
TissUUmaps

Release 3.0

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This page hosts the documentation for TissUUmaps 3.0. 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!

This page is mostly empty for now. We are working actively on writing this documentation, more content will be available soon!

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 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 PIP installation (for Linux and Mac)

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 libvips  
conda activate tissuumaps_env
```

2. Install the TissUUmaps library using pip:

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

3. Start the TissUUmaps user interface:

```
tissuumaps
```

4. 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 3.0.8.9

- 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

1.4.2 3.0.8.5

- Minor fixes.

1.4.3 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.

1.4.4 3.0.8.3

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

1.4.5 3.0.8.2

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

1.4.6 3.0.8.1

- Fix multiple dataset alignment when no background image

1.4.7 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

1.4.8 3.0.7

- Add menu to load plugins through an update-site

1.4.9 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

1.4.10 3.0.5

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

1.4.11 3.0.4

- Add filtering of markers

1.4.12 3.0

- Add tissuumaps.jupyter module

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:

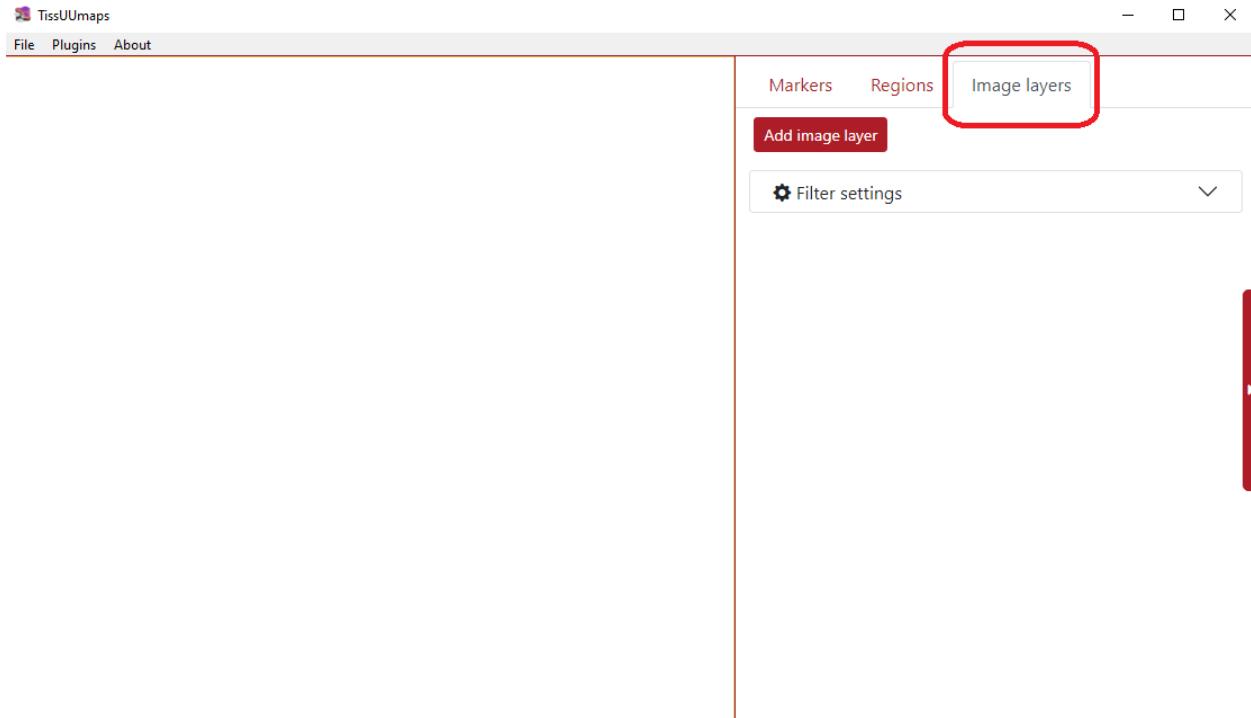
- Aperio (.svs, .tif)
- Hamamatsu (.ndpi, .vms, .vmu)
- Leica (.scn)
- MIRAX (.mrxs)
- Philips (.tiff)
- Sakura (.svslide)
- Trestle (.tif)
- Ventana (.bif, .tif)
- Generic tiled TIFF (.tif)

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

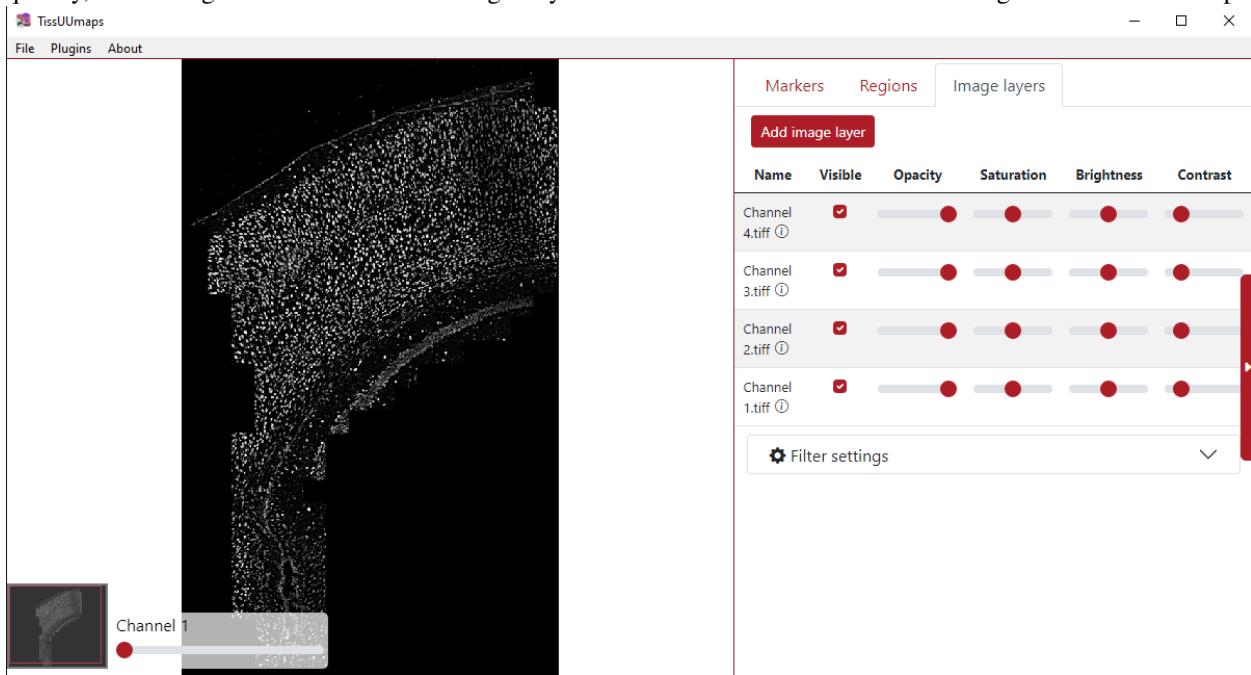
If your image fails to open, try converting it to `tif` format using an external tool.

