Immagine che contiene testo

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Master’s degree in

Data Science for Economics

Cultivating insights: Prediction models for plant growth

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## Abstract

Accurately predicting plant growth is critical to optimizing agricultural productivity in an era of growing food demand and climate challenges. This thesis, titled **"Cultivating Insights: Prediction Models for Plant Growth"**, explores advanced techniques for plant phenotyping, leveraging digital image processing and time-series models to provide deeper insights into plant growth patterns. The focus is on automating leaf detection and surface area estimation using computer vision algorithms and improving the predictive capacity of plant growth models through statistical and machine learning approaches.

The research begins by addressing the limitations of traditional phenotyping methods and highlighting the role of modern data-driven approaches in improving the precision and efficiency of plant growth monitoring. A key contribution of the thesis is the development of an enhanced leaf detection algorithm, which addresses challenges like leaf occlusion and varying lighting conditions, improving the accuracy and robustness of existing methods. Additionally, a new approach for calculating plant surface area is introduced, providing an alternative metric for growth analysis alongside traditional leaf count techniques.

This thesis also presents the implementation of two time-series models—ARIMA and Bayesian approaches—for forecasting plant growth based on hourly data collected through controlled experiments. These models are compared in terms of accuracy, computational efficiency, and applicability to real-time plant monitoring systems.

The results demonstrate that, while surface area estimation is a robust alternative, leaf detection remains the most precise metric for monitoring growth. Time-series models provide strong predictive capabilities, with the Bayesian model outperforming in terms of accuracy. This work not only advances the current state of plant phenotyping but also offers practical applications for precision agriculture, where the integration of predictive models can lead to optimized farming practices and resource management.

# Introduction

## Background and motivation

The global challenge of food security has placed unprecedented importance on the study of plant growth. As climate change, population growth, and sustainability concerns intensify, the agricultural sector is facing increasing pressure to optimize crop yields and manage resources efficiently. Traditional agricultural methods, while effective, are limited in their ability to predict and analyze plant growth in real-time. This has led to the integration of modern technologies, particularly data-driven models, into plant phenotyping and growth prediction efforts.

Phenotyping—the measurement of observable characteristics such as leaf count, surface area, and overall plant size—provides critical insights into the health and development of plants. However, manual methods for acquiring this information are often labor-intensive, time-consuming, and prone to human error. With advances in computer vision and machine learning, researchers are now equipped with powerful tools to automate the collection and analysis of plant growth data. These tools enable the extraction of detailed phenotypic information from images, providing a more accurate and scalable approach to monitoring plant development.

In this context, the current research focuses on developing predictive models for plant growth using digital image processing and time-series analysis. By leveraging data from controlled experiments, this work aims to contribute to the broader goal of improving agricultural practices through data-driven insights.

## Plant growth prediction

Predicting plant growth is a complex task that requires an understanding of numerous factors, including environmental conditions, plant genetics, and developmental stages. Traditional approaches rely heavily on empirical data gathered through manual observation. However, modern data-driven methods are increasingly gaining traction due to their ability to analyze large datasets and uncover patterns that would otherwise go unnoticed.

The ability to predict plant growth accurately has numerous applications in agriculture. For instance, it can help optimize water and nutrient use, predict harvest times, and even detect early signs of stress or disease. The integration of time-series models and machine learning algorithms into plant growth analysis allows for more precise predictions, enhancing decision-making processes for farmers and researchers alike.

## Problem statement

Despite significant advancements in computer vision and machine learning, the accurate prediction of plant growth remains a challenging problem. One of the primary difficulties lies in the variability of plant phenotypes, which can be influenced by both genetic and environmental factors. Furthermore, the occlusion of leaves in densely packed plant structures and varying lighting conditions complicate the task of accurately detecting and quantifying leaves in images. While several algorithms exist for leaf detection, their accuracy and robustness vary across different plant species and conditions.

Another challenge arises in predicting the growth trajectory of plants based on historical data. Time-series models, such as ARIMA and Bayesian approaches, offer promising methods for forecasting plant growth. However, the accuracy of these models depends heavily on the quality of the input data and the tuning of model parameters. Thus, developing an effective pipeline for data acquisition, image processing, and growth prediction remains a critical research objective.

## Objectives

The primary objective of this thesis is to develop and evaluate a comprehensive pipeline for predicting plant growth using image-based phenotyping and time-series analysis. Specifically, the research aims to:

* **Develop an improved leaf detection algorithm** that enhances accuracy and robustness in different environmental conditions.
* **Design a method for calculating plant surface area** from images, providing an alternative metric for growth analysis.
* **Implement time-series models**, including ARIMA and Bayesian approaches, for predicting plant growth over time.
* **Compare and evaluate the performance** of different predictive models to determine the most effective approach for plant growth forecasting.

## Thesis contributions

This thesis builds on existing research in the field of plant phenotyping while introducing several novel contributions:

* **Enhanced Leaf Detection Algorithm**: A refined version of the existing leaf detection algorithm that improves accuracy under challenging conditions such as occlusion and varying lighting. This algorithm extends the work from previous research, particularly in its application to densely packed plant structures.
* **Surface Area Calculation**: A new approach for estimating plant surface area based on image data, offering a complementary metric to leaf count for analyzing plant growth. This method, while less precise than leaf counting, provides a robust measure that can be used in cases where leaf detection is unreliable.
* **Time-Series Growth Prediction Models**: The integration of ARIMA and Bayesian models for predicting plant growth, using hourly data collected from controlled experiments. These models offer insights into the temporal dynamics of plant development and provide a foundation for future work in real-time plant monitoring systems.

## Thesis structure

The remainder of this thesis is organized as follows:

* **Chapter 2** presents a comprehensive review of the literature on plant growth models, leaf detection techniques, and time-series analysis. The chapter highlights the current state of the art in these areas and identifies key gaps that this research addresses.
* **Chapter 3** provides an in-depth discussion of digital image processing techniques used in plant phenotyping, with a particular focus on leaf detection and surface area calculation.
* **Chapter 4** describes the experimental setup and data collection process, including the use of Raspberry Pi and Pi3 camera systems for image acquisition and the sensors used for environmental data collection.
* **Chapter 5** details the development and implementation of the improved leaf detection and surface area calculation algorithms, with a focus on their accuracy and robustness under different conditions.
* **Chapter 6** introduces the time-series models used for plant growth prediction, including ARIMA and Bayesian approaches, and presents a comparison of their performance.
* **Chapter 7** presents the results of the experiments and discusses their implications for plant growth prediction, as well as the limitations and challenges encountered during the research.
* **Chapter 8** concludes the thesis by summarizing the key contributions and suggesting directions for future research.

# Literature overview

## Overview of plant growth models

Plant growth modeling has progressed from traditional empirical methods toward more sophisticated data-driven techniques, thanks to advances in machine learning and computer vision. Traditional models, such as logistic growth models, rely on equations to describe plant growth patterns, often based on a few input variables like time and environmental conditions. These models are helpful but inherently limited in handling complex, multi-dimensional data, such as images that reveal detailed structural information about plants【1】【2】.

