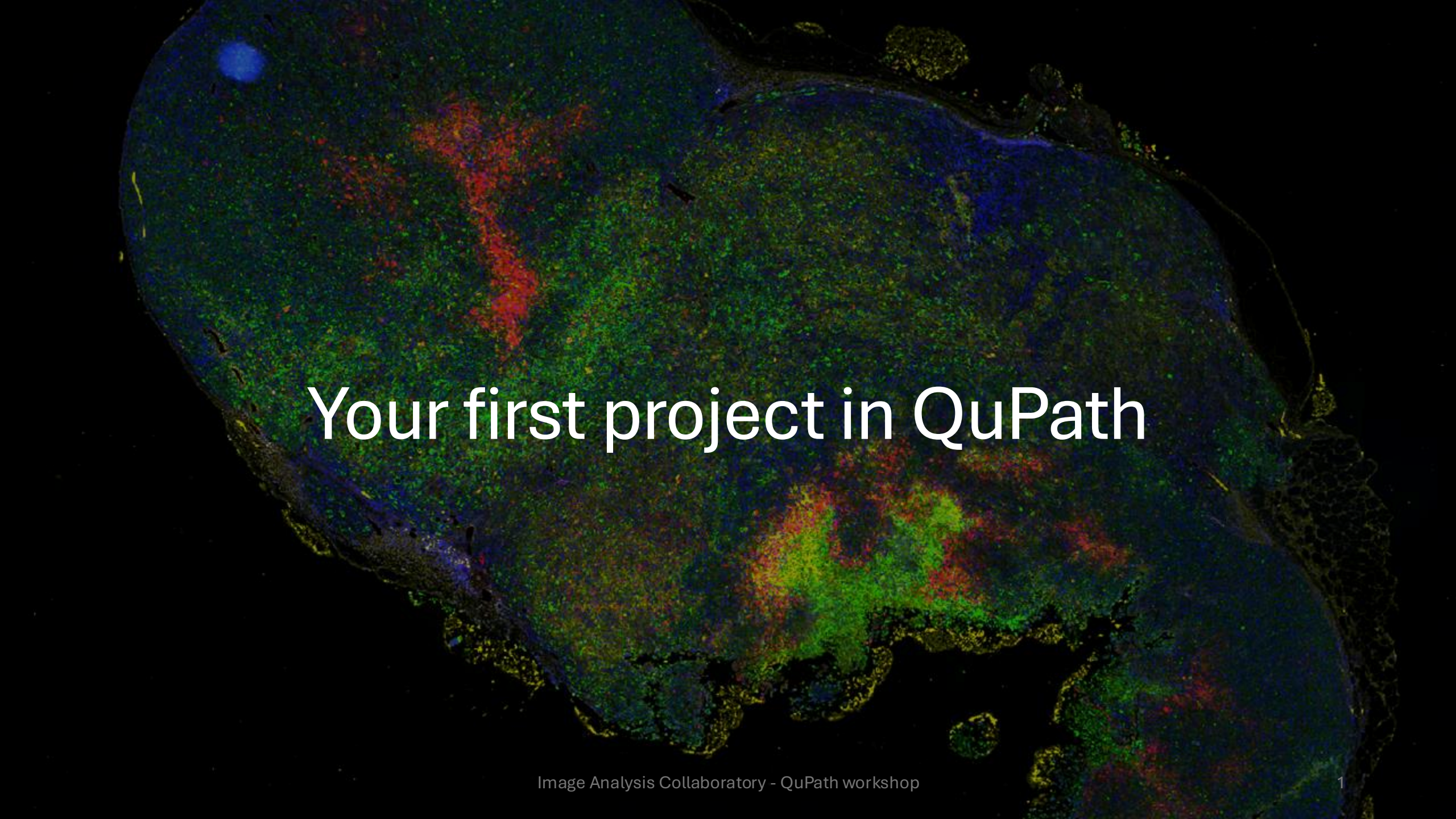
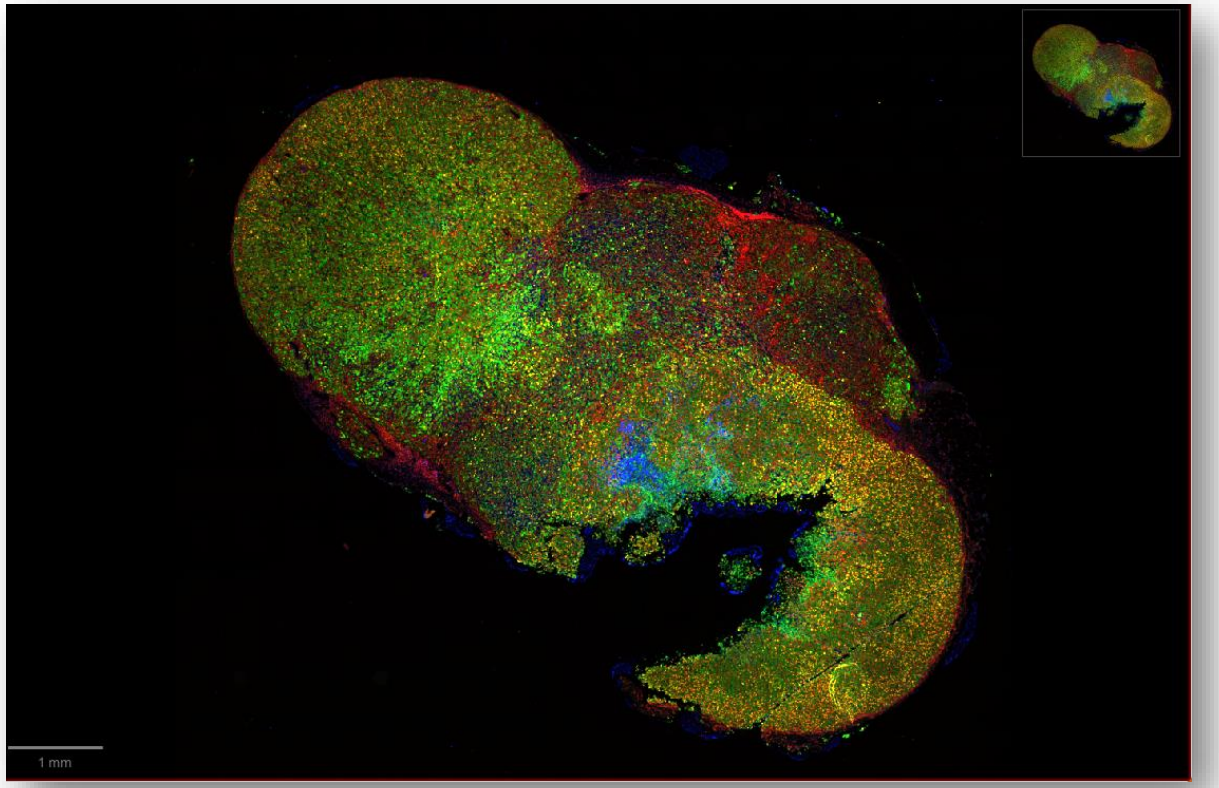


Your first project in QuPath

Classification of proliferating cancer cells in solid tumors

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

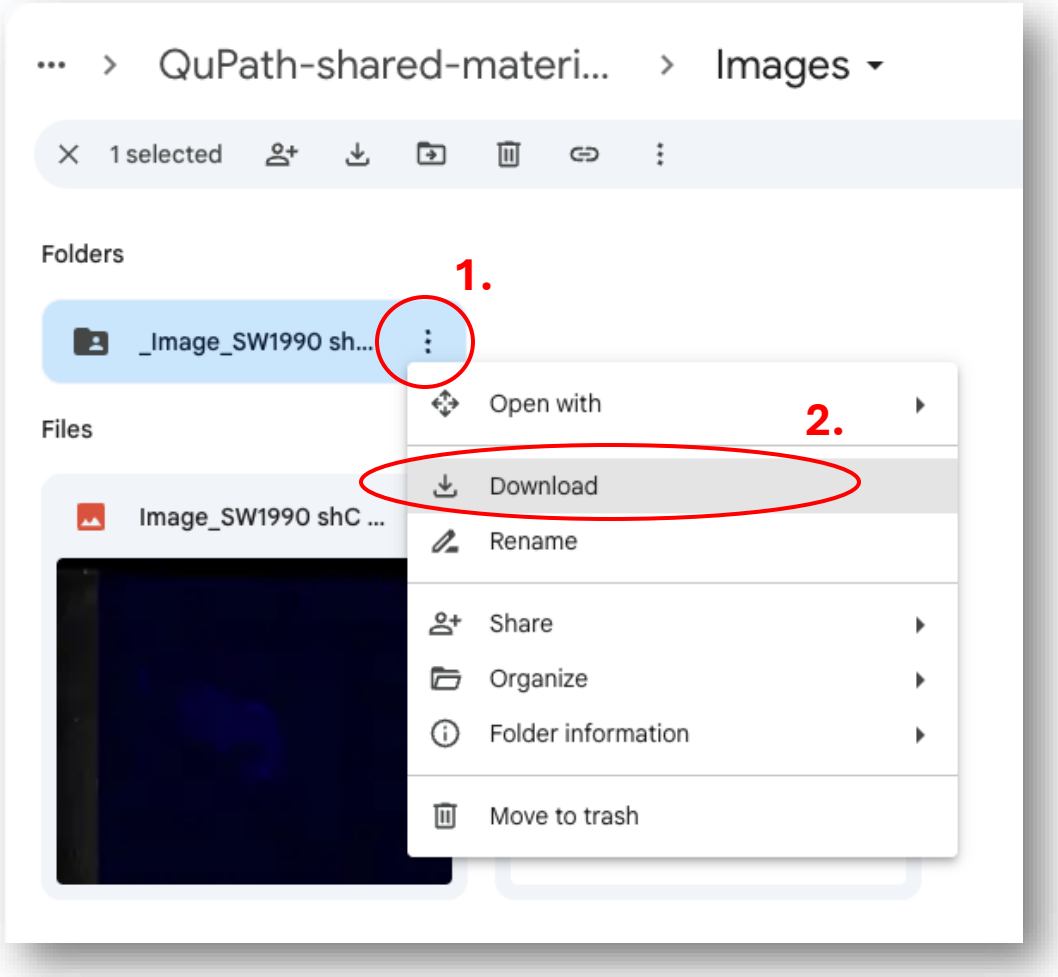
<u>At the end of this course:</u> you will have classified proliferating (Ki67) cancer cells and reveal their spatial distribution to regions with high-fibronectin content



