**Plant Leaf Dark Adaptation - How long is long enough?**

**Dark adaptation** is a technique used in some chlorophyll fluorescence measurements to fix a reference point under known, stable and repeatable conditions. (Baker 2008). Deciding where to put that reference is based on an understanding plant mechanisms that can affect measurements, and what one wants to measure. Recommended times can vary by chlorophyll fluorescence test type, and environmental conditions.

Due to recent research on chloroplast migration (Cazzaniga 2013, Dall’Osta 2014), times should be extended to at least 20 to 35 minutes, if samples are tested, and 35 minutes if samples are not tested. Dark adaption times of forty minutes and sixty minutes are also common for terrestrial plants, and some researchers only use pre-dawn values. Some research journal reviewers have their own ideas, and will only accept the equivalent of overnight dark adaptation. Researchers may want to check with target journal reviewers when designing experiments.

To obtain reliable modulated Fv/Fm or OJIP test values, decisions need to be made for control and test measurements. The plant mechanisms listed below will lower Fm, and possibly raise Fo, changing OJIP and Fv/Fm measurements downward like other types of plant stress. One must decide which mechanisms are of concern for specific types of plant stress measurement and dark adapt accordingly.

\[ \text{Fv/Fm} \] is affected by both photochemical and non-photochemical factors. If a leaf is dark adapted and measured, then subjected to very high light levels for a period of time, then dark adapted and re-measured, the first measurement will be higher then the second measurement. The decline in \( \text{Fv/Fm} \) measurement may be due to a decrease in reaction centers capable of photochemistry or un-reversed non-photochemical quenching. (Baker N.R., Oxborough K. 2004)

Papageorgiou reports that results may vary greatly depending on how long dark adaptation is done. A few minutes of dark adaptation is enough to re-oxidize the plastoquinone pool and the CaMn4OxCly 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. Tismmilli-Michael M. Stamatakis K. 2007). Under high actinic light, or near saturating light conditions, cell chloroplasts migrate from the tops of plant cells to the sides of plant cells, increasing leaf light transmission and decreasing leaf light absorptance. This process significantly affects both light and dark adapted fluorescence measurements. During dark adaptation, chloroplasts migrate back to the tops of cells. This process takes between 20 minutes to 35 minutes (Cazzaniga S. 2013). See the application note on qM chloroplast migration at [www.optisci.com](http://www.optisci.com) for more information.

Full activation of Rubisco takes between three and four minutes in vascular plants as well as photoplankton. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some photoplankton. The longer deactivation is thought to offer an advantage for species subjected to erratic bright light for maximum utilization of light (MacIntyre 1997).

Rapid acting photo-protective mechanisms activated by exposure to variable light intensities (designated in the parameters \( q_e \) and \( Y(NPQ) \) are controlled by the xanthophyll cycle and thylakoid lumen \( \Delta \text{ph} \). They relax in a several seconds to few minutes during dark adaptation. (Muller, Niyogi 2001),(Kramer D. M., Johnson G., Kiirats O., Edwards G. (2004). According to Lichtenthaler (1999)
this time is 4-6 minutes. Baker (2008) indicates that the adjustment and relaxation times can be longer in field plants, up to 7 minutes.

The affects of state transitions on chlorophyll fluorescence have recently been shown to be more complex than previously thought. Classical state transition theory saw state transitions as a low light survival mechanism that allowed light balance between Photosystem II and Photosystem I. F$_{M}$' or maximum fluorescence under light adapted conditions would decrease over a fifteen to twenty minute time frame, and then relax during dark adaptation over a fifteen to twenty minute time frame. State I – State 2 transition quenching relaxation (called q$_{T}$) was considered to be most significant at lower light levels in terrestrial plants and could represent more than 60% of quenching at low light levels. It was also thought that at high light levels it represents about 6% of total quenching. (Lichtenthaler H. Burkart S 1999). Recent evidence shows that the fluorescence change thought to be the result of state transitions is in fact caused by chloroplast migration at least at higher light levels and near saturating light levels in land plants. Chloroplast migration takes between 20 minutes and 30 minutes to adjust and to relax in wild plants and up to 35 minutes in mutants. See the application note on q$_{M}$ and q$_{T}$ for more information (Cazzaniga S. 2013), (Dall’Osta 2014).

In the past, it was thought that the effects of acute photo-inhibition caused by exposure to high light intensities for an hour or two, could be reversed with 20 to 30 minutes of dark adaption (Theile, Krause & Winter 1998). Recent evidence indicates quenching relaxations in the dark, for these time periods, are likely to be caused by chloroplast migration instead (Cazzaniga S. 2013) (Dall’Osta).

**Quenching and quenching relaxation measurements:**

When making longer quenching and quenching relaxation parameter measurements related to photo-inhibition and photodamage mechanisms that are common in chronic high light stress, high heat stress, cold stress and over wintering stress, one should understand that it can take days for full relaxation or repair of the non-photochemical quenching parameter, q$_{I}$ to pre-stress conditions. Reversal or relaxation of chronic photo-inhibition caused by several hours of high light exposure starts to relax at about 40 minutes and may take 30 to 60 hours to fully relax under dark adaptation (Lichtenthaler H. & Babani F. (2004) (Theile, Krause & Winter 1998).

To get an accurate control value for F$_{M}$ and F$_{O}$ under chronic photo-inhibition conditions, (Maximum fluorescence, and minimum fluorescence components of non-photochemical quenching parameters) it is common to dark adapt for a full night using pre-dawn values. In some cases, it may make sense to dark-adapt for longer periods of time. (Maxwell and Johnson 2000). It is understood that in plants with a recent high light history that there will likely be some residual photoinhibition built into all dark adapted measurements. This is alright as long as the light history of measured samples has been built in to the experimental design. Unless light stress is the focus of the experiment, it is important to compare samples with similar light history. In addition, quenching measurements of different samples should not be compared unless the F$_{V}$/F$_{M}$ values of the samples are identical. This is necessary because F$_{V}$/F$_{M}$ is the yard stick used to gauge other quenching parameters (Baker 2008) See the quenching application note for more details. If studying photoinhibition, it may be helpful to partially shade test plants for more than 60 hours to get a reliable F$_{V}$/F$_{M}$.

In Aquatic Plants Gorbunov (2001) is a good source for corals, and Consalvey (2004) is a good source for Algae. For information regarding dark adaption for rapid light curves Rascher 2000 is a good source.

The use of far-red pre-illumination that is available on some fluorometers is designed to rapidly re-oxidize PSII by activating PSI. While this can be valuable in fieldwork (Maxwell and Johnson 2000),
it does not affect the relaxation of other non-photo-chemical quenching mechanisms Consalvey (2004).

Dark adaptation can be accomplished by using dark adaptation leaf clips or cuvettes. Some researchers use hundreds of inexpensive clips to make measurements on larger population quickly. Shrouds, darkened rooms, and darkened growth chambers may also be used.

It is useful to use a PAR clip to measure the actinic light level and to maintain a stable light intensity level during quenching measurements to ensure steady state photosynthesis has been achieved before measurement. A dark shroud may be used with the PAR clip in this case, or it may be used in a darkened room.

In review, it is important to take a few things into account. Reliable dark adaptation times can vary by species, plant photo-history, the fluorescence parameter of interest, and the type of stress that needs to be measured. When dealing with a new species, or an unknown photo-history it is probably best to test for maximum and stable $F_v/F_M$ at different dark-adapted times for best results. When testing for optimal dark adapting times it is important to use samples that have been exposed to the maximum light conditions that will occur during the experiment for reasons discussed above.

Note: Due to the recent chloroplast migration studies (Cazzaniga S. 2013 and Dall’Osta 2014), it makes sense to use 35 minutes for dark adaption or longer when measuring samples that can not be tested for optimal dark adaptation time. Only compare samples with a similar light history. Evidence shows that plants tested reach a stable known reference dark adaptation state by this time. This is especially true when samples have been subjected to high actinic light levels. It is common to use overnight pre-dawn dark adaptation for quenching measurements.

While it is not set in stone, it is common to use the newest fully mature leaf blade for diagnosis of deficiencies in plants (Reuter and Robinson 1997)

For a complete free “Stress Guide” that deals with research, references, and recommendations on all kinds of plant stress contact Opti-Sciences by phone or E-mail.

Dark Adaptation Tests:

OS30p+ - Dark-adapted tests available: $F_v/F_M$, $F_v/F_o$ and advanced OJIP with graphing overlays. It includes $P_{I_{ABS}}$ – performance index, and all of the Strasser protocol plant stress parameters.

OS1p - Dark-adapted tests available: $F_v/F_M$, $F_v/F_o$ NPQ, $Y(NPQ)$, $Y(NO)$. Adjustments for far-red pre-illumination and post illumination are included. Rapid Light curves with Eilers and Peters curve fitting software. It also included light adapted tests: $Y(II)$, ETR, PAR, and leaf temperature.

OS5p+ - Dark-adapted tests available: $F_v/F_M$, $F_v/F_o$, Kramer $Y(NPQ)$, $Y(NO)$, $qL$, $F_o’$; Hendrickson $Y(NPQ)$, $Y(NO)$ & NPQ; Strasser OJIP; Vredenberg OJIP quenching; puddle model NPQ, $qN$, $qP$ & $F_o’$; quenching relaxation protocol includes: $qE$, $qT$, $qM$, $qZ$, $qI$. Rapid Light curves with Eilers and Peters curve fitting software include $ETR_{MAX}$, $I_k$, $I_m$, and $\alpha$. It also included light adapted tests: $Y(II)$, ETR, PAR, and leaf temperature.
References:


