

The Cell Classification tutorial with QuPath

Cédric Hassen-Khodja, Volker Baeker, Jean Bernard Fiche, Clément Benedetti

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1. Materials.

You will work with only 48 images. These images are located into /exercises/QuPath/images. The plate map is a standard 96-well plate, containing 12 concentration points in columns and 4 replica rows. In addition to these images, an excel file called “BBC014_v1_platemap.xls” is provided, containing information about where on the 96-well plate the wells were located, and how the cells were treated. This file is located into /exercises/QuPath/metadata.

2. Exercise: Using the QuPath software to identify features and obtain measurements from cellular images.

Creating a QuPath project.

Start QuPath and Click on Project... -> Create project to choose an empty directory.

Import your images.

- a) Select all images and dragging the files into QuPath's main window.
- b) Set image type to Fluorescence and click-on the Import button.
- c) Double-click an image in your list to open it in the viewer.
- d) Click-on the Image tab to find a list of the image properties.

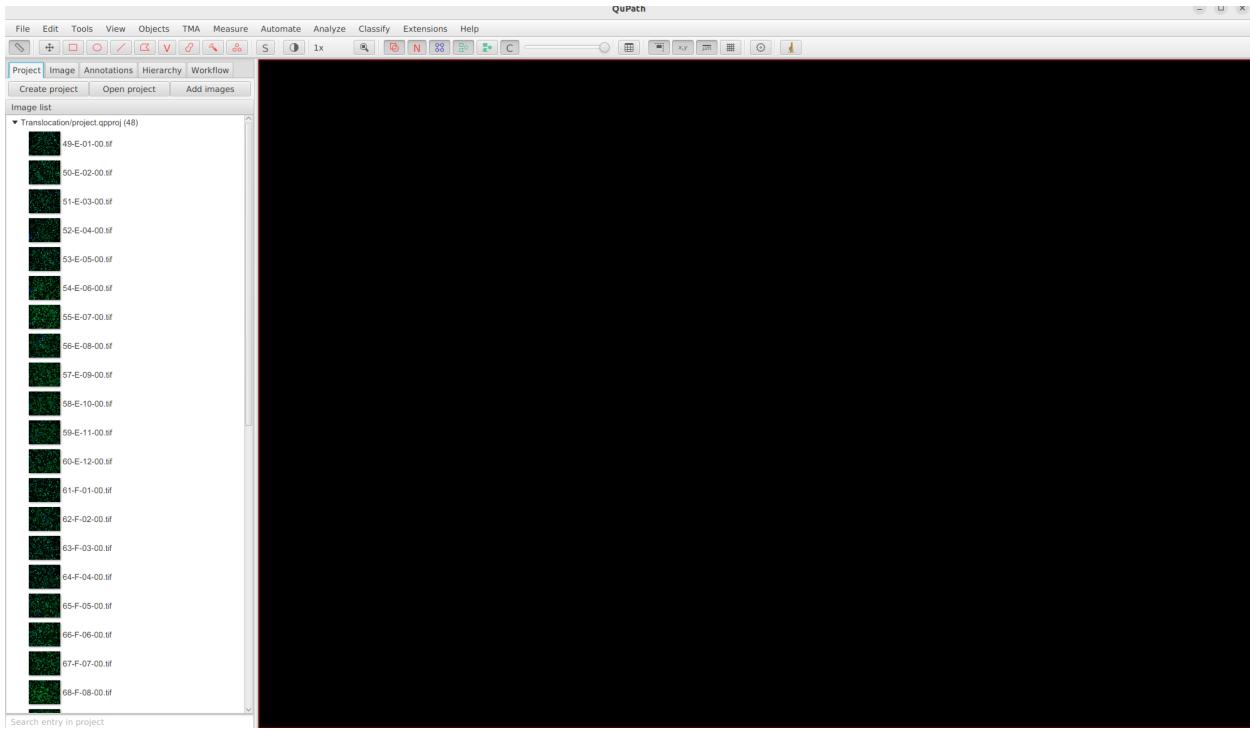


Figure 1: QuPath Interface

Set image display.

