

```
if (roiManager("count")>0 )
{roiManager("delete")};
if (roiManager("count")>0 )
{roiManager("delete")};
run("Clear Results");
run("Colors...", "foreground=white background=black selection=yellow");
run("Set Measurements...", "area mean integrated area_fraction redirect=None
decimal=3");
```

```
//home directory selection
rep=getDirectory("choose a directory");
Dialog.create("ma boite");
list=getFileList(rep);
```

```
//Creation of a Results folder
File.makeDirectory(rep+"Mask");
```

```
numero=1;
extension=".TIF";
```

```
//Counting the number of images in the folder
liste=getFileList(rep);
nombre_images=0;
for (i=0;i<liste.length;i++)
{
    x = indexOf(liste[i], ".TIF");
    if(x>=0){
        nombre_images=nombre_images+1;
    }
}
```

```
//Creation of results tables
T_aire_ROI=newArray(100);
T_aire_Bacteries=newArray(100);
T_int_moy=newArray(100);
T_Nom=newArray(100);
```

```
// Loop on all pictures
numero=0;
for (numero=0;numero<liste.length;numero++){
```

```
if (indexOf(liste[numero], ".TIF")>0)
{
    compteur=compteur+1;
    //Determination of paths for each image
    ImageName2=liste[numero];
```

```

chemin1=rep+ImageName2;
open(chemin1);
ImageName=File.nameWithoutExtension;
T_Nom[numero]=ImageName;

//Choice of the region of interest and measurement of its area
waitForUser("Draw area of interest");
run("Clear Results");
run("Measure");
T_aire_ROI[numero]=getResult("Area",0);
run("Clear Outside");
run("Select All");
run("Duplicate...", "title=Image");
run("Duplicate...", "title=Image2");
run("Duplicate...", "title=Masque");
run("Duplicate...", "title=IntensiteBacteries");

```

```

selectWindow("Masque");
//Using the Color Threshold function
// Color Thresholder 2.0.0-rc-61/1.52n
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=47;
max[0]=255;
filter[0]="pass";
min[1]=0;
max[1]=255;
filter[1]="pass";
min[2]=104;
max[2]=255;
filter[2]="pass";
for (i=0;i<3;i++){
selectWindow(""+i);
setThreshold(min[i], max[i]);
run("Convert to Mask");
}

```

```

        if (filter[i]=="stop") run("Invert");
    }
    imageCalculator("AND create", "0","1");
    imageCalculator("AND create", "Result of 0","2");
    for (i=0;i<3;i++){
        selectWindow(""+i);
        close();
    }
    selectWindow("Result of 0");
    close();
    selectWindow("Result of Result of 0");
    rename(a);
    // Colour Thresholding-----

```

```

imageCalculator("Multiply create 32-bit", "Image","Masque");
rename("Bacteries");

```

```

//Measurement of the area covered by bacteria + recording of masks

```

```

run("Clear Results");
selectWindow("Masque");
run("Select All");
run("Measure");
T_aire_Bacteries[numero]=(getResult("Area",0)*getResult("%Area",0))/100;
saveAs("tif", rep+"Mask"+File.separator+ImageName+"-MASQUE_Binaire");
rename("Masque");

```

```

//Measurement of the average intensity of bacteria in the surface of interest

```

```

run("Clear Results");
selectWindow("IntensiteBacteries");
run("Split Channels");
selectWindow("IntensiteBacteries (green)");
imageCalculator("AND create", "IntensiteBacteries (green)","Masque");
rename("IntensiteBacteries");
run("Select All");
run("Measure");
T_int_moy[numero]=getResult("RawIntDen",0)/T_aire_Bacteries[numero];
print(getResult("IntDen",0));
print(T_aire_Bacteries[numero]);
saveAs("tif", rep+"Mask"+File.separator+ImageName+"-MASQUE_Bacteries");
rename("IntensiteBacteries");

```

```

//Drawing of the areas corresponding to the bacteria in the image

```

```

selectWindow("Masque");
run("Analyze Particles...", " show=[Bare Outlines] clear include summarize add");
rename("Masque2");
run("Invert");
run("Magenta");

```

```

run("RGB Color");
selectWindow("Image2");
run("RGB Color");
imageCalculator("Add create", "Image2","Masque2");
saveAs("tif", rep+"Mask"+File.separator+ImageName+"-Outlines");
rename("Masque3");

//Control image creation
selectWindow("IntensiteBacteries");
run("Select All");
run("Copy");
selectWindow("Masque3");
run("Add Slice");
run("Paste");
selectWindow("Masque");
run("Select All");
run("Copy");
selectWindow("Masque3");
setSlice(2);
run("Add Slice");
run("Paste");
saveAs("tif", rep+"Mask"+File.separator+ImageName+"-MASQUES");

run("Close All");

}
}

// Results display

run("Clear Results");

for (i=0;i<numero;i++)
{

    setResult("Label",i,T_Nom[i]);
    setResult("ROI Area",i,T_aire_ROI[i]);
    setResult("Bacteria area",i,T_aire_Bacteries[i]);
    setResult("% area occupied by bacteria",i,100*(T_aire_Bacteries[i]/T_aire_ROI[i]));
    setResult("Avg intensity of bacteria",i,T_int_moy[i]);
    updateResults();
}
updateResults();

saveAs("Results", rep+"Mask"+File.separator+"Results");

```