$OS30p_+ \ \text{Research Chlorophyll Fluorometer}$

The best of both worlds

Hand held pulse modulated fluorometer for $F_V/F_M / F_V/F_O$, along with an advanced *OJIP* protocol with auto-calibration of actinc light source at 3,500 µmols m⁻²s⁻¹



Pulse Modulated Tests:

 F_V/F_M - A Measure of Maximum Quantum Efficiency

 F_V/F_M is by far the most used chlorophyll fluorescence measuring parameter in the world. Research has proven it to be a robust way to measure early plant stress that affects photosystem II. The parameter and its protocol also provide the advantage that samples are measured and compared at the same known dark adapted state.

 F_V/F_M has been shown to correlate with carbon assimilation for many types of plant stress in $\rm C_3$ plants, $\rm C_4$ plants & CAM plants. The OS30p_+ measuring trace is graphically displayed in color, and $\rm F_0$ 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, with calibrated actinic light source - Advanced OJIP



Enhanced Strasser OJIP

"JIP" Test – Direct readout of important stress detecting parameters and the overlay of measuring traces, are now immediately possible in the field. This plant stress testing method provides a high time resolution image of the Kautsky induction curve against a logarithmic time scale. It is possible to display and overlay curve traces for comparison. They may also be easily recreated from the data file information to evaluate plant stress.

The OS30p₊ provides a direct read out of the following parameters: OJIP, t100 :s, t300 :s (or K), tFm (or time to F_M), A (or area above the curve), M_O (or RC/ABS), PI_{ABS} (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.

F_V/F_M - The most accepted & used chlorophyll fluorescence parameter in the world.

The reason why it is the most accepted chlorophyll fluorescence measuring protocol is because results correlate to carbon assimilation measurements and because all samples are compared at the same known dark adapted state.

 F_V/F_M is a normalized ratio that requires a modulated fluorometer for reliable measurement. The protocol was developed by Kitajima and Butler (1975) and it has withstood 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.

After dark adaptation, usually with dark clips, modulated fluorometers allow the accurate measurement of minimum fluorescence or F_0 . This is done by using a weak modulated light, that is too low to drive photosynthesis, but high enough to excite prephotosynthetic antenna fluorescence. In this state, photosystem II is maximally oxidized. The xanthophyll cycle, Δ ph of the thylokoid lumen, state transitions and chloroplast migration have all relaxed to their inactive states (Lichtenthaler 1999, 2004), (OptiSciences dark adaptation application note available upon request at www.optisci.com).

The modulated fluorometer then irradiates the plant sample with an intense saturation light that is both high and long enoug, to fully reduce or close all available PSII reaction centers. The maximum fluorescence output during this saturating light radiation called F_M represents a fully reduced PSII.

It has been found that healthy plants have an F_V/F_M value in the range of 0.79 to 0.84 (Maxwell and Johnson 2000). Lower values indicate plant stress.

To make sure that the OS30p+ is the easiest to use and most reliable instrument it it's class, It has the following design advantages:

1. Modulated light intensity setup bar.

2. An algorithum that finds the highest F_M as long as the saturation flash is wide enough. This eliminates saturation pulse NPQ as an issue.

3. A calibrated saturation flash up to 6,000 umols m⁻²s⁻² for reliable results under all temperature conditions.

Graph of $F_V\!/F_M$ or $F_V\!/F_O$ saturation pulse



$$F_V/F_M = (F_M-F_O) / F_M$$
$$F_V/F_O = (F_M-F_O) / F_O$$

No more getting up way before dawn to make field measurements!



Inexpensive, long lasting, very light weight dark adaption clips allow measurement of statistically significant plant populations very quickly and at reasonable times of day. Each measurement takes between 3-5 seconds. *Made of light weight plastic & stainless steel components*.

Each instrument comes with 10 dark clips. However, more may be purchase as singles or in *packages of 50*.

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). He used high time resolution of the rise in variable chlorophyll fluorescence, after dark adaption, using a saturating actinc light. He found a distinct curve shape with multiple steps that represent chemical functions in PSII oxidation-reduction reactions. 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. In addition, special measuring parameters have been developed as sensitive stress detectors such as PI $_{ABS}$ or performance index.

Viewing graphic OJIP 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.

Reto Strasser did much of pioneering work using OJIP for plant stress measurement.

Importantly, Wim Vredenberg (2011) found that <u>OJIP</u> results vary by actinic light intensity. Strasser originally used 3,000 μ mols m⁻² s⁻¹ in his early work, but he used 3,500 μ mols m⁻² s⁻¹ in his later work. For that reason, The OS30p₊ actinic light source goes through an auto-calilbration routine every time measuring protocol selection occurs. This ensures reliable measurements at every temperature. The OS30p₊ may be used at either 3,000 μ mols m⁻² s⁻¹ or 3,500 μ mols m⁻² s⁻¹



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.

USB output allows the OS30p+, to becomes a hard drive for your computer for simple transfer of data. No special external computer software is necessary.

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

- Settings are shown in the upper left window
- A color graph of the measuring trace is shown using a logarithmic scale or a linear scale in the upper right window.
- Direct read out of the most used OJIP measuring parameters is shown in the lower window.

The last 100 measurements can be reviewed and compared as shown on screen to the right.

"JIP" test traces can now be view in the field without using a computer. Up to 32 traces can be overlaid on the viewing screen or in the data file. Colors will start to repeat after 16 traces have been overlaid.

The default calibrated red light actinic intensity is $3,500 \mu$ mols, 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 spreadsheet. Sampling rate times are listed in column A of the spreadsheet and each successive trace reports to the following columns, one after the other.

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_{O}

Rugged field instrument designed for hand held use.

The OS30p+ 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 of dark clips allows the purchase of large quantities to fit most application requirements & budgets.

Standard accessories included are a hard shell carrying case, a battery charger, 10 dark adaptation clips, a manual on a USB drive and a USB cable.

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Technical Specifications:

OS30p +

Modulated Fluorometer

F_V/F_M , F_V/F_O

Saturation intensity
Saturation light source600- 6000 umols set from 10% to 100%
Array of red LEDs 660 nm.
red 0.2 to 1.0 μmols.Detection methodPulse modulated

Detector and Filter Pin photodiode with 700-750 nm b/p filter

 F_V/F_M 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 F_O , and F_M , making it ideal for both algae and land plants.

Modulated light manual adjustment from 30% to 80%

Parameters measured and graphed $F_O, F_M, F_V/F_M, F_V/F_O$

JIP Test

Actinic light intensities - 6000 μ mols, 4500 μ mol, **3500** μ mols, **3000** μ mols, 2500 μ mols, 1000 μ mols, 875 μ mols, 525 μ mols. 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, tF_M, A (area above the curve), M₀ (or RC/ABS), PI_{ABS} (or performance index) F₀, F_M, F_V/F_M, F_V/F₀,

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. *Fo <u>is measured</u>, not estimated.*

Measured parameters reported to the data file: ABS/RC, TR₀/RC, DI₀/CS, ET₀/RC, TR₀/ABS, ET₀/TR₀, ET₀/CS, RC/CS₀, RC/CS₀, RC/CS_M, 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

Auto Calibration of actinic light source when opening measuring protocols: Ensures that the actinic light intensity is the same even under hot or cold measuring conditions. It also ensures that OJIP measurements all occur at referenced actinic intensities.

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.

Technical references:

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