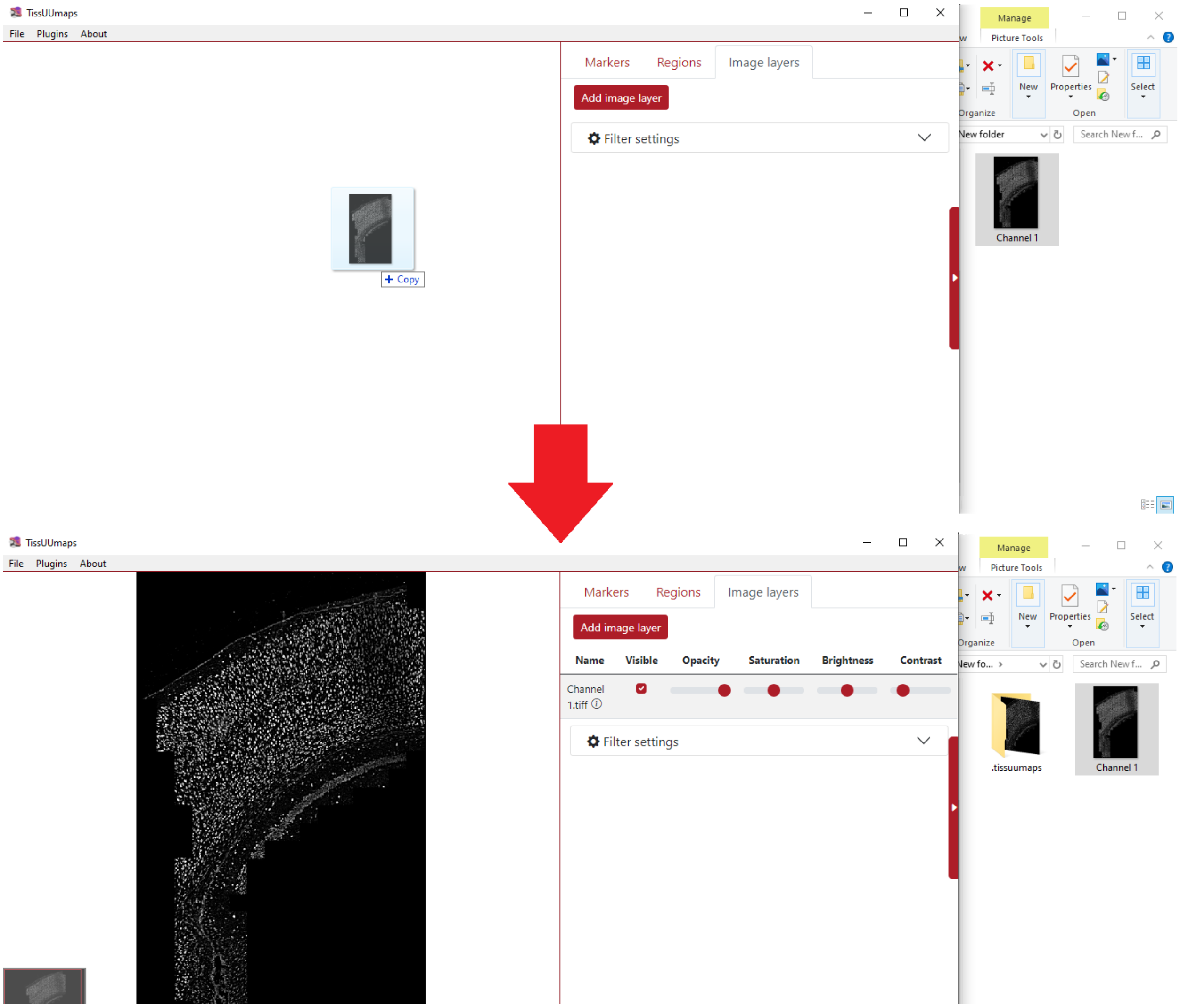
You can load the images when you select the Image layer tab as you can see in the figure below:



Then click the button Add image layer and select the desired image from your computer. Subsequently, the image is listed in the Image layer tab. You can load several images into TissUUmaps.



You can also drag and drop the image from file explorer into TissUUmaps.

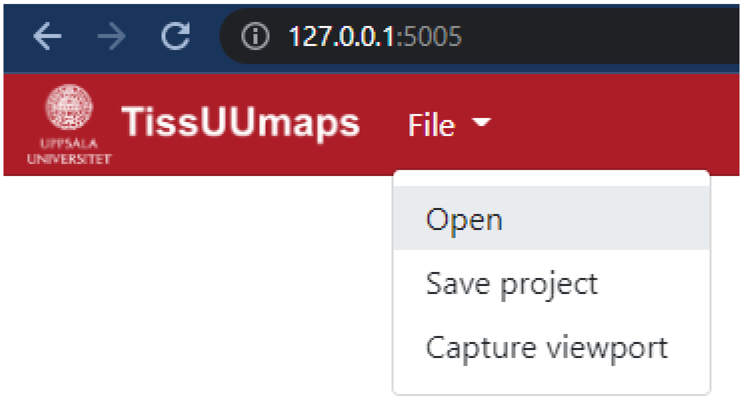


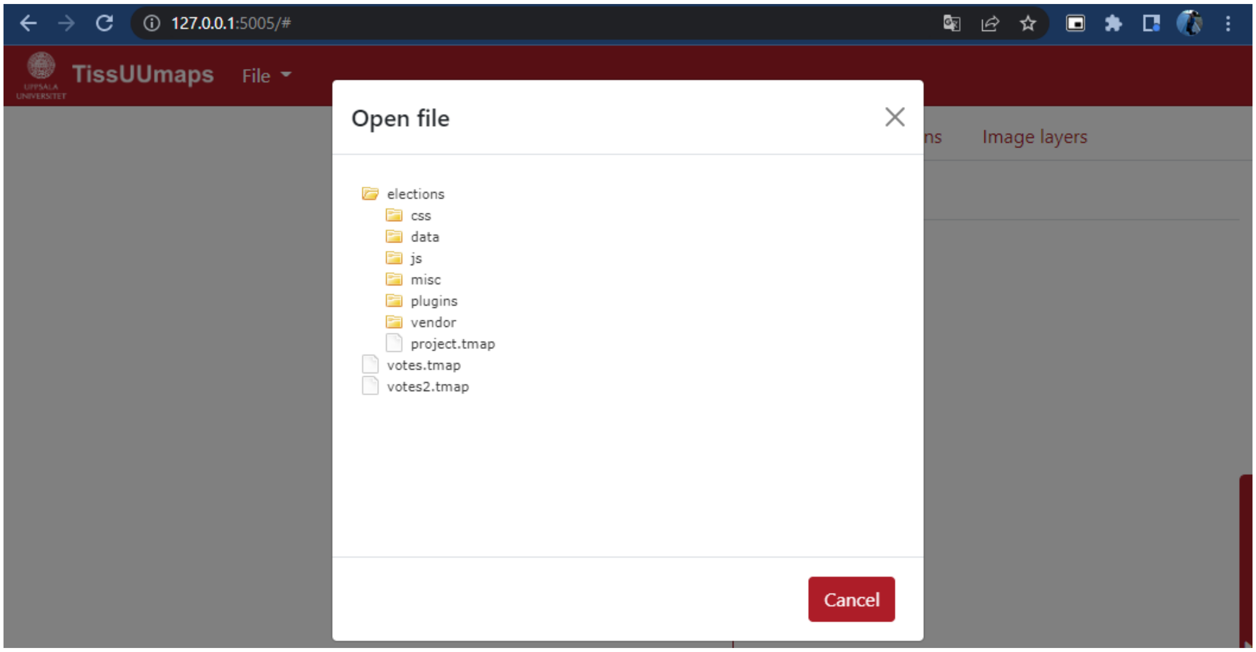
2.1.3 Load images using TissUUmaps server

If you are running TissUUmaps in server mode and not through the GUI, you must specify an image folder in the command line:

```
python -m tissuumaps "/home/username/Documents/myImages/" -p 5005
```

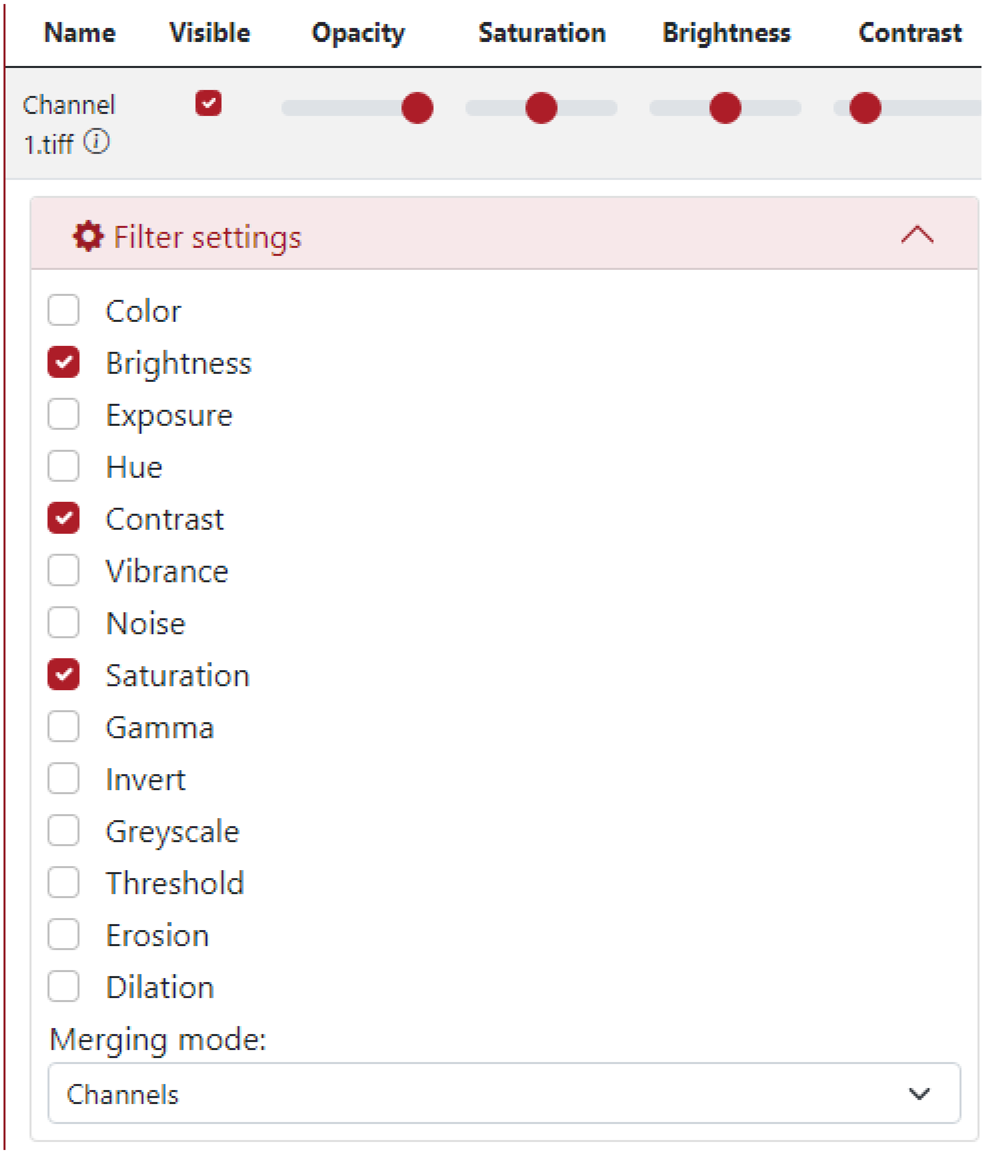
You can then access your images from your web browser by accessing the url http://localhost:5005, and using the `File > Open` menu.





2.1.4 Apply filters

You can apply several filters to the images. The ones we can be adjusted by default are saturation, brightness, and contrast. Additionally, when opening the Filter settings menu, there are various other filters, such as exposure, noise, erosion, etc. When you check their box, they are automatically added to the filter panel above. The filter's sliders can be adjusted so that the filter is applied at the desired intensity. Another option in filter settings is merging mode (bottom part), where you can merge the channels as a composite.



Name	Visible	Opacity	Saturation	Brightness	Contrast
Channel 1.tiff	☑				

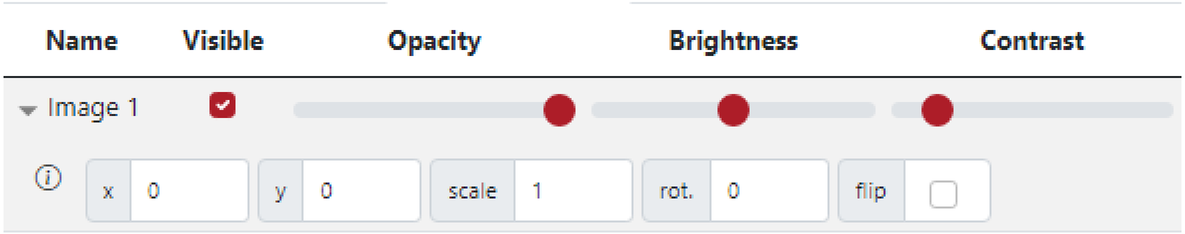
Filter settings

- ☐ Color
- ☑ Brightness
- ☐ Exposure
- ☐ Hue
- ☑ Contrast
- ☐ Vibrance
- ☐ Noise
- ☑ Saturation
- ☐ Gamma
- ☐ Invert
- ☐ Greyscale
- ☐ Threshold
- ☐ Erosion
- ☐ Dilation

Merging mode: Channels

2.1.5 Apply transformations

From the image list in the right menu, you can apply several transformations to each image individually: translation, scale, rotation and flip. Markers and regions linked to the image will be transformed accordingly.



Name	Visible	Opacity	Brightness	Contrast
Image 1	☑			

x: 0 · y: 0 · scale: 1 · rot.: 0 · flip: ☐

2.2 Markers

2.2.1 Supported marker format

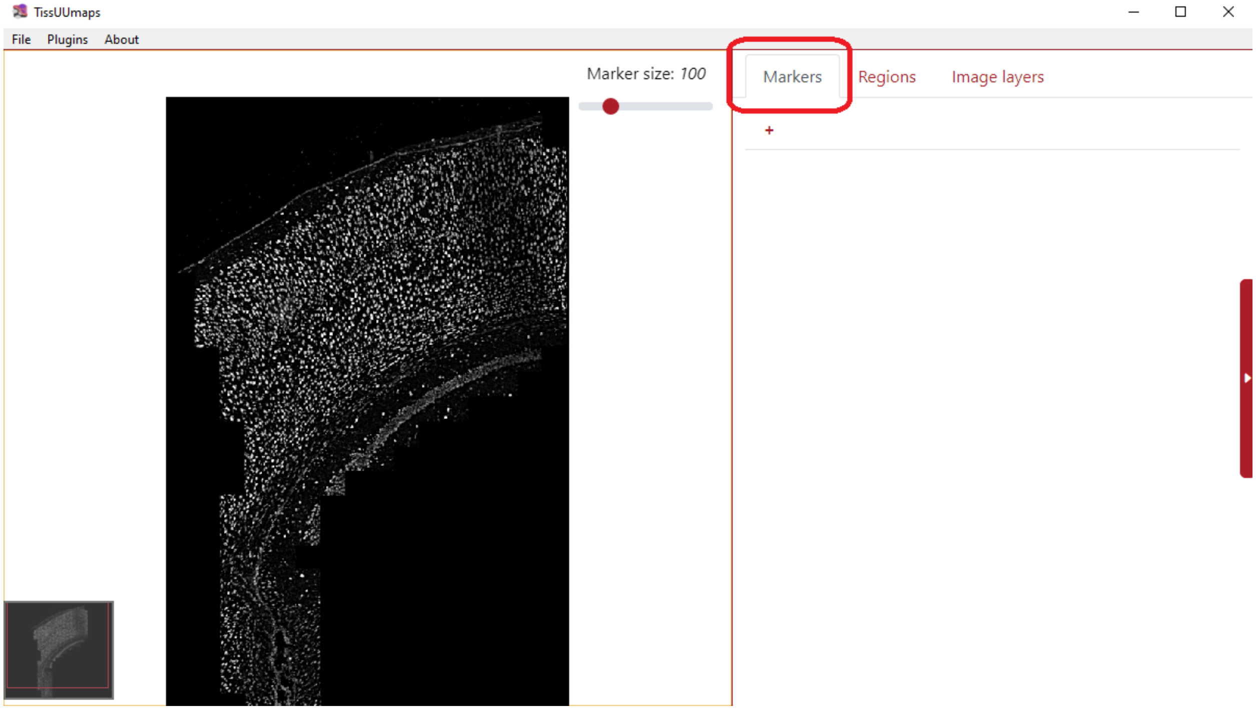
TissUUmaps can read CSV (Comma Separated Values) files with a header row, and at least spatial coordinate columns (X and Y). CSV files are not limited in the number of columns or number of rows. Other columns can contain information for displaying markers (key to group markers, color, size, shape, piecharts, etc.)

CSV files can be exported from any spreadsheet program, or any programming language (Python, R, etc.)

TissUUmaps can also read hdf5 files, which is a hierarchical file format commonly used in scientific computing. In the case of TissUUmaps, it is used to store the markers in a more efficient way than CSV files. TissUUmaps will give access to the markers stored in the hdf5 file in a similar way as for CSV files.

2.2.2 Load markers

You can load the markers when you select the *Markers* tab and click the button + as you can see in the figure below. You can click the plus several times to load various marker files.



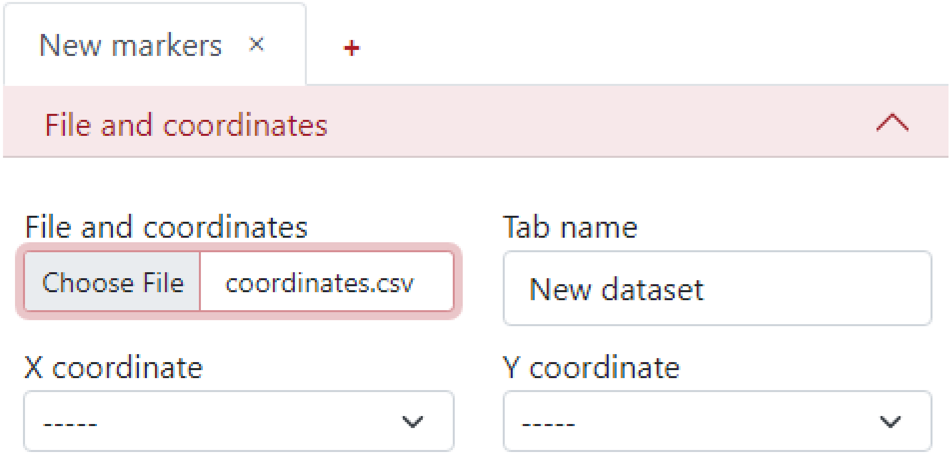
You can also load markers directly using drag and drop from a File Explorer if you are using the TissUUmaps GUI.

2.2.3 Markers settings

Before the markers are displayed you have to set up the markers settings.

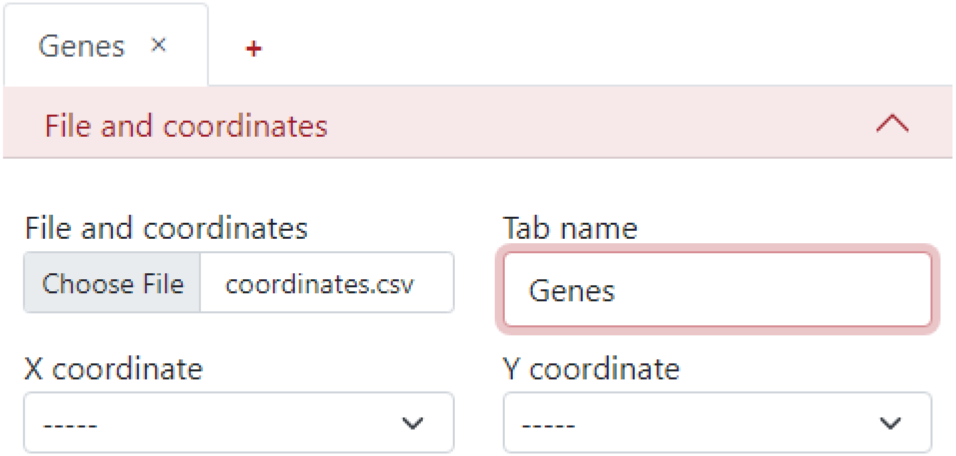
File and coordinates

The first step is to select the desired file from your computer under the tab *File and coordinates - Choose file*.



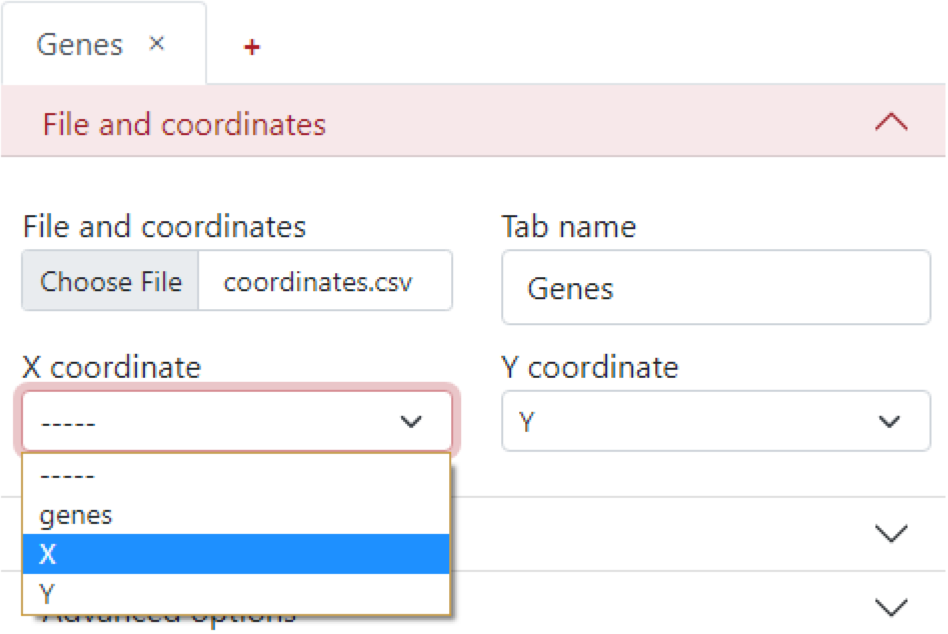
File and coordinates: coordinates.csv
Tab name: New dataset
X coordinate: -----
Y coordinate: -----

You can change the *Tab name* to the desired name, so it is easier to navigate between them when there are more tabs.



File and coordinates: coordinates.csv
Tab name: Genes
X coordinate: -----
Y coordinate: -----

The next step is to select the column names from the .csv file corresponding to the X and Y coordinates.



File and coordinates: Choose File coordinates.csv

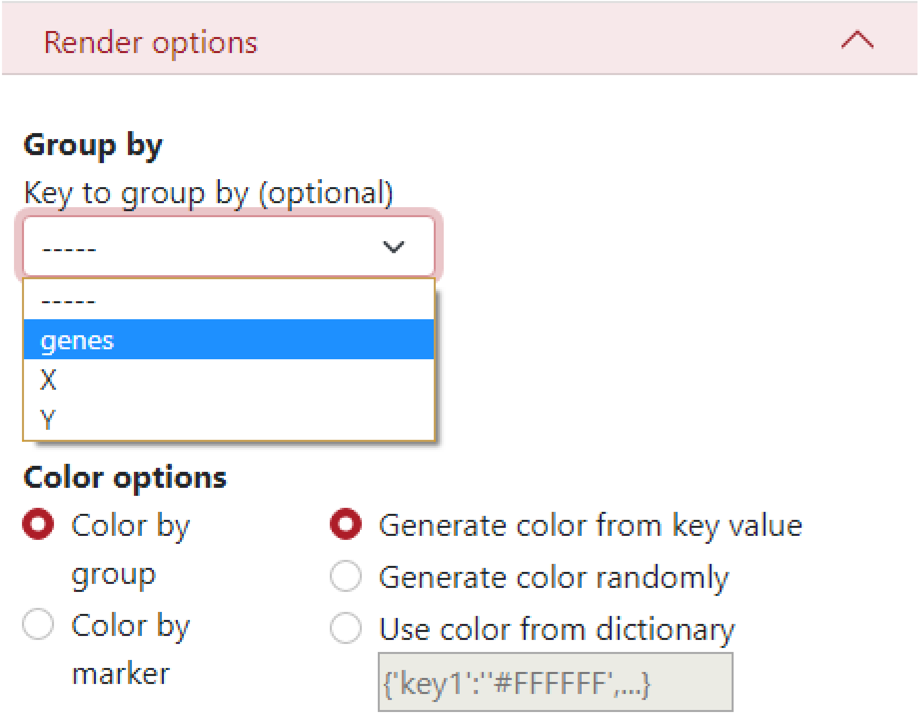
Tab name: Genes

X coordinate: -----
- -----
- genes
- X
- Y

Y coordinate: Y

Render options

Here, you can define a *key to group by*, what is a column from the .csv file which will be used to display the dataset grouped by different colors and shapes of the markers. In this example, we use the column *genes*, where different colors and shapes of markers represent different genes.



Group by

Key to group by (optional): -----
- -----
- genes
- X
- Y

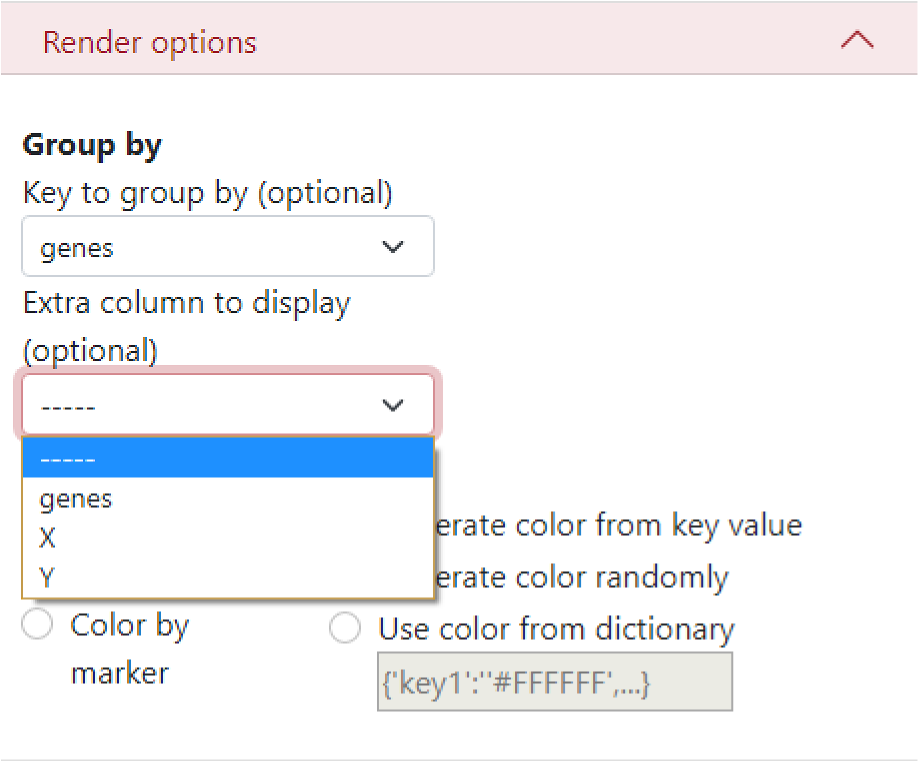
Color options

● Color by group
○ Color by marker

● Generate color from key value
○ Generate color randomly
○ Use color from dictionary

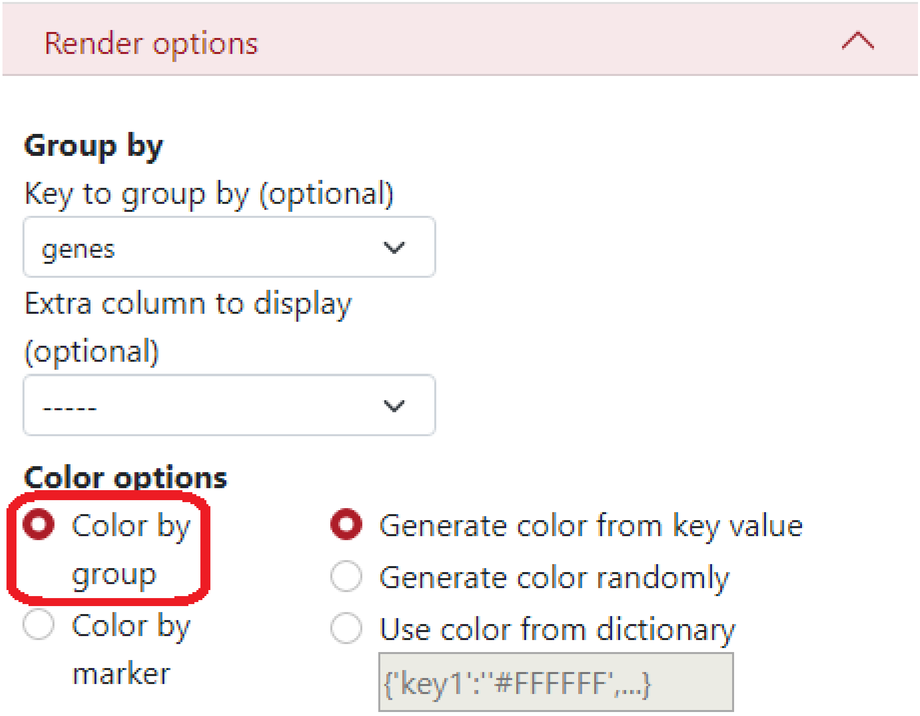
{'key1':'#FFFFFF',...}

There is an option to display an extra column, for example when the data are clustered but you want to see the original genes and also the cluster names.



