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Analyst™ AD & HT

Assay Detection Systems

Operator's Manual

BioSystems
The High Throughput Company™

PN 90-000-0004 Rev. C

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Revision History:

Date	Revision	Version	Description
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1 System Description

1.1 INTRODUCTION

The Analyst™ AD Assay Detection System is designed for use in assay development laboratories. The Analyst™ HT Assay Detection System is designed for use in high-throughput screening laboratories.

Both systems can read 96- and 384-well microplate formats in seven modes:

- Fluorescence Intensity (F)
- High Efficiency Fluorescence Polarization (HEFP™)
- Time-Resolved Fluorescence (TRF)
- Luminescence (L)
- Focused Luminescence (FLUM)
- Absorbance (A)
- Epi-Absorbance (EA)

The systems also provide Multi-Method mode, which makes two readings at each well using any two measurement modes except fluorescence polarization.

Like other Criterion™ instruments, both systems employ the SmartOptics™ optical system, a flexible system of light sources, optics, and detectors that delivers the highest performance available using a microplate format. Since SmartOptics is identical on both systems, assay methods developed using the Analyst AD can be used in the Analyst HT without requiring revalidation or method reoptimization.

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The Analyst AD and Analyst HT systems differ in the following respects:

- **Robot control**: The Analyst HT can be used with a lab robot, which controls the instrument using Open Protocol commands; the Analyst AD cannot.
- **Speed**: The Analyst HT is faster than the Analyst AD.
- **Control panel**: The Analyst HT has a control panel for executing certain basic functions such as starting and stopping the system; the Analyst AD does not. (On the Analyst AD, those functions are executed using the Windows® 95/98/NT user interface running on a host computer, and a green LED on the system indicates power-on status.)
- **Ports**: Both the Analyst HT and the Analyst AD have an RS232 serial port for connecting to a host computer; the Analyst HT also has a second serial port for transferring data (if desired) to a second computer or a laboratory information system. The Analyst HT has an auxiliary port for connecting to an optional external control box; the Analyst AD does not.
- **External control box**: An optional external control box – which can be used to alert the operator to a fault or service condition in the instrument via connection to a peripheral device – can be connected to the Analyst HT. It is not available with the Analyst AD.

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Analyst AD System

The instrument is connected via a serial port to a Pentium-class computer running the Windows® 95/98/NT operating system and the CriterionHost™ application software.

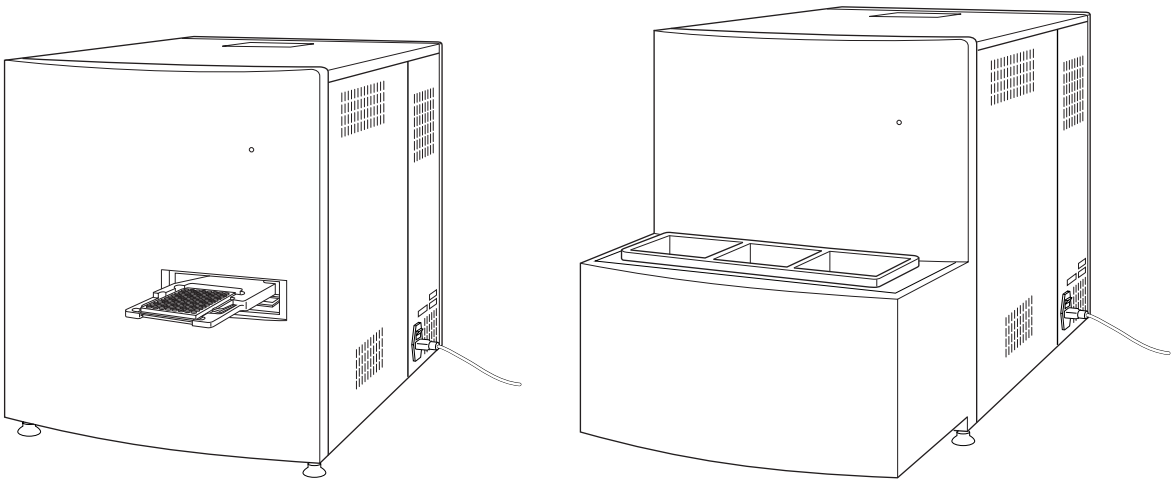
Control is on a master/slave basis – the instrument relies on the host computer for instructions and communicates status and results information back to the host. The instrument uses a single serial port for both host commands and transmitting results.



Interface to Host Computer

The operator uses the host computer to specify the detection mode, plate format, filters, integration time, and other parameters. Once the system is properly configured, the operator can start and stop plate reading from either the host computer or the instrument control panel.

The instrument is available in two models, as shown below. Without the plate stacker, the operator loads one microplate at a time. With the plate stacker, the instrument can accept magazines of 20 or 40 microplates for automatic processing.



Without Stacker **With Stacker**

Analyst AD Assay Detection System

The instrument presents a plate gripper into which a standard microplate (in 'portrait' orientation) is placed by the operator for reading. As the plate moves into the instrument, it is precisely positioned by the gripper mechanism to ensure accurate alignment relative to the optical head. All detection occurs within a light-tight enclosure. For further information, see section 6.4, 'Technical Specifications.'

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Analyst HT System

The instrument is connected via a serial port to a computer running the Windows® 95/98/NT operating system, or to a robot controller.

Control is on a master/slave basis – the instrument relies on the host computer for instructions and communicates status and results information back to the host. If desired, data from the instrument can be transferred to a separate computer or laboratory information system.

The operator uses the host computer to specify the detection mode, plate format, filters, integration time, and other parameters. Once the system is properly configured, the operator can start and stop plate reading from either the host computer or the instrument control panel.

The host computer may be either a Pentium-class computer running the Windows-based CriterionHost™ application software, or a robot controller. The robot control software communicates with the instrument using a text command-and-response format called Open Protocol. An ActiveX control is also available for communication with a robot controller.

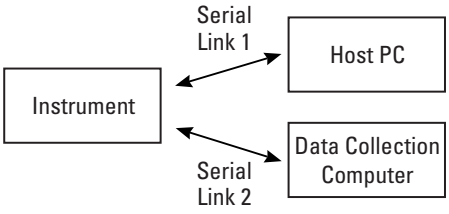
The Open Protocol uses strings of ASCII text characters to change instrument parameters, and report results. Open Protocol commands can be sent to the instrument from any program that can transmit ASCII text characters via a serial port. Although the CriterionHost application has a large range of features, its total functionality under Windows does not include all options provided by the Open Protocol.

The instrument's two serial ports allow several ways to interface with the laboratory's computers. In the most basic configuration, the instrument uses a single serial port for both host commands and transmitting results.



Single Interface to Host Computer

With the second port, it is also possible to use a second computer for data collection.

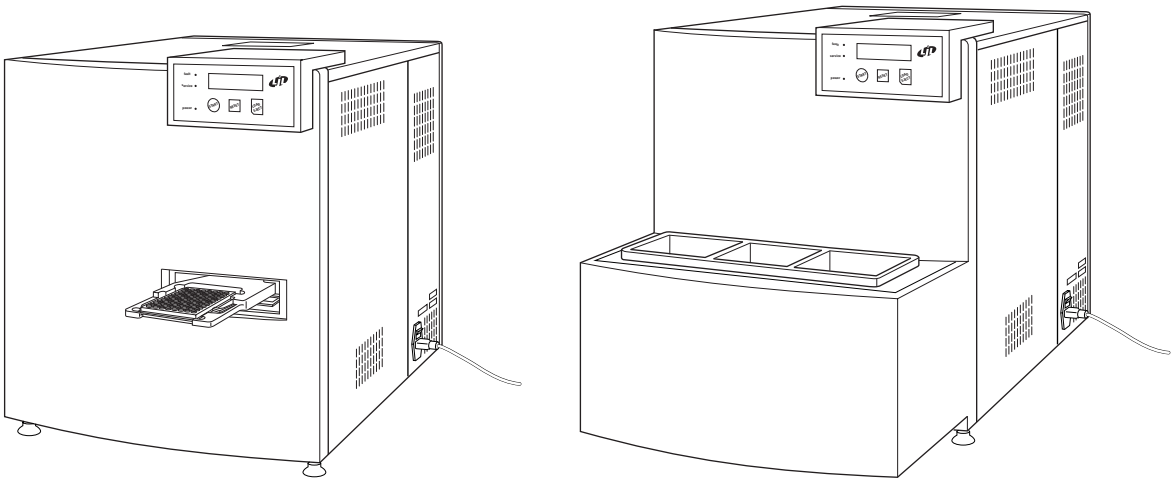


Dual Interface to Host and Data Collection Computers

Data management can be automated by transferring results to a spreadsheet or database application.

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The instrument is available in two models, as shown below. Without the plate stacker, the operator or a lab robot loads one microplate at a time. With the plate stacker, the instrument can accept magazines of 20 or 40 microplates for automatic processing.



Without Stacker **With Stacker**

Analyst HT Assay Detection System

The instrument presents a plate gripper into which a standard microplate (in 'portrait' orientation) is placed by the operator or a lab robot for reading. As the plate moves into the instrument, it is precisely positioned by the gripper mechanism to ensure accurate alignment relative to the optical head. All detection occurs within a light-tight enclosure. For further information, see section 6.4, 'Technical Specifications.'

What's New in CriterionHost 2.0

CriterionHost 2.0 includes the following changes from release 1.5:

- There are three new measurement modes – Focused Luminescence, Absorbance, and Epi-Absorbance – plus Multi-Method mode, which makes two readings at each well using any two modes except fluorescence polarization.
- The specification of the wells to be read in the method definition can now be entered graphically on a row/column matrix.
- The instrument can subtract readings in background wells from readings in sample wells. The method definition can specify up to two sets of sample and background wells on each plate (one set for Absorbance methods).
- The comparator conversion method is now called SmartRead™.
- There is a new conversion method, SmartRead™ Plus. This method operates like SmartRead, except that the read head stays at each well only until the reading is complete.

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- The analog conversion method is not available as a separate option. If SmartRead or SmartRead Plus is selected, the system will automatically use analog conversion depending on the signal level and sensitivity setting.

  Note: *For more information about SmartRead and SmartRead Plus, see 'Detector Counting Options (PMT Setup),' page 36. (In this manual, the term 'detector counting option' is used to refer to what was called 'detector conversion method' in the manual for release 1.5.)*

- There are new method parameters that specify a time delay between starting a run and the beginning of processing the first plate, a time delay between the end of processing each plate and the beginning of processing the next plate, and the number of times each plate is to be read before moving on to the next plate.
- There is a choice of three number formats for showing results on the Run screen and in reports: decimal integer, floating point, and scientific.
- The plate reports have been improved.
- The Command Line terminal emulation feature has been discontinued. Users wishing to control the instrument via Open Protocol commands can still do so using a separate terminal emulation application.

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1.2 SAFETY INFORMATION

When operated *properly* in a safe environment according to the instructions in this manual, there are no known hazards associated with the Analyst AD or Analyst HT Assay Detection System. However, proper use requires an understanding of routine situations that are potentially dangerous and can result in serious injury.

All users must be familiar with the guidelines in this section before working with the system.

Conventions Used in this Manual

This manual uses the following conventions to provide technical and safety information of special interest.

Note: *Background information provided to clarify a particular step or procedure.*

Caution: *An instruction that, if not followed, can result in damage to the system.*

Important! *An instruction provided to ensure correct results and optimal performance.*

Warning! *An instruction that, if not followed, can result in a hazardous condition.*

Electrical Safety

Observe the following warnings and precautions:

High internal voltages. Always turn off power switch and unplug system power cord before removing control panel and side or top panels.



Warning labels. The symbol shown at left appears on certain instrument component labels. The purpose of the marking is to alert you to use caution when servicing a component or the instrument. Be aware that ignoring the instructions on the label can result in a hazardous condition that can cause injury.

Xenon-arc lamp. Do not look directly at the continuous lamp or flash lamp while illuminated. The lamps emit ultraviolet radiation at levels that can injure the eye if viewed directly.

Electrical grounding. Never use a two-prong plug or extension cord to connect primary power to the system. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to a three-prong receptacle with a functional ground.

Spilled liquids. Avoid spilling liquids on the system. Fluid spilled into internal components creates a potential shock hazard. Wipe up all spills immediately. Do not operate the system if internal components have been exposed to spilled fluid. Unplug instrument if there is a fluid spill in the instrument and contact your local LJL BioSystems agent.

Replacement fuses. Use replacement fuses with the required current rating and specification. Improper fuses or short-circuiting the fuse holders can cause fire or damage the instrument.

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Power rating. Ensure the system is connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle can create electrical shock and fire hazards.

Remove watches and jewelry before removing any panels from the instrument.

Electrostatic discharge (ESD). A wrist strap is recommended when servicing the electronics. If a wrist strap is not available, be sure to touch a ground point in the system.

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1.3 HOW TO USE THIS MANUAL

This manual was written to ensure safe and proper use of the instrument. Before use, read this manual carefully in order to realize the full capabilities of the system. Also, if something is unclear during daily use or if a problem occurs, please refer to this manual.

This manual is organized as follows:

Part 1: System Description provides background information on the system, that is, the principal components and how the system functions.

Part 2: Operating Procedures provides instructions for operating the system with a Windows-based PC running the CriterionHost application software.

Part 3: Robot Integration provides instructions for establishing the mechanical and software interface between the robot and the instrument.

Part 4: Maintenance Procedures provides instructions for routine maintenance including replacing the lamp, filters, dichroic mirrors, as well as adjusting the luminescence aperture.

Part 5: Troubleshooting provides instructions for diagnosing and solving common problems, as well as a list of error conditions.

Part 6: Appendices provides instructions for moving the instrument to a new location, technical specifications, list of spare parts, index, and warranty statement.

Throughout this manual, sections, subsections, or statements that pertain only to the Analyst HT or only to the Analyst AD are signified by the notation '(Analyst HT)' or '(Analyst AD)' afterwards.



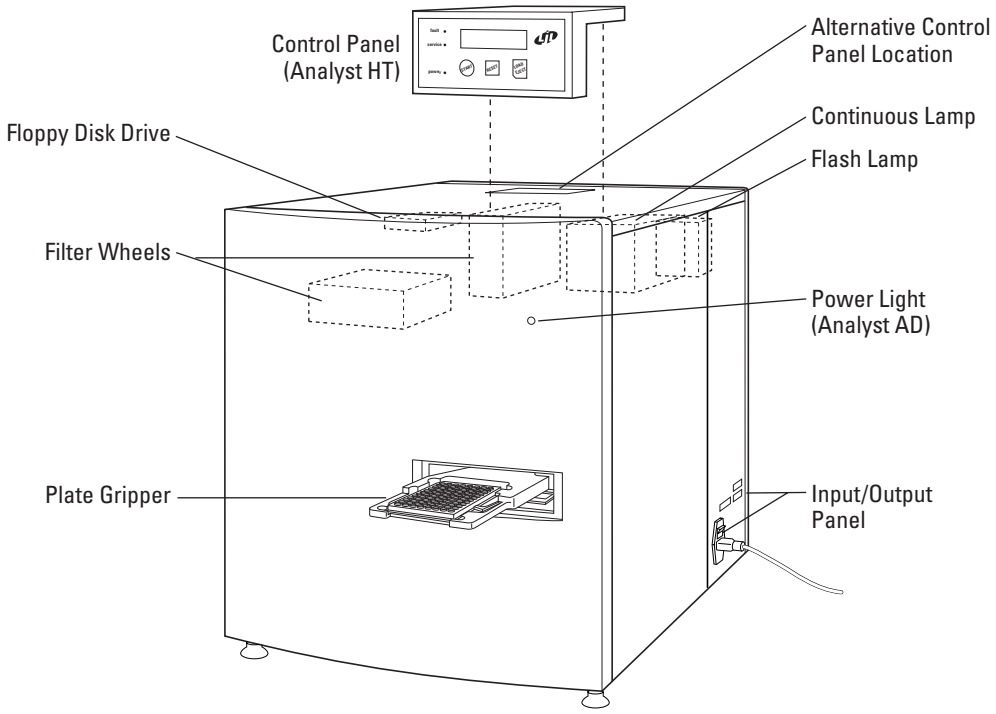
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1.4 SYSTEM COMPONENTS

This section describes the major instrument components listed below.

- Control Panel (Analyst HT)
- Power Light (Analyst AD)
- Microplate Gripper
- Microplate Stacker
  – Microplate Magazines
  – Barcode Reader
- Optical Components
  – Continuous Lamp
  – Flash Lamp
  – Filters
  – Dichroic mirrors
  – Attenuators (neutral density filters)
  – Polarizers
- Input/Output Panel
- External Control Box (Analyst HT)
- CriterionHost application and Instrument Control Program

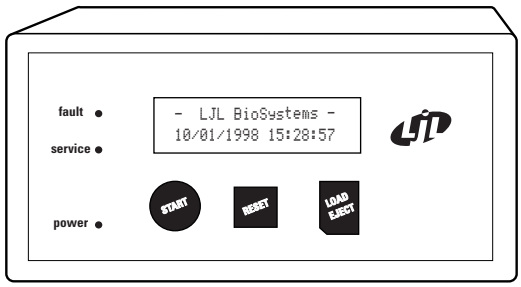


System Components

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Control Panel (Analyst HT)

The control panel consists of a gas-plasma display, keypad, indicator lights, and an audible alarm.



Control Panel

The control panel provides a convenient means for executing certain basic functions such as starting and stopping the system. This functionality can be useful for automated operations that require manual operator intervention.

The control panel is used in conjunction with the host computer. Once you have set up the system with the host, plate reading can be initiated from the control panel.

For convenience, the control panel can be mounted on the front or rear of the instrument. For further information, see section 6.1, 'Relocating the System.'

Display. The display provides current instrument status messages.

Keypad. The keypad below the display has four keys used as follows:

- The **START** key begins the reading process.
- The **LOAD/EJECT** key commands the gripper to either load or eject a plate, depending upon the current status of the instrument.
- The **RESET** key sends all motors to their home positions, reinitializes the instrument, and turns off the audible alarm.
- The **LJL** logo key turns the continuous lamp on or off. For further information, see section 3.2, 'Open Protocol Commands' (**lampenable** command).

Indicator Lights. The three lights have the following functions:

- The **POWER** light comes on whenever power is supplied to the instrument.
- The **FAULT** light comes on whenever a critical fault has occurred that requires intervention by the operator.
- The **SERVICE** light comes on when a service procedure is needed, for example, when nearing the end of the life of the lamp.

Audible Alarm. The alarm sounds in the event of a fault or service condition.

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Power Light (Analyst AD)

The Power Light comes on whenever power is supplied to the instrument.

Microplate Gripper

The plate gripper precisely positions the microplate in the mechanism and carries the plate into the instrument for analysis. Precise plate positioning is essential for good performance.

Microplate Stacker

Systems provided with the integrated stacker allow loading a number of microplates at a time. The additional functionality includes:

- Input and output magazines that allow loading 20 microplates at a time.
- Separate input and output positions, which on the Analyst HT can improve robot operation.
- Separate transfer position for manual operation when the magazines are installed.
- Barcode reader with automated reading of multiple symbologies.

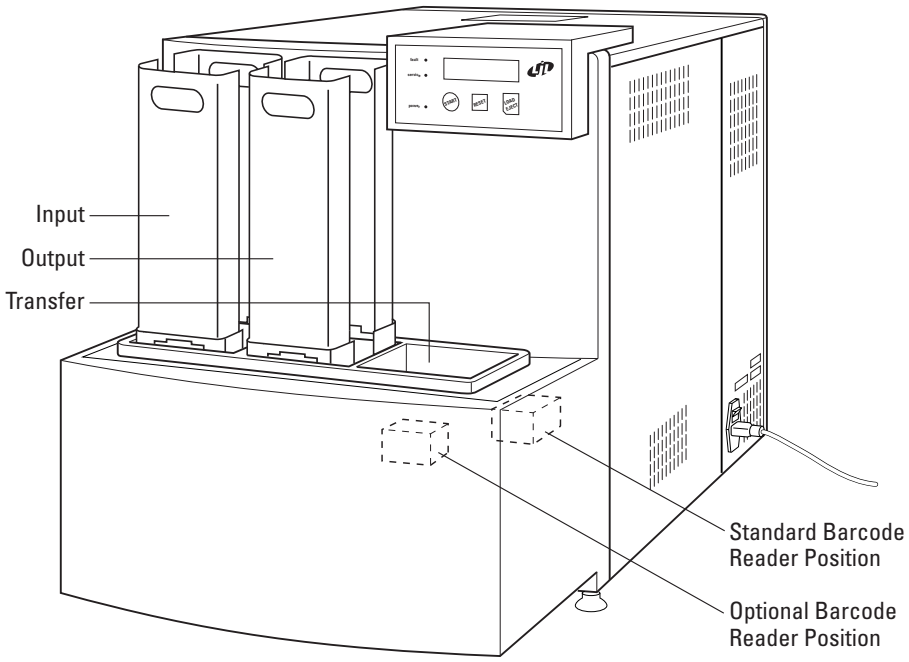


Plate Stacker with Magazines

Microplate Magazines

Magazines have a capacity of 20 microplates with SBS standard height of 15 mm. The stacker is plug-compatible with LJL BioSystems 40-plate magazines and TiterTek®

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S20 magazines. This gives the user the convenience to dispense, wash and read without restacking microplates.

Microplates are stored in the input magazine before reading and then transferred to the output magazine after reading. The user also has the option to deliver or pick up a microplate directly to or from the transfer position without removing the magazines.



In robotic operation, the stacker can reduce robot hand travel by providing separate landing (input) and pickup (output) positions. The system is designed to allow a plate to be inserted and another removed by a robot in one pass, a feature known as Process Compression. For more information on robot operation, see part 3, 'Robot Integration.'

Barcode Reader

The stacker includes an integrated barcode reader that can be used with the magazines to read the identity of a labeled microplate automatically. The barcode reader decodes the following symbologies:

- Code 39
- Code 93
- Code 128 (A, B, and C)
- Codabar
- Interleaved 2 of 5
- Standard 2 of 5
- MSI – Plessey
- WPC: EAN, JAN, UPC

In the factory-installed configuration, barcodes are read on the long edge of the microplates. (The barcode reader can be moved to read on the short edge. For further information, contact your LJL BioSystems agent.) Barcodes are read while the plate moves from the input magazine into the light-tight enclosure. Barcodes cannot be read when microplates are delivered directly to the transfer position.

You can configure the system to either 1) simply report the barcode string or 2) use it to name the report file. For further information, see section 2.5, 'Reading Microplates, Saving Results Files.'

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Optical Components

Lamps

The system has two lamps:

- For F, HEFP, A, and EA applications, the instrument can be programmed to use either 1) a xenon-arc lamp as a continuous light source, or 2) a xenon flash lamp.
- For TRF applications, the instrument always uses a xenon flash lamp.

The lamps are user-replaceable and self-aligning for convenient installation. For information on replacing the lamps, see sections 4.6 and 4.7.

Filters and Dichroic Mirrors

Each filter wheel holds eight 25-mm diameter filters. For information on selecting filters appropriate for common fluorophores, see section 6.6, 'Selecting Filters and Dichroic Mirrors.' For information on installing filters, see section 4.3, 'Filter Removal and Replacement.'

Each optics head can hold one replaceable dichroic mirror. A 50:50 beamsplitter is installed in the bottom optics head when the system is shipped. For information on installing dichroic mirrors, see section 4.4, 'Dichroic Mirror Removal and Replacement.'

The 50:50 beamsplitter transmits 50 percent of incident light at all wavelengths and reflects 50 percent of the light at all wavelengths. This property enables it to be used with the widest possible range of fluorophores, but limits the sensitivity of the instrument in some applications. To maximize sensitivity, the user can install a fluorophore-specific dichroic mirror.

Attenuators

Sensitivity and linear range are maximized by using a neutral density filter that blocks light in the optical path of the fluorescence PMT. The fluorescence attenuator can be set to one of three positions to block different amounts of light.

Mode	Attenuation Factor	Optical Density
Out (O)	1	0
Medium (M)	100	2
High (H)	10,000	4

The luminescence attenuator can be set to either the Out or Medium position. For further information, see section 2.4, 'Defining Methods.'

Note: *Attenuation factor and optical density values cited are approximate and vary over the spectrum.*

Polarizers

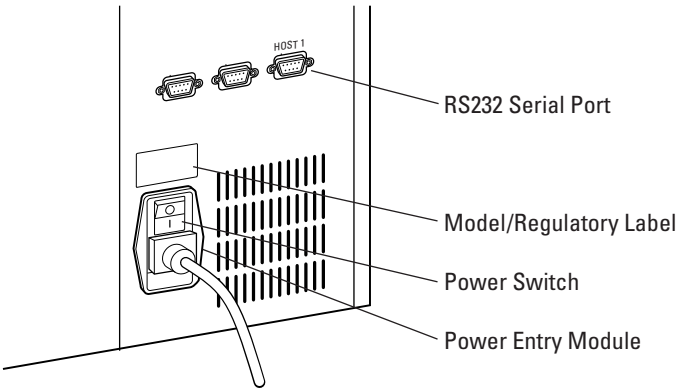
Two sets of polarizers are mounted in the optical path. One set is mounted in the excitation path, and the other in the emission path. For F and HEFP methods, you

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can specify that the polarizers be in either the S (horizontal) or P (vertical) orientations, or that the polarizers be out of the optical path. For further information, see section 2.4, 'Defining Methods.'

Input/Output Panel (Analyst AD)

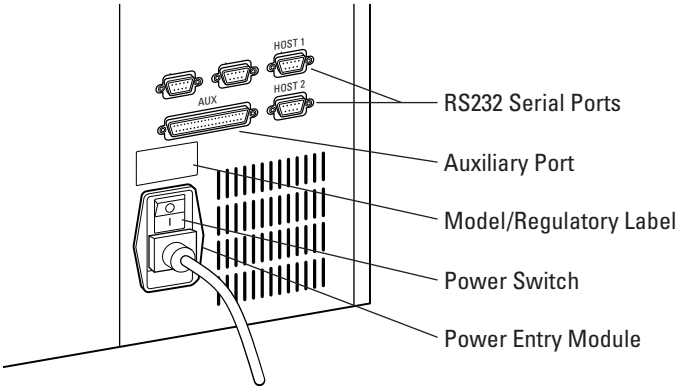
The input/output panel includes the power switch, power entry module, and three RS232 serial ports (see figure). The HOST 1 serial port is used for connecting to the host computer. (The other two serial ports are intended for future instrument options.)



Input/Output Panel (Analyst AD)

Input/Output Panel (Analyst HT)

The input/output panel includes the power switch, power entry module, auxiliary port, and four RS232 serial ports (see figure). The two HOST serial ports have identical capabilities, and provide flexibility in lab configurations. (In an early release, the instrument had two serial ports labeled COM 1 and COM 2.) The instrument can receive or send data through one serial port or use the other serial port to send data to a second computer. (The other two serial ports are intended for future instrument options.)



Input/Output Panel (Analyst HT)

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External Control Box (Analyst HT)

The optional external control box provides an alternative method for alerting the user to fault and service conditions. Instead of using a serial port connection, the instrument is connected to a peripheral device through a double-pull, double-throw contact closure. The box consists of a 37-pin connector that plugs into the 'AUX' (auxiliary) port on the instrument, a six-foot cable and terminal block enclosed in a plastic box.

The contact closures can be wired as normally-open or normally-closed. In addition, a 24-volt source is provided for powering peripherals that require an external power source. The following outputs are provided:

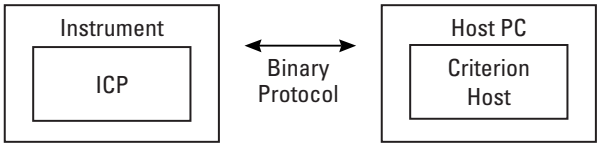
- **Fault**. Opens or closes when a fault condition occurs. Faults include all conditions listed in section 5.3, 'Fault Conditions.'
- **Service**. Opens or closes when a service condition occurs. For further information, see section 5.2, 'Service Conditions.'

For details on electrical specifications and pin connections, please refer to section 6.2, 'Technical Reference Information, External Control Box.'

CriterionHost Application and Instrument Control Program (Analyst AD)

The CriterionHost application, which runs under Windows 95, 98, or NT on the host computer, provides an easy-to-use graphical user interface (GUI) for setting up the system, defining plates and methods, and reading plates. Although a broad range of options is provided, the CriterionHost application represents a subset of the full functionality of the instrument.

The Instrument Control Program (ICP) runs on the instrument, controlling the optical, mechanical, and electronic systems within the instrument. The CriterionHost application converts the user's field entries and button selections into commands that are sent to the ICP. Results from the ICP are translated into a graphical representation of the data report. The ICP communicates with the CriterionHost application using a binary protocol.



Instrument Control Program Interface

The ICP can be upgraded by qualified service personnel using the internal floppy disk drive.

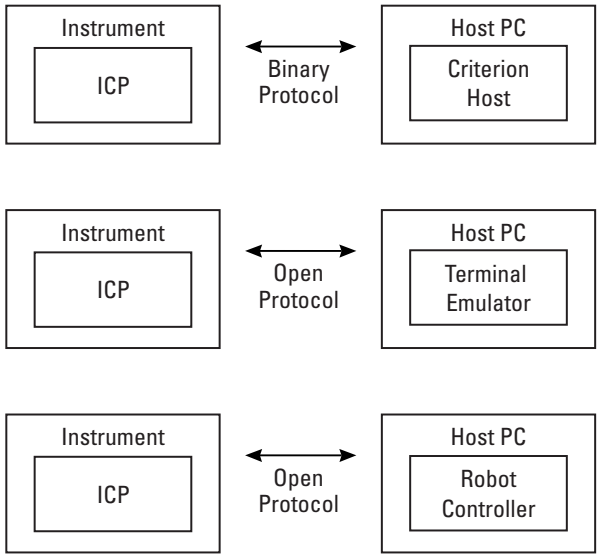
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CriterionHost Application and Instrument Control Program (Analyst HT)

The CriterionHost application, which runs under Windows 95, 98, or NT on the host computer, provides an easy-to-use graphical user interface (GUI) for setting up the system, defining plates and methods, and reading plates. Although a broad range of options is provided, the CriterionHost application represents a subset of the full functionality of the instrument.

The Instrument Control Program (ICP) runs on the instrument, controlling the optical, mechanical, and electronic systems within the instrument. The internal floppy disk drive is used by qualified service personnel to upgrade the ICP.

The ICP uses the Open Protocol to communicate with a terminal-emulation program or Robot Controller. The Open Protocol uses strings of ASCII text characters to change instrument parameters and query for results.



Instrument Control Program Interface Options

The ICP also communicates with the CriterionHost application using a binary protocol. The CriterionHost application converts the user's field entries and button selections into commands that are sent to the ICP. Results from the ICP are translated into a graphical representation of the data report.

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1.5 OVERVIEW OF OPERATION

In everyday operation, the operator performs three types of procedures:

- Setting Up the Instrument
- Defining Methods
- Running Assays

The host computer must be used to set up the instrument and define the detection methods. Once this is done, plate processing can be initiated from either the host computer or the control panel.

Setting Up the Instrument: Before using the system, the operator must configure the instrument, in particular, specifying which filters and dichroic mirrors are installed, the types of plates that will be used, and several other parameters (see sections 2.2, 'Setting Up the System,' and 2.3, 'Defining Microplates').

Defining Methods: Once the instrument has been configured, the operator can begin defining methods. First, the operator selects one of the seven modes (F, HEFP, TRF, L, FLUM, A, or EA), assigns a name to the method, and then specifies the parameters that the instrument will use when reading the plates. Each of the seven modes has a distinct set of parameters (see section 2.4, 'Defining Methods'). The system allows the user to runs two methods on each well using Multi-Method mode (see 'Defining Multi-Methods,' page 80).

Running Assays: Once a method has been defined, microplate processing can be initiated from either the host computer or the instrument control panel (see section 2.5, 'Reading Microplates').

Plate Transfer Options (Analyst AD)

Microplates can be delivered and picked up from the instrument in two ways:

- Traditional Transfer
- Magazine Transfer

Traditional Transfer (Stacker Not Present or Disabled): Microplates are delivered to and picked up from a single position known as the transfer position. Traditional Transfer occurs:

- With any instrument without a stacker, and
- With any instrument with a stacker that has been disabled.

Magazine Transfer (Stacker Enabled): Microplates are delivered by a plate magazine to the input position and ejected at the output position.

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The following table summarizes the plate transfer options:

Model	Stacker Enabled?	Magazines In Use?	Operational Mode	Load/Eject Positions
Instrument without Stacker	NA	NA	Traditional Transfer	Transfer Position
Instrument with Stacker	No	NA	Traditional Transfer	Transfer Position
	Yes	Yes	Magazine Transfer	Input/Output Positions

Plate Transfer Options (Analyst HT)

Microplates can be delivered and picked up from the instrument in three ways:

- Process Compression Transfer
- Traditional Transfer
- Magazine Transfer

Process Compression Transfer (Stacker Enabled): Microplates are delivered by the robot to the input position and picked up from the output position. (See page 12 for an illustration of the stacker positions.) This can reduce robot travel time because the robot does not have to wait for the microplate to be read. Process Compression is available only for instruments that include the stacker, and the stacker must be enabled. For further information, see section 2.2, 'Setting Up the System.'

Traditional Transfer (Stacker Not Present or Disabled): Microplates are delivered to and picked up from a single position known as the transfer position. Traditional Transfer occurs:

- With any instrument without a stacker, and
- With any instrument with a stacker that has been disabled.

Magazine Transfer (Stacker Enabled): Microplates are delivered by a plate magazine to the input position and ejected at the output position.

The following table summarizes the plate transfer options:

Model	Stacker Enabled?	Magazines In Use?	Operational Mode	Load/Eject Positions
Instrument without Stacker	NA	NA	Traditional Transfer (Manual or Robot)	Transfer Position
Instrument with Stacker	No	NA	Traditional Transfer (Manual or Robot)	Transfer Position
	Yes	No	Process Compression (Manual or Robot)	Input/Output Positions
	Yes	Yes	Magazine Transfer	Input/Output Positions

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Certain Open Protocol commands (**eject**, **load**, **reset**, **reverse**, **start**) are affected by whether the stacker is enabled or disabled. For example, the **start** command instructs the instrument as follows:

- With the stacker enabled, the instrument loads a microplate from the *input* position, reads, and ejects the microplate at the *output* position (Process Compression).
- With the stacker disabled or not present (Traditional Plate Transfer), the instrument accepts a microplate at the *transfer* position, reads, and ejects the plate at the *transfer* position.

For further information, see section 3.2, 'Open Protocol Commands.'

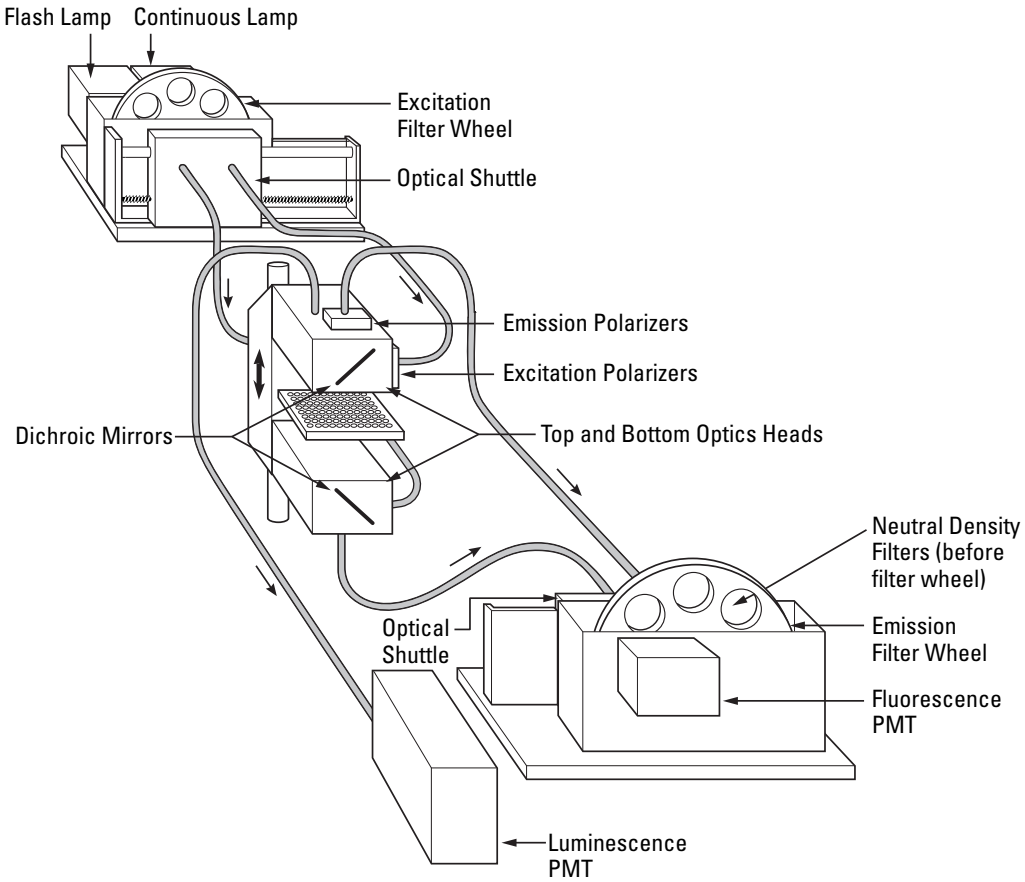
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1.6 THEORY OF OPERATION

The instrument's modular optical and electronic design combines high-quality components for each of four detection modes into a single, rugged, high-performance mechanical system. Each mode (F, HEFP, TRF, L, FLUM, A, and EA) is optimized for high sensitivity and extended dynamic range by using separate components when necessary. For instance, fluorescence and luminescence modes use different photomultiplier tubes (PMT). The luminescence PMT has relatively low dark counts and a response shifted to the blue-green for enhanced performance in typical luminescence assays.

Optical System

The heart of the instrument is the SmartOptics™ optical system, a flexible system of light sources, optics, and detectors that delivers the highest performance available using a microplate format. (Patents are pending on various aspects of the SmartOptics design.) A diagram of the optical system is shown below.



Optical System

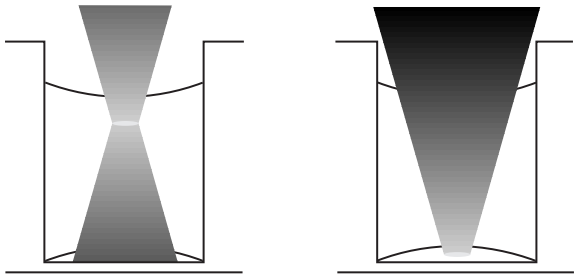
SmartOptics starts with two light sources. A high-intensity, xenon-arc lamp provides the light required for fluorescence intensity and fluorescence polarization modes. The continuous light source provides more total photons than the flash source, resulting

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in both higher sensitivity and shorter read times. The continuous source has a hot mirror that blocks heat-producing infrared light and limits ultraviolet light with wavelengths below 380 nm from entering the system. The flash source is used with the time-resolved fluorescence mode, producing light during a brief interval before the signal from the sample well is integrated.

During operation, light from the continuous lamp or flash lamp first passes through an excitation interference filter. After passing through the filter wheel, the light enters a fiber optic cable, which can be positioned by the optical shuttle to direct the light to either the top or bottom optics head (depending on the assay). Light is transmitted through and reflected by a dichroic mirror that directs light into the assay well. The top and bottom optics are epifluorescent, with the excitation and emission light traveling the same path in the optics head.

The focal height or 'z-height' of the top and bottom optics heads is controlled by a motor drive system and can be adjusted for optimum assay performance, a feature called 'Dynamic Z.' Light from the top or bottom optics head is focused into the assay well. Using Dynamic Z, the sensed volume can be precisely moved within the assay volume, optimizing the signal-to-noise (S/N) and signal-to-background (S/B) ratios. For further information, see 'Dynamic Z' later in section 1.6.



Focal height can be optimized for specific assays.

Light emitted from the assay well passes through the dichroic mirror and into a fiber optic cable that runs to another shuttle that positions the appropriate fiber optic cable in front of the detection system. The light may pass through one of two attenuators (neutral density filters). The user has the option to specify which neutral density filter, if any, is in the light path. The emitted light then passes through an emission interference filter. Finally, the light is detected by the fluorescence or luminescence photomultiplier tube.

A microplate sensor is mounted on the top optics head to prevent the plate from contacting the optics head in case the plate is misaligned, not properly specified, or the z-height is set incorrectly. If this 'top-of-plate' sensor detects a fault, the plate will be ejected prior to reading.

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Measurement Modes (F, HEFP, TRF, L, FLUM, A, EA)

There are seven optical measurement modes:

- Fluorescence Intensity (F)
- High Efficiency Fluorescence Polarization (HEFP)
- Time-Resolved Fluorescence (TRF)
- Luminescence (L)
- Focused Luminescence (FLUM)
- Absorbance (A)
- Epi-Absorbance (EA)

Each of these modes is described below.

Fluorescence Intensity Mode

Fluorescence intensity (F) measurements typically use the continuous light source. Alternatively, the flash lamp can be used for faster read times when high sensitivity is not required.

After passing through a fluorophore-specific excitation filter, the light is routed through a low-fluorescence fiber optic cable to the read head. A dichroic mirror reflects light into the assay well. Within the read head, the light emitted from the assay well travels back through the same path as the excitation light. The emitted light is transmitted through the dichroic mirror and through a fiber optic cable to an emission filter that conditions the light before detection by the photomultiplier tube.

