

Development of a Computer Vision-Based Platform for Leaf Size Estimation

Pengembangan Platform Berbasis Visi Komputer untuk Estimasi Ukuran Daun

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ABSTRACT

This study aimed to develop a platform to estimate leaf sizes based on digital vision. Although existing platforms provide valuable approach for leaf area estimation, the proposed platform extends functionality by extracting additional morphological parameters, including perimeter, length, and width. By capturing single lime leaves, the dataset comprising 86 images was split into 60% for calibration purposes and 40% for testing. The acquired leaf images were processed to extract size features, consisting of area, perimeter, length, and width. The actual sizes were measured by placing the leaf samples on a millimeter graph paper and counting the grids inside the leaf's edge. The estimated leaf sizes were predicted using image analysis by calibrating pixels per mm in the image. Simple linear regression was performed to check the strong relationship between the actual and predicted sizes. For all size parameters, the R^2 reached very close to 1.0, while the RMSEs were lower than 0.10. According to Bland-Altman (B&A) plots, the differences between the actual and predicted sizes are normally distributed, and almost all the points lie inside the 95% limit of agreement. Finally, our platform could be an alternative to estimate leaf sizes, which provides rapid, accurate, and non-destructive measurement.

Keywords: Single leaves; computer vision; size prediction; regression analysis; LeafSizeEst

ABSTRAK

Penelitian ini bertujuan untuk mengembangkan platform visi digital untuk memprediksi ukuran daun. Meskipun platform yang ada telah unggul dalam estimasi luas daun, platform yang dikembangkan mencoba memperluas fungsionalitas dengan mengekstraksi parameter morfologi lainnya, antara lain keliling, panjang, dan lebar. Dengan mengambil citra daun jeruk nipis tunggal, set data terdiri dari 86 gambar dan dibagi menjadi 60% untuk kalibrasi dan 40% untuk pengujian. Citra daun diproses untuk mengekstraksi fitur ukuran, yang terdiri dari luas, keliling, panjang, dan lebar. Ukuran aktual daun diukur dengan menempatkan sampel daun pada kertas grafik milimeter dan menghitung jumlah grid di dalam garis tepi daun. Ukuran daun diprediksi menggunakan analisis citra dengan mengkalibrasi piksel per mm. Regresi linier sederhana digunakan untuk mengetahui hubungan kuat antara data ukuran aktual dan terprediksi. Nilai R^2 yang dihasilkan mendekati 1,0, sementara RMSE kurang dari 0,10. Berdasarkan analisis plot Bland-Altman (B&A), perbedaan antara data ukuran aktual dan terprediksi terdistribusi secara normal, dan hampir semua titik berada di dalam batas agreement 95%. Platform dapat menjadi alternatif untuk memprediksi ukuran daun dengan menawarkan fitur pengukuran yang cepat, akurat, dan non-destruktif.

Katak unci: Daun tunggal; visi komputer; prediksi ukuran; analisis regresi; LeafSizeEst

INTRODUCTION

Rapid and accurate measurement of leaf area is crucial in various fields of plant science, including agronomy and forestry (Easlon & Bloom, 2014)(Hu et al., 2018)(Katabuchi, 2015). In the field of agriculture, leaf area estimation is an important aspect of physiological studies of plants concerning evapotranspiration, photosynthetic efficiency, heat transfer, fertilizer and irrigation response, and plant growth (Pandey & Singh, 2011). Several studies recorded the study of leaf area measurement for various purposes, such as to estimate the development of plant leaf area during the growing period (Elings, 2000), to differentiate species or cultivars based on different leaf shapes (Yu, Shi, Schrader, & Niklas, 2020), and plant growth characteristics of different seeds (Li et al., 2020). Moreover, another study documented

the measurement of leaf area in combination with other size measurements, such as width, length, perimeter, and weight (Huaccha-Castillo et al., 2023; Schrader et al., 2021; Yu et al., 2020).

Traditionally, leaf area has been measured using conventional methods, such as tracing the leaf outline on paper or using specialized devices like leaf area meters. These methods might be appropriate for plants that have sparse and non-fragile leaves. However, these methods can be time-consuming, labor-intensive, and may cause damage to the plant (Pandey & Singh, 2011). To address these limitations, researchers have explored the use of digital image processing techniques as a non-destructive and efficient alternative for leaf area estimation (Qiu, Fang, Zhang, & He, 2006). The linear relationships between leaf area measured manually and leaf dimension estimated from

digital images could be useful to develop equations for nondestructive estimation of leaf area. Previous studies conducted by (Easlon & Bloom, 2014; Jadon, Agarwal, & Singh, 2016; Li et al., 2020; Pandey & Singh, 2011) proposed a new automatic and easy method for leaf area estimation based on digital images of leaves.