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

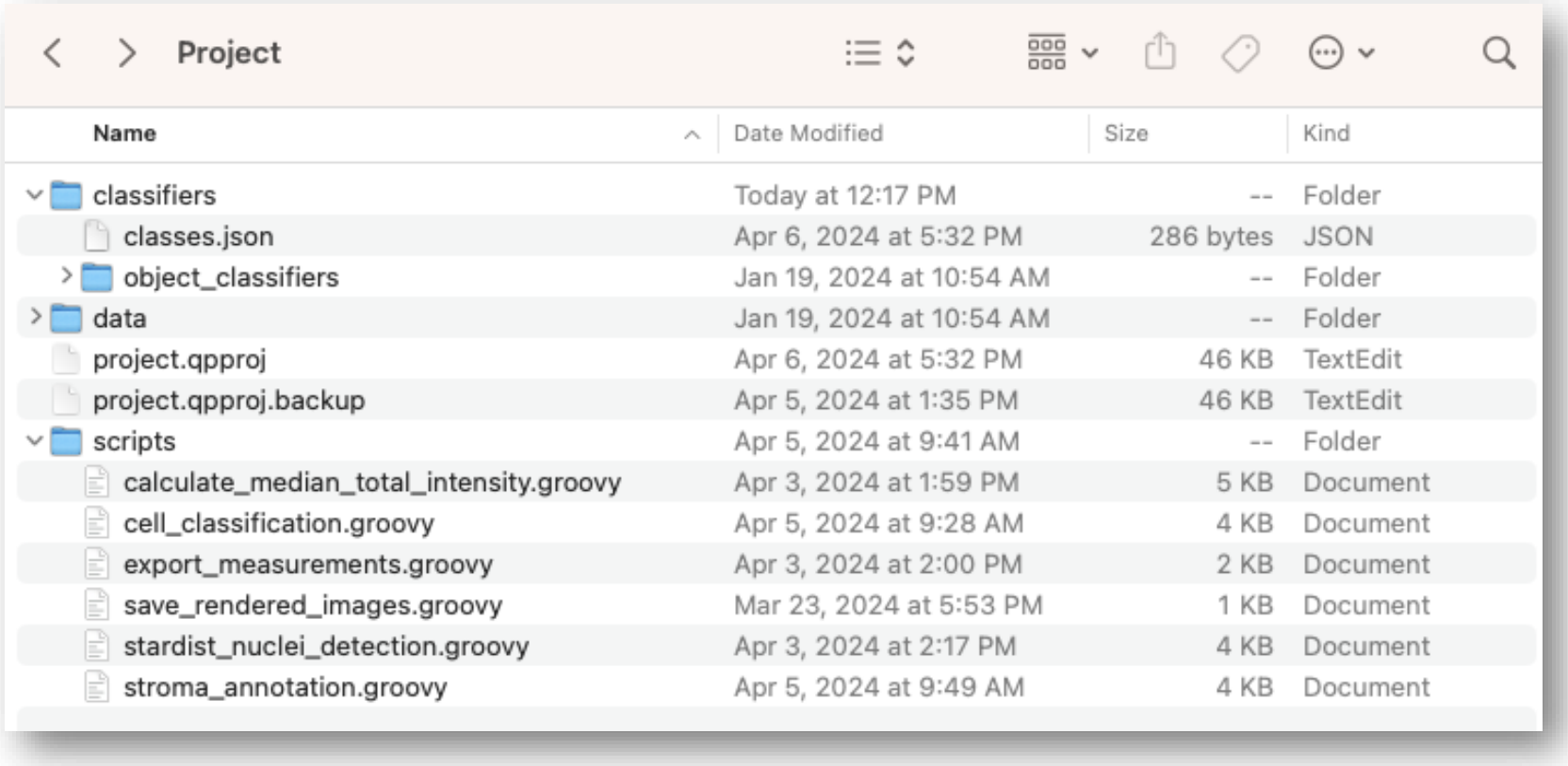
  or

- 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

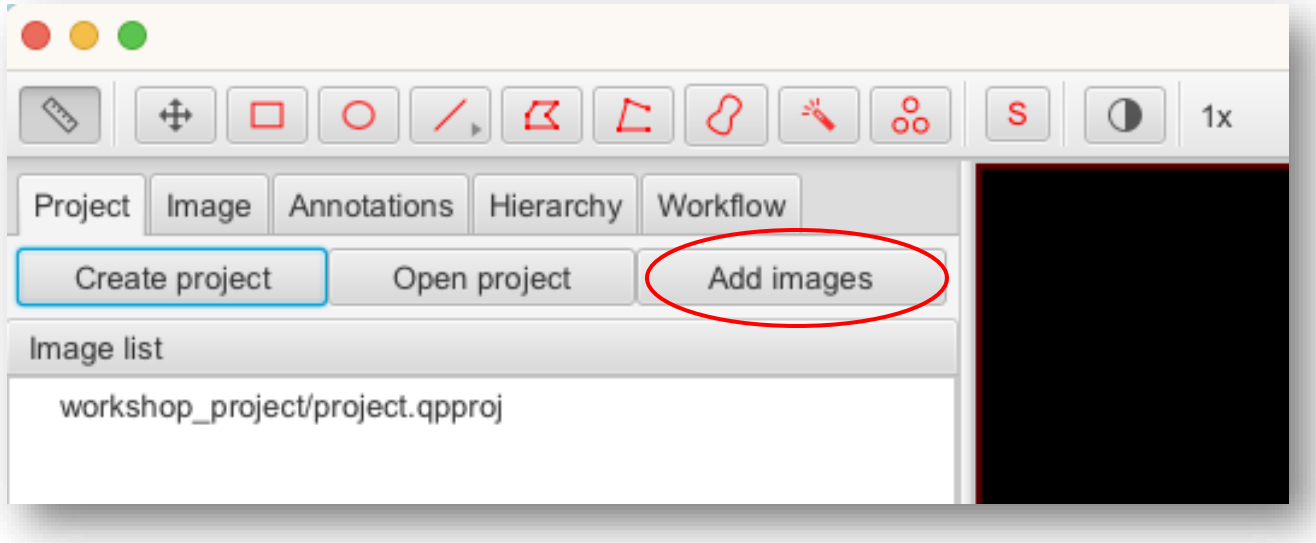
After a bit of time working on it…



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

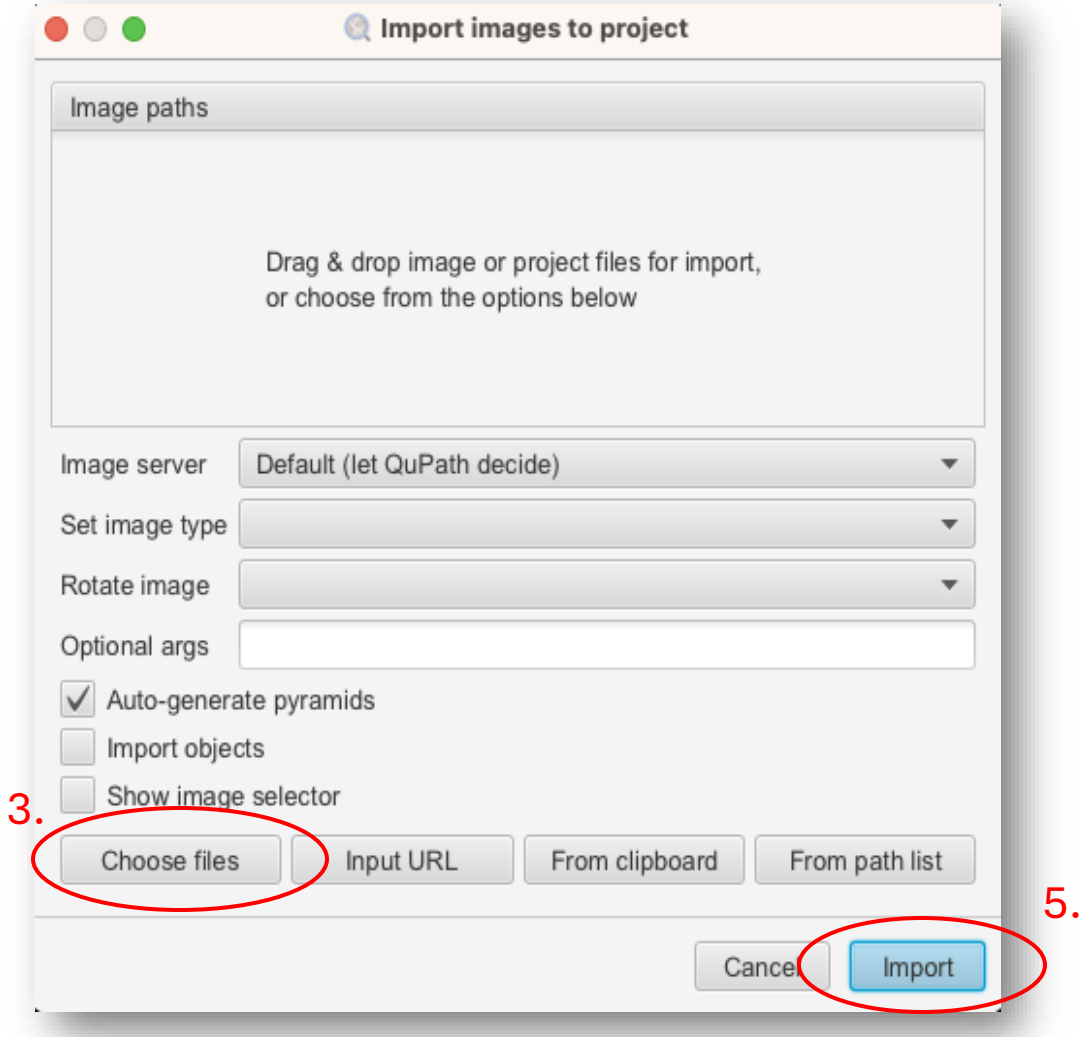


Add an image to your project

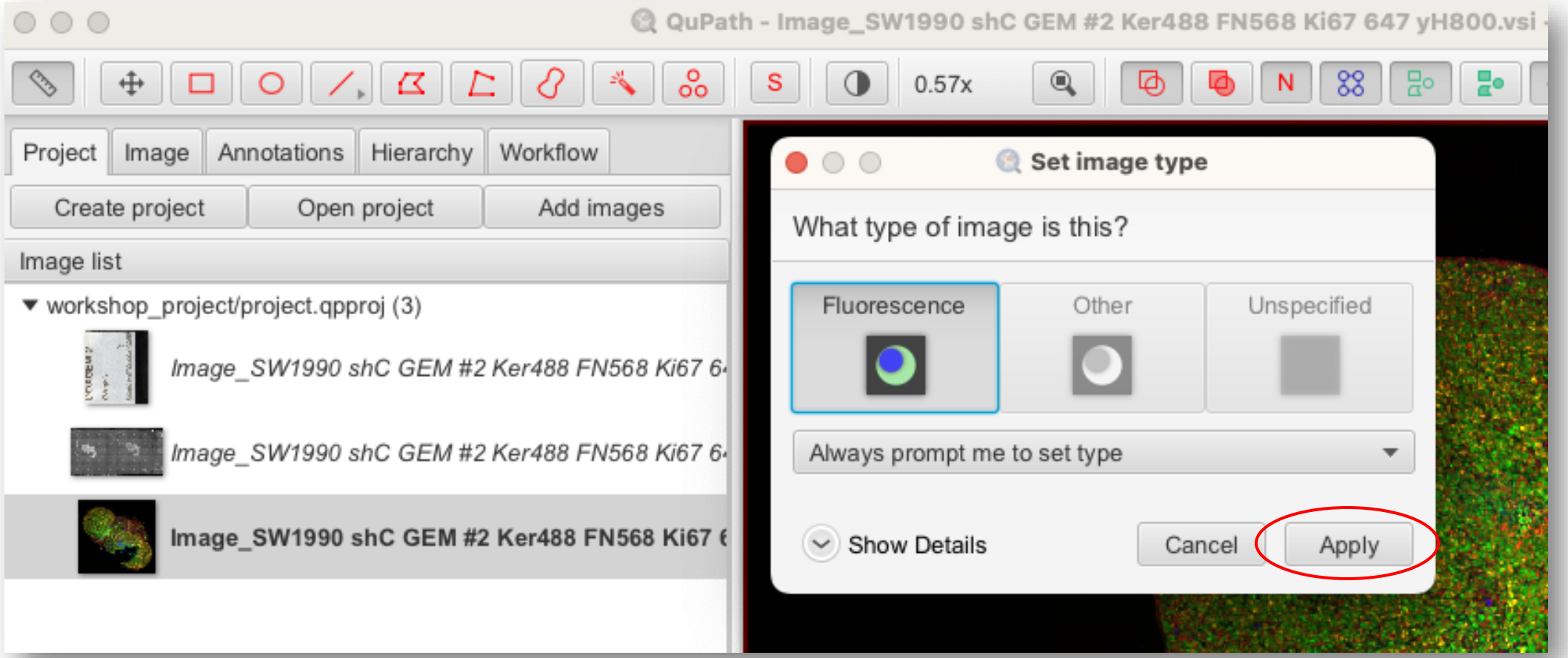
3. Select the .vsi image using *Choose files* or drag-and-drop

