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OPTI-SCIENCES

OS-500

Modulated Fluorometer

Operation Manual



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CHAPTER 1 • INTRODUCTION

OVERVIEW

This chapter provides you with a list of the equipment you should have received in your OS-500 package. Information about the fundamental theories behind chlorophyll fluorescence is also presented, in addition to scientific references.

WELCOME !

Congratulations on your purchase of the OS-500 Modulated Fluorometer. Please check the carton for any visible external damage. If you notice any damage, notify the freight carrier immediately. 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.

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List of Equipment

Carefully unpack the carton. You should have received the following items:

- OS-500 Fluorometer
- 12V 3.4 Ah lead acid battery
- AC battery charger (either 110V or 220V)
- Trifurcated fiber optic light guide with built-in trigger switch
- 9-pin serial port cable
- Five dark-adaptation cuvettes
- One open body cuvette
- OS-500 owner's manual (this document)
- 3.5 in. floppy disk with OSDATA software
- Nylon field bag

If any item is missing, please contact your authorized Opti-Sciences agent.

Getting Started

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-500.

The user interface consists of a high-resolution graphic LCD panel having four soft-keys to the right side and five more to the bottom. A data selection wheel is located below the PAR sensor plug. The bulk of all menu options are presented as icons or text legends correspondingly placed above (or to the right) of the respective key. In this way, the function of a key is determined by the users location within the program flow. Whenever possible a common method of user interaction is preserved in general key function location. Keys of similar function within the test group are assigned to the same spot for all test groups. Certain keywords are used to identify common functions. For example, the word or icon "ESC" will always step you to the previous program screen, ultimately ending in the "Main Menu".

Two common methods are used for parameter editing. On the screens where there are few settings and / or where other non-editable information is simultaneously displayed, a "Change" key or representative icon (in the case of multiple setup groups) will be present to initiate the change option. In screens possessing

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parameters needing to be user defined or frequently set (the "Test Setup" screen, for example) entry into the screen automatically activates the edit mode. In either case, the number of fields to edit will determine the method used to step between selections. When many options are present (as in the "System Setup" screen) up and down arrows will appear next to the lower right keys, these are used to step between parameters. When editing in this mode the new selection is accepted when either the field is left, using the up / down arrows or, when the "ESC" key is pressed (ending the edit session). The exception to this is the "Set Clock" function discussed in detail in the system setup chapter. When few fields are present, the "Change" key (or icon) is used to step forward through the fields (as in the serial port parameter select in the "Output to RS-232" screen). In most cases the "ESC" key will exit the edit mode.

The actual selection of parameters within a field is accomplished by turning the selection wheel. The wheel has automatic sensing of acceleration and tailors the step rate based on the users physical spinning of the wheel and, on the nature and range of the data to be entered. The current field to be edited is recognized by a blinking cursor either on or to the left of the first character within a field. A tone is sounded (if enabled) to indicate key-presses, run time status, and warning alarms.

Data is stored in on-board system memory. This is battery backed-up so no data will be lost if the main battery is removed. Stored data may be transferred to other systems through use of the built-in 1.44Mb 3.5" floppy drive, or the RS-232 compatible serial port. The disk drive uses a DOS 3.0 or greater compatible format operating system directly readable by IBM compatible machines. The data is output (disk or serial port) in comma delimited and carriage return separated ASCII strings easily importable to most spreadsheet programs. An unprocessed analog fluorescence signal is also available.

Chlorophyll Fluorescence

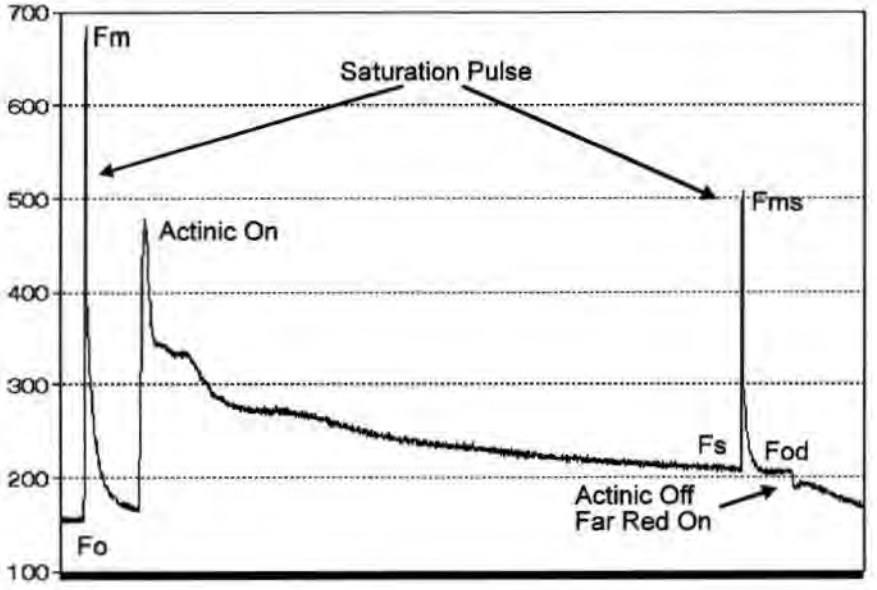
Light energy utilized in photosynthesis by higher plants and algae cells is absorbed by a number of photosynthetic pigments with absorption spectra covering a large range of the available light energy. The most prominent pigments that absorb this energy are chlorophyll-a and chlorophyll-b. The light energy absorbed by the chloroplast first excites pigment molecules of the light harvesting chlorophyll proteins (LHC). These LHC proteins transfer their energy to either Photosystem I (PSI) or Photosystem II (PSII). These photosystems contain the reaction center pigments for the conversion of absorbed light energy to oxidation and reduction potential to drive dark electron transport. Light energy absorbed initially by the LHC and transferred to the reaction centers is lost by a number of different mechanisms. Approximately 3%-9% of the light energy absorbed by chlorophyll pigments is re-emitted from the first excited state as fluorescence. The emission peak is of a longer wavelength than the excitation energy. This effect was first observed more than 100 years ago, when N.J.C. Müller (1874) by visually using

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colored glass filters. He also noted that fluorescence changes that occur in green leaves are correlated with photosynthetic assimilation. Lack of appropriate technical equipment prevented a more detailed investigation of this phenomena. The light energy absorbed by the reaction center drives photosynthetic electron transport through PSII and PSI leading to the oxidation of water, oxygen evolution, the reduction of NADP+ to NADPH, membrane proton transport and eventually to ATP synthesis.

The loss of light energy from the reaction center as fluorescence comes primarily from the PSII reaction. When the chloroplast or leaves have been dark-adapted, the pools of oxidation or reduction intermediates for the electron transport pathway return to a common level. Upon illumination of a dark-adapted leaf, there is a rapid rise in light emission from PSII fluorescence followed by a series of slow oscillations. This is referred to as the "Kautsky Effect". Named after the researcher who did detailed studies on the phenomena (Kautsky and Hirsch 1931). Fig 1-1 shows the usual onset kinetics of fluorescence emission from a typical dark-adapted leaf. Changes in the intensity of the fluorescence emission from dark-adapted leaves are sensitive to changes in the photosynthetic apparatus. Following many years of study of chlorophyll fluorescence to analyze its relationship to photosynthesis and characterize photosynthesis, it has been shown (see reference list) that any unusual change in the overall bioenergetic status of the plant can be detected by a change in chlorophyll fluorescence. This includes all the reactions from the oxidation of water through electron transport, development of the electrochemical gradient, ATP synthesis, and eventually the series of enzymatic reactions for CO_2 reduction to carbohydrate in the leaf. Even changes in the plant that affect stoma opening and gas exchange with the atmosphere are reflected by changes in the fluorescence characteristics of a leaf.

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Values Calculated:

$$Fv/Fm = \frac{Fm-Fo}{Fm} \qquad Y = \frac{Fms-Fs}{Fms} \qquad NPQ = \frac{Fm-Fms}{Fms} \qquad ETR = Y \times PAR \times .5 \times .84$$

$$qP = \frac{Fms-Fs}{Fms-Fo} \qquad qN = \frac{Fm-Fms}{Fm-Fo} \qquad \text{or} \qquad qP = \frac{Fms-Fs}{Fms-Fod} \qquad qN = \frac{Fm-Fms}{Fm-Fod}$$

(With dark-adapted Fo Value) (With post-actinic far-red illumination)

Figure 1-1 • Chlorophyll Fluorescence

The OS-500 is equipped to measure both dark-adapted fluorescence and "Yield" of energy conversion (first described by Genty, Briantais & Baker, 1989). Fig 1-1 shows the standard measured parameters from a typical test.

Parameters Measured

- Fo and Fm are the minimal and maximal fluorescence yields of a "dark adapted" sample, with all PSII reaction centers fully open and closed, respectively. The need for strict "darkness" is only critical to the extent of preventing sufficient accumulation of reduced PSII acceptors to cause an increase in fluorescence. Shielding the sample will verify this condition. The

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intensity of the measuring signal at 500 Hz is low enough to prevent any significant affect on the sample.

- Fs and Fms are the steady state and maximal fluorescence values of a sample during exposure to a source of light driving photosynthesis.
- Fod is a measure of the possible quenching of Fo. (see qP & qN)
- Ft is the instantaneous fluorescence signal.

Values calculated from measured parameters

- Fv/Fm is the ratio of the "variable" to maximal fluorescence.
- Yield is calculated from the steady state parameters Fs and Fms. It is an indicator of quantum yield of photosynthesis. A practical advantage of this measure is that Fo does not need to be known.
- ETR represents the apparent photosynthetic electron transport rate in uMols electrons m-2 s-1, which is calculated based on the measured values of Yield and PAR. The following assumptions are made:

  1. PAR corresponds to the flux density of incident photosynthetically active radiation which is measured in uMols quanta m-2 s-1.
  2. Yield represents the overall photochemical quantum yield.
  3. The transport of one electron requires the absorption of two quanta, as there are two photosystems involved. (the 0.5 factor in fig. 1)
  4. An average of 84% of the incident quanta are absorbed by the leaf (the 0.84 factor in fig. 1).

  In practice, the last assumption is not always true. The value of .84 has been reported of leaves of numerous species, though the value depends on several variables such as the spectral composition of light, chlorophyll content, and leaf surface reflectance. The previously mentioned factors must be noted when utilizing the ETR value. If a more specific absorption value is known, a correction can be introduced into the PAR calibration factor.

  The ETR may be compared to the rate of CO_2 - assimilation or of O_2 - evolution. In these comparisons the following aspects are important.

  1 4 electrons must be transported for every CO_2 assimilated or O_2 evolved

  2 Fluorescence data originates primarily from the top-most chloroplast layers, but gas exchange data integrates through all layers.

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3 The value of ETR/4 is not necessarily identical to the CO_2 fixation or O_2 evolution rates; differences may arise from photorespiratory electron flow or electron cycling in PSII.

- qP and qN are defined as the coefficients of photochemical and non-photochemical fluorescence quenching. The original definition of these terms implied that fluorescence quenching affects primarily the 'variable fluorescence' (Fv) and not the minimal fluorescence (Fo). In cases where qN is greater than 0.4 this may not be a good assumption. This will affect the calculated qN and qP values. By using the Far-Red source for post illumination, the PSII acceptors may be reoxidized through the illuminations affect on PSI. A new Fod value may be measured and used for corrections to the quenching coefficients. This assumes the PSI acceptors are properly activated, which may not be the case in a dark adapted sample. therefore the Fod determination should be done after induction of photosynthesis has be done for several minutes.

- NPQ is an alternate expression of non-photochemical quenching. It provides an estimate of quenching without knowledge of Fo. The advantage of NPQ over qN depends on the specific application. NPQ is more heavily affected by non-photochemical quenching that reflects heat-dissipation of excitation energy in the antenna system. So it may be thought of as an indicator of 'excess excitation energy'. Alternatively, NPQ is relatively insensitive to the part of non-photochemical quenching associated with qN values lower than 0.6 This range of qN is affected by 'thylakoid membrane energization' which is an important aspect of photosynthetic regulation .

- T½ (fast actinic test only) is defined as the time in mS from the point Fo to the point on the rising curve corresponding to ½Fv. This value relates to the slope of the rise to Fm in the Kautsky induction curve.

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CHAPTER 2 • THE OS-500

Introduction

The OS-500 Modulated Fluorometer is a versatile measuring instrument designed to precisely measure chlorophyll fluorescence. It uses a pulse modulated detection principle 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. This chapter provides general information about the OS-500 Modulated Fluorometer.

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Key Features

The OS-500 includes the following features:

- A modulated detection system which allows measurements to be made in variable test conditions, even in bright daylight
- A wide range of modulation intensity and gain settings, allowing for measurement in plant species with low chlorophyll content
- On-board fully adjustable actinic and far red illumination sources
- Inputs for leaf temperature and PAR (using OS-OBCP leaf clip)
- Light weight design excellent for field studies
- Fully portable battery operation
- Full measurement control from on-board computer system
- User input consists of 9 soft keys and data wheel.
- Graphic 320 by 240 pixel LCD screen
- IBM ™ compatible 3½" floppy disk drive (1.44Mb)
- Versatile software, optimized for variations in testing procedures
- OSData Windows ™ software allows detailed viewing of traces and data

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Physical Features

Front Panel Connections

The front panel of the OS-500 contains the following fiber optic connectors, electrical jacks and user interface:

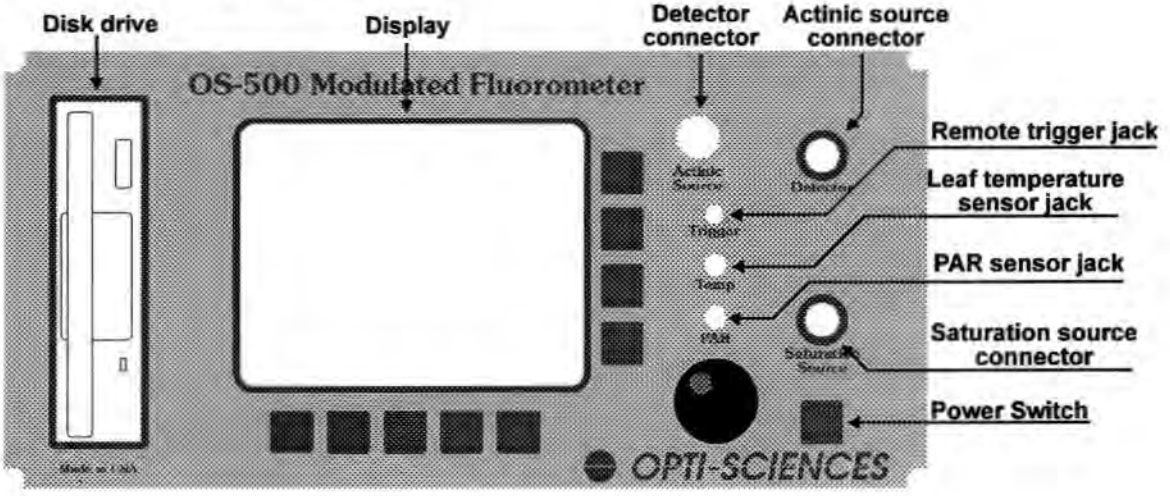


Figure 2-1 • Front Panel of OS-500

Actinic source connector — Here the modulated light source is coupled into the fiber bundle.

Detector connector — Signal from the leaf surface returns to the detector through this connector.

Remote trigger jack — The electrical plug (1/16 in.) on the fiber bundle is connected here to enable the remote trigger switch.

Leaf temperature sensor jack — The optional cuvette's (OS-OBCP) temperature probe connects to this jack.

PAR sensor jack — The optional cuvette's (OS-OBCP) PAR sensor is connected here to allow ambient light measurements.

Saturation source connector — This is where the saturation pulse is coupled into the fiber bundle.

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Front Panel

The front panel contains many specialized user-interface features.

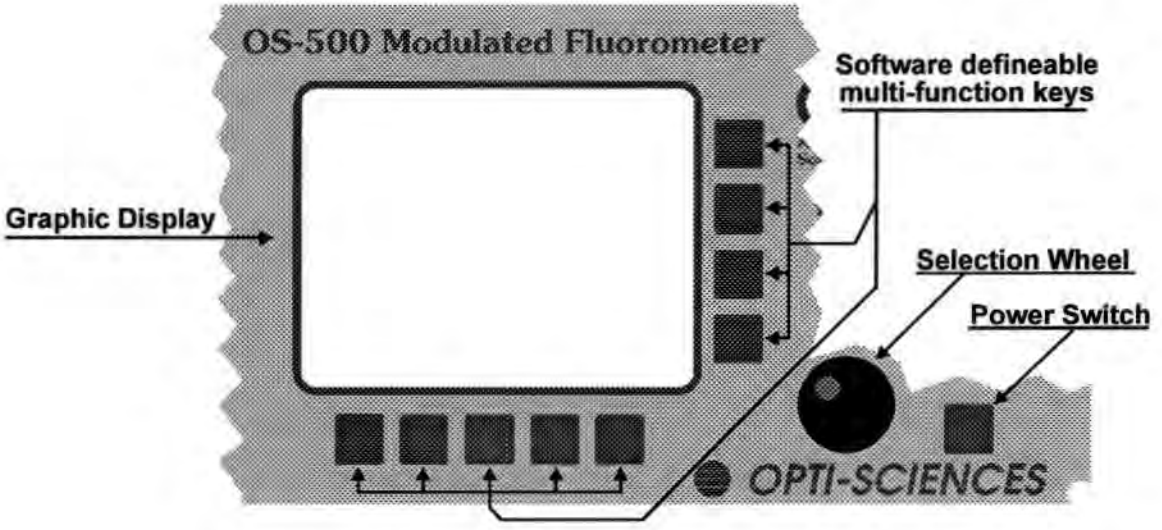


Figure 2-2 • OS-500 Keypad and LCD Panel

Disk drive — Accepts standard IBM compatible 3.5" diskette for data storage.

Graphic display — An 320 x 240 pixel graphic liquid crystal panel with LED back lighting provides a clear readout of system functions, parameters, and graphs.

Power switch — Turns the OS-500 system power ON and OFF.

9 key software programmable keypad — The front panel contains 5 tactile-feedback keys under the display and 4 more immediately to it's right.

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Rear Panel

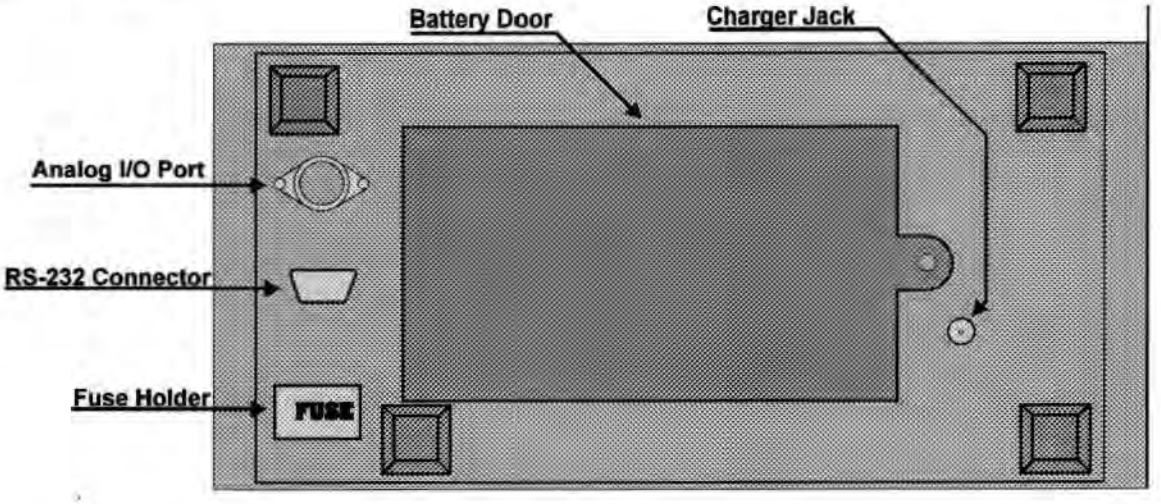


Figure 2-3 • Rear Panel of OS-500

Battery Door — This door provides access to the 12V battery compartment.

Charger jack — The battery charger plugs into this jack to allow the battery to be charged.

Analog I/O Port — Gives user access to the raw fluorescence signal. Provides a chart recorder output (0 ~ 3 Volts) and 2 analog inputs (±15 Volts)

RS-232 connector — Standard RS-232 serial port.

Fuse Holder — Unit uses a 4 Amp fast-blow fuse. (note: The fuse holder has a compartment for a spare fuse).

Hardware Overview and Measurement Principles

The OS-500 is a portable modulated chlorophyll fluorescence measurement system. It is completely self contained, eliminating the need for any external hardware or computing device (palmtop computer, etc.). All detection and data logging components are on-board.

The fluorescence is excited by a 655 nm solid-state light source with filters blocking radiation longer than 700 nm. The average intensity of this modulated light is adjustable from 0 to 1 uMol/m^2/s. The source output is optically monitored inside its assembly to correct for variations in output due to changes in ambient temperature.

Detection is done in the 710 to 760 nm range using a PIN silicon photodiode with appropriate filtering to narrow the optical response. A low-noise preamplifier is mounted in the detector assembly. The detection method is a modified lock-in

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amplifier design which allows the use of a very low average modulated light energy. This allows monitoring of the fluorescence level without exciting any noticeable variable fluorescence. In effect, the system measures the change in fluorescence due to effects other than the measuring beam. The detection rate is switchable by the computer for maximum signal to noise ratios and for accurate sensing of rapidly changing signals. An added benefit of this system is its ability to disregard extraneous ambient light.

Excitation sources

There are four excitation sources present in the OS-500:

The Modulation Source

The modulation source is a 660nm output used exclusively for detection of the fluorescence signal. It's intensity may be varied to adjust for cuvette and sample dynamics.

The Saturation Source

The saturation source is a 35W halogen lamp (with appropriate filters). This source provides the high light level (>10kuE) used to guarantee photosystem saturation. Its level is also adjustable, and is dynamically monitored and controlled. Experimentation with different pulse intensities are repeatable. It may also be used as a drive source for the continuous fluorescence measurement.

The Actinic Source

The peak wavelength of this source is ~670nm. It is a variable intensity, general purpose photosystem drive source for the kinetic and continuous tests. It is a solid state source boasting very good conversion efficiency when compared to a halogen source.

The Far Red Source

The peak wavelength for the Far Red source is ~735nm. This wavelength provides largely selective excitation of Photosystem I with the side effect of an enhanced re-oxidation rate in Photosystem II acceptors. The affects of this light are greatest under low actinic lighting levels (~50uE) or right after exposure to high actinic illumination. Usually a short exposure to this light will cause a re-oxidation of the acceptor pool similar to that produced with a 'dark adaption' period.

The optical signals are transferred to and from the sample by a custom-designed trifurcated fiber optic light guide, with randomly mixed fibers. One end of this fiber bundle has three BNC (twist-lock) connectors for the three optical elements and a

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1/16 in. plug. In addition to the trigger switch, the other end provides a comfortable fiber bundle grip and an interface section designed to fit into the many cuvette options.

The CPU board holds the computer circuitry, the main power supply, and the user interface hookup (display, keypad, RS-232 port, disk drive controller). The computer is an 16-bit CMOS microprocessor with 256Kb RAM, battery backup and 128Kb of firmware memory. This board also contains the real time clock and accompanying backup power circuitry. No data will be lost due to loss of power in the 12V battery.

The OS-500 is housed in a durable ABS plastic case and comes with a nylon field bag which protects the unit and provides an easy way to carry it in the field.

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CHAPTER 3 • USING THE OS-500

Introduction

The OS-500 is shipped with the battery disconnected. See section 5.0, Maintenance, for installation instructions. It is a good idea to give the battery a full overnight charge before the first use.

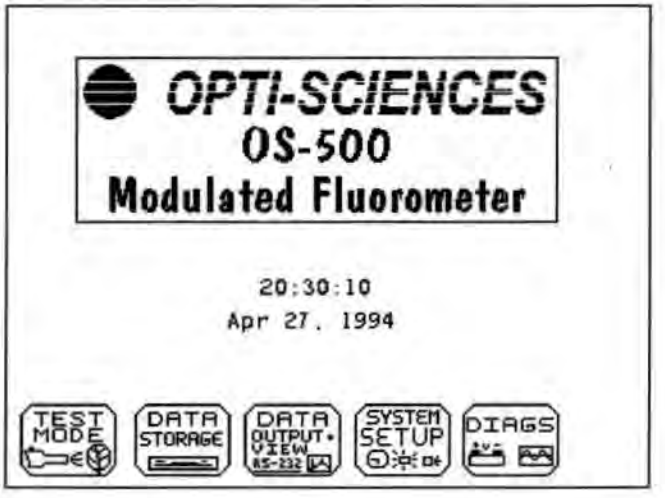
All the OS-500's optical ports will have protective caps: remove and save them.

Connect the three fiber optic light guide ends to their respective ports on the OS-500. The remote trigger switch (the 1/16" plug on the fiber) connects to the jack labeled "Trigger" on the OS-500 (see figure 2-1 Front Panel).

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Introduction to running tests

The OS-500 has some of the most advanced software ever to be found in a self contained, portable modulated fluorometer. The software is set up into several graphical screens with 9 software programmable function keys. Each screen is made to perform a specific task. When you turn on the OS-500, the following screen will appear.



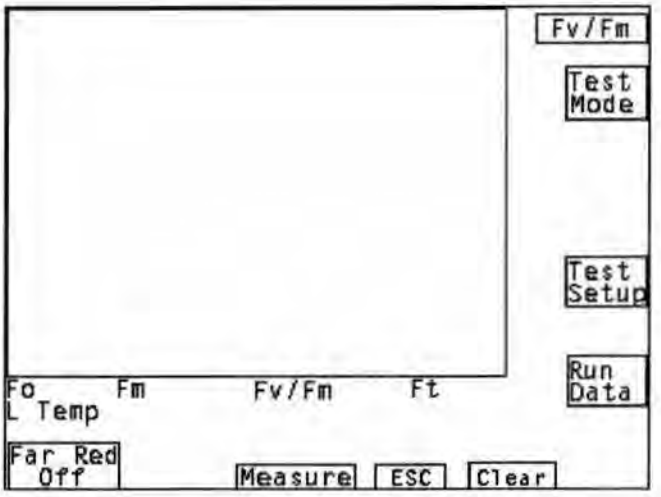
Main Menu

The time and date are set for US eastern standard time (24 hour format).

Test Modes

The OS-500 has 4 different test modes, each mode providing the necessary setup and data logging for each type of test. Every test mode is accompanied by 3 additional screens, providing the display of system settings and gathered data. The main test screen is displayed after pressing the **Test Mode** key.

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Fv/Fm test screen

The large box provides a graphical display of the sampled data. Any test data will be shown directly below the graph. The keys on the bottom of the screen control measurement operations and basic functions. The keys to the right side of the screen provide access to common functions (i.e. "Test Mode", "Test Setup", and "Run Data").

Current Test Mode

The box in the upper right corner displays the current test mode.

Test Mode

The current test mode may be changed by pressing **Test Mode** and scrolling through the choices with the selection wheel. To accept the appropriate test mode, press **Test Mode** again. The four test modes available are:

1. Fv/Fm — Dark adapted Fv/Fm test
2. Yield — Yield of photosynthetic efficiency (PSII)
3. Kinetic — quenching coefficients & yield
4. Fst Act — Continuous fluorescence (Kautsky curve)

Run Data

This key will display the data logging control screen. The current memory status, working file information, and data logging mode are displayed at the top of this screen. The currently active file number, and mode of data logging are chosen here.

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Test Setup

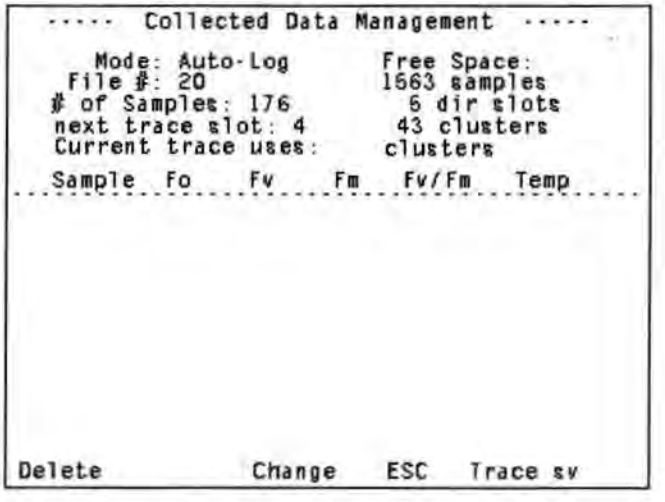
Allows the user to change the test parameters. The "Test Setup" screen displays the current test parameter settings. The user can change any of the settings by using the up/down keys and selection wheel. Pressing **ESC** will activate the new settings, and the user will be returned to the main test screen.

Error messages common to the test modes

There are several common errors that can occur in the test modes.

- If the system runs out of sample space during a test run it will automatically switch to the "Run Data" screen where the user can see the memory status.
- If the battery voltage is 10.5 or less, the warning message "!! Battery Low !!" will appear at the bottom of the test screen under the measured values area and a long beep will be sounded.
- If the amount of in-band IR radiation is above set limits thereby saturating the pre-detector the error message "!! IR to High !!" will be displayed.
- Keep in mind that the maximum signal value is 2000. If a test is started with too high an Ft value, the variable fluorescence induced with a saturation pulse may exceed this value.

Run Test Data



Typical run data Screen

This screen displays the data logging settings and memory status and as well as the most recent 20 measurements taken. On the 21st sample, the data view area will clear and start again, displaying any data after the 20th consecutive measurement.

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This feature allows the user to view data taken without having to step back to the "Data View" menu. It is important to remember that the data shown in this screen is not necessarily being logged. The user must be sure the "Auto-Log" feature is enabled or that each measurement is individually logged from the "Test Mode" screen if data is to be recorded. If the "Auto-Log" feature is off, the sample number will not increment (unless individually logged).

Pressing **Change** will allow the user to alter the settings under the "Collected Data Management" box. These functions are the same for all four test modes. Once all necessary changes have been made, press **ESC** to exit the edit mode.

Mode:
"Auto-Log" - Data from each saturation pulse will be added to memory with the sample number being automatically incremented.

"Manual" - The main test screen menu will have the key **Log** added. Pressing this key will save the displayed data and increment the sample number.

File #
Displays the current file number. This number assigns all stored data to a specific group that is later referenced for all data manipulation. A new number can be entered with the selection wheel. The range of possible file numbers is from 1 to 99.

# of Samples
Displays the number of samples stored in the current file number.

next trace slot
Displays the next free trace data entry number. It is not user configurable and will depend upon the last trace erased or saved.

Free Space:
Statistics on memory usage is listed under this heading.

Samples
Displays the number of remaining sample slots (from a possible 2048).

dir slots
Displays the number of remaining directory slots.

clusters
Displays the number of remaining clusters.

Current trace uses: ## clusters
Displays the number of clusters needed to store the current trace.

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A cluster is a 1Kb block of memory. The OS-500 has 128 clusters set aside for trace storage. A directory slot is analogous to a file name on a "MS-DOS" disk. The significance of showing these separately comes from the greatly varying nature of possible test runs and their complex data formats. For example a 'dark adapted' trace of 0.8 sec will take 2 clusters to save but there are only 27 possible trace directory spaces. This means that directory space will be used up before cluster space. However, a Kinetic run of 45min will use 54 clusters, leaving 74 unused (giving a maximum of 2 - 45 minute traces with 20 clusters left over for shorter trace storage). If there are enough free directory slots and clusters the current trace data may be saved. If not enough space is free, the current trace data will be lost. It is therefore important note the amount space available before beginning a test run.

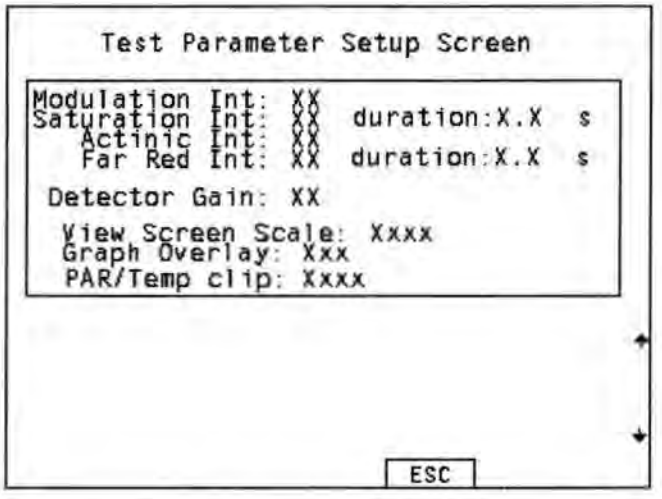
Delete
Allows the erasure of the last stored sample point

Trace save
Saves the current trace data assuming enough space exists.

ESC
Returns control to the main test screen.

Setting up a test

To set the parameters of a test, press the **Test Setup** key



Typical Test Setup Screen

The system test settings are shown and changed on this screen. The top box is common to all four test modes, any additional test parameters will be displayed in a

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box under the common one. The settings shown in this screen may be edited at any time in test modes 1, 2 and 4 and when not running a test in test mode 3. To skip between settings use the up/down arrows (to the right side of the screen) and the selection wheel to change the value. **ESC** will return you to the main test screen.

Modulation Int: ###

Modulation intensity - sets the drive level for the detection beam (note: the apparent beam intensity also depends on the current detection frequency). The setting of this value is dependent upon the size, distance from, and nature of your sample. The optimum setting is the highest possible before variable fluorescence is induced, which will cause a noticeable drop in measured Fv/Fm values. The effect of the amplitude can be seen in test mode 3 as a slow rise in Ft caused by the beam. The factory setting is 40 for an approximate value of 0.1uE and 0cm from the end of the fiber. This has shown to be a good starting value for most tests. When setting this you should also be aware of the current detector gain setting as the combination of the two should set Ft at approximately 150-250 counts. This will usually give the best results, with sufficient headroom for the maximal fluorescence peak. The range of this control is 0 to 255 counts in steps of 5. The setting is linear.

Saturation Int: ### Duration: #.# s

Saturation intensity - The saturation source intensity is controlled with this setting, allowing for experimentation with different pulse amplitudes to determine the saturation point. The setting is in steps of 5 from 0 (off) to 255 (~10kuE) and is linear.

Duration - The length of the saturation pulse. Set to ensure maximal fluorescence is reached and to prevent over-driving the photosystem. This may be verified by observing the trace shape in test modes 1 and 2. The plateau should be relatively flat. A climbing plateau may indicate insufficient saturation pulse duration, or the saturation intensity is too low, while a falling slope would indicate the reverse. The range of this setting is from 0.4 to 3.0 seconds in steps of 0.1 seconds.

Actinic Int ###

Actinic Intensity - This is set for desired actinic illumination intensity. The setting is from 0 (off) to 255 (~450uE) in steps of 5. This setting is only used in test modes 3 and 4.

Far-Red Int ### Duration ##.# s

Far-Red intensity - The far red (735nm) illumination intensity is adjusted here. The setting is from 0 (off) to 255 (~15mW per sr) in steps of 5.

Duration - The duration is interpreted differently depending on the test mode. In test modes 1, 2 and 4, it is used as the pre-run illumination time. In test mode 3 it is the post illumination time for Fod measurements. If this value is set to 0, the

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operation of the far-red source is turned over to manual control (see specific test type) and may be turned on and left on during all phases of a test. The range of this setting is from 0.0 to 10.0 seconds in steps of 0.5 seconds.

Detector Gain ##

Sets the amplification of the raw detected fluorescence signal. It should be set as low as possible while still reaching recommended Ft value (see Modulation Int) to give best signal-to-noise performance. The range of this setting is 0 to 255 in steps of 5. The total span amounts to a 100 fold gain difference.

View Screen Scale:

The trace view window in test modes 1, 2, and 4 can auto-scale to best fit gathered data. If several measurements are to be overlaid it can be set at a fixed scale of a-d tics / 1 graphic pixel. Should a trace's height exceed a fixed scale setting, the auto-scale will override to prevent "clipping". This setting has 3 options, "Auto Fit", "Fixed 2", "Fixed 5" and "Fixed 10".

Graph Overlay

This switch enables the overlay feature. Simply put, it turns off the auto-clear screen between measurements and lets multiple traces fall over one-another. The screen can be cleared using the clear key on the main test screen. This setting is only used in test modes 1, 2, and 4.

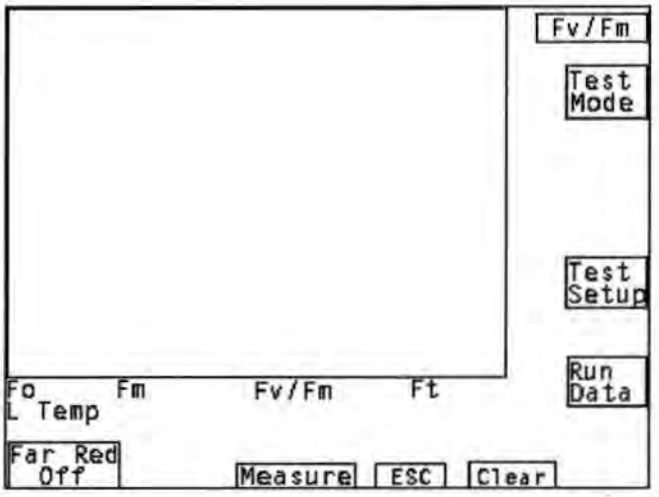
PAR/Temp clip

If you have the optional PAR / temperature clip (OS-OBCP) connected, select "Present" to activate it. If the optional clip is not attached, or you do not wish to use it, select "Not Present" to de-activate it.

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Fv/Fm

The first test type is optimized for 'dark adapted' (Fv/Fm) measurements. In this mode you will see all test parameters and data logging setup for this type of measurement. The basic test consists of monitoring the samples minimal fluorescence (Fo) for 0.2 sec (20 sample points). Then, in close order, switching the measuring system to take 1000 pts/sec and activating the saturation lamp. A period of Far red illumination will precede the gathering of Fo data (if selected to do so). The resultant data is then processed to find Fo, Fm, and Fv/Fm. Fluorescence is graphed on the main test screen, and numeric data is displayed. If the optional clip (OS-OBCP) is installed and activated, the leaf temperature will be taken just before the saturation pulse.



Fv/Fm test screen

There are several run-time test options available under this screen.

Far Red (On / Off / Auto)
Press this key to change the status of the far red source

"On" indicates that the far red source is currently on. It will remain on until **Far Red** is pressed again

"Auto" will appear if, under the "Test Setup" screen, "Duration" for the far red source was set to a value greater than zero. When auto is selected, the far red source will be turned on automatically for the specified amount of time prior to the "Fo" determination.

"Off" indicates that the far red source is off.

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Log
Available at run time if "Mode" (in the "Run Data") screen was set to "Manual". Displayed only when there is un-saved data available to be saved. Pressing this key will add this data to the current file group and increment the current sample number. **Log** will disappear after the current data has been saved.

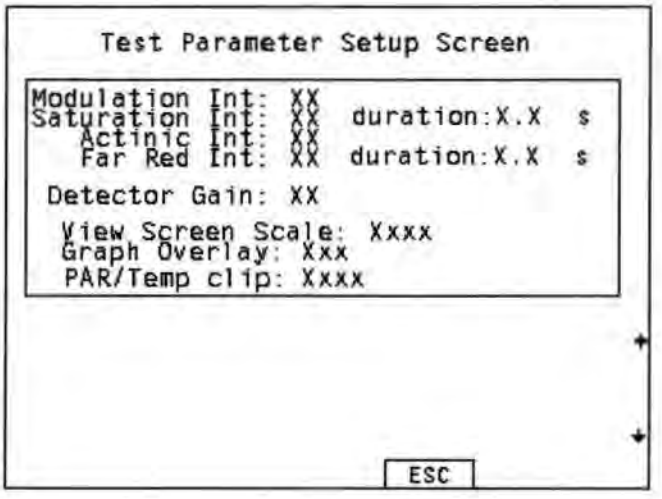
Measure
If "Ft" is greater than 50, pressing this will initiate a test run. Same as pressing the remote trigger switch.

ESC
Returns control to the "Main Menu"

Clear
Pressing this key will clear the graphical display area of any trace data. It will not change the displayed numerical data.

Running a test example
A typical test run may be setup as follows:

The "Test Setup" screen is accessed and the following settings are chosen:



Test Setup screen (Fv/Fm)

Modulation Int: 40
The sample is in close proximity and this gives an acceptable 0.1uE illumination level.

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Saturation Int: 190 Duration : 0.8 s
The sample requires reasonable amount of light to reach PS saturation. The duration is set to a starting value.

Actinic Int: XXX
Not used.

Far-Red Int XXX Duration: X.X s
Not used.

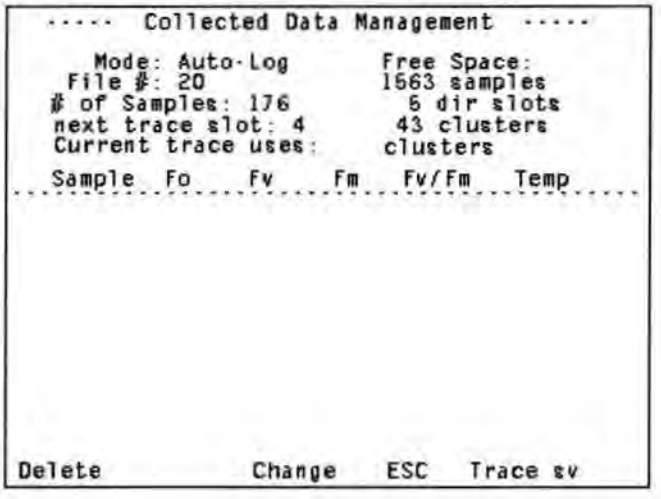
Detector Gain: 80
Set to give a minimal Ft of about 210.

View Screen Scale: Auto-Fit

Graph Overlay: Off

PAR/Temp clip: Not Present

With these parameters set, go to the "Run Data" screen by pressing **ESC** and then **Run Data**. If a parameter needs to be changed in this screen, first press **Change**.



Fv/Fm Run Data Screen

Mode: Auto-Log

File #: 1
Or any other empty file.

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If **Change** was pressed, pressing **ESC** will return control to the screen. Press the **ESC** key to exit the "Run Data" screen and return to the main test screen.

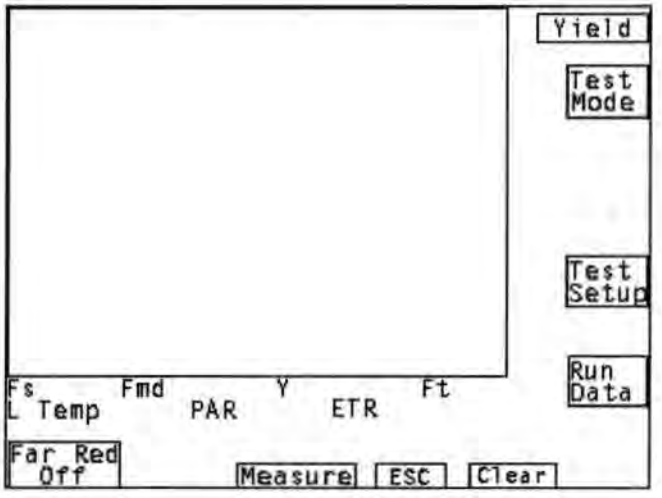
A set of 'dark adaption' clips is provided with each system. They are small, and made from a lightweight plastic. They also feature a convenient sliding shutter to prevent introduction of light during insertion of the fiber probe. Any other means of dark adaption may also be used. The time and degree of darkness required will vary with the type, and previous exposure conditions of the sample.

Having allowed the sample to dark adapt, place the probe in the clip (if used) and slide the shutter back. The Ft reading should jump up. You may press **Measure** or the trigger switch (on the fiber) at any time to start the test. An audible beep will signal the start of the test (if "Tone:" was set to "On"; see "System Setup") and another will sound to mark the end. The measured parameters will appear below the graph area and the fluorescence data will be plotted in the graphic window. Observing the shape of the pulse response is helpful in determining if sufficient saturation intensity and pulse width was used. The typical sample should have a fast rising trend at the start of the pulse, leveling out with a relatively flat plateau for the remainder of the pulse. A possible sign of insufficient excitation time or intensity is a curving initial rise and a plateau with a continuously rising slope. A possible sign of excessive drive (or pulse width) is a steady decrease in the plateau. If you want to save the fluorescence data from this run you may do so by entering the "Run Data" screen and pressing **Trace SV**. Once the range of intensity and duration settings are found satisfactory, the rapid screening of a large group of samples is a 'one key' operation with the "Auto-Log" feature set to "On".

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Yield

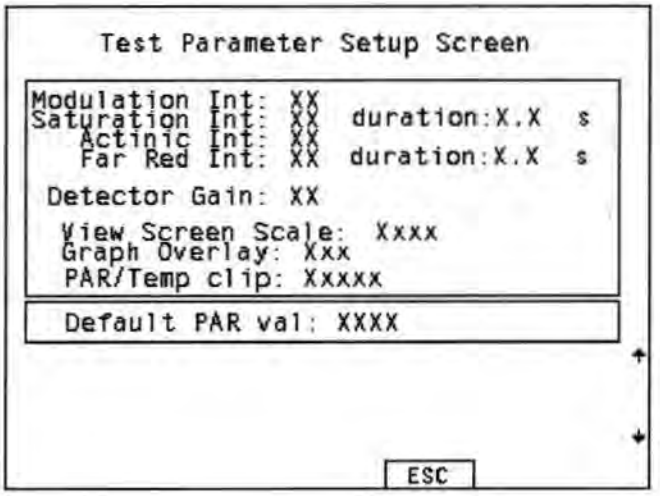
The next type (type 2) is optimized for "light adapted" yield (Y) of photosynthetic efficiency measurements. In this test mode it is assumed that the sample is being illuminated by an external source (for the use of internal sources see "Kinetic" mode). As with the "Fv/Fm" test, this basic test consists of possible far red pre illumination, followed by monitoring the samples minimal fluorescence (Fo) for .2 sec (20 sample points). Then, in close order, switching the measuring system to take 1000 pt/sec and activating the saturation lamp. The resultant data is then processed to find Fs and Fms, graphed on the main screen, and numerical data is displayed. The parameters Y and ETR are calculated.



Yield test screen

The bottom row keys function identically to the ones described in "Fv/Fm" (test mode 1). The calculation of ETR requires knowledge of the intensity of the illuminating light. Two possible options exist for getting this data, the first is to use the optional open body clip with integral PAR/Leaf Temp sensor (OS-OBCP). When this is present "PAR" and temperature data will automatically be gathered along with "Fs" just before the saturation pulse. The second option is to use an external sensor and input the reading in the "Test Setup" screen under "Default PAR val:".

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Yield Test Setup Screen

The first box contains all of the same settings as found under "Fv/Fm", "Test Setup" with the addition of:

Default PAR val: XXXX
With "PAR/Temp clip:" set to "Not Present", this number sets the PAR reading of post saturation pulse calculations. It has a range from 0 to 2000 uE in steps of 1 uE. This value is not permanent and will change when "PAR/Temp clip:" is set to "Present".

With these parameters set, go to the main test screen by pressing **ESC**.

Taking a measurement example

A typical test run may be setup as follows:

The "Test Setup" screen is accessed and the following settings are chosen:

Modulation Int: 200
Assuming the sample is in the 60^0 clip approximately 1 cm distant, this gives an acceptable 0.1uE illumination level.

Saturation Int: 230 Duration 0.8 s
The sample is under a high natural light.

Actinic Int: XXX
Not used.

Far-Red Int: XXX Duration: XX s
Not used.

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Detector Gain: 80
Set to give a minimal Ft of about 210.

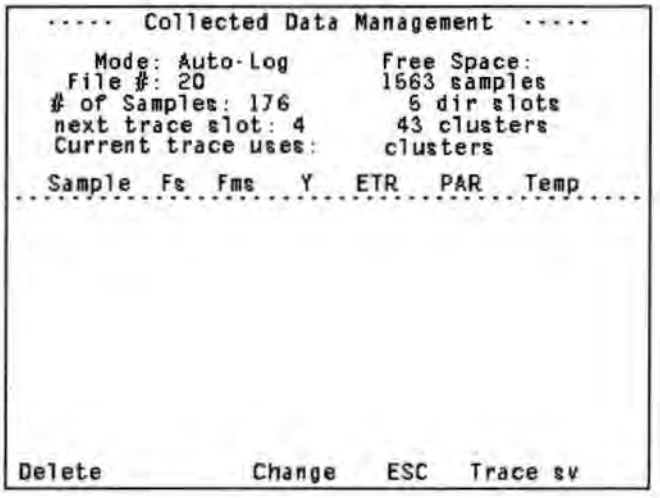
View Screen Scale: Auto-Fit

Graph Overlay: Off

PAR/Temp clip: Not Present
For this example, PAR is measured with an independent meter and found to be about 1100 uE.

Default PAR val: 1100
(see above)

With these parameters set, go to the "Run Data" screen by pressing **ESC** and then **Run Data**. If a parameter needs to be changed in this screen, first press **Change**.



Yield Run Data Screen

Mode: Auto-Log

File #: 1
Or any other empty file.

If **Change** was pressed, pressing **ESC** will return control to the screen. Press **ESC** to exit the "Run Data" screen and return to the main test screen.

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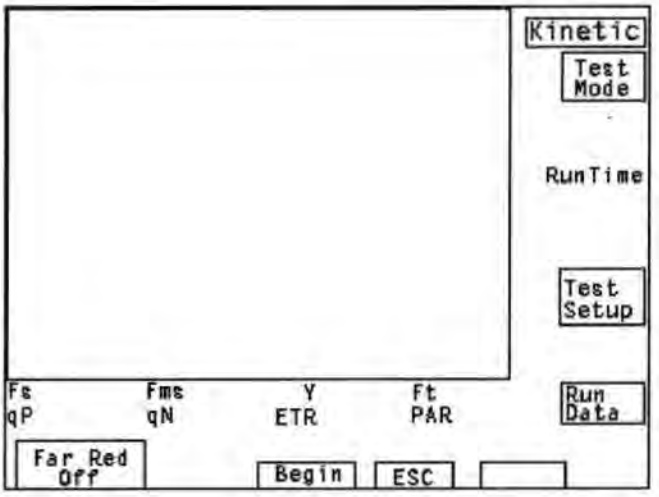
This test is often performed under outdoor or other external lighting conditions. A special leaf clip is provided to hold the fiber at a fixed angle with respect to the sample surface. This allows the fiber to look at an area of the sample with minimum blocking of external light. An optional version of this clip has a built in temperature and PAR sensor (OS-OBCP). When the fiber is in proximity to the sample, the "Ft" reading should jump up. Pressing **Measure** (or the trigger switch on the fiber) at any time to start the test. An audible beep will signal the start of the test (if "Tone:" was set to "On"; see "System Setup") and another will mark the end. The measured parameters will appear below the graph area and the fluorescence data will be plotted in the graphic window.

Observing the shape of the pulse response is helpful in determining if sufficient saturation intensity and pulse width was used. The typical sample should have a fast rising trend at the start of the pulse, leveling out with a relatively flat plateau for the remainder of the pulse. A possible sign of insufficient excitation time or intensity is a curving initial rise and a plateau with a continuously rising slope. A possible sign of excessive drive (or pulse width) is a steady decrease in the plateau. If you want to save the "raw data" from this run you may do so by entering the "Run Data" screen and pressing **Trace SV**. Once the range of intensity and duration settings is found satisfactory, the rapid screening of a large group of samples is a 'one key' operation with the "Auto-Log" feature set to "On".

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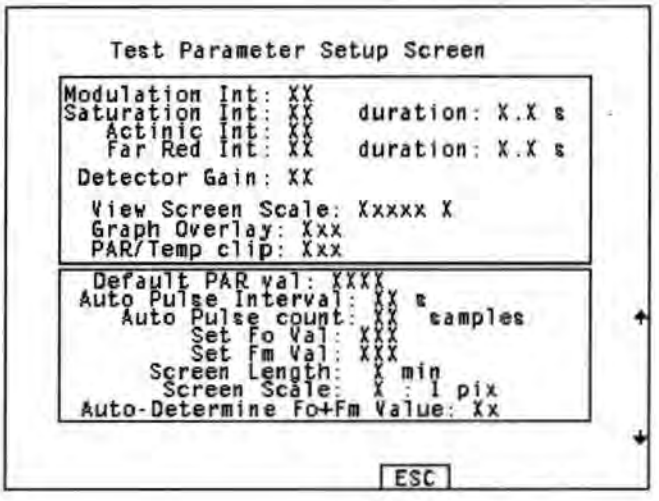
Kinetic

The third and by far the most versatile of the test modes is the Kinetics test. This test is setup to run either automatically, operating sources and firing saturation pulses at preset intervals, or, manually with system control up to the user.



Kinetic test screen

To set up the Kinetic test's parameters, press **Test Setup**.



Test Setup for Kinetic test

The first box contains all of the same settings found under "Fv/Fm" , "Test Setup" with the addition of the following (in the second box):

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Note: Unless otherwise stated, the settings function identically in both manual and auto modes.

Default PAR val: XXXX
With "PAR/Temp clip:" set to "Not Present", it sets the PAR reading of post saturation pulse calculations. It has a range from 0 to 2000 uE in steps of 1 uE. This value is not permanent and will change when "PAR/Temp clip:" is set to "Present". If the clip is present, the OS-500 will automatically enter in the correct PAR value for every test.

Auto Pulse Interval: XX s
Sets the time between saturation pulses in "Auto-Run" mode. It's range is 5 to 600 seconds in steps of 5 sec.

Auto Pulse count: XX samples
Sets the number of pulses to be triggered in an "Auto-Run" test. It's range is 0 to 200 pulses. A setting of "0" places the test in "Manual" mode.

Set Fo Val: XXX & Set Fm Val: XXX
The quenching parameters require some "dark adapted" data. This data may be measured at the beginning of a test or the values may be manually entered here. These values are not permanent, but reflect the last dark adapted test run whether it was done in test modes 1, 3, or 4. These settings will remain until a "dark adapted" test is made.

Screen Length: XX min
This setting applies only to the "manual" mode of operation. It sets the X axis (time) for the graph window. When the set value is reached, the screen is cleared and re-starts from the left. No test data is lost when this occurs.

Screen Scale: X:X pix
This will determine how many a-d tics are in each screen pixel (Y-axis) This is not necessary in the other test modes because the test data is plotted after it has been gathered and the maximal value is known for setting the graph scale. There are 200 pixels on the Y-axis. The maximum a-d tic value is 2000. The "Screen Scale" value has the range from 2 a-d tics/pixel to 10 a-d tics/pixel.

Auto-Determine Fo+Fm Value: XXX
This switch tells the "auto-run" mode to make a dark adapted measurement first then, after allowing "Ft" to settle to within ~1.2 of "Fo", begin the kinetic pulse run. When in "manual" mode, this determines the first function of the fifth key (in the bottom row, from the left). When set to "On", key 5 will first allow the user to make a "dark adapted" measurement, then switch to the "Fod" determination feature. When set to "Off", the key will be for "Fod" measurements only.

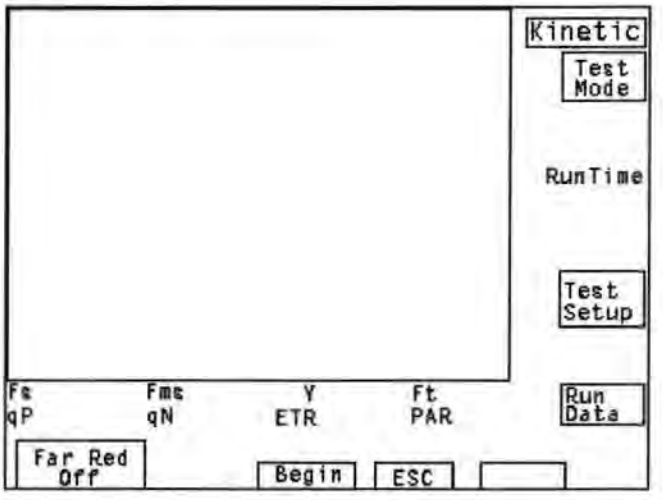
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Before running a test verify that you have chosen all the desired options from the above list. In other tests data logging may be switched off, in this mode it is always active regardless of the "Mode" setting displayed in the "Run Data" screen. Be sure to select a new file (from the "Run Data" screen - see Fv/Fm test) if you wish this data to be kept separate from previous runs.

The maximum length of a test run (in either mode) is 45 min due to memory limits.

With these parameters set, go to the main test screen by pressing **ESC**.

Taking a measurement example



Kinetic Main Test screen, manual mode

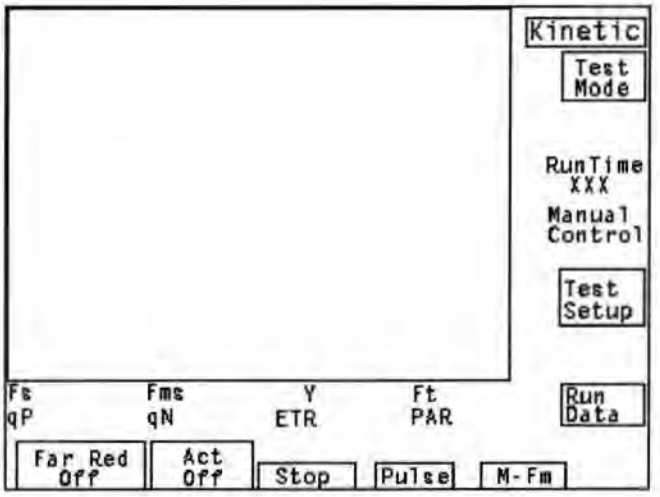
Manual mode operation

Manual mode is designed to allow the user to completely control the sequence of the test run. It is activated by setting the "Auto Pulse count" to "0" in the kinetic "Test Setup" screen.

Begin

This key starts the manual run session. After **Begin**, the key legends will change.

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Kinetic main test screen, after pressing begin

Far Red & Act
These keys control the far-red and actinic sources. They are strictly on/off switches. Their intensity cannot be changed from this screen.

Stop
Allows the user to stop the test. When pressed, the key will change to **Begin**.

Pulse
Pressing this key, or the trigger switch (on the fiber) fires a saturation pulse and uses the information to do standard yield and quenching calculations.

M-Fm or M-Fod
"M-Fm" - If the "Auto-Determine Fo+Fm Value:" switch (in the "Test Setup" screen) is set to "On", pressing "M-Fm" will measure the "dark adapted" parameters. This will fire a saturation pulse and uses the data to find Fo and Fm values (which will be used for further quenching calculations). The gathered values will be displayed above the runtime counter, and in the "Run Data" screen. The data is also saved as special test data types under the current file number.

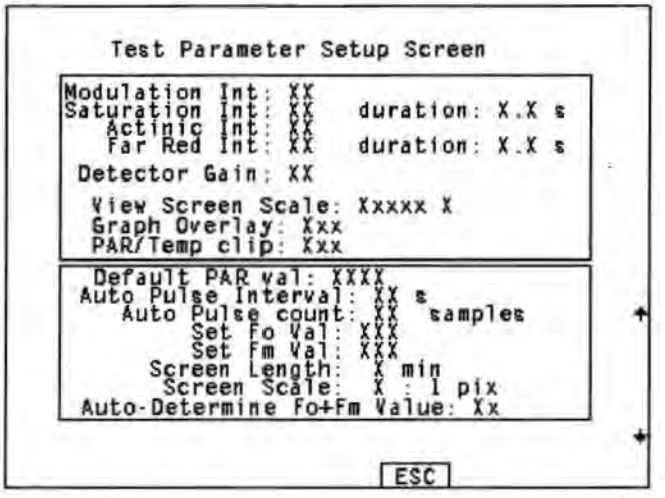
"M-Fod" - After a "M-Fm" operation, or, if "Auto-Determine Fo+Fm Value:" is set to "Off", the key will change to "M-Fod". Pressing this will take the post Far-Red exposure of "Ft" ("Fod") at the end of a run. As with "Fo" and "Fm", this data is displayed and stored. Back calculations of quenching parameters using this value (instead of Fo) may be done using the "OS-DATA" Windows ™ software provided with the system.

The three keys to the right of the display are inactive during a run.

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Running a test Example of a manual run:

The "Test Setup" screen is accessed and the following chosen:



Test setup screen kinetic test

Modulation Int: 200

Assuming the sample is in the 60^0 clip approximately 1 cm distant, this gives an acceptable 0.1uE illumination level.

Saturation Int: 190 Duration: 0.8 s

The sample requires reasonable amount of light to reach PS saturation. This duration at this saturation intensity was checked on this type of sample previously in test mode 1 (Fv/Fm) and the curve and values looked proper.

Actinic Int: 125

Test level of ~ 225 uE desired.

Far-Red Int: 50 Duration XXX

Chosen to be sufficient to produce re-oxidation without overexcitation. Duration is not used for this test.

Detector Gain: 80

Should give a minimal Ft of ~ 210.

View Screen Scale: XXXX

Not used.

Graph Overlay: XXXX

Not used.

PAR/Temp Clip: Not Present

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Default PAR val: 225
Calculated from the actinic intensity

Auto Pulse Interval: XXXX
Not used.

Auto Pulse count: 0
Used to select "manual" operation mode.

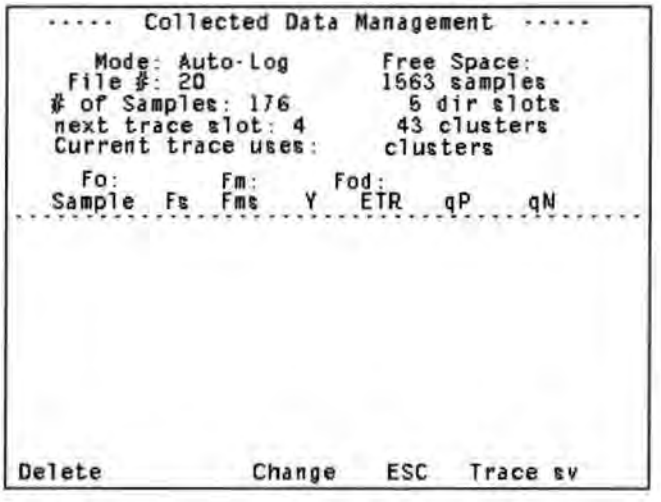
Set Fo Val: XX & Set Fm Val: XX
These values will be determining during the test.

Screen Length: 4 min
Estimate of run time. No data will be lost if this value is too long or too short, it is only used for the graphical view window.

Screen Scale: 6 : 1 pix
The expected average size of the variable fluorescence peak.

Auto-Determine Fo+Fm Value: On
Set to measure "Fo" and "Fm" during the test.

With these parameters set, go to the "Run Data" screen by pressing **ESC** and then **Run Data**. If a parameter needs to be changed, first press **Change**.



Run data for manual mode kinetic test

Mode: XXX
Not used.

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File # 2
or any empty file

If **Change** was pressed, pressing **ESC** will return control to the screen. Press **ESC** to exit the "Run Data" screen and return to the main test screen.

The fiber and clip are placed on the sample, and "Ft" is observed to be about 200. To start the test, press **Begin** (or the trigger switch on the fiber). A trace will begin to move across the graph window displaying the current value of "Ft". The "Runtime" counter will appear in the upper right of the screen.

To find "Fo" and "Fm", press **M-Fm**. A pulse will be fired and the values for "Fo" and "Fm" will appear above the "Runtime" counter. The key will change to **M-Fod** after this operation is completed. The saturation pulse will appear in the graph window.

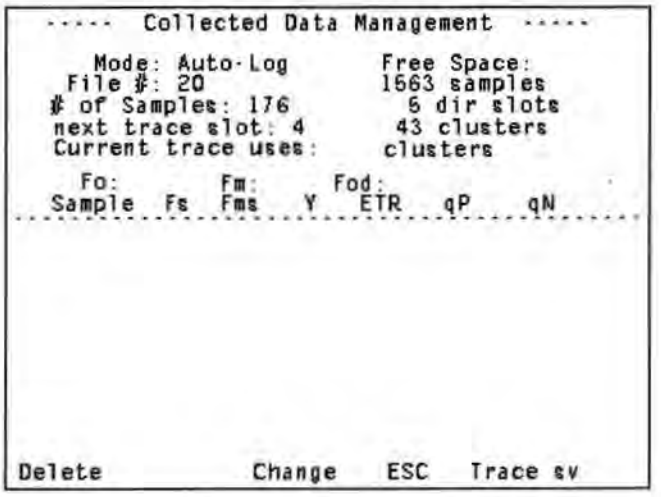
Observe the trace until "Ft" settles to approximately the value of "Fo". When this has occurred, press **Act** (to turn on the actinic source), and watch "Ft" rise. After "Ft" settles, saturation pulses can be fired by pressing **Pulse**. For this test, fire 5 pulses at the 10, 20, 30, 40, and 50 second intervals. With each pulse the relevant calculated data appears at the bottom of the screen.

Switch off the actinic source (by pressing **Act**) and turn on the Far-Red source (by pressing **Far Red**). Observe "Ft" drop and settle. Once it settles, turn off the Far-Red source (by pressing **Far Red**). To obtain the "Fod" parameter, press **M-Fod**. The value of "Fod" will be displayed directly under the values of "Fm" and "Fo" (upper right of the screen).

Press **Stop** to end the test run.

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To view the data, press **Run Data**.



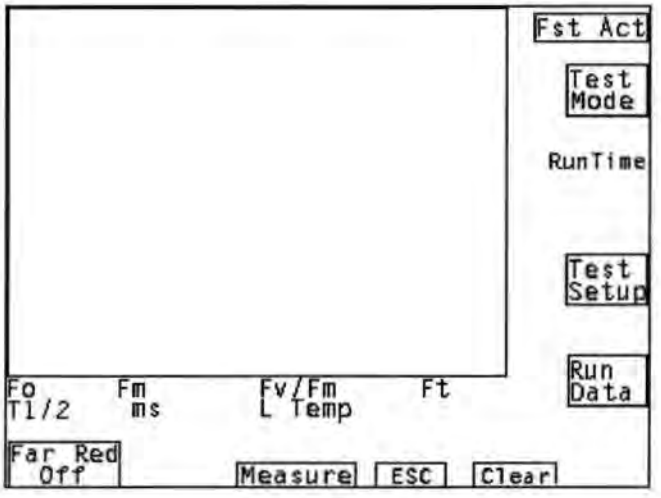
Kinetic run-data screen showing data

The saturation points will be sequentially listed. Note that the first listing is sample number 2. This is because sample number 1 is the "Fo" and "Fm" data. The last sample (sample number 7 in this example) will be the "Fod" measurement. These special case measurements are displayed above the normal data area. From this screen you have the option to save the "Ft" points gathered during the run (if sufficient memory exists). The data is currently in the test run memory and will be lost if another run is started, you exit to the "Main Menu", or switch test types without pressing **Trace sv**.

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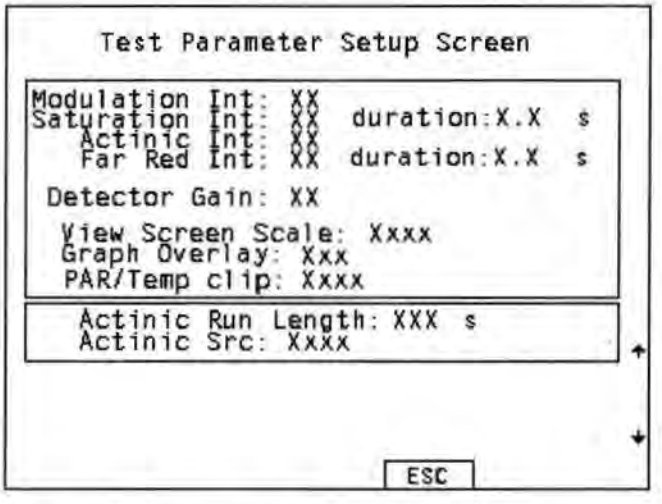
Fast Actinic (Continuous excitation fluorescence measurements)

This test mode is intended to measure the typical relaxation (Kautsky) curve. The basic test consists of monitoring the change in fluorescence during exposure of the sample to one of the built in sources (or, if preferred, an external source). The data gathered is similar to that of test mode 1 ("dark adapted"). Additional parameters have been added to further categorize transient features. The internal light source may be either the LEDs, or the saturation lamp, depending on the intensity and spectral content desired. The power consumption of the lamp is exponentially greater than that of the LEDs. The far red source is used as described in test modes 1 and 2.



Fast Actinic test screen

To set up the Fact Actinic test's parameters, press **Test Setup.**



Test Setup for Fast Actinic test

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This contains all of the same parameters found under Fv/Fm. With the addition of:

Actinic Run Length: XXX s
Sets the length of the test run and can be from 5 to 900 seconds in steps of 5 seconds.

Actinic Src: XXXX
Allows the selection of either "LEDs", or "Lamp" as the excitation source.

With these parameters set, go to the main test screen by pressing **ESC**.

Taking a measurement example

A typical test run may be set up as follows:

The "Test Setup" screen is accessed and the following setting are chosen

Modulation Int: 50
Assuming the dark adaption clip is used, this gives an acceptable 0.1uE illumination level.

Saturation Int: XXX Duration: X.X s
Not used.

Actinic Int: 125
Test level of ~ 225 uE desired.

Far-Red Int: XX Duration XXX
Not used.

Detector Gain: 80
Should give a minimal Ft of ~ 210.

View Screen Scale: Auto-Fit

Graph Overlay: Off

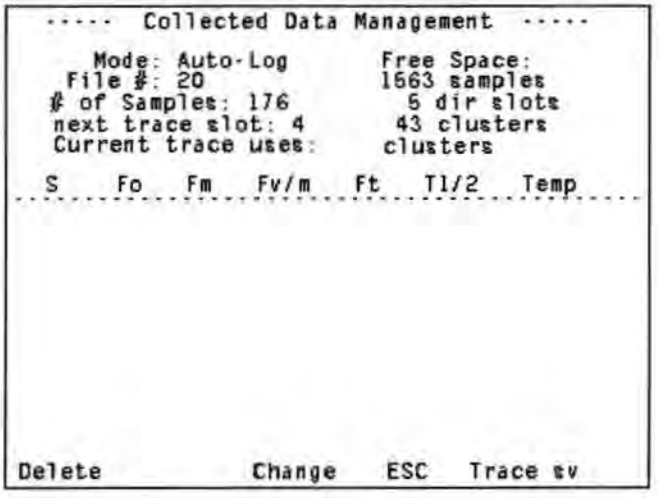
PAR/Temp Clip: Not Present

Actinic Run Length: 60 sec
Sets the desired run time.

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Actinic Src: LEDs
Sets the LEDs to be used as the acidic source.

With these parameters set, go to the "Run Data" screen by pressing **ESC** and then **Run Data**. If a parameter needs to be changed, first press **Change**.



Run data for fast actinic test

Mode: Auto-Log

File # 6
or any empty file

If **Change** was pressed, pressing **ESC** will return control to the screen. Press **ESC** to exit the "Run Data" screen and return to the main test screen.

This test is often run on a dark adapted sample, so the clip described in test mode 1 may be used. The test is started by pressing **Measure** (or the trigger switch on the fiber). After the first two seconds (fast stage), the "runtime" counter will appear in the upper right of the screen, and **Measure** changes to **Abort**. Pressing this key will stop the test.

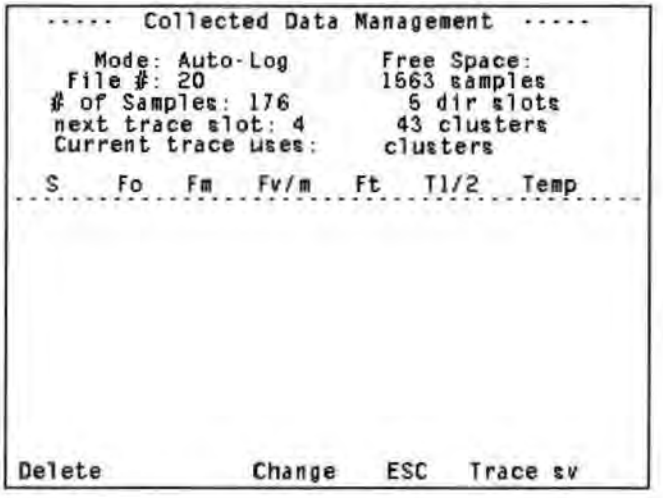
A typical series of events for this test are:

- The minimal fluorescence is sampled for 0.2 seconds at a rate of 100 pts/sec.
- The chosen actinic source is switched on and the sampling rate speeds up to 1000 pts/sec for 2 seconds. This will capture the rapidly changing initial reaction to illumination.

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- The data rate switches to 10 pts/sec for the remainder of the up to 900 seconds of test run time.
- When the test stops, automatically or when **Abort** is pressed, "Ft" is saved and the excitation source is switched off.
- The numerical and trace data is displayed.

To re-view several measurements, press **Run Data**



Run Data screen for Fast Actinic

The data points will be sequentially listed. The trace data is currently in the test run memory and will be lost if another run is started or you exit to the "Main Menu", or switch test types without pressing **Trace sv**.

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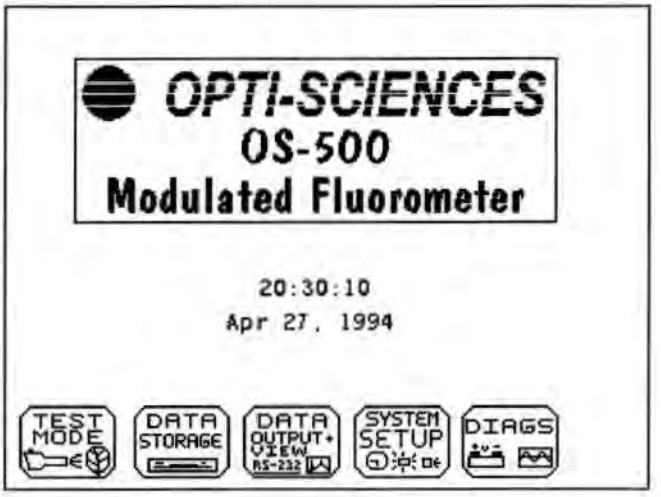
© 1995 BY OPTI-SCIENCES, INC.

CHAPTER 4 • OS-500 SYSTEM OPERATION

INTRODUCTION

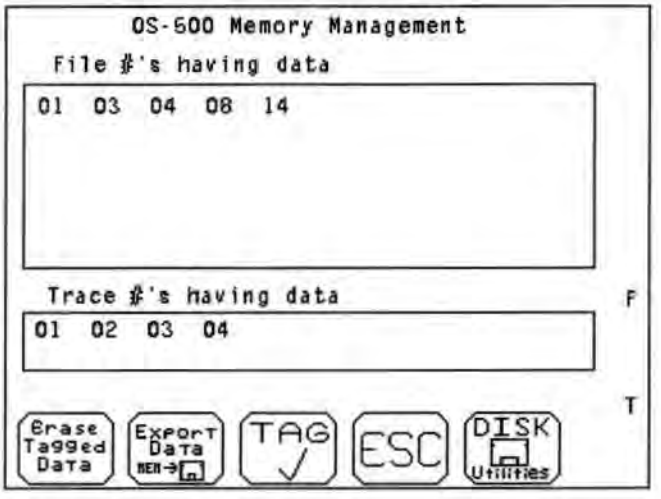
System operation options deal primarily with gathered data manipulation and viewing. A brief menu is also provided for setting system wide features such as time and date, and quick diagnostics of system hardware.

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Main Menu

Data Storage



Data Storage screen

Upon entering this screen a list of every file and trace number containing data is displayed. By tagging one or more files or traces the data can be erased or transferred to floppy disk. When the **T** (select trace box) or **F** (select file box) option is selected, a cursor will appear to the left of the first entry of the selected group. Pressing **Tag** will place a check mark next to the item. This signifies selection for a later operation. Turning the data wheel will move the cursor around the different entries. Any number of entries may be tagged at one time for subsequent action. This is the same mode of selection used in the "Data Output" screen. These

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screens are linked so that tagged items in this screen will remain tagged in the other if, upon pressing **ESC** to the main menu, "Data Output" screen is next entered. Entering another menu ("Diag", "Setup" or "Test") will reset the tags.

ESC
This will return you to the main menu.

F
Select file group for tagging

T
Select trace group for tagging

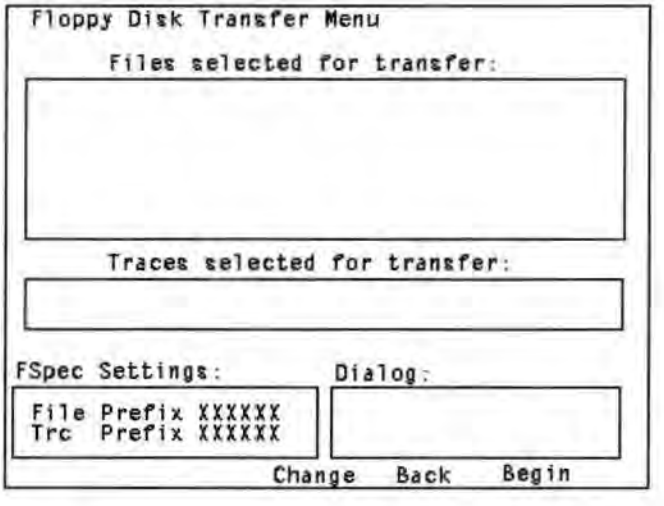
Tag
Select a file / trace number for action (view, output, etc.)

Erasing Data

The **Erase Tagged Data** key is used to clear out old files and traces from memory. Pressing this with tagged data will erase that data. A prompt will appear warning of the erasure. If the data tagged is correct, pressing **erase** again will delete the selected items from memory. Any other key press will abort the erasure.

Export Data to Disk

The **Export Data** key activates the disk transfer screen.



Export data to disk screen

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All selected items are shown in the top two main boxes. As the disk transfers are DOS compatible, each selected item is given a unique file name. It consists of three parts

1. A 6 character prefix is used as the group identifier. The prefix is automatically generated from date / time information (unless it is edited by the user. See below) in the format month:day:hour.
2. Two characters represent the actual file (or trace) number.
3. The extension is type specific ".dat" for file data and ".plt" for trace data.

Example:

A file transferred on April 4 at 2:20 PM would have the following file name

data number 2:

04041402.dat

trace number 2 would be:

04041402.plt

ESC
Returns control to the "Export Data to Disk" screen

Change
Edits the file / trace prefix selections. Upon pressing **Change** a cursor will appear on the proposed file name, and the keys **Enter**, ←, and → will appear. These are for the text editor feature. Turning the selection wheel will select a character, while the arrows select the field location. Pressing **Enter** will accept the currently displayed value and step to the trace prefix entry. Pressing **Enter** again will exit the edit mode.

Begin
After ensuring that there is a correctly formatted disk in the drive, pressing this key will initiate the disk transfer.

A transfer has three stages that are displayed in the dialog box:

1. "Loading Disk Information"
2. "Transferring Data". As each item is transferred a check is placed next to that item.

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3. "Setting Disk Tables"

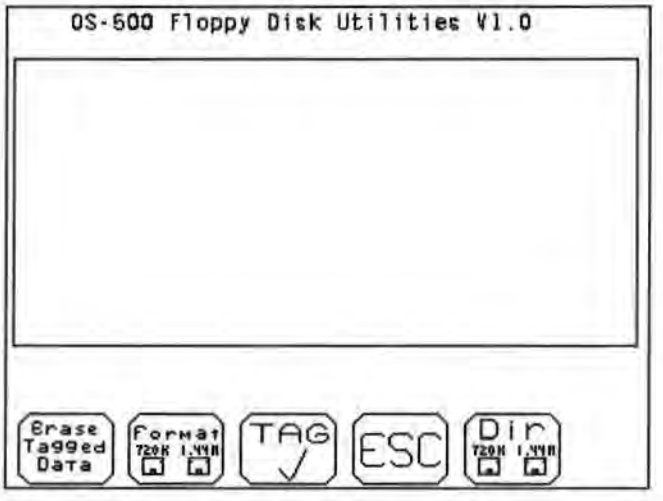
When completed, "Transfer Complete" is displayed.

Remove the disk after the green light (on the disk drive) goes off.

Should the disk already contain a file of the chosen name the prompt: "! File Exists Overwrite? !", a **Yes** and **No** key will appear. If **Yes** is selected, the file is over written. If **No** is chosen, the item that had the matching name is not saved (no check is placed next to it's name). This item must be tagged separately and renamed on another try.

Disk Utilities

Several important utilities have been provided for management of disk storage without having to rely on an external computer for disk setup.



Disk Utilities screen

Erasing Tagged Data

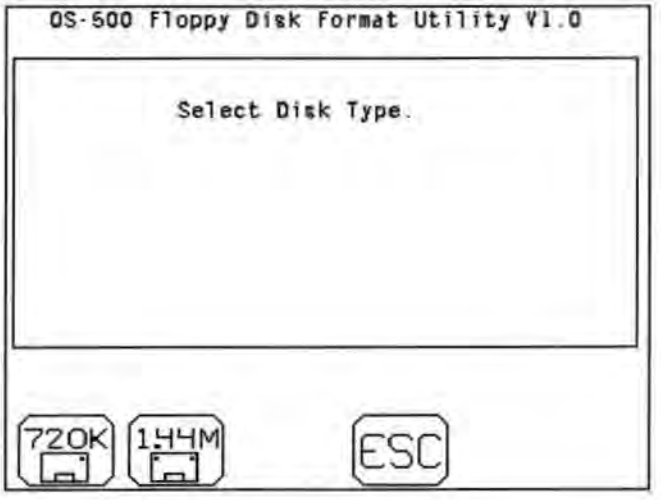
This is similar to the previously mentioned version, but in this case a directory of all the erasable files (no system, hidden, sub-directory or write protected files) are displayed in the center box. The selection wheel is used to move the cursor through the entries and the pressing the **Tag** key will place a check next to the files to erase. Once all the unwanted files have been selected, press **Erase Tagged Data**. A prompt will be displayed asking for confirmation of the operation. Pressing **Erase** again will start the process while any other key will abort it. Upon

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completion of an erasure a **Dir** must be done if you wish to see the results (though this is not required).

Formatting a floppy

To format a floppy disk, press the **Format** key.



Format screen

This function works just like DOS, and will wipe out all data on an unsuspecting disk if used accidentally. The format process is simple, the disk to format is inserted into the drive and the proper selection key is pressed depending on the disk type. The choices are either Hi-Density (1.44Mb) or Low-Density (720Kb). The format routine displays a bargraph showing the progress of the operation. When completed you will be prompted for a volume name. One may be entered using the same method described for setting filenames.

Should the format routine encounter any damaged surface area on the disk, a message reporting this condition will be displayed above the Volume name prompt. While the format routine makes an effort to lock bad tracks out of general use a use-at-your-own-risk rule applies. If it is really necessary to use the disk it would be advisable to format it on a system running a recent version of MS-DOS (or equ.) as their bad track management schemes are more advanced. Another note of some importance to those who have Low-Density Floppy drives in their main computers; you must format your 720k disks on that machine. While the OS-500 will be able to reliably read and write to a 720k disk formatted with the OS-500 (or other computing device), it has a 1.44Mb drive and will produce an incompatible format. (this limitation applies generally to all systems having a 1.44Mb drive) This does not mean that 720K disk can not be formatted. The format will be fine as long as the destination machine also has a 1.44Mb drive.

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Directory

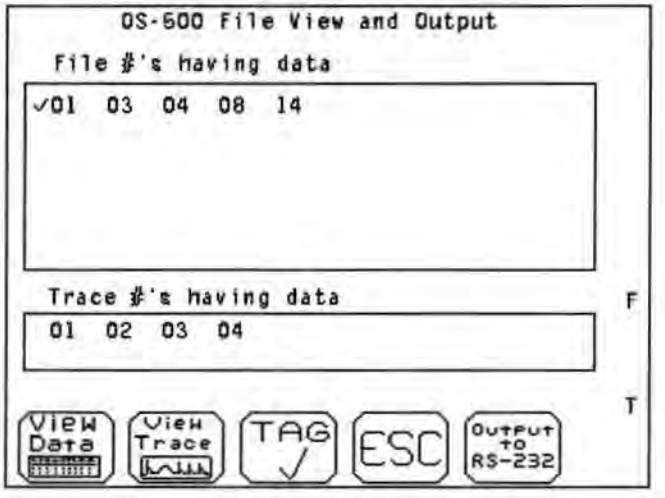
The **Dir** key is used to take a directory of a disk's contents. This feature simply displays a list (which may be scrolled through using the data wheel if more than 16 entries are present) of all files on a disk. Also displays the amount of free space available.

Disk errors

When a disk becomes full during a transfer the disk tables are closed saving only the items it was able to transfer before running out of space.

Call you're nearest authorized distributor for assistance for any other error.

Data Output and View



Data output and view screen

Upon entering this screen a list of every file and trace number containing data is displayed. By tagging one or more files or traces the data can be reviewed. Output via the RS-232 port is accessed from this screen. When the **T** (tag file) or **F** (tag trace) option is selected, the cursor will appear to the left of the first entry of the selected group. Pressing **Tag** will place a check mark next to the item, signifying selection for a later operation. Turning the selection wheel will move the cursor around the different available entries. Any number of entries may be tagged at a time for subsequent action. This same mode of selection is used in the "Data Storage" screen. These screens are linked together; tagged items in this screen will remain tagged in the other if, upon pressing **ESC** to the main menu, "Data Storage" screen is next selected. Entering another menu ("Diag", "Setup" or "Test") will reset the tags.

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ESC
This will return you to the main menu.

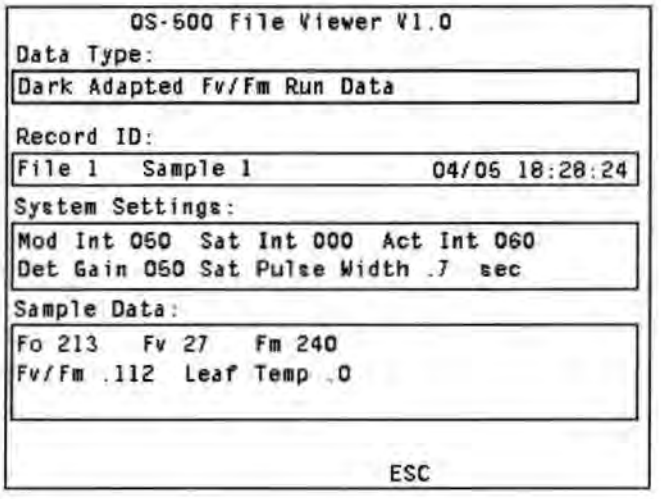
F
Select file group for tagging

T
Select trace group for tagging

Tag
Select a file / trace number for action (view, output, etc.)

View Data

The view data icon allows the user to scroll through data in tagged files.



View data screen

In the View data screen, recorded sample information for a given file is displayed. The selection wheel will step through each sample and on through each file, if multiple files are selected.

One data record is displayed at a time. The view screen is broken into four sections:

Data Type:
The record test type is indicated in this box. This corresponds to one of the four test modes available in the run test screen.

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Record ID:
The file and sample number plus its recording date are displayed here.

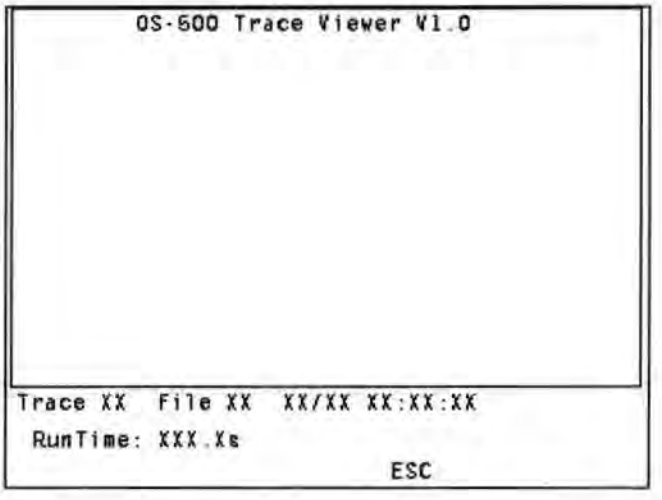
System Settings:
Displays several of the regularly altered system values as set at the time of the measurement.

Sample Data:
The actual data gathered in a test operation. The type of test will determine the exact contents of this area.

ESC
Returns control to the "Data Output and View" screen

View Trace

The view trace icon allows the user to scroll through tagged traces.



View Trace screen

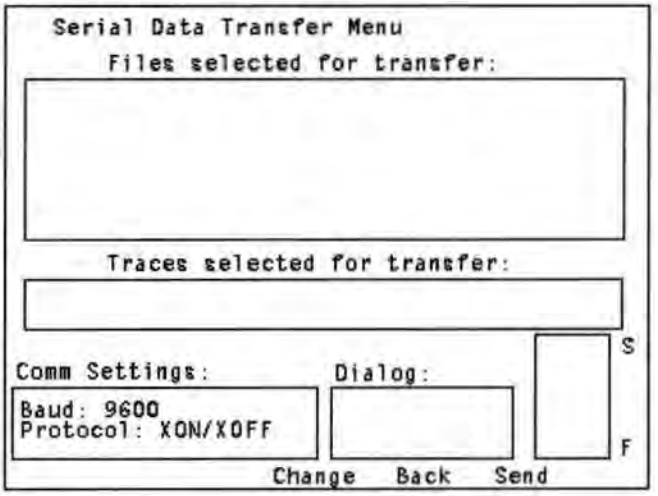
In the View trace screen, raw data is graphed in the view box. All necessary time corrections are made to the trace data. The trace is auto-scaled to best fit the available view area. Relevant accompanying data is displayed below the graphic box. The selection wheel will step through each trace if multiple traces are selected.

ESC
Returns control to the "Data Output and View" screen

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Output to RS-232

Activates the serial transfer routine for all tagged files and traces



Output to RS-232 screen

ESC (Back)
Returns control to the "Data Output and View" screen.

Change
Edits current port settings. The first press will edit the Baud rate. The next press will edit the protocol mode. And the final press exits the edit mode. A cursor will appear to the left of the selected field and the value may be changed with the selection wheel.

Send
Begins data transfer.

The output to serial port screen displays a list of the items selected for transfer. A status bar (in the lower right) will report transfer progress. Upon completion of each transfer, a check will appear next to the item sent.

When all transfers are completed the message "Transfer Complete" appears in the dialog box.

Should a handshake error occur, the message "Timeout" will appear in the dialog box. When this occurs, the keys below will appear:

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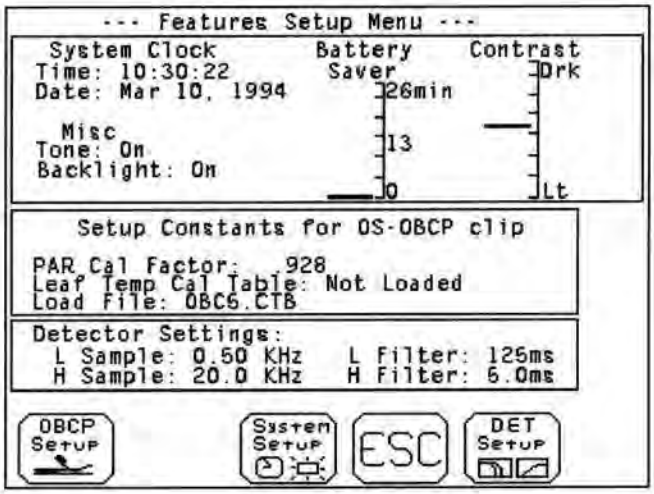
Abort
Stops sending data and returns control to the "Data Output and View" screen.

Retry
Attempts to re-start the transfer from where the error occurred.

Depending on the cause of the error, **Abort** is often the best choice.

System Setup

The system setup provides access to three main types of functions.



System Setup screen

1. General system setup.(clock, backlight, etc.)
2. Provisions for installation of the OBCP
3. The detection system's operation settings may be altered.

ESC
Returns control to the "Main Menu".

Setup Constants for OBCP clip

Each OBCP sensor clip will have a set of calibration constants that are entered here. (See Appendix B for detailed information.)

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General System Setup

This function is accessed by pressing **System Setup**.

In the case of time and date, the complete group field must be edited and stepped through of to accept the new value.

Setting the Time

Time - Hours
The hour time is set in a 24-hr format.

Time - minutes
The minute range is 0-59

The seconds will be set to 00 and started when the cursor is stepped to the date.

Setting the Date

Date - Month
The month range is 1 to 12.

Date - Day
The day range is 1-31.

Date - Year
The year range is 1994 to 2091 (by 2091 the new model will be out).

Miscellaneous settings

Tone
Switches the audible tone on or off

Backlight
Turns the LCD LED backlight on or off (the backlight consumes about 25% of the static power).

Battery Saver (bargraph)
Enables and selects the auto shut-off feature. Should the machine be idle for more than the set time (i.e. no key pressed or any test running, etc.) the system will shut off. The Range is from 0 (battery saver off) to 26 minutes.

Contrast (bargraph)
Changes the contrast of the display (LCD).

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Detector Settings

The OS-500 has several user-programmable settings for it's lock in detector. These settings are displayed in the "Detector Settings" box. Improper settings will not damage the system, but will seriously affect accuracy of the readings. Alter these settings with care!

Pressing the **Det Setup** key will allow the user to change these settings. Pressing **ESC** will save any changes and exit the edit mode.

L Sample
Sets the low sampling rate. Important to insure minimum excitation of photosystem. Default setting is 0.50 KHz

H Sample
Sets the detection rate during fast kinetics. Higher rates insures better fast transient capture. Default setting is 30.0 KHz

L filter
Sets the time constant for filtering the lower detecting rate. Default is 125ms

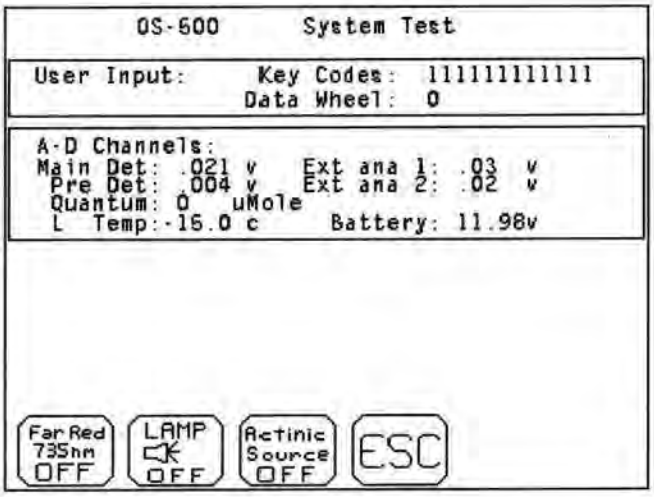
H Filter
Sets the time constant for filtering the higher sample rate. Default is 10ms

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Diagnostics

This screen is accessed by pressing the **Diags** key from the "Main Menu".

The diagnostics screen shows the status of all analog signal channels and will allow sources to be turned on and off to verify their operation.



Diagnostic screen

The top box has information on the operation of the keyboard and selection wheel. The lower box displays the a-d channel voltages and sensor inputs (if installed). The modulation source and gain are set to an average value so that deflection of the main detector can be seen next to "Main Det". The "Pre Det" value represents the offset introduced into the detector by extraneous in-band IR light. The detector can tolerate up to -9.8 Volts (with some signal degradation) before pre-amp saturation occurs. Whenever setting up external artificial actinic sources, it is wise to check this value and add heat filters to the source if the reading is high. Both the PAR sensor "Quantum" and leaf temperature "L Temp" values will be displayed if the optional OBCP clip is installed. The external ports are for future expansion features. Battery Voltage is also displayed. A value of 10.5V or less is considered too low for reliable operation.

ESC

Returns control to the "Main Menu".

Far Red (735nm)

Toggles the far red source on and off

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Lamp
Toggles the saturation source on and off. Its intensity is set to a minimum for this test.

Actinic
Toggles the actinic source on and off. Its intensity is set to a minimum for this test.

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CHAPTER 5 • MAINTENANCE

INTRODUCTION

Your OS-500 contains no user serviceable components. Please contact your closest authorized agent for periodic preventative maintenance and calibration information.

Cleaning

The OS-500 is made from durable materials, but some organic solvents can damage the surface finishes. Use a damp cloth with some mild detergent to clean the outside. The display window and keypad legend are lexan, while the case is an ABS plastic. Should dirt get into the optical ports, (remember to use the protective caps supplied) clean the glass windows with a cotton swab and isopropyl alcohol. If the unit becomes submerged in water, return it to the factory for maintenance.

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Miscellaneous Maintenance

Battery

The 12V 3.4 Amp-Hour Sealed Lead-Acid Battery will provide about four to six hours of average use.

The typical recharging period is 14 hrs. Leaving the unit on "charge" for longer than this will not damage the battery.

WARNING: Do not connect anything other than the provided charger or you may damage the OS-500!

As the battery ages, it loses its ability to hold a charge. When this gets to about 1/2 its original life time or about two ~ three hours, replace the battery. It is a good idea to keep a spare battery charged, especially if you expect to run a large number of tests.

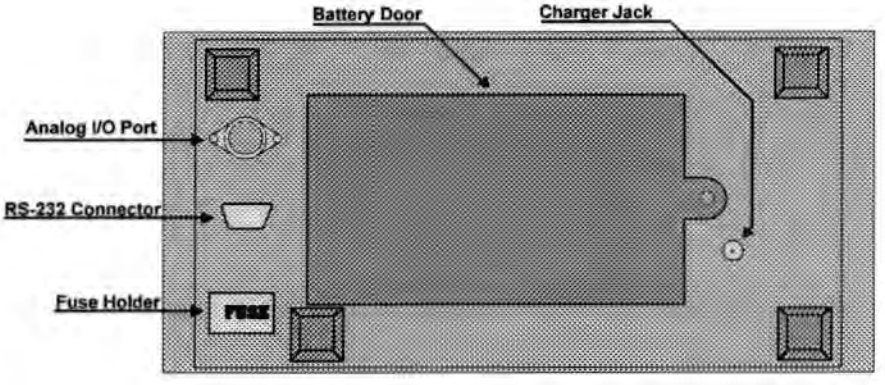


Figure 5-1 Battery Door

To change the battery, unscrew and remove the battery door, disconnect the wire terminals, slide out the battery, connect the new battery (the red or white striped wire goes to the + terminal), reinsert battery and secure back cover.

Note: you cannot run the OS-500 off the battery charger alone, it will not provide the power needed for the saturation lamp.

Fuse Replacement

The OS-500 uses a 4A fast-blow 5x20mm fuse. You should not need to change this fuse under normal conditions. However, connecting the battery backwards will instantly blow the fuse.

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To change the fuse, press downward on the fuse holder cover. After the cover "pops" out, pull gently outward to expose the fuse. There is a spare fuse directly behind the cover. Replace the blown fuse with the new one, and press the cover back onto the holder. If the new fuse blows, call you're nearest authorized dealer for service information.

Saturation Lamp Replacement

The 35W halogen lamp is part of an aligned optical assembly. Replacing the lamp is somewhat involved, and so is offered as a low-cost agent service option. Contact your nearest authorized agent for more information.

Note: Under normal use you may expect about five years service from a lamp.

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Trouble Shooting Table

Symptom	Probable Cause	Cure
Unit will not turn on	• Dead battery • Poor connection to battery • Battery connected backwards and blew internal fuse • fuse blown	• charge battery • check connections • change fuse • change fuse
Unit resets when a test is run	• battery insufficiently charged • battery has become "weak" and needs replacement	• charge battery • replace battery
Display dims or changes contrast during a test	• battery low	• charge battery • replace battery
Battery does not charge	• charger connector loose • charger is defective • dead battery	• check connections • if not then replace charger • replace battery
Fiber-optic cable will not fit into the optical ports	• obstruction in one of the ports	• clear obstruction
Remote trigger does not initiate a test	• remote trigger wire is not plugged in correctly • OS-500 is not in proper test mode	• check connections • fluorescence signal not >40. raise modulation
Saturation light does not trigger	• fluorescence signal (Ft) below 30 counts • if display also blanks, battery is low • saturation bulb has blown out	• increase modulation intensity • charge battery • call nearest authorized agent for lamp replacement
Cannot output data to computer - or - Output to computer garbled	• serial cable is loose • mismatch in baud rates • hardware handshake protocol on host • OS-DATA	• secure cable connections • check port and baud settings • OS-DATA • OS-DATA
Unit consistently looses data	• internal coin cell dead	• call nearest authorized agent for cell replacement

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Fluorescence signal is noisy	• battery is insufficiently charged • external actinic light source is heavily modulated (pulsed xenon) • external source has too much IR radiation • fiber not steady on sample	• charge battery • use actinic illuminator with a continuous output • filter out excessive IR • use open body clip
Incorrect PAR reading with optional OS-OBCP cuvette attached	• Incorrect calibration constant entered • PAR sensor not connected	• Check the OS-OBCP manual for correct calibration constant
Incorrect temperature reading with optional OS-OBCP cuvette attached	• Temperature sensor not connected • Calibration table incorrect	• See OS-OBCP manual for correct loading of calibration table.

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APPENDIX A • DATA FORMATS

OVERVIEW

Each test has a unique data output format, tailored to the changing measured parameters. The differences between the data groups are listed in this section. OS-DATA automatically adjusts for these differences in data types, providing spreadsheet, graphical view, and export capabilities.

