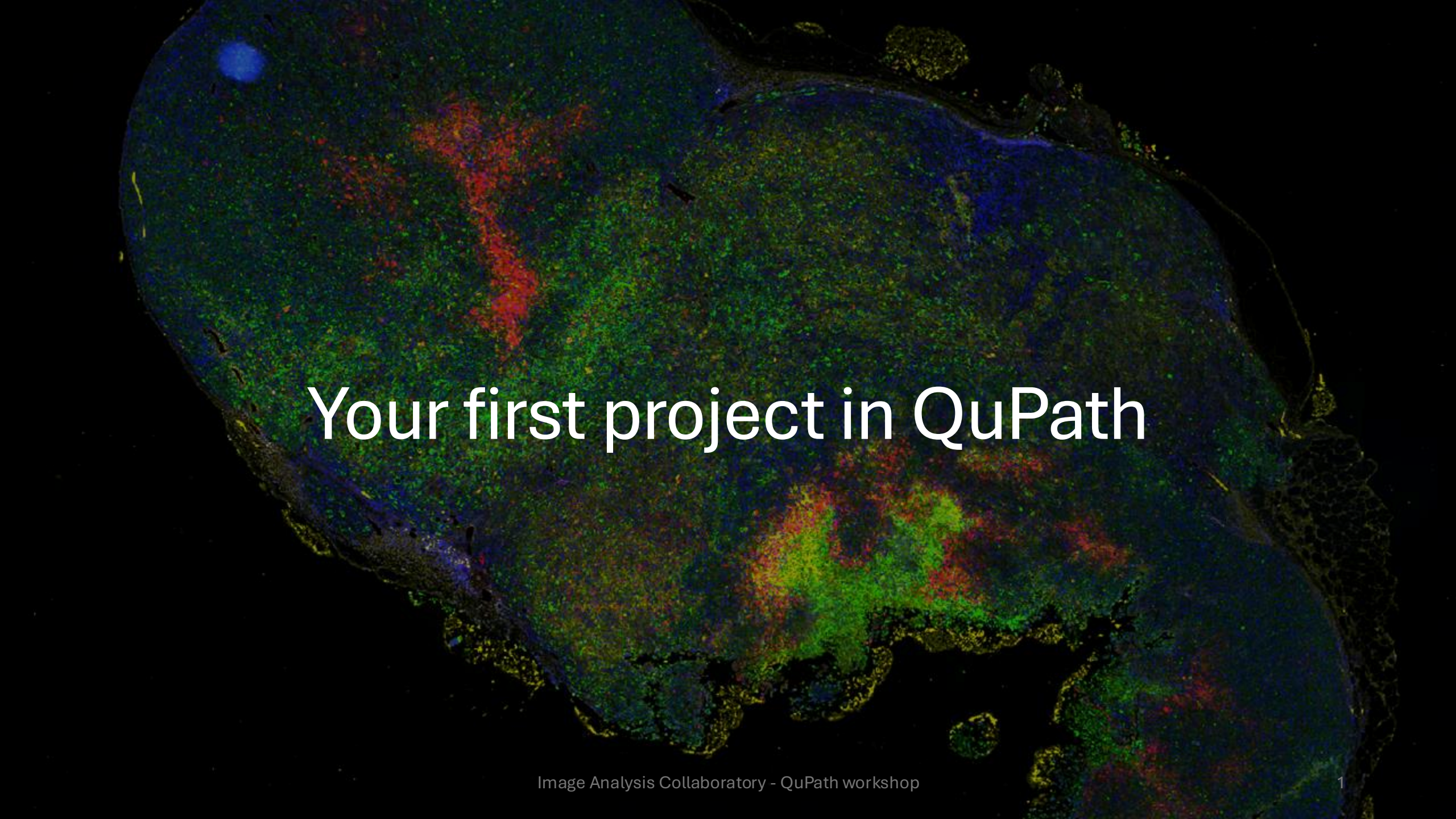
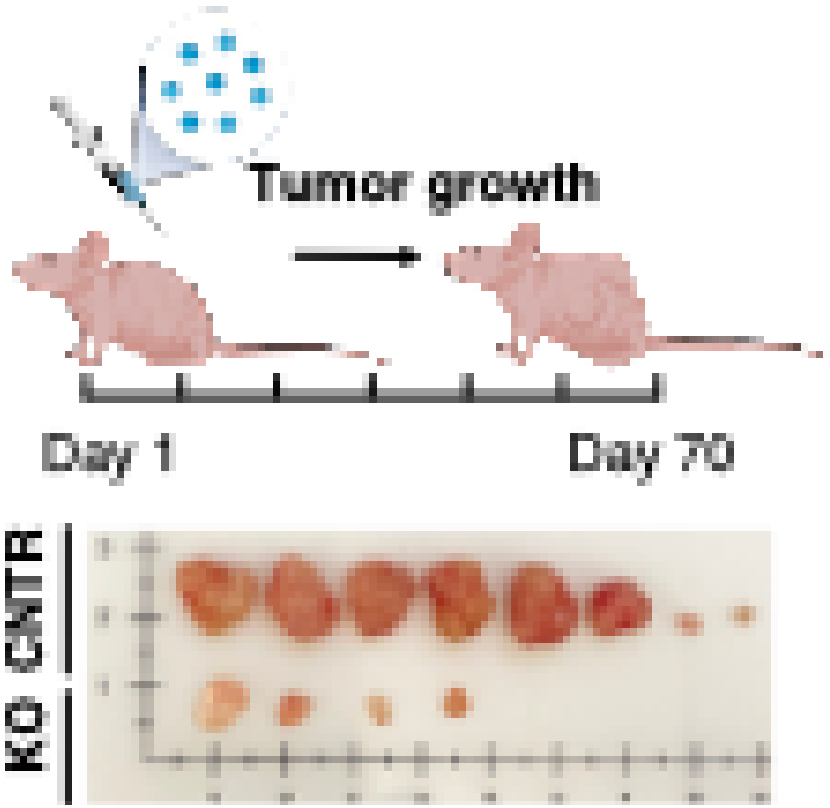


Your first project in QuPath

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)

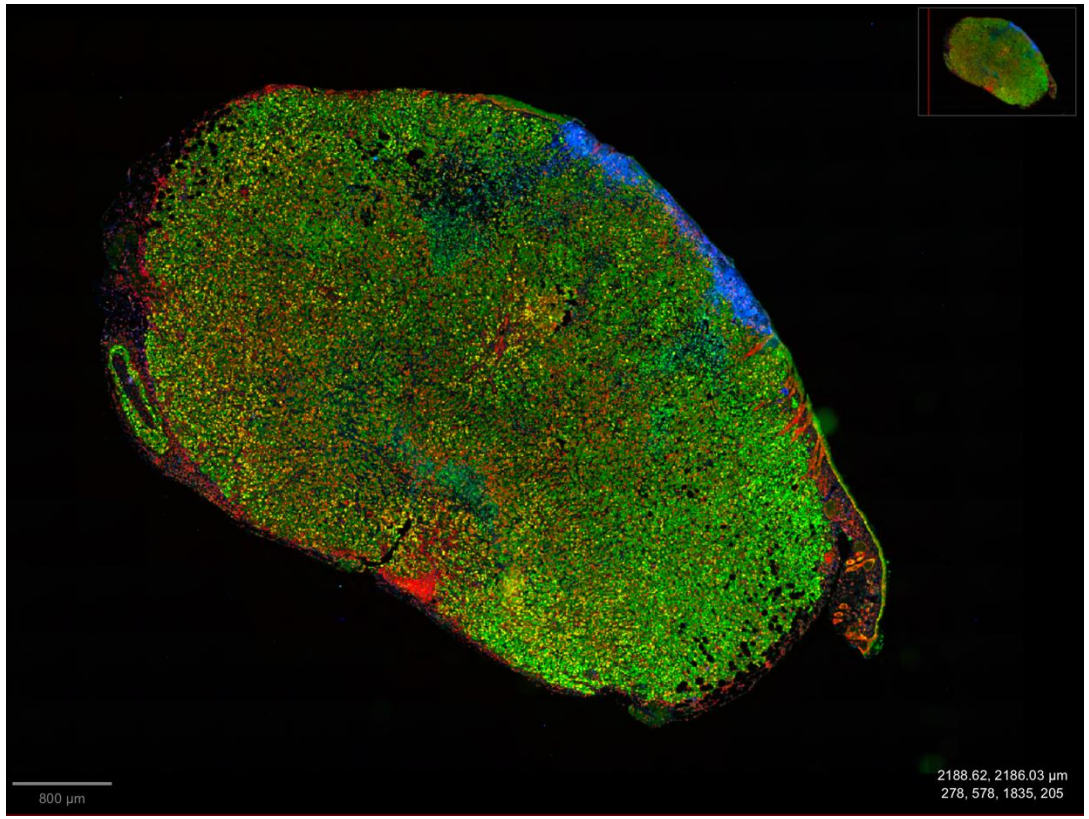
Case study

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

Choice of markers

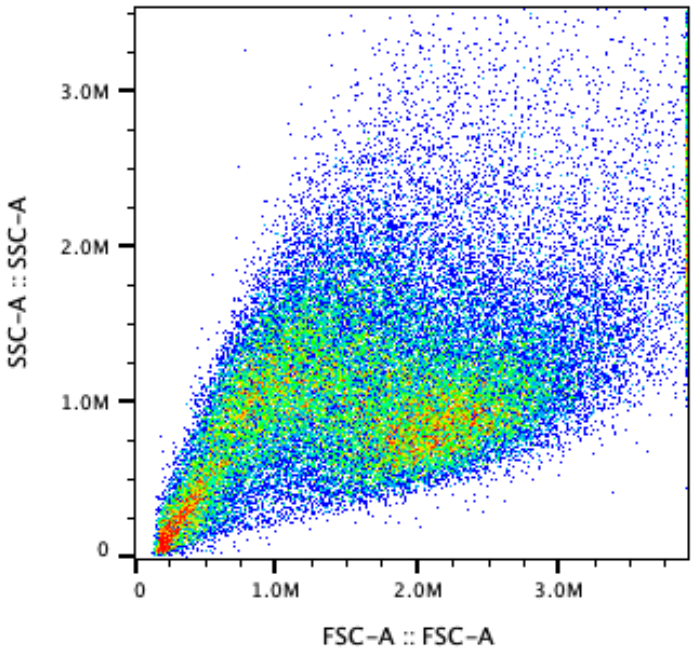
- DAPI to segment nuclei
- Epithelial cells (Pan-cytokeratin)
- Proliferating cells (Ki67)
- Stroma (Fibronectin)

