Rosette Tracker

Requirements

Rosette Tracker was developed for *ImageJ* 1.45 in java 1.6.018. The program has been extensively tested on a Intel i7 pc with 4Gb RAM. The program has been tested on Windows 7, Windows xp, Linux and mac.

Installation

Rosette Tracker is a plugin for *ImageJ*. *ImageJ* 1.45 or a later version is advised. You can download and find installation instructions for *ImageJ* on the official *ImageJ* website.

After installing *ImageJ*, you can install the plug in by copying Rosette_Tracker.jar to the correct directory. *ImageJ* will have created a directory in the program directory of you operating system (e.g. "Program Files" on windows). This "ImageJ" folder has a subdirectory named "Plugins". Copy Rosette_tracker.jar into this "Plugins" directory.

The latest version of *Rosette Tracker* can be found at the homepage of Jonas De Vylder (http://telin.ugent.be/jdvylder/RosetteTracker).

Getting Started

Start *ImageJ*, click on "Plugins" in the menu and select "Rosette Tracker". This opens a panel with three buttons: "import images", "settings" and "run".

1. Importing images

There are several possibilities to import time lapse sequences depending on the type of sequence to process, e.g. VIS images, fluorescence images or thermal image.

- Choose the first option, "Good contrast images", if the image sequence has good contrast between the background, such as is generally the case with VIS and fluorescence images. Select the first frame of the time lapse sequence. Rosette Tracker will automatically detect subsequent frames in the same directory. Note that all images in the same directory will be considered as subsequent frames, so it's necessary that all time lapse sequences are stored in separate directories.

- If the images have low contrast or bad resolution, such as NIR images, it is still feasible to perform reliable measurements on them if good contrast images of the same rosettes are also available, e.g. VIS images. The detected rosettes from the good contrast images are mapped on the low contrast images. This makes it possible to measure the average leaf temperature in low quality NIR images based on the rosette segmentation of good quality VIS images. Note that good and bad time lapse sequences have to be stored in different directories in order for Rosette Tracker to be able to analyse them. In order to calculate the exact mapping between good and bad contrast images it is necessary to register the images (see subsection about calibration)

2. Settings

Rosette tracker has several settings which can be set in order to optimize the measurement results and to optimize the users comfort.

Personalizing output

- First the user can select which measurements should be calculated: area, average intensity (for fluorescence or thermal images), compactness, the maximum diameter and or the "relative rosette growth rate".

- The user can choose how to visualize the measurements: as a table and or as a plot. The table can automatically be saved as a csv-file. Aside of the measurements the segmentation result can also be saved. The segmentation result shows which pixels are considered as foreground pixels and to which rosette it belongs. These segmentation images also show the labels, so the user can see which measurements correspond to which plant. Figure 1 shows an example of a VIS input image and the corresponding segmentation image. The segmentation images are important to check, especially in the case of outlier measurements, since all measurements are dependent on the quality of the rosette segmentation. Both measurements and segmentation images are stored in the same directory, which can be chosen by the user (by clicking on the button "choose output directory").

Optimizing measurements

A wide variety of plant monitoring setups exist, ranging from expensive robotized multispectral monitoring with different light sources to homemade setups based on reflex cameras. In order to cope with this variety of setups, a set of variables can be tweaked to get optimal performance. Most of these variables are easy to interpret or are calculated using a straightforward user interface. Note that these settings should not be optimized for each sequence individually. In general the settings can be chosen once for a given setup and then be saved either as a config file or as the default settings. Each sequence captured with this setup can then load these same settings.
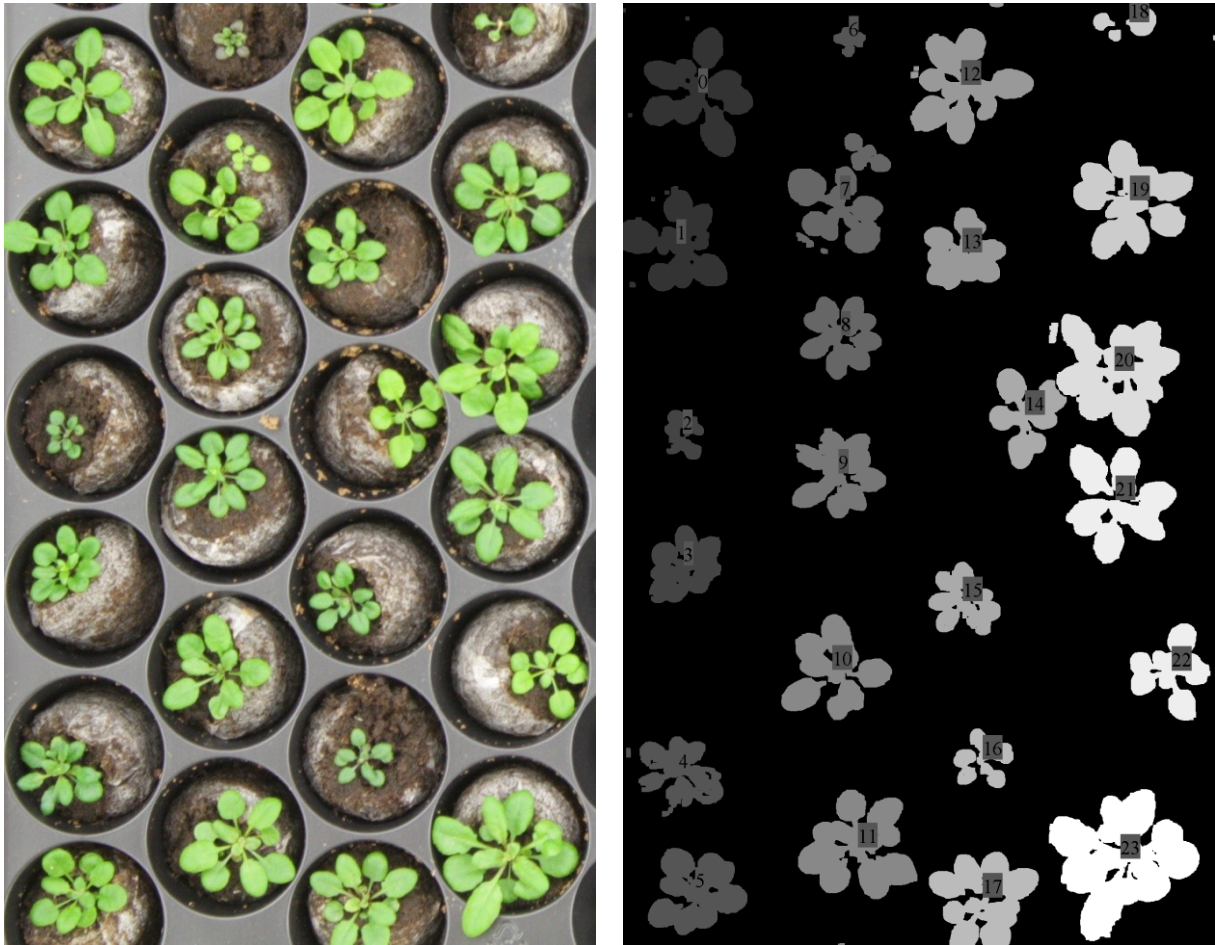
Figure 1: On the left, a VIS image of a tray with rows oriented in a vertical way. On the right, the detected rosettes are shown, as can be seen the rosettes are labelled column by column, from top to bottom

- Tray optimization:
  - the user should define the amount of rosettes to detect. Note that rosettes should not touch each other! If they do touch, they will be detected as a single rosette and might detect a different rosette as two rosettes. To avoid this situation, the user should crop out plants touching each other. This can be done for all frames at once using free image processing software such as irfanview. Of course, anticipating rosette overlap and hence preventing it from happening, before image acquisition, also helps.
- *Rosette Tracker* will try to label all rosettes with a numerical label in a logical order: from top to bottom, from left to right. In order to do so, the user has to define how many rows the trays have. The rows should be oriented in a vertical orientation, i.e. from top to bottom. If the rows are horizontal instead of vertical, the sequences should be rotated before processing. This can be done using software such as irfanview. Figure 1 show an example of a vertically oriented tray and the corresponding labelling.
- Calibration:
  - By default all measurements related to distance, e.g. area, diameter and relative rosette growth rate are expressed in pixels. In order to measure in millimetre, the image scale has to be set. This can be done in multiple ways:

- by manually entering the scale if it is known
- by selecting two points in an image of which the real distance between them is known, e.g. by selecting two points in the image of a ruler. *Rosette Tracker* will then automatically calculate the real resolution

  o In order to calculate the relative rosette growth rate, the time resolution should be set, i.e. how many seconds are there between the capturing of consecutive frames.

