Liz Dunn

Qupath IFNg CD56 CD3 analysis/Qupath Pipeline with scripts

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on

Sep 12, 2025 @02:14 PM AEST

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20250912 note:

QuPathVersion: 0.5.1Build time: 2024-03-04, 12:12Latest commit tag: '4e6bfc3'

Qupath Pipeline with scripts

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Rationale of using Qupath for analysis

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Qupath is an open source software used for whole slide imaging analysis. This enables efficient image analysis on large image files, such as those used in these IFNg analysis experiments. Qupath is recommended by ACDbio for RNAscope analysis. Additionally, this can be combined with other tools available in Quapath, in particular the ability to train object classifiers to detect CD56 and CD3+ cells. By combining these methods, I can quantify IFNg+ cells between conditions. Qupath offers benefits when working with large amounts of files, by organising the analysis as a 'project'. The images loaded into the project can be collated together and easy found under the project tab. This also means ease of scripting. Quapath has extensive scripting capabilities, so I can extract more information from my images to suit my analysis needs and questions. The scripts can be run in batch mode to the whole project, keeping analysis consistent and efficient.

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I recommend having a read of the Qupath basics here: https://qupath.readthedocs.io/en/0.5/

This will help if you're reading this for the first time. I've tried to explain everything simply, but if something doesn't make sense, understanding the basics of the software will help.

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Also in general, if you are following along with these steps, I reccomended after running each script, opening up your images and checking the script has worked and is doing what you want. Always good to double check the images look right!

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General Steps

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prep steps:

1. make classifier to find NK cells and T cells based off of CD56 and CD3 staining

2. determine cut offs for IFNg thresholding, and also determine parameters for IFNg speck counting

3. set up measurements script

4. set up export script

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1. segment with cell pose

2. classify cells

3.count IFNg spots

4. make measurements

5. export measurements

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Detailed Steps and Scripts

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Pre-step 1

Add in images.

For this I used my IFNg CD56 CD3 stained slides, as well as my batch images for IMC (IFNg no CD3 or CD56, can use to calculate overall percentages of positivity)

Can drag and drop .vsi files

use bioformats image server

do not change the directory of the images after this!

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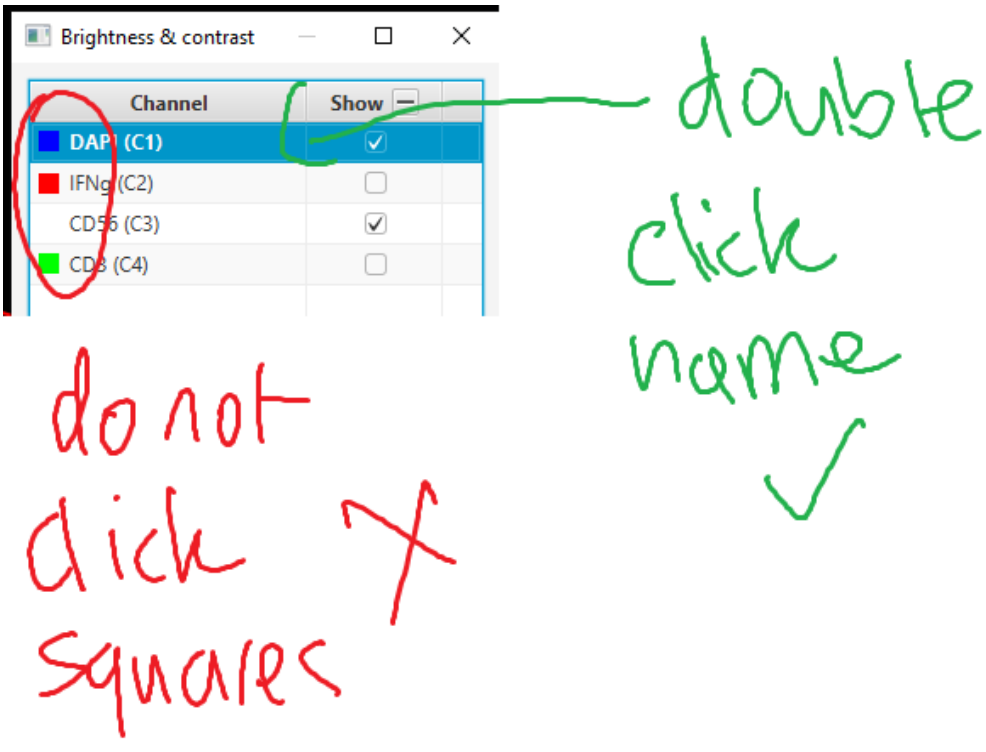
Step 1

Rename image channels to reflect staining used with the below script. Use this for CD56 CD3 stained images.

