//Ventricle and epicardial segmentation in cropped and deconvolved images

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

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");

n = lengthOf(wells);

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

well = wells[i];

run("Image Sequence...", "open=[D] file="+well+" sort");

run("Stack to Hyperstack...", "order=xyzct channels=4 slices=41 frames=1 display=Color");

run("Split Channels");

//run("Channels Tool...");

selectWindow("C1-image\_per\_macro");

//run("Channels Tool...");

run("Grays");

selectWindow("C2-image\_per\_macro");

run("Grays");

selectWindow("C3-image\_per\_macro");

run("Grays");

selectWindow("C4-image\_per\_macro");

run("Grays");

selectWindow("C3-image\_per\_macro");

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

rename("MAX\_red\_original");

selectWindow("C1-image\_per\_macro");

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

rename("Max\_green\_original");

selectWindow("C4-image\_per\_macro");

run("Z Project...", "projection=[Sum Slices]");

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

run("Gaussian Blur...", "sigma=2");

//ventricle segmentation with MorphoLibJ----------------------------------------------------------------------

run("Morphological Segmentation");

selectWindow("Morphological Segmentation");

wait(1000);

//setTool("multipoint");

call("inra.ijpb.plugins.MorphologicalSegmentation.setInputImageType", "object");

call("inra.ijpb.plugins.MorphologicalSegmentation.setGradientRadius", "5");

call("inra.ijpb.plugins.MorphologicalSegmentation.setGradientType", "External");

call("inra.ijpb.plugins.MorphologicalSegmentation.segment", "tolerance=15000.0", "calculateDams=true", "connectivity=8");

call("inra.ijpb.plugins.MorphologicalSegmentation.setDisplayFormat", "Watershed lines");

wait(1000);

call("inra.ijpb.plugins.MorphologicalSegmentation.createResultImage");

run("Create Selection");

//run("Make Inverse");

roiManager("Add");

roiManager("Split");

roiManager("Combine");

run("Create Mask");

rename("red\_mask");

run("Set Measurements...", "area mean standard min center perimeter bounding fit shape feret's redirect=None decimal=2");

run("Analyze Particles...", "size=5000-Infinity show=Masks display exclude clear add");

//run("Invert");

run("Create Selection");

run("Enlarge...", "enlarge=10");

run("Create Mask"); //ventricle mask-------------------------------------------------------------------------------------

rename("red\_enlarged"); //ventricle mask used for epicardial segmentation----------------------------------

//GFP nuclei segmentation with Stardist-----------------------------------------------------------------------------

selectWindow("C2-image\_per\_macro");

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

run("Enhance Contrast...", "saturated=0.1 normalize");

wait(1000);

run("Command From Macro", "command=[de.csbdresden.stardist.StarDist2D], args=['input':'MAX\_C2-image\_per\_macro', 'modelChoice':'Versatile (fluorescent nuclei)', 'normalizeInput':'true', 'percentileBottom':'13.700000000000001', 'percentileTop':'100.0', 'probThresh':'0.05', 'nmsThresh':'0.2', 'outputType':'Both', 'nTiles':'1', 'excludeBoundary':'5', 'roiPosition':'Automatic', 'verbose':'false', 'showCsbdeepProgress':'false', 'showProbAndDist':'false'], process=[false]");

wait(1000);

selectWindow("Label Image");

roiManager("XOR");

wait(1000);

run("Create Mask");

run("Watershed"); // final segmentation of GFP nuclei---------------------------------------------------------------

imageCalculator("AND create", "red\_enlarged","Mask"); //mask gfp image with heart area--------------

selectWindow("Result of red\_enlarged");

run("Set Scale...", "distance=1 known=0.65 unit=um");

run("Set Measurements...", "area mean standard min center perimeter bounding fit shape feret's redirect=Max\_green\_original decimal=2");

run("Analyze Particles...", "size=4.23-90 circularity=0.0-1.00 show=Masks display exclude clear add");

run("Invert");

roiManager("Deselect");

roiManager("Delete");

well="e012";

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

saveAs("Results", Dir+well+"\_deconvolved\_epic\_cells"+".csv"); //number of epicardial cells

run("Clear Results");

selectWindow("Mask of red\_mask");

run("Create Selection");

roiManager("Add");

selectWindow("SUM\_C4-image\_per\_macro");

roiManager("Select", 0);

run("Flatten");

run("Set Scale...", "distance=1 known=0.65 unit=um");

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

saveAs(".tiff", Direc+well+"\_red\_overlay");

selectWindow("Mask of red\_mask");

run("Set Scale...", "distance=1 known=0.65 unit=um");

saveAs(".tiff", Direc+well+"\_red\_mask");

run("Set Measurements...", "area mean standard min center perimeter bounding fit shape feret's redirect=MAX\_red\_original decimal=2");

run("Select None");

run("Analyze Particles...", " show=Masks display exclude clear add");

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

saveAs("Results", Dir+well+"\_deconvolved\_heart"+".csv"); //save ventricle area

selectWindow("Mask of Result of red\_enlarged");

run("Create Selection");

roiManager("Deselect");

roiManager("Delete");

roiManager("Add");

selectWindow("MAX\_C2-image\_per\_macro");

roiManager("Select", 0);

run("Flatten");

run("Set Scale...", "distance=1 known=0.65 unit=um");

saveAs(".tiff", Direc+well+"\_green\_overlay");

selectWindow("Result of red\_enlarged");

run("Set Scale...", "distance=1 known=0.65 unit=um");

saveAs(".tiff", Direc+well+"\_green\_maskTOT");

selectWindow("Mask of Result of red\_enlarged");

run("Set Scale...", "distance=1 known=0.65 unit=um");

saveAs(".tiff", Direc+well+"\_green\_maskCell");

roiManager("Deselect");

roiManager("Delete");

run("Clear Results");

close("\*");

}