SpectraMax® M3
SpectraMax® M4
SpectraMax® M5
SpectraMax® M5e

Multi-Mode Microplate Readers

User Guide

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October 2018

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Safety Information

Information about the safe use of the instrument from Molecular Devices® includes an understanding of the user-attention statements in this guide, the safety labels on the instrument, precautions to follow before you operate the instrument, and precautions to follow while you operate the instrument.

Make sure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understood all Safety Data Sheets (SDS) for all materials being used.

Read and observe all warnings, cautions, and instructions. The most important key to safety is to operate the instrument with care.

WARNING! If the instrument is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

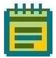
Warnings, Cautions, Notes, and Tips

All warning symbols are framed within a yellow triangle. An exclamation mark is used for most warnings. Other symbols can warn of other types of hazards such as biohazard, electrical, or laser safety warnings as are described in the text of the warning. Follow the related safety information.

The following user attention statements might be displayed in the text of Molecular Devices user documentation. Each statement implies the amount of observation or recommended procedure.

WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed.

CAUTION! A caution indicates a situation or operation that could cause damage to the instrument or loss of data if correct procedures are not followed.



Note: A note calls attention to significant information.

Tip: A tip provides useful information or a shortcut, but is not essential to the completion of a procedure.

Symbols on the Instrument

Each safety label found on the instrument contains an alert symbol that indicates the type of potential safety hazard.

Symbol	Indication
	Consult the product documentation.
	Potential electrical-shock hazard from a high-voltage source. All safety instructions must be read and understood before you proceed with the installation, maintenance, and service of all modules. Power off the instrument and disconnect the power cord before you do maintenance procedures that require removal of a panel or cover or disassembly of an interior instrument component.
	Risk of electrical shock. Refer servicing to qualified personnel only.
	Potential lifting hazard. To prevent injury, use a minimum of two people to lift the instrument.
	Required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. It indicates that you must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

- - California proposition 65 requires businesses to provide warnings to Californians about significant exposures to chemicals that cause cancer, birth defects, or other reproductive harm.

Electrical Safety

To prevent electrical injuries and property damage, inspect all electrical equipment before use and report all electrical deficiencies. Contact Molecular Devices technical support for equipment service that requires the removal of covers or panels.

WARNING! HIGH VOLTAGE. Within the instrument is the potential of an electrical shock hazard existing from a high voltage source. Read and understand all safety instructions before you install, maintain, and service the instrument.

To prevent electrical shock, use the supplied power cord and connect to a properly grounded wall outlet.

To protect against fire hazard, replace the fuses only with the same type and rating as the original factory installed fuses.

To ensure sufficient ventilation and provide access to disconnect power from the instrument, maintain a 20 cm to 30 cm (7.9 in. to 11.8 in.) gap between the rear of the instrument and the wall.

Power off the instrument when not in use.

Chemical and Biological Safety

Normal operation of the instrument can involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When you use such materials, observe the following precautions:

- Handle infectious samples based on good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original containers of solutions before their use.
- Dispose of all waste solutions based on the waste disposal procedures of your facility.
- Operate the instrument in accordance with the instructions outlined in this guide, and take all the required precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. Take applicable safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials, flammable solvents, toxic, pathological, or radioactive materials in or near a powered-up instrument.

WARNING! Never use the instrument in an environment where potentially damaging liquids or gases are present.

CAUTION! Use of organic solvents can cause harm to the optics in the instrument. Extreme caution is recommended when you use organic solvents. Always use a plate lid and do not place a plate that contains these materials in the plate chamber for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

CAUTION! When you use aggressive or corrosive reagents, you should have the plate automatically move out of the instrument after a read.

Moving Parts Safety

The instrument contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts.

WARNING! If the instrument is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

To prevent injury:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.
- Keep the instrument work area clear to prevent obstruction of the movement. Provide clearance in front of the instrument of 18 cm (7.1 in.) for the plate drawer.

CAUTION! To prevent damage to the instrument, the height of the plate must not exceed 17 mm, including the lid if the plate is lidded.

WARNING! Do not attempt to access the interior of the instrument unless specifically instructed to do so. The moving parts inside the instrument can cause injury. Do not operate the instrument with any covers or panels removed.

Note: Observe all warnings and cautions listed for all external devices attached to or in use during the operation of the instrument. See the applicable user guide for the operating and safety procedures of that device.

Chapter 1: Introduction

The SpectraMax® M3 Multi-Mode Microplate Reader, SpectraMax® M4 Multi-Mode Microplate Reader, SpectraMax® M5 Multi-Mode Microplate Reader, and SpectraMax® M5e Multi-Mode Microplate Reader are dual monochromator, multi-detection instruments with a triple-mode cuvette port and a 6-well to 384-well plate read capability.

The flexibility and high sensitivity makes these instruments appropriate for applications in 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.

Read Modes

Read Modes	SpectraMax M3	SpectraMax M4	SpectraMax M5	SpectraMax M5e
Absorbance	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓
Time-Resolved Fluorescence		✓	✓	✓
Fluorescence Polarization			✓	✓

The SpectraMax M5e is certified for use with Cisbio Bioassays' HTRF (Homogeneous Time-Resolved Fluorescence) technology. HTRF is a proprietary Time-Resolved Fluorescence technology that overcomes many of the drawbacks of standard Fluorescence Resonance Energy Transfer (FRET) techniques, such as the requirements to correct for autofluorescence and the fluorescent contributions of unbound fluorophores.

Read types include: See Read Types on page 37.

- Endpoint
- Kinetic
- Spectrum
- Well Scan

The instrument light source is a high-powered Xenon flash lamp. Two holographic diffraction grating monochromators allow for individual optimization of wavelengths for both excitation and emission in the Fluorescence Intensity read mode. Mirrored optics focus the light in to the sample volume, and cutoff filters reduce stray light and minimize background interference. You can vary the number of lamp flashes per read to optimize the sensitivity or read speed.

The instrument dynamic range of detection is from 10^{-6} to 10^{-12} molar fluorescein. Internal compensation for detector sensitivity, photomultiplier tube voltage and sensitivity, as well as excitation intensity, effectively eliminates variations in measured fluorescence values. The photometric range is 0–4 ODs with a resolution of 0.001 OD.

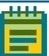
Dual monochromators enable you to select absorbance wavelength between 200 nm and 1000 nm and excitation wavelengths:

- Between 250 nm and 850 nm for reads in:
  - Fluorescence Intensity read mode
  - Time-Resolved Fluorescence read mode (SpectraMax M4, SpectraMax M5, and SpectraMax M5e)
  - Luminescence read mode
- Between 350–750 nm for Fluorescence Polarization read mode (SpectraMax M5 and SpectraMax M5e)

Computer Integration

Each Molecular Devices microplate reader is shipped with a license key for the SoftMax® Pro Data Acquisition and Analysis Software that you install on the computer that you use to operate the instrument. The SoftMax Pro Software provides integrated instrument control, data display, and statistical data analysis.

You should install the SoftMax Pro Software on the computer before you set up the instrument. Please be aware that some updates to the SoftMax Pro Software require a purchase. Contact Molecular Devices before you update the software. To download the latest version of the software, visit: https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software#Order.

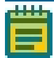


Note: For information about the computer specifications that are required to run the software, the software installation and licensing instructions, and the directions to create the software connection between the computer and the instrument, see the *SoftMax Pro Data Acquisition and Analysis Software Installation Guide*.

To prevent data loss, turn off all sleep and hibernation settings for the hard disk, the CPU, and the USB ports. Disable automatic Windows updates. Update Windows manually when you do not use the computer to control an instrument. You can set these options in Windows Control Panel.

Supported Plates and Cuvettes

The instrument accommodates both plates and cuvettes.



Note: The supplied plate adapter is required for some applications. See Plate Adapter on page 20.

Supported Plates

You can use 6, 12, 24, 48, 96, and 384-well plates that conform to ANSI/SBS standard microplate footprint and dimensions in the instrument. The plate list in the software includes half area and low- volume plates. The software enables you to define a new plate type based on the manufacturer's specifications for well size, spacing, and distance from the plate edge.

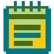
- Use polystyrene plates for absorbance wavelengths above 340 nm.
- To allow transmission of the deep UV spectra when you do a read at wavelengths below 340 nm, you must use special UV-transparent plates or quartz plates.
- Endpoint, Kinetic, Spectrum, and Well Scan read types are available for the Absorbance and Fluorescence read modes with any plate model.
- Top and bottom reads are available for the Fluorescence, Time-Resolved Fluorescence, and Luminescence read modes.
- One plate drawer adapter is provided with the instrument. The plate adapter is required for optimum performance with standard 96-well and 384-well format plates for all top-read applications.

Note: Although not recommended, you can also use strip well plates in the instrument. When you use strip-wells, use high quality support frames or holders. To avoid warping, you should fill the frame or holder with empty strips.

Supported Cuvettes

For a cuvette read, you must ensure that the meniscus is above the light beam in a standard cuvette and that the sample chamber is aligned properly with the beam in a microcuvette. The light beam is 0.625 in (15.87 mm) above the cuvette bottom.

- Use the cuvette port for Absorbance, Fluorescence, and Luminescence read modes.
- To allow transmission of the deep UV spectra when you do a read at wavelengths below 340 nm, you must use special UV-transparent cuvettes or quartz cuvettes.
- Endpoint, Kinetic, and Spectrum read types are available for the Absorbance and Fluorescence read modes with cuvettes.



Note: You can use a 12 x 75 mm test tube with a test tube cover. You can order the test tube cover from Molecular Devices. See Accessories on page 57.

Shake

The software enables you to define settings to mix of the contents of the wells by shaking the plate linearly along the long axis at preset intervals.

- For the Endpoint read type, you can shake the plate for 0 to 999 seconds before the read at each wavelength you set.
- For the Kinetic read type, you can shake the plate for 0 to 999 seconds before the initial read, and/or before each subsequent read.

