//This ImageJ macro will correct fluorescence microscopy movies for background.

// It takes into account the dark count from the camera and the beam profile from the laser.

sourceFolder = "K:/170331\_1/"; //define source folder

files = getFileList(sourceFolder); //find filenames

run("Set Measurements...", "min redirect=None decimal=0"); //set measurements

for (i = 0; i < files.length;i++)

{

IJ.log("Open " + files[i]);

run("Bio-Formats Importer", "open=" + sourceFolder+files[i] + " color\_mode=Grayscale view=Hyperstack stack\_order=XYCZT");

rename("temp1");

run("32-bit"); //Important, since we will be dividing pixel values.

run("Z Project...", "start=4 projection=[Average Intensity]");

run("Gaussian Blur...", "sigma=40");

run("Subtract...", "value=300"); //300 is measured dark count.

run("Measure");

IJ.log(i);

max =(getResult("Max",i));

IJ.log("max =" + max);

run("Divide...", "value=max");

selectWindow("temp1");

run("Subtract...", "value=300 stack");

imageCalculator("Divide 32-bit stack", "temp1","AVG\_temp1"); //correction by dividing.

selectWindow("AVG\_temp1");

close();

selectWindow("temp1");

setMinAndMax(0, 65535); // revert back to 16 bit. Important for further analysis.

run("16-bit");

filename=("Corrected\_"+files[i]);

IJ.log("save : " + sourceFolder+ "corrected\_"+files[i]);

saveAs("Tiff", sourceFolder + "corrected\_"+files[i]);

run("Close All");

}