- a) To reset the image display, go to View -> Brightness/Contrast (Shift+C) or click on  on the tab panel.
- b) You can rename channels by double-clicking on it.
Rename the Channel 1 -> Cytoplasm and the Channel 2 -> Nuclei.
- c) Highlight the channel you want to adjust by clicking on the channel.
- d) To adjust the Minimum and Maximum display, double-click on either Min display or Max display to manually input a number or drag the slide bar in the histogram to achieve the optimal visual display.

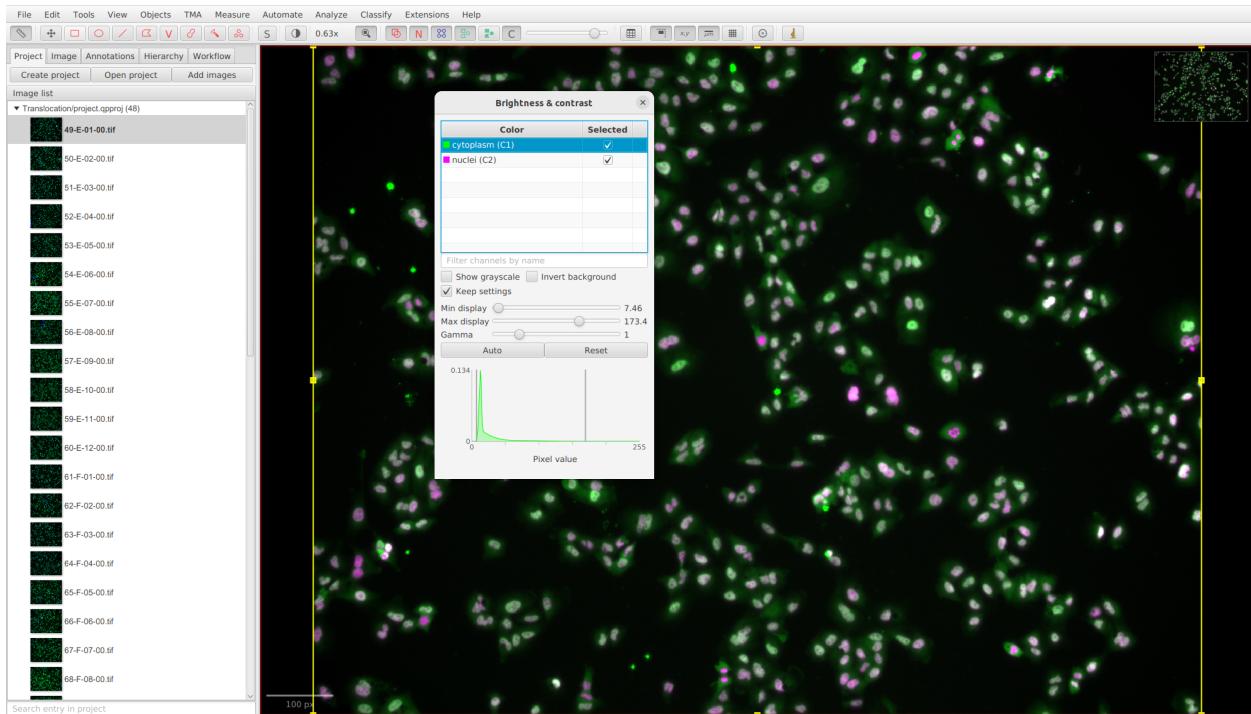


Figure 2: Brightness & contrast dialog

Cell detection.

- a) Create an annotation representing the full width and height of the current image with Ctrl+Shift+A.
- b) To perform cell segmentation, go to Analyze -> Cell detection -> Cell detection.
- c) Adjust the threshold under Intensity Parameters to maximize the detection results. Watershed cell detection uses the intensity threshold to identify cell nuclei. Start with the default settings.
 - Choose a detection channel for nucleus detection. In the following example, DAPI staining is in Channel 2 (Nuclei).
 - For suboptimal results, first adjust the Threshold value under Intensity parameters. If the value is too high, nuclei are not detected. If the value is too low, more false positives are detected.

