



Illustration reprinted from Pete Bankhead.

# Introduction to Bioimage Analysis using QuPath

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# Acknowledgments

- **Pete Bankhead et al.**
  - QuPath and its amazing documentation

# Reasons to learn image processing

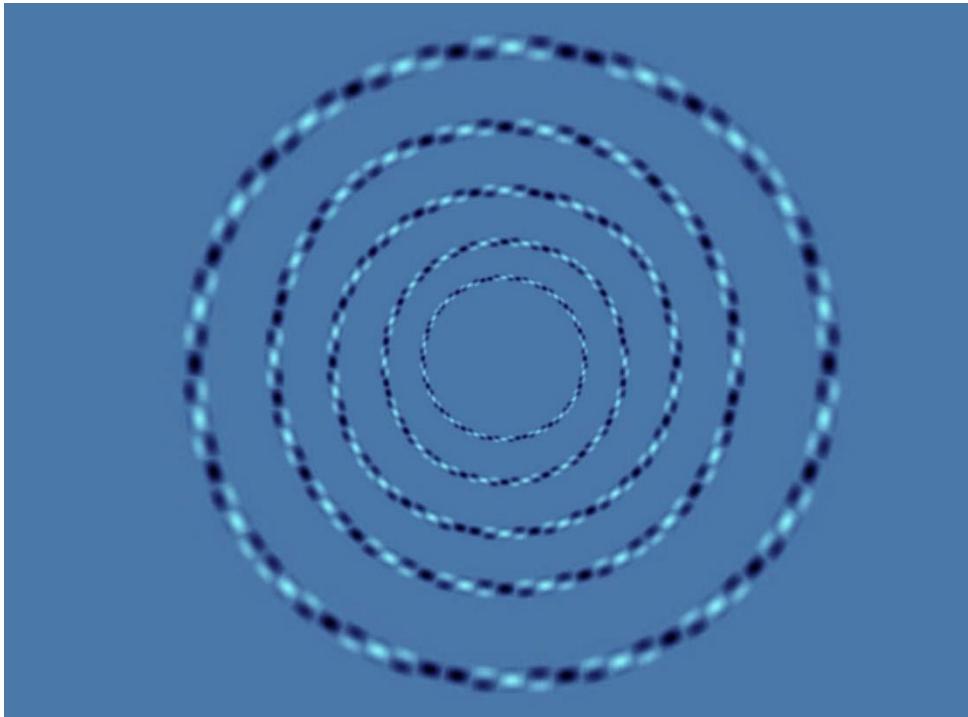
- Make pretty pictures (processing)
  - publications, talks, websites, ...
- Get numbers out of pictures (analysis)
  - cell sizes, vessel lengths, GFP expression level, ...
- Make experiment possible (automation)
  - whole-genome screen: millions of images
- Objectivity and Reproducibility
  - in science,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

# Reasons not to learn image processing

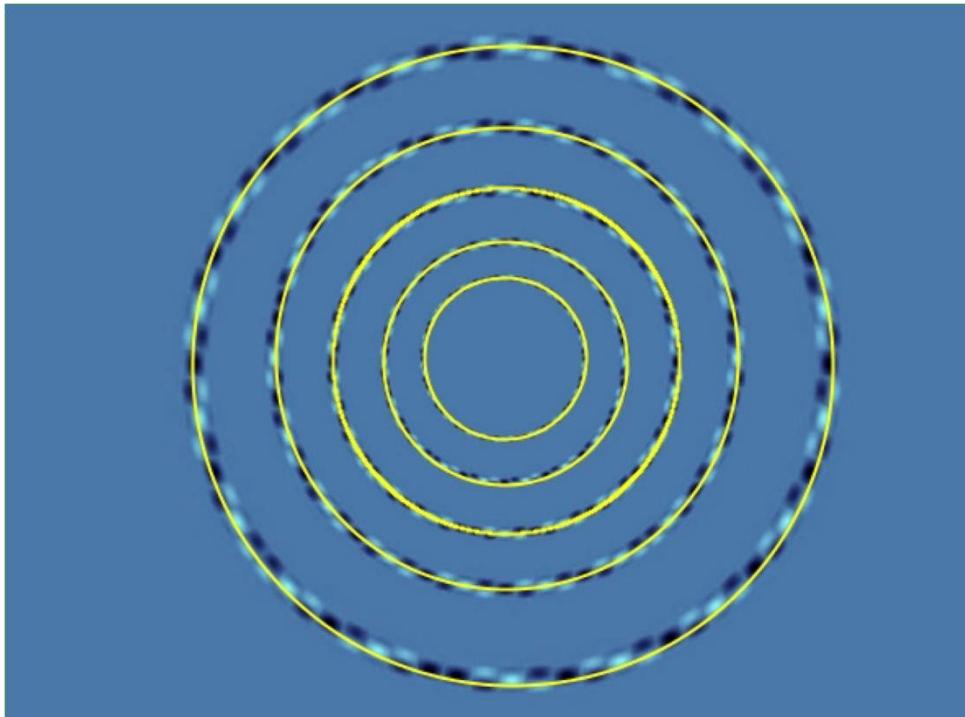
none

# Why should we analyze images with computers at all?

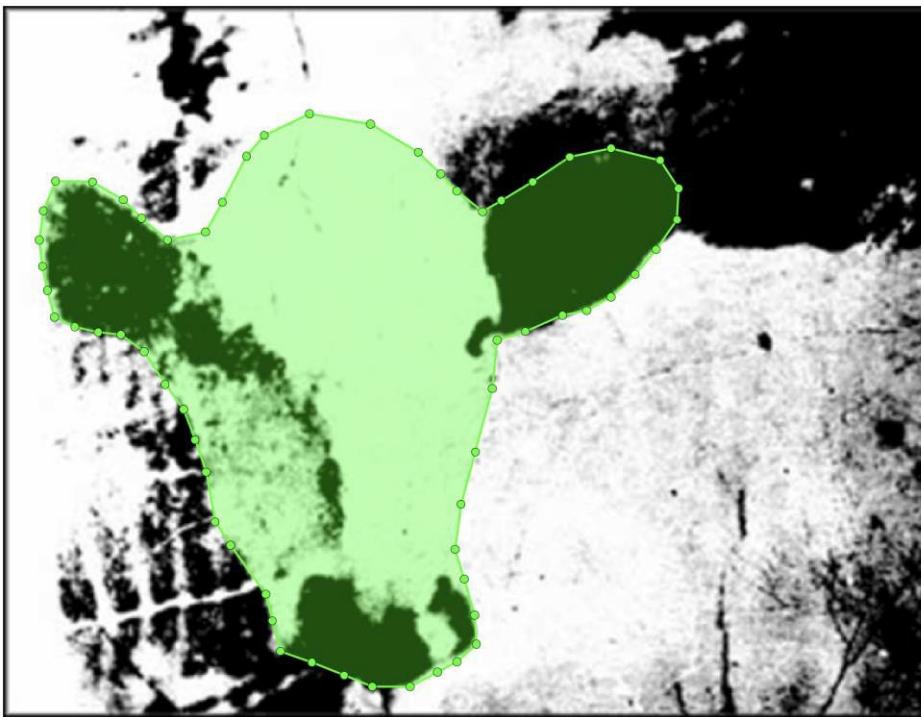
Color perception and pattern recognition is individual - Science less so



Color perception and pattern recognition is individual - Science less so



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



<http://www.brainbashers.com>

# 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



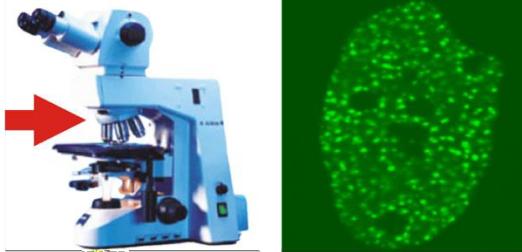
Think of this even ***before*** you acquire the images!

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

# Image processing vs analysis

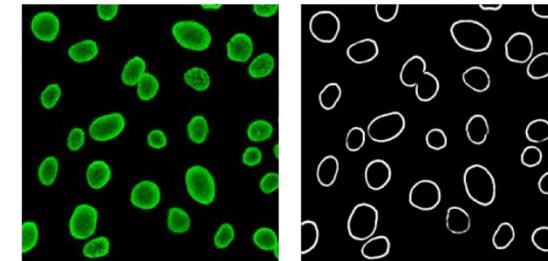
## Image Formation

object in → image out



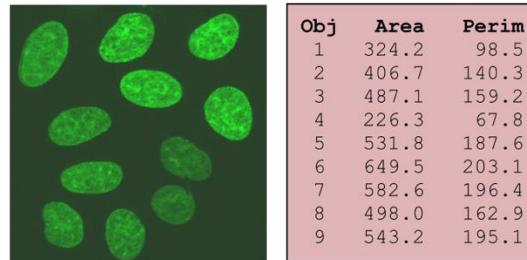
## Image Processing

image in → image out



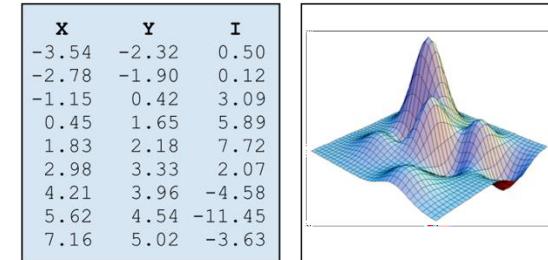
## Image Analysis

image in → features out



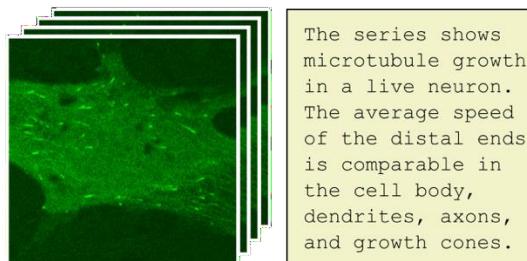
## Computer Graphics

numbers in → image out



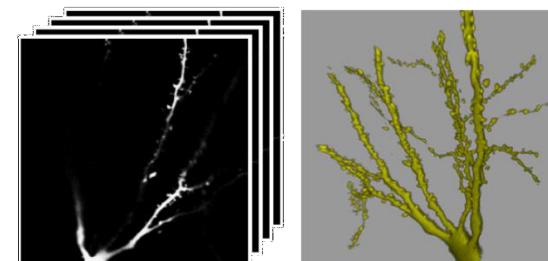
## Computer Vision

image in → interpretation out



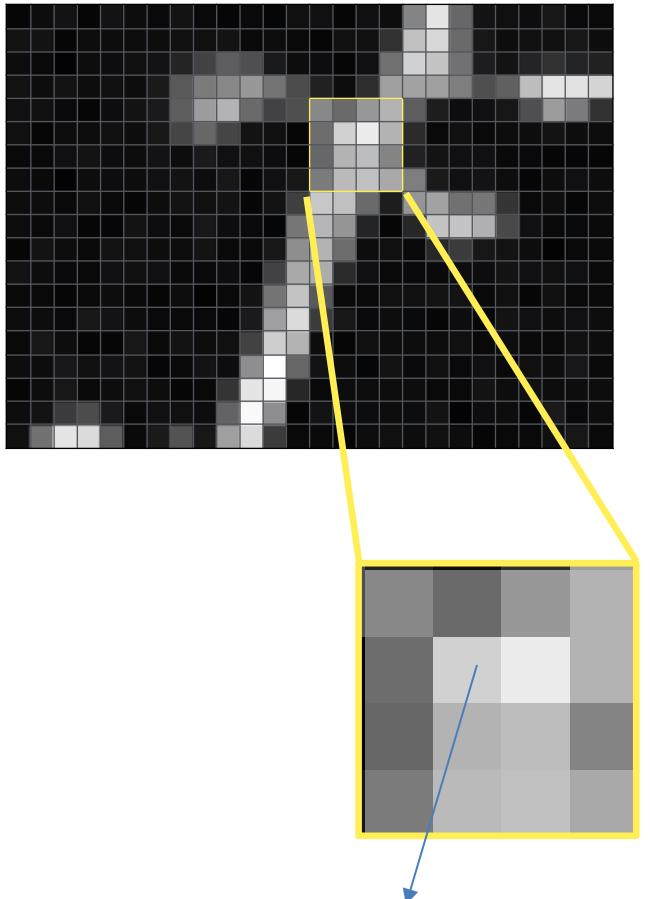
## Visualization

image in → representation out



# What is an image?

# A digital image is a matrix of numbers!



=

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	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	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	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	19	4	13	13	6	26	9	13	
16	113	229	219	93	9	26	83	23	159	219	59	9	6	13	16	13	16	13	6	9	9	16	23	9	

=

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

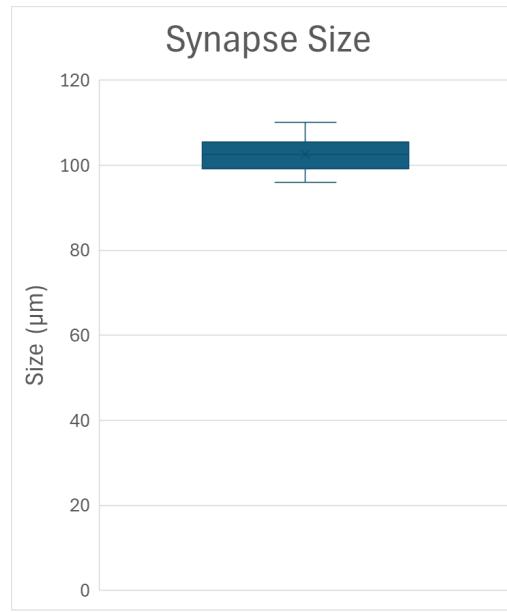
**Pixel = Picture Element**

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

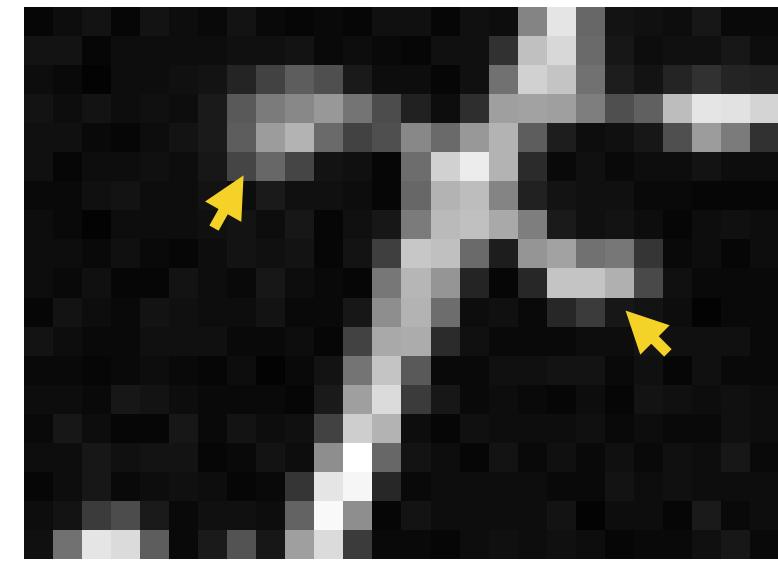
this is your **data**

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	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	19	4	13	13	6	26	9	13	9	
16	113	229	219	93	9	26	83	23	159	219	59	9	6	13	16	13	13	6	9	9	16	23	9	9	9	

this is your **result**

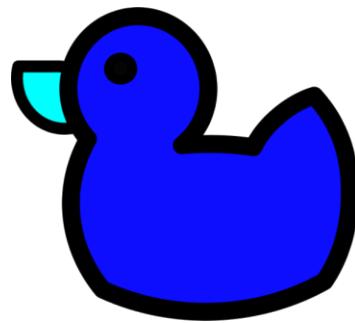


this just helps to  
**communicate** the result

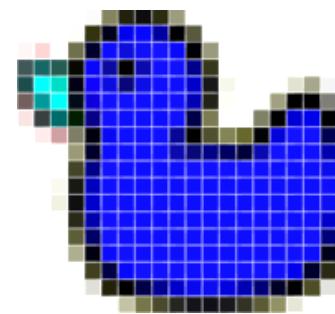


# Acquiring Images

# Sampling

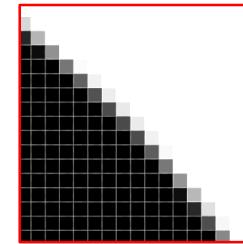
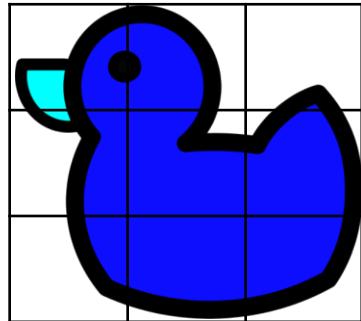
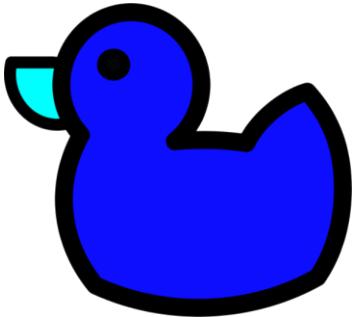


Object



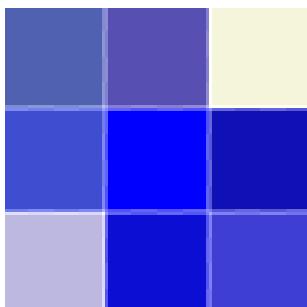
Pixels

# Sampling

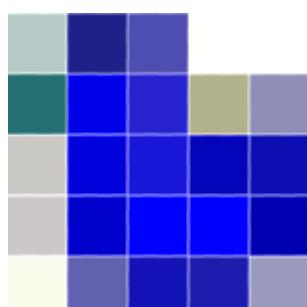


Frame size

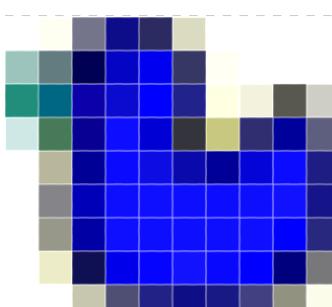
3x3



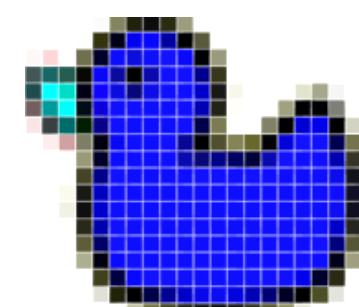
5x5



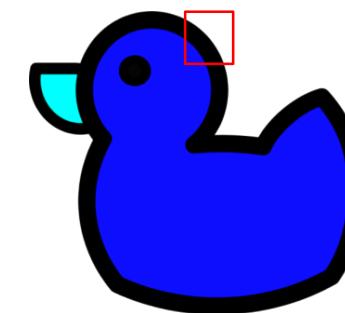
10x10



20x20



300x300



Pixel size

$1/3 = 0.3$

0.2

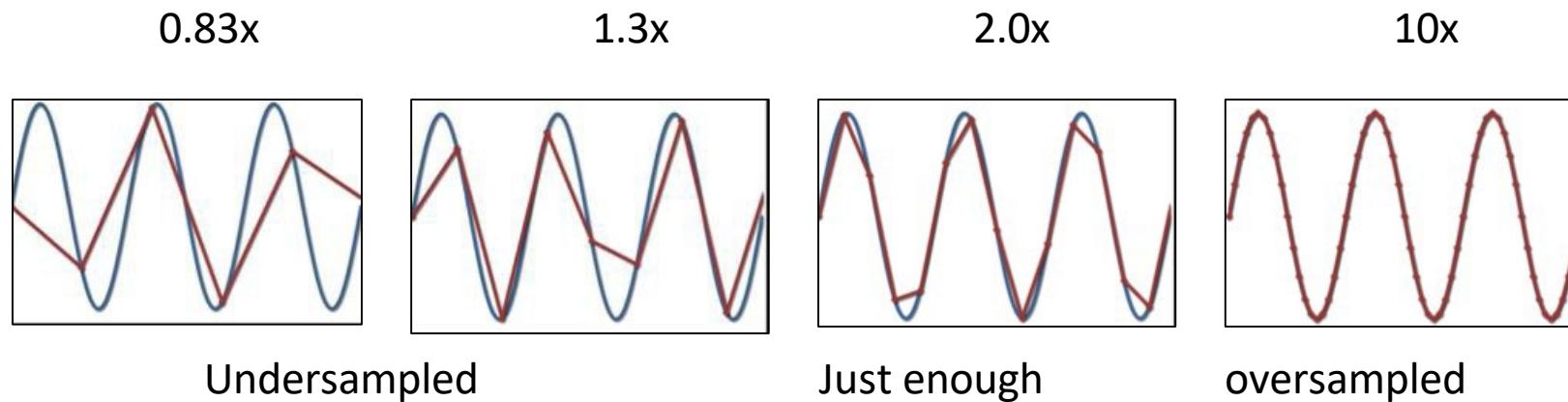
0.1

0.05

0.003

# Sampling, Nyquist

In order to convert analogue signal to digital the signal needs to be sampled **at least 2 times** of half a cycle (from top to bottom)



# Sampling, Nyquist

Pixel dimensions ( $\mu\text{m}$ ) = Theoretical axial resolution/2

Magnification	NA	Type	405 nm	488 nm	561 nm	633 nm
5 x	0.1	Air	704	848	975	1100
10 x	0.3	Air	235	282	325	367
20 x	0.5	Air	141	169	195	220
40 x	1.25	Oil	57	68	78	88
63 x	1.4	Oil	50	60	70	79
100 x	1.4	Oil	50	60	70	79

# Keep RAW data

## SAVE

Always save as the raw file extension

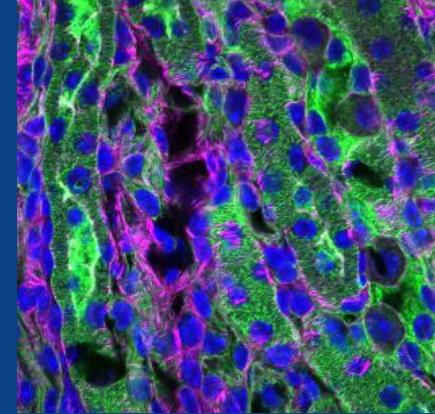
.lif , xlef(Leica)

.czi (Zeiss)

etc.....

