

Supplementary Software. *Quantitative image analysis scripts*

STEP 1 SCRIPT

```
// Script sets the image type, clears out any objects, creates a box around the entire image,
// searches it for cells using the DAPI channel, then looks within all cells for any channel 1
// regions above a manually determined threshold
setImageType('FLUORESCENCE');
clearAllObjects()
createSelectAllObject(true);
runPlugin('qupath.imagej.detect.cells.PositiveCellDetection', '{"detectionImage": "Ch1-T4",
"requestedPixelSizeMicrons": 0.5, "backgroundRadiusMicrons": 0.0, "medianRadiusMicrons":
1.0, "sigmaMicrons": 2.0, "minAreaMicrons": 40.0, "maxAreaMicrons": 400.0, "threshold":
400.0, "watershedPostProcess": true, "cellExpansionMicrons": 5.0, "includeNuclei": true,
"smoothBoundaries": true, "makeMeasurements": true, "thresholdCompartment": "Nucleus:
Ch1-T4 mean", "thresholdPositive1": 3500.0, "thresholdPositive2": 200.0,
"thresholdPositive3": 300.0, "singleThreshold": true}');
selectAnnotations();
runPlugin('qupath.imagej.detect.cells.SubcellularDetection', '{"detection[Channel 1]": 300.0,
"detection[Channel 2]": -1.0, "doSmoothing": false, "splitByIntensity": false, "splitByShape":
false, "spotSizeMicrons": 1.0, "minSpotSizeMicrons": 0.1, "maxSpotSizeMicrons": 2.0,
"includeClusters": true}');
//Manually remove any artifacts, then run "Subcellular_Summaries.groovy"
```

STEP 2 SCRIPT

```
// Script to find average intensity of spots in cells
// Cell expansion in QuPath is blind and fixed to a certain distance,
// so a threshold was set to eliminate empty background from reducing the mean or median
intensity per cell.
```

```
//Test detections for another channel.
//selectAnnotations()
//runPlugin('qupath.imagej.detect.cells.SubcellularDetection', '{"detection[Channel 1]": -1.0,
"detection[Channel 2]": 1200.0, "doSmoothing": false, "splitByIntensity": false,
"splitByShape": true, "spotSizeMicrons": 1.0, "minSpotSizeMicrons": 0.5,
"maxSpotSizeMicrons": 2.0, "includeClusters": true}');
```

```
hierarchy = getCurrentHierarchy()
```

//The primary part of the script that calculates the mean intensity of the positive parts of the estimated cell area

```
for (annotation in getAnnotationObjects()){
  //Collect all of the cells which are children of the box around the image
  totalCells = annotation.getChildObjects()
  runningTotalArea = 0
  runningTotalIntensity = 0
  totalCells.each{
    //For each cell, collect information from all child subcellular objects
    subCellArea = 0
    subCellTotalIntensity = 0
    it.getChildObjects().each{c->

      subCellTotalIntensity += measurement(c, "Subcellular cluster: Channel 1:
Area")*measurement(c, "Subcellular cluster: Channel 1: Mean channel intensity")
      subCellArea += measurement(c, "Subcellular cluster: Channel 1: Area")
    }
    //Add this information to the running total for all cells
    runningTotalArea+=subCellArea
    runningTotalIntensity += subCellTotalIntensity
    //Add a measurement to the cell with the total intensity from all subcellular objects
    it.getMeasurementList().putMeasurement("Intensity Sum", subCellTotalIntensity)
    //prevent divide by zero errors and report the average intensity for the cell
    if(subCellArea){
      it.getMeasurementList().putMeasurement("Intensity Mean",
subCellTotalIntensity/subCellArea)
    } else {it.getMeasurementList().putMeasurement("Intensity Mean", 0)}
  }
  //Once all cells are processed, calculate the mean intensity across all cells.
  annotation.getMeasurementList().putMeasurement("Average positive area",
runningTotalArea/totalCells.size())
  annotation.getMeasurementList().putMeasurement("Average total intensity per cell",
runningTotalIntensity/totalCells.size())
}
```

//Uncomment these lines to remove the subcellular detections. Only do this after the accuracy of the subcellular detections has been verified!

```
//selectAnnotations()
//runPlugin('qupath.imagej.detect.cells.SubcellularDetection', '{"detection[Channel 1]": -1.0,
"detection[Channel 2]": -1.0, "doSmoothing": false, "splitByIntensity": false, "splitByShape":
true, "spotSizeMicrons": 1.0, "minSpotSizeMicrons": 0.5, "maxSpotSizeMicrons": 2.0,
"includeClusters": true}');
```

```
println("Exporting results")
```

//The rest of the script looks complicated, but is simply to export the results as a text file per image, and then combine those into a single text file.

