Image Analysis with IAP

Example Analysis Workflow for Detached Leaves

This is a part of the user manual for the IAP (Integrated Analysis Platform). This documentation describes especially the analysis workflow for loading phenotypic image data, which was acquired with a manual scanner system or a digital camera. The the setup for the image analysis of detached leaves data will be shown in detail. Finally, the result export function will be shown shortly. The detached leaves analysis requires the installation of an external Add-On. Following this link http://iap.ipk-gatersleben.de/#addons you can find a short introduction.

(To use this manual it is recommend to have already downloaded IAP. If not done so far, please visit our website http://iap.ipk-gatersleben.de to get any information about setup and installation of the software. Also many example workflows and example datasets are available.)

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Load Image Data and Create an IAP experiment

The first part will be describing how to load any phenotypic image data (commonly: the image files saved in a folder on your computer, this images could be acquired with a digital camera or scanner and need no special preparation) and how to create an IAP experiment for image analysis. In this example manual scanned root images will be analysed.

1. Start IAP

(for more details take a look in the User documentation http://iap.ipk-gatersleben.de/documentation.pdf) and click "Load or Create Dataset".
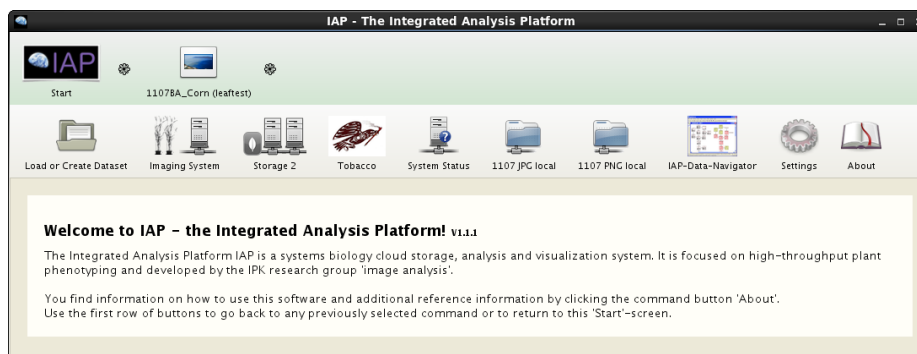

Start Window of the IAP. (first row bookmarks and actual navigation, second row action buttons)

2. Create Dataset from Image data

In our example we will load some locally saved image data from the hard drive. Therefore you click the first button "Create IAP Dataset from Files" and navigate to the images as shown in the following images. Finally mark all images you want to load and click open.
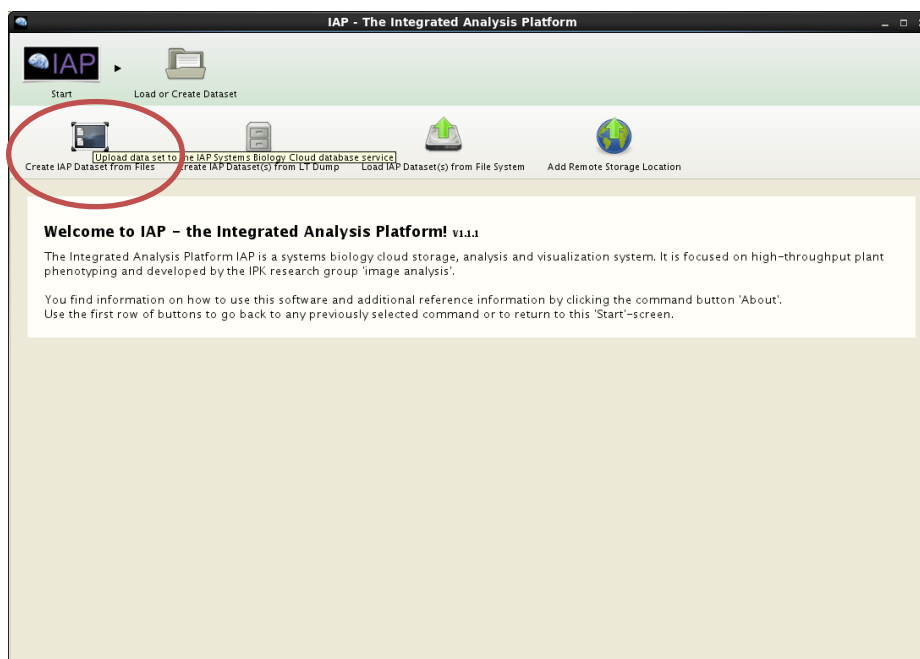
Overview window about the different creating/loading methods. Click the first to create an IAP dataset.
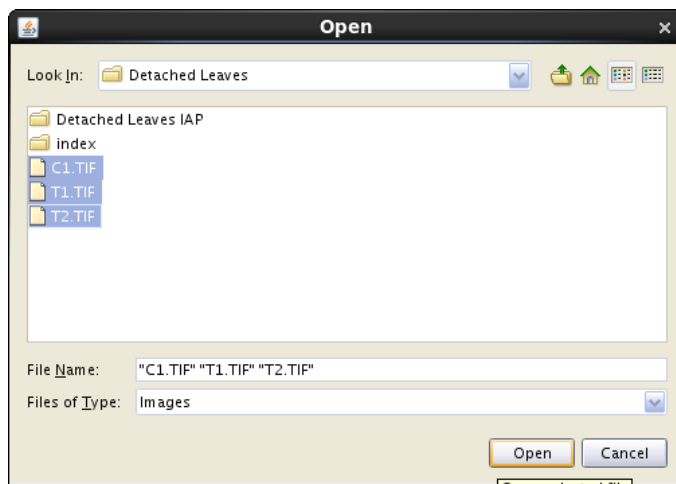
Dialogue to choose target image files.

