

An ImageJ based measurement setup for automated phenotyping of plants

J. Kokorian^{a,c}, G. Polder^b, J.J.B. Keurentjes^a, D. Vreugdenhil^{a,c}, M. Olortegui Guzman^a

^aLaboratory of Plant Physiology, Wageningen UR (University & Research centre),
PO Box 658, 6700 AR, Wageningen, The Netherlands

^bBiometris, Wageningen UR (University & Research centre), PO Box 100, 6700 AC,
Wageningen, The Netherlands;

^cCentre for Biosystems Genomics, 6708 PB Wageningen, The Netherlands



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An ImageJ based measurement setup for automated phenotyping of plants

J. Kokorian^{a,c}, G. Polder^b, J.J.B. Keurentjes^a, D. Vreugdenhil^{a,c}, M. Olortegui Guzman^a

^aLaboratory of Plant Physiology, Wageningen UR (University & Research centre),
PO Box 658, 6700 AR, Wageningen, The Netherlands

^bBiometris, Wageningen UR (University & Research centre), PO Box 100, 6700 AC,
Wageningen, The Netherlands;

^cCentre for Biosystems Genomics, 6708 PB Wageningen, The Netherlands

Abstract

The objective of plant phenotyping studies is to understand the relationship between a plant's genotype and phenotype. In our study we focus on the leaf morphology of *Arabidopsis* seedlings. Our experimental setup consists of a table of 0.9 m × 3.8 m with a matrix of about 1500 small cubes of rockwool, each containing an *Arabidopsis* seedling with a different genotype. The table is equipped with an automated supply for water and nutrients. The entire setup is placed in a climate-chamber with carefully controlled lighting and atmospheric conditions.

We will present a fully automated measurement system that monitors the development of each individual seedling by high resolution time-lapse photography. ImageJ is used to analyze the photographs. We will discuss the challenges concerning the image processing involved, including the automatic calibration of the image dimensions by marker recognition, segmentation of the photograph into regions that contain the individual plants, and the measurement of the leaf shape and area.

1. INTRODUCTION

Plant phenotyping experiments aim to detect the links between a plant's genome and its physiological traits.¹ A plant of particular interest for phenotyping studies is *Arabidopsis Thaliana* (figure 1). Its small size, rapid growth and the fact that its entire genome has been sequenced, make it an ideal model-species for plant sciences.



Figure 1. *Arabidopsis Thaliana* after 15 days of development.

In our *Arabidopsis* phenotyping setup, more than 3000 *Arabidopsis* seedlings are grown simultaneously in a large matrix. Their growth is being monitored by manually photographing the entire matrix every 24 hours. The images are then processed in ImageJ to extract the individual plants and quantify their visible traits, such as leaf morphology. Besides the data extraction itself, a particular challenge is to make sure that the measured data in consecutive images is linked to the corresponding plants in the matrix.

Phenotyping experiments like this, require a great deal of manual labor that impedes the experiment and the progress of the entire research. To overcome this obstacle, we are developing a flexible, fully automated phenotyping setup. Its design is highly modular, so it can be easily modified to host other experiments in the

future. Because most phenotyping experiments take at least several weeks, the system has to be able to cope with power failures, unexpected crashes and other inconveniences without crippling the experiment.

The image processing task of extracting an individual plant from an image showing many plants, measuring its traits and linking the results to the correct plant, is performed by a fully automated ImageJ script.

In this project, we aim to develop a system that is as 'general-purpose' and as flexible as possible, focusing mainly on the software architecture. The project was inspired by the work of Tsafaris and Noutsos,² who developed a similar automated phenotyping setup, but focusing on using off-the-shelf low-cost hardware, rather than flexibility.

2. EXPERIMENTAL SETUP

2.1 Arabidopsis growth setup

Our complete phenotyping setup consists of two $0.89\text{ m} \times 3.6\text{ m}$ tables that are fitted with an automated supply of water and nutrients. On both tables, small $4\text{ cm} \times 4\text{ cm} \times 4\text{ cm}$ rockwool cubes, each containing an Arabidopsis seedling with a slightly different genome, are arranged in five identical 18×17 matrices (figure 2). The five-fold replication improves the statistical power of the experiment. The tables are placed in a growth chamber with ambient conditions that are accurately controlled.

