UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE AGRARIE E AMBIENTALI - PRODUZIONE, TERRITORIO, AGROENERGIA
DiSAA

Dottorato di Ricerca in Agricoltura, Ambiente e Bioenergia - Università degli studi di Milano

**Short Course:** ***From -omics to phenotyping for crop improvement***

# Imaging techniques for plant phenotyping

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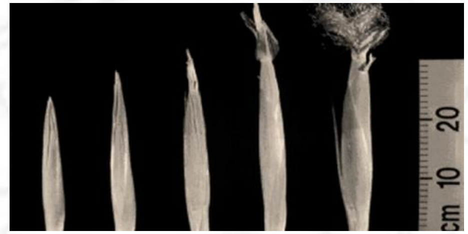
# From pioneering to high-throughput

Phenotyping relies on quantitative description of plant attributes (morphological, physiological, biochemical etc)




Involves direct measurements:

- sizing (length/height)
- weighing
- counting

In the last decade: development of automated systems for growth chambers, greenhouses (current deployment to field plots)

high-throughput phenotyping (i.e. simultaneous evaluation on $10^2 \div 10^4$ plants per cycle) enabled by:

- automation of plant handling and growing management
- non-invasive sensor technologies for repeated measurements (data acquisition, processing, storing/warehousing)

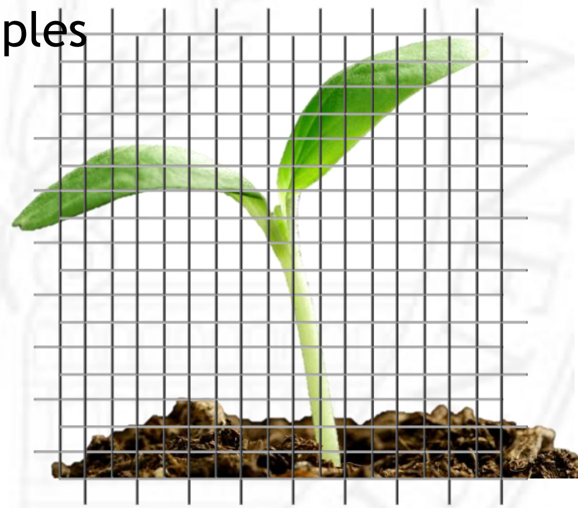
# Imaging is the reference sensing technique

Optical sensing (imaging in particular) has several desirable features:

- fast, from any distance, at any scale (almost)
- non destructive (can be repeated at any time)
- no contact (not disturbing, can be on the go)

Specific to images which are formed by pixels (picture-elements):

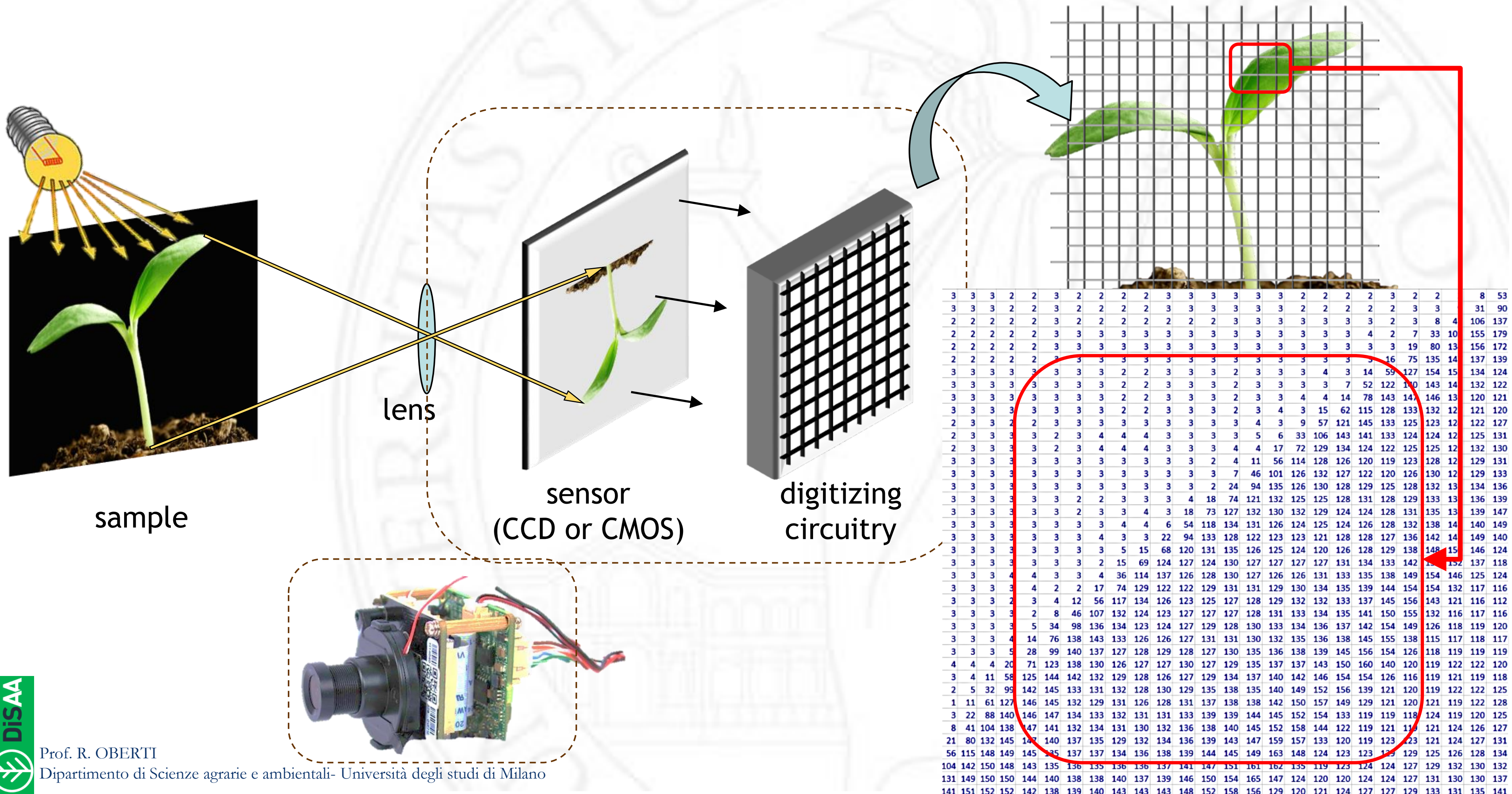
- suitable for heterogeneous (i.e. not homogenous) samples
  - ❑ detect differences (i.e. classify)
  - ❑ measure sizes and shapes



# What is a (digital) image ?

A digital image (digit=numeric) is formed:

- by collecting the radiation from the viewed sample;
- by converting it in a train of electric signals
- by digitizing them (binary conversion) in an array of numbers (eventually a file)