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

Temperature Regulation

The temperature in the plate chamber and cuvette chamber is isothermal, both at ambient and when you turn on the incubator. When you turn on the incubator, you can set the temperature from 2°C above ambient to 60°C. To achieve regulation at temperatures greater than 50°C, the instrument’s ambient temperature may need to be increased to 25°C or higher. The operating humidity must be non-condensing, and can range from 0 to 70%.

Chapter 2: Setting Up the Instrument

Before you unpack and set up the instrument, prepare a dry, flat work area that is away from direct sunlight, dust, drafts, vibration, and moisture. The work area must have sufficient space for the instrument, host computer, and required cables. To provide access for disconnecting power from the instrument, maintain a 20 cm to 30 cm (7.9 in. to 11.8 in.) gap between the rear of the instrument and the wall. To ensure sufficient ventilation, do not block the ventilation grids on the front and rear of the instrument.

The package contains the instrument and the following accessories:

- SoftMax Pro Software, product key, and installation guide
- Instrument installation guide
- USB computer connection cable
- AC power adapter
- Plate adapter

For a complete list of the package contents, see the enclosed packing list.

Before you set up the instrument, you should install the SoftMax Pro Software on the computer that you plan to use to operate the instrument. See the *SoftMax Pro Data Acquisition and Analysis Software Installation Guide*.

Unpacking the Instrument

The packaging is designed to protect the instrument during shipment. Tape is placed on the cuvette door to protect the instrument from damage during shipment.

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

The instrument is packed in a specially designed carton. Before you unpack the instrument, check the box for any damage that might have occurred during transportation. If any damage is noted, inform the supplier immediately and keep the damaged packaging.

Note: Retain the shipping box and all packing materials for future transport needs.

To unpack the instrument:

1. Open the top of the box.
2. Lift the accessories tool box and the instrument from the package, and then place the instrument on a level surface.
3. Remove the cardboard divider from the top of the instrument.
4. Remove the packing material from both ends of the instrument, remove the instrument from the plastic bag, and then set the instrument down carefully on a level surface.
5. Remove the tape from the cuvette door.

Connecting Instrument Cables

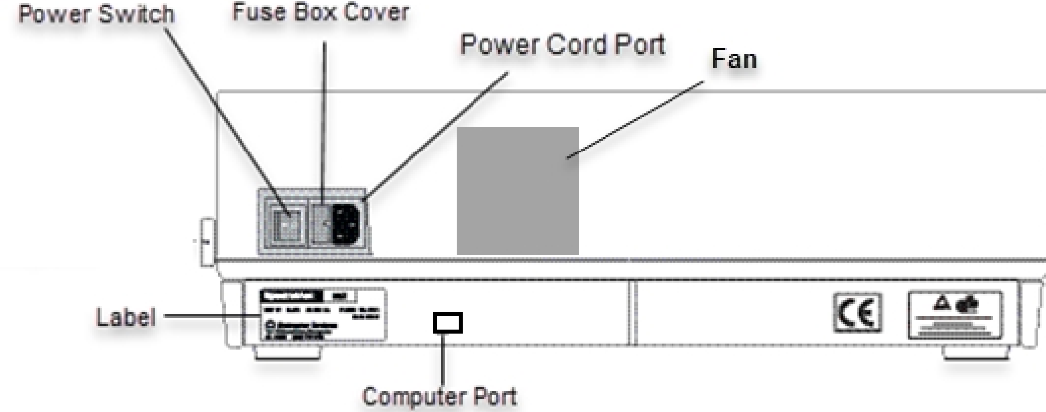
The power cord and USB cable connect to the ports on the rear of the instrument.

Part Number	Description
1-2100-0548	USB computer connection cable, 3 meter (9.8 foot)
4400-0002	Power cord, 1 meter (3.3 foot) See Accessories on page 57 for international power cord part numbers.

Note: Before you connect or disconnect the power cord, make sure that the power switch that is on the rear of the instrument is in the Off position.

To connect the instrument cables:

1. Turn the instrument around so that the rear of the instrument faces you.



2. Make sure that the power switch is in the Off position.
3. Connect the appropriate end of the supplied USB cable to the USB port, and then connect the other end to a USB port on the computer.
4. Connect the supplied power adapter to the power cord port, and then connect the other end to a grounded electrical wall outlet.
5. Turn the instrument around so that the front of the instrument faces you.

Note: Ensure no cables run beneath the instrument.

Chapter 3: Getting Started

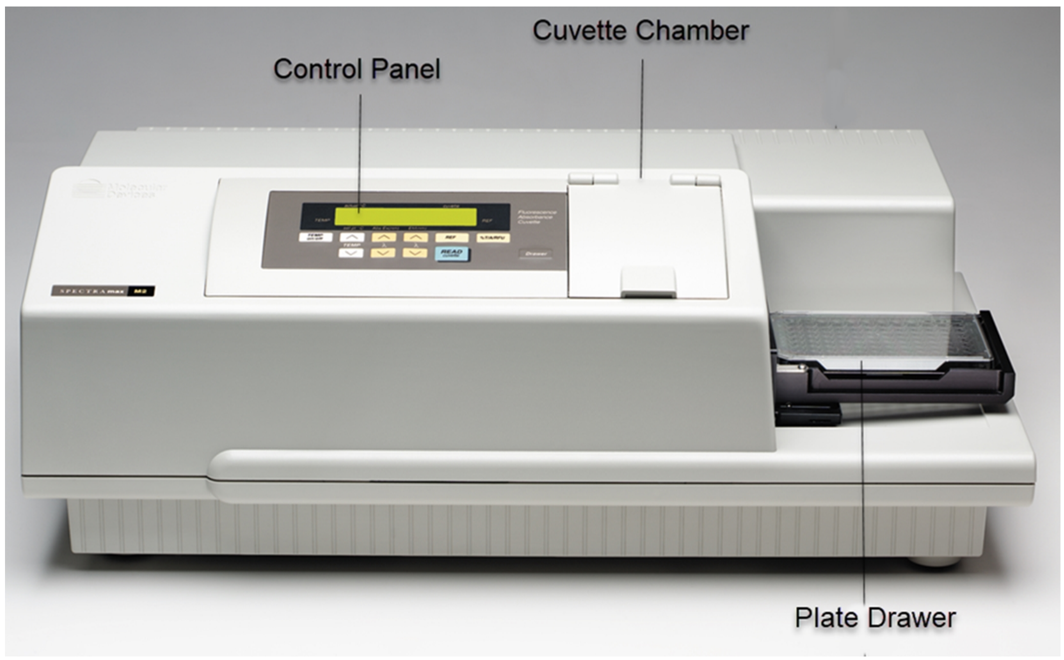
Now that you have installed the SoftMax Pro Software software on the computer, removed the tape from the cuvette port, and connected the cables, it is time to get started.

Turn on the instrument power switch located on the rear of the instrument. Wait for the instrument to complete its diagnostic check and the plate drawer opens.

The following are the main components of the instrument:

- Control panel: Enables you to open the plate drawer, control the read chamber temperature, and set the wavelengths.
- Cuvette chamber: Enables you to read the contents of a cuvette.
- Plate drawer: Enables you to insert the plate to read.

CAUTION! Never touch the internal optic mirrors, lenses, filters, or cables. The optics are extremely delicate, and critical to the function of the instrument.



Control Panel

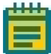
The instrument control panel works in conjunction with the SoftMax Pro Software to open the plate drawer, adjust the temperature, and to set the wavelengths for cuvette reads. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide*.

To change the control panel display contrast, press **Mode**, and then press **Temp** ▲ or ▼.

The left side of the control panel displays the actual temperature inside the cuvette chamber above the display of the temperature you set for the cuvette chamber. If the temperature inside the cuvette chamber is not at the set temperature, the indicator blinks. The temperature inside the plate chamber lags slightly behind the temperature inside the cuvette chamber. The temperature inside the plate chamber displays in the software.

The center of the control panel displays the absorbance/excitation and emission wavelengths the instrument uses for a cuvette read.

The right side of the control panel displays the data the instrument receives from a cuvette reference read as percent transmission, absorbance, relative fluorescence units, or relative luminescence units. If you do not do a reference read, the indicator blinks.



Note: Settings you enter in the software override the corresponding control panel setting.

Temp On/Off

The **Temp On/Off** button enables or disables the incubator that controls the temperature inside the cuvette chamber and the plate chamber.

- When the incubator is on, the control panel displays the temperature inside the cuvette chamber above the display of the temperature you set.
- For the Kinetic and Spectrum read types, the temperature buttons on the control panel are disabled.

Temp

The **Temp** ▲ and ▼ buttons enable you to enter the set point at which to regulate the temperature inside the cuvette chamber and the plate chamber. The temperature for the instrument chambers is isothermal at ambient temperature as well as at elevated temperatures. The cuvette chamber temperature displays on the instrument control panel and the plate chamber temperature displays in the software.

You cannot set a temperature beyond the upper (60°C) or lower (2°C above ambient) instrument limits. To set the temperature at 60°C, the ambient temperature of the room must be at least 20°C.

Wavelength (λ)

The **λ** ▲ and ▼ buttons enable you to set the wavelengths to read the cuvette. The control panel contains two sets of **λ** wavelength buttons. The left buttons enable you to set the absorbance/excitation (fluorescence) wavelength and the right buttons enable you to set the emission (fluorescence) wavelength.

Note: The control panel does not display the wavelength if you use the software to set the wavelength.

Mode

The **Mode** button enables you to change the display of the cuvette reference read data between percent transmittance (%T), absorbance (A), relative fluorescence units (RFU), or relative luminescence units (RLU).

Read Cuvette

The **Read Cuvette** button enables you to start a cuvette read.

Note: The **Read Cuvette** button is disabled when the software does a read.

