

Multiplexed data analysis using QuPath

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Today's planning

- What is QuPath?
- Preprocessing: unmixing, removing autofluorescence

Live demo

- QuPath Overview
- Cell detection and classification
- Pixel classifier
- Measurements
- Spatial analysis
- Scripting
- Advantages and drawbacks

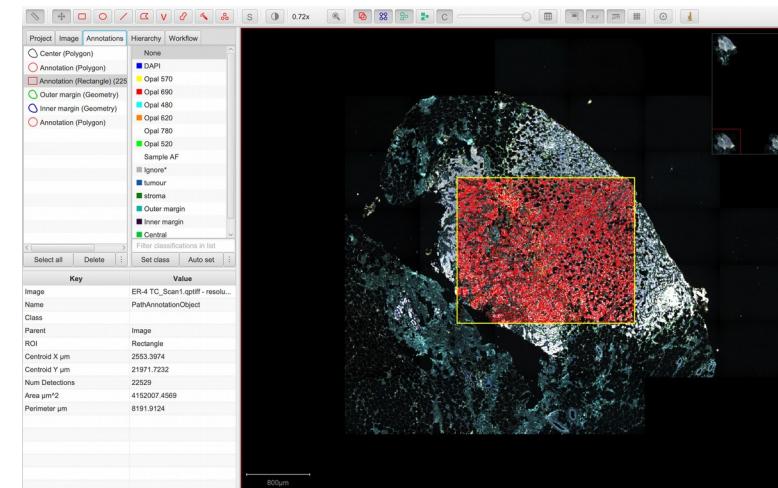
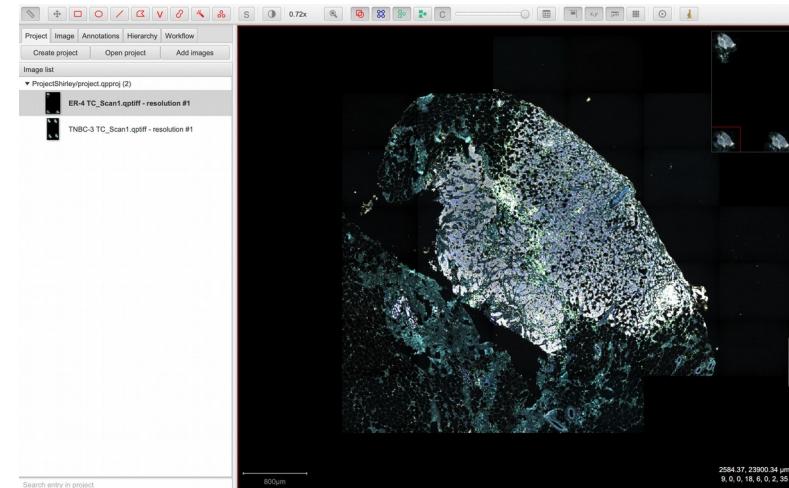


What is QuPath?

- Open source for bioimage analysis
- Powerful annotation and visualisation tools
- Built-in algorithms for common tasks
- Interactive machine learning
- Compatibility
- Support for many file formats
- Scripting for deeper data interrogation



QuPath approach



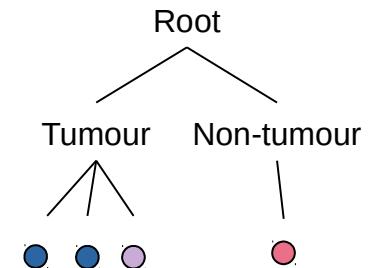
Start with pixels
 Billions of numbers



Identify objects
 Cells, structures, regions...