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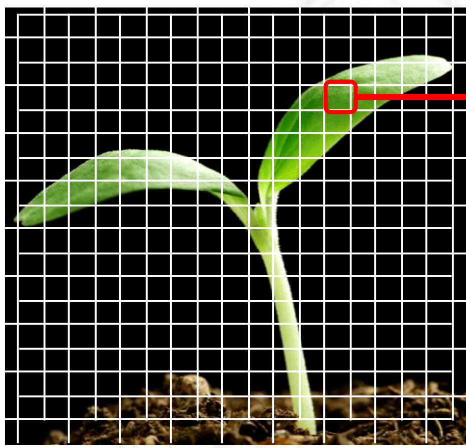
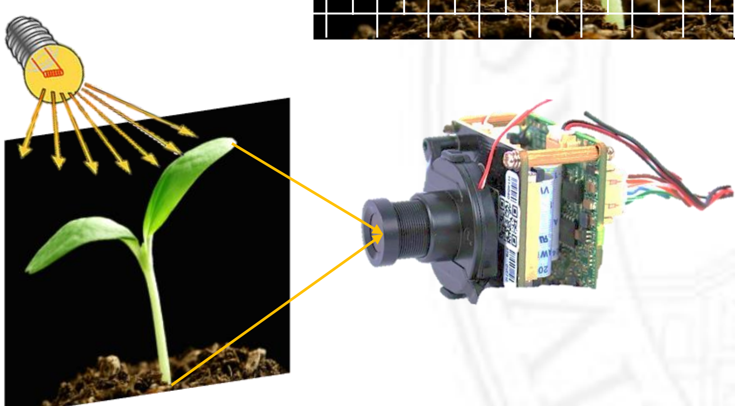
# What is a (digital) image ?

Key concept: a digital image is an array of numbers that represent a map of the intensity of the radiation reflected/emitted by the plant and captured by the camera system



$$I_{i,j} = f[R(x,y)]$$



Different technologies used for camera detectors allow to acquire images in different ranges of the electromagnetic spectrum

# RGB images

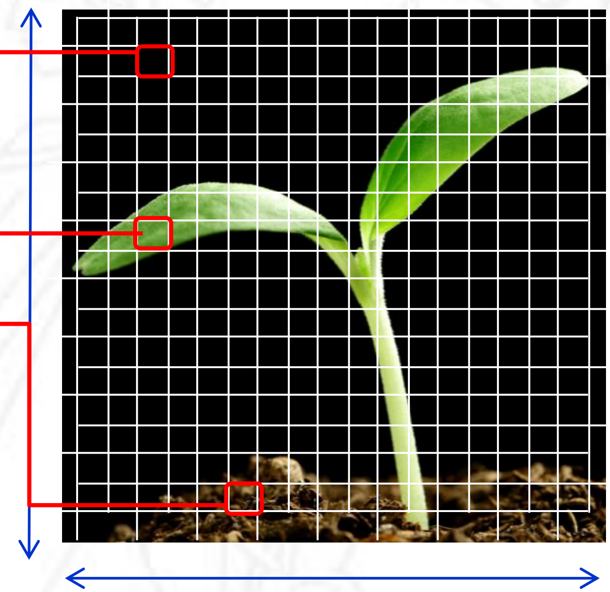
Color images are arrays of intensity values of reflected light (i.e. visible radiation) in the red, green, and blue (RGB) bands of visible window (400-700 nm) of the electromagnetic spectrum

Most RGB cameras have a digital resolution of 8-bits ($2^8$= 256), i.e. the intensity is coded from 0 to 255 levels in the three channels

RGB= (5, 6, 4)

RGB= (110, 194, 82)

RGB= (86, 44, 13)



The spatial resolution refers to the number of pixels of the image (i.e. number of elements of the detector array), e.g. 3200p x 2600p ≅ 8 Mpix

If the imaged region is 0,6m x 0,5m wide, the spatial resolution is about (in practice it depends on the lens quality):

$$res = \frac{0{,}6\ m}{3200\ pix} = 0{,}18\ mm/pix$$

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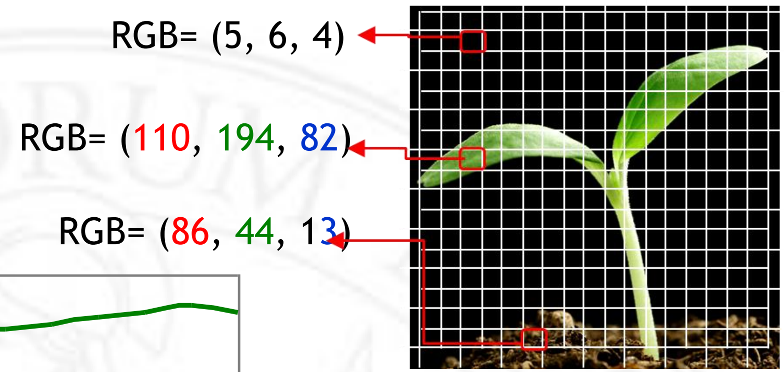
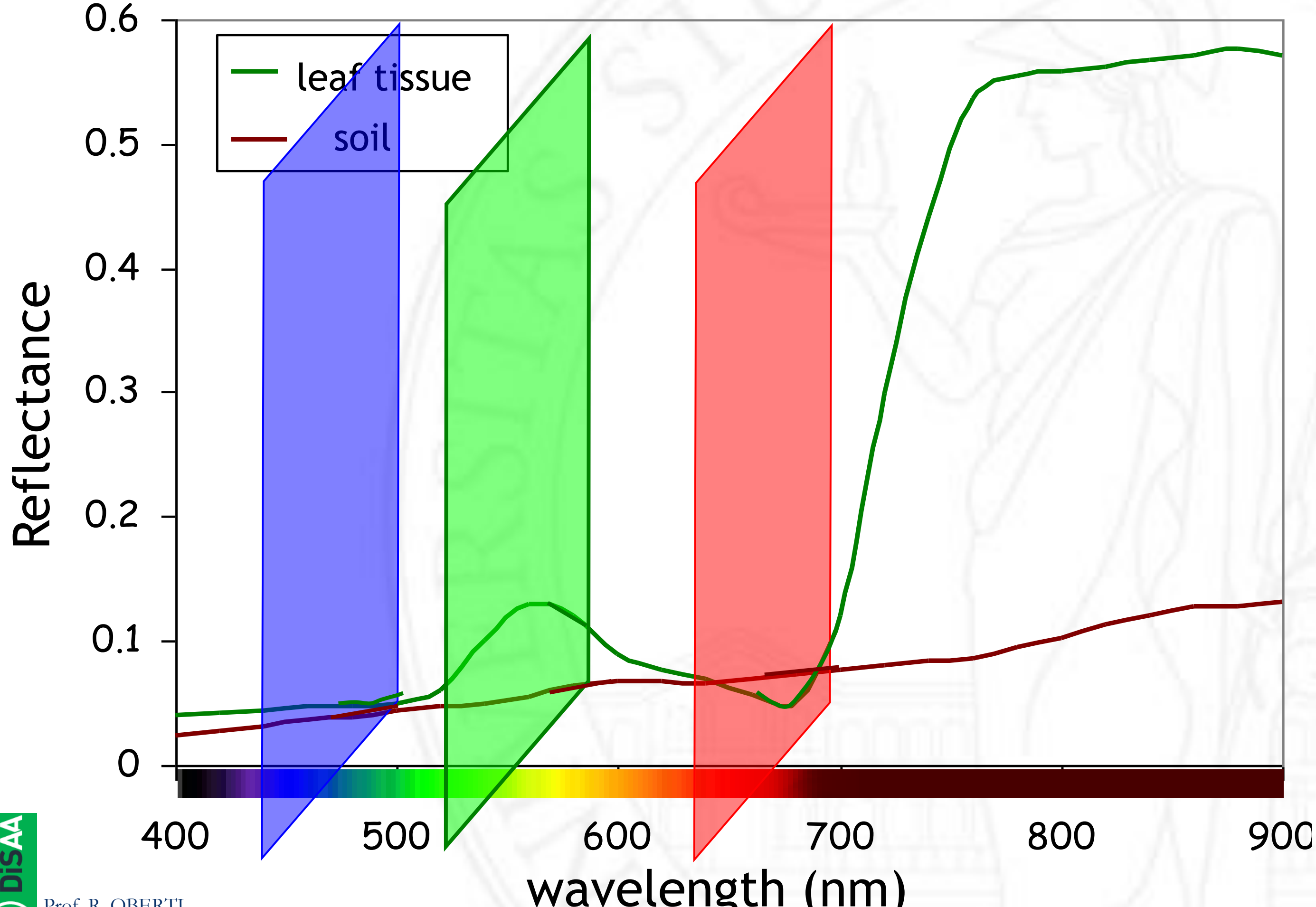
# RGB images and visible spectrum

RGB values are linked to spectral reflectance properties in the visible window

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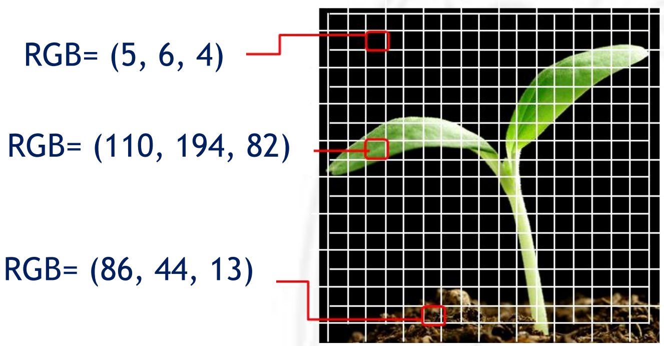
# Plant segmentation

Intensity values in the different channels are used to define pixels conditions able to segment (i.e. identify) plants/canopy pixels from background (foreign regions)

$$for\ any\ pixel\ [i,j]:\quad R_{i,j} + G_{i,j} + B_{i,j} \begin{cases} \geq Threshold \rightarrow keep\ [i,j] \\ < Threshold \rightarrow discard\ [i,j] \end{cases}$$




background removed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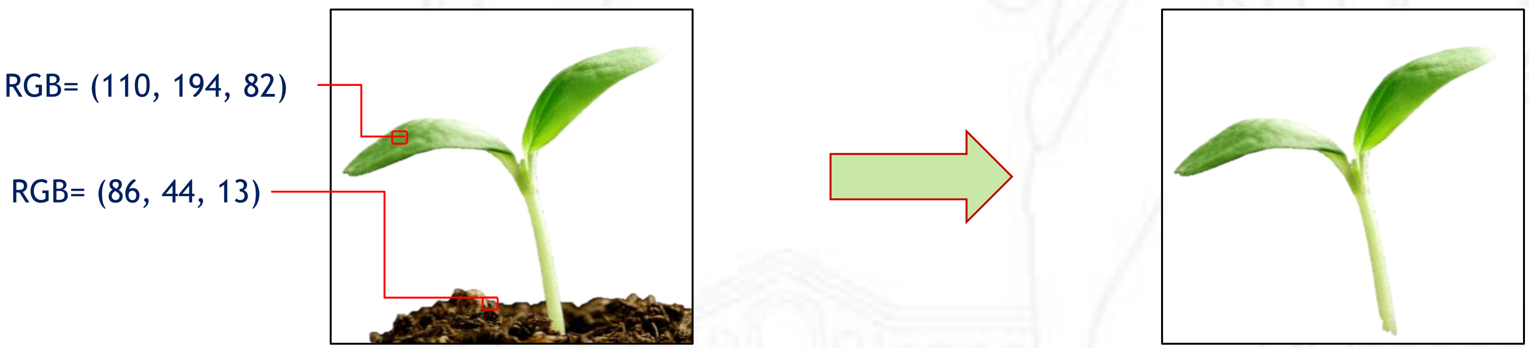
# Plant segmentation

Intensity values in the different channels are used to define pixels conditions able to segment (i.e. identify) plants/canopy from background (foreign regions)

Spectral indices are algebraic combinations of intensity values in different spectral channels

EG=excess green

$$for\ any\ pixel\ [i,j]: \frac{\left(2\, G_{i,j} - R_{i,j} - B_{i,j}\right)}{\left(R_{i,j} + G_{i,j} + B_{i,j}\right)} \begin{cases} EG \geq Threshold \rightarrow plant\ [i,j] \\ EG < Threshold \rightarrow discard\ [i,j] \end{cases}$$

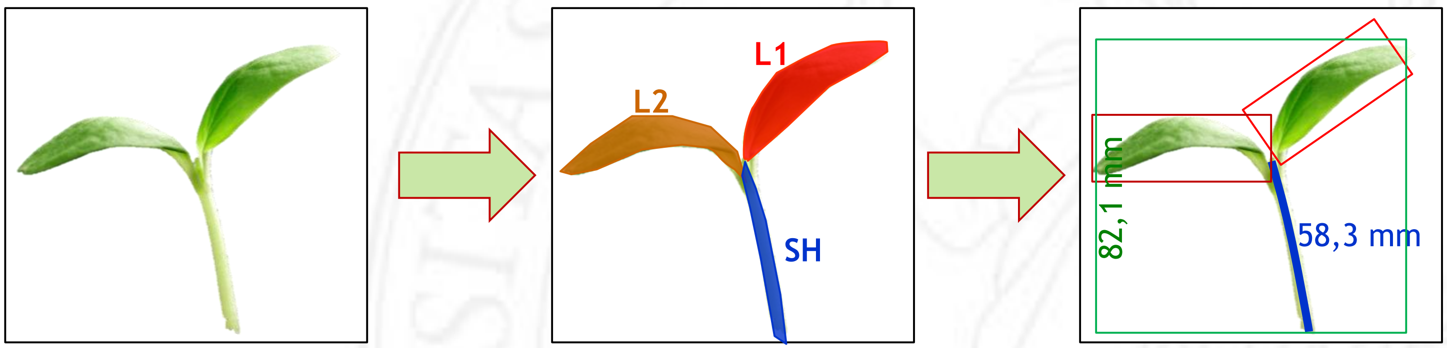



detected vegetation

# Size analysis

Morphological analysis on segmented vegetation can identify the plant architecture and enable size analysis

