EMBO Practical Course

Insights into plant biological processes through phenotyping

Image analysis tools:
software and libraries used in plant trait measurement

Guillaume Lobet

Université of Liège, PhytoSYSTEMS

@guillaumelobet

Plant Systems Biology
A VIB-UGENT DEPARTMENT

Université de Liège

UCL Université catholique de Louvain

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Insights into plant biological processes through phenotyping

1. What is **an image ?**

2. What is **image analysis ?**

3. What is **ImageJ ?**

4. What are **macros and plugins ?**

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Insights into plant biological processes through phenotyping

1. What is **an image ?**

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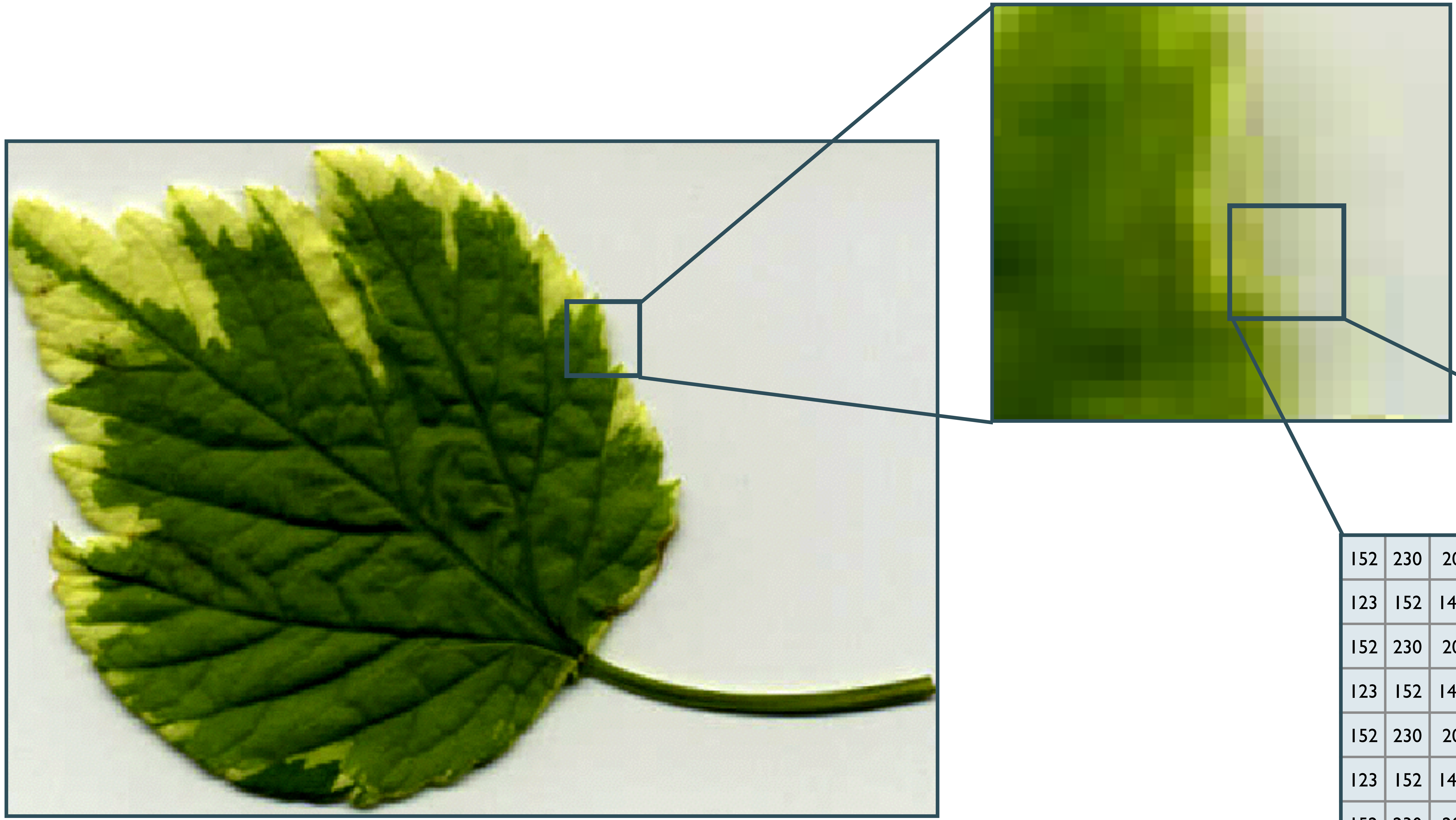
What is an image?



Matrix of values

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

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An image is a matrix of values

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

RGB

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

Greyscale

8-bit
integer [0-255]

32-bit
real values

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What is image analysis?

Image analysis **IS NOT** image manipulation

ImageJ **IS NOT** Photoshop

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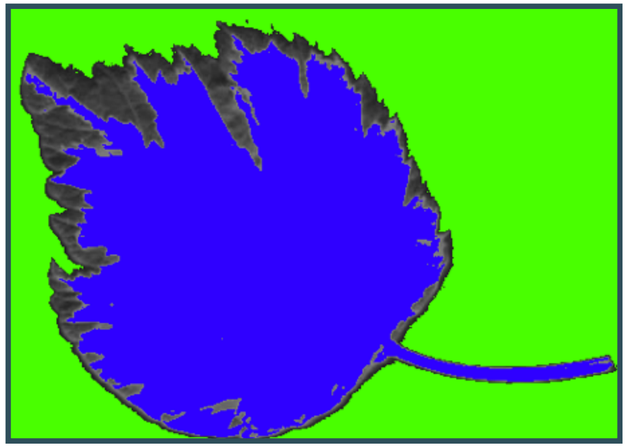
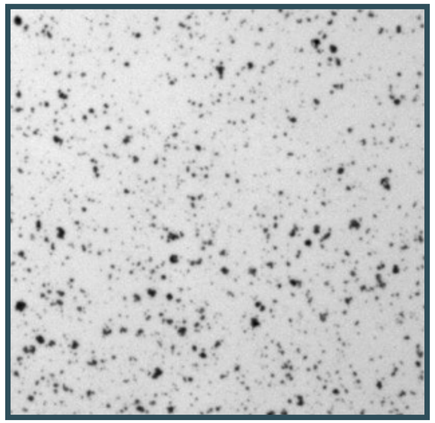
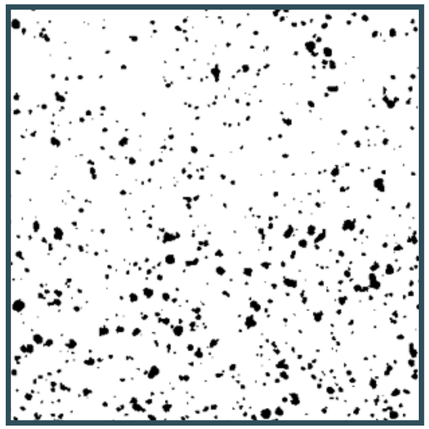
Image analysis is the **extraction of information** from images