2.1.2 Load images

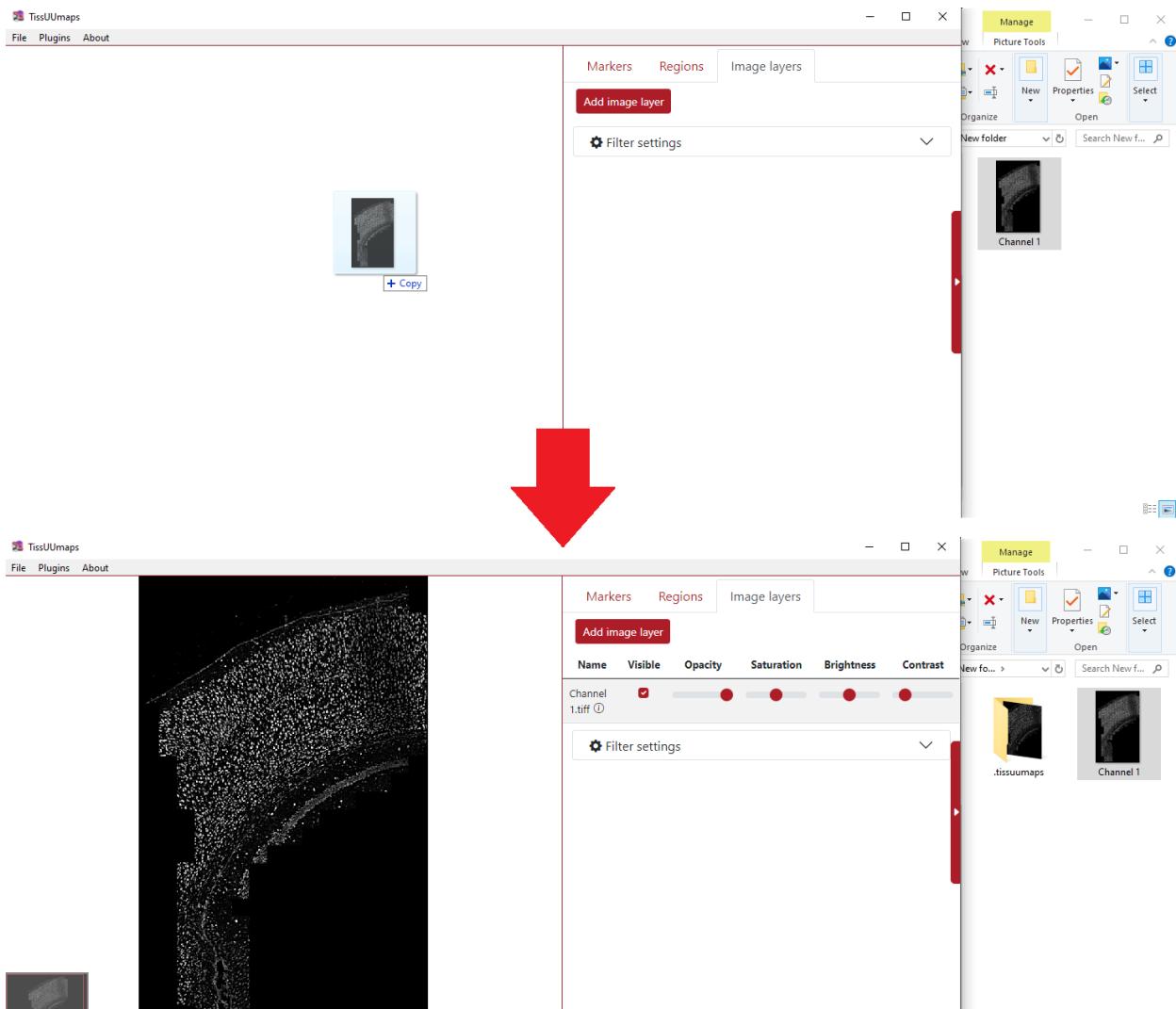
You can load the images when you select the Image layer tab as you can see in the figure below:



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 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.tif (i)	<input checked="" type="checkbox"/>	<input type="range"/>	<input type="range"/>	<input type="range"/>	<input type="range"/>

Filter settings

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

Merging mode:

Channels

2.2 Markers

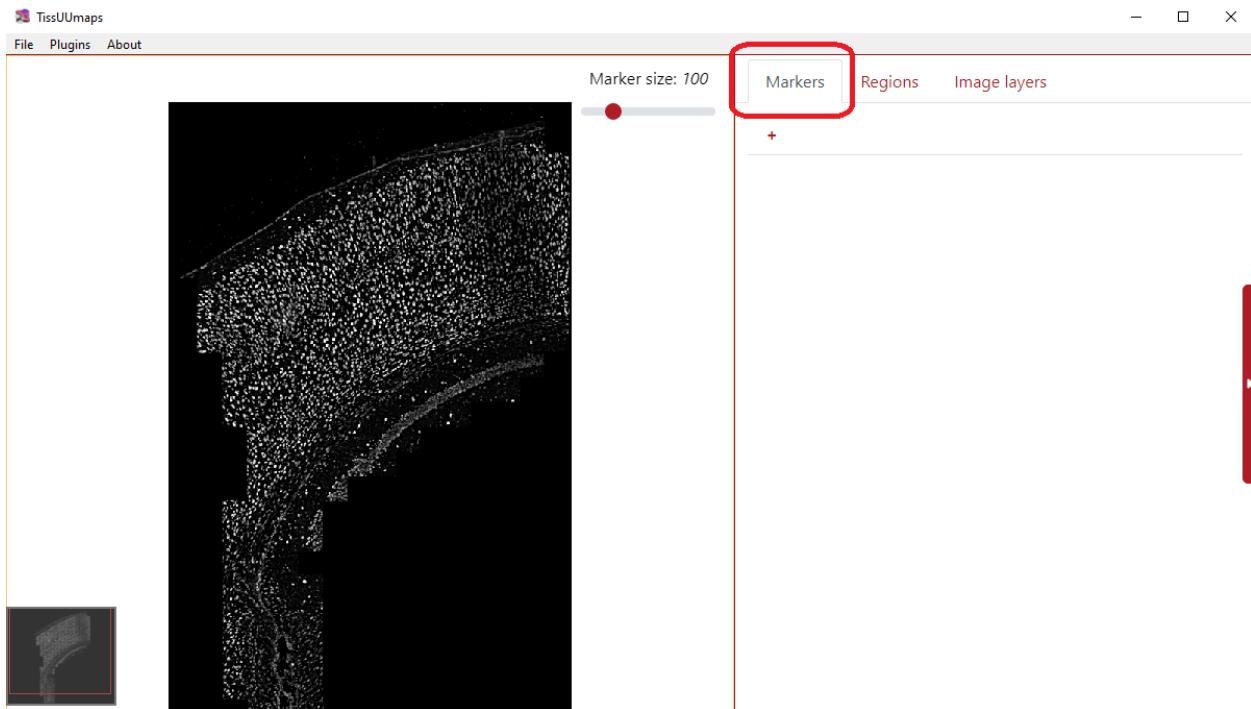
2.2.1 Supported marker format

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.)

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.



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*.

New markers **x** +

File and coordinates ^

File and coordinates

Choose File 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.

Genes x +

File and coordinates ^

File and coordinates

Choose File 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.

Genes x +

File and coordinates ^

File and coordinates

Choose File coordinates.csv

Tab name Genes

X coordinate ----

Y coordinate Y

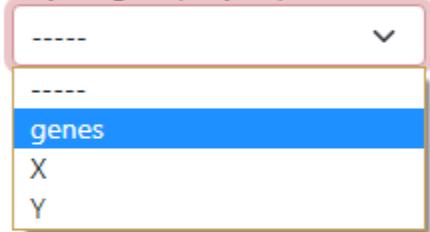
Render options

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.

Render options ^

Group by

Key to group by (optional)



Color by group Generate color from key value
 Color by marker 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.

Render options ^

Group by

Key to group by (optional)



Extra column to display (optional)



Color by group Generate color from key value
 Color by marker Generate color randomly
 Use color from dictionary
 {'key1':'#FFFFFF',...}

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

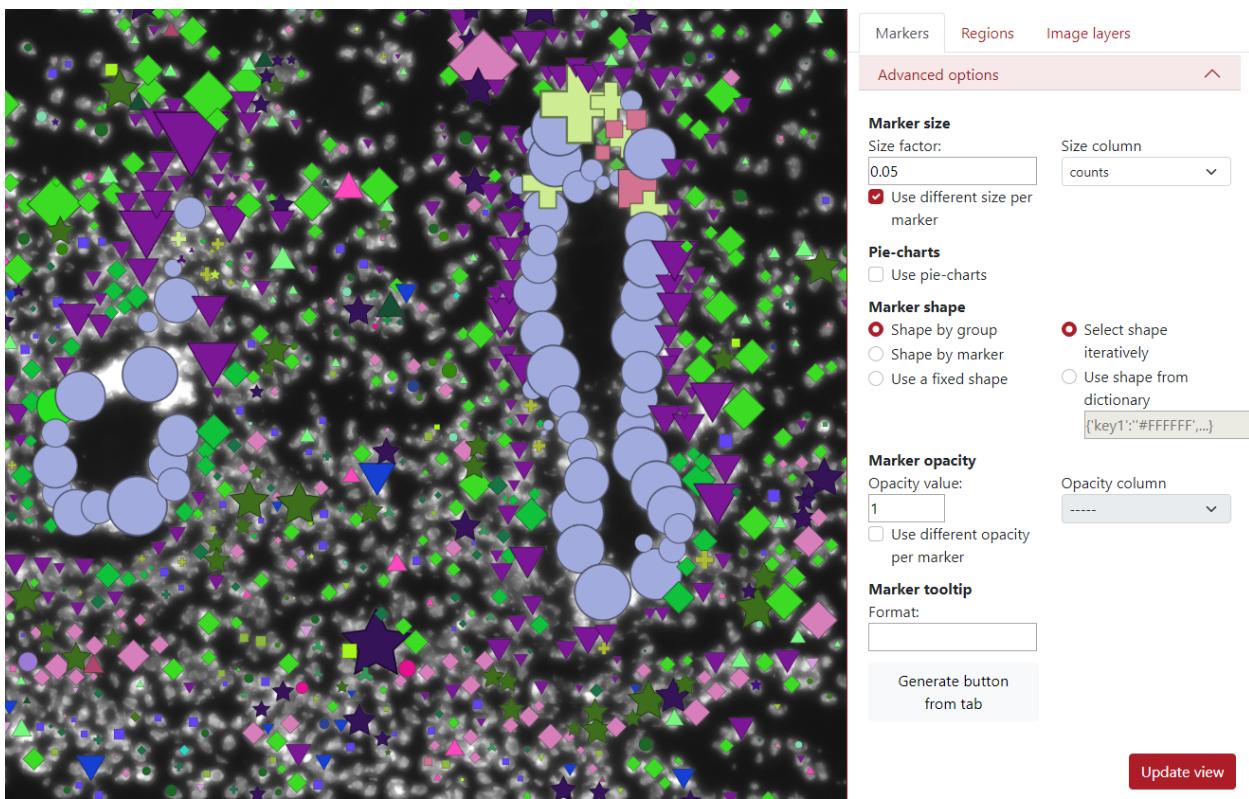
Size factor:

1

Size column

Use different size per marker

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.



