Image Analysis with IAP

Example Workflow for Root Analysis

Image Analysis Group - Leibniz Institute of Plant Genetics and Crop Plant Research IPK, Corrensstr. 3, 06466 Gatersleben

Jean-Michel Pape

Rev 1 for IAP version 1.1, November 2013

This is a part of the user manual for the IAP (Integrated Analysis Platform). This documentation describes especially the workflow for loading phenotypic image data, which was acquired with a manual scanner system or a digital camera. In the second part, the setup for the image analysis of root data will be shown in detail. Finally the result export function will be explained shortly.

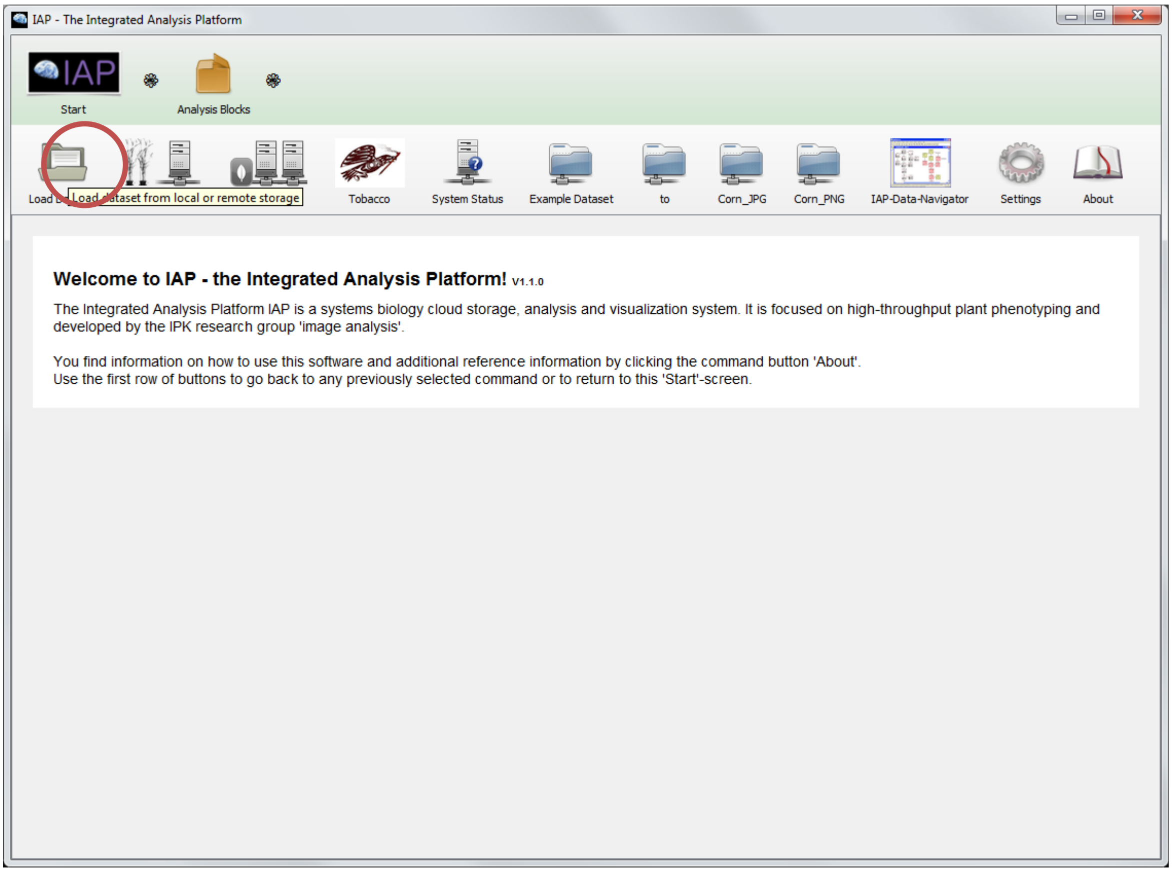
For this analysis an external Add-On is required. An Explanation is provided on our website in the section Add-ons http://iap.ipk-gatersleben.de/#addons.

(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.)

Load image data and create 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”.



Welcome to IAP - the Integrated Analysis Platform! v1.1.0

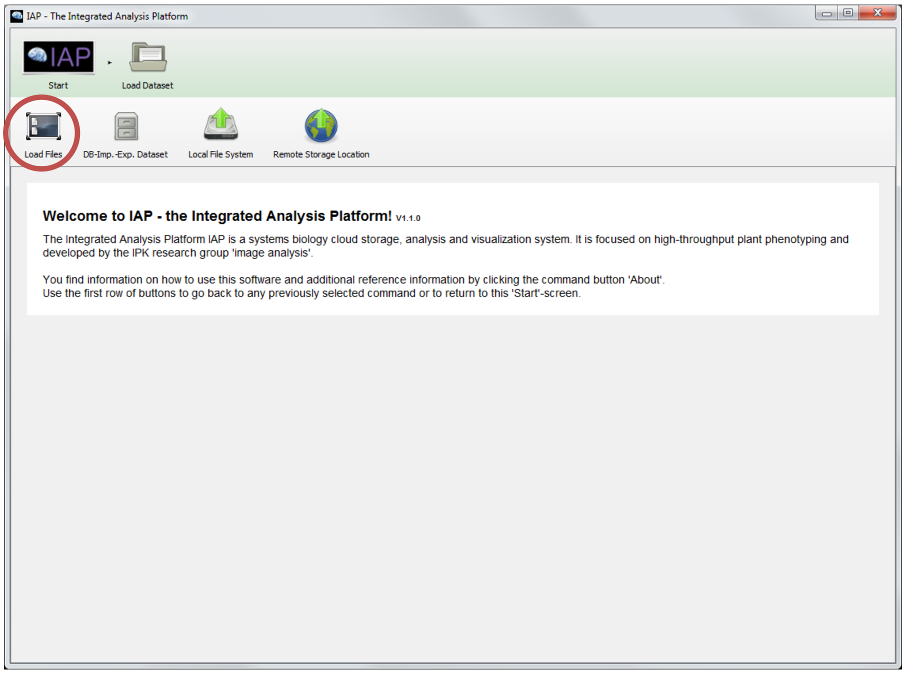
The Integrated Analysis Platform IAP is a systems biology cloud storage, analysis and visualization system. It is focused on high-throughput plant phenotyping and developed by the IPK research group 'image analysis'.

