Gemini EM
Gemini XPS

Dual Scanning Microplate Spectrofluorometer User Guide

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Molecular Devices Corporation
Gemini EM/XPS Manual

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Gemini EM Patents 6,097,025, 6,232,608, 6,236,456, 6,313,471, 6,316,774, and 6,693,709.

Gemini XPS Patents 6,097,025, 6,232,608, 6,236,456, 6,313,471, and 6,316,774**.**

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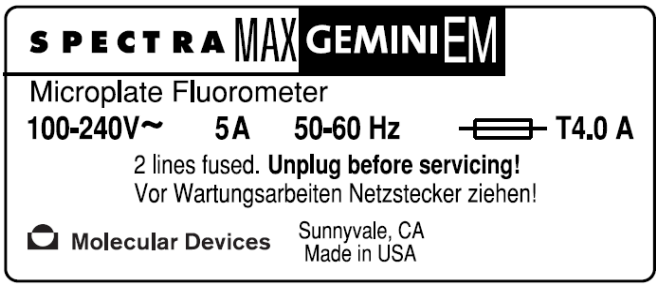

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1. Description

1.1. FEATURES

The Gemini EM and Gemini XPS Dual-Scanning Microplate Spectrofluorometers can perform a variety of fluorescent applications. The extreme flexibility and high sensitivity of Gemini readers make them appropriate for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology.

1.1.1. DUAL MONOCHROMATORS

The right pair of excitation and emission wavelengths is always available because the dual monochromators allow the selection of any wavelength in 1 nm increments. New fluorophores can easily be evaluated without purchasing additional filters.

The Gemini EM and Gemini XPS microplate readers use two holographic diffraction grating monochromators, which allow for individual optimization of wavelengths for both excitation and emission. The dual-scanning capability can also be used to determine excitation and emission settings for new fluorescent probes.

1.1.2. OPTICS

Mirrored optics focus the light into the sample volume, and cutoff filters are used to reduce stray light and minimize background interference. The light source is a high-powered Xenon flash lamp; additional flexibility is provided by allowing a variable number of lamp flashes per read.

1.1.3. WAVELENGTH SCANNING

The most sensitive results are achieved by using optimal excitation and emission wavelengths. Literature wavelengths are often based on results from wavelength-limited, filter-based readers. Wavelength scanning ensures that the most sensitive assay conditions are used.

1.1.4. WELL SCANNING

Gemini EM and Gemini XPS can report a single point from the well center, or multiple data points from the bottom of large well tissue culture plates to provide high sensitivity for cell-based assays.

1.1.5. AUTO PMT GAIN

Because a single microplate often presents a range of fluorescence intensities greater than three orders of magnitude, Gemini EM and Gemini XPS feature “Auto PMT Gain” to

avoid saturating the photomultiplier tube. The signal is calibrated against an internal standard, so the reported RFU values of individual samples can be accurately compared.

1.1.6. TOP AND BOTTOM READING OPTICS—GEMINI EM ONLY

The top/bottom-reading optical design of the Gemini EM allows for measurements for both solution and cell-based assays. With the click of a button, the Gemini EM can be switched between top- and bottom-reading modes.

1.1.7. SUPPORTED PLATES

Microplates having 6, 12, 24, 48, 96, and 384 wells can be used in Gemini readers.

One plate carrier adapter is provided with the instrument. The adapter is required for optimum performance with standard 96- and 384-well format microplates when reading from the top of the microplate.

1.1.8. DYNAMIC RANGE

The dynamic range of detection is from 10^{-6} to 10^{-11} molar fluorescein. Variations in measured fluorescence values are virtually eliminated by internal compensation for detector sensitivity, photomultiplier tube voltage and sensitivity, as well as excitation intensity.

1.1.9. TEMPERATURE CONTROL

Temperature in the microplate chamber is isothermal, both at ambient and when the incubator is turned on. When the incubator is on, the temperature may be controlled from 4°C above ambient to 45°C.

1.1.10. AUTOMIX

The contents of the wells in a microplate can be mixed automatically by shaking before each read cycle, which makes it possible to perform kinetic analysis of solid-phase, enzyme-mediated reactions such as a kinetic ELISA.

1.1.11. COMPUTER CONTROL

Gemini readers are controlled by an external computer running SoftMax® Pro software which provides integrated instrument control, data display, and statistical data analysis. Gemini readers cannot be operated without the computer and SoftMax Pro software.

1.1.12. SECONDARY MODES

The Gemini EM and Gemini XPS have two secondary modes that can be used for limited development of glow luminescence or time-resolved fluorescence assays. The performance of these two modes is not comparable to dedicated luminescence or time-resolved fluorescence instruments.

Figure 1.1: Gemini EM.

1.2. COMPONENTS

The main components of Gemini readers described in this manual are:

- Control panel
- Microplate drawer
- Optical system
- Back panel (connections and power switch)

1.2.1. THE CONTROL PANEL

The control panel consists of a 2-×-20-character LCD and four pressure-sensitive membrane keys that can be used to initiate and regulate the temperature and to open and close the drawer. When you press a control panel key, the Gemini performs the associated action.

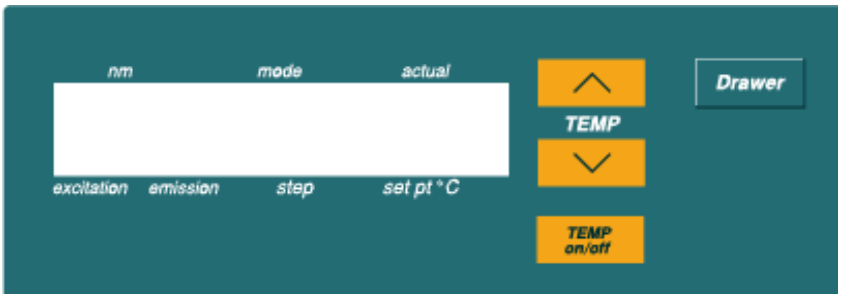

Figure 1.2: Control Panel.

TEMP

The TEMP keys allow you to enter a set point at which to regulate the microplate chamber temperature.

Pressing this key scrolls the temperature up or down, starting at the previous temperature setting (or the default of 37.0°C, if no setting had been made):

- Pressing the up (▲) or down (▼) arrow once increments or decrements the displayed temperature by 0.1°C.
- Pressing and holding either arrow increments or decrements the displayed temperature by 1°C until it is released.

You cannot set a temperature beyond the upper (45°C) or lower (15°C) instrument limits.

Temp On/Off

The TEMP on/off key enables and disables the incubator.

- When the incubator is on, the set temperature and actual temperature are shown on the front panel LCD display.
- When the instrument is performing a kinetic or spectral scan, the temperature keys on the front panel are disabled.

Drawer

The DRAWER key opens and closes the microplate drawer.

1.2.2. THE MICROPLATE DRAWER

The microplate drawer, located on the right side of the Gemini, slides in and out of the microplate chamber. A small plastic pusher, located in the front left corner of the drawer, holds the plate securely in place when the drawer is closed. The drawer remains in the reading chamber during read cycles.

One plate carrier adapter is provided with the instrument. The adapter is required for optimum performance with standard 96-well and 384-well format microplates in top read mode. The adapter is required when using the SpectraTest FL validation plate to test the Gemini XPS and when testing the Gemini EM top read optics. To test the Gemini EM bottom reading performance, remove the purple adapter and then turn the validation plate upside down by rotating it from top-to-bottom so that column 1 remains on your left.

Figure 1.3: Microplate drawer (with adapter inserted).

The adapter must be removed to read 6-well, 12-well, 24-well, or 48-well plates.

Microplate drawer operation varies, depending on the incubator setting:

- If the incubator is off, the drawer remains open.
- If the incubator is on, the drawer closes after approximately 10 seconds to assist in maintaining temperature control within the microplate chamber.

To add reagents during a kinetic read, it is necessary to open the drawer by pressing the DRAWER key. The drawer only opens, however, if the interval between readings is equal to the minimum read interval originally shown by SoftMax Pro software plus an additional 45 seconds. If you plan to open the drawer during a kinetic read, first determine the minimum read interval allowed and then increase the setting by a minimum of 45 seconds. The drawer closes automatically after this interval before the next read.

Do not obstruct the movement of the drawer. If you must retrieve a plate after an error condition or power outage and the drawer does not open, it is possible to open it manually (see Chapter 6, *"Troubleshooting"*).

1.2.3. MICROPLATES

Gemini readers can accommodate standard 6-well, 12-well, 24-well, 48-well, 96-well, and 384-well microplates. Black-walled, clear-bottom or all-black microplates are generally recommended for fluorescence assays because they have lower backgrounds than clear plates. White plates may be preferred for luminescence assays to optimize light collection.

Not all manufacturers' microplates are the same with regard to design, materials, or configuration. Some plastics, most notably polystyrene, also have significant native fluorescence and can cause moderate to severe background fluorescence, especially in the UV range. If high sensitivity is required, it may be appropriate to use microplates that are designed to reduce background fluorescence.

1.2.4. THE OPTICAL SYSTEM—GEMINI EM

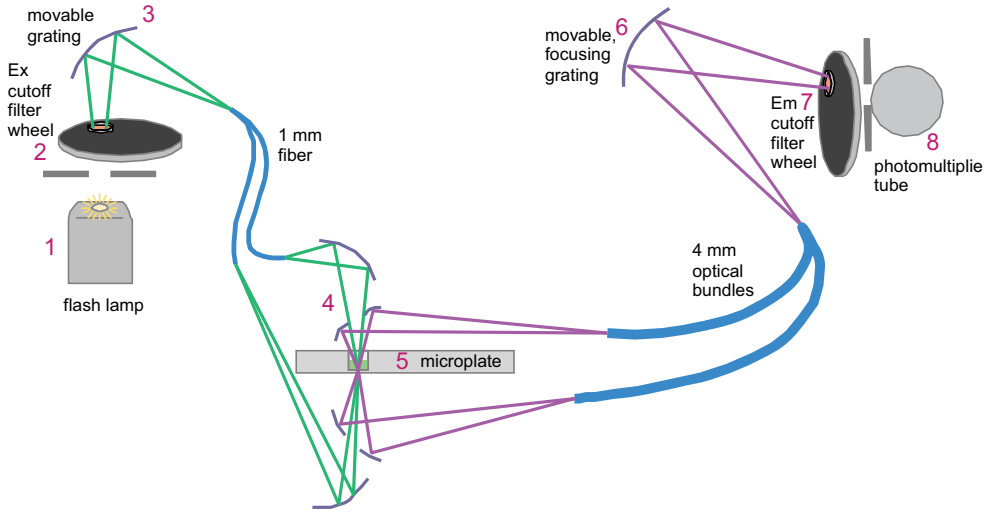

Figure 1.4: Components of the Gemini EM optical system.

