# **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.

2.3. Regions 7

The tmap format uses json, with the following specifications:

# **Tmap specifications**

e	object				
perties	***				
• filename	Name of the project				
mename	type	string			
• layers	type	array			
layers	default				
	items	LJ			
	Items	Layer			
	•	Luyer			
layerOpacities	type	object			
, 1	patternProperties				
	• ^[0-9]+\$	type	integer		
• layerVisibilities	type	object	I		
,	patternProperties				
	T · · · · · · · · · · · · · · · · · · ·	type	boolean		
	• ^[0-9]+\$				
layerFilters	type	object	'		
•	patternProperties				
	• ^[0-9]+\$	LayerFilter			
• filters	type	array			
	default				
	items	П			
	•	Filter			
• compositeMode	type	string			
markerFiles	type	array			
	default				
	items	LU			
	•	MarkerFile			
• regions	GeoJSON object, see Regions section.				
-	type	object			
	default				
• regionFile	type	string			
Č	default				
• regionFiles	type	array			

continues on next page

Table 1 – continued from previous page

	default					
	items	·				
• plugins	type	array	array			
	default	[]	0			
	items					
		type	string			
	•					
hideTabs	Hide tabs of markers dataset. Only use when you have a unique marker tab.					
	type	boolean	boolean			
	default	false	false			
• settings	type	array	array			
	default	[]				
	items	items				
		Setting				
	•					

# Layer

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

# LayerFilter

TODO. Required properties	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 supp	ports most of the color scales available in the D3.js library. See https://github.com/d3/			
d3-scale-chromatic	d3-scale-chromatic for reference. Note: the colors for 'interpolateRainbow' is currently overridden by a custom			
Turbo-like color so	cale in version 3.0.x of TissUUmaps.			
enum	interpolateCubehelixDefault, interpolateRainbow, interpolateWarm, interpolateCool, interpo-			
	lateViridis, interpolateMagma, interpolateInferno, interpolatePlasma, interpolateBlues, inter-			
	polateBrBG, interpolateBuGn, interpolateBuPu, interpolateCividis, interpolateGnBu, inter-			
	polateGreens, interpolateGreys, interpolateOrRd, interpolateOranges, interpolatePRGn, inter-			
	polatePiYG, interpolatePuBu, interpolatePuBuGn, interpolatePuOr, interpolatePuRd, interpo-			
	latePurples, interpolateRdBu, interpolateRdGy, interpolateRdPu, interpolateRdYlBu, interpo-			
	lateRdYlGn, interpolateReds, interpolateSinebow, interpolateSpectral, interpolateTurbo, inter-			
	polateYlGn, interpolateYlGnBu, interpolateYlOrBr, interpolateYlOrRd			

# Shape

TissUUmaps supports most of the marker shapes that are	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	so 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

type	object			
type	Објест			
properties	N C 1 1			
• title	Name of marker buttor			
	type	string		
<ul> <li>comment</li> </ul>	Optional description text shown next to marker button			
	type	string		
	default			
• name	Name of marker tab			
	type	string		
<ul> <li>autoLoad</li> </ul>	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		
• 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			
	type	string		
• settings	type	array		
-	default			
	items	10		
	•	Setting		

# ExpectedHeader

Input field values for settings in a m	arker tab. Required properties are shown	in <b>bold</b> 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	
	default	null	
• gb_name	Name of CSV column to display for groups instead of group key value		
	type	string	
	default	null	

continues on next page

# Table 2 – continued from previous page

• ch cman	Name of D3 color scale to h	be used for color mapping. See <i>ColorScale</i> for			
со_стар		valid string values.			
	type	string			
	default	string			
• ch col		aining scalar values for color mapping or hex-			
• 60_601	adecimal RGB colors in for				
	type default	string null			
l di					
• cb_gr_dict		JSON string specifying a custom dictionary for mapping group keys to group colors. Example: '{"key1": "#ff0000", "key2": "#00ff00", "key3":			
		key1: #110000, key2: #001100, key3:			
	"#0000ff"'}'				
	type	string			
	default				
• scale_col		ining scalar values for changing the size of mark-			
	ers				
<ul> <li>cb_cmap</li> <li>cb_col</li> <li>cb_gr_dict</li> <li>scale_col</li> <li>scale_factor</li> <li>pie_col</li> <li>pie_dict</li> <li>shape_col</li> <li>shape_fixed</li> <li>opacity_col</li> <li>tooltip_fmt</li> </ul>	type	string			
	default	null			
<ul><li>scale_factor</li></ul>	Numerical value for a fixed	scale factor to be applied to markers			
	type	string			
	default	1			
• pie_col	Name of CSV column conta	aining data for pie chart sectors			
	type	string			
	default	null			
<ul><li>pie_dict</li></ul>	TODO				
	type	string			
	default				
• shape_col	Name of CSV column conta	aining a name or an index for marker shape. See			
	also Shape.				
	type	string			
	default	null			
<ul><li>shape_fixed</li></ul>	Name or index of a single fi	xed shape to be used for all markers. See Shape			
-	for valid string values.	•			
	type	string			
	default	cross			
• shape gr dict		stom dictionary for mapping group keys to group			
1 -2 -		shapes. Example: '{"key1": "square", "key2": "diamond", "key3": "trian-			
	gle up"}'. See also Shape.	1,			
	type	string			
	default	37776			
opacity col		aining scalar values for opacities			
	type	string			
	default	null			
• opacity		opacity factor to be applied to markers			
opacity	type	string			
	default	1			
• tooltin fmt		-			
- toolup_mit	Custom formatting string used for overlay displayed over selected markers; see (TODO).				
	type	string			
	default	string			
	uciauit				

# ExpectedRadios

rpe	object		
roperties			
• cb_col	If markers should be colored by data in CSV column		
	type	boolean	
	default	false	
• cb_gr	If markers should be col	ored 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	
_v = 'V	type	boolean	
	default	true	
• pie_check	If markers should be ren	dered 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		
555F 5_5 55	type	boolean	
	default	false	
• shape_gr	If markers should get their shape from group		
shup•_gr	type	boolean	
	default	true	
shape_gr_rand	If group shape should be		
2-11-12-12-13-13-13-13-13-13-13-13-13-13-13-13-13-	type	boolean	
	default	true	
• shape_gr_dict		e read from custom dictionary	
2-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1	type	boolean	
	default	false	
• shape_fixed		nould be used for all markers	
shape_inted	type	boolean	
	default	false	
opacity_check		eir opacities from data in CSV column	
opacity_check	type	boolean	
	default	false	
• _no_outline		be rendered without outline	
_no_ounne	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"
            }
        ],
        "1": [
            {
                "name": "Color",
                "value": "0,100,0"
            }
        ]
    "filters": [
```

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

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```
"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

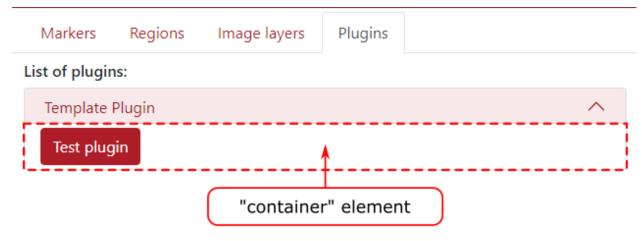
# 2.6.1 Load plugins

### 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"
}

(continues on next page)
```

(continued from previous page)

```
/**
  * 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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