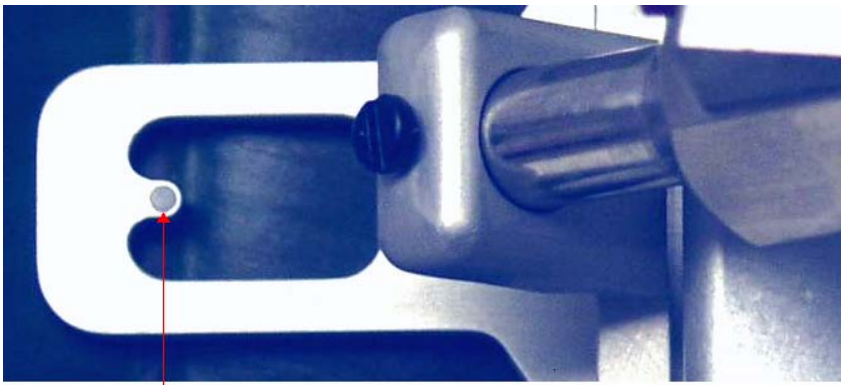


Light (PAR) Measurement with Fluorescent Yield Measurements - Benefits and Limitations.

Yield can vary significantly with light level and with temperature. Without controlling irradiation and temperature it is possible to misinterpret results. In fieldwork where both light and temperature can vary, a lower Yield measurement on one plant as compared to another could be misdiagnosed as stress when it may only be an increase in irradiation or a change in temperature on the leaf. When a PAR Clip is used to take Yield measurements, the combination can be formidable.



PAR or PPF sensor



PAR Clip on adjustable stand

A *PAR Clip* is a leaf cuvette that allows the holding of the sample leaf at a repeatable angle and distance from the measuring probe while measuring *Yield*, *ETR*, *PAR*, and *leaf temperature*. These values are usually contained in same data file with a time and date stamp. *PAR* (*Photosynthetically Active Radiation*) is irradiated light between the wavelengths of 400 nm and 700nm and it is measured very near the sample measuring area. When *PAR* is measured using a *PAR Clip* and the dimensions per square meter per second ($\text{m}^{-2} \text{s}^{-1}$) are used, the value becomes *PPFD* (*Photosynthetic Photon Flux Density*), (When using a *PAR Clip* the terms *PAR* and *PPFD* are interchangeable).

Some *PAR Clips* offer the option of using a predetermined light intensity level from an internal fluorometer light source to measure *Yield* and *ETR*. In this case, irradiation levels can be forced to be consistent for each sample. Note: When using internal illumination it is important to allow time for a plant to reach steady state photosynthesis before measurement or errors will result. Even small changes in irradiance level can change the measurement of both yield and *ETR*. Changes of as little as 100 μmol can cause errors if one does not allow for photoprotective changes, and state transitions (Opti-Sciences unpublished). Maxwell and Johnson (2000) found that it takes between 15 minutes and 20 minutes for plants to reach steady state. (For more information on this topic see Opti-Sciences App note # 0509, “*Yield value & Limitations*”.) Some *PAR Clips*, such as the digital *PAR clip* for Opti-Sciences OS5p, allow *PAR* measurement while an internal light source is being used, thus providing a reliable *PAR* or *PPFD* values over time. Of course in this case the *PAR* sensor should be calibrated to the internal actinic illumination as well as to sun light. It should also be calibrated for the location difference between the *PAR* sensor and the leaf sample (Rascher 2000).

Relative Electron Transport Rate or ETR.

ETR or Relative *Electron Transport Rate* is a parameter that is measured with a PAR Clip. ETR is a relative measurement that provides comparative electron transport rates for PSII at different light or radiation levels. It is considered *relative* because chlorophyll fluorescence does not correlate exactly with absolute gas exchange measurements. While most fluorescence occurs in the upper most layers of the leaf, gas exchange measures the effects of photosynthesis in deeper layers as well. (Schreiber 2004).

Even with this in mind, relative ETR can be extremely valuable. While exact correlation to gas exchange carbon assimilation is not possible, linear correlation is possible with C4 plants (Genty 1989) and a curvilinear correlation is possible with C3 plants (Genty 1990). While four electrons must be transported for every CO₂ molecule assimilated, or O₂ molecule evolved, differences from gas exchange measurements can occur under conditions that promote of photorespiration, cyclic electron transport, and nitrate reduction (Schreiber 2004) (Baker, Oxborough 2004). **For more detailed information concerning the relationship between fluorescence and gas exchange measurements again refer to Opti-Sciences application note #0509 on Yield measurements.**

The equation for Relative ETR is **ETR = (Yield or $\Delta F/F_m'$) (0.84) (0.50) (PPFD or PAR)**

In this equation, Yield represents overall PSI and PSII yield. It assumes an average leaf light absorbance to be 84%, and the portion of light provided to PSII to be 50%. PPFD is PAR irradiation measured very near the leaf in micromoles or microeinsteins (equivalent units). The end result is a close approximation of PSII ETR that can be used for relative evaluation of different samples.

The absolute amounts for leaf and PSII light absorption can vary at steady state with species, stress and water content. Terrestrial leaf absorbance has been found to vary between 70% to 90% (Eichelman H., Oja V., Rasulov B., Padu E., Bichele I., Pettai H., Niinemets O., Laisk A. 2004), and the percentage of light absorbed by PSII has been found to range from 40% to 60% (Laisk A. and Loreto F. 1996), (Edwards GE and Baker NR 1993).

Other areas of value:

Besides the ability to control and relate yield measurements to PPFD, leaf temperature, and the ability to relate ETR to carbon assimilation, PAR Clips can provide additional specialized high value uses.

1. **C4 plant Water stress measurement** The ratio of ETR to carbon assimilation, or ETR/A, is known to be consistent in C4 plants. **This is not true in C3 plants.** *ETR requires a PAR Clip.* (J Cavender-Bares & Fakhri A. Bazzaz 2004) (Cerovic 1996) For measurement of early water stress in C3 plants see (Burke 2007) or the Opti-Science App note on Water Stress.
2. **The relationship between the light reaction and the dark reaction may not be straightforward under certain circumstances.** In some cases light reaction electron transport from PSII is significantly higher than predicted by gas exchange measurements. When combined with gas exchange equipment, ETR can be used to improve the study of these effects in areas such as photorespiration (Roseqvist and van Kooten 2006), C3 plant water stress (Ohashi 2005), cold stress (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998) and mild heat stress (Haldimann 2004). For more information on issues related to the relationship between fluorescence measurements and gas exchange, please review the OSI application note on “Yield, value and limitations”.
3. **ETR** is good indicator of **Nickel** stress and light reaction activity (Joshi & Mohanty2004), (Tripathy 1981).

4. **Light response curves**- These are charts that show the relationship of ETR and PPFD at different irradiation values at steady state photosynthesis. They can be used to study plant range limitations, identify stress resistant plants, study the response of shade leaves vs. sun leaves, identify mutants, study plant protective mechanisms under stress conditions, and other applications. (Rascher U. 2000)
5. **Rapid light curves** – this is usually a partially dark adapted test that can take less than two minutes. Usually dark adaptation clips are used without a PAR Clip. Internal fluorometer look up tables usually supply PAR values. (With the OS5p and soon the OS1p, this test can be done using a PAR clip for actual measurement of PAR values.) Steady state photosynthesis is not reached, instead, measurements are made after 10 seconds, or 30 seconds or one or two minutes. Actinic light is stepped up or down. Data from several measurement curves at different times of day are required for reliable results as results change at different times of the day. This is a test that is typically used for aquatic plants and under canopy plants where light is constantly variable and other methods of testing prove difficult. Light fitting curves are typically used to determine cardinal point such as ETRmax (Rascher U. 2000), (Ralph P.J., Gademann R. 2005). It can be easy to misinterpret results (Rascher 2000).
6. **CAM plant light reaction measurement**. It is not possible to use gas exchange equipment during the daytime to measure photosynthesis in CAM plants. ETR may be used to study electron transport. (Edwards GE and Baker NR 1993), Rosenqvist E., van Kooten O., (2006), (Laisk A and Loreto F 1996).

The greater portability of chlorophyll fluorometers with PAR clips and the speed of yield and ETR measurements provide greater flexibility for field applications and larger populations.

PAR and PPFD

Both the PAR and PPFD terms have used extensively in biology. microeinsteins, and micromoles have also been used extensively in biology. The (SI) International System of Units is umols.

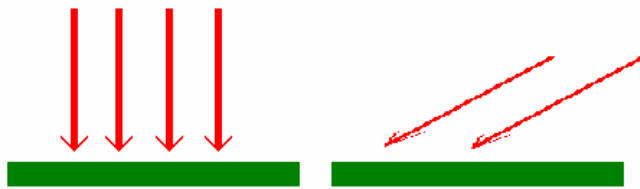
$$\begin{array}{ll}
 1 \text{ einstein} = & 6.022 \times 10^{23} \text{ photons m}^{-2} \text{ s}^{-1} & 1 \text{ micro einstein} = & 6.022 \times 10^{17} \text{ photons m}^{-2} \text{ s}^{-1} \\
 1 \text{ mol} = & 6.022 \times 10^{23} \text{ photons m}^{-2} \text{ s}^{-1} & 1 \text{ micromol} = & 6.022 \times 10^{17} \text{ photons m}^{-2} \text{ s}^{-1} \\
 \\
 \text{microeinstein} = & \mu\text{E} & \\
 \text{micromol} = & \mu\text{mol m}^{-2} \text{ s}^{-1} = \mu\text{mols} = \mu\text{M} & \\
 \\
 1 \mu\text{E} = 1 \mu\text{mol} = & 6.022 \times 10^{17} \text{ photons m}^{-2} \text{ s}^{-1} &
 \end{array}$$

In some satellite studies of PAR radiation, units are given in Watts per square meter, or W m^{-2} . To convert this value to PAR in micro-moles and micro-einsteins the Watts per meter PAR value should be multiplied by 4.6. (It should be noted that other light sources will require a different conversion value.)

Other important considerations when using a PAR Clip

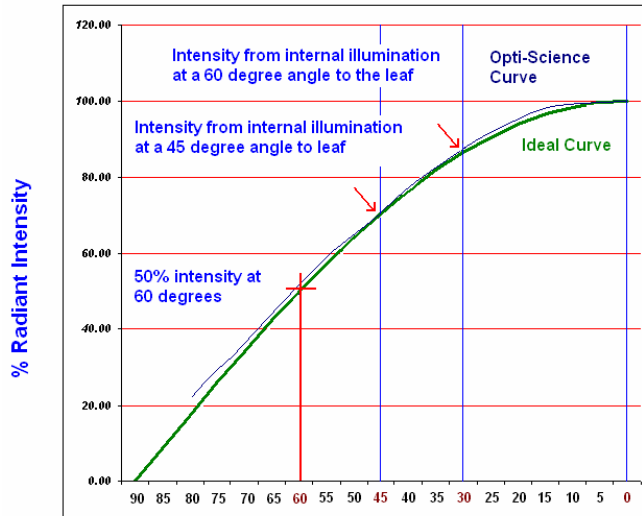
Sources of instrument error:

1. **Spatial error** – This is an error related to light angle incident on the sensor. It is made up of “cosign error” and “angular error” which are related to error caused by different radiation incident angles and the diffuse filter section used to correct for cosign error. The error is less at angles closer to normal or perpendicular. Opti-Sciences digital PAR Clips such as those used with the OS5p and OS1p chlorophyll fluorometers are Cosign corrected. At 60 degrees light irradiance is cut in half.



Less light strikes the leaf surface at steeper angles

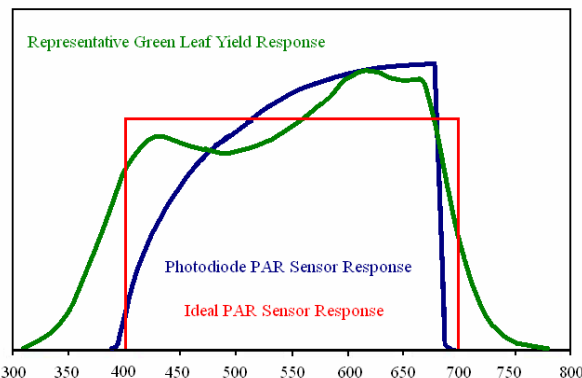
Lamberts Cosine Law
Ideal response from a cosine corrected sensor.



Angle Variation From Perpendicular (or Normal)

As the angle of irradiation increases from perpendicular, the irradiation per unit area per second decreases.

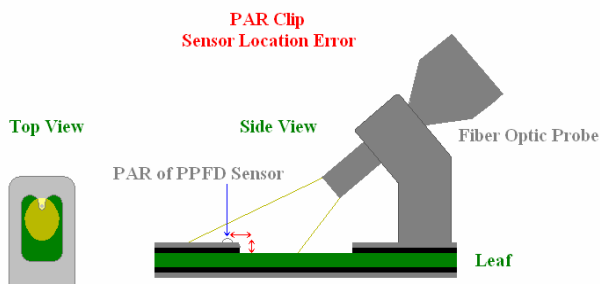
- Spectral response error** – The type of illumination used can cause an error in PAR measurement. This is due to the different spectral radiation given off by the sun and sky, halogen lamps, arc lamps, or other lamps used in growth chambers and green houses. The most accurate way to measure PAR is with a spectroradiometer, an instrument that costs thousands of dollars. To provide a field portable, reliable compromise, various photodiodes are used to measure PPF with fluorometers and PAR Clips. The Opti-Sciences sensor underestimates PPF in the blue range and overestimates PPF in the red range as compared to an ideal PAR sensor. Because the Sun and built in halogen light sources used to drive photosynthesis offer a wide spectrum of light over the PAR range, these spectral errors offset each other, minimizing overall spectral error.



The OS5p sensor is calibrated to daylight at mid-day using the Licor 190 sensor. In the next software release, the internal halogen and internal LED actinic light sources will also be calibrated

to a Licor 190 quantum. The Licor sensor is traceable to the National Institute of Standards and Technology that uses a special halogen lamp at 3150 degrees Kelvin as a standard.

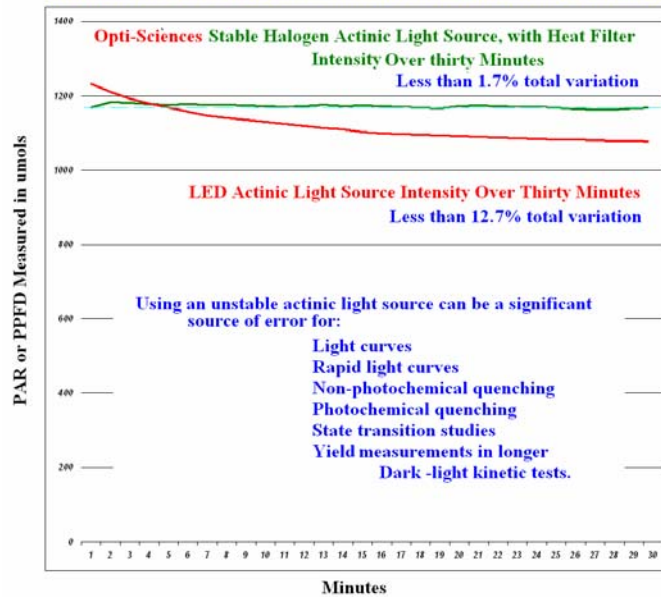
3. **Absolute error** - this is caused by error in the special halogen light source used by National Institute of Standards and Technology.
4. **Stability error** – Solid state sensors do not change significantly over time, it is recommended that a PAR Clip Calibration should occur every two years.
5. **Temperature error** in a range of 15 degrees centigrade to 35 degrees centigrade the error is very small.
6. **Readout error** caused by the digital readout is very small.
7. **PAR sensor location error.** While this is not significant when using a PAR Clip for ambient sunlight measurements, *it can be significant when making measurements using a fluorometer with an internal light source.* The height difference between the PAR sensor and the leaf can cause a significant error in the range of 10% (Rascher 2000). This can be corrected by calibrating the PAR Clip PAR sensor to a PAR sensor located at the leaf plane. The intensity relationship is linear (Rascher 2000). It can also be overcome by using internal fluorometer PPFD look-up tables for pre-measured values that correct for location error. However, if look up tables are used, the values can change over short periods of time due to heat, and over longer periods of time due to changes in lamps or LEDs. (The OS5p PAR Clip measures PPFD from internal actinic light sources. Internal look up tables are available and can be used when a PAR Clip is not being used. *In the next software release for the OS5p and OS1p, separate calibrations will be made for sun light, the internal actinic LED, and the internal actinic halogen light sources at the leaf plane. There will also be a programmable factor that can be used for other laboratory light sources.*



8. **Internal actinic lamp temperature stability error.** This is most important when using internal look up tables for irradiation intensity at the leaf. Par Clips that actually measure PPFD from an internal source during measurement will detect variation in light level due to increased instrument heat levels. The PAR output of most internal fluorometer illuminators will change over time due to increased heat caused by the lamp. This can also be of concern when making light curves and even rapid light curves. Lamp output falls off most dramatically during the first few minutes after turning on the internal actinic illuminator. If lookup tables are used for PAR or PPFD values, as when dark adaptation clips are used for light curves or rapid light curves, the values may not be accurate if the light source intensity changes.

When using the OS5p it is recommended that the Halogen actinic illuminator be used for longer light curves, rapid light curves, dark – light kinetic tests, and quenching tests. The Halogen light was designed to be very stable over time. Tests have shown that there is less than a 2% variation of intensity over time. The LED actinic light source can be used for shorter yield measurements.

PAR Irradiance at the Leaf Plane - The Effects of Heat on Actinic Light Sources



User error can be unlimited. Trying to measure samples that have momentary flecks of sunlight and short periods of shade caused by wind or cloud cover can cause momentary PAR measurement to be almost meaningless. *For best results it is important not to change the orientation of a leaf and to avoid shading the sample measuring area with the PAR clip or by other means. Extraneous reflections and breathing on the sample should also be avoided* (Rosenqvist and van Kooten 2006). *Failing to clean the sensor can also cause errors.*

Under Canopy Errors occur when using a single small sensor to measure radiation within a crop canopy, in or under other plant canopies, or within a growth chamber. The values measured on a given plane below canopy can vary considerably due to shadows, reflections, and sunflecks. Neglecting this factor in measurements can introduce errors up to 1000%. A Study comparing instrumentation and methods for under canopy irradiation measurements claim that accuracy is within 20% except when gaps in the canopy are non-random (Welles and Cohen 1996).

Many schemes and methods have been used to solve the issues related to under canopy light irradiation PAR measurement. Some involve meter long sensors and others use point sensors. In these cases *averaging the PAR value over time and in some cases averaging yield over time is a method that has worked.*

Mechanical issues

Feedback from customers of various brands of instruments has also added concern about PAR clips opening at unwanted times, such as when used for high leaf measurement while reaching over ones head, and when used with a tripod for quenching studies.

The new Standard PAR clip has been designed to open from the bottom and not the top as other PAR clips have done. This prevents the opening of the clip with high leaf measurements caused by tension of the fiber optic sensor on the clip, and it also prevents the opening of the PAR clip on tripods due to fiber optic weight.

References:

- Allen J. F., Mullineaux C.W., (2004) Probing the mechanism of State Transitions in Oxygenic Photosynthesis by Chlorophyll Fluorescence Spectroscopy, Kinetics and Imaging. From Chapter 17, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, pages 447-460
- Baker N. R., Oxborough K., (2004) Chlorophyll fluorescence as a probe of photosynthetic productivity. From Chapter 3, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, pages 66-79
- Burke J. (2007) Evaluation of Source Leaf Responses to Water-Deficit Stresses in Cotton Using a Novel Stress Bioassay, *Plant Physiology*, Jan. 2007, Vol 143, pp 108-121
- Cavender-Bares J. & Fakhri A. Bazzaz 2004 – "From Leaves to Ecosystem: Using Chlorophyll Fluorescence to Assess Photosynthesis and Plant Function in Ecological Studies". Jeannine Cavender Bares, Fakhri A. Bazzaz, From Chapter 29, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 746-747
- Cerovic Z, Goulas Y. Gorbunov M, Britantais J-M, Camenen L, & Moya I., (1996) Fluorescing of water stress in plants. Diurnal changes of mean lifetime and yield of chlorophyll fluorescence, measured simultaneously at distance with a Lidar and modified PAM-fluorometer, in maize, sugar beet and Kalanchoe. *Remote Sens Environment* 58:311-321
- Cheeseman J.M., Herendeen L.B., Cheeseman A.T., Clough B.F., (1997) Photosynthesis and photoprotection in mangroves under field conditions. *Plant Cell Envir* 20: 579-588
- Earl H., Said Ennahli S., (2004) Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. *Photosynthesis Research* 82: 177–186, 2004.
- Edwards GE and Baker NR (1993) Can Co₂ assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Res* 37:89-102
- Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. (1998) Relationship between CO₂ assimilation, photosynthetic electron transport and active O₂ metabolism in leaves of maize in the field during periods of low temperature. *Genty B., Briantais J. M. & Baker N. R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, Biochimica et Biophysica Acta* 990, 87-92
- Genty B, Harbinson J., Baker N.R. (1990) Relative quantum efficiencies of the two photosystems of leaves in photo respiratory and non-photo respiratory conditions. *Plant Physiol Biochem* 28: 1-10
- Haldimann P, & Feller U. (2004) Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant, Cell and Environment* (2004) 27, 1169–1183
- Maxwell K., Johnson G. N, (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* Vol. 51, No. 345, pp.659-668- April 2000
- Joshi M.K., Mohanty P. (2004): Chlorophyll *a* fluorescence as a probe of heavy metal ion toxicity in plants. In: Papaqeorgiou G.C. (eds.): *Chlorophyll Fluorescence: A Signature of Photosynthesis Advances in Photosynthesis and Respiration*. Springer, Dordrecht: 637–661.
- Laisk A and Loreto F (1996) Determining photosynthetic parameters from leaf CO₂ exchange and chlorophyll fluorescence. Ribulose-1,5-bisphosphate carboxylase / oxygenase specificity factor, dark respiration in the light, excitation distribution between photosystems, alternative electron transport rate, and mesophyll diffusion resistance. *Plant Physiol* 110: 903–912 .42-6
- Maxwell K., Johnson G. N, (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* Vol.51, No. 345, pp. 659-668- April 2000

- OHASHI Y., NAKAYAMA N., SANEOKA H., FUJITA K., (2006) Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. *BIOLOGIA PLANTARUM* 50 (1): 138-141, 2006
- Rascher U (2000). Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field U. RASCHER, M. LIEBIG & U. LÜTTGE *Plant, Cell and Environment* (2000) 23, 1397–1405
- Ralph P.J., Gademann R. (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany* 82 (2005) 222-237
- Rascher U (2000). Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field U. RASCHER, M. LIEBIG & U. LÜTTGE *Plant, Cell and Environment* (2000) 23, 1397–1405
- Rosenqvist E., van Kooten O., (2006) Chlorophyll Fluorescence: A General Description and Nomenclature. From Chapter 2 “Practical Applications of Chlorophyll Fluorescence in Plant Biology”. by Jennifer R. DeEll (Editor), Peter M.A. Toivonen (Editor) Kluwer Academic Publishers group, P.O Box 322, 3300 A.H. Dordrecht, the Netherlands, pages 39-78
- Schreiber U, Pulse-Amplitude-Modulation (PAM) Fluorometry and Saturation Pulse Method: An Overview From Chapter 11, “Chlorophyll a Fluorescence a Signature of Photosynthesis”, edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 279-319
- Tripathy BC, Bhatia B., Mohanty P., (1981) Inactivation of chloroplast photosynthetic electron transport activity by Ni ++. *Biochim Biophys Acta* 638:217-224
- Wells M., Cohen S. (1996) Canopy structure measurement by gap fraction analysis using commercial instrumentation *Journal of Experimental Botany*, Vol. 47, No. 302, pp. 1335-1342, September 1996