Illustration reprinted from Pete Bankhead.

Introduction to Bioimage Analysis using QuPath

Antoine A. Ruzette & Simon F. Nørrelykke

Image Analysis Collaboratory

Image Analysis Collaboratory

Get the course materials

https://hms-iac.github.io/qupath-workshop

One-stop resource for everything we'll cover today

Let’s start the download an example image

1. Browse to the workshop website >
2. Download the .vsi whole-slide image
3. Once done, unzip it
4. Save it
4. Right-click on the installer file > Open > Confirm Open

Workshop plan

1. Introduction to digital image analysis
2. Installing QuPath and your first project
3. GUI layout and toolbars
4. Introducing objects: annotations and detections
5. Saving, sharing and receiving QuPath projects
6. Nuclei detection and measurements (incl. StarDist)
7. Cell classification
8. Automating tissue annotations (pixel classifier)
9. Advance topic: scripting and workflows

Acknowledgments

- **Pete Bankhead** et al.
  - QuPath and its amazing documentation
- **Peter Sobolewski**
  - *Introduction to QuPath* workshop at the The Jackson Laboratory
- **Nina Kozlova**
  - Whole-slide image used in this workshop

Self-introductions

1. My **name** is *Antoine*
2. My **position** is as an *Associate in Systems Biology*
3. My **lab** is *the Image Analysis Collaboratory and the Megason Lab*
4. I have *confocal microscopy* **images** of *cancer tissues, embryos, …*
5. A **fun fact** about me is *I used to be a brewer*

Self-introductions

1. **Motivate** the use of algorithms in image analysis
2. **Introduce** some image-analysis nomenclature
3. **Learn** to use QuPath effectively and reproducibly

Reasons to learn image processing

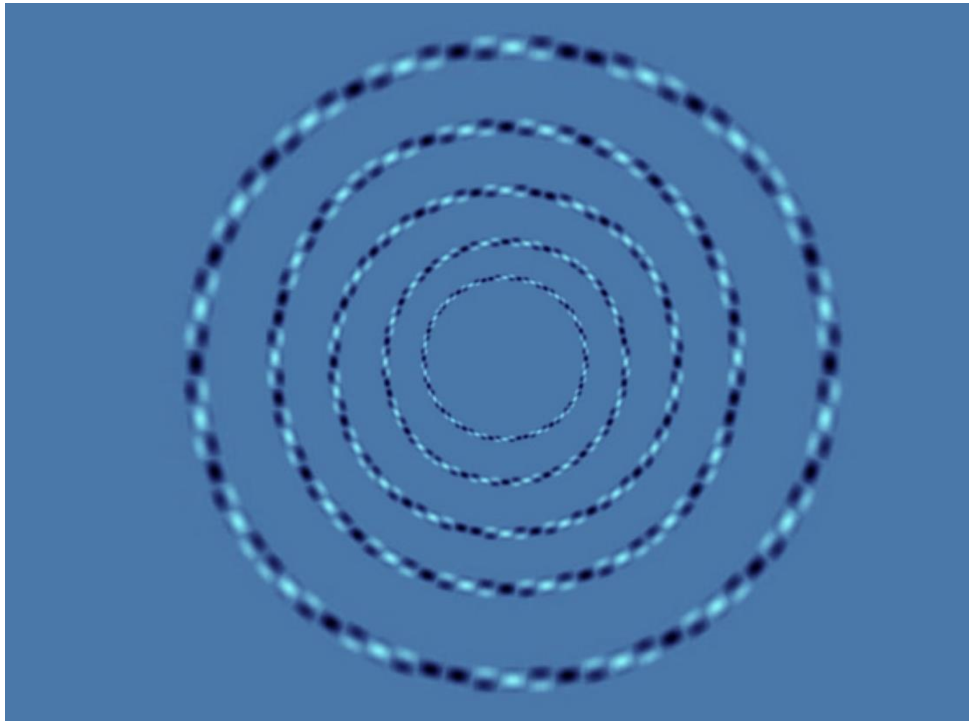
- Make pretty pictures (processing)
  - publications, talks, websites, …
- Get numbers out of pictures (analysis)
  - cell sizes, vessel lengths, GPF expression level, …
- Make experiment possible (automation)
  - whole-genome screen: millions of images
- Objectivity and Reproducibility
  - in science, it's your duty!

Reasons **not** to learn image processing

none

Why should we analyze images with computers at all?

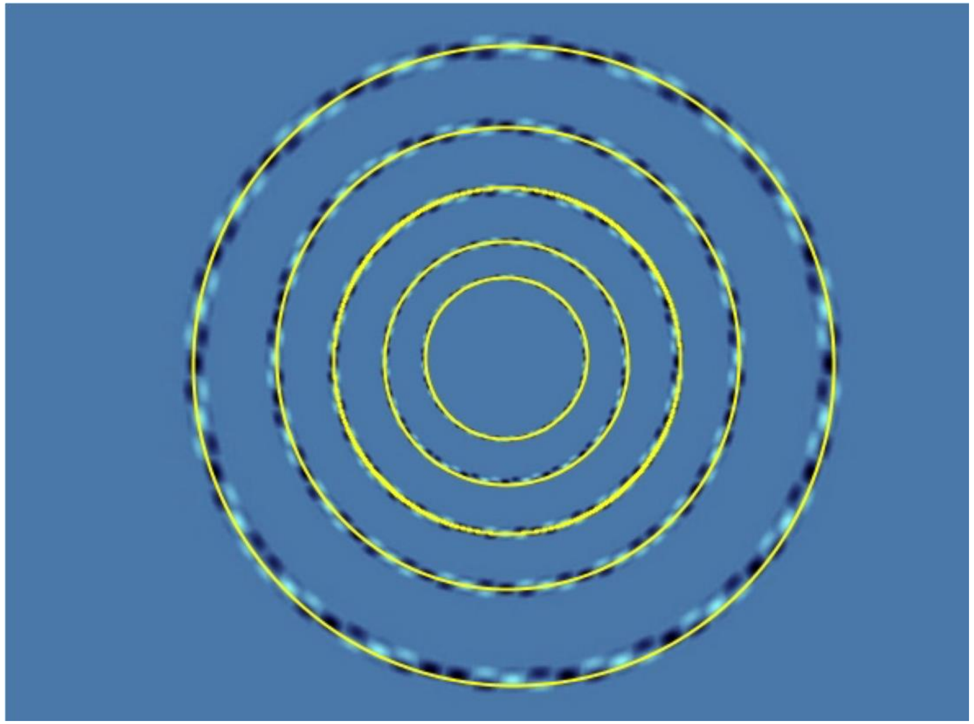
Color perception and pattern recognition is individual – science less so



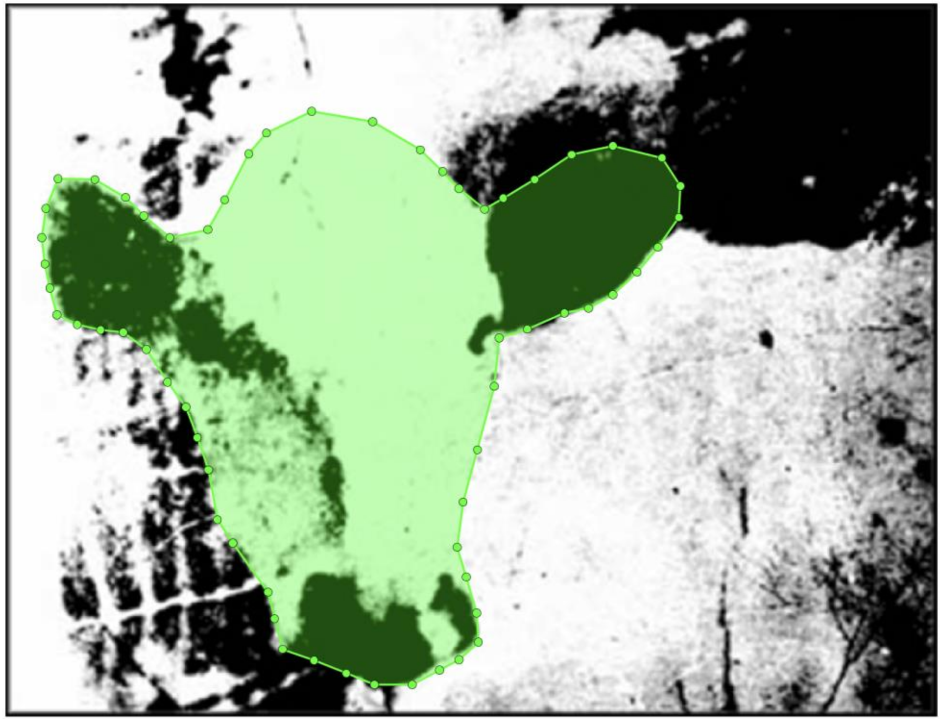
https://www.moillusions.com/perfect-circles-optical-illusion/

http://www.brainbashers.com

Color perception and pattern recognition is individual – science less so



https://www.moillusions.com/perfect-circles-optical-illusion/



http://www.brainbashers.com

In other words,

> “Each human brain is a very complex neural network trained on different data – predictions will vary”
>
> Antoine

A typical image analysis workflow

- There are typically *five* steps in an image analysis
- Often a good idea to structure work along these lines before starting

Pre-processing → Processing → Post-processing → Measuring → Analysing & Visualising

Think of this even ***before*** you acquire the images!

otherwise image analysis may become only a *post-mortem* on your experiment

Image processing vs analysis

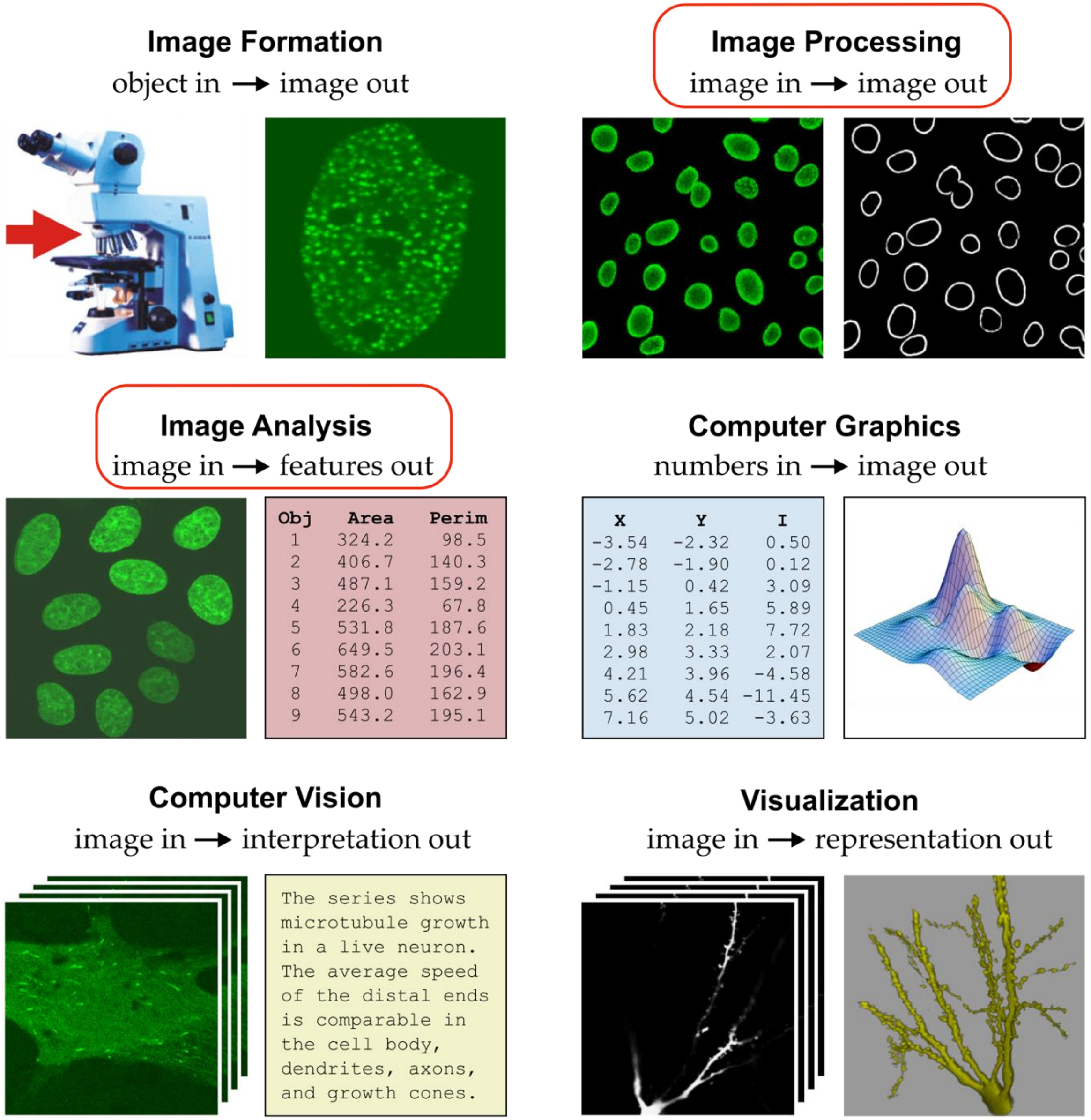


Image Formation
object in ⟶ image out

Image Processing
image in ⟶ image out

Image Analysis
image in ⟶ features out

Obj	Area	Perim
1	324.2	98.5
2	406.7	140.3
3	487.1	159.2
4	226.3	67.8
5	531.8	187.6
6	649.5	203.1
7	582.6	196.4
8	498.0	162.9
9	543.2	195.1

Computer Graphics
numbers in ⟶ image out

X	Y	I
-3.54	-2.32	0.50
-2.78	-1.90	0.12
-1.15	0.42	3.09
0.45	1.65	5.89
1.83	2.18	7.72
2.98	3.33	2.07
4.21	3.96	-4.58
5.62	4.54	-11.45
7.16	5.02	-3.63

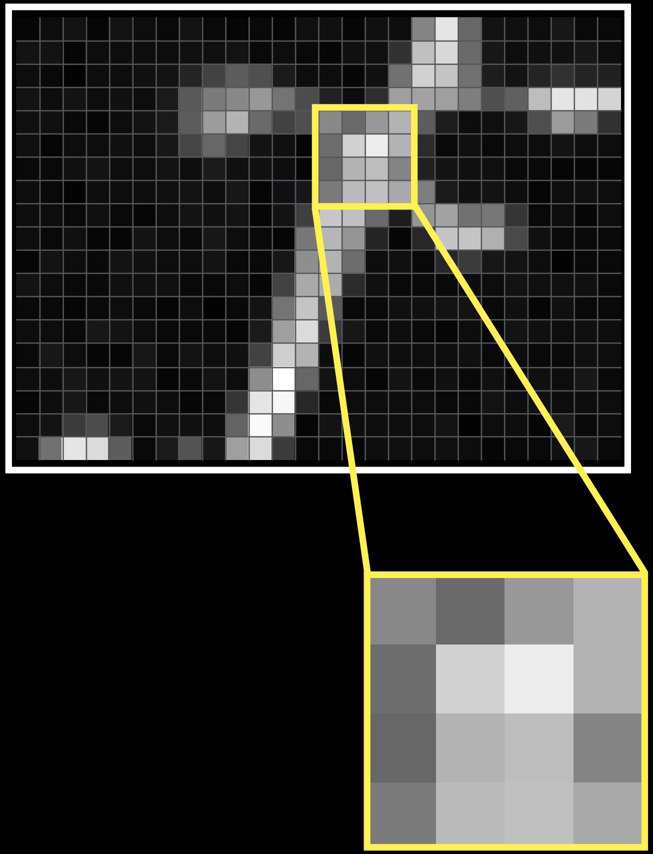
Computer Vision
image in ⟶ interpretation out

The series shows microtubule growth in a live neuron. The average speed of the distal ends is comparable in the cell body, dendrites, axons, and growth cones.

Visualization
image in ⟶ representation out

What is an image?

A digital image is a matrix of numbers!



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6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

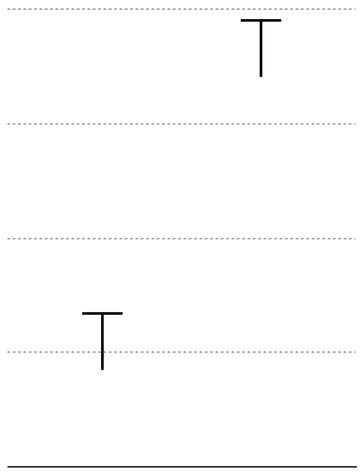
Pixel = Picture Element

Images in publications and presentations should be used to ***communicate*** a finding... not ***be*** the finding

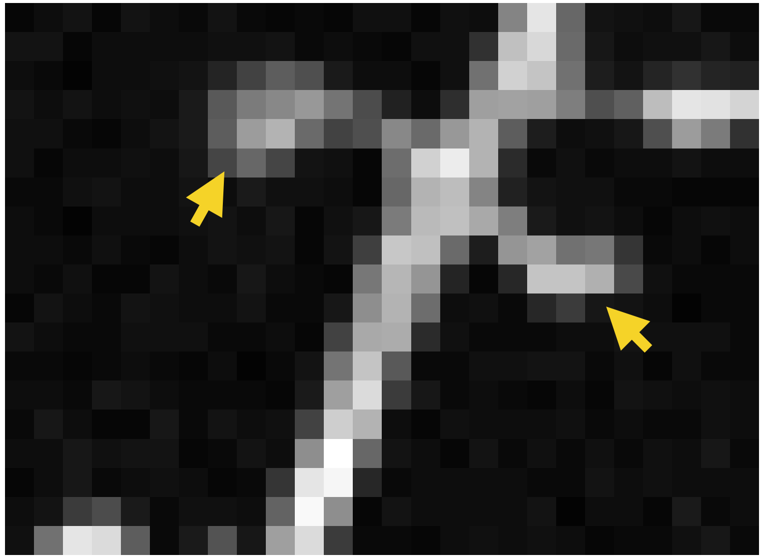
this is your **data**

6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

this is your **result**



this just helps to ***communicate*** the result

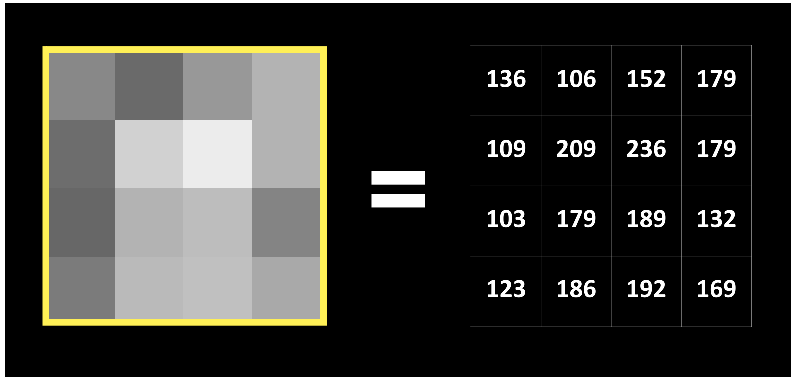


Display your images

Mapping Image Intensity to Monitor Intensity (LookUp Tables)

LUT = how the grey values are displayed

LUTs do not change the pixel values



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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

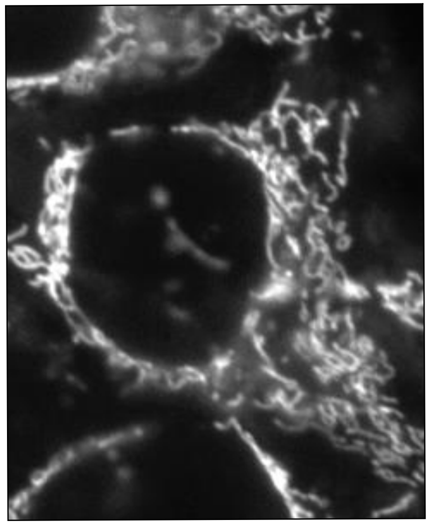
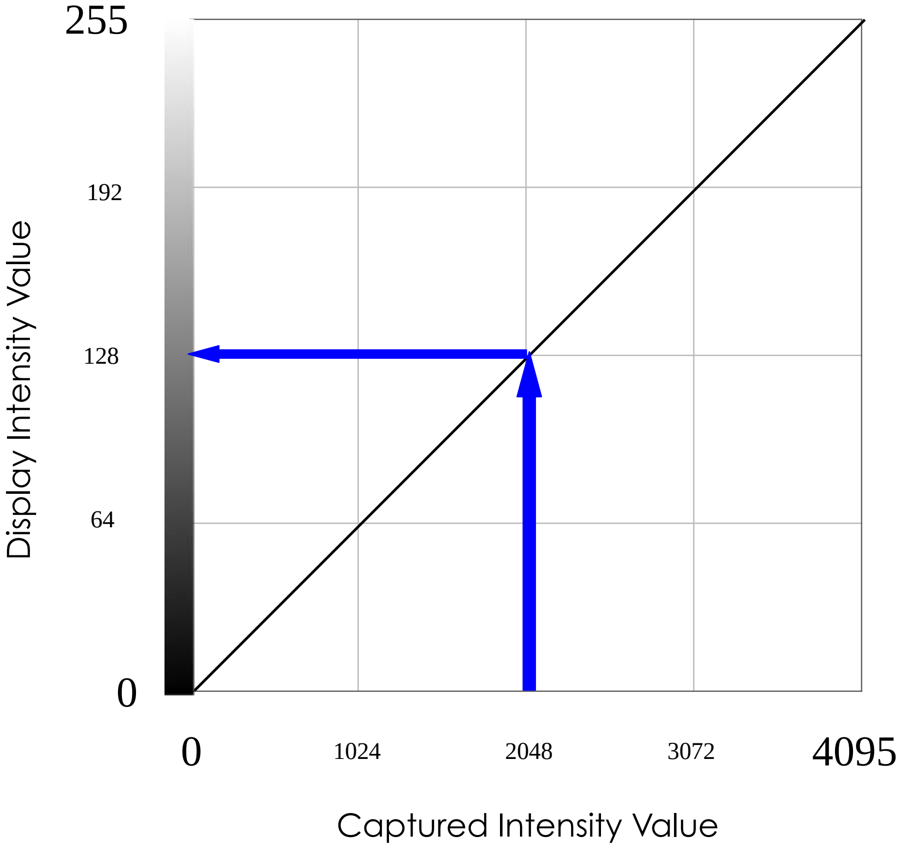


Image (12 bit)	Displayed color
0 1 … 2000 … 4095	 grey LUT



Images and Colors

Lookup Tables (LUTs)

LUT = how the grey values are displayed

LUTs do not change the pixel values

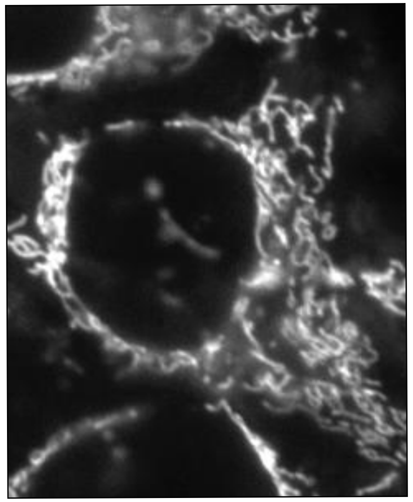
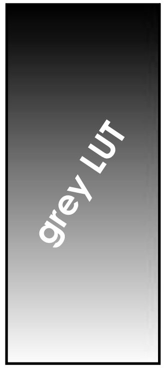


Image (8 bit)	Displayed color
0	 grey LUT
1	
…	
100	
…	
255	

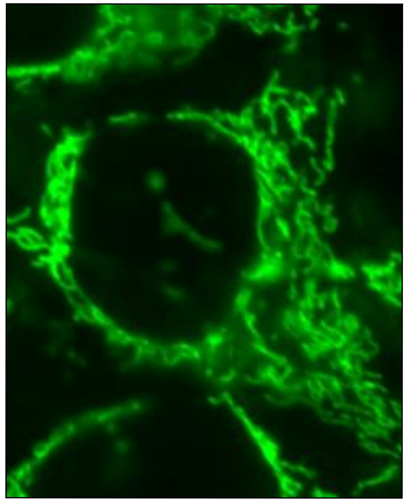
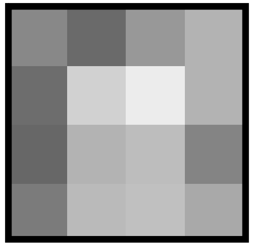


Image (8 bit)	Displayed color
0	 green LUT
1	
…	
100	
…	
255	



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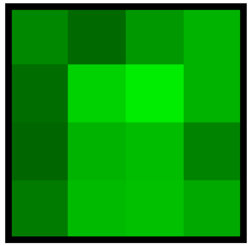
136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169



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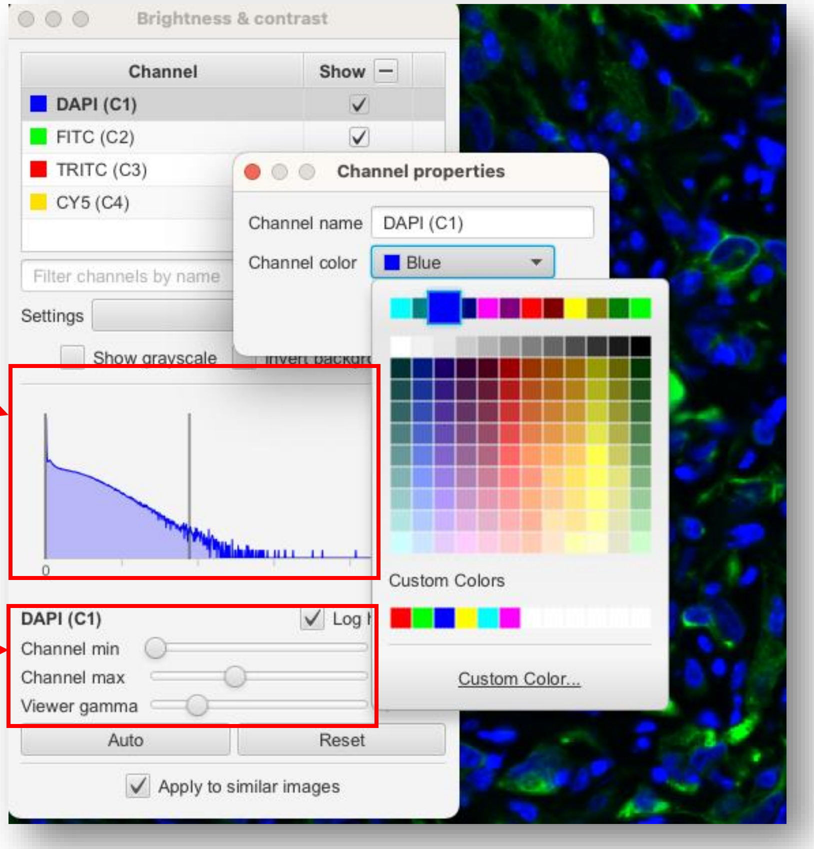
136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Display images: color, brightness & contrast

- If you are imaging a blue fluorophore, you are not forced to display it in blue!
- Pixel histogram represents the distribution of pixel values in the image
- LUT range

**You are NOT changing the pixels values, you are just changing how the image is displayed (unless you click on the “Apply” button).*

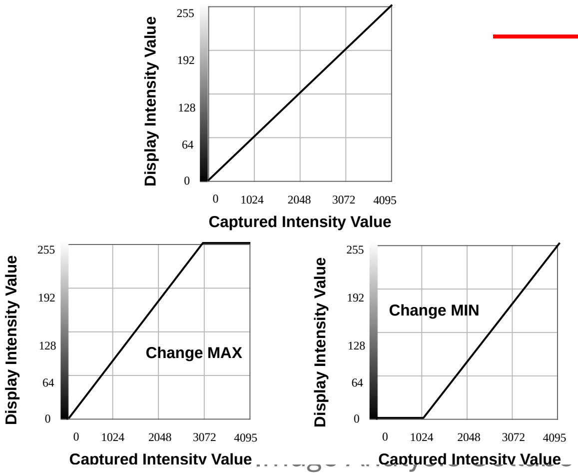

Brightness & contrast

Channel	Show
DAPI (C1)	☑
FITC (C2)	☑
TRITC (C3)	
CY5 (C4)	

Channel properties

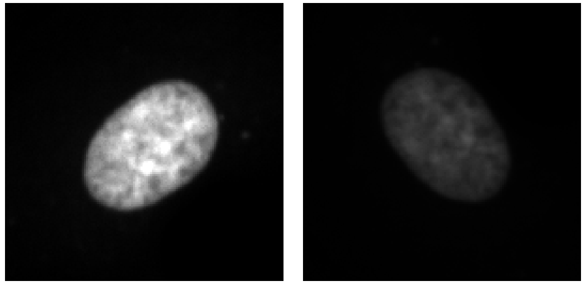
Channel name: DAPI (C1)

Channel color: Blue

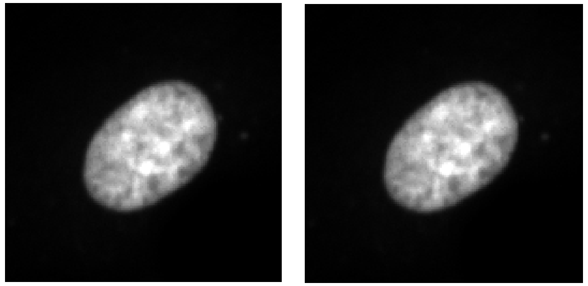


Display a file: Brightness & Contrast

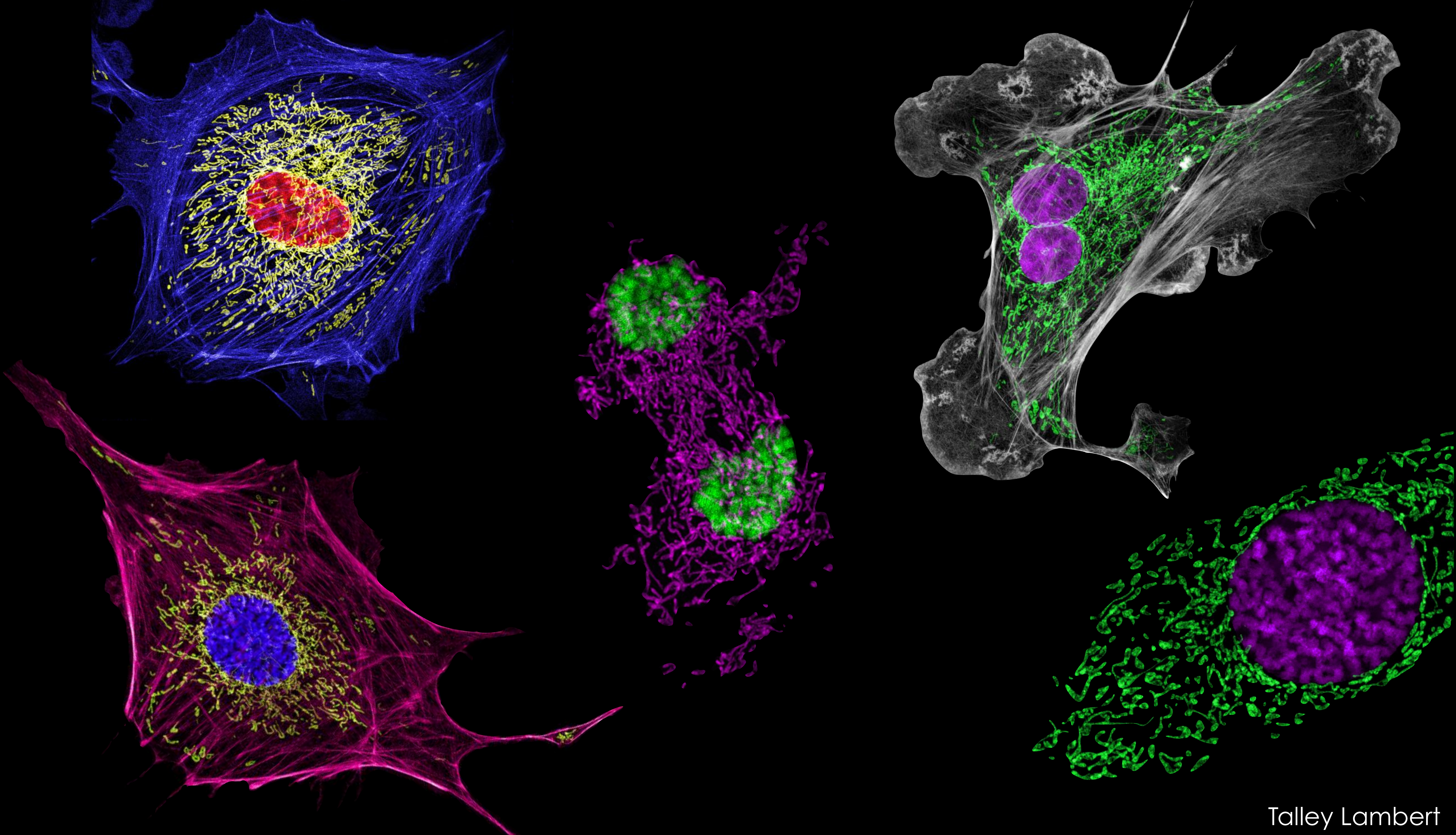
Which image has more fluorescence?



Mean:	**4803**	**4803**
Display range:	188-**16828**	188-**45514**



Mean:	**4803**	**4803**
Display range:	188-**16828**	188-**16828**



Talley Lambert

Introduction to QuPath

You before this workshop

You after this workshop

Illustration reprinted from Pete Bankhead.

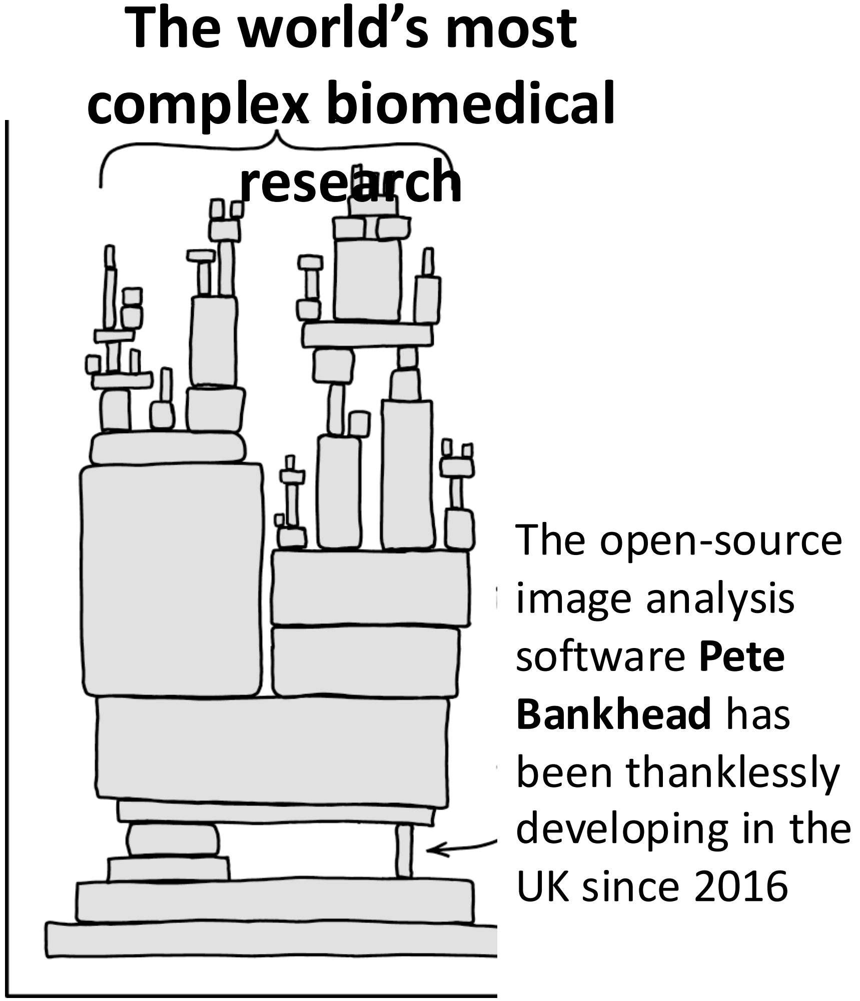
What is QuPath?

QuPath is an open-source software for bioimage analysis

- Developed and maintained by Pete Bankhead and his team at the University of Edinburgh

Key features:

1. Performant when working with very large 2D images, like those produced by slide scanner
2. Extremely well maintained



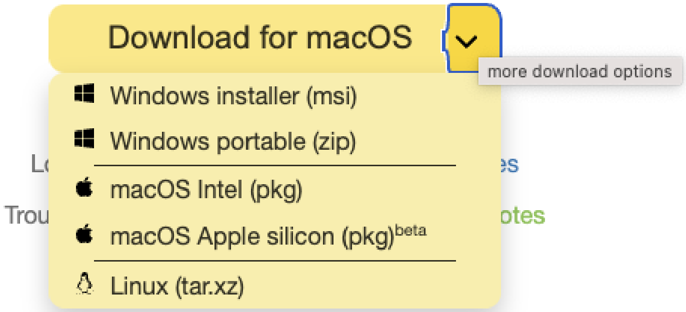
Adapted from https://xkcd.com/2347/

What is QuPath good at?

- It has a nice graphical user interface (GUI)
- It was designed to handle very large 2D images
- It supports common image analysis tasks: segmentation, annotation, feature analysis, and classification
- It support extensive visualization options
- It integrates with many other existing tools (Stardist, ImageJ, …)
- It support scripting (Groovy, akin to Java)

What is QuPath **not** good at?

- Limited to 2D images
  - Only supports the visualization of single planes
- Does not support all file format (e.g. zarr/NGFF)



Installing Qupath

INSTALL.SH

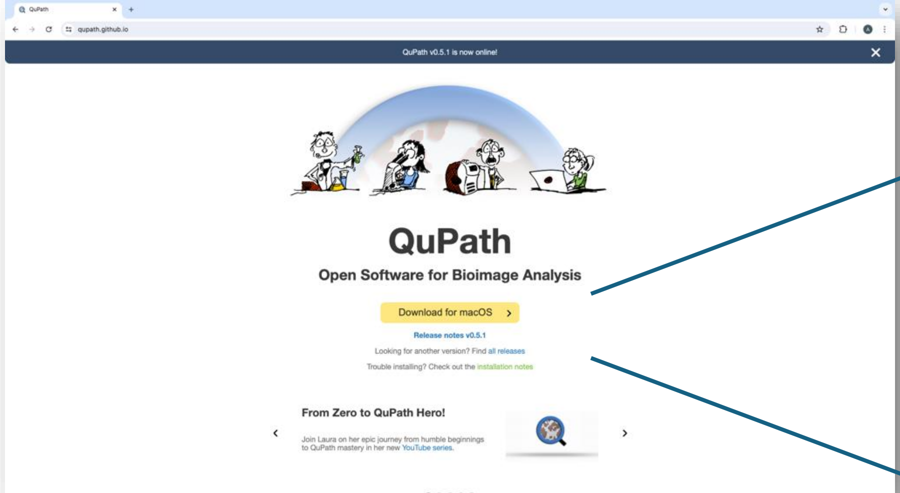
```
#!/bin/bash

pip install "$1" &
easy_install "$1" &
brew install "$1" &
npm install "$1" &
yum install "$1" & dnf install "$1" &
docker run "$1" &
pkg install "$1" &
apt-get install "$1" &
sudo apt-get install "$1" &
steamcmd +app_update "$1" validate &
git clone https://github.com/"$1"/"$1" &
cd "$1";./configure;make;make install &
curl "$1" | bash &
```

https://xkcd.com/1654/

Download QuPath

1. Go to https://qupath.github.io/ (*see useful links on website*)
2. Download the installer for the latest version

NB: we recommend the .msi file for Windows users



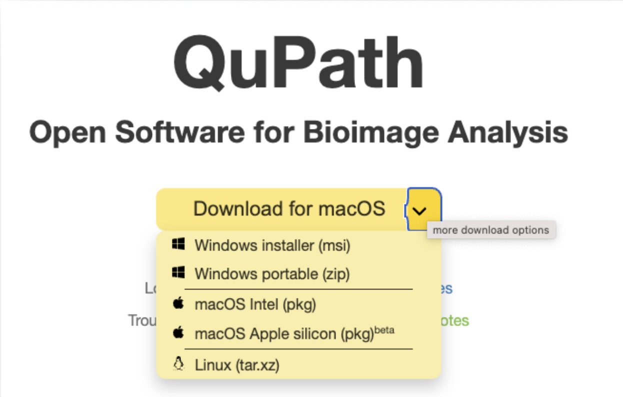
QuPath v0.5.1 is now online!

Looking for another version? Find all releases

Trouble installing? Check out the installation notes

Join Laura on her epic journey from humble beginnings to QuPath mastery in her new YouTube series.

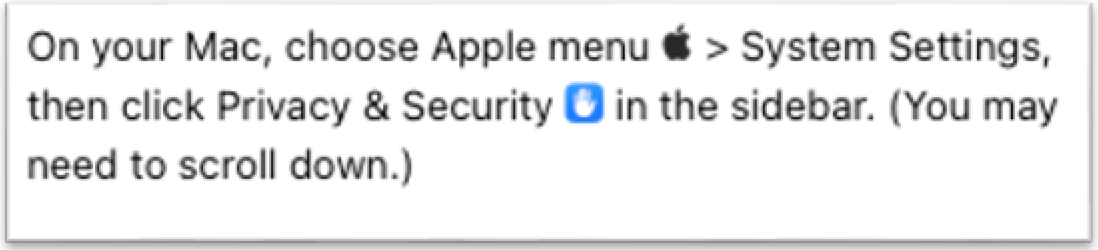
3. Choose your OS



4. Right-click on the installer file > Open > Confirm Open

For macOS users

Confirm that QuPath installer is not a malware



On your Mac, choose Apple menu > System Settings, then click Privacy & Security in the sidebar. (You may need to scroll down.)

Security

Allow applications downloaded from

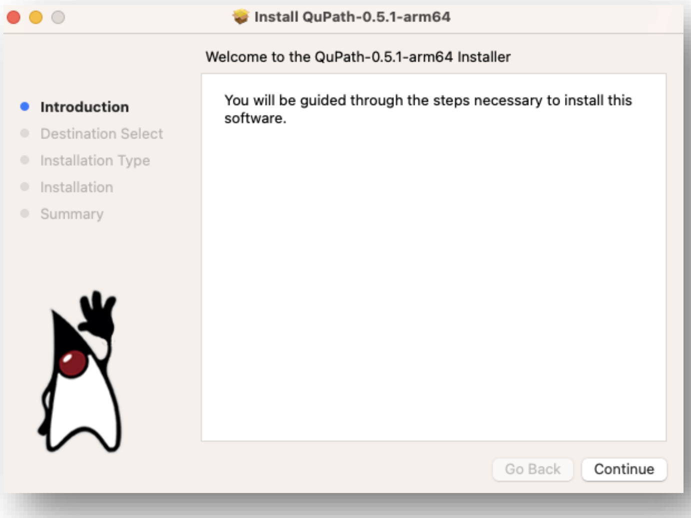
○ App Store

● App Store and identified developers

"QuPath-v0.5.1-Mac-x64.pkg" was blocked from use because it is not from an identified developer.

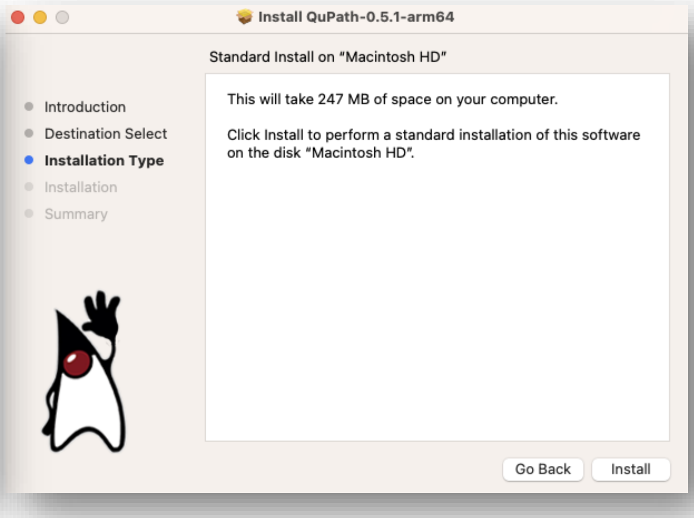
Download QuPath

Follow the steps of the installer


Install QuPath-0.5.1-arm64

Welcome to the QuPath-0.5.1-arm64 Installer

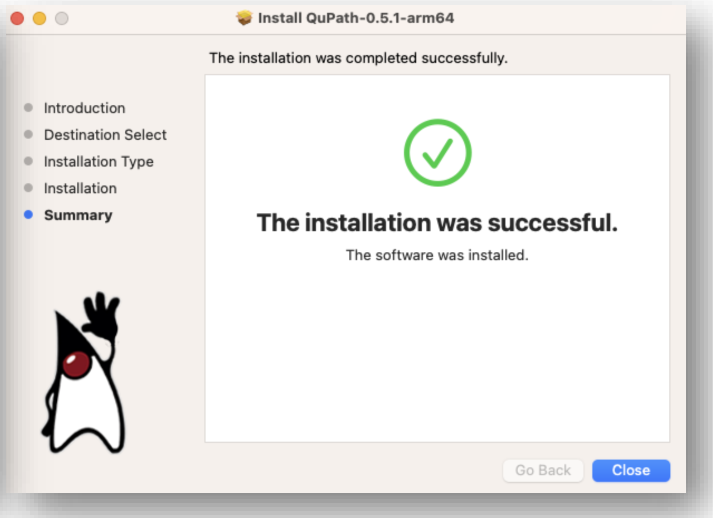
You will be guided through the steps necessary to install this software.


Install QuPath-0.5.1-arm64

Standard Install on "Macintosh HD"

This will take 247 MB of space on your computer.

Click Install to perform a standard installation of this software on the disk "Macintosh HD".


Install QuPath-0.5.1-arm64

The installation was completed successfully.

The installation was successful.

