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ABSTRACT

For plant variety testing many plant characteristics need to be measured to assess distinctness between varieties and uniformity and stability of a variety. The characteristics are described in guidelines and protocols for each crop. These guidelines are established by international organizations. Several characteristics, related to shape and form are tedious to measure by hand and should be measured in an objective and reproducible way. The use of image analysis leads to a significant decrease of time needed for measuring the characteristics. Moreover, the results obtained by using image analysis are much more accurate than results of manual measurements. In the Netherlands, a user-friendly image analysis system has been established for recording and analysis of images using a standard camera. An ImageJ plugin is developed for offline processing of the recorded images. The following crops are implemented: flax seeds, flax bolls, pods of pea and beans, cotyledons of sugar beets and carrot. Measured features include length, width and area based on the skeleton, distance transform, and derivative of the outer contour. Calibration is done using a calibration disc in a separate part of the image. Identification of the images is done using a QR code label. The software is proven to be very robust, where disturbing factors on the objects, such as small crown leaves are removed automatically. We conclude that ImageJ as popular open source imaging platform is a good choice for implementing an image analysis system for plant variety testing to automatically and reliably measure a large variety of plant characteristics.

Keywords: shape-features, calibration, labeling, user-friendly

1. INTRODUCTION

For plant variety testing many plant characteristics need to be measured to assess distinctness between varieties and uniformity and stability of a variety. The characteristics are described in guidelines and protocols for each crop, e.g. wheat, onion or carrot. These guidelines are established by the International Union for the Protection of New Varieties of Plants (UPOV)¹ and the Community Plant Variety Office (CPVO).² Several characteristics, related to shape and form are tedious to measure by hand and should be measured in an objective and reproducible way. The use of image analysis leads to a significant decrease of time that is needed for measuring the characteristics. Moreover, the results obtained by using image analysis are more accurate than results of manual measurements.³ In the Netherlands, a user-friendly image analysis system has been established for recording and analysis of images. For image recording a digital single-lens reflex (DSLR) camera was used. The camera is mounted on a repro stand with front and back illumination. For the analysis of these images an ImageJ plugin was written. This paper describes the main features of this software, after a brief description of the acquisition setup. Section 3 focuses on the structure of the plugin and the calibration and identification of the images. Section 4 gives for all crops (flax seeds, flax bolls, pods of pea and beans, cotyledons of sugar beets and carrot) a description of the measured features and how these measurements are implemented in software.

2. IMAGE ACQUISITION

Image acquisition is done using off the shelf commercial available equipment. A small darkroom is equipped with a standard repro stand, a digital single-lens reflex (DSLR) camera (Nikon D90) with 50mm lens, high frequency fluorescence back light and adjustable high frequency fluorescence front light. Figure 1 shows the acquisition setup. The lamps are fixed on the adjustable mounting arms so it is possible to choose the proper position of

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Figure 1. The image acquisition setup, consisting of a standard repro stand, a digital single-lens reflex (DSLR) camera (Nikon D90) with 50mm lens and adjustable high frequency front and back light.

lamps. The intensity of the lamps can be adjusted for improving the contrast in the images, while the QR code and calibration disk still can be decoded. For some crops the orientation of the plant parts is important where for others it does not matter how they are oriented in the image. A detailed protocol describes how to take high quality images for each crop. The NKRemote software package (Breeze systems) controls the camera and images are captured and saved for offline processing.

3. ANALYSIS PLUGIN

The key requirements for the analysis software are robustness and ease of usage. Therefore an ImageJ plugin was developed with a friendly user interface. Furthermore the amount of parameters and settings for the user are kept to a minimum. All plugin modules and corresponding files, such as button images and configuration file are kept in a single jar file. A new version of the plugin can easily be installed by replacing this jar file in the plugins folder by a new one. Prerequisites for this plugin are besides the installation of ImageJ,⁴ the Analyse Skeleton plugin⁵ and the Morphological Operators from Gabriel Landini.⁶ When running the plugin first an ImageJ version check is performed. To safeguard the user from the rest of ImageJ, the main menu is hidden on startup of the macro. A button is added to the menu to activate the main menu in case it is needed. The plugin can be opened automatically on starting ImageJ by adding the auto run command to the StartupMacros.txt, which maximizes the usability for the users. The measurements are done on all images in a directory. The plugin outputs a spreadsheet with measurements, a log file and optionally processed images with label, scaling and measurement information.

3.1 User Interface

Figure 2 shows the user interface for the plugin, which is based on Action Bar by Jerome Mutterer.⁷ The top row buttons starts the analysis of the different crops, the right three bottom row buttons respectively toggle the ImageJ menu, show the About box and Quit the application.



