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!

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 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 3.0.8.5

- Minor fixes.

1.4.2 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.

1.4.3 3.0.8.3

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

1.4.4 3.0.8.2

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

1.4.5 3.0.8.1

- Fix multiple dataset alignment when no background image

1.4.6 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

1.4.7 3.0.7

- Add menu to load plugins through an update-site

1.4.8 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

1.4.9 3.0.5

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

1.4.10 3.0.4

- Add filtering of markers

1.4.11 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

2.1.3 Apply filters

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

2.2.3 Markers settings

File and coordinates

Render options

Advanced options

Table of markers

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

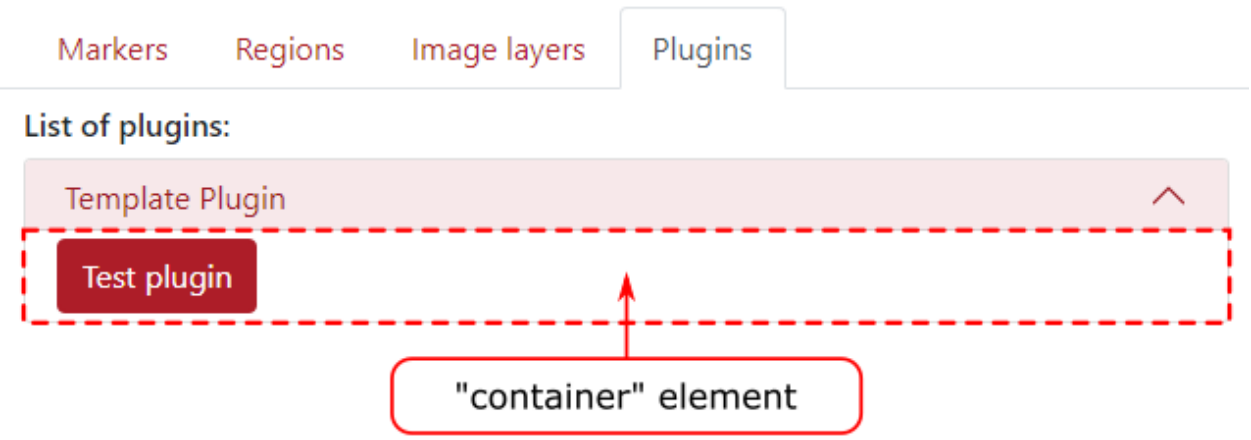
2.6.1 Load plugins

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.



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.

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

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.

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

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			
type	*object*		
properties			
• **filename**	Name of the project		
	type	*string*	
• layers	type	*array*	
	default	[]	
	items		
	•	*Layer*	
• layerOpacities	type	*object*	
	patternProperties		
	• ^[0-9]+$	type	*integer*
• layerVisibilities	type	*object*	
	patternProperties		
	• ^[0-9]+$	type	*boolean*
• layerFilters	type	*object*	
	patternProperties		

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Table 1 – continued from previous page

	• ^[0-9]+$	*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.	
	type	*string*
	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	*float*
	default	null
• boundingBox	Bounding box used to set initial zoom and pan on the view when loading the project.	
	type	*object*
	default	null
	properties	
	• **x**	Left coordinate of the bounding box in pixels
		type: *float*
	• **y**	Top coordinate of the bounding box in pixels
		type: *float*
	• **width**	Width of the bounding box in pixels
		type: *float*
	• **height**	Height of the bounding box in pixels
		type: *float*
• 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, see *Regions section*.	
	type	*object*
	default	{}
• regionFile	type	*string*
	default	
• regionFiles	type	*array*
	default	[]
	items	
• plugins	List of plugins to load with the project. See also the *Plugins section*.	
	type	*array*
	default	[]
	items	

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	•	type	*string*
• hideTabs	Hide tabs of markers dataset. Only use when you have a unique marker tab.		
	type	*boolean*	
	default	false	
• settings	type	*array*	
	default	[]	
	items		
	•	*Setting*	

Layer

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

LayerFilter

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

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	*object*	
properties		
• **title**	Name of marker button	
	type	*string*
• comment	Optional description text shown next to marker button	
	type	*string*
	default	
• **name**	Name of marker tab	
	type	*string*
• 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	*boolean*
	default	false
• 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	
	type	*string*
• **expectedHeader**	*ExpectedHeader*	
• 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.	
	type	*string* / *array*
• settings	type	*array*
	default	[]
	items	
	•	*Setting*

ExpectedHeader

Input field values for settings in a marker tab. Required properties are shown in **bold** text.		
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	
	type	*string*

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Table 2 – continued from previous page

	default	null
• gb_name	Name of CSV column to display for groups instead of group key value	
	type	*string*
	default	null
• cb_cmap	Name of D3 color scale to be used for color mapping. See *ColorScale* for valid string values.	
	type	*string*
	default	
• cb_col	Name of CSV column containing scalar values for color mapping or hexadecimal RGB colors in format '#ff0000'	
	type	*string*
	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	*string*
	default	
• scale_col	Name of CSV column containing scalar values for changing the size of markers	
	type	*string*
	default	null
• scale_factor	Numerical value for a fixed scale factor to be applied to markers	
	type	*string*
	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	*string*
	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	*string*
	default	
• shape_col	Name of CSV column containing a name or an index for marker shape. See also *Shape*.	
	type	*string*
	default	null
• shape_fixed	Name or index of a single fixed shape to be used for all markers. See *Shape* for valid string values.	
	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"}'. See also *Shape*.	
	type	*string*
	default	
• opacity_col	Name of CSV column containing scalar values for opacities	
	type	*string*
	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	*string*
	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 grammer and keywords. If no string is specified, TissUUmaps will show default metadata depending on the context.	
	type	*string*
	default	

ExpectedRadios

Radio button state and checkbox state for settings in a marker tab. Required properties are shown in **bold** text.		
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

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• 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*
	default	false

Setting

[Add description]. Required properties are shown in **bold** text.		
type	*object*	
properties		
• **function**	type	*string*
• **module**	type	*string*
• **value**	type	*number*

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"
            }
```

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```
        ],
        "1": [
            {
                "name": "Color",
                "value": "0,100,0"
            }
        ]
    },
    "filters": [
        "Color"
    ],
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