The software was installed.

Manage different QuPath versions

macOS users:

- *Applications* > multiple versions of QuPath installed > Choose the latest one
- Cmd + space, then choose from the list of available versions

Windows users:

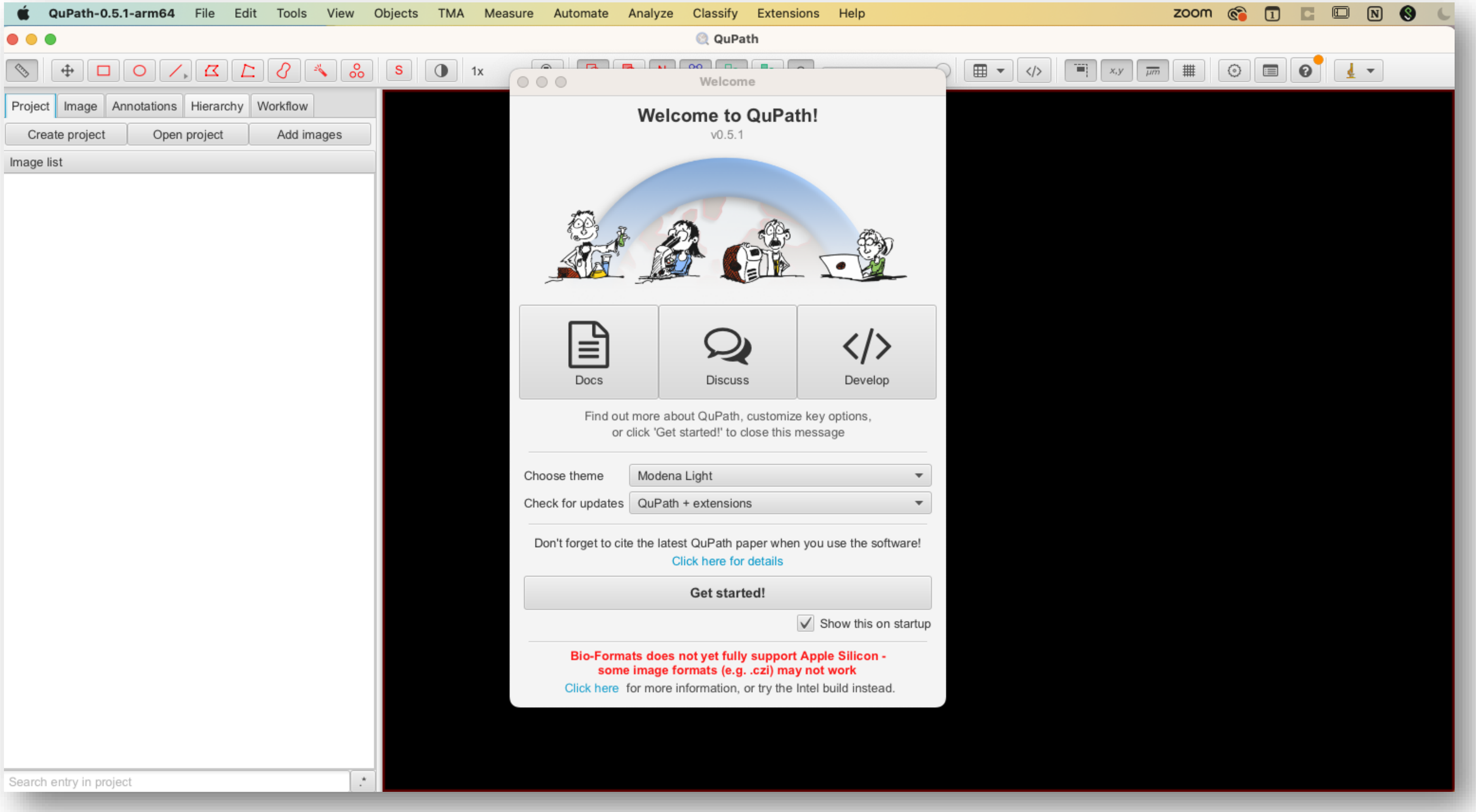
- C:\Program Files\QuPath
- Opening your application manager should prompt you the different version of QuPath that have been downloaded

Linux users:

- I don’t know

Trick: *using multiple versions of QuPath allows to easily run more than one app at the same time on your laptop (i.e. doing so from a single app would require launching each instance from a separate terminal on macOS).*

Open the QuPath application



Welcome to QuPath!
v0.5.1

Find out more about QuPath, customize key options, or click 'Get started!' to close this message

Choose theme: Modena Light

Check for updates: QuPath + extensions

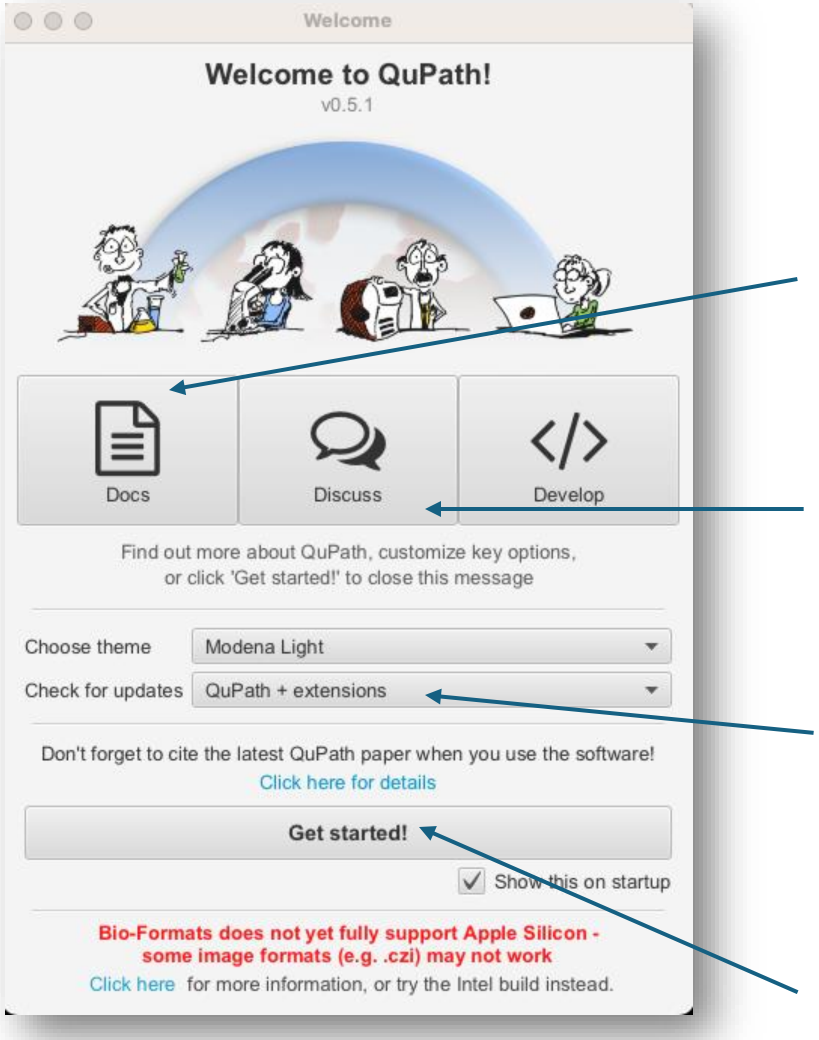
Don't forget to cite the latest QuPath paper when you use the software!
Click here for details

☑ Show this on startup

Bio-Formats does not yet fully support Apple Silicon - some image formats (e.g. .czi) may not work

Click here for more information, or try the Intel build instead.

Welcome to QuPath!



Welcome to QuPath!

v0.5.1

Find out more about QuPath, customize key options,
or click 'Get started!' to close this message

Choose theme: Modena Light

Check for updates: QuPath + extensions

Don't forget to cite the latest QuPath paper when you use the software!
Click here for details

☑ Show this on startup

Bio-Formats does not yet fully support Apple Silicon - some image formats (e.g. .czi) may not work

Click here for more information, or try the Intel build instead.

<u>Useful resources:</u>

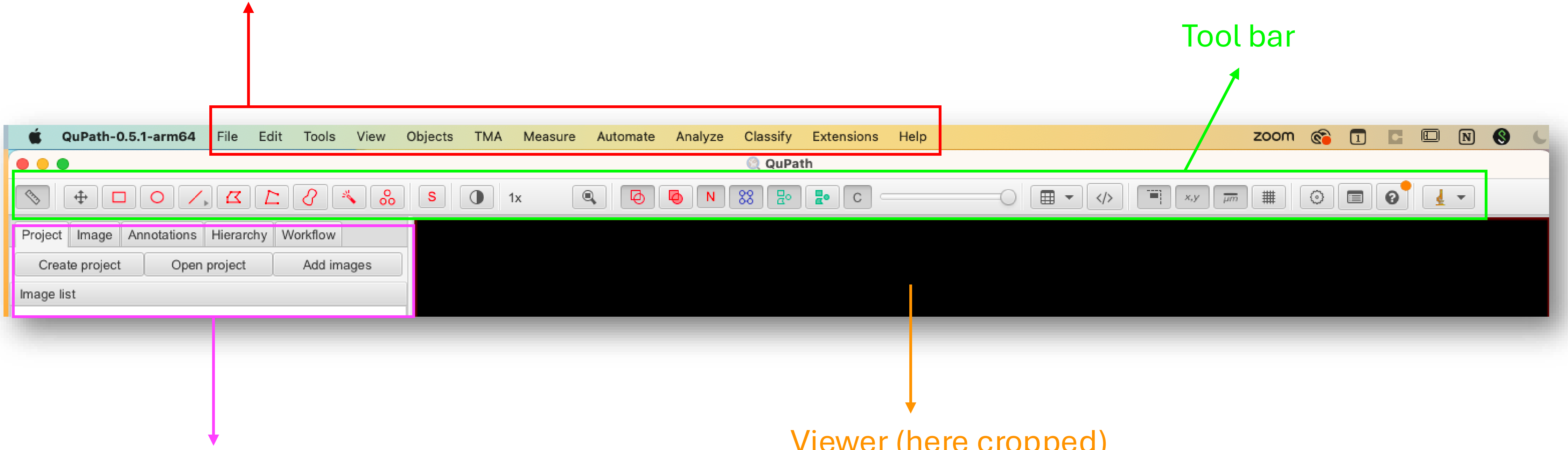
Documentation:
https://qupath.readthedocs.io/en/0.5/

QuPath-speficic place in ‘The Forum’:
https://forum.image.sc/tag/qupath

Updater

For now, let’s get started

Graphic User Interface (GUI) – intro



Menu bar

Tool bar

Analysis panel

Viewer (here cropped)

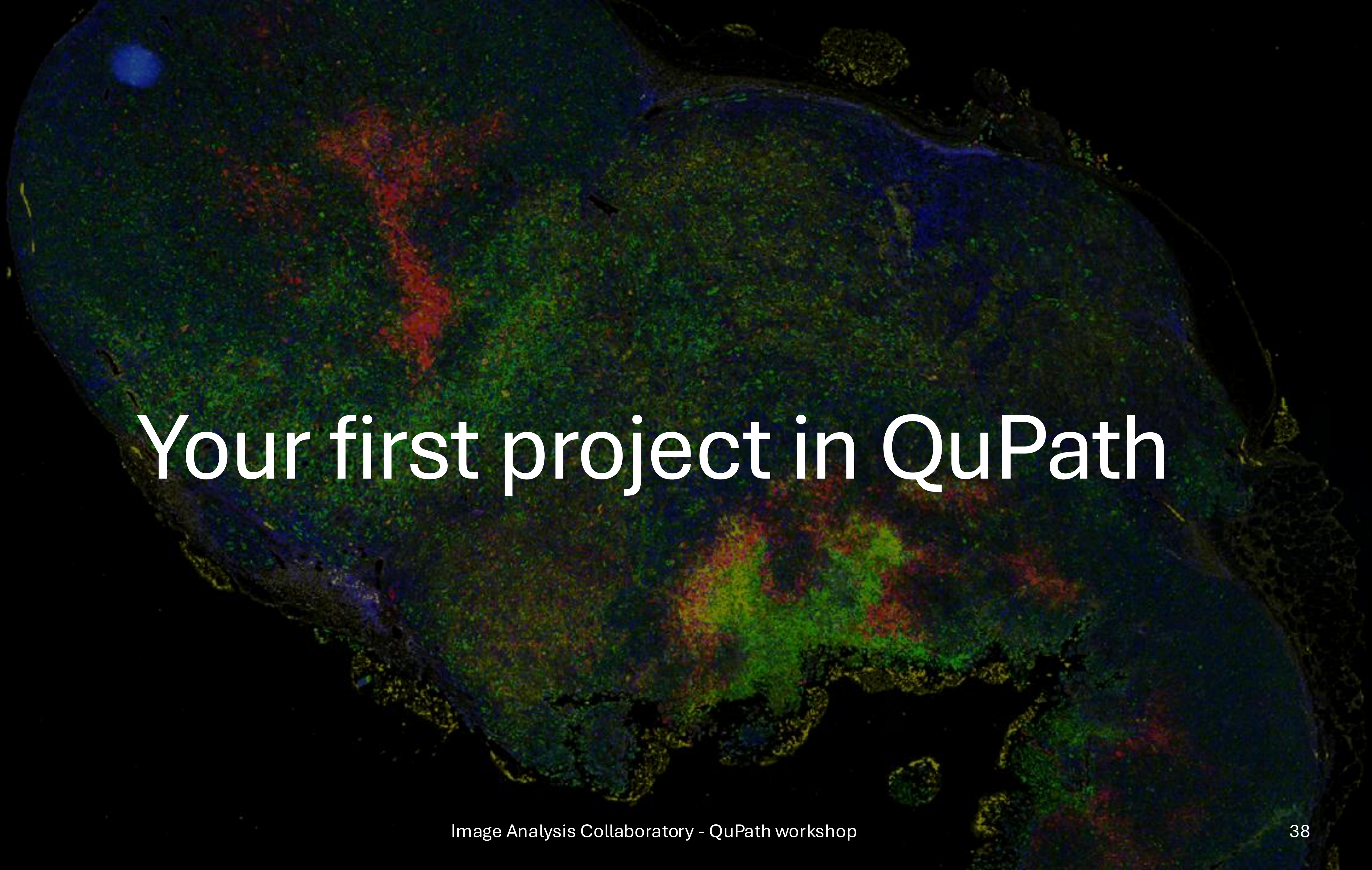
Getting help

- In-app documentation: Help menu

- QuPath documentation: https://qupath.github.io/

- The Forum: https://forum.image.sc/ image.sc

- During this workshop, ask questions to your neighbors, the TA’s and me!

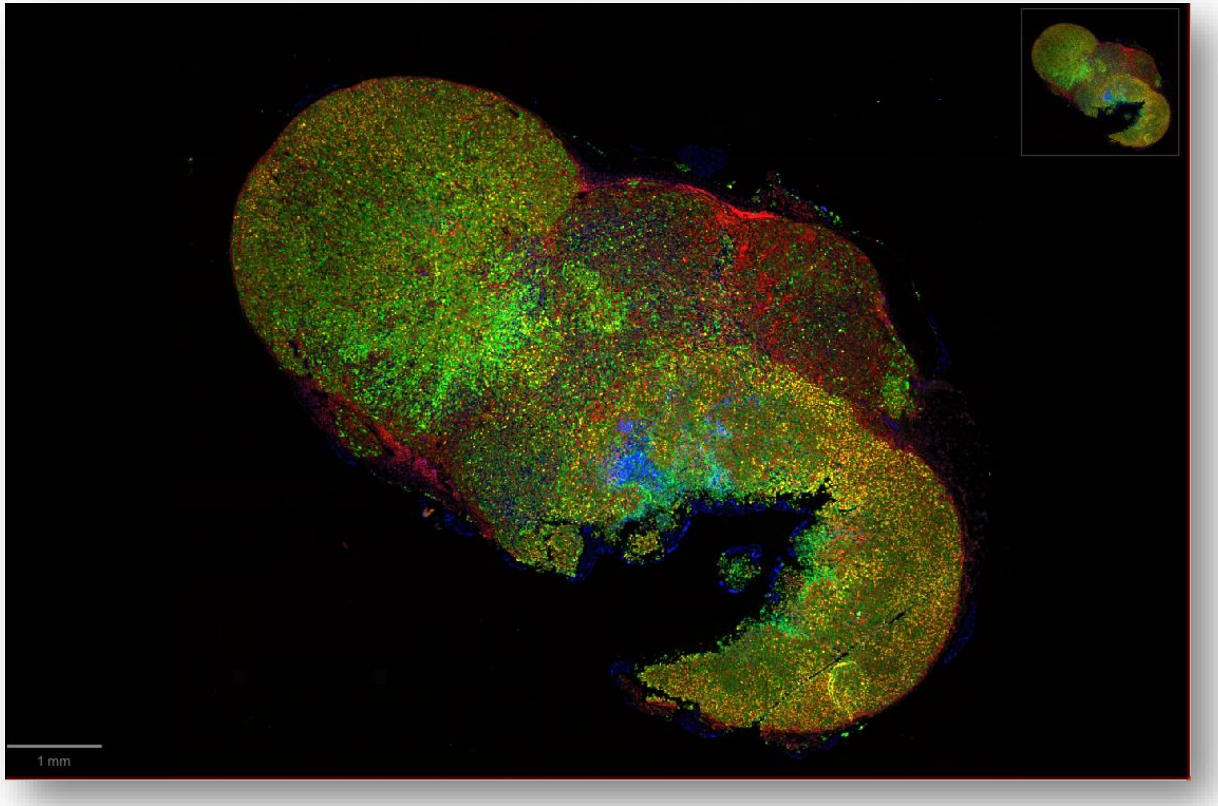


Your first project in QuPath

Classification of proliferating cancer cells in solid tumors

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

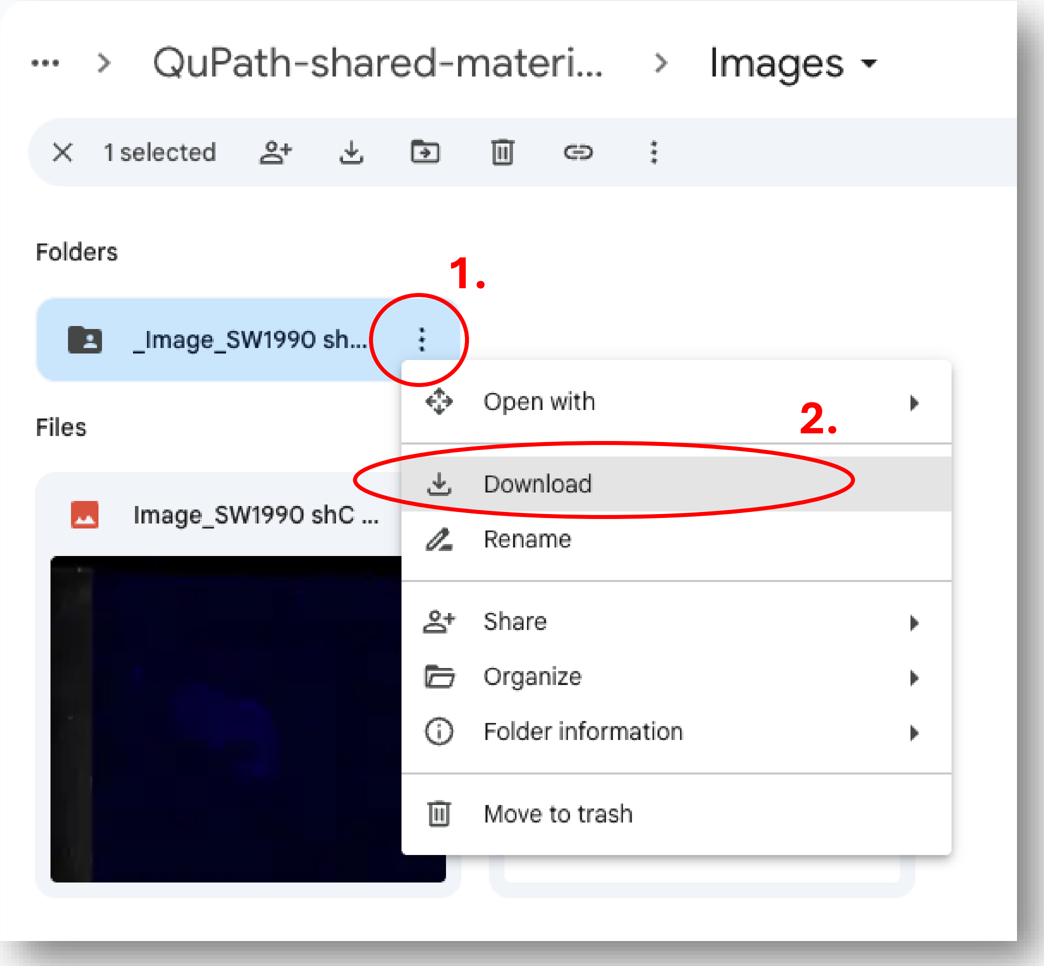
At the end of this course:
you will have classified proliferating (Ki67) cancer cells and reveal their spatial distribution to regions with high-fibronectin content



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

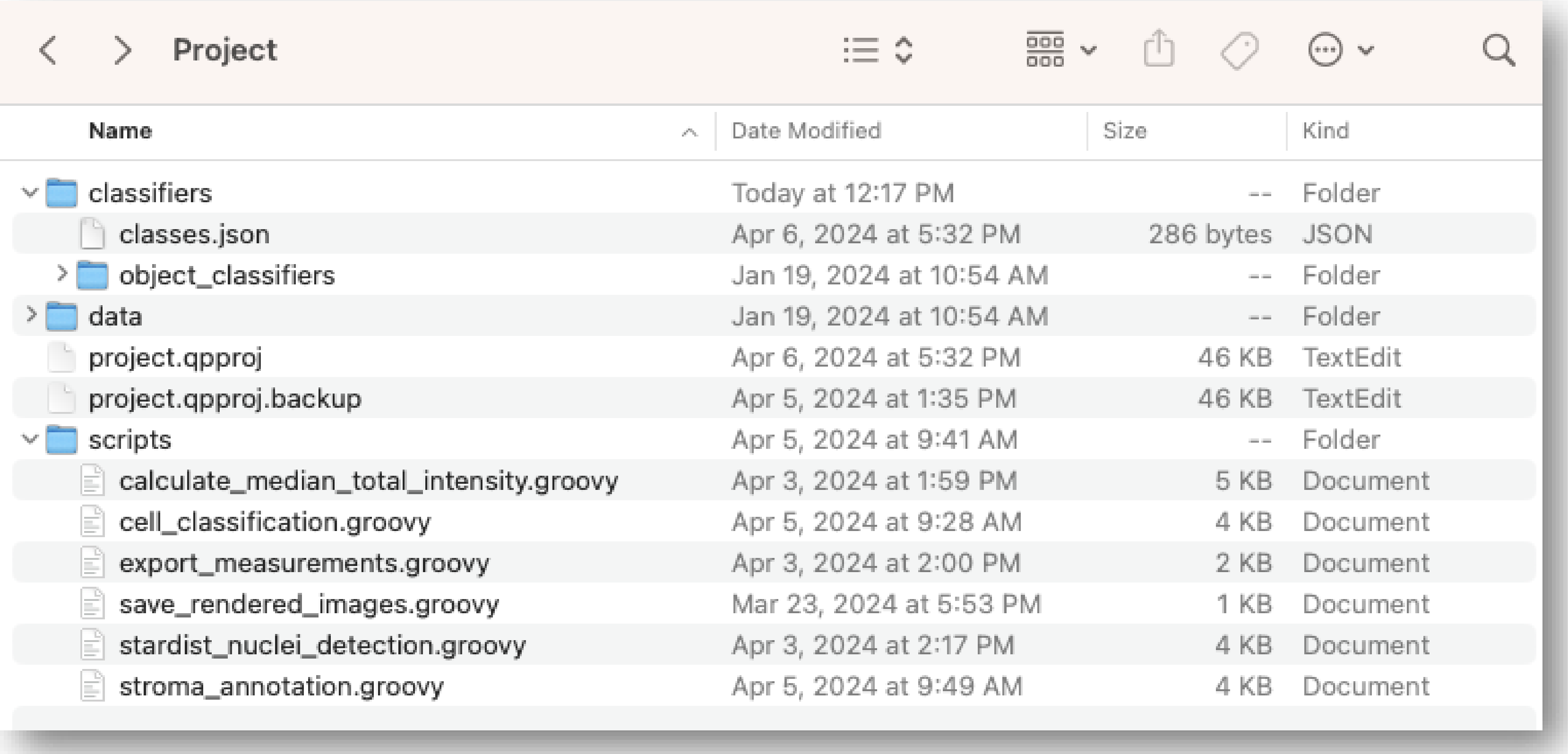
or

- 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…

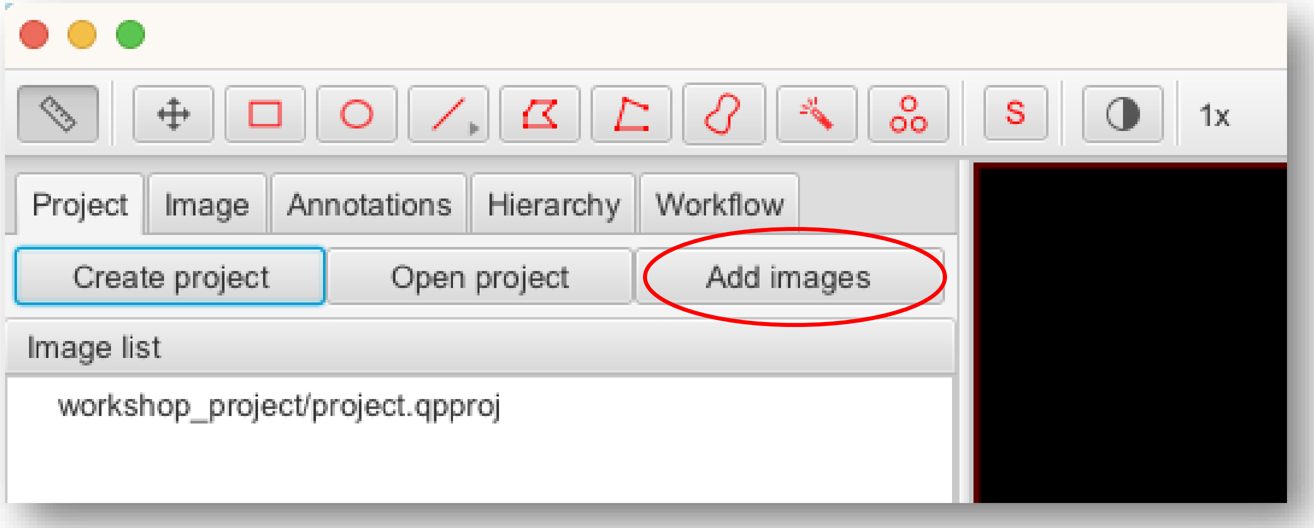


Name	Date Modified	Size	Kind
classifiers	Today at 12:17 PM	--	Folder
classes.json	Apr 6, 2024 at 5:32 PM	286 bytes	JSON
object_classifiers	Jan 19, 2024 at 10:54 AM	--	Folder
data	Jan 19, 2024 at 10:54 AM	--	Folder
project.qpproj	Apr 6, 2024 at 5:32 PM	46 KB	TextEdit
project.qpproj.backup	Apr 5, 2024 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

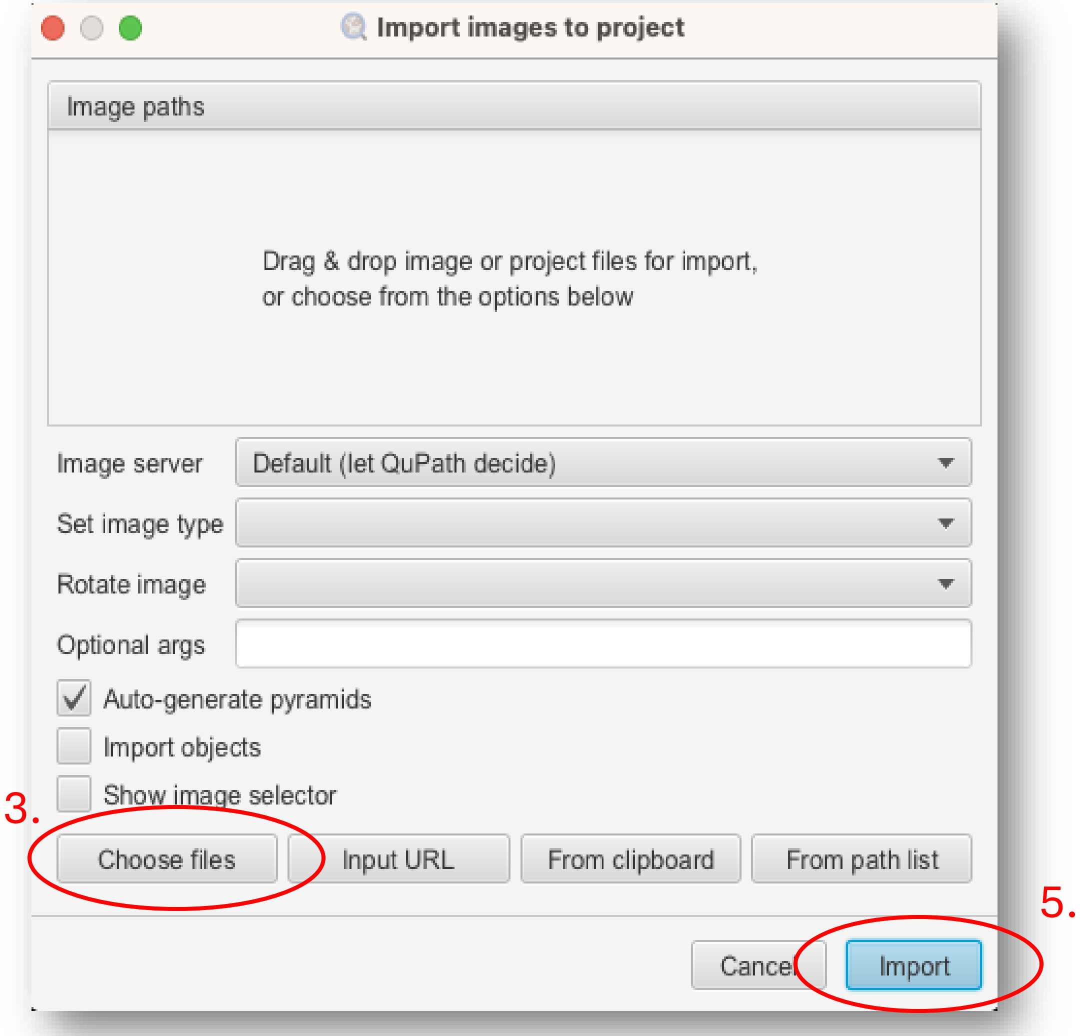


Add an image to your project

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

4. Use default settings

5. Click *import*

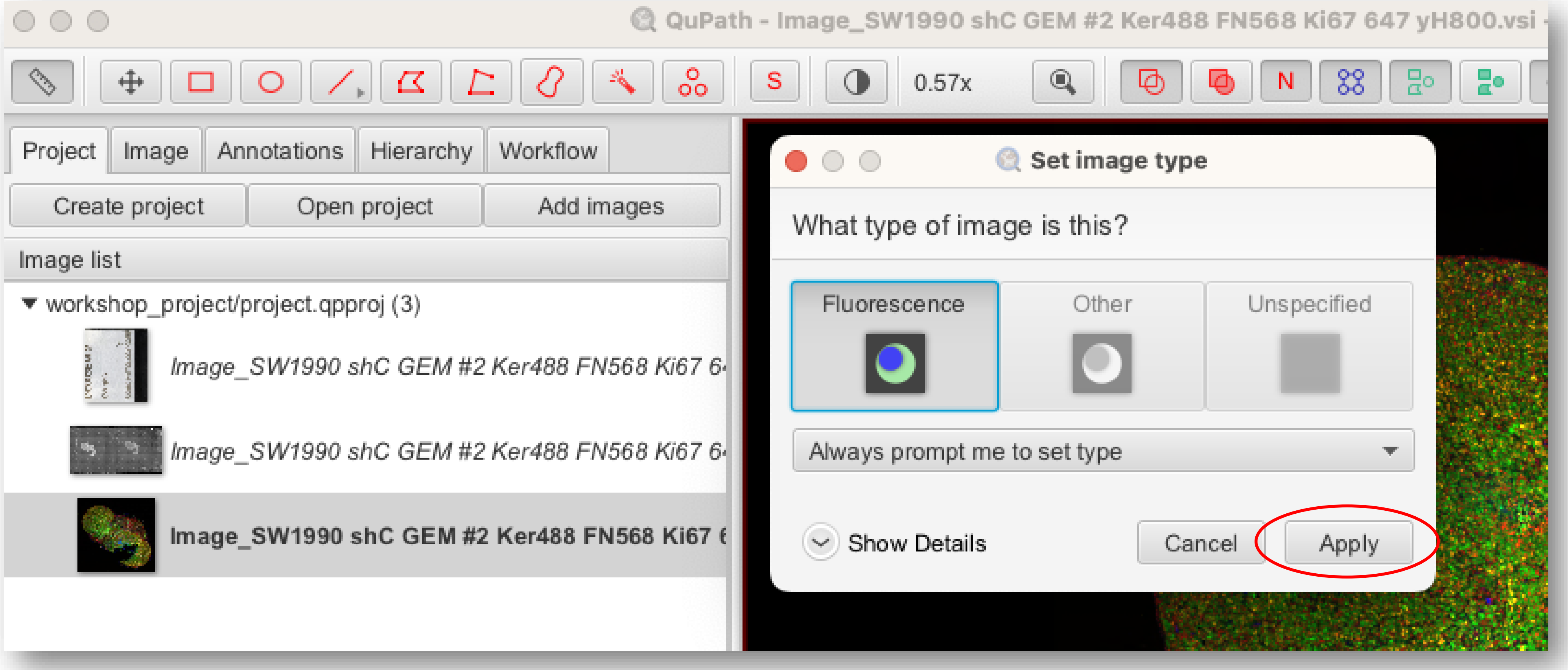


Drag & drop image or project files for import,
or choose from the options below

Image server: Default (let QuPath decide)

☑ Auto-generate pyramids
☐ Import objects
☐ Show image selector

Set image type



QuPath - Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi

workshop_project/project.qpproj (3)

Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 6

Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 6

Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 6

Set image type

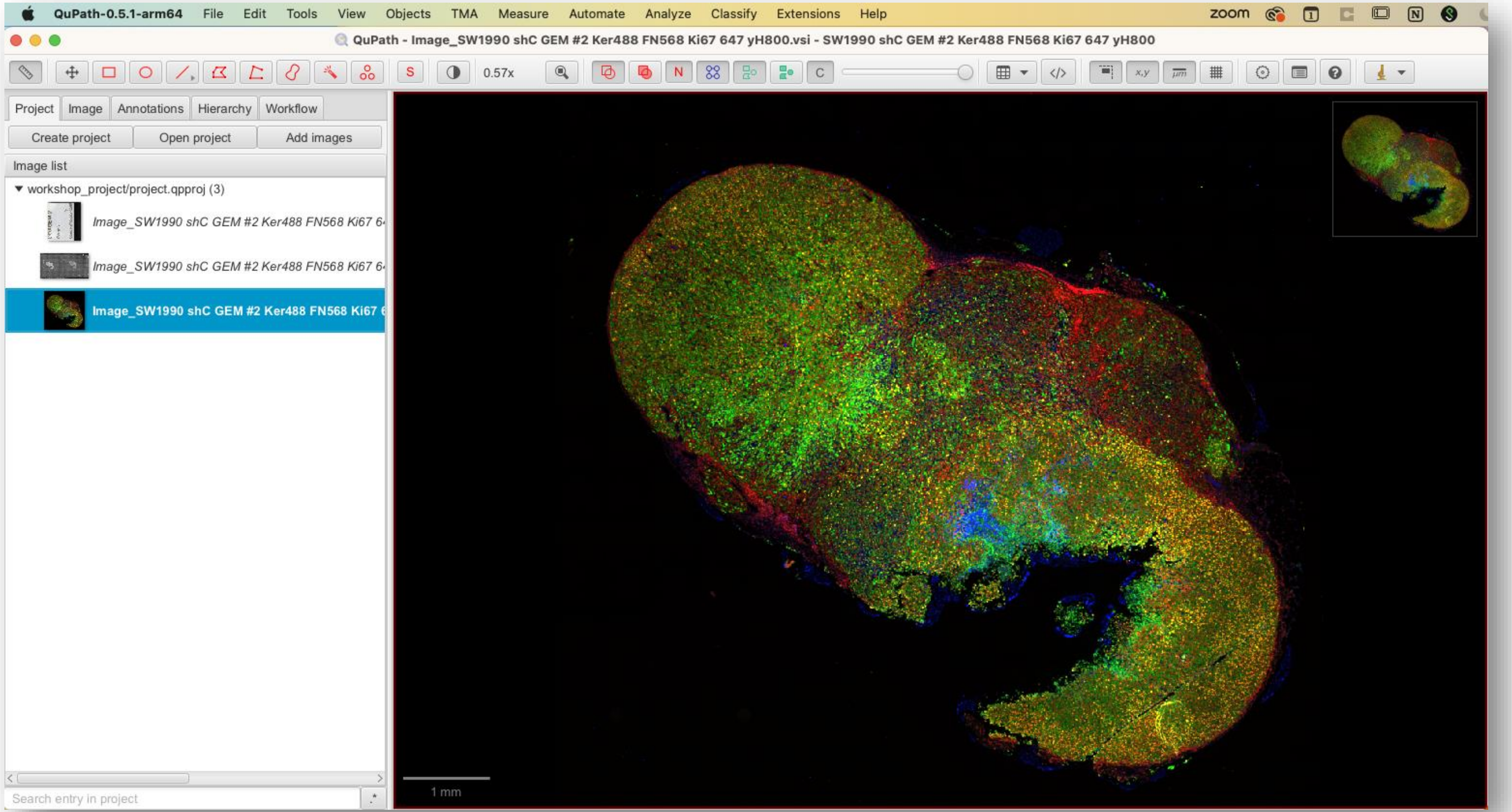
What type of image is this?

Always prompt me to set 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 - Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800

workshop_project/project.qpproj (3)
- Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 6
- Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 6
- **Image_SW1990 shC GEM #2 Ker488 FN568 Ki67**

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

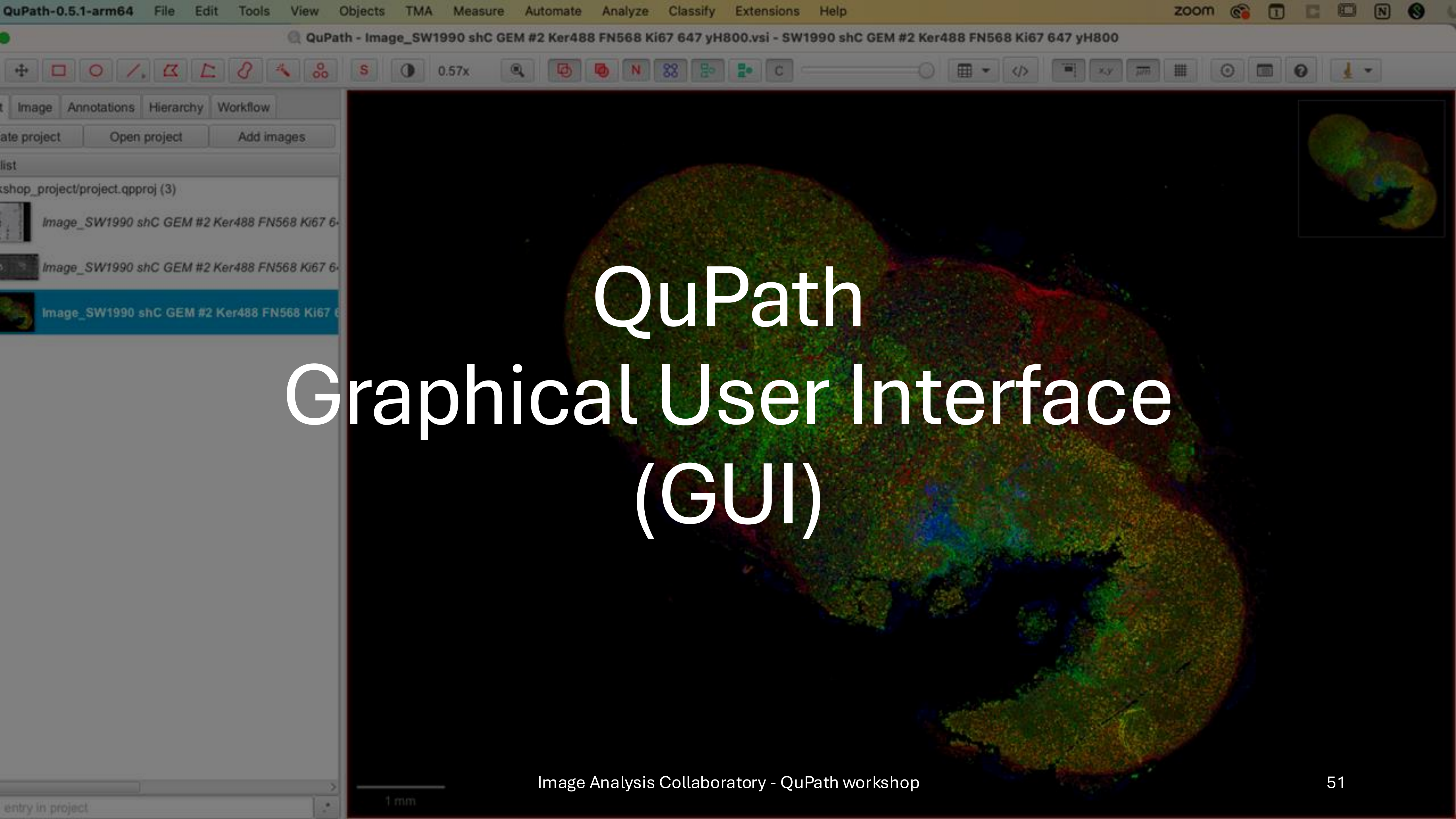
QuPath projects are portable

- 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.