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

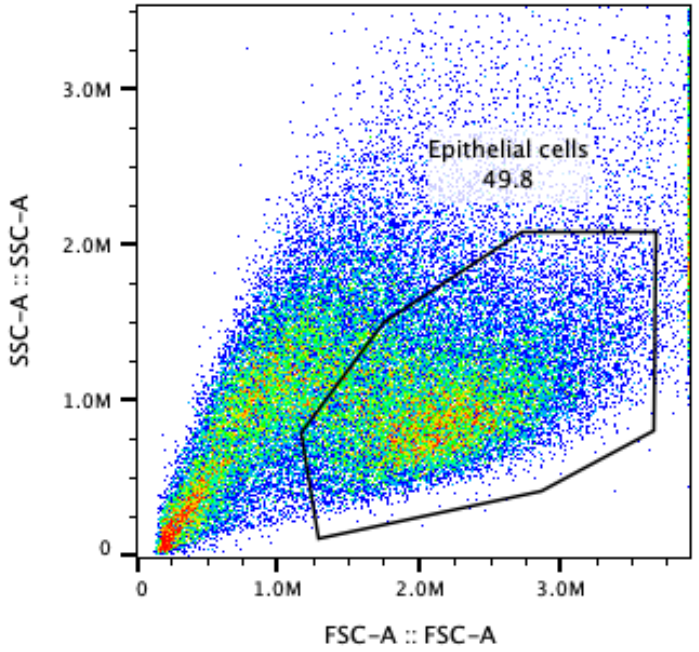


Few ideas before we move forward

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

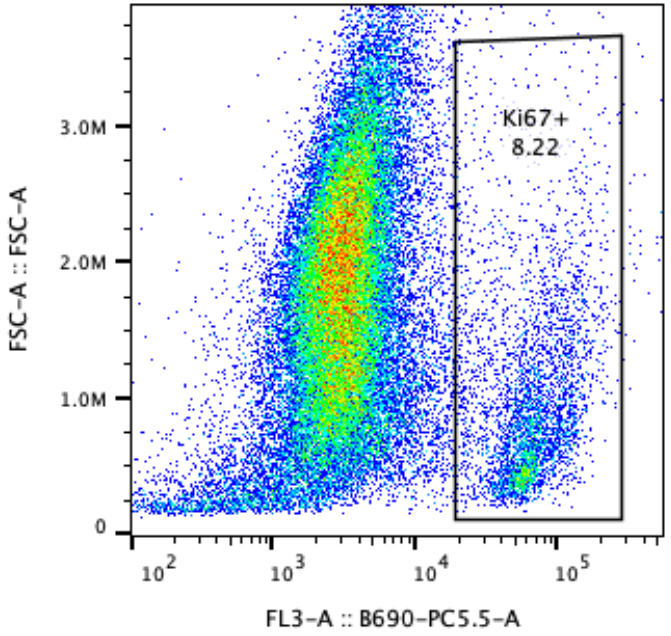


DAPI



Pan-cytokeratin

Pan-CK positive



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

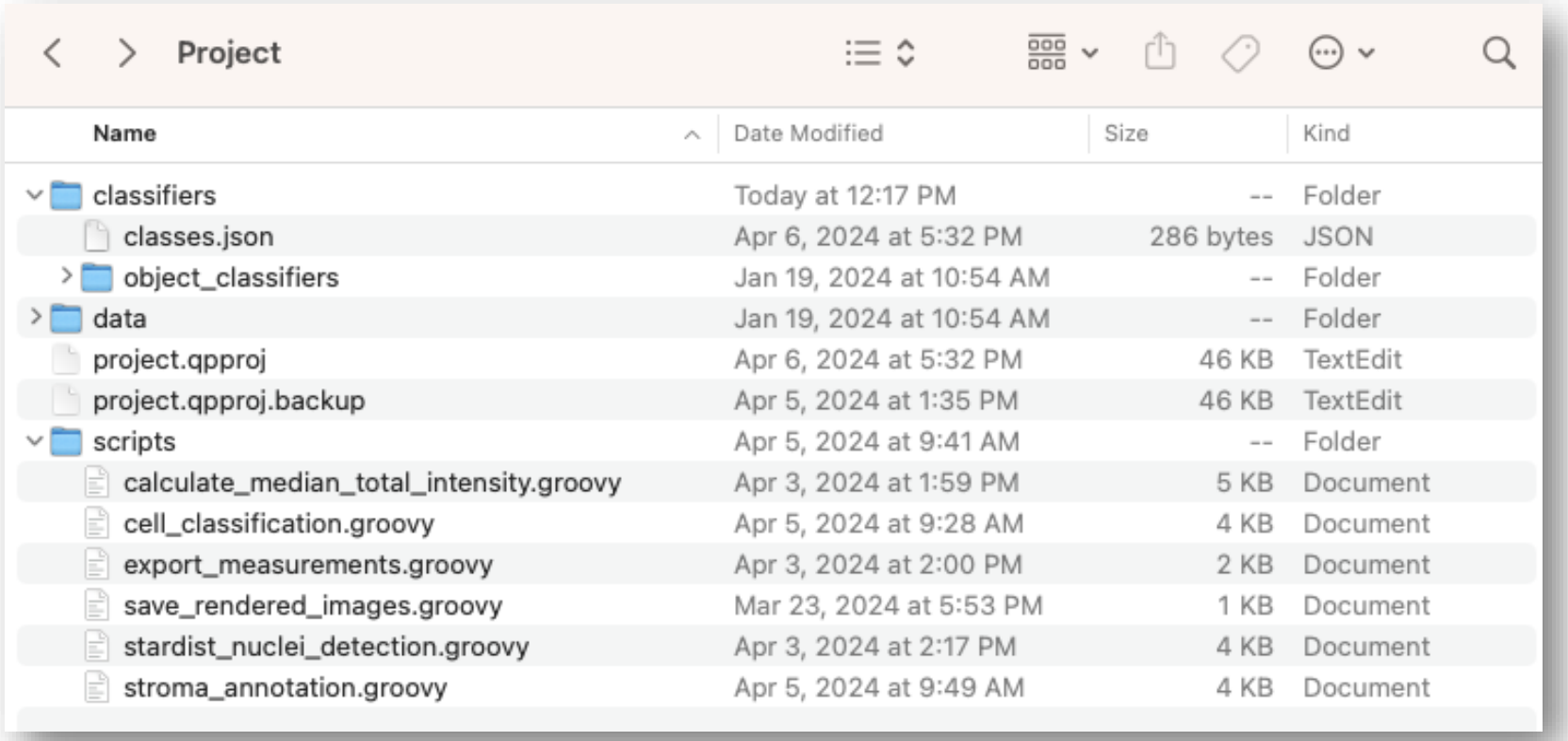
  or

- 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

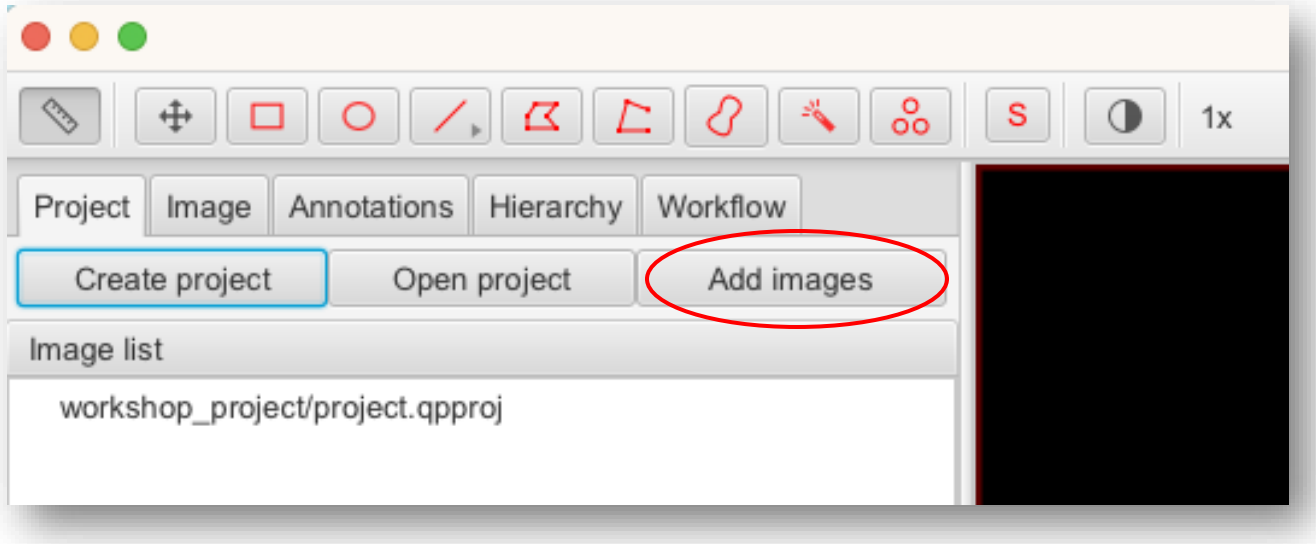
Anatomy of a QuPath project

After a bit of time working on it…



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

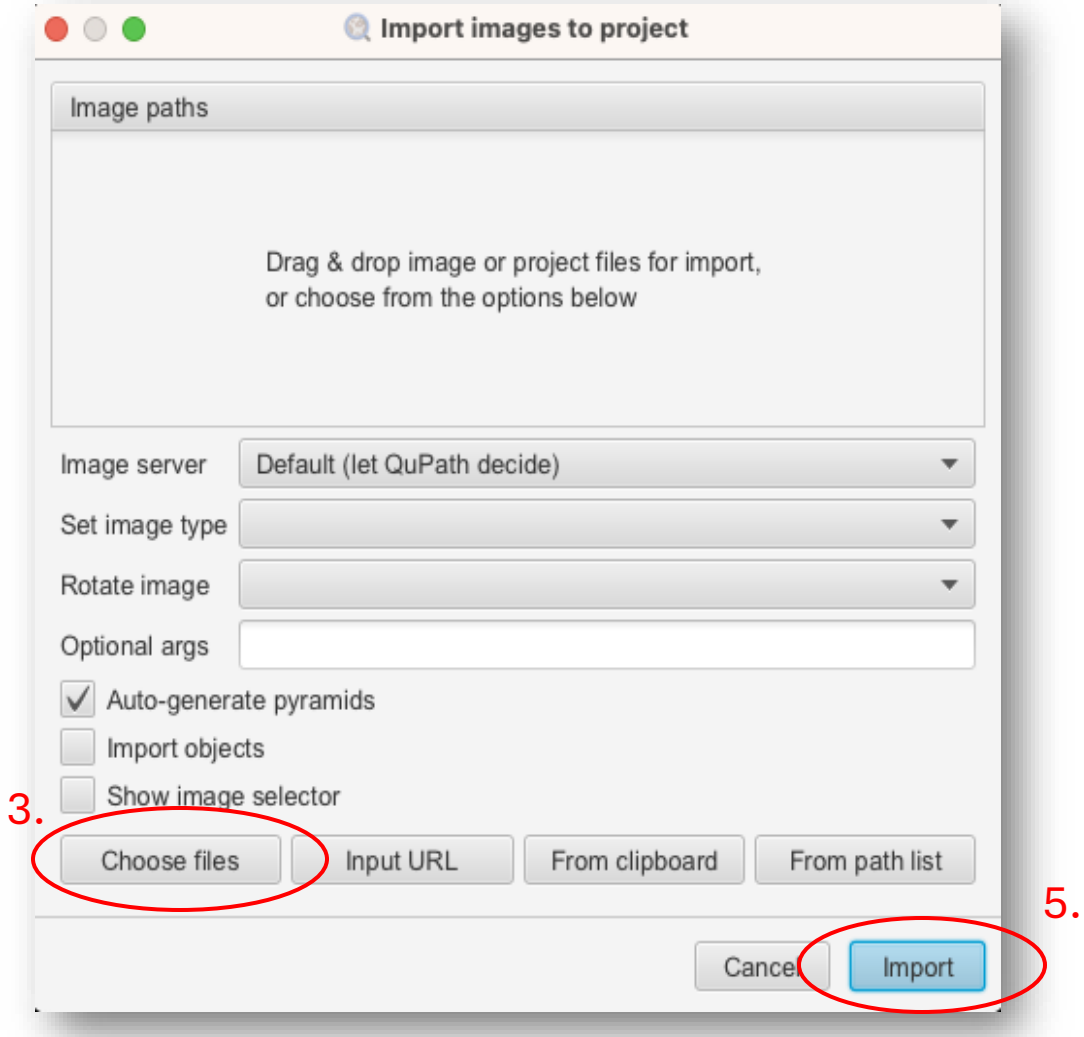


Add an image to your project

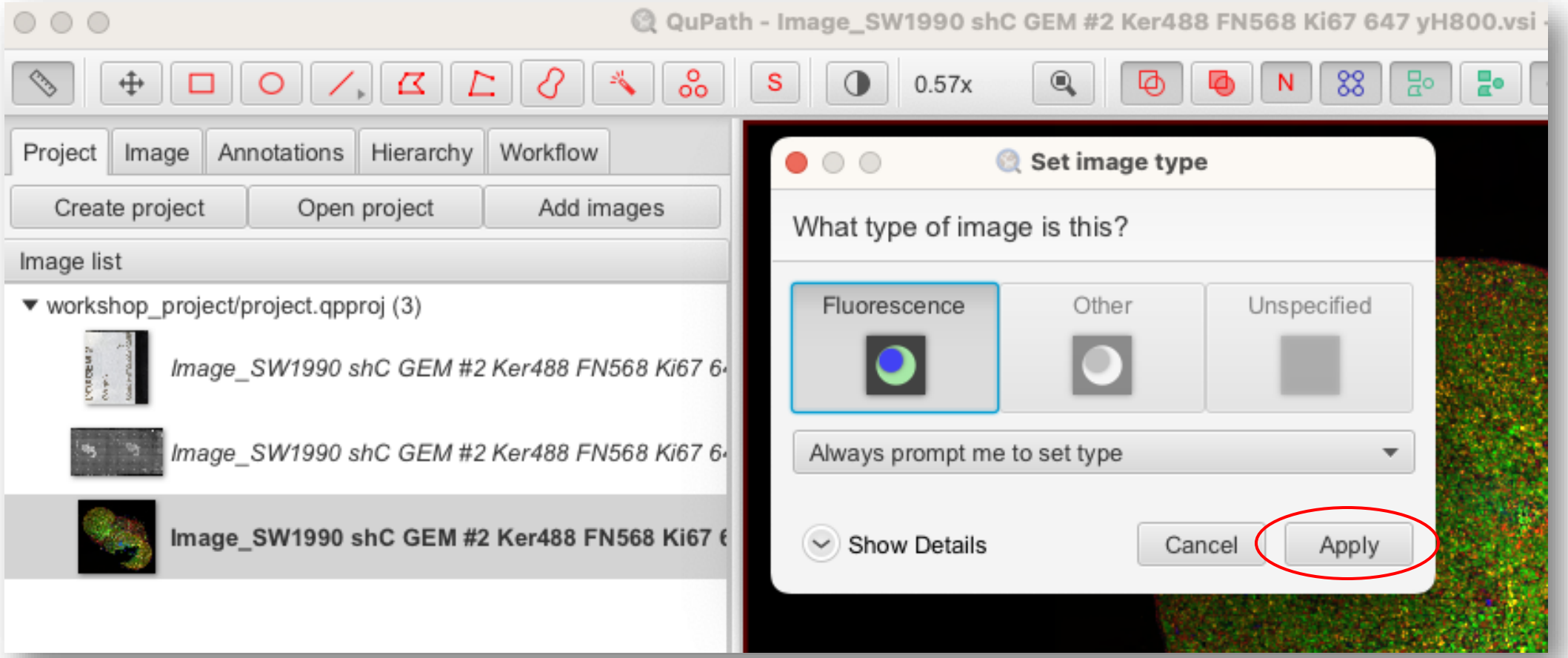
3. Select the .vsi image using *Choose files* or drag-and-drop

