



# FlexStation® 3

Multi-Mode Microplate Reader

## 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.

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**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.

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**Note:** A note calls attention to significant information.

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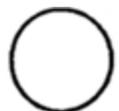


**Tip:** A tip provides useful information or a shortcut, but is not essential to the completion of a procedure.

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## 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.
	Power switch indicates power on.
	Power switch indicates power off.
	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.

## Service Trained Users

Most procedures required to operate and troubleshoot the instrument can be done by a user who reads the instructions in this user guide and is familiar with the system. However, all installation procedures and some complex service and troubleshooting procedures require the expertise of a service trained user. When Molecular Devices installs the instrument, part of the setup procedure is to provide training for the people in your company who are to be your company's service trained users. Contact Molecular Devices to arrange additional training.

Whenever the following warning message appears, a service trained user must do the procedure to ensure user safety and to prevent instrument damage. Example:



**WARNING! The following procedures must be completed by a service trained user. Do not attempt the following procedures if you have not received proper training by Molecular Devices personnel.**

---

## 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, unless a procedure specifically instructs you to remove a cover or panel.



**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.**

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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.**

---

### **Xenon-Arc Lamp Safety**

The system is equipped with a Xenon-arc flash lamp. Do not look directly at the flash lamp while it is illuminated. The lamp emits ultraviolet radiation at levels that can injure the eye if viewed directly.

## Chapter 1: Introduction

The FlexStation® 3 Multi-Mode Microplate Reader combines the performance of a dual-monochromator, multi-mode microplate reader and an integrated 8-channel or 16-channel pipettor into one compact bench top reader. This integrated system provides a multi-detection platform capable of increasing liquid-handling throughput and flexibility for biochemical-based and cell-based assays. The system is equally amenable to agonist and antagonist assay formats. The instrument combines fluid transfer with multi-detection optics to provide a microplate reader capable of doing a broad span of applications for the drug discovery and research environments.

The extreme flexibility and high sensitivity of the instrument make it appropriate for applications within the fields of biochemistry, cell biology, immunology, toxicology, molecular biology, and microbiology. Fluidic integration expands the capabilities of the instrument to include fast fluorescence (calcium mobilization), luminescence, and absorbance assays, in addition to applications that include ELISA, nucleic acid and protein quantitation, homogeneous and heterogeneous enzyme activity assays, and microbial growth, endotoxin testing, and reporter gene assays.

Supported read modes:

- [Absorbance Read Mode on page 52](#) (UV and Visible)
- [Fluorescence Intensity Read Mode on page 57](#)
- [Luminescence Read Mode on page 65](#)
- [Time-Resolved Fluorescence Read Mode on page 68](#)
- [Fluorescence Polarization Read Mode on page 73](#)

Supported read types, see [Read Modes and Read Types on page 50](#):

- Endpoint
- Kinetic
- Well Scan
- Spectrum
- Flex

### Instrument Features

The instrument can have the following features:

- Dual monochromators
- Reads 6, 12, 24, 48, 96, and 384-well plates for all reads modes
- 8-channel pipettor to do plate-to-plate fluid transfers from a 96-well compound plate to the assay plate 8 wells at a time
- 16-channel pipettor to do plate-to-plate fluid transfer from a 384-well compound plate to the assay plate 16 wells at a time
- Top detection for Fluorescence, Luminescence, Time-Resolved Fluorescence, and

Fluorescence Polarization read modes.

- Bottom detection for all read modes except Fluorescence Polarization



The instrument's dual monochromators enable you to target the optimal assay excitation and emission wavelengths and eliminate the need to change expensive band pass filters between experiments. The two holographic diffraction grating monochromators enable you to select any wavelength in the following ranges:

- Absorbance read mode from 200 nm to 1000 nm
- Fluorescence Intensity, Luminescence, and Time-Resolved Fluorescence read modes from 250 nm to 850 nm
- Fluorescence Polarization read mode from 300 nm to 750 nm

Integrated pipetting provides flexibility and offers parameters to optimize assay robustness. Trituration, mixing through aspiration and dispensing with the pipettor, encourages mixing to either resuspend compounds or mix reagents to promote a rapid response with minimal assay variability. In addition to trituration during fluid transfer, the instrument can shake the well contents before each read cycle for kinetic analysis of solid phase, enzyme mediated reactions.