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

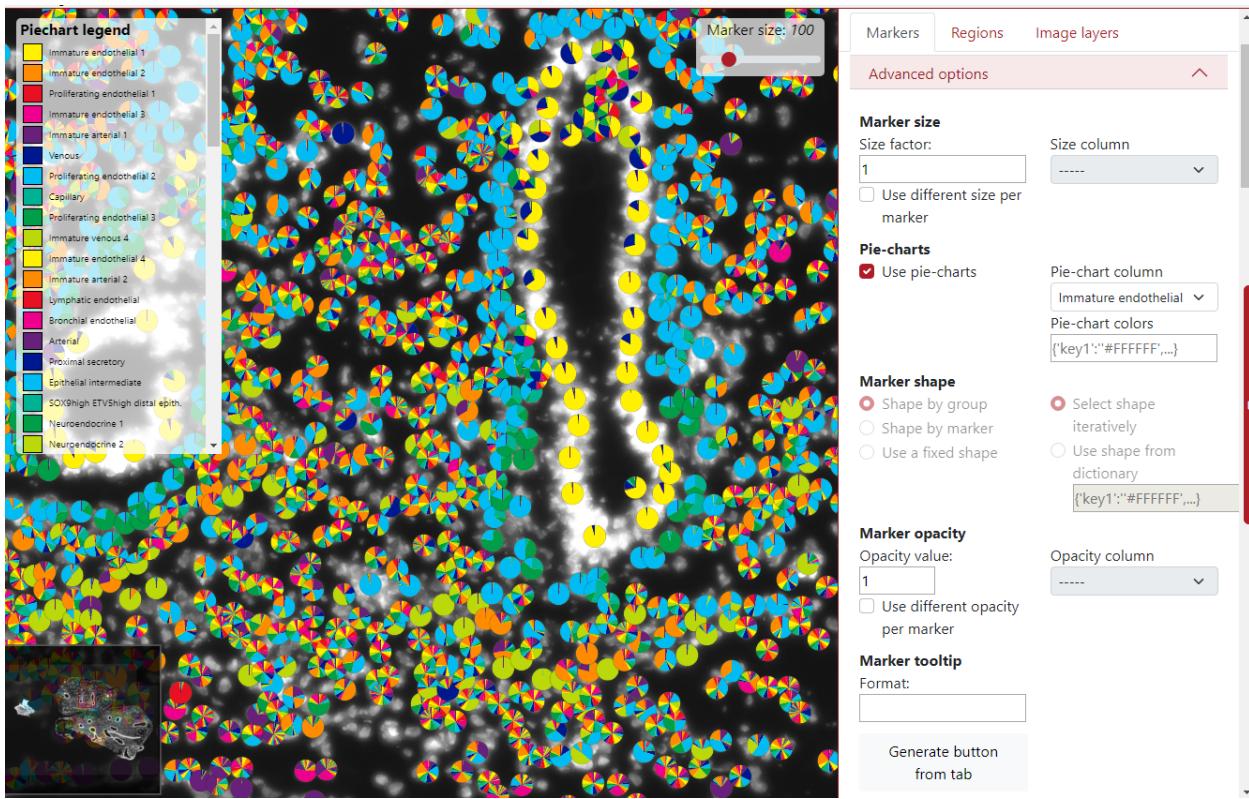
Use pie-charts

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.



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

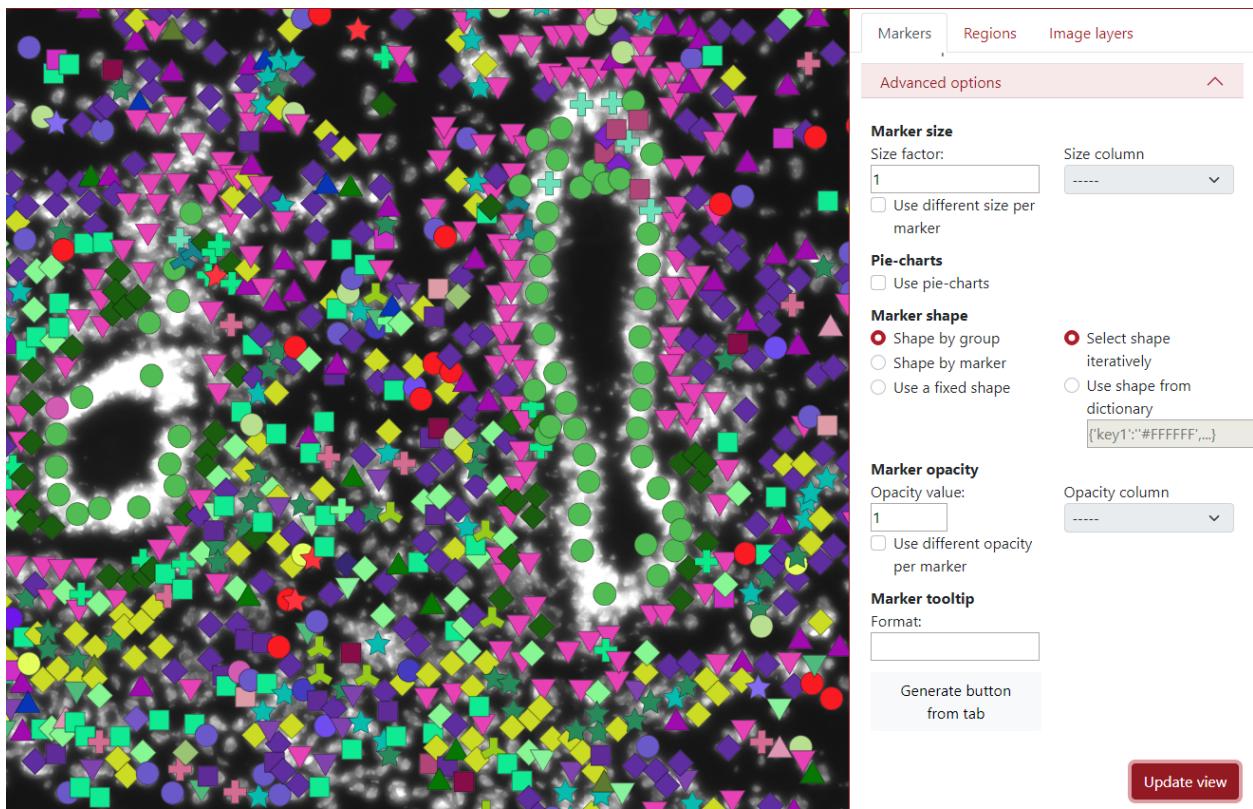
- Shape by group
- Shape by marker
- Use a fixed shape

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.



