BRAT Manual

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Brat Segmentation (Fiji Plugin Brat):

The segmentation plugin of the Brat pipeline reads a set of input images and tries to detect the objects of interest (plants) in the image. It also measures some basic traits (e. g. root length, root width, …) and writes the results into a file. The plugin is able to process both, independent images and time series images. There are only very few parameters, the user has to provide, before the plugin starts it's work.

Memory consumption:
The memory used by BRAT depends on the image size and resolution. Processing one of our example images (resolution = 1200dpi) uses about 1GB of RAM. If time series are processed, each image of the time series adds this amount to the total amount of memory used. (If you want to process a time series containing 5 time points, the total memory would be about 5GB). Please make sure, that Fiji's maximum available memory is set to an appropriate value. You can change this value at: Edit → Options → Memory & Threads (restart Fiji after changing this value).
Note: By default Fiji uses 2/3 of the available memory.

GUI parameters and actions:

File Extension	Specifies the file extension of the images. All images in the base directory which match the given file extension will be used in image processing.
Base Directory	Location, where the plugin looks for images. Choose appropriate folder by clicking "..." button.
Flip horizontal	Flip images horizontal before processing. This takes into account, that the images are scanned from below (e. g. if flatbed scanners are used), and are therefore laterally reversed.
Equalize histogram	Use histogram equalization to improve contrast in the images.
Process time series	If selected, the plugin assumes the images to be the same plate over a series of days. The filenames must match our specific filename pattern in this case. It sorts the images into time series automatically. Time dependent traits (e. g. growth rate) can be measured only if this option set. If this option is not selected, each image is assumed as an independent scan. The images' names need not to match a specific pattern. Even if they match our filename pattern, they will be processed independently. *Note: If you want to process time series, the file naming has to match a specific pattern. See Section: Filename Pattern.*
Start	Start processing.
Log Area	Log entries, created while image processing is going on, will be printed into this area.
Write Log	Write log messages to a file.

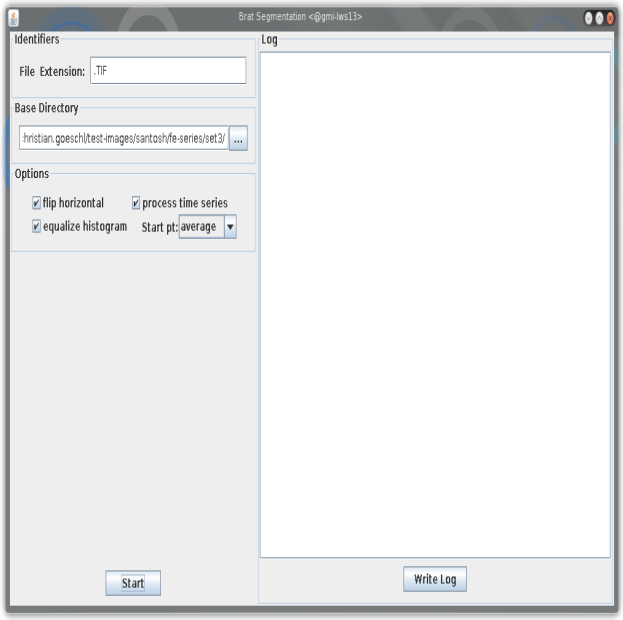

Figure 1: Screenshot of the BRAT plugin.

Filename Pattern:

If you want to process time series images, the filenaming has to match a special pattern. This makes sure that the images can be organized and each detection is assign to the correct group.
Note: Processing time series is a very memory intensive task. Make sure the computer you will use for time series processing has enough memory.

