run("In [+]");

//setTool("rectangle");

makeRectangle(41, 38, 100, 92);

makeRectangle(41, 32, 100, 98);

makeRectangle(38, 32, 103, 98);

run("Duplicate...", "duplicate channels=1 frames=7");

selectWindow("21\_11\_19 BY4741\_pEB11\_CLIs-001 (series 01)-cell2.tif");

selectWindow("21\_11\_19 BY4741\_pEB11\_CLIs-001 (series 01)-cell2-1.tif");

run("In [+]");

run("Measure");

run("Measure");

run("Measure");

run("Measure");

run("In [+]");

//setTool("freehand");

run("Measure");

run("3D Manager Options", "volume surface compactness fit\_ellipse integrated\_density mean\_grey\_value std\_dev\_grey\_value minimum\_grey\_value maximum\_grey\_value distance\_to\_surface centre\_of\_mass\_(pix) bounding\_box radial\_distance exclude\_objects\_on\_edges\_xy distance\_between\_centers=10 distance\_max\_contact=1.80 drawing=Contour");

run("3D Manager");

selectWindow("21\_11\_19 BY4741\_pEB11\_CLIs-001 (series 01)-cell2-1.tif-3Dseg");

Ext.Manager3D\_Segment(245, 4095);

run("In [+]");

run("In [+]");

run("In [+]");

run("In [+]");

Ext.Manager3D\_AddImage();

Ext.Manager3D\_Measure();

close();

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

selectWindow("c:1/2 z:13/31 t:7/8 - BY4741\_pEB11\_CLIs-001.nd2 (series 01)");

run("In [+]");

run("In [+]");

run("In [+]");

setAutoThreshold("Default dark");

//run("Threshold...");

setThreshold(245, 65535);

//setTool("wand");

doWand(42, 47);

setBackgroundColor(0, 0, 0);

run("Clear Outside");

resetThreshold();

run("Close");

doWand(69, 49);

setMinAndMax(0, 4095);

setOption("ScaleConversions", true);

run("8-bit");

run("Auto Local Threshold", "method=Niblack radius=5 parameter\_1=0 parameter\_2=0 white");

//run("Threshold...");

run("Analyze Particles...", "size=6-Infinity pixel display exclude clear add");

resetThreshold();

roiManager("Deselect");

run("Close");

//setTool("rectangle");

run("Fill Holes");

setAutoThreshold("Default dark");

//run("Threshold...");

run("Analyze Particles...", "size=6-Infinity pixel display exclude clear add");