In *Color options*, you can select to color by groups where each group has a different color. Then on the right side, you can select the color palette:

- Key value - Colors are generated from the name of the group (first 4 letters). Groups starting with the same letter have similar colors.
- Randomly - Colors are generated randomly.
- Dictionary - you can insert a specific dictionary in the text area which will be used for generating the colors.


Render options

Group by

Key to group by (optional): genes

Extra column to display (optional): -----

Color options

● Color by group

○ Color by marker

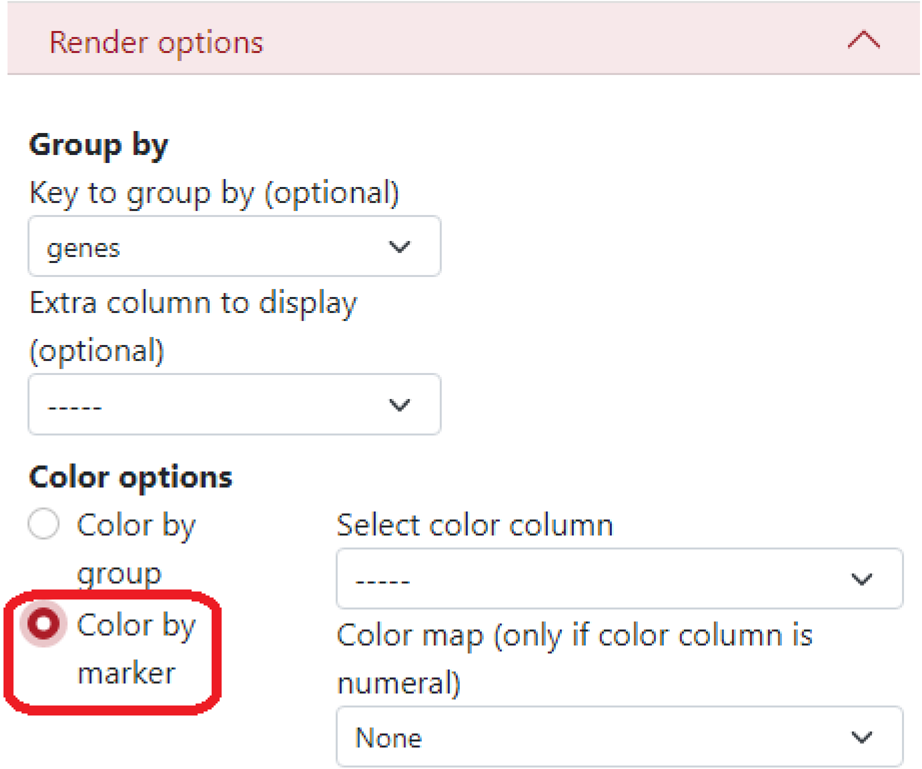
● Generate color from key value

○ Generate color randomly

○ Use color from dictionary

{'key1':"#FFFFFF',...}

If you want to *color by markers*, you have to select the column from the .csv file which will be used to create the colors, and the colormap, but only if the color column is numeral.


Render options

Group by

Key to group by (optional): genes

Extra column to display (optional): -----

Color options

○ Color by group

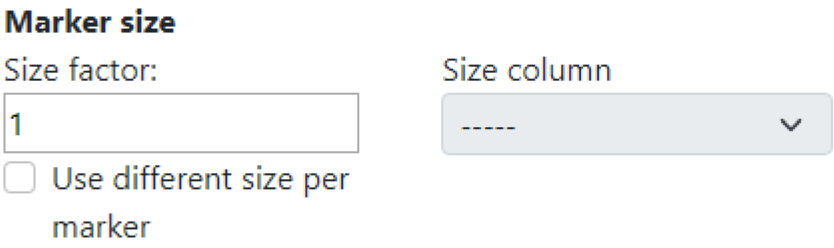
● Color by marker

Select color column: -----

Color map (only if color column is numeral): None

Advanced options

TissUUmaps tool contains also advanced options when working with the data. The first one is adjustable marker size. This is usually done in the right upper corner of the visualization panel. However, in the advanced setting, the user can change the size factor of the slider to any value.



Marker size

Size factor: 1

☐ Use different size per marker

Size column: -----

Additionally, there can be used a different size per marker based on a selected column. In the example below, I chose column *counts* which represents the number of counts in that cell (marker). This means that a larger marker represents a cell that contains more counts in it.

Marker size

Size factor: 0.05

Size column: counts

☑ Use different size per marker

Pie-charts

☐ Use pie-charts

Marker shape

● Shape by group

○ Shape by marker

○ Use a fixed shape

☐ Remove Outline

● Select shape iteratively

○ Use shape from dictionary

{'key1':"#FFFFFF',...}

Marker opacity

Opacity value: 1

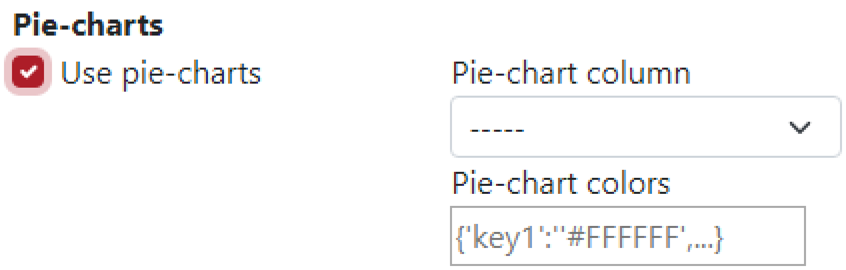
Opacity column: -----

☐ Use different opacity per marker

Marker tooltip

Format:

Another advanced option is the choice to display markers as pie-charts, it can represent the probability of that marker belonging to different groups. The user needs to select the *pie-chart column*, which contains mentioned probabilities for all the markers. All the probabilities for that specific marker need to be in a row divided by a semicolon.



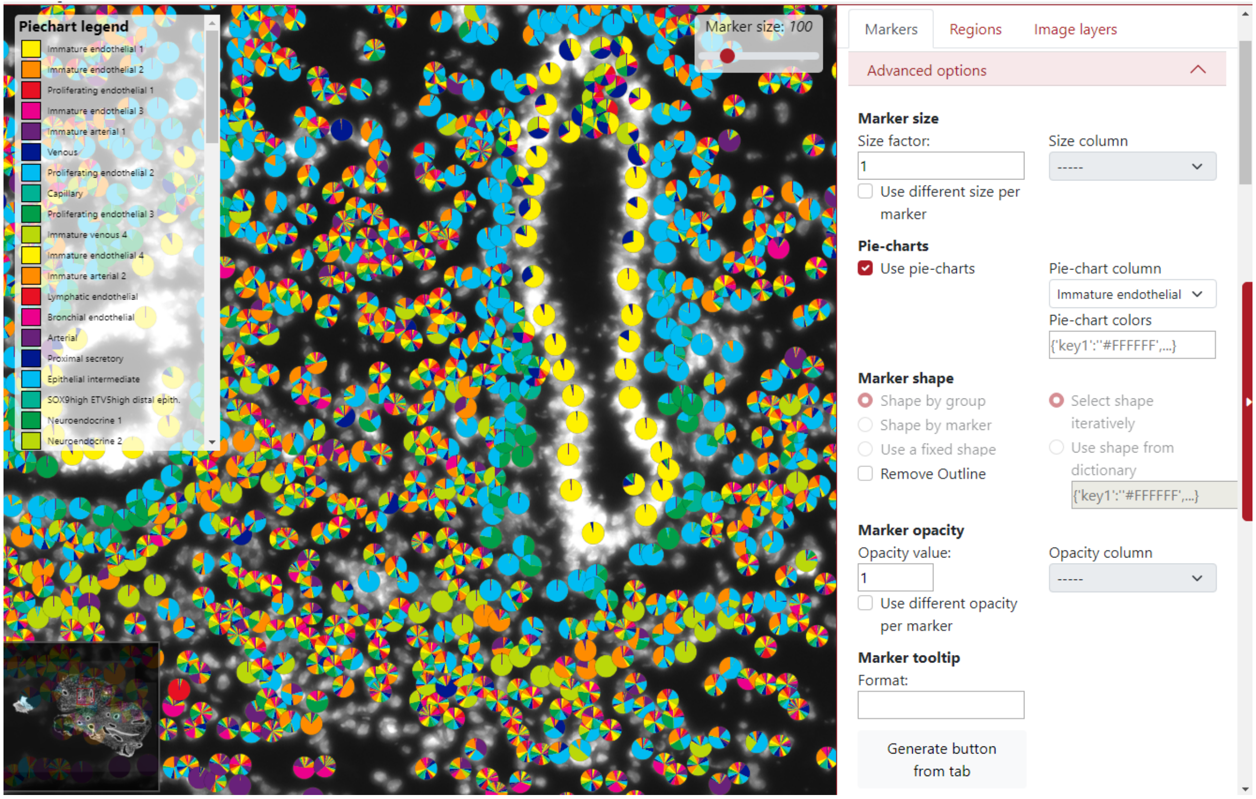
Pie-charts

☑ Use pie-charts

Pie-chart column: -----

Pie-chart colors: {'key1':"#FFFFFF',...}

In the example below the pie-charts represent the probability of the marker being of each cell type. In the left upper corner can be seen the legend of the cell types. By default, there are only 10 colors so the colors are used in the loop. This can be changed by using pie-chart colors from a dictionary.



Marker size: 100

Marker size

Size factor: 1

Size column: -----

☐ Use different size per marker

Pie-charts

☑ Use pie-charts

Pie-chart column: Immature endothelial

Pie-chart colors: {'key1':"#FFFFFF',...}

Marker shape

● Shape by group

○ Shape by marker

○ Use a fixed shape

☐ Remove Outline

● Select shape iteratively

○ Use shape from dictionary

{'key1':"#FFFFFF',...}

Marker opacity

Opacity value: 1

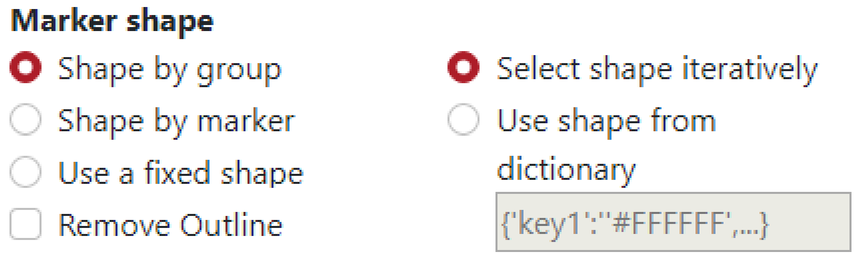
Opacity column: -----

☐ Use different opacity per marker

Marker tooltip

Format:

The shape of the markers can also be changed. By default, it is set up to be selected by the group, which means that each group has a different marker shape chosen from the list of shapes iteratively. The user can also pre-define the shapes from the dictionary to ensure visualization robustness in different sessions.



Marker shape

● Shape by group

○ Shape by marker

○ Use a fixed shape

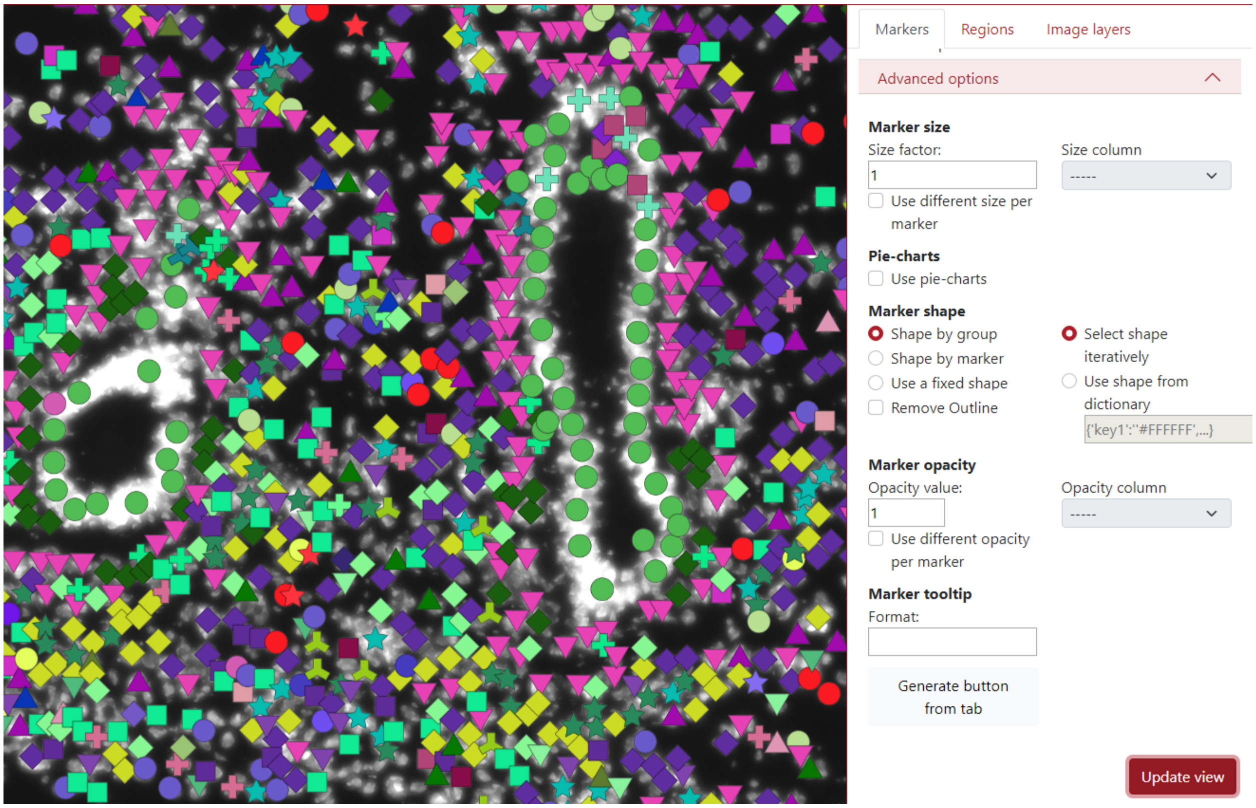
☐ Remove Outline

● Select shape iteratively

○ Use shape from dictionary

{'key1':"#FFFFFF',...}

In the example below each group has specific color as well as a specific marker shape.



Marker size

Size factor: 1

Size column: -----

☐ Use different size per marker

Pie-charts

☐ Use pie-charts

Marker shape

● Shape by group

○ Shape by marker

○ Use a fixed shape

☐ Remove Outline

● Select shape iteratively

○ Use shape from dictionary

{'key1':"#FFFFFF',...}

Marker opacity

Opacity value: 1

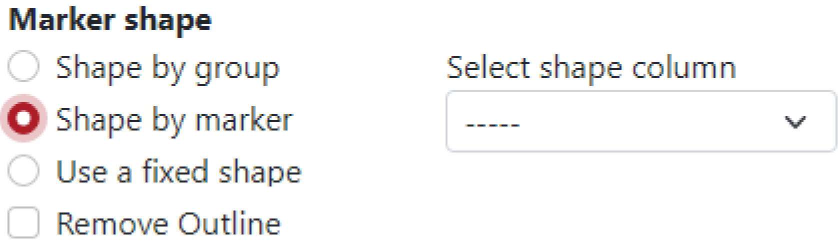
Opacity column: -----

☐ Use different opacity per marker

Marker tooltip

Format:

Another option for the marker shape is *shape by marker*. Here, the user needs to select a column with category values, and each category is used for a different shape.



Marker shape

○ Shape by group

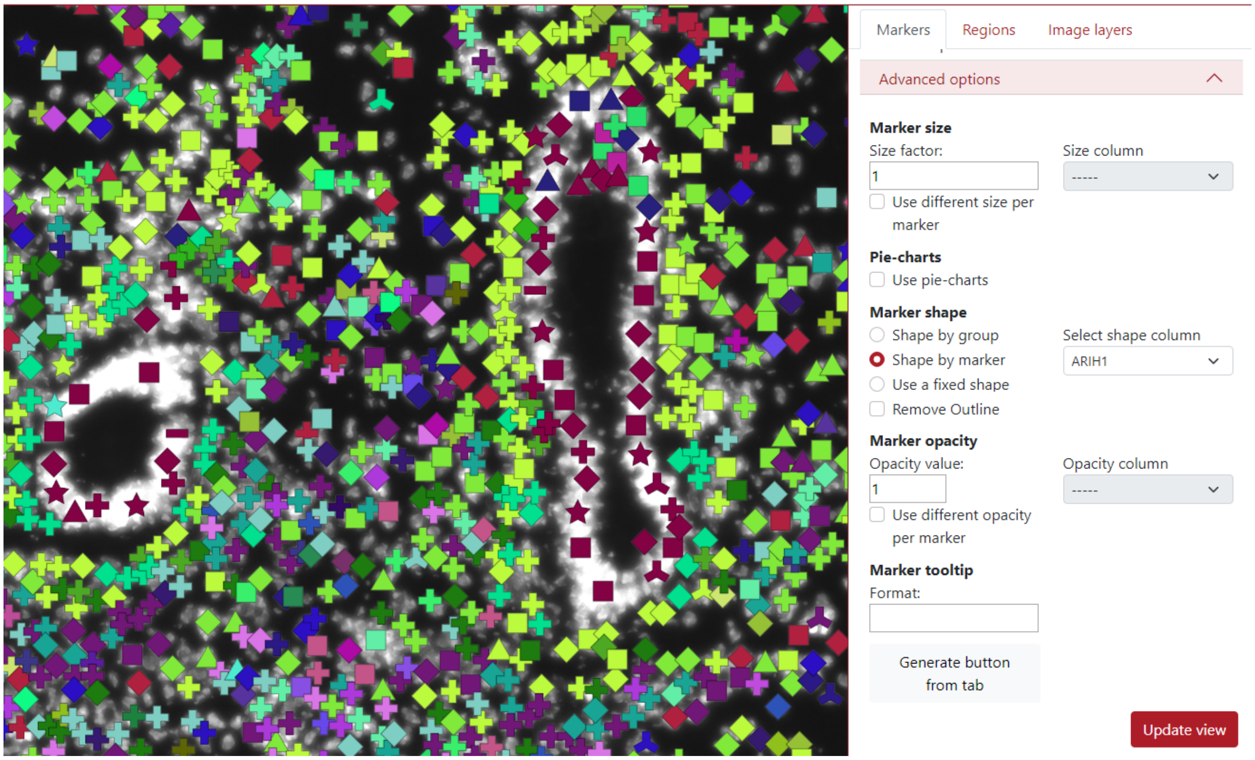
● Shape by marker

○ Use a fixed shape

☐ Remove Outline

Select shape column: -----

In the example below, the selected column is ARIH1, which contains 10 categories, so you can see that there are 10 shapes in the visualization.



Marker size

Size factor: 1

Size column: -----

☐ Use different size per marker

Pie-charts

☐ Use pie-charts

Marker shape

○ Shape by group

● Shape by marker

○ Use a fixed shape

☐ Remove Outline

Select shape column: ARIH1

Marker opacity

Opacity value: 1

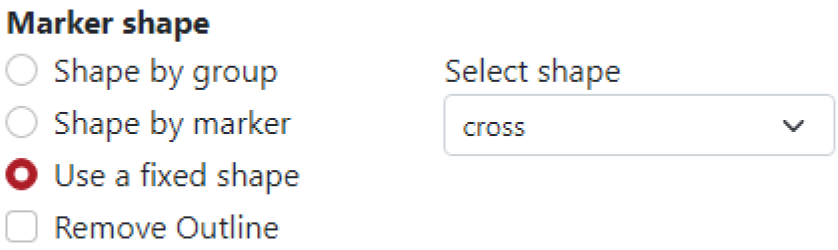
Opacity column: -----

☐ Use different opacity per marker

Marker tooltip

Format:

The third option in the marker shape is to *use a fixed shape*. This can be used if the user is not happy with all the different marker shapes and wants to make it homogeneous.



Marker shape

○ Shape by group

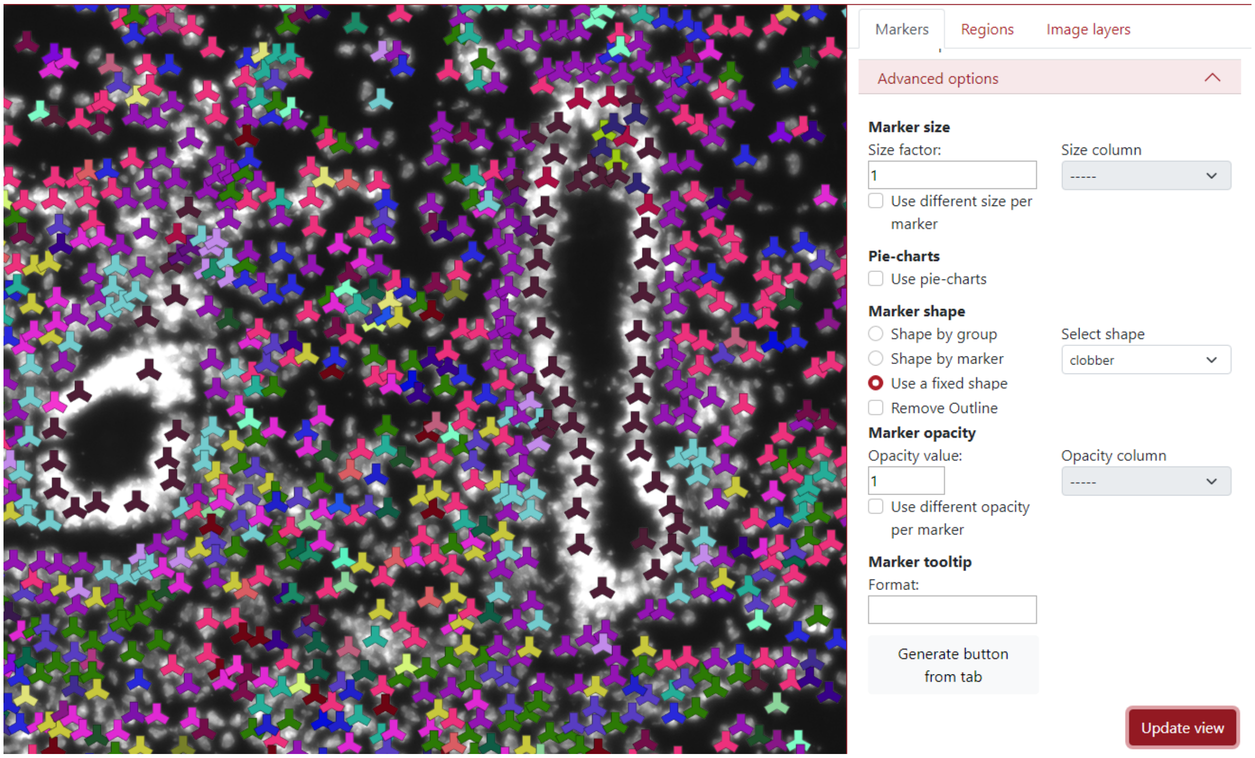
○ Shape by marker

● Use a fixed shape

☐ Remove Outline

Select shape: cross

In the example below, the selected shape is a clobber and you can see that all the markers are in the shape of a clobber.



Marker size

Size factor: 1

Size column: -----

☐ Use different size per marker

Pie-charts

☐ Use pie-charts

Marker shape

○ Shape by group

○ Shape by marker

● Use a fixed shape

☐ Remove Outline

Select shape: clobber

Marker opacity

Opacity value: 1

Opacity column: -----

☐ Use different opacity per marker

Marker tooltip

Format:

The last option in the marker shape is to *remove outline*. This can be used to remove the dark outline of markers when the check box is checked.

Marker shape

● Shape by group

○ Shape by marker

○ Use a fixed shape

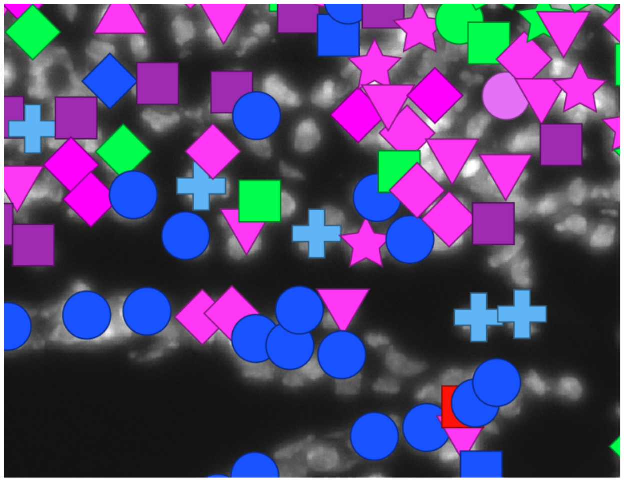
☑ Remove Outline

● Select shape iteratively

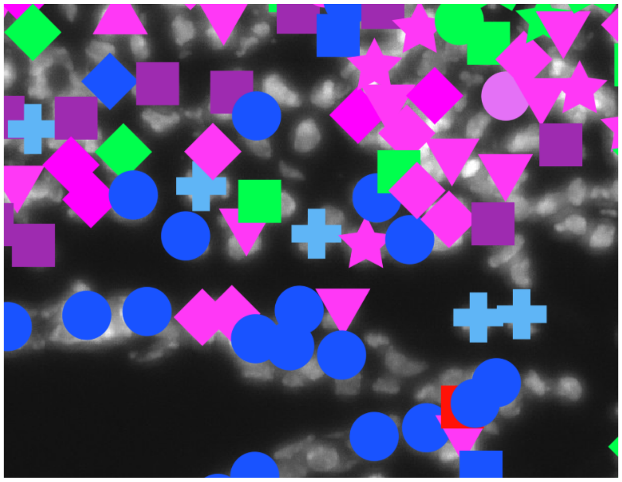
○ Use shape from dictionary

{'key1':"#FFFFFF',...}

In the example below, the outline is included on the left side and the outline is removed on the right side.

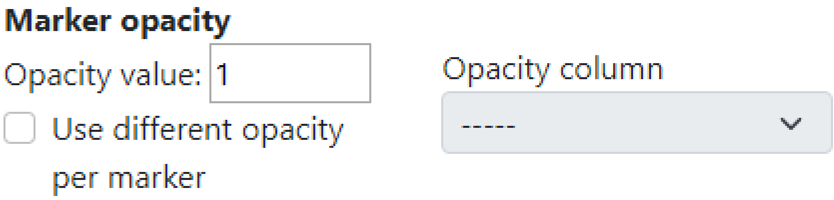


☐ Remove Outline



☑ Remove Outline

The next advanced option is *marker opacity* which is adjustable. The user can change the opacity in order to display things underneath.