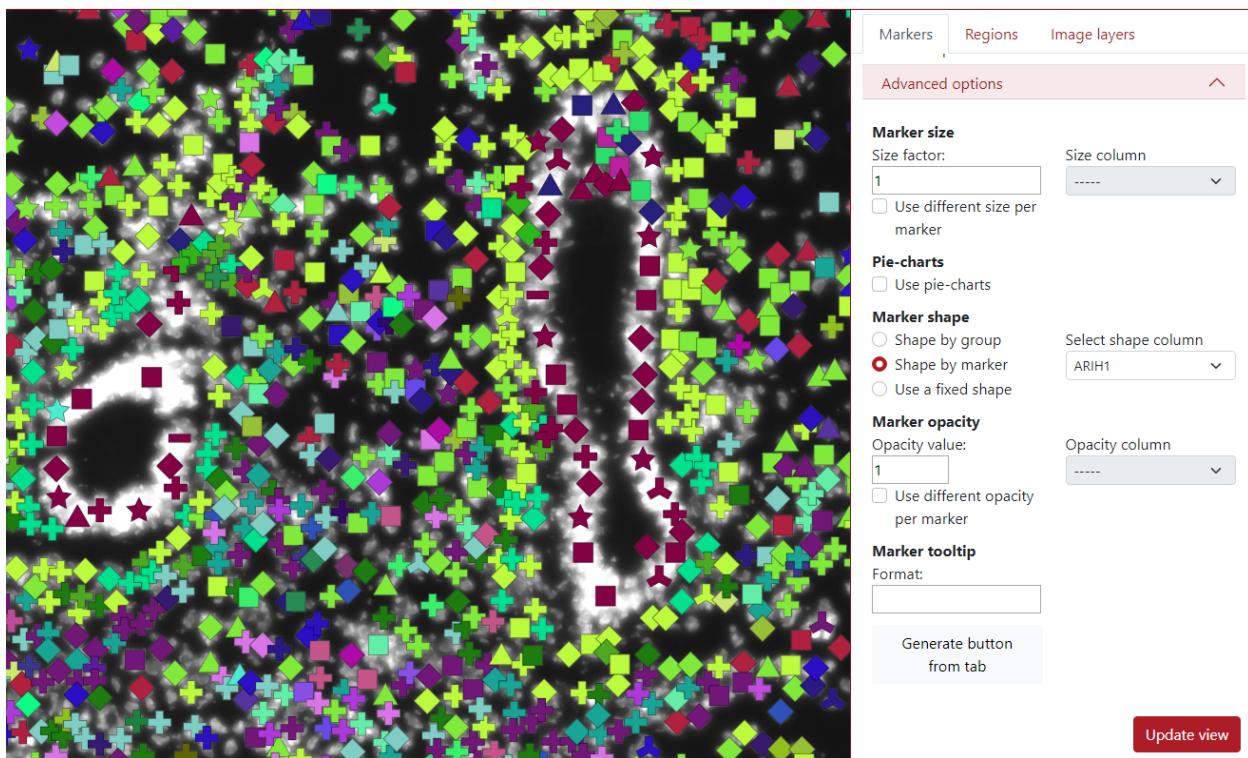
Another option for the marker shape is *shape by marker*. Here, the user needs to select a column with category values, and each category is used for a different shape.

Marker shape

- Shape by group
- Shape by marker
- Use a fixed shape

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.



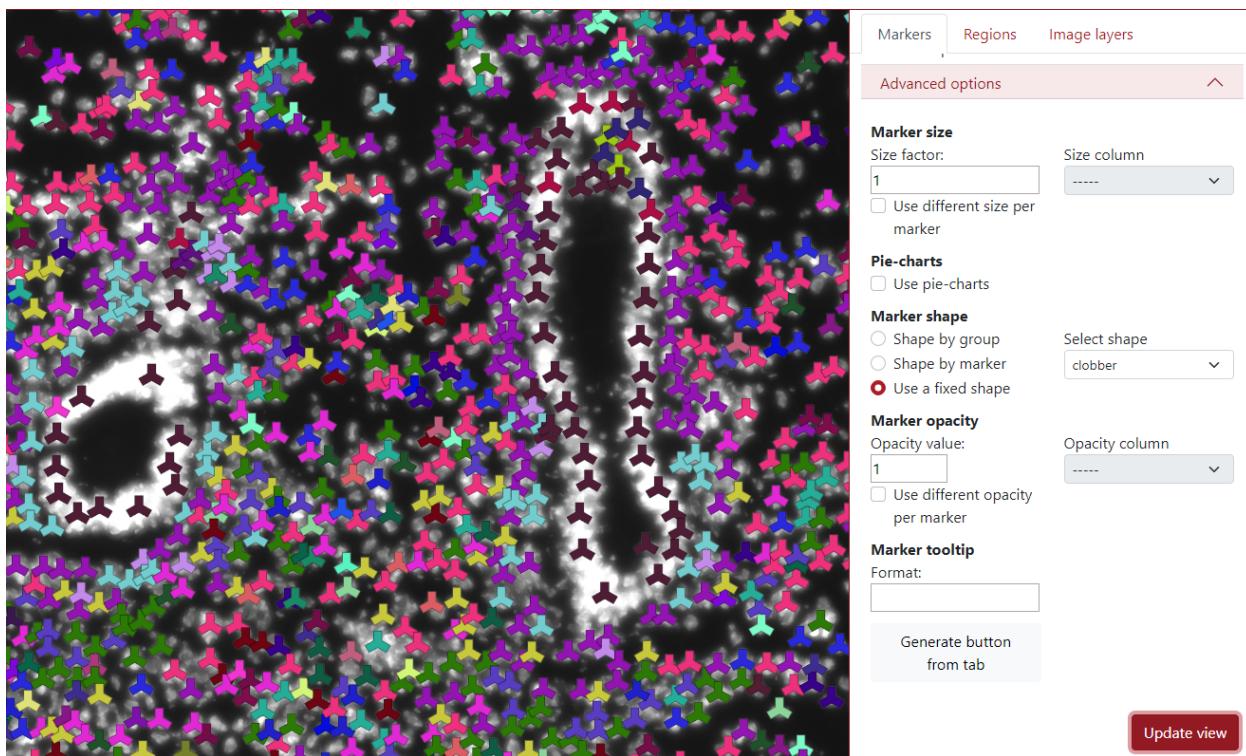
The last option in the marker shape is to *use a fixed shape*. This can be used if the user is not happy with all the different marker shapes and wants to make it homogeneous.

Marker shape

- Shape by group
- Shape by marker
- Use a fixed shape

Select shape

