//// MACRO\_TIRF\_Spot\_Dendity

roiManager("reset");

dir1 = getDirectory("Choose Source Directory for Channel1 ");

dir2 = getDirectory("Choose Source Directory for Saving channel1\_Spot");

list1 = getFileList(dir1);

//setBatchMode;

for (i=0; i <list1.length; i++) {

open(dir1+list1[i]);

name1= getTitle();

rename("channel1");

selectWindow("channel1");

//makeRectangle(0, 0, 427, 684);

//run("Crop");

run("Z Project...", "projection=[Standard Deviation]");

run("Enhance Contrast", "saturated=0.35");

run("Set Measurements...", "area display redirect=None decimal=5");

run("8-bit");

run("Sharpen");

run("Sharpen");

run("Auto Threshold", "method=Shanbhag white");

run("Despeckle");

setTool("polygon");

waitForUser("Select zones");

run("Measure");

setBackgroundColor(0, 0, 0);

run("Clear Outside");

run("Analyze Particles...", "size=2-35 circularity=0.80-1.00 show=Masks summarize");

selectWindow("channel1");

close();

selectWindow("STD\_channel1");

close();

selectWindow("Mask of STD\_channel1");

run("Invert");

saveAs("Tiff", dir2+list1[i]);

close();

}