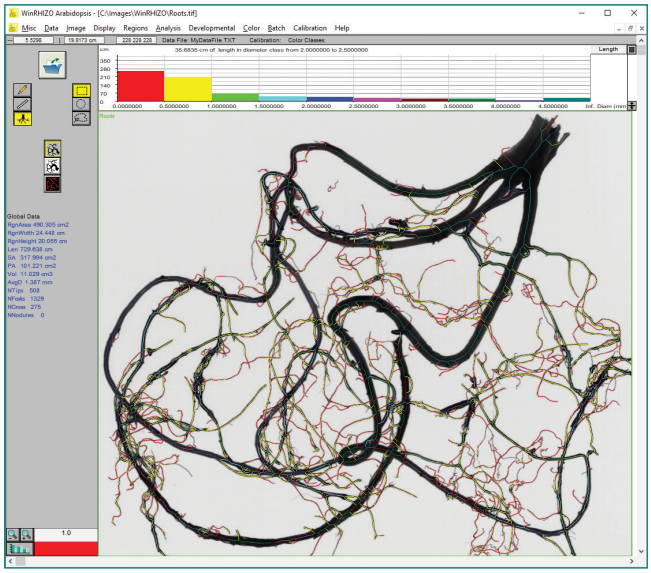
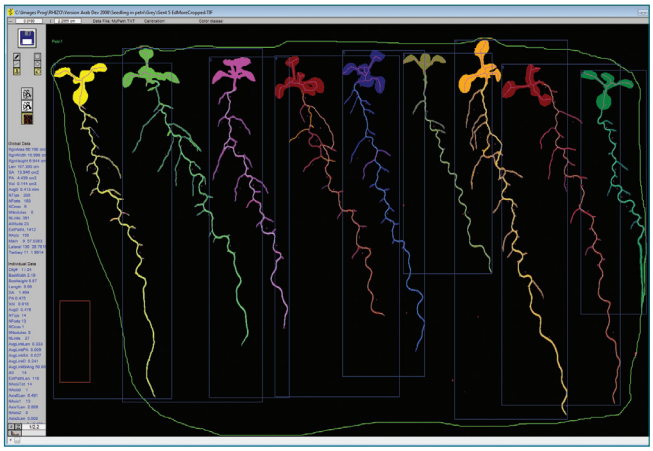
Image analysis systems for plant scientists

WinRHIZO™ 2021

Analyser of Washed Roots and Arabidopsis Seedlings

WinRHIZO performs automatic analyses such as morphology, topology, architecture and color on washed roots* and Arabidopsis seedlings. It is an image analysis system that includes a software program and image acquisition components that can be combined to meet different needs and budgets.





The image above shows an analysed washed root sample.

- The root diameter distribution graphic above the image displays the root length, area, volume or number of tips as a function of root diameter or color. The number and the width of the classes are user-definable and can be changed at any time.

- **WinRHIZO** displays the analysis over the image. The color used to draw the root skeleton indicates into which diameter class the part of the root has been classified. The same color is used for drawing the root distribution graphic.

- Measurement data of the sample under analysis are summarized on the left of the screen and are available in detail in data files.

WinRHIZO software program is offered in 4 different versions depending on your needs.

For instance, if you work on seedlings growing in Petri dishes or trays, you might be interested in the Arabidopsis version which is optimized for young seedlings. It can differentiate and analyse separately several non-touching seedlings, root systems or objects per image. Each one is represented by a distinctive color as shown in the image above.

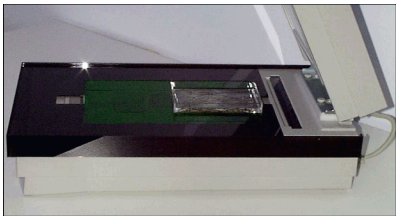
The Arabidopsis version is our high-end program and includes all features of the other lower versions.

