EZ-Rhizo

Software for the detection and measurement of plant root system architecture
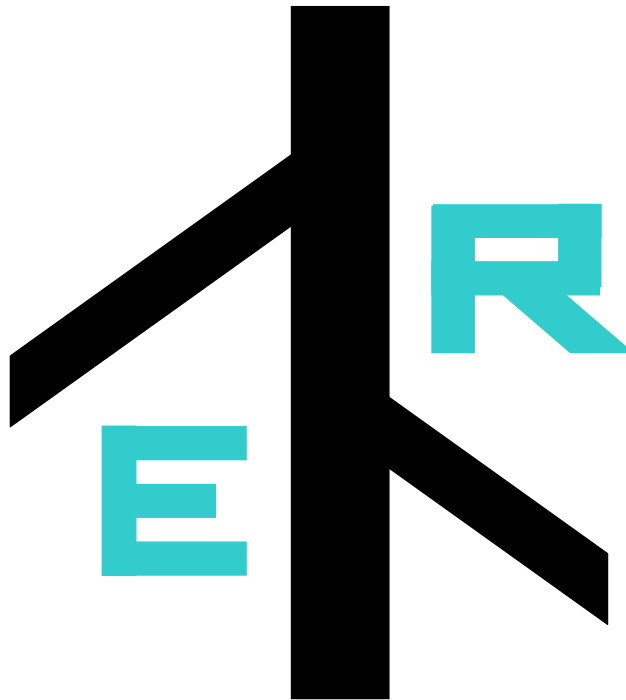
User manual

Program installation:

The entire installation procedure only takes a few minutes.

Double click EZ-Rhizo setup.exe, and follow the wizard for installation.

Choose full installation (including Help file and sample picture).

After installation you are given the option to launch the program (checked), and to install the MySQL interface EasyPHP (checked). If EasyPHP is already installed on your computer uncheck the latter.

Otherwise follow the wizard for installation of EasyPHP.

Minimize the EasyPHP window but keep the program open if you want to use EZ-Rhizo now.

EasyPHP is only required for the data management part of the program.

If you have ticked the launch the program option EZ-Rhizo will now launch.

Otherwise open the program through your Windows Program manager.

EZ-Rhizo: Semi-automated RSA measurement

Toolbar

The Easy Rhizo toolbar allows you to supervise individual operations for image preparation, root detection, and data storage and mining. The entire operation from opening the image to saving the data in a text file takes only a few minutes. The data is subsequently integrated in a SQL database and selected data are extracted from the database into a csv file. Again this is accomplished within a few minutes.

The figure below shows the EZ-Rhizo toolbar and explains essential icons.
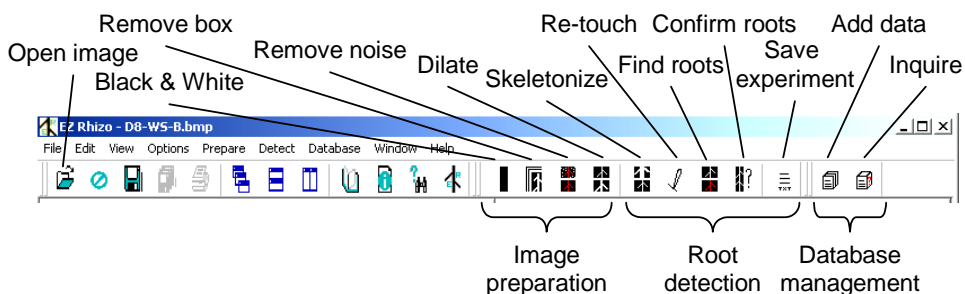
Note: EZ-Rhizo operations are one-way. Once you have decided on an action you cannot go back and change it without opening the image again. The current operation can be recognized by a pressed icon. For successful RSA measurement you have to follow the order of the operations (use icons from left to right). The only exception is optional re-skeletonization after re-touching (see below).

Open image

Click the 'Open image' icon, or choose 'Open' in the File menu..
Find the file containing your image using the browser, and open it.
The image appears in the *EZ-Rhizo* window.