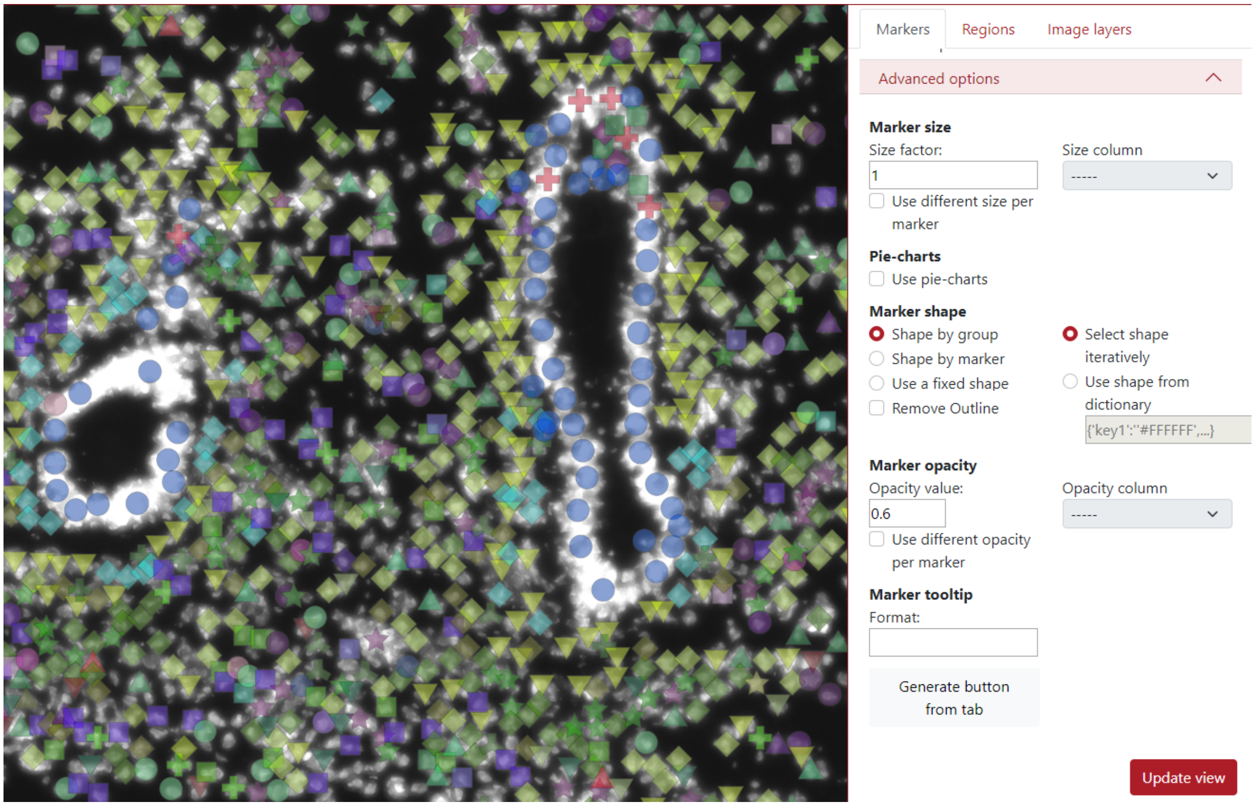
Marker opacity

Opacity value: 1

☐ Use different opacity per marker

Opacity column: -----

In the example below, the opacity value was set to 0.6 which made the markers a bit transparent.



Marker size

Size factor: 1

☐ Use different size per marker

Size column: -----

Pie-charts

☐ Use pie-charts

Marker shape

● Shape by group

○ Shape by marker

○ Use a fixed shape

☐ Remove Outline

● Select shape iteratively

○ Use shape from dictionary

{'key1':"#FFFFFF',...}

Marker opacity

Opacity value: 0.6

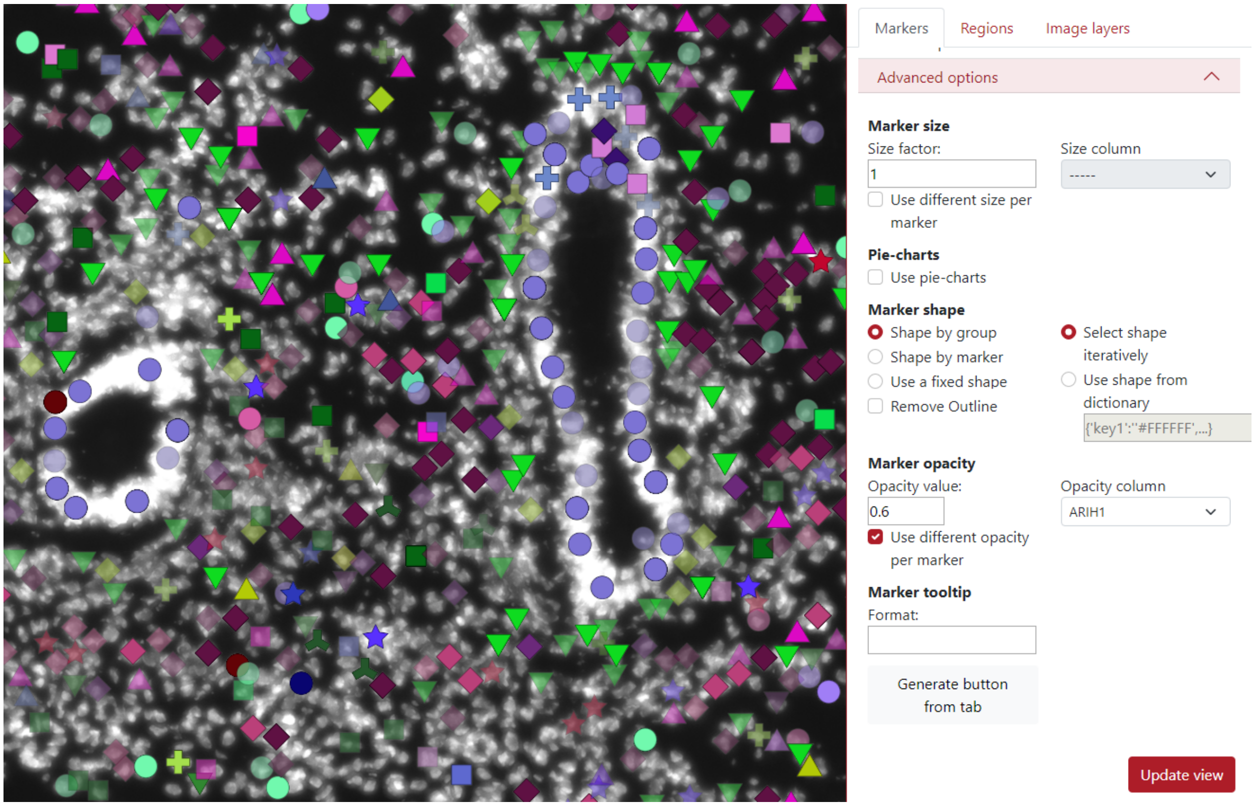
☐ Use different opacity per marker

Opacity column: -----

Marker tooltip

Format:

In the example below, we checked *use different opacity per marker*. The user needs to select opacity column which will be used for displaying different opacities in markers.



Marker size

Size factor: 1

Size column: -----

☐ Use different size per marker

Pie-charts

☐ Use pie-charts

Marker shape

● Shape by group
○ Shape by marker
○ Use a fixed shape
☐ Remove Outline

● Select shape iteratively
○ Use shape from dictionary
{'key1':"#FFFFFF',...}

Marker opacity

Opacity value: 0.6

Opacity column: ARIH1

☑ Use different opacity per marker

Marker tooltip

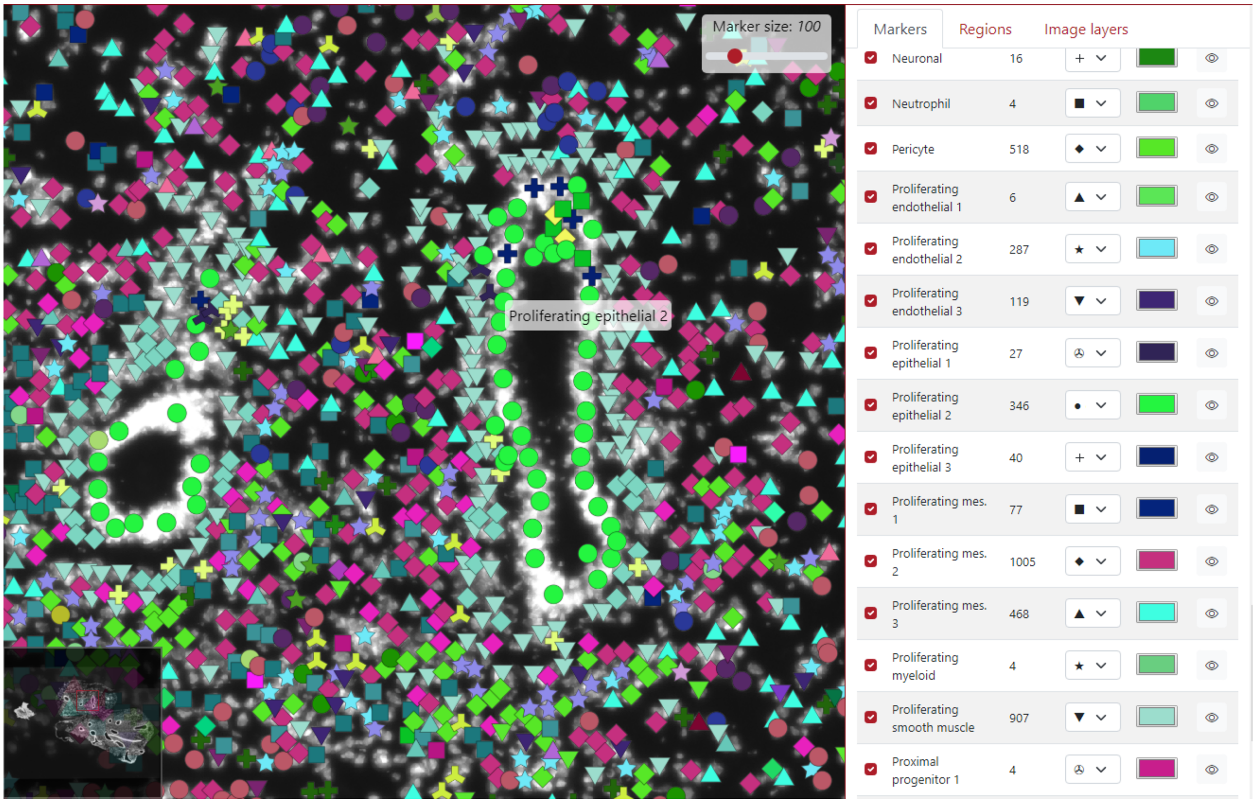
Format:

The next option is the *marker tooltip*, which is the text which is displayed when the user clicks on the marker. By default, it displayed the key group the marker belongs to.

Marker tooltip

Format:

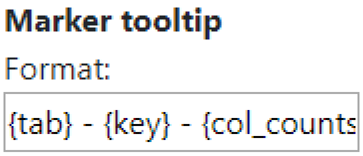
As you can see in the example below, the green marker we clicked on belongs to the cell type Proliferating epithelial 2.



Marker size: 100

☑	Neuronal	16	+	🟩
☑	Neutrophil	4	■	🟩
☑	Pericyte	518	◆	🟩
☑	Proliferating endothelial 1	6	▲	🟩
☑	Proliferating endothelial 2	287	★	🟦
☑	Proliferating endothelial 3	119	▼	🟪
☑	Proliferating epithelial 1	27	✇	🟪
☑	Proliferating epithelial 2	346	●	🟩
☑	Proliferating epithelial 3	40	+	🟦
☑	Proliferating mes. 1	77	■	🟦
☑	Proliferating mes. 2	1005	◆	🟥
☑	Proliferating mes. 3	468	▲	🟦
☑	Proliferating myeloid	4	★	🟩
☑	Proliferating smooth muscle	907	▼	🟩
☑	Proximal progenitor 1	4	✇	🟥

However, this can be modified by writing text into the text area. In this example, we wrote {tab} - {key} - {col_counts}. *{tab}* represents the tab name that we set when we loaded the markers, *{key}* represents the key group to which the marker belongs and *{col_counts}* represents the value of that marker in the column called counts. The word counts can be replaced by any column name in order to display it on the tooltip.

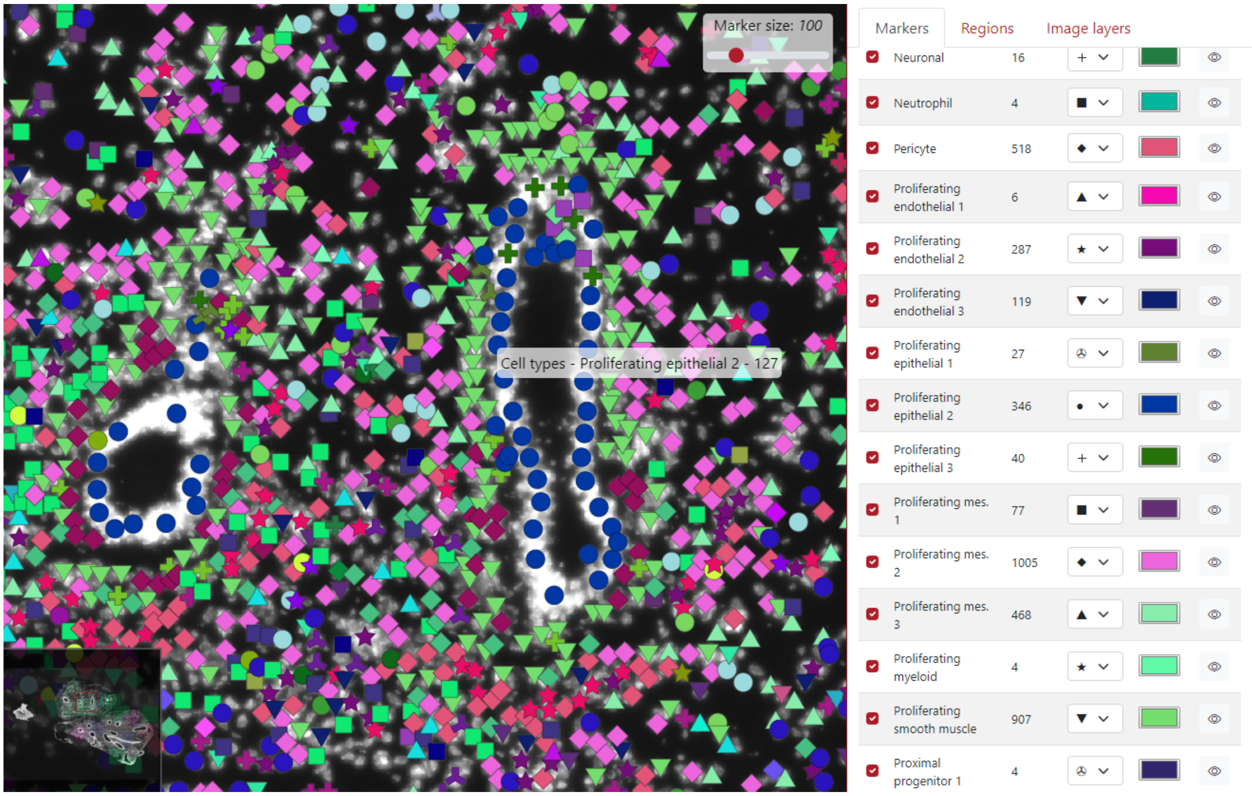


Marker tooltip

Format:

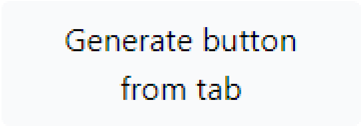
{tab} - {key} - {col_counts

In the example below, the green dot which we clicked on is from the tab Cell types, belongs to the cell type Proliferating epithelial 2 and it has 127 counts in it.

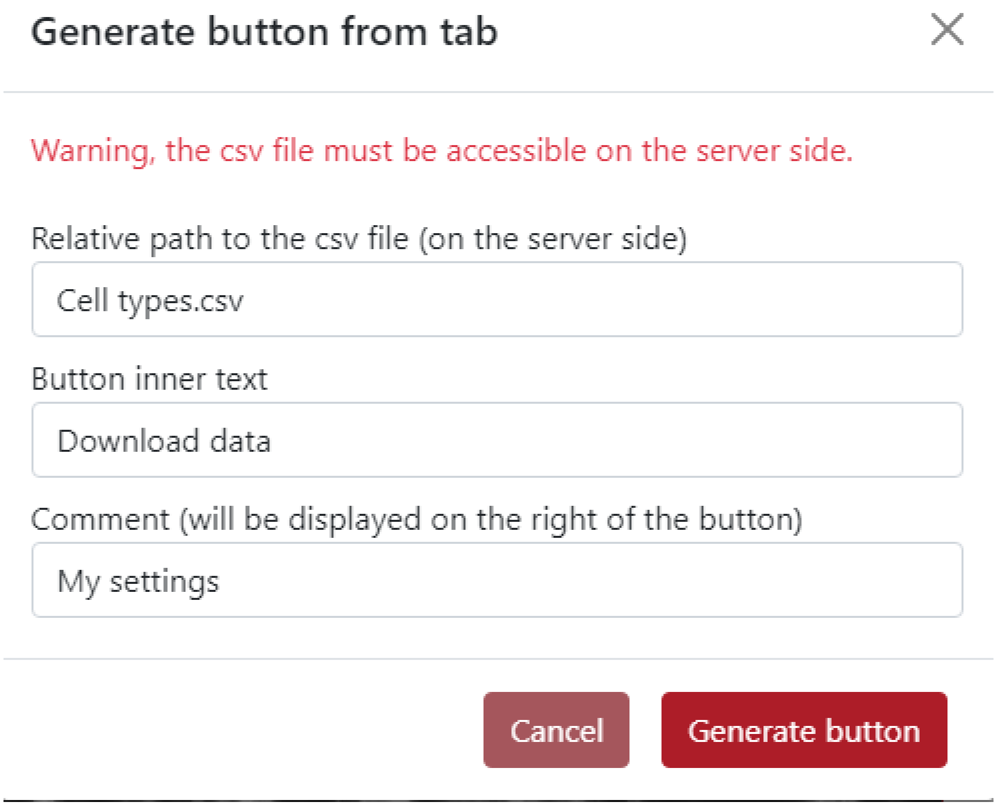


☑	Neuronal	16	+
☑	Neutrophil	4	■
☑	Pericyte	518	◆
☑	Proliferating endothelial 1	6	▲
☑	Proliferating endothelial 2	287	★
☑	Proliferating endothelial 3	119	▼
☑	Proliferating epithelial 1	27	☮
☑	Proliferating epithelial 2	346	●
☑	Proliferating epithelial 3	40	+
☑	Proliferating mes. 1	77	■
☑	Proliferating mes. 2	1005	◆
☑	Proliferating mes. 3	468	▲
☑	Proliferating myeloid	4	★
☑	Proliferating smooth muscle	907	▼
☑	Proximal progenitor 1	4	☮

The last advanced option is the button *Generate button from tab*. This button incorporates all the display settings the user set up into a single button.



The user can choose the relative path to the csv file, button inner text and comment which will be displayed next to the button.



Generate button from tab

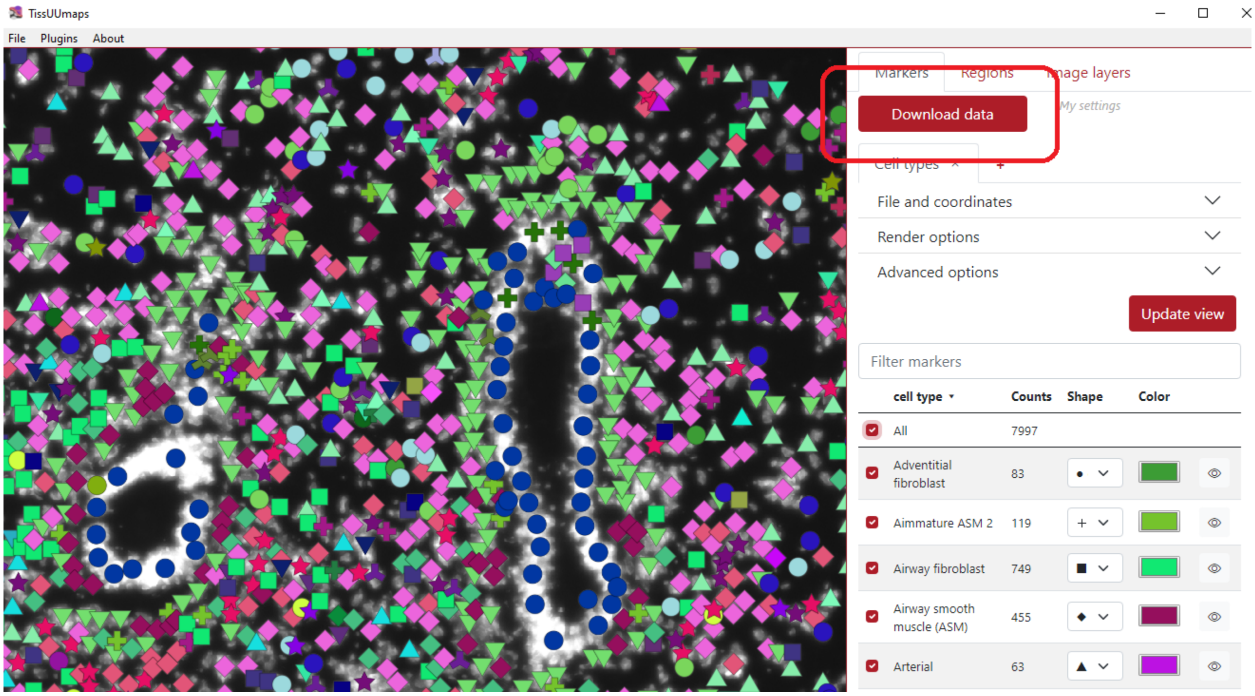
Warning, the csv file must be accessible on the server side.

Relative path to the csv file (on the server side): Cell types.csv

Button inner text: Download data

Comment (will be displayed on the right of the button): My settings

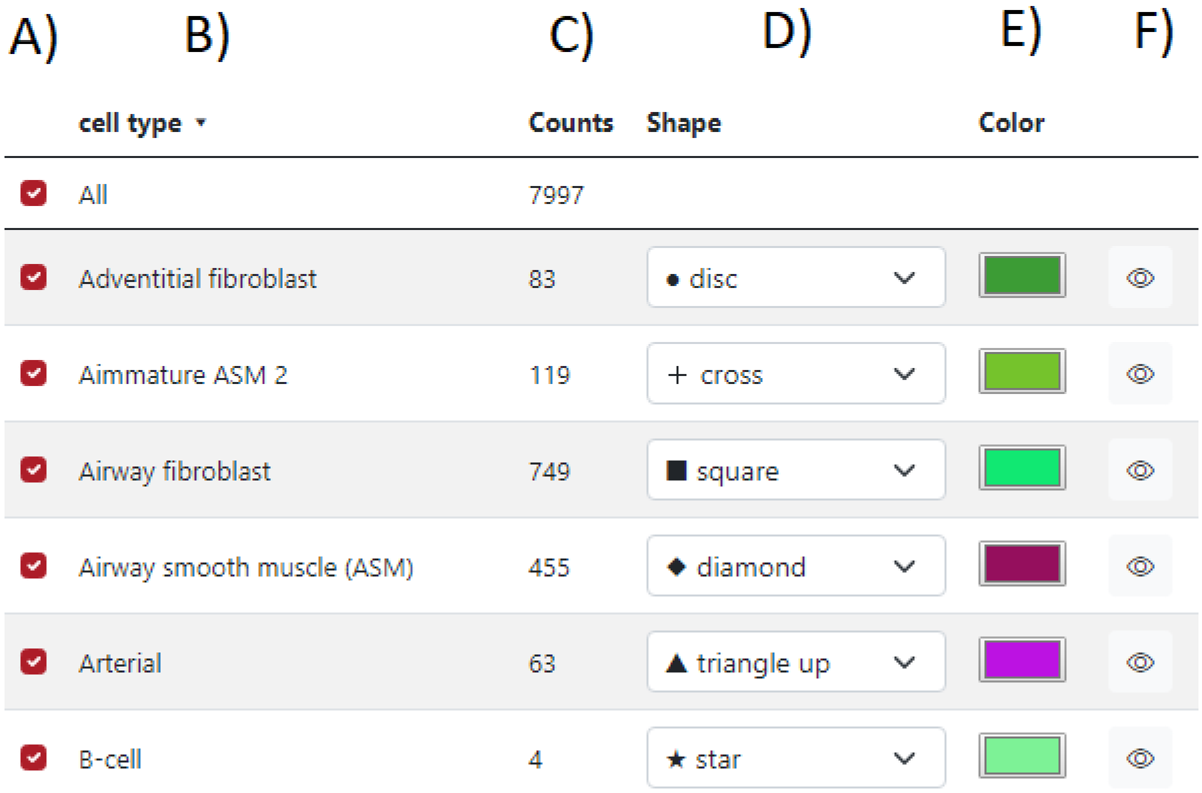
In the example below, you can see the generated button *Download data* placed on the top of the tabs panel. On the right of the button is placed text *My settings*.



	cell type	Counts	Shape	Color	
☑	All	7997			
☑	Adventitial fibroblast	83	●	🟩	👁
☑	Aimmature ASM 2	119	+	🟩	👁
☑	Airway fibroblast	749	■	🟩	👁
☑	Airway smooth muscle (ASM)	455	◆	🟥	👁
☑	Arterial	63	▲	🟪	👁

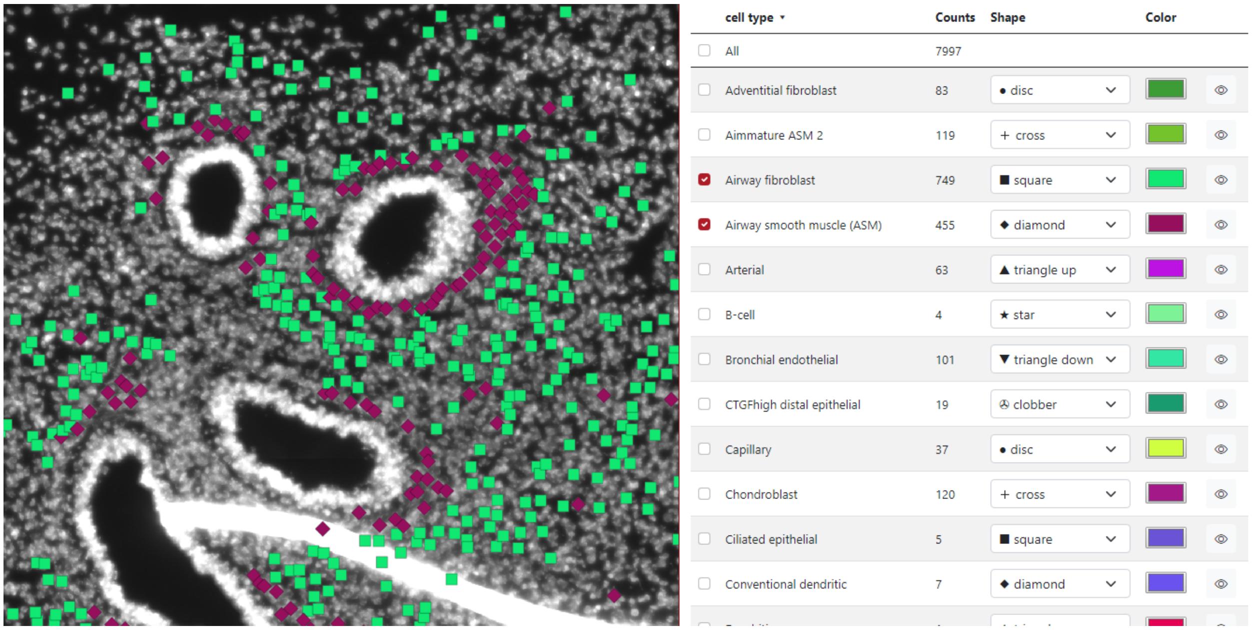
Table of markers

When the markers are loaded, a table of markers will appear in order to interact with the marker. Each row represents a group of markers with a specific color and shape. In the figure below, column *A)* represents if a specific row of markers is displayed or not, the second column *B)* represents the list of groups, the third column *C)* represents group counts, the fourth column *D)* represents the shape of the group markers, the fifth column *E)* represents the color of the group markers and the sixth column *F)* can display specific group when the cursor is on the eye icon.



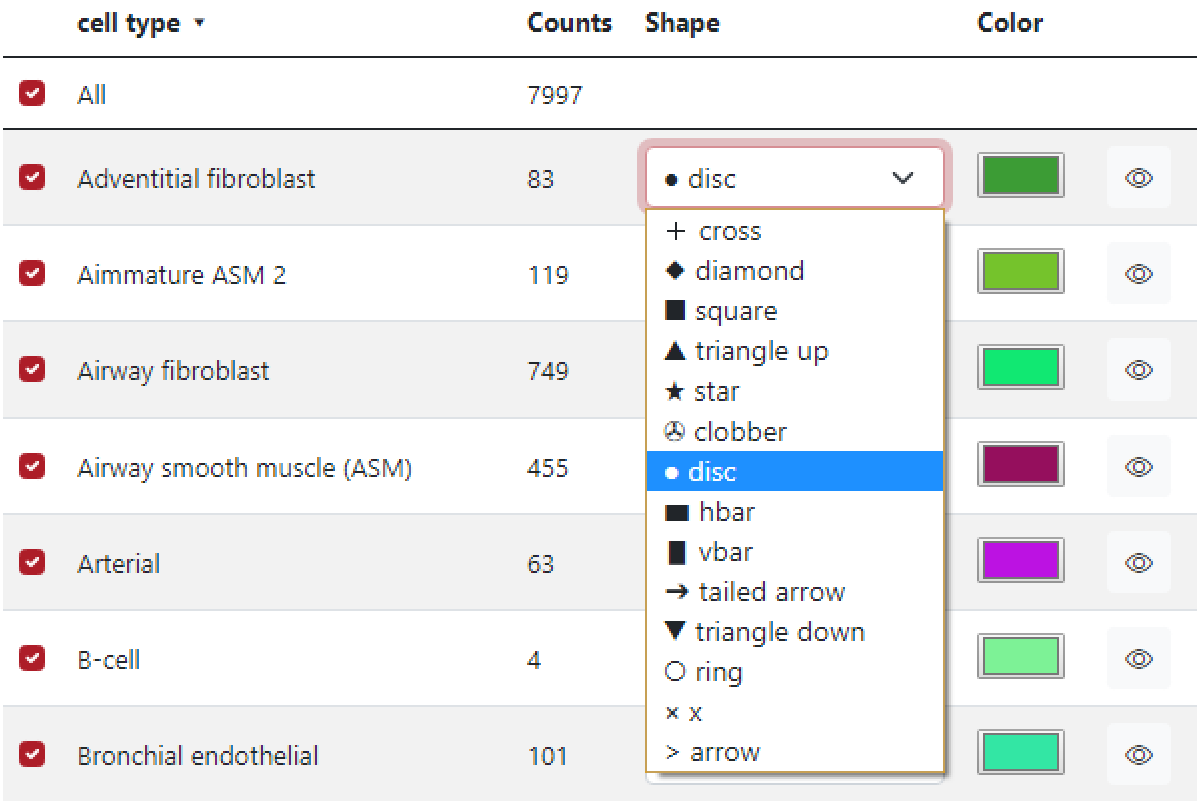
	cell type ▾	Counts	Shape	Color	
☑	All	7997			
☑	Adventitial fibroblast	83	● disc	🟩	👁
☑	Aimmature ASM 2	119	+ cross	🟩	👁
☑	Airway fibroblast	749	■ square	🟩	👁
☑	Airway smooth muscle (ASM)	455	◆ diamond	🟥	👁
☑	Arterial	63	▲ triangle up	🟪	👁
☑	B-cell	4	★ star	🟩	👁

If the check box is checked - the group is displayed, if the check box is unchecked - the group is not displayed. In the example below, we checked two groups of cell types: Airway Fibroblast and Airway smooth muscle, and only these two groups are displayed on the left visualization panel. The first checkbox *All* ensures displaying of all the markers.