4. Use default settings

5. Click *import*



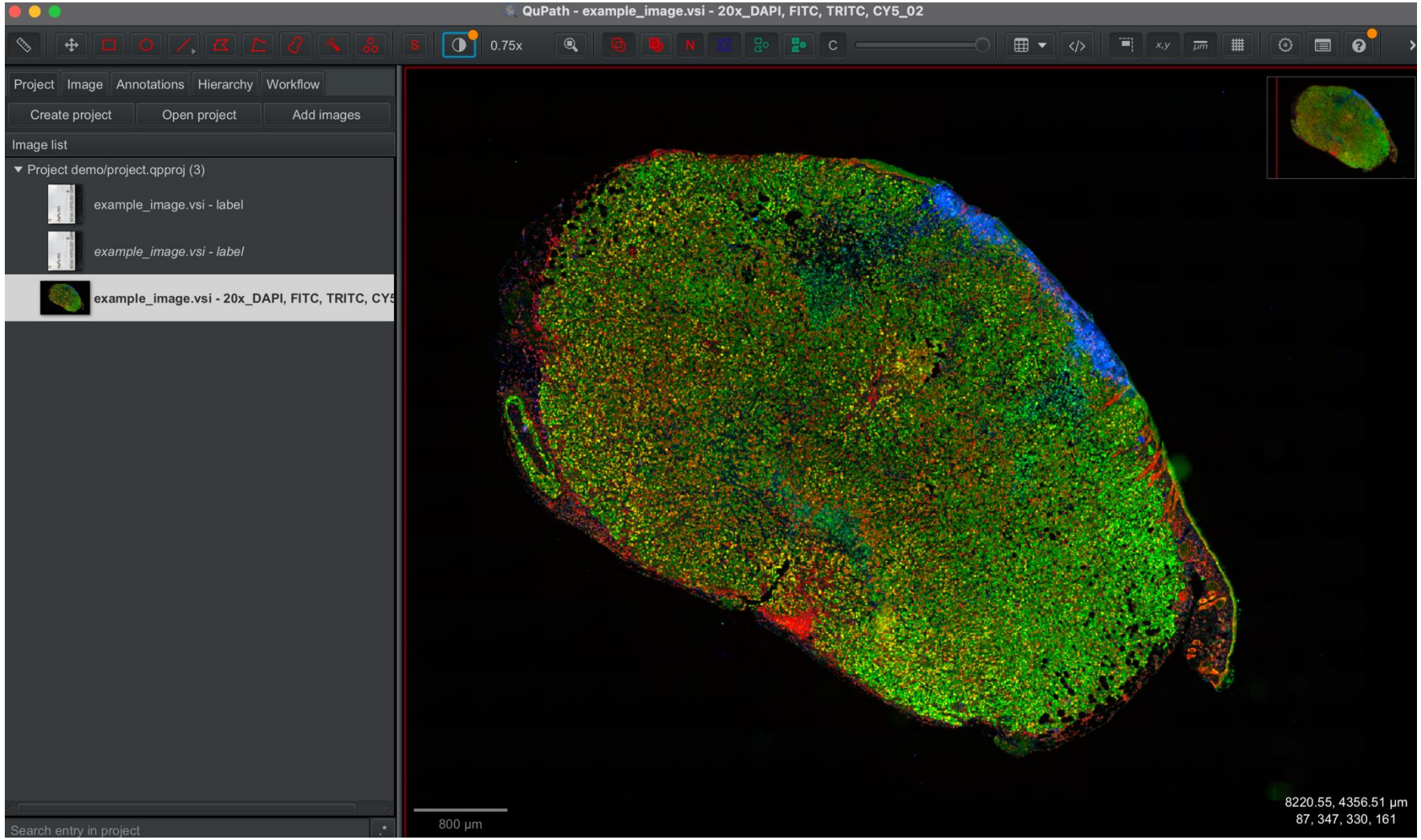
Set image type



- *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

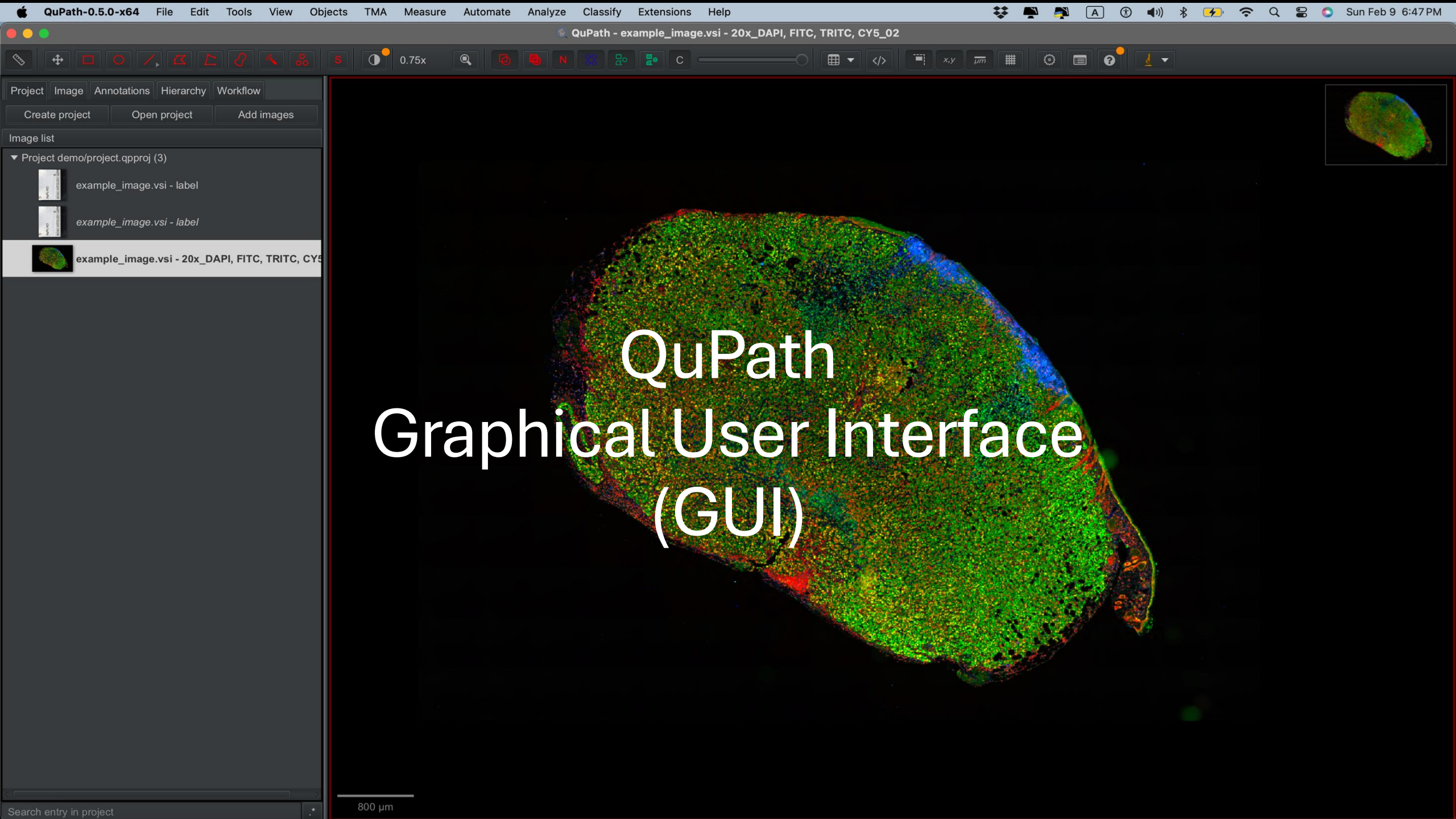
QuPath projects are portable

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

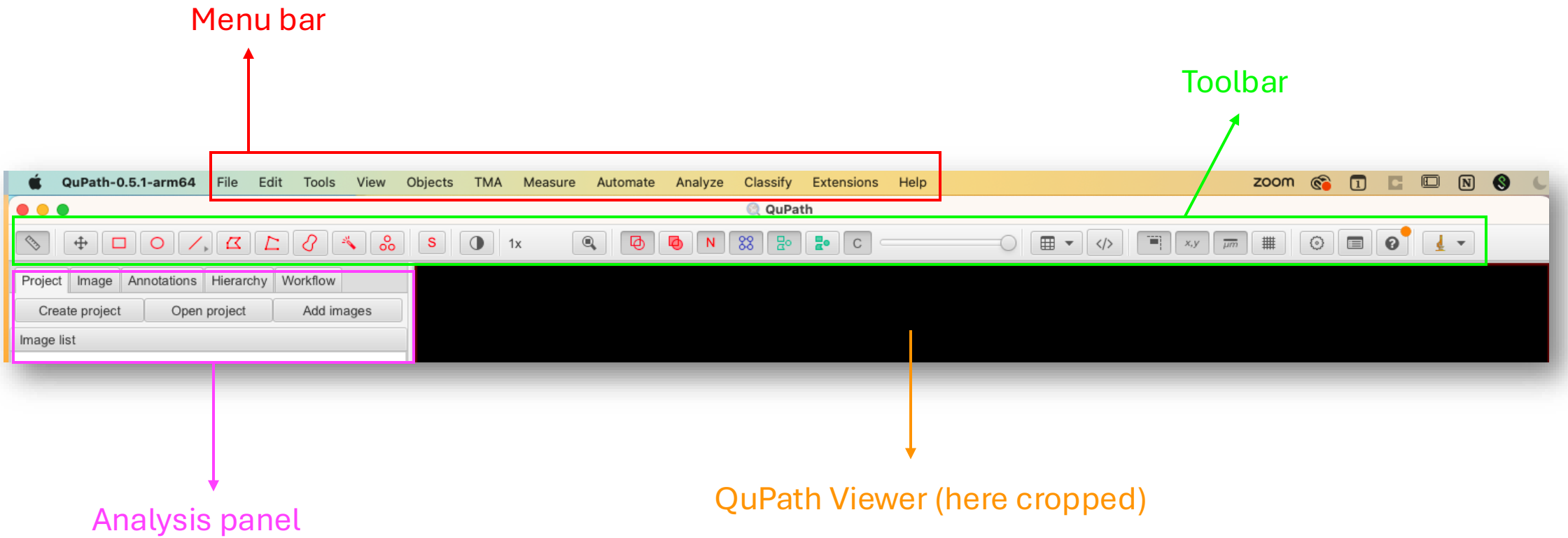
The project folder only contains QuPath objects and data, unless you had placed them there. Ensure that they can access the actual image files.

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

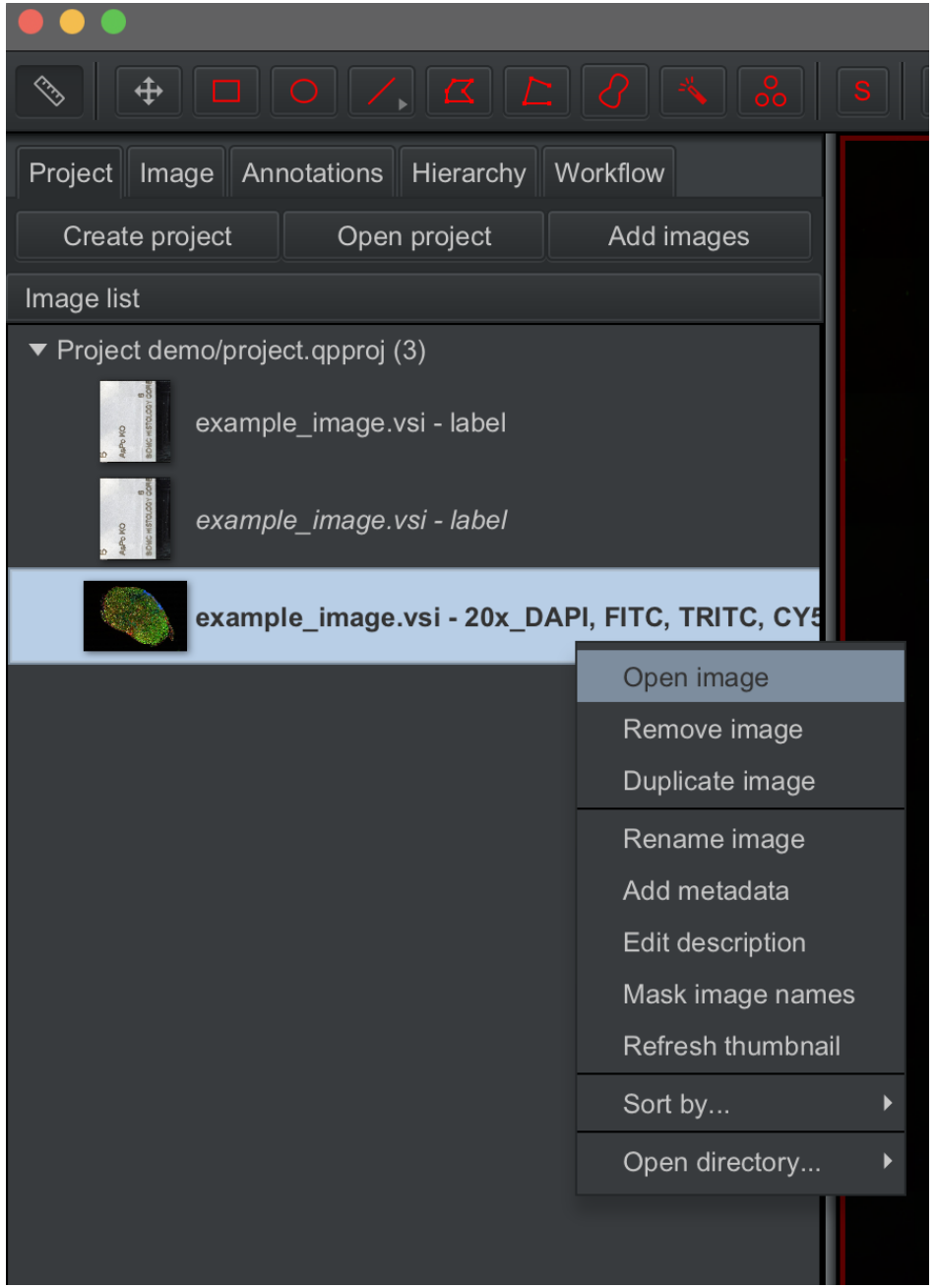

QuPath-0.5.0-x64 File Edit Tools View Objects TMA Measure Automate Analyze Classify Extensions Help
Sun Feb 9 6:47 PM
QuPath - example_image.vsi - 20x_DAPI, FITC, TRITC, CY5_02
0.75x
Project Image Annotations Hierarchy Workflow
Create project Open project Add images
Image list
Project demo/project.qpproj (3)
example_image.vsi - label
example_image.vsi - label
example_image.vsi - 20x_DAPI, FITC, TRITC, CY5
Search entry in project
QuPath
Graphical User Interface
(GUI)
800 µm

Graphic User Interface (GUI)



