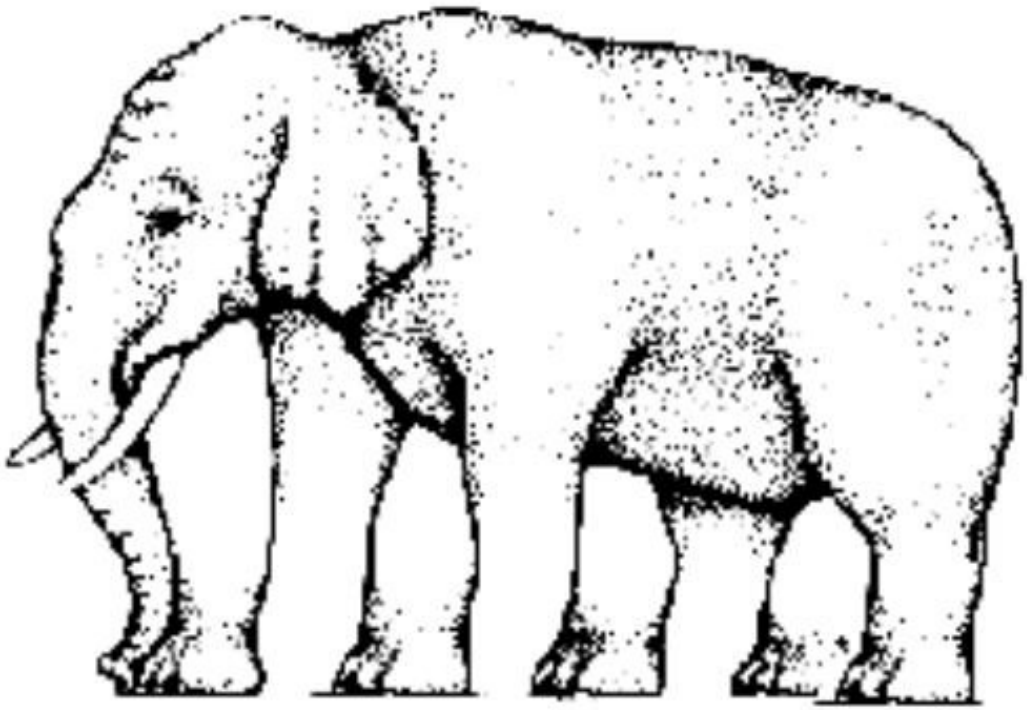
Get an impression of your plant!

NCERA 101
Cocoa Beach, March 10, 2008
Dr. Jörg Vandenhirtz

Integrated solutions for biology

- founded May 1998 in Aachen, Germany
- interdisciplinary team (biology, physics, engineering)
- development of image processing software
- development of hardware
- plant pathology, effect screening, ecotox, qc
- development of integrated solutions for research and industry in compliance with int'l standards

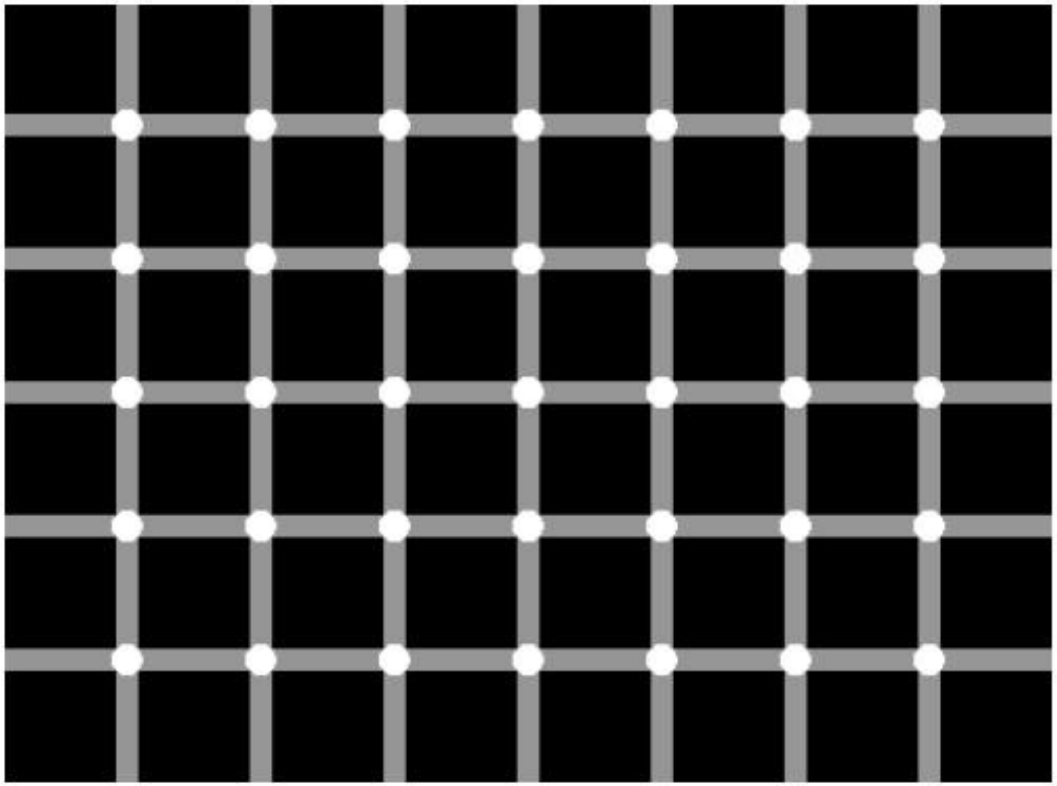
The human eye



How many legs does this elephant have ?

LemnaTec

not suited for scientific measurements

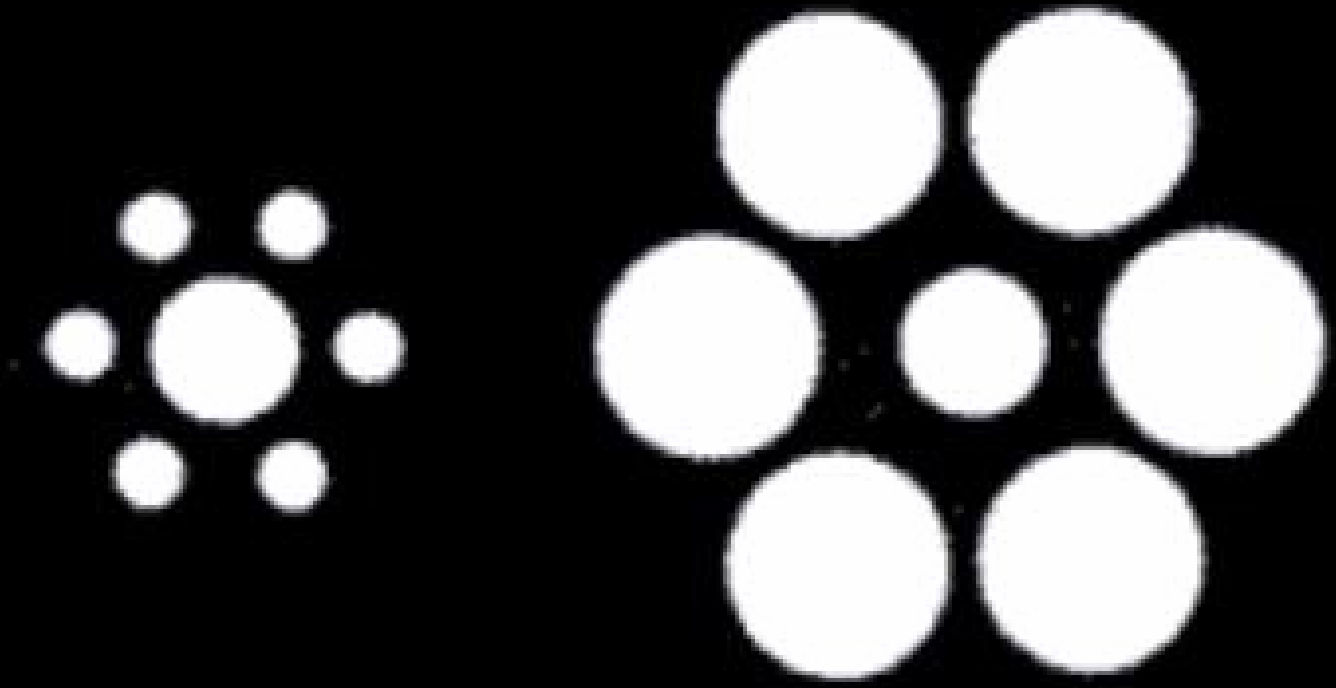


Count the number of black dots ...

LemnaTec

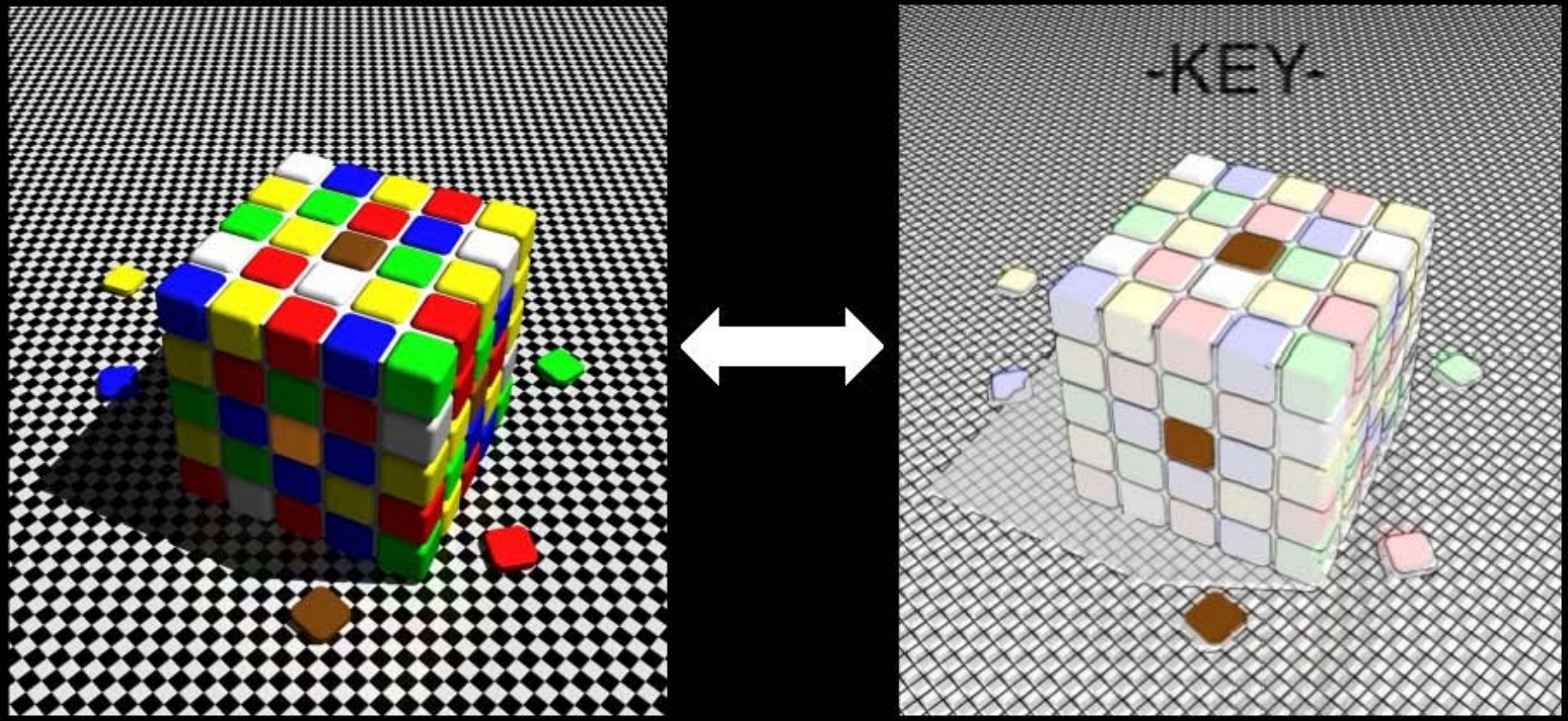
not suited for scientific measurements

the circles in the center...

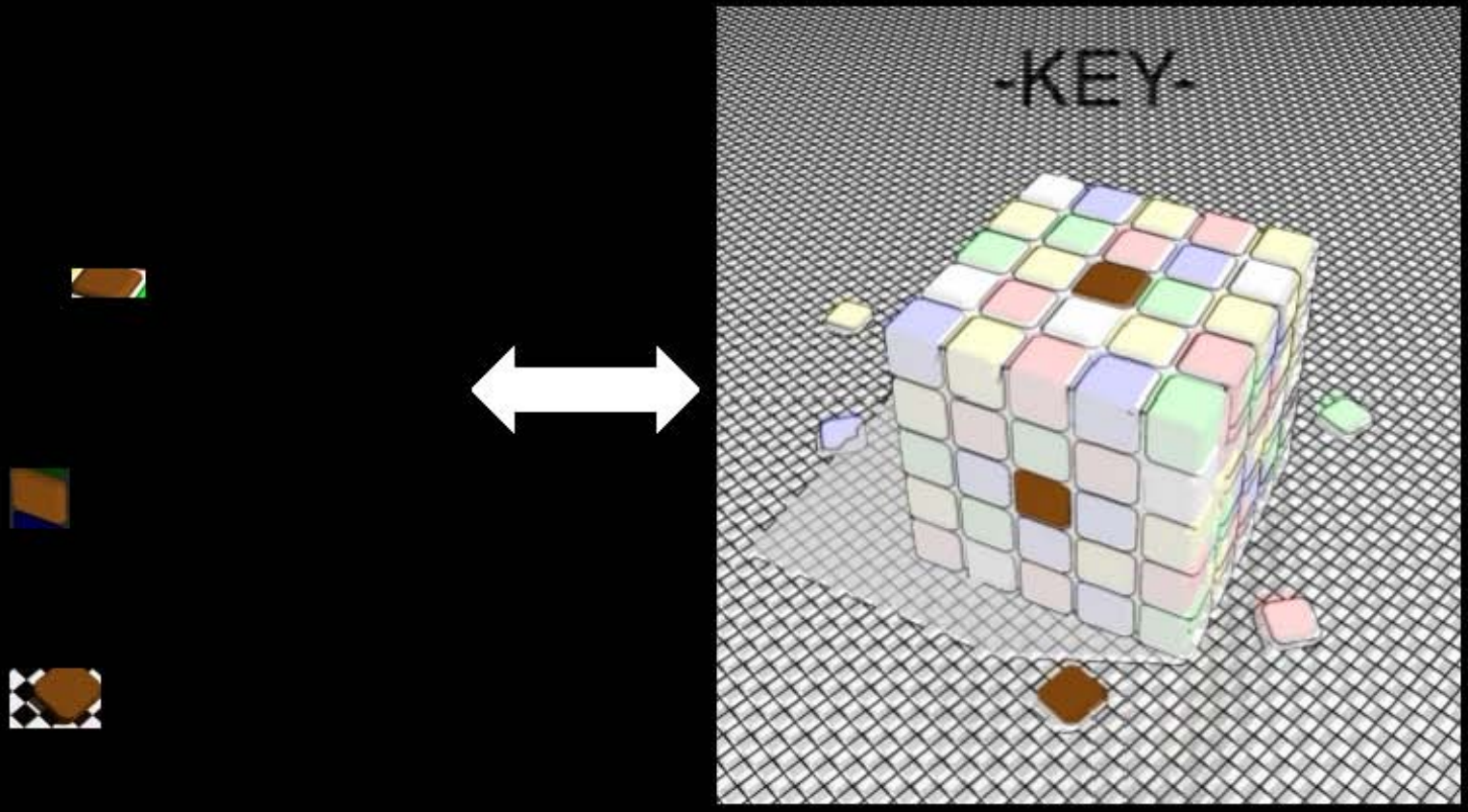


...are exactly the same size!