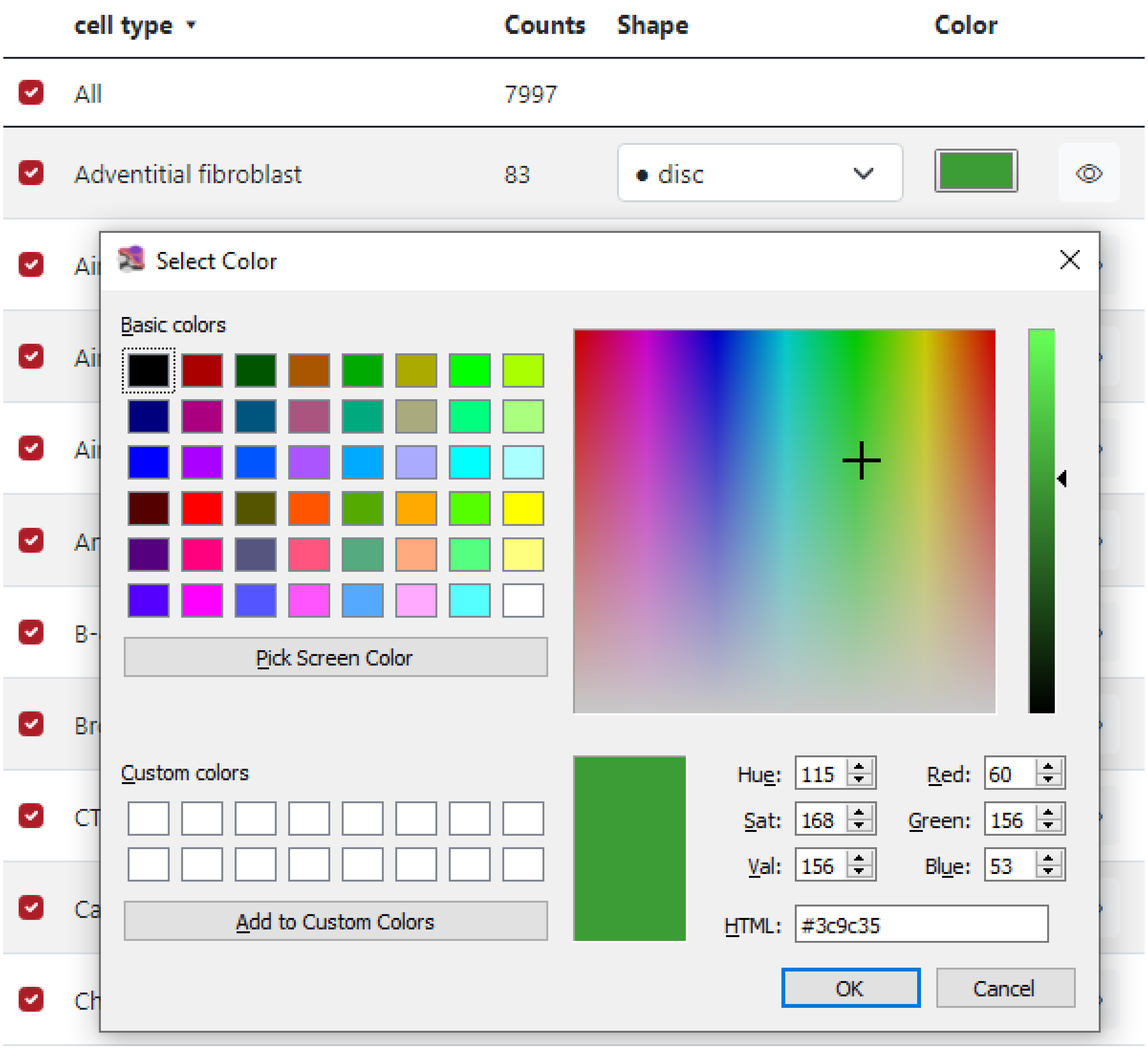
☐	cell type ▾	Counts	Shape	Color	
☐	All	7997			
☐	Adventitial fibroblast	83	● disc	🟩	👁
☐	Aimmature ASM 2	119	+ cross	🟩	👁
☑	Airway fibroblast	749	■ square	🟩	👁
☑	Airway smooth muscle (ASM)	455	◆ diamond	🟥	👁
☐	Arterial	63	▲ triangle up	🟪	👁
☐	B-cell	4	★ star	🟩	👁
☐	Bronchial endothelial	101	▼ triangle down	🟩	👁
☐	CTGFhigh distal epithelial	19	✇ clobber	🟩	👁
☐	Capillary	37	● disc	🟨	👁
☐	Chondroblast	120	+ cross	🟪	👁
☐	Ciliated epithelial	5	■ square	🟪	👁
☐	Conventional dendritic	7	◆ diamond	🟪	👁

In the fourth column *Shape*, the user can select which shape is preferred for each marker group. In the figure below, there is a list of 14 different shapes which can be used.



☑	cell type ▾	Counts	Shape	Color	
☑	All	7997			
☑	Adventitial fibroblast	83	● disc	🟩	👁
☑	Aimmature ASM 2	119		🟩	👁
☑	Airway fibroblast	749		🟩	👁
☑	Airway smooth muscle (ASM)	455		🟥	👁
☑	Arterial	63		🟪	👁
☑	B-cell	4		🟩	👁
☑	Bronchial endothelial	101		🟩	👁

In the fifth column *Color*, the user can select which color is preferred for each marker group. In the figure below, it is possible to choose from some list of basic colors, select a specific color by the cursor from the palette and also use numbers to generate color, either RGB, HSV, or HTML.

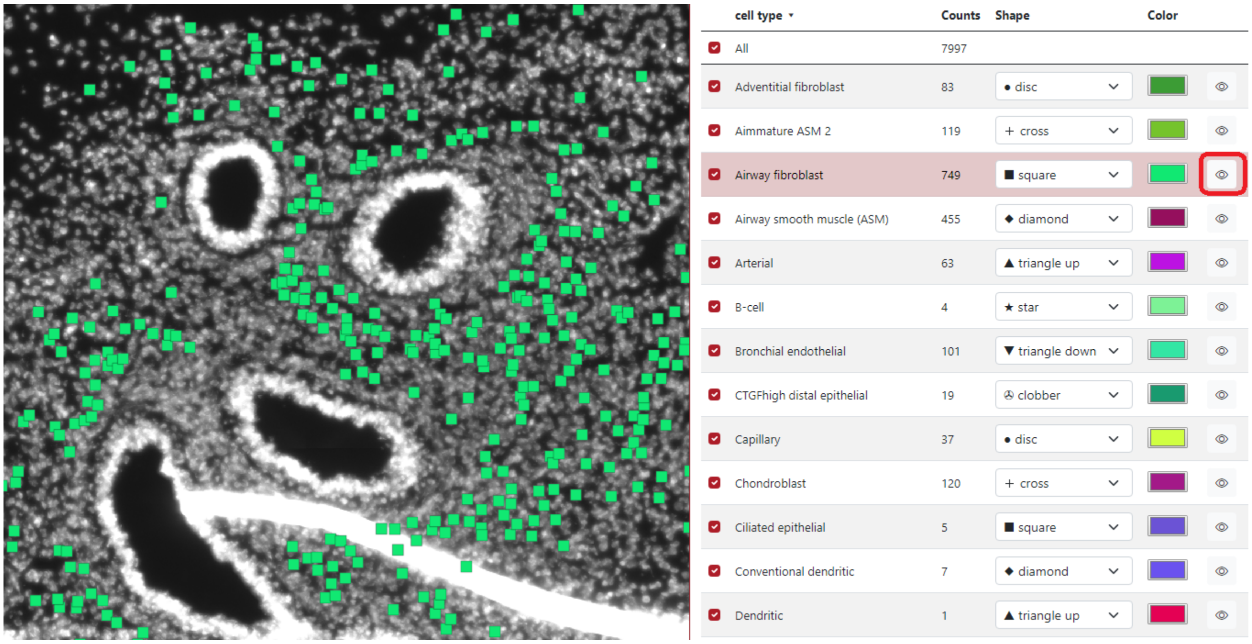


	cell type ▾	Counts	Shape	Color	
☑	All	7997			
☑	Adventitial fibroblast	83	● disc	🟩	👁
☑	Ai				
☑	Ai				
☑	Ai				
☑	Ar				
☑	B-				
☑	Br				
☑	CT				
☑	Ca				
☑	Ch				

Select Color

Hue: 115
Sat: 168
Val: 156
Red: 60
Green: 156
Blue: 53
HTML: #3c9c35

In the example below can be seen that if the cursor is placed on the eye icon in the row Airway fibroblast, only markers of this group are displayed on the visualization panel.



	cell type ▾	Counts	Shape	Color	
☑	All	7997			
☑	Adventitial fibroblast	83	● disc	🟩	👁
☑	Aimmature ASM 2	119	+ cross	🟩	👁
☑	Airway fibroblast	749	■ square	🟩	👁
☑	Airway smooth muscle (ASM)	455	◆ diamond	🟥	👁
☑	Arterial	63	▲ triangle up	🟪	👁
☑	B-cell	4	★ star	🟩	👁
☑	Bronchial endothelial	101	▼ triangle down	🟩	👁
☑	CTGFhigh distal epithelial	19	✇ clobber	🟩	👁
☑	Capillary	37	● disc	🟨	👁
☑	Chondroblast	120	+ cross	🟪	👁
☑	Ciliated epithelial	5	■ square	🟪	👁
☑	Conventional dendritic	7	◆ diamond	🟦	👁
☑	Dendritic	1	▲ triangle up	🟥	👁

2.3 Regions

2.3.1 Supported region formats

TissUUmaps can read and write region files in the GeoJSON format.

Only a subset of the GeoJSON format is supported, as TissUUmaps uses only polygonal regions:

Main types:

- Feature
- FeatureCollection
- GeometryCollection

Geometries:

- Polygon
- Multipolygon

The coordinate system must be the same as the image and marker coordinate systems.

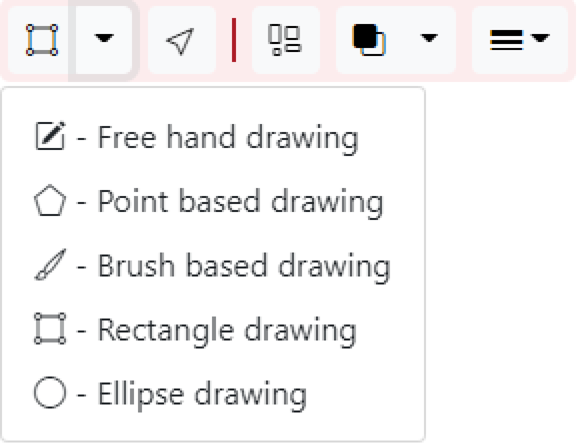
TissUUmaps is a powerful annotation tool designed to facilitate the annotation process for biological tissues.

2.3.2 Toolbar Features

When entering the Regions tab, the user will see the following toolbar:

Drawing Tools

When the user clicks on the drawing tool, a dropdown menu will appear with five different drawing tools:



1. Free Hand Drawing

The free hand drawing tool allows users to annotate regions with free-form shapes.

2. Point-Based Drawing

Use the point-based drawing tool to create annotations by placing individual points.

3. Brush-Based Drawing

The brush-based drawing tool enables users to draw annotations using a brush-like tool. Press `Shift` to erase and `Ctrl` to add to selected region.

4. Rectangle Drawing

Create rectangular annotations by selecting the rectangle drawing tool. Pressing Shift while dragging makes it a square, and Ctrl centers it around the cursor.

5. Ellipse Drawing

Similar to the rectangle tool, the ellipse drawing tool allows users to create ellipses. Press Shift for a circle and Ctrl for centering.

Other Tools

- **Selection Tool**: Click on regions to select them. Press Shift to select multiple regions.
- **Show Instance**: Color each region randomly to distinguish between polygons.
- **Fill Opacity**: Control if regions are filled, and the opacity of the filling.
- **Line Width**: Adjust line width and determine if it adapts when zooming.

Selected Region Tools

When a region is selected, additional tools become available:

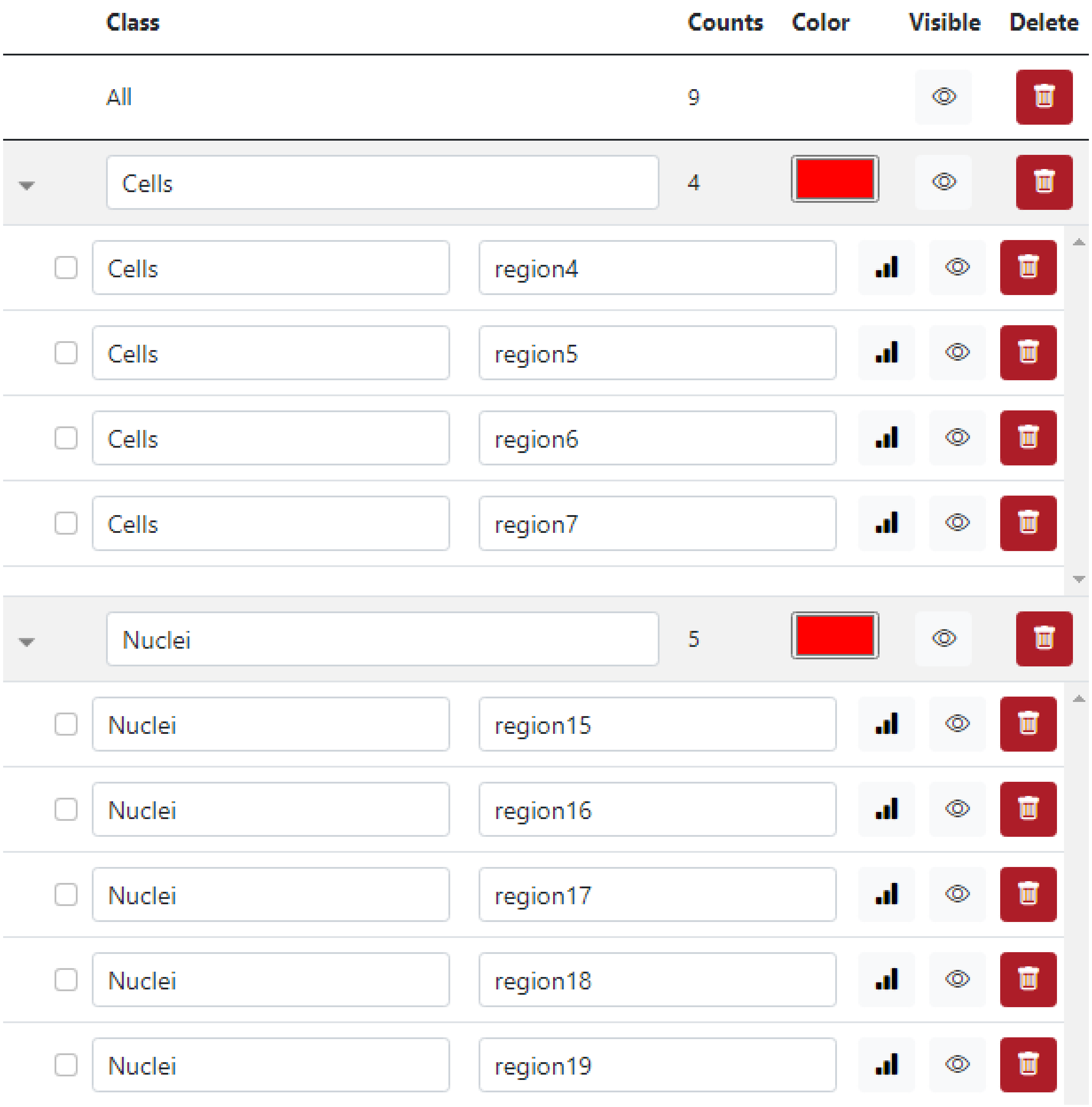
- **Zoom to Selected Regions**
- **Unselect All Regions (Shortcut: Escape)**
- **Delete Selected Region**
- **Duplicate Region**
- **Scale Region**
- **Erode/Dilate Regions**
- **Split Multipolygons into Multiple Regions**
- **Fill Holes in Regions**

Multiple Selected Regions

When multiple regions are selected, access the "Boolean Operation" dropdown with options like:

- **Merge Selected Regions**
- **XOR Selected Regions**
- **Intersect Selected Regions**

2.3.3 List of regions in the right menu



	Class		Counts	Color	Visible	Delete
	All		9			
	Cells		4	🟥		
☐	Cells	region4				
☐	Cells	region5				
☐	Cells	region6				
☐	Cells	region7				
	Nuclei		5	🟥		
☐	Nuclei	region15				
☐	Nuclei	region16				
☐	Nuclei	region17				
☐	Nuclei	region18				
☐	Nuclei	region19				

On the right side, there is a menu listing all regions ordered by class. For each region:

- **Select**: Click to select the region in the viewer.
- **Change Class**: Modify the class of the region.
- **Change Name**: Edit the name of the region.
- **Statistics**: View region statistics.
- **Hide**: Toggle the visibility of the region.
- **Delete**: Remove the region.

For groups of regions of one class:

- **Rename Class**
- **Change Class Color**
- **Hide All Regions**
- **Delete All Regions**

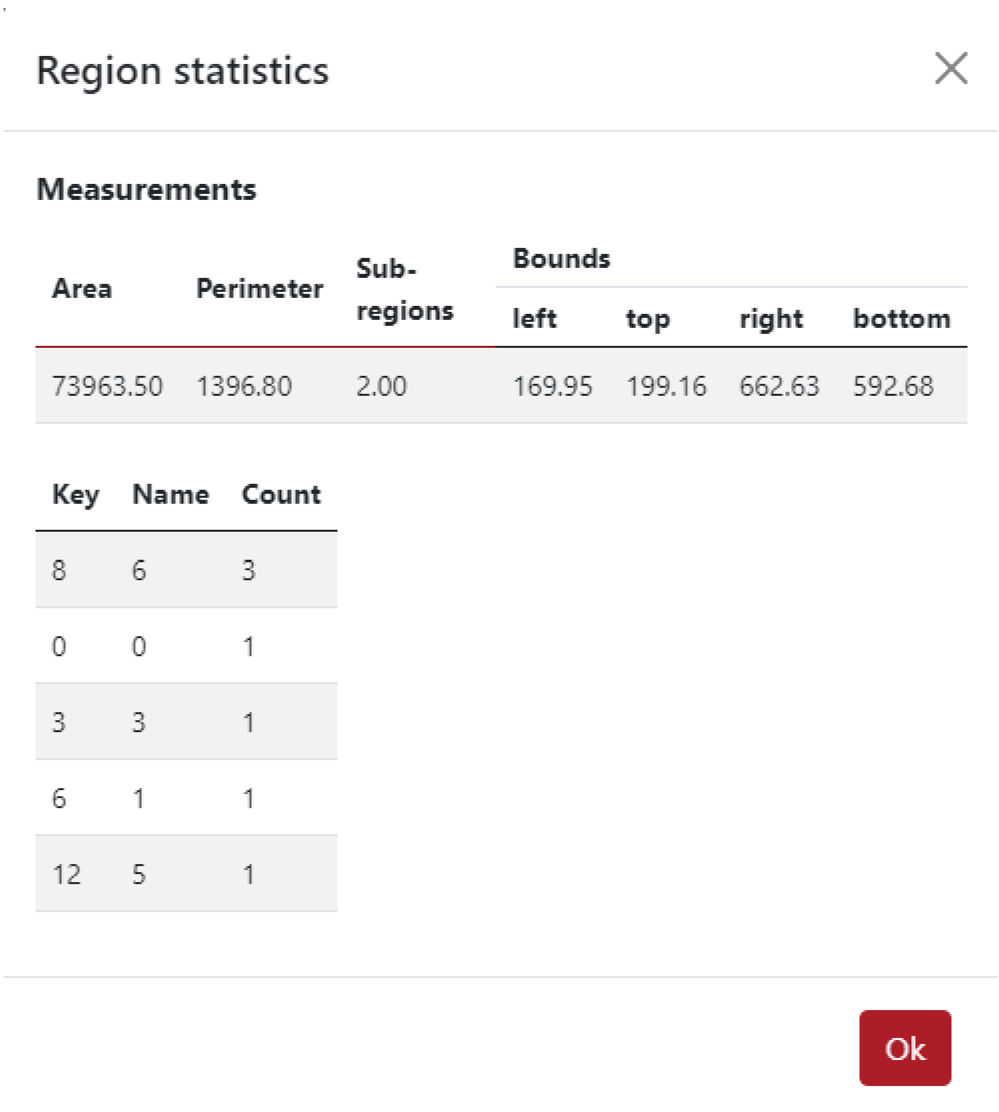
For all regions:

- **Hide All**
- **Delete All**

2.3.4 Region Statistics

Clicking on the statistics button for a specific region reveals:

- **Area**
- **Perimeter**
- **Number of Sub-regions**
- **Bounds (left, top, right, bottom in pixel coordinates)**
- **Number of Each Type of Markers**



Region statistics

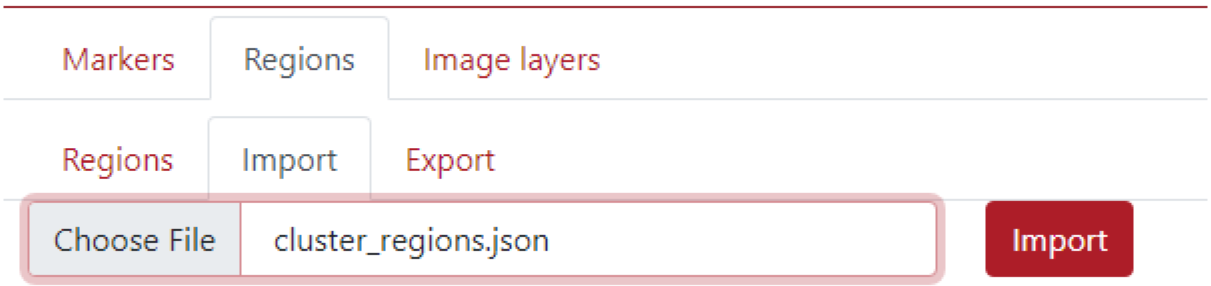
Measurements

Area	Perimeter	Sub-regions	Bounds			
			left	top	right	bottom
73963.50	1396.80	2.00	169.95	199.16	662.63	592.68

Key	Name	Count
8	6	3
0	0	1
3	3	1
6	1	1
12	5	1

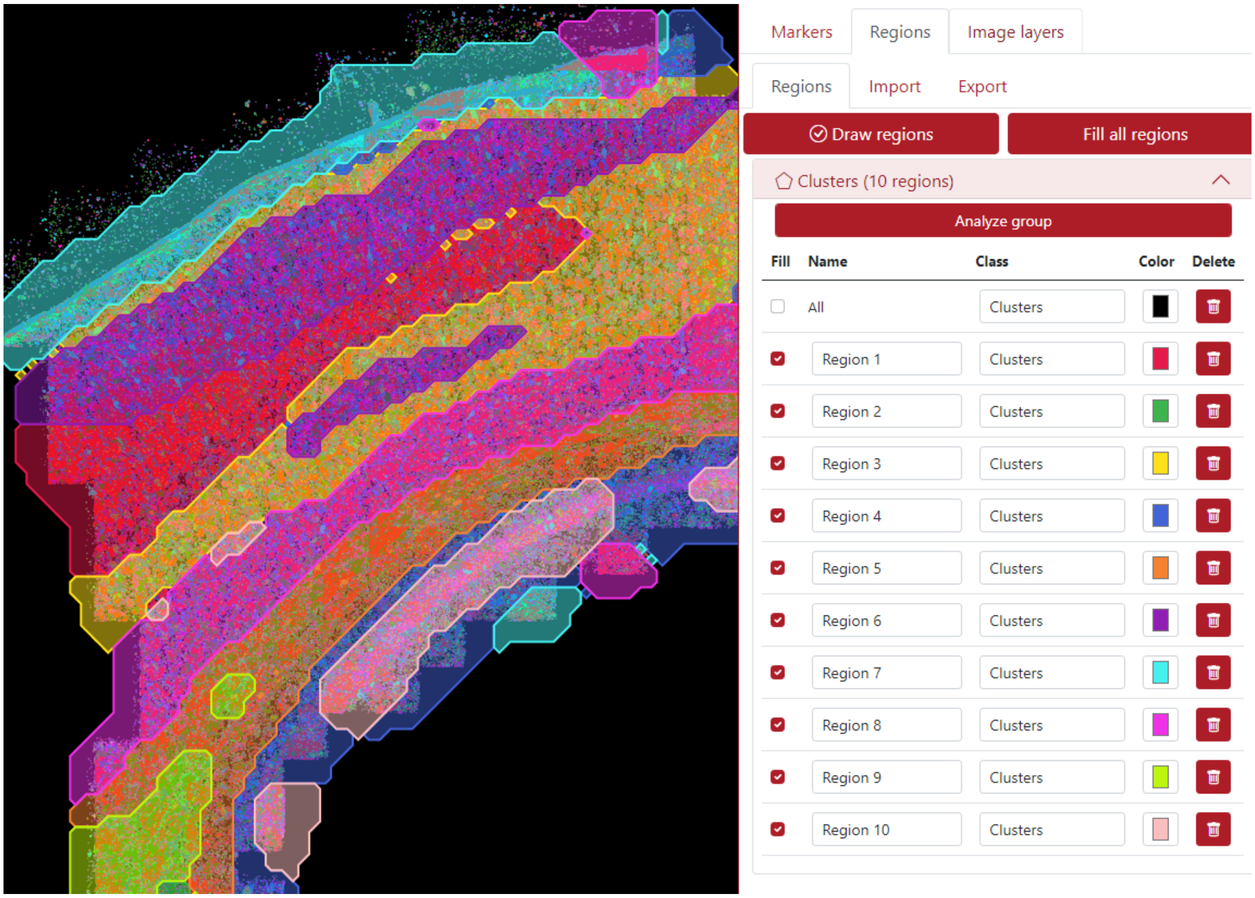
2.3.5 Import Regions

Regions can be imported from .json file, which could be achieved from an external software or also from TissUUmaps' plugin *Points2Regions*. The user just click on the tab *Import* -> *Choose File* and press the button *Import*.



cluster_regions.json

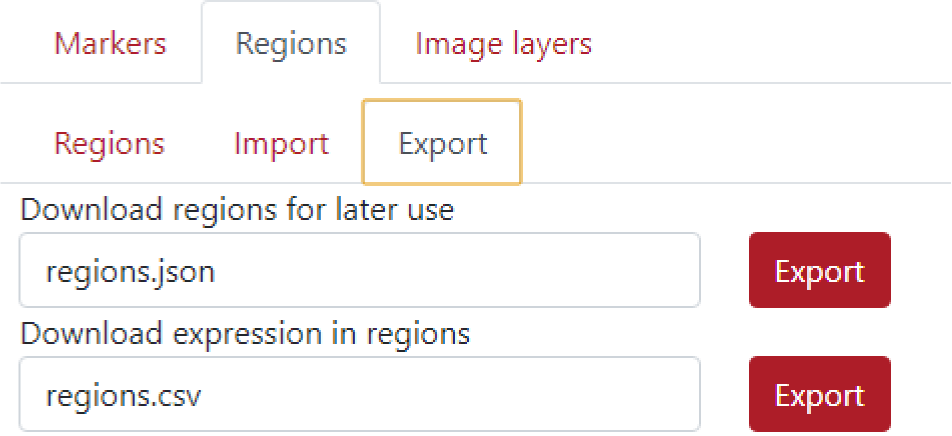
After that, the displayed regions appear in the left panel and the list of regions in the right panel as you can see in the example below. In this case, there are 10 different regions, called clusters. The user can change the color, the name, and the class of the regions if necessary. The user can as well draw some extra regions. These regions can be analyzed to observe the marker expression.



Fill	Name	Class	Color	Delete
☐	All	Clusters	⬛	🗑
☑	Region 1	Clusters	🟥	🗑
☑	Region 2	Clusters	🟩	🗑
☑	Region 3	Clusters	🟨	🗑
☑	Region 4	Clusters	🟦	🗑
☑	Region 5	Clusters	🟧	🗑
☑	Region 6	Clusters	🟪	🗑
☑	Region 7	Clusters	🟦	🗑
☑	Region 8	Clusters	🟪	🗑
☑	Region 9	Clusters	🟩	🗑
☑	Region 10	Clusters	🟥	🗑

2.3.6 Export Regions

The regions can be exported by clicking the tab *Export*, there the user can export two types of files. The first one is the .json file and the name can be selected. The second file is the marker expression in the regions which can be exported as .csv file (this is exported only if the regions were analyzed).



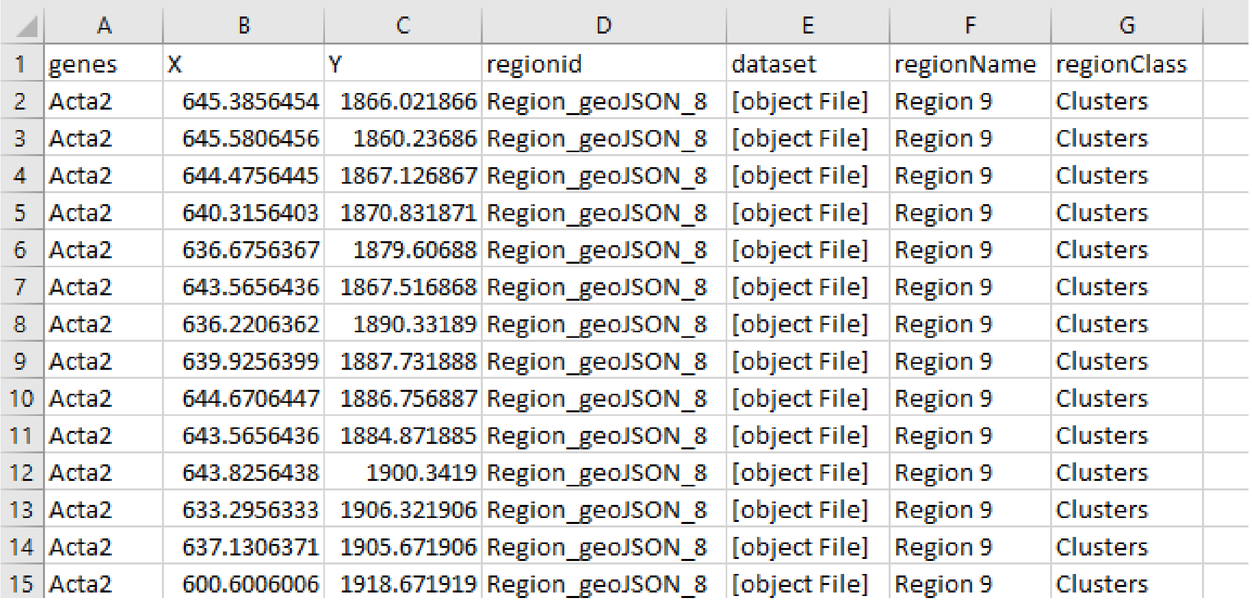
Download regions for later use

regions.json

Download expression in regions

regions.csv

In the figure below can be seen an example of the exported .cvs file.

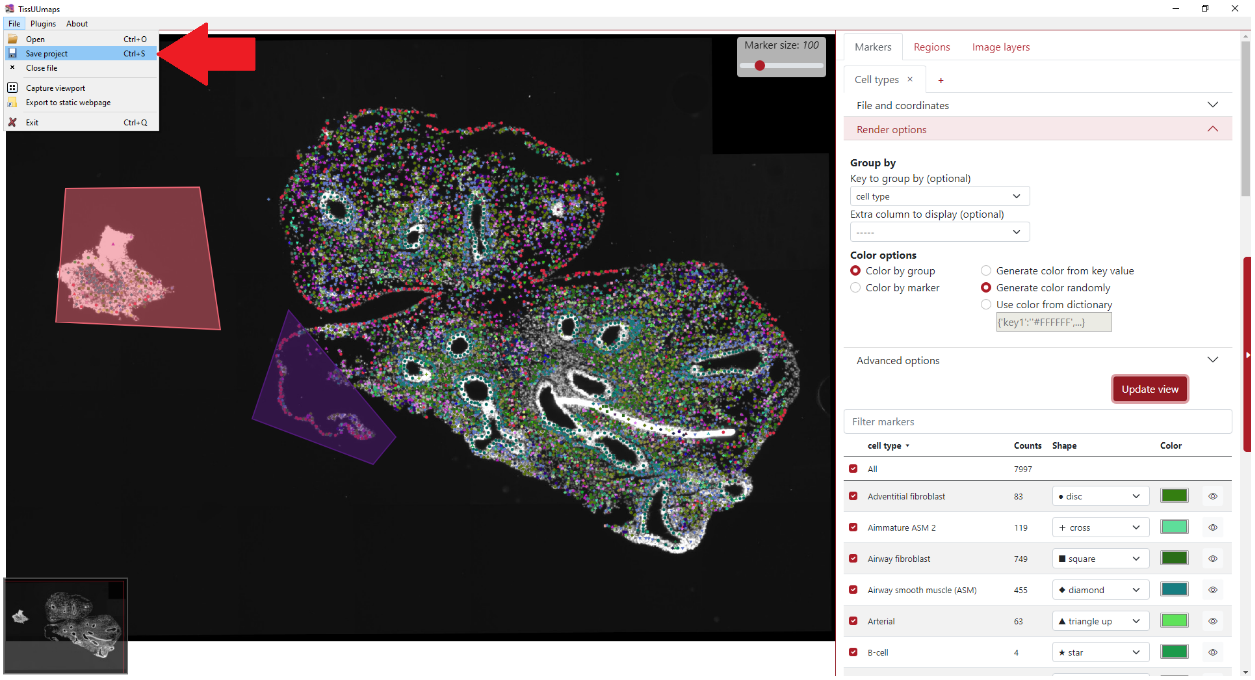


	A	B	C	D	E	F	G
1	genes	X	Y	regionid	dataset	regionName	regionClass
2	Acta2	645.3856454	1866.021866	Region_geoJSON_8	[object File]	Region 9	Clusters
3	Acta2	645.5806456	1860.23686	Region_geoJSON_8	[object File]	Region 9	Clusters
4	Acta2	644.4756445	1867.126867	Region_geoJSON_8	[object File]	Region 9	Clusters
5	Acta2	640.3156403	1870.831871	Region_geoJSON_8	[object File]	Region 9	Clusters
6	Acta2	636.6756367	1879.60688	Region_geoJSON_8	[object File]	Region 9	Clusters
7	Acta2	643.5656436	1867.516868	Region_geoJSON_8	[object File]	Region 9	Clusters
8	Acta2	636.2206362	1890.33189	Region_geoJSON_8	[object File]	Region 9	Clusters
9	Acta2	639.9256399	1887.731888	Region_geoJSON_8	[object File]	Region 9	Clusters
10	Acta2	644.6706447	1886.756887	Region_geoJSON_8	[object File]	Region 9	Clusters
11	Acta2	643.5656436	1884.871885	Region_geoJSON_8	[object File]	Region 9	Clusters
12	Acta2	643.8256438	1900.3419	Region_geoJSON_8	[object File]	Region 9	Clusters
13	Acta2	633.2956333	1906.321906	Region_geoJSON_8	[object File]	Region 9	Clusters
14	Acta2	637.1306371	1905.671906	Region_geoJSON_8	[object File]	Region 9	Clusters
15	Acta2	600.6006006	1918.671919	Region_geoJSON_8	[object File]	Region 9	Clusters

2.4 Projects

2.4.1 Saving projects

When the user has finished the visualization adjustments, region drawings, etc., the project is ready to be saved in order to continue working on it later or just basically to save it as it is for further consistency. The user needs to press *File* in the menu and then *Save project* or Ctrl + S.



Marker size: *100*

Group by

Key to group by (optional): cell type

Extra column to display (optional): -----

Color options

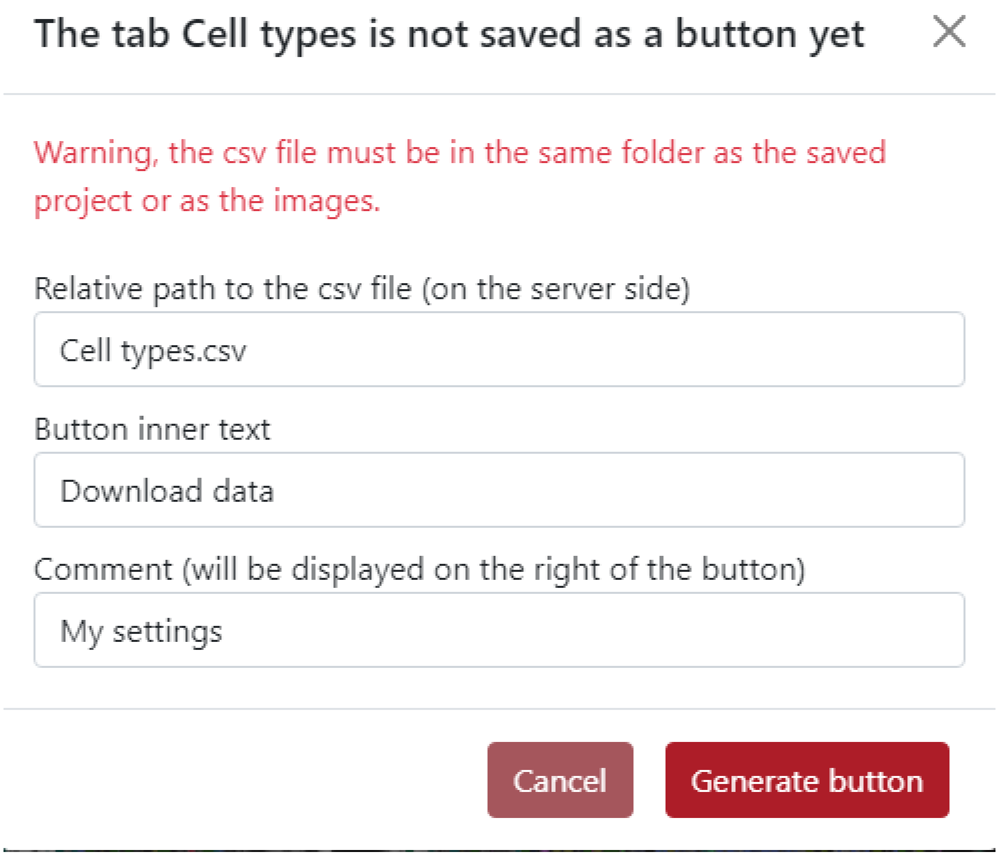
● Color by group ○ Generate color from key value

○ Color by marker ● Generate color randomly

○ Use color from dictionary {'key1':'#FFFFFF',...}

	cell type ▾	Counts	Shape	Color
☑	All	7997		
☑	Adventitial fibroblast	83	● disc	🟩
☑	Aimmature ASM 2	119	+ cross	🟩
☑	Airway fibroblast	749	■ square	🟩
☑	Airway smooth muscle (ASM)	455	◆ diamond	🟦
☑	Arterial	63	▲ triangle up	🟩
☑	B-cell	4	★ star	🟩

In order to save the project together with the .csv file, it is necessary to generate a button first. The warning window below appears and the user needs to generate the button. The path to the .csv file needs to be relative to the path of the image. In this example, the image layer and the .csv file are in the exact same directory.



The tab Cell types is not saved as a button yet

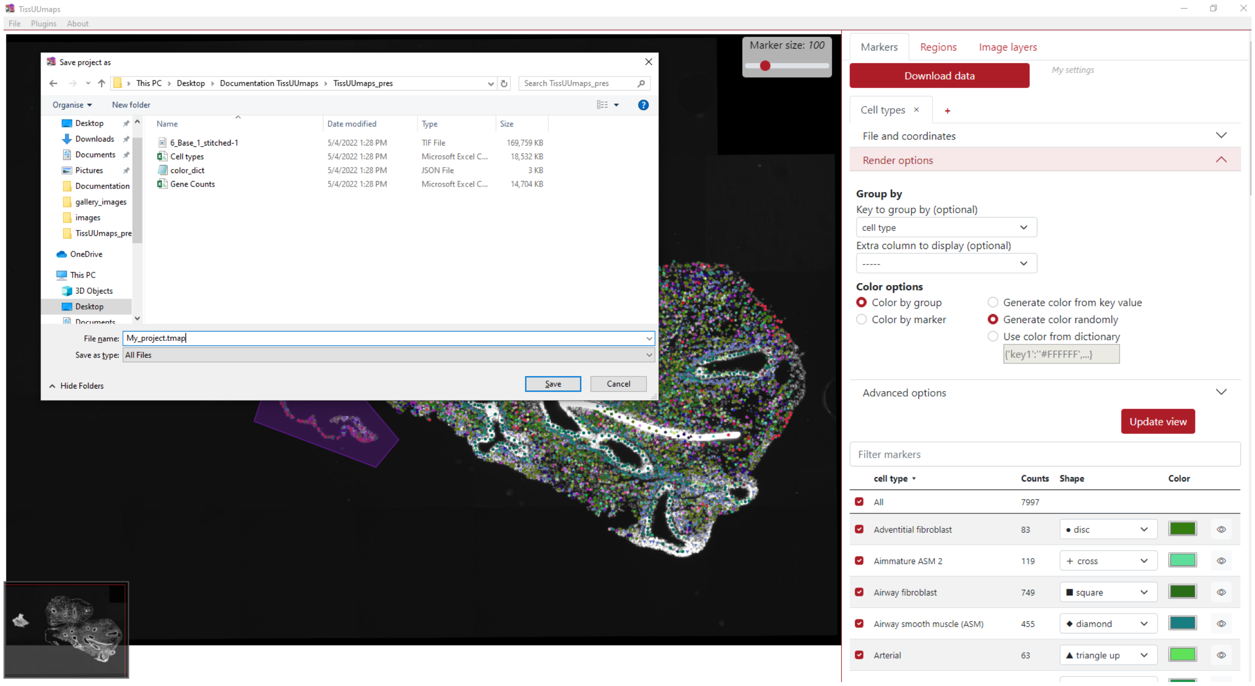
Warning, the csv file must be in the same folder as the saved project or as the images.

Relative path to the csv file (on the server side): Cell types.csv

Button inner text: Download data

Comment (will be displayed on the right of the button): My settings

Then the user selects a suitable directory to save the project and writes the project file name, i.e. My_project.tmap, and the project is saved.



Name	Date modified	Type	Size
6_Base_1_stitched-1	5/4/2022 1:28 PM	TIF File	169,759 KB
Cell types	5/4/2022 1:28 PM	Microsoft Excel C...	18,532 KB
color_dict	5/4/2022 1:28 PM	JSON File	3 KB
Gene Counts	5/4/2022 1:28 PM	Microsoft Excel C...	14,704 KB

File name: My_project.tmap

Save as type: All Files

Group by

Key to group by (optional): cell type

Extra column to display (optional): -----

Color options

● Color by group ○ Generate color from key value

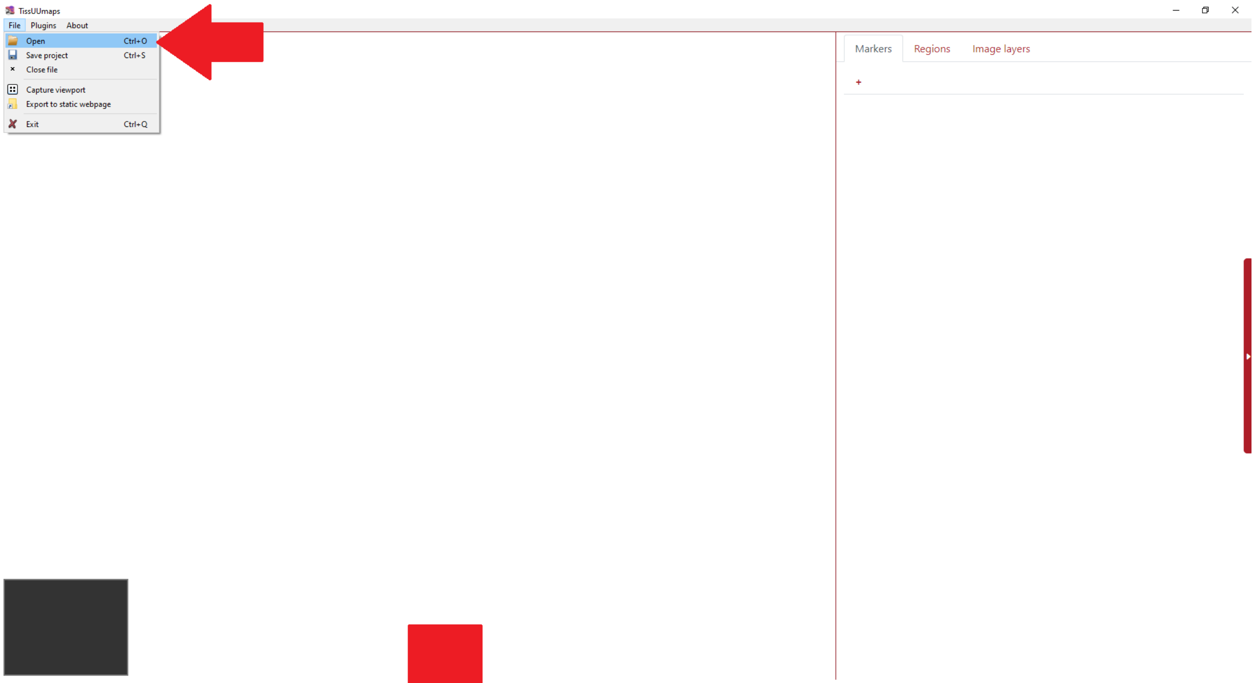
○ Color by marker ● Generate color randomly

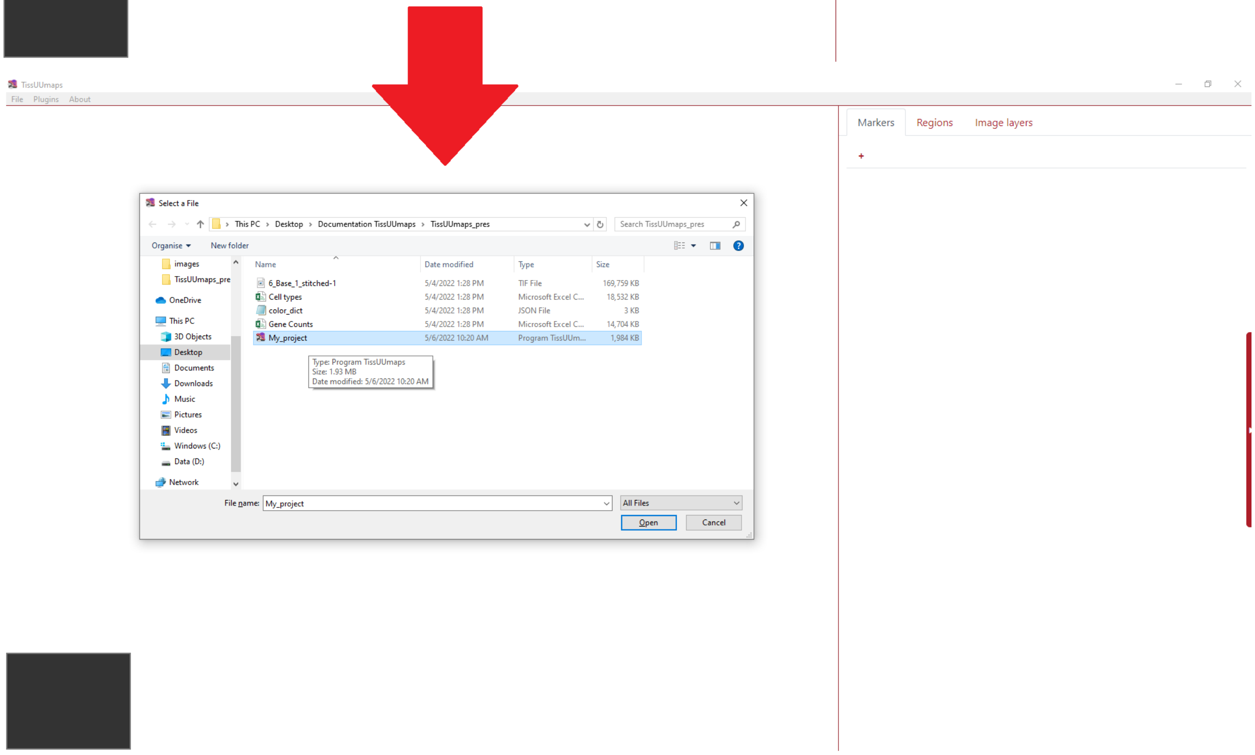
○ Use color from dictionary {'key1':'#FFFFFF',...}

	cell type	Counts	Shape	Color
☑	All	7997		
☑	Adventitial fibroblast	83	● disc	🟩
☑	Aimmature ASM 2	119	+ cross	🟩
☑	Airway fibroblast	749	■ square	🟩
☑	Airway smooth muscle (ASM)	455	◆ diamond	🟦
☑	Arterial	63	▲ triangle up	🟩

2.4.2 Loading projects

The .tmap project can be loaded by two approaches. The first one is opening the TissUUmaps program, click *File* in the menu and then *Open* or Crtl + O. Then the user navigates in the directory and selects the .tmap file. By default, the directory navigates in the recent .tmap project.



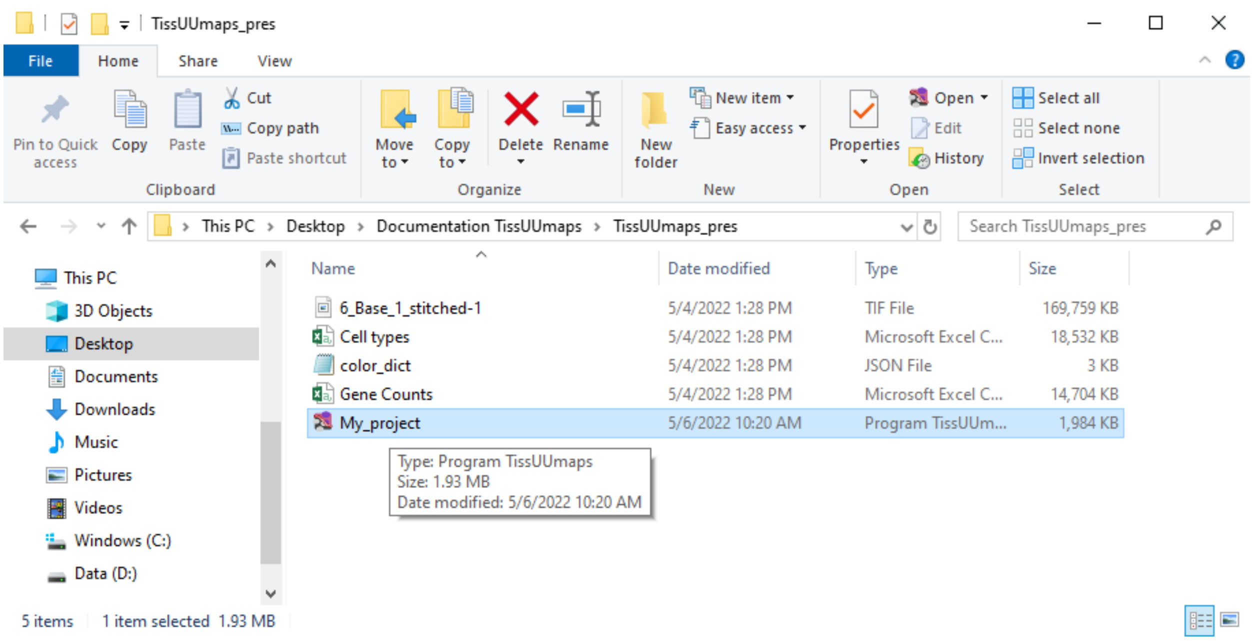


Name	Date modified	Type	Size
6_Base_1_stitched-1	5/4/2022 1:28 PM	TIF File	169,759 KB
Cell types	5/4/2022 1:28 PM	Microsoft Excel C...	18,532 KB
color_dict	5/4/2022 1:28 PM	JSON File	3 KB
Gene Counts	5/4/2022 1:28 PM	Microsoft Excel C...	14,704 KB
My_project	5/6/2022 10:20 AM	Program TissUUm...	1,984 KB

Type: Program TissUUmaps
Size: 1.93 MB
Date modified: 5/6/2022 10:20 AM

File name: My_project

The second option is directly double click on the .tmap file in file explorer in your computer.

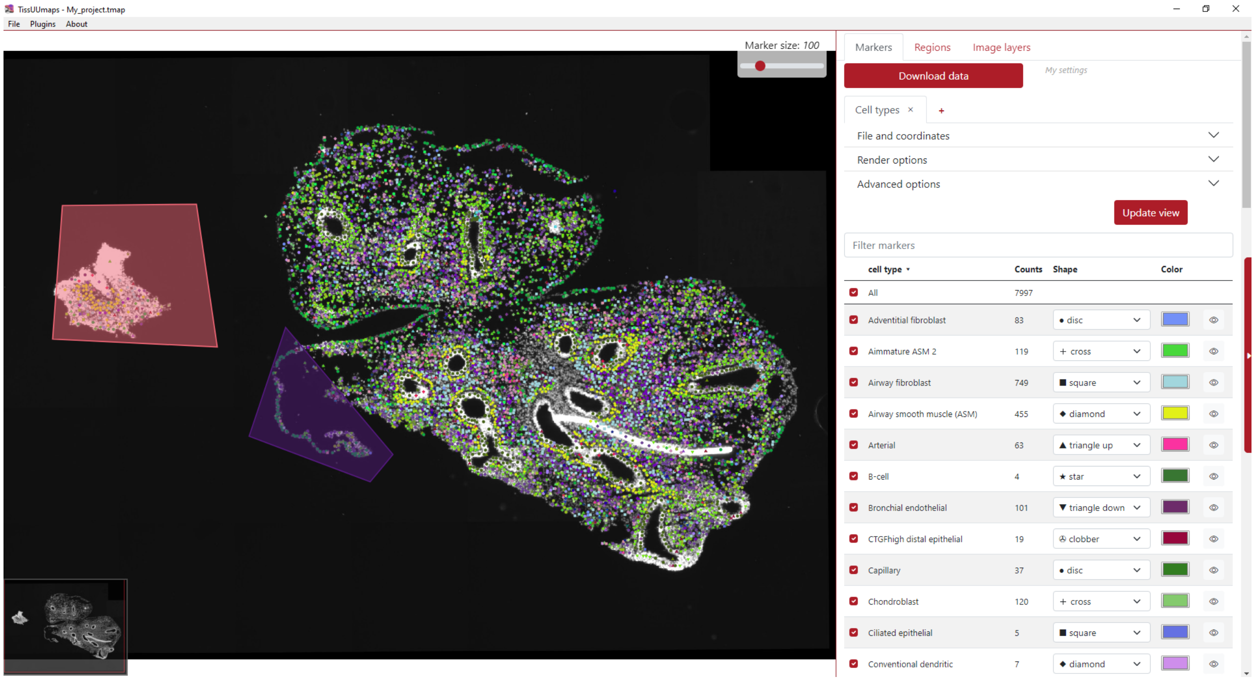


Name	Date modified	Type	Size
6_Base_1_stitched-1	5/4/2022 1:28 PM	TIF File	169,759 KB
Cell types	5/4/2022 1:28 PM	Microsoft Excel C...	18,532 KB
color_dict	5/4/2022 1:28 PM	JSON File	3 KB
Gene Counts	5/4/2022 1:28 PM	Microsoft Excel C...	14,704 KB
My_project	5/6/2022 10:20 AM	Program TissUUm...	1,984 KB

Type: Program TissUUmaps
Size: 1.93 MB
Date modified: 5/6/2022 10:20 AM

5 items 1 item selected 1.93 MB

After clicking the button *Download data*, both these approaches will lead to loading the project as can be seen in the example below.



	cell type ▾	Counts	Shape	Color	
☑	All	7997			
☑	Adventitial fibroblast	83	● disc	🟦	👁
☑	Aimmature ASM 2	119	+ cross	🟩	👁
☑	Airway fibroblast	749	■ square	🟦	👁
☑	Airway smooth muscle (ASM)	455	◆ diamond	🟨	👁
☑	Arterial	63	▲ triangle up	🟥	👁
☑	B-cell	4	★ star	🟩	👁
☑	Bronchial endothelial	101	▼ triangle down	🟪	👁
☑	CTGFhigh distal epithelial	19	✇ clobber	🟥	👁
☑	Capillary	37	● disc	🟩	👁
☑	Chondroblast	120	+ cross	🟩	👁
☑	Ciliated epithelial	5	■ square	🟦	👁
☑	Conventional dendritic	7	◆ diamond	🟪	👁

For more information on the tmap file format and specifications, see *The TMAP file format*.