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.



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

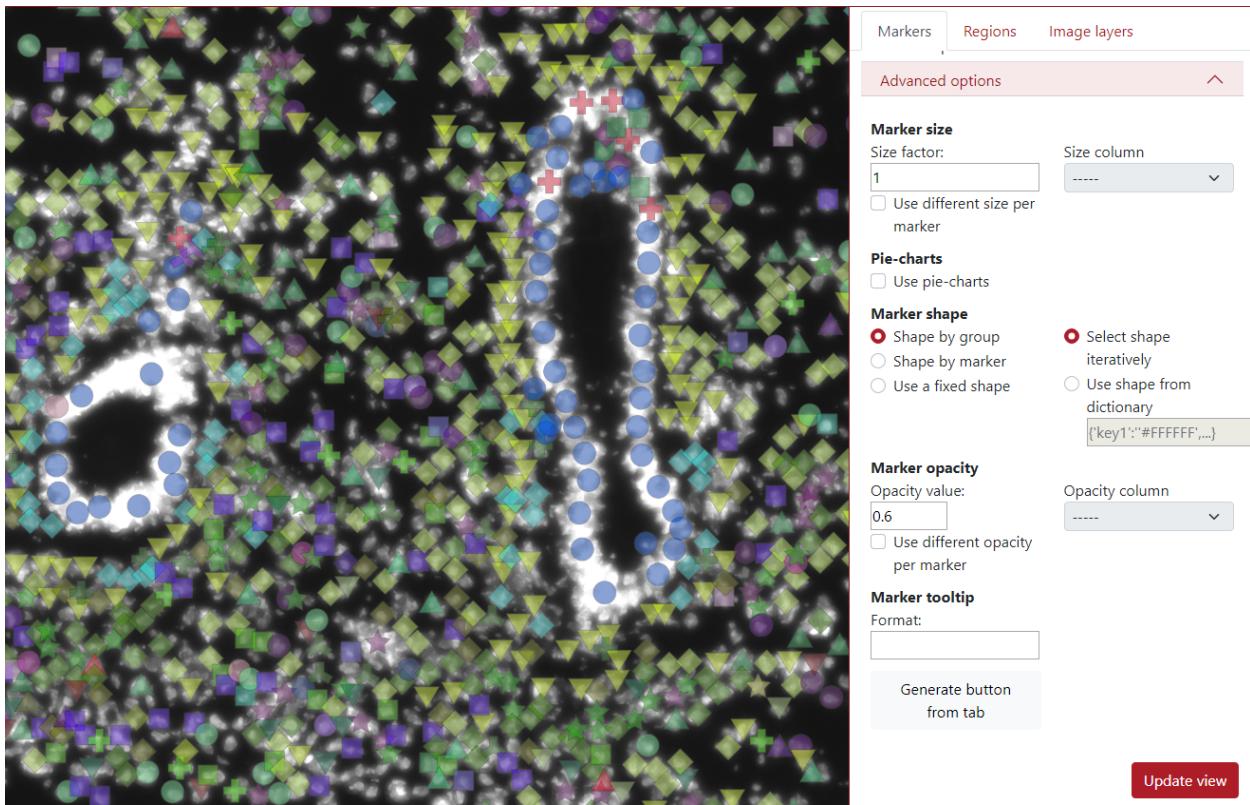
Marker opacity

Opacity value:

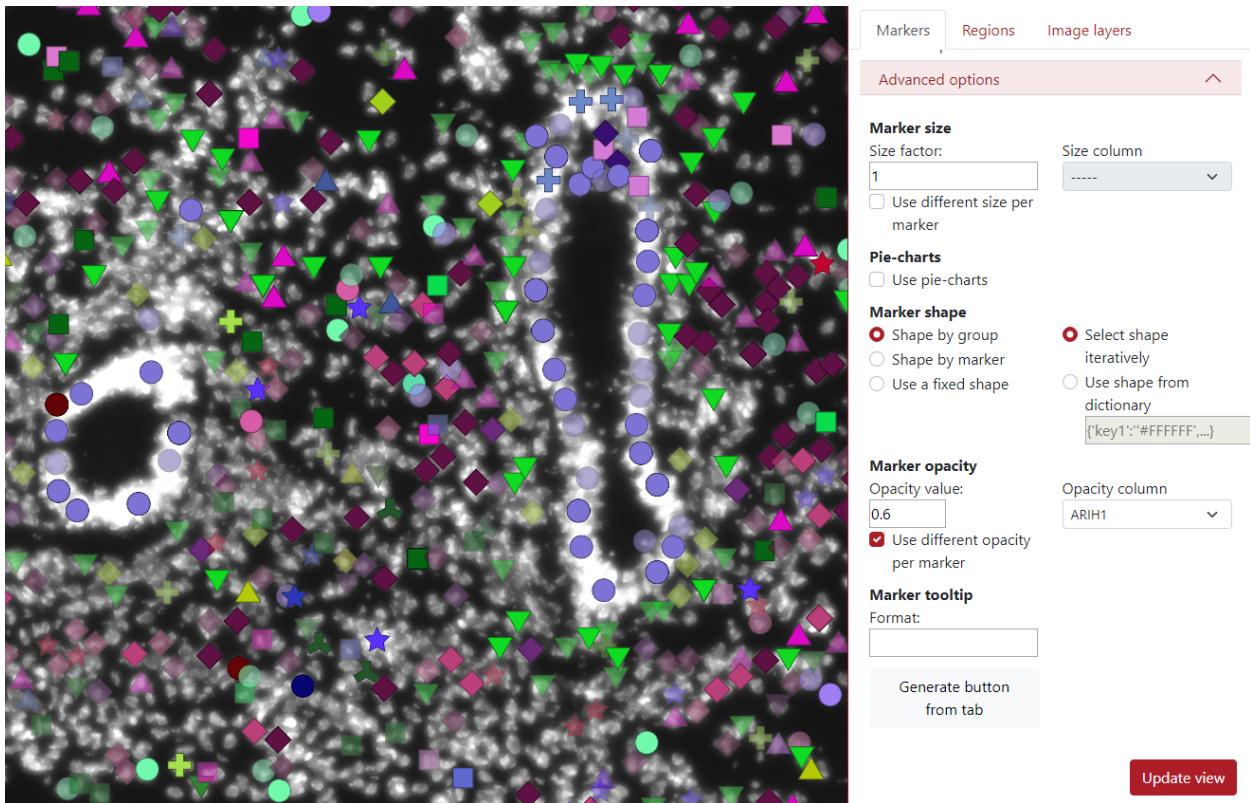
Use different opacity per marker

Opacity column

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



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.

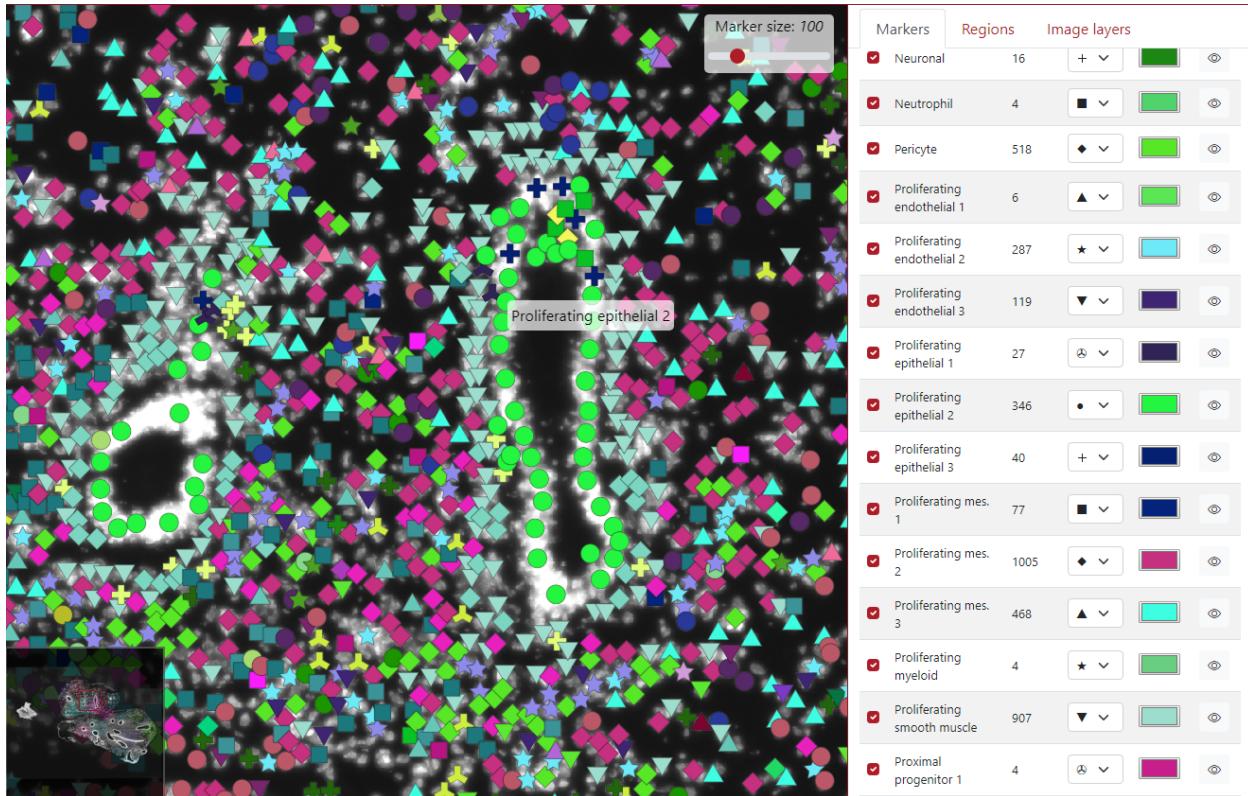


The next option is the *marker tooltip*, which is the text which is displayed when the user clicks on the marker. By default, it displayed the key group the marker belongs to.

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.