** For rhizotron or in-situ root analysis, please see our WinRHIZO Tron product.*

Digitize and Analyse Roots in Four Steps with **WinRHIZO**

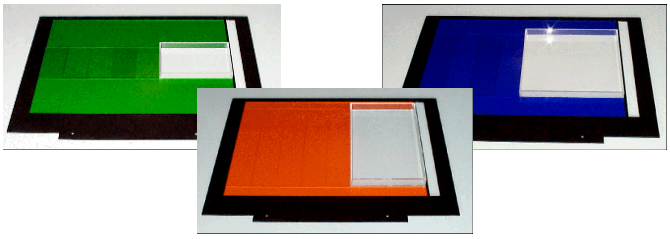
1. PREPARE AND POSITION ROOTS

Note that roots can be measured automatically by **WinRHIZO** only if they are extracted from the soil and washed of debris and soil particles.



Simply place the washed roots directly on the scanner glass or ideally in Regent's water-proof trays, as shown above. These trays allow you to scan immersed roots, which are easier to spread than dry roots. Roots can overlap and do not need to be randomly distributed*.

Our trays come with a root positioning system designed to fit perfectly on Regent's scanners. It consists of plastic blocks that can be installed and removed quickly to accommodate different scan area sizes. Together they form a semi-opaque area with a hole, i.e. the scanned area, that match our water-proof trays.



We have designed the positioning system to accelerate root positioning and scanning, and thus increasing productivity:

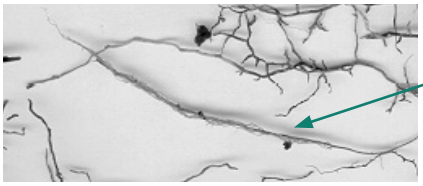
- Once you have determined the sample positioning scheme, you simply insert the tray containing your sample in the open area on the glass for the acquisition of subsequent images. **WinRHIZO** program has pre-defined positioning options for you to choose from so that you can bypass the traditional scanner Preview step. You save 10 to 20 seconds for each scan. That's a lot of time after thousands of scans!
- While a sample is being scanned or analysed, the next root sample can be prepared in another tray away from the scanner.

2. ACQUIRE THE IMAGE

WinRHIZO standard systems uses a desktop optical scanner as the image acquisition device. Optical scanners are well adapted to image acquisition of macroscopic objects like roots or leaves. Regent's scanners come with:

- Root positioning system
- Special lighting system to avoid shadows (see below)
- Permanent calibration to increase the measurement precision
- Manual that explains how to scan biological samples, e.g. root, leaves, or seeds for analysis with our programs. (Scanning for scientific analysis is different than for artistic applications.) It also gives tips specific to the scanner purchased such as scanning speed vs. quality issues.

Without a good image of the object to measure, an unnecessary complex and lengthy root detection algorithm is required. Root image acquisition with an optical scanner without proper attention to the lighting system might produce artifacts that make root identification tedious and imprecise. For instance, shadows have grey levels close to those of the roots. Determining the position of the root boundary is much more difficult when they are present (see image on the right).



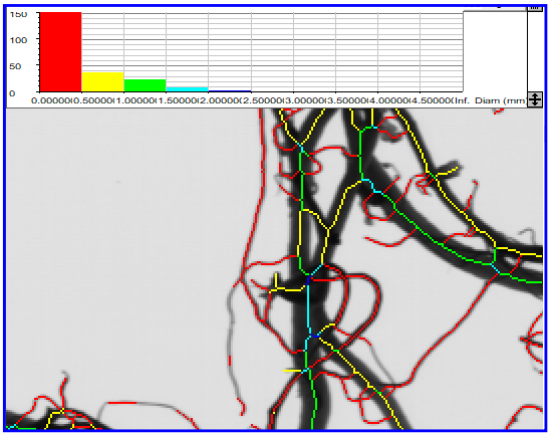
WinRHIZO program controls the scanner directly. Once you have selected the pre-defined position of your sample on the scanner glass, click the scanner icon in **WinRHIZO** menu to digitize the roots. After a few seconds, the scanned image appears on the screen.

WinRHIZO is TWAIN compatible, meaning that it can get images from many scanners (or cameras). It can also analyse images stored in tiff or jpeg files.