Figure 2. The main menu with buttons for all measurement modules.

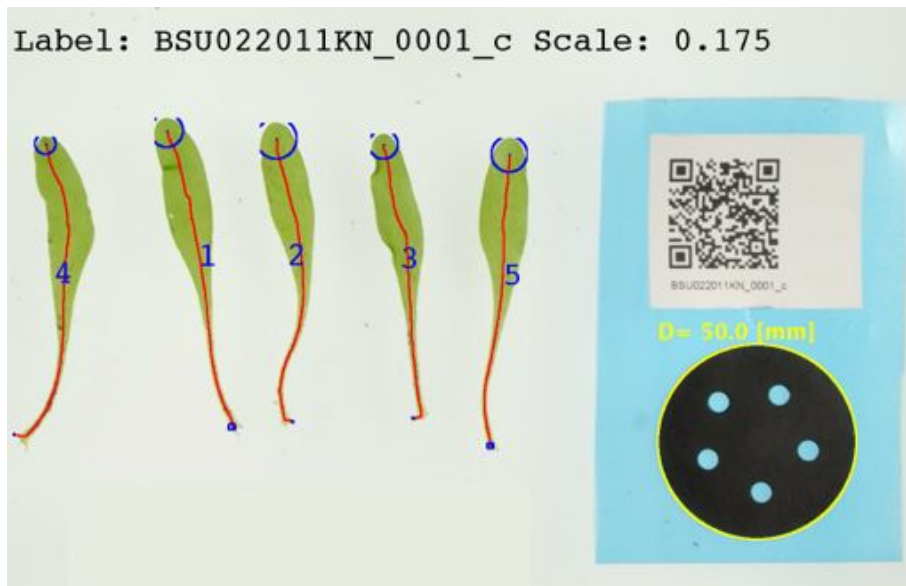


Figure 3. Example result image of cotyledon analysis with on the right the blue area containing the calibration disk and QR code. For the cotyledon the red line plus the radius of the blue circles denotes the total length.

3.2 Calibration and labeling

Calibration is done using a calibration disc in a separate part of the image. A blue background makes it possible to extract this part from the main image for automatic processing of the calibration and identification code. Different calibration disks can be used, each coded with a number of holes, which indicates the disk diameter in units of 10 mm. Identifying the images using different filenames is cumbersome and error prone, therefore in-image coding is used to identify the images using a QR code label in the same blue part. It shows information about the variety, plot and other experimental data. For decoding the QR code the ZXing library⁸ is adapted to ImageJ.

Figure 3 shows an example result image of cotyledon analysis with on the right the blue area containing the calibration disk and QR code. The measured disk size is drawn in yellow and the true size is displayed. On the



Figure 4. Example of flax boll analysis, left the original image, right the image overlays with the detected direction of the peduncle and the minimum enclosing rectangle of the boll.

top of the image the detected QR code and image scale is displayed.

4. CROP MEASUREMENT MODULES

Each measurement module is a separate plugin that measures the key features for each crop using different approaches.

4.1 Flax seeds

For flax seeds the most important UPOV features are the length and width. It was shown that the major and the minor axis of the fitting ellipse from the ImageJ measurements module has the best correlation with the real values. Since in all cases the length of the seed is longer than the width, the major axis corresponds to the length and the minor to the width of the seed.

4.2 Flax bolls

For flax bolls the measurements are more difficult, since the width (perpendicular on the peduncle) can be larger than the length. Furthermore small crown leaves often hamper determination of the width. Solution to these difficulties is to harvest the flax bolls with a small piece of the peduncle, which was used to detect the orientation of the boll. The crown leaves are removed using a binary opening. The length and width of the minimum-enclosing rectangle determines the length and width of the flax boll. Figure 4 shows the original image and an overlay of the detected direction of the peduncle and the minimum-enclosing rectangle of the boll.

4.3 Pods of pea and beans

For pods of peas and beans, the main features are length, width and bending of the pod along with the size of the beak. They are measured based on skeletonization of the binary image. For the length of the pod, the length of the main skeleton is determined, using the longest shortest path algorithm⁹ of the analyze skeleton plugin.⁵ The bending is determined by the ratio of the width (minimum width) and diameter (maximum width) of the convex hull of the skeleton. A detailed description of the method can be found in Vooren et al. (1993).¹⁰ Figure 5 shows some example results. Overlaid are the red line which denotes the length of the pod and the blue area which is the beak.

4.4 Sugar beet cotyledon

UPOV characteristics for sugar beet cotyledons are length, width and area. The length is similar to the bean analysis determined by the longest shortest path of the skeleton. Since the top of the leaf is round and smooth, the skeleton does not touch the contour of the object as with the beans. Therefore the length is extended with the value of the distance transform at the endpoints of the skeleton. Figure 3 shows some examples. The endpoint distance is visualized with a blue circle. The width is determined by the maximum value of the distance skeleton.



