

WinRHIZO

For Root Analysis

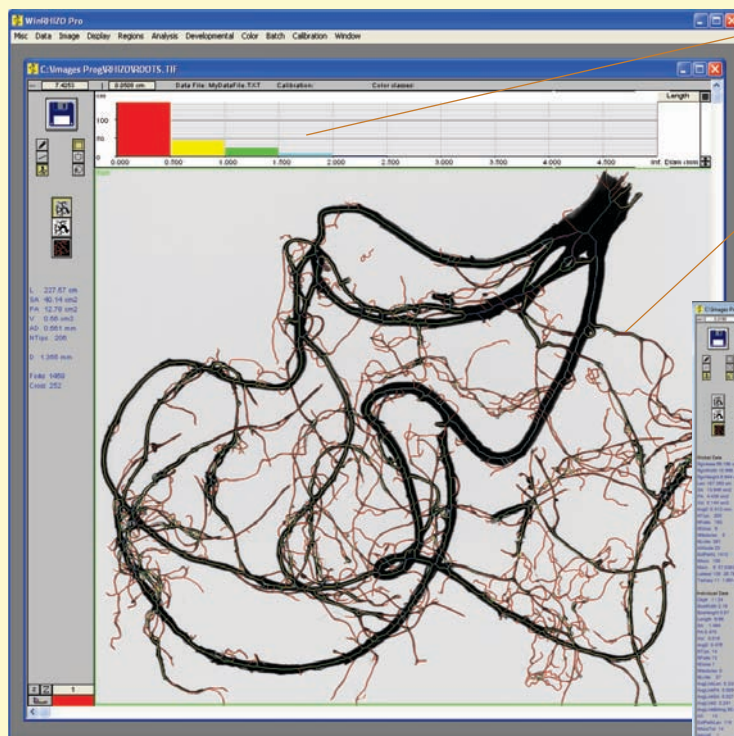
WinRHIZO is an image analysis system specifically designed for root measurement in different forms. It can do morphology (length, area, volume...), topology, architecture and color analyses. It is made of a computer program and image acquisition components that can be combined to meet different needs and budgets.



WinRHIZO automatically analyses washed roots.

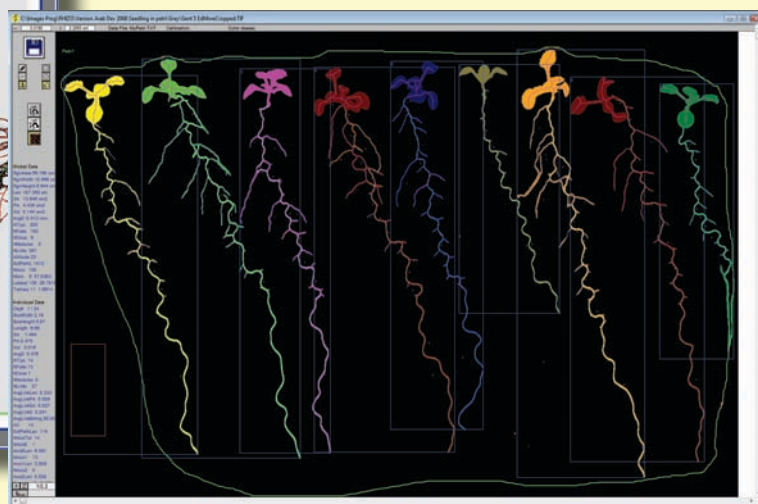
For rhizotron or in-situ root analysis, see our **WinRHIZO Tron** product.

WinRHIZO look and feel



The root diameter distribution graphic 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 above the image.



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

The Arabidopsis version can analyse more than 100 root system per image. It is optimized for young seedling analysis.

WinRHIZO is a member of a family of related products sold by Regent Instruments for plant science research. Among the other products you will find WinFOLIA and WinSEEDLE for leaf analysis and WinDENDRO for tree ring analysis. All these products work on Windows (XP to Vista) based pc computers. See our web site for more information.



REGENT INSTRUMENTS INC.

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WITH ONE MOUSE CLICK YOU CAN DIGITIZE AND ANALYSE ROOTS!

1. ROOT POSITIONING

Simply place the roots directly on the scanner glass or in Regent's water-proof trays. Root positioning is easy and fast with Regent's positioning system for optical scanners (see below). Roots can overlap and do not need to be randomly distributed.

