Full-sentence description of the script for performing staining intensity analysis over a large batch of images. If you’ve never done any scripting before, start by reading this.

Note: Anything in green (or starting with // ) is a comment. These will be simply ignored like they are not there.

1. Tell QuPath that this is an H&E slide (options: Hematoxylin & Eosin, Hematoxylin & DAB, Fluorescence, Other). QuPath usually guesses correctly, but it is good practice to set it yourself.
2. Teach QuPath what hematoxylin and what eosin looks like in your hands. [To find these values, use “estimate stain vectors” on one slide. Then, copy the values from the images tab, or use workflow-to-script and copy then entire line].
3. Find the area of the entire tissue, using the Simple Tissue Detection function. The most important parameter here is “threshold”, which is the difference between tissue and whitespace. Further to the right, you can set "maxHoleAreaMicrons”, which is the size of the largest region of all background pixels that will still be called tissue. In this case we disabled smoothing and median filtering to get a tight fit to the tissue.
4. Select the tissue region we just created to tell QuPath that the next instruction will be done on this annotation.
5. Measure the intensity of the entire selected region. We are measuring the channels labeled “true” (optical density (OD), hematoxylin, eosin, residual, red, green, and blue) and skipping the ones labeled “false” (hue, saturation, brightness). In the selected channels, we are measuring the mean intensity, but not the standard deviation, min and max intensities, median intensity, or Haralick (texture) features.
6. Now that we have the intensity measurements we want, we need to export them into a text file. First, we’re going to figure out what the name of that text file should be, and store it in the variable “txtname”. QuPath looks up the original name of the image that we are processing in its Project Entry and appends “.txt” to the image name.
7. Next, we have to tell QuPath where we will save the text file. PROJECT\_BASE\_DIR is a variable that stores the folder that you made the project in. We’re going to make a subfolder inside that directory called “intensity QC”. The function buildFilePath() puts in whatever characters your computer needs to define a subfolder (i.e. it does PROJECT\_BASE\_DIR\intensity QC on a windows or PROJECT\_BASE\_DIR/intensity QC on a mac). We creatively call this location “folder”.
8. Mkdirs() checks to see if that subfolder (the one called “folder”) already exists on your computer, and if it does not, creates it.
9. We put the folder we just defined and the text file name we generated in Step 6 into a single variable called “path”. We use buildFilePath() again to get the \ or / correct.
10. saveAnnotationMeasurements(path) saves all of the measurements made on the tissue annotation to the file “path”
11. This line simply reports to the screen that script is finished and where the results are saved to.