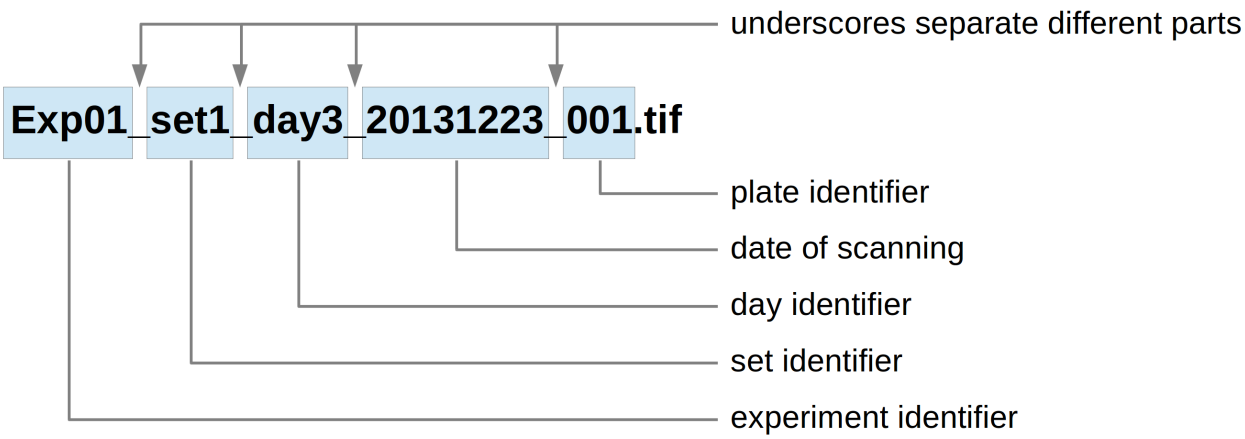

Figure 2: Filename pattern for time series processing.

- The **experiment identifier** is an arbitrary character sequence which you can use to identify your image set.
- You can use the **set identifier** to group images within a experiment. It contains the string “set” followed by an integer number.
- The **day identifier** will be used to sort the images of a time series. The string “day” followed by an integer number defines the time point. It does not matter if the day numbers start with “1” or any other number as long as the number are increased by the same amount for each time point. (“0” should not be used because we reserved this number for a day-zero-image, which is scanned directly after placing the seeds).

- The **date field** stores information about the time the plate was scanned. It is in YYYYMMDD format.
- The last three digits are used as **plate identifier**, which has to be unique for each plate.

Brat Quality Control (Plugin Brat QC):
We designed our segmentation plugin to work without having a complex parameter set adjusted by the user. Depending on the quality (mostly contrast between foreground and background) of your images the segmentation process will produce more or less of false-positives (detections, which are not a root) and false-negatives (roots, that are not detected). In some cases the segmentation will contain errors as well. These wrong segmentations could affect your measurements. While there is no ability to eliminate false-negatives (i.e. increase your sensitivity to detect plants), the user can manually discard false-positives or insufficient detections. We provide a simple quality control interface, which enables the user to efficiently iterate over the set (or a subset) of roots detected by the segmentation plugin and to discard any of the detections the user does not want to include in trait evaluation. This step is optional but can help significantly to improve the accuracy of the results.

GUI parameters and actions:

Base Dir	Directory, where the results of the segmentation process are saved. The QC-plugin will use the "Plant_<n>_Object_Diagnostics_...jpg files, where <n> is some integer number representing the detection ID. Choose the appropriate folder by clicking the "..." button.
Identifiers [Day,Plant,Plate,Set]	Regular expressions (Java regular expression) which are used to search for the according files and to define the order in which the iteration over all images is done. The expressions used in the screenshot are the default values and fit with our naming pattern.
Iteration [time series set, independent set]	This option defines the order in which the images will be visited. If "time series set" is selected, the plugin will iterate over all time points of the same plant (same plant ID) with same plate number and same set number. Then the next plant ID is taken and the plugin will show all time points of detections with this ID while plate and set numbers are unchanged. This is the recommended order for classifying time series. If "independent set" is selected, the iteration will be done on a per plate basis. Each detection on a specific plate will be shown before switching to the next plate.
Start	Start the quality control process.

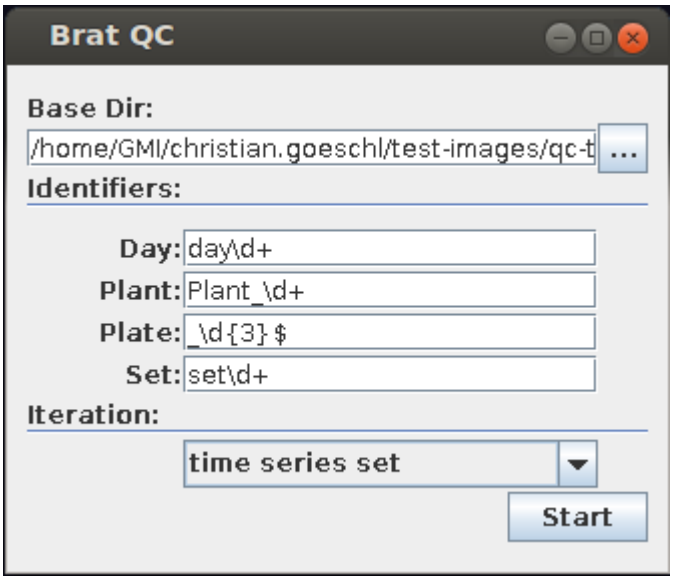

Figure 3: Screenshot of QC interface

For each detected object, a diagnostic image will be shown. You can resize the image according to your needs (and your screen resolution) by dragging the image corners with your mouse.

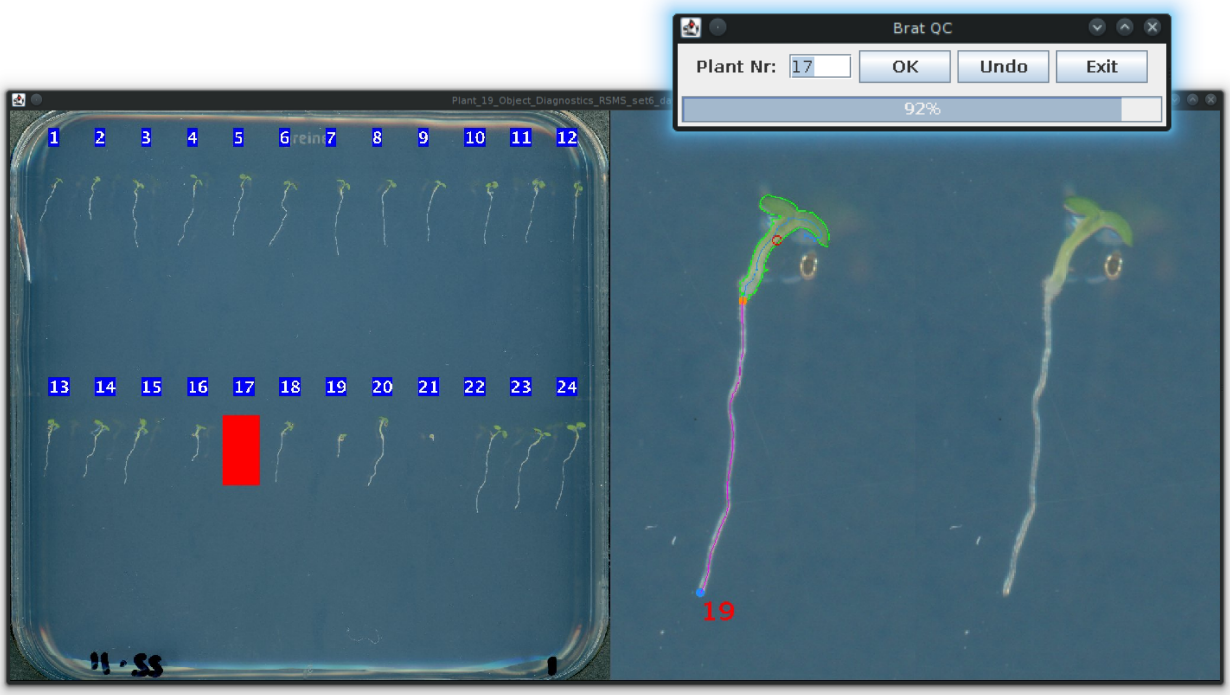

Figure 4: Screenshot of an example QC step

On the left side you can see the position of the detected object. The right side shows the detection in more detail. With green color the outline of the detected shoot part is drawn. A orange and a blue circle mark the start and end point of the main root respectively. The path of the main root is drawn with pink color and the detection ID is printed with red numbers. The very right part of the diagnostic image shows the same region of the original image without any overlay.

A second window enables the user to input his choice, whether to use or discard the according detection.

Plant Nr	Input the correct position of the root if you want to include the detection in the results. By typing anything which can not converted to a integer number (e.g. character “x”), you will discard this detection.
Ok	Click “Ok” button (or press ENTER on the keyboard) to confirm your input.
Undo	Click “Undo” button (or press CTRL-Z on the keyboard) to go back to the last image.
Exit	Click “Exit” button (or press ESC on the keyboard, or close this window) when ever you want to exit the plugin. This will save the decision you have made so far. (Automatic saving will be done every 60 seconds.) You can continue quality control from this stage by just opening the plugin again with the same options (same base directory and sorting fields).
Progress bar	The progress bar shows the percentage of images which have already been classified.

Assignment of accessions and calculation of average/median and additional trait values (plugin "Brat Eval"):
In the last step of the Brat pipeline, all detections will be tested for plausibility and get assigned a specific to a specific accession. Then average and mean values of traits as well as some derived traits get calculated. The results will be written to files afterwards.

Base Tab:

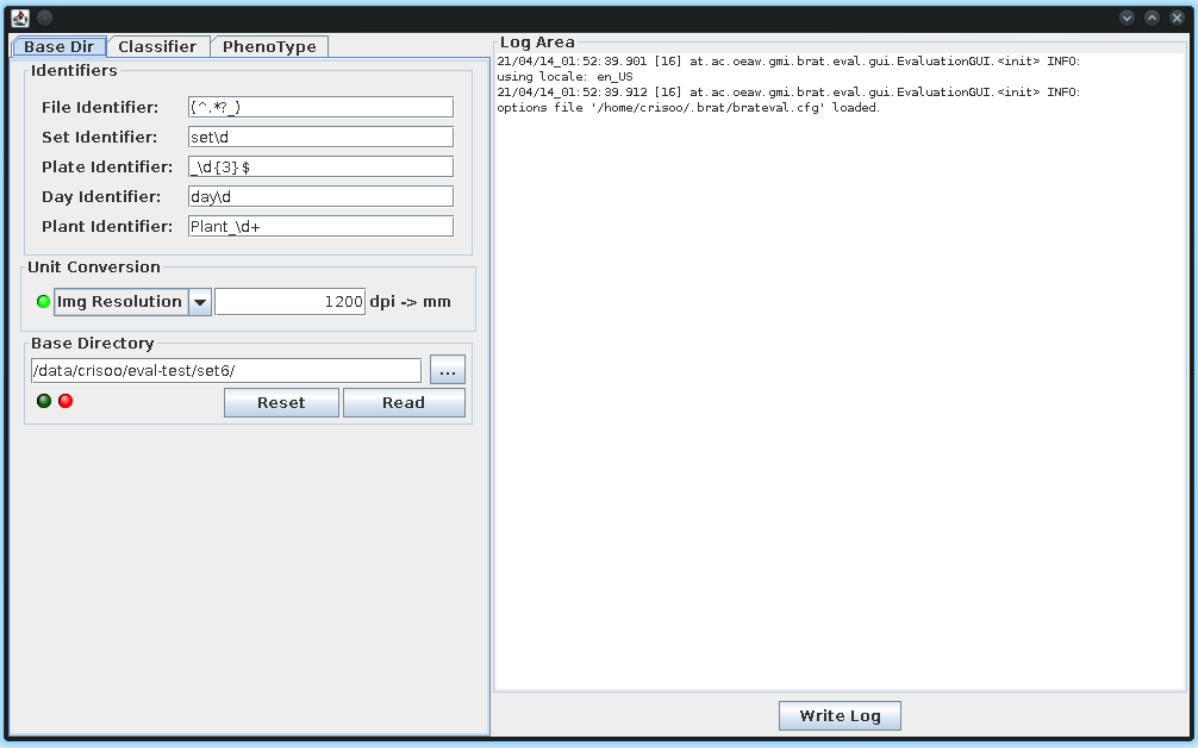

Figure 5: Screenshot of Brat – Evaluation Base Tab

Identifiers	Regular expressions to identify the files
file	arbitrary character sequence in all files (example: any String until the first "_" is reached)
Set	arbitrary character sequence in all files (example: set<n>, where <n> is an integer number [1-9])
Plate	plate number (example: last 3 integer numbers in the filename without extension)
Day	time point identifier (example: day<n>, where <n> is an integer
Plant	Character sequence specifying the ID of the detection (example: Plant_<nn>, where <nn> is an integer number of arbitrary length.
Unit Conversion	Choose, if the internal pixel units should be converted to other units. Possible choices are: • **None:** no conversion • **Img Resolution:** The image resolution is specified (e.g. 1200 dpi); the converted units will be millimeters. • **Custom Factor:** The internal pixel units will be multiplied with a given factor.