REF

The **REF** button enables you to do a cuvette reference read of buffer, water, or air. The software uses this value as I_0 to calculate Absorbance or % Transmittance. If you do not do a reference read, the instrument uses the I_0 value that is stored in the instrument.

Note: The **REF** button is disabled when the software does a read.

Drawer

The **Drawer** button enables you to open and close the plate drawer.

Cuvette Chamber

The cuvette chamber is located to the right of the control panel. Lift up the lid over the chamber to insert or remove a cuvette. The chamber contains springs that position the cuvette in the proper alignment for a read. You must close the cuvette door before you start a read.

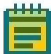
The instrument can accommodate standard height (45 mm), 1 cm cuvettes and 12 x 75 mm test tubes when you use a test tube cover. You can also use semi-microcuvettes and ultra-microcuvettes with an adapter. See Supported Plates and Cuvettes on page 11.

Cuvettes for the Absorbance read mode are frosted on two sides. Handle the cuvettes on the frosted sides only. Place the cuvette in the cuvette chamber so that the read (clear) sides face left and right.

Cuvettes for the Fluorescence read mode are clear on all four sides and you should handle these cuvettes carefully.

Plate Drawer

The plate drawer is located on the right side of the instrument and slides in and out of the plate chamber. An internal latch positions the plate on the drawer as it closes. You can use 6, 12, 24, 48, 96, and 384-well plates that conform to ANSI/SBS standard microplate footprint and dimensions in the instrument. See Supported Plates and Cuvettes on page 11.



Note: The supplied plate adapter is required for some applications. See Plate Adapter on page 20.

The **Drawer** button enables you to open and close the plate drawer, or you can use the software. The drawer closes for each read. Subsequent plate drawer operation is dependent on the incubator setting:

- When you open the drawer, if the incubator is off, the drawer remains open.
- When you open the drawer, if the incubator is on, the drawer closes after approximately 10 seconds to maintain temperature control within the plate chamber.

Drainage ports protect the plate drawer mechanism and plate chamber from accidental spills.

CAUTION! Do not obstruct the movement of the plate drawer. If you must retrieve a plate after an error condition or power outage and the plate drawer does not open, you can open the drawer manually. See Opening the Drawer Manually on page 47.

CAUTION! To prevent damage to the instrument, the height of the plate must not exceed 17 mm, including the lid if the plate is lidded.

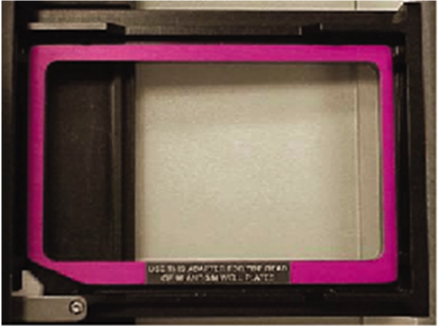
Plate Adapter

Before you read standard 96-well or 384-well plates from the top, you must insert the plate adapter in the plate drawer. Before you do a bottom read, or use high profile (6-well, 12-well, 24-well, or 48-well) plates, you must remove the plate adapter from the plate drawer.

CAUTION! Incorrect insertion or removal of the plate adapter can cause damage to the instrument plate drawer. To ensure that you do not damage the plate drawer, do the following procedures.

To insert the plate adapter:

1. Open the plate drawer.
2. Hold the plate adapter so that the label is on the front side facing up.
3. Place the top back (Row A) of the plate 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 plate adapter onto the drawer.



To remove the plate adapter:

1. Open the plate drawer.
2. In the lower left corner where the plate pusher is located, carefully lift the front of the plate adapter at the corner cutout. As you lift the plate adapter, make sure to also lift from the other end of the front edge, and then completely remove the plate adapter from the plate drawer.

Chapter 4: Operating the Instrument

You do the same basic steps to operate the instrument when you read a plate or you read a cuvette.

Note: For detailed software instructions, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

Temperature Settings

When you use the incubator to set the temperature in the cuvette chamber and the plate chamber, you should wait at least 60 minutes for the chamber temperature to reach the set point before you do the read. You cannot set a temperature beyond the upper (60°C) or lower (2°C above ambient) instrument limits. You can use the instrument control panel or the software to change the temperature.

- When you turn on the incubator, the plate drawer closes after approximately 10 seconds to maintain temperature control.
- The incubator maintains the chamber temperature at the set point until you press Temp On/Off again or you change the temperature from the Temperature Control dialog in the software. When you turn off the incubator the chamber temperature gradually returns to ambient.
- When you turn the incubator back on after a momentary shutdown, allow about ten minutes for the control algorithm to fully stabilize the chamber temperature.

Software Temperature Settings

In the software, in the Ribbon, on the Home tab, click **Temperature** to display the Temperature Control dialog. The Temperature Control dialog enables you to turn the incubator on and off and to set the chamber temperature.

Instrument Control Panel Temperature Settings

1. On the instrument control panel, press **Temp On/Off** to turn on the incubator.

   The control panel indicates that incubator is on and displays the temperature inside the cuvette chamber above the display of the set temperature. The plate chamber temperature displays in the software, in the Ribbon, on the Home tab.
2. To change the temperature:
   - Press **Temp** ▲ or **Temp** ▼ once to increase or decrease the temperature by 0.1°C.
   - Press and hold **Temp** ▲ or **Temp** ▼ to rapidly adjust the temperature setting. The temperature is set when you release the button.

Wavelength Settings

To read a cuvette, you can use the instrument control panel or the software to set the wavelengths. To read a plate, you must use the software to set the wavelengths.

Note: For a cuvette reference read, if you select A or %T, you can set only the absorbance and excitation wavelengths.

Software Wavelength Settings

In the software, in the Ribbon, on the Home tab, click **Acquisition Settings** to display the Settings dialog. The Settings dialog enables you to set the wavelengths for the Plate section or the Cuvette Set section of the experiment.

Instrument Control Panel Wavelength Settings

For a cuvette read, the left side wavelength buttons on the instrument control panel enable you to set the absorbance/excitation (fluorescence) wavelength and the right side wavelength buttons enable you to set the emission (fluorescence) wavelength.

To change the wavelength:

- Press **λ ▲** or **λ ▼** once to increase or decrease the wavelength by 1 nm.
- Press and hold **λ ▲** or **λ ▼** to adjust the wavelength by 10 nm. The wavelength is set after you release the button.

Common Wavelengths

The SpectraMax M5 and SpectraMax M5e enable you to scan the fluorophore of interest to determine the optimal excitation and emission wavelengths. Excitation and emission wavelengths listed by fluorophore manufacturers are determined in methanol and do not reflect actual values because of changes in pH, salt content, and so on. The following tables list common wavelengths based on current literature.

Luminescence Probes Wavelengths

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

Time-Resolved Fluorescence Fluorophores Wavelengths

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

Fluorescence Fluorophores Wavelengths

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 Homodimer (DNA)	530	620
TRITC, Ethidium Bromide	544	590
Texas Red	584	612
TAMRA	547	580
Tryptophan	280	340

Read a Plate

CAUTION! Before you read standard 96-well or 384-well plates from the top, you must insert the plate adapter into the plate drawer. Before you do a bottom read, or when you use high profile (6-well, 12-well, 24-well, or 48-well) plates, you must remove the plate adapter from the plate drawer. See Plate Adapter on page 20.

Before you place a plate in the drawer, the underside of the plate must be dry. If the plate has fluid on the underside, you must dry it with a lint-free cloth before you place it in the drawer.

1. On the rear of the instrument, turn on the power switch.

   The instrument carries out a diagnostic check. During this check, the plate drawer opens, and then closes. When the diagnostic check completes, the plate drawer opens and remains open.
2. Power on the computer.
3. Start the SoftMax Pro Software and connect the software to the instrument.
4. In the software, in the Ribbon, on the Home tab, click **Acquisition Settings** to display the Settings dialog where you define the acquisition settings for the Plate section.

5. Place the plate onto the plate drawer, matching well A1 with position A1 in the drawer. Note the following:
   - If you read 6, 12, 24, or 48-well plates, make sure that the plate is flat against the drawer bottom.
   - If you do a top read for 96 or 386-well plates, make sure that the plate is flat against the plate adapter.
6. Do one of the following in the software:
   - Open a data file or protocol file that contains the experiment settings for the read.
   - Create a new protocol file with the settings for the read.
7. In the software, in the Ribbon, on the Home tab, click **Read** to read the plate.

   After the read completes, the plate drawer opens and you can remove the plate.

Note: If the incubator is on, the drawer closes after approximately 10 seconds. If you return to the instrument after a read and the plate drawer closed, press **Drawer** to open the drawer and remove the plate.

Read a Cuvette

Handle a cuvette on the frosted sides only.

1. On the rear of the instrument, turn on the power switch.

   The instrument carries out a diagnostic check. During this check, the plate drawer opens, and then closes. When the diagnostic check completes, the plate drawer opens and remains open.
2. Power on the computer.
3. Start the SoftMax Pro Software and connect the software to the instrument.
4. In the software, in the Ribbon, on the Home tab, click **Acquisition Settings** to display the Settings dialog where you define the acquisition settings for the Cuvette Set section.
5. Place the cuvette in the chamber so that the read (clear) sides face left and right. Make sure that the cuvette is completely seated in the chamber. (Springs hold the cuvette in place.)
6. Close the cuvette chamber door.
7. Do one of the following:
   - If the cuvette is a reference cuvette, press **REF** to acquire the reference read of either buffer, water, or air.

     The instrument calibrates in less than two seconds, closes the plate drawer if it is open, and then reads the cuvette.
   - If the cuvette is a sample cuvette, to acquire the sample read, press **Read Cuvette**.

     The plate drawer closes if it is open, and the instrument reads the cuvette according to the software settings.
8. After the read, remove the cuvette from the chamber.

Chapter 5: Read Modes and Read Types

Absorbance Read Mode

The instrument uses the Absorbance (ABS) read mode to measure the Optical Density (OD) of the sample solutions.

Absorbance is the quantity of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. If there is no turbidity, then absorbance = optical density.

$$A = \log_{10}(I_0 / I) = -\log_{10}(I/I_0)$$

where I_0 is intensity of the incident light before it enters the sample divided by the light after it passes through the sample, and A is the measured absorbance.

The temperature-independent PathCheck® Pathlength Measurement Technology normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

The instrument enables you to choose whether to display absorbance data as Optical Density (OD) or %Transmittance (%T) in the Reduction dialog.

Optical Density

Optical density (OD) is the quantity of light passing through a sample to a detector relative to the total quantity of light available. Optical Density includes absorbance of the sample plus light scatter from turbidity and background. You can compensate for background using blanks.

A blank well contains everything used with the sample wells except the chromophore and sample-specific compounds. Do not use an empty well for a blank.

Some applications are designed for turbid samples, such as algae or other micro-organisms in suspension. The reported OD values for turbid samples are likely to be different when read by different instruments.

For optimal results, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will be subtracted is the average value of all blanks.

% Transmittance

%Transmittance is the ratio of transmitted light to the incident light for absorbance reads.

$$T = I/I_0$$

$$\%T = 100T$$

where I is the intensity of light after it passes through the sample and I_0 is incident light before it enters the sample.

Optical Density and %Transmittance are related by the following formulas:

$$\%T = 10^{2-OD}$$

$$OD = 2 - \log_{10}(\%T)$$

The factor of two comes from the fact that %T is expressed as a percent of the transmitted light and $\log_{10}(100) = 2$.

When in %Transmittance analysis mode, the instrument converts the raw OD values reported by the instrument to %Transmittance using the above formula. All subsequent calculations are done on the converted numbers.

Applications of Absorbance

Absorbance-based detection is commonly used to evaluate changes in color or turbidity, permitting widespread use including ELISAs, protein quantitation, endotoxin assays, and cytotoxicity assays.

Optimizing Absorbance Read Mode

You can adjust the wavelength of the transmitted light in 1-nm increments between 200 nm and 1000 nm. You can also use the instrument for reading up to six wavelengths per plate, which allows for reference wavelength readings such as A260 and A280 for nucleic determination.

For an assay blank, you should use appropriate plate blanks or group blanks in a template that you define in the software. You can also use the PathCheck Pathlength Measurement Technology feature to normalize the data to a 1 cm pathlength.

PathCheck Pathlength Measurement Technology

The temperature-independent PathCheck® Pathlength Measurement Technology normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

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

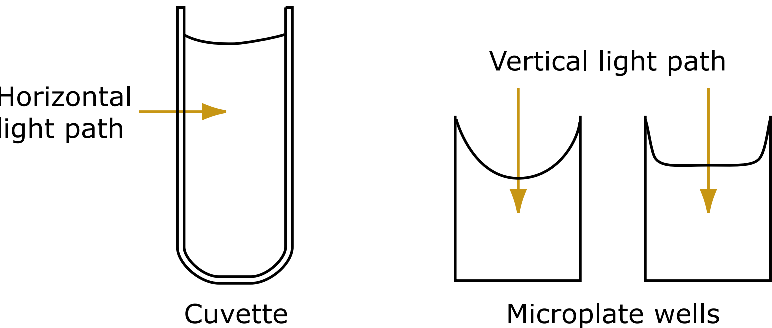
$$A = \varepsilon c L$$

where A is the absorbance, ε is the molar absorptivity of the sample, c is the concentration of the sample, and L is the pathlength. 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 do extinction-based assays and makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis to quantify the unique absorptivity properties of compounds in solution. Quantitative analysis can be done on the basis of extinction coefficients, without standard curves (for example, NADH-based enzyme assays). When you use a cuvette, the pathlength is known and is independent of sample volume, so absorbance is directly proportional to concentration when there is no background interference.

In a plate, 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 occur from pipetting the samples and standards. The PathCheck technology determines the pathlength of aqueous samples in the plate and normalizes the absorbance in each well to a pathlength of 1 cm. This way of correcting the microwell absorbance values is accurate to within ±4% of the values obtained directly in a 1 cm cuvette.



PathCheck technology normalizes the data acquired from an Absorbance read mode Endpoint read type to a 1 cm pathlength, correcting the OD for each well to the value expected if the sample were read in a 1 cm cuvette. The instrument uses the factory installed Water Constant to obtain the 1 cm values. For the SpectraMax M series you can read a cuvette that contains deionized water or buffer to use the Cuvette Reference correction method (typically not necessary when you use aqueous solutions with minimal alcohol, salt, or organic solvent content).

Water Constant

The PathCheck technology is based on the absorbance of water in the near infrared spectral 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 correction method is sufficient. The Water Constant is determined for each instrument during manufacture and is stored in the instrument.

Cuvette Reference

Note: The Cuvette Reference correction method that the software uses with the PathCheck Pathlength Measurement Technology is different from the reference read of a cuvette that occurs when you click the Ref button in the Cuvette Set section tool bar.

Use the Cuvette Reference correction method if the sample contains an organic solvent such as ethanol or methanol. When you add a non-interfering solvent 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. To minimize the error, put the same water/solvent mixture in a cuvette and use the Cuvette Reference.

Place a standard 1 cm cuvette that contains the aqueous/solvent mixture you use for the plate samples into the cuvette port. The cuvette must be in place when you read the plate. When you click Read, the instrument first makes the 900 nm and 1000 nm measurements in the cuvette and then makes the designated measurements in the plate. The software temporarily stores the cuvette values and uses the cuvette values in the PathCheck calculations for the plate samples.

The Cuvette Reference that the software uses with the PathCheck Pathlength Measurement Technology is different from the reference read of a cuvette that occurs when you click the Ref button in the Cuvette Set section tool bar. The software uses the Cuvette Reference data for PathCheck calculations does not produce data that displays in a Cuvette Set section. The software uses the Cuvette Reference data for the PathCheck calculations in plates, not cuvettes. However, you can use the accessors on the Formula Editor dialog to obtain these values. See the !PathCheckLm1000 and !PathCheckLm900 accessor in the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.

Note: After you read a plate with PathCheck technology turned on, the software stores PathCheck information permanently in the document. You can apply or not apply PathCheck technology to the absorbance values. If you do select to use PathCheck technology for the plate read, you cannot apply the PathCheck Pathlength Measurement Technology feature after the read.

Eliminating Pathlength Independent Component

Raw OD measurements of plate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of plate material). The pathlength-independent component must be eliminated from the calculation to get valid results that have been normalized by the PathCheck technology. You can do this using a plate blank or using a plate background constant.

Use a Plate Blank

You can use this method if all samples in the plate are the same volume and the read does not depend on the PathCheck technology to correct for variability in volumes.

1. Designate a minimum of one well (preferably several) as Plate Blank.
2. Pipette buffer (for example, your sample matrix) into those wells and read along with the samples. Do not use an empty well for a blank.

   The instrument automatically subtracts the average of the blank wells from each of the samples. The OD of the plate material is subtracted as part of the blank.
3. Select the Use Plate Blank check box in the Data Reduction dialog.

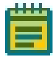
Use a Plate Background OD

If your sample volumes are not identical or if you choose not to use a Plate Blank, then you must use a Plate Background OD. Omitting a Plate Background OD results in artificially high values after being normalized by the PathCheck technology.

To determine the Plate Background OD:

1. Fill a clean plate with water.
2. Read at the wavelengths you will use for the samples.

The average OD value is the Plate Background OD. If you intend to read your samples at more than one wavelength, there should be a corresponding number of Plate Background OD values for each wavelength.



Note: It is important that you put water in the wells and do not read a dry plate for the Plate Background OD. A dry plate has a slightly higher OD value than a water filled plate because of differences in refractive indices. Use of a dry plate results in PathCheck technology normalized values that are lower than 1 cm cuvette values.

Interfering Substances

Material that absorbs in the 900 nm to 1000 nm spectral region could interfere with PathCheck technology measurements. Fortunately, there are few materials that do interfere at the concentrations generally used.

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

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

To determine possible color interference:

- Measure the OD 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 you should not use the PathCheck technology.

Organic solvents could interfere with the PathCheck technology if they have absorbance in the region of the NIR water peak. Solvents such as ethanol and methanol do not absorb in the NIR region, so they do not interfere, except for causing a decrease in the water absorbance to the extent of their presence in the solution. If the solvent absorbs between 900 nm and 1000 nm, the interference would be similar to the interference of highly colored samples. If you add an organic solvent other than ethanol or methanol, you should run a Spectrum scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck technology.

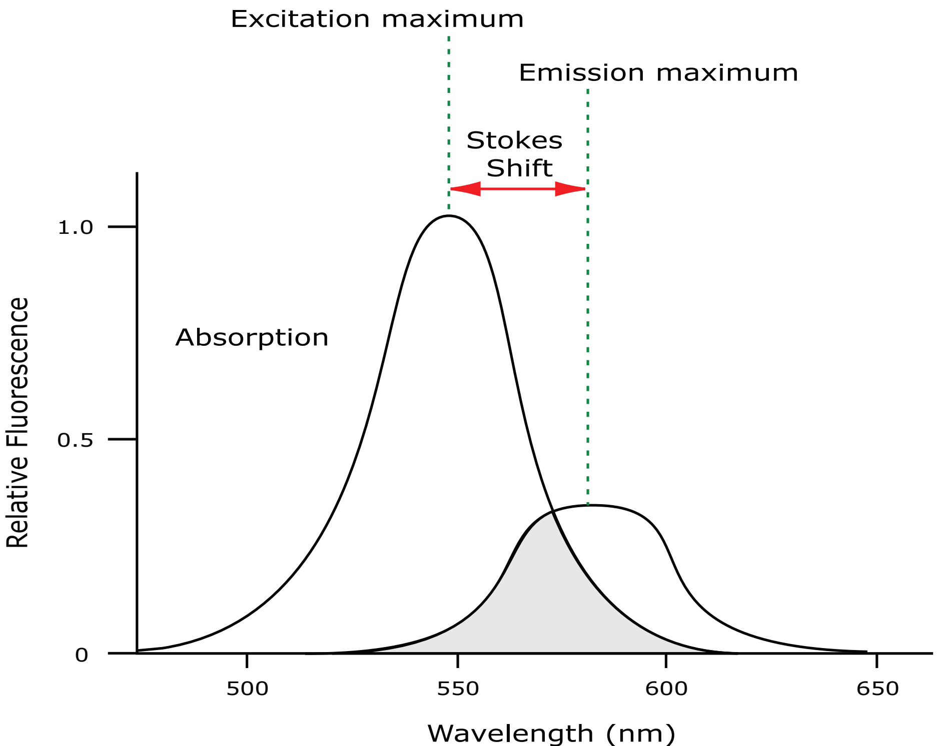
Fluorescence Intensity Read Mode

Fluorescence occurs when absorbed light is re-radiated at a longer wavelength. In the Fluorescence Intensity (FL) read mode, the instrument measures the intensity of the re-radiated light and expresses the result in Relative Fluorescence Units (RFU).

