



EARTH & LIFE INSTITUTE

ImageJ training



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UCL

UNIVERSITÉ CATHOLIQUE DE LOUVAIN

Outline

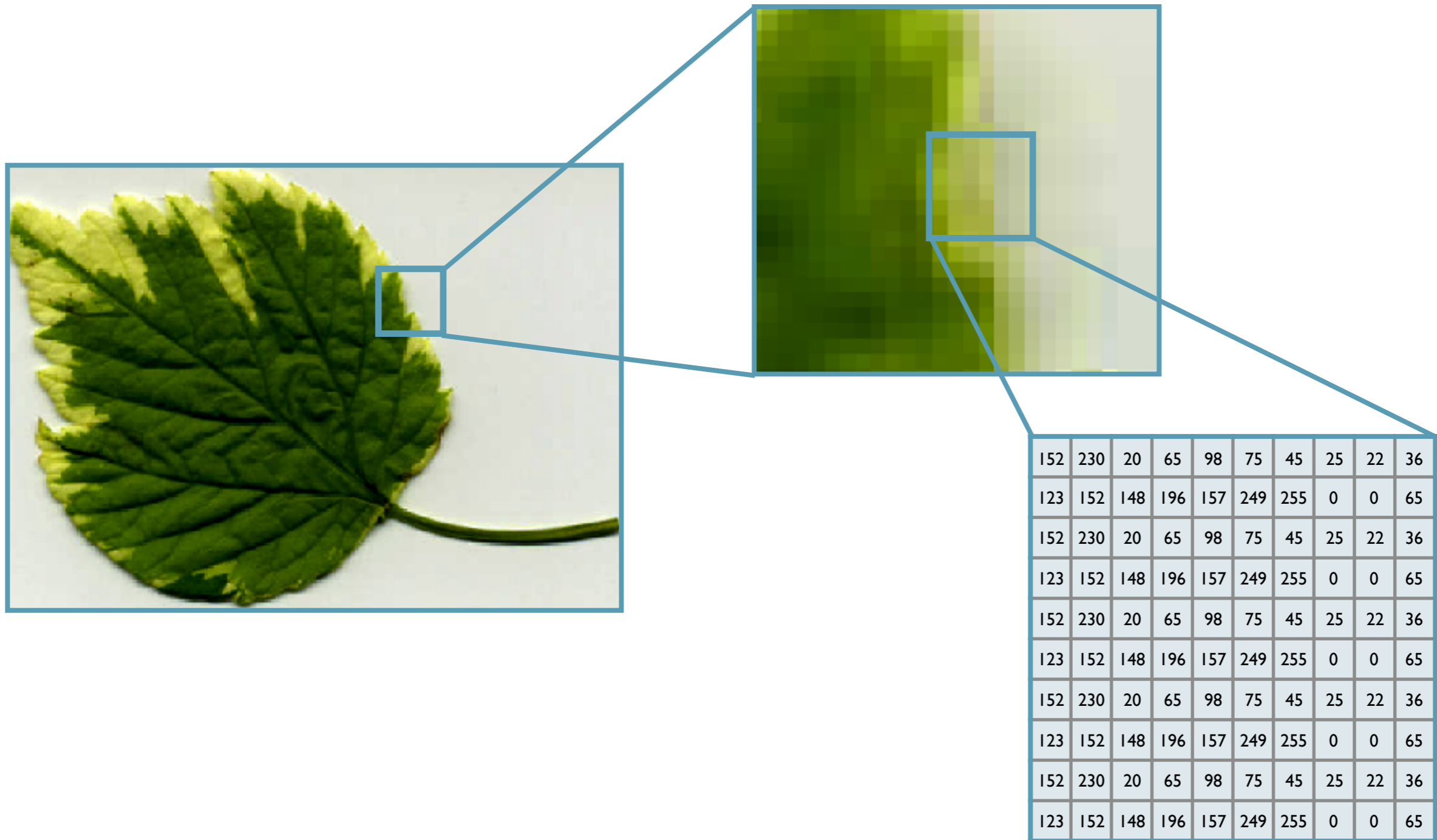
- What is an image?
- What is image analysis?
- What is ImageJ
- What are macros and plugins?



What is an image?

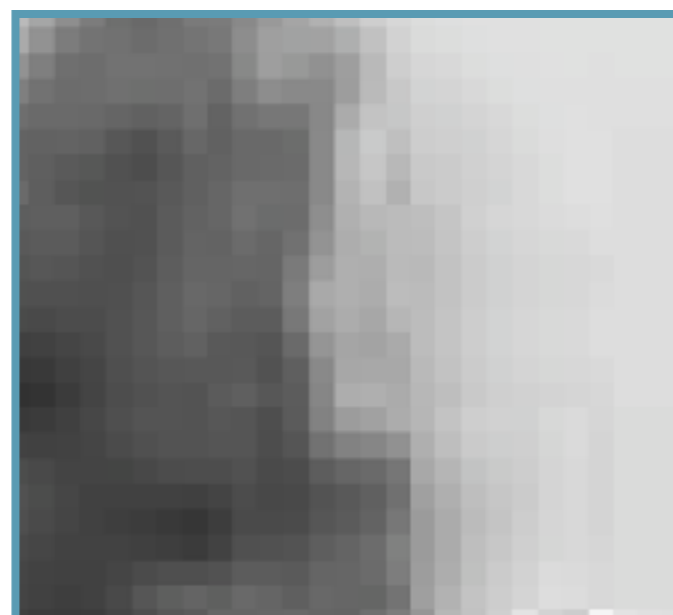
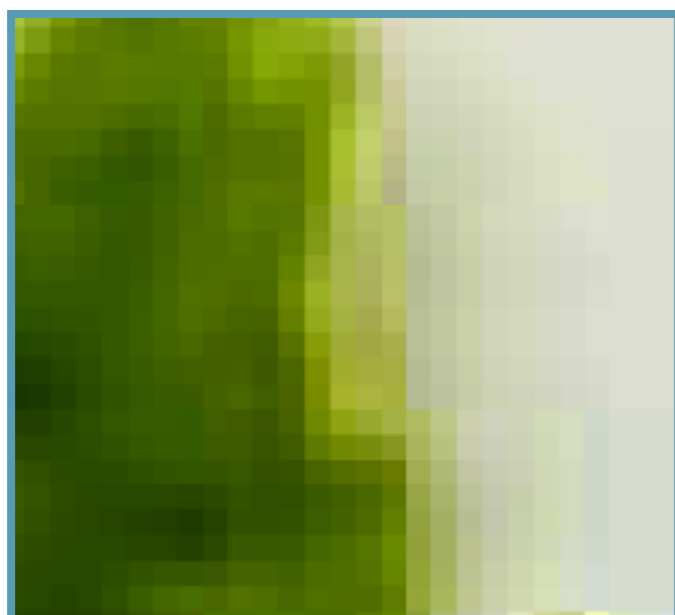


What is an image?



