//Click Run and a window will open for you to select your tomogram, select the raw data tiff exported from SuRVoS

filepath = File.openDialog("Select tomogram");

open(filepath);

filename = File.getName(filepath);

// // Create dialog box - A dialogue box will pop up where you can select to enhance the contrast on your data

//Dialog.create("Enhance Contrast?");

//Dialog.addCheckbox("Run enhance contrast?", false);

// Get dialog inputs

//runEnhancecontrast = Dialog.getCheckbox();

//if (runEnhancecontrast == true) {

//run("Enhance Contrast...", "saturated=50 normalize process\_all"); //enhance contrast on the data if ticked checkbox at start

//}

//selectWindow("data.tif");

//setThreshold(0, 150);

run("Convert to Mask", "method=Default background=Light calculate black"); //the tomogram will be made binary

//A window will open for you to select your survos level 0 annotation .tif file

filepath = File.openDialog("Select survos level 0 annotation");

open(filepath);

annotation = File.getName(filepath);

//Create dialog box - A dialogue box will pop up where you can select parameters

// select the radius you would like for the 'Closing' morphological filter on the SuRVoS annotation

//this will smooth the edge of the annotation and fill gaps

// select the radius you would like for the morphological filters on the objects

//this is the amount of erosion and dilation that will be used to smooth your objects

// select the dynamic for the Distance Transform Watershed 3D

//a lower dynamic will give more separations

// select the radius you would like for the ersoion of the watershed image

//the watershed image must be slightly eroded so that the 3D Objects Counter counts touching objects (default 1)

// select the circularity range

//here you can filter out objects that are not circular (e.g.speckle), 1 is a perfect circle

// choose to run a final despeckle if there is shadow and noise on the tomogram

//this prevents background intensities contributing to the volume of your objects

// select the size range of objects you want the 3D Objects Counter to count

//this will not count objects that are too small or too large to be bacteria

Dialog.create("Choose parameters");

Dialog.addNumber("Radius for Closing Filter on the SuRVoS annotation (pixels):", 2);

Dialog.addCheckbox("Fill holes on SuRVoS annotation?", false);

Dialog.addNumber("Radius for Erosion and Dilation of the objects (pixels):", 1);

Dialog.addNumber("Dynamic for Distance Transform Watershed 3D (Lower=more separations):", 1);

Dialog.addNumber("Radius for Erosion of the watershed (pixels):", 1);

Dialog.addNumber("Circularity min/max: ", 0.30);

Dialog.addToSameRow();

Dialog.addNumber("", 1.00);

Dialog.addCheckbox("Run final despeckle?", true);

Dialog.addNumber("Size min/max: ", 250);

Dialog.addToSameRow();

Dialog.addNumber("", 50000);

Dialog.show();

// Get dialog inputs

radiusValue = Dialog.getNumber();

runFillholes = Dialog.getCheckbox();

radiusValue = Dialog.getNumber();

dynamicValue = Dialog.getNumber();

radiusValue = Dialog.getNumber();

circularityMin = Dialog.getNumber();

circularityMax = Dialog.getNumber();

runDespeckle = Dialog.getCheckbox();

sizeMin = Dialog.getNumber();

sizeMax = Dialog.getNumber();

run("Make Binary", "method=Default background=Default calculate black"); //the SuRVoS annotation is made binary

run("Morphological Filters (3D)", "operation=Closing element=Cube x-radius=" + radiusValue + " y-radius=" + radiusValue + " z-radius=" + radiusValue); //smooths edges of inclusion

if (runFillholes == true) {

run("Fill Holes", "stack"); //fill holes in annotation if ticked checkbox at start

}

//not needed for 11 but helps 1

imageCalculator("AND create stack", filename, annotation); //combines the binary data and annotation to black-out the cytosol

selectWindow("Result of " + filename);

run("Fill Holes", "stack"); //fills gaps within bacteria cells

run("Morphological Filters (3D)", "operation=Erosion element=Ball x-radius=" + radiusValue + " y-radius=" + radiusValue + " z-radius=" + radiusValue);

run("Morphological Filters (3D)", "operation=Dilation element=Ball x-radius=" + radiusValue + " y-radius=" + radiusValue + " z-radius=" + radiusValue);

//erode away speckle/bumps and edge of bacteria but then dilate back to full volume of bacteria without bringing the speckle/bumps back

// this will be completed using the radius chosen at the start

run("Distance Transform Watershed 3D", "distances=[Borgefors (3,4,5)] output=[16 bits] normalize dynamic=" + dynamicValue + " connectivity=6");

//separates touching objects

run("Morphological Filters (3D)", "operation=Erosion element=Ball x-radius=" + radiusValue + " y-radius=" + radiusValue + " z-radius=" + radiusValue);

//makes the separations thicker such that touching objects are not counted as one object

Dialog.addNumber("Choose morphological radius", 1);

run("8-bit");

selectImage("Result-Erosion-Dilationdist-watershed-Erosion");

z=nSlices;

for (i=0; i>z; i++ ) {

run("Analyze Particles...", " circularity=" + circularityMin + "-" + circularityMax + " show=Masks display stack");

run("Invert LUT");

}

//removes non-circular obejcts e.g. background speckle that otherwise contributes to volume of bacteria

// this will be completed using the circularity range chosen at the start

if (runDespeckle == true) {

run("Despeckle", "stack"); //final despeckle if ticked checkbox at start

}

run("3D Objects Counter", "threshold=1 slice=76 size=" + sizeMin + "-" + sizeMax + " objects surfaces statistics summary");

//default min.=250 max.=50000

//counts objects, gives statistics window with volumes and objects map with numbered objects

//A window will open for you to select your raw tomogram for merge with the numbered surface map

filepath = File.openDialog("Reselect tomogram for Merge");

open(filepath);

filename = File.getName(filepath);

run("Merge Channels...", "c1=[Surface map of Result-Erosion-Dilationdist-watershed-Erosion] c4=data-1.tif");

//c4=data-1.tif is universal if using the raw exported data from SuRVoS as specified at start

//observe outlined and numbered bacteria