1 The excitation light source is a xenon flash lamp. (Note that the lamp is off when luminescence mode is selected.)

2 The light passes through a band-pass filter that reduces the amount of stray light to the excitation monochromator.

3 The holographic diffraction grating monochromator selects the desired excitation wavelength.

4 The excitation beam is focused by a grating to a 1.0-mm diameter fiber into the upper or lower optics read head (selectable) before entering the sample in the microplate well. This focusing helps to prevent part of the beam from striking adjacent wells.

5 The light beam enters the well and, if fluorescent molecules are present, light of the emission wavelength is emitted back out to mirrors that focus it and send it to an optical bundle.

6 The emission monochromator (also a holographic diffraction grating monochromator) allows light of the chosen emission wavelength to pass to the emission filter wheel.

7 A long-pass filter further conditions the light prior to detection by the photomultiplier tube (PMT). This filter may be set automatically by the instrument or manually by the user.

8 The PMT detects the emitted light and passes a quantitative signal to the instrument's electronics that then send the data to the computer.

1.2.5. THE OPTICAL SYSTEM—GEMINI XPS

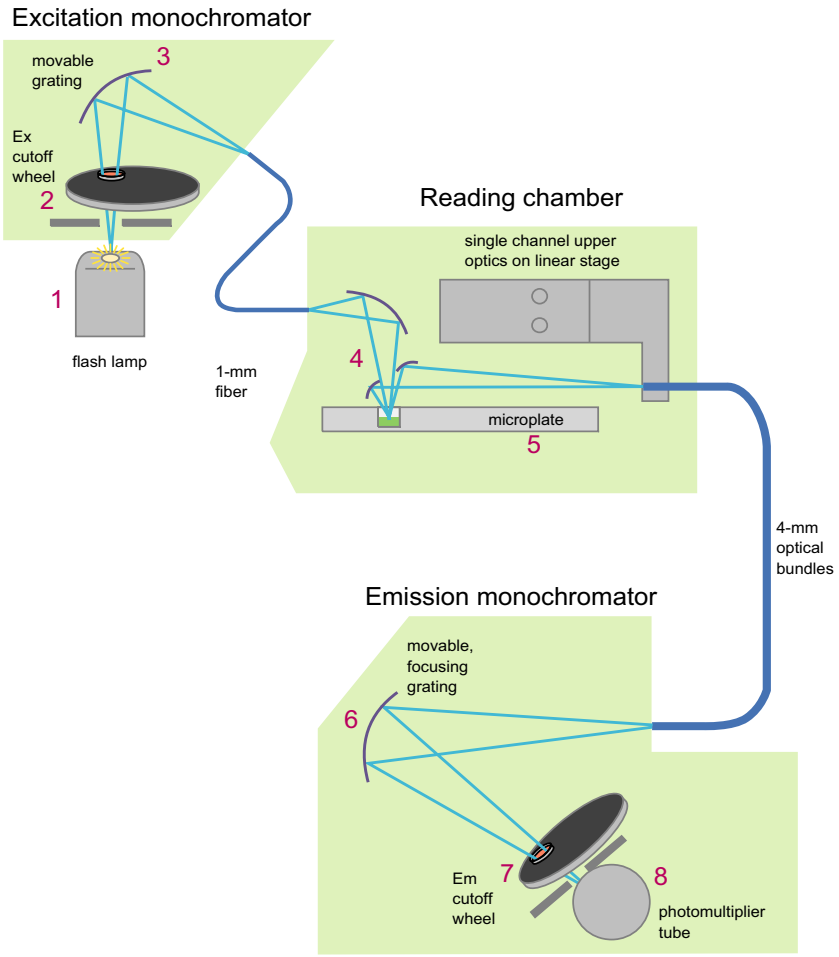

Figure 1.5: Components of the Gemini XPS optical system.

1 The excitation light source is a xenon flash lamp. (Note that the lamp is off when luminescence mode is selected.)

2 The light passes through a band-pass filter that reduces the amount of stray light to the excitation monochromator.

3 The holographic diffraction grating monochromator selects the desired excitation wavelength.

4 The excitation beam is collimated by a mirror to a 1.0-mm diameter fiber before entering the sample in the microplate well. This focusing helps to prevent part of the beam from striking adjacent wells.

5 The light beam enters the well and, if fluorescent molecules are present, light of the emission wavelength is emitted back out to mirrors that focus it and send it to an optical bundle.

6 The emission monochromator (also a holographic diffraction grating monochromator) allows light of the chosen emission wavelength to pass to the emission filter wheel.

7 A long-pass filter further conditions the light prior to detection by the photomultiplier tube (PMT). This filter may be set automatically by the instrument or manually by the user.

8 The PMT detects the emitted light and passes a quantitative signal to the instrument's electronics which then send the data to the computer.

1.2.6. THE BACK PANEL

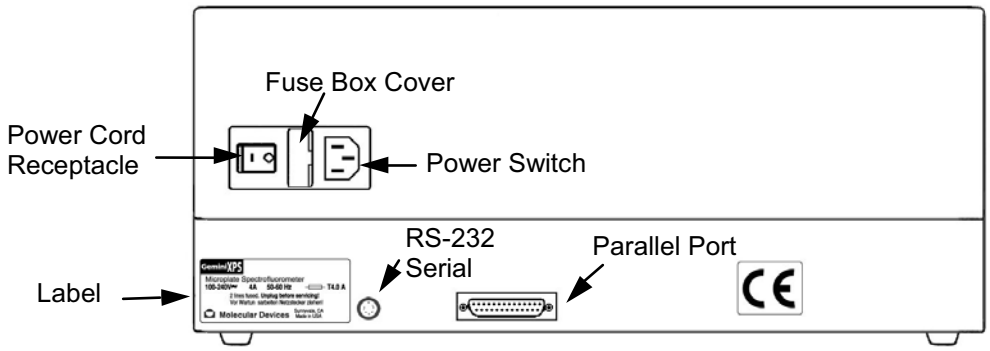

Figure 1.6: Schematic of the back panel of a Gemini reader.

The following components are located on the back panel of Gemini readers:

- **Power switch:** a rocker switch, labeled I/O (for on and off, respectively).
- **Power cord receptacle:** plug the power cord in here.
- **Fuse box cover:** cannot be opened while the power cord is plugged in. When opened, it provides access to the fuse box containing two fuses that are required for operation.
- **Parallel port:** present but not used in this model of reader.
- **Serial port** (double-shielded RS-232, for use with an external computer): plug one end of an 8-pin DIN serial cable into this port; the other end attaches to the serial (modem) port of the computer.
- **Label:** provides information about the Gemini, such as line voltage rating, cautionary information, serial number, etc. Record the serial number shown on this label for use when contacting Molecular Devices Technical Support.

2. Principles of Operation

2.1. FLUORESCENCE

Fluorescent materials absorb light energy of a characteristic wavelength (excitation), undergo an electronic state change, and instantaneously emit light of a longer wavelength (emission). Most common fluorescent materials have well-characterized excitation and emission spectra. Figure 2.1 shows an example of excitation and emission spectra for a fluorophore. The excitation and emission bands are each fairly broad, with half-bandwidths of approximately 40 nm, and the wavelength difference between the excitation and emission maxima (the Stokes shift) is typically fairly small, about 30 nm. There is considerable overlap between the excitation and emission spectra (gray area) when a small Stokes shift is present.

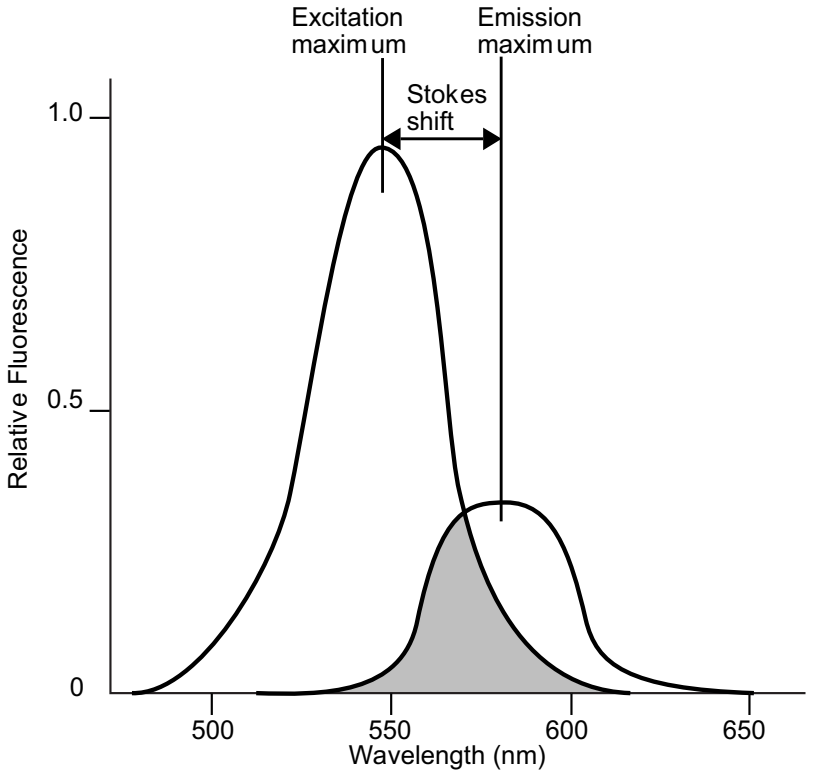

Figure 2.1: Excitation and emission spectra.