Regent's scanners can be used for other applications, e.g. document or photo scanning.

For specifications of our most recent scanner models, see our website at: www.regentinstruments.com

3. ANALYSE THE ROOTS



A few seconds after the roots have been scanned, the analysis is completed. Roots found by **WinRHIZO** are identified by colored lines (as shown on the left) according to their diameter class. Some analyses, such as nodules counting, color and Topology, require interactions from the operator before proceeding.

Root length and diameter are measured with Regent's unique method** and with Tennant's statistical method***. With the former, measurements are made continuously at each point along the root. Root overlap at forks and tips are taken into account to provide accurate measurements of length and area. Some measurements made by the system can be overridden by the operator.

4. SAVE THE MEASUREMENT DATA

Data are saved automatically once the analysis is completed. Data files are in ASCII (text) format and easily readable by many programs including spreadsheet style like Excel. Images and their analyses can also be saved to files for later validations, reanalyses or for visualization in other software programs.

REFERENCES

* "WinRHIZO™, a root-measuring system with a unique overlap correction method", Arsenault, J.-L., S. Pouleur, C. Messier, and R. Guay. 1995. HortScience 30: 906. (Abstract).
** "Accuracy of Measurements with Mac/WinRHIZO™". Stephan Pouleur, REGENT INSTRUMENTS Technical note #3, 1995, pp. 1-4.
*** "A test of a modified line intersect method for estimating root length". Tennant D. 1975, J. Ecol. 63. pp. 995-1001.

WinRHIZO Software Program is Offered in 4 Versions:

BASIC - low cost entry level version which produces only global measurements: average root diameter, total root length, area, volume and number of tips

REGULAR - besides the Basic features, this version performs root morphology measurement as a function of user definable diameter classes • a root (length, area, volume) distribution graphic is generated above the image

PRO - in addition to the Regular features, this version produces also link, topology, architecture and color analyses • root morphology can be done in function of color • it can also be used as a color area meter (see next page)

ARABIDOPSIS - high-end level version which has all the Pro features plus the ability to analyse individually several objects in an image such as seedlings and leaves • it can do multiple root analyses per image or per regions of it (see next page)

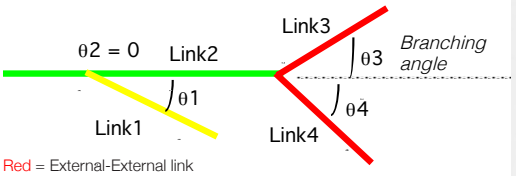
Note:
It is possible to upgrade from a low to a higher program version at any time.

The following tables list and explain the measurements and features offered by each version:

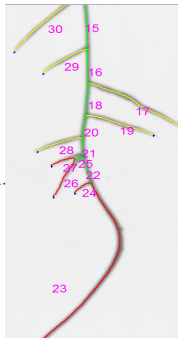
Measurement	Version		
Root Morphology	Basic	Regular	Pro & Arabidopsis
Global (Total or Average for the image)			
Total Length	Yes	Yes	Yes
Average Diameter	Yes	Yes	Yes
Total Area, Volume	Yes	Yes	Yes
Number of tips, forks & crossings	Yes	Yes	Yes
In function of root diameter (per diameter class)			
Length	No	Yes	Yes
Area, Volume	No	Yes	Yes
Number of tips	No	Yes	Yes
In function of root color (see Color Analysis below)			
Length	No	No	Yes
Area, Volume	No	No	Yes
Number of tips	No	No	Yes
Root Architecture with Fractals	No	No	Yes
Color Analysis (see next page)	No	No	Yes

Link Analysis	Basic	Reg	Pro & Arabidopsis
Globally (for the whole image)			
Total number of links	No	No	Yes
Average link length, diameter, area, volume, branching angle	No	No	Yes
Per link (individually)			
Length, Average diameter, Area, Branching angle	No	No	Yes
Basic Connectivity	No	Yes	Yes

Link analysis is a study of the morphology and basic connectivity of root segments. It can be done on incomplete or complete root systems.



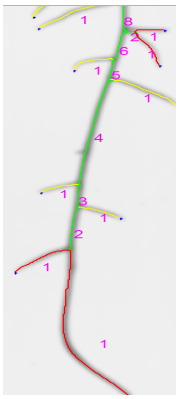
Red = External-External link
Green = Internal-Internal link
Yellow = External-Internal link



Topology	Basic	Reg	Pro & Arabidopsis
Globally (for the whole image)			
External path length	No	No	Yes
Altitude	No	No	Yes
Per link (individually)			
Magnitude	No	No	Yes
Path length	No	No	Yes
Altitude	No	No	Yes
Structured Connectivity	No	No	Yes

Topology analysis is an extensive link connectivity analysis.

- Magnitude: the number of external links extending from a link
- Path length: the number of links between a link and the base link (inclusively)
- External path length: the sum of path lengths of all external links. It is the value for the complete root system (not per link).
- Altitude: the largest path length



Note: Topology and developmental analyses require an integral root system. It is meaningless if the connectivity of the root system's links has been destroyed by manipulation or the imaging process. It is not recommended to do link analyses on dense root systems. If you cannot visually track root segments in an image, it is unlikely that WinRHIZO will do so either. Measurements made on such images will not be precise and might contain errors.

Developmental classification	Basic	Reg	Pro & Arabidopsis
Number of links (per order)	No	No	Yes
Total length (per order)	No	No	Yes
Total Area (per order)	No	No	Yes
Average link length (per order)	No	No	Yes
Average link area (per order)	No	No	Yes
Average diameter (per order)	No	No	Yes
Link order (per link)	No	No	Yes

The developmental analysis identifies the order in which links are born from the base link as the plant grow.

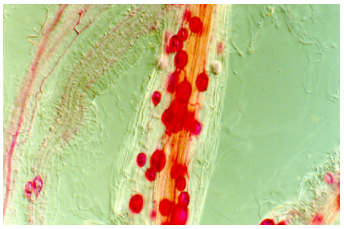
Links of the same order can also be grouped per axis. An axis is a group of connected links of the same order. Morphological data can be obtained for all orders and axes.

Color Analysis with Pro & Arabidopsis Versions

Color analysis is used to quantify areas of specific colors or groups of colors and to measure root morphology as a function of color. The operator first indicates the colors of the objects to be analysed and those of the surrounding background by clicking the mouse in the image. During the image analysis process, **WinRHIZO** classifies the colors present in the image into different classes before making the morphological measurements.

There are many possible applications for color analysis. Some are given below:

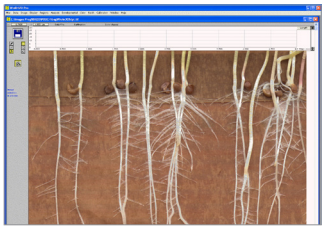
Leaf area meter, quantification of leaf disease and insect damage.



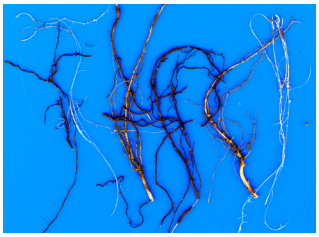
Mycorrhizae quantification (area). Images from a camera and proper sample preparation required.

Color analysis will work if there are minimal color contrasts between the feature(s) you want to quantify and the surrounding background.

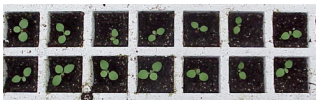
To ensure that **WinRHIZO** meets your needs, please discuss your application with our sales department before purchasing a system.



Measure roots in front of different backgrounds, i.e., in a growing pouch.



Root morphology (length, area) in function of color.



Shoot growth quantification

More about Arabidopsis Version

This version differentiates, counts and analyses separately non-touching seedlings, root systems or objects. Each object has its own measurements in addition to global measurements which encompass all the objects analysed in the image (or region of image).

Measurement of an object can translate into:
1) plant height and width when the image is a view of the seedling side, or
2) leaf (or other object) length and width when seen from above.

Objects' individual area can be measured without having to make individual selections. Overlapping regions are not detected and touching leaves are analysed as a single object unless the image is edited.

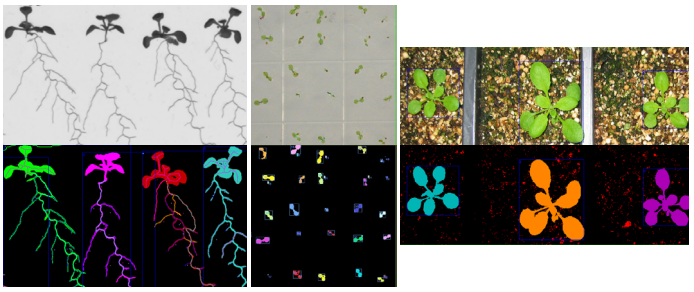
You can measure the hypocotyl (leaf) length and area separately from roots. Just click at their junction to start the root developmental analysis. Area is less precise than length (± 20%).

Measurements available per object (multiple analyses per image):

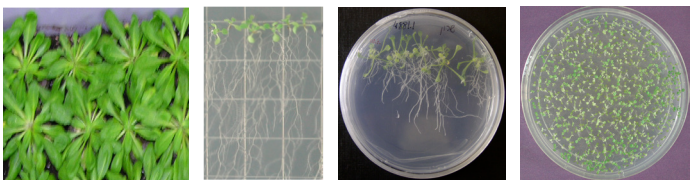
- Number of tips, forks, crossing
- Link analysis (global average, total and individual link length, diameter, area, volume, tips, color).
- Topology and Developmental analysis (axis, main & laterals number and length).
- Nodules per object (interactive count)
- Seedlings (objects) counting
- Area per color class or group

Measurements not available per object (one global analysis per image):

- Fractals
- Root distribution histogram data (length, area, volume, tips) in function of diameter
- Root morphology (length, area...) per color



Non-Touching objects are automatically identified by disctinctive colors and analysed individually (area, length, width, color). They must contrast with their surrounding background.

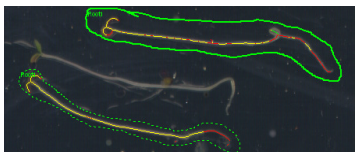


Touching objects and too dense root systems (above) cannot be analysed individually. Only total data is available. You can manually trace an outline around each seedling roots to get individual estimates, but it will include some neighboring roots. When the root system is very dense, it might not be possible to track down the main root and differentiate it from the laterals or it can be possible but time consuming (requiring interactive modifications). Also it might not work on all growing media (minimal contrast required) and with all scanners (we strongly recommend those we sell).

Miscellaneous Features

- User-defined regions can be selected for analysis or exclusion from it (Reg, Pro & Arabidopsis versions)
- Image edition to remove artifacts or image defects
- Interactive or in batch (without operator supervision) analysis. Note: Not all analyses can be done in batch
- Pre-defined analysed regions allow to create a specified number of equidistant regions at different vertical positions (soil depth) in an image • size and distance between these analysed regions are specified by the operator (Reg, Pro & Arabidopsis versions)
- Print and save to a file the images with or without their analysis marks
- Filter-out debris: based on area, shape or color (Pro & Arabidopsis versions)

Circular regions can be used to analyse roots in petri dishes.



Irregularly shaped regions permit separate analysis of roots that are close to each other.

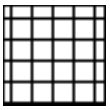
- Prompt and competent technical support by Regent's technicians
- Printed and pdf color manuals abundantly illustrated

Why Use an Optical Scanner instead of a Video Camera?

1- Scanners produce images of many times the resolution of a camera

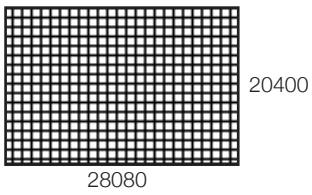
SCANNER

A good 2400 dpi scanner (true optical resolution) produces images which have 2400 by 2400 pixels per inch (2.5cm). Some can go up to 4800 dpi.



