

Light curves

Light Response Curves an overview:

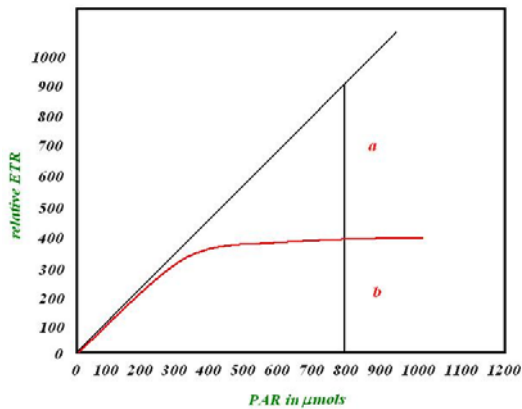
Light response curves are charts that show Photosynthetic Yield or Relative Electron Transport Rate on one axis and PPFD, or PAR (light irradiation between 400nm and 700nm) on the other axis. Light response curves are done at steady state photosynthesis after a leaf has completely adjusted itself to a specific light level and photochemical yield is stable. The time required to reach steady state photosynthesis is species dependant. Maxwell and Johnson (2000) found that twenty two English land plants reached steady state in times ranging between fifteen and twenty minutes. Light curves can measure a leaf's ability to adjust to different light levels, so they can be valuable in the study of plant range limitations, the study of differences between sun leaves and shade leaves, and to study the effect of other types of plant stress on leaves.

Light response curves are different from **(RLC) rapid light curves** in that rapid light curves measure yield and ETR before steady state is reached. Rapid light curves have been used for many applications, but have been used most frequently in the study of a plants ability to adapt and tolerate rapidly fluctuating light levels in relation to its light history. They have been heavily used in the study of aquatic plants, and under canopy measurements where light levels are constantly changing. The value of rapid light curves and some of the methods used are more controversial than normal light curves. Rapid light curves do provide different physiological information and they do have their supporters. It is stated that RLCs provide relevant information on the saturation characteristics of electron transport. (Schreiber 2004) Rapid light curves may involve dark adaptation for various periods of time. The results and limitations are discussed by Rascher (2000). Momentary dark adaptation is discussed by Ralph (2005). *Rapid light curves will be discussed in detail in the next application note.*

N-SLC or non-sequential light curves also provide different physiological information than rapid light curves, and standard light curves. These are tests that are done at different light levels but they are not stepped up or down so that there is no cumulative light history effect or no cumulative NPQ. According to Herlory (2007), actinic exposures of 50 seconds or greater are required for stable results. N-SLC. Rapid light curves provide higher initial ETR values, and lower ETR_{max} values. Consult Herlory (2007) for more information on N-SLR.

Light Response Curve Graph

In the graph below a black diagonal line is drawn from zero through the first ETR value measurements providing the initial slope of the light curve. A vertical line is drawn through a selected PAR intensity value. In this case, 800 umols has been selected, but any intensity can be used. The red line is a curve determined by measured ETR values. The equation $a/(a+b)$ is a measure of ETR limitation at any given PAR value. In this case $a/(a+b) = 0.56$ is a measure excessive light intensity.



α is the initial slope of line at low PAR values created by relating ETR to PAR. It is a measure of potential maximum quantum yield of PSII. (Schreiber 2004)

ETR_{max} is a measure of a leaf's photosynthetic capacity. (Schreiber 2004)

$I_k = \alpha/ETR_{max}$ is a measurement of the point where light saturation dominates over increases ETR as PAR values increase. (Schreiber 2004)

$a / (a+b)$ is a measure of ETR rate limitation at any given PAR value, or a measure of excessive light at a given PAR value. (Schreiber 2004)

Light history

The time required for a leaf to reach steady state involves a number of plant processes. When there is a change in light level, the rapid acting photo-protective mechanisms adjust. They include the xanthophyll cycle and thylakoid lumen ΔpH . This usually takes between two and four minutes (Lichtenthaler 1999). State transitions affect fluorescence measurements more significantly at lower and middle range intensities and less at high light intensities. State transitions take between fifteen and twenty minutes to adjust to new light levels. According to Allen and Mullineau (2004) state transitions can affect photosynthetic yield measurements in a range of 10% to 30% depending on species if care is not taken.

When measuring field plants with a photo-inhibition light history of several hours, Lichtenthaler (1999, 2004) found that photo-inhibition mechanisms starts to relax after about forty minutes and can take up to 60 hours to fully relax. While this mechanism is not considered a steady state change, it is something to keep in mind when making light curves on field plants because photo-inhibition can be part of a plant's photo-history and it will affect results.

