USER MANUAL

MyROOT
Root Length Measurement Software

Software developed by:
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1. Installation instructions

Unzip the MyROOT.zip file. As a result, you will obtain a file called `MyROOT_Installer_mcr.exe` and a folder called `HypocotylDetection`.

Execute `MyROOT_Installer_mcr.exe`:

![Installation Program Welcome Screen](image)

The installation program will start. Follow the installation process by clicking on **Next** button on the MyROOT installation program welcome screen (Fig. 1).

![Installation Process](image)

**Figure 1.** MyROOT installation program welcome screen
The next window asks for the folder where MyROOT will be installed. The default folder is \Program Files\MyROOT. In this window, select the **Add shortcut to the desktop** option (Fig. 2).

![MyROOT installation program options](image1)

**Figure 2.** MyROOT installation program options

MyROOT requires the installation of MATLAB Runtime (a freely-available standalone set of libraries that enables the execution of compiled MATLAB applications, like MyROOT, on computers that do not have MATLAB installed). MyROOT installer includes the MATLAB Runtime installer. The user just needs to select the folder in which MATLAB Runtime will be installed (Fig. 3). By default, this folder is \Program Files\MATLAB\MATLAB Runtime. Finally, the user must accept the MATLAB Runtime license (Fig. 4).

![Installation of MATLAB Runtime start window](image2)

**Figure 3.** Installation of MATLAB Runtime start window
Figure 4. Installation of MATLAB Runtime finish window

Once all directories are selected and licenses are accepted, click on the **Install** button to start the installation process (Fig. 5).

Figure 5. MyROOT installation ready to start window
2. MyROOT user manual

2.1. MyROOT starting and graphical interface

Prior to starting MyROOT, copy the HypocotylDetection folder (that is included with the software installer) to your computer desktop (as shown in the scheme below).

Click the MyROOT desktop icon to start the software. MyROOT main screen will show up. The displayed graphical interface is divided in seven different blocks (Fig. 6). Each of these blocks contains several parameters that control MyROOT functioning. Default values for these parameters are provided when the program starts. However, these parameters can be freely tuned by the user for adapting the software performance to specific image characteristics.

Figure 6. MyROOT graphical interface
The seven areas of MyROOT main screen and their functionalities are:

1) Load Image: It loads the image to process and sets the scale factor for resizing the image. In the case of high resolution images, a downscaling (i.e. scale factor <1) is recommended to speed up the processing.

2) Root Extraction: It detects and extracts the pixel-mm equivalence from the measuring tape, detects the area where roots are located, and performs root extraction.

3) Hypocotyl Detection: It loads models for detecting the hypocotyls, performs hypocotyl detection, allows the manual addition of hypocotyls, and performs root length refinement thanks to hypocotyl detection.

4) Visualization canvas: this is where intermediate processing results are presented to the user.

5) Save Results: it saves the results of the root length measurement, including images, measurement results and MATLAB variables.

6) Root Removal: it allows removing roots (using their assigned ID) from the measurement results.

7) Process Folder: it allows performing batch processing of several images contained in a folder.

2.2. Root length measurement

2.2.1. Load the image

Click the **LOAD IMG** button. A pop-up window will appear for selecting the image to process (Fig. 7). Once it is selected, it will be displayed in the visualization canvas (Fig. 8). By default, images will be processed at 60% of its original size (the Scale parameter is equal to 0.6). The value of this parameter could be modified by changing the Scale parameter, which must range between 0 and 1. It is worth to mention than lower values will process the image quickly but with a lower resolution (which may affect the accuracy of the measurements). On the other hand, higher values will increase processing time while ensuring accurate results.
2.2.2. Ruler identification

Click on the **Ruler ID** button. The measuring tape detection and analysis will start. A progress bar will indicate the end of the process. Once it is completed, the results of the pixels-mm equivalence will be shown in the visualization canvas (Fig. 9).
In the case that the measuring tape detection is not satisfactory, the user must change the *Ruler threshold* parameter. Misdetections are caused by the illumination conditions of the input image. If it is too clear (i.e. saturated), the user should set a higher value in this parameter (whose maximum value is 1). If the image is dark, the value of this parameter must be reduced (its minimum value is 0). To re-run the ruler identification process with a new parameter value, the user just needs to type the new value in the *Ruler threshold* box and press the Enter key.