Note: If adjusting the Threshold value does not work, adjust other parameters as follows:

- To remove noise, increase the median filter radius.
- To capture more faintly stained nuclei, increase the background radius parameter.

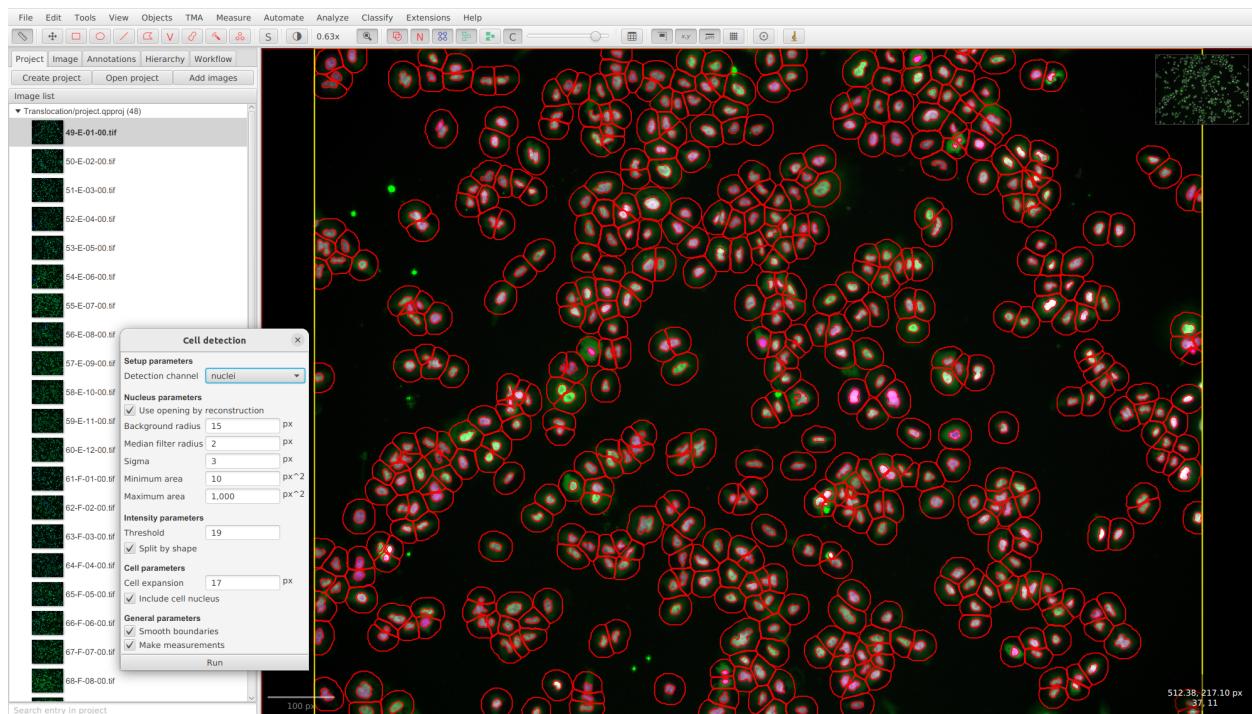


Figure 3: Cell detection image result

Apply the same workflow to other images.

- a) To create a script, choose Create a workflow from the workflow tab. A window appears with a list of processing steps.
- b) Keep the parameter that gives the best results and delete the others.
- c) Click on Create script, and save the results using File -> save.
- d) Apply the script to other images using Automate -> show script editor -> Run for project.

