
CI-710 MINIATURE LEAF SPECTROMETER

Operation Manual



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About this manual

This manual provides instructions for installation, operation, and trouble-shooting of the CI-710-V.1.1, as well as the theory and applications of spectroscopy.

What's new in this edition

The CI-710-V.1.1 has the location of the transmission port changed from the top of the leaf clip to the side of the clip (same side as the reflectivity port) - March, 2010

Chapter 1. Introduction

The CI-710 is an extremely portable leaf spectrometer that measures leaf reflectivity, transmission, or absorption spectrum, either in the field or in the lab. The instrument is powered by a PC through a USB cable. No extra battery or power supply is required to operate the instrument. The measurement results are displayed real-time on the PC screen, and can be saved on the hard drive.

Unpacking the CI-710

The CI-710 is shipped with the following components:

- A spectrometer (USB4000, Ocean Optics)
- A leaf probe (pre-installed on the spectrometer)
- A 30-cm bifurcated fiber optic cable
- An USB cable
- A software installation CD (SpectraSuite, Ocean Optics)
- A reflectance standard
- An operation manual

Caution! Please handle the reflectance standard with care. The surface property of the white reflective material is very important for reflectivity measurement. Avoid touching or contaminating the surface of the white reflective material



Figure 1. Components of the CI-710

The configuration of the CI-710

The CI-710 consists of two modules, a leaf probe and a CCD-based spectrometer, and is powered by a PC through an USB cable. A computer program, SpectraSuite, is used to set measurement parameters and display the spectral data.. The leaf probe is equipped with a tungsten-LED dual light source that provides a broad range of wavelengths of light, suitable for visible and near infrared spectroscopy. The light then passes through a bifurcated fiber optic cable and connects to one of the two sampling light ports on the side of the leaf probe for the transmission or the reflectivity measurements.

Installation guide

This section shows the step-by-step installation instructions. Please follow the steps carefully to install your new instrument.

- 1. Attach the leaf probe to the spectrometer.** Skip to the next step if the leaf probe is already attached to the spectrometer (the original form of the instrument from the manufacture). If the leaf probe is separated from the spectrometer, install the leaf probe on the spectrometer by the following steps.

Step 1. Screw the optical aligner onto the spectrometer (Fig. 2a). The color of the optical aligner may be different from the picture. Align the leaf probe to the optical aligner and align the 10-pin connector to the spectrometer. Push the leaf probe straight down to the spectrometer (Fig. 2b).

Step 2. Tighten the screws on both end of the leaf probe to the spectrometer with the attached Allen wrench (Fig. 3).

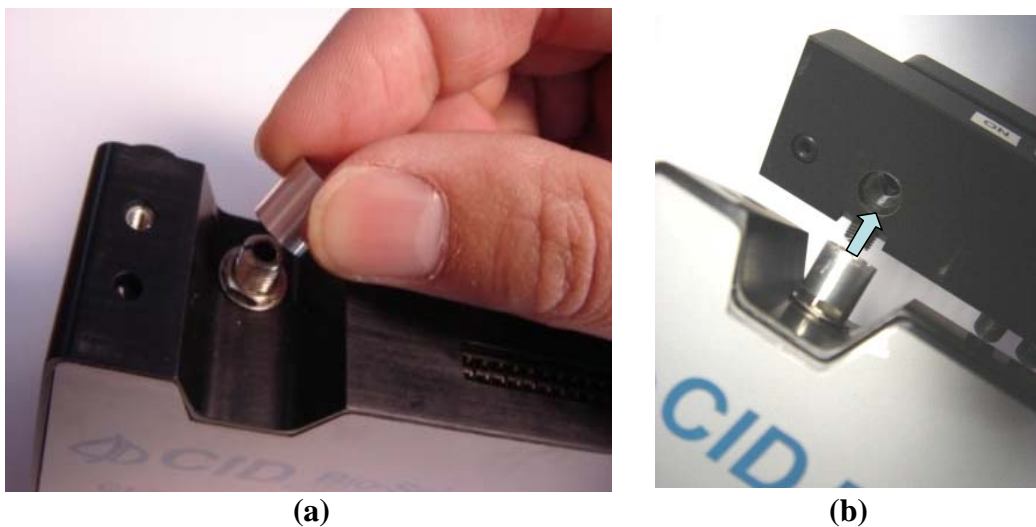


Figure 2. (a) Attaching the optical aligner to the spectrometer. (b) Aligning the leaf probe to the optical aligner to attach the leaf probe to the spectrometer

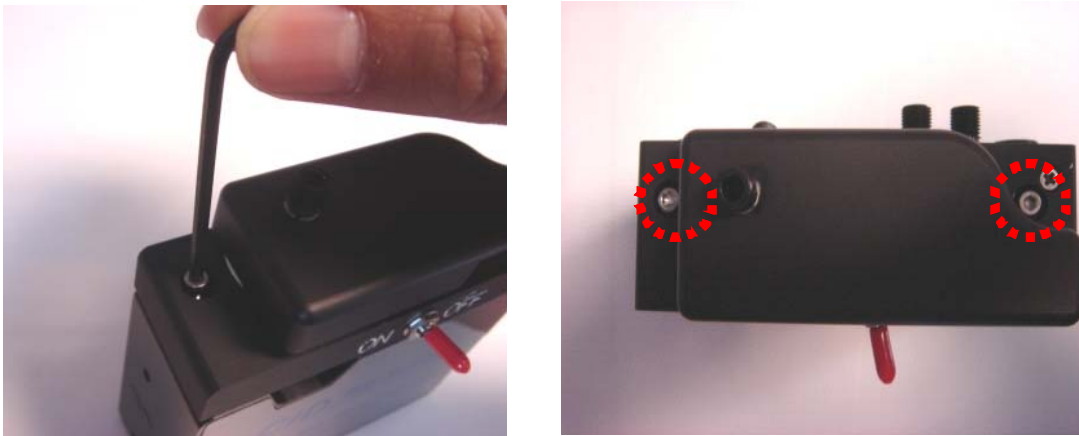


Figure 3. Tightening the screws on both ends of the leaf probe to secure the leaf probe on the spectrometer

2. **Connect the bifurcated fiber optic cable to the leaf probe.** Hold the end of the cable with two connectors (SMA 905 connectors) and insert both connectors to the light source ports, as shown in Fig. 4. Tighten the screws of the fiber optic cable to the leaf probe.

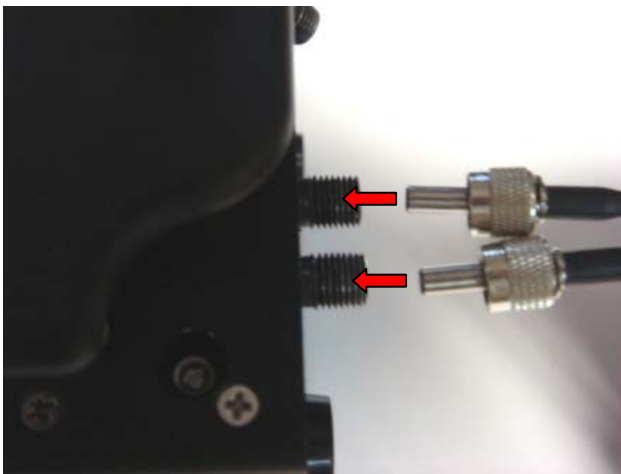


Figure 4. Connecting the bifurcated fiber optic cable to the leaf probe

3. **Install the operating software, SpectraSuite.** Follow the steps below to install the operation software on the PC.

Caution! : Install the software BEFORE connecting the spectrometer to your PC. The SpectraSuite software installs the drivers required for spectrometer installation. If you do not install SpectraSuite first, the system will not properly recognize the spectrometer.

- a. Close all other applications running on the computer.

