run("Clear Results");

roiManager("reset");

dir1 = getDirectory("Choose Source Directory for Image DAY1");

dir2 = getDirectory("Choose Source Directory for Image DAY2");

dir3 = getDirectory("Choose Source Directory for Image DAY3");

dir4 = getDirectory("Choose Source Directory for Image DAY4");

dir5 = getDirectory("Choose Source Directory for Image TOP");

dir6 = getDirectory("Choose Source Directory for Image BOT");

list1 = getFileList(dir1);

list2 = getFileList(dir2);

list3 = getFileList(dir3);

list4 = getFileList(dir4);

for (i=0; i<list1.length; i++) {

open(dir1+list1[i]);

name1= getTitle();

rename("channel1");

run("16-bit");

open(dir2+list2[i]);

name2= getTitle();

rename("channel2");

run("16-bit");

open(dir3+list3[i]);

name3= getTitle();

rename("channel3");

run("16-bit");

open(dir4+list4[i]);

name4= getTitle();

rename("channel4");

run("16-bit");

run("Images to Stack", "TOP" );

setTool("rectangle");

makeRectangle(168, 286, 6360, 2826);

run("Crop");

run("MultiStackReg", "stack\_1=Stack action\_1=Align file\_1=[] stack\_2=None action\_2=Ignore file\_2=[] transformation=[Rigid Body]");

run("8-bit");

saveAs("Gif", dir5+list4[i]);

run("Close All");

open(dir1+list1[i]);

name1= getTitle();

rename("channel1");

run("16-bit");

open(dir2+list2[i]);

name2= getTitle();

rename("channel2");

run("16-bit");

open(dir3+list3[i]);

name3= getTitle();

rename("channel3");

run("16-bit");

open(dir4+list4[i]);

name4= getTitle();

rename("channel4");

run("16-bit");

run("Images to Stack", "TOP" );

setTool("rectangle");

makeRectangle(144, 3764, 6360, 2826);

run("Crop");

run("MultiStackReg", "stack\_1=Stack action\_1=Align file\_1=[] stack\_2=None action\_2=Ignore file\_2=[] transformation=[Rigid Body]");

run("8-bit");

saveAs("Gif", dir6+list4[i]);

run("Close All");

}