Multiplexed data analysis using QuPath

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github.com/ninatubau/QuPath_scripts

Today's planning

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

Live demo

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

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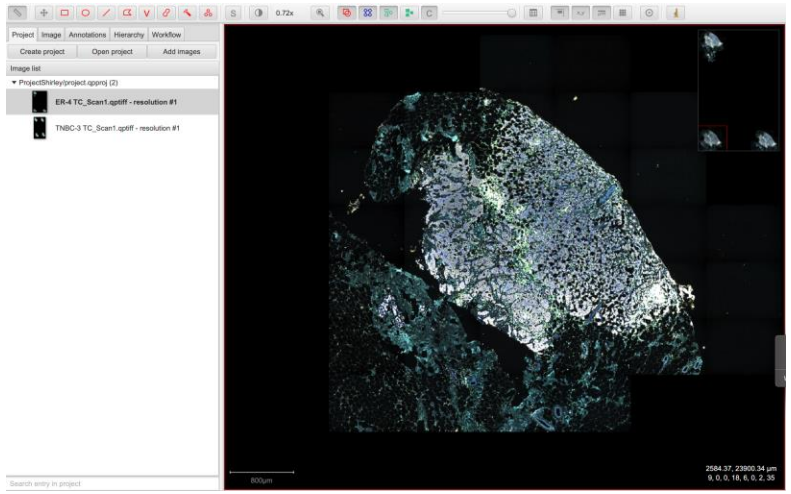
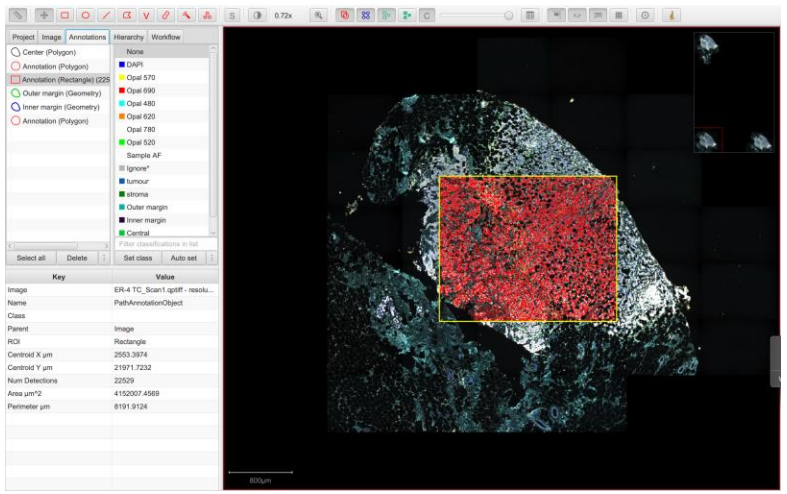
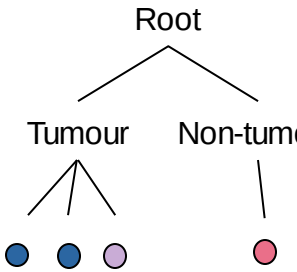
What is QuPath?

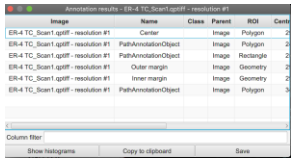
- Open source for bioimage analysis
- Powerful annotation and visualisation tools
- Built-in algorithms for common tasks
- Interactive machine learning
- Compatibility
- Support for many file formats
- Scripting for deeper data interrogation