The evolution of phenotyping practices began with the adoption of image-based techniques. Using digital images captured through advanced imaging systems, researchers can measure phenotypic traits such as plant height, leaf count, surface area, and biomass in a non-invasive, efficient manner【1】. Deep learning models, such as Convolutional Neural Networks (CNNs), enable automated analysis of these images by learning complex features that distinguish various traits【3】【5】.

Recently, machine learning algorithms, particularly CNN-based architectures, have demonstrated remarkable accuracy in plant phenotyping. These models can handle large-scale data and complex plant structures, outperforming traditional models in scalability and precision. For instance, deep learning architectures like U-Net and Mask R-CNN provide not only the ability to detect individual leaves but also to accurately segment them from cluttered backgrounds【5】【6】.

## Leaf counting and surface area estimation

### Leaf counting approaches

Leaf counting plays a fundamental role in plant phenotyping as it correlates strongly with plant health and yield potential. Manually counting leaves is labor-intensive and prone to human error, especially for large datasets. Deep learning approaches automate this task by using models that can accurately estimate the number of leaves from a single image【2】【5】.

Segmentation-based approaches, such as U-Net, focus on extracting leaf regions by identifying pixel-level boundaries between leaves and their surroundings. The Mask R-CNN model further enhances this by using region proposal networks to locate objects and segment them simultaneously, allowing for leaf counting even in complex plant structures where leaves may overlap each other【5】【6】.

An alternative to segmentation is direct leaf counting, which bypasses the need to detect and segment each leaf. For instance, multi-scale regression approaches leverage varying image resolutions to estimate leaf counts without pixel-level annotations. The multi-scale fusion technique proposed by Itzhaky et al. (2018) has been particularly useful for counting leaves in densely populated images where traditional detection methods may fail【3】.

Moreover, YOLO-based detection algorithms have been shown to be highly effective in real-time leaf counting tasks. YOLO (You Only Look Once) is a high-speed object detection model that divides an image into a grid and predicts bounding boxes and confidence scores for each object. This model is particularly efficient for leaf counting in dynamic environments, as it processes the entire image in a single pass, making it suitable for real-time applications【2】【9】.

### Surface area estimation

While leaf counting provides discrete information about plant growth, surface area estimation offers a continuous metric, giving a better sense of the plant’s overall health and photosynthetic potential. Surface area estimation techniques rely on accurate segmentation of leaf regions, followed by calculations of pixel-based measurements that are then converted into real-world units【3】.

Deep learning models like ResNet50 have been employed for estimating surface area from segmented leaf images. By combining convolutional layers that capture hierarchical image features with fully connected layers, these models achieve high accuracy in surface area estimation across various plant species. Additionally, methods that employ region proposals, like Faster R-CNN, can be adapted to measure surface area by detecting leaf boundaries and extracting spatial dimensions of the plant canopy【6】【7】.

## Image processing techniques in agriculture

The accuracy of automated plant phenotyping heavily relies on the quality of image processing techniques. Here, segmentation and object detection are crucial in ensuring that the extracted features accurately represent plant traits such as leaf number and surface area.

* **Segmentation**: Accurate segmentation is essential for isolating individual leaves from their background. U-Net and Mask R-CNN are two popular models in this domain, providing state-of-the-art performance for tasks such as leaf segmentation. U-Net’s encoder-decoder architecture allows for precise delineation of leaf edges, even in challenging conditions like shadowing or occlusion【5】【6】. In parallel, Mask R-CNN enhances object detection by using region-based segmentation, which is beneficial for identifying overlapping or hidden leaves【5】【6】.
* **Background Removal**: Removing the background noise from images is a critical preprocessing step in leaf detection and surface area estimation. Classical methods, like thresholding and color space transformations, are combined with deep learning techniques to improve accuracy. These methods are especially important in greenhouse environments where lighting conditions may vary【6】【8】.
* **Object Detection**: YOLOv3 and its variants are widely used in real-time leaf counting applications. YOLOv3’s ability to simultaneously detect multiple leaves in a dense arrangement makes it a preferred choice for fast and accurate phenotyping. The model’s grid-based detection strategy allows it to handle complex scenarios, where traditional methods might struggle to separate overlapping leaves【9】【8】.

## Time series analysis in agriculture

### Overview of ARIMA models

Time-series models, such as ARIMA (Auto-Regressive Integrated Moving Average), have long been employed to predict plant growth by analyzing historical data trends. ARIMA models are particularly effective for short-term forecasting, such as daily or weekly growth increments, because they model temporal dependencies in the data. In agricultural contexts, ARIMA is widely used to model growth patterns over time. It’s extension to ARIMAX can also provide insights into how external variables such as temperature, humidity, and light intensity influence plant development【4】【8】.

Moreover, ARIMA models have been combined with other techniques to improve accuracy in forecasting plant phenotypes under fluctuating environmental conditions. For example, hybrid ARIMA models, which integrate machine learning techniques like support vector regression (SVR), have been explored to handle more complex, non-linear growth patterns. This hybrid approach has shown improved performance in agricultural forecasting by accounting for seasonal variations and external factors【10】.

### Bayesian models in growth prediction

Bayesian methods provide a flexible framework for predicting plant growth, particularly when dealing with uncertainty or limited data. Bayesian time-series models, such as those implemented using the PyMC3 library, allow for the incorporation of prior knowledge about the system, which can then be updated dynamically as new data becomes available. This is particularly useful in agriculture, where growth patterns are often non-linear and affected by various unpredictable environmental factors【3】【4】.

Bayesian approaches have also been extended to hierarchical models, where the growth of different plants or groups of plants can be modeled together, sharing information across levels of the hierarchy. This type of model improves predictive accuracy, particularly in scenarios where data is sparse or when modeling plants under different environmental conditions. The flexibility of Bayesian models also allows them to handle missing or noisy data more effectively than traditional methods like ARIMA【11】.

### Advanced deep learning technics

Beyond ARIMA and Bayesian methods, newer time-series techniques such as Long Short-Term Memory (LSTM) networks have been introduced to predict plant growth more accurately. LSTMs, a type of recurrent neural network (RNN), are particularly well-suited for time-series forecasting as they capture long-term dependencies in sequential data. LSTMs have shown great promise in predicting agricultural time-series data, especially for long-term growth forecasts where traditional models like ARIMA may struggle【12】.

Another advanced approach is Gaussian Process Regression (GPR), which has been applied to plant growth prediction. GPR models provide a probabilistic framework that can quantify uncertainty in predictions, making them useful for agricultural applications where precise predictions are challenging due to the complexity of biological processes【13】.

# Image analysis in Plant Science

## Introduction to Digital Image Processing

Digital image processing plays a crucial role in modern plant phenotyping, enabling the automated analysis of plant growth and health. The key advantage of digital imaging is its non-invasive nature, allowing researchers to observe phenotypic traits without disturbing the plant's growth process. In agricultural science, image analysis provides tools to extract valuable information, such as leaf count, surface area, and growth dynamics, which are critical for understanding and predicting plant development. Image-based phenotyping systems rely on capturing high-quality images and applying sophisticated processing algorithms to extract meaningful information. The increasing availability of affordable hardware and open-source software has made image processing more accessible to the agricultural community, paving the way for precision farming practices.