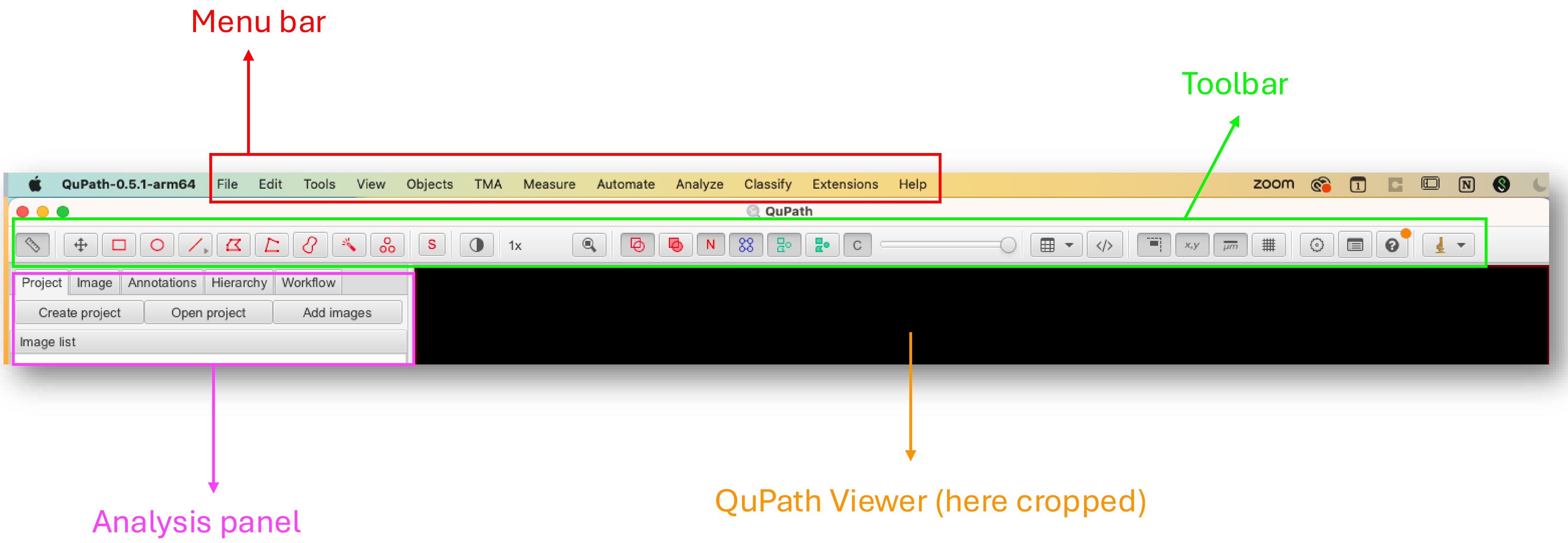
QuPath projects are portable

- 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



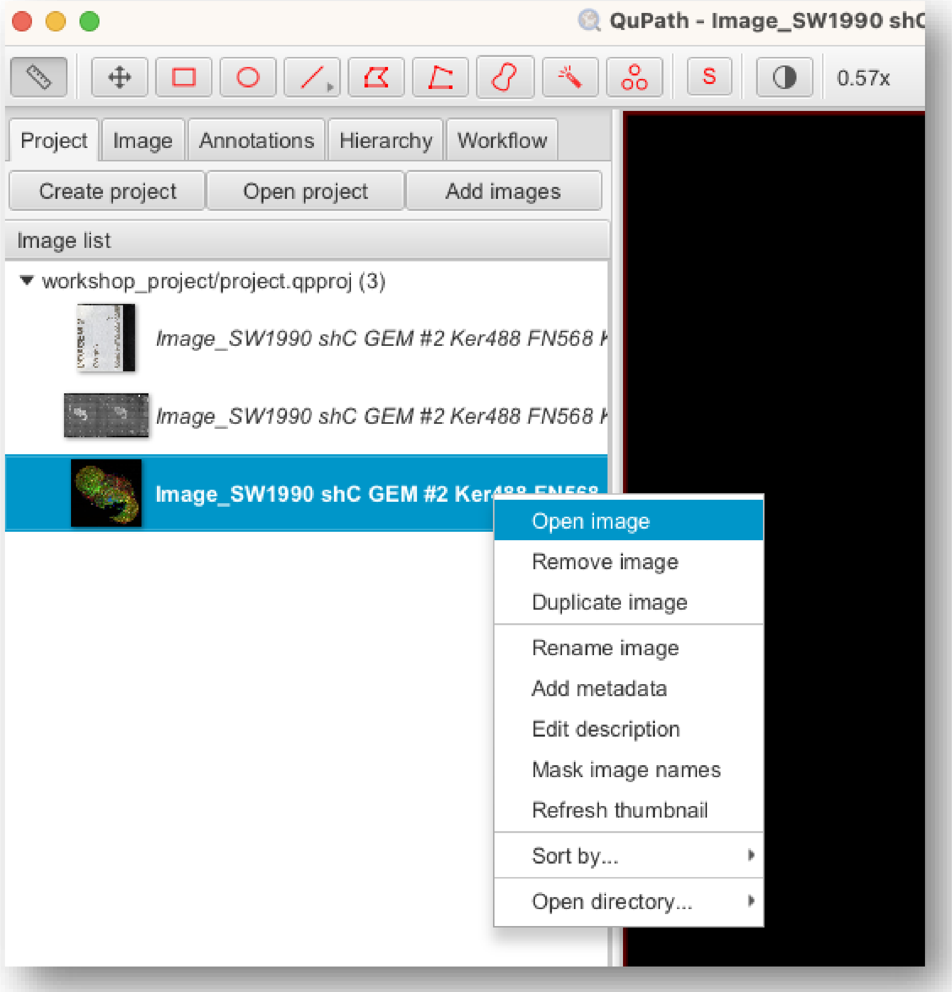
QuPath
Graphical User Interface
(GUI)

Graphic User Interface (GUI)



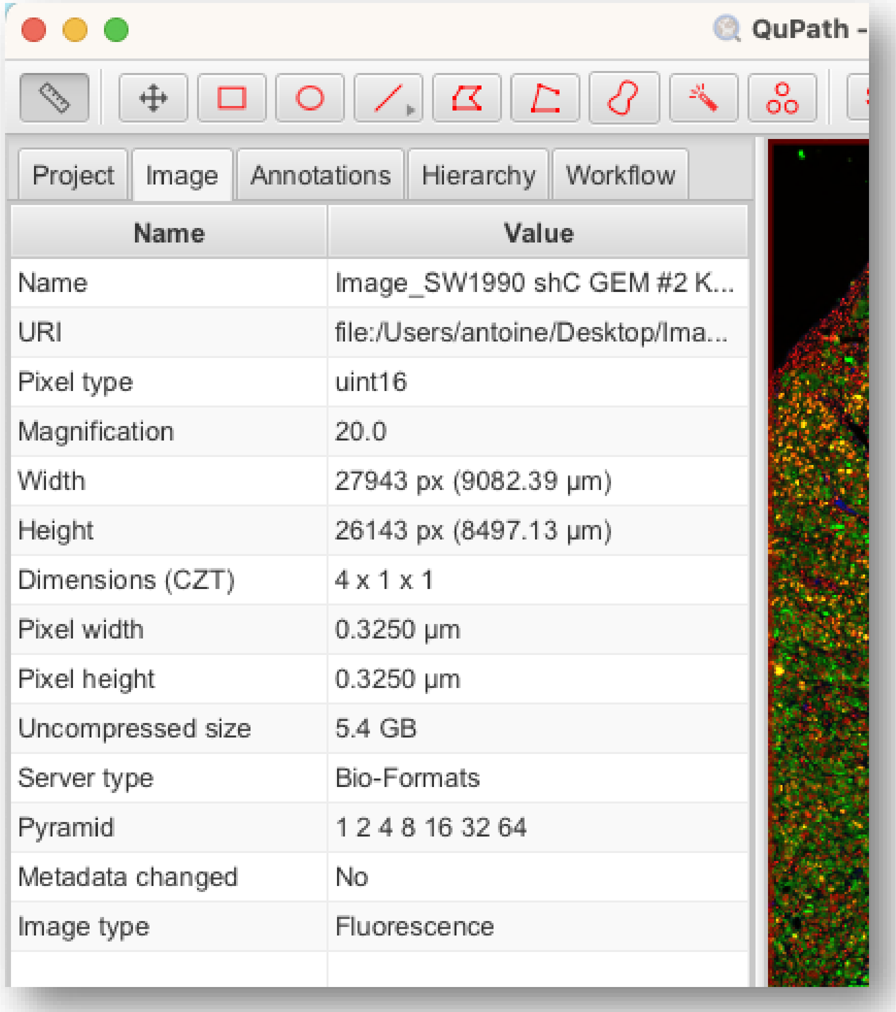
Analysis Panel

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



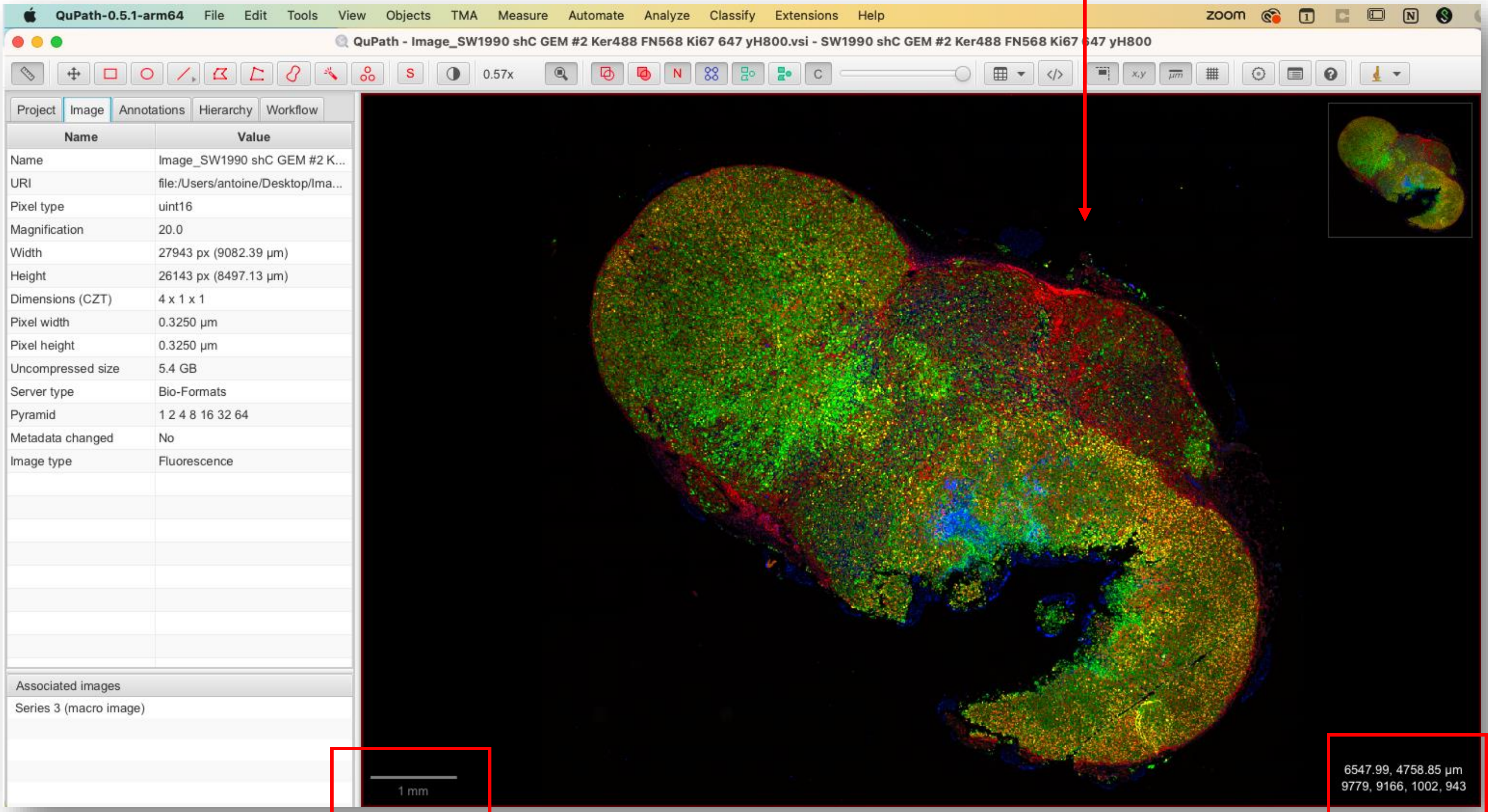
Analysis Panel

- *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



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 µm)
Height	26143 px (8497.13 µm)
Dimensions (CZT)	4 x 1 x 1
Pixel width	0.3250 µm
Pixel height	0.3250 µm
Uncompressed size	5.4 GB
Server type	Bio-Formats
Pyramid	1 2 4 8 16 32 64
Metadata changed	No
Image type	Fluorescence

QuPath viewer



QuPath - Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800

Name	Value
Name	Image_SW1990 shC GEM #2 K...
URI	file:/Users/antoine/Desktop/Ima...
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Server type	Bio-Formats
Pyramid	1 2 4 8 16 32 64
Metadata changed	No
Image type	Fluorescence

Associated images

Series 3 (macro image)

6547.99, 4758.85 µm
9779, 9166, 1002, 943

Viewer

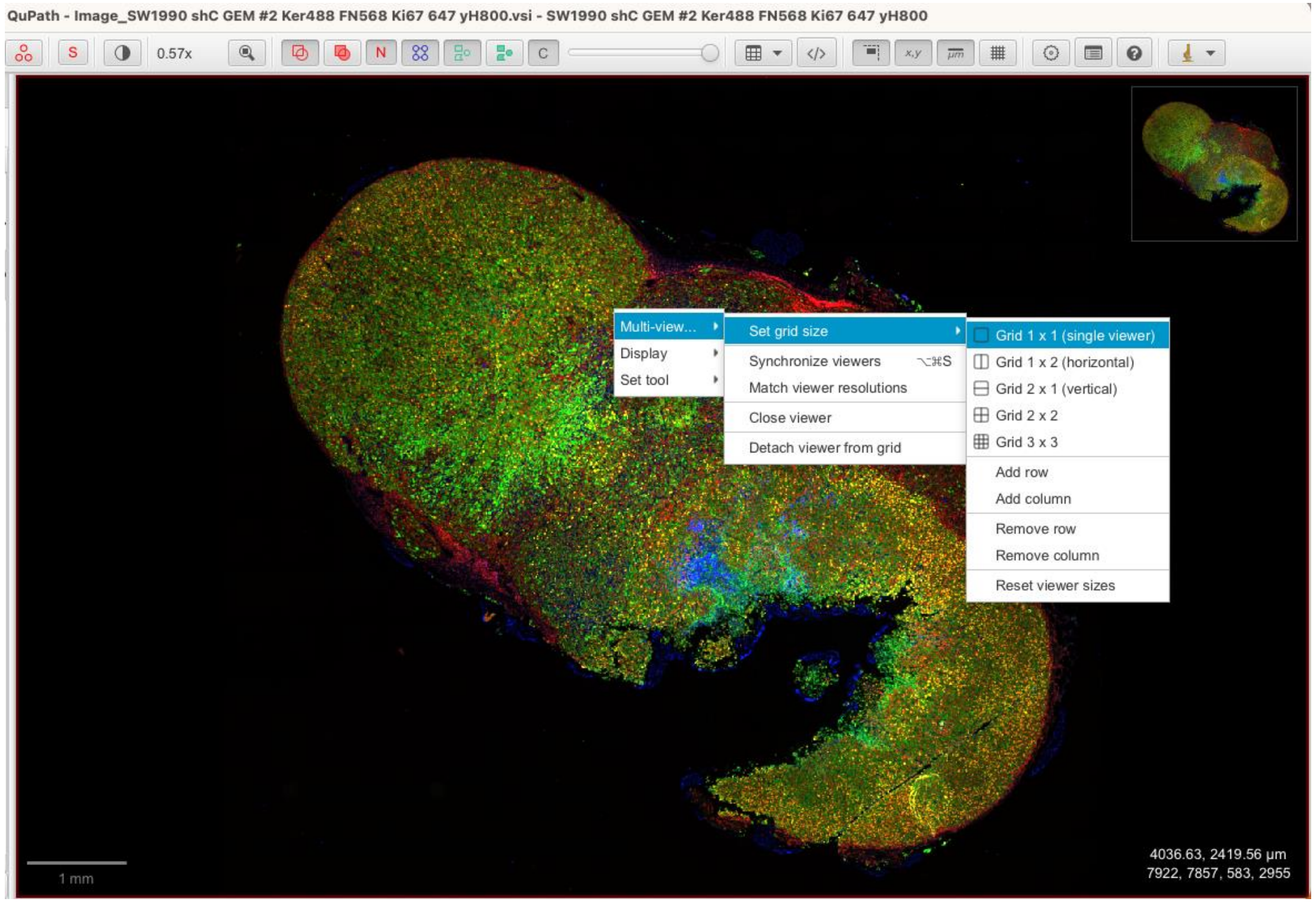
Mini-map: overview

Info bar: pixel coord and value

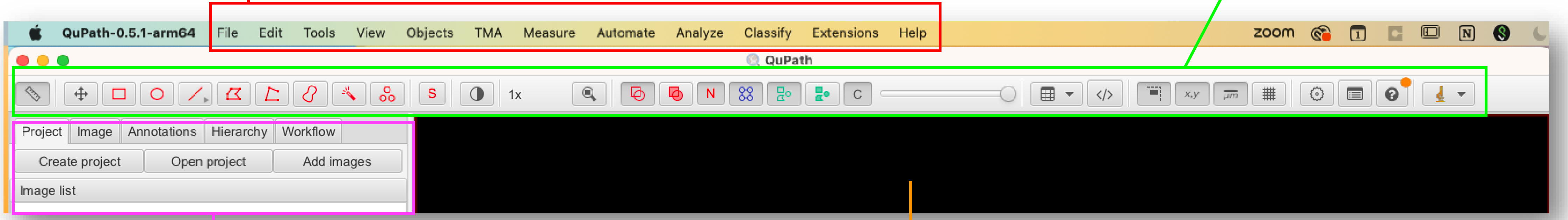
Scale bar

Multi-viewer

- Right-click in the viewer



Graphic User Interface (GUI)



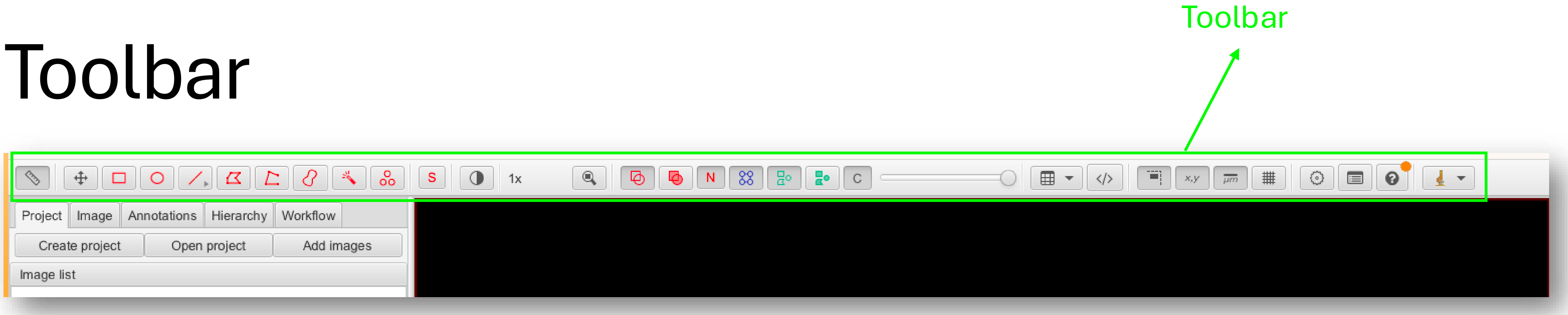
Menu bar

Toolbar

Analysis panel

QuPath Viewer (here cropped)

Toolbar

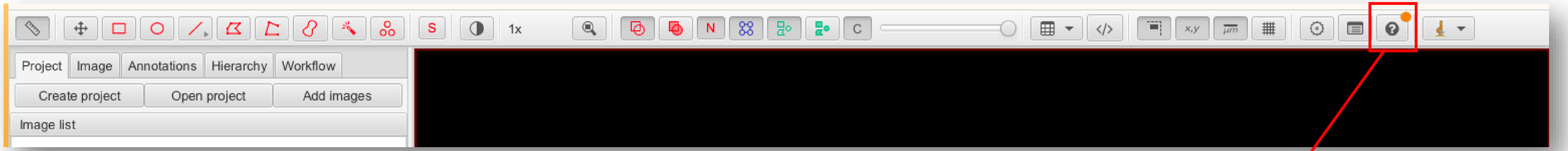

Toolbar
S
1x
N
C
Project
Image
Annotations
Hierarchy
Workflow
Create project
Open project
Add images
Image list

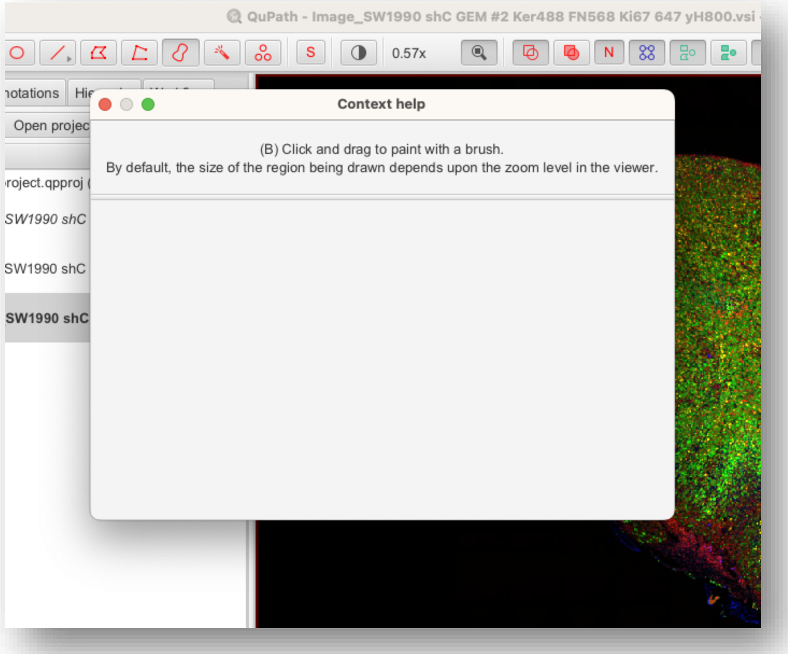
Toolbar

Preferences

Settings, GUI customization, extensions, ...

Toolbar



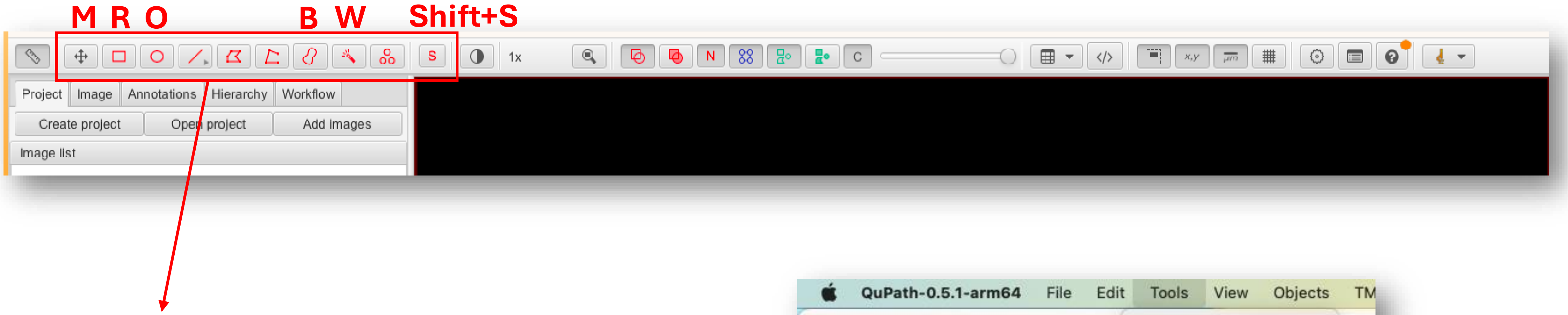


(B) Click and drag to paint with a brush.
By default, the size of the region being drawn depends upon the zoom level in the viewer.

Interactive Help
Provides contextual help based on your cursor location

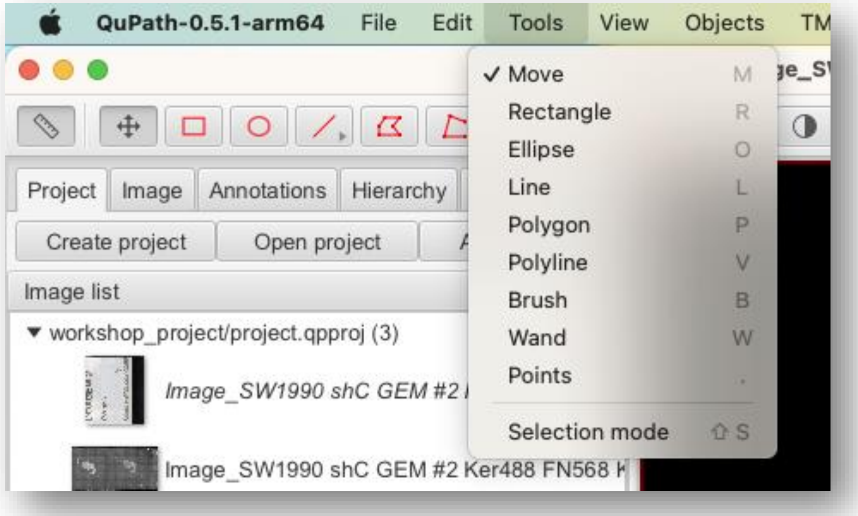
Example when my cursor is on the paint brush tool

Toolbar



Annotation tools

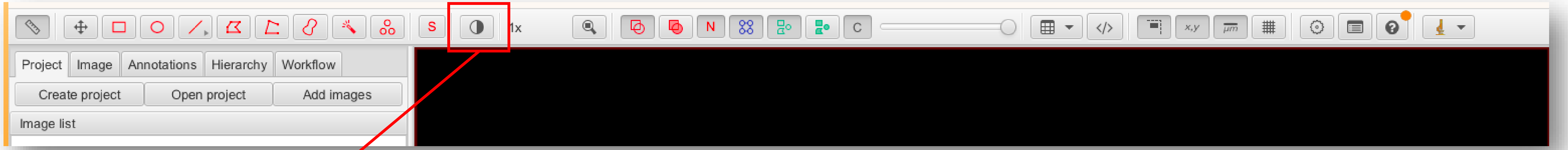
- **M** key: move tool
- **R** key: draw a rectangle annotation
- **O** key: draw an ellipse annotation
- **B** key: paint with a brush
- **W** key: draw with a wand tool
- And many more!



Annotation tools are also accessible in the *Tools* menu

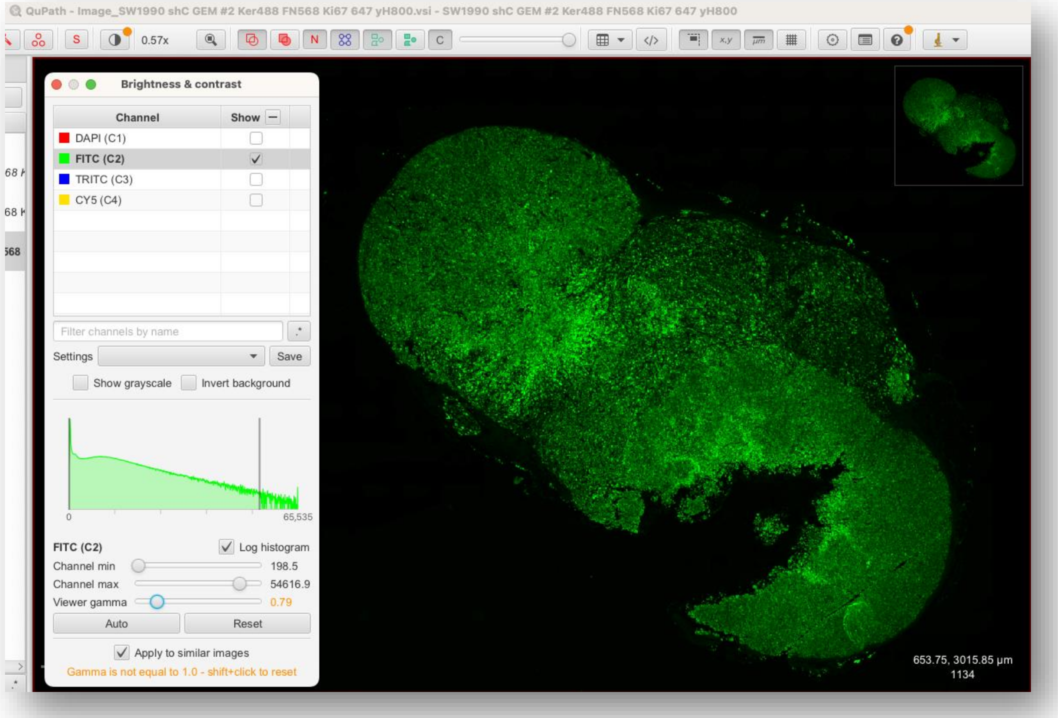
Toolbar

Shift+C



Brightness and contrast

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


QuPath - Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800

Brightness & contrast

Channel	Show
🟥 DAPI (C1)	☐
🟩 FITC (C2)	☑
🟦 TRITC (C3)	☐
🟨 CY5 (C4)	☐

☐ Show grayscale ☐ Invert background

FITC (C2) ☑ Log histogram

Channel min 198.5

Channel max 54616.9

Viewer gamma 0.79

☑ Apply to similar images

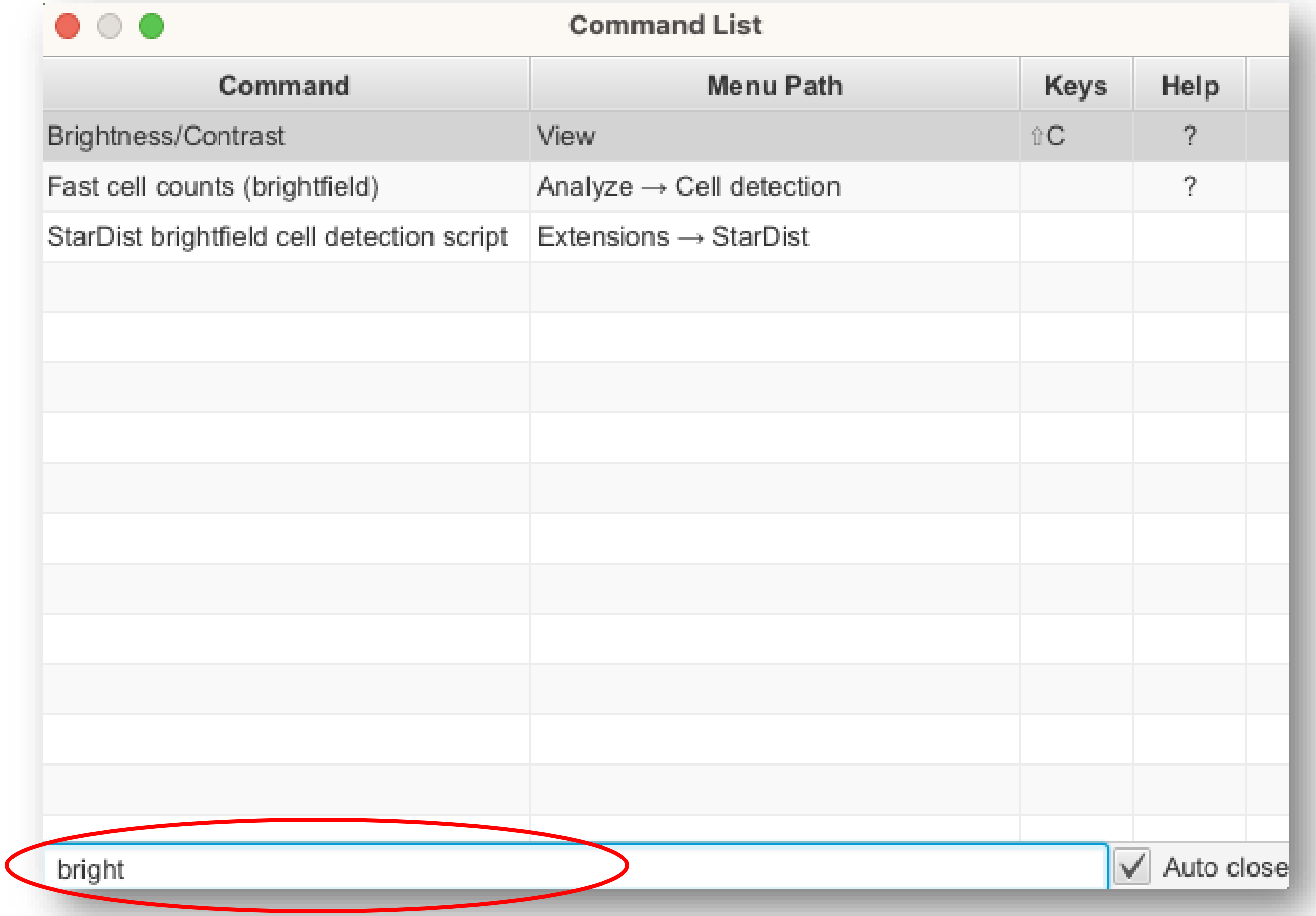
Gamma is not equal to 1.0 - shift+click to reset

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’



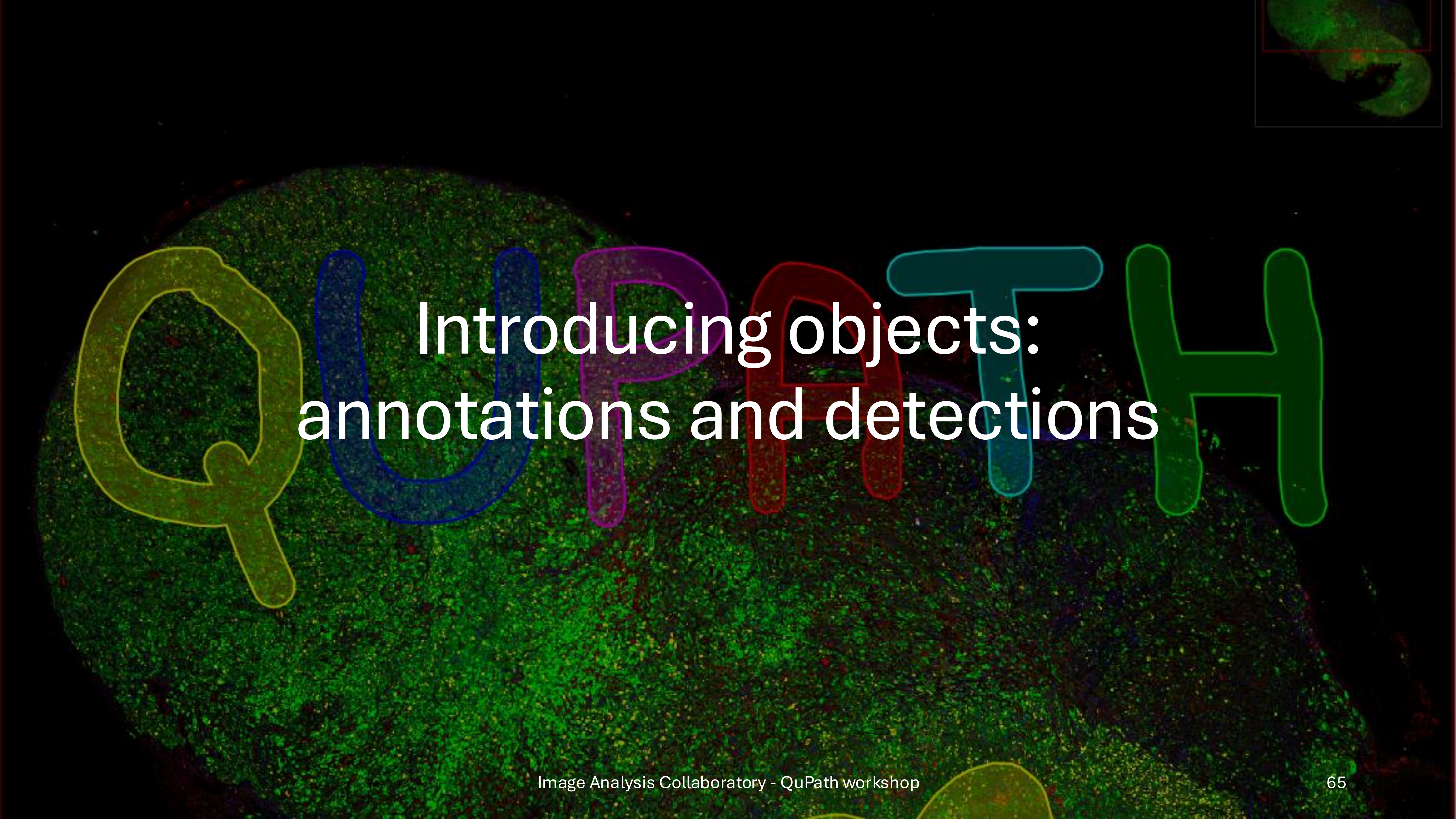
Command	Menu Path	Keys	Help
Brightness/Contrast	View	⇧C	?
Fast cell counts (brightfield)	Analyze → Cell detection		?
StarDist brightfield cell detection script	Extensions → StarDist		

bright

☑ Auto close

Practice time

Exercises 1: QuPath projects and GUI



Introducing objects: annotations and detections

Key concept: QuPath objects

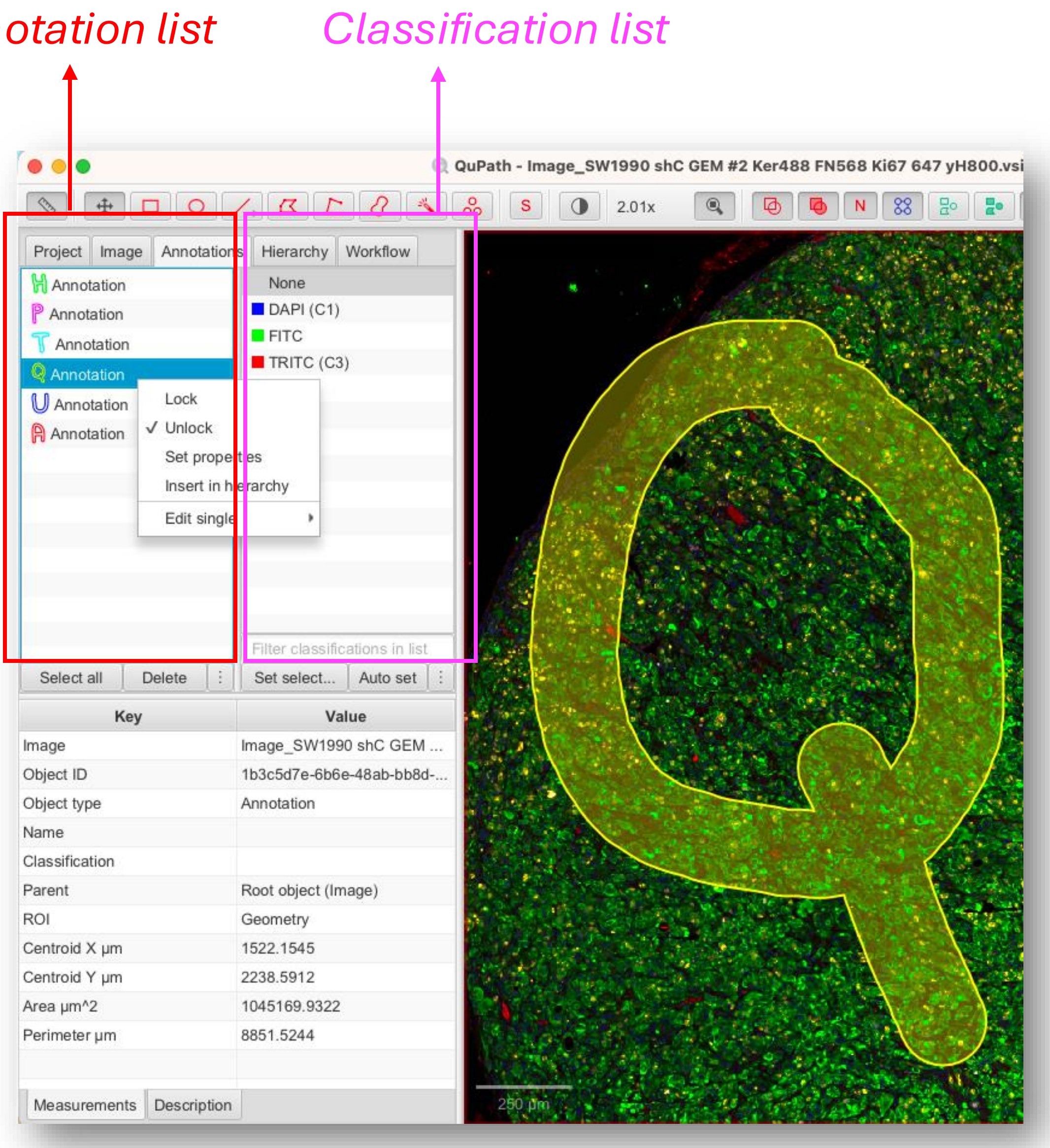
- **Objects** are a ‘thing’ in an image which encapsulates not only its shape but also some properties about it

  - **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



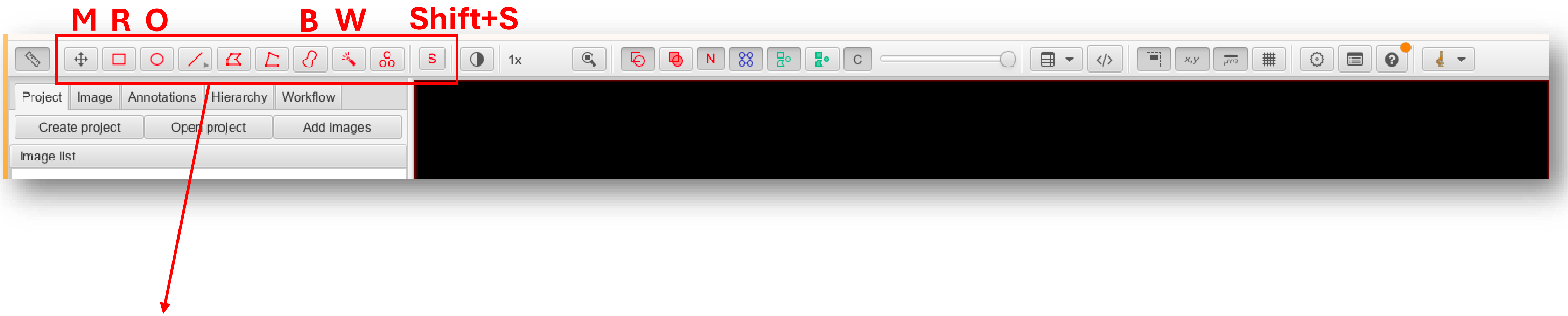
Annotation list

Classification list

Key	Value
Image	Image_SW1990 shC GEM ...
Object ID	1b3c5d7e-6b6e-48ab-bb8d-...
Object type	Annotation
Name	
Classification	
Parent	Root object (Image)
ROI	Geometry
Centroid X µm	1522.1545
Centroid Y µm	2238.5912
Area µm^2	1045169.9322
Perimeter µm	8851.5244

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!

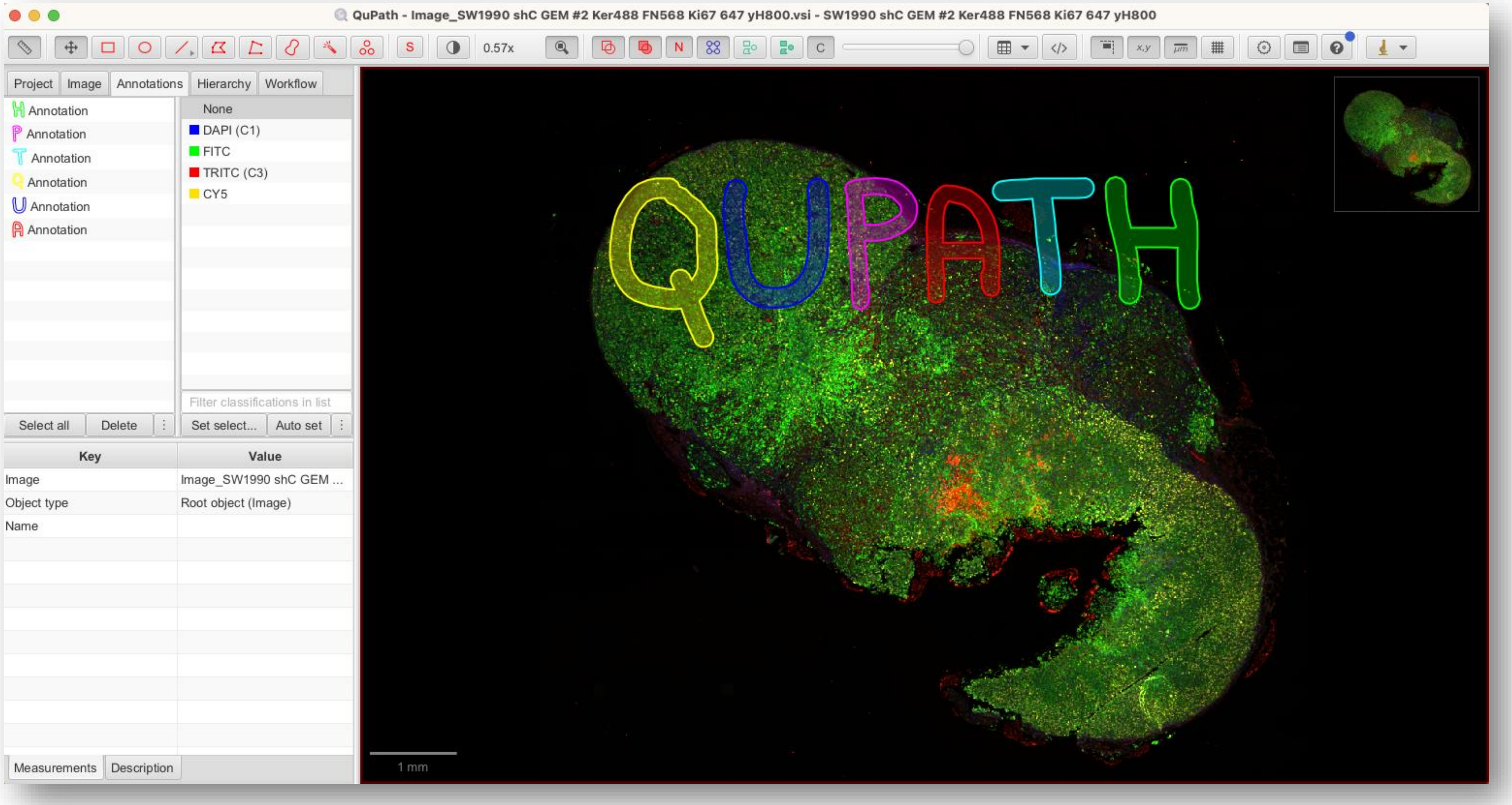
Remember to always lock your annotation to prevent accidental editing!