2. ACQUIRE THE IMAGE

Click the scanner icon to digitize the roots and wait a few seconds for the scan to complete and the image to appear on screen. There is no need to use another program to digitize the images, WinRHIZO controls the scanner (or a digital camera) directly. It 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.

3. ANALYSE THE ROOTS

A few seconds later, the analysis is complete and roots found by WinRHIZO are identified by colored lines in the image. The colors used for drawing them are coded according to the root diameter.

Some analyses (Nodules counting, Color and Topology for example) 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

The last step of the analysis is data saving. WinRHIZO knows when data are ready to be saved and does this automatically. Data files are in ASCII (text) format 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.

IMAGE ACQUISITION

Roots can be measured automatically by WinRHIZO when they are extracted from the soil and washed of debris and soil particles. Before analysis, the roots must first be digitized with a scanner or camera.

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:

- Accessories to accelerate root positioning and scanning,
- A special lighting system to avoid shadows (see below),
- A permanent calibration to increase the measurement precision,
- And a manual that explains how to scan biological samples (root, leaves, 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) .

Regent's scanners can be used for other applications (document or photo scanning). They are available in two different sizes of scan area:

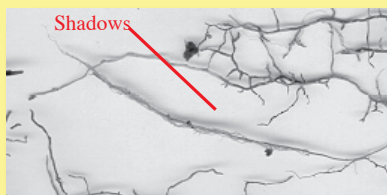
- 22 x 30 cm for Standard Area (STD) scanners,
- 30 x 42 cm for Large Area (LA) scanners.

You can find the specifications of our most recent models on our web site at: www.regentinstruments.com

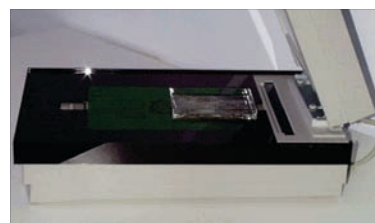
Why an additional lighting system?

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. Shadow (below) is one example.

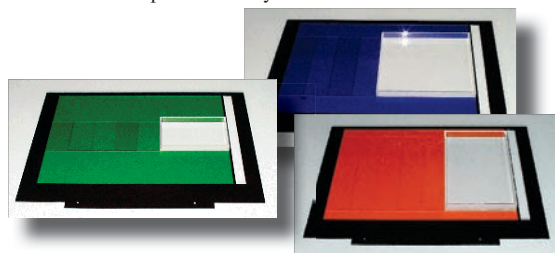
Shadows have grey levels close to those of the roots. Determining the position of the root boundary is much more difficult when they are present.



See our web site for specifications and pricing of the latest scanners and cameras sold by Regent



Our positioning systems significantly increase your productivity for a very low cost. It is comprised of plastic blocks that can be installed and removed quickly to accommodate up to 12 or 24 different scan area sizes (depending on the scanner model). Together they form a semi-opaque area with a hole (the scanned area) for sample placement. Trays that match these rectangular scan areas are supplied with a complete system. They can also be purchased at any time. These trays allow you to scan immersed roots, which are easier to spread than dry roots.



How does it increase the productivity?

- You don't have to worry where to place the samples on the scanner glass. Once you have determined the sample positioning scheme, you simply insert the samples in the open area on the glass for the acquisition of subsequent images. The WinRHIZO program has pre-defined positioning options for you to choose from.
- Because WinRHIZO knows the size and position of the sample on the scanner glass, 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, you (or a second operator) can prepare the next root sample in another tray away from the scanner.

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.

The WinRHIZO program is offered in 4 versions (Basic, Reg, Pro & Arabidopsis)

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

WinRHIZO Basic is a low cost entry level version. It produces only global measurements (average root diameter, total root length, area, volume and number of tips).

WinRHIZO Regular has all features of the Basic model plus root morphology measurement as a function of user definable diameter classes. A root (length, area, volume) distribution graphic is automatically generated and is visible above the image. This graphic can also be printed.

WinRHIZO Pro has all features of the Regular version plus link, topology, architecture and color analyses. The color analysis can produce root morphology as a function of color. It can also be used as a color area meter (see next page).