Colour – a very complicated parameter

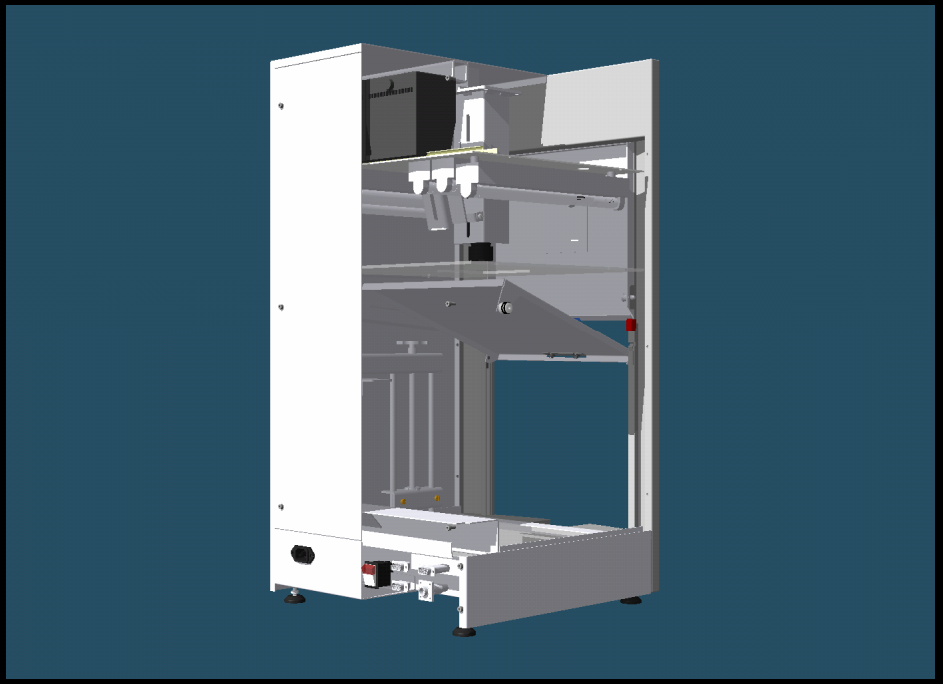


Colour – a very complicated parameter



The LemnaTec Scanalyzer System Basic

- objects up to 20 cm x 30 cm
- microplates up to 96 wells
- manual change of samples



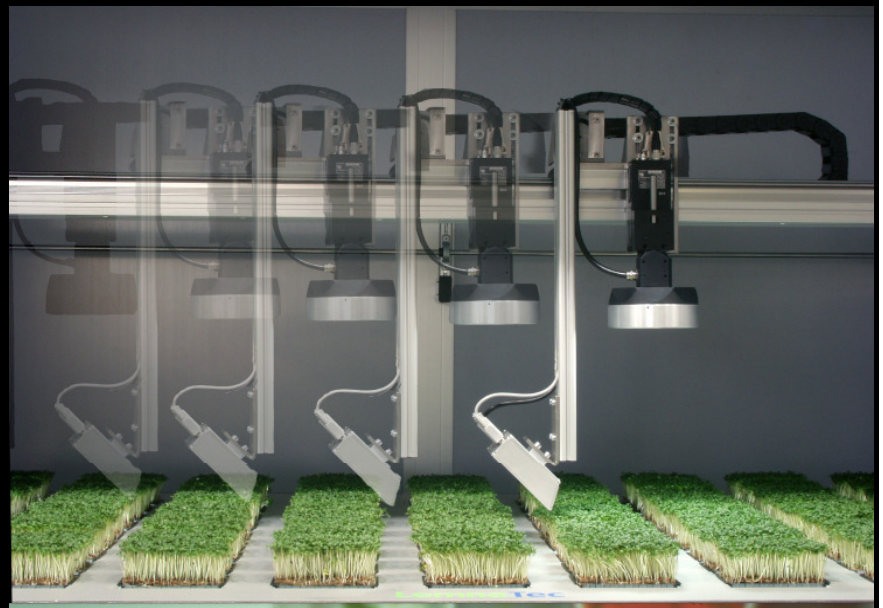
The LemnaTec Scanalyzer System Plant

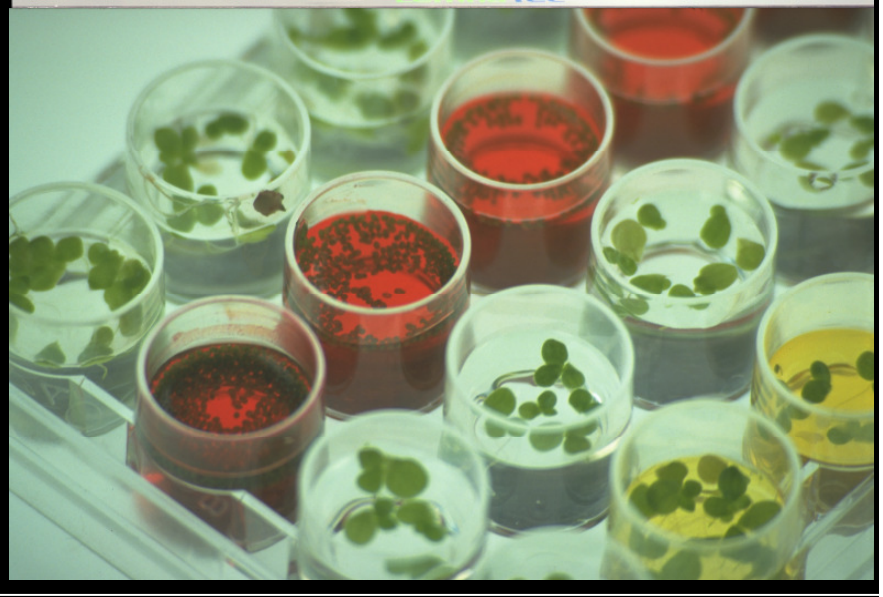
- objects up to 25 cm x 30 cm
- max. object height 25 cm
- MTPs up to 96 wells
- manual change of samples

The LemnaTec Scanalyzer System HTS

Scanalyzer HTS features

- flexible size
- high number of samples
- large units
- high resolution available esp. for 96 well plates
- automated image acquisition for mobility tests

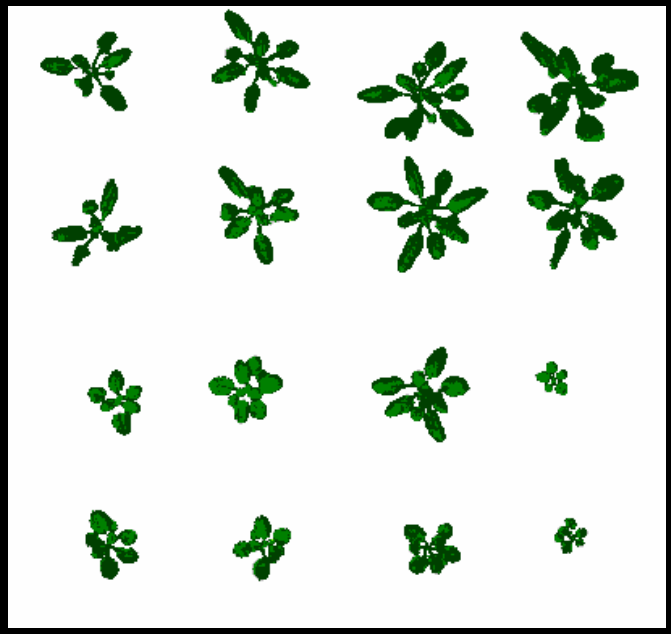


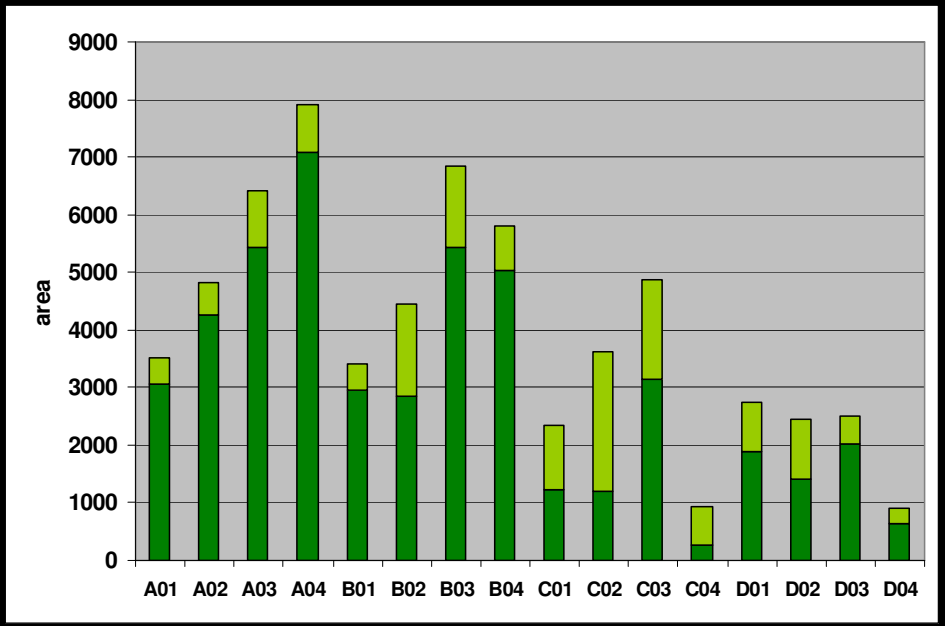


The LemnaTec Scanalyzer System HTS

Single point assessment

Image processing provides reliable quantitative data allowing e. g. any kind of statistics and a calculation of mean values and significances.

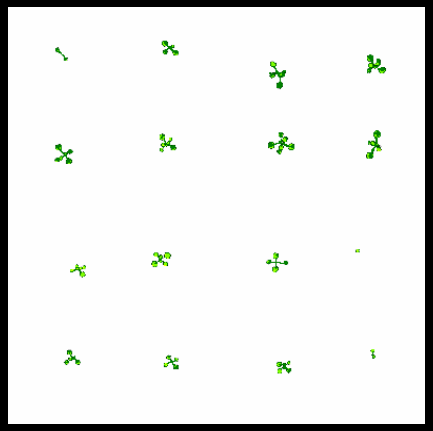




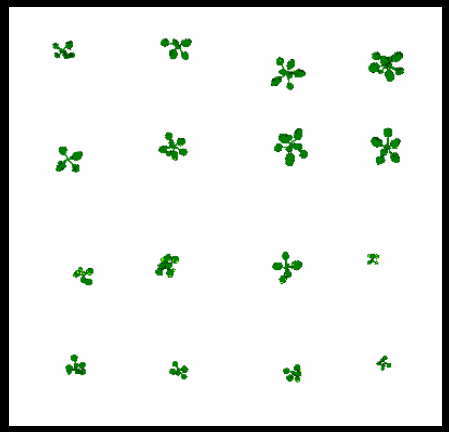
But information describing growth remains limited.

Imaging in time series

t=0d



t=4d



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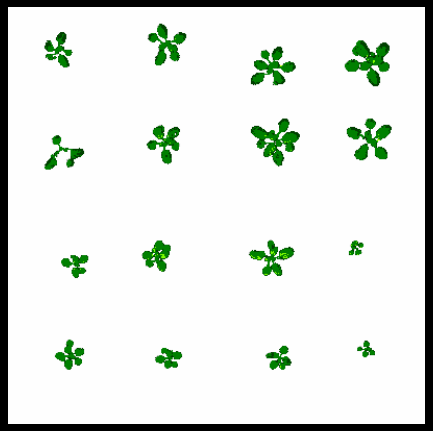
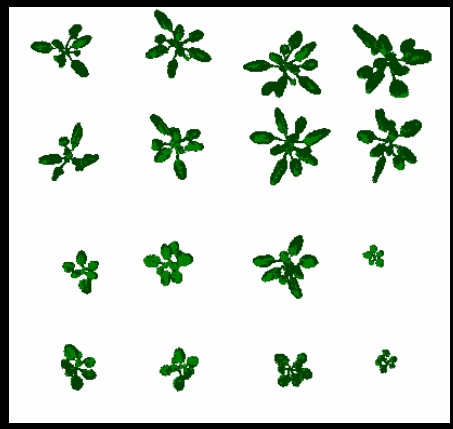
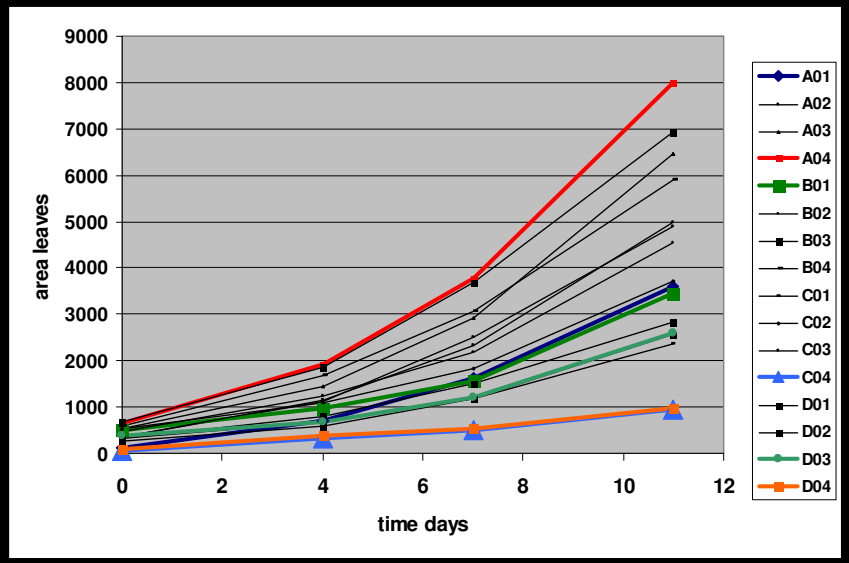


t=11d

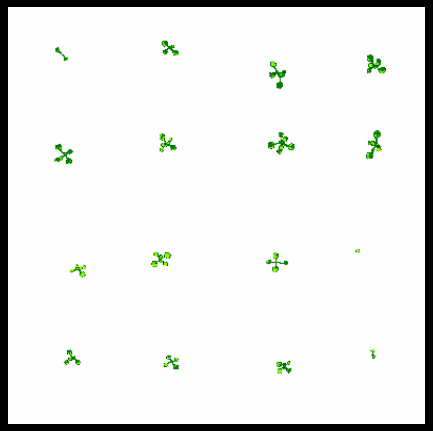


LemnaTecs image processing allows highly automated imaging in time series.
But area based growth curves only show a minor part of the imformation available. **How homogeneous was growth?**



Imaging in time series

t=0d



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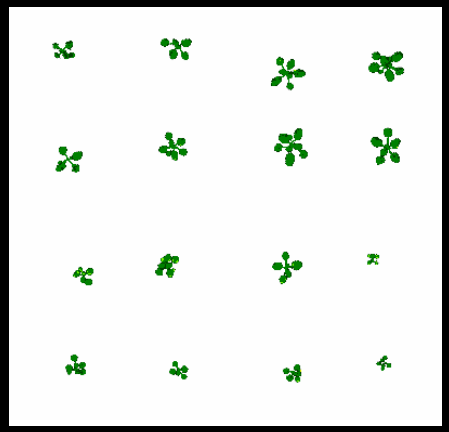

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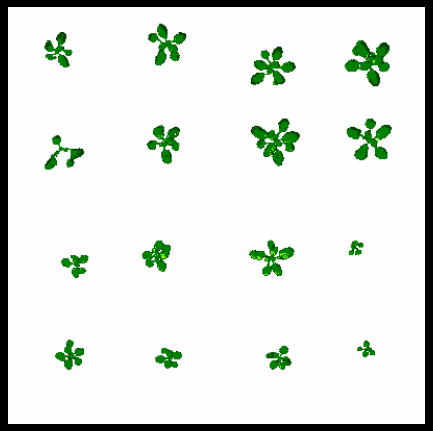

