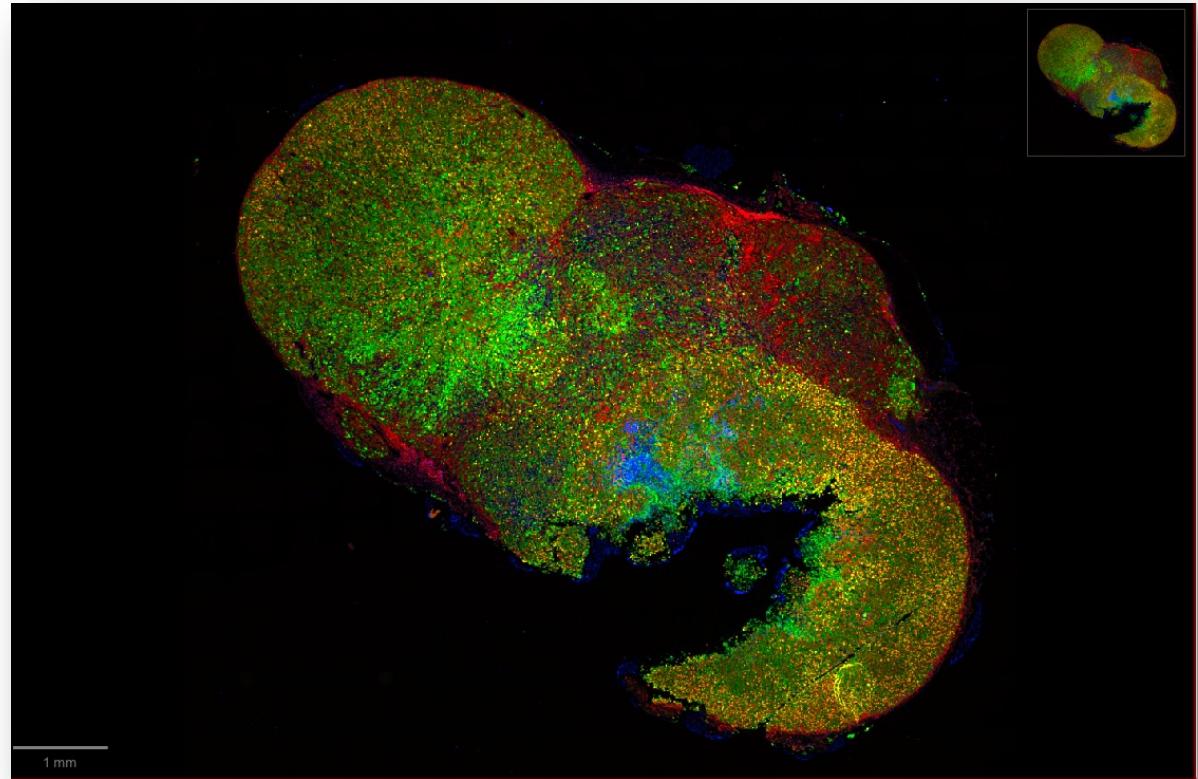
A fluorescence microscopy image showing a cross-section of tissue. The image is stained with multiple colors: green, red, blue, and yellow. The green signal is widespread, while red, blue, and yellow signals are more localized, possibly indicating different cellular components or markers. The tissue has a layered structure with some darker, irregular areas.

# 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)

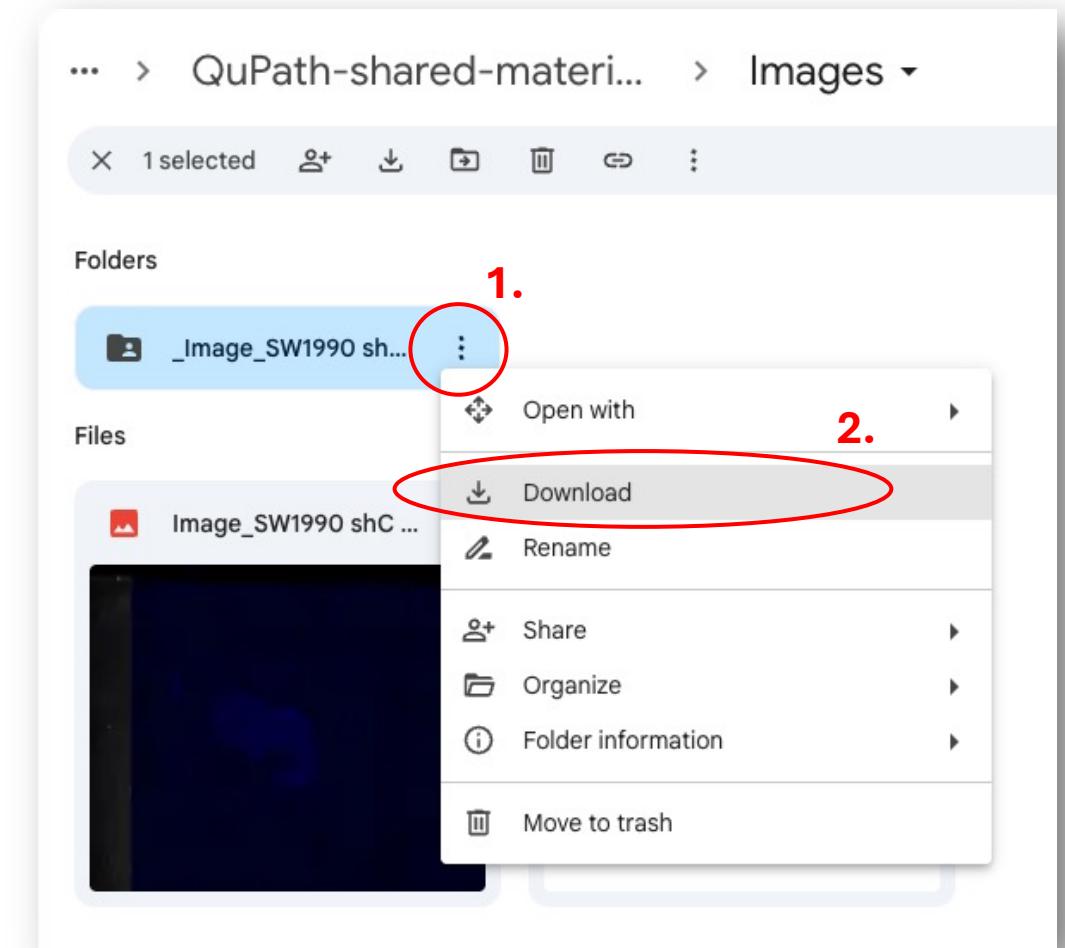
**At the end of this course:**  
you will have classified  
proliferating (Ki67) cancer  
cells and reveal their spatial  
distribution to regions with  
high-fibronectin content



Courtesy of Nina Kozlova, PhD

# Download the image from the shared folder

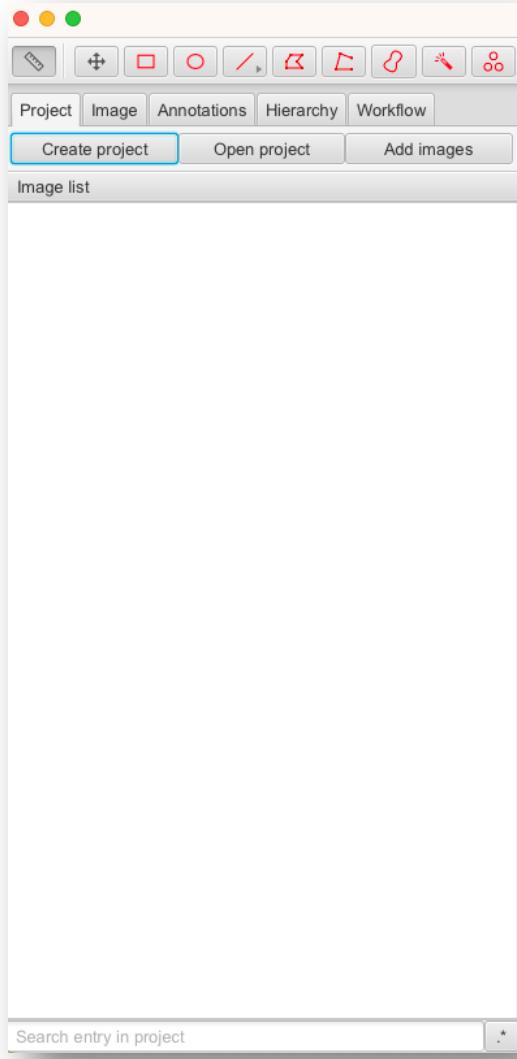
1. Download the whole folder from the Google Drive
  1. Image size: ~3GB; it will take a minute or two to download
2. Unzip it
3. Transfer the image in a new **Images** folder in your QuPath project folder