QuPath approach









Start with pixels

Billions of numbers

Identify objects

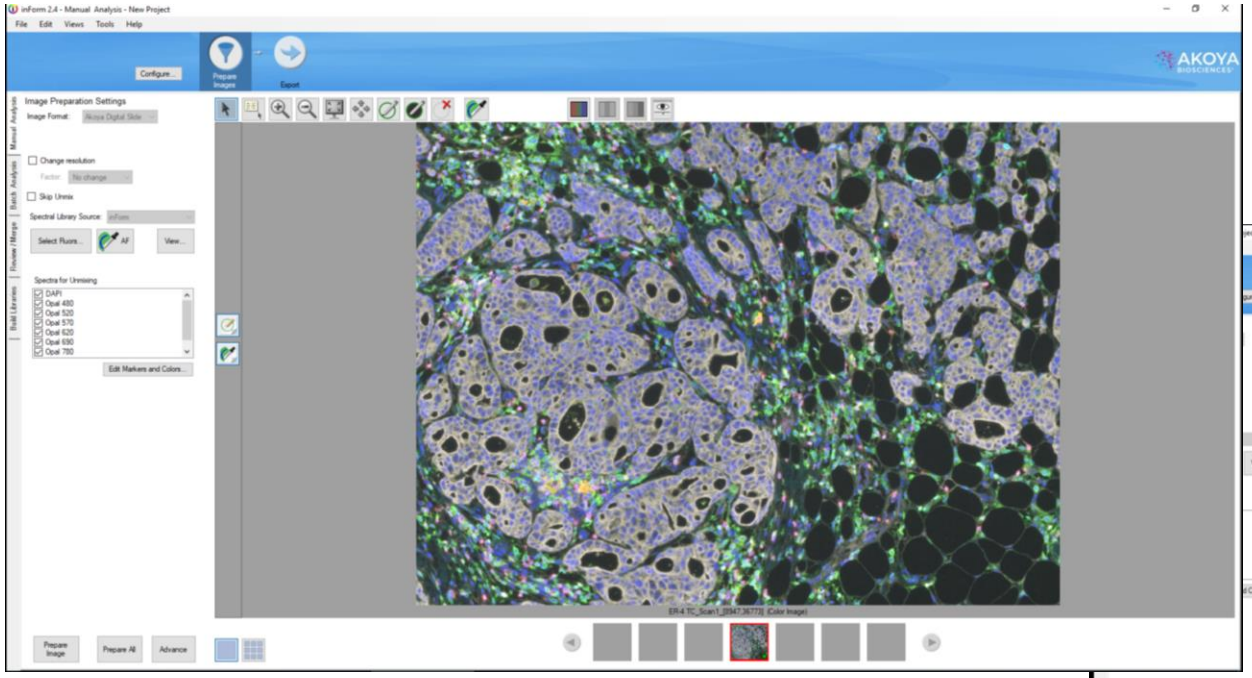
Cells, structures, regions…

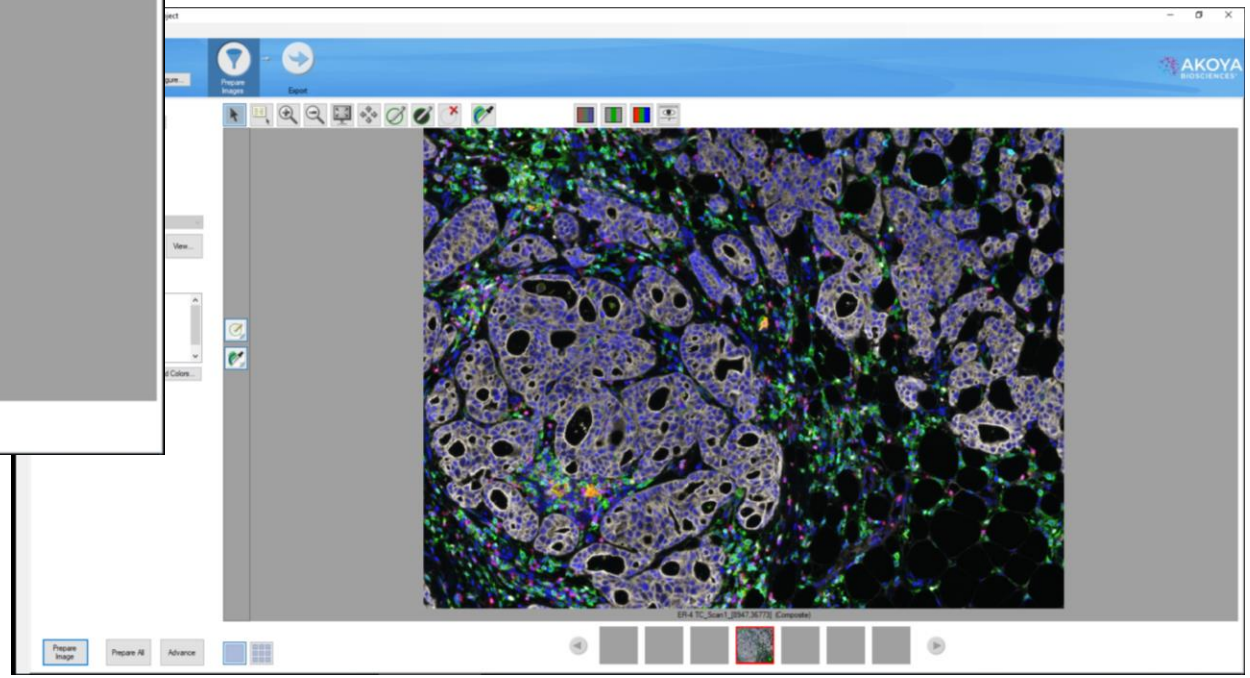
Query the objects

Shape, size, number, intensity, location, classification

Pre-processing

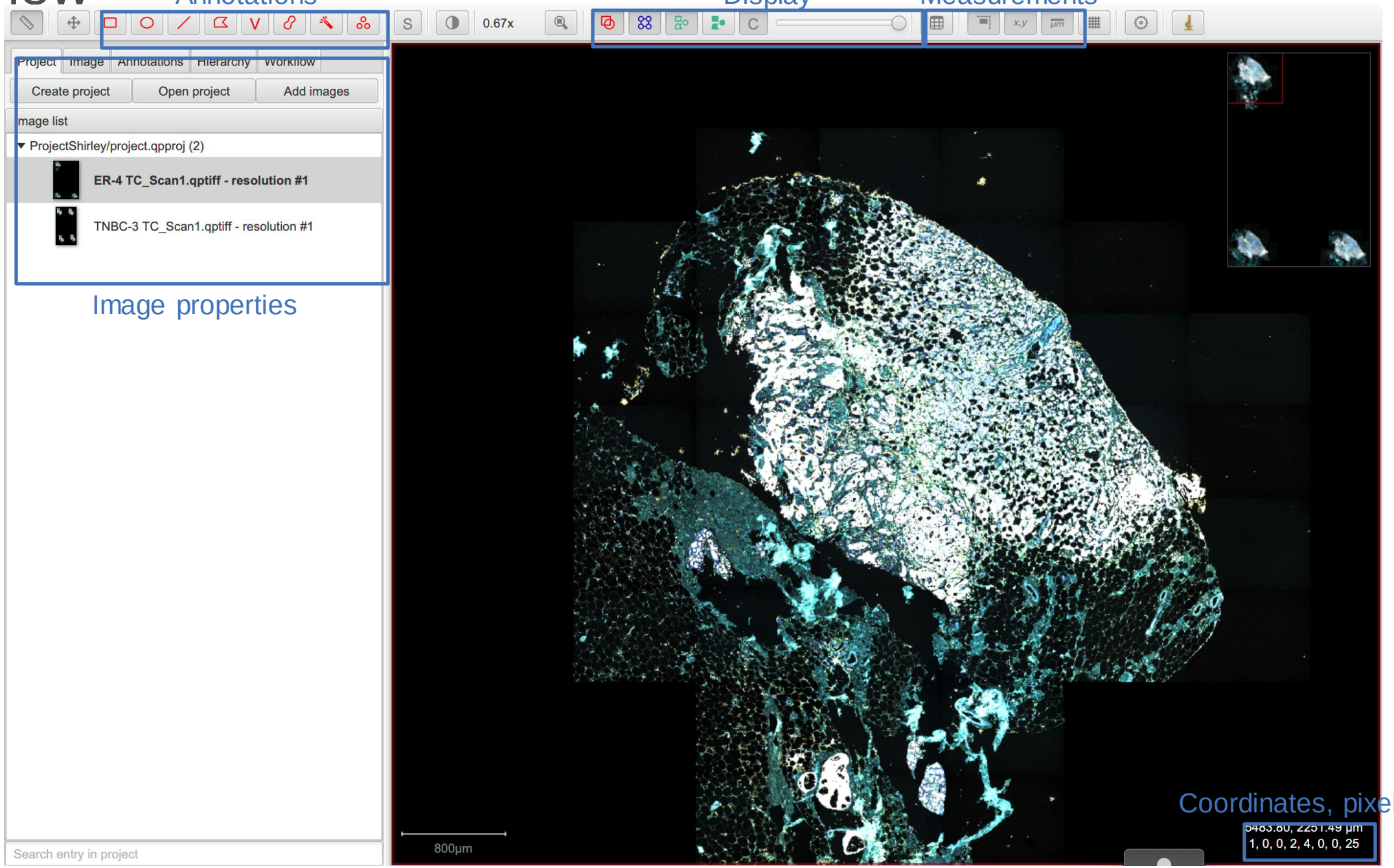
- Phenochart and InForm: Unmix fluorophores and autofluorescence





