

# Recognition and quantitative analysis of transformation in tissue cultures using hyperspectral imaging and machine learning

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**THE SOCIETY FOR IN VITRO BIOLOGY**

*FOSTERING THE EXCHANGE OF KNOWLEDGE OF IN VITRO BIOLOGY OF CELLS, TISSUES AND ORGANS*

*PLANT AND ANIMAL BIOTECHNOLOGY AND GENOMICS*



PhD Candidate Michael Nagle

PPT creator

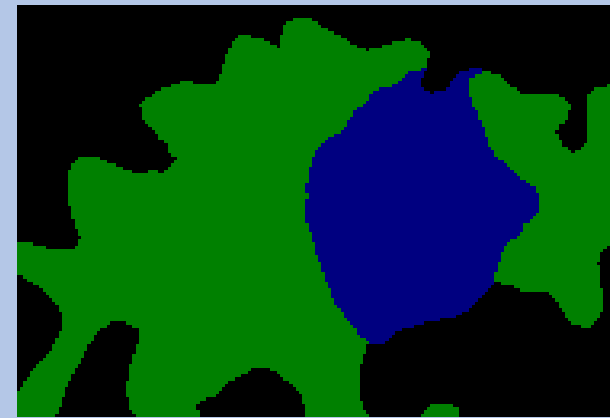
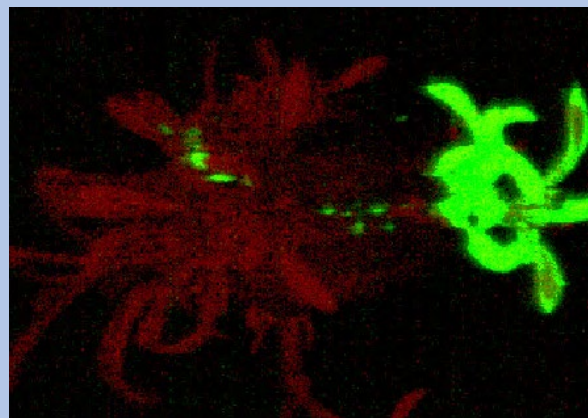
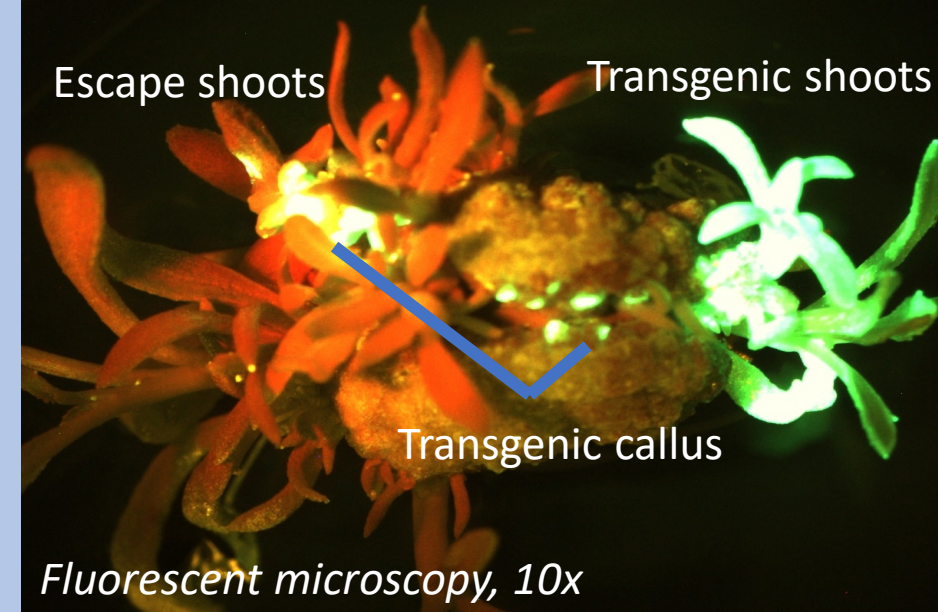
Key innovator and integrator  
of our phenomics system

Co-lead author of SIVB poster  
showing applications of  
phenomic system for in vitro  
optimization



# Presentation Overview

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



## III. Example experiments

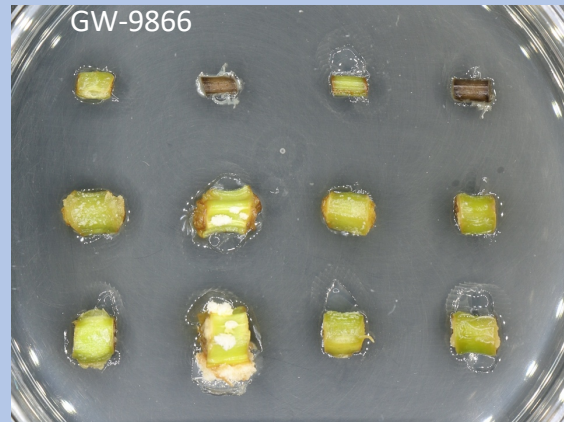
- GWAS of *in planta* regeneration
- Study of CIM treatments across genotypes



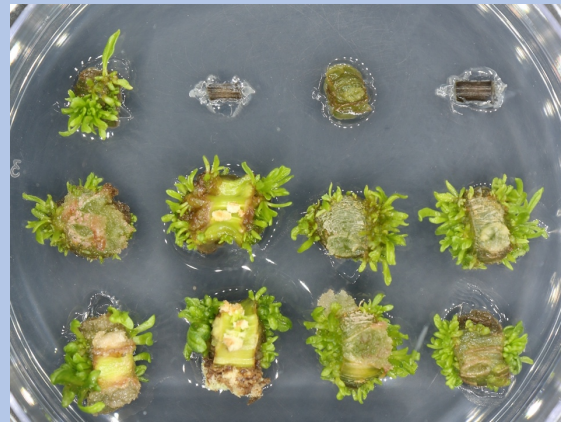
# Plant regeneration and transformation traits

–critical to agricultural biotechnology, challenging to quantify

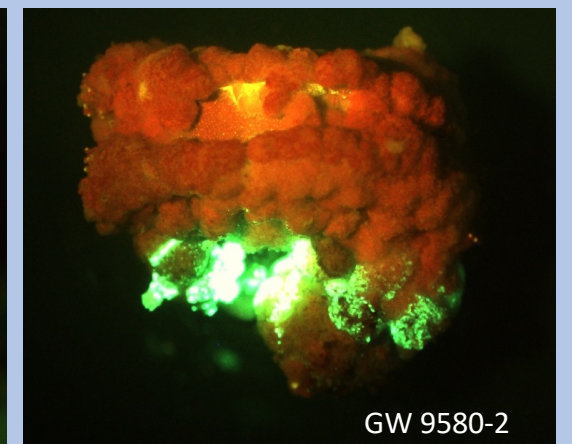
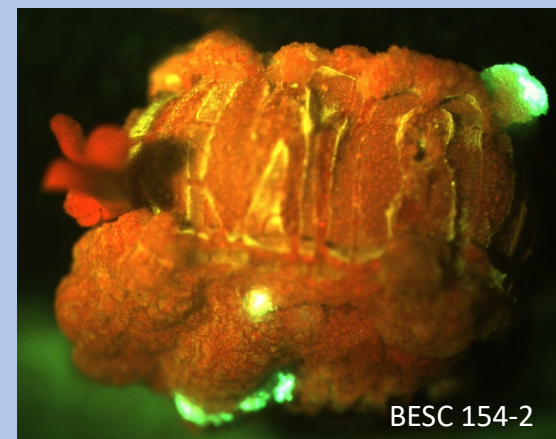
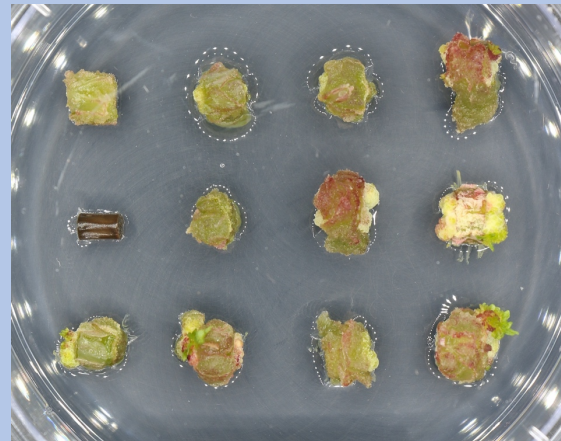
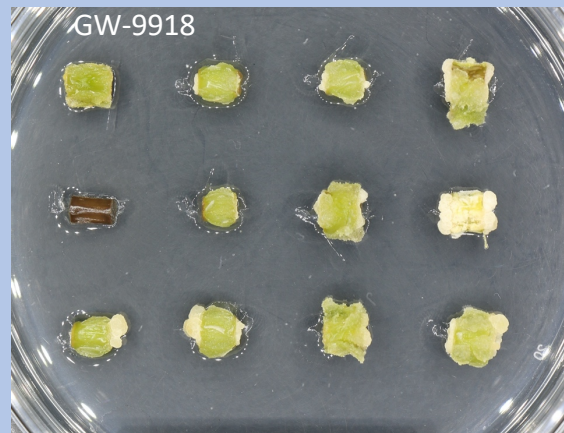
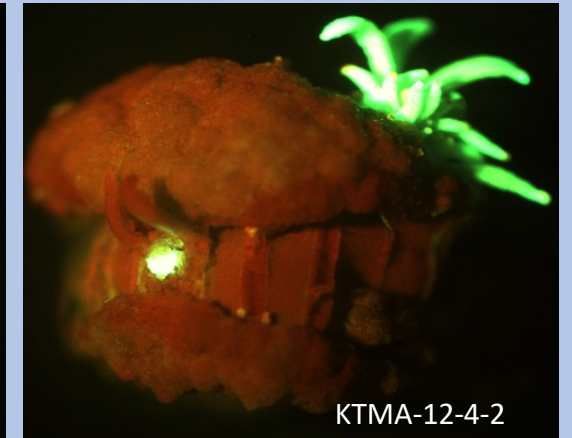
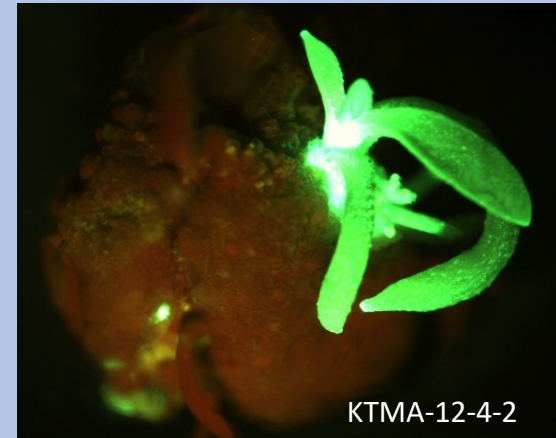
Callus induction



Shoot induction

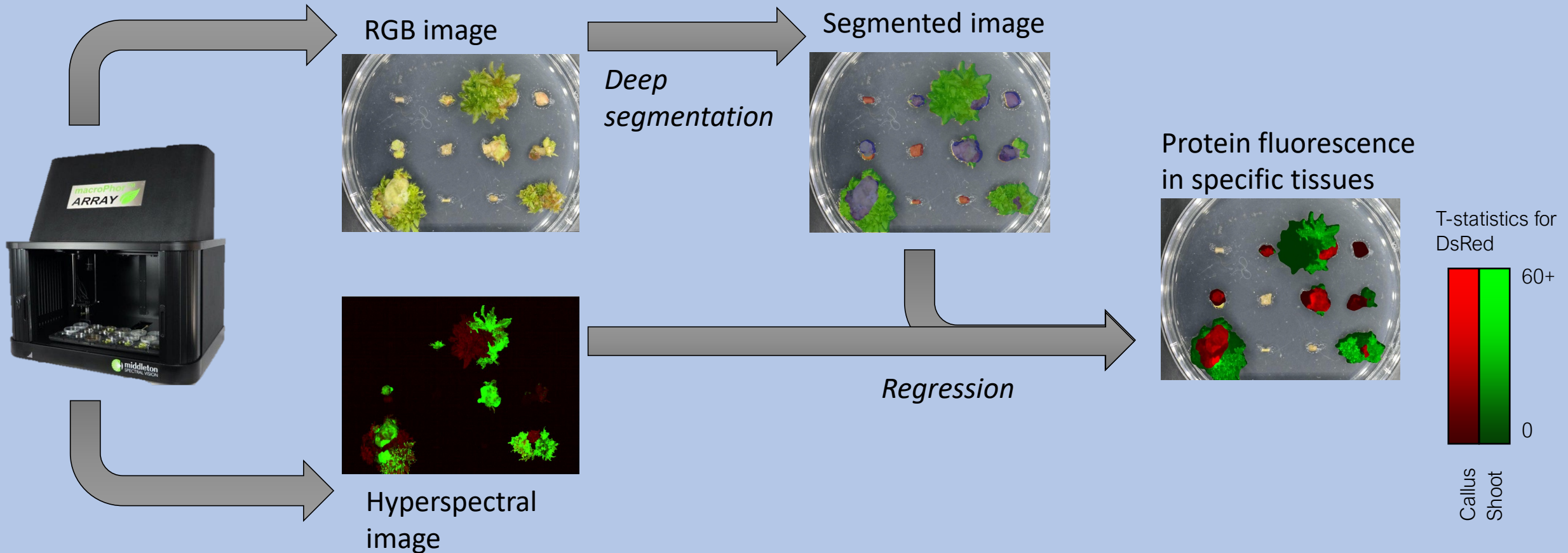


Transformation with GFP plasmid





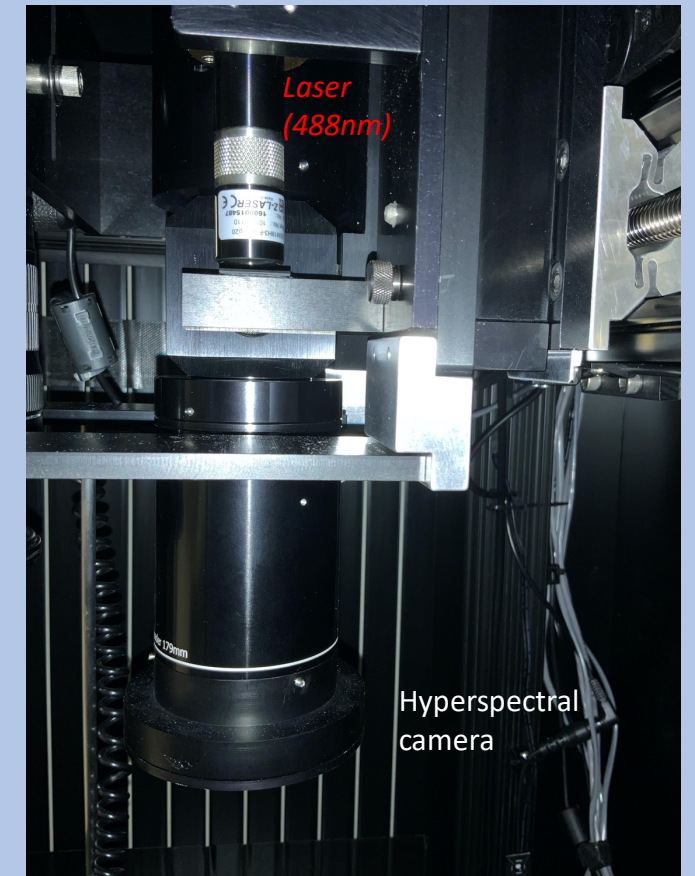
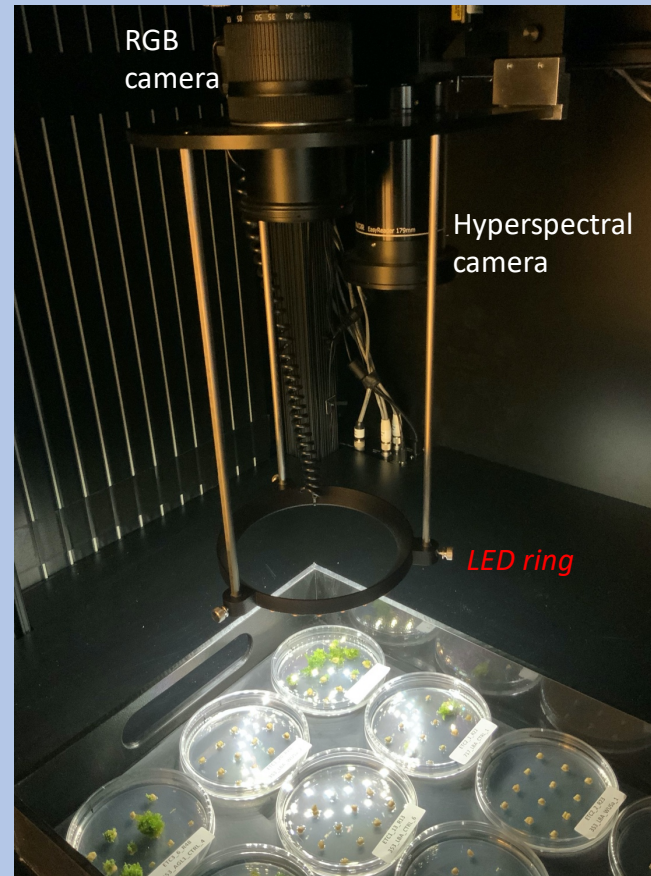
# Overview of phenomics methods developed



# 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



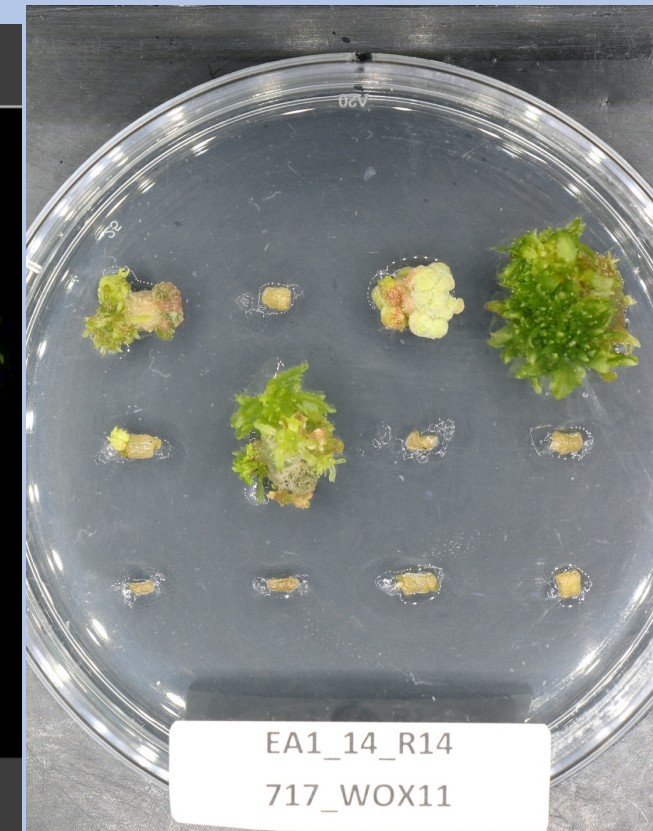
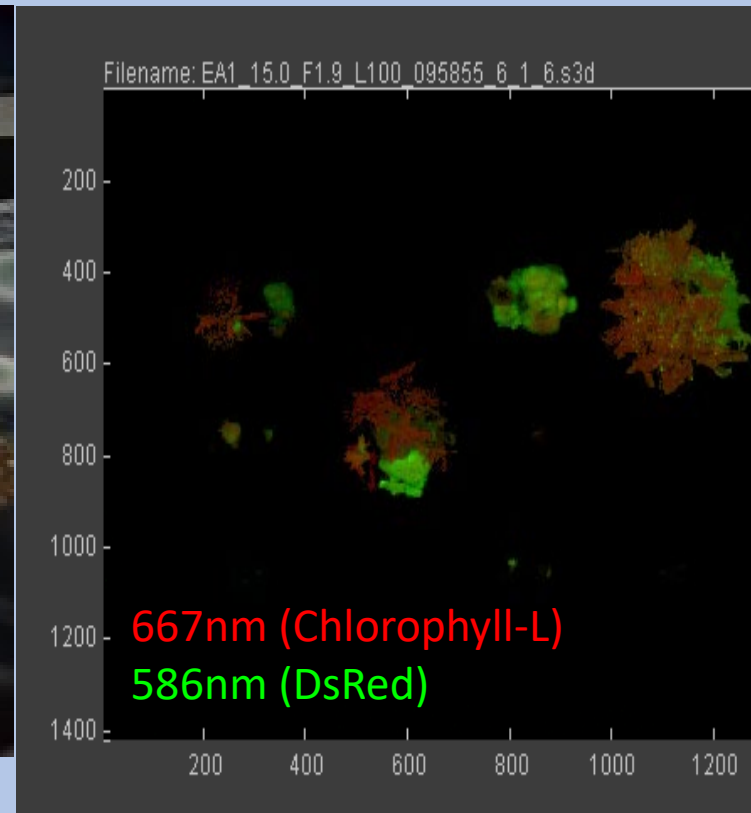
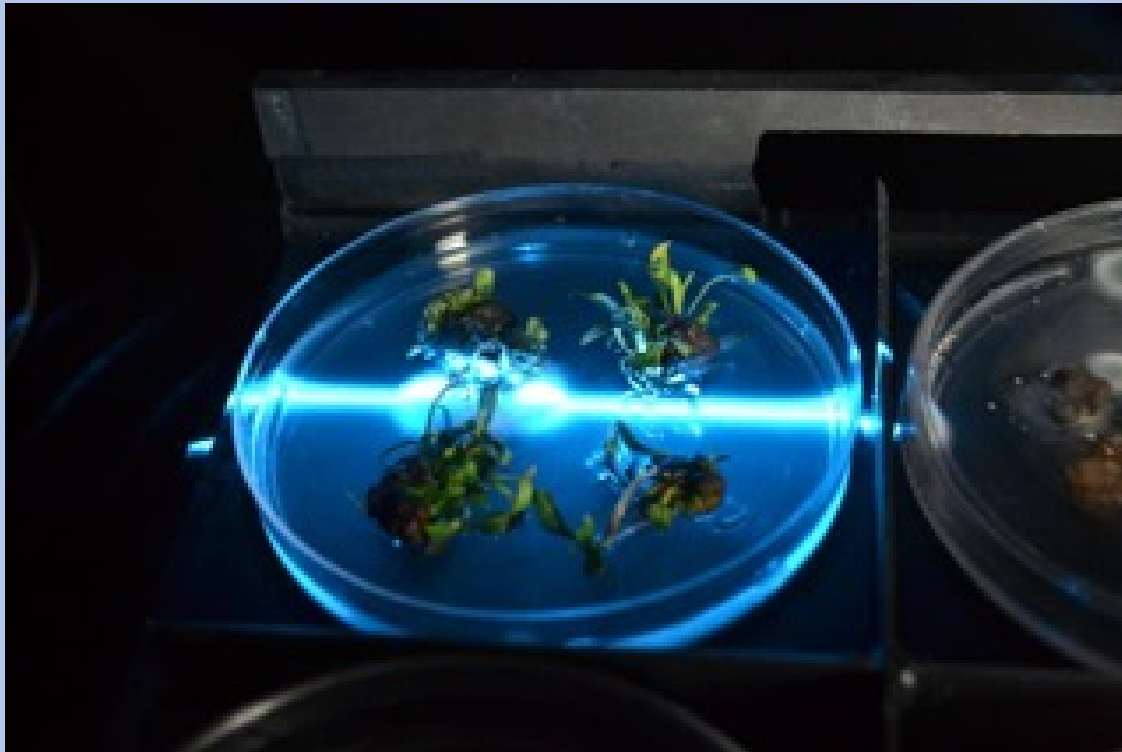


# Collection of RGB and hyperspectral images

Laser excitation

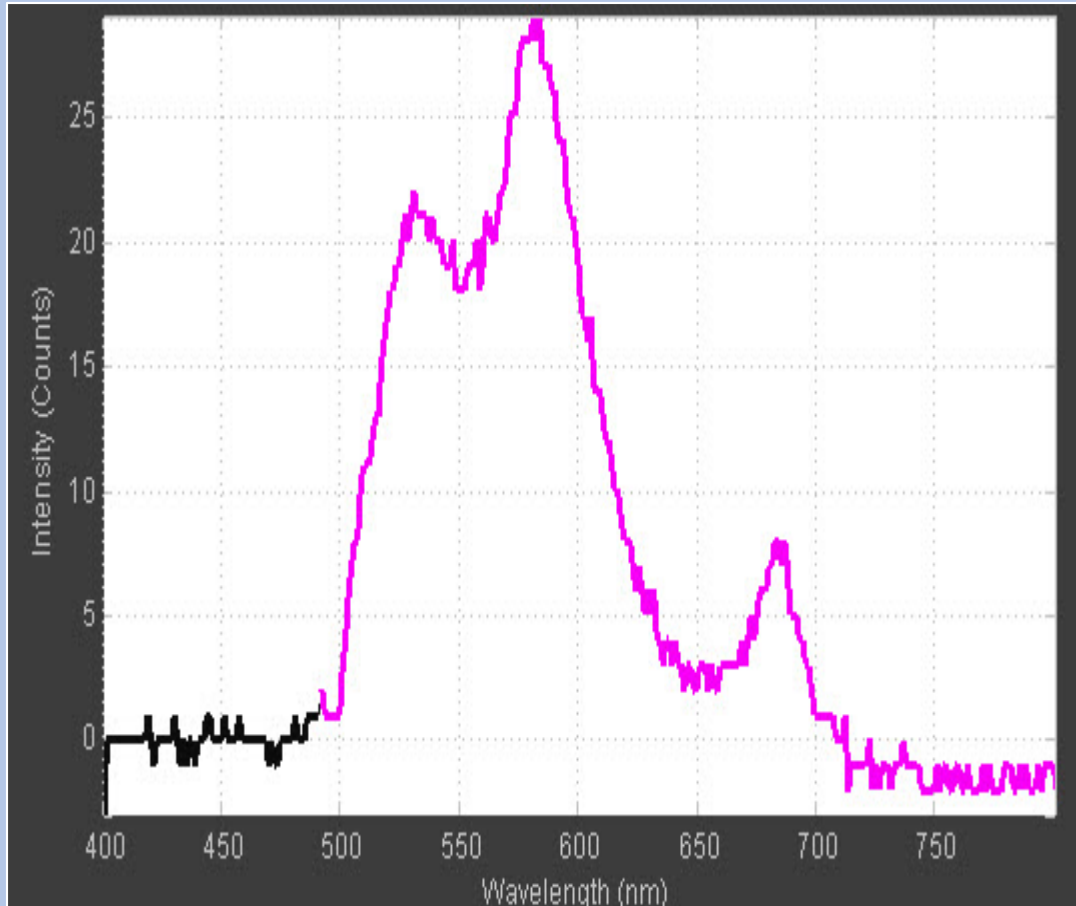
Hyperspectral image  
(visualized with false color)

RGB image

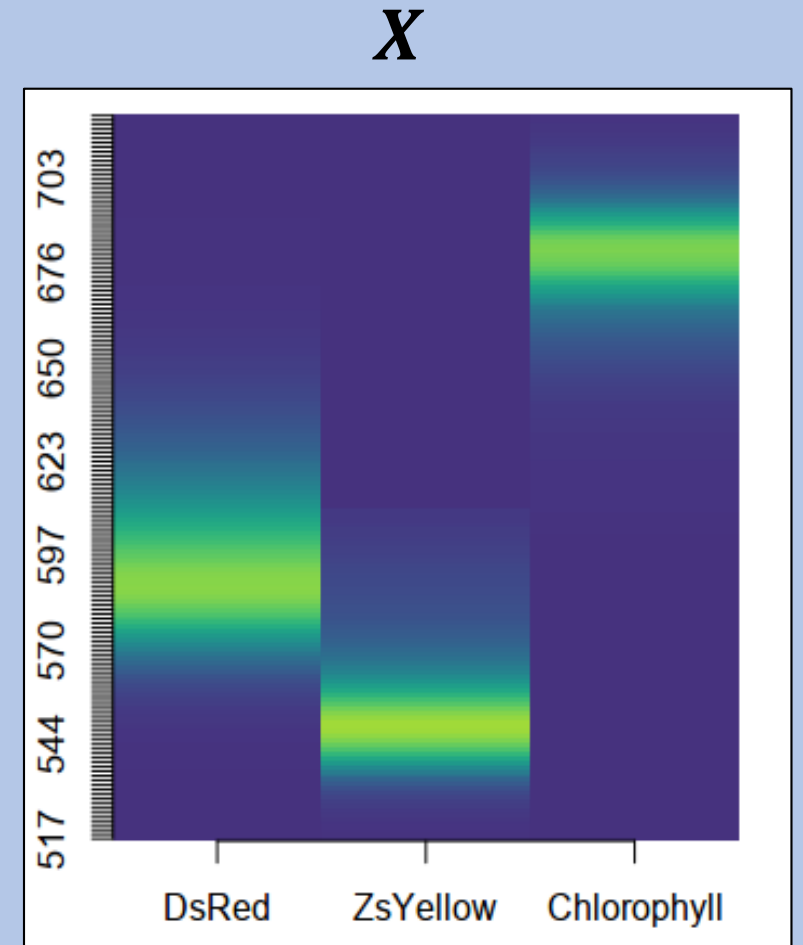
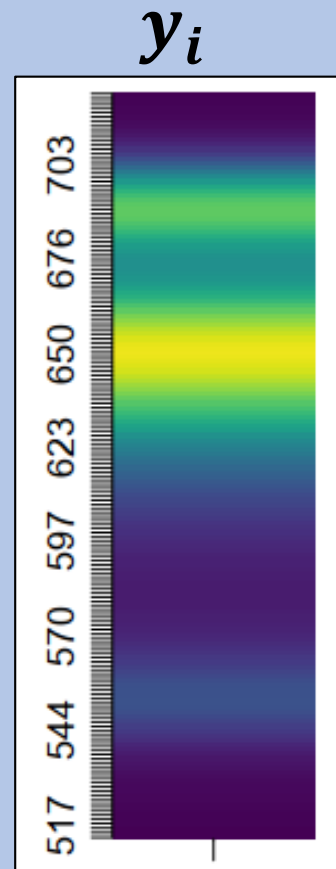


# Quantification of fluorophores

Fluorescence spectrum for a pixel



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





# GUI for annotation of training set



**Jialin Yuan**  
PhD Student,  
Machine Vision



**Fuxin Li**  
Co-PI, Professor, Machine  
Vision

IDEAS: Intelligent DEep Annotator for Segmentation

Toolkit  
posPen negPen Rectangle

line width 1 mode DL-ObjectSelect Process

Class Object

Class Panel  
enter a class name  
Pick a color  
add  
clear

Stem  
Callus  
Shoot

add to  
Stem1  
delete  
delete all

Zoom In Canvas Label Zoom Out

History Panel  
undo  
redo

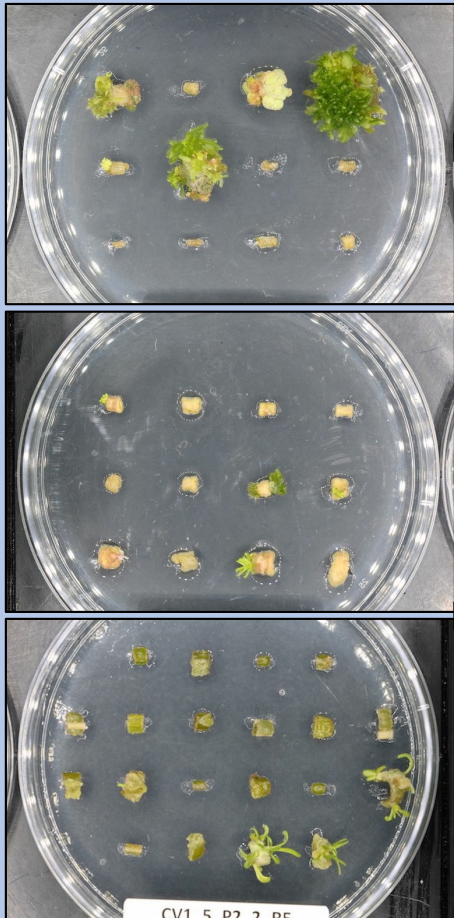
Action	Thumbnail
negPen	
negPen	
negPen	
negPen	
negPen	
negPen	

clearPositivePoints  
clearNegativePoints  
clearRectangle

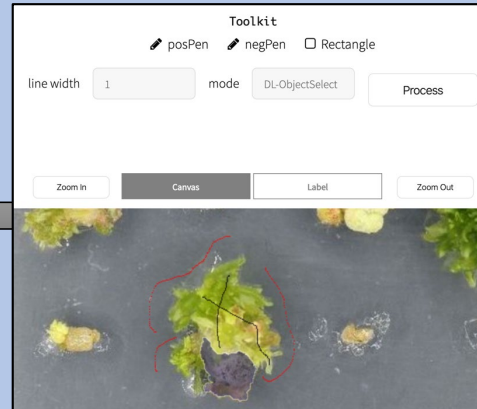
add images clear gallery importConfig xml exportAllXML exportConfigXML save label

# Deep segmentation workflow

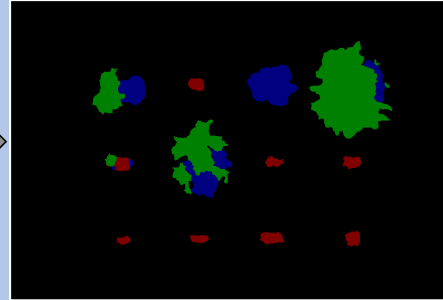
RGB images



Annotation GUI



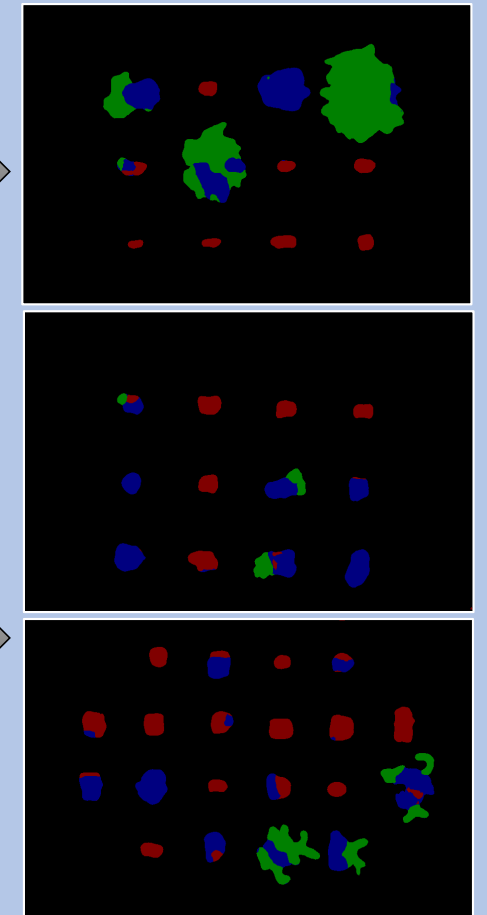
Training labels



Production of a training set  
by user annotation of  
partial dataset

Deployment of trained model to segment full dataset

Neural  
network for  
segmentation  
(Deeplab)



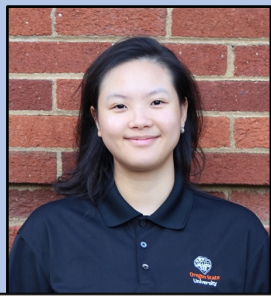


# Stacking of image layers from RGB, hyperspectral cameras requires alignment

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



**Damanpreet Kaur**  
Master's Student,  
Machine Vision

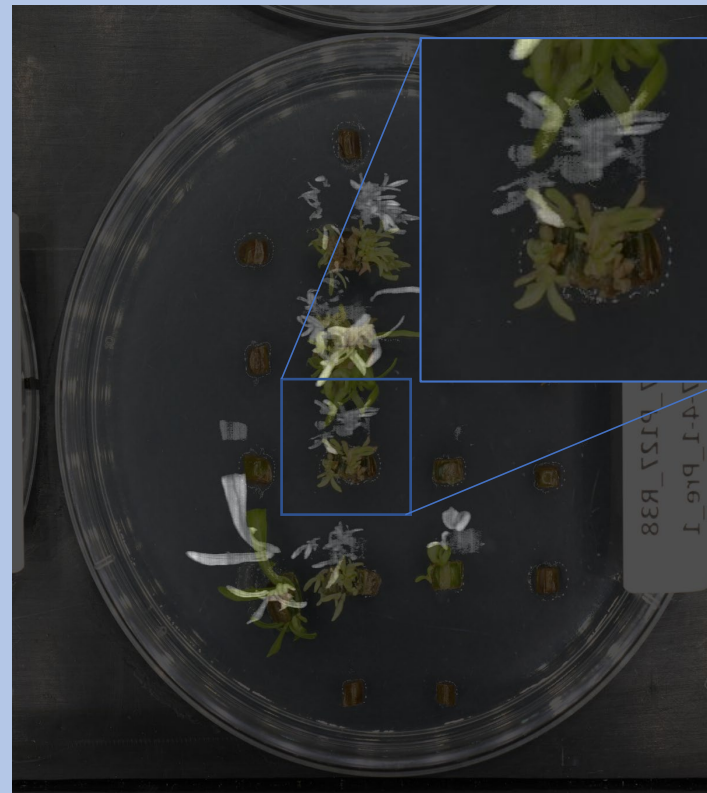


**Jia Yi Li**  
Undergraduate Student,  
Machine Vision

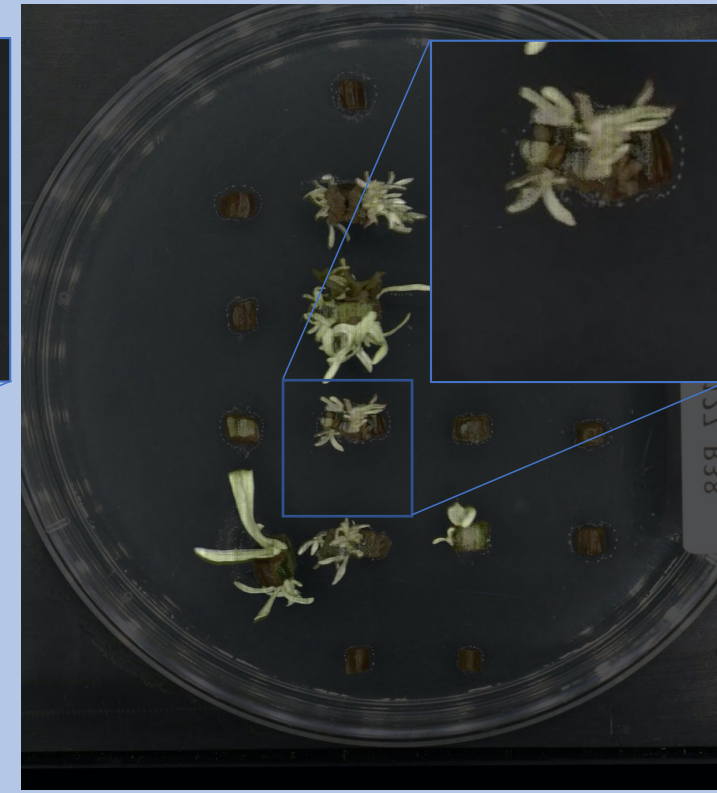


**Fuxin Li**  
Co-PI, Professor, Machine  
Vision

- 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

**GMOdetectorR**

Chroma standard  
Browse... No file selected

Sample image  
Browse... No file selected

Grid position  
18

Reporter protein  
 DsRed  ZsYellow  GFP

Plot cropping  
 Whole plate  Single explant

Plots to build  
 Hyperspectral  CLS  PCA

Denoising threshold for Chlorophyll:  
0 95 200

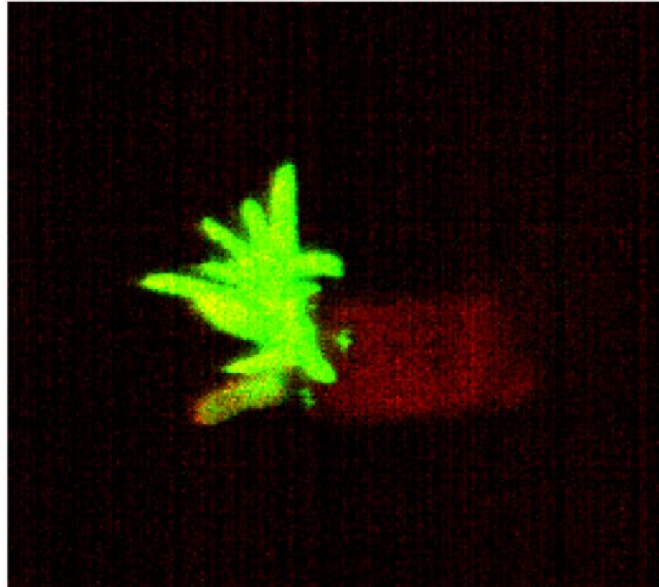
Denoising threshold for reporter protein:  
0 122 200

Maximum intensity for Chlorophyll:  
1 200 1,000

Maximum intensity for reporter protein:  
1 300 1,000

Chlorophyll signal  
0 1

Hyperspectral Plots CLS plots PCA plots



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

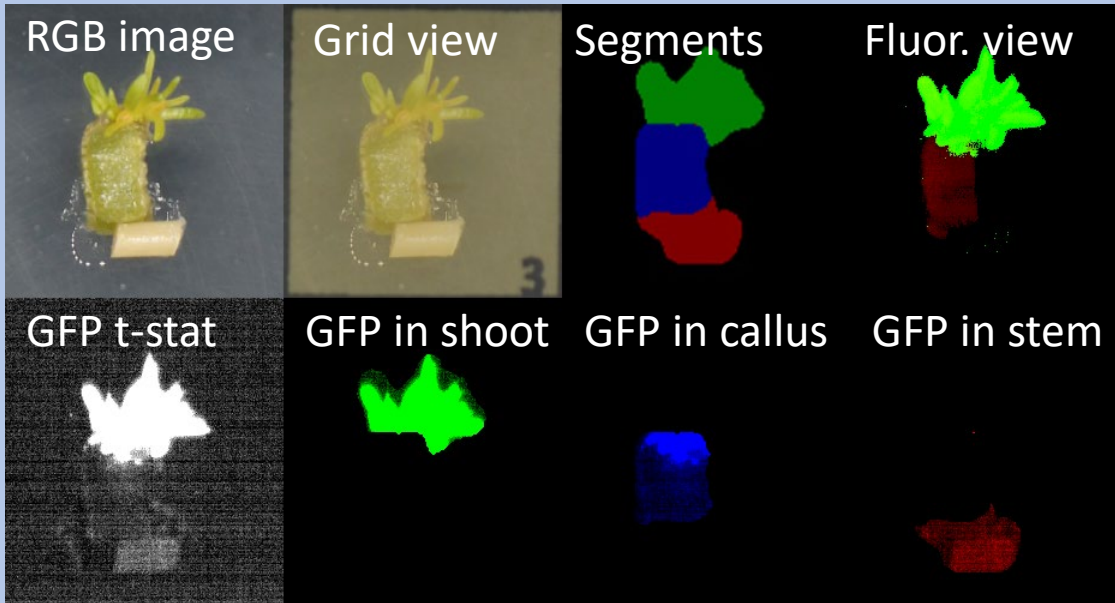
DsRed in segment: 102k



Chl-A in segment: 19k



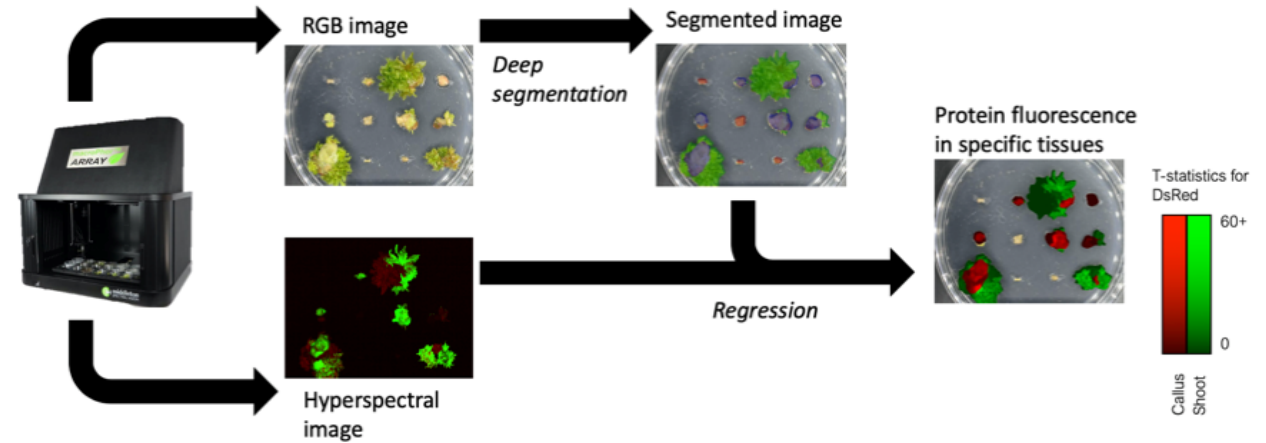
Each dataset analyzed with workflow integrating hyperspectral data, segmentation data for each explant



## GMOnotebook

*Run phenomics workflow over a new dataset*

Notebook template v0.1.45 (May 10, 2020)



Instructions for running this workflow

1. Enter information for the experiment below
2. Set [variables](#) for data paths and parameters
3. "Save as" with filename describing experiment and anything special about this analysis (e.g. T18\_OD\_TAO\_wk7\_automation\_test\_attempt2.ipynb)
4. Run notebook from console
5. Wait for email

Experiment ID and quick description:

CT, CU and CV: Three replicates testing WUS plasmids from multiple species in cottonwoods

Parameters for analysis:

[1]: `data_folder="CT_CU_CV_raw/wk6/"`

[2]: `email=michael.nagle@oregonstate.edu`



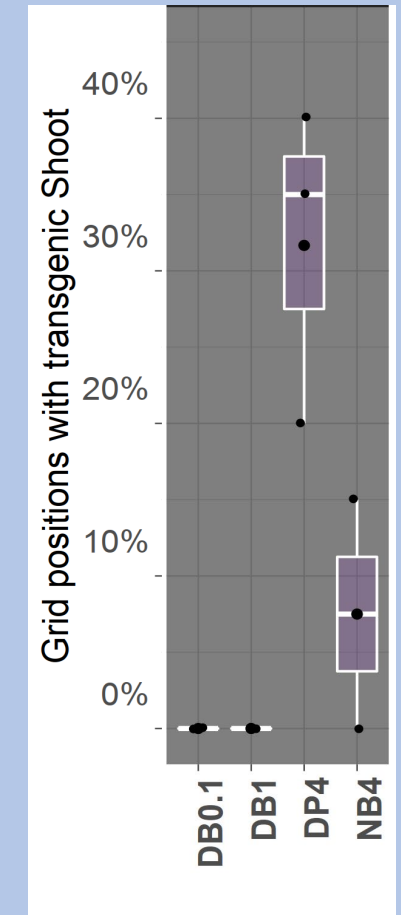
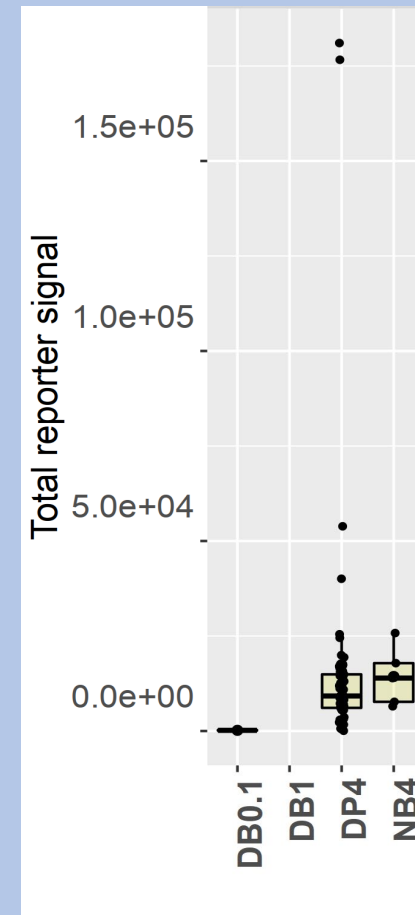
# Calculation of statistics familiar to the study of *in vitro* regeneration

- Hyperspectral statistics not as easily interpretable as % of (transgenic) explants with callus or shoot
- Classification of tissues as transgenic based on thresholding of  $x$  pixels with  $y$  reporter protein test statistic
  - Thresholds optimized for statistical power and specificity (via brute force)

Reporter signal in tissues classified as shoot (by explant)

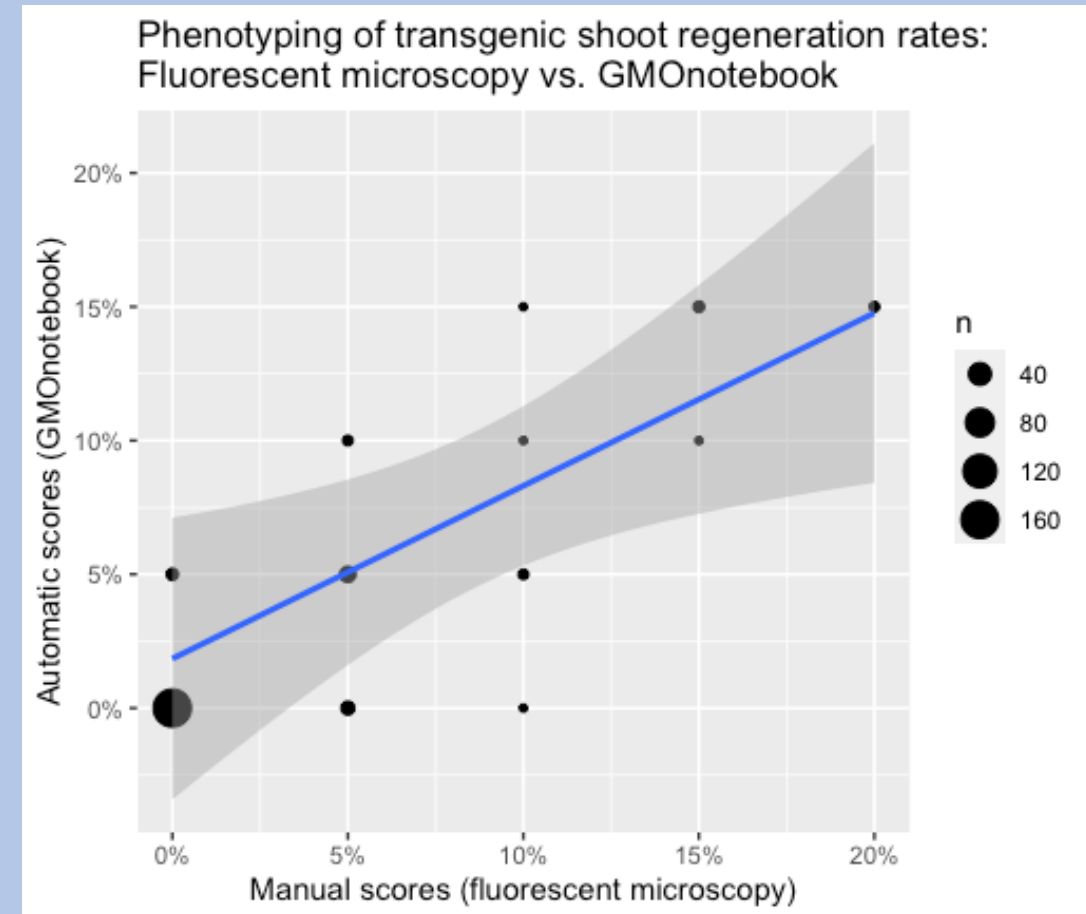
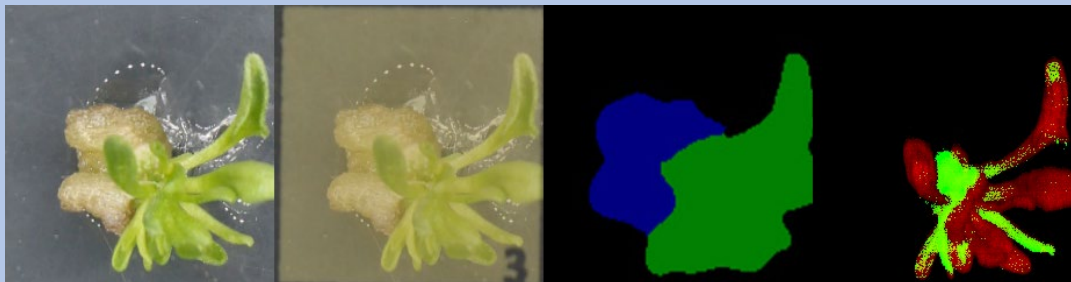


Reporter signal used to classify explants as transgenic (by plate)



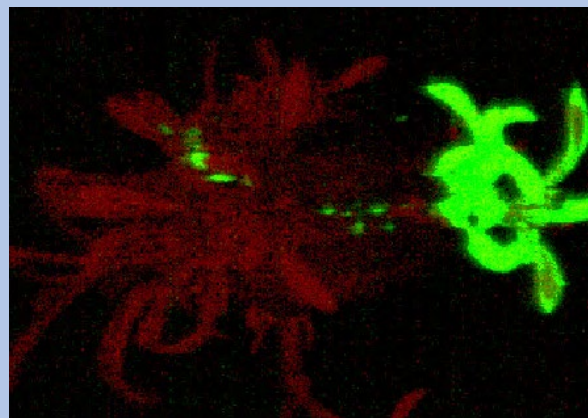
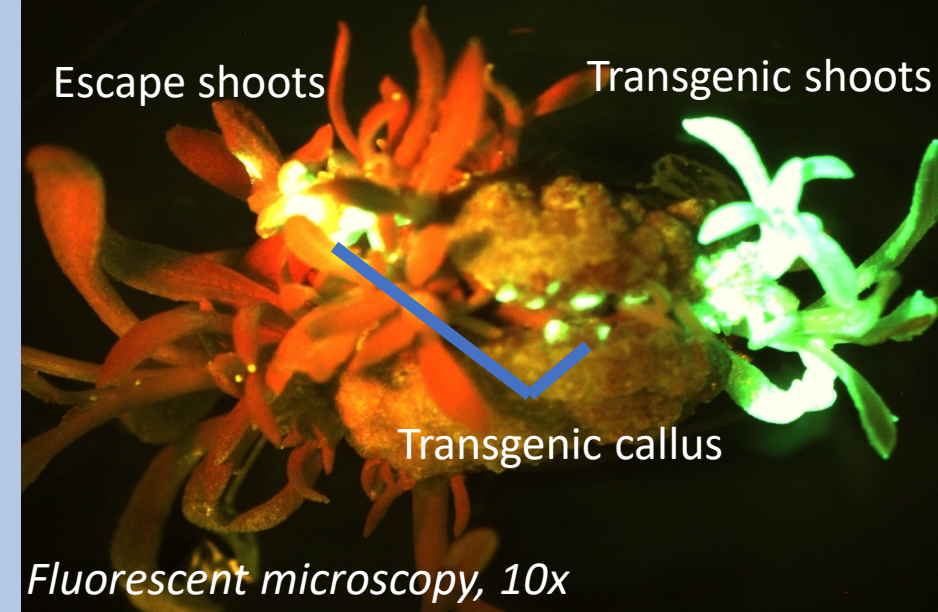
# Hyperspectral, segmentation analysis effectively substitutes for fluorescent microscopy

- Experimental materials: 204 plates, most with 20 explants each (3,988 total explants), transformed with DsRed reporter
- Phenotyped via both
  - Fluorescent microscopy (by human)
  - GMOnotebook (automated cross-analysis of hyperspectral, segmentation data)
- $R^2 = 0.87$
- Common sources of disagreement:
  - Explants invading space of adjacent explant
  - Tissues misclassified by segmentation model

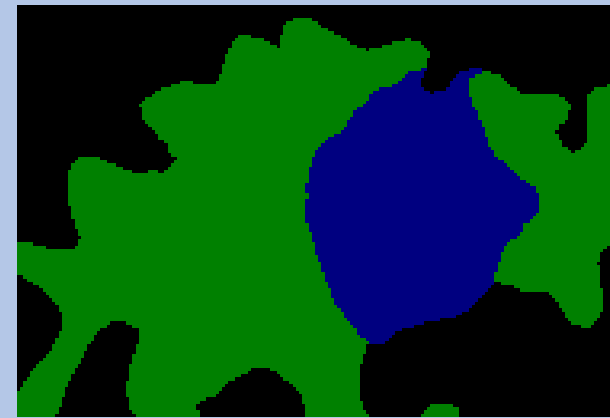


# Presentation Overview

- 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

Blue	Callus
Green	Shoot

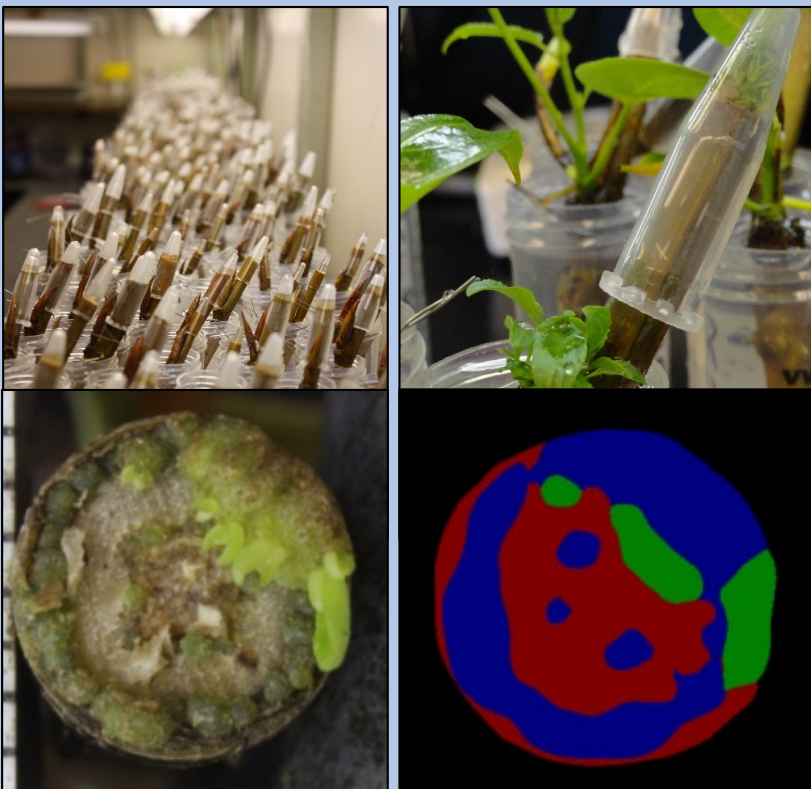
## III. Example experiments

- GWAS of *in planta* regeneration
- Study of CIM treatments across genotypes



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

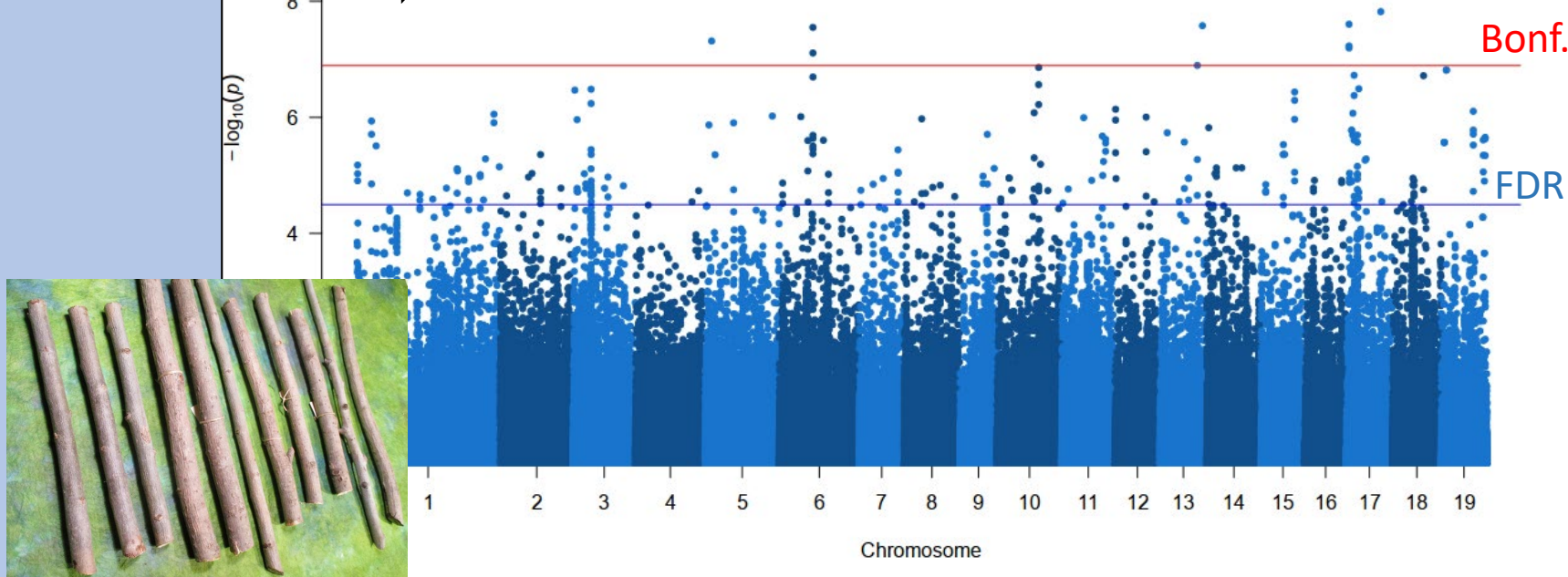
Regeneration induced in stem tips by wounding, cytokinin treatment



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



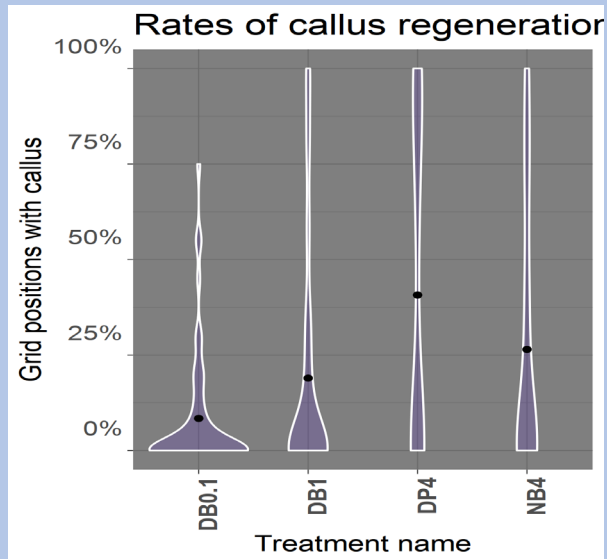
# Exploring myriad treatments across diverse genotypes

- Find ideal treatments for individual genotypes
- Find highly heritable treatment effects which provide opportunity for genetic discovery via GWAS
- Two examples:
  1. Testing of phytohormones in callus induction media (CIM)
  2. Testing effects of CIM pre-culturing prior to transformation

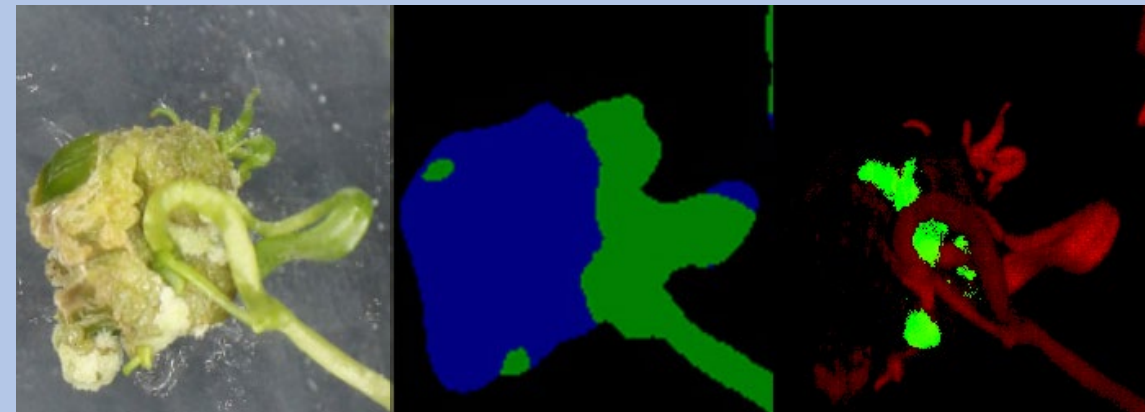
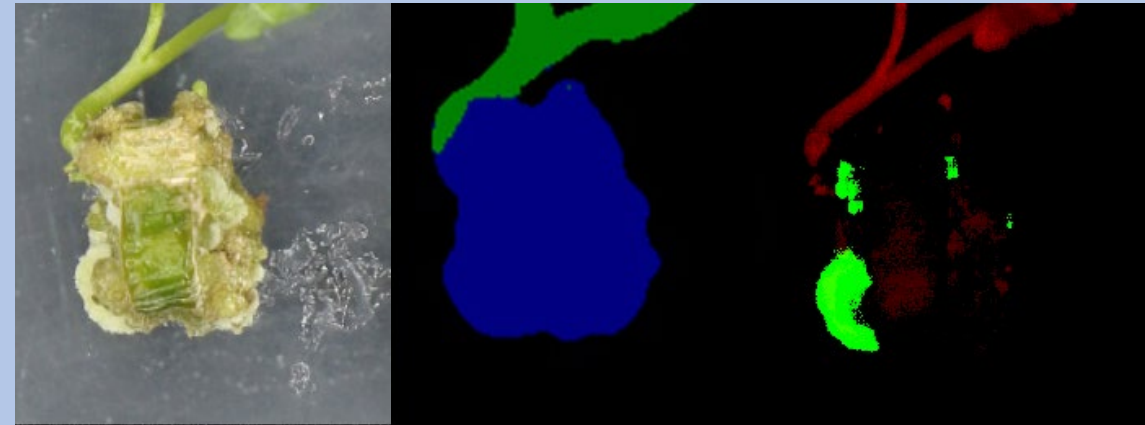
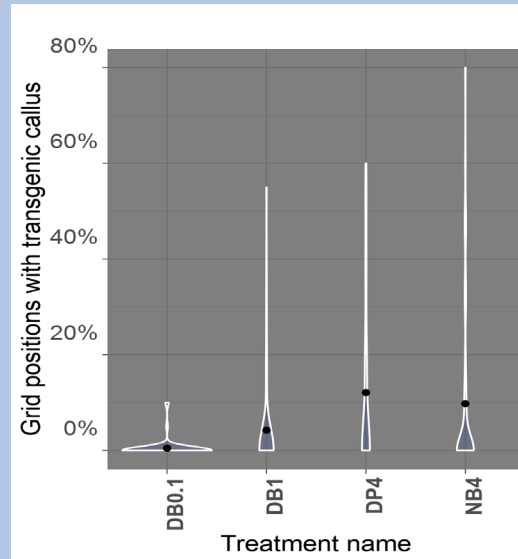
# Rates of callus formation after different CIM treatments

## Rates of callus regeneration

All callus (including escapes)