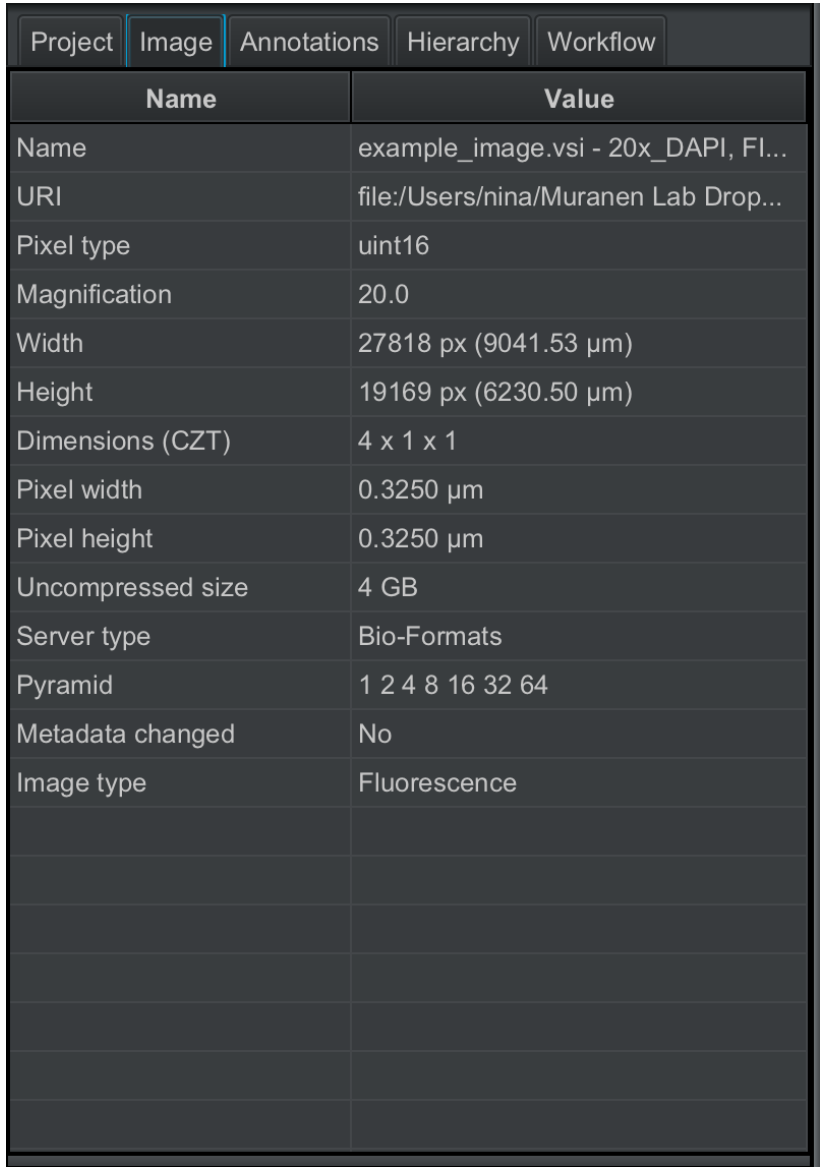
Analysis Panel

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

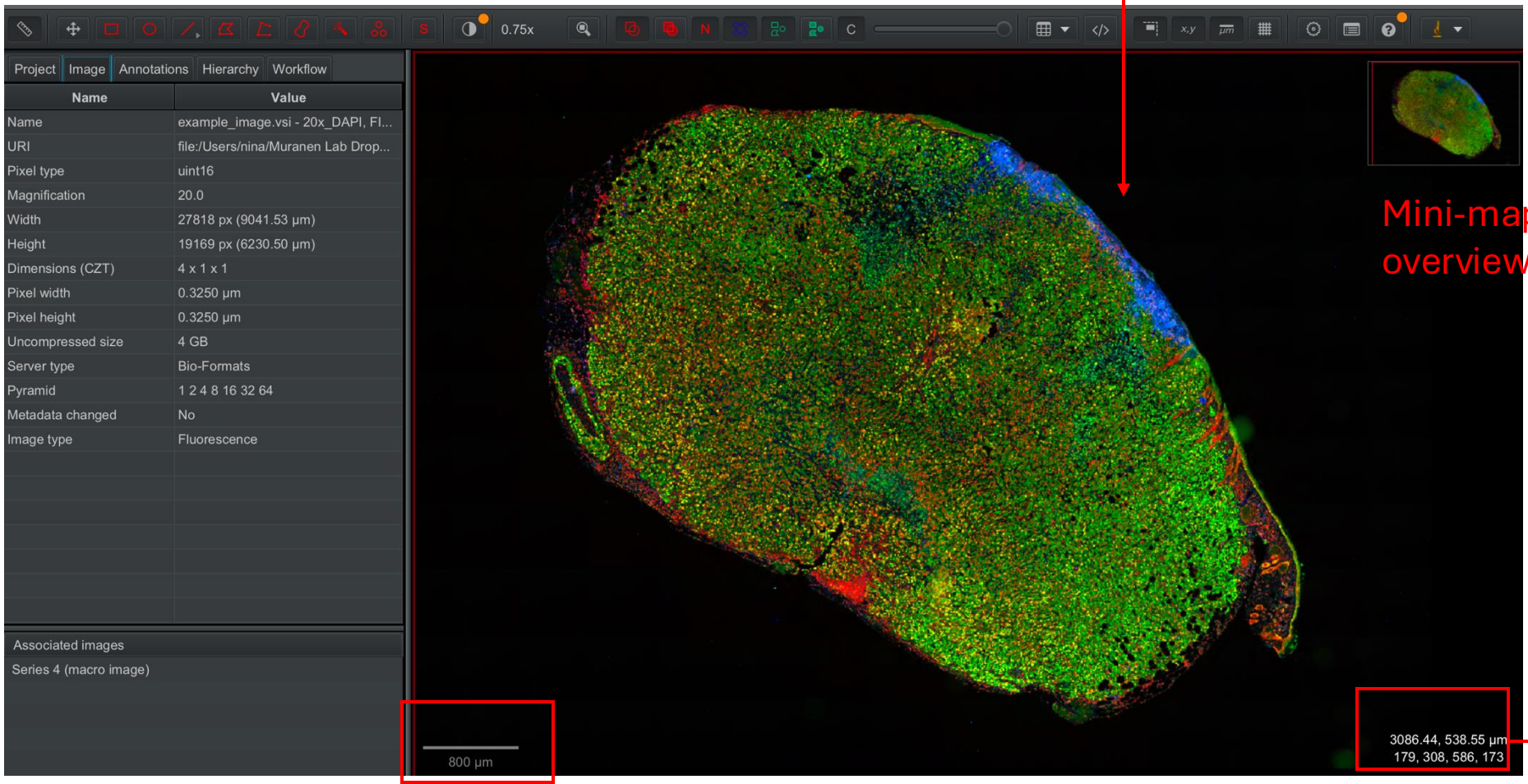


Analysis Panel

- *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

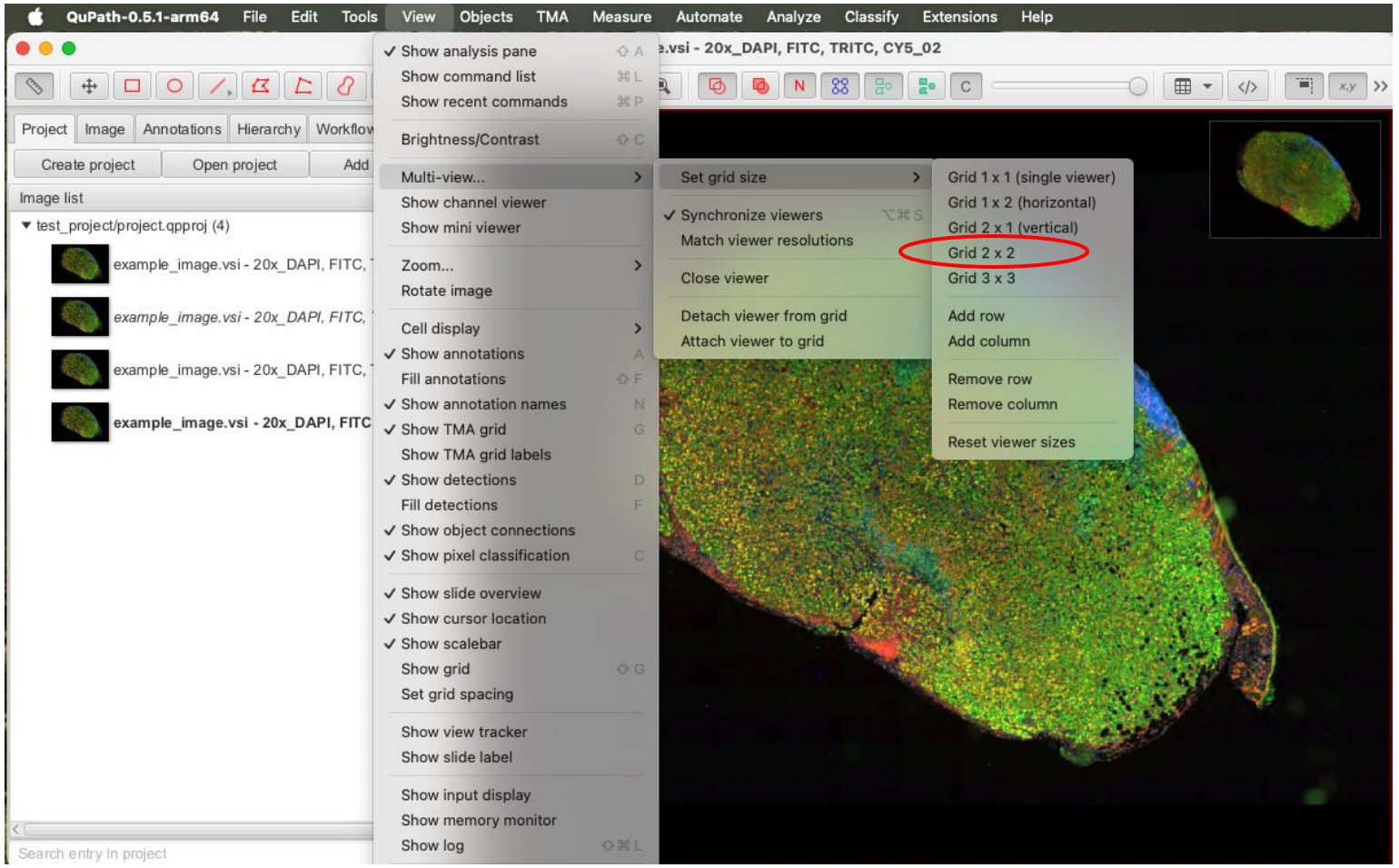


QuPath viewer



Multi-viewer

- 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* > M*ulti-view* > *Set grid size* > *Grid 2x2*