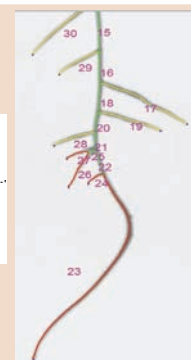
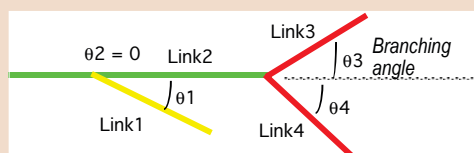
WinRHIZO Arabidopsis has all features of the Pro version plus the ability to separate and analyse separately individual objects such as seedlings and leaves. It can do multiple root analyses per analysed image or regions of it (see next page).

Measurement	Model		
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, 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

Note: It is possible to upgrade from a low to a high-end program version at any time by paying the difference in cost between the two versions.

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 (definitions & colors below refer to figure to the right)			
Red	= External-External link		
Yellow	= External-Internal link		
Green	= Internal-Internal link		
Blue	= Isolated link (not connected to any link)		

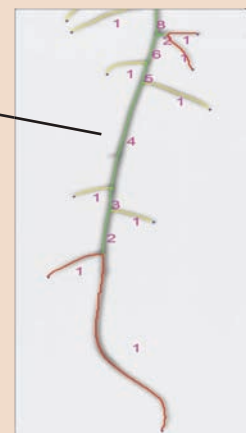
Link analysis is a study of the morphology and basic connectivity of root segments. It can be done on incomplete or complete root systems.



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 a value for the complete root system (not per link).
- Altitude, the largest path length.

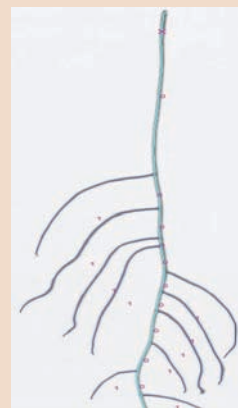


Note: Topology and developmental analyses require an integral root system. It is meaningless if the connectivity of the root system's links has been destroyed by manipulation or the imaging process. It is not recommended to do link analyses on dense root systems. If you cannot visually track root segments in an image, it is unlikely that RHIZO will be able to 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 were born from the base link as the plant grew.

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.

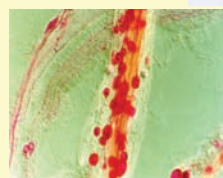


Root Architecture with Fractals	No	No	Yes
Color Analysis	No	No	Yes (see next page)

COLOR ANALYSIS WITH THE PRO & ARABIDOPSIS VERSIONS

Color analysis allows you to quantify areas of specific colors or groups of colors and to measure root morphology as a function of color. The operator first indicates to WinRHIZO the colors of the objects to be analysed and those of the surrounding background. This is done interactively 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:

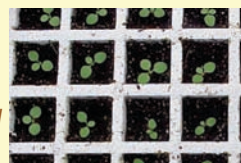
*Leaf area meter.
Quantification of
leaf disease and
insect damage.*



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



Seeds classification



Shoot growth quantification



*Root
morphology
(length, area)
in function of*

*Measure roots in
front of different
backgrounds (in a
growing pouch for
example).*



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.

WINRHIZO ARABIDOPSIS

It includes all features and measurements of the Pro version plus:

- The ability to differentiate and analyse 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).
- It can measure the object's bounding box width and length which 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.
- It can measure the object's individual area 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:

- 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 (might be different in future versions), only for the image:

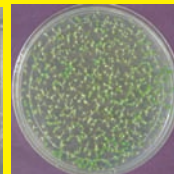
- Fractals
- Root distribution histogram data (length, area, volume, tips) in function of diameter
- Root morphology (length, area...) per color



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



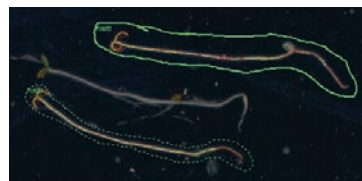
Touching objects and too dense root systems (below) cannot be analysed individually (only total data is available). You can manually trace an outline around each seedling roots to get individual estimates but it will include some neighboring roots. When the root system is very dense it might not be possible 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 of any shape can either be selected for analysis or exclusion from it (Reg, Pro & Arabidopsis versions)