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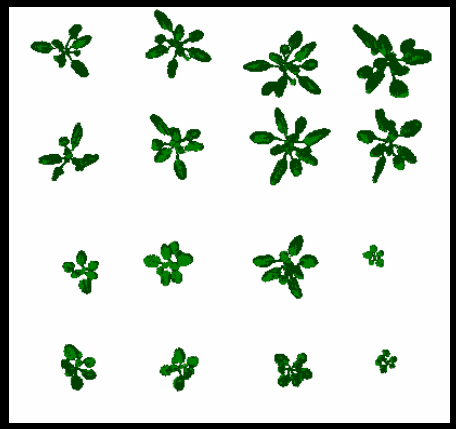
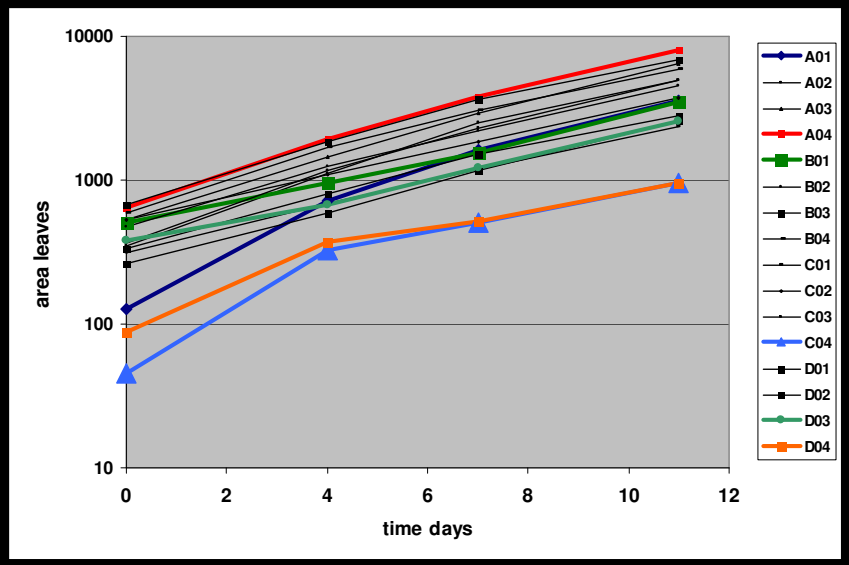


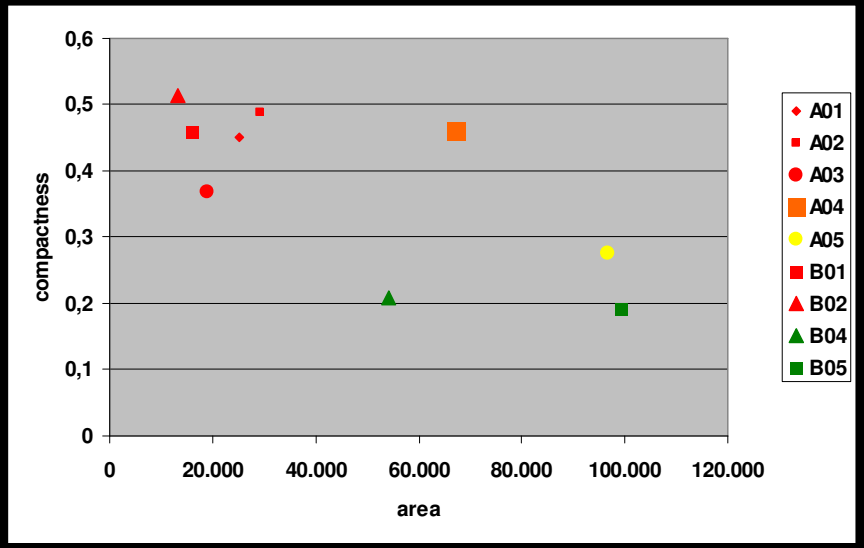
The slope of the curves displaying growth rates show that most plants grew at a relatively constant rate but just started at different sizes e. g. due to delay in germination.

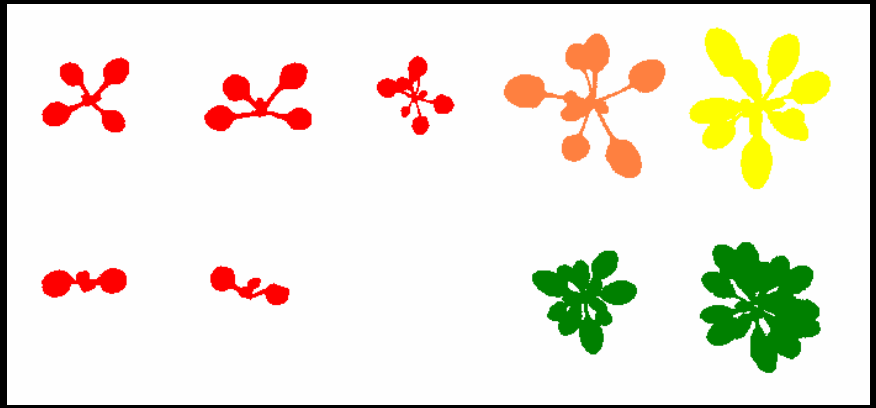


Morphological assessment

- compactness -

- compactness is calculated based on the size independent rotational momentum of the plant
- it describes if the leaves are nearer around the centroid or farther outside e. g. showing longer stipes





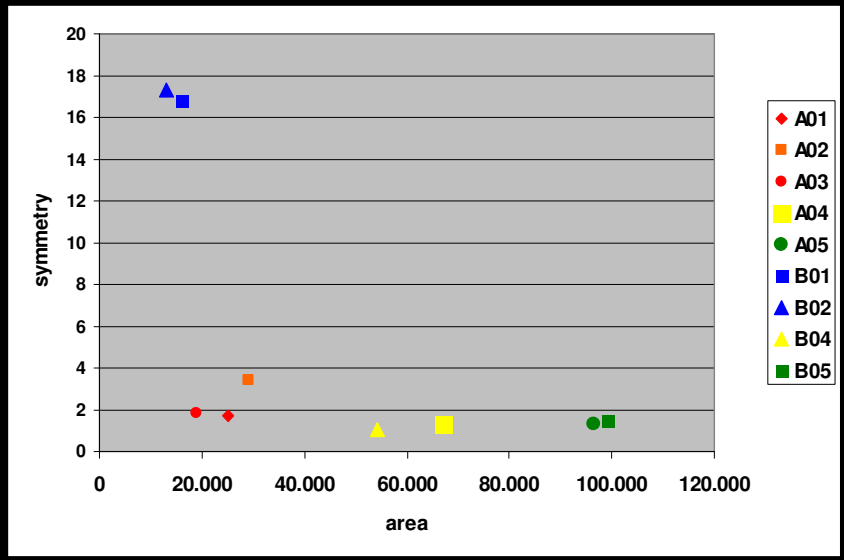
Morphological assessment

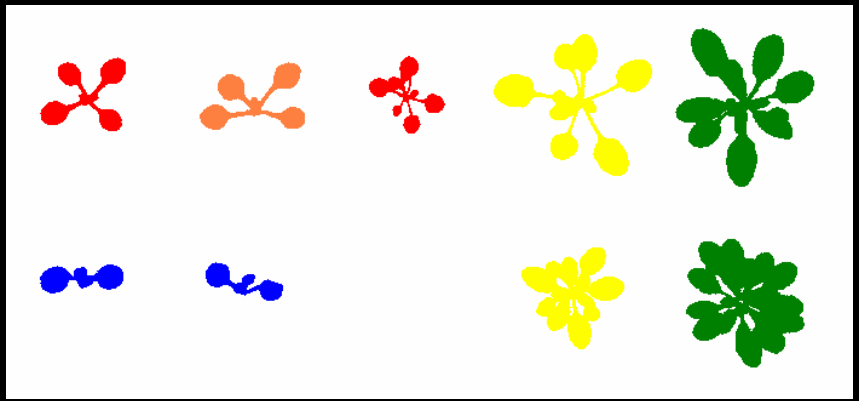
- rotational symmetry -

- rotational symmetry is calculated based on the size independent 2nd moment principal axis ratio

- it describes in how far the leaves alltogether show a symmetric plant

- this may lead to completely other grouping than before-mentioned compactness



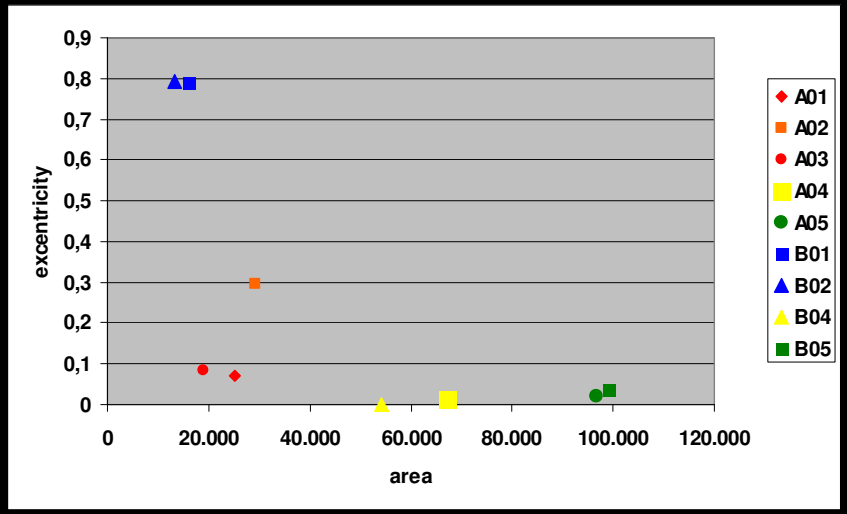


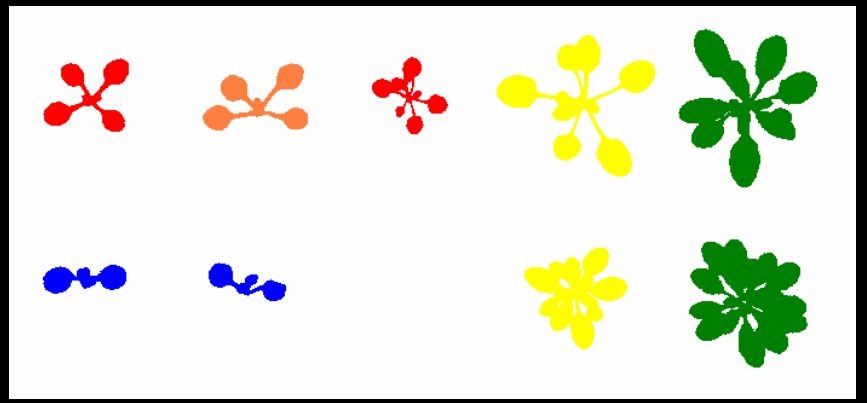
Morphological assessment

- excentricty -

- while calculated with a different algorithm excentricity provides here quite similar grouping results as rotational symmetry

- nevertheless plant A02 shows more significant distance to A01 and A03 than with rotational symmetry

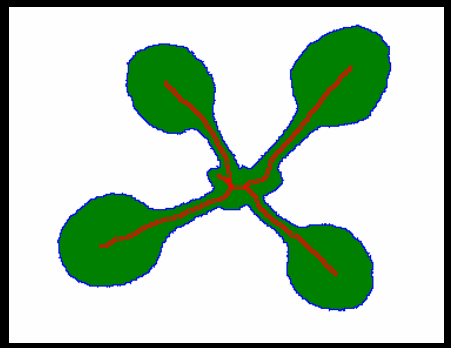


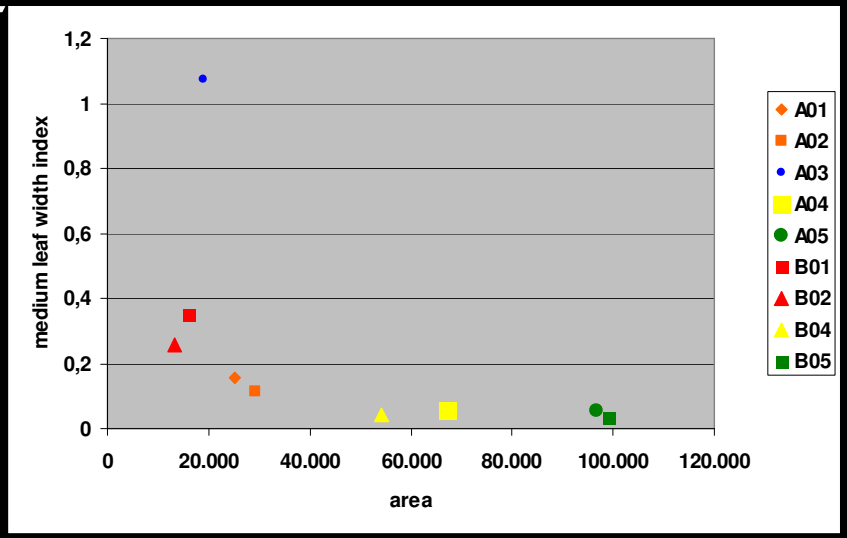


Morphological assessment

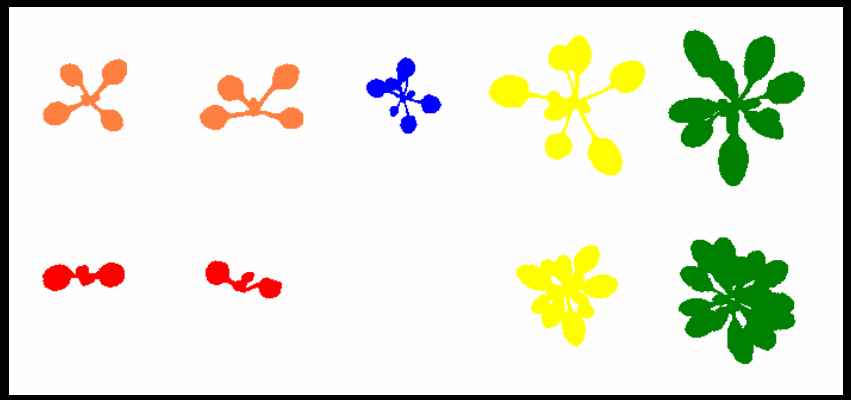
- medium leaf width index -

- the medium leaf width index is calculated from the square length of the plant skeleton divided by the leaf area





- it describes size independent differences in „leaf width“ integrating stipes, leaves and overlapping effects.

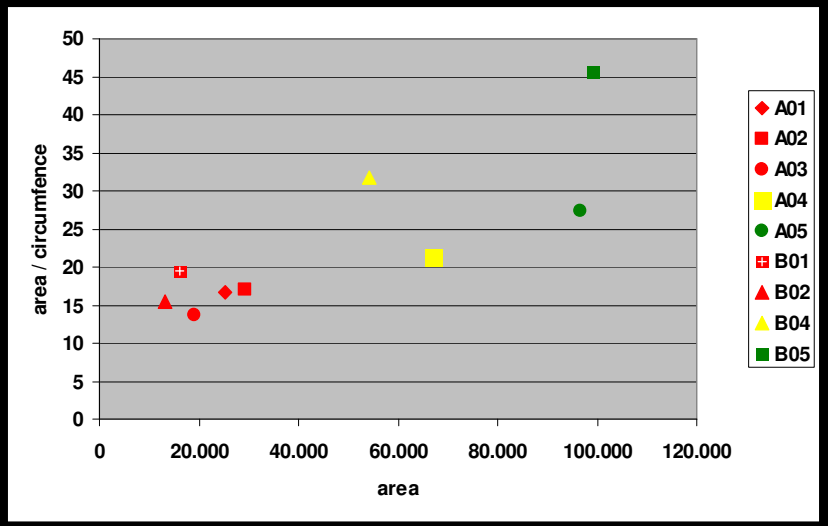


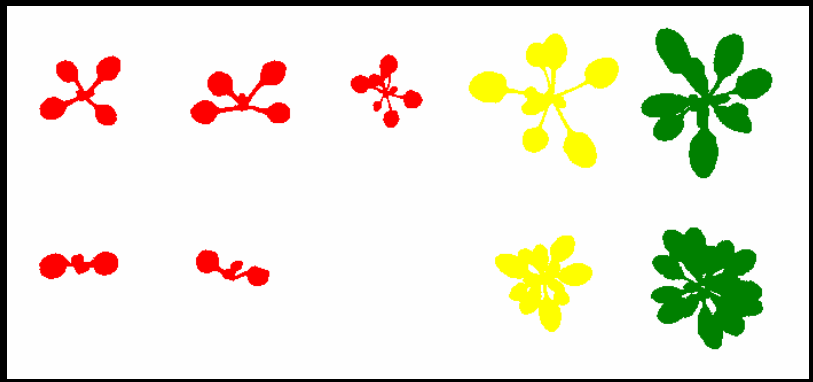
Morphological assessment

- area/circumference -

- while having some size dependency left the ratio of leaf area divided by plant circumfence may allow additional classification of morphological traits.