The Flex read type enables you to transfer fluid from the compound plate to the assay plate while immediately reading a fast kinetic assay that uses the Absorbance, Fluorescence, or Luminescence read modes. Alternatively, you can have the pipettor head transfer fluid before an Endpoint or Kinetic read type assay to automate fluid transfer and minimize user interaction.

PathCheck® Pathlength Measurement Technology allows normalization of variable well volumes for the Absorbance read mode. In addition, the PathCheck Technology allows for pipettor validation, including the online 8-channel and 16-channel pipettors, and to compare experiments from different days. See [PathCheck Pathlength Measurement Technology on page 53](#).

The fluidics module interfaces with the read chamber in the detector module, which has a high-powered Xenon-arc flash lamp as the light source. You can vary the number of lamp flashes per read to optimize the sensitivity (more flashes) or read speed (fewer flashes).

The temperature in the read chamber is isothermal, both at ambient and when you turn on the incubator. When the incubator is on, the instrument can maintain the temperature inside the read chamber at 2°C (3.6°F) above ambient to 45°C (113°F).

## 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>.



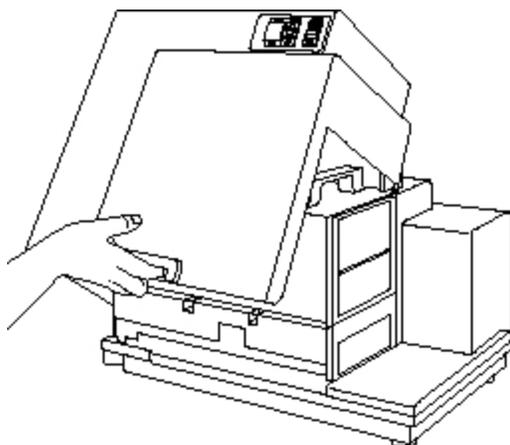
**Note:** For information about the computer specifications that are required to run the software, the software installation and licensing instructions, and the directions to create the software connection between the computer and the instrument, see the *SoftMax Pro Data Acquisition and Analysis Software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

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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.

## Instrument Covers

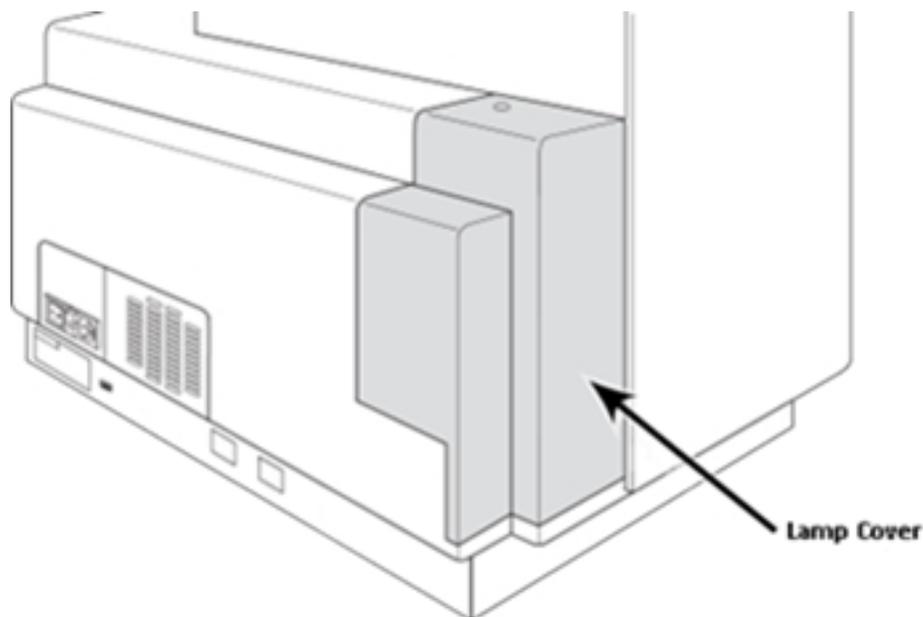
The instrument is protected by a molded plastic housing. The large top cover protects the fluidics module and the exposed portions of the detection module. You can open the top cover for certain limited maintenance and troubleshooting procedures. See [Opening the Instrument on page 81](#).



