

RootTrace V2

Technical Version 0.9

Document History:

0.87 Released with RootTrace v1

0.9 Updated for RootTrace v2

Available from <https://sourceforge.net/projects/roottrace>

This is a working document, and it will evolve: PLEASE CHECK SOURCEFORGE FOR THE LATEST VERSION OF THIS DOCUMENT.

See end of document for licence.

Requirements

RootTrace has been built for use in Windows (XP, Vista, 7). Sorry, its Windows only! Tracing can take a while (e.g. a long sequence with many roots might take a few hours) – faster processors are better. I would think 1Gb+ of memory should be fine.



Note on use

While we believe the results to be accurate, this program is constantly being developed and improved, and it is possible it contains bugs. It is good practice to compare some results with results

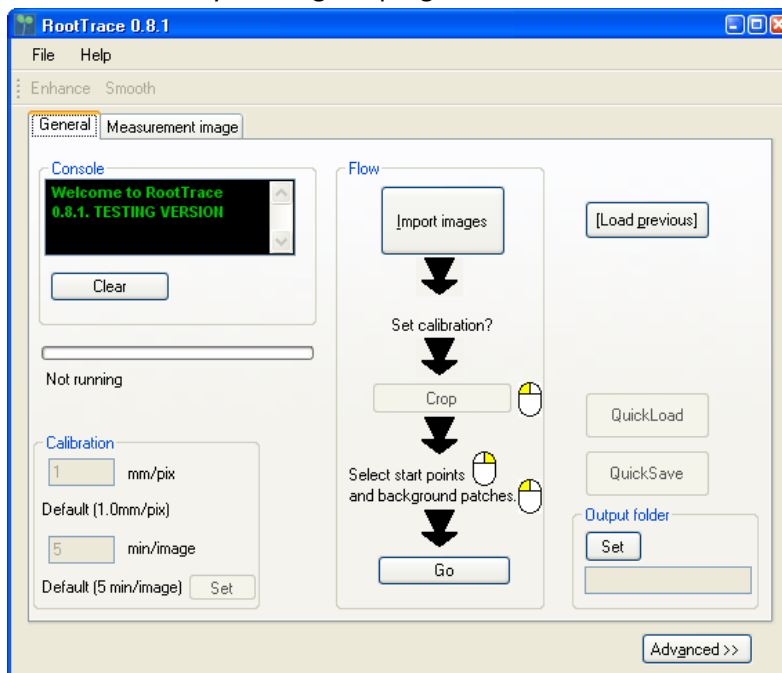
determined using other methods, to help ascertain the program is working as you expect. If you find or suspect a problem, please either email me, or report a bug on the SourceForge website. (https://sourceforge.net/tracker/?group_id=243086). Feature requests can be posted here too.

Please see the **Version 2 Notes** section below for some information about the Version 2 download of this software.

Getting started

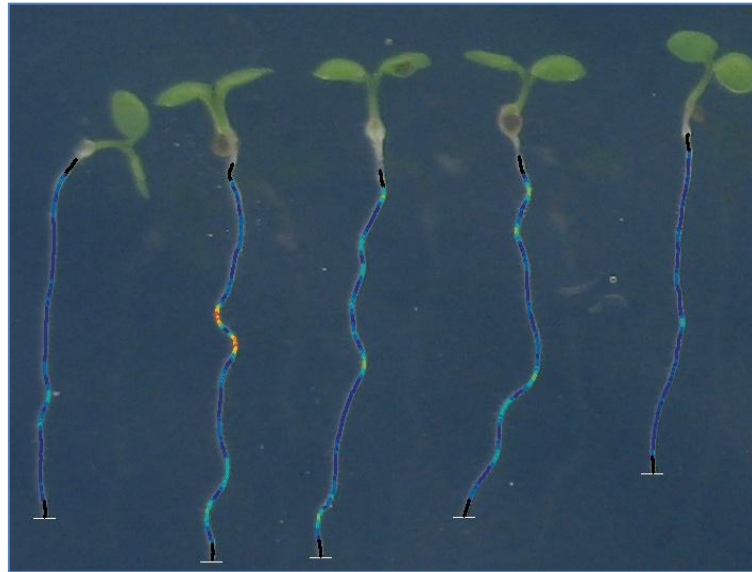
Some example images have been supplied with the software for you to play with.