- as with many parameters each one provides new aspects for grouping plants which may be especially suitable for statistical QTL analysis



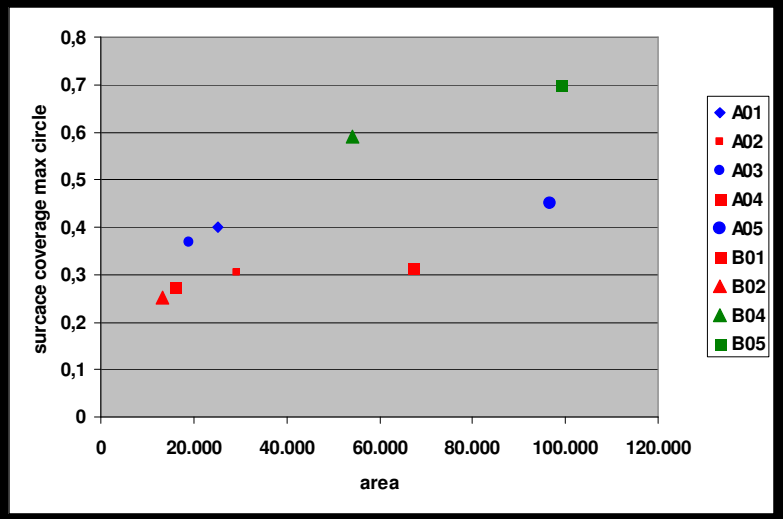


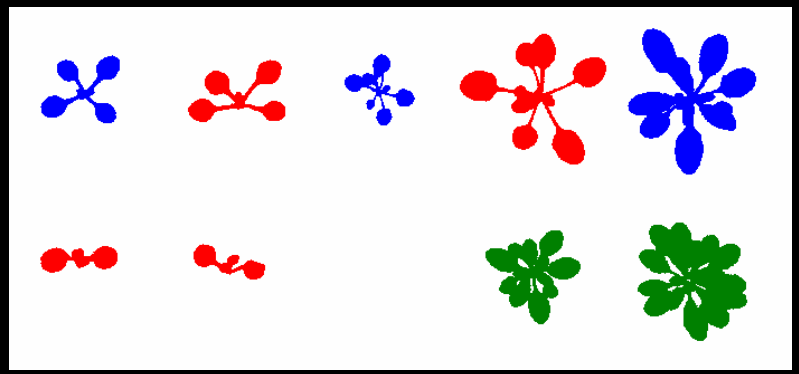
Morphological assessment

- surface coverage -

- surface coverage compares the measured plant area to the area of a circle covering the whole plant

- this parameter is intended to provide a calculation how dense the plant covers the soil in its immediate growth area



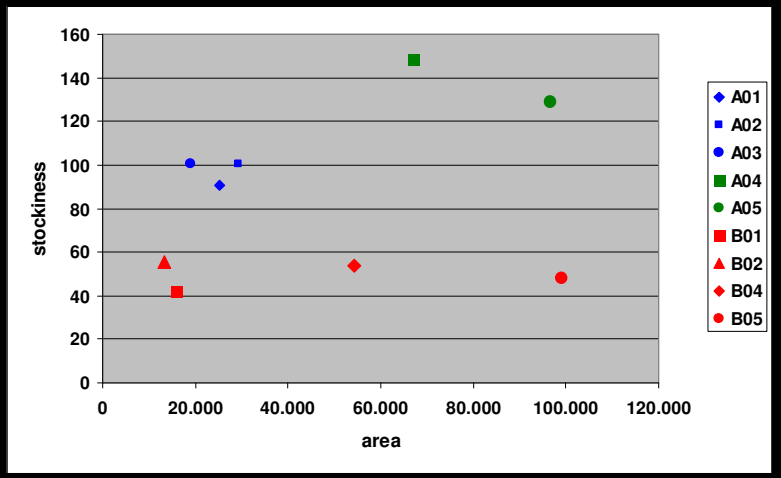


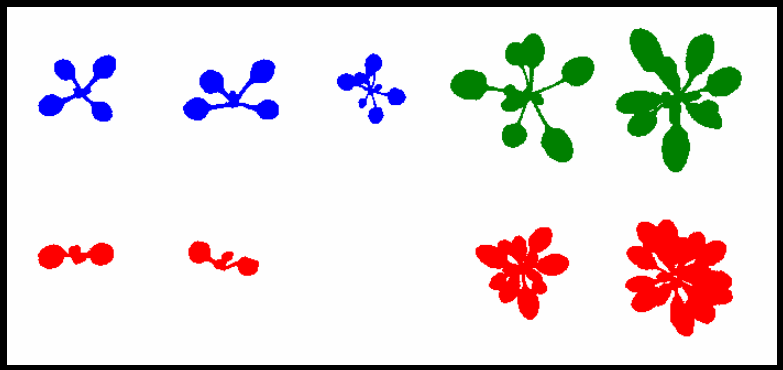
Morphological assessment

- stockiness -

- stockiness is mathematically the description of roundness.

- applied to Arabidopsis images it separates plants with invisible or relatively short broad stipes from plants with long small stipes





3-D Measurement of trays

Only in combination
With HTS Scanalyzer

▪Max field of view	810 mm
▪Max field of view in height	400 mm
▪Resolution in height	0.2 mm

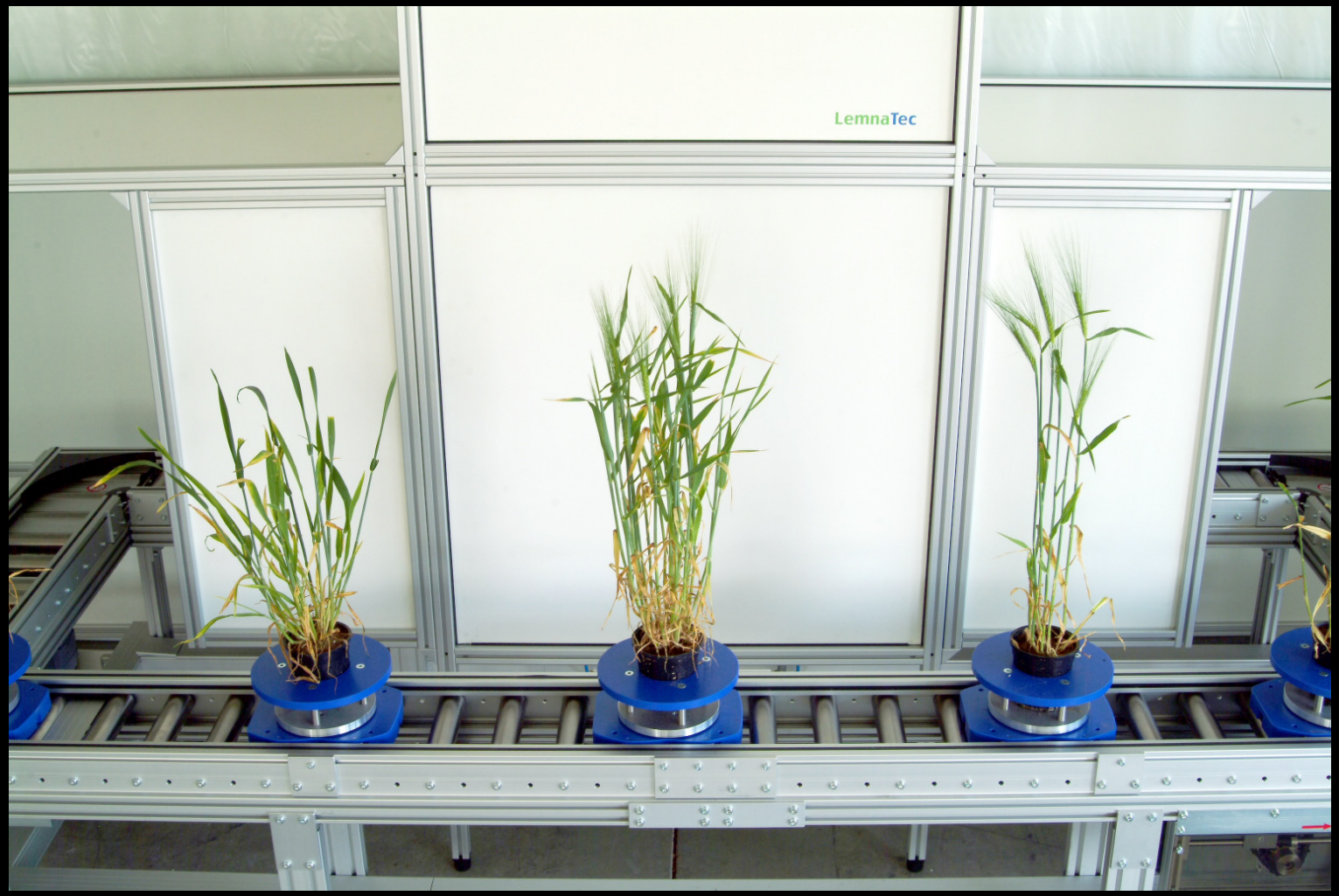
3-D Measurement of trays

▪Max field of view	810 mm
▪Max field of view in height	400 mm
▪Resolution in height	0.2 mm

Scanalyzer 3-D

Scanalyzer 3-D

Scanalyzer 3-D



Scanalyzer 3-D

Scanalyzer 3-D
With scale and watering
All IP 65

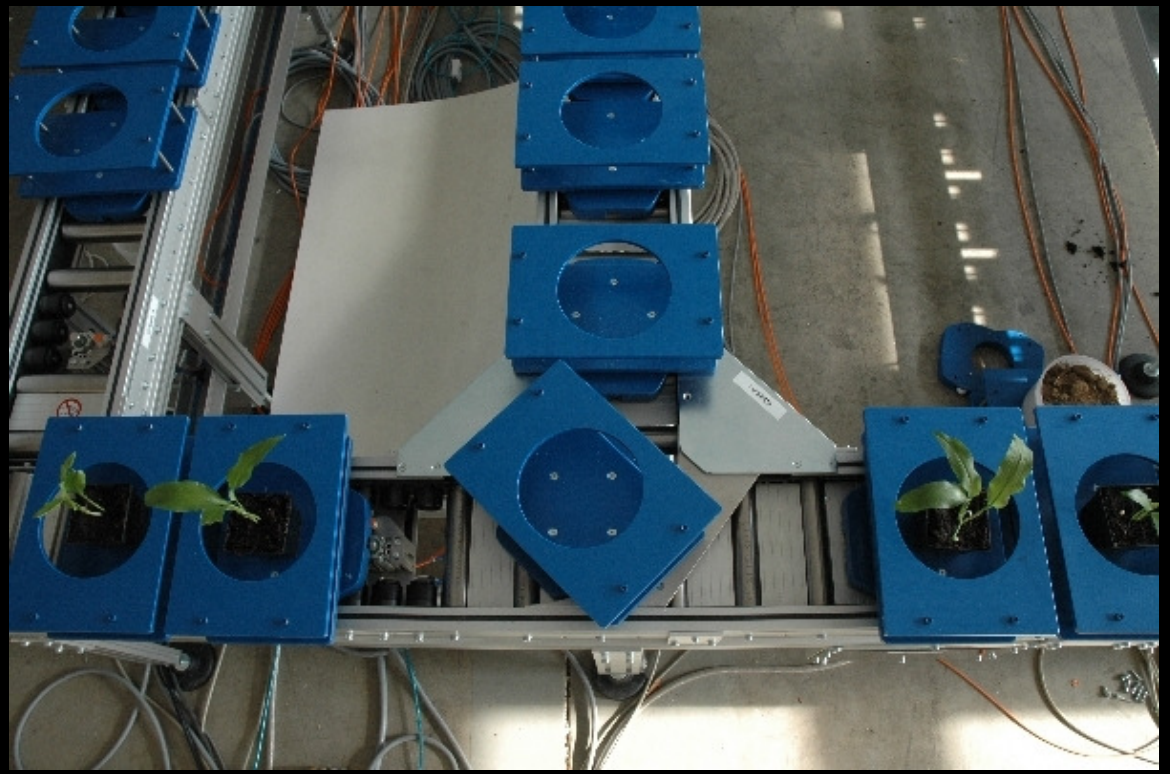
Scanalyzer 3-D

Several Loops to sort plants by:

- Hit
- Size
- BC
- Weight
- Age
- Etc.

LemnaTec

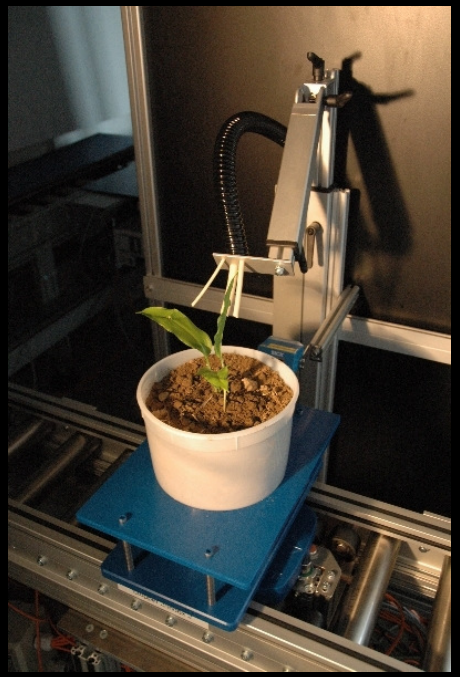
Scanalyzer 3-D



Scanalyzer 3-D

LemnaTec

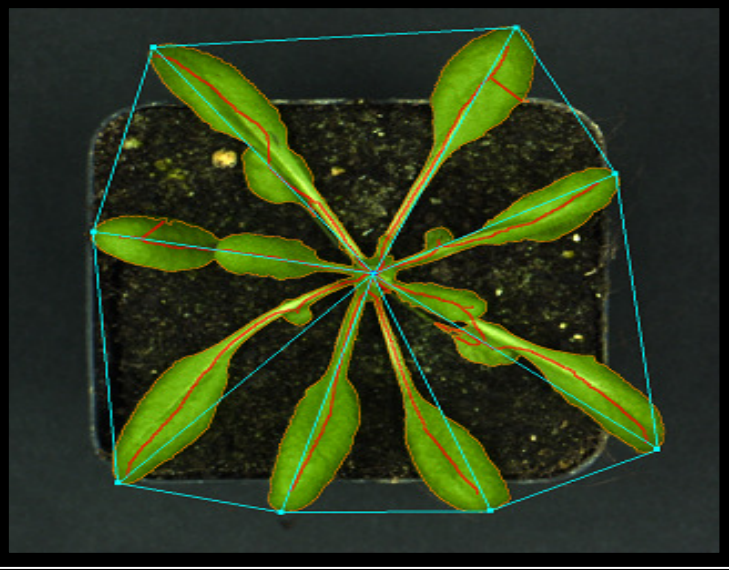
Scanalyzer 3-D



LemnaTec combines today's technology in

- robotic plant handling systems in greenhouses in the plant production industry

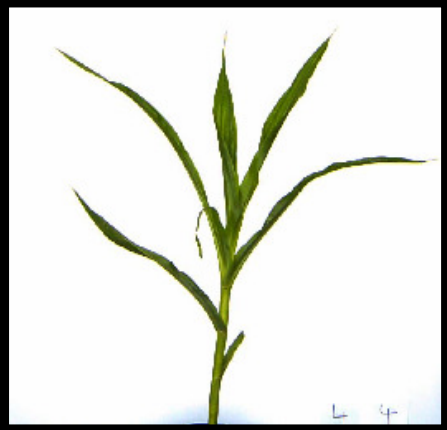
- digital imaging with modern image processing and database integration for large datasets



High variation of growth patterns

A key issue especially for GMO screening



High morphological variability makes comprehensive quantification of leaves and stem absolutely important