**Note:** To achieve optimal performance during reads, you must operate the system with the top cover closed.

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The plastic lamp cover provides access to the Xenon-arc flash lamp assembly on the right side of the reader, as viewed from the rear.



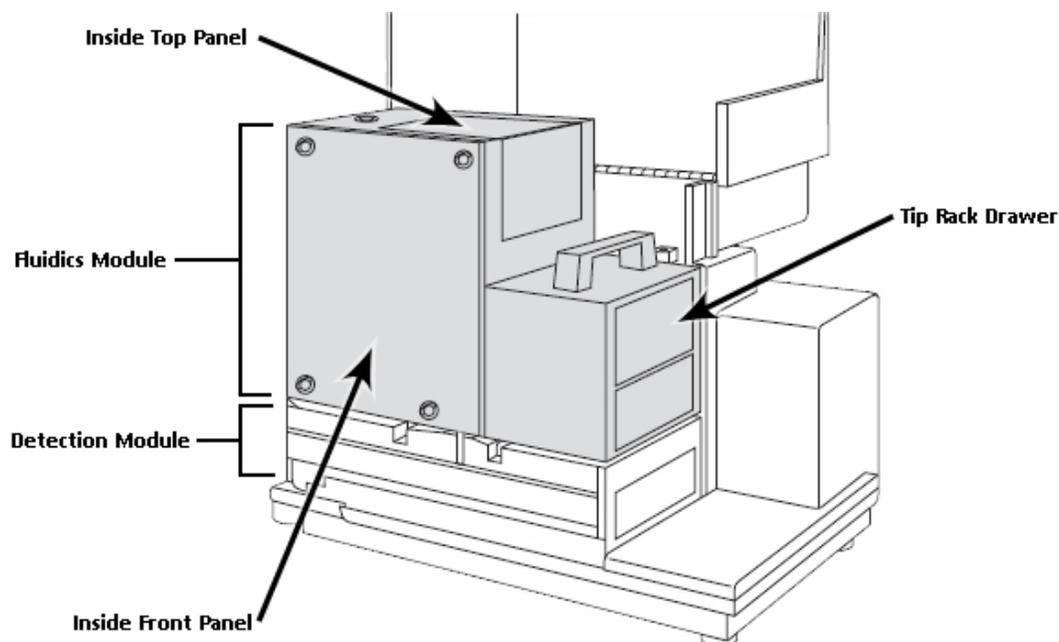
**CAUTION!** There are no user servicable parts inside the lamp cover. If the lamp needs to be replaced, contact Molecular Devices service and support.

## Fluidics Module

The instrument's fluidics module uses an integrated 8-channel or 16-channel pipettor to increase assay flexibility by transferring reagents from 96 or 384 distinct wells in the compound plate to the assay plate. The software enables you to define individual reagents or compound concentrations to deliver to each well during the assay.

The instrument can transfer up to three compounds from columns in the compound plate to a single column in the assay plate, at different points during or before the total read time. The direct transfer from the compound plate reduces consumption and allows you to explore more assay conditions in a single plate. These additions can occur either concurrently with kinetic analysis of reactions or before an assay to automate reagent additions.

The software enables you to define the specific tip from the tip drawer to aspirate a specific volume of reagent from a specific well in the compound plate and to dispense the reagent to a specific well in the assay plate. In addition, kinetic cell-based assay throughput (for example, calcium mobilization) is increased when you analyze 8 or 16 wells of the assay plate together rather than individually. You can optimize dispense parameters for each experiment to accommodate cells with different adherence characteristics to prevent cell dislodging.



The fluidics module houses the pipettor head, several motors, the fluidics components, and two drawers. One drawer is for the pipette tip rack and the other drawer is for the compound plate. The pipettor head moves vertically between the drawers.

## Avoiding Mechanical Problems

To prevent problems of a mechanical nature, read all sections in this user guide before you attempt to use the instrument for reads with fluidics.

The complex mechanical nature of the FlexStation 3 includes both fluidics and optical reads. Smooth and reliable operation of the system depends on both good experimental design and operator knowledge.

The intended function of the instrument requires it to have many moving parts. There are many interactions with the instrument including introduction of various types of plastic disposable plates, tips, and compound plates. Molecular Devices has made every effort to ensure smooth and reliable operation of the instrument. However, problems can occur. The design of the FlexStation 3 instrument provides the means to successfully recover from most problems.

Before you use the instrument, you should disconnect the power and open up the instrument to become familiar with the observable mechanical parts of the fluidics module. It is possible to manually (and always gently) move the drawers, doors, and pipettor head. See [Manually Open Drawers on page 79](#).



**WARNING!** Only service trained users should open the panels on the fluidics module to access the internal mechanical parts.

There are several opportunities for mechanical problems within the fluidics module. Fortunately, these problems are rare if you understand how to avoid them. See [Troubleshooting the Fluidics System on page 81](#).

- Pipette tips can jam in the rack, possibly due to imperfectly molded tips.
- After transfer, an unreleased tip can remain on the nose cone and be jammed against one of the lower drawers or into another tip during a subsequent transfer operation.
- If the tips are released from the nose cones during operation, they might fall outside of the rack. To help prevent frequent tip drops, periodically clean the pipettor barrels.
- If the power fails during a pipetting step, serious damage can result to the instrument if appropriate corrective steps are not taken. See [In Case of Power Failure on page 23](#).



**Note:** The software cannot confirm the presence, absence, or configuration of tips and plates in the instrument drawers.

Follow these procedures to help avoid problems:

- Use only tips from Molecular Devices that are specified for the FlexStation 3 instrument in a 96-tip or 384-tip rack configuration.

#### Molecular Devices Pipettor Tips

96-Well Configuration	384-Well Configuration
Clear tips: P/N 9000-0912	Clear tips: P/N 9000-0763
Black tips: P/N 9000-0911	Black tips: P/N 9000-0764

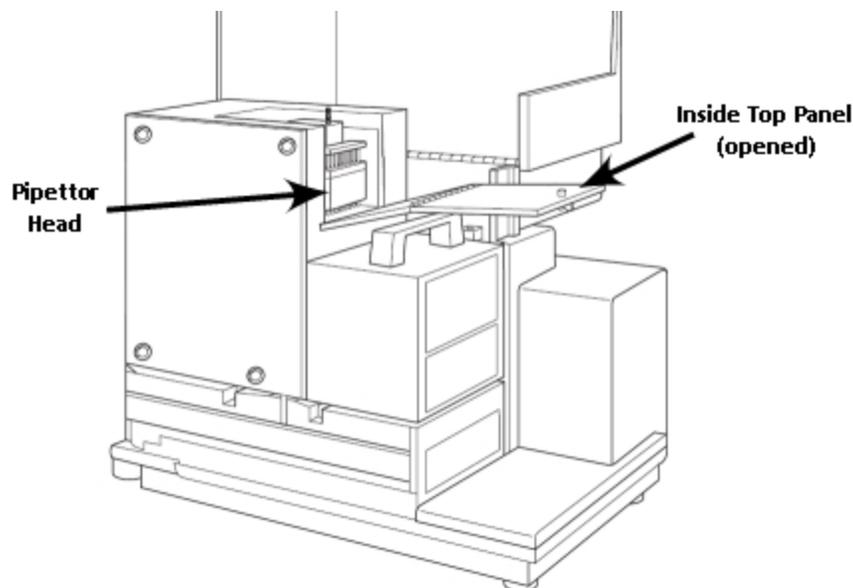
- Remove the tip rack lid before you place the tip rack in the tip rack drawer.
- Make sure you seat the tip rack, plates, and compound plate securely in the correct drawers.
- Make sure the layout of the tips you specify in the software accurately represents what you have in the tip rack.
- Make sure the compound plate and the assay plate you specify in the software accurately represent the plates you actually use.
- Make sure the pipettor configuration, compound plate type, and assay plate type are compatible with each other. For example, the 16-channel pipettor uses 384 tips with a 384-well compound plate and a 384-well assay plate.
- Make sure that the red knob that holds the pipettor head in place is securely tightened.
- Periodically clean the pipettor barrels to help prevent tip drops.

## Pipettor Head

The fluidics module incorporates an 8-channel or 16-channel pipettor that automatically changes tips and transfers reagents from the compound plate to the assay plate to be read by the instrument. The software enables you to adjust the pipette height and dispense rate. The instrument can add reagents within milliseconds of a column being read to enable fast kinetic assays of transient responses.

The fluidics module uses disposable pipette tips to minimize reagent cross contamination between wells or experiments.

Use the 8-channel pipettor head with 96-well plates and use the 16-channel pipettor head with 384-well plates.



**Note:** The pipettor head, pipette tip rack, compound plate, and assay plate must be compatible with each other. For example, use an 8-channel pipettor head with a 96-well pipette tip rack, a 96-well compound plate, and a 96-well assay plate.

Service trained users can remove the pipettor head to switch between the 8-channel head and the 16-channel head and for periodic cleaning to remove silicone lubricant, dust, and other miscellaneous contamination. See [Pipettor Head Cleaning, Maintenance, and Troubleshooting on page 87](#).

## Mixing

The instrument can use either the Trituration function or the Shake function to mix the contents of the wells. Trituration uses the pipettor to alternately aspirate the fluid from a well and then dispense it back into the well to mix the contents of the well in a plate. Trituration is available during assays that include fluid transfer and can be done in either the compound plate or the assay plate. In the compound plate, trituration can resuspend compounds that might have crashed out of solution. In the assay plate, trituration can promote prompt mixing when you deliver small reagent volumes for fast kinetic cell-based assays, such as calcium mobilization.