Matrix of values

Matrix of value



**8-bit
integer [0-255]**

		152	230	20	65	98	75	45	25	22	36		
	152	230	20	65	98	75	45	25	22	36	65		
152	230	20	65	98	75	45	25	22	36	65	36		
123	152	148	196	157	249	255	0	0	65	36	65		
152	230	20	65	98	75	45	25	22	36	65	36		
123	152	148	196	157	249	255	0	0	65	36	65		
152	230	20	65	98	75	45	25	22	36	65	36		
123	152	148	196	157	249	255	0	0	65	36	65		
152	230	20	65	98	75	45	25	22	36	65	36		
123	152	148	196	157	249	255	0	0	65	36	65		
152	230	20	65	98	75	45	25	22	36	65			
123	152	148	196	157	249	255	0	0	65				

R**G****B**

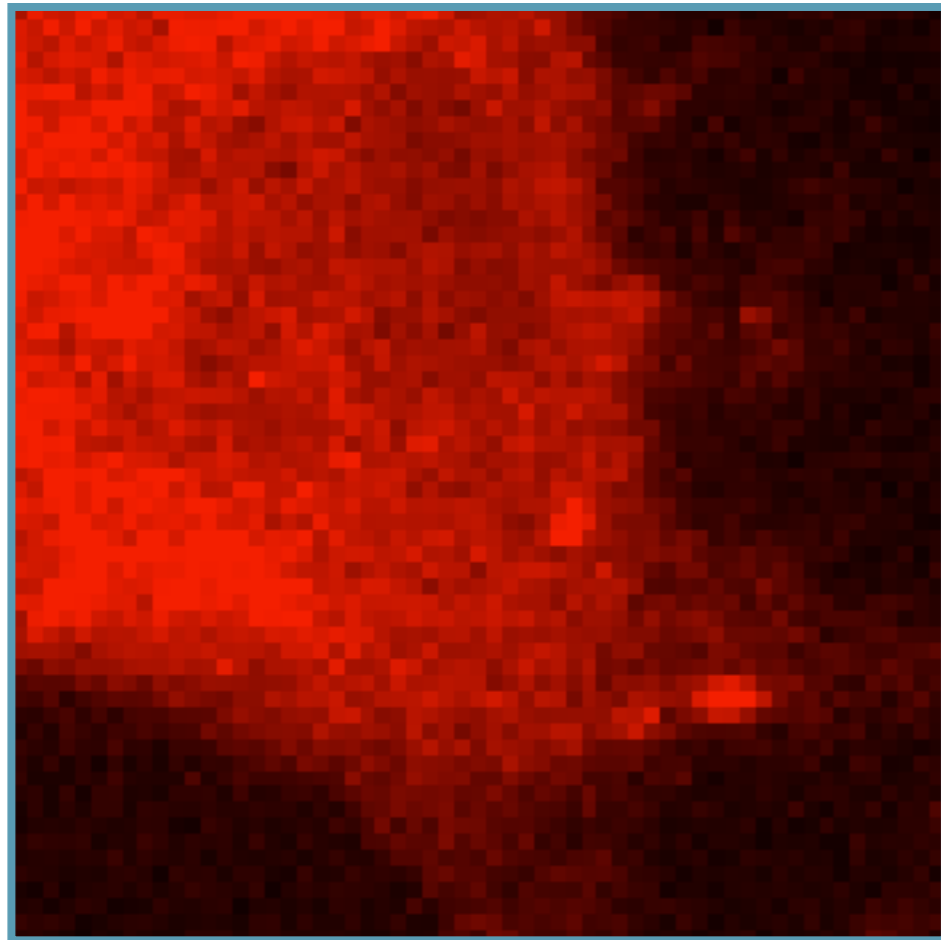
152	230	20	65	98	75	45	25	22	36
123	152	148	196	157	249	255	0	0	65
152	230	20	65	98	75	45	25	22	36
123	152	148	196	157	249	255	0	0	65
152	230	20	65	98	75	45	25	22	36
123	152	148	196	157	249	255	0	0	65
152	230	20	65	98	75	45	25	22	36
123	152	148	196	157	249	255	0	0	65
152	230	20	65	98	75	45	25	22	36
123	152	148	196	157	249	255	0	0	65

**32-bit
real values**

Greyscale

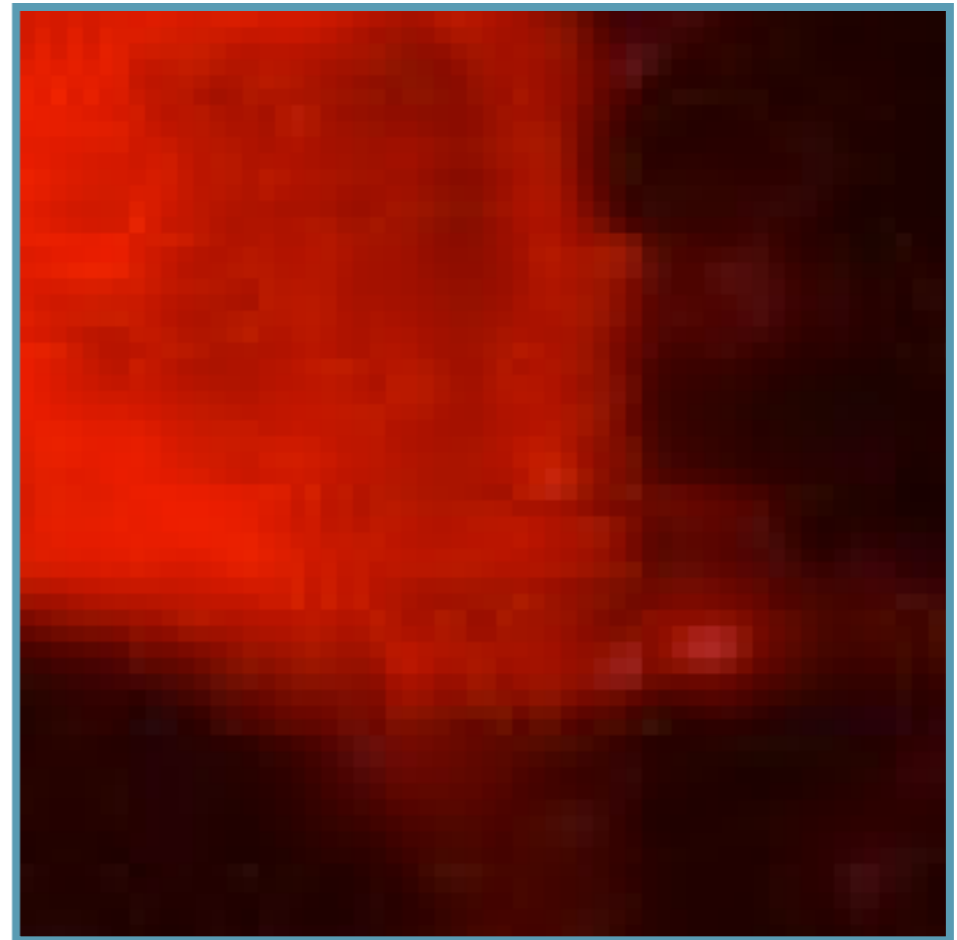
Types of images

TIFF



530K

JPEG



18K



What is image analysis?



What is image analysis?