Scanalyzer 3-D

Top View

Side View

Side View 90°

Automatic zoom-in triggered by seed-date in barcode / RFID or database

Identification of first leaf appearing from soil

Plant colour classification

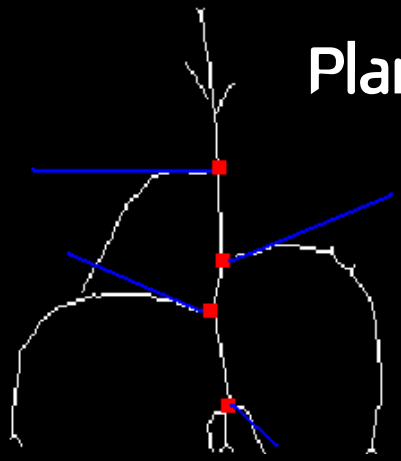
- Key to plant health -

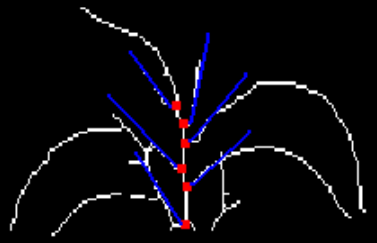
Plant skeleton analysis

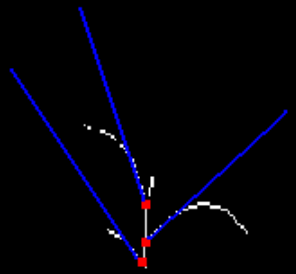
- Key to growth dynamics and morphology -

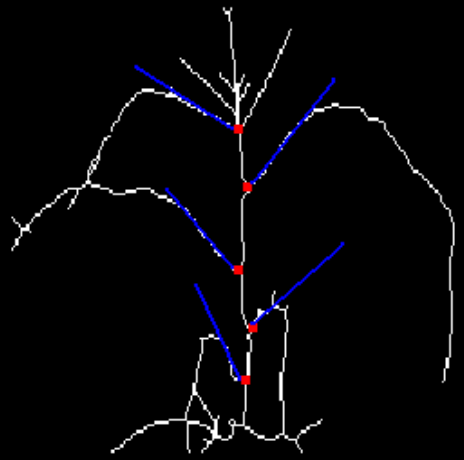
- separation of stem and leaves
- information about nodes, length of leaves
- morphology
- plant growth phases

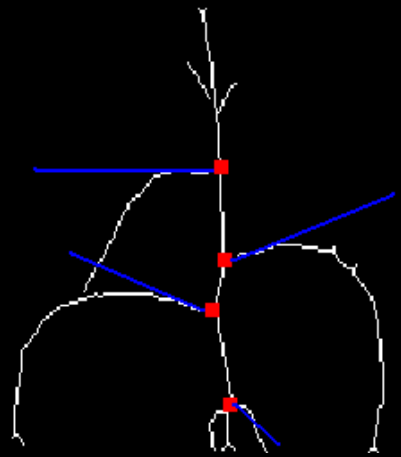
Plant architecture analysis





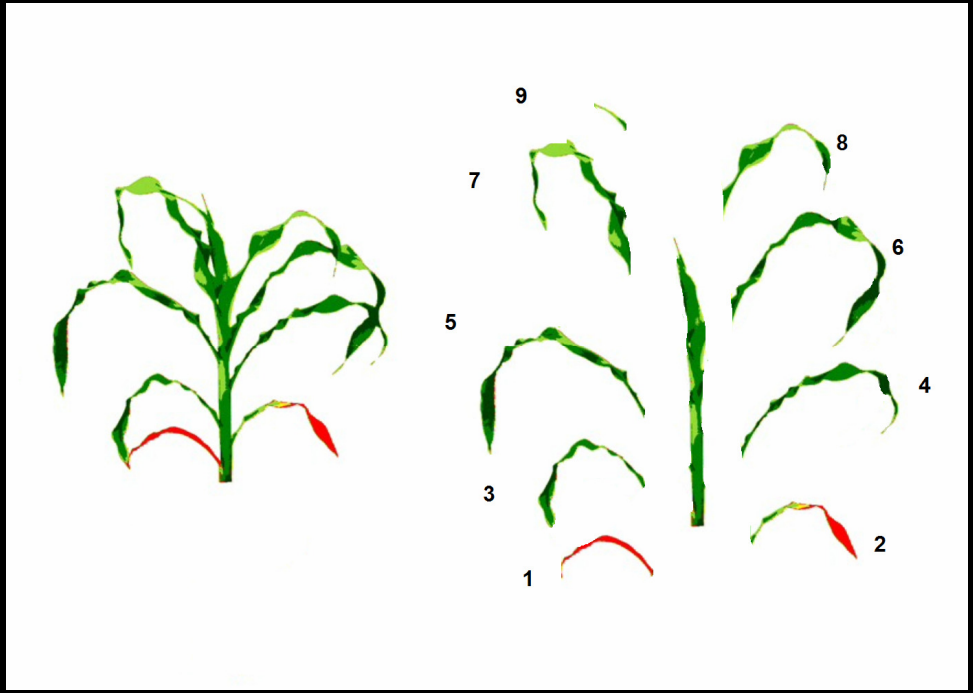






Partial analysis of corn plants

- Individual leaf length
- Individual leaf area
- Mean leaf width
- Stem length
- Stem width
- Stem volume
- Bent index
- Leaf curling index
- Leaf orientation
- Individual leaf colour classification



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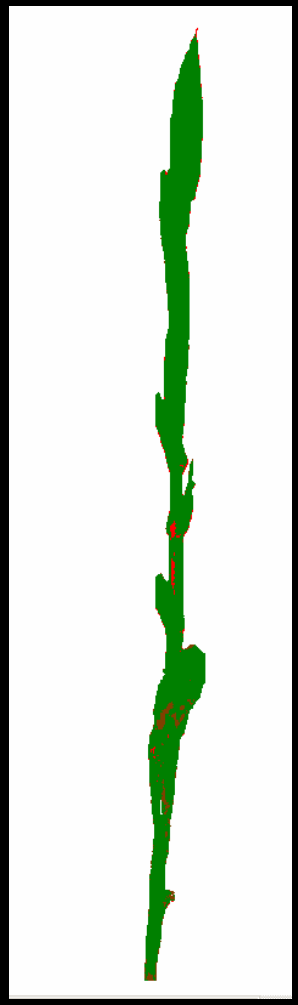
Details of three-dimensional plant screening



Identification of

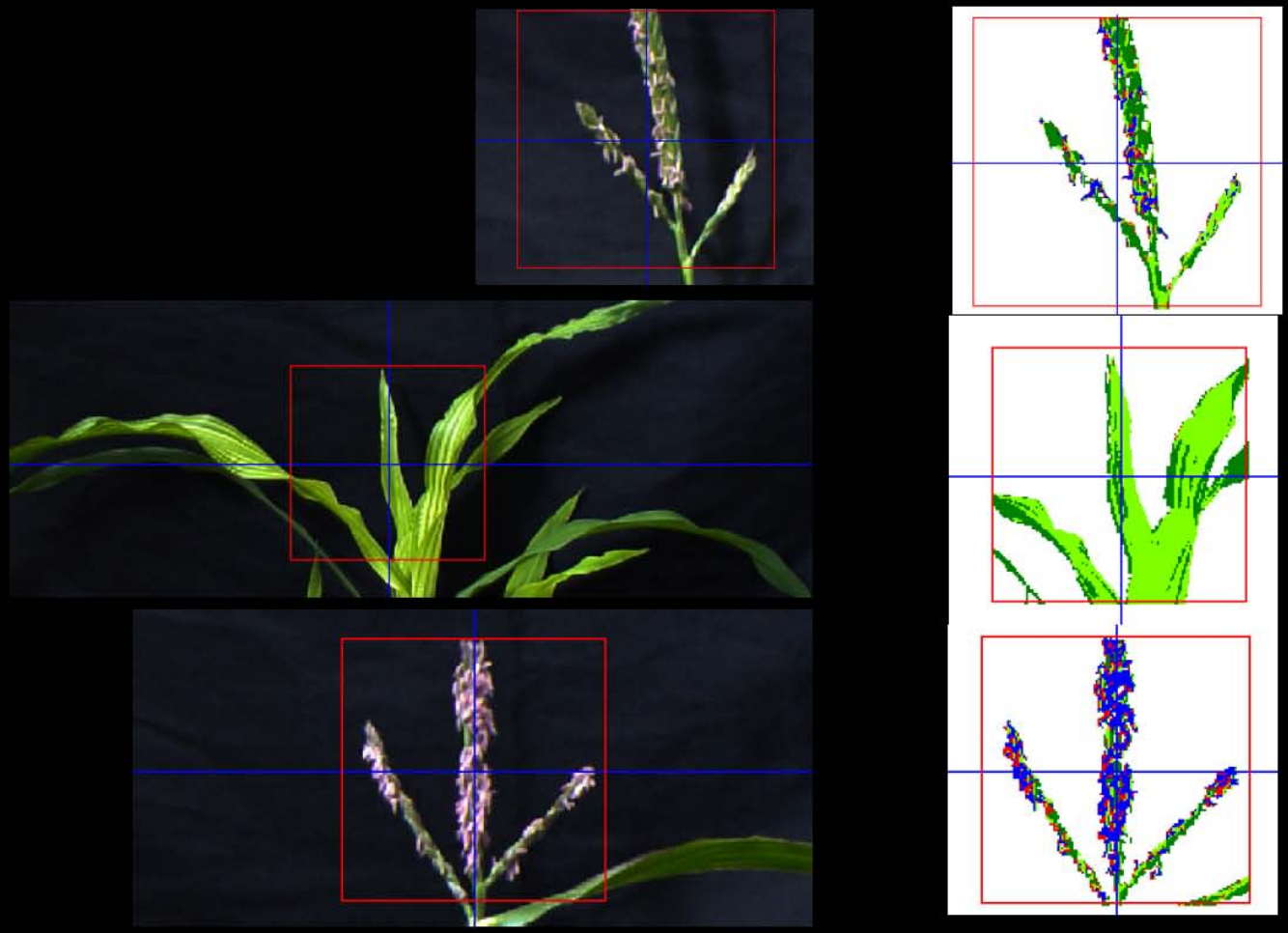
- main axis
- stem width

allows
better quantification
of biomass

Biomass estimation based on images from x,y,z-axis

For best estimation of biomass of the plants images were taken from all 3 axis

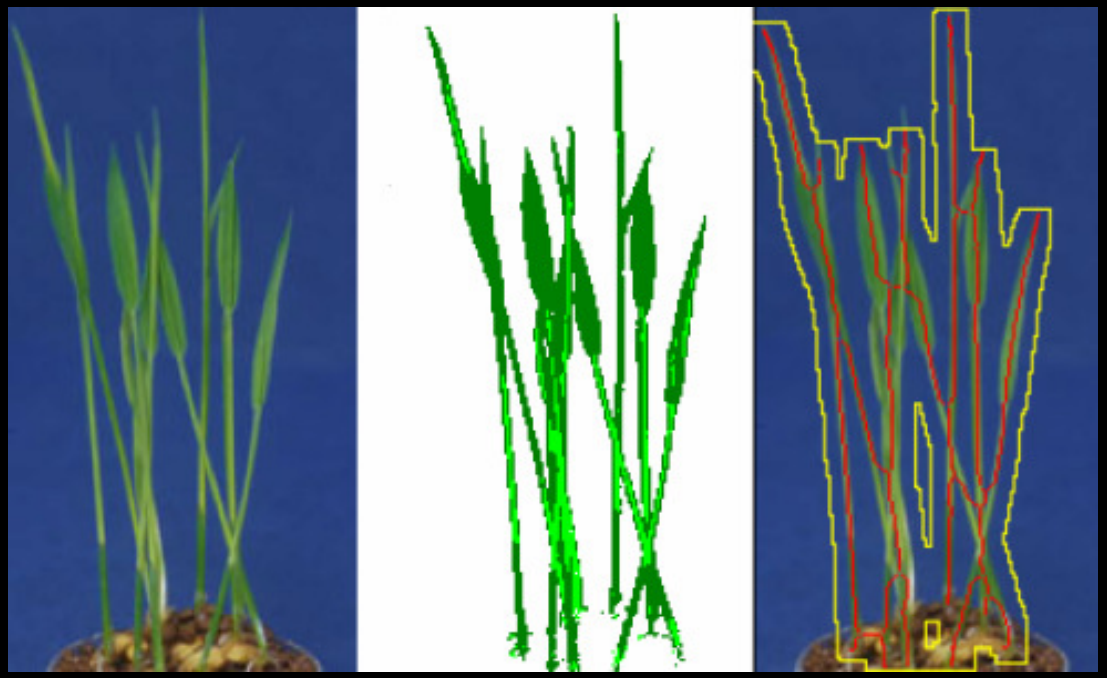
total green	total green top	total green broad side	edgewise	Cubic /10e9
	5420	4480	3162	77
	8914	4955	3342	148
	18473	16243	14691	4408
	43356	42450	41071	75590
	51428	39292	40086	81002
	54743	36399	56102	111788
	83590	43380	87281	316493
	85483	72301	98308	607593
	139829	84230	135345	1594066



Identification of active male flower

Rice

- Leaf length even with crossings
- Leaf area
- Leaf colours
- Plant height
- Plant width
- Leaf density
- Leaf orientation



Pepper diurnal movement

Day

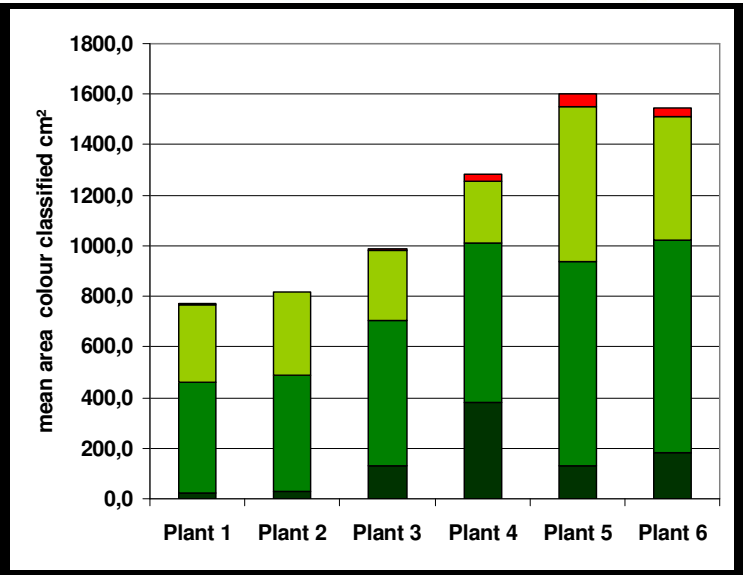
Evening

- leaf area
- leaf colour
- plant height
- plant width
- top/side ratio.

