A Deep Learning Approach to Automate the Analysis and Prediction of Function for Poplar Stomata

Connor McKeown, Philip Gillett, Katherine E. McCallum, Dr. Nora Mitchell, Dr. Rahul Gomes
Department of Computer Science; Department of Biology

University of Wisconsin Eau Claire

Introduction

Stomatal Functioning

Understanding the changes in climate across large regions is necessary to understand how plants adapt to different conditions. The characteristics of plant stomata, which are small pores whose main function is regulating water loss and gas exchange, tell us important information about how plants respond to their environment. Poplars are an emerging model system for studying adaptation to climate change, including stomatal functioning.[1] Previously, biologists gathered information about stomata by individually measuring microscope images by hand.

Automated Morphometry

Manual morphometry has some significant limitations, most notably the lengthy amount of time it takes and inconsistent accuracy. Automated Morphometry aims to use deep learning to provide fast and accurate morphometry. Stomata images are represented as a 2D matrix of values, that represent either the pore, guard cell, or a value that is neither. From here, the numbers of pixels under each value is computed, and then the data on height, width, and area of each stoma is calculated.[2]

Project Goals

In our study, we sought to automate the collection of stomata data using machine learning to optimize this process and make it more repeatable.

Figure 1. Poplar common garden in Eau Claire, Wisconsin, containing two blocks of 47 different *Populus trichocarpa* / *P. balsamifera* genotypes collected across Canada and Alaska.

Biological Samples

One leaf per plant was harvested in July 2022 and stomatal impressions were taken using New Skin adhesive bandage and lifted off the leaf with tape. Raw images of each imprint were taken using a compound light microscope at 40× magnification in two haphazardly chosen areas of 0.069mm^2 each.

Figure 2. Setup for taking stomatal leaf impressions from poplar samples using New Skin adhesive bandage.

Materials and Methods

Dataset for training

A dataset of 140 (1600px by 1200px) RGB images of stomatal impressions were collected. To train the deep learning model, manual segmentation masks were created with one color corresponding to each part of the image: pore, guard, or background. For preliminary results only 12 images were masked and used for training as this is a highly time-consuming task. Segmentation masks were done using two different open-source graphics editors: paint.net (Windows) and GIMP (Mac). Each region of interest was outlined by hand and marked with the proper color.

While training, images must be a manageable size. Each image and mask was sliced into 256px-by-256px images with 50% overlap. This produced 108 images for each original image and mask producing a 1296 images and 1296 masks, each of size 256px by 256px. The dataset was read into a NumPy array and split for training into 80% for training and 20% for testing.

Experimental set-up

Tensorflow, Keras, and Scikit learn were used for building the U-Net model and analysis of results. The preliminary model was trained using Google Colab free GPU resources. In order to provide variation in input data, images were randomly augmented and cropped. While maintaining it's data, this allows for the model to artificially see a larger variety in the original dataset.

Deep Learning Algorithm

A U-Net model with five encoding and five decoding layers trained for 100 epochs was implemented. A kernel size of 3x3, dropouts of .07 and max pooling were applied. The optimizer used was Adam and the loss function was sparse categorical cross entropy. With segmentation, accuracy values are typically high due to lots of correctly predicted background space. To represent the accuracy of our model we use the IoU value to give a better indication of the predictions