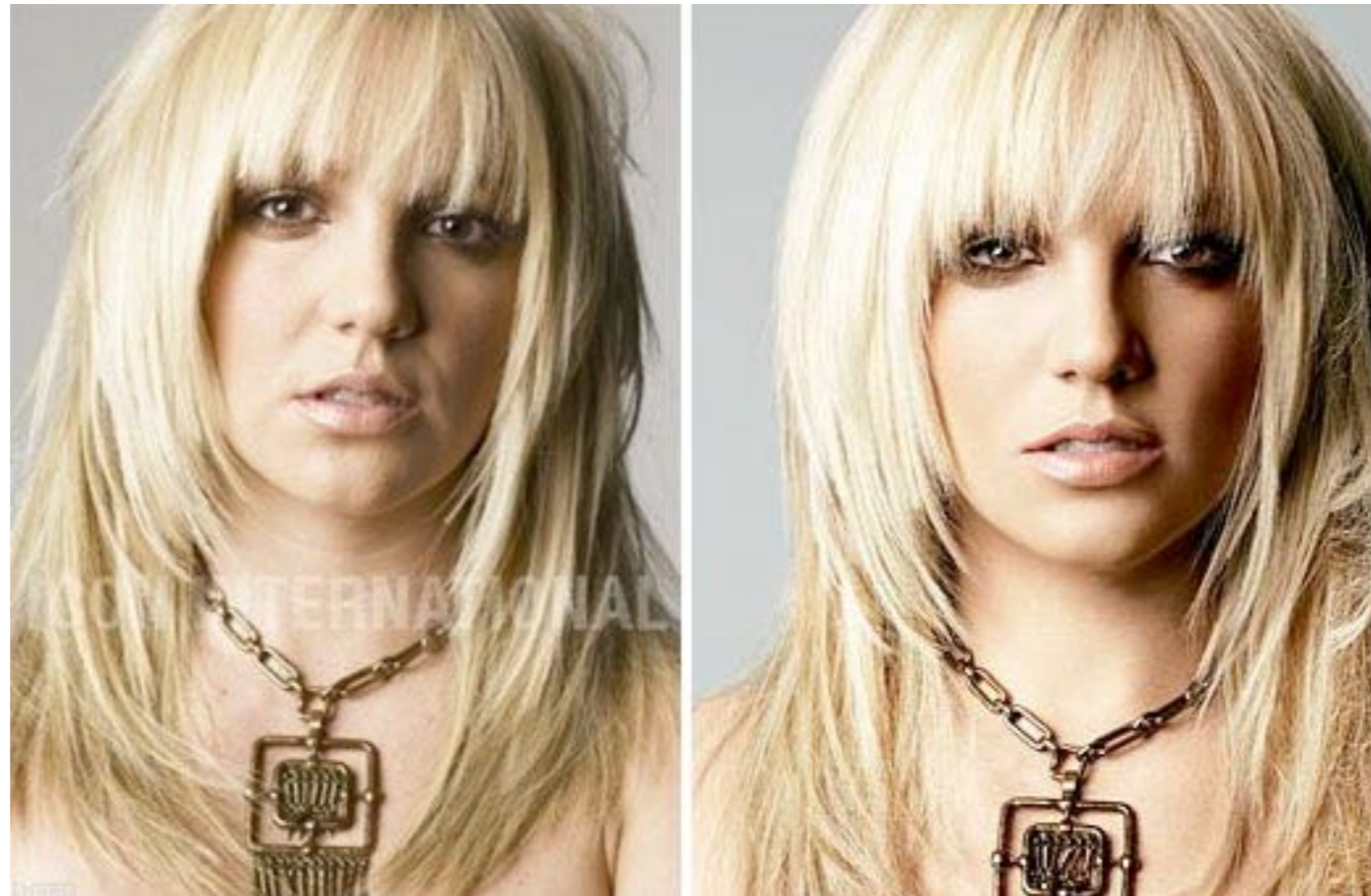
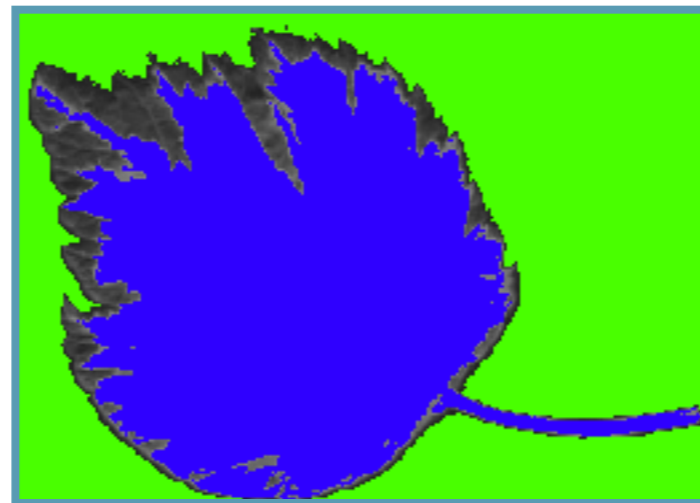


Image analysis **IS NOT** image manipulation
ImageJ **IS NOT** Photoshop

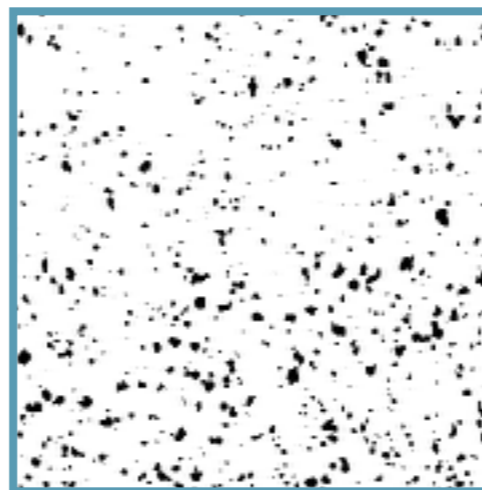
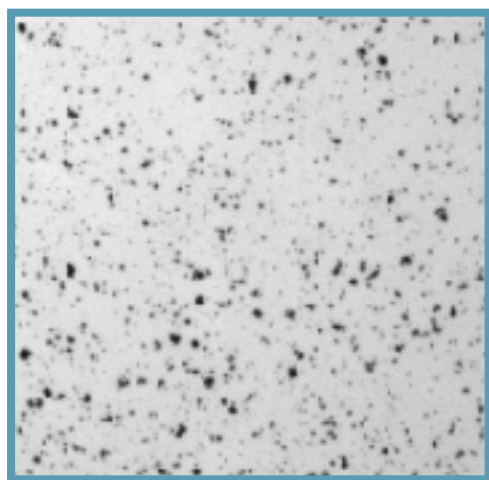


What is image analysis?

