

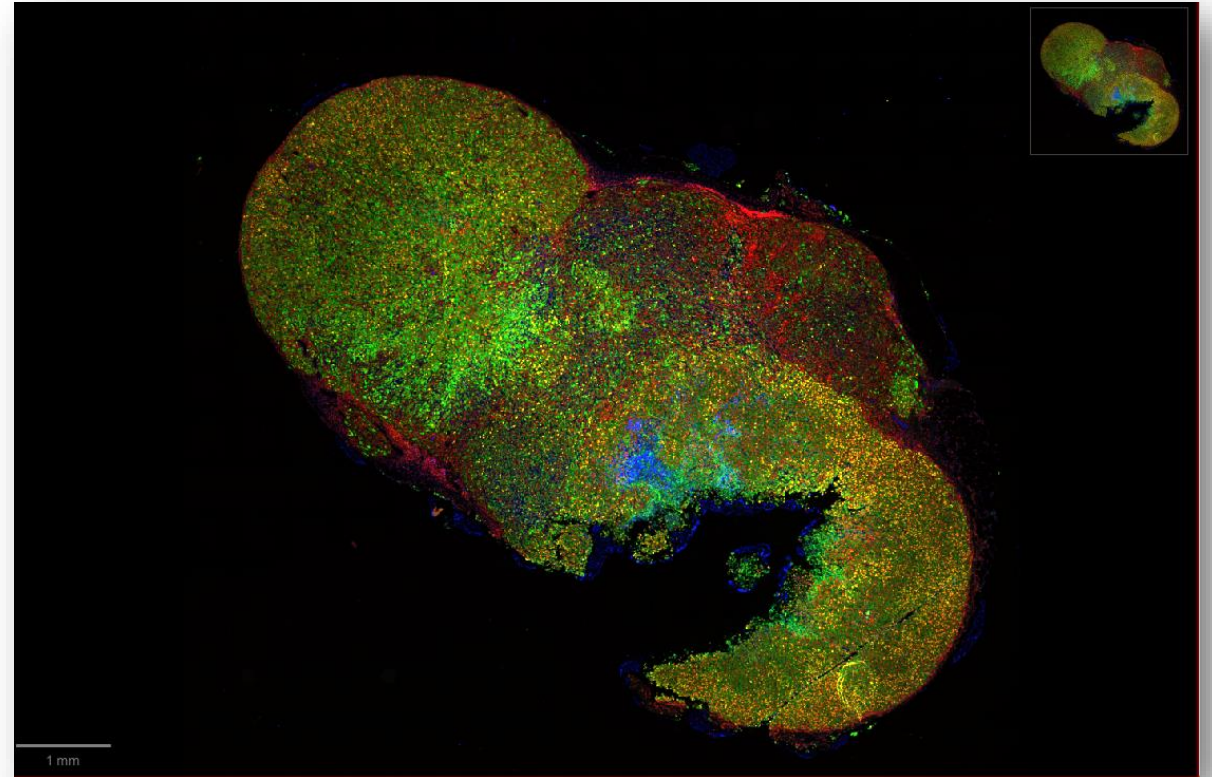
A fluorescence microscopy image of a tissue section. The image shows a complex pattern of colors: a large blue area on the left, a green area in the center, a red area on the right, and a yellow area at the bottom. The background is black. The text "Your first project in QuPath" is overlaid in white.

# Your first project in QuPath

# Classification of proliferating cancer cells in solid tumors

- Whole-slide image
  - Already been stitched
- 4 channels
  - DAPI
  - Keratin (FITC)
  - Fibronectin (TRITC)
  - Ki67 (CY5)

**At the end of this course:**  
you will have classified  
proliferating (Ki67) cancer  
cells and reveal their spatial  
distribution to regions with  
high-fibronectin content

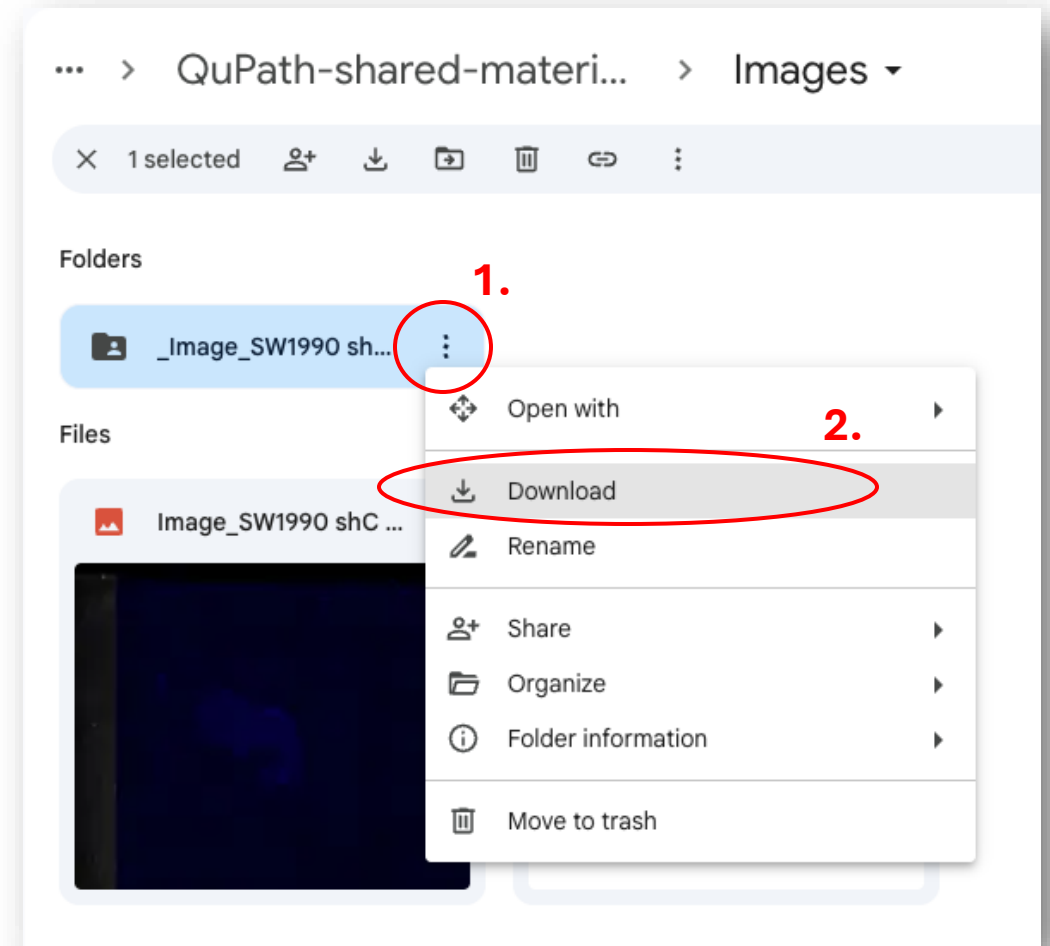


Courtesy of Nina Kozlova, PhD



# Download the image from the shared folder

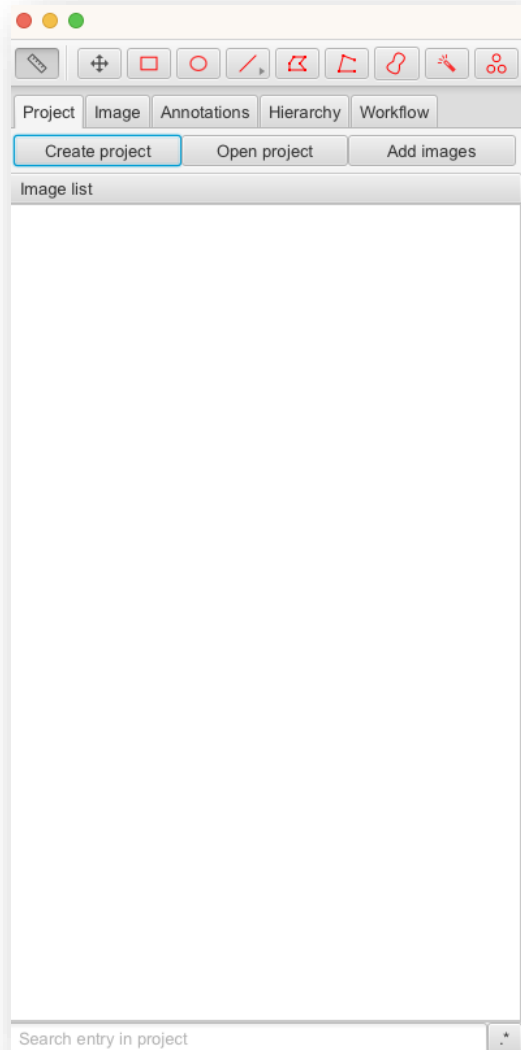
1. Download the whole folder from the Google Drive
  1. Image size: ~3GB; it will take a minute or two to download
2. Unzip it
3. Transfer the image in a new **Images** folder in your QuPath project folder



# Key concept: QuPath project

- Projects are the way to organize your work in QuPath
- In other words, they are folders
  - Group together images
  - Organize data, scripts, classifiers, etc
  - They only save data, not the original images
- Allow you to share your work with other QuPath users
  - Always send the images along!