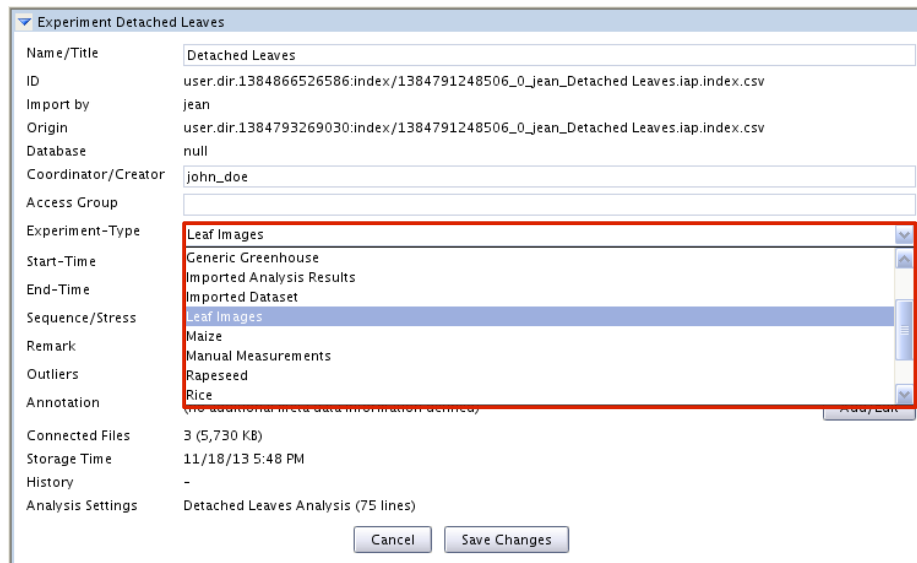**3. Adding some Additional Information**

Now you can add some additional information about the experiment. Most important information is the experiment name and coordinators name (no need for other information, it is recommend to do this later if needed).

Annotation window for image data.

After finishing your entries just click "OK". Now you created an IAP experiment, it has to be copied on the hard drive for further use.
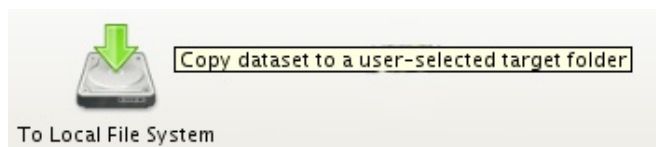
4. Copy and Save IAP dataset to Hard Drive

Before copying it is important to select a proper experiment type (Leaf Images).

Save the experiment on the hard drive by clicking the "Copy" button. To save on local file system choose the following option as shown below.


Now choose your desired target directory and voilà the IAP experiment will be saved. This may be taking some time depending on the number of images, image quality and your system speed.

## *Load and Analyse an Detached Leaves Experiment*

In the next part it will be shown how to load and analyse an experiment with the IAP software. Several steps are recommended, these are pipeline assigning, checking the pipeline performance (plus possibly parameter adaptation), analysing of the whole experiment and finally result data browsing and export for further analysis.