- b. Insert the CI-710 CD into a CD ROM driver. Double-click on the SpectraSuiteSetup_Windows.exe file located in SpectraSuite folder to run the software executable.
- 4. Connect the spectrometer to the computer.** Connect one end of the USB cable to the spectrometer and the other end to a computer. For Windows PC users: The PC will find new hardware USB4000 and launch the New Hardware Wizard. Follow instructions in the Wizard so that the PC will properly recognize the spectrometer and install the driver. **Note that the same USB port should be used every time.**
- 5. Turn on the light source switch on the side of the CI-710.** A bright light should be observed at the end of the fiber optical cable. The installation is now successful and is ready to make measurements! Proceed to the next chapter “Making measurements for the first time” section to set up spectroscopic parameters. *If no light is observed, please make sure the USB cable is securely connected and the leaf probe is properly attached to the spectrometer, or refer to the trouble-shooting guide for further information.*

Chapter 2. Making measurements for the first time

This chapter provides step-by-step guides to set up the CI-710 to perform transmission measurements for absorption spectrum or transmission spectrum, as well as reflectivity measurements for reflectivity spectrum. The next chapter will describe how to optimize the parameters for the measurements.

Making transmission measurements for the first time

This section will guide you through setting up the CI-710 for transmission measurements. The reflectivity measurement follows the similar procedure and is shown in the following section.

1. Insert the fiber optical cable to the upper sampling port on the side of the leaf probe (Fig. 5), and tighten the screw of the fiber optical cable connector. Block the light source port on the side of the leaf probe with the attached port block to prevent the stray light from entering the spectrometer. Leave the light source switch at the OFF position.

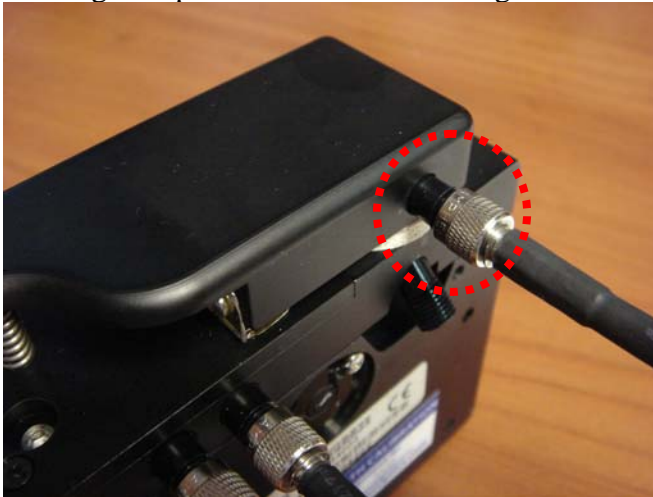


Figure 5. Connecting the fiber optic cable to the upper sampling port for transmission measurements

2. Double-click the SpectraSuite icon to start the operating software. The program is automatically measuring the light intensity entering the spectrometer when it first starts. With the light source turned off, the curve in the spectral area should be a flat line at a certain intensity value (Fig. 6). This value represents the background electronic noise. If there are any peaks or curves seen at this time, it is a symptom of stray light entering the spectrometer. Please check the instrument installation for possible optical leakage or the trouble-shooting guide. At this point, the program can only display the light intensity with **S** being the only highlighted measurement icon. It is because the required information is not yet ready for absorbance, transmission rate, reflectivity, and irradiance measurement. The next few steps will describe how to enter the parameters on the program to prepare for the transmission measurements.