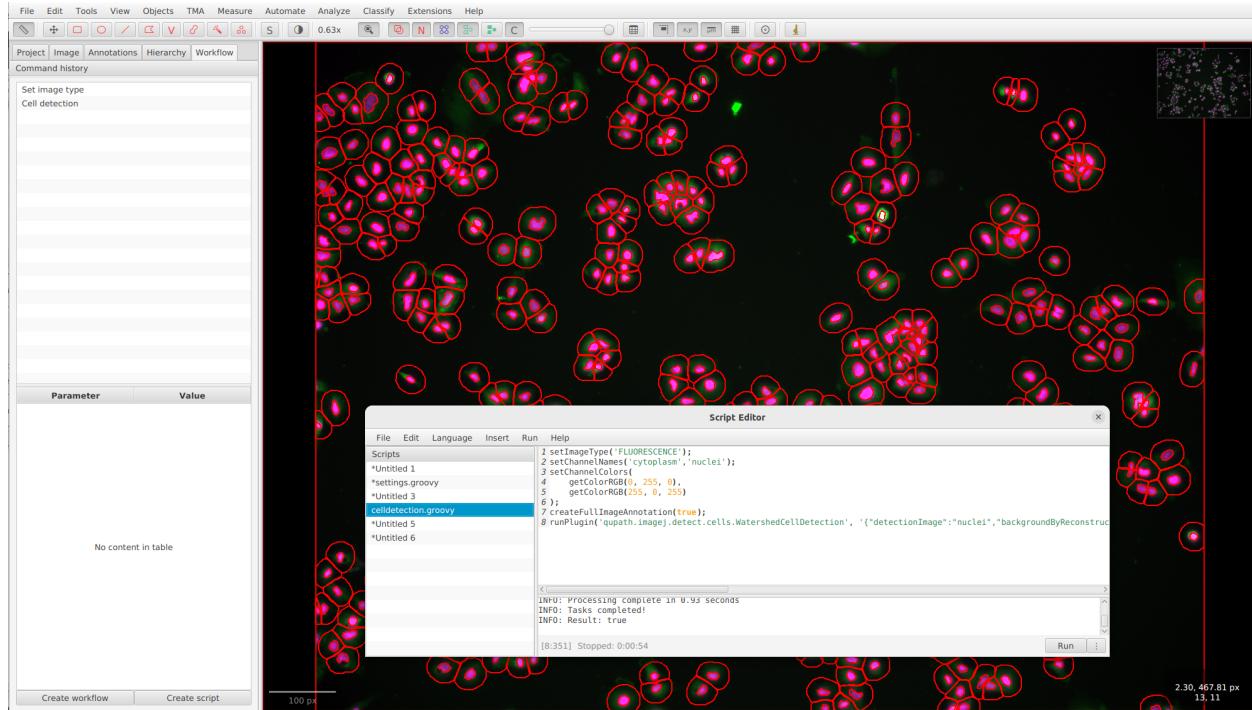


Figure 4: Workflow & Script

3. Exercise: Using the QuPath software to visualize the data and classify cells.

Visualizing the measurements.

- You can inspect the detection results detection by reviewing the measurements using Measure -> Show detection measurements. A table of cell by cell measurements results is shown. Double-click on a measurement in the table to highlight the linked cell in the image.
- You can have a measurement table that contains summary statistics and shape measurements for the annotation object using Measure -> Show annotation measurements.
- Another way to visualize cell measurements is by using the Measure -> Show measurement maps. This creates a kind of ‘heatmap’ visualization, in which each cell is color-coded according to its value for a particular measurement.