Transgenic callus

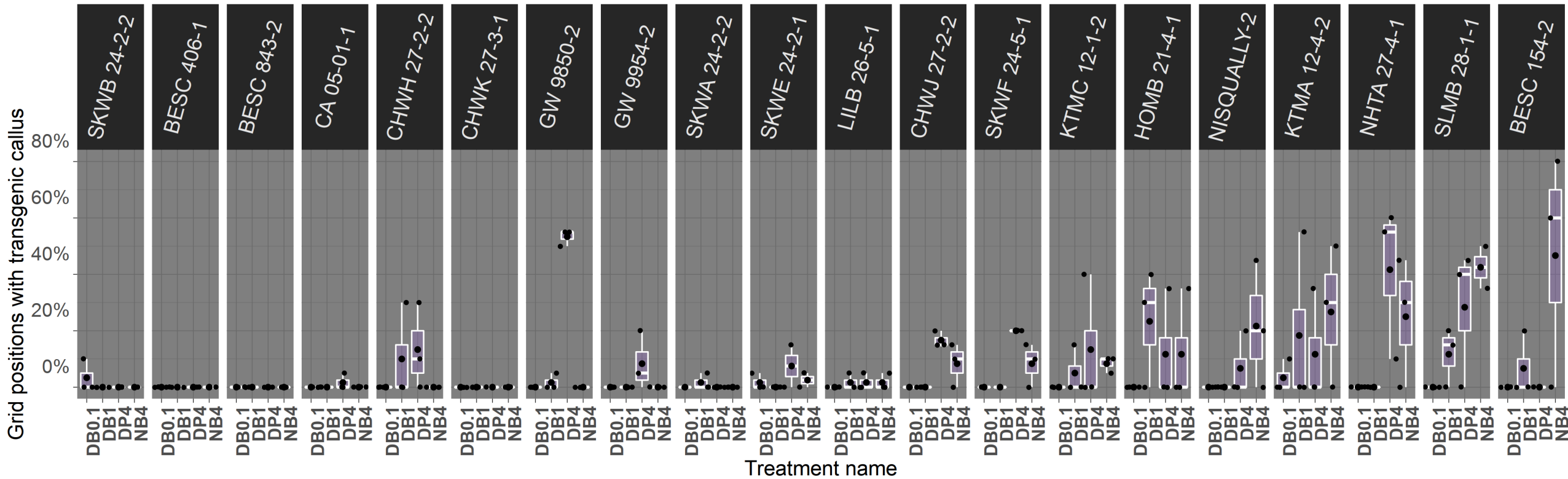


Mixture ID	2,4-D (mg/L)	NAA (mg/L)	BAP (mg/L)	2ip (mg/L)
DB0.1	0.001	0	0.5	0
DB1	0.01	0.01	0.5	0
DP4	0.1	0.1	0	1
NB4	0	0	1	0

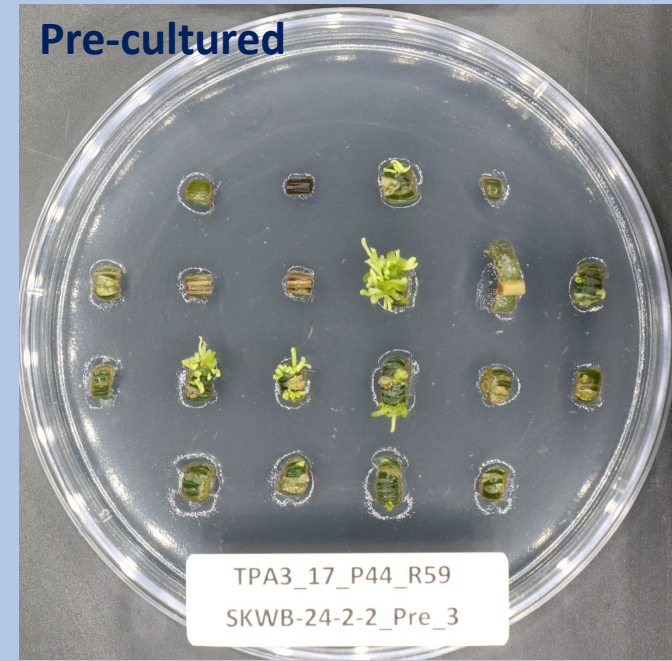
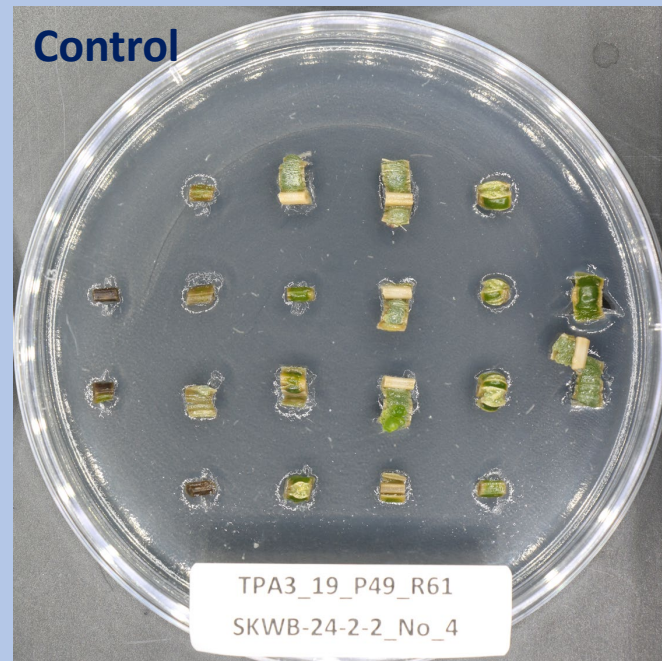


# Genotype-dependent effects of CIM treatments

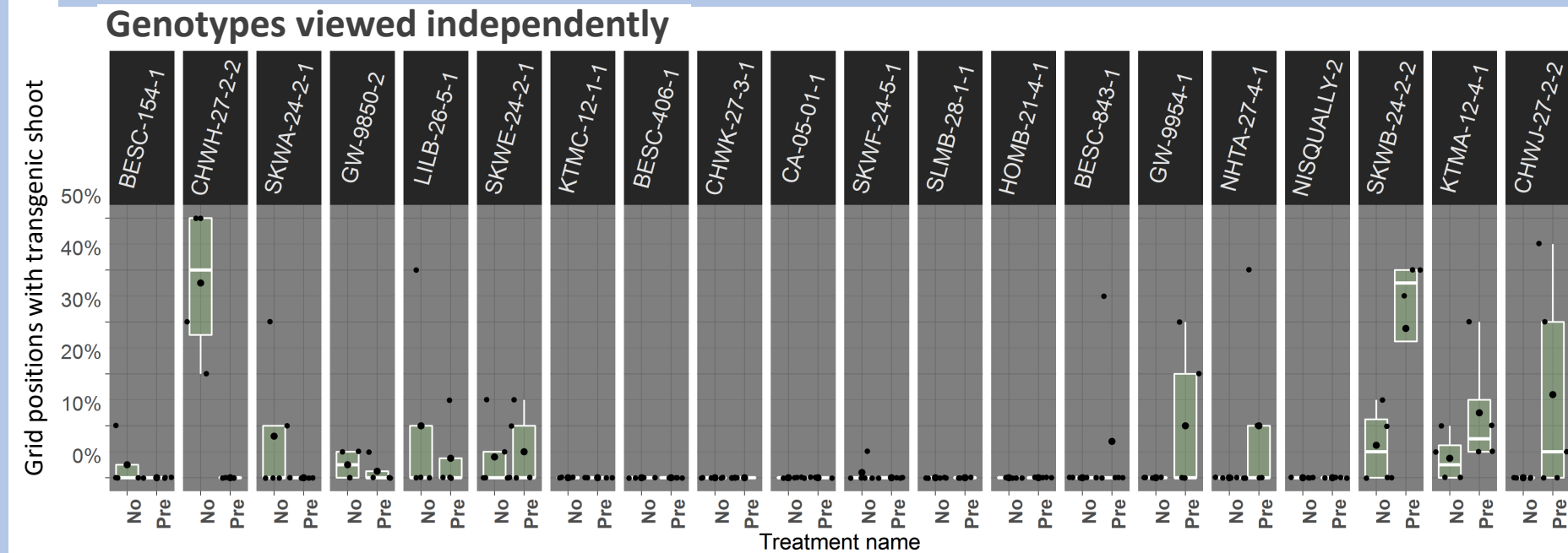
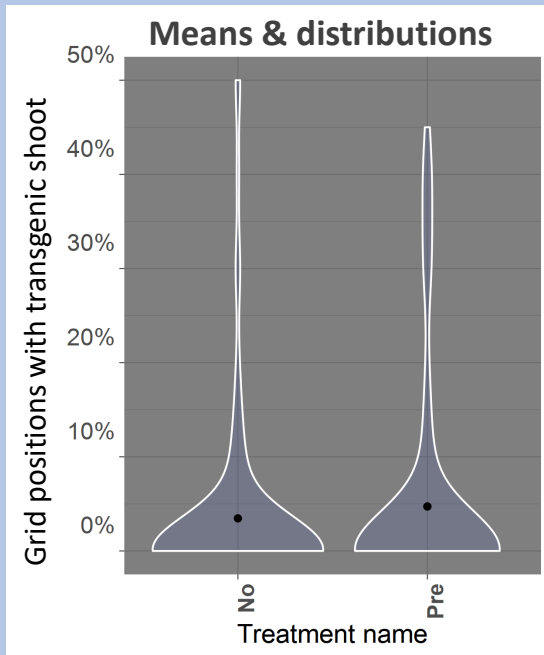
Rates of transgenic callus regeneration



# CIM pre-culturing effects on regeneration, vary strongly with genotype



## Rates of transgenic shoot regeneration



# 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
- Use of pipeline to study auxin/CIM and pre-culture effects
- System ready for large scale GWAS of *in vitro* regeneration and transformation - underway



# Acknowledgements



**Cathleen Ma**  
Transformation &  
Greenhouse  
Experiments



**Kate Peremyslova**  
GWAS,  
Transformation  
Experiments



**Julie Kucinski,**  
GWAS, in vitro  
experiments



**Steve Strauss**  
PI, Professor



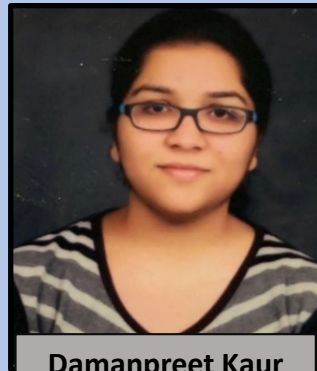
**Amanda  
Goddard**  
Program & Field  
(clone bank)  
Manager



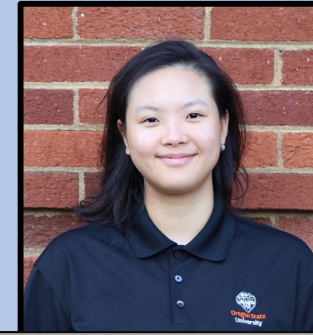
**Fuxin Li**  
Co-PI, Professor, Machine  
Vision



**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

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