

## ***HYPOTrace* User Guide**

*HYPOTrace* was created with the Matlab GUI Developer. The compiled version of *HYPOTrace* does not require Matlab provided the correct runtime library is installed. All necessary components can be downloaded by visiting this site:

<http://phytomorph.wisc.edu/download/HYPOTrace>

### **To install *HYPOTrace*:**

In the Microsoft Windows PC platform, download all files and folders to a folder on the desktop. Double click on MCRInstaller.exe to install the MATLAB Component Runtime library, which allows *HYPOTrace.exe* to run as an application without a Matlab license.

If the components are not properly installed, attempting to run the *HYPOTrace.exe* program will result in the following error message: "This application has failed to start because mclmcr76.dll was not found". Re-installing the application or restarting the computer may fix this problem.

The following message may be encountered while first installing MCRInstaller: ".NET Framework 2.0 is not installed. If you require it, select Cancel and install .NET Framework first. Otherwise, select OK to continue." This is not required for the program, so select OK.

The *HYPOTrace\_mcr* folder, *HYPOTrace.ctf* file, *note.txt* file, and folders of seedling pictures must be located in the same folder as the *HYPOTrace.exe* executable program. The *note.txt* file containing image resolution in pixels per mm (determined by photographing a ruler and determining the number of pixels in a mm with Photoshop or other software) and time interval between images in the sequence should be placed into the folder containing images. Otherwise, post-calculation will not generate correct real-world units. Screen resolutions 1280x960 or greater will allow the program window to fit properly on the screen.

For the Macintosh operating system, run the *MATLAB\_Component\_Runtime.dmg* to install the MATLAB Component Runtime library.

Then, in the terminal, type `./run_HYPOTrace.sh <mcrroot>`

<mcrroot> should be the directory where the MCR library is installed.

run\_HYPOTrace.sh is generated in the compilation.

### **Images:**

Use TIF images. Image size is not critical but 1392x1040 works well. Seedling image files in a series must be sequentially labeled 0.tif, 1.tif, 2.tif, etc.

To sequentially re-label images in a time series (assuming Windows platform):

1. Open Command Prompt and type:

2. `cd (location of folder)/(name of folder containing HYPOTrace.exe program, etc.)`

for example: `cd desktop/HYPOTrace`

3. Press Enter. Then type:

`renames (name of folder of images)`

for example: `renames 9-20-08phot1 (or ./run_renames.sh <mcrroot> 9-20-08phot1)`

Tip: The Tab key allows you to go through the names of the folders more quickly.

Caution: Images must have equal name lengths and equal gaps of time between the images (e.g. 000000, 000450, 000900, 001350, etc.) to convert the names to 0, 1, 2, 3, etc.

### **To run the program:**

1. Double click HYPOTrace.exe application for PC Windows or launch run\_HYPOTrace.sh from the Macintosh terminal.

2. After the program launches and graphical user interface appears, Click Select Path button

3. Select folder containing a time series of images

4. Select start and stop image (or first and last images to be quantified), and step size (every image, every two image, and so on)

5. Adjust the default binary threshold if the binarized last image is not clear (if the edge is not smooth or there is gap in the object). Slide the bar beneath the Binary Threshold label to adjust it. The final image can be varied for choosing a consistent well working threshold.

6. Choose one of the Run options (once they are selected, they can't be changed while the program is running through the images)

a. Manual: the user must select each end point (of the hypocotyl).

b. Semi-automatic: the user chooses the first several end points, but then the program will run automatically.

c. Automatic: the program identifies each end point.

- Display ON: the midline will be traced and displayed for each image

- Display OFF: the midline tracing won't be displayed

7. Click Run. The program will show the start and stop images overlapping.

8. Click and drag a box around a single seedling, leaving enough room for the seedling images to stay within the box. (It is OK if only part of another seedling is also included in the box).

- If the manual or semi-automatic option is used, a red circle will appear at the point where the program finds the intensity feature most likely to mark the cotyledon/hypocotyl junction.

9. After the program has run through all the images, click Save in the bottom left corner. This will save Excel documents for phototropism, growth rate, hypocotyl length and hook angle in the folder that contains the images. Once you close the *HYPOTrace* program, you will no longer be able to save a file for the phototropism calculations.

10. To make a movie of the pictures, copy the LL file, which is created by running the program, from the images' folder and paste it in the main folder (containing HYPOTrace.exe program, etc). Open Command Prompt. After entering the location and folder name, press Enter and type: check (or `./run_check.h <mcrroot>` for Mac).

This will produce a video clip as well as Excel documents for phototropism, growth rate, hook angle and hypocotyl length.

**Tips:**

- Running the program semi-automatically allows the user to see where the program would have chosen the starting point, and the user can choose points for later images if the program selects a point that may be slightly off from the ones in previous images.
- The program will run much faster if Display OFF is selected.

Errors may occur if:

- the seedling images move beyond the area of the selected box
- another seedling is too close to the first and is mostly inside the selected box