Molecular Devices

SpectraMax® M2/M2e user guide

A MULTI-DETECTION MICROPLATE READER WITH TWO-MODE CUVETTE PORT

SpectraMax® M2
SpectraMax® M2^{e}

Multimode Plate Readers
User Guide

Molecular Devices

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1. Description

1.1. INTRODUCTION

The SpectraMax M2 and SpectraMax M2^e Multi-detection Readers are monochromator-based microplate readers that have 6-well to 384-well microplate reading capability as well as a built-in absorbance and fluorescence cuvette port. The spectrophotometric performance of the SpectraMax M2 and SpectraMax M2^e is similar to the SpectraMax Plus, a dedicated absorbance plate reader. The fluorometric performance of the SpectraMax M2 is similar to that of the Gemini XPS, a dedicated top-read fluorescence microplate reader. The fluorometric performance of the SpectraMax M2^e is similar, but slightly superior to that of the Gemini EM, a dedicated top and bottom read fluorescence microplate reader.

SpectraMax M2 and SpectraMax M2^e readers can acquire absorbance as well as fluorescence data for samples by issuing a single read command in SoftMax® Pro. Dual monochromators allow selection of any absorbance wavelength between 200 nm and 1000 nm, any excitation wavelength between 250 nm and 850 nm, and any emission wavelength between 360 nm and 850 nm (SpectraMax M2) or 250 n and 850 nm (SpectraMax M2^e) for readings of both microplates and cuvettes.

1.1.1. 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 M2 and SpectraMax M2^e 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.

The SpectraMax M2 and SpectraMax M2^e also have two secondary modes that can be used for limited development of glow luminescence or time-resolved fluorescence assays. The performance of these two modes is not comparable to dedicated luminescence or time-resolved fluorescence instruments, or multimode readers such as the SpectraMax M5, Analyst HT or Analyst GT.

1.1.2. OPTICS

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

1.1.3. 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.

1.1.4. PATHCHECK

SpectraMax M2 and SpectraMax M2^e with PathCheck® Sensor allow normalization of variable well volumes to 1-cm cuvette readings. PathCheck allows for multichannel pipettor validation and for experiment comparison from different days.

1.1.5. AUTOMIX

Using SoftMax Pro, 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).

1.1.6. TEMPERATURE CONTROL

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

1.1.7. SUPPORTED PLATES

Microplates having 6, 12, 24, 48, 96, and 384 wells can be used in the SpectraMax M2 and SpectraMax M2^e. Top detection is available for fluorescence detection on the SpectraMax M2, while top and bottom reads are possible on the SpectraMax M2^e. 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.

1.1.8. COMPUTER CONTROL

SpectraMax M2 and SpectraMax M2^e are controlled by an external computer running SoftMax Pro software which provides integrated instrument control, data display, and statistical data analysis. Cuvette port functionality can be accessed using SoftMax Pro software.

The on-board microprocessor calculates and reports the absorbance, % transmittance, and/or fluorescence for each well of a microplate or from the cuvette port. Data from multiple wavelengths can be acquired and ratio analysis can be performed during a single reading, if desired, and different calculations can be made based on this data using SoftMax Pro software, including the subtraction of blanks, use of standard curves, etc.

For detailed reader specifications, refer to the chapter "Specifications" in this guide.

1.2. COMPONENTS

The main components of the SpectraMax M2 and SpectraMax M2^e are:

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

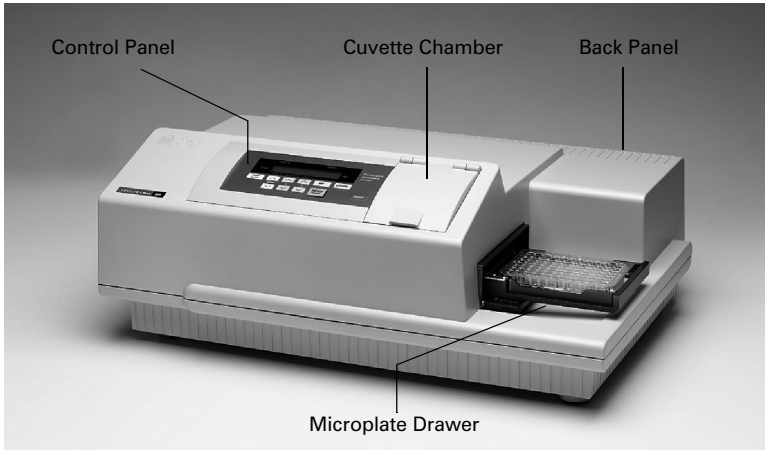


Figure 1.1: SpectraMax M2 and SpectraMax M2^e components.

1.2.1. THE CONTROL PANEL

The control panel consists of a 2x20-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 that settings made in SoftMax Pro software override control panel settings.

The left side of the display shows the cuvette temperature, both actual and set point, and whether or not the temperature is at the set point (enunciator blinks if not at set point). To see the microplate chamber temperature, you must use SoftMax Pro software.

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 on the control panel, press %T/A/RFU and the temperature up or down setting keys.

Figure 1.2: SpectraMax M2 and SpectraMax M2^e control panel.

Temp On/Off Key

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 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 Key

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

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

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

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

λ Key

Selects the wavelength to be used for reading the microplate manually. The control panel does not display the wavelength selected through SoftMax Pro.

