SpectraMax® M3, M4, M5, and M5e Multi-Mode Microplate Readers

User Guide

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Molecular Devices

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Contents

1 Description

Introduction

The SpectraMax® M3, M4, M5, and M5e Microplate Readers are a series of dual-monochromator, multidetection, multi-mode instruments with a triple-mode cuvette port and 6-well to 384-well microplate reading capability. Detection modalities are shown in Table 1-1.

Table 1-1 SpectraMax® Multi-Mode Microplate Readers and Applicable Modes

Modes	SpectraMax M3	SpectraMax M4	SpectraMax M5	SpectraMax M5e
Absorbance	X	X	X	X
Fluorescence intensity	X	X	X	X
Fluorescence polarization			X	X
Time-resolved fluorescence		X	X	X
Luminescence	x	x	X	X

Note: In this user guide, all references to SpectraMax Multi-Mode Microplate Readers include the M3, M4, M5, and M5e models. When a feature or capability applies to only certain readers, this exception is noted.

The optical performance is comparable to a top-of-the-line dedicated spectrophotometer or spectrofluorometer with no trade-off between instrument performance and the number of read modes.

The built-in cuvette port can be used for absorbance, fluorescence and luminescence readings. Dual monochromators allow selection of any absorbance wavelength between 200 nm and 1000 nm, and any excitation wavelength between 250 nm and 850 nm for readings in fluorescence intensity, time-resolved fluorescence (M4, M5, M5e models only) or wavelength-selectable luminescence modes, and 400–750 nm for readings in fluorescence polarization mode (M5, M5e models only).

Assays requiring a read in two or more modes can be combined and run on the SpectraMax Multi-Mode Microplate Readers by issuing a single command in SoftMax® Pro Software, Molecular Devices' leading microplate data acquisition and analysis software platform.

Applications

Endpoint, kinetic, spectrum, and multi-point well-scanning applications combining absorbance and fluorescence in 6-well to 384-well microplates, as well as endpoint, kinetic, and spectrum applications in absorbance and fluorescence using cuvettes, can be run with little to no optimization.

The extreme flexibility and high sensitivity of the SpectraMax Multi-Mode Microplate Readers make them appropriate for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology.

Typical applications include ELISA, nucleic acid, protein, enzymatic type homogeneous and heterogeneous assays, microbial growth, endotoxin testing, and pipettor calibration.

Certified SpectraMax® M5^{e}-HTRF Readers

The SpectraMax M5^{e} reader has the same performance specifications as the M5 but is certified for use with Cisbio Bioassays' HTRF (Homogeneous Time-Resolved Fluorescence) technology. HTRF is a proprietary time-resolved fluorescence technology that overcomes many of the drawbacks of standard Fluorescence Resonance Energy Transfer (FRET) techniques, such as the requirements to correct for autofluorescence and the fluorescent contributions of unbound fluorophores.

Optics

The use of two holographic diffraction grating monochromators allows for individual optimization of wavelengths for both excitation and emission in fluorescence readings. 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. Sensitivity or read-speed can be optimized by varying the number of lamp flashes per read.

Dynamic Range

The dynamic range of detection is from 10^{-6} to 10^{-12} 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. The photometric range is 0–4 ODs with a resolution of 0.001 OD.

PathCheck® Pathlength Measurement Technology

A SpectraMax Multi-Mode Microplate Reader with PathCheck Pathlength Measurement Technology allows normalization of variable well volumes to 1-cm cuvette readings. PathCheck Pathlength Measurement Technology allows for multichannel pipettor validation and for experiment comparison from different days.

Automix

Using the Automix feature of the SoftMax Pro Software, the contents of the wells in a microplate can be mixed automatically by linear shaking before each read cycle, making it possible to perform kinetic analysis of solid-phase, enzyme-mediated reactions (mixing is not critical for liquid-phase reactions).

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 2°C above ambient to 60°C.

Supported Plates

Microplates having 6, 12, 24, 48, 96, and 384 wells can be used in the SpectraMax Multi-Mode Microplate Readers. Top and bottom reads are available for fluorescence, time-resolved fluorescence and luminescence detection. When reading optical density at wavelengths below 340 nm, special UV-transparent, disposable or quartz microplates and cuvettes that allow transmission of the far UV spectra must be used.

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 for all top-read applications.

Computer Control

An external computer running SoftMax Pro Software, which provides integrated instrument control, data display, and statistical data analysis, controls the SpectraMax Multi-Mode Microplate Readers. Cuvette port functionality can also be controlled using SoftMax Pro Software.

SoftMax Pro Software provides the following functionality:

Instrument Control

SoftMax Pro Software allows you to set up and run a complete protocol for the SpectraMax Multi-Mode Microplate Reader, as well as all other Molecular Devices' microplate readers. Instrument settings can be saved as a protocol file and used repeatedly for reading different microplates or cuvettes. All stand-alone instrument functions can be controlled using the software. In addition, SoftMax Pro Software provides capabilities that are not available when using an instrument in stand-alone mode such as user-defined kinetic run times, read intervals, Automix parameters, etc.

Data Collection and Display

SoftMax Pro Software collects and stores all raw data received from the instrument. Data is displayed in a grid format that corresponds to the wells in a microplate or individual cuvettes.

SoftMax Pro Software can collect data from one or more microplates or cuvettes and store it in a single data file, using the same or different instrument settings for different microplates or cuvettes. For example, microplates containing different samples can be read using the same or different modes, all within the same experiment.

Data Reduction and Plotting

You can manipulate or “reduce” the raw data using dozens of built-in formulas or define your own analysis structure to quickly and easily summarize the raw data. More than one reduction can be shown, and results from different microplates or cuvettes can be compared within the same experiment.

Immediate Results Reporting and Analysis

Once you have defined instrument settings, and have customized a SoftMax Pro Software data file with assay information, reduction settings, custom columns in Group sections, and summary objects, you can save this information to create an assay protocol. Protocols can be used throughout a department or company for highly repeatable data collection and analysis that is completed the second the plate read has completed.

Reader Components

The main components of the SpectraMax Multi-Mode Microplate Readers are:

- **Control panel:** for cuvette chamber control.
- **Microplate drawer:** used for all five read modes and four read types.
- **Cuvette chamber:** used for absorbance, fluorescence intensity, and luminescence read modes for endpoint, kinetic, and spectrum scanning.
- **Back panel:** connections and power switch.

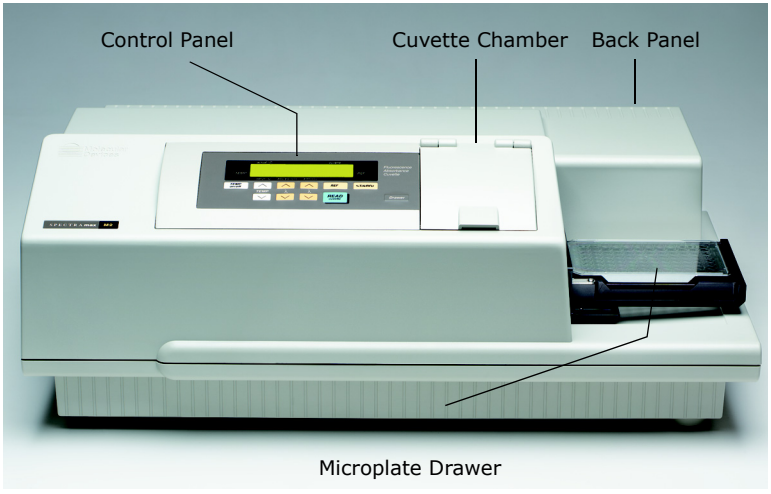


Figure 1-1 SpectraMax® components.

The Control Panel

Figure 1-2 The control panel.

The control panel consists of a 2-x-20-character LCD and eleven pressure-sensitive membrane keys that can be used to control some functions of the instrument. When you press a control panel key, the instrument performs the associated action.

Note: Settings made in SoftMax Pro Software override control panel settings.

The left side of the display shows the temperature inside the cuvette chamber, both actual and set point, and whether or not the temperature is at the set point (the enunciator blinks if it is not at set point). The temperature of the microplate chamber lags slightly behind the temperature in the cuvette chamber. The temperature in the microplate chamber is reported in the SoftMax Pro Software interface display.

The middle of the display shows the wavelengths for absorbance/excitation and emission.

The right side of the display shows the data received from the reading as absorbance, percent transmission, fluorescence emission or excitation, or luminescence, and indicates whether or not a reference measurement was made (enunciator blinks if no reference reading was taken).

To change the contrast of the display, press MODE and the temperature up (▲) or down (▲) setting keys.

Temp On/Off

The TEMP on/off key enables and disables the incubator that controls the temperature within both the microplate chamber and the cuvette port.

- When the incubator is on, the set temperature and actual temperature (cuvette chamber only) 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.

Temp

The TEMP keys allow you to enter a set point at which to regulate the cuvette and microplate chamber temperature. However, remember that the cuvette temperature only is reported on the LCD display, while the microplate chamber temperature is reported in the SoftMax Pro Software interface display.

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 (60°C) or lower (15°C) instrument limits.

Wavelengths (λ)

Selects the wavelength to be used for reading the cuvette manually. Two sets of λ up or down arrow keys are available for setting absorbance/excitation (fluorescence) wavelengths and emission (fluorescence) wavelengths.

The control panel does not display the wavelength selected through the SoftMax Pro application.

Pressing the up or down arrow key scrolls up or down through the available wavelengths, starting at the previous setting:

- Pressing the up (▲) or down (▼) arrow once increments or decrements the displayed wavelength by 1 nm.
- Pressing and holding either arrow increments or decrements the displayed wavelength by 10 nm until it is released.

Ref

A reading of buffer, water, or air taken in the cuvette that is used as I_0 to calculate Absorbance or % Transmittance. If no reference reading is taken, the instrument uses the I_0 values stored in the NVRAM (non-volatile memory) of the instrument.

This key is disabled during a computer-controlled run.

Read Cuvette

Initiates the sample reading of the cuvette.

This key is disabled during a computer-controlled run.

Mode

A toggle switch used to display cuvette data as percent transmittance (%T), absorbance (A), relative fluorescence units (RFU), or relative luminescence units (RLU).

Drawer

The DRAWER key opens and closes (toggles) the microplate drawer.

The Microplate Drawer

The microplate drawer is located on the right side of the instrument and slides in and out of the reading chamber. An internal latch positions the microplate in the drawer as it closes (allowing for better robot integration-no springs or clips are used).

The drawer remains in the reading chamber during read cycles.

Figure 1-3 The microplate drawer.

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.

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 Troubleshooting on page 61).

Microplates

The SpectraMax Multi-Mode Microplate Reader can accommodate SBS-standard 6-well to 384-well microplates and strip wells. When reading optical density at wavelengths below 340 nm, special UV-transparent, disposable or quartz microplates allowing transmission of the deep UV spectra must be used.

Not all manufacturers' microplates are the same with regard to design, materials, or configuration. Temperature uniformity within the microplate may vary depending on the type of microplate used.