Base Directory	Directory, where the result files from the segmentation plugin (Object_Measurements...txt and Object_Coordinates...txt) are saved. Click "..." button to select the folder.
Read	Click "Read" button to read the measurements and coordinates from the given base directory.
Reset	Reset the Brat evaluation plugin to initial state.

When the input files a read, proceed to the **Classifier Tab:**

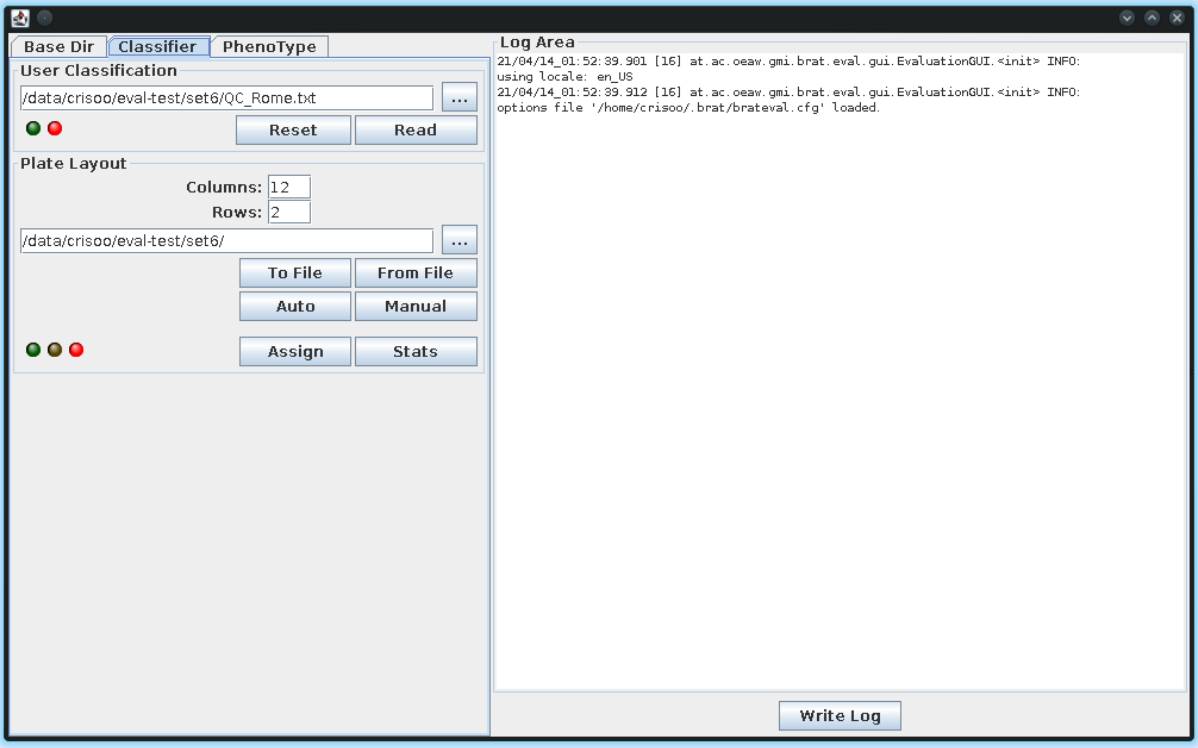

Figure 6: Screenshot of Brat – Evaluation Classifier Tab

At the classifier tab you can choose different options:

User classification	If you have done manual quality control, you can specify the output file from the Brat QC plugin by pressing the "..." button. Click "Read" button to read the selected file. Click "Reset" button to reset a previously read user classification.
Plate Layout	This section enables to create a spatial layout (semi-) automatically. Enter the columns and rows of expected plants on the plates.
Auto	By clicking "Auto" button a spatial layout is calculated from user classification data. (A user classification file has to be read before). This options makes sense, when only a subset of the segmentation results was manually classified.
Manual	Define a spatial layout manually. By clicking this button a dialog will appear where you can select on of your original images. (Select one of the images you used as input for the segmentation plugin from the current experiment). When the image is shown, click at the expected start point

location for each root. (The currently selected root will be marked in the shown table. You can select a different root, by clicking on the according row of this table). Click “Done”, when you finished selection of start points for each root.
Note: Do not forget to flip the image horizontal (if necessary) by right-clicking on the image!