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 Key

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.

Read Cuvette Key

Initiates the sample reading of the cuvette.

%T/A Key

A toggle switch used to display cuvette data as percent transmission or absorbance.

Drawer key

The DRAWER key opens and closes the microplate drawer.

1.2.2. 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.

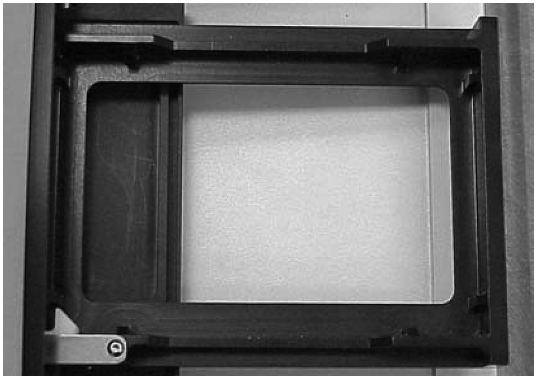


Figure 1.3: The microplate drawer.

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 (refer to the chapter “Troubleshooting” in this guide).

1.2.3. MICROPLATES

The SpectraMax M2 and SpectraMax M2^e 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 SoftMax Pro 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 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.

1.2.4. THE CUVETTE CHAMBER

Located at the right front of the SpectraMax M2 and SpectraMax M2^e, 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.

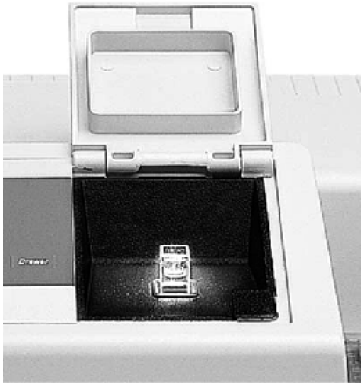


Figure 1.4: The cuvette chamber.

Cuvettes

The SpectraMax M2 and SpectraMax M2^e can accommodate standard-height (45 mm), 1-cm cuvettes and 12 x 75 mm test tubes when used with the test tube cover (Figure 1.5).

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.

Figure 1.5: Test tube cover.

1.2.5. THE BACK PANEL

The following components are located on the back panel of the SpectraMax M2 and SpectraMax M2^e:

- **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 in the SpectraMax M2 or SpectraMax M2^e.
- **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.

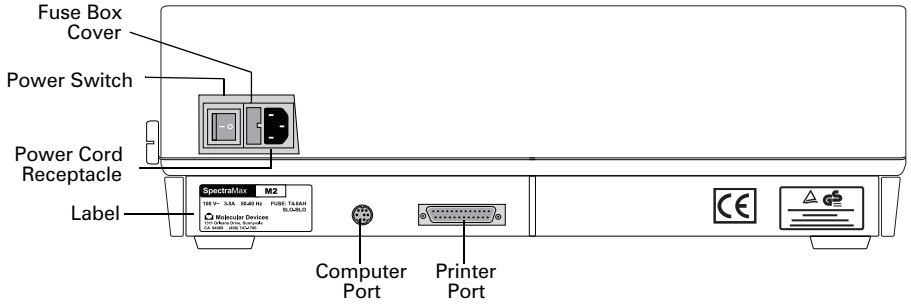


Figure 1.6: Schematic of the back panel of SpectraMax M2 and SpectraMax M2^e.

2. Principles of Operation

2.1. ABSORBANCE

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.

$$A = log(I_0/I)$$

where I_0 is incident light, and I is transmitted light.

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

2.2. 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.

2.3. TRANSMITTANCE

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

$$T = (I_0/I)$$
$$\%T = 100T$$

where I_0 is incident light, and I is transmitted light.

2.4. PATHCHECK

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 absorbtivity 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 (*e.g.* 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 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.

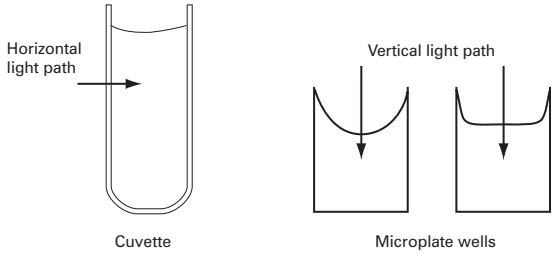


Figure 2.1: Cuvette and microwell light paths.

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.

PathCheck pathlength correction is accomplished only when using the SoftMax Pro software. PathCheck is patented by Molecular Devices and can be performed only on an MDC plate reader.

The SpectraMax M2 and SpectraMax M2^e offer 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 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 can also be used with samples containing small amounts of organics or high buffer concentrations by using the Cuvette Reference (below).

2.4.1. WATER CONSTANT OR CUVETTE REFERENCE?

The PathCheck 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-interfering 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 SoftMax Pro, 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 calculations for the microplate samples.

Use of Cuvette Reference with PathCheck 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 SoftMax Pro). The cuvette reference used for PathCheck 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.

2.4.2. BACKGROUND CONSTANT SUBTRACTION AND BLANKING 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 calculation in order to get obtain PathCheck-normalized results. There are three ways to accomplish this—plate blanks, plate background constants, and plate pre-reads—all of which are described in the PathCheck section of the SoftMax Pro User Guide.