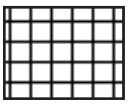
Over a scan area of 8.5 by 11.7 inches, it produces an image of 20400 by 28080 pixels.

This scanner image is equivalent to 47 camera images!

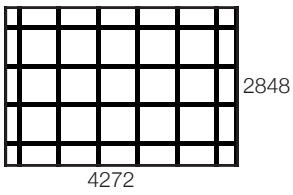


CAMERA

A good digital camera (12 Megapixels) can produce images of 4272 by 2848 pixels.



Over an area of 8.5 by 11 inches, unlike the scanner it still produces an image of 4272 by 2848 pixels, an equivalent resolution of approximately 350 dpi. The pixels are too large to measure very thin roots. Therefore, you must reduce the area and take more images.



2- It is easy to get good images using a scanner

Lighting is uniform over the entire scan area and it is not necessary to adjust the position, orientation or intensity of the light source. There are no focus or aperture rings to adjust.

3- Calibration is permanent

Unlike a camera, the object-to-camera distance and zoom are always the same.

4- Scanners are reliable and last for a long time

NOTES

A camera is better adapted when extremely high magnification is required. By adding proper lenses or mounting it on a microscope, you can see more details than with a scanner but over a much smaller area. Mycorrhizae and root hair are better analysed with such setups. A camera is also better when portability (like image acquisition in field) is required. **WinRHIZO** can analyse images taken with a camera with a means of calibration.

Desktop scanners cannot be used in the field but are transportable and usable in remote locations where electrical power is available.

Why Buy a Scanner from Regent?

1. Our scanner models have passed our scientific quality control test. They are fast, precise and reliable for long term repetitive scientific measurements. Our imaging experts have a long experience in scientific image processing with scanners and choose the best models for our customers.
2. All our scanners have a dual lighting system which produces shadow-free images when scanning roots.
3. Their TWAIN driver is compatible with our products. Unlike some other models on the market, our scanners support the dual lighting system recommended for **WinRHIZO**.
4. Each scanner comes with a root positioning system designed to fit perfectly on its glass surface (see page 2).
5. We calibrate our scanners against precise standards to obtain more accurate dimensional measurements. This calibration is supplied with the scanner and is automatically used by our programs.
6. Our scanners come with a competent and prompt technical support from people who not only sell the product but also use it.
7. We include a manual that illustrates how to scan biological samples for analysis with our programs. It helps you to obtain the best images for accurate measurement and gives some tips specific to the scanner that you have ordered.

Note:

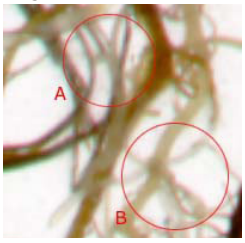
We do not provide technical support for scanners we have not sold, nor do we guarantee their compatibility with our products. In case of incompatibility, you can scan the images with your scanner manufacturer's program, save them in tiff files, then open and analyse them in **WinRHIZO**.

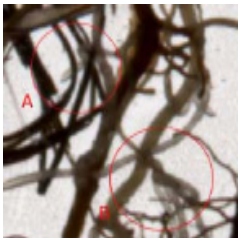
Not All Scanners are Created Equal

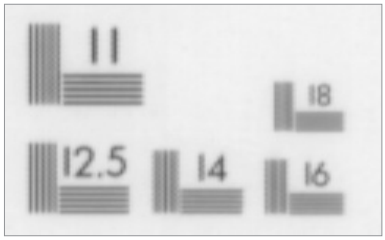
Images below show the same roots and resolution targets scanned with two different scanners at the same resolution. As you can see, not only dpi (resolution) is important. The quality of optical, electronic and mechanical components have a great influence on what can be seen in an image and hence, the precision of measurements you make from it.

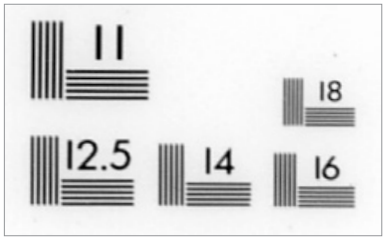
Scanners are made for different applications, the graphics industry and home use being the major ones. Requirements for scientific usage are different. Eye-pleasing images are good, but it's better to accurately reproduce reality. Therefore, before selling scanners for scientific use, we test them carefully to make sure they have minimal qualities. You cannot rely on theoretical specifications alone.

