

# SOP: Manual Segmentation of Tissue

Generating manual segmentations of anatomical structures in  
HuBMAP image data

**Authors:** Leah Scherschel, Yingnan Ju, Yashvardhan Jain

**Approved by:** Katy Börner (June 9, 2022)

HIVE MC-IU Team, Indiana University

PI: Katy Börner

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## Introduction

This document describes the process used by subject matter experts (SMEs) for downloading HuBMAP image data, generating manual segmentations of structures such as functional tissue units (FTUs), and uploading the segmentations to a storage location. The audience for this SOP is the group of subject matter experts (SMEs) who are tasked with segmenting FTUs in image data, typically a histologist recommended by the PI associated with the organ of interest. The current tool preferred for this process is [QuPath](#).

## Roles and Responsibilities

The **MC-IU Contact** is responsible for coordinating with Tissue Mapping Centers to obtain image data for segmentation, empowering the Segmentation Expert to create segmentation data, and organizing the resulting data in a safe storage location.

The **Globus Access Contact** is responsible for approving access requests for the R3 Globus data repository.

The **Segmentation Expert** is responsible for generating segmentation data as described in this SOP.

**Table 1.** Roles, names, and email addresses of key personnel.

Role	Name	Email
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MC-IU Contact	Yashvardhan Jain	ccfinfo@indiana.edu
Globus Access Contact	Melissa Schwenk	help@hubmapconsortium.org
Segmentation Expert	Varies by Tissue	

## Download Data

### Globus data acquisition - as needed

#### Creating a Globus user ID

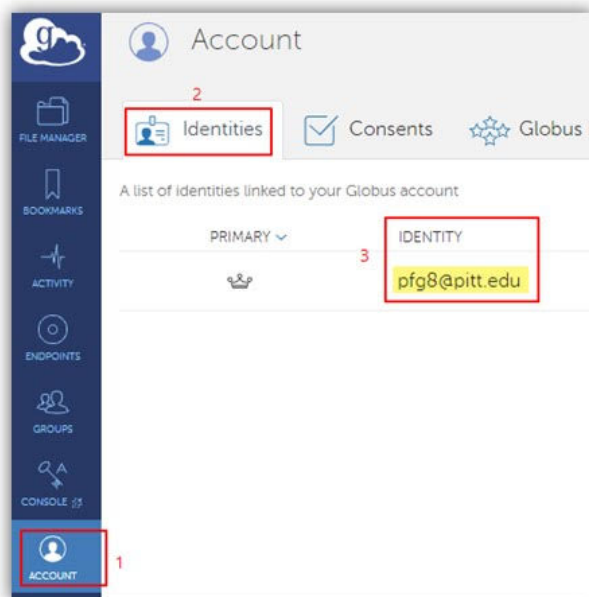
If you don't have a Globus account, please create a Globus user ID by navigating to <http://app.globus.org>. On the login screen, use your University (choose this in the dropdown) login credentials to log in.

#### Log in with an existing identity on Globus

Visit [www.globus.org](http://www.globus.org) and click *Log In* at the top-right of the page. On the Globus login page, choose an organization you're already registered with, such as your school or institution.

#### Gaining access to the R3 Globus data repository

If you need to gain access to the R3 Globus data repository, after creating a Globus user ID and logging in, email [Melissa Schwenk](mailto:Melissa.Schwenk@hubmapconsortium.org) the email address displayed in *Account* → *Identities* → *IDENTITY* as shown below.



## Choose a collection from Globus

A collection is a named location containing data you can access with Globus. Collections hold data on different types of storage systems, on a server at different institutions, in the cloud, or on the local computers.

Collection	<input type="text" value="HuBMAP Landing Zone"/>
Path	<input type="text" value="/"/>

## Set up and use Globus Connect Personal

Follow [these instructions](#) to download Globus Connect Personal and set up an endpoint and collection on your own local computer, including Mac, Linux, or Windows system.

## Request a file transfer

Navigate to the File Manager page and request a transfer between your collection and the chosen collection. Click *Transfer* or *Sync to...* in the command panel on the right side of the page. A new collection panel will open, with a "Transfer or Sync to" field at the top of the panel.

For more help with Globus, see <https://docs.globus.org/how-to/>.