Practice time

Exercises 2: QuPath manual annotations

Recreate these annotations

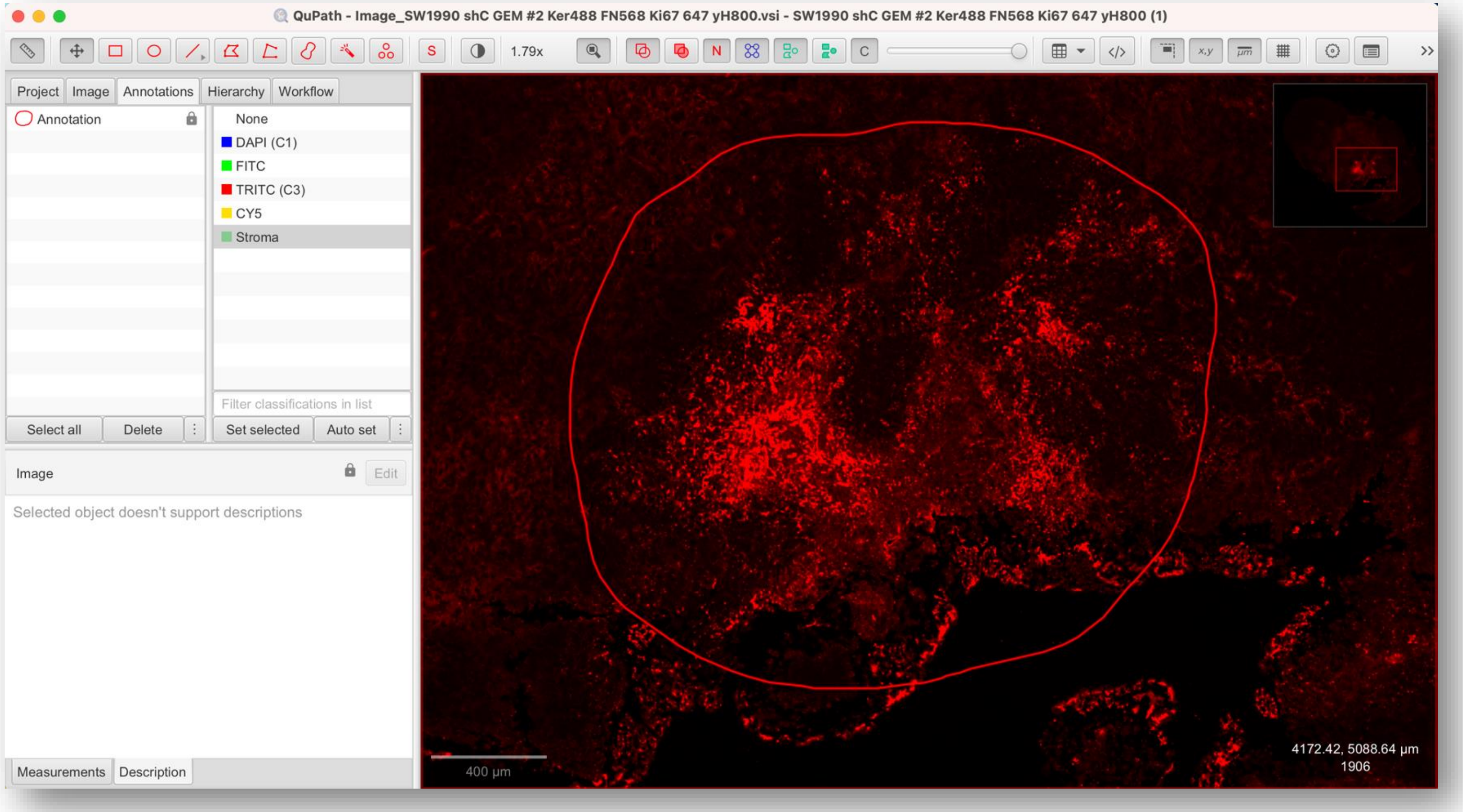
Decide on which annotations tool from the toolbar is best to do so



Key	Value
Image	Image_SW1990 shC GEM ...
Object type	Root object (Image)
Name	

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



Selected object doesn't support descriptions

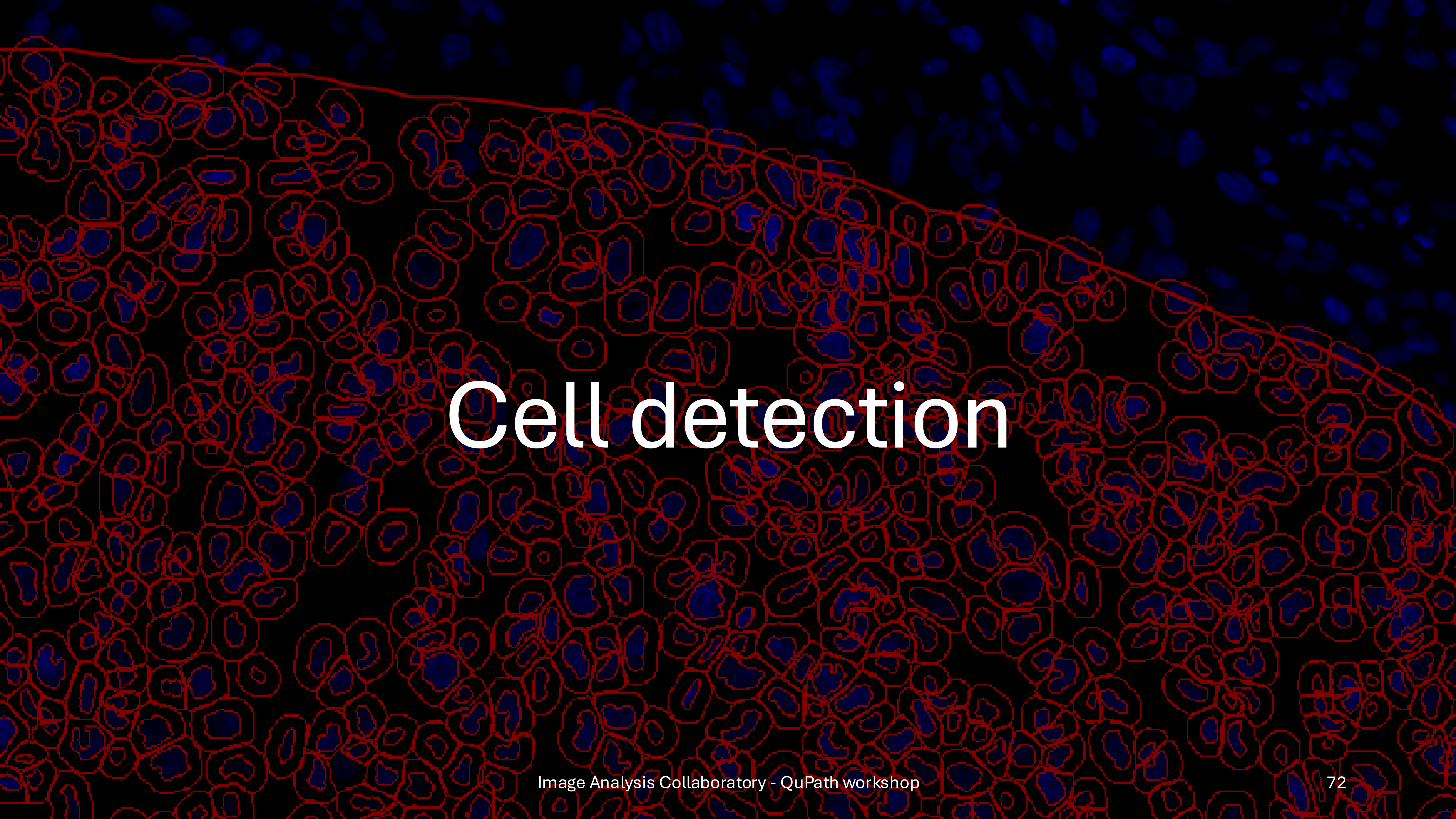
Once you have finished your annotation, **lock** it:

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

or

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

Cell detection



Cell detection

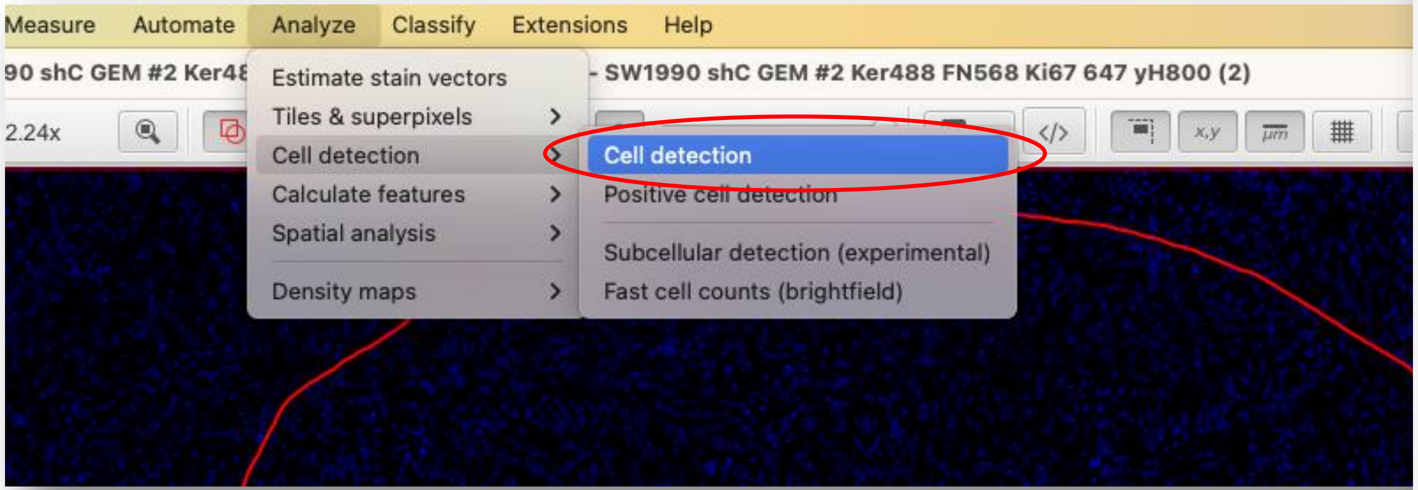
- QuPath offers three main options:
  1. Built-in cell segmentation algorithm, based on nucleus thresholding and cell body expansion
  2. StarDist as an extension (DL)
  3. Cellpose as an extension (DL) – not covered here

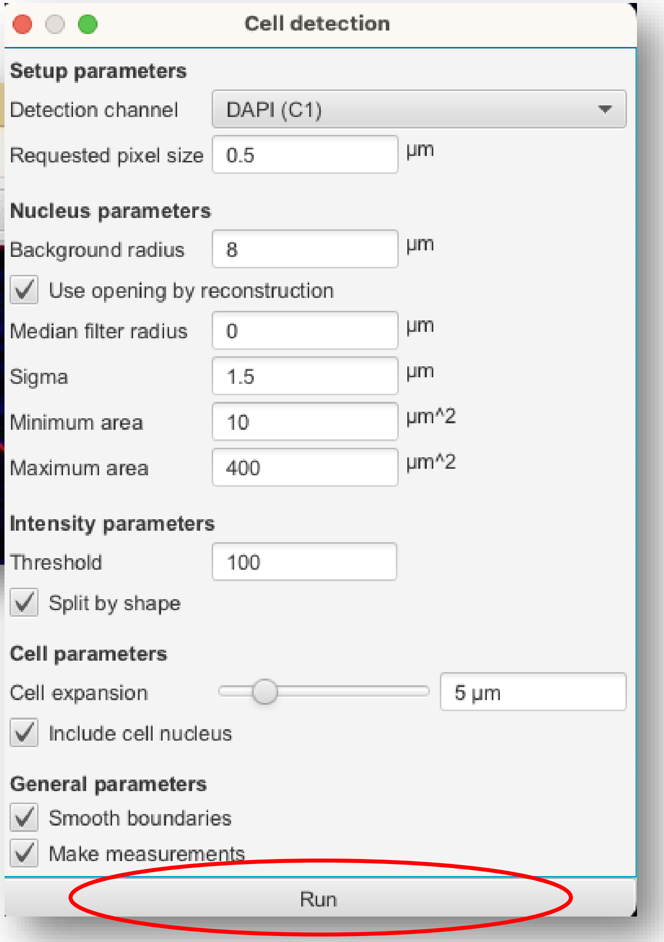
- All yield *Cell Detections* objects that will have shape and intensity measurements for nucleus, cell and membrane

- Detection can be computationally intensive so we will start from the region of interest

Cell detection

1. Built-in cell segmentation algorithm, based on nucleus thresholding and cell body expansion





Setup parameters

Detection channel: DAPI (C1)

Requested pixel size: 0.5 µm

Nucleus parameters

Background radius: 8 µm

☑ Use opening by reconstruction

Median filter radius: 0 µm

Sigma: 1.5 µm

Minimum area: 10 µm^2

Maximum area: 400 µm^2

Intensity parameters

Threshold: 100

☑ Split by shape

Cell parameters

Cell expansion: 5 µm

☑ Include cell nucleus

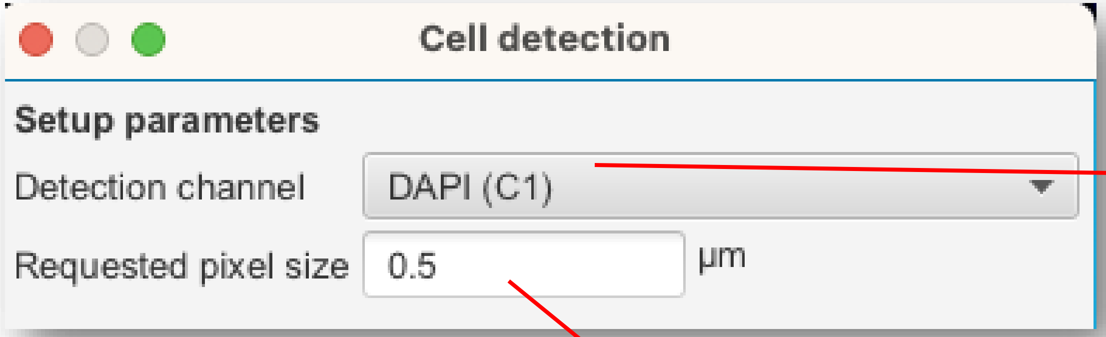
General parameters

☑ Smooth boundaries

☑ Make measurements

Note that positive cell detection allows for multi-class segmentation on the fly

Cell detection parameters



Setup parameters

Detection channel: DAPI (C1)

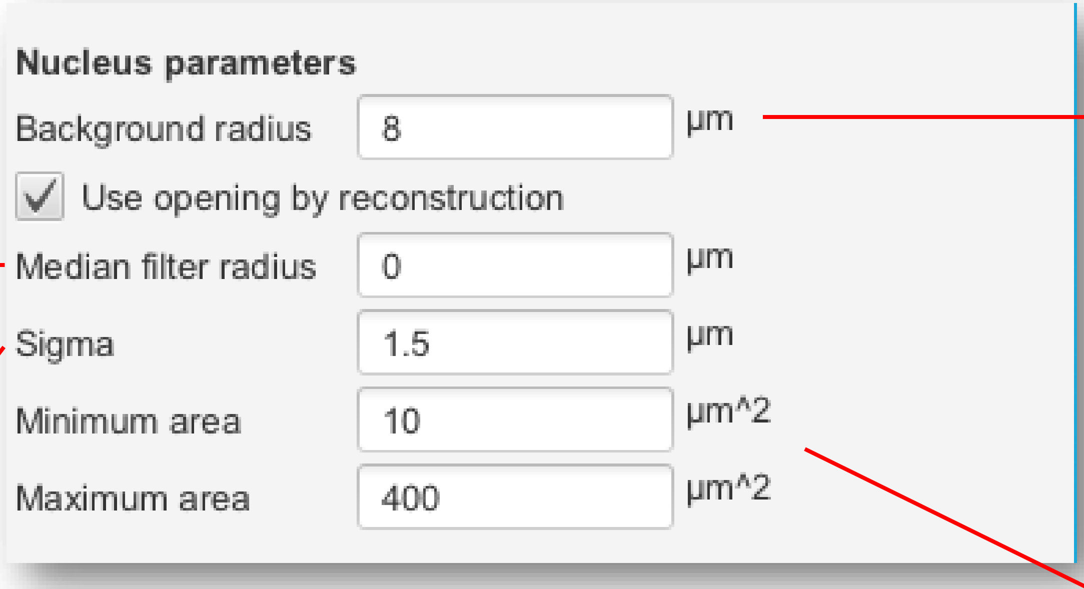
Requested pixel size: 0.5 µm

Channel e.g. DAPI

The resolution of the image used in the segmentation algorithm

- Enter 0 for full resolution
- Default 0.5 typically good trade-off between cost and details

Cell detection parameters



Nucleus parameters

Background radius 8 µm

☑ Use opening by reconstruction

Median filter radius 0 µm

Sigma 1.5 µm

Minimum area 10 µm^2

Maximum area 400 µm^2

Radius of area used for background subtraction

Radius of median filter (smoothing):

- Enter 0 to disable
- Higher values will smooth off details

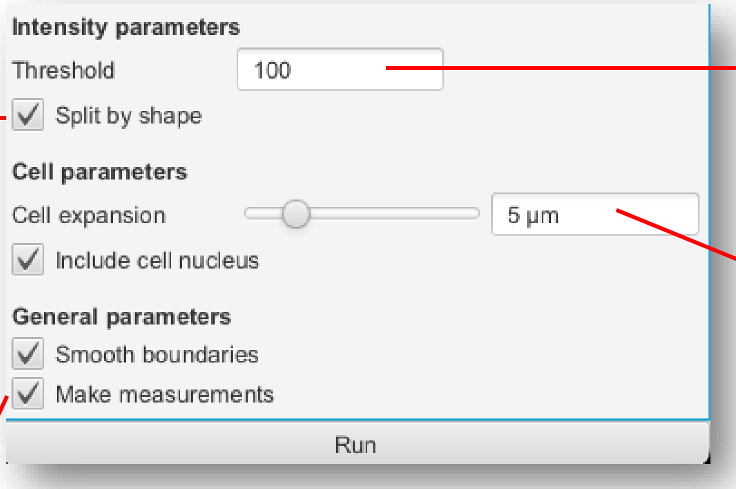
Radius of gaussian filter (smoothing):

- Enter 0 to disable
- Higher values will smooth off details

Allowed area interval for detections; nuclei detection is removed if outside of the interval

Cell detection parameters

Uses roundness of detections shape to split clusters/clumps; keep it ticked for most usages



Intensity parameters

Threshold 100

☑ Split by shape

Cell parameters

Cell expansion 5 µm

☑ Include cell nucleus

General parameters

☑ Smooth boundaries

☑ Make measurements

Minimum signal intensity of nuclei relative to background

How much to expand nuclei to get cell boundaries

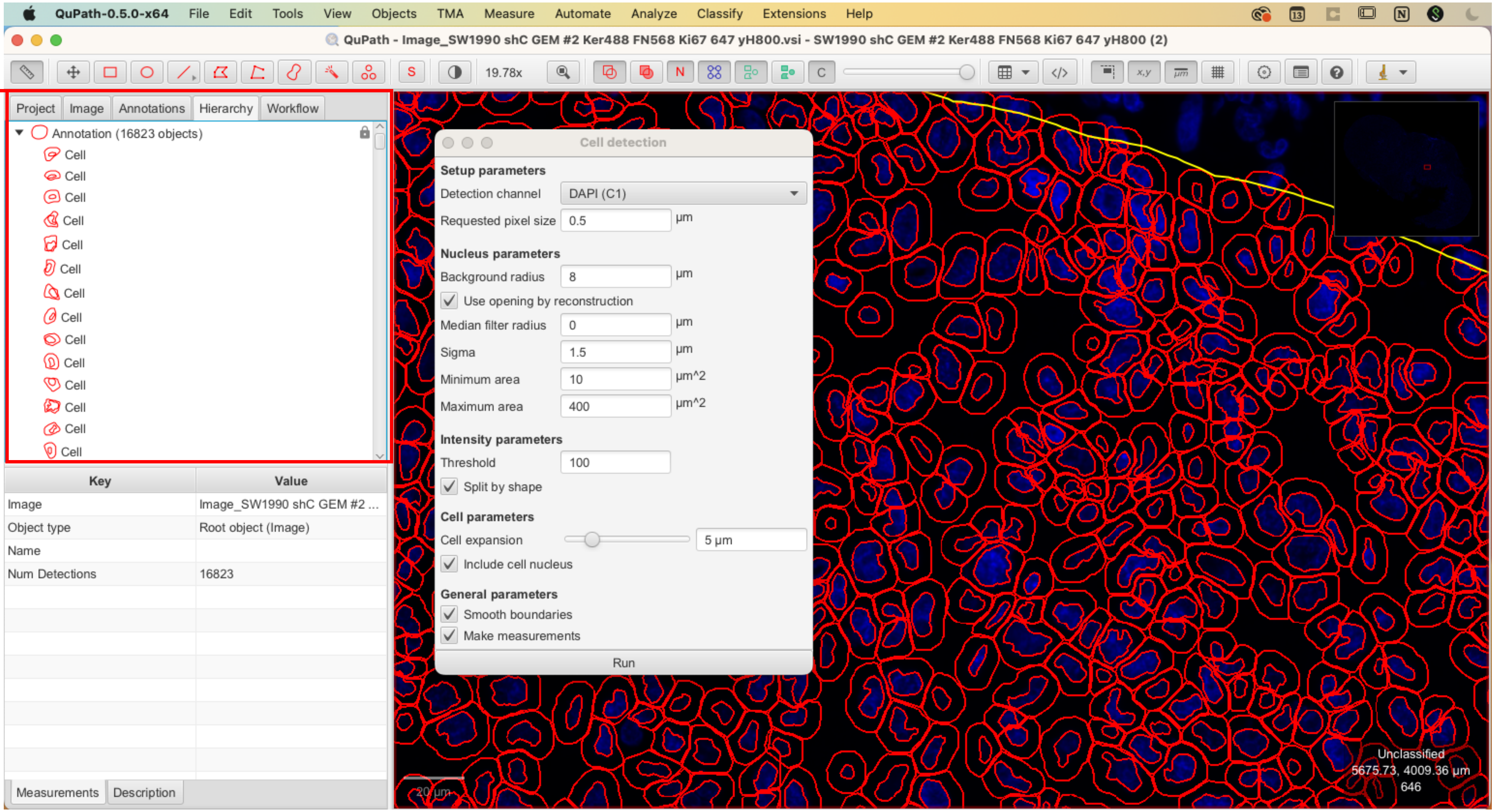
- Enter 0 to disable
- Enter small values 0 – 2 for peri-nuclear measurements
- Enter values ~5 for cytoplasm measurements, depending on tissues

If ticked, will generate measurements specific to each detected nuclei and inferred cytoplasm

Cell detection with default parameters

Hierarchy tab

- Detection list
- Nested in its parent annotation (ROI)
- Note the cell count



QuPath - Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800 (2)

Annotation (16823 objects)
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell
- Cell

Key	Value
Image	Image_SW1990 shC GEM #2 ...
Object type	Root object (Image)
Name	
Num Detections	16823

Cell detection

Setup parameters
Detection channel: DAPI (C1)
Requested pixel size: 0.5 µm

Nucleus parameters
Background radius: 8 µm
☑ Use opening by reconstruction
Median filter radius: 0 µm
Sigma: 1.5 µm
Minimum area: 10 µm^2
Maximum area: 400 µm^2

Intensity parameters
Threshold: 100
☑ Split by shape

Cell parameters
Cell expansion: 5 µm
☑ Include cell nucleus

General parameters
☑ Smooth boundaries
☑ Make measurements

Run

Note on the hierarchy of objects in QuPath

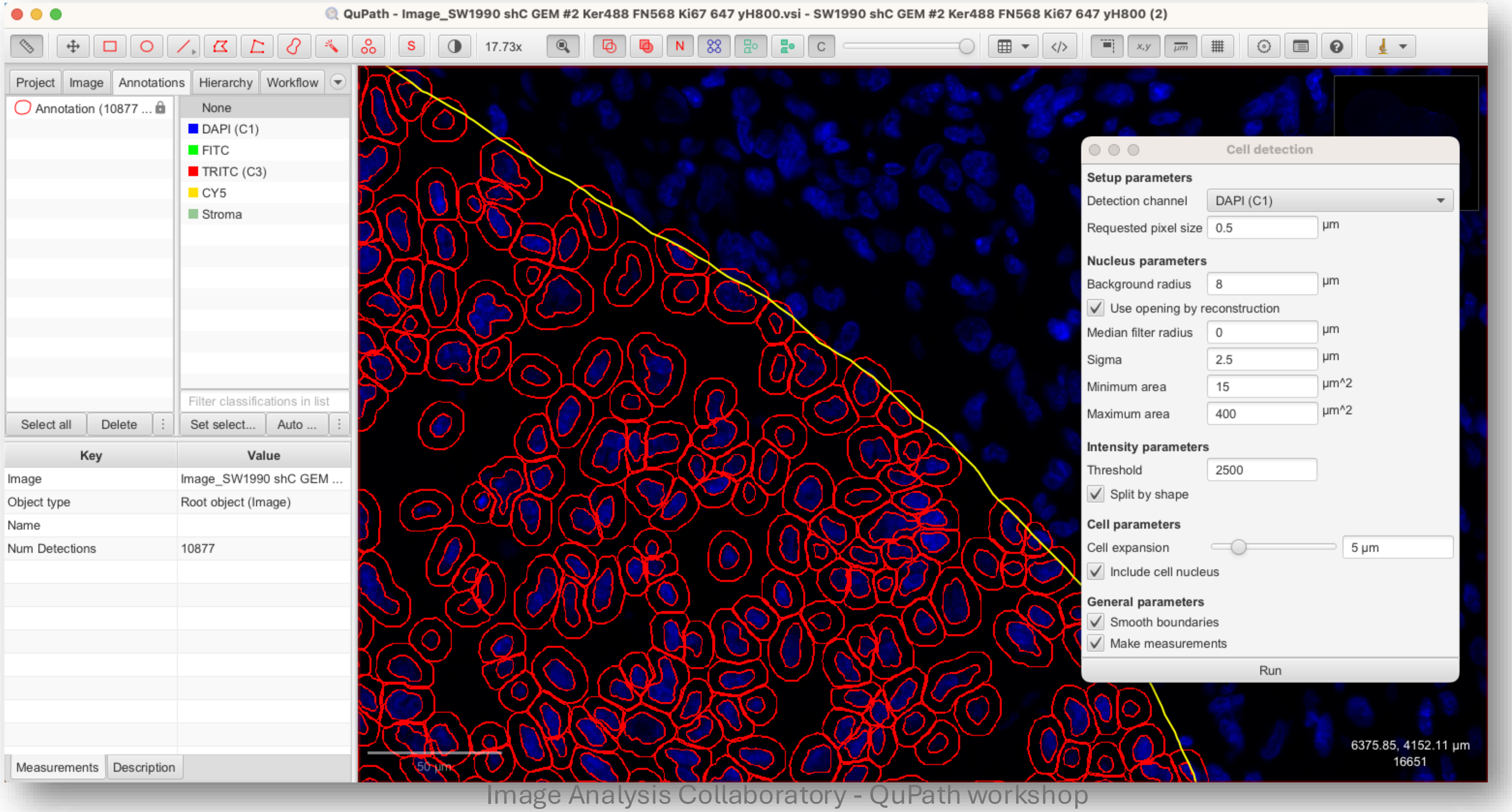
- QuPath allows to nest objects in one another to organize your projects
  - Child-parent link
  - Very useful to organize and restrict the analysis to parts of an image
  - Can be used to restrict image processing within a ROI or a detected tissue region

Practice time

Exercise 3.a: QuPath cell detection

Exercise: explore parameters

- I found that default parameters tend to over-segment nuclei so adapted the parameters to be slightly stricter (min area and threshold increased)



Key	Value
Image	Image_SW1990 shC GEM ...
Object type	Root object (Image)
Name	
Num Detections	10877

Setup parameters

Detection channel: DAPI (C1)

Requested pixel size: 0.5 µm

Nucleus parameters

Background radius: 8 µm

☑ Use opening by reconstruction

Median filter radius: 0 µm

Sigma: 2.5 µm

Minimum area: 15 µm^2

Maximum area: 400 µm^2

Intensity parameters

Threshold: 2500

☑ Split by shape

Cell parameters

Cell expansion: 5 µm

☑ Include cell nucleus

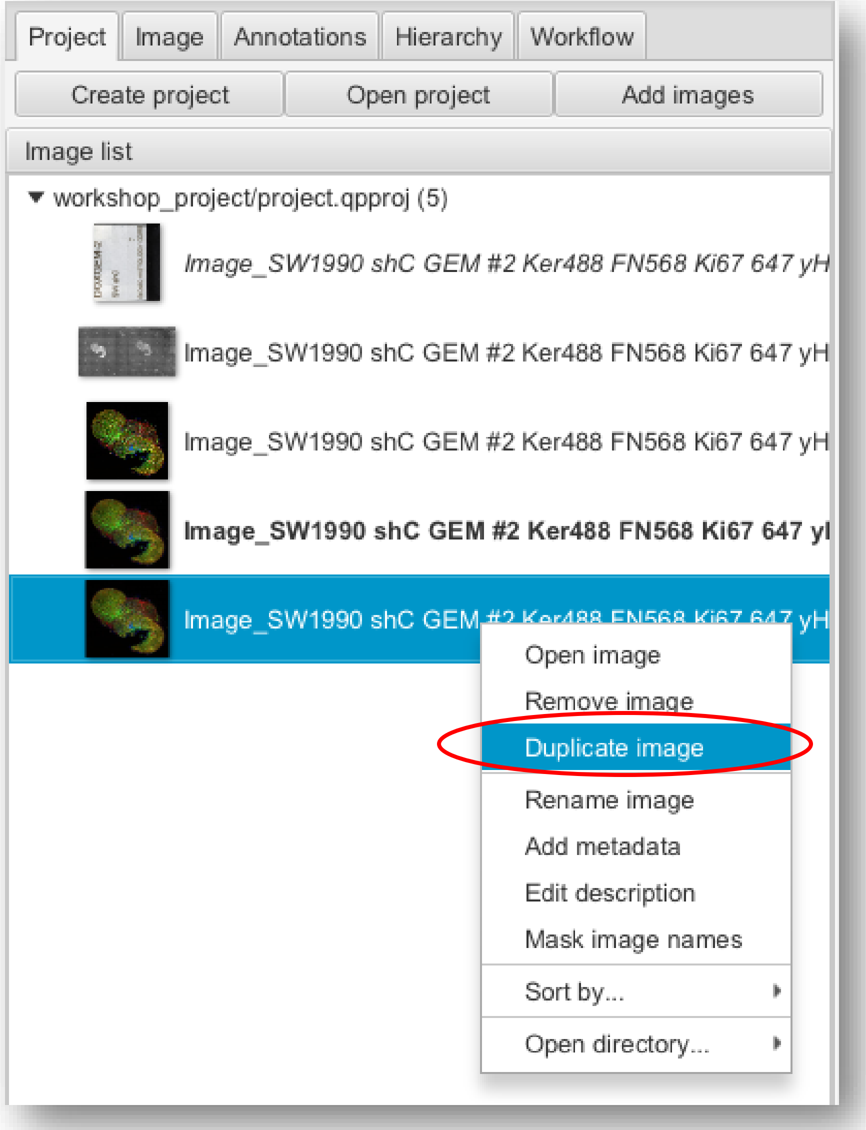
General parameters

☑ Smooth boundaries

☑ Make measurements

Duplicate your image

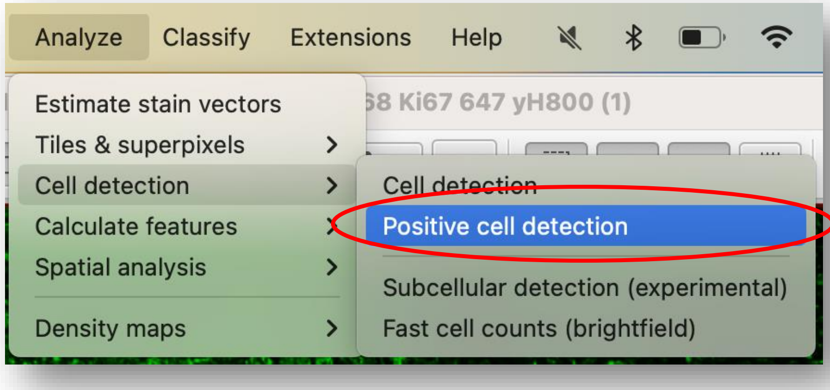
- Copy your cell detection results for future work on it
- *Project tab* > *Image list* > Option+click or *right-click* on the image name > *Duplicate image*



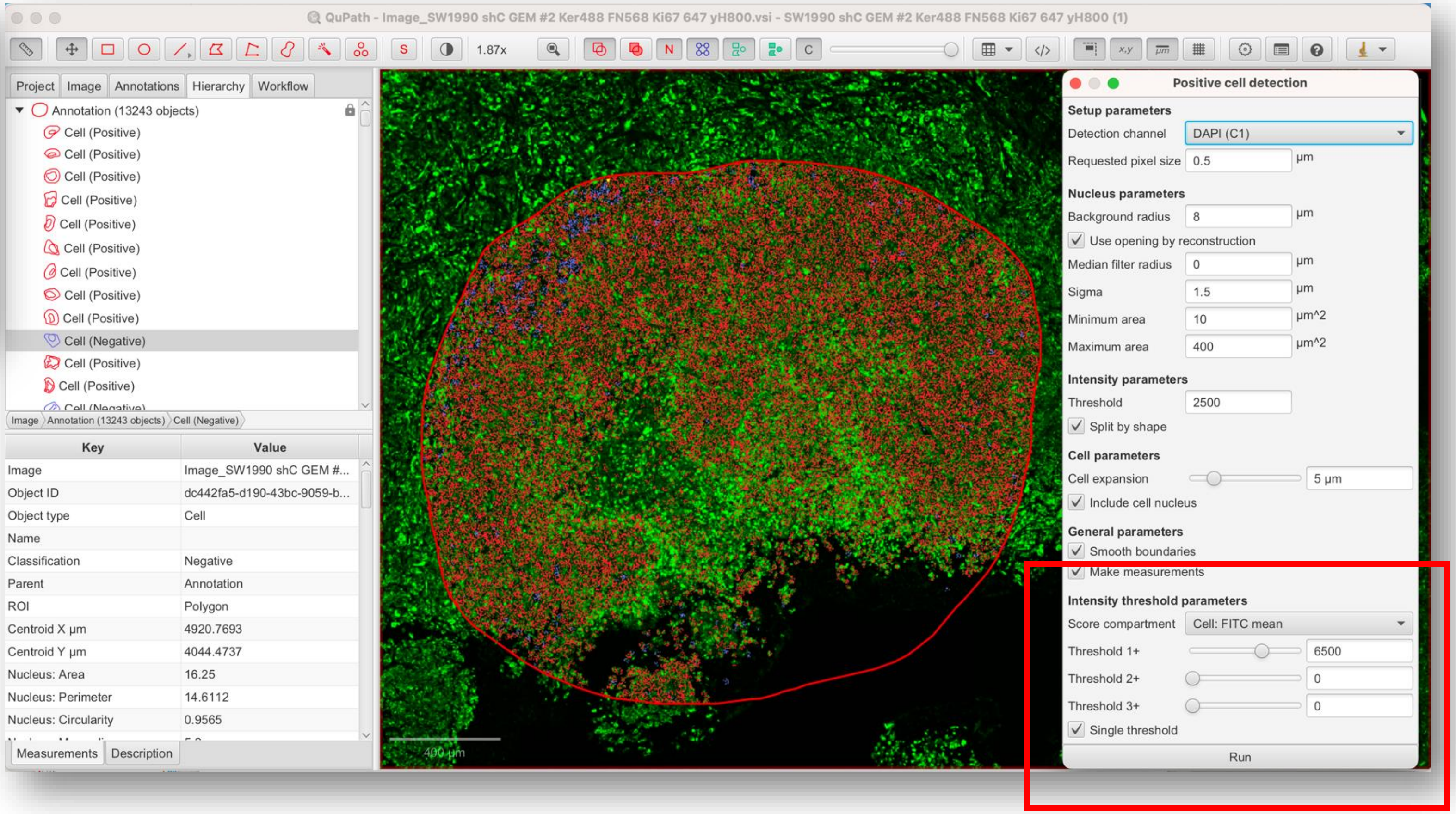
It duplicates QuPath objects, not the actual image

Detecting cells with an extra condition

- *Analyze > Cell detection > Positive cell detection*



- Adds an extra step of classifying all cells as positive or negative immediately according to staining intensity



Key	Value
Image	Image_SW1990 shC GEM #...
Object ID	dc442fa5-d190-43bc-9059-b...
Object type	Cell
Name	
Classification	Negative
Parent	Annotation
ROI	Polygon
Centroid X µm	4920.7693
Centroid Y µm	4044.4737
Nucleus: Area	16.25
Nucleus: Perimeter	14.6112
Nucleus: Circularity	0.9565

Setup parameters
Detection channel: DAPI (C1)
Requested pixel size: 0.5 µm

Nucleus parameters
Background radius: 8 µm
☑ Use opening by reconstruction
Median filter radius: 0 µm
Sigma: 1.5 µm
Minimum area: 10 µm^2
Maximum area: 400 µm^2

Intensity parameters
Threshold: 2500
☑ Split by shape

Cell parameters
Cell expansion: 5 µm
☑ Include cell nucleus

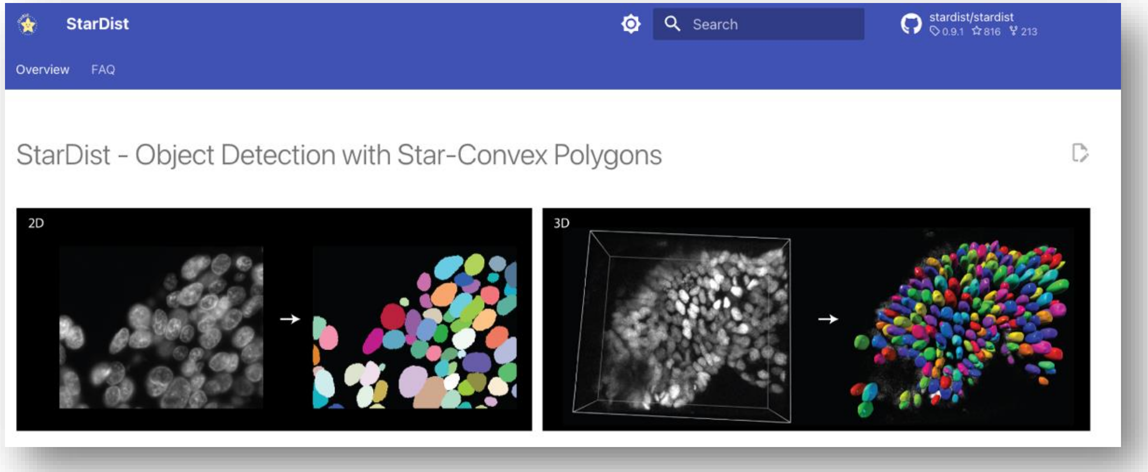
General parameters
☑ Smooth boundaries
☑ Make measurements

Intensity threshold parameters
Score compartment: Cell: FITC mean
Threshold 1+: 6500
Threshold 2+: 0
Threshold 3+: 0
☑ Single threshold

Deep learning-based cell segmentation

- DL-based methods can typically capture more complex patterns, tend to mitigate human bias such as threshold hand-picking
- **However**, they are more computationally expensive and often need fine-tuning or re-training for specific applications

- StarDist is a deep learning model trained to detect specific kinds of nuclei in different kinds of image

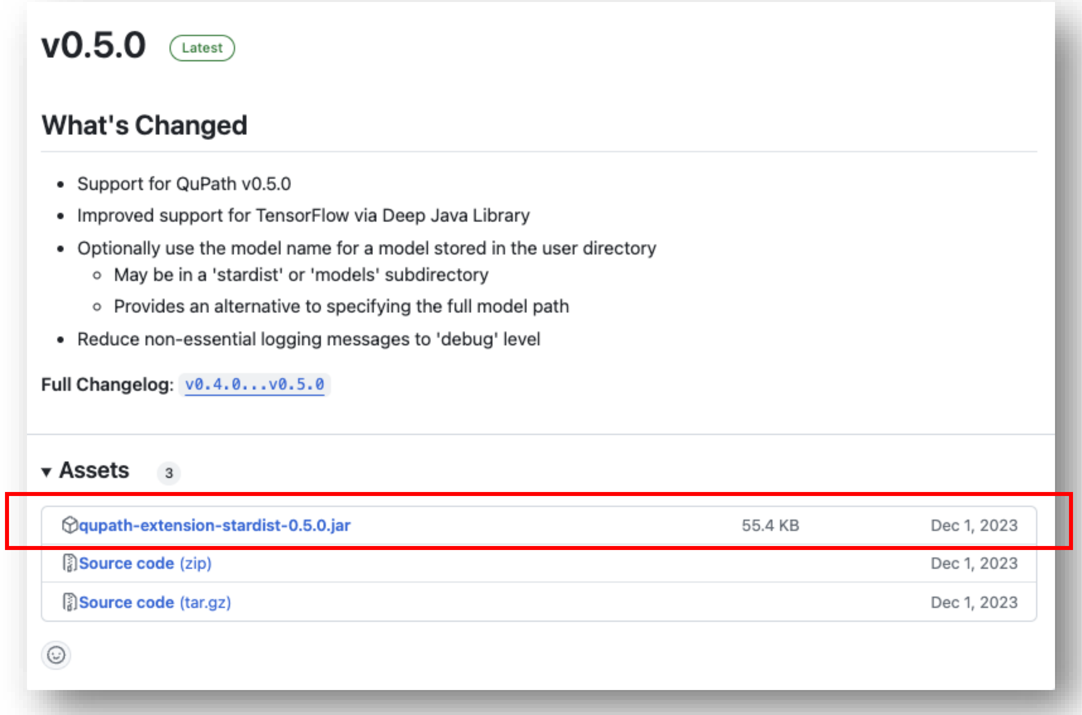


StarDist - Object Detection with Star-Convex Polygons

Installing StarDist extension in QuPath

- Browse to https://github.com/qupath/qupath-extension-stardist/releases
- Download the .jar file compatible with your QuPath version
    - For this workshop, get [qupath-extension-stardist-0.5.0.jar]()

- Drag and drop the .jar file onto QuPath main window, and… that's it!



v0.5.0 Latest

What's Changed

- Support for QuPath v0.5.0
- Improved support for TensorFlow via Deep Java Library
- Optionally use the model name for a model stored in the user directory
    - May be in a 'stardist' or 'models' subdirectory
    - Provides an alternative to specifying the full model path
- Reduce non-essential logging messages to 'debug' level

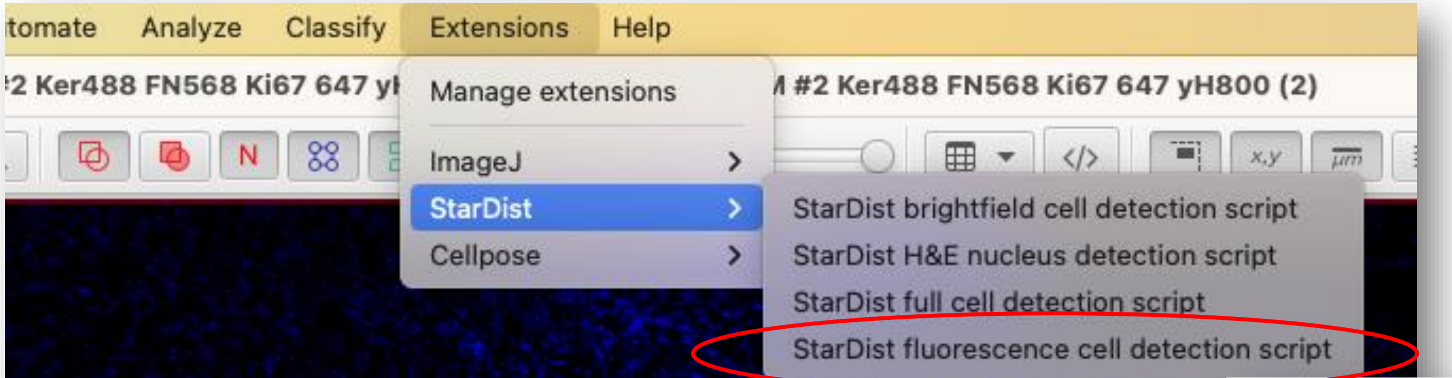
Full Changelog: `v0.4.0...v0.5.0`

▾ Assets 3

qupath-extension-stardist-0.5.0.jar	55.4 KB	Dec 1, 2023
Source code (zip)		Dec 1, 2023
Source code (tar.gz)		Dec 1, 2023

Using StarDist extension in QuPath

- Go to *Extensions* tab > *StarDist* > *StardDist fluorescence cell detection script*

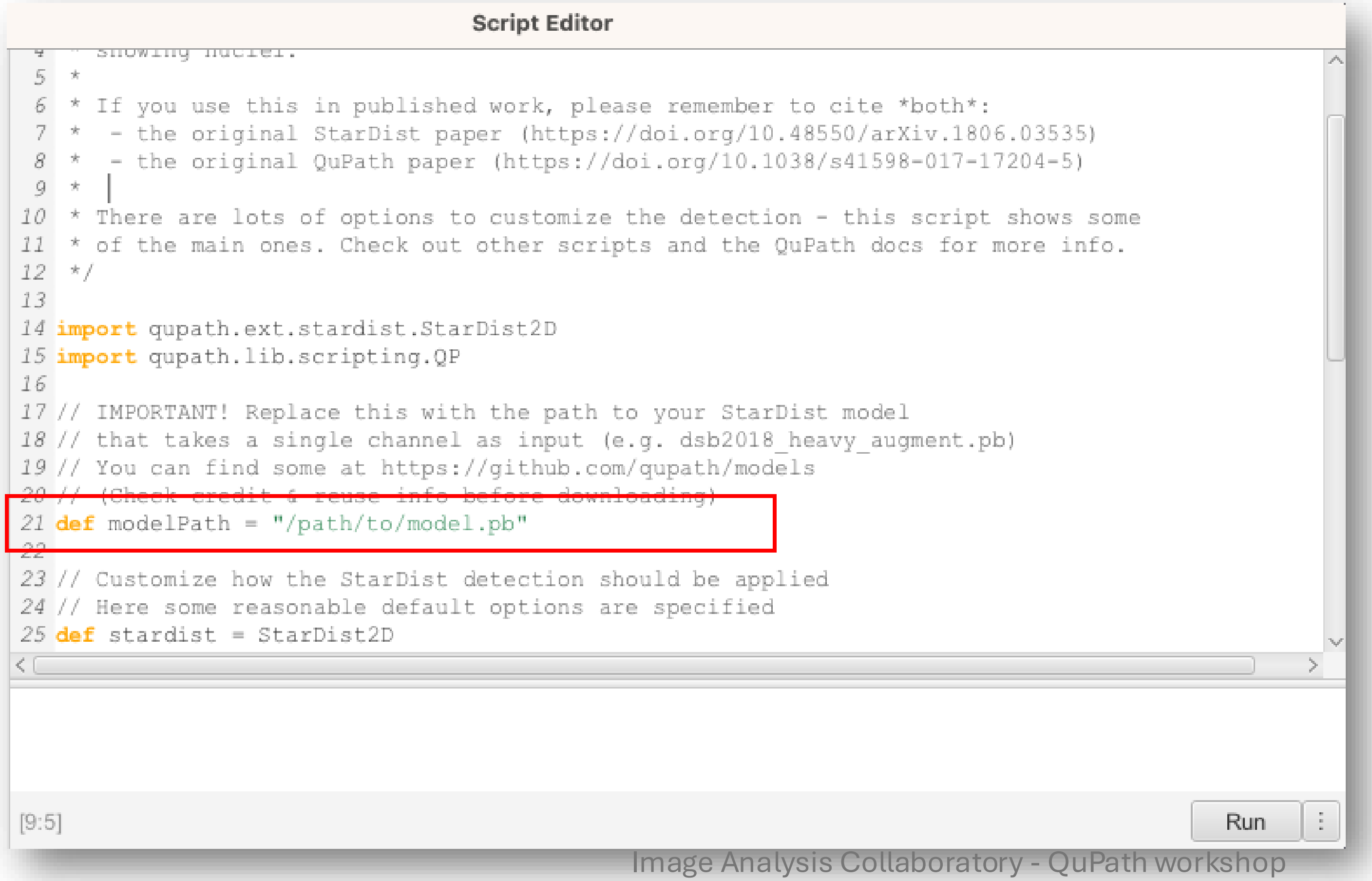


```
/**
 * This script provides a general template for cell detection using StarDist in QuPath.
 * This example assumes you have fluorescence image, which has a channel called 'DAPI'
 * showing nuclei.
 *
 * If you use this in published work, please remember to cite *both*:
 *  - the original StarDist paper (https://doi.org/10.48550/arXiv.1806.03535)
 *  - the original QuPath paper (https://doi.org/10.1038/s41598-017-17204-5)
 *
 * There are lots of options to customize the detection - this script shows some
 * of the main ones. Check out other scripts and the QuPath docs for more info.
 */

import qupath.ext.stardist.StarDist2D
import qupath.lib.scripting.QP

// IMPORTANT! Replace this with the path to your StarDist model
// that takes a single channel as input (e.g. dsb2018_heavy_augment.pb)
// You can find some at https://github.com/qupath/models
// (Check credit & reuse info before downloading)
def modelPath = "/path/to/model.pb"
```

No click-based solution yet!
But scripts are already written for you…

Using StarDist extension in QuPath

- Requires to load a pre-trained model (basically the weights)

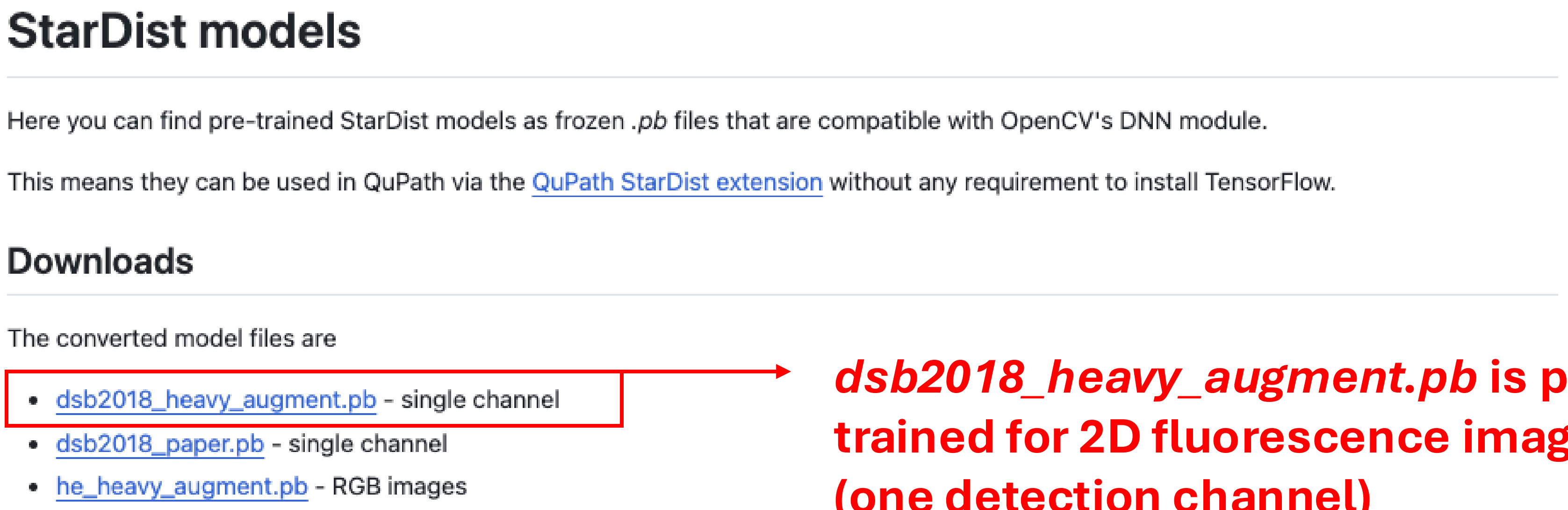


```
 * showing nuclei.
 *
 * If you use this in published work, please remember to cite *both*:
 *  - the original StarDist paper (https://doi.org/10.48550/arXiv.1806.03535)
 *  - the original QuPath paper (https://doi.org/10.1038/s41598-017-17204-5)
 *
 * There are lots of options to customize the detection - this script shows some
 * of the main ones. Check out other scripts and the QuPath docs for more info.
 */

import qupath.ext.stardist.StarDist2D
import qupath.lib.scripting.QP

// IMPORTANT! Replace this with the path to your StarDist model
// that takes a single channel as input (e.g. dsb2018_heavy_augment.pb)
// You can find some at https://github.com/qupath/models
// (Check credit & reuse info before downloading)
def modelPath = "/path/to/model.pb"

// Customize how the StarDist detection should be applied
// Here some reasonable default options are specified
def stardist = StarDist2D
```

Note: StarDist is rather computationally expensive, typically can take ~ 5 min for 100k detections

StarDist for 2D segmentation of DAPI-stained nuclei

- Some pre-trained StarDist models are freely available as .*pb* files (frozen)
- Go to https://github.com/qupath/models/raw/main/stardist and download the dsb2018_heavy_augment.pb model



StarDist models

Here you can find pre-trained StarDist models as frozen .*pb* files that are compatible with OpenCV's DNN module.