If the image is blurred in the measuring tape area, it is recommended to take a new photo, or trying with a higher image scale to reduce the blurring effect of scaling.

![Figure 9. Measuring tape and pixel-mm equivalence extraction and display](image)

### 2.2.3. Manual introduction of pixels-to-mm equivalence

If the image the user wants to process has no measuring tape on it, MyROOT offers the chance of introducing the image scale (equivalence between pixels and millimeters) manually.

To that end, the user must only type the number of pixels equivalent to 1 cm (10 mm) in the box marked in green in Figure 9. Pressing Enter will make MyROOT use that scale for subsequent root length computations, skipping the measuring tape detection process entirely.
2.2.4. Root extraction

MyROOT can automatically detect the area in the image where rows of seedlings are located. To perform automatic root area detection, click the **Automatic Region** button. As a result, MyROOT will display the image with rectangular polygons enclosing the rows of roots (see Fig. 10). The vertices of the polygons are draggable, so the user can modify them if necessary.

![Figure 10. Automatic root area detection](image)

Alternatively, the user can draw by hand the polygons enclosing the rows of roots. To that end, click the **Manual Region** button. A popup window will appear asking the user to indicate how many root rows will be drawn. Then, MyROOT will prompt the user to select the image area containing each of the root rows to be measured. By clicking on the OK button of the popup window, the user will start the area definition process by clicking on the image to create the vertices of a polygon that encloses the roots to be measured (Fig. 11). The polygon is closed by clicking on its first vertex again. If the user is not satisfied with the shape of the polygon, each vertex can be moved by clicking and dragging. To end the area definition process, the user must double-click on any of the polygon vertices.
After the (either automatic or manual) root area selection, the root extraction process starts by clicking the **Root Mask** button. A popup progress bar will appear showing the progress of the internal processes. When the progress bar disappears, the root extraction process has been completed. Immediately, the root extraction results appear in the visualization canvas (Fig. 11). At this point, it is expected that roots appear as **white continuous lines** clearly differentiated against a black background, and **separated among themselves**. This black-and-white image is called the root mask. If the roots do not appear separated in the root mask, then the user can increase the **Root Threshold** parameter (whose default value is 0.32) up to a maximum of 1. If the roots do not appear as continuous lines in the root mask, the user should decrease the same parameter down to a minimum value of 0. To generate a new root mask with a new parameter value, the user must type the new parameter value in the **Root Threshold** box and press the Enter key.
2.2.5. Root detection and measurement

Click on the **Root Detection** button to generate the mask from which the roots will be detected and measured. A popup progress bar indicates the progress of the internal processes. Once the progress bar disappears, the detected roots and their measured length are shown on the visualization canvas (Fig. 13). In this process, the roots with a length shorter than a percentage of the longest one are eliminated. This percentage can be modified by the user using the **Root Length Threshold** parameter (by default this percentage is 30%, 0.3 in the corresponding edit box).
2.2.6. Hypocotyl detection

Click on the **Hypocotyl Detection** button to start the hypocotyl detection process. A popup progress bar will appear showing internal processes progress. Once this popup window disappears, the hypocotyl detection results are displayed in the visualization canvas. In particular, detected hypocotyls are shown as black circles, and the hypocotyl regression curve is shown in blue (Fig. 14). Each root will be “cut” at the point it intersects with this curve.