2.4.3. PATHCHECK AND INTERFERING SUBSTANCES

Any material that absorbs in the 900 nm to 1000 nm spectral region could interfere with PathCheck 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 PathCheck. 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 PathCheck. 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 PathCheck.

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 feature. Use of Cuvette Reference does not correct for the interference with the current calculation scheme in SoftMax Pro. Currently, Cuvette Reference involves a single (automated) read at 900 nm and 1000 nm and the automated calculations in SoftMax Pro 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 PathCheck if they have absorbance in the region of the NIR water peak. Solvents such 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 previously. 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 PathCheck.

2.4.4. MAKING ABSORBANCE MEASUREMENTS NORMALIZED TO A 1-CM PATHLENGTH

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

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.

2.5. 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. The figure below 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.

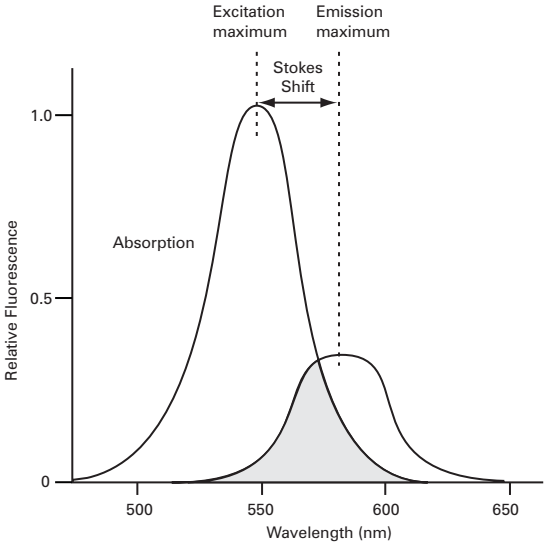


Figure 2.2: Excitation and emission spectra.

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 M2 and SpectraMax M2^e incorporate many features designed to restrict interference from reflected excitation light. Among these features is a set of long-pass emission cutoff filters that can be set automatically by the instrument or manually by the user. If the Stokes shift is small, it may be advisable to choose an excitation wavelength that is as far away from the emission maximum as possible while still being capable of stimulating the fluorophore so that less of the excited light overlaps the emission spectrum, allowing better selection and quantitation of the emitted light.

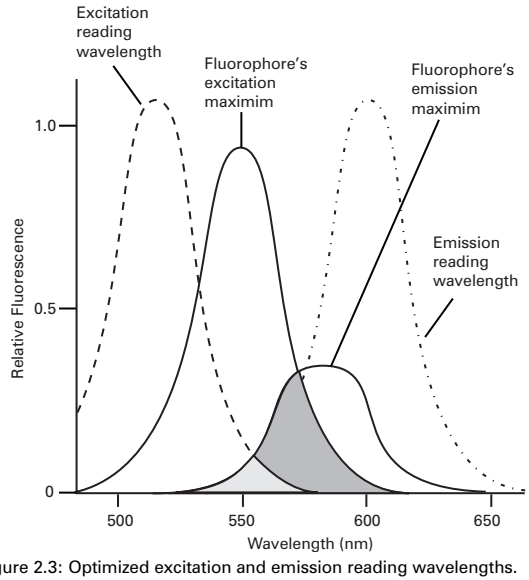


Figure 2.3: Optimized excitation and emission reading wavelengths.

The figure above 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 M2 and SpectraMax M2^e allow scanning of both excitation and emission wavelengths, using separate tunable monochromators. One benefit of being able to scan emission spectra is that you can assess more accurately whether the emission is, in fact, the expected fluorophore, or multiple fluorophores, and not one generated by a variety of background sources or by contaminants. Another benefit is that you may be able to find excitation and emission wavelengths that avoid interference when interfering fluorescent species are present.

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

For more information regarding optimizing excitation and emission wavelengths using the spectral scanning capabilities of the SpectraMax M2 and SpectraMax M2^e, refer to the section "Optimizing Fluorescence Assays" of the chapter "Operation" in this guide.

2.6. TIME-RESOLVED FLUORESCENCE

Time-resolved fluorescence is a secondary mode for the SpectraMax M2 and SpectraMax M2^e, so performance is not comparable to instruments that specialize in time-resolved fluorescence, such as the SpectraMax M5, Analyst HT and Analyst GT.

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

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

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

2.7. LUMINESCENCE

The SpectraMax M2 and SpectraMax M2^e are microplate spectrofluorometers with photomultiplier tube detection. Some luminescence applications, such as gene reporter assays, may require a luminometer with photon counting detection for greater sensitivity, such as the SpectraMax M5, Analyst GT or Analyst HT, or LMax II.

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

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

2.8. FUNCTIONAL DESCRIPTION

The full power of a SpectraMax M2 and SpectraMax M2^e 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 User Guide.

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

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

2.8.1. TEMPERATURE REGULATION

The SpectraMax M2 and SpectraMax M2^e have been designed to regulate the temperature of the microplate chamber from 4°C above ambient to 45°C. Upon power up, when the incubator is off, the temperature in the microplate chamber is ambient and isothermal. Turning on the incubator by pressing the TEMP on/off key causes the instrument to begin warming the microplate chamber. The temperature set point defaults to 37.0°C at start-up.

