

## Reviewer Report

**Title: Technical workflows for hyperspectral plant image assessment and processing on the greenhouse and laboratory scale**

**Version: Original Submission**    **Date: 4/15/2020**

**Reviewer name: Yufeng Ge**

### Reviewer Comments to Author:

This is a review paper focusing on close-range hyperspectral imaging for plant assessment in the greenhouse and laboratory scales. Given the broad interest of using hyperspectral imaging for plant phenotyping research, as well as the complexity of data structure and analysis method, this manuscript is quite timely and relevant. The hyperspectral image is known for its large data volume. The topic thus is appropriate for the journal. The paper covered the topics including camera and measurement setup, data preprocessing, and data analysis/interpretation. The authors' argument is that a standardized workflow for image acquisition, processing and analysis is needed to make the data comparable among various labs, which is a valid point. The paper provides a good technical summary of hyperspectral imaging (such as camera and imaging stage setup, white referencing), and gives a good compilation of its applications on plant assessment that can be useful for the phenotyping research community. My major comments for the authors to consider improving the manuscript are in the following.

Section of spectral smoothing. The authors only discussed Savitzky-Golay method and missed many other methods that are common for spectral preprocessing. In addition to spectral averaging (binning) that the authors also discussed, other methods like Multiplicative Signal Correction and Standard Normal Variate are also widely used. Other preprocessing such as first and second order derivative are also common. Note Savitzky-Golay can also be used for differentiation. I think you need to mention these methods rather than just Savitzky-Golay.

Preparation for ML. Your discussion of calibration set, validation set, and test set are not correct. In machine learning, calibration set is for model calibration (to calibrate model parameters), validation is for model hyper-parameter tuning, and the test set is to evaluate the performance of the developed model. Please make sure you express this correctly. In some implementations, an explicit validation set is not used where model calibration and hyper-parameter tuning are conducted together. In these implementations, test set is also referred to as validation set. I would recommend the authors to read some of the literature on NIRS analysis, as when the images are reduced to the spectrum level, the (pre)processing and analysis share commonalities. There are quite a few publications recently on using VIS-NIR-SWIR for leaf analysis in the context of plant phenotyping. Please study those so you can see calibration/validation schemes and spectral preprocessing.

The explanation following Equation 1 was poor. I cannot understand it. Please revise.

There is significant room for the authors to improve the writing and presentation of the manuscript.

There are quite a few places where the wording and phrases can be improved. Please see my comments on the attached document.

## Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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Are the conclusions adequately supported by the data shown? Choose an item.

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