

# An automated image-processing pipeline for high-throughput analysis of root architecture in OpenAlea

J. Diener<sup>1</sup>, P. Nacry<sup>2</sup>, C. Périn<sup>3</sup>, A. Dievart<sup>3</sup>, X. Draye<sup>4</sup>, F. Boudon<sup>1</sup>, A. Gojon<sup>2</sup>, B. Muller<sup>5</sup>, C. Pradal<sup>1</sup>, C. Godin<sup>1</sup>

(1) **Virtual Plants**, INRIA - CIRAD - INRA, Montpellier, 34398, France

(3) **DAR**, CIRAD, Montpellier, 34060, France

(5) **LEPSE**, SPIC, INRA, Montpellier, 34060, France

(2) **BPMP**, Intégration des fonctions nutritives, INRA, Montpellier, 34060, France

(4) **Earth and Life Institute**, Louvain-la-Neuve, 1348 Belgium

## Abstract

Automated acquisition systems of Root System Architecture (RSA) are now readily available for developmental research and provide high-throughput image data of roots. Existing acquisition systems provide many types of data, from images of dispersed root pieces to full 3d scans of underground root systems. Here we consider RSA grown in Petri plates. This is a traditional experimental protocol used for high-throughput image acquisition. Their analysis is thus a major challenge for researches on root development.

In this poster, we present a solution for extracting the full root architecture automatically and on large-scale data sets.

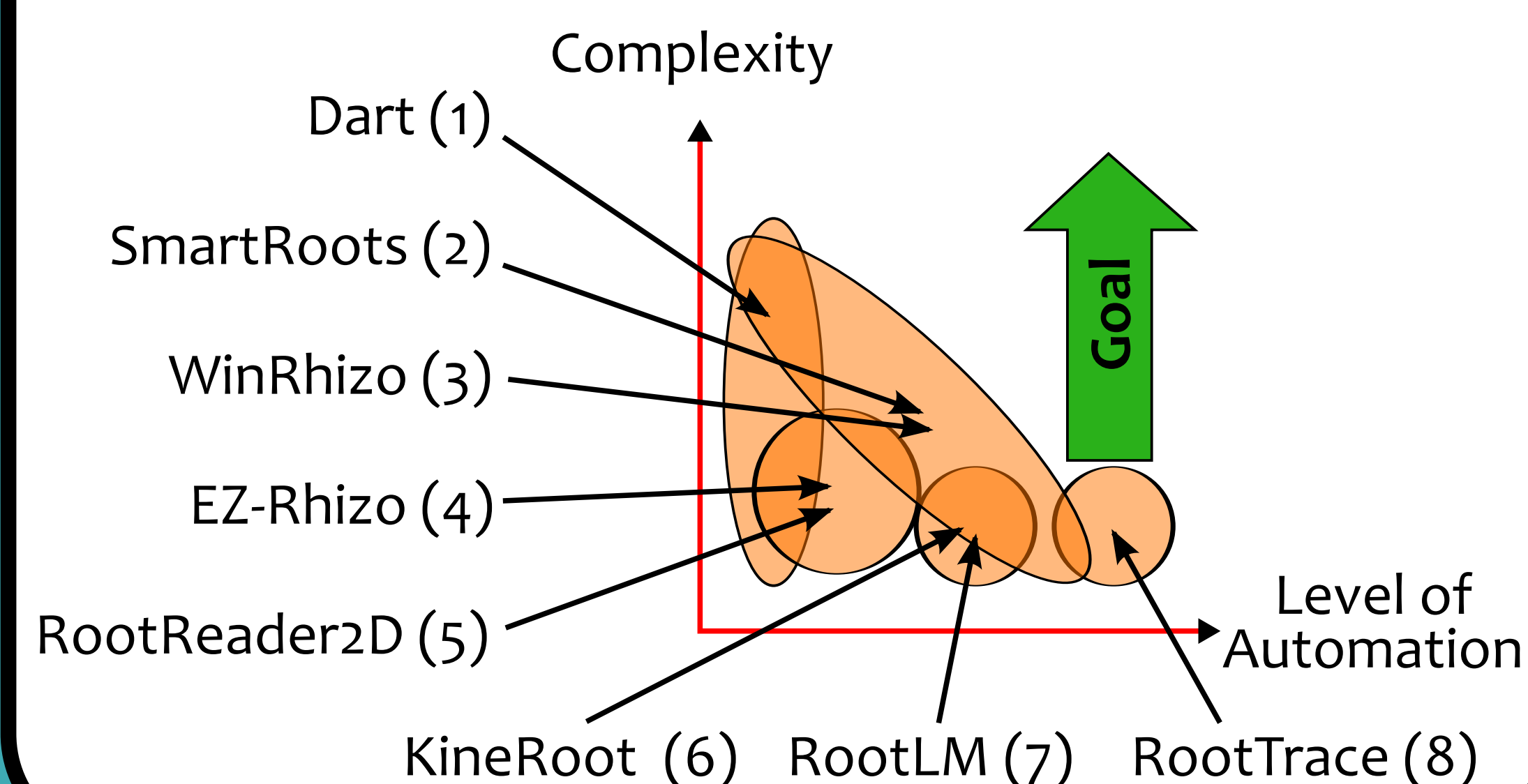
Because our framework is included in the OpenAlea platform, in addition to traditional data analysis such as the ones provided by existing software, the extracted data can also be used seamlessly as input of all the architectural analysis packages already contained in OpenAlea.

