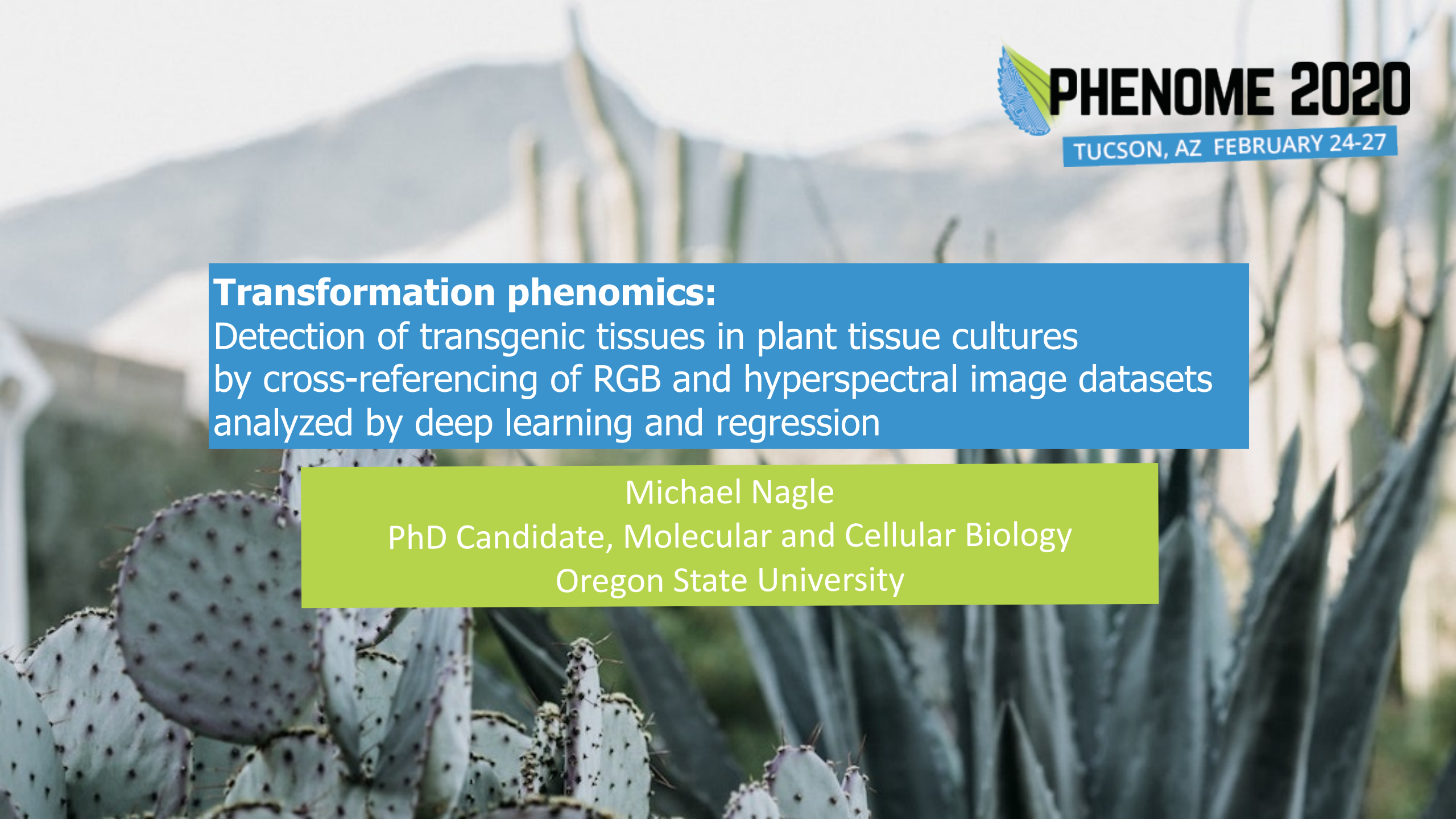
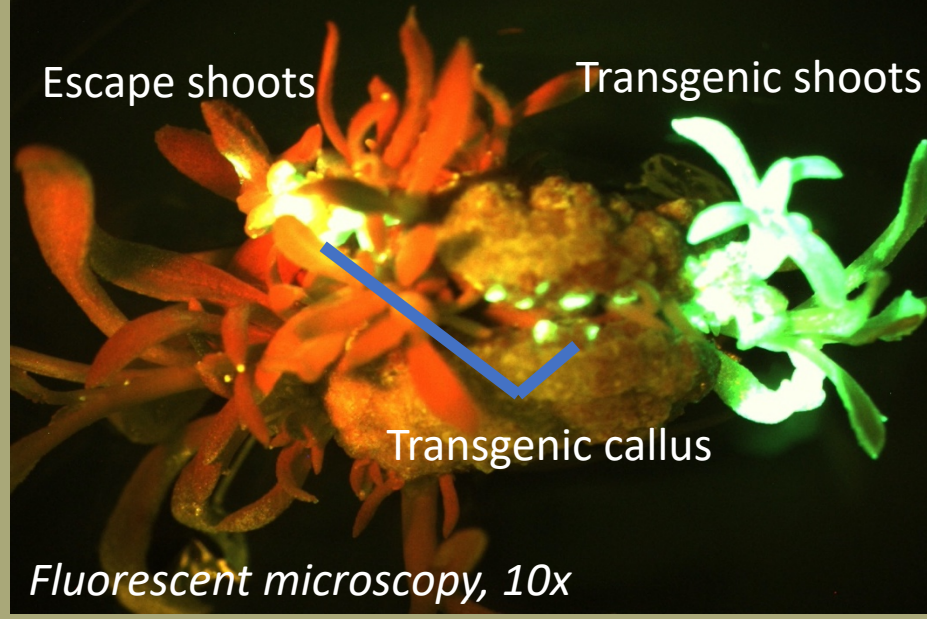


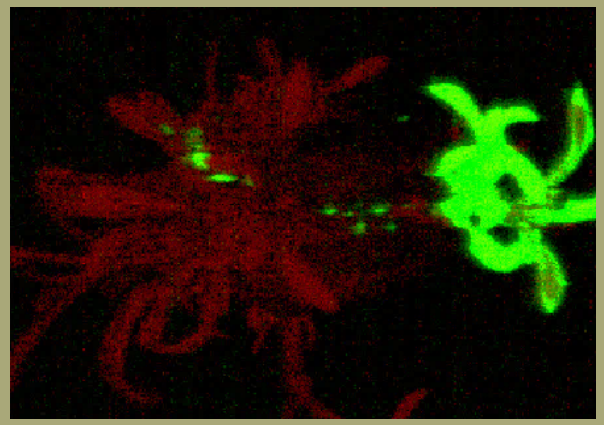
Transformation phenomics:
Detection of transgenic tissues in plant tissue cultures by cross-referencing of RGB and hyperspectral image datasets analyzed by deep learning and regression

Michael Nagle
PhD Candidate, Molecular and Cellular Biology
Oregon State University

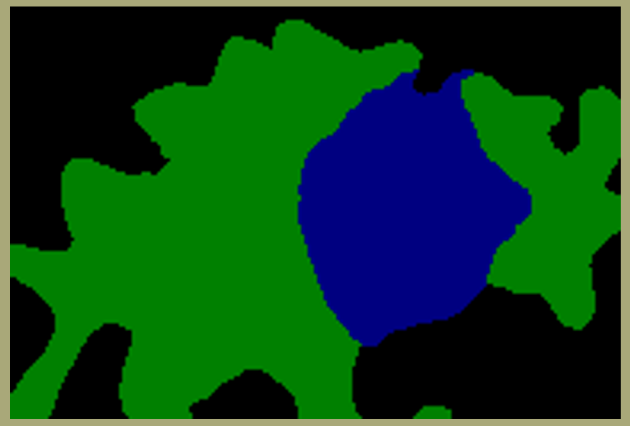
Presentation Overview

I. Introduction to phenotyping of regeneration and transformation (RT) and need for next-generation phenomics
II. Methods





Hyperspectral imaging



Deep segmentation

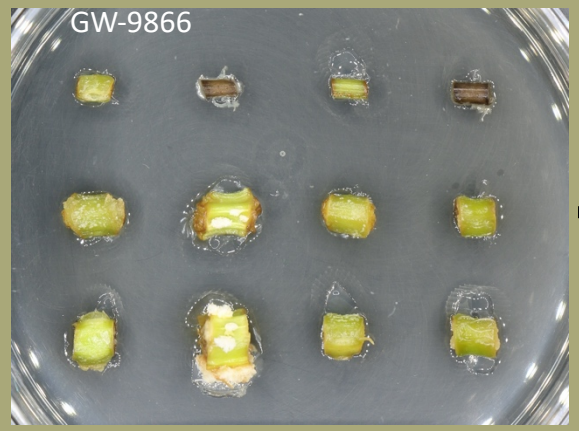
III. Example experiments
- GWAS of *in planta* regeneration
- Developmental genes to enhance transformation

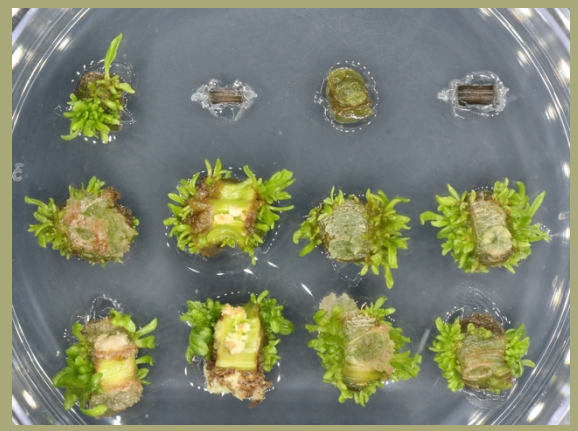
Plant regeneration and transformation traits
–critical to agricultural biotechnology, challenging to quantify