# 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!

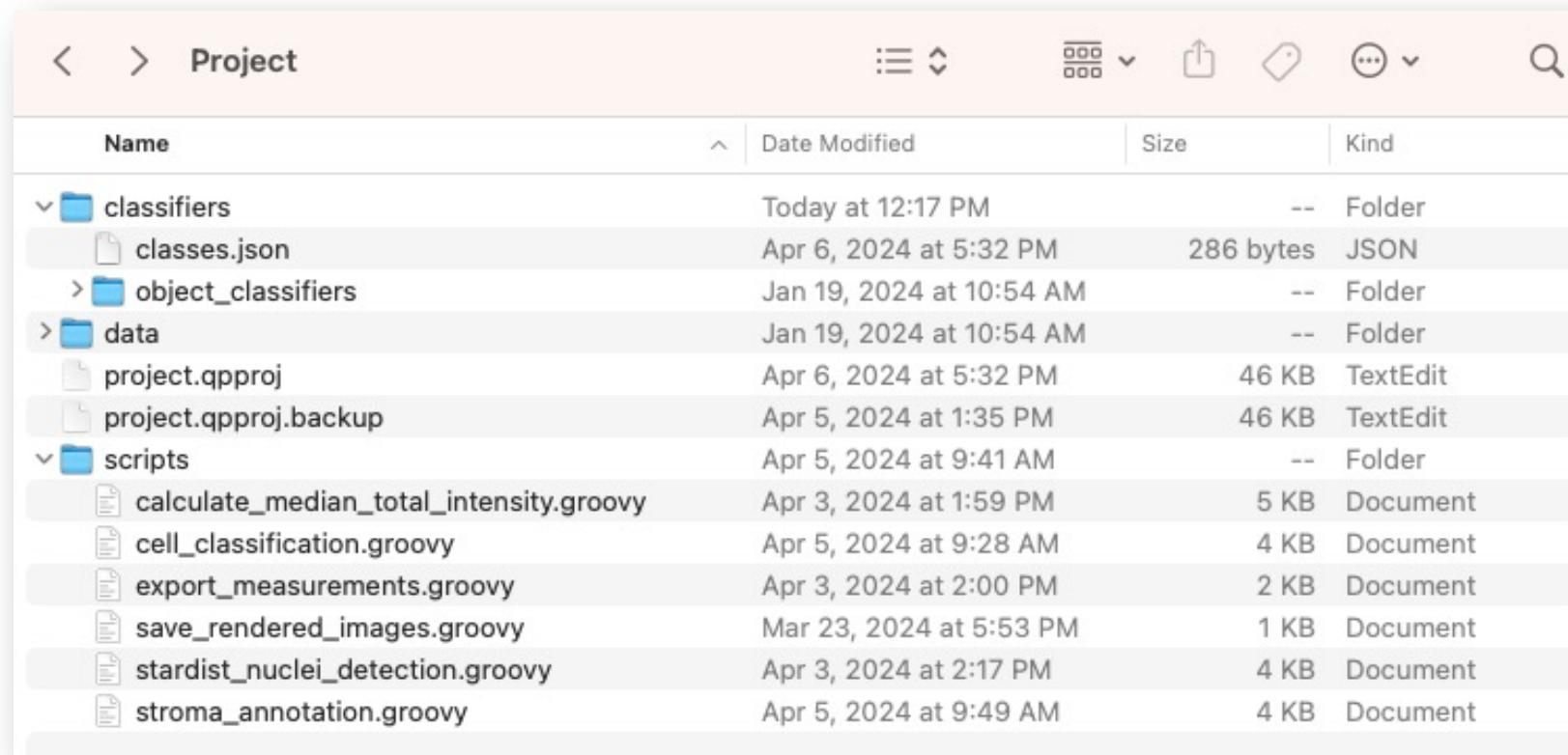
# How to create a project?



- *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...

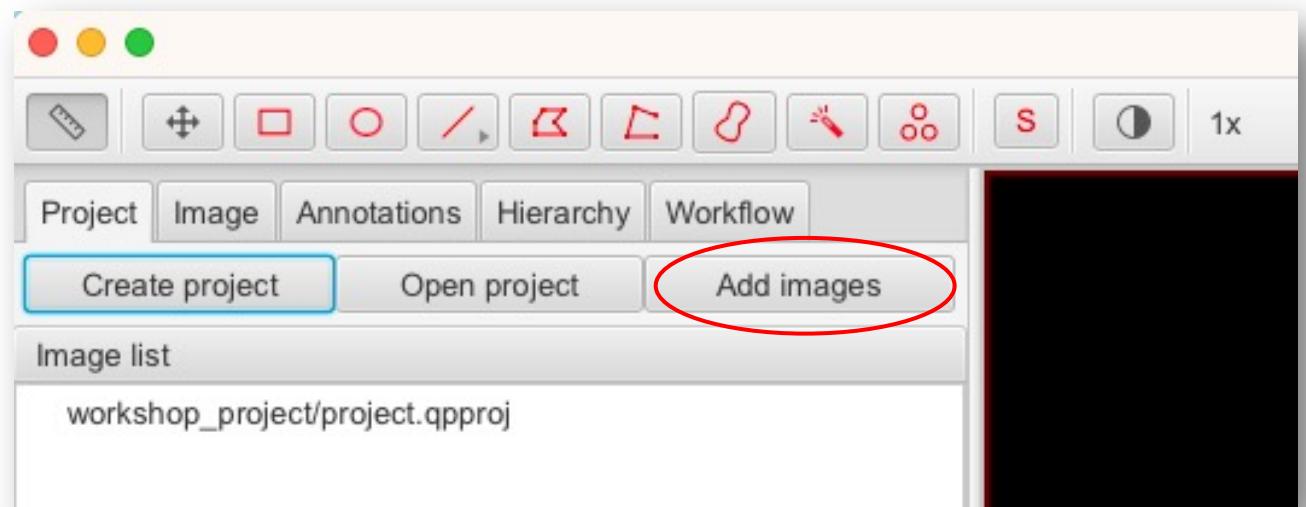


The screenshot shows the QuPath Project browser interface. The title bar says "Project". The toolbar includes icons for back/forward, search, and file operations. The main area is a table with columns: Name, Date Modified, Size, and Kind. The table lists the following items:

| 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

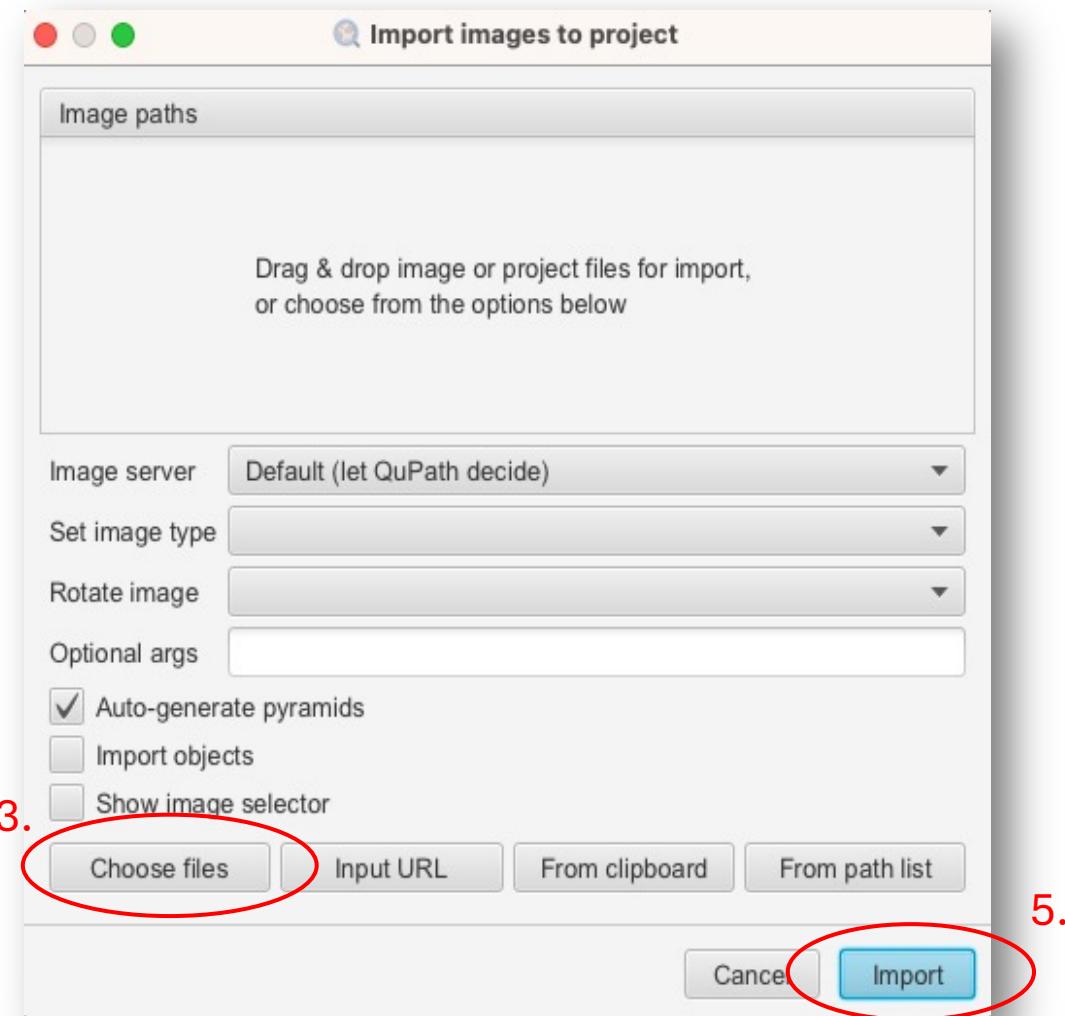


# Add an image to your project

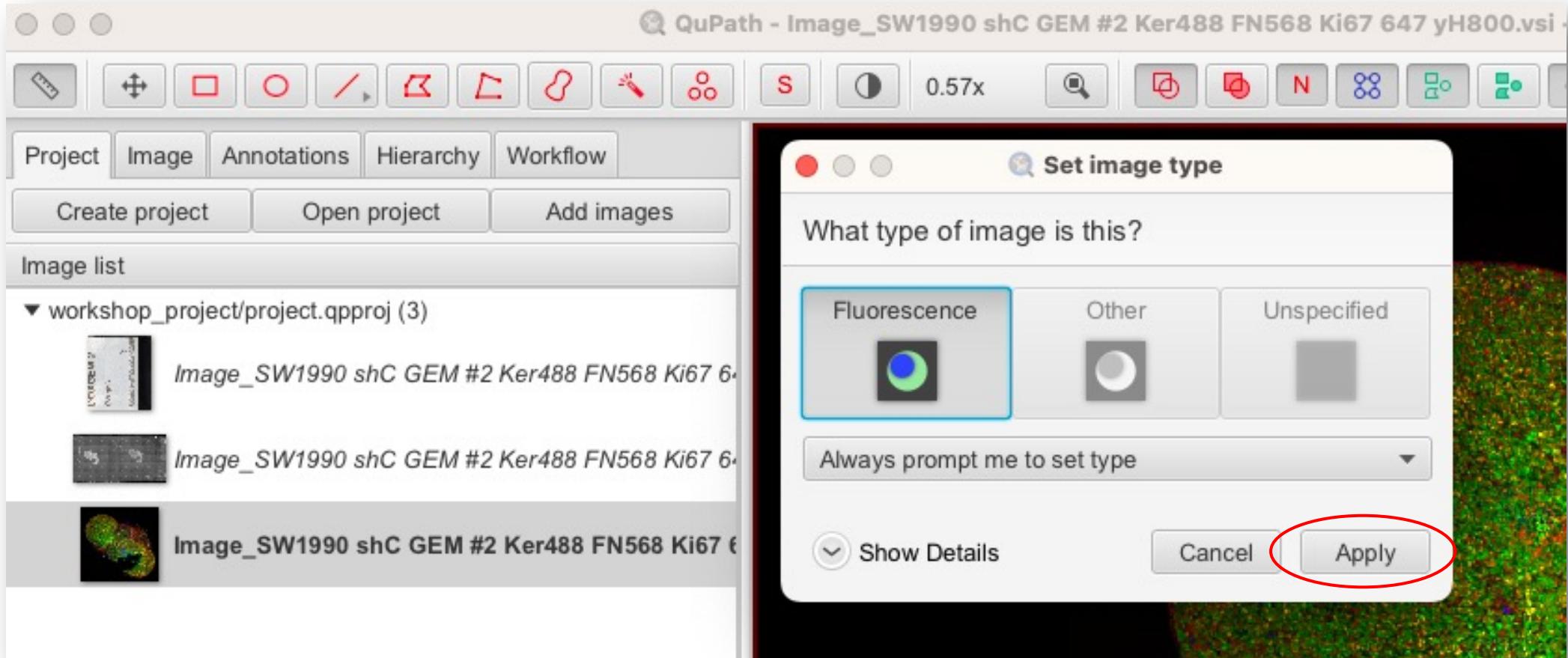
3. Select the .vsi image using *Choose files* or drag-and-drop

4. Use default settings

5. Click *import*



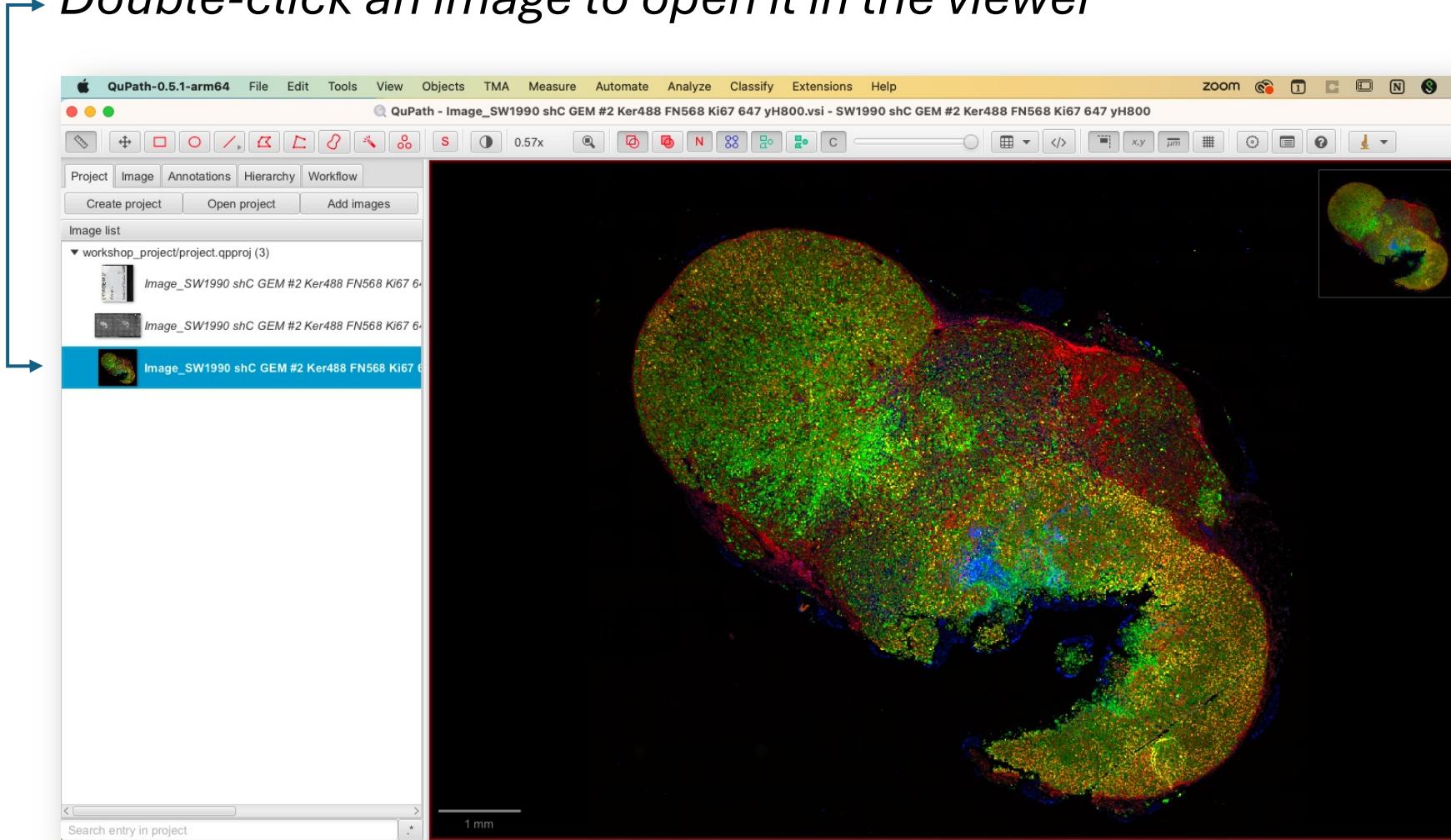
# Set image type



- 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

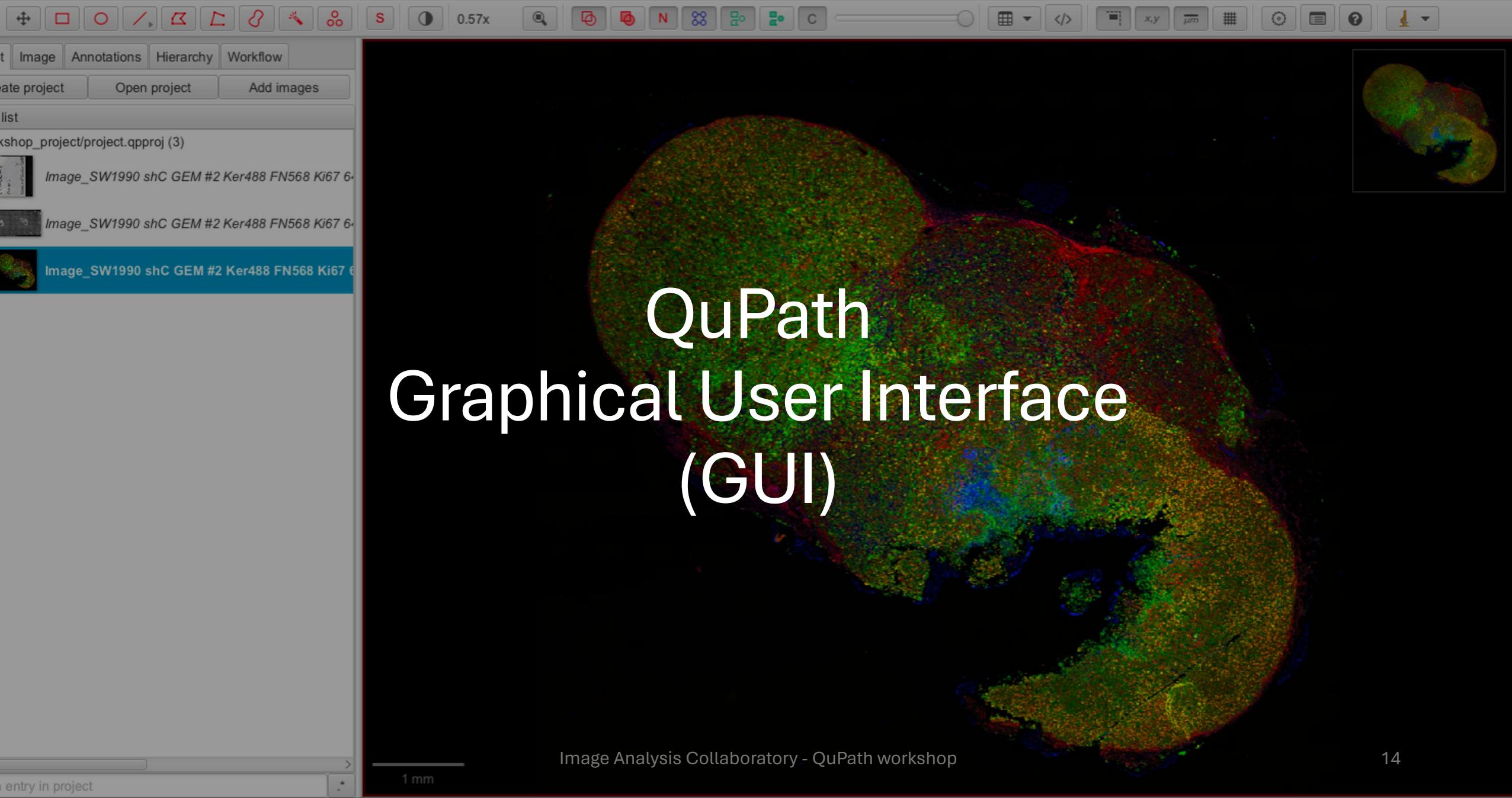
- Sharing a project:
  - Zip up the entire project directory
  - Email it to your collaborators

