Batch Analysis for measuring fluorescent signal of cells

Usage: *Run it in FIJI (www.fiji.sc)

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## This is how to apply the code for multiple files in one directory

`get Directory` *function* will give a string variable (input_path) which is the path of your directory. `FileList` will be an *array* which is contained inside the *variable* called input_path

```
input_path = getDirectory("input files");
fileList = getFileList(input_path);
```

Now we create a `for loop` to be able to access to each file in the directory. the *function* `.legth` gives the number of compartments in my array fileList.

```
for (f=0; f<fileList.length; f++){
        open(input_path + fileList[f]);
        print(input_path + fileList[f]);
```

```
> C:\Users\mdp18pm\Desktop\Test\Image 1_Out.czi
```
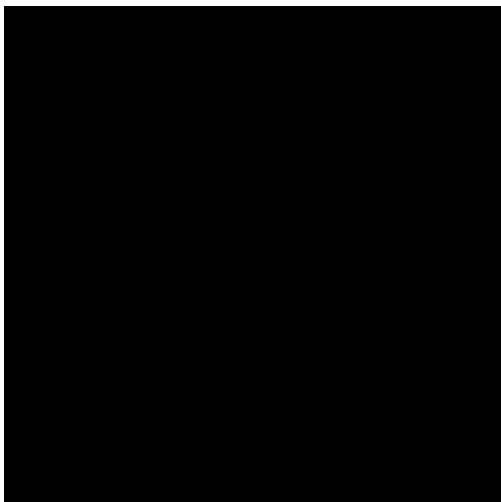

Let's create a duplicated image that we can work with

Step1: Normalise the data name and Remove scale to work with pixels

```
title = getTitle();
run("Set Scale...", "distance=0 known=0 pixel=1 unit=pixel");
```

Step 2: Duplicate original image and enhance contrast in both original and duplicated image

```
run("Duplicate...", " ");
duplicated = getImageID();

selectImage(title);
rename("original");
selectImage("original");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");

selectImage(duplicated);
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
```
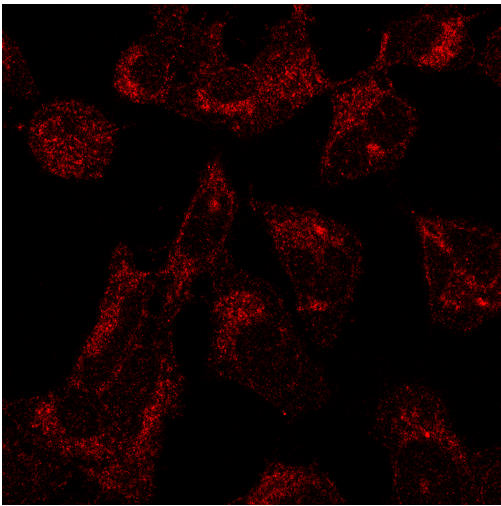

Let's start our Connected Components Analysis

(This will be done on the duplicated image only) We will use a median filter (edge preserving filter) for facilitating the threshold that we'll apply later

```
selectImage(duplicated);
run("Median...", "radius=8");
```

By default we'll use the `Huang threshold` method.

```
setAutoThreshold("Huang dark");
setOption("BlackBackground", true);
run("Convert to Mask");
```

By default we'll use dilation to refine the threshold.

```
run("Dilate");
```

Here, we'd like to apply `fill holes`. However, often times, holes of hollow space between cells is consider and measured. Of course we don't want to measure this hollow space. We therefore take advantage of the command `waitForUser` and let the user create some lines to separate cells so that the `fill holes` will work well subsequently.

```
setTool("line");
waitForUser("before filling the holes, make sure to separate cells.

Draw specific lines among cells");
```

Now we can apply the `fill holes`

```
run("Fill Holes");
```

Now we use the particle analyser to detect the objects and we measure them

```
run("Set Measurements...", "area mean min integrated redirect=None decima
run("Analyze Particles...", "size=10000.00-Infinity add");
```

Now we go to the original image and overlay the thresholded contours.

```
selectImage("original");
roiManager("Show None");
roiManager("Show All");
roiManager("Measure");
```

Area	Mean	Min	Max	IntDen	RawIntDen

|					
430131	8.88	0	250	3817523.00	3817523.00
150877	8.71	0	116	1314628.00	1314628.00
37102	5.60	0	80	207836.00	207836.00
199850	7.92	0	100	1583422.00	1583422.00
145924	10.66	0	119	1555766.00	1555766.00
173437	7.34	0	261	1273277.00	1273277.00
218187	7.10	0	120	1548879.00	1548879.00
201698	7.46	0	127	1504134.00	1504134.00
641016	7.13	0	106	4568241.00	4568241.00
260973	5.91	0	145	1542162.00	1542162.00
256082	5.40	0	169	1383537.00	1383537.00
83407	9.24	0	80	770275.00	770275.00

Save the results

Now we save the results.

```
saveAs("results", input_path+title+"results.xls");
```

To have a check for the ROIs that fiji selected I want to save them.

```
selectImage("original");
run("Capture Image");
saveAs("Tiff", input_path+title+"original.tif");
```
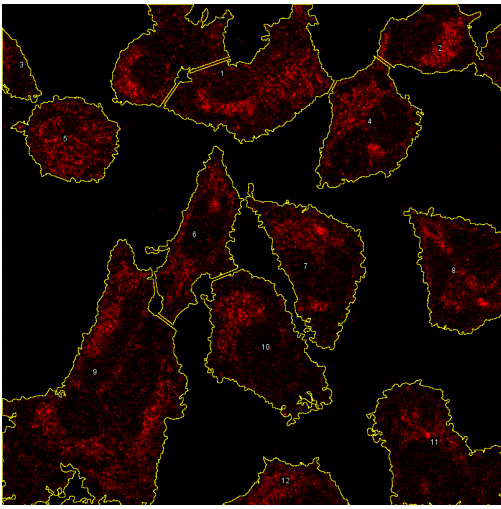
Final step: Clean-up to prepare for next image

```
roiManager("reset");
run("Close All");
run("Clear Results");

}
```