Query the objects
 Shape, size, number, intensity,
 location, classification

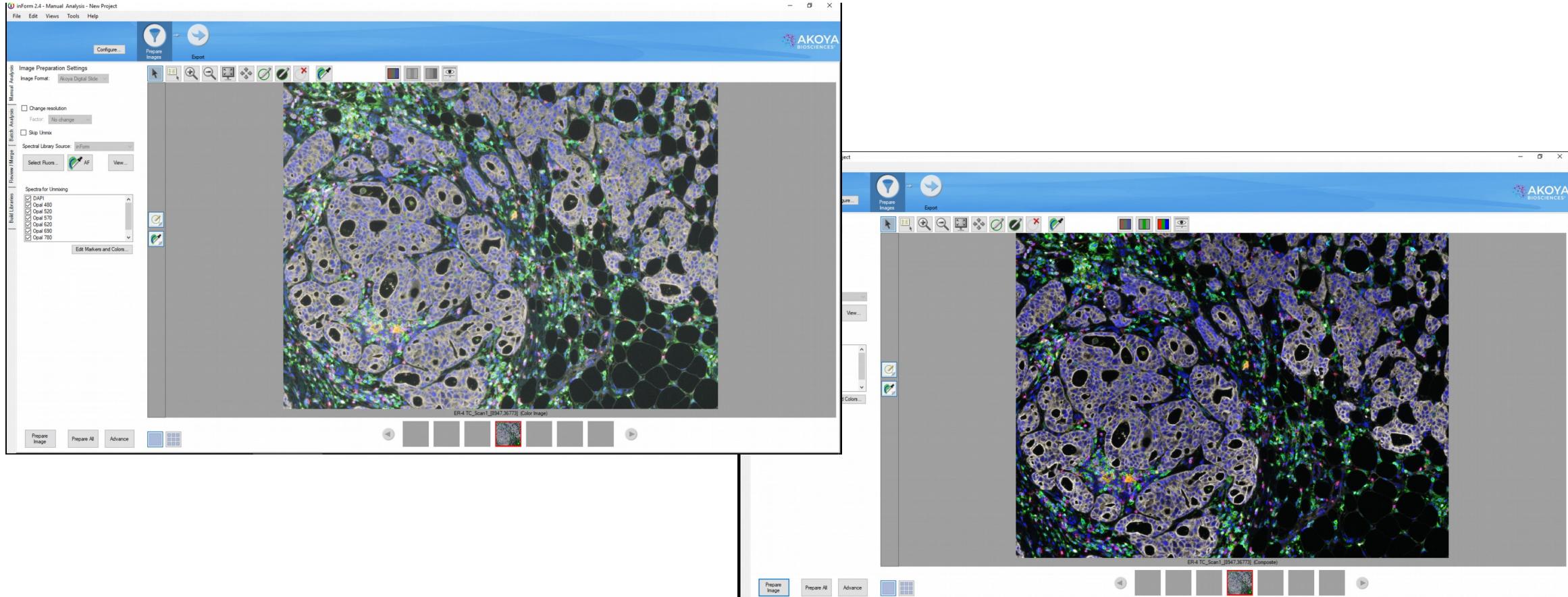


Annotation results - ER-4_TC_Scan1.qptiff - resolution #1					
	Image	Name	Class	Parent	ROI
1	ER-4_TC_Scan1.qptiff - resolution #1	Center	Polygon	2	
2	ER-4_TC_Scan1.qptiff - resolution #1	PathAnnotationObject	Image	Polygon	2
3	ER-4_TC_Scan1.qptiff - resolution #1	PathAnnotationObject	Image	Region	2
4	ER-4_TC_Scan1.qptiff - resolution #1	Outer margin	Image	Geometry	2
5	ER-4_TC_Scan1.qptiff - resolution #1	Inner margin	Image	Geometry	2
6	ER-4_TC_Scan1.qptiff - resolution #1	PathAnnotationObject	Image	Polygon	3

Column filter
 Show histograms | Copy to clipboard | Save

Pre-processing

- Phenochart and InForm: Unmix fluorophores and autofluorescence



Let's get started !

Overview

Annotation

Display

Measurements

Project | Image | Annotations | Hierarchy | Workflow

Create project | Open project | Add images

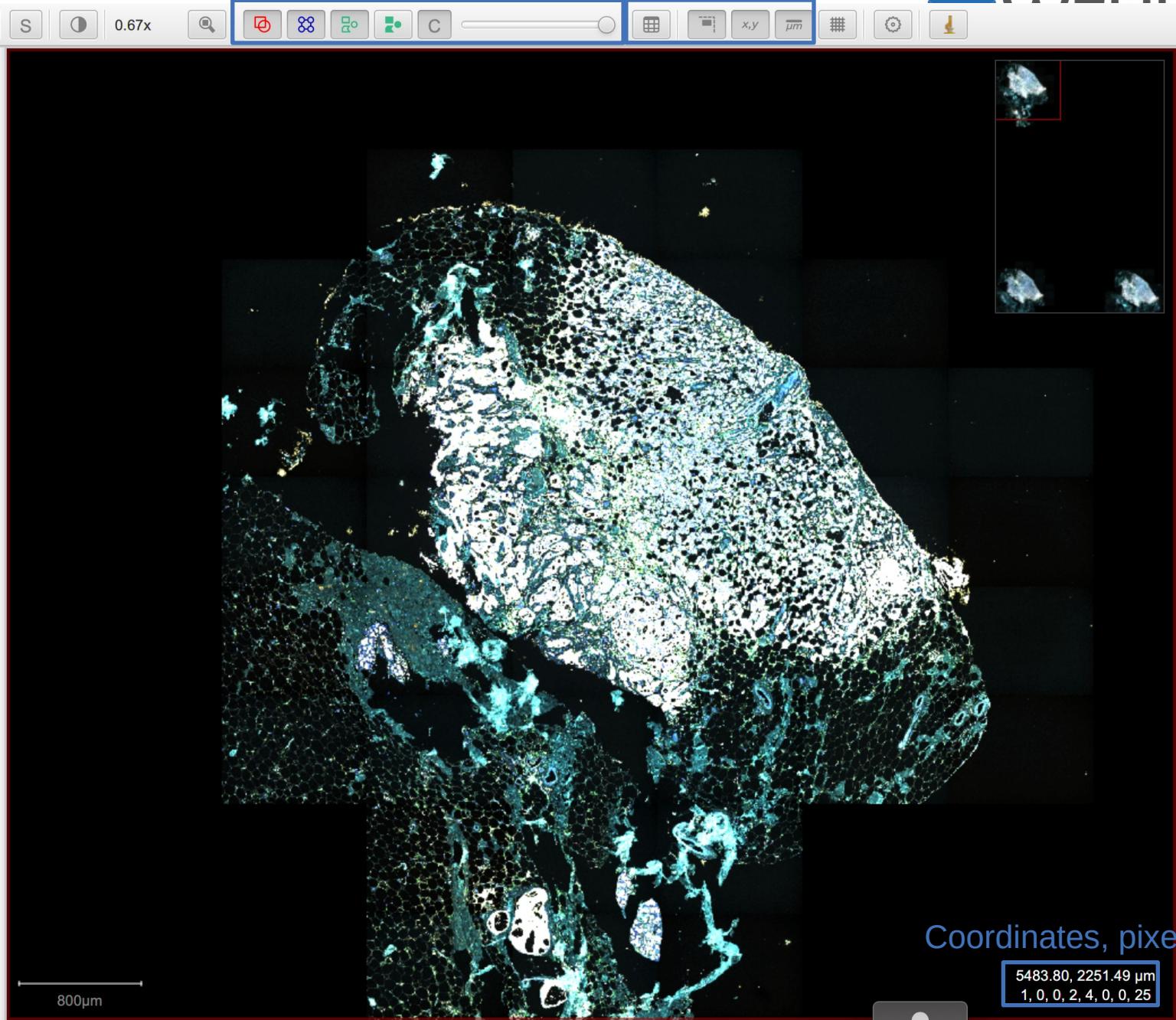
Image list

▼ ProjectShirley/project.qpproj (2)