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

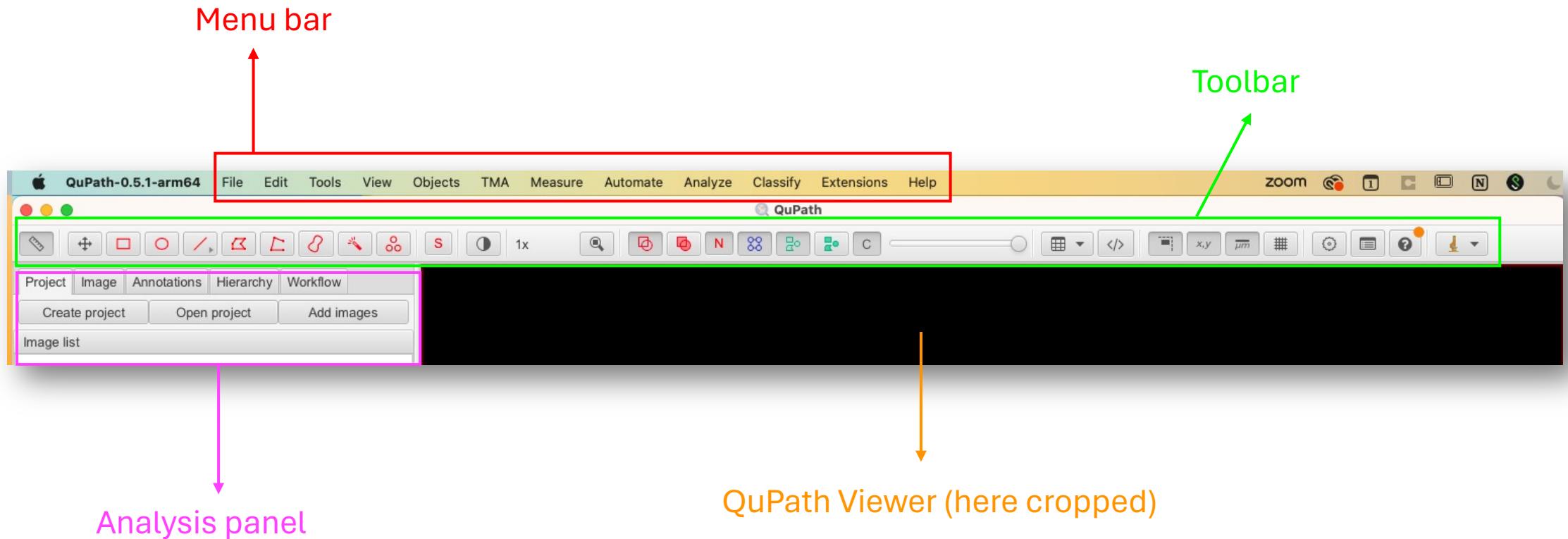
# QuPath projects are portable

- 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 - Image\_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800