Because the intensity of the excitation light is usually many tens of thousands of times greater than that of the emitted light, some type of spectral separation is necessary to reduce the interference of the excitation light with detection of the emitted light. The Gemini readers incorporate many features designed to restrict interference from reflected excitation light. Among these features is a set of long-pass emission cutoff filters that can be set automatically by the instrument or manually by the user. If the Stokes shift is small, it may be advisable to choose an excitation wavelength that is as far away from the emission maximum as possible while still being capable of stimulating the fluorophore so that less of the excited light overlaps the emission spectrum, allowing better selection and quantitation of the emitted light.

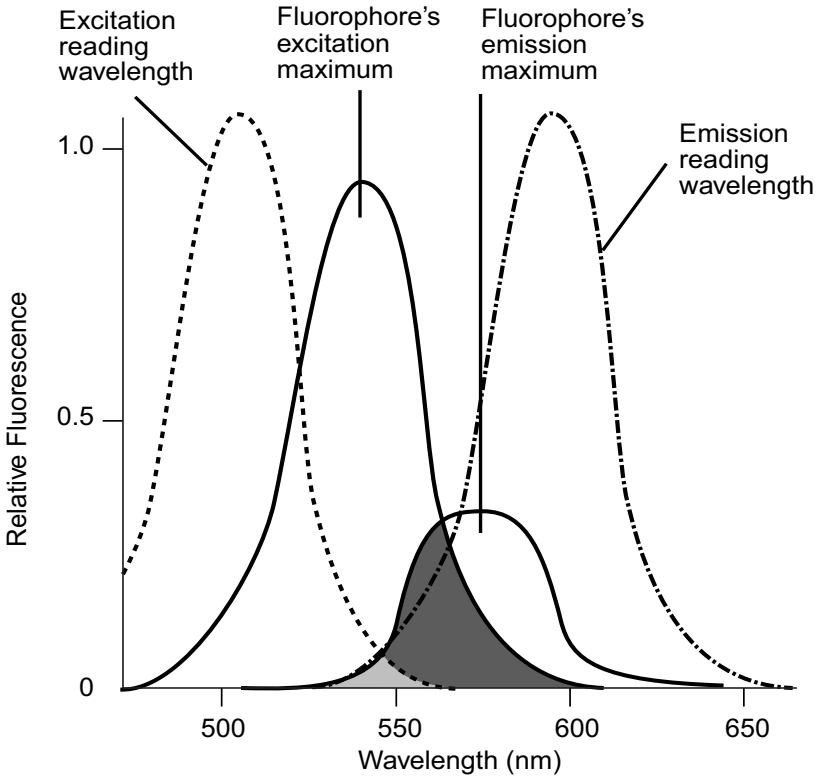

Figure 2.2: Optimized excitation and emission reading wavelengths.

Figure 2.2 shows that the best results are often obtained when the excitation and emission wavelengths used for reading are not the same as the wavelengths of the excitation and emission spectra of the fluorophore. When the reading wavelengths for excitation and emission are separated, a smaller amount of excitation light passes through to the emission monochromator (gray area) and on to the PMT, resulting in a purer emission signal and more accurate data.

The Gemini readers allow scanning of both excitation and emission wavelengths, using separate tunable monochromators. One benefit of being able to scan emission spectra is

that you can assess more accurately whether the emission is, in fact, the expected fluorophore, or multiple fluorophores, and not one generated by a variety of background sources or by contaminants. Another benefit is that you may be able to find excitation and emission wavelengths that avoid interference when interfering fluorescent species are present.

For this reason, it may be desirable to scan emission for both an intermediate concentration of labeled sample, as well as the background of unlabeled sample. The optimum setting is where the ratio of the sample emission to background emission is at the maximum.

For more information regarding optimizing excitation and emission wavelengths using the spectral scanning capabilities of the Gemini, see *"Optimizing Assays "* on page 21.

2.2. TIME-RESOLVED FLUORESCENCE

In normal fluorescence mode, readings are taken while the lamp is on. The most common limitation to sensitivity in normal fluorescence is excitation energy or background fluorescence that cannot be eliminated from the emission signal. Since the lamp is the source of excitation energy, turning it off provides the best means of eliminating background excitation.

Time-resolved fluorescence is performed by flashing the excitation lamp and, after it is off, collecting the delayed emission for a period of time before the lamp is flashed again. Lanthanide dyes are frequently used to delay the fluorescence long enough to measure it after the lamp is turned off.

To assist with proper collection of data, you can also select when to start and end data collection (within the limits of the system—the minimum is 50 µs and the maximum is 1450 µs in 200-µs steps).

2.3. LUMINESCENCE

In luminescence mode, no excitation is necessary as the species being measured emit light naturally. For this reason, the lamp does not flash, so no background interference occurs. A dark estimate is done over a dark reference, and multiple readings are averaged together into one reading per well.

You can choose the wavelength where peak emission is expected to occur. In addition, multiple wavelength choices allow species with multiple components to be differentiated and measured easily. In luminescence read mode, no emission cutoff filter is used. The default setting for luminescence is the "zero order" position where the grating monochromator acts as a mirror that reflects all light to the PMT detector.

The Gemini readers are microplate spectrofluorometers with photomultiplier tube detection. Some luminescence applications, such as gene reporter assays, may require a luminometer with photon counting detection for greater sensitivity.

2.4. FUNCTIONAL DESCRIPTION

The Gemini readers are designed to be operated using SoftMax Pro software running on a computer connected to the instrument. Standalone functions are limited to setting and enabling temperature control and opening or closing the microplate drawer.

The information contained in this section provides an overview of the instrument capabilities. For a complete description of the modes of operation, how to choose instrument settings, etc., refer to the SoftMax Pro User's Manual.

2.4.1. READ MODES

The Gemini EM and Gemini XPS can read in three modes: fluorescence, secondary luminescence, and secondary time resolved fluorescence.

2.4.2. READ TYPES

Within each read mode, Gemini readers can perform four types of read: endpoint, kinetic, spectrum, and well scan. Instrument setup parameters for each read type are discussed in the SoftMax Pro User's Manual.

Endpoint Read

In an Endpoint read, a reading of each microplate well is taken at a single or multiple wavelengths.

Depending on the read type selected, values can be reported as relative fluorescence units (RFU) or relative luminescence units (RLU).

Kinetic Read

In a Kinetic read the data are collected over time with multiple readings taken at regular intervals. To achieve the shortest possible interval for Kinetic readings, choose wavelengths in ascending order.

Kinetic analysis can be performed for up to 99 hours. The kinetic read interval depends upon the instrument setup parameters chosen in SoftMax Pro.

Kinetic analysis has many advantages when determining the relative activity of an enzyme in different types of microplate assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis is capable of providing improved dynamic range, precision, and sensitivity relative to endpoint analysis.

Spectrum Read

Spectral analysis measures fluorescence or luminescence across a spectrum of wavelengths. The Gemini EM reader allows excitation and emission wavelength scanning from 250 nm to 850 nm. The Gemini XPS reader allows excitation scanning from 250 nm to 850 nm and emission scanning from 360 nm to 850 nm.

When reading using fluorescence, you can set a fixed wavelength for excitation and scan the emission wavelengths, or vice versa. The default value reported for each well is the wavelength of maximum fluorescence.

When luminescence is chosen, only the emission wavelengths are scanned, and the default value reported for each well is the wavelength of maximum luminescence.

All spectrum readings are made using the scanning monochromators of the Gemini reader.

Well Scan Read

Some applications that involve the detection of whole cells in large-area tissue culture plates may require the use of well scanning mode. As many cell lines tend to grow in clumps or in the corner of microplate wells, this non-confluent growth pattern may require multiple reads in a well at different locations.

When used with 6-well, 12-well, 24-well, 48-well, or 96-well plates, well scanning allows maximum surface area detection for whole cell assays. No plate adapter is required when using large-area tissue culture plates.

For more information on well scanning, please review the appropriate section in the SoftMax Pro User's Manual.

2.4.3. TEMPERATURE REGULATION

The Gemini readers have been designed to regulate the temperature of the microplate chamber from 4°C above ambient to 45°C. Upon power up, when the incubator is off, the temperature in the Gemini microplate chamber is ambient and isothermal. Turning on the incubator by pressing the TEMP on/off key causes the Gemini to begin warming the microplate chamber. The temperature set point defaults to 37.0°C at start-up.

Accuracy of the temperature set point is guaranteed only if the set point is at least 4°C above ambient. If the temperature set point is lower than the ambient temperature, the chamber temperature remains at ambient. Temperature regulation is controlled by heaters only and, therefore, cannot cool the temperature to a setting lower than ambient. Additionally, the highest setting (45°C) can be achieved only if the ambient temperature is greater than 20°C.

Typically, the microplate chamber reaches 37.0°C in less than 30 minutes. The microplate chamber temperature is maintained at the set point until you press the incubator TEMP on/off key again, turning temperature regulation off.

Should you turn the incubator back on after a momentary shutdown, allow about ten minutes for the control algorithm to fully stabilize the microplate chamber temperature.

Temperature regulation and control of the microplate chamber is achieved through electric heaters, a fan, efficient insulation, and temperature sensors. The heaters are

located in the microplate chamber, which is insulated to maintain the temperature set point. The sensors are mounted inside the chamber and measure the air temperature.

The temperature feedback closed-loop control algorithms measure the chamber air temperature, compare it to the temperature set point, and use the difference to calculate the regulation of the heating cycles. This technique results in accurate, precise control of the chamber temperature with a temperature variation of the air inside the chamber of less than 1.0°C. The temperature uniformity within the microplate depends on its design and composition.

2.4.4. AUTOMIX

The Automix function permits automatic shaking of the microplate at preset intervals, thereby mixing of the contents within each well. Automix must be selected before beginning a reading. The actions associated with the Automix setting depend on the read mode chosen:

- Endpoint mode: Automix shakes the plate for a definable number of seconds and then reads at all selected wavelengths.
- Kinetic mode: two types of Automix can be enabled: Automix can shake the plate for a definable number of seconds before the initial reading, and/or for a definable number of seconds before each subsequent reading.
- Use of Automix is strongly recommended for ELISAs and other solid-phase, enzyme-mediated reactions to enhance accuracy.

2.4.5. COMPUTER CONTROL

The Gemini is equipped with an 8-pin DIN RS-232 serial port through which the computer communicates with the instrument. (Different types of cables are available for connecting to different types of computers—see Appendix A, *"Cables"* and *"Accessories"*.)