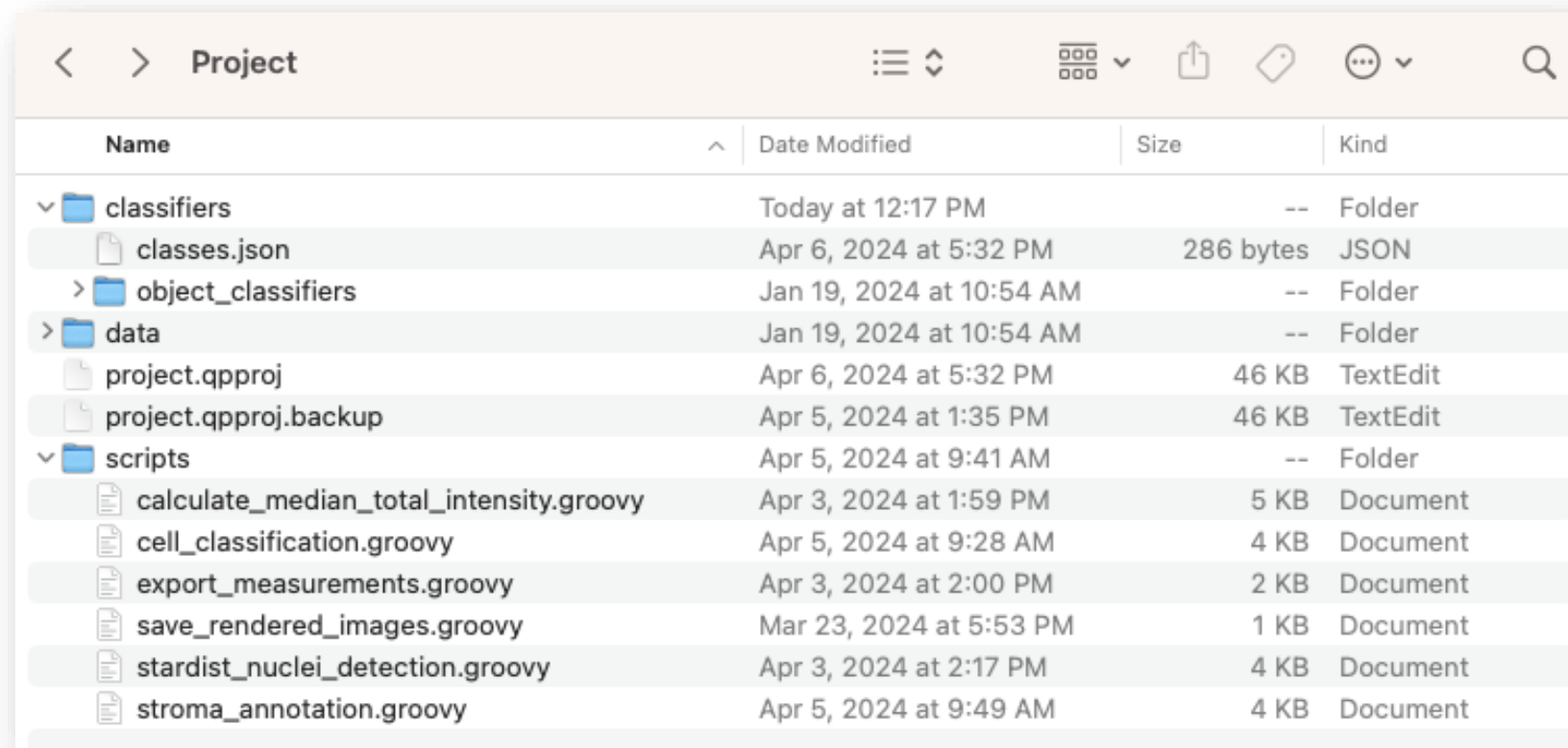
# How to create a project?



- *Create project* button  
or
- File > Project... > Create project
- ! Make sure to create an **empty** folder for your project
  - Sometimes, you have to do this twice in the empty folder

# Anatomy of a QuPath project

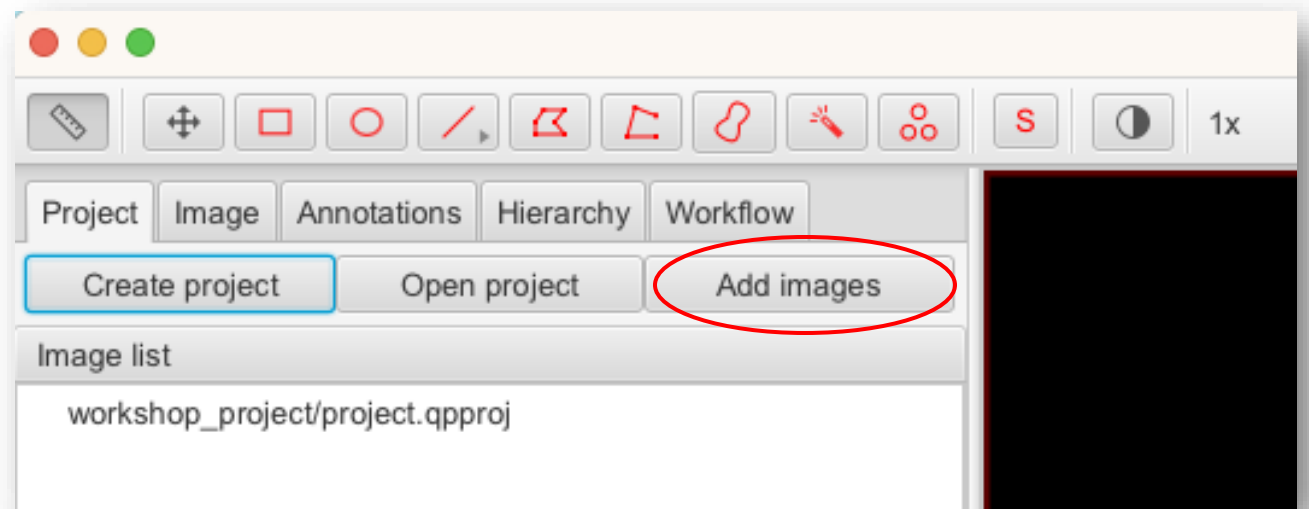
After a bit of time working on it...



Name	Date Modified	Size	Kind
classifiers	Today at 12:17 PM	--	Folder
classes.json	Apr 6, 2024 at 5:32 PM	286 bytes	JSON
object_classifiers	Jan 19, 2024 at 10:54 AM	--	Folder
data	Jan 19, 2024 at 10:54 AM	--	Folder
project.qpproj	Apr 6, 2024 at 5:32 PM	46 KB	TextEdit
project.qpproj.backup	Apr 5, 2024 at 1:35 PM	46 KB	TextEdit
scripts	Apr 5, 2024 at 9:41 AM	--	Folder
calculate_median_total_intensity.groovy	Apr 3, 2024 at 1:59 PM	5 KB	Document
cell_classification.groovy	Apr 5, 2024 at 9:28 AM	4 KB	Document
export_measurements.groovy	Apr 3, 2024 at 2:00 PM	2 KB	Document
save_rendered_images.groovy	Mar 23, 2024 at 5:53 PM	1 KB	Document
stardist_nuclei_detection.groovy	Apr 3, 2024 at 2:17 PM	4 KB	Document
stroma_annotation.groovy	Apr 5, 2024 at 9:49 AM	4 KB	Document

# Add an image to your project

1. Check your emails! Download this folder containing an example whole-slide image
2. Add an image
  - *Add images* button
    - Select the .vsi file