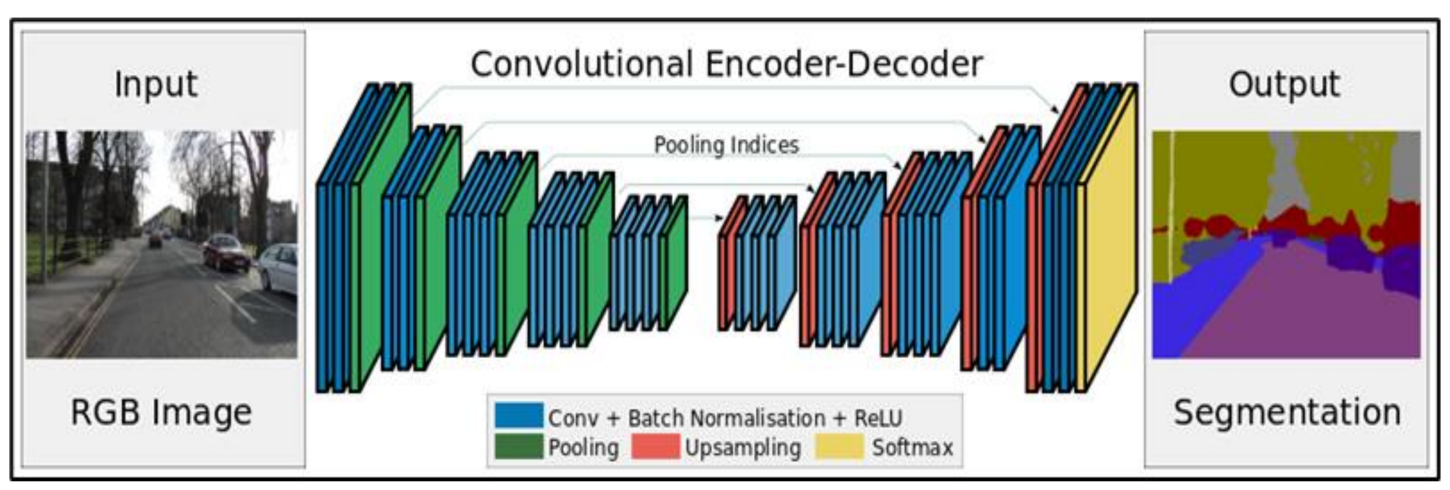


Figure 3. Visual representation of how Semantic Segmentation using U-Net architecture is done using Deep Learning. Image adapted from [3]

Results

Pore	0.9933
Background	0.8990
Guard	0.7861

Table 1. Jaccard score comparison between true masks and predicted masks. A score closer to one indicates a higher similarity.

	Training	Validation
Accuracy	0.9932	0.9889
Loss	0.0054	0.0303
Mean IoU	0.9082	0.8831

Table 2. Accuracy, Loss and Mean IoU data. Accuracy is a biased metric when doing a segmentation problem and produces a high value, hence the use of the Jaccard score in Table 1.

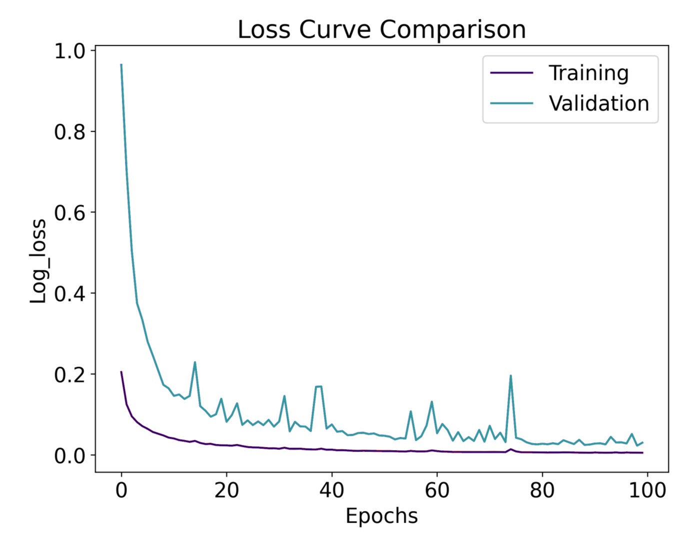


Figure 4. The loss comparison represents how bad a model's prediction is. A downward trend represents a model that is learning.