## Material

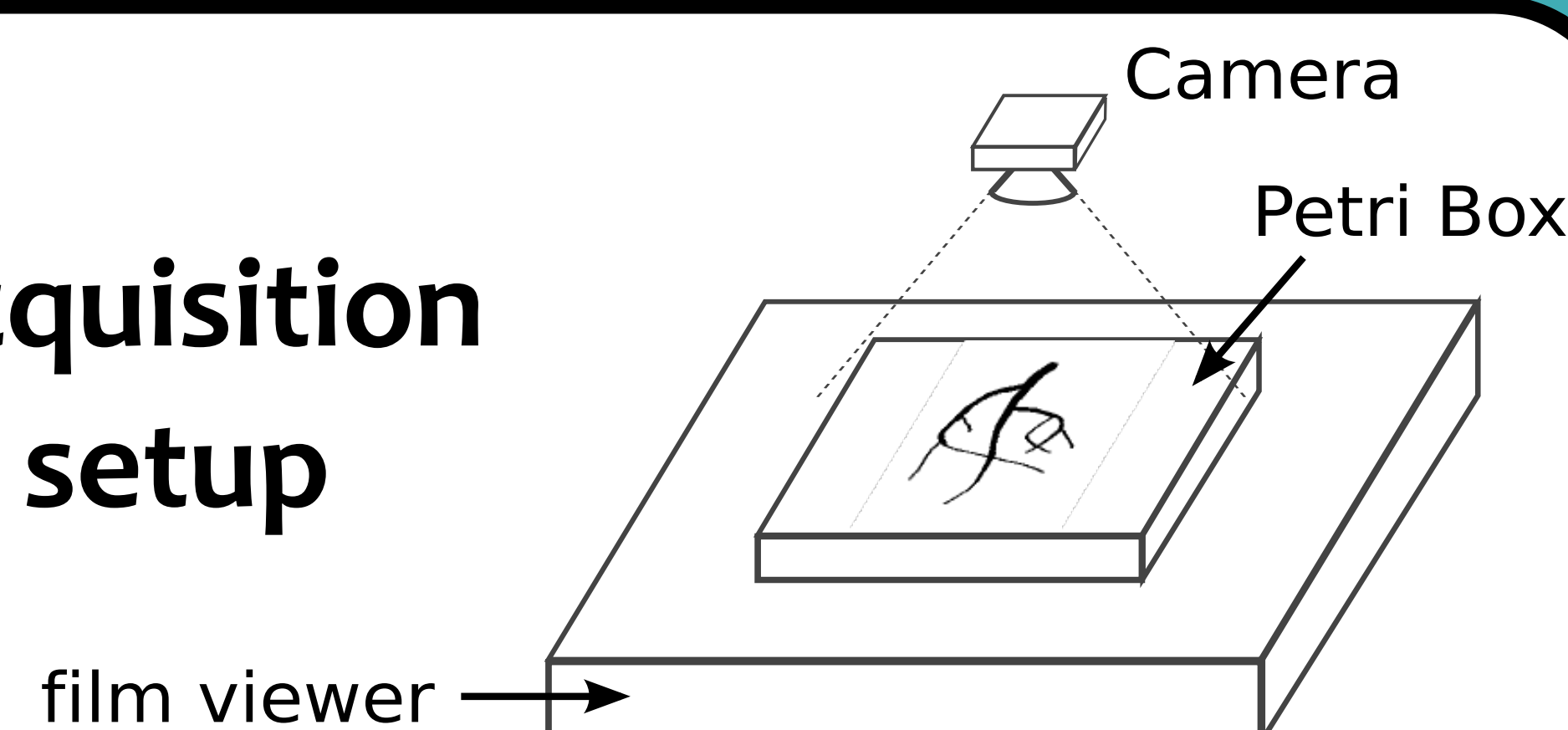
The goal of the presented work is to provide a computer software which is able to process experimental image data of roots obtained using practical imaging protocol. We consider images of root grown in petri plates acquired using a scanner or with a digital camera (see the acquisition setup scheme).

For the purpose of development and validation, two data sets have been used. The first set studies the growth of nine genotypes of *Arabidopsis thaliana* in three different nitrate concentrations. For each of these experimental modalities, four Petri boxes containing each five root systems are daily imaged during six days. The second data set contains images of rice (*Oryza sativa* of the nipponbarre genotype) in eight Petri boxes, each containing five root systems, which were scanned once per day for a 6 days period. In total both data sets represent 688 images and 3440 imaged root systems. Comparisons with expert measurements have been done for around 300 plants.

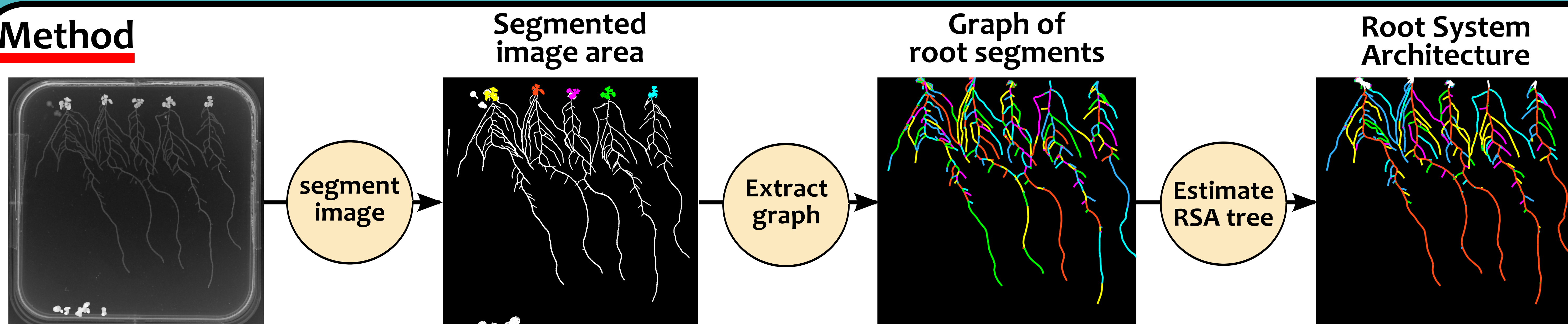
## Root architecture complexity and system automation



## Acquisition setup



## Method



### Image segmentation:

Initial root images have to be segmented. First the smooth but non-uniform background lighting is estimated based on minimum pixel intensity with large-scale distribution. It is then removed from the original images. Second, the image is segmented into three areas:

- the petri plate
- the seed and leaves, which are treated as the start of the root architecture
- the pixel area containing root axes

The segmentation is done using the Expectation Maximization algorithm on pixel luminosity, and taking advantage of relative shape attributes (e.g. the Petri plate contains all the plants).

### Extraction of the root graph:

The segmented root pixels are clustered into root sections (between branching and crossing) using a thinning algorithm (skeletonization). Then each linear curve of the skeleton is further divided into a set of line segments by fitting a 1st order spline on the curve pixels.

A graph is then created containing the set of root segments as vertices, and the set of all pairs of segments that are contiguous as edges. At this stage, the constructed graph is not necessarily a tree structure as it usually contains loops.

### Estimation of the RSA tree graph:

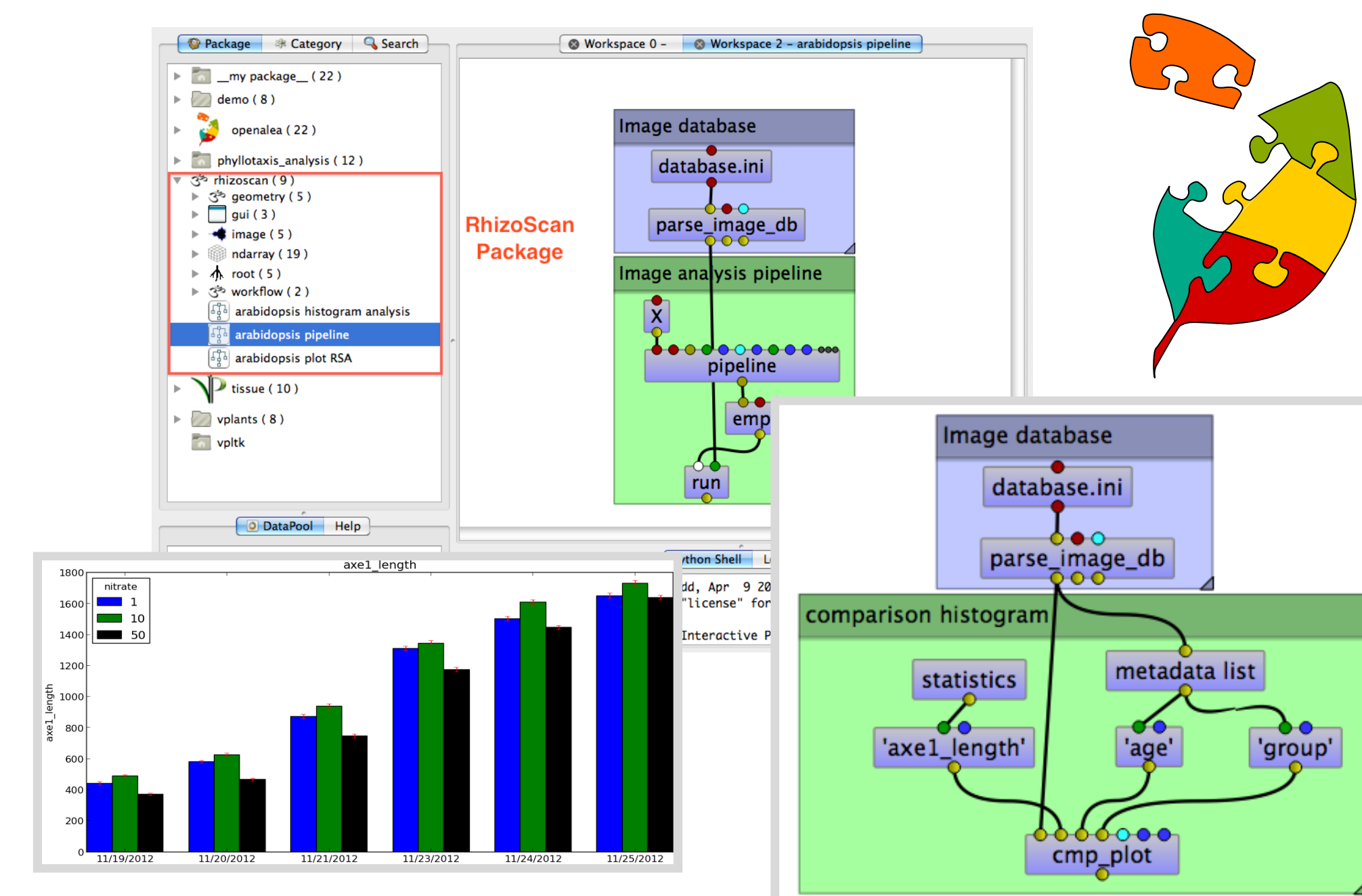
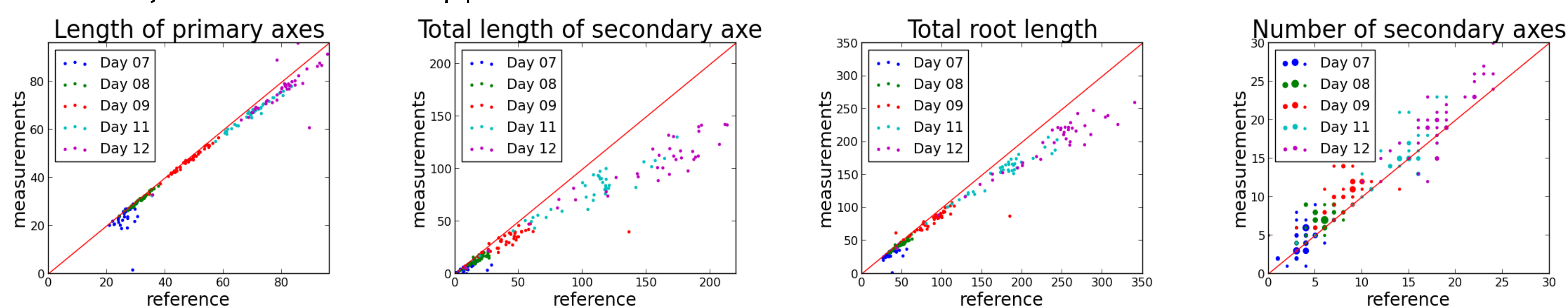
The root architecture is obtained by converting the root graph to an axial tree structure. First the graph is converted to a tree rooted at the detected seeds using the shortest path algorithm. The distance between two segments of the graph is defined as the turning angle generating tree structures with the straightest possible path. This conversion breaks graph loops, meaning that it solves the axe crossing ambiguities.

