Quigly's Circle App

Version 1.1

Readme Document

Summary

This app will find and measure the radius of bright circles on dark backgrounds, and vice versa, using Circle Hough Transform. The general flow is that it will first look for capsules (bright circles on white backgrounds) since they're easier to find. Then it will iterate through every discovered capsule, crop the image to just that region, and detect the cell body (dark circle on bright background). The rest is just math and bookkeeping to organize the resulting data and output it to an excel sheet.

To get the best results you want to maximize the contrast between each circle type. I've noticed there is a certain plane of focus where the cell body is a thick dark band, clearly visible against the cell and capsule, aim for that. It also helps to have the India Ink making as uniform a coating as possible. If you end up with too loose a distribution you see clumps of black ink interspersed with dots of white background. The script will normalize the contrast of the image so that the brightest pixel is white and darkest pixel is black. If the capsule is the brightest pixel this will work better.

Finally, while the script is pretty robust it will still make mistakes. It will generate a folder labelled "Processed" where you can see a visual representation of each detected circle, make sure you check those images to look for incorrect measurements. Otherwise, common issues include:

- The script detects a capsule but no cell body. These will be obvious in the resulting dataset and don't actually require any manual fixing. Most of the time you'll just be copying the Capsule and Body radius columns and empty capsules will not have data in those columns.
- Very small crypto are not detected. These tiny cells tend to be faded/blurry, out of focus, and extremely small. There's really nothing to do about this (I've found) so you may have to measure those yourself. Even so, if you automatically measure what you can and just manually catch the stragglers you should still save a lot of time using this script.

Detailed Instructions

1. Ensure the correct file extensions are entered into the file type text box. Do NOT include a period. Separate multiple types by a semicolon only. For example if you have both .tif and .jpeg files you would input tif;jpeg
2. Press the "Select Directory" button and navigate to the folder which contains your images. It will NOT search recursively. I am adding this functionality but it is not yet finished. The directory will be displayed in the text box below the button and the file list will be displayed in the console log text box on the right.
3. Click "Select Random Image" and a randomly selected image from your set should appear in the middle. The path of the selected file will be written in the console log.
4. Click "Run Test" to test out the current detection settings on the selected image. If it looks good you can proceed. If it doesn't, adjust the settings and try again.
    a. Capsule/Body Min/Max – The minimum and maximum radius the program will use to look for capsules or cell bodies (in pixels). The smaller the range, the better the results. There is no Body Maximum because the body could not be larger than the capsule, so I just use the detected capsule size as the max to minimize the range and maximize quality.
    b. Capsule/Body Sensitivity – How sensitive the circle detection is for each object. The more sensitive, the less perfectly round an object has to be to count.
5. Click "Run Analysis". Progress will be output to the console log, just wait. Results will be output to the same directory as the images. A spreadsheet sheet labelled "outputFinal" will contain the numerical data and resulting radii. These values will be in pixels so make sure you convert according to your microscope. There will also be a folder labelled "Processed" which contains copies of all the images with each detected circle superimposed (green for capsules, blue for cell bodies).