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

1.4. Changelog 4

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

1.4. Changelog 5

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 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.

2.3. Regions 7

The TMAP format uses JSON, with the following specifications:

TMAP project specifications

		ettings of a project. Requ	uired properties are shown in bold tex	
pe	object			
roperties				
• filename	Name of the project			
	type	string		
 layers 	type	array		
	default			
	items			
		Layer		
	•			
. 1 0	4	1:		
 layerOpacities 	type	object		
	patternProperties	4	54	
	φ. ΙΟ ΟΙΑ	type	integer	
	• ^[0-9]+\$			
layerVisibilities	type	object		
1 layer visionities	patternProperties	Object		
	patterm roperties	type	boolean	
	• ^[0-9]+\$	is pe	booteun	
	[0 >]. 4			
layerFilters	type	object		
,	patternProperties			
		LayerFilter		
	• ^[0-9]+\$			
• filters	List of filters shown as active filters in the GUI under the Image layers tab			
	type	array		
	default	["Saturation", "B	rightness", "Contrast"]	
	items			
		Filter		
	•			
	36.1.10.1.1.			
 compositeMode 			ed (composited) with each other. Valid	
			which correspond to 'Channels' and	
	'Composite' in the GU			
	type default	string		
markerFiles		source-over		
• markerines	type default	array		
	items			
	TUIIIS	MarkerFile		
	•	Markerrite		
• regions	GeoJSON object, see <i>b</i>	Regions section.		
• regions	GeoJSON object, see <i>I</i> type	Regions section. object		

continues on next page

Table 1 – continued from previous page

• regionFile	type	string	
	default		
• regionFiles	type	array	
	default		
	items		
 plugins 	List of plugins to load with	the project. See also the Plus	gins section.
	type	array	
	default		
	items		
		type	string
	•		
 hideTabs 	Hide tabs of markers dataset. Only use when you have a unique marker tab.		
	type	boolean	
	default	false	
 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 <i>Images</i>			
	section.			
	type	string		

LayerFilter

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

polateYlGn, 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.		

MarkerFile

type	object			
type	Објест			
properties	N C 1 1			
• title	Name of marker buttor			
	type	string		
 comment 		xt 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 of	the TMAP project is opened. If this is false, the user instead has to click on		
	the marker button in th	e GUI to load the dataset.		
	type	boolean		
	default	false		
• uid	A unique identifier use	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			
	•	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-co	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			
	type	string		
	default	null		

continues on next page

Table 2 – continued from previous page

1	Table 2 – continued from pr			
• cb_cmap	Name of D3 color scale to be used for color mapping. See <i>ColorScale</i> for valid string values.			
	type	string		
	default			
• cb_col		taining scalar values for color mapping or hex-		
	adecimal RGB colors in for	rmat '#ff0000'		
	type	string		
	default	null		
• cb_gr_dict	group colors. Example: '{' "#0000ff"}'	custom dictionary for mapping group keys to "key1": "#ff0000", "key2": "#00ff00", "key3":		
	type	string		
	default			
• scale_col	Name of CSV column conta	aining scalar values for changing the size of mark-		
	type	string		
	default	null		
 scale_factor 	Numerical value for a fixed	l scale factor to be applied to markers		
	type	string		
	default	1		
• pie_col		aining data for pie chart sectors. TissUUmaps ex-		
		pects labels and numerical values for sectors to be separated by ':' characters		
	in the CSV column data.	in the CSV column data.		
	type	string		
	default	null		
• pie_dict	indices to colors. Example	custom dictionary for mapping pie chart sector: '{0: "#ff0000", 1: "#00ff00", 2: "#0000ff"}'. d, TissUUmaps will use a default color palette string		
	default	50.008		
shape_col		taining a name or an index for marker shape. See		
	type	string		
	default	null		
• shape_fixed	Name or index of a single f for valid string values.	fixed shape to be used for all markers. See Shape		
	type	string		
	default	cross		
• shape_gr_dict	I	ustom dictionary for mapping group keys to group		
shape_gr_aret		shapes. Example: '{"key1": "square", "key2": "diamond", "key3": "trian-		
	type	string		
	default	0		
opacity_col		taining scalar values for opacities		
. ry	type	string		
	default	null		
opacity		l opacity factor to be applied to markers		
T	type	string		
	default	1		
	uciauit	1		

continues on next page

Table 2 – continued from previous page

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, TissU-	
	Umaps will show default metadata depending on the context.	
	type	string
	default	

ExpectedRadios

Radio button state and checkb	ox state for settings in a marker	tab. Required properties are shown in bold text.		
type	object			
properties				
• cb_col	If markers should be co	olored by data in CSV column		
	type	boolean		
	default	false		
• cb_gr	If markers should be co	olored by group		
	type	boolean		
	default	true		
 cb_gr_rand 	If group color should b	be generated randomly		
_	type	boolean		
	default	false		
• cb_gr_dict	If group color should b	oe read from custom dictionary		
_5 _	type	boolean		
	default	false		
• cb_gr_key	If group color should b	pe generated from group key		
-c - ;	type	boolean		
	default	true		
• pie_check	If markers should be re	If markers should be rendered as pie charts		
1 –	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			
1 -	type	boolean		
	default	false		
• shape_gr	If markers should get their shape from group			
1 –	type	boolean		
	default	true		
shape_gr_rand	If group shape should	be generated randomly		
1 -6 -	type	boolean		
	default	true		
shape_gr_dict	If group shape should be read from custom dictionary			
2	type	boolean		
	default	false		
shape_fixed		If a single fixed shape should be used for all markers		
~ F ~ -	type	boolean		
	default	false		
opacity_check		heir opacities from data in CSV column		
spacity_encor	type	boolean		
	5712	continues on next pag		

continues on next page

Table 3 – continued from previous page

	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	object	
properties			
• function	type	string	
• module	type	string	
• value	type	number	

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": [
```

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(continued from previous page)

```
{
            "name": "Color",
            "value": "0,100,0"
        }
    1
},
"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",
        "uid": "uniquetab"
```

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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

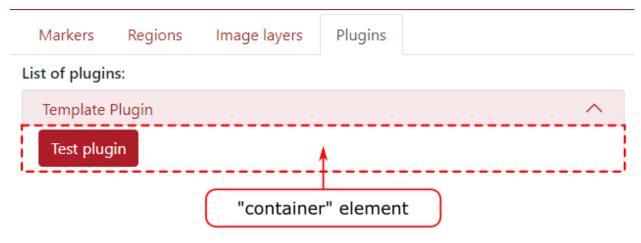
2.6.1 Load plugins

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.

2.6. Plugins 17

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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