//// Macro MATT quantification plasma mb vs cytosol A thaliana roots

run("Clear Results"); roiManager("reset");

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

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

dir3 = getDirectory("Choose Destination Directory Saving Channel2\_PM");

dir4 = getDirectory("Choose Destination Directory Saving Histogram");

list1 = getFileList(dir1);

list2 = getFileList(dir2);

//setBatchMode;

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

open(dir1+list1[i]);

name1= getTitle();

rename("channel1");

selectWindow("channel1");

run("8-bit");

//setTool("Polygone Selections");

//waitForUser("dessiner contour zone");

//run("Clear Outside");

/// Application filtres nettoyage du bruit

run("Auto Local Threshold", "method=Otsu radius=45 parameter\_1=0 parameter\_2=0 white");

run("Median...", "radius=2");

run("Despeckle");

run("Select None");

/// detection des spots

run("Analyze Particles...", "size=0-200 pixel circularity=0-1.00 show=[Bare Outlines]");

run("Create Selection");

run("Create Mask");

run("Invert");

run("Fill Holes");

run("Create Selection");

selectWindow("channel1");

run("Restore Selection");

close("\\Others");

setForegroundColor(0, 0, 0);

run("Fill", "slice");

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

run("Select None");

setTool("Polygone Selections");

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

Dialog.create("Image quality");

Dialog.addCheckbox("Continue process?", true);

Dialog.show();

yoff=Dialog.getCheckbox();

if (yoff==true)

{

waitForUser("dessiner contour zone");

run("ROI Manager...");

roiManager("Add");

setBackgroundColor(0, 0, 0);

run("Clear Outside");

run("Convert to Mask");

run("Create Selection");

open(dir1+list1[i]);

name1= getTitle();

rename("channel2");

selectWindow("channel2");

run("Restore Selection");

run("Histogram");

saveAs("PNG", dir4+list1[i]);

selectWindow("channel2");

run("Measure");

close("\\Others");

setForegroundColor(0, 0, 0);

run("Fill", "slice");

saveAs("Tiff", dir3+list1[i]);

roiManager("Select", 0);

run("Measure");

run("Close");

open(dir1+list1[i]);

name1= getTitle();

rename("channel3");

selectWindow("channel3");

roiManager("Select", 0);

run("Measure");

run("Close");

roiManager("Delete");

}

}