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

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

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

Temperature regulation and control of the microplate chamber is achieved through electric heaters, a fan, efficient insulation, and temperature sensors. The heaters are located in the microplate chamber, which is insulated to maintain the temperature set point. The sensors are mounted inside the chamber and measure the air temperature.

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

2.8.2. READ TYPES

The SpectraMax M2 and SpectraMax M2^e can perform four types of read: endpoint, kinetic, spectrum and well scan. Instrument setup parameters for each read type are discussed in the SoftMax Pro User Guide.

Endpoint Read

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

Depending on the read mode selected, 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.

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 190 nm to 1000 nm. All spectrum readings are made using the scanning monochromators of the instrument.

Well Scan

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).

2.8.3. AUTOMIX

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

- **Endpoint mode:** Automix shakes the plate for a definable number of seconds and then reads at all selected wavelengths.
- **Kinetic mode:** two types of Automix can be enabled: Automix can shake the plate for a definable number of seconds before the initial reading, and/or for a definable number of seconds before each subsequent reading.

Use of Automix is strongly recommended for ELISAs and other solid-phase, enzyme-mediated reactions to enhance accuracy.

2.8.4. COMPUTER CONTROL

The SpectraMax M2 and SpectraMax M2^e are equipped with an 8-pin DIN RS-232 serial port through which the computer communicates with the instrument. (Different types of cables are available for connecting to different types of computers (refer to the section "Cables" in the Appendix of this guide).

3. Installation

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

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

⚠ **WARNING:** Do not operate the instrument in 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.

3.1. UNPACKING

The SpectraMax M2 and SpectraMax M2^e 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 M2 and SpectraMax M2^e weigh approximately 35 pounds (15.75 kg) and should be lifted with care. It is recommended that two persons lift the instrument together, taking the proper precautions to avoid injury.

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

3.2. SETTING UP THE INSTRUMENT

1. Place the 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.

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 , “Cables” 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.

3.3. INSTALLING THE DRAWER ADAPTER

⚠ **CAUTION:** Incorrect insertion or removal of the adapter may cause damage to the microplate drawer of the SpectraMax M2 or SpectraMax M2^e. The corner cutout must be in the lower left corner where the plate pusher is located.

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

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

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

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

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

Figure 3.1: Adapter inserted in microplate drawer.

3.4. REMOVING THE DRAWER ADAPTER

If the adapter is in the drawer and you are either reading from the bottom (SpectraMax M2^e only) or using "high profile" (6-well, 12-well, 24-well, or 48-well) plates, you need to remove the adapter.

Incorrect insertion or removal of the adapter may cause damage to the microplate drawer of the SpectraMax M2 or SpectraMax M2^e.

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

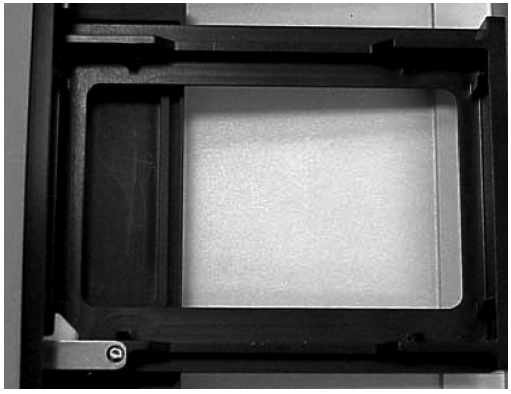


Figure 3.2: Microplate drawer without adapter.

4. Operation

4.1. 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 M2 or SpectraMax M2^e:

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 along with the temperature set point. To change the set point (to any setting from ambient +4° to 45°C), press the up or down arrow keys.
4. Select 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.

4.2. 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 M2 or SpectraMax M2^e:

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 along with the temperature set point. To change the set point (to any setting from ambient +4° to 45°C), press the up or down arrow keys.

4 Select the desired instrument settings (read mode, type of analysis, template, etc.) using SoftMax Pro software on the external computer.

5 If you are performing kinetic analysis, add substrate at this time.

6 Load the prepared microplate into the drawer, being sure to match well A1 with the A1 mark on upper left-hand corner of the drawer.

7 Using SoftMax Pro, start the reading.

4.3. PREPARING FOR A CUVETTE OR MICROPLATE READING

4.3.1. 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.

4.3.2. 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 here).

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

To enable the incubator:

› Press the incubator TEMP on/off key.

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

To change the temperature set point:

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

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.

4.3.3. SELECT THE WAVELENGTH

For a cuvette reading, press the up or down arrow keys above and below the λ to increment or decrement the wavelength setting in 1 nm increments.

4.4. 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 calibrate 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.

4.5. READ THE MICROPLATE

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

- 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, refer to the section “Installing the Drawer Adapter” in the chapter “Installation” in this guide for more information).
- You must have SoftMax Pro software running on a computer connected to the instrument. Press the READ button in SoftMax Pro to start the plate read.
- 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.
- If you return to the SpectraMax 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 plate reading, please consult the SoftMax Pro User Guide.

4.6. OPTIMIZING FLUORESCENCE ASSAYS

4.6.1. INTRODUCTION

The optimum instrument settings for detection of a particular fluorophore depend on a number of different factors. Settings that can be adjusted for assay optimization include the excitation and emission wavelengths, emission cutoff filter, readings per well, the PMT voltage, 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 (250–850 nm) and emission (SpectraMax M2: 360–850 nm; SpectraMax M2^e: 250–850 nm) wavelengths may be set in 1-nm increments within the range of the instrument. A procedure to optimize excitation and emission wavelengths for a given assay is outlined in the next section.

