

# HypoPhen User Manual (version 0.4)

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## ***Preliminary note***

This short manual is in the process of being written and may be modified in the future, along with the software. The latest version is available on <http://www.unil.ch/cbg/index.php?title=HypoPhen>. Like the software, the manual is released under the latest version of the General Public Licence (GPL) (see <http://www.gnu.org/licenses/licenses.html#GPL>)

## ***Introduction***

HypoPhen is an open-source software for the semi-automated phenotyping of Arabidopsis seedlings. It takes as input sequences of images of Arabidopsis seedlings and can measure the length and orientations of hypocotyls at the given time points.

## ***Installation from binaries – Windows and Mac OS***

### **Windows**

1. Download `HypoPhen_win.zip` and unzip it (for example using 7-Zip).
2. Put the resulting folder (`hypoPhen`) where you want it to be.

### **Mac OS**

1. Download the dmg file from: <http://www2.unil.ch/cbg/images/2/24/HypoPhen.dmg.zip>
2. Unzip the file open the dmg file and put the “hypoPhen” file (actually an `hypoPhen.app` folder) somewhere.
3. Open a terminal and using the “cd” command get into the `hypoPhen.app/Contents/MacOS` directory

## ***Installation from source – Linux and Mac OS***

### **Installing CMake**

CMake is a software for cross-platform software compilation. It basically simplifies the process of installing HypoPhen.

If CMake is not already installed on your system, it is recommended to install it. It can be found here:

<http://www.cmake.org>

### **Installing OpenCV**

You also need to install the OpenCV library. OpenCV is an open source computer vision library and can be found here: <http://opencv.willowgarage.com/wiki/>

On *Mac OSX* you first need to install XCode before being able to install OpenCV.

On *linux and Mac*, OpenCV can be installed like this:

1. Download the source file from <http://sourceforge.net/projects/opencvlibrary/>.  
The file is called something like OpenCV-X.Y.tar.bz2 where X.Y is the OpenCV version you are using.
2. Put this file in the directory where you want to install OpenCV, for example /opt
3. Type the following commands in a terminal

```
cd /opt
sudo tar -xjf OpenCV-X.Y.tar.bz2
cd OpenCV-X.Y
sudo cmake .
sudo make
sudo make install
```

If everything went well (the make command can take some time) OpenCV is now installed on your system.

Some versions (like 2.2) of OpenCV for mac have a bug that impairs window closing. While it is still possible to use HypoPhen, it can become annoying so it is recommended to apply the following patch (see patch command): <https://code.ros.org/trac/opencv/ticket/631>. After applying the patch you can recompile OpenCV with 'sudo make install'.

## Installing hypoPhen

1. Download the source files `hypoPhen.tar.gz` and save it in the directory where you want to install the software (say `mydir`).
2. Open a terminal and go to this directory by typing

```
cd mydir
```

3. Unpack the file by typing

```
tar -zxvf hypoPhen.tar.gz
```

4. Go to the `hypoPhen` directory by typing

```
cd hypoPhen
```

5. Ideally, typing the following in the terminal should install the software:

```
cmake .
make hypoPhen
```

If it does not work, you should check what `cmake` tells you and tune the `CMakeLists.txt` file.

## Usage

### Prerequisite

**Images:** In order to use this application, you must have a set of images featuring hypocotyls displayed on a regular grid. The camera should be moved between images, the frame must remain identical. Images can be of format `png`, `tif`, `jpg`. The format must be indicated by a 3-letter extension in the file name. The hypocotyls must be dark on a light background. Each image corresponds to a different time point. The image file names must be the same, except for a number at the end (before the format extension) indicating the order in which they were taken. The file names may contain no

white space. All images of a given series must be located in the same folder. For example the following set of images would be fine:

```
mydearhypocotyls001.tif,  
mydearhypocotyls002.tif,  
mydearhypocotyls003.tif, ...
```

**Hardware:** You need a mouse with working right and left buttons. On mac, you need to check in the *System Preferences* that both buttons are enabled.