Non satisfactory hypocotyl detection results are mainly due to illumination conditions or the shape of the hypocotyls. MyROOT includes different hypocotyl models and by clicking on the corresponding checkbox the user can select other models. By checking the **only color** checkbox, MyROOT uses a detection model that does not take into account the shape of the hypocotyls. Alternatively, by checking the **Norm Color** checkbox, a hypocotyl model with an extreme color normalization of color will be employed. Another configurable parameter is the threshold of the detector (edit box on the right of the **Norm Color** checkbox), which affects the number of detected hypocotyls (its default value is 1). The lower this threshold, the more hypocotyl detections, thus making the detector more sensitive to hypocotyls but also to noise.
MyROOT allows the manual introduction of hypocotyls by clicking the add button. This action will allow the user to click on the image, adding a hypocotyl with each click in the exact position of the click (Fig. 15). To end the process of adding hypocotyls, the user must press the Enter key. If, by mistake, the user clicks in a wrong position, the added hypocotyl can be removed by pressing the Del key. As result of this process, the hypocotyl curve is modified, forcing it to pass through the added hypocotyls.
2.2.7. Root length refinement

Click on the **Root Refinement** button to recalculate the root length measurements taking into account the hypocotyl curve previously calculated. As mentioned earlier, this consists in cutting roots at the intersection with the defined hypocotyl curve (Fig. 16).

![Refined root length measurement display](image)

**Figure 16.** Refined root length measurement display
2.2.8. Root removal
MyROOT allows the elimination of a measured root. To perform root removal, the user must click on the **Visualize** button to visualize the current roots and their numeric identifier (ID); then, by typing the ID of the root to remove on the edit box and by clicking the **Remove** button, the software will eliminate the selected root (Fig. 17). It is important to note that once a root is removed, it cannot be recovered unless the whole measurement process is done again.

![Figure 17. Root removal](image)

2.2.9. Saving the results
When the root length measurement is completed, the user can save the results by clicking on the **SAVE** button.

MyROOT allows to choose which items to save: a) an Excel spreadsheet with the measurement of each root, along with the mean and standard deviation of the measurements, b) a text file with the same information, c) the images of the measured roots, d) the Matlab variables, and e) the root architectural data following the RootSystemML (RSML) standard file format (Fig. 18).

Additionally, the user can type a free-text label in the **Root Label** edit box, which can be useful for particular identifications of the measured set of roots. The data to be saved is stored in a folder that is created automatically on the desktop called **result_root_images**.
2.2.10. Batch processing

MyROOT offers the user the chance to process a set of images contained in the same folder (batch processing). To perform batch processing, the user must inform MyROOT of the parameters values that must be used on the entire batch of images. To that end, the user must first process one of the images in the folder, to define: 1) the equivalence between pixels and millimetres, and 2) the root threshold parameter value (steps described in sections 2.2.1 to 2.2.4). In each image of the batch, automatic root region detection will be performed.

Once the user is satisfied with the parameter values, he/she must type, in the Folder Path text box, the path of the folder containing the set of images. Then, press the Process button (Fig. 19).
A dialog box will appear, asking the user if root length refinement based on hypocotyl detection must be performed on each image (Fig. 20). Note that if “Yes” is selected, root length will be more accurate, but the process will be slow due to the execution of hypocotyl detection on each single image. In contrast, if “No” is selected, the process will be faster but less accurate measurements will be obtained.

From that point, MyROOT will process all the image files in the folder, generating the selected type of output results (Excel spreadsheet, text file, RSML file, etc.) for each one of the image files. These results will be stored in a folder with the same name of the batch folder, but terminated with the “_results” suffix.

**NOTE 1:** for batch processing to perform correctly, the image files contained in the folder must be JPG files, and their filenames must not contain spaces.
NOTE 2: as the same parameter values are applied on all the images in the folder, the user must ensure that all the images are captured under the same conditions (illumination, distance). Otherwise, MyROOT will probably fail at detecting roots, causing the program to terminate.

3. Contact

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For inquiries of biological nature, please contact Ana I. Caño-Delgado (ana.cano@cragenomica.es).