- ER-4 TC_Scan1.qptiff - resolution #1
- TNBC-3 TC_Scan1.qptiff - resolution #1

Image properties

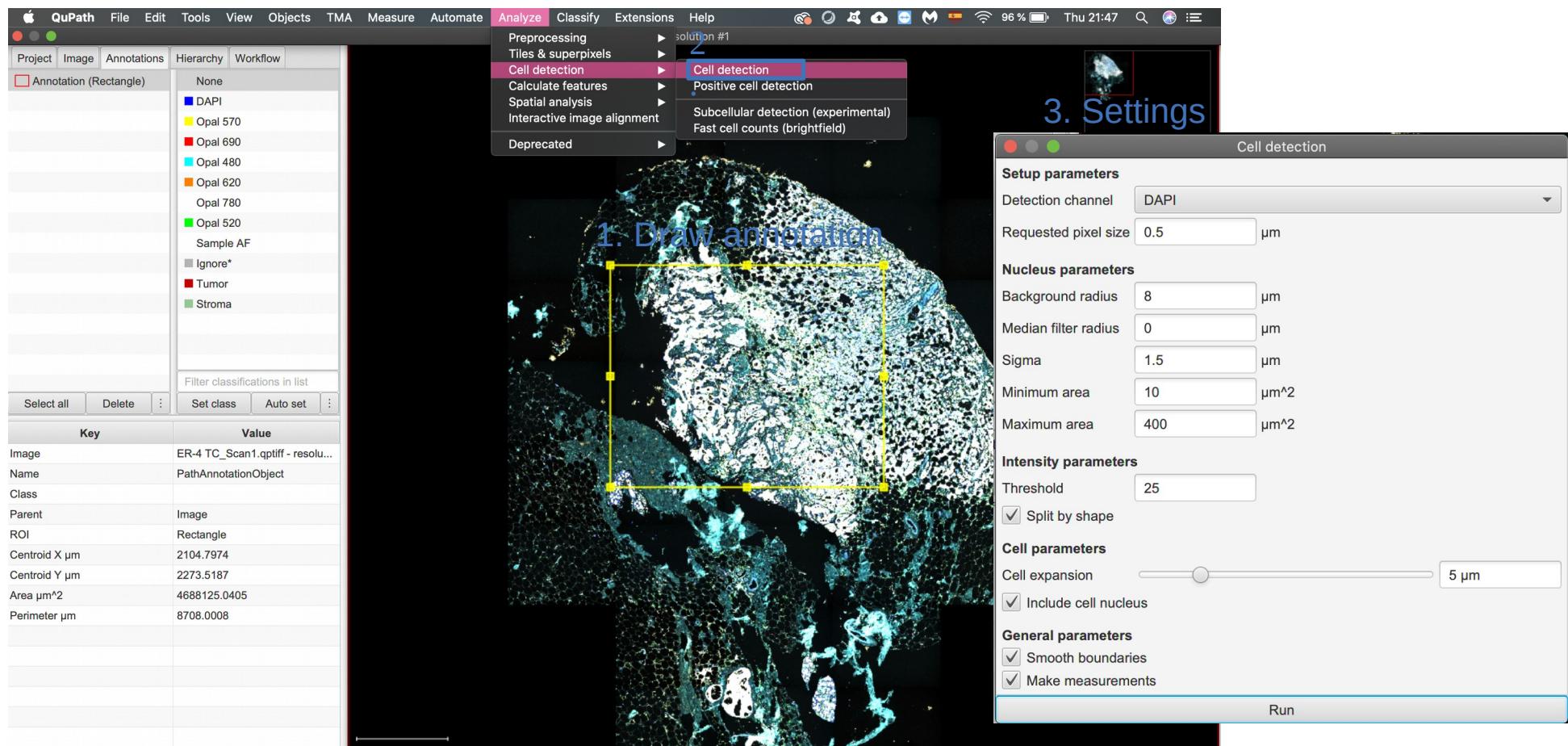
Search entry in project



Cell detection

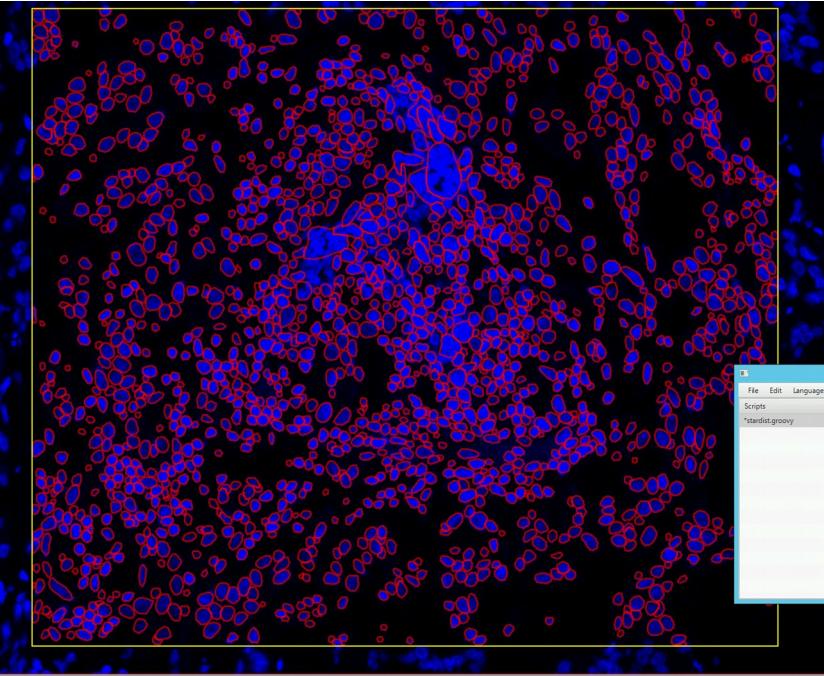