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 4.1 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 30 (for very precise measurements). The default number of readings per well varies with the read mode: for fluorescence, the default is 6, and for luminescence, the default is 30.

PMT Voltage

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

Temperature Control

The chamber of the SpectraMax M2 and SpectraMax M2^e is isothermal at ambient as well as at elevated temperatures. The temperature in the reading chamber may be adjusted from 4°C above ambient to 45°C.

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

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

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

2 Perform an excitation scan:

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

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

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

d 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.)

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

If an error message reporting missing data points occurs, it may be due to possible saturation reported by SoftMax Pro at the end of the spectral scan. Reset the PMT to "low" and re-scan 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 re-scan the sample. If the spectral scan still shows no apparent peak, adjust the Y-scale of the zoom plot so that the plot fills the graph.

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

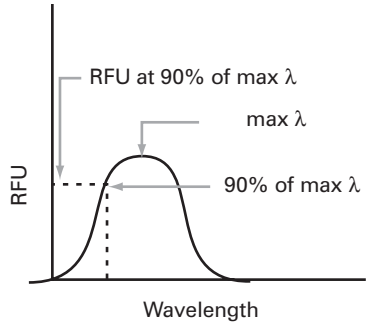


Figure 4.1: Plot of RFU vs. wavelength.

3 Perform emission scan #1:

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

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

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

d Set the step increment to 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.)

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

4 Choose the emission filter:

a Select an emission cutoff filter that blocks as much of the residual excitation light as possible without unduly reducing the fluorescence signal. The cutoff wavelength choices are 325, 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.

5 Perform emission scan #2:

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

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

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

6 The optimal excitation and emission wavelengths are those determined in steps 2f and 5b, above.

7 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 nm, experimental iteration is usually the best method of determining the optimal emission and excitation wavelengths. Begin optimization by performing steps 2–5 above. Try emission and excitation wavelength combinations with the 325 nm cutoff or with no cutoff filter. Similarly, for excitation wavelengths greater than 660 nanometers, try emission and excitation wavelength combinations with the 695 nm cutoff or with no cutoff.

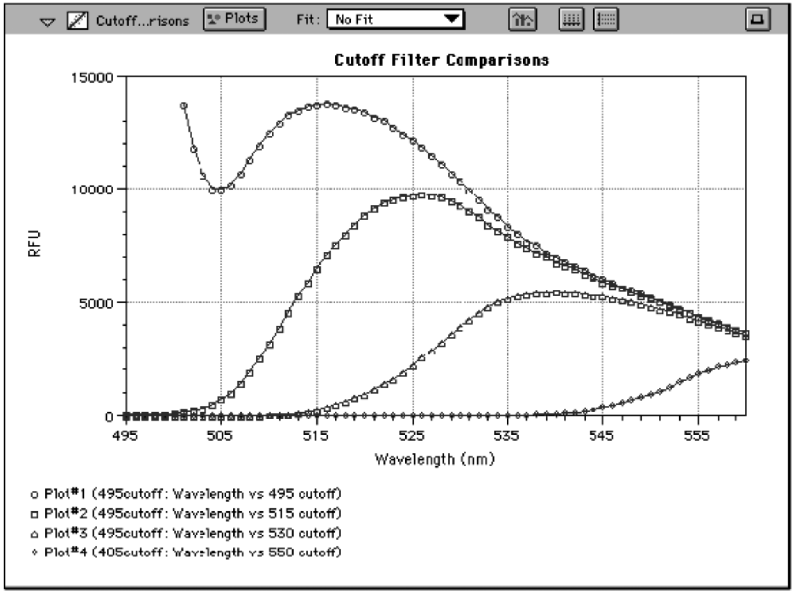


Figure 4.2: Effects of cutoff filters on fluorescein. Emission was scanned from 490 to 560 nm; excitation was fixed at 485 nm.

Figure 4.2 shows the effects of different cutoff filters on a scan of fluorescein where excitation was fixed at 485 nm and emission was scanned from 490 nm to 560 nm (buffer blanks are not shown in this plot). Table 4.1 (following) lists default settings for the emission cutoff filters. For spectrum mode, the default is "manual" (no automatic cutoff).

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
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

5. Maintenance

5.1. TECHNICAL SUPPORT

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

If you have any problems using your SpectraMax M2 or SpectraMax M2^{e}, in the U.S., contact the Molecular Devices Technical Services group at 1-800-635-5577; elsewhere contact your local representative.

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

⚠ **WARNING:** All maintenance procedures described in this user guide can be safely performed by qualified personnel. Maintenance not covered in this user guide should be performed only by a 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 use 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 the warranty.

5.2. MOVING A SPECTRAMAX M2 OR SPECTRAMAX M2^e

If you need to relocate a SpectraMax M2 or SpectraMax M2^e, follow these steps.

⚠ **WARNING:** The SpectraMax M2 and SpectraMax M2^e weigh approximately 35 pounds (15.75 kg) and should be lifted with care. It is recommended that two persons lift the instrument together, taking the proper precautions to avoid injury.

