Accelerating Grapevine Stomatal Assay: From Sample Collection to Stomatal Pore Measurement

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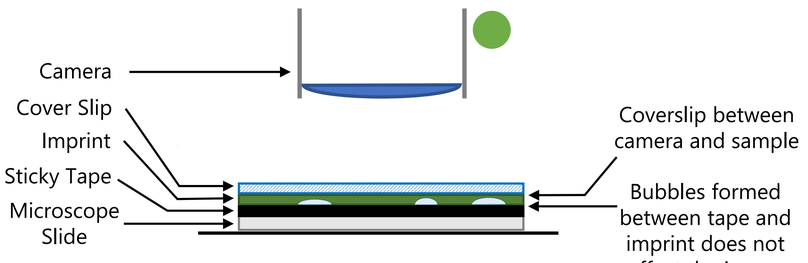
Why should we care about stomata?

The size and density of stomata are important indicators of grapevine health. Analysis of stomata using microscope images of grapevine leaf epidermis provides scientists with key information regarding the health of the plant and the surrounding environmental conditions. Accelerating this process allows stakeholders to make informed decisions in a rapid manner, paving the way to better yields and profit. In this work, improvements are introduced to sample collection, sample imaging, and stomata detection and measurement stages.

Proposed Solution

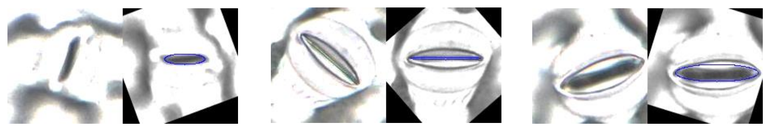
Contribution #1
Digital microscope slide scanners widely used in biomedical applications can be adopted to image large areas of leaf epidermis at once, saving both time and effort.

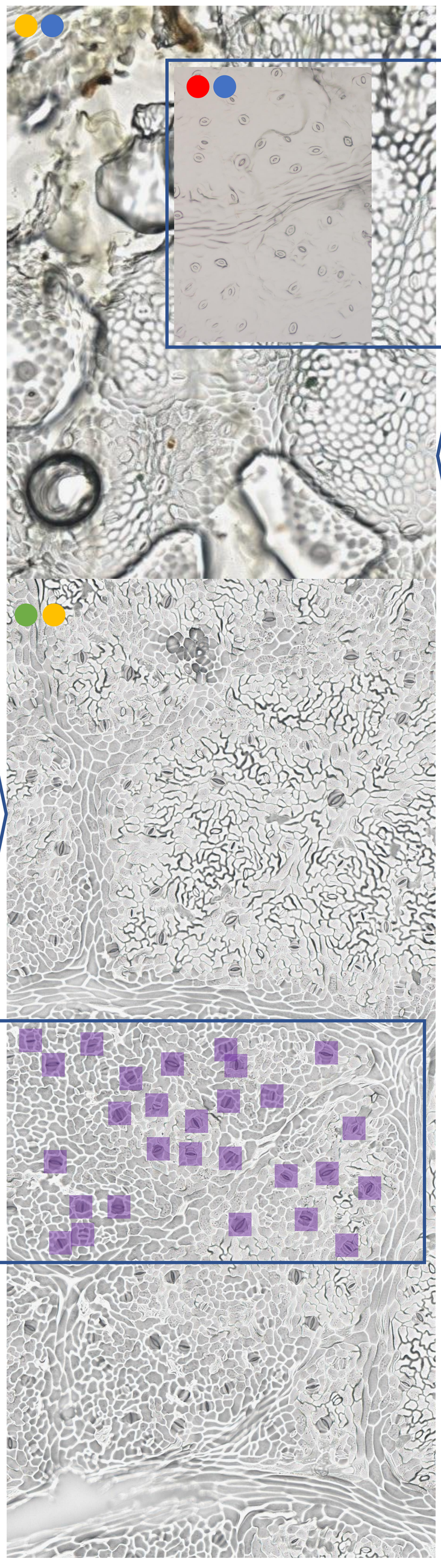
Contribution #2
The proposed modified nail polish imprint method introduces an additional coverslip which removes any unevenness of the leaf sample. The sample is also turned so that the bubbles are formed beneath the imprint.



Contribution #3
A Convolutional Neural Network (CNN) based on the pre-trained AlexNet network is deployed to automatically identify stomata in microscope images. Transfer learning allows the network to be trained with limit microscope data.

Contribution #4
A segmentation based novel stomatal cross section analysis method calculates stomatal pore area. The proposed pore estimation method is robust to stomata pore intensity.

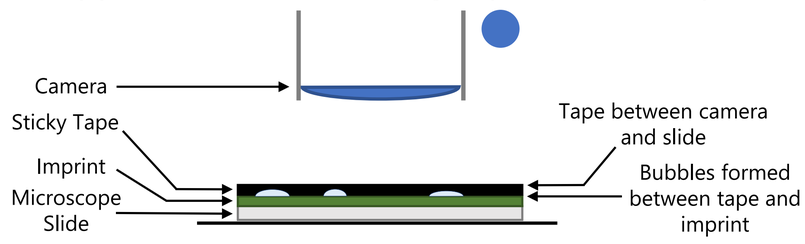




Problem

Problem #1
The traditional approach of using a manual optical microscope does not allow researchers to image a large leaf area at once.

Problem #2
The common nail polish imprint method widely used to collect leaf epidermis samples can result in low quality data due to air bubbles being trapped between the sticky tape and the imprint



Legend

- Manual Optical Microscope
- Digital Microscope Slide Scanner
- Common Nail Polish Imprint Method
- Modified Nail Polish Imprint Method

Results

Table 1: Performance of the modified nail polish imprint method and digital slide scanning

	Original nail polish imprint method with manual imaging	Modified nail polish imprint method with slide scanner
Sample collection time	8-10 minutes	**10 minutes**
Time taken to image section of sample	2 hours (+ setup time)	**10 minutes**
Time taken to image entire sample	1 day (prediction) (+ setup time)	**30-40 minutes**
Level of manual operation required	High	**Low**
Quality of images	Moderate	**High**

Table 2: Performance of AlexNet CNN based stomata detector

Number of Stomata	Accuracy	Precision	Recall	F1 score
1012	97.01	73.25	82.39	0.77

Table 3: Performance of segmentation based stomata pore estimation method

Number of Stomata Images	Number of Stomata Rejected	Accurate Pore Estimations	Inaccurate Pore Estimations	Pore Estimation Accuracy
456	103	281	72	79.6 %

Related Publications

Jayakody H, Liu S, Whitty M, Petrie P. **Microscope image based fully automated stomata detection and pore measurement method for grapevines**. *Plant Methods*. 2017;13:94. Published 2017 Nov 8. doi:10.1186/s13007-017-0244-9

Project Partners

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