Let’s get started !

Overview


Annotations
Display
Measurements
0.67x
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
800µm
Coordinates, pixel values
1, 0, 0, 2, 4, 0, 0, 25

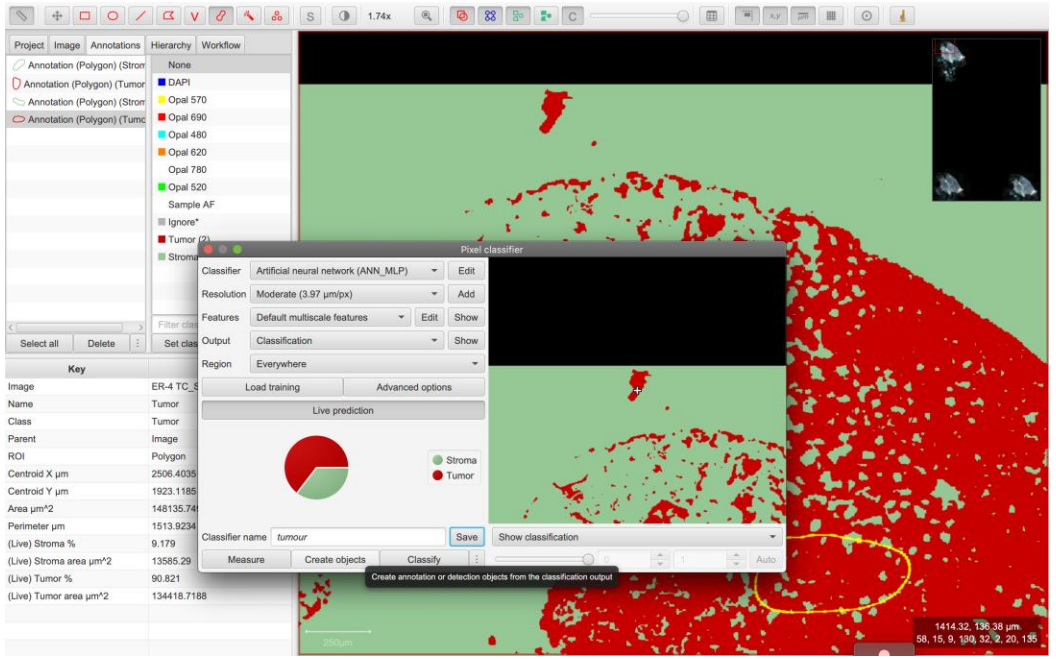
Pixel classifier

- Uses machine learning

1) Annotate a few areas

2) Run pixel classifier

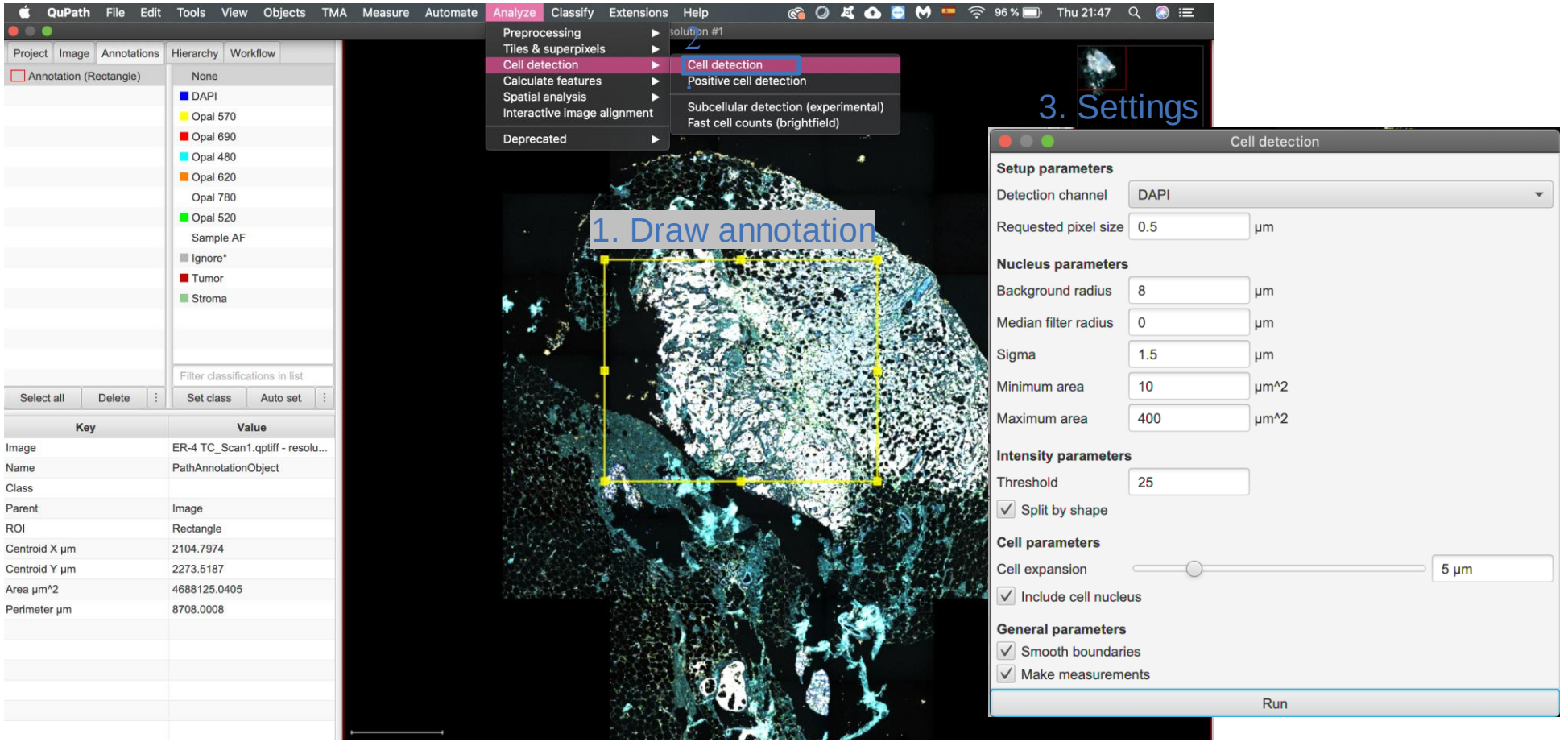
Classify > Pixel classification > Train pixel classifier



