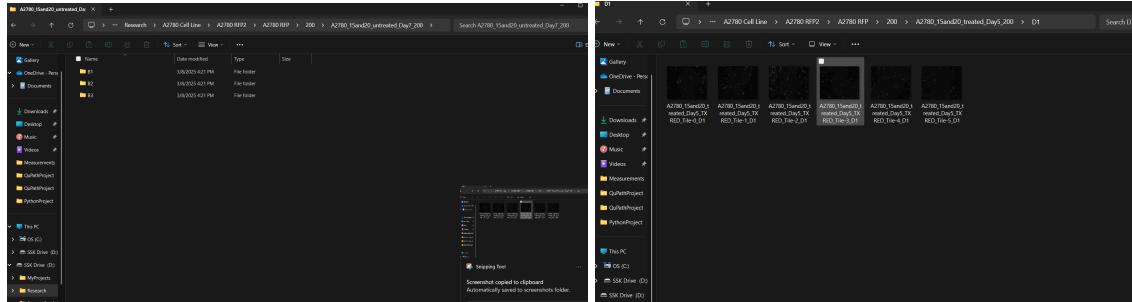


1. First step is having all your photos downloaded to your computer.

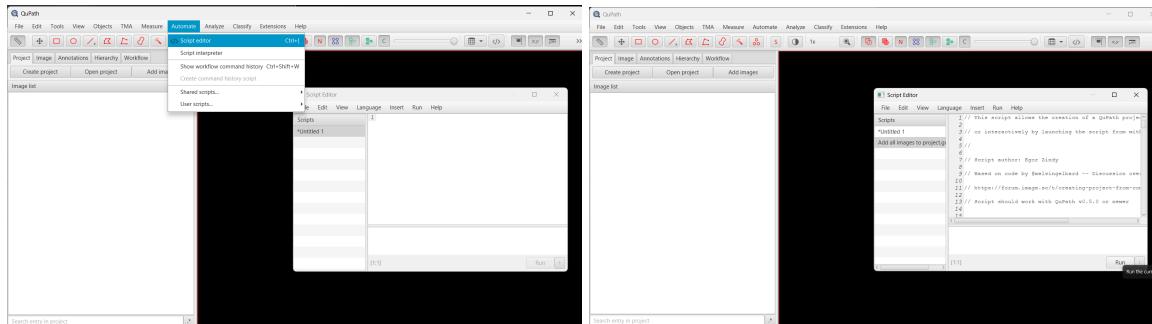
2. You need to remove all non-tiff files from your images folder. ONLY USE THESE FILES IF YOU UNDERSTAND WHAT THEY ARE SUPPOSED TO DO, TEST ON A FOLDER DUPLICATE.

The RemoveCrap.py contains two functions within it. One removes all non-tiff and unneeded wells files from a directory and the other removes all images with even number endings.

