![REGENT INSTRUMENTS logo]

Image Analysis Tools for Plant Scientists since 1991

WinRHIZO™ 2024
Analyser of Washed Roots and Arabidopsis Seedlings

WinRHIZO can perform automatic analyses such as morphology, topology, architecture and color on washed roots* and young seedlings. It is offered as a system that includes a software program and a high quality color optical scanner calibrated for accurate scientific measurement.

The image above shows an analysed washed root sample.
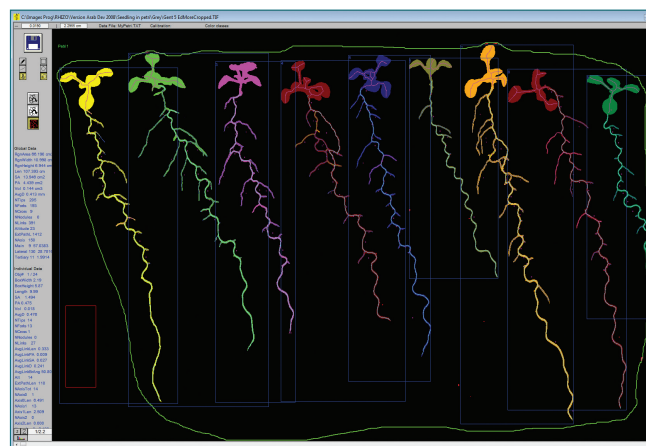
• The root diameter distribution histogram above the image displays the root length, area, surface, volume or number of tips as a function of root diameter or color.

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

• Measurements data are summarized on the left of the screen and are available in detail in data files.

WinRHIZO is offered in four different software versions depending on your needs and budget.

For instance, the Arabidopsis version is optimized for young seedlings growing in Petri dishes or trays. It can differentiate and analyse separately non-touching seedlings or root systems. 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 three other software versions.

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

* 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

Place washed roots directly on the scanner glass or in Regent's water-proof trays, as shown below. 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*.
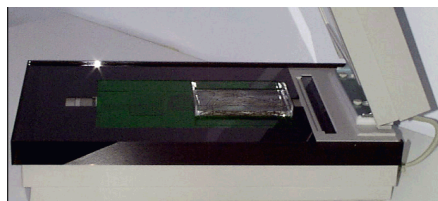
Our trays come with a root positioning system designed to fit on Regent's scanners. It consists of plastic blocks that accommodate different scan area sizes. They form a semi-opaque area with a hole, i.e. the scanned area, that match our water-proof trays.
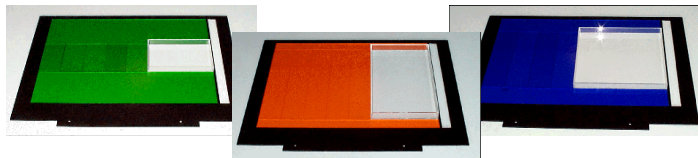
The positioning system accelerates root positioning and scanning, and thus increases productivity.

- Insert the tray containing your sample in the open area formed by the plastic blocs on the glass for the acquisition of subsequent images. **WinRHIZO** has predefined positioning options for you to choose from, so that you can bypass the traditional scanner Preview step and save about 30 seconds for each scan.

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

Our scanners are well adapted to image acquisition of macroscopic objects like roots or leaves. They come with:

- Root positioning system
- Special lighting system to avoid shadows
- Permanent calibration to increase the measurement precision
- Manual that explains how to scan biological samples.

Without a good image of the roots, an unnecessary complex and lengthy root detection algorithm is required. Root image acquisition with an optical scanner without proper attention to the lighting system produces 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, as shown below.

Shadows
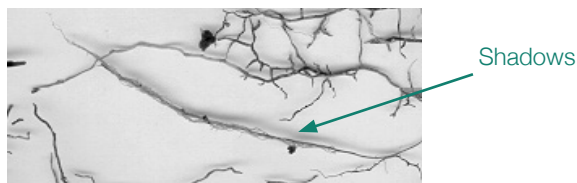
3. ANALYSE THE ROOTS
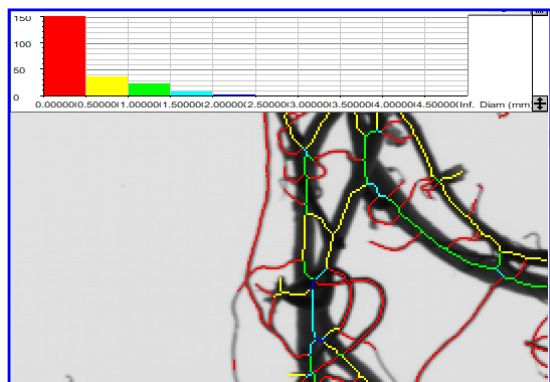
A few seconds after the roots have been scanned, the analysis is completed. Roots found by **WinRHIZO** are identified by colored lines according to their diameter class. Some analyses, such as nodules counting, color and Topology, require interactions from the operator.