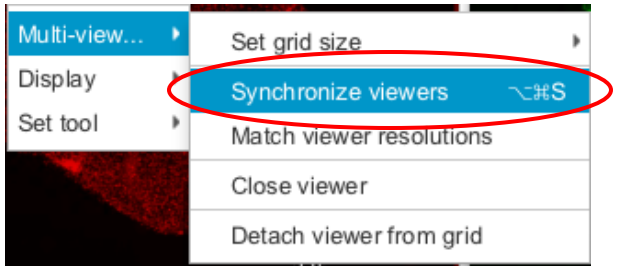


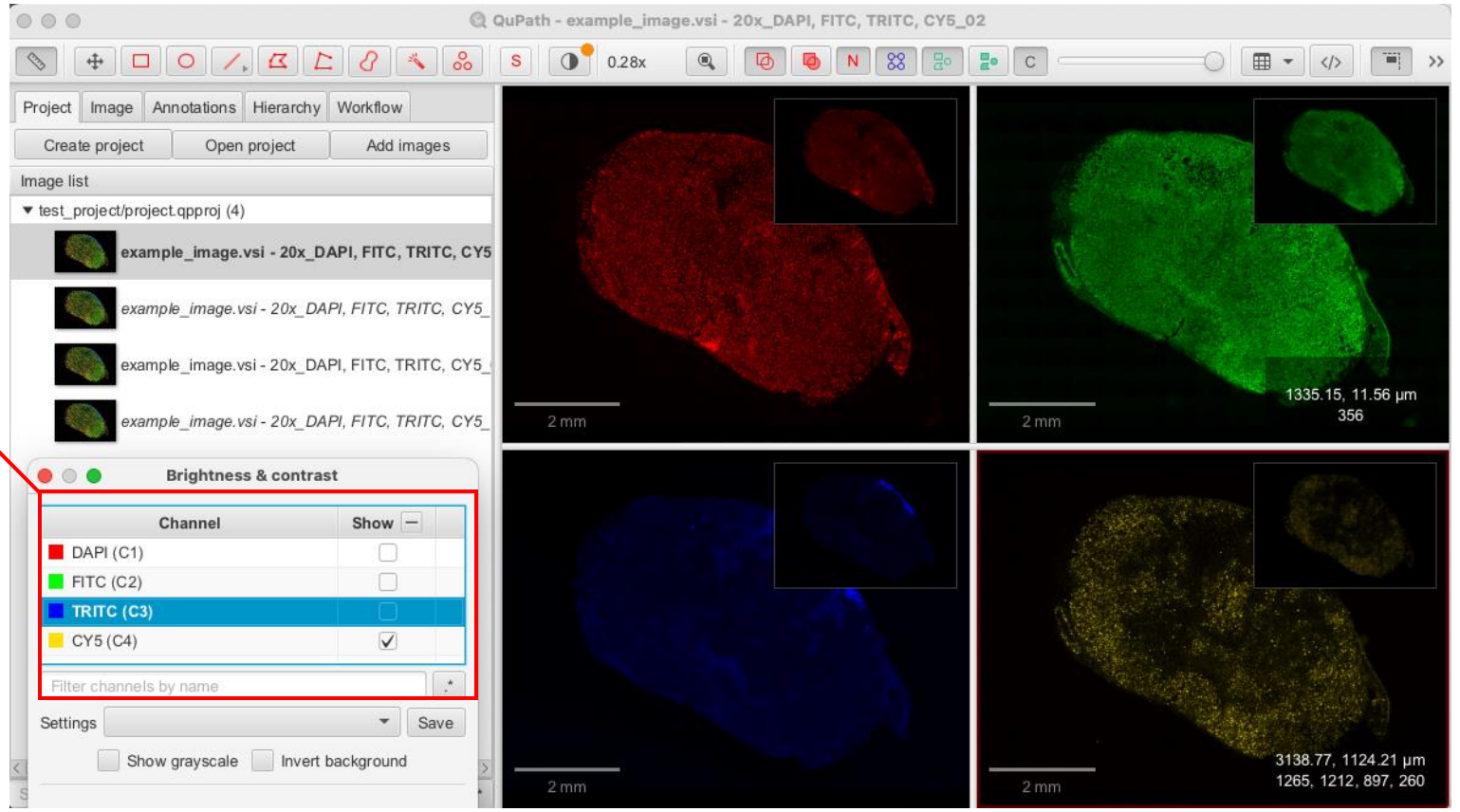
Multi-viewer

Brightness and contrast

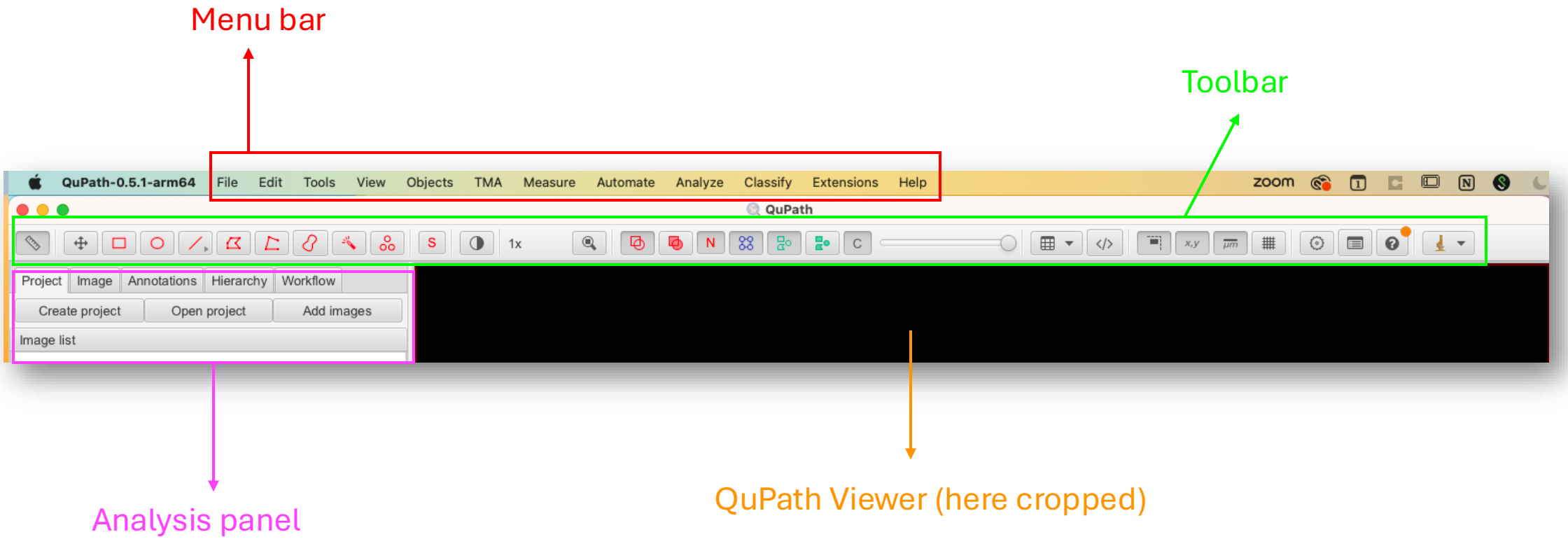
- Select a channel for each viewer

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

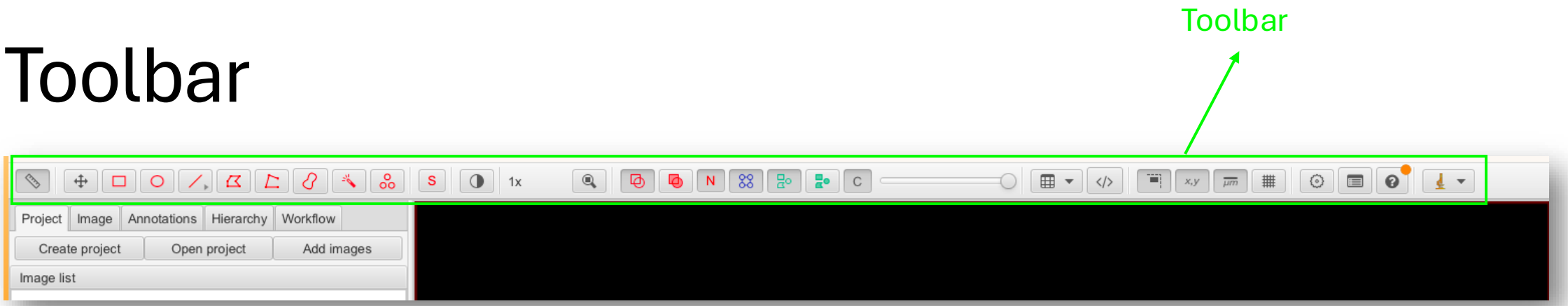




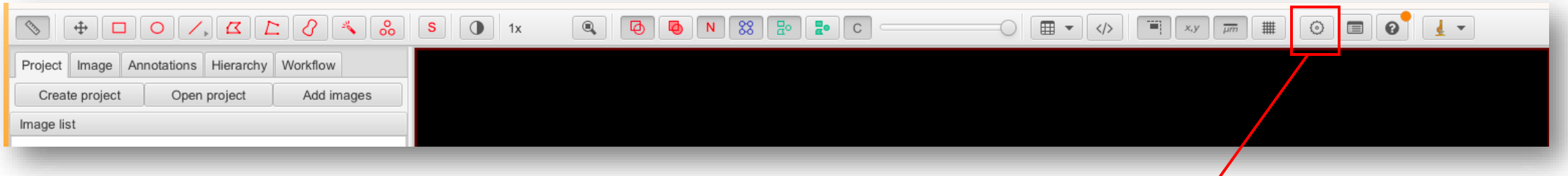
Graphic User Interface (GUI)



Toolbar

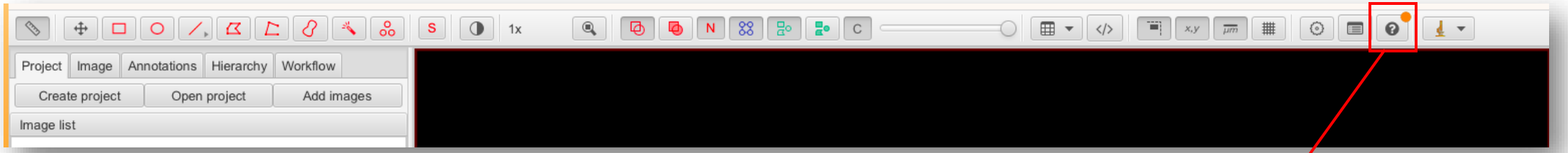

Toolbar
1x
N
C
x,y
Project
Image
Annotations
Hierarchy
Workflow
Create project
Open project
Add images