## Generate segmentations with QuPath

### Download and Install QuPath

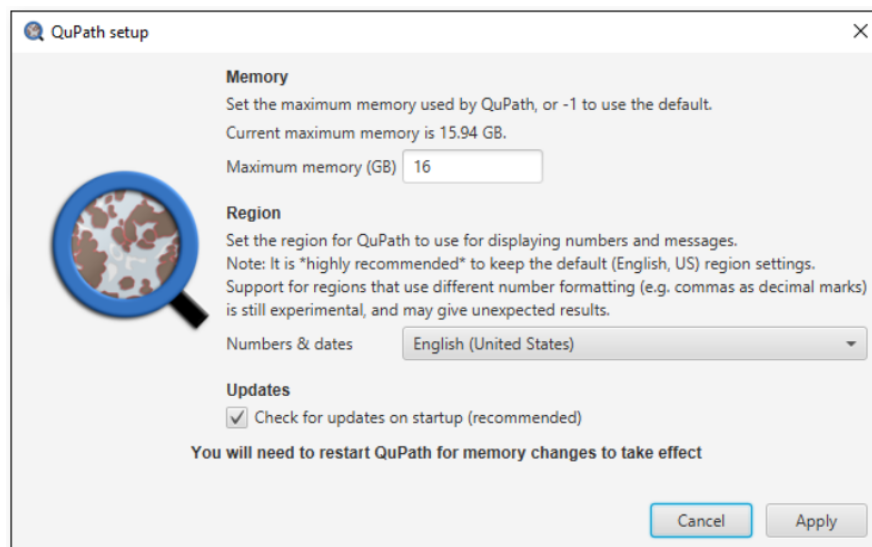
Download QuPath from <https://qupath.github.io/> (Latest stable release: v0.2.3)

Follow installation instructions found here:

<https://qupath.readthedocs.io/en/latest/docs/intro/installation.html>

### Select setup options

Upon startup, QuPath will prompt you to set Maximum memory and Region.



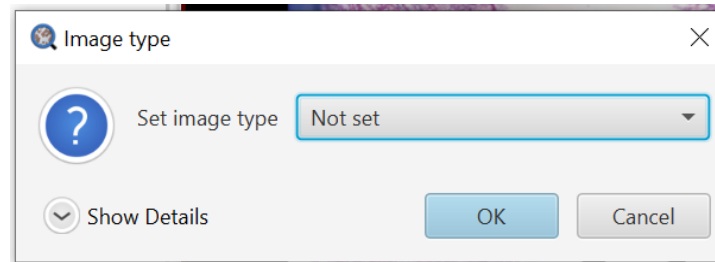
*Setup options shown on startup.*

Depending on the size of the image you are working on, you may need to increase this memory allocation. This window can also be accessed later through *Help* → *Show setup options* if you experience memory errors.

### Open image data

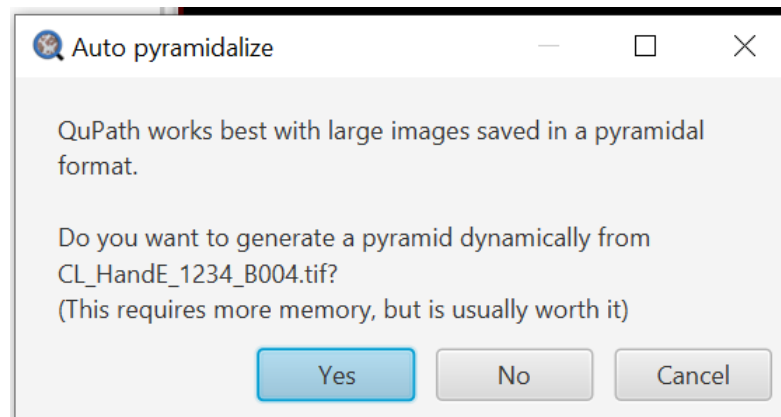
#### Basic open method

QuPath can handle a wide range of image formats through the use of [Bio-formats](#) and [OpenSlide](#). Open an image with *File* → *Open....* Upon opening the image, an Image type prompt will appear. Leave the image type as *Not set* and click *OK*.



## Pyramidal image files

If the image you want to open is not in pyramidal format, QuPath will display this prompt.

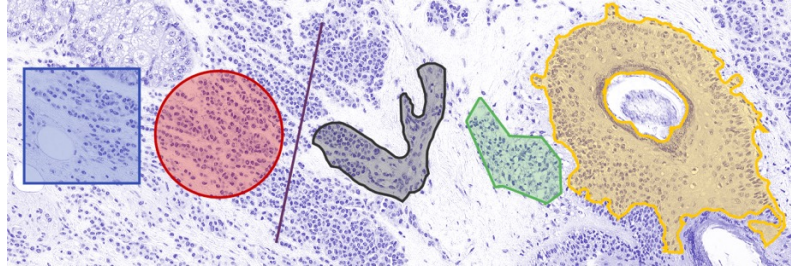


If the image will be opened in QuPath more than once, it is highly recommended that you click **Yes**, then save the pyramidal format image by using **File** → **Export images...** → **OME TIFF**, leaving the file name the same so only the extension is changed. Then, reopen the new **IMAGE\_FILENAME.ome.tiff** image file and use that for segmentation. Using this pyramid format will greatly reduce the time required for reopening the image in QuPath and help avoid future memory errors.

## Manually segment image

Drawing regions of interest (ROIs) is a frequent task within QuPath. Segmentations can be made using different drawing tools: (left to right) Rectangle, Ellipse, Line, Brush, Polygon, Wand. You can see a toolbar in the top left corner:





## Brush tool

- The Brush tool is recommended, and segmentations should be as accurate as possible.
- Each click of the brush selects a small circle, but by dragging the mouse across the image a region is “painted”.
- Brush diameter can be adjusted through *Edit* → *Preferences...* → *Drawing tools* → *Brush diameter*.
- The size of the brush adapts according to magnification.
- Holding down the *Alt* key while using the brush causes it to “subtract” regions, like an eraser.

**Note:** A touch screen and stylus make the task of segmentation much easier, but it can be completed with a mouse/trackpad as well.

## Add/Set classification for segmentation

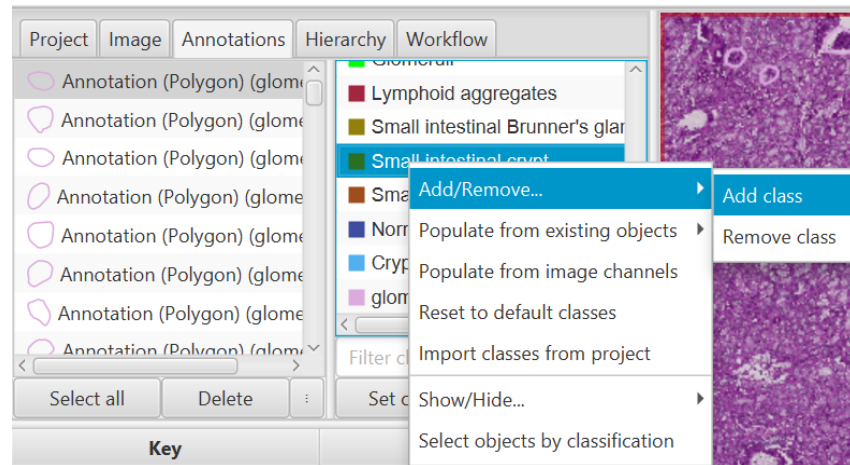
When applying analysis within QuPath, there is a default list of available classifications. These are listed in the *Annotations* tab of the analysis panel on the left side of the window.

### Classification list in the Annotations tab

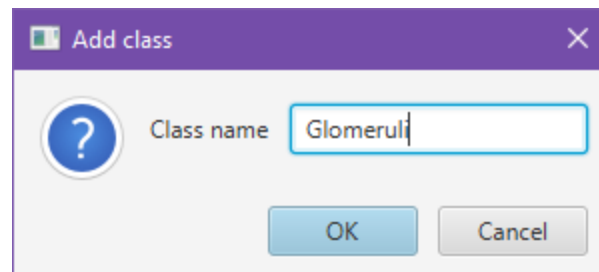
Double-clicking on any classification within this list gives the option of changing its default color, while right-clicking opens up a menu with options to change the list in some way, such as by adding or removing classes.

### Add a class

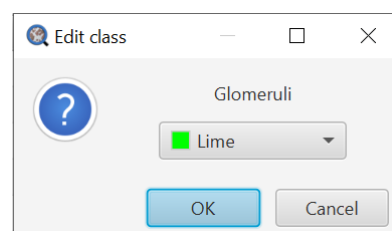
Right click on any of the classes on the right side of the *Annotations* pane to open a menu and use *Add/Remove...* → *Add class* to add a new class.



Input the name of the class in the pop up window.



Once added to the list, double click the color block in front of the class name to change the color of each class.