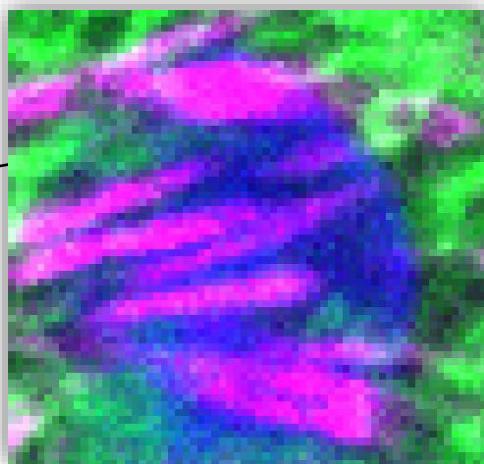
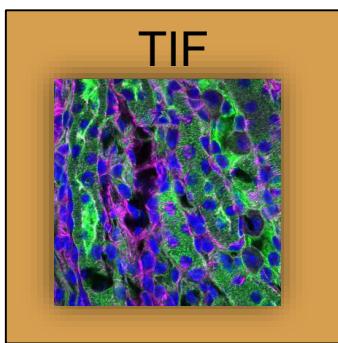
Metadata contains all image parameters, at least

- Laser wavelength
- Light path settings
- Objective lens / NA
- Pinhole
- Frame Size
- Voxel/Pixel dimension
- Gain/offset
- Bit Depth
- Scan speed
- Average

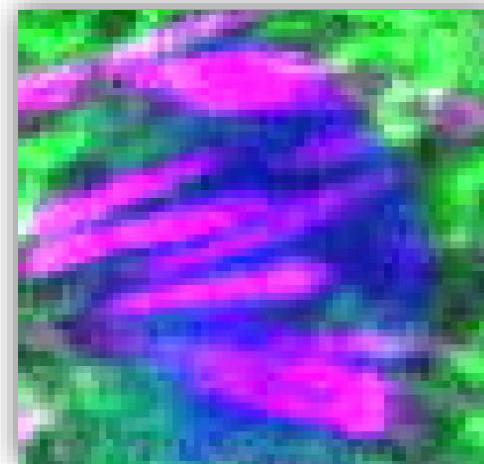
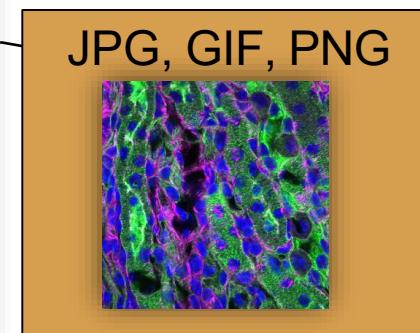
Raw file	
Image	Metadata
	Pinhole [airy] 1.00
	Size-Width 25.8 μm
	Size-Height 25.8 μm
	Size-Depth 0.0
	StepSize 0.04 μm
	Voxel-Width 50.6 nm
	Voxel-Height 50.6 nm
	Voxel-Depth 0.0 nm
	Voxel-Volume 0 nm³

# Keep RAW data

Export (only if necessary, a lot of image processing applications can import RAW data)



Keeps original information  
for data analysis (but not  
MetaData)



Compressed, lossy  
for PowerPoint etc.  
only

# Image = Scientific Data!

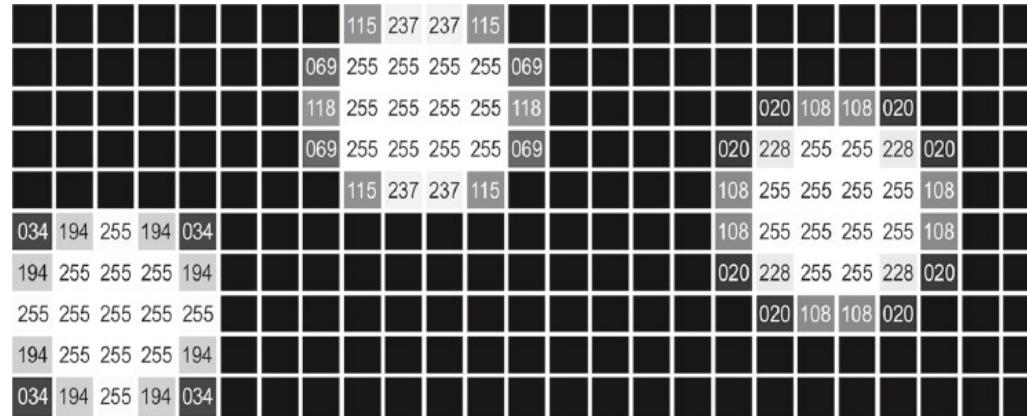


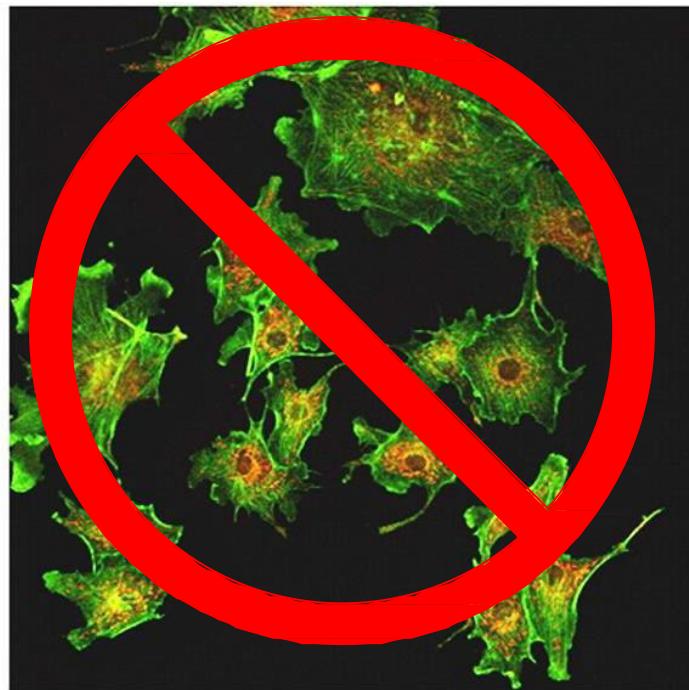
Image beautification = data manipulation

“oops, I was not aware of this”

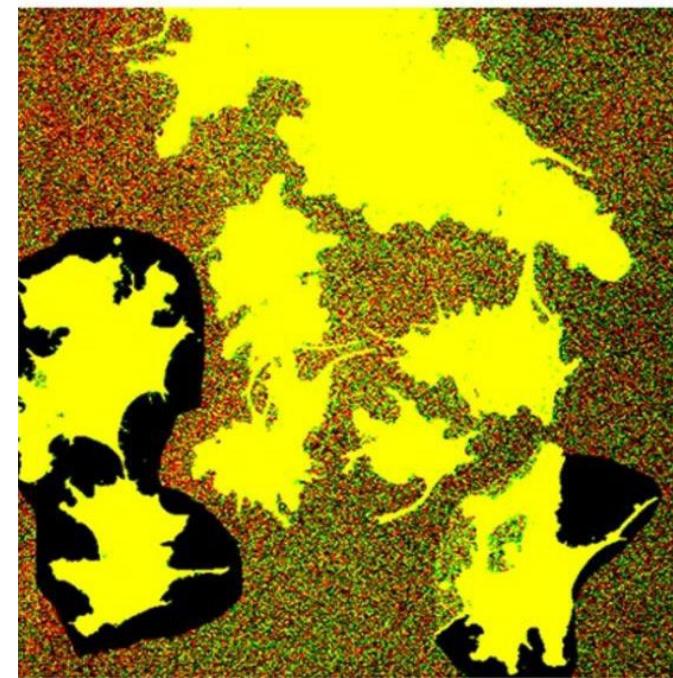
“sorry, but I thought it was okay to make the images clearer”

# Image = Scientific Data!

Manipulated image ?



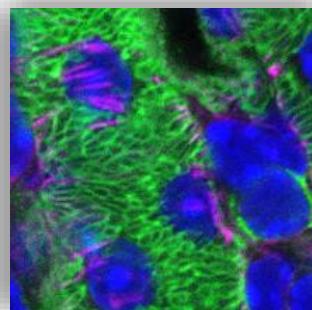
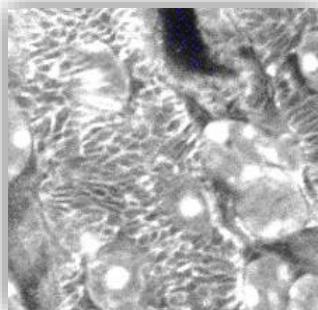
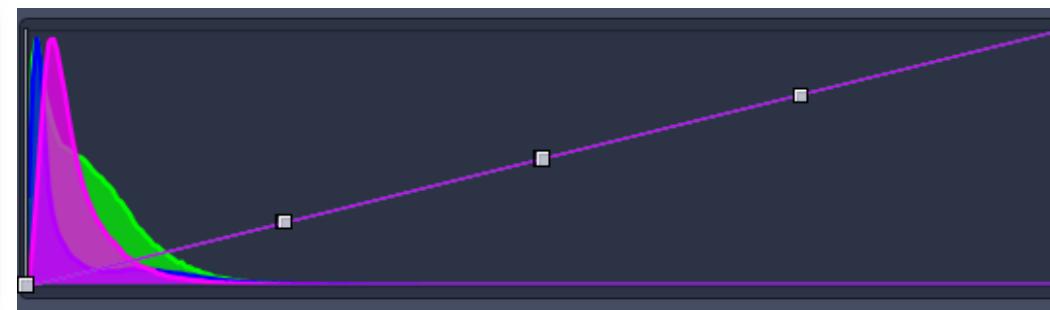
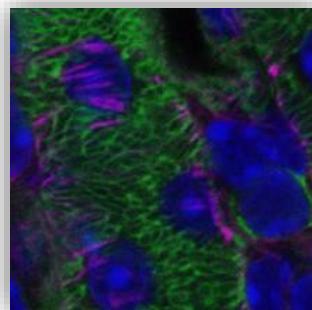
Manipulation revealed  
By contrast adjustment



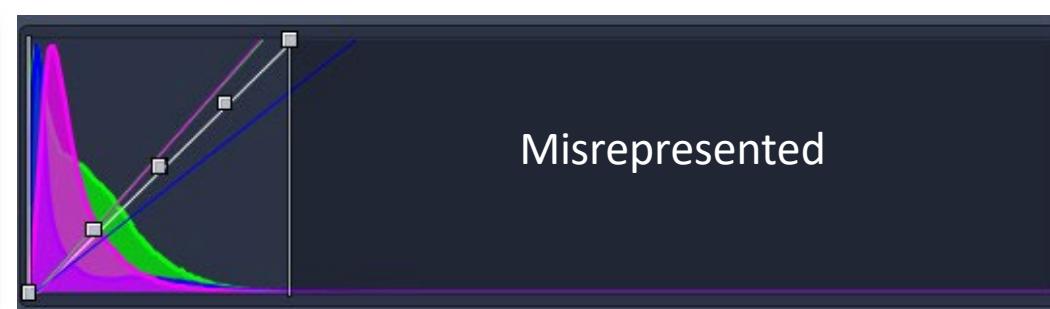
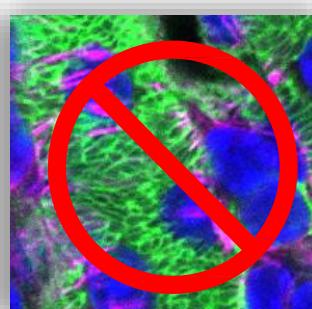
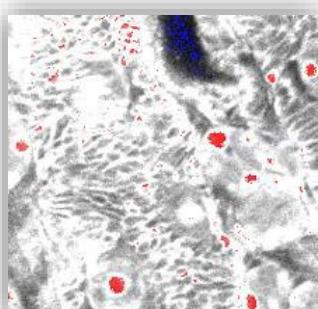
Cells from other image has been **added**

# Image = Scientific Data!

Original image



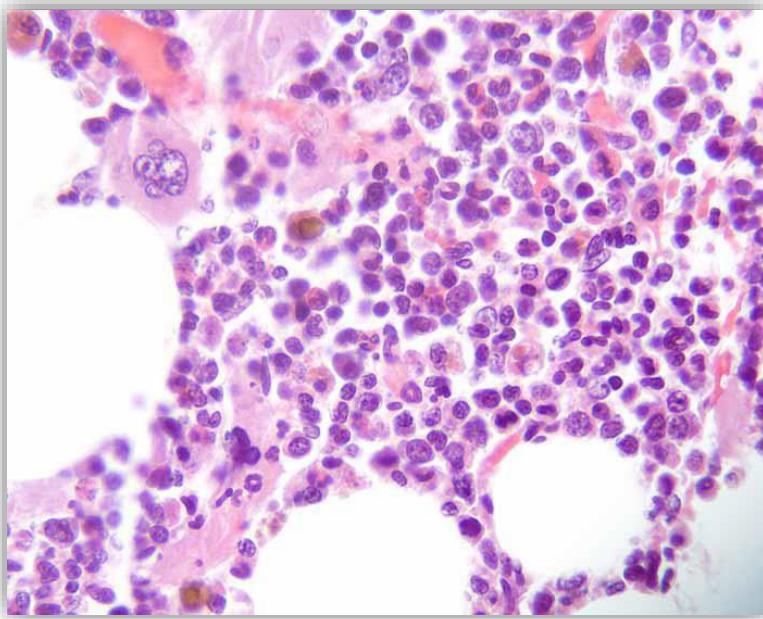
Generally  
acceptable



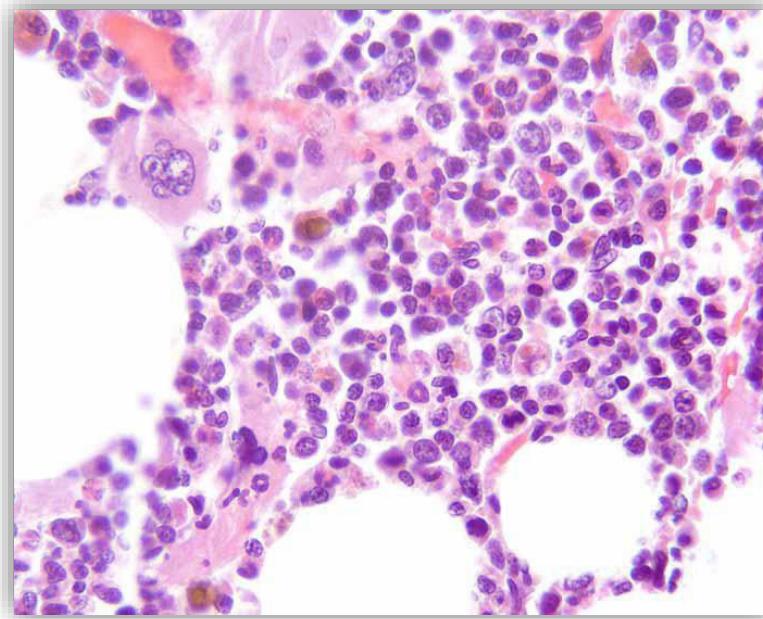
Misrepresented

# Image = Scientific Data!

Original image



Manipulated image



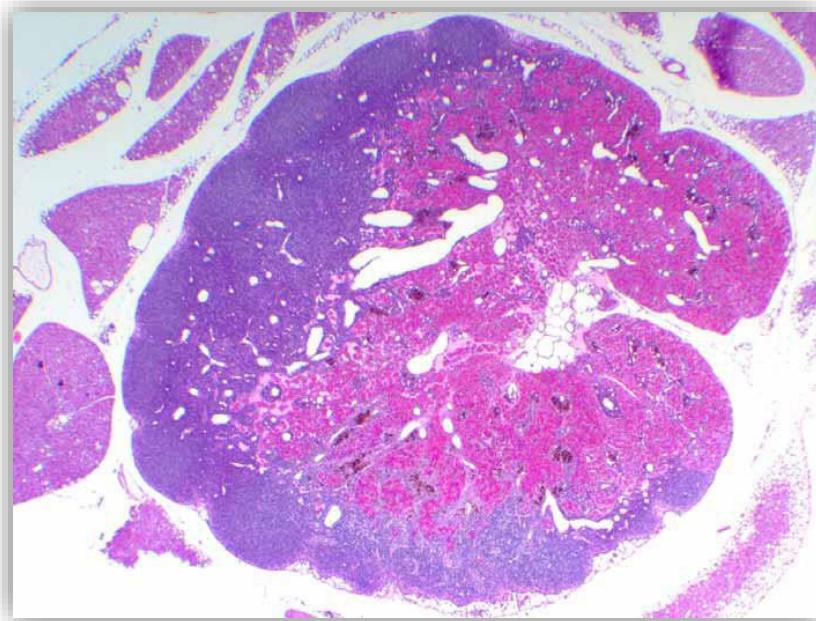
Background Shading in one corner  
**cleaned up**

Acceptable **only if**

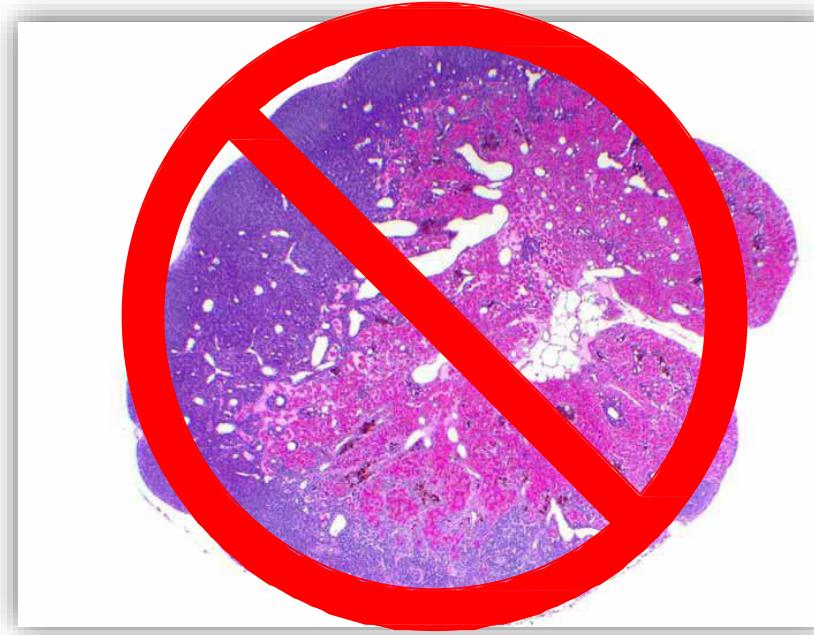
- No change occurred in the actual tissue
- A statement is made in the figure legend