Shape indexes (eccentricity, elongation, roundness etc) can be used to automatically identify shoot and leaves




Being the image resolution known, size in pixels are converted in length/Area measurements

Accuracy of the measurement strongly depends on plant/camera orientation, by leaves occlusions etc, hence accurate size plant analysis is not straightforward as it may seem

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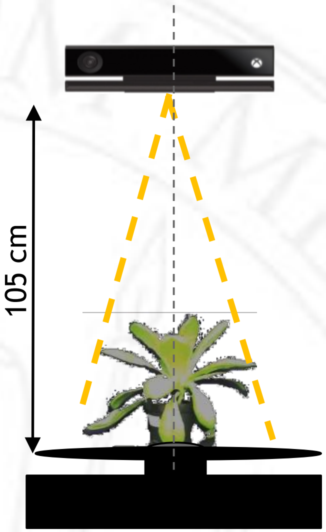
# Canopy cover

The most common method used for evaluating growth is RGB imaging at different time points followed by the extraction of the projected canopy area (canopy cover fraction of the soil)

Estimation of biomass growth in *Lactuca sativa* L. cv. Longifolia (Romain lettuce)




Projected Area=1173,21 $cm^2$

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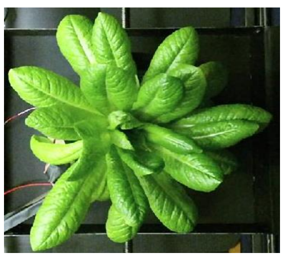
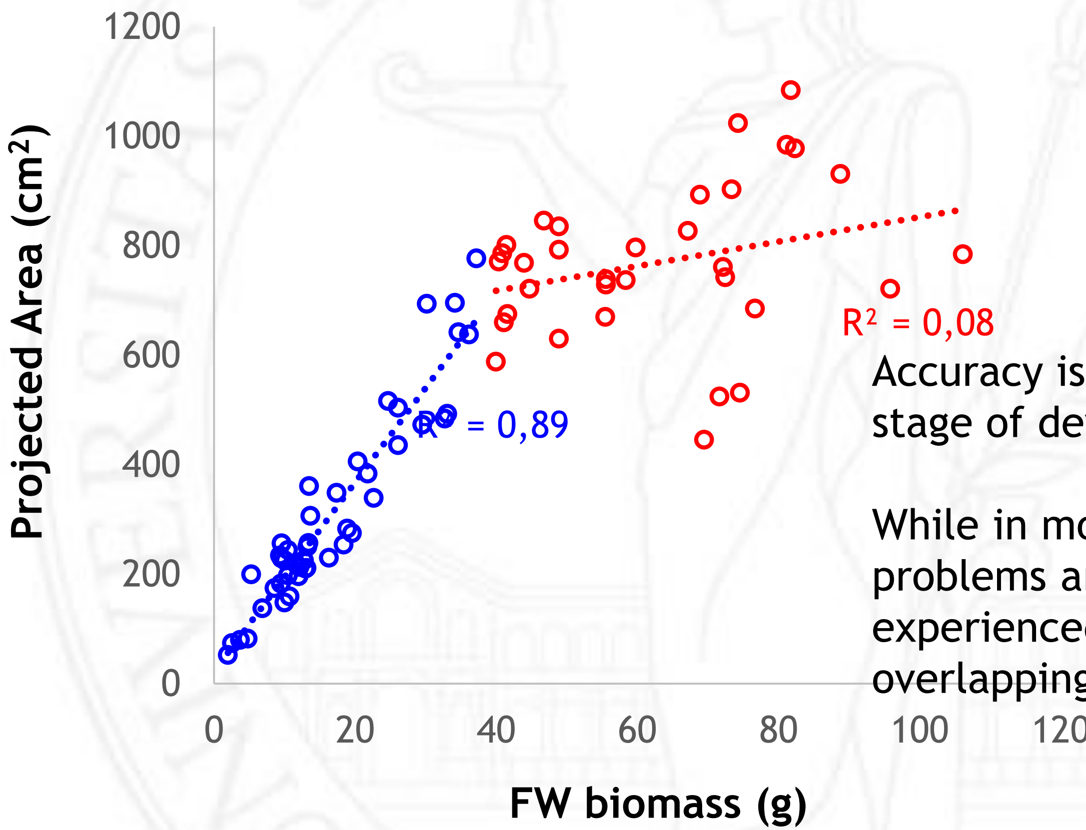
# Canopy cover

The calibration/validation of canopy cover is based on total leaf area or on biomass weight after destructive harvesting on reference samples


Estimation of biomass growth in *Lactuca sativa* L. cv. Longifolia (Romain lettuce)