- Watershed

Analyze > Cell detection > Cell detection

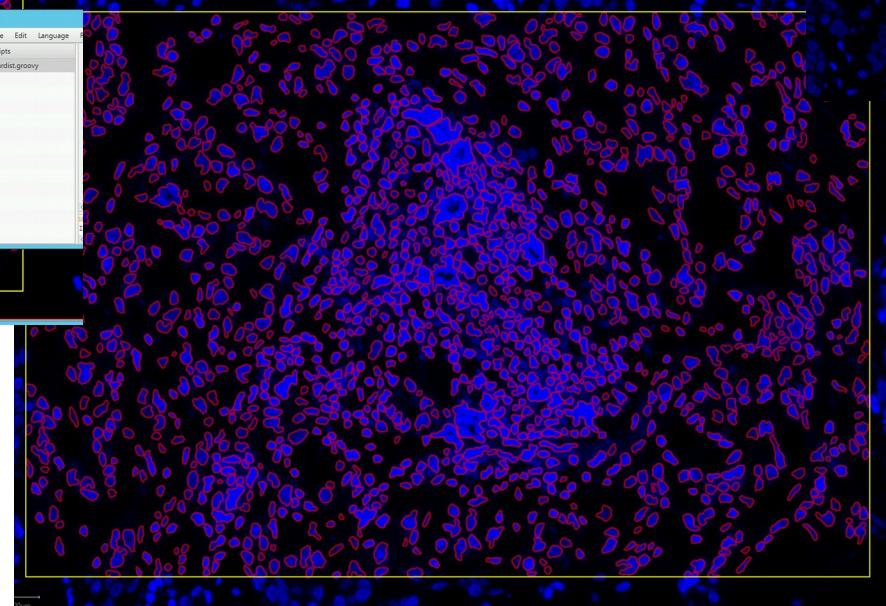


Cell detection

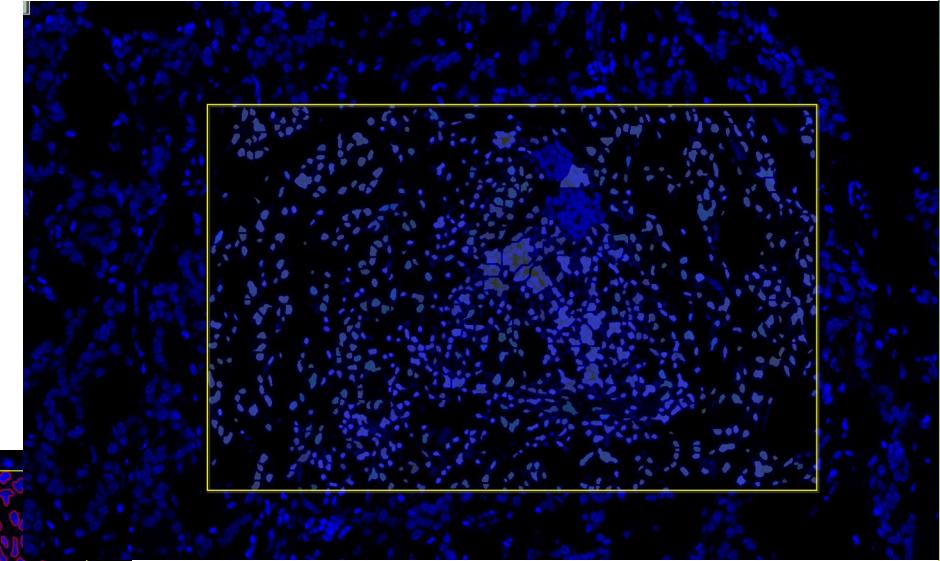
- StarDist (run stardist.groovy)



Stardist



Watershed
(QuPath)

