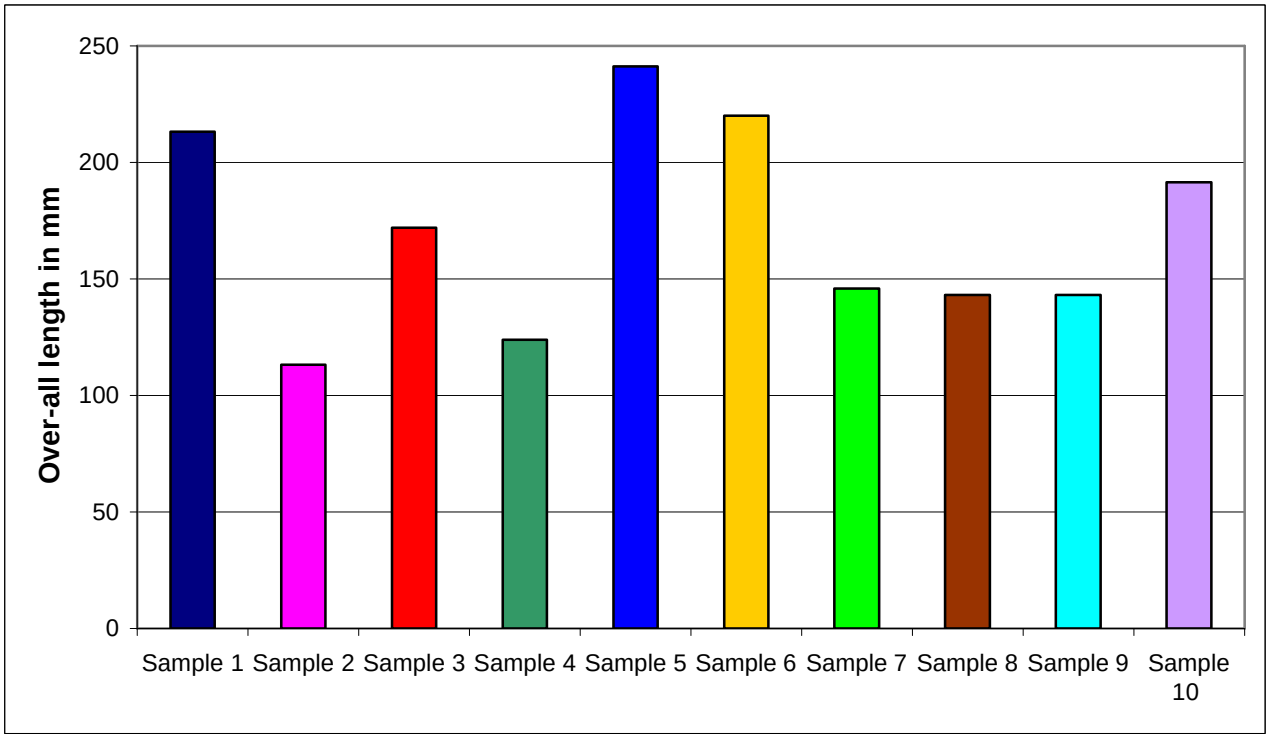
LemnaTec SAW Roots

Image Analysis for Root Quantification in 2D-Assays and Pots

Matthias Eberius
LemnaTec GmbH
Schumanstr. 18
52146 Würselen
+ 49 2405 / 4126-15
eberius@lemnatec.de
www.lemnatec.de

Image Analysis for Root Quantification with the LemnaTec Scanalyzer

The LemnaTec Scanalyzer technology makes it possible to automatically acquire multiple growth parameters of roots. The most important test parameter is the overall root length, to be identified on the sampling area after the roots are spread.