Microplates currently supported by the SoftMax Pro Software for use in this instrument are:

- 96-well Standard, 96 Costar, 96 Greiner Black, 96 Bottom Offset, 96 Falcon, 96 BD Optilux/Biocoat, 96 BD Fluoroblok MW Insert, 96 Corning Half Area, 96 MDC HE PS
- 384-well Standard, 384 Costar, 384 Greiner, 384 Falcon, 384 Corning, 384 MDC HE PS
- 48 Costar
- 24 Costar
- 12 Costar, 12 Falcon
- 6 Costar, 6 Falcon.

The SoftMax Pro Software plate list also includes half area and low-volume plates. SoftMax Pro can always be used to define a new plate type using the manufacturer's specifications for well size, spacing and distance from the plate edge.

The Cuvette Chamber

Figure 1-4 The cuvette chamber.

Located at the right front of the SpectraMax instrument, the cuvette chamber has a lid that lifts up, allowing you to insert or remove a cuvette. The chamber contains springs that automatically position the cuvette in the proper alignment for a reading. The cuvette door must be closed before initiating a reading.

Cuvettes

The SpectraMax Multi-Mode Microplate Reader can accommodate standard-height (45 mm), 1 cm cuvettes and 12 x 75 mm test tubes when used with the test tube cover.

Not all manufacturers' cuvettes are the same with regard to design, materials, or configuration. Temperature uniformity within the cuvette may vary depending on the type of cuvette used.

Cuvettes used for absorbance readings are frosted on two sides. Be sure to handle cuvettes on the frosted sides only. Place the cuvette into the chamber so that the "reading" (clear) sides face left and right.

Fluorescence cuvettes are clear on all four sides and should be handled carefully. Place a frosted cuvette into the chamber so that the "reading" (clear) sides face left and right. Semi-Micro and Ultra-Micro cuvettes can also be used with an adapter. See Cuvettes on page 78 for more information about supported cuvettes.

Figure 1-5 The test tube cover.

The Back Panel

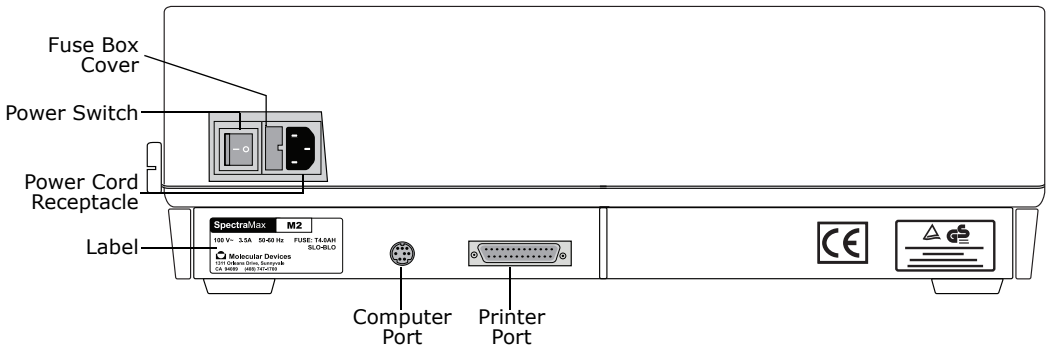


Figure 1-6 Schematic of the back panel of a reader.

The following components are located on the back panel of the SpectraMax instrument:

- **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.
- **Computer port (double-shielded 8-pin RS-232 serial, 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.
- **Printer port:** not used for the SpectraMax instrument
- **Label:** provides information about the reader, 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.

Principles of Operation

2

Absorbance

Note: In this user guide, references to the SpectraMax® readers include the M3, M4, M5, and M5**e** models. When a feature or capability applies to only certain readers, this exception is noted.

Absorbance is the amount of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. In the absence of turbidity, absorbance = optical density:

$$A = -log(I/I_O)$$

where I is transmitted light, and I_O is incident light.

In this manual, we use the terms absorbance and optical density interchangeably.

Optical Density

Optical density is the amount of light passing through a sample to a detector relative to the total amount of light available. Optical density includes absorbance of the sample plus light scatter from turbidity.

Transmittance

Transmittance is the ratio of transmitted light to the incident light.

$$T = (I/I_O)$$
$$\%T = 100T$$

where I is transmitted light, and I_O is incident light.

PathCheck® Pathlength Measurement Technology

The Beer-Lambert law states that absorbance is proportional to the distance that light travels through the sample:

$$A = \varepsilon bc$$

where A is the absorbance, ε is the molar absorptivity of the sample, b is the pathlength, and c is the concentration of the sample. In short, the longer the pathlength, the higher the absorbance.

Microplate readers use a vertical light path so the distance of the light through the sample depends on the volume. This variable pathlength makes it difficult to perform extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a cuvette is the conventional basis for quantifying the unique absorbtivity properties of compounds in solution. Quantitative analyses can be performed on the basis of extinction coefficients, without standard curves (for example, NADH-based enzyme assays). When using a cuvette, the pathlength is known and is independent of sample volume, so absorbance is proportional to concentration.

In a microplate, pathlength is dependent on the liquid volume, so absorbance is proportional to both the concentration and the pathlength of the sample. Standard curves are often used to determine analyte concentrations in vertical-beam photometry of unknowns, yet errors can still arise from pipetting the samples and standards. The PathCheck® Pathlength Measurement Technology feature automatically determines the pathlength of aqueous samples in the microplate and normalizes the absorbance in each well to a pathlength of 1 cm. This novel approach to correcting the microwell absorbance values is accurate to within 2.5% of the values obtained directly in a 1 cm cuvette.

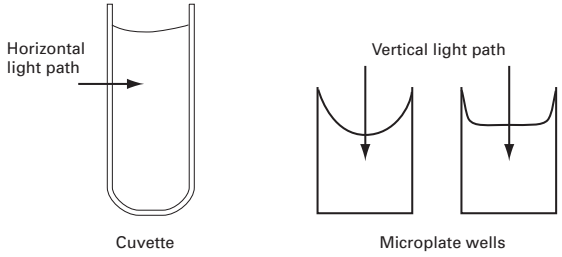


Figure 2-1 Cuvette and microwell light paths.

Reference measurements made by reading the cuvette (Cuvette Reference) or using factory-stored values derived from deionized water (Water Constant) can be used to normalize the optical density data for microplate wells.

Pathlength correction is accomplished only when using the PathCheck Pathlength Measurement Technology with SoftMax® Pro Software. PathCheck Pathlength Measurement Technology is patented by Molecular Devices and can be performed only on an Molecular Devices plate reader.

The SpectraMax Multi-Mode Microplate Reader offers both the Cuvette Reference and the Water Constant methods.

The actual pathlength, *d*, of a solvent is found from the following equation:

$$d(cm) = \frac{Sample(OD_{1000} - OD_{900})}{k}$$

When a Cuvette Reference is used for pathlength correction, the value of *k* is obtained by taking optical density measurements on the fluid in the cuvette at two wavelengths, 1000 and 900 nm:

$$k = Cuvette(OD_{1000} - OD_{900})$$

When the Water Constant is used for pathlength correction, the value of k is obtained from the instrument. This constant is saved in the instrument in the factory and may differ slightly from instrument to instrument.

Once the pathlength *d* is found, the following equation is used for the pathlength correction:

$$\frac{OD}{cm} = \frac{OD_{Sample}}{d(cm)}$$

PathCheck Pathlength Measurement Technology is applicable to almost all biological/pharmaceutical molecules in aqueous solution because they have little or no absorbance between 900 nm and 1000 nm at concentrations normally used. PathCheck Pathlength Measurement Technology can also be used with samples containing small amounts of organics or high buffer concentrations by using the Cuvette Reference. See Water Constant or Cuvette Reference? on page 22.

Water Constant or Cuvette Reference?

The PathCheck Pathlength Measurement is based on the absorbance of water in the near infrared region (between 900 nm and 1000 nm). If the sample is completely aqueous, has no turbidity and has a low salt concentration (less than 0.5 M), the Water Constant is adequate. The Water Constant is determined during manufacture and is stored in the instrument.

If the sample contains an organic solvent such as ethanol or methanol, we recommend using the cuvette reference. It is important that the solvent does not absorb in the 900 nm to 1000 nm range (to determine whether or not a given solvent would interfere, see the discussion of interfering substances below). When a non-interference solvent is added to the aqueous sample, the water absorbance decreases proportionally to the percentage of organic solvent present. For example, 5% ethanol decreases the water absorbance by 5% and results in a 5% underestimation of the pathlength. You can avoid the error by putting the same water/solvent mixture in a cuvette and using the Cuvette Reference.

To use the Cuvette Reference, place into the cuvette port a standard 1 cm cuvette containing the aqueous/solvent mixture that is used for the samples in the microplate. The cuvette must be in place when you read the microplate. When you click the Read button in the SoftMax Pro program, the instrument first makes the 900 nm and 1000 nm measurements in the cuvette, and then makes the designated measurements in the microplate. The cuvette values are stored temporarily and used in the PathCheck Pathlength Measurement Technology calculations for the microplate samples.

Use of Cuvette Reference with PathCheck Pathlength Measurement Technology is different from a reference reading of a cuvette in a CuvetteSet section (by clicking the Ref button in the CuvetteSet section tool bar in the SoftMax Pro program). The cuvette reference used for PathCheck Pathlength Measurement Technology calculations (measurements at 900 nm and 1000 nm) does not produce data that can be viewed in a CuvetteSet section and is used only with data in microplates, not cuvettes.

Background Considerations

Raw optical density measurements of microplate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of microplate material). The latter must be eliminated from the PathCheck Pathlength Measurement Technology calculation in order to obtain PathCheck Technology-normalized results. There are 3 ways to accomplish this: plate blanks, plate background constants, and plate pre-reads, all of which are described in the PathCheck Pathlength Measurement Technology section of the *SoftMax Pro User Guide*.

PathCheck Pathlength Measurement Technology and Interfering Substances

Any material that absorbs in the 900 nm to 1000 nm spectral region could interfere with PathCheck Pathlength Measurement Technology measurements. Fortunately, there are few materials that do interfere at the concentrations typically used.

Turbidity is the most common interference: if you can detect any turbidity in your sample, you should not use the PathCheck Technology feature. Turbidity elevates the 900 nm measurement more than the 1000 nm measurement and causes an erroneously low estimate of pathlength. Using Cuvette Reference does not reliably correct for turbidity.

Samples that are highly colored in the upper visible spectrum may have absorbance extending into the near infrared (NIR) and can interfere with the PathCheck Pathlength Measurement Technology. Examples include Lowry assays, molybdate-based assays and samples containing hemoglobins or porphyrins. In general, if the sample is distinctly red or purple, you should check for interference before using the PathCheck Pathlength Measurement Technology.

To determine possible color interference, do the following:

- Measure the optical density at 900 nm and 1000 nm (both measured with air reference).
- Subtract the 900 nm value from the 1000 nm value.
- Do the same for pure water.

If the delta OD for the sample differs significantly from the delta OD for water, then it is advisable not to use the PathCheck Technology feature. Use of Cuvette Reference does not correct for the interference with the current calculation scheme in the SoftMax Pro program. Currently, Cuvette Reference involves a single (automated) read at 900 nm and 1000 nm and the automated calculations in the SoftMax Pro program do not compensate for color or solvent interference. However, you could correct for such interference by taking two cuvette measurements and using a different set of calculations. For further information, contact Molecular Devices Technical Support.

