A CID Bio-Science, Inc. White Paper

CI-700 Spectrometer and Leaf Probe Attachment

Description and Applications

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Portable Instruments for Precision Plant Measurement

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CI-700 Spectrometer and Leaf Probe Attachment

Introduction

The CI-700 Spectrometer with the leaf probe attachment is a cost-effective instrument that provides qualitative spectral information of light from all sources and the interaction of light with biological substances. A biological substance may absorb light efficiently at a certain range of wavelengths and transmit/reflect other wavelengths of light. For example, most photosynthetically active green leaves strongly absorb blue/blue-green and red light and reflect green and yellow light. The presence of different pigments, organic compounds, or inorganic chemicals in biological substances exhibit distinct characteristics in the wavelength spectrum. Obtaining and/or monitoring these unique spectral characteristics can provide useful information for monitoring many physiological activities of biological systems, including the metabolism involving photosynthesis, status of nutrient uptake by plants, changes in chemical content or concentration in plant organs, biomass changes with respect to environmental parameters, and detection of plant diseases.

Due to the naturally rough surfaces on biological substances such as leaves, accurate measurement of absorption or reflectivity spectrum requires expensive optical apparatus and complicated measurement procedure. CI-700 offers a low-cost and easy to use alternative that allows users to quickly obtain the substance's spectroscopic characteristics within a wide range of wavelengths that cover UV, Visible, and Near Infra-Red (NIR) light.

Application of Spectroscopy in Plant Physiology

When a substance is illuminated with light, only a portion of the wavelengths of the light may be absorbed by the substance. The unabsorbed light is either transmitted through, or reflected/scattered off the substance without interacting with the substance (Fig. 1). The interactions of light are strongly influenced by the types and structures of chemicals present, and the electrostatic interactions between the chemicals of the substance. Aromatic hydrocarbons (such as benzene), conjugated organic compounds (such as chlorophyll (Fig. 2(a)), or dyes, absorb in the mid UV to visible range (200~700 nm). Proteins, containing certain amounts of aromatic amino acids, exhibit strong absorption around 280 nm. The protein concentration is thus commonly determined by measuring the absorbance in the near UV range.

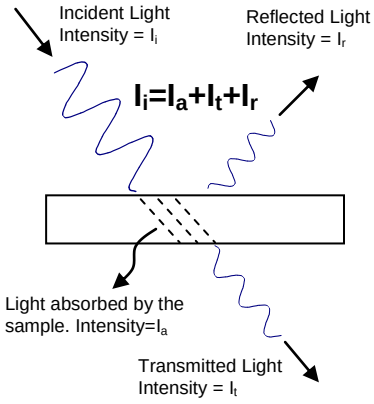


Figure 1. Schematic showing the interaction of light and a sample

For plants, light is absorbed by pigments, primarily chlorophylls and carotenoids that are responsible for photosynthesis. These pigments in leaves effectively absorb light at the blue/blue-green range (400-500 nm) and the red range (650-700 nm), while reflecting most of the green and yellow light (500-600 nm), making the leaves appear green (Fig. 2 (b)). Therefore, for photosynthetic studies with a

broadband light source, it is important to measure the irradiance of the light at wavelengths which the plant absorbs, instead of the total irradiance of the light.

In photosynthetically active biological organisms, the light-absorbing pigments are bound to proteins. Absorption of light by the pigments initiates a series of structural changes in proteins, the end result of which is the conversion of light energy into chemical energy that can be utilized in plant metabolism. The electrostatic interaction between pigments and their surrounding proteins affects the absorption spectrum of the pigments. The absorption spectrum of the pigments may change due to the changes in protein structure, which is sensitive to environmental parameters such as temperature, pressure, humidity, the water content in the plant, the presence of certain chemicals, or changes in ionic concentration. For example, chemicals in the plant nutrient, such as N, or P, are known to affect the color of leaves, and the soil pH has a strong effect on the color of certain flowers. Measuring and monitoring the absorption spectrum of certain organisms can be useful in monitoring nutrient uptake by a plant*. The increase or decrease in pigment concentration, and thus the absorption spectrum, can reflect the health status of organisms. Therefore, absorption spectrum measurement and analysis can be useful in identifying key environmental parameters for photosynthesis or plant growth status, studying the plant response to the environmental stress, or early detection of diseases.

