A Cookbook Checklist before making reliable $F_v/F_M$ measurements

Sometimes I hear this question from researchers, and I thought that it would be a good topic for an application note.

**Accuracy** is the ability to hit the bull’s eye.

In many types of measurements, accuracy is determined by calibrating to a measuring a standard that is traceable to the National Agency. With such measurements, tolerances are always involved.

**Repeatability** is the ability to achieve the same measurement again and again to a certain tolerance level.

A **Reliable** measurement is one that is accurate and repeatable.

**With Chlorophyll fluorometers, accuracy is determined in a different way.**

In this application note, will provide a cookbook style checklist of issues that musts be considered to get reliable $F_v/F_m$ measurements.

**First – $F_v/F_M$**
The biggest advantage of $F_v/F_M$ is that it is a measure of PSII performance that puts all samples in the same known dark adapted state before measurement. $F_v/F_m$ is a normalized ratio that does not use a traceable standard. Instead, it’s accuracy is determined by properly using the instrument and following the lessons learned about plant physiology by several great researchers. For most species, the optimal $F_v/F_m$ reading for stress free plants is in the range of 0.79 to 0.84 (Maxwell and Johnson 2004).

To get a reliable measurement, one has to follow tested guidelines.

1. **Dark-adapt properly** knowing the plant’s light history. It takes only a few minutes for the xanthophyll cycle and the $\Delta$ph of the thylacoid lumen to return to a dark-adapted state. It can take up to 4 minutes with indoor and green house plants, and it can take up to 7 minutes in field plants (Baker 2008). Recently Cazzaniga (2013) found that the intermediate fluorescence change previously attributed to state transitions, and acute photoinhibition, were in fact due to chloroplast migration in $C_3$ plants. Maai (2011) found that chloroplast migration was also a mechanism present in in $C_4$ plants. It was found that it takes from 20 minutes to 35 minutes for complete chloroplast migration to occur. In lower plants there is evidence that state transitions occur. (State transitions however, take between fifteen to twenty minutes (Ruban 2009) (Lichtenthaler 1999). These times can vary somewhat in field plants, and can take slightly longer. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some phytoplankton (MacIntyre 1997). In addition, field plants and other plants that have been exposed to photoinhibition conditions for a number of hours, will retain a certain amount of NPQ for up to 60 hours (Lichtenthaler 2004). This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into most summer field measurements of $F_v/F_m$. This is all right if one is measuring “light stress” and comparing results, but when measuring other types of plant stress, light history should be taken into account when comparing samples. It is common for researchers to choose dark adaptation times anywhere from twenty minutes to overnight, using pre-dawn values. Shorter times may be used to study the effects of plant protective mechanisms. For more information contact OSI for the Dark adaptation application note. (These guidelines are different for quenching measurements and for Rapid Light Curves.) If possible, testing should be done to find the time required to reach a **stable steady dark adapted state**. If not, then 35 minutes is safe for $F_v/F_M$ measurements with a similar light history.
2. **Modulation light intensity setting**  
\[ F_{v/Fm} = (F_m - F_o)/F_m \]  
Minimum fluorescence, is a “pre-photosynthetic” a dark adapted value measured by exposing the leaf antennae to a very low intensity modulated light. The intensity must be set properly to allow detection, but not high enough to drive photosynthesis. If it is set too high, it will drive photosynthesis and provide an Fo value that is too high. When setting the modulating light intensity, the Ft value or fluorescence signal should not rise over a 30 second period when a leaf is used. If it does, the intensity must be lowered. **OSI now offers an automated modulated light set up routine for its new OS1p and the OS30p.**

3. **Shade leaves vs. Sun leaves.** – The Fv/Fm ratio will be slightly higher on sun leaves than on shade leaves (Lichtenthaler 2004).

4. **Fv/Fm will be higher with a white saturation pulse than a red saturation pulse.**  
Some fluorometers use a red saturation pulse. This is not an issue for comparative measurements of plant stress with similar instruments, but values measured on a fluorometer with a white saturation pulse should not be directly compared to measurements of a fluorometer with a red saturation pulse. There is evidence to show that systems with a red saturation pulse correlate but measure consistently lower than systems with white light saturation lights. (Cessna 2010)

5. **Maximum Fv/Fm values vary with species.** The average maximum Fv/Fm value is between 0.79 - 0.84 (Maxwell and Johnson 2000).