Organic solvents could interfere with the PathCheck Technology feature if they have absorbance in the region of the NIR water peak. Solvents such as ethanol and methanol do not absorb in the NIR region, so they do not interfere, except for causing a decrease in the water absorbance to the extent of their presence in the solution. Their passive interference can be avoided by using the Cuvette Reference. If, however, the solvent absorbs between 900 and 1000 nm, the interference would be similar to the interference of highly colored samples described above. If you are considering adding an organic solvent other than ethanol or methanol, you are advised to run a spectral scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck Technology feature.

Normalizing Absorbance Measurements

SoftMax Pro Software automatically reports absorbance values normalized to a 1-cm pathlength. SoftMax Pro Software automatically reports absorbance values normalized to a 1-cm pathlength. The table below shows results obtained with 75 µL to 300 µL yellow reagent.

Table 2-1 Yellow reagent results.

Well Volume (µL)	Pathlength (cm)	Raw Absorbance	Absorbance/ cm	SD	CV%
75	0.231	0.090	0.390	0.006	1.6
100	0.300	0.116	0.387	0.005	1.2
150	0.446	0.172	0.385	0.003	0.8
200	0.596	0.228	0.383	0.002	0.4
250	0.735	0.283	0.384	0.002	0.5
300	0.874	0.336	0.384	0.001	0.3
Absorbance in 1-cm cuvette = 0.386					

Optical pathlengths and raw absorbance values were directly proportional to well columns. After normalization to a 1-cm pathlength, all absorbance values, regardless of the volume in the wells, were within 1% of the value obtained by measuring the same solution in a 1-cm cuvette.

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 2-2 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.

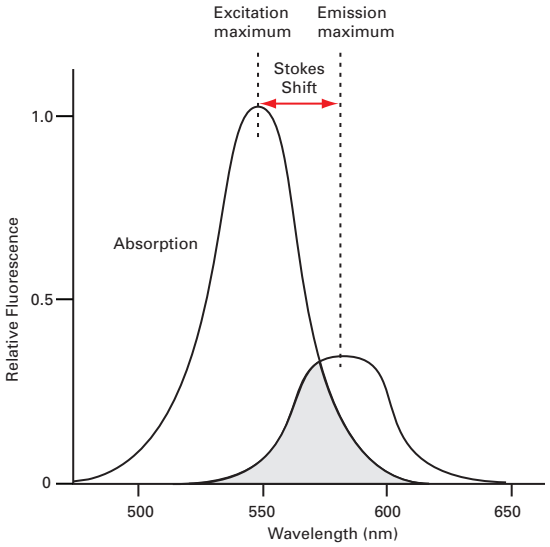


Figure 2-2 Excitation and emission spectra.

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 SpectraMax Multi-Mode Microplate Reader incorporates 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.

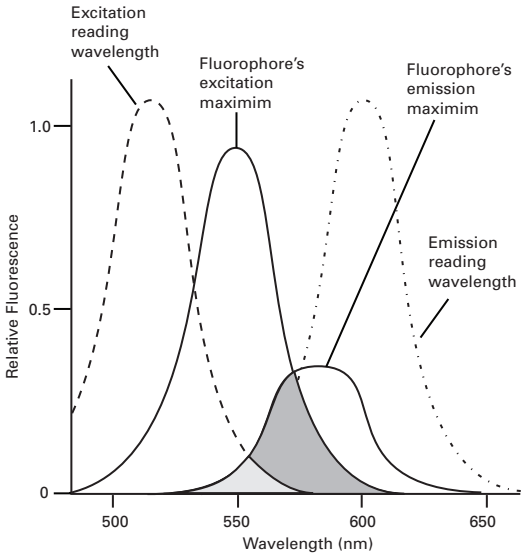


Figure 2-3 Optimized excitation and emission reading wavelengths.

Figure 2-3 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 SpectraMax Multi-Mode Microplate Reader allows 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 SpectraMax series readers, see Optimizing Absorbance Assays on page 44.

Time-resolved Fluorescence (M4, M5, and M5e only)

In normal fluorescence mode, the SpectraMax 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. The elimination of background excitation is the critical difference between fluorescence intensity measurements and TRF measurements.

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. Long-lifetime rare-earth lanthanide dyes are typically used to provide a long-lived fluorescent signal that persists after the lamp is turned off. Background fluorescence usually fades after 50 µs, while lanthanide chelates and cryptates have fluorescent lifetimes between 100 µs and 2 ms.

To optimize data collection for a particular assay, the user can select when to start and end data acquisition-the minimum is 50 µs after the lamp has been turned off, and the maximum is 1450 µs, in 50- or 200-µs steps.

Some examples of TRF assays are:

- IMAP® TR-FRET
- Cisbio HTRF
- LanthaScreen TR-FRET
- LANCE TR-FRET
- DELFIA TRF

Fluorescence Polarization (M5 and M5e only)

By using a fluorescent dye to label a small molecule, its binding to a large molecule can be monitored through its speed of rotation.

Fluorescence Polarization mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. These S and P values are used to calculate the polarization (mP) and anisotropy (r) values in SoftMax Pro Software. Although the Raw S&P value is the true raw data returned from the instrument, the calculated polarization (mP) and anisotropy (r) values are treated as the raw data, and these values become the basis for further reduction calculations in SoftMax Pro Software.

Polarization (mP) is calculated as follows:

$$mP = \frac{\text{parallel} - (G \times \text{perpendicular})}{\text{parallel} + (G \times \text{perpendicular})}$$

Anisotropy (r) is calculated as follows:

$$r = \frac{\text{parallel} - (G \times \text{perpendicular})}{\text{parallel} + (2G \times \text{perpendicular})}$$

Luminescence

Luminescence is 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.

When the SpectraMax Multi-Mode Microplate Reader is 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.

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. If wavelength selection is desired, 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.

Functional Description

The full power of a SpectraMax Multi-Mode Microplate Reader can only be harnessed when the instrument is controlled using SoftMax® Pro Software running on a computer connected to the instrument. For a complete description of the modes of operation, how to choose instrument settings, etc. refer to the *SoftMax® Pro Software User Guide*.

However, some functionality is available directly on the instrument without having to use SoftMax Pro Software:

- Temperature control
- Wavelength control
- Fixed-point cuvette readings

Temperature Regulation

The SpectraMax Multi-Mode Microplate Readers have been designed to regulate the temperature of the cuvette and microplate chamber from 2°C above ambient to 60°C. Upon power up, when the incubator is off, the temperature in the chambers is ambient and isothermal. Turning on the incubator by pressing the TEMP on/off key causes the instrument to begin warming the cuvette and microplate chambers. 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 2°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 (60°C) can be achieved only if the ambient temperature is greater than 20°C.

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

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

Temperature regulation and control 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.

Read Types

The SpectraMax Multi-Mode Microplate Reader 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 Software User Guide*.

Endpoint Read

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

Depending on the data mode selected in the Reduction window, values can be reported as optical density or % Transmittance.

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 Software.

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 optical density or % Transmittance across a spectrum of wavelengths 200 nm to 1000 nm. For fluorescence or luminescence mode, relative fluorescence units (RFU) or relative luminescence units (RLU) values are reported.

All spectrum readings are made using the scanning monochromators of the instrument.

Well Scan Read

A well scan read takes one or more readings of a single well of a microplate at single or multiple wavelengths. Every option available for endpoint reads is available for well scans.

Some applications involve the detection of whole cells in large-area tissue culture plates. Well scan mode can be used with such microplates to allow maximum surface area detection in whole-cell assays. Since many cell lines tend to grow as clumps or in the corners of microplate wells, you can choose from several patterns and define the number of points to be scanned to work best with your particular application.

Values reported are optical density, % Transmittance, relative fluorescence units (RFU), or relative luminescence units (RLU).

Automix

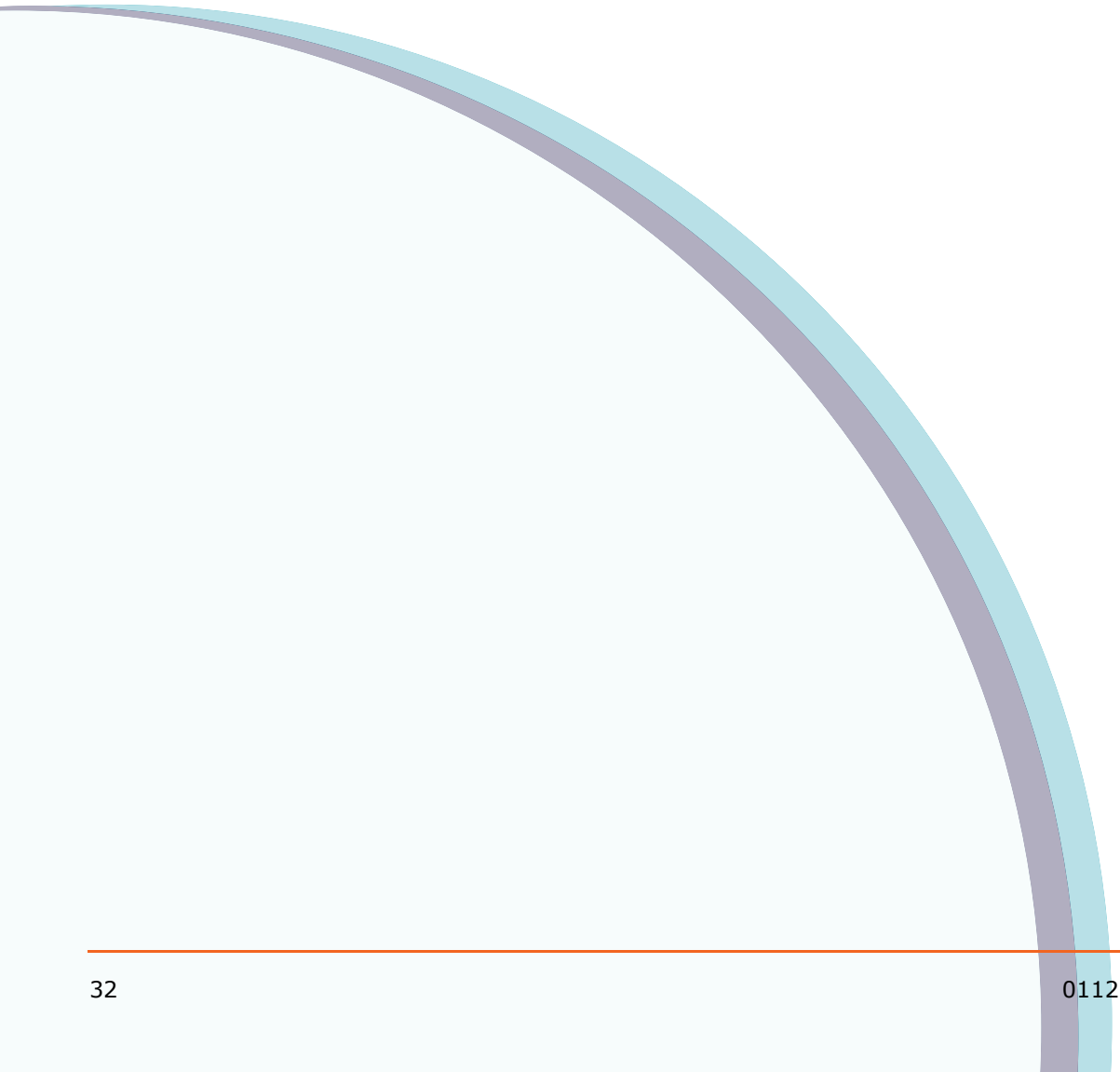
The Automix function permits automatic linear shaking along the long axis of the microplate at preset intervals, thereby mixing the contents within each well. Automix must be selected before beginning a reading. The actions associated with the Automix setting depend on the read mode chosen:

- **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.