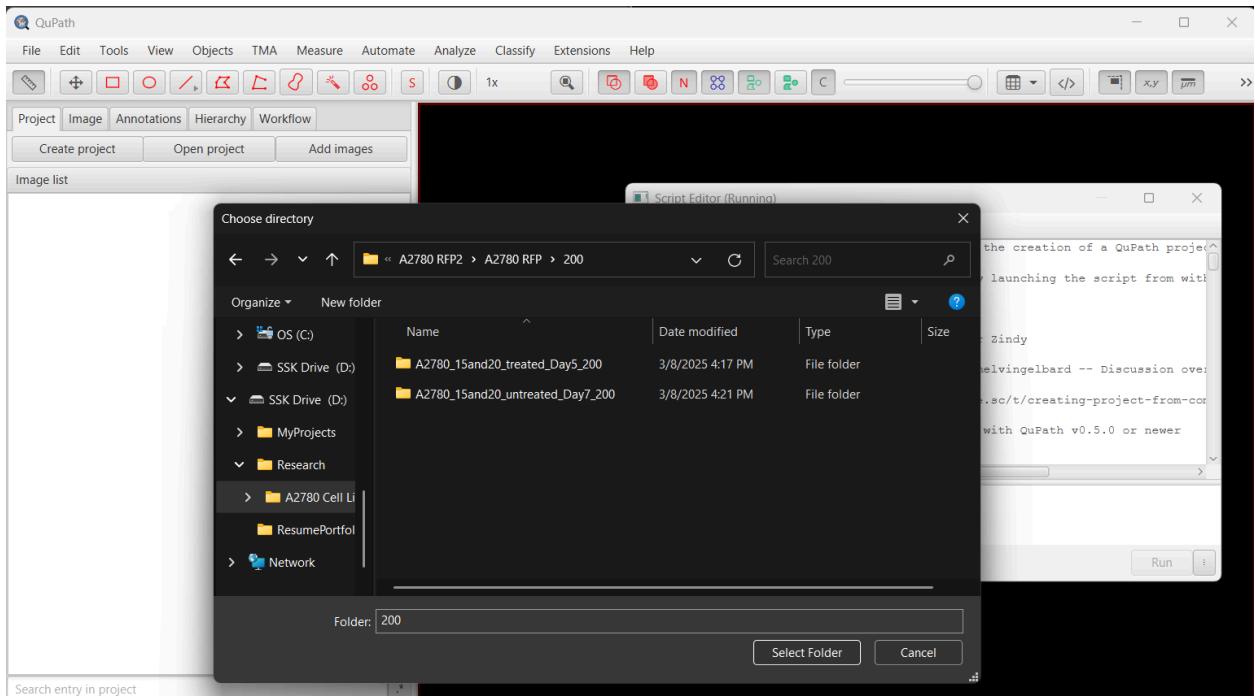


3. Open QuPath and click automate, (No need to open a project). Before starting this please download "Add all images to project.groovy".

- a) Please select script editor
- b) Please select open script from file
- c) Please select the "Add all images to file script"



d) Click run and when prompted select the outermost directory in which you plan to run analysis upon a batch (I.E. I have an images folder but within that I will run to separate analysis types upon resistant and sensitive cells. I should go one directory further and select the resistant folder).

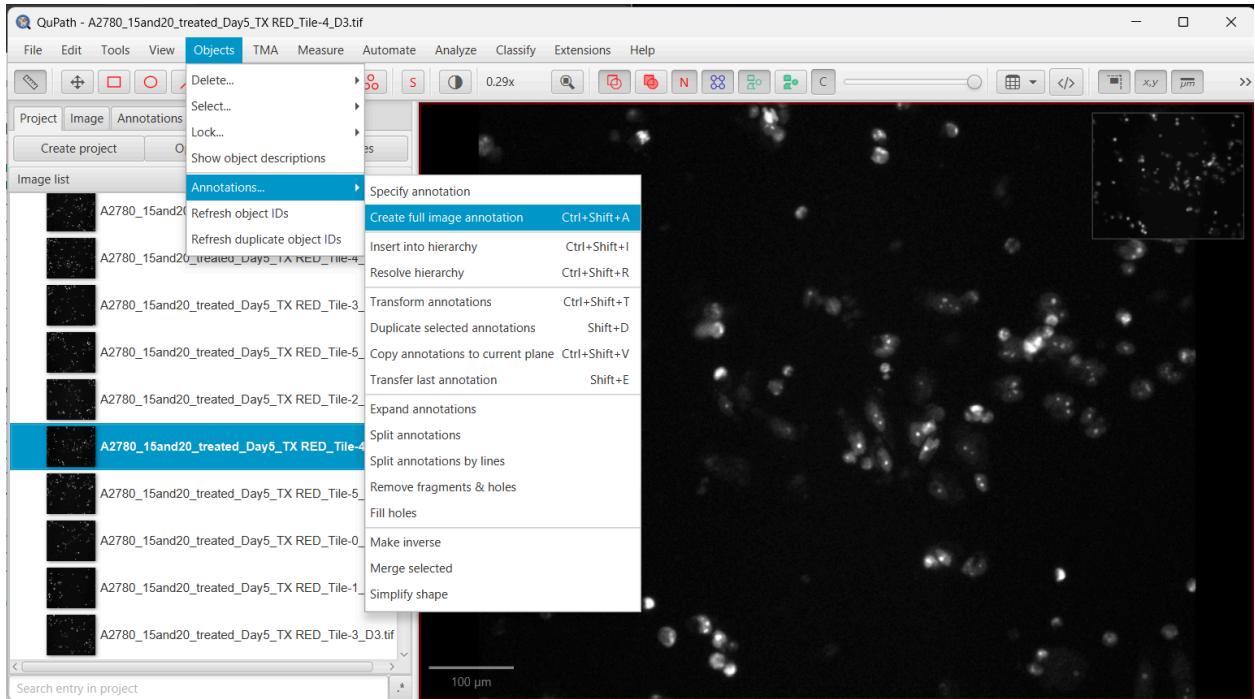


- e) Wait roughly 1 minute per 100 photos
- f) Now minimize the script editor and click back to QuPath main editor.
- g) Click file and open project and then navigate and open the previous folder you selected. You should see a new QuPath folder. Click this and then select the project file (It has a unique extension).

4. TWO OPTIONS: OPTION ONE IS FOR CELL AREA WHILE OPTION 2 IS FOR CELL COUNTING INDIVIDUALLY. So we are going to be creating a threshold and then running our workflow as an automation script.

OPTION 1

- a) First hover click Objects -> Create full image annotation



b) Next go to Classify -> Pixel Classification -> Create Threshold

c) A new window shows up. Adjust the settings as follow:

Resolution; Very High

Channel; Channel 1

Prefilter; Gaussian

Smoothing sigma; 0.5

Threshold; Please choose a threshold that highlights most of your cells. An overlay should display this. If images were done properly 4000 should do the trick.

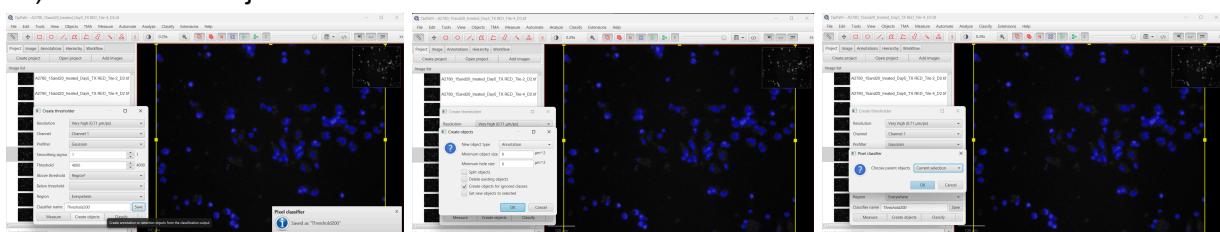
Above threshold; Region

Below threshold; Blank

Region; Everywhere

Classifier Name; "INSERT APPROPRIATE NAME" MAKE SURE TO CLICK SAVE

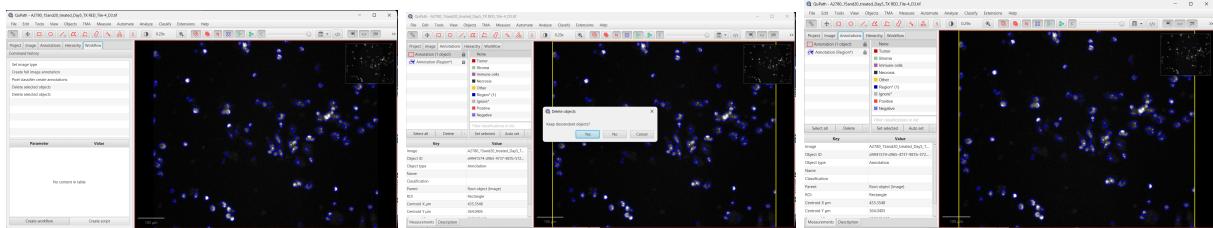
d) Click create object



e) Navigate to annotations and click select annotation, delete it with backspace, Make sure to KEEP dependencies. If you do not see an extra annotation just skip this step.

