A microscopy image showing a tissue section. The nuclei of the cells are stained blue, and there are red punctate markers, likely antibodies, indicating specific cellular components or locations. The cells are arranged in a layered, somewhat organized pattern.

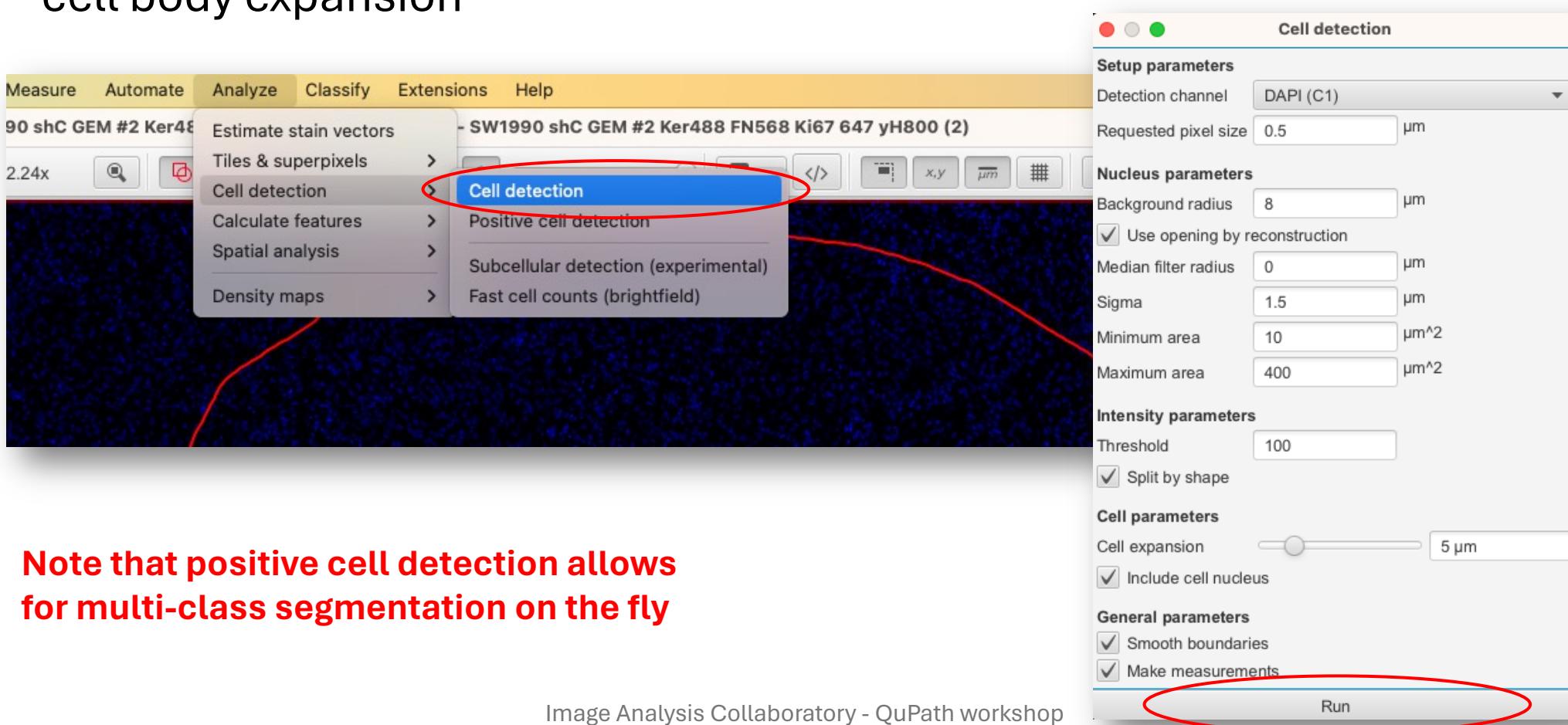
Cell detection

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



Cell detection parameters

Cell detection

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

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

Nucleus parameters

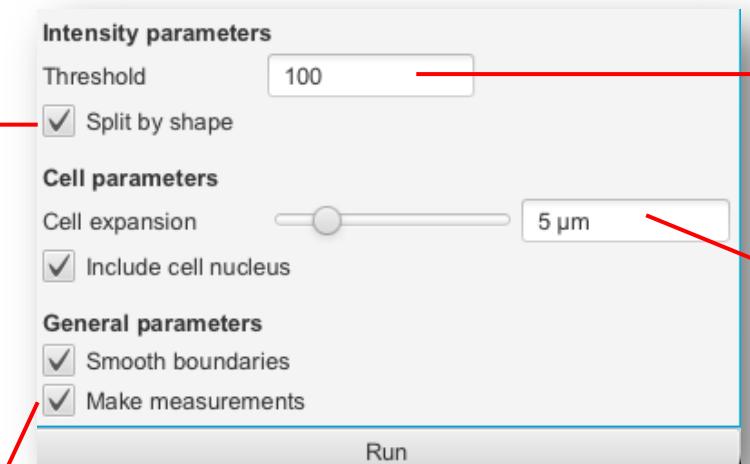
Background radius	8	µm
<input checked="" type="checkbox"/> Use opening by reconstruction		
Median filter radius	0	µm
Sigma	1.5	µm
Minimum area	10	µm ²
Maximum area	400	µm ²