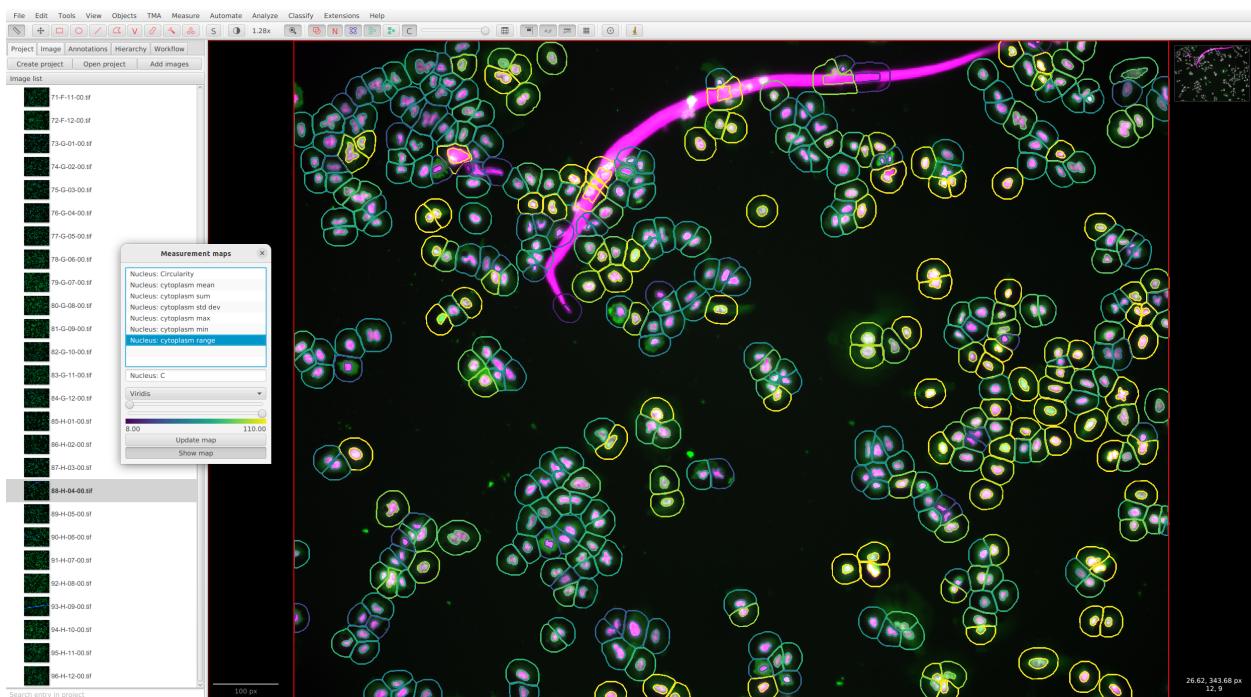


Figure 5: Measurement maps

- You can visualize the data per object by selecting “Nucleus: cytoplasm range” feature and viridis as color map.

Do we have homogeneous values or heterogeneous values for this measure? (check several wells)
Do you have an idea of the wells that have mostly a positive cell phenotype?

Annotate regions.

- The next step is to begin annotating regions by classifying clearly positive cells as positive and clearly negative cells as negative using by example the brush annotation tool. After an annotation has been drawn, right-click on the mouse button then click-on “set class” to select the appropriate class. Note: Choose an image that contains the two classes, negatives and positives cells for more accuracy.
- Get two regions of each.