4. Use default settings

5. Click *import*



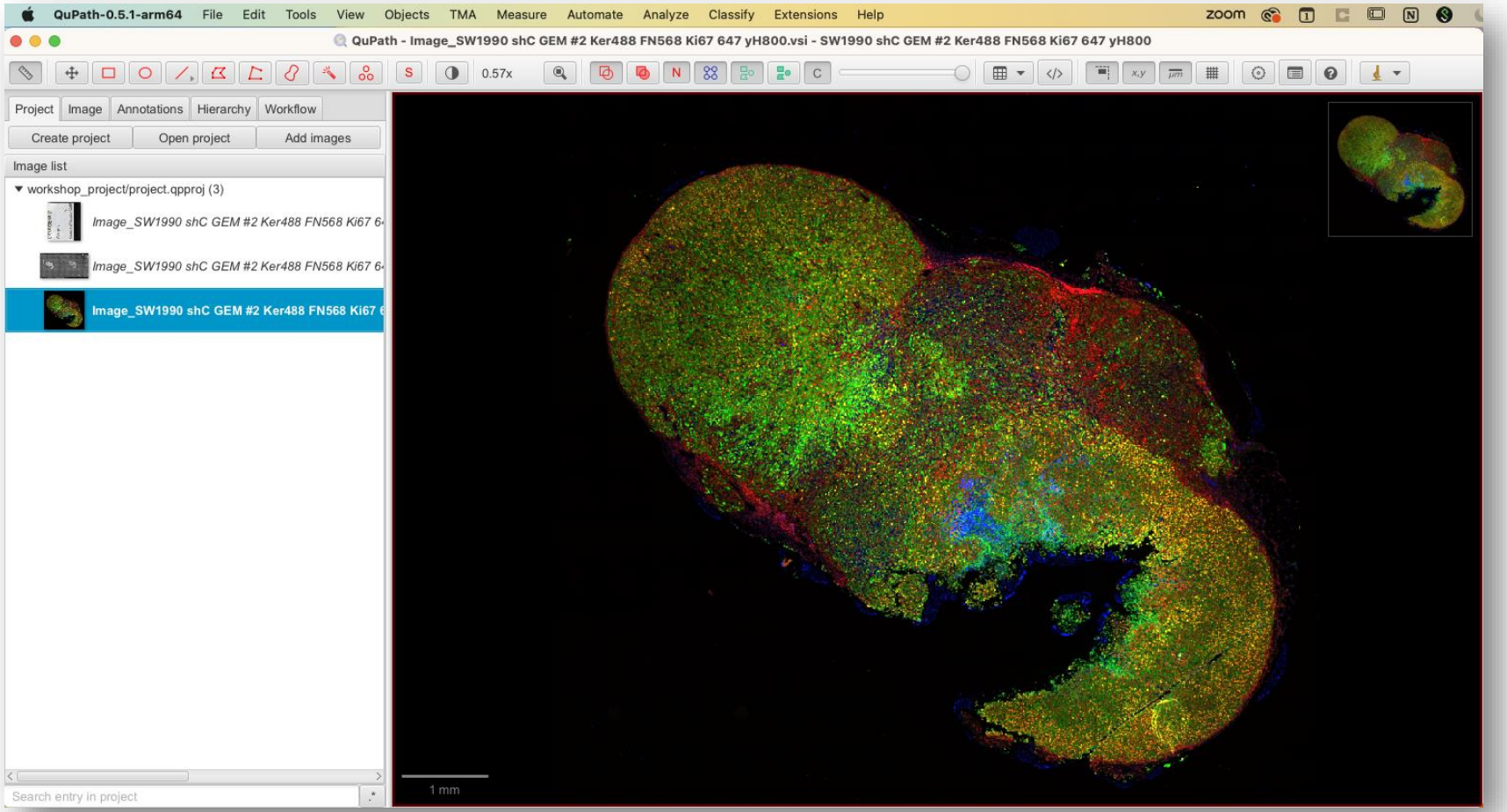
Set image type



- *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

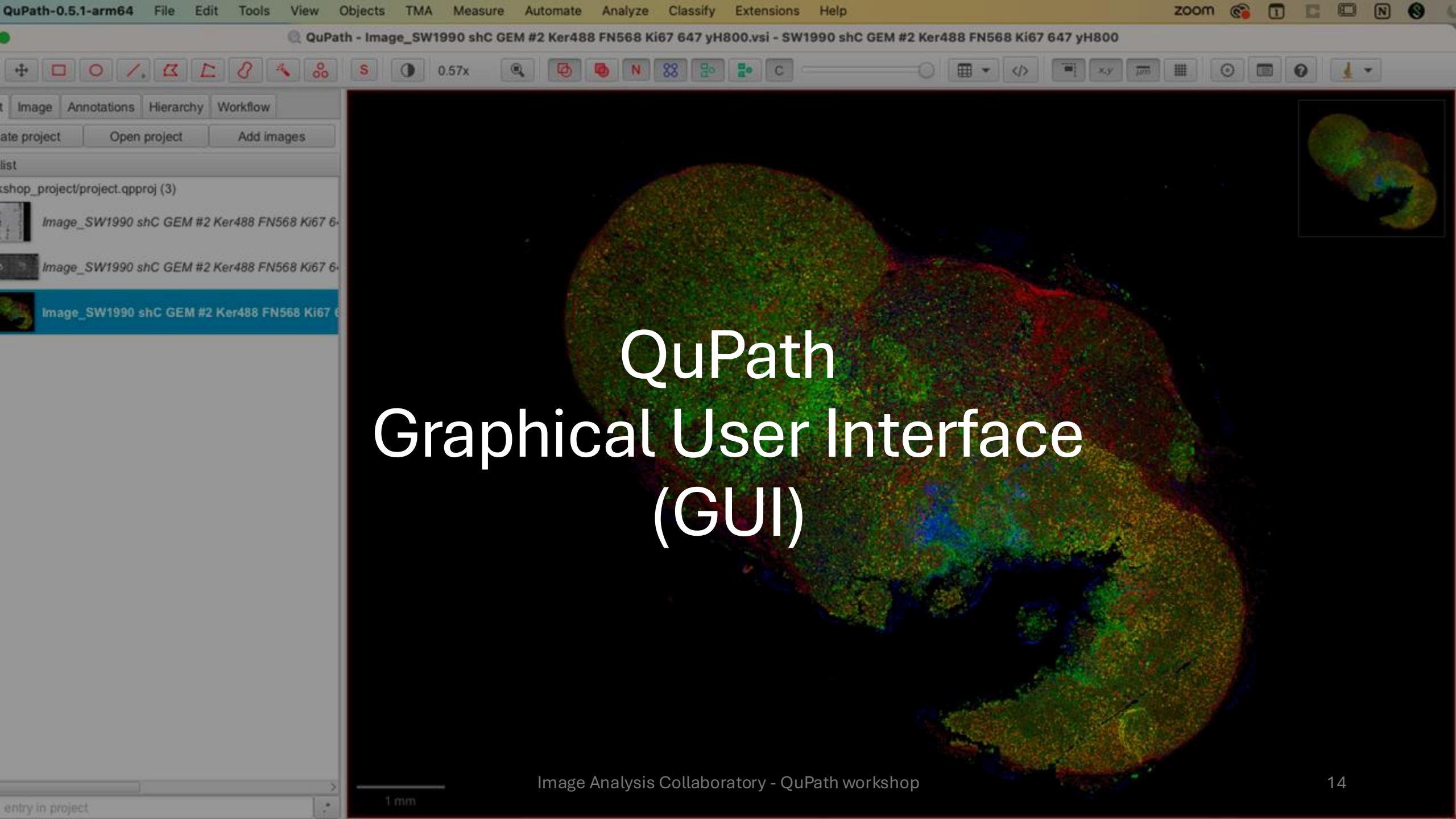
QuPath projects are portable

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

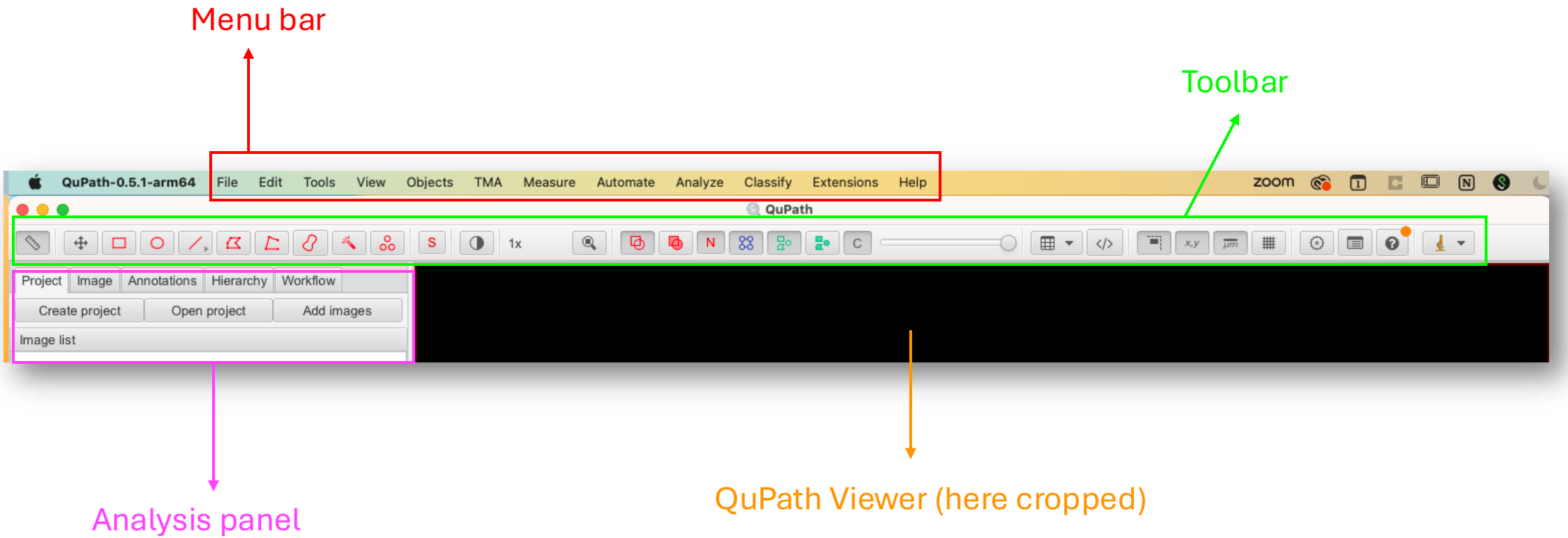