Skeleton analysis

Big hybrid poplars

- Height
- Width
- Leaf areas
- Leaf colours
- Tip Colours
- Growth rates
- Stem width
- Stockiness
- Circumference/height
- Image leaf volume (cylindrical)
- Mean vertical moment
- Mean covered area width
- Vert. moment assymetry
- 2nd moment pricipal axis ratio
- Compactness sourround area %



Poplar

image based compactness

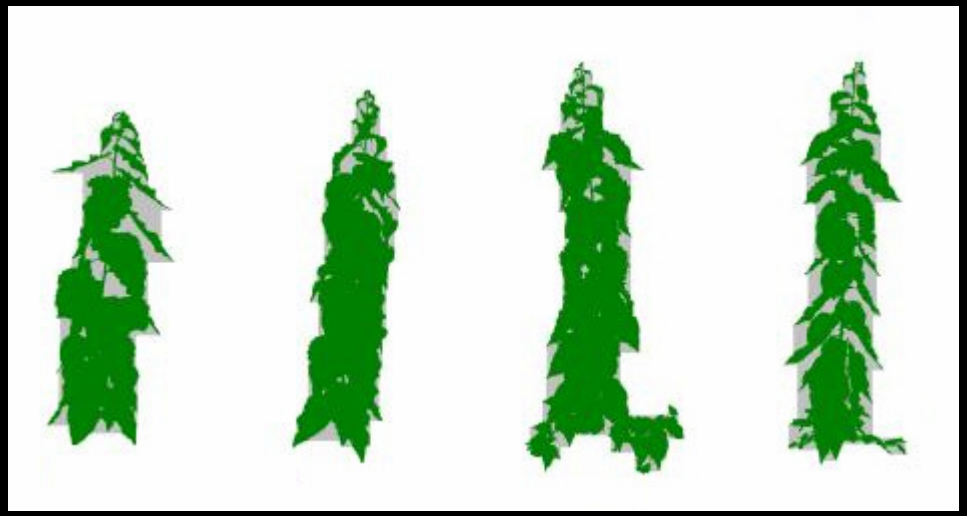
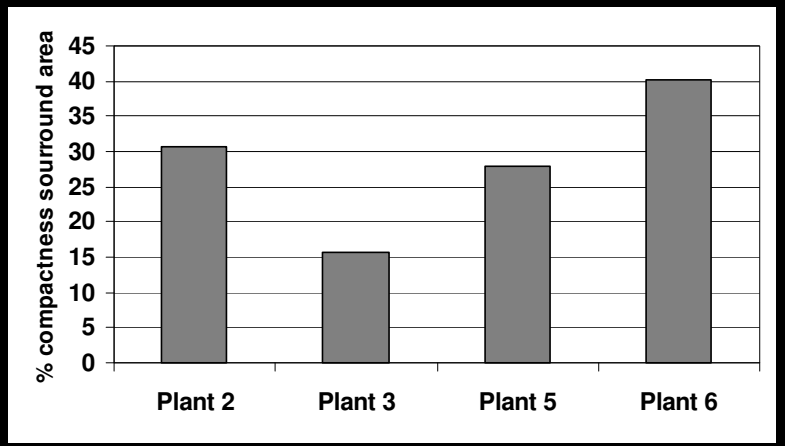


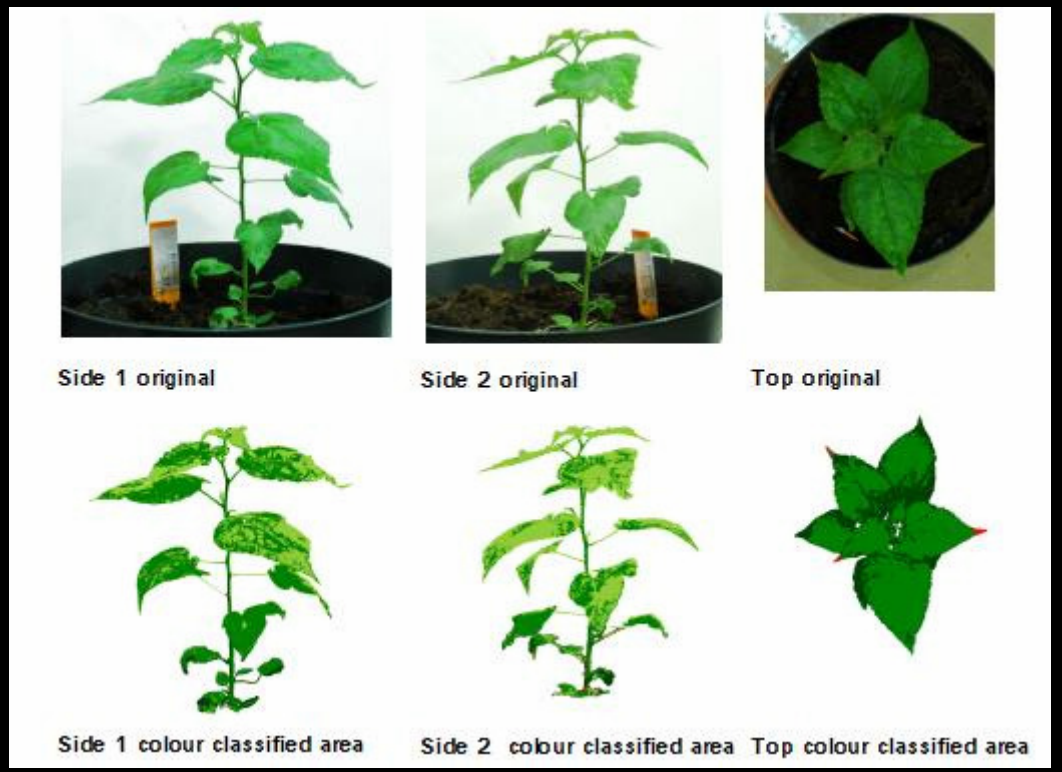
Image processing-based automatic identification of bay regions and holes to characterise compactness of growth.

The higher the compactness sourround-area the lower the compactness.



Small hybrid poplars

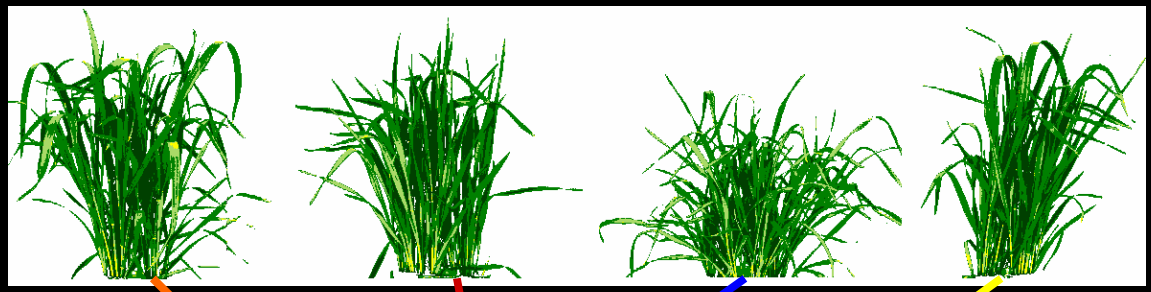
- Height
- Width
- Top area
- Mean side area
- Colour classified areas
- Top/side ratio
- Image plant volume
- Top compactness
- Side compactness
- Top symmetry
- Side vertical momentum

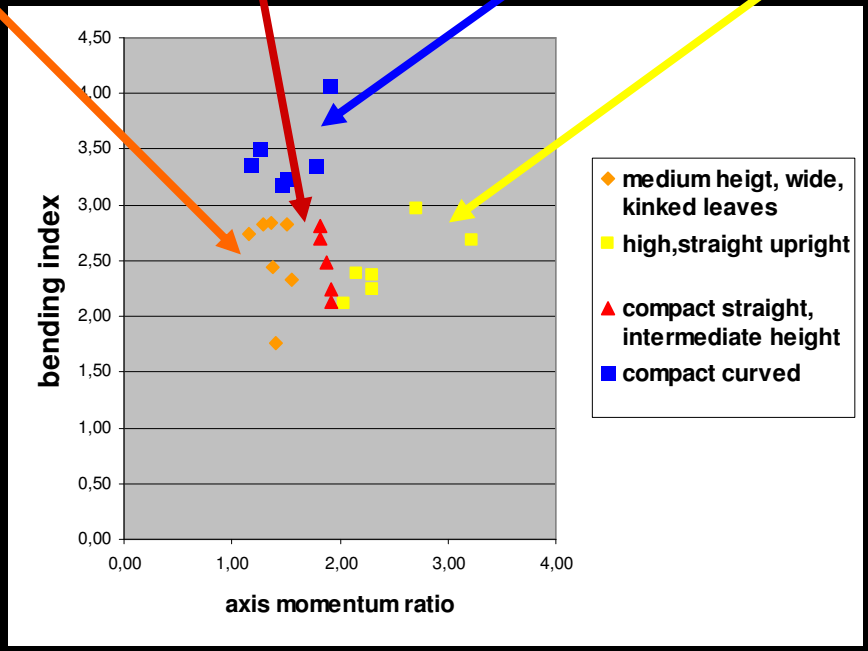


Phenotyping of growth under field-density growth conditions

- Criteria of plant growth
- Height
- Compactness
- Leaf orientation (curved, straight, kinked) – bending index
- Density
- Symmetry
- Mean plant width per height

Phenotyping of growth under field-density growth conditions



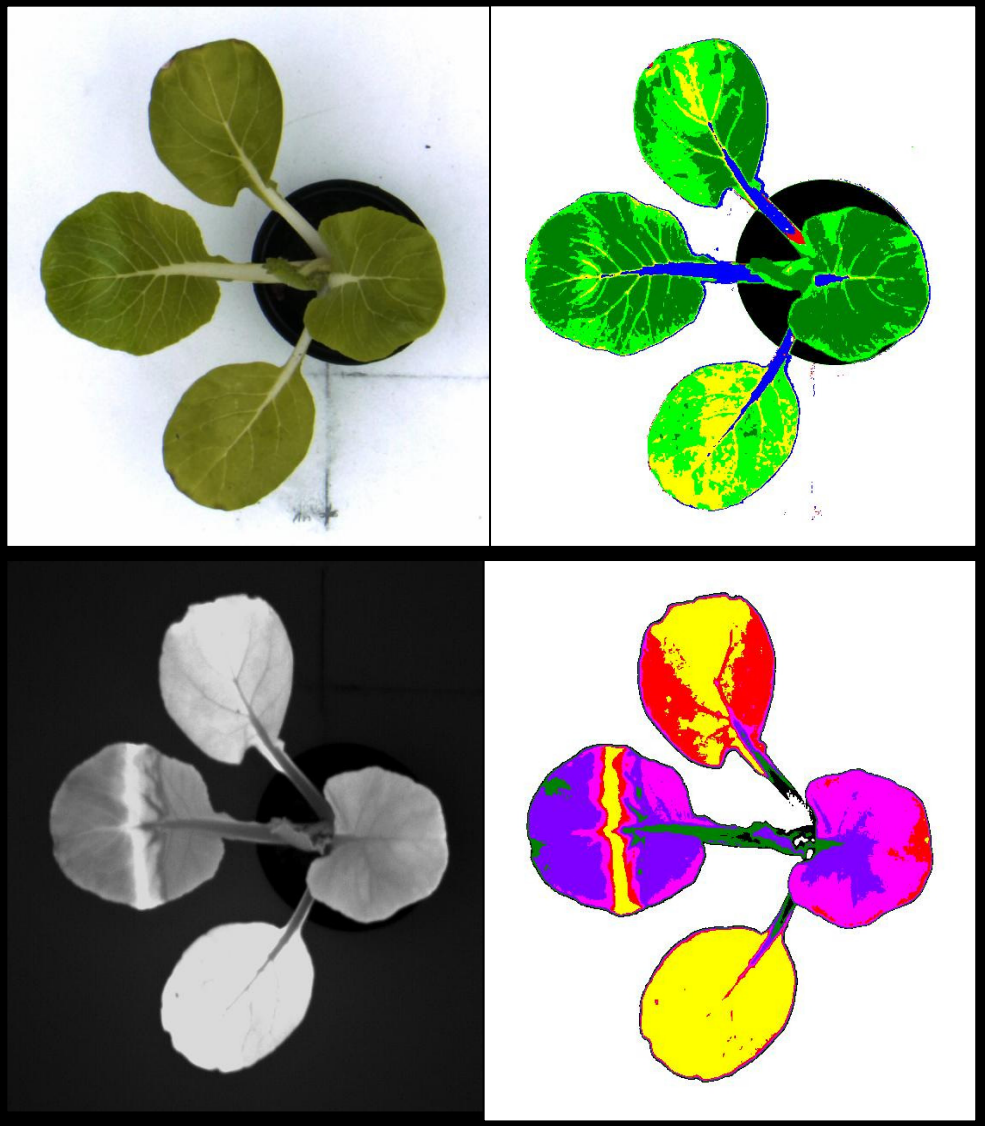


Phenotyping based on complex morphological criteria Like:

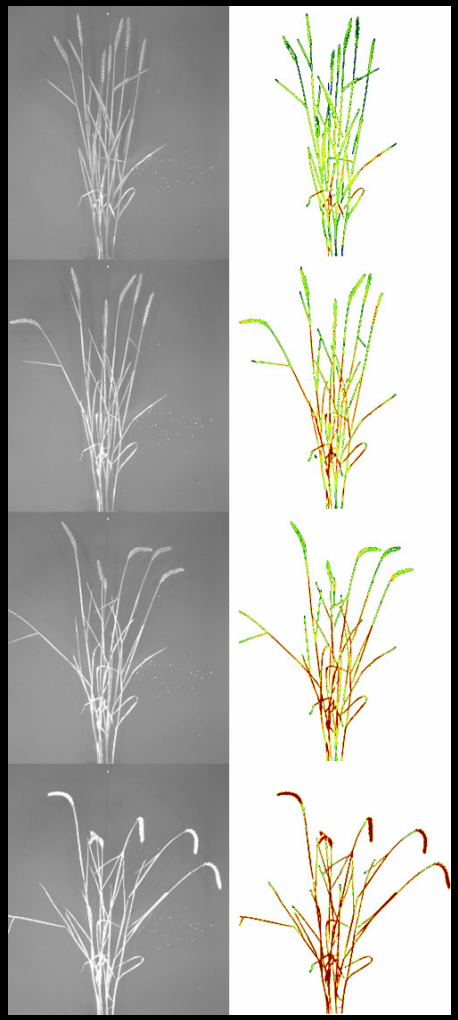
- structure orientation,
- momentum of inertia,
- height,
- width,
- roundness,
- Compactness.

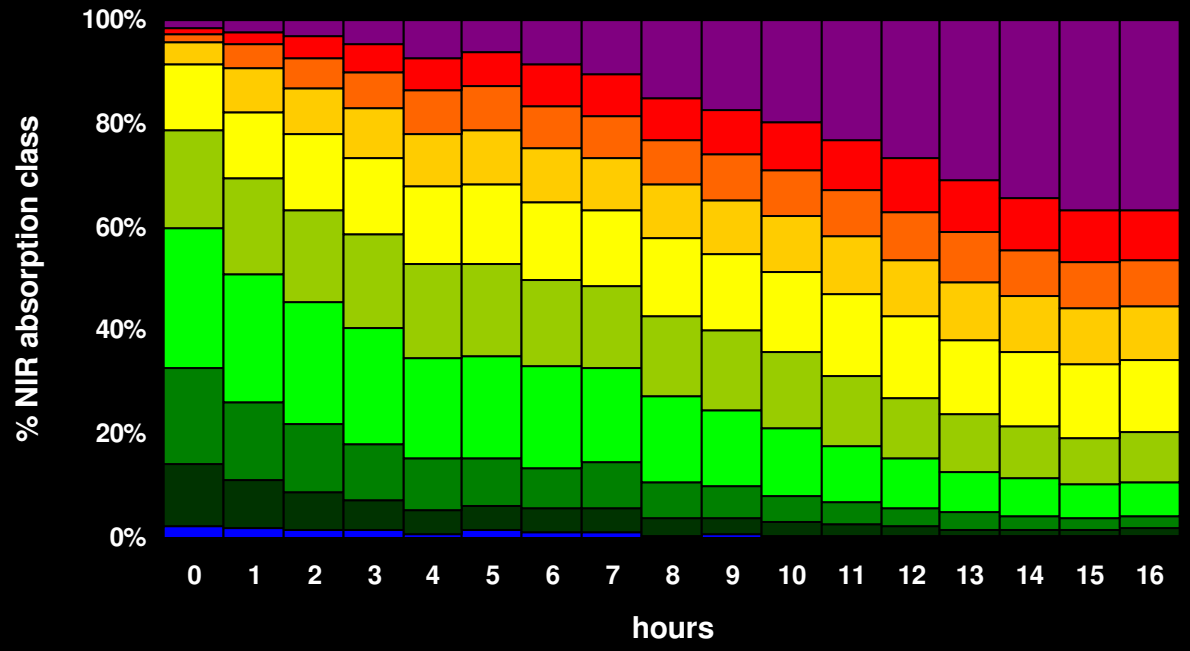
Fluorescence (UV)

