Backward Tracking and Instance Segmentation for Automated Leaf Emergence Ordering in Arabidopsis

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Abstract— **This study proposes and evaluates a method for automatically recording the timing and order of leaf emergence using only timelapse images that can be obtained in a typical plant experimental environment. A series of top-view images of *Arabidopsis thaliana* were processed using instance segmentation with the deep learning framework Detectron2, and the extracted leaf regions were then tracked based on positional information. Approximately 2,400 segmented leaf samples obtained from growth sequences were used for model training, and leaf association between consecutive frames was performed using the Hungarian algorithm. Furthermore, by incorporating backward tracking and a loss-recovery procedure that leverage structural constraints of plant growth, the stability of leaf identification was improved.**

Experimental results showed that the proposed method successfully reproduced leaf orientation and spatial arrangement during later growth stages, demonstrating its potential for automatically estimating the order of leaf emergence. In contrast, early-stage leaves tended to exhibit tracking fluctuations due to unstable segmentation results, indicating room for improvement. Nevertheless, because the primary aim of this study is to provide a low-cost and easily adoptable framework for acquiring important plant phenotypic information—such as leaf emergence order and growth tendencies—without specialized equipment, the proposed method is considered highly useful. Future work includes improving imaging conditions and segmentation models to achieve more accurate and fully automated plant growth recording.

Keywords— Plant phenotyping, leaf emergence order, instance segmentation, leaf tracking, timelapse imaging, Arabidopsis thaliana, low-cost automated analysis

I. INTRODUCTION

In plant science research, a wide variety of plant species are used as experimental subjects. Regardless of the target species, many experiments share a common workflow in which genetic modifications or environmental treatments are applied and the resulting phenotypic changes are observed and recorded. Traditionally, these observational tasks have relied heavily on manual inspection, and the creation of detailed growth records has been reported to require substantial time and labor [1], [2]. Minervini et al. [1] pointed out that many steps in plant phenotyping remain manual, limiting experimental throughput, while Fahlgren et al. [2] emphasized the growing demand for automated and high-throughput phenotyping approaches.

To address this issue, a variety of automated plant phenotyping systems have been developed. For example, Fujita et al. [3] proposed RIPPS, a fully automated phenotyping platform that constructs a dedicated imaging environment and controls environmental factors such as temperature, humidity, light intensity, and watering. The system enables precise and reproducible measurements of plant traits but requires specialized equipment and a large-scale experimental setup. Similarly, Li et al. [4] proposed a plant growth tracking method based on skeleton extraction from three-dimensional point cloud data acquired using structured light and turntable-based imaging. Although such 3D approaches provide detailed structural information, they often involve high equipment costs and complex operation, limiting their accessibility for routine use in many laboratories.

Recognizing these limitations, several studies have explored methods for analyzing plant growth under simpler imaging conditions. Nagahara et al. [5] demonstrated that plant structural changes can be tracked using time-series images combined with a high-precision 3D model obtained at a later growth stage, reducing the need for specialized facilities. In parallel, advances in image processing and machine learning have enabled accurate estimation of plant morphological traits—such as leaf size, number, color, and skeletal structure—from two-dimensional images [6]. In particular, deep learning–based instance segmentation has been successfully applied to leaf segmentation tasks in Arabidopsis and other plant species [7].

In recent years, increasing attention has been paid to low-cost and easily deployable plant phenotyping approaches based on consumer-grade cameras and time-lapse imaging. These approaches offer practical solutions for improving experimental throughput while maintaining accessibility in standard laboratory environments. However, an essential aspect of plant growth analysis—namely, determining when each leaf emerges and in what sequence—remains difficult to automate. Granier et al. [8] and Walter et al. [9] reported that leaf emergence timing is a critical physiological indicator related to plant development and environmental response, yet automatically estimating emergence order from image data alone remains challenging. Even methods based on 3D reconstruction or multi-view imaging often struggle to robustly identify the temporal sequence of leaf emergence, particularly during early growth stages.

Despite recent progress in deep learning–based plant image analysis, most existing approaches either require complex imaging systems or depend on extensive manual annotation to determine leaf emergence order. Therefore, there is a growing need for a lightweight and cost-effective framework that can automatically record leaf emergence timing and sequence using simple imaging setups.