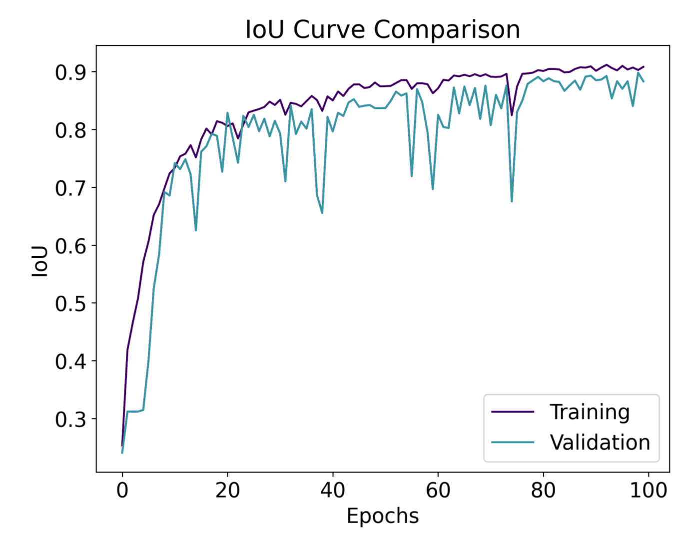
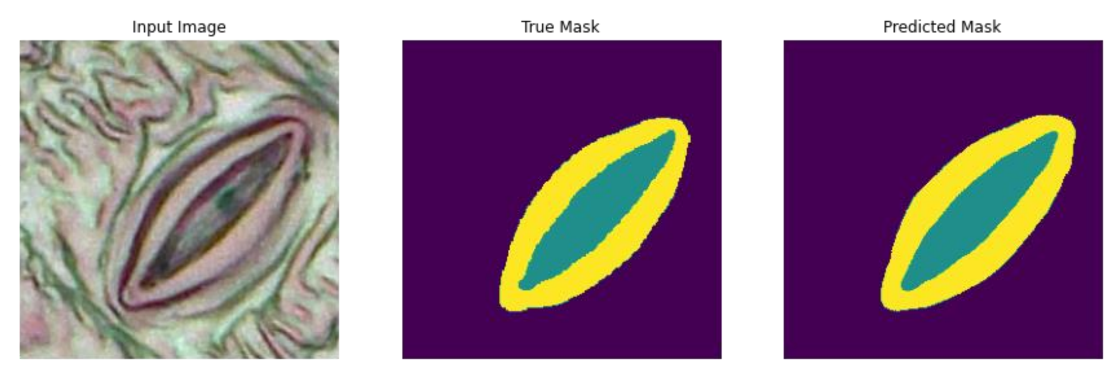


Figure 5. The intersection over union comparison between measures the accuracy of predicted segmentation masks.

Figure 7. An example of an exemplary mask prediction.



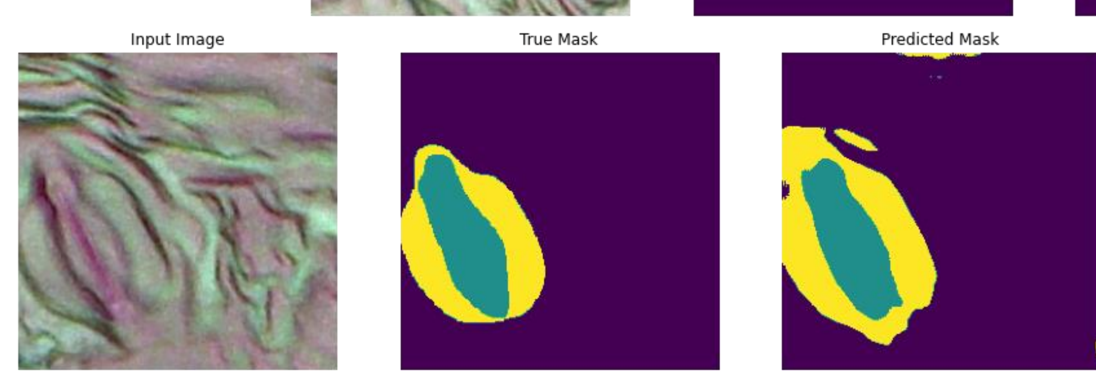


Figure 8. An example of an imperfect predicted mask.

Conclusions and Future Work

Major conclusions plus future directions, bring it back out to BIG PICTURE, see the ORSP proposal
You can also talk about object detection
^we plan to test additional parameter modifications?
We plan to use our work to help study the actual gas exchange of the stomata on the physiological level. Our U-Net model will help us efficiently process data for future and more complex stomatal analysis.

References

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2. Gibbs, Jonathon A., et al. "A Deep Learning Method for Fully Automatic Stomatal Morphometry and Maximal Conductance Estimation." *Frontiers in Plant Science* 12 (2021): 780180.
3. Badrinarayanan, Vijay, Ankur Handa, and Roberto Cipolla. "Segnet: A deep convolutional encoder-decoder architecture for robust semantic pixel-wise labelling." *arXiv preprint arXiv:1505.07293* (2015).

We thank the Office of Research and sponsored Programs for supporting this research and Learning & Technology Services for printing this poster.