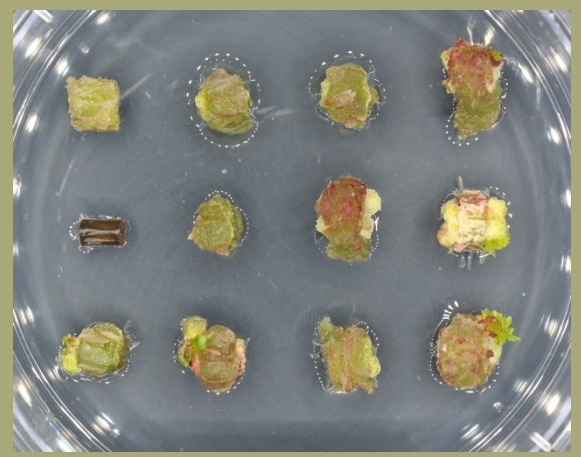
Callus induction

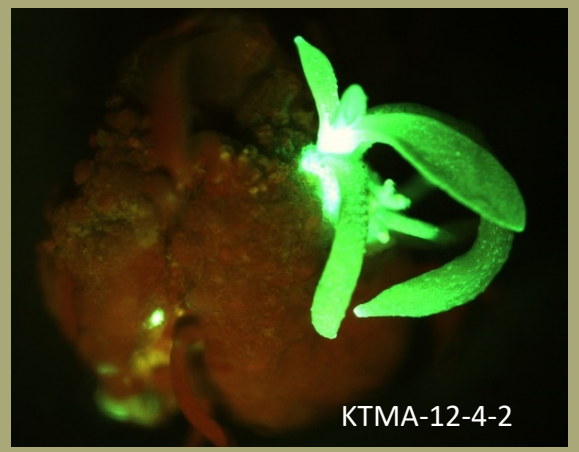
Shoot induction

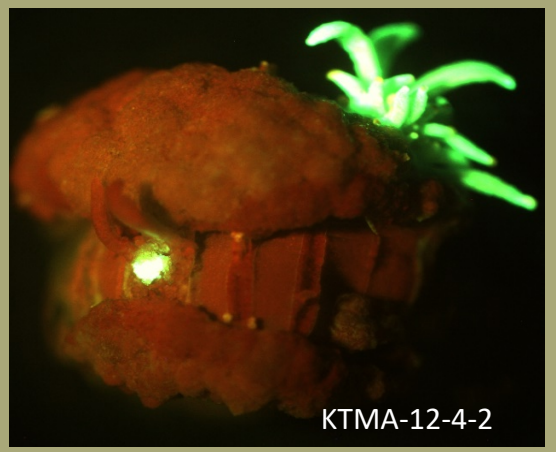
Transformation with GFP plasmid

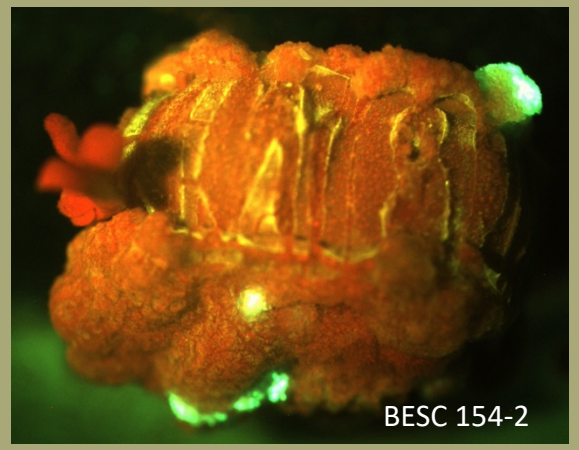


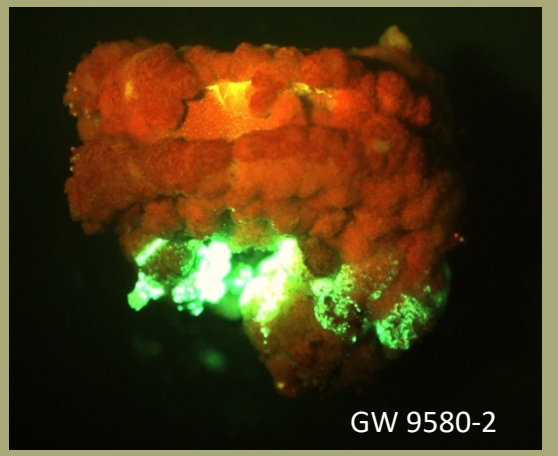










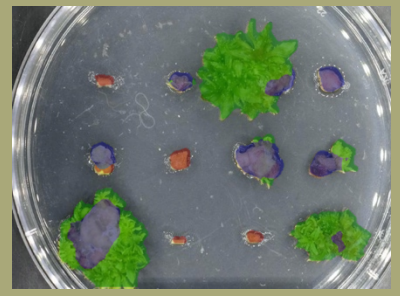


Overview of phenomics methods developed

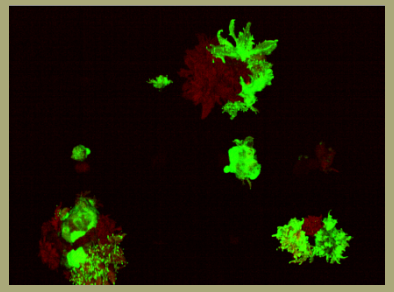
RGB image

Deep segmentation

Segmented image

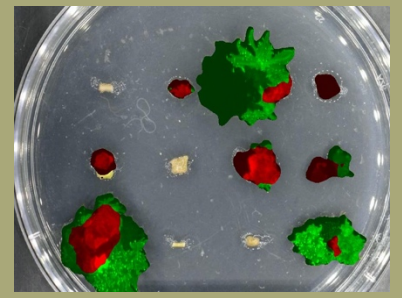


Hyperspectral image

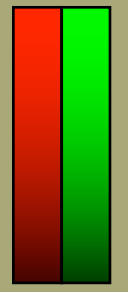


Regression

