Research Article

A comparative study of plant phenotyping workflows based

on three-dimensional reconstruction from multi-view images

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Abstract

With the world facing escalating food demand, limited agricultural land, and rapid environmental change, there is a growing need for data-driven sustainable agricultural management approaches. The proliferation of next-generation sequencers and sensor networks has reduced the cost of acquiring genomic and environmental data, respectively. However, collecting phenotypic data, which is crucial for monitoring plant growth trajectories and detecting pests and diseases, continues to be a labor-intensive endeavor. Technological advances have enabled an efficient collection of three-dimensional (3D) data, yet this process currently involves a set of intricate steps. Therefore, the development of an effective phenotyping method is essential. In this study, we developed a phenotyping process based on 3D reconstruction, including mask image generation using deep neural network models, 3D reconstruction using the Structure from Motion/Multi-View Stereo (SfM/MVS) pipeline, and leaf surface reconstruction for leaf area estimation. Our investigation using soybean datasets into the optimal magnification for input images in mask generation models revealed that a 1/5.4× magnification was most effective for segmenting thin structures. Using mask images in the SfM/MVS pipeline limited the region of interest, and this could decrease the processing time and improve the quality of the point-cloud data. We assessed four scenarios regarding mask image usage and found that the scenario that set the mask images

of soybeans and stages before SfM and soybeans only after SfM yielded the highest-quality

point-cloud data and was the second fastest in processing. Finally, we compared the Poisson

reconstruction and B-spline surface fitting for leaf surface reconstruction from point clouds.

B-spline fitting shows a greater correlation with destructive measurements. We proposed an

optimal workflow for estimating leaf area based on these results. Additionally, to contribute

to the development of plant phenotyping methods, we provided a web application for mask

generation, a command-line tool for leaf surface reconstruction, and validation datasets.

Keywords: photogrammetry, multi-view image, phenomics, morphometrics, open science

Introduction

The rise in global food demand [1–3], limited agricultural land [4–6], and climate change [7–9] have heightened the necessity for the quantitative evaluation of crop phenotypic information [10–12]. This approach would facilitate the development of efficient and robust agricultural management approaches, which are essential for addressing the abovementioned challenges in a data-driven manner [13,14]. Recent technological breakthroughs, particularly in next-generation sequencing, have facilitated the accumulation of omics data, including genomic and transcriptomic information [15–17]. In addition, advancements in Internet of Things (IoT) sensor technologies have improved the acquisition of environmental data [18,19]. However, despite the significance of phenotypic information in understanding plant growth and physiological responses in various environments [20–22], the acquisition of phenotypic data remains labor-intensive and yields limited data [23–25]. Consequently, there has been increasing interest in plant phenotyping technologies among researchers and agricultural producers [11,12,24–26]. Several studies have reported that the monitoring of three-dimensional morphological properties of plants provides insights for improving cultivation management, including optimizing the timing of pesticide and fertilizer application [27–29].

High-resolution 3D morphological data can be acquired efficiently and cost-

effectively using 3D laser scanners [30–32], depth cameras [33–35], and photogrammetry

[36–38]. A pipeline using structure from motion (SfM) [39,40] and multi-view stereo (MVS)

[41,42], which reconstructs a 3D surface as point cloud data from a set of two-dimensional

(2D) images captured from different angles, is widely available and implemented in several

libraries and software products (e.g., [39,42–44]). Based on the resultant high-resolution

point cloud data, a three-dimensional morphological model can be constructed to yield

phenotypic data (e.g., leaf number, plant height, and leaf area index (LAI) [45–47]).

Optimizing the measurement process for three-dimensional morphological data

directly facilitates the efficient acquisition of phenotypic data. Various approaches have been

proposed for the acquisition of high-quality three-dimensional point cloud data using the

SfM/MVS pipeline (e.g., [48–50]). Mask images offer several advantages in the SfM/MVS

pipeline, such as reduced processing time by limiting the region of interest (ROI) and

suppressing noise derived from background textures [51–53]. Moreover, there are several

choices for creating morphological models for estimating phenotypic data from point cloud

data acquired using SfM/MVS and 3D laser scanners, depending on the assumptions (e.g.,

target traits, precision requirements, and data acquisition conditions). Several methods have

been proposed to provide robust quantification of leaf morphology, such as modeling the

leaf surface as a triangular mesh [54], a set of small patches [55], and a piecewise polynomial

function [56]. Although Poisson surface reconstruction [57] is often used to reconstruct the

target surface based on point cloud data in various fields [58], it is not ideal for leaf surface

reconstruction [59,60]. This is because it assumes that the target surface is closed (i.e.,

topologically equivalent to a sphere). Although various techniques for improving the

phenotyping processes have been proposed, as noted above, it is difficult to say that methods

for optimizing each step in the process or the entire process for the target trait are well

established.

In this study, we aimed to optimize the processes for acquiring phenotypic

information based on 3D morphological data of plants. Through comparative analyses of

various procedures on individual soybean plants, including mask image generation,

SfM/MVS pipelines, and leaf surface reconstruction to estimate the leaf area (Fig. 1), we

formulated an optimal phenotyping process. To generate mask images using a DNN-based

semantic segmentation model, we proposed an ideal scale of input images for the two types

of mask images. Within the SfM/MVS pipeline, we compared the processing time and

reconstructed point-cloud data quality for the four mask image usage scenarios. Finally, we

evaluated the leaf surface reconstruction methods to estimate the area of each leaf by

comparing the two general methods. We propose an optimal 3D reconstruction-based plant

phenotyping process and provide tools and data to accelerate these comparisons. Such

comprehensive comparisons for optimizing plant phenotyping processes can also be applied

to other phenotyping procedures. Overall, these optimization processes can yield insights

that contribute to the improvement of crop yield, quality, and the cultivation of various target

crops.

Fig 1. A phenotyping workflow based on the 3D reconstruction. From multi-view images

of a target plant individual, mask images are generated for the (A) SfM/MVS pipeline (B).

The 3D morphological data is obtained as point cloud data using SfM/MVS pipeline. Several

phenotypic values (e.g., leaf area) are estimated through morphological modeling (C).

Materials and Methods

Multi-view image datasets of individual soybeans

111   We used three multi-view image datasets of soybean (*Glycine max*) plants to train the DNN-

112   based semantic segmentation model ($dataset_t$), evaluate different SfM/MVS scenarios based

113   on the quality of the reconstructed point cloud data and their processing time ($dataset_m$), and

114   evaluate the accuracy of leaf area estimation with two different morphological modeling

115   methods against destructive measurements ($dataset_v$).

116   The $dataset_t$ consisted of four individuals from four different cultivars (Enrei, Zairai

51-2, Aoakimame, and Saga zairai), with each individual represented using 256 or 264

multiview images. All images were annotated in a pixel-wise manner across four classes

(soybean, stake, stage/pot, and background) using Labelbox (Labelbox, Inc., San Francisco,

CA, USA), a web application for generating training data.

The $dataset_m$ consisted of six individuals of three cultivars, namely, Seita, N2295,

and Keumdu, across different growth stages (4, 8, and 12 weeks after sowing, respectively).

The cultivars each included two replicates, with each individual represented using 256, 264,

or 272 multi-view images (Table 1).

Table 1. Specimen list in the $dataset_m$.

The $dataset_v$ consisted of 256 or 264 images per soybean individual obtained from

two cultivars (Hime daizu and Shiro mitsu mame) at two growth stages (6 and 12 weeks

after sowing) with two replicates. After acquiring multi-view images, all leaves were

sampled from individual plants, and their positions were recorded. The sampled leaves were

captured as two-dimensional images at 600 dots per inch (DPI) using a flatbed scanner

(CanoScan LiDE 400; Canon, Tokyo, Japan). The region of interest (ROI) for each leaf was

set semi-manually using the Selection Brush Tool in Affinity Photo 2 (Serif (Europe) Ltd.,

Nottingham, UK) to generate mask images for destructive leaf area measurements. These

mask images were processed using OpenCV via Python; specifically, this processing

involved binarization and the counting of pixels corresponding to each leaf for leaf area

measurements. The leaves of Shiro mitsu mame tended to have a smaller area

(approximately 1000 mm^2) than those of Hime daizu (1000–3000 mm^2).

All multiview images were obtained using a simple photogrammetry studio

consisting of a rotating table (MT320RL40, ComXim, Shenzhen, China), eight digital

single-lens reflex (DSLR) cameras (EOS Rebel SL1, Canon, Tokyo, Japan), coded markers,

and blue screen-like background sheets (S1 Fig). The focal length of each camera was set to

24 mm, and the images were captured approximately every 11° until the rotating table

reached 360 °(256, 264, or 272 images per soybean individual).

Mask image generation using U-Net

U-Net [61], a DNN model for semantic segmentation, was used to generate the mask images

in the SfM/MVS pipeline. The U-Net was trained for two classification tasks: three-

(soybean, stake, and background) and four-class classification (soybean, stake, stage, and

background) (Fig 2A). These different classes of mask images can be used across different

SfM/MVS scenarios (see **SfM/MVS pipelines** for details).

Fig 2. Mask image generation using U-Net model. Results of the semantic segmentation

using the U-Net models for (A) three-class segmentation (soybean, stake, and background)

and (B) four-class segmentation (soybean, stake, stage, and background) on an individual

soybean (Saga zairai). Ground truth data was generated by manual annotation. Each color in

the ground truth and the prediction corresponds to each class. (C) Each image in multi-view

images was divided and resized into multiple 512×512 tiles with the six different

magnifications (1/2×, 1/3×, 1/4×, 1/5.4×, 1/6× and 1/6.75×), for input images to the U-Net

models. Lower magnification input image contains larger region (lower row). (D) Soybean

mask images (foreground: soybean, background: stake and background) and soybean/stage

mask images (foreground: soybean and stage, background: stake and background) were

generated based on the predictions of three- and four-class segmentation models,

respectively. The examples were mask images of a soybean individual (N2295 #1).

We used U-Net with ResNet34 [62] pre-trained on ImageNet as the encoder block

and the softmax function as the activation function on the last layer. To train and predict

using high-resolution images, each input image was divided into a certain number of tiles,

depending on magnification, and resized to 512 × 512 pixels; each tile was segmented into

three or four classes and merged into an entire image. An overlap-tile strategy that excluded

outer pixels that did not exceed a threshold during merging was adopted to avoid border

effects in each tile (Fig 2B). Class-weighted dice loss was used as a loss function, and we

adopted the weights calculated based on the occurrences of the pixels corresponding to each

class in the training data (Table 2). The number of epochs is set to 200. Adam, with a

learning rate of 0.0001, was initially used as the optimizer; this rate was then changed to

0.00001 after 150 epochs.

Table 2. Cross-validations of the U-Net models for three- and four-class segmentation.

For each fold, different values of class weights were used for calculating class-weighted

Dice loss.

Performance, i.e., intersection over union (IoU) and processing time, of U-Net, were

evaluated across six different magnifications of the input images ($1/2\times$, $1/3\times$, $1/4\times$, $1/5.4\times$,

$1/6\times$ and $1/6.75\times$) (Fig 3); each image was divided into 30, 15, 6, 4, 4, and 2 tiles on the

magnifications $1/2\times$, $1/3\times$, $1/4\times$, $1/5.4\times$, $1/6\times$ and $1/6.75\times$, respectively (Fig 2B). We

conducted a group four-fold cross-validation on a dataset_t; images belonging to a single

individual were excluded as test images, and other images were randomly divided into

training (80%) and validation (20%) images. In the training step, we used the Python

package Albumentations [63] for data augmentation, including random resizing and

cropping (i.e., while retaining the aspect ratio of the original images), geometrical

transformation (i.e., vertical and horizontal flipping, and random rotation by 90° zero or

more times), changing color, elastic transformation, and random noise (i.e., Gaussian noising,

blurring, motion blurring, and color jittering). The abovementioned steps were conducted

using a CPU (Intel(R) Core(TM) i7-6950X CPU @ 3.00GHz) and a GPU GeForce GTX

1080 Ti ×2.

Fig 3. The performance of U-Net models on different magnifications of input image.

The IoU scores three-class segmentation for (A) all classes, and each class; (B) soybean, (C)

stake, and (D) background. (E) The processing time of three-class segmentation for an

individual plant. The IoU scores four-class segmentation for (F) all classes, and each class;

(G) soybean, (H) stake, (I) stage, and (J) background. (K) The processing time of four-class

segmentation for an individual soybean.

In subsequent analyses, we generated mask images using the U-Net model trained

on the entire dataset$_t$.

Web application for generating mask images

We developed a web application for mask generation based on the trained U-Net models

(Fig 4). This application allows users to easily manipulate several parameters (scale, overlap,

and mask type) through a simple user interface using Streamlit, which is a Python framework

for developing web applications (https://streamlit.io). The web application, operating

without a GPU, is hosted on the Streamlit Community Cloud (https://mask-prediction-

app.streamlit.app/), and its source code will be made publicly available on the GitHub

repository.

Fig 4. User interface of the web application for mask image generation. (1) Images for

generating masks are uploaded via file uploader widget. User sets (2) input image size and

(3) overlapping ratio, and (4) selects an image for showing an example for dividing. Finally,

(5) one of the mask types is selected, and (6) start prediction. Once processing is complete,

the mask images can be downloaded as a zip file.

SfM/MVS pipelines

We adopted the Structure from Motion (SfM) and Multi-View Stereo (MVS) methods to

generate 3D point cloud data. SfM is a photogrammetric method for simultaneously

estimating the camera parameters and depth of the corresponding points (i.e., sparse point

clouds) from multiview images. The SfM was conducted as per the following steps: (1) key

points (characteristic points to be candidates of tie points) were obtained from each image

in multi-view images using local features (e.g., SIFT [64], AKAZE [65], ORB [66]); (2) the

tie points, which are corresponding points between images (based on key point matching

and outlier removal with epipolar constraints), were selected; (3) an essential matrix for each

image pair based on tie points was calculated, and then the extrinsic camera parameters

(position, orientation) and the depth of the tie points (sparse point cloud) were estimated;

finally, (4) the bundle adjustment was conducted to optimize the extrinsic camera parameters

and the depth of the tie points by minimizing the reprojection error. After SfM, the MVS

generates dense point-cloud data based on the estimated camera parameters and tie points.

Several algorithms have been proposed, e.g., SUrface REconstruction from imagery (SURE)

[67] and patch-based multiview stereo (PMVS) [68]. We used Metashape v1.8.4 (Agisoft,

St. Petersburg, Russia), to conduct the SfM/MVS pipeline. Metashape adopts a SURE-like

algorithm to implement the MVS. SURE can offer a depth map, which is the distance from

each pixel in a 2D image to the object or scene captured in each image using a semiglobal

matching (SGM) -based algorithm [69], and convert the depth maps into 3D coordinates

using a fusion method based on geometric constraints [70].

We evaluated four scenarios based on SfM/MVS: (i) no mask, (ii) masks before

SfM, (iii) masks after SfM, and (iv) two masks (Fig 5). The basic procedure for the

SfM/MVS implementation for each scenario was as follows: (1) multi-view images of a

soybean individual were imported, (2) the coded markers were detected, (3) scale bars were

created from the pairs of detected coded markers, (4) sparse point clouds were generated

based on SfM, with the maximum number of key points set to 200,000 and the maximum

number of tie points set to 80,000 in this study, (5) a depth map was created, and (6) dense

point clouds were generated based on MVS. In scenarios (ii) and (iii), the soybean mask

images were imported before and after step (4), respectively. In scenario (iv), the

soybean/stage mask images were imported before step (4) and replaced with the soybean

mask images before step (6). These four scenarios were implemented using the Metashape

Python API.

Fig 5. The 3D reconstruction based on SfM/MVS pipeline with the four different mask

usage scenarios. Each column corresponds to (i) no masks, (ii) masks before SfM, (iii)

masks after SfM, and (iv) two masks. The reconstructed point clouds of a soybean individual

(Keumdu #1) at 12 WAS (the second row), 8 WAS (the third row), and 4 WAS (the fourth

row). The black bars represent 20 cm. (A-L) Leaflets corresponding to red circles in the first

left column. (M-P) Key points and tie points detected in the four different scenarios.

In addition, the abovementioned four scenarios were evaluated for each soybean

individual of dataset$_m$. In the implemented script, the processing time for each step was

measured using the time function in the time module of the standard Python library. The

Mann-Whitney U test with Bonferroni correction was subsequently performed to investigate

the differences in processing times among the four scenarios.

Leaf surface reconstruction

To estimate phenotypic values from the reconstructed point-cloud data, we employed two

prevalent methods for leaf surface reconstruction: (1) Poisson surface reconstruction and (2)

B-spline surface fitting.

Poisson surface reconstruction [57] is widely used to estimate surfaces from point-

cloud data using their normal vectors (i.e., oriented points). Assume that we will reconstruct

the boundary ∂A of a target structure A from the given oriented points. If a function (called

indicator function χ), which returns one if the point is located inside of the A, otherwise 0,

is given, the boundary is approximated by its isosurface. The best-fitted boundary will be

estimated by minimizing the differences between the gradient of the indicator function $\nabla \chi$

and the vector field defined by the oriented points $\vec{P}$ (i.e., $\min_{\chi} ||\nabla \chi - \vec{P}||$). To solve the

problem, this equation is transformed into the Poisson equation as follows: $\Delta \chi = \nabla \cdot \vec{P}$.

Finally, the isosurface is extracted from the estimated χ. Additionally, the reconstructed

surface is filtered based on the point density (i.e., only parts of the surface supported by a

sufficient number of points remain) because the method potentially reconstructs a surface in

which points are distributed with low density, including the outer region of the leaf surface

(i.e., extrapolation). In this study, we used the create_from_point_cloud_poisson function

implemented in Open3D [71] to estimate the leaf surface from the point-cloud data using

Poisson surface reconstruction. Vertices of the reconstructed surface were filtered if less

than 6.5 points supported them.

B-spline surface fitting is widely used for surface reconstruction from point-cloud

data. In this study, we adopted the B-spline surface fitting and B-spline curve trimming

approaches [72] to reconstruct the leaf surface. A B-spline basis function of degree d is

defined by the de Boor-Cox recursion formula [73]:

$$b_{i,\,0}(l) = \begin{cases} 1 & \text{if } \tau_i \leq l < \tau_{i+1} \\ 0 & \text{otherwise} \end{cases},$$

$$b_{i,d}(l) = \frac{l - \tau_i}{\tau_{i+d} - \tau_i} b_{i,\,d-1}(l) + \frac{\tau_{i+d+1} + l}{\tau_{i+d+1} - \tau_{i+1}} b_{i+1,\,d-1}(l),$$

296   where $(\tau_1, \tau_2, \cdots, \tau_{n+d+1})$ is a knot vector that divides the parameter space into subintervals,

297   and n is the number of basis functions used for the approximation. A continuous surface is

298   approximated by a linear combination of the tensor products of the B-spline basis functions,

299   as follows:

$$\mathbf{p}_s(u,v) = \sum_{i}^{n_u} \sum_{j}^{n_v} \mathbf{w}_{ij} b_{i,d}^u(u) b_{j,d}^v(v) \, ,$$

where $\mathbf{w}_{ij}$ is a coefficient vector called a control point, and $b_{i,d}^u(u)$ and $b_{j,d}^v(v)$ are B-spline

basis functions of degree d along the independent parameters (u, v) of the parameter space.

In this study, we parameterized the leaf surface by applying a principal component analysis

(PCA), and the 2D parameter space was defined using the 1st and the 2nd PC axes. The $\mathbf{w}_{ij}$

is estimated by minimizing the sum of the squared distances between the B-spline surface

and point cloud data. Based on the reconstructed surface, the parameter space is trimmed

using a closed B-spline curve to exclude the outer region of the leaf. The closed B-spline

curve was fitted to the point cloud data projected into the parameter space using the

asymmetric distance with a penalty for concavity and error-adaptive knot insertion (EAKI)

[72]. In this study, we used the surface/on_nurbs submodule [74] in the Point Cloud Library

(PCL; [75]) to reconstruct leaf surfaces using B-spline surface fitting and B-spline curve

trimming. In the B-spline surface fitting, we estimated the 3-degree B-spline surface by

incrementally adding knots three times using the following parameter values: the surface

smoothness, boundary smoothness, and boundary weight were 0.8, 0.5, and 0.2, respectively.

In the B-spline curve trimming, the 3-degree B-spline curve was estimated with a maximum

of 100 iterations using the following parameter values for the penalty for concavity (0.2) and

for EAKI: iterations without knot insertion and the maximum number of control points were

4 and 100, respectively.

Based on both methods, leaf area was estimated using dataset$_v$ through scenario iv.

Each leaflet was cropped from the point-cloud data using Cloud Compare v2.12.4

(CloudCompare), software for visualizing and manipulating point-cloud data. We then

applied color thresholding (i.e., excluding points with $0.3 < hue < 0.7$, saturation < 0.2, and

value > 0.8 in the HSV color space) and statistical outlier removal (i.e., excluding outlier

points for which the mean distance calculated from their 30 neighbor points is greater than

three standard deviations by using the statistical_outlier_removal function in Open3D).

Results

Optimizing image magnification for mask image generation

We identified the optimal magnification of input images for multiview images of soybean

individuals by evaluating the IoU and processing time of segmentation.

For both three- and four-class segmentations, the IoU increased with decreasing

magnification, peaking at approximately 1/4×, followed by a gradual decrease (Fig 3A, F).

The IoU by class showed the same pattern (Fig 3B-D, G-J); however, several classes peaked

at 1/5.4×: stake (three-class segmentation) 0.8670, background (four-class segmentation)

0.9939, and stage (four-class segmentation) 0.9903 (Fig 3C, H-J). There is a tradeoff: as the

magnification of the input images decreases, a single input tile to the U-Net covers larger

plant structures; conversely, as the magnification increases, the resolution decreases. In our

dataset, a magnification of 1/4× or 1/5.4× reached the optimal IoU.

The processing times for both the three- and four-class segmentations decreased

with decreasing magnification. This is because the total number of input tiles decreased (Fig

2C, 3E, and 3K). Although a marginal increase in processing time was shown to be linked

to reductions in the magnification (i.e., the expansion of the field of view), almost all the

processing times per image fall between 0.015–0.02 s (S2 Fig).

The U-Net models for the three- and four-class segmentations were trained using

the images of all four cultivars in the dataset$_t$ (see S3 Fig). These models generated high-

resolution mask images for SfM/MVS, in which the background was excluded (Fig 2D):

soybean mask images (soybean pixels were the foreground, whereas others were the

background) and soybean/stage mask images (soybean and stage pixels were the foreground,

whereas others were the background).

Moreover, we developed a web application for mask generation based on the trained

U-Net models. This application helps generate mask images with arbitrary scales and

overlapping thresholds via a simple user interface (Fig 4). The processing time was 126 s on

the GPU device for predicting 256 mask images of 5184×3456 pixels divided into two

columns and two rows of 2900×2900 pixel tiles (approximately $1/5.4\times$) with at least 10%

overlap using a four-class segmentation model.

Quality of reconstructed point cloud data through SfM/MVS with

four mask image usages

We reconstructed the dense point cloud data of six individuals of three cultivars at three

different growth stages of the dataset_m based on SfM/MVS, following four possible

scenarios of mask image usage (Fig 5). Masking during SfM/MVS effectively suppressed

the noise derived from the background textures (Fig.5B-D, 5F-H, 5K, and 5L) compared to

scenario (i) without mask images (Fig. 5A, 5E, 5I), as noted in previous studies (e.g., [51–

53]).

The quality of the reconstructed point-cloud data varied depending on the mask type

and timing of the setting. Although in scenario (ii), setting soybean mask images before SfM

was able to suppress the noise derived from the background textures, the reconstructed points

along the leaf edges seemed inaccurate (Fig 5B, 5F) and often failed to reconstruct the leaf

surfaces for the small soybean plants (Fig 5J). These inaccurate reconstructions without

background texture-derived noise were a consequence of the low accuracy of camera

extrinsic parameter estimation in SfM, not using markers on the stage and corresponding

points on the pot, because the ROIs based on the mask images were limited to soybeans. In

scenario (ii), key points and tie points were located only in the region corresponding to

soybean (Fig 5N), and their detected numbers were lower than those in the other scenarios

(Table 3). There were few qualitative differences between scenarios (iii) and (iv), except for

the noise derived from the abaxial side of the leaves and the background textures (Fig 5C,

5D, 5G, 5H, 5K, and 5L). In both scenarios, leaf surfaces were well-reconstructed because

SfM precisely estimated extrinsic camera parameters using the markers on the stage and the

corresponding points on the pot.

Table 3. The average numbers of key points and tie points. The number of key points

and tie points detected in SfM/MVS pipeline were counted for $dataset_m$ across four scenarios.

Evaluation of the throughput of SfM/MVS on four mask image

usages

The processing times, including those for mask image generation, SfM, and MVS, were

measured for the four scenarios (Fig 6). Scenarios (ii), (iv), and (iii) achieved the first, second,

and third shortest processing times, respectively (Fig 6A). The decrease in the area of the

ROIs caused by setting mask images contributed to the processing time. The longest mean

processing time was 34.0 min for scenario (i), and the shortest one was 15.8 min for scenario

(ii).

Fig 6. The processing times on the four different mask usage scenarios. The processing

times of (A) total of mask image generation (MIG), SfM, and MVS, (B) MIG, (C) SfM, and

(D) MVS. Multiple comparison was conducted on the processing times among four scenarios

(Mann-Whitney *U* test, **: $0.001 < p <= 0.01$, ***: $0.0001 < p <= 0.001$, ****: $p <= 0.0001$).

The processing time of mask image generation for scenario (iv) was 3.61 min,

approximately double that for 1.74 min of scenario (ii) and 1.74 min of scenario (iii) (Fig

6B), because two types of mask images (soybean and soybean/stage) were generated.

The processing times of SfM and MVS increased in the order of the ROIs, except

for scenarios (ii) and (iii) of MVS (Fig 6C and 6D). There was no significant difference

between the processing times of SfM in scenarios (i) and (iii), and the mean processing times

were ~5.4 min. The shortest mean processing time was 3.3 min for scenario (ii). The majority

of processing times came from the MVS; the longest mean processing time was 27.6 min in

scenario (i) and the shortest one was 9.6 min in scenario (ii). Although the ROIs for MVS

were the same between scenarios (ii) and (iii), scenario (ii) showed a shorter processing time

for MVS because fewer key points were detected in SfM in scenario (ii) (Table 3).

We adopted scenario (iv) for the following analysis because (1) it generated the

highest-quality point cloud data, and (2) its entire processing time was the second shortest.

Leaf surface reconstruction methods for evaluating phenotypic values

We reconstructed the leaf surfaces of 190 leaflets from $dataset_v$, excluding dead leaves, using

both Poisson surface reconstruction and B-spline surface fitting, and then estimated their

leaf areas (S1 Table). The estimated leaf area had the following correlation coefficients with

the results of the destructive measurements: Poisson surface reconstruction ($\rho = 0.9123$; Fig

7A) and B-spline surface fitting ($\rho = 0.9943$; Fig 7B). The approach based on B-spline

surface fitting enabled accurate estimation of leaf area ($R^2 = 0.9871$; Fig 7B). Using the

Poisson surface reconstruction, the leaf area was overestimated, particularly for larger leaves.

Leaf surface reconstruction based on B-spline surface fitting achieved higher

accuracy than Poisson surface reconstruction for the leaf area estimation in almost all cases.

Both methods successfully reconstructed leaf surfaces and estimated areas on high-quality

point cloud data (Fig 7C); the Poisson surface reconstruction method enabled a more detailed

reconstruction of leaf surfaces, yet showed nearly equivalent performance in leaf area

estimation. Although the Poisson surface reconstruction occasionally produced the leaf

surface as dual layers for the point cloud data to reconstruct the abaxial side over the adaxial

side, the B-spline surface fitting consistently reconstructed a singular surface (Fig 7D).

However, when a single leaflet was reconstructed as multi-leaf-like point cloud data, neither

method could accurately reconstruct the surface, resulting in an overestimation of the area

(Fig 7E); we found four leaflets corresponding to this case (Table 4). Three of the four

leaflets were leaflets of the same individual plant, which implied blurring associated with

the motion of the target plants while capturing the multi-view images, which caused low

accuracy of the camera parameter estimation. This is different from the decrease in accuracy

in camera parameter estimation owing to the mask images observed for the small individuals

in scenario (ii) (Fig 5J); in fact, the quality of the reconstructed point cloud data is similar

across scenarios (ii) and (iv) (Fig 7F).

Fig 7. Leaf surface reconstruction and leaf area estimation. Scatter diagrams of leaf area

estimated by (A) Poisson surface reconstruction and (B) B-spline surface fitting, against the

destructive measurements. (C) Both methods estimated leaf area consistent with the

destructive measurements (Hime daizu #7 S01_t). (D) Poisson surface reconstruction

overestimated leaf area while B-spline surface fitting estimated accurately (Hime daizu #7

S07_t). (E) Both methods failed to reconstruct leaf surface well, then overestimated leaf area

(Shiro mitsu mame #3 S06_r). (F) The reconstructed 3D point cloud data (Shiro mitsu mame

#3 S07_t) based on scenario (i) and (iv).

Table 4. The four leaflets which were overestimated leaf area using B-spline surface

fitting. The leaf areas of 190 leaflets were overestimated when B-spline surface fitting

against the destructive measurements was used.

452   Discussion

Even though using mask images improves the quality of point cloud data reconstructed via

the SfM/MVS pipeline and decreases the processing time, it remains crucial to understand

which objects should be masked and in which operations these mask images should be

applied. As noted in previous studies [51–53], the reconstructed point-cloud data based on

SfM/MVS were improved for large individuals by masking the target objects (Fig 5A-H).

However, the point cloud was not comprehensively reconstructed by setting the mask images

before SfM for small individuals (Fig 5J), although they were reconstructed properly without

mask images (Fig 5I). We found that appropriate point clouds were reconstructed either by

setting soybean mask images after SfM (scenario iii) or by setting soybean/stage mask

images before SfM and then soybean mask images after SfM (scenario iv) (Fig 5C, D, G, H,

K, and L). This implies the importance of setting mask images, which ensures a sufficient

number and accuracy of detected key points and tie points (Fig 5M-P, Table 3). There are

several considerations for generating the mask images used in this study, one of which is the

magnification rate of the input images. As a result of different scales of input images to the

U-Net, a magnification of 5.4× achieved the first or second optimal IoU for all classes (Fig

3). However, there was a trade-off between low magnification (i.e., long-range context is

available with low resolution) and high magnification (i.e., only local context is available

with high resolution) (Fig 2C). Setting a larger input image size could resolve this issue;

however, in practice, balancing them for the target objects is necessary because of various

constraints (e.g., available GPU memory).

In plant phenotyping, it is advantageous to introduce known morphological

constraints to the target shape. In this study, we assumed that a leaf constitutes a single

bounded surface in a three-dimensional space during leaf surface reconstruction, which

enabled more robust measurements of leaf area (Fig 7). Thus, the surface was reconstructed

using Poisson surface reconstruction; this approach resulted in an overestimation of leaf area

(Fig 7A). It is worth noting that point cloud data derived from actual plants often contain

noise that is difficult to eliminate, even with denoising filters. In cases where B-spline

surface fitting is ineffective for reconstruction, methods exclusively derived for

characterizing leaf morphology could prove useful (e.g., [54–56,59,60]). The results of leaf

area estimation using B-spline surface fitting showed a high correlation with the results of

destructive measurements (Fig 7B), indicating its utility in the phenotyping process. In some

cases, the B-spline surface fitting did not reconstruct precisely (Fig 7E). However, this issue

often stems from the difficulty underlying camera parameter estimation in SfM (Fig 7F),

demonstrating that improvements in measurement devices can yield improvements in

accuracy. Concerning the qualitatively noted shortcomings in previous studies of the B-

spline surface fitting approach in reconstructing leaf surfaces [59,60], it is considered that

these issues do not significantly impact leaf area estimation as long as the point cloud is

reconstructed with high accuracy. Consequently, we propose an optimal workflow for

estimating leaf area of soybean plants, including mask image generation using U-Net with

1/5.4× magnification, 3D point cloud reconstruction based on scenario (iv) of SfM/MVS,

and leaf surface reconstruction by B-spline surface fitting to estimate leaf area. The optimal

values and methods depend on the target objects and constraints on the measurement

processes, and the optimal configurations are explored through comparative experiments, as

demonstrated in this study. The reconstructed leaf surfaces also allow for quantification of

leaf orientation in three-dimensional space; the resultant outputs can be used for functional

assessments, including light interception [76,77]. Utilizing this method in functional

structural plant models (FSPM) and other areas is a subject for future research on sustainable

food systems using data-driven approaches [11,12,78].

Moreover, we offer a tool and data that can be applied toward enhancing the

efficiency at which plant phenotyping can be conducted. Specifically, our web application

facilitates the generation of mask images through a trained U-Net model, and is designed to

allow the replacement of models, enabling its use in various subjects (Fig 4). We also

published the multiview soybean images used in model training as open data, including their

mask images and images from destructive measurements. These data will be available to

developers of future methodologies as benchmark datasets. We believe that disseminating

such tools and datasets will contribute to the field of phenomics, where data acquisition is

currently labor-intensive and slow.

Conclusion

Here, we investigated 3D reconstruction-based phenotyping processes including

mask image generation using U-Net models, 3D reconstruction based on the SfM/MVS

pipeline, and leaf surface reconstruction for leaf area estimation. Through comparative

experiments using soybean data, we proposed an optimal workflow for estimating leaf area

and generating mask images. The workflow entailed using U-Net models with 1/5.4×

magnification, 3D point cloud reconstruction based on the SfM/MVS pipeline setting

soybean/stage mask images before SfM and soybean mask images after SfM (scenario iv),

and leaf surface reconstruction by B-spline surface fitting to estimate leaf area. However, it

is important to note that in some cases, greater accuracy was achieved with 1/4 magnification

in the U-Net model, and that high-quality point clouds can also be obtained by setting only

the soybean mask images after SfM (scenario iii). The processes outlined in this study can

be adapted as required. Moreover, we offer a web application for mask generation, a

command-line tool for leaf surface reconstruction, and validation datasets to facilitate future

plant phenotyping research and methodological development.

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Supplementary Information

S1 Figure. Photogrammetry system. This figure was created based on [79] (Licensed under CC BY 4.0).

S2 Figure. Processing times of mask image generation per single 512×512 tile. Box plots of processing times among six magnifications of (a) three-class and (b) four-class segmentation.

S3 Figure. Time series data of class-weighted Dice loss and IoU of the U-Net models during training on the entire dataset$_t$. Line plots of class-weighted Dice losses of (a) three-class and (b) four-class segmentation models. Line plots of IoU scores of (c) three-class and (d) four-class segmentation.

S1 Table. Leaf area. The leaf area data of 190 leaflets (Hime daizu 87 leaves, Shiro mitsu mame 103 leaves) in dataset$_v$ were obtained by destructive measurement, Poisson surface reconstruction, and B-spline surface fitting. NA indicates missing values.

multi-view images

point cloud data

phenotypic values

- ITEM A
- ITEM B
- ITEM C

A mask image generation

mask images

B SfM/MVS

C morphological modeling

e.g. leaf surface reconstruction

A

original   ground truth   prediction

B

original   ground truth   prediction

C

1/2×   1/3×   1/4×   1/5.4×   1/6×   1/6.75×

D

original   mask: soybean   mask: soybean/stage

Soybean Mask Prediction

Please upload your image files

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📄 SSP_032_00255_05_31074008483.JPG 5.9MB		✕
📄 SSP_032_00254_04_71074003363.JPG 6.1MB		✕

Showing page 1 of 86   ‹ ›

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The input number is `512`

Input an overlap ratio from 0.05 to 0.95

0.05   − +

Select an image of all you uploaded

0   ⌄

Each cropped image will be resized into 512x512

Image Name : SSP_001_00001_08_71074003452.JPG

Image Size : (width, height) = (5184 px, 3456 px)

The number of cropped images with blue color : n_column = 11, n_row = 8

The size of the cropped image with red color : (width, height) = (512 px, 512 px)

The overlapping value : horizontal direction = 44 px, vertical direction = 91 px

Please select which mask type you want to predict

🔴 soybean
⚪ soybean & stage

You selected "soybean".

Start prediction

A Poisson surface reconstruction

- HIME DAIZU
- SHIRO MITSU MAME

Case 1
Case 2
Case 3

destructive measurement (mm²)

estimated area (mm²)

y = 0.63x+579.67
R² = 0.7337

B B-spline surface fitting

- HIME DAIZU
- SHIRO MITSU MAME

Case 2
Case 1
Case 3

destructive measurement (mm²)

estimated area (mm²)

y = 1.01x+17.98
R² = 0.9871

C Case 1   Poisson surface reconstruction   B-spline surface fitting

D Case 2

E Case 3

F scenario. (i)   scenario. (iv)

SfM
MVS

soybean/stage
SfM
soybean
MVS

Cultivar	Individual num.	Sowing date	Observation date		
			4 WAS	8 WAS	12 WAS
Seita	1	5/24/2022	6/21/2022	7/19/2022	8/16/2022
Seita	2	5/24/2022	6/21/2022	7/19/2022	8/16/2022
N2295	1	6/1/2022	6/29/2022	7/27/2022	8/24/2022
N2295	2	6/1/2022	6/29/2022	7/27/2022	8/24/2022
Keumdu	1	6/29/2022	7/27/2022	8/24/2022	9/21/2022
Keumdu	2	6/29/2022	7/27/2022	8/24/2022	9/21/2022

Model	Fold num.	Train/Validation	Test	Weights			
				background	soybean	stake	stage
three-class segmentation	1	Zairai 51-2, Aoakimame, Saga zairai	Enrei	0.02	0.484	0.496	-
	2	Enrei, Aoakimame, Saga zairai	Zairai 51-2	0.019	0.486	0.496	-
	3	Enrei, Zairai 51-2, Saga zairai	Aoakimame	0.016	0.488	0.496	-
	4	Enrei, Zairai 51-2, Aoakimame	Saga zairai	0.019	0.486	0.495	-
four-class segmentation	1	Zairai 51-2, Aoakimame, Saga zairai	Enrei	0.098	0.323	0.33	0.249
	2	Enrei, Aoakimame, Saga zairai	Zairai 51-2	0.095	0.324	0.33	0.25
	3	Enrei, Zairai 51-2, Saga zairai	Aoakimame	0.094	0.326	0.33	0.25
	4	Enrei, Zairai 51-2, Aoakimame	Saga zairai	0.094	0.324	0.33	0.252

Scinario	Average number of points	
	Key points	Tie points
(i) No Masks	169,246	84,034
(ii) Masks before SfM	59,978	29,683
(iii) Masks after SfM	169,246	84,040
(iv) Two Masks	107,532	48,759

Cultivar Name	Individual Num.	Leaf ID	Leaf area (mm²)	
			Destructive measurements	B-spline surface fitting
Hime daizu	7	S05_t	6287.910363	6741.43262
Shiro mitsu mame	3	S07_t	2568.184828	3214.35689
Shiro mitsu mame	3	S06_t	3557.466003	4076.095297
Shiro mitsu mame	3	S06_r	2898.617859	4396.995716