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4
5 macro "Batch Object Analyser"{
6     format = ".tif"; // specify image format
7     filterRadius = 1.0;
8     morphIterations = 3;
9     minSize = 10.0;
10    channel1Prefix = "C1";
11    channel1Title = "";
12
13    directory = getDirectory("Choose input files"); // get input directory
14    print("Input: " + directory);
15    fileList = getFileList(directory); // get file list
16    print(fileList.length + " files.");
17
18    outputDirectory = getDirectory("Choose output directory"); // get output directory
19    print("Output: " + outputDirectory);
20
21    setBatchMode(true); // supresses windows opening
22    run("Set Measurements...", "area shape display redirect=None decimal=3"); // set measurements for morphological analysis
23    minSize = getNumber("Enter minimum object area (" + getInfo("micrometer.abbreviation") + "^2): ", minSize); // ask user to specify minimum object size
24    for (i = 0; i < fileList.length; i++) {
25        file = directory + File.separator + fileList[i];
26        if(endsWith(file, format)){ // check if file is correct format
27            print("\nFile " + file + " is a recognised format - processing.");
28            run("Bio-Formats Importer", "open=[" + file + "] color_mode=Default rois_import=[ROI manager] view=Hyperstack"); // open image with bioformats
29            getPixelSize(unit, pw, ph); // get pixel dimensions
30            getDimensions(width, height, sizeC, slices, frames); // get image dimensions
31            print("Number of channels: " + sizeC);
32            if(sizeC > 1){
33                run("Split Channels"); // split the image into constituent channels
34            }
35            titles = getList("image.titles"); // get the list of open images i.e the individual channels
36            for(j = 0; j < titles.length; j++){
37                if(startsWith(titles[j], channel1Prefix)){
38                    channel1Title = titles[j];
39                } else {
40                    close(titles[j]);
41                }
42            }
43            selectWindow(channel1Title);
44            analyseImage(filterRadius, morphIterations, minSize, outputDirectory);
45            close(""); // close all images
46        } else {
47            print("\nFile " + file + " is not a recognised format - skipping.");
48        }
49    }
50
51    print("\nFinished");
52    showStatus("Finished.");
53    setBatchMode(false);
54
55    function analyseImage(filterRadius, morphIterations, minSize, outputDirectory){
56        run("Gaussian Blur...", "sigma=" + filterRadius); // apply Gaussian filtering
57        setAutoThreshold("Intermodes dark"); // set thresholding options
58        setOption("BlackBackground", false);
59        run("Convert to Mask"); // convert image to binary
60        run("Options...", "iterations=" + morphIterations + " count=1 do=Open"); // apply morphological opening
61        run("Analyze Particles...", "size=" + minSize + "-Infinity show=Outlines display exclude include"); // run particle analyser
62        titles = getList("image.titles");
63        for(i = 0; i < titles.length; i++){
64            if(startsWith(titles[i], "Drawing")){ // find the image window that the particle analyser output
65                selectWindow(titles[i]);
66                filename = outputDirectory + File.separator + titles[i] + "_outlines.png";
67                print("Saving " + filename);
68                saveAs("PNG", filename); // save the particle analyser output image
69            }
70        }
71    }
72 }

```