Application Note

Quantifying Chlorophyll in Leaves Using a Non-destructive Method with the **UniSpec-SC**

Chlorophyll is a driver of the physiological function of leaves. Quantifying chlorophyll content can provide information regarding the physiological state of leaves. Chlorophyll tends to decline rapidly when plants are under stress or during leaf senescence (Gitelson & Merzlyak 1994). It is very vital to understand the pattern of the pigment content change if we are to understand plant/ecosystem function.

Most of traditional methods of pigment analysis (e.g. high performance liquid chromatography (HPLC)) require destruction of the measured leaves which are not ideal to obtain long term data (Sims and Gamon, 2002). It is also time consuming and expensive to process the samples. A research group directed by Dr. John A. Gamon at California State University, Los Angeles has applied a non-destructive method using **UniSpec-SC** to measure the spectral reflectance of intact leaves. They compare the leaf spectral reflectance with leaf pigment (e.g. chlorophyll) content, leaf water content and the rate of the photosynthesis. They have used this approach to measure the leaves of different species including evergreen shrub species and annual grasses across different seasons. Leaf structural differences among species (i.e. thickness, density, cuticle thickness and pubescence) will have significant effects on the relationships between leaf spectral reflectance and leaf physiological pattern (Sims and Gamon, 2002).

Researchers from California State University are using the **UniSpec-SC** to measure chaparral species spectral response at Sky Oaks Biological Field Station.

Figure 1. Close up of the **UniSpec-SC** connected to a bifurcated fiber optic and a leaf clip for monitoring spectral response of chaparral species at Sky Oaks Biological Field Station in Northeast San Diego County in southern California.

The **UniSpec-SC** can be set up as both a reflectometer to measure the spectral reflectance and as a spectrophotometer to measure the absorbance. A reflectometer consists of a **UniSpec-SC** (with integral light source), a bifurcated fiber optic and a leaf clip (Gamon and Surfus 1999). To calculate the leaf spectral reflectance, a white standard (99% reflective) is needed to set the reference. The reflectance is calculated as the spectrum of the leaf divided by the spectrum of the standard (equation 1):

$$R_{target} = \frac{I_{target}}{I_{99\%\,panel}} \qquad (1)$$

where R_{target} is the percent reflectance of the target, $I_{99\%panel}$ is the response of the **UniSpec-SC** sensor when the leaf clip is clipped over the 99% reflectance panel, I_{target} is the response of the **UniSpec-SC** sensor when the leaf clip is clipped over the target. Different vegetation indices can be calculated using different combination of the reflectance at a certain wavelength based on the optical properties of the target studied. The $NDVI_{750}$ (also called chlorophyll index) is generally used to estimate chlorophyll content and is calculated using equation 2:

$$NDVI_{750} = \frac{(R_{750} - R_{705})}{(R_{750} + R_{705})} \qquad (2)$$

where $NDVI_{750}$ is normalized difference vegetation index calculated using spectral reflectance at 750nm and 705nm, R_{XXX} indicates reflectance at a particular wavelength (indicated by XXX, in nm) (Gitelson and Merzlyak 1994).

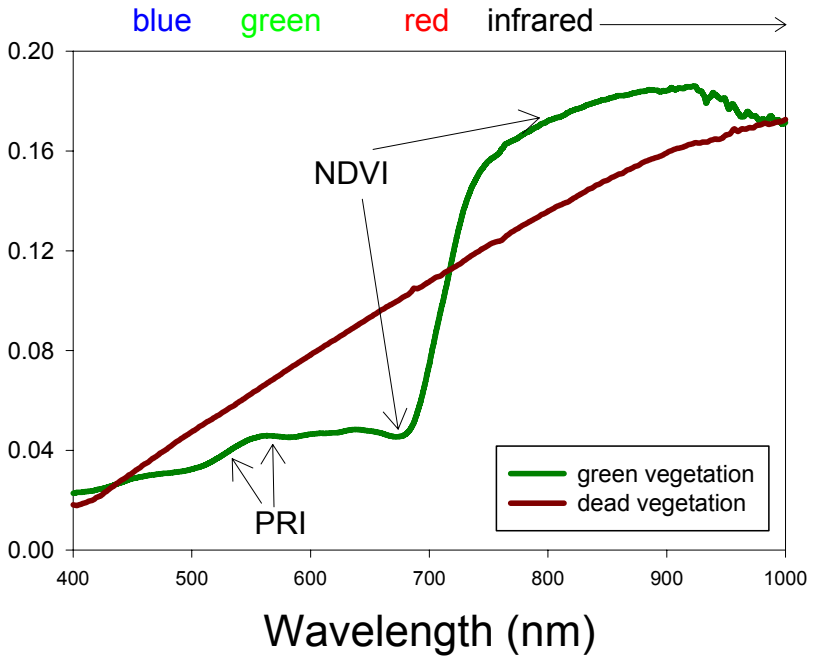

Figure 2. Spectral response monitored using **UniSpec-SC**. Note the difference of green leaf versus dead leaf. This figure is courtesy of Sims D when working at California State University, Los Angeles.

The **UniSpec-SC** can be easily set up as a spectrophotometer to measure the pigment absorbance. In this case, a clear solution serves the reference and the solution of leaf extract (from frozen sample) of known volume is the target. Similarly to the equation 1, the absorbance of a leaf sample is calculated from the ratio of the response of leaf extract solution to the reference standard.

The equations below (derived from Porra et al. 1989) can be used to estimate the concentration of chlorophyll a, chlorophyll b, and total chlorophyll. Note that A_{XXX} refers to absorbance at a specific wavelength (XXX, in nm.). The unit of the pigments concentration is nanomoles per milliliter.

$$\text{Chl a concentration} = (13.71 * A_{663.6}) - (2.85 * A_{646.6}) \quad (3)$$

$$\text{Chl b concentration} = (22.39 * A_{646.6}) - (5.42 * A_{663.6}) \quad (4)$$

$$\text{Chl a+b (total chl) concentration} = (8.29 * A_{663.6}) + (19.54 * A_{646.6}) \quad (5)$$

References:

Gamon JG, Surfus JS (1999) Assessing leaf pigment content and activity with a reflectometer. *New Phytologist* 143:105-117.

Gitelson A, Merzlyak MN (1994) Spectral reflectance changes associated with autumn senescence of Aes*culus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. *Journal of Plant Physiology* 143:286-292.

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta,* 975:384-394.

Sims, DA., and Gamon, JA (2002). Relationship between pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment, 81, 337-354.*

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