Article

An Efficient Preprocessing Algorithm for Image-based Plant Phenotyping

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Abstract: Plants are such important keys of biological part of our environment, supply the human life and creatures. Understanding how the plant's functions react with our surroundings, helps us better to make plant growth and development of food products. It means the plant phenotyping gives us bio information which needs some tools to reach the plant knowledge. Imaging tools is one of the phenotyping solutions which consists of imaging hardware such as the camera and image analysis software analyses the plant images changings such as plant growth rates. In this paper, we proposed a preprocessing algorithm to eliminate the noise and separate foreground from the background which results the plant image to help the plant image segmentation. The preprocessing is one of important levels has effect on better image segmentation and finally better plant's image labeling and analysis. Our proposed algorithm is focused on removing noise such as converting the color space, applying the filters and local adaptive binarization step such as Niblack. Finally, we evaluate our algorithm with other algorithms by testing a variety of binarization methods.

Keywords: plant phenotyping; noise filtering; binarization; accuracy evaluation; connected components

1. Introduction

Plants are one of the main source of nature. Thus, for having durable and healthier herbal products, researches and experts make effort to identify the key features of the plant growth to amend the rate of their growth and resistance to diseases.

Understanding the biological yield and processes engaged in plant development relies on knowledge of the plants genetic basis and phenotyping which related to appearance or behavior. Considering to have this information such as gene activity at any stage of plant growth, it will allow us to relate to the science of plant life which we model, thus some prerequisites should be met.

The field of computer vision, where the digital images automatically in a very structured environment are measured by the acquired scene features, is applicable to measure the plant growth [1]. Until recently phenotype is related to plant growth came in destructive ways or involved in human studies with low throughput and high-cost efficiency. Destructive ways lead to harm plant components and minimal user interaction solutions lead to gain low phenotyping information. As a result, one of the solutions is nondestructive imaging with automated image analysis using the camera in fluorescence or reflected light and automatic software analysis. Plant phenotyping methods such as European and international networks (IPPN and EPPN) and collaborative projects

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[2] iPlant to view and analyze the phenotype speed recovery and enhance our understanding of biology was built [3-5].

An Automatic image analysis of plants biology needs an Algorithm with high accuracy, low cost of time expense and imaging hardware. Base of the most image analysis software is separation of foreground from background called image segmentation. Most of these Image segmentation algorithms include some steps: removing noise from background. Boundary detection of fore-ground. Labeling the extracted boundaries.

In the segmentation techniques [6] there are some methods (e.g. Active Contour, Markov Random Field, Edge Detection, Thresholding, Clustering, Level Set and Random Walk) to extract objective image. These are wide-spreading subjects therefore they have some requirements. Information acquisition with Removing noise from images is one of the important preprocessing phase because some images due to capture have extra components affect the determination boundary, result in erroneous segmentation. Separation techniques depend on kind of image complexities and dataset. There are many kinds of noise in the pictures like illumination, document degradation and unwanted impurities. One of preprocessing task in segmentation is image binarization. Binarization helps the segmentation process to identify the objective foreground in black and white environment. Why do we do binarization instead of applying filters? To answer this question, we have some filters to remove noise, but filters don't act as binarization. Binarization methods categorized in global and local adaptive or mix of both. Filters remove noise in whole image and some content of the aim object may be disappeared.

A. Phenotyping detection

First, we review how researchers detect the phenotype by some tools. The introduction to automation and digital imaging, allows to collect time-laps images of plants in non-destructive mode [7,8]. The system is a non-intrusive method for monitoring and evaluating the amount of plant growth and provides growth rates. It is applicable for large-scale methods, such as screening for changes in growth (rate) in a series of mutants. The new system provides highly accurate tool for determining the rate of individual plant growth, even in the early stages of development, requires little time as a week. They measured plant size and growth rates by "Surface area estimate" and proved plant leaves overlapping affects the measurement of plant age. These images are analyzed in the offline mode by an analyst expert via analysis imaging software manipulation or semi-automated methods. Most of these customized solutions, which are commercial (LemnaGrid) [9], analyze the obtained images of plant growth to develop an experimental pipeline for the analysis of growth rates parameters. This solution is using the Arabidopsis plants as a model.