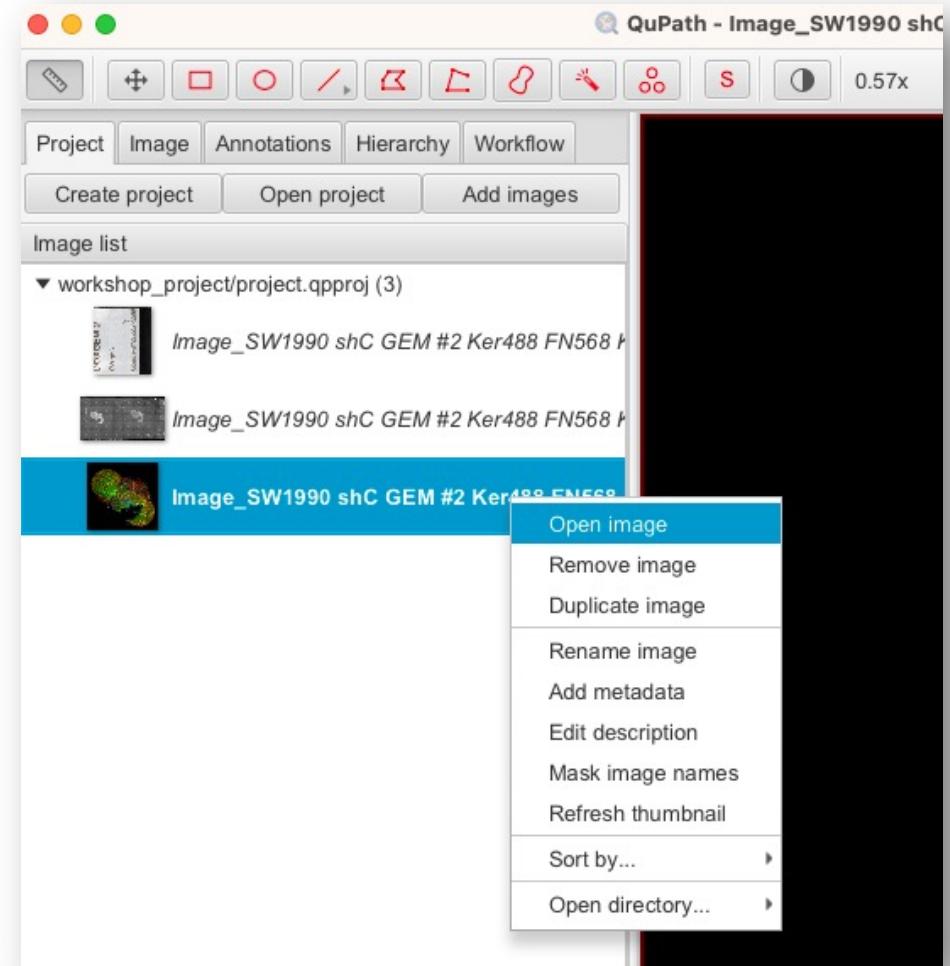


# Graphic User Interface (GUI)



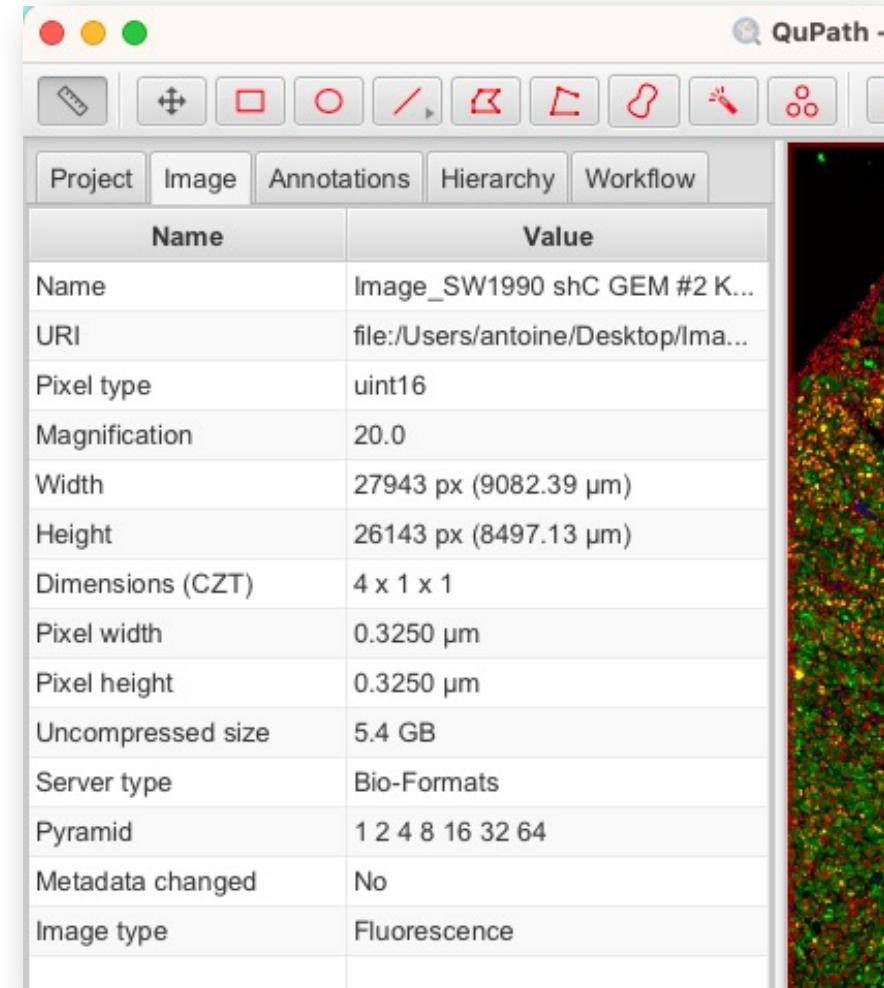
# Analysis Panel

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

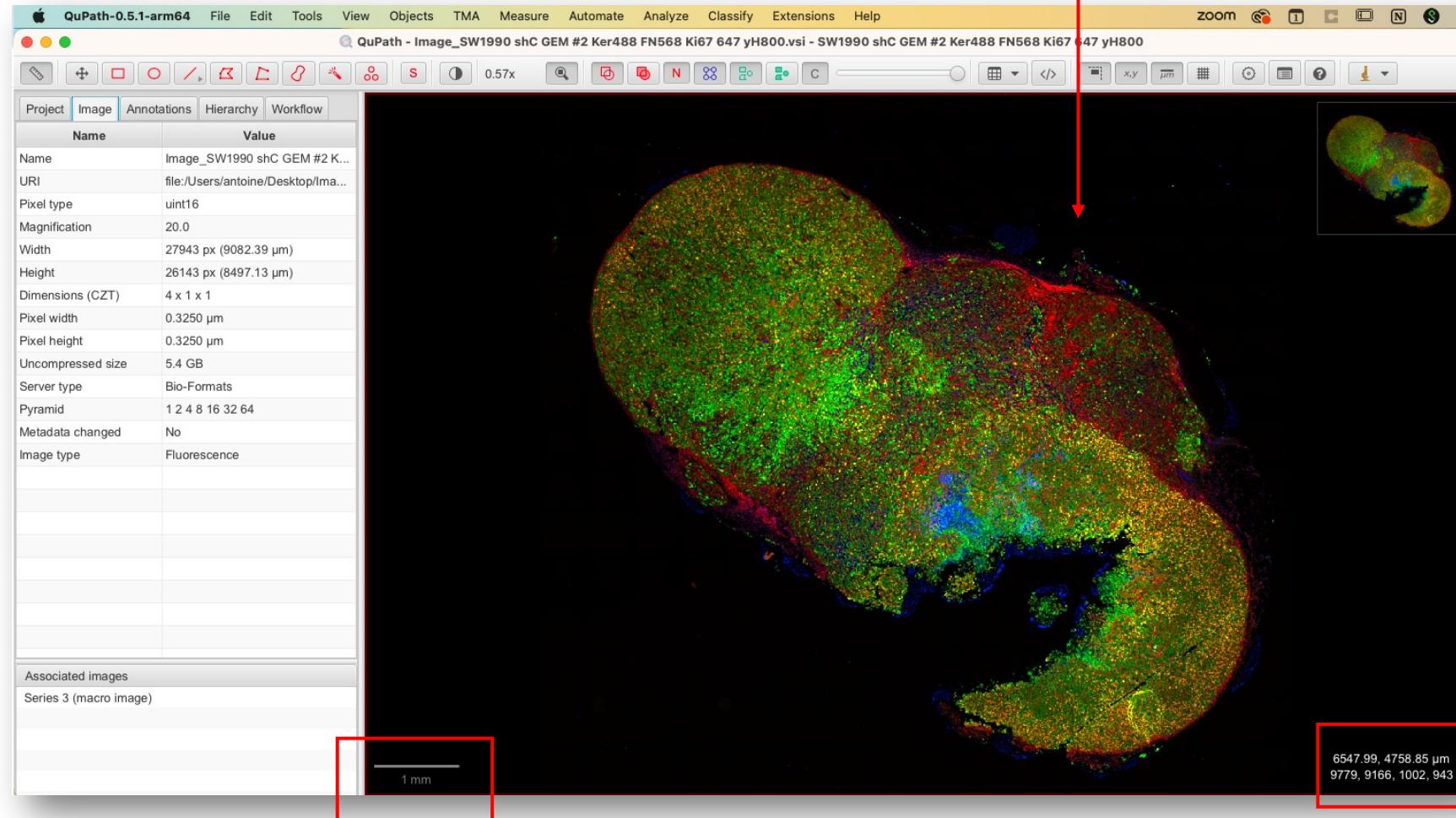


# Analysis Panel

- *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

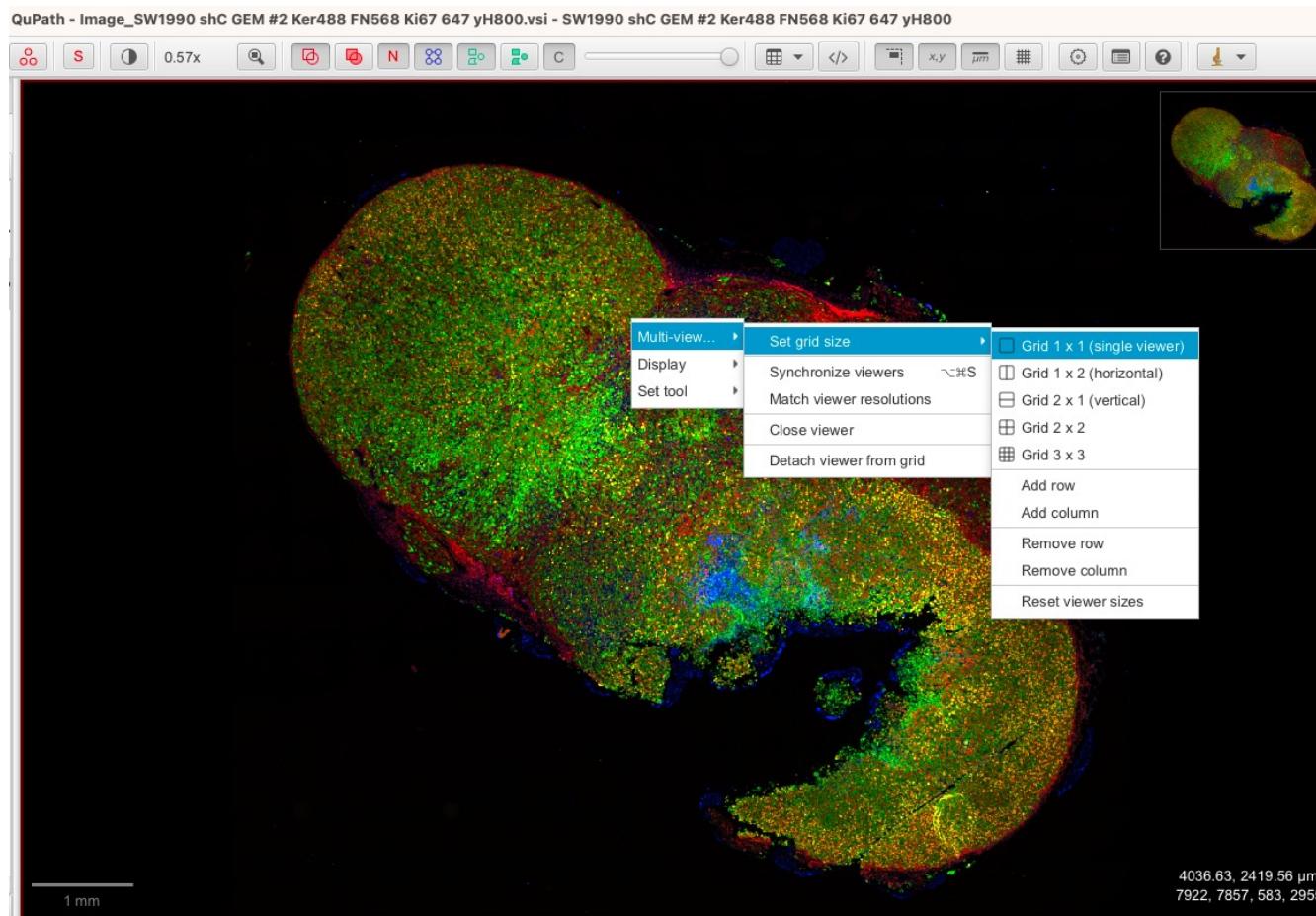


# QuPath viewer

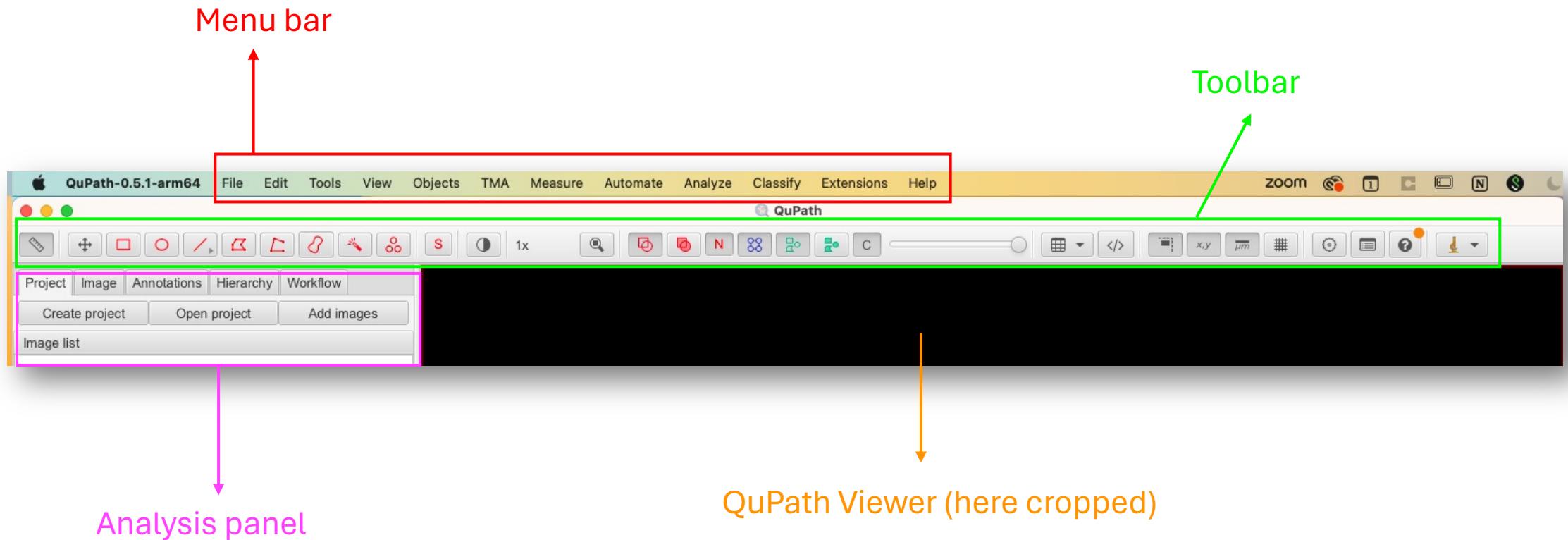


# Multi-viewer

- Right-click in the viewer



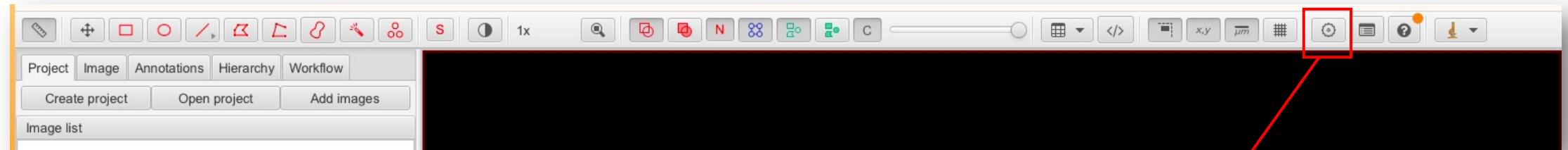
