

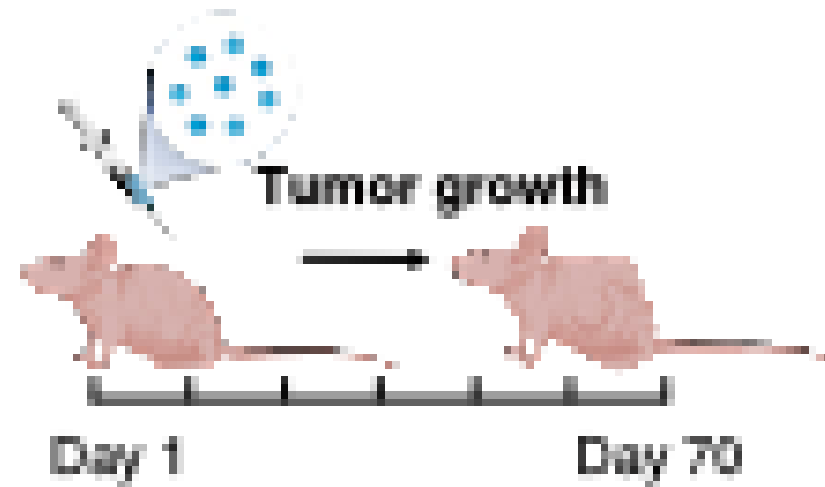
A fluorescence microscopy image of a tissue section. The image shows a complex pattern of colors: a large blue area on the left, a green area in the center, a red area on the right, and a yellow area at the bottom. The background is black. The text "Your first project in QuPath" is overlaid in white.

Your first project in QuPath

Case study

Pancreatic cancer cell line AsPC has a knockout of gene X.

Assess the effect of a gene X KO on a tumor formation and growth.



Choice of markers

- DAPI to segment nuclei
- Epithelial cells (Pan-cytokeratin)
- Immune population (CD4, CD8 etc)
- Proliferating cells (Ki67)
- Apoptotic cells (Cleaved Caspase 3)

Case study

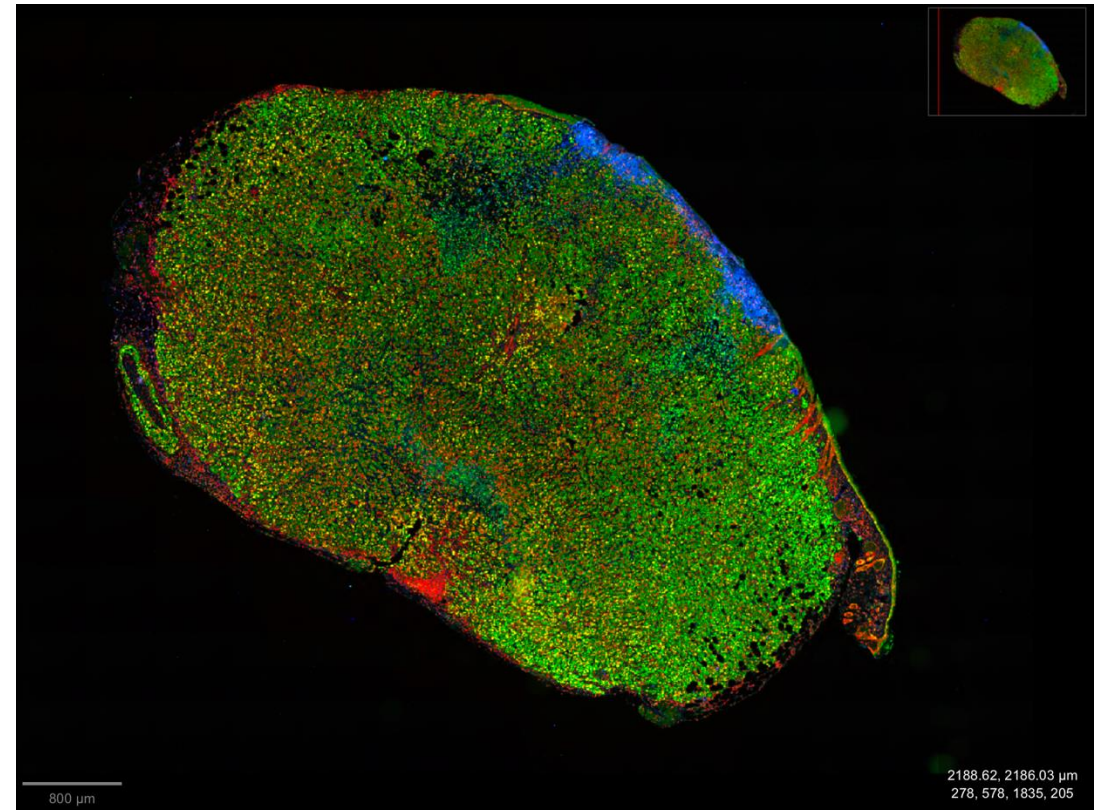
Reviewer: Assess the correlation between proliferation and distance from the stromal edge within the tumor.

Choice of markers

- DAPI to segment nuclei
- Epithelial cells (Pan-cytokeratin)
- Proliferating cells (Ki67)
- Stroma (Fibronectin)

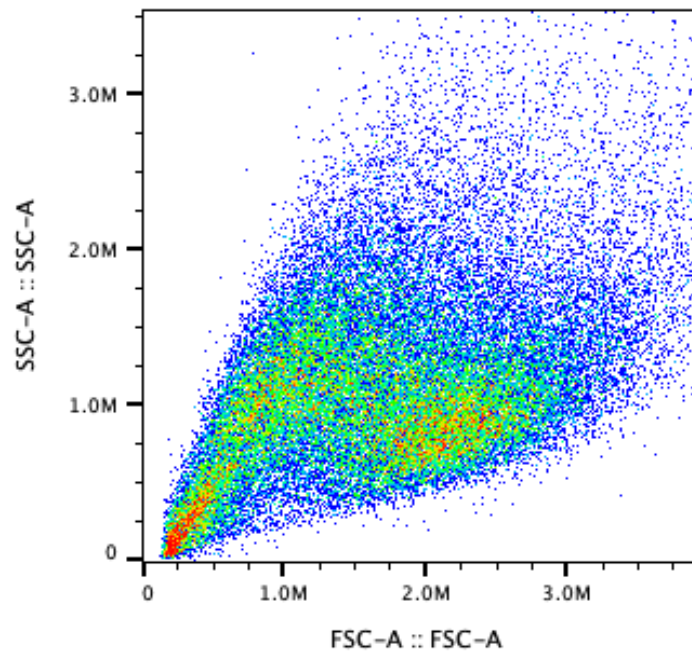
By the end of this course:

you will be able to classify proliferating (Ki67) cancer cells and analyze their spatial distribution to regions with high-fibronectin content

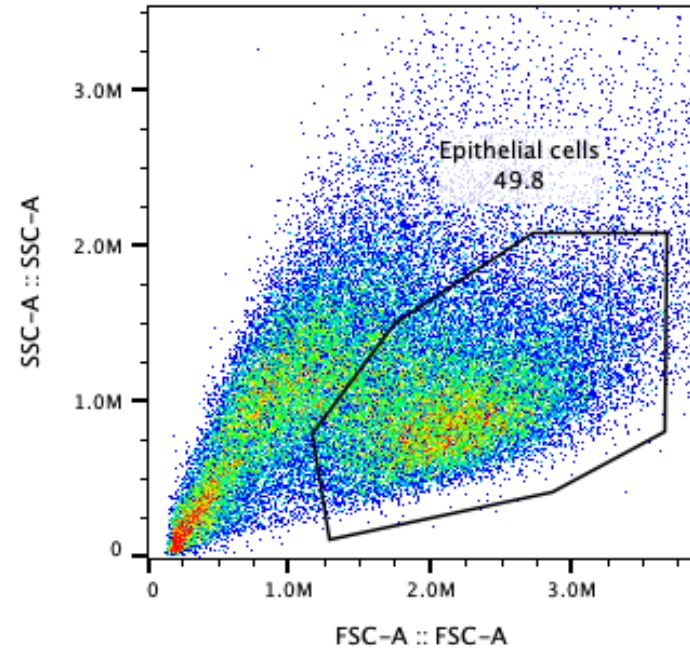


Few ideas before we move forward

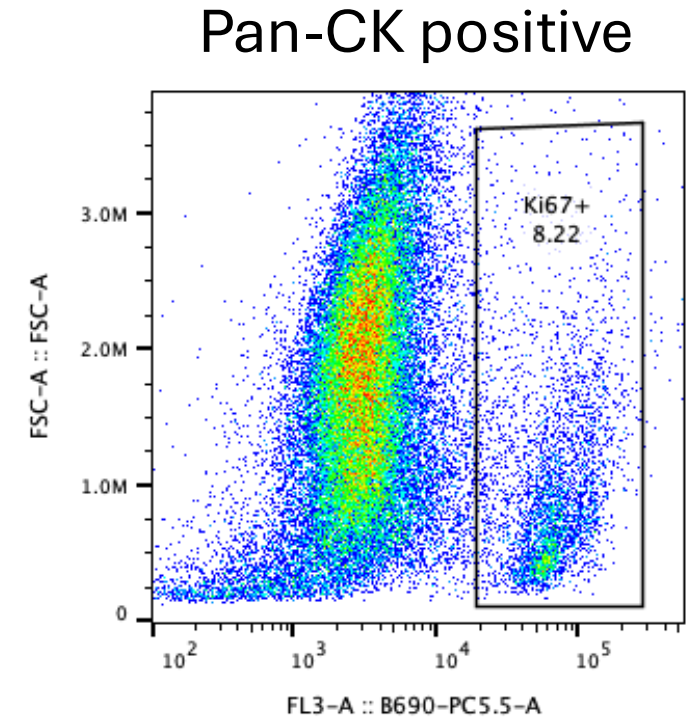
The idea behind image analysis in QuPath is very similar to the concept of ‘gating’ used in flow cytometry.