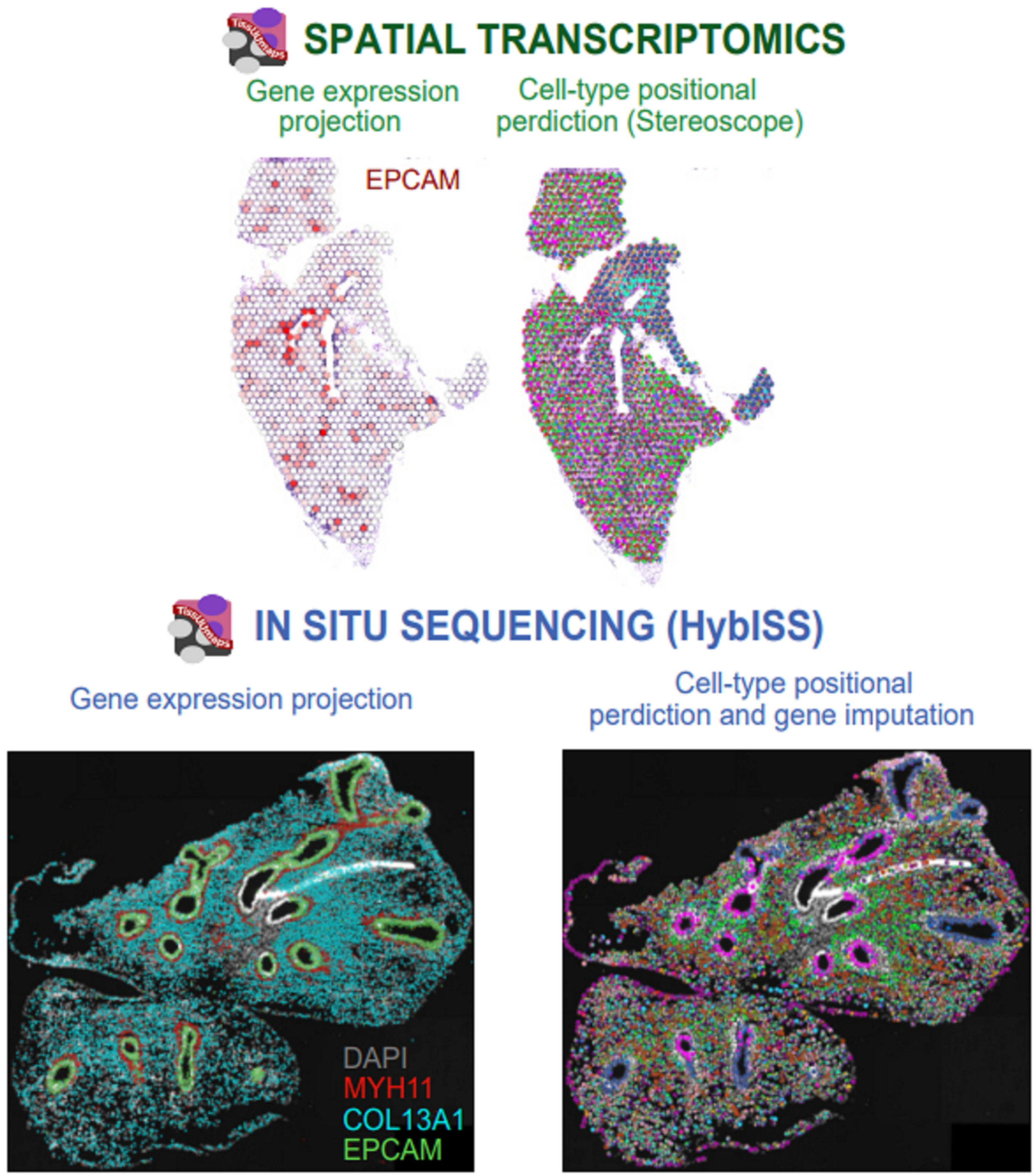
2.4.3 Editing .tmap file manually

You can edit the `.tmap` file manually in a text editor. The `.tmap` file is a JSON file, which is a human-readable format. The `.tmap` file contains all the information about the project, including the image layers, regions, and annotations. You can read the specification of the `.tmap` file in *The TMAP file format*.

2.4.4 Existing projects

Human Developmental Lung Cell Atlas (pcw 5- pcw 14)

The human lung is a highly complex tubular organ, whose main function is the gas exchange between blood and breathed air. In contains a large number of specialized cell-types of epithelial, endothelial, neuronal, stromal and immune cells that are necessary for normal organ function and structural integrity. To understand how this cell heterogeneity develops to create a healthy mature lung, we focused on the 1st trimester of gestation and applied state of art technologies to capture the gene expression profiles of all the cells in the developing organ, in time and space.



TissUUmaps interactive viewer: Single-cell RNA-sequencing UMAP representation of single-cell clusters and sub-clusters, gene expression and metadata.

In situ sequencing data (ISS) - TissUUmaps interactive viewer: pcw 5 pcw 6 pcw 13In situ sequencing data. Spot location + identity, per bin pie chart view of cell type probabilities and imputed genes.

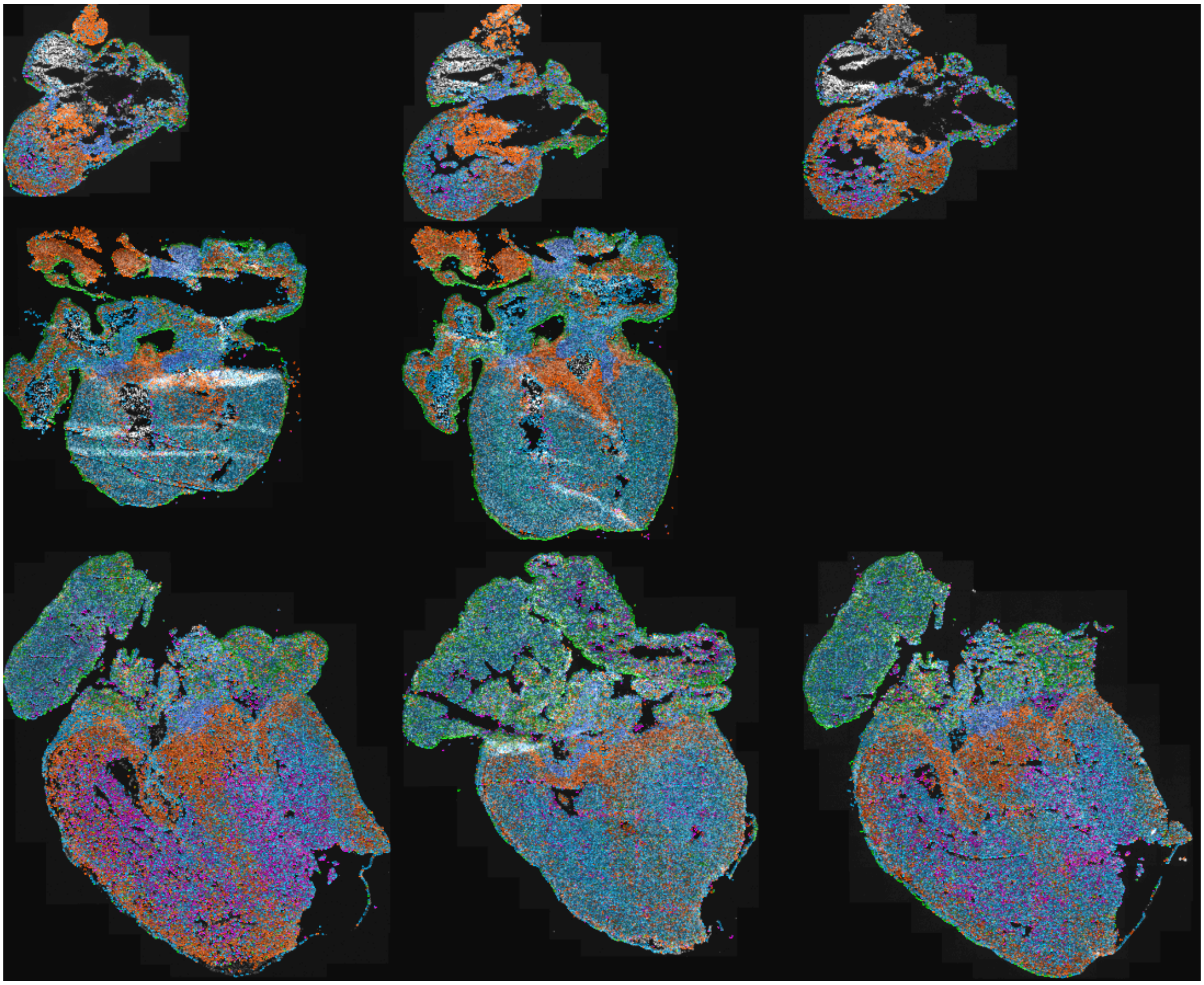
SCRINSHOT data - TissUUmaps interactive viewer: pcw 6 pcw 8 pcw 11 pcw 14SCRINSHOT data. Spot location + identity.

Spatial Transcriptomics data - TissUUmaps interactive viewer: pcw 6 pcw 8 pcw 10 pcw 11Per gene or pie chart view of gene expression.

More information is available in the original publication: A. Sountoulidis, S.M. Salas, E. Braun, C. Avenel, J. Bergenstråhle, M. Vicari, P. Czarnewski, J. Theelke, A. Liontos, X. Abalo, Ž. Andrusivová, M. Asp, X. Li, L. Hu, S. Sariyar, A.M. Casals, B. Ayoglu, A. Firsova, J. Michaëlsson, E. Lundberg, C. Wählby, E. Sundström, S. Linnarsson, J. Lundeberg, M. Nilsson, C. Samakovlis. Developmental origins of cell heterogeneity in the human lung. BioRxiv doi: https://doi.org/10.1101/2022.01.11.475631

Modelling of cell-type signatures in the developmental human heart

With the emergence of high throughput single cell techniques, the understanding of cellular diversity in biologically complex processes has rapidly increased. The next step towards comprehension of e.g. key organs in the mammal development is to obtain spatiotemporal atlases of the cellular diversity. However, targeted cell typing approaches relying on existing single cell data achieve incomplete and biased maps that could mask the molecular and cellular heterogeneity present in a tissue slide. Here we applied spage2vec, a de novo approach to spatially resolve and characterize cellular diversity during human heart development. Data from the original in situ sequencing experiment as well as identified cell types can be viewed in TissUUmaps.

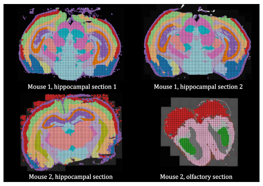


TissUUmaps interactive viewer: Human heart

More information is available in the original publication: RS. Marco Salas, X. Yuan, C. Sylven, M. Nilsson, C. Wählby and G.Partel. De novo spatiotemporal modelling of cell-type signatures identifies novel cell populations in the developmental human heart. BioRxiv doi: https://doi.org/10.1101/2021.07.10.451822

Automated identification of the mouse brain's spatial compartments from in situ sequencing data

Neuroanatomical compartments of the mouse brain are identified and outlined mainly based on manual annotations of samples using features related to tissue and cellular morphology, taking advantage of publicly available reference atlases. However, this task is challenging since sliced tissue sections are rarely perfectly parallel or angled with respect to sections in the reference atlas and organs from different individuals may vary in size and shape and requires manual annotation. Here, we show how in situ sequencing data combined with dimensionality reduction and unsupervised clustering can be used to identify spatial compartments that correspond to known anatomical compartments of the brain. Here we show results on four different sections of mouse brains.



TissUUmaps interactive viewer: Mouse brain

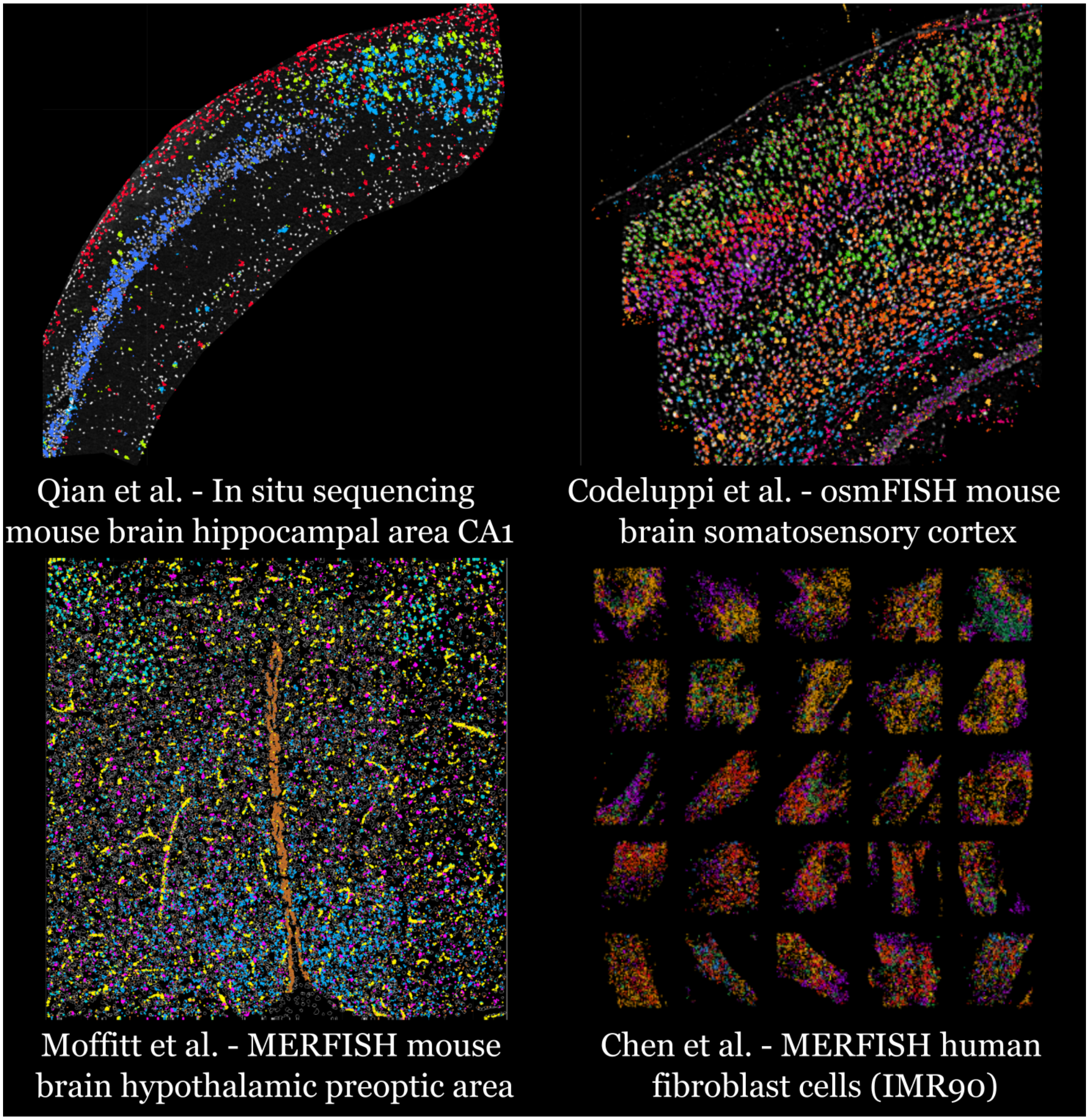
More information is available in this publication: G. Partel, M.M. Hilscher, G. Milli, L. Solorzano, A.H. Klemm, M. Nilsson, and C. Wählby. Automated identification of the mouse brain's spatial compartments from in situ sequencing data. BMC Biology, https://doi.org/10.1186/s12915-020-00874-5, Oct 2020.

The original raw ISS data was published in Qian, X., Harris, K. D., Hauling, T., Nicoloutsopoulos, D., Muñoz-Manchado, A. B., Skene, N., … & Nilsson, M. (2020). Probabilistic cell typing enables fine mapping of closely related cell types in situ. Nature methods, 17(1), 101-106.

Data and code availability: All software was developed in Python 3 using open source libraries, and data processing of pipeline workflows was carried out using Anduril2 analysis framework. The processing pipelines, data, and the software version used to generate the analysis results and figures presented in this paper are available at https://doi.org/10.5281/zenodo.3928219 or from our github repository https://github.com/wahlby-lab/graph-iss.

Spage2vec: Unsupervised representation of localized spatial gene expression signatures

Spage2vec is an unsupervised segmentation free approach for decrypting the spatial transcriptomic heterogeneity of complex tissues at subcellular resolution. Spage2vec represents the spatial transcriptomic landscape of tissue samples as a graph and leverage powerful machine learning graph representation technique to create a lower dimensional representation of local spatial gene expression. Here we visualize spage2vec localized gene expression signatures of different spatial transcriptomic datasets. We thank Mats Nilsson, Sten Linnarsson and Xiaowei Zhuang for making their datasets publicly available.



TissUUmaps interactive viewer 1: In situ sequencing mouse brain hippocampal area CA1 Qian, X., Harris, K. D., Hauling, T., Nicoloutsopoulos, D., Muñoz-Manchado, A. B., Skene, N., … & Nilsson, M. (2020). Probabilistic cell typing enables fine mapping of closely related cell types in situ. Nature methods, 17(1), 101-106.

TissUUmaps interactive viewer 2: osmFISH mouse brain somatosensory cortex Codeluppi, S., Borm, L. E., Zeisel, A., La Manno, G., van Lunteren, J. A., Svensson, C. I., & Linnarsson, S. (2018). Spatial organization of the somatosensory cortex revealed by osmFISH. Nature methods, 15(11), 932-935.

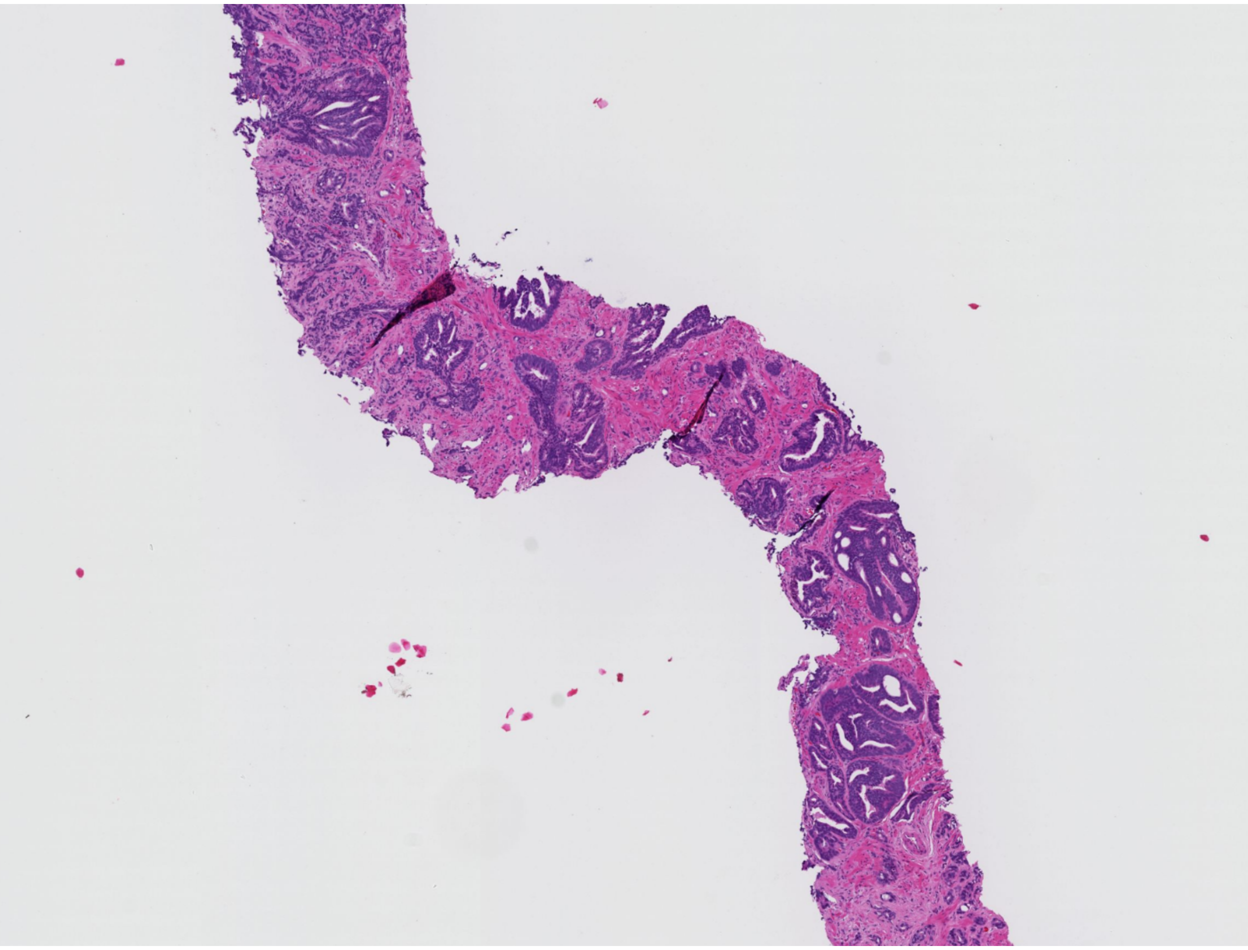
TissUUmaps interactive viewer 3: MERFISH mouse brain hypothalamic preoptic area Moffitt, J. R., Bambah-Mukku, D., Eichhorn, S. W., Vaughn, E., Shekhar, K., Perez, J. D., … & Zhuang, X. (2018). Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. Science, 362(6416), eaau5324.

TissUUmaps interactive viewer 4: MERFISH human fibroblast cells (IMR90) Chen, K. H., Boettiger, A. N., Moffitt, J. R., Wang, S., & Zhuang, X. (2015). Spatially resolved, highly multiplexed RNA profiling in single cells. Science, 348(6233), aaa6090.

Data and code availability: Spatial gene expression data are available in Zenodo database at https://doi.org/10.5281/zenodo.3897401. Source code for reproducing analysis results and figures is available in Zenodo database at http://www.doi.org/10.5281/zenodo.4030404.

Artificial intelligence for diagnosis and grading of prostate cancer in biopsies: a population-based

An increasing volume of prostate biopsies and a worldwide shortage of urological pathologists puts a strain on pathology departments. Additionally, the high intra-observer and inter-observer variability in grading can result in overtreatment and undertreatment of prostate cancer. To alleviate these problems, we aimed to develop an artificial intelligence (AI) system with clinically acceptable accuracy for prostate cancer detection, localisation, and Gleason grading. Here we show examples of full-resolution digitized biopsies and corresponding AI-based grading.



An overview of all sample **datasets** can be found here: Prostate cancer in biopsies

More information is available in this publication: P. Ström, K. Kartasalo, H. Olsson, L. Solorzano et al. Artificial intelligence for diagnosis and grading of prostate cancer in biopsies: a population-based, diagnostic study. The Lancet Oncology, Volume 21, Issue 2, 2020, Pages 222-232, ISSN 1470-2045, doi: 10.1016/S1470-2045(19)30738-7, url: https://www.sciencedirect.com/science/article/pii/S1470204519307387

2.5 Exporting screenshots

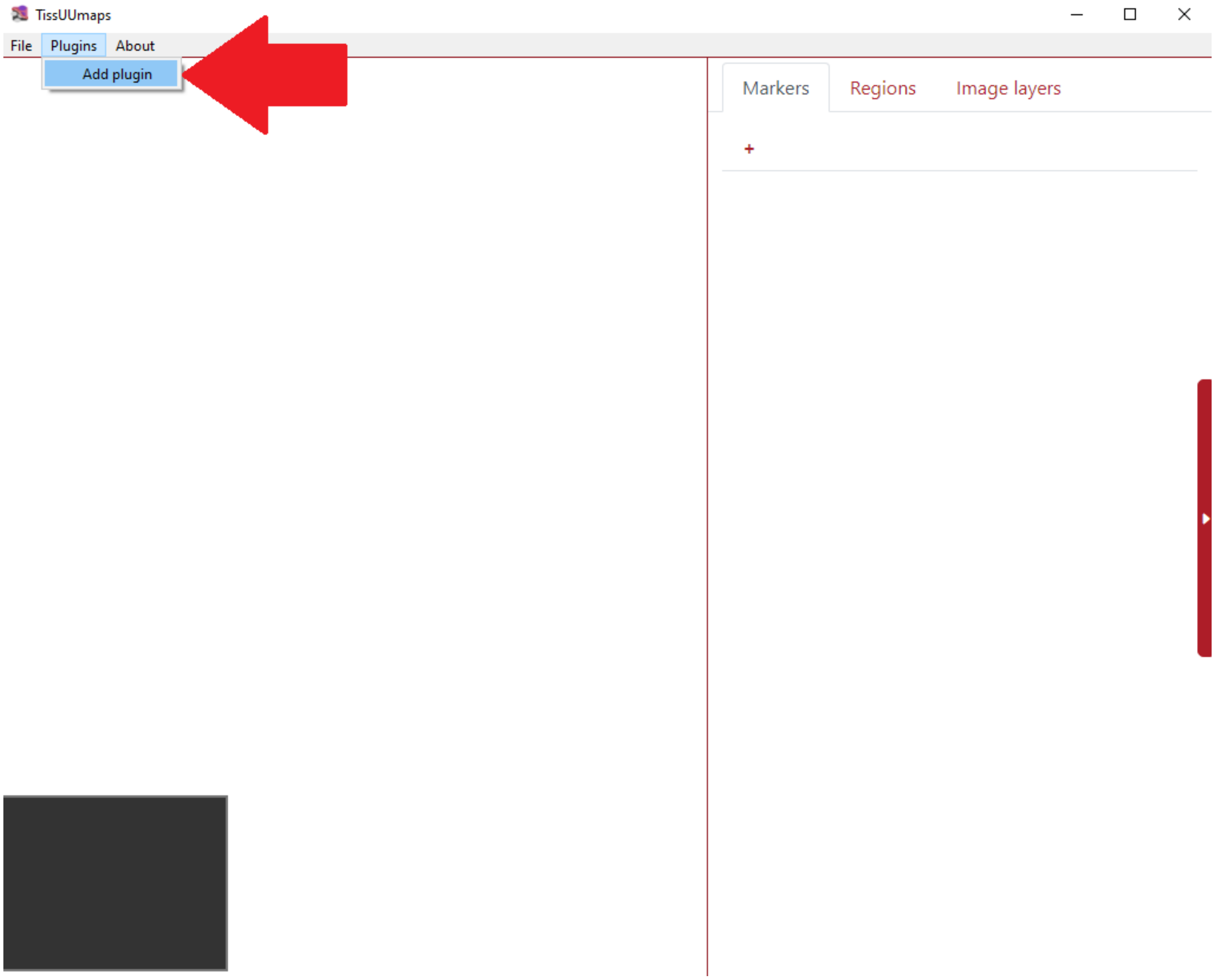
TissUUmaps allows high resolution capture of the image viewport. Go to `Menu > File > Capture viewport` and chose a zoom factor for export (1 = screen resolution).

The screen capture will contain all filtered layers, markers, and regions. Note that legends will not be part of the export and must be added manually.