21% of the leaf surface is infected

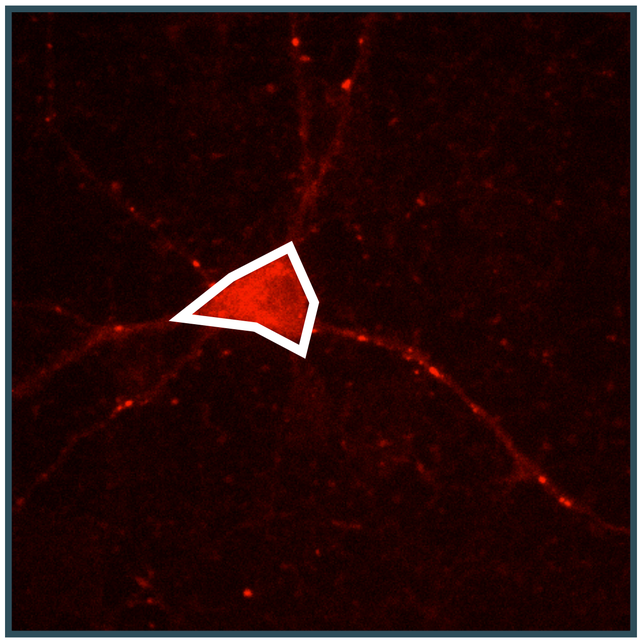


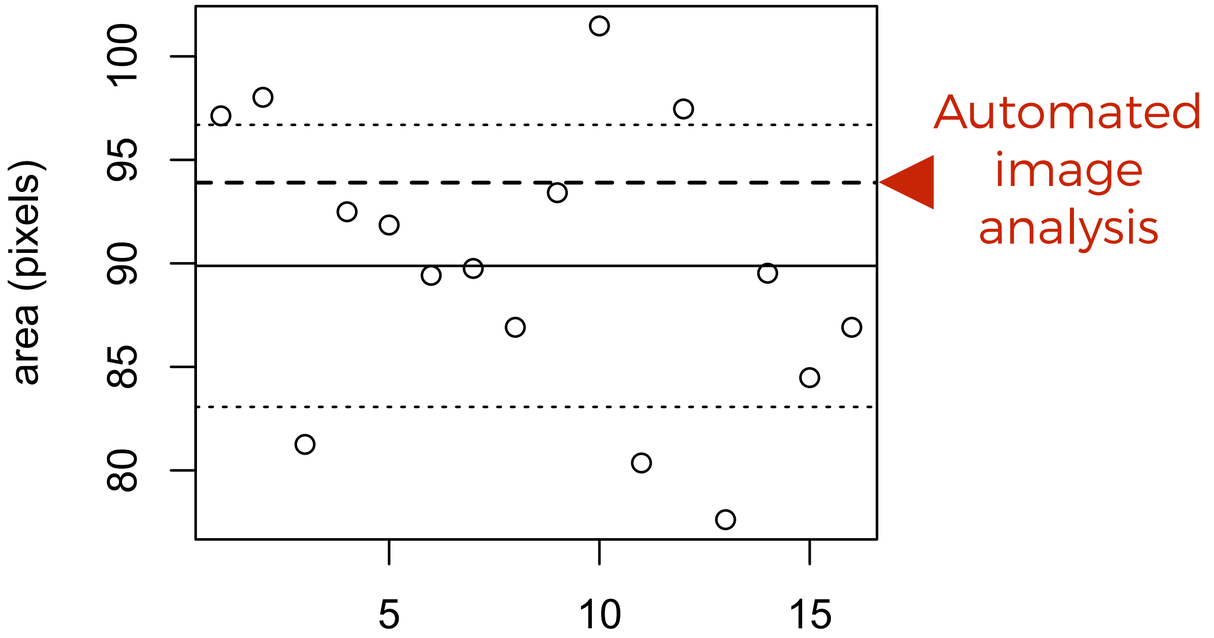


710 cells
average size = 15 px²

Main **advantages** of automated image analysis

Removes human appreciation + Automation of processes





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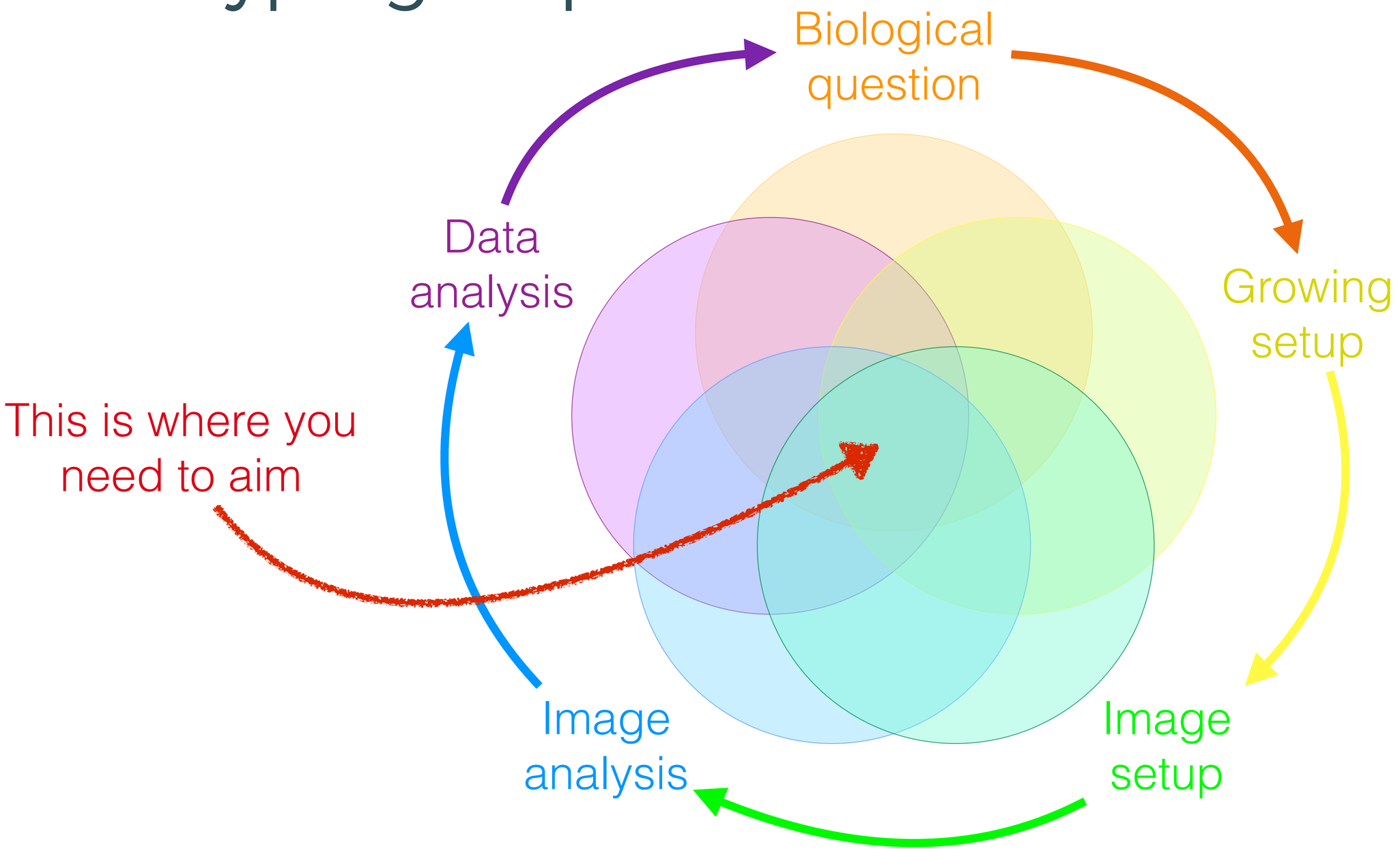
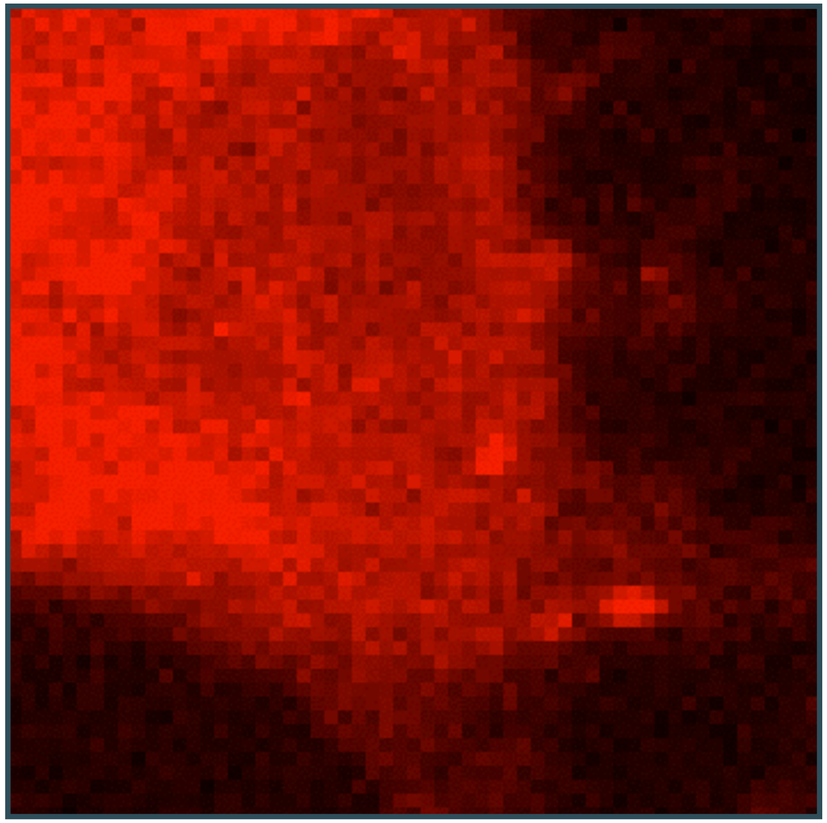
Phenotyping steps