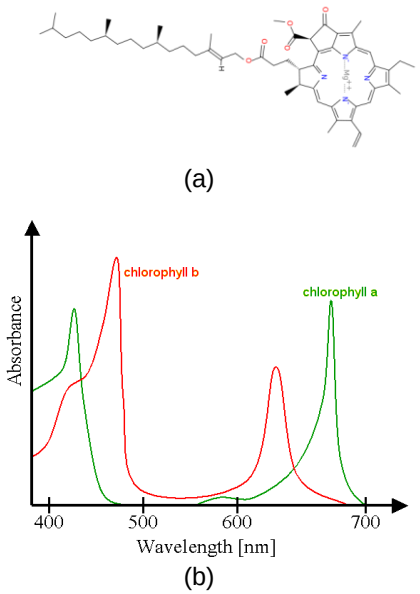


Figure 2. (a) molecular structure of chlorophyll a, and (b) absorption spectrum of Chlorophylls

In addition to the applications in the UV and Visible range, the absorption, or reflectivity spectrum in the IR range (above 700 nm) also offers a wide range of applications in plant physiology. Each chemical bond in a molecule vibrates at unique frequencies, called the vibrational states of that bond. The CI-700 covers the wavelengths up to 1100 nm in the Near IR range and is useful in detecting certain chemical bonds, such as the O-H and C-H bonds. Detection of these chemical bonds enables monitoring of the concentration water, sugar, or other organic chemicals in biological organisms[+].

*Keskin, et al, found spectral changes at the green band (520-580 nm) and NIR region (770-1050 nm) as N concentration increased in creeping bentgrass. (Keskin, M. et al. Assessing nitrogen content of golf course turfgrass clippings using spectral reflectance , Annual American Society of Agricultural Engineers, Abstract, 2001

[+] Walsh, Kerry B., et al., (2000) "Applications of Commercially Available, Low-cost Miniaturised Spectrometers to the Assessment of the Sugar Content of Intact Fruit". *Australian Journal of Plant Physiology*, vol. 27, No. 1, pp. 1175-1186

Theory and Operation

The CI-700 is comprised of two main modules: a USB (Universal Serial Bus) powered spectrometer with operating software, and a leaf probe attachment module. The leaf

probe's attached broadband light source can be conveniently placed in two positions for transmissive or reflective measurements. The spectrometer module transmits the light from the leaf probe attachment through a fiber optic cable, disperses the light by a diffraction grating, and projects the wavelength-dispersed light onto a CCD array. Each pixel of the CCD array corresponds to a specific wavelength of light. The operating software (SpectraSuite from Ocean Optics) displays the light intensity of each pixel of the CCD array. Common spectroscopic measurements are described in detail in the following.

Intensity (I) shows the electrical response of the CCD pixels to light transmitted through the fiber optic cable. Since each CCD pixel is correspondent to a specific wavelength, the response from the entire CCD array represents the intensity spectrum of the incident light at the full wavelength range (specified by the grating specifications). As is mentioned below, the transmittance, absorbance, and reflectivity of samples are calculated based on the intensity of the light source, it is essential to record at least one intensity spectrum of the light source before measuring any of these properties.

Transmittance (T) is the fraction of the incident light passing through a sample, such as a leaf. When the intensity of the incident light is $\mathbf{I_o}$, and the intensity of light passing through the sample is $\mathbf{I_t}$, the transmittance, **T**, is then expressed as $\mathbf{T = I_t / I_o}$. SpectraSuite can automatically calculate and plot the transmission spectrum with the reference spectrum (the intensity profile of the light source) stored (see the application tips below or refer to the operation manual of SpectraSuite).