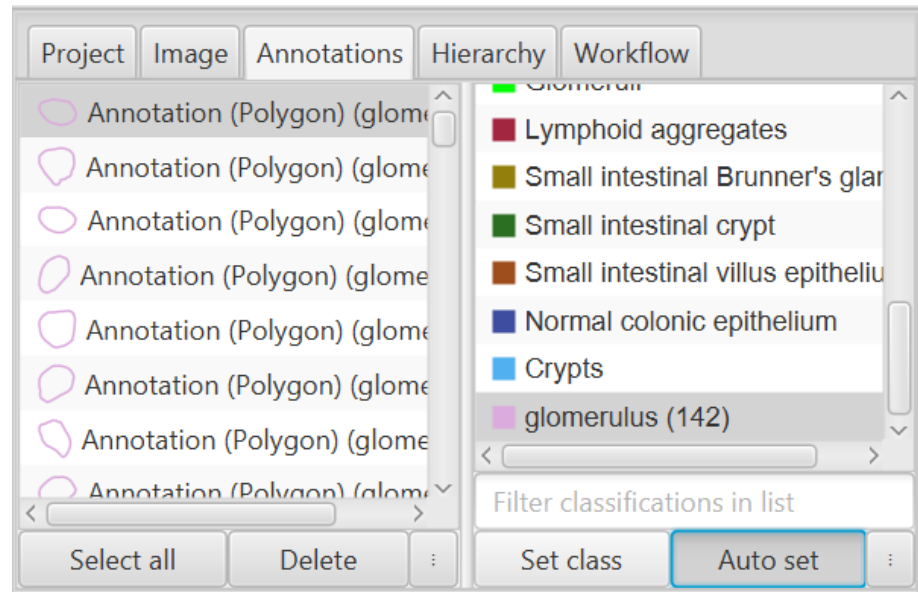


## Automatically set class for future segmentations

The *Auto set* button can be used to ensure that all new segmentations are automatically set to have the selected class. This can help when quickly drawing large numbers of segmentations that should all have the same class.

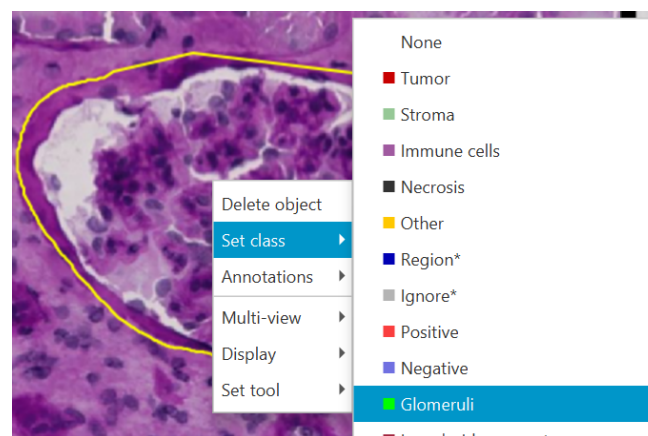
## Classify selected segmentation using *Set class* button

Classifications can be assigned to selected segmentations directly. The *Set class* button below the classification list can be used to explicitly set the class of the selected segmentation to whichever class is selected in the list.



## Classify selected segmentation by right-click

Right-clicking on the image whenever a segmentation is selected brings up a context menu through which the classification can be set.





## Delete a segmentation

Select a segmentation from the list and use the *Delete* button at the bottom of the *Annotations* pane to delete the segmentation. Alternatively, right-click the image with the desired segmentation selected and use the *Delete object* option from the pop up menu.

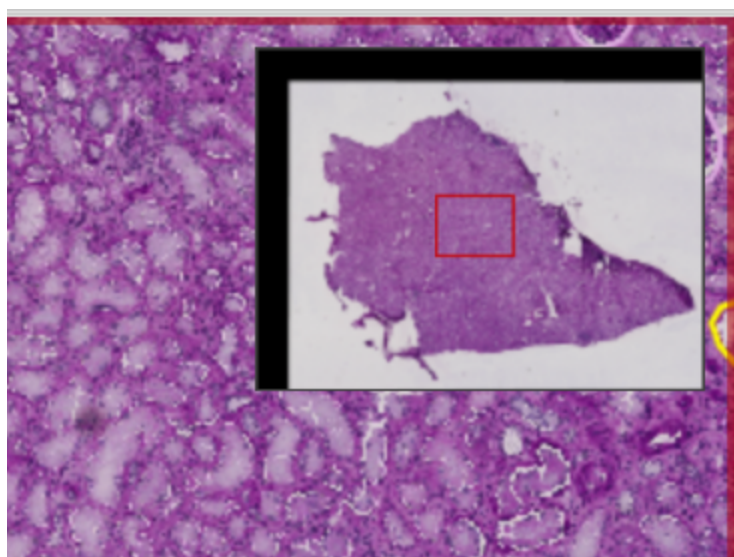
## Navigating the image



Move tool

With the *Move* tool selected from the toolbar, your cursor will show as a hand icon over the image. This will allow you to click and drag the image to move your field of view. Alternatively, you can click and drag the red field of view outline on the preview of the whole image located in the top right corner to move your field of view.