Computer Control

The SpectraMax Multi-Mode Microplate Readers are equipped with an 8-pin DIN RS-232 serial port through which the computer communicates with the instrument using the SoftMax Pro Software. Different types of cables are available for connecting to different types of computers. See Appendix B: Cables and Accessories on page 77.



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 operate the instrument in a cold room with a temperature below 15°C.

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.

Unpacking

Note: In this user guide, references to the SpectraMax® readers include the M3, M4, M5, and M5e models. When a feature or capability applies to only certain readers, this exception is noted.

The SpectraMax Multi-Mode Microplate Readers are 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 SpectraMax Multi-Mode Microplate Reader weighs approximately 36 pounds (16.4 kg) and should be lifted with care. It is recommended that two people 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 accessory kit out. Open the accessory kit box and check that all parts are accounted for:

- Plate adapter (purple)
- Reader dustcover
- Test tube cover
- Hex wrench, 3/32″, ball drive, L
- Mouse pad, SpectraMax instrument
- Cable, PC–SpectraMax, 9 pin–8 pin mini
- Country-specific Power cord
- Fuses, 4-amp (2 ea.)
- SpectraMax User Guide
- Applications guide to microplate systems

Make sure all these items are present before proceeding.

Remove the cardboard divider from the top of the SpectraMax. Lift the reader up and out of the shipping box and set it down carefully.

Setting up the Instrument

1. Place the instrument 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 *Schematic of the back panel of a reader. on page 18*.
3. Insert the round end of the serial cable into the RS-232 serial port on the back panel of the instrument. (A Keyspan USB adapter is necessary for a Macintosh computer or a Windows computer without a serial port; see Appendix B for more information on adapter cables.) Attach the other end to your computer.
4. Insert the female end of the power cord into the power receptacle at the rear of the instrument. 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.
5. Turn the instrument 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.
6. Remove the tape from the cuvette door.
7. Turn on the power to the instrument, wait for the microplate drawer to open, and remove the tape and protective covering from the drawer subplate.

Installing the Drawer Adapter

CAUTION! Incorrect insertion or removal of the adapter may cause damage to the microplate drawer of the SpectraMax instrument. 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 select the Control > Open Drawer 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.

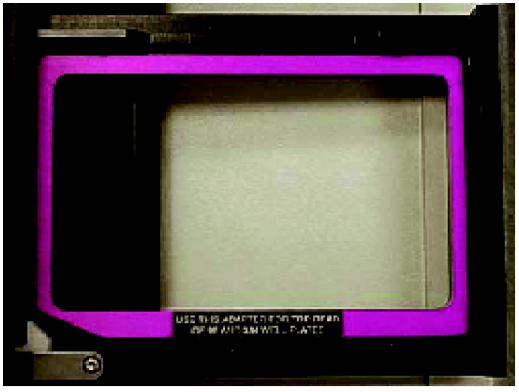


Figure 3-1 Adapter inserted in microplate drawer.

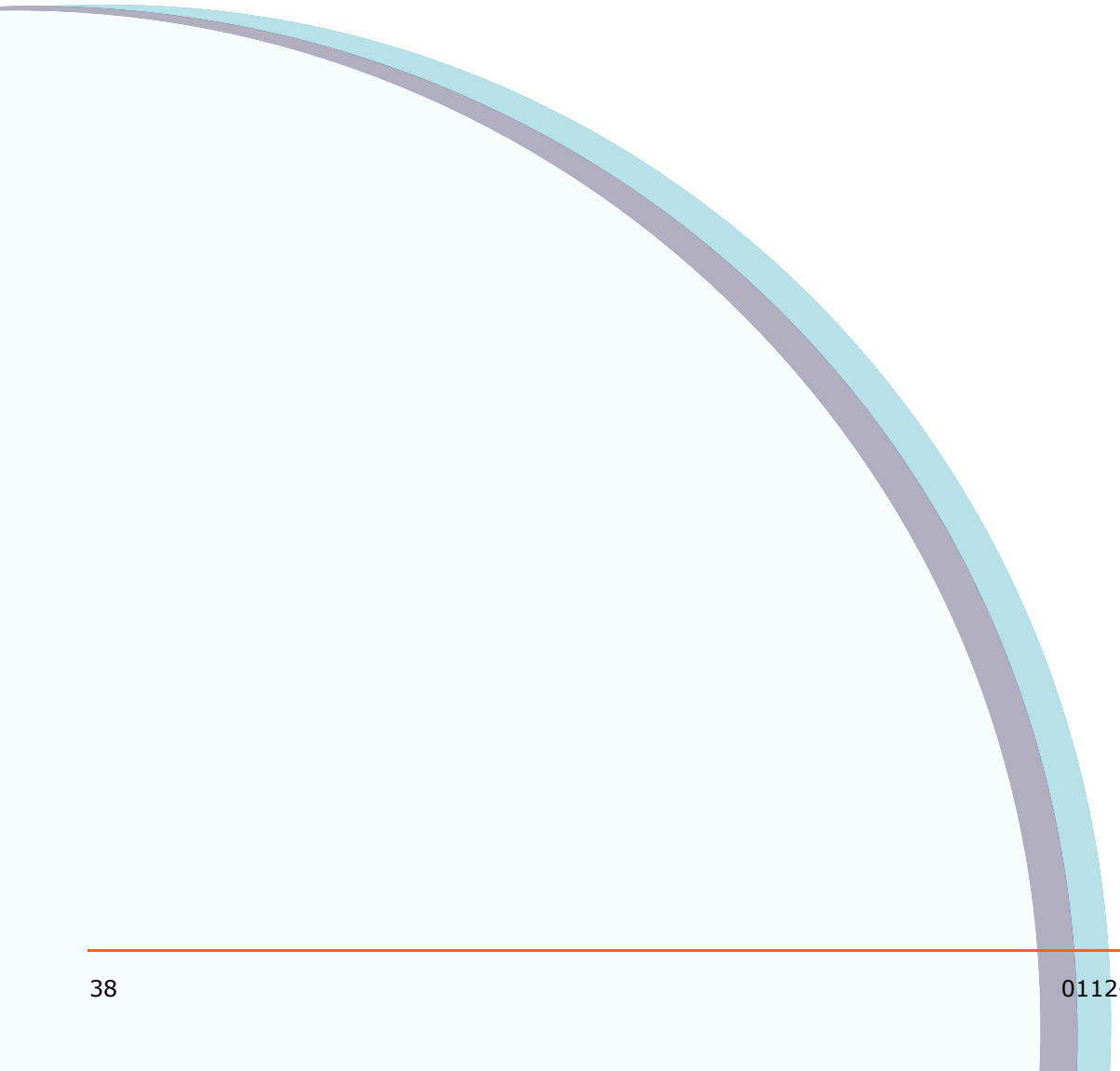
Removing the Drawer Adapter

If the adapter is in the drawer and you are either reading from the bottom 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 SpectraMax instrument.

1. Power on the instrument using the switch on the back panel.
2. Press the DRAWER button on the front panel or select the Control > Open Drawer command in SoftMax Pro Software.
3. Remove the adapter plate.

Figure 3-2 The microplate drawer without adapter.



Cuvette Read—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 read a cuvette using a SpectraMax Multi-Mode Microplate Reader:

1. Turn on the power switch (located on the back panel). The microplate drawer opens automatically.
2. If you want to regulate the temperature inside the chamber, touch the TEMP on/off (incubator) key to turn the incubator on and bring the 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 of the cuvette chamber along with the temperature set point. To change the set point (to any setting from ambient +2°C to 60°C), press the up or down arrow keys.
4. Select the desired measurement wavelength by pressing the up or down arrow near λ.
5. Load the prepared cuvette into the chamber, being sure that the clear sides are left and right (when facing the instrument).
6. Press the REF or READ CUVETTE key.

Note: In this user guide, references to the SpectraMax® readers include the M3, M4, M5, and M5e models. When a feature or capability applies to only certain readers, this exception is noted.

Microplate Read—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 read a microplate using a SpectraMax Multi-Mode Microplate Reader:

1. Turn on the power switch (located on the back panel). The microplate drawer opens automatically.
2. If you want to regulate the temperature inside the chamber, touch the TEMP on/off (incubator) key to turn the incubator on and bring the chamber to the default temperature of 37.0°C. The microplate drawer closes.
3. If the incubator is on, the LCD shows the temperature set point, but the current temperature in the microplate chamber is reported in SoftMax® Pro only—the LCD reports the cuvette chamber temperature. To change the set point to any setting from ambient +2°C to 60°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 the upper left-hand corner of the drawer.
7. Using SoftMax Pro Software, start the reading by selecting the **Control > Read** command or clicking the Read button on the Plate section tool bar.

Preparing for a Cuvette or Microplate Reading

Turn the Instrument and Computer On

The power switch 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.

Set the Temperature (Optional)

To set the temperature within the microplate or cuvette 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 below). However, only the cuvette temperature is reported on the front panel; the SoftMax Pro program reports the current microplate chamber temperature, which lags very slightly behind the cuvette temperature.

Temperature cannot be regulated at a set point that is lower than 2°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 cuvette chamber only.

To change the temperature set point, press the up or down arrow keys until the desired temperature set point is shown in the display.

To control the temperature from the SoftMax Pro Software, use the Control > Incubator dialog box both to enable the incubator and to set the temperature.

The 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 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 chamber temperature.

Select the Wavelength

The absorbance wavelength, or the excitation or emission wavelengths for a fluorescence or luminescence read, can be selected for cuvette reading using either the control panel or the SoftMax Pro Software. The wavelengths must be selected using the software when reading microplates.

To select the wavelength using the control panel

1. Select the desired measurement wavelength by pressing the up or down arrow near λ.
2. Scroll through the wavelengths shown on the LCD to increment or decrement the wavelength setting (in1-nm increments) until the desired measurement wavelength is reached.
3. If A or %T type is chosen, only the Abs/Ex wavelength can be set.

To select the wavelength using SoftMax Pro Software

1. Select the Plate > Settings command, or click the Settings button in the appropriate Plate or CuvetteSet section.
2. In the Settings dialog box, select Wavelengths from the list of settings on the left-hand side.
3. Specify the number of wavelengths to be read in each well or cuvette.
4. Specify the excitation and emission wavelengths to use.
5. Select Auto-cutoff if you would like the reader software to automatically select the emission cut-off filter.