### Types of Digital Images

Digital images used in plant phenotyping can be broadly categorized based on the spectral range in which they are captured. The most common image types include:

* **RGB (Red-Green-Blue) Images**: These are standard color images captured using conventional cameras. RGB images are widely used due to their simplicity and ease of processing. They provide enough information for tasks like leaf counting and surface area estimation.
* **Hyperspectral Images**: Hyperspectral imaging captures data across multiple bands of the electromagnetic spectrum, allowing for the detection of subtle changes in plant physiology. These images are particularly useful in identifying stress factors, such as nutrient deficiencies or disease.
* **Thermal Images**: Thermal cameras capture infrared radiation, offering insights into plant transpiration and heat stress. These images are valuable in identifying areas where plants may be experiencing drought stress or irregular water distribution.

### Image Acquisition

Acquiring high-quality images is the foundation of successful plant image analysis. In plant phenotyping, cameras are typically mounted on fixed stations, drones, or robots to capture images at regular intervals. Raspberry Pi-based systems, as used in this research, are gaining popularity due to their affordability and flexibility. Equipped with a Pi camera, these systems can capture time-lapse images at set intervals to monitor plant growth continuously. Additionally, environmental data such as temperature and humidity are often recorded alongside images to provide context for growth variations. High-resolution cameras are essential for ensuring the accuracy of subsequent image analysis steps, such as leaf segmentation and surface area calculation.

## Image pre-processing techniques

Image pre-processing is a critical step in preparing raw images for analysis. It enhances image quality, corrects distortions, and ensures that the plant features are more distinguishable from the background. Common pre-processing steps include background removal and noise reduction.

### Background removal

Background removal is essential in plant image analysis as it isolates the plant from the surrounding environment. Without effective background removal, the image analysis algorithms may incorrectly identify non-plant elements, reducing the accuracy of metrics such as leaf count and surface area. Various techniques are employed to remove backgrounds, including thresholding and color-space transformations. For instance, converting an image from RGB to the HSV (Hue-Saturation-Value) color space can make it easier to separate plant material from the background based on color differences. Machine learning methods, such as Mask R-CNN, can also be used to segment plants and remove backgrounds more accurately in complex scenarios【5】【6】.

### Noise reduction and Filtering

Images captured in natural environments often contain noise due to factors like uneven lighting, shadows, and reflections. Noise reduction techniques, such as Gaussian filtering, help in smoothing images while preserving important features like edges. Median filtering is another popular technique used in plant image processing, particularly when dealing with images that contain salt-and-pepper noise. These methods improve the quality of the image, ensuring more accurate leaf detection and surface area estimation【5】【6】.

## Leaf detection algorithms

Leaf detection is a core task in plant phenotyping, as the number of leaves is a key indicator of plant health and growth. Various algorithms have been developed for detecting leaves, ranging from traditional computer vision techniques to advanced deep learning approaches.

### Traditional approaches

Traditional leaf detection approaches primarily rely on edge detection and contour-based methods. Algorithms such as Canny edge detection and Hough Transform are used to identify the boundaries of leaves. Once the edges are detected, further processing, like contour finding, can be applied to count the number of distinct leaves. These methods, although effective in simple scenarios, often struggle in complex images where leaves overlap or have irregular shapes. Additionally, varying lighting conditions and occlusions make traditional methods less reliable.

### Deep Learning Approaches

In recent years, deep learning techniques have revolutionized leaf detection by significantly improving accuracy in complex scenarios. Models like U-Net and Mask R-CNN have been widely adopted in plant phenotyping. U-Net, with its encoder-decoder architecture, excels at segmenting individual leaves from an image, even when they overlap. Mask R-CNN, on the other hand, extends this capability by performing both object detection and instance segmentation, allowing it to accurately detect each leaf in dense foliage. These methods use large labeled datasets for training, which enable them to generalize well across different plant species and environmental conditions【5】【6】.

## Feature extraction for Plant Growth

Feature extraction is the process of identifying and quantifying phenotypic traits that can be used to assess plant growth. Leaf count and surface area are two primary features extracted from plant images.

### Leaf count and surface area

Leaf count is a straightforward but highly informative metric for assessing plant health. Automated leaf counting can be performed using deep learning models like Mask R-CNN or YOLO, which detect individual leaves even in cluttered images. Surface area, on the other hand, provides a more comprehensive measure of plant size and health. After detecting the leaves, surface area is estimated by calculating the number of pixels that correspond to the leaves and converting this value to a real-world measurement using calibration techniques【5】【6】.

### Challenges in Leaf Segmentation

Leaf segmentation presents several challenges, particularly in images where leaves overlap or are partially occluded. In such cases, traditional segmentation methods often fail to distinguish individual leaves. Variations in leaf shape and size also add complexity to the task. To address these challenges, deep learning models like Mask R-CNN and U-Net have been developed to handle dense foliage by learning from large datasets. However, these methods require extensive computational resources and high-quality labeled data for training【6】【7】.

## Image Calibration and Cropping

Image calibration ensures that the measurements extracted from images are accurate and consistent. Calibration typically involves correcting distortions caused by the camera lens and converting pixel-based measurements into real-world units. Calibration is particularly important for tasks like surface area estimation, where accurate scaling is crucial. Cropping, on the other hand, removes unnecessary parts of the image, focusing the analysis on the region of interest, such as the plant canopy.

## Summary

This chapter has outlined the key steps in image processing for plant phenotyping, from image acquisition and pre-processing to leaf detection and feature extraction. The advancements in deep learning techniques, particularly in leaf segmentation and surface area estimation, have significantly improved the accuracy and scalability of plant phenotyping. However, challenges such as occlusion and variability in plant structure continue to present obstacles, highlighting the need for further research and innovation in this field.

# Experiment setup and data collection

## Overview of plant type and Growth stages

For this experiment, we selected **lettuce (Lactuca sativa)**, a popular leafy vegetable known for its fast growth, ease of cultivation, and robustness in various environmental conditions. Lettuce is a versatile crop commonly used in salads and sandwiches, with varieties such as romaine, butterhead, and leaf lettuce being widely cultivated across the globe. It has a relatively short growth cycle, which makes it an ideal candidate for growth prediction and phenotyping experiments.

Figure 1 - Romaine Lettuce example

Lettuce was chosen for this study due to several key factors. First, its growth cycle is relatively short, typically ranging from 30 to 50 days, which makes it suitable for experimental setups where time is a limiting factor. Furthermore, lettuce is resilient and can adapt to different growing environments, from outdoor soil-based cultivation to controlled indoor conditions. This robustness reduces variability in the experimental conditions, allowing for more consistent data collection. Lettuce is also relatively easy to grow from seed, requiring basic care such as consistent watering, adequate sunlight, and protection from extreme temperatures, which simplifies the setup and ensures a successful harvest.

In this experiment, lettuce plants were grown from seeds, following standard practices to ensure proper germination and growth. **Seeds were planted in individual pots filled with nutrient-rich soil** to prevent overcrowding and ensure each plant had enough space to develop fully. In total, eight plants were grown, each in a separate pot, to monitor individual growth patterns and avoid competition for resources.