**Note:** Clicking on the preview with a tool other than *Move* selected will create a segmentation. Be careful not to generate unintended segmentations accidentally.



## Zooming in and out

Scroll up/down to zoom in/out, respectively. This can be performed while tools other than the *Move* tool selected, allowing you to zoom out, then zoom in on a different section of image (centered over your cursor) without having to switch tools.

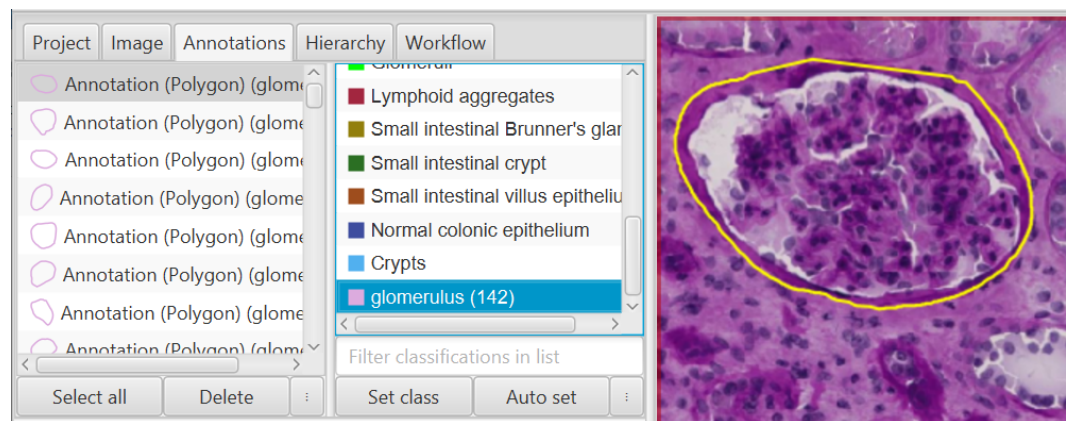
## Saving the segmentations

After segmentations have been made, click on *File* → *Save* to save the segmentation mask for the image. In the pop up window, save the file with the name: IMAGE\_FILENAME.qpdata. The file name should by default be the same as the original image file (**IMPORTANT**).

## Upload the results

Submit .qpdata files to the online sharing space provided by MC-IU. If you created a pyramidal version of the image to segment, upload it along with the segmentation mask file.

## Example segmentation



**Note:** The segmentation is a “Polygon” and the outline is very accurate.

## References and Glossary

The below references and definitions were used in writing this Standard Operating Procedure. When available, definitions were taken from the [HuBMAP Dictionary](https://docs.globus.org/), and aligned with standard terminologies used in relevant fields.

### References

- Globus Documentation: <https://docs.globus.org/>
- QuPath Documentation: <https://qupath.readthedocs.io/en/latest/>

## Glossary

**Functional tissue unit (FTU):** Functional tissue units such as glomeruli in the kidney or crypts in the colon are subregions of tissue that serve a specific function; they are important for bridging the organ level to single cell level.

**Globus:** Globus is software-as-a-service for research data management, used by hundreds of research institutions and HPC facilities worldwide for secure, reliable file transfer, sharing and publication. (Globus.org)

**QuPath:** QuPath is open source software for bioimage analysis. QuPath is often used for digital pathology applications because it offers a powerful set of tools for working with whole slide images - but it can be applied to lots of other kinds of image as well. (<https://qupath.readthedocs.io/en/latest/docs/intro/about.html>)

**Standard Operating Procedures (SOPs):** SOPs are issued to specifically instruct team members in areas of responsibility, procedural steps, appropriate specifications and required records. SOPs outline procedures, which must be followed to support the reproducibility of scientific research. Procedures can take the form of a narrative, a flow chart, a process map, computer screen printouts or combination of all or any other suitable form, however must be written in appropriate, effective grammatical style. (e.g. plain English).

**Subject Matter Expert (SME):** An SME is a person with extensive knowledge about a given topic. In our case, SMEs lend us their expertise to ensure that 3D models are built not only factually correctly but also in a manner that requires minimal correction and design iterations.