Image analysis is the extraction of information from images



21% of the leaf surface is infected



710 cells
avg size = 15 px²

Main advantages of automated image analysis

Removes human appreciation



Automation of processes

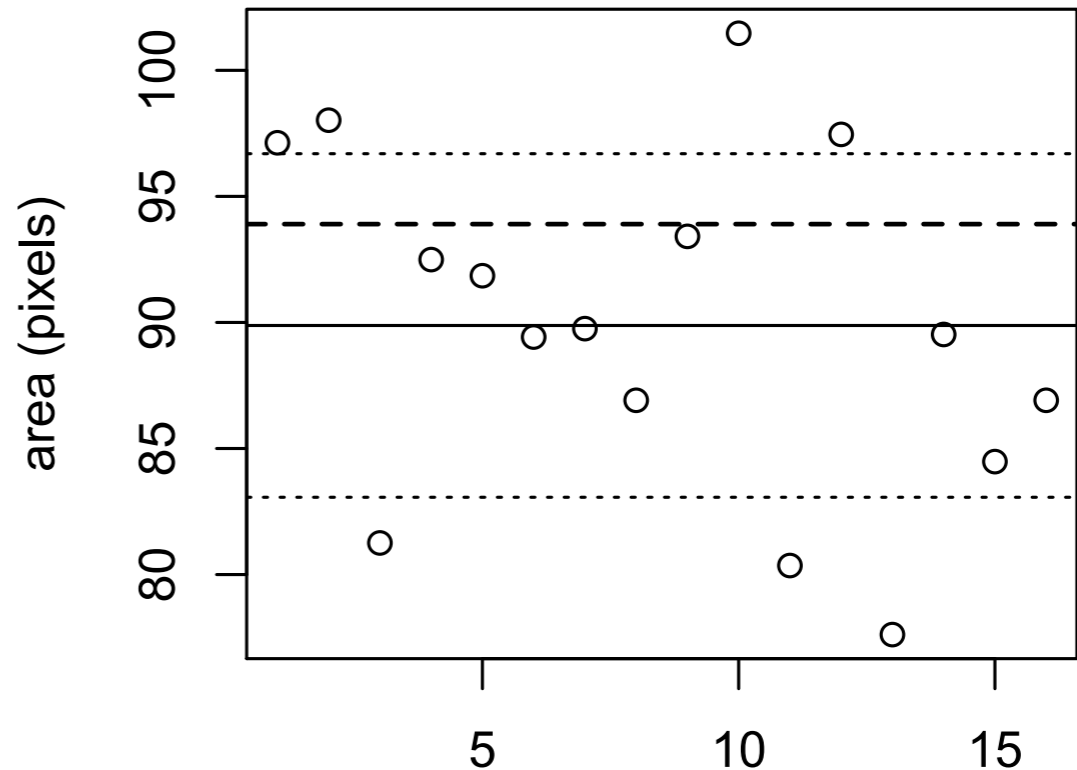
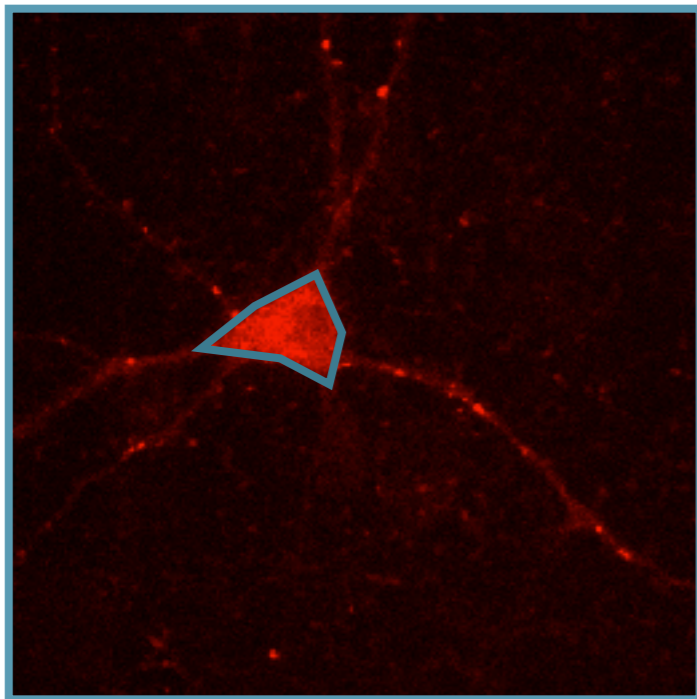


Image analysis steps

Isolate objects



Measure objects



Image analysis basics:

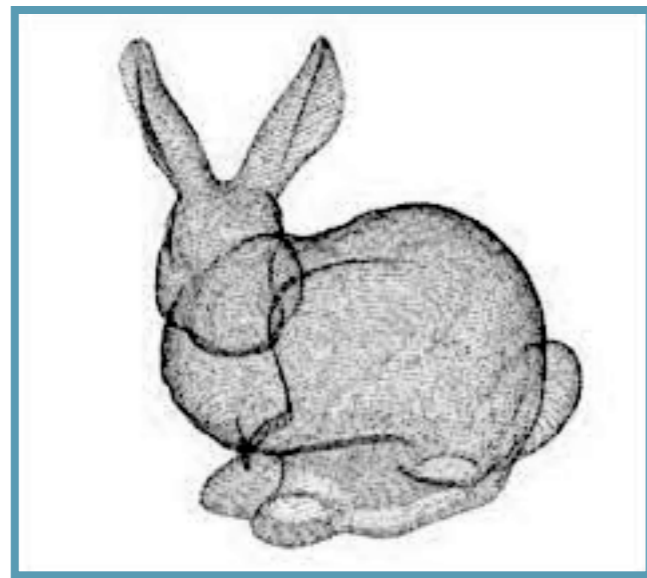
Image scale

Principle

Link between pixel
and physical size

DPI

Dots Per Inch
Pixels Per 2.54 cm



Pixels

200

cm

10

scale

20 px/cm

50 DPI

Image analysis basics: Histogram

Principle

Distribution of pixel values

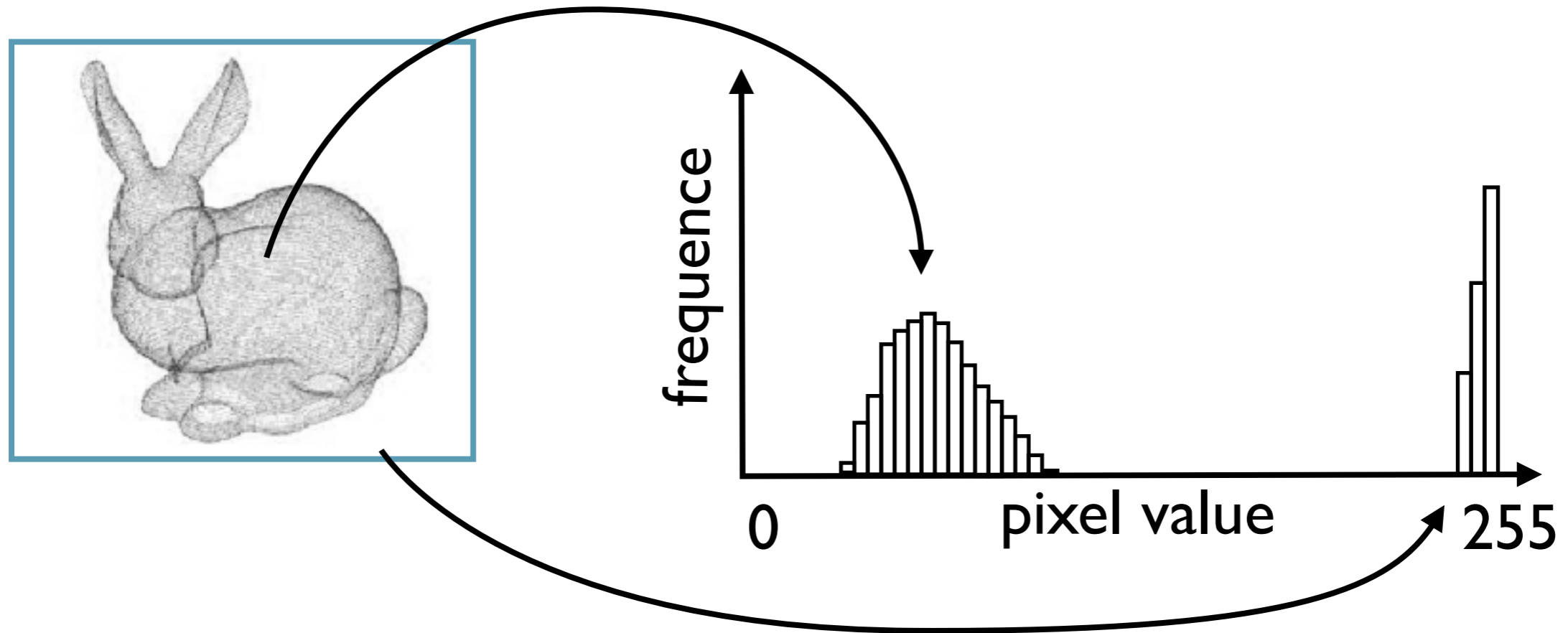
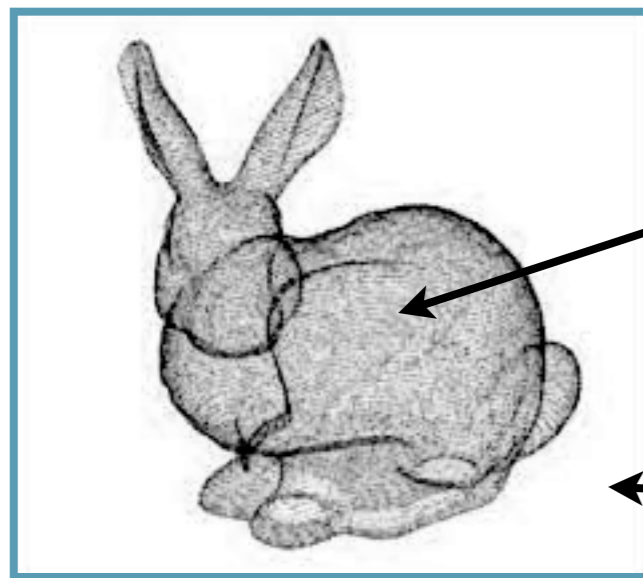