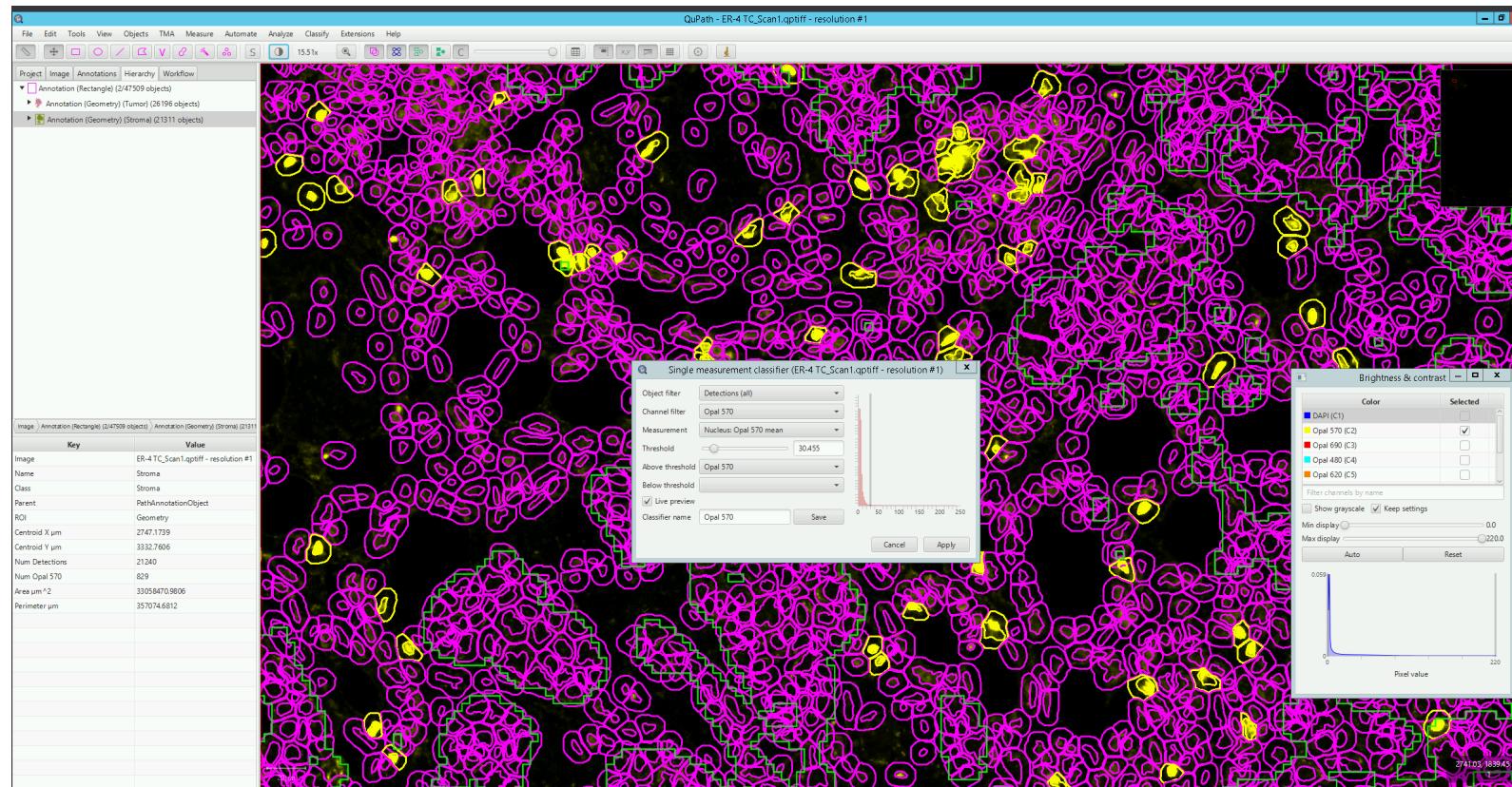


HALO

Cell classification

- Single measurement classifier

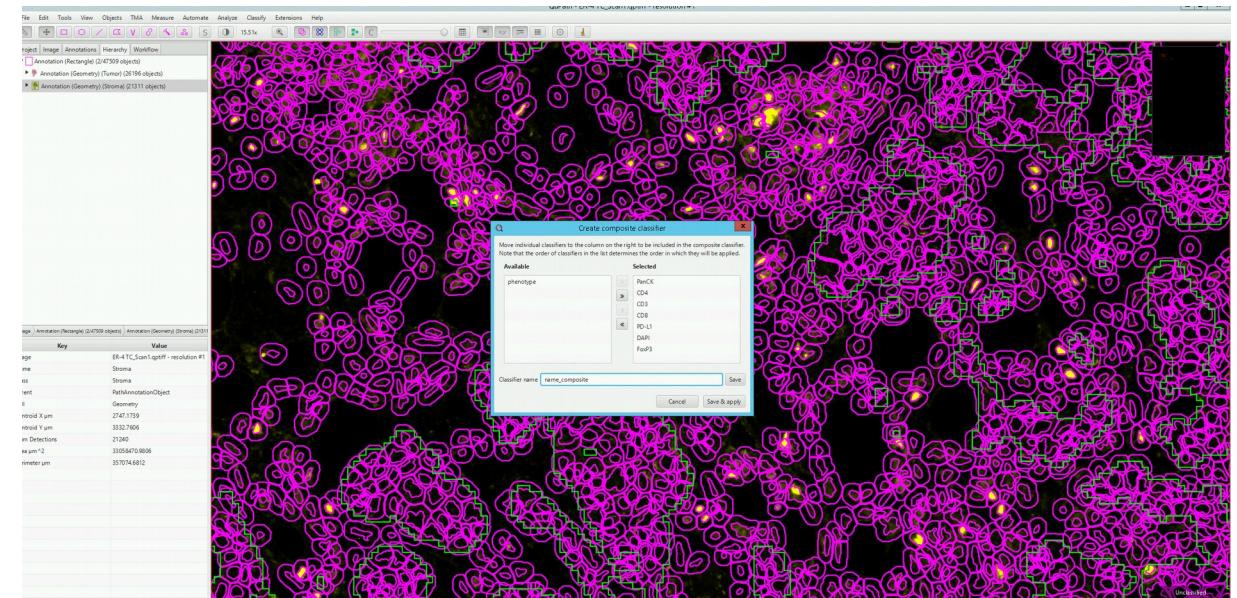
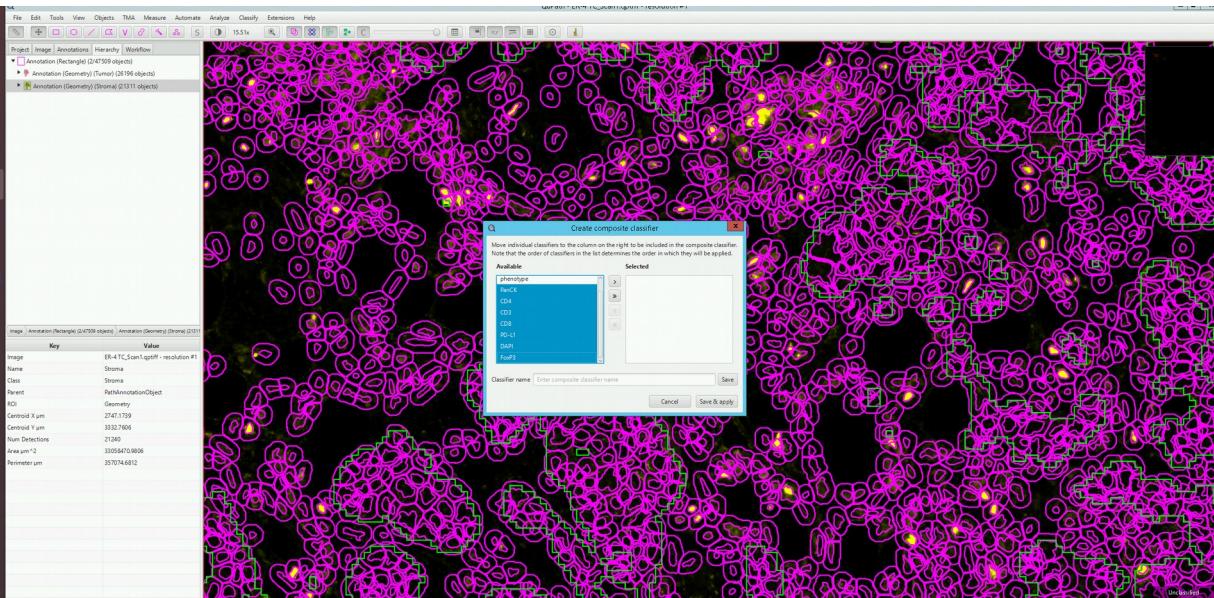
Classify > Object classification > Create single measurement classifier