To utilize digital images for leaf area estimation, practical procedures in a computer vision system are adopted. The computer vision system (CVs) practically includes an image acquisition device, i.e., RGB camera, image pre-processing, object segmentation, object measurement for feature extraction, and finally calibration and prediction (Du & Sun, 2004). It will quantitatively characterize complex properties such as detailed size and shape that cannot be observed by the human eye (Zhu, Spachos, Pensini, & Plataniotis, 2021). Size analysis plays a vital role in measuring physical dimensions that characterize the appearance of an object. The most basic convenient measurement for size is the area, perimeter, diameter, length, width, and thickness, which represent the number of pixels within them, which is straightforwardly determined by counting (Du & Sun, 2004). By assuming that the number of pixels is proportional to the actual size of an object, hence, a representative model which has a linear relationship between proposed methods to estimate leaf sizes can be constructed.

Therefore, the objective of this study was to develop a non-destructive technique based on image analysis for measuring leaf surface area and other size parameters, such as leaf perimeter, width, and length. Lime leaves were selected because they offer a morphological consistency with their relatively uniform shape, clear venation pattern, and moderate size. However, geometric variability across samples makes the determination of leaf sizes difficult, time-consuming, and subject to larger errors, when measured using a conventional method—a millimeter graph paper. The digital images of the leaves were acquired using a smartphone camera, which was connected to a local computer. The leaf images were analyzed to obtain leaf dimensions using two empirical approaches: (a) from calibration of pixels per mm of the leaf surface and (b) referring to the distance between the image sensor-to-object and sensor specifications. Another objective was to compare the manually measured and digitally estimated leaf sizes, develop linear equations, and observe the relationship between the two estimates. This study developed 'LeafSizeEst' to process digitally leaf samples, remove the background, extract the size parameters, save results to a

spreadsheet-ready CSV file, and estimate leaf sizes using the proposed empirical approaches.

METHODOLOGY

Materials

In this study, LeafSizeEst was developed as an automated digital image analysis of leaf samples to facilitate digital measurement of leaf sizes, including area, perimeter, width, and length. It used a combination of image acquisition, pre-processing, object segmentation, and measuring size parameters of individual leaf images. Finally, it predicted the size parameters based on calibrated images. The LeafSizeEst was built in Python (<http://www.python.org/>); the results were tabular data generated while shape-based feature analysis. The predicted leaf sizes were also obtained by LeafSizeEst from calibrated linear models; the exact linear equations are available in the Python code (<https://github.com/agdhr/LeafSizeEst>). The GUI of the LeafSizeEst is shown in Figure 1.

Image Acquisition

A total of 86 single lime leaves were captured using a computer vision system (CVs) to obtain standard images. The CVs developed in this study consisted of (Figure 2):

- A dark chamber (20x20x20-cm): to minimize background light and eliminate interference from outside light;
- A smartphone-based image sensor (rear camera, Galaxy A14 5G, Samsung Electronics Co. Ltd., 12.5 MP, 1.3 μm pixel length, 4.0 mm focal length, f/1.8 aperture, 1/20-s exposure, ISO 200 speed), located in the center of the chamber and 50 cm above the sample plane, followed with the following settings: no flash use and no autofocus;
- An intense lighting system: four neon LED tube lamps (30-cm length), 5 watts each, color temperature of 6000-6500 K. The lamps were placed 20 cm above the samples at an angle of 45° to the sample plane, giving a uniform light intensity over the leaves' samples;
- A personal computer (Intel(R) Core (TM) i5, CPU 8.00 GB (RAM), 512 GB SSD capacity with Ms. Windows 11 Home Single Language, and 64-bit operating system), equipped with Visual Studio Code 1.96.4 as Python 3.10 IDE for OpenCV and PyQt5, to analyze and process the images. ImageJ v1.54g was employed to determine the calibrated pixel-per-millimeter value using a ruler within the image.

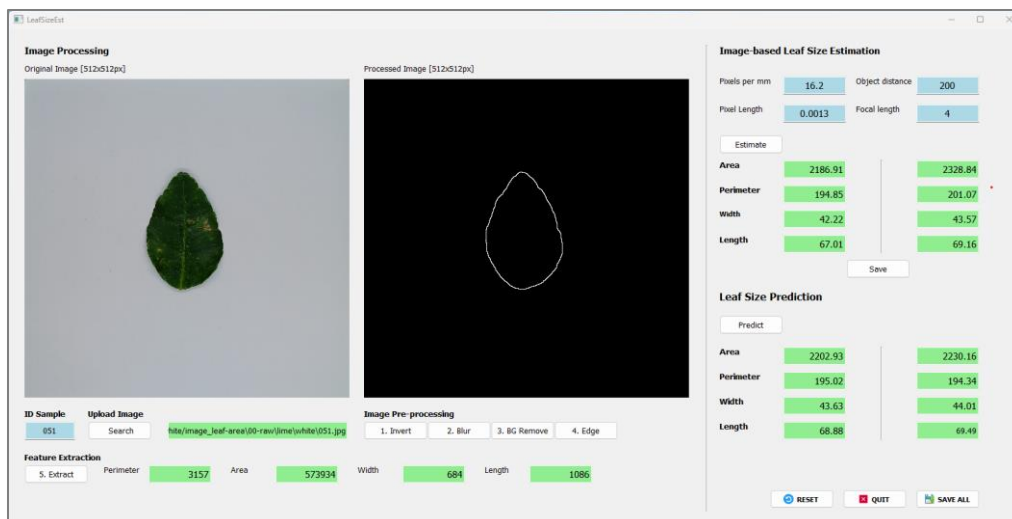


Figure 1. LeafSizeEst, a rapid and accurate measurement platform for leaf size estimation

- e) Iriun Webcam v2.8.10 software, connect and use the phone's camera to acquire and store lime leaf images into the PC.
 f) A white background.

All samples were stored in the RGB-colored image (Figure 3a) with .JPEG file format and a 3056×3056 pixels size (or 1:1 aspect ratio resolution).

Extraction of Image Features

All acquired images were processed in digital image processing to analyze images and identify physical characteristics that humans require a large amount of time to extract. A filter method was applied to eliminate noise appearing on the images. This filter used a 2D Gaussian smoothing 7×7 kernel (García, Candelo-Becerra, & Hoyos, 2019). The images' colors were inverted so that they are easy to perform image binary operations, where green was reversed to magenta and white was reversed to black. Using an Otsu thresholding method, a segmentation process was applied to remove the background and objects not part of the images (Koklu & Ozkan, 2020; Oliveira, Cerqueira, Barbon, & Barbin, 2021). To extract physical information from images, all images were converted into binary images and transformed into two colors: the object should be white, and the background should be black (Figure 3c). Image contouring then identifies an object's structural lines by combining all continuous points along the boundary to find its contour. A contour typically refers to edge pixels with a similar color intensity. Edge detection was a required step to determine which pixels should be considered edge pixels using one of the most popular edge detection techniques, the Canny operation. Figure 3d depicts a generated image after performing edge detection.

Further analysis was to extract features describing each leaf based on image dimensional attributes using shape feature analysis. This used OpenCV to identify and extract geometric features in images. The extracted features represented values in pixel count. A total of four features were determined as follows:

- Area (A): the number of pixels taken up by the surface area of an object.
- Perimeter (or circumference) (p): the number of pixels that form the distance/arch length or around the edge of an object.
- Major axis length (l): the pixel distance between the endpoints of the longest line that can be drawn through the object.

Minor axis length (w): the pixel distance between the endpoints of the longest line that can be drawn through the object while standing perpendicular to the major axis.

Extraction of Leaf Sizes

Referring to (Pandey & Singh, 2011; Santra, Mansuri, Dhaka, & Mahla, 2020), the leaf area of each leaf (A_A) was measured manually using a millimeter graph paper, see Figure 3b. Each leaf was spread over the paper (grid size = 1 mm) and scanned using a camera attached to a desktop computer while acquiring images. A leaf area was obtained by counting and summing squares or grids on the millimeter graph paper covered by the leaf. The number of grids is counted in three types: fully covered grid (FG), three-fourths covered grid (TFG), half-filled covered grid (HG), and quarter-filled covered grid (QG). The areas which were covered less than QG were either neglected or aggregated. A total covered leaf area (in mm²) was calculated as follows (Equation 1):

$$LA_{\text{measured}} = FG + \left(\frac{3}{4} \times TFG\right) + \left(\frac{1}{2} \times HG\right) + \left(\frac{1}{4} \times QG\right) \quad (1)$$

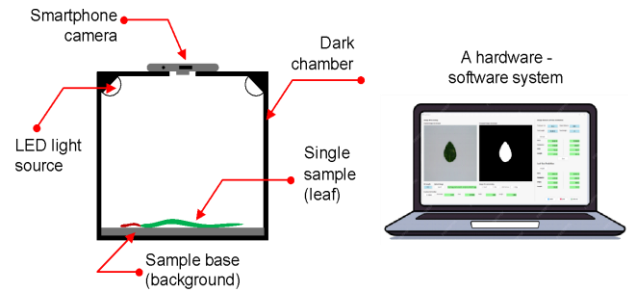


Figure 2. Image acquisition setup

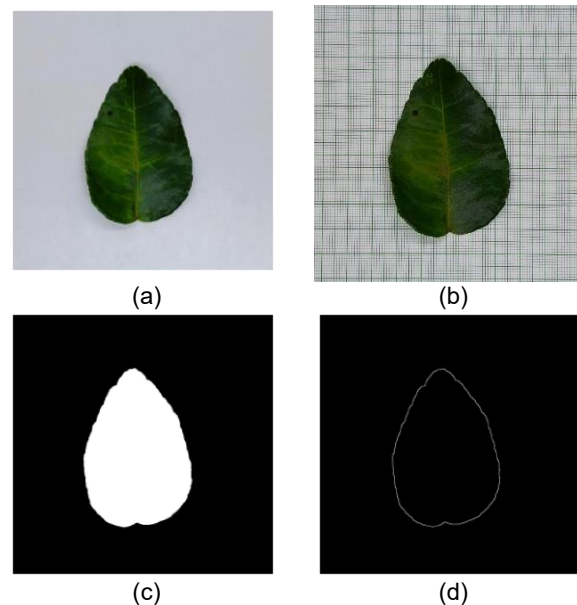


Figure 3. Results of the image processing stages

To obtain an object's perimeter (p_{measured}), a ruler was used to measure the distance (or circumference) of the object, where the outline curves away from a picked starting point and ends at that point. Leaf length (l_{measured}) was measured as the distance from the leaf base to the apex. Leaf width (w_{measured}) was identified as the maximum distance between two points on the leaf perimeter forming a straight line perpendicular to the straight line connecting the leaf base and apex (Yu et al., 2020). Both leaf length and width were measured using a caliper (with an accuracy of ±0.1 mm).

To quantify leaf area on digital images, two empirical methods were employed. The first method was by calibrating the number of pixels per millimeter of the image. By using ImageJ, a straight line was drawn horizontally (or vertically) over the grid paper with a size of 10 mm on ten leaves, a sample image is depicted in Figure 3-b. The line's scale was set with a known distance per length-unit of 10 mm and obtained an average+SD of 16.2±0.1 pixels/mm (denoted as n_A). The area (in mm²) of the leaf was estimated by the area of digital leaves (A_i) per squared value of n_A , see Equation 2. The leaf perimeter, width, and length could also be determined by dividing their pixel values ($p_{\text{estimated}}$, $l_{\text{estimated}}$, and $w_{\text{estimated}}$, respectively) by n_A .

$$LA_{\text{estimated1}} = \frac{A_i}{(n_A)^2} \quad (2)$$

Another method followed a proposed model described by (Li et al., 2020) using Equation 3, where PL is the individual pixel length (1.3 μm; value is specific to the sensor, see Section 3.1) and MF is the magnification factor.

$$LA_{\text{estimated2}} = A_i \times (PL \times MF)^2 \tag{3}$$

Based on a standard lens equation, MF is calculated as follows (Equation 4):

$$MF = \frac{OD - F}{F} \tag{4}$$

OD is object distance, which is the linear length between the camera and the leaf, and F is the focal length of the camera (4 mm; value is specific to the camera, see Section 3.1). The estimated perimeter, width, and length are equal to the product of the object sizes (p_i , w_i , and l_i , respectively) and a quantity of $PL \times MF$.

Prediction

A total of 86 images was partitioned, with 60% allocated for calibration and the remaining 40% reserved for testing. The relationship between the measured leaf area and the predicted leaf area was explained by fitting a simple linear regression model (Equation 5), where y is the actual leaf area measured by a millimeter graph paper and x is the leaf area predicted from digital images. The intercept β_0 and the slope β_1 are regression coefficients estimated using the method of least-squares (Montgomery & Runger, 2014). The linear model was not only used to predict leaf area, but also to predict the perimeter, length, and width of lime leaves. Prior to prediction, the dataset was separated into two categories, with a total of 60% of the data being used to train the models and the remaining 40% for testing the models. A paired t -test was used to identify whether the two-sample data were statistically different on average. An F -test was also used to compare the variances of measured and estimated samples to see if they were equal. Both the t - and F -tests take a confidence interval (CI) of $100(1 - \alpha) \%$, where $\alpha = 0.05$. A Bland–Altman (B&A) plot was used to analyze the agreement between these two datasets (Bland & Altman, 2007).

$$\hat{y} = \beta_0 + \beta_1 x \tag{5}$$

To evaluate the goodness and absolute fit of the models, the coefficient of determination (denoted as R-squared or R^2) was evaluated. The comparative statistical parameters for prediction were also evaluated, including the root mean squared error (RMSE), mean absolute error (MAE), and mean absolute percentage error (MAPE) (Mansuri, Gautam, Jain, Nickhil, & Pramendra, 2022). The R-squared is often used to judge the adequacy of a regression model (Montgomery & Runger, 2014). It describes the proportion of the variation in the response data that is predicted by the model using the predictor variable, calculated as Equation 6. RMSE measures the differences between values that are predicted by a model and values that are observed (Sharma, Chatterjee, Kaur, & Vavilala, 2022). It is defined as the standard deviation of the prediction errors (another name for residuals) around the best-fit regression line (Elavarasan, Vincent, Sharma, Zomaya, & Srinivasan, 2018), see Equation 7 for computing RMSE. MAE, a measure used to determine the mean of the error magnitudes ignoring the direction, can be defined as the average of the absolute difference between the predicted and the actual value (Elavarasan et al., 2018), see Equation 8. MAPE, with a relative value in the range of $[0, 1]$, is used to determine the percentage deviation of the estimation results and, further, to indicate how much error in prediction is compared to the actual value, computed in Equation 9.

$$R^2 \text{ score} = 1 - \frac{\text{RSS}}{\text{TSS}} = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \tag{6}$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \tag{7}$$

$$\text{MAE} = \frac{\sum_{i=1}^n (\hat{y}_i - y_i)}{n} \tag{8}$$

$$\text{MAPE} = \frac{1}{n} \sum_{i=1}^n \left(\frac{y_i - \hat{y}_i}{y_i} \right) \tag{9}$$

where, \hat{y}_i are the values of the estimated leaf sizes, y_i are the measured leaf size values, \bar{y} is mean of y_i , $i = 1, 2, \dots, n$, and n is the number of samples.

RESULTS AND DISCUSSION

In this study, the relationship between the leaf area of lime leaves measured by a millimeter graph paper method and that estimated by digital images acquired with a smartphone camera is examined. In the first method, the leaf area from images is calibrated to determine how many pixels in a certain image dimension correspond to a single millimeter in a real physical dimension. The second approach reveals the fact that the area in an image of an object and the actual area of an object are equal if we can correlate it with specific values stated on the product specifications of the image sensor and the distance between the sensor and the object. The mean, standard deviation, and maximum and minimum values of measured leaf area, as well as the estimated leaf area from digital images of lime leaves, are shown in Table 1. By estimating the leaf area, we can also find other leaf sizes, such as perimeter, width, and length.

Table 1. Statistical size parameters of 86 leaf images

Size parameters	Results of descriptive statistics			
	mean	stdev	min	max
Measured				
Area	1549.49	528.30	539.31	2838.97
Perimeter	159.90	29.89	93.06	213.94
Width	36.56	6.69	20.71	54.22
Length	54.09	9.89	31.05	75.34
Estimated				
Area ¹⁾	1582.03	537.37	554.53	2788.17
Perimeter ¹⁾	161.63	28.78	95.39	217.35
Width ¹⁾	38.16	6.95	22.42	51.76
Length ¹⁾	56.18	10.27	32.79	72.35
Area ²⁾	1650.05	562.59	574.31	2969.12
Perimeter ²⁾	165.01	30.84	96.04	30.84
Width ²⁾	37.73	6.91	21.38	53.41
Length ²⁾	55.82	10.21	32.05	74.66

¹⁾ Predicted from calibration of pixels per mm; ²⁾ Predicted refers to the distance between the sensor-object and the sensor specifications

Table 2. Established linear equations for the prediction of leaf area (LA_{measured}), perimeter (p_{measured}), width (w_{measured}), and length (l_{measured}) measured by a millimeter graph paper that estimated which refers to the distance between the sensor-to-object and the sensor specifications

Size parameters	Calibration		Prediction		
	Linear Equations	R^2	RMSE	MAE	MAPE
Area	$y = 6.120 + 0.955x$	1.000	0.016	2.10	0.002
Perimeter	$y = 11.572 + 0.909x$	0.996	0.014	1.64	0.011
Width	$y = 0.002 + 1.010x$	0.990	0.000	0.58	0.016
Length	$y = 0.606 + 0.996x$	0.989	0.001	0.69	0.012

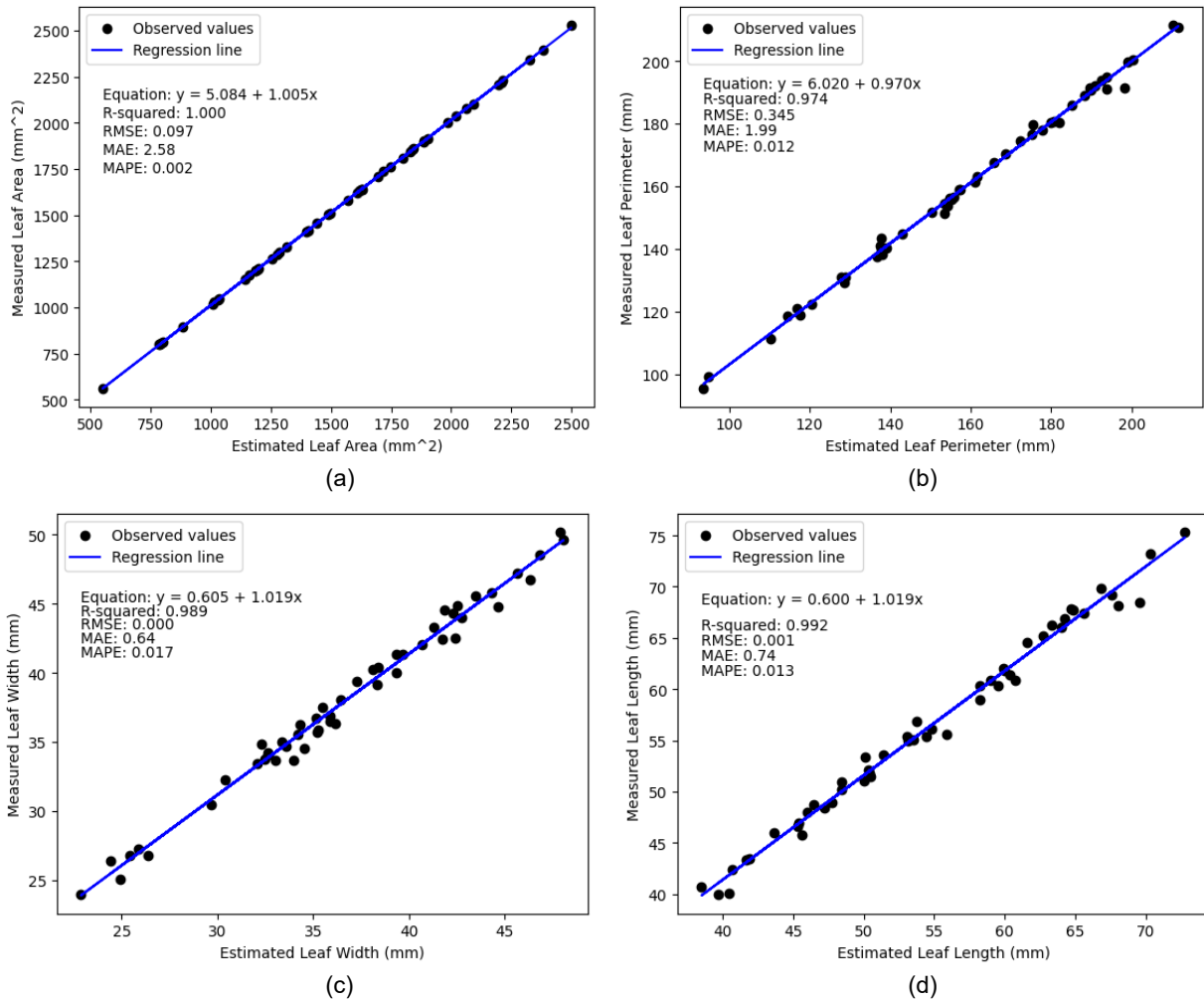


Figure 4. Linear relationship between measured leaf area (LA_{measured}), perimeter (p_{measured}), width (w_{measured}), and length (l_{measured}) using millimeter graph paper and estimated leaf area ($LA_{\text{estimated}}$), perimeter ($p_{\text{estimated}}$), width ($w_{\text{estimated}}$), and length ($l_{\text{estimated}}$) using smartphone-based image analysis, which was predicted from calibration of pixels per mm.

Table 3. Paired-sample t -test and F -test between estimated and measured size values

Estimated Size parameters	Paired t -test				F -test		
	t -statistic	p -value	95% confidence interval of the mean difference		F -statistic	p -value	
			Lower	upper			
1	Area	-0.400	0.689	-192.95	127.86	0.966	0.437
	Perimeter	-0.387	0.699	-10.56	7.10	1.078	0.635
	Weigth	-1.537	0.126	-3.65	0.45	0.926	0.362
	Length	-1.363	0.174	-5.13	0.94	0.928	0.367
2	Area	0.810	0.418	-97.59	233.63	1.096	0.663
	Perimeter	0.742	0.459	-5.60	12.36	1.148	0.737
	Weight	-0.409	0.683	-2.52	1.65	0.986	0.474
	Length	-0.236	0.814	-3.45	2.71	0.989	0.479

Estimated size values from: (I) pixel per mm calibration, and (II) obtained from sensor specifications and distance of image acquisition.

For other size attributes such as perimeter, width, and length of lime leaves, the coefficient of determination between the two sets of estimates was achieved in the range of 0.988 – 1, while the RMSE varied from 0 to 0.012. The best value for MAPE is 0. A higher value of MAPE indicates that the predictions are not accurate enough. Referring to Table 2, the predicted leaf sizes were good enough because there were 0.2 – 1.7% errors in the predicted leaf sizes based on the leaf sizes estimated from digital images. MAE obtained small differences (errors) in the range of 0.59 – 2.58.

Figure 4 depicts that the two sets of nondestructive leaf sizes estimated from calibrating image pixels per mm were strongly related to those obtained destructively by millimeter graph paper. Whereas, the linear relationship between the measured leaf sizes and that estimated using smartphone-based image analysis, which refers to the distance between the sensor-to-object and the sensor specifications, is shown in Table 2. The R^2 value, which is always between 0 and 1, indicates that the model works excellently to fit the estimated leaf sizes with the actual data. Referring to Figure 4a, the correlation between estimated leaf area and reference leaf area is superior; the first-order regression equation gives an $R^2 = 1.0$. The RMSE, which measures the differences between predicted values that are generated by the model and values that are observed, gives 0.02. It indicates that the model is considered to have the best performance. RMSE is closest to zero for perfect estimates.

The visualization of scatter diagrams in Figure 4b-d indicates that there is a strong indication that all points lie scattered randomly around a straight line. The prediction accuracy varies among the samples due to the complexity of the leaf shapes. On the other hand, the method using image processing may also affect the accuracy due to the lack of proper segmentation. The conventional measurement of an object's perimeter with its irregular leaf margin drawn on millimeter graph paper contributes to the lack of precision in counting the grid crossed by the borderline.

The next measure is a paired t-test to examine whether a statistical significance exists between the means of the two datasets (Ross & Willson, 2018). Table 3 shows the obtained p-values of paired t-tests that are used to check whether the means of two estimates are equal or not. The obtained p-values 0.689 [$LA_{\text{predicted1}}$, LA_{measured}] and 0.418 [$LA_{\text{predicted2}}$, LA_{measured}] proved that the estimated leaf area and the actual leaf area are not statistically significant at the $\alpha = 5\%$ level due to p-value > 0.05. Therefore, we conclude that the leaf area prediction from the two methods yielded almost similar results or equal. According to the datasets for estimating leaf perimeter, width, and height, the p-values that correspond to the test statistic t with 170 degrees of freedom were higher than a significance level of 0.05. Hence, we fail to reject the null hypothesis; there is insufficient evidence to prove that the means of leaf perimeter, width, and length between the two estimates are different.

Furthermore, an F-test statistic is crucial to know whether the variabilities of the sample data obtained from two methods of leaf area estimation are equal or not. It is calculated by the ratio of the variances between two leaf area datasets with 86 observations each. In this study, we will check whether the two methods ($LA_{\text{predicted1}}$ and $LA_{\text{predicted2}}$) have the same variance or are significantly different (at a significance level of 0.05). The calculated F-statistics are 0.966 ($LA_{\text{predicted1}}$, LA_{measured}) with the respective p-value equals 0.437 and 1.096 ($LA_{\text{predicted2}}$, LA_{measured}) with a p-value of 0.663, see Table 3. Hence, we will accept the null hypothesis since the p-values are larger than 0.05, indicating that the two datasets have equal variance. Because the obtained p-values for the two methods estimating the leaf perimeter, width, and length, respectively, are greater than

0.05 (a significance level) in Table 3, the null hypothesis is accepted, and the conclusion is that the two variances for the two estimates are not equal.

The R^2 explains the proportion of the variance (or differences) that two sets of data have in common. The R-squared values of the regression plots (Figure 4) are perfectly close to 1, indicating that the model explains almost all of the response variables. The models have a very strong fit to the data. However, the high correlation does not imply that there is good agreement between the two datasets. The B&A plot tries to quantify this agreement between two sets of data by constructing limits of agreement (Giavarina, 2015).

Figure 5 depicts the B&A plot generated by two methods to estimate leaf area, perimeter, width, and length, where the x-axis is the average and the y-axis is the difference between the two corresponding estimates of leaf size. The dashed line represents the limits of agreement between the two datasets. The left plots represent the B&A plot where the estimated leaf sizes form calibrating pixel per mm calibration, and the right plots are the estimated sizes obtained from digital images, which refers to sensor specifications and the distance of image acquisition.

Figure 5a compares two highly correlated datasets with an outstanding determination coefficient (R^2) of 1.00. The B&A plot defines a bias of -32.54 units with an agreement range from -51.44 to -13.65 (Figure 5a-left) and 68.02 with an agreement range between 18.14 and 117.89 (Figure 5b-right). The constant coefficient of variation across the range of the area values indicates that the two estimates have proportional difference variability. The B&A plot reported a widening trend of the agreement range with increasing area values. According to Figure 5a, the mean differences and the mean confidence interval (light gray stripe) are higher (or lower) than 0, indicating that the levels of retention (or bias) are significantly different. Points placed above or below 0 on the y-axis are largely determined by the differences between the two estimates. Due to that $-32.54 < 0$ (Figure 5a-left) means that $LA_{\text{measured}} > LA_{\text{predicted1}}$, and as a result, LA_{measured} provides a bigger retention to $LA_{\text{predicted1}}$. Conversely, due to that $68.02 > 0$ (Figure 5a-right) means that $LA_{\text{predicted2}} > LA_{\text{measured}}$, the $LA_{\text{predicted2}}$ gives a bigger retention to LA_{measured} .

In Figure 5b, the variability of the differences between the two estimates is constant, and the spread of differences remains consistent across the range of perimeter values. However, referring to Figure 5c-d, the difference plots allow us to comprehend a negative trend of differences, which is proportional to the magnitude of leaf width and length. The level of retention (or bias) of $W_{\text{predicted1}}$ VS W_{measured} and $l_{\text{predicted1}}$ vs l_{measured} are significantly different.

The two light blue bars represent the confidence intervals of the agreement limits for two estimates at 95%, stating that this study is convinced that 95% of the difference of area values will be from -51.44 to -13.65 in Figure 5a-left and between 18.14 and 117.89 in Figure 5a-right. There are two points placed below the agreement limits in Figure 5a-left, which is $(2/86) = 0.023 < 0.05$ due to 95% of the value being within the two boundaries (the upper and lower dashed lines). The three points above the upper dashed line (Figure 5a-right), which is $0.034 > 0.05$, in our case, 3.4% is perfectly normal. It concludes that the differences between the measured leaf area and the estimated leaf area are normally distributed because almost all of the points lie inside the outer boundary lines (95% limit of agreement).

Discussion

Leaf size, such as leaf area, is one of the most important attributes to gain insights into the growth and development of plants and how they interact with the environment. Manually, the researchers calculated the leaf areas using various

approaches, such as regression equations, a grid count method (Santra et al., 2020), a gravimetric method, planimeters, and scanners (Katabuchi, 2015). Many studies (Chaudhary, Godara, N. Cheeran, & K. Chaudhari, 2012; Easlon & Bloom, 2014; Santra et al., 2020) have successfully developed digital image processing-based estimation methods for leaf size estimation. In this study, we first integrate the smartphone camera and PC to acquire and store RGB images of lime leaves. The real-world size of lime leaves is calibrated with pixels per mm in the image and refers to pixel length, focal length, and object distance of the camera. We further designed a platform, namely

'LeafSizeEst.', using PyQT5 in Python code for automated prediction. The platform provides a user-friendly, automated tool for measuring leaf size from the images. Our findings proved that the developed platforms achieved a prediction performance (R^2) larger than 0.98, as detailed in Table 2. A key challenge employed this platform was image distortion caused by illumination. The presence of small particles that are captured by the camera also affected the segmentation process. Despite these issues, the platform maintained high prediction, demonstrating its robustness in practical applications.