Projected Area=1173,21 cm²



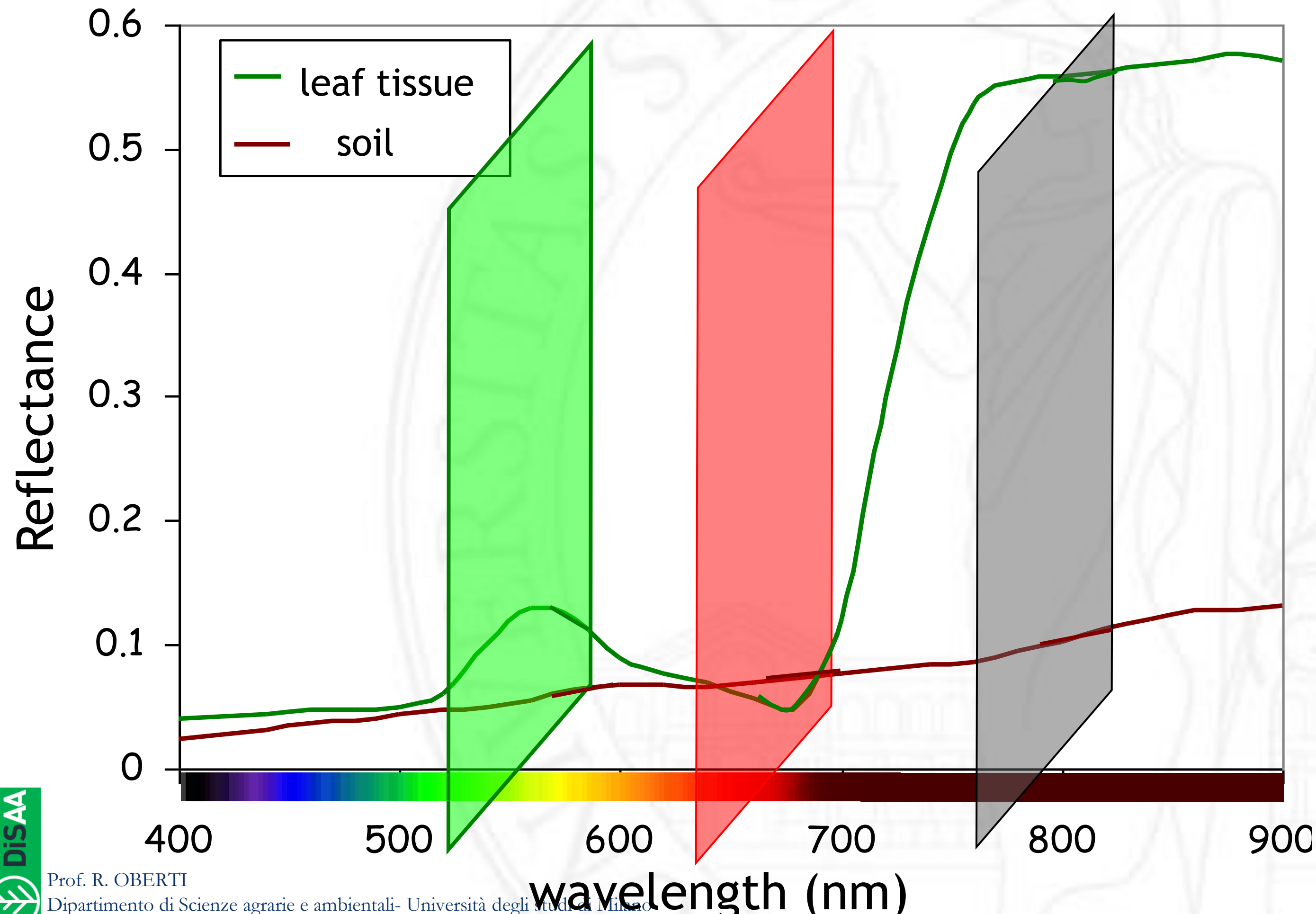

Accuracy is satisfactory at early stage of development

While in more developed plants problems are commonly experienced due to the overlapping of leaves

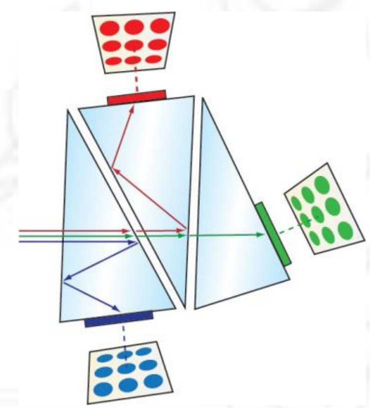
# Multispectral imaging

The same principles of RGB can be obtained at different wavelengths by adopting specific pass-bands filters in front of the detectors (one per band)

A common multispectral configuration adopted with plants in field is R-G-NIR imaging





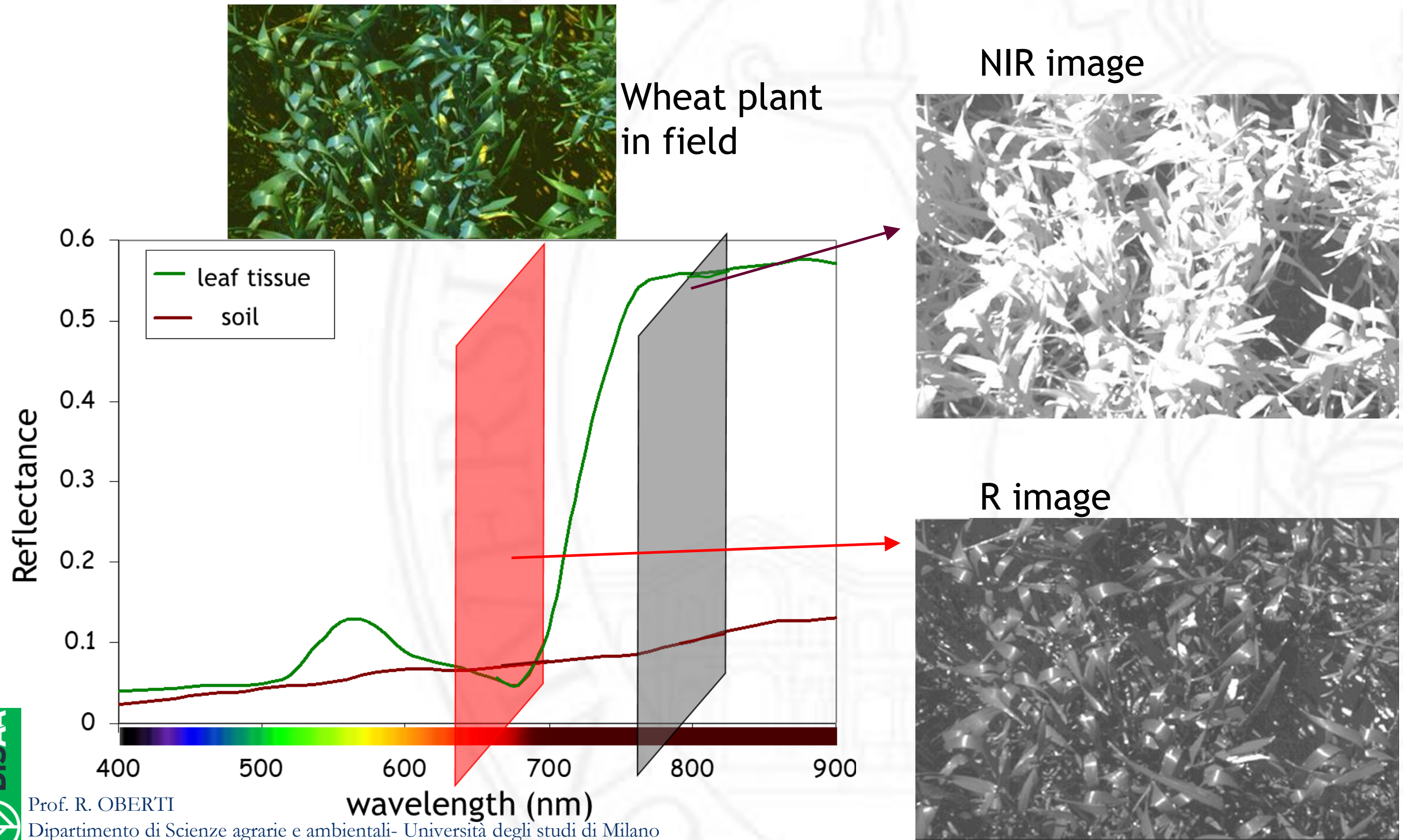


# R-G-NIR images

Normalized differential vegetation index or other NIR based spectral indexes can are very accurate in discriminating plants to estimate biomass