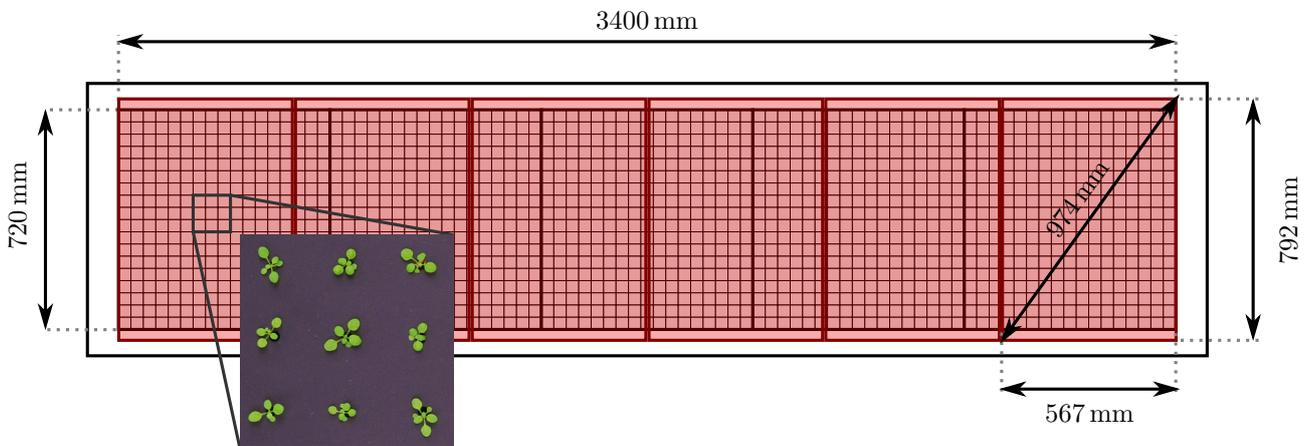


Figure 2. Schematic representation of five 17×18 matrices of Arabidopsis plants as they are being captured by six cameras.

2.2 Software architecture

A schematic overview of the camera setup and host computer is shown in figure 3. In this particular phenotyping experiment, both growth tables are photographed by six 10MP IDS uEye UI-1496LE cameras at regular intervals.

The software running on the host pc relies on three main services that function independently: camera drivers, scheduling and storage. The services are configured by means of plain-text configurations files that can be altered manually, or through a graphical user interface.

The scheduling service is responsible for triggering each camera at preset times. All cameras can be set to a different schedule and are assigned a specific folder on the hard-drive, where the camera driver places the fresh images. The folders are scanned at frequent intervals by the storage and archiving service. It moves the fresh images to another folder designated to the experiment they belong to.

All subsystems communicate with each other exclusively by placing image files in a folder, the relevant metadata of the image being contained in the filename. This architecture ensures that all subsystems are able to function independently and the image files are available to the user in a transparent way at all times. All services regularly broadcast an 'I'm still alive' message by placing a file containing the service's name and timestamp in a designated folder. Each service scans this folder regularly to make sure that the others are still working fine. If a service is detected to have crashed, it is immediately restarted by one of the others.