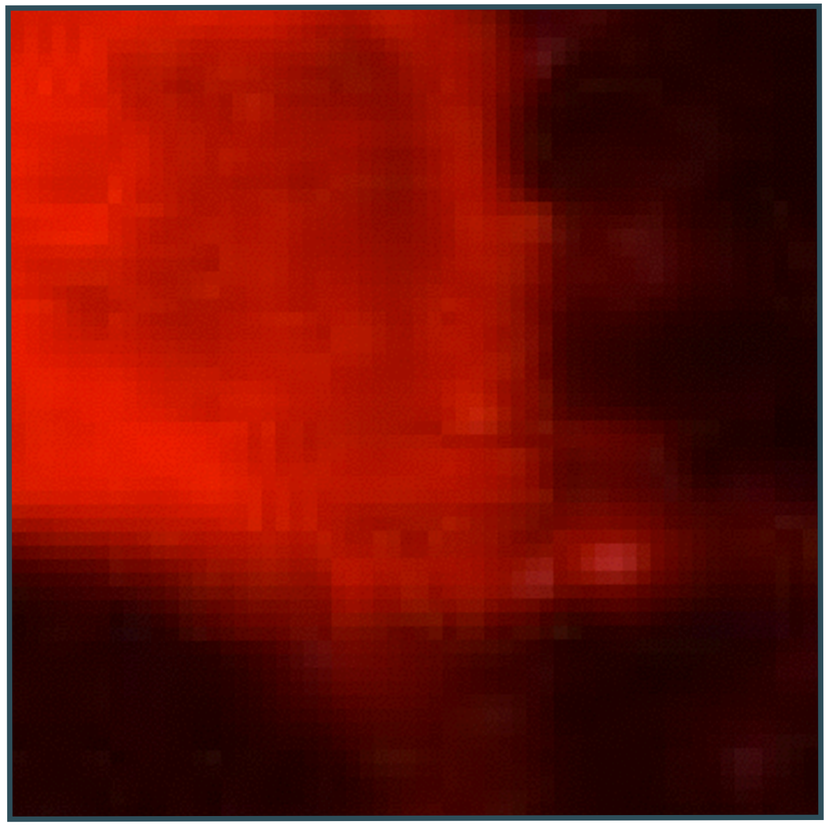
Types of images

TIFF



530K

JPEG



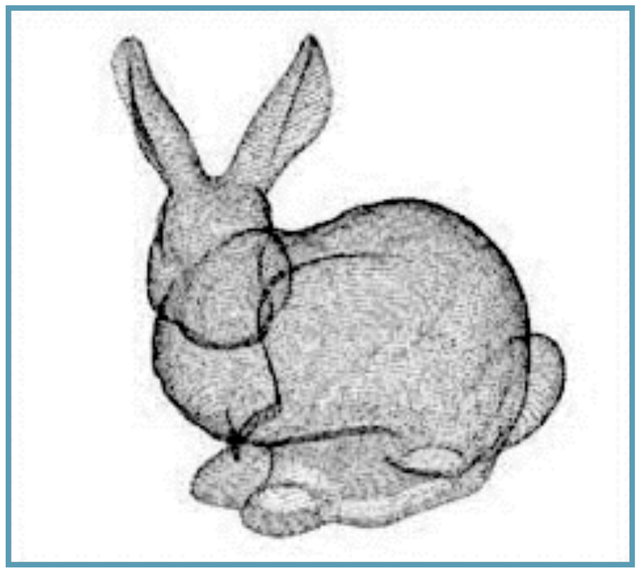
18K

Minervini et al. (2015). The significance of image compression in plant phenotyping applications. Functional Plant Biology, 1–18

