

/*

QUANTIFICATION OF SYNAPTOPHYSIN-POSITIVE PUNCTA PER CELL AREA

Definitions:

c1= z-stack images. **c2**= Vimentin composite images. **c3**= Amytracker composite images.

The threshold (*min* and *max*) and pixel size (*min* and *max*) are set manually for each experiment and subsequently applied to all images.

/*

// Counting the number of synaptophysin-positive puncta

//Automation

```
inputfile = "C:\\Input\\";
```

```
outputfile = "C:\\Output\\";
```

```
list = getFileList(inputfile);
```

```
setBatchMode(true);
```

```
for (i=0; i<list.length; i++) {
```

```
    showProgress(i+1, list.length);
```

```
    open(inputfile+list[i]);
```

//Measurement

```
run("Set Scale...", "distance=1 known=0.65 pixel=1 unit=micrometer");
```

```
run("Subtract Background...", "rolling=50 sliding");
```

```
run("16-bit");
```

```
setAutoThreshold("Default dark");
```

```
setThreshold(min, max);
```

```
setOption("BlackBackground", true);
```

```
run("Convert to Mask");
```

```
run("Despeckle");
```

```
run("Watershed");
```

```
run("Analyze Particles...", "size=min-max pixel circularity=0.10-1.00 display summarize add");
```

```
}
```

// Measurement of cell area using β 3-Tubulin signal

//Automation

```
inputfile = "C:\\Input\\";
outputfile = "C:\\Output\\";
list = getFileList(inputfile);
setBatchMode(true);
for (i=0; i<list.length; i++) {
  showProgress(i+1, list.length);
  open(inputfile+list[i]);
```

//Measurement

```
run("Set Scale...", "distance=1 known=0.65 pixel=1 unit=micrometer");
run("Subtract Background...", "rolling=50 sliding");
run("16-bit");
setThreshold(min, max);
setOption("BlackBackground", true);
run("Set Measurements...", "area limit display redirect=None decimal=2");
run("Measure");

}
```