In this study, we propose a practical framework for estimating leaf emergence order by integrating instance segmentation and tracking applied to top-view time-lapse images acquired in a typical laboratory environment. By combining deep learning–based leaf segmentation with backward tracking and loss recovery strategies, the proposed method aims to robustly identify individual leaves over time without relying on specialized equipment. This approach enables automatic extraction of important growth-related information, such as leaf emergence order and orientation, and provides a low-cost and easily adoptable solution for plant phenotyping studies.

II. OBJECTIVE

he objective of this study is to establish a cost-effective method for automatically recording when each leaf emerges and in what order it develops, using timelapse images captured in a typical laboratory environment. Many existing approaches in plant phenotyping require large-scale specialized equipment or rely on complex 3D point cloud analysis, making them difficult to adopt in standard plant biology laboratories. Moreover, only limited methods exist for automatically determining the order of leaf emergence, and current analyses still depend heavily on manual annotation by researchers. Therefore, developing a technique capable of estimating leaf emergence order under simple imaging conditions represents an important challenge that may reduce observational workload and improve research throughput in plant science.

In this study, instance segmentation is applied to top-view timelapse images to extract leaf regions at each time point. Based on the segmentation results, individual leaves are tracked across frames, and their emergence timing and emergence sequence are estimated from the resulting trajectories. This approach allows us to examine the potential for automating growth record acquisition, which has traditionally required detailed manual observation. In addition, information obtained through tracking—such as changes in leaf morphology and orientation—is analyzed to explore its potential as a novel indicator of plant growth characteristics.

III. PROPOSED METHOD

In this study, we propose a method for tracking individual leaves and estimating the order of leaf emergence by utilizing leaf region information obtained through instance segmentation, as illustrated in Fig. 1.

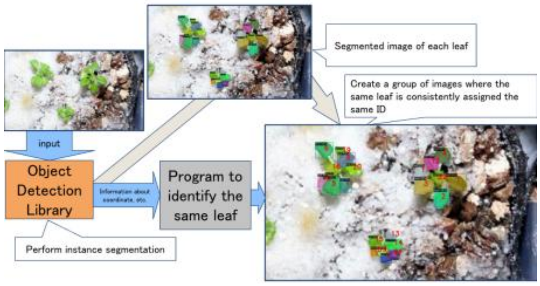

Fig. 1. Overview of the proposed framework for leaf segmentation, tracking, and emergence order estimation from top-view timelapse images. The pipeline consists of instance segmentation, tiled inference–based enhancement, backward tracking, and loss correction.

First, timelapse videos are recorded from the moment the first leaf emerges. Images are captured every 30 minutes from a top - view perspective. The analysis focuses on the early growth stage commonly examined in plant experiments, spanning from germination to the development of approximately ten true leaves. Frames captured during nighttime without illumination, which appear nearly black, are excluded from analysis.

Next, instance segmentation is applied to all frames extracted from the video to obtain leaf regions at each time point. It is known that very small early-stage leaves are often missed under standard inference settings due to their size [10]. To mitigate this issue, the present study incorporates a tiled inference strategy in which each image is divided into multiple subregions, and segmentation is performed on each tile. Although tiled inference is typically used for high-resolution images to improve small object detection, here it serves as an auxiliary technique to increase the detection rate of small emerging leaves.

Based on the extracted leaf masks and positional information, leaf tracking is performed by associating leaf instances across successive frames. This enables continuous identification of each leaf while accounting for natural changes in shape and relative position during growth. From the resulting trajectories, both the emergence timing and the emergence sequence of individual leaves are estimated. In addition, the tracking results allow further analysis of accompanying characteristics such as leaf orientation and growth rate, offering the potential to derive new phenotypic indicators.

By integrating segmentation and tracking in this manner, the proposed method aims to automate the recording of leaf emergence order—traditionally performed manually—while maintaining low cost and requiring minimal modifications to standard experimental environments.

In this study, we propose an approach that focuses on plant leaves, as illustrated in Fig. 1. The method utilizes instance segmentation to detect individual leaves and track them across time-lapse images, with the goal of extracting growth-related data.

First, a time-lapse video is prepared to capture the entire growth process of a plant starting from the emergence of the first leaf. The video is recorded at 30-minute intervals, using a top-down camera angle. In this study, we target a typical growth phase in plant experiments—specifically, the period from germination to the point where the plant has developed approximately ten leaves.

Next, each frame extracted from the time-lapse video is processed using instance segmentation to detect and segment each leaf individually. Since nighttime frames appear nearly black due to the lack of lighting, such frames are excluded from the dataset.

By combining the coordinate information of each detected leaf—obtained through a deep learning segmentation library—with the segmented leaf images, we aim to consistently identify and match the same leaves across multiple frames, even as the plant continues to grow. Through this matching process, we effectively track individual leaves. This enables us to trace the origin of each leaf, determine when it first appeared, and ultimately visualize the chronological order of leaf emergence by assigning intuitive labels (e.g., numbered annotations) to each leaf in a given image. This visualization helps distinguish older leaves from newly emerged ones.

Moreover, this tracking process can compensate for temporary segmentation errors, such as missed detections in individual frames, by leveraging temporal continuity in the data. Finally, by integrating the segmentation and tracking results, we aim to automatically determine the emergence order of leaves and facilitate the automatic extraction of various growth-related features.

IV. EXPERIMENTS

A. Preparation of Time-Lapse Video

In this study, *Arabidopsis thaliana*, a widely used model organism in plant biology, was selected as the experimental material. Arabidopsis is well known for its small size, short life cycle, and suitability for genetic and developmental analyses. To observe growth dynamics in detail, timelapse videos were recorded starting from the emergence of the first leaf. Images were captured from a top-view perspective at 30-minute intervals. Multiple videos were acquired, and a representative example used in this study is shown in Fig. 2.

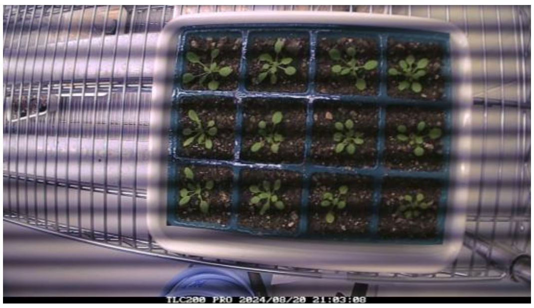

Fig. 2. Representative frame from the top-view timelapse images of *Arabidopsis thaliana* used in this study. The video sequence was captured at 30-minute intervals starting from the emergence of the first leaf. (1280 × 780)

B. Selection of a Platform for Leaf Segmentation

Accurate extraction of individual leaf regions from each frame is essential for estimating leaf emergence order. Early-stage leaves are particularly small, and overlapping leaves often lead to false detections or missed detections. Therefore, stability, reproducibility, and the ability to learn from a relatively small dataset were key considerations in selecting the segmentation model.

For these reasons, Detectron2[11] was adopted in this study. Detectron2, developed by Meta AI, is a PyTorch-based segmentation library that provides robust baseline models such as Mask R-CNN. Mask R-CNN has been widely applied to leaf segmentation in plant images[12] and is known to achieve stable inference performance even with modest amounts of training data. Although more advanced segmentation methods exist, the aim of this study is not to perform large-scale benchmarking but rather to validate a new framework for estimating leaf emergence order. Thus, employing a well-established and reliable platform such as Detectron2 is appropriate.

To further reduce missed detections of very small early-stage leaves, a tiled inference strategy was incorporated. In this approach, each image is divided into multiple subregions, and inference is performed on each tile independently. While tiled inference is commonly used in high-resolution image analysis to improve small-object detection, it is introduced here as a supportive measure to increase detection stability for emerging leaves.

C. Construction of the Dataset

Prior to training with Detectron2, frames were randomly selected from several timelapse videos to construct a training dataset. In the example presented in this paper, approximately 30 frames were extracted and manually annotated using COCO-annotator [13]. This resulted in a dataset containing approximately 2,400 annotated leaf segments.

