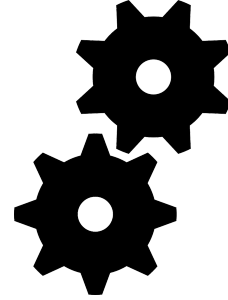


The background of the slide is an aerial photograph of the EPFL campus. In the foreground, there are several modern buildings with flat roofs, some featuring solar panels. A prominent red sculpture is visible on the left. In the middle ground, a large, white, curved building with a unique architectural design stands out. The campus is surrounded by greenery and trees. In the background, a large body of water (Lake Geneva) is visible, with mountains in the distance under a dramatic, cloudy sky.

Java & Fiji Workshop

BIO-410

Exercise solution



Q. Leaf delineation

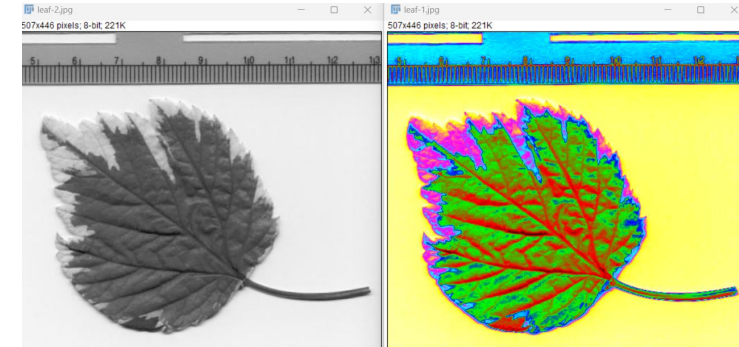
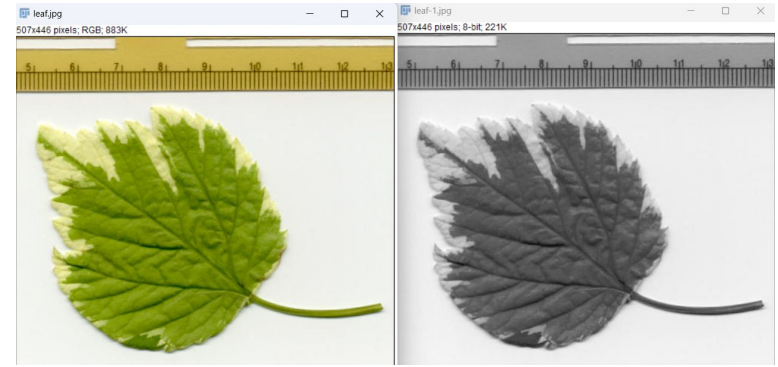
- Open the macro recorder
- Open the leaf image
- Duplicate it and convert it to 8-bits

QUESTION : is the conversion affect display ? Pixel values ? How can you see it ?

- Change the LUT of the 8-bit image to 6-shades

QUESTION : is the LUT changing affect display ?

Pixel values ?



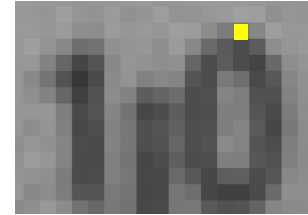
QUESTION : is the conversion affect display ? Pixel values ? How can you see it ?

- Yes, it affects the display
- Yes, it changes pixel values

value=169,145,045 (#a9912d)



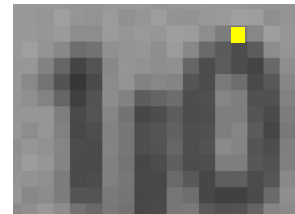
value=120



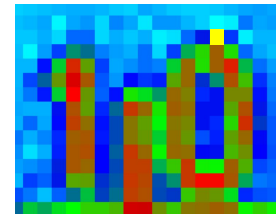
QUESTION : is the LUT changing affect display ? Pixel values ?

- Yes, it changes the display
- No, it doesn't change the pixel values

value=120



value=120



Q.Leaf delineation

- Crop the image around the leaf
- Make 2 duplications of the cropped image
- Threshold the two images such that you end-up with the 2 parts of the leaf
- Do a binary median filtering to remove small blobs

Hint: Try different thresholding methods.



R.Leaf delineation

- `selectImage("leaf-2.jpg");`
- `setAutoThreshold("Default");`
- `//run("Threshold...");`
- `//setThreshold(0, 160);`
- `setOption("BlackBackground", true);`
- `run("Convert to Mask");`
- `run("Median", "radius=3");`

```
selectImage("leaf-1.jpg");  
run("Auto Threshold", "method=Triangle white");  
//run("Threshold...");  
//setThreshold(0, 231);  
run("Convert to Mask");  
run("Median", "radius=3");
```

Q.Leaf delineation

Now we have our two binary images, we would like to create a **ROI** of the leaf borders and do some **measurements**

- First, set the measurements to analyze (Perimeter, mean, min/max, area)
- Use the **Analyze particles** plugin to create the ROI of the border
- Do it also for the second image

Have a look to the ROI manager.

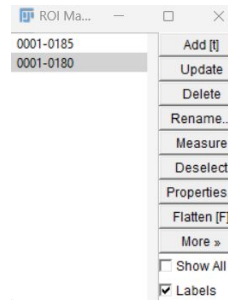
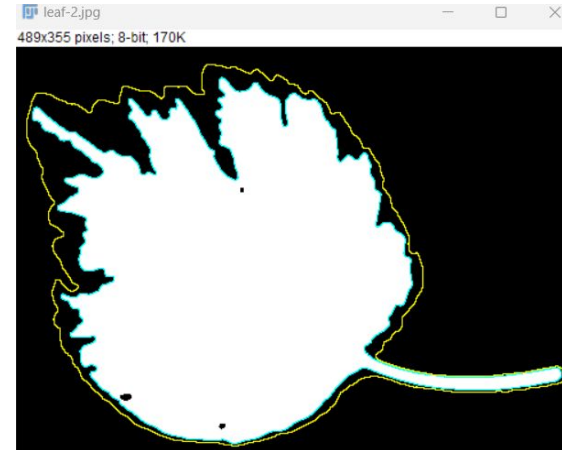
Select alternatively ROIs and images.

What is it possible to do ?



```
selectImage("leaf-2.jpg");  
run("Set Measurements...", "area mean standard min centroid center perimeter  
display redirect=None decimal=3");  
run("Analyze Particles...", "display overlay add");  
selectImage("leaf-1.jpg");  
run("Analyze Particles...", "display overlay add");
```

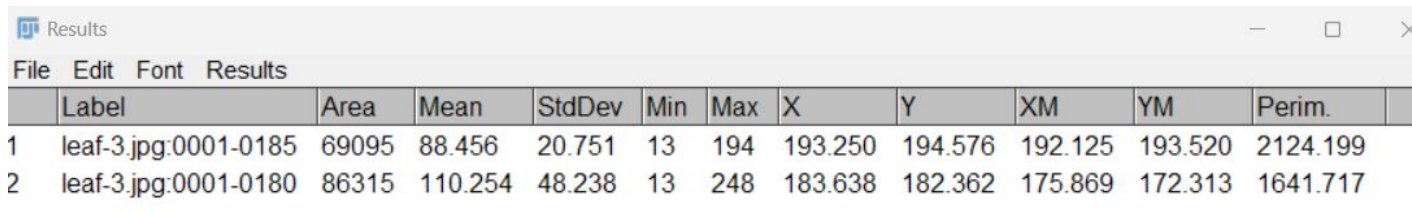
It's possible to show any ROI from
the ROI manager on any selected
images



Q.Leaf delineation

- Select the 8-bits image
- Clear the measurements
- Select one ROI and click on **Measure** in the ROI manager
- Select the second ROI and measure it.

Do you see a real difference on the mean intensity between the 2 ROIs ?

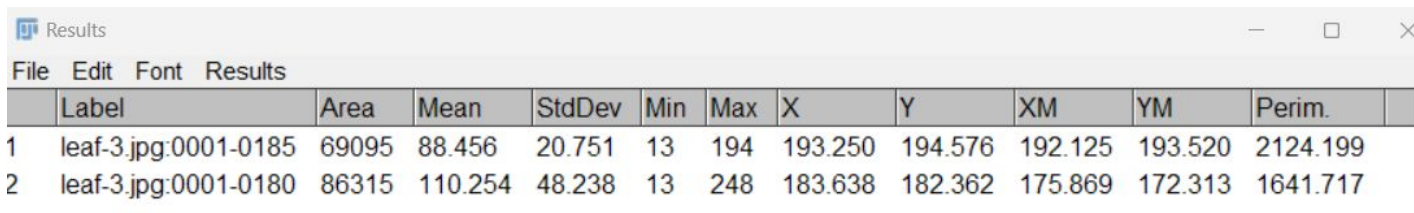


The screenshot shows a software window titled 'Results' with a menu bar (File, Edit, Font, Results) and a table of measurement data. The table has 12 columns: Label, Area, Mean, StdDev, Min, Max, X, Y, XM, YM, and Perim. There are two rows of data, labeled 1 and 2.