Cell detection

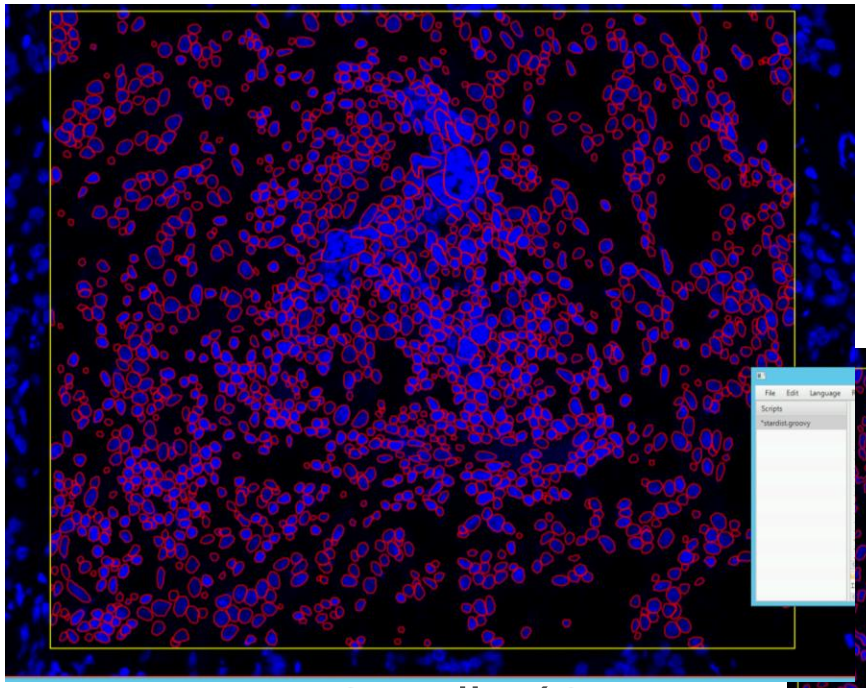
- Watershed

Analyze > Cell detection > Cell detection



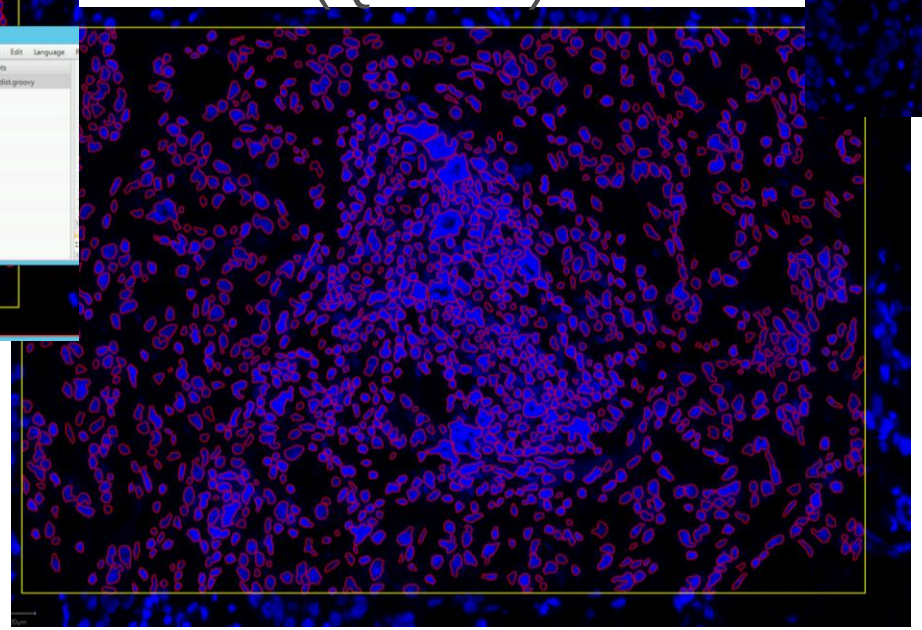
Cell detection

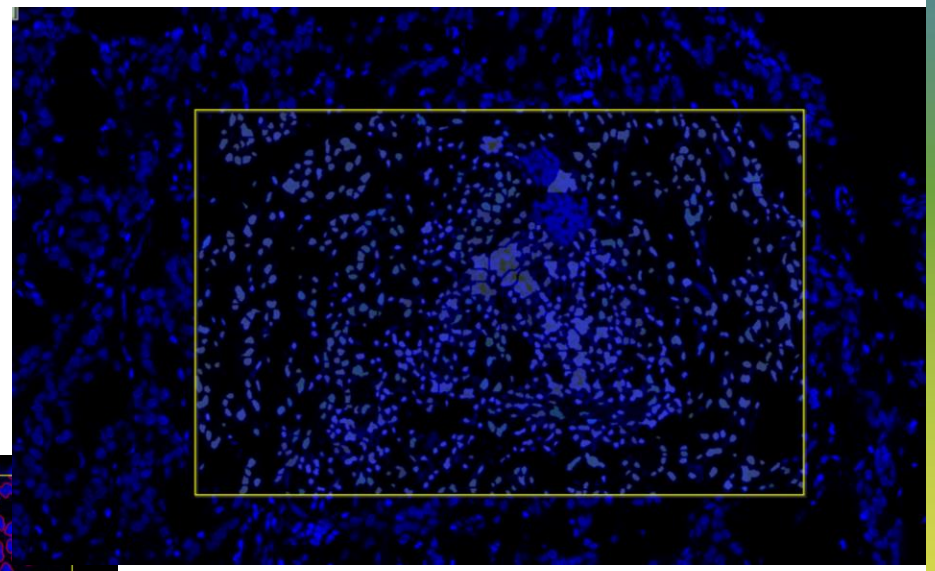
- StarDist (run stardist.groovy)



Stardist(QuPath)

Watershed (QuPath)