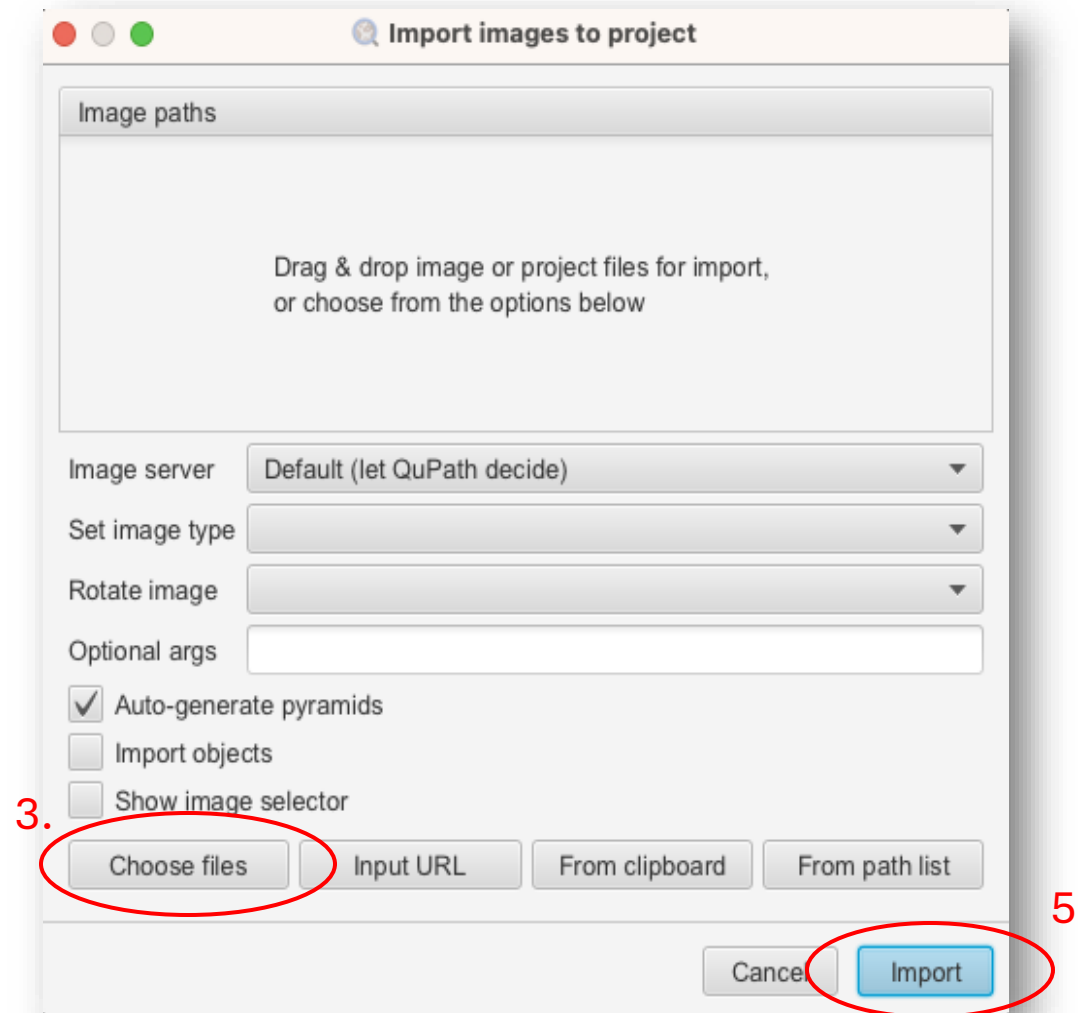


# Add an image to your project

3. Select the .vsi image using *Choose files* or drag-and-drop

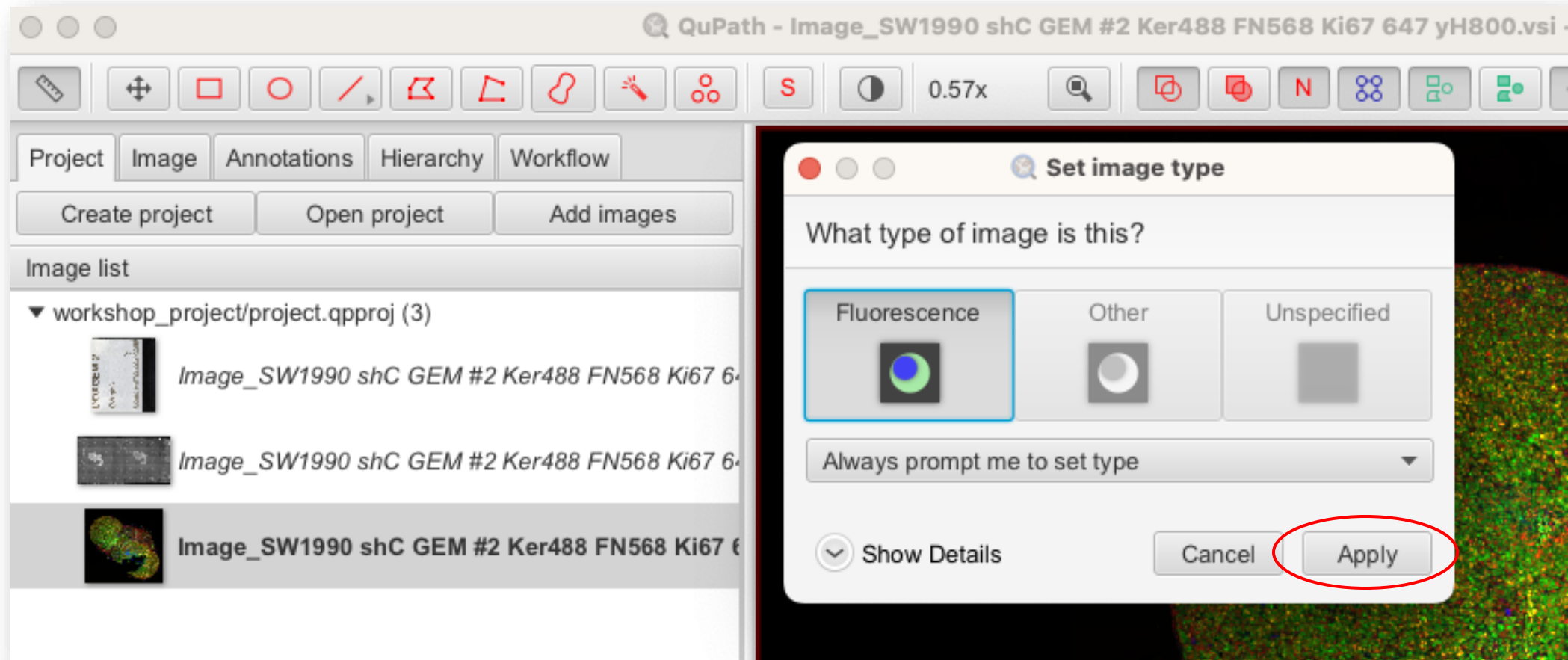
4. Use default settings

5. Click *import*





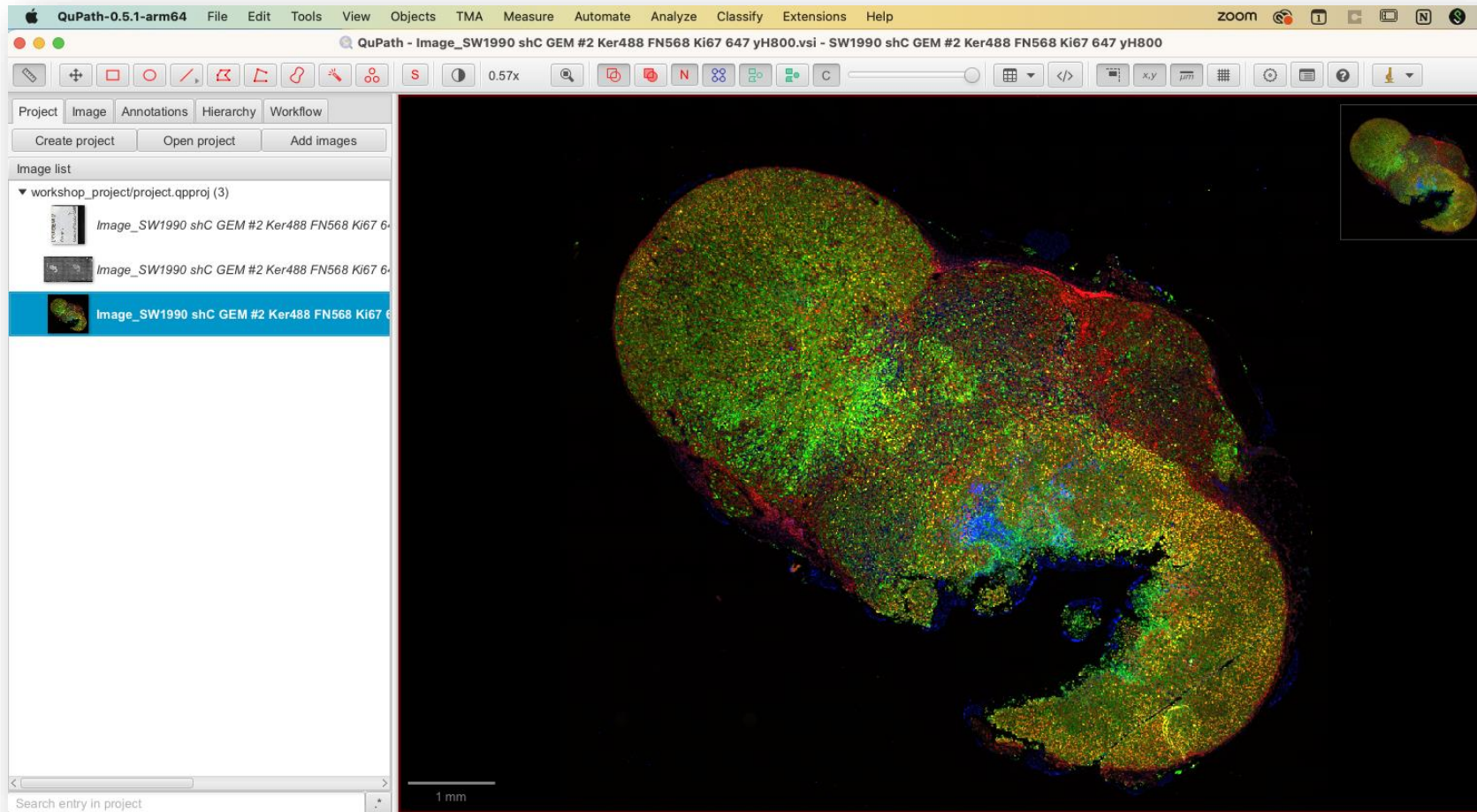
# Set image type



- *Other image types are supported: Brightfield H&E, H-DAB, other brightfield*

# Yay! We have a QuPath project with an image

→ *Double-click an image to open it in the viewer*



# QuPath works on copies of your original files

- QuPath access the image pixels and metadata via an image server
  - Akin to a copy of the original file
- Manipulating files within a QuPath project will never modify the original files or pixels
  - Deleting, duplicating, processing, etc will not be reflected in your original files