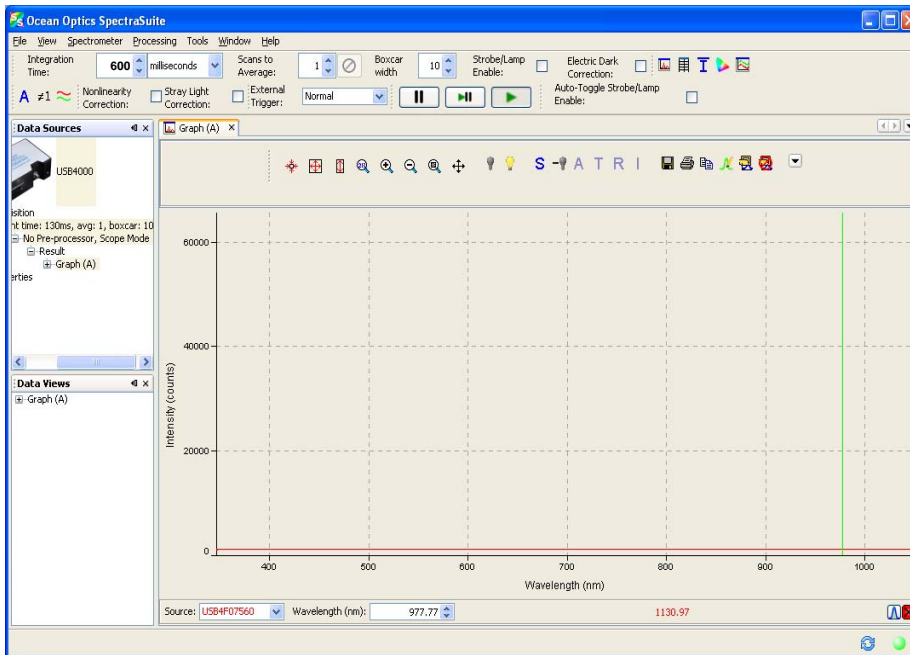





Figure 6. Dark spectrum – the background signal when the light source is turned off

3. On the Integration time text box found on the upper left corner of the program, enter 600 (millisecond) (the default value is 100 milliseconds). On the boxcar width text box, enter 10. Refer to Chapter 3 for detailed discussion on these parameters.
4. Click the dark light bulb icon , to store the “Dark spectrum”, which is the background noise. Note that the  icon now becomes visible. Click on this icon to show the light signal “minus” the background noise stored in the dark spectrum. Note that signal seen on the screen becomes a flat line at zero.
5. Turn on the light source on the leaf probe. The curve seen on the screen is the intensity spectrum of the light source, similar to the one shown in Fig. 7. Wait for about one minute when the light intensity stabilizes, then click the  icon to store the incident light intensity spectrum, or the “Reference spectrum”. Note that the rest of the measurement icons, **A T R I**, are now all visible. It is ready to make measurements.