This means they can be used in QuPath via the QuPath StarDist extension without any requirement to install TensorFlow.

Downloads

The converted model files are

- dsb2018_heavy_augment.pb - single channel
- dsb2018_paper.pb - single channel
- he_heavy_augment.pb - RGB images

***dsb2018_heavy_augment.pb* is pre-trained for 2D fluorescence images (one detection channel)**

Using StarDist extension in QuPath

- Change the value of the **modelPath** variable to an actual StarDist model path in the script

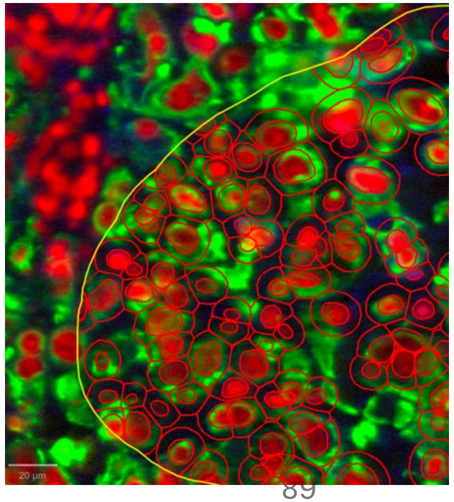
Change the channel name

```
// You can find some at https://github.com/qupath/models
// (Check credit & reuse info before downloading)
def modelPath = "/Users/antoine/Desktop/test_qupath_workshop/models/dsb2018_heavy_augment.pb"

// Customize how the StarDist detection should be applied
// Here some reasonable default options are specified
def stardist = StarDist2D
    .builder(modelPath)
    .channels('DAPI (C1)')             // Extract channel called 'DAPI'
    .normalizePercentiles(1, 99) // Percentile normalization
    .threshold(0.5)              // Probability (detection) threshold
    .pixelSize(0.5)              // Resolution for detection
    .cellExpansion(5)            // Expand nuclei to approximate cell boundaries
    .measureShape()              // Add shape measurements
    .measureIntensity()          // Add cell measurements (in all compartments)
    .build()

```

```
INFO: Done!
```

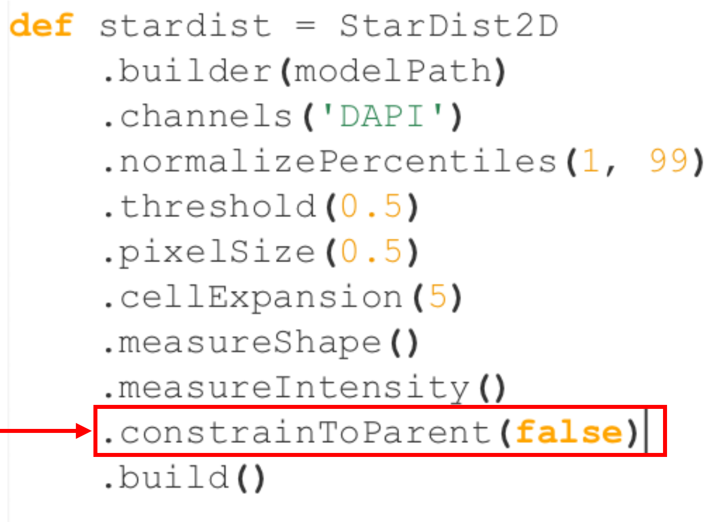
```
[27:24]   Stopped: 0:00:12
```

Make sure to select the ROI in QuPath before running the script.



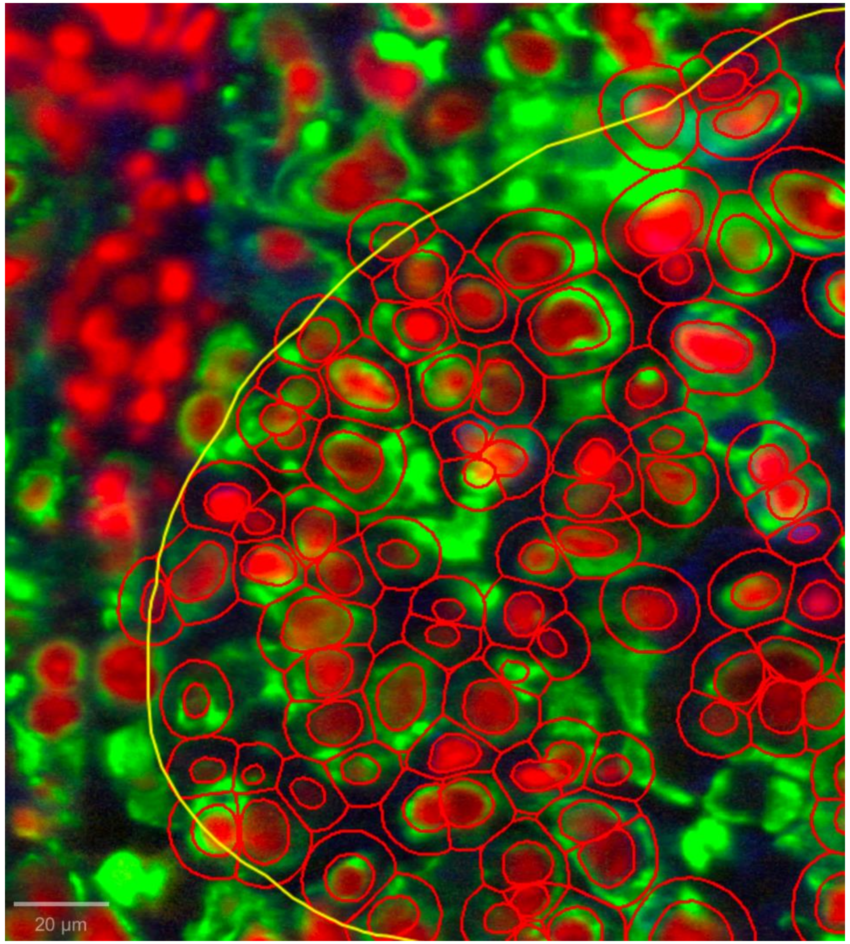
Allow cell boundaries to bleed over the ROI

- Add *.constrainToParent(false)*



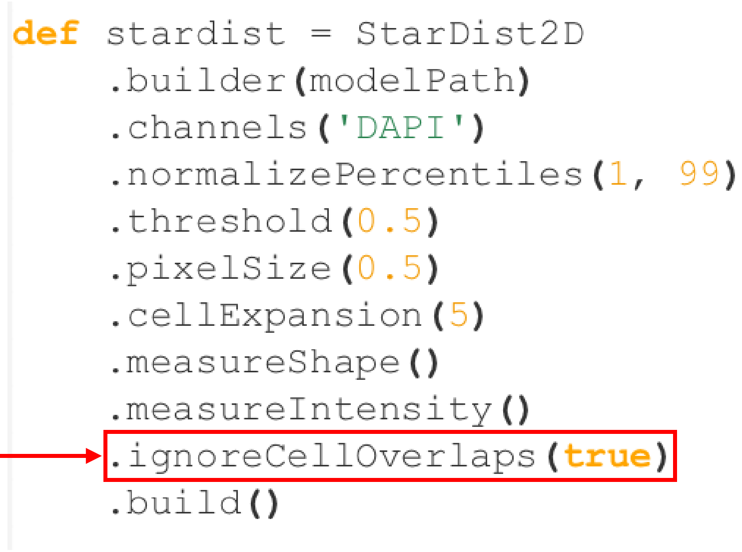
```
def stardist = StarDist2D
      .builder(modelPath)
      .channels('DAPI')
      .normalizePercentiles(1, 99)
      .threshold(0.5)
      .pixelSize(0.5)
      .cellExpansion(5)
      .measureShape()
      .measureIntensity()
      .constrainToParent(false)
      .build()
```

Add this line



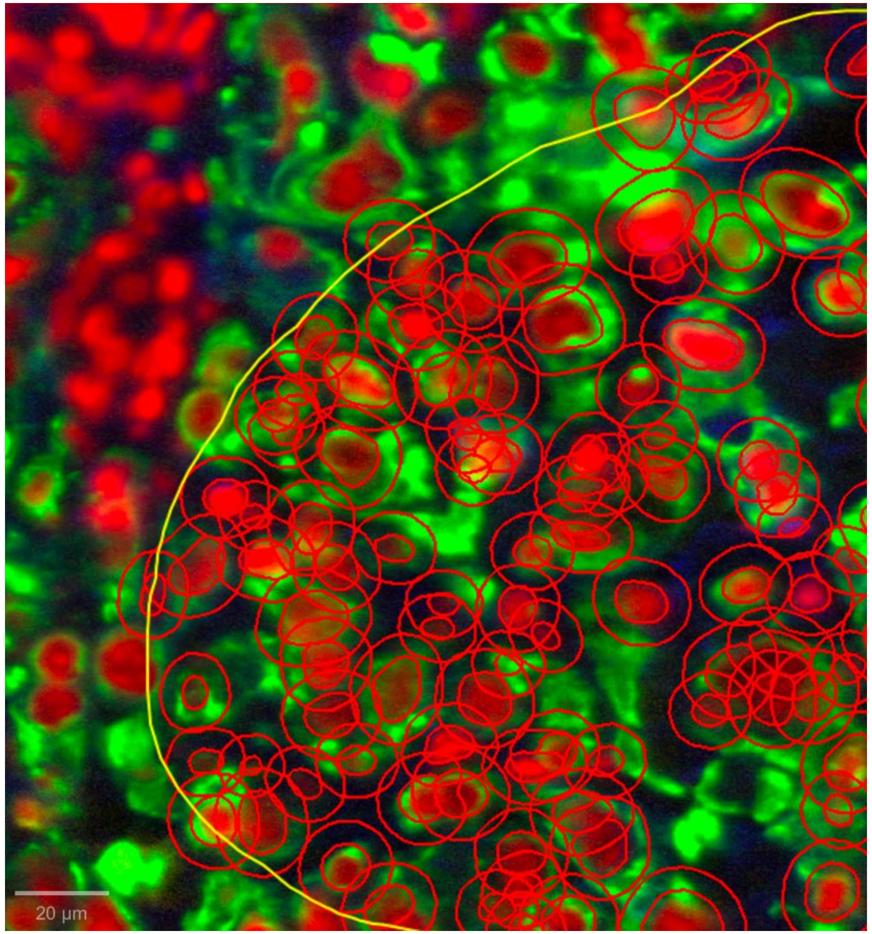
Do not constrain cell expansion with neighbors

- Add *.ignoreCellOverlaps(true)*



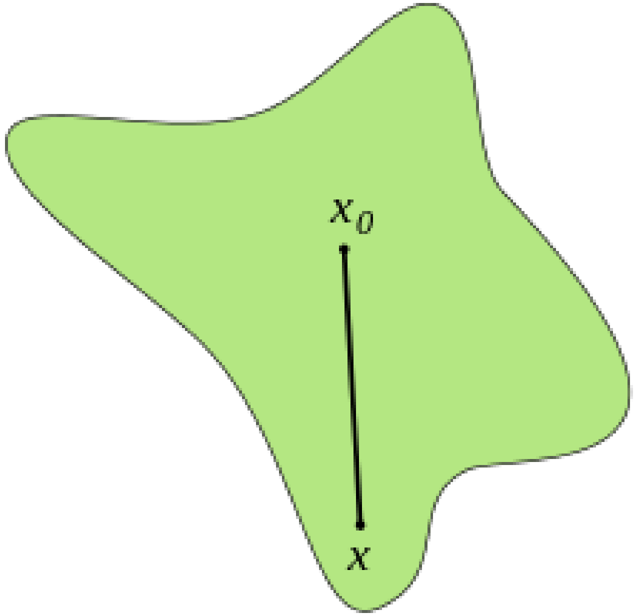
```
def stardist = StarDist2D
    .builder(modelPath)
    .channels('DAPI')
    .normalizePercentiles(1, 99)
    .threshold(0.5)
    .pixelSize(0.5)
    .cellExpansion(5)
    .measureShape()
    .measureIntensity()
    .ignoreCellOverlaps(true)
    .build()
```

Add this line

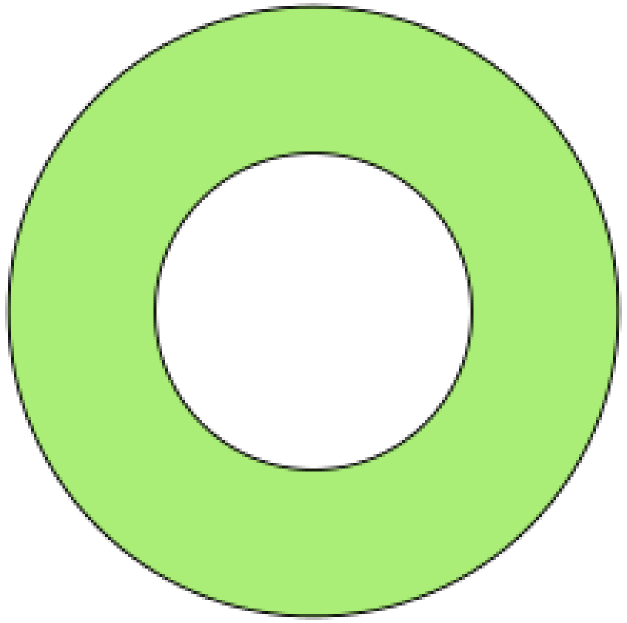


Exercise 3.b: QuPath cell detection with StarDist

Compare StarDist to threshold-based cell detection, what do you observe?



StarDist can segment



StarDist can **not** segment

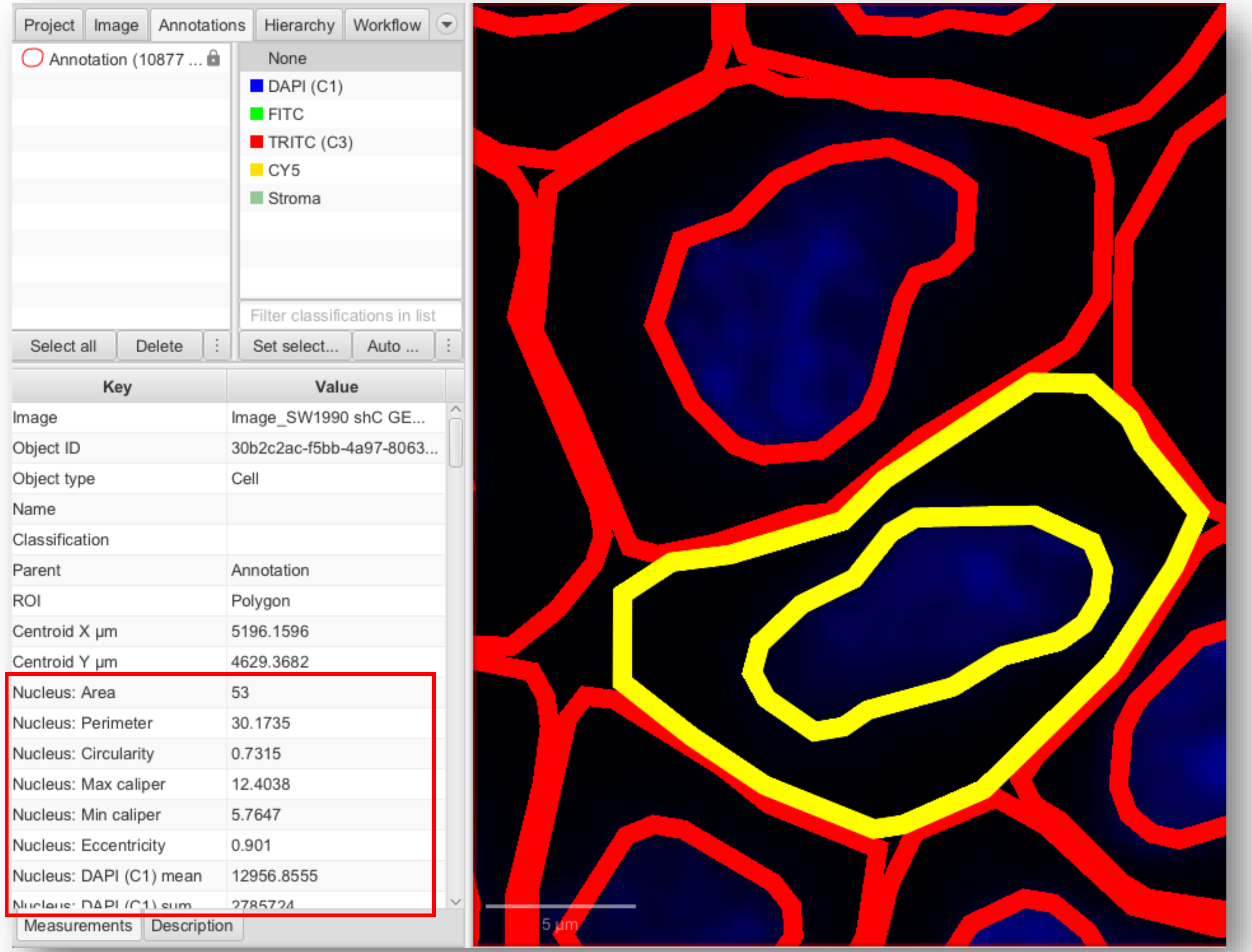
Cell detection measurements

Detection measurements

- Each detection object (i.e. a cell) has its measurement list
  - Intensity features
  - Haralick (texture) features
  - Shape features
  - Smoothed features

- *Annotations* tab > select a cell in the viewer > inspect its measurements list

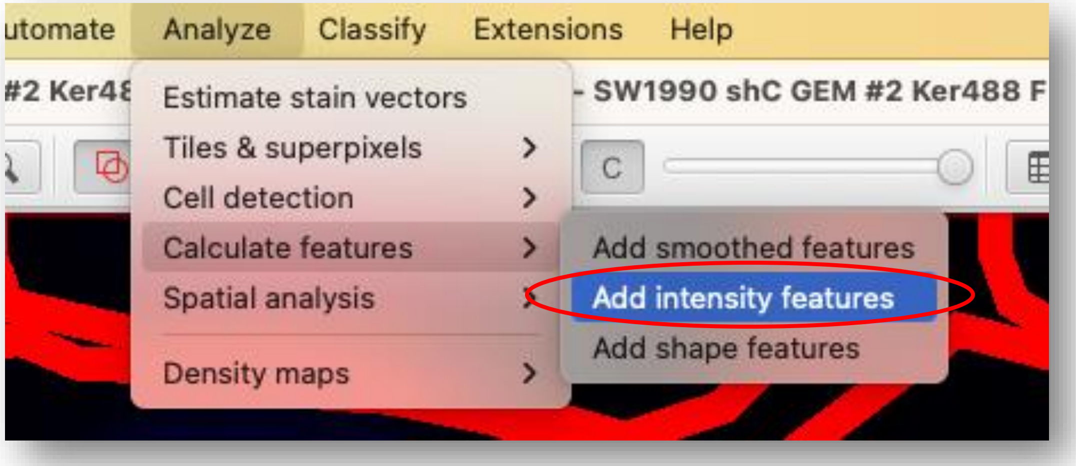
By default, basic intensity and shape features are calculated

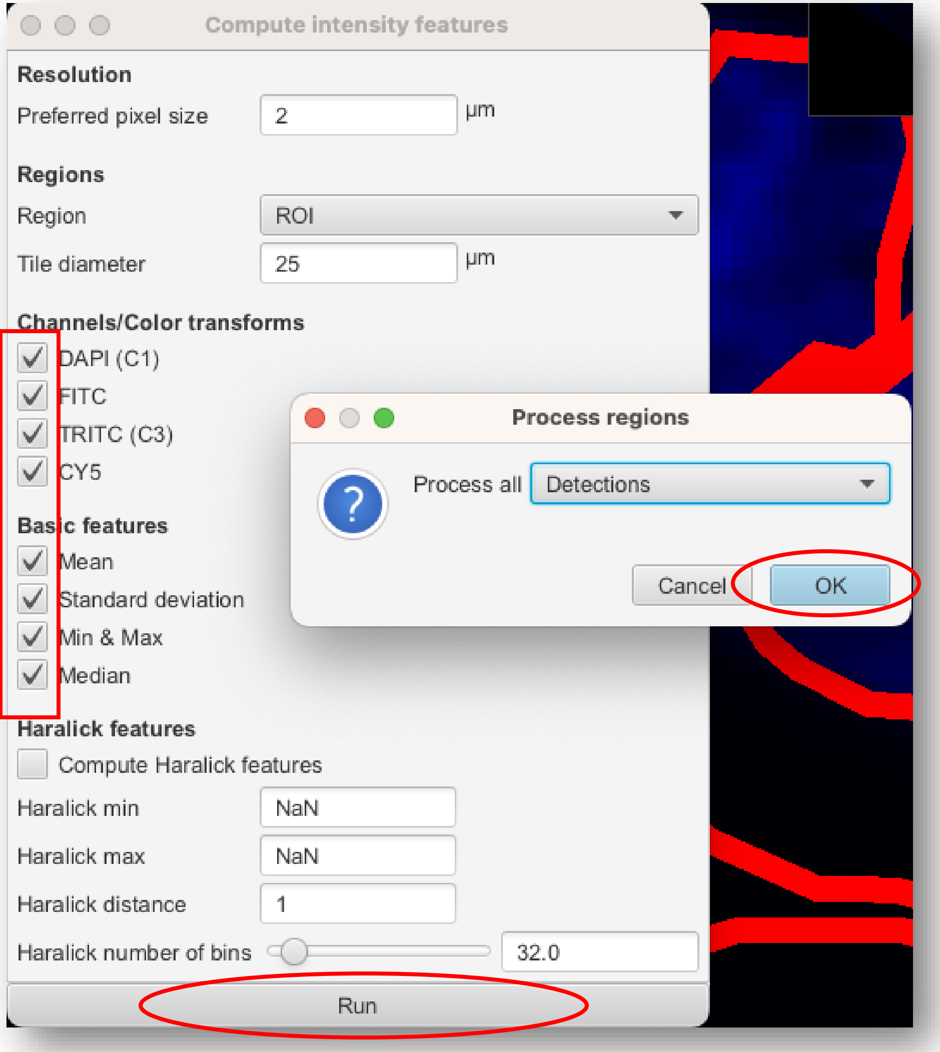


Key	Value
Image	Image_SW1990 shC GE...
Object ID	30b2c2ac-f5bb-4a97-8063...
Object type	Cell
Name	
Classification	
Parent	Annotation
ROI	Polygon
Centroid X µm	5196.1596
Centroid Y µm	4629.3682
Nucleus: Area	53
Nucleus: Perimeter	30.1735
Nucleus: Circularity	0.7315
Nucleus: Max caliper	12.4038
Nucleus: Min caliper	5.7647
Nucleus: Eccentricity	0.901
Nucleus: DAPI (C1) mean	12956.8555
Nucleus: DAPI (C1) sum	2785724

Calculating measurements

- *Analyze > Calculate features > Add intensity features*





Resolution

Preferred pixel size: 2 µm

Regions

Region: ROI

Tile diameter: 25 µm

Channels/Color transforms

☑ DAPI (C1)
☑ FITC
☑ TRITC (C3)
☑ CY5

Basic features

☑ Mean
☑ Standard deviation
☑ Min & Max
☑ Median

Haralick features

☐ Compute Haralick features

Haralick min: NaN

Haralick max: NaN

Haralick distance: 1

Haralick number of bins: 32.0

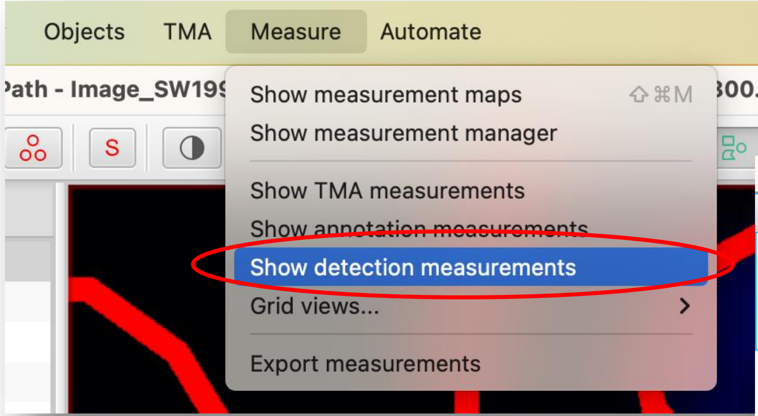
Process all: Detections

Tick boxes of the channels and features of interest

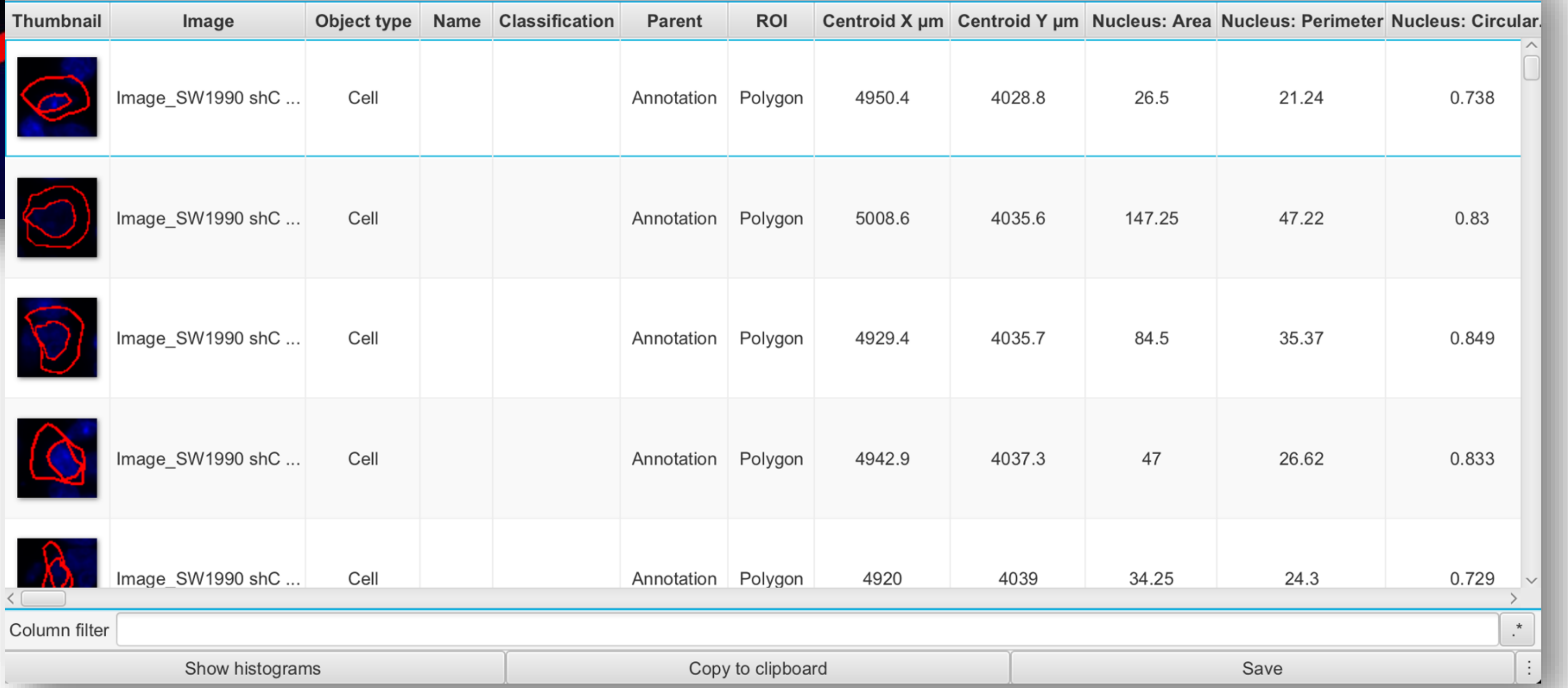
Need a custom feature? Script it!

Visualizing measurements

- *Measure > Show detection measurements*



Columns: measurements



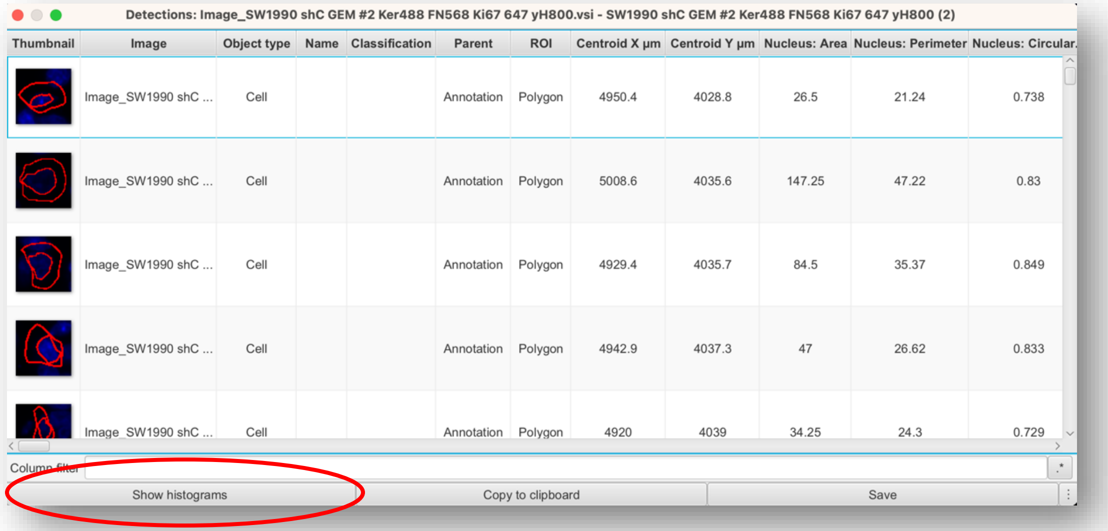
Detections: Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800 (2)

Thumbnail	Image	Object type	Name	Classification	Parent	ROI	Centroid X µm	Centroid Y µm	Nucleus: Area	Nucleus: Perimeter	Nucleus: Circular
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4950.4	4028.8	26.5	21.24	0.738
	Image_SW1990 shC ...	Cell			Annotation	Polygon	5008.6	4035.6	147.25	47.22	0.83
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4929.4	4035.7	84.5	35.37	0.849
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4942.9	4037.3	47	26.62	0.833
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4920	4039	34.25	24.3	0.729

Rows: cells

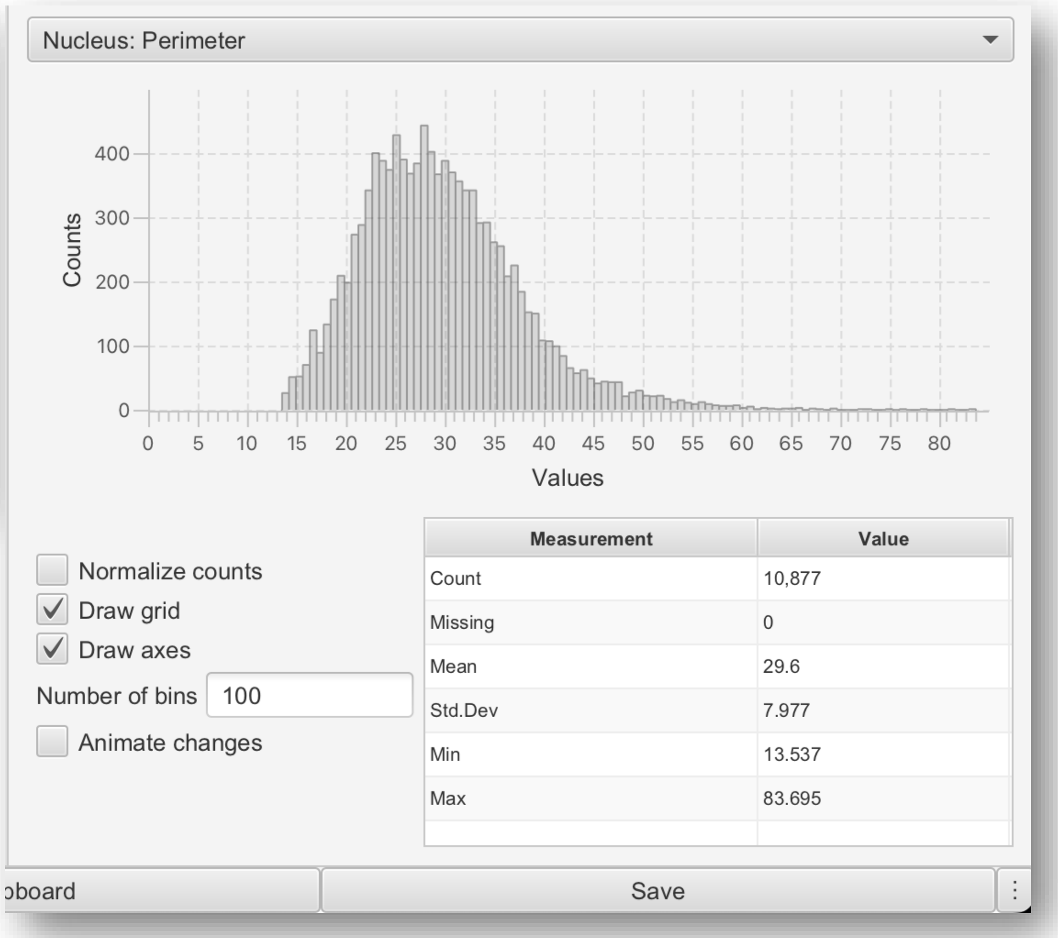
Visualizing measurement distributions

- *Measure > Show detection measurements*



Detections: Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800 (2)

Thumbnail	Image	Object type	Name	Classification	Parent	ROI	Centroid X µm	Centroid Y µm	Nucleus: Area	Nucleus: Perimeter	Nucleus: Circular.
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4950.4	4028.8	26.5	21.24	0.738
	Image_SW1990 shC ...	Cell			Annotation	Polygon	5008.6	4035.6	147.25	47.22	0.83
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4929.4	4035.7	84.5	35.37	0.849
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4942.9	4037.3	47	26.62	0.833
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4920	4039	34.25	24.3	0.729



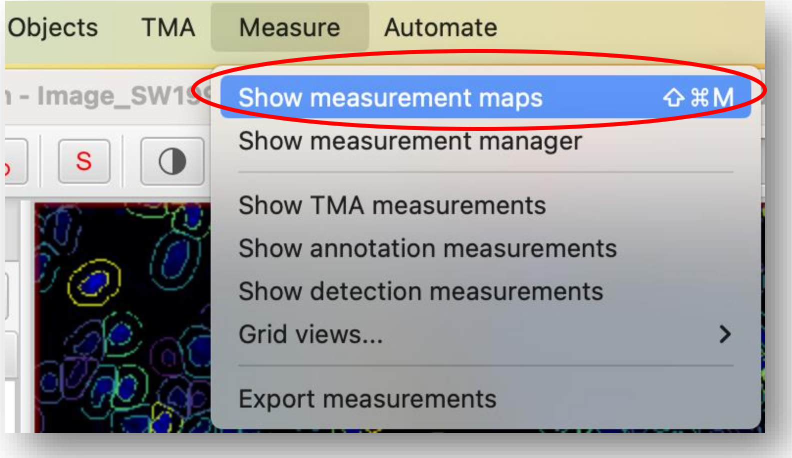
☐ Normalize counts
☑ Draw grid
☑ Draw axes
Number of bins 100
☐ Animate changes

Measurement	Value
Count	10,877
Missing	0
Mean	29.6
Std.Dev	7.977
Min	13.537
Max	83.695

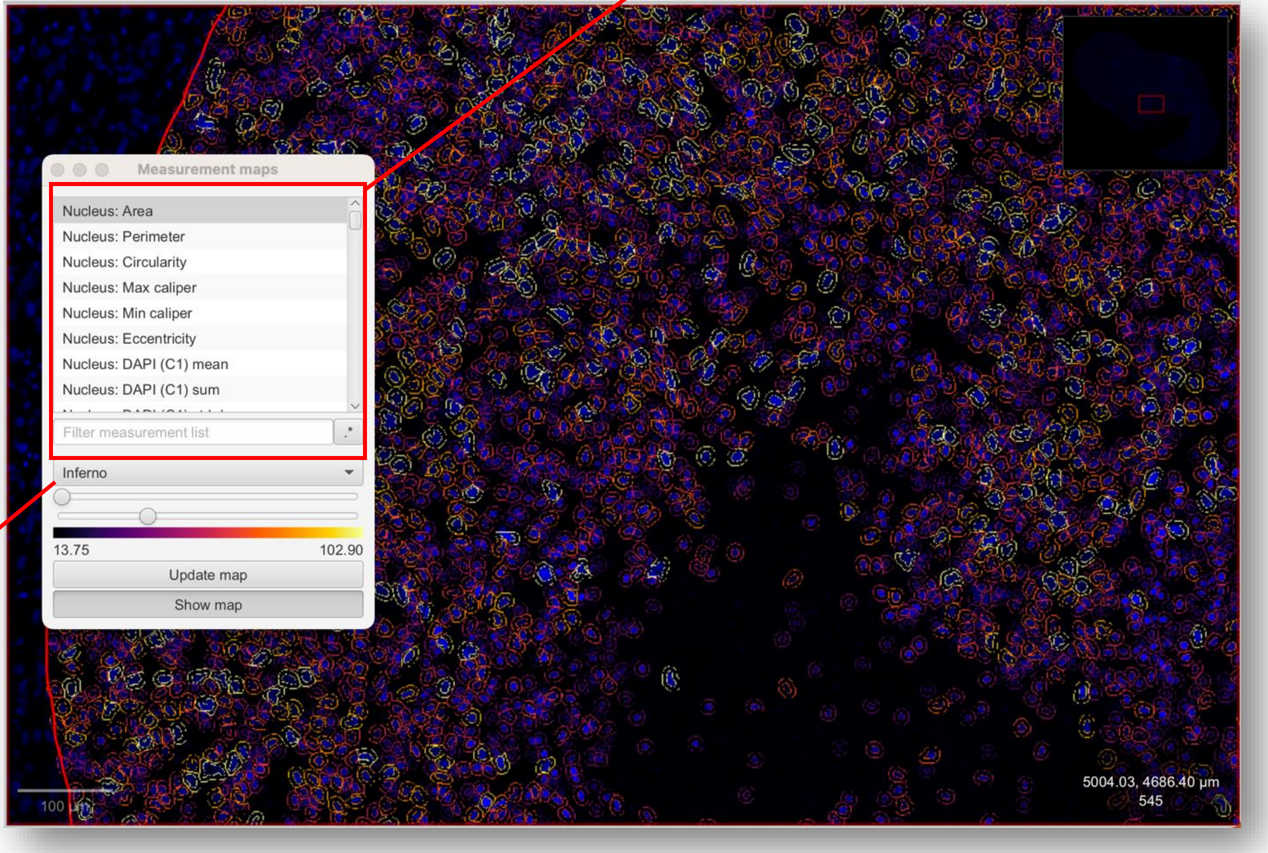
FYI, it is not possible to export distribution plots…

Visualizing measurements as heat maps

- *Measure > Show measurement maps*



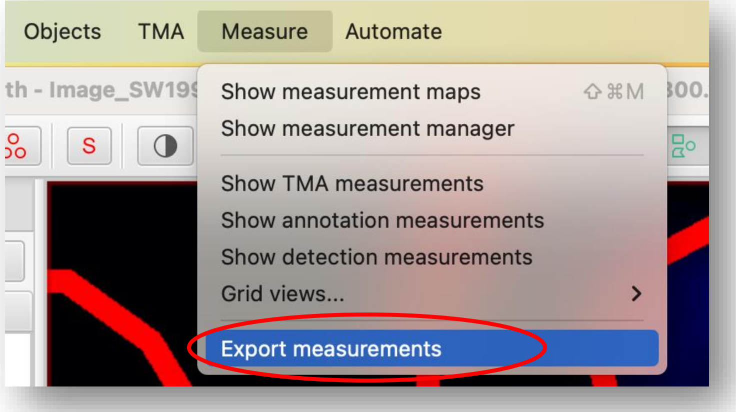
Measurements list

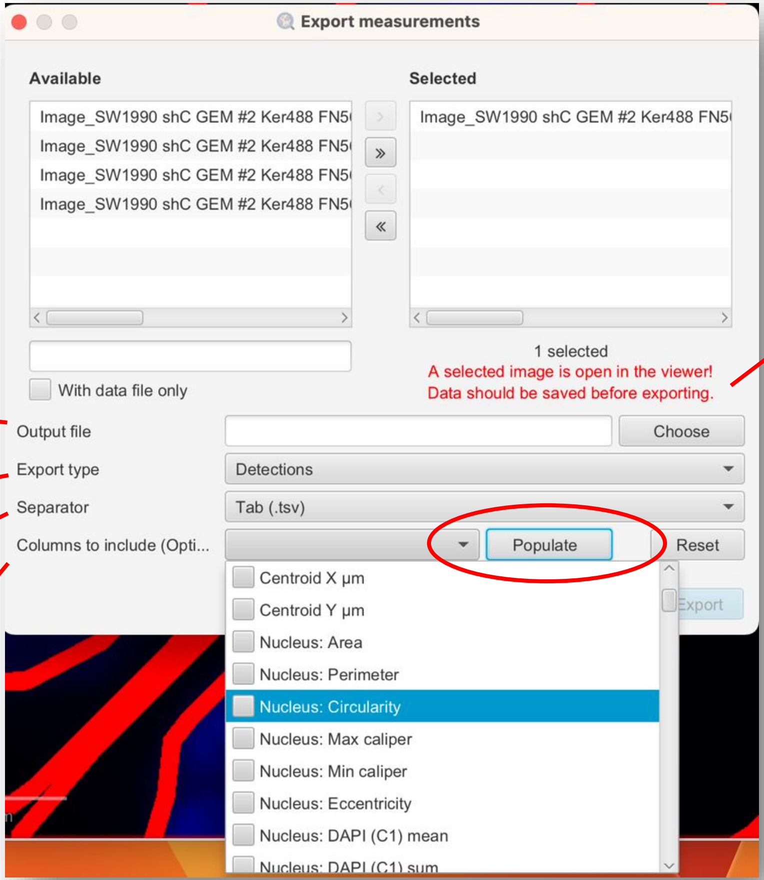


Your favorite color map,
and its range of value

Export measurements the right way

- *Measure > Export measurements*
- Drag an image from *Available* to *Selected*





Available:
Image_SW1990 shC GEM #2 Ker488 FN5
Image_SW1990 shC GEM #2 Ker488 FN5
Image_SW1990 shC GEM #2 Ker488 FN5
Image_SW1990 shC GEM #2 Ker488 FN5

Selected:
Image_SW1990 shC GEM #2 Ker488 FN5

☐ With data file only

1 selected
A selected image is open in the viewer!
Data should be saved before exporting.