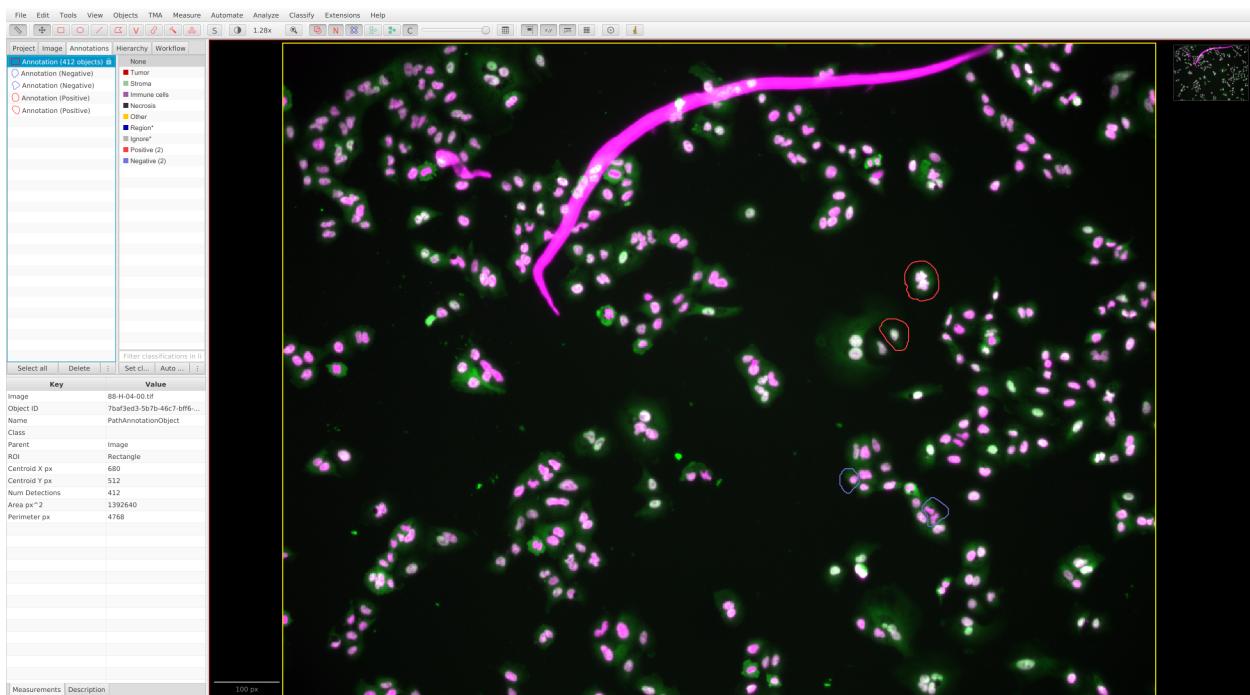


Figure 6: Training cell classification

Use the Classifier to recognize phenotypes.

- Go to Classify -> Object classification -> Train object classifier. Pressing Live update will train up a classifier that QuPath will then apply to all cells within the image.
- If you have misclassified cells, you can change the class by adding a new annotation region over the misclassified cells.

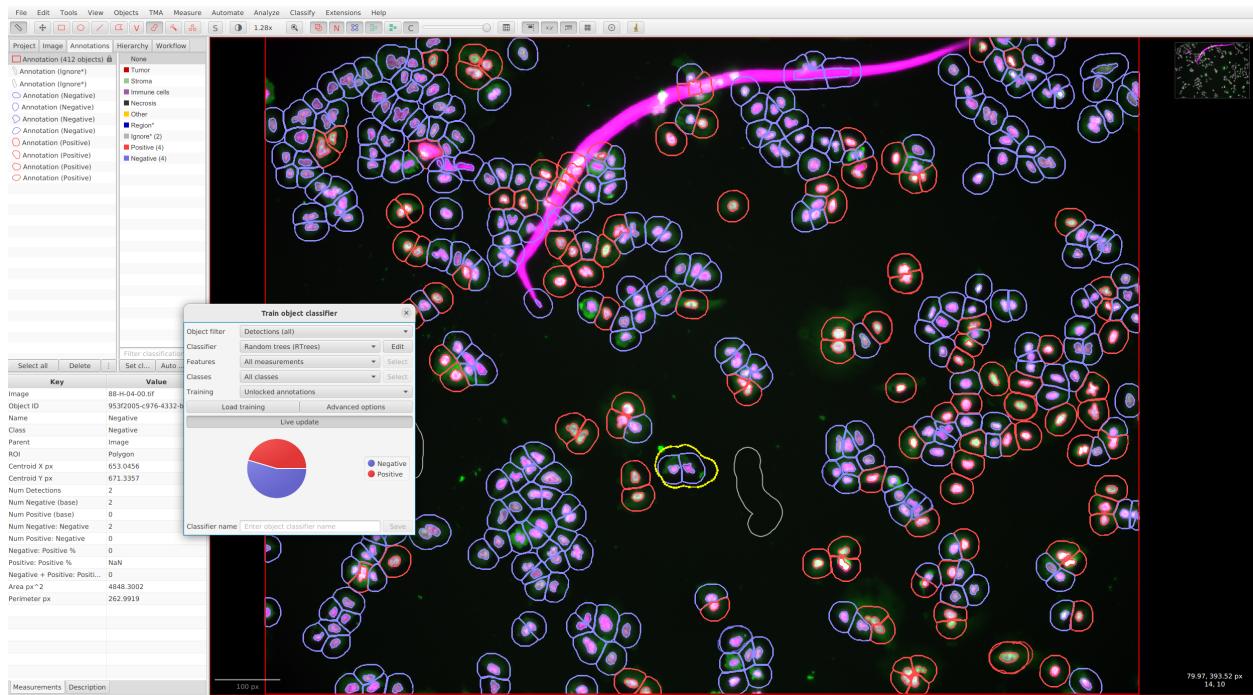


Figure 7: Training cell classification with live update

Evaluate your model.