Analysis of plant characteristics such as root growth and leaf transpiration are another side of plant phenotyping. First, measuring root dry weight, then measuring the evapotranspiration[1] using measuring changes in pots weight. Finally, data is analyzed statistically [8,10] [11-13] which imposes rigorous empirical settings. (for example, black and white background). In some cases, type of Imaging system is important in phenotype acquisition [14].

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[1] Evapotranspiration (ET) is the sum of evaporation and plant transpiration from the Earth's land and ocean surface to the atmosphere.

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B. Image analysis for phenotyping

We talked about phenotype acquisition, but the main problems are how we can extract and segment the plant images from the background accurately to analyze the phenotyping with the minimal fault. To understand this problem Figure 1 shows a top view image with different plant size and light condition. There are some algorithms to classify and segment plant leaves [15]. The authors proposed an automated leaf extract algorithm using three times watershed marker segmentation, which is a time-consuming approach for our case assumption dataset. Segmentation method [16] using a pixel-based segmentation approach known as a multi-dimensional histogram threshold (MHT) benefits the gray pixel values regardless of the neighborhood. An image consists of areas within the different levels of gray. This method can isolate the image histogram using the threshold. So subdivisions are obtained in areas defined by the user for top-view and side-view images. Due to the color similarity makes problem in segmentation, morphological operations are used. In [17] the new processing strategy to eliminate background noise and shading correction is provided. They introduced using the tooth features distinguishing the characteristic leaves with similar shapes, but with different margins. Their work feature has its drawbacks. First, time expense was not discussed. Second, the implementing cost of their algorithm is not small with such a dataset.

Figure 1. a top-view example many individual plants

These algorithms which we have discussed haven't focused on the preprocessing step. However, the preprocessing step is the most effective step which impacts on the final result such as labeling, overlapping calculation and comparison with the ground-truth. In [18] Binarization Techniques for Enhancement of Degraded Documents was reviewed as a preprocessing step while the filters such as Gaussian filters, mean filter, etc. in a way are a preprocessing step in image processing and machine vision. They compared local and global thresholding then released a hybrid algorithm. The most popular binarization [19] methods are described as follows.

In the past in many projects, Otsu's method [20] the most successful global threshold method is used. It automatically verges of hitting shape-based image histogram to reduce a grayscale image to a binary image does. This comprehensive province that minimizes the variance within the class, as a weighted sum of the variances of the two classes to be searched. The weighted variance within the class is Equation (1):

$$\sigma_w^2(t) = q_1(t)\sigma_1^2(t) + q_2(t)\sigma_2^2(t) \qquad (1)$$

Where the class probabilities of different gray level pixels are estimated as is shown in Equation (1):

$$q_2(t) = \sum_{i=t+1}^{255} p(i) \quad \text{and} \quad q_1(t) = \sum_{i=0}^{t} p(i) \qquad (2)$$

Niblack method [21] calculates a smart pixel threshold by sliding a rectangular window on the grayscale images. The method matches the threshold according to the local average m (I, J) and standard deviation σ (I, J) (I, J is the coordinates of each pixel) and calculation of the window size B×B. T threshold as listed below as Equation (3):

$$T(i, j) = m(i, j) + k \times \sigma(\mathrm{i}, \mathrm{j}) \qquad (3)$$

Here, k is a constant that determines how much of the edge of the printed object is maintained and has a value between zero and one. k and the size of the sliding window defines the quality binary. Binarization with a small amount of k gives non-specific and thick movements, and with a large k gives the thin and broken shaking. According to many applications, size m × n for sliding windows and user defined value of k as heuristic has been found desirable. Neighborhood size must be small enough to reflect the brightness level of local and large enough to be picking up objects and background.