Image analysis basics: **Image scale**

Principle
Link between pixel and physical size

DPI
Dots Per Inch
Pixels Per 2.54 cm



Pixels	cm	scale
200	10	20 px/cm 50 DPI

Image analysis basics: **Histogram**

Principle

Distribution of pixel values

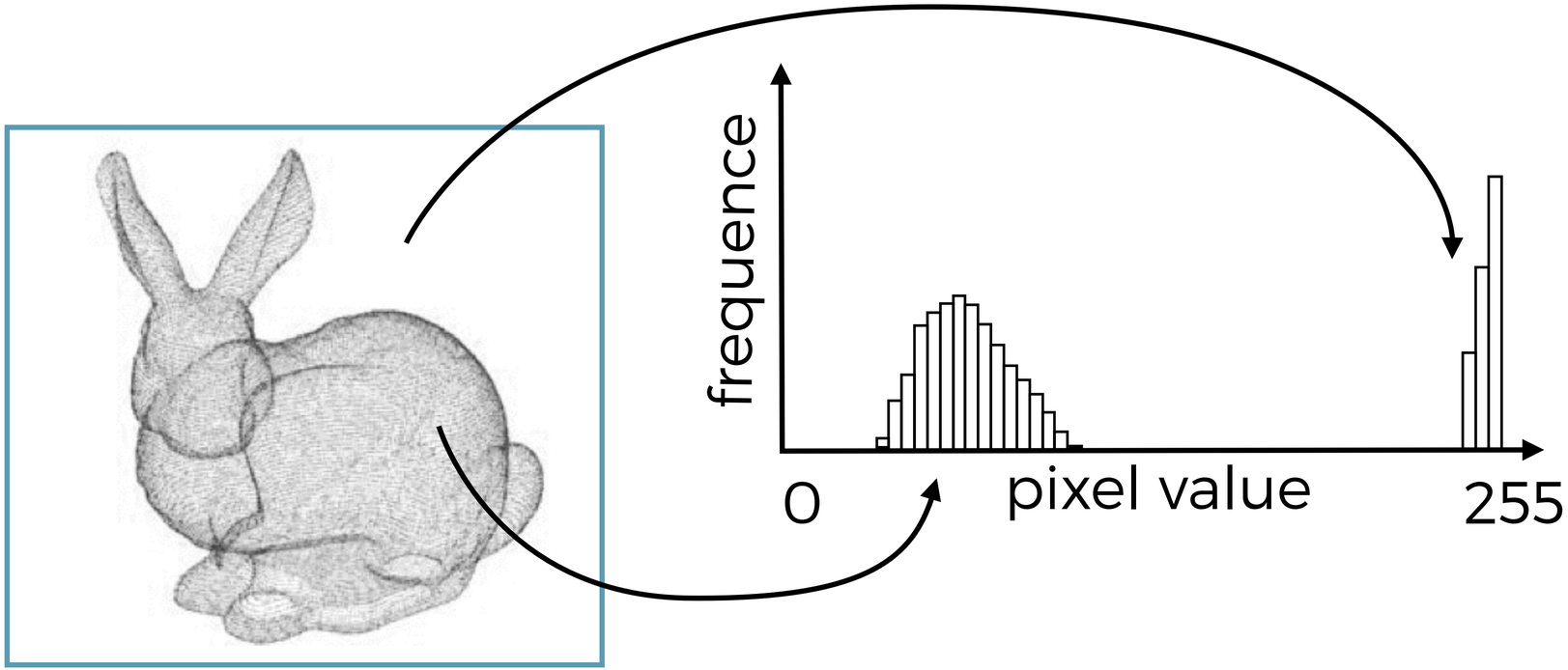


Image analysis basics: **Thresholding**

Principle

Isolate the object from the rest of the image

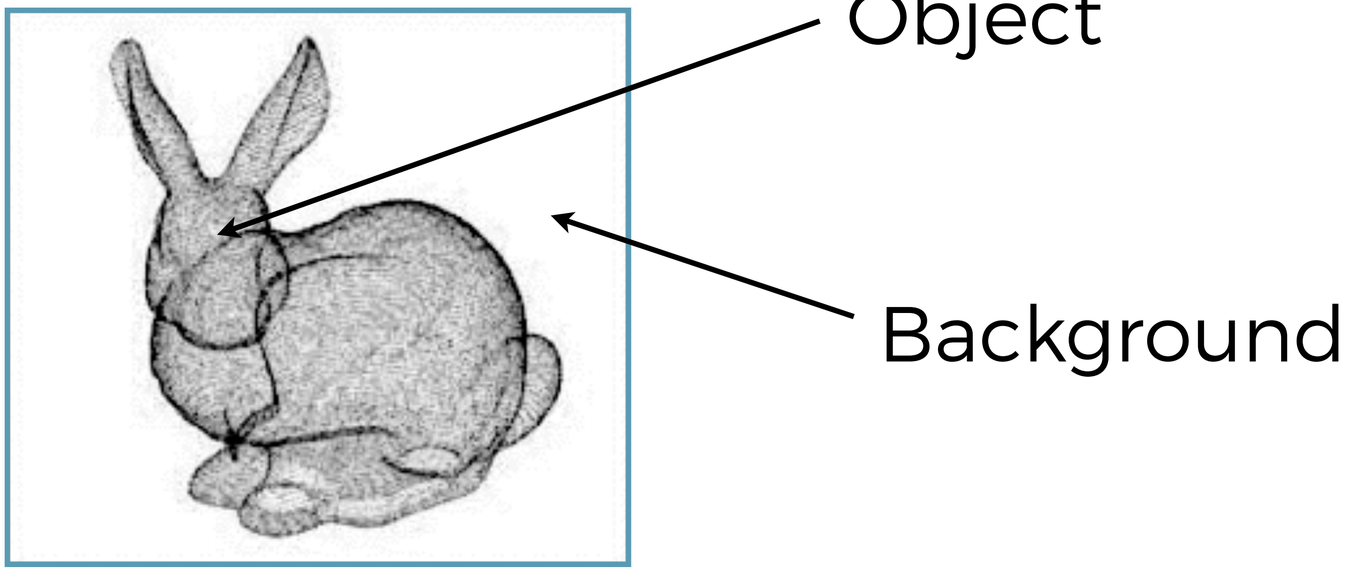
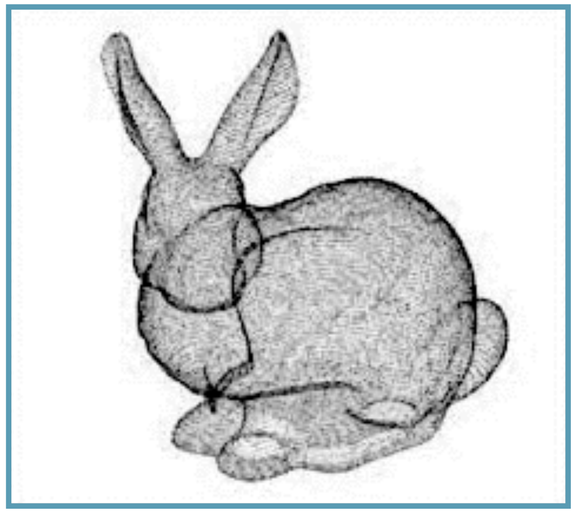
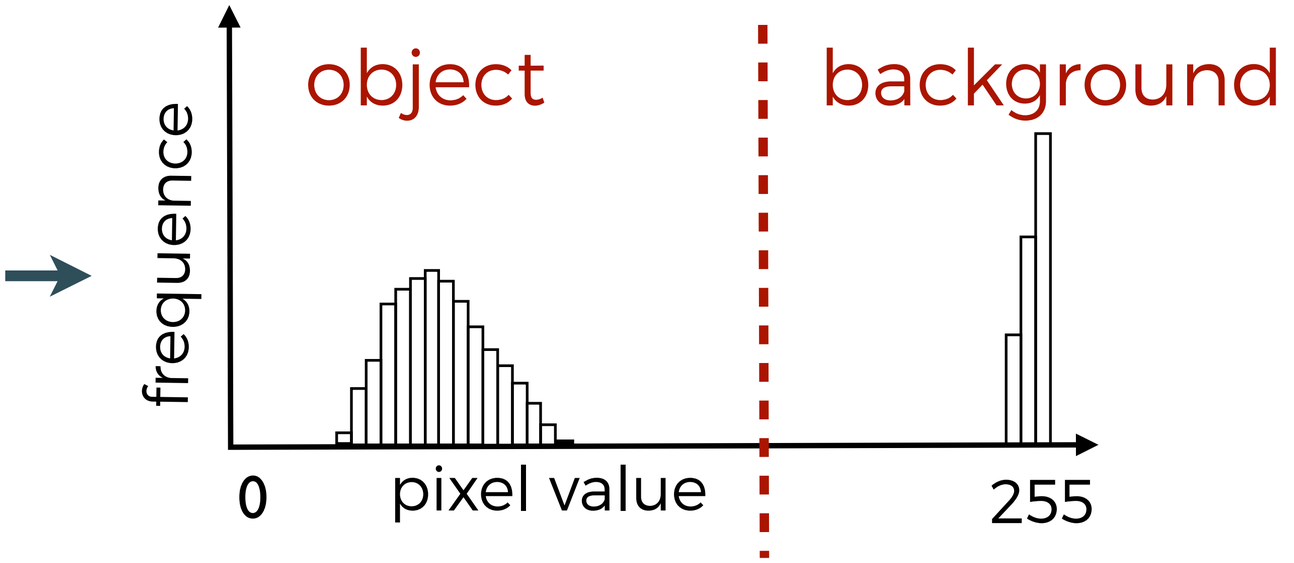


