//MACRO\_Min\_Nucleus-density

run("Clear Results");

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

dir2 = getDirectory("Choose Source Directory for Saved\_detected\_root");

dir3 = getDirectory("Choose Source Directory for Spot");

list1 = getFileList(dir1);

list2 = getFileList(dir2);

list3 = getFileList(dir3);

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

open(dir1+list1[i]);

name1= getTitle();

rename("channel1");

close("channel1");

rename("channel1");

run("Z Project...", "projection=[Average Intensity]");

selectWindow("channel1");

close();

//makeRectangle(120, 12, 609, 2412);

//run("Crop");

Stack.getDimensions(width, height, channels, slices, frames);

title=getTitle();

//run("Duplicate...", "title=\*");

//setBatchMode(true);

roiManager("reset");

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

makeRectangle((width/4), (height/4), (width/2), (height/2));

run("Find Maxima...", "noise=200 output=[Point Selection]");

//Xmax= getResult("X", 0);

//Ymax= getResult("Y", 0);

//toUnscaled (Xmax, Ymax);

run("Select All");

run("Duplicate...", "title=Wave");

run("Select All");

roiManager("Add");

run("Wavelet A Trou");

run("Stack to Images");

close("coeff-5");

close("coeff-4");

close("coeff-2");

close("coeff-3");

close("coeff-1");

roiManager("reset");

selectWindow("plan");

setAutoThreshold("Otsu dark");

run("Convert to Mask");

setOption("BlackBackground", true);

run("Create Selection");

//selectWindow("channel1");

//run("Restore Selection");

run("Measure");

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

rename("channel1");

close("channel1");

close("wave");

close("plan");

close("AVG\_channel1");

open(dir3+list3[i]);

name1= getTitle();

run("Convert to Mask");

run("Analyze Particles...", "size=0-2000000.00 circularity=0-1.00 show=Masks summarize");

run("Close");

run("Close");

}