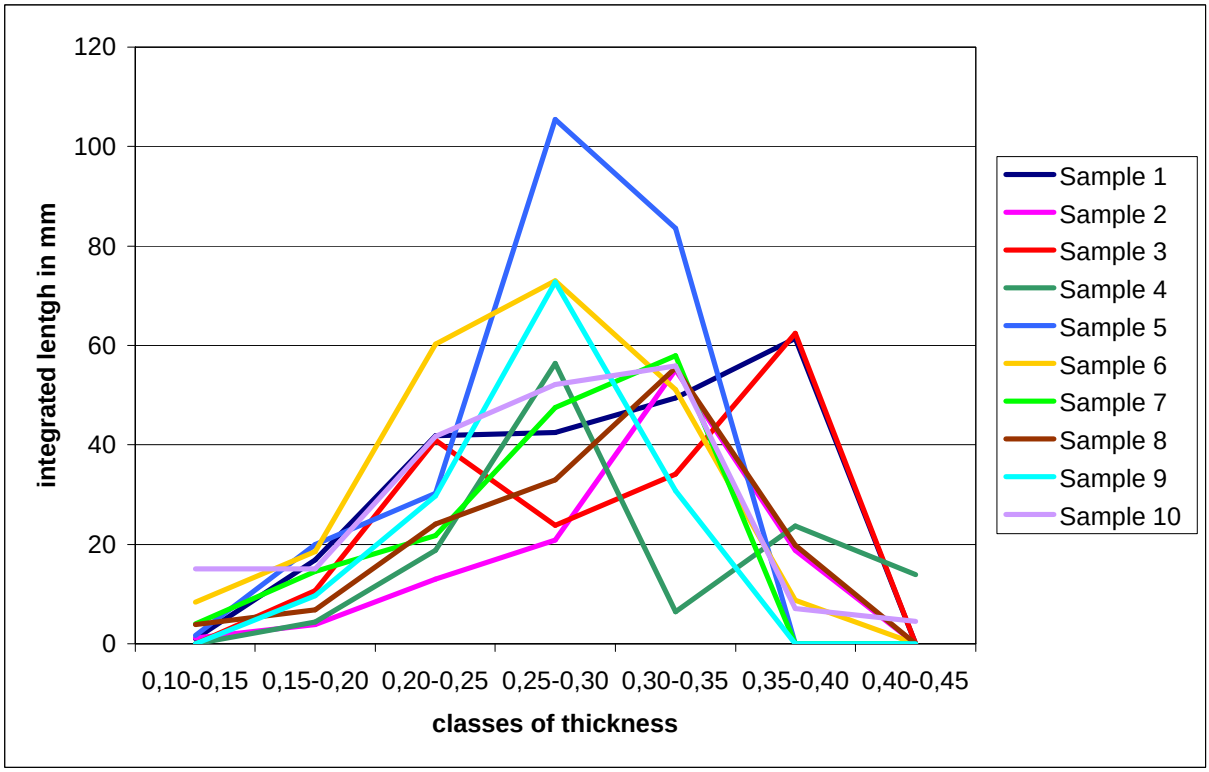


Overall length, measured with 10 samples (barley sprouts, images see below)

The minimum level of the root diameter that can be detected and evaluated by the Scanalyzer technology depends strongly on the resolution of the digital image material. A sampled image of e. g. 145 x 110 mm results in a resolution of 100 µm per pixel. The use of zoom or special fix focus optics can enhance resolution or increase the focussed area if needed. The higher the resolution, the more precise the resulting root classification.

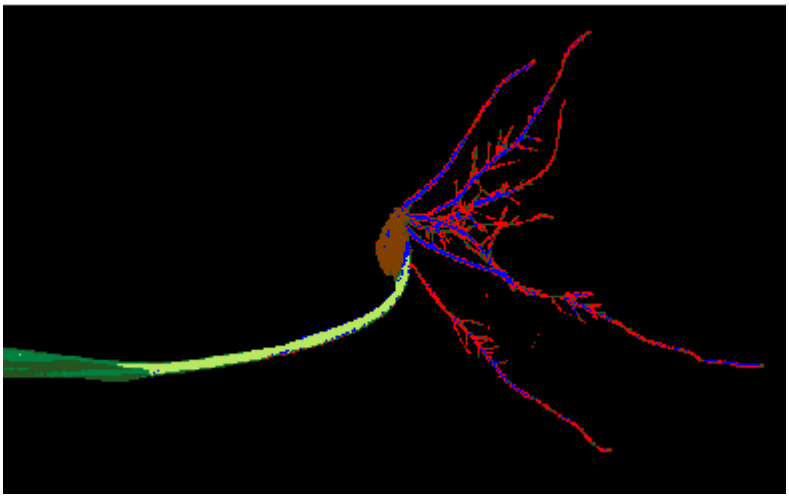
Besides root length, the average diameter of the root segments is acquired after separating them through image analysis. Primary and secondary roots can be identified by their thickness, simultaneously obtaining information on the degree