The governing equation for fluorescence is:

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

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 following figure 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 difference between the wavelengths of the excitation and emission maxima (the Stokes shift) is generally fairly small, about 30 nm. There is considerable overlap between the excitation and emission spectra (gray area) when a small Stokes shift is present.

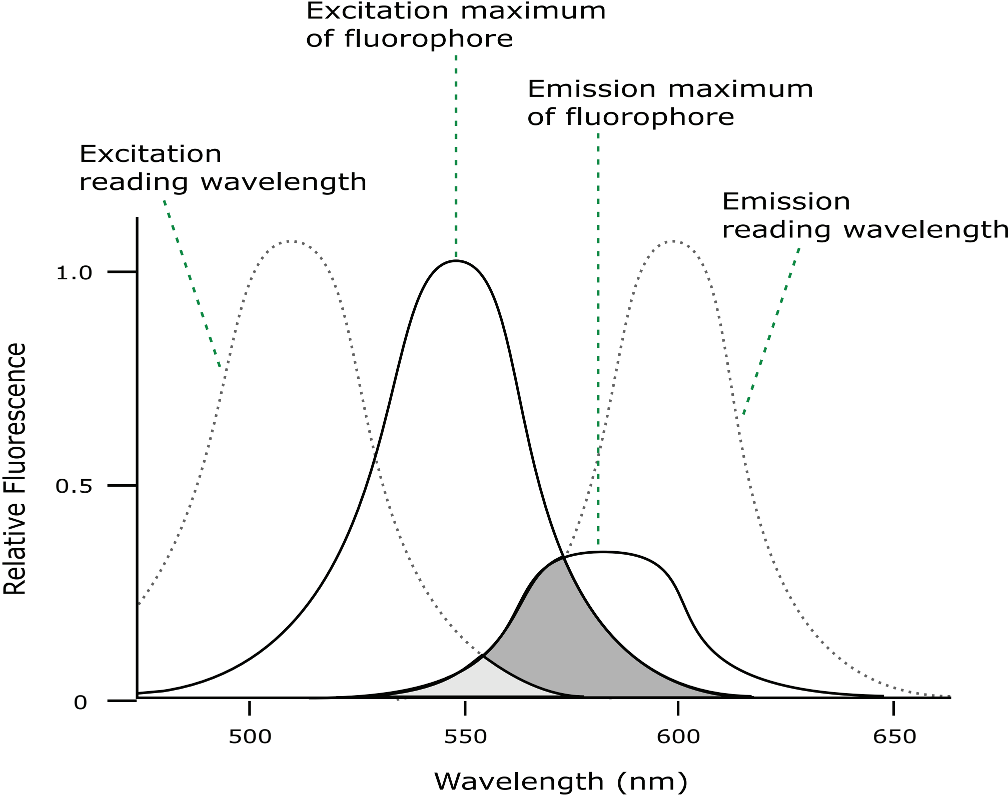


Excitation and Emission Spectra

Because the intensity of the excitation light is usually many tens of thousands of times greater than that of the emitted light, you must have sufficient spectral separation to reduce the interference of the excitation light with detection of the emitted light.

Tip: If the Stokes shift is small, you should choose an excitation wavelength that is as far away from the emission maximum as possible while still able to stimulate the fluorophore so that less of the excited light overlaps the emission spectrum, which permits better selection and quantitation of the emitted light.

The Spectral Optimization Wizard provides the best settings for maximizing the signal to background window, (S-B)/B, while minimizing the optimization time.



Optimized Excitation and Emission Read Wavelengths

The previous figure shows that the best results are often obtained when the excitation and emission wavelengths you use for the read are not the same as the peak wavelengths of the excitation and emission spectra of the fluorophore. When the read wavelengths for excitation and emission are separated, a smaller quantity of excitation light passes through to the emission monochromator (gray area) and on to the PMT, which results in a purer emission signal and more accurate data.

The instrument enables you to scan both excitation and emission wavelengths, using separate tunable dual monochromators. One benefit of scanning emission spectra is that you can determine 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. One more benefit is that you can find excitation and emission wavelengths that prevent interference when interfering fluorescent species are present.

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

Fluorescence intensity data is dependent on several variables.

Applications of Fluorescence Intensity

Fluorescence intensity is used widely in applications such as fluorescent ELISAs, protein assays, nucleic acid quantitation, reporter gene assays, cell viability, cell proliferation, and cytotoxicity. One more major application is to study the kinetics of ion release.

Some assays use a fluorescent label to selectively attach to certain compounds. The quantity or concentration of the compound can then be quantified by measuring the fluorescence intensity of the label, which is attached to the compound. Such methods are often used to quantify low concentrations of DNA or RNA, for example.

Luminescence

Luminescence is the emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation. When you use the Luminescence read mode, no excitation is necessary because the species to be 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 reads are averaged together into one read per well. The default setting for the Luminescence read mode is the zero order position where the grating monochromator acts as a mirror that reflects all light to the PMT detector. If the assay requires a wavelength selection, you can choose the wavelength where you expect the peak emission to occur. In addition, multiple wavelength choices allow species with multiple components to be differentiated and measured easily. The Luminescence read mode does not use an emission cutoff filter.

Optimizing Luminescence Read Mode

You can do top or bottom reads in either a plate or a cuvette for a Luminescence assay. You should use solid white plates or white plates with clear bottoms for Luminescence assays.

For standard Luminescence, a separate light path without monochromators carries the emitted light to a dedicated PMT. The optimum emission wavelength is between 360 and 630 nm. Make sure that the instrument emission is set to All.

For wavelength-selectable luminescence, the instrument uses the emission monochromator to differentiate the wavelengths that emit from the well. You can specify up to six different emission wavelengths between 250 nm and 850 nm. If only a single luminescent event in the well is read, you should use the standard luminescence measurement without selecting a wavelength, to achieve the best sensitivity for the assay.

You do not designate luminescence read times based on multiple reads per well, but rather by choosing the total integration time that is between 1 ms and 1,500 ms. Typical luminescence assays require between 500 ms and 1,000 ms for integration time.

If wells incubate for a long period of time, then you should use the Automix function to mix the wells before the read.

If the signal is always higher in the first wells read (for example, column A), you might need to dark adapt the plate to reduce the auto-luminescence of the white plastic. Because the auto-luminescence decreases quickly, use the control panel Drawer button to load the plate, and then wait 1–2 minutes before you initiate the read and determine if the read-out is more consistent across the plate.

Time-Resolved Fluorescence Read Mode

The Time-Resolved Fluorescence read mode is available on the SpectraMax M4, SpectraMax M5, and SpectraMax M5e.

Time-Resolved Fluorescence is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) vanishes, while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period, with the added benefit of reduced background.

In Time-Resolved Fluorescence read mode, the instrument detects the extremely long emission half-lives of rare earth elements called lanthanides, such as europium (lifetime of about 700 µs), samarium (lifetime of about 70 µs), or terbium (lifetime of about 1000 µs).

Applications of Time-Resolved Fluorescence

Time-Resolved Fluorescence is widely used in high throughput screening applications such as kinase assays, and is useful in some fluorescence immunoassays, such as DELFIA (dissociation-enhanced enzyme linked fluorescence immunoassay). TRF is also useful in some assay variants of TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer) in which the FRET acceptor label acts as a quencher only and does not emit fluorescence. The proximity between donor label and acceptor (quencher) is then quantified by the intensity decrease of the donor label.

DELFIA requires washing steps as in an ELISA, but the TR-FRET assay involving quenching is a homogeneous plate assay technique and requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

The Cisbio Bioassays HTRF® (Homogeneous Time-Resolved Fluorescence) technology is a proprietary Time-Resolved Fluorescence technology that overcomes many of the drawbacks of standard Fluorescence Resonance Energy Transfer (FRET) techniques, such as the requirements to correct for autofluorescence and the fluorescent contributions of unbound fluorophores.

Optimizing Time-Resolved Fluorescence Read Mode

The SpectraMax M4, SpectraMax M5, and SpectraMax M5e enable you to read Time-Resolved Fluorescence assays from either the top or the bottom of a plate. You should use solid white plates for top time-resolved fluorescence reads, and white plates with clear bottoms for bottom reads.

Two important settings to obtain the best results in TRF assays are integration delay and integration time.

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

Delay and integration time are usually specified in the package insert of commercially available TRF reagent kits. If you do not use a kit, then start with a delay of 50 µs and try different delays up to 400 µs with a fixed integration time of 400 µs. After you select the optimum delay (based on the highest ratio of a well that contains a fluorophore divided by wells that contain only buffer) optimize the integration time, which is typically between 400 µs and 1000 µs.