The instrument uses focusing optics to direct the excitation light into the assay well and to detect the light emitted from the well. The actual sensed volume in the well is small compared to the overall volume of the well, especially for 96-well microplates. The location of the sensed volume does not change when 384-well microplates are used. Because the sensed volume remains the same, performance in 96- and 384-well plates is similar.

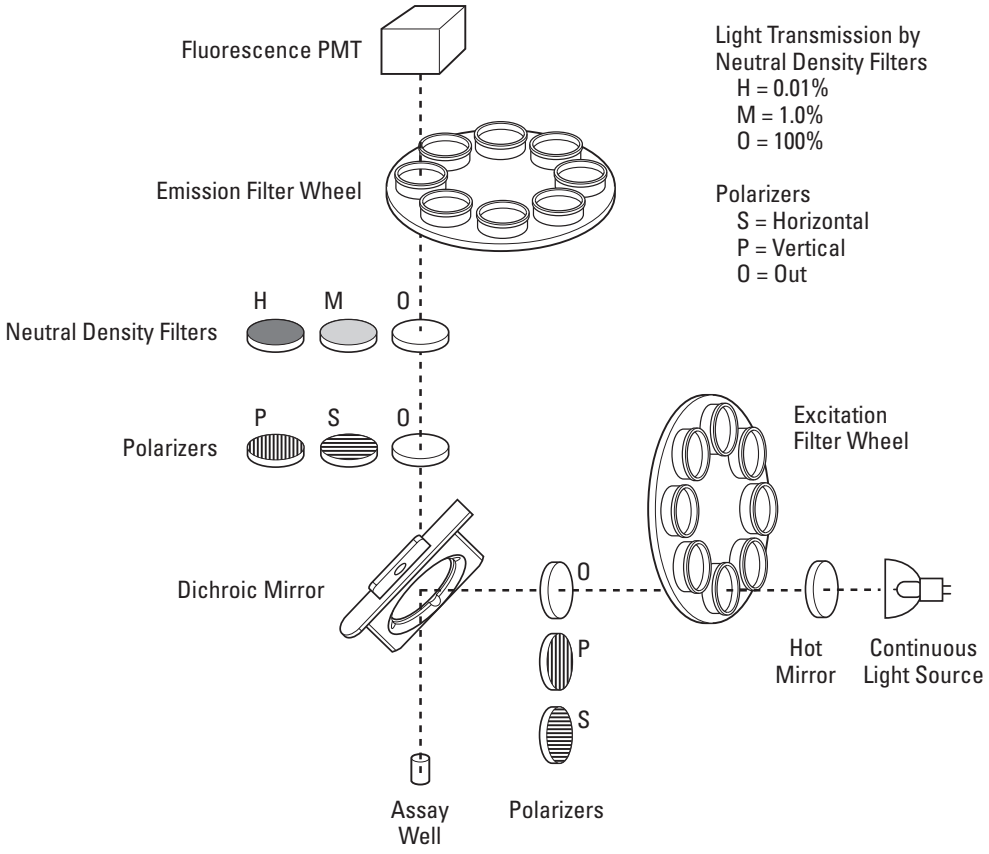
For homogeneous assays, the location of the sensed volume with the highest signal-to-noise ratio (S/N) and highest signal-to-background ratio (S/B) is in the middle of the well. For cell-based assays, better performance is typically achieved by moving the sensed volume to the bottom of the well to increase signal from fluorophores in the cells and reduce noise from fluorescence in the assay buffer. The z-height for optimal S/N and S/B can best be determined empirically. In 384-well microplates, the user can adjust the z-height to accurately position the sensed volume in the well. Due to the small geometry, the location of the sensed volume in 384-well plates stays the same.

Fluorescence measurements can be made from either the top or bottom of the microplate. Using bottom optics typically delivers a lower S/N because of increased autofluorescence from the plate plastic. However, the sensed volume is larger when using the bottom instead of the top optics. All other factors being equal, the total signal collected using the bottom optics is greater than the total signal collected using the top optics. The bottom optics are optimized for detection in cell-based assays.

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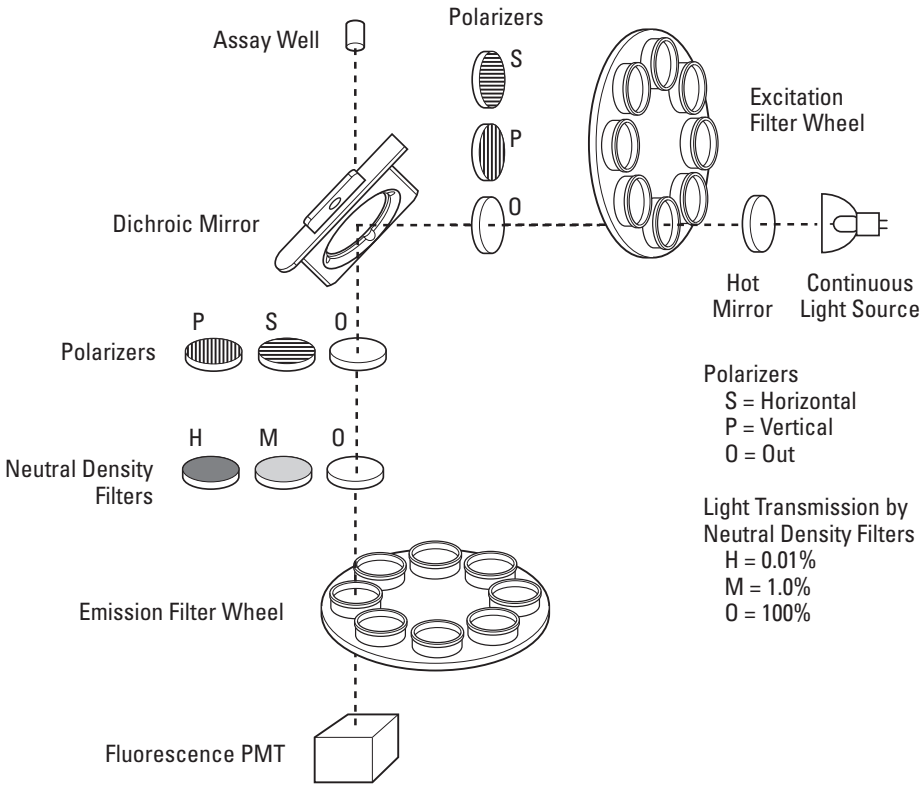
The user controls most instrument settings through the operating software. For fluorescence measurements, the user specifies top or bottom optics, lamp, plate format, excitation and emission filters, wells to be read (sample and background), z-height adjustment, detection units, attenuation factor, number of readings per well, integration (read) time, and (if the flash lamp is selected) interval between flashes and delay after flash. Other variable parameters include the polarizer orientation (if used), detector counting option and sensitivity, duration and intensity of plate shaking, plate settling time, delay before first read, delay between reads, number of reads, and (if the flash lamp is used) the flashlamp voltage.

The user has the option to manually install a fluorophore-specific dichroic mirror to maximize sensitivity or use the 50:50 beamsplitter, which is suitable for a broad range of fluorophores.



Fluorescence Intensity Mode, Top Optics

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Fluorescence Intensity Mode, Bottom Optics

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Fluorescence Polarization Mode

Fluorescence polarization (HEFP) measurements use the same optical configuration as fluorescence intensity measurements except for the addition of emission and excitation polarization filters. The instrument makes two measurements for each well. HEFP measurements typically use the continuous light source, but the flash lamp can be used for faster read times when high sensitivity is required. Light from the continuous or flash lamp light source passes through an excitation filter.

In the standard configuration, a dynamic (movable) polarization filter polarizes the light in either the S or P orientation. In all Criterion instruments, the S and P polarizers correspond to horizontal and vertical orientations respectively. (The user can select either the excitation or emission polarizers to be dynamic. The static polarizer can be set to be either S or P.)

A dichroic mirror then splits the light, reflecting the polarized light into the assay well. Epifluorescent light emitted from the assay well is transmitted through the dichroic mirror and the static polarization filter.

After passing through the emission filter, the polarized light is detected by the fluorescence photomultiplier tube. The instrument makes the second measurement after the dynamic polarization filter moves automatically into the alternate S or P position. The report lists the fluorescence intensity for the S and P orientations and the calculated polarization is expressed in mP (millipolarization units).

As in the F and TRF modes, focusing optics direct the excitation light into a small sensed volume. The location of the sensed volume can be changed using the z-height parameter. For HEFP measurements, the best signal-to-noise ratio (S/N) is typically found when the sensed volume is in the middle of each well. This minimizes spurious polarization signals from fluorophores bound to the well surfaces. The z-height for optimal S/N and S/B can best be determined empirically. Due to the small geometry, the location of the sensed volume in 384-well plates stays the same.

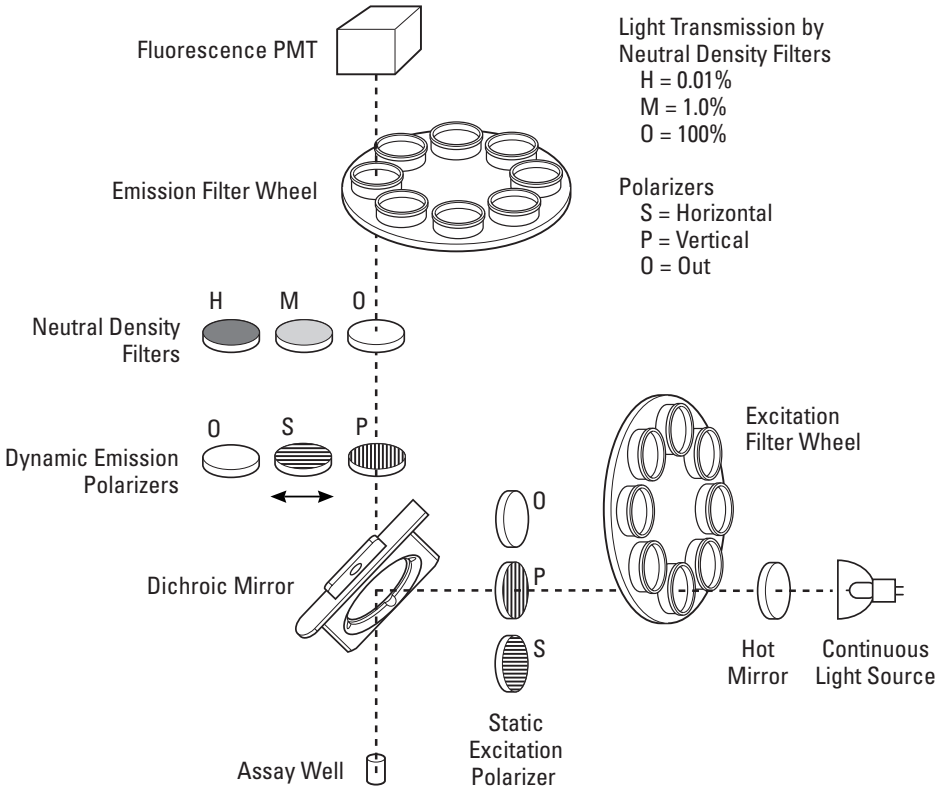
HEFP measurements are read only from the top of the plate.

The user has the option to manually install a fluorophore-specific dichroic mirror to maximize sensitivity or use the 50:50 beamsplitter, which is suitable for a broad range of fluorophores.

For HEFP measurements, the user specifies the lamp, plate format, excitation and emission filters, whether to switch polarization after each well or after each plate, the wells to be read (sample and background), G (grating) factor adjustment, z-height adjustment, detection units, attenuation factor, number of readings per well, integration (read) time, and (if the flash lamp is selected) the interval between flashes and delay after flash. Other variable parameters include which polarizer will be static, polarizer orientation, detector counting option and sensitivity, duration and intensity of plate shaking, plate settling time, number of readings per well, z-height, and (if the flash lamp is used) the flashlamp voltage.

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Using the G Factor in HEFP Mode: The G or grating factor is used in the fluorescence polarization mode to correct polarization data for optical artifacts. That is, an appropriately assigned G factor converts relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. Thus, the G factor is a correction for instrumental bias. Examples of instrumental hardware that may impact the G factor determination include excitation and emission filters, 50:50 beamsplitters, dichroic mirrors, polarizers and attenuators. For information about empirically determining the G factor, see section 2.6, 'Performance Verification.'



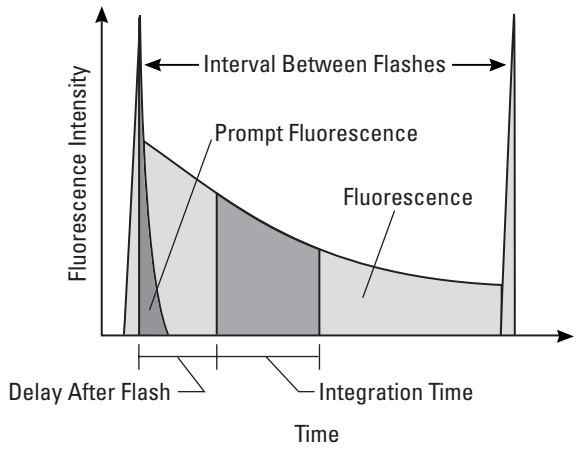
Fluorescence Polarization Mode

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Time-Resolved Fluorescence Mode

Time-resolved fluorescence (TRF) measurements use a xenon flash lamp as the light source. Since the flash lamp does not use a hot mirror, it transmits wavelengths from 250–720 nm.

After passing through an excitation filter, the light passes through a fiber optic cable to the read head where a dichroic mirror reflects light into the assay well. Epifluorescent light emitted from the assay well passes through the dichroic mirror and through a fiber optic cable to an emission filter before detection by the photomultiplier tube.



TRF Decay

As in the F and HEFP modes, focusing optics direct the excitation light into a small sensed volume. The location of the sensed volume can be changed using the z-height parameter. The z-height for optimal S/N and S/B can best be determined empirically.

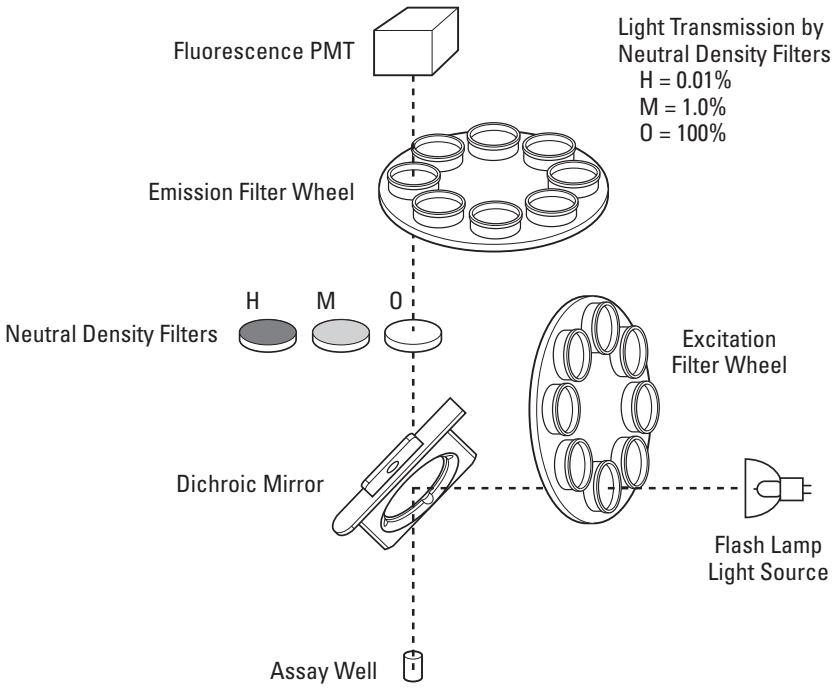
TRF measurements are made from either the top or bottom of the microplate.

The user has the option to manually install a fluorophore-specific dichroic mirror to maximize sensitivity or use the 50:50 beamsplitter, which is suitable for a broad range of fluorophores. Since almost all TRF measurements use lanthanide probes, a 400nm dichroic mirror (article number 42-0039) is recommended.

***Note**: TR-FRET measurements, which require reading the emission of two different fluorophores, is supported using Multi-Method mode (see 'Defining Multi-Methods,' page 80).*

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For TRF measurements, the user specifies top or bottom optics, plate format, excitation and emission filters, wells to be read (sample and background), z-height adjustment, detection units, attenuation factor, number of flashes per well, interval between flashes, delay time after flash, and integration (read) time. Other variable parameters include the detector counting option and sensitivity, duration and intensity of plate shaking, plate settling time, delay before first read, delay between reads, number of reads, and flashlamp voltage.



TRF Mode

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Luminescence Mode

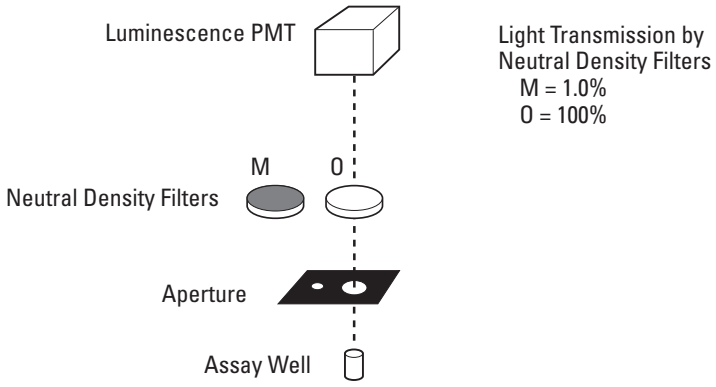
Luminescence (L) measurements use a luminescence read head with a dedicated fiber optic cable and photomultiplier tube separate from those used in fluorescence measurements. Light emitted from the assay well passes through an aperture optimized for either 96- or 384-well reading to reduce cross-talk. The light is directed through a low-fluorescence fiber optic cable to the luminescence photomultiplier tube.

Luminescence measurements are made only from the top of the plate.

The user sets the aperture for 96- or 384-well plates by manually moving a slide in the top optics head. For further information, see section 4.5, 'Setting the Luminescence Aperture.'

For luminescence measurements, the user can specify the plate format, wells to be read (sample and background), luminescence height adjustment, number of readings per well, integration (read) time, detection units, , and attenuation factor. Other variable parameters include the duration and intensity of plate shaking, plate settling time, delay before first read, delay between reads, and number of reads.

The system uses only digital counting (see 'Detector Counting Options,' page 36) for L measurements.



Luminescence Mode

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Focused Luminescence Mode

Focused Luminescence Mode (FLUM) enables low-crosstalk luminescence detection for 'low-profile' microplates (4 mm or less in height).

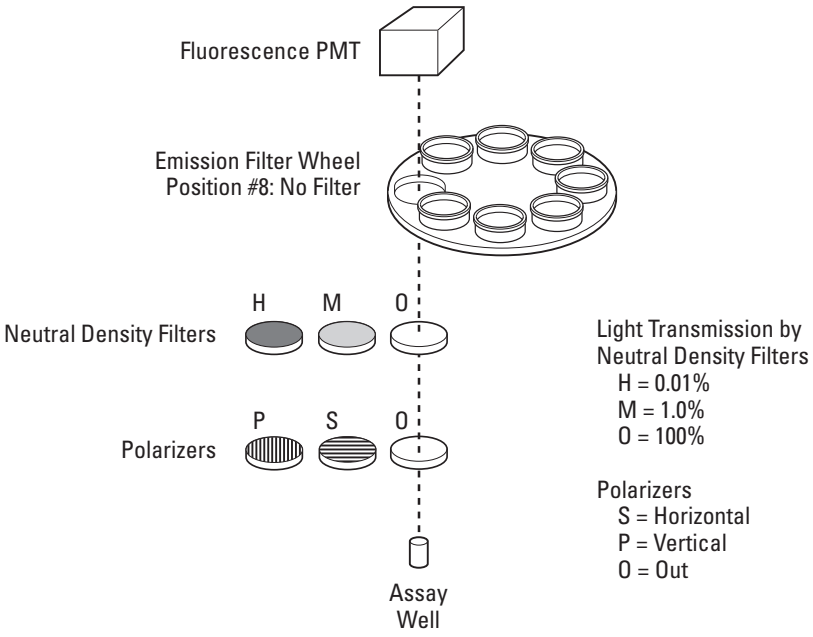
In low-profile plates, crosstalk from adjacent wells can result in inaccurate readings. To preclude this, FLUM measurements use the same optical configuration as fluorescence measurements (including the fluorescence read head and fluorescence photomultiplier tube), except that only the top optics are used and no light source is used. Light emitted from the assay well is transmitted through the fluorescence focusing lenses and then through a fiber optic cable to an emission filter that conditions the light before detection by the photomultiplier tube.

FLUM measurements are much less sensitive than L measurements of luminescence (roughly, one-quarter as sensitive), because 1) for FLUM measurements, the light goes through an optical system with lenses; for L measurements, it does not, and 2) the fluorescence photomultiplier tube used for FLUM measurements is optimized for fluorescence assays rather than for luminescence assays. Consequently, FLUM should be used only with relatively 'bright' assays and when standard L mode results in unacceptably high crosstalk (greater than 10 percent).

For FLUM measurements, the user specifies the plate format, emission filter wheel position, wells to be read (sample and background), z-height adjustment, number of readings per well, integration (read) time, detection units, and attenuation factor. Other variable parameters include the polarizer orientation (if used), detector counting option and sensitivity, duration and intensity of plate shaking, plate settling time, delay before first read, delay between reads, and number of reads.

For highest sensitivity, remove the dichroic mirror in the top optics head. Focused Luminescence methods require that emission filter-wheel position #8 be open; remove any filter or metal slug from position #8.

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Focused Luminescence Mode

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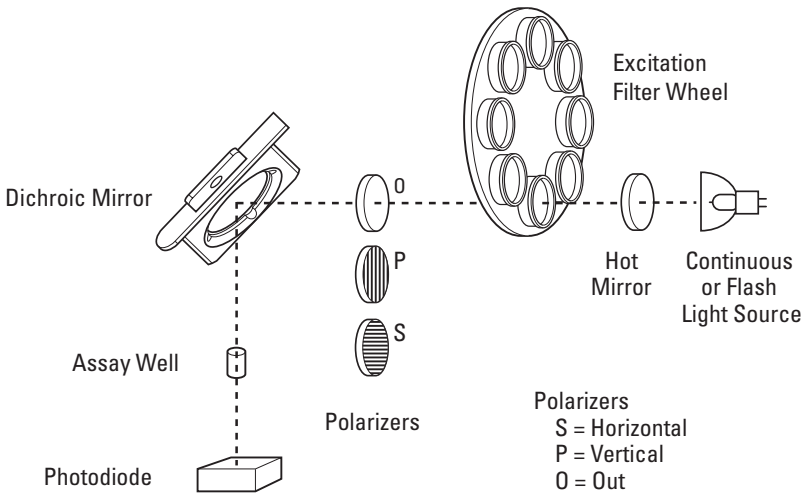
Absorbance Mode

Absorbance (A) measurements typically use the continuous light source. Alternatively, the flash lamp can be used for faster read times when high sensitivity is not required.

After passing through a bandpass filter in the excitation filter wheel, the light is routed through a low-fluorescence fiber optic cable to the read head. (Only the top optics are used.) A dichroic mirror reflects light into the assay well. The light that passes through the bottom of the assay well is sensed by a photodiode positioned beneath the assay well. According to the Beer-Lambert law, the concentration of the analyte in the well will be inversely proportional to the intensity of the light passing through the well.

Important! *Since measurements are made of light passing through the assay well, only clear microplates may be used.*

For absorbance measurements, the user specifies the lamp, plate format, excitation filter (which should be a bandpass filter of the appropriate wavelength), wells to be read (sample and background), z-height adjustment, detection units, number of readings per well, integration (read) time, and (if the flash lamp is selected) interval between flashes and delay after flash. Other variable parameters include the absorbance factor and buffer well for automatic gain setting or the coarse and fine adjustment sliders for manual gain setting; duration and intensity of plate shaking, plate settling time, delay before first read, delay between reads, number of reads, and (if the flash lamp is used) the flashlamp voltage.



Absorbance Mode

Note: *Analyst HTS Assay Detection Systems do not have absorbance capability.*

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Epi-Absorbance Mode

Epi-absorbance is similar to trans-absorbance in that the concentration of chromaphores can be deduced from the amount of light absorbed in a defined path-length as compared to a "background" or "blank" reference. Epi-absorbance differs from trans-absorbance in that the light entering the top of the well exits from the top of the well after being reflected off the bottom surface of the well.

Epi-Absorbance (EA) measurements use the same optical configuration as fluorescence intensity measurements, except only the top optics are used. Typically, the continuous light source is used; alternatively, the flash lamp can be used for faster read times when high sensitivity is not required.

After passing through a bandpass filter in the excitation filter wheel, the light is routed through a fiber-optic cable to the read head. A dichroic mirror reflects light into the assay well. The light reflected from the bottom of the assay well travels back through the same path as the excitation light. The reflected light is transmitted through the dichroic mirror and through a fiber optic cable to a neutral-density filter that reduces the amount of light before detection by the fluorescence photomultiplier tube.

As in the F, HEFP, and TRF modes, focusing optics direct the light into the well. The z-height for optimal S/N and S/B can best be determined empirically.

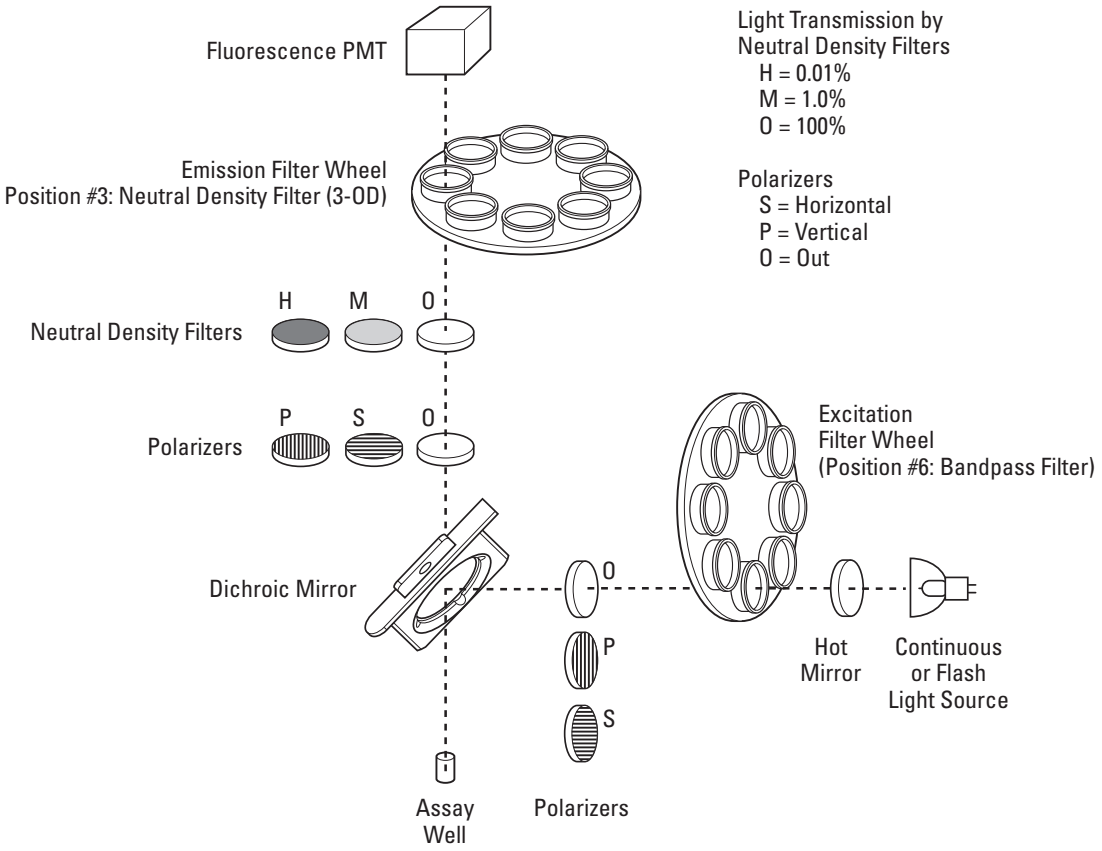
For EA measurements, white plates provide the greatest reflection of light off the bottom of the assay well. Black plates provide the next greatest reflection; clear plates the least reflection, but may be acceptable.

Because the intensity of the light reflected depends upon the particular plates that are used, the system allows the user to calibrate the readings by subtracting readings in background wells (which typically contain only buffer) from the readings in sample wells. (For more information, see 'Background Subtraction,' page 45.)

The user specifies the lamp, plate format, excitation filter wheel position and emission filter wheel position, sample and background wells to be read, z-height adjustment, detection units, attenuation factor, number of readings per well, integration (read) time, and (if the flash lamp is selected) the interval between flashes and delay after flash. Other variable parameters include the detector counting option and sensitivity, duration and intensity of plate shaking, plate settling time, delay before first read, delay between reads, number of reads, and (if the flash lamp is used) the flashlamp voltage.

In the default EA method, emission filter-wheel position #3 is designated for the 3 OD neutral density filter.

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Epi-Absorbance Mode

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Detector Counting Options (PMT Setup)

The photomultiplier tube (PMT), which is used for all measurement modes except Absorbance (which uses a photodiode instead), converts the photons (particles of light) emitted from the assay well into pulses of electrical current. These current pulses are converted into a reading of counts (the total number of current pulses) or counts per second (CPS).

The system provides three detector counting options for performing this current-to-count conversion: digital, SmartRead, and SmartRead Plus. Any of these three detector counting options can be used with the fluorescence intensity mode (F) and fluorescence polarization mode (HEFP). Time-resolved fluorescence mode (TRF) uses only the digital and SmartRead detector counting options. Luminescence (L) mode and focused luminescence mode (FLUM) use only the digital detector counting option, since luminescent signals are generally low-level and are best detected with photon counting. Epi-absorbance mode (EA) uses only the SmartRead and SmartRead Plus detector counting options, since the signal levels are too high for digital detector counting.

The operator specifies the detector counting option by first clicking the Advanced button in the Define and Edit Methods screen and then, in the Advanced Setup screen, choosing one of the options shown in the PMT Setup box.

Selecting the appropriate PMT counting option helps optimize assay performance by improving precision, increasing sensitivity, and extending dynamic range. This section describes the three PMT counting options and how to select the appropriate sensitivity setting for the SmartRead detector counting options.

Overview

Digital detector counting has a more limited dynamic range than the SmartRead detector counting options, but it achieves high sensitivity at very low signal levels. The detector circuit simply counts the individual current pulses generated as individual photons strike the PMT. Limitations of the current-pulse detector circuitry lead to nonlinearity (saturation) at about one million counts per second (CPS). Optimal uses include determination of the lower detection limit (LDL) for L, FLUM, and TRF methods.

SmartRead detector counting is less sensitive than digital counting at low signal levels but can accommodate higher signal levels and offers increased dynamic range than digital counting. The current pulses generated by the PMT are integrated (accumulated and stored) in a capacitor (a component in an electronic circuit that stores current). If the capacitor does not become fully charged before the end of the integration time (that is, the read time) specified by the operator, SmartRead uses the amount of current accumulated in the capacitor to determine the CPS. If the capacitor does become fully charged before the end of the specified integration time, SmartRead determines the time taken to fully charge the capacitor and uses that measurement to determine the CPS. Optimal uses include LDL and dynamic range readings for F, HEFP, and EA methods.

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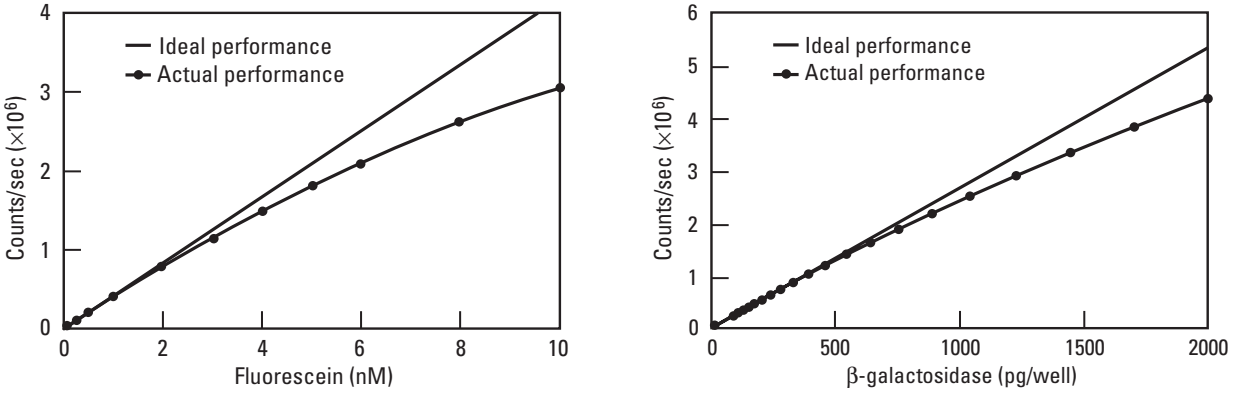
SmartRead Plus detector counting operates like SmartRead, except that the read head stays at each well only until the reading is complete. In the basic SmartRead, the read head stays at each well a fixed amount of time, regardless of how much time is required for the capacitor to reach full scale. SmartRead Plus therefore results in shorter processing time for each plate. SmartRead would be preferable to SmartRead Plus if the system is being used with a lab robot that expects a fixed read time for each plate, or if the operator attends the system periodically to unload and load stacks of plates and therefore needs to know just when each run will be complete.

Digital Detector Counting

When digital detector counting is used, the photomultiplier tube (PMT) detector circuit counts individual current pulses generated as individual photons strike the photocathode in the PMT. Since the photon-counting circuit can reject certain sources of background noise, digital counting is optimal for detecting low-level signals. Although photon counting can achieve high sensitivity, this advantage is somewhat offset by reduced dynamic range in comparison to SmartRead counting options.

With digital counting, the PMT is sensitive not only to the number of photons but also to the rate at which it receives the photons. Nonlinearity in digital counting begins at about one million counts per second (CPS) due to limitations in the photon counting circuit.

The instrument contains two PMTs, one optimized for fluorescence mode and the other for luminescence mode. The following figures show typical response curves for the fluorescence and luminescence PMTs when using digital counting.



Fluorescence **Luminescence**

PMT Response Curves (Digital Counting)

With the fluorescence PMT, non-linearity of digital counting is about 10 percent at 1.5 million CPS, 15 percent at 2 million CPS, 23 percent at 2.5 million CPS, and 27 percent at 3.0 million CPS.

With the luminescence PMT, non-linearity of digital counting is about 5 percent at 2 million CPS, 10 percent at 3 million CPS, and 20 percent at 4 million CPS.

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SmartRead and SmartRead Plus Detector Counting

When either of the SmartRead detector counting options is used, the system integrates (accumulates and stores) the PMT current in one of five capacitors, depending on the sensitivity setting. The current stored in the capacitor is proportional to the total number of photons collected by the PMT during the specified integration time. The system allows the user to select one of five sensitivity settings (0–4). The highest sensitivity (4) is achieved with the smallest capacitor; the lowest sensitivity (0) and highest full-scale signal reading are obtained with the largest capacitor.

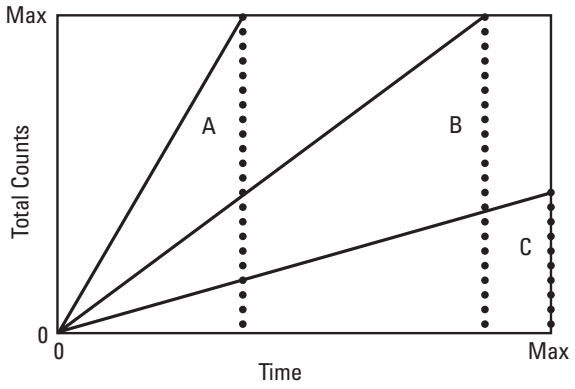
If the capacitor does not become fully charged before the end of the integration time, SmartRead uses the amount of current accumulated in the capacitor to determine the CPS. If the capacitor becomes fully charged before the end of the integration time, the CPS is calculated using the time taken for the capacitor to reach full scale (that is, to become fully charged). When the capacitor is fully charged, the total counts will be constant for each reading. This means that every reading will contain the same number of counts, even though the integration time (and therefore the count rate) will vary from reading to reading. Consequently, SmartRead and SmartRead Plus counting use CPS units.

In SmartRead and SmartRead Plus counting, saturation occurs at much higher count rates than in digital counting (typically one billion CPS) and is caused by actual PMT saturation. This differs from digital counting where the nonlinear response is due to limitations in the PMT current-pulse detector circuitry. This is because the SmartRead circuit integrates PMT current pulses rather than counting individual pulses.

The following figure illustrates SmartRead counting at three different signal levels. Measurement of CPS is based either on the time to reach maximum counts (that is, the time to fully charge the capacitor) or on the counts recorded at the maximum integration time, depending on signal intensity.

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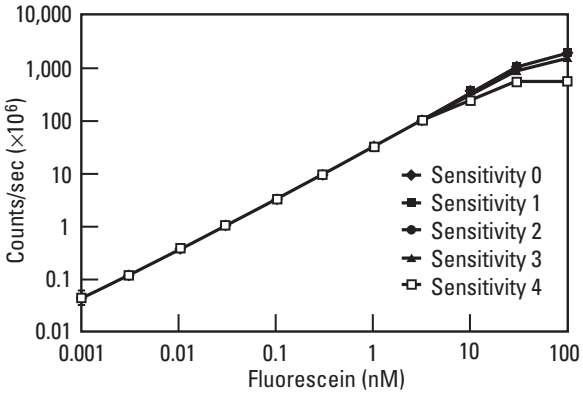
In cases A and B, the capacitor becomes fully charged before the end of the integration time; therefore, SmartRead will determine the CPS based on the time to reach maximum counts. In case C, the capacitor does not become fully charged before the end of the integration time; therefore, SmartRead will determine the CPS based on the counts recorded at the end of the integration time.



Schematic of SmartRead Counting

The time to full scale can be very short (less than 1.5 milliseconds) at high signal levels and/or small capacitor settings (high sensitivity settings). At these very short integration times, the flux from the light source is not sampled effectively and may contribute an undesirable level of imprecision. Therefore, it is important to select a sensitivity setting that will ensure an integration time of greater than 1.5 milliseconds. The recommended minimum integration time is 5 milliseconds; this accounts for limitations of the capacitor integration and fluctuations in lamp intensity.

The following figure shows PMT saturation in SmartRead or SmartRead Plus counting when using a 100-millisecond integration time. Sensitivities 0–2 afford linearity to about one billion CPS.



PMT Saturation in SmartRead or SmartRead Plus Counting

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Definitions of Counting Units

The relationship between the various unit choices is as follows:

- **Counts** = Number of raw counts acquired during the integration time. With the continuous light source, total counts equals CPS × integration time. With the flash lamp, total counts equals counts per flash × number of flashes.
- **Counts/Second** (CPS) = Counts ÷ Integration Time.
- **Intensity** = Counts/Second × Attenuator Factor.
- **RFU** = Counts/Second × Attenuator Factor.
- **RLU** = Counts/Second × Attenuator Factor.

The attenuator factor depends on the attenuator (neutral density filter) selected. When using RFUs or RLUs, the attenuator setting for each method determines the values of the attenuator factor (out = 0, medium = 100, high = 10,000). Select the neutral density filter based on the relative brightness of the well.

Table 1: Guidelines for selecting detector counting option and sensitivity setting

Detector Counting Option	Sensitivity Setting	Full-Scale Counts	Optimal Signal Range (CPS)	Saturation (CPS), 100 msec read
Digital	NA	NA	less than 1,000,000	1,000,000
SmartRead or SmartRead Plus	**0**	1,560,000	1,560,000 to 312,000,000	1,000,000,000
	1	734,000	734,000 to 146,800,000	1,000,000,000
	2	156,000	156,000 to 31,200,000	1,000,000,000
	3	15,600	15,600 to 3,120,000	300,000,000
	4	3,400	3,400 to 680,000	100,000,000

Notes:

1. *In the column 'Optimal Signal Range,' the optimal signal ranges are determined as follows:*
   - *Digital counting: signals that do not saturate the digital counting circuit.*
   - *SmartRead and SmartRead Plus counting: signals that 1) fill the capacitor greater than 10 percent with a 100-millisecond integration time and 2) do not fill the capacitor in less than 5 milliseconds of integration time.*
2. *Digital saturation is due to limitations of the photon counting circuitry and is not caused by actual PMT saturation.*
3. *SmartRead and SmartRead Plus saturation levels are approximate due to instrument-to-instrument variability.*
4. *Values in the table above apply to any fluorophore.*

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Table 2: Selecting units and detector counting option based on measurement mode

Measurement Mode	Recommended Units	Digital Detector Counting	SmartRead or SmartRead Plus Detector Counting
Fluorescence Intensity (F) with continuous light source	CPS	Use only with low-level signals (<10^6 CPS).	**Optimal performance** (with appropriate sensitivity setting). Provides sensitivity and largest dynamic range.
Fluorescence (F) Intensity with flash lamp	counts	Not recommended.	**Optimal performance** (SmartRead) with sensitivity setting 4.
Fluorescence Polarization (HEFP)	CPS	Use only for low-level signals (<10^6 CPS).	**Optimal performance** (with appropriate sensitivity setting). Provides sensitivity and largest dynamic range.
Time-Resolved Fluorescence (TRF)	counts	**Optimal performance** for low-level signals (<10^6 CPS).	**Optimal performance** (SmartRead) for high-level signals (>10^6 CPS) with sensitivity setting 4.
Luminescence (L)	CPS	**Optimal performance.**	NA
Focused Luminescence (FLUM)	CPS	**Optimal performance.**	Not recommended.
Epi-Absorbance (EA)	CPS	Not recommended.	**Optimal performance** (with appropriate sensitivity setting).