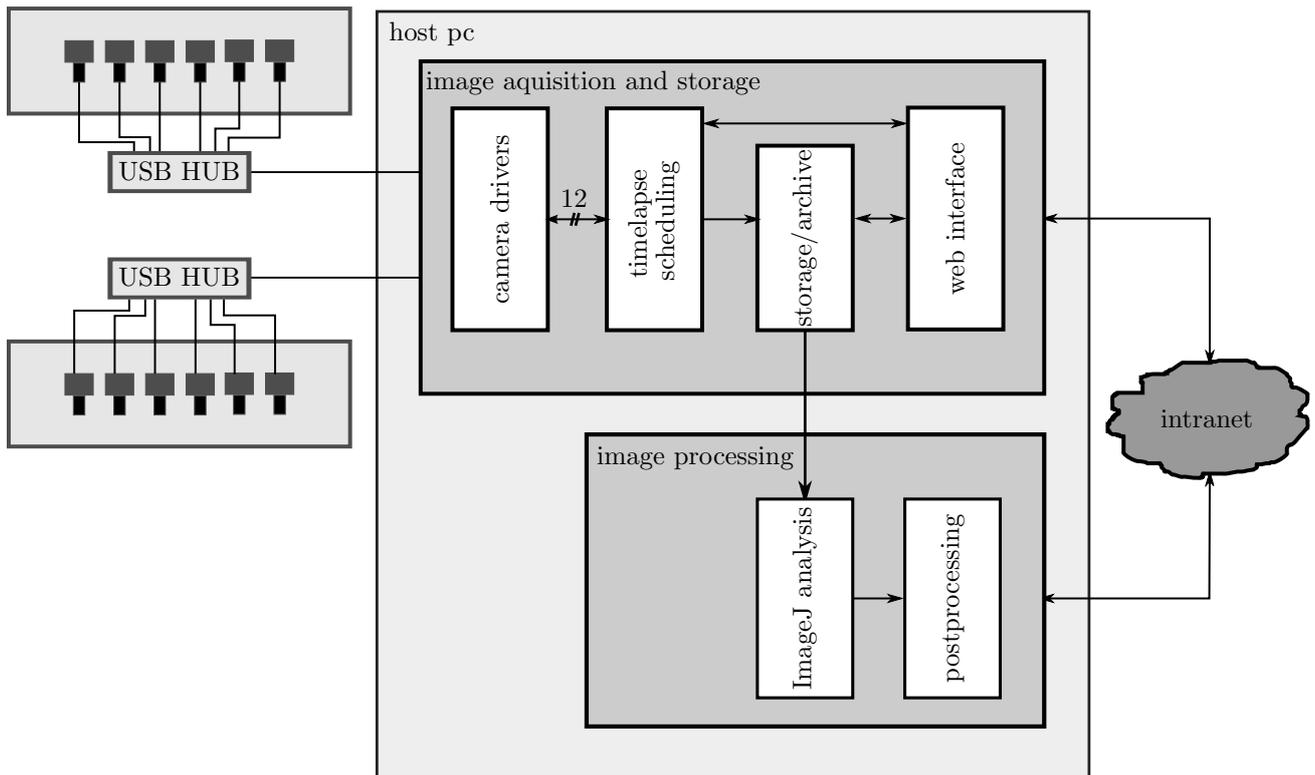


Figure 3. Schematic representation of the measurement host-pc architecture. All systems function independently and communicate with each other by exchanging image files. The image shows the twelve uEye USB cameras that are used for the phenotyping experiment discussed in this paper, but it can easily be extended with more cameras of different types.

3. IMAGE PROCESSING

All image processing tasks are implemented in ImageJ and are written specifically for this particular phenotyping setup. The script continuously scans the folder that receives the new image files and processes all new files that arrive.

Almost every computer vision task that is meant to analyze large numbers of plants in one picture involves similar process steps:

- Calibration of the image dimensions;
- segmentation of the image to separate the different plants;
- extracting the desired data from the segmented images.

Since the cameras are fixed above the setup, calibrating the dimensions just once would suffice in this particular case. However, in other experiments this might not be the case. For this reason, the first two steps have been implemented in such a way that they can be reused for any setup involving multiple plants that are photographed from the top.

3.1 Calibration of the image dimensions

To calibrate the image dimensions, four markers are placed within the field of view of each camera. The position of the markers is detected using a template-matching algorithm. Because the real distance between the markers is known, the pixel-to-width ratio can be deduced and the image can be rectified, in case the camera was off-axis when capturing it.

3.2 Image segmentation

Because the position of the markers relative to the rows and columns of the matrix is known as well, the image can now be split across each row and column, yielding a set of sub-images, containing a single plant each, labeled according to the plants row-column position in the matrix. The green plants can easily be thresholded against the black background, yielding a binary mask image for each seedling.

3.3 Measurements of leaf morphology

The morphological traits of the plant leaves, such as area, circularity and leaf density, are now determined using ImageJ's particle analysis toolbox. Additional to the standard features, the shape of the individual leaves is characterized by calculating a weighed skeleton, of which the pixel intensity corresponds to the skeleton's distance from the leaf edge. The statistical distribution of the weighed skeleton characterizes the leaf shape.