3. Installation

⚠**WARNING:** Always make sure the power switch on the instrument is in the OFF position and remove the power cord from the back of the instrument prior to any installation or relocation of the instrument.

⚠**WARNING:** Do not operate the instrument in an environment where potentially damaging liquids or gases are present.

⚠**CAUTION:** Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might cause misalignment and voids the instrument warranty.

3.1. UNPACKING

The Gemini is packed in a specially designed carton. Please retain the carton and the packing materials. If the unit should need to be returned for repair, you must use the original packing materials and carton for shipping. If the carton has been damaged in transit, it is particularly important that you retain it for inspection by the carrier in case there has also been damage to the instrument.

⚠**WARNING:** The Gemini weighs approximately 35 pounds (16 kg) and should be lifted with care. It is recommended that two persons lift the instrument together, taking the proper precautions to avoid injury.

After examining the carton, place it on a flat surface in the upright position. Open the top of the box and lift the Gemini, along with the packing materials around the ends, up and out of the shipping box. Remove the packing material from both ends of the instrument and set the instrument down carefully. The packing list that accompanies the instrument describes all components that should have been placed in the packing carton. Make sure all these items are present before proceeding.

3.2. SETTING UP THE INSTRUMENT

1. Place the Gemini on a level surface, away from direct sunlight, dust, drafts, vibration, and moisture.
2. Turn the instrument around so that the back of the instrument is facing you as shown in Figure 1.6.
3. Insert the female end of the power cord into the power receptacle at the rear of the Gemini. Connect the male end to a grounded power outlet of the appropriate voltage.

Molecular Devices recommends that you use a surge protector between the power cord and the grounded power outlet.

4 Insert the 8-pin DIN round end of the computer connection cord into the RS-232 serial port receptacle on the back panel of the instrument. Attach the other end to your computer (see Appendix A for more information).

5 Turn the Gemini around so that the control panel now faces you. Ensure no cables run beneath the instrument. Leave at least three inches between the back of the instrument and the nearest objects or surfaces to ensure proper ventilation and cooling.

3.3. INSTALLING THE DRAWER ADAPTER

⚠**CAUTION:** Incorrect insertion or removal of the adapter may cause damage to the microplate drawer of the Gemini. The corner cutout must be in the lower left corner where the plate pusher is located.

If you are reading standard 96-well or 384-well microplates from the top, you need to install the drawer adapter.

1 Power on the instrument using the switch on the back panel.

2 Press the DRAWER button on the front panel or activate the drawer open command in SoftMax Pro software.

3 Hold the adapter so that the label is on the front side facing up.

4 Place the top back (Row A) portion of the adapter into the drawer first. The corner cutout must be in the lower left corner where the plate pusher is located. While pushing against the back edge of the adapter, lower the front of the adapter into the drawer.

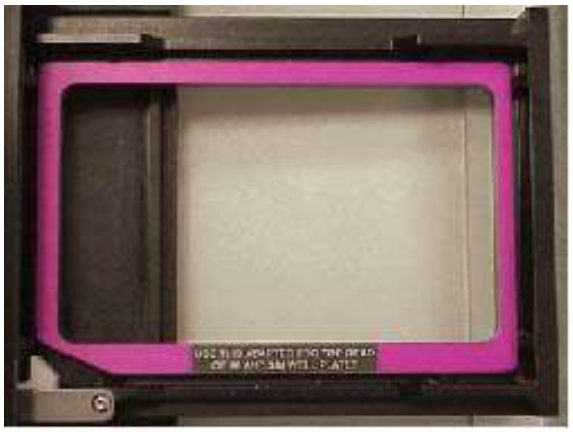

Figure 3.1: Adapter inserted in microplate drawer.

3.4. REMOVING THE DRAWER ADAPTER

If the adapter is in the drawer and you are either reading from the bottom(Gemini EM only) or using “high profile” (6-well, 12-well, 24-well, or 48-well) plates, you need to remove the adapter.

Incorrect insertion or removal of the adapter may cause damage to the microplate drawer of the Gemini.

1. Power on the instrument using the switch on the back panel.
2. Press the DRAWER button on the front panel or activate the drawer open command in SoftMax Pro software.
3. Remove the adapter plate.

Figure 3.2: Microplate drawer without adapter.

4. Operation

This chapter contains operating information for the Gemini Dual-Scanning Microplate Spectrofluorometer.

4.1. QUICK OVERVIEW

If you are an experienced user of this instrument, the following steps provide a quick reminder of the basic operating procedures required to perform an assay using the Gemini:

1. Turn on the power switch of the Gemini (located on the back panel). The microplate drawer opens automatically.
2. If you want to regulate the temperature inside the microplate chamber, touch the TEMP on/off (incubator) key to turn the incubator on and bring the microplate chamber to the default temperature of 37.0°C. The microplate drawer closes.
3. If the incubator is on, the LCD shows the current temperature along with the temperature set point. To change the set point (to any setting from ambient +4° to 45°C), press the up or down arrow keys.
4. Select the desired instrument settings (read mode, type of analysis, template, etc.) using SoftMax Pro software on the external computer.
5. If you are performing kinetic analysis, add substrate at this time.
6. Load the prepared microplate into the drawer, being sure to match well A1 with the A1 mark on upper left-hand corner of the drawer.
7. Using SoftMax Pro, start the reading.

4.2. PREPARING FOR A READING

4.2.1. TURN THE INSTRUMENT AND COMPUTER ON

The power switch for the Gemini is located on the back panel. Press the rocker switch to the ON position.

The instrument automatically performs diagnostic checks to ensure that it is functioning correctly. Turn the computer on at this time also and start the SoftMax Pro software program.

4.2.2. SET THE TEMPERATURE (OPTIONAL)

To set the temperature within the microplate chamber, you should turn on the incubator first, allowing enough time for the temperature to reach the set point before performing a reading. When you first turn the instrument on, up to 60 minutes may be required for the temperature within the chamber to reach the set point. Turning on the incubator and choosing a temperature set point can be done using the software or the front panel of the instrument (described here).

Temperature cannot be regulated at a set point that is lower than 4°C above the ambient temperature.

To enable the incubator:

1 Press the incubator [TEMP on/off] key.

2 The LCD display indicates that temperature control is on and shows the set point and current temperature of the microplate chamber.

To change the temperature set point:

1 Press the up or down arrow keys until the desired temperature set point is shown in the display.

The microplate chamber temperature is maintained at the set point until you disable temperature control by touching the incubator key again. When the incubator is off, the temperature within the microplate chamber gradually returns to ambient.

Should you turn the incubator back on after a momentary shutdown, allow about ten minutes for the control algorithm to fully stabilize the microplate chamber temperature.

4.3. READ THE MICROPLATE

⚠**BIOHAZARD:** The underside of the microplate must be dry prior to placing it in the drawer. If the microplate has fluid on the underside, dry it using a paper towel (or equivalent) before placing it in the drawer.

1 Insert the filled microplate into the drawer, matching well A1 with position A1 in the drawer. Make sure the microplate is flat against the drawer bottom (for 6-, 12-, 24-, or 48-well microplates) or against the adapter (if using top read for 96- or 386-well plates—see *"Installing the Drawer Adapter"* for more information).

2 You must have SoftMax Pro software running on a computer connected to the Gemini. Press the [READ] button in SoftMax Pro to start the plate read.

3 When reading is complete, the drawer of the instrument opens, allowing you to remove the microplate. If the incubator is on, the drawer closes again after approximately 10 seconds.

4 If you return to the Gemini and find the drawer closed after a reading has finished, press the [DRAWER] key. When the drawer opens, you can remove the microplate.

4.4. OPTIMIZING ASSAYS

4.4.1. INTRODUCTION

The optimum instrument settings for detection of a particular fluorophore depend on a number of different factors. Settings that can be adjusted for assay optimization include the excitation and emission wavelengths, emission cutoff filter, readings per well, the PMT voltage, the temperature of the reading chamber, and the length of delay time for time-resolved fluorescence.

Another important factor that is independent of the instrument but which affect assays optimization is the Stokes shift. When the Stokes' shift is very small, optimizing the excitation and emission wavelengths and correct cutoff filter choices are very important.

Excitation and Emission Wavelengths

The excitation and emission wavelengths may be set in 1-nm increments within the range of the instrument. The Gemini EM reader allows excitation and emission wavelength scanning from 250 nm to 850 nm. The Gemini XPS reader allows excitation scanning from 250 nm to 850 nm and emission scanning from 360 nm to 850 nm. A procedure to optimize excitation and emission wavelengths for a given assay is outlined in the next section.

Emission Cutoff Filter

The 15 emission cutoff filters assist in reducing background. Sources of background include stray excitation light and native fluorescence of plate materials, sample constituents, and solvents (including water). The default setting allows the instrument and SoftMax Pro software to determine which cutoff filter should be used (see Table 4.1for default settings) in endpoint and kinetic modes. The spectral scan mode default uses no cutoff filter.

Readings per well

The number of readings per well may vary between 1 (used for a quick estimate) and 30 (for very precise measurements). The default number of readings per well varies with the read mode: for fluorescence, the default is 6, and for luminescence, the default is 30.

PMT Voltage

The voltage of the photomultiplier tube may be set to low (for higher concentration samples), medium, or high (for lower concentration samples) in all read modes. In endpoint and spectrum mode, there is an additional setting, automatic, in which the instrument automatically adjusts the PMT voltage for varying concentrations of sample in the plate.

Temperature control

The chamber of the Gemini is isothermal at ambient as well as at elevated temperatures. The temperature in the reading chamber may be adjusted from 4°C above ambient to 45°C.

Delay Time

In time-resolved fluorescence mode, you may set the integration start and end time in 200-µsecond increments from the minimum 50 µs to the maximum 1450 µs.

4.4.2. USING SPECTRAL SCANNING TO OPTIMIZE EXCITATION AND EMISSION WAVELENGTHS FOR FLUORESCENCE ASSAYS

Put 200 µL of sample that includes the fluorophore and 200 µL of a buffer control into separate wells of a microplate.