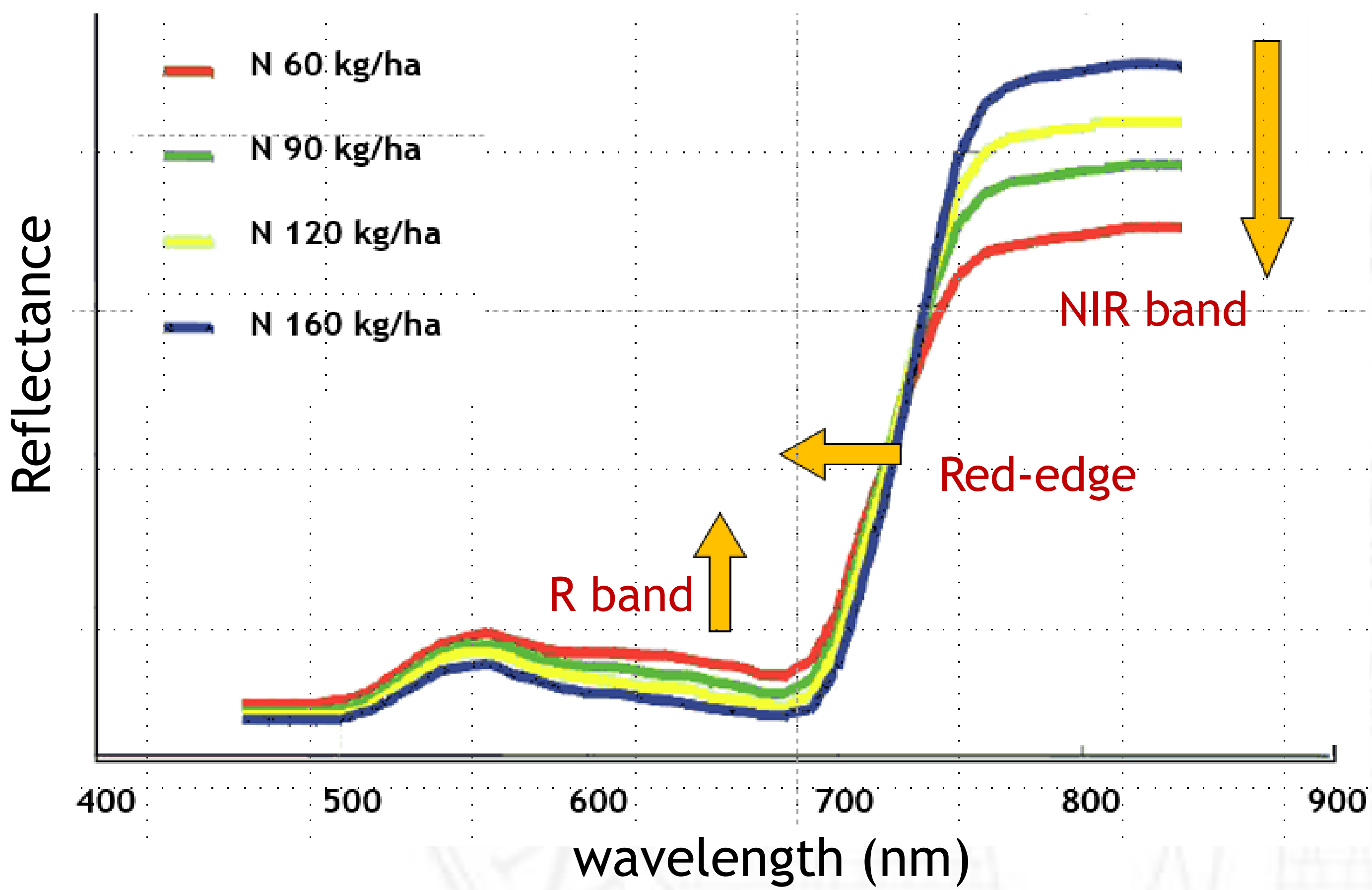
$$NDVI_{i,j} = \frac{(NIR_{i,j} - R_{i,j})}{(NIR_{i,j} + R_{i,j})}$$

$$\begin{cases} NDVI \geq Threshold \rightarrow plant\ [i,j] \\ NDVI < Threshold \rightarrow soil\ [i,j] \end{cases}$$



# Chlorophyll content

Modifications in chlorophyll content strongly affect the reflectance in R-NIR bands that can be used e.g. to estimate differences in N uptake



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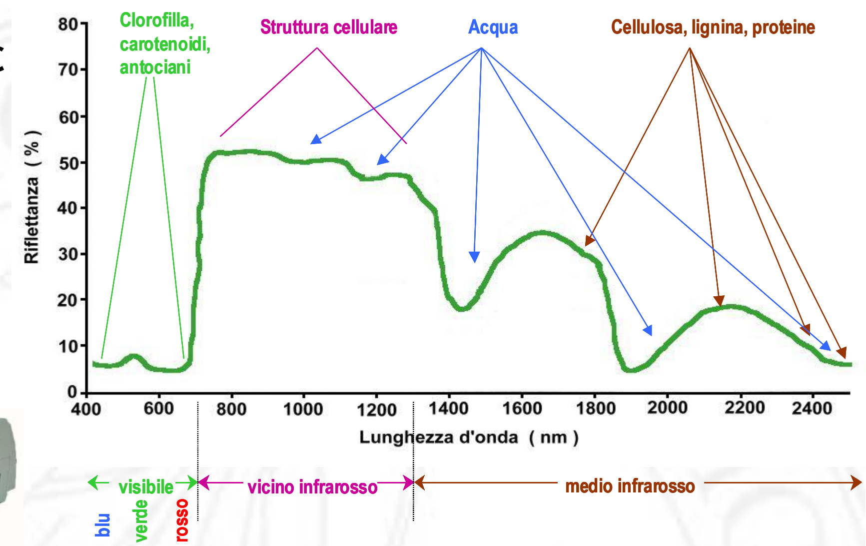
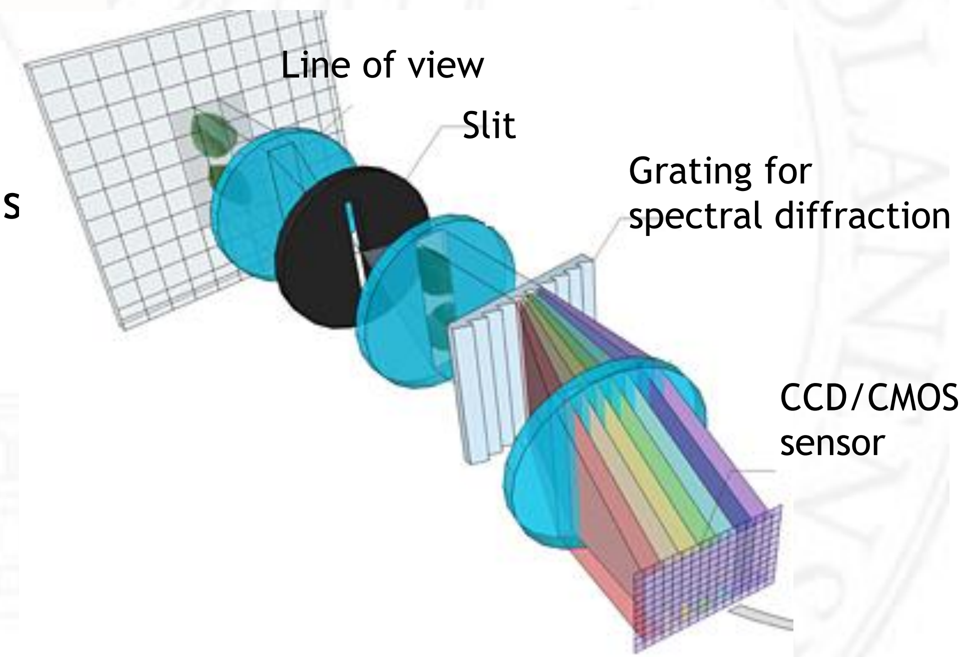
# Hyperspectral imaging