f) Navigate to workflow -> Create script

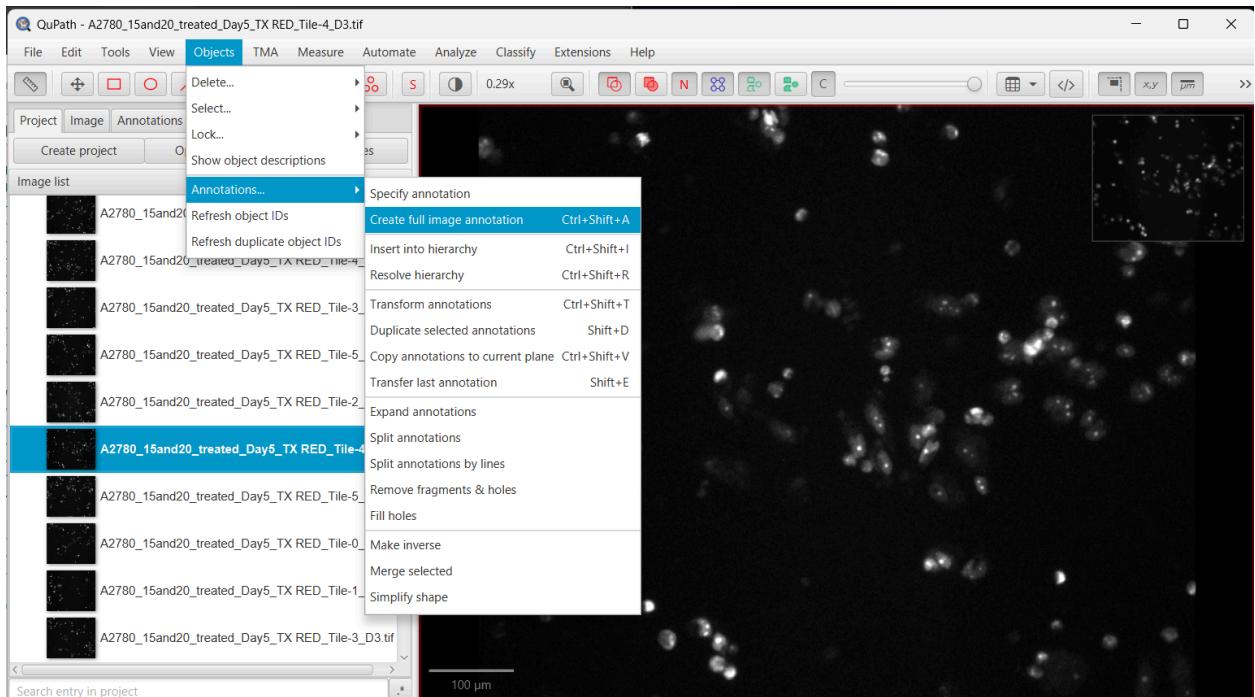
g) Click the three buttons in the bottom right of the script editor.



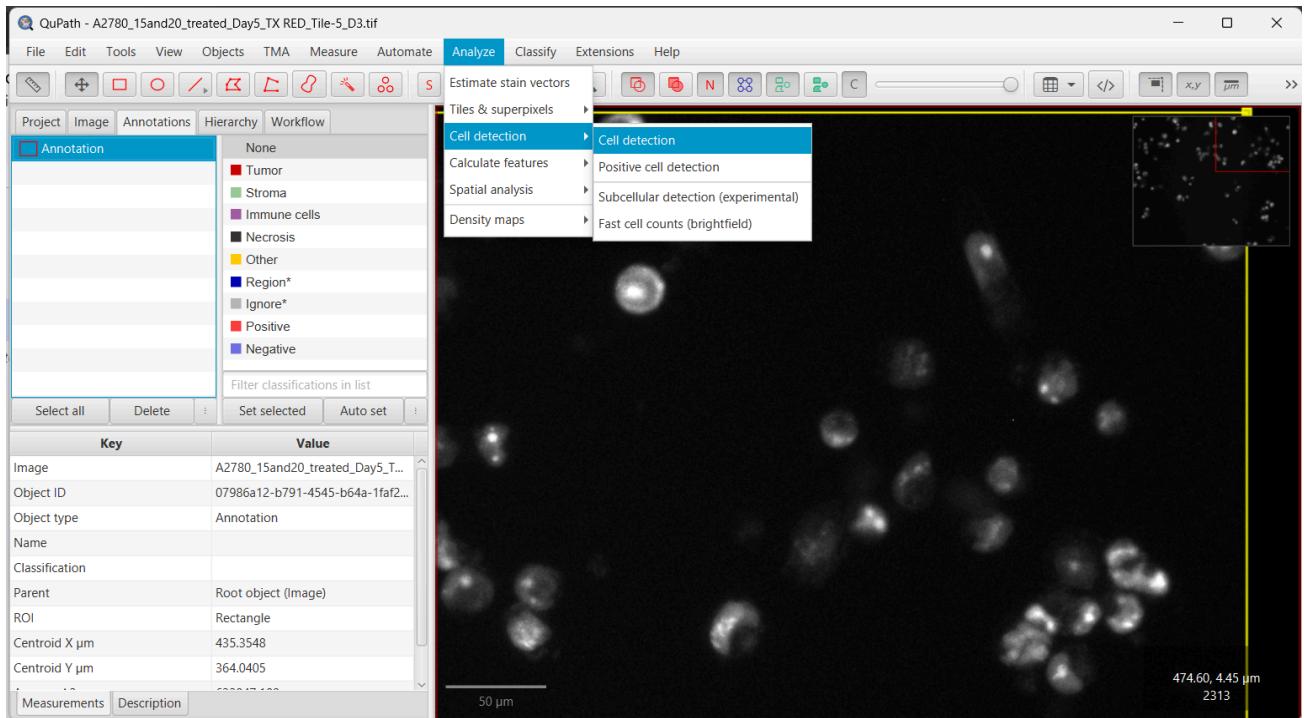
h) Click run for project.