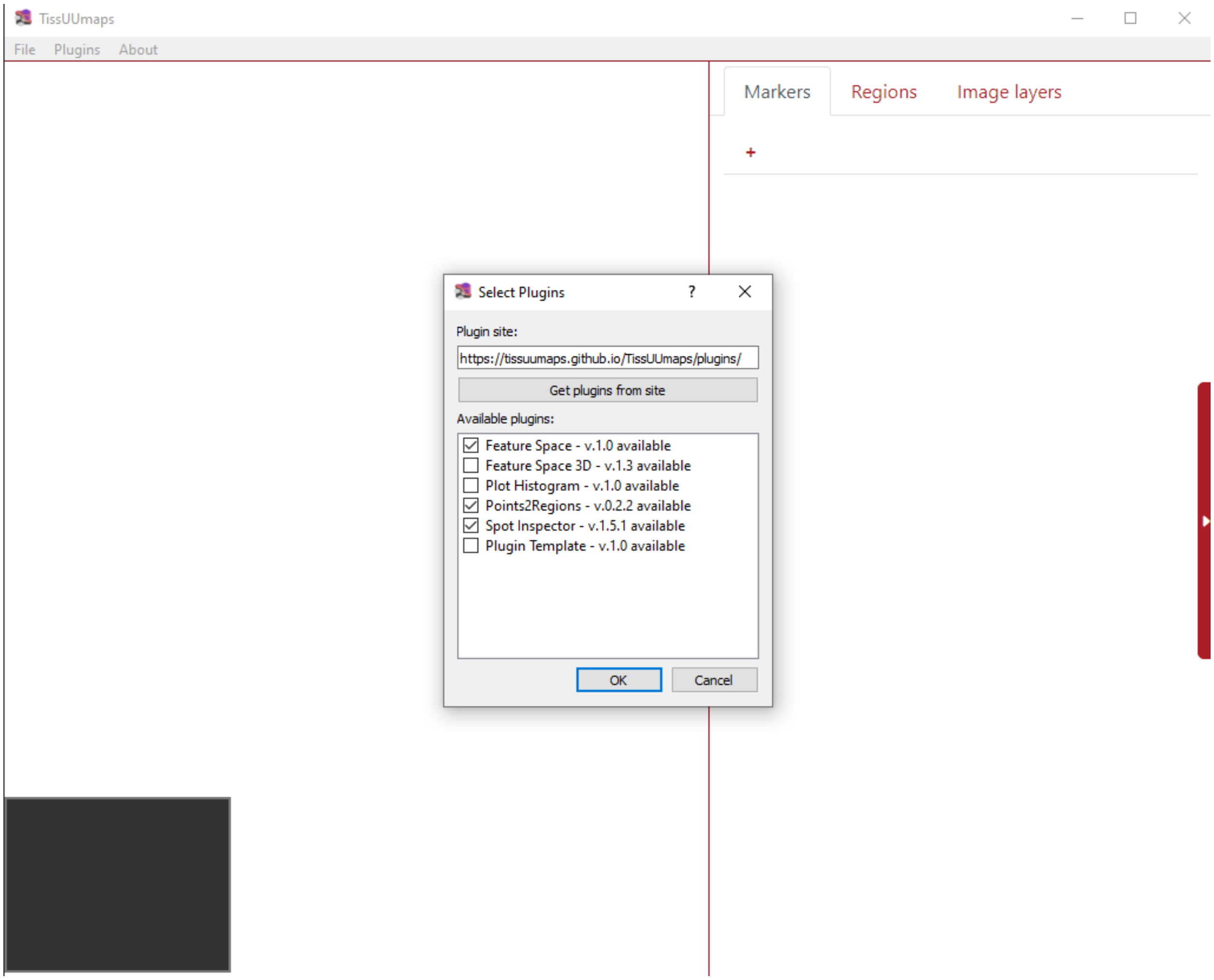
2.6 Plugins

2.6.1 Load plugins

In order to load plugins, first, they need to be installed. This can be done in the menu `Plugins > Add plugin` as can be seen in the example below.



Consequently, the user can check any number of plugins they desire and press *OK*.



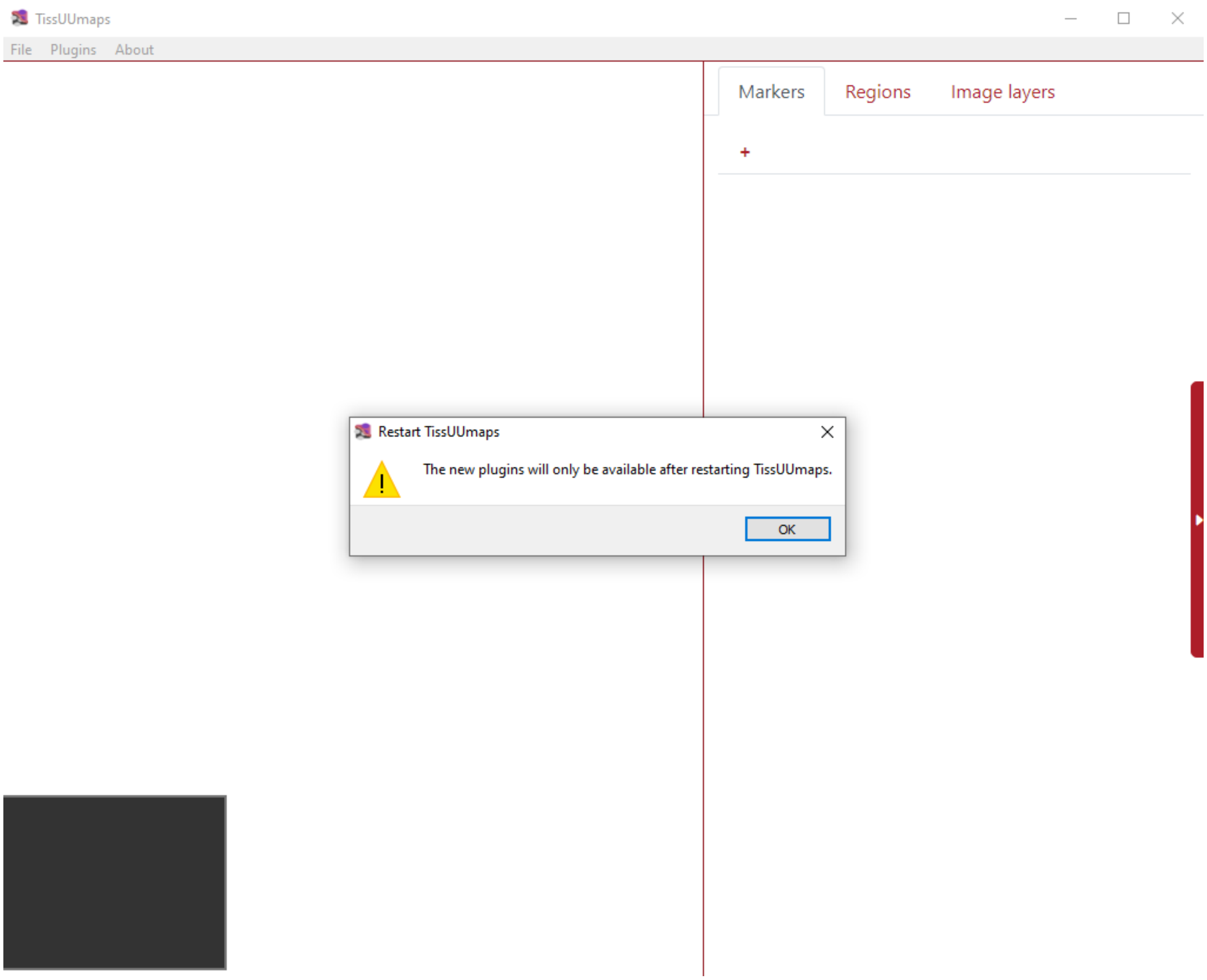
Plugin site:

https://tissuumaps.github.io/TissUUmaps/plugins/

Available plugins:

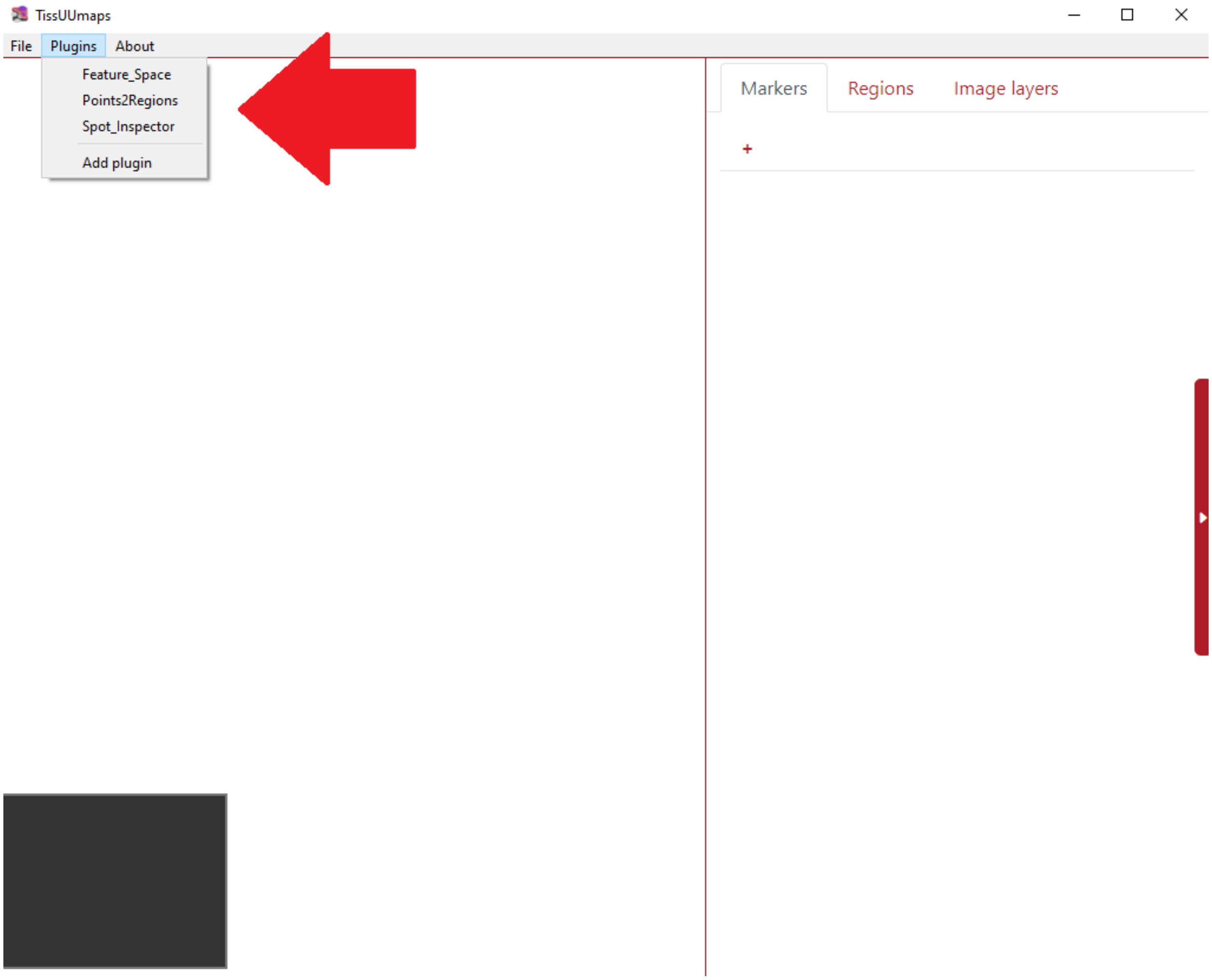
- ☑ Feature Space - v.1.0 available
- ☐ Feature Space 3D - v.1.3 available
- ☐ Plot Histogram - v.1.0 available
- ☑ Points2Regions - v.0.2.2 available
- ☑ Spot Inspector - v.1.5.1 available
- ☐ Plugin Template - v.1.0 available

Then the tool warns the user then the installed plugins will be available after restarting TissUUmaps.

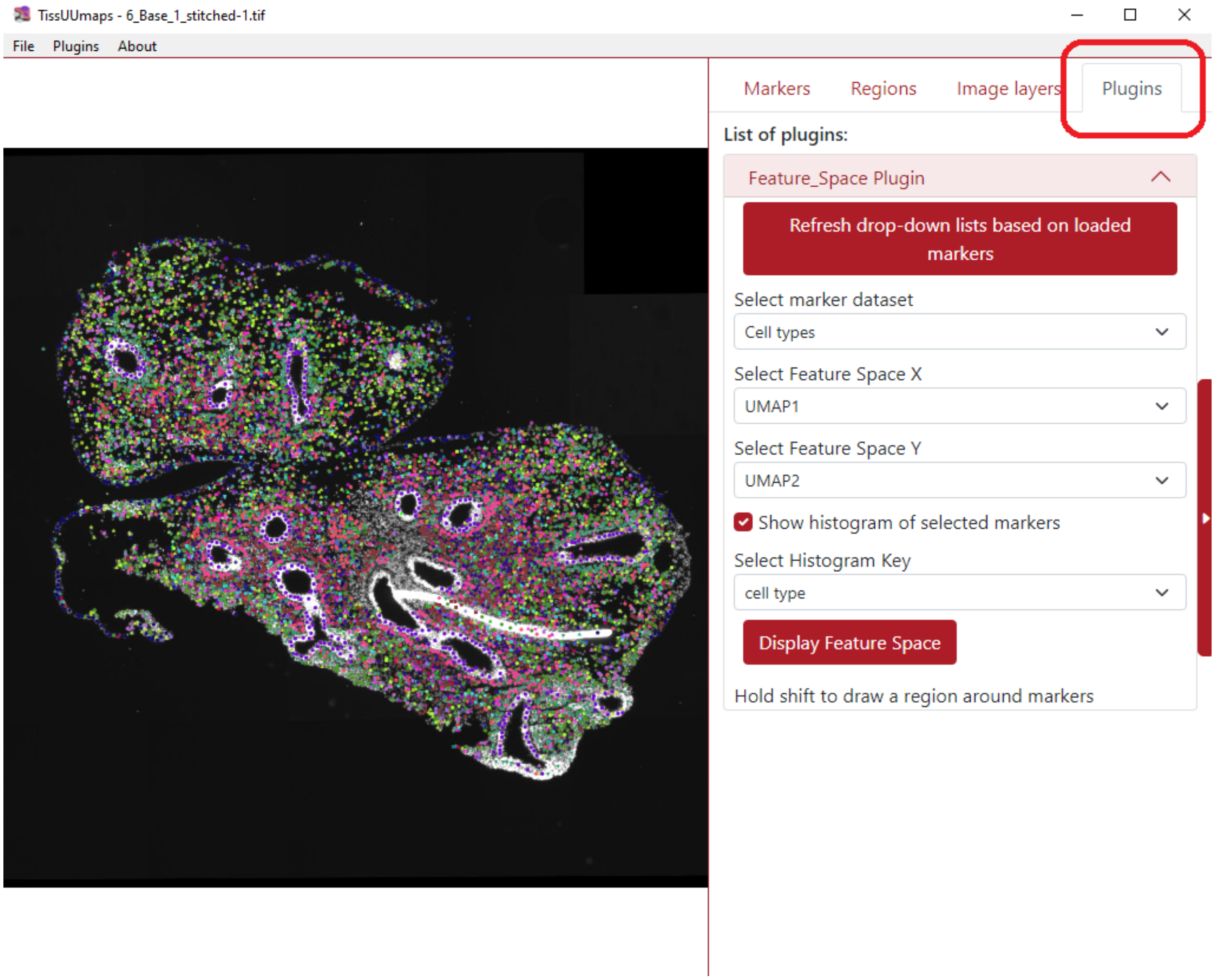


The new plugins will only be available after restarting TissUUmaps.

After restarting the TissUUmaps, all the installed plugins are listed in the menu `Plugins` as you can see in the figure below.



Once the user selects any of the installed plugins (in the example below I selected *Feature_Space*), a new tab *Plugins* appears in the upper right part of the screen with all the required boxes for filling.



List of plugins:

Select marker dataset: Cell types

Select Feature Space X: UMAP1

Select Feature Space Y: UMAP2

☑ Show histogram of selected markers

Select Histogram Key: cell type

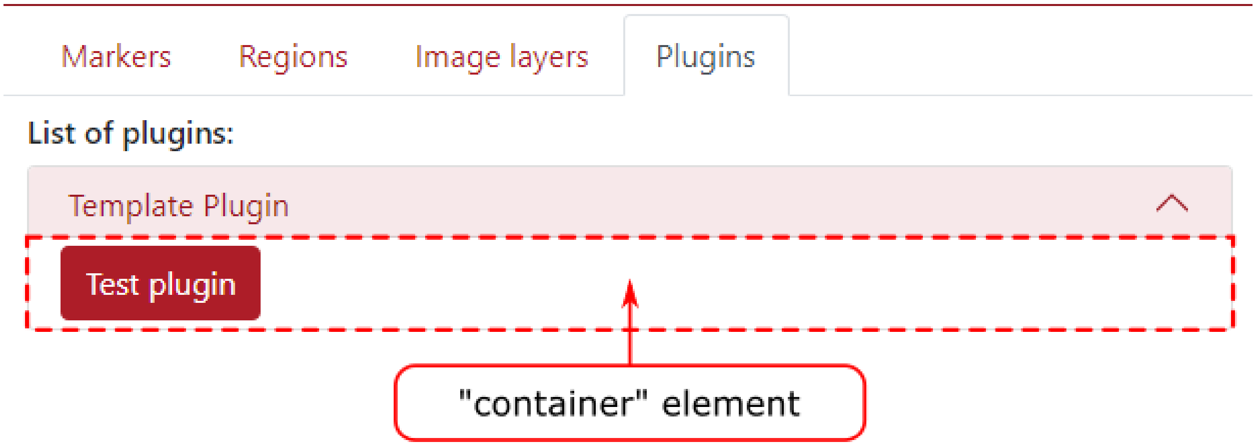
Hold shift to draw a region around markers

2.6.2 Make your own plugin

Download the Plugin Template python and javascript files from the Plugin Update Site and put both files in your local folder `$USER_PATH/.tissuumaps/plugins/`. You can then change the plugin name and add your own options and functions.

Javascript file

When loading a plugin, the function `PluginName.init(container)` will be called. The `container` is an html Element that will be added to the plugin menu. Use this element to add options and texts related to your plugin.



Javascript example:

File `Plugin_template.js`:

```
var Plugin_template;
Plugin_template = {
  name: "Template Plugin",
  parameters: {
    _section_test: {
      "label": "Test section",
      "title": "Section 1",
      "type": "section",
      "collapsed": false
    },
    _message: {
      "label": "Message",
      "type": "text",
      "default": "Hello world"
    },
    _testButton: {
      "label": "Test button",
      "type": "button",
    },
  }
};

/**
 * This method is called when the document is loaded.
 * The container element is a div where the plugin options will be displayed.
 * @summary After setting up the tmapp object, initialize it*/
Plugin_template.init = function (container) {
  interfaceUtils.alert("The plugin has been loaded");
};
```

(continues on next page)

(continued from previous page)

```
/**
 * This method is called when a button is clicked or a parameter value is changed*/
Plugin_template.inputTrigger = function (input) {
  console.log("inputTrigger", input);
  if (input === "_testButton") {
    let message = Plugin_template.get("_message");
    Plugin_template.demo(message);
  }
}

Plugin_template.demo = function (message) {
  let successCallback = function (data) {
    interfaceUtils.alert(data);
  };
  let errorCallback = function (data) {
    console.log("Error:", data);
  };
  // Call the Python API endpoint "server_demo"
  Plugin_template.api(
    "server_demo",
    {message: message},
    successCallback,
    errorCallback,
  );
};
```

Plugin parameters

The `parameters` object contains the parameters of your plugin. Each parameter is an object with the following properties:

- `label`: the text that will be displayed in front of the parameter.
- `type`: the type of parameter. It can be one of the following:
  - `button`: an input button.
  - `checkbox`: a checkbox input.
  - `text`: a text input.
  - `number`: a number input.
  - `select`: a dropdown list.
  - `label`: a text label.
  - `section`: a section that can be collapsed or expanded.
- `default`: the default value of the parameter [for `type` in `checkbox`, `number`, `select`, `text`].
- `options`: an array of options [for `type` in `select`].
- `title`: the title of a section [for `type` in `section`].
- `collapsed`: a boolean indicating if a section is collapsed or expanded by default [for `type` in `section`].

Javascript API

TissUUmaps offers helper functions to help you create your plugin:

- `My_plugin_name.set(paramName, value)`: set the value of a parameter.
- `My_plugin_name.get(paramName)`: get the value of a parameter.
- `My_plugin_name.getInputID(paramName)`: get the id of the html element associated with a parameter.
- `My_plugin_name.api(endpoint, data, successCallback, errorCallback)`: call a Python method from Javascript. The endpoint is the name of the method, and the data is an object that will be passed as a parameter to the method. The successCallback and errorCallback are functions that will be called after the method has been called. The successCallback will be called with the result of the method as a parameter, and the errorCallback will be called with the error message as a parameter.

You can access the complete TissUUmaps javascript API here.

Python file

You only need to use the Python file if your plugin needs to do processing on the server side. For pure javascript plugins, you can leave this file empty.

The python file should implement the class `Plugin`:

```
import logging
import time
from flask import abort, make_response

class Plugin:
    def __init__(self, app):
        self.app = app

    def server_demo(self, jsonParam):
        if not jsonParam:
            logging.error("No arguments, aborting.")
            abort(500)
        resp = make_response("The server received the message: " + jsonParam["message"])
        return resp
```

The `app` object being the flask application running the TissUUmaps server.

You can call a Python method inside the `Plugin` class from Javascript using Ajax and the Python API. The endpoint for a method `methodName` of the plugin `PluginName` will be: `/plugins/methodName/functionName`. Data can be transmitted through Ajax as stringified JSON, and will be available as a parameter inside the method.

See the Plugin Template for a working example of Javascript / Python communication.

2.7 Shortcuts

TissUUmaps contains following keyboard shorcuts:

- `M` - to toggle the markers
- `F` - to expand to fullscreen
- `0` - to hide right menu
- `R` - to toggle regions
- `Escape` - to exit any active region tool

When running TissUUmaps from the graphical interface, you can also use the following shortcuts:

- `Ctrl O` - to open a new image
- `Ctrl S` - to save the current project
- `Ctrl Q` - to quit TissUUmaps

CHAPTER THREE

SHARING PROJECTS

3.1 Apache server

TissUUmaps projects can be exported into static webpages, that can be uploaded to any Apache server.

1. Save your project from TissUUmaps (`menu > File > Save project`)
2. Export to static page (`menu > File > Export to static webpage`)
3. Copy the exported folder on your Apache server

3.2 Docker container

3.2.1 Start a TissUUmaps docker instance

1. Start the docker container `cavenel/tissuumaps:latest` from Docker Hub:

```
docker run -it -p 56733:80 --name=tissuumaps -v /path/to/local/images:/mnt/data cavenel/
↪tissuumaps:latest
```

1. Place your images in the local folder `/path/to/local/images/share`.
2. Open http://127.0.0.1:56733/ in your favorite browser.

3.2.2 Define TissUUmaps service in a Compose file

Compose is a tool for defining and running multi-container Docker applications. With Compose, you use a YAML file to configure your application's services. Then, with a single command, you create and start all the services from your configuration.

If you use compose, you don't need to start the docker container from the previous section.

1. Install docker-compose.
2. Create a file called `docker-compose.yml` in your project directory and paste the following:

```
version: "3.9"
services:
  backend:
    image: docker.io/cavenel/tissuumaps:latest
    volumes:
```

(continues on next page)

(continued from previous page)

```
      - type: bind
        source: /jail
        target: /mnt/data
    restart: on-failure
    ports:
      - 127.0.0.1:8050:80
```

This `Compose` file defines TissUUmaps backend service on port 8050. The web service uses an image that's built from docker.io hub. It then binds the container and the host machine to the exposed port, 8050.

3. Put your data in the source folder (here `/jail/shared/`). You can change the source path in the `docker-compose.yml` file.
4. From your project directory, start up your application by running `docker compose up`.

```
$ docker-compose up
Creating network "tmap_compose_default" with the default driver
Creating tmap_compose_backend_1 ... done
Attaching to tmap_compose_backend_1
backend_1  | [2023-03-27 11:23:57 +0000] [1] [INFO] Starting gunicorn 20.1.0
backend_1  | [2023-03-27 11:23:57 +0000] [1] [INFO] Listening at: http://0.0.0.0:80␣
↪(1)
backend_1  | [2023-03-27 11:23:57 +0000] [1] [INFO] Using worker: gevent
backend_1  | [2023-03-27 11:23:57 +0000] [7] [INFO] Booting worker with pid: 7
backend_1  | [2023-03-27 11:23:57 +0000] [8] [INFO] Booting worker with pid: 8
backend_1  | [2023-03-27 11:23:57 +0000] [9] [INFO] Booting worker with pid: 9
backend_1  | [2023-03-27 11:23:57 +0000] [10] [INFO] Booting worker with pid: 10
backend_1  | [2023-03-27 11:23:57 +0000] [11] [INFO] Booting worker with pid: 11
backend_1  | [2023-03-27 11:23:57 +0000] [12] [INFO] Booting worker with pid: 12
backend_1  | [2023-03-27 11:23:57 +0000] [13] [INFO] Booting worker with pid: 13
backend_1  | [2023-03-27 11:23:57 +0000] [14] [INFO] Booting worker with pid: 14
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
backend_1  | INFO:root: * TissUUmaps version: 3.1.0.1
```

5. Enter http://localhost:8050 in a browser to see TissUUmaps application running.

3.2.3 Configure sftp multi-user access (Optional)

Add the following lines to `/etc/ssh/sshd_config`:

```
Subsystem sftp internal-sftp
Match Group sftpusers
    ChrootDirectory /jail/shared/%u
    X11Forwarding no
    AllowTcpForwarding no
    ForceCommand internal-sftp
    PasswordAuthentication yes
```

Use the following bash script to create new sftp users (**as root**):

```
#! /bin/bash

read -p 'Username: ' uservar
# Use the path mounted in docker:
MOUNT_TMAP='/jail'

# Create sftpusers group and $uservar user
groupadd sftpusers
adduser --gecos "" --home $MOUNT_TMAP/shared/$uservar $uservar
usermod -G sftpusers $uservar

# Create home for user in $MOUNT_TMAP/shared/ with correct permissions
mkdir -p $MOUNT_TMAP/shared/$uservar/files
chmod 755 $MOUNT_TMAP/shared/$uservar/
chown root:root $MOUNT_TMAP/shared/$uservar/
chmod 750 $MOUNT_TMAP/shared/$uservar/files/
chown $uservar:$uservar $MOUNT_TMAP/shared/$uservar/files

# Make sure all files created in the files folder are writable by $uservar
setfacl -m d:u:$uservar:rwx $MOUNT_TMAP/shared/$uservar/files/

echo 'To access files placed in the /files sftp folder, use:'
echo '    http://TMAP_URL/FILENAME?path='$uservar'/files'
```

The new users will be able to connect through sftp, and only see the files in `/jail/shared/USER_NAME/files`. Files will be available on the web using `http://localhost:8050/MYFILE.tmap?path=/USER_NAME/files`

Restart the ssh daemon with `sudo service ssh restart`.

3.2.4 Password protection of files hosted on TissUUmaps docker

You can add login and password for any folder hosted on the TissUUmaps server by adding a `auth` file containing your login and password in the format:

```
login:password
```

CHAPTER

FOUR

ADVANCED USAGE

4.1 Jupyter notebooks

TissUUmaps can easily be used inside a Jupyter Notebook or Jupyter Lab.

Simple example to load an image in TissUUmaps:

```
import tissuumaps.jupyter as tj
viewer = tj.loaddata(["image.png"])

viewer.screenshot()
```

4.1.1 tissuumaps.jupyter

Module used to run TissUUmaps from a Jupyter Notebook or from Jupyter Lab.

`tissuumaps.jupyter.`**`opentmap`**(*path*, *port=5100*, *host='localhost'*, *height=700*)

Open a tmap project

Parameters

- **`path`** (*`str`*) – The path to a tmap file
- **`port`** (*`int`*) – The port to run the TissUUmaps server
- **`host`** (*`str`*) – The host to run the TissUUmaps server
- **`height`** (*`int`*) – The height of the jupyter iframe

Returns The TissUUmaps viewer

Return type *TissUUmapsViewer*

`tissuumaps.jupyter.`**`loaddata`**(*images=[]*, *csvFiles=[]*, *xSelector='x'*, *ySelector='y'*, *keySelector=None*, *nameSelector=None*, *colorSelector=None*, *piechartSelector=None*, *shapeSelector=None*, *scaleSelector=None*, *fixedShape=None*, *scaleFactor=1*, *colormap=None*, *compositeMode='source-over'*, *boundingBox=None*, *port=5100*, *host='localhost'*, *height=700*, *tmapFilename='_project'*, *plugins=[]*)

Load data in TissUUmaps

Parameters

- **`images`** (*`list | str`*) – List of images or single image to display
- **`csvFiles`** (*`list | str`*) – List of csv files or single csv file to display

- **`xSelector`** (*str*) – Name of the csv column defining the X coordinates
- **`ySelector`** (*str*) – Name of the csv column defining the Y coordinates
- **`keySelector`** (*str*) – Name of the csv column defining the grouping key
- **`nameSelector`** (*str*) – Name of the csv column defining the group name
- **`colorSelector`** (*str*) – Name of the csv column defining the group color
- **`piechartSelector`** (*str*) – Name of the csv column defining pie-charts
- **`shapeSelector`** (*str*) – Name of the csv column defining markers' shape
- **`scaleSelector`** (*str*) – Name of the csv column defining markers' scale
- **`fixedShape`** (*int*) – Name of the markers' shape
- **`scaleFactor`** (*int*) – Global scale of markers
- **`colormap`** (*str*) – Name of the colormap used if colorSelector is set
- **`compositeMode`** – (str): Composite mode used for images
- **`boundingBox`** (*list*) – [X,Y,W,H] of the bounding box to display
- **`port`** (*int*) – The port to run the TissUUmaps server
- **`host`** (*str*) – The host to run the TissUUmaps server
- **`height`** (*int*) – The height of the jupyter iframe
- **`tmapFilename`** (*str*) – Name of the project file that will be created
- **`plugins`** (*list*) – List of plugins to add to the tmap project

Returns The TissUUmaps viewer

Return type *TissUUmapsViewer*

`class tissuumaps.jupyter.`**`TissUUmapsViewer`**`(`*server*, *image*, *height=700*`)`

Class representing a TissUUmaps viewer instance

`screenshot()`

Capture the TissUUmaps viewport and display image in the Notebook.

