**Macro for image analysis**

//1. Pre-processing

run("Colors...", "foreground=black background=black selection=white");

run("Set Measurements...", "area mean standard min integrated redirect=None decimal=3");

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

rename("Master");

run("Duplicate...", "title=nucleus\_mask duplicate");

//2. Nucleus Segmentation

selectWindow("nucleus\_mask");

run("8-bit");

waitForUser("Using the channel slider, select the DAPI channel and click OK");

run("Gaussian Blur...", "sigma=1 stack");

run("Enhance Contrast...", "saturated=0.30");

selectWindow("nucleus\_mask");

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

selectWindow("nucleus\_mask");

Stack.setPosition(3, 1, 1)

setAutoThreshold("Triangle dark");

setOption("BlackBackground", true);

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

run("Watershed", "stack");

run("Cascade");

setTool("wand");

selectWindow("nucleus\_mask");

waitForUser("Choose nucleus of interest and click OK");

run("Clear Outside", "stack");

roiManager("Add");

roiManager("Select", 0);

roiManager("Rename", "nucleus\_mask");

//3. Heterochromatin Segmentation

run("Cascade");

selectWindow("forHCmask");

roiManager("Select", 0);

run("Clear Outside", "stack");

run("Threshold...");

setAutoThreshold("Triangle dark");

waitForUser("Set heterochromatin threshold and click OK");

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

run("Close");

run("Create Selection");

roiManager("Add");

roiManager("Select", 1);

roiManager("Rename", "HCmask");

//4. Heterochromatin Subtraction

selectWindow("nucleus\_mask");

roiManager("Select", 1);

run("Clear", "stack");

run("Create Selection");

roiManager("Add");

roiManager("Select", 2);

roiManager("Rename", "Nucleoplasm\_mask");

close("nucleus\_mask");

close("forHCmask");

// 5. Measure intensities in the respective ROIs

selectWindow("Master");

Stack.setPosition(1, 1, 1)

roiManager("Select", 0);

run("Measure");

roiManager("Select", 1);

run("Measure");

roiManager("Select", 2);

run("Measure");

Stack.setPosition(2, 1, 1)

roiManager("Select", 0);

run("Measure");

roiManager("Select", 1);

run("Measure");

roiManager("Select", 2);

run("Measure");

Stack.setPosition(3, 1, 1)

roiManager("Select", 0);

run("Measure");

roiManager("Select", 1);

run("Measure");

roiManager("Select", 2);

run("Measure");

waitForUser("Save Results", "Save Results and click OK");

close();

close();

close("ROI Manager");

close("Results");