If the Time-Resolved Fluorescence assay you use has low signal or gives results with high % CV, then use 100 reads per well. If a faster read speed is required, then in the Settings dialog, turn the Settling Time to Off and experiment with fewer flashes per well until you achieve an acceptable precision and speed.

Fluorescence Polarization Read Mode

The Fluorescence Polarization read mode is available on the SpectraMax M5 and SpectraMax M5e.

The Fluorescence Polarization (FP) read mode measures the relative change of polarization of emitted fluorescence compared to excitation light.

Fluorescence Polarization detection is based on Fluorescence Intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Microplate readers measure the Fluorescence Polarization of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation.

By using a fluorescent dye to label a small molecule, the binding of a small molecule to an interaction partner of equal or greater size can be monitored through its speed of rotation.

When molecules are excited with polarized light, the change in the polarization of the emitted light depends on the size of the molecule to which the fluorophore is bound (the emitted light quickly depolarizes if the fluorescent molecule is unbound). Larger molecules yield a stronger polarization of the emitted light, while smaller molecules cause less polarization because of their rapid molecular movement. Fluorescence Polarization is used for molecular binding assays in high-throughput screening (HTS).

Applications of Fluorescence Polarization

Fluorescence Polarization measurements provide information on molecular mobility and are generally used to quantify the success of a binding reaction between a smaller labeled ligand and a binding site at a much larger or immobilized molecule. Fluorescence Polarization can also be used to quantify the dissociation or cleavage of the labeled ligand from a binding site.

Fluorescence Polarization is a homogeneous plate assay technique and requires only mixing and measuring—no wash steps are required as in an ELISA. It can also be miniaturized, which makes it useful for high-throughput screening applications.

Optimizing Fluorescence Polarization Assays

The SpectraMax M5 and SpectraMax M5e enable you to read Fluorescence Polarization assays from the top of a plate. The plastic from the plate affects the light polarization, which precludes bottom reads and covered plates.

You should use solid black plates for Fluorescence Polarization reads. If the assay components seem to bind to the plate, as evidenced by poor mP dynamic range (small difference between bound and unbound tracer), then you should use plates that are treated to minimize binding, or polypropylene plates and/or add a very small amount of detergent, such as Tween-20, to the assay buffer.

You should test background wells that contain all assay components minus the fluorophore. If the signal that is in the background wells is more than 1/10 the signal that in the wells that contain fluorophore, then you should run background wells on each assay plate. Before the mP calculation is carried out, you should subtract the average raw signal from parallel and perpendicular reads for the background from the raw parallel and perpendicular reads of each sample well.

For best precision in assays that use a low amount of fluorophore (for example, <5 nm fluorescein), set the PMT sensitivity to High and the number of reads to 100. If faster read speed is required, then in the Settings dialog, turn the Settling Time to Off and experiment with fewer flashes per well until you achieve an acceptable precision and speed.

Analyzing Fluorescence Polarization Data

The Fluorescence Polarization read mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. The software uses the S and P values to calculate the Polarization (mP) and Anisotropy (r) values.

Fluorescence Polarization assays in plates are generally designed with two control samples:

- LOW control sample: minimal polarization value resulting from unbound labeled ligand only
- HIGH control sample: maximum polarization value resulting from bound labeled ligand only

The Fluorescence Polarization data for a sample is evaluated based on its relative position between the low and high control values. Total intensity can also be determined from the raw data and is proportional to the quantity of label in a sample.

Blank Correction

Many Fluorescence Polarization assays use small fluorescent label concentrations in the lower nm range. In this range, blank controls become significant when compared to samples.

A blank well contains everything used with the sample wells except the label and sample-specific compounds. Do not use an empty well for a blank.

Background wells, which contain all assay components minus the fluorophore, should be tested. If the signal in the background wells is more than 1/10 the signal in the wells that contain fluorophore, then background wells should be run on each assay plate. The average raw signal from the background's parallel and perpendicular reads must be subtracted from the raw parallel and perpendicular reads of each sample well before the mP calculation is done.

For optimal results, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will be subtracted is the average value of all blanks.

Data Reduction

Although the raw S and P values are the true actual values returned from the instrument, the calculated Polarization (mP) and Anisotropy (r) values are treated as the raw data and become the basis for further reduction calculations.

Polarization (mP) is calculated as follows:

$$mP = 1000 * \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (G * \text{perpendicular}))}$$

Anisotropy (r) is calculated as follows:

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

The G factor, or grating factor, is used in Fluorescence Polarization to correct polarization data for optical artifacts, converting relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. G factor corrects for this instrument-based bias.

Data Qualification and Validation

When you validate the data of a Fluorescence Polarization measurement and the assay, the two factors to look at are the precision value and the Z´ factor.

The FP precision value is a measure of replicate uniformity determined by the standard deviation of replicates at a label concentration of 1 nM. Since the precision of a measured signal also depends on the read time, the read time must also be specified. A longer read time leads to a lower (better) precision value.

Z´ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

The value of Z´ can be determined using the following formula:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|\, Mean_{c+} - Mean_{c-} \,|}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c–** is the negative control.

A Z´ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. You can use higher values when results are more critical.

Z´ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z´ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

The assay window is dependent on the fluorophore lifetime and relative size of the receptor to the ligand. Precision values are better (lower) at higher signals, which normally come from higher label concentrations.

For a given assay window, Z´ is a downward sloping linear function. That is, as precision values get higher (worse), the Z´ value gets lower (worse).

Precision is dependent upon assay characteristics (sample volume, label concentration) and read time. In many assays, the characteristics are defined and cannot be changed. In this case, the only way to improve precision is to increase the read time per well.

Read Types

The instrument support the following read types:

Endpoint

In an endpoint read type, a reading of each plate well is taken in the center of each well, at a single wavelength or at multiple wavelengths. Raw data values are reported as optical density (OD), % transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

Kinetic

In a kinetic read type, the instrument collects data over time with multiple reads taken in the center of each well at regular intervals. To achieve the shortest possible interval for kinetic reads, choose wavelengths in ascending order.

The software can do the following calculations based on raw data: VMax, VMax per Sec, Time to VMax, and Onset Time. Kinetic readings can be single wavelength or multiple wavelength readings.

The kinetic read type can collect data points in time intervals of seconds, minutes, or hours (up to 99 hours).

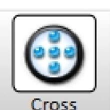
Kinetic analysis has many advantages to determine the relative activity of an enzyme in different types of plate assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis is capable of providing improved dynamic range, precision, and sensitivity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

Spectrum

The Spectrum read type measures optical density (OD) or %Transmittance across a specified wavelength range, with allowed values from 200 nm to 1000 nm. For the Fluorescence or Luminescence read mode, relative fluorescence units (RFU) or relative luminescence units (RLU) values are reported.

Well Scan

The Well Scan read type takes reads at more than one location on an evenly spaced, user defined grid or other pattern inside of each well at single or multiple wavelengths. The fill pattern is either round or square to match the well shape. The number of points that are available depend on the well size of the plate you select. Four scan patterns are available:



Chapter 6: Maintenance

Perform only the maintenance tasks described in this guide. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year. See Obtaining Support on page 48.

Before you operate the instrument or perform maintenance operations, make sure you are familiar with the safety information in this guide. See Safety Information on page 5.

CAUTION! Maintenance procedures other than those specified in this guide must be performed by Molecular Devices. When service is required, contact Molecular Devices technical support.

Cleaning the Instrument

WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before you request service by a service engineer or you return parts to Molecular Devices for repair. Molecular Devices does not accept items that have not been decontaminated where applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag that states that the contents are safe to handle and are not contaminated.

WARNING! BIOHAZARD. Always wear gloves when operating the instrument and during cleaning procedures that could involve contact with either hazardous or biohazardous materials or fluids.

Do the following before you clean equipment that has been exposed to hazardous material:

- Contact the applicable Chemical and Biological Safety personnel.
- Review the Chemical and Biological Safety information contained in this guide. See Chemical and Biological Safety on page 7.

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. Power off the instrument, and wipe up spills immediately with a lint-free absorbent wipe. Allow the unit to air dry completely before you power on the instrument.

CAUTION! Do not use abrasive cleaners. Do not spray cleaner directly onto the instrument or into any openings. Do not let water or other fluids drip inside the instrument.

Always turn the power switch off and disconnect the power cord from the main power source before using liquids to clean the instrument.

- Periodically clean the outside surfaces of the instrument using a cloth or sponge that has been lightly dampened with water, or use disinfectant wipes according to the supplier instructions, with an emphasis on the following areas you will handle when packing, unpacking, and servicing the instrument:
  - Plate carrier
  - Cuvette chamber
  - Instrument top
  - Cover edges
  - Underneath the instrument, between the feet
  - Rear edges (Do not damage the warranty seal.)
- If needed, clean the plate drawer using a cloth or sponge that has been lightly dampened with water.
- If a bleach solution has been used, wipe the instrument using a lint-free cloth that has been lightly dampened with water to remove the bleach residue.

Cleaning the Fan Filter

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

To clean the fan filter:

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

Replacing Fuses

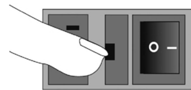
If the instrument does not seem to get power after you switch it on, check to see whether the power cord is securely plugged into a functioning power outlet and to the power port on the rear of the instrument.

If the power failed while the instrument was on, verify 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, replace the fuses. You can obtain replacement fuses from Molecular Devices.

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

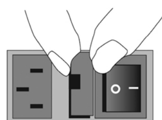
The fuses are located in the fuse carrier which is part of the power switch on the rear of the instrument.



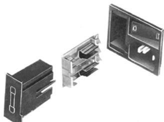
To replace the fuses:

WARNING! HIGH VOLTAGE Always turn off the power and disconnect the power cord from the main power source before you do a maintenance procedure that requires removal of a panel or cover or disassembly of an interior instrument component.