# Image = Scientific Data!

Original image



Manipulated image



uneven illumination & brown fat has been **erased**

**NOT Acceptable because**

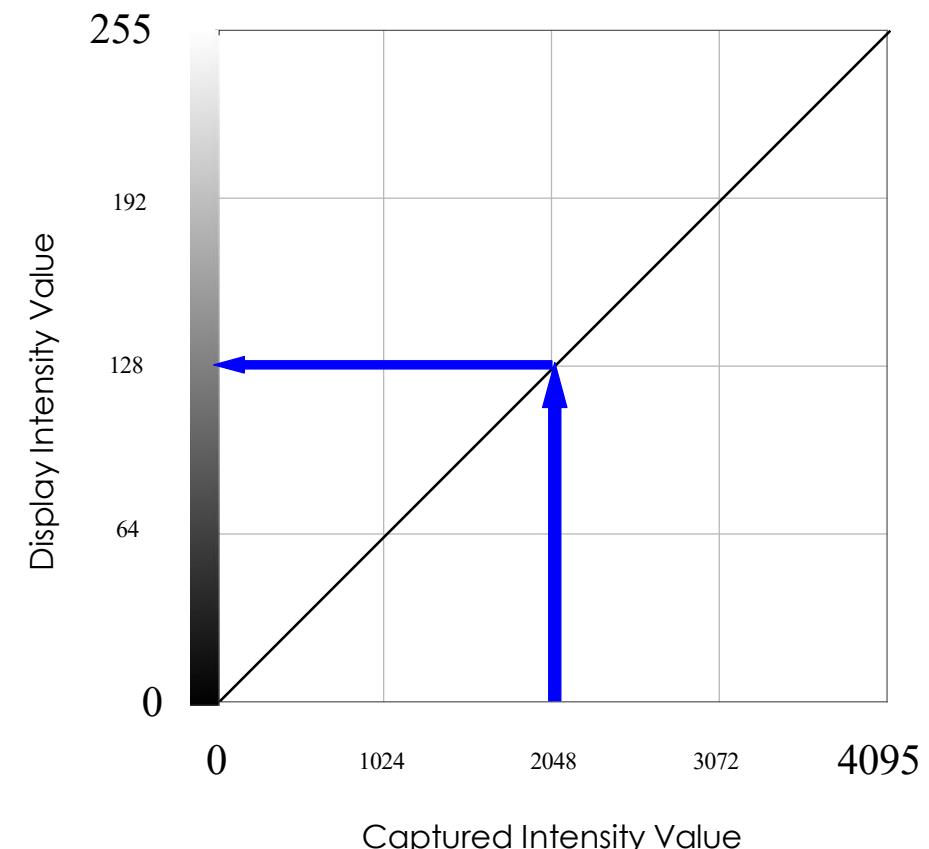
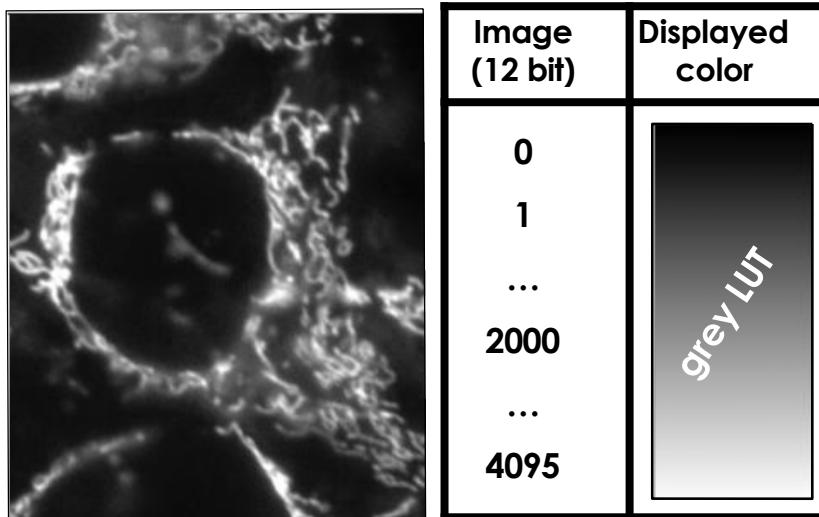
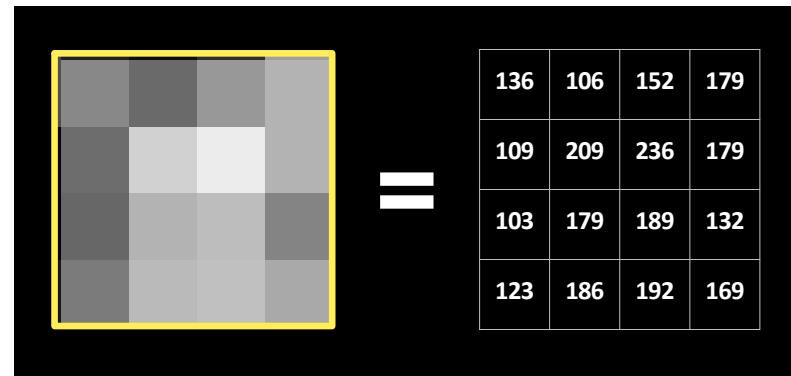
-large degree of background alteration (even if it is clarified and the interested area is not modified)

# 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*



# Images and Colors

## Lookup Tables (LUTs)

*LUT = how the grey values are displayed*

*LUTs do not change the pixel values*

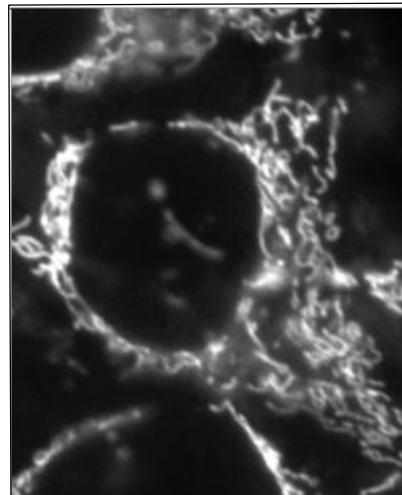


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	grey LUT

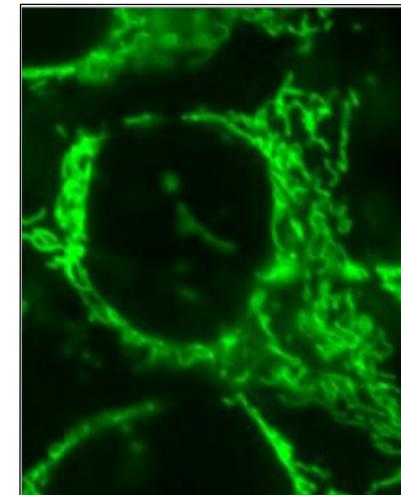
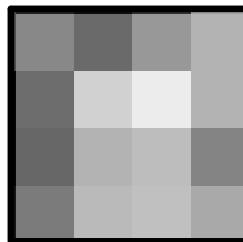
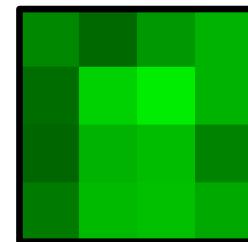


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	green LUT



=

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



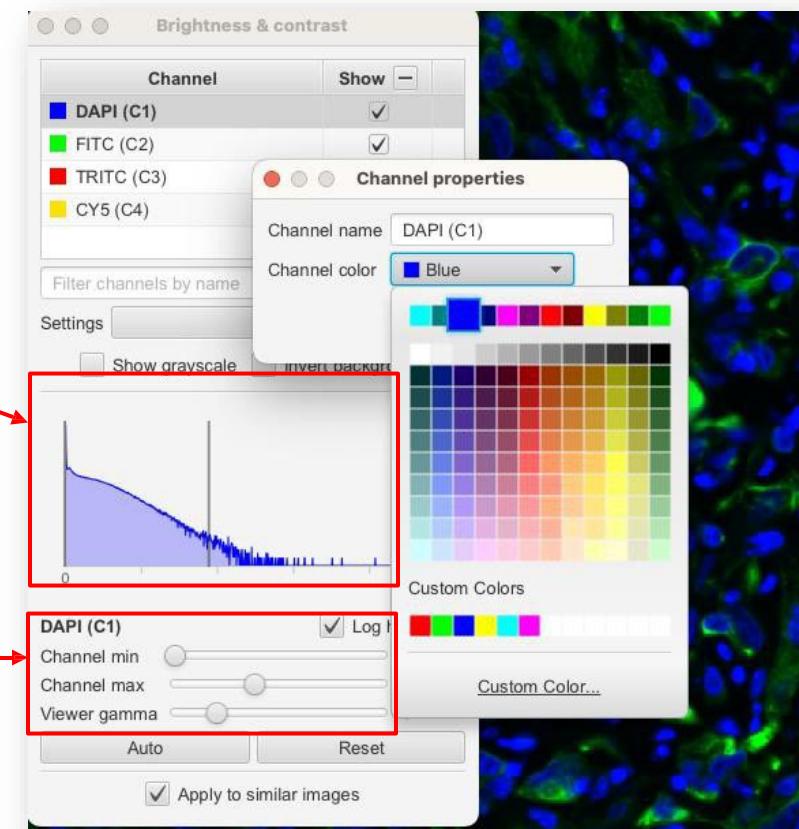
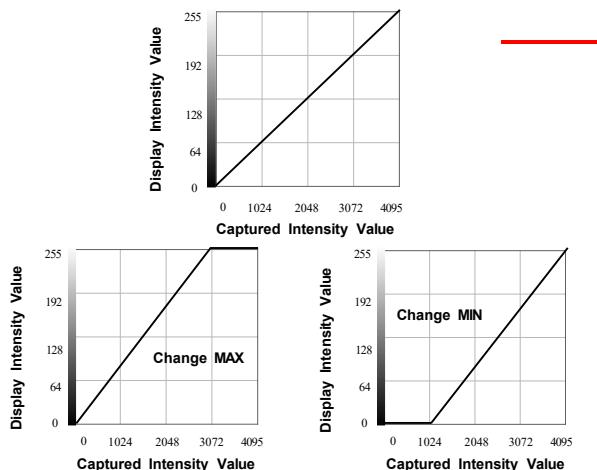
=

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

# Display images: color, brightness & contrast

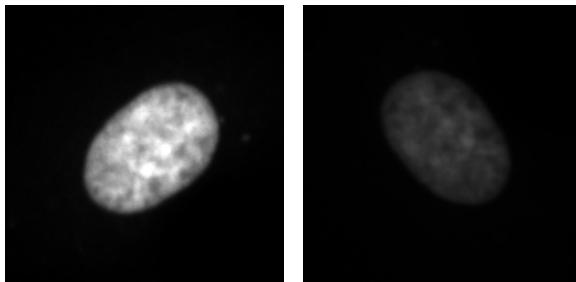
- If you are imaging a blue fluorophore, you are not forced to display it in blue!
- Pixel histogram represents the distribution of pixel values in the image
- LUT range

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

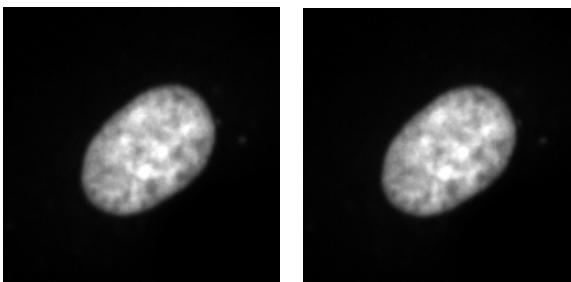


# Display a file: Brightness & Contrast

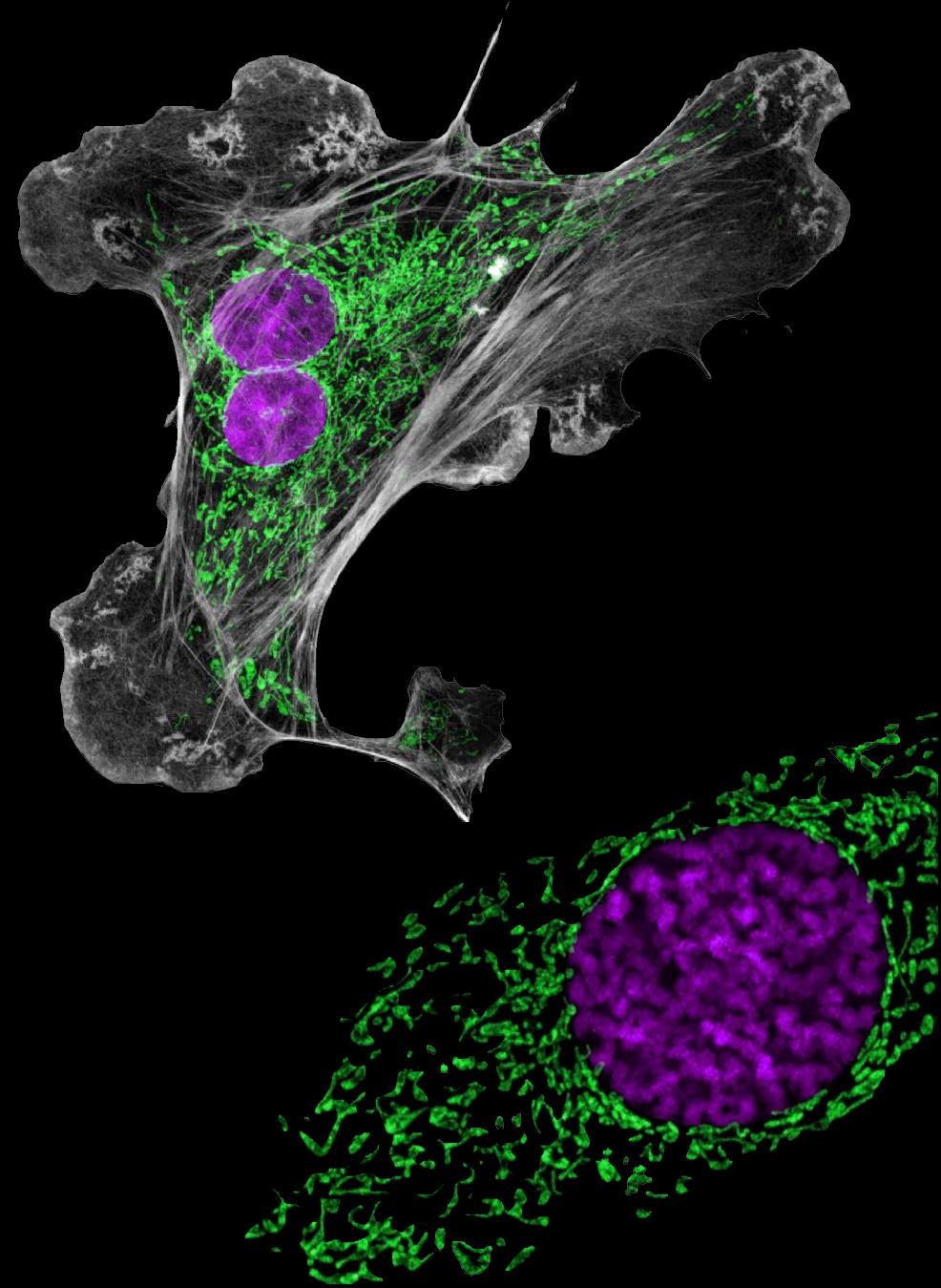
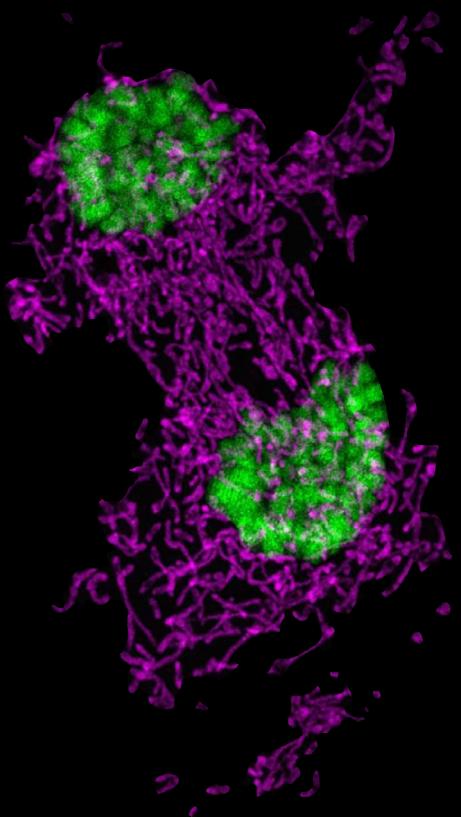
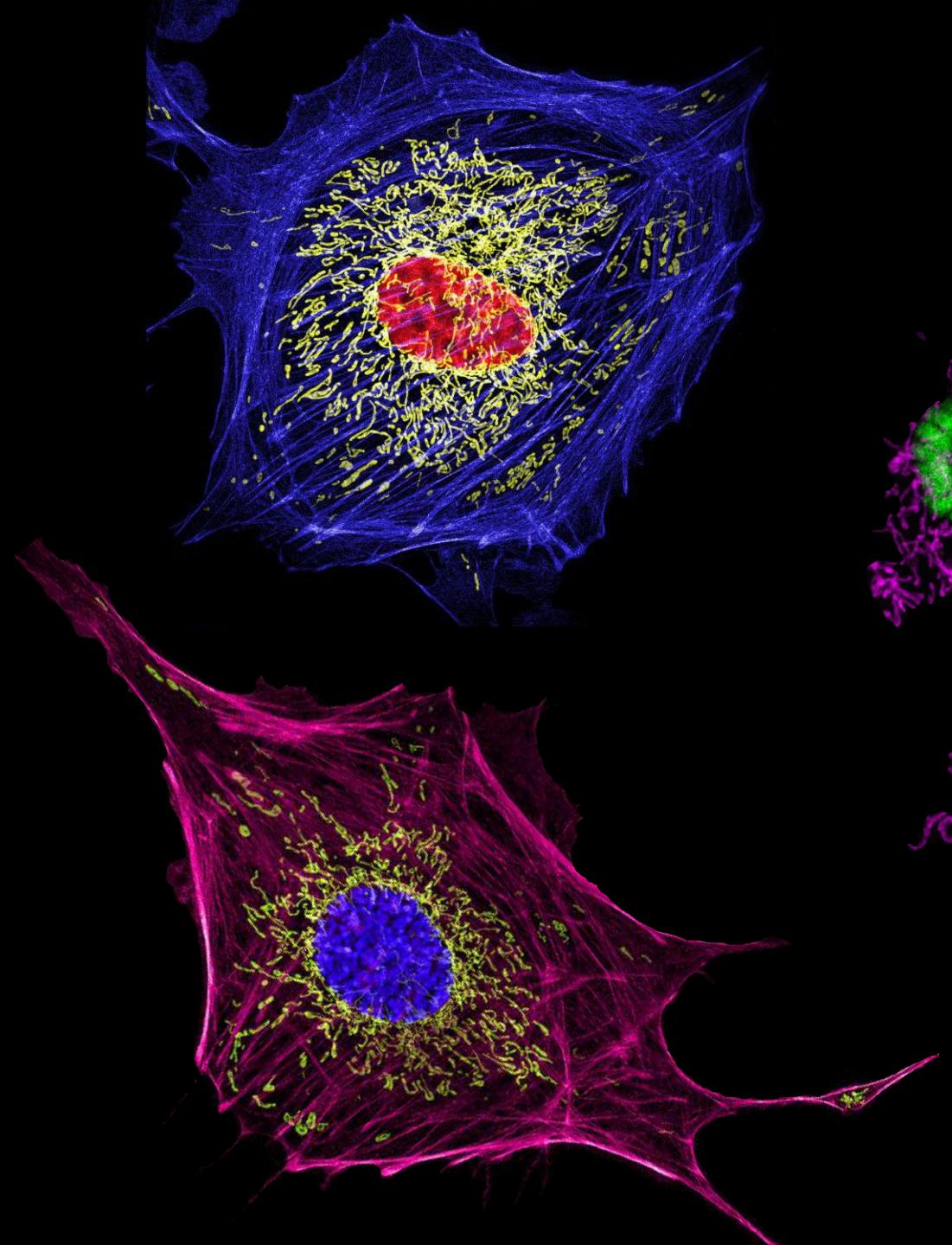
Which image has more fluorescence?