Radius of area used for background subtraction

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



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

Minimum signal intensity of nuclei relative to background

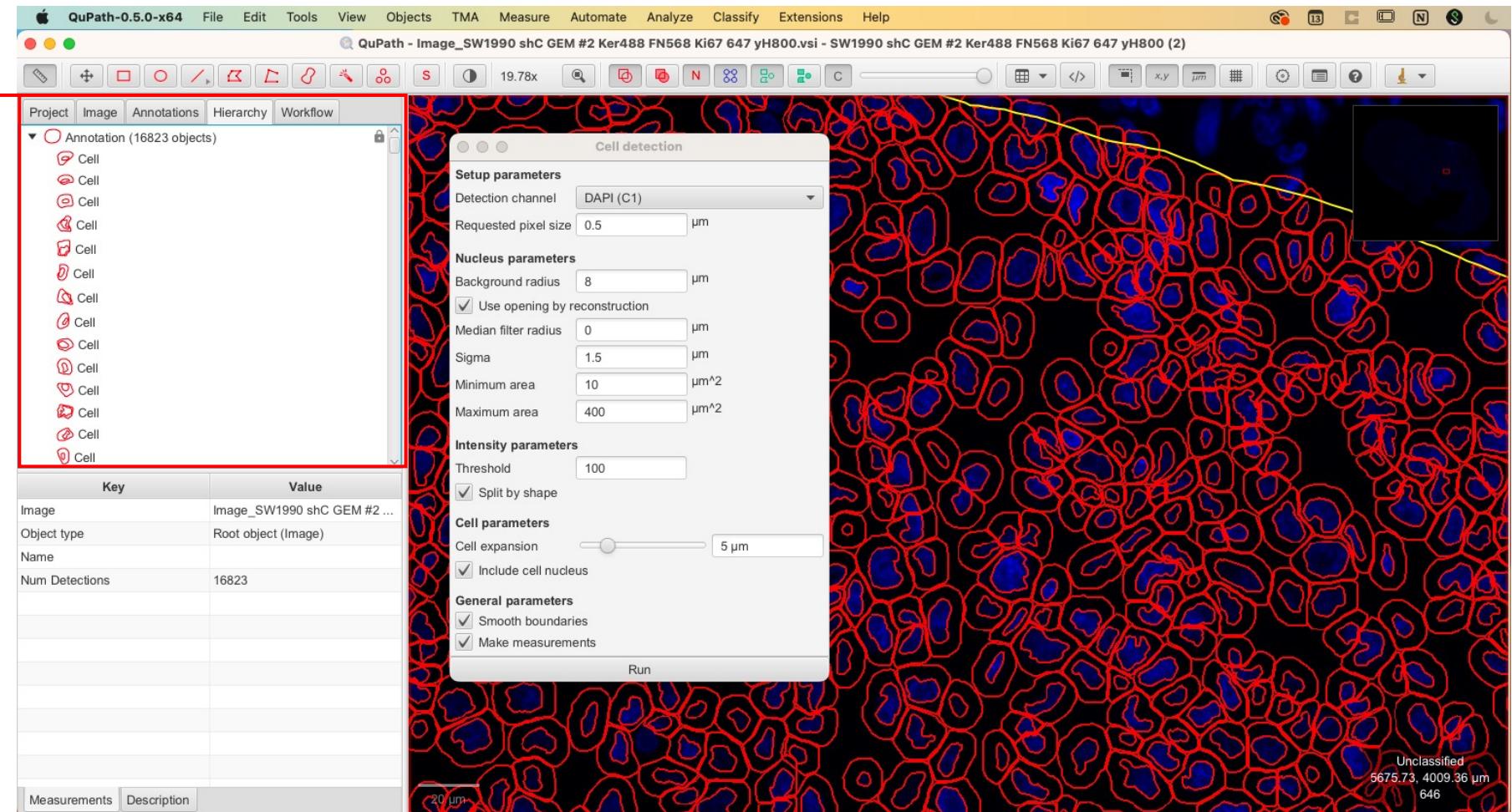
How much to expand nuclei to get cell boundaries

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

Cell detection with default parameters

Hierarchy tab

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



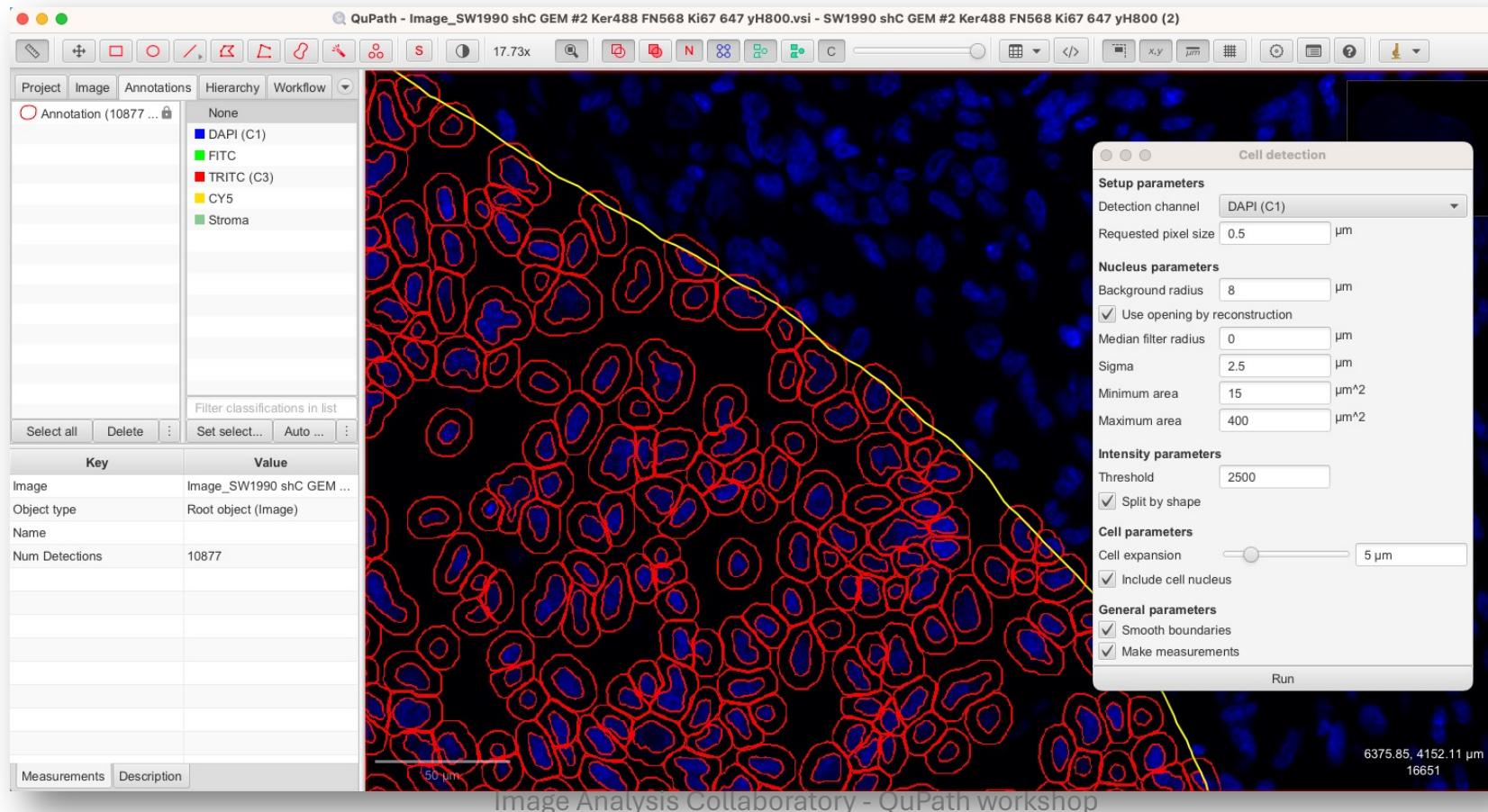
Note on the hierarchy of objects in QuPath

- 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

Exercise 3.a: QuPath cell detection

Exercise: explore parameters

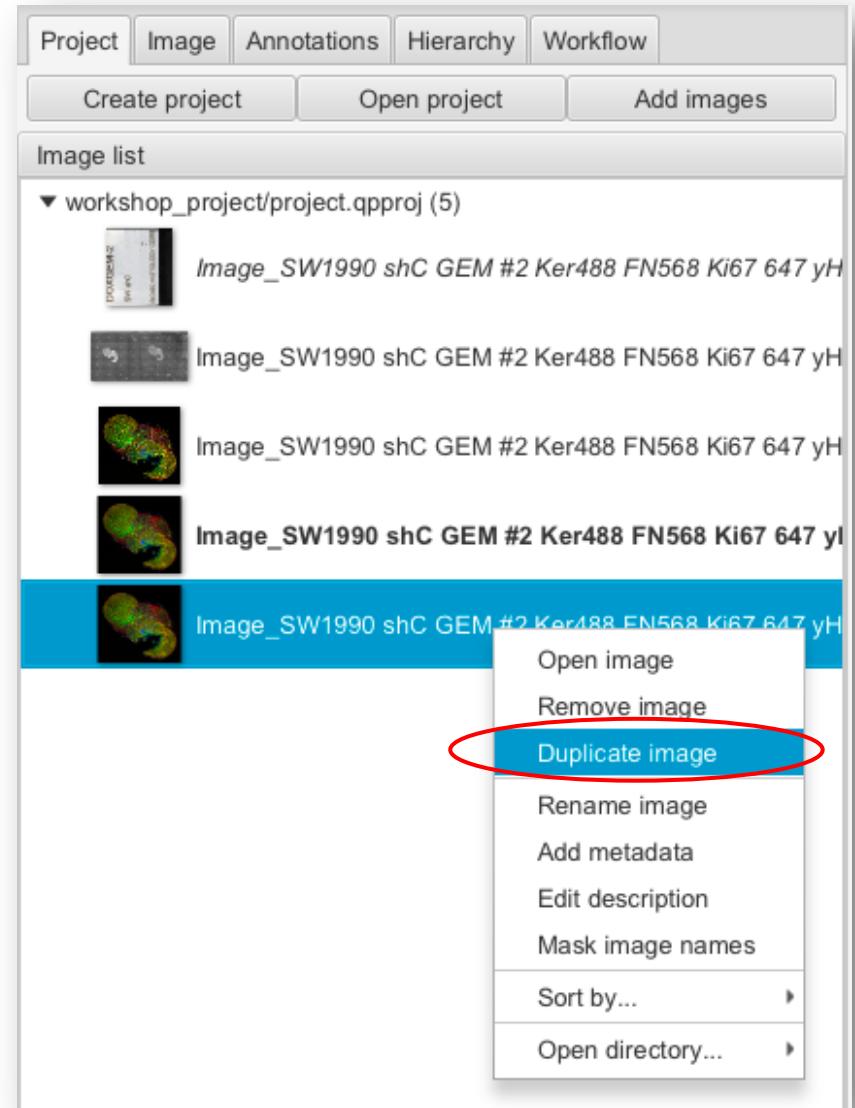
- I found that default parameters tend to over-segment nuclei so adapted the parameters to be slightly stricter (min area and threshold increased)



Duplicate your image

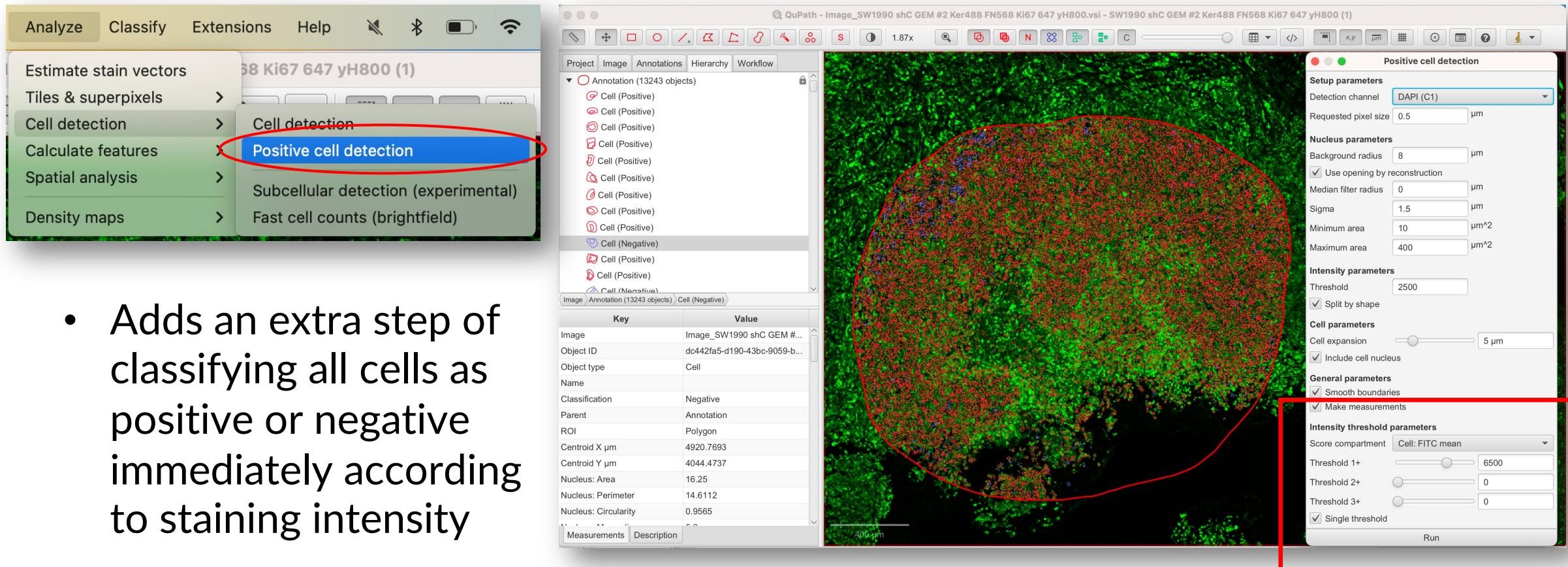
- Copy your cell detection results for future work on it
- *Project tab > Image list > Option+click or right-click on the image name > Duplicate image*