The project folder only contains QuPath objects and data, unless you had placed them there. Ensure that they can access the actual image files.

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

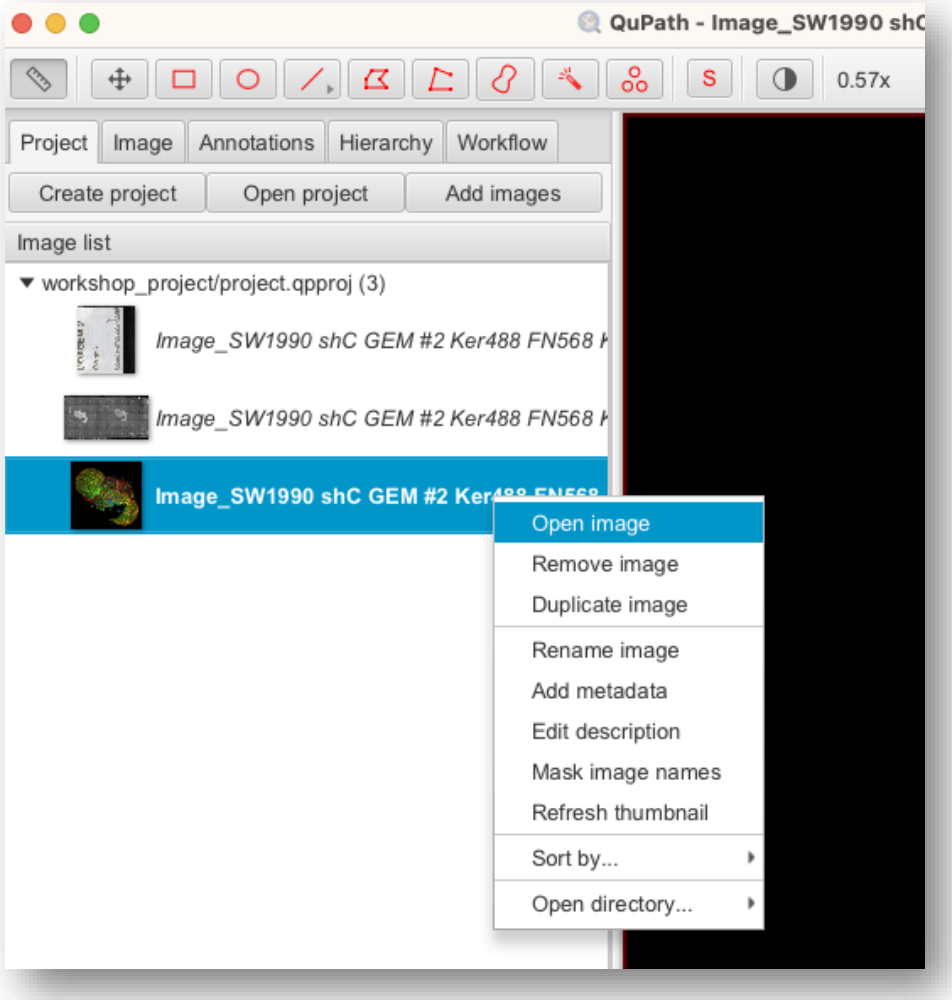

QuPath
Graphical User Interface
(GUI)

Graphic User Interface (GUI)



