

浙江农林大学环境与资源学院

Research Report

**Deep Learning-Based Volume Estimation of Callus Tissue Using Image Analysis**

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**Abstract**

This project introduces a deep learning-based approach for estimating the volume of irregular biological structures, specifically callus tissue, using image analysis. By leveraging EfficientNet, a state-of-the-art convolutional neural network, the model enhances precision in volume estimation. The methodology involves capturing images from multiple angles and zoom levels while ensuring scale consistency. To train the model, surrogate materials such as sand or crushed stones are used, with ground truth volume measurements obtained through water displacement methods. The trained model is then adapted for callus tissue volume estimation. This system aims to provide a non-invasive, accurate, and automated solution for monitoring callus tissue growth, facilitating research in cellular biology and tissue engineering.

# ****Introduction****

The ability to accurately estimate the volume of irregularly shaped objects is essential in various scientific and industrial fields. Traditional methods, such as water displacement, are precise but impractical for large-scale or automated systems. This research explores the feasibility of deep learning-based volume estimation through image processing. The key contributions of this project include dataset creation, model training using EfficientNet, and validation against ground-truth measurements.

## Background of the Study

In cellular biology research, accurately measuring the volume of callus tissue is crucial for investigating its growth rate and simulating cell proliferation dynamics. However, due to the irregular morphology of callus tissue and the necessity to maintain it in sterile and sealed environments, direct volume measurement presents a significant challenge. Traditional methods, such as displacement techniques using liquid immersion, are impractical for in situ measurements. This limitation hinders real-time growth monitoring and comprehensive studies on cell culture dynamics.

Recent advancements in **artificial intelligence (AI) and computer vision** offer a promising alternative. By leveraging deep learning models—specifically, **pretrained convolutional neural networks (CNNs) such as EfficientNet or ResNet**—it is possible to develop a system that estimates the volume of callus cell clusters based on images. This approach allows for non-invasive, real-time volume estimation, addressing the limitations of conventional methods and providing a scalable solution for biological research.

## ****Research Objectives****

The primary objective of this study is to develop a **machine learning-based system** capable of accurately estimating the volume of callus cell clusters from images, thereby facilitating the investigation of their growth patterns and enabling the generation of callus growth curves.

**Specific objectives include:**

* **Simulating Callus Tissue for Model Training:** Since callus tissue must remain in a sterile environment, obtaining a large dataset of labeled images is challenging. Thus, the first step involves simulating callus structures using **sand or crushed stones**, whose volume can be accurately measured using a graduated cylinder and water displacement.
* **Dataset Collection & Annotation:** By manually altering the shape of the sand or stone clusters and capturing images alongside reference objects of known volume (such as cubes and spheres), a diverse dataset can be created for model training.
* **Model Training & Optimization:** Implement a deep learning-based volume estimation model trained on the generated dataset, utilizing CNN architectures such as EfficientNet or ResNet. The model should learn to estimate the volume of irregular objects based on photographic data.
* **Application to Callus Tissue Analysis:** Once the model achieves sufficient accuracy in volume estimation, it will be adapted and applied to **in situ** callus tissue images, enabling non-invasive volume monitoring in controlled biological environments.

## ****Challenges and Proposed Solutions****

The primary challenges associated with this research include:

* **Sterile Environment Constraints:** Callus tissue must be maintained in a sealed, sterile environment, making direct measurement difficult.
  + **Solution:** Use a proxy dataset (sand/crushed stones) to train the model before applying it to real biological samples.
* **Irregular Morphology:** Unlike geometric objects, callus tissue has an **irregular** and constantly evolving shape.
  + **Solution:** Introduce a variety of irregular training samples to improve model generalization.
* **Scaling and Magnification Issues:** Differences in imaging conditions (camera distance, zoom, lens specifications) can affect volume predictions.
  + **Solution:** Incorporate **image scaling factors** and reference objects in training to standardize predictions across different setups.

## ****Significance of the Study****

This study introduces a novel **AI-driven methodology** for non-invasive volume estimation in cell biology research. By replacing conventional measurement techniques with an automated image-based approach, the proposed model offers a **scalable, efficient, and real-time** solution for monitoring callus tissue growth. This advancement not only **improves experimental efficiency** but also has potential applications in areas such as **tissue engineering, regenerative medicine, and bioprocess monitoring**.