Cell classification

- Composite classifier

Classify > Object classification > Create composite classifier

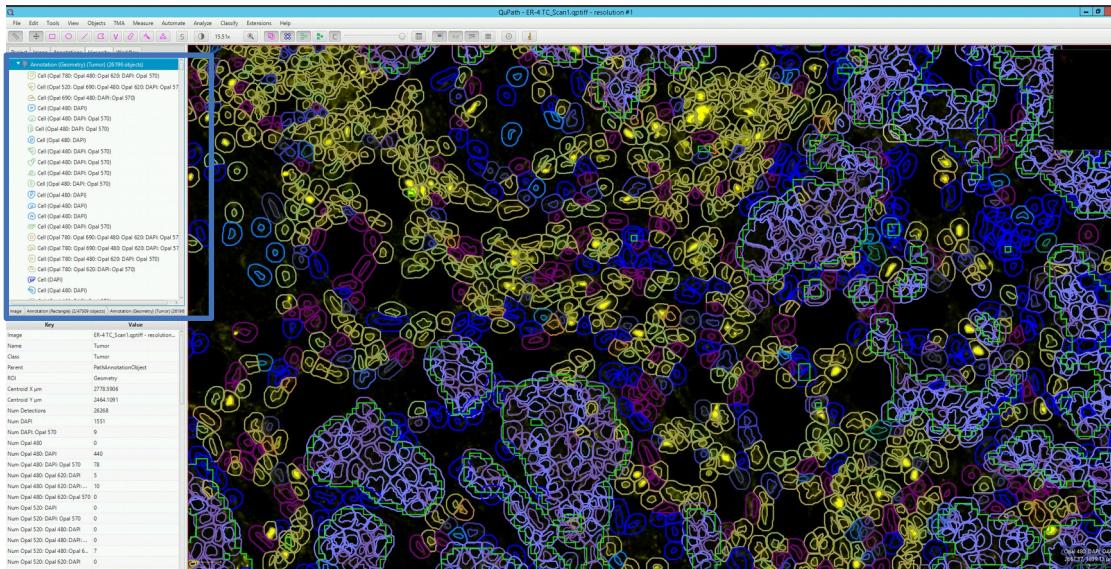


Define cell phenotypes

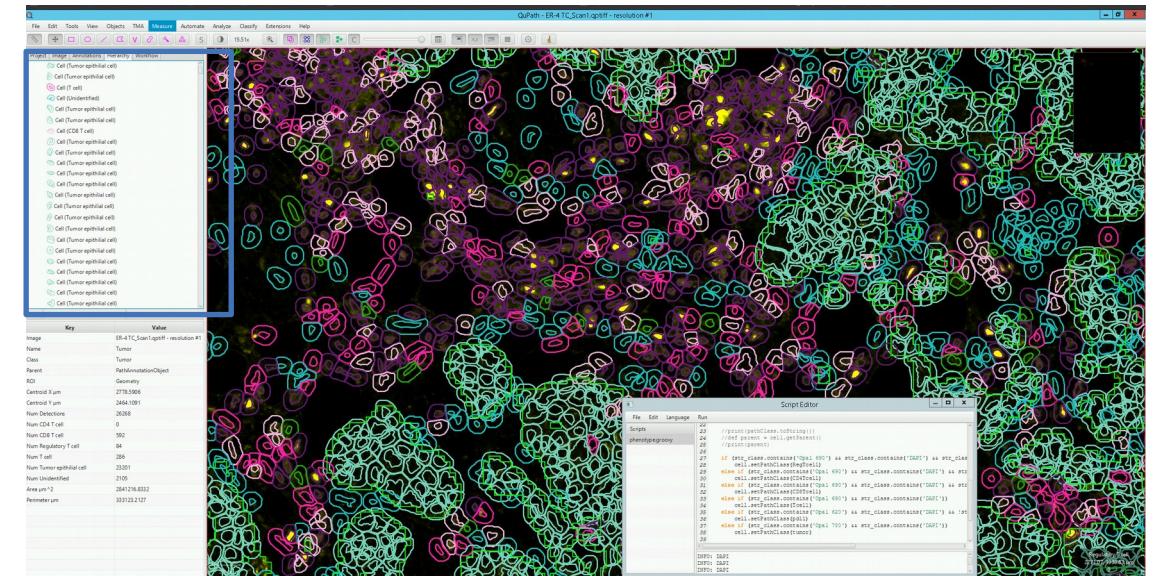


- Run `gui_phenotype.groovy`

Before running the script



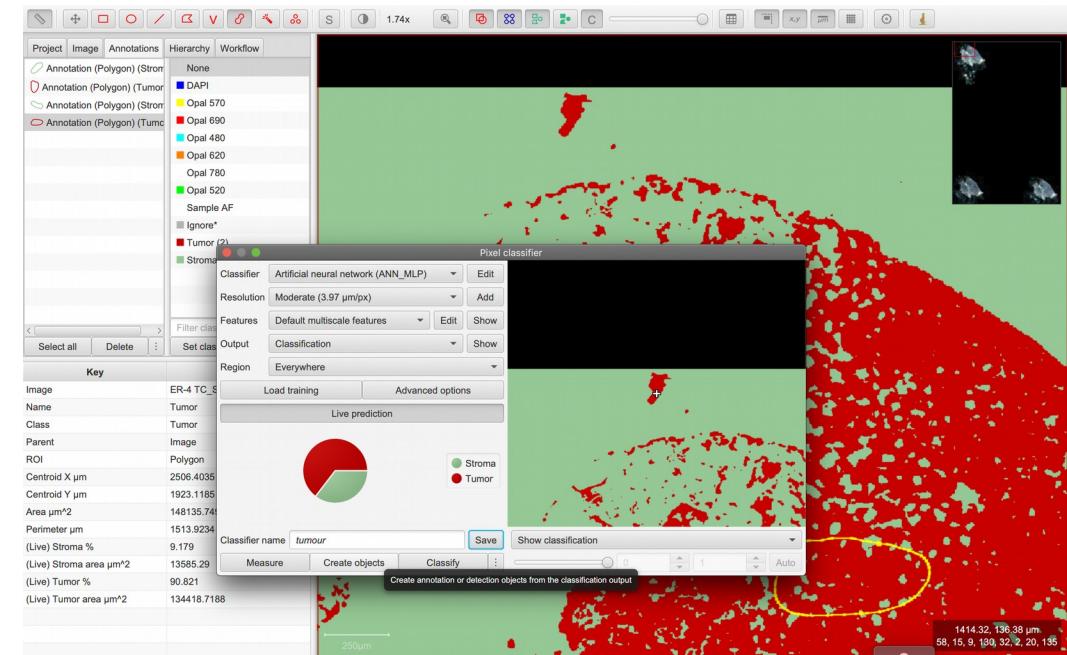
After running the script



Pixel classifier

- Uses machine learning
- 1) Annotate a few areas
 - 2) Run pixel classifier

Classify > Pixel classification > Train pixel classifier



Types of measurements

GENERAL

Automatically measured

- Area, perimeter, centroid, intensities etc.

SPATIAL

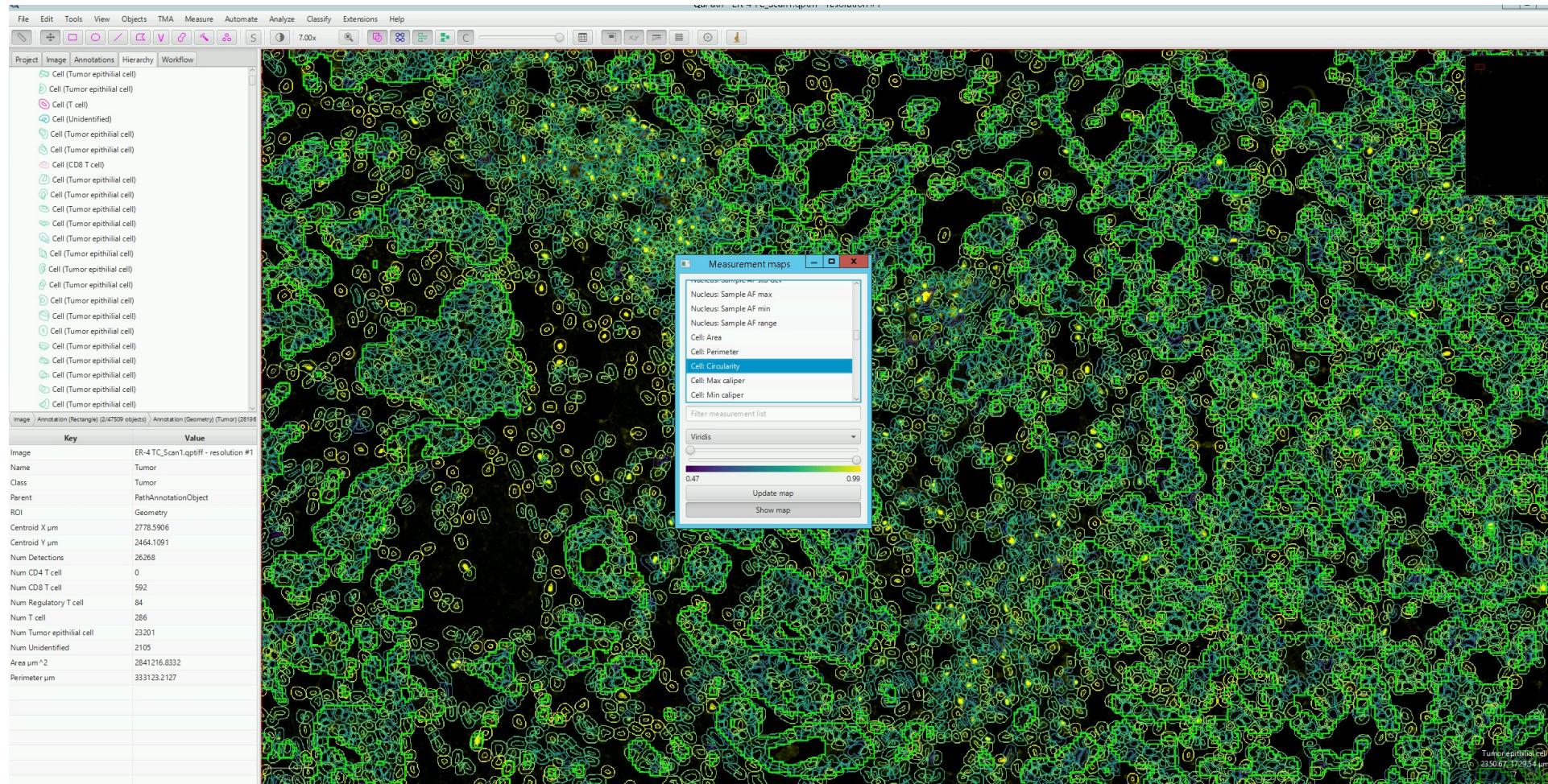
Analyze>Spatial analysis> Select the measurement that you want to perform

- Distance to annotation 2D: distances between detection centroids and the closest annotation for each classification (eg. distance from every cell to closest area of tumour)
- Detect centroid distance 2D: distances between detection centroids for each classification. (eg. this may be used to identify the closest cell of a specified type)
- Delaunay cluster features 2D: Delaunay triangulation to detect objects based on their centroid locations. This helps identify clusters of objects neighbouring one another.
- Other possibilities (scripting needed): infiltration, nearest neighbours etc