Figure 2 - Experiment setup. End of seeding stage

The experiment was conducted over a period of 42 days, during which the plants progressed through the following growth stages:

1. **Seed Stage**: The experiment began with the planting of lettuce seeds, which were sown at a shallow depth of around ¼ inch in well-drained soil. Lettuce seeds generally require a temperature of around 60–70°F (15–21°C) to germinate effectively. Under optimal conditions, germination occurred within 5 to 10 days, with tiny cotyledons—the first leaves—emerging from the soil.
2. **Seedling Stage**: Once the seeds germinated, the plants entered the seedling stage, where they developed their first true leaves. During this period, maintaining the right balance of light, temperature, and moisture was critical. The seedlings were thinned out, if necessary, to ensure each plant had enough space to grow.
3. **Rosette Stage**: As the lettuce continued to grow, the plants entered the vegetative or rosette stage. In this phase, which lasted between 25 and 50 days, the plants rapidly produced leaves, forming a circular arrangement typical of lettuce growth. Consistent watering and nutrient supply were critical during this stage to promote leaf development and ensure a healthy plant structure.
4. **Cupping Stage**: After the rosette stage, the plants entered the cupping stage, a brief period during which the outer leaves began to curl inward, preparing for the formation of a dense head in head lettuce varieties. This stage typically lasted about a week. Monitoring for pests and maintaining soil moisture were important to prevent stunted growth or early bolting.
5. **Heading Stage**: In the final growth stage before harvest, the plants formed a compact head. For leaf lettuce, this phase did not result in head formation, but the leaves were still ready for harvesting. For head-forming lettuce varieties, such as romaine, the heading stage lasted an additional 20 to 45 days, depending on environmental conditions.

At the end of the 42-day experimental period, the plants were harvested. During the final harvest, the above-ground biomass of each plant was carefully collected. The harvested plants were weighed to determine their fresh biomass, a key metric for evaluating plant growth and overall health. Lettuce is typically harvested once has formed a full rosette of leaves or, in the case of head lettuce, when the head has firmed up.

Figure 3 - End of the experiment. Harvesting plants

## Equipment setup tools

### Hardware

The **Raspberry Pi** is a versatile, single-board computer widely used for a range of tasks in both hobbyist and professional settings. We utilized the **Raspberry Pi Model 3**, which is a popular version known for its balance of performance, affordability, and energy efficiency. This model features a quad-core 1.2 GHz ARM Cortex-A53 processor and 1 GB of RAM, making it capable of handling tasks that involve data collection, image processing, and basic automation. Raspberry Pi devices are commonly used for tasks such as home automation, robotics, Internet of Things (IoT) applications, and environmental monitoring.

The Raspberry Pi 3 was chosen for this experiment due to its **low power consumption**, which is particularly important when running a 42-day continuous experiment. The device’s built-in GPIO (General Purpose Input/Output) pins also allowed seamless integration with sensors such as temperature and humidity monitors, while its USB ports enabled the connection of external peripherals, including the Raspberry Pi camera used in this setup. Furthermore, the **Raspberry Pi runs on open-source software**, making it highly customizable for specific project needs, and the Python environment it supports provides an excellent platform for running automation scripts.

**Raspberry Pi Camera Module**

For capturing images of the lettuce plants, we used the **Raspberry Pi Camera Module**, specifically the **Pi3 camera**. This camera module integrates directly with the Raspberry Pi through the dedicated camera port and provides high-quality image capture capabilities. The Pi3 camera has the following key specifications:

* **Resolution**: 8 megapixels
* **Image size**: 3280 x 2464 pixels
* **Lens**: Fixed-focus, capable of capturing detailed close-up images, which is ideal for plant phenotyping experiments.
* **Frame rate**: Supports 1080p video at 30 frames per second (fps), though for this experiment, we focused on still image capture.

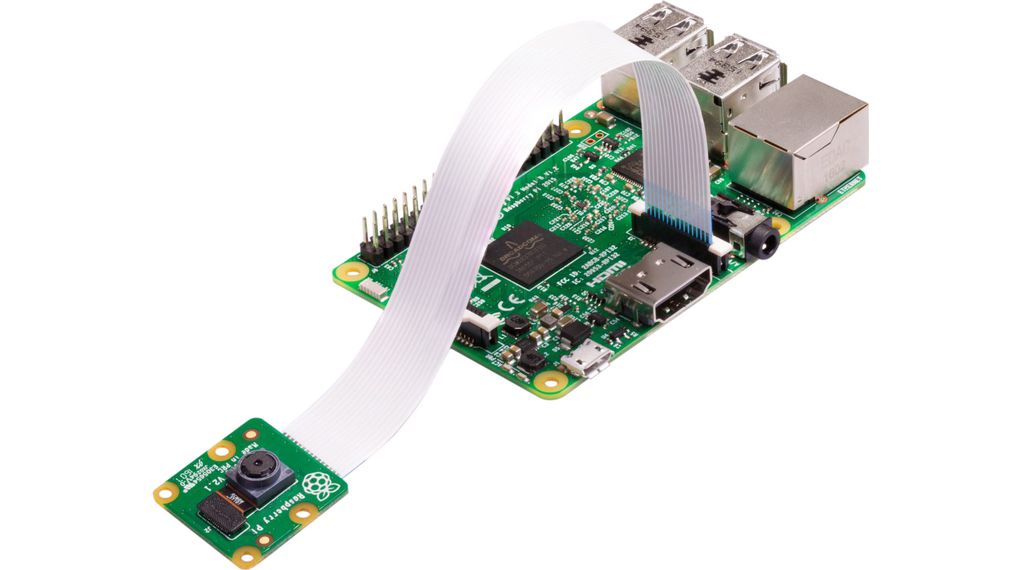
The camera was configured to capture high-resolution images of the lettuce plants at hourly intervals, providing continuous documentation of plant growth. The fixed-focus lens of the Pi3 camera ensured that each image was sharp, and the camera's compact size allowed it to be mounted in close proximity to the plants without interfering with their environment. This setup was essential for monitoring subtle changes in leaf development, which were later analyzed for growth patter

Figure 4 - Raspberry Pi 3 with Pi camera

**Temperature and Humidity Sensors**

Accurate monitoring of environmental conditions is crucial in plant growth experiments, as factors such as temperature and humidity significantly affect plant development. For this project, we used a **DHT11 temperature and humidity sensor**. The DHT11 sensor is a low-cost digital sensor that can measure:

* **Temperature**: Ranges from 0°C to 50°C with an accuracy of ±2°C.
* **Humidity**: Ranges from 20% to 90% relative humidity (RH), with an accuracy of ±5%.

The **DHT11 sensor** was connected to the Raspberry Pi via its GPIO pins, allowing real-time temperature and humidity data collection throughout the experiment. These environmental parameters were logged every hour alongside the images, providing a comprehensive dataset for analyzing the relationship between environmental factors and plant growth.

