Development of an imaging-based phenomics system for *in vitro* GWAS studies of plant regeneration and transformation

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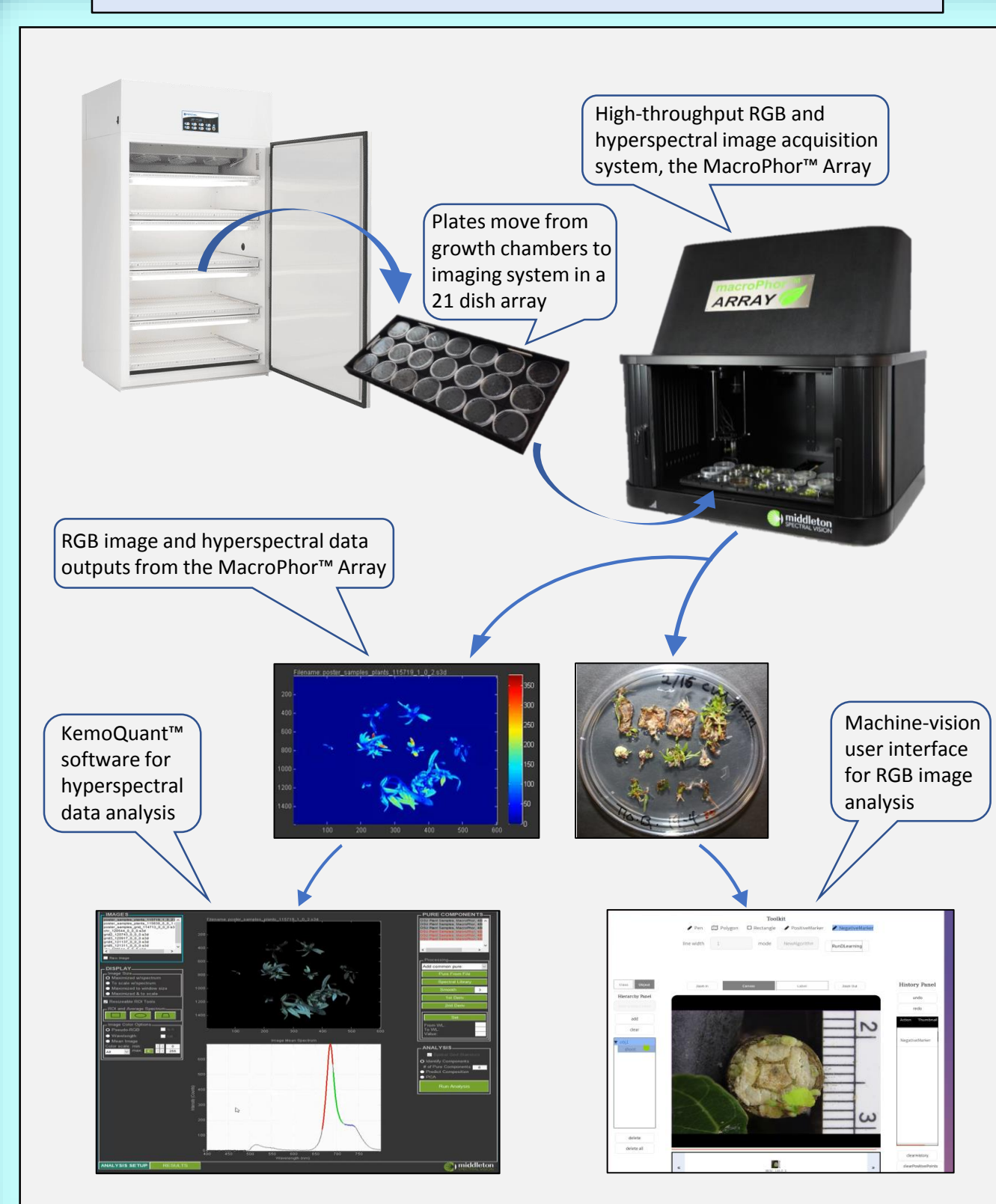
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Abstract

Collecting quantitative data on *in vitro* plant material has long been difficult as it relies on scoring systems that are subjective and time consuming. Oregon State University has partnered with Middleton Spectral Vision (MSV, a Wisconsin-based company) to develop a phenomics pipeline that can efficiently generate high-throughput precision data. The work is part of an NSF-Plant Genome Research Program funded study to conduct genome wide associate studies (GWAS) to identify genes that determine the rate of regeneration and transformation in poplar (*Populus*). The pipeline begins with an RGB and hyperspectral imaging system that is coupled with two routes of data extraction: KemoQuant™, a software developed by MSV that can analyze spectral data, and a machine-vision system to identify and quantify tissues from RGB and ultimately hyperspectral images.

Pipeline overview

High-throughput RGB and hyperspectral image acquisition system, the MacroPhor™ Array

Plates move from growth chambers to imaging system in a 21 dish array

RGB image and hyperspectral data outputs from the MacroPhor™ Array

KemoQuant™ software for hyperspectral data analysis

Machine-vision user interface for RGB image analysis

Custom imaging system — The MacroPhor™ Array

The MacroPhor™ Array is designed to capture RGB and hyperspectral images across a 21 Petri dish configuration. Hyperspectral images contain a full 400-800nm emission spectrum for each pixel which allows for early and precise detection of fluorescence signals including GFP, and quantitative analysis of spectral components within a sample.
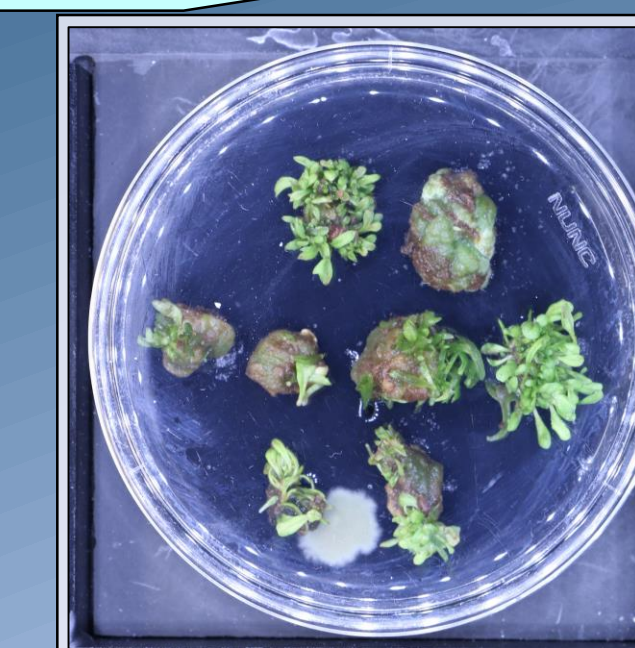

An LED light ring was developed to allow for RGB images without producing glare on the Petri dish lid (lights are visible at edges of Petri dishes, and ring is visible in chamber image to the middle-left).
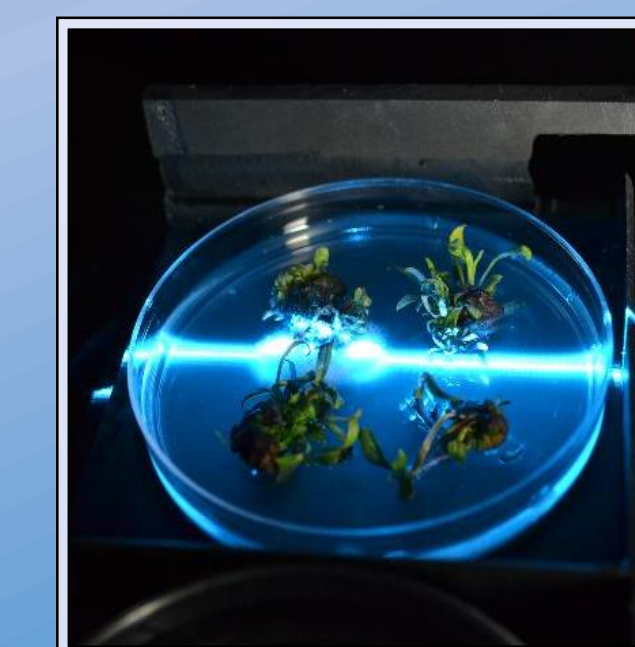
A 488nm wavelength laser scans over the area of a Petri dish to detect GFP, chlorophyll, and other fluorescence signatures in the sample.

Hyperspectral analysis

KemoQuant™ uses multivariate curve resolution to detect pure component fluorescent signals within spectra and automatically exports data on signal intensities and component ratios.
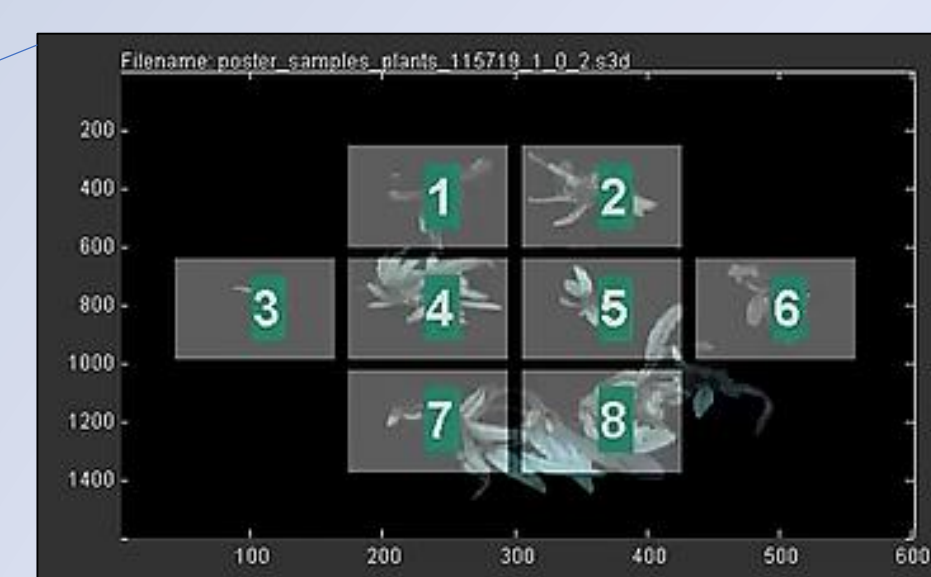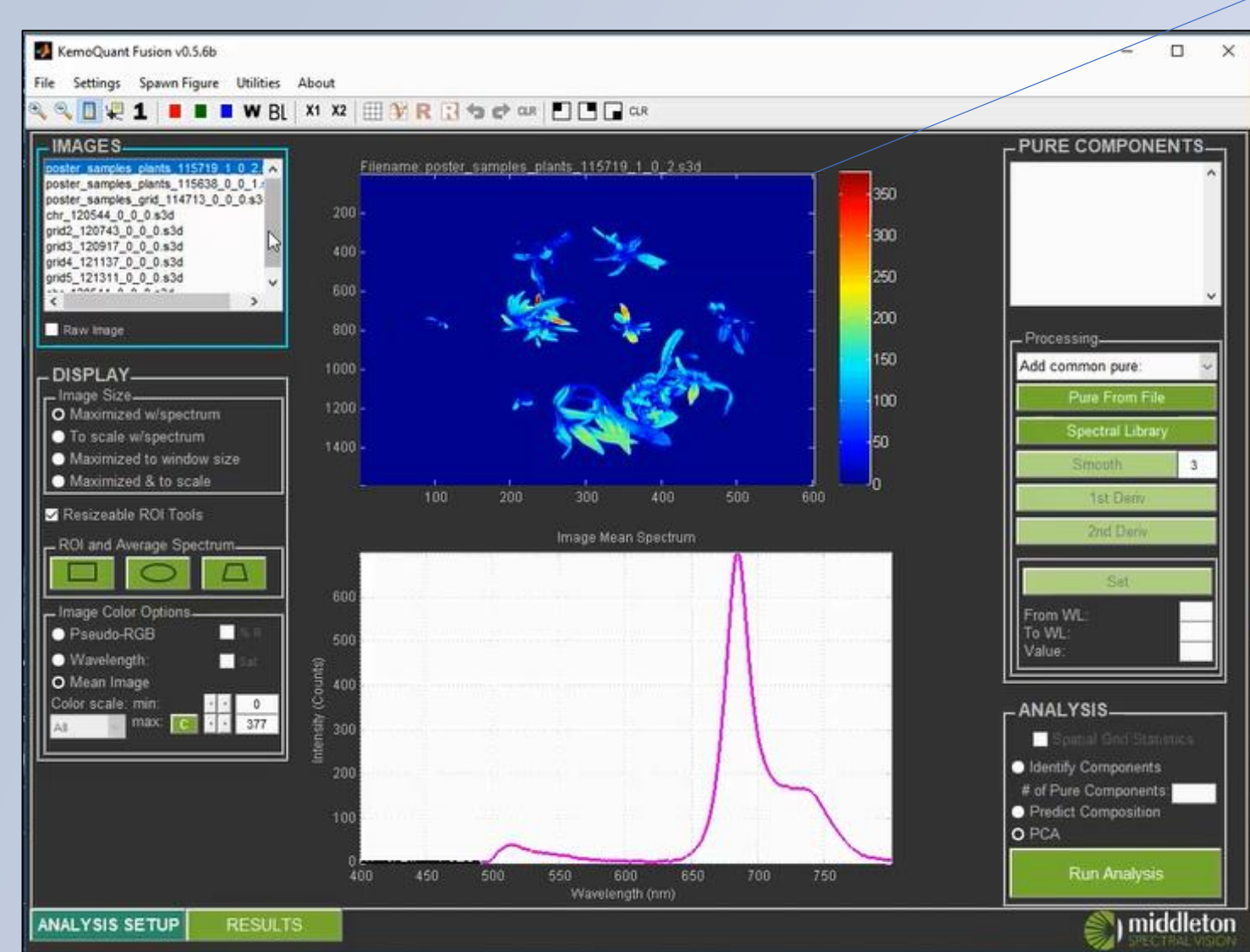
A custom grid overlay system was developed so that KemoQuant™ will recognize tissue explants on a Petri dish and output their data individually.
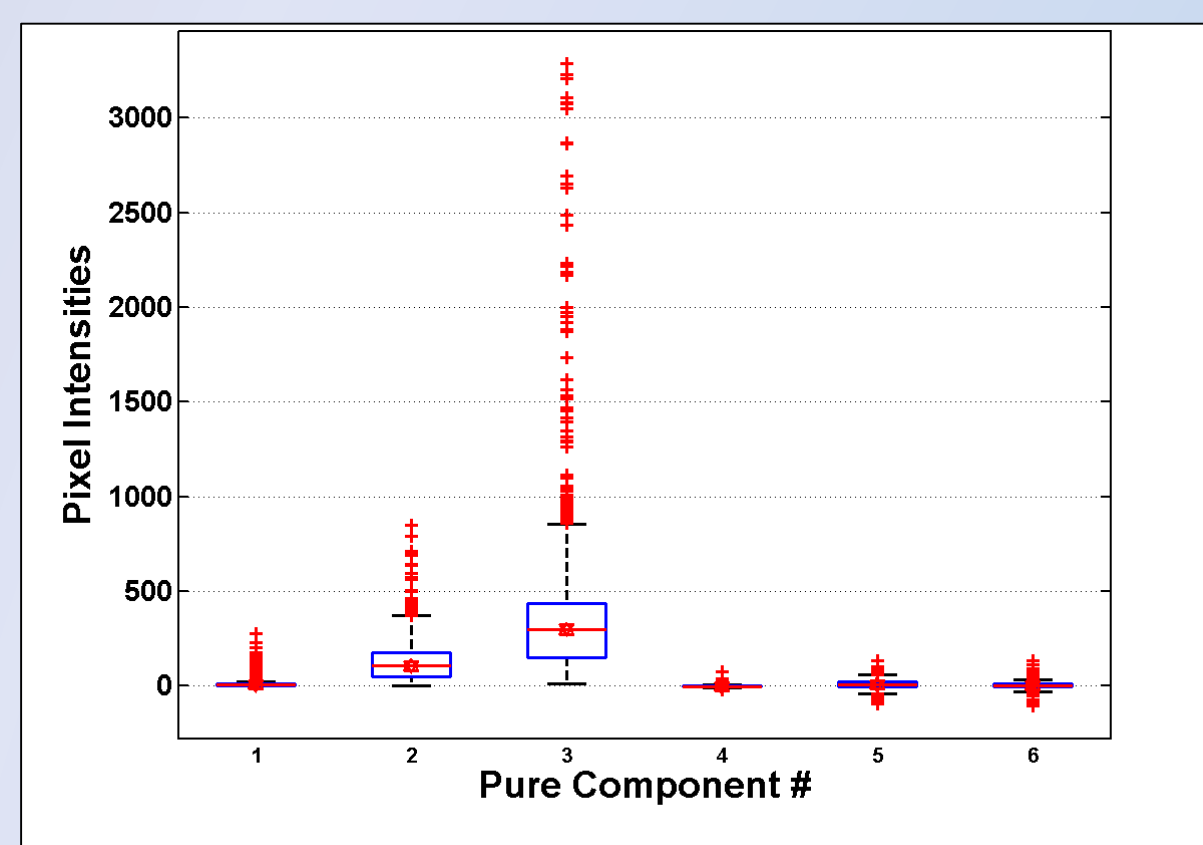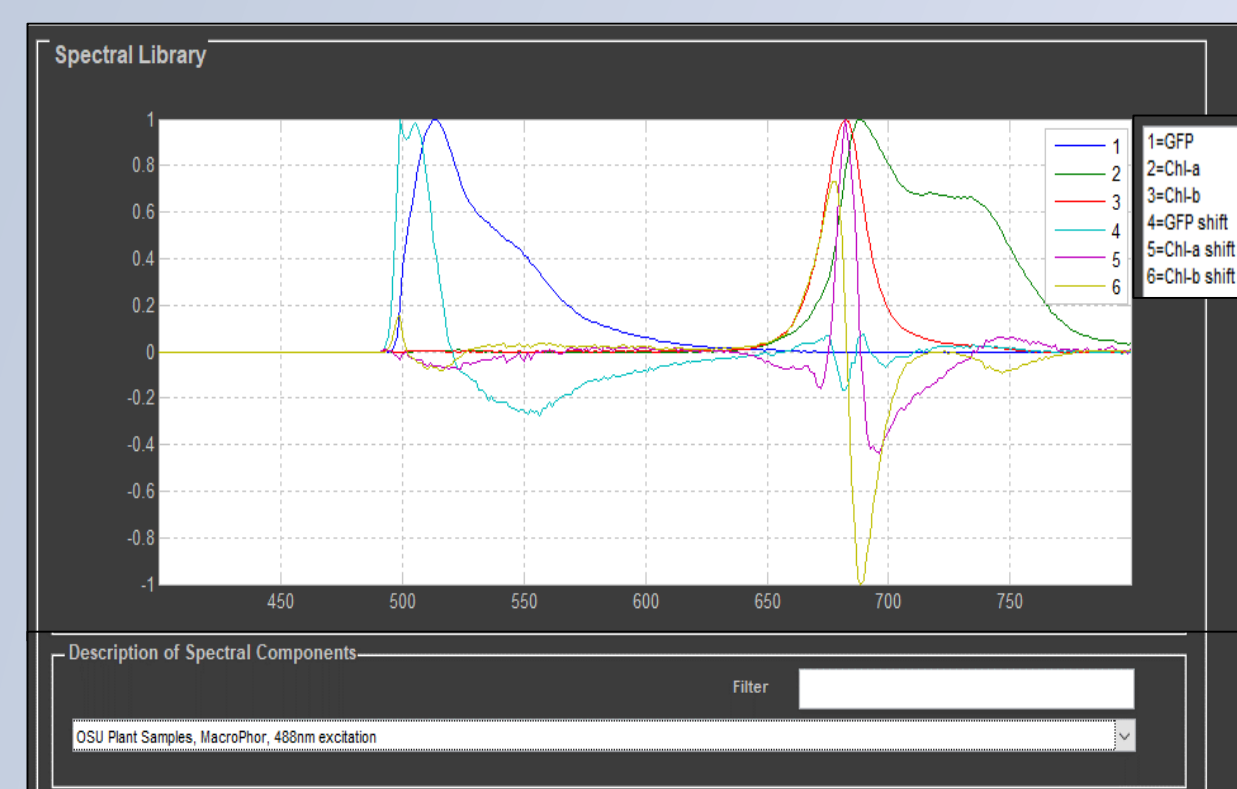
Pure components for an OSU sample include GFP, chlorophyll a, chlorophyll b, and a unique signature shift for each signal.

Machine vision

(A) Raw RGB image of *in vivo* poplar stem regenerating callus and shoots. Using a custom GUI (graphic user interface) seen in (B), it was manually annotated to train the machine vision algorithm, which is based on a deep neural network (DNN) model.
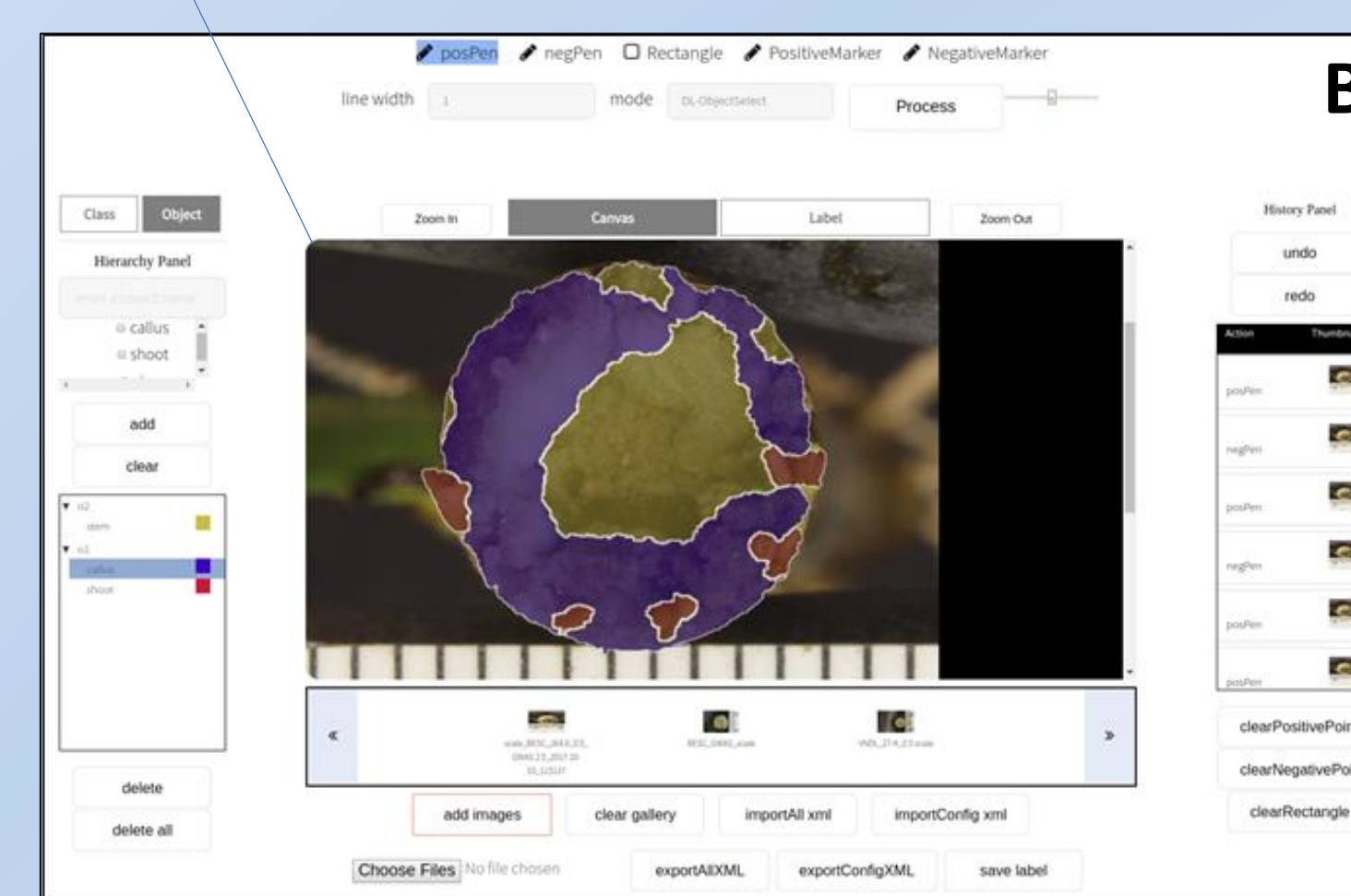
(C) Results from the first round of DNN training. The middle column shows the user annotated image to its left, and the right column contains the results from the DNN model output. The DNN model returns an estimated probability for each pixel as to whether it believes the annotation it chose is correct—its current estimate is above 90%, but as this consists mostly of background, we are working on a larger training set to further refine and increase resolution.
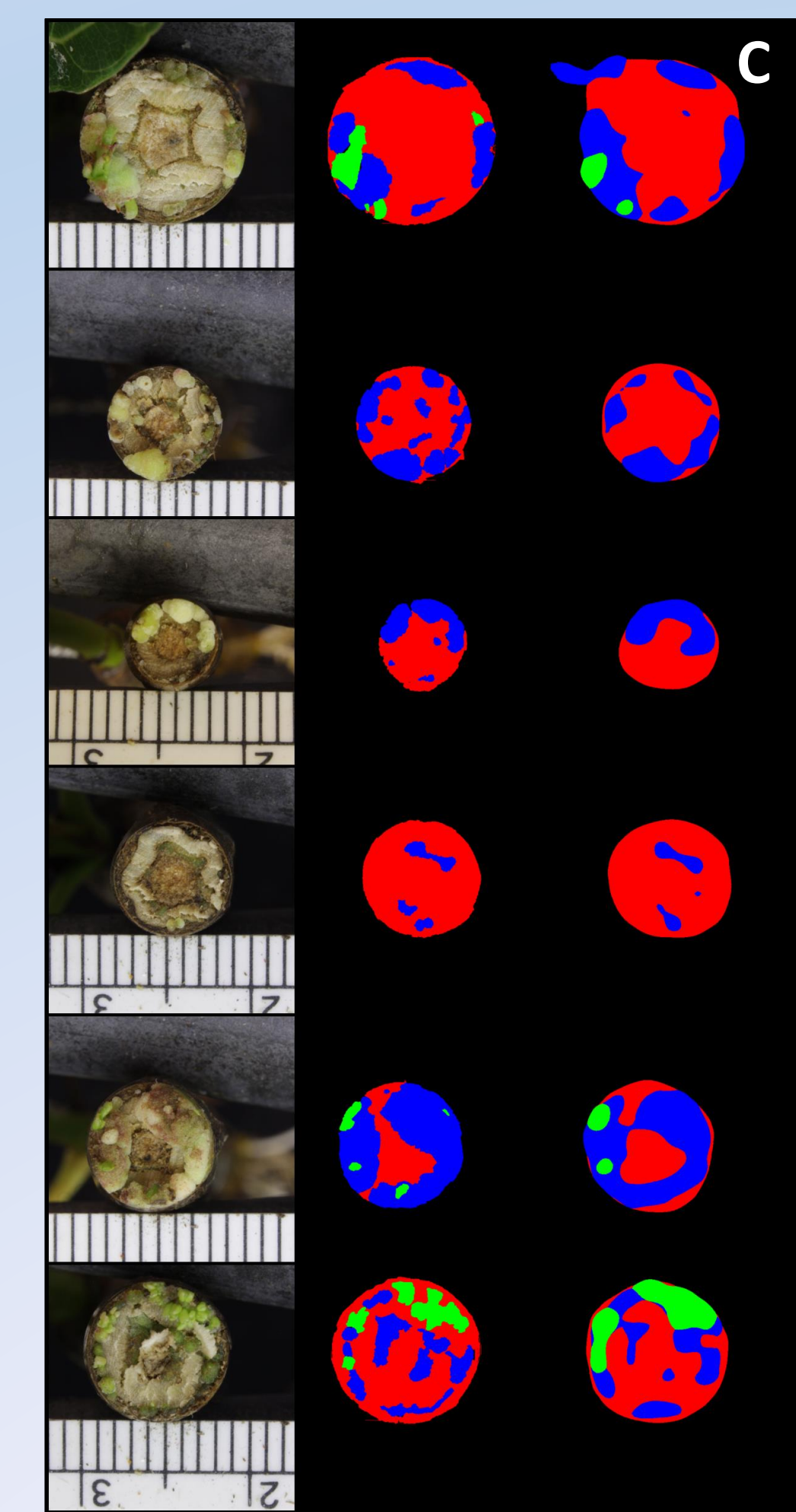
Summary

- The Middleton Spectral Vision system enables consistent, high throughput, and high resolution RGB and hyperspectral imaging of tissues in Petri dishes
- Its hyperspectral image files and software enable unique spectral signatures, including of GFP and chlorophylls, to be developed, extracted, and quantified for specific explants
- Hyperspectral imaging of fluorescence allows us to quantify transformation rate and genetic variation in hormone reactivity using with auxin- and cytokinin-responsive GFP/YFP
- A graphic user interface for annotation in machine vision has been developed that allows us to specify target classes of tissue types within explants (e.g., callus, leaves, shoots, roots)
- Training on the deep neural network model is ongoing and will ultimately allow us to obtain high-throughput data for an *in vivo* GWAS study of variation in stem regeneration and rootability that is currently in progress
- Phenotyping of *in vitro* regeneration response for GWAS is expected to begin this fall through winter

Acknowledgements