DAPI



Pan-cytokeratin

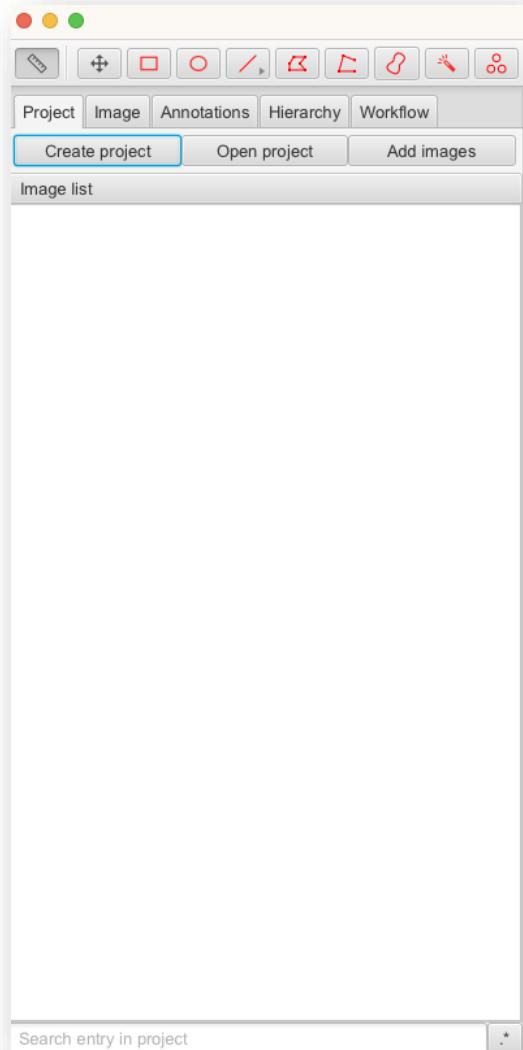


Proliferating cells (Ki67)

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!

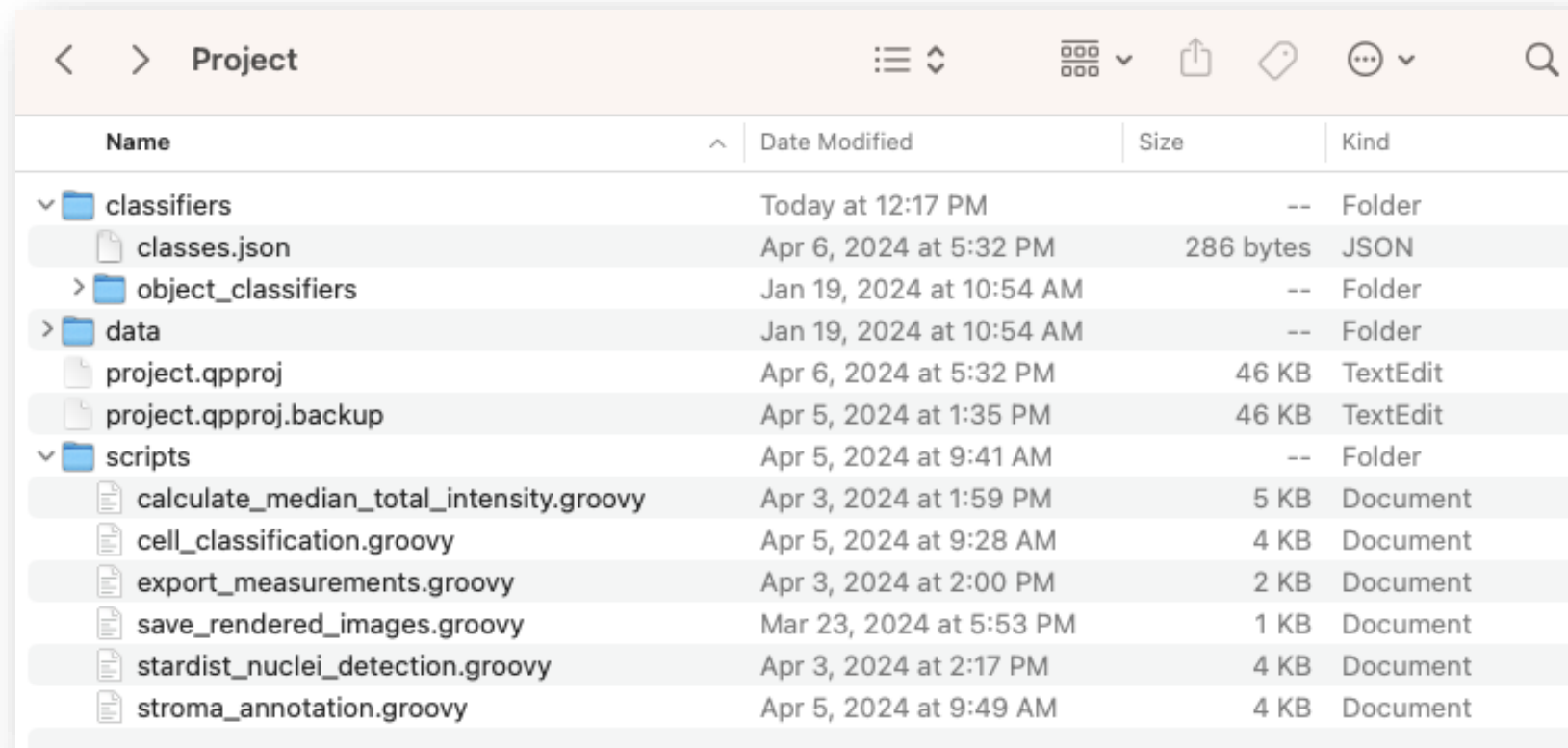
How to create a project?



- *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

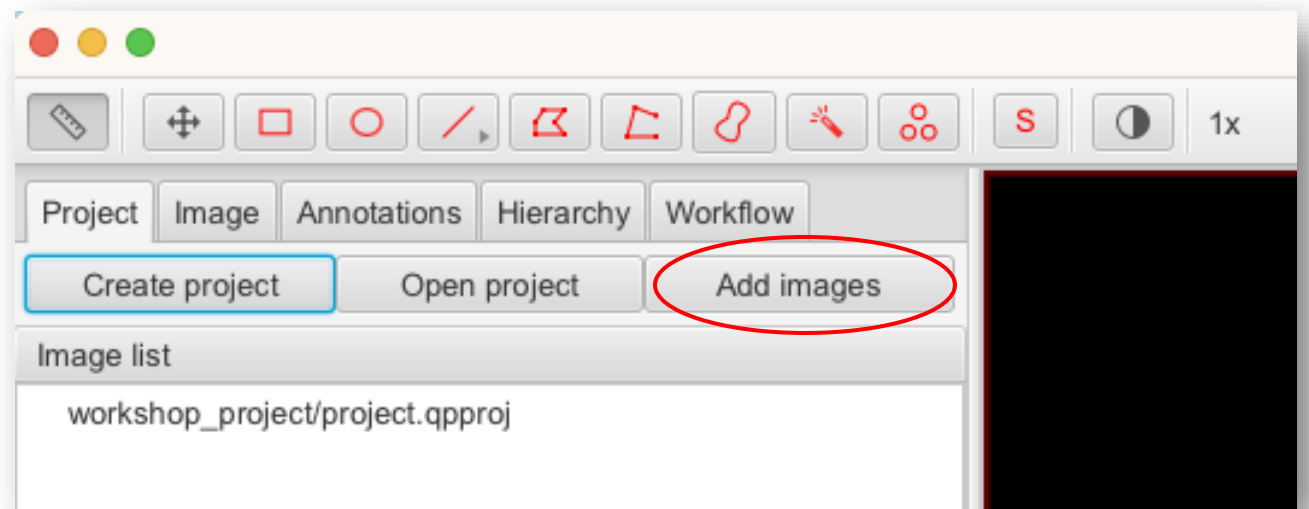
After a bit of time working on it...



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 the whole-slide image from the workshop website
2. Add an image
 - *Add images* button
 - Select the .vsi file