Protein fluorescence in specific tissues

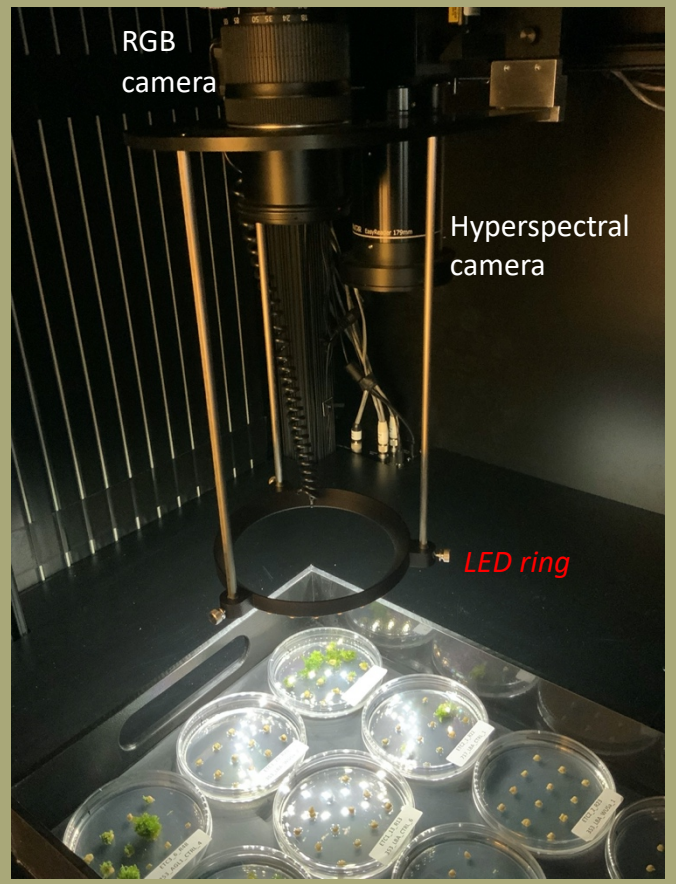


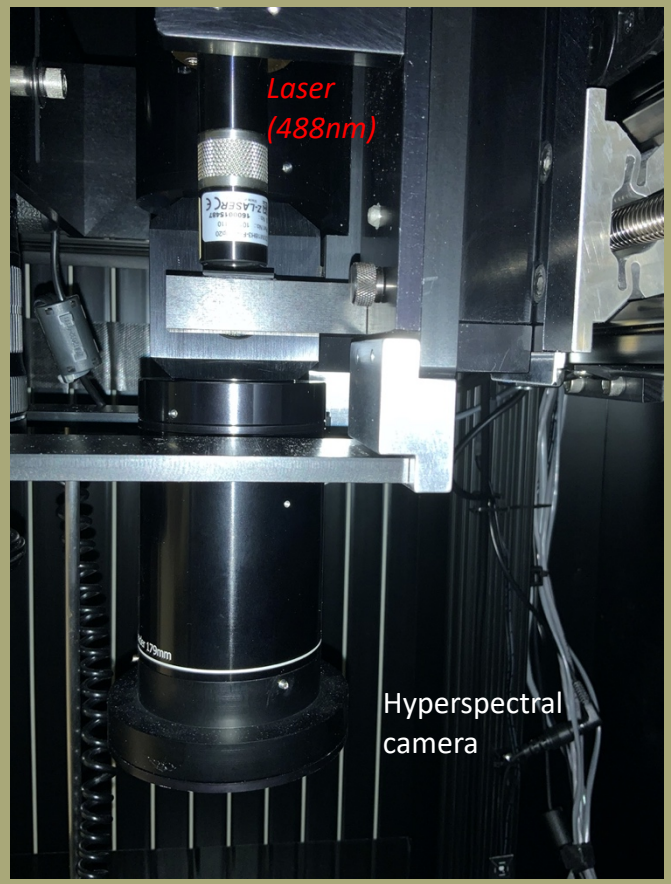
T-statistics for DsRed



Design and use of high-throughput imager of petri dishes

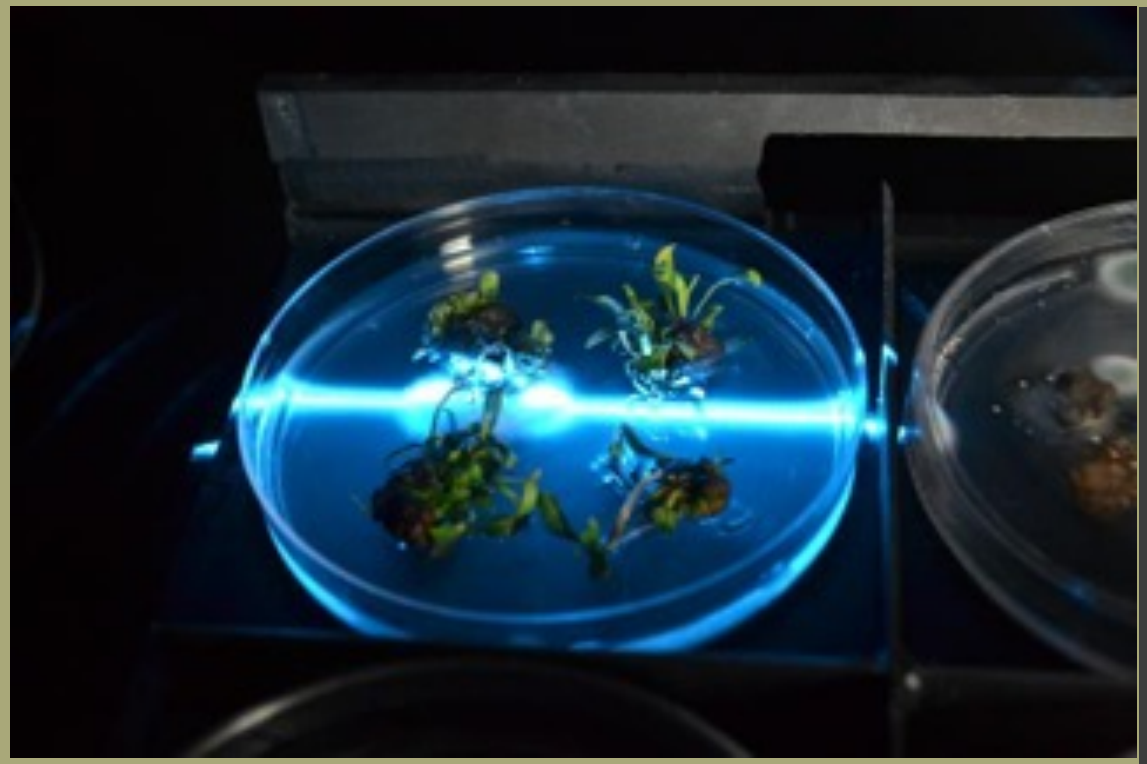
macroPhor Array (Middleton Spectral Vision)
Custom instrument for high-throughput RGB + hyperspectral imaging of petri dishes



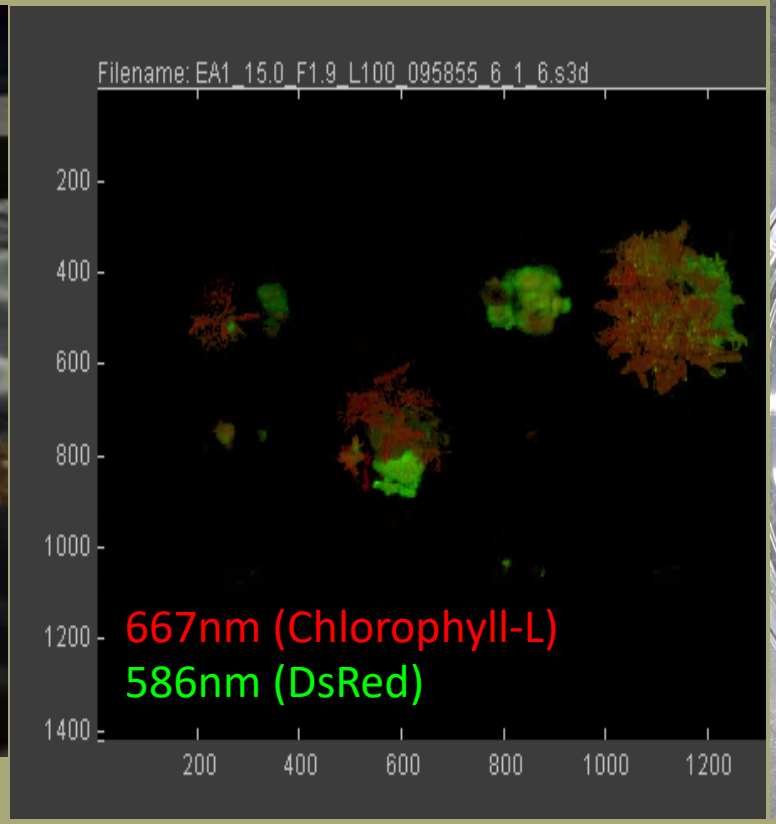


Collection of RGB and hyperspectral images

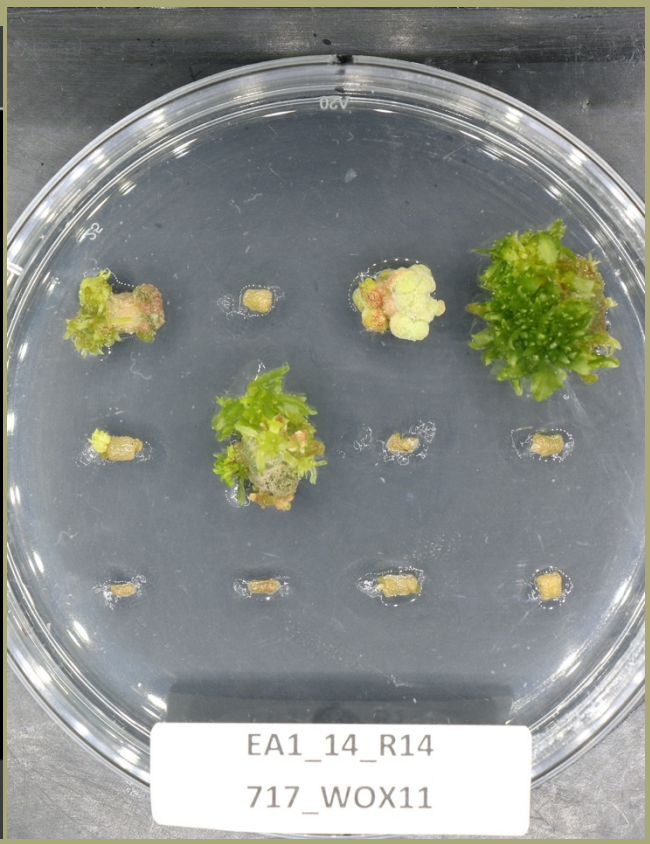
Laser excitation



Hyperspectral image
(visualized with false color)