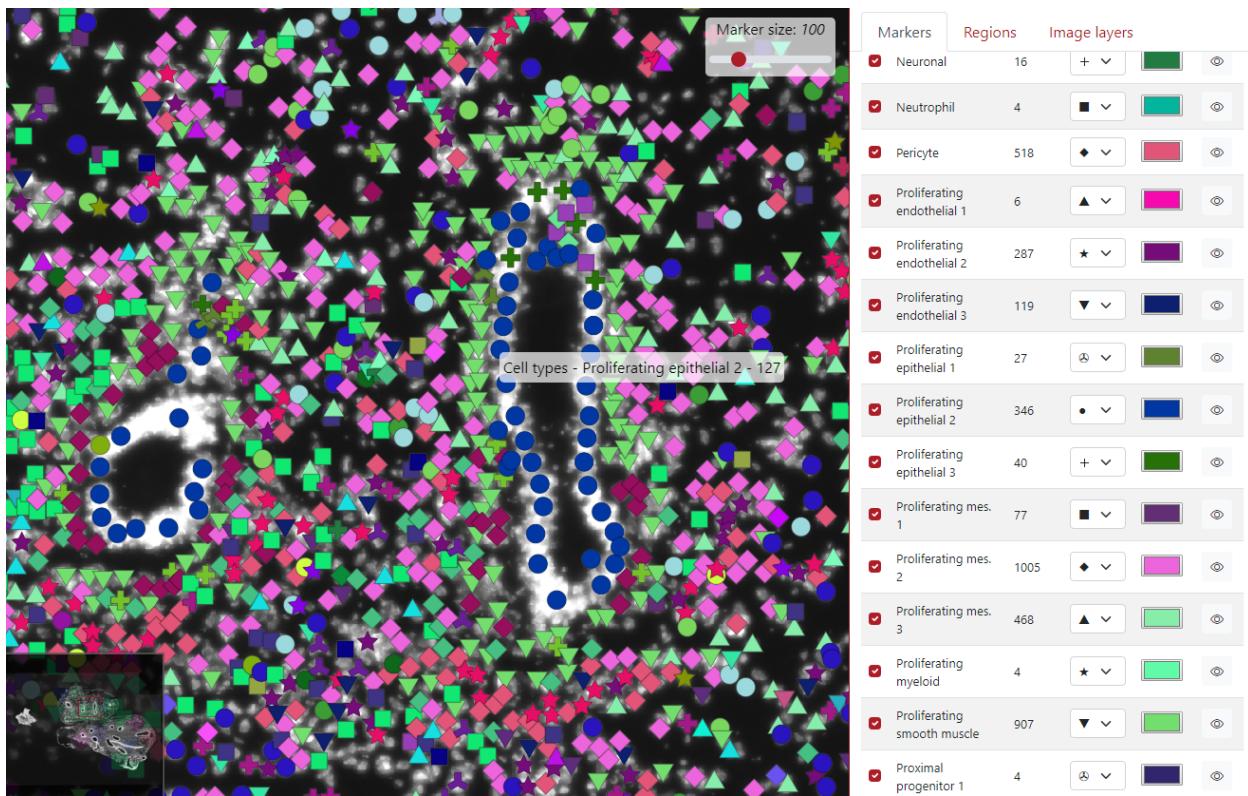


HUHU However this can be modified by writing text into the text area. In this example, we wrote `{tab} - {key} - {col_counts}`. `{tab}` represents the tab name which we set when we loaded the markers, `{key}` represents the key group to which the marker belongs and `{col_counts}` represents the value 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:

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.



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

Generate button
 from tab

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

Cancel

Generate button

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*.

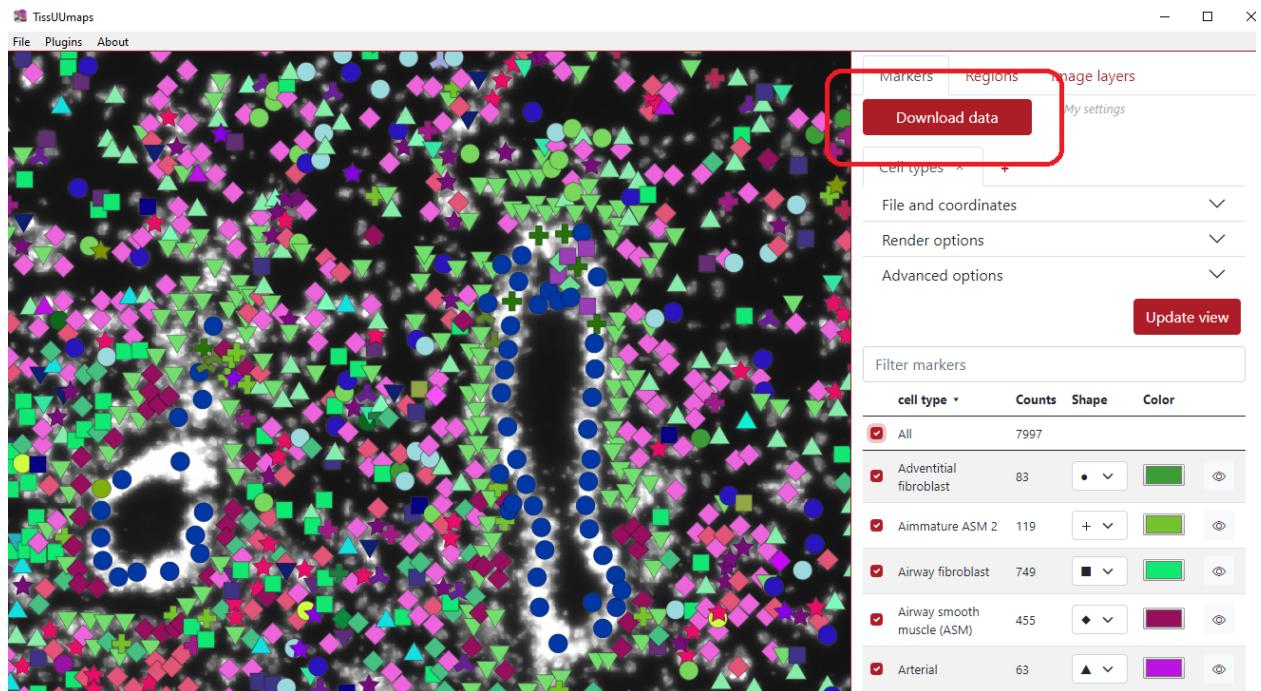
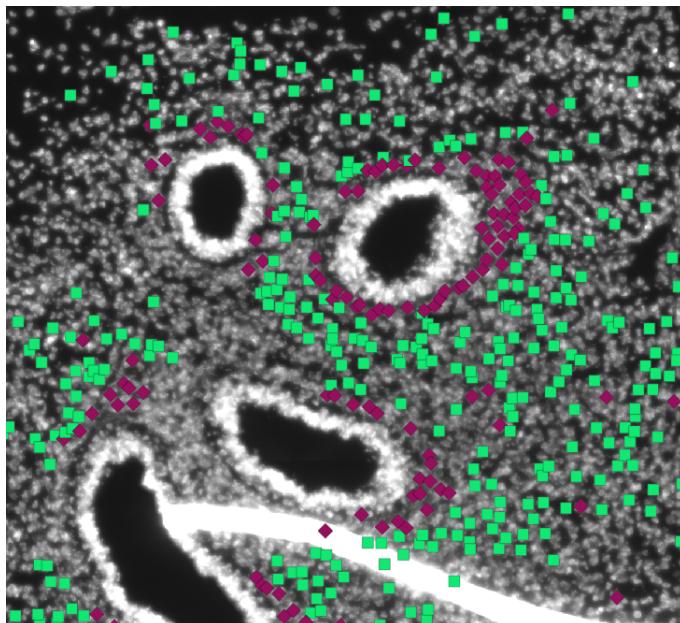


Table of markers

A)	B)	C)	D)	E)
cell type ▾		Counts	Shape	Color
<input checked="" type="checkbox"/> All		7997		
<input checked="" type="checkbox"/> Adventitial fibroblast	83	● disc		
<input checked="" type="checkbox"/> Aimmature ASM 2	119	+ cross		
<input checked="" type="checkbox"/> Airway fibroblast	749	■ square		
<input checked="" type="checkbox"/> Airway smooth muscle (ASM)	455	◆ diamond		
<input checked="" type="checkbox"/> Arterial	63	▲ triangle up		
<input checked="" type="checkbox"/> B-cell	4	★ star		



cell type ▾	Counts	Shape	Color
<input type="checkbox"/> All	7997		
<input type="checkbox"/> Adventitial fibroblast	83	● disc	
<input type="checkbox"/> Aimmature ASM 2	119	+ cross	
<input checked="" type="checkbox"/> Airway fibroblast	749	■ square	
<input checked="" type="checkbox"/> Airway smooth muscle (ASM)	455	◆ diamond	
<input type="checkbox"/> Arterial	63	▲ triangle up	
<input type="checkbox"/> B-cell	4	★ star	
<input type="checkbox"/> Bronchial endothelial	101	▼ triangle down	
<input type="checkbox"/> CTGFhigh distal epithelial	19	◎ clobber	
<input type="checkbox"/> Capillary	37	● disc	
<input type="checkbox"/> Chondroblast	120	+ cross	
<input type="checkbox"/> Ciliated epithelial	5	■ square	
<input type="checkbox"/> Conventional dendritic	7	◆ diamond	
<input type="checkbox"/> Endothelial	1	○ circle	

cell type	Counts	Shape	Color
All	7997		
Adventitial fibroblast	83	● disc	
Aimmature ASM 2	119	+ cross	
Airway fibroblast	749	◆ diamond	
Airway smooth muscle (ASM)	455	■ square	
Arterial	63	▲ triangle up	
B-cell	4	★ star	
Bronchial endothelial	101	◎ clobber	

cell type ▾	Counts	Shape	Color
<input checked="" type="checkbox"/> All	7997		
<input checked="" type="checkbox"/> Adventitial fibroblast	83	• disc	 
<input checked="" type="checkbox"/> Air...			
<input checked="" type="checkbox"/> Air...			
<input checked="" type="checkbox"/> Air...			
<input checked="" type="checkbox"/> Ar...			
<input checked="" type="checkbox"/> B...			
<input checked="" type="checkbox"/> Br...			