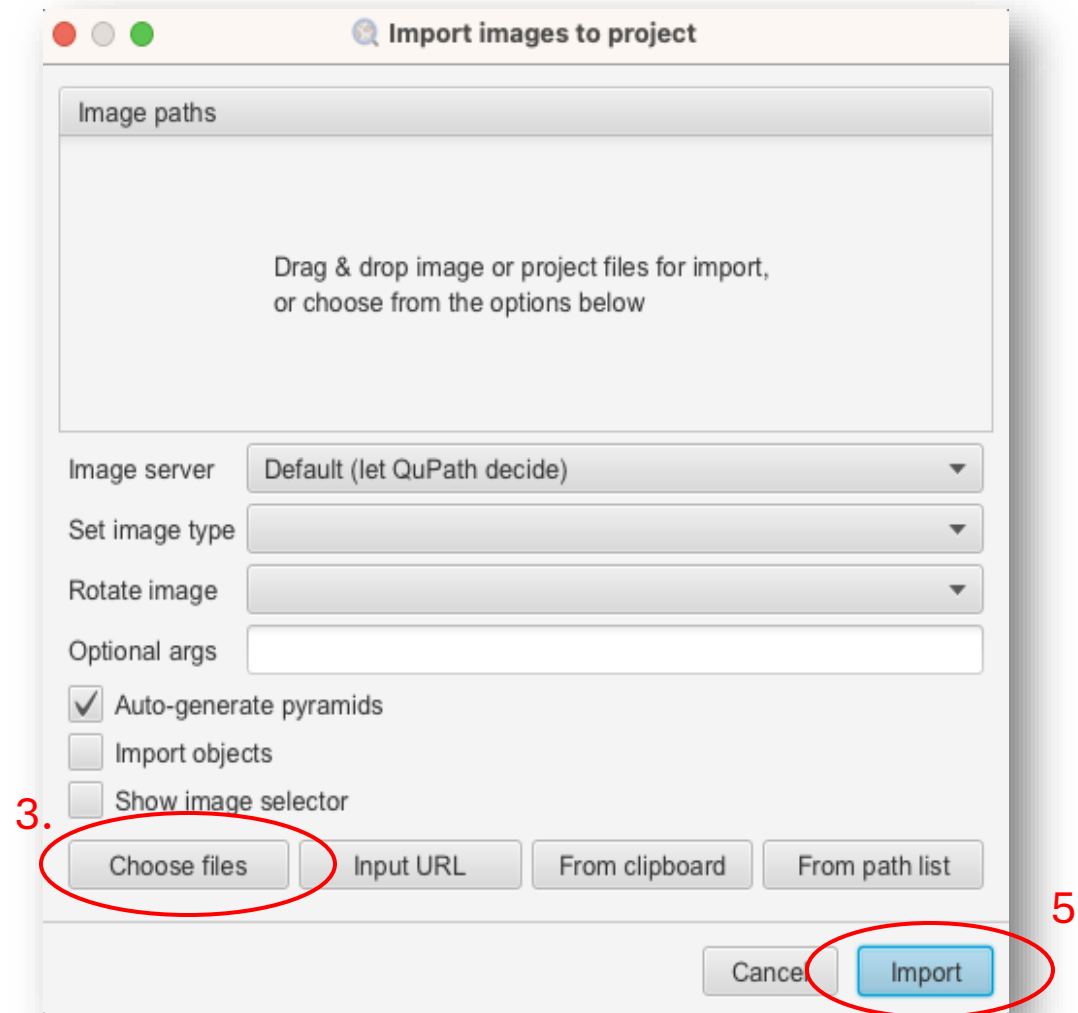


Add an image to your project

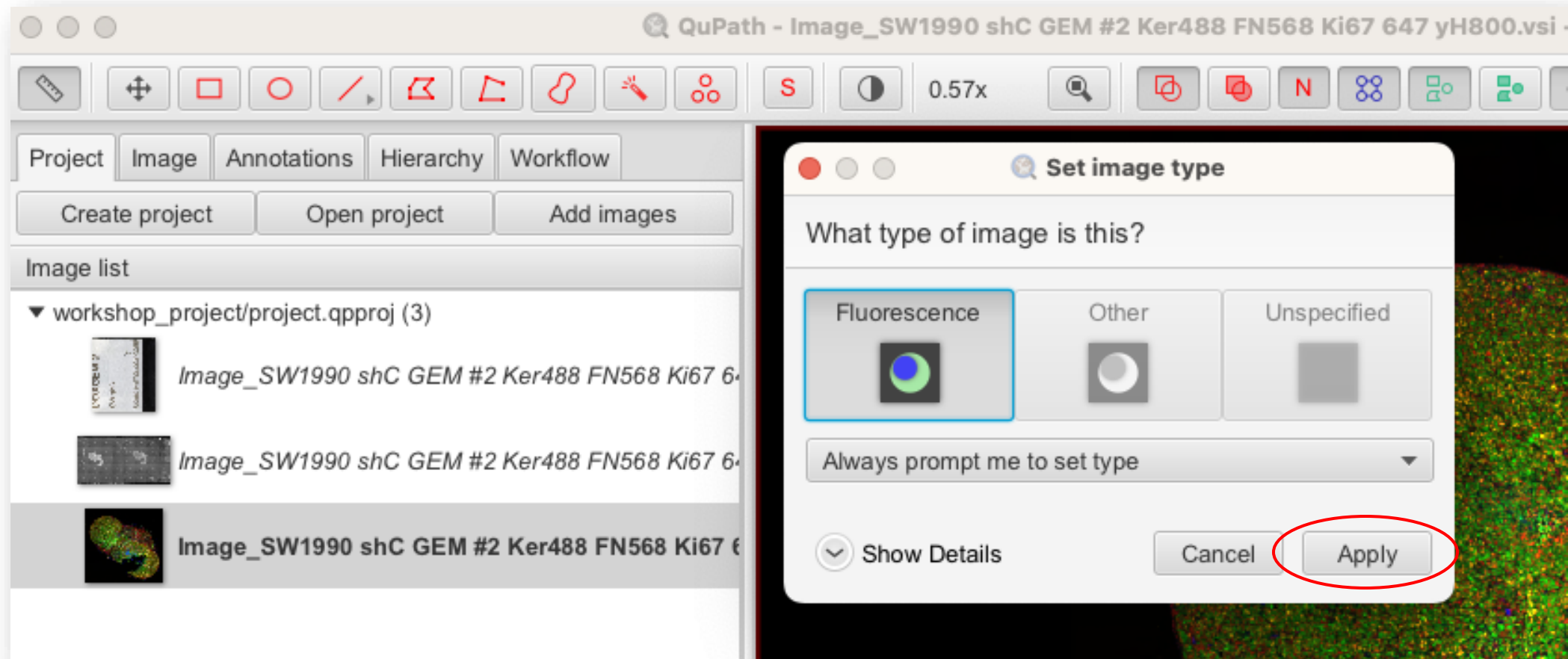
3. Select the .vsi image using *Choose files* or drag-and-drop