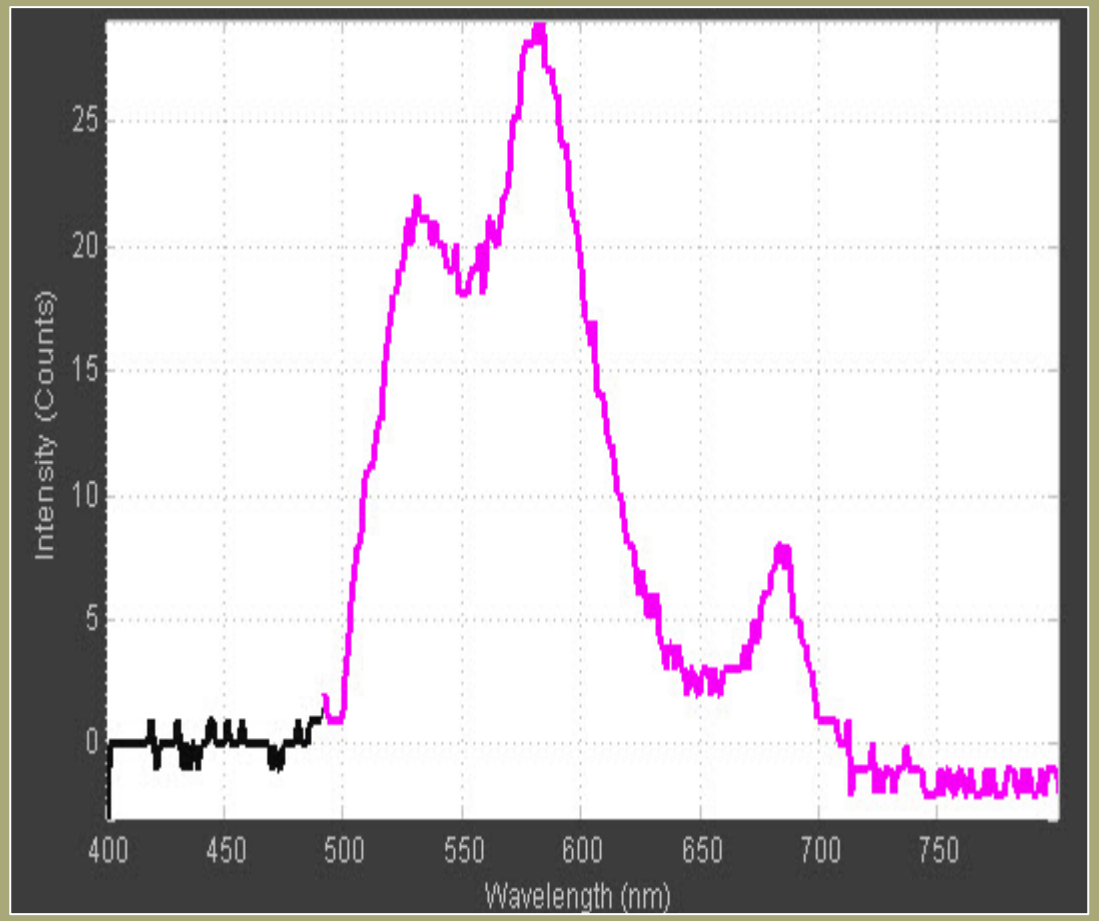
RGB image



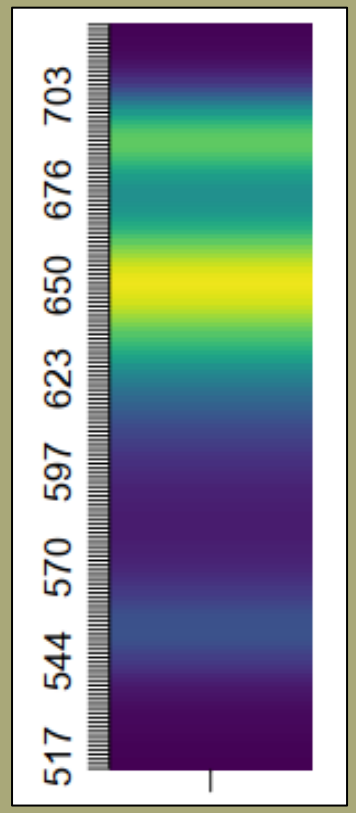
Quantification of fluorophores

Fluorescence spectrum for a pixel

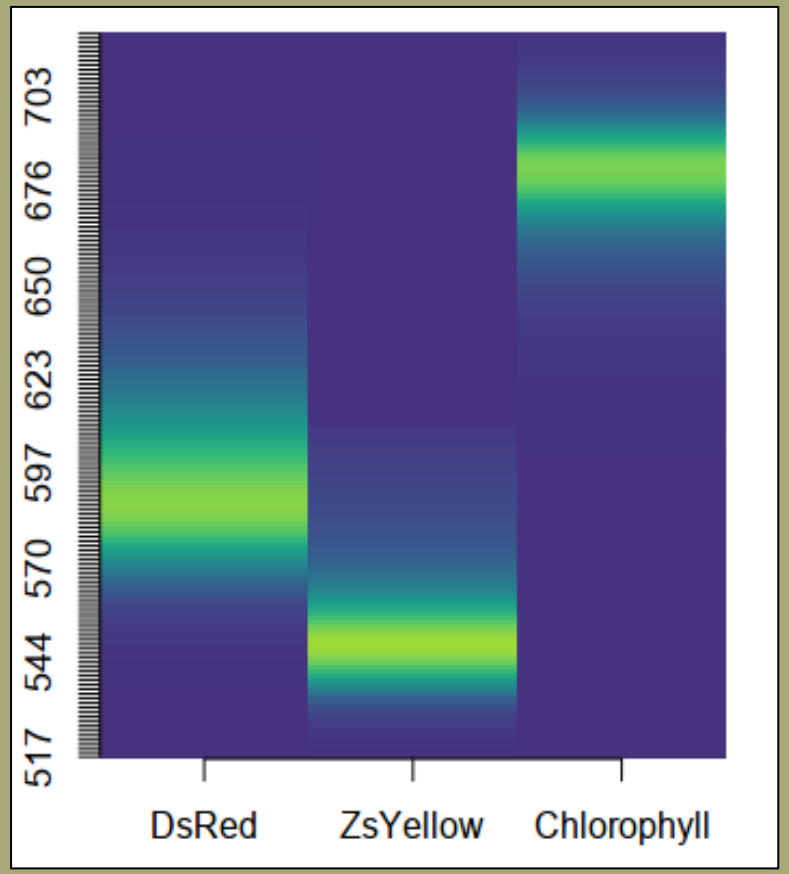
$$y_i = X^T b_i + \varepsilon_i$$



y_i



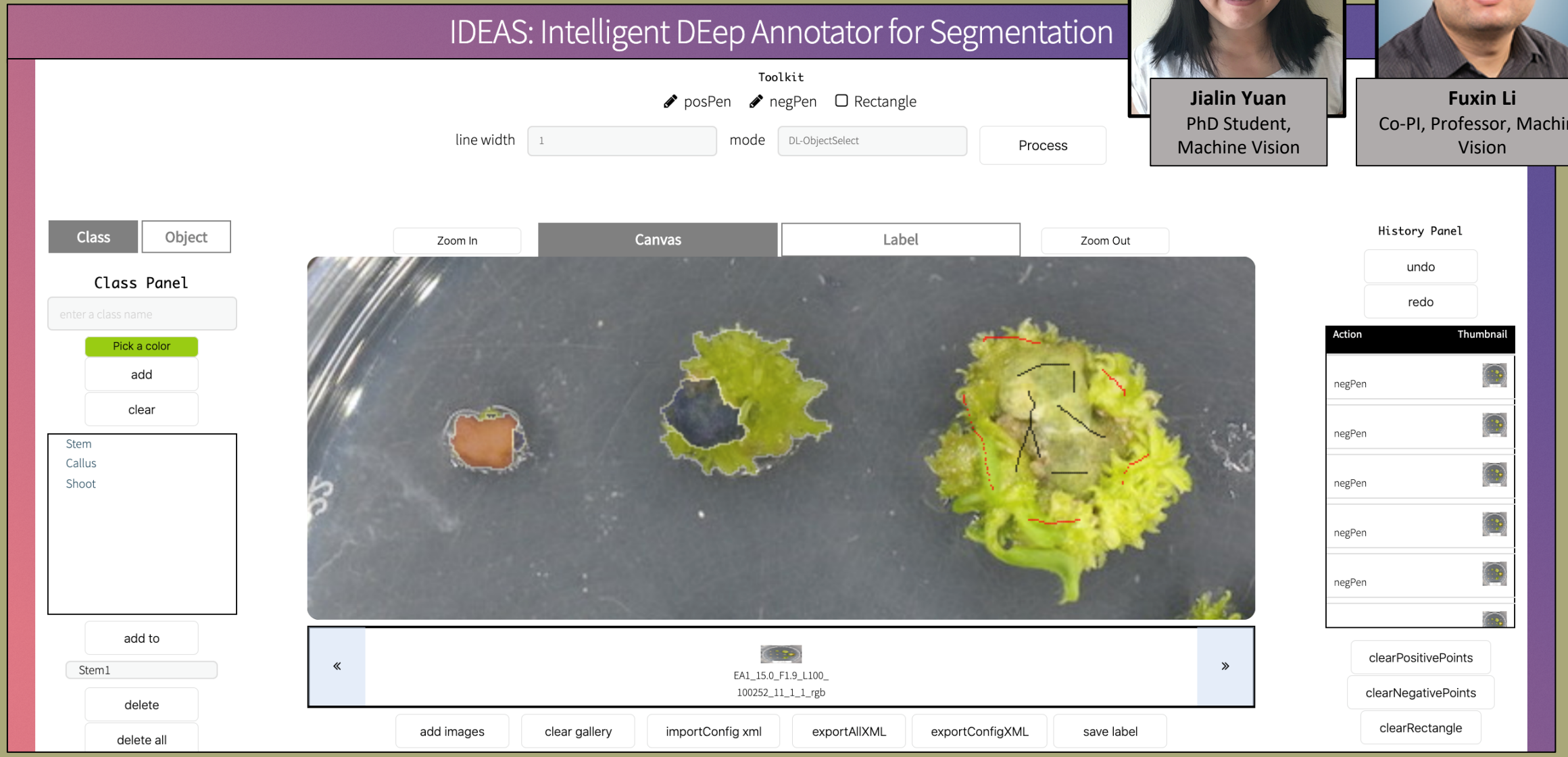
X



GUI for annotation of training set

Jialin Yuan
PhD Student, Machine Vision

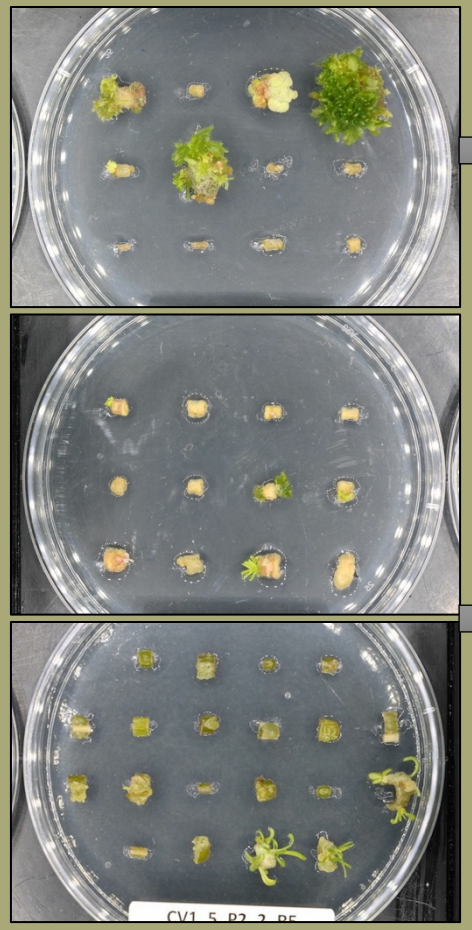
Fuxin Li
Co-PI, Professor, Machine Vision



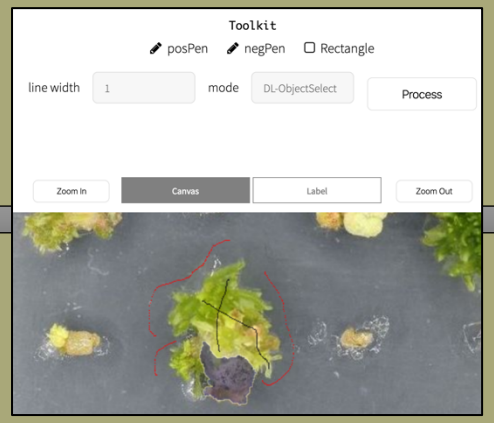
https://bitbucket.org/JialinYuan/image-annotator/