6. **Compare samples with a similar light history.** Field plants should only be compared to field plants and greenhouse plants should be compared to greenhouse plants. Due to the fact that it can take up to 60 hours for chronic photoinhibition to relax, photoinhibition can be involved in some measurements more than others. (Lichtenthaler 2004) Results after a sunny day in the summer may be different that measurements on the same plant after a few days of overcast, again because it takes a long time for photoinhibition to relax or repair.

7. **It is common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants** (Reuter and Robinson 1997)

8. **The duration of the saturation pulse** should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for Phytoplankton and cyanobacteria. (Schreiber 1995). Times outside these ranges increase the error in Fv/Fm measurements. Shorter durations prevent complete saturation of PSII regardless of the light intensity. Longer durations create a form of saturation pulse NPQ that rounds the tail end of the pulse maximum value, and reduces the average maximum saturation pulse value. Opti-Sciences provides a moving 25 ms average to determine the highest FM. This ensures that a reliable value will be measured even if the saturation pulse width or duration is too long.

9. **Saturation pulse intensity.** Dark adapted leaves saturate easily with lower saturation pulse intensities. It may take a few hundred µmols to saturate shade leaves and sun leaves will saturate below 1,500 µmols. Lower values may not fully saturate PSII, and provide an error. Higher values always work with dark adapted samples. (Ralph 2005) (Requirements are different for Y(II).)

10. **Some Fv/Fm fluorometers have the ability to pre-illuminate dark adapted leaves with far-red light.** When this feature is used for five to ten seconds before an Fv/Fm measurement takes place, it activates PSI, and ensures that all electrons have been drained from PSII before the measurement of Fo. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, or photoinhibition. **Time is still required in a darkened environment to relax all forms of NPQ and to obtain a reliable Fv/Fm measurement.** (Maxwell and Johnson 2000)

11. **Fluorescence heterogeneity** presents itself as different Y(II) measurements on different parts of the leaf. It has been found to occur under cold stress conditions, with biotic stress, and under water stress conditions. By using multiple measurements and a sampling plan, heterogeneity can be overcome (Buschmann C. in correspondence by e-mail 2008). Imaging fluorescence can also be used.
12. Part of the minimum fluorescence, the Fo parameter, in Fv/Fm (Fm – Fo)/Fm), contains PSI fluorescence as well as PSII fluorescence. With Fv/Fm, one is trying to measure the maximum variable fluorescence of PSII in a dark-adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an error. In C₃ plants, about 30% of Fo fluorescence is due to PSI, and in C₄ plants about 50% of Fo fluorescence is due to PSI fluorescence. PSI produces about 6% of the fluorescence found in Fm in C₃ plants, and about 12% of Fm in C₄ plants (Pfundle 1998). This is not a problem when comparing Fv/Fm measurements for plant stress because PSI fluorescence does not change. It remains constant.

There are fluorescence solutions and assays available that are sensitive to most types of plant stress. Fv/Fm is not as sensitive as Y(II) for many types of plant stress.

Fv/Fm is not a sensitive test for drought stress, heat stress, nitrogen stress, nickel stress, sulfur stress, zinc stress, some herbicides and salt stress in some types of plants (Opti-Sciences Plant Stress Guide 2010). It can be used effectively in most other types of plant stress. For specific research results on specific types of plant stress, see the Plant Stress Guide offered by Opti-Sciences Inc.

References:


Buschmann C. (2008) in correspondence by e-mail, Dr. Buschmann recommended taking multiple measurements per leaf to find potential infection locations as a substitute for fluorescence leaf imaging: Dr. Claus Buschmann (Priv.Doiz), Botanik 2, Universität Karlsruhe (TH), 76128 Karlsruhe (Germany).


Opti-Sciences Plant Stress Guide  www.optisci.com

Opti-Sciences Dark Adaptation Application Note  www.optisci.com