1 Excitation Scan

a Using SoftMax Pro, set up a Plate section for a fluorescence read, spectrum mode, Em Fixed/Ex Scan, with no cutoff filter (default), and medium PMT.

b Set the emission wavelength based on the tentative value from the literature (or from a customary filter set used to measure your fluorophore). If the emission wavelength is not known, select a tentative emission wavelength about 50 nanometers greater than the absorbance maximum of the fluorophore. If necessary, the absorbance maximum can be determined by performing a spectral scan in a UV/Vis spectrophotometer.

c Set the excitation scan to start/stop approximately 50 nm below/above the tentative excitation value obtained from the literature (or the customary excitation filter).

d Set the step increment to 1 or 2 nm. (You may choose to do a preliminary scan with a 10-nm increment to determine the approximate peak location, and then repeat the scan over a narrower wavelength range with a 1- or 2-nm increment.)

e Perform the scan and view the results as a plot of emission fluorescence vs. excitation wavelength. Note the excitation wavelength at the emission peak and the maximum RFU value.

If an error message reporting missing data points occurs, it may be due to possible saturation reported by SoftMax Pro at the end of the spectral scan. Reset the PMT to "low" and rescan the sample (scan the buffer blank with the PMT set to "medium" or "high"). If the error occurs after scanning with the PMT set to "low," it may be necessary to dilute the sample.

If the excitation scan shows no apparent peak, change the PMT setting to "high" and rescan the sample. If the spectral scan still shows no apparent peak, adjust the Y-scale of the zoom plot so that the plot fills the graph.

f Select the optimal excitation wavelength. If the excitation peak wavelength and emission wavelength are separated by more than 80 nm, use the excitation peak wavelength value. If the excitation and emission wavelengths are less than 80 nm apart, use the shortest excitation wavelength that gives 90% maximal emission. (Follow the plot to the left of the peak until the RFU value falls to approximately 90% of the maximum, and then drop a line from the 90% point on the plot to the x-axis—see Figure 4.1)

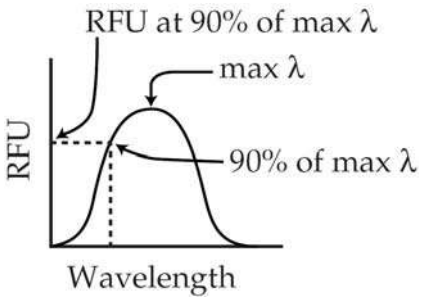

Figure 4.1: Figure 4.1: Plot of RFU vs. Wavelength.

2 Emission Scan #1

a In SoftMax Pro, set up a second plate section for a fluorescence read, spectrum mode, Ex Fixed/Em Scan, with no cutoff filter (default), and medium PMT.

b Set the excitation wavelength to the value determined in 2F above.

c Set the emission scan to start/stop approximately 50 nm below or above the tentative emission value obtained from the literature (or existing filter pair). Note: If the Stokes shift is less than 50 nm, then start the emission scan above the excitation wavelength.

d Set the step increment to 1-2 nm (or do a preliminary scan with a 10-nm increment to determine the approximate peak location and then repeat the scan over a narrower wavelength range using a 1-2 nm increment.)

e Perform the scan and view the results as a plot of fluorescence vs. emission wavelength.

3 Emission Filter

a Select an emission cutoff filter that blocks as much of the residual excitation light as possible without unduly reducing the fluorescence signal. The cutoff wavelength choices are 325(Gemini EM only), 420, 435, 475, 495, 515, 530, 550, 570, 590, 610, 630, 665, or 695 nm. The cutoff value should be near the maximum emission wavelength (preferably between the excitation wavelength and the maximal emission wavelength) but at least 35 nm greater than the excitation wavelength.

4 Emission Scan #2

a In SoftMax Pro, set up a third plate section for an emission scan as specified in Step 3 above, except selecting Manual Cutoff Filter and setting the wavelength to that determined in Step 4.

b Perform the scan and view the results as a plot of fluorescence vs. emission wavelength. Note the wavelength giving the maximum emission (the optimal emission wavelength).

c Compare the spectra of the sample containing the fluorophore to the spectra of the buffer blank to get an estimate of the signal-to-noise ratio. If there is significant background interference, repeat steps 5A and 5B with another choice of cutoff filter.

5 Results

The optimal excitation and emission wavelengths are those determined in steps 1f and 4b, above.

6 Comments

a In endpoint or kinetic fluorescence modes, the "Autofilter" feature generally selects the same cutoff filter wavelength as the above optimization method. If desired, however, you may specify the cutoff filters manually.

b For emission wavelengths less than 325 nanometers, experimental iteration is usually the best method of determining the optimal emission and excitation wavelengths. Begin optimization by performing steps 1–4 above. Try emission and excitation wavelength combinations with the 325 cutoff or with no cutoff filter. Similarly, for excitation wavelengths greater than 660 nanometers, try emission and excitation wavelength combinations with the 695 cutoff or with no cutoff filter.

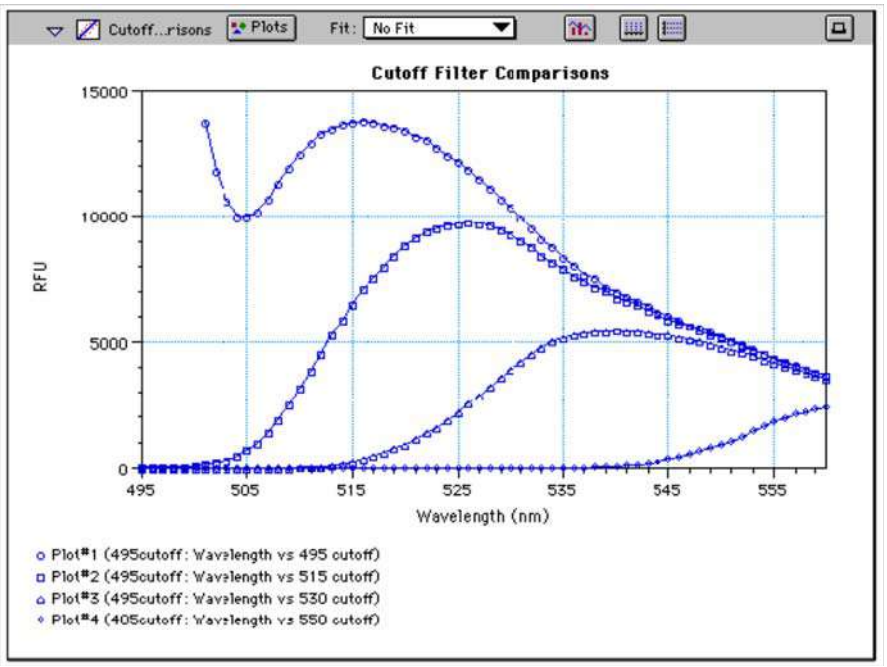

Figure 4.2: Effects of Cutoff Filters on Fluorescein. Emission was scanned from 490 to 560 nm; excitation was fixed at 485 nm.

Figure 4.2 shows the effects of different cutoff filters on a scan of fluorescein where excitation was fixed at 485 nm and emission was scanned from 490 nm to 560 nm (buffer

blanks are not shown in this plot). Table 4.1 following lists default settings for the emission cutoff filters.

Table 4.1: Gemini XPS Emission Cutoff Filter Default Setting.

Automatic Cutoff Selection		Endpoint and Kinetic Modes
#	Wavelength (nm)	Emission Wavelength (nm)
1	None	< 415
2	420	415–434
3	435	435–454
4	455	455–474
5	475	475–494
6	495	495–514
7	515	515–529
8	530	530–549
9	550	550–569
10	570	570–589
11	590	590–609
12	610	610–629
13	630	630–664
14	665	665–694
15	695	695–850

For spectrum mode, the default is "manual" (no automatic cutoff).

5. Maintenance

5.1. TECHNICAL SUPPORT

Molecular Devices Corporation is a leading worldwide manufacturer and distributor of analytical instrumentation. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service. In order to fully benefit from our technical services, please complete the registration card and return it to the address printed on the card.

If you have any problems using the Gemini EM or XPS Dual-Scanning Microplate Spectrophotometer, in the U.S., contact our Technical Services group at 1-800-635-5577; elsewhere contact your local representative.

⚠**WARNING:** All maintenance procedures described in this manual can be safely performed by qualified personnel. Maintenance not covered in this manual should be performed only by a Molecular Devices representative.

⚠**WARNING:** Turn the power switch off and disconnect the power cord from the main power source before performing any maintenance procedure that requires removal of any panel or cover or disassembly of any interior instrument component.

⚠**WARNING:** Removal of protective covers that are marked with the High Voltage warning symbol shown below can result in a safety hazard.

5.2. MOVING THE GEMINI

If you need to relocate the Gemini, follow these steps.

The Gemini weighs approximately 35 pounds (16 kilograms). To avoid injury, it is recommended that two people lift the instrument together, using proper lifting techniques.

1 Remove any microplate (and the adapter, if any) from the drawer and then close the drawer. Leaving the adapter in the drawer when moving the Gemini could cause damage to the instrument.

2 Turn off the power switch and unplug the power cord from the source and from the receptacle on the back of the instrument.

3 Depending on the distance that you are moving the instrument, you may want to repackage the Gemini in its original shipping carton. Otherwise, carry the instrument or place it on a rolling cart to transport it.

4 Ensure that the new location meets the proper specifications as described in Chapter 3, *"Setting Up the Instrument"*.

5.3. CLEANING

⚠**BIOHAZARD:** Wear gloves during any cleaning procedure that could involve contact with either hazardous or biohazardous materials or fluids.

⚠**WARNING:** Never clean the inside of the instrument.

Periodically, you should clean the outside surfaces of the Gemini using a cloth or sponge that has been dampened with water:

- Do not use abrasive cleaners.
- If required, clean the surfaces using a mild soap solution diluted with water or a glass cleaner and then wipe with a damp cloth or sponge to remove any residue.
- Do not spray cleaner directly onto the instrument.

If needed, clean the microplate drawer using a cloth or sponge that has been dampened with water.

Should fluids spill in the drawer area (when the drawer is out), they are directed to a tray at the bottom of the instrument, from which they exit to the bench or counter beneath the instrument. Wipe up any spills immediately.

Do not allow excess water or other fluids to drip inside the instrument.

5.4. CLEANING THE FAN FILTER

The fan filter on the bottom of the instrument requires periodic cleaning. The frequency of cleaning depends on how dusty your particular lab is and could range from once a month to once every six months.

1 Turn power to the instrument OFF and then remove the power cord and cables from the back of the instrument.

2 Remove any plate or adapter from the instrument drawer. Turn the instrument over so that it rests flat on the bench.

3 Pop the black fan cover off and remove the filter.

4 Clean the filter by blowing clean, canned air through it or by rinsing it—first with water and then with alcohol—and allowing it to dry completely.

5 Place the clean, dry filter over the fan and replace the black cover.

6 Turn the instrument back over. Reconnect the power cord and cables to the instrument.

5.5. CHANGING THE FUSES

Fuses burn out occasionally and must be replaced.

If the instrument does not seem to be getting power after switching it on (the LCD shows no display):

1 Check to see whether the power cord is securely plugged in to a functioning power outlet and to the receptacle at the rear of the Gemini.

If power failed while the Gemini was already on:

1 Check that the power cord is not loose or disconnected and that power to the power outlet is functioning properly.

If these checks fail to remedy the loss of power, follow the steps listed below to replace the fuses. Spare fuses (two U.S. and two metric) are shipped with the instrument. The U.S. and metric fuses are identical except for physical size. They may be taped to the back of the Gemini.

If you no longer have spare fuses, you may obtain new ones from Molecular Devices (part numbers: 4601-0013 for U.S., 4601-0014 for metric) or from a local hardware store. Make sure fuses are rated SLOWBLOW (U.S.: 4-amp time-delay; metric: 4-amp, 5 × 20 mm, time-delay).

To change fuses:

1 Switch power to the instrument off and then remove the power cord from the outlet and from the Gemini power cord receptacle.

2 Remove the computer cable from the back of the Gemini.

3 Turn the instrument around for easy access to the rear panel.

4 On the left-hand side of the rear panel (viewed from the back) is the power switch, fuse box, and power cord receptacle. As shown in the figures below, press to the left of the black plastic cover of the fuse box to release it. Pull the fuse box cover away from the instrument. The fuse box will begin to slide forward.

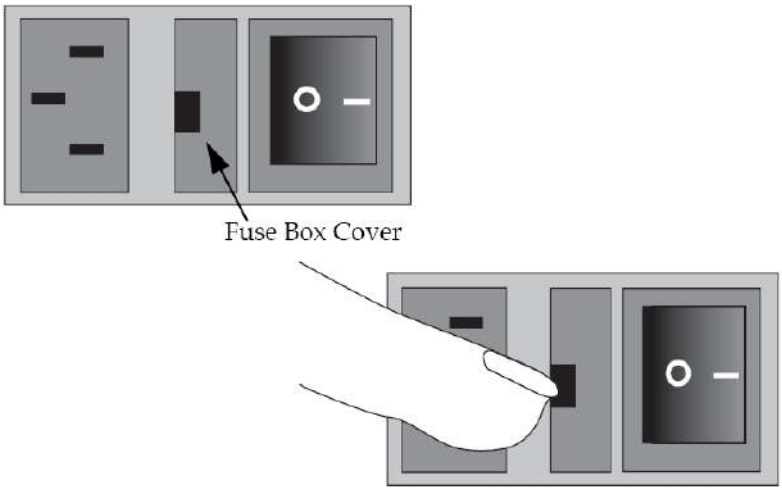

Figure 5.1: Power switch, fuse box, and power receptacle.

5 Continue gently pulling the fuse box forward until it is free of the instrument.

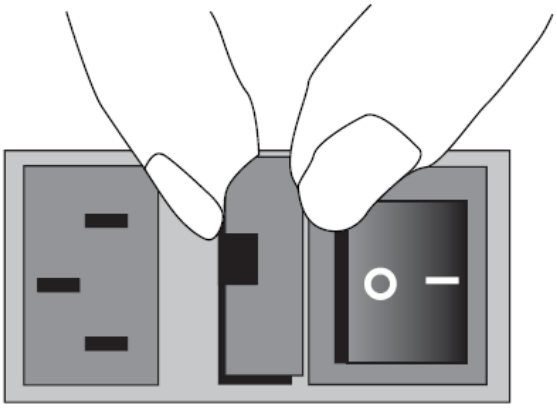

Figure 5.2: Removing the fuse box.

6 When removed, the fuse assembly will appear as shown in Figure 5.3. The holder inside contains two fuses.

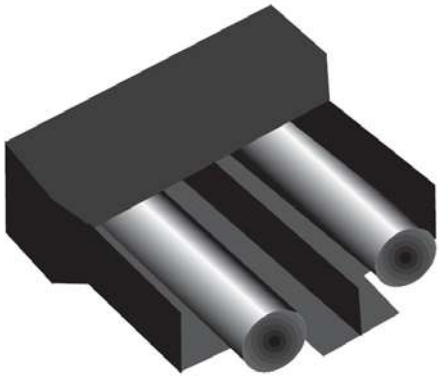

Figure 5.3: The fuse box and holder (with fuses) removed from instrument.

7 It is possible that only one of the fuses may have blown. However, Molecular Devices recommends that you replace both fuses to ensure continued proper operation. Pull both fuses out of the holder and discard them.

8 Insert new SLOWBLOW-rated fuses into the fuse holder. Either end of the fuse may be forward.

9 Insert the fuse box into the opening in the instrument, making sure that the fuses are on the left side (toward the power receptacle). Press the fuse box into place, making sure the cover snaps closed.

10 Reconnect the power cord to the instrument and to the wall outlet and reconnect other cables previously disconnected.

5. Maintenance

6. Troubleshooting

This chapter lists error codes that may occur while using the instrument, followed by their most likely causes and remedies.

Maintenance procedures are described in the previous chapter.

For problems with the Gemini EM or Gemini XPS that are not listed here, in the U.S., contact Molecular Devices Technical Services group at 1-800-635-5577; elsewhere, call your local representative.

⚠**BIOHAZARD:** It is your responsibility to decontaminate the instrument, as well as any accessories, before requesting service by Molecular Devices representatives and before returning the instrument or any components to Molecular Devices Corporation.

6.1. OPENING THE DRAWER MANUALLY

- If an error occurs while the drawer is closed and you need to remove a microplate, press the DRAWER key.
- If the drawer does not open, turn power to the instrument off and then on again. If the drawer still remains closed, turn the power off and using your thumbnail, locate the groove in the upper left side wall of the door. Open the door, and with your index finger, pull the microplate drawer out of the instrument (do not force the drawer) and remove the microplate. This action will not harm the instrument, but should only be taken if the first two options have failed to open the drawer.

If you are still unable to open the drawer, contact your local Molecular Devices representative.

6.2. ERROR CODES AND PROBABLE CAUSES

If a problem occurs during operation that causes an unrecoverable error, the instrument will stop and an error code number will be shown in the display on the front panel. To correct the problem, call your local Molecular Devices representative for assistance.

6.2.1. ERROR MESSAGES

The LCD displays Fatal Error codes when a situation arises that requires attention. Any reading in progress will stop.

Warning messages do not stop a reading but are logged in the error buffer. Warning messages indicate a situation that requires attention but is not sufficient to stop or prevent

a reading. Examples of situations that might cause warning messages are low memory, entries being out of range, or operations that could result in loss of data. These messages are generally self-explanatory.

For assistance regarding warning messages, contact your local Molecular Devices representative.

6.2.2. ERROR CODE CLASSIFICATIONS

Not all error messages are listed in this user guide. The errors are grouped in relationship to possible causes as follows:

Table 6.1: Gemini EM and Gemini XPS error code ranges.

ERROR CODE NUMBERS	POSSIBLE CAUSES
100–199	Errors possibly caused by unrecognized commands being sent from the computer to the instrument.
200–299	Errors probably due to a main board failure or an error in the firmware code. Most of these errors require the assistance of Technical Support.
300–399	Instrument errors due to either a main board failure or other system failure. Most of these errors require the assistance of Technical Support.
400–499	Errors caused by a motor motion failure. Most of these errors require the assistance of Technical Support.
500–599	Errors due to failure or improper initialization of the instruments non-volatile memory (NVRAM). All of these errors require the assistance of Technical Support.

Some errors (shown in **boldface** in the following table) are considered fatal in that if they are detected during power up, the instrument aborts the power up sequence and displays "FATAL ERROR" on the LCD panel.

Check the following list to see if there is something that you can do to change the condition of the instrument to prevent the fatal error.

After correcting the problem, leave the instrument on for about five minutes, turn it off and then back on.

If you continue to get the fatal error message on power up, record the error message number and contact Molecular Devices Technical Support or your local representative for assistance.

If the instrument is functioning normally when using SoftMax Pro, no errors should be in the buffer (except error number 100).

Table 6.2: Error codes, error messages, and notes about the errors.

ERROR CODE	ERROR MESSAGE	NOTES
100–199: UNRECOGNIZED COMMAND ERRORS SENT FROM THE COMPUTER		
100	command not found	Command string not recognized.
101	invalid argument	Command argument not recognized.
102	too many arguments	Too many arguments after command.
103	not enough arguments	Missing arguments.
104	input line too long	Too many characters in the input line.
105	command invalid, system busy	Instrument could not perform the give command because it was busy doing another task.
106	command invalid, measurement in progress	Instrument could not perform command because a measurement was in progress.
107	no data to transfer	Inputting transfer when there's no data in the buffer.
108	data buffer full	Too many data sets in the buffer. Can be caused by setting up a long kinetic and disconnecting computer or SoftMax Pro is preempted by another application.
109	error buffer overflow	More than 65 errors in the buffer, clear the buffer.
110	stray light cuvette, door open?	Cuvette door open while doing a read.
111	invalid read settings	
200–299: FIRMWARE ERRORS		
200	assert failed	Firmware error.
201	bad error number	Firmware error.
202	receive queue overflow	Caused by external device sending too much data over serial port and ignoring flow control.