Deep segmentation workflow

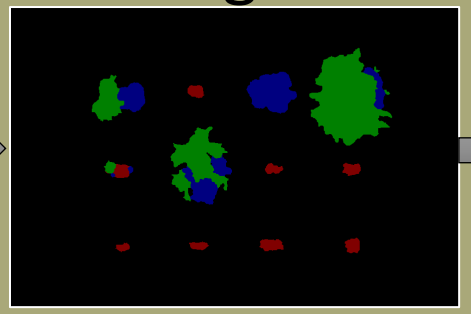
RGB images



Annotation GUI

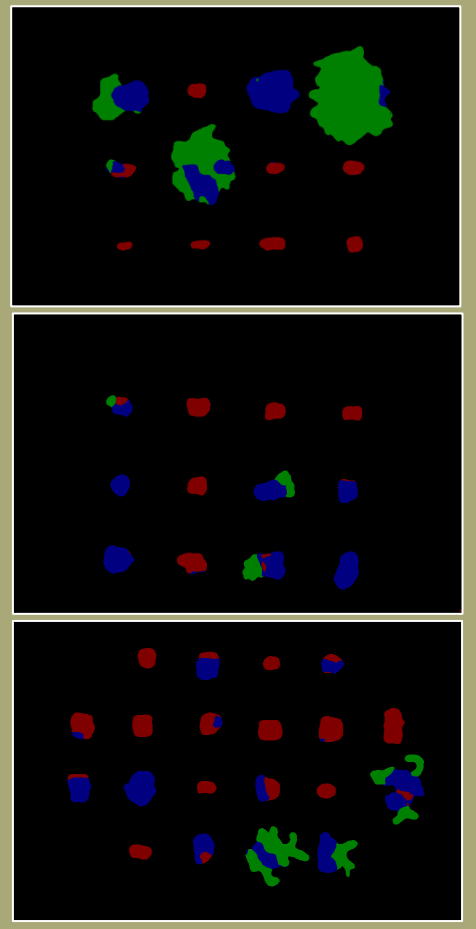


Training labels



Production of a training set by user annotation of partial dataset

Neural network for segmentation (Deeplab)

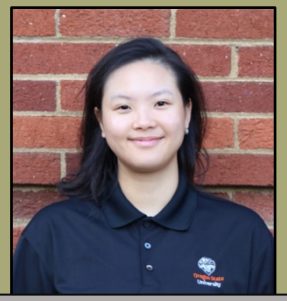


Deployment of trained model to segment full dataset

Stacking of image layers from RGB, hyperspectral cameras requires alignment

https://github.com/NSF-Image-alignment/ImageAlignment

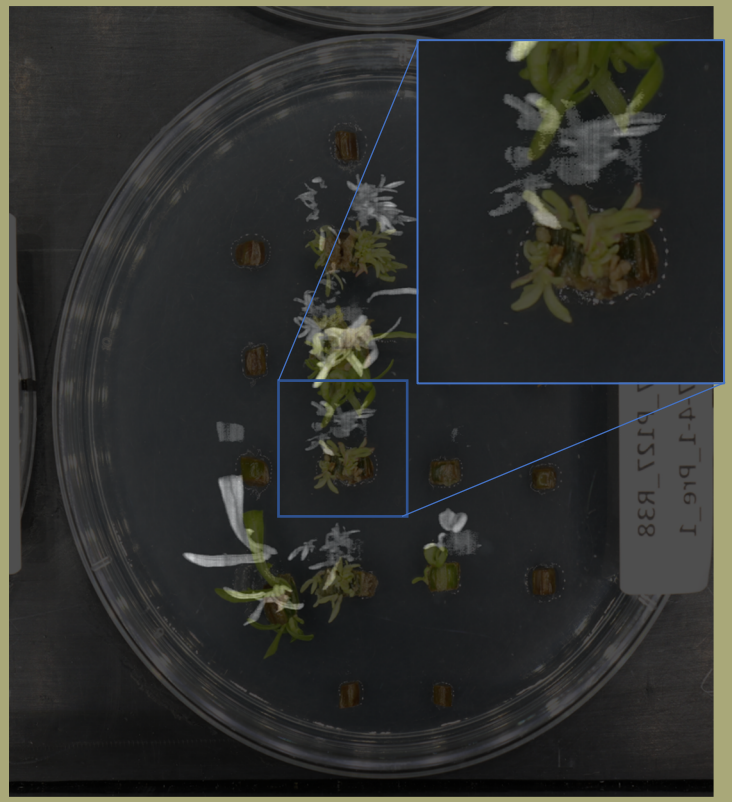
Damanpreet Kaur
Master's Student, Machine Vision



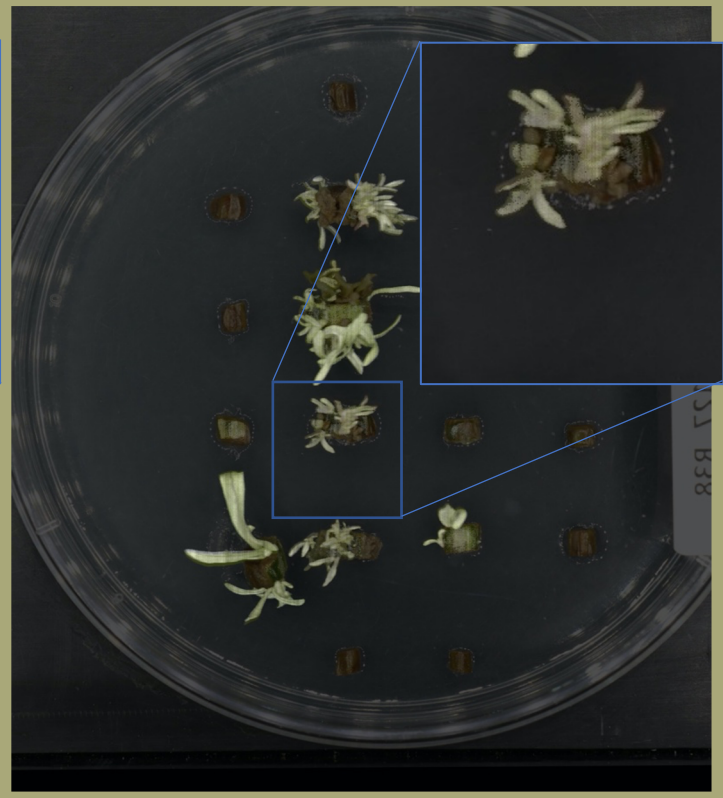
Jia Yi Li
Undergraduate Student, Machine Vision

Fuxin Li
Co-PI, Professor, Machine Vision

- Differing resolutions, proportions, frame, angle of RGB, hyperspectral image layers
- Align green from RGB images, chlorophyll from hyperspectral data
- Batch transformation of RGB images to align with hyperspectral data