# QuPath projects are portable

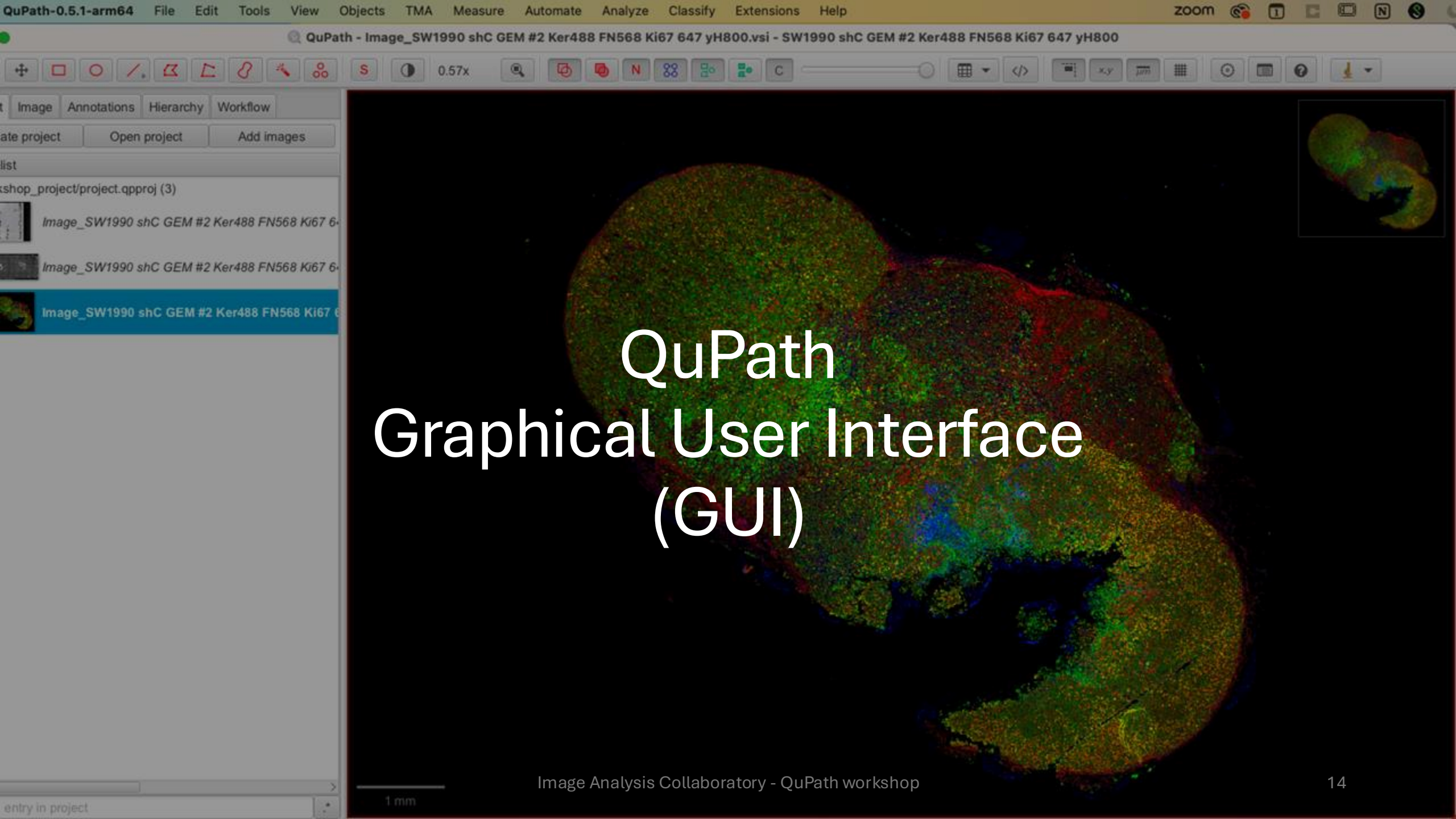
- Sharing a project:
  - Zip up the entire project directory
  - Email it to your collaborators

**The project folder only contains QuPath objects and data, unless you had placed them there. Ensure that they can access the actual image files.**

# QuPath projects are portable

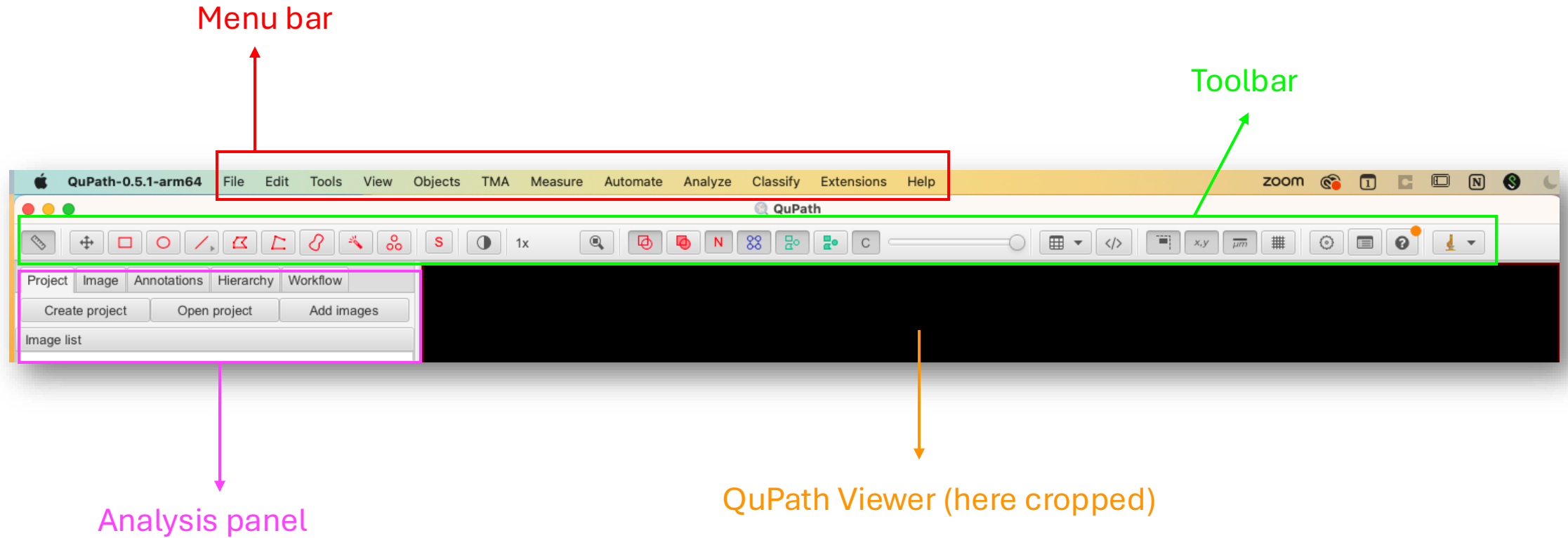
- Receiving a project:
  - The project still contains image paths specific to the local machine of the sender
  - If you move the image, you will be prompted to update the file path





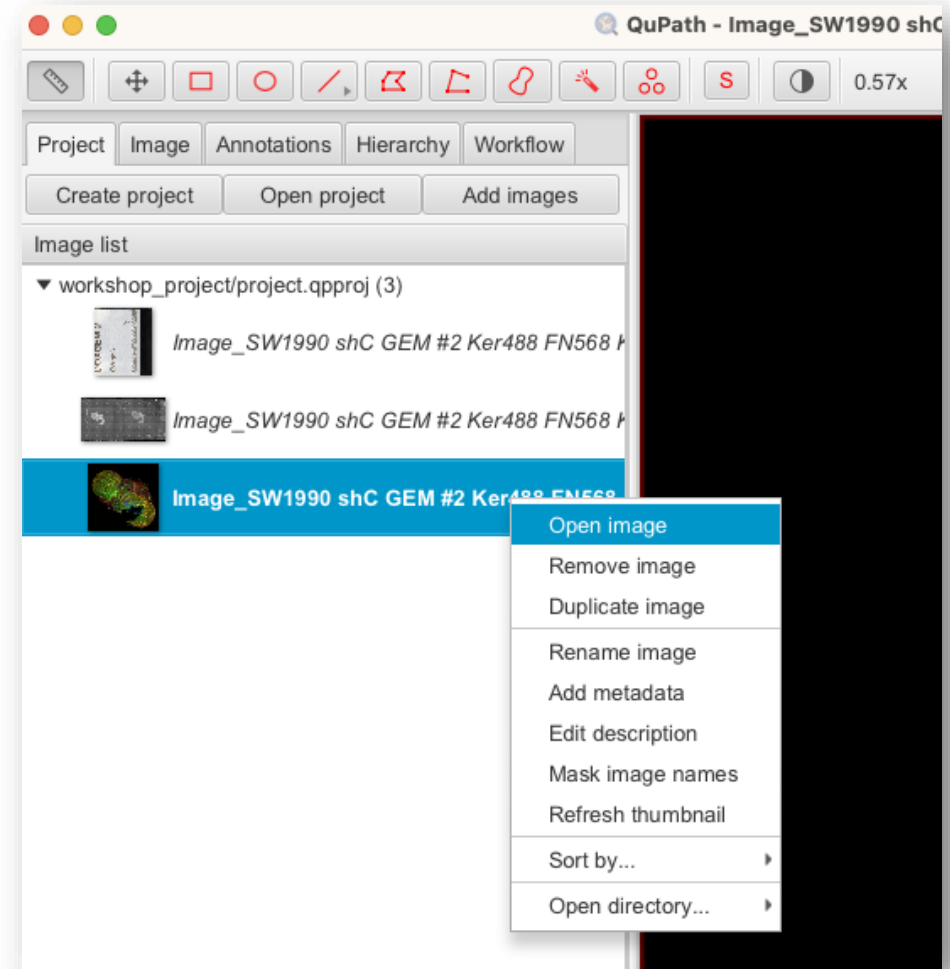
# QuPath Graphical User Interface (GUI)