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



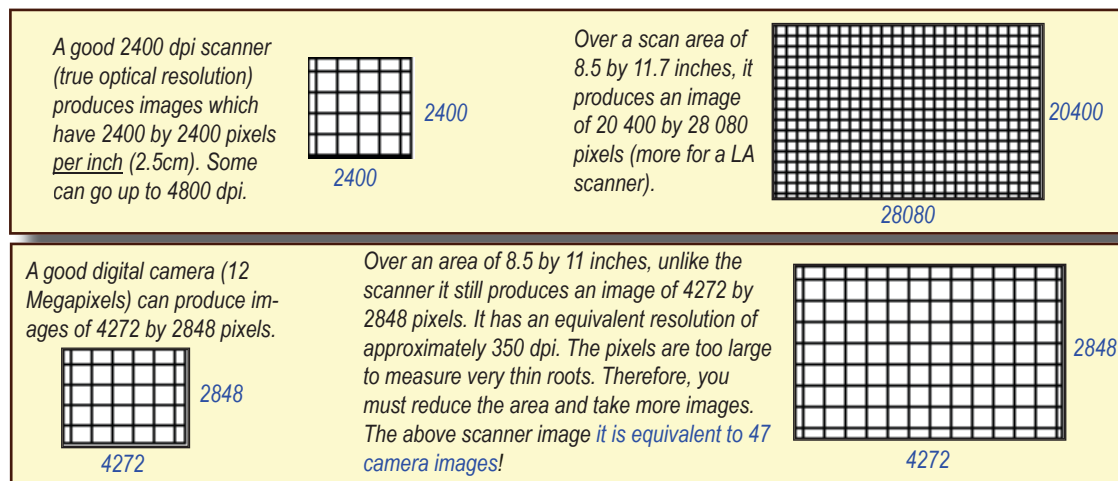
*Circular regions can
be used to analyse
roots in petri dishes.*

- **Image edition** to remove artifacts or image defects. You can pick up and edit with any color present in an image or define your own.
- **Interactive or in batch** (without operator supervision) analysis. *Note:* Not all analyses can be done in batch.
- **Pre-defined analysed regions.** They allow to create with a single command, a specified number of equidistant regions at different vertical positions (soil depth) in an image. The size and distance between these analysed regions are specified by the operator. (Reg & Pro)
- You can **print and save** to a file the images with or without their analysis marks over them.
- Debris can be filtered-out based on area, shape (all versions) or color (Pro & Arabidopsis versions).

MORE ABOUT SCANNERS AND CAMERAS

Why use an optical scanner rather than a video camera?

- Scanners can produce images of many times the resolution of a camera.



RÉGENT INSTRUMENTS imaging experts have experience in scientific image processing with scanners since 1988.

- 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.
- Calibration is permanent.
Unlike a camera, the object-to-camera distance and zoom are always the same.
- They are reliable and last for a long time.

When is a camera better adapted than a scanner?

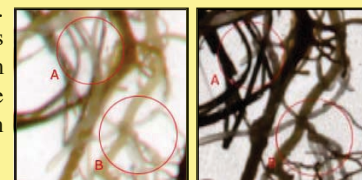
A camera is better when extremely high magnification is required. By adding proper lenses or mounting it on a microscope, you can see more details than with a scanner BUT OVER A MUCH SMALLER AREA. Mychorizae and root hair are better analysed with such setups. A camera is also better when portability (like image acquisition in field) is required. WinRHIZO can analyse images taken with a

Can Regent's scanners be used in the field or at a remote location?

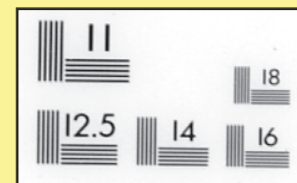
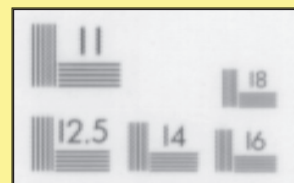
Desktop scanners cannot be used in the field but are easily transportable and usable in remote locations where electrical power is available.

Not all scanners are created equal

The images below show the same roots 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 the 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



Why buy a scanner from Regent?

Although WinRHIZO can work with many scanner models that are TWAIN compatible, it is advantageous to purchase a scanner from Regent.