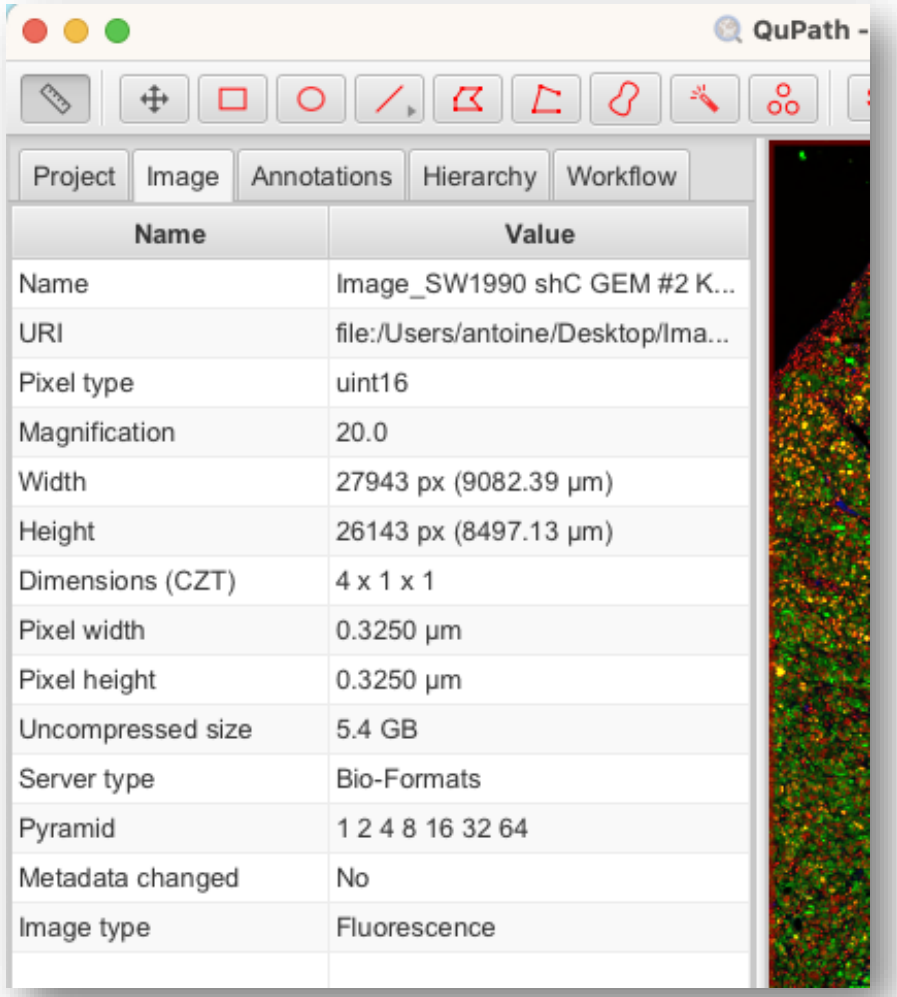
Analysis Panel

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

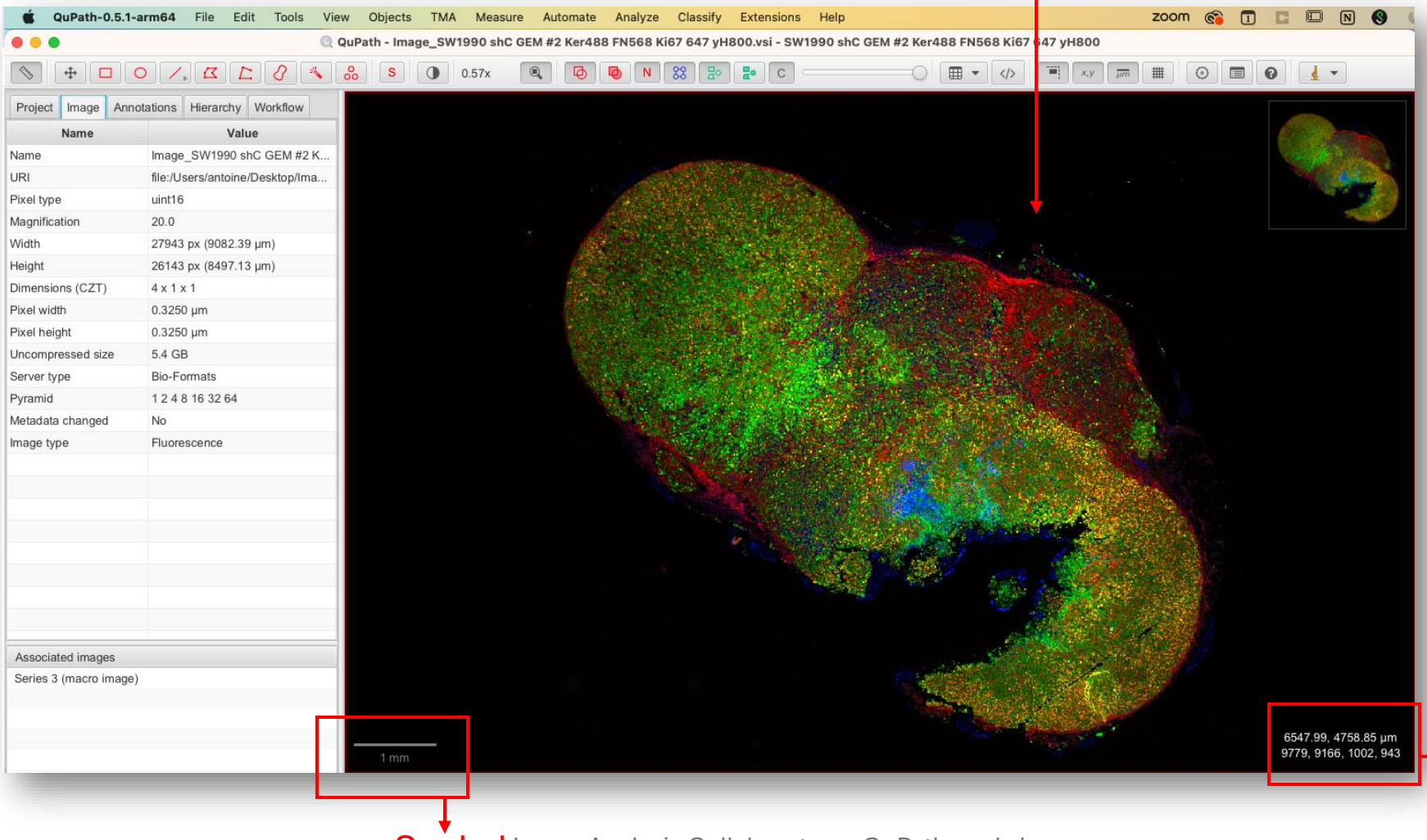


Analysis Panel

- *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

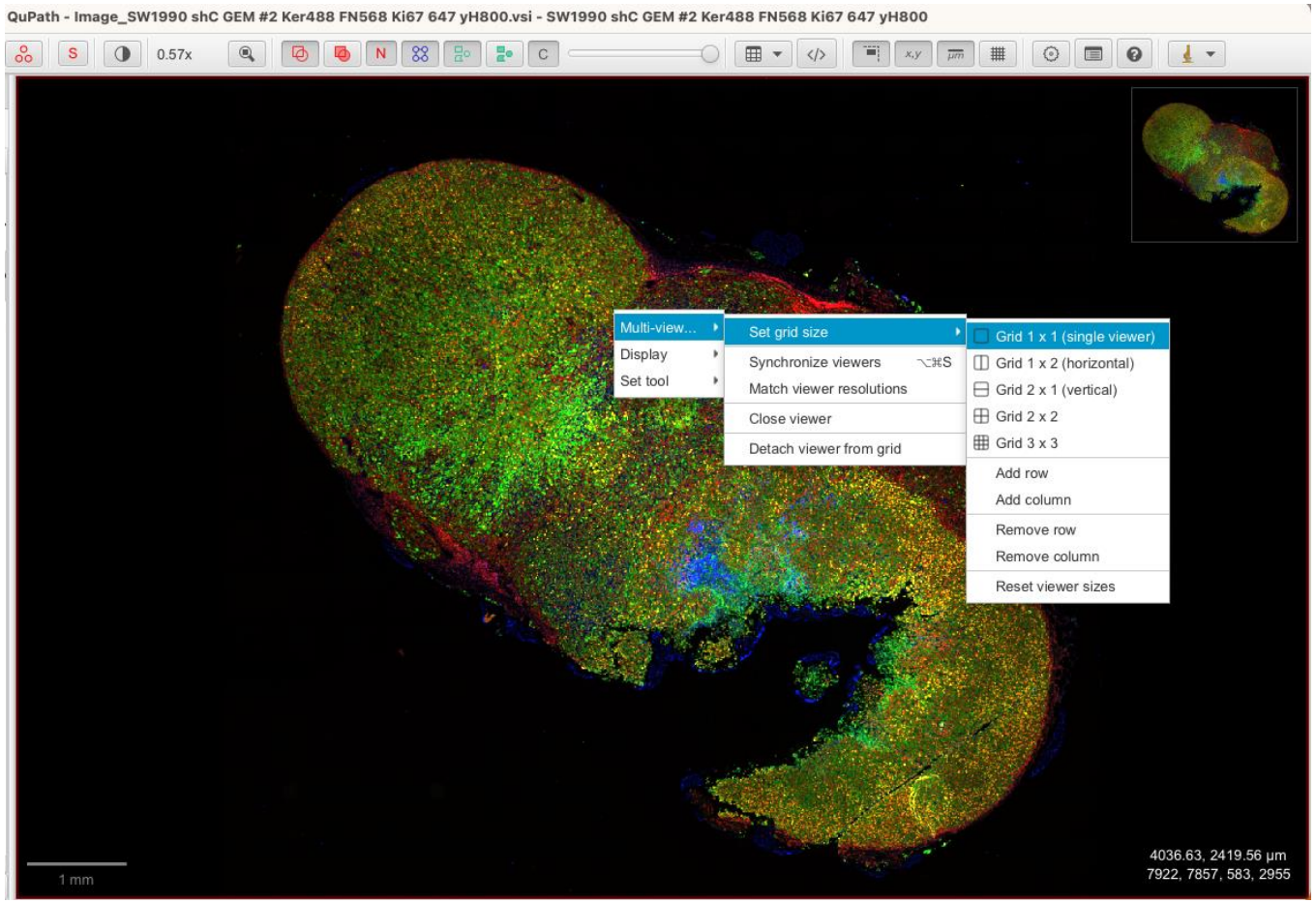


QuPath viewer

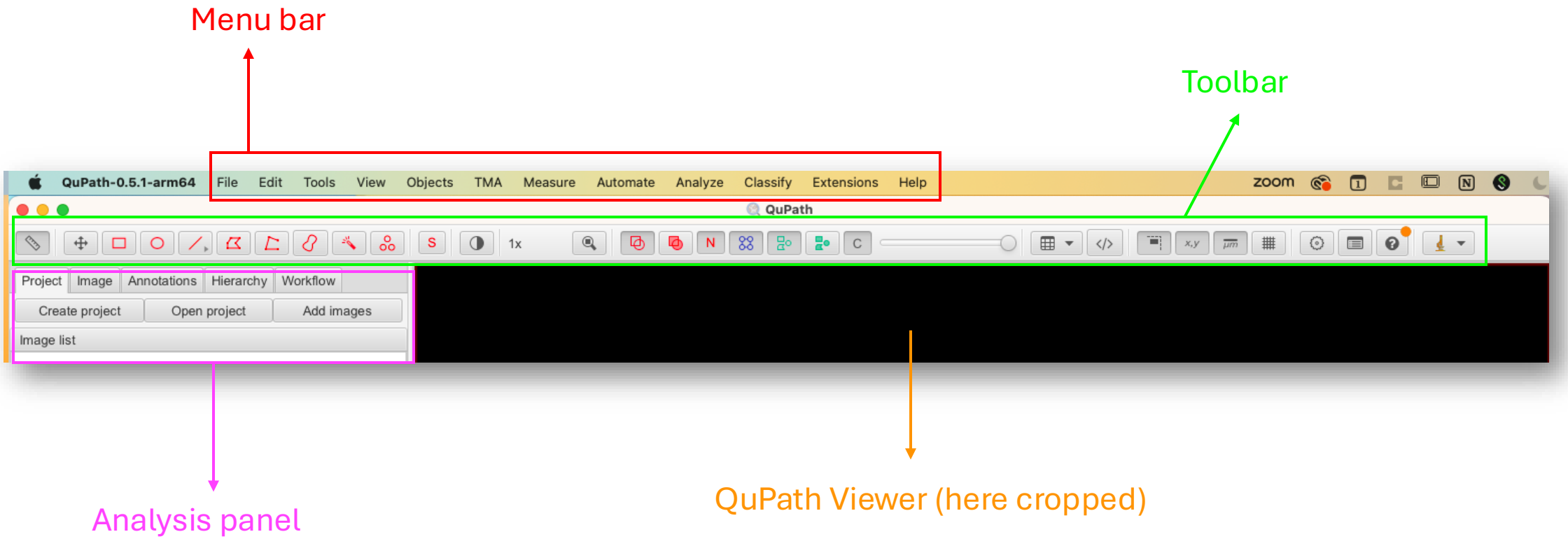


Multi-viewer

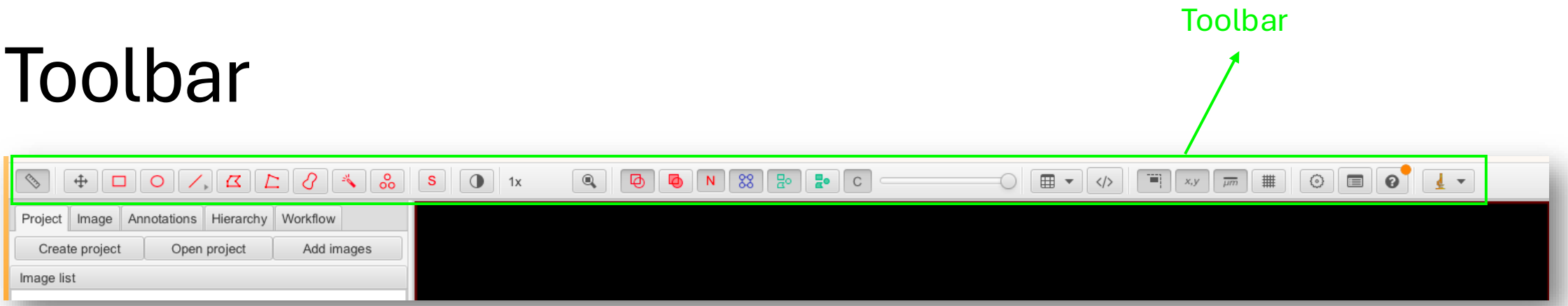
- Right-click in the viewer



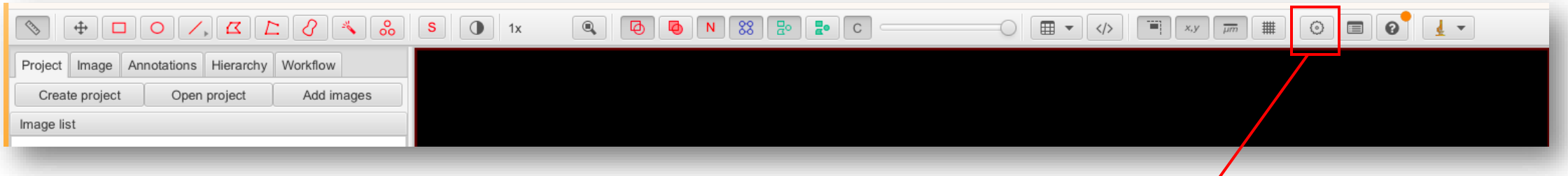
Graphic User Interface (GUI)



Toolbar

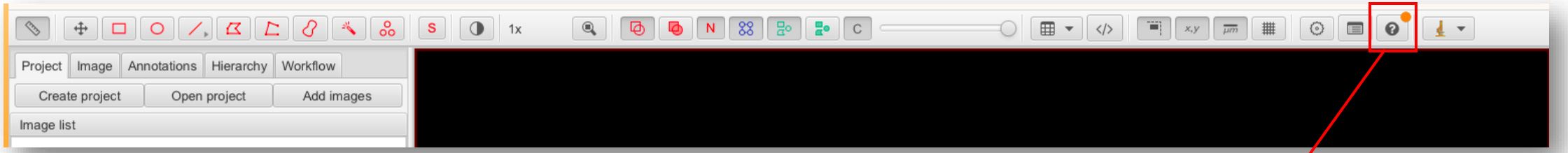

Toolbar
S
1x
N
C
x,y
Project
Image
Annotations