The second pass then identifies the branching relationship by selecting for each parent segment the direct child that belongs to the same root axe. The selection is also done in order to minimize the axes curvature.

## Results

In order to quantify the accuracy of our reconstruction, reference data were obtained by manual annotation of the analysed root systems. Experts manually specified the root architectures on a serie of images. Then selected measurements made on these reference root systems were compared with those on the structures automatically reconstructed with our pipeline.

Here the comparison of 200 root systems of the *Arabidopsis* data set are presented. On the six days period of the measurement, we can see that our measurement of the main axes length fits well to the reference data: mean percent error is less than 5% after outlier removal. The total root length (and secondary axes length) is also close to the reference measurement up to day 9 with an average percentage error less than 10%, but then scatter for older roots. This is a consequence of the increase number of root juxtaposition for which the currently employed algorithm in the pipeline last step is failing. We are currently working on an adaptation for this limitation.



## RhizoScan package in OpenAlea

(1) J. Le Bot et al, 2010, Plant and Soil, pp. 261

(2) G. Lobet et al 2011 Plant Physiology

(3) WinRhizo, Pro, 2012b. Regent Instruments Inc.

(4) P. Armengaud et al, 2009 The Plant Journal, pp. 945

(4) <http://www.plantmineralnutrition.net/rootreader.htm>

(6) P. Basu et al, 2007, Plant Physiology, pp. 305

(7) X. Qi et al, <http://digital.cs.usu.edu/~xqi/RootLM/>

(8) A. French et al 2009, Plant physiology, pp. 1784

Work founded by **Agropolis fondation** through  
the **Rhizopolis** and **OpenAlea** Projects