Image analysis basics: **Thresholding**





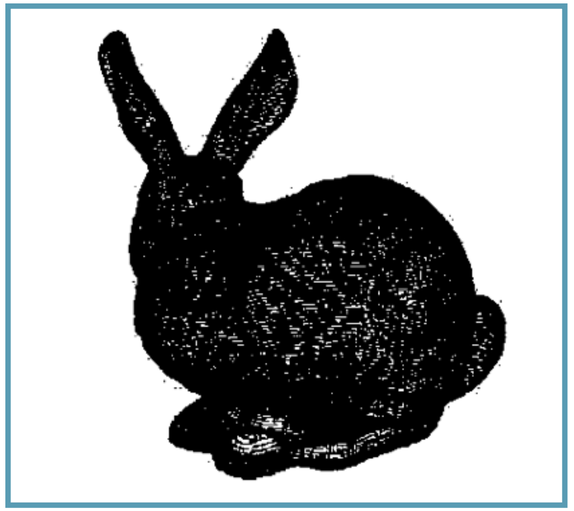
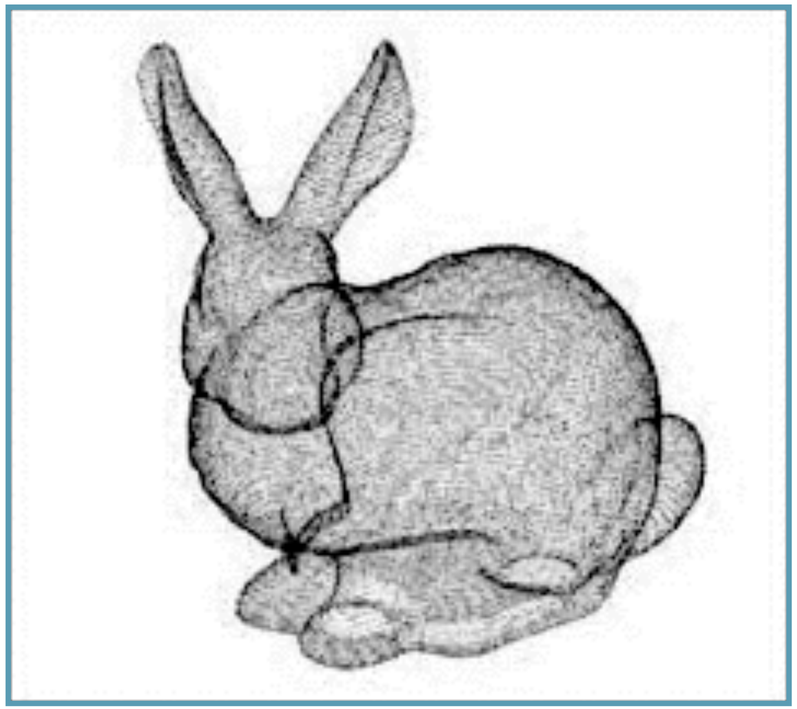


Image analysis basics: **Thresholding**



Be careful with thresholding

use a fixed threshold value
or
use an algorithm

But use always the same

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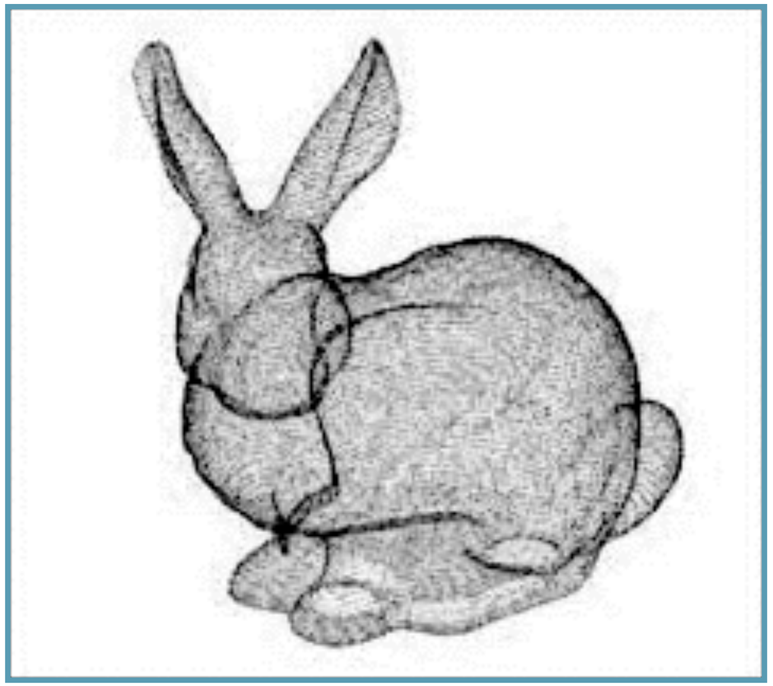
Image analysis basics: **Thresholding**