**It duplicates QuPath objects,
not the actual image**



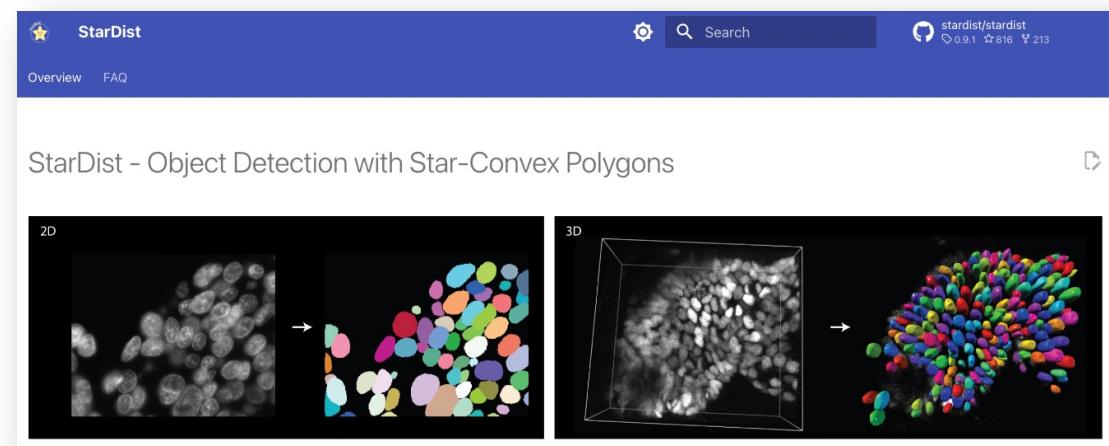
Detecting cells with an extra condition

- *Analyze > Cell detection > Positive cell detection*



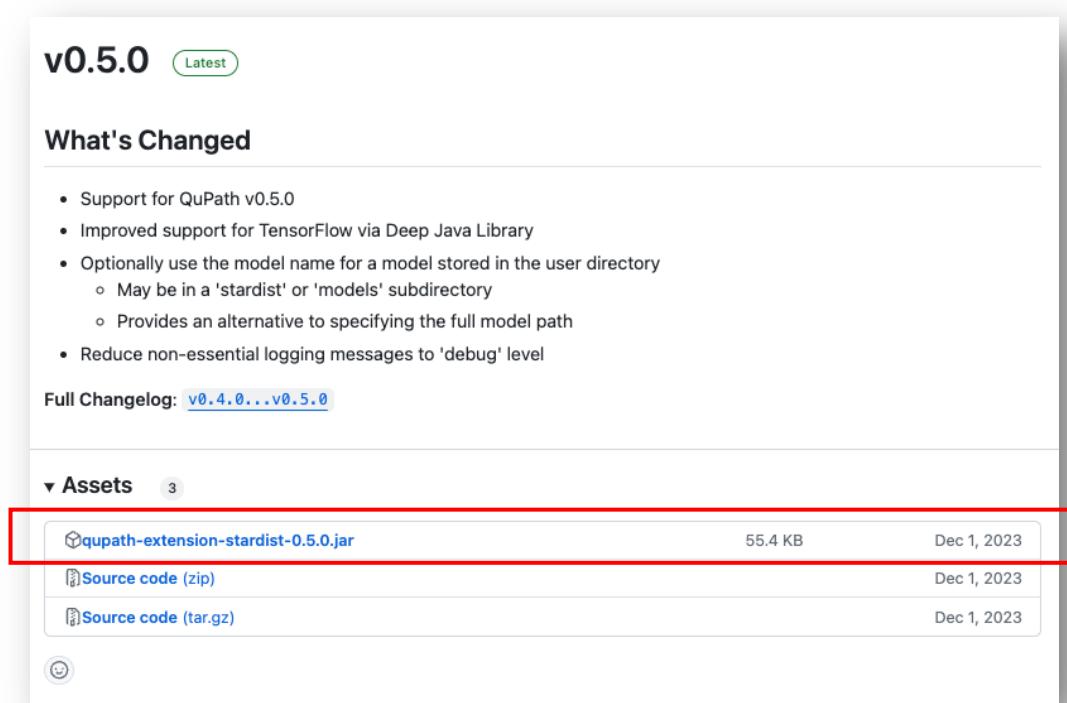
Deep learning-based cell segmentation

- 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



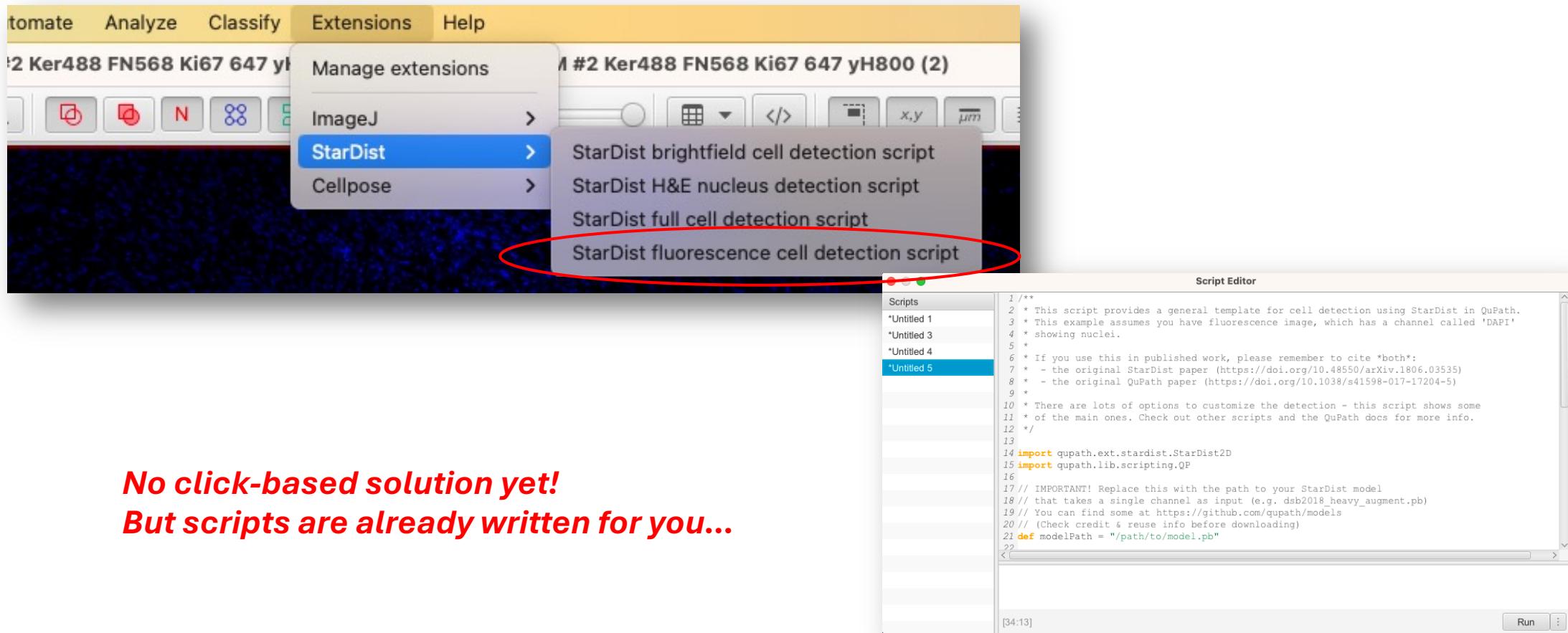
Installing StarDist extension in QuPath

- 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!