Image analysis basics: Thresholding

Principle

Isolate the object from
the rest of the image



Object

Background

Image analysis basics: Thresholding

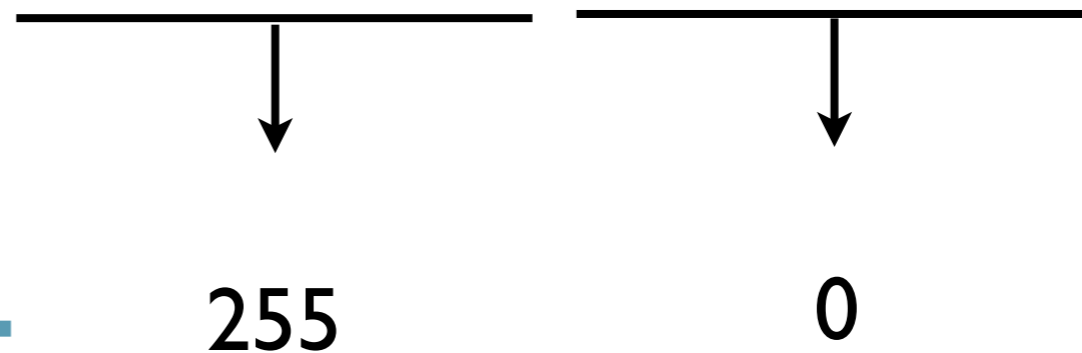
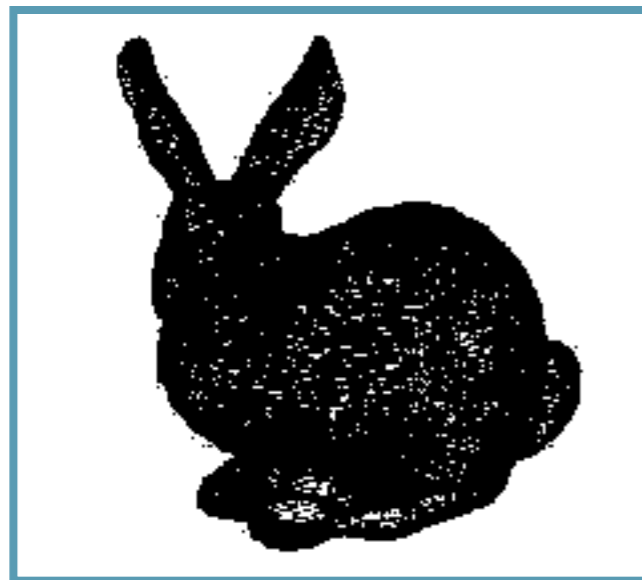
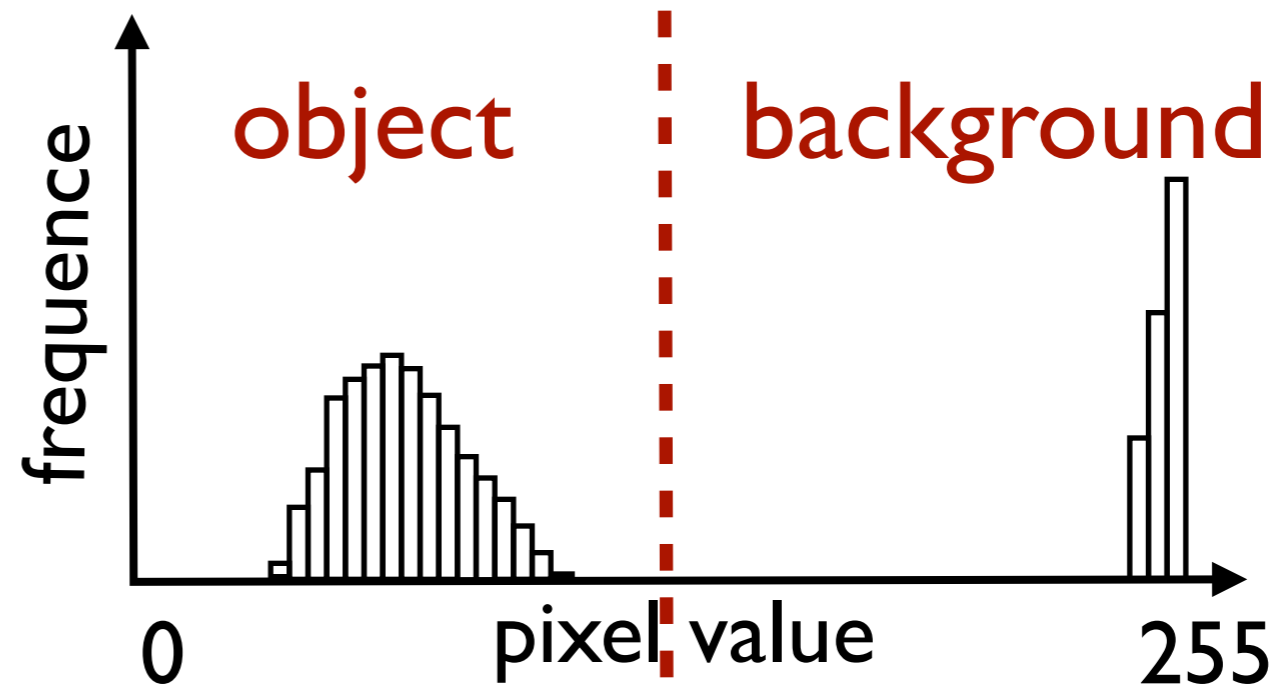
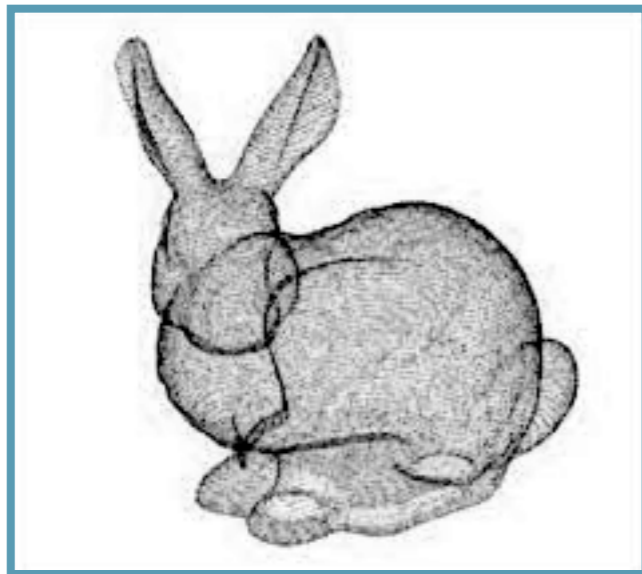
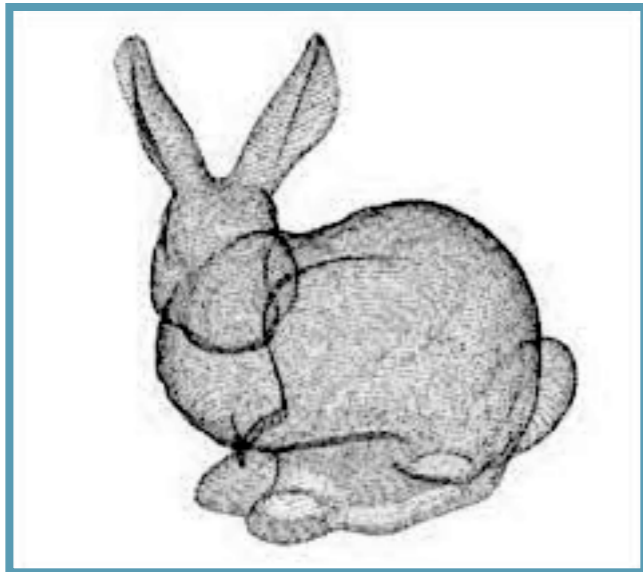