Mean:	<b>4803</b>	<b>4803</b>
<i>Display range:</i>	188- <b>16828</b>	188- <b>45514</b>



Mean:	<b>4803</b>	<b>4803</b>
<i>Display range:</i>	188- <b>16828</b>	188- <b>16828</b>



# Introduction to QuPath



# What is QuPath?

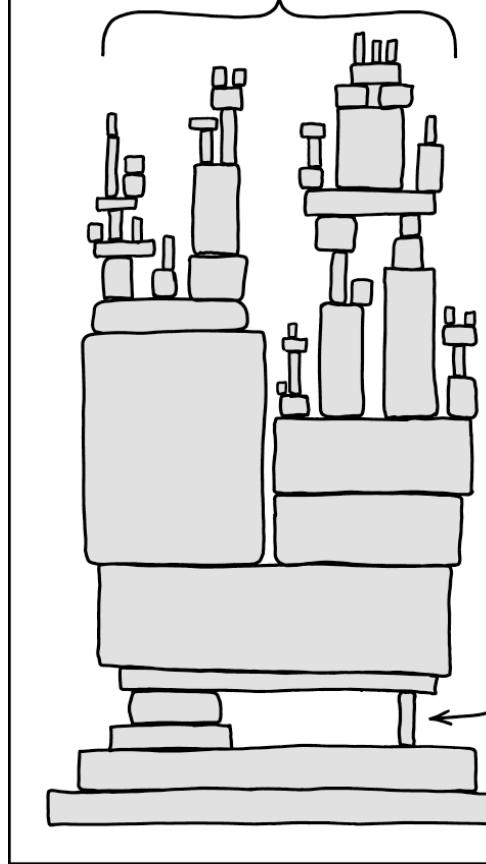
**QuPath is an open-source software for bioimage analysis**

- 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

**The world's most complex biomedical research**



The open-source image analysis software **Pete Bankhead** has been thanklessly developing in the UK since 2016

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 supports extensive visualization options
- It integrates with many other existing tools (Stardist, ImageJ, ...)
- It supports scripting (Groovy, akin to Java)

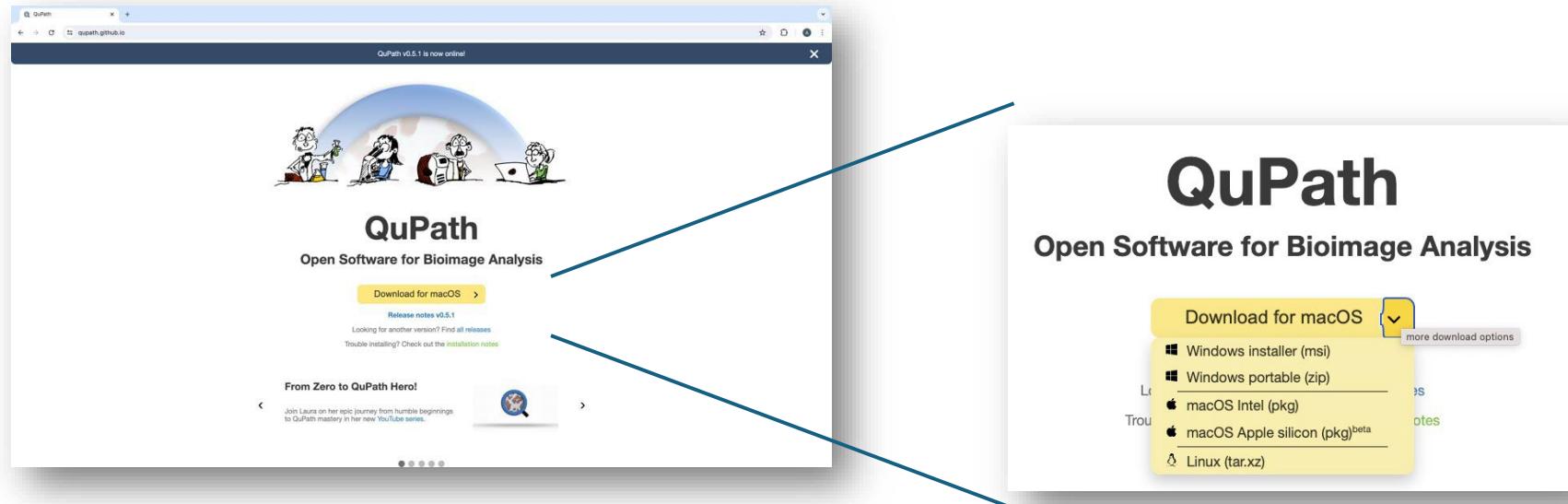
# What is QuPath not good at?

- Limited to 2D images
  - Only supports the visualization of single planes
- Image processing (image to image) is limited

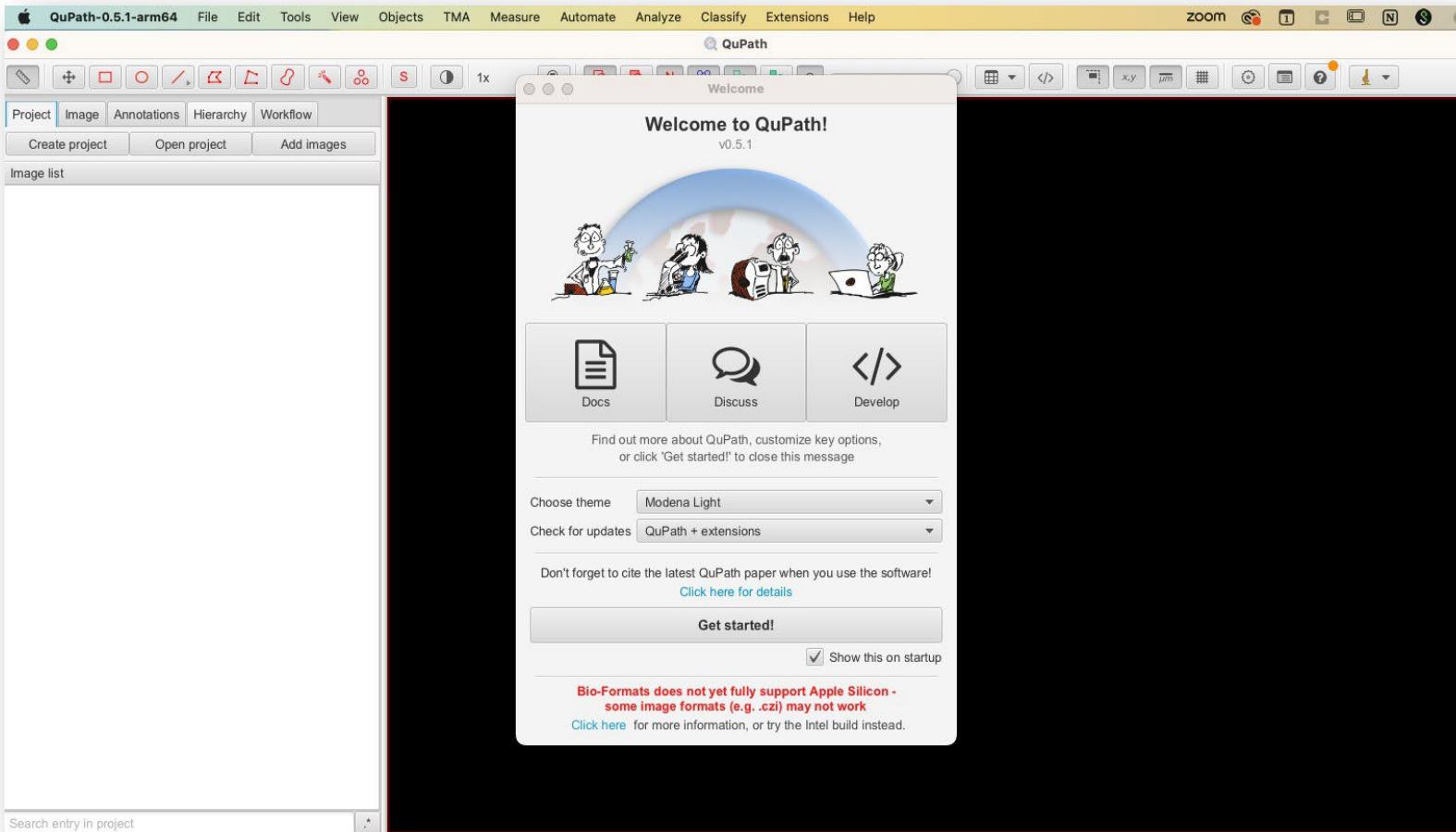
# Download QuPath (optional)

1. Go to <https://qupath.github.io/> (see useful links on website)
2. Download the installer for the latest version
3. Right-click on the installer file > Open > Confirm Open
4. (Docker Option, read the readme-qupath.md)

<https://github.com/BIOP/BIOP-desktop/tree/main/docker/QuPath>

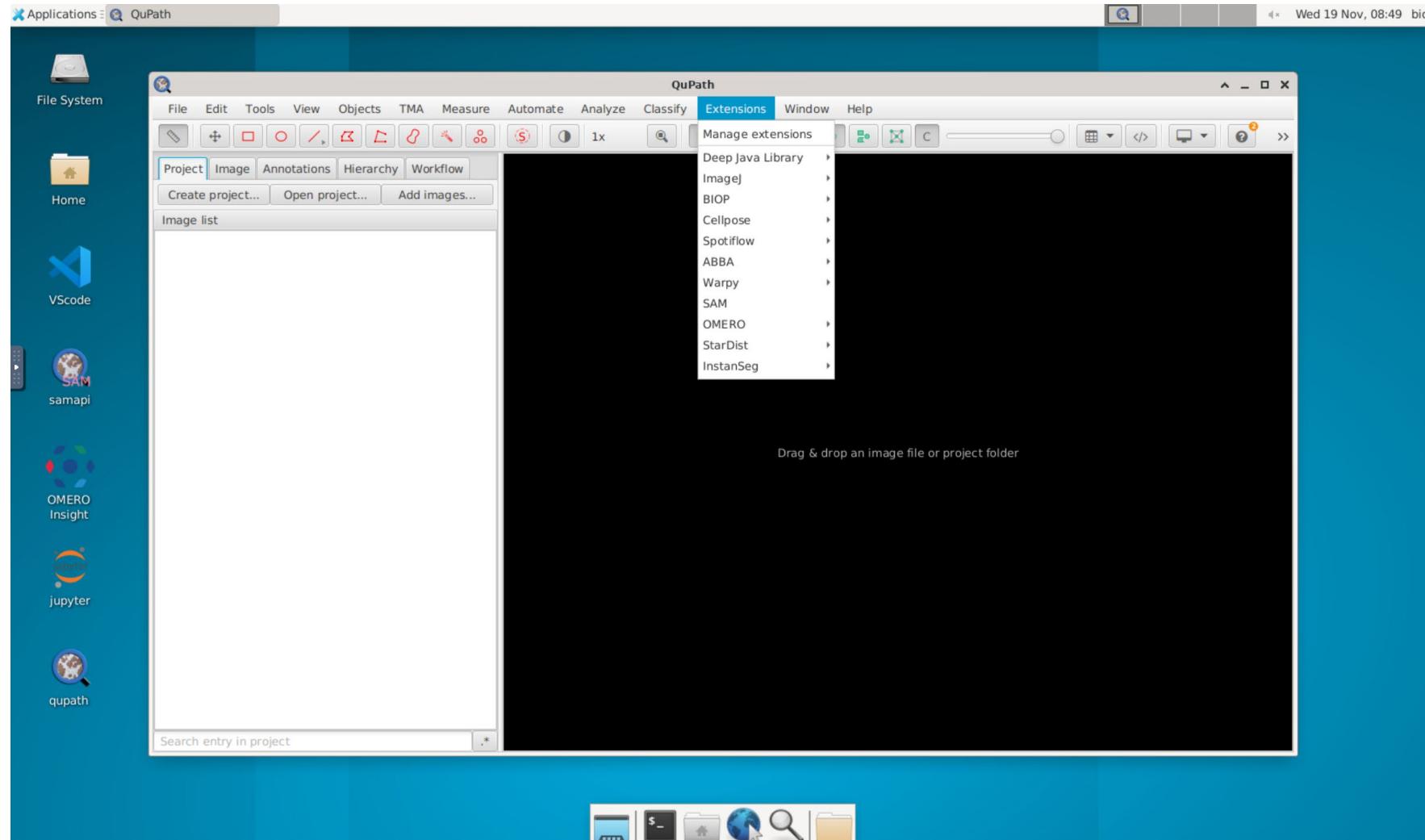


# Open the QuPath application



All Screenshots in this presentation are from QuPath 0.5, we will use QuPath 0.6, so there can be minor differences

# Running in Docker (with most Extensions)



Example on Windows (with WSL2 and Docker Desktop installed, the docker image is 45 GB so you need a lot of diskspace, and time to download)  
docker run -it --rm -p 8888:8888 --gpus device=0 --mount src=C:/,target=/home/biop/local,type=bind biop/biop-qupath:v0.6.0-full

# Welcome to QuPath!



## Useful resources:

## Documentation:

<https://qupath.readthedocs.io>

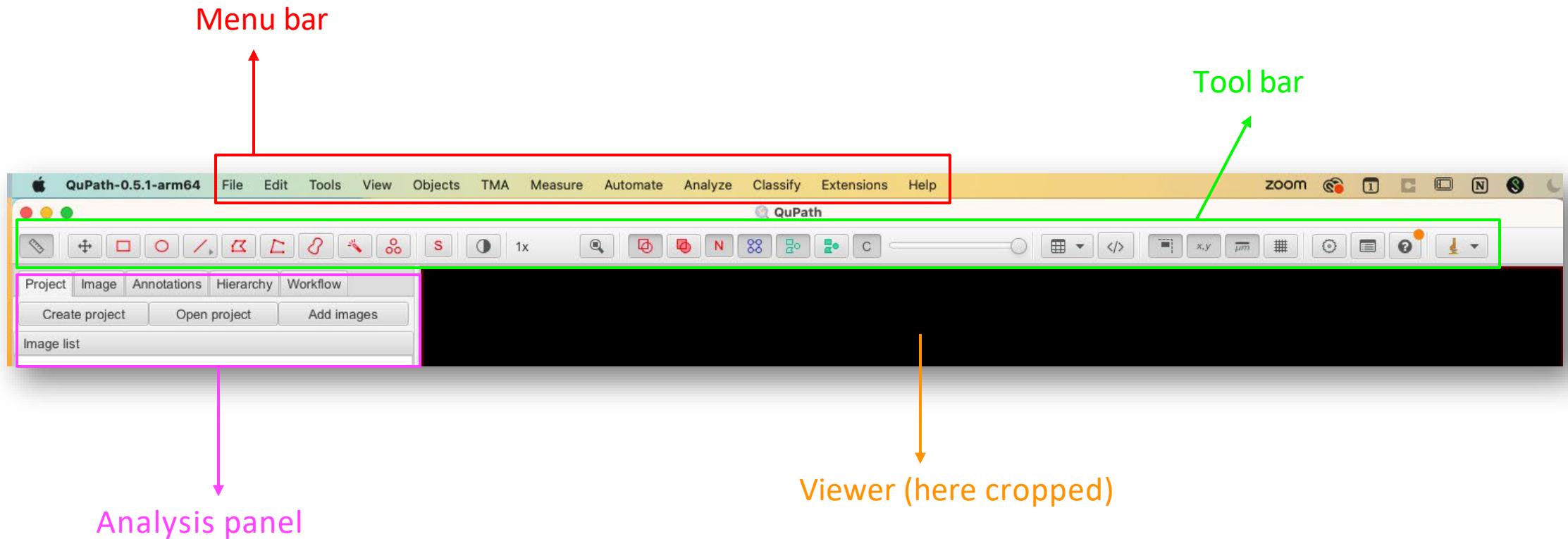
## QuPath-specific place in ‘The Forum’:

<https://forum.image.sc/tag/qupath>

## - Updater

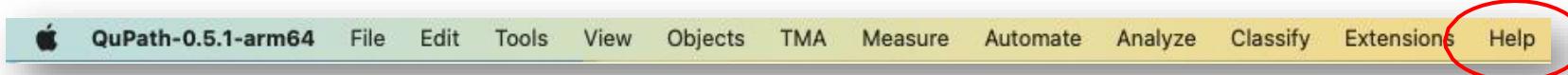
## For now, let's get started

# Graphic User Interface (GUI) – intro

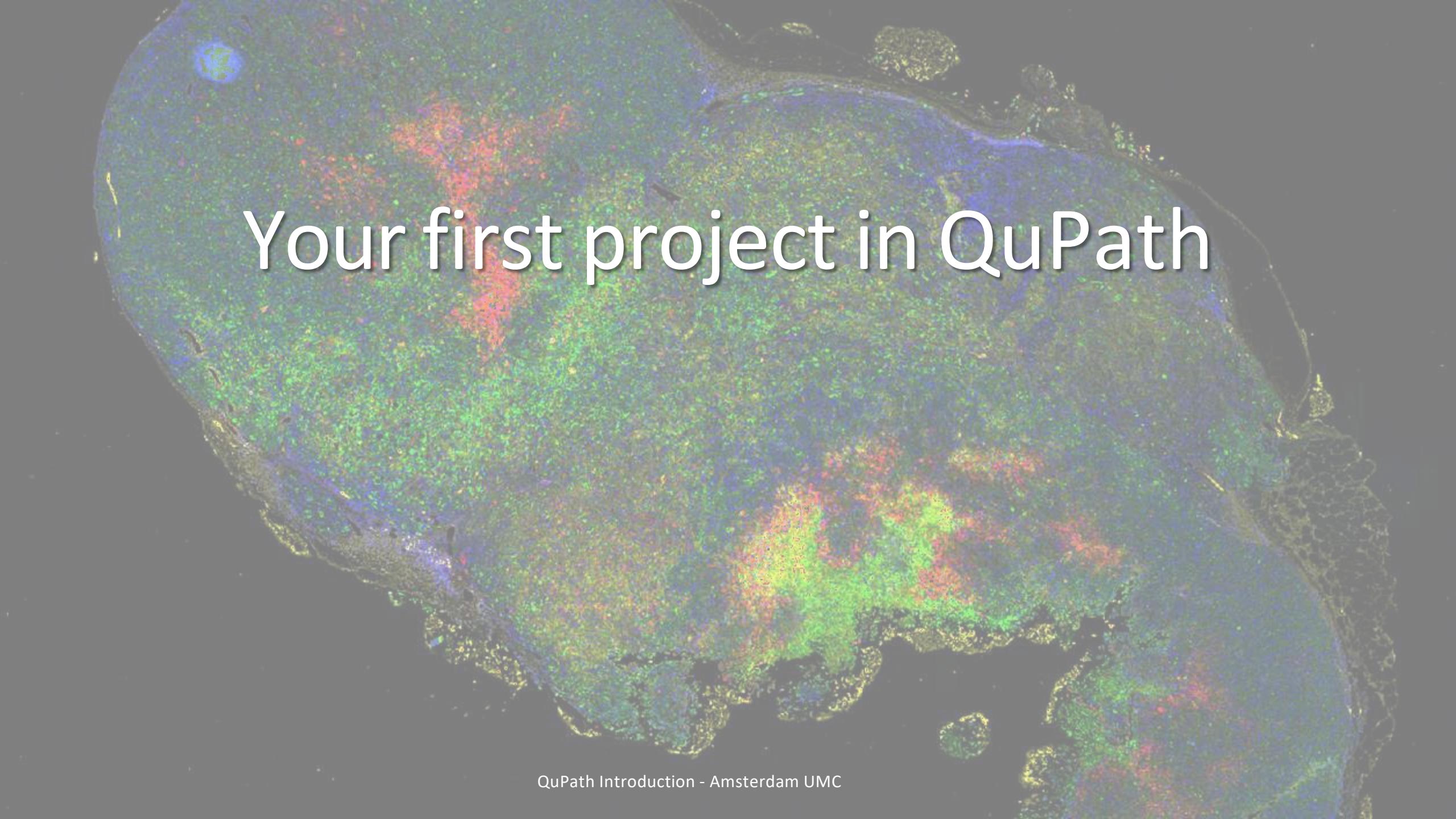


# Getting help

- In-app documentation: Help menu



- QuPath documentation: <https://qupath.github.io/>
- The Forum: <https://forum.image.sc/>  image.sc
- During this course, ask questions to your neighbors, or us!

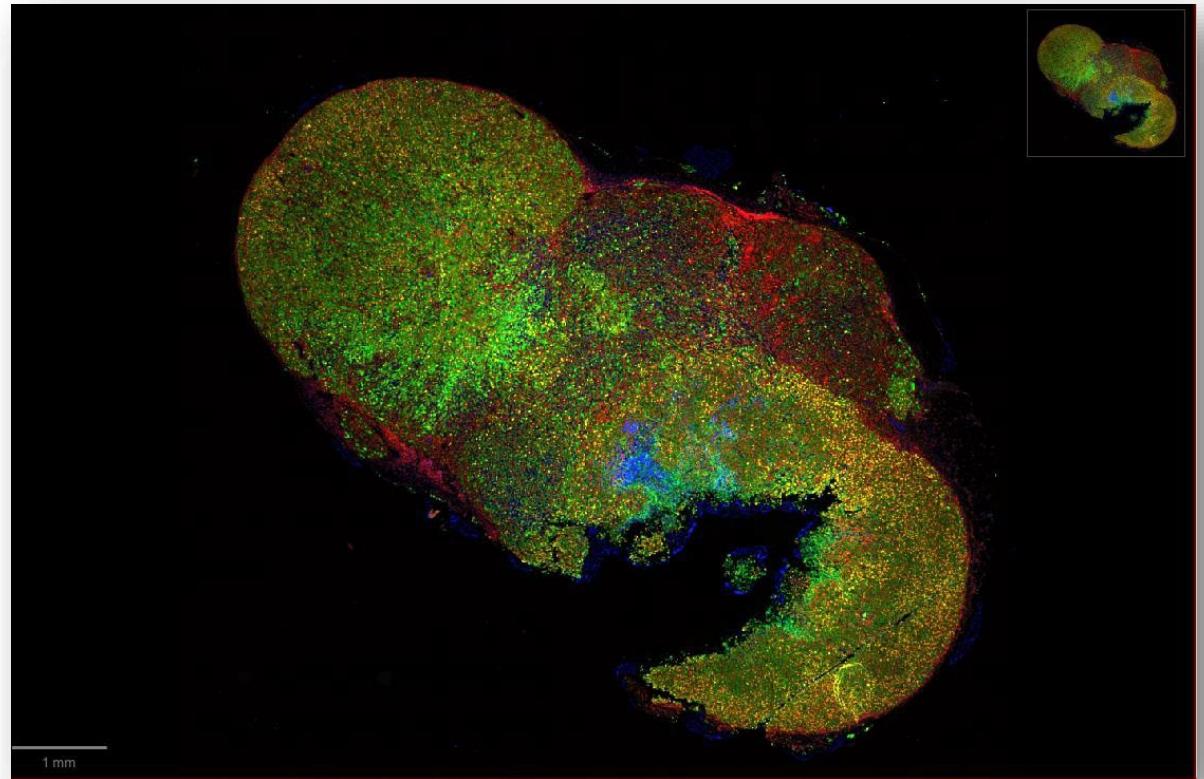


# Your first project in QuPath

# 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

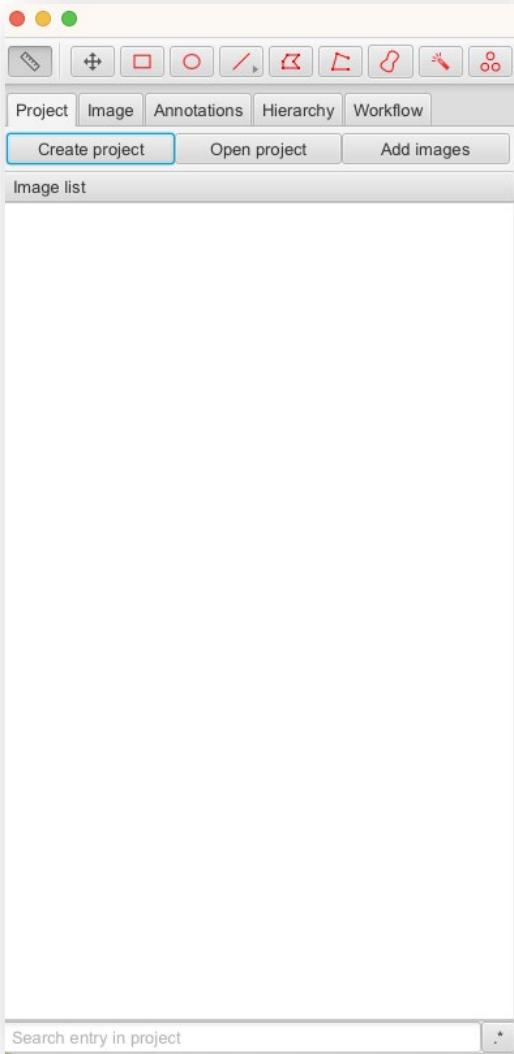


Courtesy of Nina Kozlova, PhD

# Key concept: QuPath project

- Projects are the way to organize your work in QuPath
- In other words, they are folders
  - Group together images
  - Organize data, scripts, classifiers, etc
  - They only save data, not the original images
- Allow you to share your work with other QuPath users
  - Always send the images along!