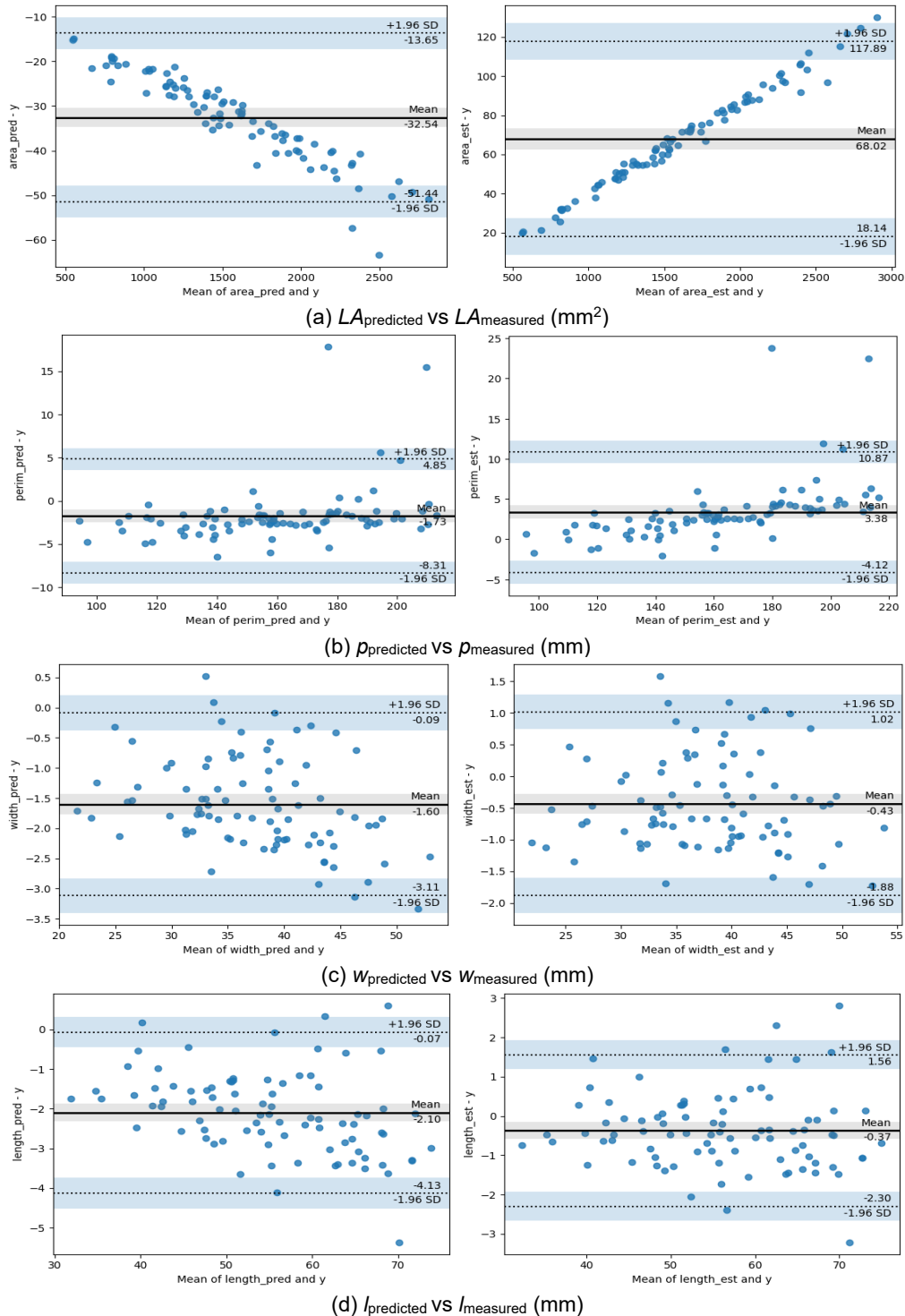


Figure 5. The Bland-Altman (B&A) plots to check the agreement between measured leaf sizes vs that estimated from, left: pixel per mm calibration and right: obtained from sensor specifications and distance of image acquisition.

CONCLUSION

In this research, we experiment with an accurate image analysis that uses a smartphone connected to a local hardware-software system to predict the area of lime leaves. The millimeter graph paper was used manually to measure the sizes of the individual leaf samples. Further, we develop a linear (first-order) model to discover the relationship between manually measured and digitally estimated leaf areas, in which the samples vary across different shapes. The linear model was superior for leaf area prediction, with an R^2 very close to 1. Another size prediction, such as leaf perimeter, width, and length, also demonstrated an excellent linear relationship, R^2 was 0.988, 0.989, and 0.991, respectively. It implies that the models are feasible to use, and the leaf size estimated from digital images has a simultaneous influence on the response variable of the actual leaf size. By using LeafSizeEst, the process of measurement and estimation of leaf sizes becomes rapid, accurate, and non-destructive.

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