D. Training of the Segmentation Model

Mask R-CNN from the Detectron2 Model Zoo was fine-tuned for leaf segmentation. The maximum number of training epochs was set to 1000, and the model was saved after confirming convergence of the loss functions. During inference, only leaf segments with a confidence score of 0.6 or higher were retained.

E. Tracking Based on Coordinate Informatio

In this study, leaf tracking is performed by associating segmented leaf instances across frames based on spatial proximity. To improve robustness against ID switching caused by temporary detection failures, a backward tracking strategy combined with a loss recovery procedure is adopted. The overall tracking procedure is summarized in Algorithm 1.

Algorithm 1. Backward Leaf Tracking with Loss Recovery

Input: Leaf instances at each frame (centroid position, mask, orientation angle)

Output: Estimated leaf trajectories and emergence order

Step 1 Apply instance segmentation to all frames to obtain leaf candidates.

Step 2 Initialize tracking from the last frame, where the number of leaves is maximal.

Step 3 Compute a cost matrix between leaf instances at frame t and t−1, where the cost is primarily defined by the Euclidean distance between centroid positions.

Step 4 Apply the Hungarian algorithm[14] to obtain optimal leaf correspondences.

Step 5 Assign leaf IDs by backward tracking from frame t to t−1.

Step 6 If a leaf is missing due to temporary detection failure, apply loss recovery by searching neighboring frames within a predefined temporal window.

Step 7 Record the first appearance of each leaf ID as its emergence timing and determine the emergence order.

In real timelapse data, temporary detection failures may still occur due to leaf occlusion, illumination changes, or segmentation instability, as illustrated in Fig. 3. Such failures can lead to fragmented tracking sequences and degrade the accuracy of leaf emergence order estimation.

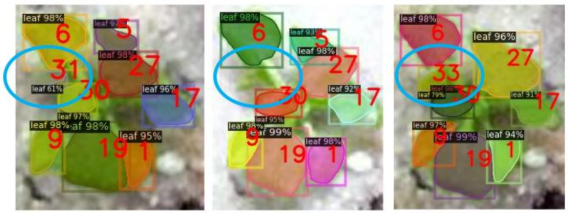

Fig. 3. Example frame illustrating temporary leaf occlusion or segmentation failure. Such situations motivate the adoption of backward tracking and loss recovery described in Algorithm 1.

By incorporating backward tracking and the proposed loss recovery procedure, the method maintains temporal consistency of leaf identities and achieves stable estimation of both leaf emergence timing and emergence order.

V. RESULTS

Using the tracking algorithm developed in this study, the temporal changes in leaf angle and growth metrics were extracted for each leaf. Figure 4 illustrates the leaf orientation obtained from the tracking results, visualized in a polar coordinate representation.

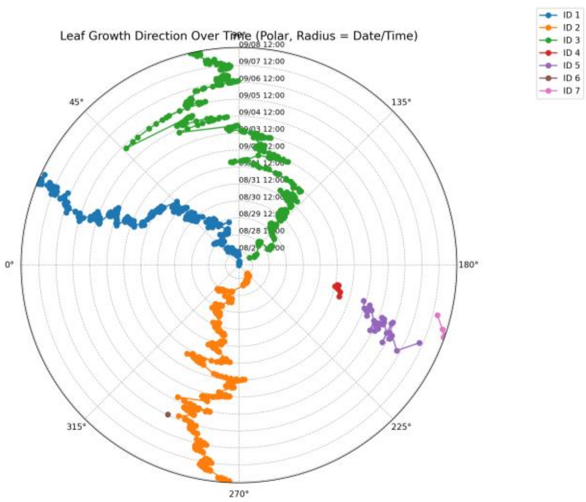

Fig. 4. Polar visualization of leaf orientation trajectories obtained by the proposed tracking method. The angular axis represents leaf orientation, while the radial axis indicates temporal progression from inner (early stage) to outer (later stage), enabling simultaneous observation of spatial arrangement and growth dynamics.

This representation enables simultaneous visualization of leaf expansion direction (angle) and temporal progression (radial axis), providing intuitive insight into both spatial arrangement and temporal dynamics of leaf development.

For quantitative evaluation of tracking performance, a consistency-based assessment grounded in plant growth characteristics was employed. Because the number of leaves increases monotonically over time, intervals in the backward tracking sequence where the leaf count increased or decreased in an implausible manner were counted as mismatches. As a result, inconsistencies were observed in 5.8% of all frames, indicating that 94.2% of the tracking sequence was structurally consistent with expected plant growth. These findings suggest that the proposed method achieves sufficient stability for temporal identification of leaves in plant timelapse imagery.

Using the obtained tracking sequences, the growth process of individual leaves was also extracted. Figure 5 shows the area change of the fifth-emerging leaf in each plant.

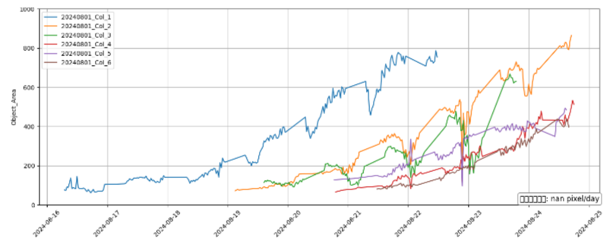

Fig. 5. Area growth curves of the fifth-emerging leaf in each plant, computed from segmented mask regions. The fifth leaf was selected as a representative example to illustrate post-emergence growth dynamics across individuals.

The leaf area, computed from the segmented mask regions, reflects how rapidly the leaf expands after emergence and how its growth rate evolves over time. This demonstrates that the proposed method can be applied not only to analyzing angular trajectories but also to extracting growth-related information.

VI. DISCUSSION

In this study, we established a framework for automatically extracting growth-related information—such as leaf emergence order and orientation—from timelapse images acquired under standard plant experimental conditions. The spatial arrangement, emergence timing, and growth direction of leaves are known to be important indicators for analyzing environmental responses, genetic differences, and stress reactions in plants [8,9]. Traditionally, these observations have relied heavily on manual inspection, which imposes substantial labor and limits experimental throughput [1,2]. The automated approach presented here provides a foundation for reducing this bottleneck and enabling more efficient phenotypic analysis.

The tracking results demonstrated that leaf orientation trajectories in later growth stages were reproduced smoothly, capturing the spatial configuration of the plant appropriately. In contrast, instability in segmentation during early growth—when leaves are small—led to occasional irregularities in tracking. These issues are likely attributable to occlusion between leaves and variations in illumination that affect segmentation performance in top-view timelapse images. However, the goal of this study is not to develop a highly precise tracking system per se, but to explore a practical framework through which researchers can readily obtain key growth descriptors—particularly leaf emergence order—without specialized equipment. From this perspective, the tracking accuracy achieved here is sufficient for capturing the overall developmental tendencies of the plant.

Furthermore, the visualization of leaf area transitions revealed distinct periods of rapid growth, indicating the potential for automatically extracting growth characteristics on a per-leaf basis.

However, when relying solely on top-view 2D images, the apparent leaf area may decrease due to leaf curling or changes in inclination, as observed in Fig. 6—even when captured on the same day.

Fig. 6. Example showing apparent leaf area fluctuation caused by leaf curling or changes in inclination under top-view imaging.

This suggests that studies requiring accurate measurements of leaf area or volume may need additional imaging angles or 3D structural information.

Possible solutions include depth estimation or geometric correction based on image cues, which are considered important future directions.

A key strength of this study is that growth information can be extracted from timelapse images obtained in a general laboratory environment, without relying on complex or specialized equipment. Leaf emergence order and orientation are known to respond sensitively to physiological changes and environmental stress [8,9], and thus the proposed method has potential utility for large-scale screening and genetic analysis by providing new quantitative traits. While further improvements — such as optimized lighting, alternative imaging angles, or integration of more advanced segmentation models—may enhance performance, such refinements must be balanced carefully against the central aim of this work: maintaining ease of implementation and low cost.