Image analysis basics: Thresholding



Be careful with thresholding

use a **fixed** threshold value

use an **algorithm**

But use **always** the same

Basic functions: Thresholding

Different fixed values

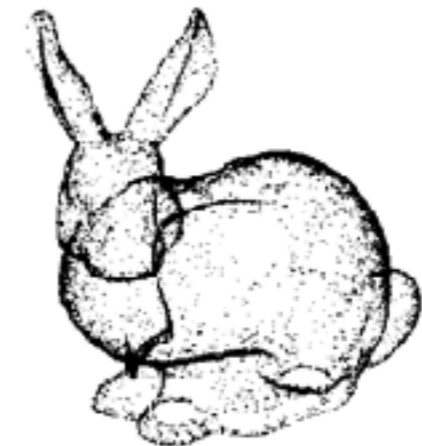
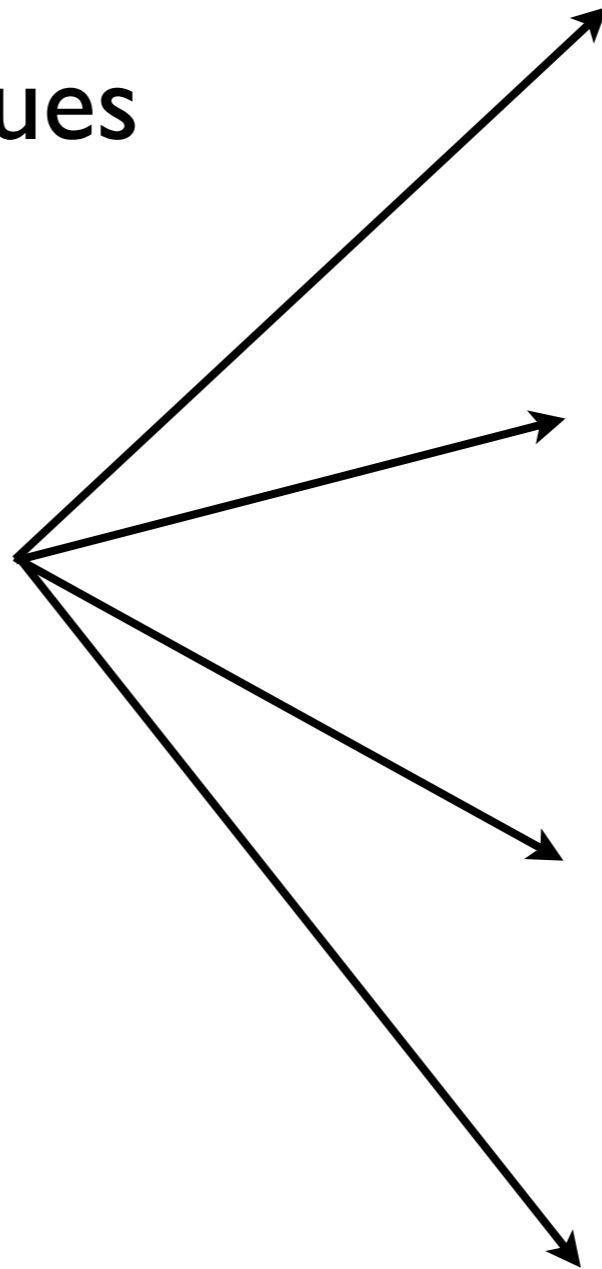
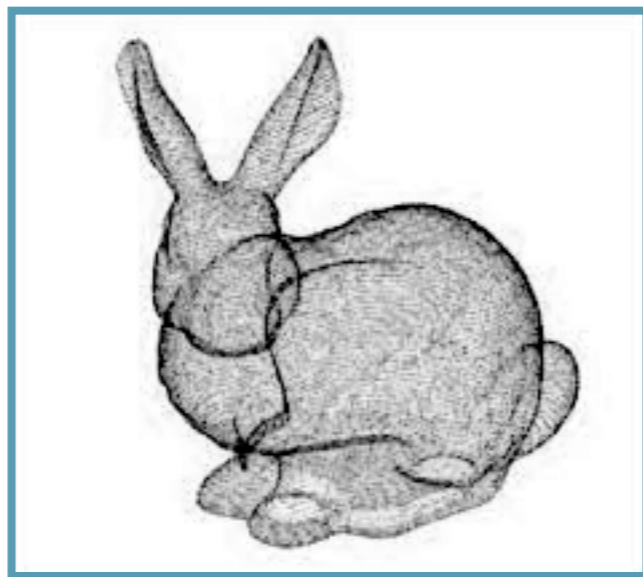
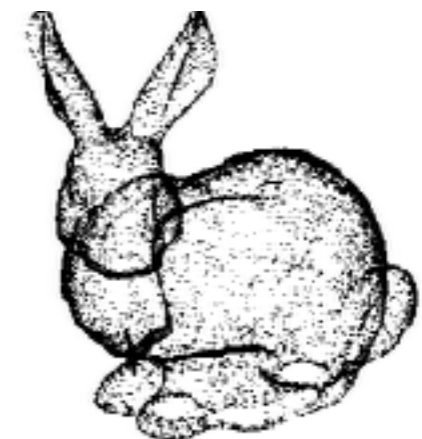
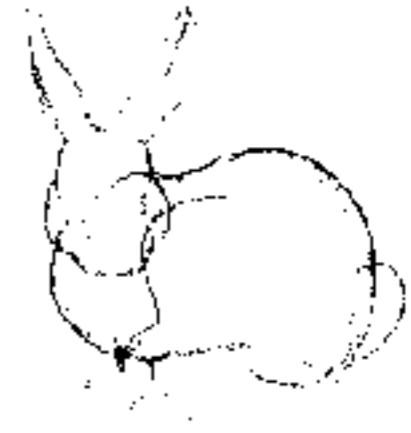
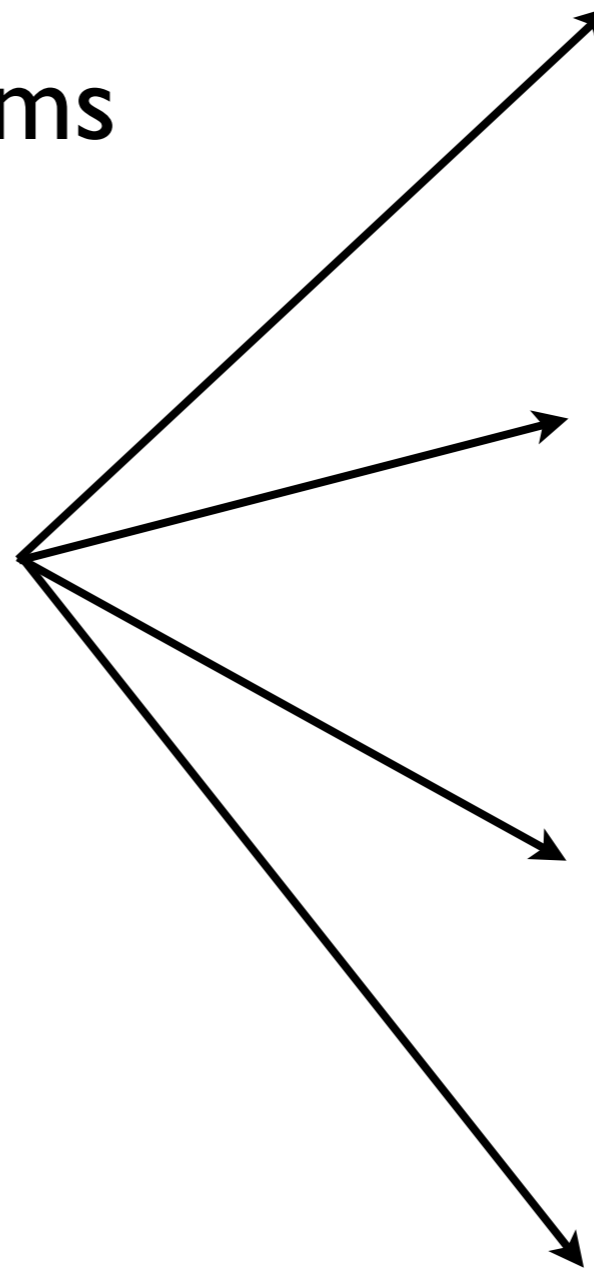
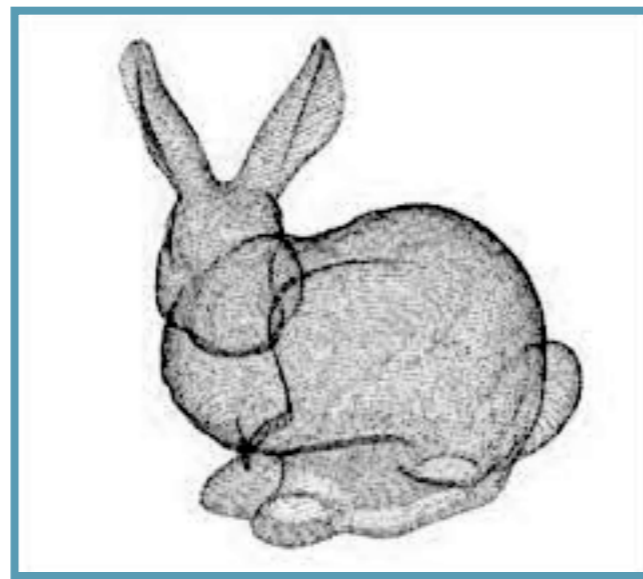


Image analysis basics: Thresholding

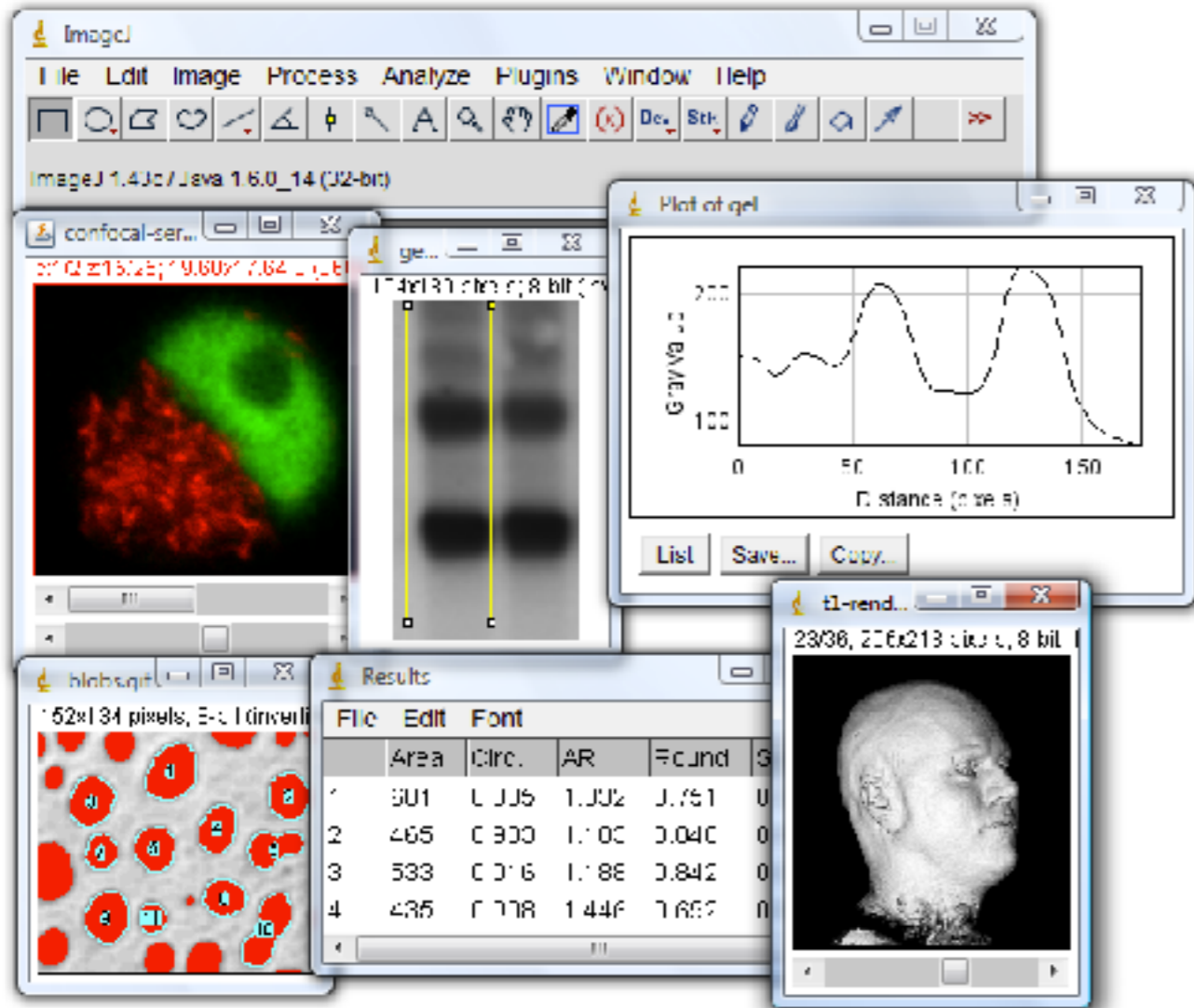
Different algorithms



What is ImageJ?



