

RhizoVision Explorer

Root image analysis for the rest of us



RhizoVision Explorer is free and open-source software developed for estimating root traits from images acquired from a flatbed scanner or camera. Root images are expected to have a high contrast of roots with the background, homogenous lighting, and minimal overlapping of roots. The software is built in C++ for speed and stability, using QT for the graphical user interface and OpenCV for image processing. Traits extracted by RhizoVision Explorer have been extensively validated using a physical copper wire ground truth image set, thousands of simulated roots, and comparisons with other image analysis software. The default “Broken Roots” mode is meant for roots washed out from soil cores or pots that are disconnected and provides length, volume, branching frequency, among other features, with the ability to bin measurements based on diameter thresholds. The “Whole Root” mode extracts additional root system architecture features of more intact root systems, especially excavated root crowns or rhizoboxes, such as the convex hull, angles, and holes. The software supports multiple regions of interest, batch mode, and user-defined export of processed images with overlaid features for use in reports.

Minimum requirements are Windows 10 or 8 operating system, an Intel or AMD x86_64 processor, and 8 GB of RAM. If the processor supports Intel AVX 2.0, the code is optimized.

You can redistribute it and/or modify it as permissible under the [GNU General Public License](#). The source code is available on [GitHub](#).

Please contact the creators with concerns or comments

Larry M. York

larry.york@gmail.com

Anand Seethepalli

anand_seethepalli@yahoo.co.in

Citing the software – We humbly ask you cite the software when reporting data it generated in presentations or manuscripts.

Software: Seethepalli A and York LM. RhizoVision Explorer - Interactive software for generalized root image analysis designed for everyone (Version 2.0.3). Zenodo. DOI: [10.5281/zenodo.3747697](https://doi.org/10.5281/zenodo.3747697)

Manuscript: Seethepalli A, Dhakal K, Griffiths M, Guo H, Freschet GT, York LM (2021). RhizoVision Explorer: Open-source software for root image analysis and measurement standardization.

User Guide




Contents

Load software	3
Overview	3
Loading an image	4
Choose appropriate root image pre-processing settings	5
Feature extraction settings	7
Features and Log pane	8
Region of interest tool	8
Save features data and batch analysis	10
Display output settings	11
Using features CSV file with external software like Excel and R	12
List of extracted features and their definitions	14
Feature validation using a novel ground truth copper wire dataset	16
Included example images and settings files	17

RhizoVision Explorer graphical user interface with displayed output image of wheat roots

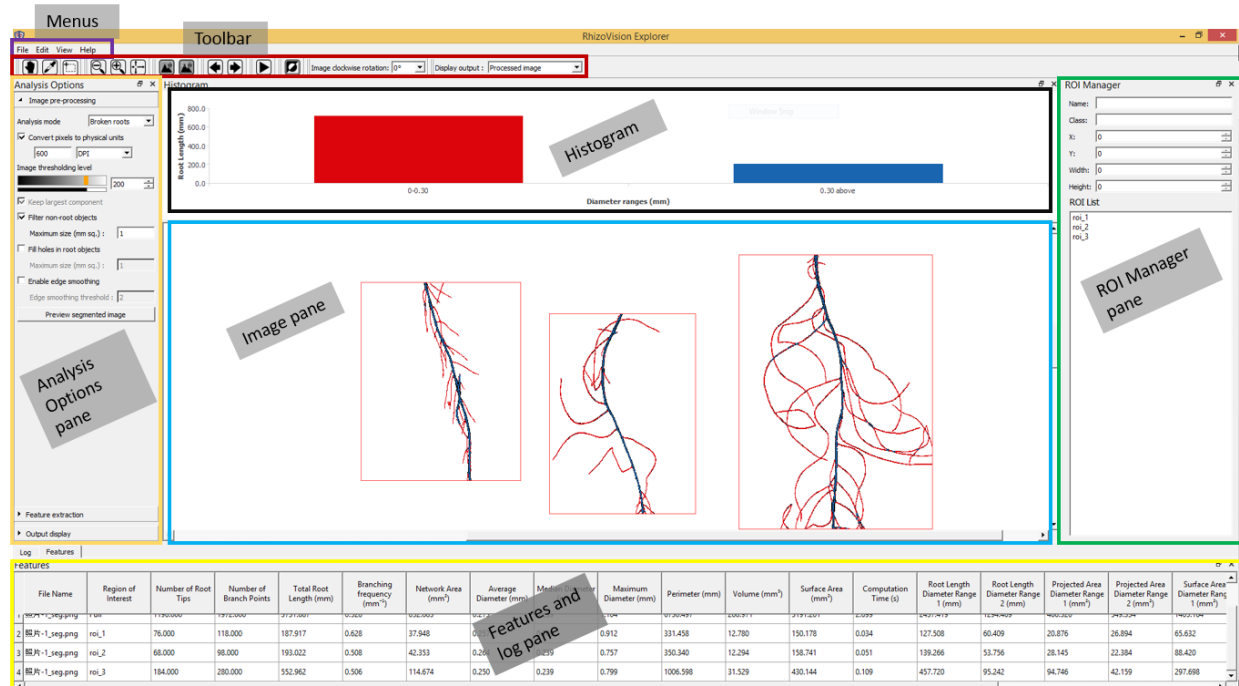


Load software – Simply double click RhizoVisionExplorer.exe

 README.md	2/5/2019 4:41 PM	MD File	3 KB
 RhizoVisionExplorer.exe	10/12/2020 5:08 PM	Application	924 KB
 RoiManager.dll	10/12/2020 2:26 PM	Application extens...	97 KB

Hot tip! Right-click on RhizoVisionExplorer.exe and choose “Pin to taskbar” for quick access

Overview

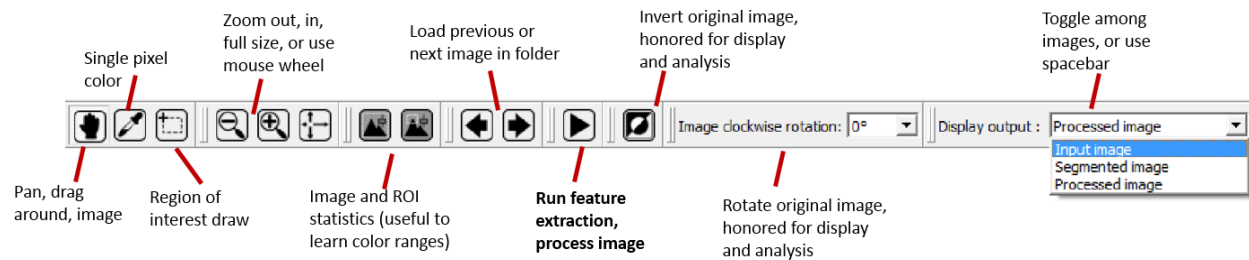


RhizoVision Explorer is divided into groups of **panes**, as shown above. The **histogram** pane only appears after feature extraction. The **overall workflow** is:

1. Load image by drag-and-drop to the **Image pane** or selecting an image from the **File menu**.
2. Use the **Analysis Options pane** for pre-processing, feature extraction, and output settings.
3. Use the **Image pre-processing area** to choose whether a **whole root** (like a root crown) or **broken roots** (usually scanned), supply image resolution in either DPI or pixels per mm, set the pixel intensity level for identifying roots, and filter out objects by relatively small sizes such as dirt.
4. Use the **Feature extraction area** to enable root pruning to erase short, erroneous root segments from the output and set diameter ranges. Use interactively to optimize the settings.
5. Use the **Output display area** to change which image features are displayed on the feature image.
6. Use **Run analysis** on the Toolbar to process the image, output the display image and numeric features.
7. This workflow may be repeated for images within a folder or continued with batch mode (see below).

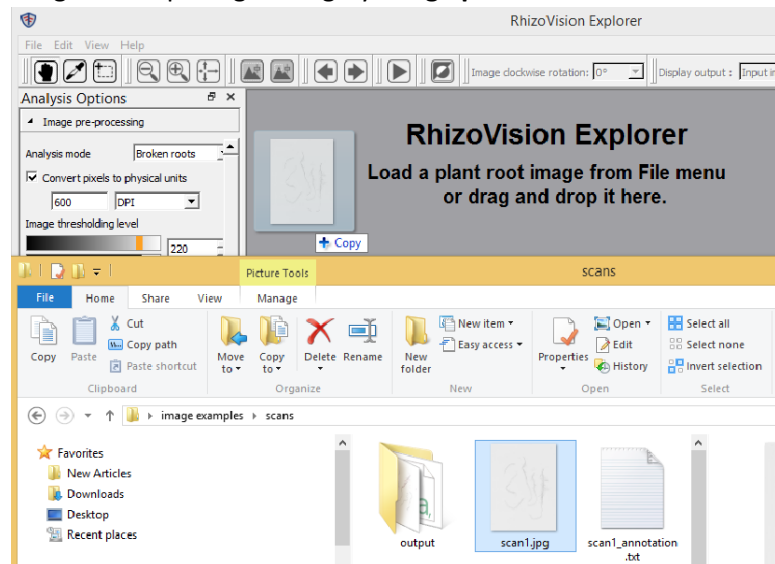
Hot tip! RhizoVision Explorer converts pixel values (default) to mm, mm², and mm³ for length, area, and volume, respectively, when the image resolution is supplied in the Image pre-processing options

The main **Toolbar**, as shown below, groups various ways to interact with the root image data.

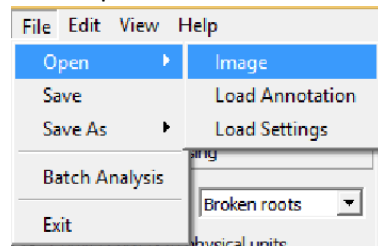


Loading an image

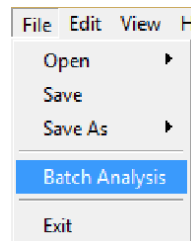
- a. Single image (useful for testing or small image sets with different regions of interest)
 - i. Drag and drop image into gray **image pane** window



- ii. File > Open



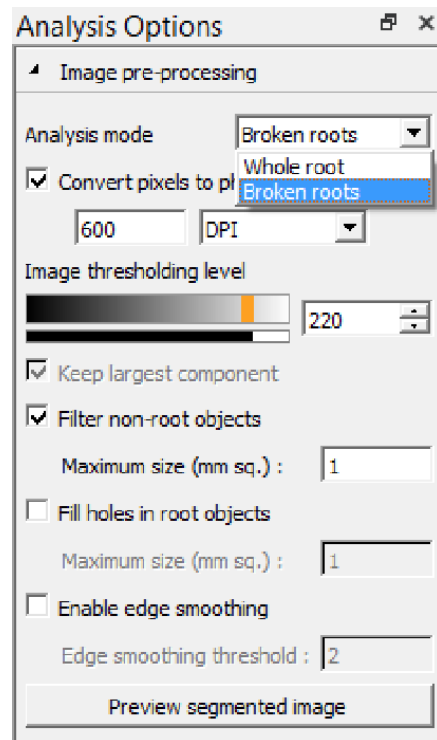
- b. Batch mode (when you know your image settings and/or fixed ROI regions)
 - i. File > Batch analysis



Choose appropriate root image pre-processing settings

Change a setting (see below) > press **Preview segmented image** > Compare Display output tab

RhizoVision Explorer expects black roots on a white background. If you have white roots on a black background, choose the **Invert image** option in the Toolbar first, then continue.

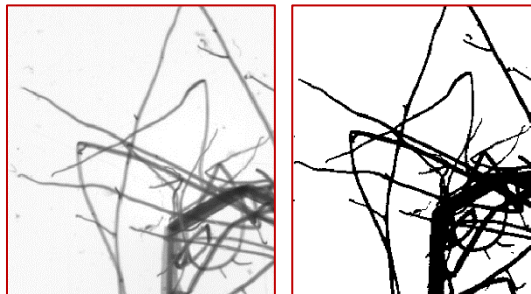


The Analysis Options pane contains tools for **Image pre-processing** (segmenting) the image, **Feature extraction** (pruning and diameter bins), and what features to overlay in **Output display**. These settings should be adjusted interactively until you are satisfied.

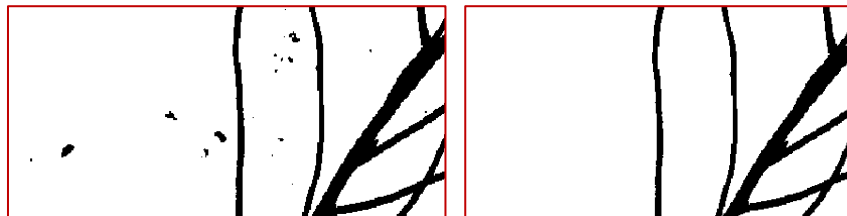
Analysis mode determines defaults for filtering objects and providing extra architectural data as described on the next page.

Convert pixels to physical units accepts DPI for scanners or pixels per mm as measured by the user for a camera system.

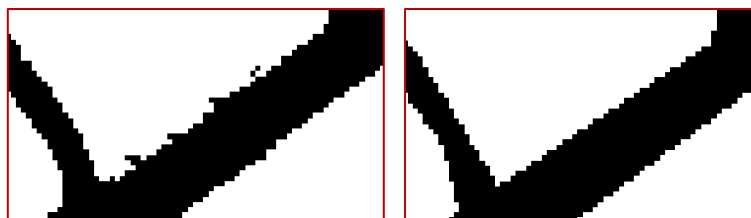
Image thresholding level should be adjusted to maximize clean root borders while limiting the appearance of noise.



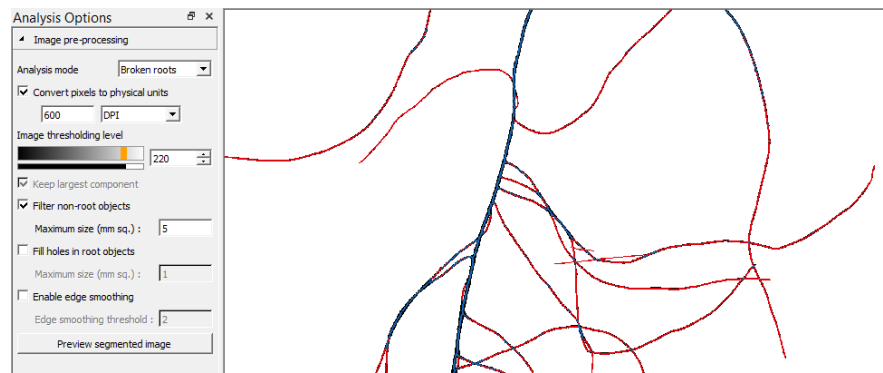
Filter non-root objects removes black specs like dirt that are not root. Objects smaller than set size are erased. Fill holes works oppositely, to fill in holes (white spaces) on the roots, but is rarely needed.



Enable edge smoothing smooths root edges to decrease false roots during skeletonization. Higher values smooth more. In general, using **Pruning** in **Feature extraction** is preferred due to distortion.



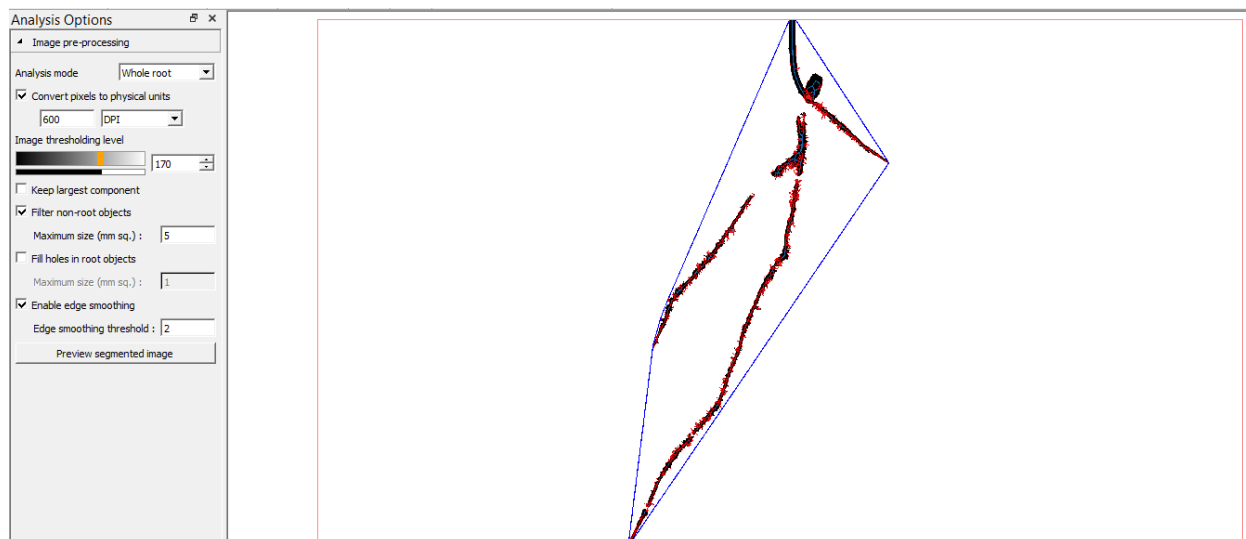
Broken roots analysis is the default because the method is common, typically for washed-out roots from pots or soil cores where the underlying architecture is lost and the root system is disconnected.



Example of a **Whole root analysis** and options using a root crown from the [RhizoVision Crown platform](#)

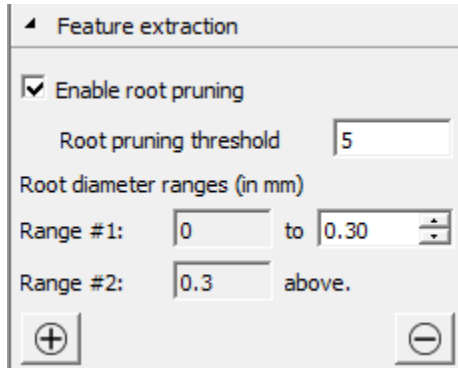


Example of **Whole root analysis** and options for a rhizobox image where roots may still be disconnected but where angles, convex hull, system width, and depth are still relevant.



Feature extraction settings

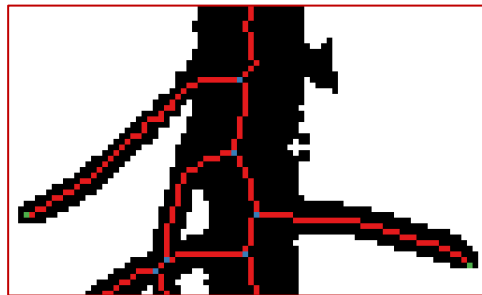
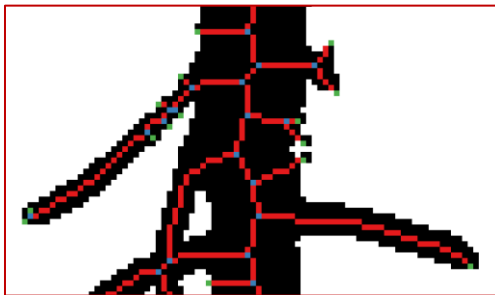
Change a setting (see below) > press **Run analysis** button to test > Compare Display output tab



Root pruning is recommended to delete false root segments where tips are longer than the diameter of the parent root plus the additional pixels supplied in the **Root pruning threshold**. Values between 1-20 are recommended to reduce unwanted tips. Higher values reduce false tips, but can reduce total length.

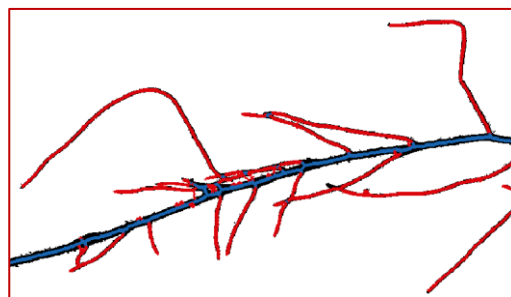
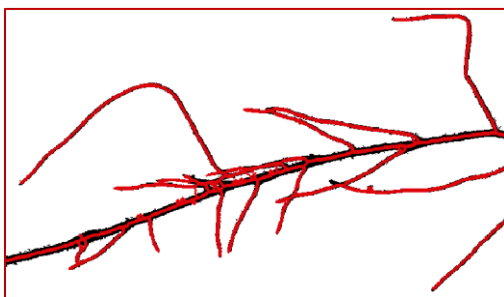
Diameter ranges can be set to capture [fine roots below 2 mm](#) or separate lateral roots from their parents through interactive adjustment. Up to 10 ranges are currently supported.

The raw skeleton on the left has many short root segments that lead to the overestimation of root parameters. By enabling pruning, segments longer than the diameter of their parent are deleted (right).



Hot tip! In the Output display setting, turn off **Color media axis by diameter** and set **Medial axis width** to 1 to display topological points like tips in green, branch points in blue, and segments in red.

The heterogeneity of root diameters within a sample can vary greatly and is determined mainly by the number of root orders or lateral branching. In many cases, the length of lateral roots and their parent roots can be quantified separated by appropriate adjustment of these diameter ranges. Ranges can be added to a maximum of 10 or deleted to a minimum of 1 (left), but in this case 2 was optimal (right).



Features and Log pane

The **Features** tab is displayed after Run analysis completes all operations, with the most recent analysis appearing as the last row of the output. The first column gives the file name, and the second the “Region of Interest” (ROI) which will list “Full” if it was the whole image, or else will use either default ROI names or the names provide in the ROI manager (see below). Every feature is given in the column headers, along with the correct units. When features are saved (see below), these names are converted to formats without spaces or exponents for loading directly into common data analysis pipelines, such as R or Python. Missing values are stored as ‘NA’. One or multiple rows can be selected to clear those or all rows by right-clicking.

Log

Features

Features

	File Name	Region of Interest	Number of Root Tips	Number of Branch Points	Total Root Length (mm)	Branching frequency (mm ⁻¹)	Network Area (mm ²)	Average Diameter (mm)
5	scan1.jpg	Root1	137.000	213.000	661.072	0.201	103.362	0.311
6	scan1.jpg	Root2	213.000	213.000	747.720	0.285	202.010	0.309
7	scan2.jpg	Full	123.000	494.000	3216.596	0.154	925.855	0.318
8	scan3.jpg	Full	125.000	529.000	2318.974	0.228	682.144	0.331

The **Log** tab is displayed to show major events or warnings. The **Features** tab will activate if another analysis is executed.

Log

Features

Log

Image "C:/Users/lmyork/Desktop/image examples/scans/scan2.jpg" loaded.

Image "C:/Users/lmyork/Desktop/image examples/scans/scan3.jpg" loaded.

Image "C:/Users/lmyork/Desktop/image examples/scans/scan1.jpg" loaded.

Region-of-interests loaded from file "C:/Users/lmyork/Desktop/image examples/scans/scan1_annotation.txt"

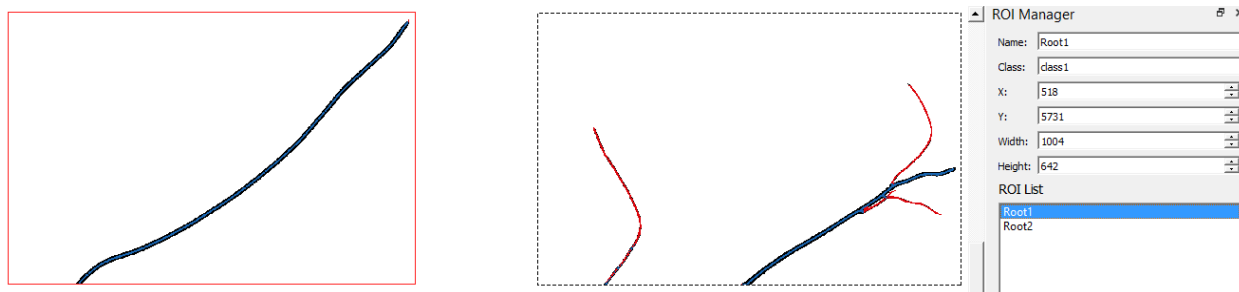
Image "C:/Users/lmyork/Desktop/image examples/scans/scan2.jpg" loaded.

Image "C:/Users/lmyork/Desktop/image examples/scans/scan3.jpg" loaded.

Region of interest tool



Use the **ROI tool** to draw new regions of interest (ROI) or to move and resize ROIs.

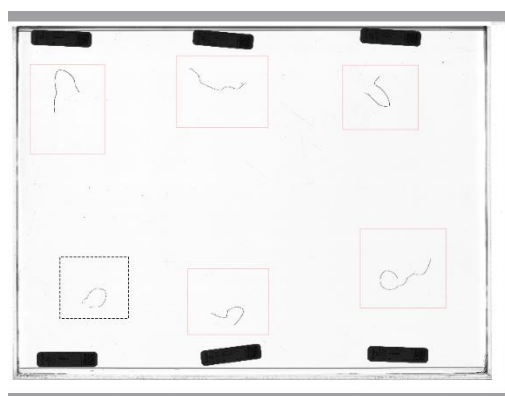


ROIs are displayed as red rectangles. An active ROI is displayed with a dashed rectangle if it has just been drawn, has been selected with the ROI tool, or has been selected in the ROI List within the ROI Manager pane. The ROI tool allows the active ROI to be resized, moved, or deleted with the 'delete' key. The ROI Manager allows an ROI to be renamed, manually updating coordinates and size, and selecting an ROI from a list. All ROIs can be cleared from the Edit menu.

Example 1. Use the ROI tool to crop out the tray in flatbed scanner images



Example 2. Create multiple ROIs, each with a different name, to separate samples in the same image. As shown in the **Features** section, a second column after File Names displays the named ROIs, which allows convenient subsequent data import and analysis.



Once a single or multiple ROIs are drawn, they may be saved for reuse using **the File > Save as > Annotations**, and loaded using **File > Open > Load annotation**

Hot tip! The ROIs drawn on an interactive image are honored for the next loaded image, and also in batch analysis. Therefore, samples should be placed consistently during imaging or scanning for ease of subsequent image analysis.

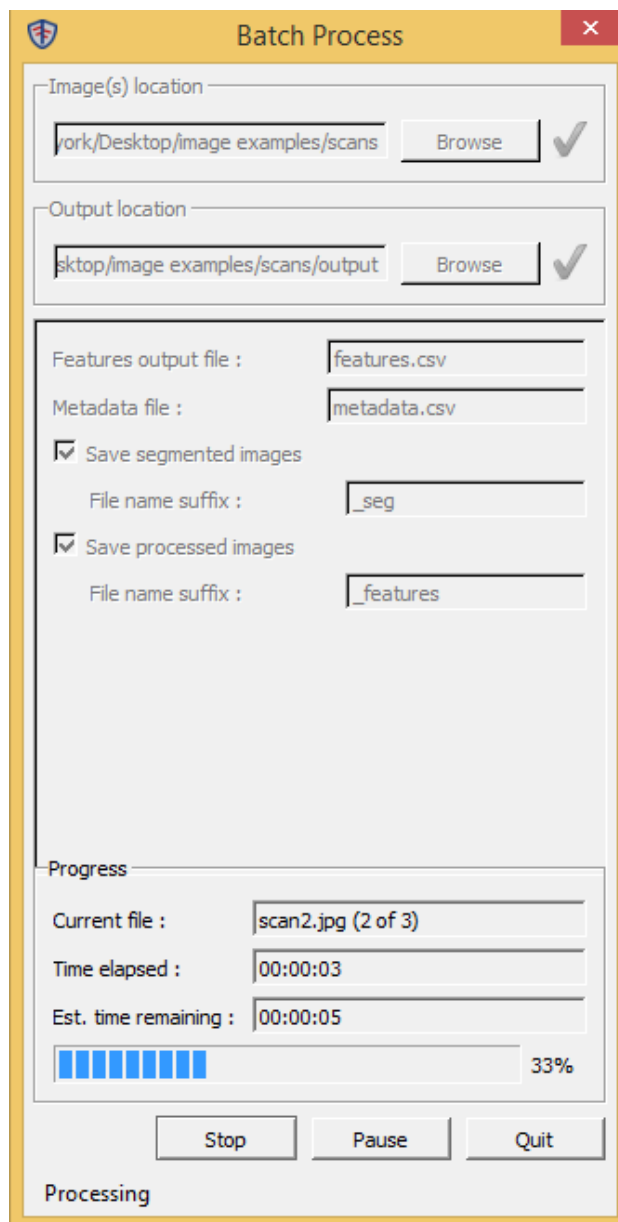
Save features data and batch analysis

Interactive data analysis is possible in RhizoVision Explorer by using the load previous and next arrows to move through a directory of images and manually clicking on Run analysis for each image. The trait features will be stored in the Features pane, and can be saved using **Menu > Save as > Features**.

However, the real strength of this software is using the interactive mode for optimize settings and then using **Batch Analysis** as described next.

Batch Analysis is found by using the menu **File > Batch Analysis**

Settings are inherited from the pre-processing, feature extraction, and output display



The screenshot shows the 'Batch Process' dialog box. It has a yellow title bar with a shield icon and a close button. The dialog is divided into several sections:

- Image(s) location:** A text field containing 'ork/Desktop/image examples/scans' and a 'Browse' button with a checkmark.
- Output location:** A text field containing 'sktop/image examples/scans/output' and a 'Browse' button with a checkmark.
- Features output file:** A text field containing 'features.csv'.
- Metadata file:** A text field containing 'metadata.csv'.
- Save segmented images:** A checked checkbox with a text field for 'File name suffix' containing '_seg'.
- Save processed images:** A checked checkbox with a text field for 'File name suffix' containing '_features'.
- Progress:** A section containing:
 - Current file:** A text field containing 'scan2.jpg (2 of 3)'.
 - Time elapsed:** A text field containing '00:00:03'.
 - Est. time remaining:** A text field containing '00:00:05'.
 - A progress bar showing 33% completion.
- Buttons:** 'Stop', 'Pause', and 'Quit' buttons.
- Status:** 'Processing' text at the bottom left.

Choose image folder location – defaults to last loaded image directory

Choose output location – Browse defaults to Image location – recommended to create an Output folder to use

Update file names for the features CSV file containing the data and the metadata CSV file, which contains all the settings used for the run. The Metadata file is the same as a Settings file, so can be loaded later, or shared for reproducibility.

Optionally choose to output either segmented or processed images, and choose a suffix to append to the file name. Images are saved as PNG.

Press Start!

Current file shows the file being processed, *Time elapsed* shows the actual time elapsed since start, and *Est. time remaining* uses the average processing time and number of remaining files to estimate. The progress bar shows the percentage of finished images.

Stop or *Pause* can be used to restart analysis. *Quit* exits without finishing.

The log shows *Processing* then *Process complete* once progress reaches 100%.

Hot tip! You can view the Output directory to see the saved images being populated. However, do not open the features CSV file until batch processing is complete because data will not be stored correctly.

Display output settings



One unique strength of this software is giving users more ability to choose output images that display features on top of the segmented images. Output display allows choosing the options and a few settings. **Show convex hull** and **Show holes** are only available for **Whole root** analysis mode.

Show distance map will show a green mist within the segmented root with the intensity defined by the distance of a pixel from the edge, more intensive in the center of the root. This distance map is what was used to identify the ridges in order to create the root skeleton.

Show medial axis shows the root skeleton.

Hot tip! Set the **Medial axis width** to higher values to artificially thicken the skeleton, or even beyond the original root size to make roots more visible for presentations or posters, as shown above.

Color medial axis based on diameter ranges uses color-blind friendly colors to represent skeleton pixels falling within the user-set diameter ranges.

Hot tip! Turn off **Color medial axis based on diameter ranges** to view the root topology points as described for root pruning above. Changing **Medial axis width** to 1 will give the user a very good idea of the skeleton topology.

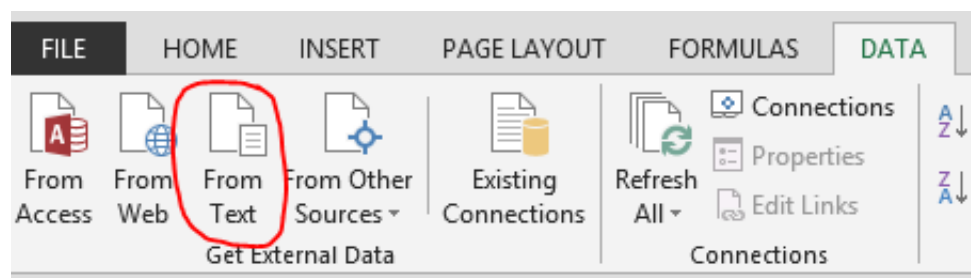
Show root contour displays the boundary of the roots or perimeter in red color, and the width can be chosen.

Using features CSV file with external software like Excel and R

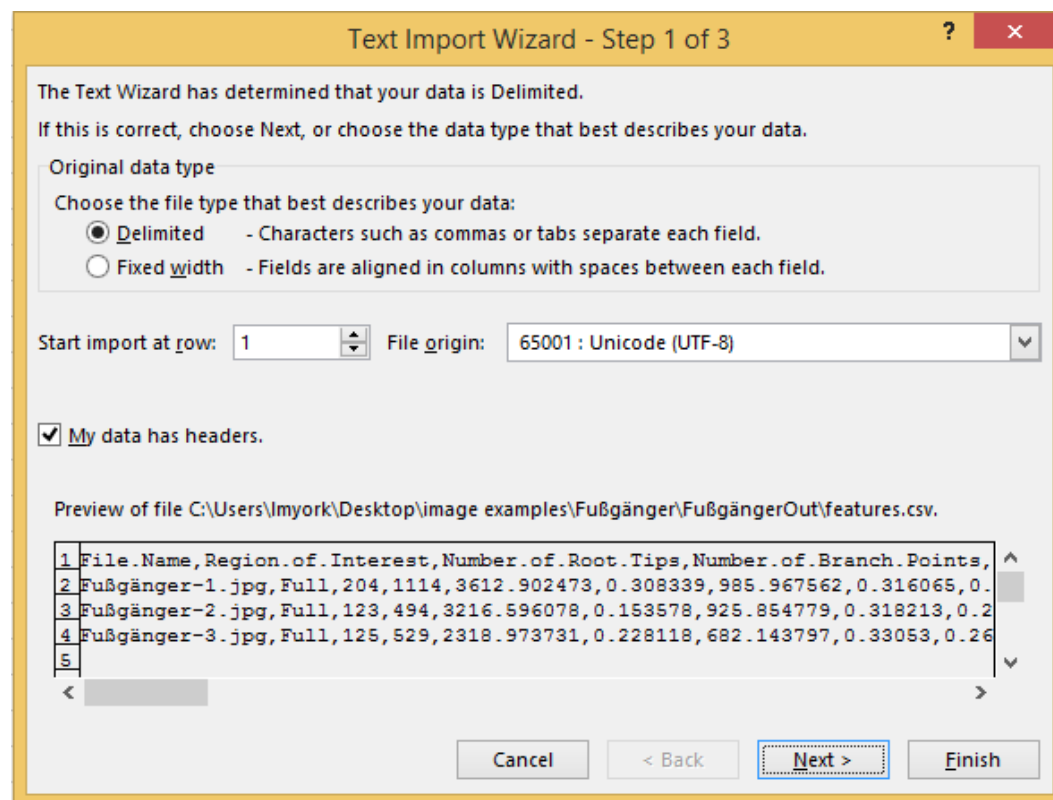
The features CSV files are standard comma separated values text files (*.csv), and characters are encoded in UTF-8 to support multiple languages. When using Latin alpha-numeric characters, generally, the files can be used without special consideration by programs like Excel and R. However, if your original image file names had non-Latin characters, please refer to the following instructions to display these correctly.

Excel

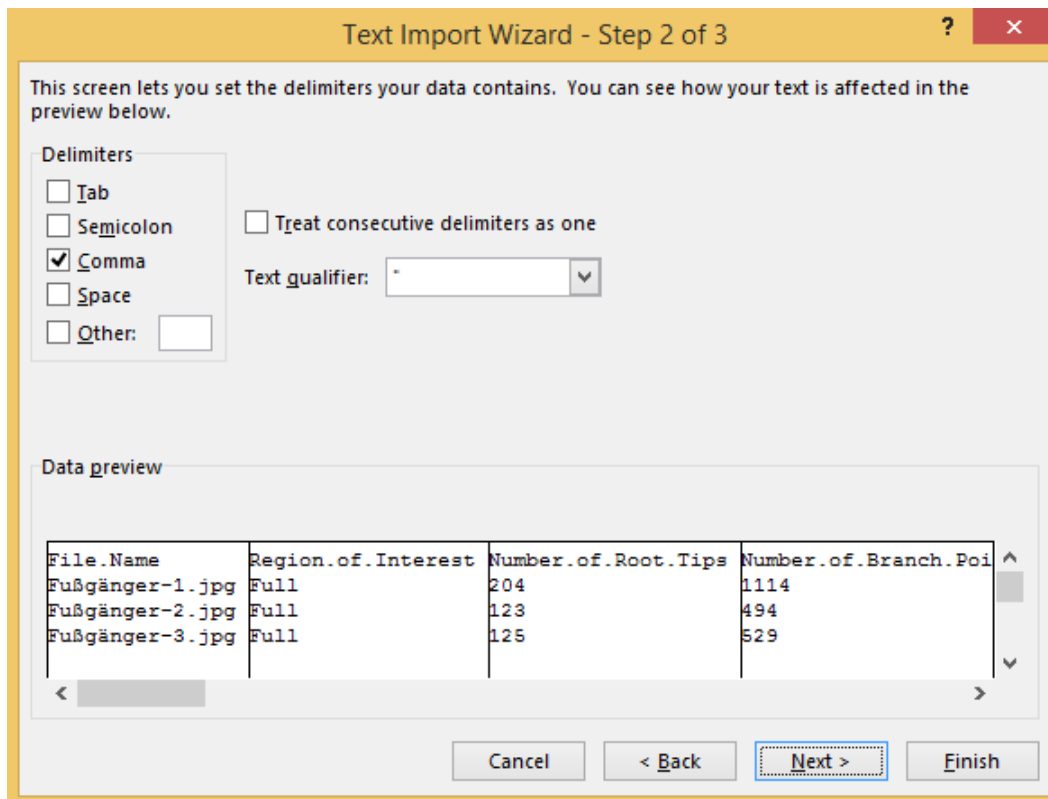
If you open the file in Excel, you may notice non-Latin characters are not rendered correctly, because Excel may not recognize the correct encoding. You can use the text import feature by going to the Data tab, in the Get External Data area, and choose From Text to start the import wizard



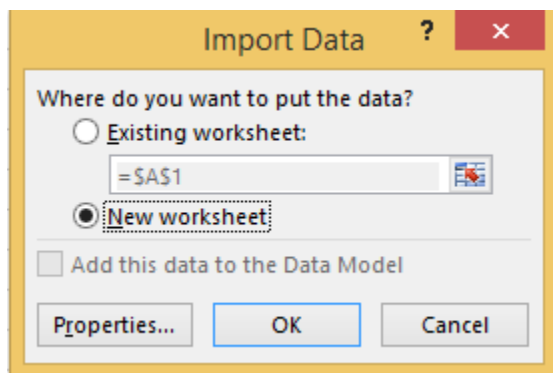
Choose delimited, import row 1, and file origin as Unicode (UTF-8), and with headers, the click next



Choose comma as the delimiter and click Finish



Choose New worksheet, then OK to display the file correctly



R and RStudio

The features CSV file exported by RhizoVision Explorer was designed for direct use in R or Python.

The new versions of R and RStudio support importing files with UTF-8 encoding in multiple languages. Older versions do not. We recommend using at least R version 4 and RStudio version 1.3

If only Latin alpha-numeric characters were used, generally the default `read.csv` function will work.

If additional characters were used, simply specify UTF-8 encoding as in the following example:

```
features_german <- read.csv("C:/Fußgänger/FußgängerOut/features.csv", encoding="UTF-8")
```

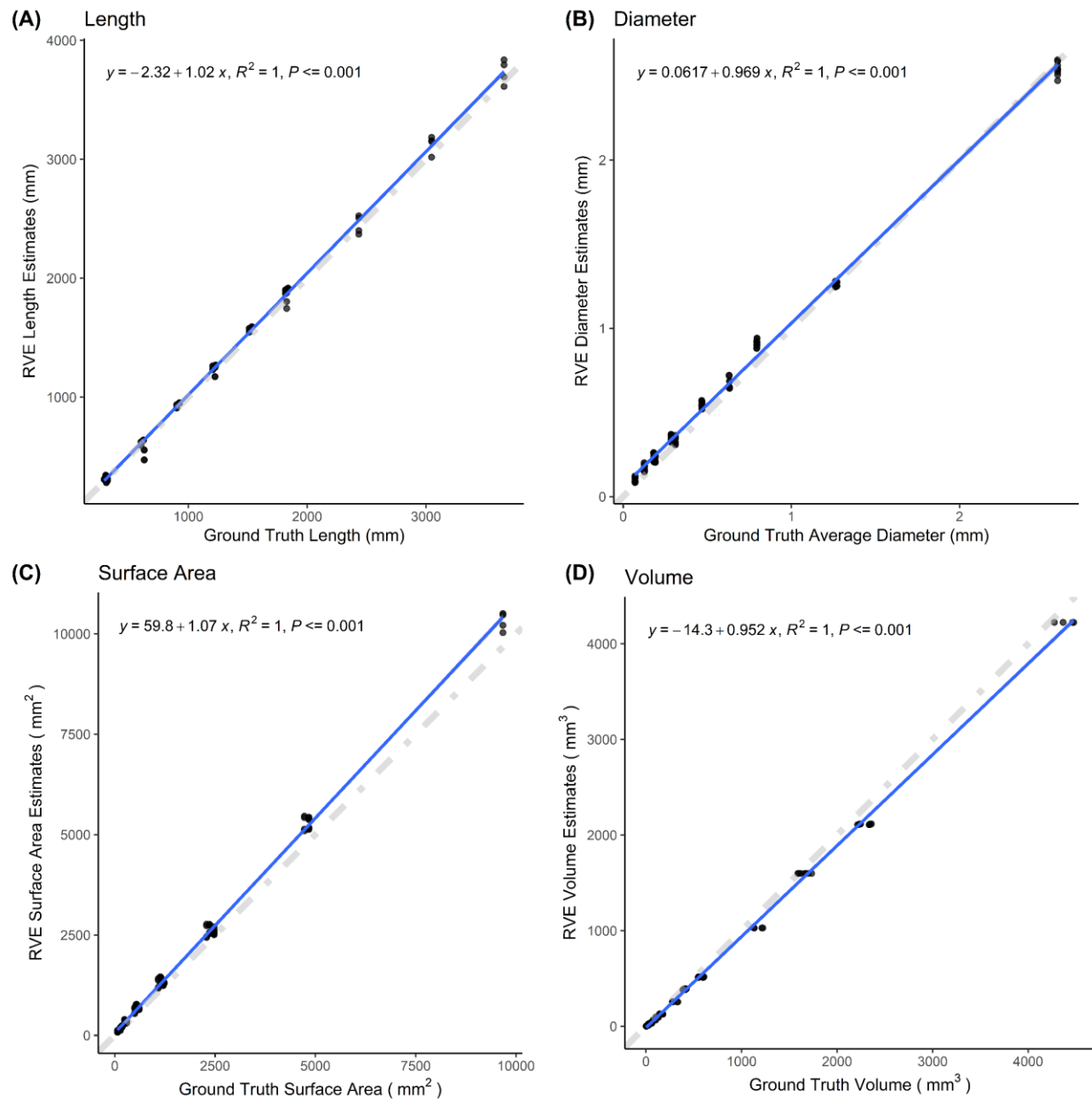
List of extracted features and their definitions

The list of features extracted from each root crown image by RhizoVision Explorer. Pixels are converted to physical units using the user-supplied conversion factors. Length, surface area, and volume are additionally computed within user-supplied diameter ranges. Features exclusive to **Whole root** mode are highlighted in red and **Broken roots** mode in blue.

Features extracted	Description
Median and maximum number of roots	The number of roots is counted by performing horizontal line scans from left to right in each row through the segmented image. In each of the line scan, we check if there is a pixel value transition from the previous pixel value to the current pixel value on its right side. If the current pixel value changes from 0 to 1, we note that a root is present. The number of roots is recorded from each row of the segmented image, and the median and the maximum number of roots is determined from these values.
Number of Root Tips	Computed by counting the total number of tip pixels in the skeletonized image with topology
Total root length	Computed by counting the total number of pixels in the skeletonized image. Diagonal pixels are counted as the $\sqrt{2}$
Perimeter	Perimeter is the count of the total number of pixels in the perimeter image.
Average, median, and maximum diameter	For each pixel on the skeletonized image, the distance to the nearest non-root pixel is computed and using this distance as radius a circle is fitted. The diameter of the circle at each pixel is noted as the diameter at that pixel. We get the list of diameters from all the medial axis pixels and determine the average, median, and maximum diameter.
Volume and surface area	Using the radii determined earlier, the sum of all cross-sectional areas across all the medial axis pixels are noted as volume and the sum of the perimeter across all the medial axis pixels are noted as surface area.
Network area	The network area is the total number of pixels in the segmented image.
Branch points	Computed by counting the total number of branch pixels in the skeletonized image with topology
Branching frequency	The number of branch points divided by the total root length
Depth, maximum width and width-to-depth ratio	The trait values for both depth and maximum width of the root in the segmented image. The ratio of maximum width to depth of the image is noted as width-to-depth ratio.

Convex area and solidity	The convex hull of a geometric shape is a minimal sized convex polygon that can contain the shape. The ratio of network area and the convex area is noted as the solidity.
Lower root area	The lower root area is the area of the segmented image pixels that are located below the location of the medial axis pixel that has the maximum radius.
Holes and Average hole size	Holes are the disconnected background components and indicative of root branching and complexity. They can be counted by inverting the segmented image. The average hole size (area) is also calculated.
Average Root Orientation	For every medial axis pixel, the orientation at the pixel is computed by determining the mean orientation of medial axis pixels in a 40x40 pixel locality. The average of all these orientations is noted as average root orientation.
Shallow Angle Freq., Medium Angle Freq., Steep Angle Freq.	Given the skeletal image, for every pixel in the medial axis, we get the locations of the medial axis pixels in a 40x40 pixel locality and determine the orientation of these pixels in the locality. This orientation is noted for every medial axis pixel. Given these orientations, we calculate the frequency in bins less than 30, less than 60, and less than 90 degrees.
Computation time	The time taken to extract traits for every plant root image.

Feature validation using a novel ground truth copper wire dataset

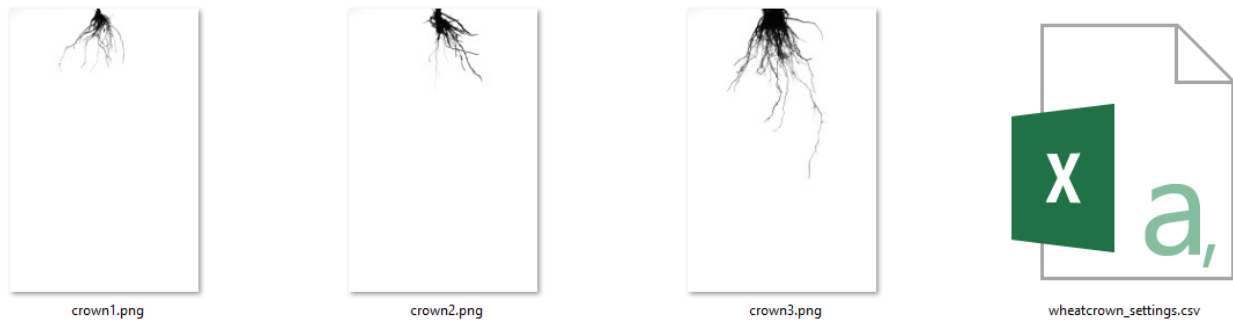


RhizoVision Explorer (RVE) has been extensively validated, with one example using a novel physical, ground truth dataset that resembles roots. Copper wires of several diameters relevant to root studies were cut to specific lengths. Length was measured using string and diameter with a digital caliper. Volume and surface area were calculated for the physical data assuming the wires are cylinders. Regression analysis was performed using the `lm()` function in R for the RVE estimates versus the ground truth data for A) length, B) average diameter, C) surface area, and D) volume. Estimates from RhizoVision Explorer match extremely closely. Points are the raw data, blue line is the regression line, grey dashed line is the 1:1 line, and the regression equations and coefficient of determination are given. Further validations and explanations are presented in a manuscript currently available as a [preprint](#).

Included example images and settings files

The RhizoVision Explorer directory structure includes a folder named *imageexamples* with images that serve as inspiration for the quality of high contrast images needed. The *crowns* folder includes excavated wheat root crown images for the **Whole root** mode, and the *scans* folder includes washed out wheat roots for the **Broken roots** mode. Settings files (CSV format) are included for each, and can be loaded as described above for correct output. Batch analysis can also be tested using these folders since there are multiple images of each type.

The root crown images were acquired using the [RhizoVision Crown platform](#) that uses a backlight.



The root scans were acquired using an Epson Perfection 12000 XL scanner with a transparency unit.



While high-contrast color images are technically supported, thresholding in free software like GIMP or ImageJ is highly recommended. For more complex images, new machine learning methods like [RootPainter](#) may be helpful for prior segmentation, with the output images passed to RhizoVision Explorer. Still, acquiring clear, high contrast images is arguably the most important step for root phenotyping, so considerable effort should be made to get the right images before imaging the samples from an entire experiment. Conveniently, imaging methods can be tested by passing the images through RhizoVision Explorer at the start of an experiment, and refining the methods to optimize output.