Absorbance (A) is the fraction of incident light absorbed by the sample. It is related to the transmittance as $\mathbf{A = - \log T = -\log(I_t / I_o)}$. Absorbance is commonly denoted in optical density (OD) as it is linearly proportional to the amount of light absorbing sample in the light path length (Beer-Lambert law). For example, in a standard 1-cm cuvette, a sample with an OD of 2 is twice as concentrated as a sample with an OD of 1. Note that using the absorbance equation above, the intensity of light transmitted **(T)** from a sample with an OD of 2 is only 1/10 of that from a sample with an OD of 1. Transmission and absorption spectrum measurements are the most common applications of spectrometers. The spectrum can be used for the quantification of chemical concentrations, color analysis, the study of photochemical reactions such as photosynthesis, and the quantification of physical or optical properties such as film thickness, index of refraction, and extinction coefficient. In SpectraSuite, absorption spectrum is obtained much the same way as the transmission spectrum.

Reflectivity (R) is the fraction of incident light that is reflected from a sample. Depending on the purposes of the study, reflectivity can be measured for reflection at a specific angle from the sample, or the entire reflected hemisphere. The leaf probe attachment of CI-700 is not designed to measure the directional reflectivity. Nonetheless, it can be used to measure the absorption spectrum of surface substance of reflective or opaque samples.

Irradiance (Ir) is the amount of energy at each wavelength from a radiant sample. The relative irradiance is related to the radiant energy per unit area (watt cm^{-2}) illuminating

the sample. Common applications include characterizing the light output of incandescent lamps, or sunlight.

Operation Procedure and Application Tips

The CI-700 is primarily operated by the application software, SpectraSuite. The following sections describe important operation procedures and application tips when working with biological substances. It is assumed that the hardware connection and software installation have been successfully completed. General software functionality and operation procedures can be found in the operation guide included with CI-700.

Operation Procedure

As described earlier, the transmittance, absorbance, and reflectivity of a substance are all related to the intensity of incident light (or light source). The CI-700 is equipped with a single CCD array; the intensity of incident light and the transmitted/reflected light cannot be measured simultaneously. To calculate transmittance, absorbance, and reflectivity, it is necessary to separately measure dark spectrum (background noise), reference spectrum (intensity of light source without sample), and the spectrum with samples.

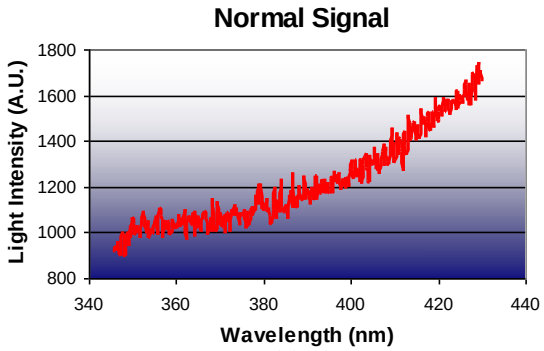
Dark spectrum – The dark spectrum is the background noise of the experimental setup with the light source turned off. Two factors could contribute to the noise: the electronic noise from the CCD array and the circuitry of the spectrometer, and/or the leakage of ambient light into the spectrometer. Electronic noise can be significant when the light intensity becomes weak or a small number of signals are averaged. The leakage of ambient light could be significant when samples with rough or structured surfaces are measured under strong ambient lighting conditions, such as bright or direct sunlight. The background noise needs to be recorded and subtracted from the "true" signal. In SpectraSuite, at least one dark spectrum has to be stored before the transmittance, absorbance, reflectivity, and irradiance measurements can be performed. However, it is a good practice to refresh the dark spectrum measurement periodically; after every change in experimental setup, such as changing samples, or when ambient lighting conditions change.

Reference spectrum – The reference spectrum records the intensity spectrum of the light source. It should be measured with the light source turned on and the sample removed from the leaf probe attachment. This spectrum is then used by SpectraSuite to calculate transmittance, absorbance, or reflectivity after the sample is placed on the leaf probe attachment. At least one reference spectrum needs to be recorded for T, A, or R spectrum. However, since the spectrometer measures the light intensity with and without a sample separately, the fluctuation of light intensity during a measurement cannot be corrected. It is strongly recommended to periodically record the reference spectrum if a series of measurements are performed over a long period of time (>5 min).

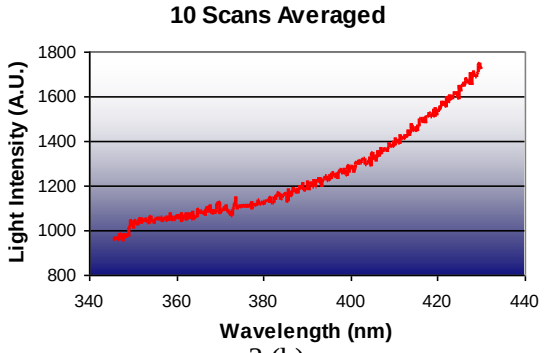
Application Tips for Highly Opaque Samples

Spectral measurements are normally easier for liquid samples as they are place in a cuvette with an optically flat surface and the concentration can be diluted or concentrated for optimal signal strength. Solid biological substances like leaves are generally more difficult to measure, because of the rough surface structure and high opaqueness weaken the intensity of light reaching the detector. The rough surface causes excessive scattering of incoming light, resulting in overestimation of sample's absorbance. For highly opaque samples (OD> 2.5), the amount of light transmitted may become too weak that the signal becomes too noisy. The tips below may be applied to improve the signal-to-noise ratio (SN ratio).

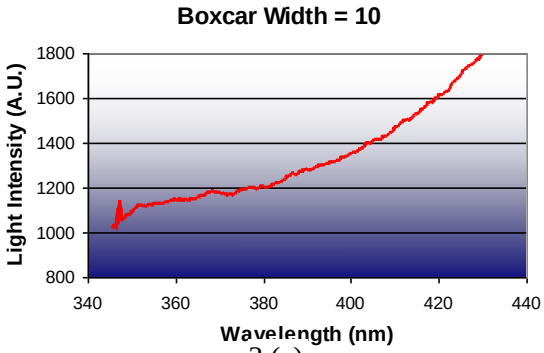
- **Increase the number of scans to be averaged**. At low light intensity levels, the electronic noise of the spectrometer becomes significant. This type of noise can be reduced by increasing the number of scans averaged, as seen in Fig. 3 (b). The number of scans to be averaged is defined in SpectraSuite.
- **Increase the boxcar width**. The boxcar width is a smoothing tool that averages the values of numbers of adjacent pixels, as shown in Fig. 3 (c). This function smoothes the signal in only one scan, which sometimes is required for certain experiments. However, if existence of sharp spikes are expected and are important in the spectrum, the boxcar width should be set to 0 as it may smooth out the spikes.



3 (a)



3 (b)



3 (c)

Figure 3. Effects of techniques in noise reduction. (a) is the original spectrum with one scan and zero boxcar width, (b) is the spectrum with 10 scans averaged, and (c) is the spectrum with a boxcar width of 10

- **Optimize the integration time.** The best way to improve the SN ratio is, of course, to increase the signal strength by increase the intensity of incident light. Although the energy output from the light source is fixed, the intensity of light detected by CCD can be increased by increasing the amount of time CCD is exposed. This can be done by increasing the "Integration Time" in SpectraSuite. However, when too much light is illuminated on CCD, the output signal from CCD can become saturated, which can be seen as portions of the intensity spectrum become flat, as shown in Fig. 4. Therefore, the Integration Time should be optimized to maximize the light intensity while avoiding saturating the CCD. Once the integration time is determined, the same integration time should be used for subsequent reference spectrum and sample spectrum measurements. Unlike the number of scans averaged and boxcar width, changes in the integration time affect the magnitude of light intensity measurements.

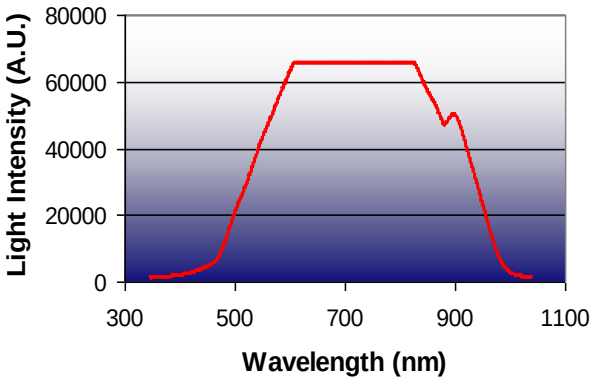


Figure 4. Saturation of CCD is seen at wavelengths in the 620-820 nm range