ERROR CODE	ERROR MESSAGE	NOTES
203	serial port parity error	Parity bit error detected with incoming serial data.
204	serial port overrun error	Caused by host computer sending too much data and ignoring the flow control signal.
205	serial port framing error	
206	cmd generated too much output	Firmware error.
207	fatal trap	Instrument error. Instrument locks up.
208	RTOS error	Firmware error.
209	stack overflow	Firmware error.
210	unknown interrupt	Firmware error.
300–399: HARDWARE ERRORS		
300	thermistor faulty	Unable to read a reasonable thermistor value. Thermistor faulty or disconnected, Main board problem, or ambient temperature out of range.
301	safe temperature limit exceeded	A temperature of over 50°C detected on one or more of the 4 thermistors. Temperature will be shut off and remain off until a successful completion of power-up reset.
302	low light	Not enough light detected to make an accurate measurement. If doing a cuvette read, the cuvette door may be open.
303	**unable to cal dark current**	**Too much stray light detected on power-up, faulty or disconnected pre-amp boards.**
304	signal level saturation	During a cuvette read, could be due to cuvette door being open.
305	reference level saturation	During a cuvette read, could be due to cuvette door being open.

ERROR CODE	ERROR MESSAGE	NOTES
306	plate air cal fail, low light	Minimum signal/reference ratio not met during air calibration.
307	cuv air ref fail	
308	stray light	Light leak in reading chamber or cuvette door open. Could also be a faulty pre-amp board.
309	front panel not responding	LCD front panel bad or disconnected.
312	**gain calibration failed**	**Power-up calibration and check of signal path gain is out of tolerance. Could be due to bad or disconnected pre-amp or excessive stray light.**
313	**reference gain check fail**	**Power-up check of the Reference amplifier's gain out of tolerance. Could be due to bad or disconnected pre-amp board or excessive stray light.**
314	**low lamp level warning**	
315	**can't find zero order**	**On power-up, grating motor could not find zero-order home position.**
316	**grating motor driver faulty**	**Grating motor didn't move to where it was commanded to in a reasonable time.**
317	**monitor ADC faulty**	
400–499: MOTION ERRORS		
400	**carriage motion error**	**Carriage did not move to either of its photo interrupts in a reasonable time, or can't find its photo interrupt.**
401	**filter wheel error**	**Filter wheel did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.**
402	**grating error**	**Grating did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.**

ERROR CODE	ERROR MESSAGE	NOTES
403	**stage error**	**Stage did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.**
500–599: NVRAM ERRORS		
500	NVRAM CRC corrupt	The CRC for the NVRAM data is corrupt.
501	NVRAM Grating cal data bad	Grating calibration data is unreasonable.
502	NVRAM Cuvette air cal data error	Cuvette air calibration data is unreasonable.
503	NVRAM Plate air cal data error	Plate air calibration data is unreasonable.
504	NVRAM Carriage offset error	Carriage offset data is unreasonable.
505	NVRAM Stage offset error	Stage offset data is unreasonable.
506	NVRAM Battery	Time to replace the NVRAM battery (U3).

For all other error messages (codes not listed here), please contact your local Molecular Devices representative for assistance.

7. Specifications

7.1. GEMINI EM SPECIFICATIONS

Technical specifications are subject to change without notice.

FLUORESCENCE PHOTOMETRIC PERFORMANCE	
Wavelength range (Excitation/Emission)	250–850 nm
Wavelength selection	Scanning monochromator tunable in 1-nm increments
Excitation wavelength bandwidth	9 nm
Emission wavelength bandwidth	9 nm
Wavelength accuracy	< ± 2.0 nm
Calibration	Self-calibrating with built-in fluorescence calibrators
Sensitivity (signal 3X STD DEV of baseline)	8.0 fmol/well FITC (bottom read) 3.0 fmol/well FITC (top read)
LUMINESCENCE PHOTOMETRIC PERFORMANCE	
Wavelength range	250–850 nm
Sensitivity (signal 3X STD DEV of baseline)	10 amol/well Alkaline Phos. (obtained with Emerald II reagent from Tropix, an Applera company)
GENERAL PHOTOMETRIC PERFORMANCE	
Microplate formats	6, 12, 24, 48, 96, 384
Light source	Xenon flash lamp (1 joule/flash)
Average lamp lifetime	2 years normal operation (estimate)
Detector	Photomultiplier (R-3896)

Read time	96 wells in < 15 seconds (measurement type may extend read time)
Shaker Time	0–999 seconds
Temperature control (chamber)	Ambient +4°C to 45°C
Ramp up to 37°C	< 20 minutes
ENVIRONMENTAL	
Robot ready	Yes
Turn-on time	< 5 min. to rated accuracy
Operating conditions	15°C to 40°C
Operating humidity	0 to 80% RH non-condensing
Storage temperature	–20°C to 65°C
Operational altitude	< 2000 m
Installation category	II
Pollution degree	2
SYSTEM VALIDATION	Internal standards for fluorescence and wavelength
SOFTWARE	Windows 95/98/NT/2000/XP compliant Macintosh 8.6–9.x; OS X
PHYSICAL	
Size (h × w × d)	13.5" (340 mm) × 16.5" (420 mm) × 16.5" (420 mm)
Weight	35 lb (16 kg)
Power consumption	500 VA maximum
Line voltage and frequency	90–240 VAC, 50/60 Hz

7.2. GEMINI XPS SPECIFICATIONS

Technical specifications are subject to change without notice.

FLUORESCENCE PHOTOMETRIC PERFORMANCE	
Wavelength range—Excitation **Wavelength range—Emission**	250–850 nm 360–850 nm
Wavelength selection	Scanning monochromator tunable in 1-nm increments
Wavelength bandwidth	9 nm
Wavelength accuracy	< ± 2.0 nm
Calibration	Self-calibrating with built-in fluorescence calibrators
Sensitivity (signal 3X STD DEV of baseline)	3.0 fmol/well FITC
TIME-RESOLVED FLUORESCENCE PHOTOMETRIC PERFORMANCE	
Data collection	50–1450 μs
Integration start/end	User-selectable in 200 μs intervals
Sensitivity (signal 3X STD DEV of baseline)	0.5 fmol/well Eu-chelate (obtained with DELFIA reagent from Perkin Elmer using 384-well plate)
LUMINESCENCE PHOTOMETRIC PERFORMANCE	
Wavelength range	360–850 nm
Sensitivity (signal 3X STD DEV of baseline)	10 amol/well Alkaline Phos. (obtained with Emerald II reagent from Tropix, an Applera company)
GENERAL PHOTOMETRIC PERFORMANCE	
Microplate formats	6, 12, 24, 48, 96, 384
Light source	Xenon flash lamp (1 joule/flash)
Average lamp lifetime	2 years normal operation (estimate)
Detector	Photomultiplier (R-3896)

Read time	96 wells in 15 seconds; 384 wells in 45 seconds (measurement type may extend read time)
Dynamic range	6 decades in 96-well black plates; auto PMT circuitry
Shaker Time	0–999 seconds
Temperature control (chamber)	Ambient +4°C to 45°C
Sample evaporation control	90% RH compartment
Air temperature uniformity (across microplate)	< 1°C when temperature set point is 37°C
Ramp up to 37°C	< 60 minutes
ENVIRONMENTAL	
Robot ready	Yes
Turn-on time	< 5 min. to rated accuracy
Operating conditions	15°C to 40°C
Operating humidity	0 to 90% RH non-condensing
Storage temperature	–20°C to 65°C
SYSTEM VALIDATION	Internal standards for fluorescence and wavelength
SOFTWARE	Windows 95/98/NT/2000/XP compliant Macintosh 8.6–9.x; OS X
PHYSICAL	
Size (h × w × d)	13.5" (340 mm) × 16.5" (420 mm) × 16.5" (420 mm)
Weight	35 lb (16 kg)
Power consumption	500 VA maximum
Line voltage and frequency	90–240 VAC, 50/60 Hz

A. Appendix

A.1. CABLES

Molecular Devices recommends that you use high-quality, double-shielded cables to connect the Gemini reader to the computer. Choose cables that meet the following requirements:

A.1.1. SERIAL INTERFACE CABLE

Contact Molecular Devices for specific pin-out requirements:

- Macintosh: Male DB8 to M ale DB8
- IBM compatible: Male DB8 to Female DB9

A.1.2. USB ADAPTER

iMac, G4 and G5 Macintosh computers, and many newer Windows computers do not have a serial port. You can connect a serial cable between these computers and the instrument using a USB-to-serial adapter.

Molecular Devices has tested many third-party serial-to-USB adapter cables and has found the Keyspan USA-19HS (Molecular Devices, PN 9000-0938) to be the most reliable. It is the only one we recommend.

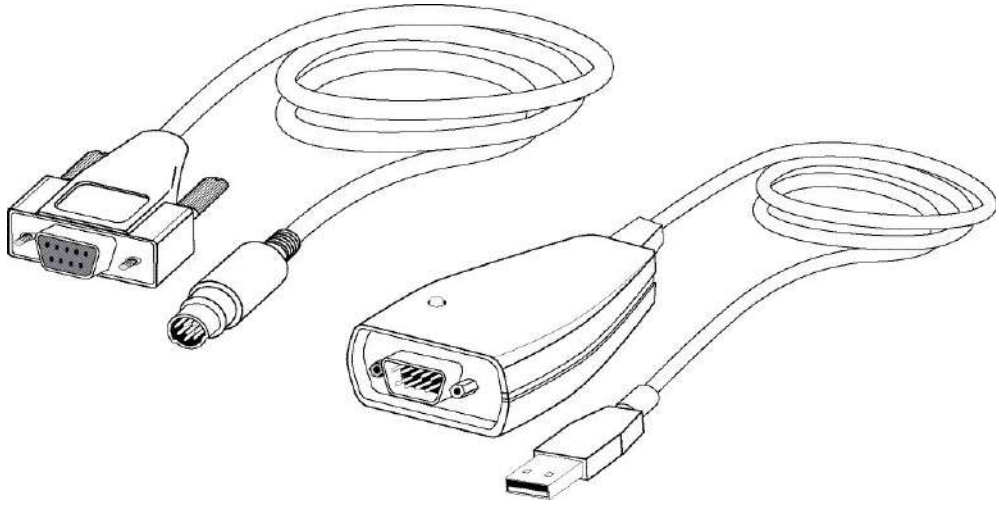

Figure A.1: Molecular Devices' custom serial cable (left) and a serial-to-USB converter (right).