Visual and chlorophyl
scan of a half boiled leaf



NIR Imaging of wheat drying

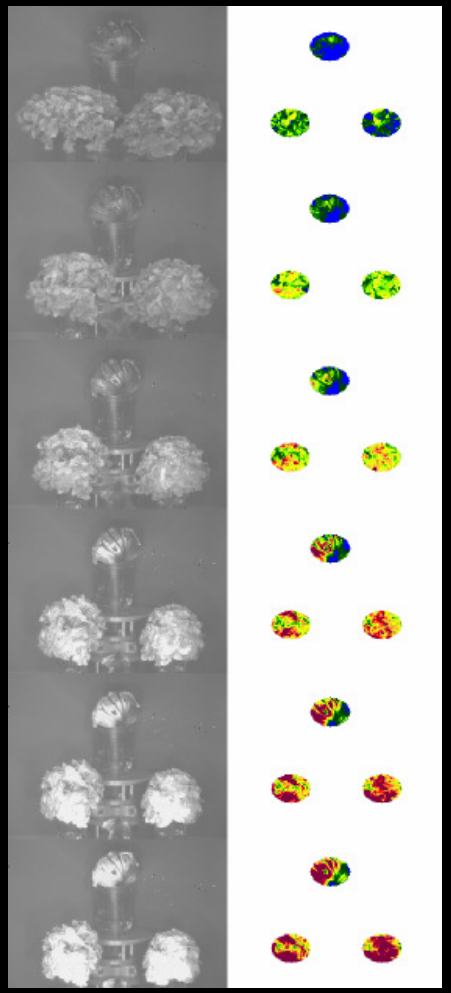




- wheat dried down over 16 h at elevated temperature

- LemnaTec NIR imaging and analysis can cover the whole water dynamics of the drying for wheat
- LemnaTec NIR cameras are suitable for detection of fine cereals structures of fully grown wheat

Lettuce evaporation dynamics - results



0 h

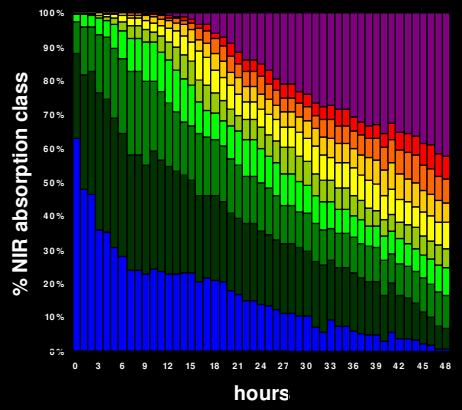
4 h

12 h

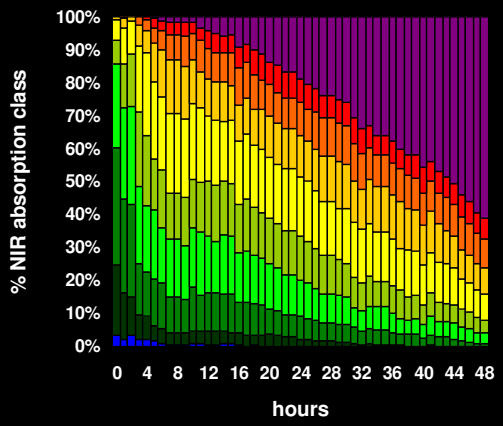
24 h

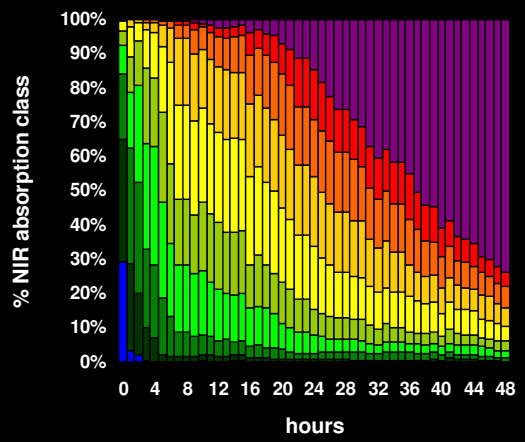
36 h

48 h



Iceberg lettuce,
- highest water content
- dries out the slowest



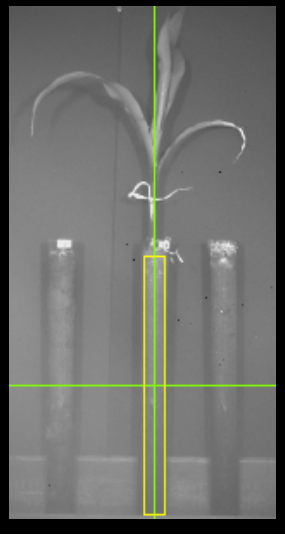


Oak leaf lettuce green

Oak leaf lettuce red

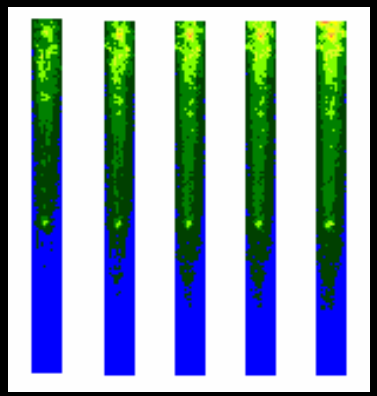
- red one dries out faster than green one
- immediate reactions already in first hour measurable

NIR imaging of corn plant in soil colums

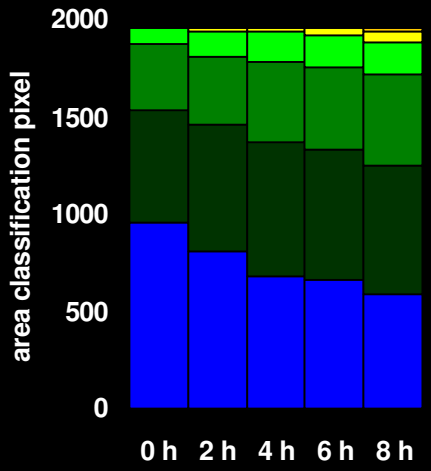


Corn plant previously shown was grown in a transparent 8 cm polyacryl column

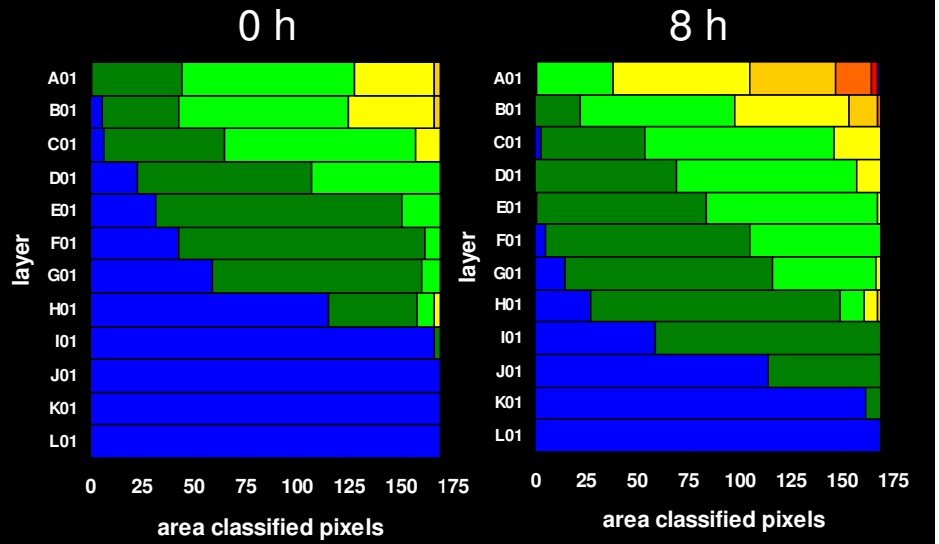
Results of NIR monitoring allow measurement of spatial distribution water content in soil



total development of drying over time

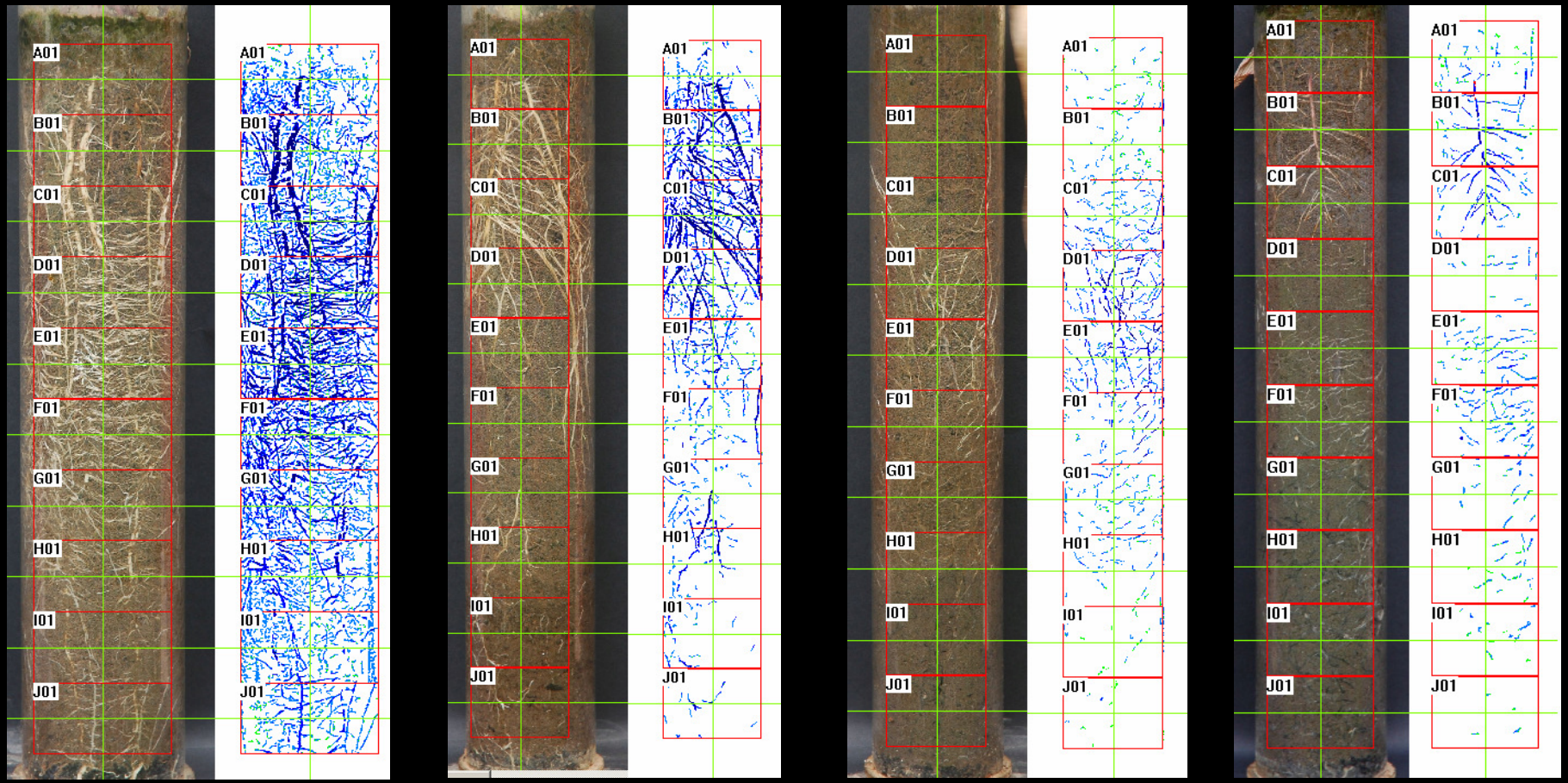


drying dynamics based on soil layer



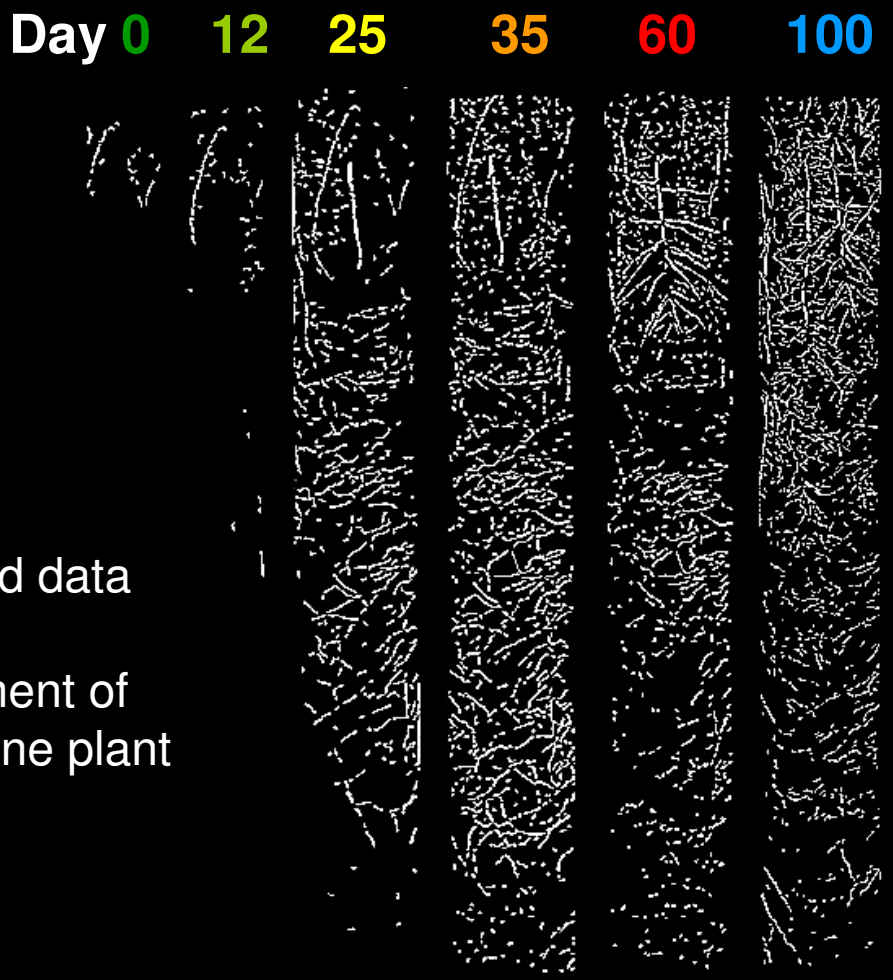
LemnaTec

Static root density profiles – growth pattern



Bangui Sweet Corn Prelude Bangui

Dynamic root growth - pattern



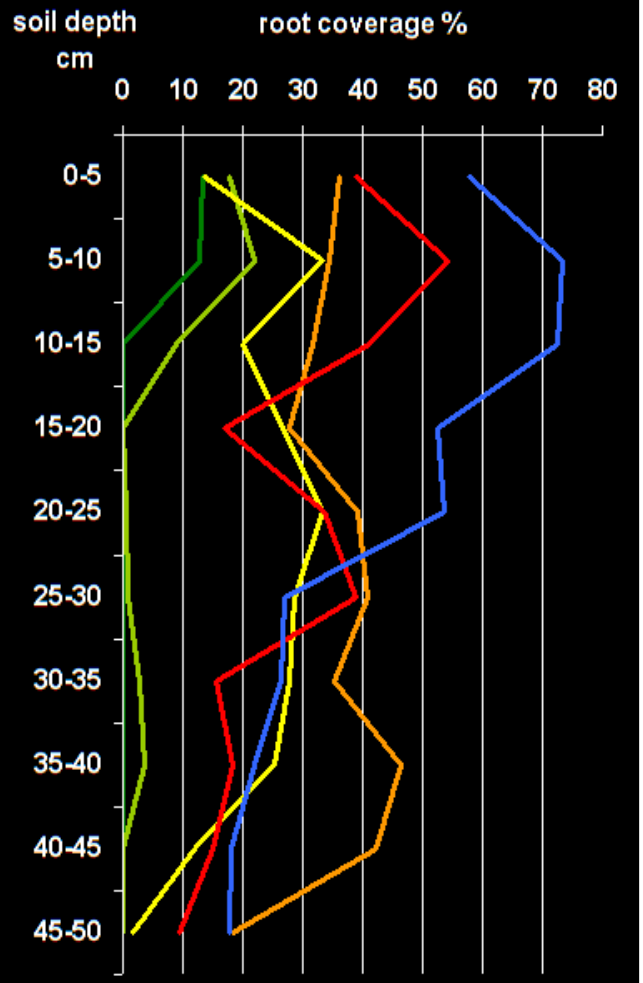
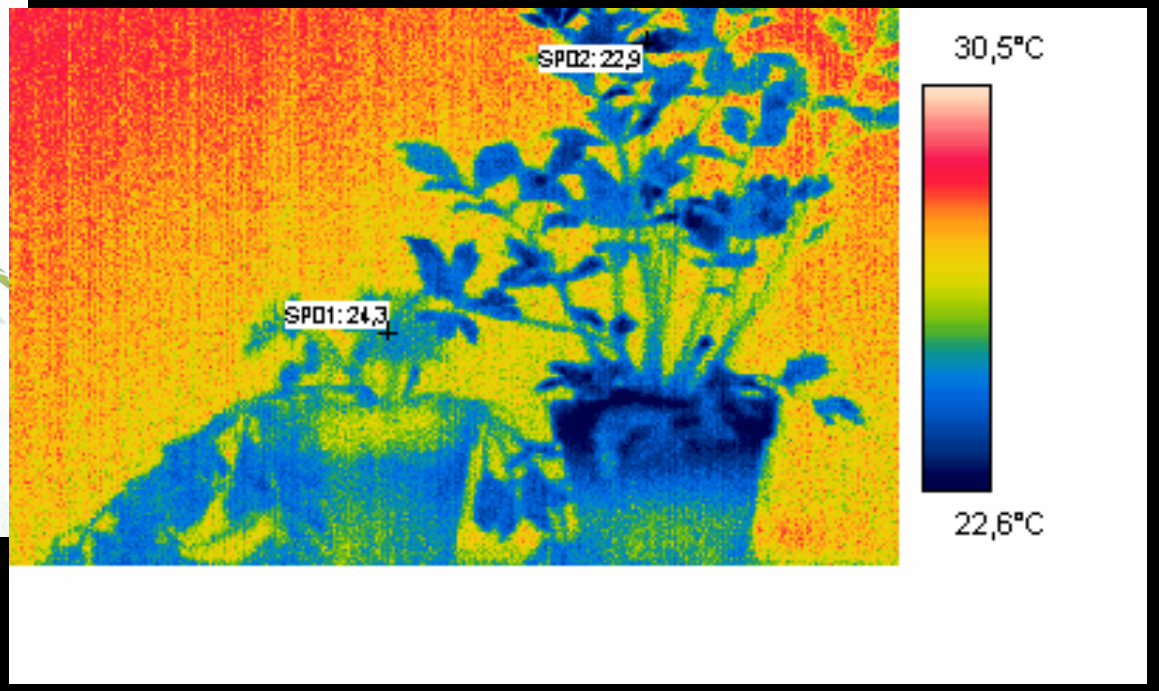