# Background

### Callus Tissue and Its Importance in Cellular Biology

Callus tissue refers to an undifferentiated mass of plant cells that forms in response to wounding or specific in vitro culture conditions. It plays a crucial role in **plant tissue culture, genetic modification, and regenerative studies**. The ability to monitor callus growth over time is essential for **understanding cellular proliferation, optimizing culture conditions, and evaluating the effects of external stimuli** on plant development.

Traditional methods for **measuring callus growth** rely on **dry weight measurements or volume displacement techniques**, both of which are invasive and impractical for continuous monitoring. The need for **a non-invasive, real-time method** for estimating callus volume has driven interest in **machine learning-based approaches** that leverage **image analysis and deep learning** for volume prediction.

### ****Challenges in Volume Measurement of Callus Tissue****

Measuring the volume of callus tissue presents several **unique challenges**:

* **Irregular Morphology:** Unlike geometric objects, callus tissue has an **amorphous and constantly changing structure**, making precise volume estimation difficult.
* **Sterile and Sealed Growth Conditions:** Callus tissue is typically cultured in a **sealed, aseptic environment**, preventing the use of direct measurement techniques such as displacement methods.
* **Lack of a Standardized Measurement Protocol:** No widely accepted protocol exists for estimating callus volume **non-invasively**, leading to inconsistencies in research methodologies.

Given these challenges, a novel approach based on **computer vision and deep learning** is proposed to **estimate callus volume from images**, enabling researchers to track growth dynamically without disrupting sterile conditions.

### ****Advances in Deep Learning for Volume Estimation****

With the rise of **deep learning and convolutional neural networks (CNNs)**, AI models have demonstrated exceptional capabilities in **object detection, segmentation, and volumetric estimation**. **Pretrained CNN models such as EfficientNet and ResNet** have been successfully applied in medical imaging and industrial applications, proving their reliability in analyzing complex visual data.

Some key advancements in the field include:

* **Computer Vision in Biology:** AI-driven **image analysis** has been widely adopted in **cell counting, segmentation, and morphological analysis**, proving its potential for biological research.
* **3D Volume Estimation Using 2D Images:** Deep learning models have been developed to predict **3D structures from 2D images**, using techniques such as **feature extraction, depth estimation, and regression-based volume prediction**.
* **Scalability and Transfer Learning:** Pretrained models can be **fine-tuned** for specific applications, allowing researchers to develop high-accuracy models even with **limited datasets**.

These advancements provide a strong foundation for the current study, which aims to apply **CNN-based models to estimate callus volume using image data**, thereby overcoming the limitations of traditional measurement methods.

### ****Justification for Using CNNs in Volume Estimation****

Compared to traditional **image processing techniques** (e.g., thresholding, edge detection), CNN-based models offer several advantages:

* **Automated Feature Extraction:** CNNs learn to extract relevant visual features without requiring **manual image preprocessing**.
* **High Accuracy and Robustness:** Deep learning models can generalize across different lighting conditions, backgrounds, and imaging setups.
* **Scalability and Adaptability:** The model can be trained on **proxy datasets (e.g., sand or crushed stones)** before being fine-tuned for **real callus tissue**.

By leveraging **deep learning, standardized image acquisition protocols, and scale correction techniques**, this research aims to establish an **accurate and non-invasive** method for measuring callus tissue volume, contributing to advancements in **plant biology and tissue culture research**.

# ****Methodology****

## ****3.1 Introduction****

This chapter outlines the methodology used in this research to develop a machine learning-based approach for estimating the volume of callus tissue from images. The study follows a structured workflow that integrates **data collection, preprocessing, model training, evaluation, and deployment** to create a reliable system for non-invasive volume measurement. Given the constraints of working with sterile environments, an alternative **synthetic dataset** approach is employed initially to facilitate model training before transitioning to real biological samples.

## ****3.2 Research Design****

This research adopts an **experimental and computational approach** combining **deep learning, image processing, and statistical analysis** to develop an AI-assisted volume estimation system.

The study is divided into five key phases:

1. **Data Collection:** Acquisition of training and testing images under controlled conditions.
2. **Data Preprocessing:** Image normalization, augmentation, and metadata extraction.
3. **Model Training:** Using a pre-trained **EfficientNet-B0** architecture for volume regression.
4. **Model Evaluation:** Assessing accuracy through standard machine learning performance metrics.
5. **Deployment & Validation:** Implementing a real-world testing environment for volume prediction.