Target size is 5x5 mm





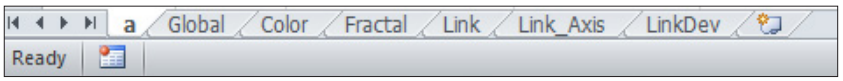




XLRhizo Companion Program for Data Analysis and Visualization

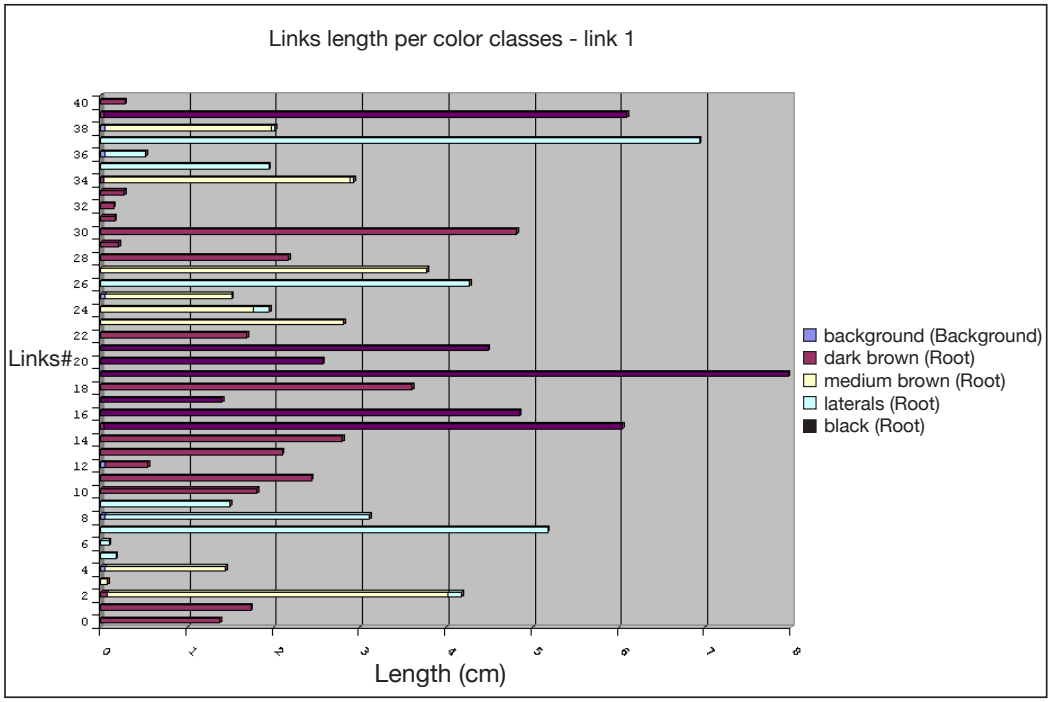
XLRhizo is a utility program written in VBA (Visual Basic Application) for Microsoft Excel (Excel not included) that allows you to visualize data produced by **WinRHIZO**. It facilitates data analysis, provides easy graphic functions, saves time and helps validate data.

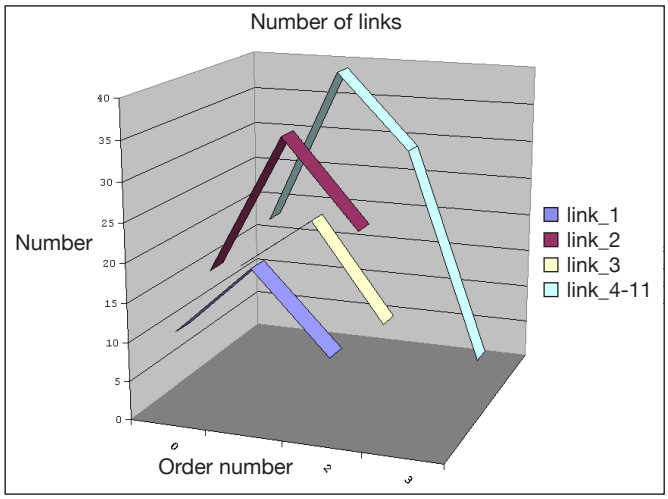
- XLRhizo can separate measurement data into different sheets as a function of their type (global, color, fractal, link, axis, developmental or manual paths analysis) for one or many images.