- You can check manually the classification or get the result table using Measure -> Show annotation measurements.

How many positive and negative cells you have ?

- You can get more information about classifier by clicking on Edit button and tick “calculate variance importance”. Then go to View -> Show log.

Write the top 5 features.

- Repeat the steps above to obtain more accuracy.

Classifying all cells in the experiment.

- a) Once the classifier is sufficiently accurate, you can save it by supplying a name and pressing Save.
- b) This will allow you to load it again later on via Classify -> Object classification -> Load object classifier, whenever you wish to classify other images.

Is the number of negative cells more important from column 8 to 12? Otherwise you have probably a bad training set.

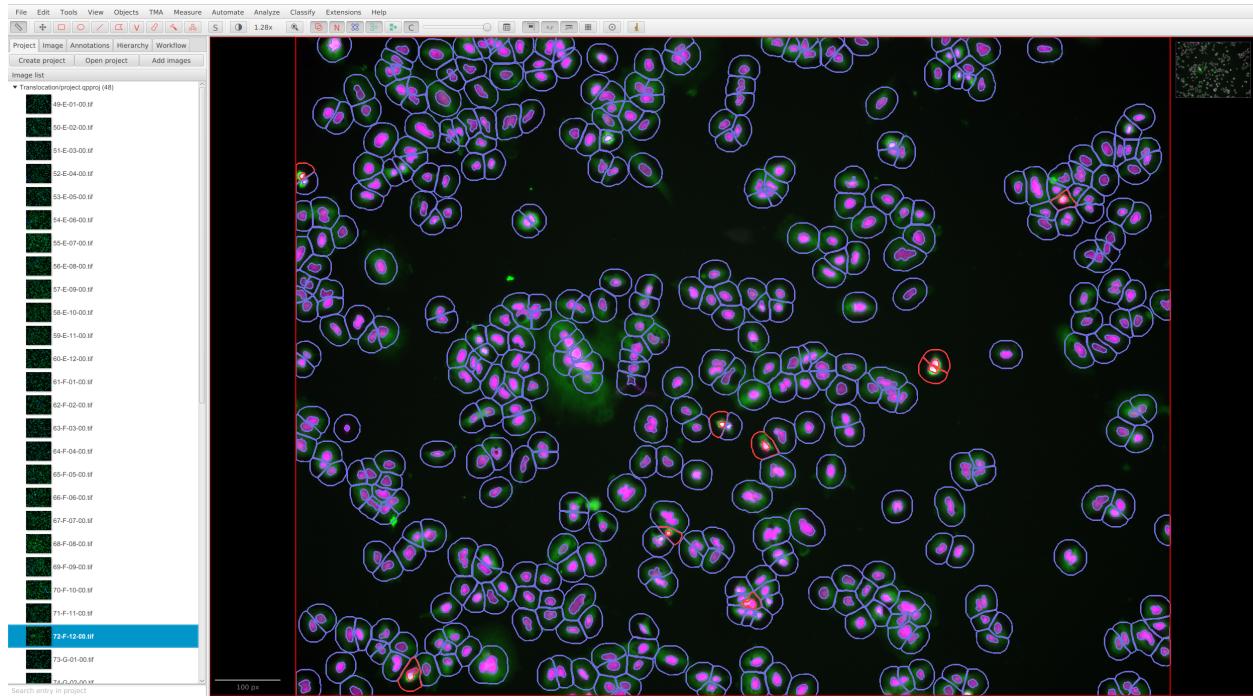


Figure 8: Result of Classifieer on specific well