### Launching the application

In its present form, the application is launched from the terminal (the command prompt in Windows) by typing the following command:

*Linux and Mac OSX:*

```
./hypoPhen <imagepath> <nrows> <ncolumns> <outputfile>  
[imagefile=<outputimage>]
```

*Windows:*

```
hypoPhen <imagepath> <nrows> <ncolumns> <outputfile>  
[imagefile=<outputimage>]
```

where the part within brackets is optional.

The following provides the meaning of the above arguments:

<imagepath>: the name of the first image of the series

<nrows>: the number of rows of hypocotyls in an image

<ncolumns>: the number of columns of hypocotyls in an image

<outputfile>: the name of the files where the results (hypocotyl length and orientations) will be written

<outputimage>: the name of the files where the images recording the tracking will be stored. This argument is optional

*Example:*

We assume that all images are in the folder `exp1/images/` and are called, `image001.tif`, `image002.tif`, ...

We also assume that we have a folder named `exp1/tracking` where we want to save the results of the tracking. Also we assumed that the hypocotyls are arranged on a rectangular grid with 3 rows and 7 columns, i.e., there is 21x hypocotyls per image.

Then, we can launch the program with the following command.

```
./hypoPhen exp1/images/image001.tif 3 7 exp1/results  
imagefile=exp1/tracking/track001.tif
```

This way, the program will write the results in four files called `expl/results_seg.txt`, `expl/results_or.txt`, `expl/results_len.txt`, `expl/results_tip.txt` containing respectively the location of the top point with respect to the bottom point, the bending, the length, and the absolute position of the tip of the hypocotyls. This last file may be the least subject to noise. The data in those files can for example be imported into spreadsheet software (like Openoffice, Numbers, Excel) for further processing.

### **Hand calibration**

The software opens a window showing the first image of the hypocotyls.

Select each hypocotyl you want to phenotype by Alt-clicking on its tip with the right button. A red string appears indicating where the hypocotyl is located.

Press <SPACE> when all hypocotyls have been selected, a blank window with a cursor appears.

Move the cursor to the left until the black hypocotyls are clearly distinguishable from the white background. You can resize the image if it is too small or **zoom in and out** by pressing the 'i' and 'o'

Press <SPACE> when you have a good separation. If, because of an inhomogeneous illumination it is not possible to have a good separation for all hypocotyls, press 'd' to do the calibration for each hypocotyl separately.

After you are done, the window shows the whole image again, and the red strings.

Move over the window with the mouse to set the white horizontal bar to the ground level for each row of hypocotyls. You can click with the left button to set the ground level for a row of hypocotyls but this step is not mandatory.

Press <SPACE> when you are done. With this ends the hand-calibration process.

### **Semi-automatic phenotyping**

You are now in the semi-automatic phase. Here are the things you can do.

Click left button to **select** a hypocotyl

Click right button to **zoom in** and press 'z' to **zoom out**

Ctrl - click-and-drag left button to **move one single point of the closest hypocotyl**.

Ctrl-Shift click-and-drag left button to **move the closest hypocotyl**.

Shift click left button to **set the tip** of the hypocotyl.

Ctrl-Shift right button to **set the base (bottom)** of the hypocotyl

Shift click right button to **redo the threshold calibration** for the closest hypocotyl. Then press 'g' to validate.

Ctrl click right button to **remove the closest hypocotyl**.

Alt click right button to **add an hypocotyl**.

Move the cursor to **adapt the smoothness of the selected hypocotyl**.

Press <SPACE> to **register those hypocotyls and move to the next image**

Press 'l' to **calibrate** the length used for the computation of **the bending angle** (using the cursor) or to stop this calibration

Press 'b' to **go back one image** (to check and possibly modify the tracking of that image)

Press 'v' to **validate** and overwrite the tracking for this image

Press 'n' to move to the **next image**

Press 'i' to **increase the window size**

Press 'o' to **decrease the window size**

Press 'q' to **quit** the application

### **Ending the application**

After you've been through all images, your application writes your results in the files specified in the command line and quits. You can now inspect and process your results. The automated measurements may be somewhat noisy and it is usually recommended to apply some filtering procedure to the data.