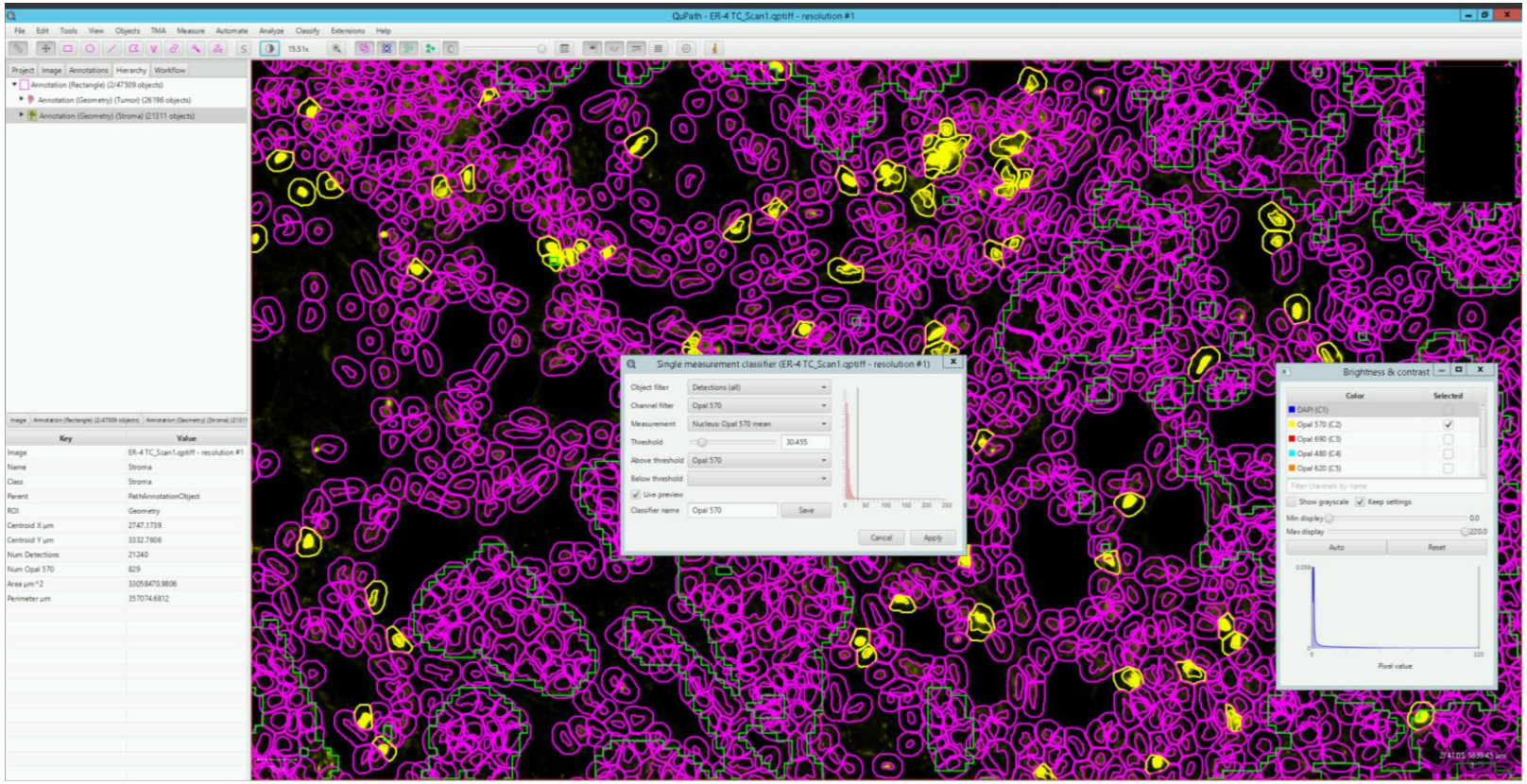


HALO

Cell classification

- Single measurement classifier

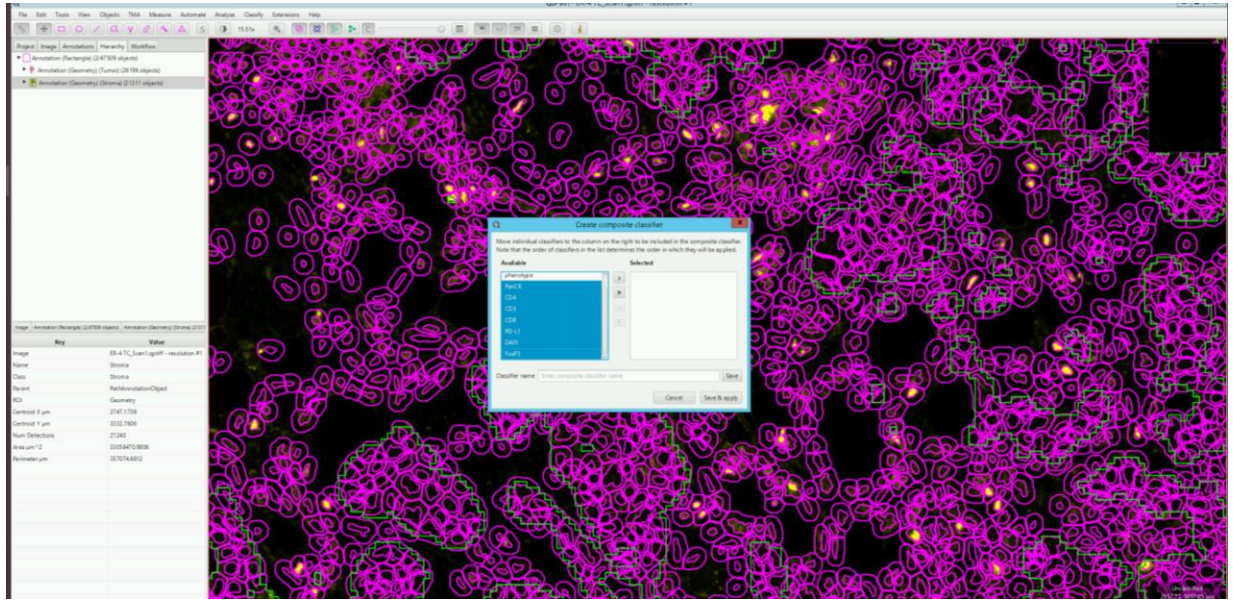
Classify > Object classification > Create single measurement classifier