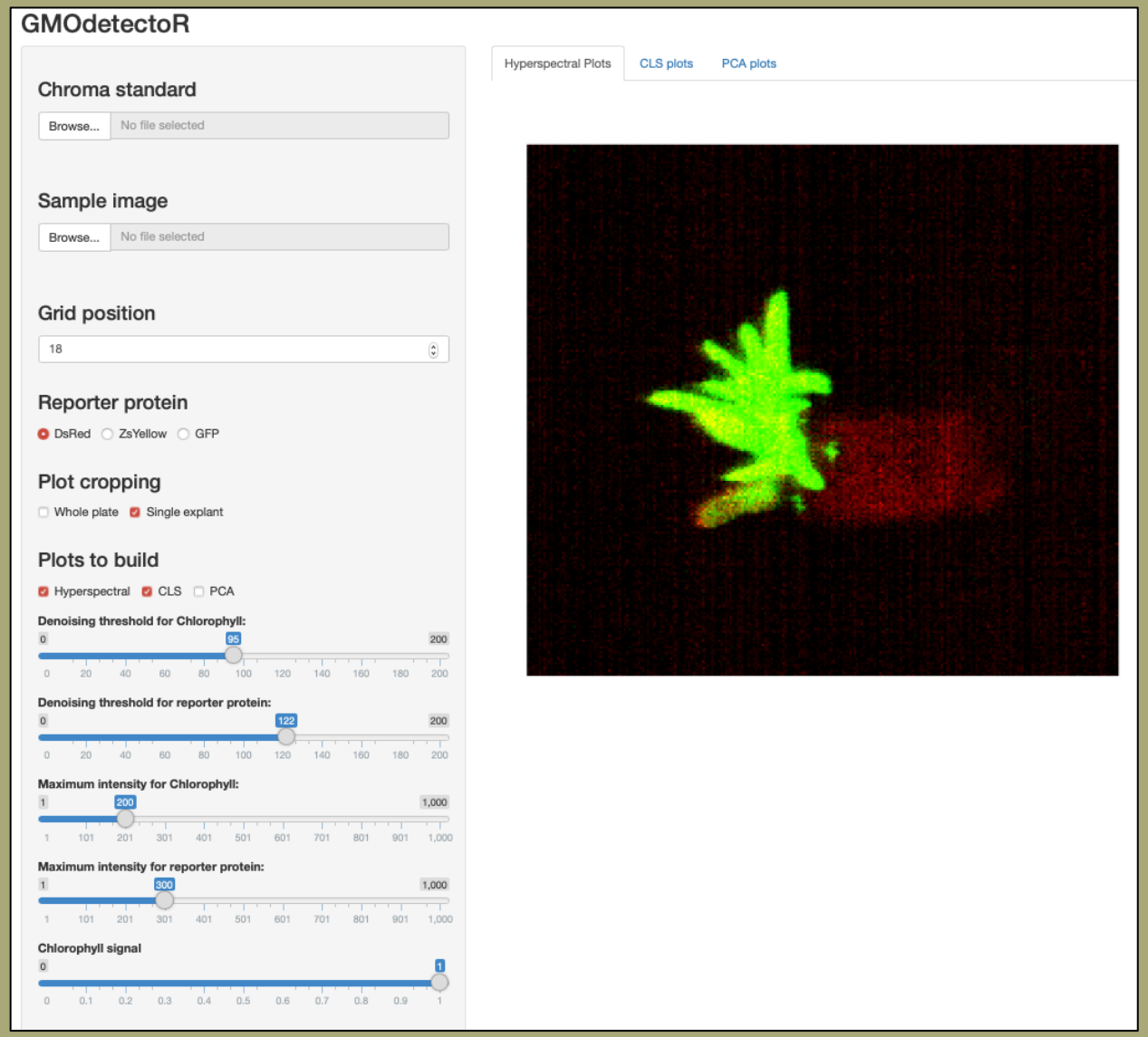


Unaligned image channels

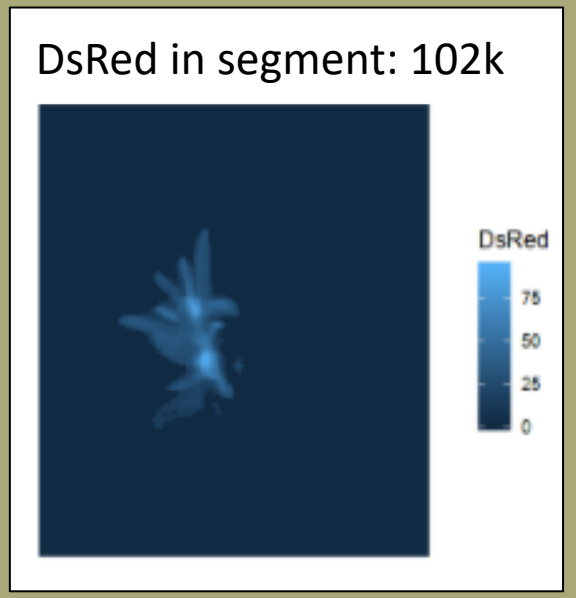


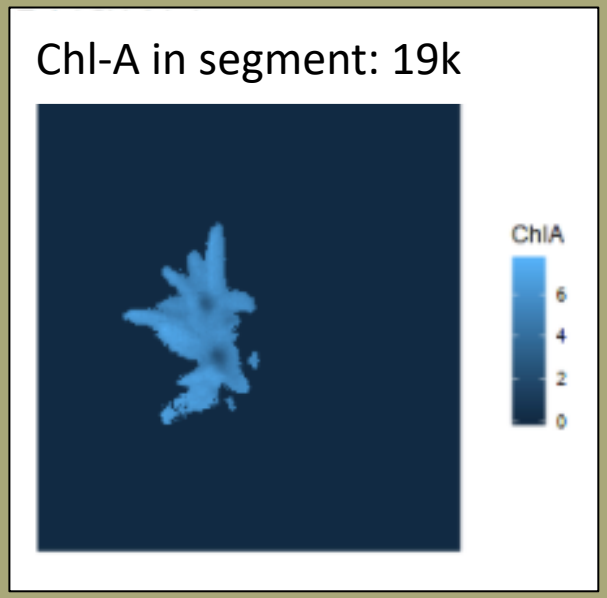
Aligned image channels

Measuring transformation rates across portions of images



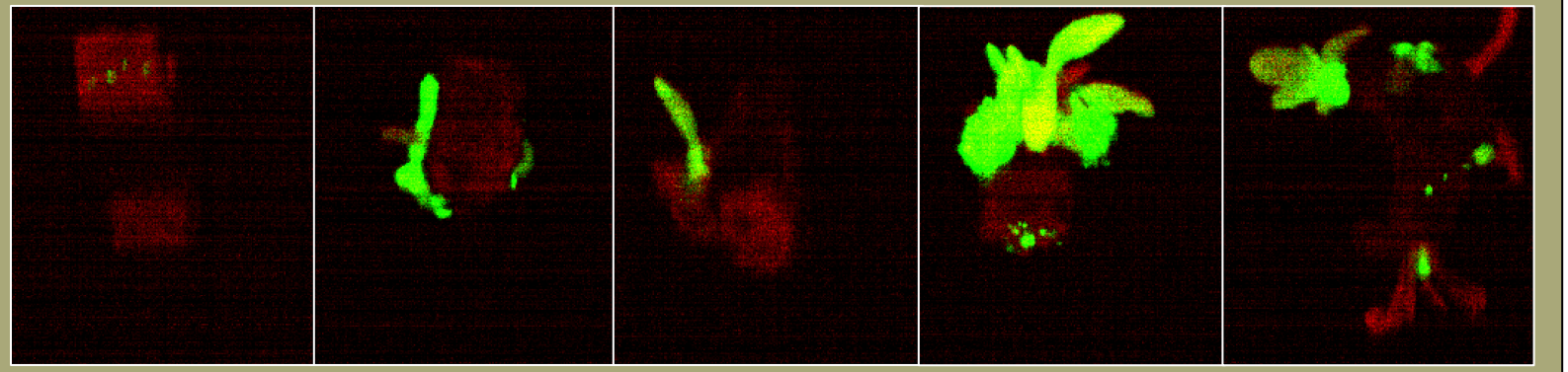
- Graphical interface for easily…
  - Tweaking parameters for hyperspectral data filtering and visualization
  - Analyzing filtered pixels by regression, PCA



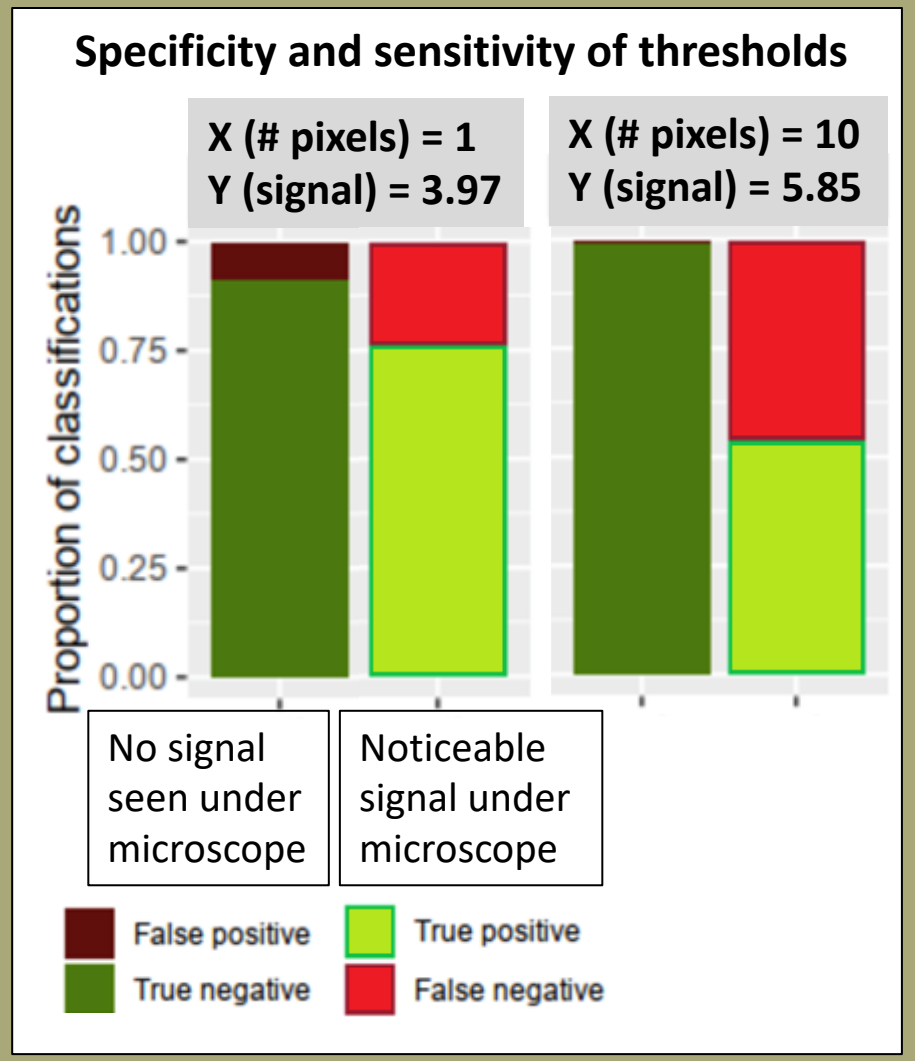


Fluorescent microscopy vs. hyperspectral analysis:
Direct comparison of ability to recognize transgenic explants

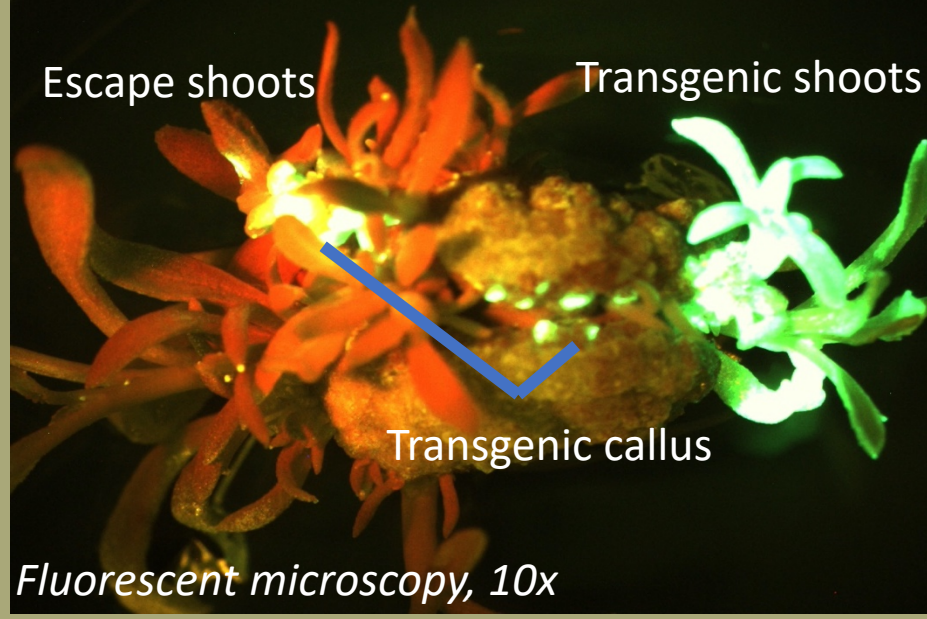
Examples of DsRed+ phenotypes observable with false color applied to hyperspectral data



- Convert hyperspectral statistics to binary for comparison to fluorescent microscopy
  If X pixels have Y signal intensity, classify explant as transgenic
- Compare to classifications by human on microscope (4,423 explants)

