

Introduction

Plants have strong potential as spectral indicators:

- Plants are naturally present in the environment and produce photosynthetic pigments that are detectable from remote distances
- Environmental exposures can lead to unique physiological responses and changes in photosynthetic pigments
- Prior SNL LDRD demonstrated the ability to reliably detect spectral changes in *Arabidopsis thaliana* upon exposure to metal stress

Approach

- Model System: **Tall fescue (*Festuca arundinacea* Shreb.)**
 - A common temperate perennial grass species found in pastures, roadsides, and cultivated turf in many areas of the world, including North and South America, Europe, Asia and Africa
 - Has high root and shoot biomass accumulation to facilitate absorption and uptake of Cr(VI).
- Brightfield and confocal fluorescence microscopy:
 - Spatial-temporal quantification of total chlorophyll in tall fescue at the microscopic level.
- Hyperspectral confocal fluorescence microscopy:
 - Identifies and quantifies chlorophyll associated with individual photosystems with subcellular level resolution.

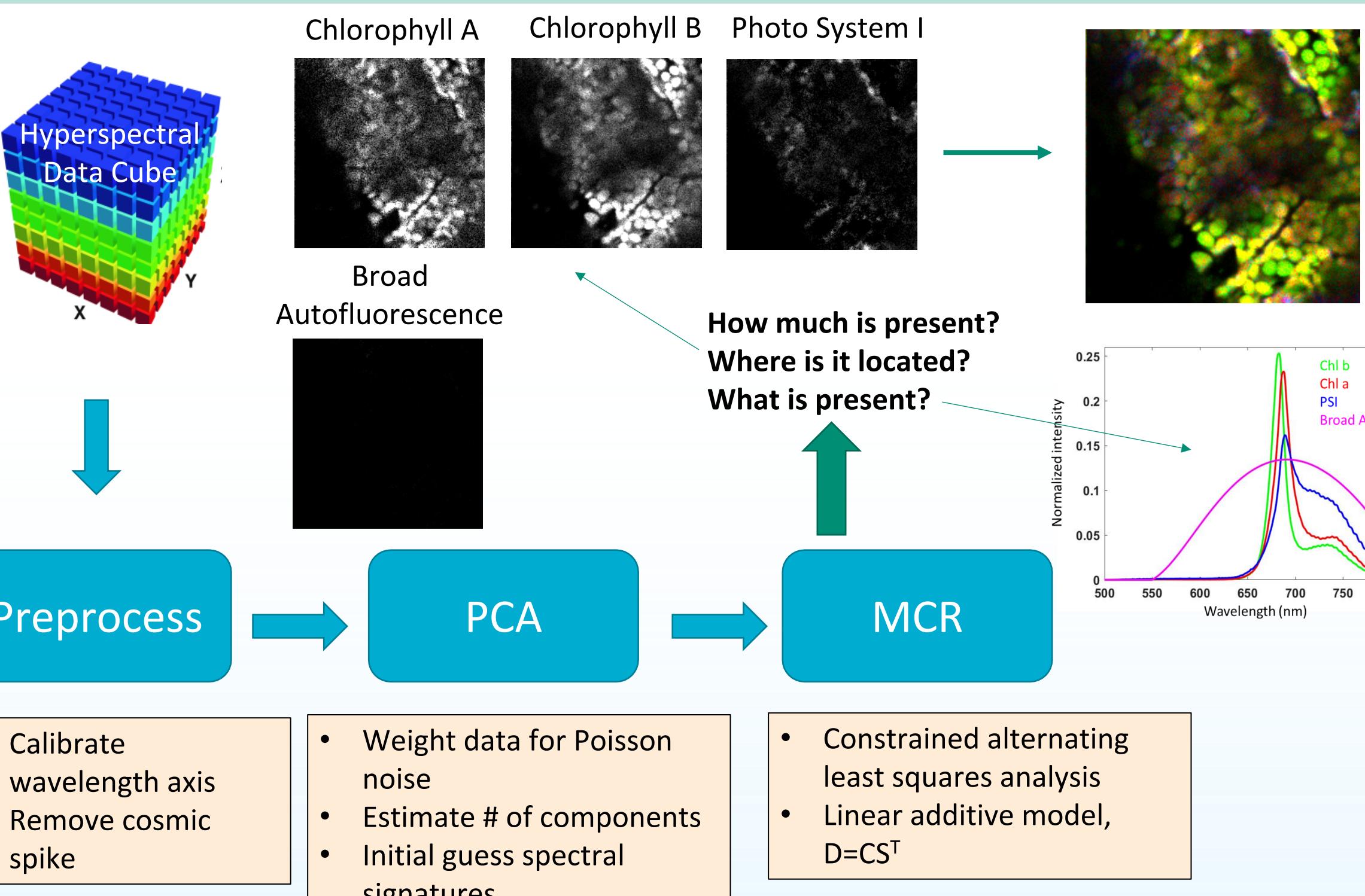


Fig 1.: Hyperspectral Confocal Fluorescence Microscopy (HCFM) Workflow: Samples were excited with 488nm laser and an entire emission spectrum was collected at each image voxel. Samples were then processed using Multi-Curve Resolution (MCR) where the pigments are mathematically isolated and quantifies all emitting species in a sample simultaneously (Jones 2012).

Experimental Overview

Chronic Chromium (VI) Exposure

- Healthy Control, Low Cr(VI): 150mL per day of 50ug Cr(VI)/L, High Cr(VI): 150mL per day of 22.5mg Cr(VI)/L
- 28-day assessment after initial application of Cr(VI)
- 2 plants per condition

Data Collection

- Brightfield and Confocal Fluorescence Microscopy images were collected on days -1, 6, 13, 20, and 27
- HCFM images were collected on days 0, 7, 14, 21, and 28 {10 leaf cross-sections were imaged per plant}
- Digital Images were collected on day 0, 2, 7, 10, 14, 21 and 24 {5 leaf cross-sections were imaged per plant}

Quantitative analysis of total chlorophyll fluorescence from confocal fluorescence images

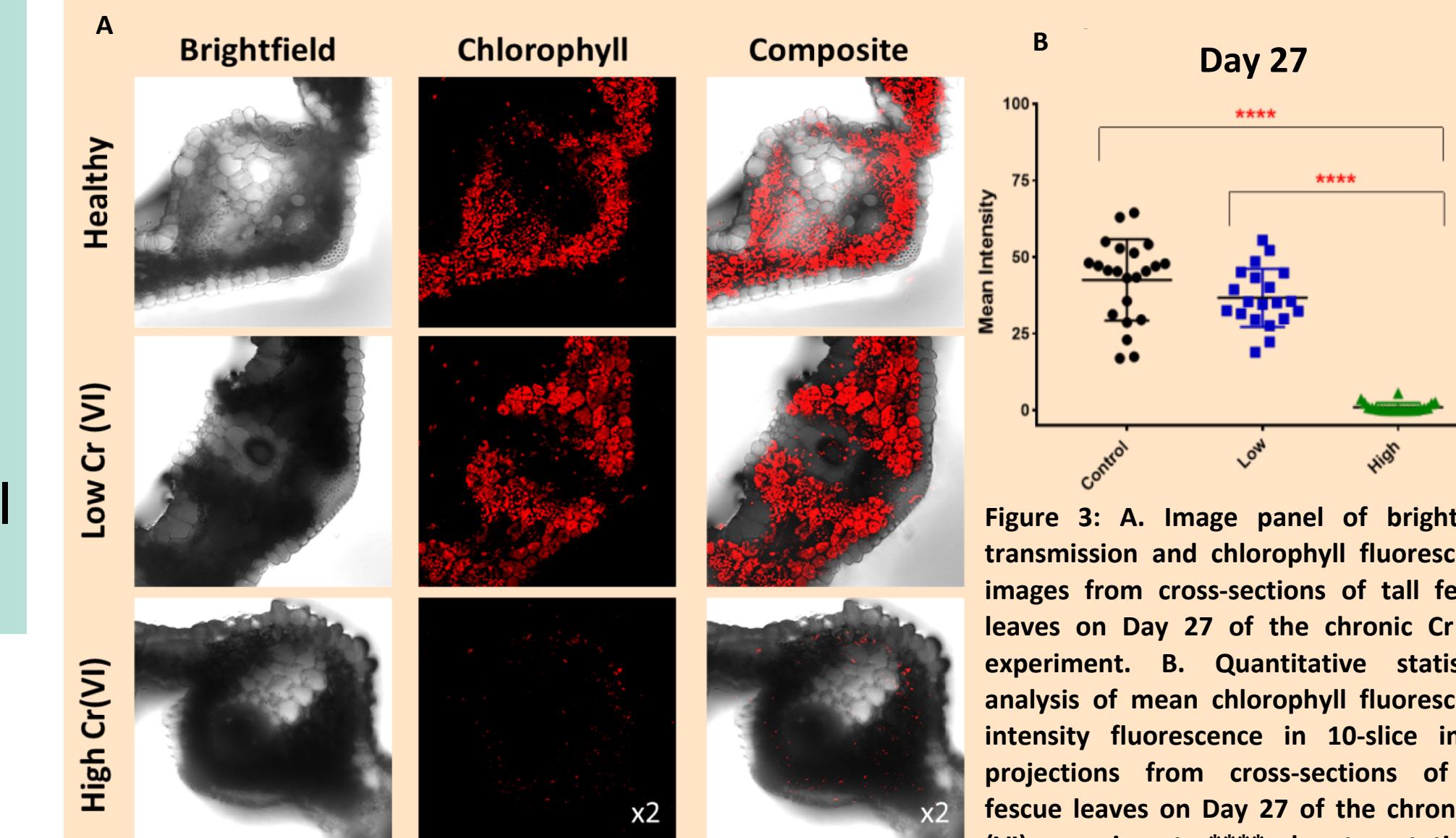


Figure 3: A. Image panel of brightfield transmission and chlorophyll fluorescence images from cross-sections of tall fescue leaves on Day 27 of the chronic Cr (VI) experiment. B. Quantitative statistical analysis of mean chlorophyll fluorescence intensity in 10-slice image projections from cross-sections of tall fescue leaves on Day 27 of the chronic Cr (VI) experiment. **** denotes statistical significance ($p<0.0001$).

Quantitative image analysis of structural features from brightfield images

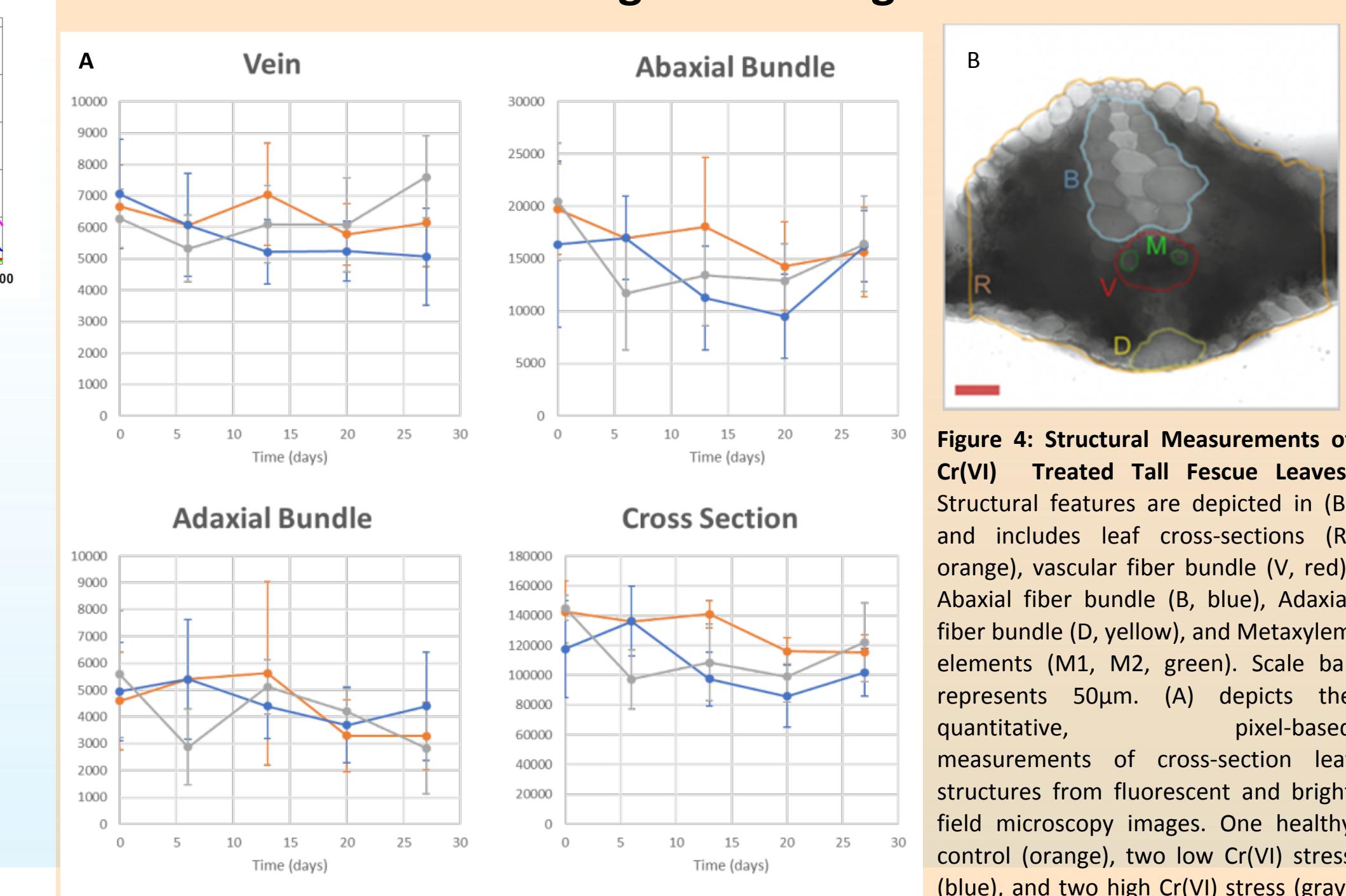


Figure 4: Structural Measurements of Cr(VI) Treated Tall Fescue Leaves. Structural features are depicted in (B) and includes leaf cross-sections (R, orange), vascular fiber bundle (V, red), Abaxial fiber bundle (B, blue), Adaxial fiber bundle (D, yellow), and Metaxylem elements (I1, M, green). Scale bar represents 50μm. (A) depicts the quantitative, pixel-based measurements of cross-section leaf structures from fluorescent and bright field microscopy images. One healthy control (orange), two low Cr(VI) stress (blue), and two high Cr(VI) stress (gray) plants were imaged and analyzed. Two representative leaf blades were chosen from each plant and cross-sectioned for imaging.

Results

- A progression of reduced chlorophyll fluorescence was observed in the high Cr(VI) stress condition and moderate chlorophyll fluorescence reduction was observed in the low Cr(VI) stress condition.
- Moderate and severe changes in leaf structure were observed under the high Cr(VI) stress condition visually, high leaf-to-leaf and plant-to-plant variability prevented any statistical significance.
- The spatial distribution of each of four spectral components show statistically significant changes as observed in the decrease of Chl-a, Chl-b, and PSI at Day 14 for the high Cr(VI) treated plants and an increase in the Broad AF feature. This trend continues and is enhanced at Day 21 and Day 27.

Spatial-temporal distribution of each spectral component identified by HCFM

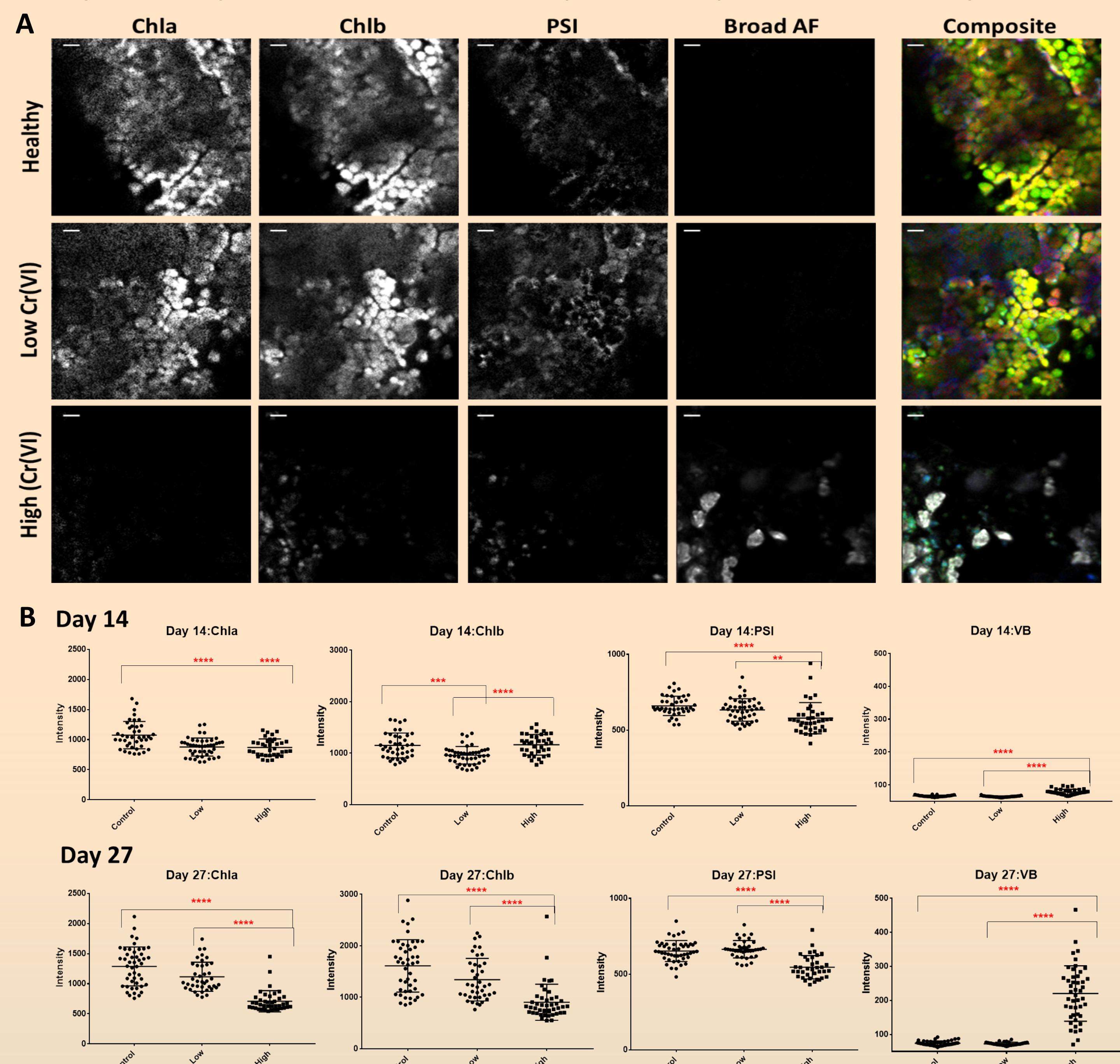


Figure 5: A) Representative image panel containing HCFM results for tall fescue leaf cross-sections on Day 28. Scale bars indicate 2 μm. B) Quantitative analysis of images of photosynthetic pigments (Chl-a, Chl-b, PSI) and the still to be identified Broad AF feature in tall fescue for each experimental condition at Days 14 and 27.

Acknowledgements: The authors also acknowledge Dr. Michael Sinclair (SNL) for his assistance and maintenance of the hyperspectral confocal fluorescence microscope, Bryce Ricken (SNL) for collection of the digital images of tall fescue, and Dr. Tessily Hogancamp (SNL) for assistance in collecting the confocal fluorescence images.