ImageJ



- Open source
- Developed at the NIH
- Created in 1986
- Plugin and macro
- Current version: 1.46

<http://rsb.info.nih.gov>

<http://fiji.sc/>



ImageJ menu

File Basic file operations (opening, saving, creating new images).

Edit Editing and drawing operations as well as global settings.

Image Conversion and modification of images including geometric transformations.

Process Image processing, including point operations, filters and arithmetic operations.

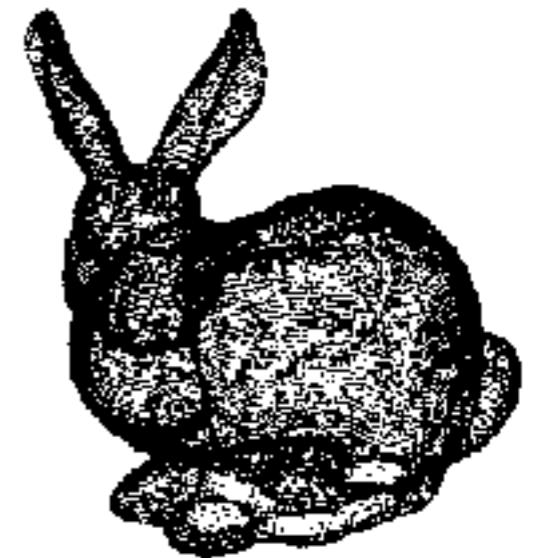
Analyze Statistical measurements, profile and histogram plotting and other operations related to image analysis.

Plugins Commands for creating, editing and managing add-ons



Exercise 1 - Thresholding

1. Open the image `bunny.tiff`
2. Duplicate the RGB image
3. Change the image type to 8-bit
4. Duplicate the 8-bit image
5. Threshold the image
6. Save the thresholded image



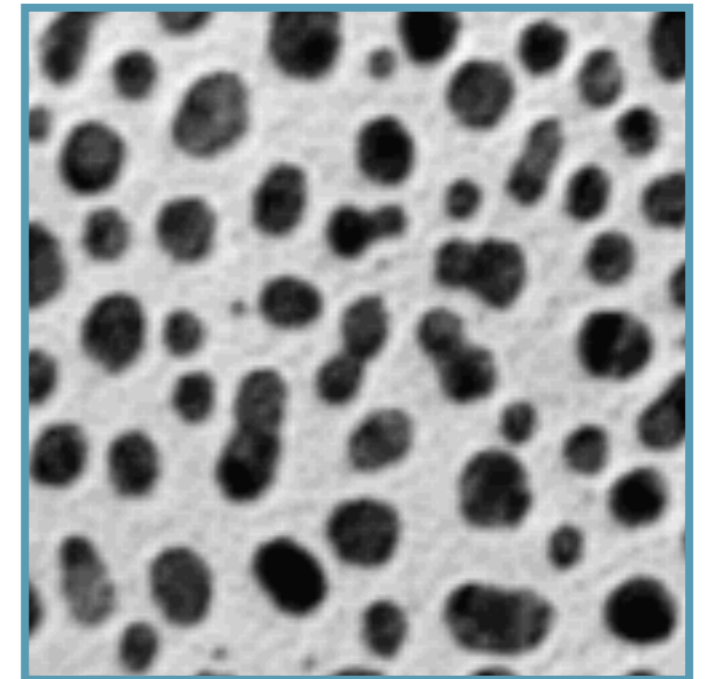
Exercise 1 - Thresholding

1. File > Open
2. Image > Duplicate
3. Image > Type > 8-bit
4. Image > Duplicate
5. Image > Adjust > Threshold
6. File > Save as



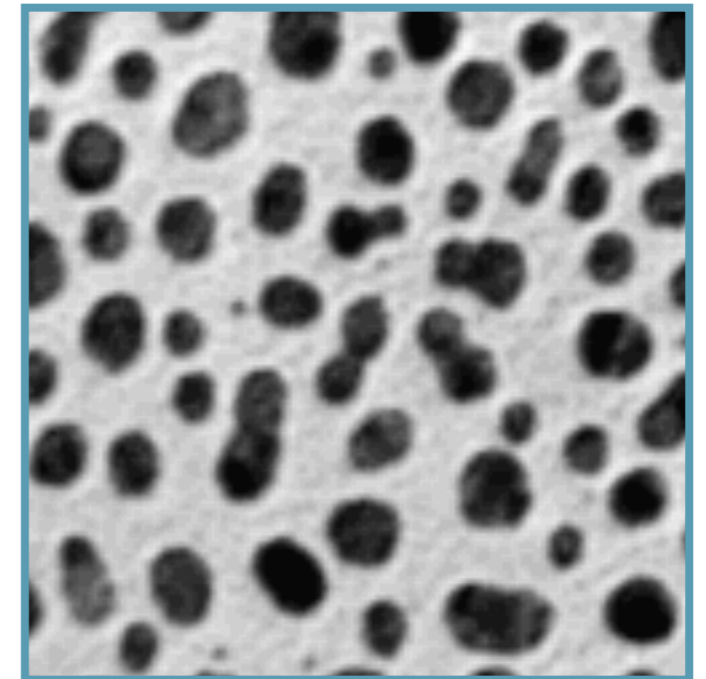
Exercise 2 - Counting objects

1. Open the image [blobs.gif](#)
2. Set the image [scale](#) to 300 DPI
3. [Threshold](#) the image
4. Create a [binary](#) image
5. [Separate](#) the objects
6. [Count](#) the objects



Exercise 2 - Counting objects

1. File > Open Samples
2. Analyze > Set scale...
3. Image > Adjust > Threshold
4. Process > Binary > Make binary
5. Process > Binary > Watershed
6. Analyze > Analyze particles



What are macros and plugins?



Macros and plugins

Macros

Set of ImageJ commands
Useful for automation

Plugins

New commands
More complex image analysis



Creating macro

ImageJ built-in macro recording tool

Plugins > Macros > Record...

Macro manual

<http://rsb.info.nih.gov/ij/developer/macro/macros.html>

Launch the macro

Plugins > Macros > Run...



My first macro

```
run("Blobs (25K)");  
run("Set Scale...", "distance=300 known=1 pixel=1 unit=cm");  
setAutoThreshold("Default");  
setThreshold(121, 255);  
run("Convert to Mask");  
run("Make Binary");  
run("Watershed");  
run("Analyze Particles...", "size=100-Infinity  
circularity=0.00-1.00 show=Nothing summarize");
```

Finish lines with ;

Comment lines with //



A bit more complex

```
setBatchMode(true);

dir=getDirectory("Where are your images");
list=getFileList(dir);
num=list.length;

for(k = 0 ;k < num ; k++){
    open(dir+list[k]);
    run("Set Scale...", "distance=300 known=2.54 pixel=1 unit=cm");
    run("Set Measurements...", "area redirect=None decimal=2");
setAutoThreshold("Default");
    run("Convert to Mask");
    run("Make Binary");
    run("Watershed");
    run("Analyze Particles...", "size=0.1-Infinity
circularity=0.00-1.00 show=Nothing display summarize");
    close();
}
```



Example of plugin: SmartRoot