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Data exists in two forms in the OS-500. The first type is the fluorescence signal over the course of a test. This "raw data" is useful when visualizing what is happening dynamically in the photosystem, as it is exposed to the different illumination sources over the course of the test. The second kind of data is the tabular data that is generated around the saturating pulses for test modes 1, 2, and 3, and the start and endpoints in test mode 4. The specifics of each of these parameters is mentioned later in this section. Storage method varies between these two types of data. The "raw data" for a trace is allotted a unique storage number based on the next open location in the storage directory, as they are saved. The user can not specify which slot number a trace is stored to. The "Tabulated Data", on the other hand, is stored in logical groupings called file numbers. Unlike traces, the current file number is a user setable option (this is set from the "Run Data" screen). When joint saving of trace and data is desired, the file number can be chosen to match the "Next Trace Slot" shown in the "Run Data" screen. This will give the file and trace the same identifying number. There can be up to 99 different files and 27 different traces stored simultaneously in the system depending on memory use. Information on free memory status is always available in the "Run Data" screen of each test mode.

There are specific formats for each type of data and trace. Each test mode will generate a slightly different data output (each test measures different parameters). The details of each type are listed at the end of this section.

All types include a common header containing information about the date, time and setup of the machine when each measurement was made.

In test modes 1, 2, and 4, the "raw data" that follows occurs at different sampling rates, depending on the phase of the test. This must be considered when graphing the raw data as a time distortion will occur between the different sample rates. For example, the first 20 points of test mode 1 (dark adapted) are at 100 pts/sec, while the saturation pulse interval is run at 1000 pts/sec. For this test, the time for first 20 points will equal that of the next 200 points. OS-DATA takes these factors into account and gives the user the ability to view the "raw data" in an accurate time format.

Data formats for each test mode are listed below. All data types and output methods (either serial port or disk) are presented in a ASCII format that is comma delimited with a character 13 (<CR.>) code at the end of each line. This type of data output format, while bulkier than binary coding, is compatible with most data analysis and spreadsheet programs "Import" feature.

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Test Mode 1 Data

A typical "dark adapted" (test mode 1) data point has the following format:

```
Data: 129,2,22,04,04,12,32,22,120,50,100,.8,100,200,600,800,.750,22.6
Word #:1  2 3  4  5  6  7  8  9   10 11  12 13  14  15  16  17   18
```

W#	Data description	Data
1	test type (129) for "Fv/Fm" data	129
2	file # id's parent file	2
3	sample # for entries within file	22
4	month	04
5	day	04
6	hour (24 hour format)	12
7	minute	32
8	second	22
9	Modulation Intensity	120
10	Detector Gain	50
11	Saturation pulse Intensity	100
12	Saturation pulse width	.8
13	Actinic Intensity	100
14	Fo value	200
15	Fv value	600
16	Fm value	800
17	Fv/m value	.750
18	Leaf Temp in deg C (if optional sensor present)	22.6

Test Mode 2 Data

A typical "light adapted" (test mode 2) data point has the following format:

```
D :130,2,22,04,04,12,32,22,120,50,100,.8,100,340,320,660,.485,40.7,200,22.6
W#:1   2 3  4  5  6  7  8  9   10 11  12 13  14  15  16  17   18   19  20
```

W#	Data description	Data
1	test type (130) for "Yield Test" data	130
2	file # id's parent file	2
3	sample # for entries within file	22
4	month	04
5	day	04
6	hour (24 hour format)	12
7	min	32
8	sec	22
9	Mod Int	120
10	Det Amp	50
11	Sat Int	100
12	Sat PW	.8
13	ActI	100
14	Fs	340
15	Fds	320
16	Fms	660
17	Yield	.485
18	ETR	40.7

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19	PAR uE	200
20	Leaf Temp deg C	22.6

Test Mode 3 Data

Kinetic (test mode 3) tests have 3 types of data points

Fo & Fm

The first type holds the dark adapted data for the start of a run (note this data is only present if a determination of Fo & Fm is done. See description of test mode 3 for more information

A typical "dark adapted" data point has the following format:

```
Data:  132,2,22,04,04,12,32,22,120,50,100,.8,100,155,1090
Word #:1   2 3  4  5  6  7  8  9   10 11  12 13  14  15
```

W#	Data description	Data
1	test type (132)	132
2	file # id's parent file	2
3	sample # for entries within file	22
4	month	04
5	day	04
6	hour (24 hour format)	12
7	minute	32
8	second	22
9	Modulation Intensity	120
10	Detector Gain	50
11	Saturation pulse Intensity	100
12	Saturation pulse width	.8
13	Actinic Intensity	100
14	Fo	155
15	Fm	1090

Saturation Pulse

The second (and most prevalent) data point is the saturation pulse information.

A typical "saturation pulse" data point has the following format:

```
D:133,2,22,04,04,12,32,22,120,50,100,.8,100,185,574,.678,56.9,.899,.928,.552,200,22.6
W:1   2 3  4  5  6  7  8  9   10 11  12 13  14  15  16   17   18   19   20   21  22
```

W#	Data description	Data
1	test type (133)	133
2	file # id's parent file	2
3	sample # for entries within file	22
4	month	04

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5	day	04
6	hour (24 hour format)	12
7	minute	32
8	second	22
9	Modulation Intensity	120
10	Detector Gain	50
11	Saturation pulse Intensity	100
12	Saturation pulse width	.8
13	Actinic Intensity	100
14	Fs	185
15	Fms	574
16	Y	.678
17	ETR	56.9
18	NPQ	.899
19	qP	.928
20	qN	.552
21	PAR uE (With optional sensor clip)	200
22	Leaf Temp deg C (With optional sensor clip)	22.6

Fod

The last type is the Fod determination data. This data is only present if a determination of Fod is done (see description of test mode 3 for more information)

A typical "Fod" data point has the following format:

```
Data:    134,2,22,04,04,12,32,22,120,50,100,.8,100,140
Word #: 1   2 3  4  5  6  7  8  9   10 11  12 13  14
```

W#	Data description	Data
1	test type (134)	134
2	file # id's parent file	2
3	sample # for entries within file	22
4	month	04
5	day	04
6	hour (24 hour format)	12
7	minute	32
8	second	22
9	Modulation Intensity	120
10	Detector Gain	50
11	Saturation pulse Intensity	100
12	Saturation pulse width	.8
13	Actinic Intensity	100
14	Fod	140

In a typical kinetic run where Fo, Fm, and Fod are found, the file used will contain a type 132 for the first entry then a type 133 for every subsequent saturation pulse fired and lastly, a single type 134 for the measurement of Fod.

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Test Mode 4 Data

Fast actinic (test mode 4) is the continuous fluorescence measurement test.

```
Data:  135,2,22,04,04,12,32,22,120,50,100,.8,100,220,400,620,320,.645,112,22.6
Word #:1   2 3  4  5  6  7  8  9   10 11  12 13  14  15  16  17  18   19  20
```

W#	Data description	Data
1	test type (135)	135
2	file # id's parent file	2
3	sample # for entries within file	22
4	month	04
5	day	04
6	hour (24 hour format)	12
7	minute	32
8	second	22
9	Modulation Intensity	120
10	Detector Gain	50
11	Saturation pulse Intensity	100
12	Saturation pulse width	.8
13	Actinic Intensity	100
14	Fo	220
15	Fv	400
16	Fm	620
17	Ft	320
18	Fv/m	.645
19	T1/2 in milliseconds	112
20	Leaf Temp deg C	22.6

Data Headers

Traces are different from files in that the bulk of the data consists of single points with one header at the beginning of the trace holding the general information.

Each test type has a slightly different header and, as mentioned, the point rate can vary over the run of the test. In trace types 1, 2, and 4 relevant tabulated data is included in the trace header as these tests usually involve one set of numerical data per transient. This allows you to make single detailed measurements without needing to store another line of data for a single point. Because a typical kinetic run usually involves multiple numerical data points per run, no numerical data is included in the trace headers for this type of file. All information is output in ASCII format with the header being comma delimited with a character 13 end mark. Each trace point is separated by a chr 13.

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Test mode 1 header

```
Data:  1,1,4,22,4,4,21,50,2,110,50,100,.8,150,820,199,700,899,.778,21.5
Word #:1 2 3 4  5 6 7  8  9 10  11 12  13 14  15  16  17  18  19   20
```

W#	Header description	Data
1	trace number corresponds to trace # as seen in the selection box	1
2	trace type 1 for this one	1
3	current file # id's parent file	4
4	current sample # when "trc save" key pressed (usually the next one in a file)	22
5	month	4
6	day	4
7	hour (24 hour format)	21
8	minute	50
9	second	2
10	Modulation Intensity	110
11	Detector Gain	50
12	Saturation pulse Intensity	100
13	Saturation pulse width	.8
14	Actinic Intensity	150
15	number of sample points in the following trace	820
16	Fo	199
17	Fv	700
18	Fm	899
19	Fv/m	.778
20	Leaf Temp deg C	21.5

Trace points data rates:

Point numbers	Rate
1 to 19	100 pts / sec
20 to EOT	1000 pts /sec

Test mode 2 header

```
D : 1,2,4,22,4,4,21,50,2,110,50,100,.8,150,820,299,400,699,.572,60.1,250,23.5
W#: 1 2 3 4  5 6 7  8  9 10  11 12  13 14  15  16  17  18  19   20   21  22
```

W#	Header description	Data
1	trace number corresponds to trace # as seen in the selection box	1
2	trace type 2 for this one	2
3	current file # id's parent file	4
4	current sample # when "trc save" key pressed (usually the next one in a file)	22

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5	month	4
6	day	4
7	hour (24 hour format)	21
8	minute	50
9	second	2
10	Modulation Intensity	110
11	Detector Gain	50
12	Saturation pulse Intensity	100
13	Saturation pulse width	.8
14	Actinic Intensity	150
15	number of sample points in the following trace	820
16	Fs	299
17	Fds	400
18	Fms	699
19	Yield	.572
20	ETR	60.1
21	PAR uE	250
22	Leaf Temp deg C	23.5

Trace points:

Point numbers	Rate
1 to 19	100 pts / sec
20 to EOT	1000 pts /sec

Test mode 3 header

```
Data:     1,3,4,22,4,4,21,50,2,110,50,100,.8,150,820
Word #:   1 2 3 4  5 6 7  8  9 10  11 12  13 14  15
```

W#	Header description	Data
1	trace number corresponds to trace # as seen in the selection box	1
2	trace type 3 for this one	3
3	current file # id's parent file	4
4	current sample # when "trc save" key pressed (usually the next one in a file)	22
5	month	4
6	day	4
7	hour (24 hour format)	21
8	minute	50
9	second	2
10	Modulation Intensity	110
11	Detector Gain	50
12	Saturation pulse Intensity	100
13	Saturation pulse width	.8

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14	Actinic Intensity	150
15	number of sample points in the following trace	820

Trace points:

Point numbers	Rate
1 to EOT	10 pts / sec

Test mode 4 header

```
D: 1,4,4,22,4,4,21,50,2,110,50,100,.8,150,2080,199,700,899,280,.778,103,20.5,0
W#:1 2 3 4  5 6 7  8  9 10  11 12  13 14  15   16  17  18  19  20   21  22  23
```

W#	Header description	Data
1	trace number corresponds to trace # as seen in the selection box	1
2	trace type 4 for this one	4
3	current file # id's parent file	4
4	current sample # when "trc save" key pressed (usually the next one in a file)	22
5	month	4
6	day	4
7	hour (24 hour format)	21
8	minute	50
9	second	2
10	Modulation Intensity	110
11	Detector Gain	50
12	Saturation pulse Intensity	100
13	Saturation pulse width	.8
14	Actinic Intensity	150
15	number of sample points in the following trace	2080
16	Fo	199
17	Fv	700
18	Fm	899
19	Ft	280
20	Fv/m	.778
21	T1/2 in milliseconds	103
22	Leaf Temp deg C	20.5
23	Actinic source selection 0-LEDs 1-Lamp	0

Trace points:

Point numbers	Rate
1 to 19	100 pts / sec
20 to 2019	1000 pts / sec
2020 to EOT	10 pts / sec

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APPENDIX B • OBCP LEAF CLIP

Overview

The OBCP leaf clip is designed to provide data to the OS-500 system on ambient conditions. This clip resembles the standard 60^0 angle clip provided with the OS-500, with two added features, a thermistor for measuring leaf temperature and a PAR sensor for measuring light intensity. The PAR sensor is mounted to a small white support extending into the leaf view area. The thermistor is tethered below the support to measure leaf temperature.

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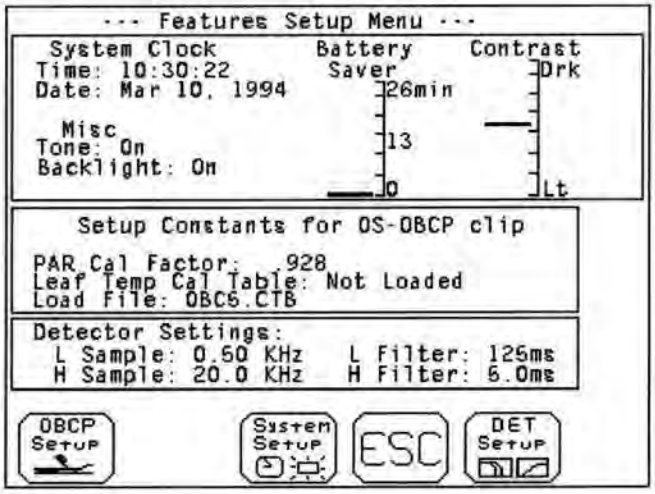
OS-500 Connection

The fiber is positioned in the clip in the same manner as the 60^0 clip. A nylon locking screw secures the fiber end. A cable exiting the clip carries all electrical signals back to the OS-500. At the end of this cable are two plugs, one is labeled **PAR** and the other **Temp**. Jacks bearing the same label are present on the OS-500 just below the fiber port **Detector**. Swapping the two signal plugs will cause incorrect readings but no permanent damage.

Loading calibration data

The **ESC** key can be used at any time to exit the calibration load.

1. Enter the **SYSTEM SETUP** menu.



System Setup screen

First, the PAR Cal Factor will be entered:

1. Press **OBCP Setup** to accesses the coefficient load box. A cursor will appear beside the value displayed on the line PAR Cal Factor x.xxx.
2. Use the data wheel to enter the coefficient.

Next, to load the calibration table for the leaf thermistor:

3. Press **OBCP Setup** key again.
4. The message prompt "*Insert Disk*" will appear on the line Load File: OBC5.CTB Place the disk provided with the clip into the OS-500's disk drive.

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5. Press the **OBCP Setup** key again. The OS-500 will attempt to load the calibration file. If successful, the *Insert Disk* message will disappear and the line Leaf Temp Cal Table: Loaded should be seen. This will conclude the load procedure.
6. Press **ESC** to step back to the main menu.

Note: If the OS-500 couldn't read the file or found that the file read contained an error then the message *Insert Disk* would be replaced by the message *Not a Cal Table*. The table status line will display the error condition as Leaf Temp Cal Table: Not Loaded.

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