# Graphic User Interface (GUI)



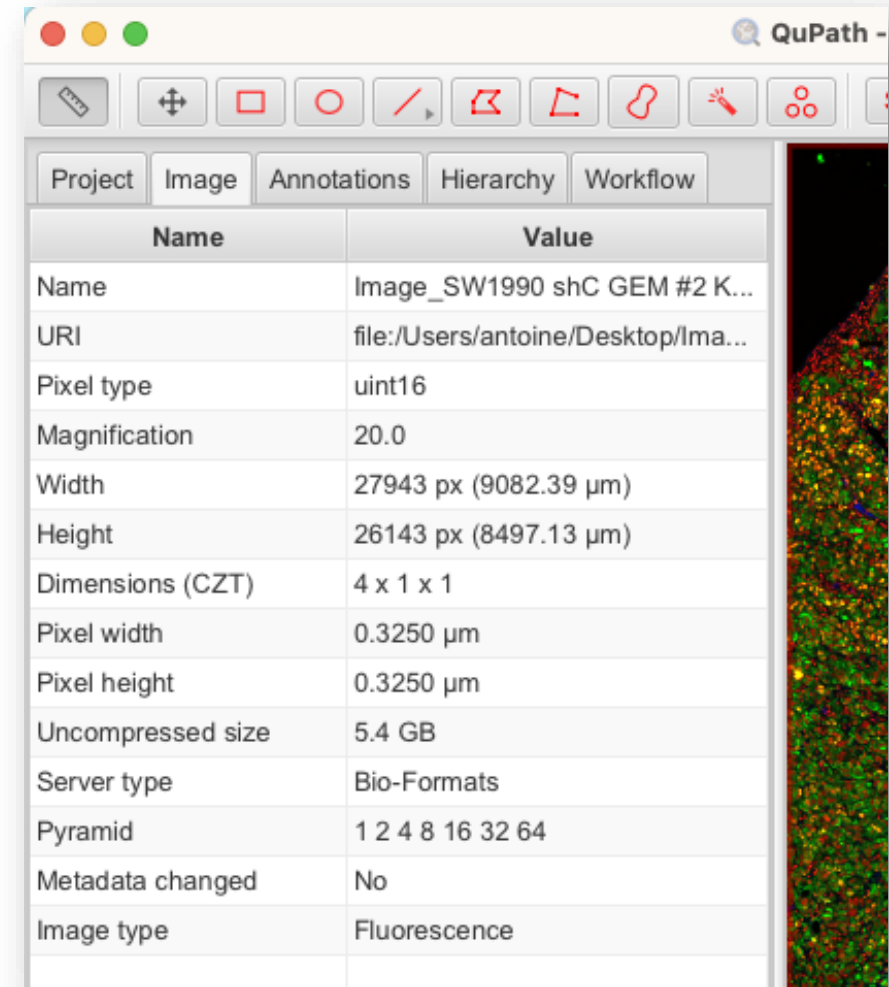
# Analysis Panel

- *Project* tab > right-click on an image
  - *Open, remove, rename and duplicate images*
  - *Edit metadata*



# Analysis Panel

- *Image* tab
  - Name and image file path
  - Magnification: 20x
  - Pixel type, width and height are crucial for scale calibration
  - Dimensions: 4 channels + 2D
  - Pyramid: level of downsampling in the viewer
  - Image type: previously set to fluorescence



# QuPath viewer

Viewer

Mini-map: overview

Info bar: pixel coord and value

Scale bar

The screenshot displays the QuPath viewer interface. The main window shows a fluorescence microscopy image of a tissue section, likely a brain, with various colored regions (green, red, blue, yellow) representing different cell types or markers. The interface includes a menu bar at the top with options like File, Edit, Tools, View, Objects, TMA, Measure, Automate, Analyze, Classify, Extensions, and Help. Below the menu bar is a toolbar with various icons for image manipulation. On the left side, there is a sidebar with tabs for Project, Image, Annotations, Hierarchy, and Workflow. The 'Image' tab is active, showing a table of image metadata.

Name	Value
Name	Image_SW1990 shC GEM #2 K...
URI	file:/Users/antoine/Desktop/ima...
Pixel type	uint16
Magnification	20.0
Width	27943 px (9082.39 $\mu\text{m}$ )
Height	26143 px (8497.13 $\mu\text{m}$ )
Dimensions (CZT)	4 x 1 x 1
Pixel width	0.3250 $\mu\text{m}$
Pixel height	0.3250 $\mu\text{m}$
Uncompressed size	5.4 GB
Server type	Bio-Formats
Pyramid	1 2 4 8 16 32 64
Metadata changed	No
Image type	Fluorescence

Associated images

Series 3 (macro image)

1 mm

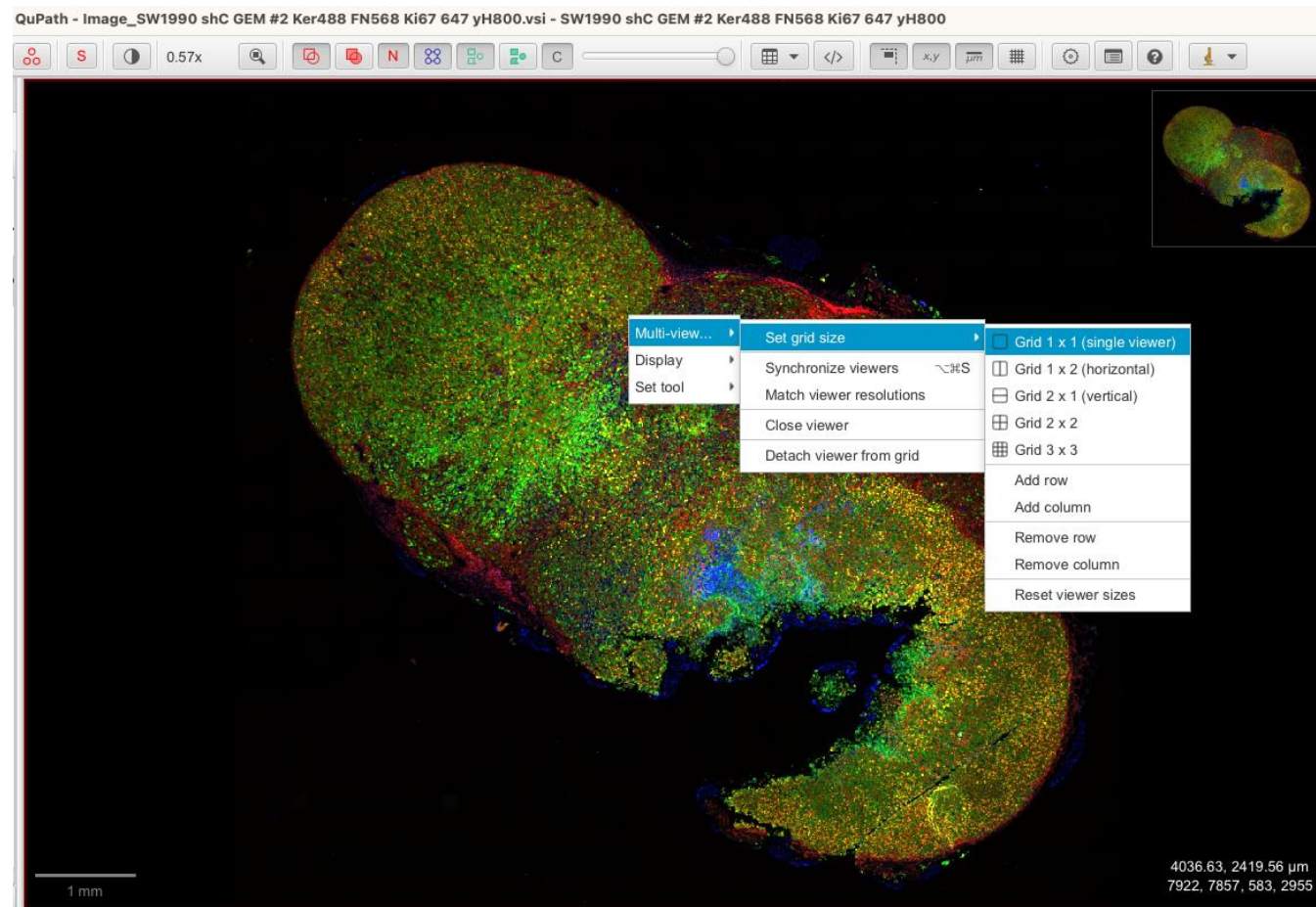
6547.99, 4758.85  $\mu\text{m}$   
9779, 9166, 1002, 943