4. Use default settings

5. Click *import*



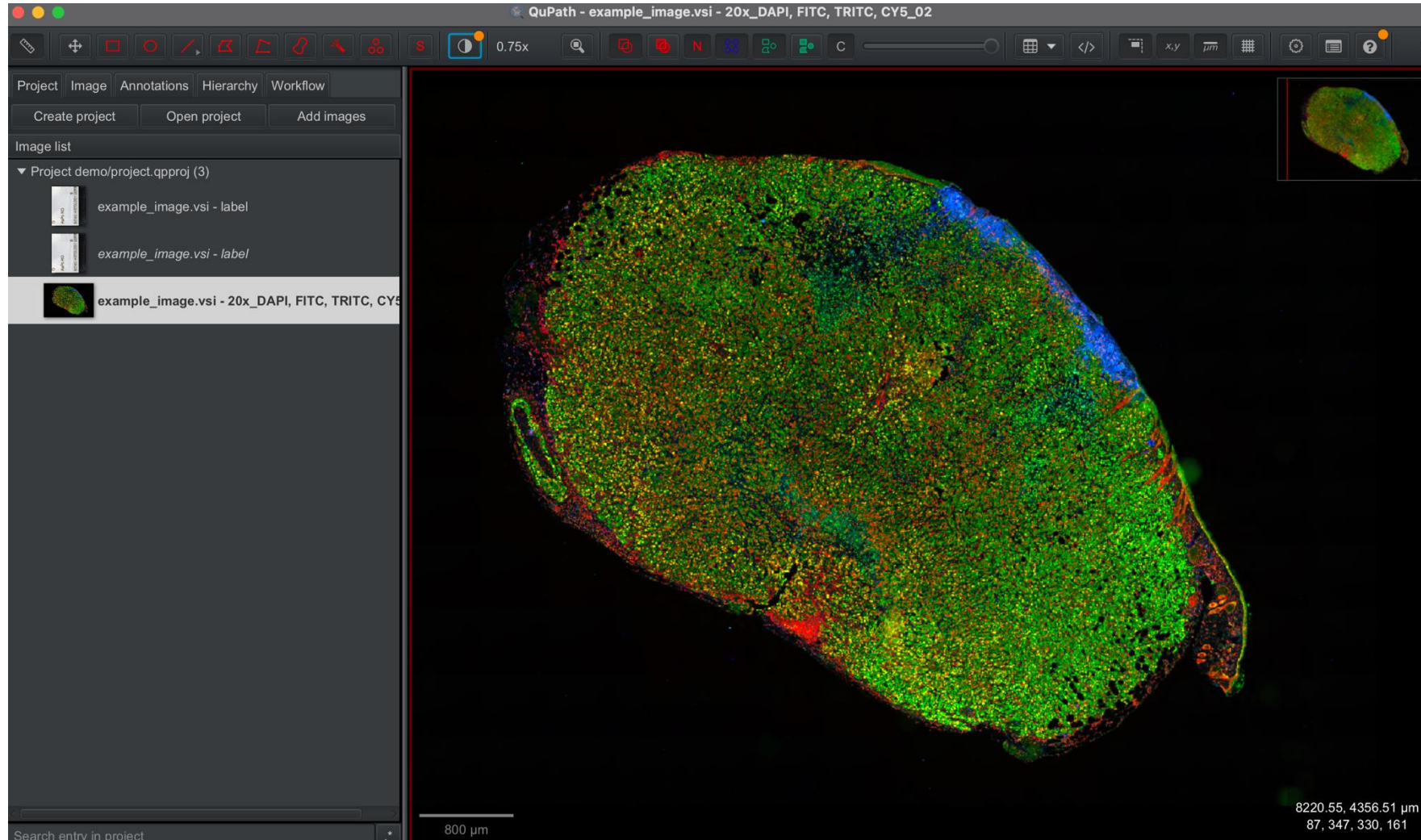
Set image type



- *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

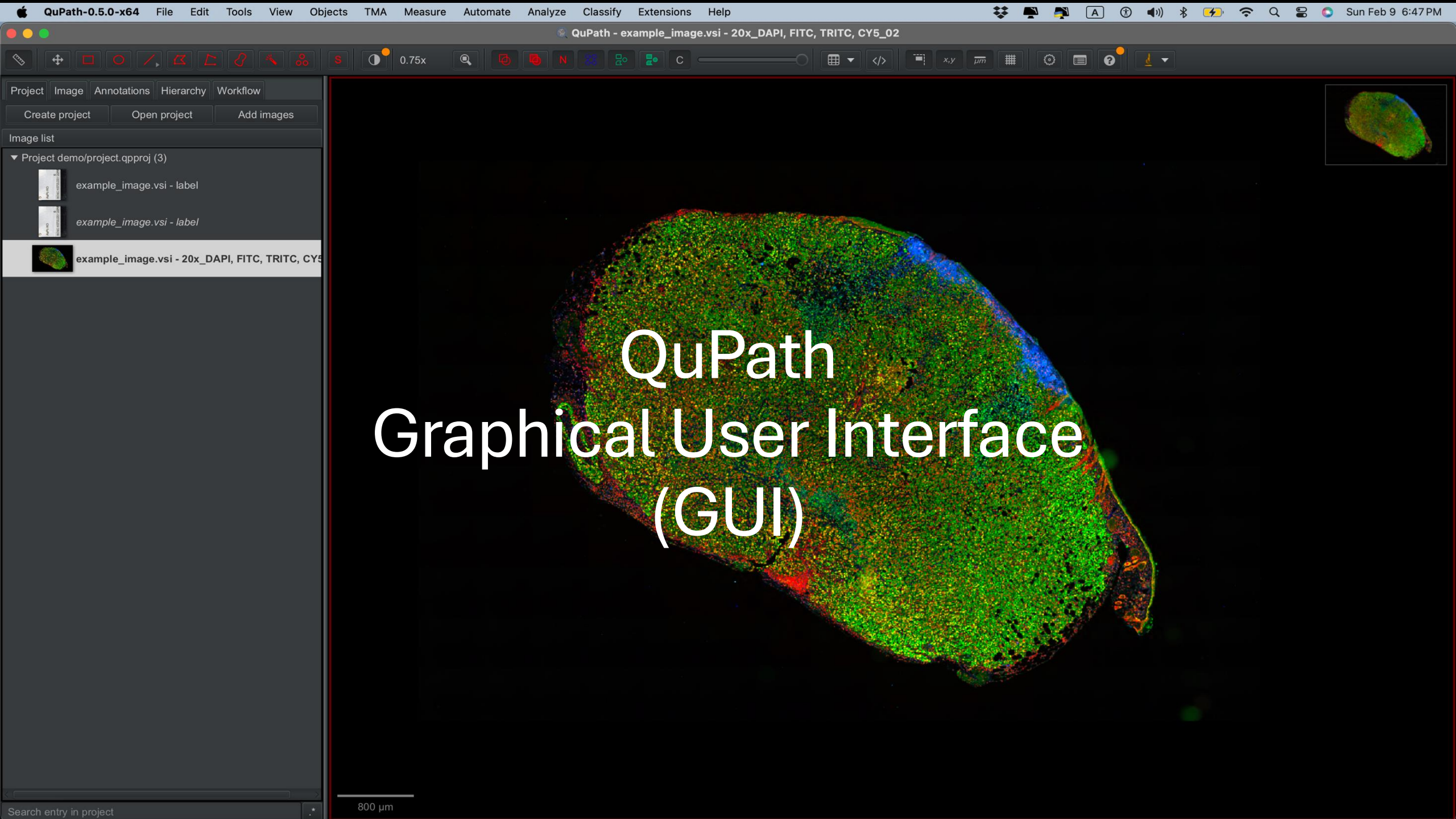
QuPath projects are portable

- 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.

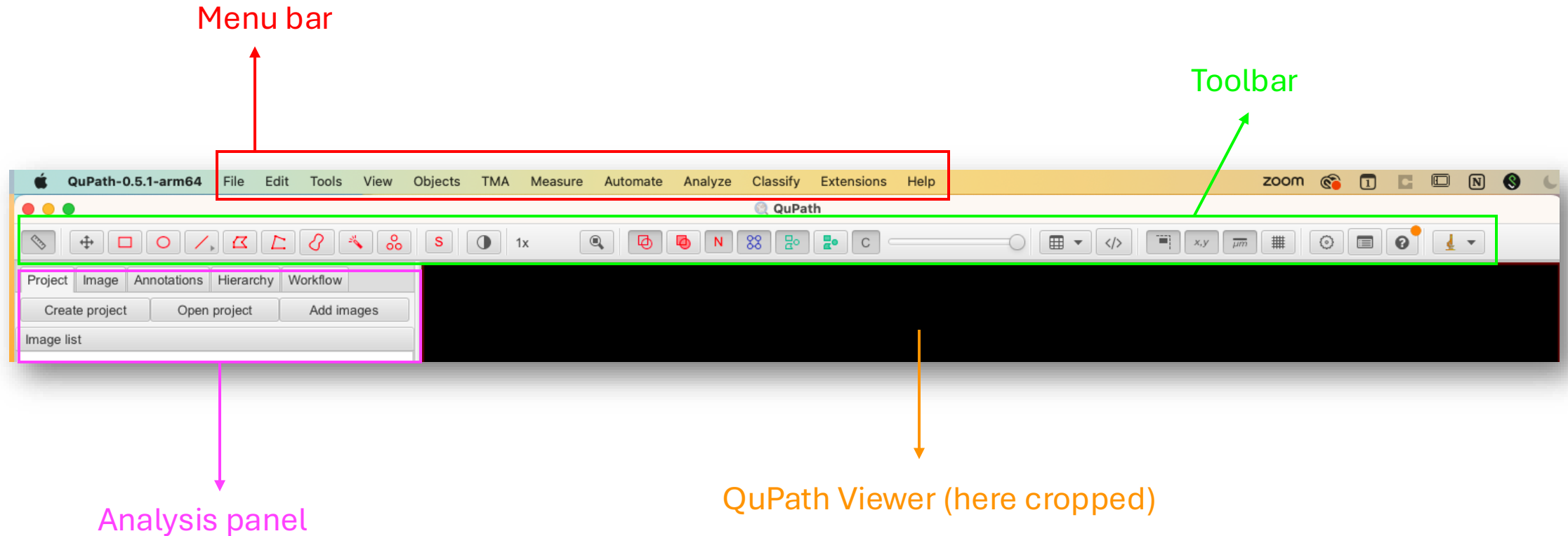
QuPath projects are portable

- 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



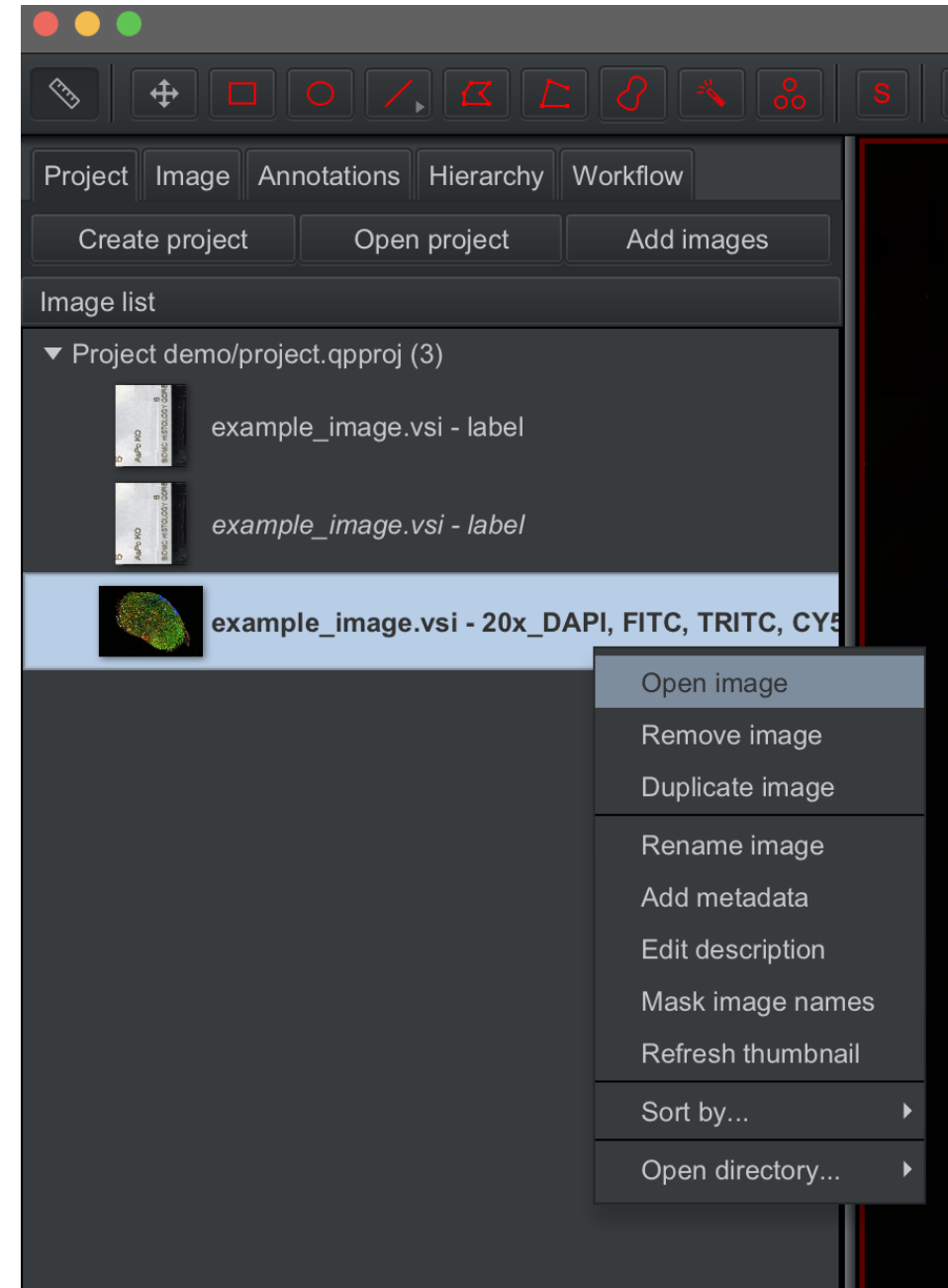
QuPath Graphical User Interface (GUI)

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

Project Image Annotations Hierarchy Workflow	
Name	Value
Name	example_image.vsi - 20x_DAPI, FI...
URI	file:/Users/nina/Muranen Lab Drop...
Pixel type	uint16
Magnification	20.0
Width	27818 px (9041.53 µm)
Height	19169 px (6230.50 µm)
Dimensions (CZT)	4 x 1 x 1
Pixel width	0.3250 µm
Pixel height	0.3250 µm
Uncompressed size	4 GB
Server type	Bio-Formats
Pyramid	1 2 4 8 16 32 64
Metadata changed	No
Image type	Fluorescence

QuPath viewer

Viewer

Mini-map: overview

Info bar: pixel coord and value

Scale bar

The screenshot displays the QuPath viewer interface. On the left, a sidebar shows project and image metadata. The central area contains a large fluorescence microscopy image of a tissue section, primarily green with some blue and red regions. A red arrow points to this main image area. In the top right corner, a mini-map provides an overview of the entire image. Another red arrow points to this mini-map. At the bottom left, a scale bar indicates 800 μm . A red arrow points to this scale bar. At the bottom right, an info bar displays pixel coordinates and values. A red arrow points to this info bar.