of branching. Characteristic differences between the samples can be illustrated by plotting the cumulated length of roots in thickness classes.



Distribution of root length in thickness classes

Additionally to the length, the area of the roots in a class of thickness can be determined. This evaluation gives additional information about colours to be illustrated similarly, in case specific patterns of discolouring have to be determined in a quantitative analysis.

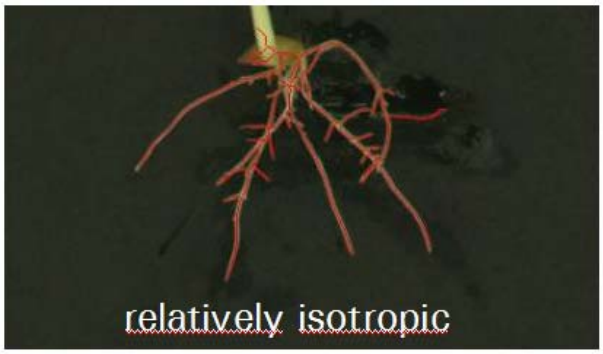


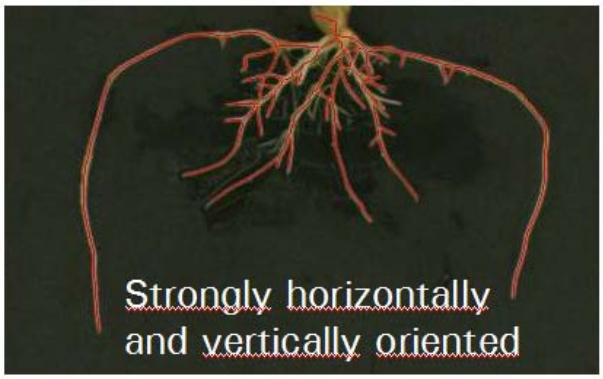
Colour-classification in false colours

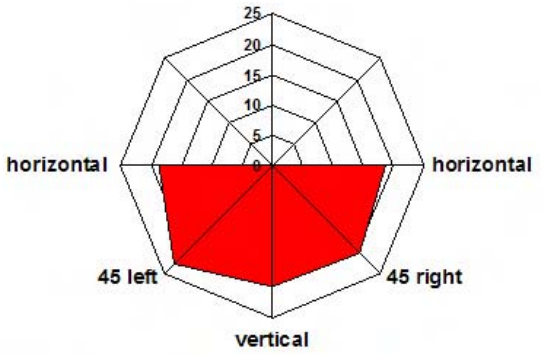
Analysed roots, samples: false-coloured illustration of two classes of root thickness, giving a clear view of primary and secondary roots

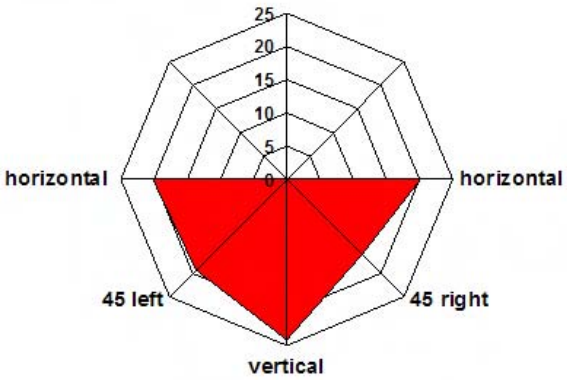
Root orientation

Analysing the orientation of roots in two-dimensional assays becomes more and more important as root architecture influences the ability of plants to extract water and nutrients from the soil. The following results show a quantified









Direction plots of root orientation

orientation pattern based on a comprehensive analysis of all changes in direction of each root.