Dark adaptation

Dark adaption *may or may not* be included in a leaf light curve. The same leaf mechanisms that come in to play as light intensity increases, come in to play in dark adaptation. In this case, photoprotective mechanisms relax in two to four minutes, and state transitions shift back toward state 1 in terrestrial plants after fifteen to twenty minutes. The affects of Dark adaptation on light curves and rapid light curves is discussed in more detail in Rasher (2000)

Other changes also occur. A few minutes of dark adaptation is enough to re-oxidize the plastoquinone pool and the $CaMn_4O_xCl_y$ cluster, while longer periods deplete respiratory substrates through respiration in cyanobacteria and chlororespiration in higher plants and algae. Longer times will also deplete ATP pools, and trans-membrane ion concentration gradients. Dark adaptation also shifts higher plants and algae toward state 1 conditions and cyanobacteria to state 2 conditions. (Papageorgiou G.C. Tismilli-Michael M. Stamatakis K. 2007)

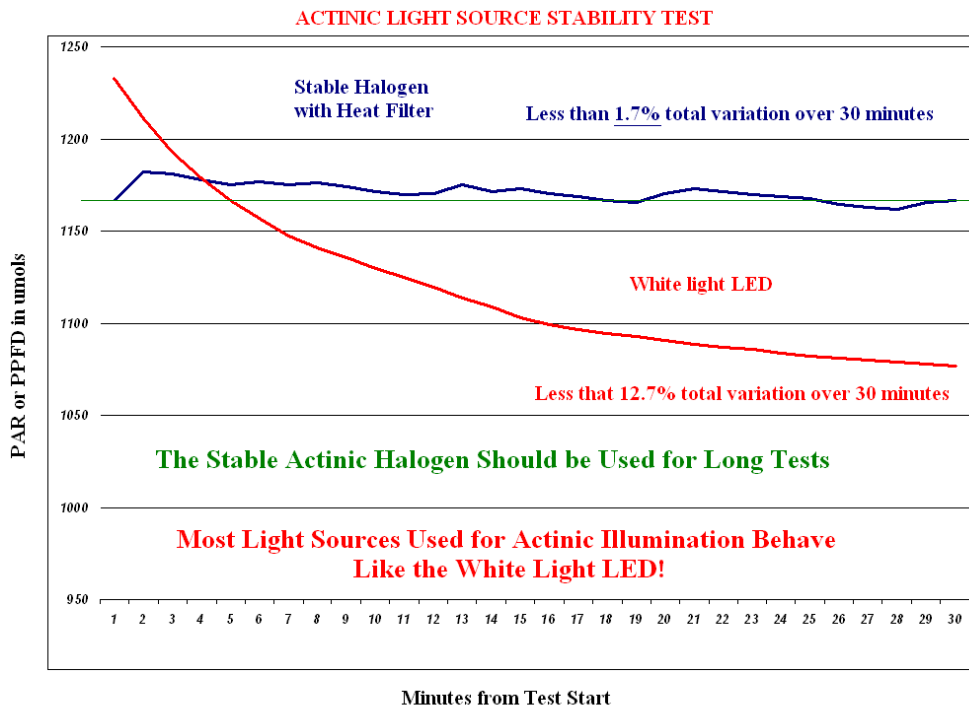
Requirements

Since light intensity must be stepped up or down in known and stable increments, some advanced Chlorophyll fluorometers allow the uses of an internal actinic light source to create these curves. Other manufacturers recommend the use of external light sources only, for some models of fluorometer. The reason for the concern is that most internal fluorometer light source intensities will change significantly in a short period of time as the fluorometer heats up. The light source itself is the source of the heat. The warmer the fluorometer becomes, the more the light source intensity is reduced. This is true of halogen light sources and LED light sources.

One manufacturer states that their internal actinic illuminator should only be used for less than two minutes, and that light intensities may change by as much as 10% to 20%. In this case the leaf does not reach steady state.

When using the OS5p, the halogen light source is designed to provide a stable intensity over time. Tests have shown that the intensity of the halogen actinic light source changes less than $\pm 2\%$ over a thirty-minute period, making this light source the one to use for light curves.

The graph below shows the intensity variation of the stable actinic halogen light source and a white light LED that is also included with the OS5p.



**Important for light curves, rapid light curves,
quenching tests, and quenching relaxation tests**

Light curves may be done using a dark adaptation clip and stepping the light intensity up or down using pre-measured, predetermined light intensities. However, a greater error in the actual PAR intensity is to be expected with this method due to the heat issue, and lamp age. The intensity of halogen lamps change over time due to vaporization of tungsten from the lamp filament that deposits on the cooler lamp envelope and darkens the bulb. Halogen light sources are designed to prevent this at higher intensities and heat levels, but the process does not work at low and medium intensity values.

A better way to make light curves is by using a PAR Clip that measures PAR during the light curve. In this way, even slight light changes are measured. The OSI halogen is the light source that is recommended as an actinic light source. A cover, a dark cloth or a darkened room can be used for dark adaptation if desired.

Accurate PAR measurement is another issue. Rasher (2000) found that when using internal fluorometer illuminators, it is important to compensate for the difference in PAR sensor location vs. leaf location. He found that an error in the range of 10% can occur when measuring PAR at the sensor location compared to measuring PAR at the leaf plane. This error is not significant when using ambient sun light.