Using StarDist extension in QuPath

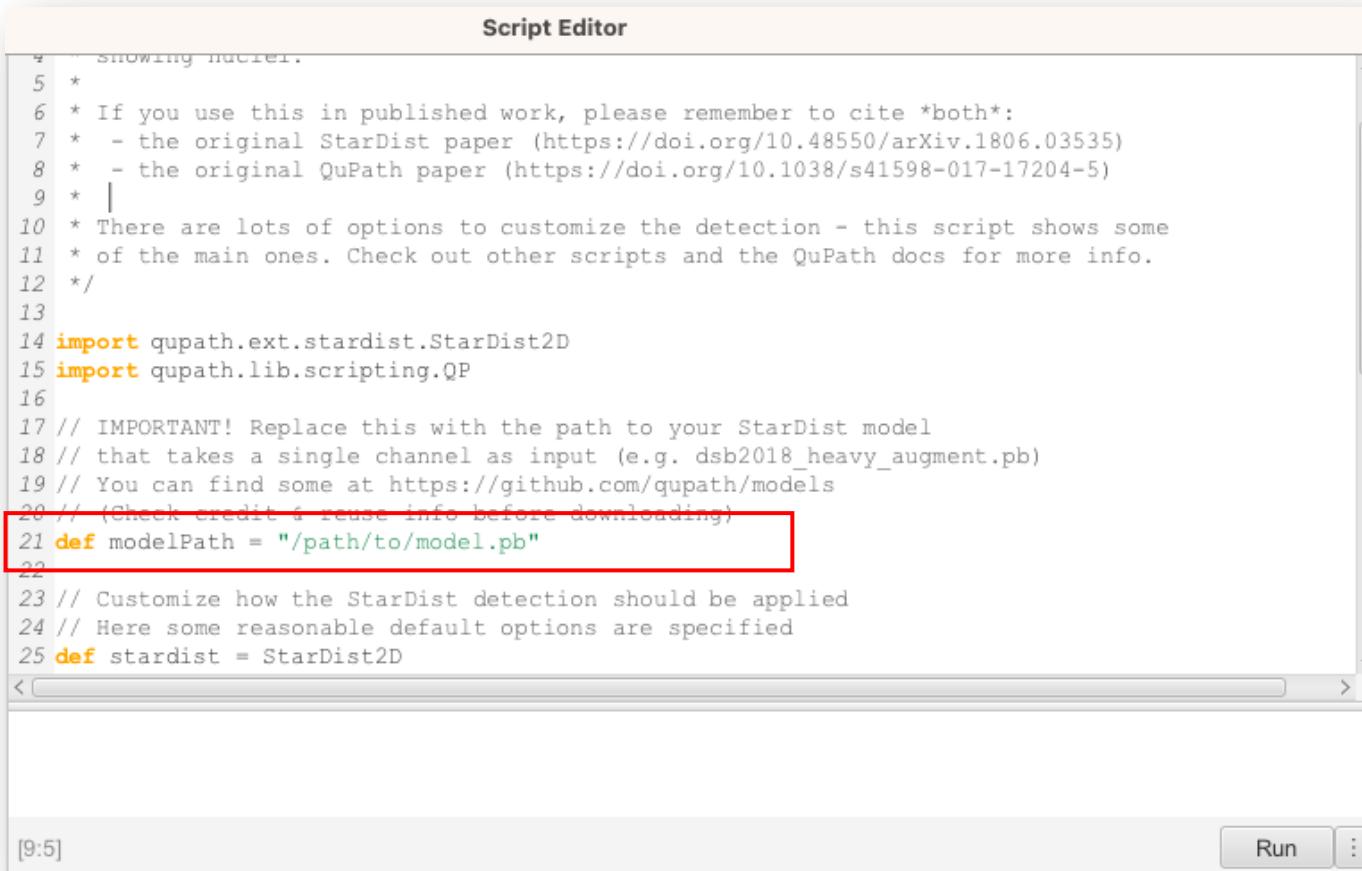
- Go to *Extensions* tab > *StarDist* > *StarDist fluorescence cell detection script*



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)