Output file:
Export type: Detections
Separator: Tab (.tsv)
Columns to include (Opti...

☐ Centroid X µm
☐ Centroid Y µm
☐ Nucleus: Area
☐ Nucleus: Perimeter
☐ Nucleus: Circularity
☐ Nucleus: Max caliper
☐ Nucleus: Min caliper
☐ Nucleus: Eccentricity
☐ Nucleus: DAPI (C1) mean
☐ Nucleus: DAPI (C1) sum

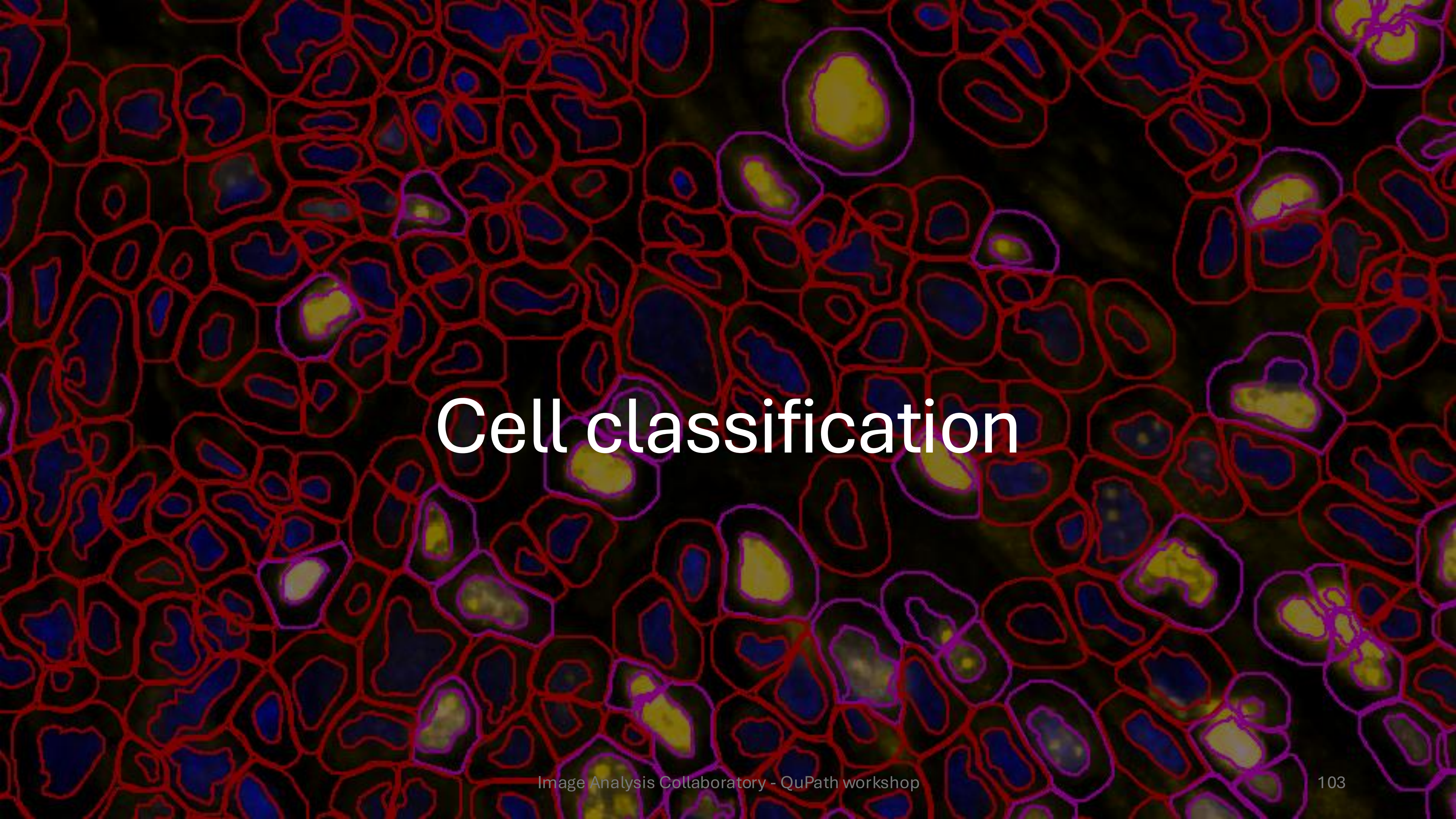
Save your results first!

Output file location

Measurement type to be exported

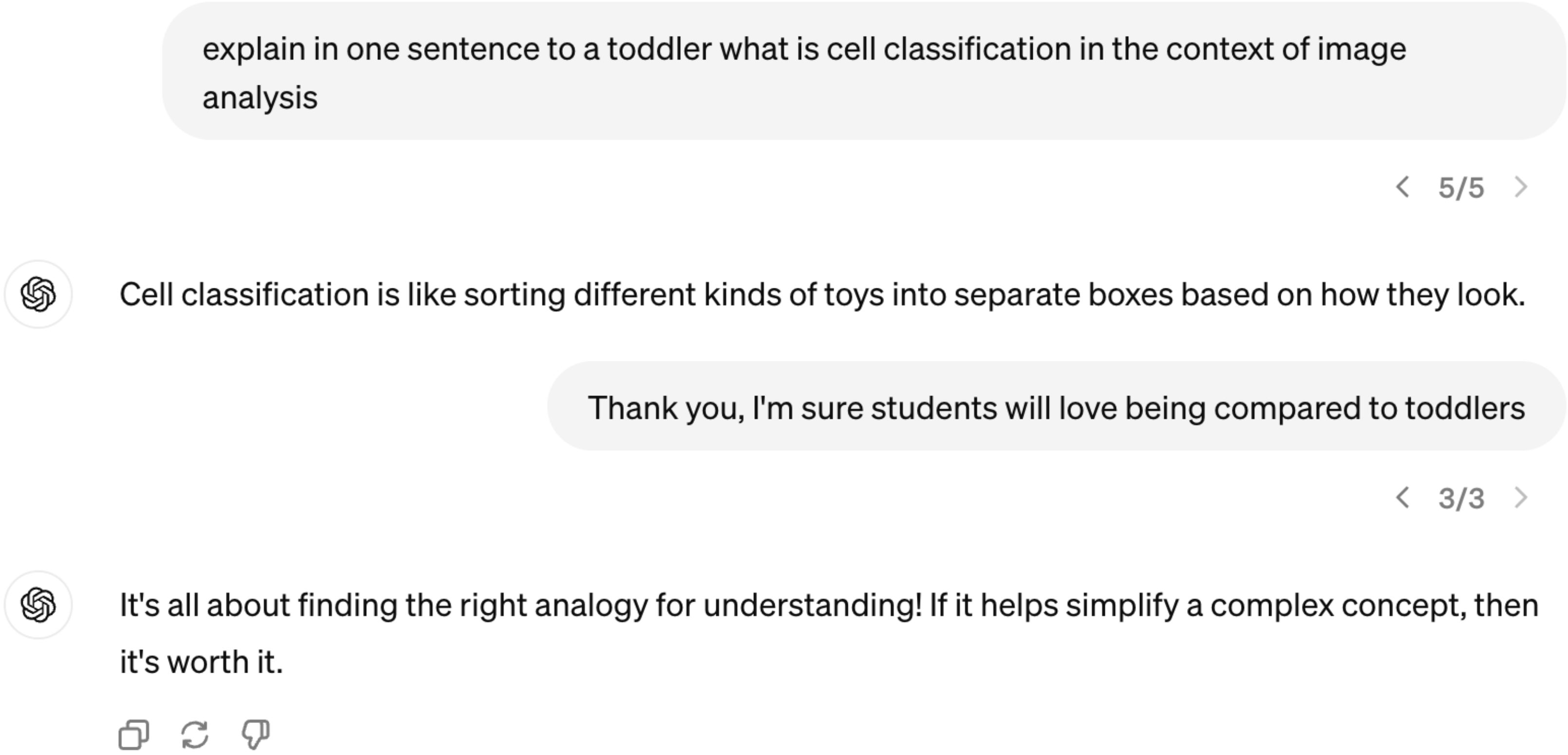
File type (.tsv, .csv)

List of measurements to include in the export



Cell classification

What is cell classification?



explain in one sentence to a toddler what is cell classification in the context of image analysis

Cell classification is like sorting different kinds of toys into separate boxes based on how they look.

Thank you, I'm sure students will love being compared to toddlers

It's all about finding the right analogy for understanding! If it helps simplify a complex concept, then it's worth it.

What is cell classification?

- Cell classification in image analysis is sorting different types of cells into groups based on their features or characteristics, such as shape, color, position, neighbors, etc.

- If visually you can’t discriminate cells in your images, then your algorithm might be separating cell on very fine differences (careful of *overfitting*)

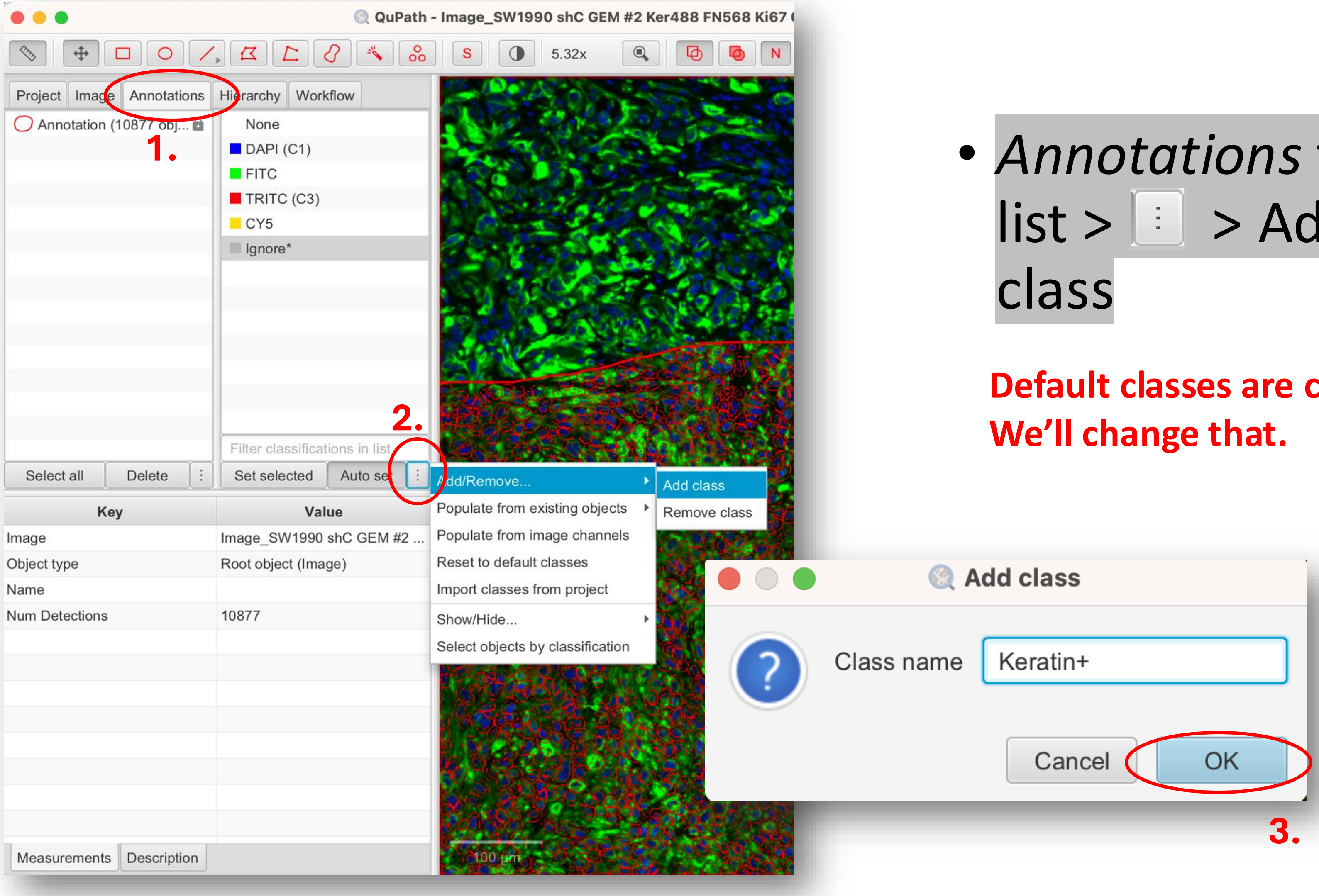
Cell classification in QuPath

- Single measurement classifier

- Composite thresholder: combine single measurement thresholders together

- Train a machine learning classifier

Create a class named ‘Keratin+’

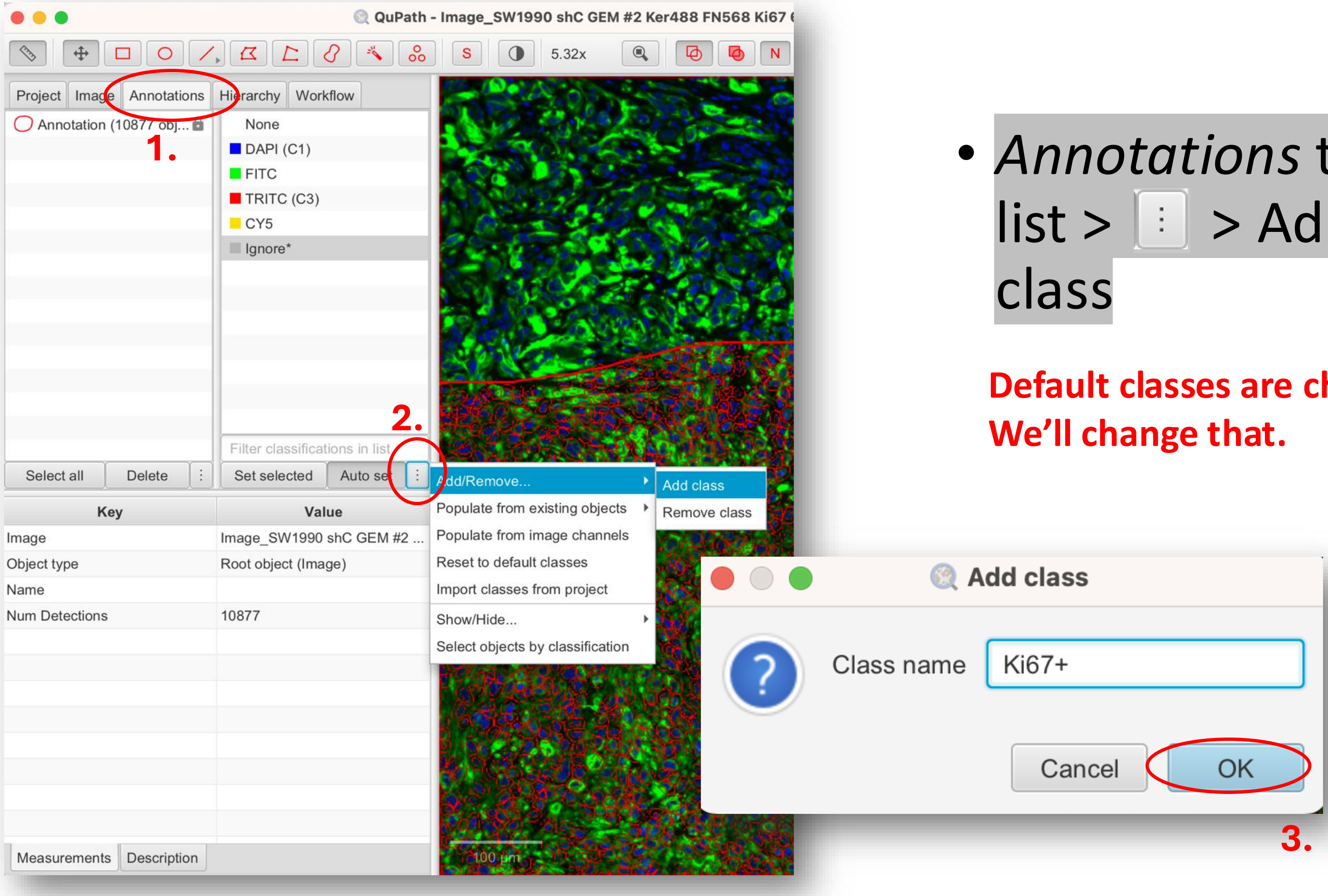


Class name: Keratin+

- *Annotations* tab > Classification list > ⋮ > Add/Remove... > Add class

Default classes are channel names.
We’ll change that.

Create a second class named ‘Ki67+’



Key	Value
Image	Image_SW1990 shC GEM #2 ...
Object type	Root object (Image)
Name	
Num Detections	10877

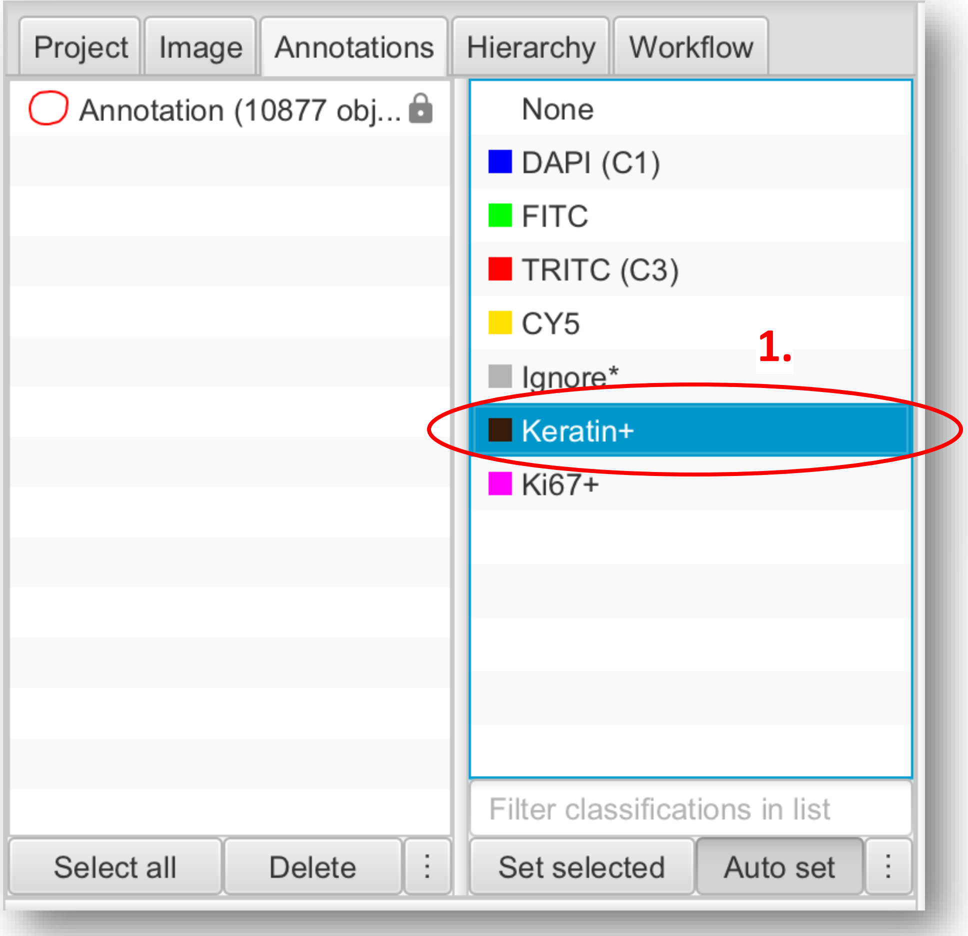
Class name: Ki67+

- *Annotations* tab > Classification list > ⋮ > Add/Remove... > Add class

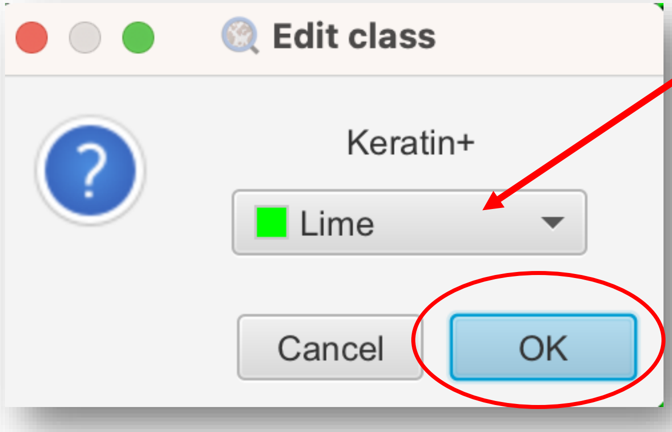
Default classes are channel names.
We’ll change that.

Change the color of a class

- Double click on the class > Edit class > Choose a new color > *OK*



1.

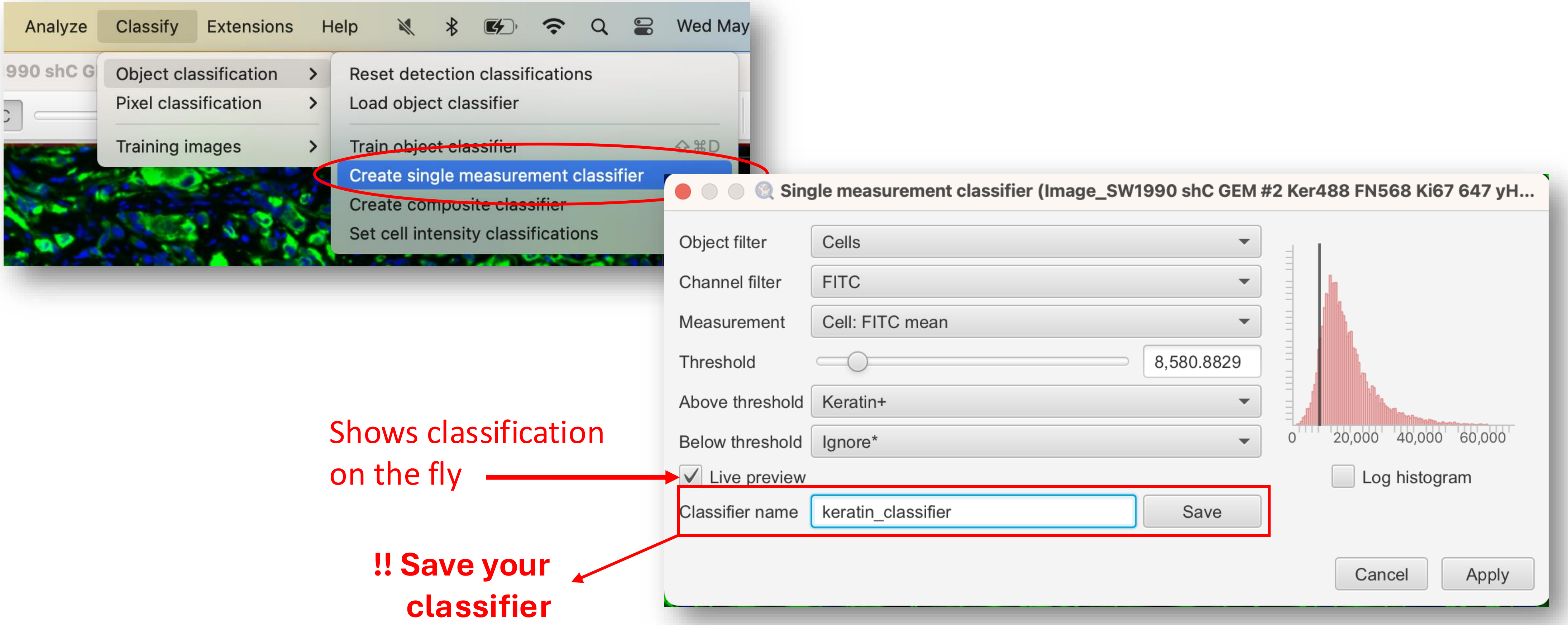


Your favorite color.

By default, classes are populated by image channels.

Simple measurement classifier on Keratin signal intensity (FITC channel)

- *Classify > Object classification > Create single measurement classifier*



Single measurement classifier (Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH...

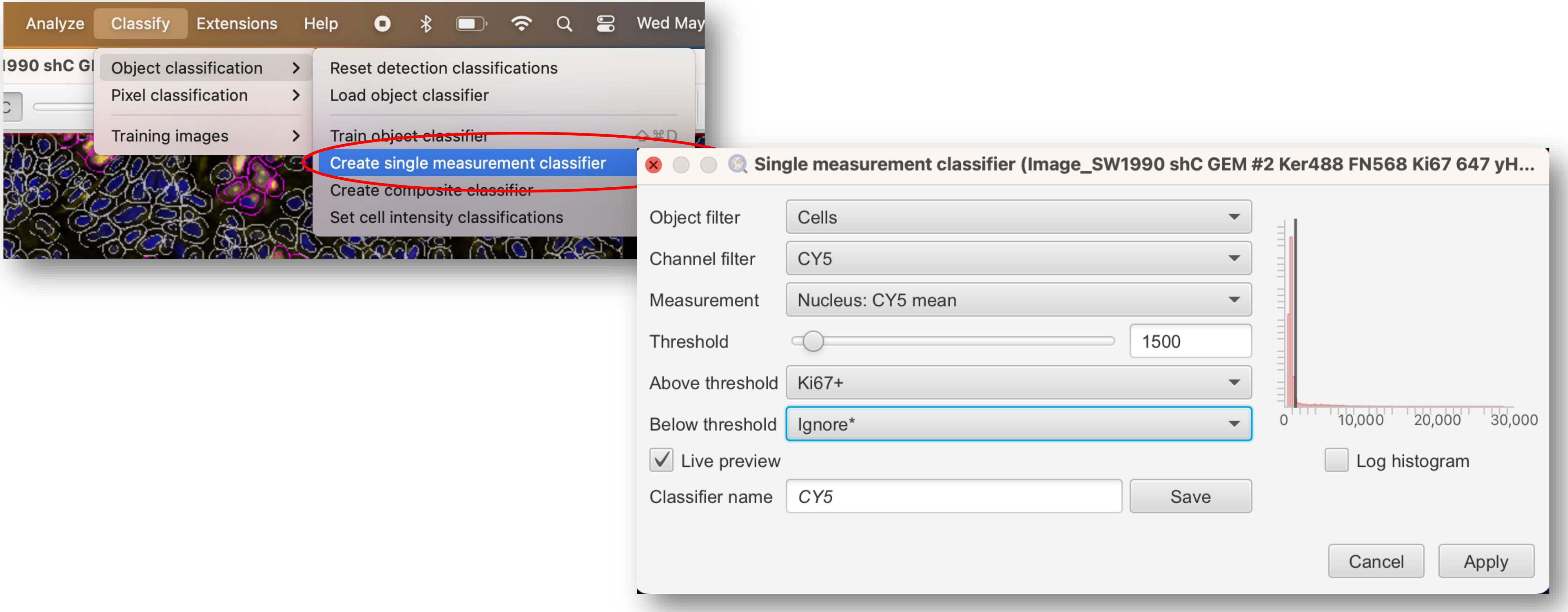
Object filter: Cells
Channel filter: FITC
Measurement: Cell: FITC mean
Threshold: 8,580.8829
Above threshold: Keratin+
Below threshold: Ignore*
☑ Live preview
Classifier name: keratin_classifier

Shows classification on the fly

!! Save your classifier

Simple measurement classifier on Ki67 signal intensity (CY5 channel)

- *Classify > Object classification > Create single measurement classifier*



Single measurement classifier (Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH...

Object filter: Cells

Channel filter: CY5

Measurement: Nucleus: CY5 mean

Threshold: 1500

Above threshold: Ki67+

Below threshold: Ignore*

☑ Live preview

☐ Log histogram

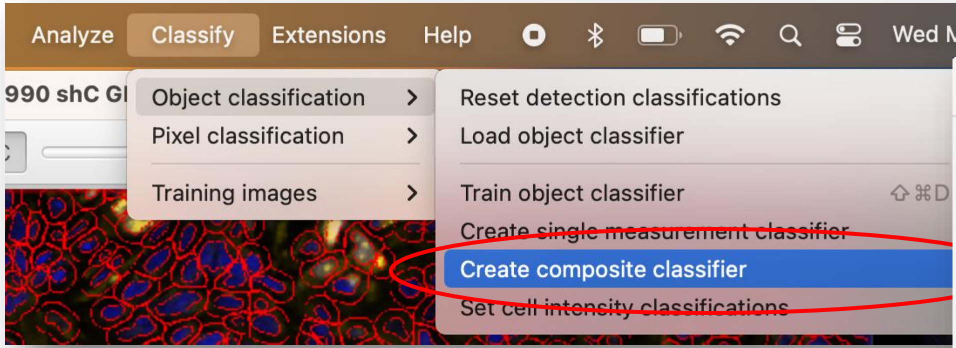
Classifier name: CY5

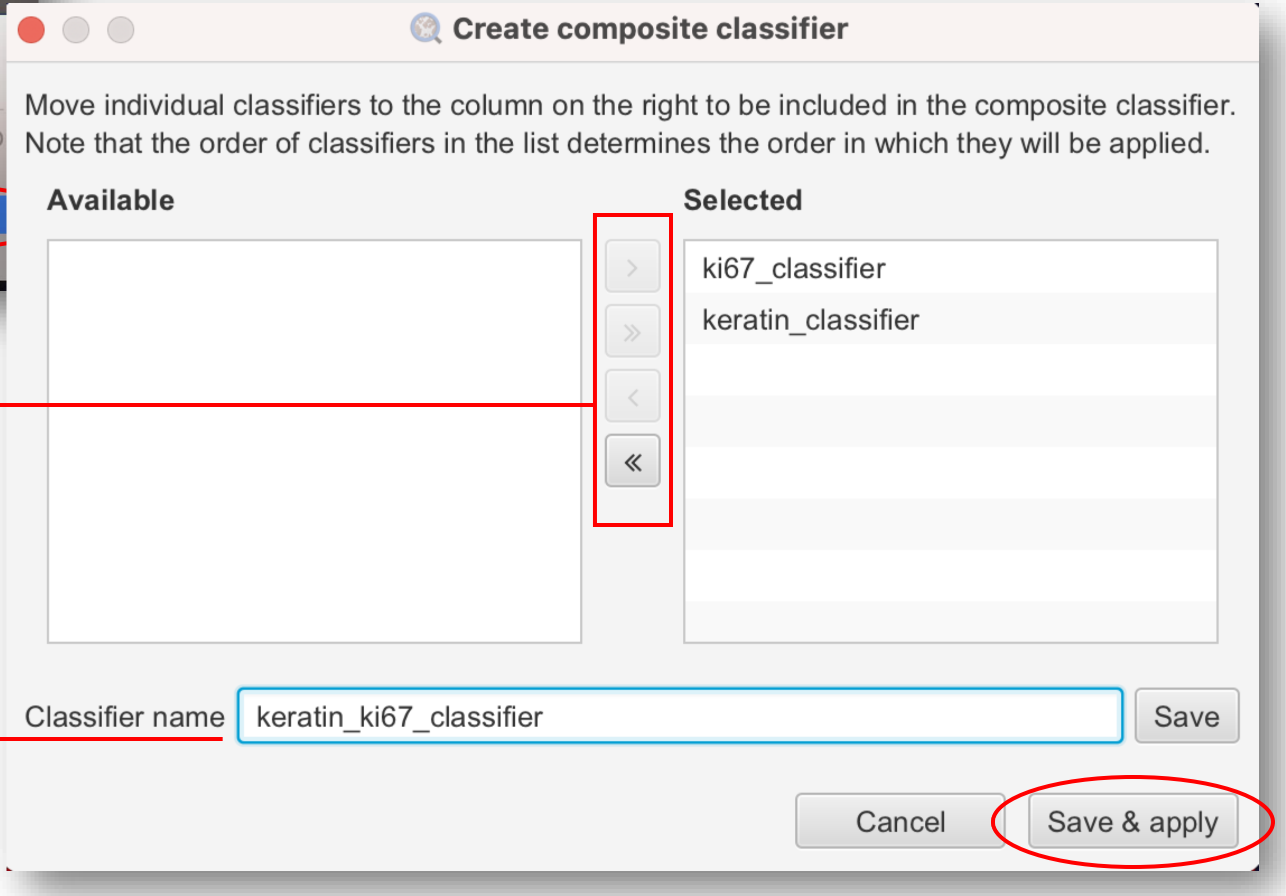
Practice time

Exercise 4.a: single-measurement classifier

Combine single measurement classifiers into a composite classifier

- *Classify > Object classification > Create composite classifier*





Move individual classifiers to the column on the right to be included in the composite classifier. Note that the order of classifiers in the list determines the order in which they will be applied.

Classifier name: keratin_ki67_classifier

Select a classifier by moving it onto the 'Selected' list.

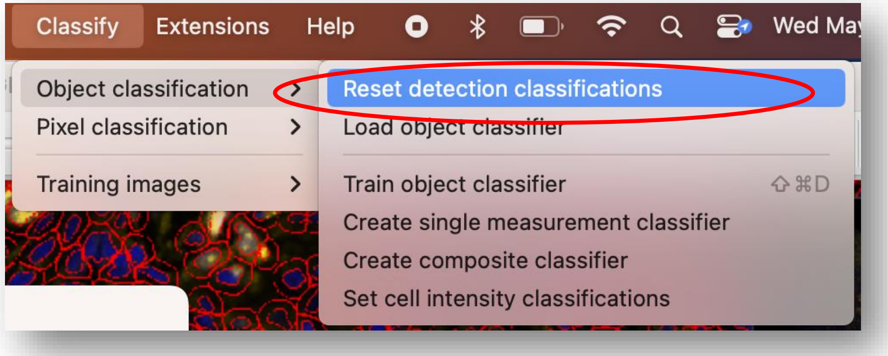
A name is required to 'Save & apply'

Practice time

Exercise 4.b: composite classifier

Reset detection classes

- *Classify > Object classification > Reset detection classifications*



Populate classes in the classification panel

- *Annotations tab >* Classification list > ⋮ > *Populate from existing objects > All classes (including sub-classes)*

Keep existing available classes?

Object classification using machine learning

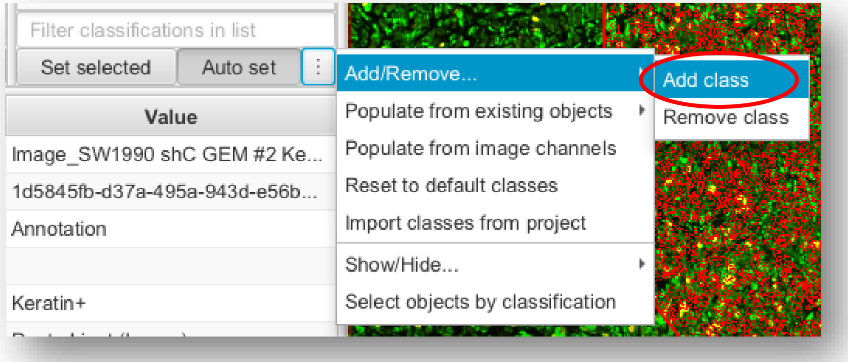
- Detections (and annotations) can be classified into classes using a ML classifier
- Classification requires measurements!
- Object classifiers are trained using manual annotations of 2 or more classes
  - Need to create some training data points

- **Live demo of object classification using ML**

Reset your detection classes!

Train an object classifier: create classes

- *Annotations* tab > Classification list > ⋮ > Add/Remove... > Add class



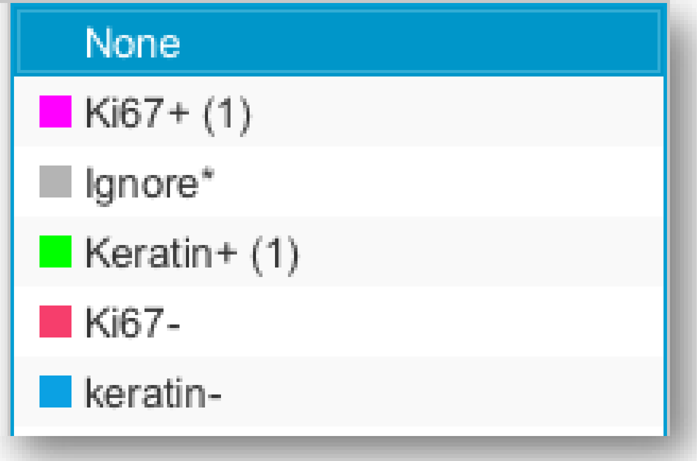
Image_SW1990 shC GEM #2 Ke...

1d5845fb-d37a-495a-943d-e56b...

Annotation

Keratin+

- Create 4 classes:
  - Keratin+
  - Keratin-
  - Ki67+
  - Ki67-



None

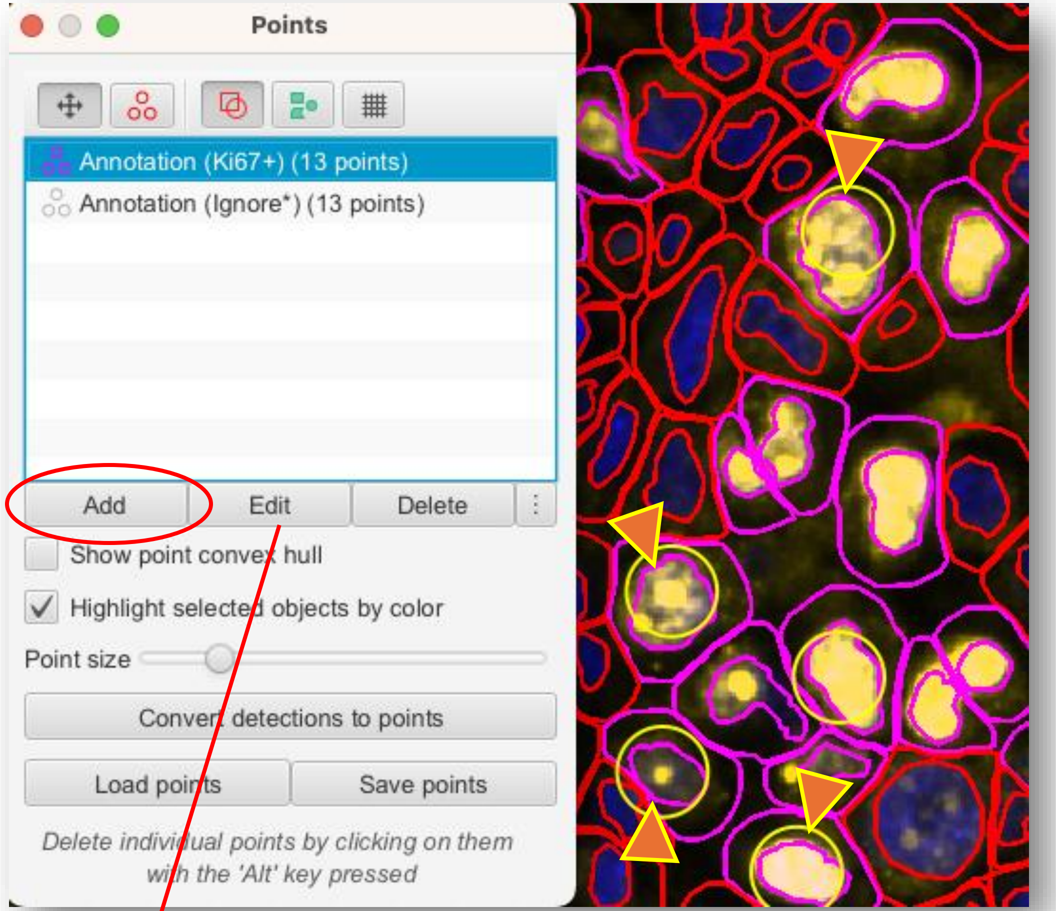
- 🟪 Ki67+ (1)
- ⬜ Ignore*
- 🟩 Keratin+ (1)
- 🟥 Ki67-
- 🟦 keratin-

Train an object classifier: training data points

- > *Add* > Label ~10 for each class

To remove a single point:
Option + click (Mac) or left-click

- Assign each training data set a class:
  - Select the annotation set
  - Select the class



Annotation (Ki67+) (13 points)
Annotation (Ignore*) (13 points)

☐ Show point convex hull

☑ Highlight selected objects by color

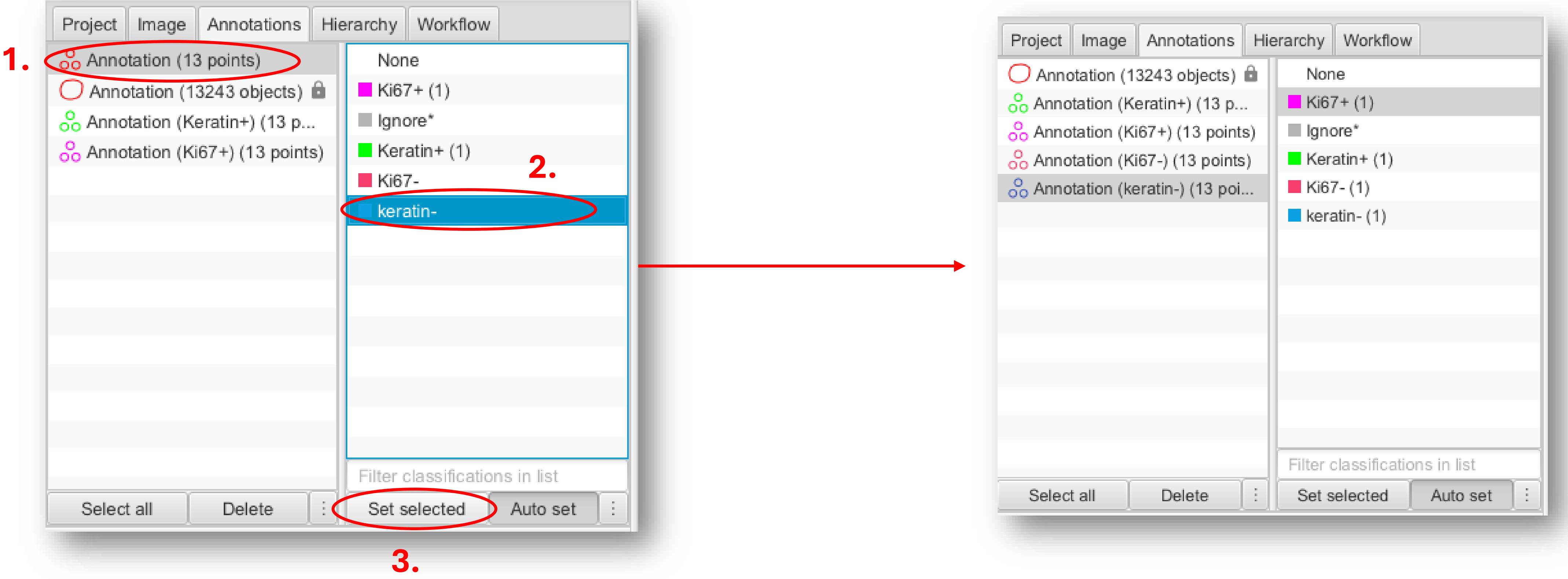
Delete individual points by clicking on them with the 'Alt' key pressed

Click edit to change color

▼ Training data

Train an object classifier: training data points

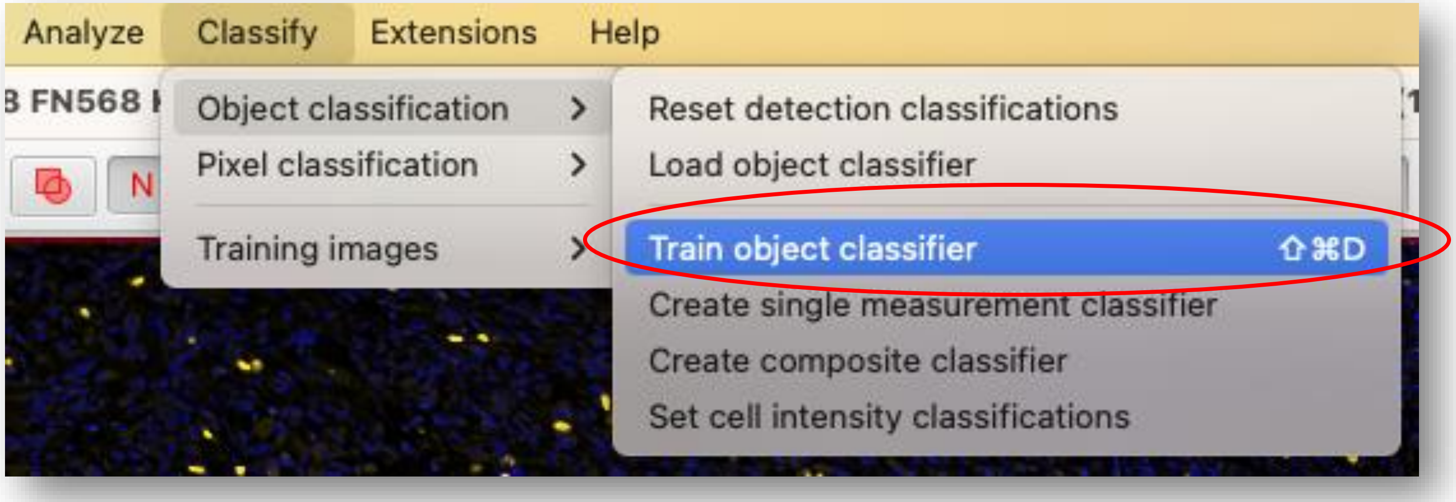
- Assign each training data set a class in the *Annotations* tab



Make sure to lock your annotation: Ctrl+click > Lock

Train an object classifier

- *Classify > Object classification > Train object classifier*

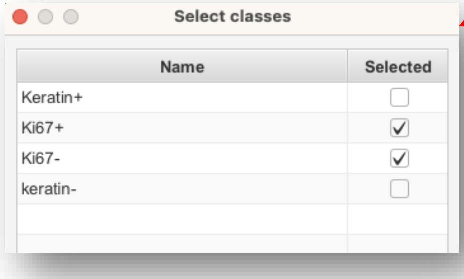


Train an object classifier

- *Classify > Object classification > Train object classifier*

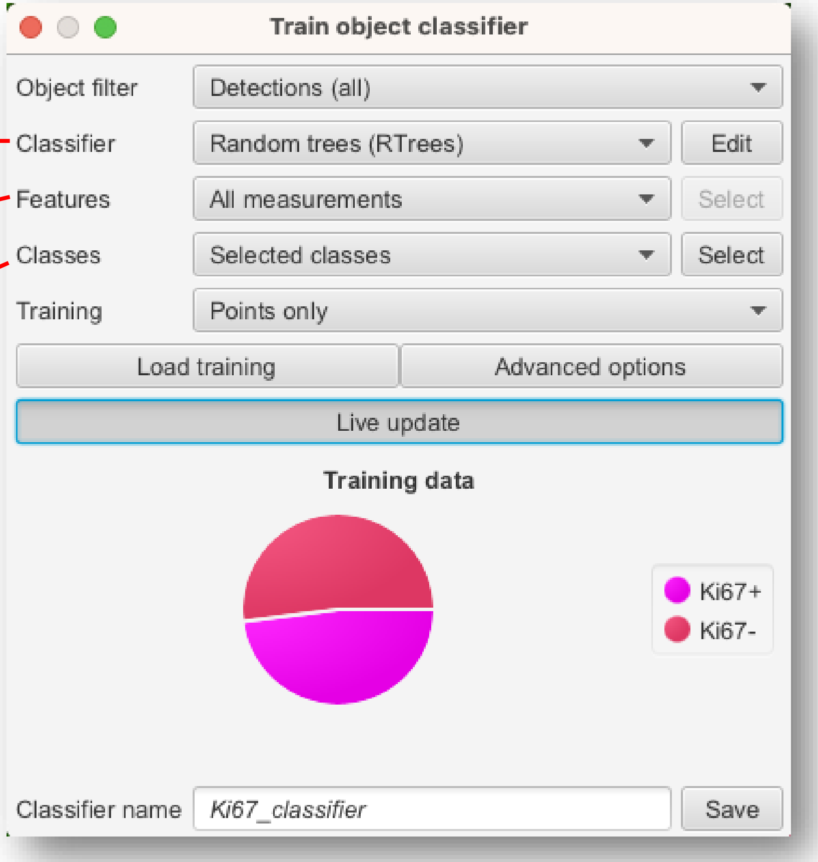
Model type (RT, ANN, k-NN)

Features: choose Selected measurements and click Select to restrict the feature space



Select classes

Name	Selected
Keratin+	☐
Ki67+	☑
Ki67-	☑
keratin-	☐



Train object classifier

Object filter: Detections (all)

Classifier: Random trees (RTrees)

Features: All measurements

Classes: Selected classes

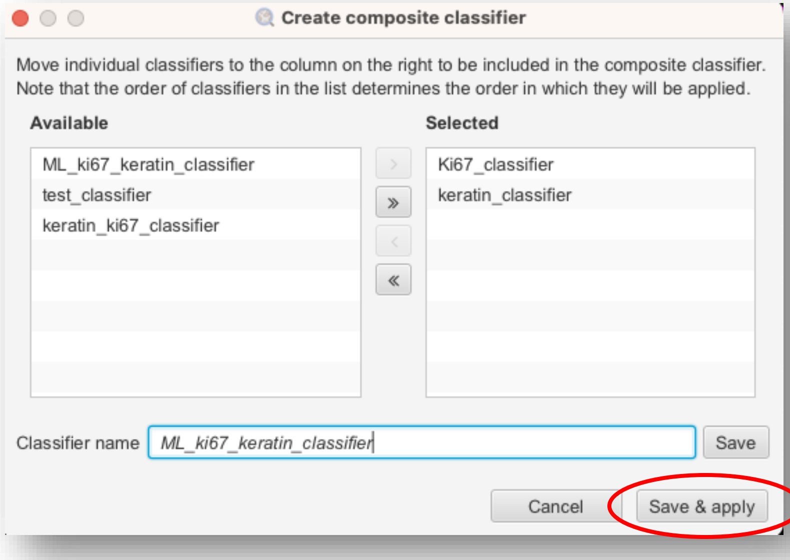
Training: Points only

Classifier name: *Ki67_classifier*

Name it to save it.