Examples of SmartRead or SmartRead Plus Detector Counting

Example 1: A fluorescence-polarization assay is read using the continuous light source with a 100-millisecond integration time (100,000 microseconds). The average maximum signal levels for both S and P values is 4 million CPS. What is the appropriate detector counting option and sensitivity setting? Why not read the plate using digital counting?

Solution:

1. In Table 1, refer to the column 'Optimal Signal Range' to determine which SmartRead or SmartRead Plus sensitivity setting will accommodate 4,000,000 CPS without saturating. The table indicates that sensitivity settings 0–2 meet the criteria. The optimal setting depends on the percentage of full-scale counts used and on the integration time required for each sensitivity setting (that is, the capacitor) to reach full-scale counts. Performance is best when the percentage of full-scale counts is greater than 50 percent (though acceptable down to 10 percent) and the integration time is greater than 5 milliseconds.
2. For each sensitivity range, calculate the percentage of full-scale counts used by dividing the counts collected by the full-scale counts (shown in Table 1) and multiplying by 100. In this example, 4,000,000 CPS results in 400,000 counts in the 100-millisecond integration time (4,000,000 CPS × 0.1 second); therefore, sensitivity setting 0 uses 26 percent (400,000 counts ÷ 1,560,000 counts × 100), sensitivity setting 1 uses 54 percent (400,000 counts ÷ 734,000 counts × 100), and sensitivity setting 2

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uses 256 percent of the full-scale counts (400,000 counts ÷ 156,000 counts × 100). When the percentage of full-scale counts used is less than 100 percent, the counts are determined by the current stored in the capacitor; when the percentage of full-scale counts is greater than 100 percent, the counts are determined by the time to reach full-scale.

3 Calculate the time required to fill the capacitor associated with sensitivity setting 2 by dividing the full-scale counts by the signal level (156,000 counts ÷ 4,000,000 CPS = 39 milliseconds).

Sensitivity setting 1 or 2 will be optimal in this case because they both meet the criteria of greater than 50 percent full-scale counts and an integration time greater than 5 milliseconds.

***Note**: For SmartRead, sensitivity setting 2 will offer the same level of precision even if the integration time is decreased to 39 milliseconds, since any signal collected after 39 milliseconds of the specified 100-millisecond integration time will not be utilized (because the capacitor has already reached full-scale counts). For SmartRead Plus, sensitivity setting 2 will offer the same level of precision with a shorter read time, since the read head will move to the next well once the capacitor has reached full-scale counts.*

If read with digital detector counting, these signals would saturate the photon-counting circuit and therefore require the use of an attenuator to prevent saturation and consequent non-linearity. Reading with the medium attenuator would result in only a few thousand counts during an integration time of 100 milliseconds. This reduced count level results in higher CV's and translates into noisier mP data. The extended dynamic range of SmartRead detector counting allows the low CVs to be preserved since the attenuator is not required.

Example 2: A fluorescence-polarization assay has both high (specific signal) and low (background) signal levels on the same microplate. The signal levels range from 40,000 CPS to 20,000,000 CPS. Why read with SmartRead (or SmartRead plus) counting? What is the optimal sensitivity setting and integration time?

Solution:

When there are both high and low signal levels, SmartRead (or SmartRead Plus) counting is recommended over digital counting. For the high signal levels, SmartRead will measure the time required to charge the capacitor; for low signal level, it will measure the total charge collected in the integration capacitor at the end of the integration period. The best overall performance is obtained when both the low and high level signals are considered. Optimal performance will be achieved when the lowest signal levels fill the capacitor to at least 10 percent of full-scale counts and the highest signal levels do not fill the capacitor in less than 5 milliseconds of signal integration.

1 In Table 1, refer to the column 'Optimal Signal Range' to determine which SmartRead or SmartRead Plus sensitivity setting will accommodate 20,000,000 CPS without saturating and 40,000 CPS utilizing greater than 10 percent of the full-scale counts. The table indicates that none of the settings meet the criteria for both signals.

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In this case, evaluate the impact a 500-millisecond read time will have on the optimal ranges.

2 Calculate the time required to fill each capacitor (that is, for each sensitivity setting) with 20,000,000 CPS, the high signal level. The time required to fill a capacitor is calculated by dividing the full scale counts for the sensitivity setting by the assay signal level in CPS. Sensitivity setting 0 will require 78 milliseconds (1,560,000 counts ÷ 20,000,000 CPS); sensitivity setting 1 will require 37 milliseconds (734,000 counts ÷ 20,000,000 CPS). Similar calculations can be done for sensitivity settings 2–4.

3 Calculate the percentage of full-scale counts utilized for each sensitivity setting with an assay signal of 40,000 CPS (low signal level) and a 100- and 500-millisecond integration time. The percentage of full-scale counts utilized is calculated by dividing the counts collected by the full-scale counts for a particular sensitivity setting and multiplying by 100. The PMT collects 4,000 counts during a 100-millisecond read (40,000 CPS × 0.1 second) and 20,000 counts during a 500-milliseccond read (40,000 CPS × 0.5 second). Therefore, during the 100-milliseccond read, sensitivity setting 2 utilizes 2.6 percent of the full scale counts (4,000 counts ÷ 156,000 counts × 100), while the 500-milliseccond read utilizes 12.8 percent (20,000 counts ÷ 156,000 counts × 100). Similar calculations should be done for the other sensitivity settings.

Table 3: Summary Results for Example 2

Sensitivity Setting	Time (Milliseconds) Required to Fill Capacitor With 20×10^6 CPS	Percentage of Full-Scale Counts Used With 4×10^6 CPS	
		100-Millisecond Read	500-Millisecond Read
0	78	0.26	1.3
1	37	0.54	2.7
2	7.8	2.6	12.8
3	0.78	27	128
4	0.17	118	588

According to Table 3, none of the sensitivity settings meet the criteria for optimal signal detection with a 100-milliseccond integration time. That is, the high signal level must have an integration time greater than 5 milliseconds and the low signal level must utilize greater than 10 percent of the full-scale counts. However, when the integration time is increased to 500 milliseconds, there is a single sensitivity setting that will meet our criteria for optimal signal detection for both signal levels. Sensitivity setting 2 maintains a 7.8-milliseccond integration time for the high signal level while utilizing 12.8 percent of the full-scale counts for the low signal level.

Note: *With SmartRead, 500-millisecond integration times greatly increase overall plate-read times, since the read head will dwell over all wells for 500 milliseconds regardless of signal level. With SmartRead Plus, the read will be significantly faster, since the reading of high signal wells takes just 7.8 milliseconds, after which the read head moves on to the next well. The full 500-millisecond integration time is utilized only for low signal levels.*

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Example 3: A flash-fluorescence assay is read using the flash lamp source, with 100 flashes. The maximum signal level is 78,000 counts. What is the appropriate sensitivity setting?

Solution:

1. Calculate the number of counts per flash collected by the PMT by dividing the total counts by the number of flashes per well (78,000 ÷ 100 = 780).
2. In Table 1, refer to the column 'Full-Scale Counts' to determine which SmartRead or SmartRead Plus sensitivity setting will accommodate 780 counts and utilize greater than 10 percent of the full-scale counts. The table indicates that only sensitivity setting 4 satisfies the criteria. Choosing sensitivity settings 0–3 would result in a loss of precision because the percentage of full-scale counts would be less than 10 percent.
3. Calculate the percentage of full-scale counts used by dividing the counts collected by the full-scale counts (shown in Table 1) for a particular sensitivity setting and multiplying by 100. In this example, sensitivity setting 4 uses 23 percent of the full-scale counts (780 counts ÷ 3,400 counts × 100). Therefore, this is the optimal setting.

Note: *For flash fluorescence measurements, most of the signal is generated during the flash pulse, which lasts about one microsecond. However, integration times of 50 to 100 microseconds (with delay equal to zero) are used to ensure that all the fluorescent signal is captured and the circuit has time to respond. PMT saturation occurs above 1,000 counts per flash or 1 billion CPS (1000 counts ÷ 1 µsec flash duration). Therefore, flash fluorescence measurements are limited by PMT saturation and not by sensitivity setting since the smallest capacitor (sensitivity 4) will hold 1,000 counts per flash. Flash-fluorescence measurements, therefore, have at best a 1000-fold dynamic range.*

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Background Subtraction

In any measurement mode, the instrument can subtract readings in background wells from readings in sample wells. To specify that this be done, the user selects up to two sets of sample and background wells (one set for Absorbance methods) in the method definition. If the user selects multiple background wells, the system will average the background-well readings.

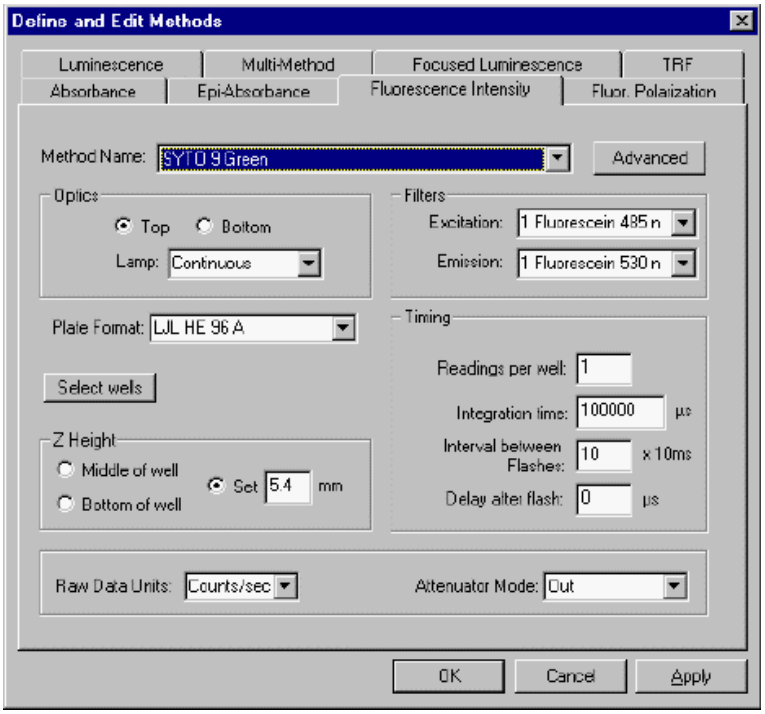
During the plate read, only raw data are reported; in the final report, the background-subtracted data will appear following the raw data. For multi-methods, the user can specify whether the background-subtracted data will appear before or after the raw data.

For HEFP methods, the instrument calculates, subtracts, and reports separate results for S and P backgrounds.

For details about specifying the background wells and sample wells on a plate, see 'Selecting Wells,' page 69.

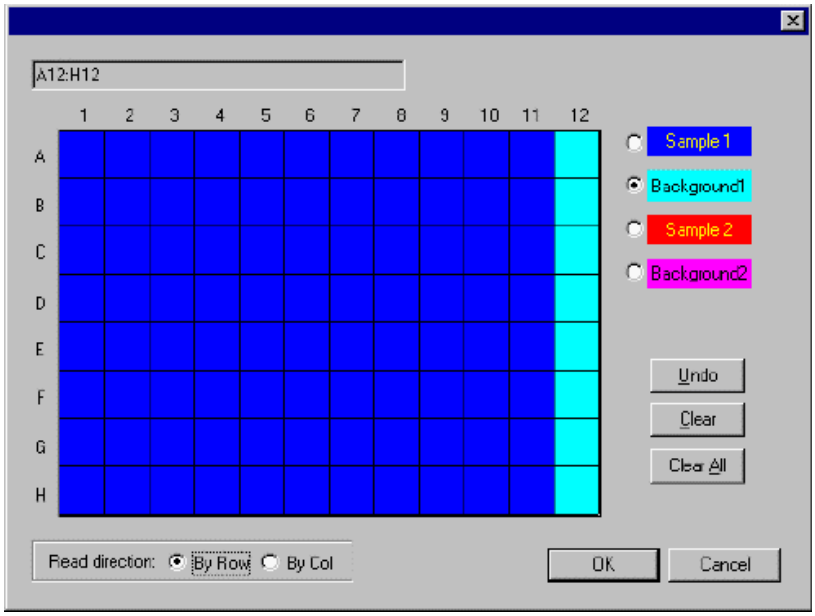
Example: An assay distinguishes between live and dead bacteria by utilizing SYTO 9™ green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain propidium iodide, and determining the ratio of the fluorescence at 530nm (live) to the fluorescence at 620nm (dead). The assay can be performed in a single run using Multi-Method mode with two methods, SYTO 9 Green and SYTO 9 Red, which differ only in that SYTO 9 Green uses the Fluorescein 530nm emission filter while SYTO 9 Red uses the Europium 620nm emission filter.

The illustration below shows the basic parameters for the SYTO 9 Green method:



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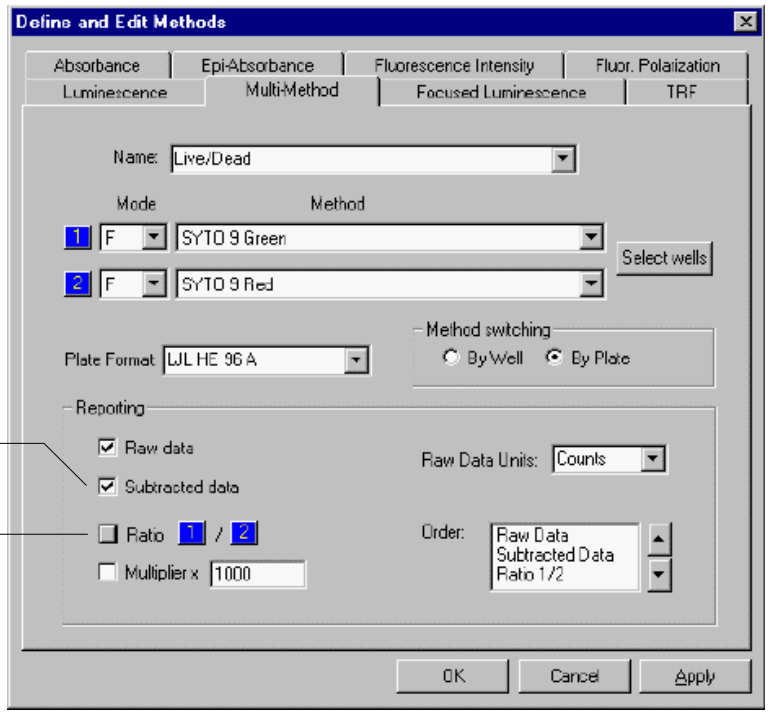
To factor out the endogenous fluorescence of the cells, both methods read a column of background wells (A12 through H12) that contain cells in buffer without stain:



The Multi-Method used to run the assay reads the fluorescence in each sample well, subtracts the average of the readings from the background wells, and calculates the ratio of the background-subtracted readings from the green-stained wells to the background-subtracted readings from the red-stained wells:

Specifies subtracted data to be calculated

Specifies ratio of method 1 data to method 2 data



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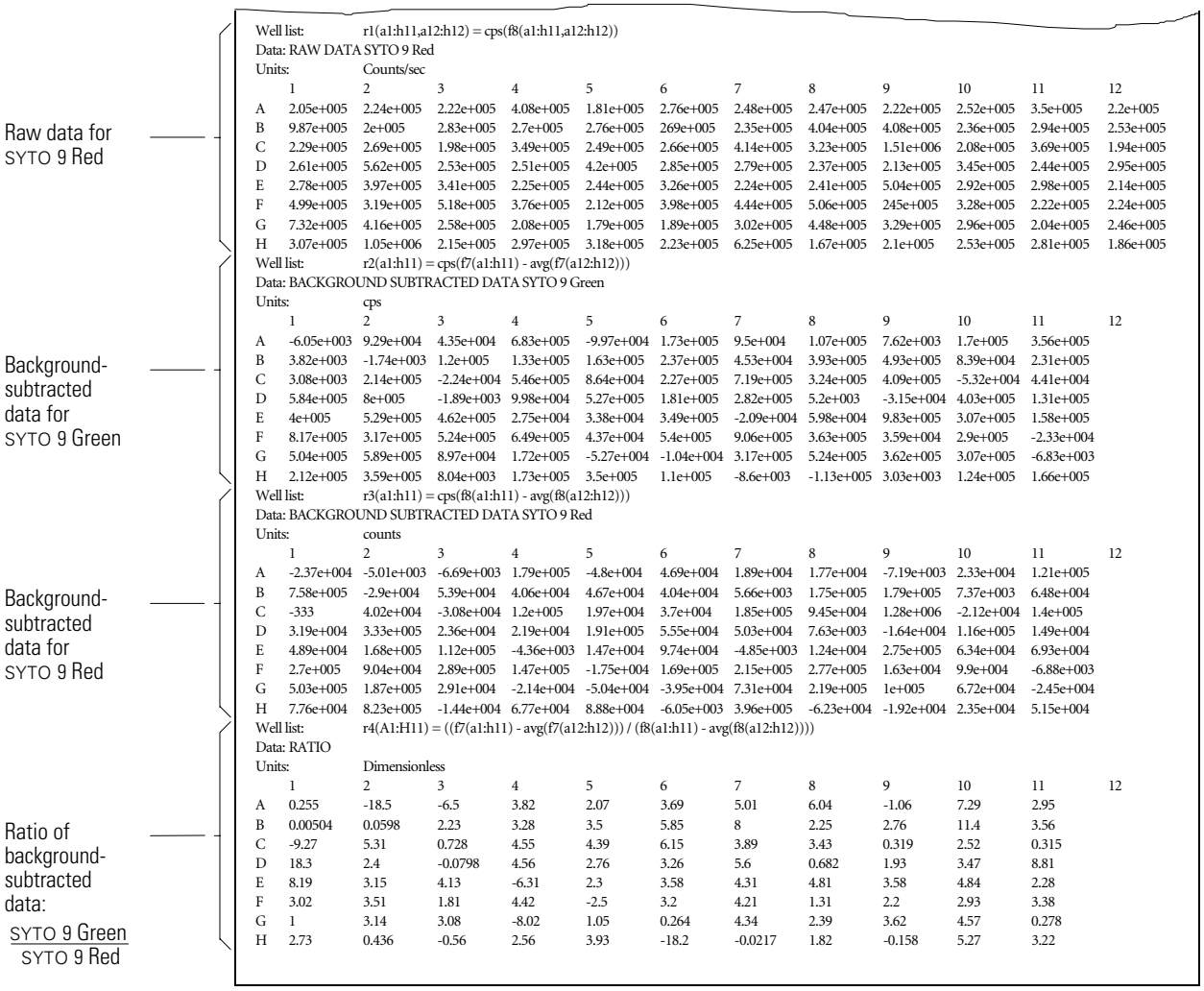
The report generated by the system for this assay is shown below:

Read time:	07/19/1999 14:14:50		
Kinetic read cycle:	1 of 1		
Read start delay time:	0	min	
Time delay between kinetic reads:		<n/a>	
Barcode:	<n/a>		
Method ID:			
Plate ID:	plate #5848		
Comment:			
Microplate format:	LJL HE 96 A		
Shake time:	0	s	
Temperature:	25.6	C	
Instrument tag:			
Serial number:	AN0097		
Read sequence:	row		
Mode sequence:	plate		
Detection mode:	f7		
Light sensor:	HC-120		
Excitation side:	Top		
Emission side:	Top		
Lamp:	Continuous		
Flash lamp voltage:		<n/a>	
Readings per well:	1		
Time between readings:		<n/a>	
Integration time:	100000	us	
Delay after flash:	<n/a>		
Attenuator mode:	o		
Motion settling time:		150	ms
Z Height:	5.4	mm	Numeric
Excitation filter:	1	Fluorescein 485 nm	
Emission filter:	1	Fluorescein 530 nm	
Beamsplitter:	Top	50/50	
Excitation polarizer filter:		o	
Emission polarizer filter:		o	
Detector counting:	Digital		
Sensitivity setting:	<n/a>		
A/D converter gain:		<n/a>	
Integrating gain:	<n/a>		
Max cps:	1439860	cps	
Min counts:	34353	counts	
Dark Count reading:		628340	cps
Detection mode:	f8		
Light sensor:	HC-120		
Excitation side:	Top		
Emission side:	Top		
Lamp:	Continuous		
Flash lamp voltage:		<n/a>	
Readings per well:	1		
Time between readings:		<n/a>	
Integration time:	100000	us	
Delay after flash:	<n/a>		
Attenuator mode:	o		
Motion settling time:		150	ms
Z Height:	5.4	mm	Numeric
Excitation filter:	1	Fluorescein 485 nm	
Emission filter:	2	Europium 620 nm	
Beamsplitter:	Top	50/50	
Excitation polarizer filter:		o	
Emission polarizer filter:		o	
Detector counting:	Digital		
Sensitivity setting:	<n/a>		
A/D converter gain:		<n/a>	
Integrating gain:	<n/a>		
Max cps:	1511290	cps	
Min counts:	16672	counts	
Well list:	r0(a1:h11,a12:h12) = cps(f7(a1:h11,a12:h12))		
Data: RAW DATA SYTO 9 Green			
Units:	Counts/sec		

Raw data for SYTO 9 Green

	1	2	3	4	5	6	7	8	9	10	11	12
A	4.51e+005	5.5e+005	5.01e+005	1.14e+006	3.57e+005	6.3e+005	5.52e+005	5.64e+005	4.65e+005	6.27e+005	8.13e+005	4.7e+005
B	4.61e+005	4.55e+005	5.77e+005	5.9e+005	6.2e+005	6.94e+005	5.02e+005	8.5e+005	9.5e+005	5.41e+005	6.88e+005	4.37e+005
C	4.6e+005	6.71e+005	4.35e+005	1e+006	5.43e+005	6.84e+005	1.18e+006	7.81e+005	8.66e+005	4.04e+005	5.01e+005	3.5e+005
D	1.04e+006	1.26e+006	4.55e+005	5.57e+005	9.84e+005	6.38e+005	7.39e+005	4.62e+005	4.25e+005	8.6e+005	5.88e+005	7.15e+005
E	8.57e+005	9.86e+005	9.19e+005	4.85e+005	4.91e+005	8.06e+005	4.36e+005	5.17e+005	1.44e+006	7.64e+005	6.15e+005	4.34e+005
F	1.27e+006	7.74e+005	9.81e+005	1.11e+006	5.01e+005	9.97e+005	1.36e+006	8.2e+005	4.93e+005	7.47e+005	4.34e+005	4.42e+005
G	9.61e+005	1.05e+006	5.47e+005	6.29e+005	4.04e+005	4.47e+005	7.74e+005	9.81e+005	8.19e+005	7.64e+005	4.5e+005	4.48e+005
H	6.69e+005	8.16e+005	4.65e+005	6.3e+005	8.07e+005	5.67e+005	4.48e+005	3.44e+005	4.6e+005	5.81e+005	6.23e+005	3.59e+005

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Raw data for SYTO 9 Red

Well list: r1(a1:h11,a12:h12) = cps(f8(a1:h11,a12:h12))
Data: RAW DATA SYTO 9 Red
Units: Counts/sec

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.05e+005	2.24e+005	2.22e+005	4.08e+005	1.81e+005	2.76e+005	2.48e+005	2.47e+005	2.22e+005	2.52e+005	3.5e+005	2.2e+005
B	9.87e+005	2e+005	2.83e+005	2.7e+005	2.76e+005	269e+005	2.35e+005	4.04e+005	4.08e+005	2.36e+005	2.94e+005	2.53e+005
C	2.29e+005	2.69e+005	1.98e+005	3.49e+005	2.49e+005	2.66e+005	4.14e+005	3.23e+005	1.51e+006	2.08e+005	3.69e+005	1.94e+005
D	2.61e+005	5.62e+005	2.53e+005	2.51e+005	4.2e+005	2.85e+005	2.79e+005	2.37e+005	2.13e+005	3.45e+005	2.44e+005	2.95e+005
E	2.78e+005	3.97e+005	3.41e+005	2.25e+005	2.44e+005	3.26e+005	2.24e+005	2.41e+005	5.04e+005	2.92e+005	2.98e+005	2.14e+005
F	4.99e+005	3.19e+005	5.18e+005	3.76e+005	2.12e+005	3.98e+005	4.44e+005	5.06e+005	245e+005	3.28e+005	2.22e+005	2.24e+005
G	7.32e+005	4.16e+005	2.58e+005	2.08e+005	1.79e+005	1.89e+005	3.02e+005	4.48e+005	3.29e+005	2.96e+005	2.04e+005	2.46e+005
H	3.07e+005	1.05e+006	2.15e+005	2.97e+005	3.18e+005	2.23e+005	6.25e+005	1.67e+005	2.1e+005	2.53e+005	2.81e+005	1.86e+005

Background-subtracted data for SYTO 9 Green

Well list: r2(a1:h11) = cps(f7(a1:h11) - avg(f7(a12:h12)))
Data: BACKGROUND SUBTRACTED DATA SYTO 9 Green
Units: cps

	1	2	3	4	5	6	7	8	9	10	11	12
A	-6.05e+003	9.29e+004	4.35e+004	6.83e+005	-9.97e+004	1.73e+005	9.5e+004	1.07e+005	7.62e+003	1.7e+005	3.56e+005	
B	3.82e+003	-1.74e+003	1.2e+005	1.33e+005	1.63e+005	2.37e+005	4.53e+004	3.93e+005	4.93e+005	8.39e+004	2.31e+005	
C	3.08e+003	2.14e+005	-2.24e+004	5.46e+005	8.64e+004	2.27e+005	7.19e+005	3.24e+005	4.09e+005	-5.32e+004	4.41e+004	
D	5.84e+005	8e+005	-1.89e+003	9.98e+004	5.27e+005	1.81e+005	2.82e+005	5.2e+003	-3.15e+004	4.03e+005	1.31e+005	
E	4e+005	5.29e+005	4.62e+005	2.75e+004	3.38e+004	3.49e+005	-2.09e+004	5.98e+004	9.83e+005	3.07e+005	1.58e+005	
F	8.17e+005	3.17e+005	5.24e+005	6.49e+005	4.37e+004	5.4e+005	9.06e+005	3.63e+005	3.59e+004	2.9e+005	-2.33e+004	
G	5.04e+005	5.89e+005	8.97e+004	1.72e+005	-5.27e+004	-1.04e+004	3.17e+005	5.24e+005	3.62e+005	3.07e+005	-6.83e+003	
H	2.12e+005	3.59e+005	8.04e+003	1.73e+005	3.5e+005	1.1e+005	-8.6e+003	-1.13e+005	3.03e+003	1.24e+005	1.66e+005	

Background-subtracted data for SYTO 9 Red

Well list: r3(a1:h11) = cps(f8(a1:h11) - avg(f8(a12:h12)))
Data: BACKGROUND SUBTRACTED DATA SYTO 9 Red
Units: counts

	1	2	3	4	5	6	7	8	9	10	11	12
A	-2.37e+004	-5.01e+003	-6.69e+003	1.79e+005	-4.8e+004	4.69e+004	1.89e+004	1.77e+004	-7.19e+003	2.33e+004	1.21e+005	
B	7.58e+005	-2.9e+004	5.39e+004	4.06e+004	4.67e+004	4.04e+004	5.66e+003	1.75e+005	1.79e+005	7.37e+003	6.48e+004	
C	-333	4.02e+004	-3.08e+004	1.2e+005	1.97e+004	3.7e+004	1.85e+005	9.45e+004	1.28e+006	-2.12e+004	1.4e+005	
D	3.19e+004	3.33e+005	2.36e+004	2.19e+004	1.91e+005	5.55e+004	5.03e+004	7.63e+003	-1.64e+004	1.16e+005	1.49e+004	
E	4.89e+004	1.68e+005	1.12e+005	-4.36e+003	1.47e+004	9.74e+004	-4.85e+003	1.24e+004	2.75e+005	6.34e+004	6.93e+004	
F	2.7e+005	9.04e+004	2.89e+005	1.47e+005	-1.75e+004	1.69e+005	2.15e+005	2.77e+005	1.63e+004	9.9e+004	-6.88e+003	
G	5.03e+005	1.87e+005	2.91e+004	-2.14e+004	-5.04e+004	-3.95e+004	7.31e+004	2.19e+005	1e+005	6.72e+004	-2.45e+004	
H	7.76e+004	8.23e+005	-1.44e+004	6.77e+004	8.88e+004	-6.05e+003	3.96e+005	-6.23e+004	-1.92e+004	2.35e+004	5.15e+004	

Ratio of background-subtracted data: SYTO 9 Green / SYTO 9 Red

Well list: r4(A1:H11) = ((f7(a1:h11) - avg(f7(a12:h12))) / (f8(a1:h11) - avg(f8(a12:h12))))
Data: RATIO
Units: Dimensionless

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.255	-18.5	-6.5	3.82	2.07	3.69	5.01	6.04	-1.06	7.29	2.95	
B	0.00504	0.0598	2.23	3.28	3.5	5.85	8	2.25	2.76	11.4	3.56	
C	-9.27	5.31	0.728	4.55	4.39	6.15	3.89	3.43	0.319	2.52	0.315	
D	18.3	2.4	-0.0798	4.56	2.76	3.26	5.6	0.682	1.93	3.47	8.81	
E	8.19	3.15	4.13	-6.31	2.3	3.58	4.31	4.81	3.58	4.84	2.28	
F	3.02	3.51	1.81	4.42	-2.5	3.2	4.21	1.31	2.2	2.93	3.38	
G	1	3.14	3.08	-8.02	1.05	0.264	4.34	2.39	3.62	4.57	0.278	
H	2.73	0.436	-0.56	2.56	3.93	-18.2	-0.0217	1.82	-0.158	5.27	3.22	

***Note**: The data shown above is for illustrative purposes only and does not represent the results of an actual "live/dead" assay.*

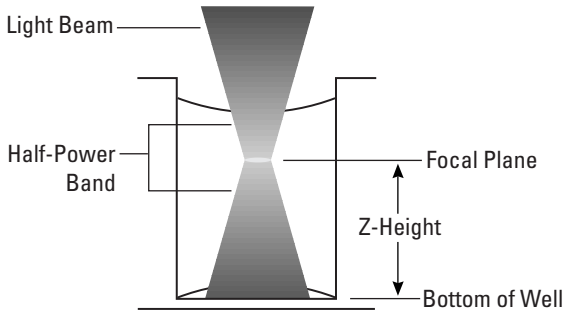
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Dynamic Z

This section describes how Dynamic Z, which is available for all measurement modes except Luminescence, can be used to optimize the signal-to-noise and signal-to-background ratios (S/N and S/B) in 96-well microplates.

The system allows you to adjust focal height to accommodate a variety of different plate formats and sample types.

The following illustrates the instrument's light beam as it illuminates a well. The portion of the light beam that coincides with the liquid in a partially filled well is the *sensed volume*. The light beam is shaped like an hourglass; its diameter is narrowest at its *focal plane* or *waist*, and is wider both above and below this point. Furthermore, the instrument does not sample the liquid uniformly; it is more sensitive to liquid within the *half-power* band, which is the volume within approximately one waist diameter of the focal plane. For fluorescence modes, z-height is the distance between the focal plane and the bottom of the well.



Cross-Section of 96-Well Microplate

Z-Height Positioning

Ideally, for homogeneous assays, the half-power band of the light beam should be positioned such that it is in the middle of the liquid volume, and does not impinge on the meniscus or on the bottom or sides of the well.

In homogeneous assays, as a general rule, the optimal S/N and S/B are achieved by setting the z-height so the meniscus and the bottom of the well falls outside the half-power band.

In cell-based assays, and other assays where the fluorophores are near the bottom of the well, the optimal S/N and S/B are achieved by setting the z-height such that the bottom of the well falls within the half-power band.

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Meniscus Effects

Because light is refracted as it crosses the boundary between air and sample (Snell's law), the meniscus shape affects the geometry and shape of the light beam. This in turn affects optimization of z-height settings.

When the meniscus is bowl-shaped (concave), the half-power band elongates and moves to a lower than nominal position. With a less concave meniscus, the half-power band moves up and is less stretched. Even with a flat meniscus, there is still some stretching and lowering of the half-power band. With a slightly lens-shaped meniscus (convex), the focal plane moves further towards the nominal position. When the meniscus is sufficiently convex, the half-power band can be raised above the nominal position and can become compressed rather than stretched.

Changes in meniscus shape can occur due to changes in protein or detergent concentration in the assay. The best results are typically achieved by setting the z-height such that the meniscus does not fall in the half-power band. This is best determined empirically.

Well Geometry Effects

A further consideration in setting z-height is the geometry of the microplate well. The instrument is capable of focusing at sufficiently low z-heights that part of the beam impinges on the upper surface of the microplate, a condition that should generally be avoided.

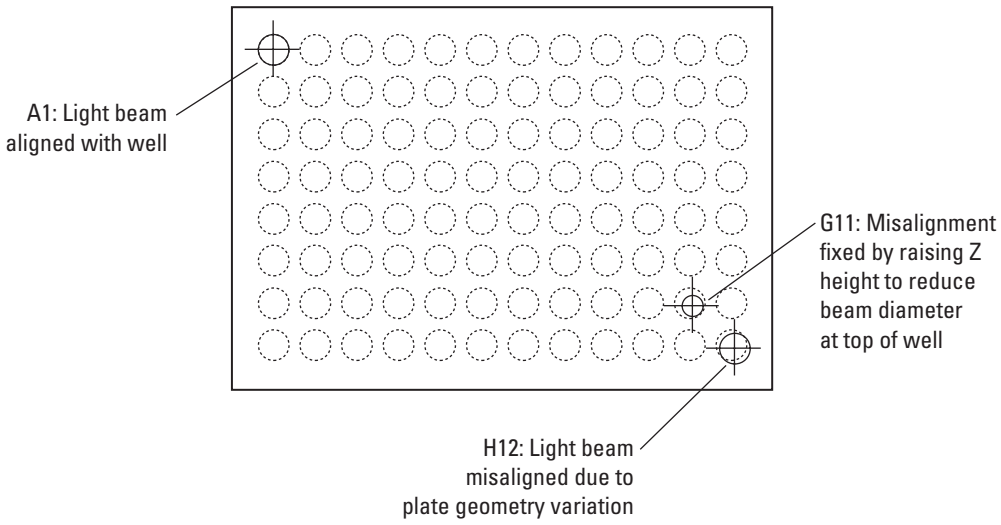
Setting the z-height too low can reduce sensitivity because of the following:

- Decrease in desired signal because less light enters the well.
- Increase in background (undesired signal) because the light beam illuminates the tops of wells. Many microplates are made from materials that are fluorescent, and the instrument will detect the fluorescence from materials at the tops of wells.

Despite these factors, a low z-height can still result in an optimal S/N, depending upon assay conditions.

Another manifestation of setting the z-height too low is cross-plate drift. The system is configured with the dimensions (plate heights, interwell distances, etc.) for several microplate styles. These plate parameters are nominal values and do not account for plate-to-plate or lot-to-lot variations in plate geometry. The following diagram illustrates the effect of a slight variation in interwell distance. When the z-height is set too low, the light beam can be perfectly centered on well A1, but can be off-center on other wells. This is more critical in 384-well plates and can also occur if the improper plate dimensions are entered.

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Cross-Plate Drift

Under these conditions, cross-plate drift of fluorescence readings may be observed as the instrument scans across the plate, because as it moves farther from well A1, misalignment generally increases, causing drift in the readings. The drift is often worst at well H12, the well farthest from A1. However, the user can minimize the effects of misalignment by setting the z-height correctly. Increasing the z-height, as shown for well G11, accurately places the light beam back in the well, reducing drift.

Optimizing Z-Height

To optimize z-height for a particular plate and chemistry, first prepare a test plate with representative chemistry (blanks, positive and negative controls, dilution series, etc.). Read the plate multiple times at different z-heights to determine the z-height that gives the best signal-to-background data. Some combinations of chemistry and plate are relatively insensitive to z-height, while others demonstrate a distinct optimum.

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2 Operating Procedures

Part 2 explains how to start up the system and how to use the CriterionHost application software to set up the instrument, define microplate formats and methods, and read microplates.

2.1 STARTING UP THE SYSTEM

Normally, you do not need to switch off power at the end of the day. If the system will not be used for more than one day, it is best to turn off the instrument. Use the following procedure only if the system has been switched off.

1 Switch on instrument power.

Turn on the instrument power switch. The green **POWER** light should come on immediately. After about 90 seconds, the control panel should display:

```
- LJL BioSystems -
10/01/1998 15:28:58
```



Caution: *Never remove or attach the control panel while the instrument power is on.*

Note: *For F, HEFP, A, and EA methods that use the continuous lamp, the instrument performs best after the continuous lamp has warmed up for 10 minutes. To warm up the lamp, check the 'Lamp On' checkbox in the Continuous Lamp Status dialog box (see 'Configuring the Continuous Lamp Settings,' page 61).*

2 Switch on the host computer and click on the CriterionHost icon.

Note: *Be sure to switch on the instrument first and allow it to complete the initialization process. Otherwise, when you start the CriterionHost application, it will not be able to detect the instrument.*

Turn on the host computer and allow Windows to start up. Click on the CriterionHost icon to start the program.

3 View the Run screen.

Depending upon how you left the program when you last used it, the Run screen appears in one of two views.

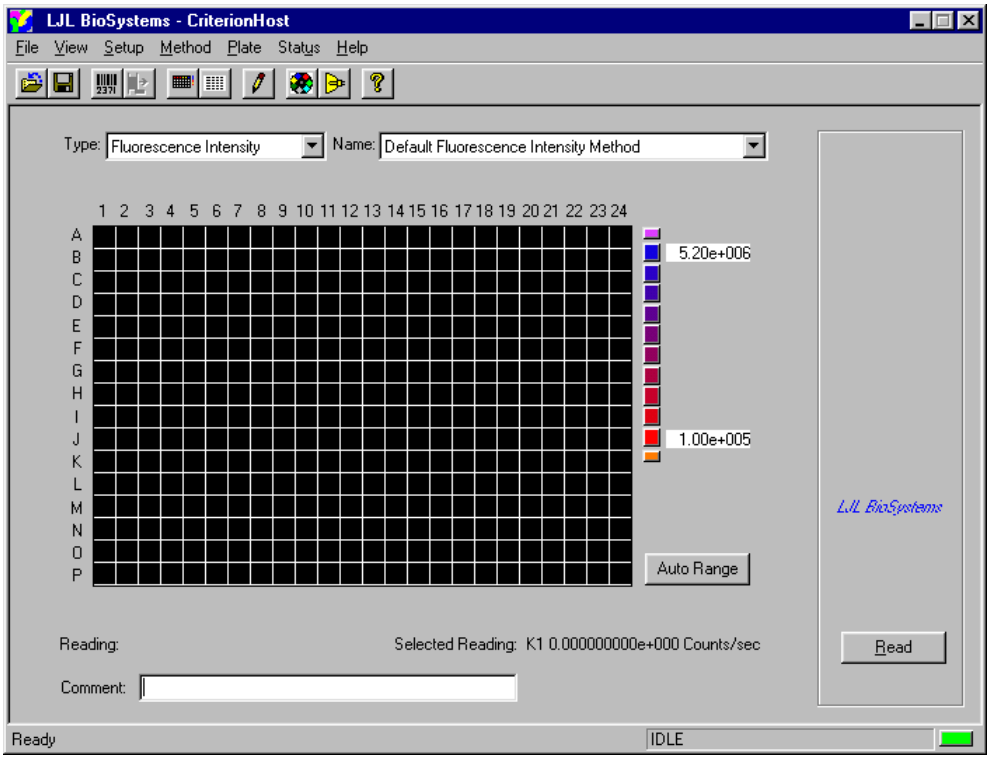
- **Plate View** shows a graphical representation of the microplate readings.
- **Report View** shows a numeric representation of the microplate readings.

To select another view, click on the View menu, and select the desired format.

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Run Screen

The Run screen is the starting point for all operations, whether you wish to configure the system or begin reading microplates. An example of the Plate View screen for a 384-well microplate is shown below.



The Status Indicator at the lower right provides the following information:

Green Status is normal.

Yellow A service procedure is needed, for example, when nearing the end of the life of the lamp (same function as the control panel **SERVICE** light on the Analyst HT).

Red A critical fault has occurred that requires intervention by the operator (same function as the control panel **FAULT** light on the Analyst HT).

Black There is no communication with the instrument.

To begin reading microplates, you first select a reading mode followed by a method name from the drop-down menus. Then you load one or more microplates and click the Read button.

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2.2 SETTING UP THE SYSTEM

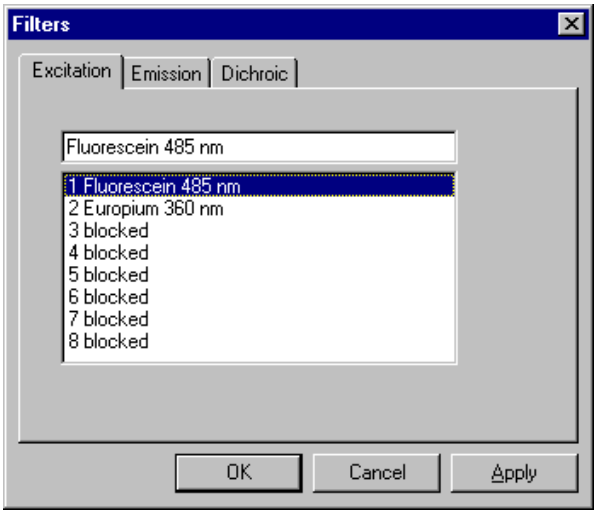
This section explains how to use the Setup menu to configure the system with parameters that are not specific to any mode or method. These include:

- Entering names for filters and dichroic mirrors
- Setting the instrument date and time
- Configuring the continuous lamp settings
- Selecting the number format used for showing results
- Selecting the report format
- Enabling or disabling the stacker and barcode reader

Entering Names for Filters and Dichroic Mirrors

The CriterionHost application allows you to enter a name for each filter and dichroic mirror installed in the instrument. Once entered, you can select the filters when you define a new method. To enter a name for a new filter or dichroic mirror:

1 From the Setup menu, click on Edit Filter Names.



The dialog box shows the filter names for each of the eight positions in the excitation filter wheel.