Image Analysis Collaboratory - QuPath workshop

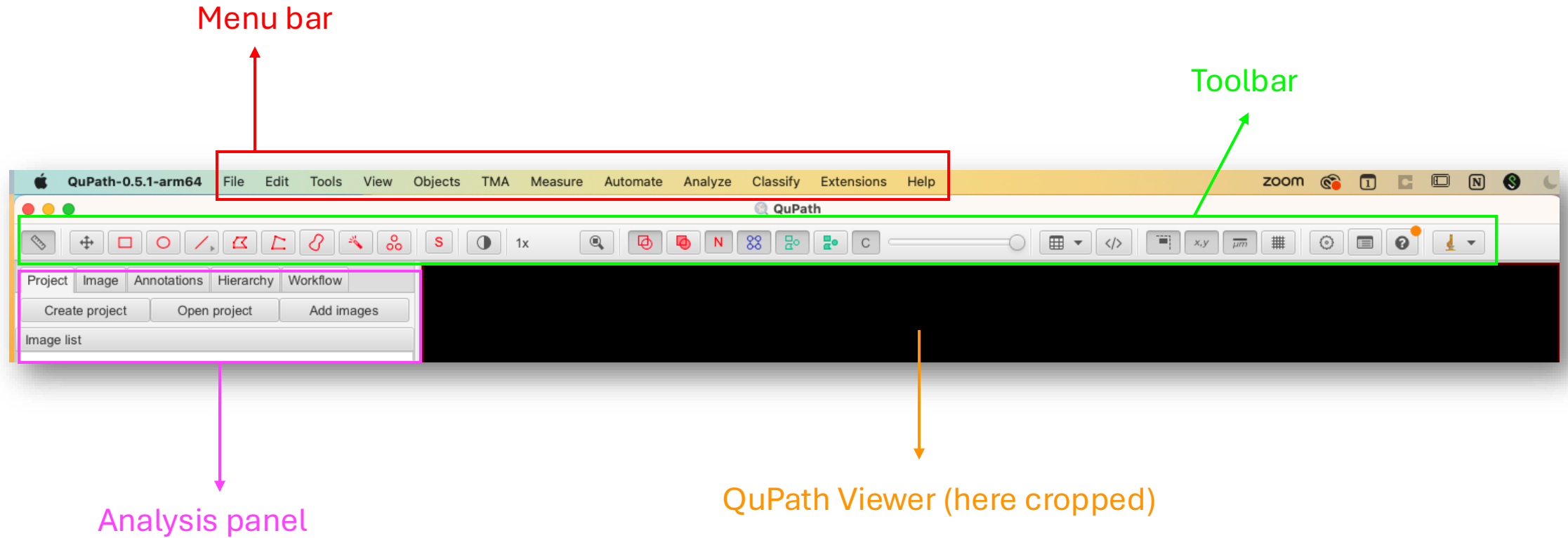


# Multi-viewer

- Right-click in the viewer

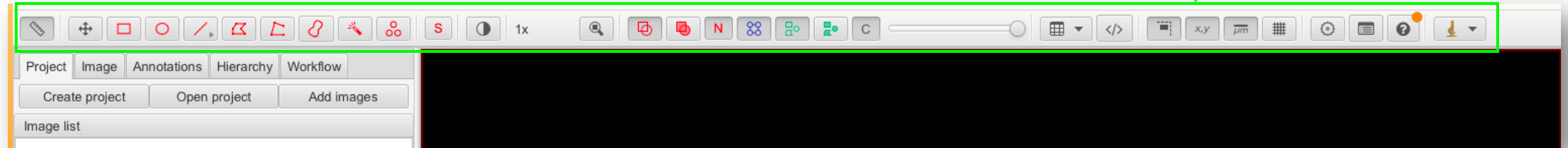


# Graphic User Interface (GUI)

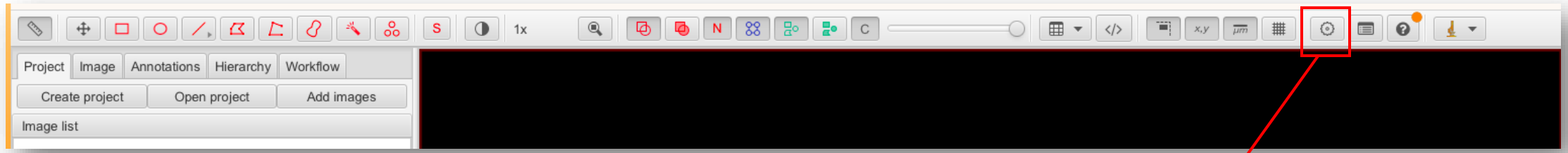


# Toolbar

Toolbar

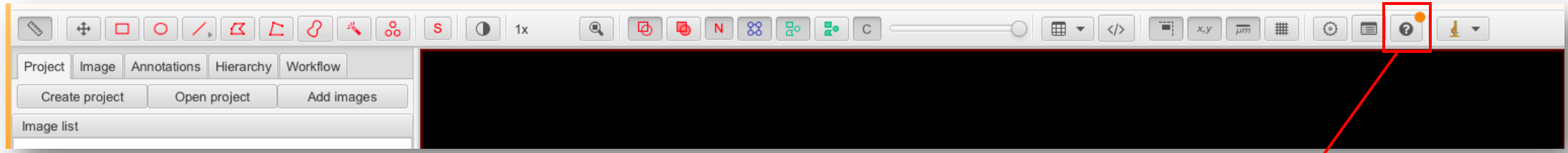


# Toolbar

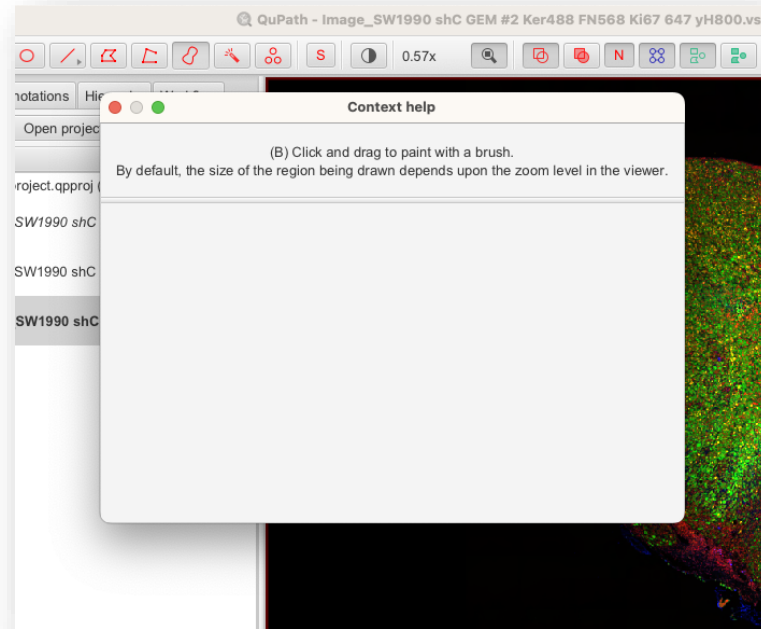


**Preferences**  
Settings, GUI  
customization,  
extensions, ...

# Toolbar



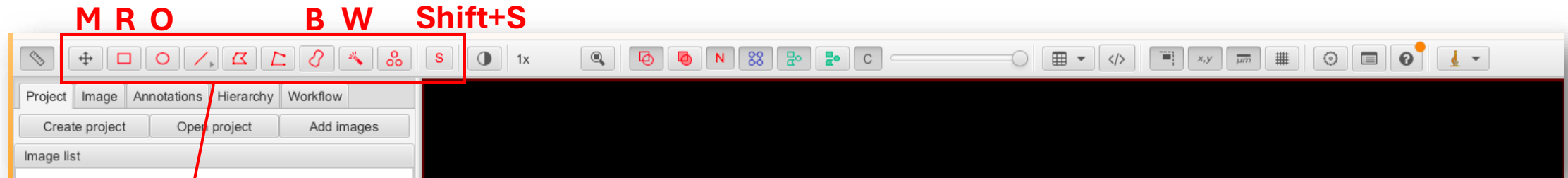
**Interactive Help**  
Provides contextual  
help based on your  
cursor location



Example when my cursor is on the paint brush tool

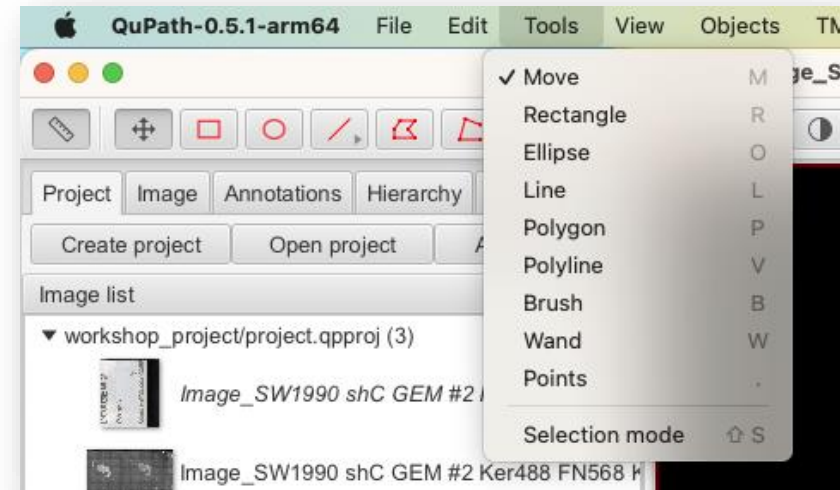


# Toolbar



## Annotation tools

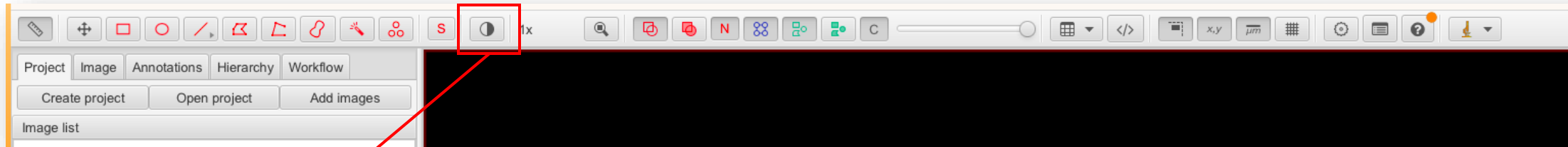
- **M** key: move tool
- **R** key: draw a rectangle annotation
- **O** key: draw an ellipse annotation
- **B** key: paint with a brush
- **W** key: draw with a wand tool
- And many more!