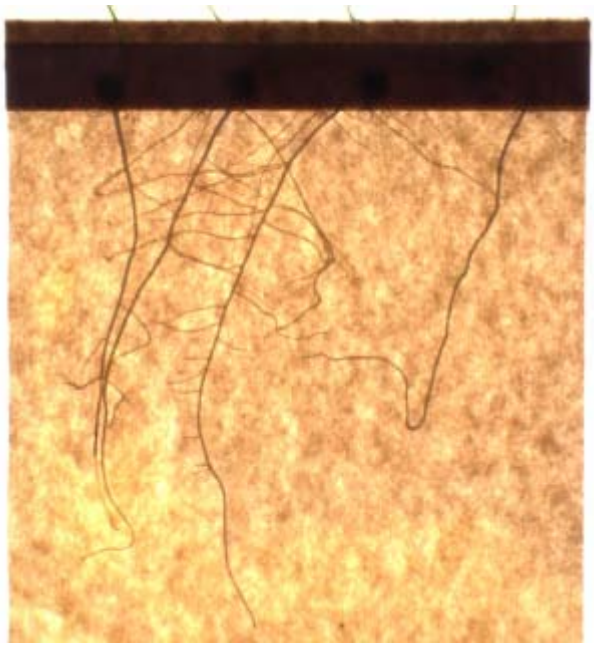
Advanced Cultivating Options for Roots

While root samples (as displayed above) normally need manual handling and separation before being analysed, there are some root cultivating systems that permit non-destructive root quantification without human interference.

Roots in germination pouches

Root germination pouches, provided for example by Mega international (**www.mega-international.com**), consist of a special paper in a plastic bag. They and seem to be quite successfully used quite successfully for various applications. For analysing roots in these pouches, specific illumination conditions and advanced image processing are needed to overcome reflections and differentiate even fine root structures from the very inhomogeneous (paper) background.

The following images show how roots can be analysed in these pouches.



Original

Contour

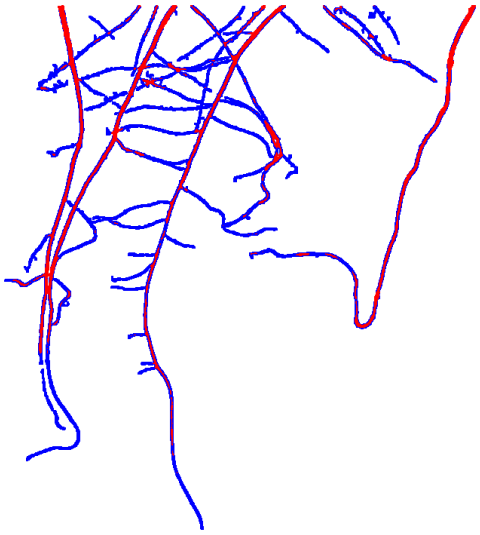
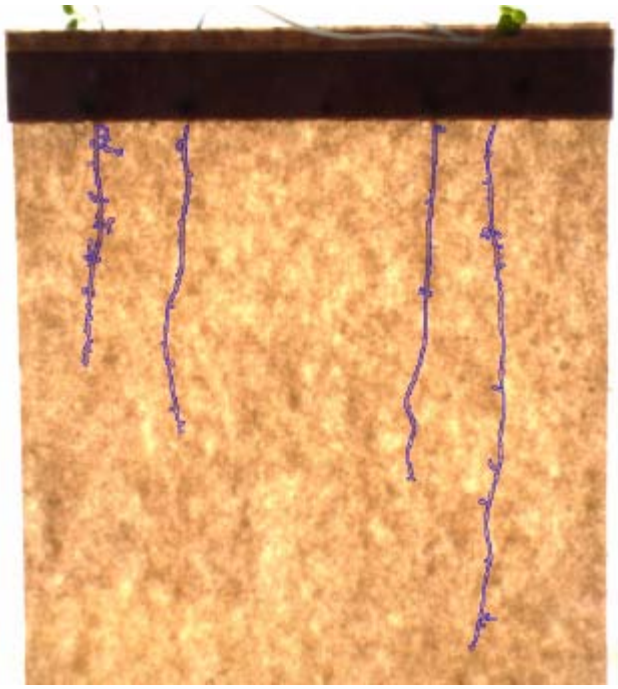


