TissUUmaps

Release 3.0

Nicolas Pielawski Axel Andersson Christophe Avenel Andrea Behanova Eduard Chelebian Anna Klemm Fredrik Nysjö Leslie Solorzano Carolina Wählby

CONTENTS:

1	Intro	oduction
	1.1	About TissUUmaps
	1.2	Installation
	1.3	Citing TissUUmaps
	1.4	Changelog
2	Getti	ing started
	2.1	Images
	2.2	Markers
	2.3	Regions
	2.4	Projects
	2.5	Exporting screenshots
	2.6	Plugins
3	Shar	ring projects 1
	3.1	Apache server
	3.2	Docker container
4	Adva	anced usage
	4.1	Jupyter notebooks
	4.2	Napari
	4.3	AnnData
Ind	dev	2

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!

CONTENTS: 1

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 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

type	object		
properties			
• filename	Name of the project		
mename	type	string	
• layers	type	array	
layers	items	итиу	
	Items	Layer	
		Luyer	
layerOpacities	type	object	
• layerOpacities	type patternProperties	object	
	patternirioperties	typo	intagan
	• ^[0-9]+\$	type	integer
	• ~[0-9]+\$		
. 1	4	-1-:4	
 layerVisibilities 	type patternProperties	object	
	patternProperties	4	1 1
	• ^[0-9]+\$	type	boolean
	• ^[0-9]+\$		
1T'14	4	7	
 layerFilters 	type	object	
	patternProperties	T T'I	
	ATO 01. ¢	LayerFilter	
	• ^[0-9]+\$		
• filters	4		
• niters	type	array	
	items	T:1.	
		Filter	
	•		
. 3.6.1	type	string	
 compositeMode 			
 markerFiles 	type	array	
	items		
		MarkerFile	
	•		
 regions 	GeoJSON object, see		
	type	object	
	type	string	
 regionFile 			
 regionFiles 	type	array	
	items		
 plugins 	type	array	
	items		
			continues on poyt page

continues on next page

Table 1 – continued from previous page

1 10			
		type	string
	•		
• hideTabs	Hide tabs of markers dataset. Only use when you have a unique marker		
	type	boolean	
 settings 	type	array	
items			
		Setting	
	•		

Layer

type	object	
properties		
• name	type	string
• tileSource	type	string

LayerFilter

type	array	array		
items	·			
•	type	object		
	properties	properties		
	• name	Filter		
	• value	type	string	

Filter

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

MarkerFile

Description of settings and GUI objects for a marker dataset loaded from CSV file			
type	object		
properties	properties		
• title	Name of marker button		
	type	string	
• comment	Optional description text shown n	ext to marker button	
	type	string	
	default		
• name	Name of marker tab		
	type	string	
 autoLoad 		aset 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 data	the marker button to load the dataset.	
	type	boolean	
	default	false	
• uid	A unique numeric identifier for the marker dataset		
	type	string	
• expectedHeader	ExpectedHeader		
• expectedRadios	ExpectedRadios		
• path	Relative file path to CSV file for marker dataset		
	type	string	
• settings	type	array	
	items		
	•	Setting	

ExpectedHeader

Input field values for settings in a marker tab			
ype object			
properties			
• X	type	string	
• Y	type	string	
• gb_col	type	string	
• gb_name	type	string	
• cb_cmap	type	string	
• cb_col	type	string	
• cb_gr_dict	type	string	
• scale_col	type	string	
• pie_col	type	string	
• pie_dict	type	string	
• shape_col	type	string	
shape_fixed	type	string	
shape_gr_dict	type	string	
opacity_col	type	string	
• opacity	type	string	
• tooltip_fmt	type	string	
	l .	I	

ExpectedRadios

ype	object	
roperties		
• cb_col	type	boolean
• cb_gr	type	boolean
• cb_gr_rand	type	boolean
• cb_gr_dict	type	boolean
• cb_gr_key	type	boolean
• pie_check	type	boolean
• scale_check	type	boolean
• shape_gr	type	boolean
• shape_gr_rand	type	boolean
• shape_gr_dict	type	boolean
• shape_col	type	boolean
• shape_fixed	type	boolean
• opacity_check	type	boolean
• _no_outline	type	boolean

Setting

type	object		
properties	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": [
    "Color"
```

(continues on next page)

(continued from previous page)

```
],
"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,
"settings": []
```

(continues on next page)

(continued from previous page)

}

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

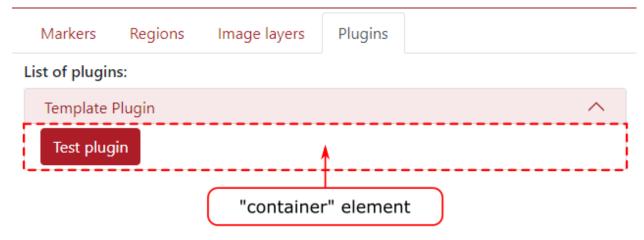
2.6.1 Load plugins

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.

2.6. Plugins 16

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

4.2. Napari 19

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

4.3. AnnData 20

INDEX

```
L
loaddata() (in module tissuumaps.jupyter), 18

M
module
    tissuumaps.jupyter, 18

O
opentmap() (in module tissuumaps.jupyter), 18

S
screenshot() (tissuumaps.jupyter.TissUUmapsViewer
    method), 19

T
tissuumaps.jupyter
    module, 18
TissUUmapsServer (class in tissuumaps.jupyter), 19
TissUUmapsViewer (class in tissuumaps.jupyter), 19
```