- If you select the Emission tab, you will see the filter names for the emission filter wheel.
- If you select the Dichroic tab, you will see the names of the dichroic mirrors for the top and bottom optics.

2 Click on the filter or dichroic mirror position and enter a new name. When done, click Apply.

3 When you finish entering or editing all the filter and dichroic mirror names, click OK.

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Important! *Be sure the name you enter corresponds correctly to the position of the filter in the instrument. The system has no way to verify the filter's identity.*

For information on installing filters and dichroic mirrors, see sections 4.3, 'Filter Removal and Replacement,' and 4.4, 'Dichroic Mirror Removal and Replacement.'

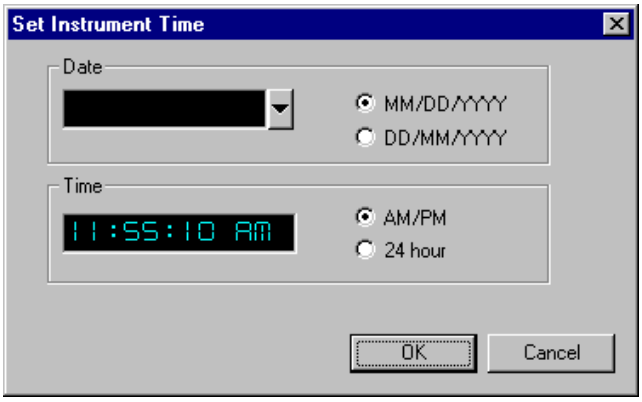
Setting the Instrument Date and Time

Whenever the instrument generates results, it includes a date and time stamp. You must use the host computer to set the correct date and time for the instrument's internal computer. Once set, you only need to reset the time when daylight savings or standard time begins.

The date and time settings explained here are independent of the date and time set in Windows.

To set the date and time that appears with test results:

1 From the Setup menu, select Date and Time. The program displays:

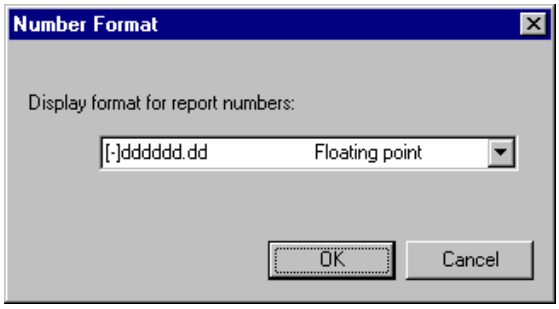


2 Click on the field you want to reset and type the new date or time. You can also change the date and time formats. When done, click OK to confirm the new settings.

Selecting the Number Format

To select the number format used for showing results on the Run screen and in reports:

1 From the Setup menu, select Number Format. The program displays:



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2 Click on one of the number formats shown in the table below. When done, click OK to confirm the new setting.

Number Format	Example
Decimal integer	112793608
Floating point	112793608.00
Scientific	1.127936080e+008

Setting Up the Stacker

For systems with the integrated stacker, the Setup menu allows you to configure the instrument to use the stacker. To do so, click Stacker on the Setup menu. A check mark will appear indicating that the device is enabled.

- If the stacker is *disabled*, then the instrument assumes that plates will be loaded and picked up at the *transfer* (right) position. Plates can be managed either manually or (on the Analyst HT) by a laboratory robot. The plates are always transferred to and removed from the plate gripper.
- If the stacker is *enabled*, then the instrument assumes that plates will be loaded at the *input* (left) position and picked up at the *output* (middle) position. Plates can be managed either manually, with a laboratory robot (on the Analyst HT), or with the magazines.



Note: *To use the Process Compression feature, the stacker must be enabled. Process Compression allows a plate to be inserted at the input position and another removed at the output position by a robot in one pass, thereby improving robot efficiency.*

Setting Up the Barcode Reader

The Setup menu allows you to configure the instrument to use the barcode reader. To do so, click Barcode Reader on the Setup menu. A check mark will appear indicating that the device is enabled, and the instrument will look for an identifying barcode on the side of each microplate.

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Selecting the Report Format

When you select Report Format from the Setup menu, you can select one of four formats:

No Header: Provides the following data:

- number of read in the total specified by **mrc** command
- well read list in the format specified by **read** command
- data description field in the format specified by **rtinfo** command
- units description field in the format specified by **rtinfo** command
- number format
- well read values in a row/column matrix in units specified by read list with display format specified by **rtinfo** command

```
Kinetic read cycle:          1 of 1
Well list:       r0(a1:c12) = cps(f1(a1:c12))
Data:            RAW DATA
Units:           cps
Display format: %d
346963   404982   521884   753673   1485481   3983709   11515540   38096524   112664200   366849152   1037790528   1831252864
359718   386282   454083   729026   1462944   3966172   11627346   38409444   112535088   366934816   1071770176   1765410048
359910   390406   482086   743699   1455081   3979986   11495405   38097448   112793608   366934816   1048175424   1829121024
```

Columns: Provides the following data in four columns:

- number of read in the total specified by **mrc** command
- well read list in the format specified by **read** command
- data description field in the format specified by **rtinfo** command
- units description field in the format specified by **rtinfo** command
- number format
- date/time stamp for each well read in the format specified by **tf** command
- plate identification specified by **plateid** command
- well number
- corresponding read value in units specified by read list with display format specified by **rtinfo** command

```
Kinetic read cycle:          1 of 1
Well list:       r0(a1:c12) = cps(f1(a1:c12))
Data:            RAW DATA
Units:           cps
Display format: %d
06/29/1999 03:18:32 PM    fluorescein dilution series    A1    346963
06/29/1999 03:18:32 PM    fluorescein dilution series    A2    404982
06/29/1999 03:18:33 PM    fluorescein dilution series    A3    521884
06/29/1999 03:18:33 PM    fluorescein dilution series    A4    753673
06/29/1999 03:18:34 PM    fluorescein dilution series    A5    1485480
06/29/1999 03:18:35 PM    fluorescein dilution series    A6    3983710
```


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Terse: Provides the following data:

- date/time stamp of start of read sequence in the format specified by **tf** command
- plate identification specified by **plateid** command
- barcode (if enabled and read)
- number of read in the total specified by **mrc** command
- well read list in the format specified by **read** command
- data description field in the format specified by **rtinfo** command
- units description field in the format specified by **rtinfo** command
- number format
- well read values in a row/column matrix in units specified by read list with display format specified by **rtinfo** command

```
07/13/1999 11:23:10 AM    Plate #74
Kinetic read cycle:       1 of 1
Well list:    r0(a1:b12) = cps(fl(a1:b12))
Data:         RAW DATA
Units:        cps
Display format: %d
346963  404982  521884  753673  1485481  3983709  11515540  38096524  112664200  366849152  1037790528  1831252864
359718  386282  454083  729026  1462944  3966172  11627346  38409444  112535088  366934816  1071770176  1765410048
359910  390406  482086  743699  1455081  3979986  11495405  38097448  112793608  366934816  1048175424  1829121024
```

Verbose: Provides all data generated from the run. The full report format is dependent upon the detector counting mode used for the reading. For further information, see the **rl** command in section 3.2, 'Open Protocol Commands.'

```
Read time:    07/13/1999 11:25:27 AM
Kinetic read cycle:          1 of 1
Read start delay time:       0              min
Time delay between kinetic reads:           <n/a>
Barcode:      <n/a>
Method ID:                   Fluorescence Intensity Method
Plate ID:     Plate #75
Comment:
Microplate format:           LJL HE 384 A
Shake time:   0              s
Temperature:0                C
Instrument tag:              Set by customer
Serial number:
Read sequence:               row
Mode sequence:               well
Detection mode:              fl
Light sensor:  HC-120
Excitation side:             Top
Emission side:               Top
Lamp:         Continuous
Flash lamp voltage:          <n/a>
Readings per well:           1
Time between readings:       <n/a>
Integration time:            100000         us
Delay after flash:           <n/a>
Attenuator mode:             o
Motion settling time:        25             ms
Z Height:     2.12           mm             Middle
Excitation filter:           1              Fluorescein 485 nm
Emission filter:             1              Fluorescein 530 nm
Beamsplitter: Top            Fluorescein 505
Excitation polarizer filter: o
Emission polarizer filter:   o
Detector counting:           SmartRead
Sensitivity setting:         2
```


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A/D converter gain: x1
Integrating gain: x1
Max cps: 18000010 cps
Min counts: 10001 counts
Well list: r0(a1:h12) = cps(f1(a1:h12))
Data: RAW DATA
Units: cps
Display format: %d

	1	2	3	4	5	6	7	8	9	10	11	12
A	346963	404982	521884	753673	1485481	3983709	11515540	38096524	112664200	366849152	1037790528	1831252864
B	359718	386282	454083	729026	1462944	3966172	11627346	38409444	112535088	366934816	1071770176	1765410048
C	359910	390406	482086	743699	1455081	3979986	11495405	38097448	112793608	366934816	1048175424	1829121024
D	368349	422148	495991	749741	1453642	3939274	11471150	37920908	111386280	362867168	1060199040	1763428608
E	354539	406709	485442	765564	1451436	3972097	11550758	38410380	114495008	371007072	1037790528	1829121024
F	339291	394146	459741	719245	1464575	3987916	11620553	38230004	112923312	366849152	1071039552	1763428608
G	331332	388488	477099	712628	1464670	3962331	11463116	37905352	112398232	369697632	1048875072	1831252864
H	349840	357992	437588	759618	1464191	4002045	11467132	38038420	113576320	366934816	1095686912	1765410048

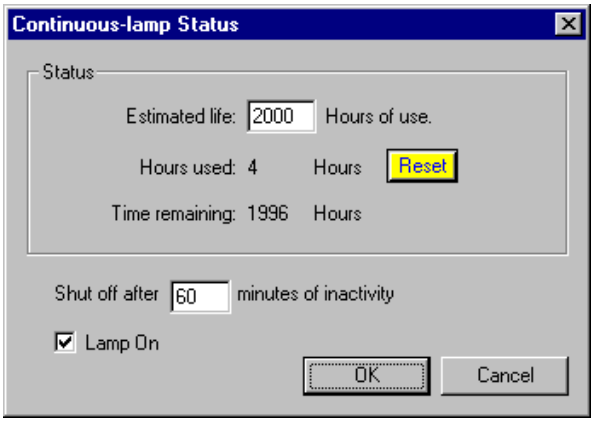
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Configuring the Continuous Lamp Settings

After replacing the continuous lamp, you can use the Continuous Lamp Status dialog box to:

- Enter the expected life of the new lamp.
- Monitor hours remaining before the lamp must be replaced again.
- Configure the lamp to shut off after a specified interval of inactivity.

If you select Lamps from the Status menu, the system displays:



Estimated Life: Enter the expected life of the lamp in hours. The continuous lamp is rated for 2000 hours of use before it must be replaced. The 'Estimated life' value can be changed with the **lamplife** command.

Hours Used, Time Remaining: After replacing the lamp, click the Reset button to re-start the lamp monitor. For example, if you enter 2000 as the estimated lamp life and then click Reset, the Hours used will return to '0' and the Time remaining will return to '2000.' The 'Hours used' value can be changed with the **lamphours** command.

Shutoff Interval: To prolong lamp life, you can have the continuous lamp automatically shut off after a specified interval. To enter a new shutoff interval, click in the lower window and type the number of minutes desired for the lamp shutoff. The 'Shut off' value can be changed with the **lamptimeout** command.

Note: *LJL BioSystems recommends setting the lamp shutoff interval to 120 minutes. Do not set the interval to less than 10 minutes – optimal system performance for F, HEFP, A, and EA methods that use the continuous lamp requires a 10-minute lamp warm-up.*

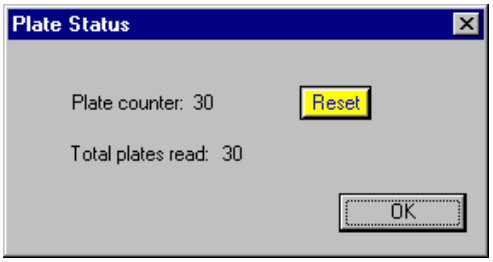
Lamp Service Message: The system reports a service message when the continuous lamp has been on for a specified interval. When shipped, the system is configured to post the message when there are 400 hours of lamp life remaining. Although this value does not appear on the Continuous Lamp Status screen, the value can be changed with the **lampservice** command. For further information, see section 3.2, 'Open Protocol Commands.'

When done, confirm your entries by clicking OK.

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Monitoring Plate Status

If you select Plates from the Status menu, you can review the total number of plates read by the instrument, as well as the number of plates read since the last reset ('Plate Counter'). To reset 'Plate Counter,' click the Reset button.



Note: *'Plate Counter' and 'Total Plates Read' are incremented by 1 each time a plate is ejected.*

The 'Plate ID' field in the Columns, Terse, and Verbose report formats shows the number of plates processed since 'Plate Counter' was reset. The number that appears in the report for 'Plate ID' is the current value of the plate counter when the plate is done being read. When the plate is then ejected, the number shown for 'Plate Counter' is incremented by 1, but the number shown in the report for 'Plate ID' is not. Therefore, the number shown for 'Plate Counter' will always be at least 1 greater than the number shown for 'Plate ID.' Note that if a plate is loaded and ejected without being read, the difference between the numbers shown for 'Plate Counter' and 'Plate ID' will increase by 1.

With a new instrument ('Total Plates Read' = 0), 'Plate Counter' and 'Total Plates Read' will both show 2 after the first plate is ejected.

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2.3 DEFINING MICROPLATES

The CriterionHost application is shipped with a number of predefined standard microplate formats. However, some methods may utilize microplates that do not conform with any of the predefined formats.

Check the ClubHT web site (www.ljlbio.com/clubht) for a cross-reference table containing currently defined microplate formats and vendor article numbers. Many plates share the same dimensions. The web site also lists dimensions for new plates not currently defined in the software.

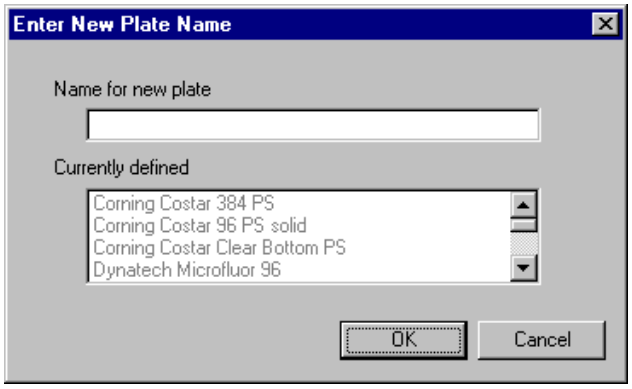
This section explains how to use the Plate menu to enter precise dimensions for any microplate. With the Plate menu, you can:

- **Define** a new microplate format.
- **Edit, Copy**, or **Rename** an existing microplate definition.
- **Delete** a microplate definition.

Defining a New Microplate Format

To create a new microplate format, you enter a name for the plate; the plate length, width, and height; the number of rows and columns; and precise dimensions for the alignment of the wells.

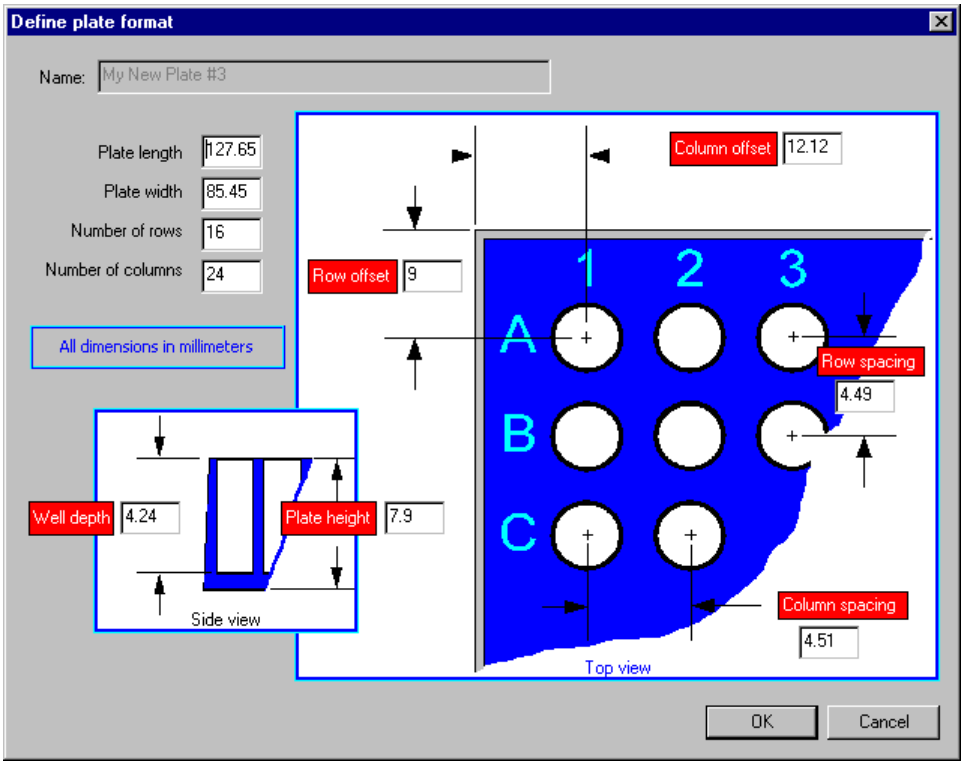
1 From the Plate menu, select New… The program displays:



2 Enter a name for the new microplate format in the Name box. This name will appear in the Plate Format drop-down list when you define or edit a method. When done, click OK.

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A schematic representation of the microplate appears:



3 Continue by entering the plate dimensions indicated on the screen. All measurements should be in millimeters.

4 When done, confirm your entries by clicking OK.

Modifying a Microplate Format

You may wish to start with a predefined plate format and create a new one using the Plate menu options:

- The Edit command allows you to select a previously defined plate format and modify any of the plate dimensions. Although you can edit your own plate formats, you cannot edit plate formats predefined by LJL BioSystems.
- The Copy command allows you to copy all parameters from an existing plate format to a new plate format.

Deleting a Microplate Format

To delete a microplate format:

1 From the Plate menu, select Delete. The program displays the current list of microplate formats.

2 Select the microplate format you want to delete. When the program prompts you confirm the selection, click Yes to delete.

Note: *Microplate formats predefined by LJL BioSystems cannot be deleted.*

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2.4 DEFINING METHODS

The Method menu allows you to define a wide variety of new methods for use with the system. With the Method menu, you can:

- **Define** a new method.
- **Copy** a method.
- **Edit** an existing method. (In some cases, you may want to start with a predefined method and create a new one using the Copy option on the Method menu.)
- **Delete** a method. For further information, see 'Deleting a Method Definition' later in section 2.4.
- **Rename** a method.

When you define a new method, you will edit default parameter values that appear in the Define and Edit Methods screen. Each of the six assay modes has a set of predefined default parameters. For a complete listing of programmable method parameters including parameter ranges and defaults for each of the seven detection modes, see section 6.3, 'Method Parameter Cross-Reference.'

Overview of Method Parameters

This section provides information on the parameters used to define methods.

Basic Parameters

The following parameters are set using the Define and Edit Methods screens, which are accessible through the Method menu.

Method Name: Specify a name for each new method by selecting New from the Method menu. The method name appears in the Method ID field on the verbose report format.

Optics: For F and TRF methods, select either the top or bottom optics. HEFP, L, FLUM, and EA methods use only the top optics.

Lamp: F, HEFP, A, and EA methods use the continuous lamp by default, but the flash lamp option is also available for these modes. TRF methods use the flash lamp exclusively. L and FLUM methods do not use either lamp.

Filters: For F, HEFP, and TRF methods, select one excitation and one emission filter from the drop-down menu. L and FLUM methods do not use filters; for FLUM methods, select emission filter-wheel position #8, which must be open. For A and EA methods, select one excitation filter (a bandpass filter of the appropriate wavelength). For EA methods, select emission filter-wheel position #3, which must contain the 3 OD neutral density filter. To identify the installed filters, select Edit Filter Names from the Setup menu.

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Dichroic Mirrors: Identify the installed dichroic mirrors by selecting Edit Filter Names from the Setup menu. The dichroic mirrors are not included within the method definition. Note that L and FLUM methods do not use dichroic mirrors.

Plate Format: For each method, select a plate format from the drop-down list. The system is provided with a number of predefined standard plate formats. In addition, you can define custom formats by using the options on the Plate menu.

Select Wells: The system lets the user define up to two sample regions and two background regions (one of each for Absorbance methods). For details about how to specify the wells to be read, see 'Selecting Wells,' page 69.

Z-Height: For F, HEFP, TRF, FLUM, A, and EA methods, the z-height is the height of the focal plane volume relative to the bottom of the well.

Luminescence Height: For L methods, the luminescence height is the position of the top optics head relative to the top of the plate.

Timing, Continuous Lamp: The system allows the following settings:

- **Readings per Well**: Enter from 1 to 65,535 readings.

  If there is more than one reading, the system will report either 1) the average of the readings (in the counts per second mode) or 2) the sum of the readings (in the counts mode). Specify the mode with the Raw Data Units parameter described below.

- **Integration Time**: Enter the duration of the read time (the interval during which the PMT collects data) in microseconds.

  ***Note**: With the basic SmartRead, the length of time the read head stays at each well is equal to the integration time plus the plate settling time. With SmartRead Plus, the read head stays at each well only until the reading is complete; therefore, for SmartRead Plus the specified integration time is ignored.*

Timing, Flash Lamp: The system allows the following settings:

- **Flashes per Well**: This parameter specifies the number of readings per well.
- **Interval between Flashes**: Enter the time between flashes in 10-millisecond increments.
- **Delay after Flash**: Enter the time between the end of the flash and the beginning of the integration time in microseconds.
- **Integration Time**: Enter the duration of the read time (the interval during which the PMT collects data) in microseconds.

Switch Polarization: For HEFP methods, specify 'By Well' to have the system get readings with both polarizers on each well before moving on to the next well; specify 'By Plate' to have the system get readings with one polarizer on all wells on the plate and then get readings with the other polarizer on all the wells.

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G Factor: For HEFP methods only, the G factor is used to correct polarization data for optical artifacts. For further information, see sections 1.6, 'Theory of Operation, Fluorescence Polarization Mode,' and 2.6, 'Performance Verification.'

Raw Data Units: RFUs (Relative Fluorescence Units) are used only with F, HEFP, and TRF methods, while RLUs (Relative Luminescence Units) are used only with L methods. Intensity units are used only with FLUM, A, and EA methods. All methods allow you to select counts or counts per second. For a discussion of the different units, see 'Detector Counting Options' in section 1.6.

Attenuator Mode: For F, HEFP, TRF, FLUM, and EA methods, select High, Medium, or Out. For L methods, select either Medium or Out.

Advanced Method Parameters

The following parameters are set using the Advanced Setup screens, which are accessible from the Define and Edit Methods screens by clicking the Advanced button.

F, HEFP, FLUM, and EA Polarizers: For F, HEFP, FLUM, and EA methods, you can select polarizing excitation filters and emission filters. Polarizing filters can be used to reduce background noise. Static polarizers are almost never used in other than HEFP applications; however, they may be useful in reducing background signal in certain assays with high counts per second. Both excitation and emission polarizers can be placed in the light path.

HEFP Polarization: For HEFP methods, you can specify 1) either the excitation or the emission filter as the dynamic (moving) polarizer, and 2) the static polarizer in either the S or P orientation.

PMT Setup: F, HEFP, TRF, FLUM, and EA methods allow you to select one of three measurement methodologies: digital, SmartRead, or SmartRead Plus. In addition, for SmartRead and SmartRead Plus you can select one of five sensitivity settings (based on the rating of the integration capacitor). For further information, see 'Detector Counting Options (PMT Setup)' in section 1.6.

Flashlamp Voltage: TRF methods allow you to adjust the voltage to the flash lamp. To decrease light intensity, lower the flash lamp voltage. If you select the flash lamp for F, HEFP, or EA methods, the system also allows you to adjust the voltage.

Note: *It is almost never necessary to lower the flashlamp voltage. For further information, consult your LJL BioSystems agent.*

Caution: *Do not set flashlamp voltage to less than 360 volts.*

Plate Agitation: The instrument can agitate the plate by moving it in a circular motion. You can specify the following parameters:

- **Shaking Time**: The duration of the plate agitation.
- **Intensity:** The intensity of the plate agitation.

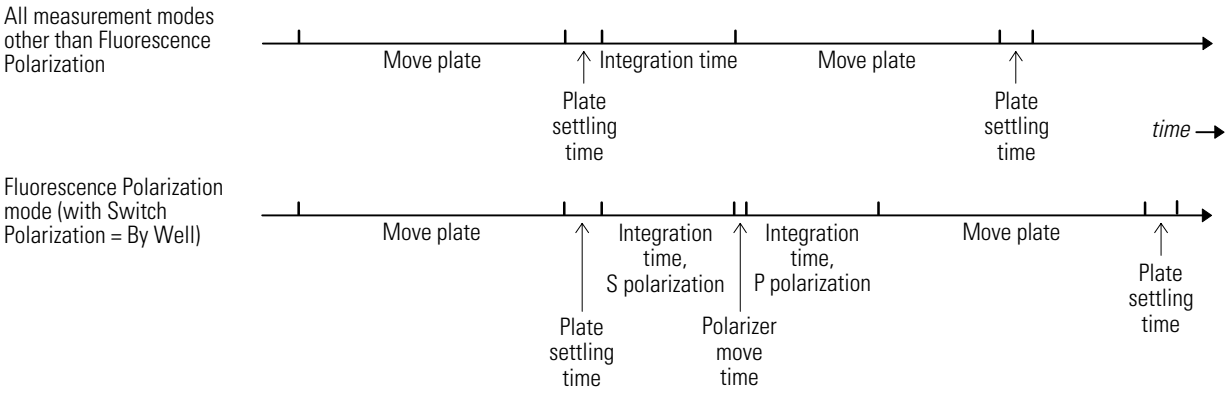
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Kinetic Timing: The system allows the following settings:

- **Delay Before First Read**: Enter the time between starting the run and the beginning of processing the first plate, in seconds.
- **Delay Between Reads**: Enter the time between the end of processing each plate and the beginning of processing the next plate, in seconds.
- **Number of Reads** : Enter the number of times each plate is to be read before moving on to the next plate.

Note: *If the number of reads is greater than 1 and SmartRead Plus detector counting is selected, SmartRead will be used instead.*

Plate Settling Time: All methods allow you to define the duration of the interval that begins when the plate reaches the read position for the next well and ends when the next reading starts. This time allows movement of liquid in the well to subside.



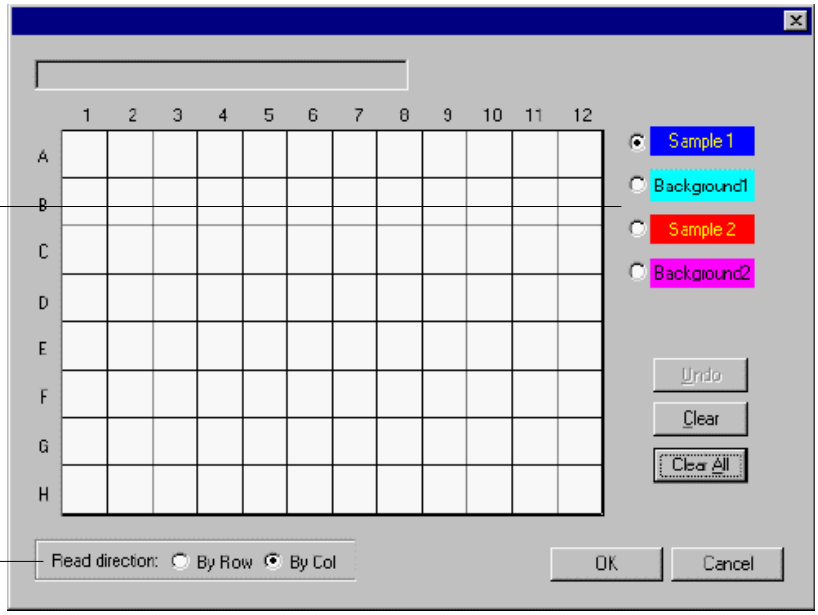
Well Processing Time

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Selecting Wells

To specify the wells to be read:

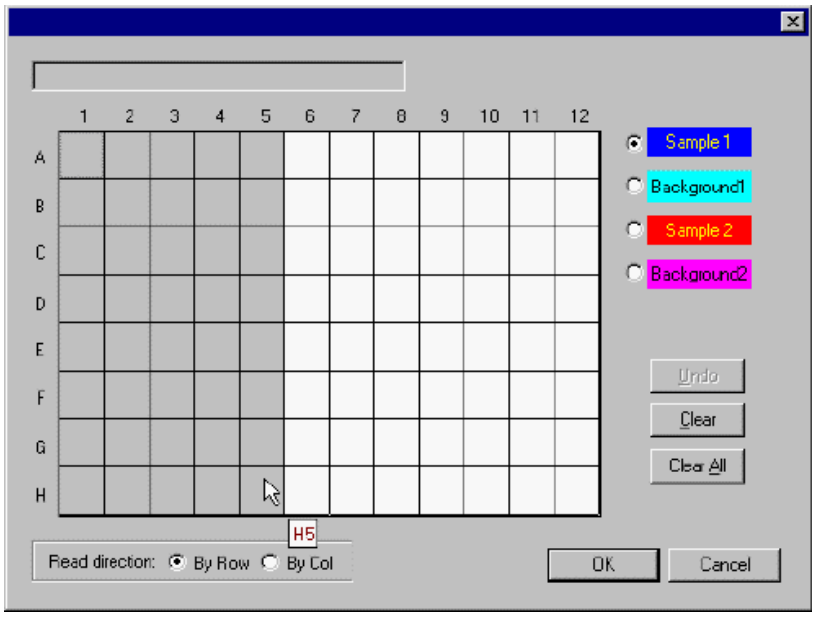
1 Click the Select wells button.



2 Select reading across rows or down columns.

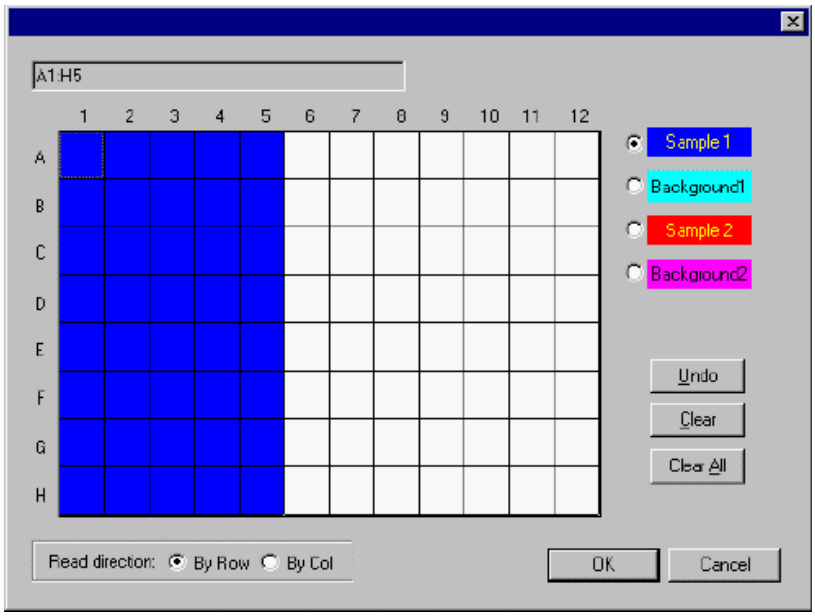
3 Specify the region to be defined.

4 Select the wells for this region by clicking and then dragging across them on the grid.

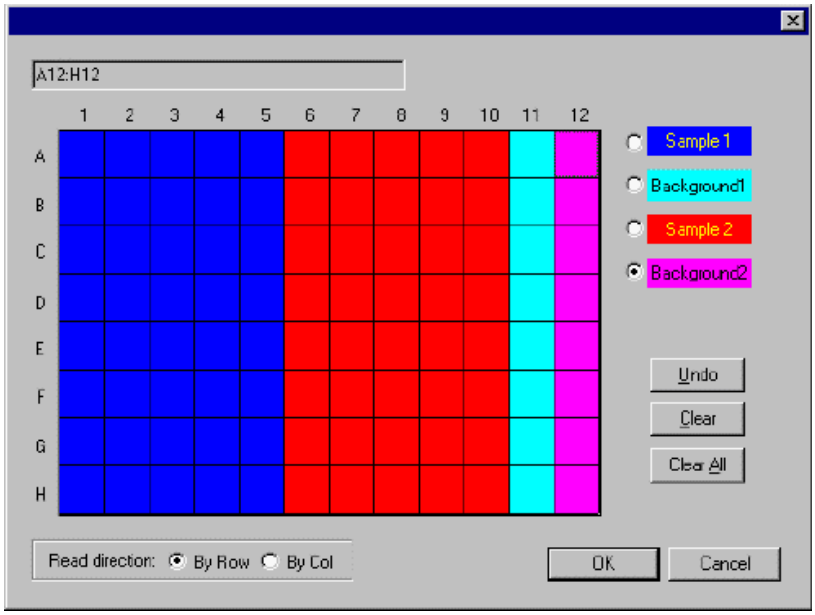


When you release the mouse button, the system displays the region in the color corresponding to the region selected.

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5 Repeat steps 3–4 for the other regions to be defined.

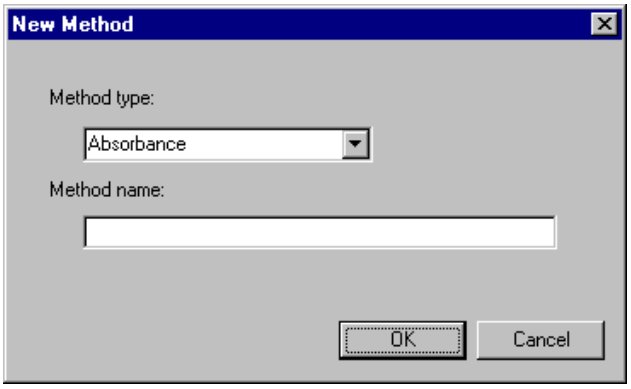


6 Click OK to accept the regions defined.

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Defining a New Method

When you select New from the Method menu, the system displays the New Method dialog box:



First select the desired method type from the drop-down list. Then enter a name for the new method. When you click OK to confirm the new method name, the Define and Edit Methods screen appears. The new method is assigned the default parameters for the selected method type.

For examples of the Define and Edit Methods screens, see the appropriate method type in the following pages.

For a complete listing of programmable method parameters, including parameter ranges and defaults for each of the detection modes, see section 6.3, 'Method Parameter Cross-Reference.'

Deleting a Method Definition

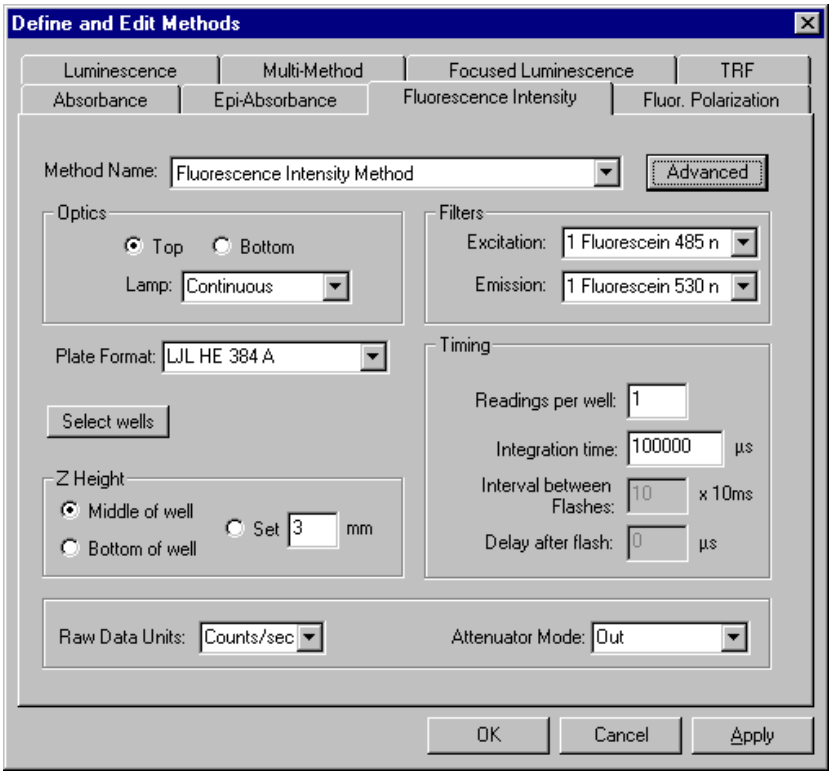
To delete a method definition:

1. Select Delete from the Method menu. The program displays the current list of method types and names.
2. Select the method you want to delete. When the program prompts you confirm the selection, click Yes to delete.

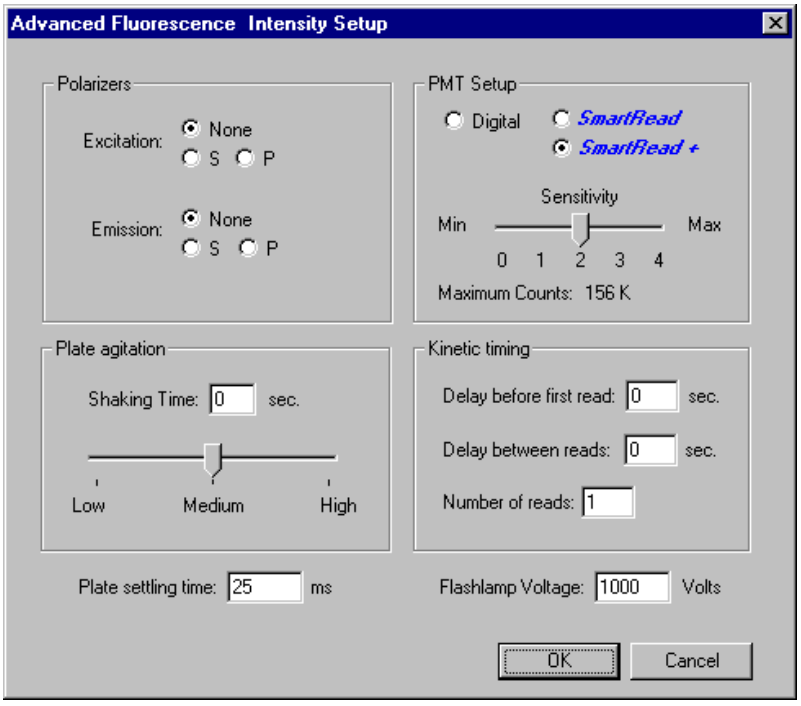
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Defining a Fluorescence Intensity Method

The CriterionHost application allows you to specify the Fluorescence method parameters shown in the following example screens.