Annotation tools are also accessible in the *Tools* menu

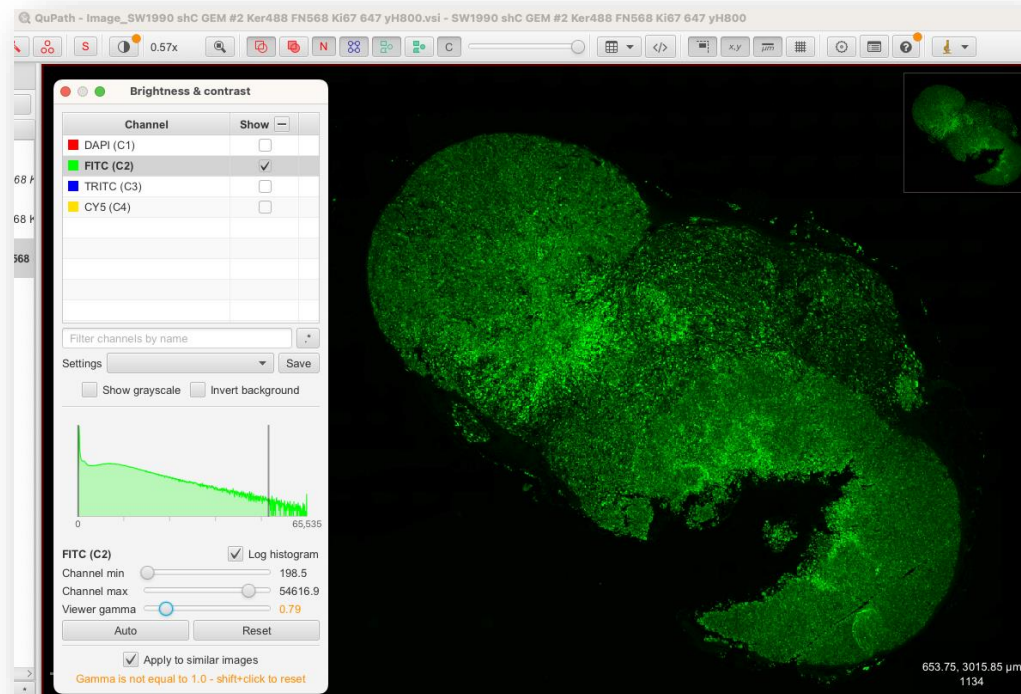
# Toolbar

Shift+C



## Brightness and contrast

- Toggle on/off channels
- Adjust LUT range
- Visualize intensity histogram



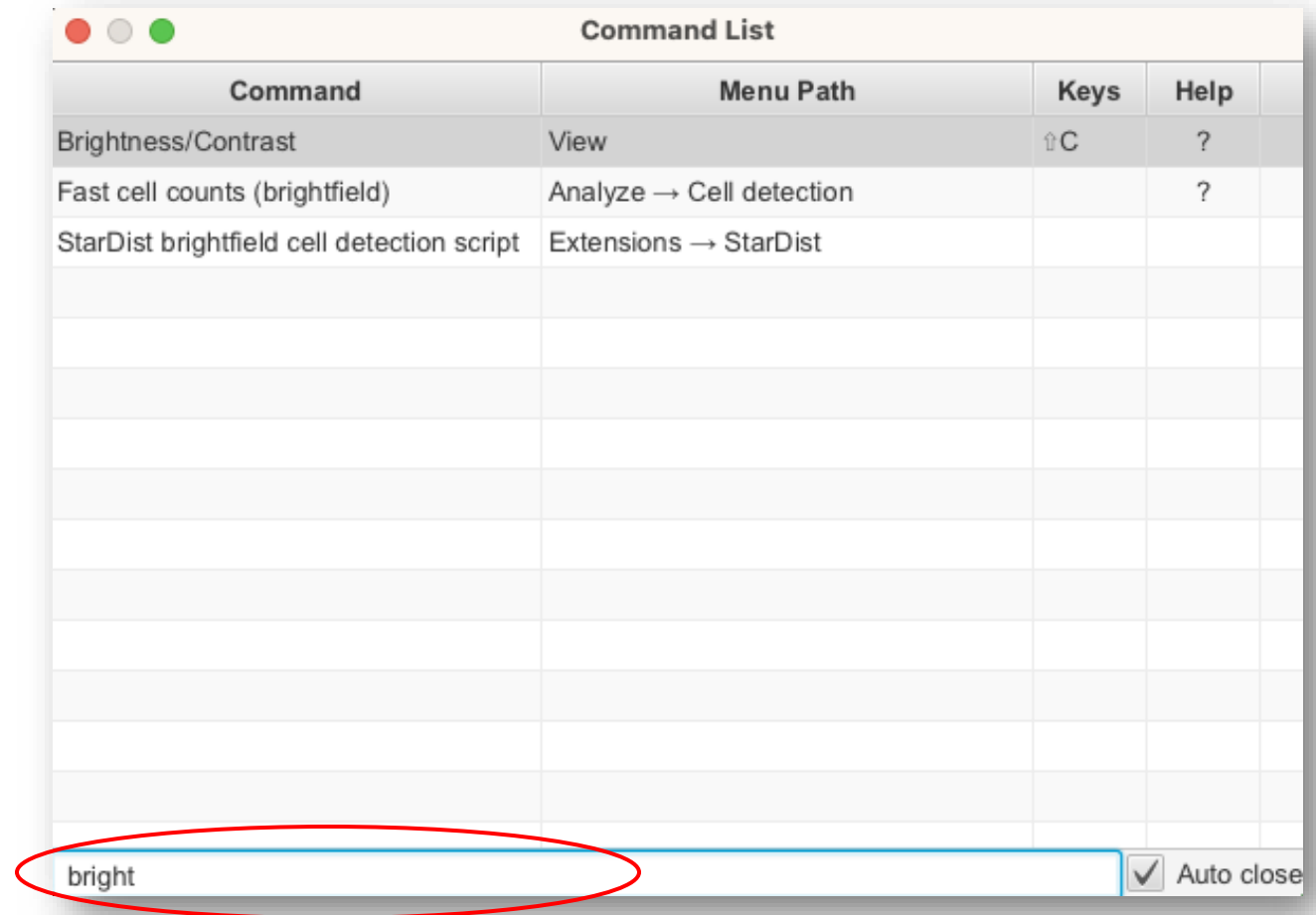
Example for FITC (Keratin) channel

# QuPath pro-tip: command list

## Command/Control + L

Opens a dialogue to  
search for any command  
using keyword

For example, search for  
'brightness'



*Practice time*

# Exercises 1: QuPath projects and GUI





# Introducing objects: annotations and detections



# Key concept: QuPath objects

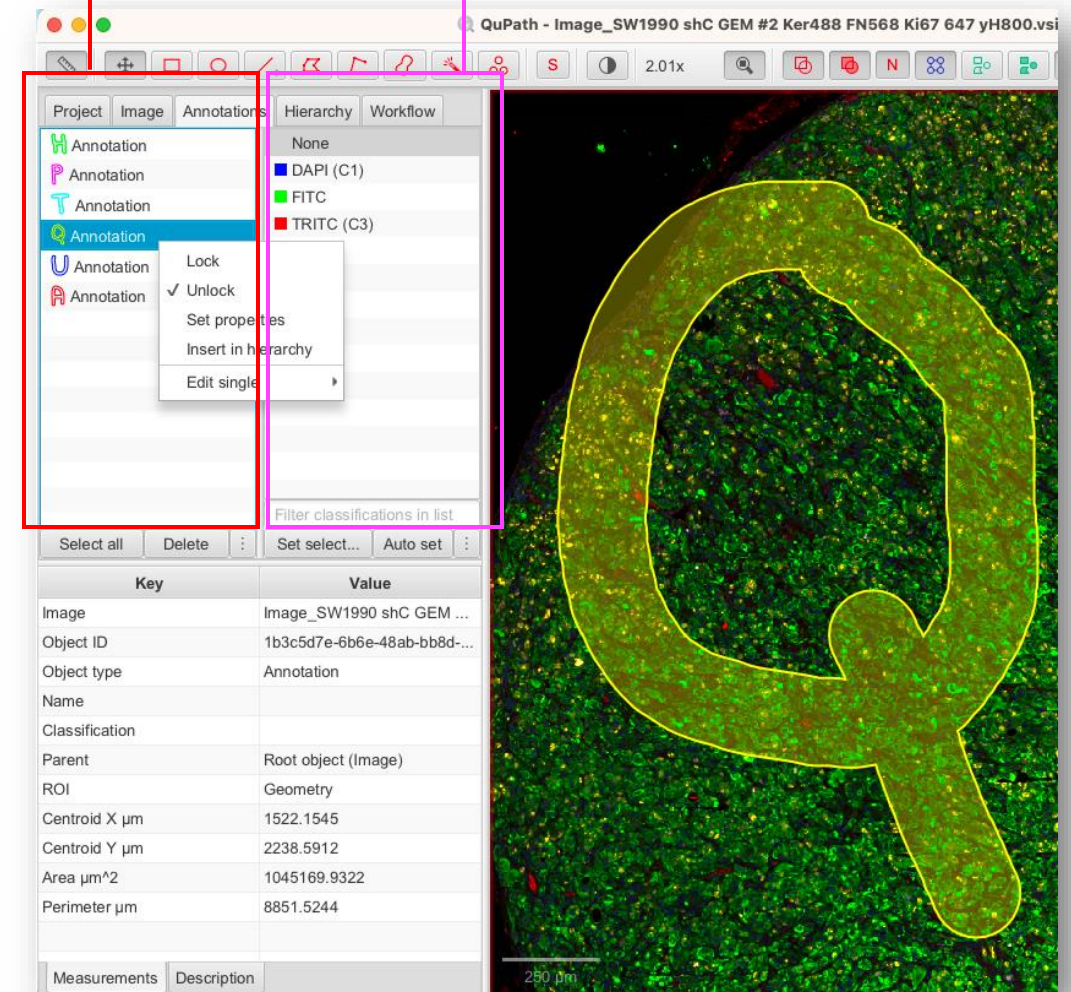
- **Objects** are a ‘thing’ in an image which encapsulates not only its shape but also some properties about it
  - **Annotations:** Objects that you usually create yourself, by drawing on the image
    - They are flexible, up to ~100 per image
    - Can be edited
    - Often used to define regions
  - **Detections:** Objects that QuPath usually creates for you
    - They are efficient, up to ~millions per image
    - Can be deleted but not edited
    - Often used to define cells

# Analysis Panel

- *Annotations* tab
  - Annotation list lets you select, delete
  - Right-click to **lock** or edit properties (name, color)
  - Shift or Command/Control to multi-select

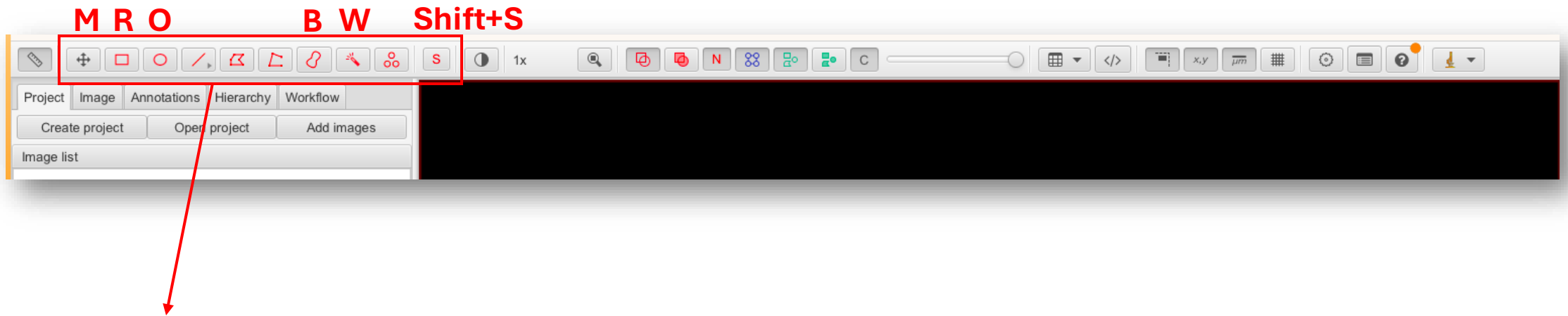
*Annotation list*

*Classification list*



# How to create manual annotations?

Select one of the annotation tools from the toolbar then scribble on the image!