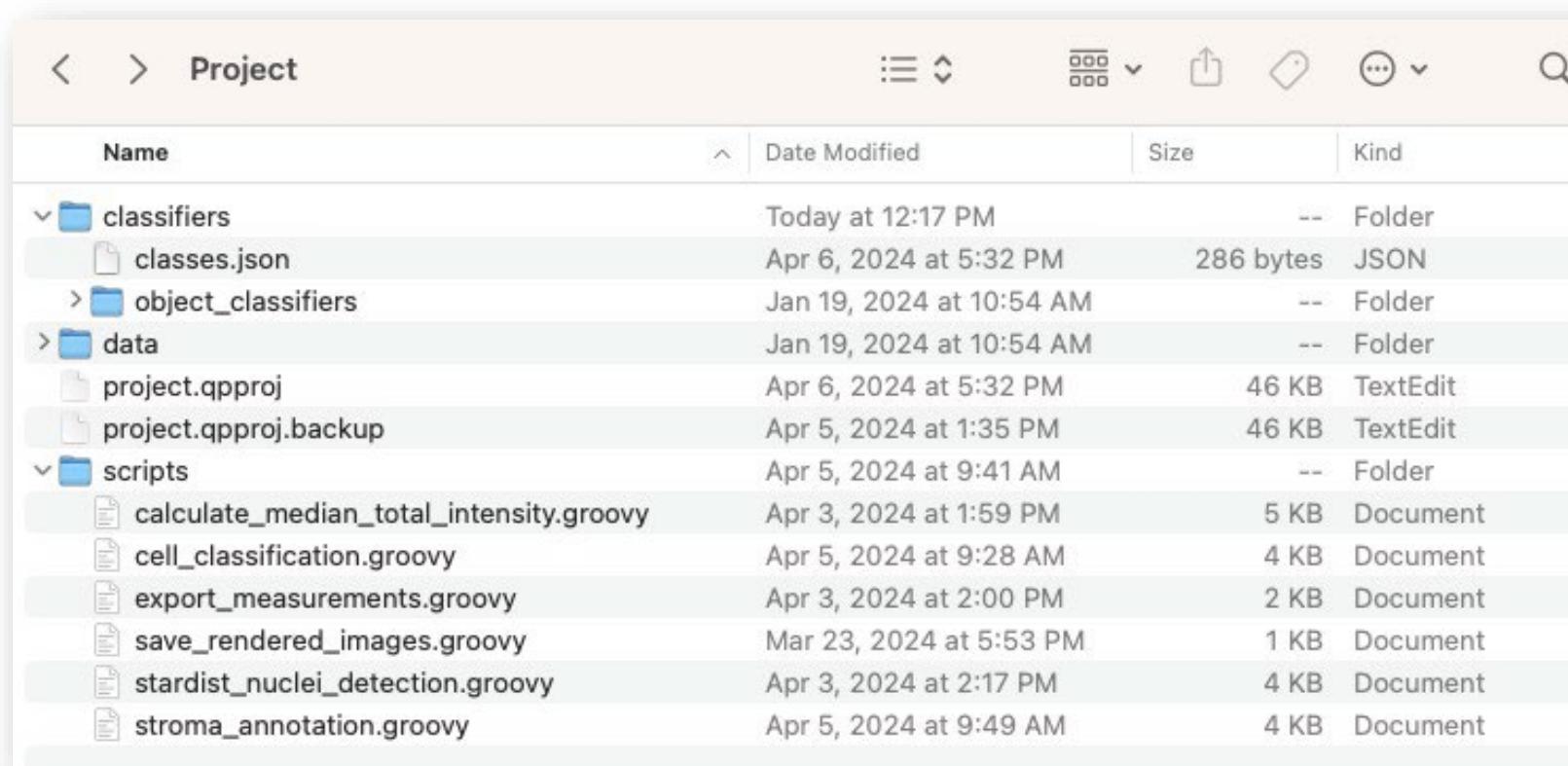
# How to create a project?



- *Create project* button  
or
- File > Project... > Create project
- ! Make sure to create an **empty** folder for your project
  - Sometimes, you have to do this twice in the empty folder

# Anatomy of a QuPath project

After a bit of time working on it...



The screenshot shows the QuPath Project browser interface. The title bar says "Project". The main area is a table with columns: Name, Date Modified, Size, and Kind. The table lists the following files and folders:

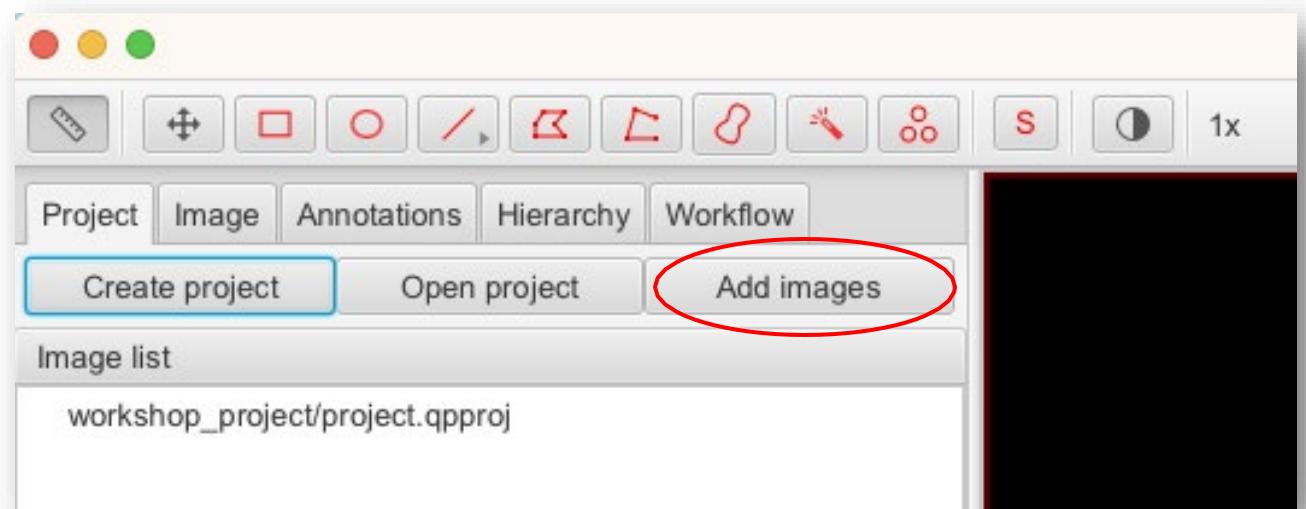
Name	Date Modified	Size	Kind
✓  classifiers	Today at 12:17 PM	--	Folder
classes.json	Apr 6, 2024 at 5:32 PM	286 bytes	JSON
>  object_classifiers	Jan 19, 2024 at 10:54 AM	--	Folder
>  data	Jan 19, 2024 at 10:54 AM	--	Folder
project.qpproj	Apr 6, 2024 at 5:32 PM	46 KB	TextEdit
project.qpproj.backup	Apr 5, 2024 at 1:35 PM	46 KB	TextEdit
✓  scripts	Apr 5, 2024 at 9:41 AM	--	Folder
calculate_median_total_intensity.groovy	Apr 3, 2024 at 1:59 PM	5 KB	Document
cell_classification.groovy	Apr 5, 2024 at 9:28 AM	4 KB	Document
export_measurements.groovy	Apr 3, 2024 at 2:00 PM	2 KB	Document
save_rendered_images.groovy	Mar 23, 2024 at 5:53 PM	1 KB	Document
stardist_nuclei_detection.groovy	Apr 3, 2024 at 2:17 PM	4 KB	Document
stroma_annotation.groovy	Apr 5, 2024 at 9:49 AM	4 KB	Document

# Add an image to your project

1. Check your emails! Download this folder containing an example whole-slide image

2. Add an image

- *Add images* button
  - Select the .vsi file

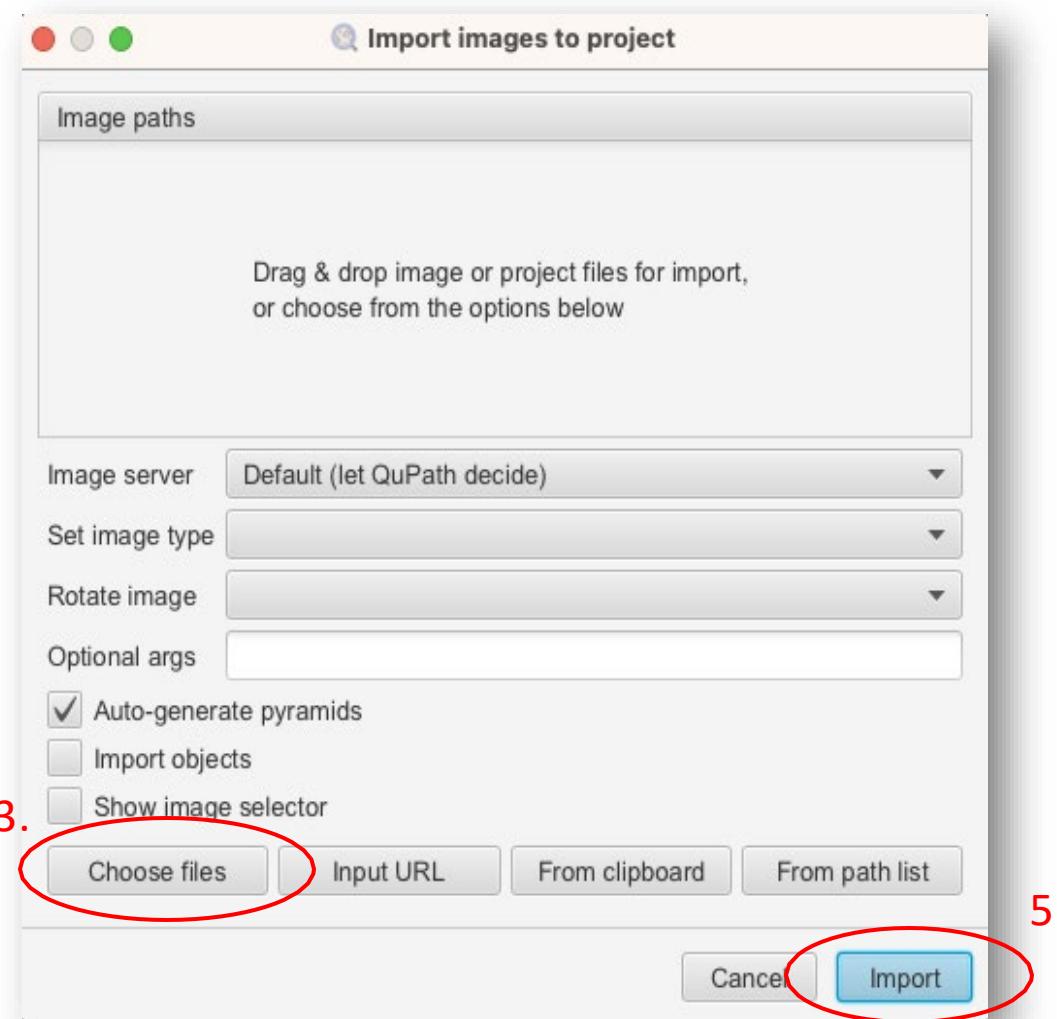


# Add an image to your project

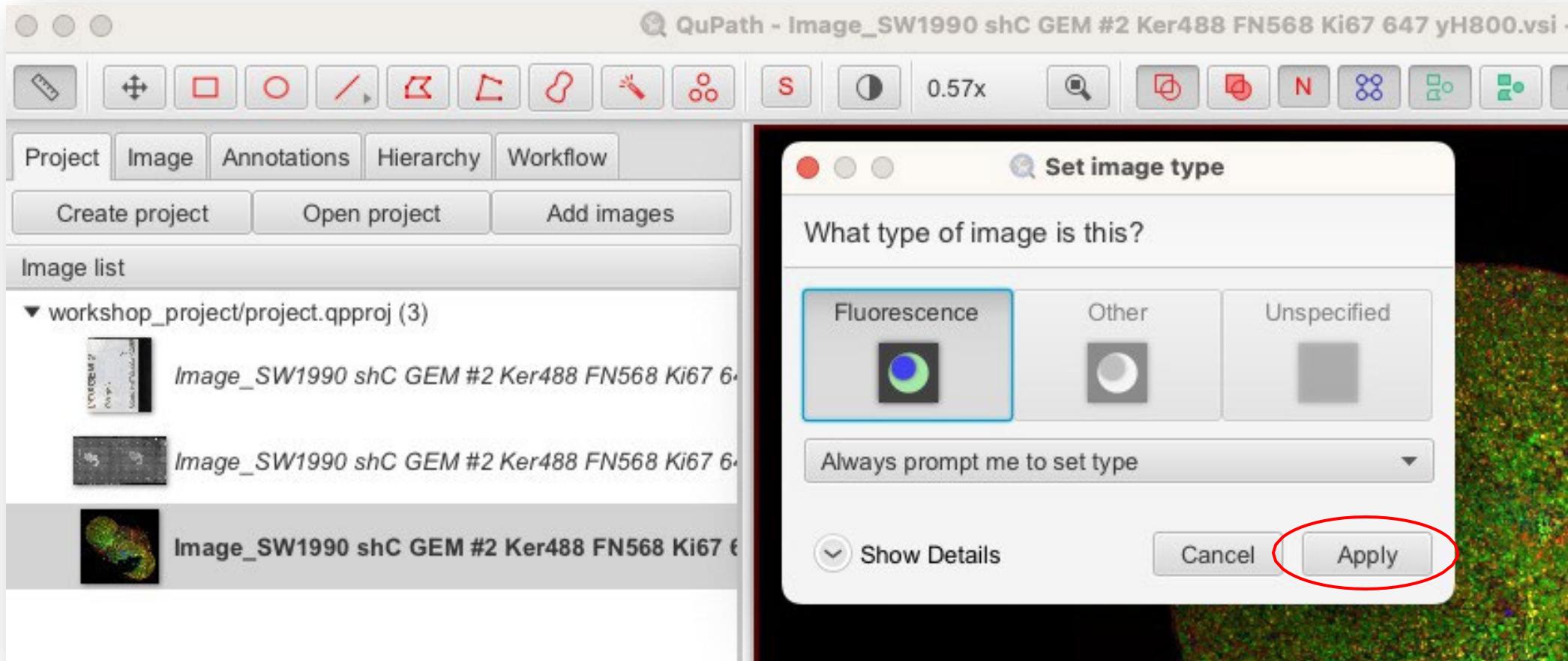
3. Select the .vsi image using *Choose files* or drag-and-drop

4. Use default settings

5. Click *import*



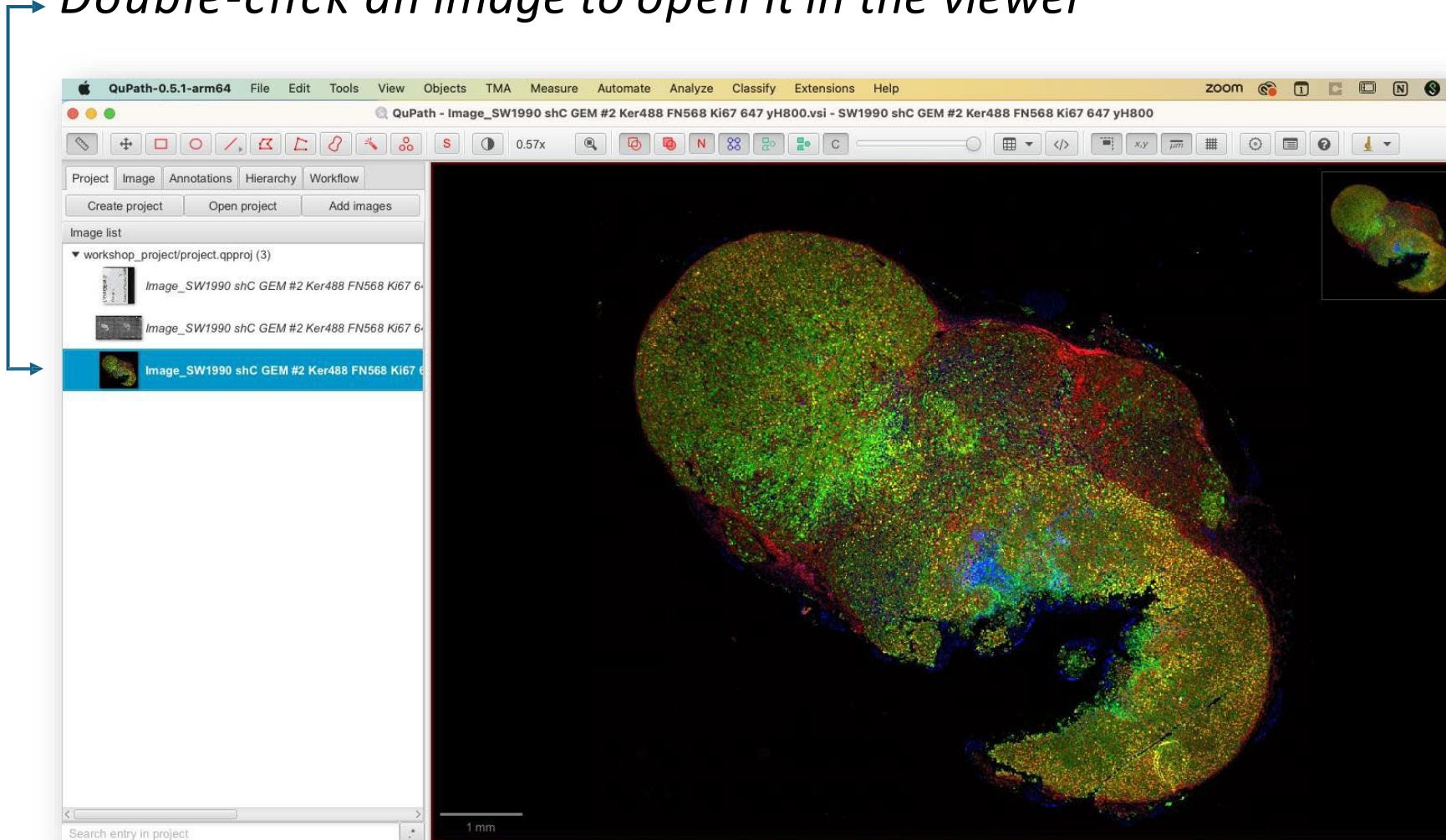
# Set image type



- *Other image types are supported: Brightfield H&E, H-DAB, other brightfield*

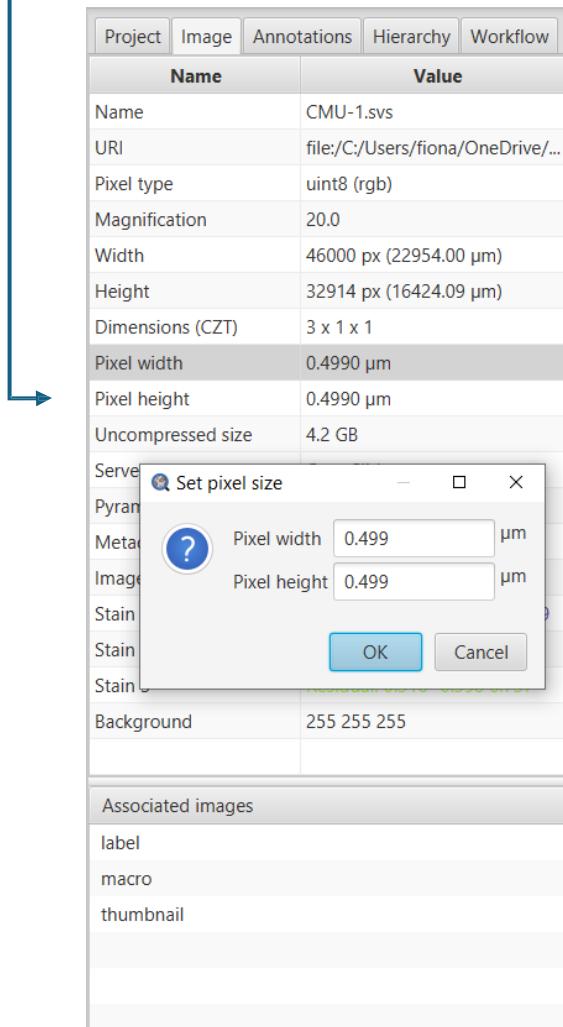
# Yay! We have a QuPath project with an image

*Double-click an image to open it in the viewer*



# Check the Pixel Size of the image

*Double-click pixel width to change if needed.*



The pixel size is used extensively by QuPath. This is why it is good practice to make sure it's correct or any measurements could be incorrect.