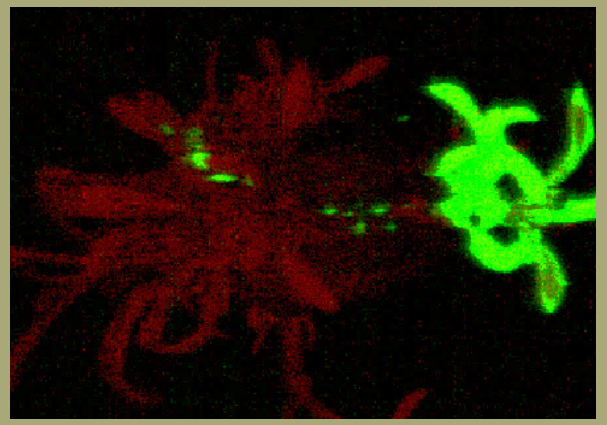


Presentation Overview

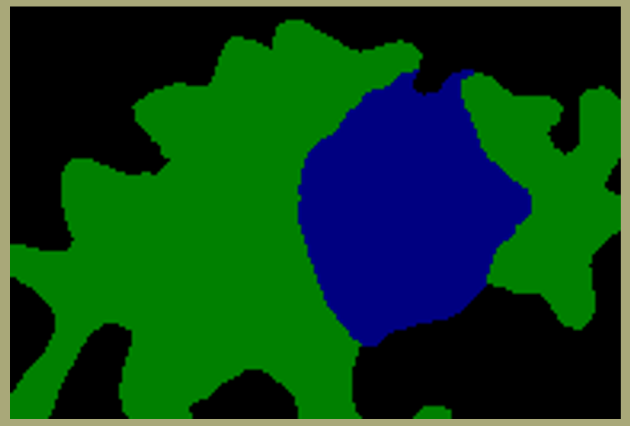


I. Introduction to phenotyping of regeneration and transformation (RT) and need for next-generation phenomics

II. Methods



Hyperspectral imaging to quantify fluorescent proteins



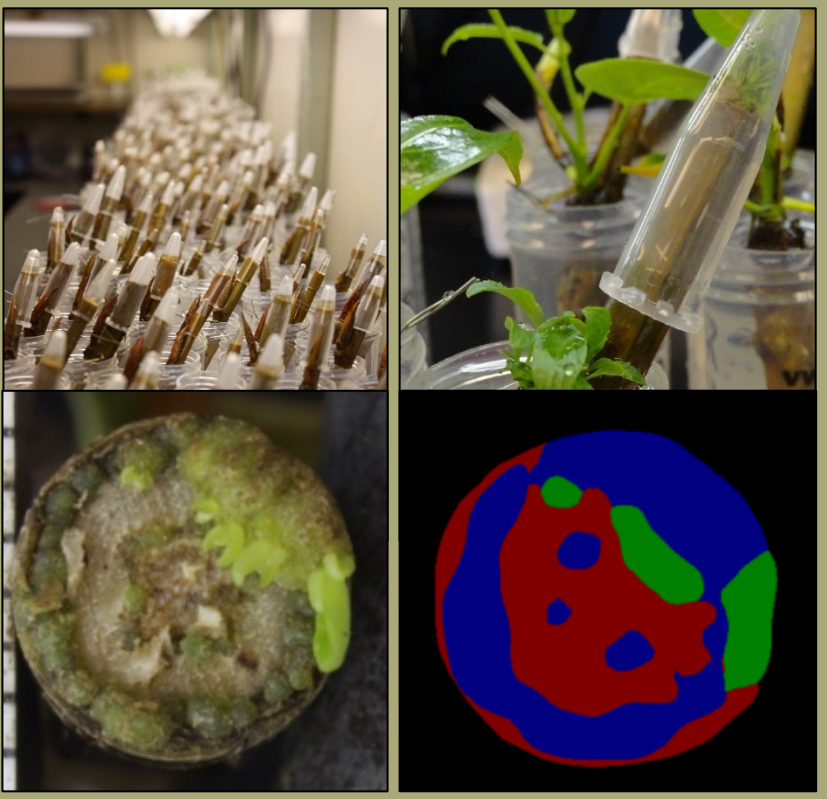
Deep learning for segmentation of plant tissues

III. <u>Example experiments</u>

- GWAS of *in planta* regeneration
- Developmental gene experiments

Demonstration of machine vision workflow in Genome-Wide Association Study of *in planta* regeneration

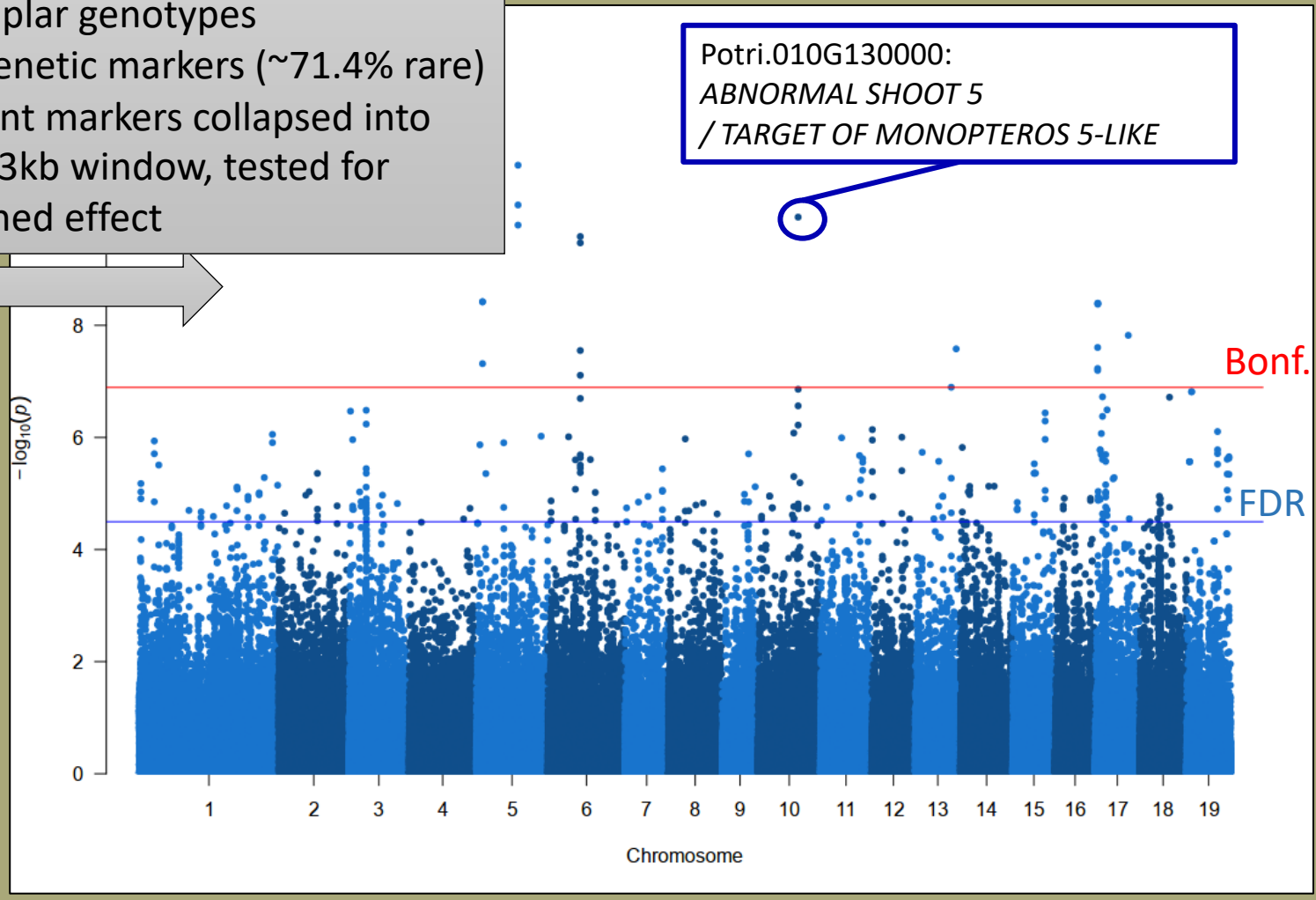
Regeneration induced in stem tips by wounding, cytokinin treatment



Tissue class	Percent of area
Stem	45%
Callus	43%
Shoot	12%

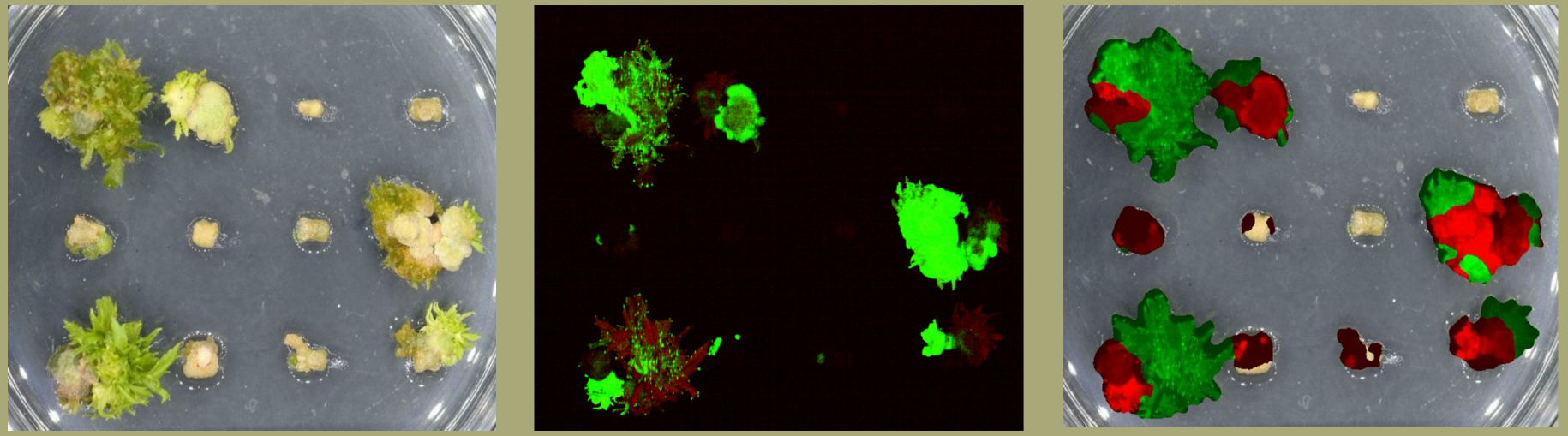
Sequence-Kernel Association Test

- 874 poplar genotypes
- 28M genetic markers (~71.4% rare)
- Adjacent markers collapsed into ~390k 3kb window, tested for combined effect