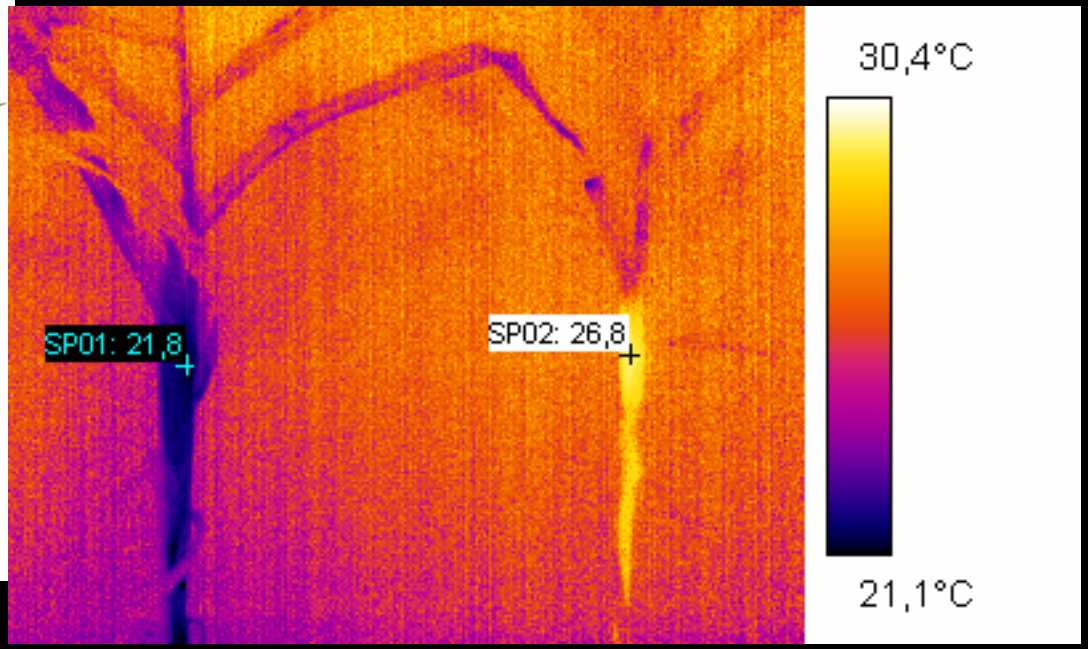
image and data show the development of roots of one plant in time.

IR detection of leaf temperature



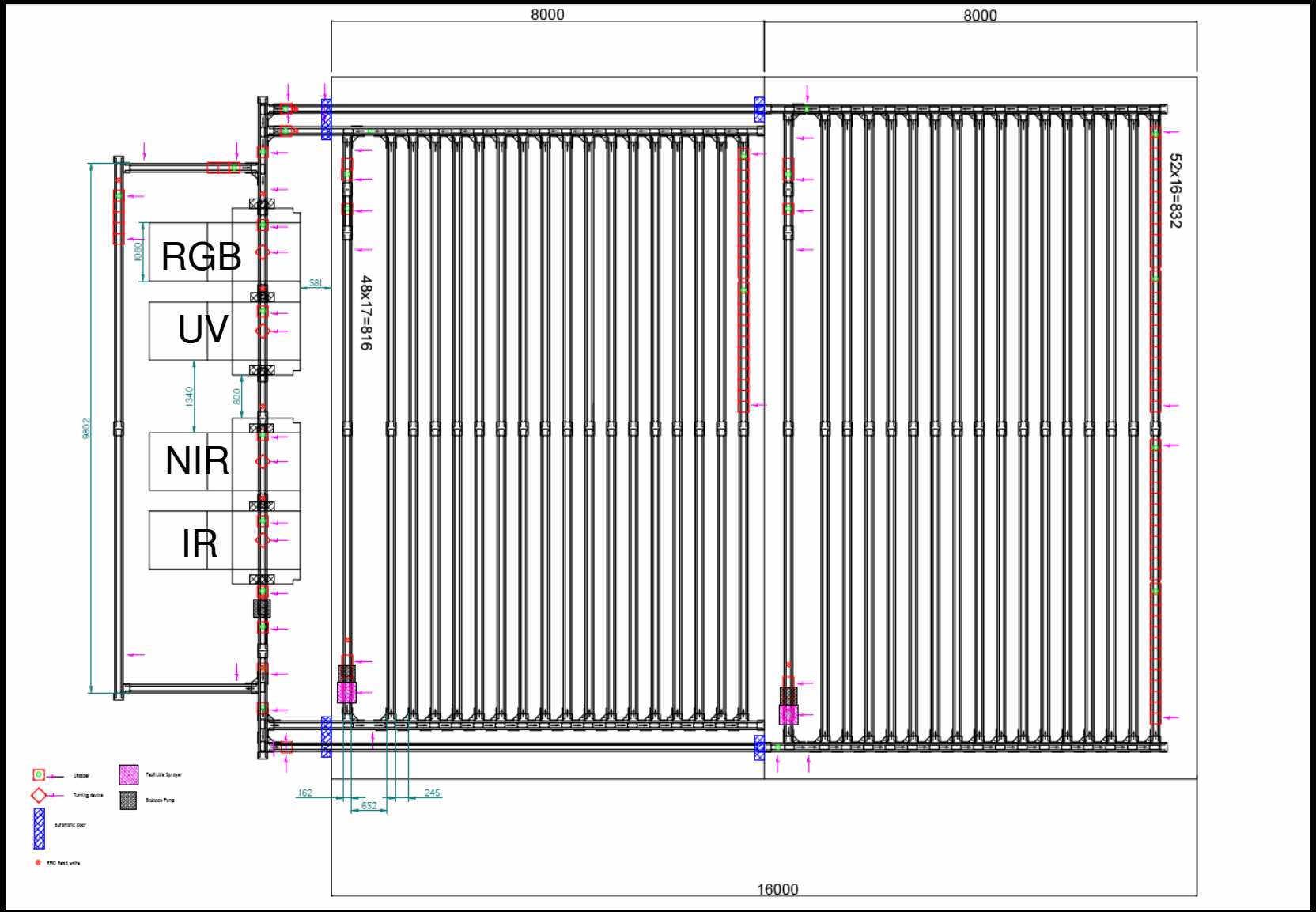
The right plant shows significantly lower leaf temperatures due to better watering. IR camera used has a resolution of 0.1 ° C at a resolution of 320 to 240 pixels

IR Cameras
to detect differences in plant temperature



While looking quite similar in visible light the right plant is much drier resulting in higher temperature due to lower evaporation. Temperature of the right plant is very near to ambient temperature while Is significantly cooler.

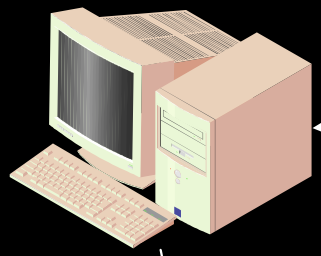
Layout


8000
8000
RGB
UV
NIR
IR
48x17=816
52x16=832
16000
9802
1340
800
581
162
652
245

LemnaTec

Measurement PC

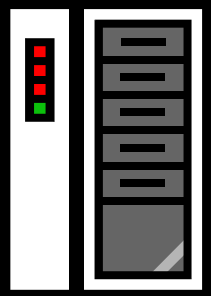
Data
RS232 or TCP/IP



Export:
*.html
*.bmp
*.xls
etc

Scanalyzer

Server

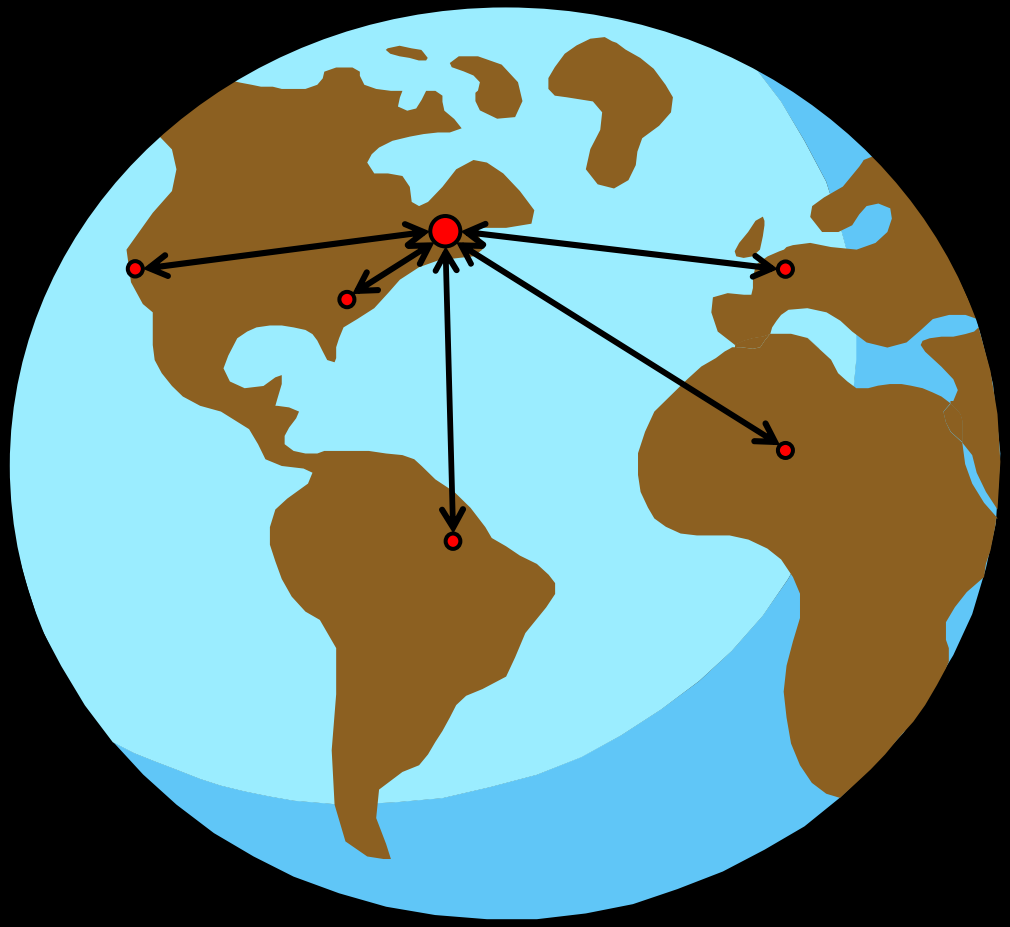


Input via SQL, *.txt, *.csv
RS232 or TCP/IP

Access via php module

LemnaTec

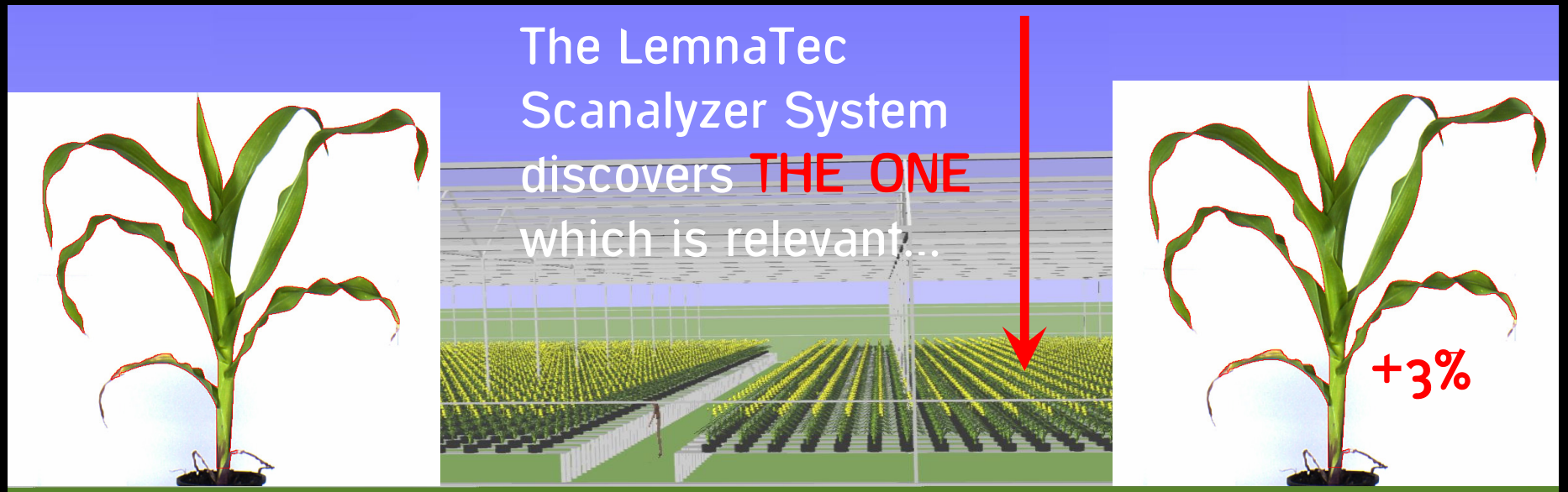
Research independent from Screening Location



The LemnaTec Public Database Server

Finding the needle in the haystack

Modern breeding or GMO methods are able
to produce **THOUSANDS** of new varieties per week



For example, **THE plant** with 3% increase in value is worth
$50m if it obtains 10% market penetration and released 2 yr earlier

Automatic screening 10x more efficient than human screening

Non Destructive Plant Measurements:

- 3D Plant Architecture
- Phenotype
- Biomass
- Stress Tolerance
- Disease Resistance
- Time of Flowering
- Fluorescence (UV)
- Water use efficiency (NIR
- Thermal Imaging (IR)

The Scanalyzer System
discovers the relevant variability
Relevant for the scientist
+ Relevant for the breeder

- Future Development: X-Ray or NMRI Root Architecture etc.

Applied Research!

Most Global Players in Breeding and GMO are already using the LemnaTec Technology:

- BASF
- Pioneer Dupont
- Bayer CropScience
- Monsanto
- Syngenta
- Dow Agro
- Keygene
- IPK
- etc.