If stored in the image file, QuPath should automatically fetch the pixel size and display it under the **Image** tab as seen in the figure above ('Pixel width' & 'Pixel height').

If not, you can set the pixel size manually by double-clicking on either row and type the correct values.

# 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

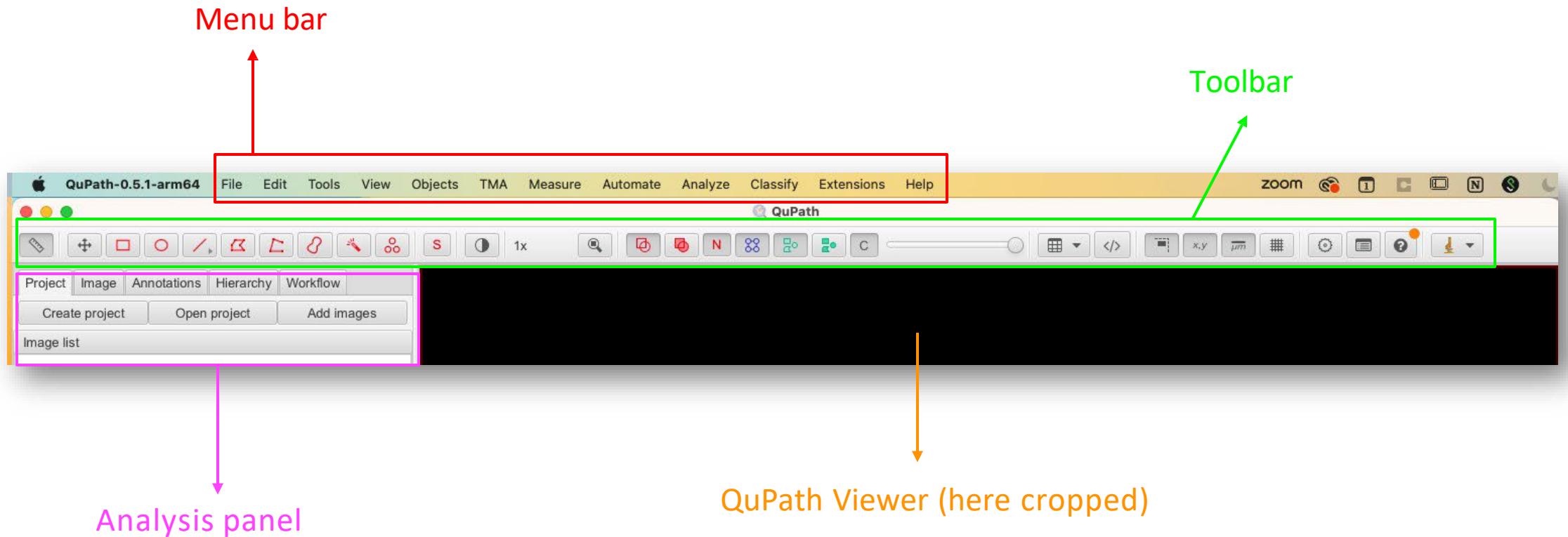
**The project folder only contains QuPath objects and data, unless you had placed them there. Ensure that they can access the actual image files.**

# 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

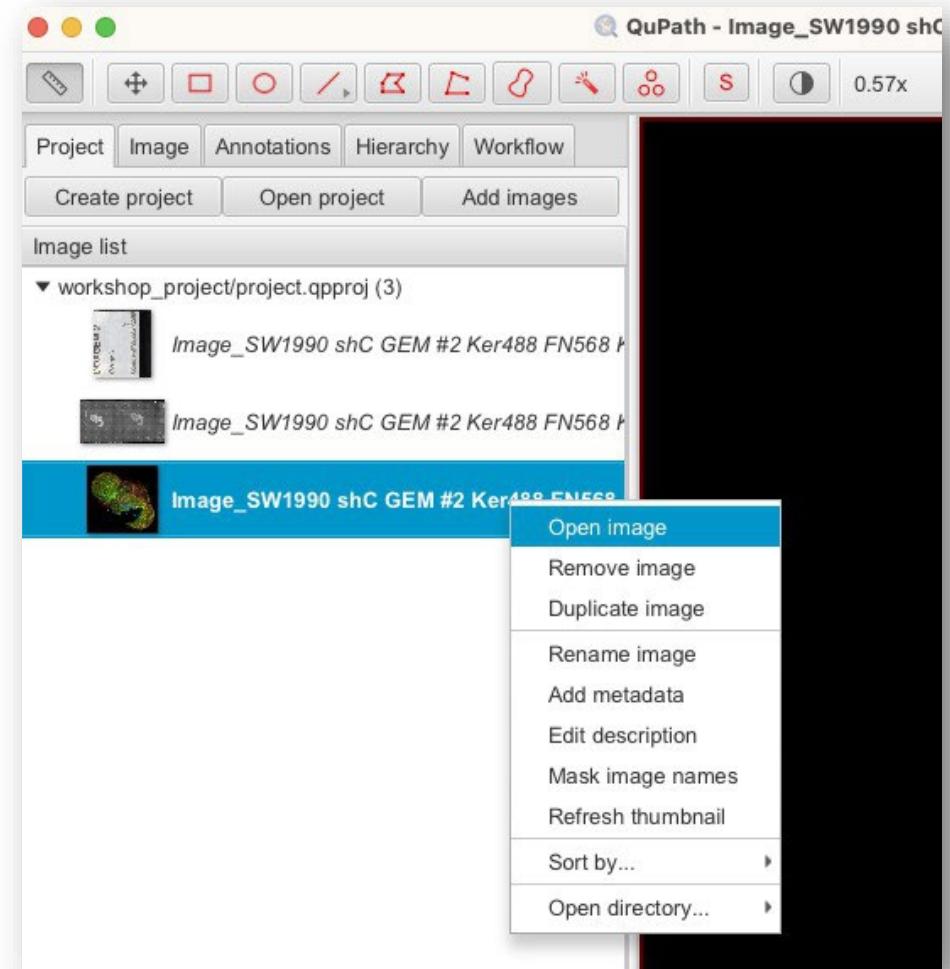
# QuPath Graphical User Interface (GUI)

# Graphic User Interface (GUI)



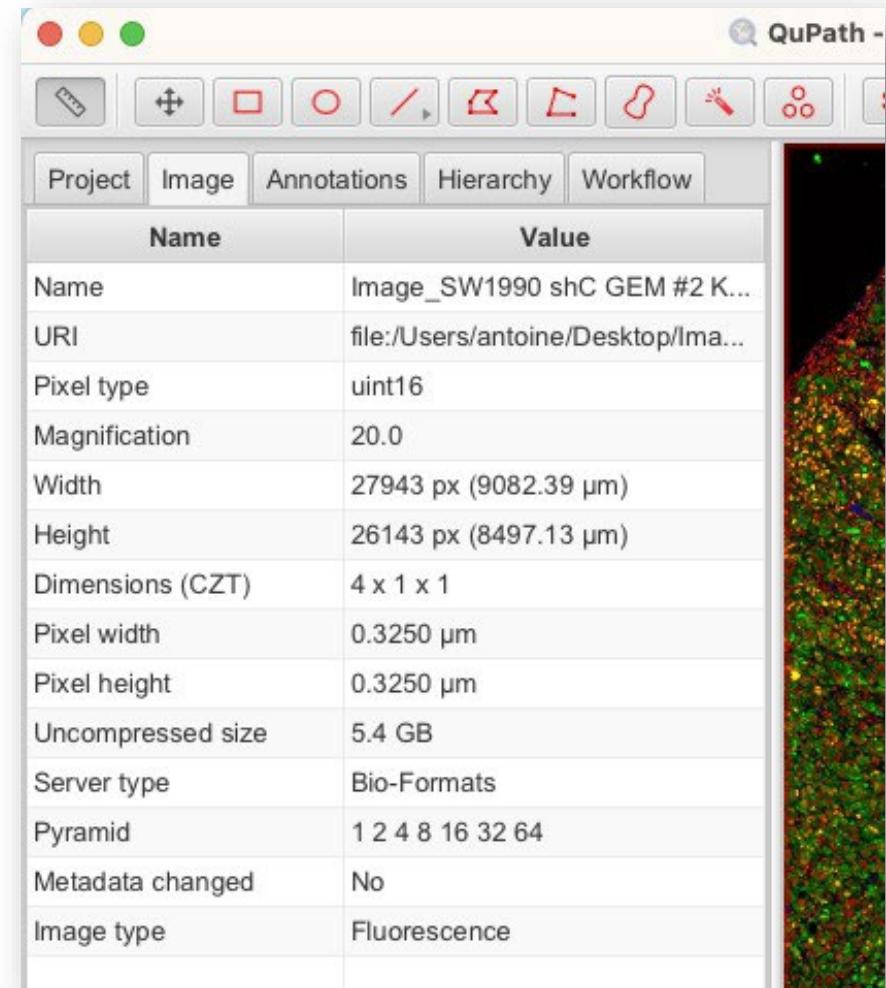
# Analysis Panel

- *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

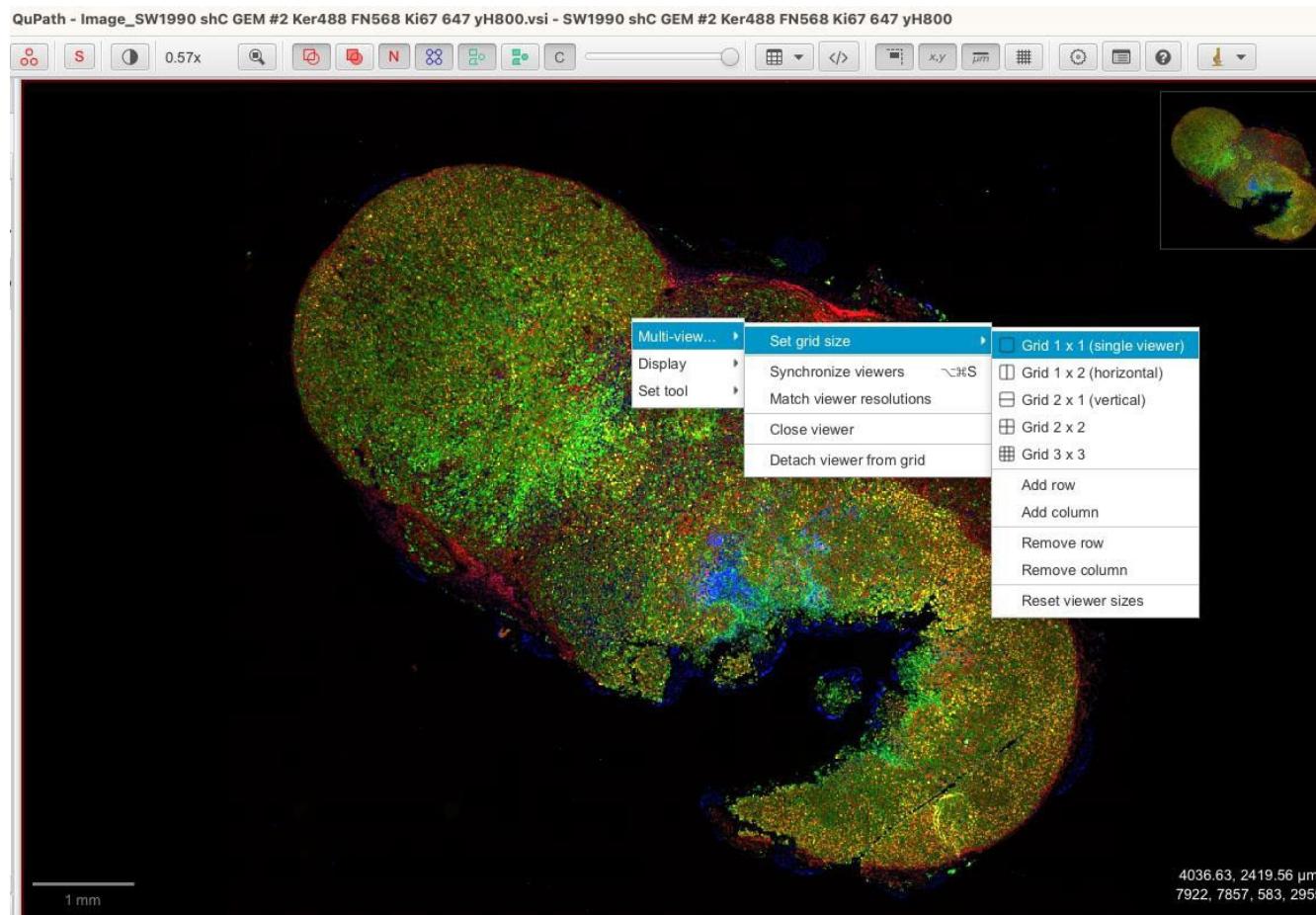


# QuPath viewer

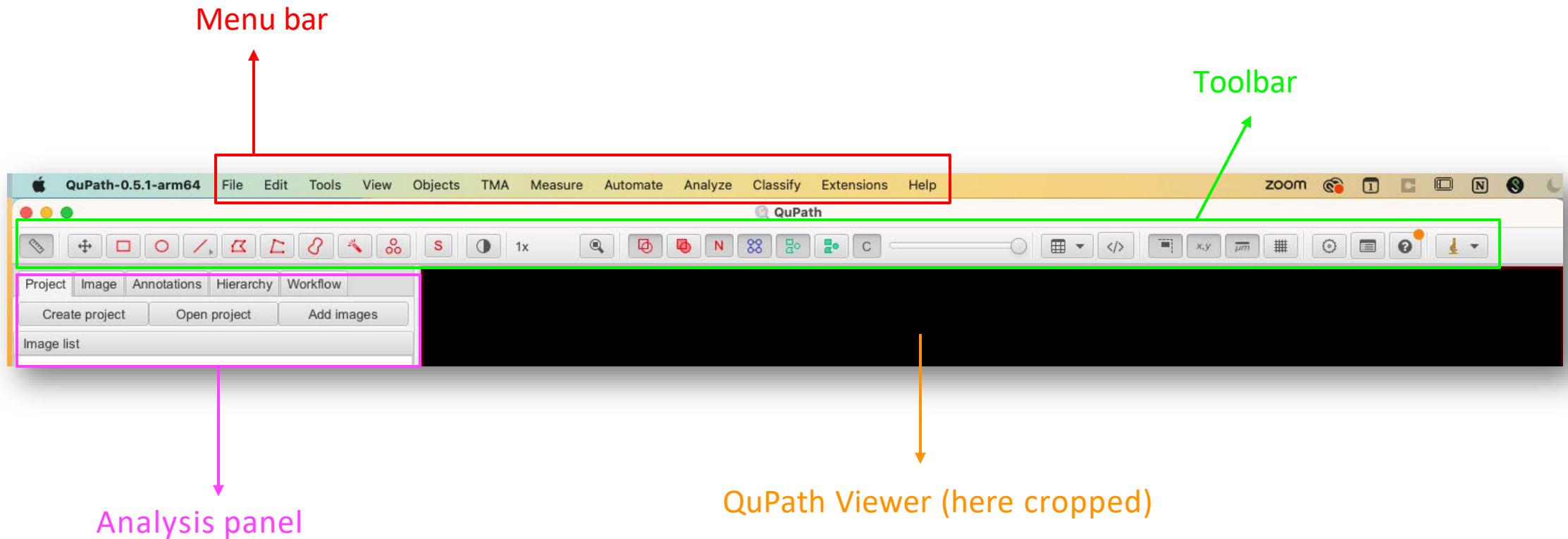


# Multi-viewer

- Right-click in the viewer



# Graphic User Interface (GUI)



# Toolbar



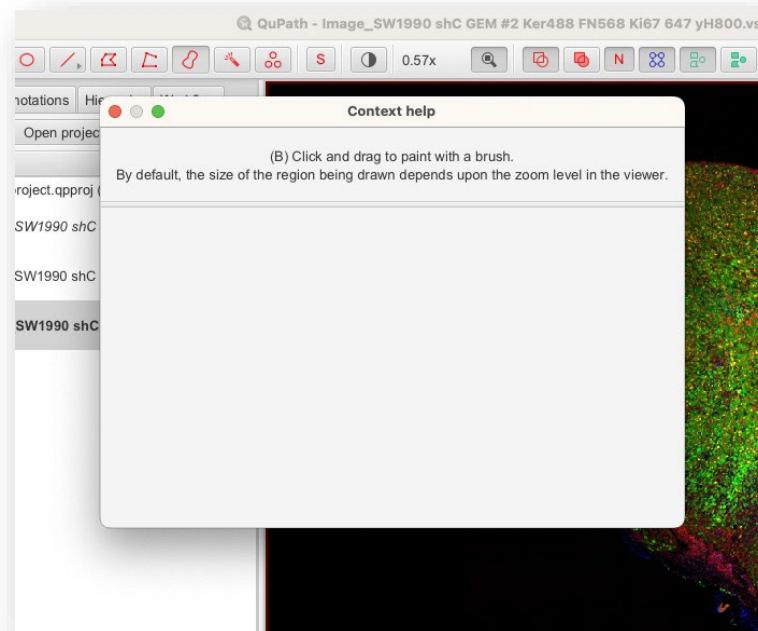
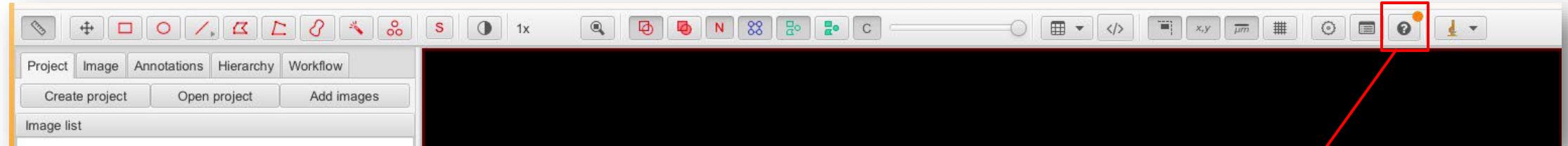
Toolbar

# Toolbar



Preferences  
Settings, GUI  
customization,  
extensions, ...

# Toolbar



**Interactive Help**  
Provides contextual  
help based on your  
cursor location

Example when my cursor is on the paint brush tool

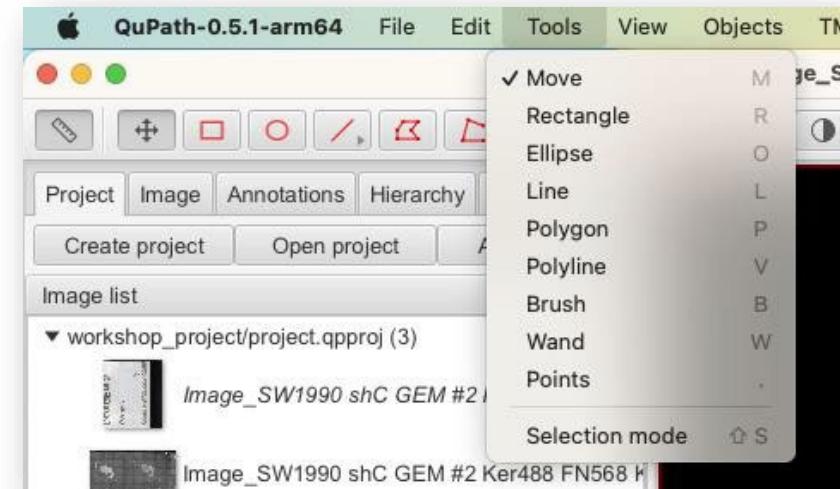
# Toolbar

M R O      B W      Shift+S



## Annotation tools

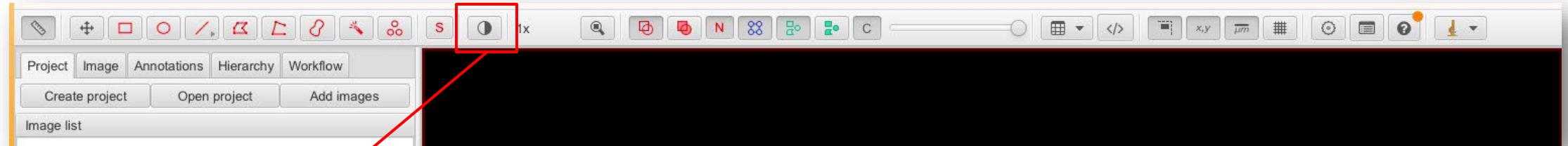
- **M** key: move tool
- **R** key: draw a rectangle annotation
- **O** key: draw an ellipse annotation
- **B** key: paint with a brush
- **W** key: draw with a wand tool
- And many more!