Hierarchy
Workflow
Create project
Open project
Add images
Image list

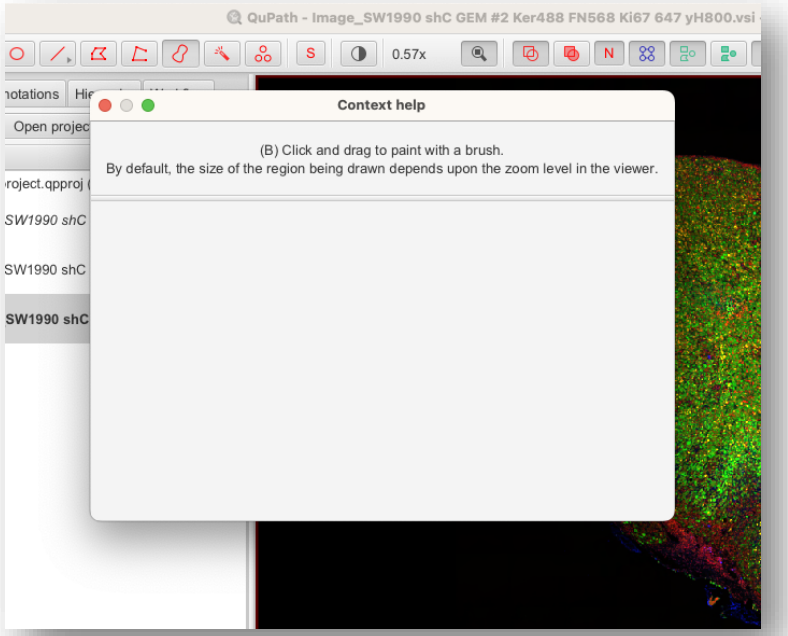
Toolbar



Preferences
Settings, GUI customization, extensions, …

Toolbar





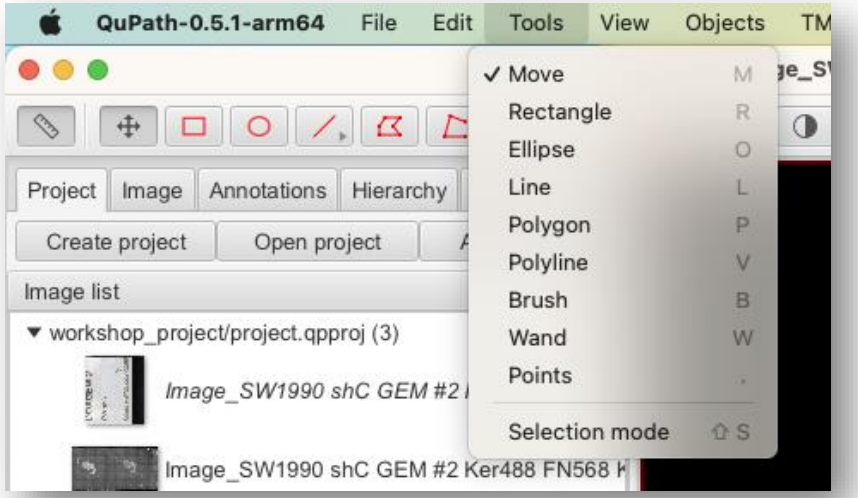
Example when my cursor is on the paint brush tool

Interactive Help
Provides contextual help based on your cursor location

Toolbar

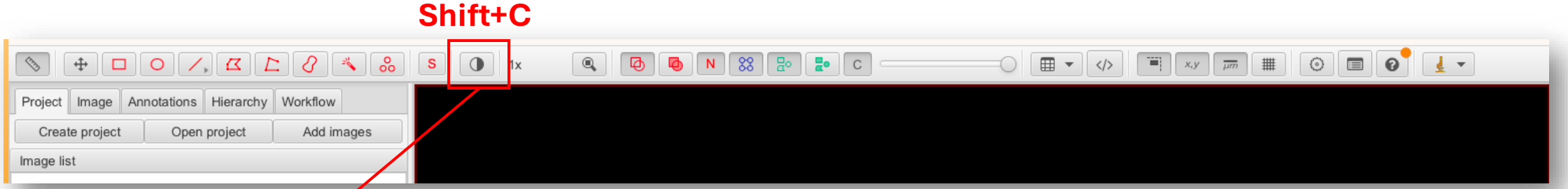
Annotation tools

- **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!



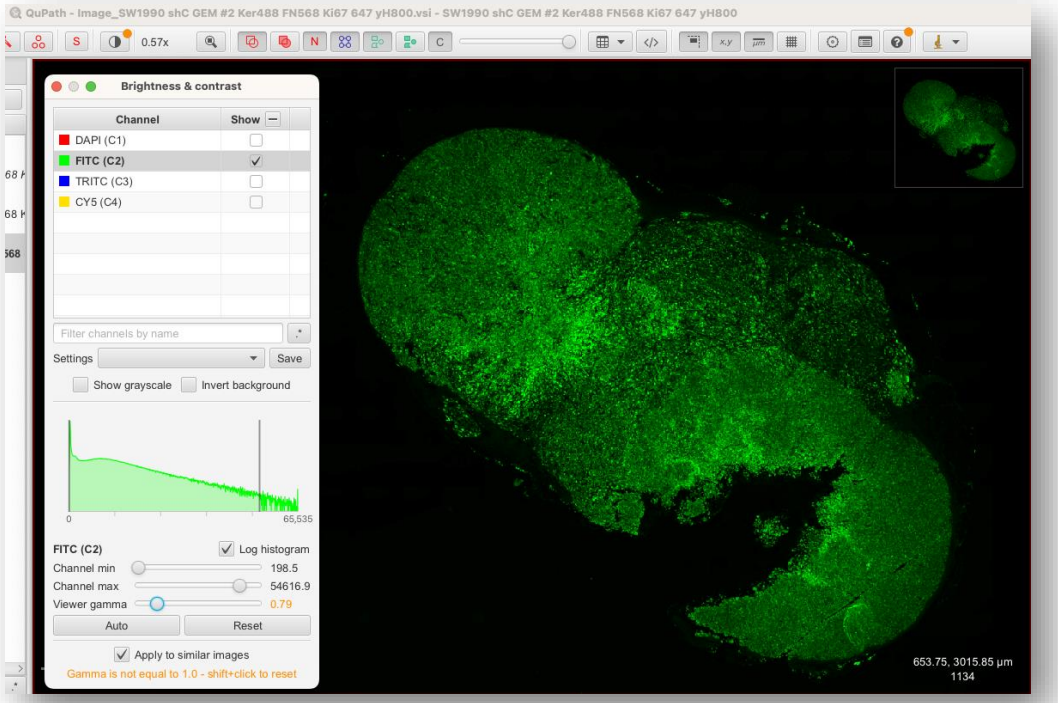
Annotation tools are also accessible in the *Tools* menu