VII. CONCLUSION

In this study, we developed a method for automatically tracking and visualizing leaf position and growth dynamics using timelapse images acquired under standard plant experimental conditions. A key contribution of this work is that essential physiological traits—such as leaf emergence order and growth direction—can be extracted at low cost without the need for complex equipment or specialized imaging conditions.

The proposed method successfully reproduced leaf orientation and spatial configuration during later growth stages, whereas challenges remained in the early growth phase due to limited detection stability for very small leaves. However, these limitations are primarily attributable to controllable factors such as imaging conditions and the choice of segmentation model, rather than to the framework itself. Importantly, the method demonstrated practical utility in achieving the primary objective of this study: automating the determination of leaf emergence order and growth tendencies, which previously required manual annotation by researchers.

Future work will focus on improving leaf detection accuracy and developing automated estimation of plant center position, enabling more stable and comprehensive growth analysis. Applying the proposed approach to large numbers of individuals will allow high-frequency growth data to be collected at a scale difficult to achieve manually, potentially leading to the discovery of new developmental traits and advancing research in genetics and environmental response.

Overall, this study represents a foundational step toward automating plant phenotyping workflows. By reducing manual workload and increasing research throughput, the proposed framework has the potential to play an important role in future developments in plant science.

[1] M. Minervini, H. Scharr, and S. A. Tsaftaris, "Image-based plant phenotyping: A review," *Plant Methods*, vol. 11, no. 1, pp. 1–11, 2015.

[2] N. Fahlgren, M. A. Gehan, and I. Baxter, "Lights, camera, action: High-throughput plant phenotyping is ready for a close-up," *Plant Physiology*, vol. 169, no. 3, pp. 1532–1540, 2015.

[3] M. Fujita and K. Shinozaki, "Development of 'RIPPS,' an automated phenotyping system for evaluation of environmental stress response," *BSJ Review Shokubutsu Kagaku Saizensen*, vol. 10, pp. 59–66, 2019.

[4] E. P. Spalding and N. D. Miller, "Image-based sensing for phenotyping," *Plant Physiology*, vol. 161, no. 2, pp. 439–447, 2013.

[5] Y. Li, X. Fan, N. J. Mitra, D. Chamovitz, D. Cohen-Or, and B. Chen, "Analyzing growing plants from 4D point cloud data," *ACM Transactions on Graphics*, vol. 32, no. 6, pp. 157:1–157:10, 2013.

[6] T. Nagahara, F. Okura, and Y. Yagi, "Spatiotemporal plant tracking by vertex optimization," *Research Reports on Computer Vision and Image Media*, vol. 2022, no. 20, pp. 1–8, 2022.

[7] L. Yang, Y. Fan, and N. Xu, "Video Instance Segmentation," in *Proc. IEEE/CVF International Conference on Computer Vision (ICCV)*, 2019, pp. 5188–5197.

[8] C. Granier and D. Vile, "Phenotyping and beyond: Structuring phenotyping efforts for the future," *Trends in Plant Science*, vol. 19, no. 11, pp. 712–721, 2014.

[9] A. Walter, F. Scharr, M. Gilmer, A. Zierer, and U. Rascher, "Automated image-based phenotyping of plant shoot architecture," *Plant Methods*, vol. 11, no. 1, pp. 1–13, 2015.

[10] A. Dobrescu, H. Valerio Giuffrida, and S. A. Tsaftaris, "Detecting tiny plant organs using deep learning," *Plant Methods*, vol. 15, no. 1, pp. 1–13, 2019.

[11] Y. Wu, A. Kirillov, F. Massa, W.-Y. Lo, and R. Girshick, "Detectron2,"Meta AI, 2019. Available: https://github.com/facebookresearch/detectron2

[12] X. Zhu, M. Wang, H. Liu, and L. Yu, "Leaf segmentation in *Arabidopsis thaliana* using Mask R-CNN," *Plant Phenomics*, vol. 4, pp. 1–10, 2022.

[13] C. Broaddus, "COCO Annotator," 2019. Available: https://github.com/jsbroks/coco-annotator

[14] H. W. Kuhn, "The Hungarian method for the assignment problem," Naval Research Logistics Quarterly, vol. 2, no. 1–2, pp. 83–97, 1955.