OS30p+ Chlorophyll Fluorometer

The best of both worlds
Hand held pulse modulated fluorometer for $F_{v}/F_{m}$ & $F_{v}/F_{o}$, along with advanced OJIP protocols

Pulse Modulated Tests:

$F_{v}/F_{m}$ - A Measure of Maximum Quantum Efficiency

$F_{v}/F_{m}$ is the most used parameter for plant stress detection in the world. Research has proven it to be a robust way to measure plant stress that affects photosystem II. The parameter and its protocol also provides the advantage that samples can be measured and compared in the same known dark adapted state. $F_{v}/F_{m}$ has been shown to correlate with carbon assimilation for many types of plant stress. The OS30p+ measuring trace is graphically displayed in color, and $F_{o}$ is accurately measured using red modulated light.

$F_{v}/F_{o}$ - While it does not directly correlate with carbon assimilation, it is a very sensitive stress detector that is more sensitive than $F_{v}/F_{m}$. It also allows comparison of samples in the same known dark adapted state.

The “JIP” test, - Advanced OJIP

“JIP” Test - This plant stress testing method provides a high time resolution image of the Kautsky induction curve against a logarithmic time scale. The curves are displayed, and traces can be overlaid and compared on the color instrument screen, or easily recreated from the data file for comparison, to evaluate plant stress. Direct readout of important stress detecting parameters and the overlay of measuring traces, are now immediately possible in the field.

The OS30p+ provides a direct read out of the following parameters: OJIP, t100 μs, t300 μs (or K), tFm (or time to $F_{m}$), A (or area above the curve), $M_{o}$ (or RC/ABS), PIABS (or performance Index), $F_{o}$, $F_{m}$, $F_{v}/F_{m}$, and $F_{v}/F_{o}$. In addition, OptiSciences goes the extra mile, by measuring $F_{o}$ instead of estimating the parameter.
FV/FM - The most used chlorophyll fluorescence parameter in the world.

FV/FM is a normalized parameter that is measured most accurately by a modulated fluorometer. Developed by Kitajima and Butler (1975) and others, it has stood the test of time for measuring maximum quantum efficiency of PSII in plants. It has shown the robust capability to measure plant stresses that affect PSII. In addition, it offers the advantages that it measures plants in a known and repeatable dark adapted state, and that the measurement was designed to correlate with carbon assimilation (Baker 2004).

After dark adaptation, modulated fluorometers allow the accurate measurement of minimum fluorescence or FO. This is done by using a weak modulated light, that is too low to drive photosynthesis, but high enough to excite pre-photosynthetic antenna, minimum fluorescence. In this state, photosystem II is maximally oxidized. The xanthophyll cycle, Δph of the thylakoid lumen, and state transitions have all relaxed to their inactive states (Lichtenthaler 1999, 2004), (OptiSciences dark adaptation application note www.optisci.com).

The modulated fluorometer then irradiates the plant sample with an intense saturation light that is high enough, and long enough, to fully reduce all available PSII reaction centers. The maximum fluorescence output during this saturating light radiation, or FM, is also measured.

The result is \( \frac{(F_{\text{Maximum fluorescence}} - F_{O}(\text{minimum fluorescence}))}{F_{\text{maximum fluorescence}}} \) or \( \frac{F_V}{F_M} \).

It has been found that healthy plants have an \( \frac{F_V}{F_M} \) value in the range of 0.79 to 0.84 (Maxwell and Johnson 2000). Plants subjected to different types of plant stresses, that affect PSII, measure lower.

The OS30p has added a special automated routine. It uses a rolling 8 point average that ensures that only the highest 25 milliseconds of maximum fluorescence is used, so that saturation duration time is never an issue for land plants, or for algae. This routine help ensure mistake free use.

**Graph of a 0.8 second \( \frac{F_V}{F_M} \) or \( \frac{F_V}{F_O} \) saturation**

\[ \frac{F_V}{F_M} = \frac{(F_M - F_O)}{F_M} \]

\[ \frac{F_V}{F_O} = \left( \frac{F_M - F_O}{F_O} \right) \]
The “JIP” Test - OJIP

OJIP or the “JIP” test is another dark adapted test that has been used for detecting and measuring plant stress. It was discovered by Kautsky (1957) that if the rise in fluorescence, caused by illumination after dark adaptation, was analysed, at high time resolution, there was a distinct curve shape with multiple steps. Using this approach, plant stress that affects PSII can be measured.

The latest research shows that O-J is caused by photochemical quenching, J-I is caused by photoelectrochemical quenching, and I-P is associated with the electric trans-thylakoid potential generated by the proton pump fueled by Cyclic Electron Transport (CET) in PSI (Vredenberg 2011).

It has also been shown that some types of plant stress affect specific parts of the OJIP curve. For example, nitrogen stress, at higher levels, has been shown to display a K step at 300 μs (Strasser 2004). In addition, special measuring parameters have been developed as sensitive stress detectors such as PI$_{ABS}$ or performance index.