Image list

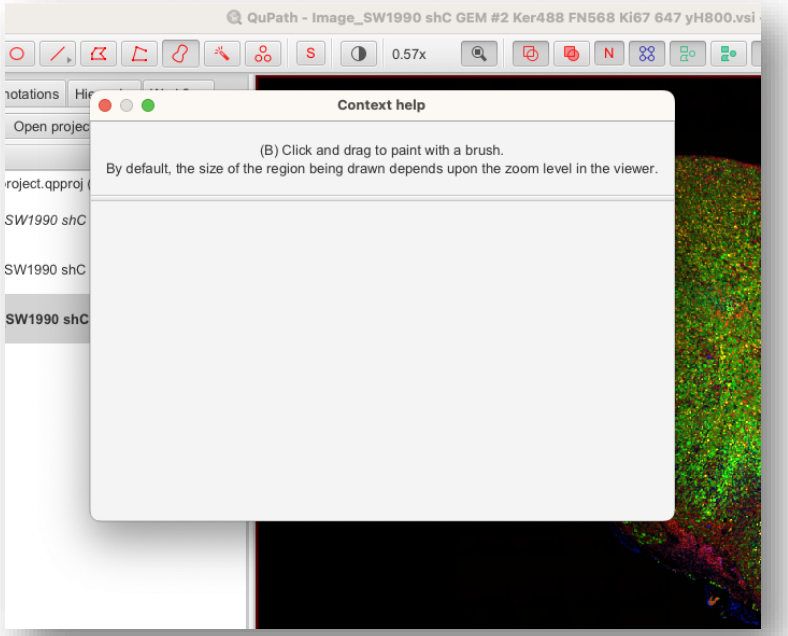
Toolbar



Preferences
Settings, GUI customization, extensions, …

Toolbar





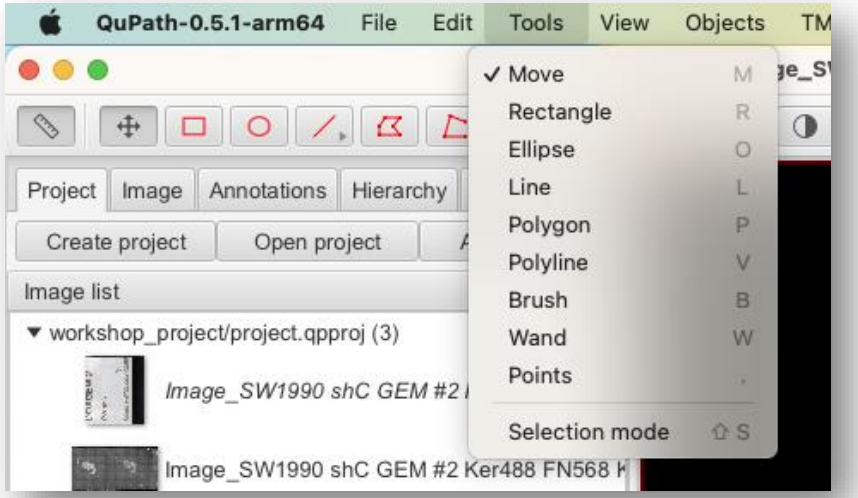
Example when my cursor is on the paint brush tool

Interactive Help
Provides contextual help based on your cursor location

Toolbar

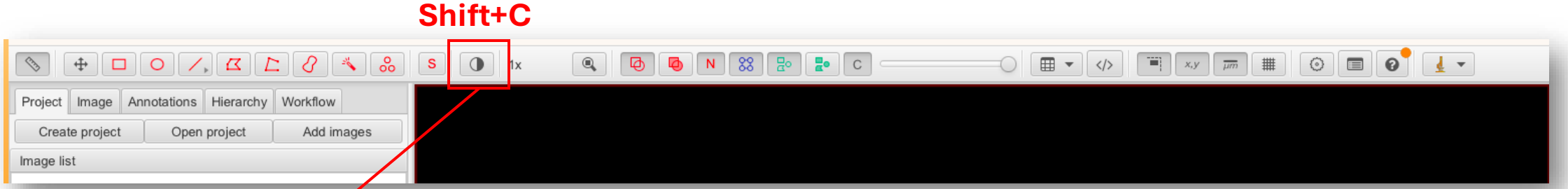
Annotation tools

- **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!



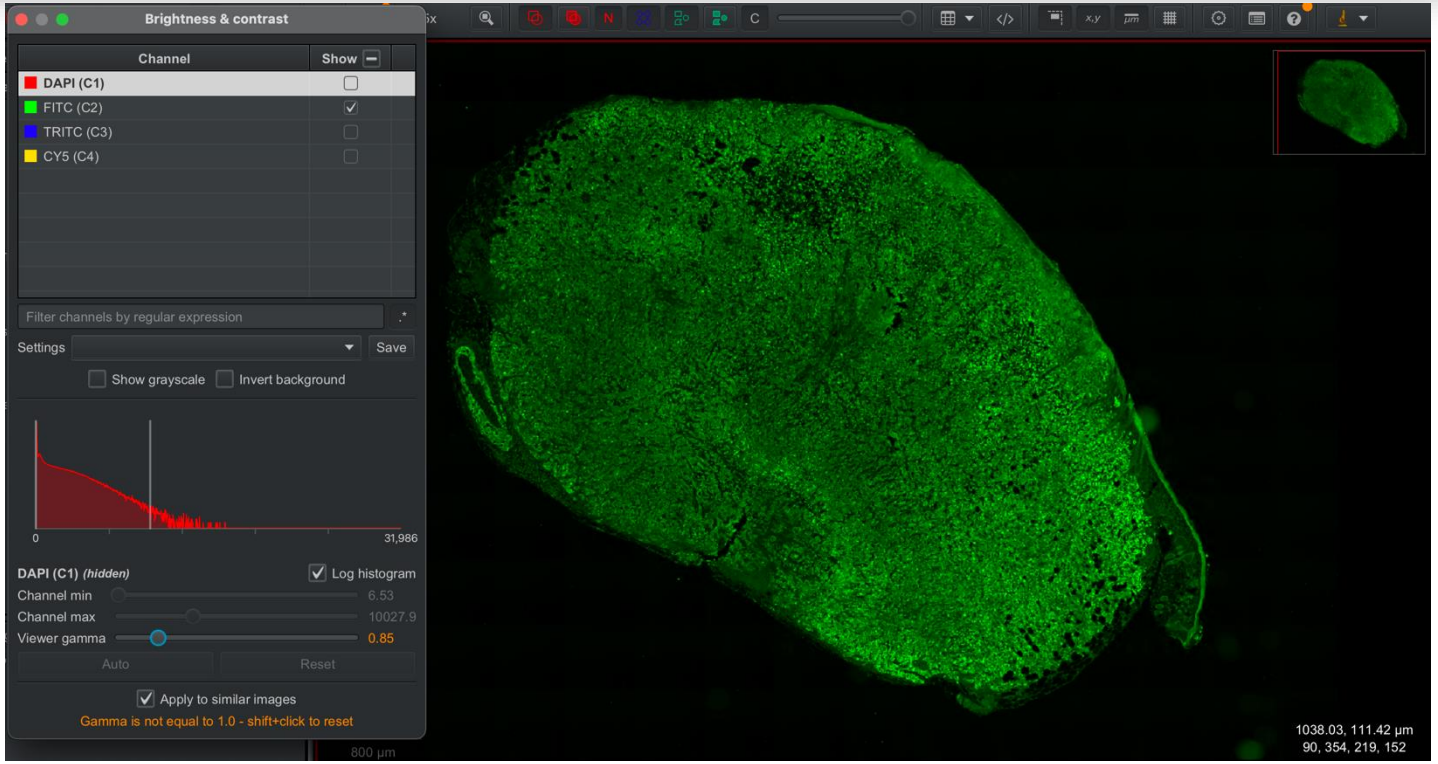
Annotation tools are also accessible in the *Tools* menu