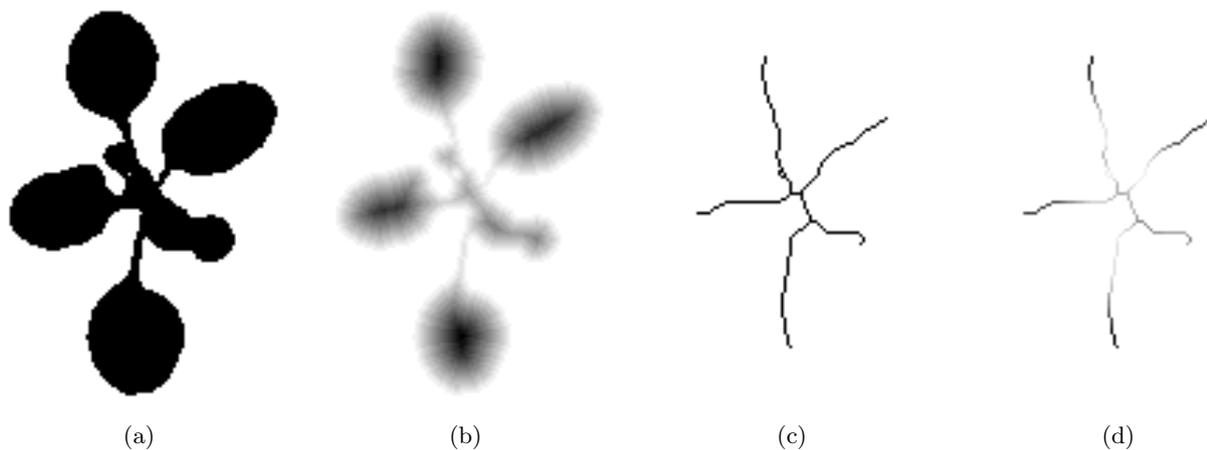


Figure 4. The plant shown in figure 1 after conversion to binary (a), the distance map (b), the binary skeleton (c) and the weighed skeleton (d).

3.4 Determination of the circadian rhythm of leaf movement

The entire system is based on two dimensional image analysis. The leaves, as they appear on the images, are projected on a two-dimensional canvas and the measured values therefore deviate from the actual value. To be accurate, all measured quantities are given the prefix 'projected'. Arabidopsis leaves are relatively flat and the projected leaf area has been found to correlate reasonably well with the actual area. However, in the early growth stages, the leaves are regularly tilted in an approximately 24 hour long cycle, called the circadian rhythm. When the angle is studied as a function of time, the exact frequency and amplitude of the circadian rhythm can be determined. The projected leaf area varies proportionally to the cosine of the leaf angle, so the leaf angle can be calculated. The minimum leaf angle of a cycle is close to zero, so the actual leaf area can be found as well.

3.5 Postprocessing data

All measured data is written to a csv-file so it can be analyzed by standard statistical data-analysis software. On top of this, graphs are automatically generated every time the image processing script is executed, enabling the progress of every plant to be followed in 'real-time'. The graphs are generated by a Python script that is called from ImageJ.

4. DISCUSSION AND CONCLUSION

Due to the modular structure of the software, the system can easily be extended with additional cameras and can be adapted to accommodate another experiment or multiple experiments simultaneously. Compared to the phenotyping setup designed by Tsafaris,² the setup we designed for the phenotyping experiment described in

this paper is more expensive, due to the professional computer vision cameras that were used. However, because of the modular design of the software, it is straightforward to replace the expensive camera's with off-the-shelf consumer types, as was done by Tsiftaris.² Moreover, we have followed up on Tsiftaris' recommendation of developing the software on open-source platforms: Python and ImageJ. This implies that no license fees have to be paid and the price of our system is lowered even further.

Because we are still in the process of developing the system, no measurement results are currently available. When the system is completed, the amount of manual labor required for the phenotyping experiment we described and similar experiments in the future, will be greatly reduced. Since all data is acquired and processed fully automatically, the amount of time spent by the user to analyze the images is reduced to zero. Because the data is processed and visualized in real-time, intermediate results can be interpreted, presented and discussed much quicker than before, altogether enhancing the scientific process considerably.

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