## Annotation tools

- **M** key: move tool
- **R** key: draw a rectangle annotation
- **O** key: draw an ellipse annotation
- **B** key: paint with a brush
- **W** key: draw with a wand tool
- And many more!

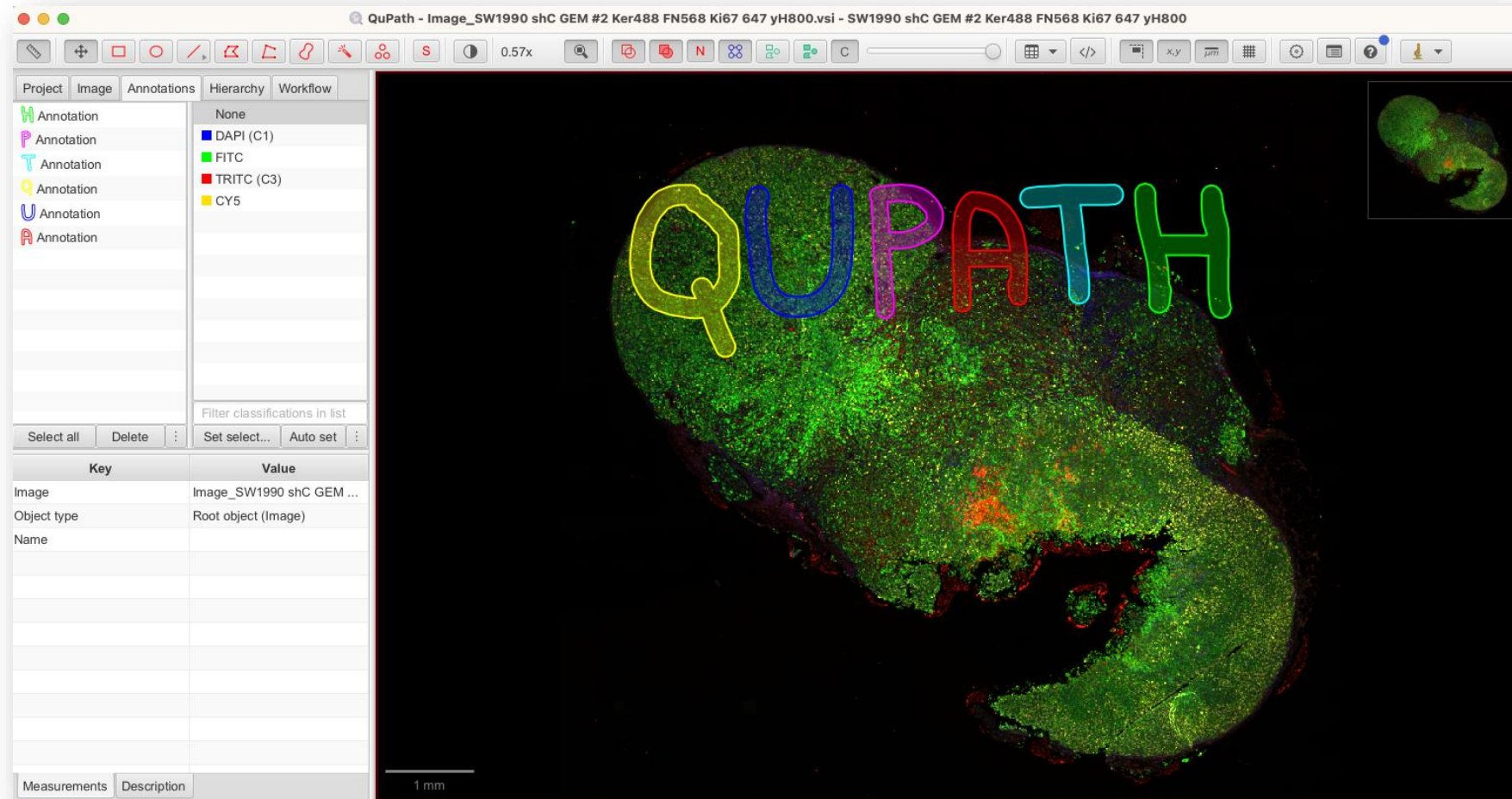
**Remember to always  
lock your annotation  
to prevent accidental  
editing!**

*Practice time*

## Exercises 2: QuPath manual annotations

# Recreate these annotations

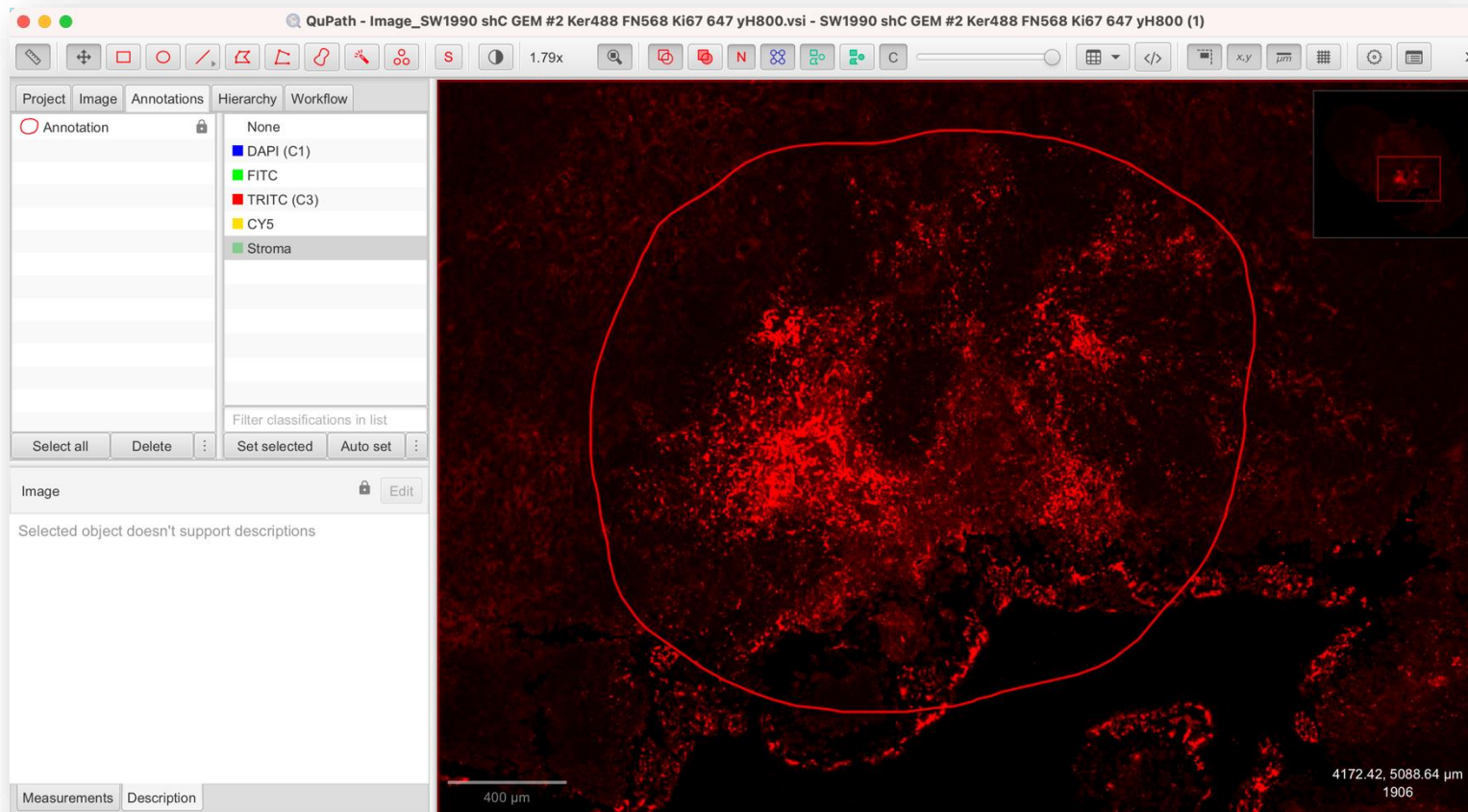
Decide on which annotations tool from the toolbar is best to do so





# Create a region of interest with the annotation tools

In the TRITC channel (fibronectin), create a region of interest that enclose high-fibronectin content regions



Once you have finished your annotation, **lock** it:

Right-click in the viewer  
> *Annotations* > *Lock*

or

Right-click on the  
annotation in the  
analysis panel > *Lock*