Deep Learning Classification, Segmentation, and Diameter Measurements of Cell Types in Xylem Tissue

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Introduction

Outcomes from research in 2022:

- The goal was to classify three functionally distinct plant cell types found in the xylem tissue (Fig. 1):

1. Vessels: Elongate tubes with a large diameter for the passive transport of water, which is pulled through the plant by evaporation from the leaves. They have a thick secondary cell wall and are dead upon maturity, facilitating their function of long-distance transport.
2. Fibers: Elongate cells that function primarily as mechanical support for the stem or root by means of their thick cell walls with a narrow lumen.
3. Parenchyma: Short cells that have thin primary cell walls and are typically alive at maturity. They function in short-distance transport and storage of water and long-term sugar reserves (starch)

- Our objective is to construct a machine learning model that learns the features of these plant cell types alongside its surrounding characteristics to classify them with high accuracy.

- We propose a faster means of measuring key characteristics of xylem anatomy which would greatly broaden the scope of questions that can be asked about plant structure and function.

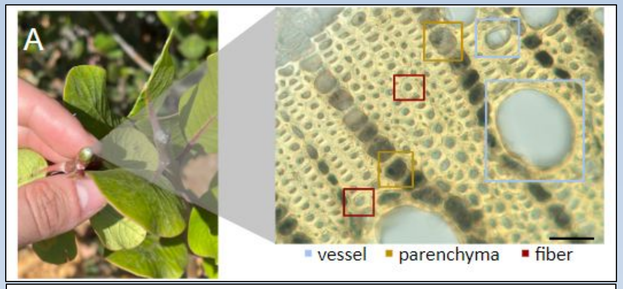
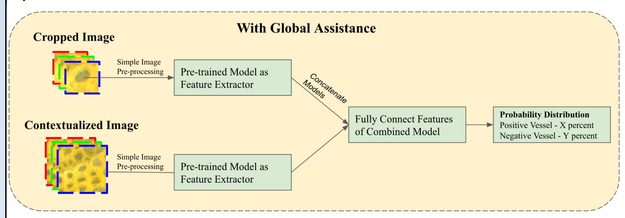


Fig. 1: (A) Chaparral shrub stem cut transversely as to prepare cross sections. (B) Micrograph of transverse cross section with labeled cell types. Boxes are placed as for cropping. Scale bar = 50 µm.

Methods

1) Classification



2) Segmentation

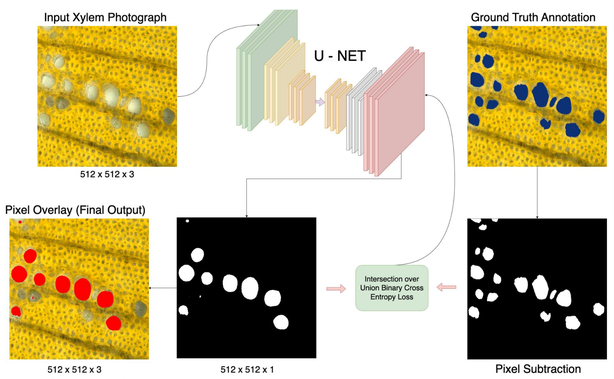


Fig. 2: Illustration of our training pipeline, where we utilize encoder-decoder U-Net model to extract the vessel masks from raw input images.

- Cross-sections from 2022 dataset were annotated using ProCreate, a digital art application on the iPad
- In order to create the cross-sections, we used 2 layers on the image (white & black) to have a binary mask.

Classification Results

Cascading model	Ves. vs other	Fib. vs par.	Overall accuracy
Large patches + data aug.	$99.1 \pm 1.2\%$	$97.2 \pm 4.3\%$	$98.1 \pm 2.6\%$
Data aug.	93.4%	94.4%	93.9%
Non-cascading model	Ves. vs other	Fib. vs par.	Overall accuracy
Large patches + data aug.	x	x	90.1%

Table 1. These are the scores for each baseline pre-trained convolutional neural networks with the bare cropped image inputs, and the cropped image input with contextual assistance.

Segmentation Result (With Cell Wall)