Annotation tools are also accessible in the *Tools* menu

# Toolbar

Shift+C



## Brightness and contrast

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

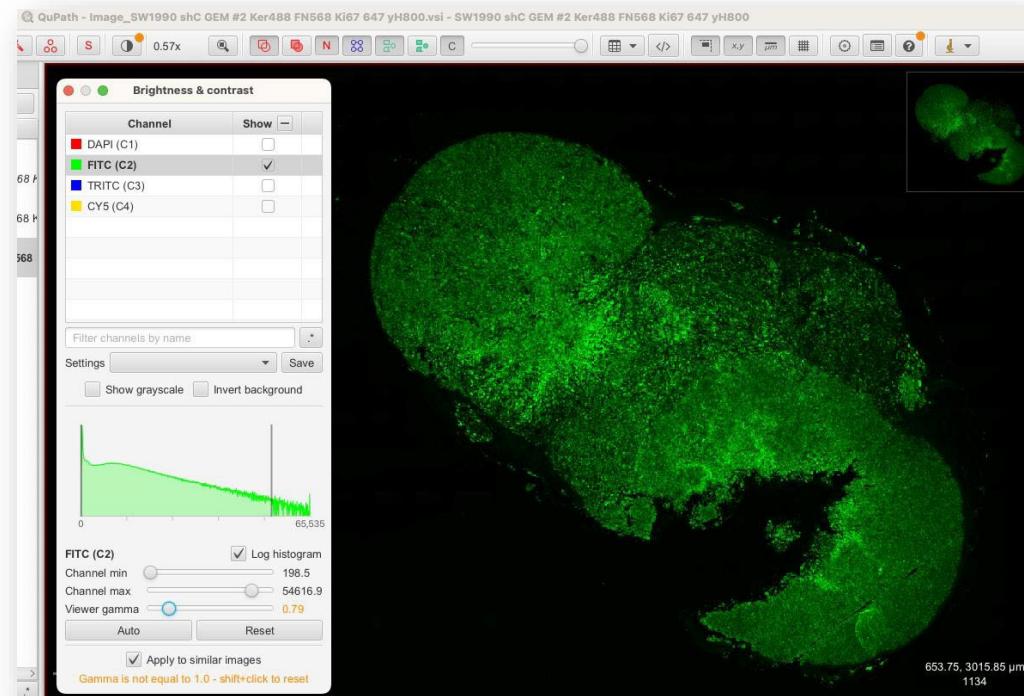
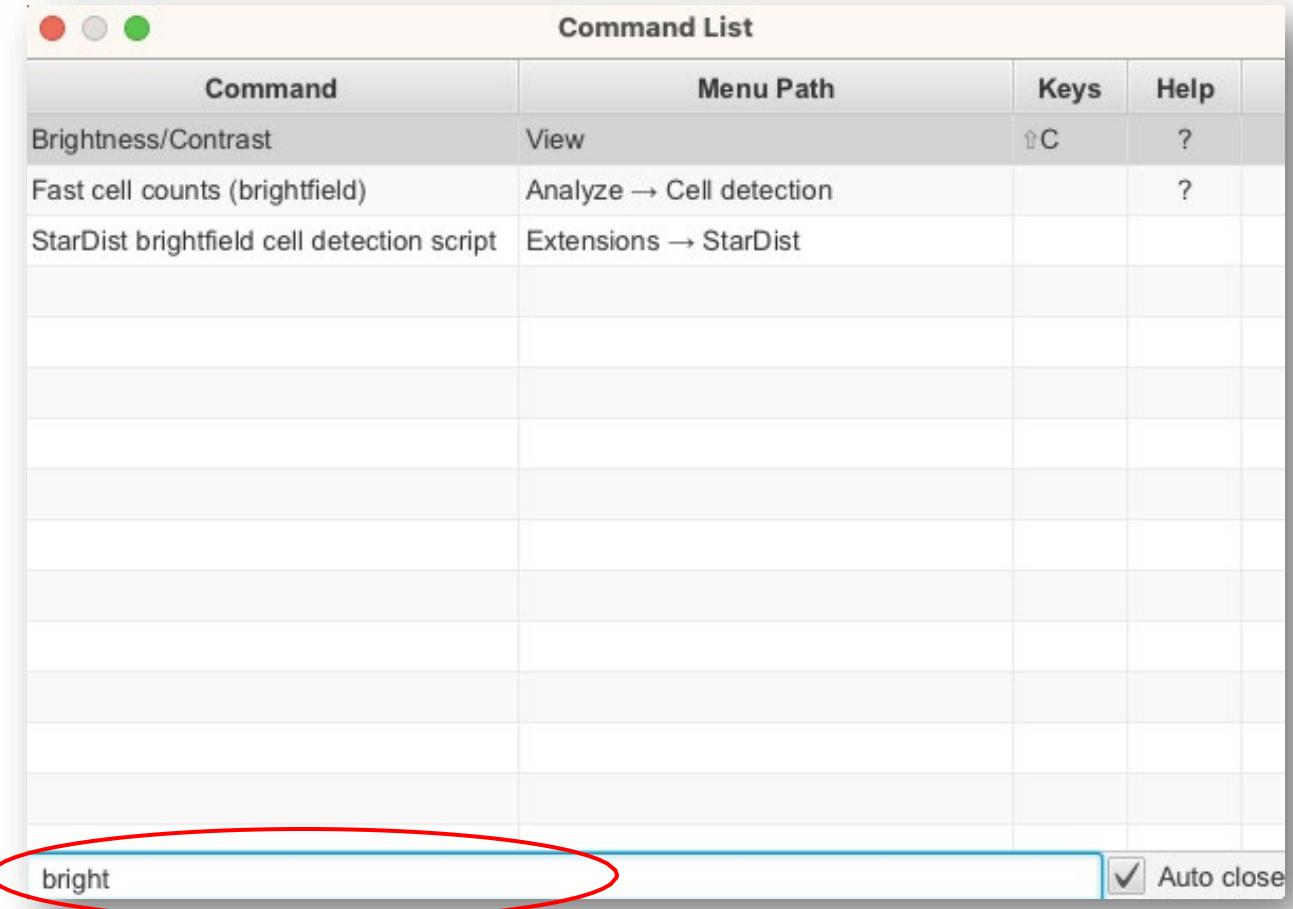


Image Analysis Collaboration - QuPath workshop  
Example for FITC (Keratin) channel

# 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

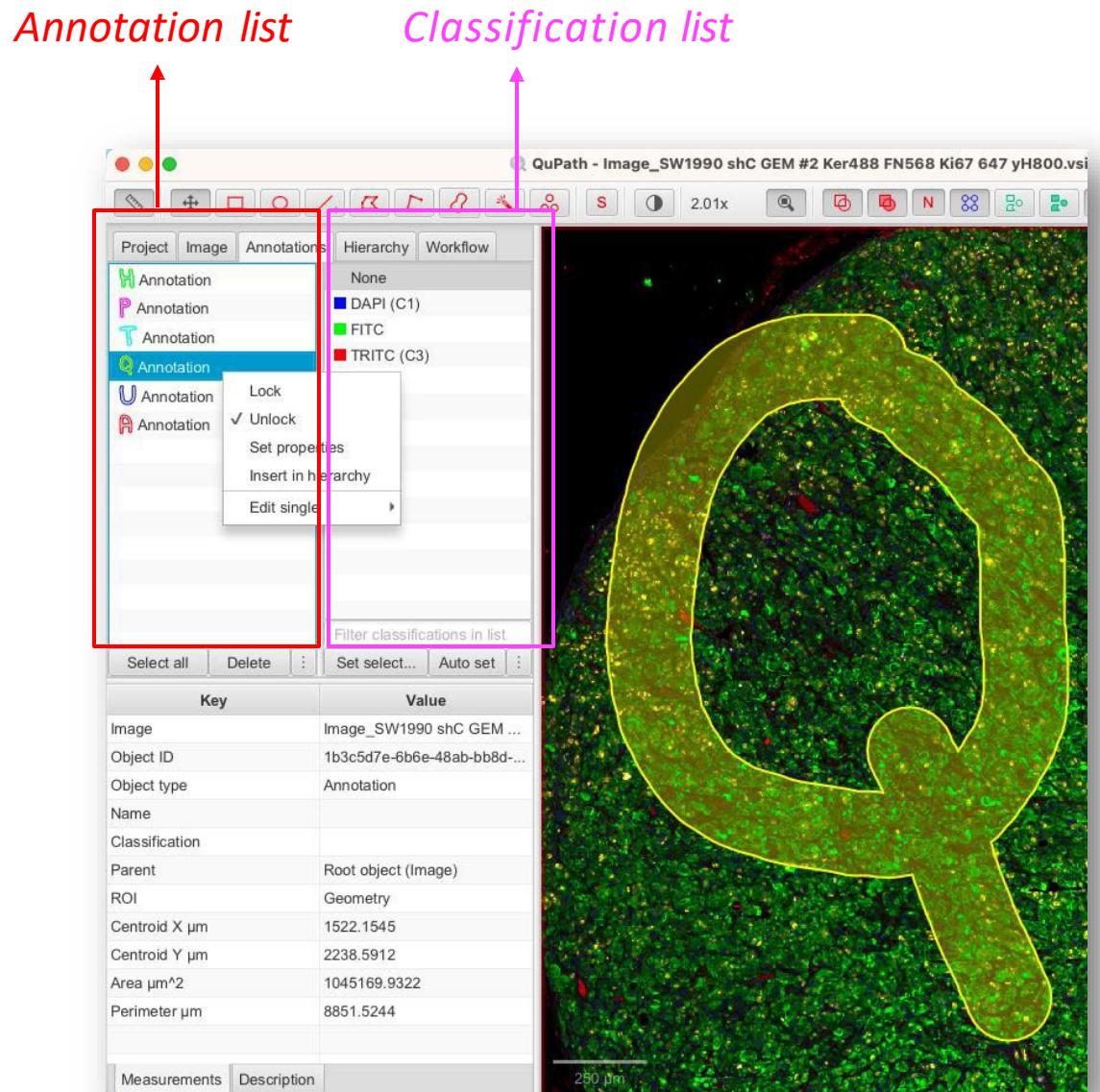
# Introducing objects: annotations and detections

# 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

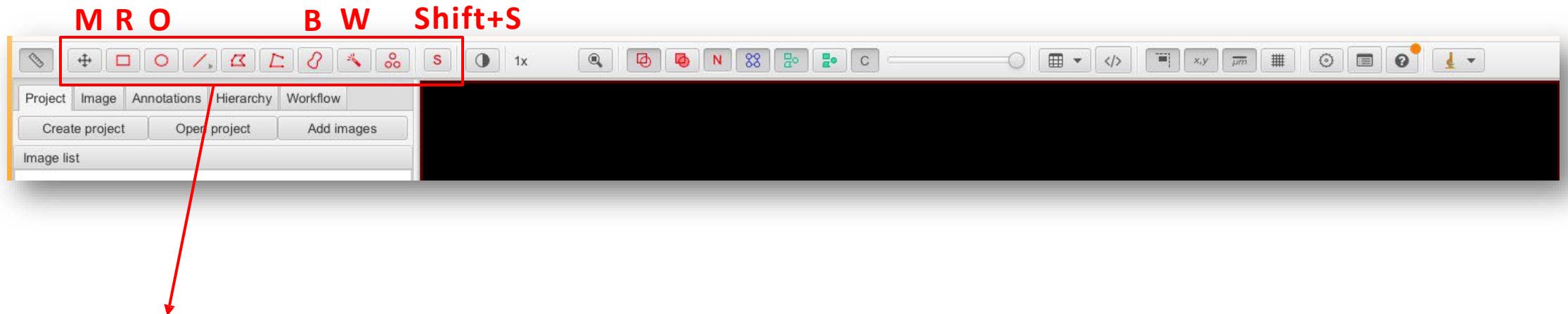
# Analysis Panel

- *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!

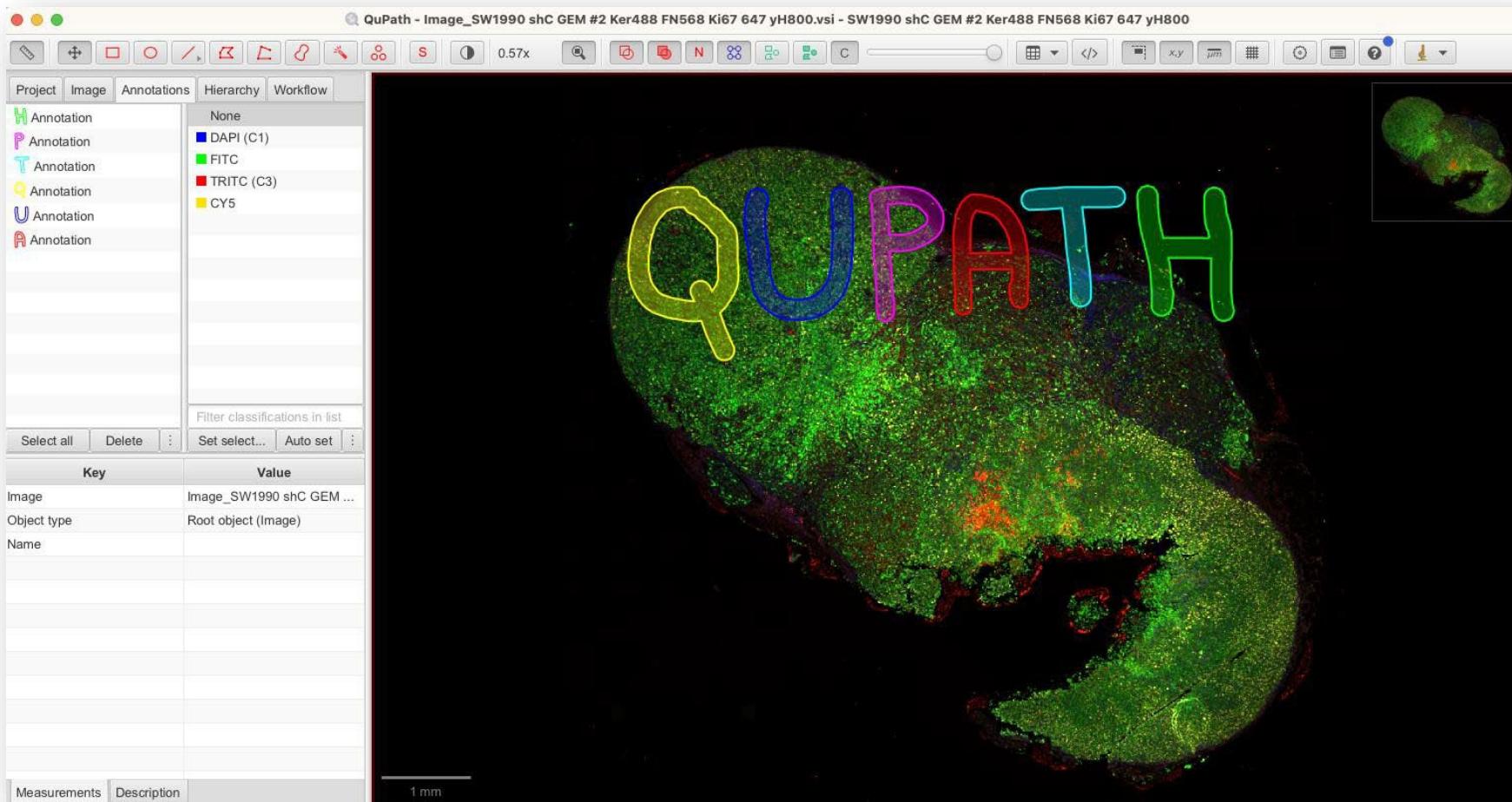
**Remember to always  
lock your annotation  
to prevent accidental  
editing!**

*Practice time*

## Exercises 2: QuPath manual annotations

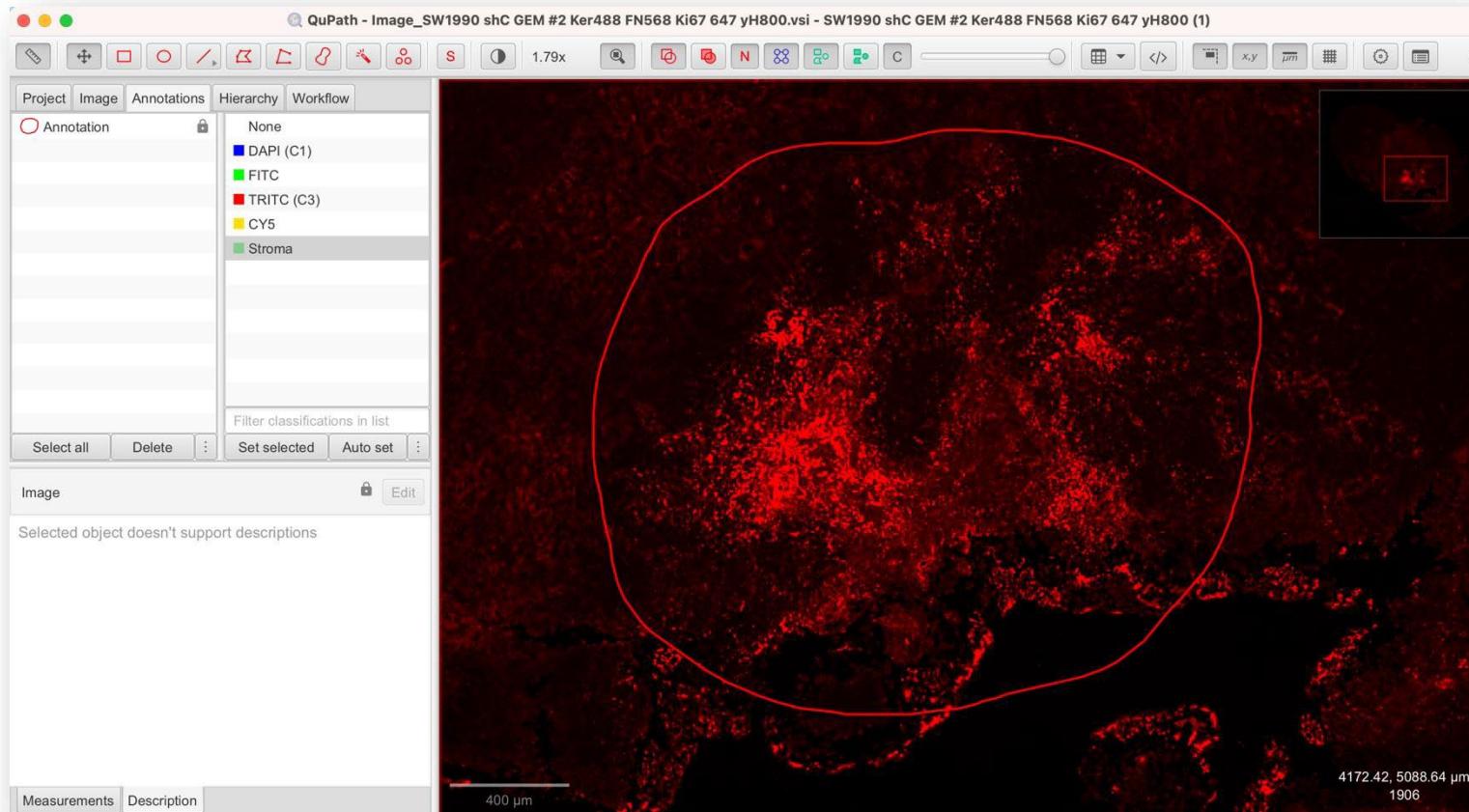
# Recreate these annotations

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

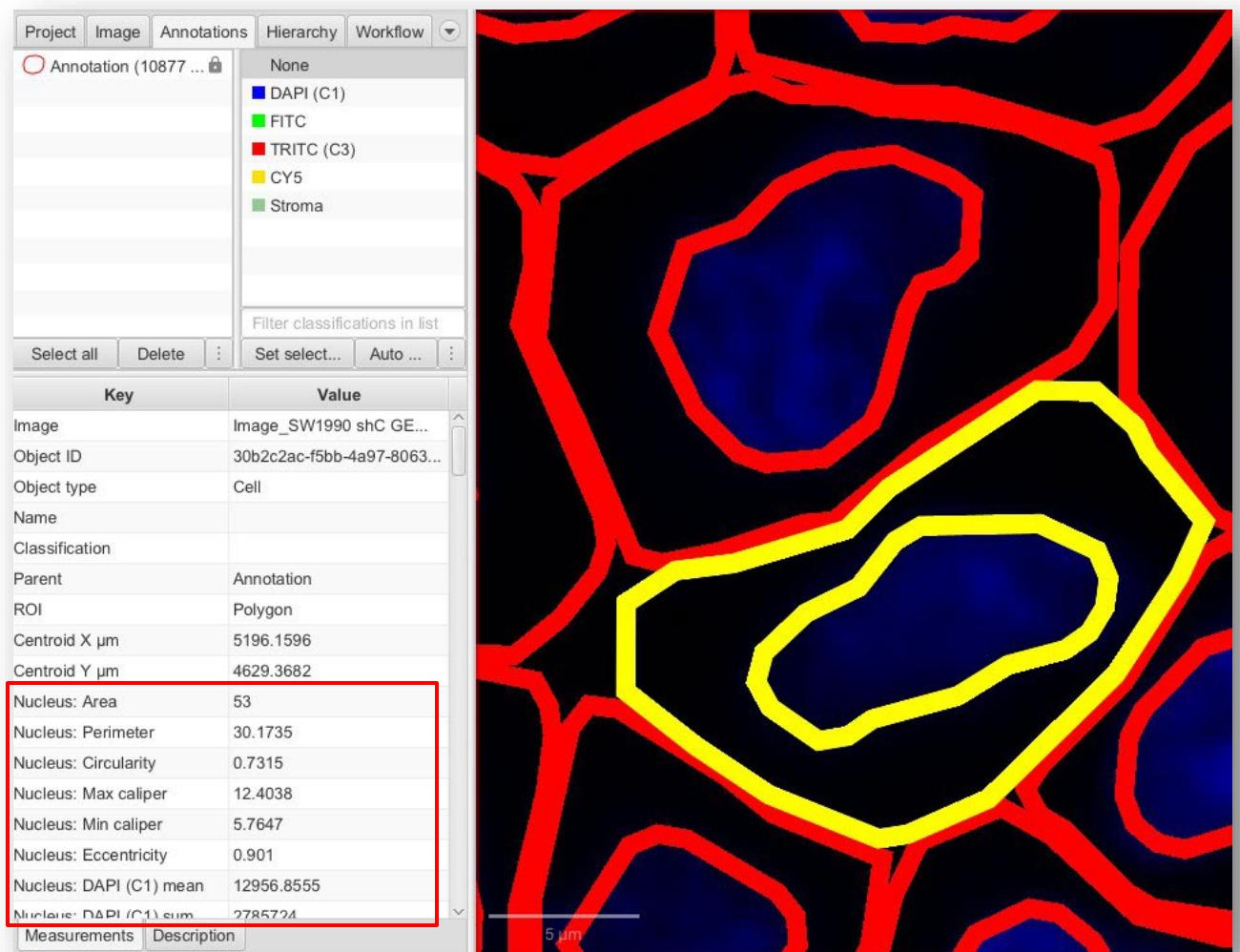
Right-click on the annotation in the analysis panel > *Lock*