Toolbar



Brightness and contrast

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

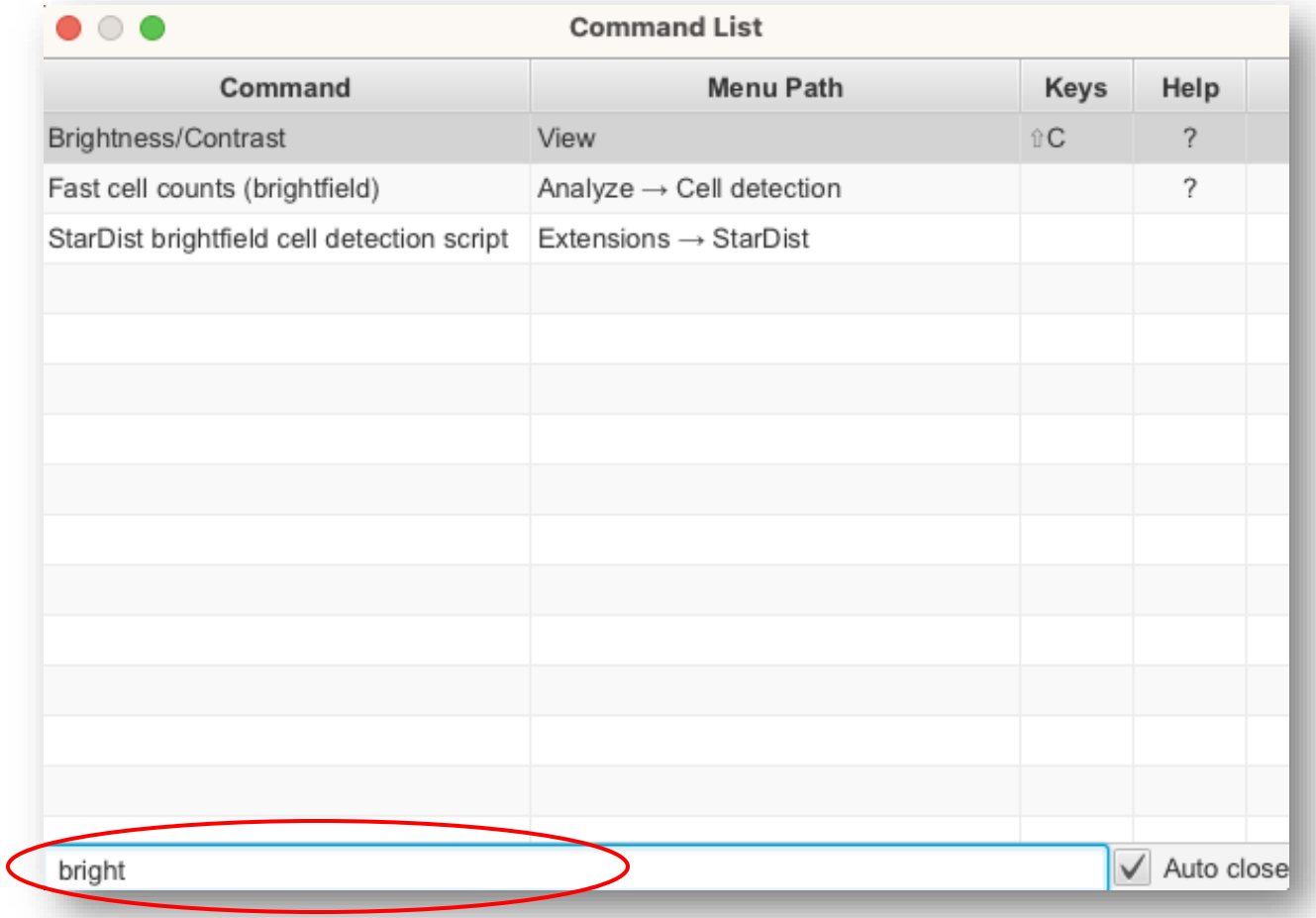


Example for FITC (Keratin) channel

QuPath pro-tip: command list

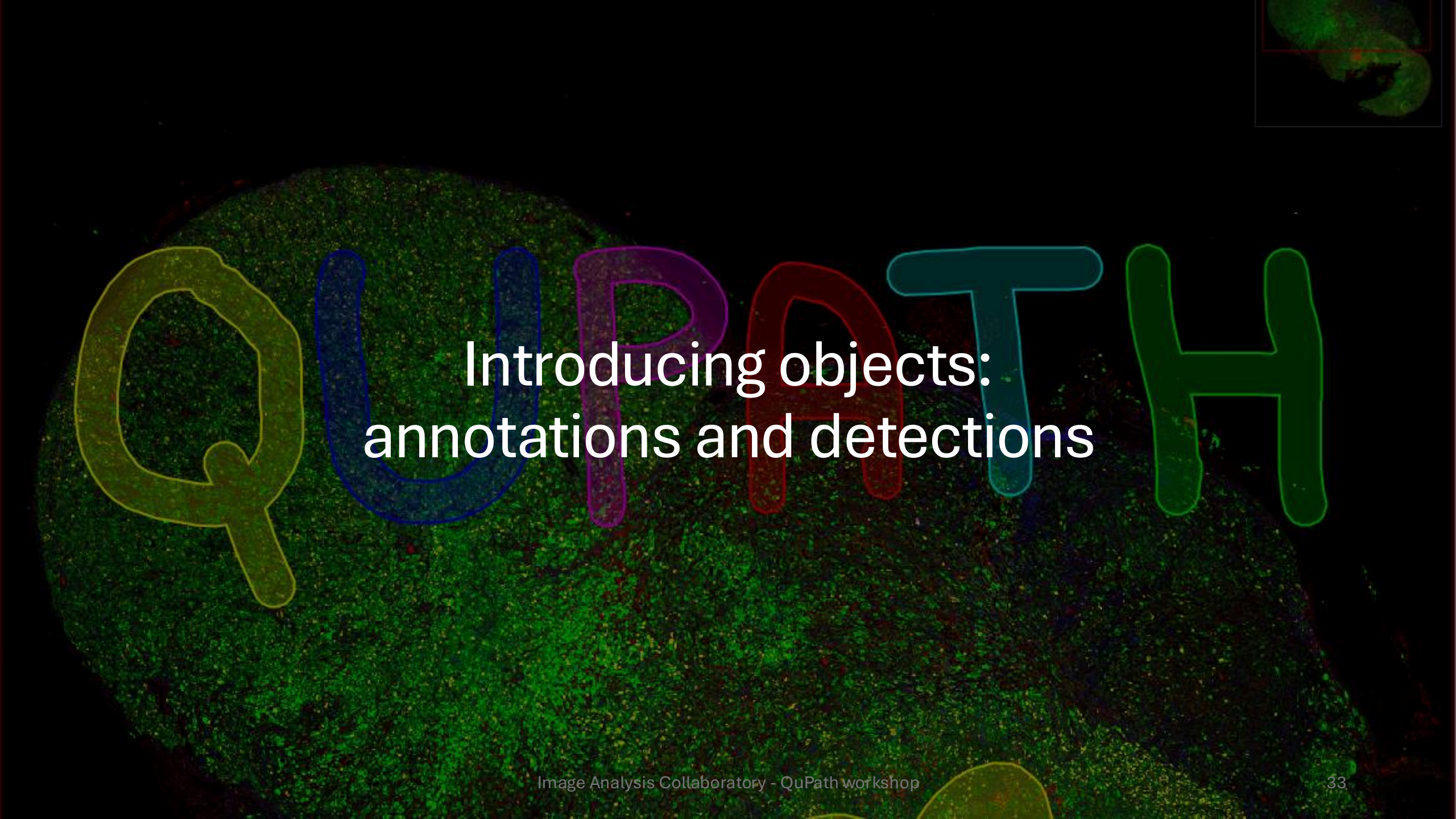
Command/Control + L
Opens a dialogue to search for any command using keyword

For example, search for 'brightness'



