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Fluorescent Ratio Chlorophyll Content Measurements

Non-destructive measurement of very small leaves and difficult samples.

Possible applications include conifer needles, immature rice, filamentous algae on rocks, lichens, turf grasses, prickly pear cactus, Agave, pineapple, Arabidopsis, fruit, moss, leaf stems and petioles.

Today, chlorophyll content is usually measured either by chemical assay methods or estimated by non-destructive, 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 the instrument's 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.*
- *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, it was decided that it was time to engineer an affordable solution for these difficult applications. This article describes and explains this technology.

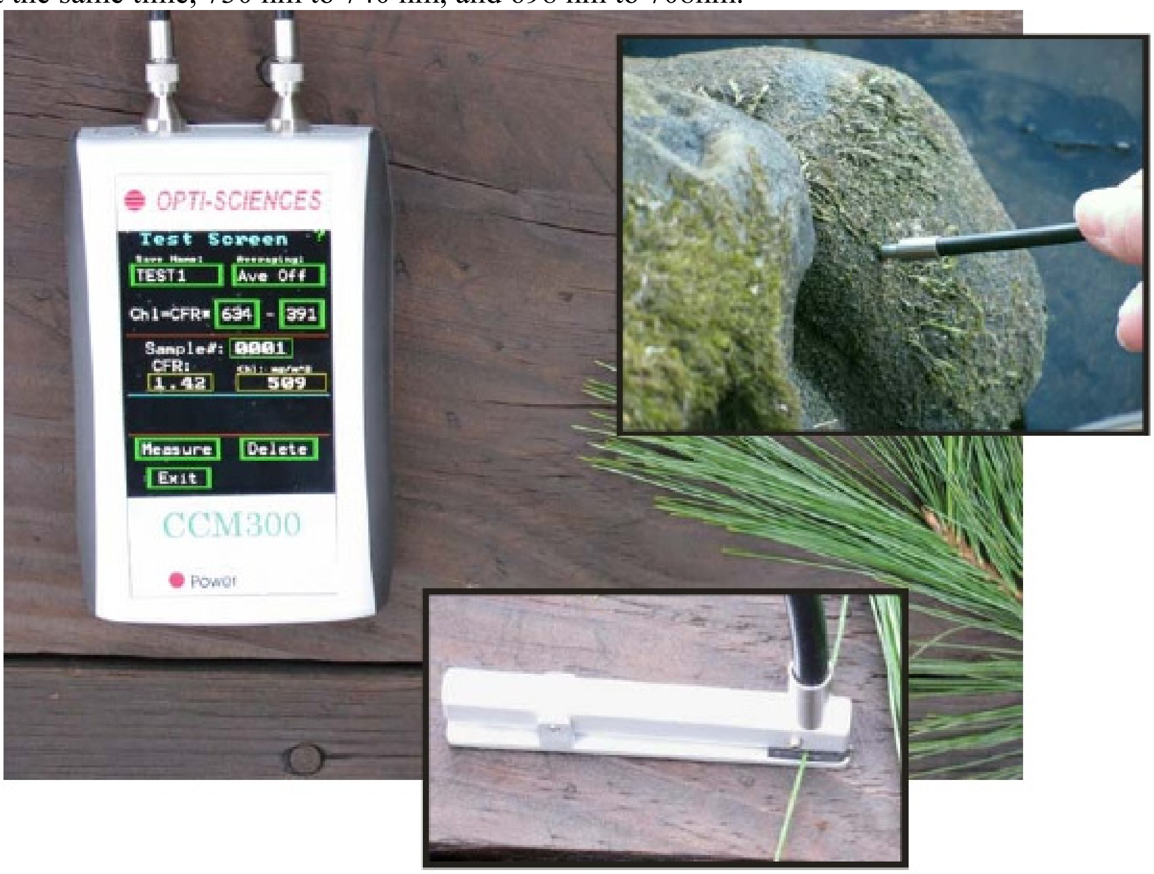
Research regarding the use of ratio fluorescence to measure chlorophyll content has been established for some time, and the experimental protocols and results are well understood. However, until now, the cost of such systems has been prohibitive.

Research by Gitelson A. A., Buschmann C., Lichtenthaler H. K. (1999) was used 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}. Research regarding the use of ratio fluorescence to measure chlorophyll content has been peer reviewed and the technique has now been well established.

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 thick samples can be measured. 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 difficult to measure 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.

The advantage of this ratio is that it works exceptionally well above chlorophyll content levels of 200mg m^{-2}, a limitation of previous fluorescence ratios. By raising the lower fluorescent emission measuring range away from chlorophyll absorption bands near 680nm or 685nm, to 700nm, the amount of fluorescence light that is reabsorbed and emitted again as chlorophyll fluorescence is minimized. This significantly extends the useful, linear measurement range of the device.

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



The instrument also provides two different readouts, one is the F735nm / F700nm 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, incase 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.

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