The screenshot shows the QuPath Script Editor window. The title bar says "Script Editor". The code editor contains a Python script for performing StarDist detections. The script includes comments explaining the purpose and options of the code. A specific line of code, "modelPath = '/path/to/model.pb'", is highlighted with a red rectangular box. The status bar at the bottom left shows "[9:5]" and the bottom right has "Run" and "..." buttons.

```
# ... showing nuclei.
5 *
6 * If you use this in published work, please remember to cite *both*:
7 * - the original StarDist paper (https://doi.org/10.48550/arXiv.1806.03535)
8 * - the original QuPath paper (https://doi.org/10.1038/s41598-017-17204-5)
9 *
10 * There are lots of options to customize the detection - this script shows some
11 * of the main ones. Check out other scripts and the QuPath docs for more info.
12 */
13
14 import qupath.ext.stardist.StarDist2D
15 import qupath.lib.scripting.QP
16
17 // IMPORTANT! Replace this with the path to your StarDist model
18 // that takes a single channel as input (e.g. dsb2018_heavy_augment.pb)
19 // You can find some at https://github.com/qupath/models
20 // (Check credit & reuse info before downloading)
21 def modelPath = "/path/to/model.pb"
22
23 // Customize how the StarDist detection should be applied
24 // Here some reasonable default options are specified
25 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

The screenshot shows the QuPath Script Editor window. The code is as follows:

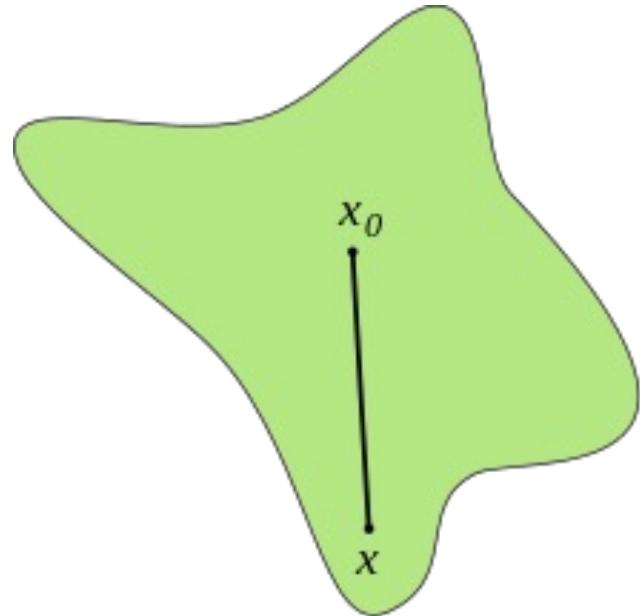
```
19 // You can find some at https://github.com/qupath/models
20 // (Check credit & reuse info before downloading)
21 def modelPath = "/Users/antoine/Desktop/test_qupath_workshop/models/dsb2018_heavy_augment.pb"
22
23 // Customize how the StarDist detection should be applied
24 // Here some reasonable default options are specified
25 def stardist = StarDist2D
26     builder(modelPath)
27     .channels('DAPI (C1)')           // Extract channel called 'DAPI'