Name	Value
Name	example_image.vsi - 20x_DAPI, FI...
URI	file:/Users/nina/Muranen Lab Drop...
Pixel type	uint16
Magnification	20.0
Width	27818 px (9041.53 μm)
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Dimensions (CZT)	4 x 1 x 1
Pixel width	0.3250 μm
Pixel height	0.3250 μm
Uncompressed size	4 GB
Server type	Bio-Formats
Pyramid	1 2 4 8 16 32 64
Metadata changed	No
Image type	Fluorescence

Associated images

Series 4 (macro image)

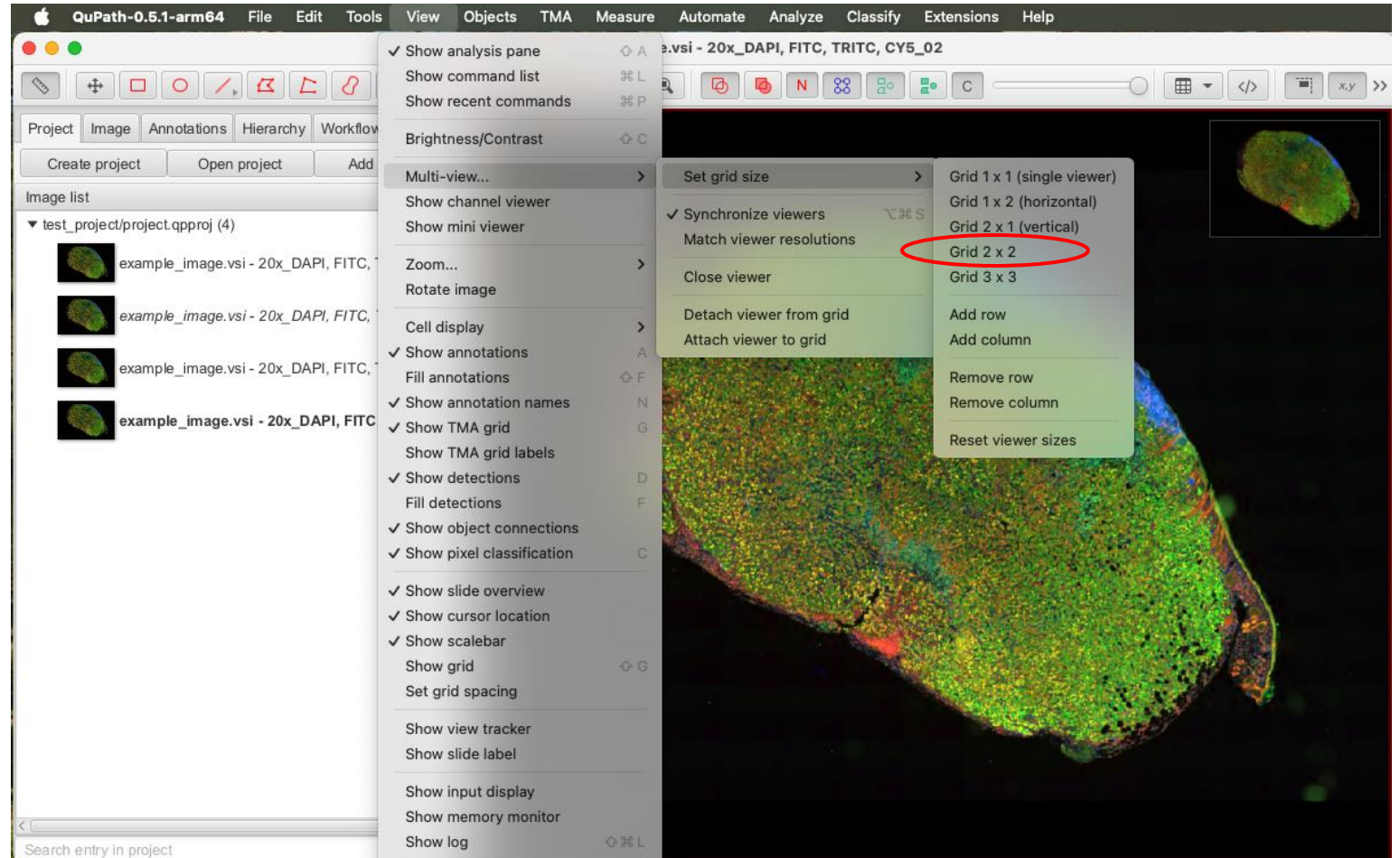
800 μm

3086.44, 538.55 μm
179, 308, 586, 173

Image Analysis Collaboratory - QuPath workshop

Multi-viewer

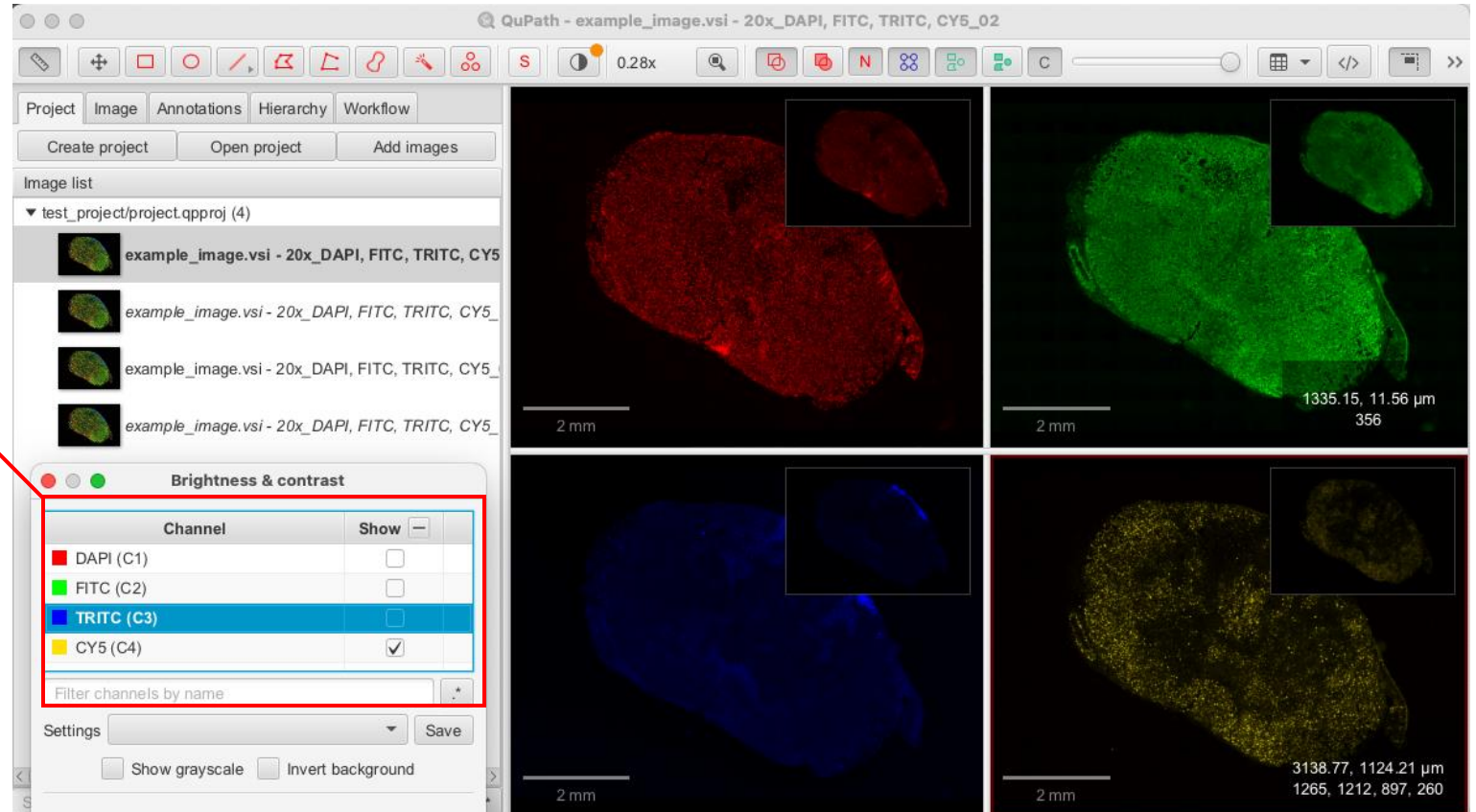
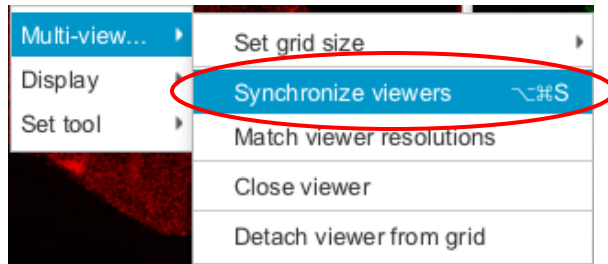
- Allows to **view multiple images**, or can be used to view each channel of a single image at the same time
- To do so, you actually need to import the image 4 times
- *View > Multi-view > Set grid size > Grid 2x2*