Corrections can be made by one of two methods. The first method is to add a correction factor to measurements. By comparing PAR values at the leaf plane to PAR values at the sensor location, Rasher found that the difference was linear through the intensity range. So a factor can be used to multiply by PAR Clip PAR measurements to get PAR measurements at the leaf plane. The second way is to use the next version of New OS5p software that corrects for this discrepancy by calibrating the PAR clip sensor to a Licor 190 PAR sensor at the leaf plane. Internal light sources are calibrated to the PAR sensor and ambient sunlight is also calibrated.

Most light curves are made in equal light incremental steps, but it is not a requirement. Most light curves start at low light levels and go beyond saturation levels or start beyond saturation levels and go to low light level, but it is also not a requirement.

When making light curves, a researcher may use a single saturation pulse or multiple saturation pulses at a given light level. Multiple saturation pulses allow one to observe and measure the changes to steady state as they occur, however a single pulse at steady state is all that is necessary.

Saturation Pulse intensity duration, and frequency According to Roseqvist and vanKooten (2004) a spacing of one to two minutes between saturation pulses is required for complete elimination of NPQ caused by saturation pulses. If shorter spacing is used a small amount of NPQ will accumulate with each additional pulse. The Ideal Saturation duration for land plants is between 0.8- 1.0 seconds. (It is less for Algae). Longer pulses will cause NPQ to provide a reduce yield value, and shorter durations will not allow full saturation of reaction centers. Roseqvist and vanKooten (2004) The OS5p duration is fixed at 0.8 seconds.

Intensity should be high enough to completely close all PSII reaction centers. If dark adaptation is used, intensity values as low as 2000 to 3000 umls can be used for saturation intensities. However, higher intensities are required to close all reaction centers at high actinic light levels, an effect thought to be related to NPQ. (Note: When measuring Yield and ETR in plants that have been exposed to high light levels for an extended period of time it may not be possible to fully saturate PSII reaction centers with any amount of light. A method has been developed to correct for this issue by Hughes (2004) and it is available with the OS5p. For further information contact OSI.

While studies have found that photodamage can occur when using very high saturation pulse intensities in a dark environment at night, for relaxation curves, the evidence also shows that high saturation intensity damage does not occur when actinic light is applied. One can test for adequate saturation intensities by looking at the peak of the saturation pulse that is used. If the top is flat then the intensity is adequate, if it is curved on both sides then probably low. If it is curved only at the back of the peak, then NPQ is occurring, and the intensity is too high or the duration is too long. Peaks can be graphed in Excel by highlighting some of the Fs values and all of the saturation values of a single saturation pulse, and then using chart Wizard to graph and magnify the peak... (Intensities lower than about 8000 umls has been found to prevent photodamage in a dark environment (Roseqvist and vanKooten 2004)).

ETR:

ETR is an estimate of relative electron transport rate. The standard equation used is:

$ETR = (PAR) \times (Photosynthetic\ Yield) \times (0.84)$ (the average leaf absorbance value for red light) $\times (0.5)$ (the average amount of light that is absorbed and channeled to PSII) The actual absorption values vary with species and water content (Eichelman H., Oja V., Rasulov B., Padu E., Bichele I., Pettai H., Niinemets O., Laisk A. 2004) (Laisk A. and Loreto F. 1996), (Edwards GE and Baker NR 1993). In addition, fluorescence is measured from only the top leaf layers as compared to gas exchange measurements that measure the lower layers as well (Schreiber 2004). While ETR is a relative measurement, it is very useful when comparing different samples, and a single sample at different parameter values. (**More information on ETR our Yield are available in the Yield and PAR measurement application notes from OSI.**)

Relating light curves to photosynthesis gas exchange:

In 1989, Genty developed the yield measurement and provided strong evidence of a linear correlation between Yield measurements, Electron Transport Rate, and CO₂ assimilation for C₄ plants (Baker and Oxborough 2004) and many others have confirmed the relationship. It was found that a curve-linear correlation between Yield and CO₂ assimilation exists for C₃ species where photorespiration can also use significant products of electron transport (Genty 1990), (Baker and Oxborough 2004). While linear correlation and curvilinear correlation are possible (Genty 1989), (Genty 1990), (Baker Oxborough 2004), *exact* correlation between fluorescence ETR and gas exchange ETR is not possible due to the fact that fluorescence comes from only the upper most layers of the leaf while gas exchange measurements measure lower layers as well (Schreiber 2004)

Correlation Issues related to light curves:

1. Green algae - Results show that ETR_{max} was 20% higher than expected when compared to O₂ polarography measurements. It is thought the difference was due to Mehler-Ascorbate Peroxidase reactions (Gilbert (2000) (Schreiber 2004).
2. Cyanobacteria – Result show errors of up to 100% compared to O₂ polarography. It is thought that the differences were due to either O₂ respiration or the unique state transition make up of cyanobacteria (Gilbert (2000) (Allen and Mullineau 2004) (Schreiber 2004).

The OS5p is recommended for light curve work because it offers programmable automation for various types of light curves, and just as importantly, a stable actinic light source and a PAR clip designed to measure the internal stable light irradiance values in a reliable way.

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