The method proposed by Sauvola [22] which is based on a local variance. This is an improvement in the method proposed by Niblack, especially when the background includes a light texture, great changes, paint and clear documents is irregular. It adapts the share of standard deviation. For example, the text on dirty or stained paper, the threshold is reduced. The Threshold is calculated as Equation (4):

$$T(i, j) = m(i, j) \times \left[1 + k\left(\frac{\sigma(i, j)}{R} - 1\right) \right] \qquad (4)$$

A local adaptive method provided by Bernsen [23] which is based on image contrast. Some articles have innovative method or combination of methods which described above. For example, in [24], a learning framework for optimizing the binarization techniques have been introduced to determine parameter values for an image document. This framework can work with any binary method that performs three main steps: Extract the features, estimates the optimal parameters and learn the relationship between features and optimal parameters. A method suggested for producing feature vectors of two-dimensional numeric data. Different Statistics mappings are extracted and then to final feature vectors, are combined with a non-linear manner.

We have reviewed related works about phenotyping methods and important level for image-based phenotyping. Thus, we realized the important level of segmentation is the preprocessing cause of elimination of image details. We reviewed popular binarization methods as an effective approach to avoid errors of image analysis.

In this paper, we test the binarization methods for plant image segmentation. We show binarization is very important task in the image-based phenotyping. If we select suitable binarization method, in the contouring step, we would not have extra unwanted objects beside the plant (moss

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and soil) and even there will be no mislabeled plant component in the labeling step due to leave cutting and leave details elimination. These are challenges to segmentation for researches just not in plant phenotyping, in any field of image processing and machine vision.

Here we implement our test on the Arabidopsis thaliana dataset that is time-lapse top view plant images from the phenotyping experiments (as the **Error! Reference source not found.** shows). I n section 1 we introduced the plant phenotyping and image-based analysis and we discuss recent works in binarization. In section 2 we propose our algorithm. In section 3 we implement and evaluate our algorithm. Also, we discuss the results in section 4 and in section 5 we discuss the conclusion.

2. Materials and Methods

In this paper, we propose an algorithm mix of noise removal and binarization as a preprocessing level. Scene image of plants includes spectral light and neon light illumination, physical texture such as soil, moss and pot shapes. In the other side separation of plant green color from another scene feature is one of the main challenges. At first, to eliminate issues of lighting distortion related to neon lights and shadowing, we convert the image from RGB to NTSC which shows YIQ map (The Y component represents the luma information. I and Q represent the chrominance information.). Then, we apply the element-wise binary multiplication to the image array (which is converted to lab). Our conversion algorithm (this is obtained by test and error.) with manipulation [25] shows as follows:

Table 1. The color space converting algorithm

Line	**Code**
1	lab ← rgb2ntsc(Image)
2	f ← 2
3	d ← concatenate (3,1-f,f/2,f/2)
4	box ← d × lab
5	wlab ← Converting the box array in 3 columns
6	S ← convert the lab to wlab
7	J ← pick the Q color from the S
8	grayImage ← (J-min(J))/(max(J)-min(J))

After the converting to NTSC in line 1, we start manipulation phase. Line 2 defines the constant number f=2, which is changeable to detecting image features. In line 3 we use the concatenate function to concatenate the f in three-dimensional matrix, which the first parameter is the number of dimensions, second and other parameters are arrays dimensions which the operation is applied to evaluate array size. We applied element-wise binary multiplication to image array lab with d in line 4. This task includes the element wise multiply, first and second parameters are arrays which the task is applied. In line 5 we convert the box array in shape of 3 columns 2D array. We convert the matrix shape of lab to wlab in line 6. One dimension of array S is picked in line 7. Finally, the gray image result is obtained by the line 8 as is shown in the Equation (5):

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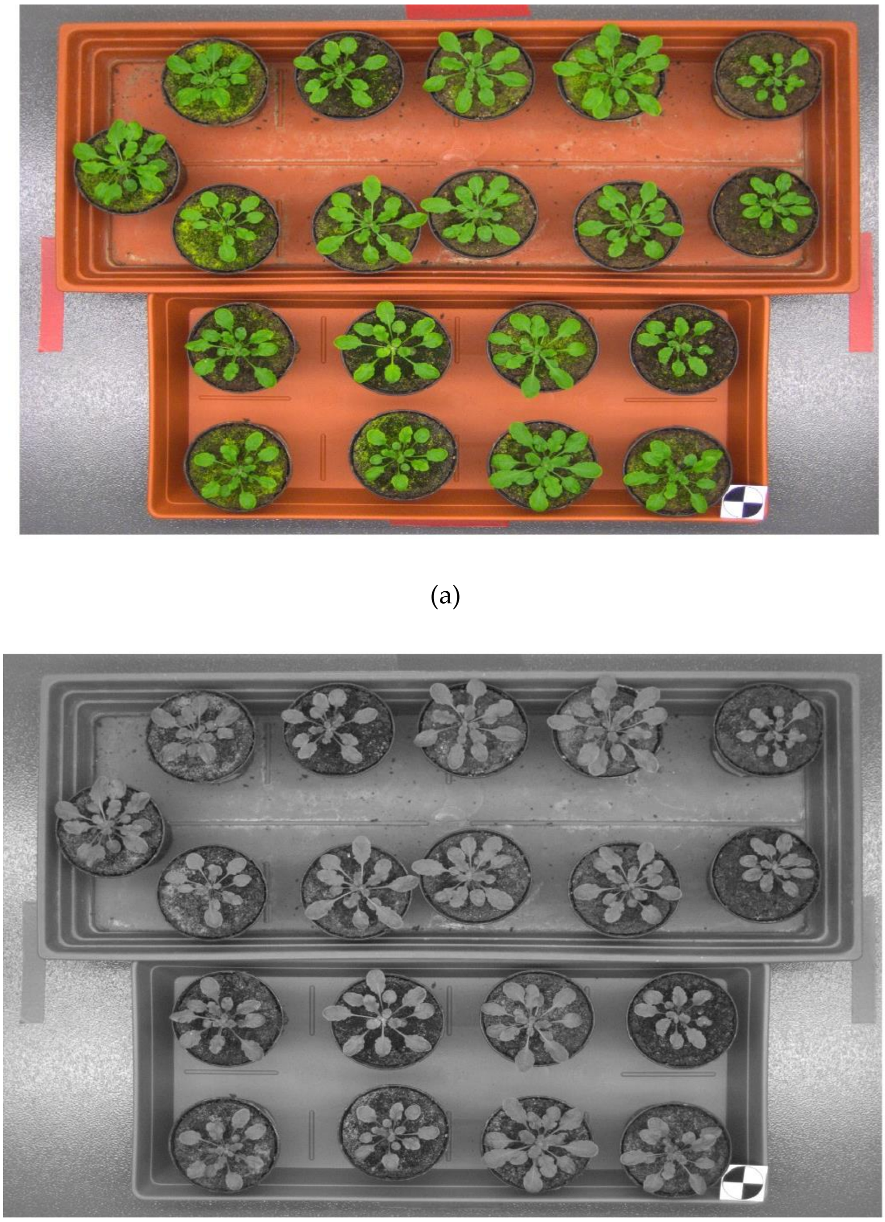
$$GrayImage = \frac{S - min(S)}{max(S) - min(s)} \qquad (5)$$

When a gray image is obtained, Q channel is extracted to separate plant appearance. Although moss and soil aren't omitted completely, but it is acceptable in our algorithm. Figure 1 shows the converted RGB image to NTSC color space.

To measure plant size accurately, we consider on plant pots locations. A rectangle mask (it is user defined for the first time and grow automatically by plant growth) is created for eliminating unwanted areas which have negative effects in comparison with ground-truth.



(a)

(b)

Figure 1. Converting RGB image (a) to NTSC color space similar to lab 1976 (b)

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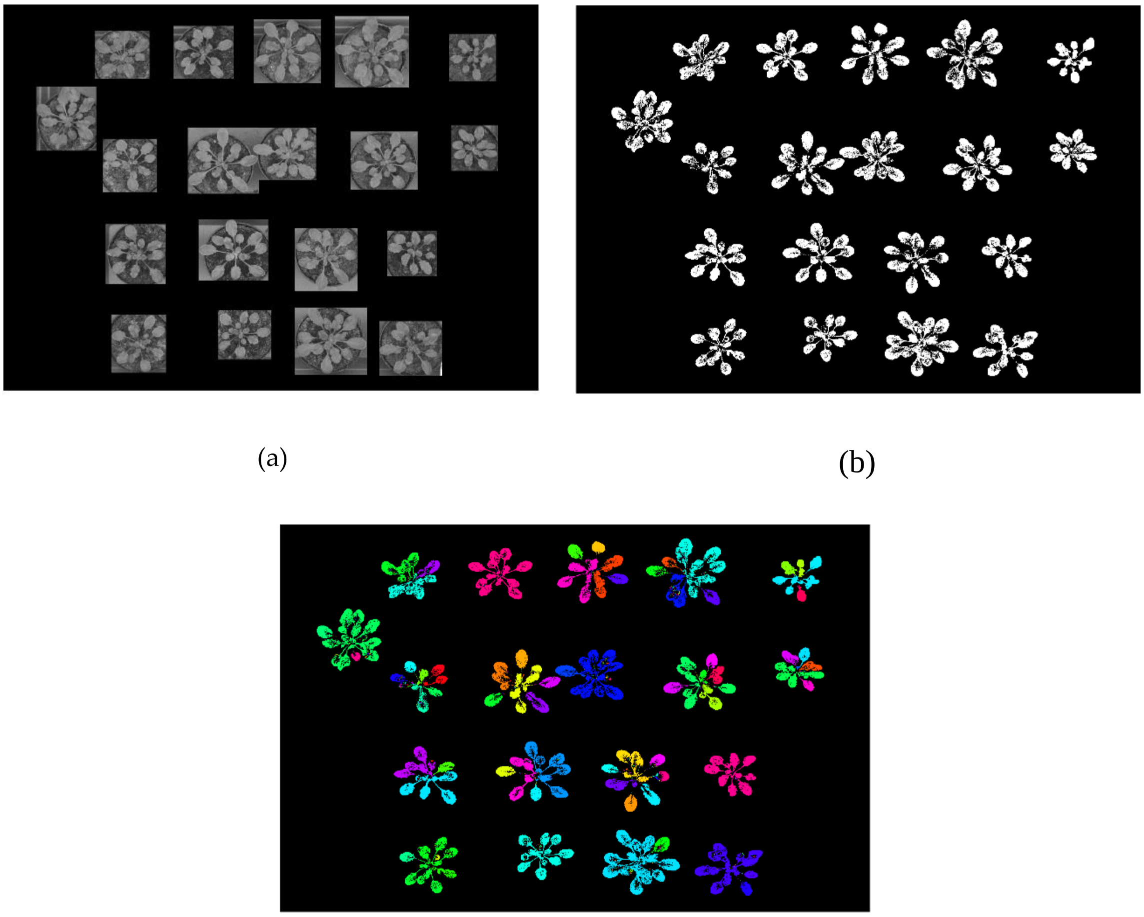
The Niblack binarization is implemented on the masked image which NIBLACK (IMAGE, [M N], K) performs the local thresholding with M-by-N neighborhood. We set parameters M=30, N=30, k=0.2 and offset=4.



(a) (b)

(c)

Figure 2. Image (a) shows masked grey image by rectangle positions. Image (b) shows active contours after binarization without considering moss and soil. The bottom image shows labeling plants shapes donating by colors which indicate connected/disconnected components

After that, we use the pillbox filter, which is linearly combined with a Difference of Gaussians (DoG) filter. The pillbox filter is a circular averaging filter within the square matrix of size 2*radius+1 (radius is 2). In the simple case of grayscale images, the blurred images are obtained by convolving the original grayscale images with Gaussian kernels having differing standard deviations. As the Figure 2(a) indicates the multiple masks on gray scaled scene image, it is required to consider the plant area to applying filters just on probability foreground. In Figure 2(b) active contour applied on plants area after applying filters and binarization. Labeling is implemented in Figure 2(c) shows the connected components in different colors. Small objects which are beside the larger one shows the binarization's impact by leaves cut. In plant growth times, the number of connected components will increase automatically because the structure of the rosettes which have thin stems during increasing leaves size. We assume that we segment and extract the images of plants one by one correctly and we don't focus on the group labeling cause of leaves cut in plant structure. If we implement these steps in whole of image we should solve the problem of the group labeling. Therefore, we apply the one of the solutions such as label clustering, region growing or distance transformation. Although Figure 2 has active contours and labeling step, but it just measures connected components after binarization,

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contour and labeling. So, we extract plant images one by one per plant's tray, which is captured in specific time.

Finally, the results of filtered images are compared with ground-truth. In the next section, we compare the results with ground-truth in recall, precision, Jaccard and Dice Similarity criteria.

3. Results

We implemented our system in Matlab (release 2016b), on a machine equipped with Intel Core i7 6500u 2.5GHz and 16 GB memory, running 64-bit Windows 10 pro. We devised our test on Arabidopsis thaliana dataset. Several challenging situations, such as water and moss growth were allowed to occur in scene image. The scene consists of top-view images of $N = 19$ Arabidopsis thaliana Columbia (Col-0) wild-type rosettes, acquired over a period of 12 days [26] and plants were imaged with a 7 megapixel commercial camera (Canon PowerShot SD1000) following the setup discussed in [10]. The images were stored and processed in raw format to avoid any distortion introduced by compression. Figure 5 shows an example image from the dataset, illustrating the arrangement of the plants and the complexity of the scene.

Ground-truth segmentations were obtained manually in [27]. According to [26], to quantify the accuracy of the preprocessing algorithms, we adopt the following metrics:

$$\text{Precision}(\%) = \frac{TP}{TP + FP} \tag{6}$$

$$\text{Recall}(\%) = \frac{TP}{TP + FN} \tag{7}$$

$$\text{Jaccard}(\%) = \frac{TP}{TP + FP + FN} \tag{8}$$

$$\text{Dice}(\%) = \frac{2.TP}{2.TP + FP + FN} \tag{9}$$

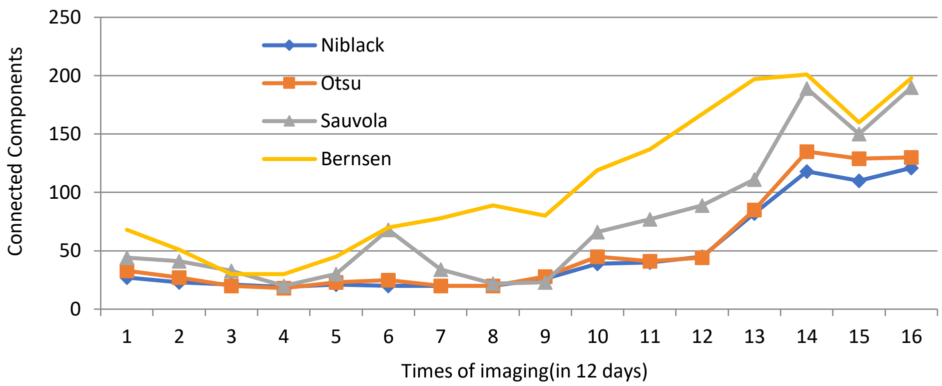

Figure 3. Shows which binarization method has less destruction in plant shape by indicating how many connected components appears after binarization cause of leaves cut and other components.

Precision is the fraction of pixels in the segmentation mask that matches the ground truth, whereas recall is the fraction of ground-truth pixels contained in the segmentation mask. The Jaccard and Dice similarity coefficients are used to measure the spatial overlap between algorithmic result and ground truth. All of these

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metrics are expressed in percentages, with larger values representing higher agreement between ground truth and algorithmic result [**Error! Bookmark not defined.**].

shows the connected component result of the proposed algorithm and other methods. All of the preprocessing steps except binarization same as proposed algorithm. It shows Niblack binarization is more efficient than the other binariztion algorithms. After that, Ostu global thresholding make less connected components than Bernsen and Sauvola. Finally, Sauvola is better than bernsen.

Although we don't consider group labeling, but the performance integrity of the plant's shape is measured. To calculate how a plant structure is eliminated, we don't consider moss and soil particles around the plant, so we multiply ground truth images with the results. It means TP is obtained because it recovers parts of the ground-truth mask (pixels correctly segmented as foreground). FP is number of recovered pixels are existing, but don't belong to the plant (pixels falsely segmented as foreground). TN is number of recovered pixels do not belong to plant (pixels correctly detected as background). FN is the number of pixels belong to the plant but are not recovered (pixels falsely detected as background).

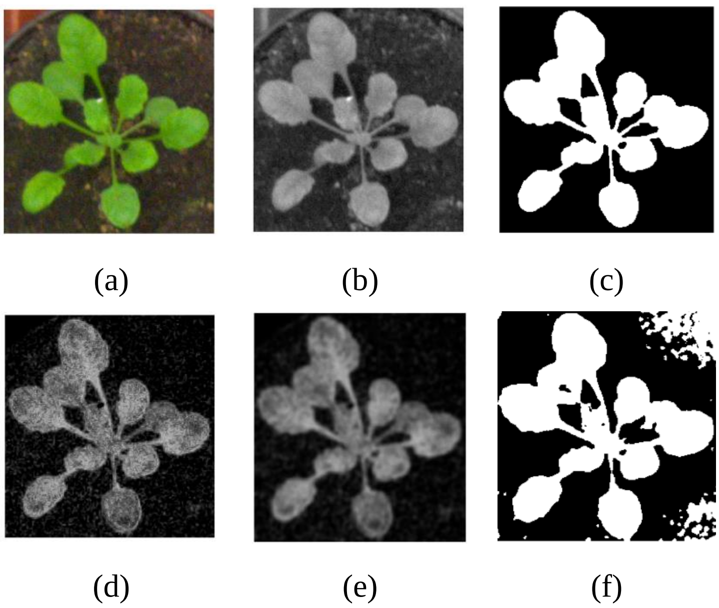

Figure 4. (a) The cropped original RGB image. (b) The converted image to NTSC YIQ color space (near the lab a*b*l* 1976 color space). (c) The cropped ground-truth image. (d)The q channel is extracted. (e) A pillbox filter is applied. (f) The Niblack binarization is applied.

As the Figure 4 shows, first we applied changeable mask on plant location image by image and then applied filters and binarization. Then precision, recall, Jaccard and dice similarity coefficient are computed for each plant. Finally, sum of precision, recall, Jaccard and dice is computed for all of image plant. It has some benefits such as comparing plant images day by day or even how the whole of tray is changed during specific time. Of course, except binarization step, all of the preprocessing levels are the same. shows the binarization accuracy of proposed method and other binarization methods.

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Table 2. Binarization accuracy is reported for proposed (Niblack), Otsu,Sauvola and bernsen methods

Method's name	Recall	Precision	Jaccard	Dice
Niblack (proposed)	0.76	0.95	0.73	0.84
Otsu	0.72	0.90	0.64	0.75
Sauvola	0.67	0.91	0.55	0.58
Bernsen	0.45	0.76	0.41	0.52

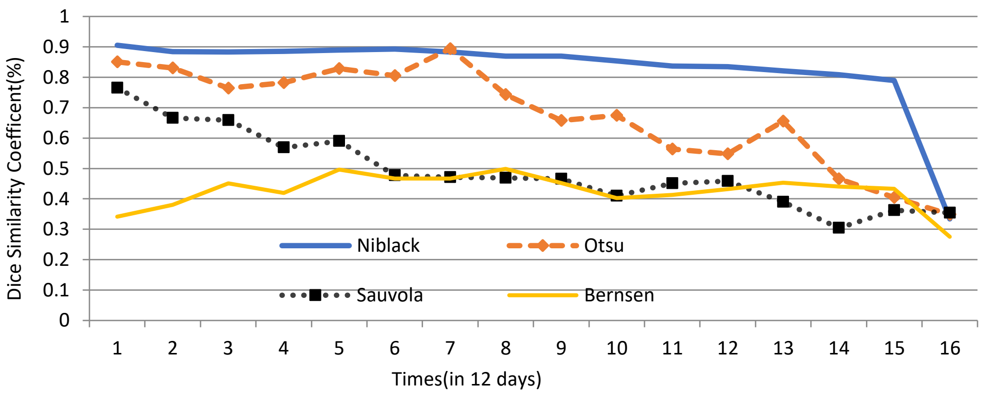

Figure 5. Segmentation accuracy over time estimated using the dice similarity coefficient for the proposed (Niblack), Sauvola, Otsu and Bernsen's binarization methods.

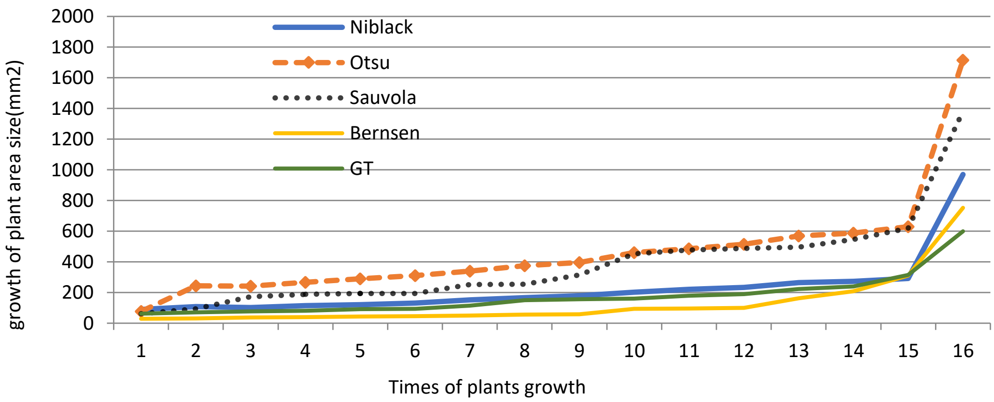

Figure 6. Plant growth size in scale mm^2 is shown by binarization methods(each pixel is 0.264 mm)

4.Discussion

Niblack's method (proposed method) has the better result of Recall, Precision, Jaccard and Dice in comparison with Otsu, Sauvola and Bernsen. Otsu's method has the better result than Sauvola and bernsen, and Sauvola is more accurate method than Bernsen. Global thresholding approaches such as Otsu, eliminate some parts of the foreground or develop white areas near the foreground. We expect the Sauvola binarization results better than Niblack, but in practice it is not happened. Due to the Bernsen's method works with contrast and our dataset has not high contrast, it results in the lowest level. We show Dice similarity coefficient by changing plant pots in the tray over period of 16 days using .

Nibalck's method has more efficient in our result, but Otsu's method has less performance in accuracy measurement. In the last part of this section we measure the plant growth rates of the 19 plant objects over the tray in 16 times of the image capture during the 12 days (number of plants is fixed by the user). Figure 7 shows the comparison plant growth's size (converted from pixel to mm2) between Niblack binarization method (proposed method), Otsu, Sauvola, Bernsen and ground-truth. It shows the Niblack's method result is nearer to the ground-truth than others which consider the difference of white pixels between ground-truth and proposed binarization method

5. Conclusions

We proposed an effective approach for the preprocessing level to remove unwanted objects in the foreground related to the image-based plant phenotyping. We proposed an NTSC color space (near the lab 1976) to prepare the grayscale scene image before applying Dog filter. We created the changeable rectangle masks to limit plant's area for applying Dog filter. We tested applied Dog filter to remove moss and soil beside the plant area. After that, we tested binarization methods to separate foreground and background. We measured the number of connected components which results by binarization methods and we concluded Niblack's method is more effective than the other binarization such as Otsu, Sauvola, and Bernsen. Also, the accuracy and performance are measured by calculating the precision, recall, Jaccard and Dice in plant segmentation. We found the Niblack's binarization is more accurate than the other binarization methods. In plant growth rates, Niblack binarization results are nearer to ground-truth than the others.

Our work is suitable for agricultural industries want to have automated maintenance tools such as powerful digital cameras, drones on the farms or even images captured by satellites to monitor the climate. Testing on group labeling algorithms is left for future work.

6. Patents

Supplementary Materials: The dataset of paper is downloaded from www.plant-phenotyping.org/datasets-home.

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