By enlarging the portion of the electromagnetic spectrum, different components (water, proteins, nitrogen etc.) and corresponding plant traits can be studied




Derived from applications in remote sensing, hyperspectral cameras are capable of scanning continuous wavebands at high spectral resolution (1 nm or less)

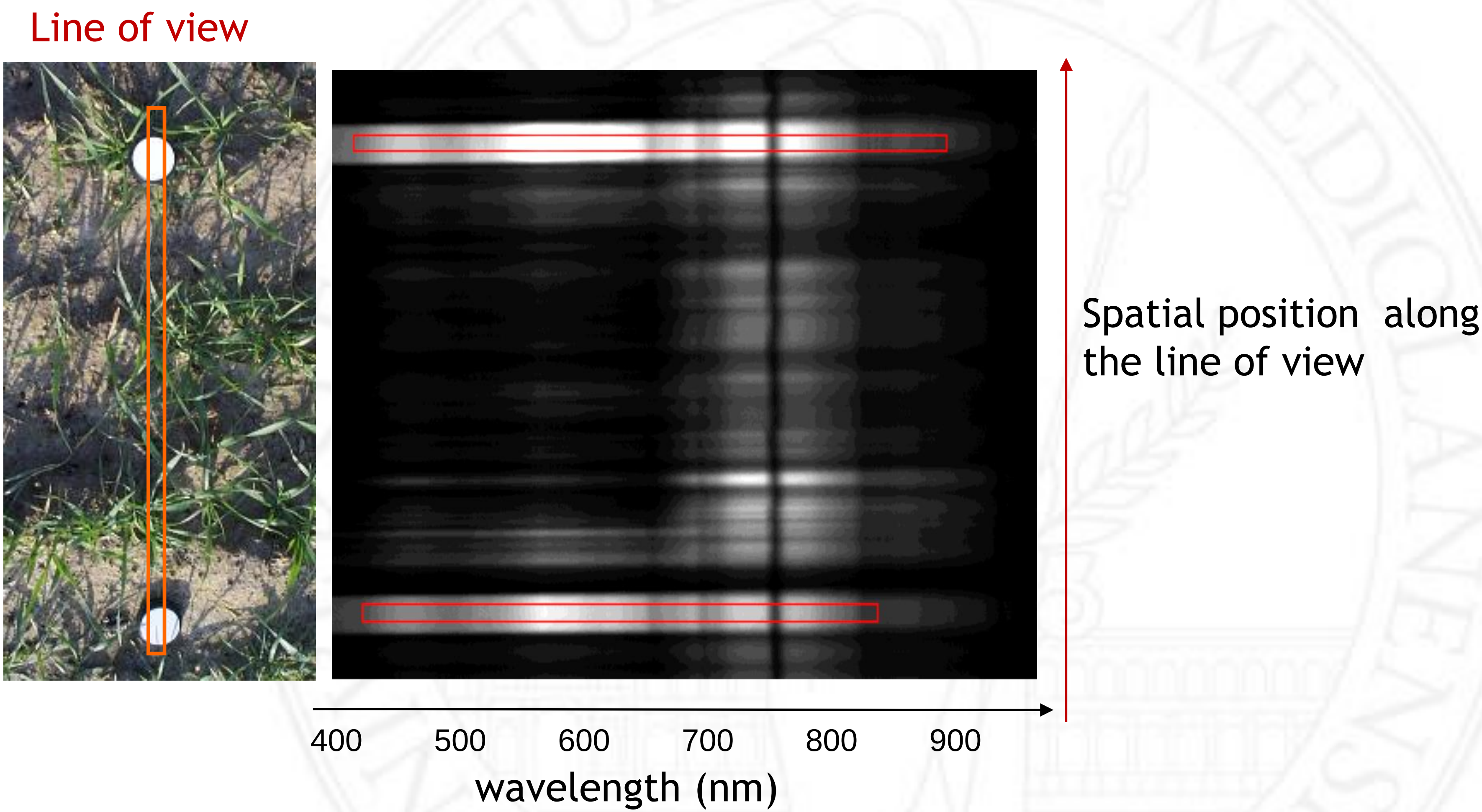
A line of view is acquired, and for each spatial pixel a spectrum line is obtained



# Hyperspectral imaging

In the hyperspectral image:

- each row corresponds to the spectrum of a spatial point
- each column corresponds to a transect (line of view) seen at a specific wavelength

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# Hyperspectral imaging

In the hyperspectral image:

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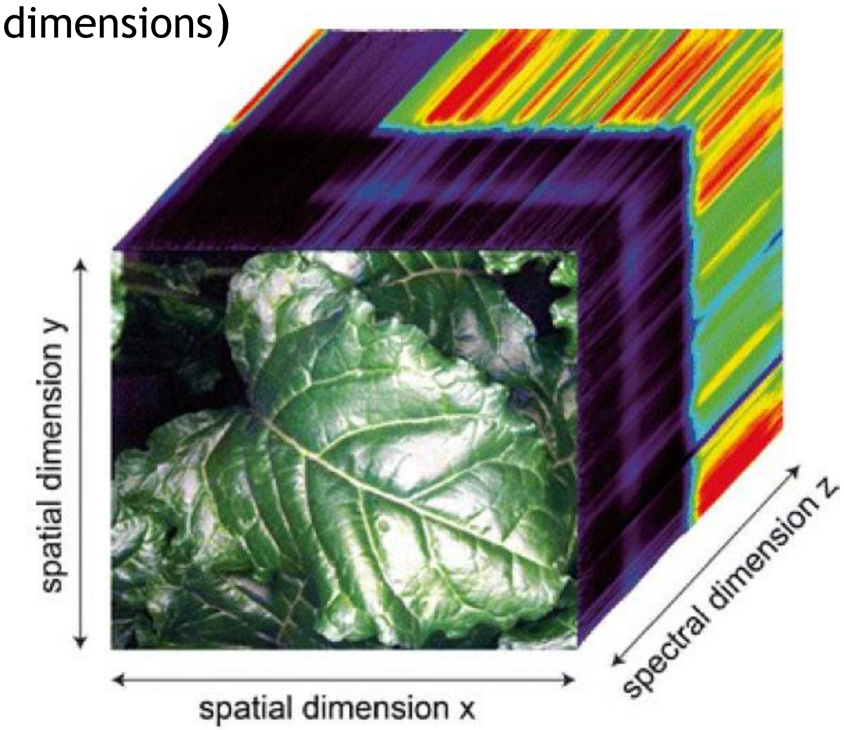
Line of view

