OPTI-SCIENCES
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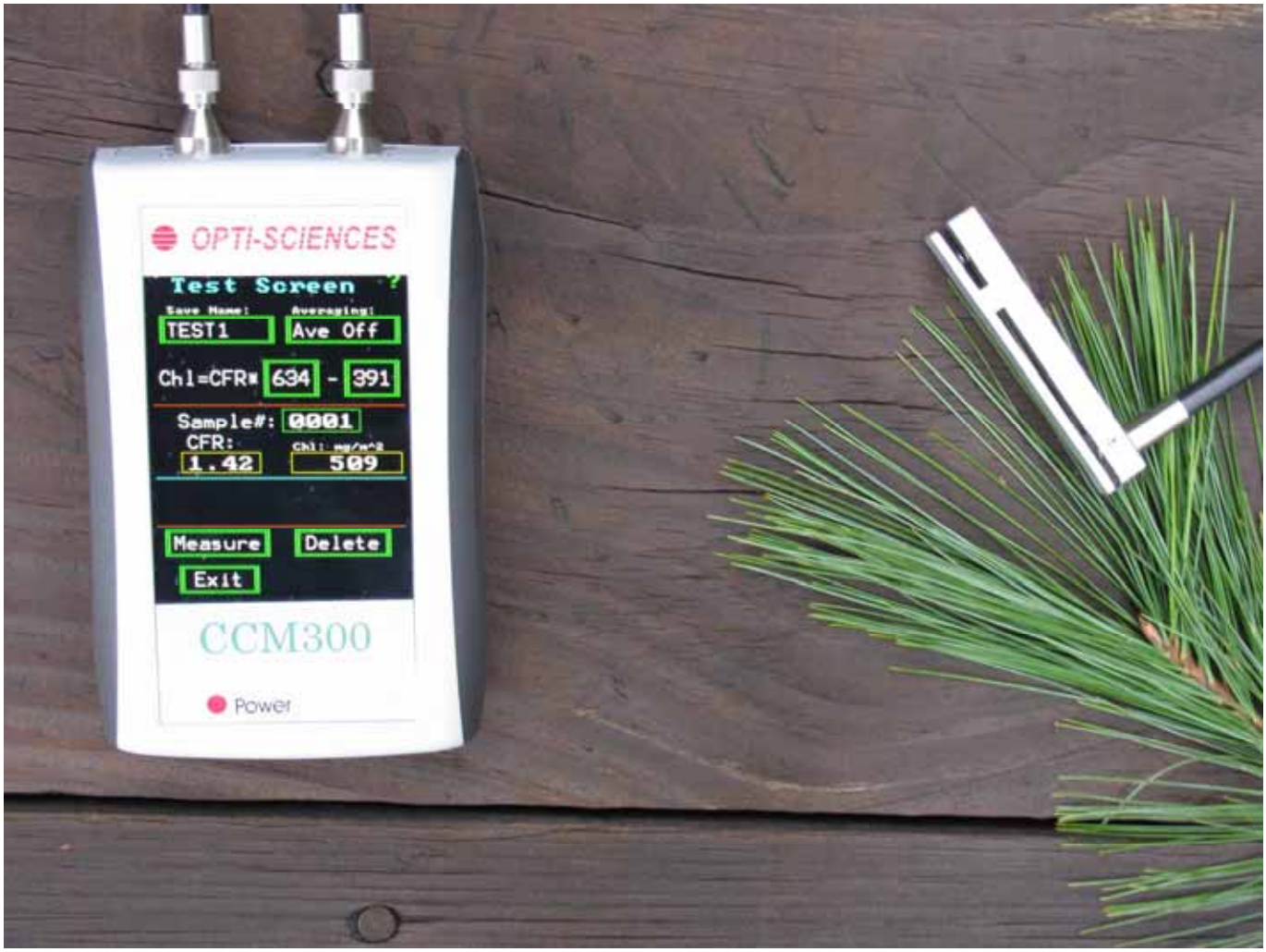
CCM-300
Chlorophyll Content meter for small leaves and difficult samples
Operation manual



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Introduction

The CCM-300 Chlorophyll Content Meter is a modulated fluorometer that uses the emission ratio of red chlorophyll fluorescence at 700nm to the far red emission fluorescence value at 735nm., utilizing a fiber optic probe. The source and detector wavelengths were selected from the Science developed by Gitelson A. A., Buschmann C., Lichtenthaler H. K. (1999) "The Chlorophyll Fluorescence Ratio F735/F700 as an Accurate Measure of Chlorophyll Content in Plants" Remote Sens. Enviro. 69:296-302 (1999). It is a handheld, battery powered device with almost unlimited built in data storage.

The instrument is powered by 2 AA batteries. NiMH or alkaline types may be used. Two sets of NiMH batteries and a NiMH charger are included with the unit. The unit will typically run for 8-10 hrs on a freshly charged set of NiMH batteries. Ambient temperature and battery charge will affect runtime.

The unit has a 320x480 pixel display with touch sensitive screen. While finger touch may be used successfully for most functions, A stylus is provided for easier use of some of the input and control buttons, such as entering file names.

Items included

- CCM-300 Chlorophyll Content Meter, with a filter set (source & detectors) installed.
- Bifurcated fiber optic light guide
- Sample clip
- USB data cable
- Touch screen stylus
- 4 NiMH AA rechargeable batteries
- Charger for NiMH batteries
- Users guide CD
- Stylus

Initial Setup

Completely charge all 4 NiMH batteries before using them. The GFPIII uses two batteries at a time. Make sure to install batteries with the polarity shown below.

Install 2 AA batteries as shown

The fiber optic probe is attached to the unit with two threaded connections. Use caution when attaching the fiber, do not force the threads. The fiber should easily attach to the unit, and seat securely. If it does not, try reseating the fiber connection.

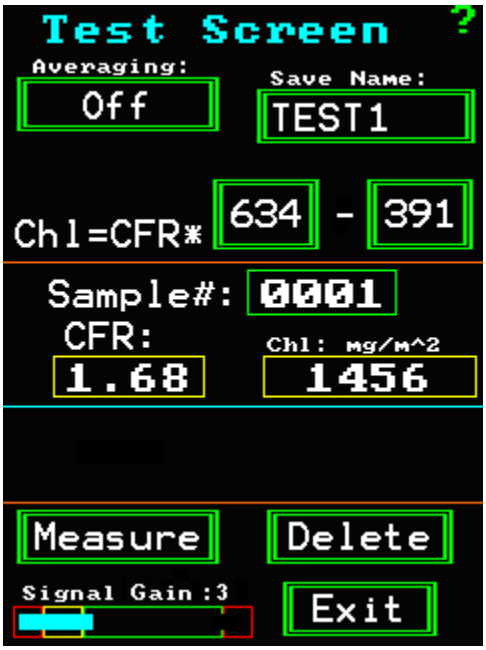
Fibers should seat securely

The sample end of the fiber can be used with the sample clip if thin flat samples (such as leaf tissue) are to be tested. The set screw has a nylon tip. It has been set at the factory for a firm press fit. If the fiber is loose, or does not fit correctly, the set screw may be adjusted. The fiber optic can be used without the leaf clip. See the section on measurement mode for more information.

Sample leaf clip with fiber installed.

User interface:

The CCM-300 uses a touch panel for all user input. Green boxes typically indicate a control or an editable variable. Controls that are on the screen are usually rendered inactive if a popup window is active. Popup windows include parameter change boxes, and warning messages. The screen can be used by tapping with a finger, in some cases it may be desirable to use the stylus provided. This is especially true when using the on-screen keyboard. Screens having a green "?" character in the upper right corner include help information that may be viewed by tapping it. The help box will display information about the current screen.

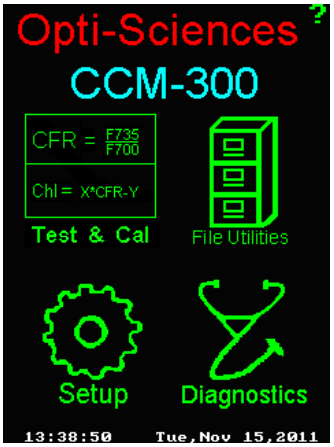


An option to capture a "snapshot" of the current screen is available in the help box, "PScrn". This can be useful when diagnosing a system problem. When this feature is used, a box will appear asking for a file name to save the picture to.

Opening screen:

Power switch

Press the Power switch to turn on the unit. The screen above to the right will appear. Each icon will lead to a specific part of the program when pressed. The current date/time is shown along the bottom



Measurement overview:

This technology shows a strong linear correlation to chemical chlorophyll content tests between 41 mg m^{-2} and 675 mg m^{-2}. It is ideal for precise measurements of chlorophyll content in this range. Direct read out of Chlorophyll content in mg m^{-2} is possible using the Gitelson equation included on the instrument or the fluorescence ratio F735/F700 can be used .

Today, chlorophyll content is usually measured by either by chemical assay methods or estimated by nondestructive absorption techniques. While absorption instruments, have been proven over time to work well as a non-destructive test on many types of samples, they do have limitations.

Absorption technique limitations:

- *The sample to be measured must completely cover instruments aperture, with no holes.*
- *Samples must be thin enough to allow transmission of the measuring wavelengths of light.*
- *Surfaces must be relatively flat and uniform.*
- *Variable fluorescence (the Kautsky induction effect) caused by the red wavelength of absorption instruments, limits the repeatability of measurements at the same location without waiting 2 minutes.*
- *Selection of the measuring area, on smaller leaves, can cause measurement variations due to internal leaf structure variation. Veins and midribs can cause significant variation on samples.*
- *Wavelengths used in absorption methods, typically limit linear correlation with chemical chlorophyll content methods to concentration levels below 300 mg m^{-2}. (Gitelson 1999)*

As a result, absorption instruments do not work with conifer needles, turf grasses, Arabidopsis leaves, moss, most CAM plants such as prickly pear cactus, and Agave, fruit, stems, petioles, lichens, and algae on rocks. Furthermore, it is difficult to get reliable readings on very small leaf plants such as immature rice and wheat.

With this in mind, Opti-Sciences decided that it was time to engineer an affordable solution for these difficult applications. This article describes and explains this technology.

Research regarding the use of ratio fluorescence to measure chlorophyll content has been established for some time, and the limitations are well understood. However, until now, the cost of such systems has been prohibitive.

With this technology, chlorophyll samples absorb a blue fluorescence excitation light, and emit a range of fluorescing light at longer wavelengths. The research shows that by comparing the ratio of fluorescence emission at 735nm and at 700 nm, there is a linear response to chlorophyll content in a range from 41 mg m^{-2} to 675 mg m^{-2}. Since this method does not compare transmission through a leaf at two different wavelengths, the measuring aperture does not have to be completely covered. In addition, the fluorescence is measured on the same side of the sample as the excitation light. For these reasons, fluorescence will work with leaves smaller than the measuring aperture like immature rice and turf grasses, measure samples with curved surfaces like white pine needles, and measure difficult samples like cactus, fruit, lichens, and algae on rocks. In other words, the instrument is designed to work well with all of the types of samples listed above, that are a problem for existing absorption systems.

Opti-Sciences decided to use the research by Gitelson A. A., Buschmann C., Lichtenthaler H. K. (1999) as a blue print for an instrument design because of the excellent linear correlation as compared to chemical testing from 41 mg m^{-2} to over 675 mg m^{-2}. The advantage of this ratio is that it works exceptionally well above chlorophyll content level of 200 mg m^{-2}, a limitation of previous fluorescence ratios. By raising the lower fluorescent emission measuring range away from chlorophyll absorption band near 680 nm or 685 nm, to 700 nm, the amount of fluorescence light that is reabsorbed and emitted again as chlorophyll fluorescence is minimized, significantly extending the useful linear measuring range of the instrument.

The instrument is called the CCM 300 chlorophyll content meter. It uses a fluorescence excitation wavelength with a peak at 460 nm and a half band width of 15 nm. It measures two different emission wavelength ranges at the same time, 730 nm to 740 nm, and 698 nm to 708nm.

The instrument also provides two different read-outs. One is the F735nm / F700 ratio reported by Gitelson, and the other is a precise direct readout of chlorophyll content according to Gitelson’s formula. The option also exists for changing formula parameters in case a researcher should want to modify the formula for some unexpected non-conforming plant species.

Measuring options include, single point measurement, averaging from between 2 and 30 samples, averaging with 2 sigma outlier removal, and median value readout.

Research overview:

As with all things, it is also important to know a technique's limitations, and these are detailed in Gitelson 1999. To a lesser degree, Buschmann C. (2007)" Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves." Photosynthesis Res.(2007) 92:261-271, explores the limitations of a different ratio technique that uses fluorescence near the peak of PSII fluorescence. The ratio in Buschmann (2007) is F690nm /F735nm.

Starting with Gitelson (1999): " The ratio between chlorophyll fluorescence at 735 nm and the wavelength range 700nm to 710 nm, F735/F700 was found to be linearly proportional to the chlorophyll content (with determination coefficient, r^2, more than 0.95) and thus this ratio can be used as a precise indicator of chlorophyll content in plant leaves."

Bushmann (2007) comes to the following conclusion regarding red and far red ratio fluorescence:

"Thus, the Chl fluorescence ratio red/far-red represents an ideal tool for detecting differences and changes of Chl content in plant species and leaf tissues, to monitor changes in Chl content and photosynthetic activity caused by changing environmental conditions, strain and stress events, and to detect stress tolerance, mineral deficiency, diseases, and other constraints. It can be applied for non-destructive monitoring of terrestrial vegetation in basic photosynthesis research as well as in agriculture, horticulture, and forestry."

The variables:

Fluorescence emission response to Chlorophyll content:

As chlorophyll content levels increase, the intensity of the far red emission fluorescence at F735 nm increases slightly and then declines slightly. At the shorter Red fluorescence emission wavelength of F700 nm, the value increases and then it declines dramatically. The ratio of the two fluorescence emission values provides a highly linear correlation to chlorophyll content in the range of 41 mg m^{-2} to 675 mg m^{-2} with a determination coefficient of $r^2 = 0.95$ value or higher. gitelson tests different species including Beach, Elm, and Wild Vine.On the other hand, correlation of absorbance style measuring systems starts to fall off above chlorophyll content levels of 300 mg m^{-2} .

The Gitelson paper (1999) compares results using various excitation wavelengths and emission wavelengths. They find good results using various excitation wavelengths; however on the emission side, the best results are found using F700 (700nm-710nm)/ F735 values. Blue excitation tends to only penetrate the leaf slightly and so re-absorption of emitted fluorescent light, causing additional fluorescence, is less of a problem. The CCM-300 uses a 460 nm diode with a half band width of 15 nm for fluorescence excitation. According to Bushmann, (2007) with blue excitation, the fluorescence comes primarily from the mesophyll layer. By raising the red fluorescence wavelength away from the peak of fluorescence emission, at about 685 nm, Gitelson (1999) extends the measuring range of linear correlation to chlorophyll content.

The Kautsky induction effect:

Unlike the CCM-300 F735 / F700 fluorescence ratio that uses modulated light, at very low intensity levels, to make measurements, other ratios and methods, such the method used by Buschmann (2007) have shown Kautsky induction effects. It has been found that the fluorescence emission ratio F690/ F735 is affected by the onset of photosynthesis and exposure to light (The Kautsky effect). Buschmann (2007) shows that the shorter wavelength fluorescence emission spectra changes faster, during the Kautsky induction, than the longer wavelength emission spectra. With the CCM-300, the Kautsky induction effect is minimized by using a very low intensity modulated light source, and by averaging measurements over a five second period. As a result, the Kautsky induction effect is less significant in the CCM-300.

Re-absorption of the emitted chlorophyll fluorescence by the leaf chlorophylls:

Chlorophyll fluorescence emission can be re-absorbed by other chlorophyll and re-emitted as additional fluorescence. While this is a relatively small error source in the F735nm / F700 ratio, re-absorbance when other ratio components have been tried, such as 685 nm and 690 nm wavelengths, have limited the reliability of chlorophyll measurement to below 200 mg m-2 according to Gitelson (1999). The F735nm / F700 ratio, on the other hand, has been shown to be linear from 41 mg m^{-2} to 675 mg m^{-2}. Because 700 is relatively far from the chlorophyll absorption peak near 685 nm, the re-absorption issue is minimized.

Furthermore, since longer fluorescence excitation wavelengths reach deeper into the leaf, causing greater fluorescence re-absorption, shorter excitation wavelengths minimize this effect. At shorter wavelengths, most fluorescence comes from mesophyll cells and re-emitted fluorescence is minimized (Gitelson 1999). The CCM-300 uses a 460nm diode for fluorescence excitation, to take advantage of this fact.

Temperature:

Buschmann reports that the ratio of F690/F735 (a similar ratio but not F735/F700) can drop by up to 25% as temperatures drop from 23^{o}C to 4^{o}C. This is thought to be influenced by state transitions, and PSI fluorescence. This shows that the instrument can be used for plant stress measurement, but it also shows that for direct chlorophyll content comparison measurement applications, temperature differences should be considered when comparing samples.

When using the CCM 300 with white pine needles, the measured ratio was 1.72 at 20^{o}C using an average of ten measurements. At 3^{o}C, the measurements dropped to 1.68 with an average of ten measurements (data not shown).

Fluorescence emission signal strength:

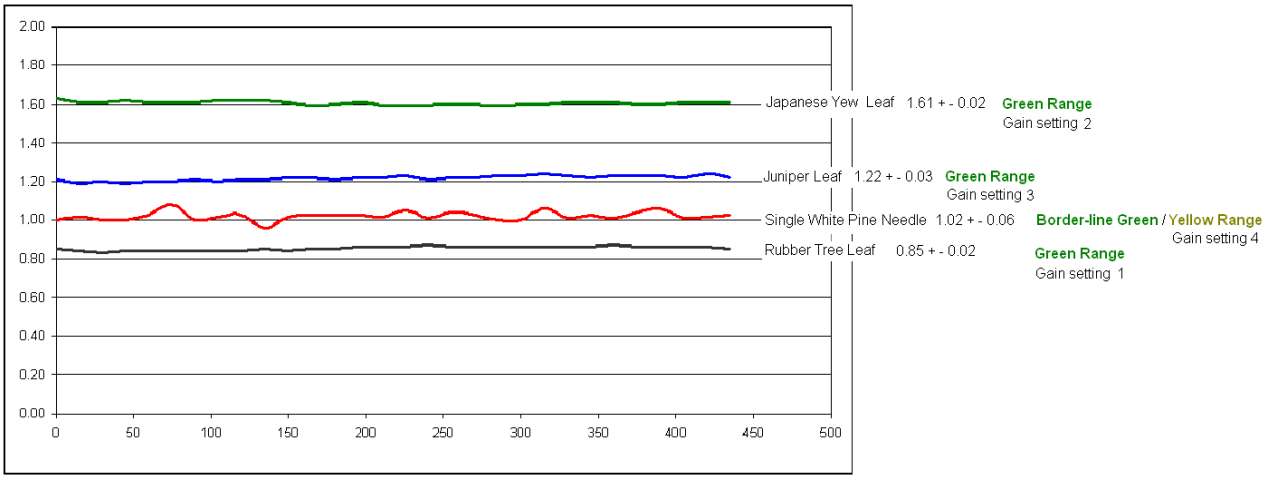
It has been found that measurement variation is effected by fluorescence emission signal strength. A special signal strength color gauge is provided on the bottom of the measuring screen. If the signal is in the green range the variation is less, and if it is in the yellow range it is more. Samples with lower signal

strength such as individual white pine needles may require multiple measurements and measurement averaging to provide the most reliable results.

Green signal range **Green – Yellow border line signal range**

Graph of variation due to fluorescence emission signal strength

Each sample was measured 30 times at the same location.



Samples with greater variation may require an average of multiple measurements for the best results. For this reason, measuring options are included to provide averaging from 2 to 30 measurements, or averaging with outlier removal beyond 2 sigma, or median determination.

State transitions and chloroplast migration:

State transitions "…state transitions are an essential strategy for photosynthetic organisms helping them utilize every photon". State transitions are a low light plant survival mechanism that take up to twenty minutes to fully adjust (Ruban, Johnson 2009).

Recently research with single, double and triple mutant Arabidopsis plants show that the intermediate chlorophyll fluorescence change, thought to be due to state transitions, and acute photoinhibition, was actually due to chloroplast migration in C_3 plant. C_4 plants also exhibit chloroplast migration but the fluorescence link is still being investigated. (Cazzaniga 2014, & Dall O'sta 2014) The research shows that

it takes between 20 to 30 minutes for plant chloroplast migration to fully adjust. Some mutants can take up to 35 minutes (Cazzaniga 2014, & Dall O'sta 2014).

After measuring outdoor sun leaves, outdoor shade leaves, and indoor plants grown at low light levels, the CCM-300 fluorescence ratio did not significantly change over the longer periods of times (20 to 30 minutes) involved in chloroplast migration for most samples. It was found, however, that with a plant grown indoors at low light levels, the F735/F700 ratio started to rise after about three minutes and it continued for about twenty minutes. While this effect is usually very small it is thought to be the result of chloroplast migration.

For this reason, it is suggested that when averaging multiple measurements with the instrument, all measurements should be completed within three minutes of the initial measurement on the same leaf. If measuring for longer periods of time is desired, it is recommended that the sample in question be measured over a 30 minute period to map any possible variation due to q_E, the zanthophyll cycle (3-4 minutes in green house plants or up to 7 minutes in field plants Baker 2008), or q_M, chloroplast migration 20 -30 minutes (Cazzaniga 2013). After this characterization, a reliable sampling plan can be created. If there is no significant variation, time is not an issue.

For more information on chloroplast migration, request the Opti-Sciences application note on the subject.

The CCM-300 with leaf clip

Because chlorophyll content can be measured from a single side with this method, the opportunity to measure difficult samples now exists. This nondestructive method can be used to measure chlorophyll content of algae on rocks, of lichens, of cactus, of fruit, of seeds, and of moss. Because the aperture does not need to be filled, small samples such as turf grasses, rice, and Arabidopsis can be measured, and because the method uses ratio fluorescence, difficult shapes can be measured such as conifer leaves.

References:

Gitelson A. A., Buschmann C., Lichtenthaler H. K. (1999) "The Chlorophyll Fluorescence Ratio F735/F700 as an Accurate Measure of Chlorophyll Content in Plants" Remote Sens. Enviro. 69:296-302 (1999)

Baker N.R., (2008) Chlorophyll Fluorescence:A Probe of Photosynthesis In Vivo Neil R. Baker Annu. Rev. Plant Biol. 2008. 59:89–113

Buschmann C. (2007)" Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves." Photosynthesis Res.(2007) 92:261-271

Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013) "Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis", The Plant Journal, Volume 76, Issue 4, pages 568–579, November 2013 DOI: 10.1111/tpj.12314

Dall'Osto L., Cazzaniga S., Wada M. and Bassi R. (2014) On the origin of a slowly reversible fluorescence decay component in the Arabidopsis npq4 mutant, Phil. Trans. R. Soc. B 2014 369, 20130221, published 3 March 2014, http://rstb.royalsocietypublishing.org/content/suppl/2014/02/25/rstb.2013.0221.DC1.html

Ruban A.V., Johnson M.P., (2009) Dynamics of higher plant photosystem cross-section associated with state transitions. Photosynthesis Research 2009 99:173-183

Setup Mode

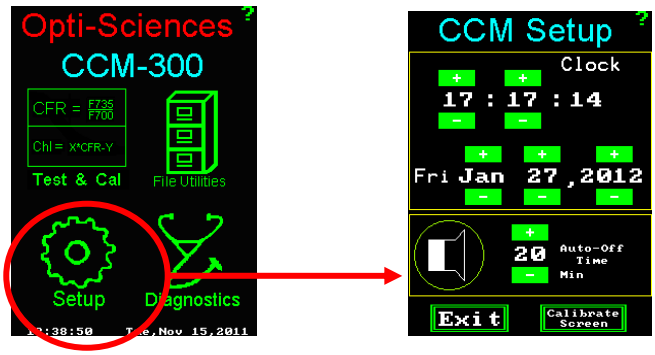
After assembling the instrument, go to the **Setup** mode to set the time and date stamp for all measurements. Use your finger or the stylus to tap the + or – boxes to set the date and time.

The **Auto – Off Time** feature is preset to 20 minutes. This is a battery saving feature that turns off the instrument if a button or box has not been tapped for the period selected. It is adjustable from 5 minutes to 20 minutes.

The horn icon contains the controls for the beeper and auto-off feature. Tapping the speaker icon will toggle the beep tone on/off.

The **Calibrate Screen** button will adjust the touch panel. **The screen is pre-calibrated. A calibration is only needed if a key location and its active press area do not coincide.** This may be most apparent when entering in file names using the on screen keyboard. Should pressing a key consistently result in the selection of an adjacent key, then a calibration may be necessary. With normal use this should not occur.

Changes in this screen are automatically saved.



Measurement Mode

Measure is for measuring samples. Go to the **setup** mode and adjust the time and date before using the measuring mode.

In the measuring mode, a few parameters should to be setup before measuring begins.

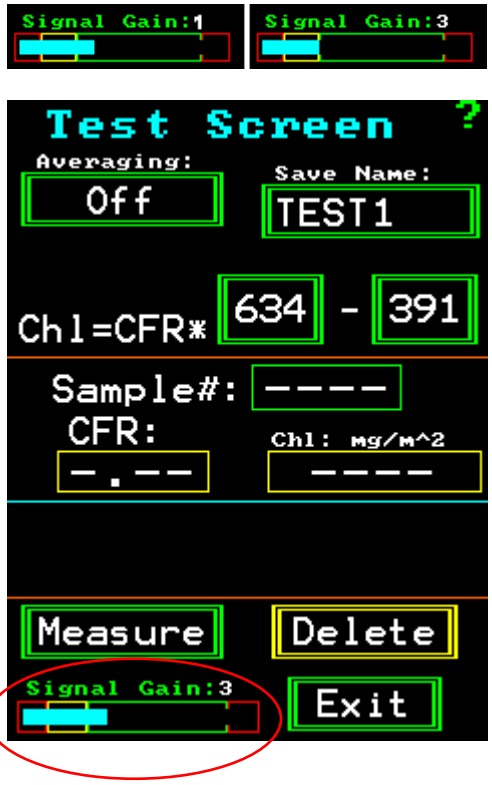
First, a data file should be selected, or created.

1. The file name is shown in the **Save Name:** box. Tap on this box to change the file name. A pop up will appear with three choices, **Create** (to create a new file), **Cancel** (cancel) and **Open** (appends data to an existing file). File names are restricted to 1-8 characters. The default extension ".CSV" (comma separated value) is used with the chosen name. This name will become the default name until it is changed again.

2. Set the averaging function to the desired method and quantity. In the **Averaging:** box, tap on this box to make changes. A new box will appear with arrow and quantities. Tap the arrows to scroll through the options. The options include **off** for single point measurements, Tot **Ave** that provides an average value for the number of measurements selected from two to thirty. The number can be changed by tapping the number shown in the box. **Std Dev** is a method that provides an average of the number of measurements selected and removes outlining measurements beyond a 2 sigma standard deviation. This method works

using five to thirty measurements. **Median** is the final option. Here, measurements from two to thirty can be selected and the Median is reported after the measurements are taken.

3. Adjust the instrument gain by using your finger or the stylus on the green signal gain words on top of the signal gain graph. The signal must be in the yellow or green range to make measurements. By tapping the words, the gain scrolls from 1 to 4. Place a sample to be measured in front of the fiber optic probe and watch the signal graph. Adjust the gain so that the light blue bar is as close to the middle of the green range window as possible. Samples like White Pine needles will need a gain of 4 and may still be in the yellow or low green range, while standard leaves can usually be measured with a gain of 1. Electronic noise increases somewhat with a higher gain setting. There is less measurement variability in the green window range than in the yellow window. Measurements made in the yellow window range may require an averaging of multiple measurements to obtain the desired results.



For most applications, the instrument is now ready to make measurements.

Other options include the ability to change the Gitelson chlorophyll content equation for direct readout in chlorophyll content values. The Equation worked for Elm, Beech, and Wild Vine. However, this option provides maximum flexibility in case researchers want to make adjustments for other species. These values can be changed by tapping the **Chl+CFRx** windows.

The Gitelson equation used is **Chlorophyll in mg m^{-2} = 634*F_{735}/F_{700} - 391** from Gitleson (1999). For more information see the Gitelson (1999) paper.

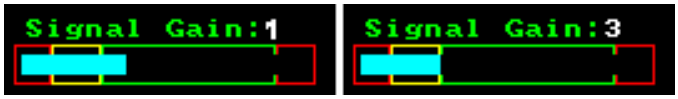
You are now ready to make measurements.

For measurements with the leaf clip:

Place the end of the fiber optic completely into the leaf clip and tighten the Allen screw so that it is snug.

Place the sample in such a way that it is located directly under the middle of the end of the fiber optic. The fiber optic aperture does not have to be completely filled by the sample. Tap the **Measure** box.

It takes *five seconds to make a measurement*. The Ratio and direct chlorophyll content values are displayed along with the sample number and the number of measurements left before an average is reported. (This is true when one of the averaging or median modes is being used). CFR: is the ratio of fluorescence or fluorescence emission at 735nm / 700 nm. Chl: is direct read out of chlorophyll content in mg m^{-2} according to Gitelson's equation. **Pts Lft** is the number of points left before an average in displayed. The larger yellow box displays the measurements that have been made to calculate the average value. One can scroll through the values with a finger or a stylus if larger numbers of samples are averaged. Measurements may be deleted by tapping the **Delete** box.



The signal window on the bottom left corner can be very valuable. While it does not provide a chlorophyll content indication, it does indicate combined signal measuring strength. If the blue bar is in the green range, a single measurement is probably adequate for measurement. If the signal strength is in the yellow range, it is recommended that an average of values be used for measurement. In the lower yellow range, it is recommended that several measurements be made, and an average value determined. The instrument will not measure in the red range. The detector gain may be adjusted from 1 to 4 by tapping on the green "Signal Gain" words. Adjust the gain so that the sample is as close to the middle of the green range as possible. For a more detailed look at this variable, see the graph on page ten. After using the instrument for a while, predicting the number of measurement required for reliable measurement becomes easy. The gain remains the same until changed

For measurements without the leaf clip:

When measuring without the leaf clip, it is recommended that the user adjust the fiber optic tip so that it is close to perpendicular to the sample surface. This will help ensure repeatable results. Direct contact with the sample surface is recommended for repeatable results. Dark adapting or light adapting do not appreciably affect most measurements. Please see the section on state transitions & chloroplast migration to view the exception. *Calibration will be significantly different using this method. It is therefore recommended that the unit be calibrated without the leaf clip, using the calibration slide, and holding it perpendicular to the slide. Movement of the fiber can cause some variation. Be carful that the area under the calibration slide is not laying on something that auto-fluoresces in the red range.*

Here again, the signal bar will help determine the number of measurements required for best results.

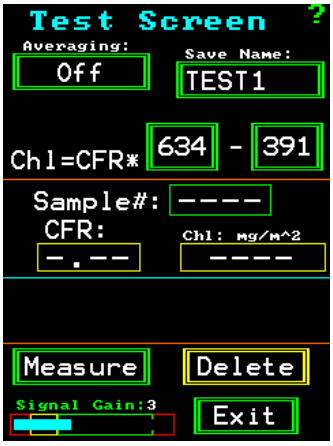
The signal window on the bottom left corner can be very valuable. While it does not provide a chlorophyll content indication, it does indicate combined signal measuring strength. If the blue bar is in the green range, a single measurement is probably adequate for measurement. If the signal strength is in the yellow range, it is recommended that an average of values be used for measurement. In the lower yellow range, it is recommended that several measurements be made, and an average value determined. The instrument will not measure in the red range. The detector gain may be adjusted from 1 to 4 by tapping on the green “Signal Gain” words. Adjust the gain so that the sample is as close to the middle of the green range as possible. For a more detailed look at this variable, see the graph on page ten. After using the instrument for a while, predicting the number of measurement required for reliable averaging measurement becomes easy if values are in the yellow range. The gain remains the same until changed.

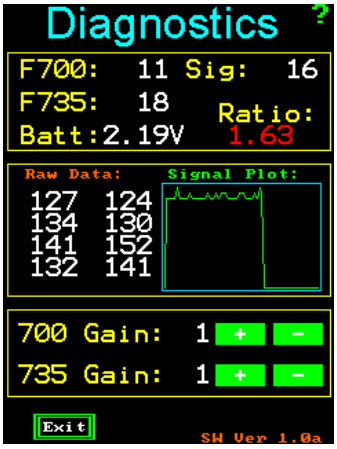
Viewing the fluorescence ratio components:

For most applications the information on the measuring “Test Screen” is ideal. However, to view the individual ratio components separately, or to look at the graph of the combined signal strength while the instrument is working, go to the “Diagnostics” main screen. The Top window displays the fluorescence reading for both the F735nm emission intensity, and the F700nm emission intensity, as well as the ratio.

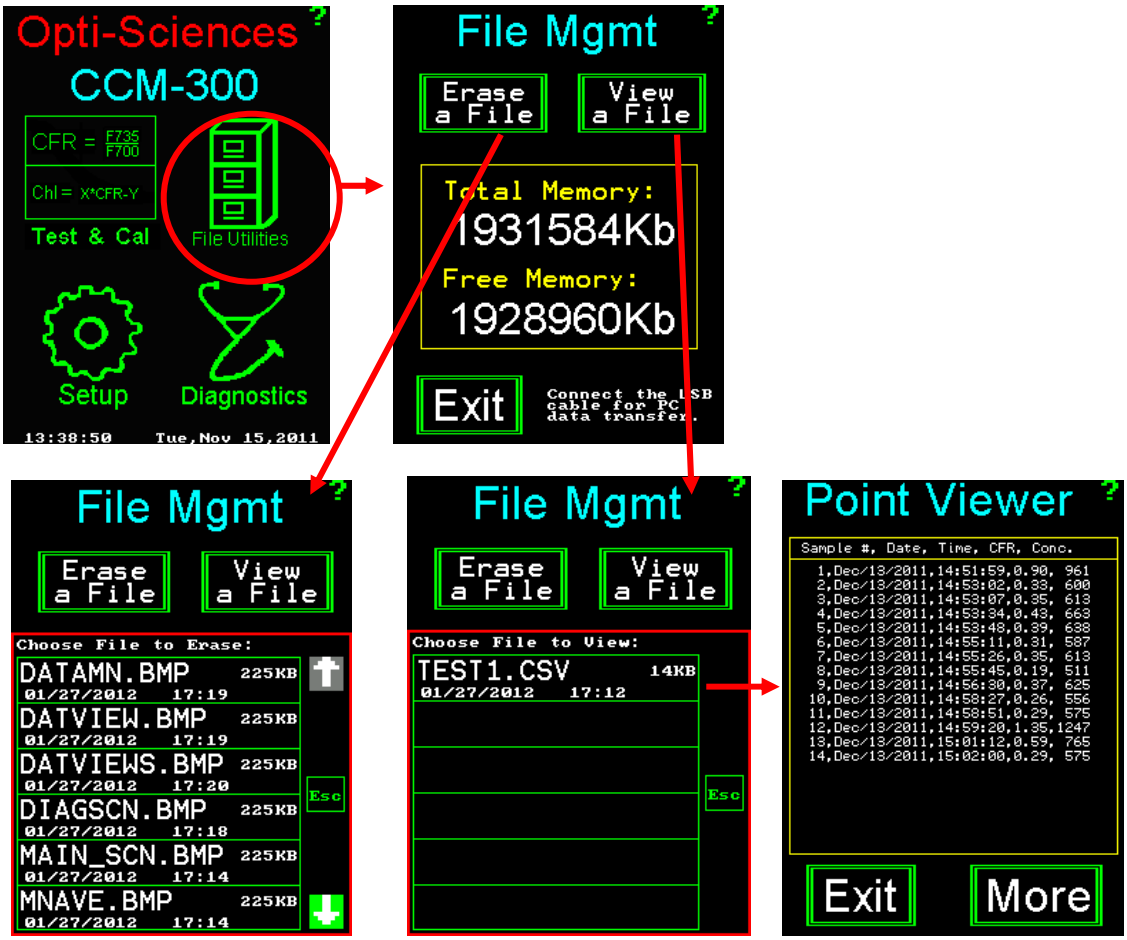
In the middle window, the combined signal strength can be viewed in the graph window during measurement. The small spikes are electronic noise. The instrument integrates the signal over a five second period to provide an individual measurement.

For more information on the Diagnostics screen, see the section covering Diagnostics.





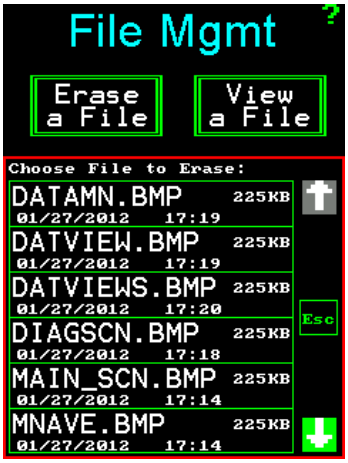
Data Management – File Utilities



Erasing a file on the instrument

Data saved on the unit may be erased or viewed from this menu. Data may be moved between the unit and a host PC in the menu also. The unit will appear, on the pc, as a Removable Memory device if a USB cable is connected now.

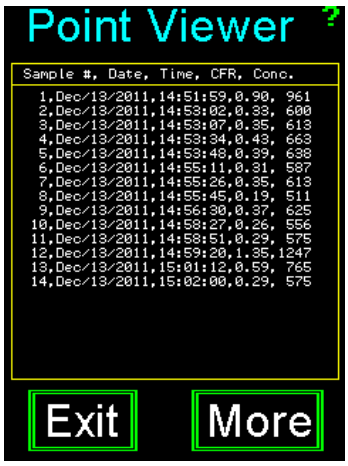
Tapping **Erase a File** will display a list of files that are on the machine. The Up/Down buttons will change color to indicate if any more data exists in that direction. Tapping on a file name in the list box will display a smaller window to confirm the erase action. Once erased, a file cannot be restored.



Viewing a file on the instrument

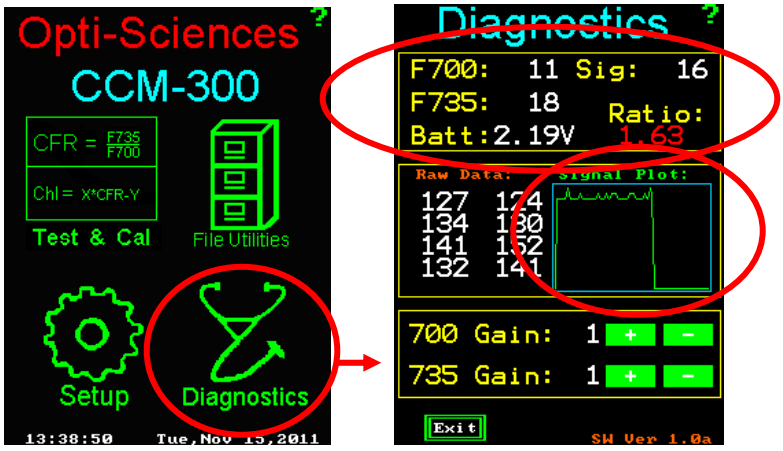
Tapping on **View a File** will display a list of files stored in the instrument.

Tap on the file of interest or scroll up and down using the arrow buttons on the right hand side. When the file of interest is found tap on the file name and the data in the file may be viewed. Detailed analysis of data is best performed on a PC.



Diagnostics Menu

Instrument operational values are shown here. This information can be useful for checking system functionality. In the event of trouble, the readings can be given to a service person.



The top box shows current system values, including the independent F700 and F735 values along with the ratio. The Signal reading is a measure of the fluorescence intensity detected. The Ref reading shows the source feedback amplitude. Batt displays the battery voltage. When the unit has NiMH batteries installed, a voltage of less than 2.20V indicates that the charge in the battery is almost used up. A freshly charged set should be installed before beginning a new round of measurements. A voltage greater than 2.50V is recommended for alkaline batteries. The middle box shows preprocessed signal (raw data) info and a graph of the signal value over a 1 minute period. This information is useful in verifying proper operation of the instrument.

The bottom box provides access to system settings that can be changed for diagnostics purposes.

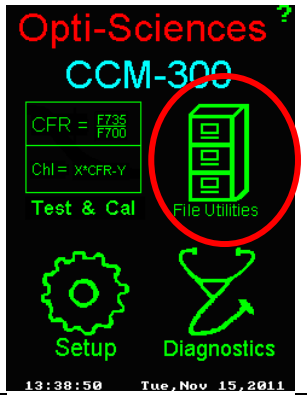
Current software version is displayed in the lower right corner.

Retrieving data:

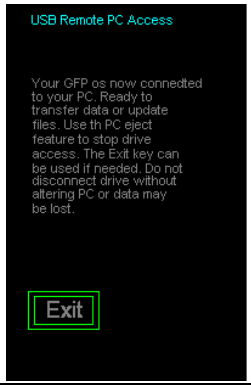
The CCM-300 behaves as a mobile storage device when connected to a USB equipped PC.

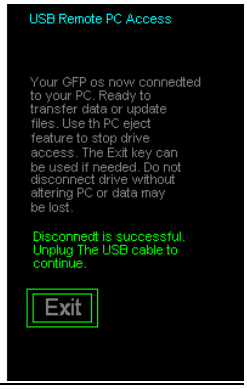
1. Connect the included USB cable to the instrument and a host PC.
2. Tap the **File Utilities** icon on the instrument. The instrument will sense the PC and display the remote access mode screen. No special drivers are required to transfer files to a PC. Files can be moved on and off the instrument in the same manner as with any other mobile storage device.

Attach USB cable to PC



File Utilities





Remote access screen is displayed

Note: Do not disconnect the instrument without first using the eject function on the PC. Failure to use the eject function may result in the loss of data, and possible corruption of the CCM-300 file system.

To use the eject function, select the device from Windows Explorer, right mouse click, and select eject. The instrument should beep, and display the message; "Disconnect is successful. Unplug the USB cable to continue." To complete the process, unplug the USB cable from the instrument. The file management screen will then appear.

Remote access screen

Data formats:

The format is stored in a comma separated variable format that can be easily imported into popular spreadsheet programs. A header in each file identifies the specific data field.

Sample Test Format:

Sample #, Date, Time, Raw Signal, CFR (Chlorophyll fluorescence ratio), Chlorophyll content in mg m^{-2}

1,Oct/11/2011,11:49:43,751,0.33, 600

2,Oct/11/2011,11:50:02,796,0.35, 613

Specifications:

- Source:
  Solid state modulated LED device providing excitation wavelengths from 460 nm with a half bandwidth of 15 nm
- Detector:
  Two solid state detectors . One filtered for detection from 698nm to 708 nm and the other filtered for a range from 730nm to 740nm.
- Adjustable detector gain with four different ranges. 1 is for high signal samples and 4 is for low signal samples.
- Noise:
  Typically < 2% of full scale, but is dependant upon calibrated range. Individual measurements are integrated over 5 seconds. Noise may be further reduced for samples with low fluorescence signal by averaging multiple measurments.
- Sensitivity:
  Signal integration over 5 seconds. Adequate to measure most single white pine needles.
- Sensing probe:
  Fiber has 0.10” diameter active region contained within a 0.15" diameter stainless steel tip
- Calibration:
  Solid state sensors and light sources provide no significant variation over the life of the instrument. The measurement represents a ratio of fluorescence at two different emission wavelengths. The research shows that this ratio provides a precise measurement of Chlorophyll content from 41mg m-2 to 675 mg m-2 using Beech, Elm or Wild Vine. Calibration may be checked by comparing chemical tests to read-out values. Parameters in the direct chlorophyll content formula may be changed to conform with chemical test results for any unexpected non-conforming species. The values and equation in the instrument conform with the equation supplied by Gitleson (1999).
- Repeatability is dependent on the emission signal strength. Measurements in the yellow signal strength range may require multiple measurements and averaging for more reliable results.

- User Interface:
  320x480 color TFT LCD screen with touch panel surface for user input
- Data capacity:
  Internal, non volatile 2GB memory for storage of calibration and data files
- PC interface:
  Unit appears as USB storage device to host PC
- Power Supply:
  2 AA batteries. Can use either NiMH or alkaline batteries. A charger is supplied.
- Operating Life:
  Typically 8 to 10 hrs with freshly charged batteries
- Operating Temperature:
  0 degrees to 120 degrees F (-18 degrees to 48 degrees C)
- Dimensions:
  4.7” x 3.5” x 1.2” (12cm x 9cm x 3cm)
- Weight:
  0.60 lbs (275g)