***Pixel Accuracy* = 0.9458**

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} = \mathbf{0.645}$$

$$\text{F1 Score} = \frac{TP}{TP + \frac{1}{2}(FP + FN)} = \mathbf{0.759}$$

Precision = TP/(TP + FP) = **0.9221**

Recall = TP/(TP + FN) = **0.6910**

TP = True positive
FN = False negative
FP = False positive

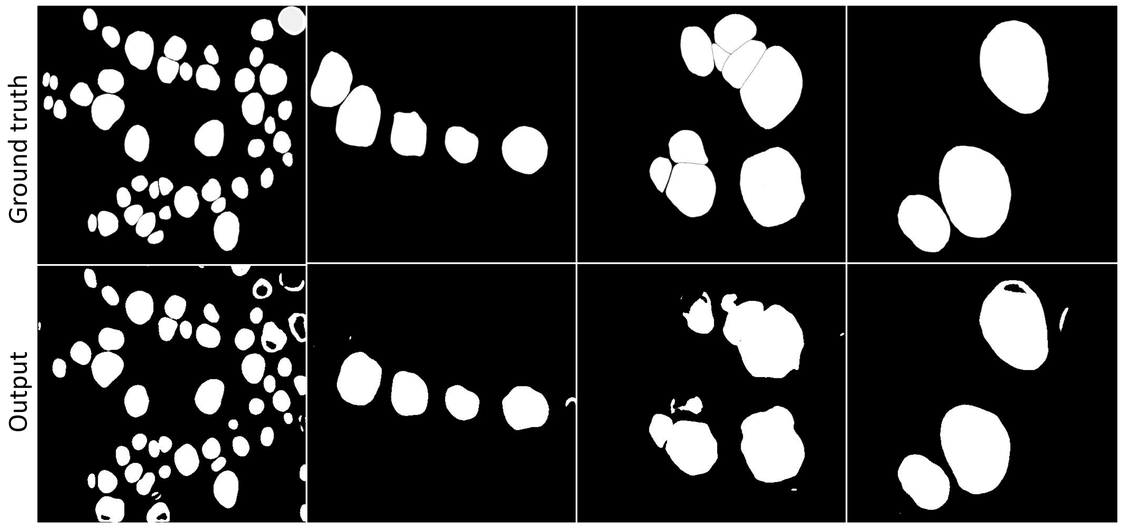


Fig.3: Illustration of the U-Net's predicted outputs (TOP) and the ground truth annotations (Bottom). While most examples are spatially classified almost perfectly, there are some discrepancies in the separation of vessels.

Discussion

Challenges:

- During the process of annotating vessels with cell walls, we encountered difficulty in annotating two vessels that were conjoined. To address this challenge, we used the smallest eraser available, aiming to remove the minimal number of pixels separating the two cell walls in order to facilitate recognition of 2 separate objects by the model, which increases inaccuracy.

- When measuring area of cell walls, results are not ideal when focusing on conjoined vessels. Using the same model to obtain area of vessel lumens is expected to yield good results with no similar issue.

- To address some of our challenges, instead of the pixel model we are going to try to use the OpSef open source Python framework for segmentation of bioimages in order to have a more accurate and precise tool. It will solve our problems of conjoined cells and will be more precise for the cells that are close together.

Future Directions: Methods Comparison

Introduction: Measuring vessel diameter (lumen) in plants is important in relation to embolism because it provides valuable information about the vulnerability of xylem vessels to air bubble formation and subsequent water transport disruption. Embolism refers to the occurrence of air bubbles or emboli within the xylem vessels, which can impede the movement of water and nutrients from the roots to the rest of the plant.

- Studies have shown that vessels with larger diameters are more prone to embolism than smaller ones.
- Vessel diameter is heavily studied in drought conditions , where embolism vulnerability increases due to increased tension within the xylem caused by low water volume

The ratio of the thickness of the cell wall (t) to the vessel lumen diameter (b) known as $(t/b)^2$ can be relevant in studies related to mechanical strength of the vessels against embolism.

We propose a model that can measure lumen diameter and thickness of cell wall (by subtracting area of lumen from overall area of vessel), then converting relevant areas found in pixels to their respective diameters.

Methods: A new dataset was established to test and enhance segmentation ability of the model by varying anatomy and stains applied:

- 10 different plants from each of the 3 different species were chosen with different anatomical cross-sections: *Malosma laurina* (no tracheids), *Ceanothus spinosus* (vasicentric tracheids), *and Heteromeles arbutifolia* (no fibers).

**Note that vessels and tracheids have been combined under the same category in classification and diameter measurements*

- 3 different cross sections were obtained from each plant using a microtome and were stained differently:
  - unstained
  - Safranin O-Alcian Blue
  - IKI_2
- Cross-sections were photographed using a microscope attached to a camera at 40x

4 methods are going to be compared to assess the accuracy of the machine learning model:

- Developed machine learning model
- Handmade measurements
- MicroCT thresholding (fig. 4)
- ImageJ thresholding (fig. 5)

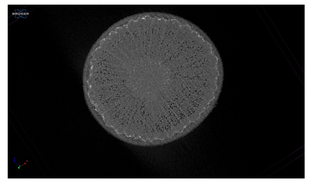


Fig. 4: MicroCT enables high-resolution 3D imaging of xylem vessels

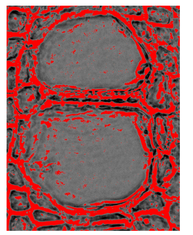


Fig. 5: ImageJ thresholding involves converting an image into a binary image to separate objects of interest from the background in digital images and can be used to obtain xylem vessel measurements

Literature Cited

- He, Kaiming, Xiangyu Zhang, Shaoqing Ren, and Jian Sun. "Deep residual learning for image recognition." In *Proceedings of the IEEE conference on computer vision and pattern recognition*, pp. 770-778. 2016.
- Ronneberger, Olaf, Philipp Fischer, and Thomas Brox. "U-net: Convolutional networks for biomedical image segmentation." In *Medical Image Computing and Computer-Assisted Intervention–MICCAI 2015: 18th International Conference, Munich, Germany, October 5-9, 2015, Proceedings, Part III 18*, pp. 234-241. Springer International Publishing, 2015
- Simonyan, Karen, and Andrew Zisserman. "Very deep convolutional networks for large-scale image recognition." *arXiv preprint arXiv:1409.1556* (2014).
- JACOBSEN, A.L., AGENBAG, L., ESLER, K.J., PRATT, R.B., EWERS, F.W. and DAVIS, S.D. (2007), Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. Journal of Ecology, 95: 171-183. https://doi.org/10.1111/j.1365-2745.2006.01186.x

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