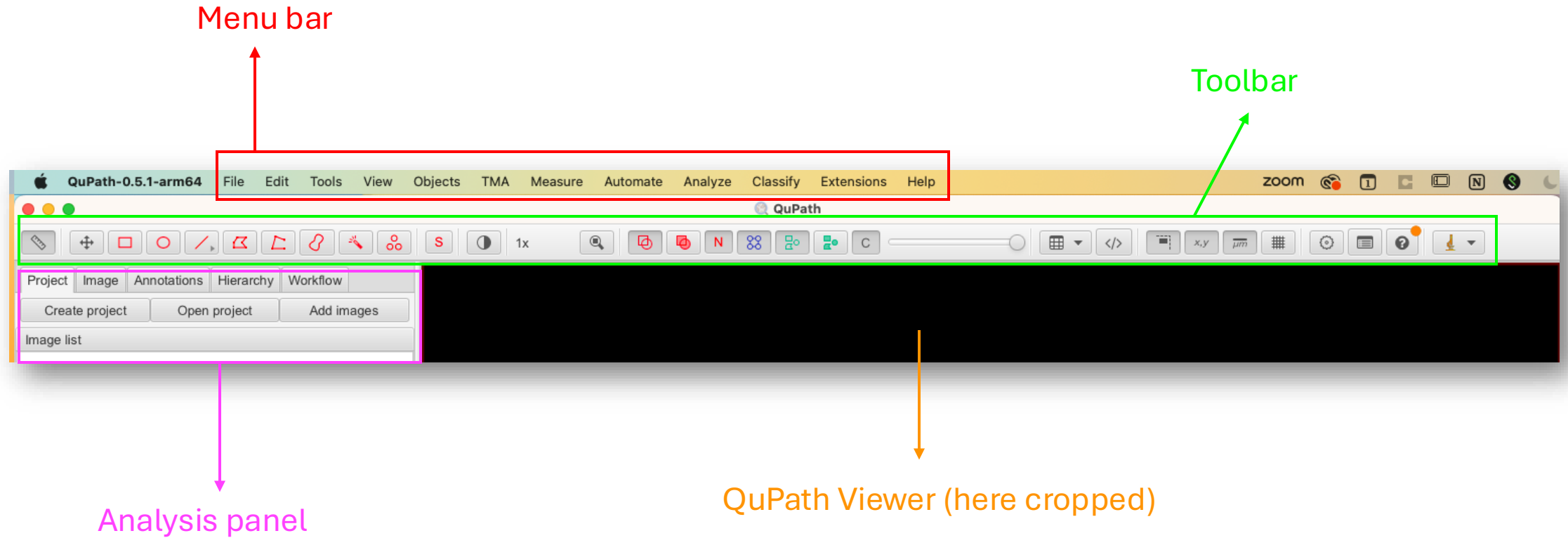
Multi-viewer

Brightness and contrast

- Select a channel for each viewer
- Control/Right + click in one of the viewer > *Synchronize viewers*
- Make sure images are aligned

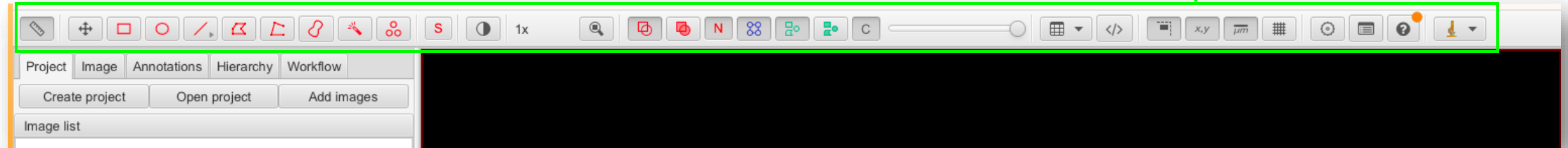


Graphic User Interface (GUI)

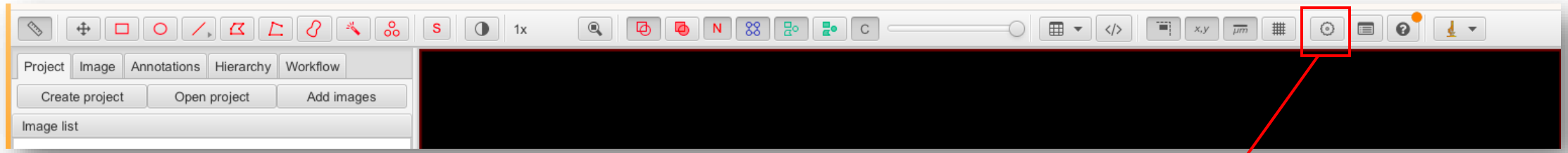


Toolbar

Toolbar

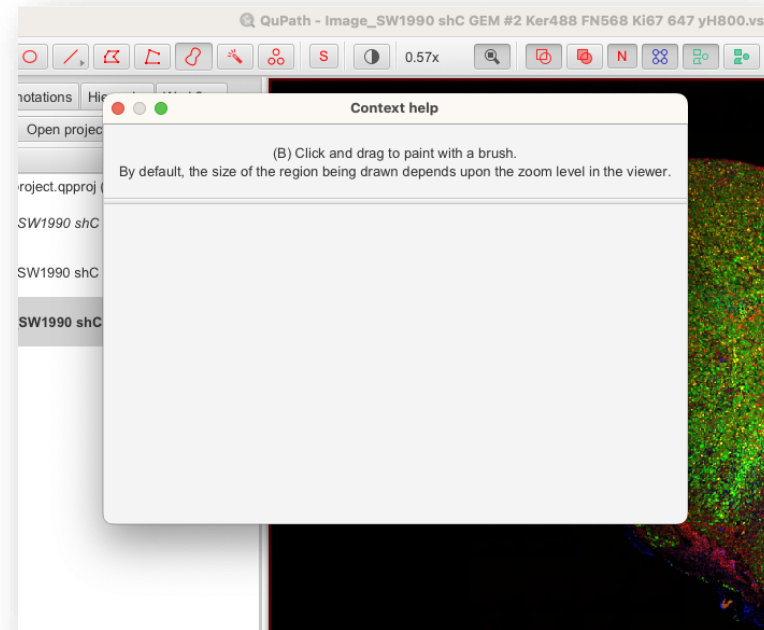
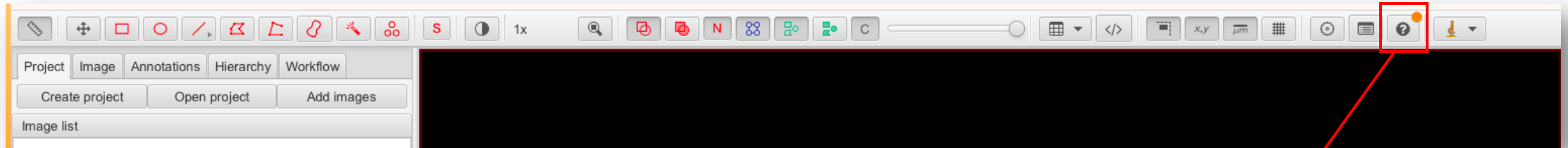


Toolbar



Preferences
Settings, GUI
customization,
extensions, ...

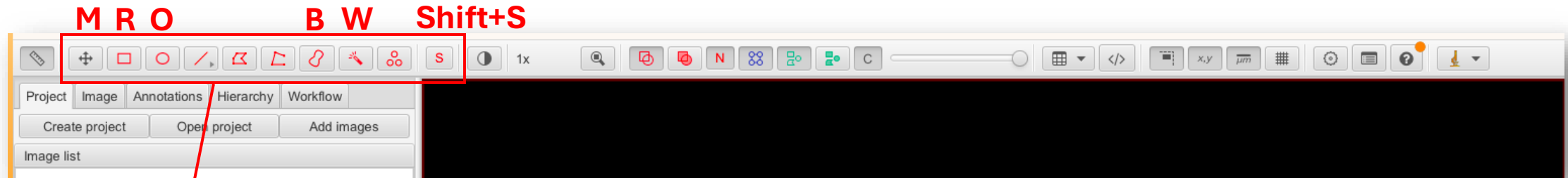
Toolbar



Interactive Help
Provides contextual
help based on your
cursor location

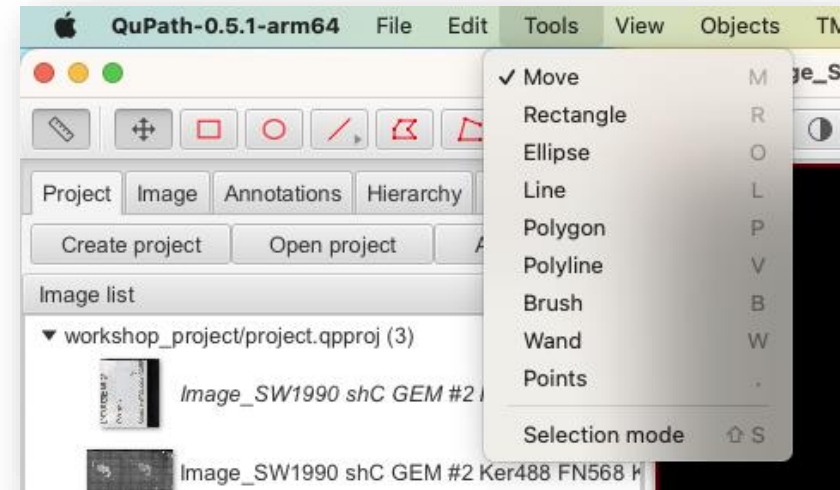
Example when my cursor is on the paint brush tool

Toolbar



Annotation tools

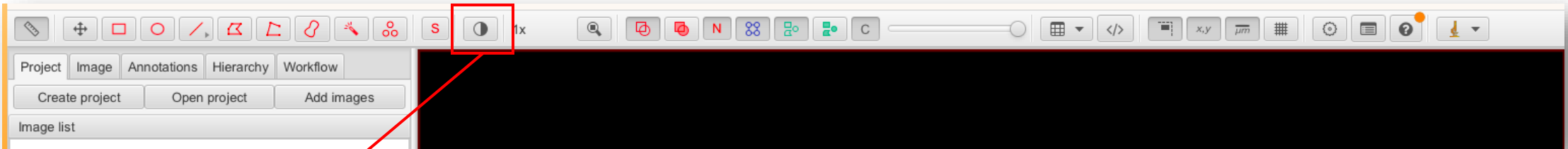
- **M** key: move tool
- **R** key: draw a rectangle annotation
- **O** key: draw an ellipse annotation
- **B** key: paint with a brush
- **W** key: draw with a wand tool
- And many more!