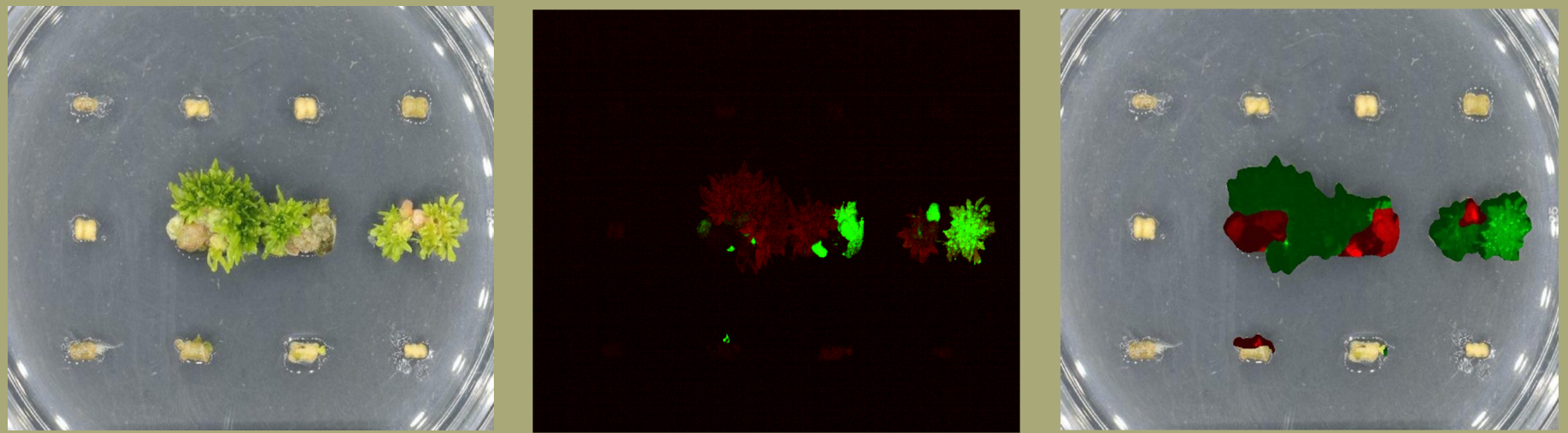


Treatments to enhance regeneration: Comparison of morphogenic genes in poplar

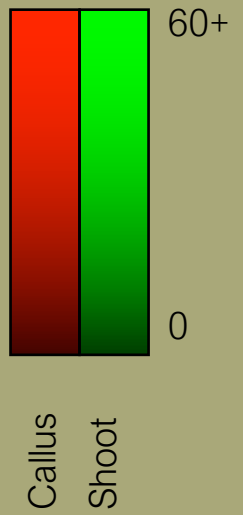
Transformed with ***WOX5a***



Transformed with ***WOX11***

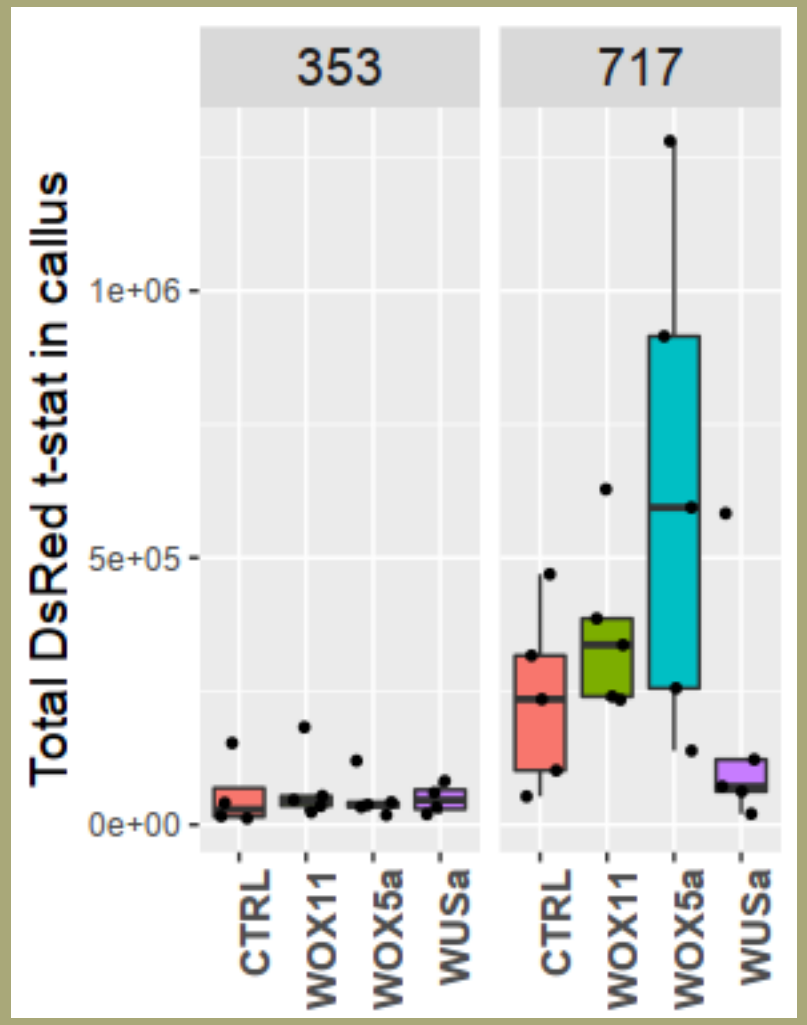


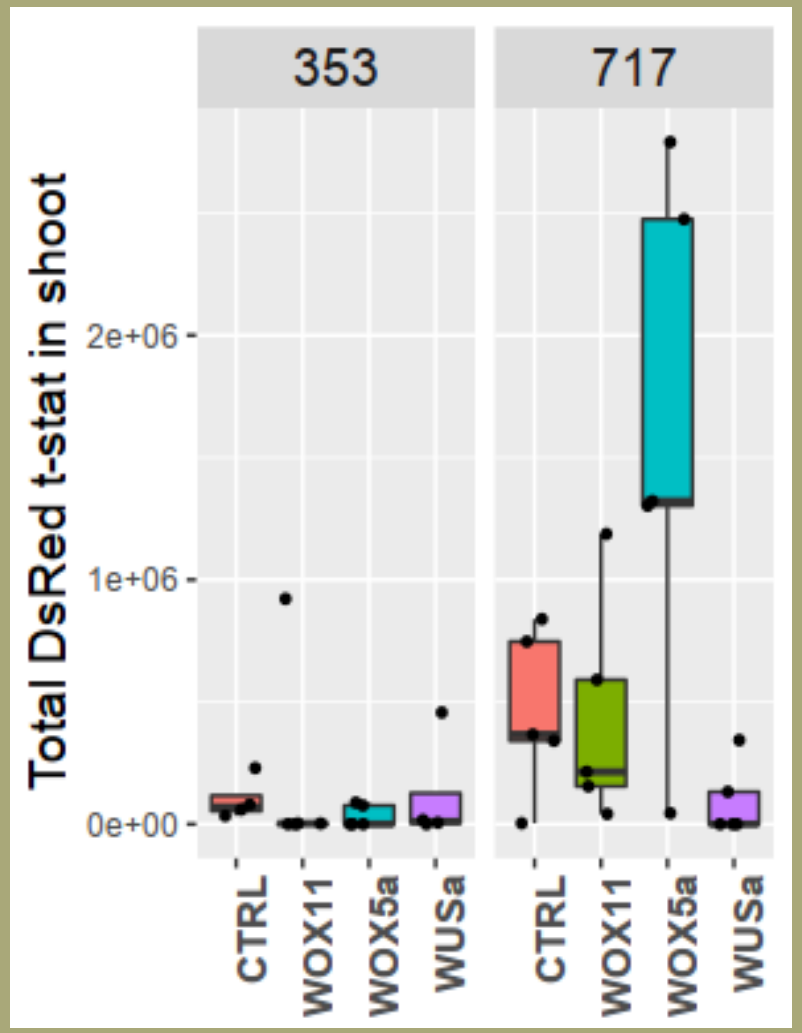
T-statistics for DsRed in tissues



Effects of *WUSa*, *WOX5a*, *WOX11* on regeneration in two poplar hybrids

Preliminary results for 456 explants across 38 plates





- Ongoing replication
  - More genotypes
  - More co-treatments
  - >10k explants total

Summary

- High-throughput RGB + hyperspectral imager (*macroPhor Array*)
- Annotation interface to build training set for deep segmentation
- Deep segmentation of RGB images into specific tissues
- Hyperspectral analysis of fluorescent protein content by pixel
- Alignment, integration of deep segmentation and hyperspectral data
- GWAS of *in planta* regeneration using deep segmentation alone
- Effects of developmental genes on transformation using combined deep segmentation and hyperspectral analysis

Acknowledgements

Cathleen Ma
Transformation & Greenhouse Experiments

Kate Peremyslova
GWAS, Transformation Experiments

Julie Kucinski, GWAS, in vitro experiments

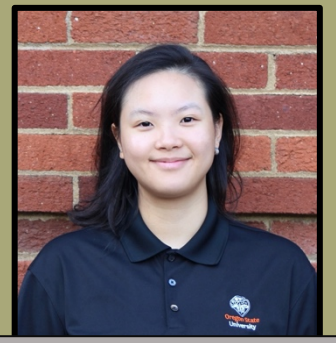
Steve Strauss
PI, Professor

Amanda Goddard
Program & Field (clone bank) Manager

Fuxin Li
Co-PI, Professor, Machine Vision

Jialin Yuan
PhD Student, Machine Vision

Damanpreet Kaur
Master's Student, Machine Vision



Jia Yi Li
Undergraduate Student, Machine Vision

Yuan Jiang
Co-PI, Professor, Statistics

We thank the National Science Foundation Plant Genome Research Program for funding "Analysis of Genes Affecting Plant Regeneration and Transformation in Poplar." IOS # 1546900.