For DAPI and IFNg only (IMC images), change the script to remove the CD56 and CD3 channel.

VERY IMPORTANT to note, there is a bug in Qupath. DO NOT change the colour of the channels by clicking the little coloured square. It will change the channel name and add a C1 to the name, not just the display. This will stop the later classifier step from working. See here: https://forum.image.sc/t/bug-channel-name-changed-when-changing-color/95010



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setting_channel_names.groovy (47 B)

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Step 2

Make object classifiers.

Need classifiers for CD3, CD56, and AF spots.

Combined classifer for CD3 and CD56 will classify our cells. Only apply this to the CD56 and CD3 stained images.

AF spots classifier will remove subcellular detections that aren't actual IFNg staining. See details in the change log page.

See tutorials here: https://qupath.readthedocs.io/en/stable/docs/tutorials/multiplex_analysis.html

https://www.youtube.com/watch?v=NIivylGXOvk

To use the classifiers I have already made in a new project. In the main project directory (where the .qproj file is located). Have a folder called 'classifiers'. In the folder put the below 'classes' .json file. Also make a folder called 'object classifiers' and put the classifiers .json files in there.

The channel names and detection measurements used in the .json file classifiers must be used, so make sure the current project names and measurements applied match.

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classes.json (1.23 kB)

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20240124_1_AF_classifier_IFNg.json (3.86 MB)

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20250121_CD3_CD56_classifier.json (4.43 MB)

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20250121_CD3_classifier.json (1.29 MB)

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20250121_CD56_classifier.json (2.57 MB)

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Step 3

Run cellpose to count all the cells.

Make an annotation of the tissue area (LN exlcuding capsule) for all tissues. Then run for batch with the script below.

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1_Cellpose_Qupath_Script_for_IF_images_20240927.groovy (5.37 kB)

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Step 4

Run subcellular detection for all batches of images. I have scripts for each batch, named by the date. The threshold is slightly different for each batch hence all the different variations of the scripts

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2_Subcellular_detection_for_IMC_and_IF_20250227_for_20250225.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240410.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_202410128.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20241018.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20241004.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240917.groovy (1.14 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240825.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240808.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240730.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240701.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240625.groovy (1.12 kB)

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240423.groovy (1.12 kB)

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Step 5

Add measurements to the cells. This will enable the classifiers to run. Make sure its adding the features to the subcellualr detections (IFNg)! Click on a subcellular detection to see if it worked, should have added the haralick features measurement.

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Step 6

Run the classifers to categorise the cells and spots

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2_Subcellular_detection_for_IMC_and_IF_20241206_for_20240417.groovy (1.12 kB)

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20250120_adding_shape_and_intensity_features_FINAL.groovy (1.07 kB)

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3.5_AF_classification_for_IF_images_Qupath_Script_20250125.groovy (540 B)

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3_Cell_classification_for_IF_images_Qupath_Script_20250227.groovy (539 B)

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Step 7

Need to add measurements so they can be exported.

run '4_counting AF and IFNg_20250121_FINAL_cleaned' first.

The above script will add measurements about the IFNg spots to the parent cell so these measurements can be exported.

Then run '4 20250127 making measurements IF and IMC images'

This will add extra measurements we want to export based on the measurements created by '4_counting AF and IFNg_20250121_FINAL_cleaned'

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4_counting_AF_and_IFNg_20250121_FINAL_cleaned.groovy (3.29 kB)

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Step 8

Export measurements to a .csv

Can edit to include what ever is needed

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4_20250127_making_measurements_IF_and_IMC_images.groovy (19.1 kB)

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5_Exporting_measurements_Qupath_Script_20250227.groovy (3.12 kB)

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Step 9

Open the .csv in excel

In excel I added the H score columns with the H score formula. I have the bin number columns, and percentage of cells per bin columns exported from Qupath so I used those in excel

H-score = Σ (ACD score or bin number x percentage of cells per bin) Bin 0 -> 4

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Step 10

Transfer the values to graphpad for analysis

Done!