Read the Cuvette

1. Insert the cuvette into the chamber, making sure that the clear sides are to the left and right (facing the instrument). Do not touch the clear surfaces of the cuvette.
2. Make sure the cuvette is completely seated in the chamber and close the cuvette door.
3. If the cuvette contains a blank (typically this solvent contains everything that the samples contain except for analyte), press the REF key to acquire the reference reading from the cuvette. The instrument automatically calibrates in less than two seconds, closes the microplate drawer (if it is open), and reads the cuvette according to the selected instrument settings.
4. If the cuvette contains a sample, touch the READ CUVETTE key to acquire the sample reading from the cuvette.
5. When the reading is complete, remove the cuvette.

Read the Microplate

Note: 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 on page 36 for more information).
2. You must have SoftMax Pro Software running on a computer connected to the instrument.

   Open a SoftMax Pro data file or protocol file that contains the appropriate experiment settings for the plate read. Alternatively, create new settings by selecting the Plate section in the SoftMax Pro program and configuring the instrument using the Plate >Settings dialog box.

3. Select the Control > Read command or press the Read button in SoftMax Pro Software to start the plate read.
4. 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.
5. If you return to the SpectraMax instrument and find the drawer closed after a reading has finished, press the DRAWER key. When the drawer opens, you can remove the microplate.

For more information about configuring the software for plate reading, please consult the *SoftMax® Pro Software User Guide*.

Optimizing Fluorescence Assays

The wavelength of the transmitted light can be adjusted in 1-nm increments between 200 nm and 1000 nm. Guke also allows reading up to four wavelengths per plate. This enables reference wavelength readings such as A260 and A280 for nucleic determination.

An appropriate plate blank should be applied. Unless the user suspects that there is significant well-to-well variability due to the thickness and optical properties of the plate, the use of Pre-Read Plate in the SoftMax Pro program is not required. Instead, we recommend using appropriate plate blanks or group blanks in the Template dialog box of the Plate section in the SoftMax Pro program. For discussion of the different types of blanking, please refer to the *SoftMax® Pro Software User Guide*.

If desired, the PathCheck Technology feature in SoftMax Pro program can be activated to normalize the data to a 1-cm pathlength.

Optimizing Absorbance Assays

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, and the temperature of the reading chamber.

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 between 250 nm and 850 nm. A procedure to optimize excitation and emission wavelengths for a given assay is outlined below.

Emission Cutoff Filter

The 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 for 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 100 (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 display shows 1 read per well.

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 SpectraMax Multi-Mode Microplate Reader is isothermal at ambient as well as at elevated temperatures. The temperature in the reading chamber may be adjusted from 2°C above ambient to 60°C.

Note that assay optimization requires the use of a computer and SoftMax Pro Software.

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. Perform the excitation scan:
   - Using SoftMax Pro Software, set up a Plate section for a fluorescence read, spectrum mode, Em Fixed/Ex Scan, with no cutoff filter (default), and medium PMT.
   - 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 an optical density spectral scan first.
   - 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).
   - Set the step increment to 2 or 3 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 2-nm or 3-nm increment.)
   - 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 the SoftMax Pro program 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.

- 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.)

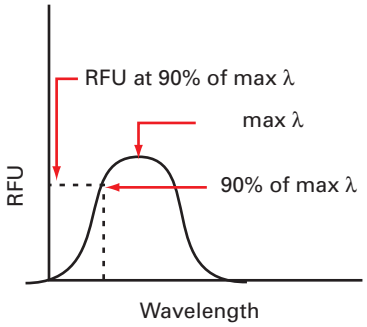


Figure 4-1 Plot of RFU vs. wavelength.

2. Perform emission scan #1:
   - In the SoftMax Pro program, set up a second plate section for a fluorescence read, spectrum mode, Ex Fixed/Em Scan, with no cutoff filter (default), and medium PMT.
   - Set the excitation wavelength to the value determined in Step 1w above.
   - 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.
   - Set the step increment to 2–3 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 2–3 nm increment.)
   - Perform the scan and view the results as a plot of fluorescence vs. emission wavelength.

3. Choose the emission filter:
   - 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, 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 10 nm less than the emission wavelength. If you have questions about this procedure please contact MDC Technical Support and ask to speak to an applications scientist.
4. Perform emission scan #2:
   - In the SoftMax Pro Software, set up a third plate section for an emission scan as specified in Step 2 above, except selecting Manual Cutoff Filter and setting the wavelength to that determined in Step 3.
   - 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).
   - 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 3 and 4 with another choice of cutoff filter.
5. Results

   The optimal excitation and emission wavelengths are those determined in Steps 1 and 4, above.
6. Comments
   - 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.
   - 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.

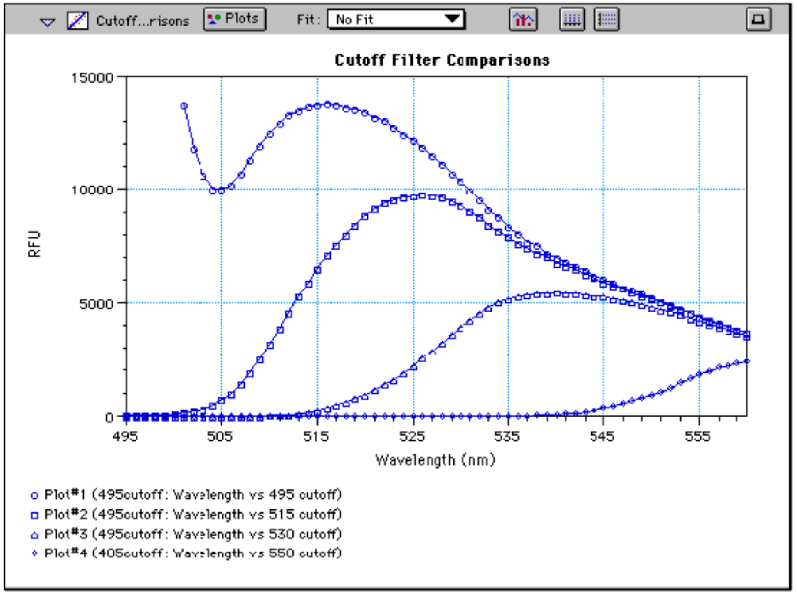


Figure 4-2 Effects of cutoff filters on fluorescein. Emission was scanned from 490 to 560 nm; excitation was fixed at 485 nm.

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 following lists default settings for the emission cutoff filters.

Table 4-1 Emission cutoff filter default settings.

#	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

Table 4-1 Emission cutoff filter default settings.

#	Automatic Cutoff Selection	Endpoint and Kinetic Modes
	Wavelength (nm)	Emission Wavelength (nm)
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).

Optimizing Time-resolved Fluorescence Assays

Time-resolved fluorescence assays on a SpectraMax M4, M5, and M5e reader may be read from the top or bottom of a microplate. Solid white plates are recommended for top time-resolved fluorescence reads, and white plates with clear bottoms are recommended for bottom reads.

If the time-resolved fluorescence assay you are using has low signal or gives results with high %CV, use 100 readings per well. If a faster read speed is required, be sure Settling Time is "Off" in the SoftMax Pro Plate Settings dialog box, and experiment with fewer flashes per well until acceptable precision and speed are achieved.

Important settings for obtaining the best results in TRF assays are integration delay and integration time:

- The integration delay is the amount of time that elapses between the flash of the lamp (excitation) and the beginning of data acquisition from the well.
- The integration time is the amount of time the well is read.

Delay and integration time are usually specified in the package insert of commercially available TRF reagent kits. If a kit is not used, start with a delay of 50 µs and try different delays up to 400 µs with a fixed integration time of 400 µs. Once the optimum delay is chosen (based on the highest ratio of a well containing a fluorophore divided by wells containing only buffer) optimize the integration time, which is usually between 400 µs and 1000 µs.

Optimizing Fluorescence Polarization Assays

Fluorescence polarization for SpectraMax M5 and M5e readers may only be read from the top of a microplate. The plastic from a microplate will affect the light polarization, precluding bottom reads and reading a covered plate.

Solid black plates are recommended for fluorescence polarization reads. If the assay components seem to bind to the microplate, as evidenced by poor mP dynamic range (small difference between bound and unbound tracer), we suggest using plates treated to minimize binding, or polypropylene plates and/or adding a very small amount of detergent, such as Tween-20, to the assay buffer.

Background wells, containing all assay components minus the fluorophore, should be tested. If the signal in the background wells is more than 1/10 the signal in the wells containing fluorophore, then background wells should be run on each assay plate. The average raw signal from the background's parallel and perpendicular readings should be subtracted from the raw parallel and perpendicular readings of each sample well before the mP calculation is performed. See the *SoftMax® Pro Software User Guide* for set-up of background subtraction in fluorescence polarization.

For best precision in assays using a low amount of fluorophore (for example, <5 nm fluorescein), set the PMT sensitivity to High and the number of readings to 100. If faster read speed is required, be sure Settling Time is “Off” in the SoftMax Pro Plate Settings dialog box, and experiment with fewer flashes per well until acceptable precision and speed are achieved.

Optimizing Luminescence Assays

Luminescence may be read from the top or the bottom of a microplate or the cuvette. Solid white plates or white plates with clear bottoms are recommended for luminescence reads.

For standard luminescence a separate light path without monochromators carries the emitted light to a dedicated PMT. The optimum emission wavelength is between 360 and 630 nm. Under reader set-up the emission says “All”.

For wavelength-selectable luminescence, the emission monochromator is used to differentiate the wavelengths being emitted from the well. Up to four emission wavelengths between 250 nm and 850 nm may be specified. If reading only one luminescent event in the well, best sensitivity should be achieved using the standard luminescence measurement, without a wavelength selected.

Luminescence read times are not designated by multiple reads per well, but rather by choosing the total integration time desired between 1 ms and 1,500 ms. Typical luminescence assays require between 500 ms and 1,000 ms integration.

If wells have been incubating for a long period of time, it is a good idea to mix the plate before reading. This can be done using Automix in the reader.

If it appears that the signal is always higher in the first wells read (for example, column A), the plate may need to be “dark adapted” to reduce the auto-luminescence of the white plastic. The auto-luminescence decreases quickly, so manually load the plate from the control panel and wait for 1–2 minutes before initiating the read and determine if the read-out is more consistent across the plate.

Maintenance

5

Technical Support

Molecular Devices 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.

Note: In this user guide, all references to SpectraMax Multi-Mode Microplate Readers include the M3, M4, M5, and M5^{e} models. When a feature or capability applies to only certain readers, this exception is noted.

If you have any problems using your SpectraMax Multi-Mode Microplate Reader, in the U.S., contact our Technical Services group at 1-800-635-5577; elsewhere contact your local representative.

WARNING! 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.

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 an Molecular Devices representative.

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

WARNING! Always 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! Never perform any operation on the instrument in an environment where liquids or potentially damaging gases are present.

WARNING! Risk of electrical shock. Refer servicing to qualified personnel.

CAUTION! Use of organic solvents (such as dichloromethane) may cause harm to the optics in the instrument. Extreme caution is advised when using organic solvents. Always use a plate lid and avoid placing a plate containing these materials in the reading chamber for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty

CAUTION! Never touch any of the optic mirrors, filters, or cables or their housing, or manifold. The optics are extremely delicate, and critical to the function of the instrument.

CAUTION! Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void warranty.