Measurements

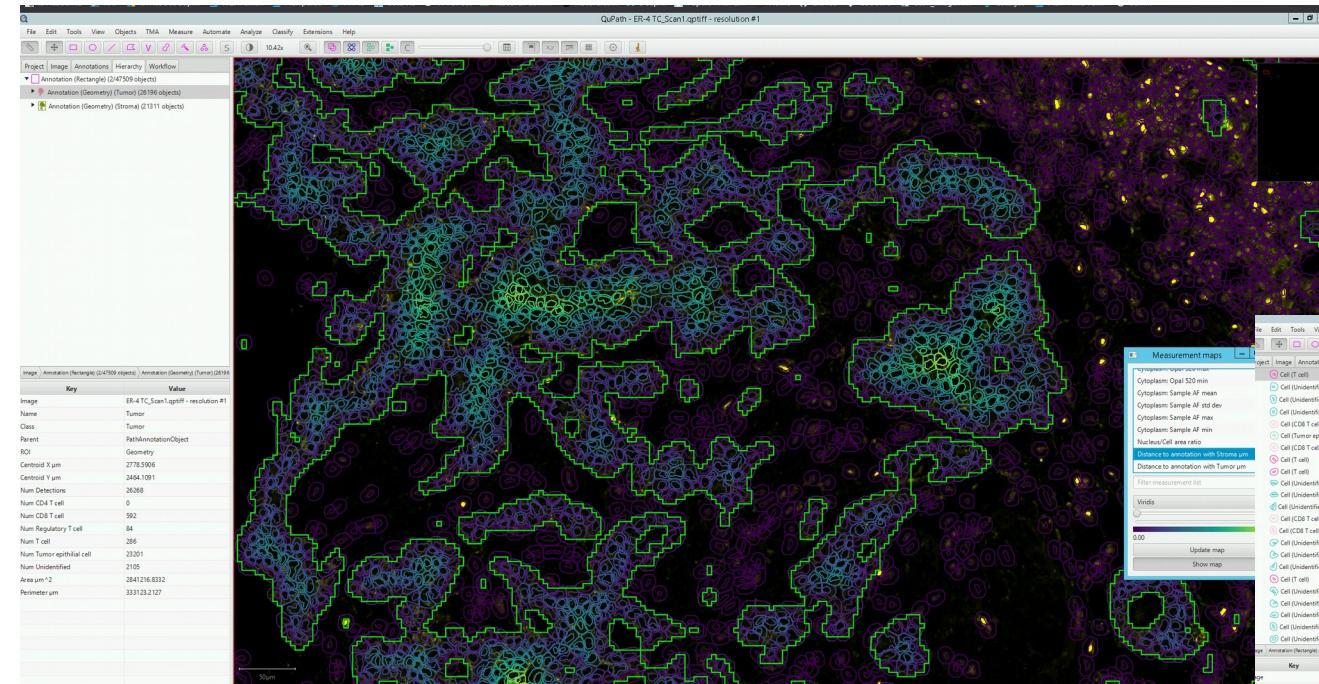
- Visualise measurements

Measure>Show measurement maps

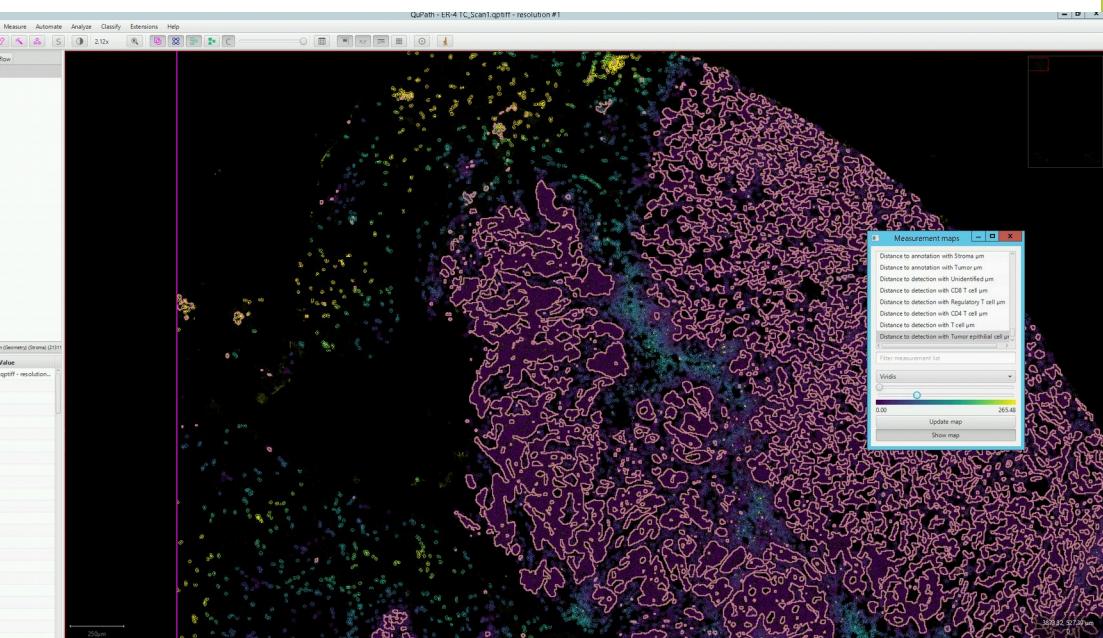


Examples

- Distance to tumour edge



- Distance to specific type of cell



Batch processing and scripting

Possibility to run custom analysis or do batch processing

Workflow > Create Script

User forum, a good source of scripts: <https://forum.image.sc/tag/qupath>

Examples



Advantages and drawbacks

	QuPath	HALO
Advantages	Better cell detection Possibility to add any custom analysis Free and accessible	User-friendly
Drawbacks	Need of scripts	Limited to modules: need to switch to R more advanced analysis Only one license

Thank you!



Thank you!