# Graphic User Interface (GUI)



# Toolbar

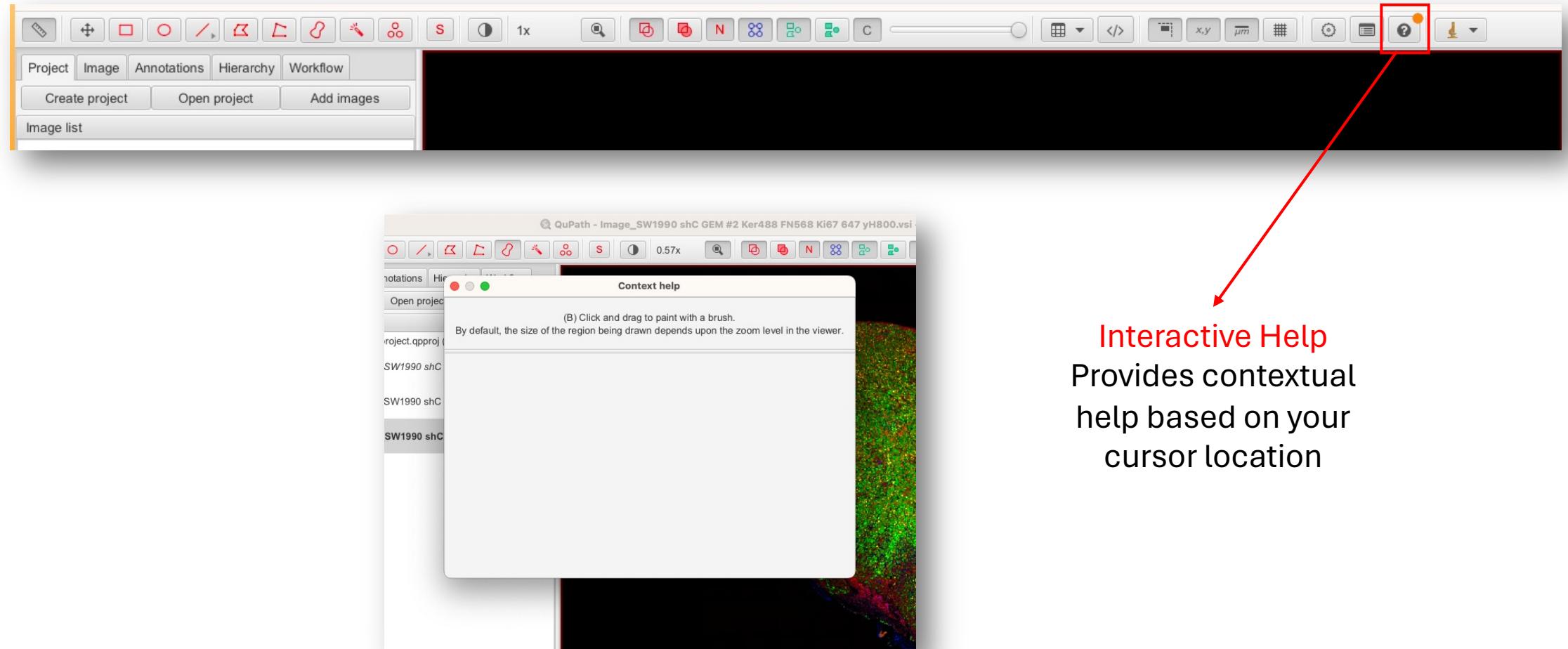


# Toolbar



Preferences  
Settings, GUI  
customization,  
extensions, ...

# Toolbar



Example when my cursor is on the paint brush tool

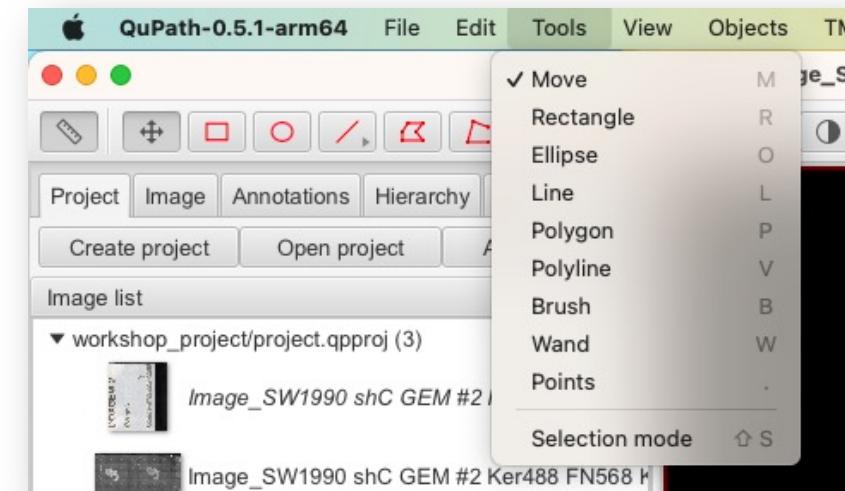
# Toolbar

M R O      B W      Shift+S



## Annotation tools

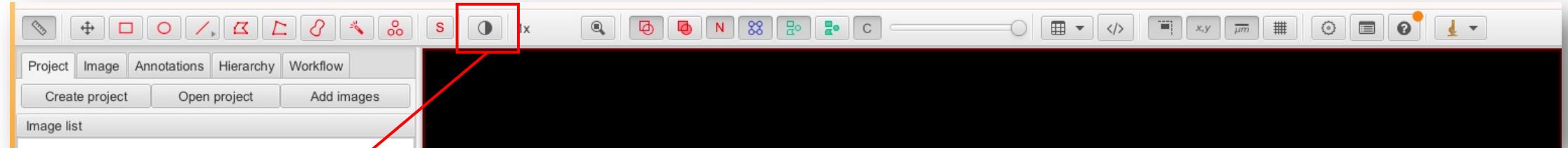
- M key: move tool
- R key: draw a rectangle annotation
- O key: draw an ellipse annotation
- B key: paint with a brush
- W key: draw with a wand tool
- And many more!



Annotation tools are also accessible in the *Tools* menu

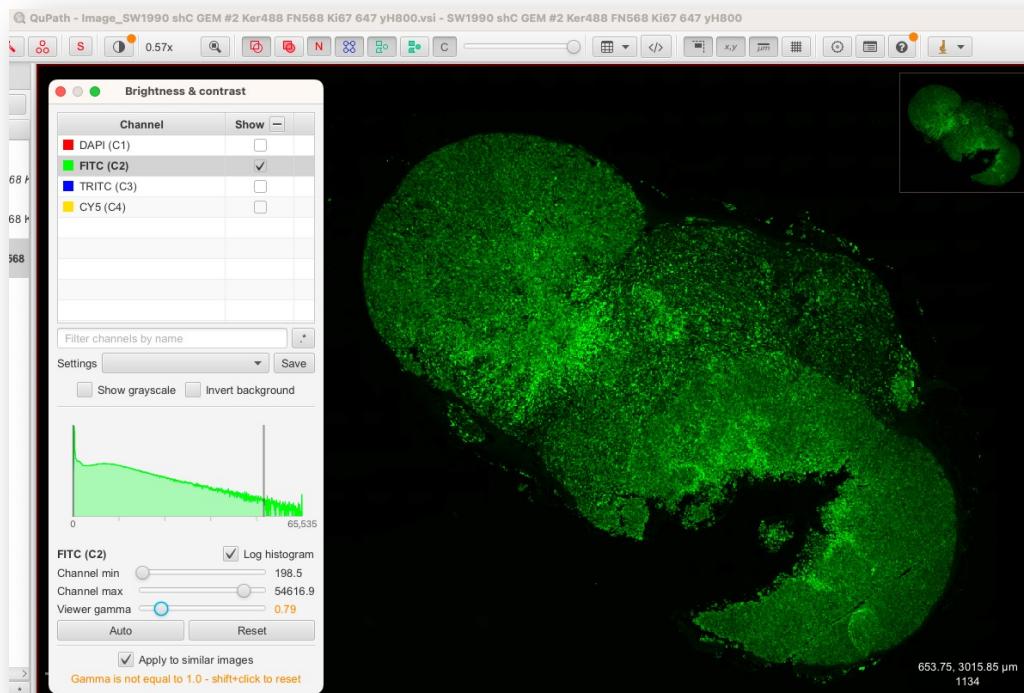
# Toolbar

Shift+C



## Brightness and contrast

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

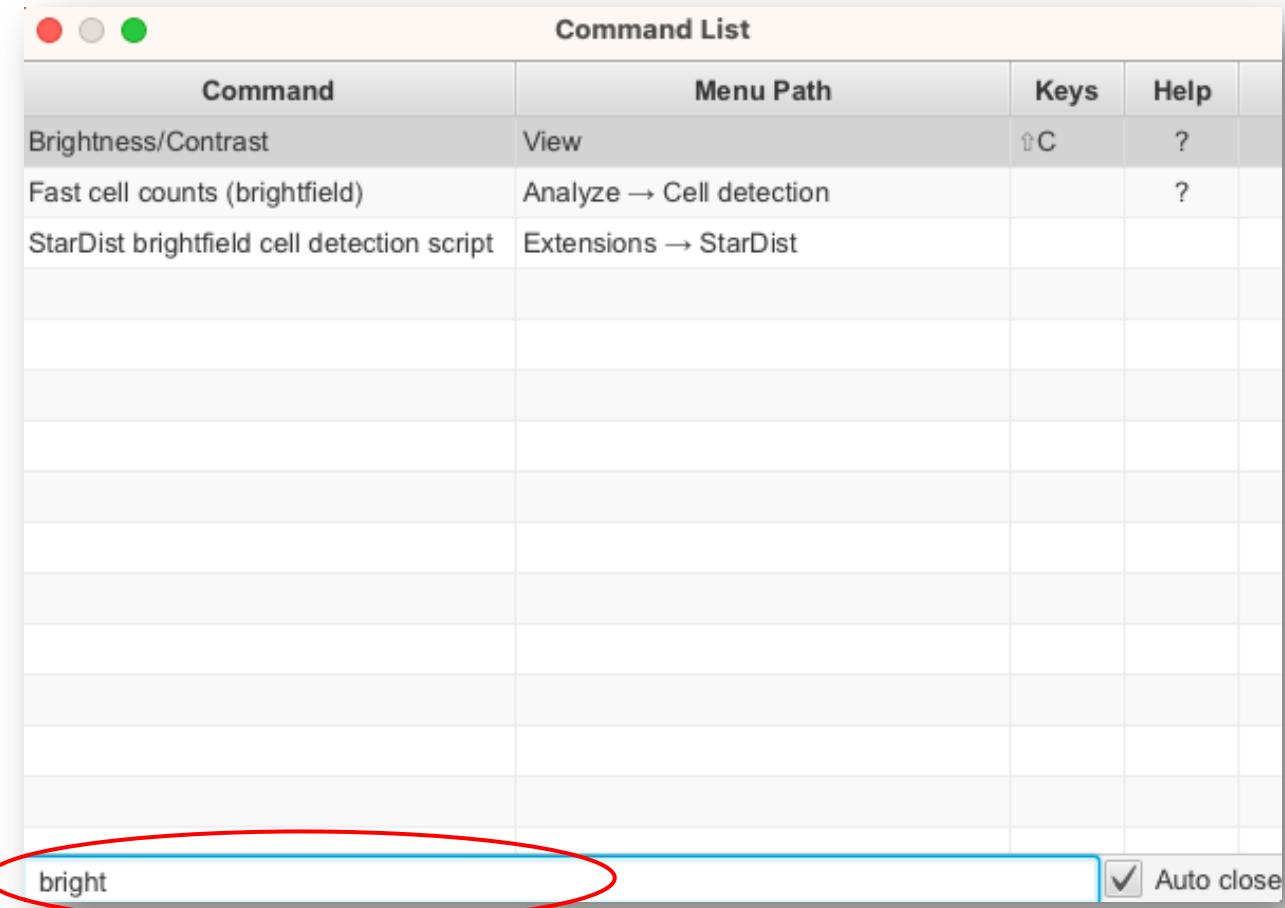


Example for FITC (Keratin) channel

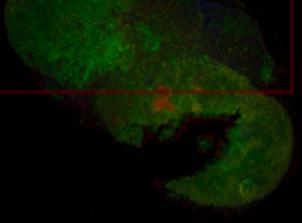
# QuPath pro-tip: command list

**Command/Control + L**  
Opens a dialogue to  
search for any command  
using keyword

*For example, search for  
'brightness'*



# Exercises 1: QuPath projects and GUI



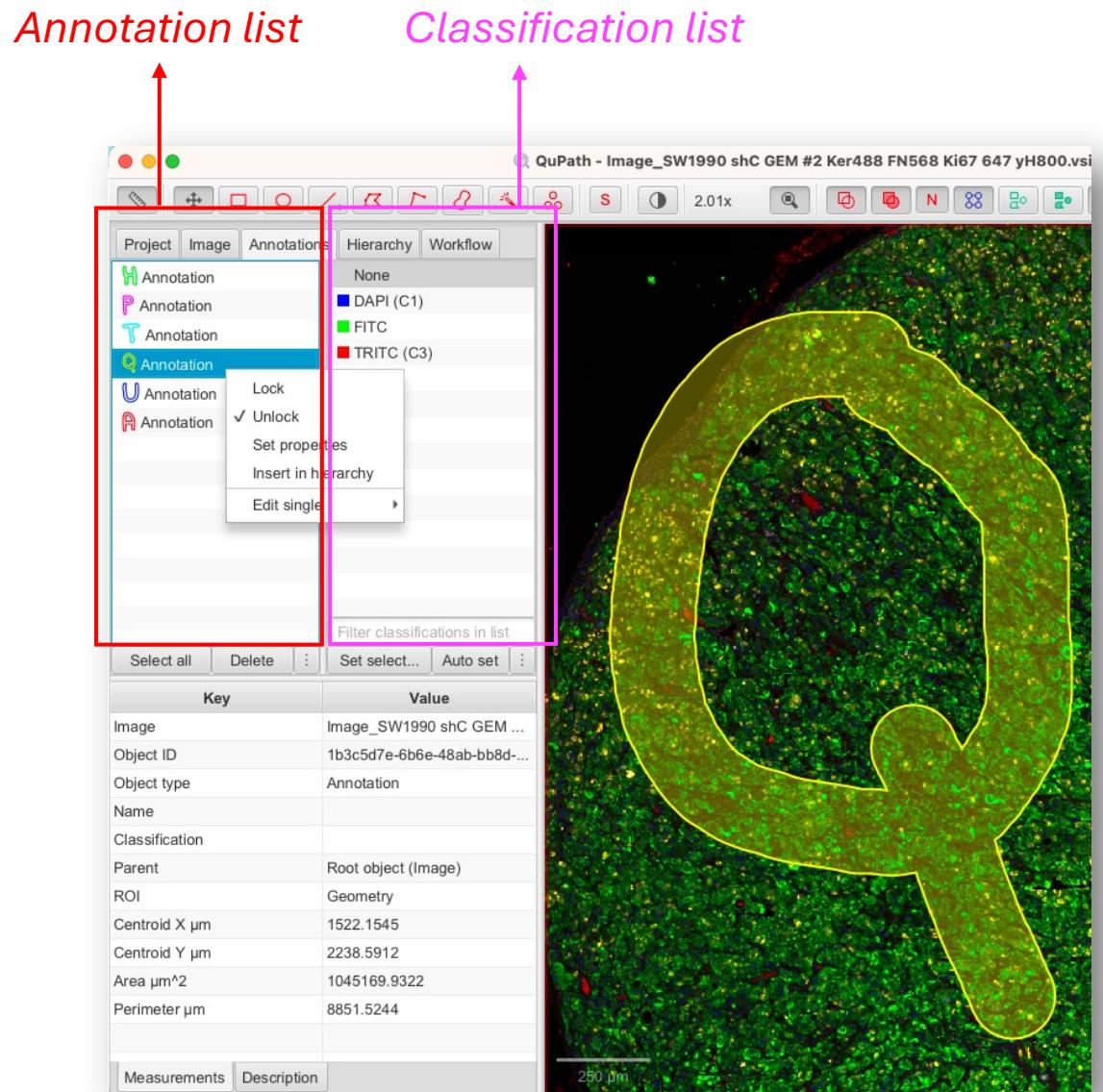
# Introducing objects: annotations and detections