- The model you buy has passed our scientific quality control test. You get a scanner that is well adapted (fast, precise and reliable) for long term repetitive scientific measurements. Before deciding which scanners to sell, we test many of the models available and we choose the best.
- You get a scanner that has a dual lighting system which produces shadow-free images when scanning roots.
- You get a scanner for which the TWAIN driver compatibility has been tested with our products. Unlike some other models on the market, our scanners support the dual light system recommended for WinRHIZO.
- You are sure that our accessories will fit on the scanner. The root positioning system is specific to each scanner we sell.
- 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.

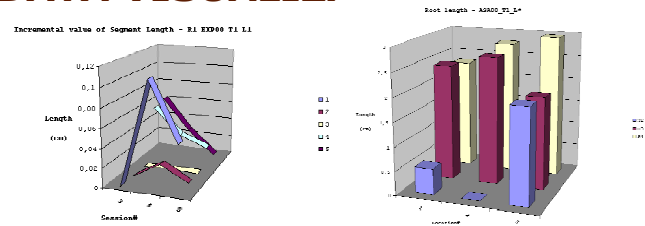
- It is much easier for you to get technical support. You get competent and prompt technical support from people who not only sell the product but also use it. If problems arise, you don't have to contact two vendors, only one is responsible for finding the solution.

- You get our manual that illustrates how to scan biological samples for analysis with our programs (WinRHIZO, WinFOLIA...). This manual helps you to obtain the best images for accurate measurement and also gives some tips specific to the scanner that you have ordered.

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

THE XLRHIZO COMPANION PROGRAM FOR DATA VISUALIZATION

When comes the time to visualize and analyse data produced by WinRHIZO, XLRhizo is greatly appreciated. This is a utility program that runs in Microsoft Excel. It allows you to manipulate, reorganize and display measurement data graphically. XLRhizo is a utility program that is optional and which can be ordered separately or with WinRHIZO. It is very affordable and can save a lot of time (and manipulation errors).



THE ALTERNATIVES TO WinRHIZO

What are the advantages of a dedicated program like WinRHIZO compared to other programs that can analyse everything?

Many image analysis programs on the market were developed for morphology analyses of complex objects. These programs can analyse many types of objects and produce hundreds of measurements for each one. The main problem with them is that they require a high level of expertise and customisation before producing the analyses that you want. This means that you must be familiar with the basics of image analysis. This complexity is easily recognisable in the structure of the commands and the manuals that come with these programs. They contain many commands that you will never use. They are designed for people who don't do repetitive measurements or for those who have image analysis experts (with spare time) in house.

In contradiction to the above approach, WinRHIZO already knows what you want to digitize and measure (we refer to WinRHIZO as being **integrated**). Without modification to its configuration, it can do root analysis. You simply indicate which analyses you want to do. This makes your work more efficient (and hopefully more pleasant!).

What are the differences between WinRHIZO and the other dedicated root measurement programs commonly found in agro-forestry instrumentation companies?

Some products are simply an interface in front of a general image analysis library of functions that can do many things. These systems are in fact complex morphology image analysis programs marketed as *Do Everything* solutions. They share the same disadvantages as the non-dedicated systems described above.

Another approach consists of subcontracting the design to an external firm which either modifies an existing product, customizes a general image analysis system or builds one from scratch. Because the contractor has to pay for each modification, it is unlikely that these systems will be upgraded on a regular basis. On the other hand, WinRHIZO is updated regularly as can be seen at the following web page: <http://www.regentinstruments.com/products/rhizo/RhizoMostRecent.html>

Can RHIZO be used for other applications?

Although WinRHIZO is optimized for root analysis, it can be used to analyse other objects. For example all versions can be used as an area meter (*i.e.* leaf analysis), while the *Pro* and *Arabidopsis* versions can be used as a color quantifier that has many applications (*i.e.* disease quantification). By default it expects to analyse roots, so you'll have to change some settings to use it for other applications. Refer to WinFOLIA for more sophisticated leaf analysis and WinCAM for generic objects morphology and color analyses.

You can get scientific publications from people who have tested and used WinRHIZO from the following web page:

<http://www.regentinstruments.com/pages/References.html>

To learn more about the latest version of WinRHIZO available see the following web page:

<http://www.regentinstruments.com/products/rhizo/RhizoMostRecent.html>

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For sales or technical information visit our web site at:

www.regentinstruments.com

To place an order or to ask questions, please contact: sales@regentinstruments.com

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