HypoPhen User Manual

Micha Hersch, last updated 02/18/11

Preliminary note

This short manual is in the process of being written and may be modified in the future, along with the software. 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

Installation

Installing cmake

Installing OpenCV

Installing hypoPhen

Down load the source files `hypoPhen.tar.gz` and save it in the directory where you want to install the software

Go to this directory (say `mydir`) :

```
cd mydir
```

Unpack the file:

```
tar -zxf hypoPhen.tar.gz
```

Go to the hypoPhen directory:

```
cd hypoPhen
```

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

Prerequesite

Images: In order to use this application, you must have a set of images featuring hypocotyls displayed on a regular grid. 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 enables.

Launching the application

In its present form, the application is launched from the terminal by typing the following command:

```
./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 wit h3 rows and 7 columns, i.e., there is 21x hypocotyls per image.

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

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

This way, the program will write the results in three files called `exp1/results_seg.txt, exp1/results_or.txt, exp1/results_len.txt,` containing respectively the top and bottom points, the bending and the length of the hypocotyls.

Hand calibration

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

Select each hypocotly you want to phenotype by Alt-clicking on it 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.

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 separetely.

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. Click with the left button the set the ground level.

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

Ctrl - click-and-drag left button to **move on 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.

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 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 'q' to **quit** the application

Alt click left button to **enter one of the following commands:**

- 'i': to **zoom in** (increase window size)
- 'o': to **zoom out** (decrease window size)

Ending the application

After you've been through all images, you 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.