APPENDIX B

HyDE software user's guide

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Abstract

HyDE (Hypocotyl Determining Engine) is a simple matlab-based program that measures hypocotyl growth in time-series images. The program will output raw length, smoothened length, and growth rate numerical data (though this feature is not implemented yet), as well as avi movies showing where the algorithm has predicted the hypocotyl to be. You can download HyDE from the web (http://cactus.salk.edu/hyde), or use a pre-compiled version installed through the iPlant collaborative (http://www.iplantcollaborative.org).

Installation

To use HyDE, you must first install the MATLAB Component Runtime compiler, which should be included with the distribution. Simply double-click the MCRinstaller.exe (windows users) or the MCRinstaller.dmg (mac users) file to begin installation. Once installed, open the HyDEv1_0win32.exe file (windows) or the HyDEv1_0macOSX.sh file (you may need to run this file in the terminal) to launch the application. Test the software on the images provided to ensure it is working properly (the software may take a while to load).

Image Pre-Processing

Input to HyDE requires one or more image stacks that have been pre-cropped in ImageJ or some similar software. Ideally, images should be cropped so that only hypocotyl tissue is visible at the very base (no interfering cotyledon or petiole tissue). Other structures can be tolerated in other areas of the image, but the very bottom row of pixels should contain only hypocotyl information. This is true for all images in the stack. The entire seedling need not be present in the picture, but the shoot apical meristem and hypocotyl must be visible in all images in each stack, as the software will use bulge information in the meristem to find the hypocotyl termination point. Please refer to the test images provided to gauge how your images should look.

Directory Type

HyDE can be operated in three different major modes (“Single Stack”, “Group of Stacks”, “Group of Groups”), reflecting the type of folder hierarchy that is input to the program. Which setting you use will depend on your data storage scheme, or the number/type of image stacks you wish to analyze at once.

“Single Stack”

In the most basic case, only the Stack folders are necessary, in which case “Single Stack” should be selected, and the input will be the name of the folder containing one image stack. Here, graphical output will be the raw length data for the single stack (no error bars included).

“Group of Stacks”

If a whole set of seedlings need to be processed, select “Group of Stacks”, and the folder containing all seedling stack folders should be selected as the input, reflecting a

complete set of, for example, 10 seedlings of one genotype across a single condition. Here, the graphical output will be a single line (raw length data) with error bars, reflecting the standard error of the mean at each time point.

“Group of Groups”

If the entire experiment needs to be analyzed at once, select “Group of Groups” as the input, and the program will process a set of sets of seedling stacks. Graphical output will be multiple lines, reflecting each a different stack type, with error bars indicating the standard error of the mean at each time point.

Input Parameters

All assay parameters are required for the program to proceed. They are described as follows:

“Start Time” This is used in defining the x-axis for the graphic (left edge of x-axis is “Start Time – Treat Time”). Usually, setting this to 0 is suitable.

“Treat Time” This is the time (in minutes) after “Start Time” that the treatment occurs, for setting the x-y intercept point. For some experiments, this may not be applicable, in which case, simply enter 0 (or the start time).

“End Time” This is the total amount of time (in minutes) that the experiment lasts. For example, for a 4 hour experiment, enter “240”. The program will expect an appropriate number of frames, given the “Minutes/Frame” parameter.

“Minutes/Frame” Simply the number of minutes between each frame (regardless of what

time step you wish to use).

“Pixels/mm” The number of pixels per millimeter in the image.

“Frames/interval” This indicates whether the program will process every, ever other, every third, etc. frame in the stack. Entering “1” will process every image, while “2” will process every other image.

“Assay Tag” This is a string that will appear in all output files from the run.

Output options

This is what the program will return once its finished analyzing the data. Check the boxes to indicate how much output is needed. If nothing is checked, the program will still return a graphic indicating the relevant curves.

“Raw Length” This is the unprocessed length that is found on every frame. Output is in csv format, with a header row indicating from which plate/seedling the curve originated from. Some values may be omitted, due to too much fluctuation.

“Smoothened Length” This is the spline-fitted length curve for each seedling, for subsequently determining growth rate.

“Growth Rate” This is the derivative of the length smoothing spline for each seedling.

“Movie” This is the single-seedling movie, a compendium of each frame in the stack, with a red line indicating where the software thinks the hypocotyl is.