From File — If you have saved spatial layout information from a previous run, you could load it by pressing the “From File” button. A dialog will open, where you can select the according file to load.

To File — Save the current spatial layout information to a file. Clicking the “To File” button will open a dialog, where you can select where to save this information.

Assign — Click this button to assign position to the detections loaded from the base directory. If the offset to the next layout point of a specific detection is too much, the detection will be marked as false positive and discarded from evaluation otherwise it gets the nearest position assigned. If a detection was already classified by the user, this setting will not be overridden. Roots, which show a negative growth rate will be discarded as well as such we change their assigned position over time.

Stats — Prints some statistics about the assigned positions. If a user classification was loaded before, a correlation matrix will be shown, showing the user classification in rows and the automatic classification shown in columns.

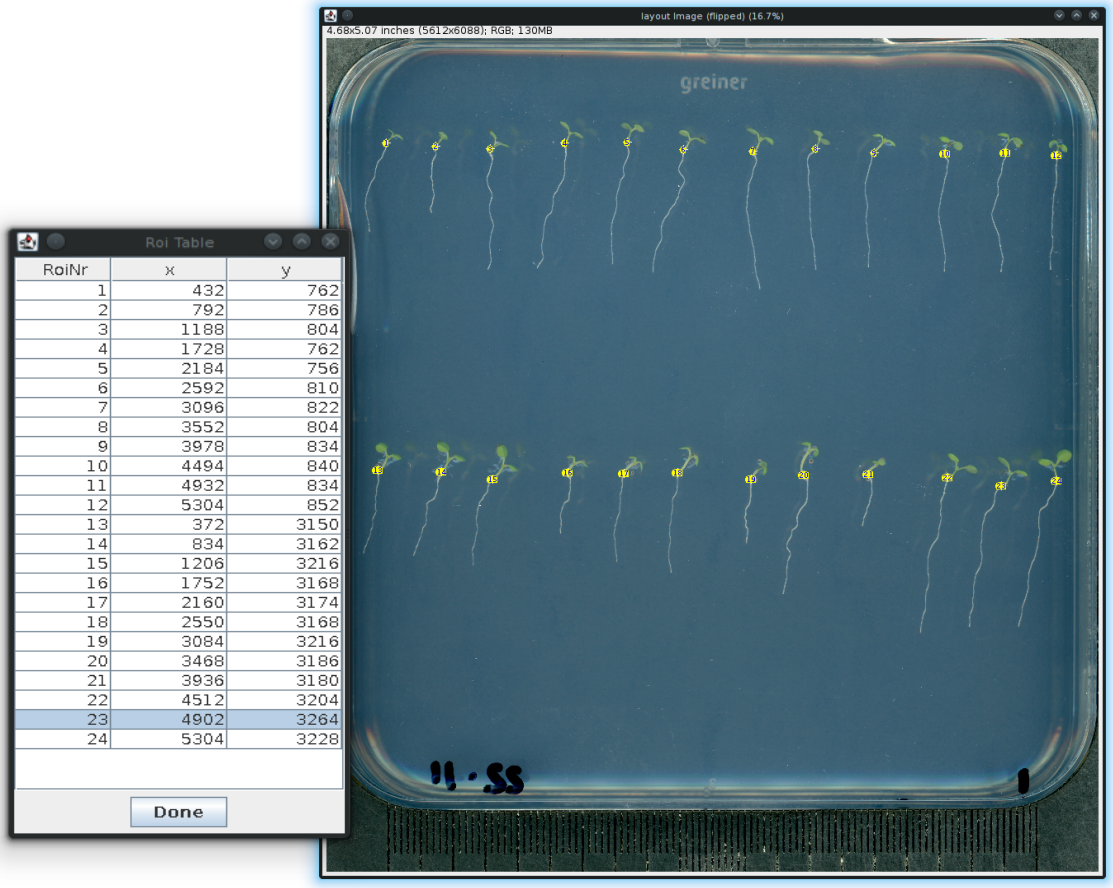

Figure 7: Screenshot of a sample spatial layout creation

Read accessions layout and write results, the **Phenotype Tab:**

When you finished the assignment of positions, you can assign a accession to each root. Some special traits and average/median values will be calculated on a per accession basis.

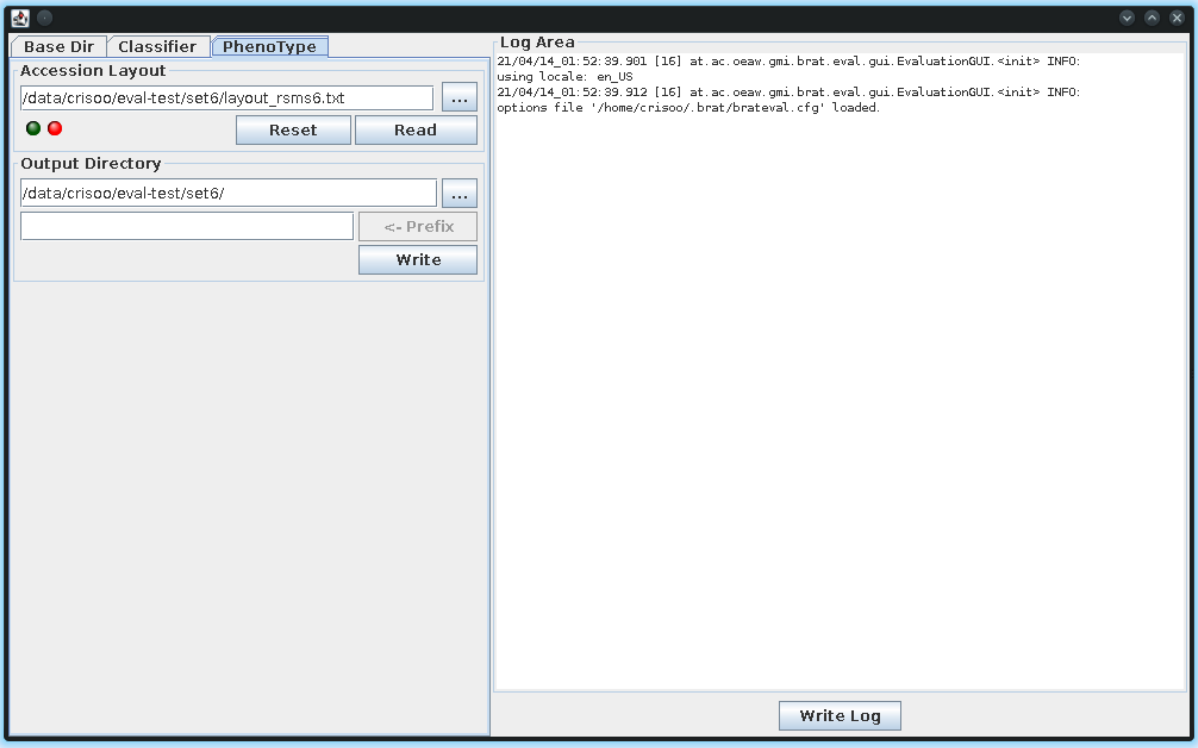

Figure 9: Screenshot of Brat-Evaluation Phenotype Tab

Accession Layout	The accession layout file provides information, which plant on a plate belongs to a specific accession. This enables the user to grow plants from different accessions on the same plate (and therefore, the same conditions) and to compare them directly. Select the appropriate accession layout file by clicking the "..." button.
Read	Read the selected accession layout file and assign the accessions to the detected plants.
Reset	Remove already assigned accession information from plants.
Output Directory	Choose a directory, where you want to save the result files by clicking "..." button.
Prefix	Input an arbitrary character sequence to name your result files. The filenames will start with the given prefix.
Write	Write the result files to the selected directory.