OPTION 2

a) First hover click Objects -> Create full image annotation



b) Please navigate to Analyze and click cell detection



c) Then fix the settings to be:

Background radius: 8-35 (Higher numbers with more clustering)

Median filter radius: 1

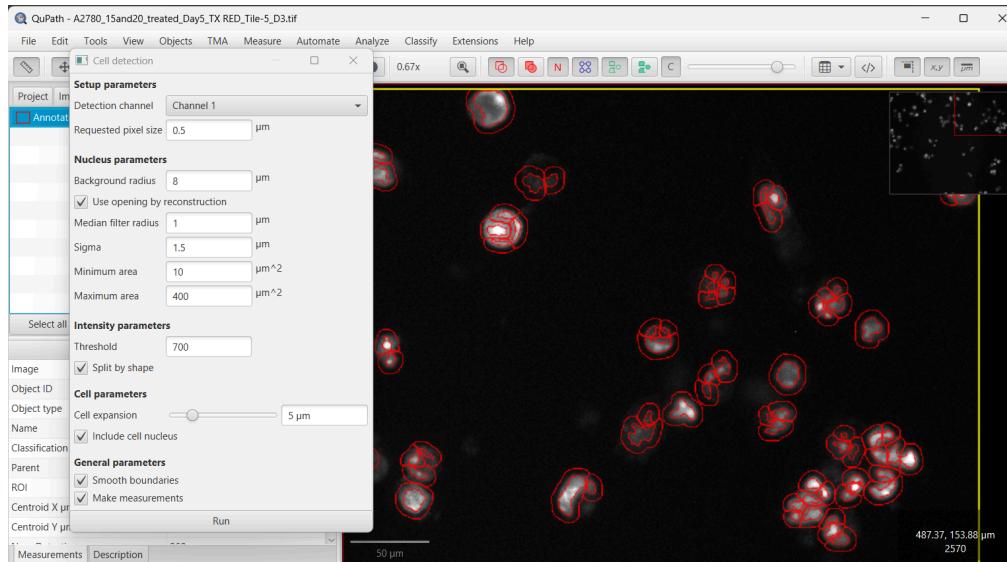
Sigma: 1

Minimum Area: 10

Maximum Area: 400

Threshold: 700

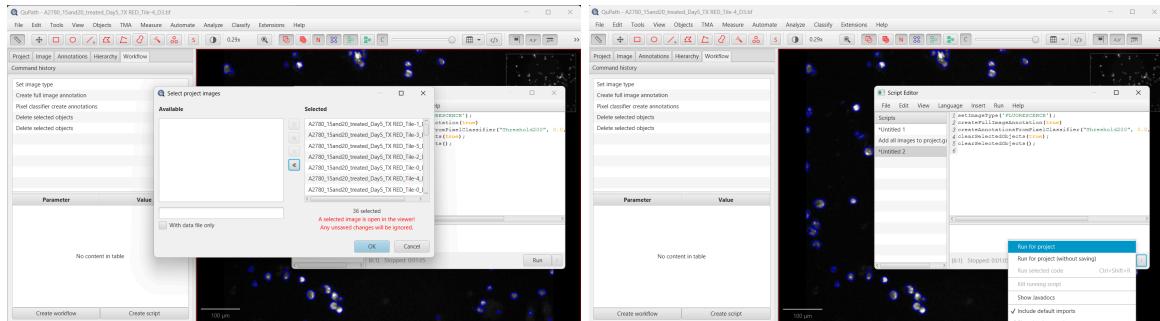
Check Split by Shape



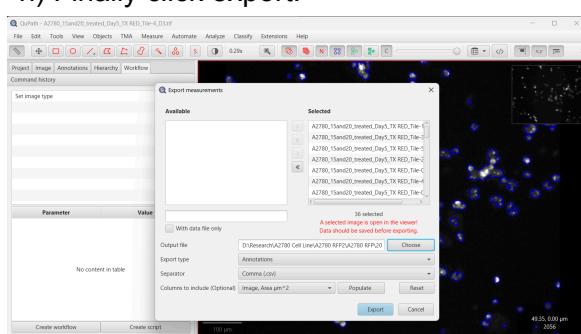
d) YOU CAN SKIP STEP 7!!!!

5. Now it is time to export our results

- a) Please hover click measure -> export measurements
