//crop RFP and GFP images from Imaging machine

directory = "name\_of\_the\_fowlder";

//print(directory);

//directory=replace(directory,"\\","/");

//print(directory);

//wells = newArray("a001", "a002", "a003", "a004", "a005", "a006", "a007", "a008", "a009", "a010", "a011", "a012", "b001", "b002", "b003", "b004", "b005", "b006", "b007", "b008", "b009", "b010", "b011", "b012", "c001", "c002", "c003", "c004", "c005", "c006", "c007", "c008", "c009", "c010", "c011", "c012", "d001", "d002", "d003", "d004", "d005", "d006", "d007", "d008", "d009", "d010", "d011", "d012", "e001", "e002", "e003", "e004", "e005", "e006", "e007", "e008", "e009", "e010", "e011", "e012", "f001", "f002", "f003", "f004", "f005", "f006", "f007", "f008", "f009", "f010", "f011", "f012", "g001", "g002", "g003", "g004", "g005", "g006", "g007", "g008", "g009", "g010", "g011", "g012", "h001", "h002", "h003", "h004", "h005", "h006", "h007", "h008", "h009", "h010", "h011", "h012");

wells = newArray("f009");

n = lengthOf(wells);

for (i = 0; i <n ; i++){

//if (File.exists(path)){

open("directory/template.tif"); //open the template image for cropping

rename("template");

wait(1000);

well = wells[i];

command = "image=[" + directory + "] channel(s)=[] z-slice(s)=[] timepoint(s)=[] sub-position(s)=[] show\_stack method=max " + well;

//print(command);

run("Hyperstack IM04", command);

rename("image");

run("Split Channels");

resetMinAndMax();

//template matching plugin to detect the heart ROI in right field channel and use this ROI to crop the GFP and RFP channel----

selectWindow("C3-image");

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

//eliminate images out of focus excluding the images with a standard deviation below 400

run("Set Measurements...", "area mean standard min center shape redirect=None decimal=0");

run("Measure");

Std=getValue("StdDev");

if (Std<400) {

close("\*");

run("Clear Results");

} else{

//template matching plugin to detect the heart ROI in right field channel and use this ROI to crop the GFP and RFP channel----

run("Clear Results");

run("Template Matching Image", "template=template image=MAX\_C3-image rotate=[] matching\_method=[Normalised cross-correlation] number\_of\_objects=1 score\_threshold=0.7 maximal\_overlap=0.6 add\_roi");

selectWindow("C2-image");

roiManager("Select", 0);

run("Duplicate...", "duplicate");

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

run("Set Measurements...", "area mean standard min center shape redirect=None decimal=0");

run("Measure");

//eliminate images gfp negative excluding the images with a gfp intensity below 600

Mean=getValue("Mean");

if (Mean<600) {

close("\*");

run("Clear Results");

roiManager("Deselect");

roiManager("delete");

}else {

run("Clear Results");

selectWindow("C1-image");

roiManager("Select", 0);

run("Duplicate...", "duplicate");

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

run("Measure");

//eliminate images rfp negative exclding the images with a rfp intensity below 425

Mean=getValue("Mean");

if (Mean<425) {

close("\*");

run("Clear Results");

roiManager("Deselect");

roiManager("delete");

} else{

selectWindow("C2-image-1");

Dir="name\_of\_the\_fowlder";

saveAs(".tiff", Dir+well+"\_green\_");

selectWindow("C1-image-1");

Dir="name\_of\_the\_fowlder";

saveAs(".tiff", Dir+well+"\_red\_CROP");

run("Close All");

roiManager("Deselect");

roiManager("delete");

run("Clear Results");

}

}

}

}