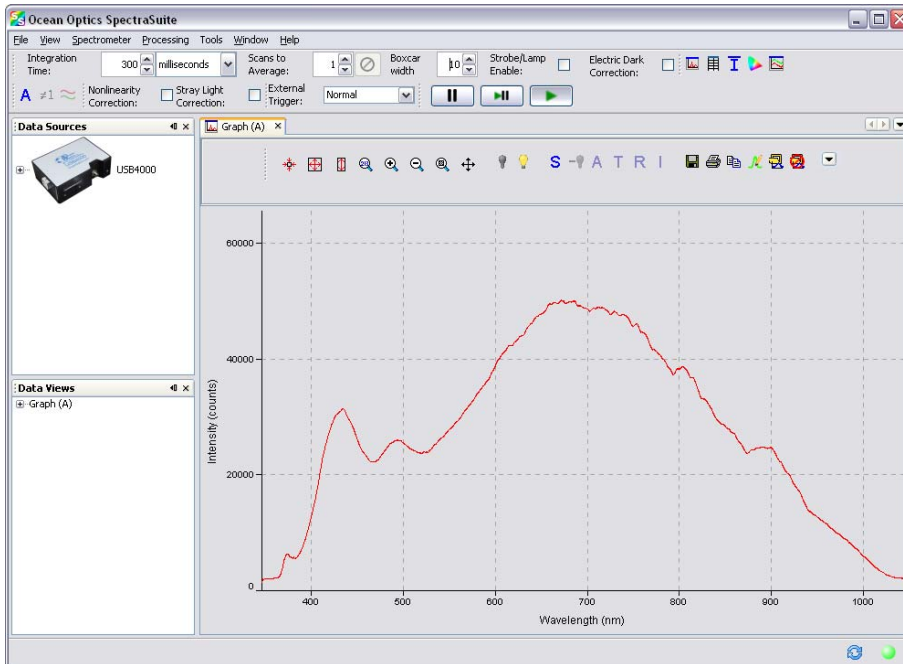





Figure 7. Intensity spectrum of the light source of the CI-710

6. Click on the **A** icon to measure absorption spectrum of a leaf. Before clipping the leaf probe on a leaf sample, the signal should be a flat line at zero absorbance level. (If any portion of the signal is not at zero, it may be due to the light intensity drift. Click the  icon again to update the reference spectrum.) Place the leaf sample on the leaf probe so that the leaf covers the entire measuring area. The absorption spectrum of the leaf sample is now displayed on the computer screen.
7. If the transmission rate is desired, repeat step 5 but click on the **T** icon, instead of **A**. Before clipping the leaf probe on a leaf sample, the signal should be a flat line at the 100% level. (If any portion of the signal is not flat and deviates from 100%, it may be due to the light intensity drift. Click the  icon again to update the reference spectrum.) Follow the same procedure as in step 6.
8. Click on the  icon to save the spectrum.



Making reflectivity measurements for the first time


This section will guide you through setting up the CI-710 for reflectivity measurements. Follow the procedure below to set up a reflectivity measurement for the first time.



1. Insert the fiber optical cable to the lower sampling port on the side of the leaf probe (Fig. 8), and tighten the screw of the fiber optical cable connector. Block the light source port on the side of the leaf probe with the attached port block to prevent the stray light from entering the spectrometer. Leave the light source switch at the OFF position.



Figure 8. Connecting the fiber optic cable to the lower sampling port for reflectivity measurements

2. The program is automatically measuring the light intensity entering the spectrometer when it first starts. With the light source turned off, the curve in the spectral area should be a flat line at a certain intensity value (Fig. 6). This value represents the background electronic noise. If there are any peaks or curves seen at this time, it is a symptom of stray light entering the spectrometer. Please check the instrument installation for possible optical leakage or the trouble-shooting guide. At this point, the program can only display the light intensity with **S** being the only highlighted measurement icon. It is because the required information is not yet ready for absorbance, transmission rate, reflectivity, and irradiance measurement. The next few steps will describe how to enter the parameters on the program to prepare for the reflectivity measurements.
3. On the Integration time text box found on the upper left corner of the program, enter 300 (millisecond) (the default value is 100 milliseconds). On the boxcar width text box, enter 10. Refer to Chapter 3 for detailed discussion on these parameters.
4. Click the dark light bulb icon , to store the “Dark spectrum”, which is the background noise. Note that the  icon now becomes visible. Click on this icon to show the light signal “minus” the background noise stored in the dark spectrum. Note that signal seen on the screen becomes a flat line at zero.
5. Place the reflectance standard into the leaf probe with the white reflective material facing down and covering the entire measurement area. Turn on the light source on the leaf

probe. The curve seen on the screen is the intensity spectrum of the light source, similar to the one shown in Fig. 7. Wait for about one minute when the light intensity stabilizes, then click the  icon to store the incident light intensity spectrum, or the “Reference spectrum”. Note that the rest of the measurement icons, **A T R I**, are now all visible. It is ready to make measurements.

6. Click on the **R** icon. Before clipping the leaf probe on a leaf sample, the signal should be a flat line at the 100% level. (If any portion of the signal is not flat and deviates from 100%, it may be due to the light intensity drift. Click the  icon again to update the reference spectrum.) Place the leaf sample on the leaf probe so that the leaf covers the entire measuring area. The reflectivity (%) curve of the leaf sample is displayed on the screen.
7. Click on the  icon to save the spectrum.

Chapter 3 Theory and Applications

The CI-710 miniature leaf spectrometer is a cost-effective instrument that provides spectral information useful to study the interaction of light with biological substances in the visible and near infrared (NIR) range. When a substance is illuminated with light, a portion of the light energy may be absorbed by the substance. The amount of light energy absorbed by the substance varies with the wavelength. The unabsorbed light either transmits through, or reflects off the substance without interacting with the substance (Fig. 9). For plants, light is absorbed by pigments in the leaves, primarily chlorophylls, which are responsible for photosynthesis. These pigments in leaves effectively absorb light at the blue/blue-green range (400-500 nm) and the red range (650-710 nm), while reflecting most of the green and yellow light (500-600 nm), making the leaves appear green.

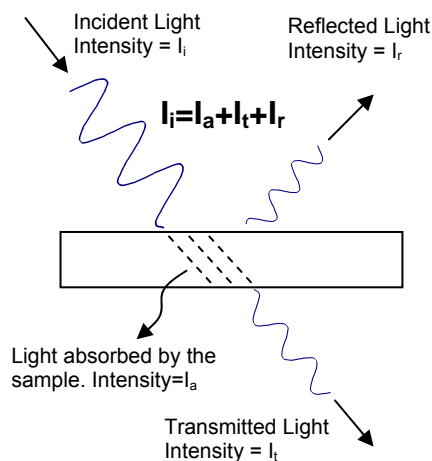


Figure 9. Schematic showing the interaction of light and a sample

The CI-710 is comprised of two main modules: a USB (Universal Serial Bus) powered spectrometer with operating software, and a leaf probe module with a broadband light source. The light source in the leaf probe is guided through a bifurcated fiber optic cable and is to be placed in one of the two ports on the leaf probe for transmission or reflectivity measurements. The spectrometer module collects the light from the leaf probe, disperses through 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. A plot of the light intensity from the response of each of the CCD pixels thus represents the light intensity vs. the wavelengths of light which is an intensity spectrum. By formulating the changes of light intensity at each CCD pixel with and without a sample, spectra of absorption, transmission, or reflectivity can be obtained. The operating software, SpectraSuite, displays and stores the light intensity of each pixel of the CCD array, and then formulates the intensity data to perform spectroscopic measurements. 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 I_0 , and the intensity of light passing through the sample is I_t , the transmittance, T , is then expressed as $T = I_t / I_0$. 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 $A = -\log T = -\log(I_t / I_o)$. 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-710 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.

As described previously, the transmission, absorbance, and reflectivity of a substance are all related to the intensity of incident light vs. the transmitted or reflected light. The CI-710 is equipped with a single CCD array; the intensity of incident light and the transmitted/reflected light cannot be measured simultaneously. To calculate absorbance, transmission, or reflectivity, it is necessary to separately measure the intensity spectrum of incident light (without samples), which is called the reference spectrum in the software, and the intensity spectrum of light with samples. For a measurement to be accurate, the background noise, which is called the dark spectrum in the software, should be recorded and subtracted from the signal.

Dark spectrum – The dark spectrum is the background noise of the experimental setup with the light source turned off. The background noise needs to be recorded and subtracted from the measured signal to obtain the “true” signal. In SpectraSuite, the dark spectrum has to be stored before the transmittance, absorbance, reflectivity, and irradiance measurements can be performed. 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 leakage of ambient light can be easily differentiated from the electronic

noise as the signal changes when rotating or move the instrument. If the leakage of ambient light is detected, make sure the optical aligner and the port block are installed. If it is impossible to completely remove the ambient light, try to keep it at the same level and store it with the electronic noise as the background noise. The noise will be subtracted from the signal by the computer program. It is a good practice to refresh the dark spectrum measurement periodically as the electronic noise can shift over time and ambient lighting conditions can 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. This spectrum is used by SpectraSuite to calculate transmittance, absorbance, or reflectivity of the sample. It is a good practice to update the reference spectrum periodically because the spectrometer measures the light intensity may drift over time and affects the measurement accuracy. The easiest way to determine whether the reference spectrum needs an update is to observe the absorbance, transmission rate, or reflectivity signal without a sample. The signal should appear as a flat line at the 100% level for transmission or reflectivity, or at the zero level for absorbance. Any portion deviating from the baseline level is an indication of spectral shifts of the light source and it is necessary to update the reference spectrum.

Factors affecting the signal-to-noise (SN) ratio

Solid biological substances like leaves are generally more difficult to measure, because of the rough surface structure and high opaqueness weakening the intensity of light reaching the detector. For highly opaque samples ($OD > 2.5$), the amount of light transmitted through becomes too weak, resulting in a poor SN ratio. The tips below may be applied to improve the SN ratio.

- **Optimize the integration time.**

One way to improve the SN ratio is to increase the signal strength of the 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. 10.

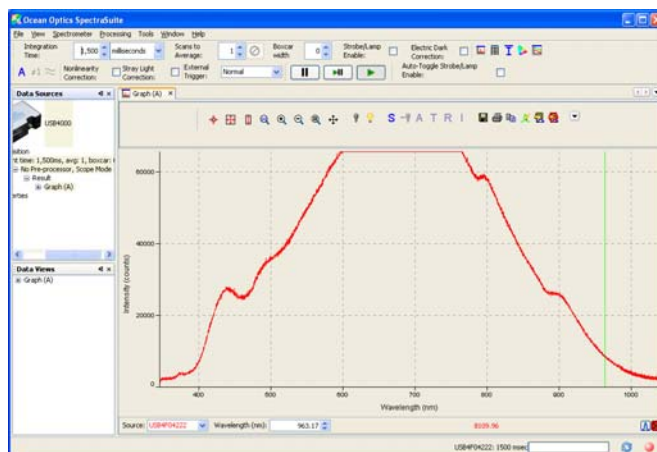
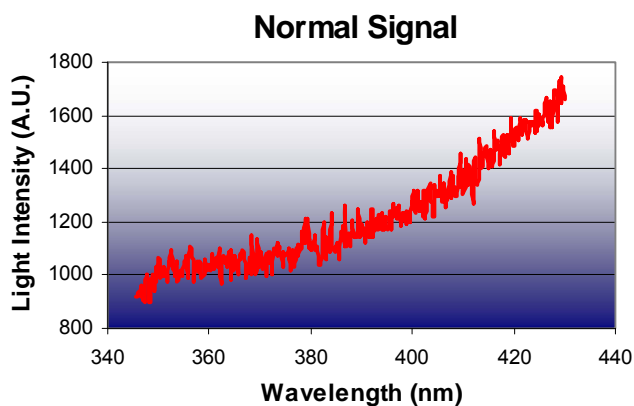


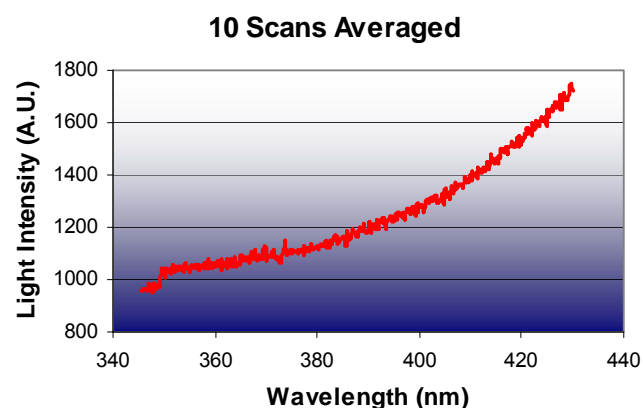
Figure 10. Saturation of CCD is seen at wavelengths in the 600-770 nm range

The integration time can be set from 3.8 ms to 10 second. Ideally, this parameter should be set so that the peak of the incident light intensity is maximized but not saturating the CCD. Once the integration time is determined, the same integration time should be used for subsequent reference spectrum and sample spectrum measurements. However, if measurement speed is more important than the SN ratio, the integration time can be set at a lower value.

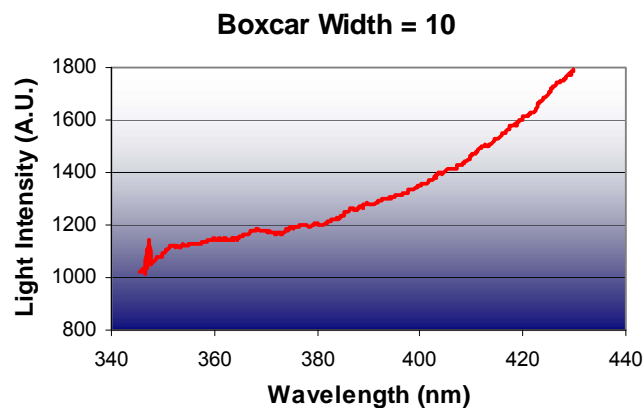
- Increase the number of scans to be averaged.** At low light intensity levels, the electronic noise of the spectrometer becomes significant. This type of noise can be reduced by averaging over a number (N) of repeatable signals (Fig. 11 (b)). To increase the number of scans to be averaged, enter the desired number in “Number of scans averaged” in SpectraSuite. The disadvantage of averaging over a number of signals is a potentially long measurement time. This could be problematic if the spectrum is expected to be dynamic, as in the study of dynamics of photochemistry that involved with different photo intermediate states.
- Increase the boxcar width.** The boxcar width is a smoothing tool that averages the values of numbers of adjacent pixels, as shown in Fig. 11 (c). This function smooths 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. For samples with broad absorption spectrum, such as leaves, a value of 10 does not cause any spectral shifts.



11 (a)



11 (b)



11 (c)

Figure 11. 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

Chapter 4 Trouble-Shooting Guide

Item	Issue	Cause	Solution
1	No spectrometer recognized or no information shown in the spectral area	<ol style="list-style-type: none"> 1. Loose USB connection 2. Incorrect USB port used 3. Driver not installed correctly 	<ol style="list-style-type: none"> 1. Check the USB connection 2. Always use the same USB port when the driver was installed 3. Uninstall and re-install the driver
2	Saturation seen in the intensity spectrum	The value in the Integration time is too high	Lower the integration time
3	A T R I are greyed out and not available in the SpectraSuite	Dark spectrum and reference spectrum are not stored	Store both spectra as described in Chapter 2
4	No intensity from the light source	<ol style="list-style-type: none"> 1. Integration time too low 2. Switch is turned off 3. Broken fiber optic cable 4. Broken light bulbs 	<ol style="list-style-type: none"> 1. Use auto zoom function 2. Turn on the switch 3. Remove the fiber optic cable and check if any light comes out of the leaf probe 4. Contact CID for further instruction
5	Strong stray light	<ol style="list-style-type: none"> 1. Missing the optical aligner 2. The sample is thick and does not cover the measurement area 	<ol style="list-style-type: none"> 1. Check the optical aligner 2. Make sure the sample covers the entire measurement area
6	Negative transmission rate	Shift in background noise	Re-store the dark reference by either removing the fiber optic cable from the sampling port or turning off the light switch

Chapter 5 Specifications

Physical Specifications

Detector	Toshiba TCD134AP Linear CCD array
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Spectroscopic

Wavelength range:	400-1000nm
Optical resolution:	~1.5 nm FWHM
Integration time:	3.8 ms - 10 seconds
Stray light:	<0.05% at 600 nm; 0.10% at 435 nm
Optical Properties:	Light intensity, absorbance, Reflectivity, Irradiance
Optical Sample Size	7.6 mm

Electronic

Power consumption:	250 mA @ 5 VDC
Data transfer speed:	Full spectrum to memory every 4 ms with USB 2.0 port
Inputs/Outputs:	Yes, 8 onboard digital user-programmable GPIOs
Analog channels:	No
Auto nulling:	Yes
Breakout box compatible:	No
Trigger modes:	4 modes
Strobe functions:	Yes
Connector:	22-pin connector
Power Supply:	USB powered via laptop or PC

Computer

Operating systems:	Windows 98/Me/2000/XP, Mac OS X and Linux with USB port
Computer interfaces:	USB 2.0 @ 480 Mbps (USB1.1 compatible)

Detector Specifications

Detector	Toshiba TCD134AP Linear CCD array
Detector Range	400-1000nm
Pixels	3648 pixels
Pixel Size	8 μm x 200 μm
Pixel well depth:	100,000 electrons
Signal-to-noise ratio:	300:1 (at full signal)
A/D resolution:	16 bit
Dark noise:	50 RMS counts
Corrected linearity:	>99.8%
Sensitivity:	130 photons/count at 400 nm; 60 photons/count at 600 nm
Dynamic range:	2 x 10 ⁸ (system), 1300:1 for a single acquisition