1. Power off the instrument.
2. Unplug the power cord from the power port.
3. Use a small slot-head screwdriver to gently press on the carrier-release tab and then pull the fuse carrier to remove it from the instrument.



4. Gently pull the old fuses from the carrier by hand.



5. Gently place new fuses into the carrier by hand.
6. Press the fuse carrier into the instrument until the carrier snaps into place.
7. Plug the power cord into the power port.
8. Turn on power to the instrument.

Note: If the instrument still does not power on after you change the fuses, contact Molecular Devices technical support.

Before You Move the Instrument

Before you move the instrument, make sure that the new location is a dry, flat work area that has sufficient space for the instrument, host computer, and required cables.

Tip: Although you can always carry the instrument, depending on the distance that you are moving it, you might use a rolling cart instead.

If you must store the instrument, then store it in a dry, dust-free, environmentally controlled area. The storage temperature can range from -20°C to 65°C.

To minimize the possibility of damage during storage or shipment, you should pack the instrument in the original packaging materials. Correctly repacking the instrument includes following applicable decontamination procedures and packing instructions.

CAUTION! When transporting the instrument, warranty claims are void if improper packing results in damage to the instrument.

Packing the Instrument

The original packaging is designed to protect the instrument during shipment and storage. You must always pack the instrument before you ship it or store it.

To pack the instrument:

1. Make sure the plate drawer and cuvette chamber are empty.
2. Place tape to hold the cuvette chamber door closed.
3. Place the instrument back in the plastic bag.
4. Place the packing material on both ends of the instrument.
5. Place the instrument and the accessories tool box into the original instrument shipping box.
6. Seal the top of the box with packing tape.

Chapter 7: Troubleshooting

The software displays messages and error codes on the instrument control panel. Warning messages indicate a situation that requires attention but is not sufficient to stop or prevent a read. Warning messages are logged in the error buffer. 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.

An error code displays when a situation arises that requires attention. Any read in progress stops. The errors are grouped in relationship to possible causes as follows:

Table 7-1: Error Code Ranges

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

Some errors are considered fatal. If these errors occur during power up, the instrument aborts the power up sequence, and if the software is connected to the instrument, the instrument control panel Fatal Error. Review the list of possible causes to see if there is something that you can do to change the condition of the instrument to prevent the error. For example, close the cuvette door during the power up sequence to prevent errors 111, 219, 302, and 310.

After you correct the problem, leave the instrument on for about five minutes, turn it off and then back on. If the error message continues to display on power up, then record the error message number and contact Molecular Devices Technical Support or your local representative for assistance.

Note: If the instrument functions normally when you use the SoftMax Pro Software, then with the exception of error #100, no errors should be in the buffer. This guide does not list all possible error codes. For assistance with all other error messages (codes not listed here), please contact Molecular Devices Technical Support or your local representative.

Note: If an error occurs while the plate drawer is closed, and you need to remove a plate, see Opening the Drawer Manually on page 47.

Table 7-2: Error Codes 100-199: Unrecognized Command Errors Sent From the Computer

Error Code	Error Message	Notes
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 is 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 read and then disconnecting computer or the SoftMax Pro Software 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	

Table 7-3: Error Codes 200-299: Firmware Errors

Error Code	Error Message	Notes
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.
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.

Table 7-4: Error Codes 300-399: Hardware Errors

Error Code	Error Message	Notes
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 preamp 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.
307	cuv air ref fail	
308	stray light	Light leak in read chamber or cuvette door open. Could also be a faulty pre-amp board
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 did not move to where it was commanded to in a reasonable time.
317	monitor ADC faulty	

Table 7-5: Error Codes 400-499: Motion Errors

Error Code	Error Message	Notes
400	carriage motion error	Carriage did not move to either of its photo interrupts in a reasonable time, or cannot find its photo interrupt.
401	filter wheel error	Filter wheel did not move to its photo interrupt in a reasonable time, or cannot find its photo interrupt.
402	grating error	Grating did not move to its photo interrupt in a reasonable time, or cannot find its photo interrupt.
403	stage error	Stage did not move to its photo interrupt in a reasonable time, or cannot find its photo interrupt.

Table 7-6: Error Codes 500-599: NVRAM Errors

Error Code	Error Message	Notes
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.

Opening the Drawer Manually

Do the following if an error occurs while the drawer is closed and you need to remove a plate.

- Press **Drawer**.
- If the drawer does not open after you press Drawer, then turn off the power to the instrument and then on again.
- If the drawer remains closed after you cycle the power, and the incubator is on, then turn off the incubator.
- If the drawer still remains closed, power off the instrument, unplug the power cord and then at the groove in the upper left side wall of the drawer, try to use a blunt, flat object, such as a spatula, to open the door. With your index finger, pull the plate drawer out of the instrument (do not force the drawer) and remove the plate. This action will not harm the instrument, but should only be taken if the first two options fail to open the drawer.

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

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website, www.moleculardevices.com/service-support, has a link to the Knowledge Base, which contains technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

Please have the instrument serial number, (on the rear of the instrument) and any related sample data files available when you call.

Appendix A: Specifications

Thermal specifications for plates used in the instrument apply to flat-bottom plates with isolated wells. All other plate specifications apply to standard 96-well polystyrene flat-bottom plates. Performance specifications for cuvette reads apply only to aqueous solutions that have solute molal concentrations less than 0.4 M.

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

Note: Technical specifications are subject to change without notice. Molecular Devices provides validation documentation for software and hardware, as well as absorbance, fluorescence, and luminescence detection test tools with its SpectraTest® solutions. The SpectraTest line of microplate reader validation packages provide automated and comprehensive validation of a microplate reader's optical performance. See Accessories on page 57.

Table A-1: Absorbance Photometric Performance

Item	Description
Wavelength range	200-1000 nm
Wavelength selection	Monochromator, tunable in 1 nm increments
Wavelength bandwidth	≤ 4.0 nm full width half maximum
Wavelength accuracy	±2.0 nm across wavelength range
Wavelength repeatability	±0.2 nm
Photometric range	0.000 to 4.000 OD
Photometric resolution	0.001 OD
Photometric accuracy linearity (plate), 0-2.0 OD	< ± 1.0% and ± 0.006 OD
Photometric precision (repeatability)	< ± 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 every endpoint read and before the first kinetic read
Optical alignment	None required
Light source	Xenon flash lamp (50 Watts)
Average lamp lifetime	1 billion flashes
Illumination	Top down (plates); horizontal (cuvettes)
Photodetector	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

Table A-2: Fluorescence Intensity Performance

Item	Description
Sensitivity	Top Read < 5 pM FITC, 1 fmol/200 µL (96 well microplate) < 20 pM, 2 fmol/100 µL (384 well microplate) Bottom Read < 20 pM FITC (96 well microplate)
Sensitivity (cuvette)	< 15 pM fluorescein
Wavelength range	250-850 nm
Wavelength selection	Monochromators, tunable in 1 nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Number of excitation/emission pairs per plate	4
Dynamic range	10^6 in 96-well black plates: auto gain circuitry
System validation	Self-calibrating with built-in fluorescence calibrators
Light source	Xenon flash lamp (1 joule/flash)
Average lamp lifetime	1 billion flashes
Detector	Photomultiplier (R3896)

Table A-3: Fluorescence Polarization Performance

Item	Description
Wavelength range (M5 and M5e models only)	300-750 nm
Wavelength selection	Monochromators, tunable in 1 nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Precision	< 5 mP standard deviation at 1 nM fluorescein in 96 and 384 wells

Table A-4: Time-Resolved Fluorescence Performance

Item	Description
Sensitivity (M4, M5, M5e models only)	100 fM europium in 96- or 384- well microplates (top read)
Wavelength range	250-850 nm
Wavelength selection	Monochromators, tunable in 1 nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Precision data collection	1–100 flashes; delay of 0–600 µs before read; integration time selectable 50–1500 µs

Table A-5: Luminescence Photometric Performance

Item	Description
Sensitivity	< 2 fg/well for firefly luciferase in 96- and 384-well microplates (top read)
Wavelength range	250-850 nm
Crosstalk	< 0.5% in 96- and 384-well microplates

Table A-6: Photometric Analysis Modes

Item	Description
Front Panel Operation	Single wavelength Absorbance, %Transmittance, Fluorescence reading of the cuvette (or test tube)
Using SoftMax Pro Software	• Express data as Absorbance, %Transmittance, Fluorescence, Luminescence • Single wavelength reading of microplate and/or cuvette • Multiple wavelength (up to four) reading of microplate or cuvette • Kinetic and kinetic graphics of microplate and/or cuvette • Spectral scan (190–1000 nm) of microplate and/or cuvette • Well scan of microplate using absorbance or fluorescence intensity

Table A-7: Measurement Time (Calibration Off)

Item	Description
Microplate read time (endpoint), Standard read	• 96 wells in 24 seconds (single wavelength, absorbance) • 96 wells in 15 seconds (single wavelength, fluorescence intensity) • 384 wells in 1:57 minutes (single wavelength, absorbance) • 384 wells in 45 seconds (single wavelength, fluorescence intensity)
Microplate read time (endpoint), Standard read with PathCheck Pathlength Measurement Technology	• 96 wells in 2:07 minutes (single wavelength, absorbance) • 384 wells in 7:19 minutes (single wavelength, absorbance)
Microplate read time (endpoint, Speed read	• 96 wells in 18 seconds (single wavelength, absorbance) • 384 wells in 49 seconds (single wavelength, absorbance)