A.2. ACCESSORIES

Description	Part #
SpectraTest FL1 Fluorescence Validation Test Plate	0200-5060
Fuse, 4-amp Time Delay	4601-0013
Fuse, 4-amp (5 x 20 mm) Time Delay	4601-0014
Power Cord (US, Canada, Japan, Mexico, India)	4400-0002
Power Cord, EC1 (Germany, France, Scandinavia, Italy, Korea)	4400-0036
Power Cord, EC2 (UK, Indonesia, Singapore, Malaysia)	4400-0037
Power Cord, AP1 (Australia, Hong Kong, China)	4400-0038
SpectraMax Mouse Pad	9000-0133
Cable, RS-232, 8-pin DIN to 8-pin DIN (instrument to pre-G3 Macintosh)	9000-0091
Cable, RS-232, 9-pin DIN to 8-pin DIN (instrument to PC serial port)	9000-0149
Adapter USB-Serial High-Speed (KeySpan adapter; instrument to USB-only instrument)	9000-0938

B. Appendix

B.1. COMMON WAVELENGTHS FOR FLUORESCENCE AND LUMINESCENCE

Values in this table are based on the literature. You may want to scan your fluorochrome of interest in the Gemini EM or Gemini XPS to determine the optimal excitation and emission wavelengths for your application.

B.1.1. FLUORESCENCE

Fluorophore	Excitation Wavelength (nm)	Emission Wavelength (nm)
HPPA	320	405
4-MeU, NADH, NADPH	355	460
Biotinidinase	355	544
PKU	390	485
Green Fluorescent Protein	390	510
Attophos /Attofluor	444	555
FITC	485	538
Ethidium Homodmer (DNA)	530	620
TRITC, Ethidium Bromide	544	590
Texas Red	584	612
TAMRA	547	580
Tryptophan	280	340
La Jolla Blue	695	705

B.1.2. TIME-RESOLVED FLUORESCENCE

Fluorophore	Excitation Wavelength (nm)	Emission Wavelength (nm)
Eu-Chelate	360	610

B.1.3. LUMINESCENCE

Probe	Wavelength (nm)
Emerald and Emerald II[a]	542
Sapphire and Sapphire II[a]	461
Ruby[a]	620

a.Emerald, Emerald II, Sapphire, Sapphire II, and Ruby are trademarks of Tropix, Inc.

B.2. GLOSSARY

Automix

The Automix function determines how often, if at all, automated shaking of the microplate is performed during a reading. This feature is covered by U.S. Patent Number 5,112,134.

Emission Cutoff Filter

A long pass filter used to condition the emission light prior to detection by the PMT. In automatic mode, the instrument sets the cutoffs automatically based upon the wavelength(s) chosen for reading; in manual mode, you can choose the filter wavelength manually.

Endpoint

A single reading made at one or more excitation/emission wavelengths.

Emission Spectral Scan

Measures fluorescence or luminescence across a spectrum of wavelengths for emitted light at a fixed excitation wavelength (or no excitation in the case of luminescence). The default value reported for each well is the wavelength of maximum fluorescence or luminescence.

Excitation Filter

Band pass filter that reduces the amount of extraneous lamp excitation light prior to the excitation monochromator. In endpoint reads and emission spectral scans, selection of excitation filter is automatic. In excitation spectral scans, the user has the choice of “no

excitation filter" (for smoother scans) or "auto excitation filter" in which case there may be slight glitches in the spectrum at the wavelengths where filter changes occur.

Excitation Spectral Scan

Measures fluorescence at a single emission wavelength across a spectrum of excitation wavelengths. The default value reported for each well is the excitation wavelength of maximum fluorescence.

Fluorescence

The light emitted by certain substances resulting from the absorption of incident radiation. To measure fluorescence accurately, it is necessary to reduce light scatter. The governing equation for fluorescence is:

> Fluorescence = extinction coefficient * concentration * quantum yield * excitation intensity * pathlength * emission collection efficiency

Fluorophore

A material that absorbs light energy of a characteristic wavelength, undergoes an electronic state change, and emits light of a longer wavelength.

Gain

The amount of increase in signal power expressed as the ratio of output to input.

Incubator

(In SoftMax Pro software) Choosing Incubator from the Control menu or clicking the incubator button opens a dialog box allowing you to start or stop temperature regulation and to select an elevated temperature for the microplate chamber.

Instrument Setup

Defines the parameters (mode, wavelengths, automatic mixing, run time, read interval, etc.) used to read the microplate.

Kinetic

During kinetic readings, data is collected over time, with multiple readings made at regular intervals. The values calculated based on raw kinetic data are Vmax, Time to Vmax, and Onset Time. Kinetic readings can be single- or multiple-wavelength readings.

LCD (Liquid Crystal Display)

The 2-×-20-character display which shows the current instrument settings.

Luminescence

The emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation.

Photomultiplier Tube (PMT)

A vacuum tube that detects light especially from dim sources through the use of photoemission and successive instances of secondary emission to produce enough electrons to generate a useful current.

Read Mode

The type of reading performed: fluorescence or luminescence.

Read Type

The method used to read the microplate: endpoint, kinetic, spectrum, or well scan.

Readings per Well

The number of times (user-definable) that readings are taken on a well in fluorescence mode or the amount of time that data is collected using the luminescence read type.

SoftMax Pro

An integrated software program from Molecular Devices Corporation that is used to control and collect data from the Gemini instrument.

Stokes Shift

The difference between the wavelengths of the excitation and emission peaks.

Time-Resolved Fluorescence

Most fluorescence substances are not suitable for this type of reading. However, the fluorescence emitted by lanthanide dyes is delayed long enough to measure fluorescence after the lamp is turned off. Time-resolved fluorescence is used to reduce the amount of background noise that interferes with fluorescence. The excitation lamp flashes and, after it is off, the delayed emission is collected for a set period of time before the lamp is flashed again.

B.3. SYSTEM DIAGRAMS AND DIMENSIONS

Dimensions are shown in inches (millimeters).

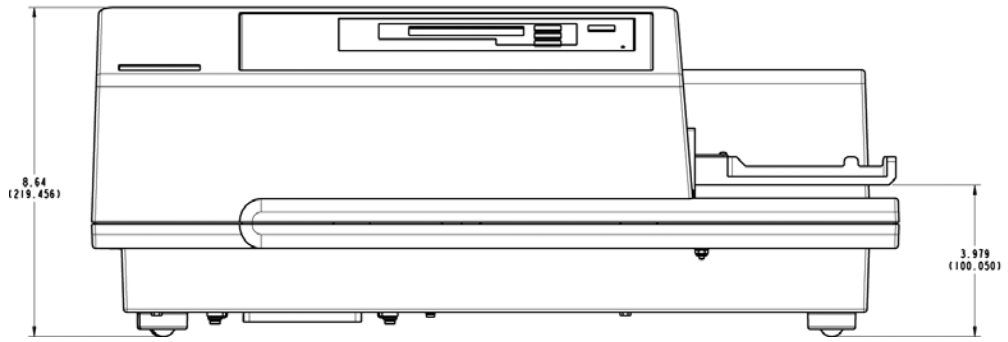

Figure B.1: Front view of Gemini EM/XPS.

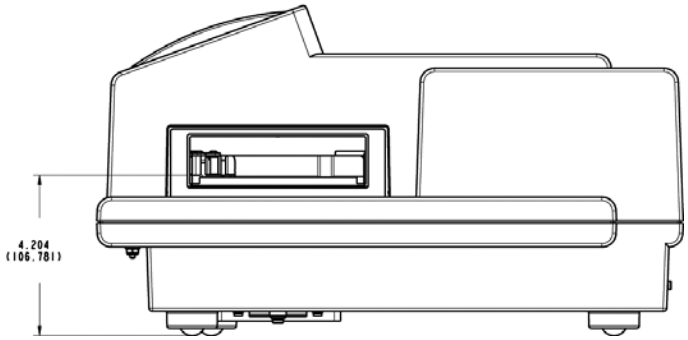

Figure B.2: Side view of Gemini EM/XPS.

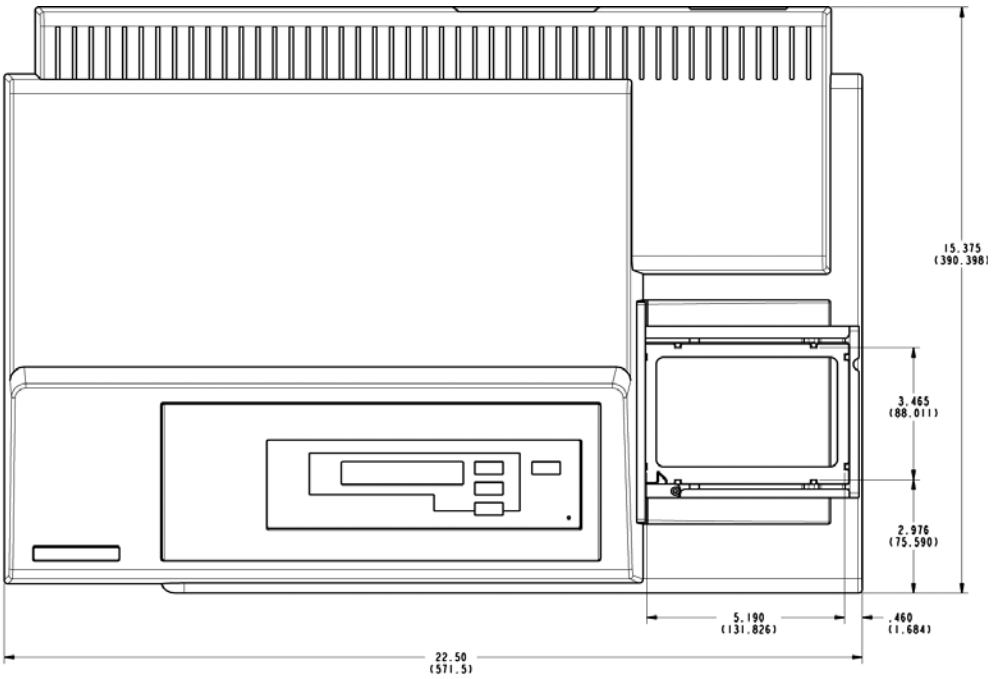

Figure B.3: Top view of Gemini EM/XPS.