WinRHIZO can also analyse images stored in files (*tiff, jpeg, png, jpeg2000 or bmp*) and uncompressed images larger than 4GB.

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.

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.

IMPORTANT NOTES ON MEASUREMENTS

WinRHIZO has two total root volume measurements. One, not invented by us and used by many researchers for years, is estimated from root projected area and length (we call it Estimated Volume). The other, our own method invented more than 28 years ago, is calculated from the measured diameter for all pixels along the root (we call it Measured Volume). In the latest version, both are displayed in the Command area and saved in adjacent columns in data files.

Similarly, it has two average diameter measurements, one estimated and one measured (the latter added in 2023).

Users of versions 2019 and older had to compute the total Measured Volume from the root volume per diameter class data (sum them). Note that total Measured Volume is independant of the number and range of the diameter classes.

WinRHIZO is Offered in Four Software Program Versions:

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

REG - Besides the Basic features, this version performs root morphology measurement as a function of user definable diameter classes. A root distribution histogram generated above the image displays root length, area, volume or number of tips per diameter.

PRO - In addition to the Regular features, this version produces 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.

ARABIDOPSIS - High-end level version which has all the Pro features plus the ability to analyse separately individual objects in an image such as seedlings and leaves. It can do multiple root analyses per image or per regions of it.

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

Measurement		Version	
Root Morphology	Basic	Reg	Pro & Arabidopsis
Global (Total or Average for the image)			
Total Length	Yes	Yes	Yes
Average Diameter	Yes	Yes	Yes
Total Area	Yes	Yes	Yes
Number of Tips, Forks & Crossings	Yes	Yes	Yes
Manual Nodules Counting (not automatic)	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 and branching angle	No	No	Yes
Per link (individually)			
Length, Average diameter, area, and Branching angle	No	No	Yes
Basic Connectivity	No	No	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
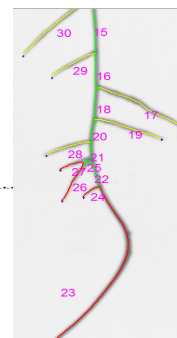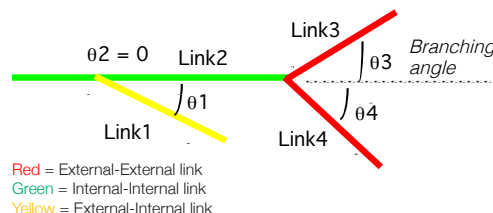
Topology	Basic	Reg	Pro & Arabidopsis
Globally (for the whole image)			
External path length	No	No	Yes
Altitude	No	No	Yes
Per link (individually)			
Magnitude	No	No	Yes
Path length	No	No	Yes
Altitude	No	No	Yes
Structured Connectivity	No	No	Yes

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. Dense root systems are not recommended. 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 link)	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

Used to quantify areas of specific colors or groups of colors and to measure root morphology as a function of color. The operator must indicates the colors of the objects to be analysed and those of the surrounding background by clicking 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.
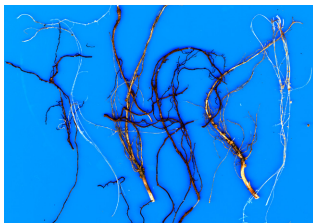
Root morphology (length, area) in function of color.

Color analysis will work if there are minimal color contrasts between the feature(s) you want to quantify and the surrounding background.
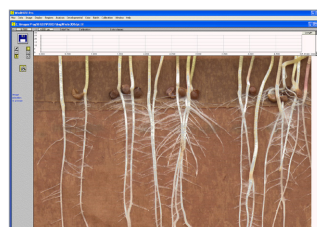
Measure roots in front of different backgrounds, i.e., in a growing pouch.