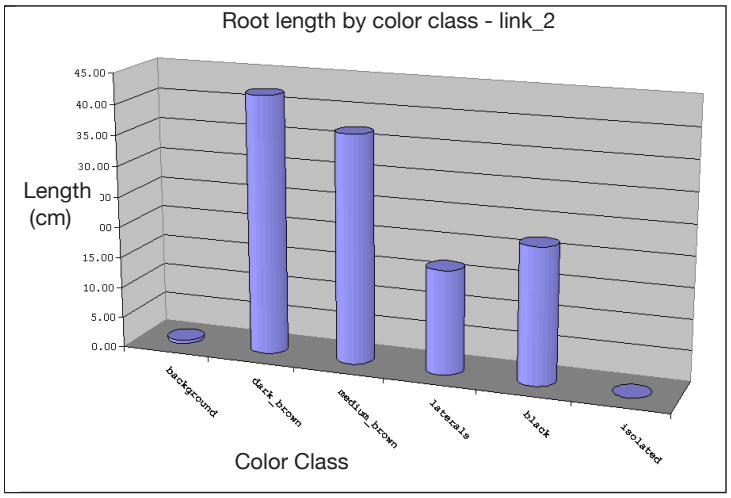


- It can merge the measurement data of a root system analyzed in more than one image when the entire root system is too large or too dense to fit on the scanner and must be analyzed in several pieces. After merging, two or more data lines acquired from the same root system will become a single one. Data will be processed as “Sum of total root length for all merged images”, “Sum of projected root area for all merged images”, and so on.

- XLRhizo can display graphically different measurements for visualization or validation. See examples below.



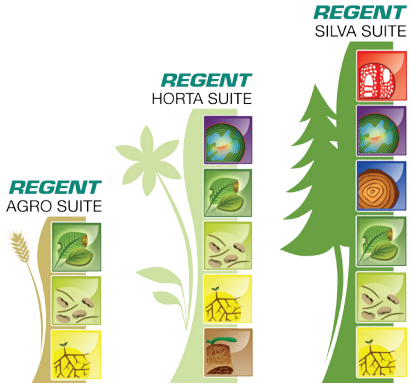




What's New About WinRHIZO™ 2021?

- Multiple analysis windows are now supported which means that users can work on more than one image at a time. Each document window contains its own image, root distribution graphic and data file.
- The 64-bit software versions can load, save and analyse uncompressed tiff images larger than 4GBytes (Big TIFF). Also, they can read Exif and GPS tags (lens focal length, camera manufacturer, software model) in JPEG files.
- Can open TIFF "compressed" and JPEG 2000 images files (24 bits color or 1, 8 or 16 bits grey levels).
- Drawing of tips, forks and crossings is faster. The effect is noticeable when analysing huge images with lots of tips, forks and crossings.
- When clicking to analyse a whole image, the sample identification window is displayed more rapidly. The effect is noticeable when analysing huge images (1TB or +) with Color Analysis.
- When using the Pro version, color classes can be edited, loaded and created after the analysis which is then updated.

WinRHIZO™ is also available in our Software Suites



	REGENT AGRO SUITE	REGENT HORTA SUITE	REGENT SILVA SUITE
WinCELL™			√
WinSCANOPY™		√	√
WinDENDRO™			√
WinFOLIA™	√	√	√
WinSEEDLE™	√	√	√
WinRHIZO™	√	√	√
WinRHIZO™ Tron		√	

REGENT INSTRUMENTS sells worldwide.
For details or to place an order, please contact us.

sales@regentinstruments.com

www.regentinstruments.com