///// SiCE FRAP analysismacro

Dialog.create("SiCE FRAP analysis Plugin");

Dialog.addMessage("This macro quantifies FRAP within Arabidopsis root tissue \n ");

Dialog.addNumber("FRAP zone lenght",5, 1,3, "pixel");

Dialog.addNumber("Membrane thickness",2, 1,3, "pixel");

Dialog.addChoice("Orientation of bleaching", newArray("lateral", "Apico basal"));

Dialog.show();

zone = Dialog.getNumber();

mbt = Dialog.getNumber();

ori = Dialog.getChoice();

//run("Image Sequence...");

//setBatchMode;

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

dir2 = getDirectory("Choose Source Directory for Saving\_txt\_File ");

list1 = getFileList(dir1);

//setBatchMode;

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

open(dir1+list1[i]);

name1= getTitle();

rename("channel1");

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

title=getTitle();

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

//setBatchMode(true);

run("Clear Results");

roiManager("reset");

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

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

run("Find Maxima...", "noise=2000 output=[Point Selection]");

run("Measure");

Xmax= getResult("X", 0);

Ymax= getResult("Y", 0);

//toUnscaled (Xmax, Ymax);

run("Select All");

//use wavelet to determine root

run("Clear Results");

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");

run ("Invert");

doWand("Xmax","Ymax");

run("Fill");

//determine root orientation

run("Measure");

run("Fit Ellipse");

angle= getResult("Angle",0);

close("plan");

close("wave");

setBatchMode(false);

//stack rotation

selectWindow(title);

run("Rotate... ", "angle="+angle+" grid=1 interpolation=Bilinear stack");

roiManager("reset");

//determine bleached ROIs

selectWindow(title);

run("Duplicate...", "duplicate range=1-1");

rename("1");

selectWindow(title);

run("Duplicate...", "duplicate range=2-2");

rename("2");

imageCalculator("Subtract create", "1","2");

selectWindow("Result of 1");

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

setAutoThreshold("Yen");

//run("Threshold..."); //setOption("BlackBackground", true);

run("Convert to Mask");

run ("Invert");

run("Analyze Particles...", "size="+(zone)+"-"+(200\*zone)+" show=Nothing display exclude summarize add in\_situ");

//selectWindow("Summary");

//run("Set Measurements...", "mean redirect=None decimal=5");

//selectWindow("channel1");

//roiManager("Multi Measure");

//roiManager("Deselect");

//roiManager("Delete");

close("1");

close("2");

close("Result of 1");

roiManager("Show All");

setTool("ellipse");

waitForUser("Draw control ROIs");

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

selectWindow("channel1");

roiManager("Multi Measure");

close("channel1");

selectWindow("Results");

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

}