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The sample picture D8-WS-B.bmp is in the following location:
C:\Program Files\EZ-Rhizo Setup\Examples
```

Note: The image has to be in .bmp format

Make B/W

Click the 'black & white' icon or choose 'Make B/W' in the Prepare menu.
The image is converted into a monochrome image using a default threshold (90).
You can choose a different threshold using the slider or typing a number between 40 and 120 into the box.
Press OK.

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Sample picture: Set the threshold to 84.
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Note: Finding the right threshold helps to avoid gaps being created during skeletonization.

Remove box

Click the 'Remove box' icon or choose 'Remove box' in the Prepare menu.
The program automatically crops the image to remove the edges of the plate.

Remove noise

Click the 'Remove noise' icon or choose 'Remove noise' in the Prepare menu. The pop-up window offers you a selection of erosion algorithms. The result of the applied algorithm is immediately visible in the image window.
When you have decided on the best algorithm press OK.

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Sample picture: Choose Gaussian-2.
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Note: The best method removes background signal from the plate while leaving root appearance unchanged.

Dilate

Click the 'Dilate' icon or choose 'Dilate' in the Prepare menu. A pixel dilation function is automatically applied to fill gaps within the roots created by noise removal.

Note: After carrying out the last four operations you can re-apply the same settings when analysing the next image by choosing 'Re-use previous' in the Prepare menu.

Skeletonize

Click the 'Skeletonize' icon or choose 'Skeletonize' in the Detect menu. Pixels are automatically removed from the edges of objects until these are reduced to single pixel-wide skeletons.

Note: Skeletonization is essential for automatic root path tracking and branch detection.

Re-touch

This function allows you to manually edit the image. Click the 'Re-touch' icon or choose 'Re-touch' in the Detect menu. An amplified image appears. Move the cursor into the window; it guides a red rectangle. Move the rectangle over the relevant areas and draw with the brush by pressing the left (white) or right (black) mouse button. The size of the brush can be adjusted with the slider in the upper left corner. The image can be further amplified by pressing the provided amplification buttons. The amplified window can be explored by using the positioning buttons in the upper right corner of the window.
Apply left mouse click to rejoin disconnected parts within the roots.
Apply right mouse click to disconnect the roots from the shoots.
Repeat these procedures until you are satisfied. Finish by pressing OK.

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Sample picture: No gaps have to be re-connected if the previous
settings have been used, but you do have to disconnect the shoots
from the roots using the right mouse button.
```

Note: Re-touching can take some time if you haven't used the program before, but you will soon get used to the operations. Experience with choosing the best settings during previous steps will also make this task easier as you will have to reconnect no or only very few areas. Don't forget to disconnect roots from shoots!

Important! If you have used the white brush during re-touch you have to go back to 'Skeletonize' and skeletonise the objects again. Then go directly to 'Find roots'.

Find roots

Click the 'Find roots' icon or choose 'Find roots' in the Detect menu.
The program automatically detects putative root objects and presents them in different colours in the image window.

Note: You can change the colours by choosing 'Color Item' in the Detect menu.

Confirm roots

Click the 'Confirm roots' icon or choose 'Confirm roots' in the Detect menu.
A dialogue box presents each detected object to be confirmed or rejected by pressing 'Yes' or 'No'. After the last object has been validated the dialogue box disappears. The discarded objects have been removed from the image. You should now be left only with the root objects for subsequent measurement of RSA parameters.

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Sample picture: The sequence for root confirmation is
No, Yes, Yes, No, No, No, No, No, No, No, No, Yes, Yes, No.
```

Save experiment

Click the 'Save experiment' icon or choose 'Save experiment' in the Detect menu. If this is the first time you save data you will receive a message 'The results folder doesn't exist' Click OK. The 'Results folder path' window will open suggesting a path for allocation of the folder. You can accept this path by pressing OK or choose a different path by pressing 'Change' The latter action opens a Browser to select your preferred path. Once you have chosen a path it will appear in the 'Results folder path' window, and you accept it by pressing OK. The program now allows you to enter relevant image metadata in an 'Image information' window. Fill in the fields and press OK. You will be informed that your data has been successfully saved by a message 'Experiment saved'. Press OK.

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Sample picture: Enter 200 in the resolution field.
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Note: The last two fields in the 'Image information' window only accept numbers.
Filling the last field in is obligatory. The unit for the resolution is dpi.

To finish close the image box.
Before you open another image close the program and re-launch it.

EZ-Rhizo: Database management

For this part of the program you must have Easy PHP open. The program allows you to incorporate your measured RSA parameters and metadata as well as a number of derived RSA parameters in a MySQL database ('Add data'). In a second (independent) operation you can query the database for all or a subset of these parameters and save the retrieved data in a comma-separated value (csv) file ('Inquire').

Add data

Click the 'Add data' icon or choose 'Add data' in the Database menu.
A MySQL administrator permission window will open asking you for a password.
You can enter a password if you want to secure the database or you can leave this field blank (default). Press OK.
The 'Image Analysis Result folder' window will open. Use the browser to find your Results folder (path as specified before under 'Save Experiment'). Click on the folder.
A 'Data correctly inserted' message appears. Press OK.

Inquire

Click the 'Inquire' icon or choose 'Inquire' from the Database menu.
The 'Save as' window will open for you to specify path and name of the file where you want to save the retrieved data. Find the path, enter the name and press Save.
The 'Query' window will open allowing you to select the data you want to retrieve.
The options are organised in four layers: General data, Main root, Secondary root and More parameters (see Table 1 for definitions).
Check all boxes in all four layers to retrieve the entire dataset.
Check only some boxes to retrieve only some of the data and/or enter value limitations as provided in the middle field (=, >, <, between, not between).
After marking your choices in all four layers, press OK.
A 'Request done' message appears. Press OK.