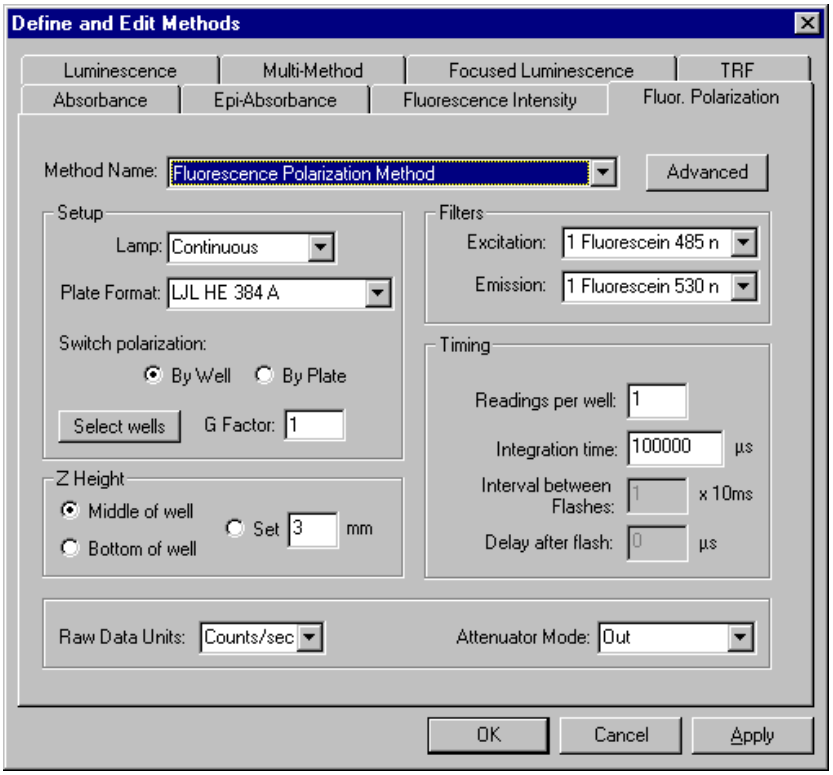
If you click on the Advanced button, the following screen appears:



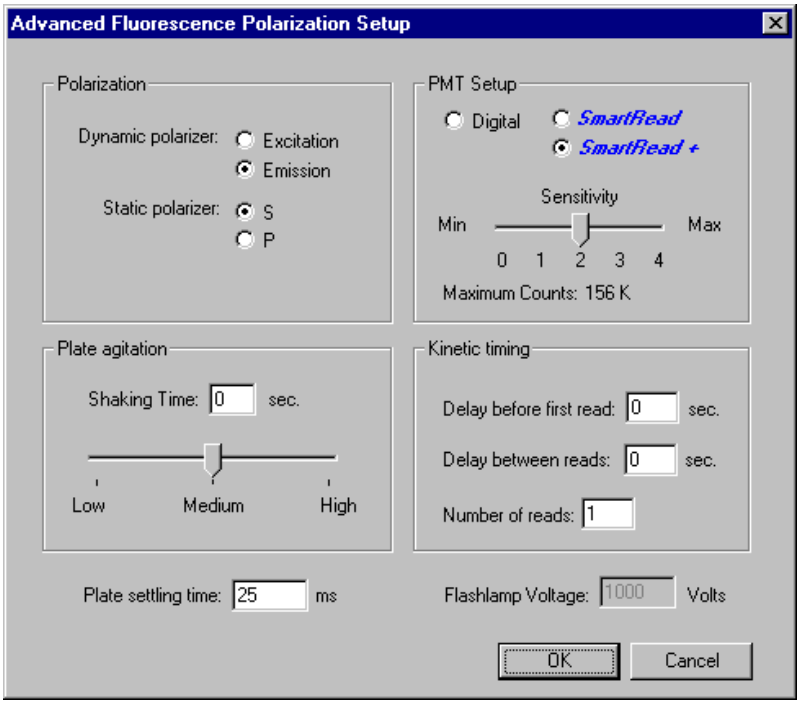
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Defining a High Efficiency Fluorescence Polarization Method

The CriterionHost application allows you to specify the Fluorescence Polarization method parameters shown in the following example screens.



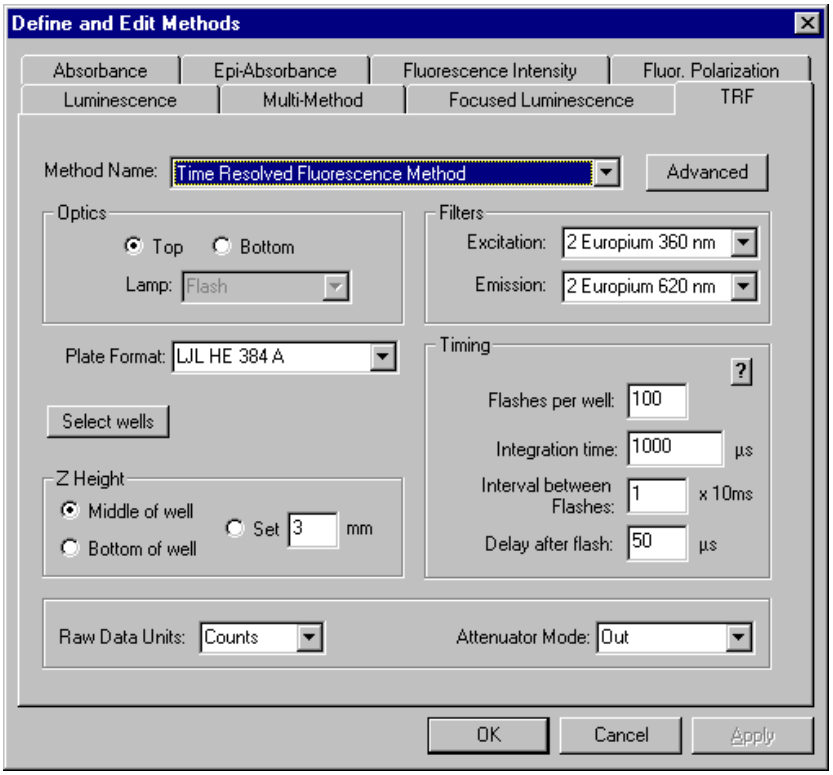
If you click on the Advanced button, the following screen appears:



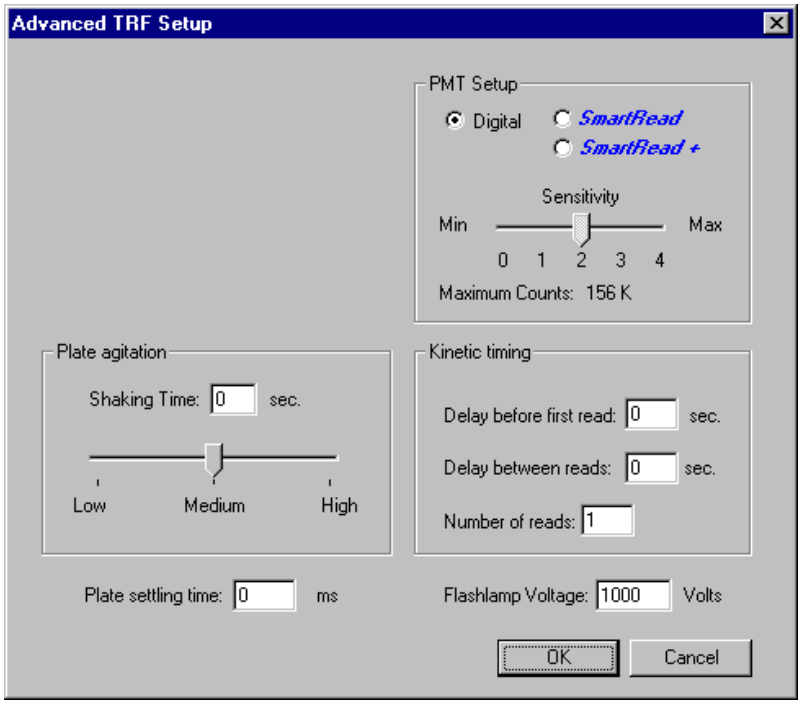
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Defining a Time-Resolved Fluorescence Method

The CriterionHost application allows you to specify the Time-Resolved Fluorescence method parameters shown in the following example screens.



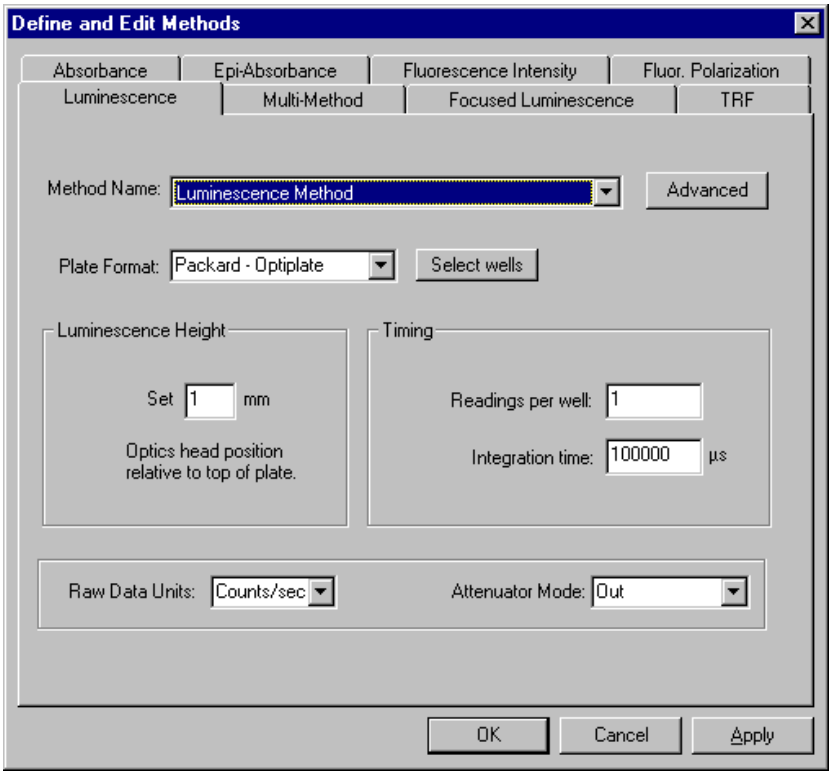
If you click on the Advanced button, the following screen appears:



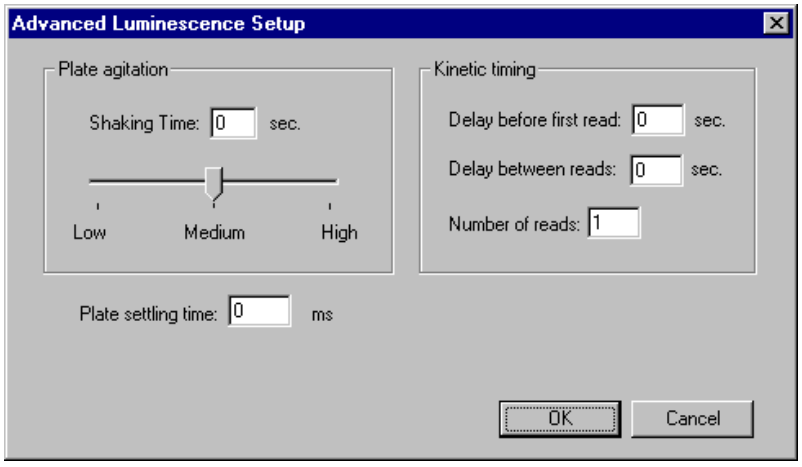
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Defining a Luminescence Method

The CriterionHost application allows you to specify the Luminescence method parameters shown in the following example screens.



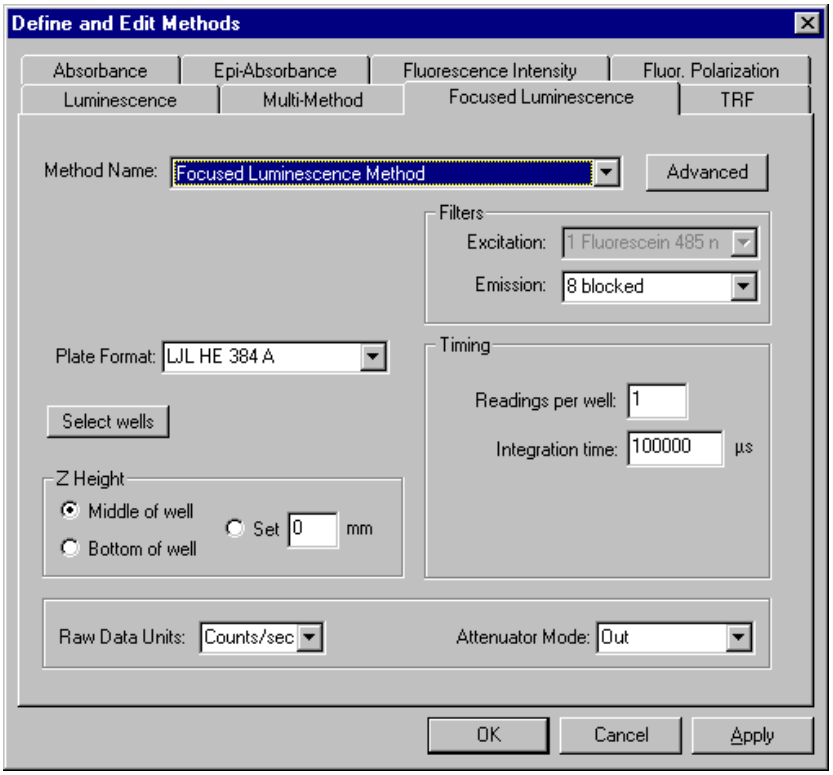
If you click on the Advanced button, the following screen appears:



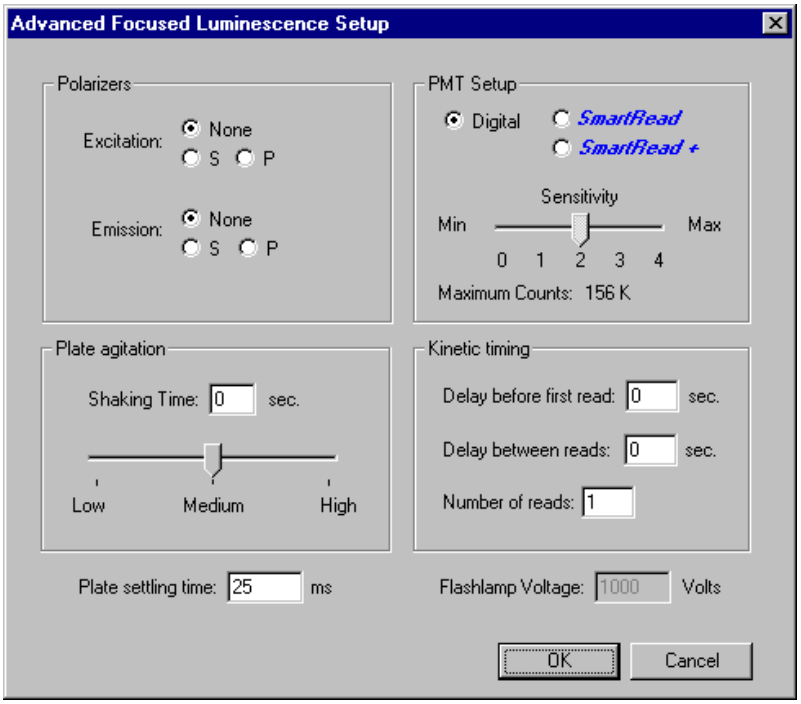
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Defining a Focused Luminescence Method

The CriterionHost application allows you to specify the Focused Luminescence method parameters shown in the following example screens.



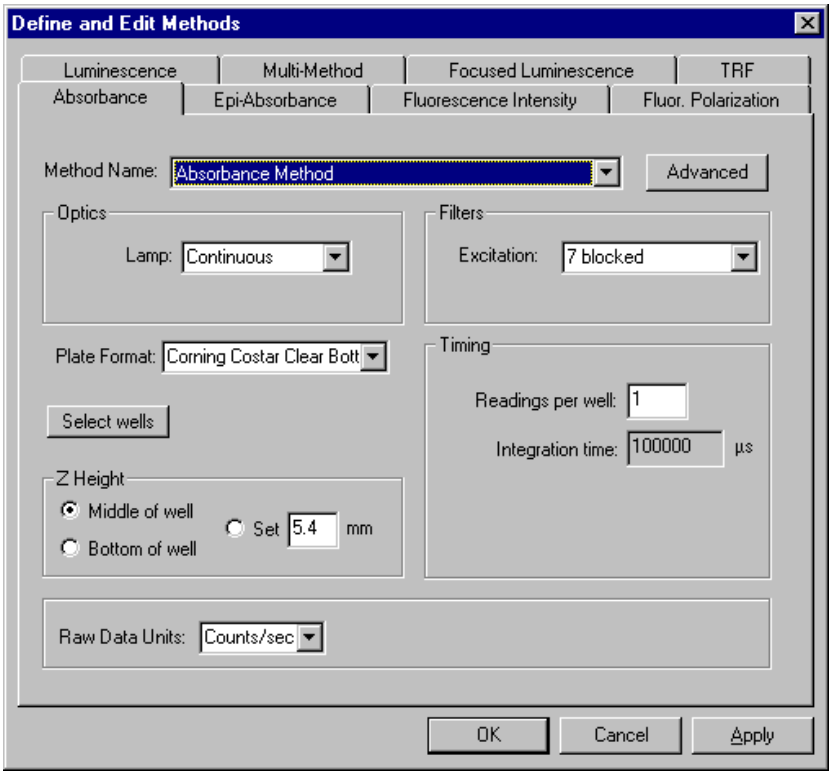
If you click on the Advanced button, the following screen appears:



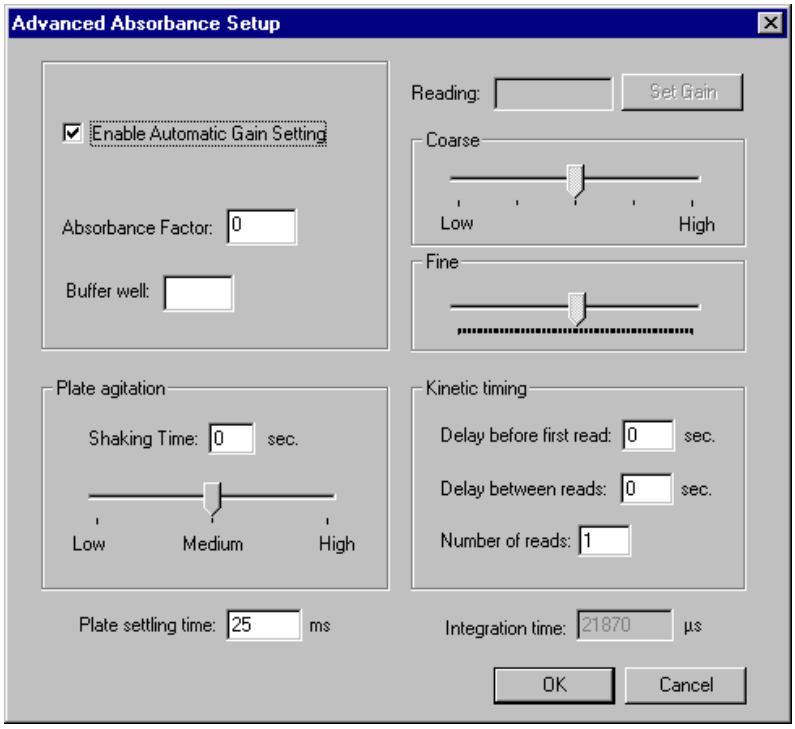
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Defining an Absorbance Method

The CriterionHost application allows you to specify the Absorbance method parameters shown in the following example screens.



If you click on the Advanced button, the following screen appears:



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Setting the Gain for Absorbance Measurements

To ensure accurate readings, it is necessary to set the gain of the photodiode used for absorbance measurements. The system provides two ways of doing this – automatically or manually. The automatic mode is intended for normal use; the manual mode is intended for reading multiple plates with the same gain setting (which increases throughput) and for diagnostic purposes as directed by an LJL BioSystems agent.

TO SET THE GAIN AUTOMATICALLY:

1. Display the Advanced Absorbance Setup screen by clicking the Advanced button on the Define and Edit Methods screen.
2. Check 'Enable Automatic Gain Setting.'
3. In 'Set Gain on Wells,' enter the wells you want to use to set the gain. The instrument will use those wells as a reference before reading each plate. If no wells are specified, the instrument will set the gain using an air blank.

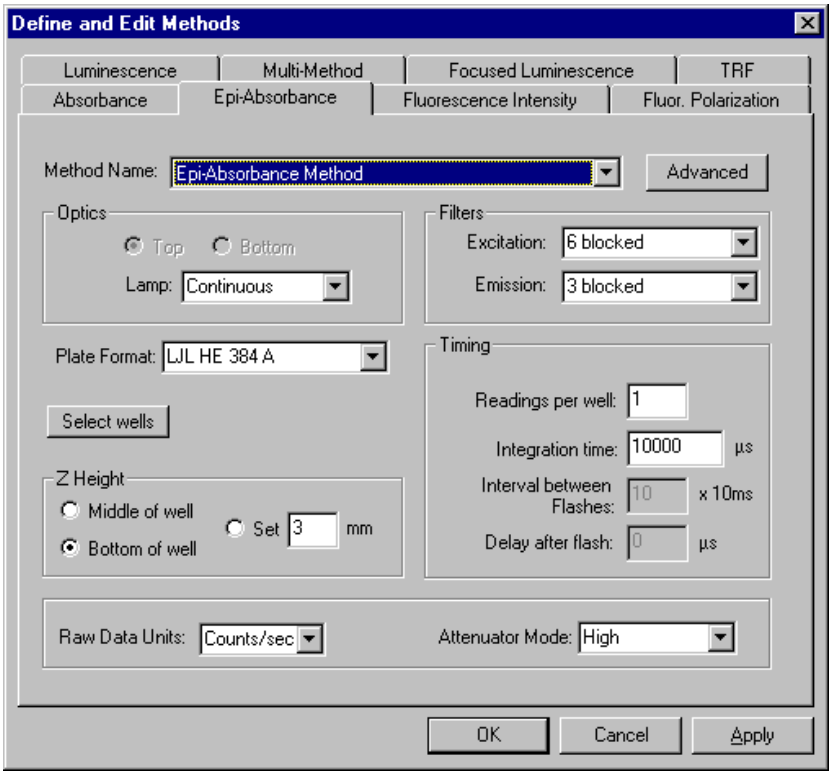
TO SET THE GAIN MANUALLY:

1. Display the Advanced Absorbance Setup screen by clicking the Advanced button on the Define and Edit Methods screen.
2. Uncheck 'Enable Automatic Gain Setting.'
3. Move the Coarse adjustment slider to the far left (lowest setting) and move the Fine adjustment slider to the far right (highest setting).
4. Click the Set Gain button. A number less than 16383 – which is the saturation value – appears in 'Reading.'
5. Move the Coarse slider to the right until 16383 appears in 'Reading.'
6. Move the Fine slider to the left until the reading is between 12000 and 15000.
7. Click the Done button.

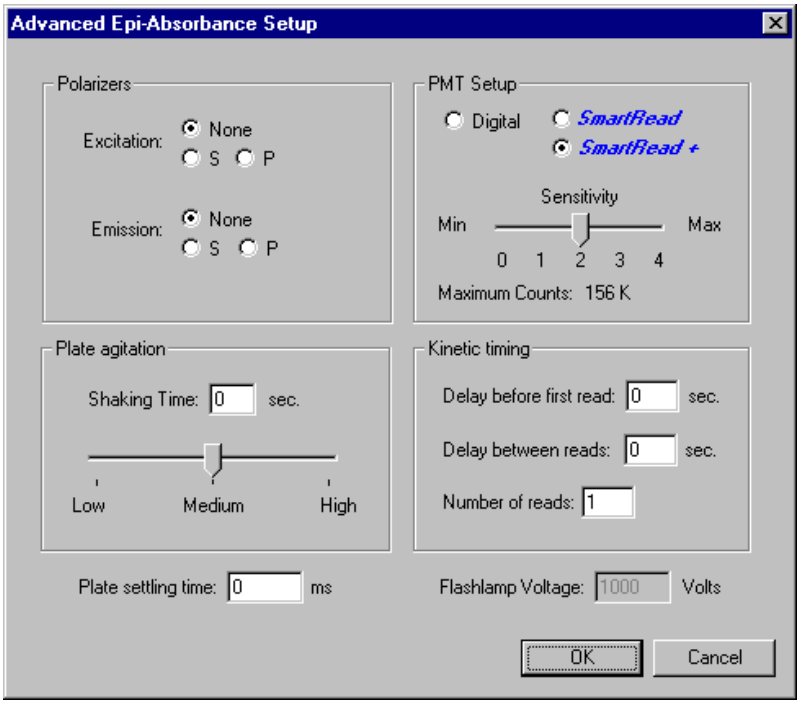
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Defining an Epi-Absorbance Method

The CriterionHost application allows you to specify the Epi-Absorbance method parameters shown in the following example screens.



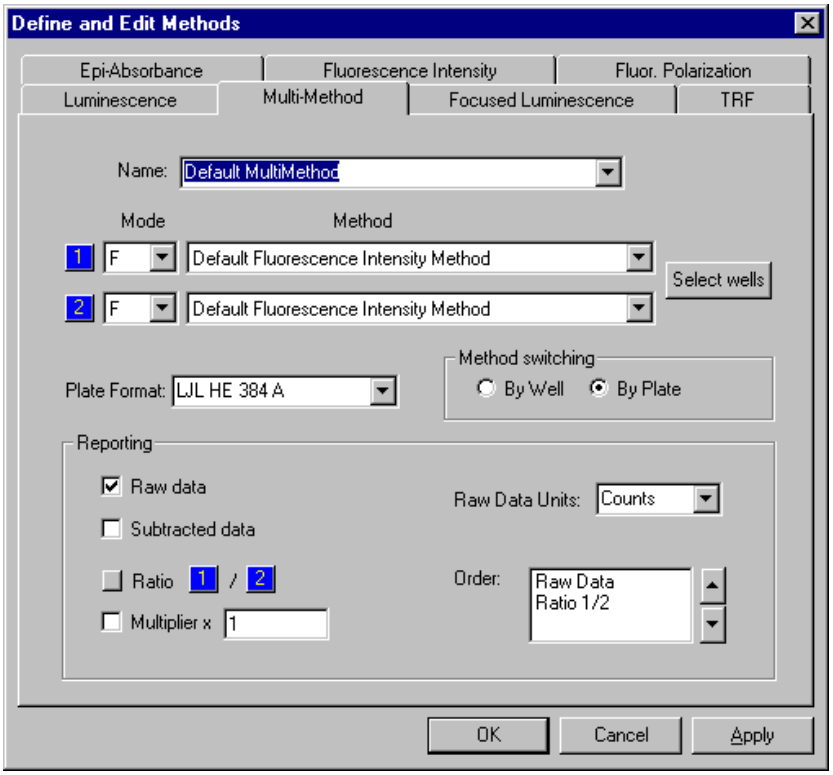
If you click on the Advanced button, the following screen appears:



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Defining Multi-Methods

The CriterionHost application allows you to specify the Multi-Method parameters shown in the following example screen.



Mode: Select the modes for the first and second methods. You can select any mode except fluorescence polarization.

Method: Select the first and second methods.

Plate Format: For each method, select a plate format from the drop-down list. The system is provided with a number of predefined standard plate formats. In addition, you can define custom formats by using the options on the Plate menu.

Method Switching: Specify 'By Well' to have both methods run on each well before moving on to the next well; specify 'By Plate' to have the first method run on all wells on the plate and then have the second method run on all the wells.

***Note**: Specifying 'By Well' will substantially diminish throughput if any of the following parameters is not the same in both methods:*

- *Z-height*
- *Type of lamp (continuous or flash)*
- *Optics used (top or bottom)*

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Reporting:

- To specify which data will appear in reports, check 'Raw data' and/or 'Subtracted data.' The readings reported in the Subtracted Data section of the report will be the results of subtracting the average of the readings in the background wells from the readings in each of the sample wells.
- To include in the report a section showing the ratio of the readings using one of the two methods to the readings using the other method, click the small button next to 'Ratio.' Each time you click the button, the specification for the Ratio section of the report switches among the following, as shown in the 'Order' box:
  - Omitted.
  - Showing the ratio of the readings using method 1 to the readings using method 2.
  - Showing the ratio of the readings using method 2 to the readings using method 1.
- To specify a multiplier for the ratio, check 'Multiplier' and enter the value.
- To specify the order in which the various sections of data will appear in the report, select them in the 'Order' box and click the arrow keys to move them up or down.

Note: *In Multi-Method mode, the kinetic timing parameters ('Delay Before First Read,' 'Delay Between Reads,' and 'Number of Reads') in the individual method definitions are ignored.*

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2.5 READING MICROPLATES

This section explains how to select a method, read microplates, and review results.

Note: *Focused luminescence methods require that position #8 in the emission filter wheel be open.*

Caution: *Replace the filter or metal slug after running a focused luminescence method (if this is not done, the instrument could be damaged).*

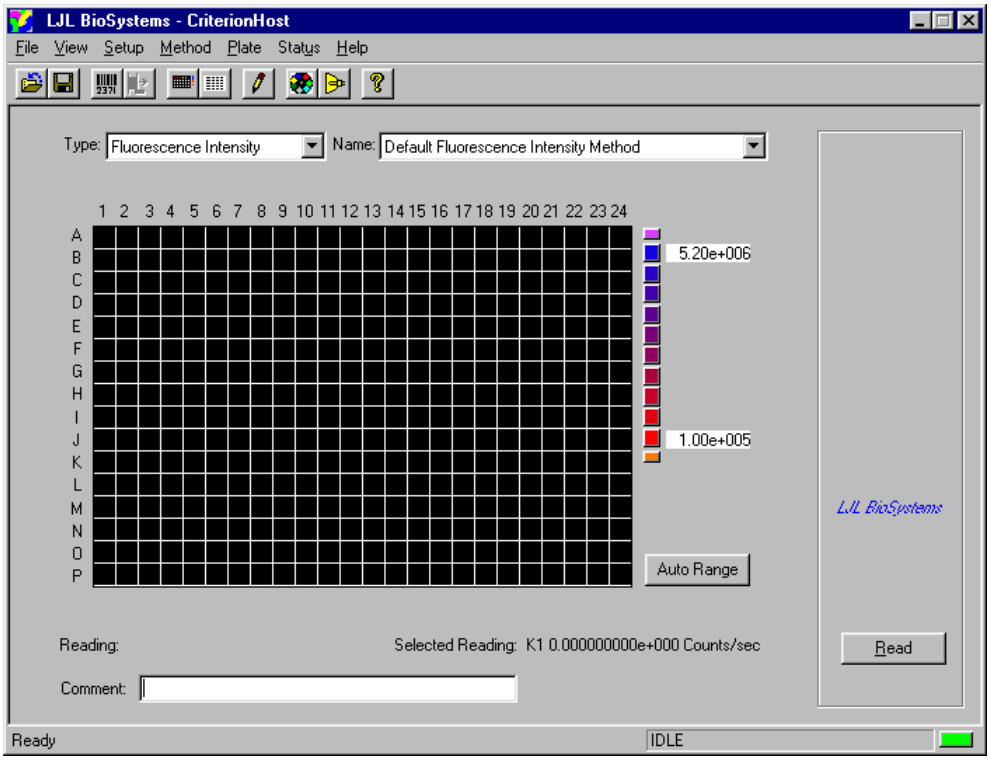
Note: *After removing or replacing the filter (or metal slug) in position #8 in the emission filter wheel, be sure to update the list of filter names using the Edit Filter Names option on the Setup menu.*

Starting a Run

From the CriterionHost application, there are three steps to starting a run:

1 Select method.

From the Run screen (in either Plate View or Report View), use the drop-down Type and Name menus to select the method. An example of the Plate View screen is shown below.



2 Load microplate(s).

- **Systems without Stacker:** Place microplate into the plate gripper.
- **Systems with Stacker**: Insert a single microplate into the input position. Or load the input magazine with a stack of up to 40 plates.

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3 Start microplate reading.

There are several options for starting the run.



- **START Key:** Press **START** on the control panel. The instrument loads and reads the microplate, and, when finished, ejects the plate automatically.

  If plates are loaded in the stacker input magazine, then pressing **START** automatically loads one plate from the input magazine, reads it, and ejects it to the output magazine.

- **Read Button:** If you click the Read button on the Run screen (lower right corner), the instrument loads and reads the microplate. If the plate is already loaded, the instrument simply reads the plate. If the stacker has been enabled with the Setup menu, then clicking the Read button will read all the plates in the stacker.

4 Monitor results during the run.

The following options are available while the run is in process:



- **LOAD/EJECT key:** To stop the run, press **LOAD/EJECT** on the control panel at any time.

- **Stop/Eject button:** When the run begins, the Read button changes to 'Stop/Eject' (Plate or Report View screens). To stop the run, click the Stop/Eject button, which has the same effect as the **LOAD/EJECT** key on the instrument control panel of the Analyst HT.

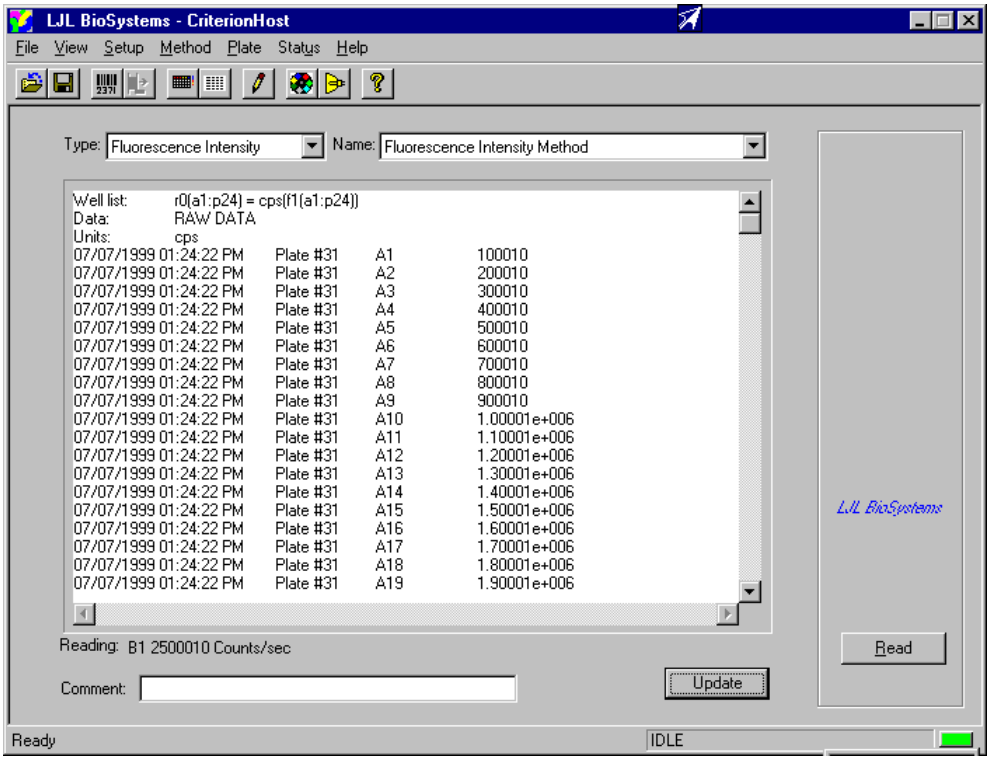
- **Update button:** In Report View, you can view results already collected by clicking the Update button.

- **Reset Instrument command and RESET key:** After the run begins, you can stop the run and reinitialize the instrument at any time by selecting Reset Instrument from the File menu or (on the Analyst HT) by pressing **RESET** on the control panel.

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Reviewing Results

When reading is complete, the instrument reports the results to the host computer. The Report View screen shows the results.



Note the following options:

- **Auto Range Button:** You can use the Plate View screen to quickly spot high and low values. The screen automatically assigns a color to each result on the plate grid. Low values are shown in red, while high values are shown in blue. (For an example of the Plate View screen, see section 2.1, 'Starting Up the System.') Use the color scale at the right of the plate grid to determine the approximate reading. If you click the Auto Range button on the Plate View screen during the reading or when the reading is complete, the top and bottom numbers on the color scale change to match the highest and lowest readings, and the colors shown for the various results on the plate grid change accordingly.
- **Reverse Button:** For systems equipped with the stacker, the Reverse button appears at the end of the run (if you used the stacker to load the plates). With the Reverse button, you can quickly restore the stack of plates to their original order:

  1 When the run ends, switch the magazines on the stacker. The magazine on the left should be loaded with plates – the one on the right should be empty.

  2 Click the Reverse button. The system automatically restacks the plates.

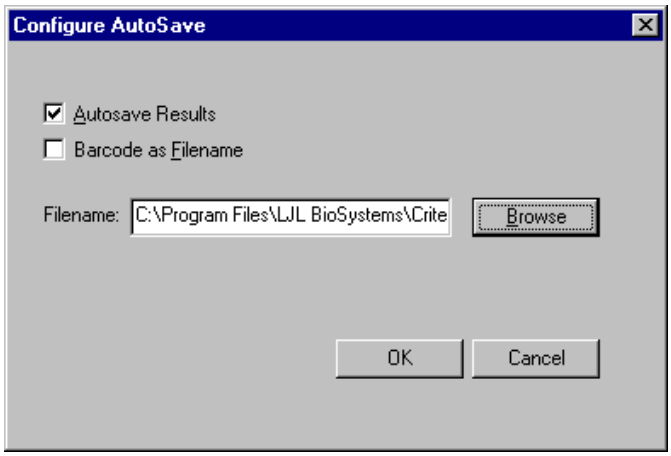
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Saving Results Files

The File menu provides the following options for saving files:

If you select…	The CriterionHost application…
File, Save	Saves current results using the current filename. If no filename is defined, then the system prompts for a new filename.
File, Save As…	Prompts for new filename for current results.

If you select File, Autosave, the following dialog box appears.



If you select…	The CriterionHost application…
☐ Autosave Results ☐ Barcode as Filename Filename: (field disabled)	Prompts for new filename at beginning of next read.
☑ Autosave Results ☐ Barcode as Filename Filename: c:filename.txt	Automatically appends microplate report to the end of current file with name 'filename.txt.'
☑ Autosave Results ☑ Barcode as Filename Filename: (field blank)	Automatically assigns barcode as filename. Saves one microplate report per file without prompting user.
☑ Autosave Results ☑ Barcode as Filename Filename: c:filenamebarcode.txt	Concatenates (joins) filename and barcode string. Saves one microplate report per file without prompting user.

Example: Suppose the barcode is 234779, you check both Autosave Results and Barcode as Filename, and you enter 'kinaseassay.txt' for the filename. The results will be saved under the filename 'kinaseassay234779.txt.'

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2.6 PERFORMANCE VERIFICATION

The performance of the instrument can be compared to published specifications for the F and HEFP modes by determining the lower detection limits (LDL) for fluorescein using the following protocol:

Filters and Dichroic Mirrors

LJL BioSystems provides two sets of excitation and emission filters and three dichroic mirrors to verify system performance.

F and HEFP Modes: For performance verification in the F and HEFP modes, the system includes an optimized set of fluorescein filters and a fluorescein dichroic mirror. When the instrument is shipped, the fluorescein filters are installed in the filter wheels and the fluorescein dichroic mirror is installed in the top optics head.

TRF Mode: For performance verification in the TRF mode, the system includes an optimized set of europium filters and a europium dichroic mirror. When the instrument is shipped, the europium filters are installed in the filter wheels. The europium dichroic mirror is provided in the accessory kit and is not preinstalled.

Materials

- Phosphate-buffered saline (PBS), pH=7.4
- Costar black 96 or 384-well plates
- Sodium fluorescein stock solution
- Polypropylene tubes
- Multichannel pipet
- Multichannel basin(s), e.g., Monoblock

Method for Fluorescence Intensity Verification

1 **Prepare a 1:2 dilution series.** Start at 512 pM and decrease to 0.5 pM. Prepare dilutions in polypropylene tubes, and then transfer them from multichannel basin(s) to the microplate using a multichannel pipet. The following is a suggested template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	Buffer	0.5 pM	1 pM	2 pM	4 pM	8 pM	16 pM	32 pM	64 pM	128 pM	256 pM	512 pM
D												
E												
F												
G												
H												

200 µL/well for 96-well plate
80 µL/well for 384-well plate

2 **Read plate.** Use fluorescein excitation/emission filters with fluorescein dichroic mirror, plate format set to Corning Costar 96 PS Solid, z-height set to 3.0, integration time set to 100,000 µsec, units set to CPS, SmartRead detection counting mode (sensitivity 2), and attenuator set to Out.

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3 **Analyze data**. Use Microsoft Excel or equivalent spreadsheet program.

a Calculate the average, standard deviation, and coefficient of variation for each set of sample readings.

b Subtract average background (buffer reading) from each averaged sample value.

c Plot concentration versus background-subtracted sample values on a log-log plot.

d For determination of the fluorescein lower detection limit (LDL), calculate a linear regression using nonsaturated readings (nonbackground-subtracted readings less than approximately 2 million counts per second) and forcing the line through the zero point.

e From the equation of the line, determine the LDL as the concentration that is three times the background standard deviation. Example equation used:

LDL = TREND($AF$8:$AF$14, AH31:AH37, AH39, FALSE)

This equations returns LDL in pM where:

- $AF$8:$AF$14 = range of concentrations in pM
- AH31:AH37 = range of background-subtracted values
- AH39 = value for three times the background standard deviation
- FALSE forces line through zero

Note: *The LDL for Fluorescence mode should be* ***less than or equal to 5 pM*** *for both 96- and 384-well microplate formats.*

f Another useful number is the R^2 value for the curve fit. It can be calculated using the following equation:

R^2 = INDEX[LINEST($AF$8:$AF$14, AH31:AH37, FALSE, TRUE), 3, 1]

- $AF$8:$AF$14 = the range of concentrations
- AH31:AH37 = range of background-subtracted values
- FALSE forces line through zero
- TRUE reports statistics
- 3 specifies the row for the R^2 value in the statistics table
- 1 specifies the column for the R^2 value in the statistics table

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Method for Fluorescence Polarization Verification

1 **Prepare a dilution series.** Start at 100 nM and decrease to 1 pM by half-log dilutions. Prepare dilutions in polypropylene tubes and then transfer them from multichannel basin(s) to the microplate using a multichannel pipet. The following is a suggested template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	Buffer	1 pM	3 pM	10 pM	30 pM	100 pM	300 pM	1 nM	3 nM	10 nM	30 nM	100 nM
D												
E												
F												
G												
H												

200 µL/well for 96-well plate
80 µL/well for 384-well plate

2 **Read plate.** Use fluorescein excitation/emission filters with fluorescein dichroic mirror, plate format set to Corning Costar 96 PS Solid, z-height set to 3.0, integration time set to 100,000 µsec, units set to CPS, SmartRead detection counting (sensitivity 2), and attenuator set to Out. Set the excitation polarizer to the S position and the emission polarizer to dynamic.

3 **Analyze data to calculate G factor.** Use Microsoft Excel or equivalent spreadsheet program. The assumption is that higher pM or nM concentrations of fluorescein have a value of 27 mP (millipolarization units).

a Calculate the average buffer reading for both the S and P raw data.

b Choose the highest concentration of fluorescein (for example, 3 nM) that reports values approximately ten times the background signal for both S and P raw data (this will be the concentration used to calculate the G factor). The values must be greater than 10,000 counts for acceptable noise.

c Subtract the respective average background from all other S and P data.

d Calculate the G factor using the following formula

G factor = S/P*(1-27/1000)/(1+27/1000)

where S and P are the background-subtracted intensity measurements for the parallel and perpendicular components at a selected concentration, and 27 is the assumed mP value for HEFP mode. The G factor is typically in the range of 0.8 to 1.2.

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Calculating the G Factor

Use the following equation to calculate the G factor for each probe:

$$G\ factor = (S/P)(1 - P^{true}) / (1 + P^{true})$$

where S and P are the background-subtracted intensity measurements for the parallel and perpendicular components and P^{true} is the literature value for the probe being evaluated.

e For calculation of actual polarization values, use the following equation:

$$= 1000*(S-G*P)/(S+G*P)$$

where S and P are background-subtracted data and G is the calculated G factor.

f For each concentration, calculate the average polarization (mP) and standard deviation. It is useful to create two plots for viewing the results: concentration vs. polarization and concentration vs. standard deviation.

Note: *The LDL (lower detection limit) for Fluorescence Polarization mode for both 96- and 384-well microplate formats should be:*

- *Less than or equal to 10 mP standard deviation at 100 pM fluorescein, and*
- *Less than or equal to 3 mP standard deviation at 1 nM fluorescein.*

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3 Robot Integration (Analyst HT)

The Analyst HT Assay Detection System has been designed for easy integration into the laboratory automation environment. For complete information on robot integration, request the 'Robot Integrator's Package' from your LJL BioSystems representative. The package includes a description of Active X Suite, a set of Active X controls for the Analyst HT System.

3.1 PLATE DELIVERY AND PICKUP

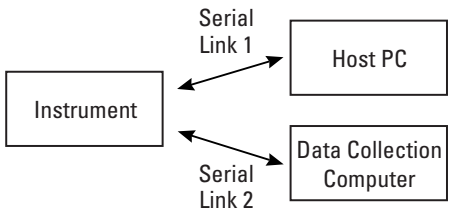
The mechanical interface between the robot fingers (end effectors) and the instrument's plate gripper has been optimized to reduce the precision and accuracy required by the robot to deliver and pick up plates. Microplates conforming to the Society for Biomolecular Screening provisional standard for 96-well microplates will have 1.24 mm clearance on the long dimension and 7.33 mm clearance on the short dimension of the plate when placed in the plate gripper. As the microplate is loaded into the instrument, positioning fingers push the plate into the upper left-hand corner of the gripper to ensure positional accuracy. This allows the robot to place the microplate anywhere in the plate gripper mechanism.

Contact Closure Relay Ports

The instrument has three double-pull, double-throw, contact closures. Two of these outputs correspond to the Fault and Service conditions. For further information, see 'Relay Port' in section 1.4, 'System Components.'

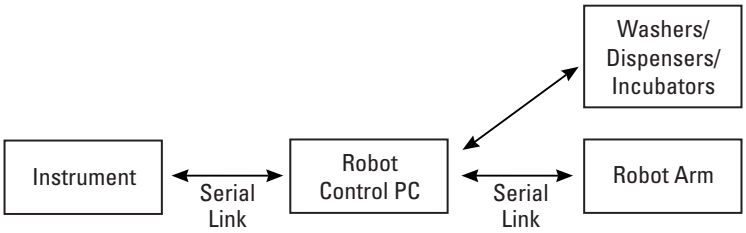
3.2 OPEN PROTOCOL COMMANDS

The Open Protocol is a simple command-and-response interface between the instrument and the host computer controlling the robot. Text commands in ASCII format are sent via an RS232C serial port to the instrument. The instrument's response can be sent back to the host computer or to another computer. Two typical configurations are shown in the following diagrams.



Dual Interface to Host and Data Collection Computers

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Single Interface to Robot Control Computer

The Open Protocol can be used with any program that can transmit and receive ASCII text over a serial port. This capability is available with any terminal emulation program, Microsoft Visual Basic®, and most robot control software.

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Open Protocol Syntax

The Open Protocol commands use the following conventions.

- **Bold** text is used for command names.
- *Italic* text in brackets '[*parameter*]' are optional user-selected variables. If omitted, the instrument gets (displays) the current value. When appropriate, this listing defines acceptable user entries as either discrete values [*on, off*] or a specified range.

A message from the user has the form: **command** *parameter...*<CR>

- **command** is a string of one or more characters representing an instrument command.
- *parameter...* represents one or more optional or variable parameters for a command.
- <CR> is the ASCII code for 'carriage return' indicating the end of a message.

All command and parameter sequences are given in lowercase. Uppercase letters can also be used with no alteration in the output unless specified in the command description (for example, see the **tf** command).

Responses from the instrument have one of the following forms:

<LF>OK<CR><LF>
<LF>data<CR><LF>
<LF>ER message<CR><LF>

- **<LF>** is the ASCII code for 'line feed' indicating the start of the reply.
- **OK** indicates the command was executed successfully.
- **data** is a text string containing data requested by the host computer.
- **ER** indicates the command failed (error)
- **message** is a text string identifying the error that caused a command to fail.
- **<CR>** is the ASCII code for 'carriage return.'
- **<LF>** is the ASCII code for 'line feed' indicating the end of a message.

Use 'exit' to exit from the program.

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Concordance of Commands Grouped by Function

Open Protocol commands are grouped below by function. For each command, a short description of the command is given. For detailed information about a command, see 'Alphabetical Command Listing' later in this section.

Instrument Configuration Commands

Command	Description
debug [*on* | *off*]	Gets or sets message reporting status. When 'on,' logs messages to the control panel.
efilters	Gets a list of currently installed emission filters.
efname [*number*] [*any string*]	Gets or sets the description for an emission filter.
formats	Gets a list of currently defined plate formats.
hwversion [*any string*]	Gets or sets the hardware version number.
la	Gets the current luminescence aperture position.
lamphours [*number*]	Gets or sets the number of hours the continuous lamp has been switched on.
lamplife [*number*]	Gets or sets the continuous lamp lifetime in hours.
lamps	Gets a list of lamps.
lampservice [*number*]	Gets or sets the warning time in hours for the remaining lifetime of the continuous lamp.
lamptimeout [*number*]	Gets or sets the continuous lamp automatic power off time in minutes.
ljltype	Identifies the instrument on a blind serial port query.
manufacturer [*any string*]	Gets or sets the manufacturer name.
model [*any string*]	Gets or sets the product model information.
platenumber [*number*]	Gets or sets the number of plates processed.
platetotal	Gets the number of plates ejected for the lifetime of the instrument.
product [*any string*]	Gets or sets the product name.
sn [*any string*]	Gets or sets the instrument serial number.
stackerenable [*on* | *off*]	Enables or disables the stacker or gets the current stacker setting.
swversion	Gets the version number of the software.
tag [*any string*]	Gets or sets a text string that identifies the instrument in the user's lab.
tf [*time format string*]	Gets or sets the current date and time display format.
time [*date time string*]	Gets or sets the current date and time in the instrument.
xfilters	Returns a list of the currently installed excitation filters.
xfname [*number*] [*any string*]	Gets or sets the description for an excitation filter.

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Microplate Configuration Commands

Command	Description
pfcolumnoffset [*number*]	Gets or sets the offset in mm from the edge of the plate to the center of column 1 (well A1).
pfcolumns [*number*]	Gets or sets the number of columns for the current plate format.
pfcolumnspacing [*number*]	Gets or sets the spacing in mm from column to column for the current plate format.
pfdelete [*any string*]	Deletes the plate format specified by *any string*.
pfheight [*number*]	Gets or sets the current plate height in mm.
pflength [*number*]	Gets or sets the current plate length in mm.
pfnew [*any string*]	Gets current plate definition or generates a new plate definition.
pfrename [*old name*, *new name*]	Renames a plate format.
pfrowoffset [*number*]	Gets or sets the offset in mm from the edge of the plate to the center of row A (well A1).
pfrows [*number*]	Gets or sets the number of rows for the current plate format.
pfrowspacing [*number*]	Gets or sets the spacing in mm from row to row for the current plate format.
pfstandard [*on*]	Gets or sets the current plate definition as a standard (uneditable) plate format.
pfwelldepth [*number*]	Gets or sets the depth in mm of a well.
pfwells [*plate format*]	Gets the number of wells for the current plate format.
pfwidth [*number*]	Gets or sets the current plate width in mm.
rf	Generates a report on the current microplate format.

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Detection Method Setup Commands

Command	Description
am [*h* | *m* | *o*] [, *method name*]	Gets or sets the neutral density filter attenuator.
bsname bottom [*any string*] **bsname top** [*any string*]	Gets or sets top or bottom dichroic mirror name.
cap [*0* | *1* | *2* | *3* | *4*] [, *method name*]	Gets or sets the integration capacitor.
cm [*analog* | *digital* | *comparator* | *smartread*] [, *method name*]	Gets or sets the photomultiplier tube (PMT) detector counting option.
cycles [*number*] [, *method name*]	Gets or sets the number of readings per well. For flash lamp methods, gets or sets the number of flash cycles.
dcenable [*on* | *off*] [, *method name*]	Enables or disables dark count reading.
dmdelete [*method name*]	Deletes an existing detection method.
dmnew [*existing method name*, *new method name*]	Creates a new detection method.
dmrename [*old method name*, *new method name*]	Renames a detection method.
efilter [*number*] [, *method name*]	Gets or sets an emission filter.
epolarizer [*s* | *p* | *o*] [, *method name*]	Gets or sets the emission polarizer position.
eside [*bottom* | *top* | *none*] [, *method name*]	Gets or sets the side of the plate for collection of the emission light.
format [*string*]	Gets or sets the currently selected microplate format.
it [*number*] [, *method name*]	Gets or sets the sample integration time in microseconds.
lamp [*continuous* | *flash* | *none*] [, *method name*]	Gets or sets the lamp source.
methodid [*any string*]	Gets or sets the method name.
mode [*f* | *fpp* | *fps* | *l* | *trf* | *a*| *fljl* | *fppljl* | *fpsljl* | *lljl* | *trfljl* | *aljl*]	Gets or sets the current detection method. Including 'ljl' after the method designation (for example, 'mode fljl,') configures the system with a set of default parameters for the method.
mrc [*number*]	Gets or sets the number of read cycles to perform.
mrt [*number*]	Gets or sets the time in seconds between multi-read cycles.

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Detection Method Setup Commands (cont'd)

Command	Description
pchead [*hc-124* | *hc-120* | *photodiode*] [, *method name*]	Gets or sets the type of light sensor for a detection method.
pdcap [*0* | *1* | *2*] [, *method name*]	Gets or sets the photodiode integrator capacitor.
pdgain [*x1* | *x2* | *x4* | *x8*] [, *method name*]	Gets or sets the signal gain at the input to the photodiode A/D converter.
pdresistor [*0* | *1* | *2* | *3* | *4* | *5* | *6* | *7*] [, *method name*]	Gets or sets the photodiode integrator resistor
pdrgain [*x1* | *x100*] [, *method name*]	Gets or sets the photodiode remote amplifier gain.
pgaadc [*number*] [, *method name*]	Gets or sets the ADC programmable gain amplifier.
pgaint [*number*] [, *method name*]	Gets or sets the integrating programmable gain amplifier.
plate	Gets the string specified by the **plateid** command.
plateid [*any string with or without %d*]	Gets or sets a plate ID string, which appears on the 'verbose' report.
pmtenable [*off* | *on*]	Gets the current PMT status or enables or disables the currently selected PMT.
rs [*row* | *column*] [, *method name*]	Gets or sets the well read sequence.
settle [*number*]	Gets or sets the plate settling time.
xfilter [*number*] [, *method name*]	Gets or sets an excitation filter.
xpolarizer [*o* | *s* | *p*] [, *method name*]	Gets or sets the excitation polarizer position.
xside [*bottom* | *top* | *none*] [, *method name*]	Gets or sets the side of the plate for transmission of the excitation light.
zh [*number*] [, *method name*]	Gets or sets the z-height in mm.
zt [*bottom* | *middle* | *numeric*] [, *method name*]	Gets or sets the z-height target.

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The lists below identify subsets of commands specific to HEFP and TRF modes. The instrument retains these parameters for each detection mode. It restores the parameters when the user switches between the detection modes. For example, if the user sets the detection filter to number 8 while in Fluorescence mode, the detection filter settings will not be changed to number 8 in Fluorescence Polarization and Time-Resolved Fluorescence modes.

Fluorescence Polarization Commands

Command	Description
dpolarizer [*emission* | *excitation* | *none*]	Gets or sets the dynamic polarizer.

Flash Lamp Commands

Command	Description
delay [*number*] [, *method name*]	Gets or sets the delay after flash in microseconds.
fv [*number*] [, *method name*]	Gets or sets the flash lamp voltage.
period [*number*] [, *method name*]	Gets or sets the sample period in milliseconds.

Barcode Reader Commands

Command	Description
bc [*any string*]	Gets or sets barcode string.
bcenable [*on* | *off*]	Enables or disables barcode reader on the stacker (or gets current setting).
bcr	Instructs the instrument to read the barcode string with the barcode reader.

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Run Time Commands

Command	Description
calc [*valid read expression*]	Computes a constant based on the *valid read expression* entered.
dal [*number*]	Gets or sets the delay in seconds between loading the plate and starting a read operation.
dalr	Gets the delay time remaining in seconds until the read operation begins.
eject	Moves the plate gripper to the eject position.
help	Gets a list of commands and descriptions.
lampenable [*off* | *on*]	Enables or disables the currently selected lamp or gets current lamp setting.
load	Loads the microplate.
mrcr	Gets the number of read cycles remaining (including the current read cycle).
mrn	Gets the number of multi-read cycles the instrument has just completed.
mrtr	Gets the delay time remaining in seconds until the start of the next read.
ms [*plate* | *well*]	Gets or sets the method sequence for multi-method reading, either by *plate* or *well*.
mw [*rc*]	Positions plate so that center of well *rc* (row/column) is positioned in the light cone.
rdc [*method name ()*]	Performs a dark count reading.
rr [*well*]	Reports well readings.
read [*valid read expression*]	Loads and reads the plate according to the *valid read expression*. When done, the instrument does not eject the plate. See also **rw** and **start** commands.
reset	Resets the instrument.
reverse	Reverses a stack of plates by moving plates one at a time from the input to the output magazine.
rlc [*method name*]	Performs an air (or *light*) count reading.
rr [*well*]	Reports well readings.
rw [*valid read expression*]	The instrument reads the last well read or the plate according to the *valid read expression*. See also **read** and **start** commands.
shake	Shakes the currently loaded plate.
shakeradius [*number*]	Gets or sets radius of circular motion used for shaking the plate.
shaketime [*number*]	Gets or sets the time in seconds to shake the plate.
shakevel [*number*]	Gets or sets the velocity for the shaking operation.
start [*valid read expression*]	The instrument loads, reads, and ejects the plate. See also **read** and **rw** commands.

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Assay Report Commands

Command	Description
comment [*any string with or without %d*]	Gets or sets the comment field for the 'verbose' microplate report.
errors	Generates a report of all errors.
ert	Gets the 'end report text' or sets it with the specified string.
report	Generates a report of the well readings for the current plate.
rl [*no header* | *terse* | *verbose* | *columns*]	Gets or sets the report layout.
rrdc	Reports the dark count reading.
rrlc	Reports the air (or *light count*) reading.
rtidelete [*name*]	Deletes the report table information with the given *name*.
rtilist [*name*]	Generates a listing for the report table information identified by *name*.
rtinfo [*name*, *label*, *units*, *display format*]	Sets the parameters for the report table information block identified by *name*.
rtirename [*existing name*, *new name*]	Renames report table information from *existing name* to *new name*.
srt [*any string*]	Gets the 'start report text' or sets it with the specified string.

Status and Troubleshooting Commands

Command	Description
autocycle [*on*| *off*]	Cycles instrument (loads, reads, and ejects plate) through the measurement modes.
beeper [*on* | *off*]	Gets or sets the beeper status.
busy	Returns the current state of busy devices.
ce	Clears the error condition bit in the status word (see **st**).
cie	Clears the stacker empty bit in the status word (see **st**).
faults	Generates a report of all active faults.
rm	Generates a report on the current detection method settings.
service	Generates a report of all active service conditions.
se	Generates an error condition for testing host computer programs.
sf	Generates a fault condition for testing host computer programs.
ss [*on* | *off*]	Generates a service condition for testing host computer programs.
st	Gets operating status as a 16-bit hexadecimal number (four hex digits).
temp [*1* | *2*]	Reports the temperature reading for sensor 1 or 2.

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Alphabetical Command Listing

am [*h* | *m* | *o*] [*, method name*]

Gets or sets the neutral density filter attenuator for the currently configured detection method or for the specified detection method (as indicated by *method name*) as follows:

h: high attenuation: 10,000 times or optical density of 4
m: medium attenuation: 100 times or optical density of 2
o: out, no attenuation

Since there are only two attenuator positions in Luminescence mode (in and out), *h* and *m* are the same position (in) with an OD of 2.

To get a list of currently defined method names, use the **modes** command.

autocycle [*on* | *off*]

Gets or sets autocycle status.

If *on* is specified, the instrument cycles automatically through plate load, shake (if specified), read, and eject operations for each of the detection modes in turn in the following sequence: F, FP, TRF, L, A. When the instrument completes the reading for the last detection mode, the sequence starts over again. Ejecting a plate while loading, shaking or reading causes a subsequent load and read using the next detection mode in the sequence.

If *off* is specified while autocycling, the instrument finishes the current plate read and ejects the tray. No further readings occur.

bc [*any string*]

Gets or sets the barcode string.

If *any string* is not specified, the last barcode read will be displayed (the barcode reader does not read a new barcode using this command).

If *any string* is specified, the last barcode read will be updated with this string. To clear the current string, type **bc** followed by two quotes ("").

Range: 1–80 characters

bcenable [*on* | *off*]

Enables or disables barcode reader on the stacker (or gets current setting). Barcode reading is performed automatically when a plate is loaded in the instrument upon issuance of a **load**, **start**, or **read** command.

If the input is *on*, the instrument turns on the barcode reader light, reads the barcode on the side of the plate, and then transfers the plate into the instrument. The light remains on with the plate in the same position until the instrument reads the barcode or five seconds elapses, whichever occurs first. At this time, the plate is transferred into the instrument.

If the input is *off*, the barcode is not read prior to transferring the plate into the instrument.

bcr

Instructs the instrument to read the barcode string with the barcode reader. The instrument turns on the barcode reader light and reads the barcode on the side of the plate. The light remains on until the barcode is read or five seconds elapses, whichever occurs first. If the barcode cannot be read within the time interval allowed or is read incorrectly, the last barcode read (stored in the instrument) will be set to 'ERROR' (see **bc** command).

beeper [*on* | *off*]

Gets or sets the beeper status as follows:

If *on* is specified, the beeper turns on.

If *off* is specified, the beeper turns off.

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bsname bottom [*any string*]
bsname top [*any string*]

Gets or sets the top or bottom dichroic mirror (beamsplitter) name.

If *any string* is specified, the name is assigned to either the top or bottom dichroic mirror depending upon the input specified. To clear the current string, type **bsname** followed by two quotes ("").

If *any string* is not specified, the name of either the top or bottom dichroic mirror is displayed.

Range: 1–32 characters

busy

Returns the current state of busy devices.

calc [*valid read expression*]

Computes a constant based on the *valid read expression* entered. The constant can be used by a remote host computer for data analysis immediately after the data has been read. Note: Any calculations must be performed prior to issuance of the next **read** or **start** command since the data will be overwritten in the instrument memory at this time.

A *valid read expression* consists of one or more parameters used to specify the detection method(s), well sequence(s), and data processing operator(s). The instrument performs the sequence in the order entered (from left to right) using precedence rules when parentheses and operators are used. Some examples of usage are listed below. Refer to the **read** command for a detailed description of defining read expressions (and functions used in the examples below).

Example 1:

- read cps (f1(a1:a12)): Reads wells a1 thru a12 in f1 detection method and stores results in cps
- calc cps (max (f1(a1:a12))): Computes the maximum of wells a1:a12 that were read in method f1

Example 2:

- read counts (trf1(a1,b1,c1)): Reads wells a1, b1, c1 in trf1 detection method and stores results in counts
- calc counts ((trf1(a1)*1000) / trf1(c1)): Multiplies well reading a1 by 1000 and divides by reading c1

Example 3:

- read fp (cps (fps(a:h)), cps (fpp(a:h)), 0.9): Reads a 96-well plate in methods fpp and fps and computes mP data
- calc fp (cps (fps(a1)), cps (fpp(a1)), 0.9): Computes mP data for well a1 that was read in fps and fpp methods

cap [*0* | *1* | *2* | *3* | *4*] [*, method name*]

Gets or sets the integration capacitor to be used by the instrument optics module for reading in analog or comparator mode (see **cm** command). The integration capacitor will be set up for the currently configured detection method or for the specified detection method (as indicated by *method name*). Selections are given below followed by the full-scale counts (in parentheses):

0: 100 nF (1,560,000)
1: 47 nF (733,000)
2: 10 nF (156,000)
3: 1 nF (15,600)
4: 0.2 nF (3,120)

To get a list of currently defined method names, use the **modes** command.

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ce

Clears the error condition bit in the status word (see **st**).

cie

Clears the stacker empty bit in the status word (see **st**).

cm [*analog* | *digital* | *comparator* | *smartread*] [*, method name*]

Gets or sets the detector counting option (conversion method) for light detection applied by the photomultiplier tube (PMT) for the currently configured detection method or for the specified detection method (as indicated by *method name*).

analog: For a given integration time (see **it** command), the system measures the voltage across the integration capacitor (see **cap** command) and uses it to determine the photomultiplier tube (PMT) current output. The system uses the current to determine accumulated counts and counts per second (normalized) taking the PMT gain (a constant) into account.

digital: Also referred to as photon counting, the system accumulates the PMT digital pulse output (1 pulse ≅ 1 photon) and normalizes it (counts per second) while taking the PMT gain (a constant) into account.

comparator: The system monitors the integrator voltage while measuring time (in 100 nanosecond increments). When 5 volts is reached, the system uses the accumulated time to determine the PMT current output. When the integration time expires, the system uses the current output to determine the accumulated counts and counts per second (normalized) taking the PMT gain (a constant) into account. If 5 volts is not reached within the integration time period (see **it** command), the system applies the analog detector counting option.

smartread: Same as comparator mode except that when the integrator voltage reaches 5 volts, reading stops (regardless of the integration time). The system measures the accumulated time and uses it to determine the PMT current output immediately.

Note: *To specify SmartRead detector counting, use* ***cm*** *comparator; to specify SmartRead Plus, use* ***cm*** *smartread.*

To get a list of currently defined method names, use the **modes** command.

comment [*any string with or without %d*]

Gets or sets the comment field for the verbose report. To clear the current string, type **comment** followed by two quotes (""). If the string contains *%d*, then the system substitutes the number of plates processed in place of the *%d* code. The current number of plates processed is managed with the **platenumber** command.

Recommended range: 1–160 characters

cycles [*number*] [*, method name*]

Gets or sets the number of readings per well for the currently configured detection method or for the specified detection method (as indicated by *method name*). For flash lamp methods, gets or sets the number of flash cycles (the number of times the flash lamp illuminates) per well.

Range: 1–1023

To get a list of currently defined method names, use the **modes** command.

dal [*number*]

Gets or sets the delay in seconds after loading the plate in the instrument, prior to starting a read operation.

Range: 0–3600 seconds

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dalr

Gets the time remaining in seconds for the delay after loading the plate prior to starting a read operation.

dcenable [*on* | *off*] [*, method name*]

Enables or disables dark count reading for the currently configured detection method or for the specified detection method (as indicated by *method name*). The instrument performs a dark count read in the following cases:

- When light from the source is blocked from the light sensor (absorbance), or
- When there is no light (luminescence), or
- When the flash lamp is used (TRF).

This measurement is used as a read offset for calibration of the optics and for establishing a baseline for subsequent well reads that can be examined in the report. The standard methods for absorbance, luminescence, and TRF have dark count reading enabled.

If *on* is specified, the instrument performs dark count reading for the detection method specified in the read sequence prior to reading the well sequence when a **start** or **read** command is issued. If *off* is specified, the instrument does not perform dark count reading.

To get a list of currently defined method names, use the **modes** command.

debug [*on* | *off*]

Gets or sets message reporting status. If *on* is specified, the program logs incoming and outgoing messages to the console and an output log file. If *off* is specified, messages are suppressed.

delay [*number*] [*, method name*]

Gets or sets the delay after flash in microseconds for the currently configured detection method or for the specified detection method (as indicated by *method name*). The delay time is the time to wait after a flash occurs to start integration. See also **period** and **it** commands.

Range: 0–13,000 µsec

To get a list of currently defined method names, use the **modes** command.

dmdelete [*method name*]

Deletes an existing detection method with the name, *method name*. If *method name* is not specified, the current method will be deleted (unless it is a default method) and the instrument will be configured to the first method in the list of methods (see **modes** command).

dmnew [*existing method name, new method name*]

Creates a new detection method with the name, *new method name*, with behavior defined by one of the currently defined methods, *existing method name*. Since detection methods may be used in arithmetic expressions, characters in the name must be alphanumeric, a space or an underscore, the first character being alphabetic. Case is not significant (i.e., names with the same characters in upper and lower case will match).

Range: 1–32 characters

dmrename [*old method name, new method name*]

Renames a detection method from *old method name* to *new method name*. See **dmnew** for detection method naming conventions.

Range: 1–32 characters

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efilter [*number*] [*, method name*]

Gets or sets an emission filter for the currently configured detection method or for the specified detection method (as indicated by *method name*). The filter *number* is in the range 1 to 8. When a filter is selected, the instrument positions the selected filter in front of the fluorescence PMT. For excitation filter, see **xfilter** command.

To get a list of currently defined method names, use the **modes** command.

efilters

Gets a list of the currently installed emission filters.

efname [*number*] [*any string*]

Gets or sets the description for an emission filter.

- If *number* and *any string* are omitted, it returns a list of the current filter descriptions.
- If *number* is present and *any string* is omitted, it returns the description for the given filter number.
- If *number* and *any string* are both present, it changes the description for the given filter number. To clear the current string, type **efname** followed by two quotes ("").

Number range: 1–8

Any string range: 1–32 characters

eject

The instrument interrupts any load, shake or read operation and moves the plate gripper to the eject position just outside the door. If the stacker is present and enabled, it transfers the plate to the output magazine once the plate is outside the instrument. This has the same effect as the control panel LOAD/EJECT key.

epolarizer [*s* | *p* | *o*] [*, method name*]

Gets or sets the emission polarizer position for the currently configured detection method or for the specified detection method (as indicated by *method name*) according to the following:

s: The emission polarizer is aligned in a horizontal orientation when reading begins.

p: The emission polarizer is aligned in a vertical orientation when reading begins.

o: The emission polarizer is moved out of the optical path; for this case, the instrument defaults to the *s* position when reading begins in HEFP mode.

To get a list of currently defined method names, use the **modes** command.

errors

Generates a report of all errors complete with a date and time stamp (in the format specified by the **tf** command) that have occurred since the instrument was reset. An error is a temporary condition resulting in a failure to perform a requested operation. Such a condition can be caused from incorrect user-initiated commands and/or data. If there are no errors, the instrument responds with 'OK.'

ert [*any string*]

Gets the 'end report text' or sets it with the specified string (see **rl** command). The string may contain nonprinting characters (for example, '\x01') that will be interpreted for printing. To clear the current string, type **ert** followed by two quotes (""). Also see **srt** command.

\a: Bel
\b: Backspace
\f: Formfeed
\n: Linefeed
\r: Return

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\t: Horizontal tab
\v: Vertical tab
\x: Two-digit hexadecimal number
\\: Backslash '\'
\": Double quote '"'

Range: 1–32 characters

eside [*bottom* | *top* | *none*] [*, method name*]

Gets or sets the side of the plate for collection of the emission light for the currently configured detection method or for the specified detection method (as indicated by *method name*).

If *top* or *bottom* is selected, the PMT fiber shuttle moves such that the top or bottom emission fiber is in alignment with the current fluorescence PMT detector filter.

If *none* is selected, the detector fiber shuttle moves such that neither emission fiber is in alignment with the PMT detector filter (e.g., the midway point between the top and bottom fiber-optic ports). To get a list of currently defined method names, use the **modes** command.

faults

Generates a report of all active faults that have occurred since the instrument was reset, complete with date and time stamps in the format specified by the **tf** command. A fault is a condition resulting from a failure in the instrument mechanics, electronics, or software that can be cleared only upon an instrument reset (see **reset** command). If there are no currently active faults, the instrument responds with 'OK.'

format [*string*]

Gets the currently selected microplate format name, or sets a new format name if the input, *string*, is provided.

Range: 1–32 characters

formats

Gets a list of currently defined plate formats. The current list is:

Corning 96 Flat Clear PS
Corning 96 Flat Opaque PS
Corning 384 Square Clear PS
Corning 384 Square Opaque PS
Corning Costar 384 PS
Corning Costar 96 PS solid
Corning Costar Clear Bottom PS
Dynatech Microfluor 96
Dynex Microfluor 96 Round Black PS
Greiner Square 384 Flat Black PP
LJL HE 384 A
LJL HE 384 A Black PS
LJL HE 96 A
LJL HE 96 A Black PS
Nunc-Immuno Flat Bottom
Nunc Maxisorp 96 Flat Clear PS
Packard 384
Packard 384 Flat White PS
Packard - Optiplate
Packard-Optiplate 96 Flat White PS
Polyfiltronics 96 Flat Black PP
PolyfiltronicsPP96

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fv [*number*] [*, method name*]

Gets or sets the flash lamp voltage for the currently configured detection method or for the specified detection method (as indicated by *method name*).

Range: 31–1000 volts

Caution: *Do not set flash lamp voltage to less than 360 volts (except when performing absorbance mode readings).*

To get a list of currently defined method names, use the **modes** command.

help

Gets a list of commands and descriptions.

hwversion [*any string*]

Gets or sets the hardware version number. To clear the current string, type **hwversion** followed by two quotes (""). A security password is required to change the hardware version number.

Range: 1–32 characters

it [*number*] [*, method name*]

Gets or sets the sample integration time in microseconds for the currently configured detection method or for the specified detection method (as indicated by *method name*). The sample integration time is the time period during which the system performs photon sampling on each sample. See also **cycles** command.

Range: 50–1,600,000 µsec

To get a list of currently defined method names, use the **modes** command.

la

Gets the current luminescence aperture position. The position is set by the user on the luminescence detector head and is dependent upon the current plate format as follows:

384: The aperture is set up for 384-well plates

96: The aperture is set up for 96-well plates

If the current aperture position does not correspond to the current plate format while operating in Luminescence mode, the system issues a service condition.

lamp [*continuous* | *flash* | *none*] [*, method name*]

Gets or sets the lamp source for the currently configured detection method or for the specified detection method (as indicated by *method name*) according to the following:

continuous or *flash*: Moves the lamp fiber shuttle such that the top or bottom excitation fiber (see **xside** command) is in alignment with the selected lamp, continuous or flash. If the current excitation fiber is *none*, then the lamp fiber shuttle remains in the current position (e.g., midway between lamp sources). In addition, the instrument performs a **lampenable on** command.

none: Moves the lamp fiber shuttle such that neither lamp source is in alignment with the excitation fiber (e.g., the midway point between lamp sources) and performs a **lampenable** *off* command.

To get a list of currently defined method names, use the **modes** command.

lampenable [*off* | *on*]

Enables or disables the currently selected lamp (see **lamp** command) or gets the current lamp setting. For the continuous lamp:

off: Turns the lamp off (if currently on).

on: Turns the lamp on (if currently off).

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For the flash lamp:

off: Disables the lamp from turning on during reading.

on: Enables the lamp to turn on during reading.

The **lampenable** *on (off)* command duplicates the function of the LJL logo key on the front panel.

lamphours [*number*]

Gets or sets the number of hours the continuous lamp has been in use (switched on). Resetting *number* to 0 (presumably after changing the bulb) removes the warning condition if active (see **lampservice** command).

Range: 0–20,000

lamplife [*number*]

Gets or sets the continuous lamp lifetime in hours.

Range: 0–20,000
Recommended setting: 2000 hours

lamps

Gets a list of the lamps (see **lamp** command for possible types).

lampservice [*number*]

Gets or sets the warning time in hours for the remaining lifetime of the continuous lamp. The system reports a service message when the continuous lamp has been on for an interval (current **lamphours** value) equal to the current **lamplife** setting minus the current **lampservice** setting. When shipped, the system is configured to post the message when there are 400 hours of lamp life remaining.

Range: 0–20,000
Recommended setting: 400 hours

lamptimeout [*number*]

Gets or sets the continuous lamp automatic power off time in minutes. The lamp shuts off this many minutes after the last **lampenable** *on* command is issued or a plate read is completed. Resetting *number* to zero disables use of the automatic power off time.

Range: 0–1440
Recommended setting: 120 minutes

ljltype

Identifies the instrument on a blind serial port query. Used by remote computer programs hunting for LJL instruments on serial ports. The instrument responds with 'leader.'

load

The instrument moves the plate from the eject position to the load position just inside the door of the instrument. This is the same effect as the control panel LOAD/EJECT key.

If the stacker is present and enabled, the system transfers the bottom plate from the input magazine into the instrument. If the barcode reader on the stacker is enabled, the system reads the barcode before moving the plate into the instrument.

manufacturer

Gets or sets the instrument manufacturer. To clear the current string, type **manufacturer** followed by two quotes (""). A security password is required to change the manufacturer.

Recommended range: 1–32 characters

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methodid [*any string*]

Gets or sets the method name. The name appears in the Method ID field on the verbose report. To clear the current string, type **methodid** followed by two quotes ("").

Recommended range: 1–160 characters

mode [*f* | *fpp* | *fps* | *l* | *trf* | *a* | *fljl* | *fppljl* | *fpsljl* | *lumljl* | *trfljl* | *aljl*]

Gets or sets the currently configured detection method. When a detection method is selected, the instrument is configured according to settings retained for that method. The method name designations are:

f = Fluorescence Intensity
fpp = Fluorescence Polarization*
fps = Fluorescence Polarization*
l = Luminescence
trf = Time Resolved Fluorescence
a = Absorbance

fljl = Fluorescence Intensity (default definition)
fppljl = Fluorescence Polarization* (default definition)
fpsljl = Fluorescence Polarization* (default definition)
lumljl = Luminescence (default definition)
trfljl = Time Resolved Fluorescence (default definition)
aljl = Absorbance (default definition)

*Two Fluorescence Polarization methods are provided for setting aligned and opposing polarizer positions such that reading with these methods in a multi-method sequence provides polarizer switching across wells.

Methods *fljl*, *fppljl*, *fpsljl*, *lumljl*, *trfljl*, and *aljl* are default methods. If one of these methods is selected, the method parameters cannot be changed during instrument use. The default methods can be useful for creating new methods which, upon creation, will have parameters set to the same values as the default one copied (see **dmnew**).

model [*any string*]

Gets or sets the product model information. To clear the current string, type **model** followed by two quotes (""). A security password is required to change the model information.

Range: 1–160 characters

modes

Gets a list of available methods (see **mode** command).

mrc [*number*]

Gets or sets the number of read cycles to perform. A single read cycle consists of a read through the entire well sequence specified by the **read**, **rw**, or **start** command.

Range: 1–100

mrcr

Gets the number of read cycles remaining (including the current read cycle) (see **mrc** command). This count is set equal to the number of read cycles to perform and is decremented when the current read cycle is complete.

mrn

Gets the number of multi-read cycles the instrument has just completed, which is equal to the number of multi-read cycles to perform minus the number of multi-read cycles remaining.

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mrt [*number*]

Gets or sets the time in seconds between multi-read cycles. This is the time from the start of one read cycle to the start of the next read cycle (see **mrc** command), provided a **report** command has been issued to retrieve the data from the first read cycle. If the **report** command has not been issued, the next read will not start. Note: A subsequent read cycle will start immediately after the end of the current read cycle if the read time for the current read is equal to or greater than this time interval.

Range: 0–28,800 seconds (or 8 hours)

mrtr

Gets the delay time remaining in seconds until the start of the next read (see **mrt** command).

ms [*plate* | *well*]

Gets or sets the sequence for multi-method reading to be selectable by *plate* or by *well*. If the sequence is by *plate*, the instrument collects the readings for a detection method for the entire plate before reconfiguring to the next method. Similarly, if the method sequence is by *well*, the instrument collects the readings for all detection methods from a single well before moving to the next well.

mw [*rc*]

Positions plate so that center of well *rc* (row/column) is positioned in the light cone. Range for *rc* is specified according to the currently selected plate format.

pchead [*hc-120* | *hc-124* | *photodiode*] [*, method name*]

Gets or sets the type of light sensor for the currently configured detection method or for the specified detection method (as indicated by *method name*) where:

hc-120 specifies the fluorescence PMT

hc-124 specifies the luminescence PMT

photodiode specifies the photodiode (for reading in Absorbance mode)

To get a list of currently defined method names, use the **modes** command.

pdcap [*0* | *1* | *2*] [*, method name*]

Gets or sets the photodiode integrator capacitor for the currently configured detection method or for the specified detection method (as indicated by *method name*). Selections are given below followed by the capacitor values (in parentheses):

0: large capacitor (1 µF)
1: medium capacitor (100 nF)
2: small capacitor (470 pF)

The nominal gain of the integrator is inversely proportional to RC (where R is the integrator resistor and C is the integrator capacitor). To get a list of currently defined method names, use the **modes** command.

pdgain [*x1* | *x2* | *x4* | *x8*] [*, method name*]

Gets or sets the signal gain at the input to the photodiode A/D converter for the currently configured detection method or for the specified detection method (as indicated by *method name*).

To get a list of currently defined method names, use the **modes** command.

pdresistor [*0* | *1* | *2* | *3* | *4* | *5* | *6* | *7*] [*, method name*]

Gets or sets the photodiode integrator resistor for the currently configured detection method or for the specified detection method (as indicated by *method name*) as specified below:

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0: 2M Ω (largest resistor)
1: 499K Ω
2: 249K Ω
3: 124K Ω
4: 61.9K Ω
5: 31.6K Ω
6: 15.8K Ω
7: 7.87K Ω (smallest resistor)

The nominal gain of the integrator is inversely proportional to RC (where R is the integrator resistor and C is the integrator capacitor). To get a list of currently defined method names, use the **modes** command.

pdrgain [*x1* | *x100*] [*, method name*]

Gets or sets the photodiode remote amplifier gain for the currently configured detection method or for the specified detection method (as indicated by *method name*) such that:

x1: Corresponds to a 10K Ω resistor yielding 10v/mA (low gain)

x100: Corresponds to 1M Ω resistor yielding 1v/µA (high gain)

To get a list of currently defined method names, use the **modes** command.

period [*number*] [*, method name*]

Gets or sets the sample period in milliseconds for the currently configured detection method or for the specified detection method (as indicated by *method name*). When the flash lamp is used, the sample period is the interval between flashes. If the flash lamp is not used (in F or HEFP methods), the sample period specifies the time interval for the next read to occur. The sample period is defined as follows:

Period ≥ Delay + Integration Time + Optics Processing Overhead

That is, the period should be greater than or equal to the sum of the delay, the integration time, and the optics processing overhead. If it is not, the instrument will force the sample period to occur at a time interval specified by the sum of these parameters. In such a case, the specified sample period is meaningless and will not be used. The optics processing overhead is dependent upon the type of reading performed (worst case about five msec). See **delay** command.

Range: 1–100 × 10 msec (10–1000 msec in 10 msec increments)

To get a list of currently defined method names, use the **modes** command.

pfcolumnoffset [*number*]

Gets or sets the offset in mm from the edge of the plate to the center of column 1 (well A1) for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 1–131 mm

pfcolumns [*number*]

Gets or sets the number of columns for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 1–36

pfcolumnspacing [*number*]

Gets or sets the spacing in mm from column to column for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 1–131 mm

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pfdelete [*any string*]

Deletes the plate format specified by *any string* provided the plate format is not standard (see **pfstandard** command). If *any string* is not specified, the command deletes the current plate format and arbitrarily forces an existing plate format to be the current plate format.

pfheight [*number*]

Gets or sets the plate height in mm for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 4–18 mm

pflength [*number*]

Gets or sets the plate length in mm for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 124.5–131 mm

pfnew [*any string*]

Gets current plate definition or generates a new plate definition with a name specified by *any string* and selects this plate definition as the current plate.

Range: 1–80 characters

pfrename [*old name, new name*]

Renames a plate format from *old name* to *new name* provided the plate format is not standard (see **pfstandard** command).

Range: 1–80 characters

pfrowoffset [*number*]

Gets or sets the offset in mm from the edge of the plate to the center of row A (well A1) for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 1–92.8 mm

pfrows [*number*]

Gets or sets the number of rows for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 1–36

pfrowspacing [*number*]

Gets or sets the spacing in mm from row to row for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 1–92.8 mm

pfstandard [*on*]

Gets or sets the current plate definition as a standard (uneditable) plate format. Standard definitions cannot be changed.

If *on* is specified, the current plate format becomes a standard plate format and, therefore, uneditable.

Note that *off* cannot be entered (read-only). New plate formats default to *off* upon creation and remain a nonstandard format until *on* is entered.

pfwelldepth [*number*]

Gets or sets the depth in mm of a well from the top of the plate to the bottom of the well for the current plate format provided the plate format is not standard (see **pfstandard** command).

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Range: 0–18 mm

pfwells [*plate format*]

Gets the number of wells for the current plate format or for the one specified (as indicated by *plate format*). This command cannot be used to change the number of wells. Instead, use **pfrows** or **pfcolumns** commands.

pfwidth [*number*]

Gets or sets the plate width in mm for the current plate format provided the plate format is not standard (see **pfstandard** command).

Range: 77.0–92.8 mm

pgaadc [*number*] [*, method name*]

Gets or sets the ADC programmable gain amplifier (PGA) to be used by the instrument optics for reading in analog or comparator mode (see **cm** command). The ADC PGA will be set up for the currently configured detection method or for the specified detection method (as indicated by *method name*). Allowable multiplicative factors for the *number* field are: *x1, x2, x4, x8, x16, x32*, or *x64*.

Default: *x1*

To get a list of currently defined method names, use the **modes** command.

pgaint [*number*] [*, method name*]

Gets or sets the integrating programmable gain amplifier (PGA) to be used by the instrument optics for reading in analog or comparator mode (see **cm** command). The integrating PGA will be set up for the currently configured detection method or for the specified detection method (as indicated by *method name*). Allowable multiplicative factors for the variable field are: *x1, x2, x4, x8, x16, x32*, or *x64*.

Default: *x1*

To get a list of currently defined method names, use the **modes** command.

plate

Gets the string specified by the **plateid** command. If the **plateid** command string contains *%d*, then the system substitutes the number of plates processed in place of the *%d* code. The current number of plates processed is managed with the **platenumber** command.

plateid [*any string with or without %d*]

Gets or sets a plate ID string, which appears on the verbose report. To clear the current string, type **plateid** followed by two quotes (""). If the string contains *%d*, then the system substitutes the number of plates processed in place of the *%d* code. The current number of plates processed is managed with the **platenumber** command.

Range: 1–32 characters

platenumber [*number*]

Gets or sets the number of plates read during instrument use or the number of plates processed. The system increments the number automatically every time it ejects a plate. When the limit is reached, the number resets to zero.

Range: 0–9999

pmtenable [*off* | *on*]

Gets the current PMT status or enables or disables the power supply for the currently selected PMT.

off: Turns off PMT power

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on: Turns on PMT power

product [*any string*]

Gets or sets the product name. To clear the current string, type **product** followed by two quotes (""). A security password is required to change the product name.

Recommended range: 1–32 characters

rdc [*method name ( )*]

Performs a dark count reading for the currently configured detection method or for the specified detection method (as indicated by *method name* and parentheses) such that the light is blocked from the photodiode when the reading is taken. This command is used to acquire dark count reads to be used for computing well read data collected in Absorbance mode. See also **rlc** and **rrdc** commands.

read [*valid read expression*]

The instrument checks for a valid plate format specification and returns an error message if it is not valid (see plate format commands). If the format is valid, the instrument performs the following:

- Loads the plate if not already loaded in the instrument (see **load**) and optionally delays the commencement of the next activity (see **dal**).
- Shakes the plate (optional, see **shake**).
- Moves the plate from the load position and begins reading using the detection method(s), well sequence(s), data processing operator(s), and report generation format specified by the *valid read expression*.
- Returns the plate to the load position once the read sequence is complete.

A *valid read expression* consists of one or more parameters used to specify the detection method(s), well sequence(s), data processing operator(s), and report generation format. The instrument performs the sequence in the order entered (from left to right) using precedence rules when parentheses and operators are used. When the sequence ends, the instrument repeats the read cycle if specified (optional, see **mrc** and **mrt**).

Below are examples of multi-method reading and data processing sequences. In each case, reading can be started by using **rw** or **start** in place of **read**.

- **read:** Reads all wells for the currently selected plate format using the currently selected detection method.
- **read a1:a5:** Reads wells a1 thru a5 using the currently selected plate format and the currently selected detection method.
- **read f, g:** Reads all wells in row f and row g for the currently selected plate format using the currently selected detection method.
- **read f ( ):** Reads all wells for the currently selected plate format using detection method f.
- **read report0(a1:b12) = f1(a1:b12) + f2(a1:b12):** Reads well matrix defined by a1:b12 using user created detection methods f1 and f2, adds them together, and stores the results in the same wells of a single report, report0.
- **read f1(a1:b12) + f2(a1:b12):** Reads well matrix defined by a1:b12 using methods f1 and f2, adds them together, and stores the results in a single report in well matrix a1:b12.
- **read r1(a1:b12) = f1(a1:b12) – avg(f2(c1:c12)):** Reads well matrix defined by a1:b12 and c1:c12 using detection method f1 and f2, respectively. Takes the average of readings c1:c12 and subtracts this average from a1:b12 well readings. A single report, r1, is created with the calculated results in well matrix a1:b12.
- **read r1(a1) = (trfx(a1:h12) + trfy(a1:h12))/2, r2(a1) = (f1(a1:h12)*100)/f2(a1:h12):** Reads all wells using trfx, trfy, f1 and f2 detection methods. Two reports will be created: The first

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report contains the average of trfx and trfy readings and the second report contains the ratio of f1 and f2 reported as a percent. The results will be stored in well a1 for both reports, r1 and r2.

- **read r0(a1:a5)=cps(f (a1:a5)):** Reads well matrix a1:a5 and stores the readings in units of counts per second in report r0.

Note: If an output report is not specified when reading a well sequence, the instrument will attempt to store the output results in all wells of the plate, since it does not know where to store the results. For example, the command 'read cps(f(1:a5))' will store data in all wells of the report. To prevent this from occurring, ensure that an output report and associated well sequence is specified in the command.

Well Sequence Specification

The instrument reads the wells identified by the given well sequence. If no well sequence is given, then it reads the entire plate. Range for well sequence is specified according to the current plate format selected. Each option specified in the sequence must be separated by a comma.

Well Sequence Options:

- *RC*: Specifies a well to read where R is the row and C is the column (i.e., h1 reads well H1)
- *Row letter*: Specifies an entire row to read (i.e., A, B, C)
- *Column number*: Specifies an entire column to read (i.e., 1, 2, 3)
- *Row range*: Specifies a range of rows to read by row or column order (see **rs** command) starting with the first row specified (i.e., a:c reads rows A, B, C)
- *Column range*: Specifies a range of columns to read by row or column order (see **rs** command) starting with the first column specified (i.e., 17:15 reads columns 17, 16, 15)
- *Well to well*: Specifies a range of wells to read by row or column order (see **rs** command) starting with the first well specified (i.e., j22:d10 reads wells within matrix specified by wells D10, D22, J10, J22)

Operator and Operand Specification

The instrument supports the operators specified below for performing calculations on well read data. Operands consist of:

- detection methods (optional; if not specified, the instrument reads in the currently selected detection method)
- well sequences (optional; if not specified, the instrument reads all wells in the currently selected plate format)
- units: the instrument displays report data according to the selected units (optional) as specified below:
  cps: counts per second
  counts: counts
  not specified: data reported in RLU or RFU (cps * attenuation)
- real numbers (±) including decimal point and exponential representation ('e' or 'E')
- integer numbers (±)

Operators consist of those listed in the table below. The usage syntax specifies the **read** command, operator, and any valid read expression. A valid read expression can be simply a detection method and optional well sequence or a compound expression containing several detection methods, well sequences, operators, and/or constants.

+ (addition)

Syntax: read <read expression 1> + <read expression 2>

Example: read f1 + f2

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Reads all wells for detection methods f1 and f2, adds corresponding readings together, and stores the results in corresponding wells in the report.

– (subtraction)

Syntax: read <read expression 1> – <read expression 2>

Example: read f1 – f2

Reads all wells for detection methods f1 and f2, subtracts readings taken for f2 from corresponding readings in f1, and stores the results in corresponding wells in the report.

**** (multiplication)***

Syntax: read <read expression 1> * <read expression 2>

Example: read trf1(a1:a4) * trf2(a1:a4)

Reads wells defined by matrix a1:a4 in trf1 and trf2 detection methods, multiplies corresponding readings together, and stores the results in all the wells in the report since no output report with corresponding well sequence is specified (see '=' operator).

/ (division)

Syntax: read <read expression 1> / <read expression 2>

Example: read trf1(a1:a4) / trf2(a1:a4)

Reads wells defined by matrix a1:a4 in trf1 and trf2 detection methods, divides trfx readings by trfy readings, and stores the results in all the wells in the report since no output report with corresponding well sequence is specified (see '=' operator).

= (set report equal to)

Syntax: read report (well sequence) = <read expression>

Example 1: read r1(a1:b12) = f1(a1:b12)

Reads wells defined by matrix a1:b12 in f1 detection method and stores the results in the same wells in report r1.

Example 2: read r1(a1:b12) = f1(a1:b12), r2(a1:b12) = f2(a1:b12)

Reads wells defined by matrix a1:b12 in f1 and f2 detection methods, and stores the results in corresponding wells in reports r1 and r2, respectively.

Example 3: read r1(a1:b12) = f1(a1:b12), r1(c1:d12) = f2(c1:d12) + f3(c1:d12)

Reads wells defined by matrix a1:b12 in f1 and matrix c1:d12 in f2 and f3 detection methods. Stores the results of f1 readings in wells a1:b12 of report r1. Stores the addition of f2 and f3 readings in wells c1:d12 of report r1.

avg (average)

Syntax: read avg (<read expression>)

Example: read r1(a1) = avg (f1 (a1:b12))

Reads wells defined by matrix a1:b12 in f1 detection method, takes the average of the readings, and stores the result in well a1 of report r1.

min (minimum)

Syntax: read min (<read expression>)

Example: read r1(a1) = min (f1 (a1:a12))

Reads wells defined by matrix a1:a12 in f1 detection method, takes the minimum of the readings, and stores the result in well a1 of report r1.

max (maximum)

Syntax: read max (<read expression>)

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Example: read r1(a1) = max (f1 (a1:a12))

Reads wells defined by matrix a1:a12 in f1 detection method, takes the maximum of the readings, and stores the result in well a1 of report r1.

sum (summation)

Syntax: read sum (<read expression>)

Example: read r1(a1) = sum (f1 (a1:a12))

Reads wells defined by matrix a1:a12 in f1 detection method, takes the sum of the readings, and stores the result in well a1 of report r1.

count (number of variables)

Syntax: read count (<read expression>)

Example: read r1(a1) = sum (f1 (a1:a12) / count (f1 (a1:a12))

Reads wells defined by matrix a1:a12 in f1 detection method, takes the sum of the readings, divides by the number of readings or the *count* (12), and stores the result in well a1 of report r1.

log10 (log base 10)

Syntax: read log10 (<read expression>)

Example: read r1(a1) = log10 (avg (f1 (a2,b2)) / f1)

Reads all wells takes the average of a2 and b2 and divides the result by readings for the entire plate. This intermediate result is converted to a logarithm (base 10) and stored in well a1 of report r1.

fp (fluorescence polarization)

Syntax: read fp(<read expression 1>, <read expression 2>, gfactor)

Example: read fp (s – avg (s (a2,b2)), p – avg (p (a2,b2)), 0.9)

Reads all wells in detection method s, takes the average of well a2 and b2 readings and subtracts that from each reading, and the resultant readings are input to the fp function via the first argument. The same algorithm is performed in detection method p, and the resultant readings are input to the fp function via the second argument. The grating factor is input to the fp function via the third argument. A single report is generated with well computations in mP for all wells as specified below:

mP = 1000 * ((s – gfactor * p) / (s + gfactor * p))

report

Generates one or more reports of the well readings for the current plate or for the last plate read if no plate is currently loaded in the format specified by the report layout command, **rl**. The number of reports generated is determined by the syntax specified for reading when the **start**, **read**, or **rw** command was last issued.

reset

Resets the instrument by performing the following:

- Reading stops (if currently active)
- Plate is ejected (if loaded)
- Stacker moves plate to output magazine (if stacker is present and enabled)
- Continuous lamp turns off and then back on (if currently on)
- Lamp fiber shuttle moves to home position and back to original position (top excitation fiber cable is in alignment with continuous lamp source)
- Excitation filter wheel moves to home position and back to original position

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- The PMT fiber shuttle moves to home position and back to original position (bottom emission fiber cable is in alignment with fluorescence PMT)
- Emission filter wheel moves to home position and back to original position
- Barcode reader light turns off (if reader is present and light is on)

reverse

If the stacker is present and enabled (see **stackerenable** command), reverses a stack of plates by moving plates one at a time from the input to the output magazine. Upon completion, the uppermost plate in the input magazine becomes the lowermost plate in the output magazine.

rf

Generates a report on the current microplate format selected which consists of the following:

- Plate format name (see **pfnew**)
- Standard indicator (see **pfstandard**)
- Number of wells (see **pfwells**)
- Number of rows (see **pfrows**)
- Number of columns (see **pfcolumns**)
- Plate width (see **pfwidth**)
- Plate length (see **pflength**)
- Plate height (see **pfheight**)
- Row offset (see **pfrowoffset**)
- Row spacing (see **pfrowspacing**)
- Column offset (see **pfcolumnoffset**)
- Column spacing (see **pfcolumnspacing**)
- Well depth (see **pfwelldepth**)

rl [*no header* | *terse* | *verbose* | *columns*]

Gets current report layout or selects the report layout according to the options specified below. A single report can contain one or more data table information blocks (depending upon the read list sequence specified), each using the report layout specified below. The actual data set reported is the most recent data read for each well specified in the read list; wells not read are left blank. Refer to the **read**, **rw**, or **start** command for a description of how to specify the read list sequences. Refer to the **rtinfo** command for specifying data table information block parameters.

no header: Includes the following:

- Start of report text (see **srt**)
- Kinetic read cycle number of total number (see **mrc**)
- Read list sequence (see **read**)
- Data description field (see **rtinfo**)
- Units description field (see **rtinfo**)
- Display format (see **rtinfo**)
- Well read values displayed in a row/column matrix in units specified in read list sequence and display format specified by **rtinfo** command
- End of report text (see **ert**)

columns: Includes the following:

- Start of report text (see **srt**)
- Kinetic read cycle number of total number (see **mrc**)
- Read list sequence (see **read**)
- Data description field (see **rtinfo**)
- Units description field (see **rtinfo**)
- Display format (see **rtinfo**)
- Date/time for each well read (see **time**)

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- Plate identification (see **plate**)
- Well number
- Corresponding read value in units specified in read list sequence and display format specified by **rtinfo** command
- End of report text (see **ert**)

terse: Includes the following:

- Start of report text (see **srt**)
- Date/time of start of read sequence (see **time**)
- Plate identification (see **plate**)
- Barcode, if enabled and read (see **bc**)
- Kinetic read cycle number of total number (see **mrc**)
- Read list sequence (see **read**)
- Data description field (see **rtinfo**)
- Units description field (see **rtinfo**)
- Display format (see **rtinfo**)
- Well read values displayed in a row/column matrix in units specified in read list sequence and display format specified by **rtinfo** command
- End of report text (see **ert**)

verbose: The full report format is dependent upon the method used for the reading, *so not all parameters may be listed for a given report.* The list below contains all possible report data parameters. Refer to command definitions for identification of method-specific parameters.

- Start of report text (see **srt**)
- Date/time of load (see **time**)
- Date/time of start of read (see **time**)
- Kinetic read cycle number of total number (see **mrc**)
- Read start delay time (see **dal**)
- Time delay between kinetic reads (see **mrt**)
- Barcode (see **bc**)
- Method name (see **methodid**)
- Plate identification (see **plate**)
- Comment (see **comment**)
- Microplate format (see **format**)
- Shake time (see **shaketime**)
- Temperature (see **temp**)
- Instrument tag (see **tag**)
- Serial number (see **sn**)
- Read sequence (see **rs**)
- Mode sequence (see **ms**)
- Detection Mode (see **mode**)
- Light sensor (see **pchead**)
- Excitation side (see **xside**)
- Emission side (see **eside**)
- Lamp (see **lamp**)
- Flash lamp voltage (see **fv**)
- Readings (or flashes) per well (see **cycles**)
- Time between readings or flashes (see **period**)
- Integration time (see **it**)
- Delay after flash (see **delay**)
- Attenuator mode (see **am**)
- Motion settling time (see **settle**)
- Z-height (includes luminescence height for L methods) (see **zt**, **zh**)
- Luminescence aperture (see **la**)
- Excitation filter (see **xfilter**)

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- Emission filter (see **efilter**)
- Dichroic mirror (see **bsname**)
- Excitation polarizer filter (see **xpolarizer**)
- Emission polarizer filter (see **epolarizer**)
- Detector counting (see **cm**)
- Sensitivity setting (see **cap**)
- A/D converter gain (see **pgaadc**)
- Integrating gain (see **pgaint**)
- Photodiode capacitor (see **pdcap**)
- Photodiode gain (see **pdgain**)
- Photodiode resistor (see **pdresistor)**
- Photodiode remote amplifier gain (see **pdrgain**)
- Max cps: maximum counts per second read
- Min counts: minimum counts read
- Dark count reading: (see **dcenable**)
- Well list: set of wells and optional parameters such as units, modes, and/or operators specified in read sequence (see **read**)
- Data: (see **rtinfo**)
- Units: (see **rtinfo**)
- Display format: (see **rtinfo**)
- Well read data displayed in a row/column matrix in units specified in read list sequence and display format specified by **rtinfo** command
- End of report text (see **ert**)

rlc [*method name ( )*]

Performs an air (or *light*) count reading for the currently configured detection method or for the specified detection method (as indicated by *method name* and parentheses) such that the light directly hits the photodiode when the reading is taken. This command is used to acquire air reads to be used for computing well read data collected in Absorbance mode. See also **rdc** and **rrlc** commands.

To get a list of currently defined method names, use the **modes** command.

rm

Generates a report on the settings for the currently configured detection method. Since the settings are dependent upon method type, it is possible that not all parameters will be listed. The list below contains all current report data parameters. Refer to command definitions for identification of method-specific parameters.

- Method (see **mode**)
- Excitation side (see **xside**)
- Emission side (see **eside**)
- Lamp (see **lamp**)
- Flash lamp voltage (see **fv**)
- Readings (or flashes) per well (see **cycles**)
- Time between readings or flashes (see **period**)
- Integration time (see **it**)
- Delay after flash (see **delay**)
- Attenuator mode (see **am**)
- Motion settling time (see **settle**)
- Z-height (includes luminescence height for L methods) (see **zt**, **zh**)
- Excitation filter (see **xfilter**)
- Emission filter (see **efilter**)
- Dichroic mirror (see **bsname**)
- Excitation polarizer filter (see **xpolarizer**)
- Emission polarizer filter (see **epolarizer**)

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- Detector counting (see **cm**)
- Sensitivity setting (see **cap**)
- A/D converter gain (see **pgaadc**)
- Integrating gain (see **pgaint**)
- Photodiode capacitor (see **pdcap**)
- Photodiode gain (see **pdgain**)
- Photodiode resistor (see **pdresistor)**
- Photodiode remote amplifier gain (see **pdrgain**)

rr [*well*]

Reports well readings. The instrument responds with the well label, its reading, and the detection mode. This command is intended for use by a remote host computer to get well readings while plate reading is in progress. The Well Reading Ready bit in the status word is set (1) if there is an unreported reading ready (see **st** command). The host computer then issues the **rr** command to get the next unreported reading specified in the well sequence. The status bit is cleared when all well readings have been reported or a plate is loaded.

rrdc

Reports the dark count reading taken with the **rdc** command. The **rrdc** command is intended for use by a remote host computer to get the unreported dark count reading when the Dark Count Reading Ready bit in the status word is set (see **st** command). The host computer then issues the **rrdc** command to get the unreported reading. The instrument clears the status bit after reporting the reading.

rrlc

Reports the air (or *light count*) reading taken with the **rlc** command. The **rrlc** command is intended for use by a remote host computer to get the unreported air reading when the Light Count Reading Ready bit in the status word is set (see **st** command). The host computer then issues the **rrlc** command to get the unreported reading. The instrument clears the status bit after reporting the reading.

rs [*row* | *column*]

Gets or sets the well read sequence such that the plate is traversed by *row* or *column*. Using the input, *row*, as an example, the plate will be read by rows such that completion of the current row shall initiate a read at the adjacent well in the next row from the well just read and continue along in the reverse order until the well sequence in the row is read. The process is repeated until all rows specified in the well sequence or the entire plate is read. This command is used in conjunction with the **rw**, **read,** or **start** commands.

rtidelete [*name*]

Deletes the report table information with the given *name*.

rtilist [*name*]

Generates a listing for the report table information identified by *name*. If *name* is omitted, it generates a list of all currently defined report table information blocks.

rtinfo [*name, label, units, display format*]

Sets the parameters for the report table information block identified by *name*. It creates a new report table information block with this *name* if one does not already exist. The parameters are:

name: The report table information block name. Range: 1–32 characters

label: This is an arbitrary string that will appear as a label for the table. This label appears just before the data table in the report in the 'Data:' field (see **rl**). *Label* must be enclosed in quotes ("*label*") if it contains comma characters. Range: 1–160 characters

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units: This is a string identifying units for the numbers in the table. This string appears as the last line in the header block before the table in the 'Units' field (see **rl**). These units are an arbitrary label assigned by the user and do not affect the reported numbers in any way (see **read** command for a description of how to specify data units as part of the read well list). Range: 1–160 characters

display format: This is a string that determines the formatting of the data in the table. This string should have the form of a C/C++ printf() format specification. For example, '%.3g' generates numeric text with three significant digits; '%.0f' prints the numbers with no digits after the decimal point. Range: 1–32 characters

Additionally, the user can specify the front panel units and data sent to a remote host computer (via the **rr** command) in the same units specified in the report using the report table information block name, *frontpanel*.

Below is an example of setting up a report table information block for the report, the front panel and remote host computer.

rtinfo r0, raw data, cps, %0g: Creates a report table information block with the corresponding report table information.

rtinfo frontpanel, 'any string,' cps, %0g: Sets up front panel units and data format ('any string' field is not used).

read r0 (a1:a5) = cps (f (a1:a5)): Performs a read using r0 as the output data table format.

As the wells are read, the front panel will display the live data in units of counts per second using the format, %0g. If a remote host computer is used to fetch the data via the **rr** command, the data will be sent over in units of counts per second using the format, %0g. The report will contain readings for wells a1 thru a5 in units of counts per second (specified as *cps* in the read list), using the format %0g for the data, a data label, 'RAW DATA' and units, 'CPS.'

rtirename [*existing name, new name*]

Renames report table information from *existing name* to *new name*.

Range: 1–32 characters

rw [*valid read expression*]

The instrument checks for a valid plate format specification and returns an error message if it is not valid (see plate format commands). If the format is valid, the instrument begins reading using the units, method(s), well sequence(s), data processing operator(s), and report generation format specified by the *valid read expression*. When done, the instrument leaves the plate at the position corresponding to the last well specified in the sequence.

The **rw** command reads wells like the **start** and **read** commands but differs from these commands in the following ways:

- The **rw** command only reads well sequences (it does not load, shake, or eject the plate).
- If the well sequence is not specified, the **rw** command reads only one well (the last well read). If no previous wells were read for the current plate format, it reads well A1.

For detailed information about and examples of *valid read expressions*, see the **read** command.

se

Generates an error condition for testing host computer programs.

service

Generates a report of all active service conditions complete with a date and time stamp (in the format specified by the **tf** command). A service condition is a persistent condition requiring user action to remedy (e.g., continuous lamp life limit reached). If there are no currently active service conditions, the instrument responds with 'OK.'

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settle [*number*]

Gets or sets the plate settling time, which is the time in milliseconds to wait after moving to a well before the instrument starts to read the PMT.

Range: 0–1000 msec
Default: 25 msec

sf

Generates a fault condition for testing host computer programs.

shake

Shakes the currently loaded plate prior to reading in a circular motion as defined by a radius, duration, and velocity (see **shakeradius**, **shaketime**, and **shakevel** commands).

shakeradius [*number*]

Gets or sets the radius of the circular motion used for shaking the plate (see **shakevel** command) in millimeters.

Range: 2–6 mm

shaketime [*number*]

Gets or sets the time in seconds to shake the plate. If *number* = 0, shaking will no longer occur upon initiation of the next plate read.

Range: 0–10 sec

shakevel [*number*]

Gets or sets the velocity, in millimeters per second, for the shaking operation. The velocity is the speed at which the plate is rotated in a circular motion.

Range: 10–40 mm/sec

sn [*any string*]

Gets or sets the instrument serial number. A security password is required to change the serial number. To clear the current string, type **sn** followed by two quotes ("").

Range: 1–32 characters

srt [*any string*]

Gets the 'start report text' or sets it with the specified string (see **rl** command). The string may contain nonprintable characters (for example, '\x01') that will be interpreted for printing. To clear the current string, type **srt** followed by two quotes (""). Also see **ert** command.

\a: Bel
\b: Backspace
\f: Formfeed
\n: Linefeed
\r: Return
\t: Horizontal tab
\v: Vertical tab
\x: Two-digit hexadecimal number
\\: Backslash '\'
\": Double quote '"'

Range: 1–32 characters

ss [on | off]

Generates a service condition for testing host computer programs. Unlike real service conditions, the simulated service condition can only be cleared via an instrument reset.

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st

The instrument replies with operating status as a 32-bit hexadecimal number (eight hex digits):

Transient Operating Status Indicators (clear condition)
0x00000001 = Busy (done with operation)
0x00000002 = Resetting (done with resetting)
0x00000004 = Loading (done loading plate)
0x00000008 = Reading (done reading plate)
0x00000010 = Ejecting (done ejecting plate)
0x00000020 = Light (or Air) Count Reading Ready (count read or plate loaded)
0x00020000 = Delaying After Loading (read starts or plate ejected)
0x00040000 = Delaying Before Reading (read starts or plate ejected)

Normal Status Indicators (clear condition)
0x00000080 = Stacker Empty (plate in input tray)
0x00000100 = Plate is Loaded (plate is ejected)
0x00000200 = Dark Count Reading Ready (count read or plate loaded)
0x00000400 = Plate Report Ready (plate report read or plate loaded)
0x00000800 = Well Reading Ready (well report read or plate loaded)
0x00080000 = Waiting for Report Query (plate report read or plate ejected)

Status Indicators Requiring Attention (clear condition)
0x00001000 = Service* (condition causing message is cleared or instrument is reset)
0x00002000 = Error* (instrument reset or **ce** command issued)
0x00008000 = Fault* (instrument reset)

Hardware Connection Indicators (clear condition)
0x00010000 = Stacker Connected (stacker disconnected)
0x00100000 = Absorbance Connected (absorbance disconnected)

*See corresponding commands for definition of exception conditions.

stackerenable [*on* | *off*]

Enables or disables the stacker or gets the current stacker setting.

If the input is *on* and the stacker is connected, plates will be loaded onto the tray from the input magazine and plates ejected from the instrument will be transferred to the output magazine. If the stacker is not connected, the instrument returns an error message indicating the stacker is not available.

If the input is *off*, the stacker will no longer be used to fetch and discard plates. However, the stacker can still be connected to the instrument without it being used. Plates can be placed into the transfer position for loading just as if the stacker was not connected.

start [*valid read expression*]

The instrument checks for a valid plate format specification and returns an error message if it is not valid (see plate format commands). If the format is valid, the instrument performs the following:

- Loads the plate if not already loaded in the instrument (see **load**) and optionally delays the commencement of the next activity (see **dal**)
- Shakes the plate (optional, see **shake**)
- Moves the plate from the load position to begin reading wells identified by the given method and/or well sequence (if specified)
- Returns the plate to the load position once the read sequence is complete
- Ejects the plate (see **eject**)

In general, **start** has the same purpose as the **read** command except that the **read** command does not eject the plate. For detailed information about and examples of *valid read expressions*, see the **read** command.

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swversion

Gets the version number of the software in the format 'vx.yy' where 'v' implies version, 'x' is the version number and 'yy' is the revision number.

tag [*any string*]

Gets or sets a text string that identifies the instrument in the user's lab. The text string appears in the Instrument tag field of the verbose report. To clear the current string, type **tag** followed by two quotes ("").

Range: 1–32 characters

temp [*1* | *2*]

Reports the temperature reading in centigrade degrees for sensors 1 and 2 or the average of the temperature readings for the two sensors. If *1* or *2* is specified, the instrument reports the reading for the sensor specified; otherwise it reports the average.

Range: 0–62.5 °C

tf [*time format string*]

Gets or sets the current date and time display format. This is a string in unix strftime() format:

%a: Abbreviated weekday name
%A: Full weekday name
%b: Abbreviated month name
%B: Full month name
%c: Date and time representation appropriate for locale
%d: Day of month as decimal number (01–31)
%H: Hour in 24-hour format (00–23)
%I: Hour in 12-hour format (00–11)
%j: Day of year as decimal number (001–366)
%m: Month as decimal number (01–12)
%M: Minute as decimal number (00–59)
%p: Current locale's AM/PM indicator for 12-hour clock
%S: Second as decimal number (00–59)
%U: Week of year as decimal number, with Sunday as first day of week (00–51)
%w: Weekday as decimal number (0–6; Sunday is 0)
%W: Week of year as decimal number, with Monday as first day of week (00–51)
%x: Date representation for current locale
%X: Time representation for current locale
%Y: Year with century, as decimal number
%z, *%Z*: Time zone name or abbreviation; no characters if time zone is unknown
%%: Percent sign

time [*date time string*]

Gets or sets the current date and time in the Analyst instrument. If a *date time string* is used to change the time, this string should be in the format defined by the **tf** command.

xfilter [*number*] [*, method name*]

Gets or sets an excitation filter for the currently configured detection method or for the specified detection method (as indicated by *method name*). The filter *number* is in the range 1 to 8. When an excitation filter is selected, the instrument positions the filter in front of the currently selected lamp. For emission filter, see **efilter** command.

To get a list of currently defined method names, use the **modes** command.

xfilters

Returns a list of the currently installed excitation filters.

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xfname [*number*] [*any string*]

Gets or sets the description for an excitation filter.

If *number* and *any string* are omitted, it returns a list of the current filter descriptions.

If *number* is present and *any string* is omitted, it returns the description for the given filter number.

If *number* and *any string* are both present, it changes the description for the given filter number. To clear the current string, type **xfname** followed by two quotes ("").

Number range: 1–8

Any string range: 1–32 characters

xpolarizer [*o* | *s* | *p*] [*, method name*]

Gets or sets the excitation polarizer position for the currently configured detection method or for the specified detection method (as indicated by *method name*) according to the following:

s: The excitation polarizer is aligned in a horizontal orientation when reading begins.

p: The excitation polarizer is aligned in a vertical orientation when reading begins.

o: The excitation polarizer is moved out of the optical path. In HEFP methods, the instrument defaults to the **s** position when reading begins.

To get a list of currently defined method names, use the **modes** command.

xside [*bottom* | *top* | *none*] [*, method name*]

Gets or sets the side of the plate for transmission of the excitation light for the currently configured detection method or for the specified detection method (as indicated by *method name*) according to the following:

top or *bottom*: Moves the lamp fiber shuttle such that the top or bottom excitation fiber is in alignment with either the flash or continuous lamp (see **lamp** command). If the currently selected lamp is *none*, then the lamp fiber shuttle remains in the current position (e.g., midway between lamps).

none: Moves the lamp fiber shuttle such that neither lamp is in alignment with the excitation fiber (e.g., midway between lamps).

To get a list of currently defined method names, use the **modes** command.

zh [*number*] [*, method name*]

Gets or sets the z-height or luminescence height in mm for the currently configured detection method or for the specified detection method (as indicated by *method name*).

For fluorescence methods, this is the height of the focal point from the bottom of the well. The maximum range is dependent upon the plate dimensions.

Recommended range: 0–7 mm

For luminescence methods, this is the height of the detector head from the top of the plate. The maximum range is dependent upon the plate dimensions.

Recommended range: 0.5–2 mm

To get a list of currently defined method names, use the **modes** command.

zt [*bottom* | *middle* | *numeric*] [*, method name*]

Gets or sets the z-height target for the currently configured detection method or for the specified detection method (as indicated by *method name*).

- For fluorescence methods, *bottom* or *middle* selects the bottom or middle of the well.
- For luminescence methods, always use *numeric*.

If *numeric* is selected, use the **zh** command to define the z-height.

To get a list of currently defined method names, use the **modes** command.

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4 Maintenance Procedures

Part 4 provides instructions for the following procedures:

- Preventive Maintenance Schedule
- Panel Removal and Replacement
- Filter Removal and Replacement
- Dichroic Mirror Removal and Replacement
- Setting the Luminescence Aperture
- Lamp Removal and Replacement

4.1 PREVENTIVE MAINTENANCE SCHEDULE

Criterion systems have been designed to provide high availability in high throughput screening environments. High availability can only be achieved if preventive maintenance schedules are followed (to maximize uptime) and if service personnel and adequate spare parts inventories are available to minimize down time. Furthermore, proper preventive maintenance schedules and procedures must be followed or the lifetime of the mechanical systems will be reduced.

Recommended preventive maintenance service intervals are given below.

Usage per Week (hours)	Service Interval
10–20	1 year
20–40	6 months
>40	4 months

Under no circumstances should the number of readings between services exceed 36,000 microplates.

Use the **platetotal** command to check the total number of load/eject cycles performed by the instrument. Keep a log to help plan preventive maintenance. Call your LJL BioSystems agent when it is necessary to schedule preventive maintenance.

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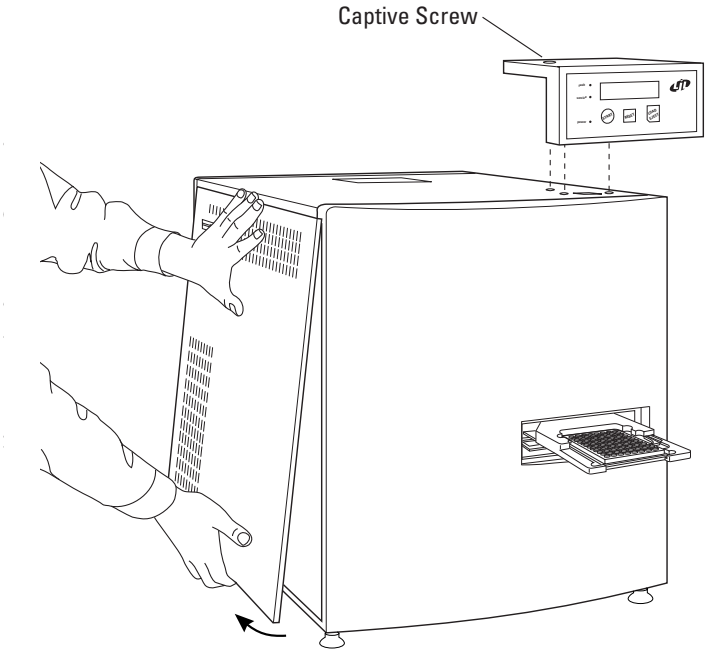
4.2 PANEL REMOVAL AND REPLACEMENT

One or more panels must be removed during certain maintenance and service procedures. If the top panel must be removed, it is easiest to remove one of the side panels first.

TO REMOVE:

1 **Switch off power and unplug instrument.**

2 **Remove side panel.** With a flat-head screwdriver, remove the screw that secures the panel to the chassis. Grip the lower edge of the side panel and pull it away from the instrument. Once the bottom edge is free, you can disengage the panel's top edge and pull the panel free.



Left Side Panel Removal

Note: *Remove the right panel in the same manner as the left panel.*



3 **Remove control panel.** With a Phillips screwdriver, loosen the captive screw that secures the control panel to the top panel. Lift control panel off of top panel.

4 **Remove top panel.** Push up on the edges of the top panel until it disengages from its fasteners.

TO REPLACE:

Reverse removal procedure.

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4.3 FILTER REMOVAL AND REPLACEMENT

The excitation and emission interference filters are housed in cartridges in two filter wheel assemblies. Mechanically, these assemblies are identical. To remove a filter, you must first remove one or more outside panels and then remove the appropriate filter wheel.

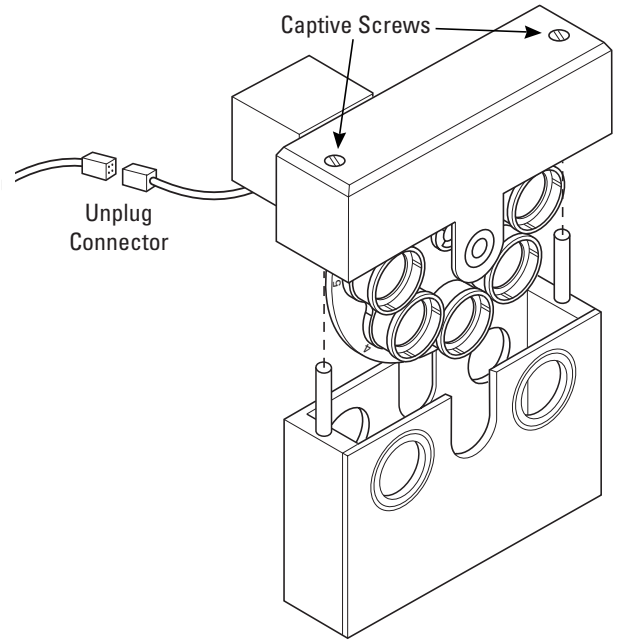
Filter Wheel Removal and Replacement

TO REMOVE:

1 **Switch off power and unplug instrument.**

2 **Remove panels and (on Analyst HT) control panel as needed.** See section 4.2, 'Panel Removal and Replacement.' The excitation filter wheel is accessible by removing the top panel of the instrument, the emission filter wheel by removing the left side panel.

3 **Unplug the drive motor connector** attached to the wheel assembly.



Filter Wheel Assembly Removal

4 **Loosen two captive screws** on the top of the wheel assembly with a flat-head screwdriver.

5 **Pull wheel assembly out** of the instrument.

Caution: *Do not touch the optical surfaces of the filters.*

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TO REPLACE:

1. **Verify that each cartridge is properly seated** in the filter wheel. The wheel should rotate smoothly when moved by hand.
2. **Seat filter wheel assembly** in the filter wheel housing. Check that the correct wheel assembly is placed in the correct housing.
3. **Secure the two captive screws.**
4. **Plug drive motor connector** back into the wheel assembly.

   Caution: *Always unplug instrument before reconnecting the drive motor. Failure to do so can damage the instrument.*
5. **Replace panels and (on Analyst HT) control panel.** See section 4.2, 'Panel Removal and Replacement.'

Filter Cartridge Removal and Replacement

Filters must be mounted into cartridges before they can be installed in the instrument. The filter wheels can accept two types of filter cartridges, known as 'tall' and 'short.'

Tall cartridges. The instrument ships with tall cartridges installed in each filter wheel position. Except for the fluorescein and europium filter cartridges, these cartridges contain metal slugs that balance the filter wheel and protect the PMT from direct exposure to the light sources. The tall cartridges can accept standard 25-mm diameter filters.

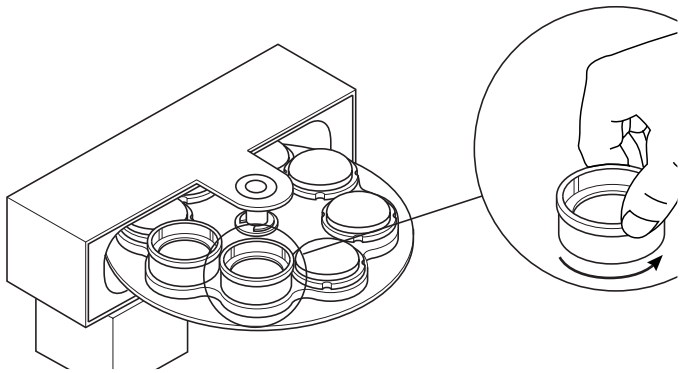
Short cartridges. These cartridges differ from tall cartridges in that they have a low profile, allowing you to quickly see the name of the filter on the rim. Filters must be glued into the short cartridges. Contact your LJL BioSystems agent for more information.

TO REMOVE:

1. **Switch off power and unplug instrument.**
2. **Remove filter wheel assembly.** See 'Filter Wheel Removal and Replacement' earlier in this section.
3. **Locate the filter wheel position** with desired filter. Each position is marked on the filter wheel.
4. **Remove filter cartridge(s)** by unscrewing. It may be easiest to remove the cartridge by holding the filter wheel assembly with one hand while unscrewing the cartridge with the other.

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Remove **tall cartridges** by hand, turning counter-clockwise.



Tall Cartridge Removal

5 **Remove filters from tall cartridges.** Place a lint-free cloth on the work surface. Remove the installed filter (or slug) by gently pushing the end of a paper clip through the holes in the side of the cartridge. Do not touch filter with fingers. The filter and friction ring (which holds the filter in place) will drop out of the cartridge.

Caution: *Do not attempt to remove filters glued into short cartridges.*

6 **Store filters** in a cool, dry place. The surfaces are delicate. Avoid scratches by placing each filter in a protective container (provided).

7 **Update filter names.** If a filter (or slug) has just been removed from the emission filter wheel for a FLUM method, select Edit Filter Names from the Setup menu in the CriterionHost application and update the filter names.

TO REPLACE:

1 **Install filter in empty cartridge.**

- For tall cartridges, see 'Installing Filters in Tall Cartridges' later in this section.
- For short cartridges, contact your local LJL BioSystems agent for information on gluing filters into the cartridges.

2 **Locate the filter wheel position** for the desired filter. Each position is marked on the filter wheel.

3 **Install filter cartridge(s)** by screwing clockwise. It may be easiest to install the cartridge by holding the filter wheel assembly with one hand while screwing the cartridge with the other.

Caution: *Except for the emission filter wheel with FLUM methods, do not leave open positions in the filter wheels. Each filter position should contain either a filter or a metal slug to balance the filter wheel and protect the PMT from direct exposure to the light sources.*

4 **Note changes in installed filters** on the label on top of the assembly.