1. Load IAP Experiment

For loading an IAP experiment click again on "Load or Create Dataset" and then "Load IAP Dataset(s) from File System" button as shown below.
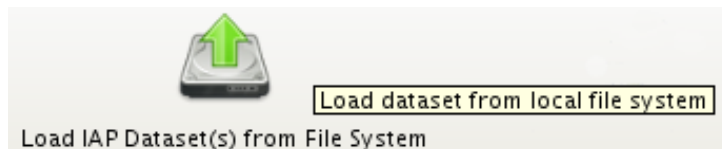

Now browse to the experiment main folder and click "Select Target Folder".
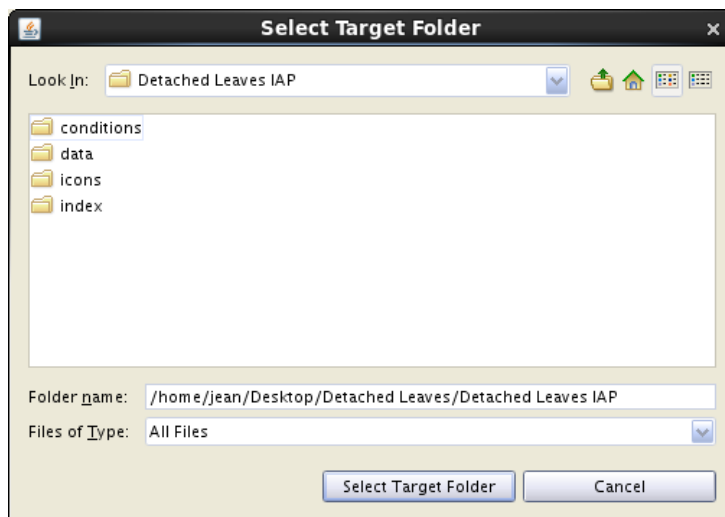
After loading the experiment navigate to the experiment overview screen by clicking on the buttons as shown in the fallowing picture.

Now you are on the "experiment overview window". Here are the options to add some additional meta-data and information. After modifying, just click "Save Options" and your changes will be added to the experiment.
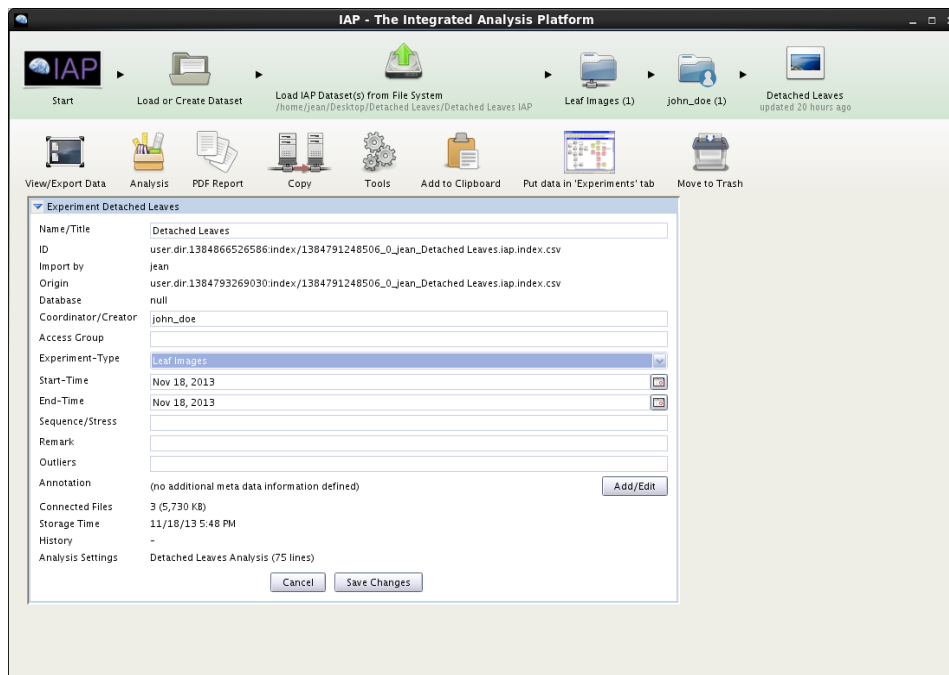
"Experiment overview screen", on top are the navigation path, in the second row some action buttons and below the "experiment information (meta-data) Field".

1. Assign Analysis Template

For assigning an analysis template to your experiment, navigate to "Analysis" > "Select analysis Template". Now choose the "Use Scanned Roots Analysis", this is an analysis template designed for detached leaves analysis.
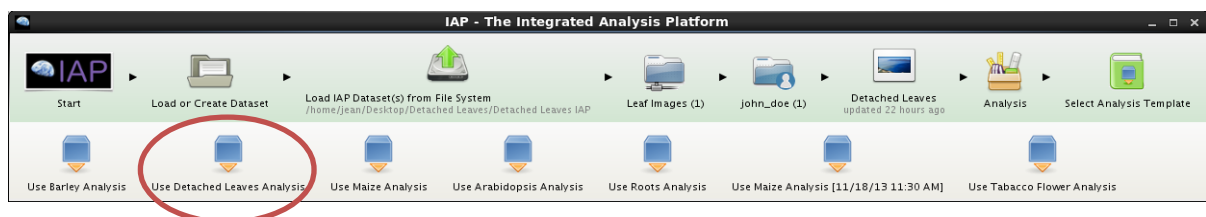
Now you have assigned a template for the image processing. A template includes an image processing pipeline which is designed to analyse a species (maize, barley, arabidopsis, ...) or a plant organ (leaves, roots, ...). A pipeline consists of several image processing blocks. Each block fulfils a special image processing task. Most pipeline parameters are predefined and should work well with different applications, but sometimes it is necessary to change some parameter (e.g. in case of strong illumination fluctuation). Also you are free to add or remove some blocks. The detached leaves pipeline uses following blocks as default.

1. ***Load Images (without reference)***

2. ***Copy Image Set to Mask Set***

3. ***Auto-tuning VIS-Segmentation (K-Means)***

4. ***Morphological Operations***

> 5. **Separate Objects**
>
> 6. **Calculate Color and Intensity Histograms**
>
> 7. **Calculate Areas**
>
> 8. **Run Post Processors**
>
> 9. **Move Mask Set**

You can get detailed information about each block in our main documentation (see documentation http://iap.ipk-gatersleben.de/documentation.pdf). A special block in the detached leaves analysis pipeline is "Separate Objects", this recognizes all detached leaves in the image area and separates them. The area, intensity and color features will be processed for each leaf.

2. Image Browsing and Testing of Pipeline Settings

Now go back to the "experiment overview" window. On the next pages the image analysis and pipeline testing will be described elementarily.

For checking the pipeline, navigate to "View/Export Data" and choose an image to run a test analysis for this single image by clicking right on the image. Choose the "Detached Leaves Analysis (Image + Reference)" option in the appearing window as shown in the next two figures.
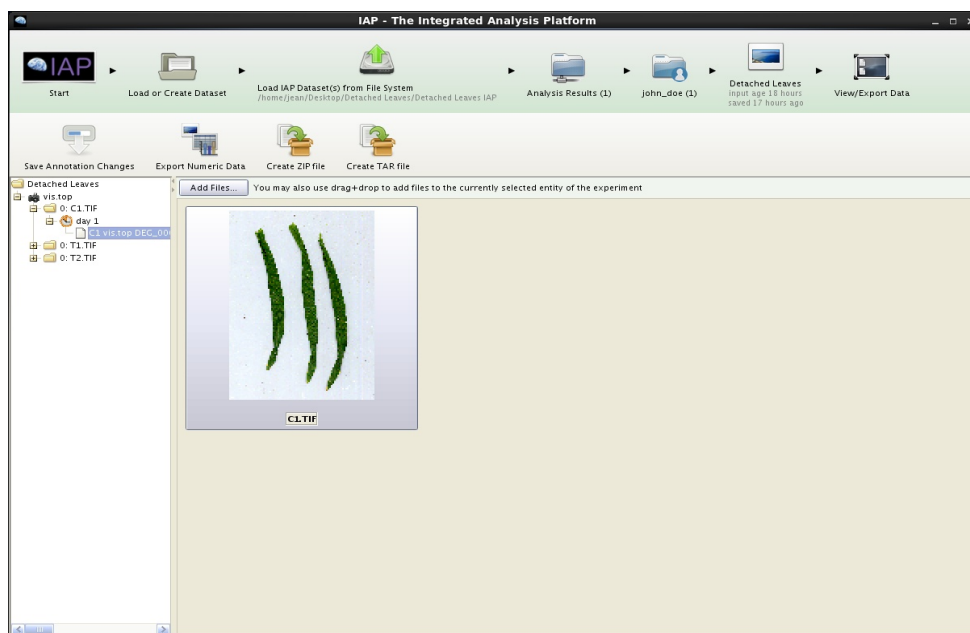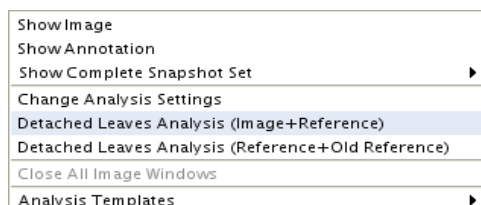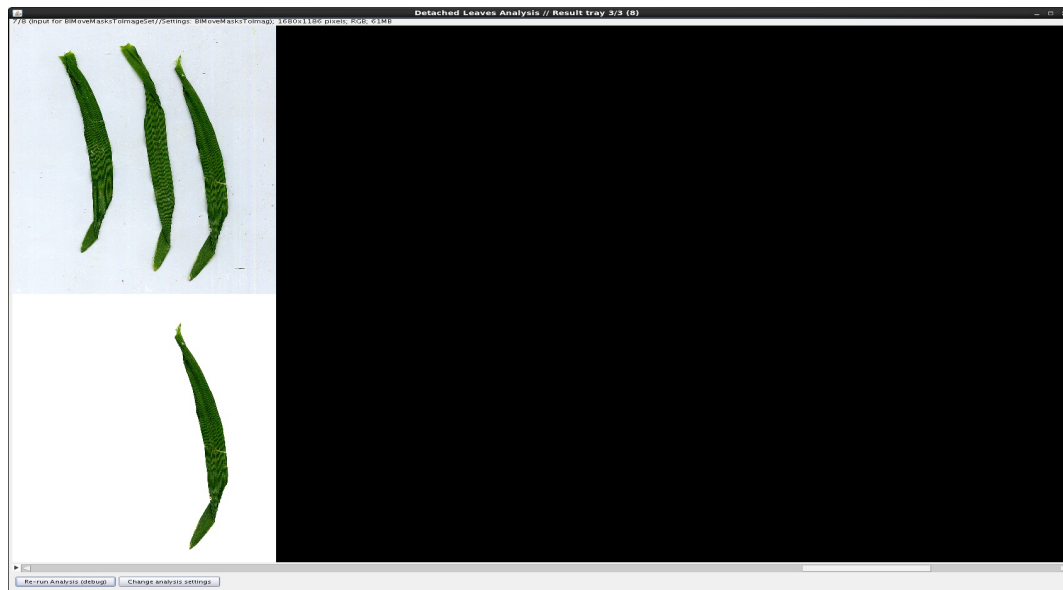

Image browsing, on left side navigation tree, right side contained images.

Menu for image analysis tasks (opened by right clicking on the image).

After calculation the result window appears. It is recommended to check each pipeline-step for any errors in the image processing.


*Result-window, navigation through the image analysis steps by moving the scroll bar.*

3. Modifying settings if needed

In some cases it is necessary to change some analysis settings, most commonly are the background/ foreground definition for the segmentation step. This example will used here as a short introduction in the pipeline modification options.

By clicking on the "Change Analysis Settings" button in the result window or by clicking on "Export/Modify settings" in the main window as shown in the image below you can open the settings window.
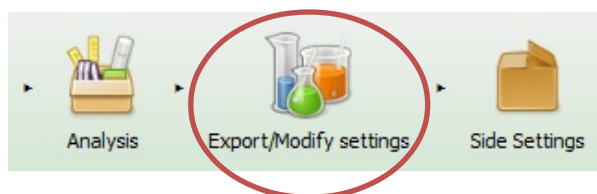
Here choose top settings (depends on your image data, default is top). The opening window includes all block settings. To change the background/foreground navigate to the segmentation options, in this case "Auto-tuning Vis-Segmentation (k-Means)".
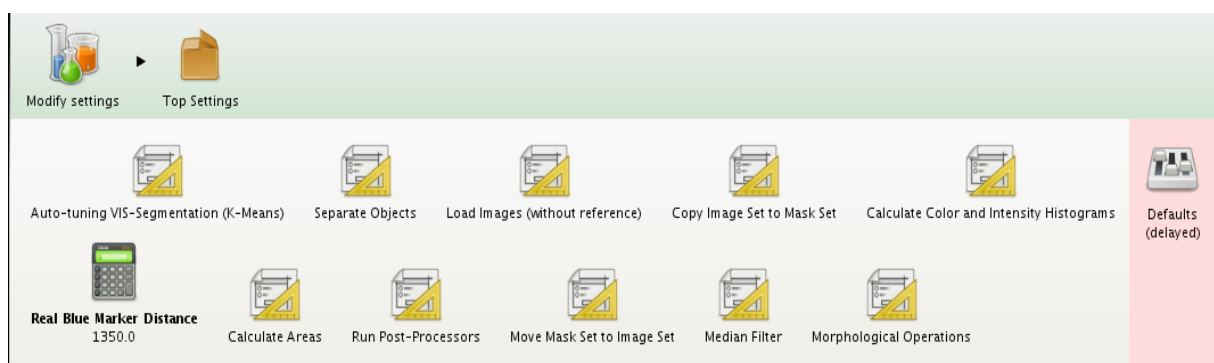
Here define the colors for background and foreground, in this case dark slate blue and dark khaki were chosen (Accepted are the color name, RGB-, HSV- or L*a*b*-values). The k-Means algorithm assign a pixel to a color class based on the euclidean distance.
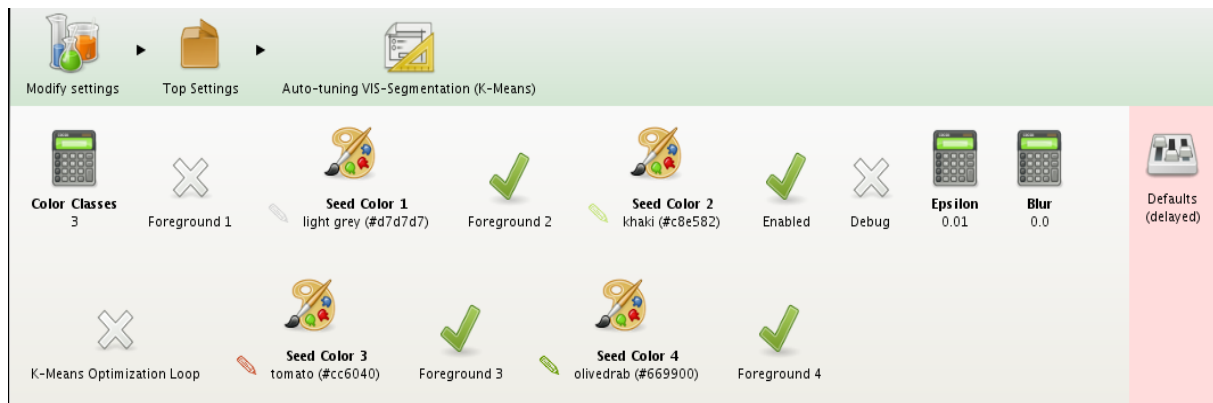

Window for changing settings of the analysis block "Auto-tuning VIS-Segmentation (k-Means)", if "Foreground" is checked for a color, all segments belonging to this color class will be processed as foreground (in this example as root). Result traits are only processed for the foreground classes.

To define the number of detached leaves on an image go back and choose the options for "Separate Objects". Here change the value of "Maximum Object Count" to the number of leaves which are visible in your image configuration (in our example this would be 3).

(To get further information about pipeline tuning and customization, please use our complete user-documentation which is published on our website. http://iap.ipk-gatersleben.de/documentation.pdf)

4. Start Analysis of the Complete Dataset
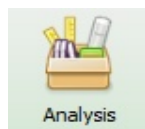
After checking the pipeline the analysis of the whole dataset can be started. Go back to "Analysis" and click on "Process Detached Leaves Analysis". Now the calculation begins. The duration of the calculation depends on the processed dataset (number/size/dimension of images) and on your computation system.
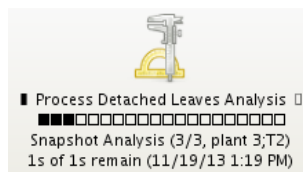
During analysis the analysis button shows the progress. After a while the last line shows the amount of time, that is left to complete the analysis and the amount of time the analysis will overall take. Finally, the date and time of the estimated finish time is shown. After the analysis is completed, the result is automatically saved to the file system location.
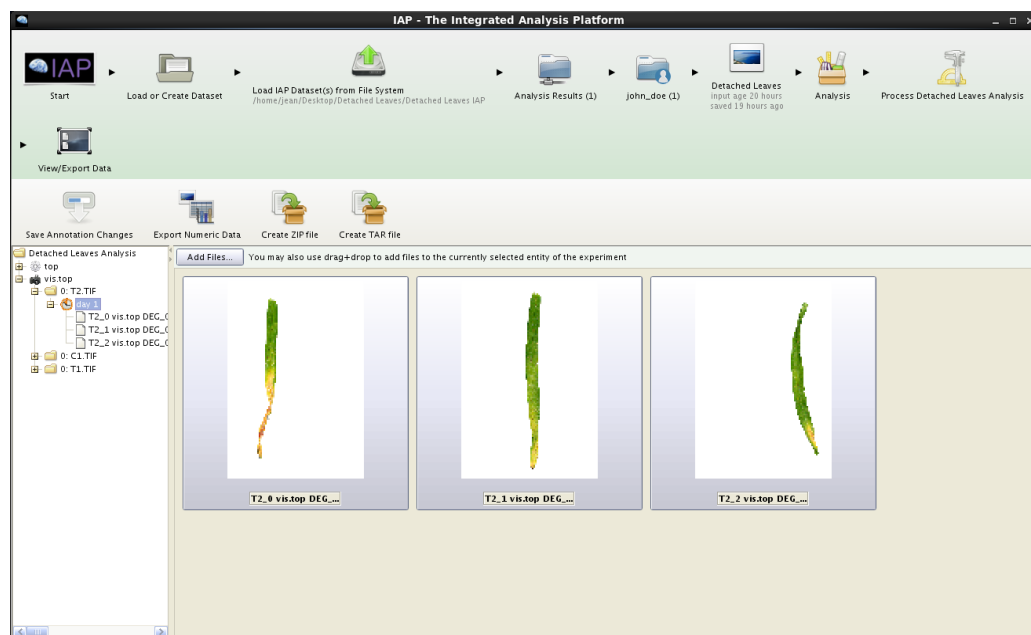
View/Export result data

The analysis result data set is automatically loaded after completion of the analysis. Now you could browse the result images or export data.
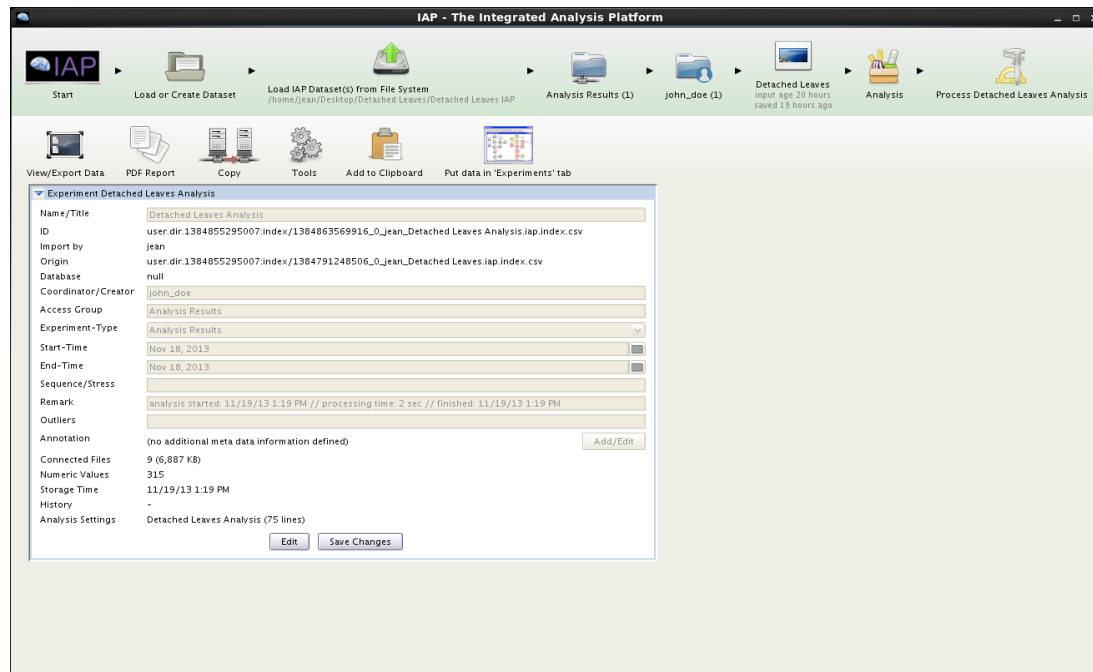
Appearing overview window after calculation including analysis results.

1. View/Export Data

Click "View/Export Data", to browse through the analysis results over time. In our example each image would be separated into 3 leaf images.

Browsing of the result data (navigating through the tree on the left, result images and numeric results can be chosen; on the right the result images will be displayed).

2. Export Numeric Data

By clicking on "Export Numeric Data" you can export the numeric data in different data formats. To export the whole data set in form of an Excel file, click "Create Spreadsheet (XLSX) (complete...)" for .csv click "Create Spreadsheet (CSV)". Other options are not useful in case of the root analysis.
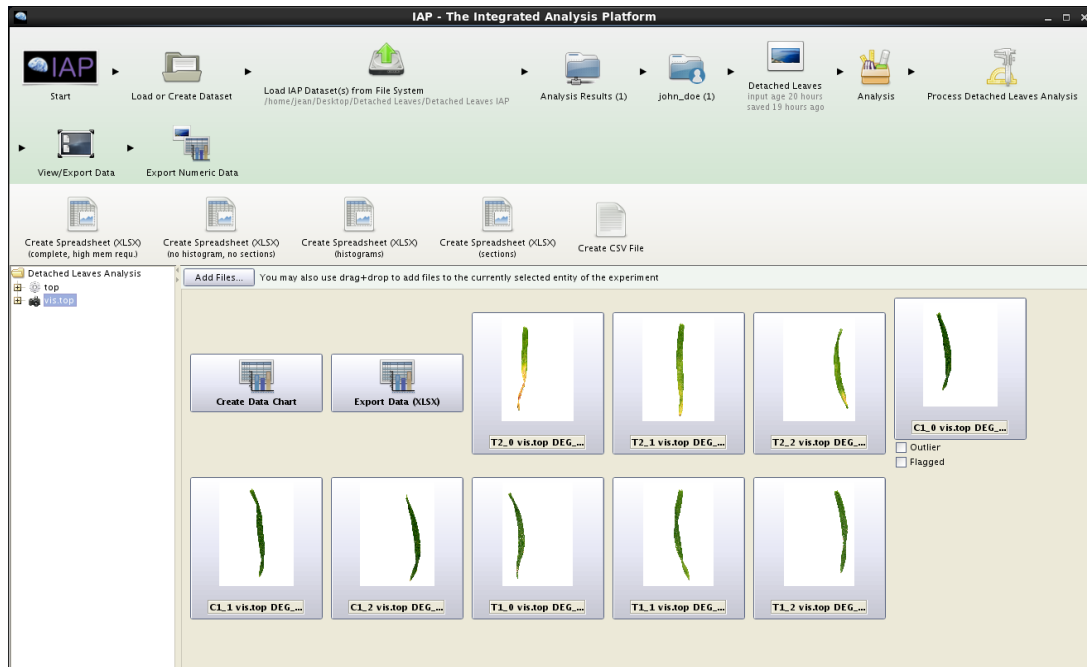
Window including the export functions, .xlsx and .csv export buttons are marked.

3. Post Processing

To investigate the result data set further and individually, it is recommended to export the data as XLSX (as previously described). Once opened, the quickest way to group, filter and plot selected traits is the 'Pivot' function of Excel.

Trait	Example value	Explanation
Plant ID	1302_01	Unique identification number for each plant and leaf index from 0 up to leaf number of an image (counted from left to right, beginning with zero).
top.vis.hvs.h.average top.vis.hsv.s.average top.vis.hsv.v.average	0.227	Mean value for the H / S / V value of the HSV color-space[1].
top.vis.hsv.v.stddev top.vis.hsv.v.stddev top.vis.hsv.v.stddev	0.041	Standard deviation for the H / S / V values of the pixel colors. A small value indicates similar leaf colors, a high value indicates a diverse set.
top.vis.area (px)	1832	Calculated leaf area in pixel.

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[1] see http://en.wikipedia.org/wiki/HSL_and_HSV