# **TissUUmaps**

Release 3.0

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This page hosts the documentation for TissUUmaps 3.0. You can find a pdf version of thie 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!

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**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

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### 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

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#### **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)

 $Tiss UU maps \ will \ automatically \ convert \ any \ other \ format \ into \ a \ pyramidal \ tiff \ (in \ a \ temporary \ .tissuumaps \ folder \ created \ in \ the \ original \ image \ folder) \ using \ vips.$ 

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

#### 2.4.2 The tmap file format

The tmap format contains image layers, saved markers, regions, and settings. It is highly recommended to create tmap files by saving projects from TissUUmaps applications, but you can also edit the files manually to add or change project's settings.

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The tmap format uses json, with the following specifications:

### **Tmap specifications**

	es are shown in <b>bold</b> text object		
pe parties	Object		
operties • filename	Nome of the arrainst		
• піепате	Name of the project	1	
	type	string	
• layers	type	array	
	items		
		Layer	
	•		
layerOpacities	type	object	
	patternProperties		
		type	integer
	• ^[0-9]+\$		
<ul> <li>layerVisibilities</li> </ul>	type	object	
	patternProperties		
		type	boolean
	• ^[0-9]+\$		
layerFilters	type	object	,
<b>y</b>	patternProperties		
		LayerFilter	
	• ^[0-9]+\$		
	[		
• filters	type	array	
	items	1 227 229	
	1001115	Filter	
	•		
	type	string	
<ul> <li>compositeMode</li> </ul>	type	Siring	
CompositeMode			
markerFiles	type	arran	
- markerfiles	type items	array	
	101115	MarkerFile	
	•	Markerrue	
:	Capicon	D :	
<ul><li>regions</li></ul>	GeoJSON object, see I		
	type	object	
. 5.1	type	string	
<ul> <li>regionFile</li> </ul>			
<ul> <li>regionFiles</li> </ul>	type	array	
	items		
• plugins	type	array	
	items		

continues on next page

# Table 1 – continued from previous page

	1 1 8		
		type	string
	•		
hideTabs     Hide tabs of markers dataset. Only use when you have a		t. Only use when you have a	unique marker tab.
	type	boolean	
<ul> <li>settings</li> </ul>	type	array	
	items		
Setting			
	•		

### Layer

TODO. Required properties are shown in <b>bold</b> text				
ype object				
properties	properties			
• name	type	string		
• tileSource	type	string		

### LayerFilter

TODO. Required properties are shown in <b>bold</b> text				
type	array			
items				
type object				
	properties			
	• name Filter			
	• value	type	string	

### **Filter**

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 'interpolatRainbow' is 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, interpolateOrRd, interpolateCividis, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolatePrBGn, interpolateRdBu, interpolateRdBu,

#### **MarkerFile**

Name of marker button type Optional description text shown next type default Name of marker tab	string to marker button string	
type Optional description text shown next type default	to marker button	
type Optional description text shown next type default	to marker button	
Optional description text shown next type default	to marker button	
type default		
default	511 1118	
Name of marker lab		
type	string	
• 1	0	
If the CSV file for the marker dataset should be automatically loaded when the TMAP project is opened. If this is false, the user has to instead click on		
the marker button to load the dataset.		
type	boolean	
default	false	
A unique identifier used internally for the marker dataset		
type	string	
ExpectedHeader		
ı		
ExpectedRadios		
Relative file path to CSV file for marker dataset		
	string	
	array	
items		
_	Setting	
•		
	ExpectedHeader  ExpectedRadios  Relative file path to CSV file for mark type type	

# ExpectedHeader

type	in a marker tab. Required propertie object	are shown in both text.	
properties	Objecti		
• X	Name of CSV column to	usa as V acordinata	
• 1			
• Y	Name of CSV column to	string	
• 1			
1 1	type	string	
• gb_col		use as key to group markers by	
	type	string	
	default	null	
• gb_name		display for groups instead of group key value	
	type	string	
	default	null	
<ul><li>cb_cmap</li></ul>		be used for color mapping. See ColorScale for	
	valid string values.		
	type	string	
	default		
• cb_col		ntaining scalar values for color mapping or hex-	
	adecimal RGB colors in for	ormat '#ff0000'	
	type	string	
	default	null	
<ul><li>cb_gr_dict</li></ul>	JSON string specifying a custom dictionary for group colors		
	type	string	
	default		
<ul><li>scale_col</li></ul>	Name of CSV column con	taining scalar values for changing the size of mark	
	ers		
	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 con	ntaining data for pie chart sectors	
-	type	string	
	default	null	
• pie_dict	TODO		
1 –	type	string	
	default		
• shape_col	Name of CSV column containing a name or an index for marker shape		
F	type	string	
	default	null	
• shape_fixed		Name of a single fixed shape to be used for all markers	
sh <b>u</b> p <b>o_</b> mou	type	string	
	default	cross	
shape_gr_dict		custom dictionary for group shapes	
511upo_51_uiot	type	string	
	default	SHUIS	
opacity_col		ntaining scalar values for opacities	
· opacity_cor	type	string	
	default	null	
	uciauit	IIuII	

continues on next page

Table 2 – continued from previous page

<ul><li>opacity</li></ul>	Numerical value for a fixed opacity factor to be applied to markers		
	type	string	
	default	1	
• tooltip_fmt	Custom formatting string used for overlay displayed over selected markers;		
	see (TODO).		
	type	string	
	default		

### **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 colo	If markers should be colored by group		
	type	boolean		
	default	true		
• cb_gr_rand	If group color should be g	generated randomly		
	type	boolean		
	default	false		
• cb_gr_dict	If group color should be r	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		
<ul><li>shape_gr_rand</li></ul>	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		
<ul><li>shape_fixed</li></ul>	If a single fixed shape sho	ould be used for all markers		
-	type	boolean		
	default	false		
<ul> <li>opacity_check</li> </ul>	If markers should get thei	r opacities from data in CSV column		

continues on next page

Table 3 – continued from previous page

	type	boolean
	default	false
• _no_outline	If marker shapes should be rendered without outline	
	type	boolean
	default	false

#### **Setting**

TODO. Required properties are shown in <b>bold</b> text.				
type	object			
properties				
• function type string				
• module	type	string		
• value	type	number		

#### **Example of 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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(continued from previous page)

```
"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
        "name": " markers",
        "path": "./istdeco_codes_n.csv",
        "title": "Download markers",
```

(continues on next page)

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```
"uid": "uniquetab"
}
],
"regions": {},
"plugins": [
    "Spot_Inspector"
],
    "hideTabs": true,
    "settings": []
}
```

# 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

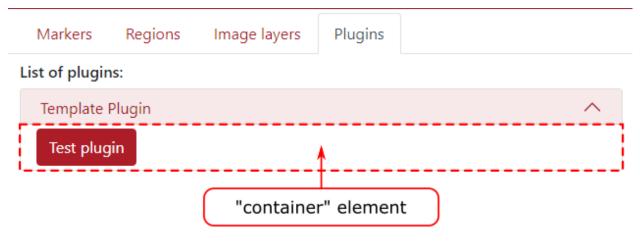
### 2.6.1 Load plugins

### 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.

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**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/\rightarrowtissuumaps: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** (1ist) [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

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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

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