Shoot growth quantification

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

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.

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 (manual 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
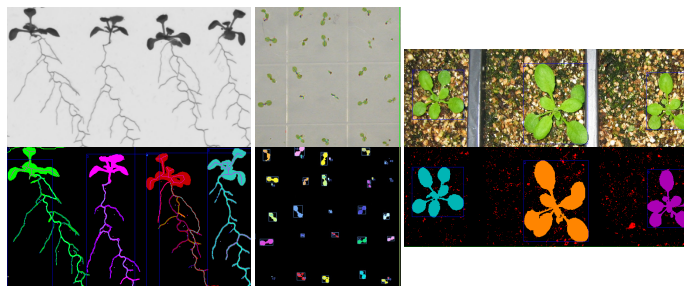
Non-Touching objects are automatically identified by disctinctive colors and analysed individually (area, length, width, color). They must contrast with their surrounding background.
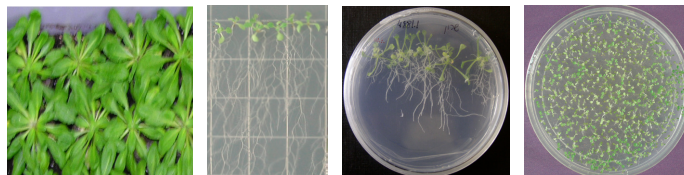
Touching objects and too dense root systems (above) cannot be analysed individually. Only total data is available. You can manually trace an outline around each seedling roots to get individual estimates, but it will include some neighboring roots. When the root system is very dense, it might not be 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 or excluded from analysis (Reg, Pro & Arabidopsis versions)
- **Image edition** to remove artifacts or image defects
- **Interactive or in batch** (without operator supervision) analysis. Not all analyses can be done in batch
- **Predefined 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** 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.
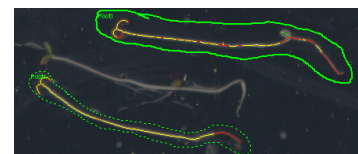
Irregularly shaped regions permit separate analysis of roots that are close to each other.

- **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
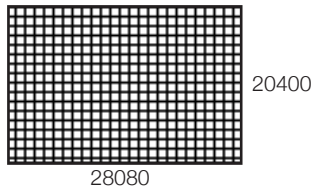
SCANNER

A good 2400 dpi scanner (true optical resolution) produces images which have 2400 by 2400 pixels <u>per inch</u> (2.5cm). Some can go up to 4800 dpi.
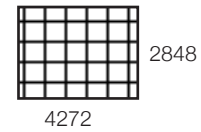
2400 by 2400
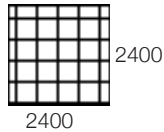
Over a scan area of 8.5 by 11.7 inches, it produces an image of 20400 by 28080 pixels.

This scanner image is equivalent to 47 camera images!

20400 by 28080

CAMERA

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

2848 by 4272

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.

2848 by 4272
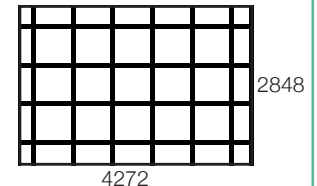
2- It is easy to get good images

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- 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 Instruments?

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.
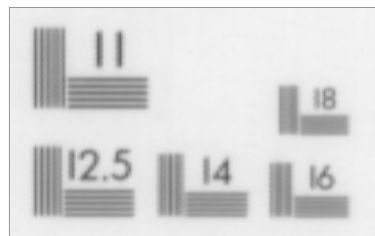
Note:

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.
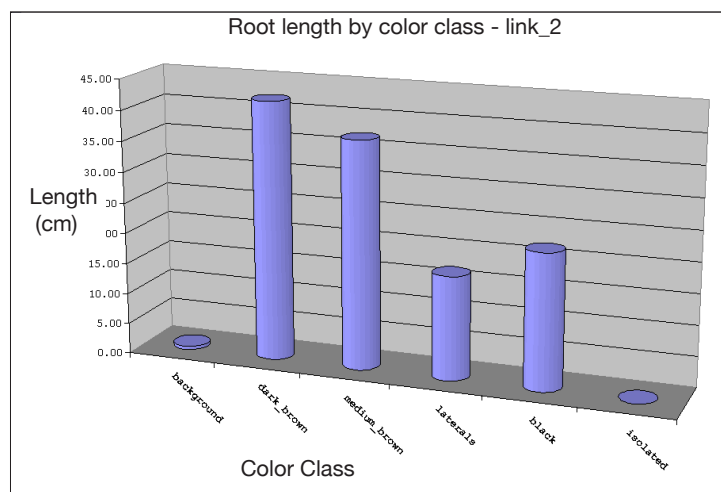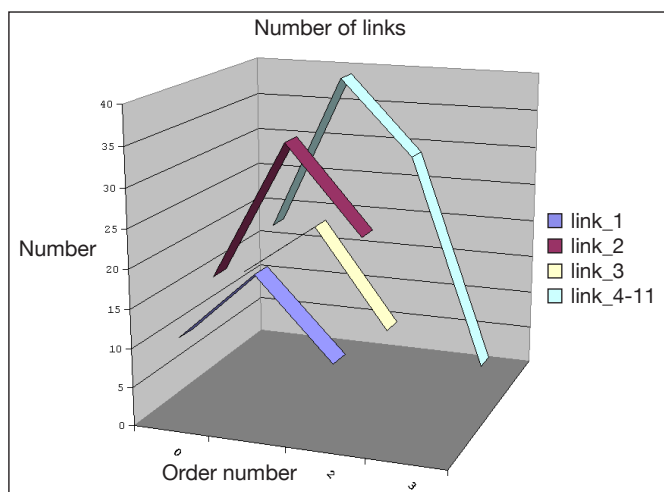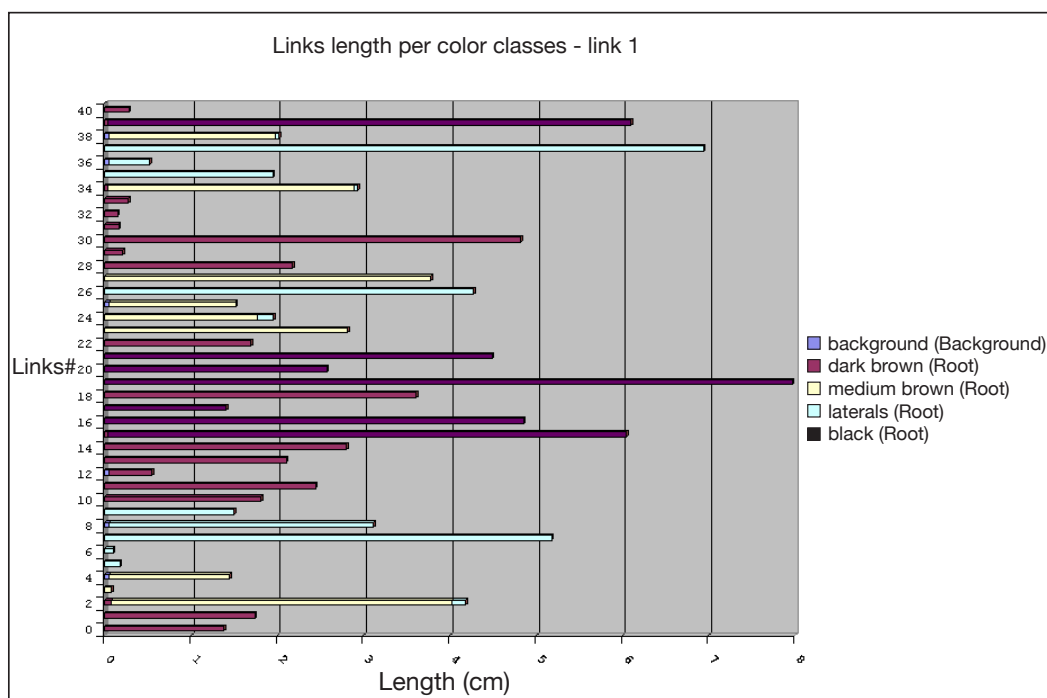
Target size is 5x5 mm

XLRhizo Companion Program for Data Analysis and Visualization

XLRhizo is a utility program that runs in Microsoft Excel (Excel not included) for visualization of data produced by **WinRHIZO**. It facilitates their analysis and provides easy graphic functions.