# Cell detection

# Detection measurements

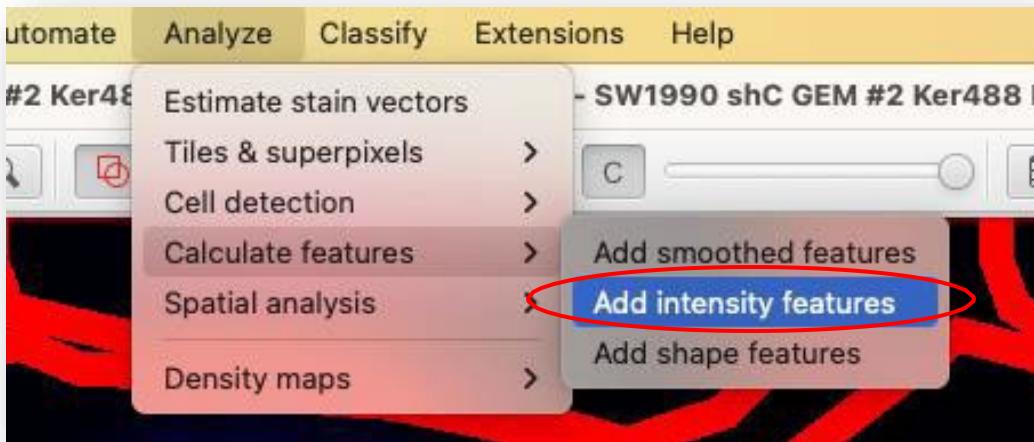
- Each detection object (i.e. a cell) has its measurement list
  - Intensity features
  - Haralick (texture) features
  - Shape features
  - Smoothed features
- *Annotations* tab > select a cell in the viewer > inspect its measurements list

By default, basic intensity and shape features are calculated



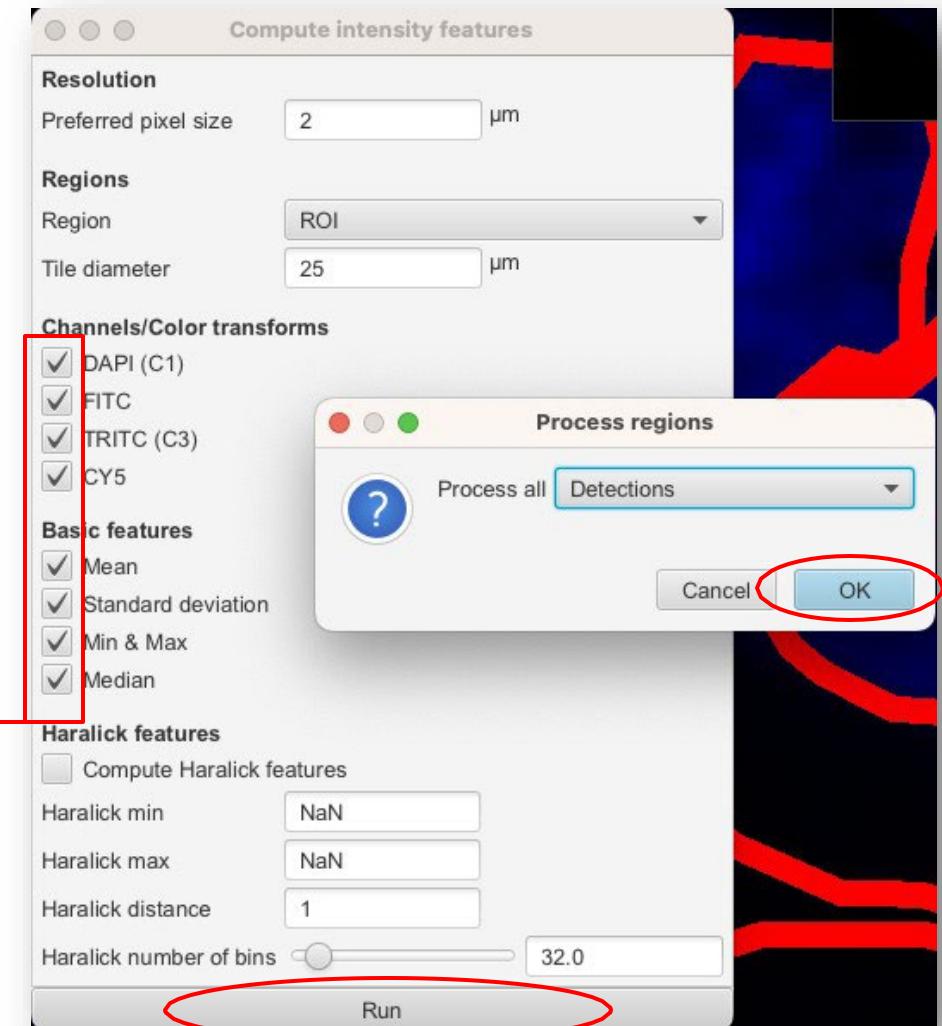
# Calculating measurements

- *Analyze > Calculate features > Add intensity features*



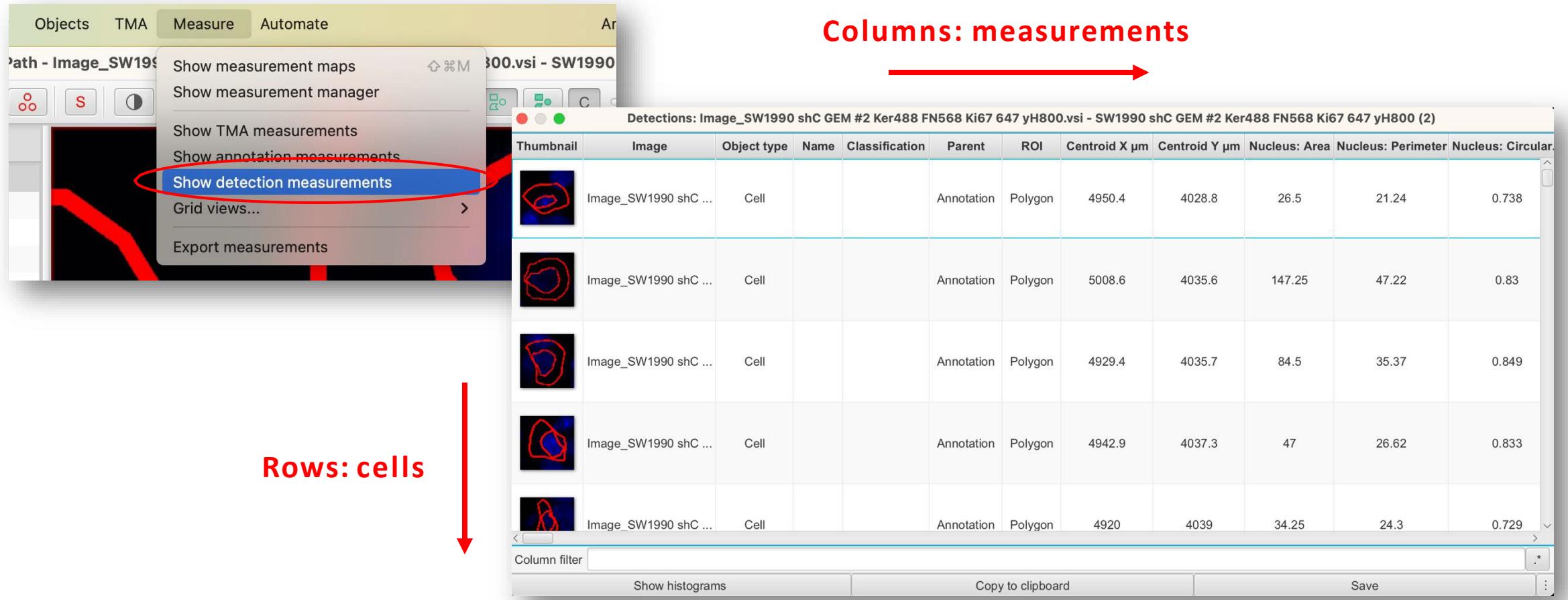
Tick boxes of the channels and features of interest

Need a custom feature? Script it!



# Visualizing measurements

- *Measure > Show detection measurements*



Objects TMA Measure Automate

Path - Image\_SW1990.vsi - SW1990

Show measurement maps

Show measurement manager

Show TMA measurements

Show annotation measurements

**Show detection measurements**

Grid views...

Export measurements

Thumbnail Image Object type Name Classification Parent ROI Centroid X  $\mu$ m Centroid Y  $\mu$ m Nucleus: Area Nucleus: Perimeter Nucleus: Circular

Thumbnail	Image	Object type	Name	Classification	Parent	ROI	Centroid X $\mu$ m	Centroid Y $\mu$ m	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

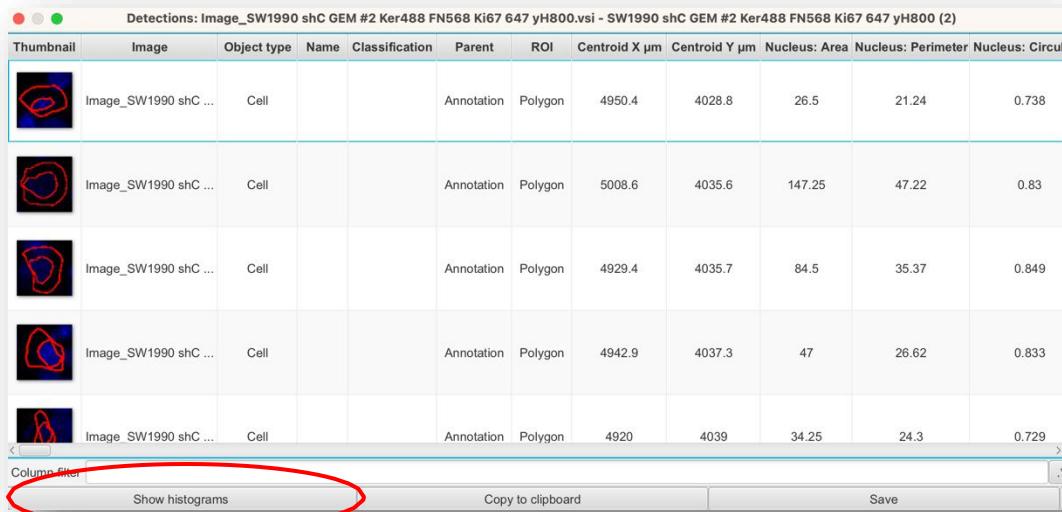
Rows: cells

Columns: measurements

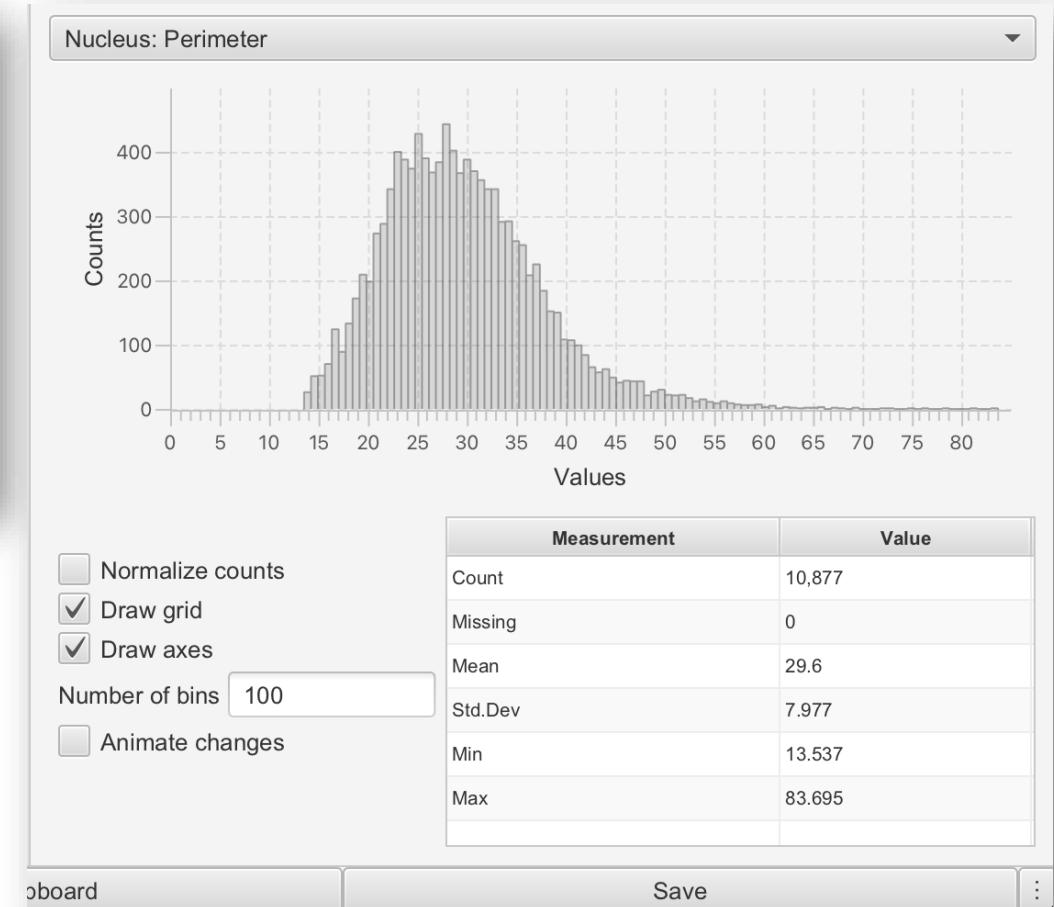
Show histograms Copy to clipboard Save

# Visualizing measurement distributions

- *Measure > Show detection measurements*

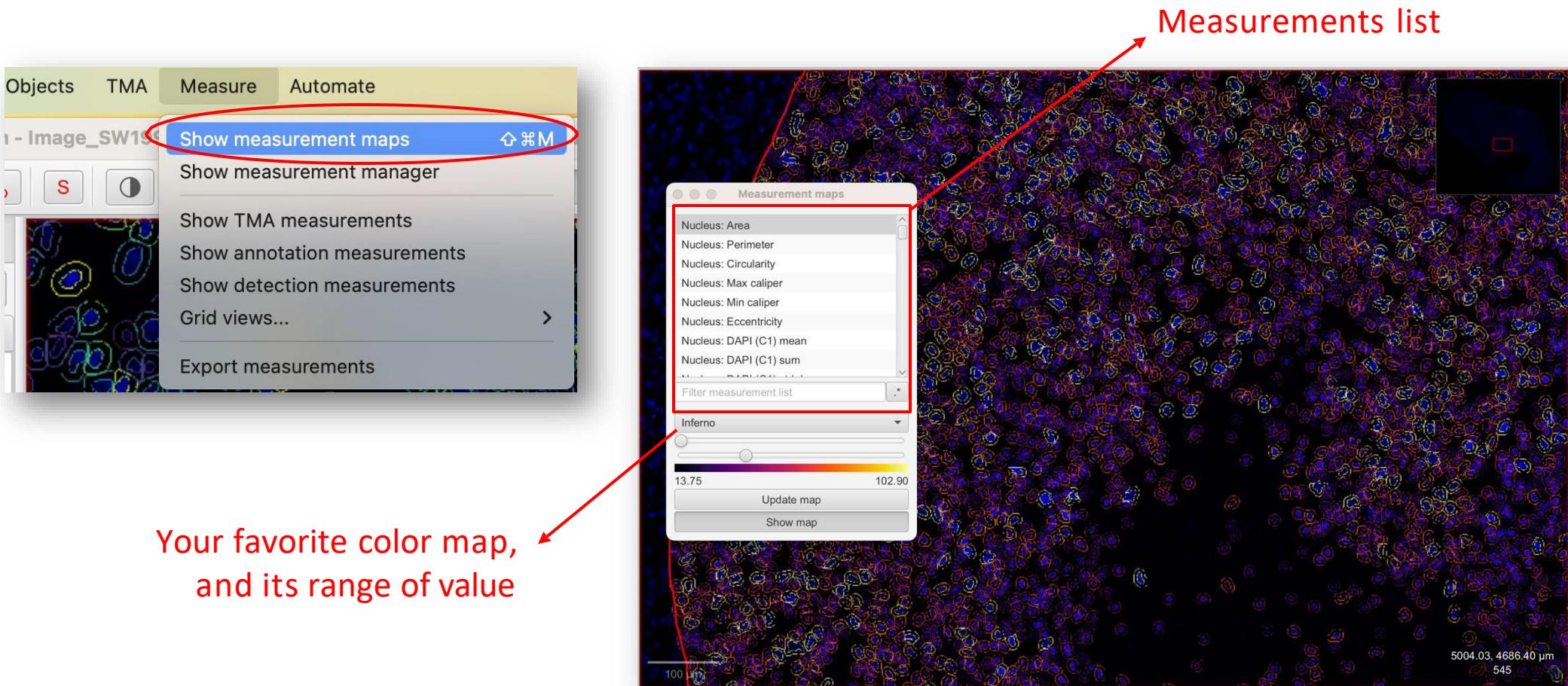


**FYI, it is not possible to export distribution plots...**



# Visualizing measurements as heat maps

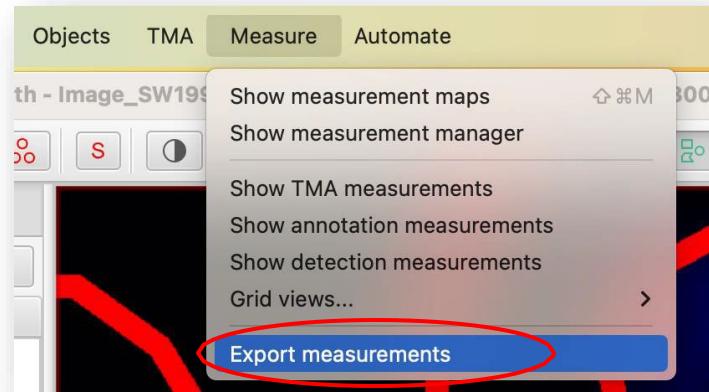
- *Measure > Show measurement maps*



Your favorite color map,  
and its range of value

# Export measurements the right way

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



Output file location

Measurement type to be exported

File type (.tsv, .csv)

List of measurements to include in the export