Image: lentils in germination pouch

The sequence above shows the original image acquired by the Lemnatec Scanalyzer, the analysed root image with contours each root and the colour classification image.
From these images all further quantitative information on root length, area, distributions and colours can be derived.

Colour

Original



Contour

Image: rapeseeds in germination pouches

The image above shows the capability of the LemnaTec SAW Roots software to recognise even fine roots in very inhomogeneous paper structures.

It is LemnaTec's basic philosophy that image analysis must adapt to the established biological test systems, and not vice versa.

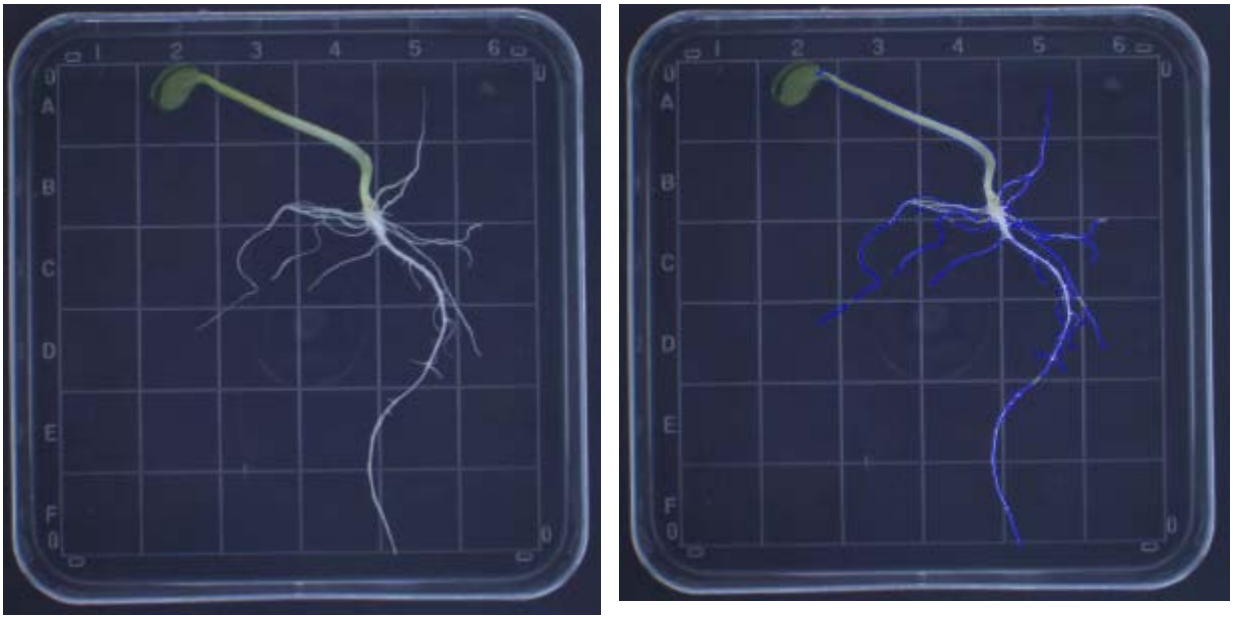
Germination of seedlings on agar plates

Another method often used to observe root development is by growing plants on agar plates. For manual counting many of these plates are provided with a grid. Using the LemnaTec SAW Root software and the LemnaTec BioGridFinder makes it possible to separate the grid even from narrow roots showing less contrast .

To avoid reflections on the agar, the special design of the LemnaTec Scanalyzer acquisition unit and adapted cloudy day illumination is of great importance.