Practice time

Exercises 1: QuPath projects and GUI



Introducing objects: annotations and detections

Key concept: QuPath objects

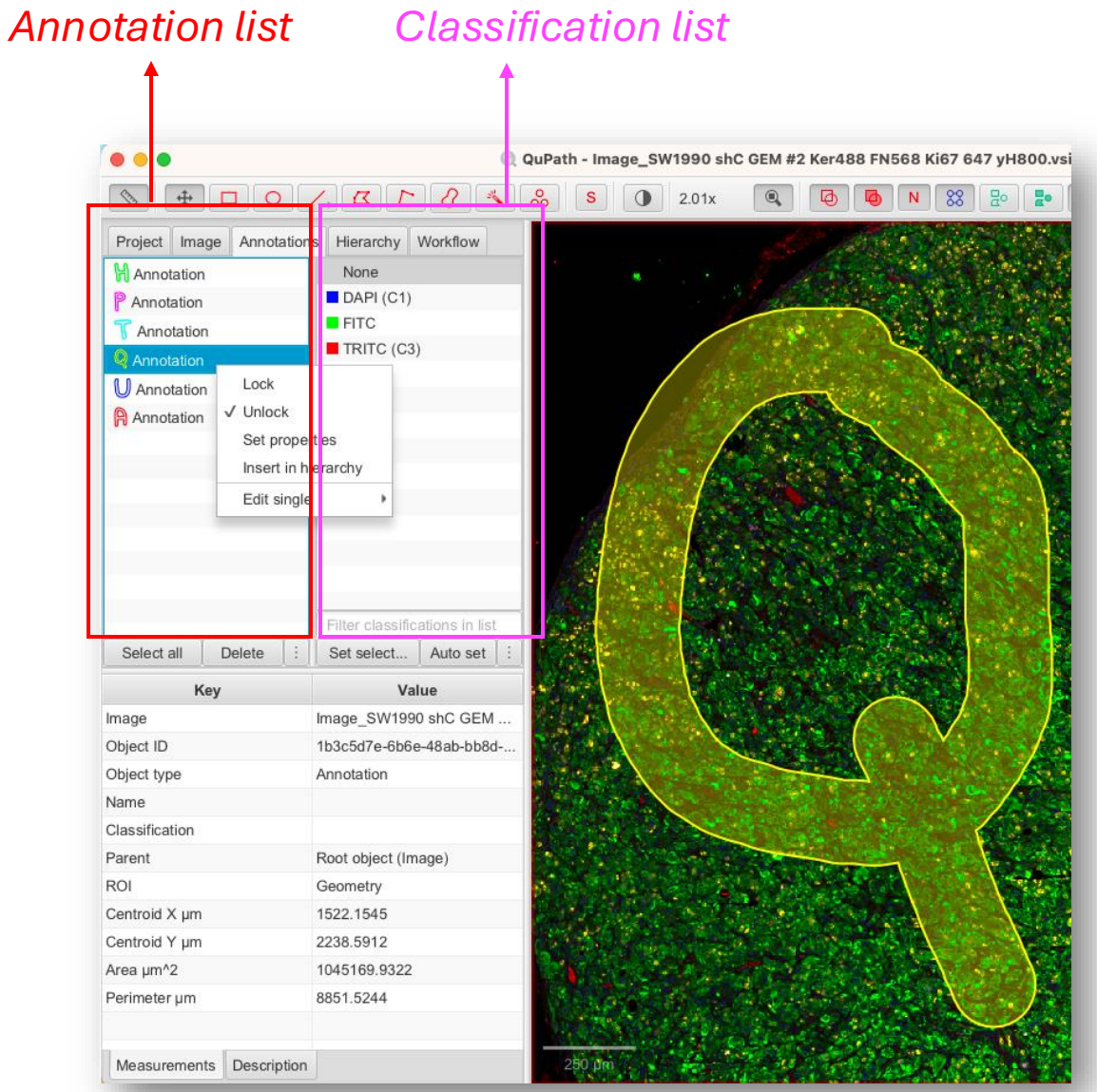
- **Objects** are a ‘thing’ in an image which encapsulates not only its shape but also some properties about it

  - **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

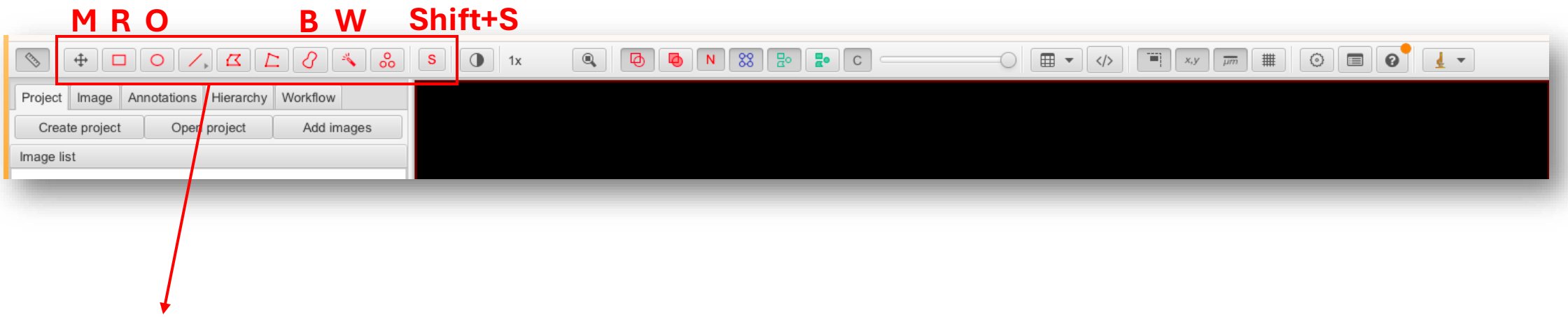
Analysis Panel

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



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!

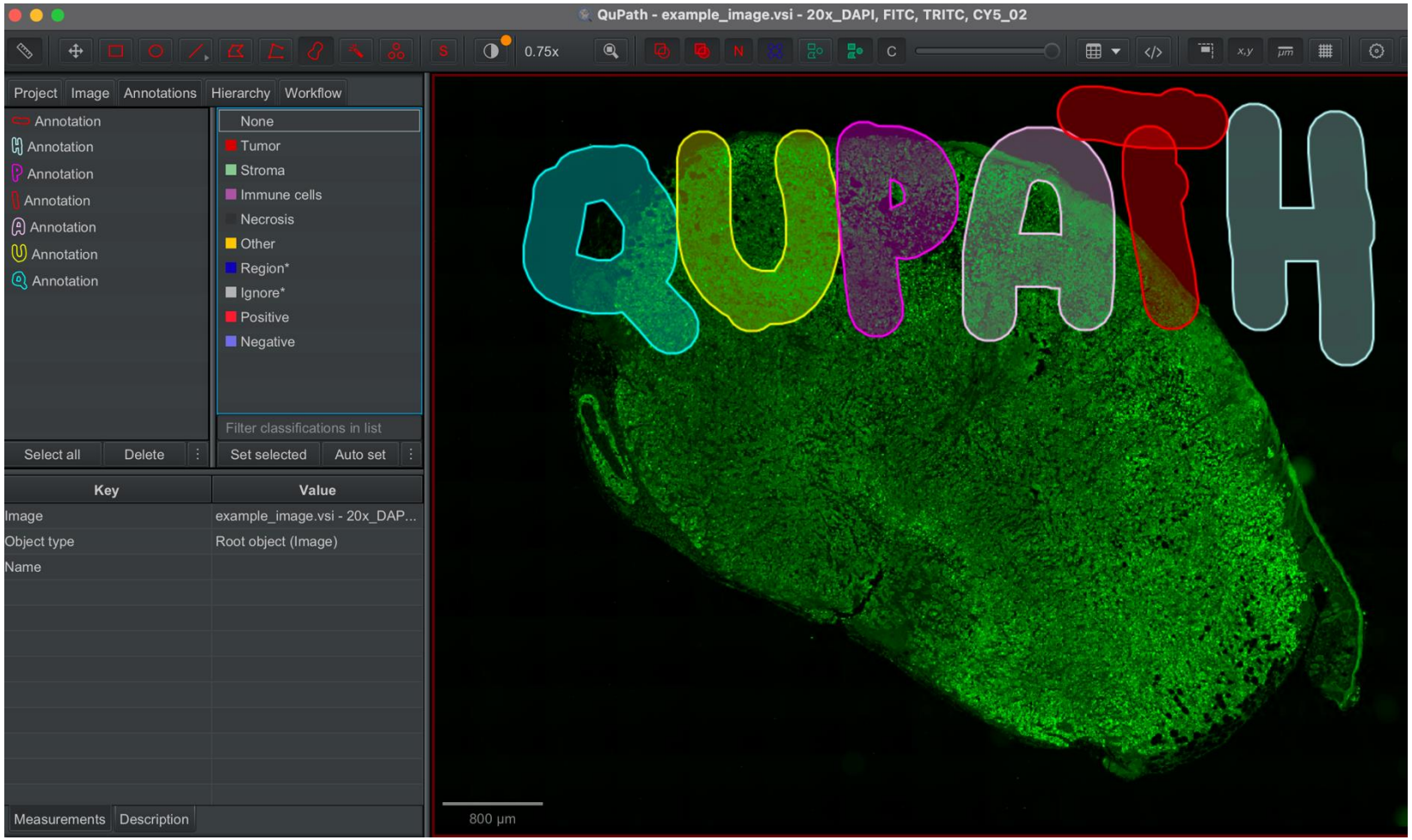
Remember to always lock your annotation to prevent accidental editing!

Practice time

Exercises 2: QuPath manual annotations

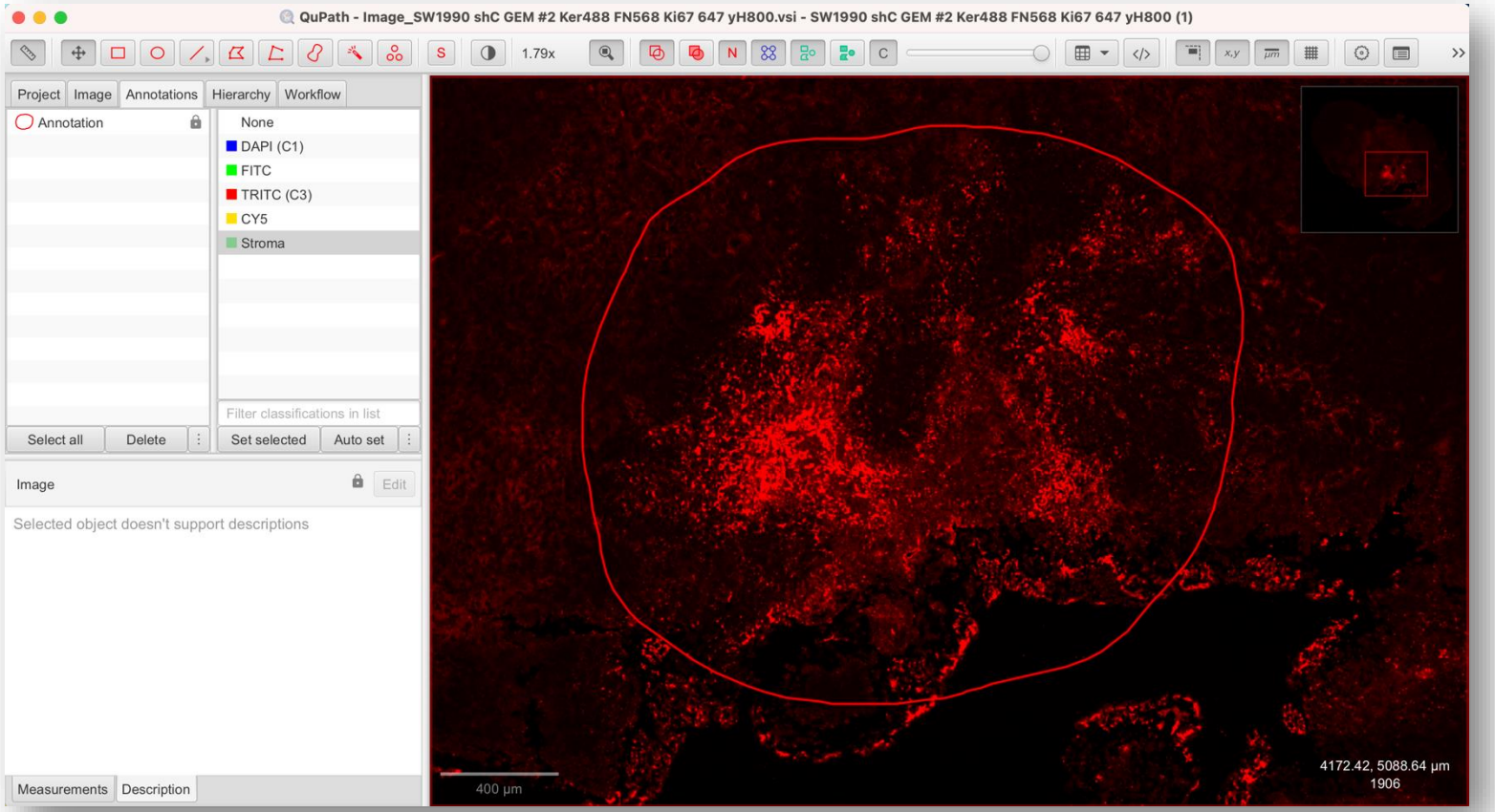
Recreate these annotations

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*