  o VIS images and NIR images can have a different scale, orientation, etc. Therefore a special calibration step is needed in order to calculate the mapping parameters. This calibration requires the user to select 16 corresponding points in a VIS and NIR image. Based on these 16 points Rosette Tracker can calculate the exact mapping. Note that slight deviation on these 16 points is acceptable, i.e. if the actual location is a couple of pixels off, Rosette Tracker will still find the exact mapping parameters.
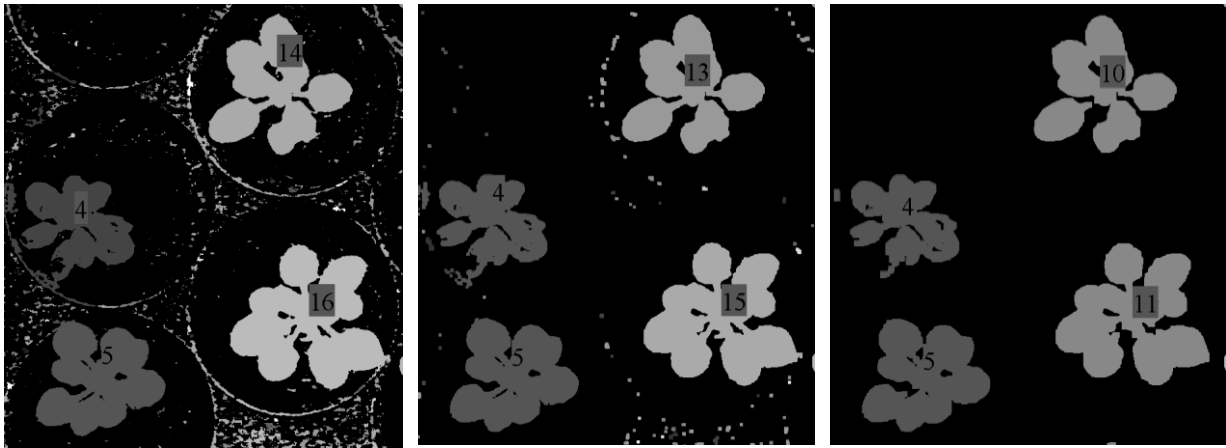

Figure 2: The segmentation result using different levels of clutter removal. From left to right clutter level 0, 2 and 4 was used.

- Segmentation parameters:
  o Rosette tracker uses colour/intensity to detect which pixels belong to the foreground. However, which hue of green corresponds to foliage is dependent on the lighting conditions of the setup, and the camera. By selecting a single pixel belonging to a leaf, the correct colour/intensity parameters used by the segmentation method can be calculated. We remind the user that this only has to be done once for the setup, not for each plant or sequence.

  o Due to bad lighting conditions (from an image processing point of view) and noise, it might be possible that isolated pixels in the rosette are not detected. A special post-processing method can be applied to correct for these misdetections. This method is activated by selecting the "fill holes" option. The size of the holes can be set as well. If the segmentation method gives good results without this post-processing, we advise not to use this option, since it might hamper the removal of clutter (see next subsection)

  o Due to noise and reflections of the leaves on the tray, Rosette tracker might detect small false segments. To remove this clutter, a special post processing method can be used, which will discard small detected segments. This method is activated by selecting the "remove clutter" option. Which object to consider as

small, i.e. discardable, of course depends on the resolution and lenses used by the setup. Therefore the user can manually set a size parameter 0 for very small object to 5 for bigger objects. The best way to set this number is to start at 0 and see if there are false detections in the segmentation results. If so, increase the size by one. Repeat this until all noisy detections have been removed. Avoid setting this size parameter to high (for example to be certain), since in the extreme case it might also remove very small leaves. In Figure 2 an example of the influence of different levels of clutter removal is shown.

o   Note that the above segmentation parameters are highly dependent on the lighting conditions. Depending on the monitoring setup it might be possible to monitor plants during night time as well, e.g. using NIR or fluorescence imaging. During night time however, different lighting conditions hold. So if nighttime frames are available, the user has to set the above parameters both for day- and night time. In order to use the proper settings, the user should also define how many frames are captured during night time (If no such definition is made, *Rosette tracker* assumes the same number of night time as daytime frames). *Rosette tracker* assumes the first frame is captured during daytime.

To avoid setting all these parameters for each sequence individually, the user can chose to save the settings. The user can choose to save them under a specific name and load them afterwards. Another option is to save the settings as default. If done so, these settings will automatically be loaded every time *Rosette Tracker* is started

3. Run

After importing the sequence and choosing or loading the right settings, the actual measurements can be calculated using the "run" button. The measurements can be represented using a table and/or in a graph.

4. Analysing measurements using Microsoft Excel

Measurements are saved in a comma separated value (csv) file, where all values are separated by a comma. Measurements csv files can be loaded into Excel using the following steps:

1.   Open a new excel worksheet
2.   Click on data>get external data>from text
3.   Choose the csv measurement file
4.   Select delimited and click next
5.   Select comma and click finished

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