Combine multiple ML classifiers together

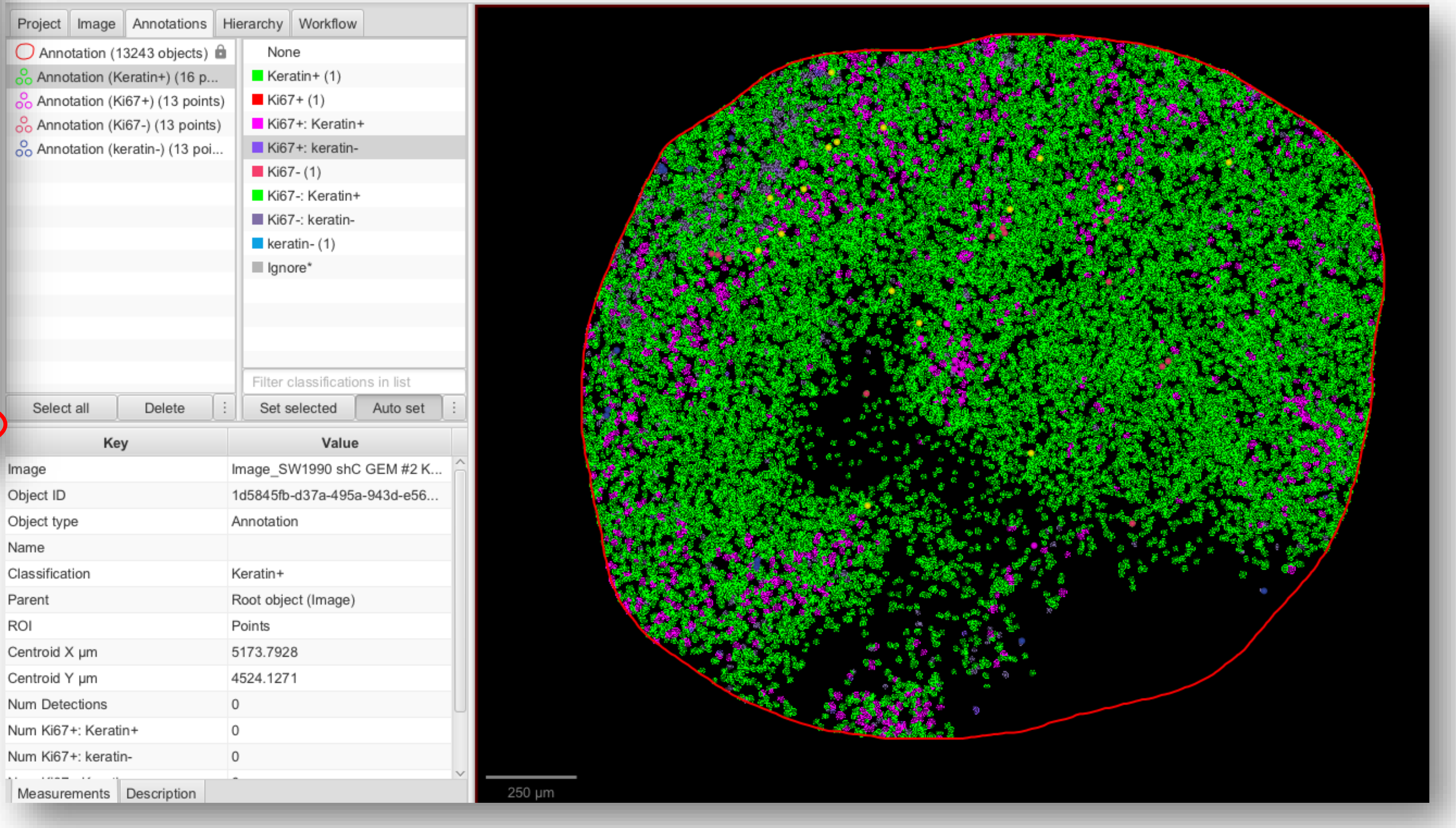
- *Classify > Object classification > Create composite classifier*



Move individual classifiers to the column on the right to be included in the composite classifier. Note that the order of classifiers in the list determines the order in which they will be applied.

Available	Selected
ML_ki67_keratin_classifier	Ki67_classifier
test_classifier	keratin_classifier
keratin_ki67_classifier	

Classifier name: *ML_ki67_keratin_classifier*



Annotation (13243 objects)
Annotation (Keratin+) (16 p...
Annotation (Ki67+) (13 points)
Annotation (Ki67-) (13 points)
Annotation (keratin-) (13 poi...

Key	Value
Image	Image_SW1990 shC GEM #2 K...
Object ID	1d5845fb-d37a-495a-943d-e56...
Object type	Annotation
Name	
Classification	Keratin+
Parent	Root object (Image)
ROI	Points
Centroid X µm	5173.7928
Centroid Y µm	4524.1271
Num Detections	0
Num Ki67+: Keratin+	0
Num Ki67+: keratin-	0

8 resulting classes!
+ ignore*

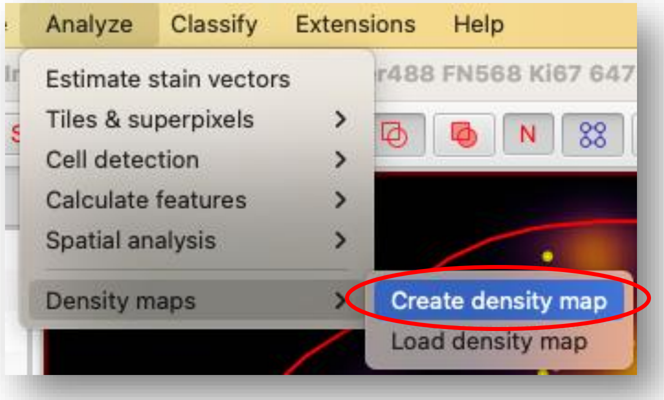
Refine your classifier

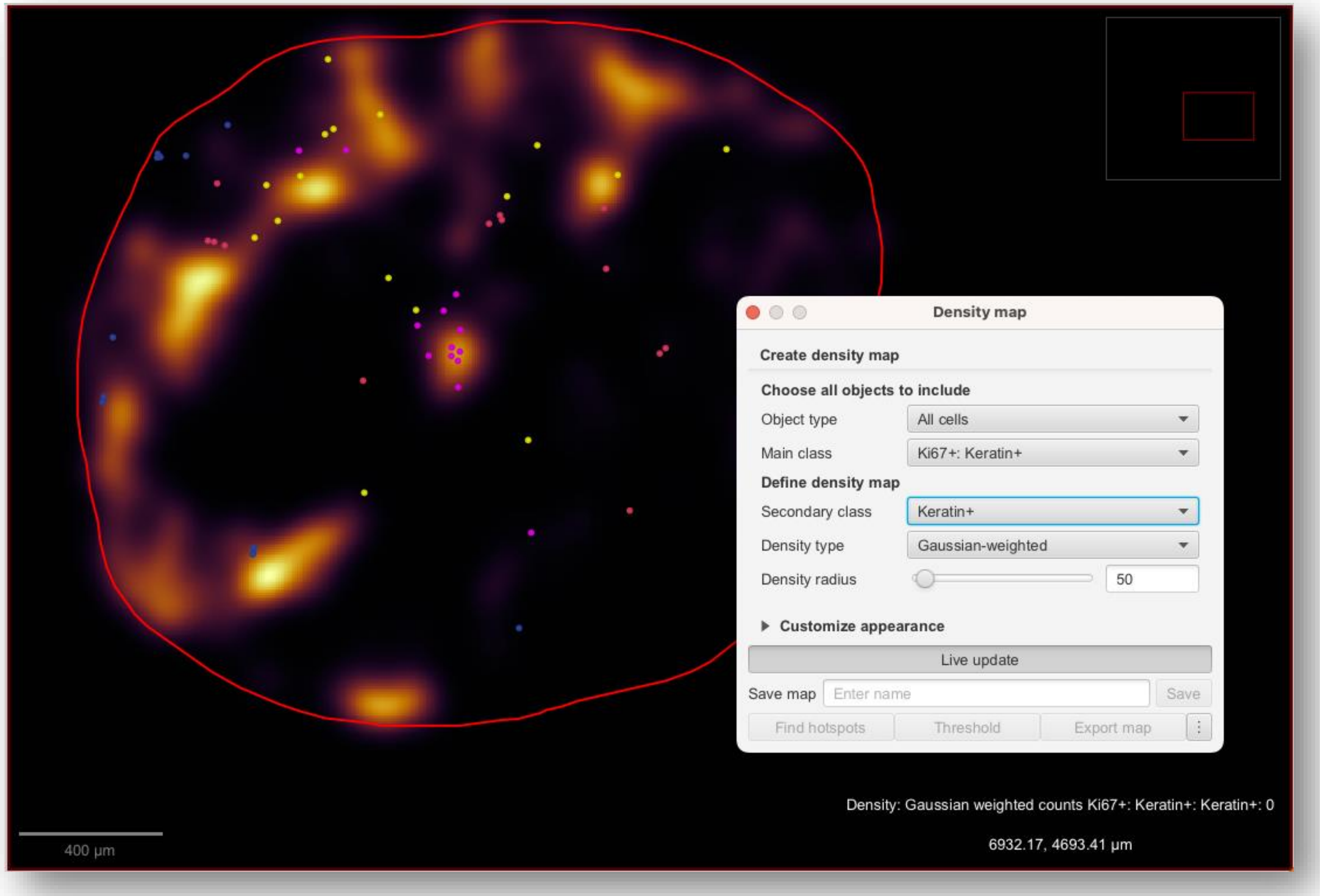
- Add more training data points
  - Classification results will change in real time if ‘Live update’ option is enabled

- Typically, *fewer*, but *well-chosen* features provides more robust results

Visualizing results using density maps

- Analyze > Density maps > Create density maps




Density map

Create density map

Choose all objects to include

Object type: All cells

Main class: Ki67+: Keratin+

Define density map

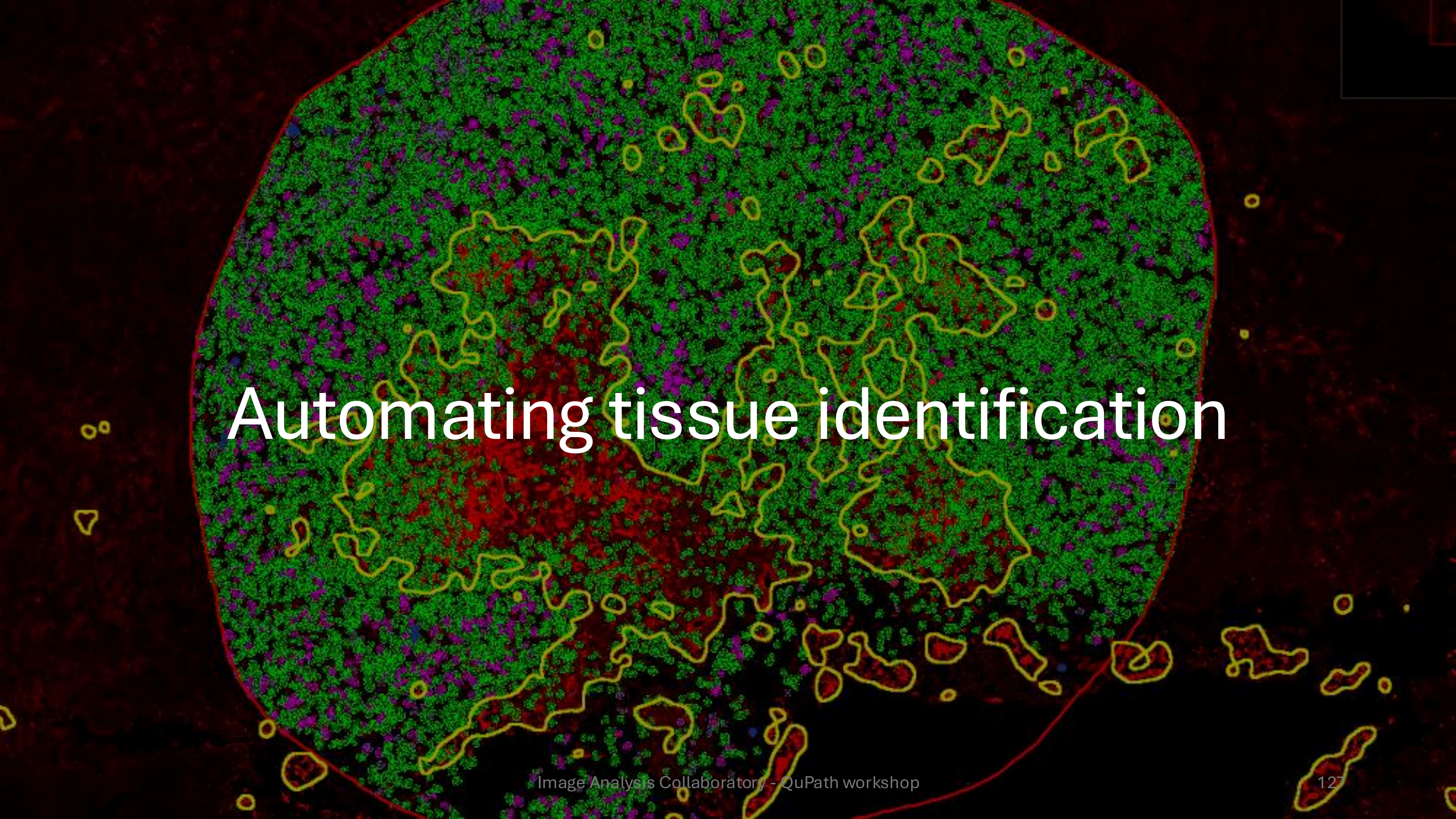
Secondary class: Keratin+

Density type: Gaussian-weighted

Density radius: 50

Density: Gaussian weighted counts Ki67+: Keratin+: Keratin+: 0

6932.17, 4693.41 µm

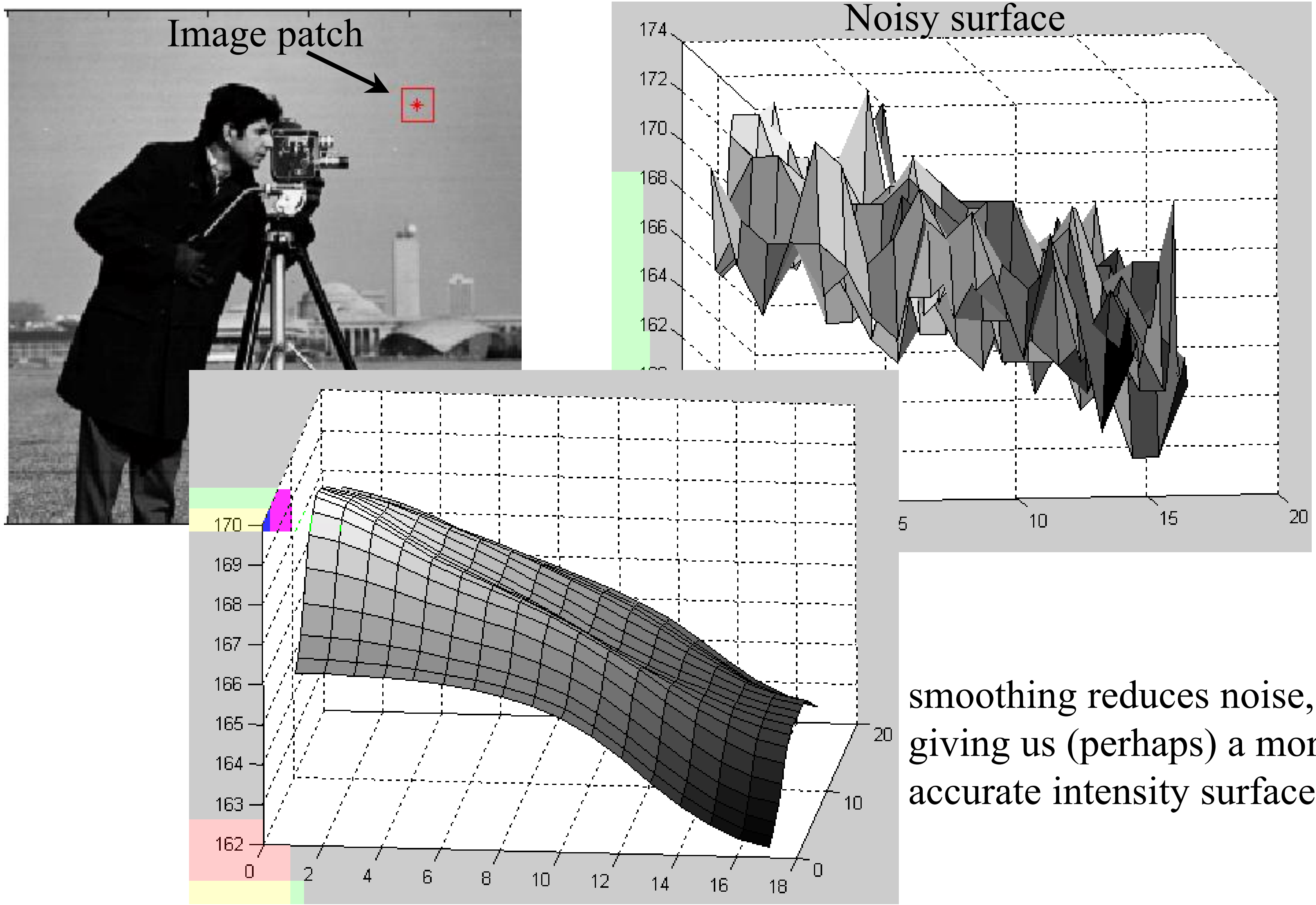


Automating tissue identification

But first, let’s talk smoothing

Intermezzo aperto

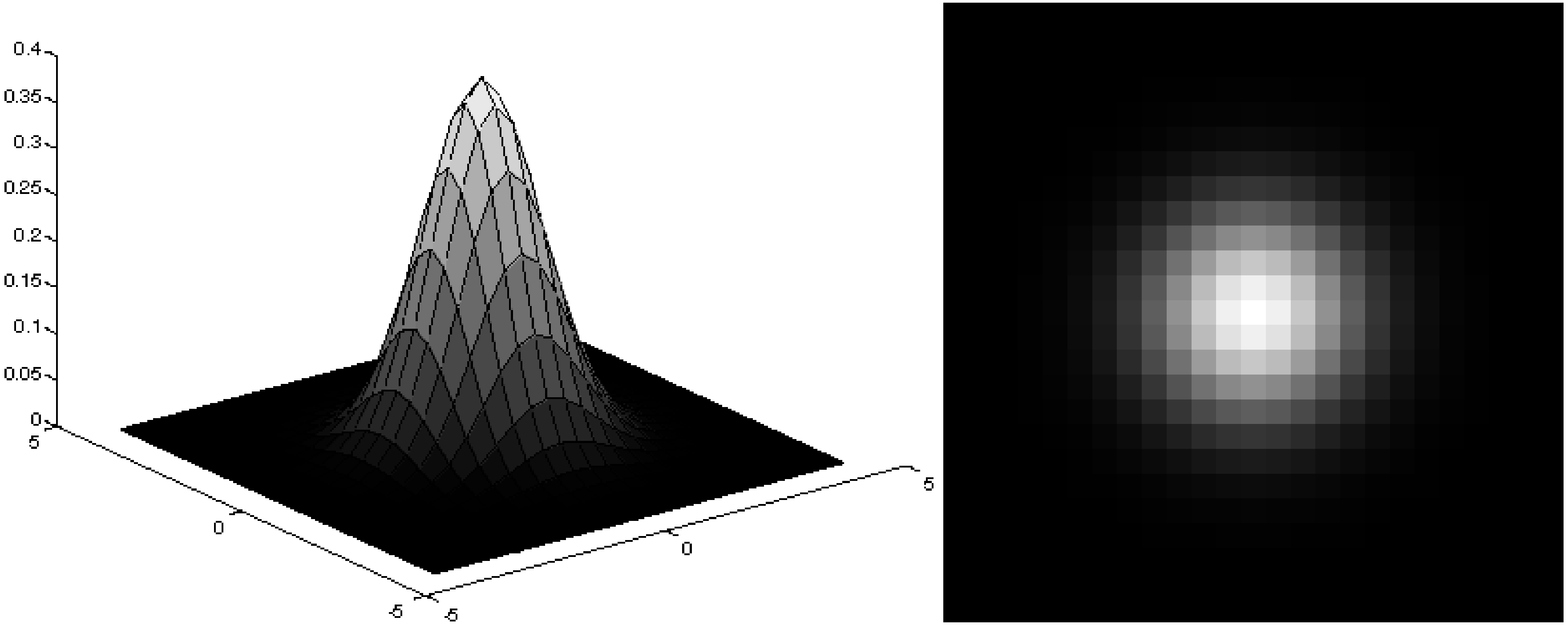
Today: Smoothing Reduces Noise



smoothing reduces noise, giving us (perhaps) a more accurate intensity surface.

Gaussian Smoothing Filter

An isotropic (circularly symmetric) Gaussian:



Gaussian Smoothing Example

original

sigma = 3

Gaussian Smoothing at Different Scales

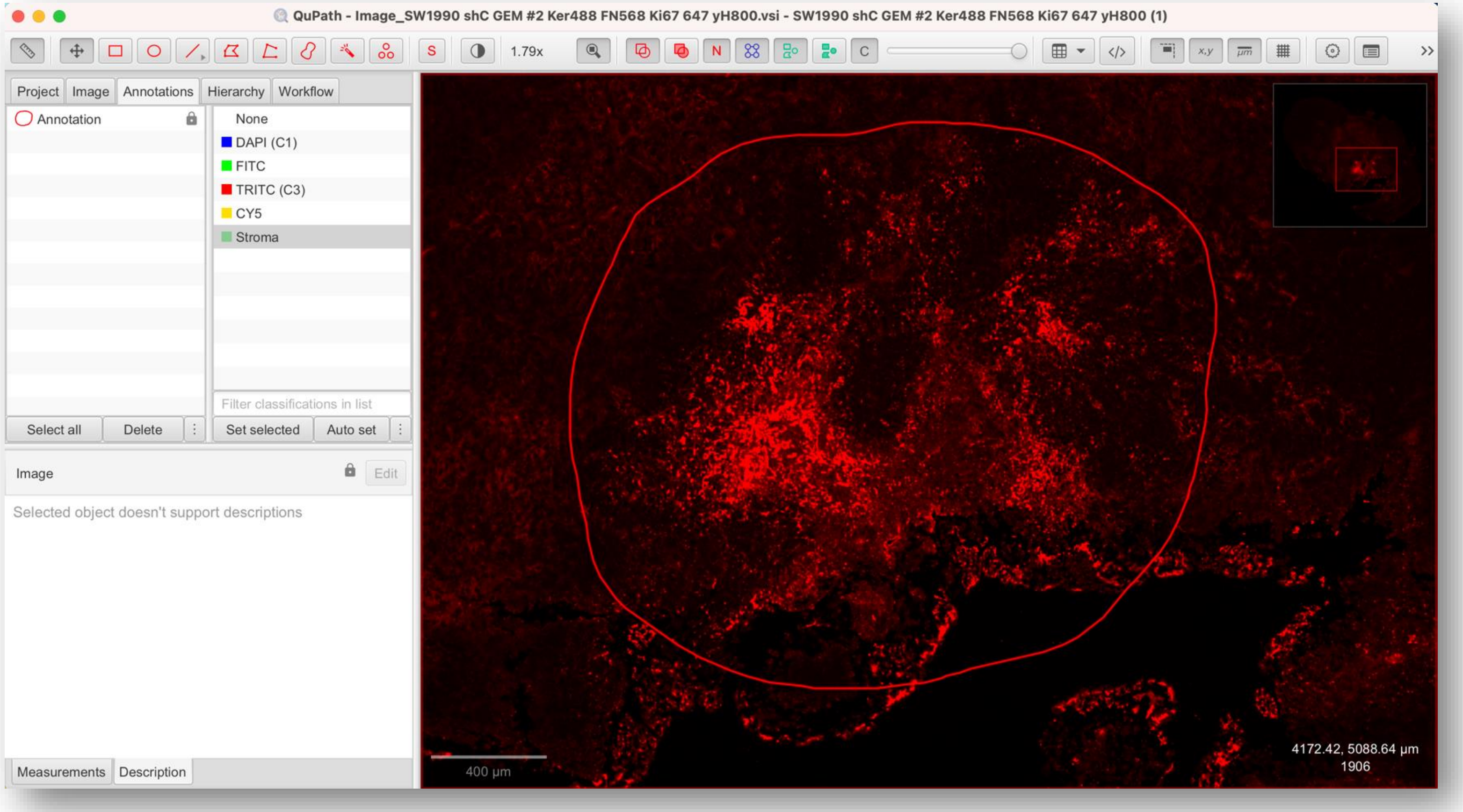
Balancing act: smooth enough to “clean up” the noise, but not so much as to remove important image gradients.

Back to QuPath

Intermezzo chiuso

Creating a region of interest

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



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

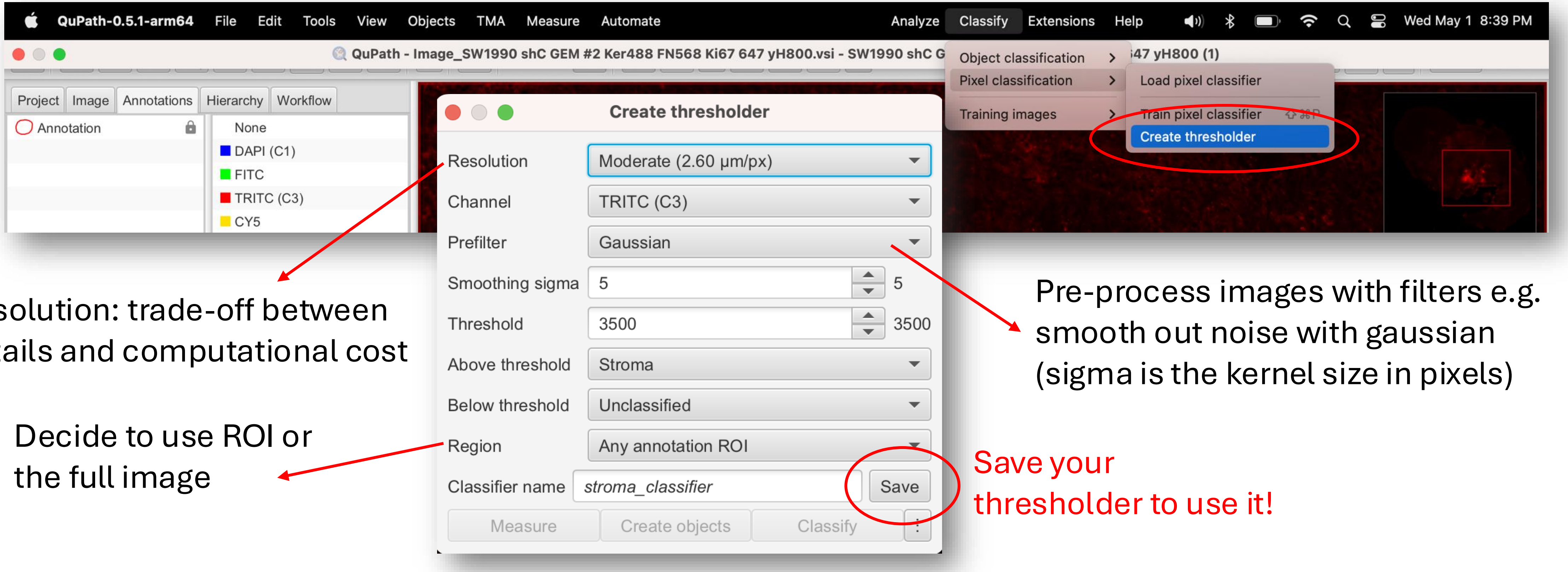
Right-click in the viewer > *Annotations* > *Lock*

or

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

Pixel-based tissue annotation

- Simplest case of annotation: every pixel get assigned a class based on its intensity value – **or is a given pixel above or below a certain numeric value?**



Resolution: Moderate (2.60 µm/px)
Channel: TRITC (C3)
Prefilter: Gaussian
Smoothing sigma: 5
Threshold: 3500
Above threshold: Stroma
Below threshold: Unclassified
Region: Any annotation ROI
Classifier name: *stroma_classifier*

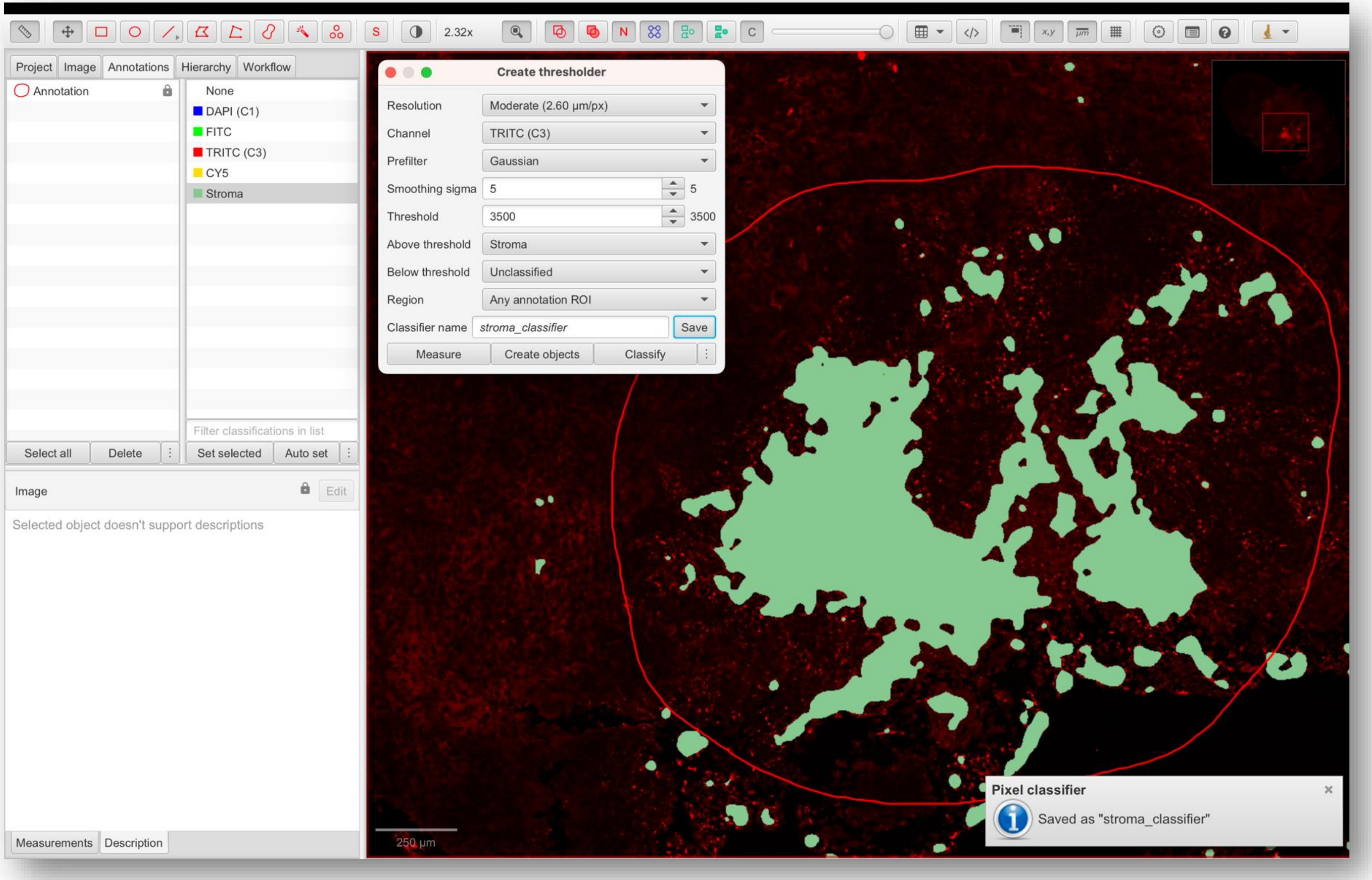
Resolution: trade-off between details and computational cost

Decide to use ROI or the full image

Pre-process images with filters e.g. smooth out noise with gaussian (sigma is the kernel size in pixels)

Save your thresholder to use it!

Interactive visualization of thresholding results



Selected object doesn't support descriptions

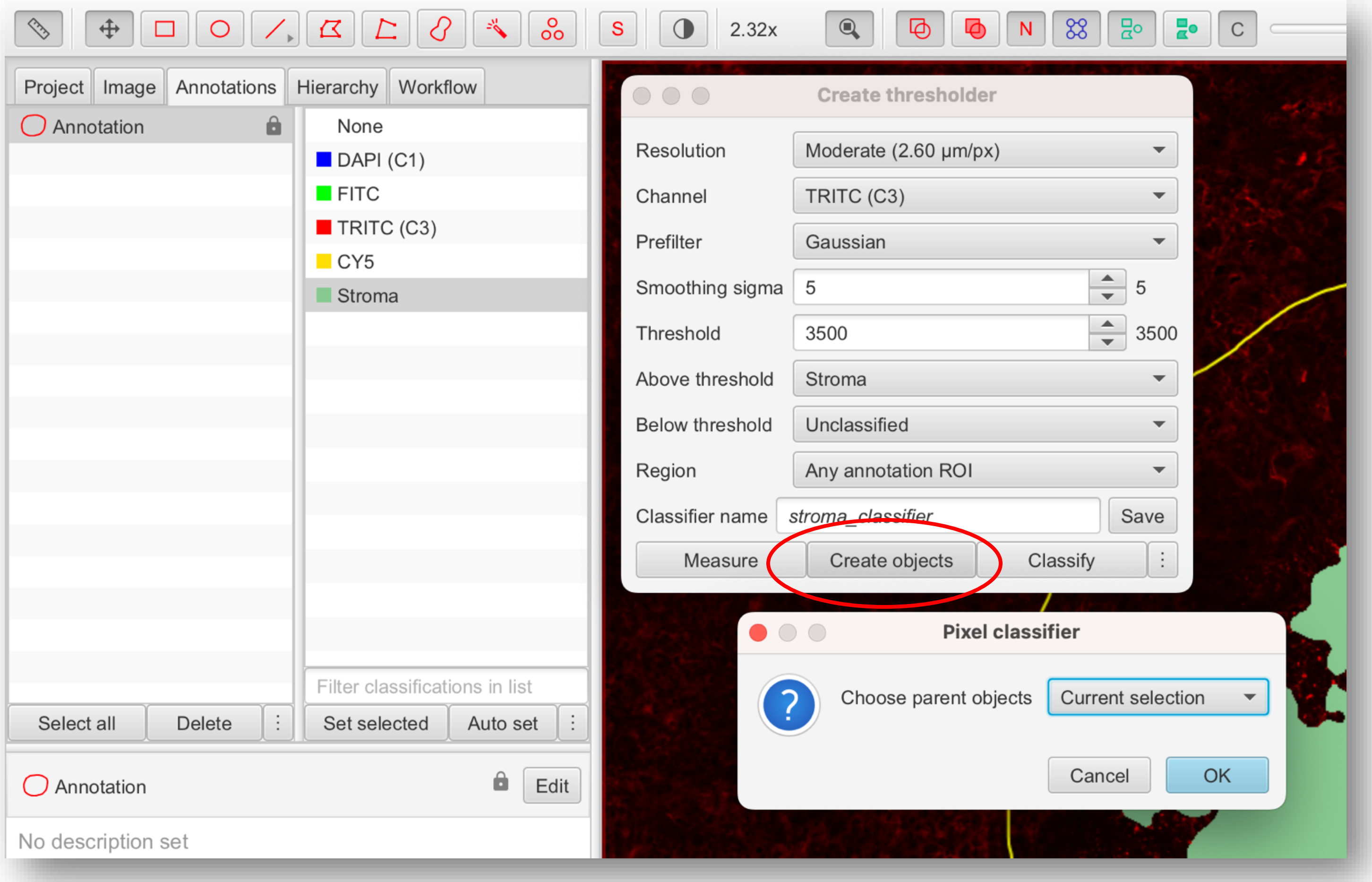
Resolution: Moderate (2.60 µm/px)
Channel: TRITC (C3)
Prefilter: Gaussian
Smoothing sigma: 5
Threshold: 3500
Above threshold: Stroma
Below threshold: Unclassified
Region: Any annotation ROI
Classifier name: *stroma_classifier*

Pixel classifier
Saved as "stroma_classifier"

Create a class ‘Stroma’

Try varying the value of the different parameters!

