

/*

QUANTIFICATION OF INTRACELLULAR AMYTRACKER SIGNAL

Definitions:

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

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

/*

//Automation

```
inputfile = "C:\\Input\\";
outputfile = "C:\\Output\\";
inputfilelist = getFileList(inputfile);
for(k=0;k<lengthOf(inputfilelist);k=k+2) {
    roiManager("Reset");
    open(inputfile + inputfilelist[k]);
    print(k);
    C2location=lastIndexOf(inputfilelist[k],"c2");
    print(C2location);
    name=inputfilelist[k];
    newname=replace(name, "c2", "c3");
    print(name);
    open(inputfile + newname);
    selectImage(name);
```

//Determination of cellular margins using vimentin signal

```
run("Set Scale...", "distance=1 known=0.65 pixel=1 unit=micrometer");
    run("Subtract Background...", "rolling=500");
    run("16-bit");
    setThreshold(min, max);
    run("Create Selection");
    roiManager("Add");
```

//Quantification of Amytracker signal

```
selectImage(newname);
```

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

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

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

```
        roiManager("Select", 0);
```

```
        run("Clear Outside");
```

```
roiManager("select", 0);
```

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

```
run("Create Selection");
```

```
run("Set Measurements...", "area mean integrated display redirect=None decimal=3");
```

```
run("Measure");
```

```
}
```