// Script for segmentation of PROX1+/LYVE-1+ structures as used in Jafree et al. eLife 2019, written in JAVA format and used in ImageJ / FIJI

// Take nuclei from Ch1, vessels from Ch2, segment each and use Morphological reconstruction

// Generates an output with just LYVE-1+ vessels with PROX1+ overlapping nuclei

// Created by Dale Moulding (UCL GOSICH Light Microscopy facility) & modified by Daniyal Jafree. August 2019

// Segment PROX1+ nuclei

 run("Duplicate...", "title=orig duplicate");

 run("Duplicate...", "title=cells duplicate channels=1");

 run("Subtract Background...", "rolling=50 stack");

 setAutoThreshold("RenyiEntropy dark stack");

 run("Convert to Mask", "method=RenyiEntropy background=Dark");

 run("3D Simple Segmentation", "low\_threshold=128 min\_size=50 max\_size=10000"); //set nuclei size, requires tweaking

 setThreshold(1, 65535);

 setOption("BlackBackground", false);

 run("Convert to Mask", "method=Default background=Dark");

 run("Invert LUT");

 rename("cellsmask");

 selectWindow("Bin");

 close();

 selectWindow("cells");

 close();

// Segment LYVE-1+ structures

 selectWindow("orig");

 run("Duplicate...", "title=vessels duplicate channels=2");

 run("Subtract Background...", "rolling=50 stack");

 setAutoThreshold("Moments dark stack");

 run("Convert to Mask", "method=Moments background=Dark");

 run("3D Simple Segmentation", "low\_threshold=128 min\_size=100 max\_size=1000000");// set vessel sizes

 setThreshold(1, 65535);

 setOption("BlackBackground", false);

 run("Convert to Mask", "method=Default background=Dark");

 run("Invert LUT");

 rename("vesselsmask");

 selectWindow("Bin");

 close();

 selectWindow("vessels");

 close();

// Exclude non overlapping regions using IJPB plugin Morpholibj Morphological reconstruction

 setBatchMode(true);

 selectWindow("cellsmask");

 n = nSlices();

 for (i=1; i<=n; i++) {

 showProgress(i, n);

 selectWindow("cellsmask");

 setSlice(i);

 selectWindow("vesselsmask");

 setSlice(i);

 run("Morphological Reconstruction", "marker=cellsmask mask=vesselsmask type=[By Dilation] connectivity=4");

 if (i==1)

 output = getImageID();

 else {

 run("Select All");

 run("Copy");

 close();

 selectImage(output);

 run("Add Slice");

 run("Paste");

 }

 }

 run("Select None");

 setBatchMode(false);

// Create a new image of the segmented PROX1+/LYVE-1+ vessels on top of the original

 selectWindow("orig");

 run("Split Channels");

 run("Merge Channels...", "c1=C1-orig c2=C2-orig c4=cellsmask-rec create");

 selectWindow("vesselsmask");

 close();

 selectWindow("cellsmask");

 close();