## ****3.3 Data Collection****

Since callus tissue must be cultivated in a **sterile and enclosed environment**, direct data acquisition presents challenges. To overcome this limitation, a two-stage data collection method is implemented.

### ****3.3.1 Synthetic Data Generation Using Sand and Gravel****

To create a training dataset, **sand or small stones** are used as a **proxy** for callus tissue due to their irregular shapes. The following process is employed:

* **Volume Measurement:** The true volume of each sample is determined using the **water displacement method** with a **graduated cylinder**.
* **Image Acquisition:** Each sample is photographed under **controlled conditions**, ensuring:
  + **Fixed camera-to-object distance**
  + **Consistent zoom settings (measured in millimeters)**
  + **Uniform lighting conditions**
  + **Reference objects (e.g., a known-volume cube or sphere) for scale calibration**
* **Metadata Recording:** Each image is labeled with its corresponding **actual volume, zoom level (mm), and scale information**.

### ****3.3.2 Real Callus Tissue Image Collection****

After training the model on synthetic data, real callus tissue images are collected following the same structured approach:

* Images are captured in **sterile conditions** without disrupting the growth environment.
* The model is tested on these images to evaluate its ability to generalize from synthetic to real-world data.

## 3.4 Data Preprocessing

To ensure high model accuracy and generalization, all collected images undergo a rigorous preprocessing pipeline before being fed into the neural network.

The key steps include:

#### ****Image Preprocessing****

* **Resolution Standardization**: All images are resized to **224×224 pixels**, as required by **EfficientNet-B0** to maintain consistency in input dimensions.
* **Normalization**: Pixel values are normalized using **mean = [0.485, 0.456, 0.406]** and **standard deviation = [0.229, 0.224, 0.225]**, aligning with standard preprocessing for pretrained convolutional neural networks.

#### ****Data Augmentation****

To enhance model robustness and prevent overfitting, various augmentation techniques are applied, including:

* **Random Rotations**: Introducing slight variations in angle to simulate different perspectives.
* **Horizontal and Vertical Flipping**: Enhancing model adaptability to positional variations.
* **Brightness Adjustments**: Simulating different lighting conditions for improved generalization.
* **Scaling Perturbations**: Introducing slight resizing to account for minor variations in object distance.

#### ****Metadata Processing****

To maintain data integrity and facilitate structured learning, all relevant metadata is stored in a CSV file, including:

* **Image Filename**: Unique identifier for each image.
* **Zoom Level (mm)**: The focal length used during image capture, ensuring proper scale interpretation.
* **True Volume (ml)**: The actual measured volume of the sample, serving as the ground truth for model training.

This preprocessing strategy ensures that the dataset remains diverse, well-structured, and optimized for training an accurate volume estimation model.

# Results and Discusion

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# Conclusion

This study successfully demonstrates the feasibility of estimating the volume of callus tissue using deep learning models trained on images. By leveraging **EfficientNet-B0**, we developed a machine learning model capable of predicting volume based on images with high accuracy. The research follows a structured methodology, beginning with the collection of training data using granular materials (such as sand and small stones) to simulate callus tissue, incorporating known volume references, and progressively refining the model through supervised learning techniques.

The results indicate that the proposed approach can provide reliable volume estimations, overcoming the challenge of irregular callus shapes and sterile cultivation environments where direct measurement is impractical. The integration of **image preprocessing techniques, metadata utilization (zoom level and scale factor), and data augmentation** played a crucial role in enhancing model robustness and generalization.

Despite these promising results, some limitations remain. The model's performance may vary when tested on actual callus tissue due to differences in texture, transparency, or structure compared to the training materials. Additionally, variations in lighting conditions and camera settings can introduce noise into the estimation process.

For future research, it would be beneficial to:

1. **Expand the dataset** by incorporating real callus tissue images labeled with precise volume measurements.
2. **Implement transfer learning techniques** using biomedical imaging datasets to improve feature extraction for biological materials.
3. **Enhance model interpretability** by exploring explainable AI methods to understand how the network correlates image features with volume.
4. **Develop a real-time application** that automates volume estimation in sterile laboratory settings, facilitating non-invasive measurement techniques for biological research.

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