28     .normalizePercentiles(1, 99)    // Percentile normalization
29     .threshold(0.5)                // Probability (detection) threshold
30     .pixelSize(0.5)                // Resolution for detection
31     .cellExpansion(5)              // Expand nuclei to approximate cell boundaries
32     .measureShape()                // Add shape measurements
33     .measureIntensity()            // Add cell measurements (in all compartments)
34     .build()
35
INFO: Done!
```

A red box highlights the line `def modelPath = "/Users/antoine/Desktop/test_qupath_workshop/models/dsb2018_heavy_augment.pb"`. A red arrow points from the text "Change the channel name" to the line `.channels('DAPI (C1)')`.

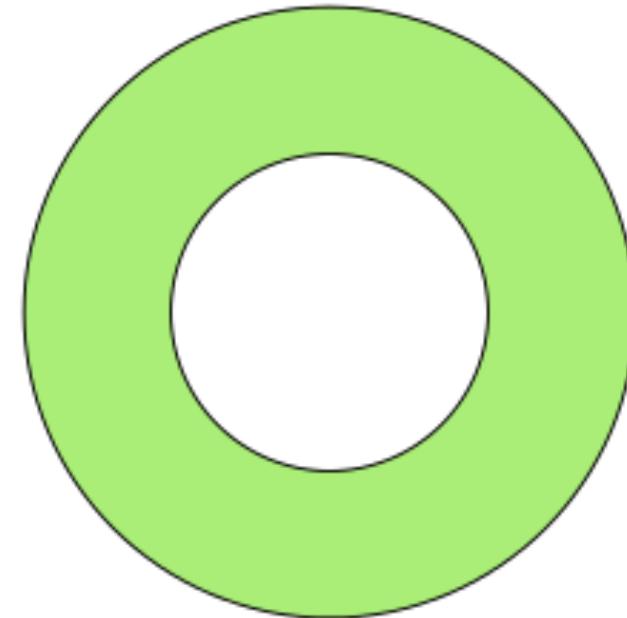
Make sure to
select the ROI in
QuPath before
running the script.

Exercise 3.b: QuPath cell detection with StarDist

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



StarDist can segment



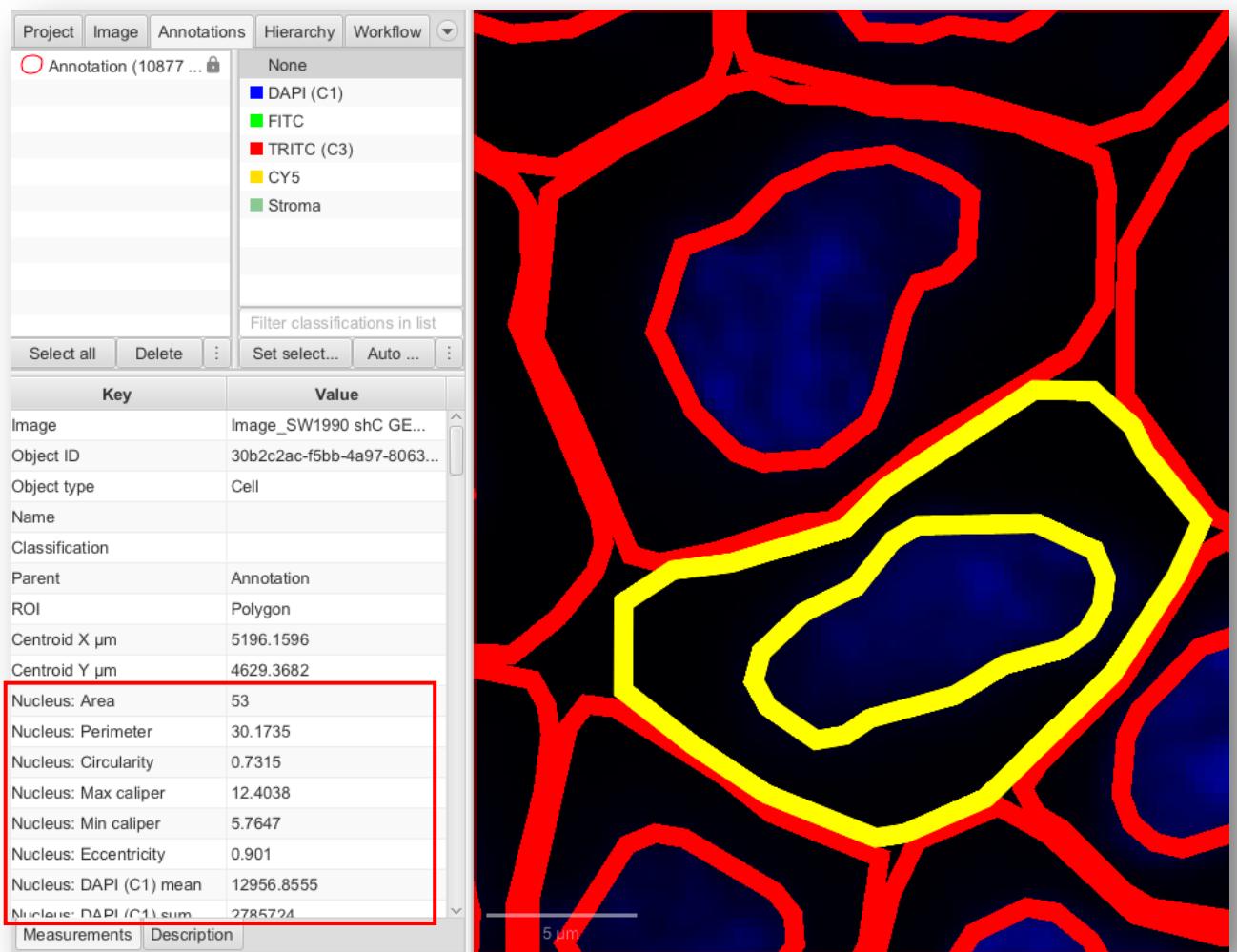
StarDist can not segment

Cell detection measurements

Detection measurements

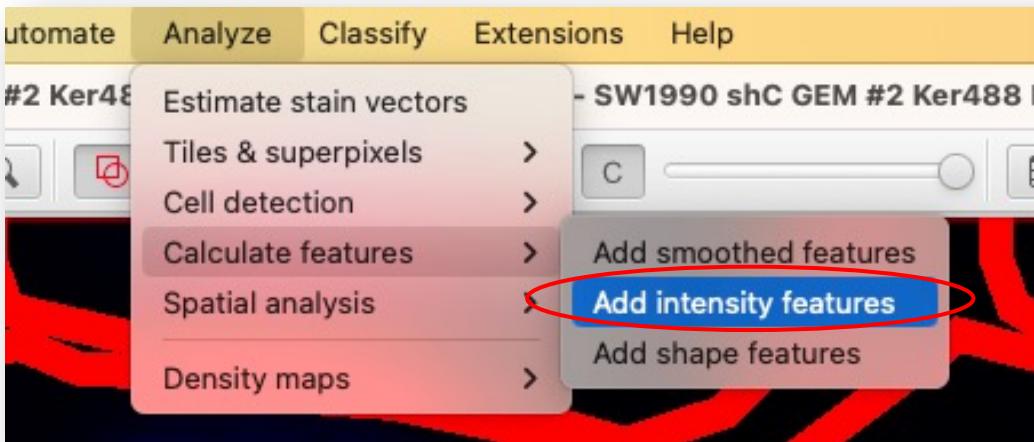
- Each detection object (i.e. a cell) has its measurement list
 - Intensity features
 - Haralick (texture) features
 - Shape features
 - Smoothed features
- *Annotations* tab > select a cell in the viewer > inspect its measurements list

By default, basic intensity and shape features are calculated



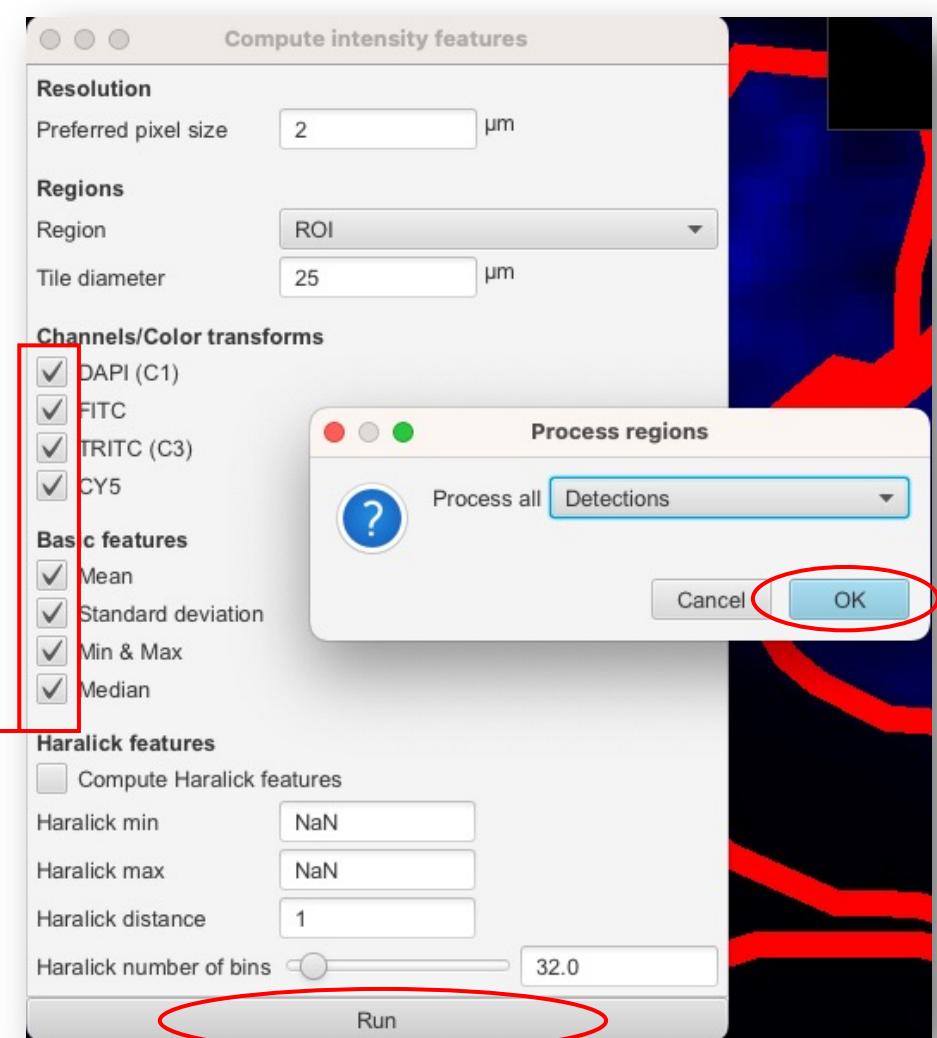
Calculating measurements

- *Analyze > Calculate features > Add intensity features*



Tick boxes of the channels and
features of interest

Need a custom feature? Script it!



Visualizing measurements

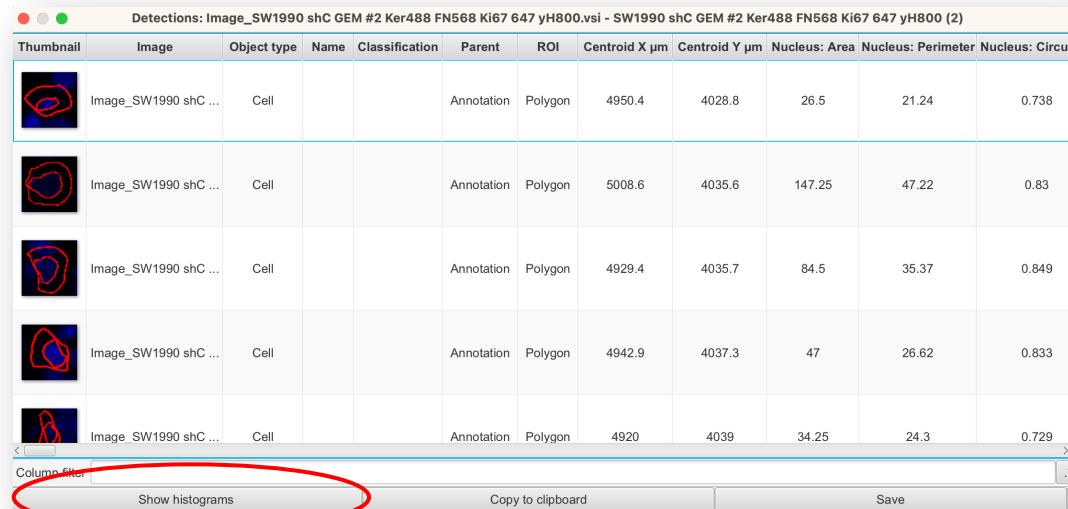
- *Measure > Show detection measurements*