Annotation tools are also accessible in the *Tools* menu

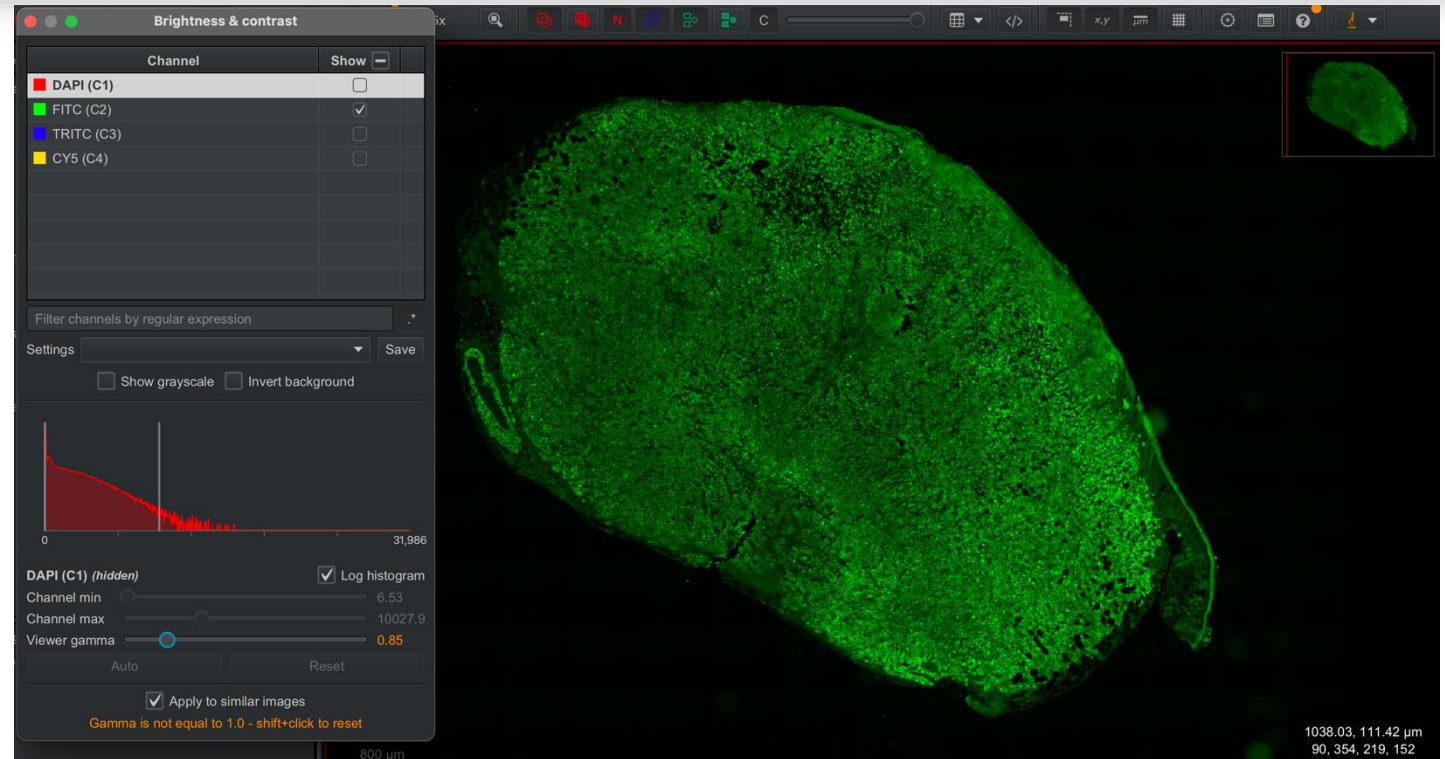
Toolbar

Shift+C



Brightness and contrast

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

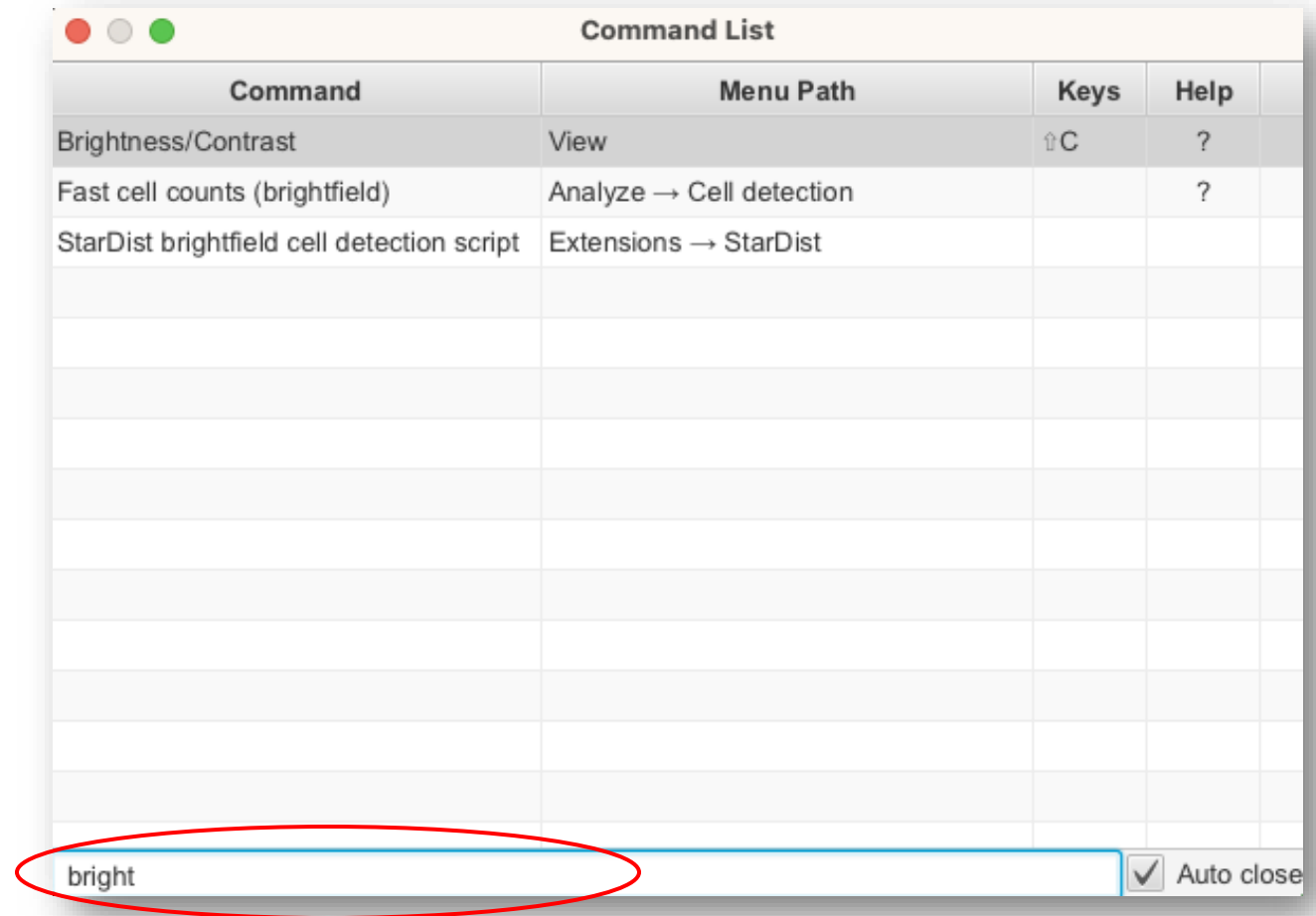


QuPath pro-tip: command list

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

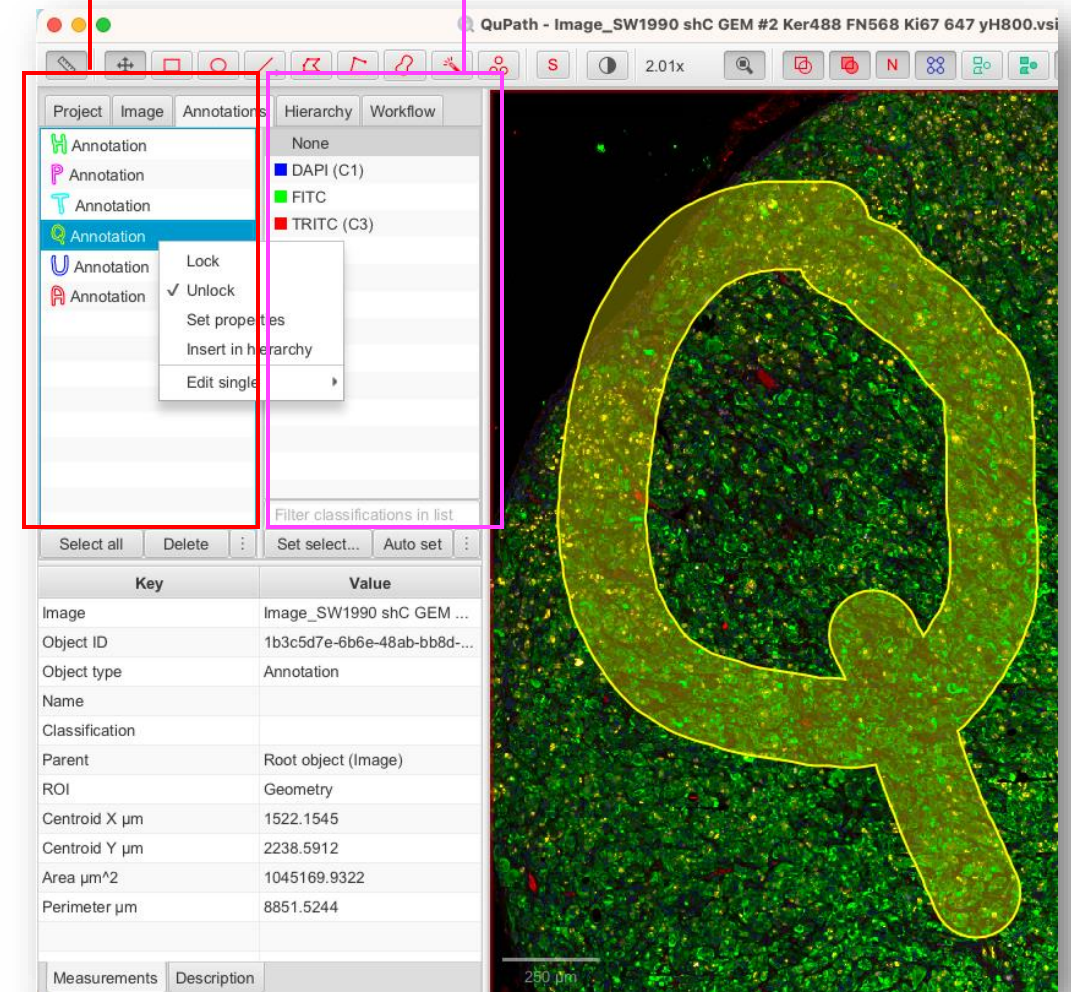
- **Objects** are a ‘thing’ in an image which encapsulates not only its shape but also some properties about it
 - **Annotations:** Objects that you usually create yourself, by drawing on the image
 - They are flexible, up to ~100 per image
 - Can be edited
 - Often used to define regions
 - **Detections:**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