Viewing OJIP graphic results can now be quickly and easily done in the field. The OS30p, provides a color graphic display of the OJIP curve with a logarithmic time scale. It is common for researchers that use this technique to overlay measuring graph traces to study the effects of plant stress, and to use the special parameters that have been created to quantify plant stress. Up to 16 traces may be overlaid on the graphic color instrument screen, and up to 32 can be overlaid from a single measuring file in software.

The parameters O, J, I, P, t100us, t300us (or K), M$_O$ (or RC/ABS), PI$_{ABS}$ (or Performance Index), A (or Area above the curve), and tF$_M$ (or time to F$_M$) are all displayed, along with F$_V$/F$_M$, and F$_V$/F$_O$ on the screen.

Overlaid OJIP graphs and Spider graphs are now easily created using standard Microsoft Excel software. The data files are specifically organized with detailed time stamps, and consecutive row organization for very easy parameter spider graphing. Detailed OJIP overlay graphing is equally as easy, with the first column providing data capture time, and all OJIP curve traces lined up next to each other in columns to the right of the time. Both linear and logarithmic graphing become very easy to create.

Since USB is now used exclusively by the OS30p, the instrument becomes a drive for your computer for simple transfer of data.
The “JIP” Test - OJIP

The instrument measuring screen shown on the right allows instant review of the most important information when taking a measurement.

- Settings are displayed.
- A color graph of the measuring trace is shown using a logarithmic scale of a linear scale.
- Direct read out of the most used measuring parameters is provided.

The last 100 measurements, and the important measuring parameters associated with these measurements, can be reviewed and compared as shown on screen to the right.

The default red light actinic intensity is 3,500 μmols, other intensities are also available:

- 525 μmols
- 875 μmols
- 1,000 μmols
- 2,500 μmols
- 3,000 μmols
- 3,500 μmols
- 4,500 μmols
- 6,000 μmols

The graph on the right was made by using a measuring data file that was output by USB port to a spread sheet. Sampling rate times are listed in column A, and each successive trace is reported, one after the other, in the remaining columns.

The X axis is in logarithmic time and the Y axis is in fluorescence units. Of course, the Y axis may also be normalized over $F_0$. 

Rugged field instrument designed for hand held use.

The OS-30p has been one of most cited research fluorometers in the world in recent years.

The system comes with 10 light weight quality, dark adaptation clips that are very affordable. Pricing allows the purchase of large quantities to fit most budgets.

Standard accessories included are a hard shell carrying case, a battery charger, 10 dark adaptation clips, and a USB cable.
Technical Specifications:

Os30p +

Modulated Fluorometer

FV/FM, FO/FV

Saturation intensity
Saturation light source - Array of red LEDs 660 nm.
Modulated light source - red 0.2 to 1.0 μmols.
Detection method - Pulse modulated
Detector and Filter - Pin photodiode with 700-750 nm band pass filter
Test duration - 0.1 seconds to 1.5 seconds. The default saturation pulse duration is set at 1.0 second; however, the software takes a rolling eight point 25 ms average to determine FO, and FM, making it idea for both algae and land plants.

Modulated light adjustment - manual adjustment from 30% to 80%

Parameters measured and graphed - FO, FM, FV/FM, FO/FV

JIP Test

Actinic light intensities - 6000 μmols, 4500 μmols, 3500 μmols, 3000 μmols, 2500 μmols, 1000 μmols, 875 μmols, 525 μmols.
An array of red LEDs at 650 nm are used for actinic illumination.

Detection method - Pin photodiode detector with 700-750 nm band pass filter using red pulse modulated light, and variable sampling rate from 10 μs to seconds.

Test duration - JIP test 3 - 300 seconds

"JIP * Test parameters measured

Direct readout parameters: O, t100μs, t300μs (or K), t2ms (or J), t30ms (or I), P, tFM, A (area above the curve), M0 (or RC/ABS), PABS (or performance index) FO, FM, FV/FM, FO/FV.
Up to 32 OJIP traces can be overlaid and displayed on the graphic display screen, Colors on the screen start to repeat after 16 traces are overlaid. Fo is measured not estimated.

Measured parameters reported to the data file:
ABS/RC, TRo/RC, Dl0/CS, ETo/RC, TRo/ABS, ETo/TRo, ETo/CS, RC/CS0, RC/CSM, S, M, T are also measured and recorded in the data file, but not on the measuring screen.

Up to 32 traces can be overlaid on the graphic display screen, and up to 32 traces, per file name, can be stored for graphic overlay of traces in a single data file. Colors start to repeat on the viewing screen after 16 traces are overlaid. This limitation does not exist in the data file. If traces are not stored, thousands of measurement parameter sets can be stored in a single data file for spider graphing. The number of data files are only limited by machine memory limits.

General Specifications

Display - Color Graphic Display
Storage Capacity - Up to 160,000 measurements. Up to 32 traces can be stored with a one data file name. Hundreds of traces can be stored in multiple data files, with different names.
Digital output - USB port
Battery - NiMH battery pack with a battery life of 8 hours between charges.
Dimensions - 18cm, 7 cm, 6cm.
Weight - 1.25 lbs. , with carrying case and accessories - 4 lbs.
Carrying case - Included std. Case dimensions: 36cm, 28cm, 15 cm.
References:


Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376:105-115