### Software

The Python script used for the experiment was designed to automate both image capture and environmental data collection, making the entire process efficient and self-sustaining. At a high level, I developed the script which performs the following tasks:

1. **Directory and File Setup**: Before collecting any data, the script checks whether the necessary directories and files exist. These include directories for storing images and CSV files for logging environmental data (temperature and humidity) and any errors that occur during execution.
2. **Sensor and Camera Initialization**: The script interfaces with a DHT11 temperature and humidity sensor using the Adafruit library. This sensor reads the temperature and humidity at regular intervals. Simultaneously, the Raspberry Pi camera is configured using the Picamera2 library, with settings to capture high-resolution still images. The camera is manually focused to ensure clarity in the images.
3. **Data and Image Capture**: The script runs an infinite loop, capturing data from the DHT11 sensor and saving it to a CSV file every hour. Alongside this, it captures an image of the lettuce plants and stores it in a specified directory. Each image and data point is timestamped for easy tracking.
4. **Error Handling**: The script includes error-handling mechanisms to manage potential issues, such as sensor failures or camera errors. If an error occurs, it is logged in a separate CSV file with the timestamp and error type, ensuring that any issues are documented for future reference.
5. **Automation**: To ensure that the data collection process continues uninterrupted, the script was configured to run as a background process on the Raspberry Pi. This means that even after a power outage or system reboot, the script automatically restarts without requiring manual intervention.

This setup allowed for continuous, automated monitoring and documentation of the experiment, providing consistent data for later analysis.

## Experiment data collection

Data collection is a critical part of this experiment, as it serves as the foundation for both the analysis of plant growth and the development of prediction models. Over the course of the 42-day experimental period, we employed a systematic approach to gather both visual and environmental data, ensuring consistency and accuracy in the dataset.

**Hourly Image Capture**

The primary data source for this experiment was the **hourly image capture** of the lettuce plants, facilitated by the Raspberry Pi Camera Module. Each plant was photographed in high resolution once every hour, capturing its growth and physical changes in real-time. This resulted in a large collection of images over the 42-day period, providing a detailed time-lapse of plant development from seedling to full maturity.

Each image was automatically saved with a timestamp, which allowed us to correlate visual growth patterns with environmental factors such as temperature and humidity. The high frequency of image capture ensured that even subtle growth changes, such as new leaf formations or minor structural adjustments, were documented. This wealth of image data would later be used to analyze growth trends, measure leaf area, and monitor overall plant health.

**Environmental Data: Temperature and Humidity Monitoring**

In addition to the visual data, **environmental conditions** were meticulously monitored throughout the experiment. Temperature and humidity levels can significantly affect plant growth, and it was essential to capture these parameters at regular intervals to establish any correlation between environmental changes and plant development. The DHT11 temperature and humidity sensor was used to record these metrics once every hour.

The Raspberry Pi was programmed to collect the temperature (in degrees Celsius) and humidity (as a percentage) at the same intervals as the image captures. This environmental data was logged into a CSV file alongside the images, ensuring that each data point had a corresponding timestamp. By synchronizing the environmental data with the image capture, we ensured that any deviations in plant growth could be cross-referenced with changes in temperature or humidity, providing a robust dataset for predictive modeling.

**Ensuring Data Integrity**

To ensure the integrity of the collected data, several measures were put in place:

1. **Backup Protocols**: The Raspberry Pi was configured to back up data automatically to an external storage device at the end of each day. This prevented data loss in case of hardware failure or power outages.
2. **Error Logging**: As previously mentioned, the Python script was designed to log any errors encountered during the data collection process. If a sensor malfunctioned or the camera failed to capture an image, these errors were documented with timestamps, allowing us to track and address any anomalies in the data collection.
3. **Manual Checks**: While the system was automated, periodic manual checks were performed to ensure that the sensors and camera were functioning correctly. These checks included verifying that images were being captured at the correct intervals and that environmental data was being logged accurately.

|  |  |  |
| --- | --- | --- |
| **timestamp** | **error\_type** | **message** |
| 2024-06-21\_16:42:17 |  | success |
| 2024-06-21\_17:24:01 |  | success |
| 2024-06-21\_18:24:03 |  | success |
| 2024-06-21\_19:24:04 |  | success |
| 2024-06-21\_20:24:05 | runtime\_error | Checksum did not validate. Try again. |

Table 2 - CSV file structure with running logs

## Raw data description

The raw data collected during the 42-day experiment consisted of two main types: **image data** and **environmental data**. Both data types were collected continuously at hourly intervals, creating a detailed record of plant growth and environmental conditions. This section provides an overview of the structure, content, and characteristics of the raw data that forms the basis for the subsequent analysis.

**Image Data**

The image dataset comprises a total of **1,000 high-resolution images**, with each of the eight plants captured once every hour throughout the 42 days of the experiment. The images were stored in separate directories for each plant to facilitate easier access and analysis.

**File Naming Convention**: Each image was saved with a unique file name indicating the timestamp of when it was captured, using the format *lattuga\_YYYY-MM-DD\_HH:MM:SS.jpg*. This allowed for easy correlation with the environmental data and precise tracking of growth over time.

**Resolution**: The images were captured at a resolution of **3280 x 2464 pixels**, providing enough detail to analyze individual leaves, detect subtle changes in leaf morphology, and calculate surface area. The high resolution was particularly beneficial for accurately monitoring the structural growth of the plants, even in the early stages.

**Image Consistency**: Throughout the experiment, the images were captured under relatively stable lighting conditions to minimize variability. However, occasional fluctuations in natural light or shadows may be present in some images, which will be addressed during the image pre-processing phase in later chapters.

**Environmental Data**

The environmental data collected during the experiment included hourly records of temperature (in °C) and humidity (in %) for the entire duration of the study. These measurements were stored in a CSV file format, making them easily accessible for further analysis and modeling.

**Data Fields**: The environmental data was logged with three key fields:

1. **Timestamp**: The exact time the measurement was taken (e.g., 2023-08-15\_14:00:00), allowing for easy synchronization with the corresponding image data.
2. **Temperature (°C):** The temperature of the environment surrounding the plants, recorded hourly using the DHT11 sensor. The temperature varied between 18°C and 25°C during the experiment, staying within the optimal range for lettuce growth.
3. **Humidity (%):** The relative humidity in the environment, which ranged between 50% and 75% depending on the day. These variations could impact plant growth, making this data valuable for understanding any correlations between environmental factors and plant development.

|  |  |  |
| --- | --- | --- |
| **timestamp** | **temperature\_c** | **humidity** |
| 2024-06-21\_17:24:01 | 21 | 73 |
| 2024-06-21\_18:24:03 | 22 | 74 |
| 2024-06-21\_19:24:04 | 22 | 74 |
| 2024-06-21\_20:24:05 | 22 | 74 |
| 2024-06-21\_21:24:06 | 21 | 73 |

Table 1 - CSV file structure with the environmental data

**CSV File Format**: The environmental data was stored in CSV format with the following structure: *timestamp, temperature\_c, humidity*. This simple structure ensured that the data could be easily imported into various analysis tools for further processing.

**Preliminary Observations**

Once the data collection phase was complete, the dataset was prepared for analysis. This involved organizing the images and environmental data into a format compatible with the analytical tools to be used in later chapters. The images were processed to ensure uniformity in terms of size, resolution, and framing, while the environmental data was cleaned to remove any errors or anomalies that were detected during the collection phase. This preparation step was crucial for ensuring that the subsequent analysis and predictive modeling would be based on accurate and high-quality data.

