WinRHIZO™ is an image analysis system specifically designed for automatic root measurement in different forms. It can do morphology (length, area, volume...), topology, architecture and color analyses of washed roots.

A system is made of a computer program and image acquisition components that can be combined to meet different needs and budgets.

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 one root system per image. It has the ability to differentiate and analyse separately non-touching seedlings, root systems or objects.

WinRHIZO™ and all other Regent Instruments’ systems work on Windows Vista to Windows 10 based PC computers.
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.

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

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.

REFERENCES
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).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Morphology</td>
<td>Basic  Reg  Pro &amp; Arabidopsis</td>
</tr>
<tr>
<td>Global (Total or Average for the image)</td>
<td>Yes  Yes  Yes</td>
</tr>
<tr>
<td>Total Length</td>
<td>Yes  Yes  Yes</td>
</tr>
<tr>
<td>Average Diameter</td>
<td>Yes  Yes  Yes</td>
</tr>
<tr>
<td>Total Area, Volume</td>
<td>Yes  Yes  Yes</td>
</tr>
<tr>
<td>Number of tips, forks &amp; crossings</td>
<td>Yes  Yes  Yes</td>
</tr>
<tr>
<td>In function of root diameter (per diameter class)</td>
<td>No  Yes  Yes</td>
</tr>
<tr>
<td>Length</td>
<td>No</td>
</tr>
<tr>
<td>Area, Volume</td>
<td>No</td>
</tr>
<tr>
<td>Number of tips</td>
<td>No</td>
</tr>
<tr>
<td>In function of root color (see Color Analysis below)</td>
<td>No  No  Yes</td>
</tr>
<tr>
<td>Length</td>
<td>No</td>
</tr>
<tr>
<td>Area, Volume</td>
<td>No</td>
</tr>
<tr>
<td>Number of tips</td>
<td>No</td>
</tr>
</tbody>
</table>

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

### Link Analysis

**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)

### Topology

**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

### Developmental classification

<table>
<thead>
<tr>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of links (per order)</td>
</tr>
<tr>
<td>Total length (per order)</td>
</tr>
<tr>
<td>Total Area (per order)</td>
</tr>
<tr>
<td>Average link length (per order)</td>
</tr>
<tr>
<td>Average link area (per order)</td>
</tr>
<tr>
<td>Average diameter (per order)</td>
</tr>
<tr>
<td>Link order (per link)</td>
</tr>
<tr>
<td>Root Architecture with Fractals</td>
</tr>
<tr>
<td>Color Analysis</td>
</tr>
</tbody>
</table>
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.

**Shoot growth quantification**

**Seeds classification**

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

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

**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

Includes all features and measurements of the Pro version plus:

- 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).
- 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.
- 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);
- It can measure the hypocotyl (leaf) length and area separately from roots (you must click at their junction to start the developmental analysis). Area is less precise than length (± 20%).

**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):

- **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-definedanalysed regions.** They allow to create with a single command, a specified number of equidistant regions at different vertical positions

(soon 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).
- WinRHIZO comes with prompt and competent technical support made by Regent’s personnel, not by subcontracted firms.
- WinRHIZO comes with printed color manuals which are abundantly illustrated.
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.

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 20 400 by 28 080 pixels (more for a LA scanner).

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. It has 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. The above scanner image is equivalent to 47 camera images!

• 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 camera with a means of calibration.

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.

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.

1. 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.

2. You get a scanner that has a dual lighting system which produces shadow-free images when scanning roots.

3. 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.

4. You are sure that our accessories will fit on the scanner. The root positioning system is specific to each scanner we sell.

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. 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.

7. 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 WinRHIZO 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/assets/references.html

Complementary products sold by Regent Instruments Inc.: