Non-destructive measurement of leaf area and leaf number of hydroponic pak-choy plants (*Brassica rapa*)

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**Abstract.** Leaf area and leaf number is an important factor in many agronomic and plant physiological studies. The measurements are commonly obtained by destructive and time consuming via laboratory. Therefore, there is a trend in utilizing fast and non-destructive methods to estimate leaf area and leaf number. The aim of this study was to estimate leaf area and leaf number of pak choy (*Brassica rapa*) under field condition. For this purpose, 180 pots of pak choy plants were captured. Afterward, the images were analysed via image processing. Distance transform-watershed segmentation was used to extract and to separate every leaf from its background. The area of each leaves were estimated according to the pixel values obtained. The area of leaves also compared to the ImageJ measurement. The number of extracted leaves was evaluated according to precision, recall, and f-measure calculation. In conclusion, our system achieves a good performance for minimal occlusion leaves and provides quick and non-destructive method. This method could potentially be applied to design phenotyping system in the greenhouse even for small scale companies.

1. **Introduction**

Leaves are one of the key plant parts required of biomass production of plant through photosynthesis. Besides, the leaf area is related to plant production and it is utilized in functional-structural plant models to mimic plant development, yet they are hard to determine [1]. Other reference expressed that leaf cover are a key parameters of a plant's development stage [2].

The accurate determination of leaf area is a main point in crop growth analysis. Accurate leaf area estimation is indicative parameters in understanding crop growth. However, complexity of leaves shapes making leaf area measurement more challenging, laborious and subject to larger mistakes. Moreover, it is not possible to make subsequent assessment of the similar leaf, due to it will injured and cause difficulties to other experiment [3].

Non-destructive measurement of leaf area and leaf number is necessary for predicting the crop yield. Some studies deals with image analysis to measure growth and shape, anatomy, and surface of plants. Several image analysis procedures [4] have been employed for providing annotated imaging data and suitable evaluation such as leaf segmentation, controlled experimental design using internet of things [5], and particular data acquisition scenarios [6]. Various computer vision and imaging procedures for non-destructive plant phenotyping have been studied [7]. Huang and Lee [8] studied an automatic identification system for Phalaenopsis tissue culture plantlets. Tang et al. [9] suggested leaf extraction with complicated background. Young and Jung [10] studied a model validation for
estimation of leaf number and leaf area of hydroponic pak-choi plants. Another study the use of depth camera for 3D phenotyping of entire plants [11]. Cerutti et al. [12] proposed active polygon for leaf segmentation and shape estimation. The disadvantages of these procedures are requiring training, need large labelled datasets, and inability of handling overlapping leaves. The current study aims to measure leaf area and leaf number of hydroponic pak choy plant in the open field.

2. Materials and methods

2.1. Pak choy seedling
Pakcoy seeds of Nauli F1 variety were sown on rock wool and stored in a dark room, after 24 hours the seeds are exposure to sun light. When the seeds have 4-5 leaves, the rock wool is transplanted into gully (DFT hydroponic system). The hydroponic nutrient concentrations are adjusted in the range of 1050-1400 ppm and a pH value of 7.

2.2. Image acquisition and capturing
The pak coy images were captured using Kinect v2 camera which has a color image resolution of 1920 x 1080 pixels. The images obtained in the form of color images (RGB images) which is connected directly to the computer (figure 1). A number of 180 pak choy images were capturing automatically in the open field using Kinect camera at two different ages (two weeks after sowing and three weeks after sowing) (figure 2). At the same time, leaf area and plant canopy area calculated manually by using the ImageJ. In order to calculate the leaf area using the ImageJ the pak choy leaves transported to laboratory for taking the images (see figure 3).

Figure 1. Images capturing system connected to computer (laptop). Pak choy samples are placed in holes of the grow ray.
2.3. Image extraction using histogram smoothing
Pak choy and background objects were extracted based on L*a*b* color space and histogram smoothing method. Color space of the b* channel and histogram smoothing were applied to distinguish between the foreground and background. Firstly, the relative histogram of the gray values was resolved. At that point, the significant minima were separated from the histogram, which were utilized as boundaries for a thresholding activity. So as to diminish the number of minima, the histogram was smoothed with a Gaussian filter [13]. The mask size was amplified until there was just a single minimum in the smoothed histogram. At that point, the threshold was set to the position of this minimum. Leaves area was calculated by following equation [14]:

\[
\text{Leaves area} = \text{leave pixels count} \times \frac{\text{area pixels count (cm}^2\text{)}}{\text{calibrated area (pixel)}}
\]

(1)

2.4. Distance transform and watershed segmentation
Distance transform operations can be classified into several categories, depending upon the way the distance of a pixel from the boundary has been calculated (distance metrics). The Euclidean metric is a suitable model for varied geometrical facts and is employed in many applications, since it’s radially symmetric and virtually invariant to rotation [15]. The watershed method was proposed by Vincent and Soille [16] which involves the sorting and the flooding step.

2.5. Leaf area calculation using ImageJ [17]
The method proposed by Ealson and Bloom [18], which provides an easy-to-use method for rapid assessment of leaf area and measurement of canopy area from digital images. Otsu’s thresholding method was utilized [19][20] binary image with a bimodal intensity histogram. Areas red were identified as leaves and a ruler used as calibrated area (figure 3).

Figure 2. Pak choy images from top view with small red rectangle as calibrated area.

Figure 3. Leaves area calculation using ImageJ.
2.6. Image processing

The MVTec Halcon HDevelop 20.05 image processing (MVTec Software GmbH) was used to analyse the images using a PC with an Intel® Core™ i7-4510U processor, 2.6 GHz, 12.0 GB RAM, 64-bit operating system. Measurement of root means square error (RMSE) [21] and mean absolute percentage error (MAPE) [22] were performed on the following experiments.

\[
RMSE = \sqrt{\text{mean}(t-m)^2} \tag{2}
\]

\[
MAPE = \text{mean}\left(\left|\frac{t-m}{t} \times 100\right|\right) \tag{3}
\]

Where, \( t \) is the target measurement histogram smoothing method, while \( m \) is the measurement using imageJ (see figure 3).

Precision and recall [23] were used for evaluating the leaf number. Precision (Eq. 4) was utilized to define the portion of extracted leaf pixels that suitable with the ground truth. Recall (Eq. 5) employed to calculate how much ratio of ground-truth pixels present in the extracted leaf region. F-measure [24] is a harmonic mean of precision and recall, providing an evaluation of both false detection and detection of foreground (Eq. 6).

\[
\text{Precision} \, (\%) = \frac{TP}{TP + FP} \times 100 \tag{4}
\]

\[
\text{Recall} \, (\%) = \frac{TP}{TP + FN} \times 100 \tag{5}
\]

\[
F\text{-measure} = 2 \frac{\text{Recall} \times \text{Precision}}{(\text{Recall} + \text{Precision})} \tag{6}
\]

Where True Positive (TP) is the number of leaf region pixels correctly identified, False Negative (FN) is the number of leaf region pixels unidentified, and False Positive (FP) is the number of partial/overlapping leaf region pixels is identified.

3. Results and discussion

In this study, the area of each leaf was calculated (figure 4) using the proposed method (step 1-3 in figure 5) compared to ImageJ measurement (see figure 3). In order to evaluate the proposed method quantitatively, two evaluation indexes, namely, the RMSE and MAPE were used. According to table 1, it was found that the mean RMSE is 3.924, whereas the MAPE with an average value of 6.437. The proposed method was accurate with a small relative error. The errors may be attributed to uneven surface leaf [25] [26], thus incomplete image resulting in a pixel count is less than the real pixel count of the selected region. This gives a wrong value for the calculated area.

| Table 1. RMSE and MAPE measurement of pak choy canopy. |
|---------|------|------|
| Replication* | RMSE | MAPE |
| 1        | 3.892 | 5.122 |
| 2        | 3.887 | 6.120 |
| 3        | 3.994 | 8.070 |
| mean     | 3.924 | 6.437 |

*Every replication contains 20 plant samples (2 weeks after transplanting).
Figure 4. Leaf area of pak choy measurement using image analysis (2 weeks after transplanting).

The experiments were carried out based on watershed segmentation using a distance transform technique. The idea is to generate a border to the extent that possible from the middle of the occlusion objects. The method involves of computing the distance transform of the binary image and applying a watershed technique to it using the original image as mask. The distance transform-watershed results are given in figure 5.

Figure 5. Leaf area measurement and leaf counting using distance transform-watershed method on pak choy plants.
Our segmentation of leaf region is given in table 2. We apply precision, recall, and F-measure to describe leaf number measurement of pak choy every week. According to the table 2, the trend of the results illustrates that the precision values increases with increasing age of the plant. This is affected by a variety of factors, such as, the structure of the pak choy is increasingly complex as well as the leaves become straight up made difficult to identify the real leaf. Due to the complexity of the leaves, the number of leaves occlusion will increase false positive. The recall values tend to be constant due to false negative value is 1 (weeks 3 and weeks 4). However, in the weeks 2 the false negative is 0.99 because some of tiny leaves are incorrectly detected. The f-measure also decreases over time. The reason is that the proposed technique cannot accurately extract the target leaves, especially for heavy occlusion regions (see figure 6). Moreover, since the plants take place in the open field, the environmental factors such as illumination could influence the segmentation of the images. In addition, the illumination could produce little difference in brightness between the leaf area and surrounding area [27].

Table 2. Performance comparison of leaf region extraction at different age of pak choy.

| Pak choy age | Replication* | Segmentation performance |
|--------------|--------------|-------------------------|
|              |              | Precision  | Recall  | F-measure |
| Weeks 2      | 1            | 0.816      | 0.986   | 0.893     |
|              | 2            | 0.882      | 1       | 0.937     |
|              | 3            | 0.800      | 0.985   | 0.883     |
|              | mean         | 0.833      | 0.99    | 0.944     |
| Weeks 3      | 1            | 0.505      | 1       | 0.671     |
|              | 2            | 0.482      | 1       | 0.650     |
|              | 3            | 0.432      | 1       | 0.604     |
|              | mean         | 0.473      | 1       | 0.642     |
| Weeks 4      | 1            | 0.345      | 1       | 0.512     |
|              | 2            | 0.375      | 1       | 0.545     |
|              | 3            | 0.349      | 1       | 0.517     |
|              | mean         | 0.356      | 1       | 0.525     |

*Every replication contains 20 plant samples (each of them has approximately 5-12 leaves).

The original watershed algorithm produces excessive object fragmentation when applied to overlapping images [28]. Other study used extended-maxima transform watershed segmentation algorithm for touching corn, the results satisfactory segmentation by the improved algorithm [29]. The over segmentation is caused mainly by spurious minima when spurious markers are generated due to an irregular object shape and overlapped [30]. The distance transform-watershed segmentation provides more correctly separating overlapping leaves than the original watershed method. The proposed method is able to decrease over segmentation and under segmentation of touching and overlapping leaves.

The figures (figure 6a–6c) show the experimental results on pak choy images with varying complexities. With our method some errors still remain, due to heavy occlusion (figure 6d) on the leaf pixels that look strongly similar to other leaf beneath. However, our method represents a good compromise since we assure a good robustness to false negatives and good precision for minimal occlusion leaves. Further study heavy leaves occlusion is needed to investigate in order to improve the recognition of partially occluded plant leaves.
4. Conclusions
The image processing was used to segment the images of the pak choy. The area of leaves measured compared to the ImageJ measurement which achieves minimal error and provides quick and non-destructive method. The number of extracted leaves was evaluated according to precision, recall, and f-measure formula. The present study represents good robustness to false negatives and also good precision for minimal occlusion leaves.

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