<input checked="" type="checkbox"/> CT...			
<input checked="" type="checkbox"/> Ca...			
<input checked="" type="checkbox"/> Ch...			

Select Color

Basic colors

Black	Red	Dark Green	Brown	Dark Blue	Cyan	Yellow	Light Green
Dark Blue	Pink	Dark Purple	Grey	Light Blue	Light Cyan	Orange	Light Yellow
Dark Brown	Red	Dark Green	Orange	Green	Light Green	Yellow	Light Yellow
Dark Purple	Pink	Dark Grey	Pink	Dark Green	Light Green	Light Orange	Light Yellow
Dark Blue	Pink	Dark Blue	Blue	Light Blue	Light Cyan	Light Orange	Light Yellow

Pick Screen Color

Custom colors

White	Light White	Very Light White	Lightest White	Lightest Light White	Lightest Very Light White	Lightest White
White	Light White	Very Light White	Lightest White	Lightest Light White	Lightest Very Light White	Lightest White

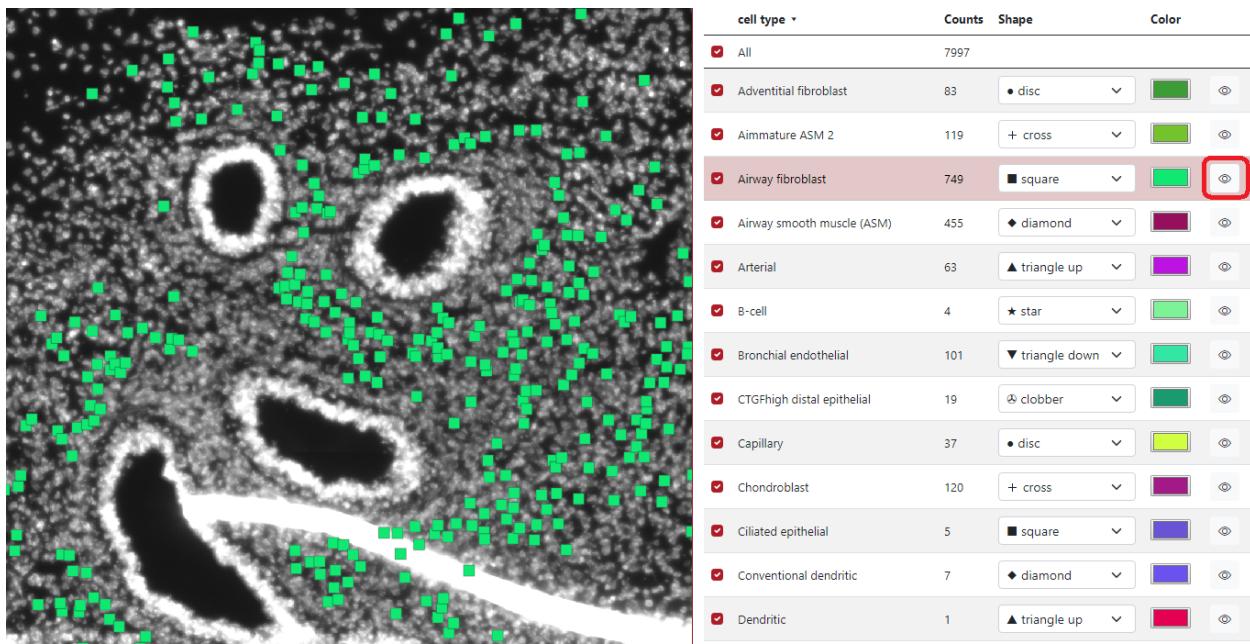
Add to Custom Colors

Color picker controls:

- Hue: 115
- Red: 60
- Sat: 168
- Green: 156
- Val: 156
- Blue: 53

HTML: #3c9c35

OK Cancel



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.

2.3.2 Draw Regions

2.3.3 Analyze Regions

2.3.4 Load Regions

2.3.5 Export Regions

2.4 Projects

2.4.1 Saving and loading projects

For more information on the tmap file format and specifications, see [The TMAP file format](#).

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

2.6.2 Make your own plugin

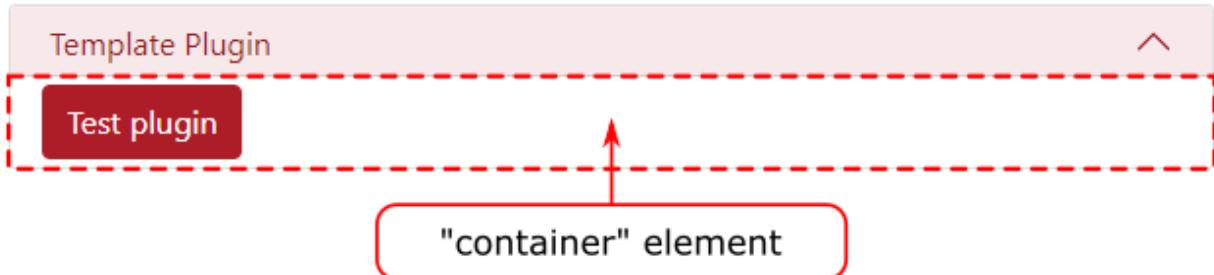
Download the Plugin Template python and javascript files from the [Plugin Update Site](#) and put both files in your local folder `$USER_PATH/.tissuumaps/plugins/`. You can then change the plugin name and add your own options and functions.

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.

Markers Regions Image layers Plugins

List of plugins:



Here is a minimal example of plugin:

```
var Plugin_template;
Plugin_template = {
    name:"Template Plugin"
}

/**
 * This method is called when the document is loaded.
 * The container element is a div where the plugin options will be displayed. */
Plugin_template.init = function (container) {
    container.innerHTML = "Hello world";
}
```

You can access the 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`:

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

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.

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

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.

ADVANCED USAGE

4.1 Jupyter notebooks

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

Simple example to load an image in TissUUmmaps:

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

viewer.screenshot()
```

4.1.1 tissuumaps.jupyter

Module used to run TissUUmmaps 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 TissUUmmaps server
- **host** (*str*) – The host to run the TissUUmmaps server
- **height** (*int*) – The height of the jupyter iframe

Returns The TissUUmmaps viewer

Return type *TissUUmmapsViewer*

```
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 TissUUmmaps

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

Work in progress

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.

The TMAP format uses JSON, with the following specifications:

4.4.1 TMAP project specifications

Description of image layers, markers, regions, and settings of a project. Required properties are shown in bold text		
<code>type</code>	<i>object</i>	
<code>properties</code>		
• <code>filename</code>	Name of the project	
	<code>type</code>	<i>string</i>
• <code>layers</code>	<code>type</code>	<i>array</i>
	<code>default</code>	<code>[]</code>
	<code>items</code>	
	•	<i>Layer</i>
• <code>layerOpacities</code>	<code>type</code>	<i>object</i>
	<code>patternProperties</code>	
	• <code>^[0-9]+\$</code>	<code>type</code> <i>integer</i>
• <code>layerVisibilities</code>	<code>type</code>	<i>object</i>
	<code>patternProperties</code>	
	• <code>^[0-9]+\$</code>	<code>type</code> <i>boolean</i>
• <code>layerFilters</code>	<code>type</code>	<i>object</i>
	<code>patternProperties</code>	

continues on next page

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	• ^[0-9]+\$	<i>LayerFilter</i>
• filters	List of filters shown as active filters in the GUI under the Image layers tab	
	type	<i>array</i>
	default	[“Saturation”, “Brightness”, “Contrast”]
	items	
	•	<i>Filter</i>
• compositeMode	Mode defining how image layers will be merged (composed) with each other. Valid string values are “source-over” and “lighter”, which correspond to ‘Channels’ and ‘Composite’ in the GUI.	
	type	<i>string</i>
	default	source-over
• 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.	
	type	<i>float</i>
	default	null
• boundingBox	Bounding box used to set initial zoom and pan on the view when loading the project.	
	type	<i>object</i>
	default	null
	properties	
	• x	Left coordinate of the bounding box in pixels
	type	<i>float</i>
	• y	Top coordinate of the bounding box in pixels
	type	<i>float</i>
	• width	Width of the bounding box in pixels
	type	<i>float</i>
	• height	Height of the bounding box in pixels
	type	<i>float</i>
• rotate	Angle of rotation of the view in degrees. Only multiples of 90 degrees are supported.	
	type	<i>integer</i>
	default	0
• markerFiles	type	
	default	<i>array</i>
	items	[]
	•	<i>MarkerFile</i>
• regions	GeoJSON object, see Regions section .	