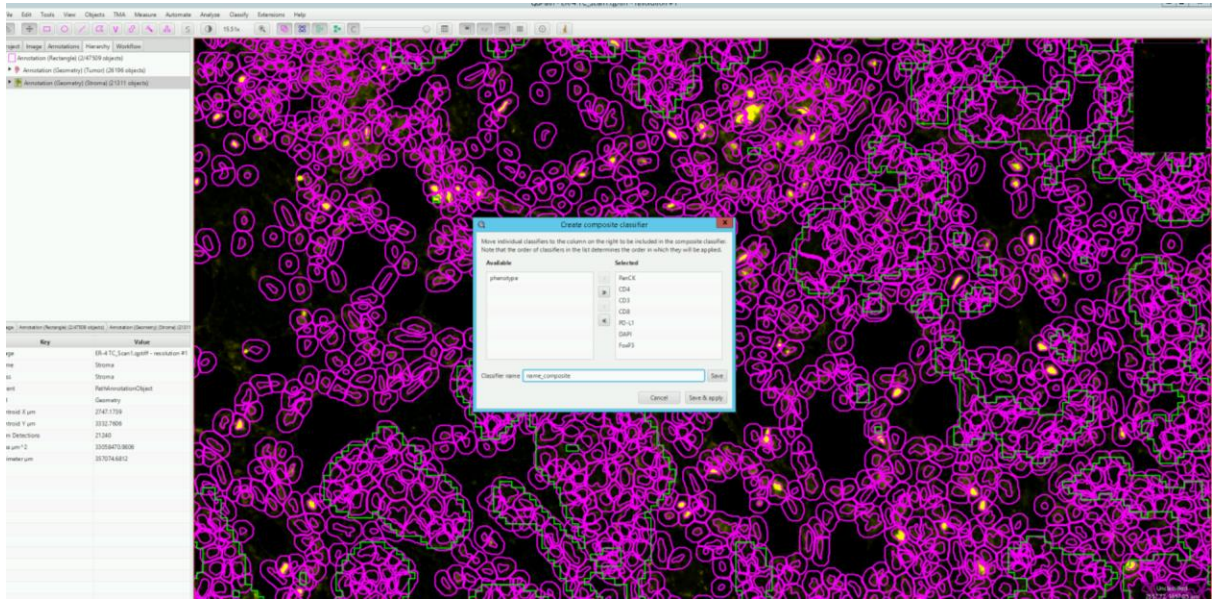


Cell classification

- Composite classifier

Classify > Object classification > Create composite classifier

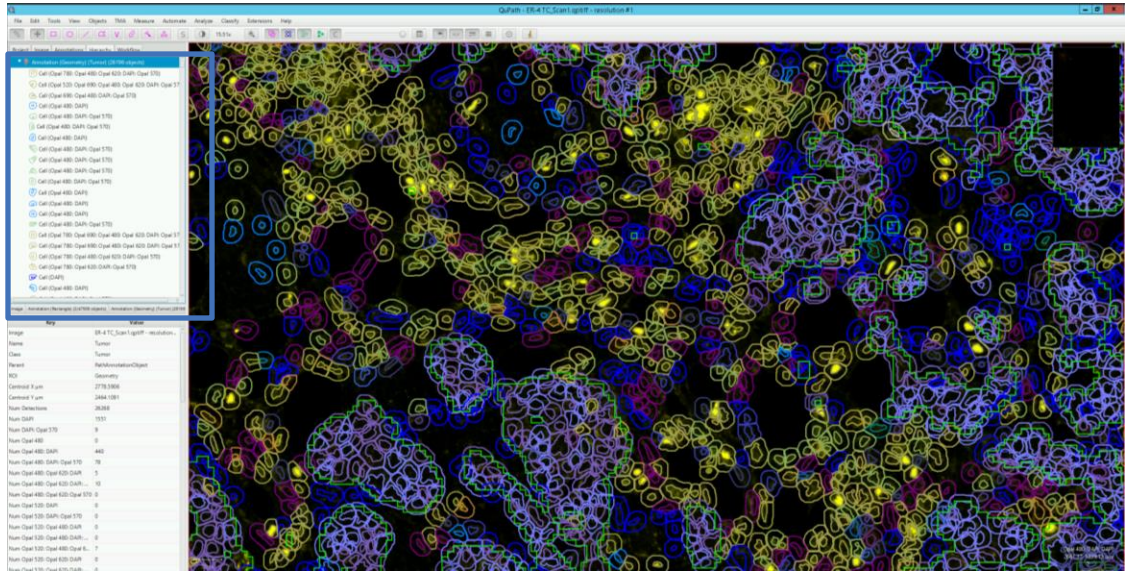




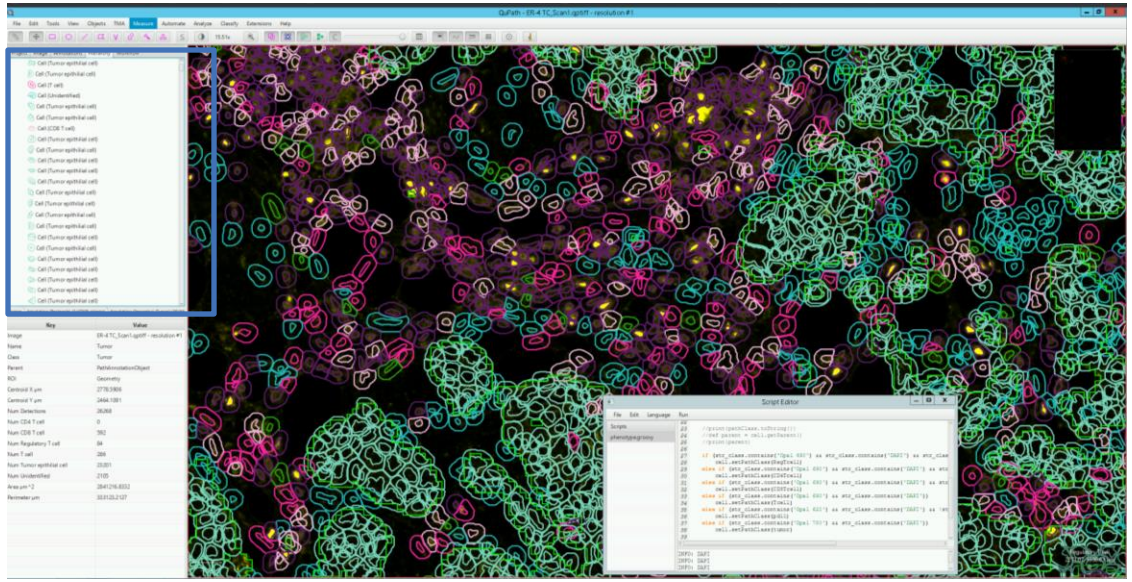
Define cell phenotypes

- *Run gui_phenotype.groovy*