Figure 5. Example of results of bean analysis, the red line denotes the length of the pod, the blue area is the beak.

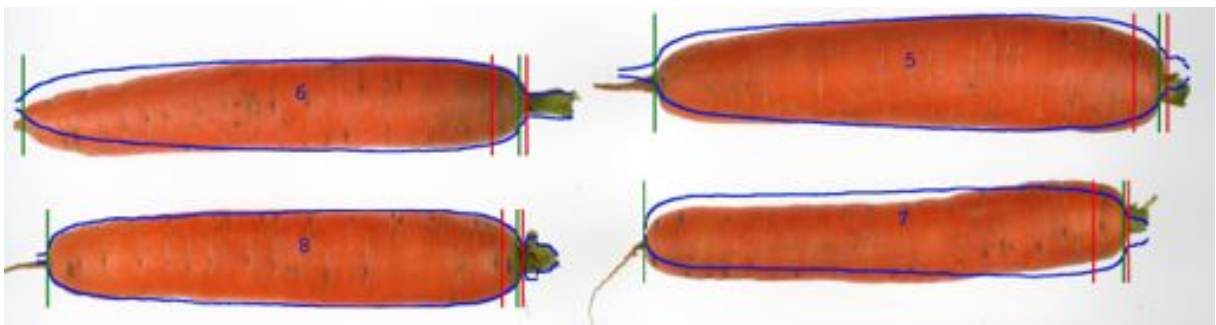


Figure 6. Example of results of carrot analysis, the blue line denotes the width distribution of the root, Between the green lines is the length, between the red lines the width of the shoulder.

4.5 Carrot root

The main characteristics for carrot roots are length, width, width of the crown, the size of the shoulder and a shape factor based on the inflection points of the outer contour. The width distribution is calculated by taking the width over the main axis of the root, resulting in a straight and symmetrical root model. The length of the root and the size of the shoulder is determined using the zero crossings of the first and second derivative of the width distribution. Figure 6 shows four examples. The blue lines denotes the width distribution of the root, between the green lines is the length, between the red lines the width of the shoulder.

4.6 General module

The general module embeds the ImageJ 'Analyze Particles' command which makes its possible to measure standard features. This module is also used to explore possibilities for crop features which are currently not yet implemented in the other modules.

5. DISCUSSION

The software is proven to be very robust, where disturbing factors on the objects, such as small crown leaves are removed automatically. Other crops can easily be added to the software, depending on the features which need to be measured and the disturbing factors which can be present in the crops. Currently only shape measurements are implemented. Because the acquisition setup is equipped with front illumination, color features can be added in the future. Standardizing the setup and calibration of the camera is very important in that case. We consider ImageJ as a popular open source imaging platform and a good choice for implementing an image analysis system for plant variety testing to automatically and reliably measure a large variety of plant characteristics.

REFERENCES

- [1] “The International Union for the Protection of New Varieties of Plants.” <http://www.upov.int> (2011). [27-August-2012].
- [2] “Community Plant Variety Office.” <http://www.cpvo.europa.eu> (2012). [27-August-2012].
- [3] van der Heijden, G. W. A. M., *Applications of Image Analysis in Plant Variety Testing*, PhD thesis, Delft University of Technology (1995).
- [4] Rasband, W., “ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA..” <http://imagej.nih.gov/ij/> (1997–2012). [27-August-2012].
- [5] Arganda-Carreras, I., “AnalyzeSkeleton [ImageJ Documentation Wiki].” <http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:analyzeskeleton:start> (2012). [27-August-2012].
- [6] Landini, G., “Morphological Operators for ImageJ.” <http://www.dentistry.bham.ac.uk/landinig/software/software.html> (2011). [27-August-2012].
- [7] Mutterer, J., “Action Bar Plugin [ImageJ Documentation Wiki].” http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:action_bar:start (2012). [27-August-2012].
- [8] “ZXing, Multi-format 1D/2D barcode image processing library with clients for Android, Java..” <http://code.google.com/p/zxing/> (2012). [27-August-2012].
- [9] Polder, G., Hovens, H., and Zweers, A., “Measuring shoot length of submerged aquatic plants using graph analysis,” *Proceedings of the ImageJ User and Developer Conference*, 172–177 (Oct. 2010).
- [10] van de Vooren, J. G. and van der Heijden, G. W. A. M., “Measuring the size of french beans with image analysis,” *Plant Varieties Seeds* **6**, 47–53 (1993).