The screenshot shows the QuPath software interface. On the left, there is a navigation bar with tabs for Objects, TMA, Measure (which is selected), and Automate. Below the navigation bar, a context menu is open over a tissue microarray (TMA) slide. The menu items include: Show measurement maps, Show measurement manager, Show TMA measurements, Show annotation measurements, Show detection measurements (this item is highlighted with a red oval), Grid views..., and Export measurements. To the right of the menu, a main workspace displays a digital image of a tissue section with several cells outlined in red. A legend at the top of the workspace shows three colored circles: red, grey, and green. Below the image, a table titled "Detections: Image_SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800.vsi - SW1990 shC GEM #2 Ker488 FN568 Ki67 647 yH800 (2)" lists the detected cells. The table has columns for Thumbnail, Image, Object type, Name, Classification, Parent, ROI, Centroid X µm, Centroid Y µm, Nucleus: Area, Nucleus: Perimeter, and Nucleus: Circular. Five rows of data are shown, each corresponding to one of the outlined cells in the image. A large red arrow points from the "Show detection measurements" menu item to the table, indicating the relationship between the selection and the resulting data view. Another red arrow points downwards from the table, labeled "Rows: cells", indicating that each row represents a single cell.

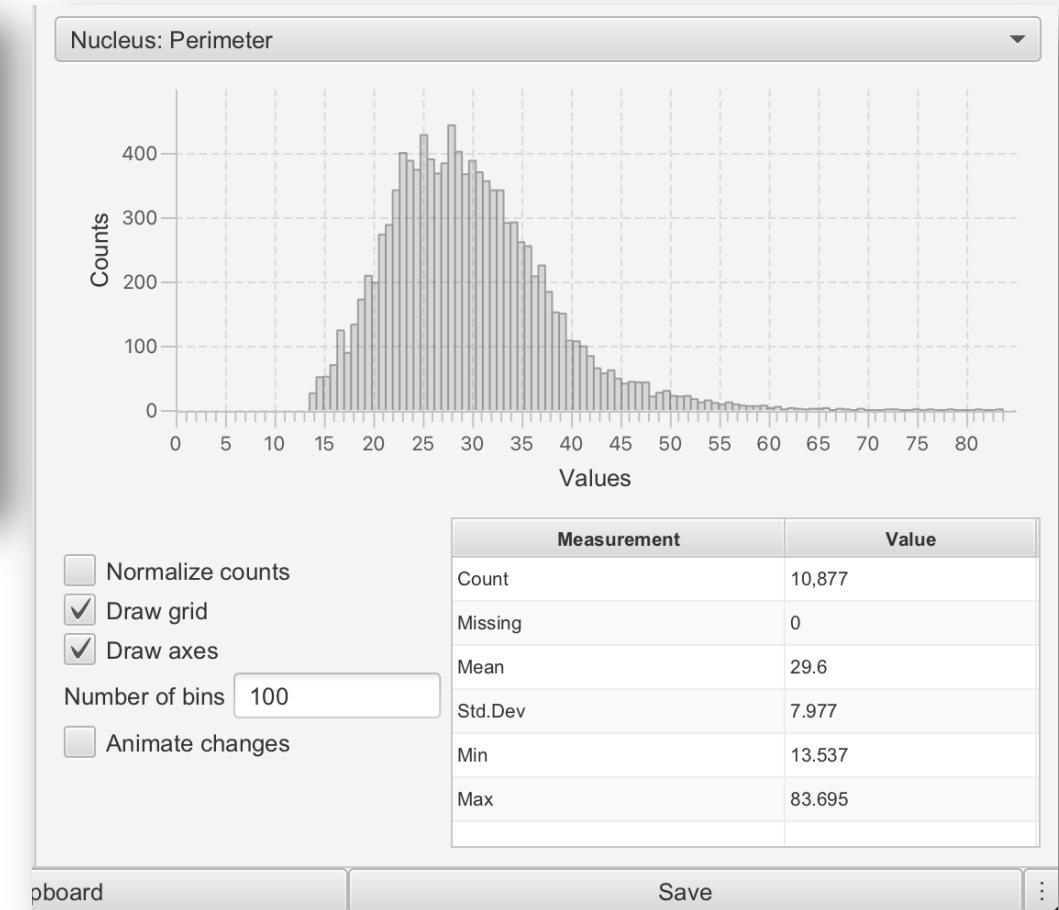
Thumbnail	Image	Object type	Name	Classification	Parent	ROI	Centroid X µm	Centroid Y µm	Nucleus: Area	Nucleus: Perimeter	Nucleus: Circular
	Image_SW1990 shC ...	Cell			Annotation	Polygon	4950.4	4028.8	26.5	21.24	0.738
	Image_SW1990 shC ...	Cell			Annotation	Polygon	5008.6	4035.6	147.25	47.22	0.83
	Image_SW1990 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

Visualizing measurement distributions

- *Measure > Show detection measurements*

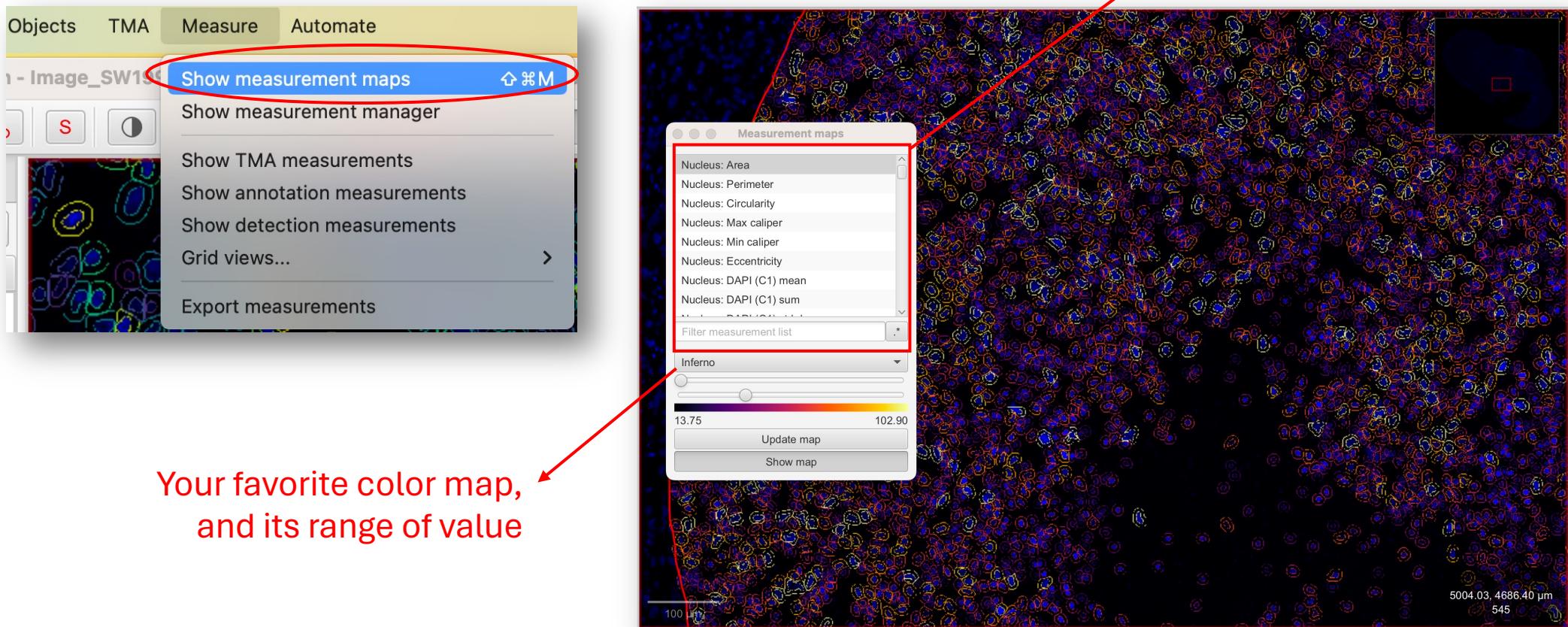


FYI, it is not possible to export distribution plots...



Visualizing measurements as heat maps

- *Measure > Show measurement maps*



Export measurements the right way

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