1. Install the OpenCV libraries from <http://sourceforge.net/projects/opencvlibrary/files/opencv-win/1.0/> (**RootTrace was developed with Version 1.0, please install *exactly* this version**). This needs to be done with administrator privileges in order for the paths to be set up correctly. VISTA USERS: if you run into problems, please see the troubleshooting guide at the end of this document.
2. Run RootTrace's setup.exe and install the software
3. Load RootTrace by locating the program in the Start menu



4. Click Import Images
 - a. Navigate to the Examples folder
 - b. Select the 3 input images ("Capture_... .jpg" - Use shift+click and ctrl+click to help you)
 - c. Click open. Some windows should open up.
5. Set the output directory. This is where the output files are stored. It will default to a 'output' subfolder if one exists, or else the folder the images are in will be used. A new folder can be created in which to store the output.
6. Load the start points and background file

- a. Click QuickLoad. Individual background and start point files can be saved/loaded on the Advanced dialog.
7. Click Go to begin tracing



8. When all three images have been traced, click on the title bar of the image to make it active, press 'Esc' to exit the images, and close the program using the cross in the top right. - **NOTE Always exit in this order.** Other methods of exiting may be unstable at the moment... To exit the software while it is still processing the images, simply close the main RootTrace window .
9. Files saved:
 - a. Generally, output files are saved in the 'output' subfolder if one exists (you can create and select one in the Set dialog under 'Output Folder'). Otherwise, they are saved in the same folder as the input files, or any other folder as specified.
 - b. Output files consist of:
 - i. **The trace images.**
These have the original filenames preceded by "_". They show the trace made by roottrace at each timepoint. Red = large curvature angle dark blue = low curvature angle (the colours are the same as for the curvature plot). They also show the largest detected curve above a threshold, if one is found (yellow circle). The black areas on the ends of the trace indicate border areas where curvature cannot be determined (because one of the calculation points extends beyond the trace); the length of these give you an idea of the scale over which curvature is calculated (the length of lines BA and AC in Figure 3).
 - ii. **Length measurements file.** This is named after the name of the first input file, with "_data.csv" tagged on the end. For each file the length of the root is presented (in pixels, or millimetres if this was entered). The order of the measurements corresponds to the order in which the start points were clicked.
 - iii. Curvature data
 1. **Curvature plots**, one for each root (created in the order the roots were traced), curvature_rootX.bmp. If a large curve (e.g.

gravitropic response) was detected, it will be marked on this chart with grey crosshairs.

2. **Gravitational response.** This is presented as a preview histogram at the end of the tracings, and saved as gravHistogram.bmp; the raw data is saved as grav_response_histogram.csv. It shows the number of roots showing a gravitational response in each time bin, calculated from the detection of the gravitropic point and root length/time data. The onset time is determined by noting the length when the angular threshold is exceeded, then looking back through the (median filtered) time-length data to determine the first time the root was recorded as being that length.
3. Each root has an associated **curvature_rootN.csv** file. This stores, for each file, the local angle measured at each point down the root trace. A value of 999 indicates that a curvature measurement was not possible at that location (the black trace ends in the trace images). Currently, this length is in pixels.

- c. Any other files are largely for debug purposes. NOTE watch out for files being overwritten each time you run the software. It's a good idea to back them up to another folder.

10. Removing the software

- a. Uninstall from the start menu, select RootTrace->Uninstall.

Manually setting start points

Right-click on the image to set start points for traces. At present it is not possible to delete start points. The user must start the software again.

Manually selecting region(s) for background statistics

A rectangular region is selected by left clicking on the image to first define one corner of a rectangle, and dragging to the opposite. The region will be drawn in white.

Two options are possible.

1. One region. Select one background region as above. The pixels in this region will be used to build a background model and used for all roots.
2. One region per root. Define exactly one background region per root, in the same order the trace start points were defined. The pixels in each region will be used to build a background model for each corresponding start point.

Cropping

The images in the sequence can be cropped by defining a crop on the first frame. Press the Crop button and then define a rectangle as per selecting a background above. All images will be cropped this way.

QuickSave and QuickLoad

This allow you to save a set of start points and a background for a particular working directory. Just define start points and background region(s), the hit QuickSave. To load, load the images and hit QuickLoad. More save and load options are available in the Advanced pane.

Calibration

The calibration panel allows you to set the number of millimetres per pixel and the number of minutes between images. This must be done after images have been loaded, but before the tracing has started. Don't forget to click Set after you have entered the values to commit the changes.

Multiple sequences

It is best to restart the software between sequences for maximum reliability.

Discussion Group

There is a RootTrace discussion group at <http://groups.google.com/group/roottrace-users>

Sign up to share comments and to get announcements of bugs and fixes.