Before running the script



After running the script



Types of measurements

GENERAL

Automatically measured

- Area, perimeter, centroid, intensities etc.

SPATIAL

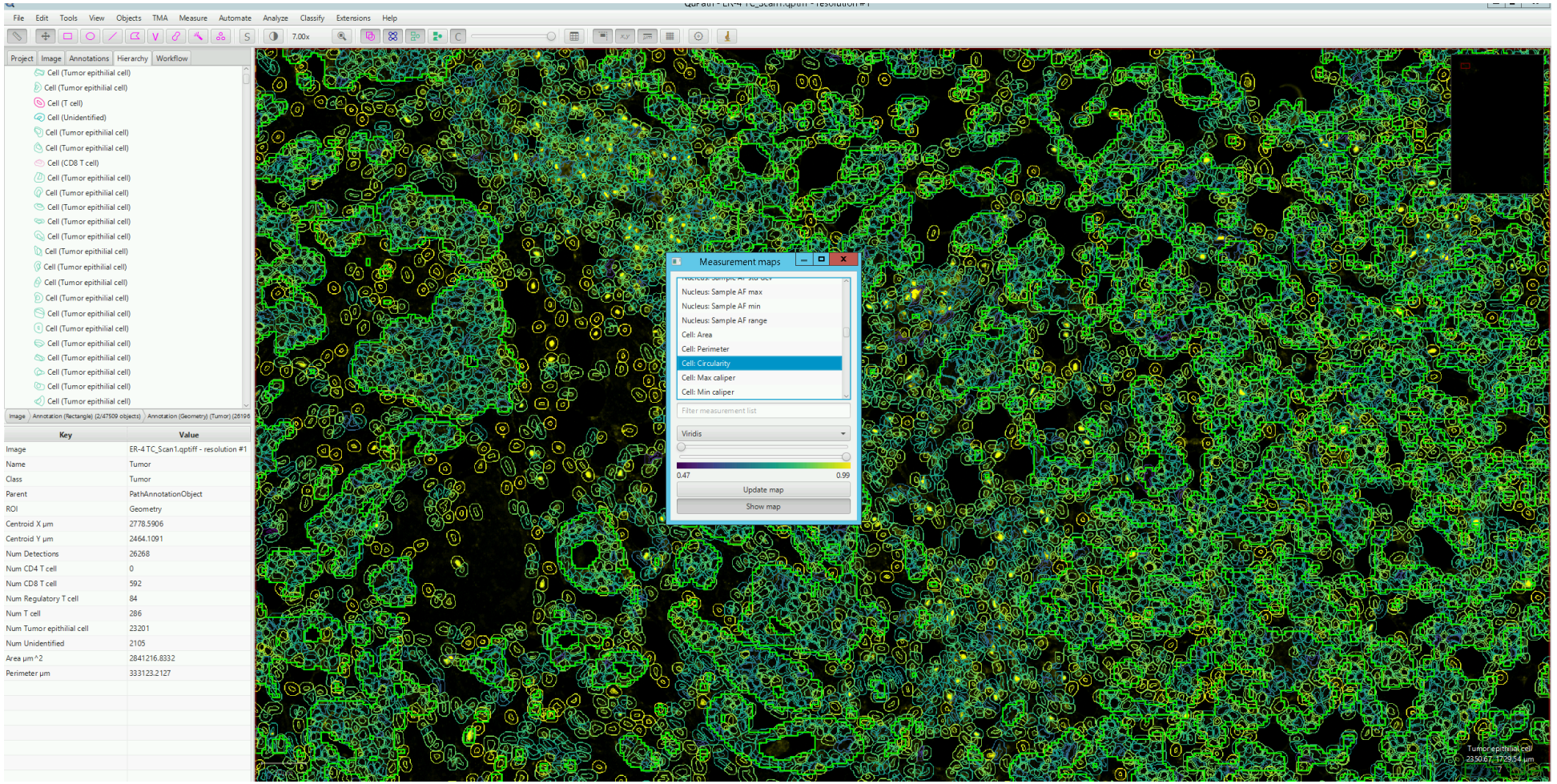
Analyze>Spatial analysis> Select the measurement that you want to perform