Different fixed values

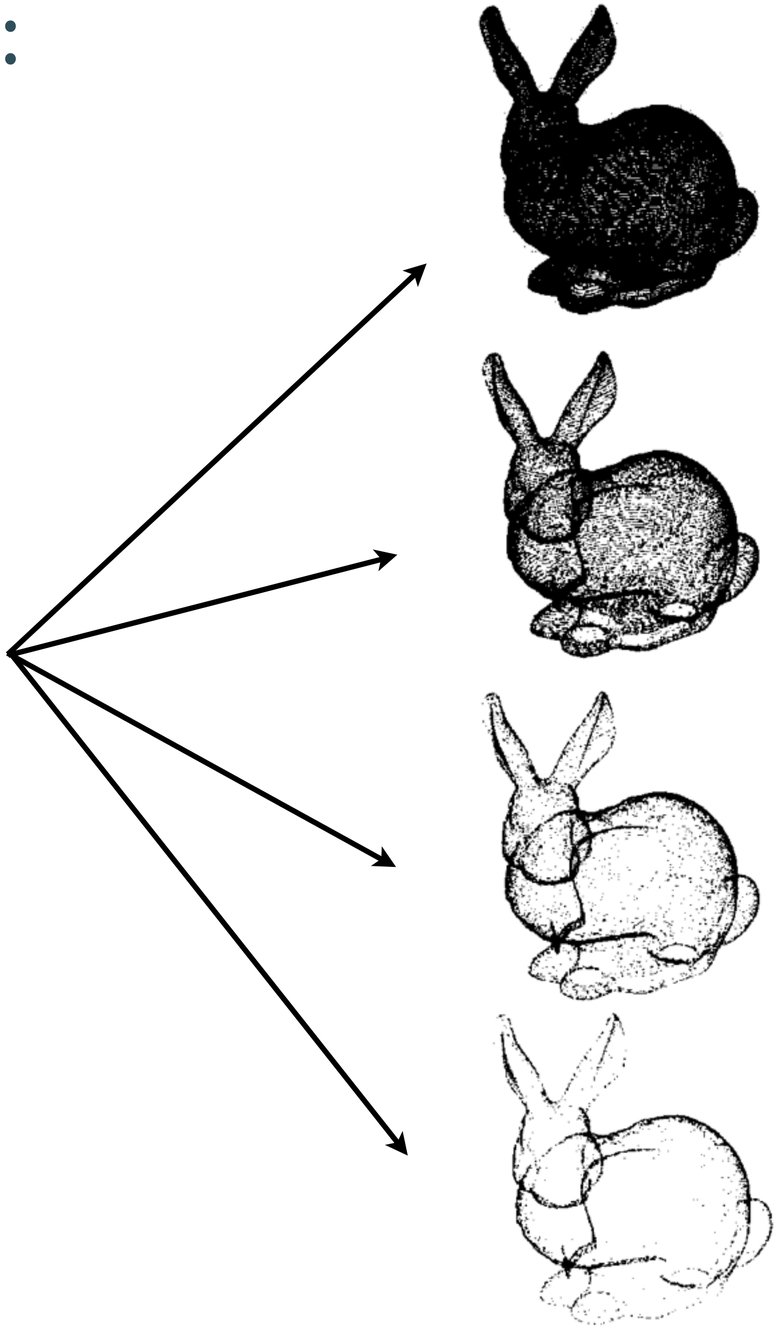
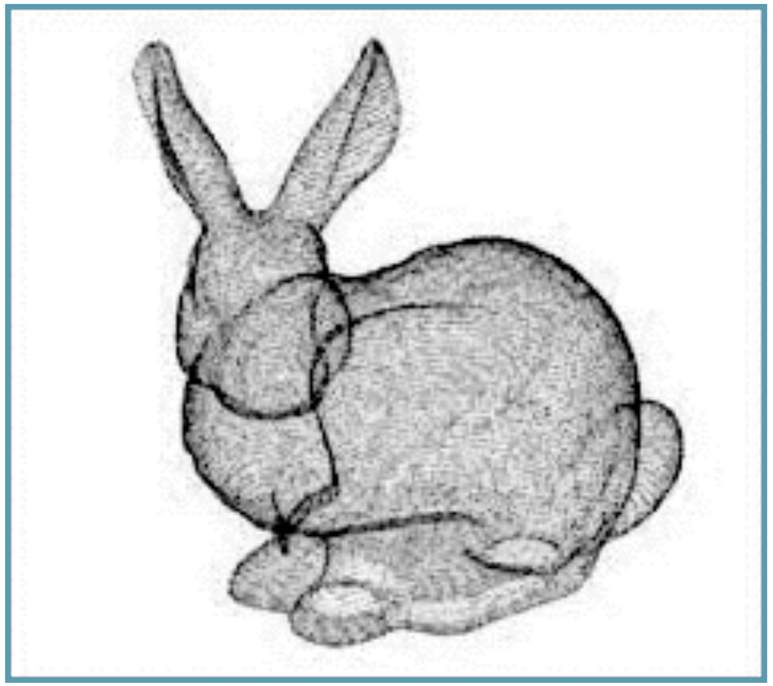
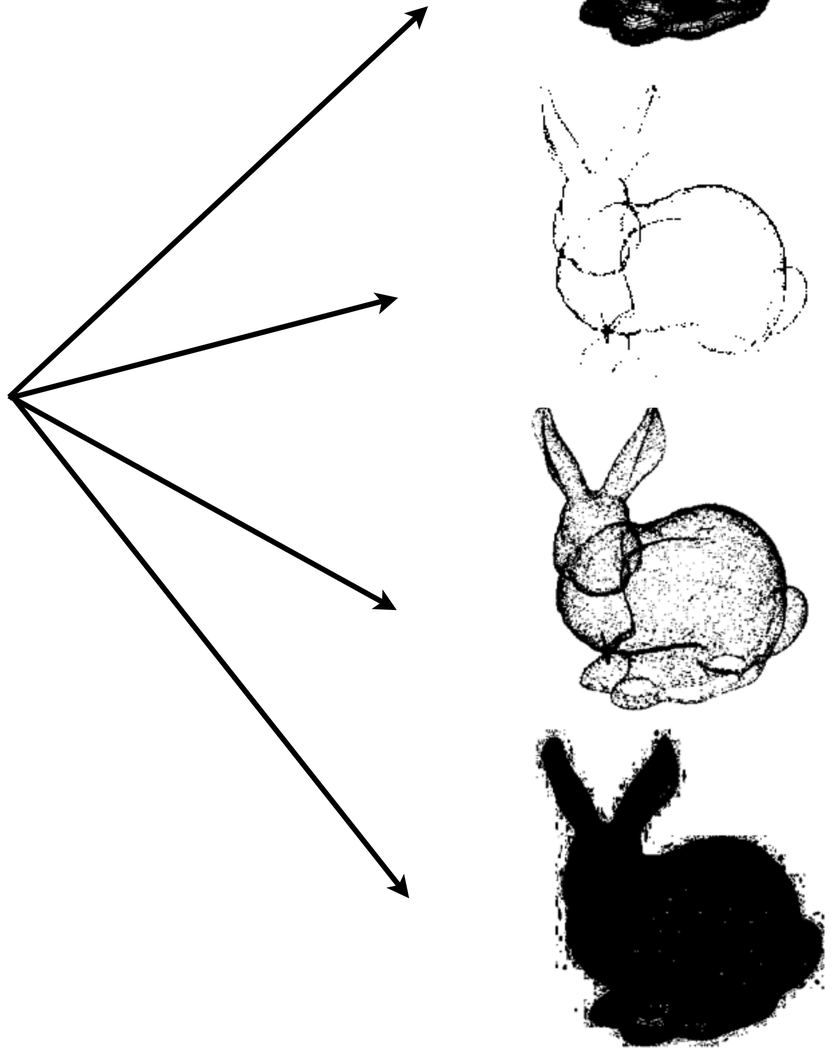


Image analysis basics: **Thresholding**





Different algorithms

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Image analysis basics: **Skeletons**

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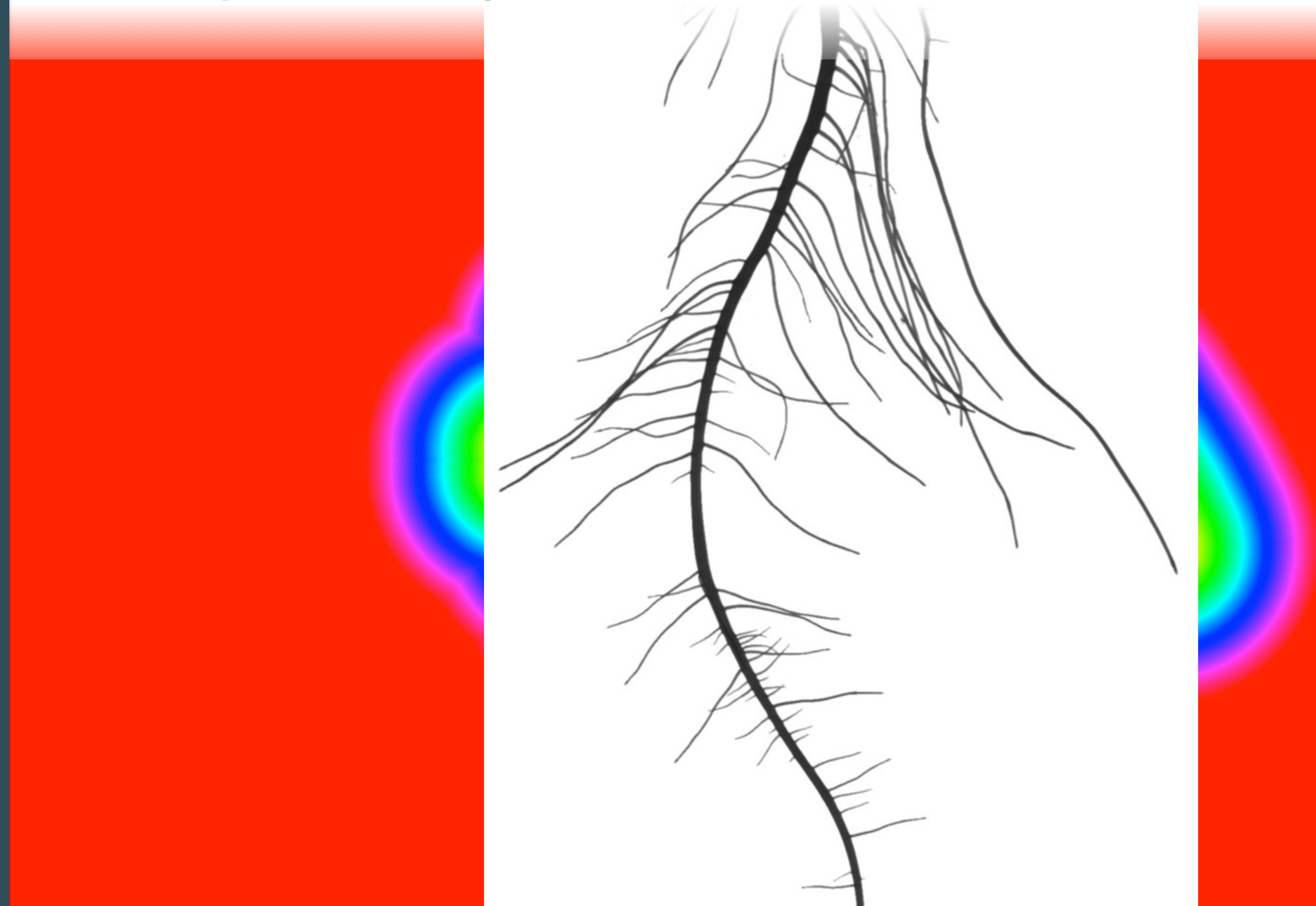
Image analysis basics: **Distance Map**