In controlled conditions (as for phenotyping) the line of view can be moved at constant speed in order to scan the whole sample

This allow to obtain an hypercube (i.e. a 3D matrix with 1 spectral x 2 spatial dimensions)

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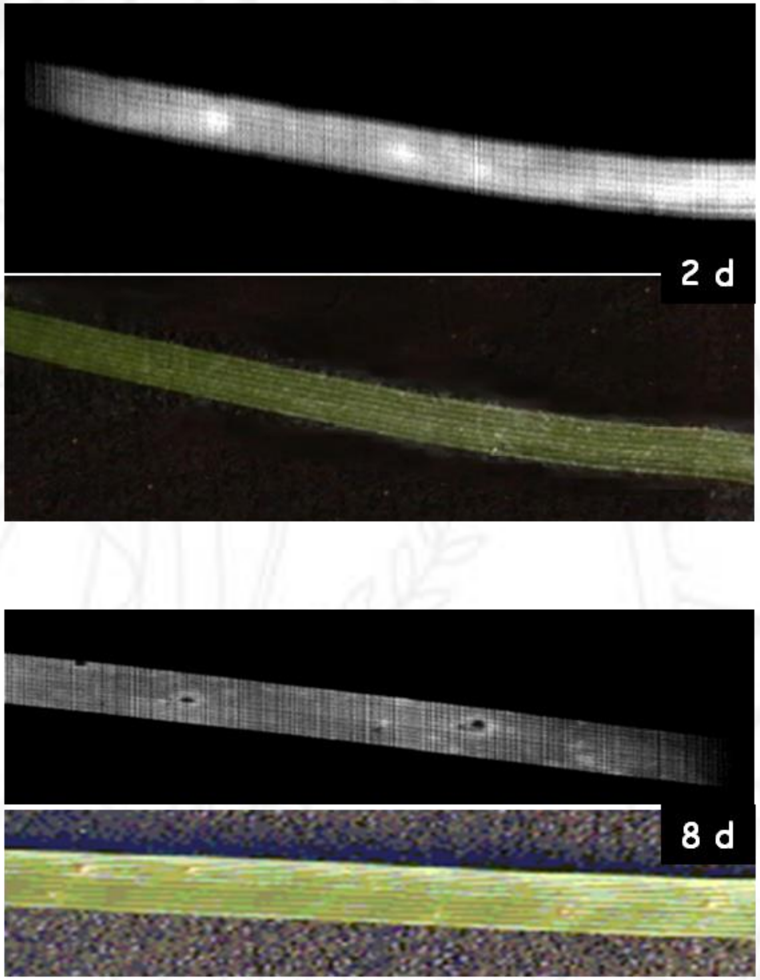
# Chlorophyll fluorescence imaging

Intensity of chlorophyll fluorescence can be imaged at 690-740 nm under suitable actinic excitation

Being a probe of the photosystem status it enable early detection of stress responses to abiotic and biotic

Heterogeneities in kinetic fluorescence parameters as Fv/Fm can be measured by acquiring a sequence of images during 1-2 s

Fluorescence on wheat ($\lambda$>690nm)

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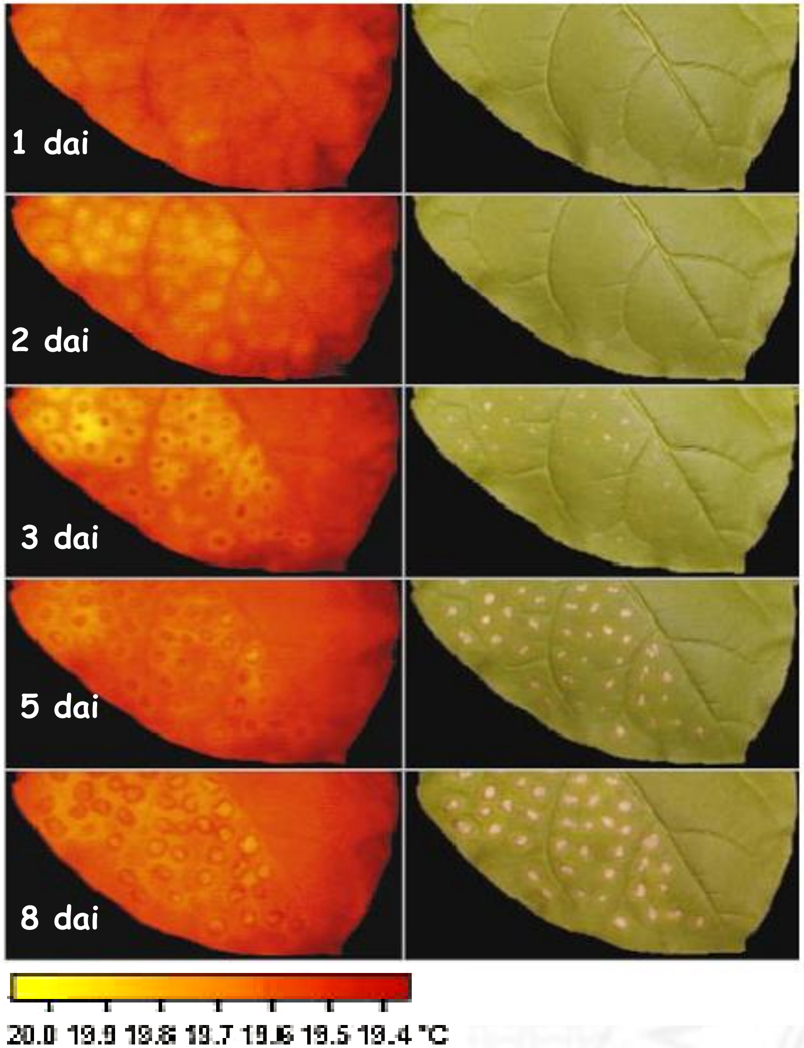
# Thermal imaging

Thermal cameras (8-10 μm spectral range) allow to map leaf and canopy temperature (differential accuracy $\Delta T \cong 0,05$ C)

Given the link with evapotranspiration, temperature difference between canopy and the environment is typically used as indicator of water status of the plant

Local spots of temperature can also be associated to early stages of pathogens infection

Influence of the canopy microclimate and sun illumination can easily cover the physiological heterogeneity making thermal imaging less obvious than seems

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# Future trends

Automatic phenotyping is rapidly growing towards new applications that were not covered here:

- 3D imaging
- seedling and germination
- root systems
- field phenotyping

Thanks for the attention and for further discussions

Short Course: *From -omics to phenotyping for crop improvement*

# Imaging techniques for plant phenotyping

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