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 the section "Setting Up the Instrument" in the chapter "Installation" in this guide.

5.3. GENERAL MAINTENANCE

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.

5.4. CLEANING

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

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

Periodically, you should clean the outside surfaces of the 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.

5.5. CLEANING THE FAN FILTER

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

1. Turn power to the instrument OFF and then remove the power cord and cables from the back of the instrument.
2. Remove any plate or adapter from the instrument drawer. Turn the instrument over so that it rests flat on the bench.
3. Pop the black fan cover off and remove the filter.
4. Clean the filter by blowing clean, canned air through it or by rinsing it—first with water and then with alcohol—and allowing it to dry completely.
5. Place the clean, dry filter over the fan and replace the black cover.
6. Turn the instrument back over. Reconnect the power cord and cables to the instrument.

5.6. 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 will appear as shown in Figure 5.2. The holder inside contains two fuses.

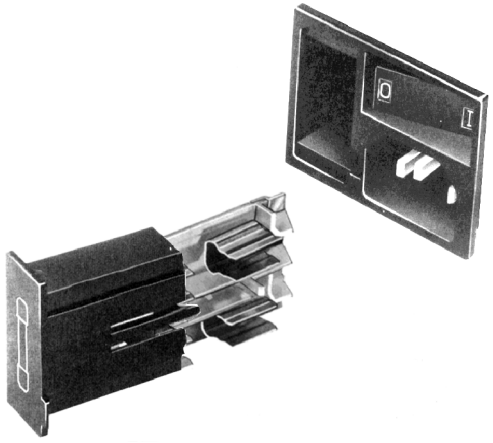


Figure 5.2: The fuse box and holder (with fuses), removed from instrument.

7 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 5.3).

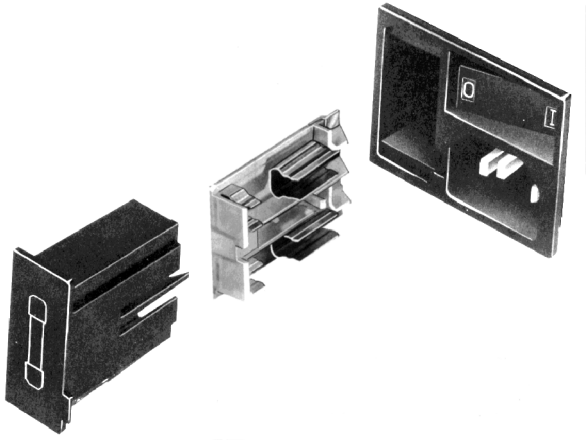


Figure 5.3: The fuse holder (with fuses), removed from the fuse box.

8 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.

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

10 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.

11 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.

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

6. Troubleshooting

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

Maintenance procedures are described in the previous chapter.

For problems with the SpectraMax M2 or SpectraMax M2^e that are not listed here, in the U.S., contact Molecular Devices Technical Services group at 1-800-635-5577; elsewhere, call your local representative.

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

6.1. OPENING THE DRAWER MANUALLY

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

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

6.2. ERROR CODES AND PROBABLE CAUSES

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

6.2.1. ERROR MESSAGES

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

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

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

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

6.2.2. ERROR CODE CLASSIFICATIONS

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

Table 6.1: SpectraMax M2 and SpectraMax M2^e error code ranges.

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

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

Check the following list to see if there is something that you can do to change the condition of the instrument to prevent the fatal error. (*e.g.*, 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, no errors should be in the buffer (except error number 100).

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

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

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

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

Error Code	Error Message	Notes
500–599: NVRAM ERRORS		
500	NVRAM CRC corrupt	The CRC for the NVRAM data is corrupt.
501	NVRAM Grating cal data bad	Grating calibration data is unreasonable.
502	NVRAM Cuvette air cal data error	Cuvette air calibration data is unreasonable.
503	NVRAM Plate air cal data error	Plate air calibration data is unreasonable.
504	NVRAM Carriage offset error	Carriage offset data is unreasonable.
505	NVRAM Stage offset error	Stage offset data is unreasonable.
506	NVRAM Battery	Time to replace the NVRAM battery (U3).

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

7. Specifications

7.1. SPECTRAMAX M2 AND M2^{e} PERFORMANCE SPECIFICATIONS

Thermal specifications for microplates used in the SpectraMax M2 and SpectraMax M2^{e} apply to flat-bottom microplates with isolated wells.

All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

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.

ABSORBANCE PHOTOMETRIC PERFORMANCE	
Wavelength range	200–1000 nm
Wavelength selection	Monochromator tunable in 1-nm increments
Wavelength bandwidth	≤ 4.0 nm full width half maximumëë
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
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 PHOTOMETRIC PERFORMANCE	
Detection limit (top read, microplate, SpectraMax M2 and SpectraMax M2^e)	3.0 fmol/well in 200 µL FITC 96 wells (15 pM) 3.0 fmol/well in 75 µL FITC 384 wells (40 pM)
Detection limit (bottom read, microplate, SpectraMax M2^e only)	5.0 fmol/well FITC 200 µL in 96 wells (25 pM) 5.0 fmol/well FITC 75 µL in 384 wells (67 pM)
Detection limit (cuvette)	15 pM fluorescein
Excitation wavelength range	250–850 nm
Emission wavelength range	360–850 nm (SpectraMax M2) 250–850 nm (SpectraMax M2^e)
Scanning provided over full range	in 1-nm increments
Number of excitation/emission pairs per plate	4
Bandwidth (excitation emission)	9/9 nm
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)