Insights into plant biological processes through phenotyping

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1. What is an image ?

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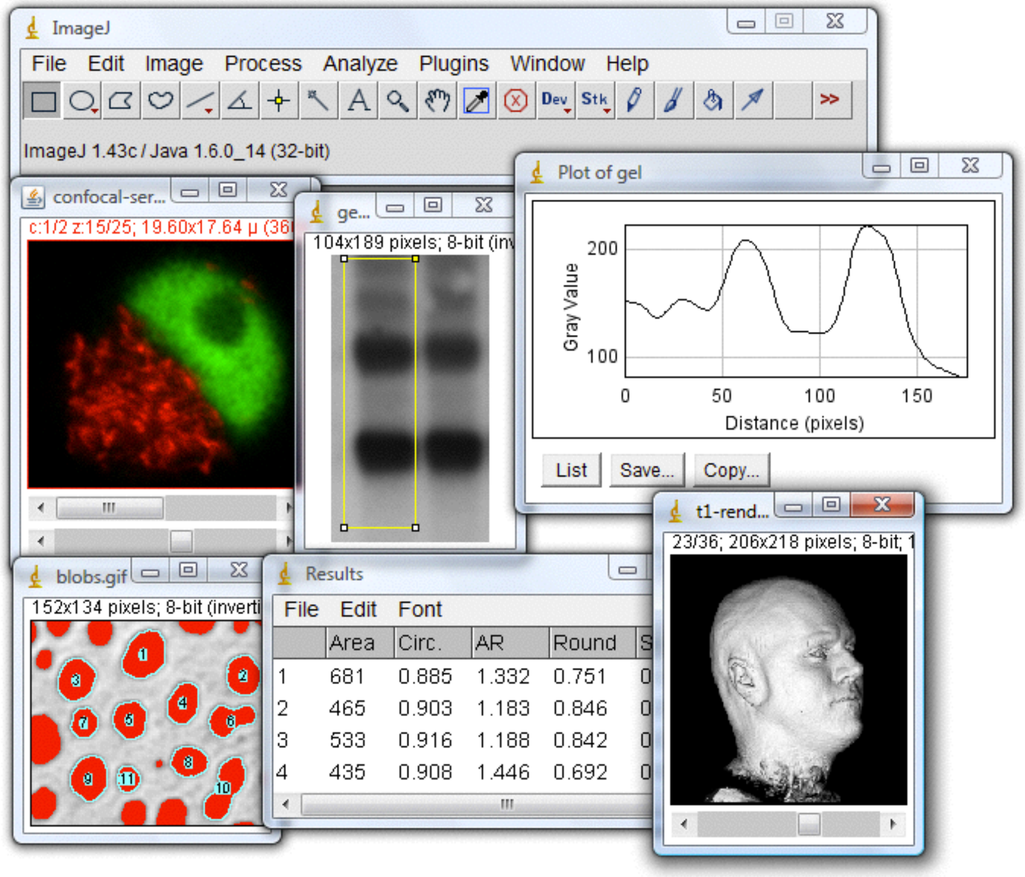
ImageJ



- Open source
- Developed at the NIH
- Created in 1986
- Plugin and macro
- Current version: 1.49

http://rsb.info.nih.gov

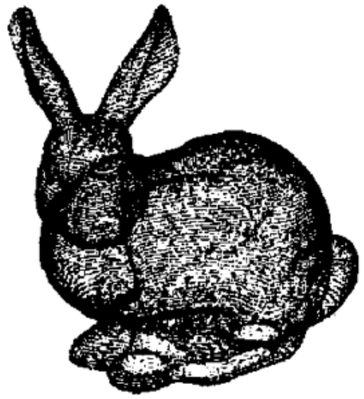
http://fiji.sc/

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

Exercice 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



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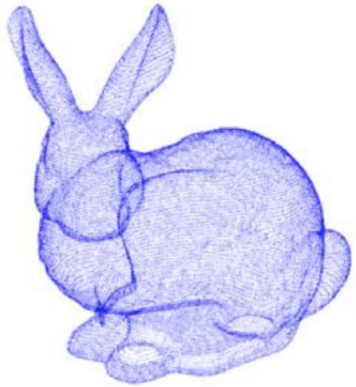
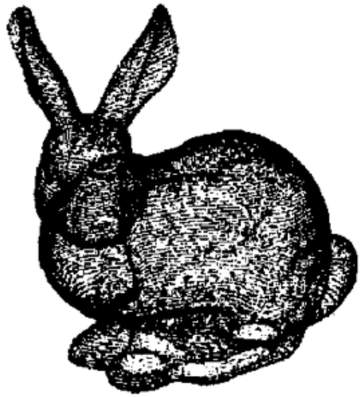
Exercice 1: **Thresholding**

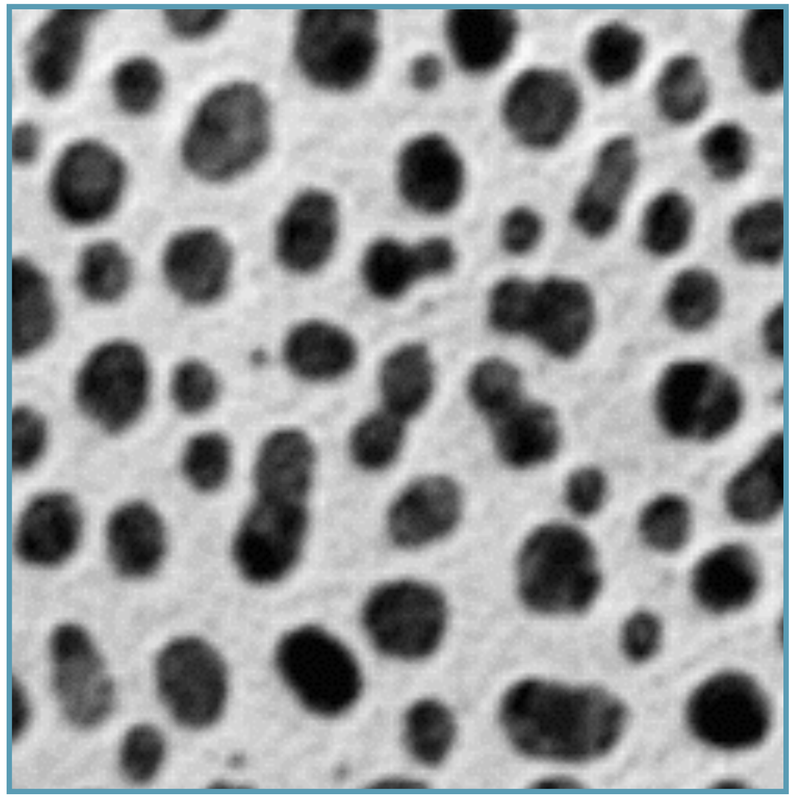
1. File > Open
2. Image > Duplicate
3. Image > Type > 8-bit
4. Image > Duplicate
5. Image > Adjust > Threshold
6. File > Save as





Exercice 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



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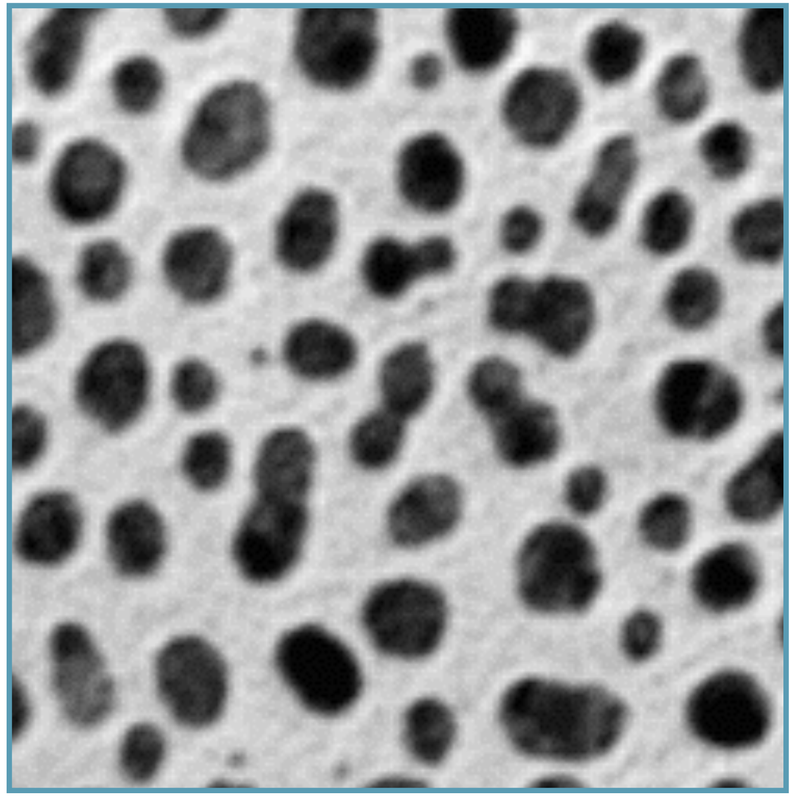
Exercice 2: **Counting objects**

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



Exercice 3: **Working with roots**

1. Open the image lupin.jpg
2. Threshold the image
3. Create a binary image
4. Estimate the length of the root system
5. Estimate the diameters of the roots

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Exercice 3: **Working with roots**

1. File > Open
2. Process > Binary > Make binary
3. Image > Duplicate
4. Select lupin.jpg
5. Process > Binary > Skeletonize
6. Analyze > Analyze particles
7. Select lupin-1.jpg
8. Process > Binary > Distance Map
9. Process > Image Calculator
10. lupin.jpg AND lupin-1.jpg

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Macros and plugins

Macros

Set of ImageJ commands

Useful for automation

Java-like

Plugins

New commands

More complex image analysis

Java

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Creating macros

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...

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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 **//**

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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();

}
```

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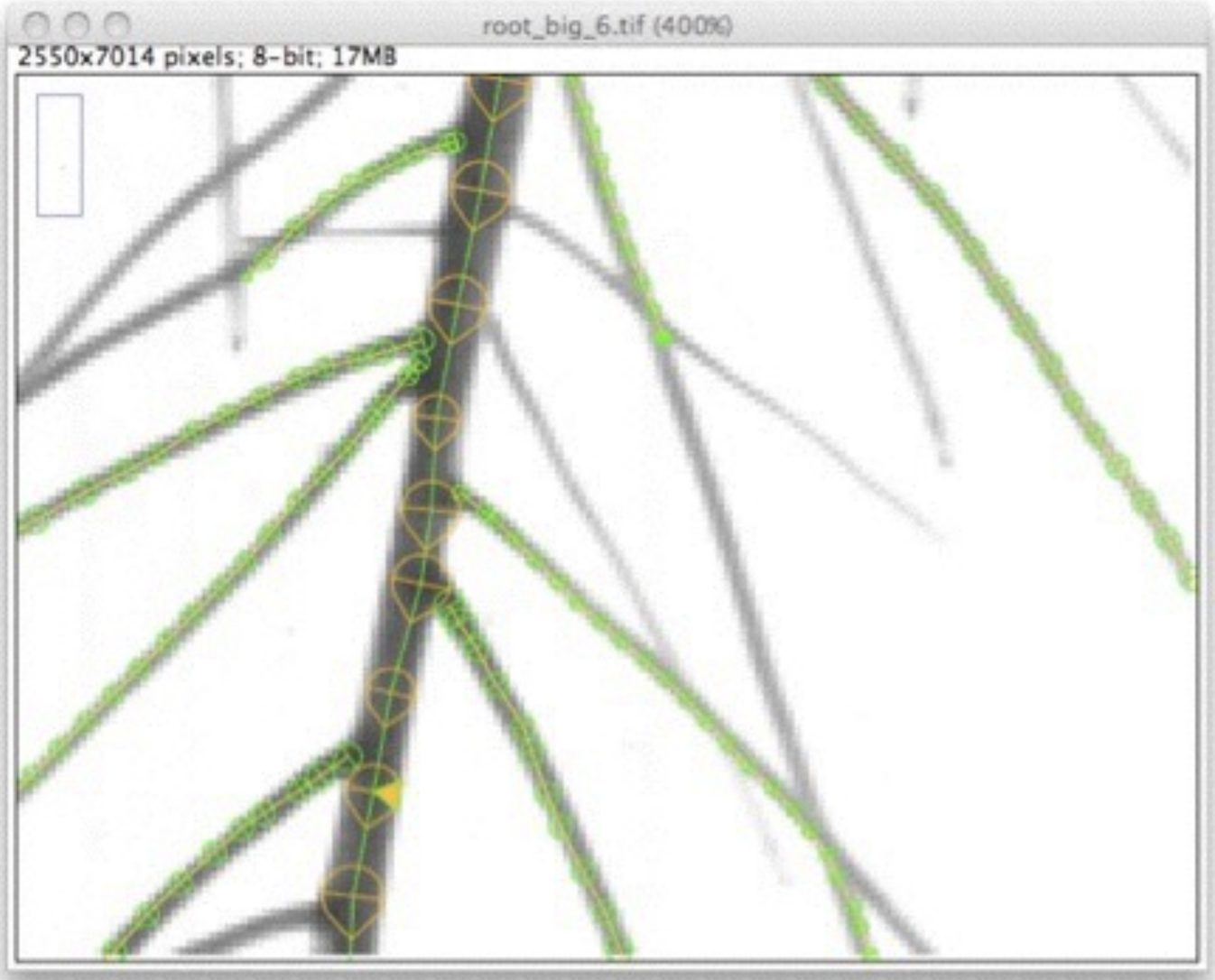
Example of plugin: **SmartRoot**



More on Wednesday...

More ressources

http://imagej.nih.gov/ij/

http://fiji.sc/

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