5 **Install filter wheel assembly.** See 'Filter Wheel Removal and Replacement' earlier in this section.

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6 **Replace panels and control panel as needed.** See section 4.2, 'Panel Removal and Replacement.'

7 **Reconnect power cord and switch on power.**

8 **Update filter names.** In the CriterionHost application, select Edit Filter Names from the Setup menu and update the filter names.

Important! *To avoid errors, always update filter names immediately after changing filters.*

Installing Filters in Tall Cartridges

Use the following procedure when installing filters in tall cartridges.

Note: *For information on installing filters in short cartridges, contact your local LJL BioSystems agent.*

1 **Place filter in empty cartridge**, noting filter orientation.

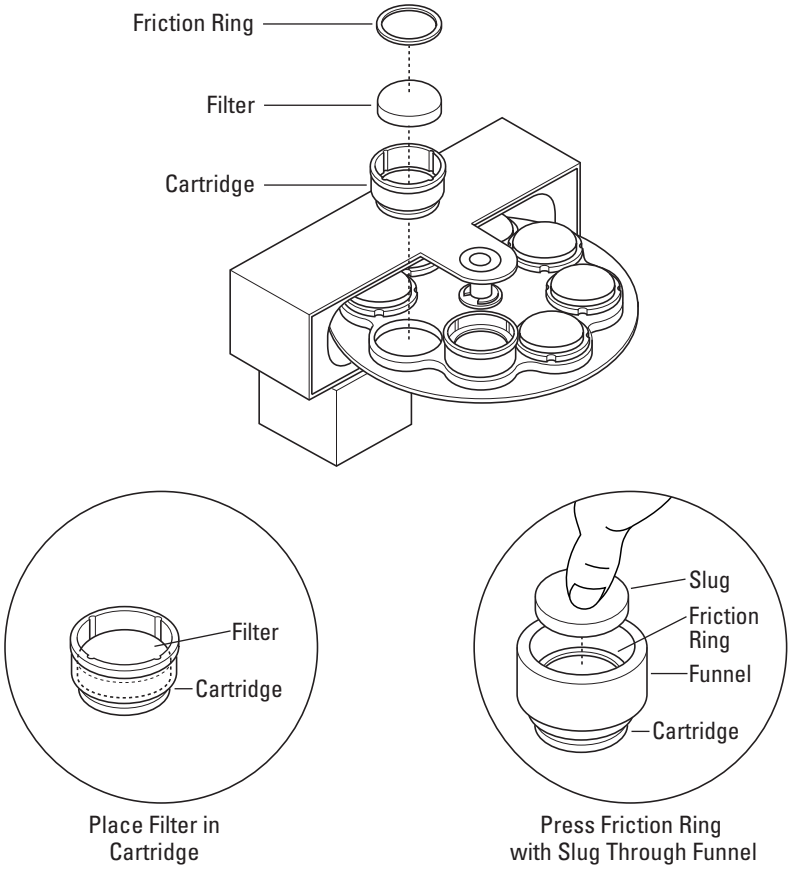
Note: *Many filters have a specific orientation with respect to the light source. In general, the mirrored surface should face the light source.*

- *If an arrow is present on the rim of the filter, be sure the arrow points* ***away*** *from the light source (in the direction of the light path).*
- *If there is no arrow, the top of the letters on the rim should be oriented* ***away*** *from the light source.*

2 **Place funnel on top of cartridge.** The funnel is used to compress the friction ring.

3 **Place friction ring and then metal slug in funnel.**

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Filter Installation in Tall Cartridge

4 **Push metal slug down** through the funnel to compress the friction ring. The friction ring should fit snugly against the filter.

5 **Remove the slug and funnel.**

6 **Reinstall the cartridge.** See 'Filter Cartridge Removal and Replacement' earlier in this section.

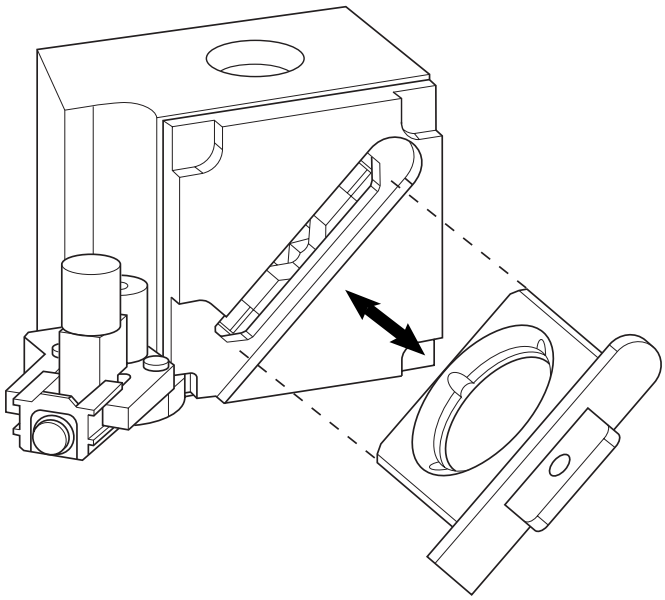
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4.4 DICHROIC MIRROR REMOVAL AND REPLACEMENT

Dichroic mirrors are installed in the instrument in the top and bottom optics heads. The dichroic mirror holder is identical for the top and bottom optics and can be used interchangeably.

TO REMOVE:

1. **Switch off power and unplug instrument.**
2. **Remove right side panel.** See section 4.2, 'Panel Removal and Replacement.'
3. **Remove enclosure hatch.** Loosen two knurled thumbscrews and then remove the light-tight enclosure hatch.
4. **Locate the top and bottom dichroic mirrors** with a flashlight. The handles are at a 45 degree angle to each other.
5. **Remove dichroic mirror** by grasping the handle and pulling it out of the instrument. Take care not to touch the surface of the optics.
6. **Store dichroic mirror** in a cool, dry place. The surfaces are delicate. Avoid scratches by placing each dichroic mirror in a protective container (provided).



Dichroic Mirror Removal

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TO REPLACE:

1. **Insert new dichroic mirror.** Note that the holder is asymmetrical and will only seat in the instrument one way. Push the dichroic mirror in as far as possible.
2. **Replace the enclosure hatch and right side panel.**
3. **Reconnect power cord and switch on power.**
4. **Update dichroic mirror names.** In the CriterionHost application, select Edit Filter Names from the Setup menu and update the dichroic mirror names using the Setup Filters dialog box.

   Important! *To avoid errors, always update dichroic mirror names immediately after changing a dichroic mirror.*

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4.5 SETTING THE LUMINESCENCE APERTURE

The luminescence aperture must be set manually for either 96-well or 384-well operation. A sensor automatically determines its position and displays it in the CriterionHost application screens.

1 **Switch off power and unplug instrument.**

2 **Remove right side panel.** See section 4.2, 'Panel Removal and Replacement.'

3 **Remove light-tight enclosure hatch.**

4 **Locate the top optics head** with a flashlight. The aperture handle is to the left of the top optics dichroic mirror.

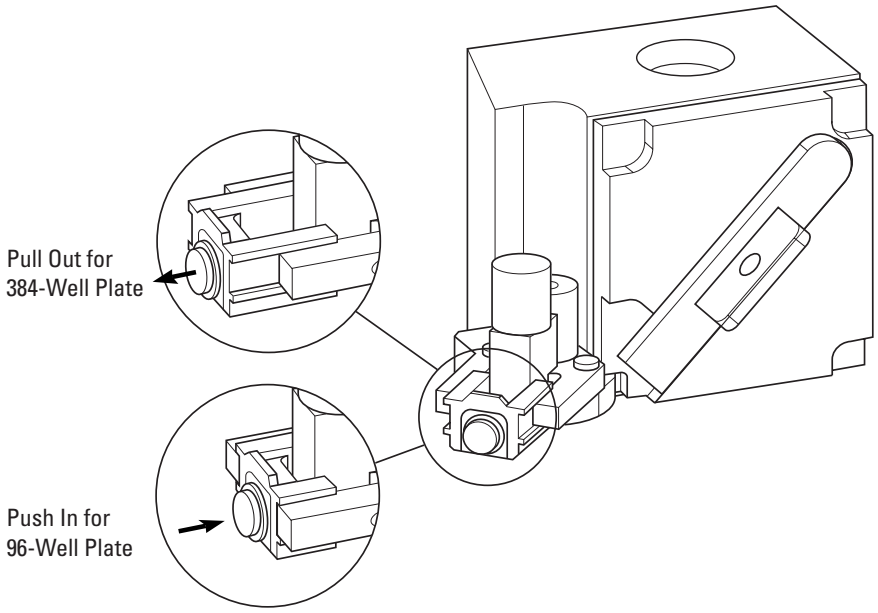
5 **Set the aperture.**

- For 96-well operation, grasp the aperture handle and push it in.
- For 384-well operation, pull it out as far as possible.

6 **Replace the hatch and panel.**

7 **Reconnect power cord and switch on power.**

8 **Check aperture status.** Using Command View, type '**la**' and press [Enter]. The system will respond with either '96' or '384,' depending upon the current aperture setting.



Setting the Luminescence Aperture

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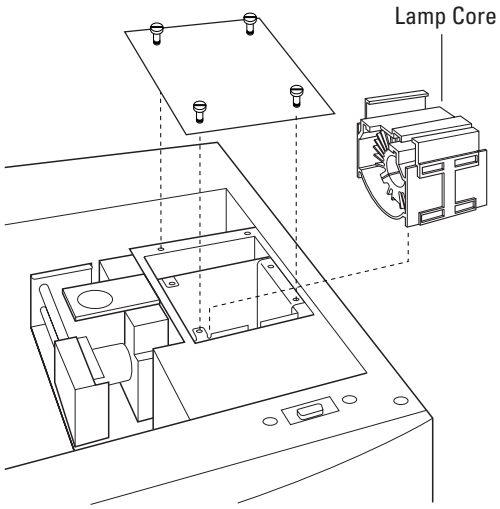
4.6 CONTINUOUS LAMP REMOVAL AND REPLACEMENT

The continuous lamp has an expected life of 2000 hours. The recommended lamp life threshold is 1600 hours. When the threshold is reached, the instrument alerts the user. Use the following procedure to replace the continuous lamp.

TO REMOVE:

1. **Switch off power and unplug instrument.**
2. **Remove control panel (on Analyst HT), left panel, and top panel.** See section 4.2, 'Panel Removal and Replacement.'
3. **Remove four screws** on lamp housing cover.
4. **Remove continuous lamp core** from the lamp housing using the holding tabs.

   Warning: *Handle with care. If the lamp has been on recently, then there are metal parts that can be very hot.*



Continuous Lamp Replacement

TO REPLACE:

1. **Insert new lamp core into housing.** Make sure that the new lamp module is positioned properly.

   Note: *There is only one position for the lamp core that allows the lamp to be fully inserted into its housing.*
2. **Tighten four screws** on lamp housing cover.
3. **Replace top panel, side panel, and (on Analyst HT) control panel.**
4. **Reconnect power cord and switch on power.**
5. **Update lamp status.** Using the CriterionHost application, reset the lamp status by selecting Lamps from the Status menu. See section 2.2, 'Setting Up the System.'

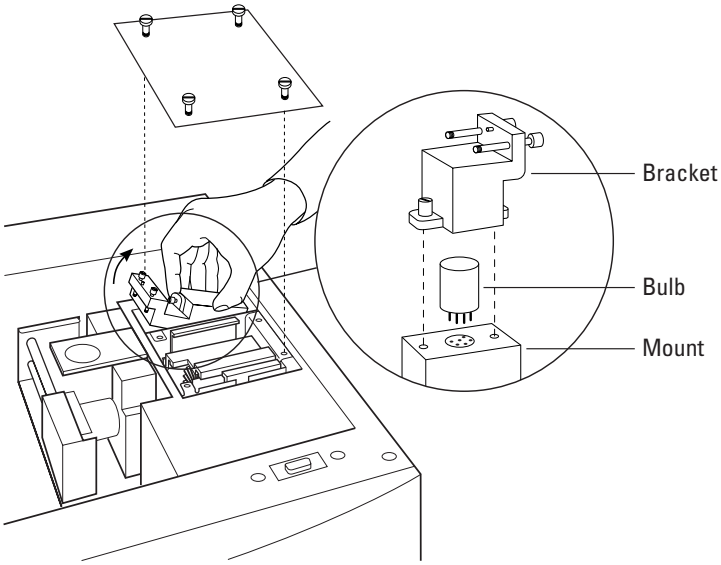
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4.7 FLASH LAMP REMOVAL AND REPLACEMENT

Use the following procedure to replace the flash lamp.

TO REMOVE:

1. **Switch off power and unplug instrument.**
2. **Remove control panel (on Analyst HT), left panel, and top panel.** See section 4.2, 'Panel Removal and Replacement.'
3. **Remove four screws** on lamp housing cover.
4. **Loosen captive screw** on the bracket and remove assembly as shown. *Do not touch* the central alignment screw.
5. **Remove bracket from mount** by loosening the two captive screws that attach the bracket to the mount.
6. **Remove bulb from mount.**



Flash Lamp Replacement

TO REPLACE:

1. **Install new bulb in mount.** *Do not touch bulb with fingers.*
2. **Secure bracket to mount** with two captive screws.
3. **Replace bracket-mount assembly** and secure captive screw.
4. **Tighten four screws** on lamp housing cover.
5. **Replace top panel, side panel, and (on Analyst HT) control panel.**
6. **Reconnect power cord and switch on power.**

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5 Troubleshooting

Part 5 provides instructions for removing jammed plates and a listing of error conditions.

5.1 OPERATION AND ERROR CODES

The Analyst software displays operation and error codes during run time. Transient operating status indicators will appear only when the event occurs. Other status indicators appear when the event occurs and are reset when the operator reboots the system. For further information, see the **st** command in section 3.2, 'Open Protocol Commands.'

5.2 SERVICE CONDITIONS

An audible alarm and (on the Analyst HT) the control panel **SERVICE** light indicate a service condition. The instrument will continue to operate normally.

Display	Comment
X hrs lamp life	Continuous lamp has exceeded threshold limit.
luminescence aperture service	Luminescence aperture does not match current plate format.

5.3 FAULT CONDITIONS

Fault conditions display a message on the control panel with a two-digit fault code and short descriptor. All faults are announced with an audible alarm and (on the Analyst HT) the control panel **FAULT** light. In all cases, if the problem persists, contact your LJL BioSystems agent.

Display	Comment
00 Door open	Door to light-tight enclosure is open, plate may be jammed. Remove obstruction and select Reset Instrument from the File menu or (on the Analyst HT) press **RESET**.
01 Stop by home signal	Select Reset Instrument from the File menu or press **RESET**.
02 Plate crash	Plate contacted optics head. Eject plate by selecting Reset Instrument from the File menu or pressing **RESET**.
03 Plate crash	Plate contacted optics head. Eject plate by selecting Reset Instrument from the File menu or pressing **RESET**.
04 Motor error	Select Reset Instrument from the File menu or press **RESET**.
05 Motor error	Select Reset Instrument from the File menu or press **RESET**.

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Display	Comment
06 Motor error	May be due to physical blockage of any motor drive mechanism or may be due to worn components.
07 Amp Fault	Select Reset Instrument from the File menu or press **RESET**.
08 Motor error	Select Reset Instrument from the File menu or press **RESET**.
09 Lost X steps	May be due to physical blockage of any motor drive mechanism or may be due to worn components.
10 XY Reset failure	Select Reset Instrument from the File menu or press **RESET**.
20 X timed out	Indicates that operating state *X* did not occur in its allotted time. The problem may be due to a mechanical obstruction that prevents a motor from reaching its destination. Check for obstructions in XY mechanism, optical shuttles and filter wheels. Select **Reset instrument** from the File menu or press **RESET**.
21 State stack error	Select Reset Instrument from the File menu or press **RESET**.
22 State stack error	Select Reset Instrument from the File menu or press **RESET**.
30 Stacker timed out	Indicates that a stacker operation did not occur in its allotted time. The problem may be due to a mechanical obstruction that prevents a motor from reaching its destination. Check for obstructions in stacker mechanism. Select **Reset instrument** from the File menu or press **RESET**.
31 Stacker motor failure	Plate may be jammed in stacker. Check for jam and select **Reset instrument** from the File menu or press **RESET**.
32 Stacker motor failure	Plate may be jammed in stacker. Check for jam and select **Reset instrument** from the File menu or press **RESET**.
33 Invalid command	Select Reset Instrument from the File menu or press **RESET**.
34 Stacker error	Select Reset Instrument from the File menu or press **RESET**.
35 Stacker error	Select Reset Instrument from the File menu or press **RESET**.
36 Stacker error	Select Reset Instrument from the File menu or press **RESET**.
37 Stacker error: X 38 Stacker error: X 39 Stacker error: X 40 Stacker error: X 41 Stacker error: X 42 Stacker error: X	Plate may be jammed in stacker. X is a string providing additional information regarding the fault. X can be one of the following cases: 1: Z motor home failed. Possible obstruction in input, output or transfer positions. Select **Reset instrument** from the File menu or press **RESET**. 2: X motor home failed. Possible obstruction on tray below bezel. Select **Reset instrument** from the File menu or press **RESET**. 3: Invalid command sent to stacker. 4: Stacker not initialized. unk: unknown stacker error. Select **Reset instrument** from the File menu or press **RESET**.
43 Stacker error	Select Reset Instrument from the File menu or press **RESET**.

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6 Appendices

6.1 RELOCATING THE SYSTEM

Moving the Instrument Within the Lab

Ensure the new location has enough room for the instrument (see 'Physical Dimensions' in section 6.4, 'Technical Specifications'). Turn off the instrument with the plate gripper in the instrument. Remove power cord and RS232 cables.

The height of the instrument can be adjusted using the four feet. Allow enough space to permit access to the filter wheels, lamps, and dichroic mirrors.

Changing the Control Panel Position (Analyst HT)

The control panel can be installed on either the front or rear. To change the control panel's position, remove the connector panel, loosen the captive screw in the control panel, and plug the control panel in the alternative location. Secure the control panel with the captive screw.

Warning! Never *remove or install the control panel when the power is on. Serious damage to the instrument will result. Also, the instrument will not work properly with two control panels installed.*

Shipping the Instrument

Please contact your LJL BioSystems agent for proper instructions on safely shipping the instrument.

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6.2 TECHNICAL REFERENCE INFORMATION

Microplates

Microplates are defined in terms of their plate height, plate width, plate depth, number of wells, well depth, and spacing between wells. These parameters are factory-set for many common types of plates.

The operator can define new plate formats for the instrument with either the CriterionHost application or the Open Protocol. The software allows the operator to define a complete range of plate parameters. Accurate plate definitions are important, especially when optimizing performance for 384-well plates using the Dynamic Z feature, which allows precise specification of the vertical positioning of the plate relative to the optics head in the F, HEFP, and TRF modes.

External Control Box (Analyst HT)

- Maximum voltage on relay connections is 30 volts.
- Maximum current from the 24-volt relay supply is 3 amperes.
- Maximum total current from the Beacon+ supplies is 1 ampere.
- Beacon+ connections are always 24 volts.
- Beacon– connections are switches to 24 volts common (*not* chassis ground).

Name and Interconnect Table

Signal and Label	37-Pin Connector Pins	Terminal Block Pins	Comment
+24V	22	1-1	Max 2 Amps
Relay 1 NO	6	1-2	Fault
Relay 1 COM	3	1-3	
Relay 1 NC	21	1-4	
Relay 2 NO	5	1-5	Service
Relay 2 COM	24	1-6	
Relay 2 NC	2	1-7	
24V COM	25	1-11	24V return
Beacon Fault+	8	2-1	24V max
Beacon Fault–	28	2-2	Switch to 24V Com
Beacon Service+	26	2-3	24V
Beacon Service–	9	2-4	Switch to 24V Com
None	–	2-7	Empty

NO – normally open, NC – normally closed

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6.3 METHOD PARAMETER CROSS-REFERENCE

The following tables list all parameters set using the Define and Edit Methods screens. For each parameter, the range of possible values is listed as well as the default value for the given detection mode. Each detection mode has a set of default values. Also listed (for the Analyst HT) is the Open Protocol command that is equivalent to the default CriterionHost application parameter.



Important! *Method parameters entered using Open Protocol commands override previous settings for any method as long as you continue to use the Open Protocol to operate the system. However, when you return to the graphical user interface (for example, the Plate View screen) and resume operation, the CriterionHost application restores the method parameters previously entered with the Define and Edit Methods screens.*

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Fluorescence (F) Parameters	Range or Option	Default	Open Protocol Command
Method Name	User-defined	NA	**mode** f **methodid** any string
Optics	Top or Bottom	Top	**xside** top (**mode** f command defaults to top optics)
Lamp	Continuous or Flash	Continuous	**lamp** continuous
Filters			
Excitation	Select one from list of eight	1 Fluorescein 485 nm	**xfilter** 1 (check current setting by entering **xfilter** with no string)
Emission	Select one from list of eight	1 Fluorescein 530 nm	**efilter** 1 (check current setting by entering **efilter** with no string)
Plate Format	Select one from list	LJL HE 384 A	**format** LJL HE 384 A
Select Wells	All or user-specified range	All	**rs** column, **rw** a:p
Z Height	Middle, Bottom, or Set 0–7 mm	Middle	**zt** middle
Timing			
Readings per Well	1–65,535	1	**cycles** 1
Integration Time	5,000–1,600,000 µsec	100,000	**it** 100000
Interval between Flashes	1–100 × 10 msec (10–1000 msec)	100*	**period** 100
Delay after Flash	0–13,000 µsec	0*	**delay 0**
Raw Data Units	RFU, Counts, Counts/sec	Counts/sec	**read** cps
Attenuator Mode	High, Medium, or Out	Out	**am** o

Advanced F Parameters	Range or Option	Default	Open Protocol Command
Polarizers			
Excitation	None, S, or P	None	**xpolarizer** none
Emission	None, S, or P	None	**epolarizer** none
PMT Setup			
Measurement Type	Digital, SmartRead, or SmartRead Plus	SmartRead	**cm** comparator
Sensitivity	0–4	2	**cap** 2
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Medium	**shakevel** 30 **shakeradius** 4
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0
Delay between reads	0–28,800 sec	0	**mrt** 0
Number of reads	1–100	1	**mrc** 1
Flash Lamp Voltage	0–1000 volts	1000*	**fv** 1000
Plate Settling Time	0–1000 msec	25	**settle** 25

**Note:* *To specify this parameter, the flash lamp must be selected.*

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Fluorescence Polarization (HEFP) Parameters	**Range or Option**	**Default**	**Open Protocol Command**
Method Name	User-defined	NA	**mode** fp **methodid** any string
Optics	Top	Top	**xside** top (**mode** fp command defaults to top optics)
Lamp	Continuous or Flash	Continuous	**lamp** continuous
Filters			
Excitation	Select one from list of eight	1 Fluorescein 485 nm	**xfilter** 1 (check current setting by entering **xfilter** with no string)
Emission	Select one from list of eight	1 Fluorescein 530 nm	**efilter** 1 (check current setting by entering **efilter** with no string)
Plate Format	Select one from list	LJL HE 384 A	**format** LJL HE 384 A
Select Wells	All or user-specified range	All	**rs** column, **rw** a:p
Z Height	Middle, Bottom, or Set 0–7 mm	Middle	**zt** middle
Timing			
Readings per Well	1–65,535	1	**cycles** 1
Integration Time	5,000–1,600,000 µsec	100,000	**it** 100000
Interval between Flashes	1–100 × 10 msec (10–1000 msec)	1*	**period** 1
Delay after Flash	0–13,000 µsec	0*	**delay 0**
Switch Polarization	By Well or By Plate	By Well	
G Factor	0.1–10	1	**read** r0(a1:h12)=fp(fpp,fps,1)
Raw Data Units	RFU, Counts, Counts/sec	Counts/sec	**read** cps
Attenuator Mode	High, Medium, or Out	Out	**am** o

Advanced HEFP Parameters	**Range or Option**	**Default**	**Open Protocol Command**
Polarizers			
Dynamic Polarizer	Excitation or Emission	Emission	**dpolarizer** emission
Static Polarizer	S or P	S	**xpolarizer** s
Polarizer Settling Time	0–1000 msec	0	**pt** 0
PMT Setup			
Measurement Type	Digital, SmartRead, or SmartRead Plus	SmartRead	**cm** comparator
Sensitivity	0–4	2	**cap 2**
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Medium	**shakevel** 30 **shakeradius** 4
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0
Delay between reads	0–28,800 sec	0	**mrt** 0

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Number of reads	1–100	1	**mrc** 1
Flash Lamp Voltage	0–1000 volts	1000*	**fv** 1000
Plate Settling Time	0–1000 msec	25	**settle** 25

****Note:** To specify this parameter, the flash lamp must be selected.*

Time-Resolved Fluorescence (TRF) Parameters	**Range or Option**	**Default**	**Open Protocol Command**
Method Name	User-defined	NA	**mode** trf **methodid** any string
Optics	Top or Bottom	Top	**xside** top (**mode** trf command defaults to top optics)
Lamp	Flash	Flash	**lamp** flash
Filters			
Excitation	Select one from list of eight	2 Europium 360 nm	**xfilter** 2 (check current setting by entering **xfilter** with no string)
Emission	Select one from list of eight	2 Europium 620 nm	**efilter** 2 (check current setting by entering **efilter** with no string)
Plate Format	Select one from list	LJL HE 384 A	**format** LJL HE 384 A
Select Wells	All or user-specified range	All	**rs** row, **rw** a:p
Z Height	Middle, Bottom, or Set 0–7 mm	Middle	**zt** middle
Timing			
Flashes per Well	1–65,535	100	**cycles** 100
Integration Time	5,000–1,600,000 µsec	1000	**it** 1000
Interval between Flashes	1–100 × 10 msec (10–1000 msec)	10	**period** 10
Delay after Flash	0–13,000 µsec	50	**delay** 50
Raw Data Units	RFU, Counts, Counts/sec	Counts	**read** counts
Attenuator Mode	High, Medium, or Out	Out	**am** o

Advanced TRF Parameters	**Range or Option**	**Default**	**Open Protocol Command**
PMT Setup			
Measurement Type	Digital, SmartRead, or SmartRead Plus	Digital	**cm** digital
Sensitivity	NA	NA	NA
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Medium	**shakevel** 30 **shakeradius** 4
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0

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Delay between reads	0–28,800 sec	0	**mrt** 0
Number of reads	1–100	1	**mrc** 1
Flash Lamp Voltage	0–1000 volts	1000	**fv** 1000
Plate Settling Time	0–1000 msec	0	**settle** 0

Luminescence (L) Parameters	**Range or Option**	**Default**	**Open Protocol Command**
Method Name	User-defined	NA	**mode** l **methodid** any string
Plate Format	Select one from list	Packard - Optiplate	**format** Packard Optiplate
Select Wells	All or user-specified range	All	**rs** row, **rw** a:h
Luminescence Height	Set 0.5–2 mm	1	**zt** numeric **zh** 1
Timing			
Readings per Well	1– 65,535	1	**cycles** 1
Integration Time	5,000–1,600,000 µsec	100,000	**it** 100000
Raw Data Units	RLU, Counts, Counts/sec	Counts/sec	**read** cps
Attenuator Mode	Medium or Out	Out	**am** o

Advanced L Parameters	**Range or Option**	**Default**	**Open Protocol Command**
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Medium	**shakevel** 30 **shakeradius** 4
Plate Settling Time	0–1000 msec	25	**settle** 25
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0
Delay between reads	0–28,800 sec	0	**mrt** 0
Number of reads	1–100	1	**mrc** 1

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Focused Luminescence (FLUM) Parameters	Range or Option	Default	Open Protocol Command
Method Name	User-defined	NA	**mode** f **methodid** any string
Filters			
Emission	Select one from list of eight	8 blocked	**efilter** 8 (check current setting by entering **efilter** with no string)
Plate Format	Select one from list	LJL HE 384 A	**format** LJL HE 384 A
Select Wells	All or user-specified range	All	**rs** row, **rw** a:p
Z Height	Middle, Bottom, or Set 0–7 mm	Bottom	**zt** bottom
Timing			
Readings per Well	1–65,535	1	**cycles** 1
Integration Time	5,000–1,600,000 µsec	100,000	**it** 100000
Raw Data Units	Counts, Counts/sec, or Intensity	Counts/sec	**read** cps
Attenuator Mode	High, Medium, or Out	Out	**am** o

Advanced FLUM Parameters	Range or Option	Default	Open Protocol Command
Polarizers			
Excitation	None, S, or P	None	**xpolarizer** none
Emission	None, S, or P	None	**epolarizer** none
PMT Setup			
Measurement Type	Digital, SmartRead, or SmartRead Plus	Digital	**cm** digital
Sensitivity	NA	NA	**NA**
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Medium	**shakevel** 30 **shakeradius** 4
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0
Delay between reads	0–28,800 sec	0	**mrt** 0
Number of reads	1–100	1	**mrc** 1
Plate Settling Time	0–1000 msec	25	**settle** 25

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Absorbance (A) Parameters	Range or Option	Default	Open Protocol Command
Method Name	User-defined	NA	**mode** f **methodid** any string
Lamp	Continuous or Flash	Continuous	**lamp** continuous
Filters			
Excitation	Select one from list of eight	7 blocked	**xfilter** 7 (check current setting by entering **xfilter** with no string)
Plate Format	Select one from list	Corning Costar Clear Bottom PS	**format** Corning Costar Clear Bottom PS
Select Wells	All or user-specified range	All	**rs** row, **rw** a:h
Z Height	Middle, Bottom, or Set 0–7 mm	7	**zt** numeric **zh** 7
Timing			
Readings per Well	1–65,535	1	**cycles** 1
Integration Time	5,000–1,600,000 µsec	3,000	**it** 3000
Raw Data Units	Counts, Counts/sec, or Intensity	Counts/sec	**read** cps

Advanced A Parameters	Range or Option	Default	Open Protocol Command
Enable Automatic Gain Setting	Checked or unchecked	Checked	NA
Absorbance Factor		0	NA
Buffer Well		a1	NA
Coarse		NA	NA
Fine		NA	NA
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Low	**shakevel** 20 **shakeradius** 6
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0
Delay between reads	0–28,800 sec	0	**mrt** 0
Number of reads	1–100	1	**mrc** 1
Flash Lamp Voltage	0–1000 volts	500*	**fv** 500
Plate Settling Time	0–1000 msec	25	**settle** 25

**Note:* *To specify this parameter, the flash lamp must be selected.*

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Epi-Absorbance (EA) Parameters	Range or Option	Default	Open Protocol Command
Method Name	User-defined	NA	**mode** f **methodid** any string
Lamp	Continuous or Flash	Continuous	**lamp** continuous
Filters			
Excitation	Select one from list of eight	6 blocked	**xfilter** 6 (check current setting by entering **xfilter** with no string)
Emission	Select one from list of eight	3 blocked	**efilter** 3 (check current setting by entering **efilter** with no string)
Plate Format	Select one from list	LJL HE 384 A	**format** LJL HE 384 A
Select Wells	All or user-specified range	All	**rs** row, **rw** a:p
Z Height	Middle, Bottom, or Set 0–7 mm	Bottom	**zt** bottom
Timing			
Readings per Well	1–65,535	1	**cycles** 1
Integration Time	5,000–1,600,000 µsec	10,000	**it** 10000
Interval between Flashes	1–100 × 10 msec (10–1000 msec)	10*	**period** 10
Delay after Flash	0–13,000 µsec	0*	**delay** 0
Raw Data Units	Counts, Counts/sec, or Intensity	Counts/sec	**read** cps
Attenuator Mode	High, Medium, or Out	High	**am** h

Advanced EA Parameters	Range or Option	Default	Open Protocol Command
Polarizers			
Excitation	None, S, or P	None	**xpolarizer** none
Emission	None, S, or P	None	**epolarizer** none
PMT Setup			
Measurement Type	Digital, SmartRead, or SmartRead Plus	SmartRead	**cm** comparator
Sensitivity	0–4	2	**cap** 2
Plate Agitation			
Shaking Time	0–10 sec	0	**shaketime** 0
Intensity	High, Medium, or Low	Medium	**shakevel** 30 **shakeradius** 4
Kinetic Timing			
Delay before first read	0–3600 sec	0	**dal** 0
Delay between reads	0–28,800 sec	0	**mrt** 0
Number of reads	1–100	1	**mrc** 1
Flash Lamp Voltage	0–1000 volts	1000*	**fv** 1000
Plate Settling Time	0–1000 msec	25	**settle** 25

**Note:* *To specify this parameter, the flash lamp must be selected.*

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Multi-Methods Parameters	Range or Option	Default	Open Protocol Command
Name	User-defined	NA	**methodid** any string
Mode 1	F, TRF, L, FLUM, A, or EA	F	**read***
Method 1	User-defined	Default Fluorescence Intensity Method	**read***
Mode 2	F, TRF, L, FLUM, A, or EA	F	**read***
Method 2	User-defined	Default Fluorescence Intensity Method	**read***
Select Wells	All or user-specified range	All	**rs** row, **rw** a:p
Plate Format	Select one from list	LJL HE 384 A	**format** LJL HE 384 A
Method Switching	By Well or By Plate	By Plate	**ms** plate
Reporting			
Raw data	Checked or unchecked	Checked	**read***
Subtracted data	Checked or unchecked	Unchecked	**read***
Ratio	Ratio 1/2, Ratio 2/1, or omitted	Ratio 1/2	**read***
Multiplier	Checked or unchecked; integer	Unchecked; 1	**read***
Raw Data Units	Counts, Counts/sec, or Intensity	Counts	**read** counts
Order	NA	Raw Data, Ratio 1/2	**read***

Note:* *This parameter is specified indirectly via the read expression for the **read command.*



Important! *Method parameters entered using Open Protocol commands override previous settings for any method as long as you continue to use the Open Protocol to operate the system. However, when you return to the graphical user interface (for example, the Plate View screen) and resume operation, the CriterionHost application restores the method parameters previously entered with the Define and Edit Methods screens.*

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6.4 TECHNICAL SPECIFICATIONS

Read Times*	**Analyst AD**	**Analyst HT**
Fluorescence Intensity	160 sec	110 sec
High Efficiency Fluorescence Polarization	255 sec	200 sec
Time-Resolved Fluorescence	505 sec	460 sec
Luminescence	160 sec	110 sec
Absorbance	160 sec	115 sec

***Note:** All read times are for 384-well plates and assume 100-millisecond integration times for Fluorescence Intensity, High Efficiency Fluorescence Polarization, Luminescence, and Absorbance, and 100 flashes for Time-Resolved Fluorescence.*

Integration Time	User-selectable from 1 µsec to 1.6 seconds in 1-µsec increments
Maximum Plate Dimensions	
Without stacker	18 × 92.8 × 129 mm (H × W × D)
With stacker	15 × 92.8 × 129 mm
Light Source, F, HEFP, A, and EA Methods	
Lamp Type	Xenon arc
Spectral Range	400–700 nm
Lamp Life	>2000 hours
Light Source, TRF Methods	
Lamp Type	Xenon flash, 170-watt
Spectral Range	250–900 nm
Lamp Life	5 years
Fluorescence Detection	
Wavelength	400–740 nm
Emission Bandwidth	Filter-dependent
Photomultiplier Tube (PMT)	Photon-counting
Linear Dynamic Range	6 Decades
Luminescence Detection	
Wavelength	450–600 nm
Crosstalk	0.5%
Detection Limit	
Fluorescence Intensity	<15 pM fluorescein
Fluorescence Polarization	<10 mP SD for 100 pM fluorescein
Time-Resolved Fluorescence	<100 fM europium
Luminescence	<10,000 photons/sec
Physical Dimensions	
Without stacker	
Weight	147 lb (66.7 kg)
Height	24.0 in (60.9 cm)
Width	20.5 in (52.1 cm)
Depth	25.5 in (64.8 cm)
With stacker	
Weight	157 lb (71.4 kg)
Height	24.0 in (60.9 cm)
Width	20.5 in (52.1 cm)
Depth	34.7 in (87.9 cm)

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Environmental Specifications

Operation humidity rage	10 to 90% Noncondensing
Operation temperature range	15 to 30 °C
Storage temperature range	5 to 50 °C
Noise level	70 dB maximum at 2 meters

Host Computer Requirements and Recommendations

Recommended Manufacturer	Dell, Hewlett-Packard
Computer Microprocessor	P5 or Pentium
Operating System	Windows 95, 98, or NT
Clock Speed	133 MHz or greater
Display	VGA (640 × 480)
Data Storage	1.2 GB Hard Drive
Memory	16 MB with Windows 95 or 98 32 MB with Windows NT
Communications Ports	2 serial, 1 parallel
Mouse	2-button Microsoft mouse or equivalent
Printer	optional

Communication Interfaces

Type	Bidirectional RS232
Number of Ports	
Analyst AD	1 (Host 1)
Analyst HT	2 (Host 1 and Host 2)
Protocols	ASCII, Binary with CRC
Baud Rate	115,200
Connectors	9-pin male sub D

Power Requirements

Voltage	90–250 VAC, 50–60 Hz
Voltage Switching	Automatic
Power	<1KW

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6.5 SPARE PARTS

When ordering parts from LJL BioSystems, please provide the following information:

- Purchase order number
- Type and serial number of the instrument for which the part is intended
- Quantity of each item ordered
- Product number and description
- Shipping and billing address

Article Number	Description
42-0028	Magazine, S20 (2 each)
42-0029	Continuous Lamp Core (new)
42-0030	Flash Lamp
42-0040	Tall Cartridge Spare Parts Kit
	Cartridge installation instructions
	Slug, 25 mm (2 each)
	Funnel
	Tall cartridge (2 each)
	Friction ring (6 each)
	O-rings (6 each)
42-0041	Short Cartridge Spare Parts Kit
	Cartridge installation instructions
	Short cartridge (2 each)
	Glue (0.5 oz)
	Short cartridge tool
	O-rings (6 each)
42-0042	Excitation/Emission Filter Label
43-0342	External Control Box

6.6 SELECTING FILTERS AND DICHROIC MIRRORS

LJL BioSystems offers optimized interference filters and dichroic mirrors for specific fluorophores. Typically, each fluorophore requires a specific excitation filter, emission filter, and dichroic mirror. The following table shows recommended wavelengths for six fluorophores.

Fluorophore	Excitation Wavelength	Emission Wavelength	Dichroic Mirror
Fluorescein	485	530	505
Europium	360	620	400
Coumarin	360	425	400
Rhodamine	530	580	565
Texas Red	580	630	505
EDANS	360	485	400

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Filters: Determine the wavelength and bandpass requirements for your fluorophore.

You may already have an appropriate filter installed. For example, all Criterion systems are shipped with a fluorescein emission filter (CW 530, FWHM 25) installed. This same filter can be manually moved to the excitation filter wheel for use with Rhodamine. If automatic filter selection is required, order an additional filter.

The filters in the table below are all supplied pretested and installed in tall cartridges. For each filter, the center wavelength (CW) is listed, then the 'full width at half maximum' (FWHM) is listed. The FWHM is the bandwidth at the point where 50 percent of the impingent light is transmitted.

Dichroic Mirrors: All Criterion systems are equipped with a fluorescein dichroic mirror and a 50:50 beamsplitter. The 50:50 beamsplitter can be used with any fluorophore. However, use of the appropriate dichroic mirror instead of the 50:50 beamsplitter increases assay sensitivity significantly (by a factor of 4).

The dichroic center cut-on wavelength falls between the excitation and emission wavelengths. The center cut-on wavelength is the wavelength in which 50 percent of the impingent light is both transmitted and reflected.

Dichroic mirrors are assembled in holders and are ready to install into either the top or bottom read heads.

Article Number	Description	Wavelength	Bandpass
42-0037	CW 360, FWHM 35	360	35
42-0045	CW 425, FWHM 35	425	35
42-0031	CW 485, FWHM 20	485	20
42-0032	CW 530, FWHM 25	530	25
42-0034	CW 580, FWHM 10	580	10
42-0038	CW 620, FWHM 35	620	35
42-0035	CW 630, FWHM 35	630	35
42-0039	Dichroic 400	400	NA
42-0033	Dichroic 505	505	NA
42-0036	Dichroic 505/600	505	NA
42-0048	Dichroic 565	565	NA
42-0049	50:50 Beamsplitter	NA	NA

Additional filters may be available. Check the LJL BioSystems web site for details (www.ljlbio.com/clubht).

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6.7 WARRANTY STATEMENT

The Analyst™ AD Assay Detection System or Analyst™ HT Assay Detection System (the 'Instrument') is warranted against defects in materials or workmanship for one (1) year following the delivery date.

LJL BioSystems, Inc. ('LJL') reserves the right to repair or replace a defective Instrument at its discretion, which shall be the sole liability of LJL under this warranty. During the warranty period, on-site service will be provided by LJL or its authorized agent. This warranty does not apply to damage resulting from misuse or operation in an environment or manner other than intended or recommended by LJL, modifications or repairs not made by LJL, accidents, or other improper maintenance, any of which will immediately void this warranty. Should service be required, please contact your local representative of LJL products.

DO NOT UNDER ANY CIRCUMSTANCES ATTEMPT TO OPERATE THIS INSTRUMENT WITH PANELS REMOVED OR ATTEMPT TO CHANGE ANY COMPONENTS OR PERFORM ANY MAINTENANCE WITH THE POWER ON.

LJL BioSystems, Inc. does not provide any other warranty, express or implied, and disclaims all other warranties, including without limitation any implied warranties for infringement, merchantability or the appropriateness of the Instrument or other products for a particular purpose, and LJL assumes no liability for the results derived from its products.

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