LUMINESCENCE PHOTOMETRIC PERFORMANCE	
Detection limit (384-well microplate)	10 amol/well alkaline phosphatase, 200 µL
Wavelength range	250–850 nm
TIME-RESOLVED FLUORESCENCE PERFORMANCE	
Detection limit (384-well microplate)	0.5 fmol/well Eu-Ch
Wavelength range	250–850 nm
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
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	• 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 45°C when temperature regulation enabled. The ambient temperature must be > 20°C to achieve temperature regulation at 45°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)
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)
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 the section "Cuvettes in SpectraMax M2 and SpectraMax M2e" in the Appendix 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	2x20-character backlit LCD
Operating panel	11-key membrane keypad
Self-diagnosis	Continuous on-board diagnostics
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 automation interface integrated with robot partners. SpectraMax and SoftMax Pro are the #1 choice of robotic partners and robots. Visit the Molecular Devices web site for more information at http://www.moleculardevices.com/pages/instruments/automation.html#spectramax.
ENVIRONMENTAL	
Operating temperature	15°C to 40°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" (380 mm)
Weight	35 lbs. (15.75 kg)
Power consumption	< 250 W
Line voltage and frequency	90–250 VAC autoranging, 50/60 Hz

A. Appendix

A.1. CABLES

Molecular Devices recommends that you use high-quality, double-shielded cables to connect your SpectraMax M2 or SpectraMax M2^e to the computer. Choose cables that meet the following requirements:

A.1.1. 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)

A.1.2. USB ADAPTER

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

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

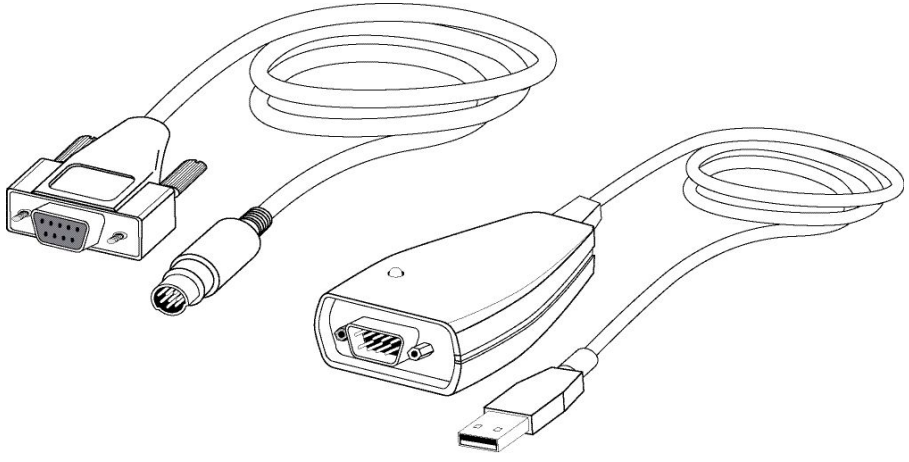


Figure A.1: Molecular Devices' custom serial cable (left) and a serial-to-USB converter (right).

A.2. ACCESSORIES

Description	Part #
SpectraTest 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

A.3. CUVETTES IN SPECTRAMAX M2 AND SPECTRAMAX M2^e

The guidelines for cuvette use in the SpectraMax M2 and SpectraMax M2^e are the same as those 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 cm x12.5 cm). Their fill volumes differ only because of their different internal width and chamber height dimensions.

A.3.1. STANDARD AND SEMI-MICRO CUVETTES

(Several brands available)[1]

Internal Width	Minimum Volume	Maximum Volume
10 mm	~ 1.8 mL	4.0 mL
4 mm	~ 0.75 mL	1.4 mL
2 mm	~ 0.40 mL	0.7 mL

A.3.2. ULTRA-MICRO CUVETTES (HELLMA)

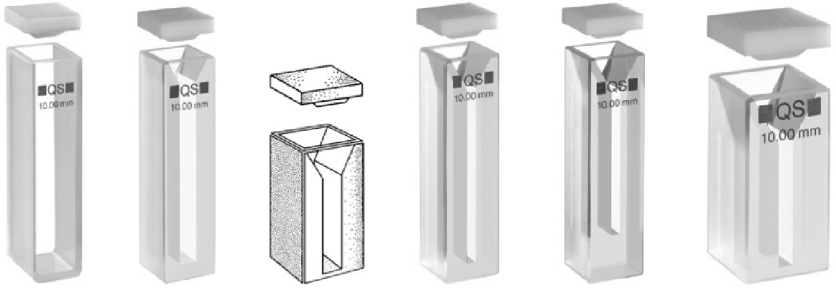
When ordering, specify the Z-dimension to be 15 mm.

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[a]	2.0 x 2.5 mm	50 µL	70 µL
105.210-QS[b]	0.8 mm diameter	5 µL	10 µL

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

1.Hellma: http://www.hellma-worldwide.com.

A.3.3. STANDARD, SEMI-MICRO AND MICROCUVETTES (HELLMA)



	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

A.3.4. ULTRA-MICRO CUVETTES (HELLMA)

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

A.4. COMMON WAVELENGTHS FOR FLUORESCENCE AND LUMINESCENCE

Values in this table are based on the literature. You must scan your fluorochrome of interest in the SpectraMax M2 or SpectraMax M2^e to determine the optimal excitation and emission wavelengths for your application. Excitation and emission wavelengths listed by

fluorochrome manufacturers are generally in methanol and do not reflect actual values due to changes in pH, salt content, etc.

A.5. FLUORESCENCE

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

A.6. TIME-RESOLVED FLUORESCENCE

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

A.7. LUMINESCENCE

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

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

A.8. GLOSSARY

Absorbance

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.

$$A = log(I_0 / I)$$

where I_0 is incident light, and I is transmitted light.

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

Automix

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

Emission Cutoff Filter

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

Endpoint

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

Emission Spectral Scan

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

Excitation Filter
Band pass filter that reduces the amount of extraneous lamp excitation light prior to the excitation monochromator. In endpoint reads and emission spectral scans, selection of excitation filter is automatic. In excitation spectral scans, the user has the choice of "no excitation filter" (for smoother scans) or "auto excitation filter" in which case there may be slight glitches in the spectrum at the wavelengths where filter changes occur.

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

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

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

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

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

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

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

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

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

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

Read Mode
The type of reading performed: absorbance, fluorescence or luminescence.

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

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

SoftMax Pro
An integrated software program from Molecular Devices Corporation that is used to control and collect data from all Molecular Devices plate readers.

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

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

A.9. SYSTEM DIAGRAMS AND DIMENSIONS

Dimensions are shown in inches (millimeters).

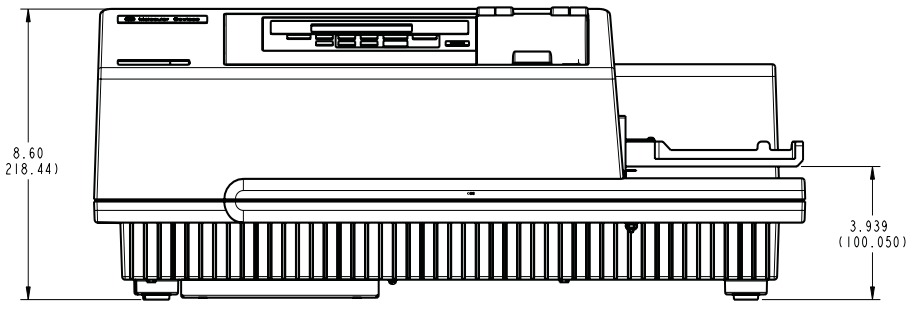


Figure A.2: Front view of SpectraMax M2.

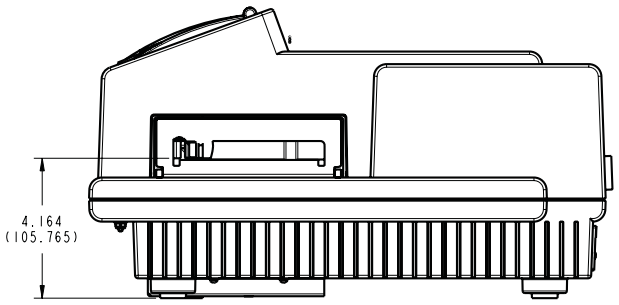


Figure A.3: Side view of SpectraMax M2.

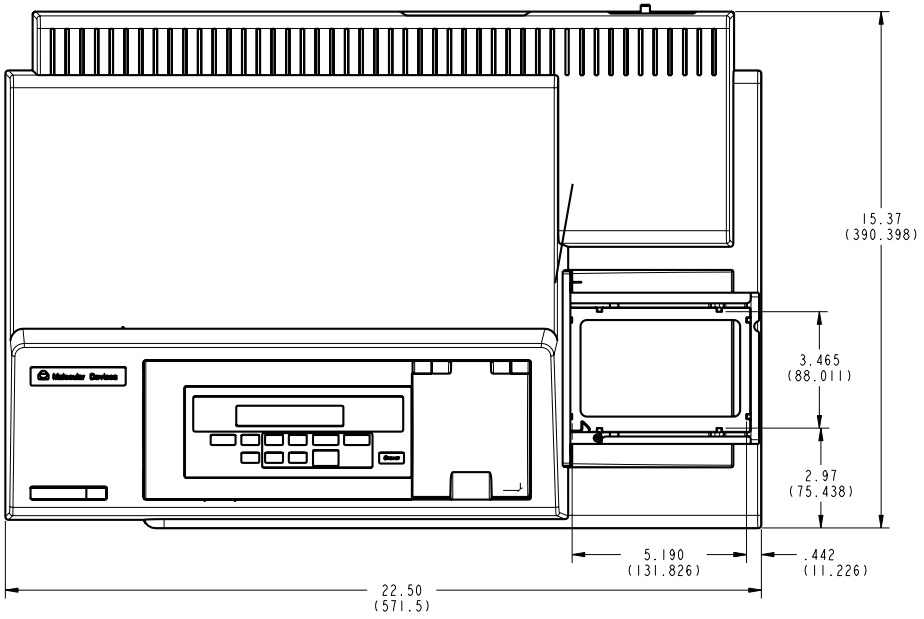


Figure A.4: Top view of SpectraMax M2.

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