Version 2 Notes

The major differences between original RootTrace version 1 and version 2 are:

- A new motion model option which is not dependent on tracing ‘down’ the image beyond the first few steps. Useful for highly curving roots
- The ability to count newly emerged lateral roots
- Some image processing ability: rotating images, filtering etc.

Details can be found in Naeem et al., Bioinformatics, 2011.

Image processing

There are now some options for pre-processing images:

- Images can be rotated clockwise (CW) and counter clockwise (CCW) by 90 degrees, as well as flipped. The check boxes for these options are in the Advanced pane, and must be selected before loading images, as the transform is applied on image load. The same transform will be applied to all images in the series.
- There is now an “Enhance Image” tab. When enabled, there are various filtering options (good for removing noise in images, as well as clutter such as dust speckles). Selecting these checkboxes applies the results live on the open image, and as any further images are opened in the series, prior to tracing.

A motion model ‘blender bar’

The alternate motion tracking model is selected using the Blender Bar (in Advanced tab).

On 0 (default), the tracking follows the RootTrace v1 gravity-driven model, and so is best for typically straighter roots growing downwards. The further this bar is moved to the right, the more capable the tracking model becomes at tracing bends, but it also is more likely to follow incorrect paths (eg. Lateral roots), as the tracking criteria has been relaxed. So the blender bar is provided to allow the user to set the most useful model for their data, assuming the default position fails.

This bar must be set before tracking begins.

Lateral Root Counting

When enabled (checkbox on the main screen), RootTrace will try to count and mark emerged lateral roots as it finds them. This option works best with plants which have largely straight main roots (and so can use the 0 option in the Blender bar – see above), and counts laterals which have emerged by at least a few millimetres. Note, if the images are noisy or contain scratches, dust speckles, bubbles in the agar etc., this option may not work reliably. Try filtering the images using the “Enhance Image” tab first.

Counts for the left and right sides of each root, for each frame, are saved in the subroottxt.txt file in the output folder.

Tip angles

NOTE: this is experimental, and still in development! Use with care.

The aim of this option is to capture the evolving angle of the root tip as the root grows.

Examples

Some example images, with quickload files and instructions are available from the SourceForge RootTrace project page.

Problems?

Problem	Solution
The program always finishes the trace before the end of the root	<ol style="list-style-type: none">1. Do the background regions you specify contain parts of roots or image noise which looks similar in colour to roots? This will cause an early termination of the trace. Choose different background regions.2. Lower the background prior in the advance settings.3. Does the root bend such that it points upwards in the image? The tracker will not move "up" the image. This is to prevent back tracking. To work around this, it may be possible to rotate your images before processing them, so the root is pointing downwards.4. If your roots simply fade out at the end, or are very thin, then there is no workaround for this. Sorry.5. There is currently a maximum length of a root trace hardwired into the program. If you are tracing long images of roots, you may encounter this.
A message complains of a missing DLL file	<ol style="list-style-type: none">1. The software needs an external library to work correctly. Download the OpenCV library for Windows from http://sourceforge.net/projects/opencvlibrary/files/opencv-win/1.0/ and install on your system and try again. Make sure you install version 1.0, and that you have all the necessary access rights for the install program to set up paths on your system. VISTA USERS: it seems the OpenCV installer does not set up the system path correctly in Vista. To fix this:<ol style="list-style-type: none">1. Open control panel -> System -> Advanced System Settings2. Click Environment Variables...3. In the lower System Variables panel, find a variable called Path, click on it, and click Edit...4. Scroll right to the end of the text, and add in: ;C:\Program Files\OpenCV\bin assuming you installed OpenCV in the default directory, otherwise amend the above as appropriate.5. Click OK and close all the windows. Re-try RootTrace.2. Email me the message if the above doesn't fix it.
An Out Of Memory message appears	Try using fewer image files as input; if possible try using every other file. Email me with the details of the message, the number of files you were trying to open, the RAM on your computer and one example input file.
I installed RootTrace in Program Files, I'm running Vista, and the examples don't work right	Vista is picky about what files can be written to folders in Program Files. To find your output files, navigate to the RootTrace examples folder, and click 'Compatibility Files'. You should then see them.

If you need more help, please email andrew.french@cpib.ac.uk. If relevant, it would be helpful to email me an example input image file as well.

LICENCE

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Iridium

Iridium is a third party open source library available from here:

<http://www.mathdotnet.com/Iridium.aspx>

You can find the licence file at this link where you can also find the sourcecode. A licence file is also provided in the RootTrace install folder.