- b) Click the double arrow in the middle of the "Available" and "Selected" windows.



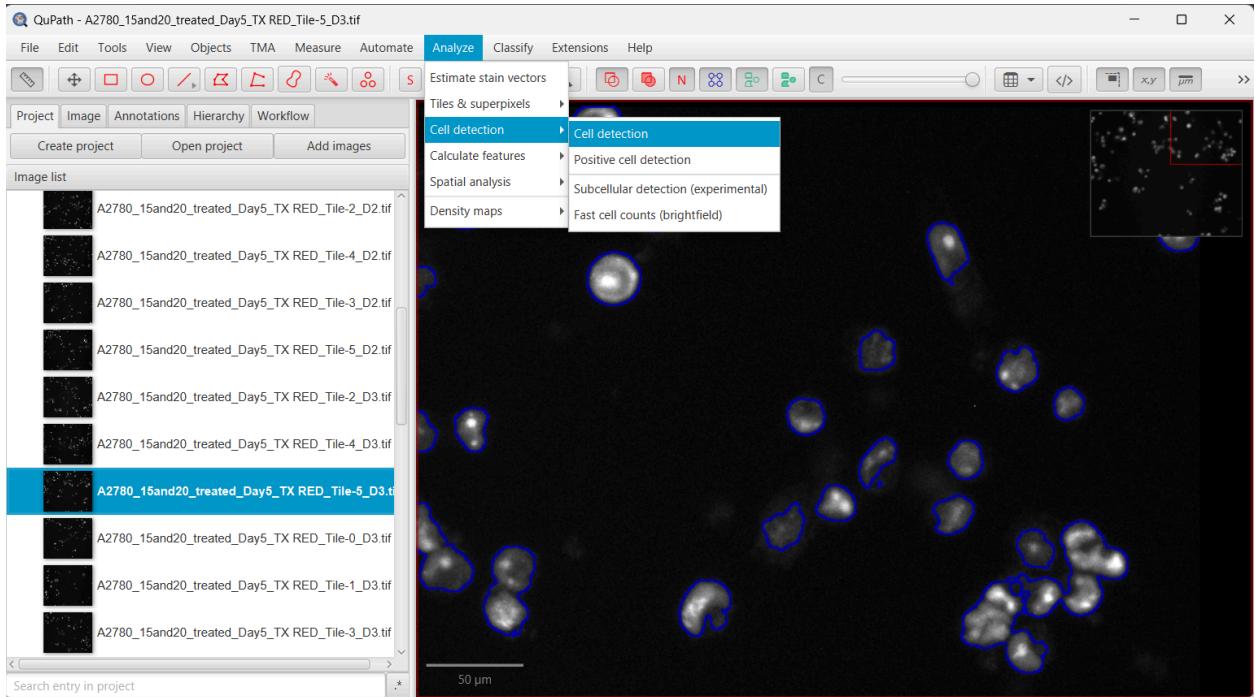
- c) Click image dropdown next to export type. Ensure annotations is clicked instead.
- d) Click on Tab (.tsv) next to Separator. Ensure Comma (.csv) is clicked instead.
- e) Select choose to see what the file will be saved as and where it will be saved. Feel free to edit these if you have high computer literacy (You know your way around your directories).
- f) Click Populate. This will take a moment to load.
- g) Now click "image" and "area" and any other columns you may want.
- h) Finally click export.