Toolbar



Brightness and contrast

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

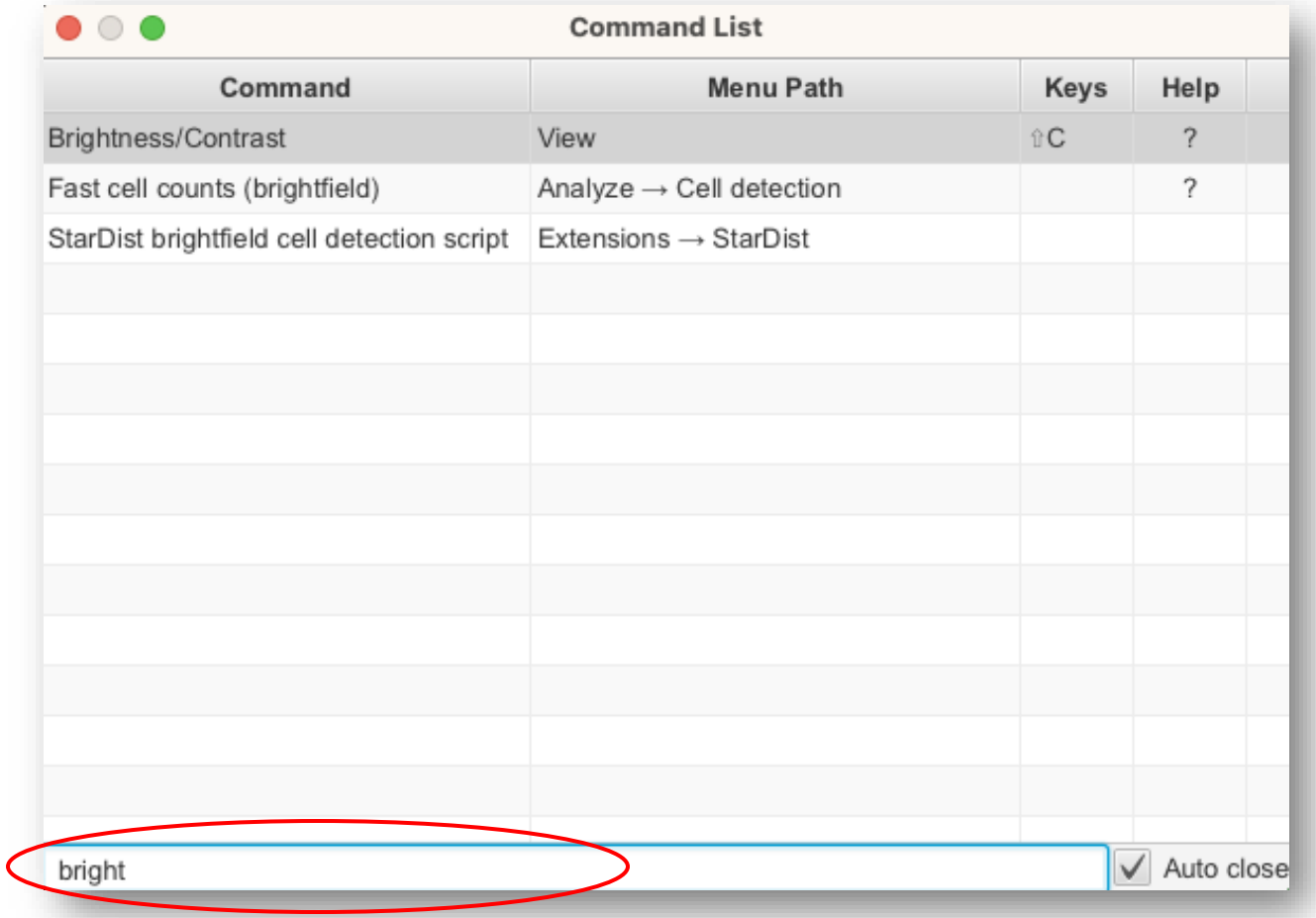


Example for FITC (Keratin) channel

QuPath pro-tip: command list

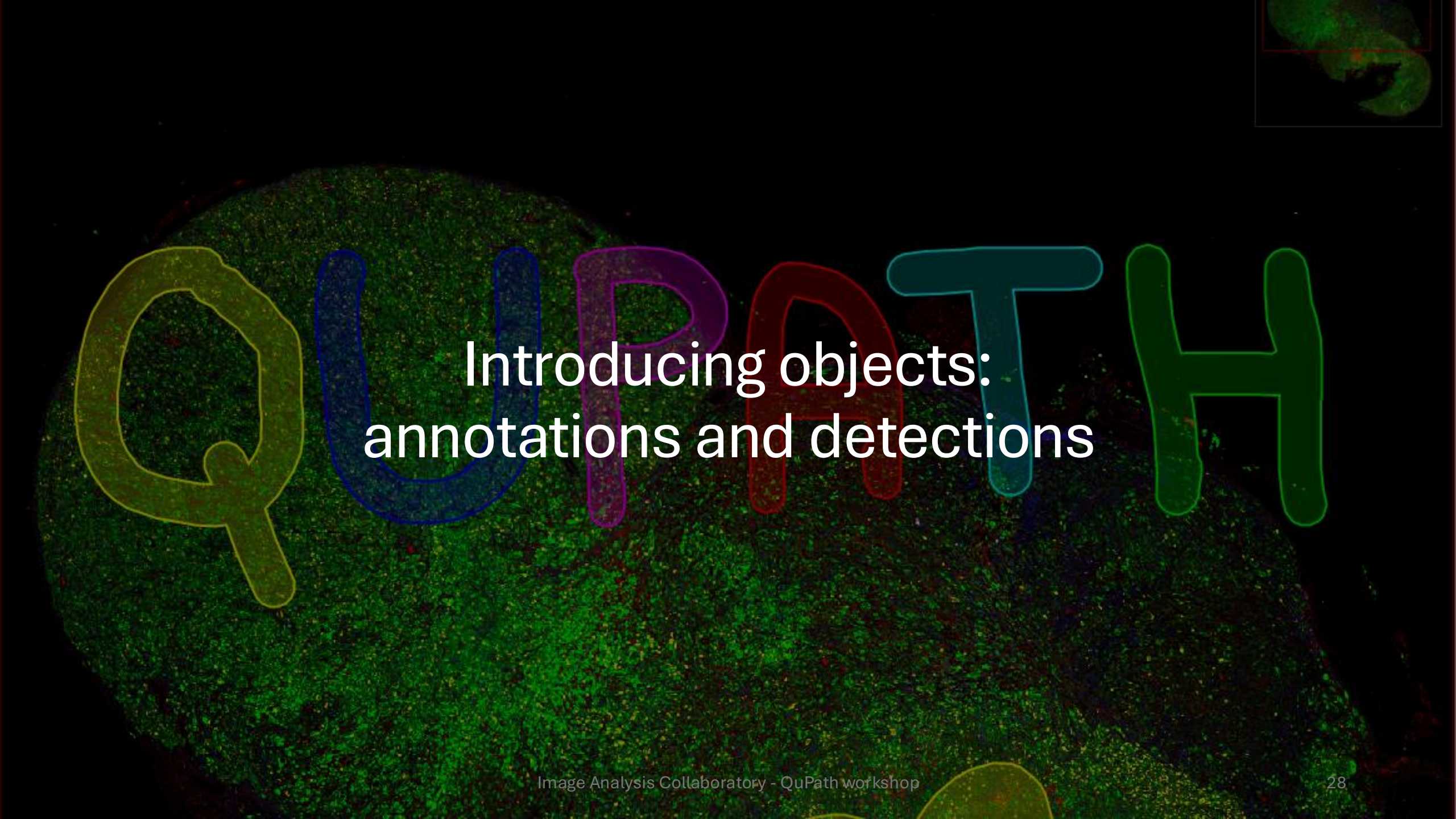
Command/Control + L
Opens a dialogue to search for any command using keyword

For example, search for 'brightness'



Practice time

Exercises 1: QuPath projects and GUI



Introducing objects: annotations and detections

Key concept: QuPath objects

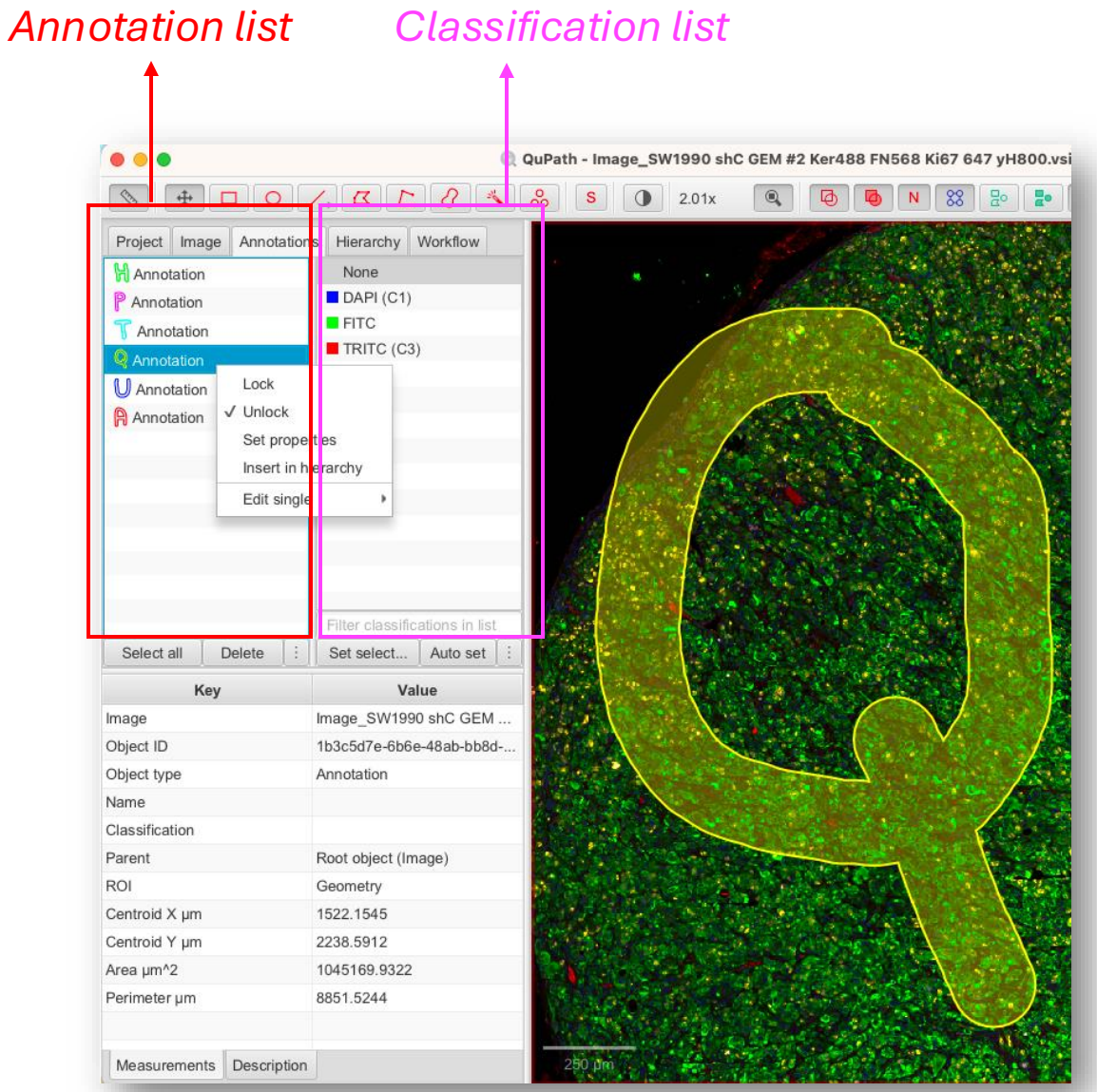
- **Objects** are a ‘thing’ in an image which encapsulates not only its shape but also some properties about it