	Label	Area	Mean	StdDev	Min	Max	X	Y	XM	YM	Perim.
1	leaf-3.jpg:0001-0185	69095	88.456	20.751	13	194	193.250	194.576	192.125	193.520	2124.199
2	leaf-3.jpg:0001-0180	86315	110.254	48.238	13	248	183.638	182.362	175.869	172.313	1641.717

- `selectImage("leaf-3.jpg");`
- `run("Clear Results");`
- `roiManager("Select", 0);`
- `roiManager("Measure");`
- `roiManager("Select", 1);`
- `roiManager("Measure");`

There is a small difference between the means but the standard deviation has doubled



The screenshot shows a window titled 'Results' with a menu bar (File, Edit, Font, Results) and a table of measurement data. The table has 13 columns: Label, Area, Mean, StdDev, Min, Max, X, Y, XM, YM, and Perim. There are two rows of data, both for the file 'leaf-3.jpg:0001-0185'.

	Label	Area	Mean	StdDev	Min	Max	X	Y	XM	YM	Perim.	
1	leaf-3.jpg:0001-0185	69095	88.456	20.751	13	194	193.250	194.576	192.125	193.520	2124.199	
2	leaf-3.jpg:0001-0180	86315	110.254	48.238	13	248	183.638	182.362	175.869	172.313	1641.717	

Q.Leaf delineation

In order to compare the 'black' part with the 'white' part, we need to create a ROI of only the white part (and not the black+white part)

- Select the 2 ROIs in the ROI manager
- Click on **More -> XOR**. The new ROI is created but yet saved in the ROI manager
- Press 't' on your keyboard to add it
- Select the new ROI and click on **Measure**

Compare the mean intensities



R.Leaf delineation

```
roiManager("Select", newArray(0,1));  
roiManager("XOR");  
roiManager("Add");  
selectImage("leaf-3.jpg");  
roiManager("Select", 2);  
roiManager("Measure");
```

Results					
File	Edit	Font	Results		
	Label	Area	Mean	StdDev	
1	leaf-3.jpg:0001-0185	69095	88.456	20.751	
2	leaf-3.jpg:0001-0180	86315	110.254	48.238	
3	leaf-3.jpg:0180-0244	17220	197.718	19.479	



Q.Leaf delineation

Finally, you would like to export a nice image of the leaf

- Show all the ROIs
- Select the outer one and change its color to Red+ stroke width = 3
- Select the inner one and change its color to Green + stroke width = 3
- Select the combined one and change its fill color to Yellow
- Go under File -> save as tiff
- Open the image you've just saved on Fiji and PowerPoint

What happens ?



R.Leaf delineation

```
roiManager("Select", 2);  
RoiManager.setGroup(0);  
RoiManager.setPosition(0);  
roiManager("Set Fill Color", "yellow");  
roiManager("Select", 0);  
RoiManager.setGroup(0);  
RoiManager.setPosition(0);  
roiManager("Set Color", "green");  
roiManager("Set Line Width", 3);  
roiManager("Select", 1);  
RoiManager.setGroup(0);  
RoiManager.setPosition(0);  
roiManager("Set Color", "red");  
roiManager("Set Line Width", 3);
```

ROIs are saved as overlay, readable on Fiji
but not on Power-point

Fiji



Power-point



Q.Leaf delineation

The ROIs are saved with the image as overlay. To have image with ROIs, readable on any software, you can use the **flatten** option

- Show all the ROIs on the image you would like to export
- Click on **flatten**
- Save the new image again

What does the flatten option ?

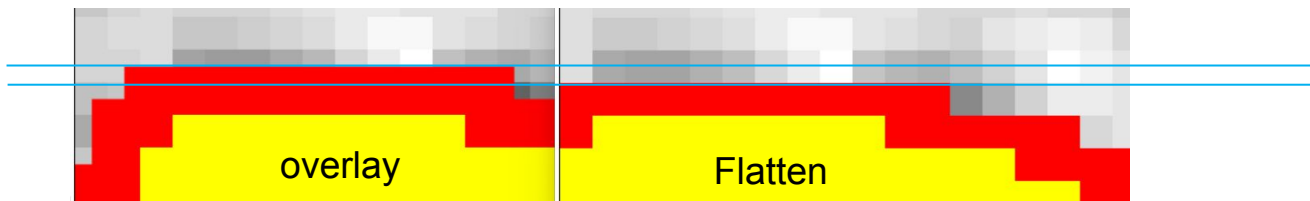
Does it modify the display ? Pixel values ?



Flatten **burns** full pixels with the color of the overlay.

It is NOT an overlay anymore

- `selectImage("leaf-3.jpg");`
- `roiManager("Show All without labels");`
- `run("Flatten");`



- Flatten doesn't change the display ; you still see the different colors
- But it changes pixel values, as it converts the 8-bits image into a RGB image