//Code source: <https://petebankhead.github.io/qupath/scripting/2018/03/04/script-annotation-export.html>

```
def name = getProjectEntry().getImageName() + '.txt'
def path = buildFilePath(PROJECT_BASE_DIR, 'annotation results')
mkdirs(path)
path = buildFilePath(path, name)
saveAnnotationMeasurements(path)
print 'Results exported to ' + path
path = buildFilePath(PROJECT_BASE_DIR, 'detection results')
mkdirs(path)
path = buildFilePath(path, name)
saveDetectionMeasurements(path)
print 'Results exported to ' + path
/**
 * Script to combine results tables exported by QuPath.
 *
 * This is particularly intended to deal with the fact that results tables of annotations can produce
 * results
 * with different column names, numbers and orders - making them awkward to combine later
 * manually.
 *
 * It prompts for a directory containing exported text files, and then writes a new file in the same
 * directory.
 * The name of the new file can be modified - see the first lines below.
 *
 * Note: This hasn't been tested very extensively - please check the results carefully, and report
 * any problems so they
 * can be fixed!
 *
 * @author Pete Bankhead
 */

// Some parameters you might want to change...
String ext = '.txt' // File extension to search for
String delimiter = '\t' // Use tab-delimiter (this is for the *input*, not the output)
String outputName = 'Combined_results.txt' // Name to use for output; use .csv if you really want
comma separators

// Prompt for directory containing the results
//def dirResults = QuPathGUI.getSharedDialogHelper().promptForDirectory()
def dirResults = new File(buildFilePath(PROJECT_BASE_DIR, 'annotation results'))
```

```

if (dirResults == null)
    return
def fileResults = new File(dirResults, outputName)

// Get a list of all the files to merge
def files = dirResults.listFiles({
    File f -> f.isFile() &&
        f.getName().toLowerCase().endsWith(ext) &&
        f.getName() != outputName} as FileFilter)
if (files.size() <= 1) {
    print 'At least two results files needed to merge!'
    return
} else
    print 'Will try to merge ' + files.size() + ' files'

// Represent final results as a 'list of maps'
def results = new ArrayList<Map<String, String>>()

// Store all column names that we see - not all files necessarily have all columns
def allColumns = new LinkedHashSet<String>()
allColumns.add('File name')

// Loop through the files
for (file in files) {
    // Check if we have anything to read
    def lines = file.readlines()
    if (lines.size() <= 1) {
        print 'No results found in ' + file
        continue
    }
    // Get the header columns
    def iter = lines.iterator()
    def columns = iter.next().split(delimiter)
    allColumns.addAll(columns)
    // Create the entries
    while (iter.hasNext()) {
        def line = iter.next()
        if (line.isEmpty())
            continue
        def map = ['File name': file.getName()]
        def values = line.split(delimiter)
        // Check if we have the expected number of columns
        if (values.size() != columns.size()) {
            print String.format('Number of entries (%d) does not match the number of columns (%d)!', columns.size(), values.size())
            print('I will stop processing ' + file.getName())
        }
    }
}

```

```

        break
    }
    // Store the results
    for (int i = 0; i < columns.size(); i++)
        map[columns[i]] = values[i]
    results.add(map)
}
}

// Create a new results file - using a comma delimiter if the extension is csv
if (outputName.toLowerCase().endsWith('.csv'))
    delimiter = ','
int count = 0
fileResults.withPrintWriter {
    def header = String.join(delimiter, allColumns)
    it.println(header)
    // Add each of the results, with blank columns for missing values
    for (result in results) {
        for (column in allColumns) {
            it.print(result.getDefault(column, ""))
            it.print(delimiter)
        }
        it.println()
        count++
    }
}

// Success! Hopefully...
print 'Done! ' + count + ' result(s) written to ' + fileResults.getAbsolutePath()

dirResults = new File(buildFilePath(PROJECT_BASE_DIR, 'detection results'))
if (dirResults == null)
    return
fileResults = new File(dirResults, outputName)

// Get a list of all the files to merge
files = dirResults.listFiles({
    File f -> f.isFile() &&
        f.getName().toLowerCase().endsWith(ext) &&
        f.getName() != outputName} as FileFilter)
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```

```

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    }
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}

// Success! Hopefully...
print 'Done! ' + count + ' result(s) written to ' + fileResults.getAbsolutePath()

import qupath.lib.gui.QuPathGUI
import qupath.lib.objects.PathCellObject

```