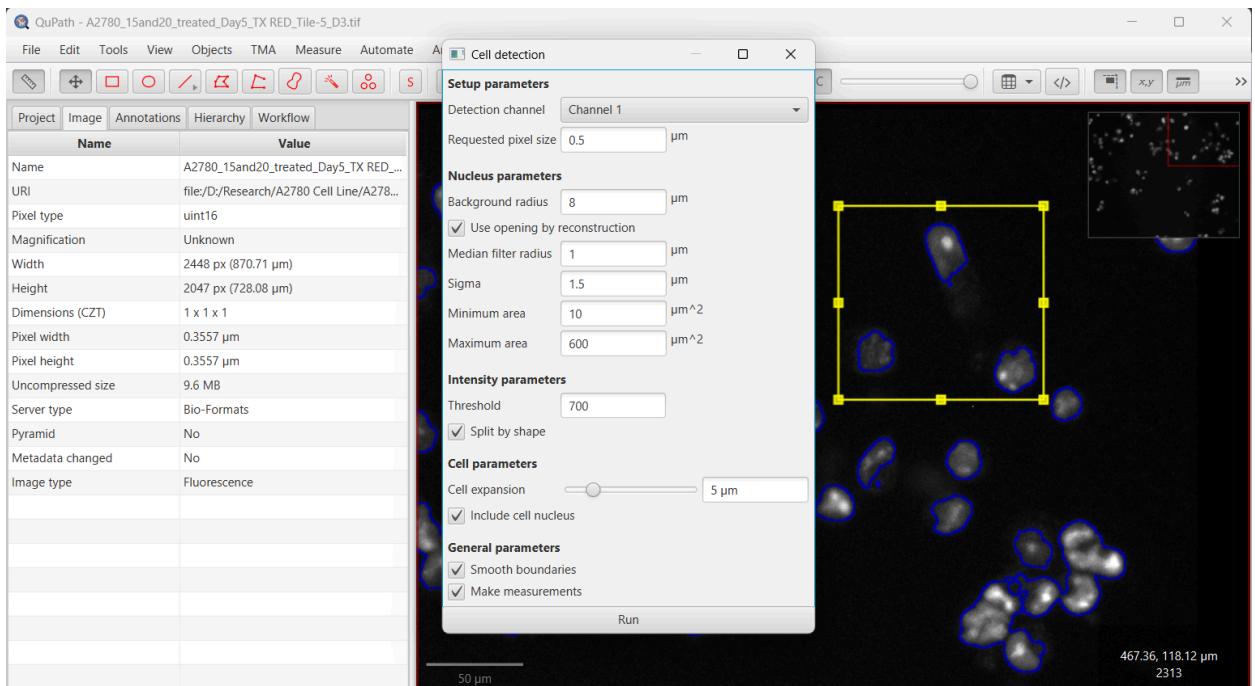
NOTE: YOU WILL NEED TO USE EXCEL, PYTHON, or JULIA to divide area by cell size.

7. HOW TO FIND CELL SIZE

- a) Find an area of an image with decent unclumped area.



- b) Find the rectangle tool and select given area.
 c) Click hover Analyze -> Cell Detection -> Cell Detection
 Change Background radius to 15
 Median filter radius at 0.5
 Sigma to 0.5
 Threshold at 400+



d) Click Run

- e) Navigate to annotations and view bottom left corner. There is a small scroll in which you need to go to bottom of.
- f) Find "Num Detections" and "Area um²"
- g) Divide "Area um²" by "Num Detections" to get an average cell size.

8. Filling in the rest of your data columns. THIS IS ONLY FOR AUTOMATICALLY FILLING ROWS. YET AGAIN BEWARE OF USING FILE IF YOU HAVE LITTLE CODING KNOWLEDGE.

OPEN pycharm or your favorite editors and open the A2780 reorder file. In it has comments to tell you how to alter the code with your cell size. There are also comments to tell you what will be added in the columns. Feel free to manually add this content if you so wish.

HOORAY YOU HAVE YOUR DATA!!!!