Moving a SpectraMax Multi-Mode Microplate Reader

If you need to relocate a SpectraMax Multi-Mode Microplate Reader, follow these steps.

WARNING! The instrument weighs approximately 36 pounds (16.4 kilograms). To avoid injury, it is recommended that two people lift the instrument together, using proper lifting techniques.

1. Remove any microplate from the drawer and then close the drawer.
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 instrument 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 “Setting Up the Instrument”.

General

Keep the drawer closed when the instrument is not in use. The drawer can be opened by pressing the DRAWER button. Always close the drawer immediately prior to switching the instrument off.

Cleaning

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

CAUTION! Never clean the inside of the instrument. Cleaning the interior may cause damage to the instrument.

Periodically, you should clean the outside surfaces of the instrument 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.

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.

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):

- 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 instrument.

If power failed while the instrument was already on:

- 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 instrument.

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 x 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 instrument power cord receptacle.
2. Remove the computer cable (if connected) from the back of the instrument.
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.
5. Continue gently pulling the fuse box forward until it is free of the instrument.

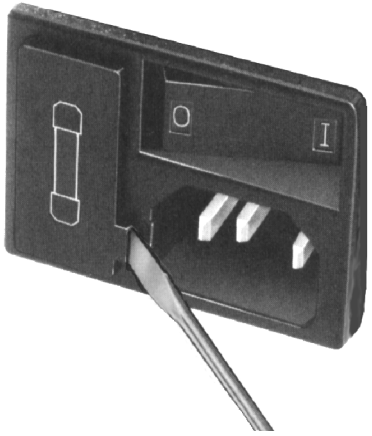


Figure 5-1 Prying open the fuse box cover.

6. When removed, the fuse assembly contains two fuses. Once the fuse box is out, you will see a holder inside containing two fuses. Pull the fuse holder out of the box (see Figure 5-2).

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.

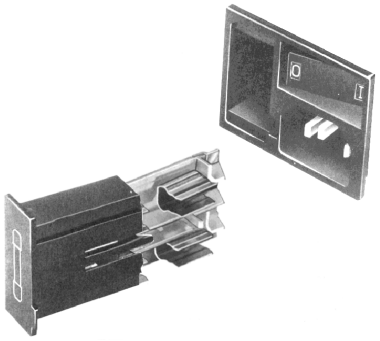
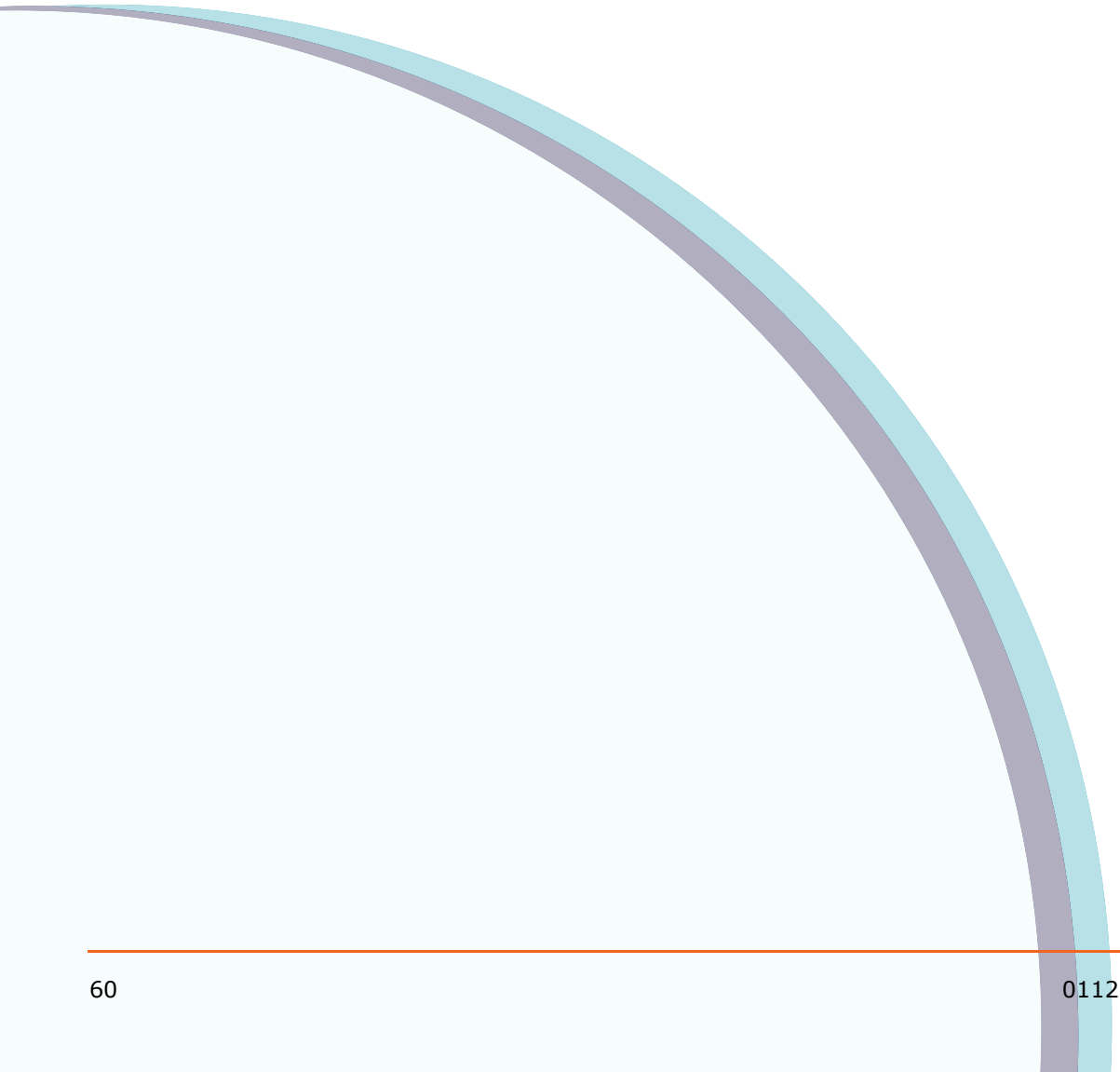


Figure 5-2 The fuse box and holder with fuses removed.

8. Insert new SLOWBLOW-rated fuses into the fuse holder. Either end of the fuse may be forward.
9. Insert the fuse holder into the fuse box, making sure that the fuses face toward the right (toward the tongue on the cover) as you insert it. Slide the fuse holder all the way into the box.
10. 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.
11. Reconnect the power cord to the instrument and to the wall outlet and reconnect other cables previously disconnected.



Troubleshooting

6

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 Chapter 1.

Note: In this user guide, SpectraMax® refers to several SpectraMax Multi-Mode Microplate Readers including the M3, M4, M5, and M5^{e}. When a feature or capability applies to only certain readers, this exception is noted.

For problems with SpectraMax Multi-Mode Microplate Readers 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.

WARNING! 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.

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.

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.

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; they 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.

Table 6-1 SpectraMax® 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 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. (for example, closing the cuvette door during the power up sequence prevents errors 111, 219, 302, and 310).

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 Software, no errors should be in the buffer (except error number 100).

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

Table 6-2 Error codes, error messages, and notes about the errors

ERROR CODE	ERROR MESSAGE	NOTES
100-199: Operator Errors		
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. Example: Request a wavelength while the monochromator is in motion.
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	assert failed	Firmware error.

Table 6-2 Error codes, error messages, and notes about the errors

ERROR CODE	ERROR MESSAGE	NOTES
200-299: Firmware Errors		
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.
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.
211	bootup fpga check failure	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 60°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 ref 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.

Table 6-2 Error codes, error messages, and notes about the errors

ERROR CODE	ERROR MESSAGE	NOTES
305	reference level saturation	During a cuvette read, could be due to cuvette door being open.
306	plate air cal fail	Minimum signal/reference ratio not met during plate air calibration.
307	cuv air ref fail	Measurement error during cuvette air reference read.
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.
310	PMT over current	PMT pre-flash value is outside of tolerated range.
311	PMT auto-range fail	When using auto PMT-A setting, PMT voltage limit was reached during read. When using auto PMT-C, even at the lowest PMT voltage levels, the read had saturated wells.
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	Lamp check upon power-up failed.
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	Error found during periodic check of ADC system.
318	PMT cal coef check failed	PMT calibration measurement is >20% different from previous good value.
320	Absorbance boot check failed	Plate or cuvette check failed.

Table 6-2 Error codes, error messages, and notes about the errors

ERROR CODE	ERROR MESSAGE	NOTES
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.
403	stage error	Stage did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.
404	shutter error	One of the shutters has problems performing its action.
405	polarization filter error	Problem with actuating the polarization filter mechanism.
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).
507	NVRAM PMT Cal Volts bad	
508	NVRAM PMT Sensitivity Cal error	

SpectraMax® Multi-Mode Microplate Reader Performance Specifications

Thermal specifications for microplates used in the SpectraMax® Multimode Microplate Reader apply to flat-bottom microplates with isolated wells. All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

Note: In this user guide, all references to SpectraMax Multi-Mode Microplate Readers include the M3, M4, M5, and M5^{e} models. When a feature or capability applies to only certain readers, this exception is noted.

Performance specifications for cuvette readings apply only to aqueous solutions having solute molal concentrations less than 0.4 M.

When pathlength compensation is applied to microplate absorbance measurements, agreement with cuvette absorbance measurements for the same solution requires that the solution volume in the microplate well is between 100 µL and 300 µL.

Technical specifications are subject to change without notice.

Table A-1 Technical Specifications

ABSORBANCE PHOTOMETRIC PERFORMANCE	
Wavelength range	200–1000 nm
Wavelength selection	Monochromator tunable in 1-nm increments
Wavelength bandwidth	≤ 4.0 nm full width half maximum
Wavelength accuracy	± 2.0 nm across wavelength range
Wavelength repeatability	± 0.2 nm
Photometric range	0.0 to 4.0 OD
Photometric resolution	0.001 OD
Photometric accuracy/linearity, 0–2.0 OD	< ± 1.0% and ± 0.006 OD
Photometric precision (repeatability), 0–2.0 OD	< ± 1.0% and ± 0.003 OD

Table A-1 Technical Specifications (cont'd)

Stray light	≤ 0.05% at 230 nm
Photometric stabilization	Instantaneous
Photometric drift	None — continuous referencing of monochromatic input
Calibration	Automatic before first kinetic read and before every endpoint reading
Optical alignment	None required
Light source	Xenon flash lamp (50 Watts)
Average lamp lifetime	1 billion flashes
Photodetectors	Silicon photodiode
Endpoint baseline noise (cuvette)	± 0.003 OD @190, 405, 850 nm
Endpoint kinetic noise (cuvette)	± 0.003 OD @190, 405, 850 nm ≥ 0.2 mOD/min and ≤ 0.2 mOD/min
FLUORESCENCE INTENSITY PERFORMANCE	
Sensitivity	Top Read < 5 pM FITC, 1 fmol/200 µL(96), < 20 pM, 2 fmol/100 µL(384) Bottom Read < 20 pM FITC(96)
Sensitivity (cuvette)	< 15 pM fluorescein
Wavelength range	250–850 nm
Wavelength selection	Monochromators, tunable in 1-nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Number of excitation/emission pairs per plate	4
Dynamic range	10^6 in 96-well black plates: auto gain circuitry
System validation	Self-calibrating with built-in fluorescence calibrators
Light source	Xenon flash lamp (1 joule/flash)
Average lamp lifetime	2 years normal operation
Detector	Photomultiplier (R3896)

Table A-1 Technical Specifications (cont'd)

FLUORESCENCE POLARIZATION PERFORMANCE	
Wavelength range (M5 and M5e models only)	400–750 nm
Wavelength selection	Monochromators, tunable in 1-nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Precision	< 5 mP standard deviation at 1 nM fluorescein in 96 and 384 wells
TIME-RESOLVED FLUORESCENCE PERFORMANCE	
Sensitivity (M4, M5, M5e models only)	100 fM europium in 96 or 384 wells (top read)
Wavelength range	250–850 nm
Bandwidth (excitation, emission)	9 nm, 15 nm
Precision data collection	1–100 flashes; delay of 0–600 µs before read; integration time selectable 50–1500 µs
LUMINESCENCE PHOTOMETRIC PERFORMANCE	
Sensitivity	< 2 fg/well for firefly luciferase in 96- and 384-well top read
Wavelength range	250–850 nm
Crosstalk	< 0.5% in 96- and 384-well microplates
PHOTOMETRIC ANALYSIS MODES	
Front Panel Operation	Single wavelength Absorbance, %Transmittance, Fluorescence reading of the cuvette (or test tube)
Using SoftMax Pro	Express data as Absorbance, %Transmittance, Fluorescence, Luminescence Single wavelength reading of microplate and/or cuvette Multiple wavelength (up to four) reading of microplate or cuvette Kinetic and kinetic graphics of microplate and/or cuvette Spectral scan (190–1000 nm) of microplate and/or cuvette Well scan of microplate using absorbance or fluorescence intensity

Table A-1 Technical Specifications (cont'd)

MEASUREMENT TIME (CALIBRATION OFF)	
Microplate read time (endpoint), Standard read	96 wells in 24 seconds (single wavelength, absorbance) 96 wells in 15 seconds (single wavelength, fluorescence intensity) 384 wells in 1:57 minutes (single wavelength, absorbance) 384 wells in 45 seconds (single wavelength, fluorescence intensity)
Microplate read time (endpoint), Standard read with PathCheck Pathlength Measurement Technology	96 wells in 2:07 minutes (single wavelength, absorbance) 384 wells in 7:19 minutes (single wavelength, absorbance)
Microplate read time (endpoint, Speed read	96 wells in 18 seconds (single wavelength, absorbance) 384 wells in 49 seconds (single wavelength, absorbance)
SCAN SPEED	
Cuvette: Normal scan	45*K nm/min (K = wavelength interval)
Cuvette: Speed scan	130*K nm/min
Wavelength repeatability	± 0.2 nm
TEMPERATURE REGULATION	
Reading chamber	Isothermal when temperature regulation is not enabled
Range	4°C above ambient to 60°C when temperature regulation enabled. The ambient temperature must be > 20°C to achieve temperature regulation at 60°C.
Resolution	± 0.1°C
Accuracy	± 1.0°C for microplate and cuvette chamber
Temperature uniformity at equilibrium	± 0.5°C at 37°C
Chamber warm-up time	15–30 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	4 sensors
Drift	± 0.2°C (regulated)

Table A-1 Technical Specifications (cont'd)

Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling
Recommended microplate	Flat-bottom microplates with isolated wells and lid
Control	Front panel reports cuvette chamber temperature only (temperature for microplate chamber reported in SoftMax® Pro Software)
AUTOMIX WITH SOFTMAX PRO	
Plate mixing modes	Selectable: off, once prior to any reading, and once prior to and between kinetic readings
Plate mixing duration	Selectable: 0 to 999 seconds (three-second default)
COMPATIBILITY	
Microplates	Standard 6- to 384-well flat-bottomed microplates. Polystyrene plates for absorbance wavelengths above 340 nm; UV-transparent plates for absorbance readings above 220 nm; quartz plates for absorbance readings above 200 nm; low-volume 384-well plates. Use purple adapter plate only with 96- and 384-well plates.
Cuvettes	Standard height (45 mm) cells with 10 mm pathlength (12.5 mm x 12.5 mm outside) with minimum inside width of 4 mm (typical for 3 mL volume cells). See Cuvettes on page 78 for more information.
Test tubes	12 x 75 mm test tubes can be used in the cuvette chamber with the test tube cover.
GENERAL INSTRUMENT	
Display	2-x-20-character backlit LCD
Operating panel	11-key membrane keypad
Self-diagnosis	Continuous on-board diagnostics

Table A-1 Technical Specifications (cont'd)

Spill control	Drawer mechanism and reading chamber assembly protected from accidental spillage by drainage ports
Computer interface	8-pin DIN RS-232 serial (double shielding required)
Printer interface	Parallel 25-pin to Centronics (double shielding required)
Microplates supported	All 6- to 384-well and strip-well microplates, including lids
ROBOTICS AND AUTOMATION	
Robot compatible drawer	Positioning and plate gripping as drawer closes
Integrated automation interface	SoftMax® Pro Software automation interface integrated with robot partners. SpectraMax® Multi-Mode Microplate Readers and SoftMax Pro Software are the #1 choice of robotic partners and robots. Please visit the Molecular Devices web site for more information: www.moleculardevices.com.
ENVIRONMENTAL	
Operating temperature	15°C to 60°C
Operating humidity	0 to 70%, non-condensing
Storage temperature	-20°C to 65°C
PHYSICAL	
Size (h x w x d)	8.6" (220 mm) x 22.8" (580 mm) x 15.3" (390 mm)
Weight	36 lb (16.4 kg)
Power consumption	< 420 W
Line voltage and frequency	100–240 VAC autoranging, 3.5 A, 50/60 Hz

System Diagrams and Dimensions

Dimensions are shown in inches (millimeters).

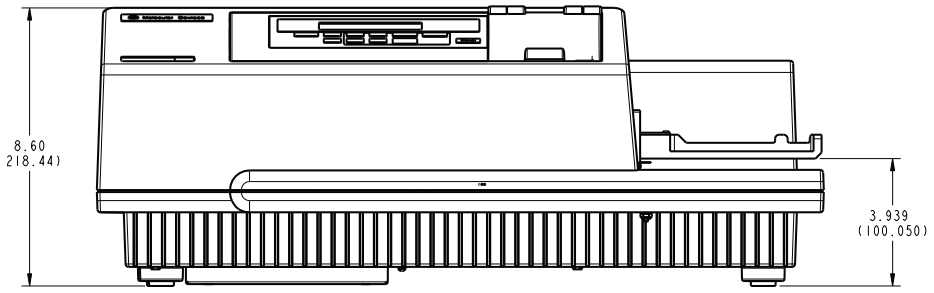


Figure A-1 Front view of a SpectraMax ® reader

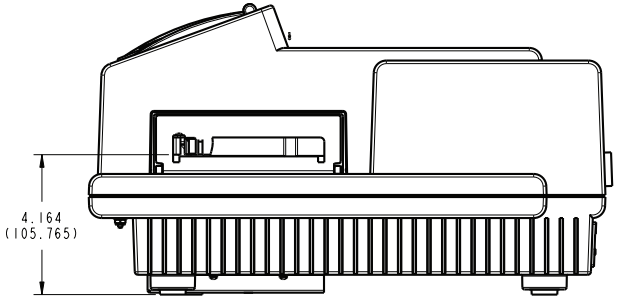


Figure A-2 Side view of a SpectraMax ® reader

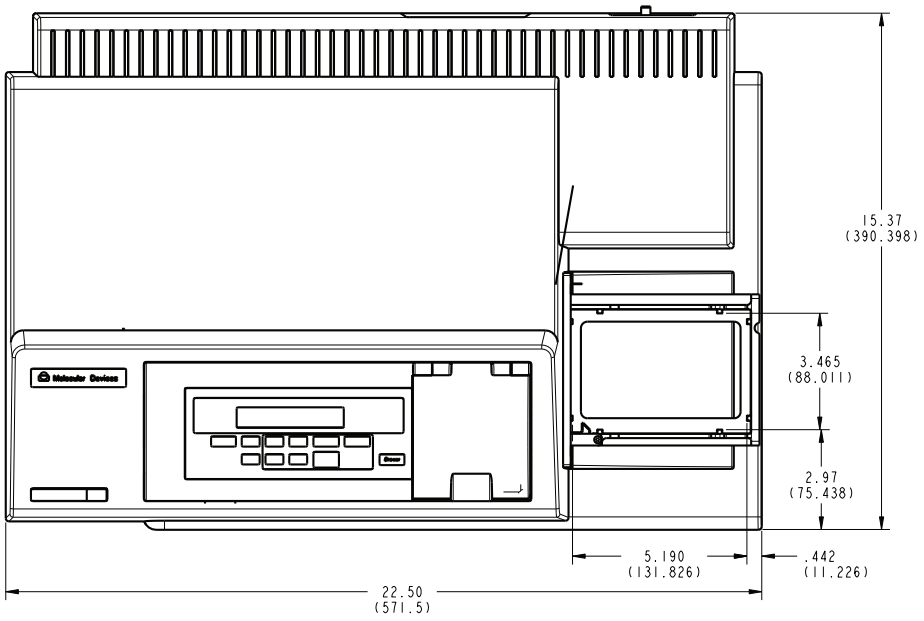


Figure A-3 Top view of a SpectraMax ® reader

Common Fluorescence and Luminescence Wavelengths

Values in the following tables are based on the literature. You must scan the fluorochrome of interest in the SpectraMax M5 or M5e reader to determine the optimal excitation and emission wavelengths for your application. Excitation and emission wavelengths listed by fluorochrome manufacturers are generally determined in methanol and do not reflect actual values, due to changes in pH, salt content, etc.