`class tissuumaps.jupyter.`**`TissUUmapsServer`**`(`*slideDir*, *port=5000*, *host='0.0.0.0'*`)`

Class representing a TissUUmaps server instance

4.2 Napari

Napari features an important hub containing 118 plugins at the time of writing, many of them expanding further the capabilities of Napari when dealing with biomedical imaging. We thus created our own plugin to allow users to work in Napari, benefit from the tools, scripting and existing plugins, and easily visualize and share the output of their research through TissUUmaps.

The Napari-TissUUmaps plugin is available on Napari Hub which makes the installation trivial: from the Napari install/uninstall plugins menu, the `napari-tissuumaps` appears in the list and can be installed with a single click. Alternatively, the plugin can be installed with the Python package manager: `pip install napari-tissuumaps`.

The plugin can export all standard Napari layers, such as images, labels, points, and shapes and preserves the metadata (opacity, visibility), but also the objects parameters (e.g.: label colors, marker colors and symbols, etc…). To export a TissUUmaps project, care must be taken to save all layers of interest and type in a name with the extension `.tmap`, e.g.: `myProject.tmap`. This is important for Napari to delegate the saving of the files to the plugin. A folder is created and contains all the necessary files and can be loaded in the TissUUmaps server, software, Jupyter Notebook, or shared with the community.

The project folders generated by the plugin contain the metadata in a `main.tmap` file, along with folders for each Napari layer types: images, labels, points and regions. Images and labels are saved as plain tif images, points are saved as CSV files, and shapes are saved as GeoJSON. We hope that the use of a simple structure and widespread file formats can simplify the modifying and updating of the TissUUmaps project when prototyping with e.g. Jupyter Notebooks. The source code is available at https://github.com/TissUUmaps/napari-tissuumaps under the permissive MIT license. A demonstration of the Cellpose plugin of Napari being exported to the TissUUmaps web viewer is available at: https://tissuumaps.github.io/tutorials/#napari.

4.3 AnnData Handling in TissUUmaps

To visualize and manipulate annotated data matrices, TissUUmaps leverages the capabilities of the **AnnData** (Annotated Data) Python package, stored in the h5ad format. AnnData provides a flexible and efficient framework for working with annotated data in memory and on disk, bridging the functionality of pandas and xarray. Notable features of AnnData include support for sparse data, lazy operations, and a PyTorch interface.

4.3.1 Loading AnnData Objects

You can load AnnData objects in the same way as you would load TissUUmaps projects or images, by dragging and dropping the file into the TissUUmaps interface. TissUUmaps will automatically detect the file type and load the AnnData object into the interface. You can also load AnnData objects by clicking on the “Open” button in the “File” menu and selecting the file from your computer.

4.3.2 AnnData Specification in TissUUmaps

TissUUmaps adheres to the specifications defined by the SCVerse community for AnnData objects. TissUUmaps can load AnnData objects stored in the HDF5 format.

The key components of the AnnData structure in TissUUmaps are as follows:

Spatial Coordinates

Spatial coordinates are crucial for spatial omics analysis. In TissUUmaps, these coordinates are stored as a 2-column matrix in one of the following locations:

- `/obsm/spatial`
- `/obsm/X_spatial`
- `/obsm/X_umap`
- `/obsm/tSNE`
- `/obsm/UMAP`

Observations

Observations are stored in the `/obs` section of the AnnData object. This section contains information related to individual observations.

Gene Expressions

Gene expressions are stored in the `X` section of the AnnData object. It's important to note that the gene expression matrix should be in sparse Compressed Sparse Column (CSC) format. If the matrix is not in CSC format, TissUUmaps will create a new AnnData object with the gene expression matrix in CSC sparse format.

Variable Information

Variable information is stored in the `/var` section of the AnnData object.

Image Loading

TissUUmaps is equipped to load images stored in the following location:

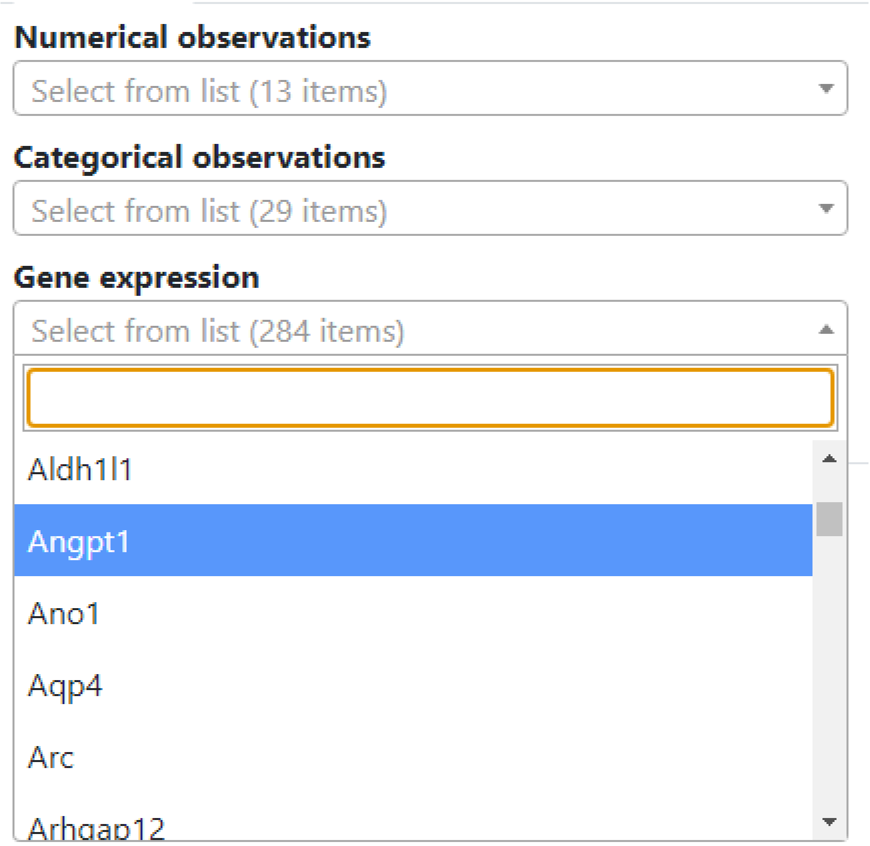
- `/uns/spatial/{library_id}/images/hires`

Here, `{library_id}` is an observation column obtained from `/obs/library_id`. Additionally, TissUUmaps utilizes a scale factor from `/uns/spatial/{library_id}/scalefactors/tissue_hires_scalef`.

4.3.3 Dropdowns

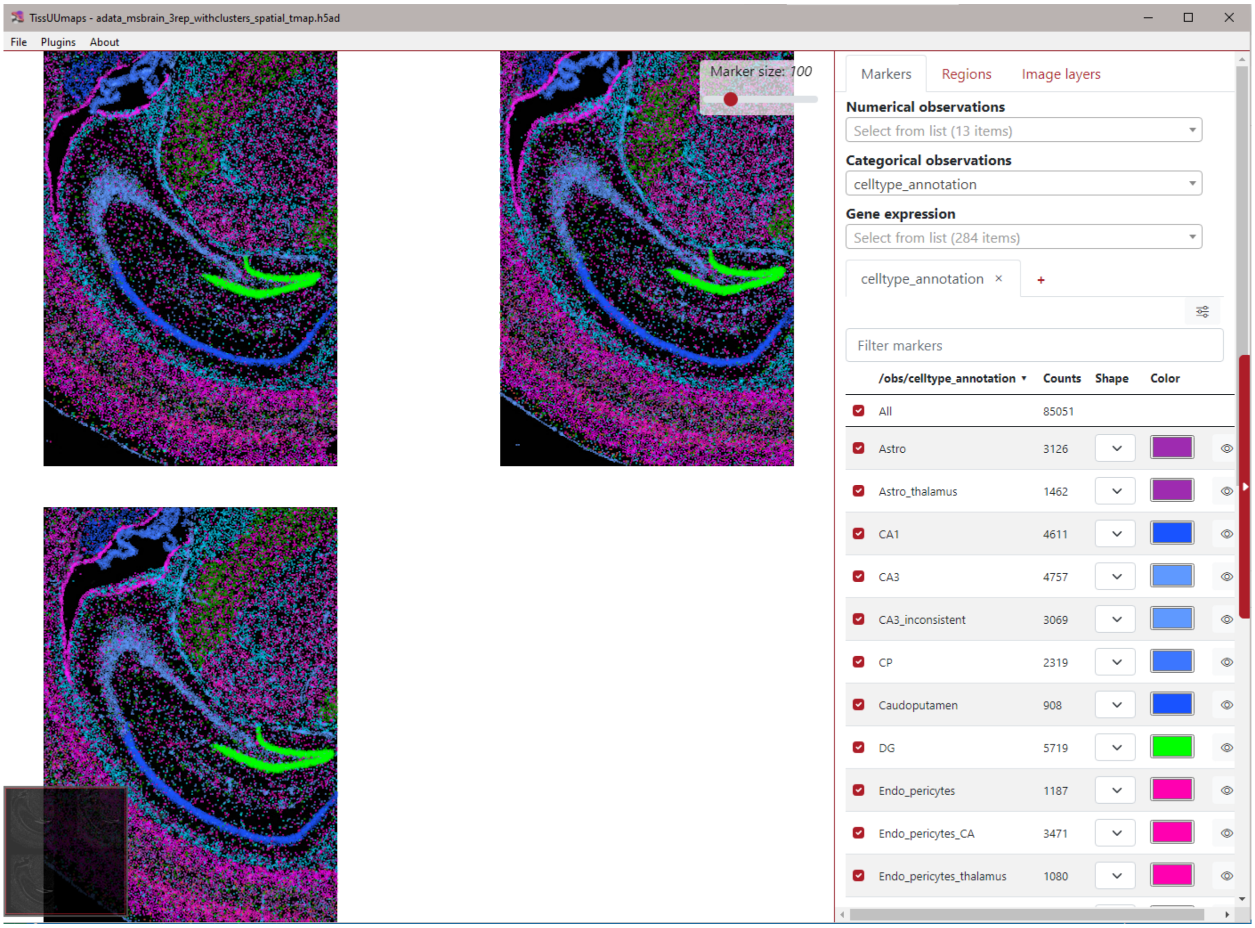
Once loaded into TissUUmaps, each observation is accessible through dropdown menus. There are separate dropdowns for numerical and categorical observations, providing a convenient interface for users to explore and analyze their spatial omics data.

The gene expression dropdown menu contains the X matrix.



4.3.4 Example

Here is an example of the TissUUmaps interface when loading an AnnData object with three samples. The dropdown menus are populated with the observations from the AnnData object. Here, the “celltype_annotation” is selected.



Numerical observations: Select from list (13 items)

Categorical observations: celltype_annotation

Gene expression: Select from list (284 items)

celltype_annotation ×

Filter markers

	/obs/celltype_annotation	Counts	Shape	Color
☑	All	85051		
☑	Astro	3126		
☑	Astro_thalamus	1462		
☑	CA1	4611		
☑	CA3	4757		
☑	CA3_inconsistent	3069		
☑	CP	2319		
☑	Caudoputamen	908		
☑	DG	5719		
☑	Endo_pericytes	1187		
☑	Endo_pericytes_CA	3471		
☑	Endo_pericytes_thalamus	1080		

4.4 The TMAP file format

The TMAP format contains a description of image layers, markers, regions, and settings. It is highly recommended to create .tmap files by saving projects from TissUUmaps, but you can also edit the files manually to add or change projects' settings, or generate them as exported data from other software for import in TissUUmaps.

For more information on the TMAPS format, see the TissUUmaps-schema github page.

The TMAP format uses JSON, with the following specifications:

type	*object*		
properties			
• schemaVersion	type	*string*	
	default	1.2	
• filename	Name of the project.		
	default	null	
	anyOf	type	*string*
		type	*null*
• link	Url to a publication or other external resource: a click on the filename will open this link.		
	default	null	
	anyOf	type	*string*

continues on next page

Table 1 – continued from previous page

		type	*null*
• layers	type	*array*	
	default		
	items	*Layer*	
• layerOpacities	type	*object*	
	default		
	additionalProperties	type	*number*
• layerVisibilities	type	*object*	
	default		
	additionalProperties	type	*boolean*
• layerFilters	Image filters to be applied to pixels in image layers.		
	type	*object*	
	default		
	additionalProperties	type	*array*
		items	*LayerFilter*
• filters	List of filters shown as active filters in the GUI under the Image layers tab.		
	type	*array*	
	default	Saturation	
		Brightness	
		Contrast	
	items	*Filter*	
• compositeMode	Mode defining how image layers will be merged (composited) with each other. Valid string values are 'source-over' and 'lighter', which correspond to 'Channels' and 'Composite' in the GUI.		
	default	source-over	
	allOf	*CompositeMode*	
• collectionLayout	Options to be passed to OpenSeadragon arrange method when in collectionmode. See (https://openseadragon.github.io/docs/OpenSeadragon.World.html#arrange)		
	default	null	
	anyOf	*CollectionLayout*	
		type	*null*
• mpp	The image scale in Microns Per Pixels. If not null, then adds a scale bar to the viewer. Set to 0 to display the scale bar in pixels.		
	default	null	
	anyOf	type	*number*
		type	*null*
• boundingBox	Bounding box used to set initial zoom and pan on the view when loading the project.		
	default	null	
	anyOf	*BoundingBox*	
		type	*null*
• rotate	Angle of rotation of the view in degrees. Only multiples of 90 degrees are supported.		
	type	*integer*	
	default	0	
• markerFiles	type	*array*	
	default		
	items	*MarkerFile*	
• regions	GeoJSON object.		
	type	*object*	
	default		
• regionFile	**[Deprecated]** GeoJSON region file loaded on project initialization. Use regionFiles instead.		
	default	null	

continues on next page

Table 1 – continued from previous page

	anyOf	type	*string*
		type	*null*
- regionFiles	type	*array*	
	default		
	items	*RegionFile*	
- plugins	List of plugins to load with the project.		
	type	*array*	
	default		
	items	type	*string*
- hideTabs	Hide tabs of markers dataset. Only use when you have a unique marker tab.		
	type	*boolean*	
	default	False	
- hideChannelRange	Hide input range of channels. Only use when you have a unique image layer.		
	type	*boolean*	
	default	False	
- hideNavigator	Hide navigator of the viewer.		
	type	*boolean*	
	default	False	
- collectionMode	If true, then the viewer will be in collection mode, which puts all layers in a grid next to each other.		
	type	*boolean*	
	default	False	
- backgroundColor	Background color of the viewer.		
	default	null	
	anyOf	type	*string*
		type	*null*
- menuButtons	List of menu items to be added to the menu bar.		
	default	null	
	anyOf	type	*array*
		items	*menuButton*
		type	*null*
- settings	type	*array*	
	default		
	items	*Setting*	
additionalProperties	False		

4.4.1 BoundingBox

type	*object*	
properties		
- **x**	Left coordinate of the bounding box in viewport coordinate.	
	type	*number*
- **y**	Top coordinate of the bounding box in viewport coordinate.	
	type	*number*
- **width**	Width of the bounding box in viewport coordinate.	
	type	*number*
- **height**	Height of the bounding box in viewport coordinate.	
	type	*number*
additionalProperties	False	

4.4.2 CollectionLayout

type	*object*		
properties			
• immediately	Whether to animate to the new arrangement.		
	default	False	
	anyOf	type	*boolean*
		type	*null*
• layout	See collectionLayout in OpenSeadragon.Options.		
	default	null	
	anyOf	*LayoutAxis*	
		type	*null*
• rows	See collectionRows in OpenSeadragon.Options.		
	default	null	
	anyOf	type	*integer*
		type	*null*
• columns	See collectionColumns in OpenSeadragon.Options.		
	default	null	
	anyOf	type	*integer*
		type	*null*
• tileSize	See collectionTileSize in OpenSeadragon.Options.		
	default	null	
	anyOf	type	*number*
		type	*null*
• tileMargin	See collectionTileMargin in OpenSeadragon.Options.		
	default	null	
	anyOf	type	*number*
		type	*null*
additionalProperties	False		

4.4.3 CompositeMode

type	*string*
enum	source-over, lighter, darken, source-atop, source-in, source-out, destination-over, destination-atop, destination-in, destination-out, copy, xor, multiply, screen, overlay, color-dodge, color-burn, hard-light, soft-light, difference, exclusion, hue, saturation, color, luminosity

4.4.4 DropdownOption

type	*object*	
properties		
• **optionName**	Name displayed in the dropdown menu.	
	type	*string*
• **name**	Name of the tab to be loaded when the option is selected.	
	type	*string*
additionalProperties	True	

4.4.5 ExpectedHeader

type	*object*			
properties				
• **X**	Name of CSV column to use as X-coordinate.			
	type	*string*		
• **Y**	Name of CSV column to use as Y-coordinate.			
	type	*string*		
• gb_col	Name of CSV column to use as key to group markers by.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• gb_name	Name of CSV column to display for groups instead of group key.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• cb_cmap	Name of D3 color scale to be used for color mapping.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• cb_col	Name of CSV column containing scalar values for color mapping or hexadecimal RGB colors in format ‘#ff0000’.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• cb_gr_dict	JSON string specifying a custom dictionary for mapping group keys to group colors. Example: `"{'key1': '#ff0000', 'key2': '#00ff00', 'key3': '#0000ff'}"`.			
	default			
	anyOf	type	*string*	
		type	*object*	
		type	*array*	
		items	type	*string*
• scale_col	Name of CSV column containing scalar values for changing the size of markers.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• scale_factor	Numerical value for a fixed scale factor to be applied to markers.			
	type	*number*		
	default	1.0		
• coord_factor	Numerical value for a fixed scale factor to be applied to marker coordinates.			
	type	*number*		
	default	1.0		
• pie_col	Name of CSV column containing data for pie chart sectors. TissUUmaps expects labels and numerical values for sectors to be separated by ‘:’ characters in the CSV column data.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• pie_dict	JSON string specifying a custom dictionary for mapping pie chart sector indices to colors. Example: `"{0: '#ff0000', 1: '#00ff00', 2: '#0000ff'}"`. If no dictionary is specified, TissUUmaps will use a default color palette instead.			
	default			
	anyOf	type	*string*	

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Table 2 – continued from previous page

		type	*object*	
		type	*array*	
		items	type	*string*
• shape_col	Name of CSV column containing a name or an index for marker shape.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• shape_fixed	Name or index of a single fixed shape to be used for all markers.			
	type	*string*		
	default	cross		
• shape_gr_dict	JSON string specifying a custom dictionary for mapping group keys to group shapes. Example: `"{'key1': 'square', 'key2': 'diamond', 'key3': 'triangle up'}"`.			
	default			
	anyOf	type	*string*	
		type	*object*	
		type	*array*	
		items	type	*string*
• edges_col	Name of CSV column containing a name or an index for marker edges in Network Diagram mode.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• collectionItem_col	Name of CSV column containing a name or an index for marker collection items in Collection mode.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• collectionItem_fixed	Name or index of a single fixed collection item to be used for all markers in Collection mode.			
	default	0		
	anyOf	type	*string*	
		type	*integer*	
• opacity_col	Name of CSV column containing scalar values for opacities.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• opacity	Numerical value for a fixed opacity factor to be applied to markers.			
	type	*number*		
	default	1.0		
• stroke_width	Numerical value for the marker stroke width.			
	type	*number*		
	default	2.5		
• sortby_col	Name of CSV column containing scalar values for sorting markers.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• z_order	Numerical value of z-order to be used for all markers.			
	type	*number*		
	default	1.0		
• tooltip_fmt	Custom formatting string used for displaying metadata about a selected marker. See https://github.com/TissUUmaps/TissUUmaps/issues/2 for an overview of the grammer and keywords. If no string is specified, TissUUmaps will show default metadata depending on the context.			

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Table 2 – continued from previous page

	type	*string*
	default	
additionalProperties	False	

4.4.6 ExpectedRadios

type	*object*	
properties		
• cb_col	If markers should be colored by data in CSV column.	
	type	*boolean*
	default	False
• cb_gr	If markers should be colored by group.	
	type	*boolean*
	default	True
• cb_gr_rand	If group color should be generated randomly.	
	type	*boolean*
	default	False
• cb_gr_dict	If group color should be read from custom dictionary.	
	type	*boolean*
	default	False
• cb_gr_key	If group color should be generated from group key.	
	type	*boolean*
	default	True
• pie_check	If markers should be rendered as pie charts.	
	type	*boolean*
	default	False
• scale_check	If markers should be scaled by data in CSV column.	
	type	*boolean*
	default	False
• shape_col	If markers should get their shape from data in CSV column.	
	type	*boolean*
	default	False
• shape_gr	If markers should get their shape from group.	
	type	*boolean*
	default	True
• shape_gr_rand	If group shape should be generated randomly.	
	type	*boolean*
	default	True
• shape_gr_dict	If group shape should be read from custom dictionary.	
	type	*boolean*
	default	False
• shape_fixed	If a single fixed shape should be used for all markers.	
	type	*boolean*
	default	False
• opacity_check	If markers should get their opacities from data in CSV column.	
	type	*boolean*
	default	False
• _no_outline	If marker shapes should be rendered without outline.	
	type	*boolean*

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Table 3 – continued from previous page

	default	False
• no_fill	If marker shapes should be rendered without filling.	
	type	*boolean*
	default	False
• collectionItem_col	If markers should get their collection item from data in CSV column.	
	type	*boolean*
	default	False
• collectionItem_fixed	If a single fixed collection item should be used for all markers.	
	type	*boolean*
	default	True
• sortby_check	If markers should be sorted by data in CSV column.	
	type	*boolean*
	default	False
• sortby_desc_check	If markers should be sorted in descending order.	
	type	*boolean*
	default	False
• edges_check	If markers should be connected by edges in Network Diagram mode.	
	type	*boolean*
	default	False
additionalProperties	False	

4.4.7 Filter

type	*string*
enum	Color, Brightness, Exposure, Hue, Contrast, Vibrance, Noise, Saturation, Gamma, Invert, Greyscale, Threshold, Erosion, Dilation, SplitChannel, Colormap

4.4.8 Layer

type	*object*		
properties			
• **name**	Name of the image layer		
	type	*string*	
• **tileSource**	Relative path to an image file in a supported format.		
	type	*string*	
• x	Left coordinate of the image in viewport coordinate.		
	default	null	
	anyOf	type	*number*
		type	*null*
• y	Top coordinate of the image in viewport coordinate.		
	default	null	
	anyOf	type	*number*
		type	*null*
• rotation	Rotation of the image in degrees.		
	default	null	
	anyOf	type	*number*
		type	*null*
• flip	Flip the image horizontally.		
	type	*boolean*	
	default	False	
• scale	Scale of the image.		
	default	null	
	anyOf	type	*number*
		type	*null*
• clip	Bounding box used to clip image in image pixel coordinate. If not specified, the whole image is shown.		
	default	null	
	anyOf	*LayerClip*	
		type	*null*
additionalProperties	False		

4.4.9 LayerClip

type	*object*	
properties		
• **x**	Left coordinate of the clip in image pixel coordinate.	
	type	*number*
• **y**	Top coordinate of the clip in image pixel coordinate.	
	type	*number*
• **w**	Width of the clip in image pixel coordinate.	
	type	*number*
• **h**	Height of the clip in image pixel coordinate.	
	type	*number*
additionalProperties	False	

4.4.10 LayerFilter

type	*object*		
properties			
• **name**	Filter name.		
	allOf	*Filter*	
• **value**	Filter parameter.		
	anyOf	type	*string*
		type	*boolean*
		type	*integer*
		type	*number*
additionalProperties	False		

4.4.11 LayoutAxis

type	*string*
enum	horizontally, vertically

4.4.12 MarkerFile

type	*object*		
properties			
• comment	Optional description text shown next to marker button.		
	default	null	
	anyOf	type	*string*
		type	*null*
• name	Name of marker tab.		
	default	null	
	anyOf	type	*string*
		type	*null*
• autoLoad	If the CSV file for the marker dataset should be automatically loaded when the TMAP project is opened. If this is false, the user instead has to click on the marker button in the GUI to load the dataset. If this is an integer, the n-th marker dataset is automatically loaded.		
	default	False	
	anyOf	type	*boolean*
		type	*integer*
• hideSettings	Hide markers' settings and add a toggle button instead.		
	type	*boolean*	
	default	False	
• uid	A unique identifier used internally by TissUUmaps to reference the marker dataset.		
	default	null	
	anyOf	type	*string*
		type	*null*
• **expectedHeader**	*ExpectedHeader*		

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Table 4 – continued from previous page

• expectedRadios	*ExpectedRadios*			
• **path**	Relative file path to CSV file in which marker data is stored. If array of string, then a dropdown is created instead of a button.			
	anyOf	type	*string*	
		type	*array*	
		items	type	*string*
• dropdownOptions	List of dropdown options. Each option is a dictionary with the keys ‘title’ and ‘path’.			
	default	null		
	anyOf	type	*array*	
		items	*DropdownOption*	
		type	*null*	
• settings	type	*array*		
	default			
	items	*Setting*		
• fromButton	If this is an integer, then the marker dataset is loaded from the n-th marker button.			
	default	null		
	anyOf	type	*integer*	
		type	*null*	
additionalProperties	False			

4.4.13 RegionFile

type	*object*			
properties				
• comment	Optional description text shown next to region button.			
	default	null		
	anyOf	type	*string*	
		type	*null*	
• autoLoad	If the regions should be automatically loaded when the TMAP project is opened. If this is false, the user instead has to click on the region button in the GUI to load the regions.			
	type	*boolean*		
	default	False		
• **path**	Relative file path to GeoJSON file in which marker data is stored. If array of string, then a dropdown is created instead of a button.			
	anyOf	type	*string*	
		type	*array*	
		items	type	*string*
• settings	type	*array*		
	default			
	items	*Setting*		
additionalProperties	False			

4.4.14 Setting

type	*object*	
properties		
• **module**	Module where the function or property lies.	
	type	*string*
• **function**	Function or property of the given module.	
	type	*string*
• **value**		
additionalProperties	False	

4.4.15 menuButton

type	*object*			
properties				
• **text**	Text of the menu item. If list, then a nested menu is created.			
	anyOf	type	*array*	
		items	type	*string*
		type	*string*	
• **url**	Url of the menu item.			
	type	*string*		
additionalProperties	False			

4.4.16 Example of a .tmap file

```
{
    "filename": "TissUUmaps_Example.tmap",
    "layers": [
        {
            "name": "Round1_A.tif",
            "tileSource": "images/Round1_A.tif.dzi"
        },
        {
            "name": "Round1_C.tif",
            "tileSource": "images/Round1_C.tif.dzi"
        }
    ],
    "layerOpacities": {
        "0": "1",
        "1": "1"
    },
    "layerVisibilities": {
        "0": true,
        "1": false,
    },
    "layerFilters": {
        "0": [
            {
                "name": "Color",
```

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```
                "value": "0,100,0"
            }
        ],
        "1": [
            {
                "name": "Color",
                "value": "0,100,0"
            }
        ]
    },
    "filters": [
        "Color"
    ],
    "compositeMode": "lighter",
    "markerFiles": [
        {
            "autoLoad": false,
            "comment": "",
            "expectedHeader": {
                "X": "global_x",
                "Y": "global_y",
                "cb_cmap": "",
                "cb_col": "null",
                "cb_gr_dict": "",
                "gb_col": "Gene",
                "gb_name": "",
                "opacity": "1",
                "opacity_col": "null",
                "pie_col": "null",
                "pie_dict": "",
                "scale_col": "null",
                "scale_factor": "0.5",
                "shape_col": "null",
                "shape_fixed": "cross",
                "shape_gr_dict": "",
                "tooltip_fmt": ""
            },
            "expectedRadios": {
                "cb_col": false,
                "cb_gr": true,
                "cb_gr_dict": false,
                "cb_gr_key": true,
                "cb_gr_rand": false,
                "pie_check": false,
                "scale_check": false,
                "shape_col": false,
                "shape_fixed": false,
                "shape_gr": true,
                "shape_gr_dict": false,
                "shape_gr_rand": true,
                "opacity_check": false
            },
```

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```
            "name": " markers",
            "path": "./istdeco_codes_n.csv",
            "title": "Download markers",
            "uid": "uniquetab"
        }
    ],
    "regions": {},
    "plugins": [
        "Spot_Inspector"
    ],
    "hideTabs": true,
    "settings": []
}
```

CHAPTER

FIVE

SUPPORT

5.1 How to seek support and discussion

If you want to ask questions about TissUUmaps please visit forum.image.sc.

5.2 How to issue an error on GitHub

If you want to report a bug, please do so at issues on GitHub.

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