Table A-8: Scan Speed

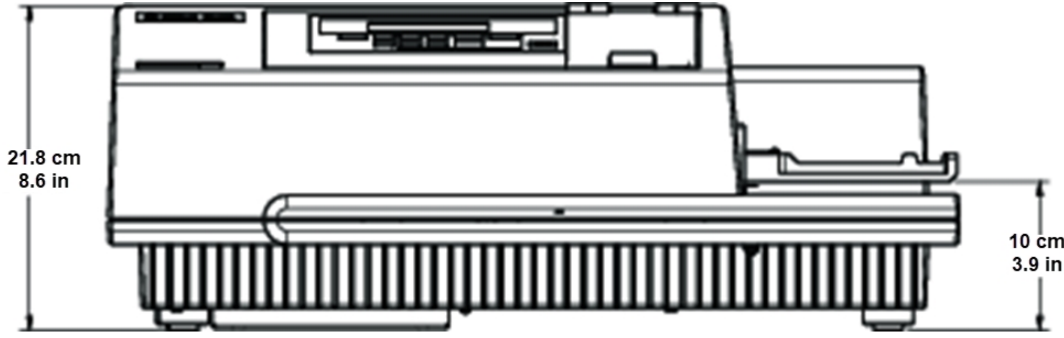
Item	Description
Cuvette	< 2 fg/well for firefly luciferase in 96- and 384-well microplates (top read)
Wavelength range	250-850 nm
Crosstalk	< 0.5% in 96- and 384-well microplates

Table A-9: Temperature Regulation

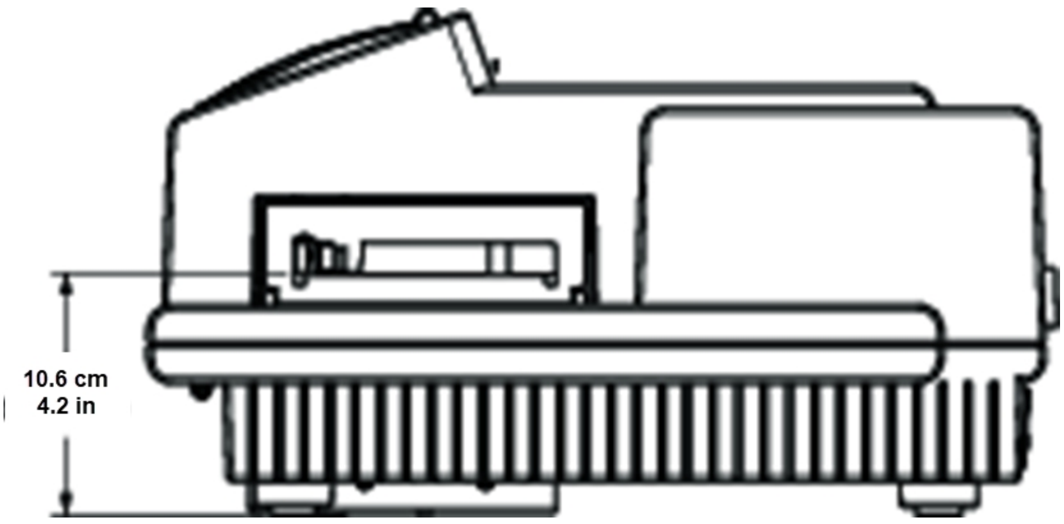
Item	Description
Reading chamber	Isothermal when temperature regulation is not enabled
Range	2°C above ambient to 60°C when temperature regulation enabled. **Note:** To achieve regulation at temperatures greater than 50°C, the instrument's ambient temperature may need to be increased to 25°C or higher.
Resolution	Resolution ± 0.1°C
Accuracy	± 1.0°C for microplate and cuvette chamber
Temperature uniformity at equilibrium	± 0.5°C at 37°C
Chamber warmup 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

System Diagrams and Dimensions

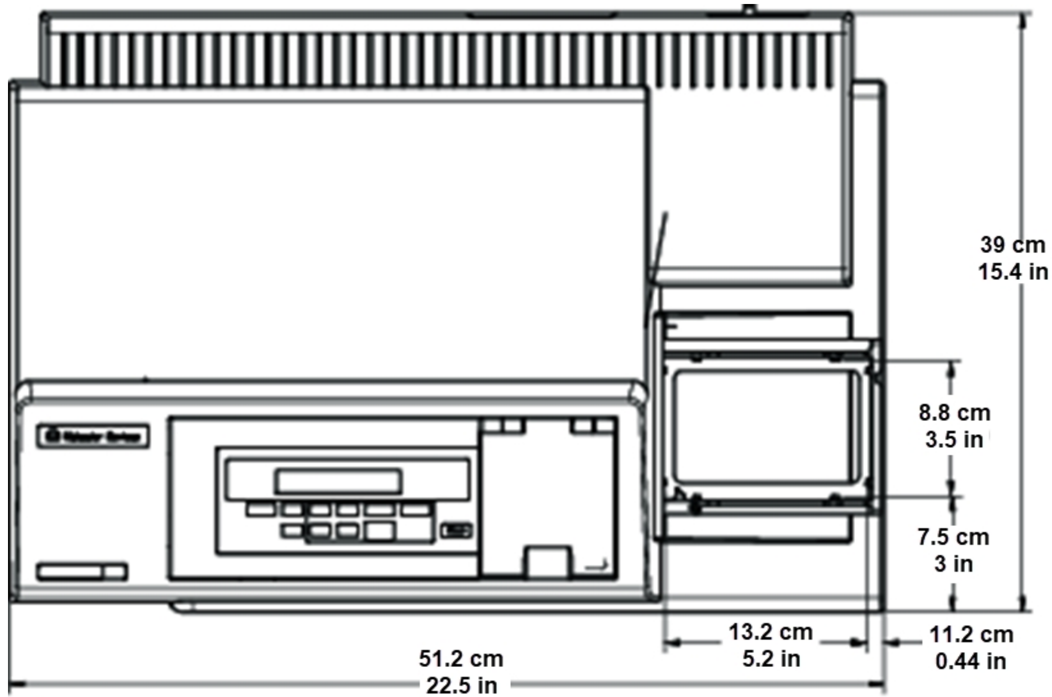
In the following drawings, the dimensions are show in centimeters and inches.



Front View with Dimensions



Side View with Dimensions



Top View with Dimensions

Appendix B: Accessories

This appendix describes supported accessories for the instrument.

Supported Plates and Cuvettes

The software supports the following plates:

- 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 MD HE PS
- 384-well Standard, 384 Costar, 384 Greiner, 384 Falcon, 384 Corning, 384 MD HE PS
- 48 Costar
- 24 Costar
- 12 Costar, 12 Falcon
- 6 Costar, 6 Falcon

The software plate list also includes half area and low volume plates.

Note: Not all manufacturers' plates and cuvettes are the same with regard to design, materials, or configuration. Depending on the type of plate that you use, temperature uniformity within the plate or cuvette can vary.

The following have been tested and have an optical pathlength of 10 mm and standard external dimensions (12.5 mm x 12.5 mm). The fill volumes differ only because of the different internal width and chamber height dimensions.

Standard and Semi-Microcuvettes

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

Ultra-Microcuvettes

Window Size	Chamber Size	Fill Volume
2.0 x 5.0 mm	100 µL	120 µL
2.0 x 5.0 mm	50 µL	70 µL
0.8 mm diameter**	5 µL	10 µL

*To match the cuvette window to the beam, you must put a riser (0.8–1 mm) on the cuvette bottom.

**To match the cuvette window to the beam, you must put a riser (0.8–1 mm) on the cuvette bottom. Provides good spectral scans (qualitative results), but because the window is smaller than the beam, quantitative results are not possible.

Note: You must specify 15 mm for the Z dimension when you order ultra-microcuvettes.

SpectraTest Microplate Reader Validation Packages

Technical specifications are subject to change without notice. Molecular Devices provides validation documentation for software and hardware, as well as absorbance, fluorescence, and luminescence detection test tools with its SpectraTest® solutions. The SpectraTest line of microplate reader validation packages provide automated and comprehensive validation of a microplate reader's optical performance.

Validation Packages Part Numbers

Part Number	Item Name	Compatible Instruments
0200-6117	SpectraTest ABS1 Absorbance Validation Plate	SpectraMax iD3, iD5, i3x, i3, M2, M2e, M3, M4, M5, M5e, Plus 384, 340PC 384, 190, ABS, ABS Plus, VersaMax, FlexStation 3
0200-5060	SpectraTest FL1 Fluorescence Validation Plate	Gemini EM, Gemini XPS, SpectraMax iD3, iD5, i3x, i3, M2, M2e, M3, M4, M5, M5e, FlexStation 3
0200-6186	SpectraTest LM1 Luminescence Validation Plate	SpectraMax iD3, iD5, i3x, i3, M3, M4, M5, M5e, SpectraMax L, FlexStation 3
0200-2420	Cuvette Absorbance Validation Set	SpectraMax Plus 384, ABS Plus, M2, M2e, M3, M4, M5, M5e
0200-7200	Multi-Mode Validation Plate	FilterMaxF3, FilterMaxF5, SpectraMax Paradigm, iD5*, i3, i3x* * Specific read modes or cartridges.

Additional Accessories

The following accessories and international power cords are compatible with the instrument.

Accessories

Item Name	Part Number
SpectraPlate—Quartz UV-transparent plate	R8024
Fuse, 4-amp Time Delay	4601-0013
Fuse, 4-amp (5 x 20 mm) Time Delay	4601-0014
Test Tube Cover	2300-0277
Power cord, USA/Canada	4400-0002
Power cord, Continental Europe/C13	4400-0036
Power cord, United Kingdom, Ireland/C13	4400-0037
Power cord, Australia/C13	4400-0038
Power cord, Argentina/C13	4400-0275
Power cord, China/C13	4400-0276
Power cord, Denmark/C13	4400-0277
Power cord, India/C13	4400-0278
Power cord, Israel/C13	4400-0279
Power cord, Italy/C13	4400-0280
Power cord, Japan/C13	4400-0281
Power cord, Switzerland/C13	4400-0282

Appendix C: Electromagnetic Compatibility

Regulatory for Canada (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est confomre à la norme NMB-001 du Canada.

ISM Equipment Classification (Group 1, Class A)

This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power supply network which supply buildings used for domestic purposes.

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