- **XLRhizo** can separate measurements 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.
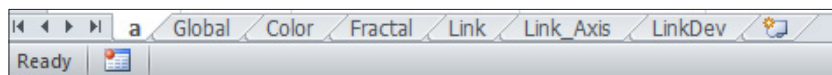
- It can merge the measurements data of a root system analyzed in more than one image when the entire root system is too large or too dense to fit on the scanner and must be analyzed in several pieces.

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

Links length per color classes - link 1

Links#

Length (cm)

- background (Background)
- dark brown (Root)
- medium brown (Root)
- laterals (Root)
- black (Root)

Number of links

Number

Order number

- link_1
- link_2
- link_3
- link_4-11

Root length by color class - link_2

Length (cm)

Color Class

What's New About WinRHIZO™ 2024?

- **WinRHIZO** 64-bit can analyse uncompressed tiff images larger than 4GBytes and can now acquire images from the STD4800 scanner.
- "Measured" average diameter is now available in addition of the previous "estimated" average diameter.
- "Measured" root volume is now saved next to "estimated" root volume.
- Two new options to detect thin and isolated roots.
- Color analysis can be done on 48 bits color images and 16 bits grey levels images for greater precision and accuracy.
- The Command Area data are more detailed and better displayed.

At Least 5 Good Reasons for Choosing our Products

1 Perpetual Software Licences
- No need to budget licence renewals each year
- Use our products as long as you wish
- No activation and no internet connection required
- 32-bit and 64-bit versions included with each licence

2 Software Updated Regularly
- Increase precision and speed of analysis
- New 64-bit versions can analyse uncompressed images larger than 4GB
- Update or upgrade software programs anytime even for old licences bought in 2000

3 Free Technical Support
- Prompt and available via email since 1991
- Provided by our competent software engineers

4 High Quality Hardware
- Optical scanners calibrated by our technicians for accurate scientific measurements
- Special lighting system for thick samples, e.g. roots, seeds, needles
- Positioning systems with trays and wood core holders for easy and rapid scanning of samples
- Blue reflectance target for wood density analysis
- Several software can share the same scanner
- Self-leveling mount with compact DSLR camera and calibrated fisheye lens for canopy analysis

5 Advantageous Discounts
- The more software programs you buy, the bigger the discount
- See all our product families on our website

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).
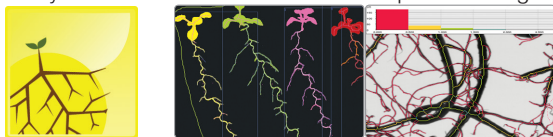** "Accuracy of Measurements with Mac/WinRHIZO™". Stephan Pouleur, REGENT INSTRUMENTS Technical note #3, 1995, pp. 1-4.
*** "A test of a modified line intersect method for estimating root length". Tennant D. 1975, J. Ecol. 63. pp. 995-1001.

Systems and Software Programs offered by Regent Instruments

WinRHIZO™
Analyser of Washed Roots and Arabidopsis Seedlings

WinRHIZO™ Tron
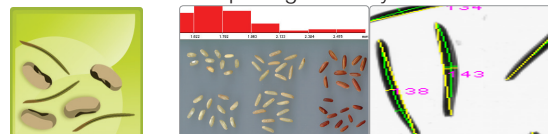Interactive Analyser of Images of Roots Growing in Soil

WinFOLIA™
Leaf Area Meter and Morphological Analyser

WinSEEDLE™
Seed and Needle Morphological Analyser and Counter

WinSCANOPY™
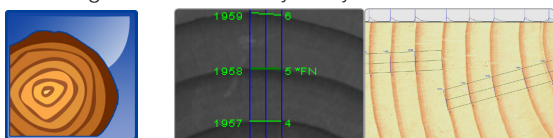Analyser of Canopy Structure and Solar Radiation

WinCAM NDVI™
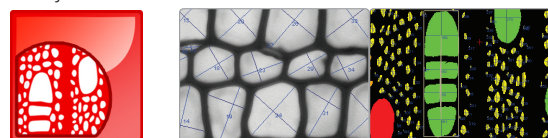Color Area Meter, NDVI & Basic Morphological Analyser

WinDENDRO™
Tree-Ring and Wood Density Analyser

WinCELL™
Analyser of Wood-Cell Structure Parameters

INSTRUMENTS
REGENT

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