

Os30p₊ Chlorophyll Fluorometer

The best of both worlds

Hand held pulse modulated fluorometer for $F_V/F_M \& F_V/F_{O_r}$ 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 allow comparison of samples in the same known dark adapted state.

The "JIP" test,





"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 μ s, t300 μ 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 used chlorophyll fluorescence parameter in the world.

 F_V/F_M 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 F_O . 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, Δ ph of the thylokoid lumen, and state transitions have all relaxed to their inactive states (Lichtenthaler 1999, 2004), (OptiSciences dark adaptation application note <u>www.optisci.com</u>).

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 F_M , is also measured.

The result is ($F_{Maximum fluorescence} - F_{O(minimum fluorescence)}$) / $F_{maximum fluorescence}$ or F_V / F_M .

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). 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 F_V/F_M or F_V/F_O saturation



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

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 μ s (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.



The OS- $30p_+$ actually measures F_o using a weak modulated red light. It is not an estimated value.

"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.



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 logarithmic 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 $3,500 \mu$ mols, other intensities are also available.

525 µmols
875 μmols
1,000 µmols
$2{,}500 \; \mu mols$
$3,000 \ \mu mols$
$3,500 \ \mu mols$
$4,500 \ \mu mols$
6,000 µmols



	JIP	Protoc	ol
File:HHJJ			
Modulation Src Power:100%			
Actinic Power:3500umol		<u></u>	
Test Length: 4S			
TLog:On Ft: 1			
	ſ		
S# 0 K J t100 M T tI	I P M Mo	Vj Area	PI Fv∕m
1 262 362 472	584 649	0.542	1.003
	477 485	0.717	0.559
248 478 0 2	30 0.331	1011	0.546
	447 449	0.758	0.544
4 191 293 436	656 765	0.426	2.888
210 703 0	40 0.427	13610	0.743
5 195 285 422	593 808	0.370	4.247
		1 6 9 6 2	

		JIP Pr	otocol
File:HHJJ	Actinic	l vl	
Power:100%	7.000000		
Actinic	<u>525</u> u	moļ	-
Power:3500	_875u	moļ	
Test Lengt	<u>1000</u> u	moļ	
TLog. On	<u>2500</u> u	moļ	
	<u>3000u</u>	mol	
S#• 5	<u>3500u</u>	mo	- 808
	4500u	moļ	1 1 6 4
	6000u	mol	104
0:195			:285
J:422			: 808
Mo:0.35			Em: 150
PI: 4 2			
1 1 . 4.2			

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

$F_V\!/F_M$, $F_V\!/F_O$



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Saturation intensity Saturation light source - Modulated light source	600- 6000 umols set from 10% to 100% Array of red LEDs 660 nm. 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 F_{O} , and F_{M} , making it idea for both algae and land plants.		
Modulated light adjustment	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 μmols, 4500 μmol, 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, tF _M , A (area above the curve), M ₀ (or RC/ABS), Pl _{ABS} (or performance index) F ₀ , F _M , F _V /F _M , F _V /F ₀ , 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. <i>Fo <u>is measured</u> not estimated</i> .		
	Measured parameters reported to the data file: ABS/RC, TR ₀ /RC, DI ₀ /CS, ET ₀ /RC, TR ₀ /ABS, ET ₀ /TR ₀ , ET ₀ /CS, RC/CS ₀ , 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			
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:

Baker N. R., Oxborough K., (2004) Chlorophyll fluorescence as a probe of photosynthetic productivity. From Chapter 3, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, pages 66-79

Kautsky H., Hirsch A. (1931) Neuw Versuche zur Kohlensaureassimilation. Naturwissenshaften 19, 964.

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

Lichtenthaler H. K., Burkart S., (1999) Photosynthesis and high light stress. Bulg. J. Plant Physiol., 1999, 25(3-4), 3-16

Lichtenthaler H. K., Babani F. (2004) Light Adaption and Senescence of the Photosynthetic Apparatus. Changes in Pigment Composition, Chlorophyll Fluorescence Parameters and Photosynthetic Activity. From Chapter 28, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 716

Maxwell K., Johnson G. N, (2000) Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany Vol. 51, No. 345, pp. 659-668- April 2000

Strasser R.J, Tsimilli-Michael M., and Srivastava A. (2004) - Analysis of Chlorophyll a Fluorescence Transient. From Chapter 12, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 340

Vredenberg Wim (2011) Kinetic analyses and mathematical modeling of primary photochemical and photoelectrochemical processes in plant photosystems, BioSystems Contents lists available at ScienceDirect journal homepage: www.elsevier.com/locate/biosystems