Additional image processing tools can be used to eliminate substructures in the agar and deal with sprouts and leaves

As image processing is based on biological shape and colour recognition, SAW Bonit Roots allows for recognition of fine roots at high densities as well as lower densities.



Original **Contour**

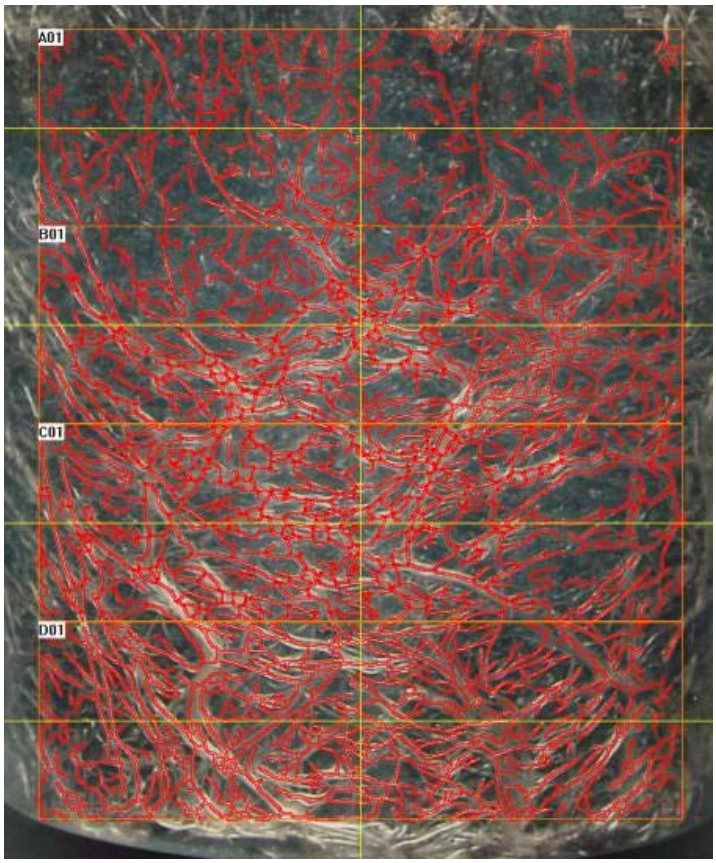
Image: roots on agar

Images above show that the special image acquisition conditions in connection with the SAW Root software allow for detecting even fine roots on plates with grids. There is no need to position the grid in a special way as the BioGridFinder automatically recognises any grid in any orientation.

Root Recognition in Transparent Pots

Recognising roots in pots becomes more and more important for scientific analysis as roots grow more natural in pot tests than in two-dimensional systems. To allow quantification of root growth dynamics in time and space, LemnaTec provides standard and customised image acquisition solutions, ranging from low to high throughput, with the Scanalyzer, Plant Scanalyzer, Scanalyzer 3-D and Scanalyzer HTS systems.

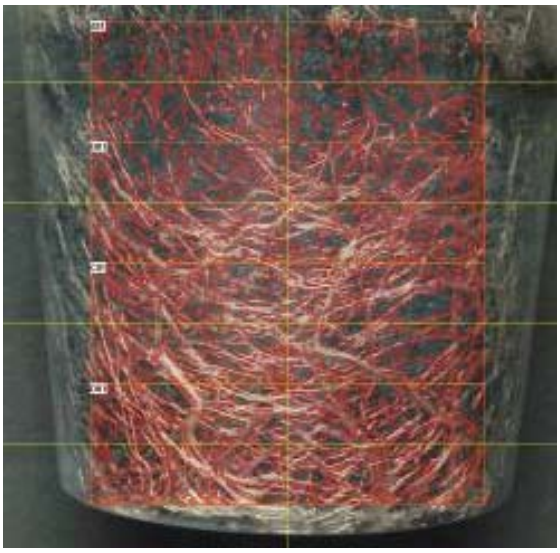
Root recognition needs strong algorithms to separate roots from earth in the pots. The following examples show some of the options available.