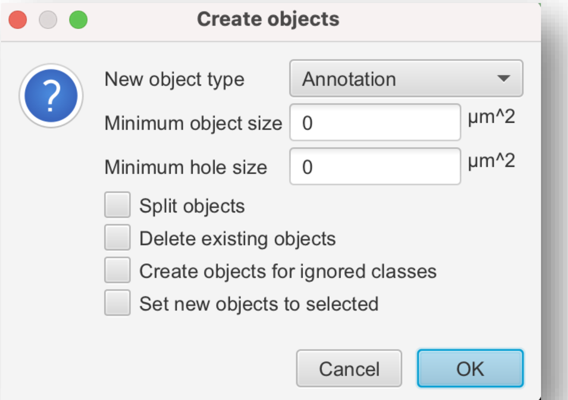
Create annotations from pixel classifier



Resolution: Moderate (2.60 µm/px)
Channel: TRITC (C3)
Prefilter: Gaussian
Smoothing sigma: 5
Threshold: 3500
Above threshold: Stroma
Below threshold: Unclassified
Region: Any annotation ROI
Classifier name: *stroma_classifier*

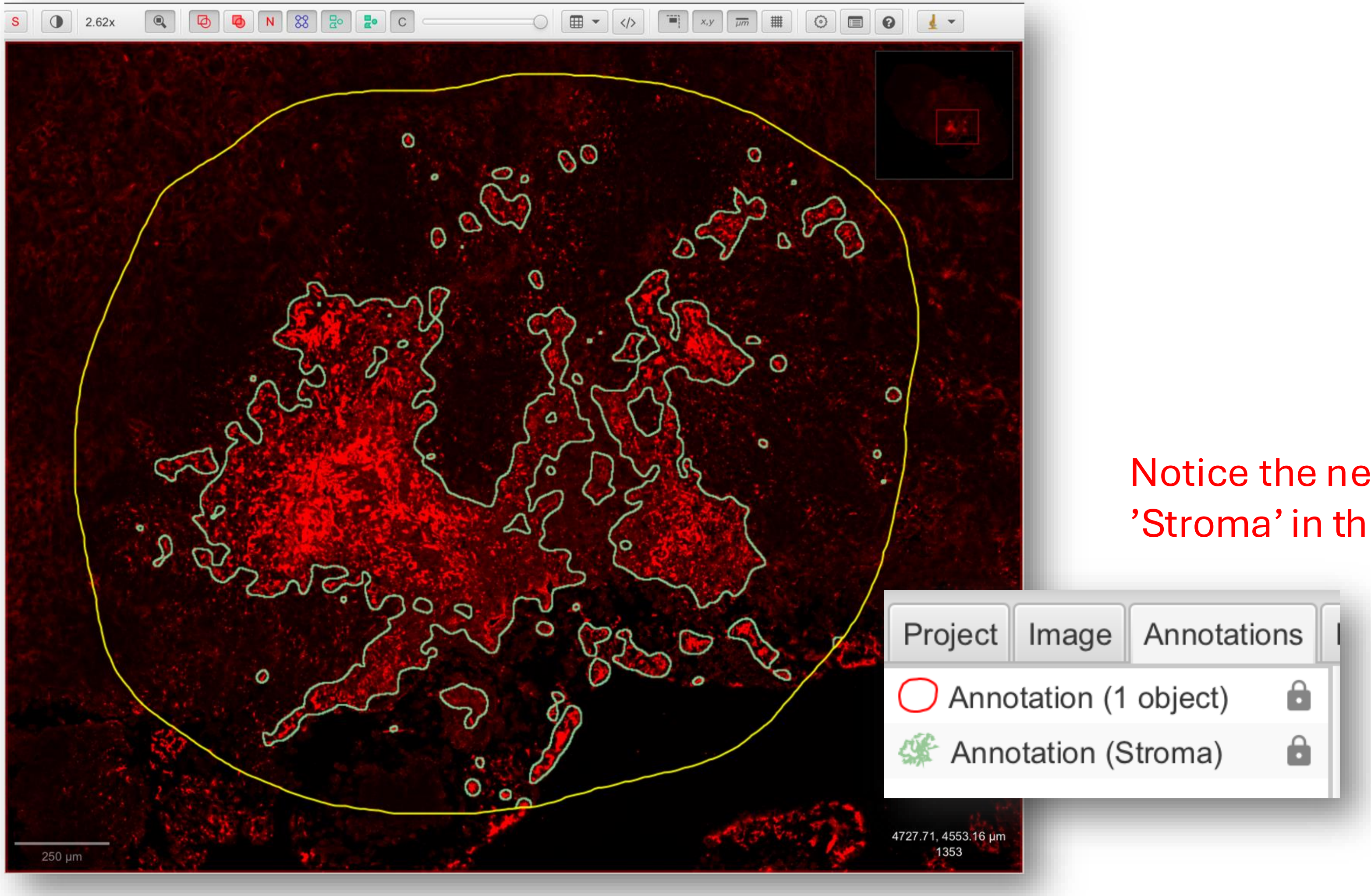
Choose parent objects: Current selection

- Real-time visualization of results, once happy with it:
  1. Save your thresholder
  2. Select ROI
  3. Click *Create objects*
  4. Keep default parameters > OK



New object type: Annotation
Minimum object size: 0 µm^2
Minimum hole size: 0 µm^2
☐ Split objects
☐ Delete existing objects
☐ Create objects for ignored classes
☐ Set new objects to selected

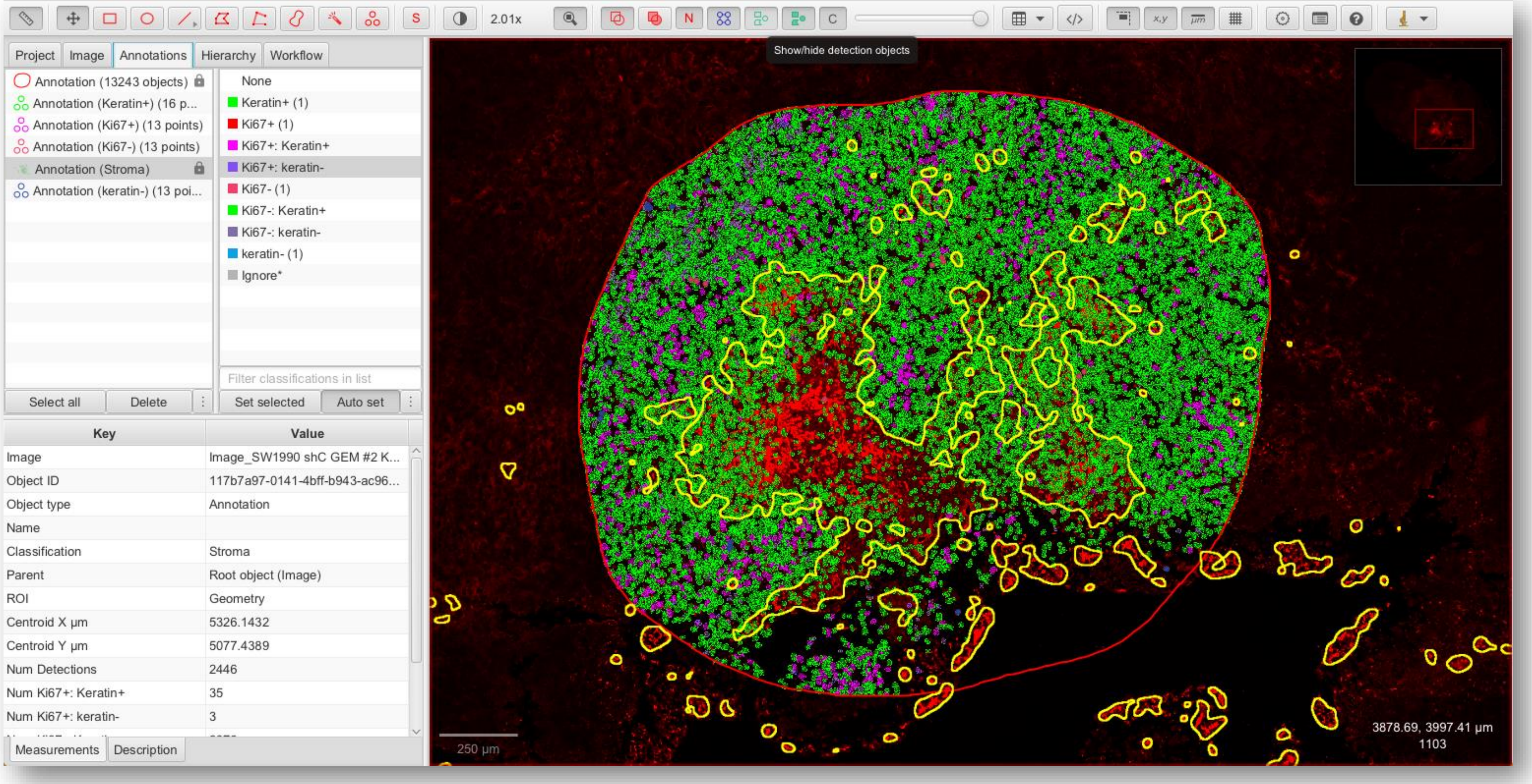
Create annotations from pixel classifier



Annotation (1 object)
Annotation (Stroma)

Notice the new annotation named ’Stroma’ in the *Annotations* list

Fully annotated image



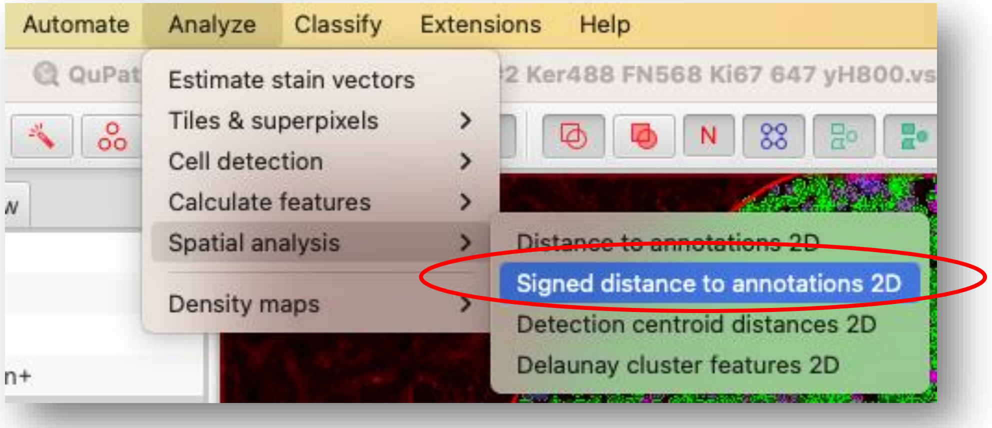
- Annotation (13243 objects)
- Annotation (Keratin+) (16 p...
- Annotation (Ki67+) (13 points)
- Annotation (Ki67-) (13 points)
- Annotation (Stroma)
- Annotation (keratin-) (13 poi...

- None
- 🟩 Keratin+ (1)
- 🟥 Ki67+ (1)
- 🟪 Ki67+: Keratin+
- 🟪 Ki67+: keratin-
- 🟥 Ki67- (1)
- 🟩 Ki67-: Keratin+
- 🟪 Ki67-: keratin-
- 🟦 keratin- (1)
- ⬜ Ignore*

Key	Value
Image	Image_SW1990 shC GEM #2 K...
Object ID	117b7a97-0141-4bff-b943-ac96...
Object type	Annotation
Name	
Classification	Stroma
Parent	Root object (Image)
ROI	Geometry
Centroid X µm	5326.1432
Centroid Y µm	5077.4389
Num Detections	2446
Num Ki67+: Keratin+	35
Num Ki67+: keratin-	3

Spatial information: signed distance

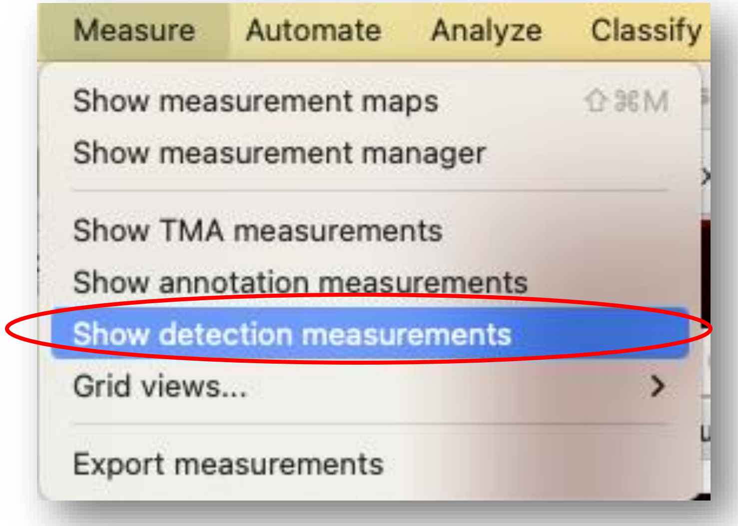
- *Analyze > Spatial analysis > Signed distance to annotations 2D*

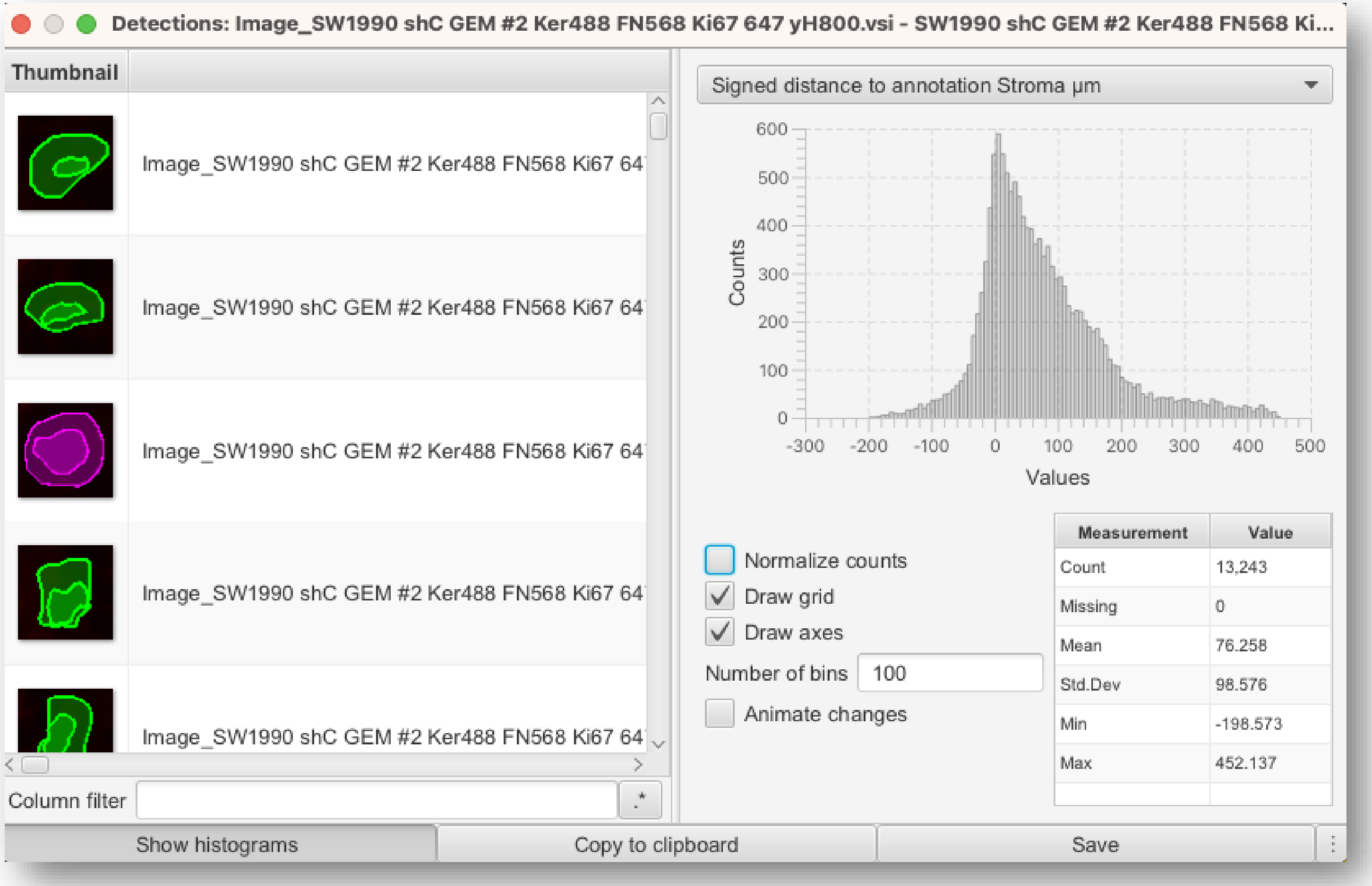


- Calculates the signed distance (2D euclidian) between cells and annotations
  - If a cell lies inside the annotation: negative distance
  - If a cell lies outside the annotation: positive distance

Spatial information: signed distance

- *Measure > Show detection measurements*

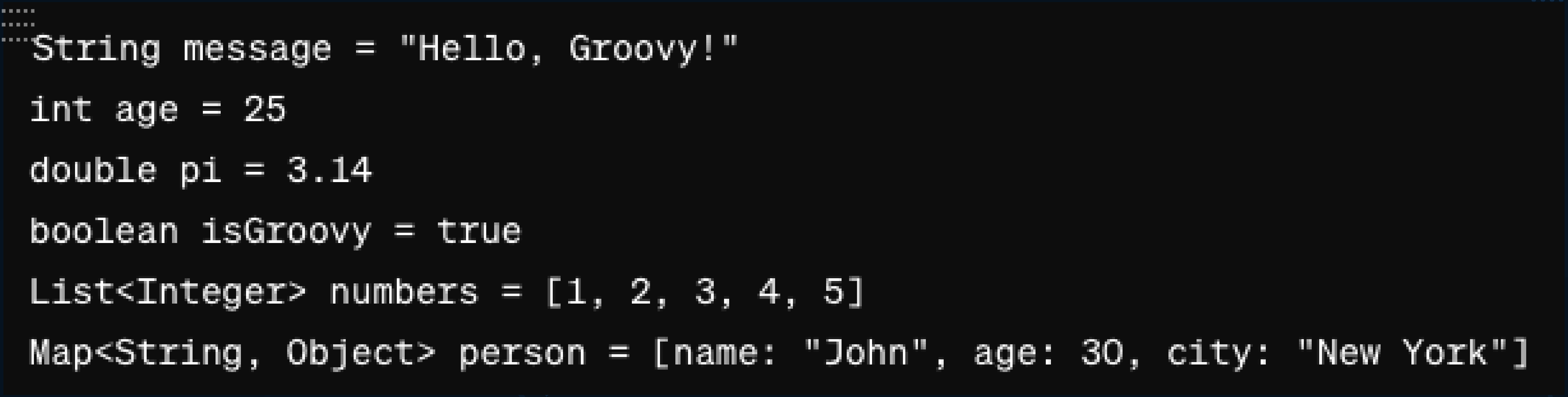




☐ Normalize counts
☑ Draw grid
☑ Draw axes
Number of bins 100
☐ Animate changes

Measurement	Value
Count	13,243
Missing	0
Mean	76.258
Std.Dev	98.576
Min	-198.573
Max	452.137

Export measurements table and use Python/R for visualization based on classes

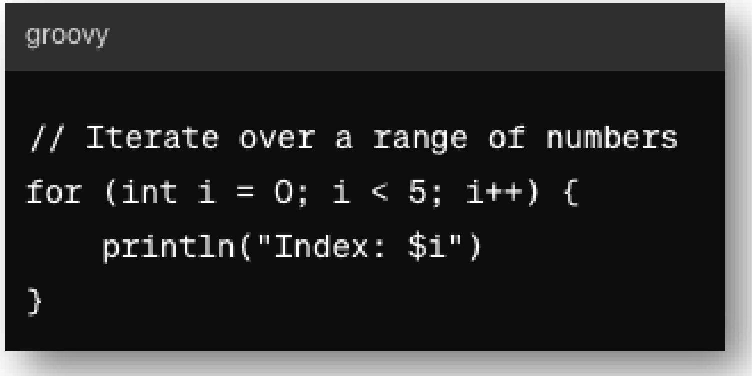


```
String message = "Hello, Groovy!"
int age = 25
double pi = 3.14
boolean isGroovy = true
List<Integer> numbers = [1, 2, 3, 4, 5]
Map<String, Object> person = [name: "John", age: 30, city: "New York"]
```

Scripting, workflows and batch processing

Scripting in QuPath

- QuPath uses **Groovy**, a scripting language with Java-like syntax
- Some **fun** facts about Groovy:
    - Created by James Strachan in 2003
    - Open-source (under the Apache License 2.0)
    - Groovy is a superset of Java and its syntax is Java-like
    - Bonus: dynamically typed (vs Java being statically typed)

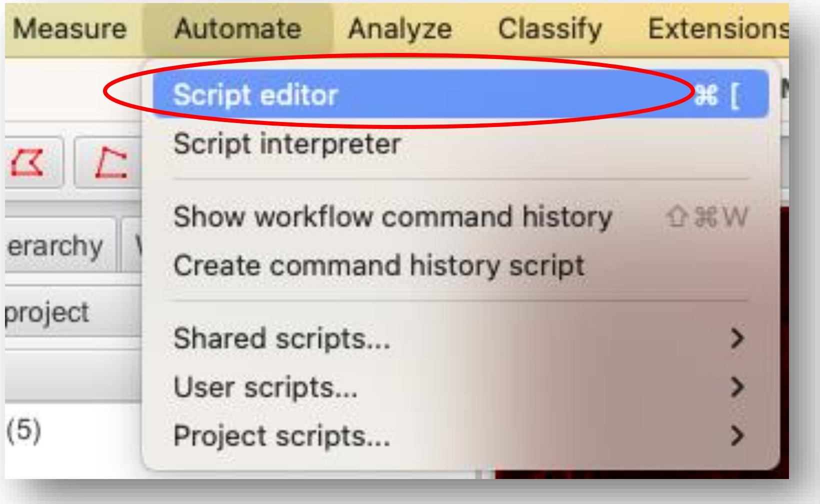


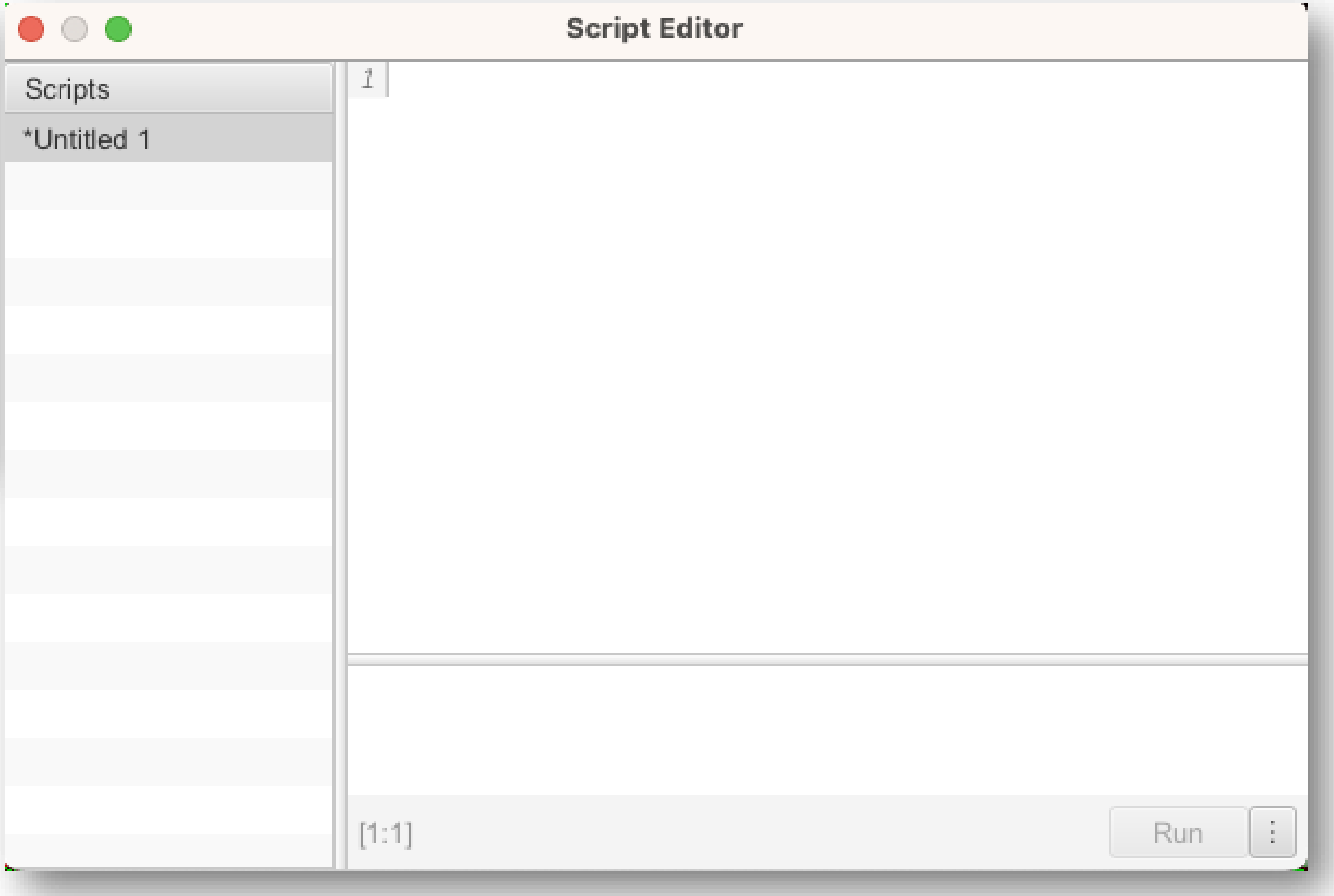
```groovy
// Iterate over a range of numbers
for (int i = 0; i < 5; i++) {
    println("Index: $i")
}
```

for *loop in Groovy*

Scripting in QuPath

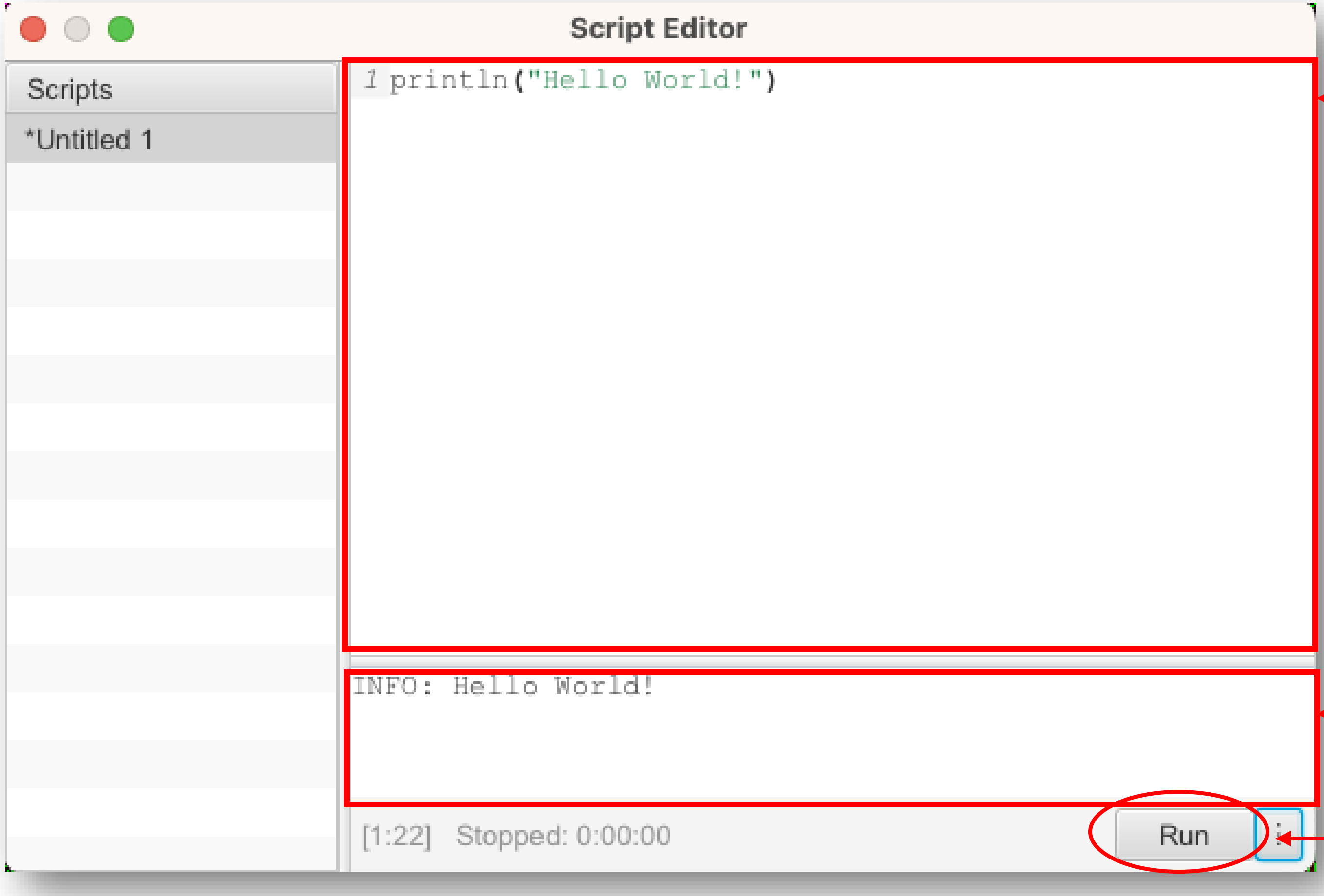
- *Automate > Script editor*





Hello World!

- *Automate > Script editor*



```
println("Hello World!")
```

```
INFO: Hello World!
```

Editor (where you write code)

Console

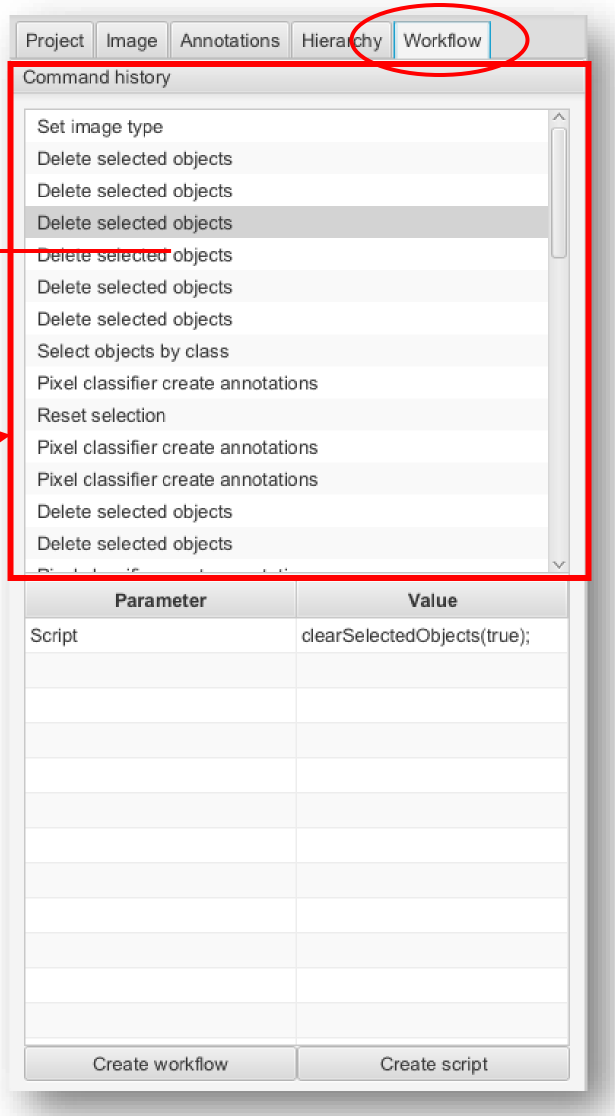
Run script

Automate your workflows without coding

QuPath uses **Workflows** to represent sequences of steps that have been applied to an image (commands run but also the parameters used).

- *Analysis panel > Workflow* tab

- The *Command history* is a record of most processing that has been done to the currently open image

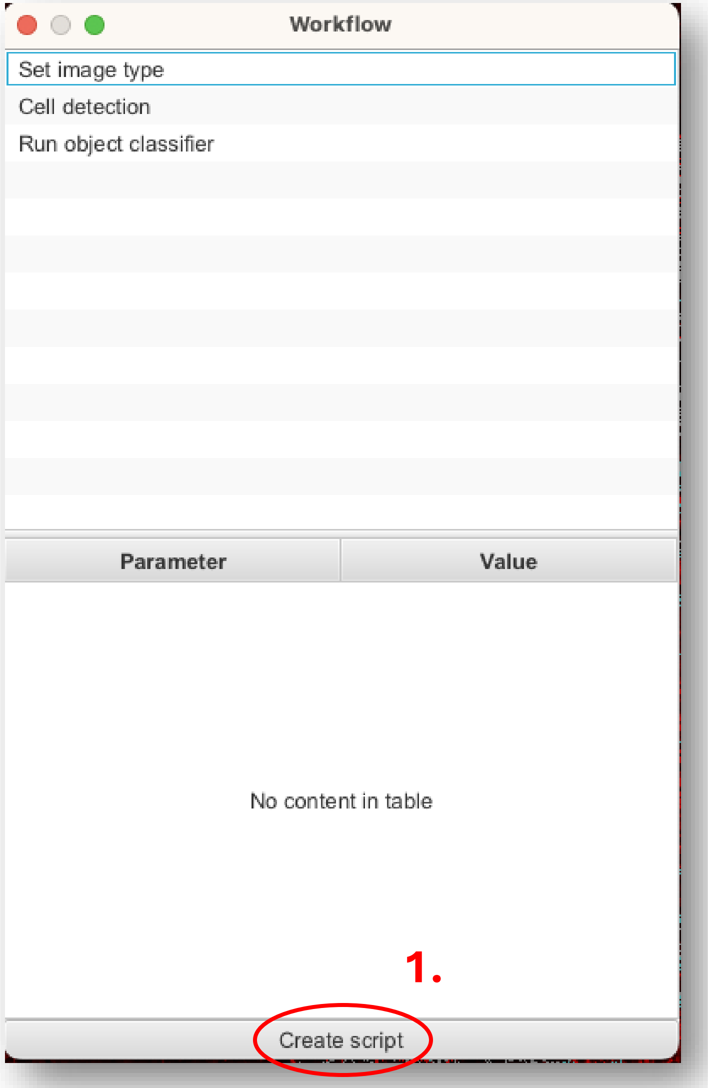


Command history

- Set image type
- Delete selected objects
- Delete selected objects
- Delete selected objects
- Delete selected objects
- Delete selected objects
- Delete selected objects
- Select objects by class
- Pixel classifier create annotations
- Reset selection
- Pixel classifier create annotations
- Pixel classifier create annotations
- Delete selected objects
- Delete selected objects

Parameter	Value
Script	clearSelectedObjects(true);

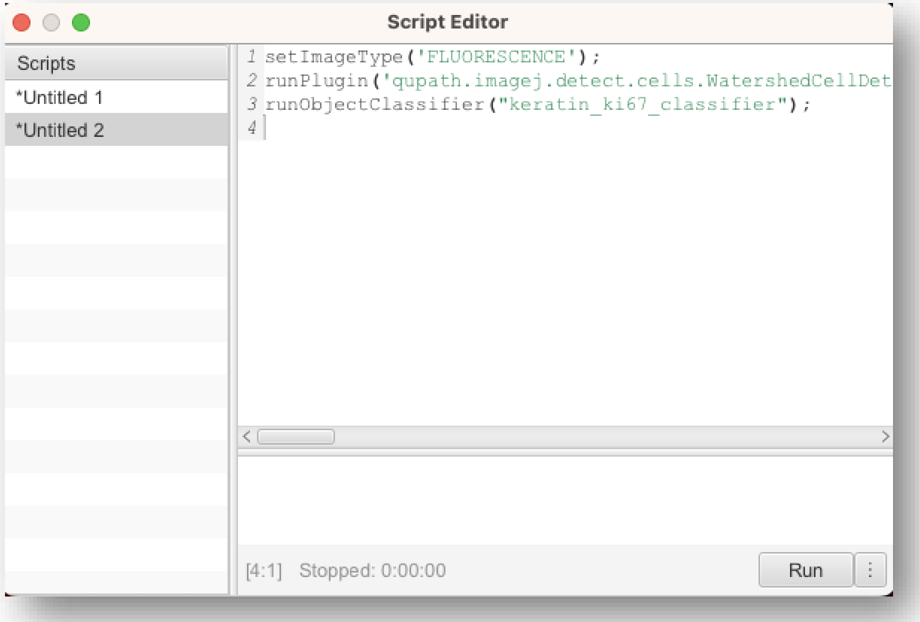
Clean your workflow for cell detection and classification



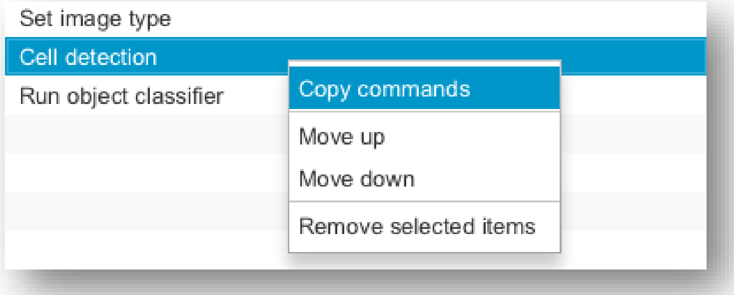
- Set image type
- Cell detection
- Run object classifier

1.

2.

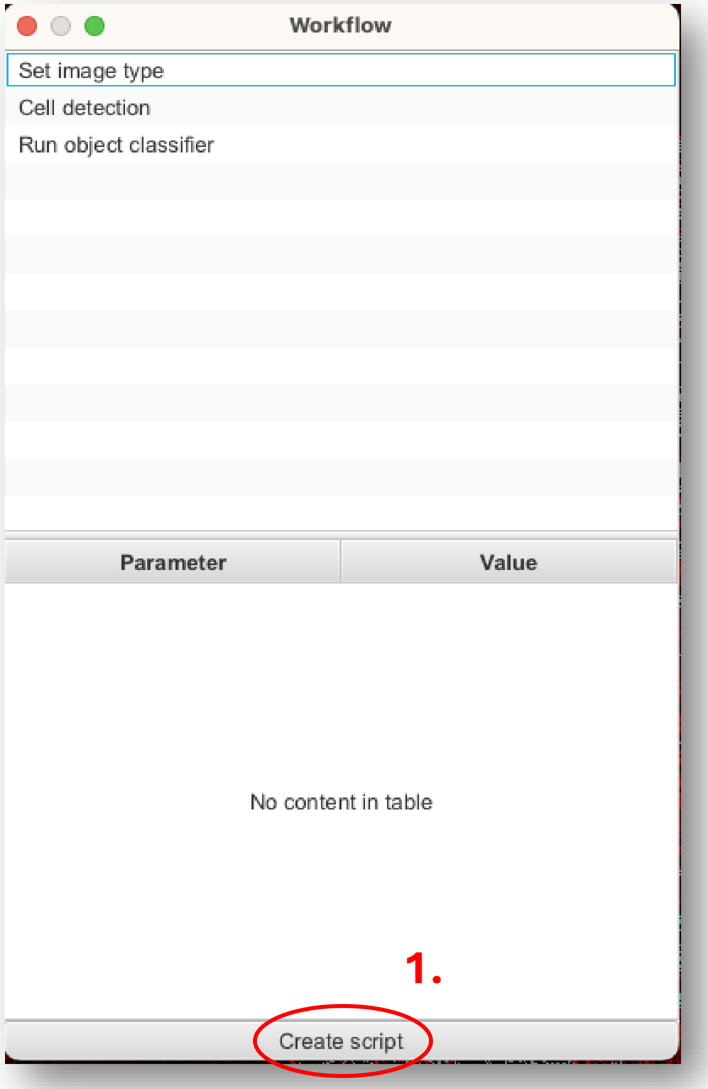


```
setImageType('FLUORESCENCE');
runPlugin('qupath.imagej.detect.cells.WatershedCellDet
runObjectClassifier("keratin_ki67_classifier");
```



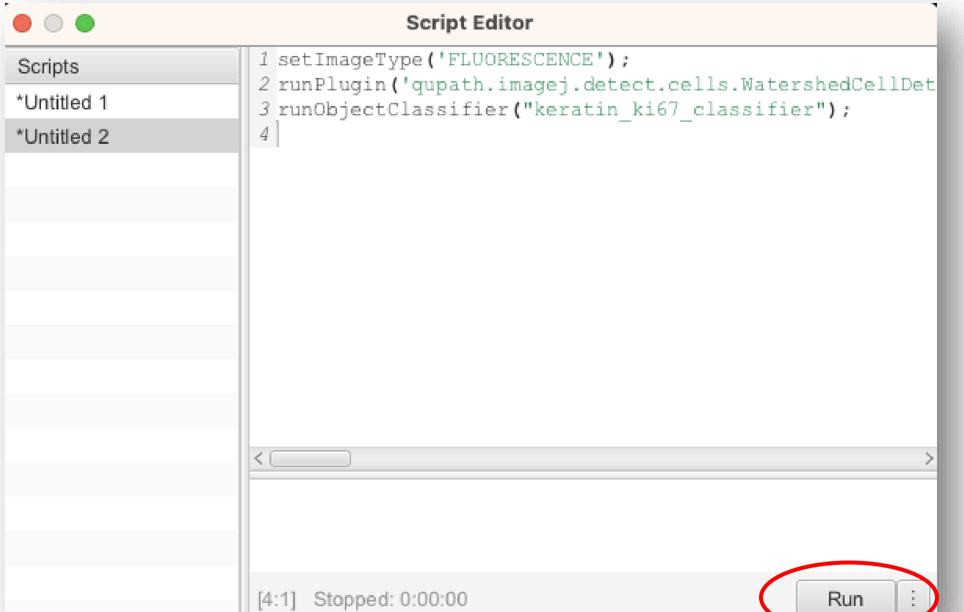
Edit the sequence of steps in the workflow using right-click

Save and run a script



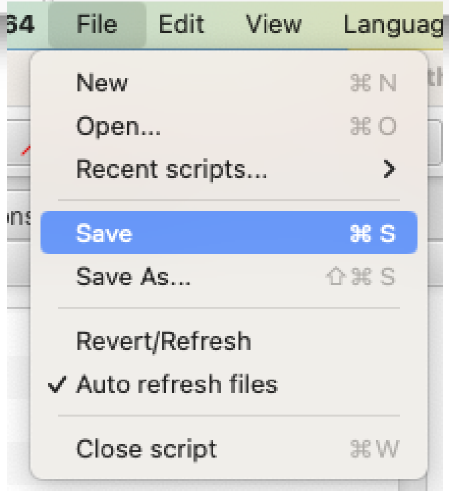
1.

2.



```
setImageType('FLUORESCENCE');
runPlugin('qupath.imagej.detect.cells.WatershedCellDet
runObjectClassifier("keratin_ki67_classifier");
```

3.

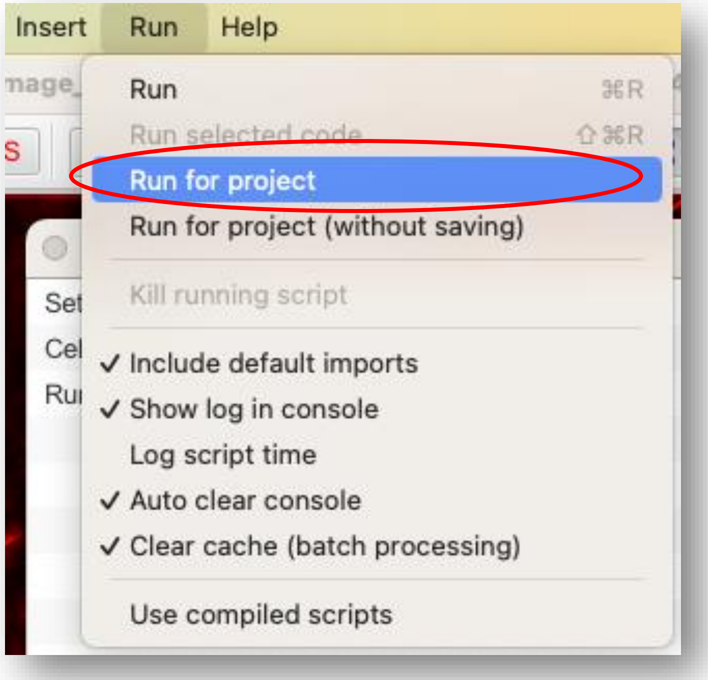


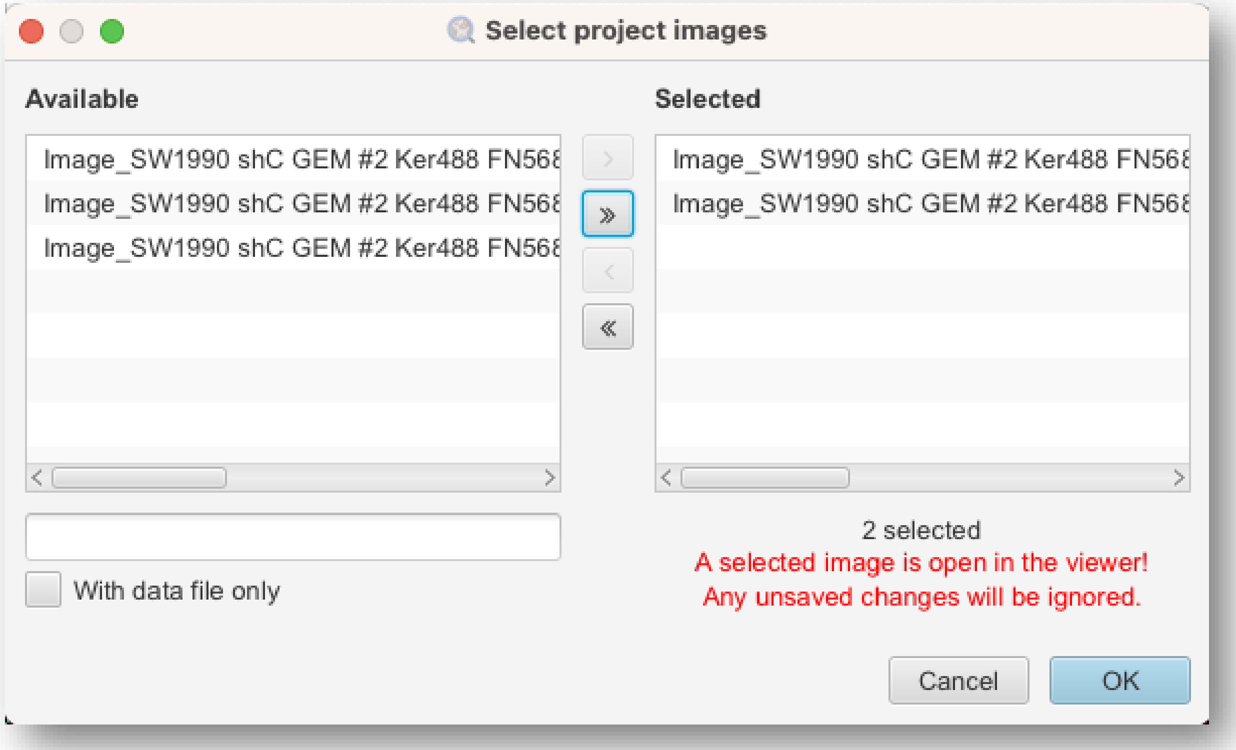
4.

Scripts can be repeated on a batch of images

QuPath allows for batch processing: scripts will run on multiple images loaded in the project.

- *Run > Run for project*





2 selected

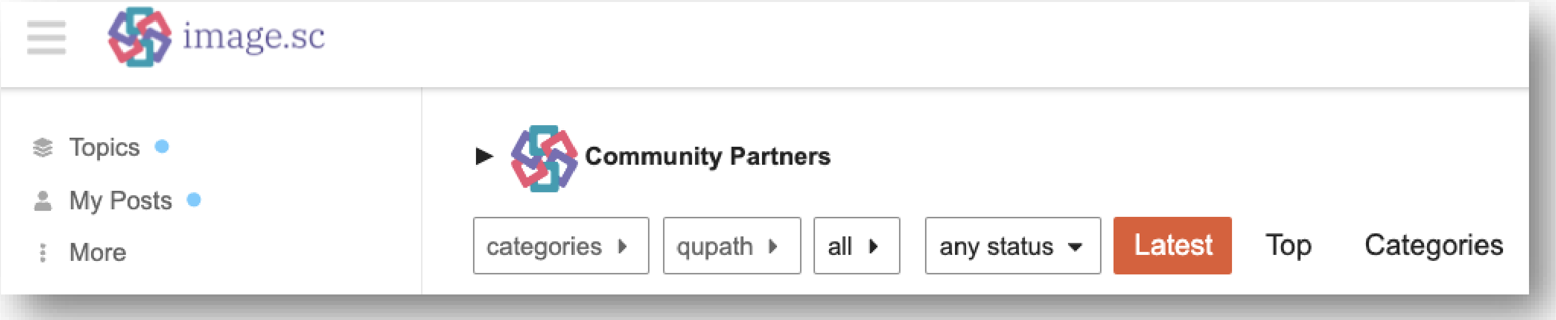
A selected image is open in the viewer!
Any unsaved changes will be ignored.

Select images you wish to run the script on.

Further resources

- QuPath documentation
  - Scripting: https://qupath.readthedocs.io/en/latest/docs/scripting/overview.html
  - QuPath’s API docs: https://qupath.github.io/javadoc/docs/

- The Forum
  - Where to contact the developers of most image analysis tools
  - If you have a question, likely someone else already asked



QuPath on O2

Ranit Karmakar

Give us your feedback!

https://tinyurl.com/52hu7bkt

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