Fluorescence

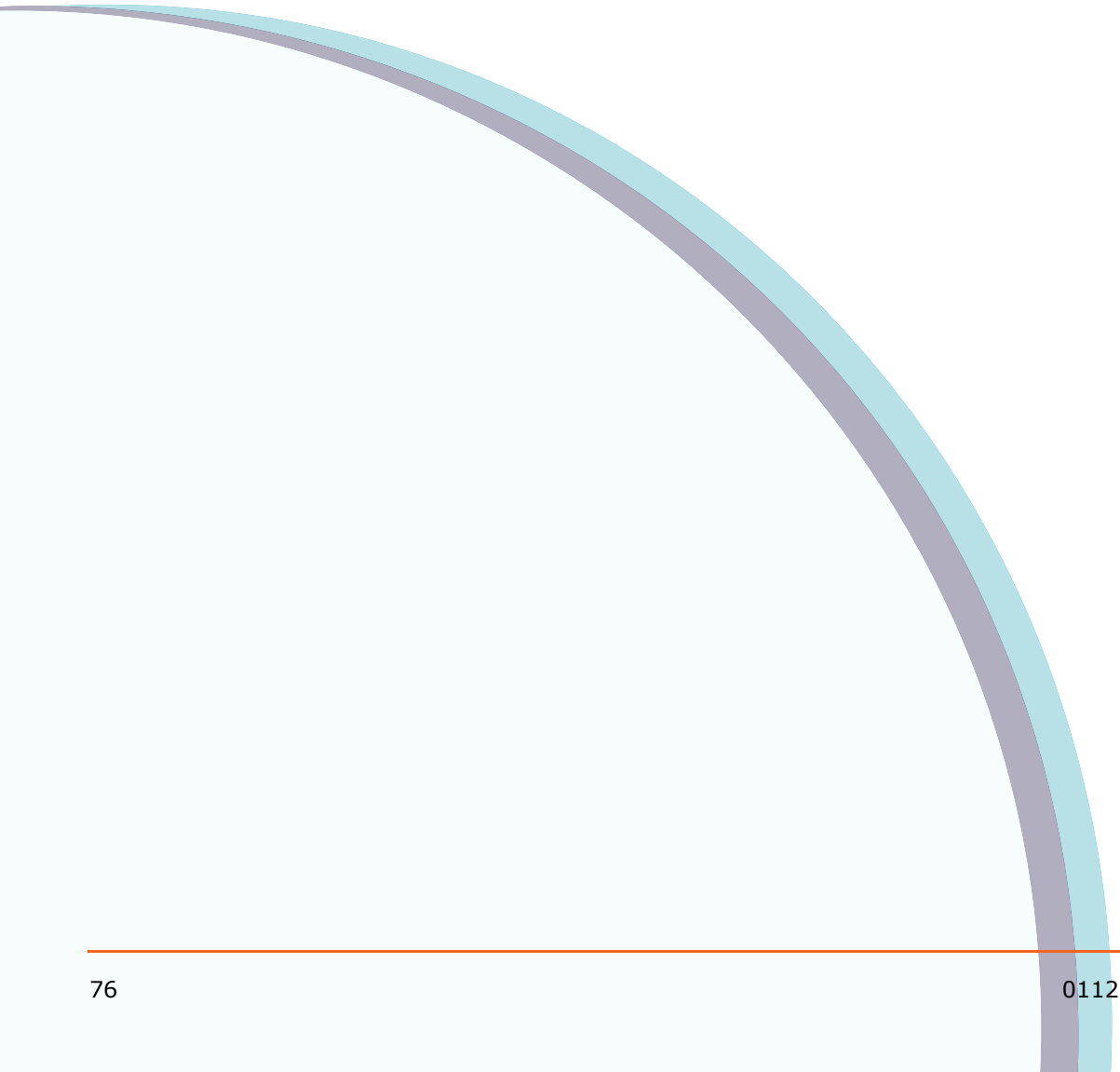
Fluorophore	Excitation Wavelength (nm)	Emission Wavelength (nm)
HPPA	320	405
4-MeU, NADH, NADPH	355	460
Biotinidinase	355	544
PKU	390	485
Green Fluorescent Protein	390	510
Attophos /Attofluor	444	555
FITC	485	538
Ethidium Homodmer (DNA)	530	620
TRITC, Ethidium Bromide	544	590
Texas Red	584	612
TAMRA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

Time-resolved Fluorescence

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

Luminescence

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



Cables and Accessories

B

Cables

Molecular Devices recommends that you use high-quality, double-shielded cables to connect your SpectraMax® reader to the computer. Choose cables that meet the requirement described in this appendix.

Note: In this user guide, all references to SpectraMax Multi-Mode Microplate Readers include the M3, M4, M5, and M5e models. When a feature or capability applies to only certain readers, this exception is noted.

Serial Interface Cable

The serial interface cable used to connect the instrument to the computer is a custom cable designed and built by Molecular Devices. Please use the cable supplied by Molecular Devices, or contact Molecular Devices for specific pin-out requirements:

Male DB8 to Female DB9 (custom cable made by Molecular Devices, PN 9000-0149)

USB Adapter Cable

Macintosh computers, and many newer Windows-based 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 B-1 Molecular Devices' custom serial cable (left) and a serial-to-USB converter (right).

Accessories

Description	Part #
SpectraTest ABS1 Absorbance Validation Test Plate	0200-6117
SpectraTest FL1 Fluorescence Validation Test Plate	0200-5060
Cuvette Absorbance Validation Kit	9000-0161
SpectraPlate—Quartz UV-transparent microplate	R8024
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
Test Tube Cover	2300-0277

Cuvettes

The guidelines for cuvette use in the SpectraMax Multi-Mode Microplate Readers are the same that apply to any high-quality spectrophotometer. The user must ensure that the meniscus is comfortably above the light beam in standard cuvettes and that the sample chamber in a microcuvette is aligned properly with the beam. The light beam is 0.625 in (15.87 mm) above the cuvette bottom.

Below are some cuvettes that have been tested. All have an optical pathlength of 1 cm (10 mm) and standard external dimensions (12.5 mm x 12.5 mm). Their fill volumes differ only because of their different internal width and chamber height dimensions.

Standard and Semi-micro Cuvettes

Note: Several brands are available at Hellma: http://www.hellma-worldwide.com.)

Internal Width	Minimum Volume	Maximum Volume
10 mm	~ 1.80 mL	4.0 mL
4 mm	~ 0.75 mL	1.4 mL
2 mm	~ 0.40 mL	0.7 mL

Ultra-micro Cuvettes (Hellma)

When ordering, specify the Z-dimension to be 15 mm.

Hellma Cat. No.	Window Size	Chamber Volume	Fill Volume
105.201-QS	2.0 x 5.0 mm	100 µL	120 µL
105.202-QS[1]	2.0 x 2.5 mm	50 µL	70 µL
105.210-QS[2]	0.8 mm diameter	5 µL	10 µL

1. You must put a riser (0.8–1 mm) on cuvette bottom to match the cuvette window to the beam.

2. You must put a riser (0.8–1 mm) on cuvette bottom to match the cuvette window to the beam. Gives good qualitative results (i.e. spectral scans), but quantitative results are impractical because the window is smaller than the beam.

Standard, Semi-micro, and Microcuvettes (Hellma)

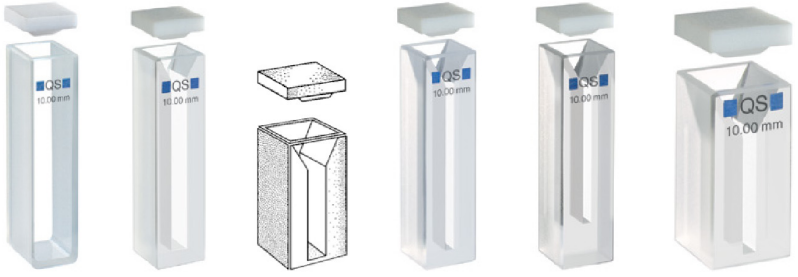


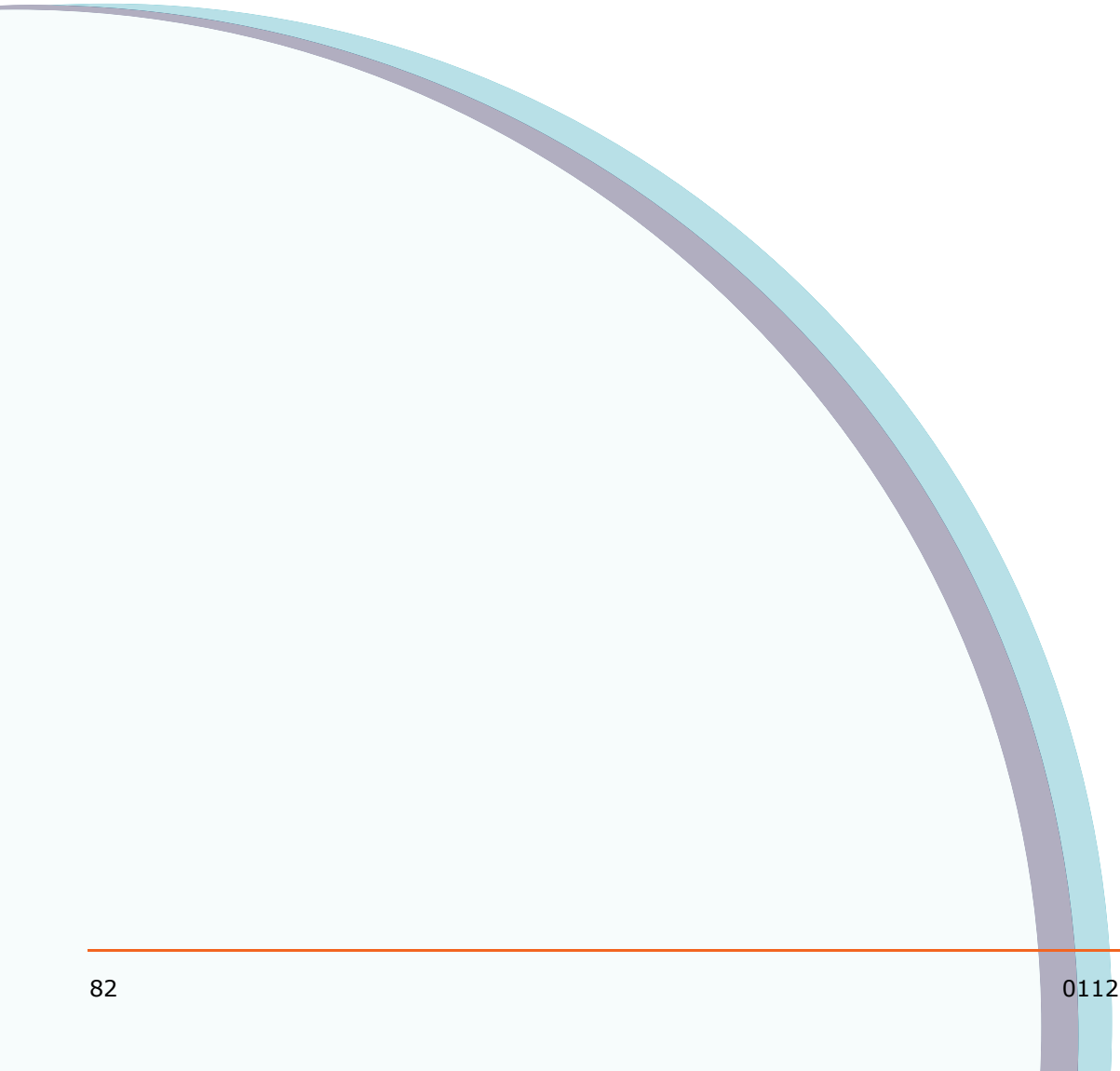
Figure B-2 Standard, semi-micro, and microcuvettes.

	Standard	Semi-micro		Micro		
Hellma Cat. No.	100	104	105.004	104.002	108.002	105
Internal Dimensions	10 x 10	4 x 10	4 x 10	2 x 10	2 x 10	2 x 10
Fill Volume	4 mL	1.4 mL	600 µL	700 µL	500 µL	300 µL

Ultra-micro Cuvettes (Hellma)

Figure B-3 Ultra-microcuvettes.

Hellma Cat. No.	105.200	105.201	105.202	105.210
Optical Pathlength	10 mm	10 mm	10 mm	10 mm
Fill Volume	180 µL	120 µL	70 µL	10 µL



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