	type	<i>object</i>
	default	{}
• regionFile	(Deprecated) GeoJSON region file loaded on project initialization. Use regionFiles instead.	
	type	<i>string</i>
	default	
• regionFiles	type	
	default	<i>array</i>
	items	[]

continues on next page

Table 1 – continued from previous page

	•	<i>RegionFile</i>
• plugins	List of plugins to load with the project. See also the Plugins section .	
	type	<i>array</i>
	default	[]
	items	
	•	type <i>string</i>
• hideTabs	Hide tabs of markers dataset. Only use when you have a unique marker tab.	
	type	<i>boolean</i>
	default	false
• settings	type	<i>array</i>
	default	[]
	items	
	•	<i>Setting</i>

Layer

Description of an image layer. Required properties are shown in bold text		
type	<i>object</i>	
properties		
• name	Name of the image layer	
	type	<i>string</i>
• tileSource	Relative path to an image file in a supported format. See also the Images section .	
	type	<i>string</i>

LayerFilter

Description of an image filter to be applied to the pixels in an image layer. Required properties are shown in bold text		
type	<i>array</i>	
items		
•	type	<i>object</i>
	properties	
	• name	Filter name. See Filter for more details.
		type <i>string</i>
	• value	Filter parameter. See Filter for more details.
		type <i>string</i>

Filter

TissUUmaps supports most filters available in OpenSeadragon via the <https://github.com/usnistgov/OpenSeadragonFiltering> plugin.

enum	Color, Brightness, Exposure, Hue, Contrast, Vibrance, Noise, Saturation, Gamma, Invert, Greyscale, Threshold, Erosion, Dilation
------	---

ColorScale

TissUUmaps supports most of the color scales available in the D3.js library. See <https://github.com/d3/d3-scale-chromatic> for reference. Note: the colors for ‘interpolateRainbow’ are currently overridden by a custom Turbo-like color scale in version 3.0.x of TissUUmaps.

enum	interpolateCubehelixDefault, interpolateRainbow, interpolateWarm, interpolateCool, interpolateViridis, interpolateMagma, interpolateInferno, interpolatePlasma, interpolateBlues, interpolateBrBG, interpolateBuGn, interpolateBuPu, interpolateCividis, interpolateGnBu, interpolateGreens, interpolateGreys, interpolateOrRd, interpolateOranges, interpolatePRGn, interpolatePiYG, interpolatePuBu, interpolatePuBuGn, interpolatePuOr, interpolatePuRd, interpolatePurples, interpolateRdBu, interpolateRdGy, interpolateRdPu, interpolateRdYlBu, interpolateRdYlGn, interpolateReds, interpolateSinebow, interpolateSpectral, interpolateTurbo, interpolateYlGn, interpolateYlGnBu, interpolateYlOrBr, interpolateYlOrRd
------	---

Shape

TissUUmaps supports most of the marker shapes that are also used by the Napari software, <https://napari.org>. In addition to the name strings listed below, shape can also be specified by a corresponding index in range 0-13.

enum	cross, diamond, square, triangle up, star, clobber, disc, hbar, vbar, tailed arrow, triangle down, ring, x, arrow
------	---

MarkerFile

Description of settings and GUI objects for a marker dataset loaded from CSV file. Required properties are shown in bold text.		
type	<i>object</i>	
properties		
• title	Name of marker button	
	type	<i>string</i>
• comment	Optional description text shown next to marker button	
	type	<i>string</i>
	default	
• name	Name of marker tab	
	type	<i>string</i>
• 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.	
	type	<i>boolean</i>
	default	false
• hideSettings	Hide markers' settings and add a toggle button instead.	
	type	<i>boolean</i>
	default	false
• uid	A unique identifier used internally by TissUUmaps to reference the marker dataset	
	type	<i>string</i>
• expectedHeader	<i>ExpectedHeader</i>	
• expectedRadios	<i>ExpectedRadios</i>	
• 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.	
	type	<i>string / array</i>
• settings	type	<i>array</i>
	default	[]
	items	
	•	<i>Setting</i>

ExpectedHeader

Input field values for settings in a marker tab. Required properties are shown in bold text.		
type	<i>object</i>	
properties		
• X	Name of CSV column to use as X-coordinate	
	type	<i>string</i>
• Y	Name of CSV column to use as Y-coordinate	
	type	<i>string</i>
• gb_col	Name of CSV column to use as key to group markers by	
	type	<i>string</i>

continues on next page

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	default	null
• gb_name	Name of CSV column to display for groups instead of group key value	
	type	<i>string</i>
	default	null
• cb_cmap	Name of D3 color scale to be used for color mapping. See <i>ColorScale</i> for valid string values.	
	type	<i>string</i>
	default	
• cb_col	Name of CSV column containing scalar values for color mapping or hex-decimal RGB colors in format '#ff0000'	
	type	<i>string</i>
	default	null
• cb_gr_dict	JSON string specifying a custom dictionary for mapping group keys to group colors. Example: '{"key1": "#ff0000", "key2": "#00ff00", "key3": "#0000ff"}'	
	type	<i>string</i>
	default	
• scale_col	Name of CSV column containing scalar values for changing the size of markers	
	type	<i>string</i>
	default	null
• scale_factor	Numerical value for a fixed scale factor to be applied to markers	
	type	<i>string</i>
	default	1
• 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.	
	type	<i>string</i>
	default	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.	
	type	<i>string</i>
	default	
• shape_col	Name of CSV column containing a name or an index for marker shape. See also <i>Shape</i> .	
	type	<i>string</i>
	default	null
• shape_fixed	Name or index of a single fixed shape to be used for all markers. See <i>Shape</i> for valid string values.	
	type	<i>string</i>
	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"}'. See also <i>Shape</i> .	
	type	<i>string</i>
	default	
• opacity_col	Name of CSV column containing scalar values for opacities	
	type	<i>string</i>
	default	null
• opacity	Numerical value for a fixed opacity factor to be applied to markers	

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Table 2 – continued from previous page

	type	<i>string</i>
	default	1
• 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 grammar and keywords. If no string is specified, TissUUmaps will show default metadata depending on the context.	
	type	<i>string</i>
	default	

ExpectedRadios

Radio button state and checkbox state for settings in a marker tab. Required properties are shown in bold text.		
type	<i>object</i>	
properties		
• cb_col	If markers should be colored by data in CSV column	
	type	<i>boolean</i>
	default	false
• cb_gr	If markers should be colored by group	
	type	<i>boolean</i>
	default	true
• cb_gr_rand	If group color should be generated randomly	
	type	<i>boolean</i>
	default	false
• cb_gr_dict	If group color should be read from custom dictionary	
	type	<i>boolean</i>
	default	false
• cb_gr_key	If group color should be generated from group key	
	type	<i>boolean</i>
	default	true
• pie_check	If markers should be rendered as pie charts	
	type	<i>boolean</i>
	default	false
• scale_check	If markers should be scaled by data in CSV column	
	type	<i>boolean</i>
	default	false
• shape_col	If markers should get their shape from data in CSV column	
	type	<i>boolean</i>
	default	false
• shape_gr	If markers should get their shape from group	
	type	<i>boolean</i>
	default	true
• shape_gr_rand	If group shape should be generated randomly	
	type	<i>boolean</i>
	default	true
• shape_gr_dict	If group shape should be read from custom dictionary	
	type	<i>boolean</i>
	default	false
• shape_fixed	If a single fixed shape should be used for all markers	
	type	<i>boolean</i>
	default	false

continues on next page

Table 3 – continued from previous page

• opacity_check	If markers should get their opacities from data in CSV column	
	type	<i>boolean</i>
	default	false
• _no_outline	If marker shapes should be rendered without outline	
	type	<i>boolean</i>
	default	false

RegionFile

Description of settings and regions loaded from GeoJSON file. Required properties are shown in bold text.		
type	<i>object</i>	
properties		
• title	Name of region button	
	type	<i>string</i>
• comment	Optional description text shown next to region button	
	type	<i>string</i>
	default	
• 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	<i>boolean</i>
	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.	
	type	<i>string / array</i>
• settings	type	<i>array</i>
	default	[]
	items	
	•	<i>Setting</i>

Setting

Advanced javascript setting, used to change the value of a property in a module, or to call a function in a module. If module.function is a function, then calls <code>module.function(value)</code> , else affects value to <code>module.function</code> . Required properties are shown in bold text.		
type	<i>object</i>	
properties		
• module	Module where the function or property lies.	
	type	<i>string</i>
• function	Function or property of the given module.	
	type	<i>string</i>
• value	type	<i>number</i>

4.4.2 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",
        "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",
      }
    }
  ]
}
```

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```

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

```

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