You find information on how to use this software and additional reference information by clicking the command button 'About'.
Use the first row of buttons to go back to any previously selected command or to return to this 'Start'-screen.

Figure 1 - 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.

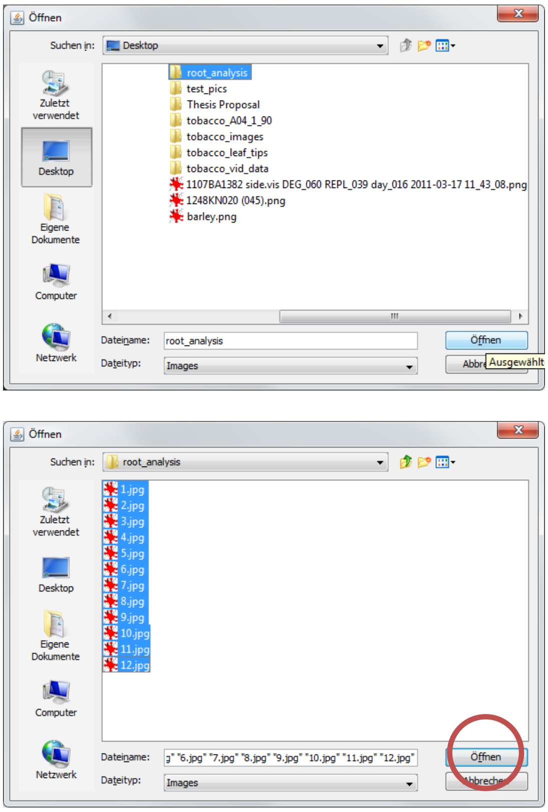


Welcome to IAP - the Integrated Analysis Platform! V1.1.0

The Integrated Analysis Platform IAP is a systems biology cloud storage, analysis and visualization system. It is focused on high-throughput plant phenotyping and developed by the IPK research group 'image analysis'.

You find information on how to use this software and additional reference information by clicking the command button 'About'.
Use the first row of buttons to go back to any previously selected command or to return to this 'Start'-screen.

Figure 2 - Overview window about the different creating/loading methods. Click the first to create an IAP dataset.



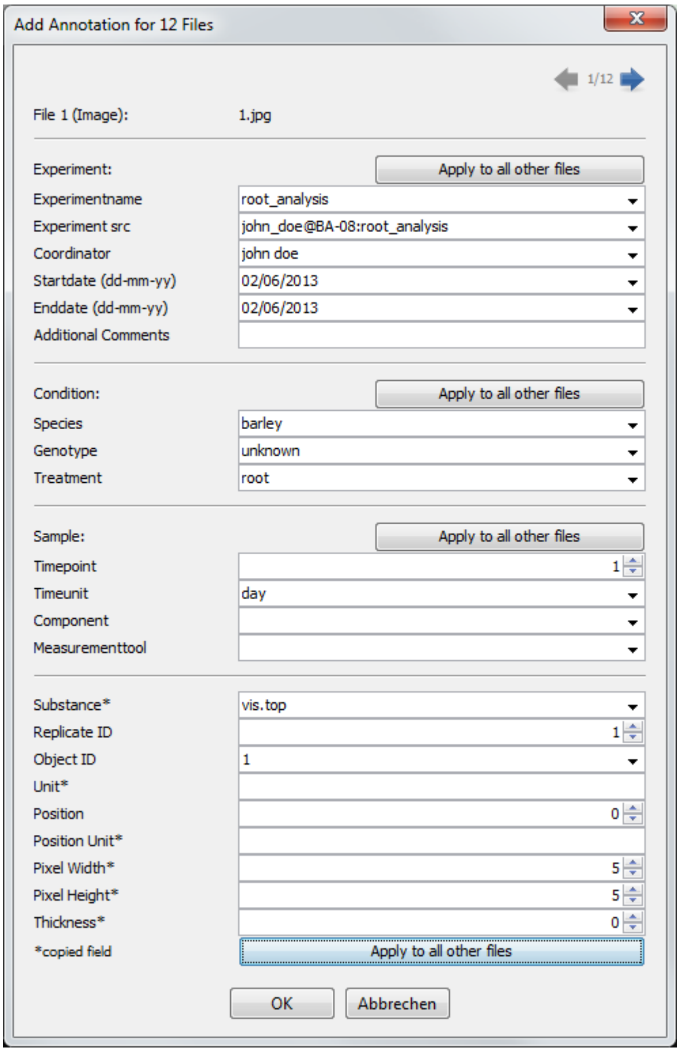
Dateiname: root_analysis
Dateityp: Images

Dateiname: g" "6.jpg" "7.jpg" "8.jpg" "9.jpg" "10.jpg" "11.jpg" "12.jpg"
Dateityp: Images

Figure 3 – Dialog to choose target image files.

3. Adding some additional Information.

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



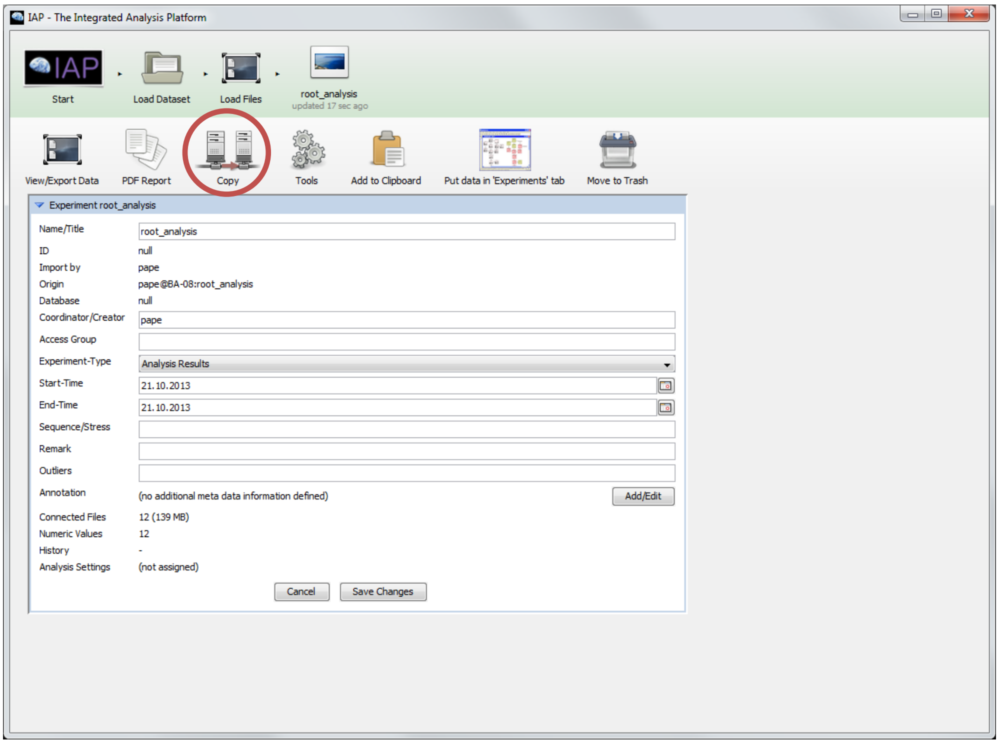
File 1 (Image):	1.jpg
Experiment:	
Experimentname	root_analysis
Experiment src	john_doe@BA-08:root_analysis
Coordinator	john doe
Startdate (dd-mm-yy)	02/06/2013
Enddate (dd-mm-yy)	02/06/2013
Additional Comments	
Condition:	
Species	barley
Genotype	unknown
Treatment	root
Sample:	
Timepoint	1
Timeunit	day
Component	
Measurementtool	
Substance*	vis.top
Replicate ID	1
Object ID	1
Unit*	
Position	0
Position Unit*	
Pixel Width*	5
Pixel Height*	5
Thickness*	0
*copied field	

Figure 4 – 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.

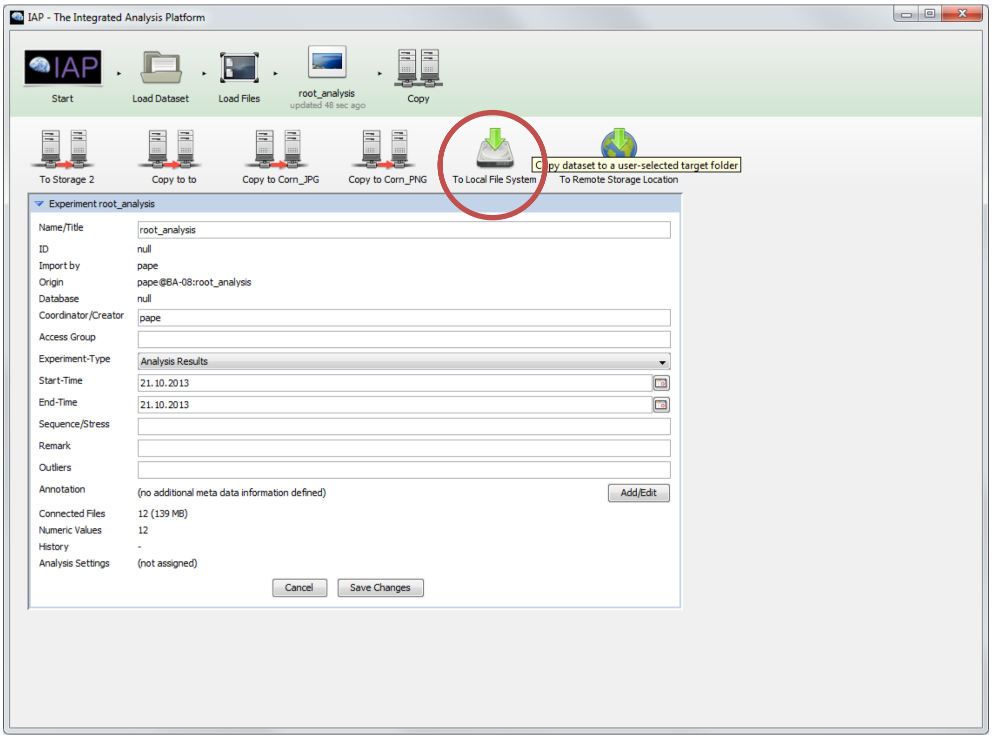
Save the experiment on the hard drive by clicking the “Copy” button as shown in the next image.



Experiment root_analysis

Name/Title	root_analysis
ID	null
Import by	pape
Origin	pape@BA-08:root_analysis
Database	null
Coordinator/Creator	pape
Access Group	
Experiment-Type	Analysis Results
Start-Time	21.10.2013
End-Time	21.10.2013
Sequence/Stress	
Remark	
Outliers	
Annotation	(no additional meta data information defined)
Connected Files	12 (139 MB)
Numeric Values	12
History	-
Analysis Settings	(not assigned)

To save on local file system choose the following option as shown below.



Experiment root_analysis

Name/Title	root_analysis
ID	null
Import by	pape
Origin	pape@BA-08:root_analysis
Database	null
Coordinator/Creator	pape
Access Group	
Experiment-Type	Analysis Results
Start-Time	21.10.2013
End-Time	21.10.2013
Sequence/Stress	
Remark	
Outliers	
Annotation	(no additional meta data information defined)
Connected Files	12 (139 MB)
Numeric Values	12
History	-
Analysis Settings	(not assigned)

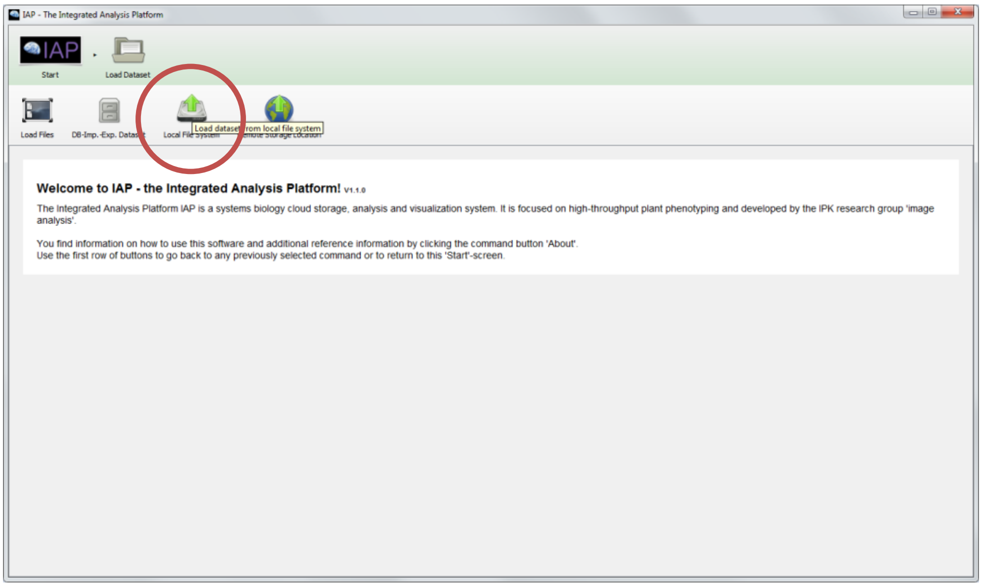
Now choose your desired target directory and voila the IAP experiment will be saved. This may be taking some time depending on the number of images, image quality and your system speed.

Load and analyse an IAP "root" 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 adaption), 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 …



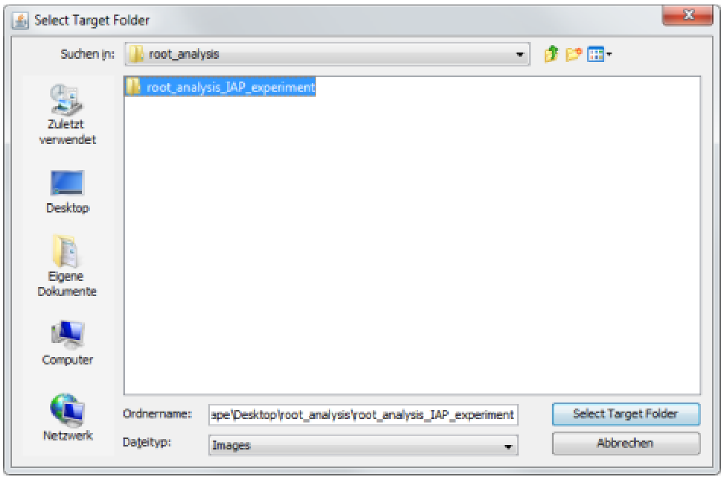
Welcome to IAP - the Integrated Analysis Platform! v1.1.0

The Integrated Analysis Platform IAP is a systems biology cloud storage, analysis and visualization system. It is focused on high-throughput plant phenotyping and developed by the IPK research group 'image analysis'.

You find information on how to use this software and additional reference information by clicking the command button 'About'.
Use the first row of buttons to go back to any previously selected command or to return to this 'Start'-screen.

Figure 5 – Again the window for loading data. Now click "Load IAP Dataset(s) from File System" instead of "Create IAP Dataset from Files".

… and choose the directory where you saved the experiment before and …



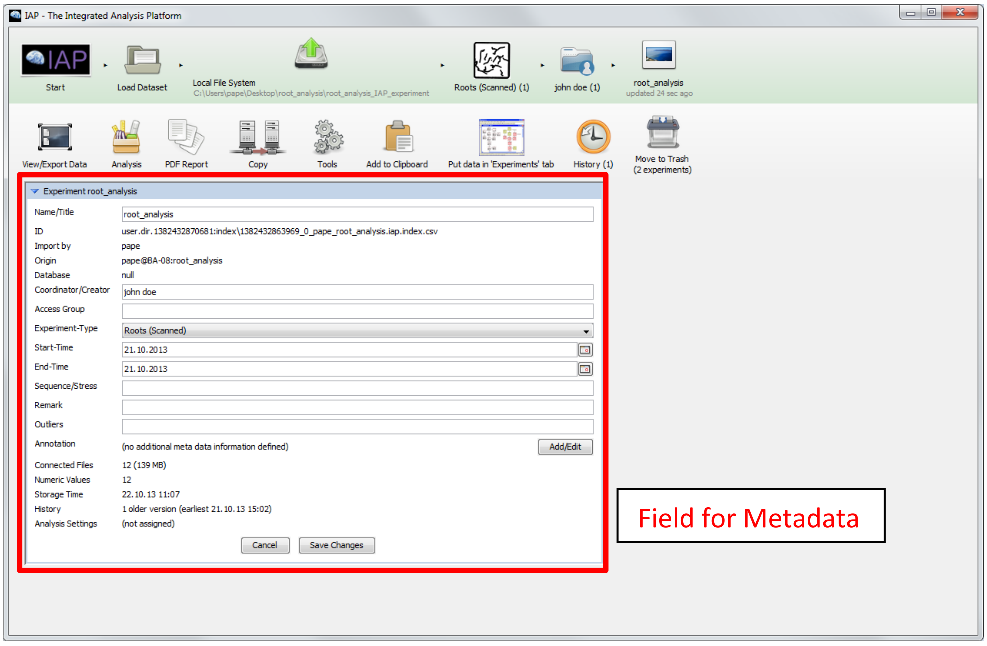
Suchen in: root_analysis

Ordnername: ape\Desktop\root_analysis\root_analysis_IAP_experiment

Dateityp: Images

… navigate to the experiment overview screen by clicking on …

Now you are on the "experiment overview window". Here are the options to add some additional metadata and information. After modifying, just click "Save Options" and your changes will be added to the experiment.



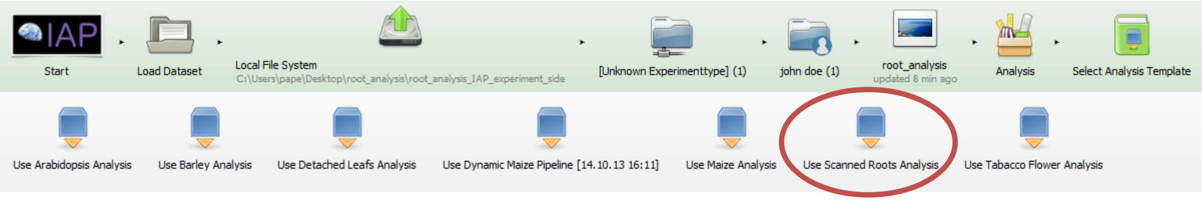
Name/Title: root_analysis
ID: user.dir.1382432870681:index\1382432863969_0_pape_root_analysis.iap.index.csv
Import by: pape
Origin: pape@BA-08:root_analysis
Database: null
Coordinator/Creator: john doe
Access Group:
Experiment-Type: Roots (Scanned)
Start-Time: 21.10.2013
End-Time: 21.10.2013
Sequence/Stress:
Remark:
Outliers:
Annotation: (no additional meta data information defined)
Connected Files: 12 (139 MB)
Numeric Values: 12
Storage Time: 22.10.13 11:07
History: 1 older version (earliest 21.10.13 15:02)
Analysis Settings: (not assigned)

Field for Metadata

Figure 6 – "Experiment overview screen", on top are the navigation path, in the second row some action buttons and below the "experiment information (metadata) Field".

2. 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 root analysis.



Now you have assigned a template for the image processing. A template includes an image processing pipeline which consists of several image processing blocks. Each block fulfils a special image processing task.

1. ***Load Images (without reference)***
2. ***Crop Sides***
3. ***Copy Image Set to Mask Set***
4. ***Auto-tuning VIS-Segmentation (k-Means)***
5. ***Morphological Operations***
6. ***Remove Small Noise Objects***
7. ***Root Skeletonization***
8. ***Run Post-Processors***
9. ***Move Mask Set***

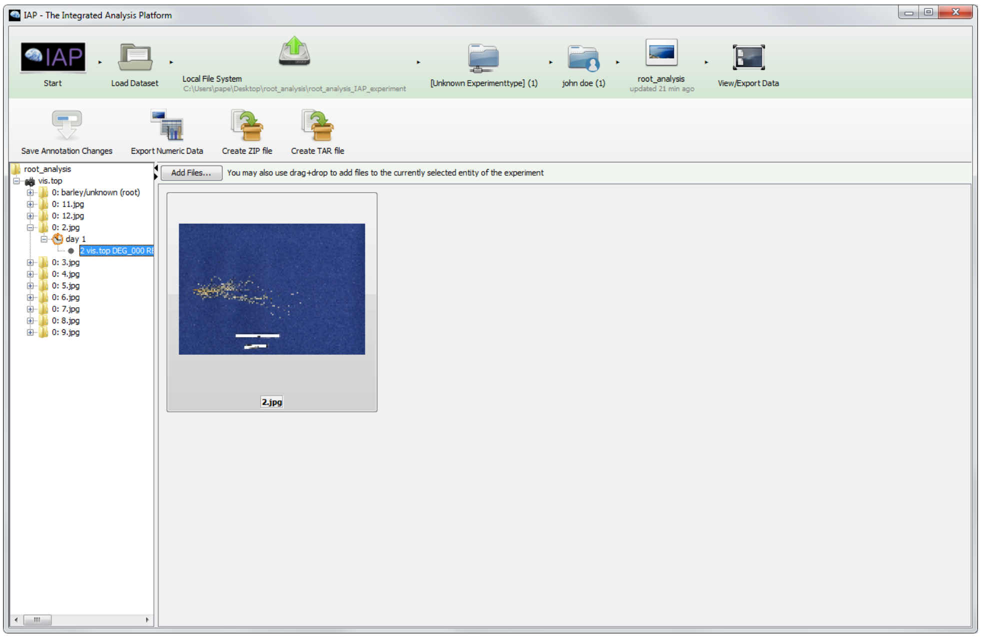
You can get detailed information about each block in our main documentation (see documentation http://iap.ipk-gatersleben.de/documentation.pdf). Special block in the root analysis pipeline is "BlRootsSkeletonize", this calculates all root traits like area, number of endpoints, and branches etc.

The algorithm calculates an image where the foreground (root) is shrinking to a one pixel width line, the so called skeleton. This is the base for further calculations.

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

3. Image Browsing and test pipeline settings.

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 “Scanned Roots Analysis” option in the appearing window (see figure 8).



You may also use drag+drop to add files to the currently selected entity of the experiment

Figure 7 – Image browsing, on left side navigation tree, right side contained images.

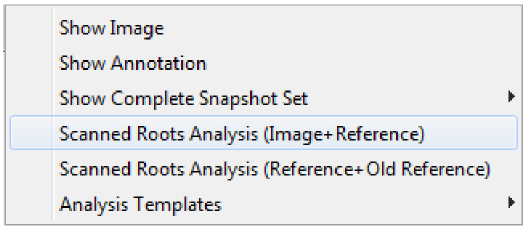


Figure 8 – menu for image analysis tasks (opened by right clicking on the image).

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

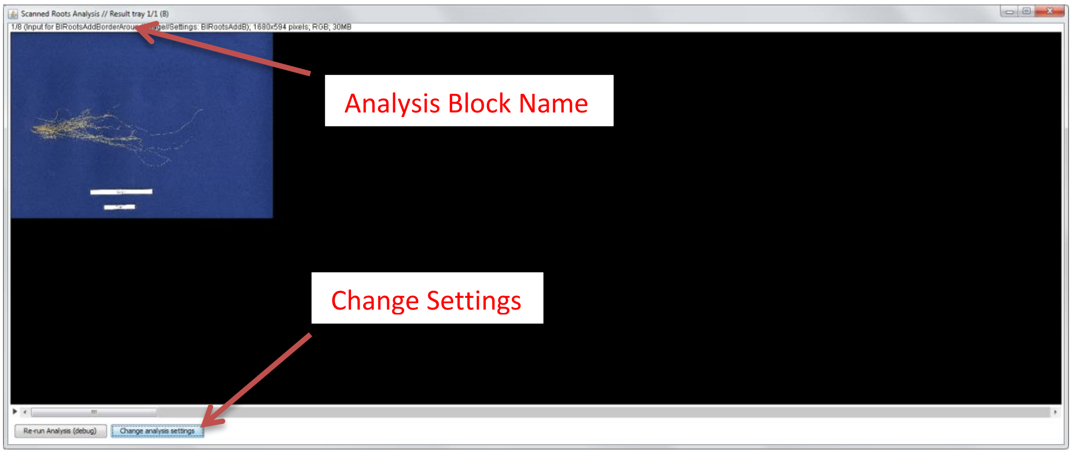
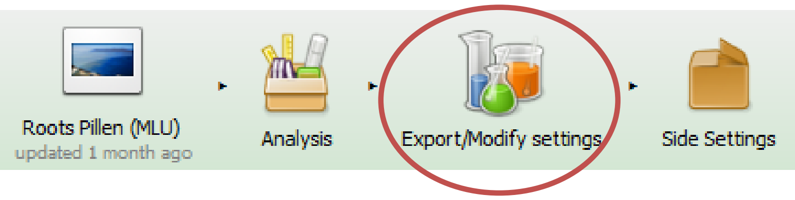


Figure 9 – Result-window, navigation through the image analysis steps by moving the scroll bar.

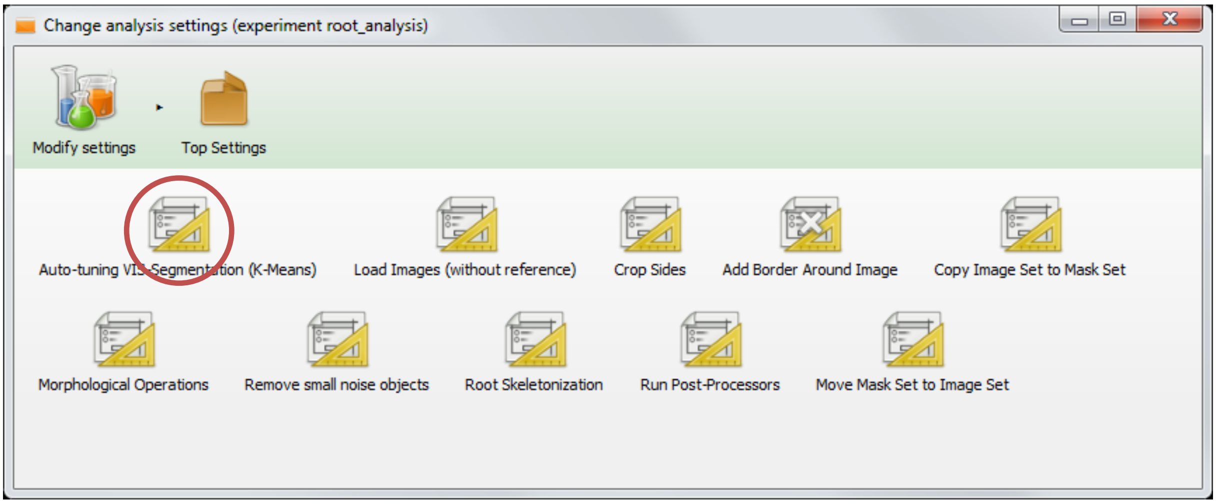
4. Modifying settings if needed.

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.

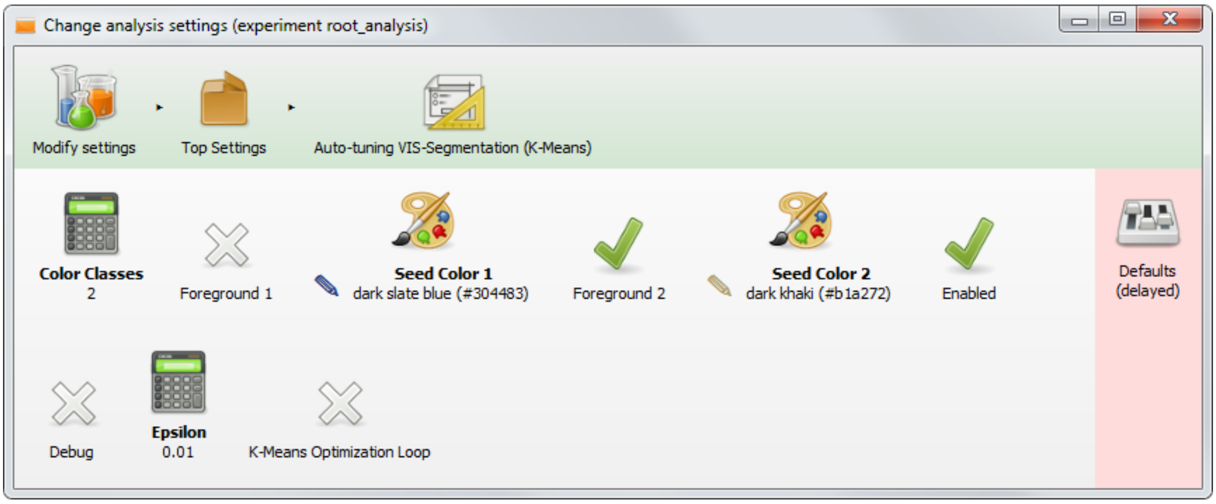
By clicking on the “Change Analysis Settings” button in the result window or by clicking on “Export/Modify settings” in the main window as shown in the image below you can open the settings window.



Here choose top- or side 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 (Acepted are the color name, RGB-, HSV- or L*a*b*-values). The k-Means algorithmen assign a pixel to a color class based on the euclidian distance.



Change analysis settings (experiment root_analysis)

Modify settings ▸ Top Settings ▸ Auto-tuning VIS-Segmentation (K-Means)

Color Classes 2

☒ Foreground 1

Seed Color 1 dark slate blue (#304483)

✓ Foreground 2

Seed Color 2 dark khaki (#b1a272)

✓ Enabled

☒ Debug

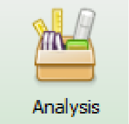
Epsilon 0.01

☒ K-Means Optimization Loop

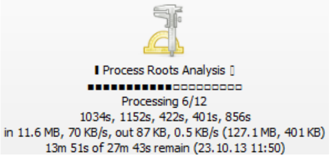
Figure 10 – Window for changing settings of the analysis block "Auto-tuning VIS-Segmentation (k-Means)", if "Foreground" is checked for a color, all segments belonging to this color class will be processed as foreground (in this example as root). Result traits are only processed for the foreground classes.

(To 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)

5. Start analysis for whole dataset.



After checking the pipeline the analysis of the whole dataset can be started. Go back to "Analysis" and click on "Process Root 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.

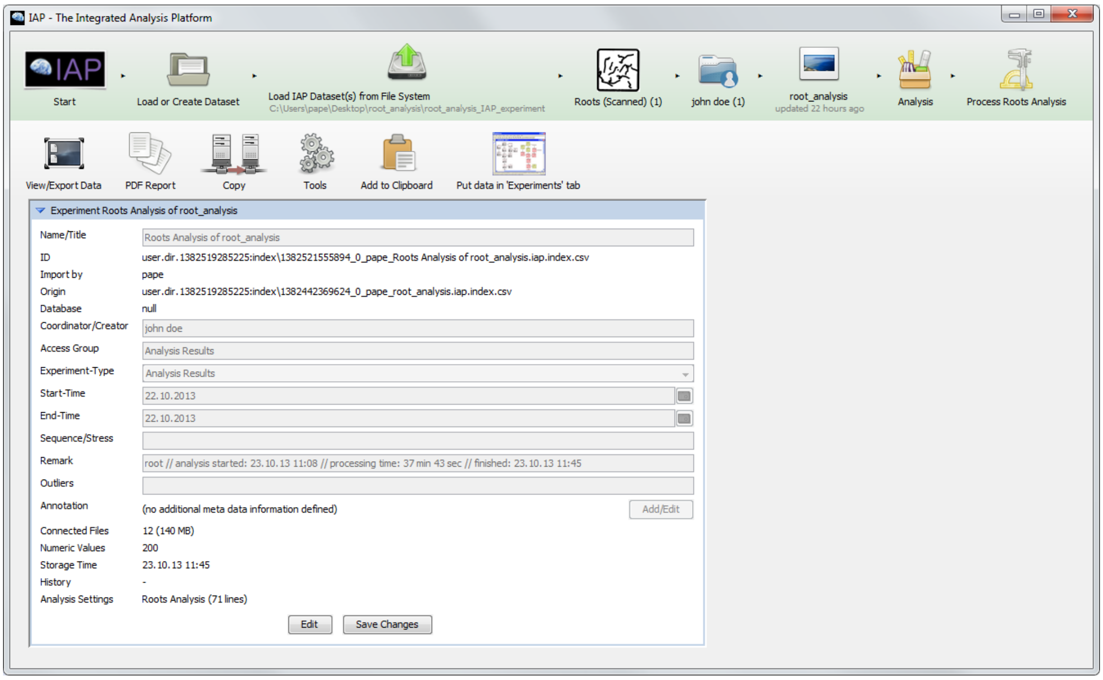


```
▌Process Roots Analysis ▯
■■■■■■■■■■■□□□□□□□□□
Processing 6/12
1034s, 1152s, 422s, 401s, 856s
in 11.6 MB, 70 KB/s, out 87 KB, 0.5 KB/s (127.1 MB, 401 KB)
13m 51s of 27m 43s remain (23.10.13 11:50)
```

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 saved to the file system location.

View/Export result data

1. The analysis result data set is automatically loaded after completion of the analysis.

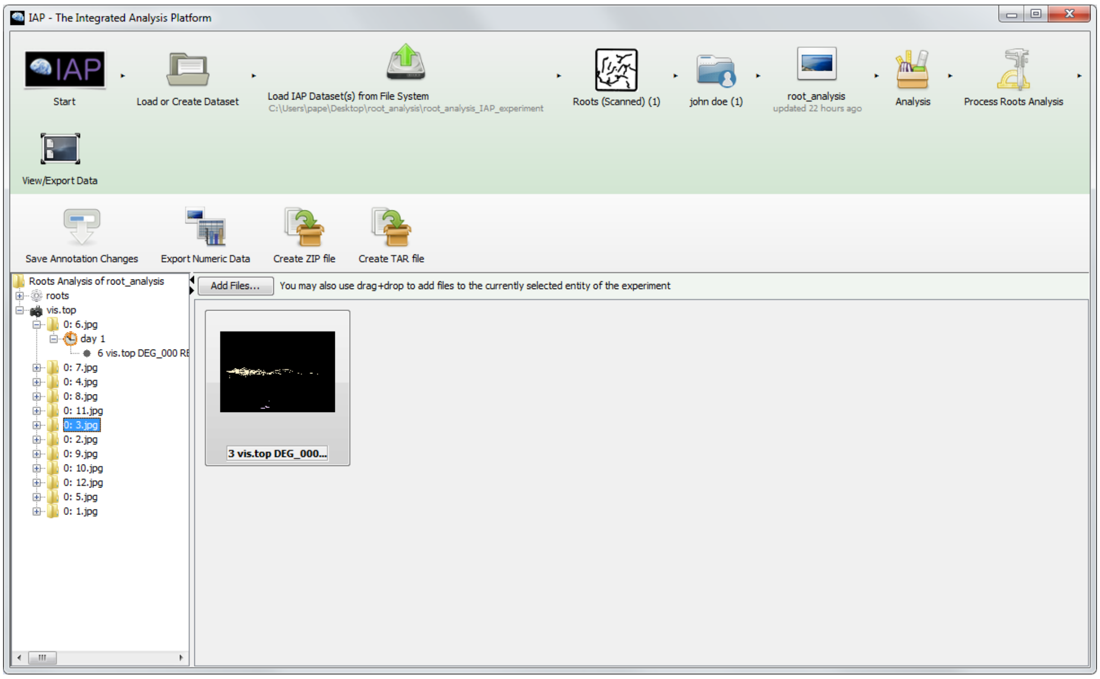


Experiment Roots Analysis of root_analysis

Name/Title: Roots Analysis of root_analysis
ID: user.dir.1382519285225:index\1382521555894_0_pape_Roots Analysis of root_analysis.iap.index.csv
Import by: pape
Origin: user.dir.1382519285225:index\1382442369624_0_pape_root_analysis.iap.index.csv
Database: null
Coordinator/Creator: john doe
Access Group: Analysis Results
Experiment-Type: Analysis Results
Start-Time: 22.10.2013
End-Time: 22.10.2013
Sequence/Stress:
Remark: root // analysis started: 23.10.13 11:08 // processing time: 37 min 43 sec // finished: 23.10.13 11:45
Outliers:
Annotation: (no additional meta data information defined)
Connected Files: 12 (140 MB)
Numeric Values: 200
Storage Time: 23.10.13 11:45
History: -
Analysis Settings: Roots Analysis (71 lines)

Figure 11 – Appearing overview window after calculation.

2. Click “View/Export Data”, to browse through the analysis results over time.

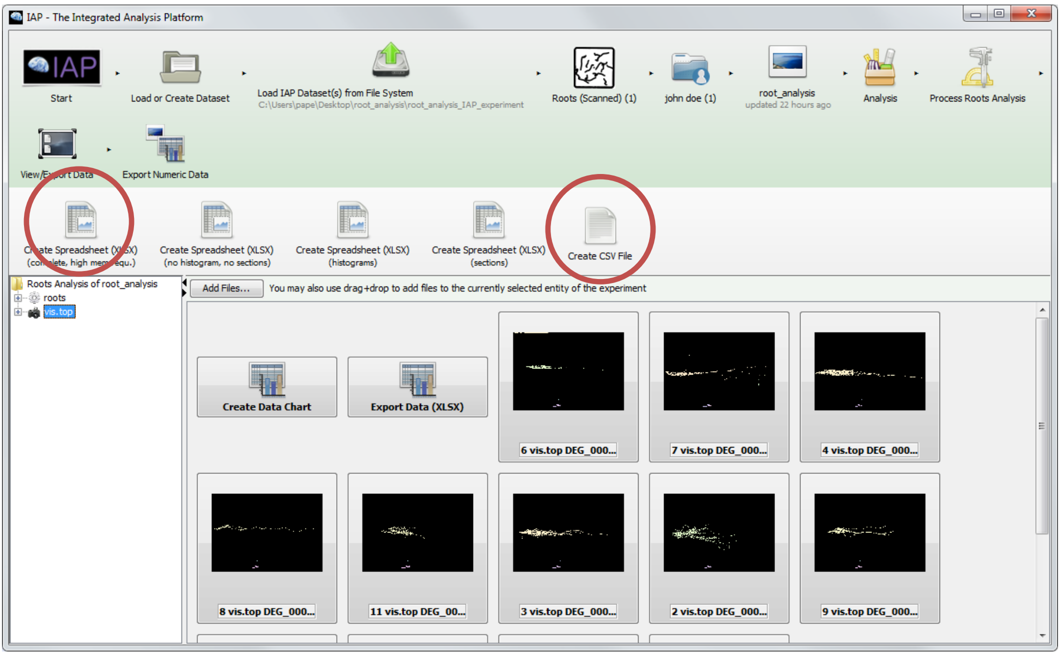


You may also use drag+drop to add files to the currently selected entity of the experiment

Figure 12 – 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).

3. 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.



You may also use drag+drop to add files to the currently selected entity of the experiment

Figure 13 – Window including the export functions, .xlsx and .csv export buttons are marked.

4. 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.

Appendix

Evaluation

The IAP root pipeline was evaluated with a representative dataset of root scans. The image data were analysed with IAP and WinRhizo, which is an established software tool in the field of biology (see http://www.regent.qc.ca/assets/winrhizo_about.html). The results show that IAP provides mostly similar results to WinRhizo:

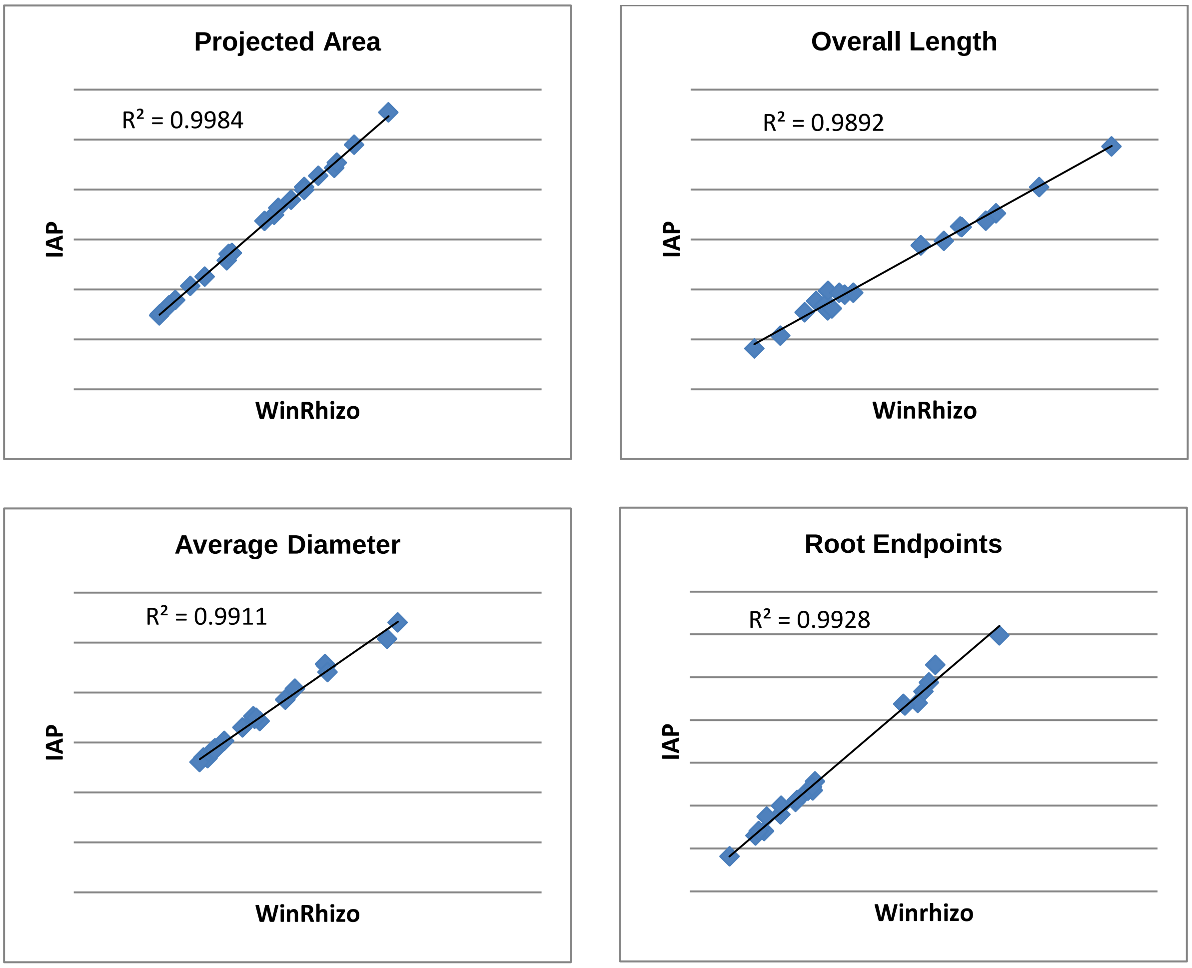


Figure 14 – Correlated results from IAP and WinRhizo (Trait names were unified for a better understanding).