While the primary analysis of the data is presented in later chapters, some preliminary observations can be made from the raw data:

* The hourly images captured distinct growth stages in the lettuce plants, from the early seedling stage through to full maturity.
* The environmental data showed some variability in temperature and humidity, particularly during the night and early morning hours. This variability could play a role in the observed differences in growth rates between the individual plants.

Overall, the raw data collected forms a comprehensive foundation for the detailed analysis of lettuce growth and the development of predictive models, as discussed in the following chapters.

## Raw data preprocessing

Raw data preprocessing is an essential step in preparing the collected image data for further analysis. This process ensures that the images are organized, cropped, and ready for downstream tasks such as feature extraction and growth analysis. The following steps outline the preprocessing tasks performed on the raw data in this experiment:

**1. Organizing and Loading Image Files**

The first step in the preprocessing pipeline involved organizing the image files by their respective timestamps and plant identifiers. The images collected during the experiment were stored in the directory data/daylight\_images, with filenames following a specific format: plantname\_YYYY-MM-DD\_HH.jpg. These filenames encoded the plant ID, the date, and the time of capture, which allowed for easy sorting and chronological arrangement of the images.

Using Python’s os library, the images were read and listed for processing. The images were sorted by filename to ensure that the chronological sequence of plant growth was maintained throughout the analysis.

**2. Defining Plant Centers and Cropping**

Given that each image captured multiple plants, it was necessary to isolate individual plants from the full image. This was achieved through **cropping**, where each plant was extracted based on its pre-determined center coordinates. The coordinates of the center for each plant were manually defined in two sets, **centers\_1** and **centers\_2**, corresponding to different phases of the experiment (before and after a specific date defined by a **breakpoint**).

* **Centroid Coordinates**: Two dictionaries, centers\_1 and centers\_2, were created to store the x and y coordinates for the center of each plant in the image.
* **Cropping Window**: Each plant’s region of interest (ROI) was defined by a square window, with the side lengths of either 900 pixels or 700 pixels, depending on the timestamp of the image.

During preprocessing, the code calculated the window around each plant based on its center coordinates. The plant image was then extracted using array slicing, isolating the specific plant from the full image.

**3. Time-Based Conditional Cropping**

A critical aspect of the preprocessing involved handling the images based on their capture time. A specific **breakpoint** (set to **July 4, 2024, at 15:00**) was defined to account for changes in the experimental setup or camera configuration that affected the size of the plant images. Based on whether an image was captured before or after this breakpoint, the appropriate side length and center coordinates were chosen for cropping.

* For images captured **before** the breakpoint, a window of size **900 x 900 pixels** was used for cropping.
* For images captured **after** the breakpoint, a smaller window of **700 x 700 pixels** was used.

This conditional cropping ensured consistency in the size and focus of the extracted plant images despite changes in the experimental conditions.

**4. Saving Preprocessed Images**

Once the plants were cropped from the raw images, the individual plant images were saved in the directory data/daylight\_preprocessed\_images. Each image was saved with a new filename, following the format lettuce{i}\_YYYY-MM-DD\_HH.jpg, where {i} represents the plant number and the rest of the filename reflects the original timestamp of the capture.

The imsave function from the skimage.io module was used to save the images, ensuring that the format and quality of the images were maintained throughout the preprocessing pipeline.

**5. Data Preparation for Analysis**

This preprocessing step generated a dataset of isolated plant images, each corresponding to a specific plant and timestamp. By organizing and cropping the images in this structured way, the resulting dataset was well-prepared for the next stages of analysis, such as feature extraction, leaf counting, and growth prediction. This preprocessing step significantly reduced the complexity of the data, enabling more focused and efficient analysis on individual plants.

1. **Filtering daytime images**

After cropping and saving the individual plant images, an additional filtering step was performed to ensure that only images captured during the daytime—when sufficient natural light was present—were included in the dataset. This step was crucial to exclude nighttime images, which did not provide meaningful visual information due to low lighting conditions.

The filtering process was conducted using an additional Python script that analyzed the brightness levels of each image. The basic approach involved the following tasks:

**Brightness Analysis for Daytime Detection**

For each image, the overall brightness was calculated by converting the image to grayscale and computing the mean pixel intensity. Images captured during the daytime exhibited higher brightness values due to the presence of natural light, whereas images captured at night had significantly lower brightness levels.

* **Grayscale Conversion**: Each RGB image was converted to a grayscale image, where pixel intensity values ranged from 0 (black) to 255 (white).
* **Thresholding**: A brightness threshold was defined based on the mean pixel intensity. Images with a mean brightness value below this threshold were considered too dark to provide meaningful information and were thus classified as nighttime images. These images were excluded from further analysis.

**Filtering and Storing Daytime Images**

Once the brightness analysis was completed, only the images with sufficient brightness (i.e., those captured during the day) were retained. The daytime images were stored in a separate folder for subsequent analysis. This step ensured that the dataset contained only high-quality images where plant growth could be clearly observed.

By filtering out nighttime images, this step helped to enhance the overall quality and consistency of the dataset, reducing noise and ensuring that the remaining images provided valuable information for the analysis of plant growth over time.

# Leaf detection and surface calculation

## Review of the existing algorithms

Leaf detection in plant phenotyping has seen significant advancements over the years, with a wide range of algorithms being developed to enhance the accuracy and efficiency of this task. From traditional image processing techniques to advanced deep learning models, each method brings unique strengths and limitations depending on the context in which they are applied. Below is an expanded review of five key algorithms for leaf detection.

**1. Canny Edge Detection and Hough Transform**

The **Canny Edge Detection** algorithm, introduced by John Canny in 1986, is one of the most widely used edge detection techniques in image processing. It detects edges by first smoothing the image to reduce noise, then identifying intensity gradients, and finally applying edge-tracing algorithms to highlight continuous boundaries. In leaf detection, this method identifies the edges of leaves based on the contrast between the plant and the background. Once the edges are detected, the **Hough Transform** can be applied to detect circular or elliptical shapes, which often approximate the shape of leaves【14】.

However, while Canny edge detection is computationally efficient and easy to implement, it has several limitations. It struggles with complex plant structures, especially when leaves overlap or lighting conditions vary. Moreover, it is sensitive to noise and background interference, requiring controlled environments for optimal performance. In agricultural applications where leaves are often occluded by others, more advanced methods are usually preferred.

**2. U-Net for Leaf Segmentation**

**U-Net** is a deep learning model that was originally developed for biomedical image segmentation. Its architecture consists of an encoder-decoder structure, where the encoder captures features from the image and the decoder reconstructs them to form precise segmentation masks. U-Net has become one of the most popular models for leaf segmentation due to its ability to accurately delineate leaf boundaries, even in densely packed and complex plant structures【15】.

One of the key strengths of U-Net is its ability to capture context at multiple scales. By using skip connections between the encoder and decoder, the model retains high-resolution information while also learning abstract features from deeper layers. This makes U-Net particularly effective at detecting individual leaves in images where leaves overlap or are partially occluded. Its success in the field of plant phenotyping is largely due to its versatility and accuracy in segmenting both simple and complex images.