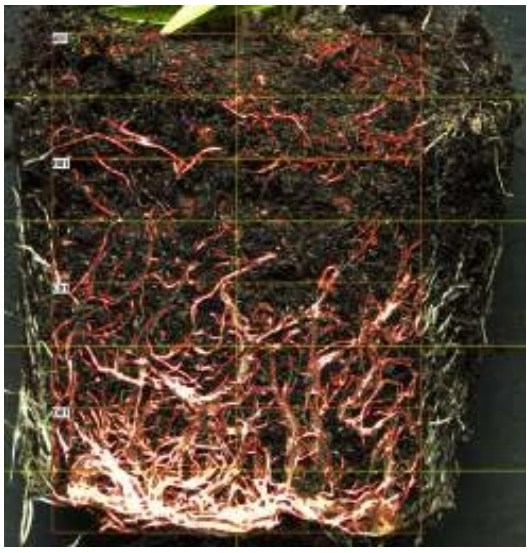
Root length analysis and spatial distribution

The LemnaTec SAW Root Software allows free definition of the areas to be analyzed separately. This may include different regions related to depth as shown above, or even separate analysis on an xy-array, e. g. of plants in multiwell-like array structures imaged from the bottom.

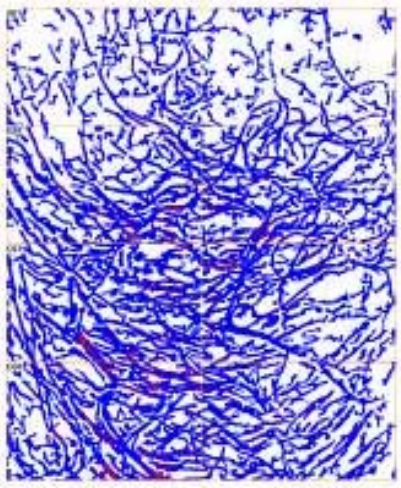
Analysing development over time allows for example to determine when the first root hits the bottom in systems where the bottom of the pots is imaged.