# 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

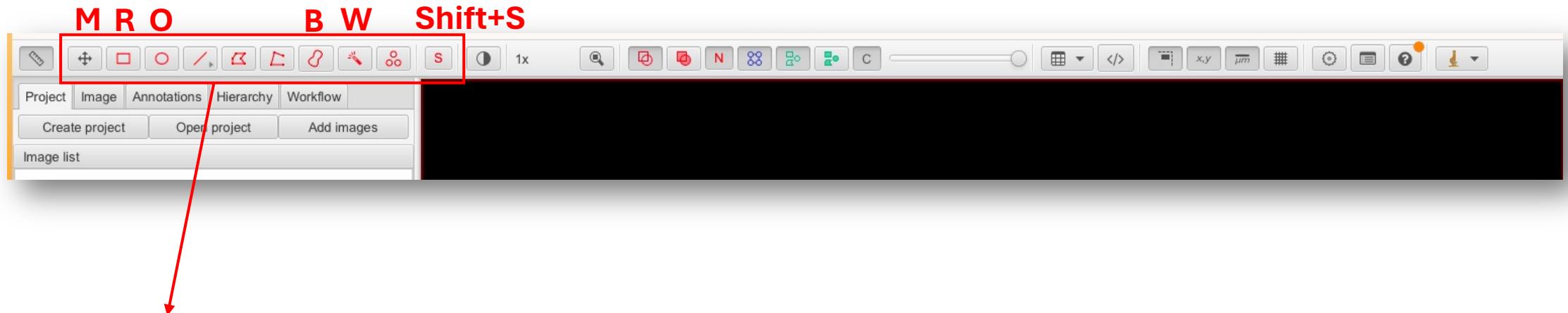
# Analysis Panel

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



# How to create manual annotations?

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



## Annotation tools

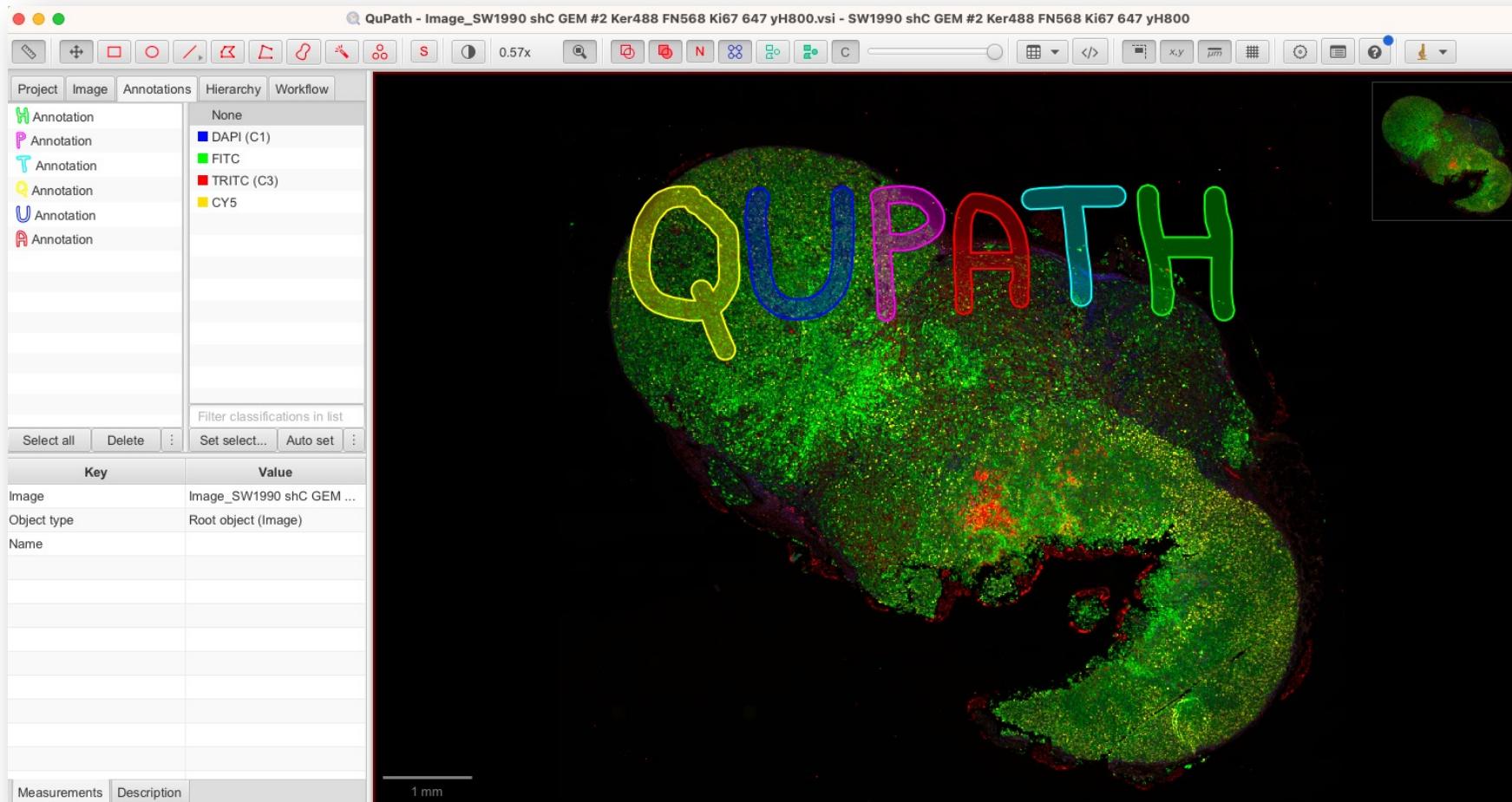
- M key: move tool
- R key: draw a rectangle annotation
- O key: draw an ellipse annotation
- B key: paint with a brush
- W key: draw with a wand tool
- And many more!

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

# Exercises 2: QuPath manual annotations

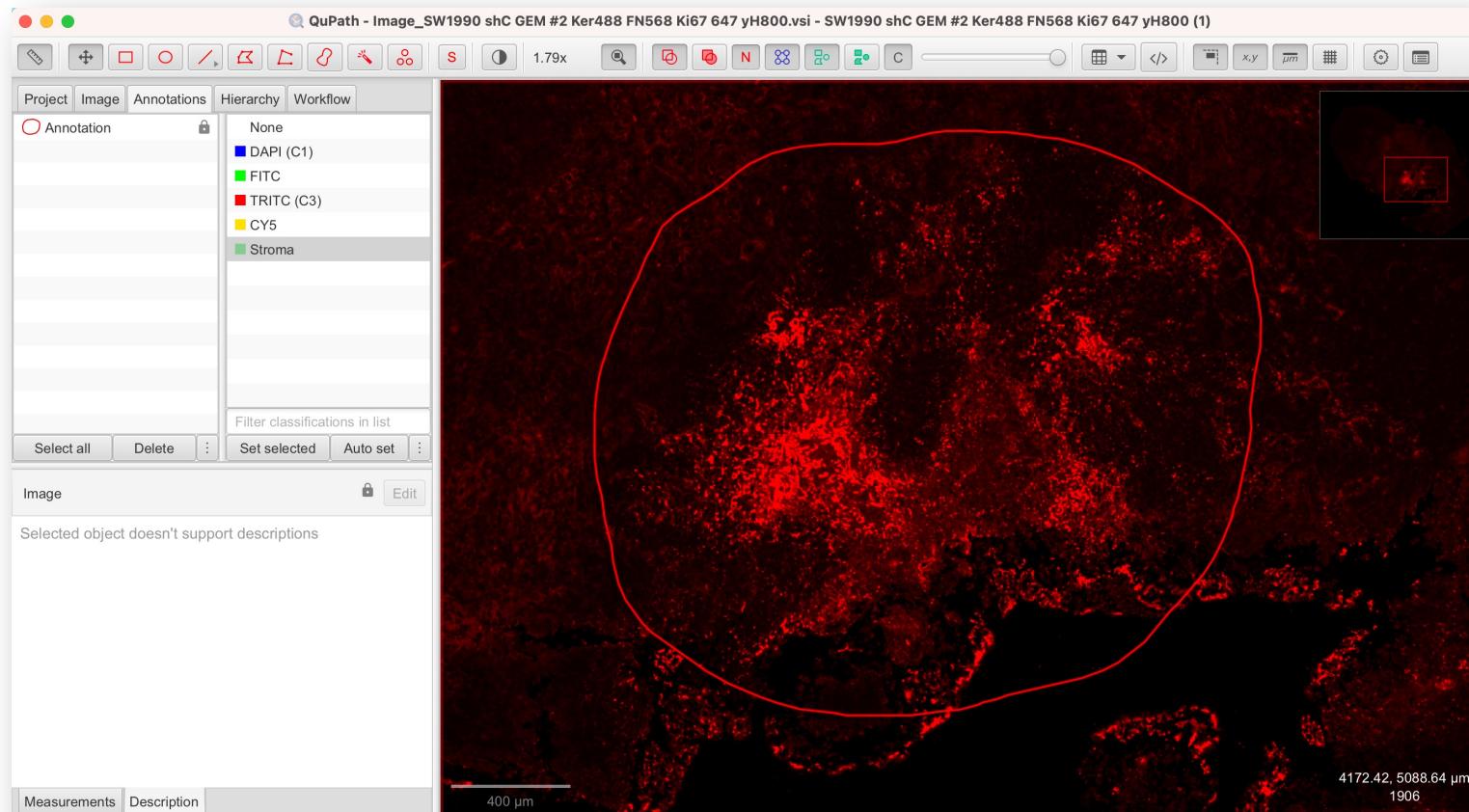
# Recreate these annotations

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