**3. Mask R-CNN for Instance Segmentation**

**Mask R-CNN** is a more advanced deep learning model that extends traditional object detection networks by adding a segmentation mask for each detected object. It was developed to address the challenge of detecting and segmenting individual instances of objects within an image. In the context of leaf detection, Mask R-CNN is particularly useful because it not only detects each leaf but also generates a pixel-wise segmentation mask, providing detailed information about the size, shape, and position of each leaf【16】.

The architecture of Mask R-CNN builds upon Faster R-CNN by introducing an additional branch for segmentation. This allows the model to handle complex plant structures, where leaves may overlap or be hidden behind others. The model’s ability to segment leaves at the pixel level makes it ideal for calculating phenotypic traits like leaf count and surface area. However, Mask R-CNN is computationally intensive and requires a large amount of labeled data for training, which can be a limitation in some agricultural settings.

**4. YOLO (You Only Look Once)**

**YOLO (You Only Look Once)** is a real-time object detection algorithm that processes an entire image in a single pass, making it exceptionally fast compared to other object detection models like Faster R-CNN. YOLO has been successfully applied in plant phenotyping for tasks like leaf detection and counting. **YOLOv3** and **Tiny-YOLOv3** versions are commonly used for detecting leaves in real-time, providing an efficient solution for large-scale agricultural applications【8】.

One of the key advantages of YOLO is its speed. By dividing the image into a grid and making predictions for each cell in a single forward pass, YOLO can detect and localize objects (leaves) much faster than traditional methods. This makes it ideal for applications where quick decision-making is crucial, such as in precision agriculture. However, YOLO’s accuracy is somewhat lower compared to more specialized models like Mask R-CNN, especially when dealing with densely packed plants or small leaves.

**5. DeepLab for Semantic Segmentation**

**DeepLab** is a deep learning model designed for semantic segmentation, which means that it assigns a class label to each pixel in the image. DeepLab’s unique approach lies in its use of **atrous (dilated) convolutions**, which allow the model to capture larger contextual information without increasing the number of parameters or sacrificing resolution. This is particularly important in leaf detection, where capturing the spatial relationships between leaves is critical for accurate segmentation【6】.

DeepLab has proven to be highly effective in leaf detection, especially in high-resolution images where fine-grained segmentation is necessary. The model’s ability to handle multiple scales of objects makes it well-suited for detecting leaves of varying sizes and orientations. Additionally, DeepLab’s flexibility allows it to be used in different agricultural environments, from controlled greenhouses to open fields. However, like other deep learning models, DeepLab requires a significant amount of computational resources and high-quality labeled data for training.

## Improvements to Leaf Detection Algorithm

In this section, we describe the refinements made to the existing leaf detection algorithm, primarily focusing on adapting simple image processing techniques to better fit the dataset used in this experiment. While the improvements made were not revolutionary in terms of novel algorithm development, they were crucial in customizing the algorithm to work effectively with the specific characteristics of the images collected during the plant growth experiment. The goal was to enhance accuracy and reliability in counting leaves across different growth stages using a series of sequential image processing filters.

### 5.2.1 Methodology of Enhancement

The core of the methodology revolved around the **sequential application of filters** from the skimage library to improve leaf segmentation and counting. The chosen approach was relatively simple but effective, relying on traditional image processing techniques rather than advanced machine learning models. These techniques included color space conversion, noise removal, morphological operations, and connected component analysis. The following steps outline the workflow:

1. **Color Space Conversion (RGB to HSV)**: The first step in the process was converting the RGB image into the HSV (Hue, Saturation, Value) color space. This conversion allowed for more effective segmentation based on color, as the hue channel in HSV is particularly well-suited for isolating green areas in plant images. In this case, specific thresholds were defined for the hue values to capture the green areas of the plants, which represented the leaves. The range of hue values was set between **0.15** and **0.2**, corresponding to the green hue spectrum in lettuce leaves.

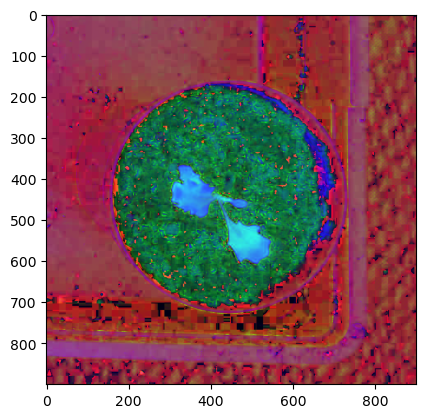


Figure 5 - Lettuce image in HSV color space

1. **Green Mask Application**: After converting the image to HSV, a **green mask** was applied to filter out non-leaf areas of the image. The mask was created by retaining pixels whose hue values fell within the specified range. This effectively isolated the plant leaves from other elements in the image, such as the soil, pot, or background noise.

A yellow and purple map

Description automatically generated

Figure 6 - Lettuce image with Green Mask applied

1. **Noise Removal and Small Object Filtering**: To ensure that small, irrelevant objects (such as small patches of dirt or random noise) were not misclassified as leaves, a morphological operation was performed to **remove small objects** from the binary mask. The minimum size of objects retained was set to 500 pixels, ensuring that only significant plant features were preserved for further analysis.

A purple and yellow square with numbers

Description automatically generated

Figure 7 - Lettuce image without noise

1. **Morphological Opening**: A **morphological opening** operation was applied to clean up the segmented image further. This step involved the use of a disk-shaped structuring element to remove small, disconnected noise while retaining the shape and structure of the leaves. Morphological opening is particularly useful for smoothing the edges of segmented regions and removing artifacts introduced by the green mask application.

A purple and yellow chart

Description automatically generated

Figure 8 - Lettuce image with morphological opening

1. **Connected Component Labeling**: Once the leaves were effectively segmented, the next step was **connected component labeling**. This technique identifies individual connected regions (i.e., leaves) in the binary mask and labels them accordingly. By counting the number of connected components, the algorithm was able to determine the number of leaves present in each image.
2. **Leaf Count Calculation**: The labeled components were analyzed to calculate the total number of leaves in the image. The algorithm identified distinct regions corresponding to individual leaves and tallied them to produce a leaf count. This count was then logged along with the timestamp and plant number for each image.

This method proved to be effective in isolating and counting leaves from the dataset of lettuce plants, despite using relatively simple techniques compared to deep learning-based approaches. The combination of color-based segmentation, morphological filtering, and connected component analysis allowed for a robust and accurate detection of leaves, even in images with challenging lighting conditions or slight occlusions.

### 5.2.2 Validation of Enhanced Accuracy

After the tailored leaf detection algorithm was implemented and integrated into the data processing pipeline, it was essential to evaluate its performance across the dataset, particularly in terms of accuracy and consistency over time. While the algorithm showed promising results, a deeper analysis revealed some variance in the leaf count estimates that required further refinement.

**Time-Series Analysis of Leaf Count Data**

A graph showing a line of blue color

Description automatically generated with medium confidenceThe leaf count data collected from the images was initially compiled into a time-series dataset, with each row corresponding to a specific plant at a given timestamp. Using this data, an aggregated analysis of the leaf count over time was performed. A **line plot** was generated to visualize the progression of leaf counts for the entire experiment period, allowing us to detect trends and any potential anomalies.

Figure 9 - Raw time series of the leave count visualization

The aggregated plot of the leaf count over time highlighted a **high variance** in the detection results, particularly in images where occlusion, lighting, or segmentation challenges arose. For instance, there were instances where the leaf count would fluctuate significantly over short time periods, which was not consistent with the biological reality of the plants' growth process.

**Application of Rolling Median to Reduce Variance**

To mitigate the high variance observed in the time-series data, a **rolling median** technique was applied to smooth the leaf count values over a 24-hour window. By calculating the median leaf count within a 24-hour time frame, the approach helped reduce the impact of outliers and sudden spikes or drops in the detected leaf count that were likely caused by inconsistencies in image conditions rather than actual changes in leaf growth.

A graph showing a line

Description automatically generatedThis smoothing process improved the overall trend visualization, revealing a more consistent and biologically plausible growth curve. The rolling median approach effectively minimized the sudden fluctuations in leaf count, providing a clearer picture of the plants' growth over time.

Figure 10 - Leaf count time series with 1-day mean rolling window

**Logical Correction: Non-Decreasing Leaf Count Rule**

One critical observation during the validation process was that, biologically, the number of leaves on a plant cannot decrease over time. However, due to segmentation errors or detection challenges, there were instances where the algorithm reported fewer leaves than had previously been detected. To address this, a logical rule was introduced to enforce **non-decreasing leaf counts** over time.

The rule applied was straightforward: if a higher leaf count was detected at an earlier time point, the algorithm would adjust any subsequent counts that were lower, ensuring that the leaf count could only remain constant or increase.

This adjustment helped align the detected leaf counts with the biological constraints of plant growth. By ensuring that the leaf count did not decrease over time, the modified time-series more accurately reflected the true growth patterns of the lettuce plants.

**Final Validation and Observations**

The combination of rolling median smoothing and the application of the non-decreasing leaf count rule significantly improved the accuracy and robustness of the leaf count data. After these adjustments, the time-series data provided a more reliable and biologically consistent representation of plant growth.

However, despite these improvements, some residual variance remained, particularly in early stages of plant growth when leaf detection is most challenging. Variability in lighting conditions, leaf occlusion, and segmentation accuracy still contributed to minor inaccuracies in certain images. Nonetheless, the overall performance of the algorithm was deemed satisfactory for the purposes of this experiment, with further improvements possible through more advanced segmentation methods or deep learning approaches.

## Plant surface calculation

In this section, we focus on the method used to calculate the surface area of each plant over time. The surface area of the plant is an important phenotypic trait that provides insights into the plant's overall health and growth potential. The approach used in this experiment was similar to the leaf detection method, relying on traditional image processing techniques with additional steps to convert the detected pixel area into real-world measurements.

### 5.3.1 Approach and Challenges

The surface area calculation followed a structured approach, combining color-based segmentation with pixel area conversion. The goal was to estimate the total area covered by the leaves in each image and convert that into a real-world unit (cm²) to track plant growth.

**Image Preprocessing and Segmentation**

Similar to the leaf detection process, the first step in calculating surface area involved converting the RGB image into the HSV (Hue, Saturation, Value) color space. This color space was chosen because it allows for a more precise segmentation of the green areas of the plant. The hue range for green was defined between 0.15 and 0.30, which helped isolate the leaves from other elements in the image, such as the background or soil. Additionally, the saturation and brightness ranges were set to filter out pixels that were too dull or too bright, further refining the mask used to detect leaves.

Once the green mask was applied, small, irrelevant objects (such as noise or tiny specks of green) were removed using morphological operations. This step ensured that only significant plant features were retained for the surface area calculation.

**Calculating Pixel-Based Surface Area**

The next step was to calculate the number of **green pixels** in the image, which corresponded to the detected leaf area. This was done by summing all the pixels in the cleaned binary mask (where green areas were represented by True values).

The total number of pixels in the image was also computed to determine the resolution of the image, which was later used to convert pixel measurements into real-world units.

**Pixel-to-Centimeter Conversion**

To translate the pixel count into an actual surface area, the size of the image frame was considered. The image frame, represented in centimeters, allowed for the conversion of pixel-based measurements into real-world units (cm²). This was achieved by calculating the area per pixel in the image, which was given by the formula:

Using this pixel area value, the surface area covered by the green pixels was calculated as follows:

**Accounting for Leaf Overlap and Changes Over Time**

One of the challenges in calculating surface area using 2D images is that leaves often overlap, resulting in an underestimation of the true surface area. To address this, a **leaf overlap factor** was introduced to adjust the surface area values based on the stage of plant growth. For example, during early growth stages, leaves are less likely to overlap, but as the plant matures and the canopy becomes denser, the algorithm was adjusted to account for potential overlap.

In addition, surface area calculations were tracked over time, allowing for adjustments based on changes in plant growth behavior as new leaves emerged and expanded.

### 5.3.2 Validation and comparison with leaf count

During the course of the experiment, it became evident that calculating the **surface area** of the plants, rather than focusing solely on leaf count, provided a more **robust and reliable metric** for analyzing plant growth over time. Unlike leaf counting, which faced significant challenges due to occlusion and the variance in leaf detection across different stages of growth, the surface area calculation consistently delivered more stable and biologically plausible results.

One of the key reasons for this reliability is that the surface area approach inherently accounts for the overall leaf coverage, rather than relying on detecting and counting individual leaves. As plants matured and their leaves began to overlap, the **leaf counting algorithm** struggled to maintain accuracy, resulting in high variation and even occasional undercounts as leaves became difficult to differentiate. This high variance in leaf count data necessitated additional post-processing, including smoothing techniques and logical rules to ensure non-decreasing leaf counts over time.

A graph with colored lines

Description automatically generatedIn contrast, the **surface area calculation** was far less susceptible to these issues. By focusing on the total number of green pixels detected in the image, the algorithm could more accurately reflect the plant's overall growth, even in cases of leaf overlap or partial occlusion. The pixel-based surface area measurement provided a more holistic view of the plant's size and growth trajectory, avoiding the fluctuations and inconsistencies that plagued the leaf count method.

Figure 11 - Time series of the plant surface in cm2

As a result, the surface area data proved to be a far more suitable input for **time-series analysis**. The surface area values exhibited a natural, smooth progression over time, with minimal variation and no need for the additional corrections or adjustments that were necessary for leaf count data. This smooth progression was particularly beneficial for modeling and predicting plant growth, as it allowed for more reliable trend detection and analysis without requiring post-processing to handle inconsistencies.

Moreover, because the surface area measurements remained stable throughout the experiment, there was no need to apply logical rules or validate the accuracy of the time-series as was done with leaf count data. The surface area metric consistently aligned with the expected growth patterns of the plants, making it a more **robust and straightforward** metric for further analysis and modeling.

In summary, the decision to focus on surface area calculation for time-series analysis was driven by its superior **reliability** and **robustness** compared to leaf counting. The surface area provided a consistent, biologically accurate measure of plant growth that required minimal post-processing, making it the preferred metric for analyzing and predicting plant development over time.

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