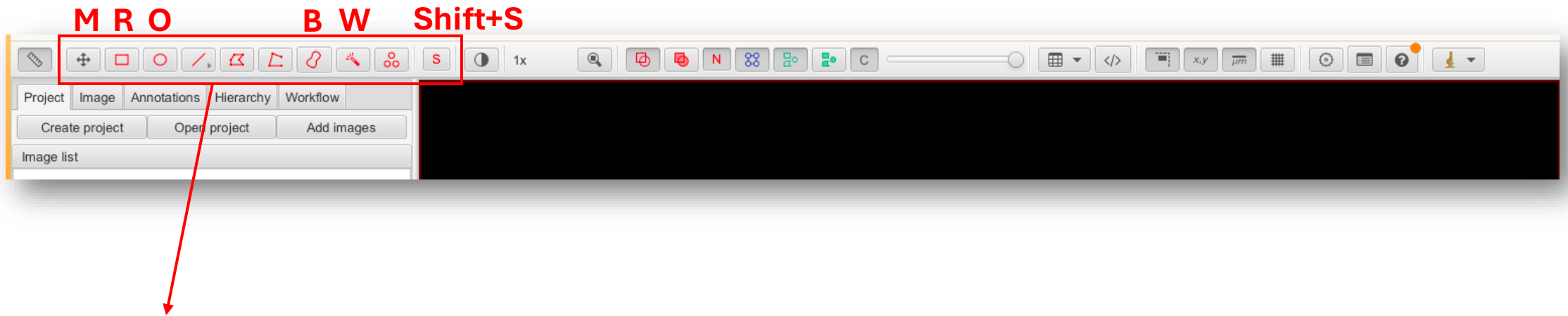
Annotation list

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!

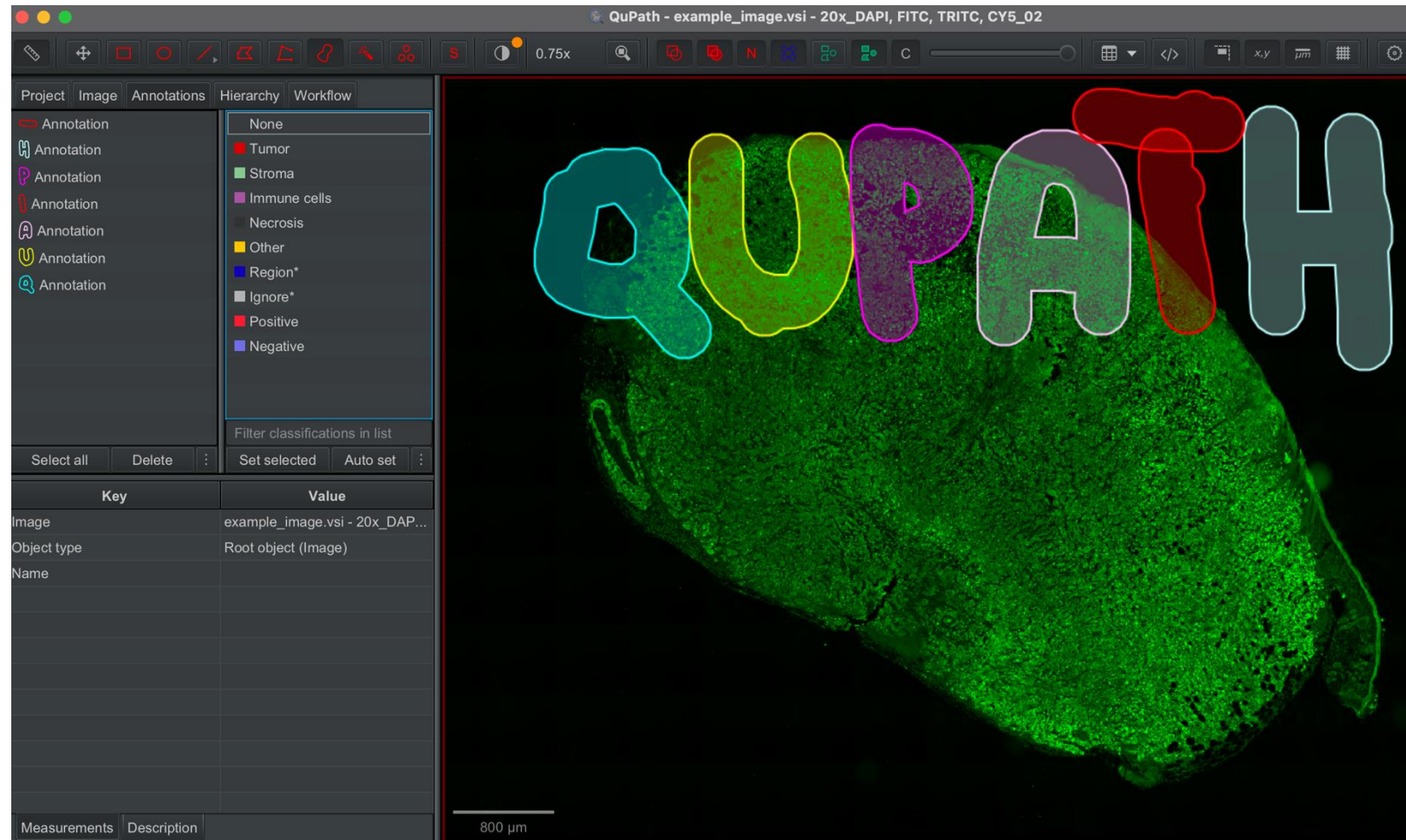
**Remember to always
lock your annotation
to prevent accidental
editing!**

Practice time

Exercises 2: QuPath manual annotations

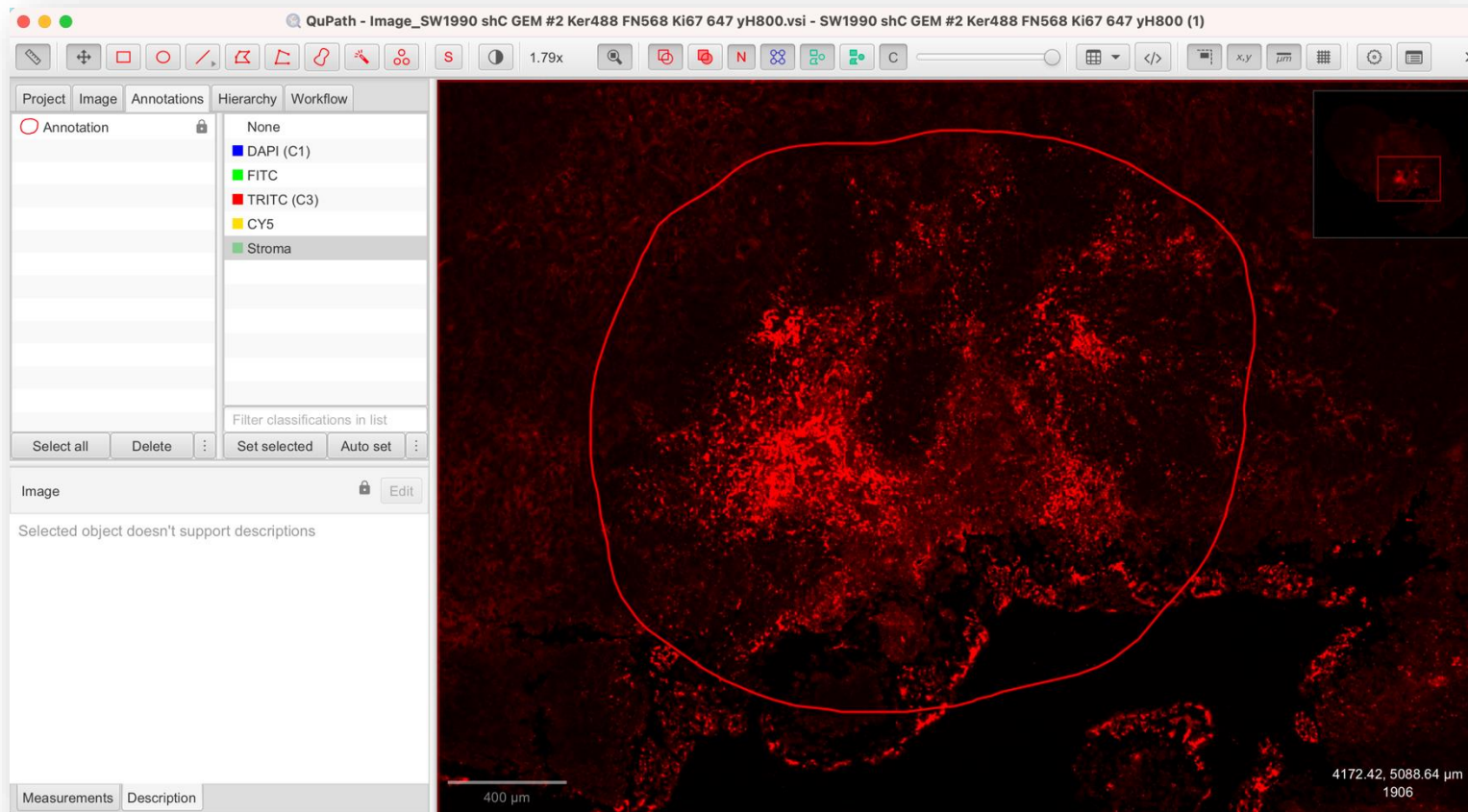
Recreate these annotations

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*