- 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

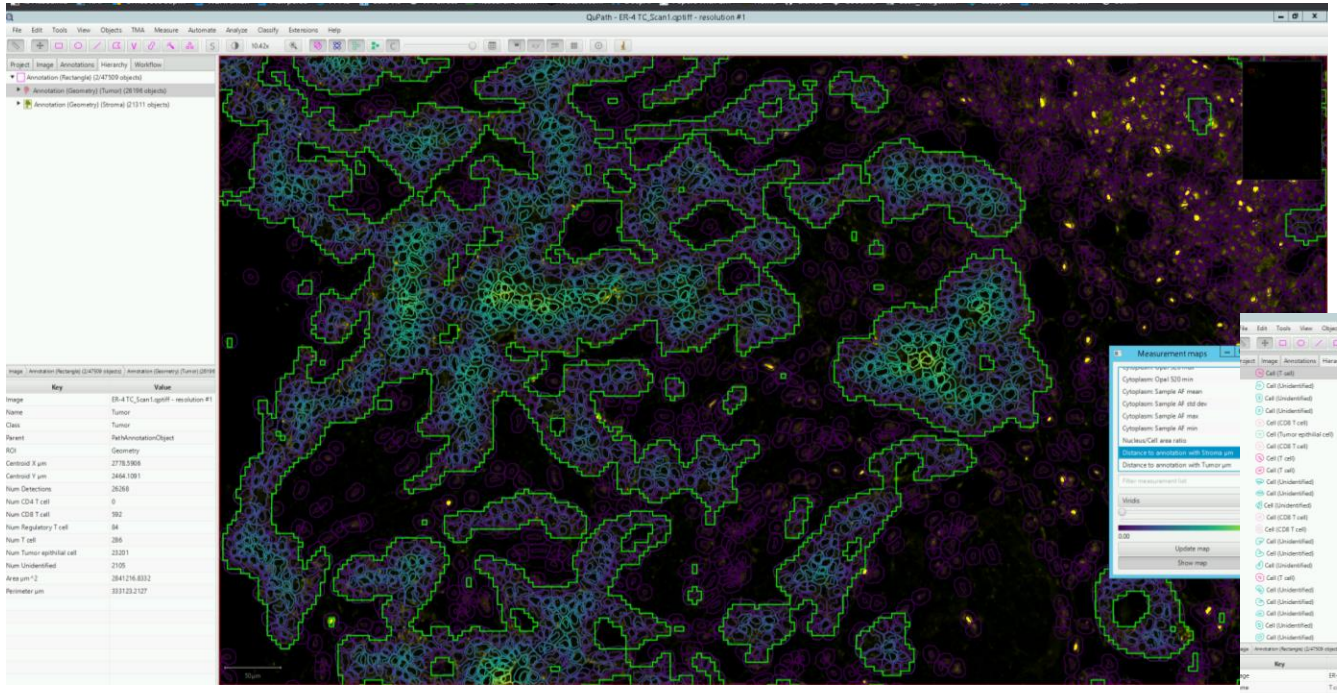
- Visualise measurements

Measure>Show measurement maps

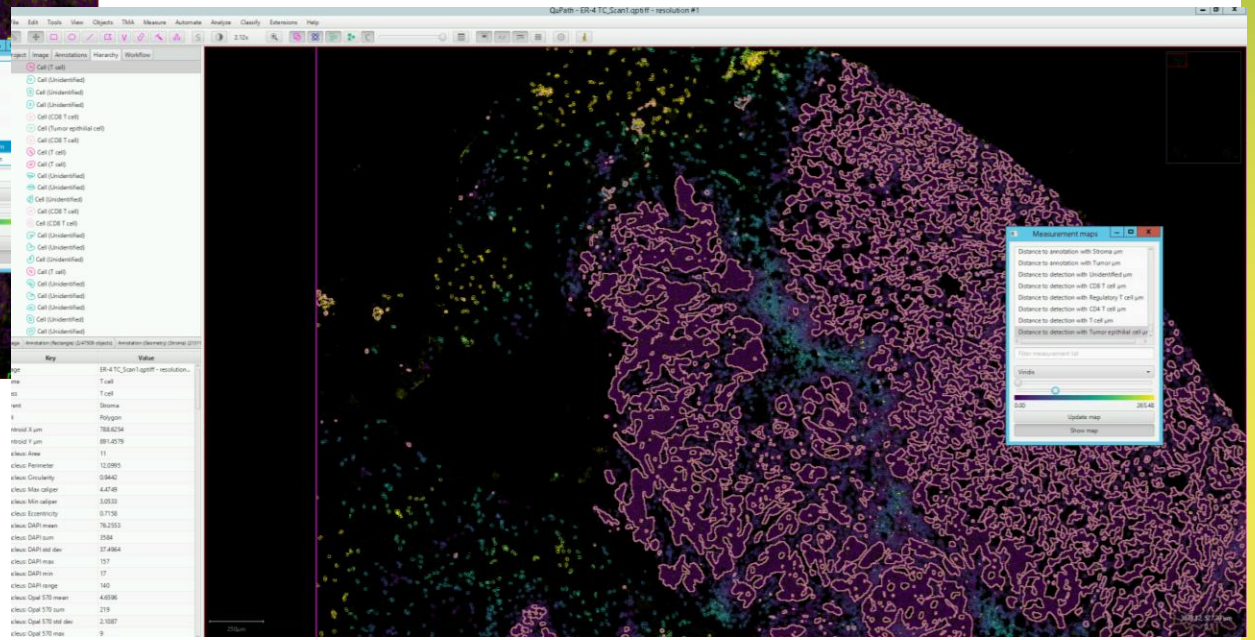


Examples

- 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

github.com/ninatubau/QuPath_scripts

Advantages and drawbacks

	QuPath	HALO
Advantages	Free and accessible Possibility to add any custom analysis	User-friendly
Drawbacks	Use of scripts	Limited to modules Only one license

Thank you!