  - **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

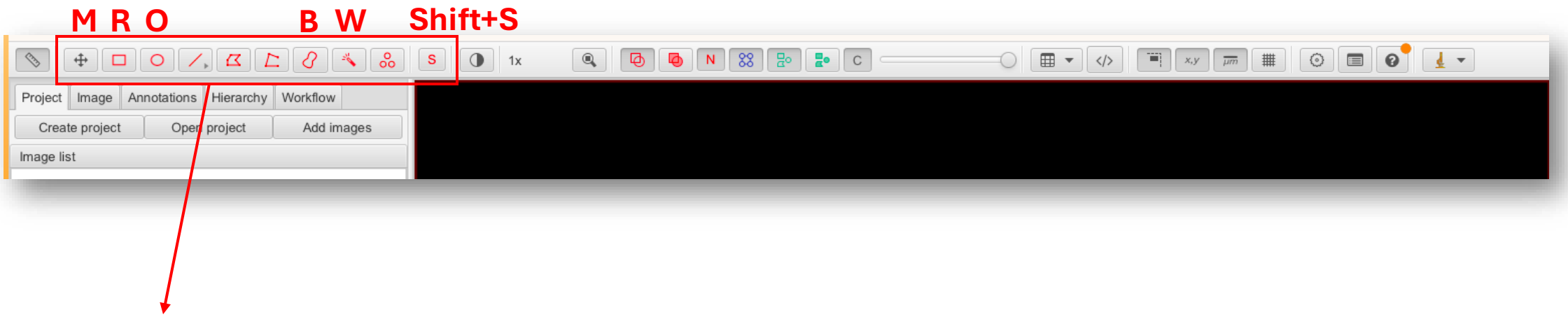
Analysis Panel

- *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!

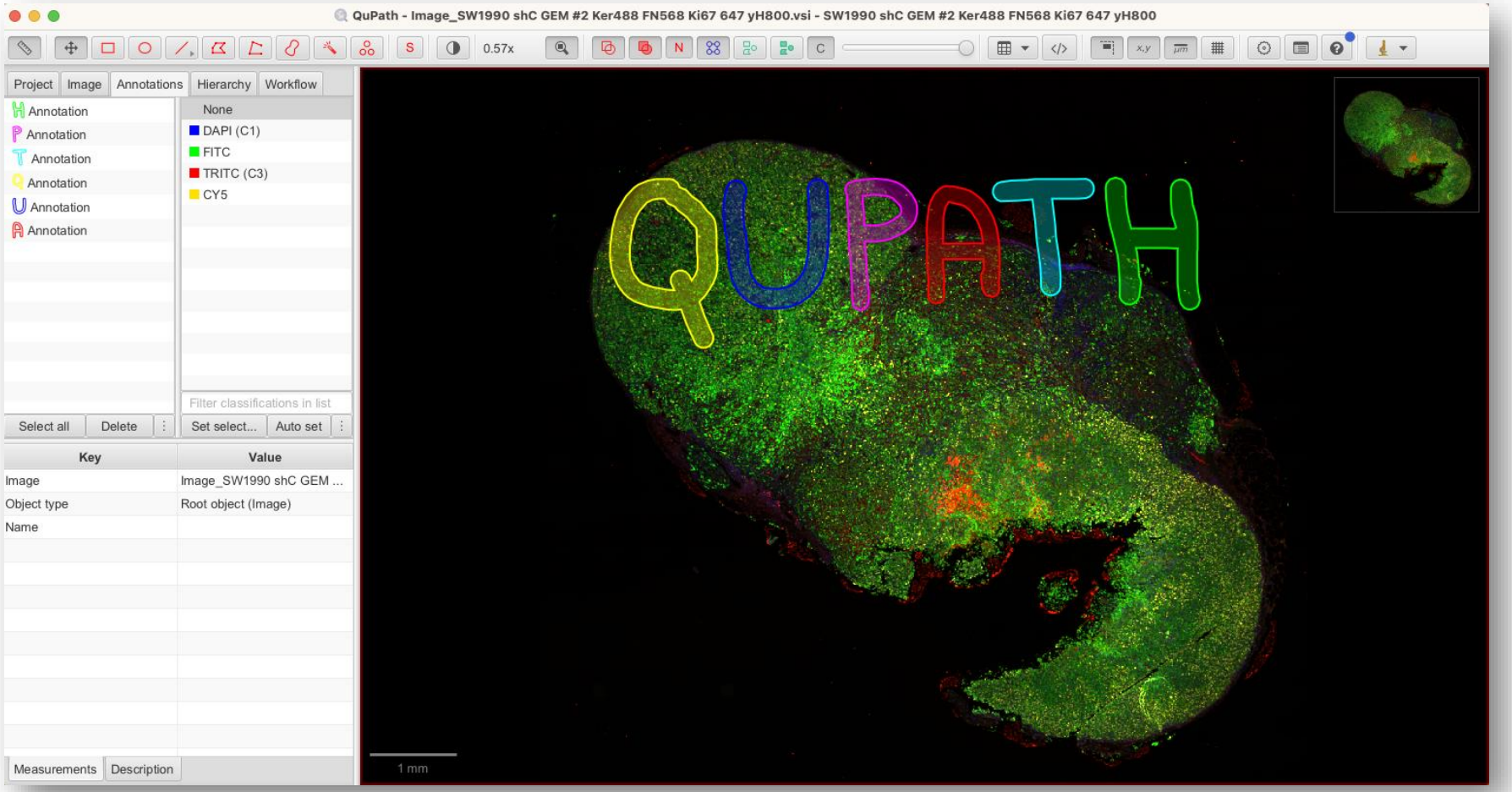
Remember to always lock your annotation to prevent accidental editing!

Practice time

Exercises 2: QuPath manual annotations

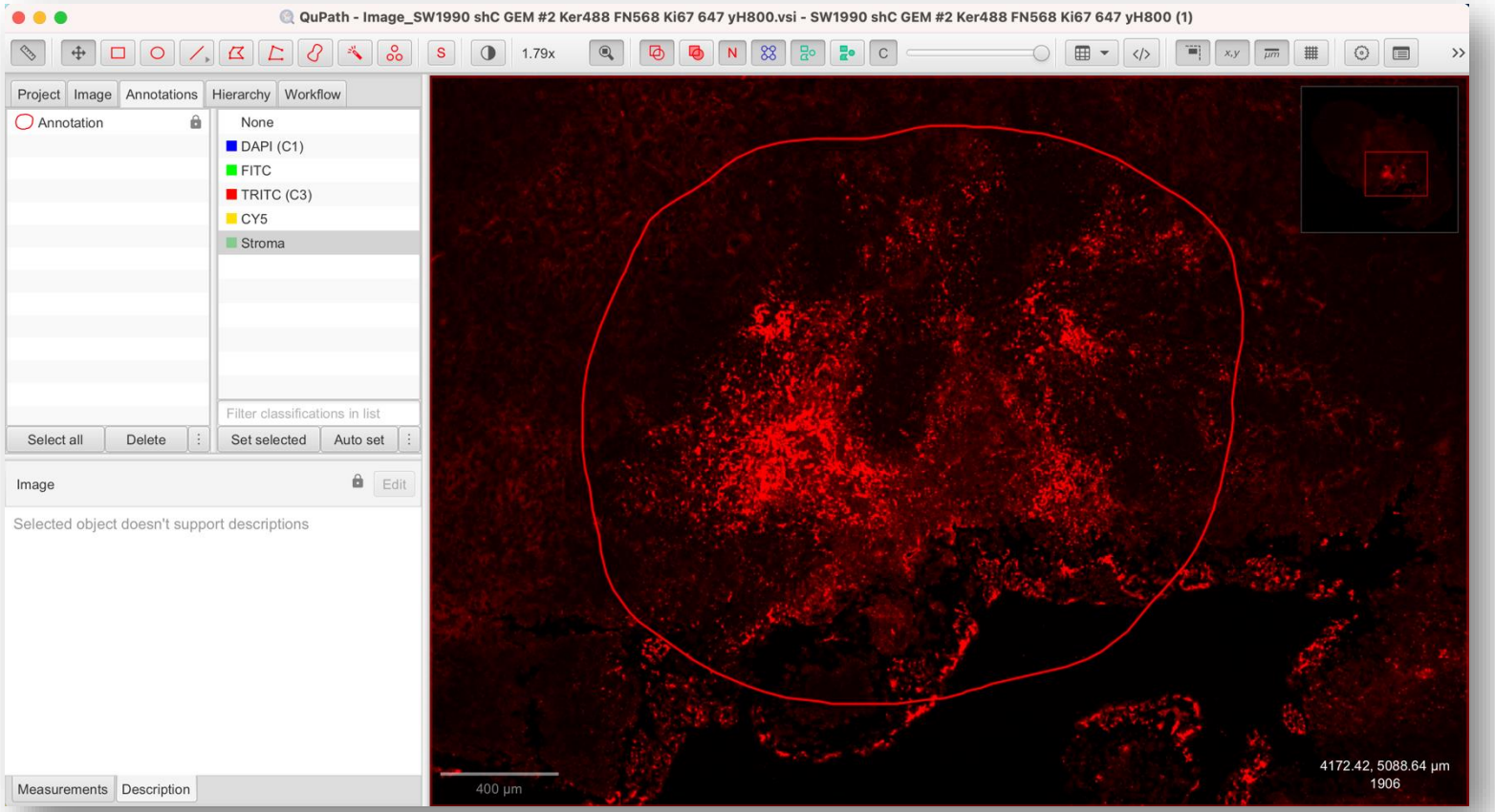
Recreate these annotations

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*