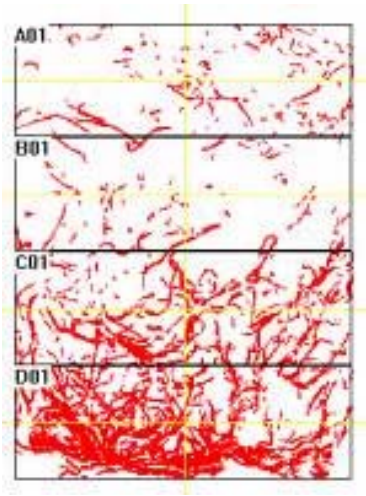
Ocimum basilicum



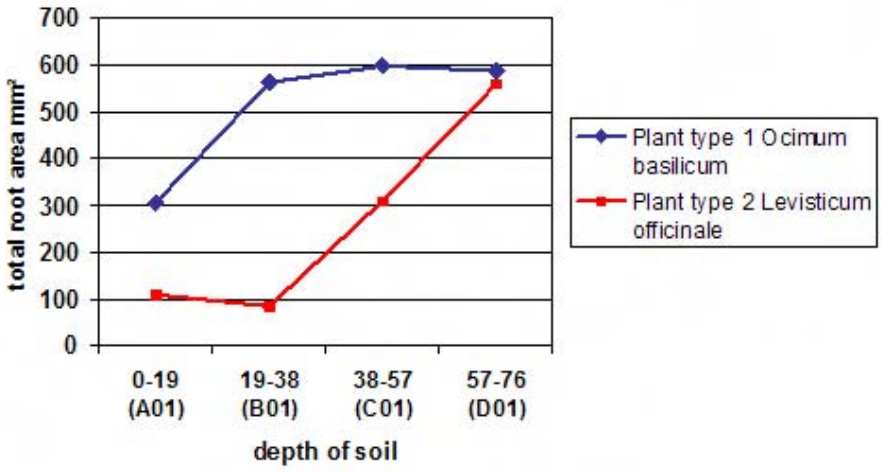
Levisticum officinale



***Ocimum basilicum* (root coverage)**

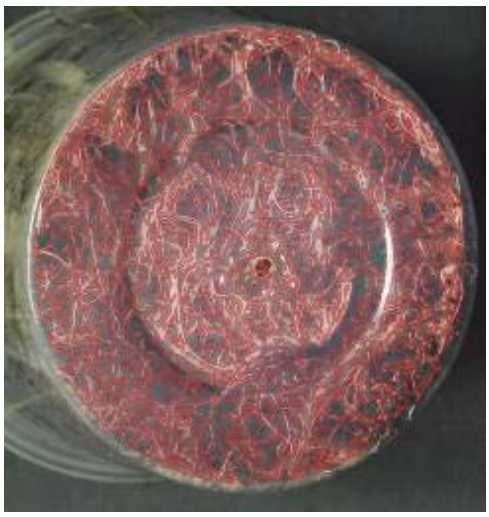


***Levisticum officinale* (root coverage)**

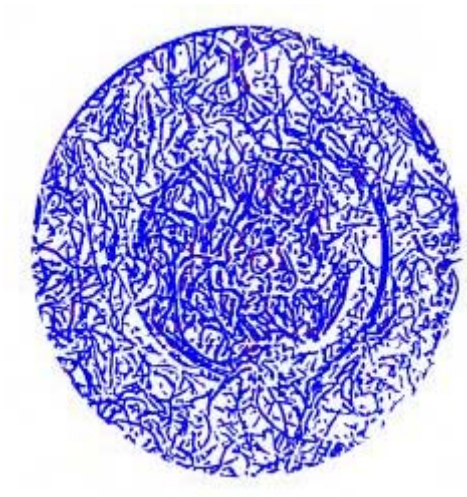


Root area depending on soil depth for both plant types. Analysis of changes over time can provide additional information

Ocimum basilicum

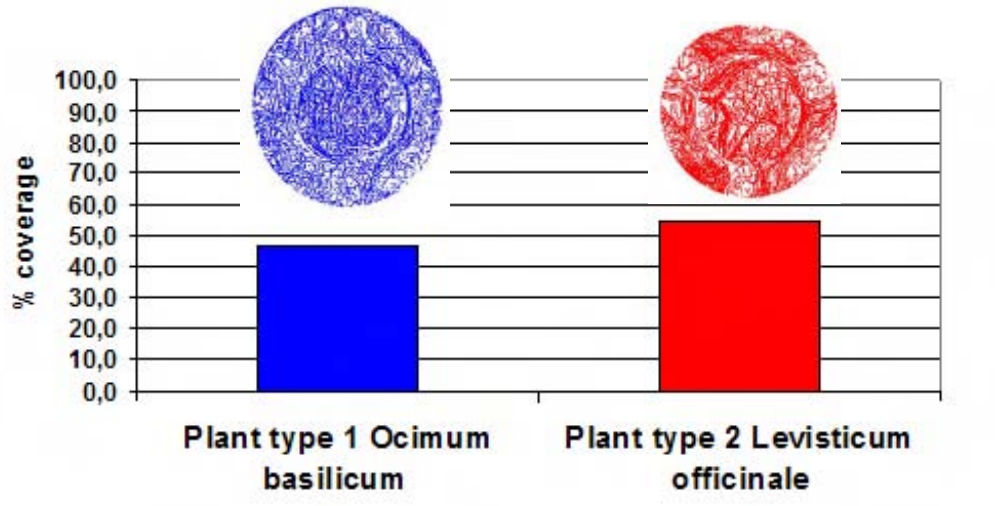


***Ocimum b*. root area recognition**



***Ocimum basilicum* (root coverage)**

Magnification



Root coverage on bottom of vessels. Analysis of changes over time can provide additional information

Summary

With the Scanalyzer product family in connection with the software unit SAW Roots LemnaTec offers powerful solution systems for the comprehensive quantification of root observation parameters.

Customised solutions for specific needs can be developed to gain maximum data output and documentation of various experiments.

For further information please do not hesitate to contact:

Matthias Eberius

LemnaTec GmbH

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+ 49 2405 / 4126-21

eberius@lemnatec.de

www.lemnatec.de