Umweltanalytische Produkte GmbH

OPTI-SCIENCES

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OS-30p+ Chlorophyll Fluorometer

Field portable hand held instrument designed for F_V/F_M , F_V/F_O and advanced OJIP measurements

Preliminary manual 7/2/2012

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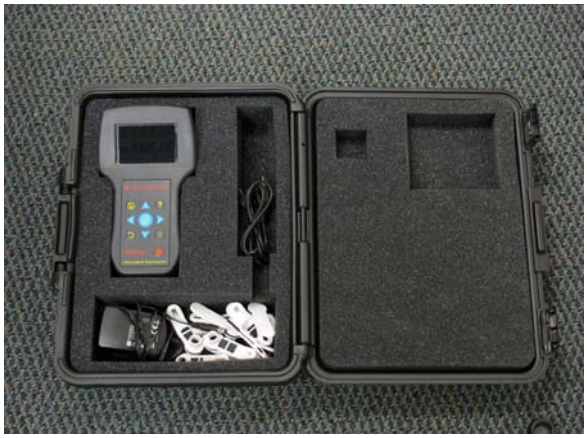
Welcome !

Congratulations on your purchase of the OS-30p$_+$ Chlorophyll Fluorometer. Please check the carton for any visible external damage. If you notice any damage, notify the freight carrier immediately to inform them about it. Follow their procedures for reporting and filing a claim. The carton and all packing materials should be retained for inspection by the carrier or insurer.



List of Equipment

Carefully unpack the carton. You should have received the following items:
OS-30p$_+$ Fluorometer
AC battery charger (Universal voltage)
Ten dark-adaptation cuvettes
USB cable
Hard shell carrying case
Instruction Manual on disc.

Note: If any item is missing, please contact Opti-Sciences, or your authorized Opti-Sciences dealer.

Getting Started

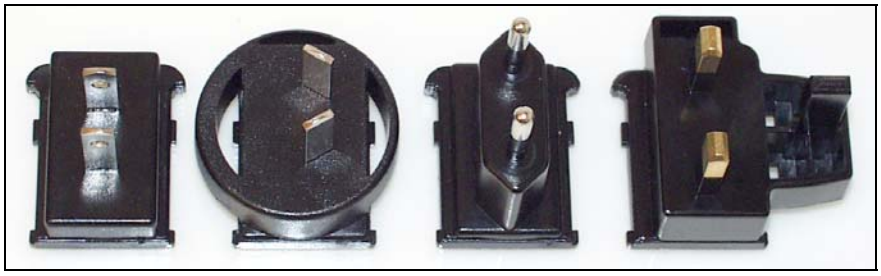
The OS-30p+ Chlorophyll Fluorometer is a versatile measuring instrument designed to precisely measure chlorophyll fluorescent parameters F_V/F_M, F_V/F_O and advanced OJIP parameters. It uses pulse modulated detection system to allow for a variety of tests to be conducted under greatly varying environmental conditions. The system is simple to use, light in weight, and battery powered, making it an excellent choice for field studies. It is the culmination of several years of testing and development and was engineered to give many years of reliable service. Its predecessor, the OS-30p is one of the most cited research chlorophyll fluorometers in the world in recent years.

Auto-Calibration routine:

The instrument has an auto-calibration routine that insures that various light source intensities remain constant over time. It happens every time the instrument is turned on, and when the intensity of the actinic light, and the modulation light are changed. Do not put a sample, leaf clip, or anything else on the optical probe until this routine is complete. Point the optical probe down toward the ground while it is happening

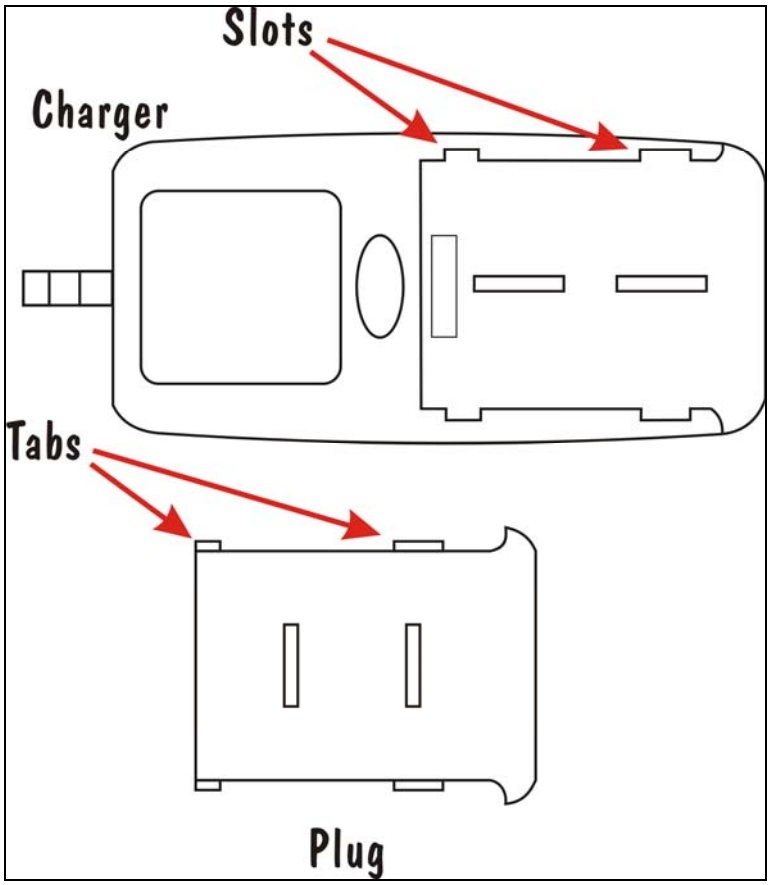
Battery Charger

The battery charger supplied with the fluorometer is a universal voltage and frequency type (100-240V AC, 47-63 Hz). Included are four common types of plugs, allowing use in most countries without additional adapters, or transformers.



From left to right - North America & Japan, Australia, Europlug, United Kingdom & Ireland.

Select the appropriate plug type and place its tabs directly over the charger slots. Press down slightly, and push the connector with the words the identification sticker. When the plug is secured, an audible click should be heard, when it locks into position. To remove the plug, press the button (located between the sticker, and the plug) and slide the plug away and up from the sticker.



The other end of the charger plugs into the charger port on the fluorometer.

The center port is for recharging

The left diode provides charging status

The covered port is for future development

OS-30p+ ports.

The status indicator light will turn on when power is applied.
Red blinking light: Charging circuitry is conditioning the battery.
Red steady light: Battery is being charged.
Green steady light: The battery is fully charged

USB port

Integrated sample probe.

The OS-30p+ features an integrated probe allowing for easier operation and faster measurement acquisition. No longer is the probe vulnerable to being dropped, damaged, or misplaced.

A set of 'dark adaption' clips is provided with the fluorometer. The clips are small, and made from a lightweight plastic. They also feature a convenient sliding shutter to prevent introduction of light during insertion of the sample probe. The time and degree of darkness required will vary with the type, and previous exposure conditions of the sample. See the section on dark adaptation for more information.

Sample clip on probe.

The OS-30p+ has been ergonomically designed to simplify use, without sacrificing measurement precision or flexibility. Menu-driven operation and data handling, combined with a membrane keypad, color graphic display, and

rechargeable battery offer easy test set-up for sophisticated measurement capability in a compact package.

? A context sensitive help system has been programmed into this fluorometer for ease of use. To view the help text, simply press the ? key from any screen. Use the arrow. To exit press The home icon or the reverse arrow on the keypad.

Throughout this manual you will be shown setup options and response messages. When an example of a program screen is given within a box you may assume that this is shown as it will appear on the OS-30p+.

Home key takes you to the main menu

The ? key is to answer basic questions on the screen that is displayed, and to allow capture of graphic screen images.

The curved arrow key allows return to a previous screen.

The Menu button allows parameter change or adjustment.

The red key is the off and on button

Blue arrow keys allow scrolling

The central blue button is the measurement button and allows acceptance of changes.

Chlorophyll Fluorescence

As ambient light irradiates a leaf, about 70% to 90% is absorbed by the leaf, and approximately 40% to 60% of that light is absorbed by the antennae associated with PSII and transferred to PSII (photosystem II) reaction centers. (The percentages are different for algae). Under normal non-stressed conditions, most light energy is channeled into photochemistry with smaller amounts of energy channeled into heat and fluorescence. In photosystem II, this process is competitive so that as plant stress occurs, mechanisms that dissipate heat, photo-protect the leaf, and balance light between photosystem II and photosystem I, change the output of fluorescence and heat.

Only photosystem II displays variable fluorescence. photosystem I fluorescence is at a significantly lower level and it does not vary with carbon assimilation, photo-protective mechanisms, or plant stress levels. For these reasons, the study of chlorophyll fluorescence of PSII has been studied in depth.

Over the years F_V/F_M has proven to be a robust and reliable test of plant stress types that affect PSII (Baker 2004).

It has been found that not all types of plant stress affect PSII to the same degree. Various researchers have found that the relationship between the light reaction and the dark reaction is not always strait forward. As a result, in some cases, special chlorophyll fluorescent assays have been developed to over come most of these limitations. For a review of this topic we suggest that you contact Opti-Sciences for a free copy of the Desk top Plant Stress Guide.

The OS30p+ is designed to measure chlorophyll fluorescence using dark adaptation protocols F_V/F_M, F_V/F_O, and the JIP test.

F_V/F_M offers a number of advantages. Values have been found to correlate to carbon assimilation in regards to plant stress types that affect PSII; samples are dark adapted to the same know state for comparison purposes, and it is the most used chlorophyll fluorescent parameter in the world, with oceans of data to support its robust capability.

F_V/F_O is a more sensitive plant stress detector for plant stresses that affect PSII. While the values do not correlate to carbon assimilation, plant stress can generally be detected earlier than with F_V/F_M.

An advanced form of the JIP test is also provided with this instrument. Like F_V/F_O, measuring parameters have been developed that provide sensitive plant stress detection capabilities for plant stress types that affect PSII. This instrument provides advanced JIP parameters like the direct readout of PI_{ABS}, or

performance index, Vj, and M_O. For more information regarding the limitations of these tests, The Desk Top Stress Guide is a good source.

It is also recommended that you read the section on dark adaptation before using the instrument. It is important to design your experiment with reliable dark adaptation times for reliable results.

A Checklist is provided as well to make sure that all of the important variables are understood, and that they are properly controlled.

Measured Parameters F_V/F_M – F_V/F_O protocol:

The OS-30p_+ automatically measures and calculates the following parameters:
Measured:

F_O: Minimal fluorescence (arbitrary units).
Pre-photosynthetic antennae fluorescence measured with a weak red modulated light source for excitation. The intensity of the resulting fluorescence is high enough for detection, but the excitation light intensity is not high enough to drive photosynthesis. Fo can vary to some degree with different dark adaptation times and light history. See the discussion about dark adaption to ensure reliable results. It is common to dark adapt for twenty to thirty minutes, however, some researchers only use overnight dark adaptation. If dark adapted properly, all available PSII reaction centers are fully oxidized, the xanthophyll cycle and state transitions have relaxed to their inactive state. See the FV/FM checklist for a list of limitations.
F_M: Maximal fluorescence (arbitrary units). Fluorescence intensity under exposure to a high light intensity saturation flash. All available PSII reaction centers have been reduced and are closed. The intensity of the saturating light must be high enough and long enough to fully saturate all available PSII reaction centers.
Ft: fluorescence at time t. (arbitrary units). Momentary fluorescence readout used to set the modulation light intensity correctly.
Calculated Parameters:
F_V/F_M: The ratio of variable fluorescence to maximal fluorescence.
Calculated as $F_V/F_M = (F_M-F_O)/F_M$. Variable fluorescence (F_V) is variable fluorescence of PSII. 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).

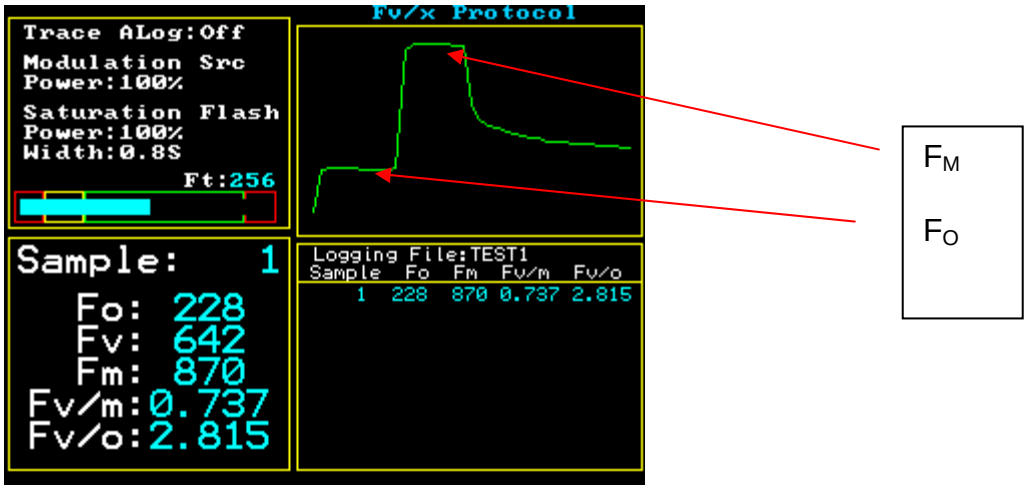
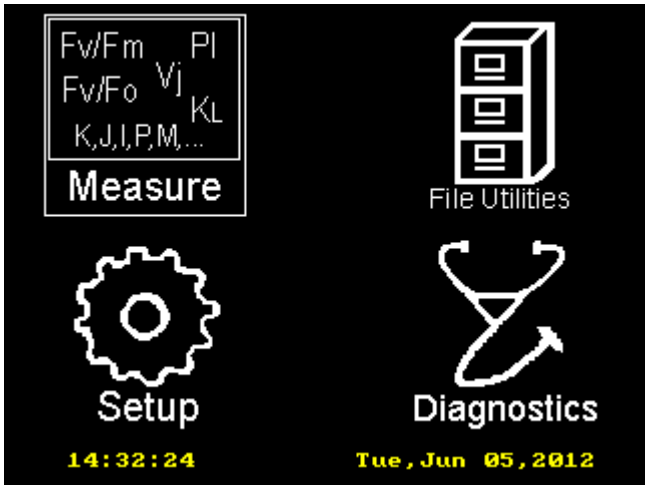


Figure #1 Dark adapted test.

F_V/F_O: This is a more sensitive stress detecting parameter than F_V/F_M because it is normalized over F_O or minimum fluorescence. $F_V/F_O = (F_M-F_O)/F_O$. 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.

Operation

When you turn on your OS-30p+, using the red button on the bottom of the instrument, this screen (referred to as the main screen) will appear:



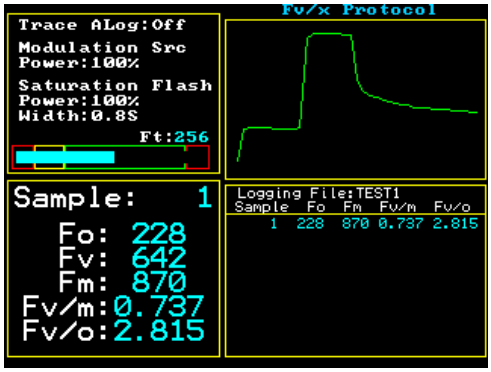
At a glance, you can tell the, system date and time.

The blue arrow keys allow navigation from measure, to file utilities, to set up, to diagnostics. Once the category has been highlighted with the arrow keys, press the round blue button with the yellow check mark to select the category of interest.

Measure F_V/F_M – F_V/F_O:

After selecting Measure, Choose between the F_V/F_M - F_V/F_O protocol or the JIP test protocol. Highlight one or the other with the blue arrow keys, and select the protocol of interest with the round blue button.

F_V/F_M , F_V/F_O



When the Fv/x protocol (F_V/F_M protocol) measuring screen appears, settings may be adjusted by pressing the menu button on the lower right hand side of the control panel.

Options appear in a smaller window that allow changes to modulation light source intensity, saturation light source intensity, saturation pulse duration time or width, the ability to save the measuring trace, delete a measurement, or change the measuring file name. The blue arrow keys allow navigation and the round button allows selection. A keyboard appears to allow alpha numeric input using the arrow keys and the central blue button. When the change has been completed, use the arrow keys to go to Done, and press the round blue button.

Setting the modulation light intensity:

Place a leaf into a dark adaptation clip with the slider closed, and dark adapt for several minutes. Place the instrument probe into the dark clip opening all the way, and slide open the dark slide. This exposes the leaf to the weak modulated light source.

The blue light intensity bar in the upper left hand corner box of the color display will either be in the red, yellow or green part of the box. This intensity bar is designed as an aid to proper modulation light setting. When properly set, the blue bar will be in the green part of the box, and the Ft value to be found in the upper left hand corner window, above the modulated light intensity bar, does not raise slowly over a 15 to 30 second period. If the value does start to rise, it means that the modulation light intensity setting is too high, and that it is high enough to drive photosynthesis. If this happens, dark adapt again for a few minutes, and try a lower modulated light intensity. The intensity must be high enough to be measured, but not high enough to drive photosynthesis. Values from 10% to 100% are input using the key board and the blue keys for navigation and selection.

Saturation light intensity:

Press the menu button and select the saturation intensity line. The saturation light intensity can be adjusted to the following settings using the up and down arrow keys: 6000 µmols, 4500 µmol, 3500 µmols, 3000 µmols, 2500 µmols,1000 µmols, 875 µmols, 525 µmols, 300 µmols, 200 µmols, 100 µmols, & 50 µmols. When done press the blue round button. It has been shown that leaves grown in low light conditions saturate at about 300 µmols and that leaves grown under high light conditions saturate at about 1,500µ mols. (Ralph P. J. 2005) One can tell if the saturation intensity is high enough by observing the

peak of the saturation trace. If the top of the trace is square, then the intensity is high enough to saturate PSII. If it looks like a mountain peak, then the intensity should be increased. It is common to use 3500 µmols for the JIP test.

Saturation Width:

Press the menu button and select the saturation width line. The saturation light width can be adjusted from 0.1 seconds to 1.5 seconds. The default is 1.0 seconds. To fully saturate PSII reaction centers, light irradiation must not only be high enough but also be irradiated for a sufficient amount of time. For higher plants this time is between 0.5 and 1.5 seconds. (Rosenqvist E., van Kooten O., 2006). For algae, this time is from 25 to 50 milliseconds. (Schreiber U. 1995). The OS30P$_+$ uses a special algorithm to minimized issues related to this parameter.

The instrument uses a rolling eight point average to determine F_O at its lowest point and F_M at its highest point. This eight point average represents 25 milliseconds. For this reason, as long as the saturation width is wide enough, the OS30p+ will provide a reliable answer. In the past, it the width was set too long saturation pulse NPQ could affect the F_M measured value. That is no longer the case. Researchers should feel comfortable setting the saturation width at 1.0 seconds for most samples.

Saving the trace:

Press the menu button and select the save trace line. The measuring trace that may be graphed in Excel must be selected, or it is discarded.

Delete:

Press the menu button and select the delete line using the blue arrow keys and the round blue button.

File Name:

Press the menu button and select the File name line using the blue arrow keys and the round blue button. A key board appears. Use the blue arrow keys and the round blue button to navigate the key board, and select alpha numeric characters fro the name. Scroll to done and press the blue round button.

Home Icon:

Pressing this Icon takes you to the main menu,

Curved Arrow Icon:

Pressing this icon takes you back to a previous screen

? Icon:

Pressing this icon allows answers to some basic questions related to the screen that is displayed. It also allows the capture of a compete display screen in memory. Press the ? icon again and a key board will appear that will allow the saving of the screen that is displayed. This is true of most screens, however some pop up screens used for parameter adjustment can not be saved.

Scrolling through previous measurements:

The measuring screen lower right hand window displays up to 10 previous measurements at a time. By using the up and down blue arrow keys, one can scroll through the previous 100 measurements. If one leaves the primary F_V/F_M measuring screen, these values disappear. They are still retained in the measurement data file and they may be reviewed later, on your PC, by transferring the measuring file to Excel or other programs.

Dark adaptation

Dark adaptation is a technique used in some chlorophyll fluorescence measurements to fix a non-stressed reference point relative to various measurements (Maxwell and Johnson 2000). Deciding where to put that reference is based on an understanding plant mechanisms that can affect measurements, and what one wants to measure.

Dark adaption times of fifteen minutes, twenty minutes, thirty minutes, forty minutes and sixty minutes are common for terrestrial plants, and some researchers use pre-dawn values.

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.

Fv/Fm is affected by both photochemical and non-photochemical factors. If a leaf is dark adapted and measured, then subjected to high light levels, then dark adapted and re-measured, the first measurement will be higher then the second measurement. The decline in 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)

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 the xanthophyll cycle and hyracoid lumen Δph. They relax in a few minutes during dark adaptation. (Muller, Neoga 2001),(Kramer D. M., Johnson G., Karats O., Edwards G. (2004). According to Lichtenthaler (1999) this time is 4-6 minutes.

State I – State 2 transition quenching (called q_T) is most significant at lower light levels in terrestrial plants and can represent more than 60% of quenching at low light levels. At high light levels it represents about 6% of total quenching. State transition quenching relaxes in ten to twenty minutes in terrestrial plants. (Lichtenthaler H. Burkart S 1999)

It has been shown that the effects of acute photo-inhibition caused by exposure to high light intensities can be reversed with 20 to 30 minutes of dark adaption (Theile, Krause & Winter 1998), where as reversal 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). For this reason, it is important to only compare samples with similar light histories unless light stress is your focus.

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 parameters, q_I and Y(NO), to pre-stress conditions. To get an accurate control value for Fm and Fo under chronic photo-inhibition conditions (components of non-photochemical quenching parameters) it is common to dark-adapt for a full night, or 24 hours. (Maxwell and Johnson 2000) In some cases longer times may be appropriate. For this reason, it is likely that there is some residual photoinhibition built into most summer field fluorescence measurements. This expected and it is alright as long as one compares samples with similar light history.

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 and Earl 2004 are good sources.

The use of far-red pre-illumination that is available on some fluorometers is designed to rapidly re-oxidize PSII by activating PSI and draining the remaining electrons from PSII. While this can be valuable in fieldwork (Maxwell and Johnson 2000), it can not be used as a substitute for the time required to relax the xanthophyll cycle, relax the Δph thylakoid lumen or state transitions of 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 or darkened rooms may also be used.

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 Fv/Fm 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.

References:

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Thiele A., Krause G.H., & Winter K. (1998) In situ study of photo-inhibition of photosynthesis and xanthophyll cycle activity in plants growing in natural gaps of the tropical forest. Australian Journal of Plant Physiology 25, 189-195

Checklist before making dark adapted measurements

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 Institute of Standards and Technology (NIST). 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.

F_V/F_M and the JIP test. Dark adaptation factors that affect F_V/F_M also affect OJIP.

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 an accurate 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 Δph of the thylakoid lumen to return to a dark-adapted state. (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 photoplankton (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. 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 read the section on dark adaptation.

2. Modulation light intensity setting must be set correctly to reliably measure F_O or minimum fluorescence($F_V/F_M = (F_M-F_O)/F_M$). 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 a higher erroneous Fo value, the Ft value or fluorescence signal should not rise over a 20 to 30 second period when a leaf is used. If it does, the intensity must be lowered. Fo is also measured in the JIP test.

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

4. F_V/F_M 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) The OS30p+ uses a red light saturation pulse.

5. Maximum F_V/F_M values vary with species. The average maximum FV/FM value is between 0.79 - 0.84 (Maxwell and Johnson 2000).

6. Field plants should only be compared to field plants and green house plants should be compared to green houseplants due to light history. (Lichtenthaler 2004) Furthermore, unless light stress is of primary interest, it is important to compare samples of similar light history, due to the fact that it can take up to 60 hours for relaxation of chronic photoinhibition (Lichtenthaler 2004).

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 in the F_V/F_M test protocol, should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for Phytoplankton and cyanobacteria. (Schreiber 1995). On some fluorometers, times outside these ranges increase the error in F_V/F_M 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. A figure of 0.8 is sometimes set at the factory on some fluorometers for higher plants, because it works for all higher plants tested. The $OS30p_+$ eliminates this issue by using a special algorithm that finds the highest 25 milisecond eight point rolling average for FM. This insures that if the saturation pulse width is wide enough, it will always provide a reliable measurement for higher plants or for algae.

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 light adapted Y(II) and ETR.) Several papers by Strasser and others use 3,500 μmols for the JIP test.

10. While the OS30p+ does not, some F_V/F_M 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 F_V/F_M 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) It is also true for OJIP

11. Part of the minimum fluorescence, the Fo parameter, in F_V/F_M $(F_M – F_O)/F_M$), 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 a small error. In C_3 plants, about 30% of Fo fluorescence is due to PSI, and in C_4 plants about 50% of Fo fluorescence is due to PSI fluorescence. PSI produces about 6% of the fluorescence found in F_M in C_3 plants, and about 12% of F_M in C_4 plants (Pfundle 1998). This not a problem when comparing Fv/Fm measurements for plant stress because PSI fluorescence does not change. It remains constant.

12. The instrument has an auto-calibration routine that insures that various light source intensities remain constant over time. It happens everytime the instrument is turned on, and when the intensity of the saturation light, the actinic light, and the modulation light are changed. Do not put a sample, leaf clip, or anything else on the optical probe until this routine is complete. Point the optical probe down toward the ground while it is happening.

The best experiments are ones that take these issues into account. PSI fluorescence is involved in all measurements. It does not vary with light level or plant stress. (Schreiber 2004). With this in mind, comparing samples with similar light histories allows comparison of many types of plant stress. The Plant Stress guide provided by Opti-Sciences references papers that deal with specific types of plant stress and limitations of different chlorophyll fluorescence parameters for measuring plant stress.

There are fluorescence solutions and assays available that are sensitive to most types of plant stress. See the “Desk Top Plant Stress Guide” available for free at www.optisci.com for a review of measuring different types of plant stress.

References:

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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

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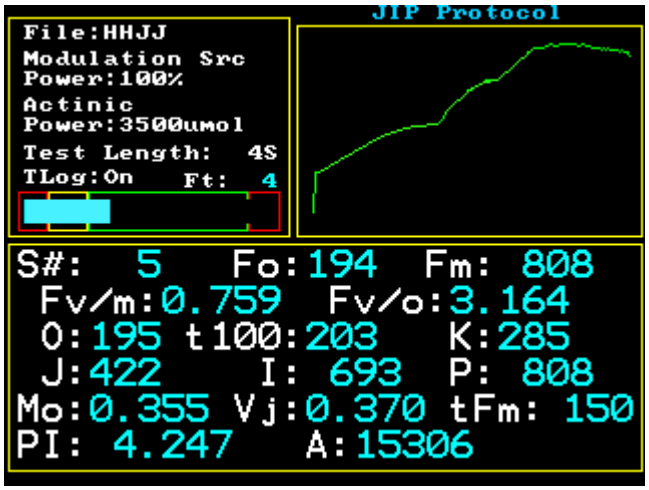
Ruban A.V., Johnson M.P., (2009) Dynamics of higher plant photosystem cross-section associated with state transitions. Photosynthesis Research 2009 99:173-

183 Schreiber U, (2004)Pulse-Amplitude-Modulation (PAM) Fluorometry and Saturation Pulse Method: An Overview From Chapter 11, “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 279-319

Schreiber U, Tsuyoshi E., Hualing M., Kozi A. (1995) Quenching Analysis of Chlorophyll Fluorescence by the Saturation Pulse Method: Particular Aspects Relating to the Study of Eukaryotic Algae and Cyanobacteria, Plant and Cell Physiology Volume 36, Issue 5 pp 873-882

Measured parameters – The JIP Test:

After selecting Measure, Choose the JIP test protocol. Highlight it with the blue arrow keys, and select the protocol of interest with the round blue button.



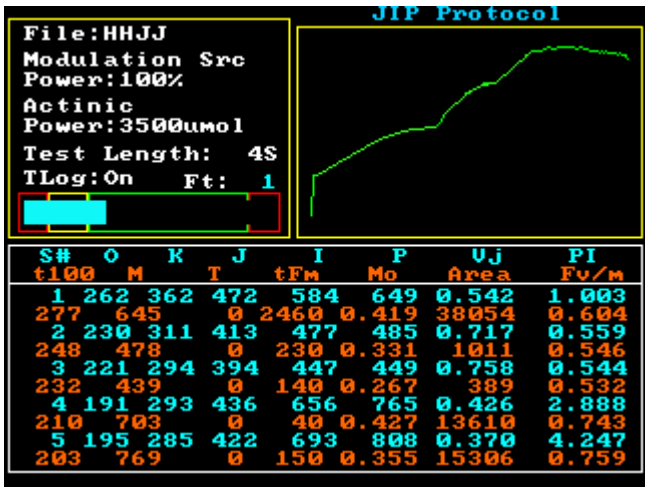
When the JIP Test protocol measuring screen appears, settings may be adjusted by pressing the menu button on the lower right hand side of the control panel. Options appear in a smaller window that allow changes to modulation light source intensity, actinic light source intensity, actinic light duration time, the ability to save the measuring trace, delete a measurement, or change the measuring file name. The blue arrow keys allow navigation and the round button allows selection. A keyboard appears to allow alpha numeric input using the arrow keys and the central blue button. When the change has been completed, use the arrow keys to go to done, and press the round blue button. Direct readout parameters include:

O at t20 µs, t100 µs, t300 µs (or K), t2ms (or J), t30ms (or I), P, tF_M, A (area above the curve), M_O (or RC/ABS), Vj, PI_{ABS} (or performance index) F_O, F_M, F_V/F_M, and F_V/F_O. *Up to 16 OJIP traces can be overlaid on the color graphic instrument screen for immediate viewing.*

F_O is measured, not estimated.

Several other parameters are reported in the measuring data file. They include:

ABS/RC, TR_O/RC, DI_O/CS, ET_O/RC, TR_O/ABS, ET_O/TR_O, ET_O/CS, RC/CS_O, RC/CS_M, S, M, and T are also measured if the test is long enough; however, they are only recorded in the data file, not on the measuring screen. Up to 32 traces can be stored for graphing per data file.



Data review:

After making multiple measurements, the JIP test parameters may be reviewed and compared instantly in the field by pressing the up blue arrow key. A chart like the one shown above is displayed and the user can scroll through the last 100 measurements to compare results. To go back to the original measuring screen touch the curved arrow icon.

JIP Test Overview:

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. Up to 16 color traces may be overlaid on the measuring screen. Up to 32 measurement traces can be stored in a single measuring file for later viewing and comparison on your computer. When the file is transferred to Excel or other spread sheets, the first column provides a detailed time stamp for the measurement fluorescence values. The remaining columns, up to 32, provide fluorescence trace values for stored measuring traces.

Originally in OJIP techniques, the overlay of graphs taken from different plants, or the same plant under varying degrees of plant stress, were used to monitor plant stress. Later, various quantitative parameters were developed to provide a more sensitive and more quantitative approach to OJIP plant stress detection. It is still common for researchers to use this overlay technique, to study the effects of plant stress, and to use the special parameters that have been created to detect and quantify plant stress.

Sampling rates and measurement points:

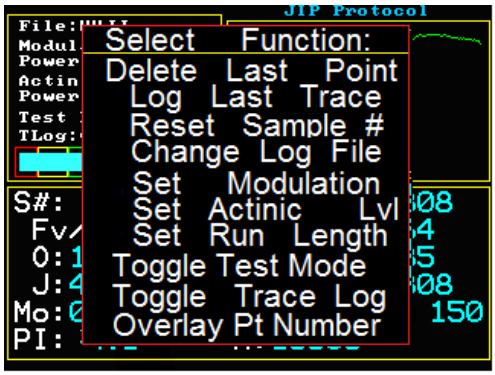
Number of Data Points	Sampling Time Interval	Total Elapsed Time	Parameter Designation
0	0	0	FO measured
2	10 µs	20 µs	O - 20 µs
8	10 µs	100 µs	t100 µs
20	10 µs	300 µs	t300 µs K step
27	100 µs	3 ms	t 2 ms J step
27	1ms	30 ms	t 30 ms I step
27	100 ms	3 secs	P = F_M variable time
27	1 sec	30 secs	
27	10 secs	300 secs	

Parameter equations or definitions:

	Direct read out parameters
F_O	Pre-photosynthetic minimum fluorescence measured with weak red modulated light. PSII is oxidized and all available reaction centers are open.
F_M	Maximum fluorescence measured or P. All available reaction centers are reduced or closed.
O	Origin –Fluorescence value at 20 μs.
t_{100}	Fluorescence value at 100 μs
t_{300} K step	Fluorescence value at 300 μs
J step	Fluorescence value at 2 ms
I step	Fluorescence value at 30 ms
P Step	Maximum fluorescence or F_M
tF_M	Time to reach P or F_M
A	Area above the curve from t0 to tF_M and from F_O to F_M
Vj	Vj = (Fj – Fo) / Fm-Fo
M_O or RC/ABS	$M_O = (F_{300} – F_{50}) / (F_M – F_{50}) / 0.25ms$
PI_{ABS}	$PI_{ABS} = (Vj / Mo) (F_V/F_M) (F_V/F_O) ((F_M-Fj)/ (Fj-Fo))$
or	$PI_{ABS} = (Vj / (dV/dto)) (F_V/F_M) (F_V/F_O) ((F_M-Fj)/ (Fj-Fo))$
	Parameters recorded in the data file only
S	Value at approximately 1 seconds
M	Value at approximately 3 seconds
T	Value at approximately 30 seconds
ABS/RC	$ABS/RC = (M_O) (1/Vj) (1/(1-(F_O/F_M)))$
TR_O/RC	$TR_O/RC = (M_O) (1-Vj)$
DI_O/CS	$DI_O/CS = (M_O) (1/Vj) (1/(1-(F_O/F_M))) – ((M_O) (1/Vj))$
ET_O/RC	$ET_O/RC = (M_O) (1/Vj) (1-Vj)$
TR_O/ABS	$TR_O/ABS = F_V/F_M$
ET_O/TR_O	$ET_O/TR_O = 1-Vj$
ET_O/CS	$ET_O/CS = (1- (F_O/F_M)) (1-Vj) (F_O)$
RC/CS_O	$RC/CS_O = F_O / (M_O) (1/Vj) (1/(1- (F_O/F_M)))$
RC/CS_M	$RC/CS_M = F_M / (M_O) (1/Vj) (1/(1- (F_O/F_M)))$

Reference :

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 340-362



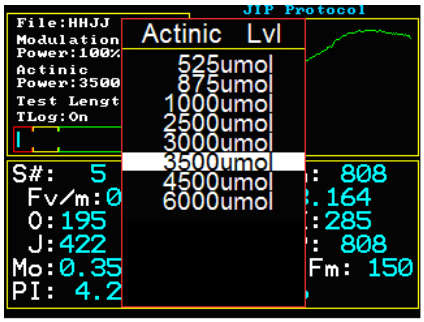
Setting the modulation light intensity:

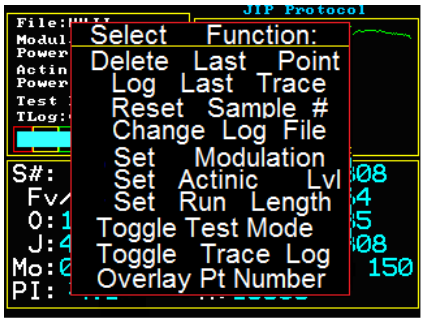
Place a leaf into a dark adaptation clip with the slider closed, and dark adapt for several minutes. Place the instrument probe into the dark clip opening all the way, and slide open the dark slide. This exposes the leaf to the weak modulated light source.

The blue light intensity bar in the upper left hand corner box of the color display will either be in the red, yellow or green part of the box. This intensity bar is designed as an aid to proper modulation light setting. When properly set, the blue bar will be in the green part of the box, and the Ft value above the bar does not raise slowly over a 15 to 30 second period. It the value does start to rise, it means that the modulation light intensity setting is too high, and that it is high enough to drive photosynthesis. If this happens, dark adapt again for a few minutes, and try a lower modulated light intensity. The intensity must be high enough to be measured, but not high enough to drive photosynthesis. Values from 30% to 80% are input by pressing the menu button, and going to Set Monduation with the blue arrow keys. Press the round blue button to select it. Using the key board and the blue arrow keys for navigation and selection. When done, go to the done key board key and push the round blue button.

Actinic light intensity:

Press the menu button and select the actinic intensity line. The actinic light intensity can be adjusted to the following settings: 6000 µmols, 4500 µmol, 3500 µmols, 3000 µmols, 2500 µmols,1000 µmols, 875 µmols, 525 µmols, using the keyboard and the blue arrow keys and the round blue button. When done using the arrow keys to go to done, and push the blue round button. The default value is 3500 µmols. It has been shown that leaves grown in low light conditions saturate at about 300 µmols and that leaves grown under high light conditions saturate at about 1,500 µmols. (Ralph P. J. 2005). It is common to use 3,500 µmols for the JIP test in plant stress testing; however, Vredenberg (2011) uses various actinic intensities to study the different phases of the OJIP induction curve.





Set Run Length:

Press the menu button and select the Set Run Length. The actinic light run length can be adjusted from 3 seconds to 300 seconds. The default is 3 seconds.

Log Last Trace:

If the instrument is set up to record only measuring parameters, and not traces, select the menu button, and then use the arrow keys to select Log Last Trace. This allows saving of the last measuring trace for further evaluation. If not, the measuring trace is discarded. Each discrete measuring file can save up to 32 separate traces for graphing in Excel, along with all of the various important stress detecting parameters. To record more than 32, a separate file name must be created. After 32 saved traces, the file only saves the measuring parameters. Toggle DMode overlay to overlay multiple OJIP traces on the instrument screen automatically, and store multiple traces to the measuring file automatically.

Toggle Test Mode:

To overlay JIP traces on instrument viewing screen and store up to 32 trace in the data measuring file automatically: Press the menu button and select toggle test mode using the arrow keys, and toggle Dmode with the round blue button. This will allow viewing and saving of traces. Up to 32 traces may be overlaid on the instrument screen, and up to 32 traces will be saved per file name in the measuring data file. The instrument screen only has 16 colors. After 16 traces are overlaid on the instrument measuring screen, they start to repeat.

Toggle Trace Log:

When not in Dmode or multiple trace overlay mode, this option allows the saving of single traces to the data file or turns it off. Use the arrow keys and the round blue button to select Toggle Trace mode and toggle this function off or on.

Overlay Pt Number:

This function allows one to select the number of traces that will appear in the data file side by side. Up to 32 traces can be stored with a single file name. The color graphic screen has 16 colors. It does allow the overlay of up to 32 traces on the instrument measuring screen, but colors repeat after 16 traces have been made.

Delete Last Point:

Press the menu button and select the delete Last Point to delete the last measurement. Use the blue arrow keys and the round blue button.

Change Log File:

Press the menu button and select the Change Log File line using the blue arrow keys and the round blue button. A key board appears. Use the blue arrow keys and the round blue button to navigate the key board, and select alpha numeric characters fro the name. Scroll to done and press the blue round button.

Home Icon:

Pressing this Icon takes you to the main menu,

Curved Arrow Icon:

Pressing this icon takes you back to a previous screen

Reset Sample Number:

There is a sample number in the data file. When not recording traces, one can make several measurement in one field, and then select Reset Sample number to start the numbering for the next measurement at one. The previous measurements are still retained, but the sample number of the next measurement will be one, and the sample numbering will start over.

? Icon:

Pressing this icon allows answers to some basic questions related to the screen that is displayed. It also allows the capture of a compete display screen in memory. Press the ? icon again and a key board will appear that will allow the saving of the screen that is displayed. This is true of most screens, however some pop up screens used for parameter adjustment can not be saved.

Data files

This is an example of a JIP test data file.

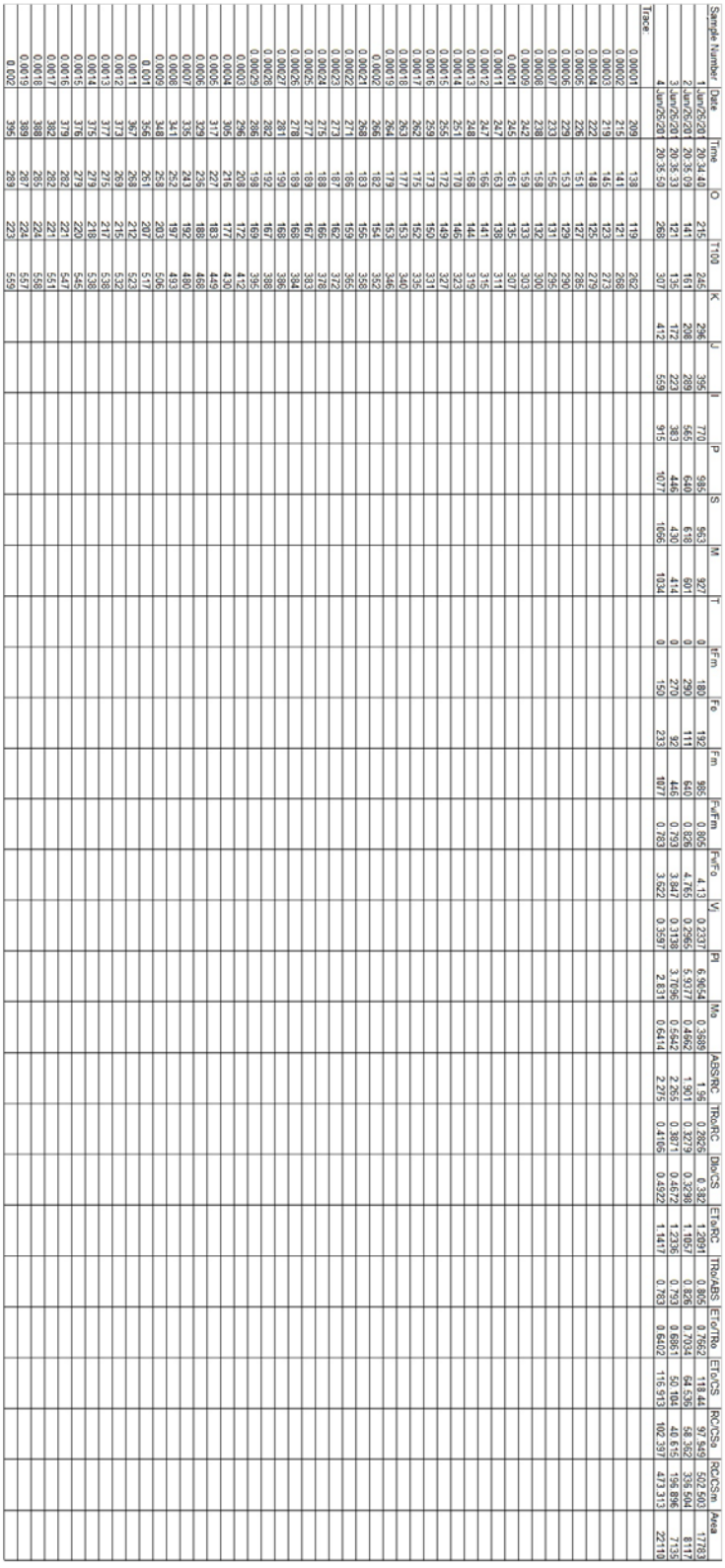
It is organized so that overlay and spider graphing are easily accomplished

The first row represents OJIP parameter headers. Each successive row represents a new measurement, and the parameters derived from that measurement are listed below the header.

Below the rows of individual measurements are the individual traces of the measurements.

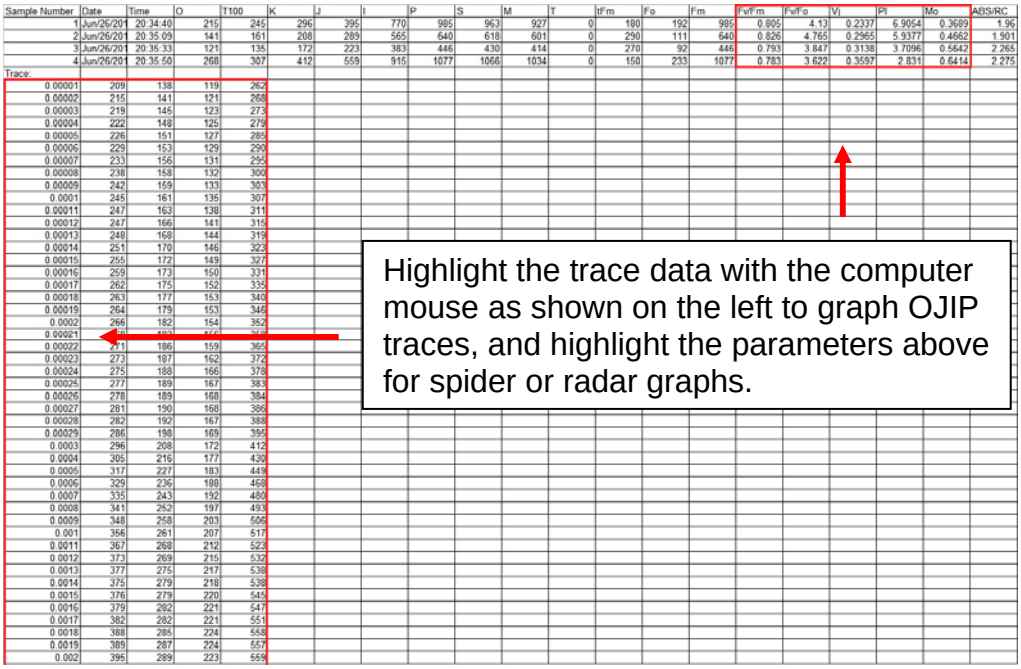
Column A, or the first column, is the time scale used for collecting the data. 0.00001 is 10 µs, O is measured at 0.00002 or 20 µs, J is measured at 0.002 or 2 ms. I is measured at 0.03 or 30 ms. P is measured as the highest value.

F_O, or minimum fluorescence, is measured with a weak modulated light before the actinic light is turned on. It is measured, it is not an estimated value.



Sample Number	Date	Time	O	T100	K	J	I	P	S	M	T	tFm	Fo	Fm	FvFm	FvFo	Vi	Pi	Mo	ABS/RC	TRo/RC	DIo/CS	ETo/RC	TRo/ABS	ETo/TRo	ETo/CS	RC/CSo	RC/CSm	Area
1	Jun/26/201	20.34.40	215	245	296	395	770	985	963	927	0	180	192	886	0.805	4.13	0.2337	6.9654	0.3889	1.96	0.2826	6.382	1.2891	0.805	0.7662	118.44	97.849	502.503	11783
2	Jun/26/201	20.35.09	141	161	208	289	565	640	618	601	0	290	111	640	0.826	4.765	0.2965	5.9277	0.4962	1.901	0.3279	0.3298	1.1857	0.826	0.7034	64.595	58.362	336.594	8117
3	Jun/26/201	20.35.33	121	135	172	223	383	446	430	414	0	270	92	446	0.793	3.847	0.3138	3.7696	0.5642	2.265	0.3871	0.4672	1.2336	0.793	0.6851	50.104	46.615	196.896	7135
4	Jun/26/201	20.35.59	268	307	412	559	915	1077	1666	1634	0	150	233	1077	0.783	3.622	0.3597	2.831	0.6414	2.275	0.4166	0.4922	1.1417	0.783	0.6402	116.913	102.397	473.313	22110
Trace																													
0.00001	209	138	119	262																									
0.00002	215	141	121	268																									
0.00003	219	145	123	273																									
0.00004	222	148	125	279																									
0.00005	226	151	127	285																									
0.00006	229	153	129	290																									
0.00007	233	156	131	295																									
0.00008	238	158	132	300																									
0.00009	242	159	133	303																									
0.0001	245	161	135	307																									
0.00011	247	161	138	311																									
0.00012	247	166	141	315																									
0.00013	248	168	144	319																									
0.00014	251	170	146	323																									
0.00015	255	172	149	327																									
0.00016	259	173	150	331																									
0.00017	262	175	152	335																									
0.00018	263	175	153	340																									
0.00021	264	179	153	346																									
0.00022	268	182	155	352																									
0.00023	271	184	159	358																									
0.00024	273	187	162	362																									
0.00025	277	189	165	368																									
0.00026	278	189	167	373																									
0.00027	281	190	169	384																									
0.00028	282	192	167	386																									
0.00029	286	193	169	395																									
0.0003	296	203	172	412																									
0.0004	305	216	177	430																									
0.0005	317	227	183	449																									
0.0006	329	235	188	468																									
0.0007	335	243	192	480																									
0.0008	341	252	197	493																									
0.0009	348	258	203	506																									
0.001	356	261	207	517																									
0.0011	367	268	212	523																									
0.0012	373	269	215	532																									
0.0013	377	275	217	538																									
0.0014	375	279	218	538																									
0.0015	376	279	220	545																									
0.0016	379	282	221	547																									
0.0017	382	282	221	551																									
0.0018	388	285	224	558																									
0.0019	389	287	224	557																									
0.002	395	289	223	559																									

Graphing



Sample Number	Date	Time	O	T100	K	J	I	P	S	M	T	tFm	Fo	Fm	Fv/Fm	Fv/Fo	Vj	Pi	Mo	ABS/RC
1	Jun/26/201	20:34:40	215	245	296	396	770	985	963	927	0	180	192	985	0.805	4.13	0.2337	6.9054	0.3689	1.96
2	Jun/26/201	20:35:09	141	161	208	289	565	640	618	601	0	290	111	640	0.826	4.765	0.2965	5.9377	0.4662	1.901
3	Jun/26/201	20:35:33	121	135	172	223	383	446	430	414	0	270	92	446	0.793	3.847	0.3138	3.7096	0.5642	2.265
4	Jun/26/201	20:35:50	268	307	412	559	915	1077	1066	1034	0	150	233	1077	0.783	3.622	0.3597	2.831	0.6414	2.275
Trace:																				
0.00001		209	138	119	262															
0.00002		215	141	121	268															
0.00003		219	145	123	273															
0.00004		222	148	125	279															
0.00005		226	151	127	285															
0.00006		229	153	129	290															
0.00007		233	156	131	295															
0.00008		238	158	132	300															
0.00009		242	159	133	303															
0.0001		245	161	135	307															
0.00011		247	163	138	311															
0.00012		247	166	141	316															
0.00013		248	168	144	319															
0.00014		251	170	146	323															
0.00015		255	172	149	327															
0.00016		259	173	150	331															
0.00017		262	175	152	335															
0.00018		263	177	153	340															
0.00019		264	179	153	346															
0.0002		266	182	154	352															
0.00021		[illegible]																		
0.00022		271	186	159	365															
0.00023		273	187	162	372															
0.00024		275	188	166	378															
0.00025		277	189	167	383															
0.00026		278	189	168	384															
0.00027		281	190	168	386															
0.00028		282	192	167	388															
0.00029		286	198	169	396															
0.0003		296	208	172	412															
0.0004		305	216	177	430															
0.0005		317	227	183	449															
0.0006		329	236	188	468															
0.0007		335	243	192	480															
0.0008		341	252	197	493															
0.0009		348	258	203	506															
0.001		356	261	207	517															
0.0011		367	268	212	523															
0.0012		373	269	215	532															
0.0013		377	275	217	538															
0.0014		375	279	218	538															
0.0015		376	279	220	543															
0.0016		379	282	221	547															
0.0017		382	282	221	551															
0.0018		388	285	224	558															
0.0019		389	287	224	557															
0.002		395	289	223	559															

Highlight the trace data with the computer mouse as shown on the left to graph OJIP traces, and highlight the parameters above for spider or radar graphs.

OJIP trace graphing

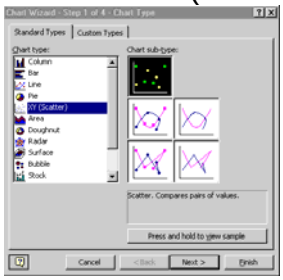
To graph single or overlay several OJIP traces, follow the directions below in Excel:

1. Highlight the entire time column and the entire traces that are to be graphed by holding down the left mouse button on your computer, and moving the screen arrow until all of the data is included. Column A is the time scale, and column B, C, D, E etc. are the JIP traces.

2. Click the Chart wizard in most Excel versions - Symbol for chart Wizard - and select XY Scatter Graph .

For Excel versions 2008 and 2009 look to the insert tab of the ribbon. Other ribbon options are also shown.

3. Select XY (Scatter)



4. Select the “Scatter option with data points connected by smoothed lines without markers” option, and click next.

5. Select columns, and click next.

6. Titles along with X and Y value titles may be added or not. Select next.

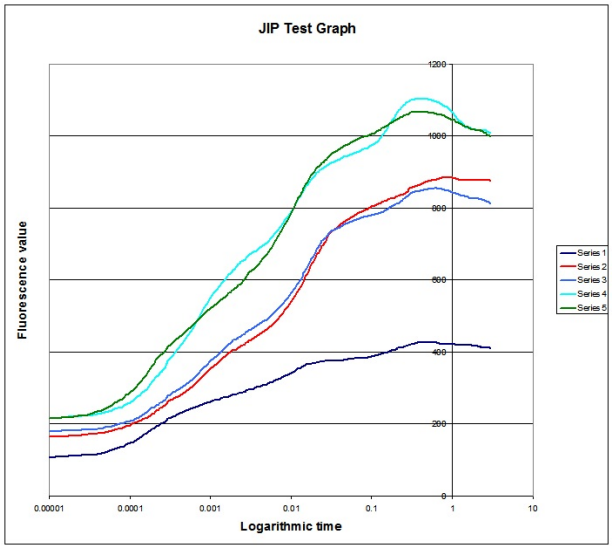
7. Select next again.

8. Select Finish.

9. To provide a logarithmic time scale double click on the lower time axis.

10. Check the logarithmic scale box and click OK.

11. Colors and line weight can be changed by left clicking on a trace and then right click to find formatting information. Here line color and weight of lines may be changed. To change the background color, left click on the background, and right click to find formatting.



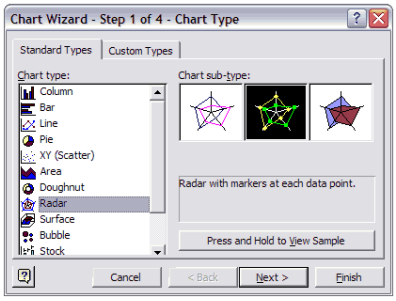
Spider or Radar Graphs

To produce “Spider” or “radar graphs” of measured parameters, follow the instructions below:

1. Highlight the parameters of interest along with the headers as shown on the top of the previous page. Group parameter of interest in columns next to each other.

2. Click on Excel Graph Wizard . (For Excel 2008 and 2009, look to the insert tab of the ribbon.)

3. Click on Radar graph



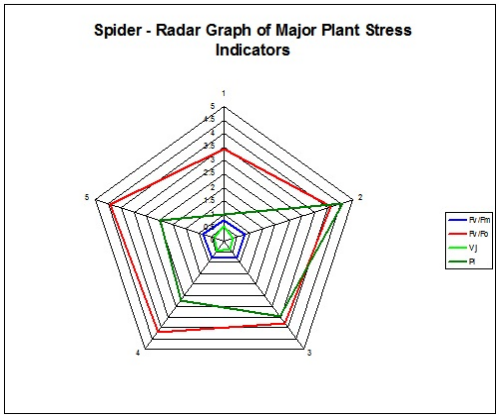
4. Choose one of the data formats without markers or fill, and click next.

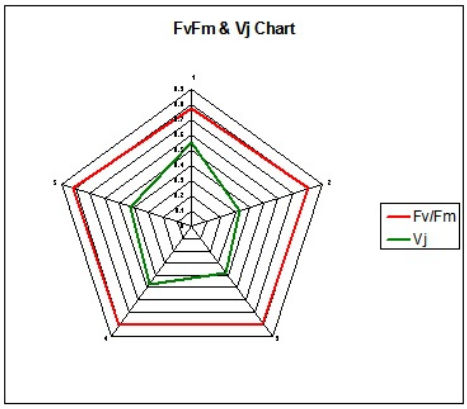
5. Choose rows; however, columns can also be used. Both are of value.

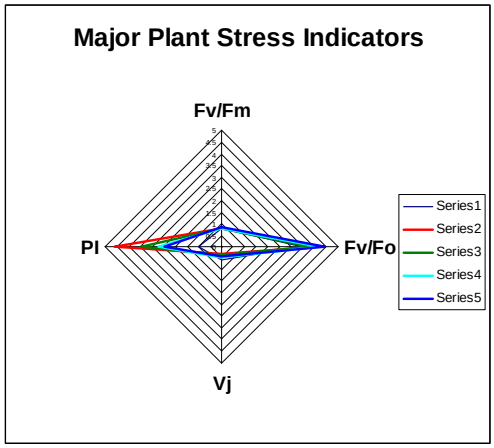
6. Titles and value may or may not be entered and click next.

7. Click finished.

8. Colors can be changed by clicking on the graphed data.

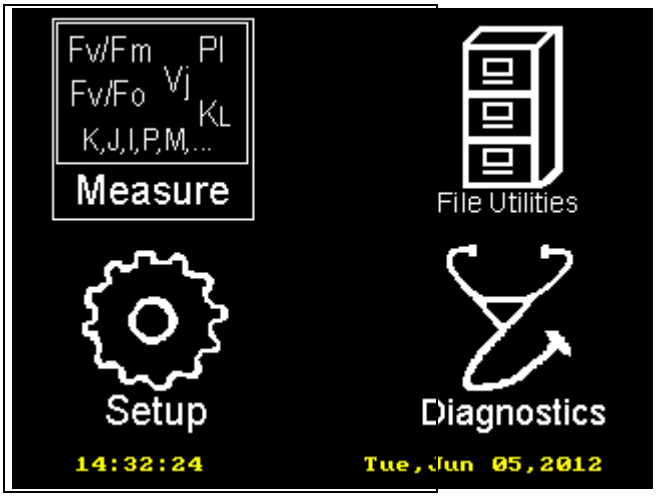






Setup

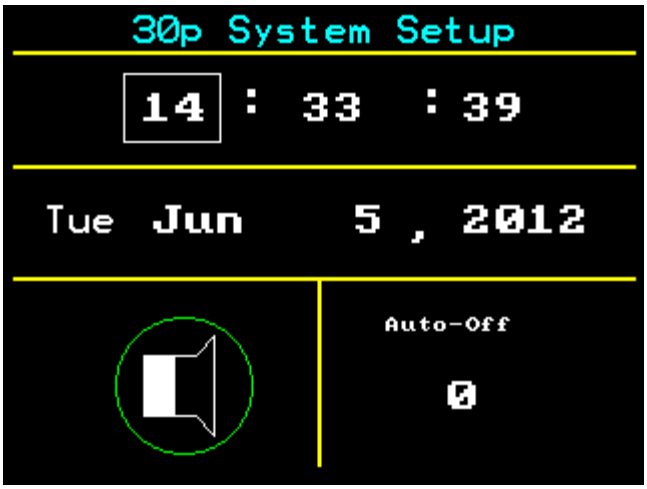
When you turn on your OS-30p+, using the red button on the bottom of the instrument, this screen (referred to as the main screen) will appear:



At a glance, you can tell the, system date and time.

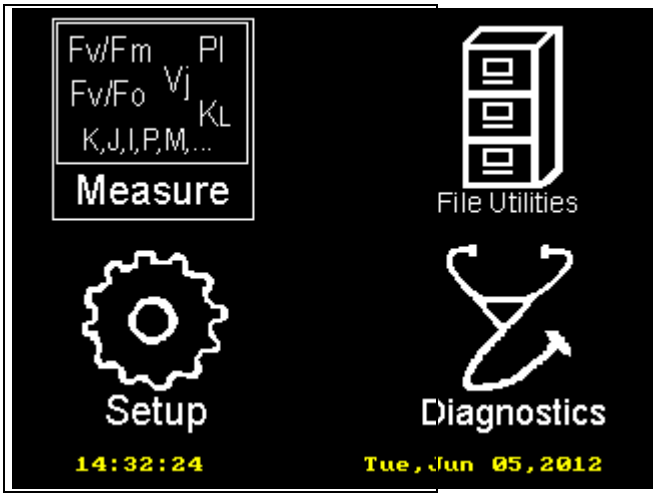
The blue arrow keys allow navigation from Measure, to File Utilities, to Setup, to Diagnostics. Once the category has been highlighted with the arrow keys, press the round blue button with the yellow check mark to select the category of interest. Navigate to Setup Icon and press the round blue button.

The setup screen, as shown below, will appear. Use the blue arrow keys to navigate and the round blue button to select times, dates, auto off times, and allowing a sound reinforcement beep of measurements. If the Auto-Off is set to 0, then the auto-off is turned off.



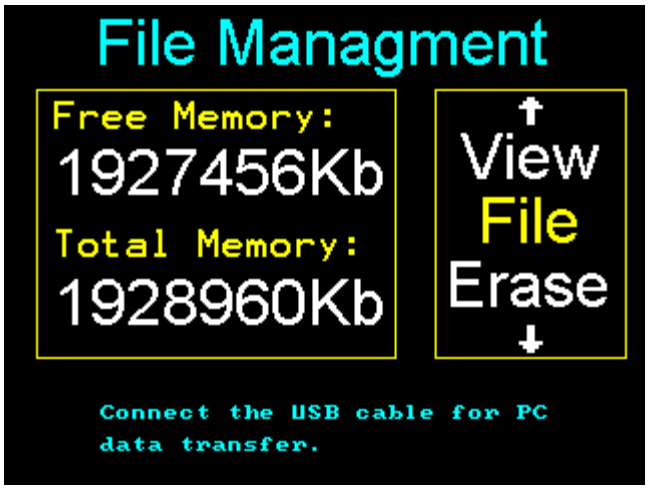
File Utilities

When you turn on your OS-30p+, using the red button on the bottom of the instrument, this screen (referred to as the main screen) will appear:



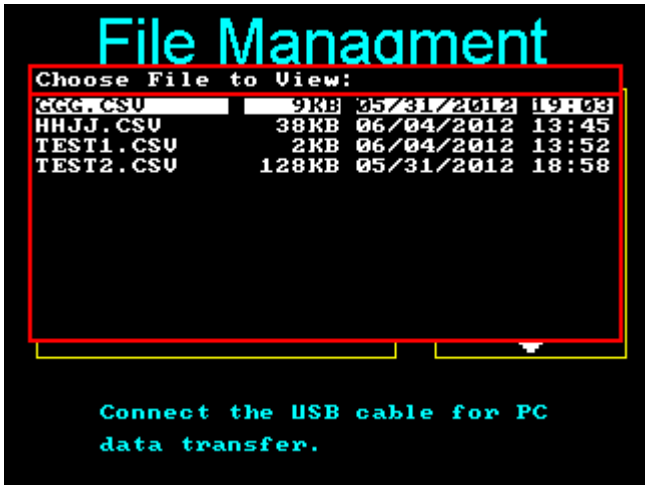
At a glance, you can tell the, system date and time.

The blue arrow keys allow navigation from Measure, to File Utilities, to Setup, to Diagnostics. Once the category has been highlighted with the arrow keys, press the round blue button with the yellow check mark to select the category of interest. Navigate to File Utilities Icon and press the round blue button. The screen shown below will appear. This part of the software is designed to allow viewing of the memory used and the amount left, allow measurement file review and selection, and it is designed to allow file transfer from the OS30p+ to a PC by USB port.



Viewing measuring files and erasing files:

To view a list of the measuring files used on the instrument, use the up blue arrow key to navigate to view files and the down blue arrow key to erase files. Files are high lighted by the up and down blue arrow keys. Press the round blue button to select the file. Before erasure occurs, a message will appear and ask if you want to erase the file selected. The horizontal blue arrows allow erasure or aborting the process. Press the Home button to leave the File Utilities section.



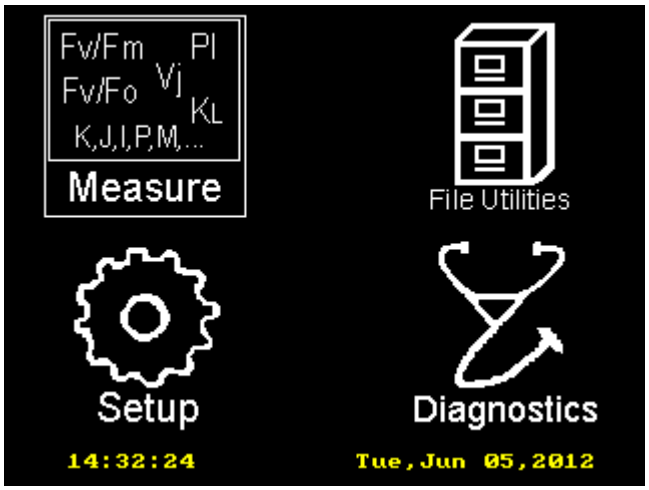
Data transfer to a PC by USB port:

Turn off the OS30p+.

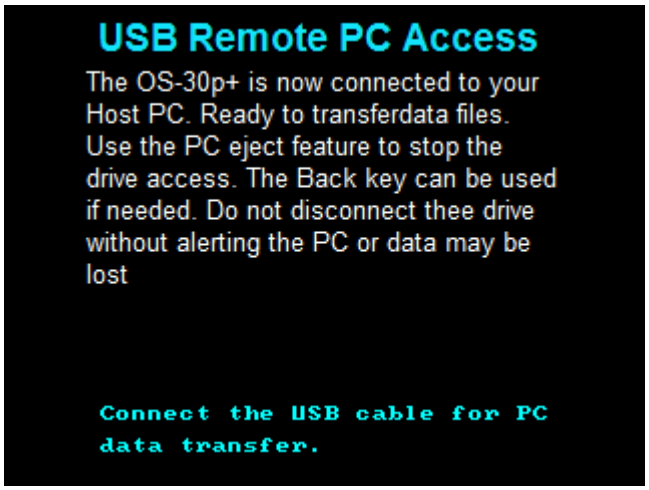
Connect the OS30p+ to your PC using the USB cable provided with the instrument.

To transfer measuring files to a PC, turn the instrument on with the red button on the bottom right hand side of the instrument. The main screen shown below will appear.

Use the blue arrow keys to navigate to Utility Files and press the round blue button to select Utility Files.



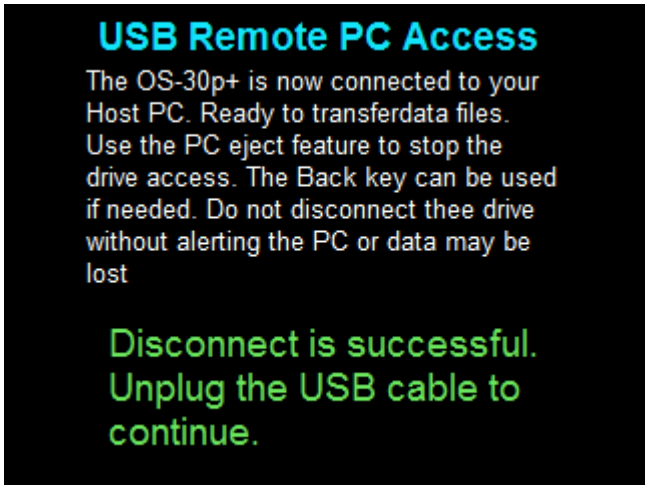
At first the screen shown below will appear.



The OS30p+ becomes a hard drive for your PC. If a screen does not appear on your PC desk top showing file opening options, go to “Windows Explorer” and select the OS30p drive listed under Computer. Once this is done, a list of files will appear. Some may be comma delineated files, or measuring files, and some may be bitmaps. Right click on the file of interest with your mouse and select “open with”, select the program that you will uses to open the file. The measuring files are comma delineated files that may be opened directly into Excel or other spread sheets. The Bitmaps may be opened directly in Paint.

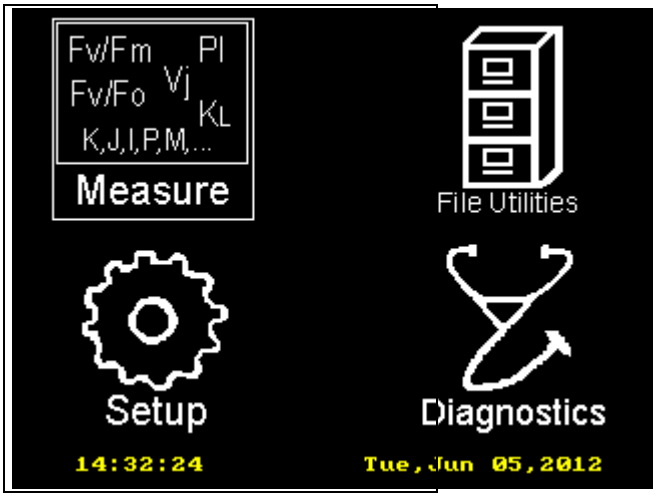
The OS30p+ allows complete screen image capture with the use of the ? icon. Images can be saved by pressing the ? icon, pressing it again, and then entering a file name.

When file transfer is complete, it is recommended that you right click on the OS30p drive in “Windows explorer” and left click Eject. Once this has been done, the screen shown below will appear.



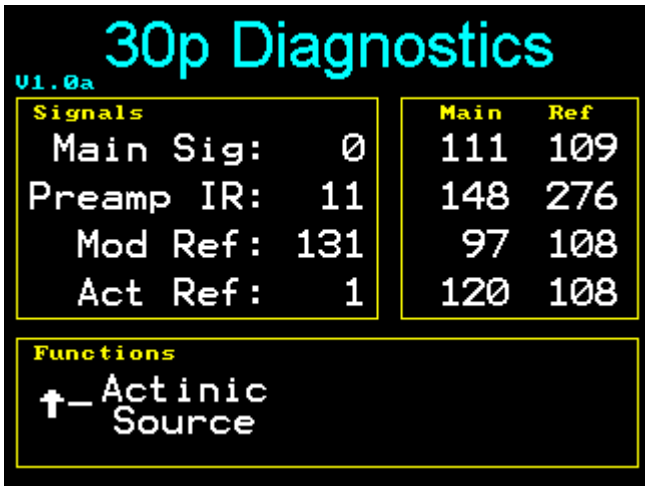
Diagnostics

When you turn on your OS-30p+, using the red button on the bottom of the instrument, this screen (referred to as the main screen) will appear:



At a glance, you can tell the, system date and time.

The blue arrow keys allow navigation from Measure, to File Utilities, to Setup, to Diagnostics. Once the category has been highlighted with the arrow keys, press the round blue button with the yellow check mark to select the category of interest. Navigate to the Diagnostics Icon and press the round blue button. The screen shown below will appear. This part of the software is designed to allow testing of the various function of the OS30P+. It is designed primarily to let Opti-Sciences personnel diagnose possible problems. It is not designed to be used by end users without the help of Opti-Sciences.



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Auto-Calibration.

The OS30p+ goes through an auto-calibration routine every time the instrument is turned on, or when a parameter is changed. A separate light meter is not necessary for light source calibration.

Light diode output can change over time, and as a result, the actinic light intensities are monitored from time to time to ensure repeatable actinic intensities. The instrument checks the actinic light source, the modulation light source, and the conditions of filters and mirrors. The intensity values are adjusted automatically to remain constant.

Appendix A - Technical Support

Opti-Sciences provides technical support for all Opti-Sciences' products, directly to end users, and through our distributors. Please contact your local representative, or contact Opti-Sciences directly for questions including technical assistance, instrument maintenance and repair. Warranty service period is determined by instrument serial number. Please have the serial number available when making service inquiries.

In North America, contact:
Opti-Sciences, Inc.
8 Winn Avenue
Hudson, NH 03051
USA
Tel: (603)883-4400
Fax: (603)883-4410

Internet: www.optisci.com
e-mail: support@optisci.com