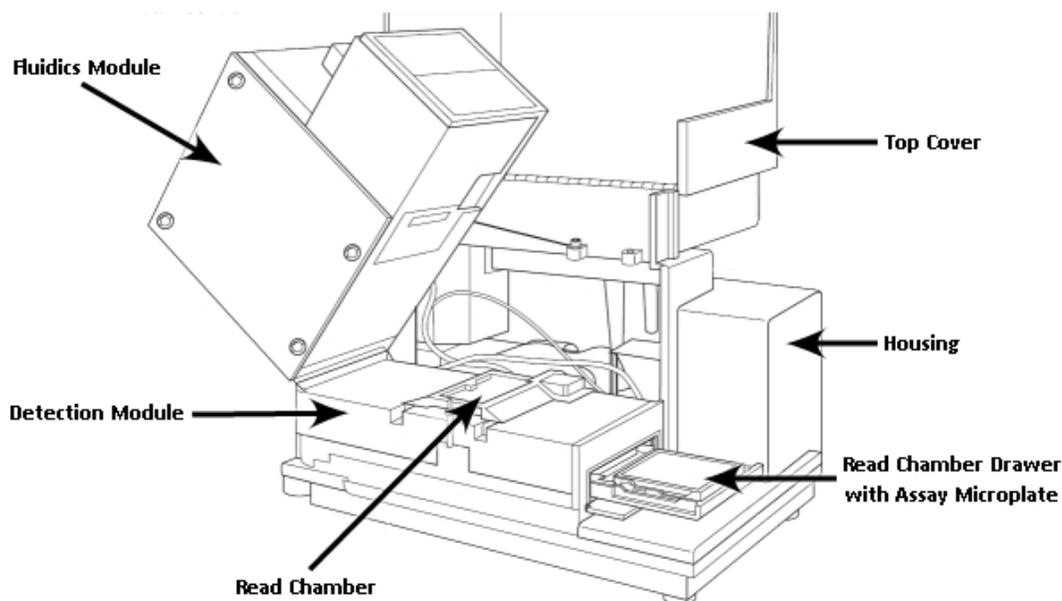


**Note:** Trituration in the assay plate can agitate the cells in the wells that cause responses not associated with the compound addition. Do assay development to determine if the assay requires trituration.

You define shake settings before you start a read to shake the plate at preset intervals and mix the contents in each well. You should use Shake for ELISA and other solid-phase, enzyme-mediated reactions to enhance accuracy.

## Detection Module

The detection module is the lower portion of the instrument and is contained in a molded plastic housing to which the top cover is attached on the left side of the instrument. The detection module houses the read chamber, the optics bench, several cables and optic fibers, the power supply, the flash lamp, and other hardware. The fluidics module attaches to the detection module. Service trained users can tilt the fluidics module off to the side to provide access to the optical system for troubleshooting or maintenance.



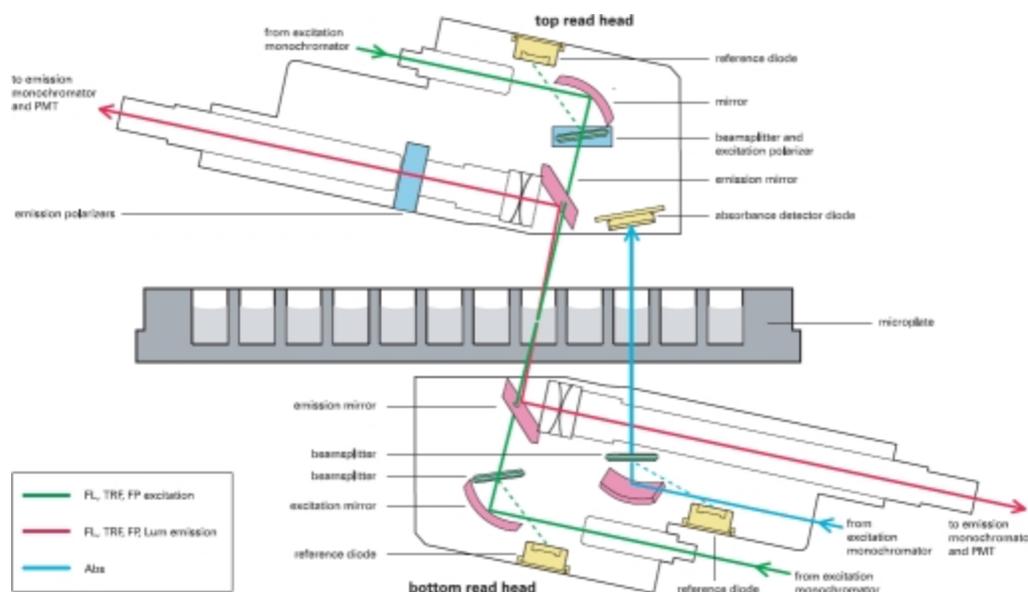
## Optical System

The instrument contains dual photomultiplier tubes (PMT) to provide flexibility and enable multiple detection modes. A separate PMT provides more sensitivity for luminescence applications. Reference diodes automatically adjust to slight fluctuations in excitation intensity to reduce measurement noise. Absorbance applications are enhanced using top quality UV grade fibers to provide high light transmission in the lowest wavelengths. These optical characteristics make the system performance comparable to a top-of-the-line dedicated spectrophotometer or spectrofluorometer.

The instrument uses excitation and emission filter wheels to decrease interference by stray light and to augment the wavelength selection provided by the monochromators. The two independent, single-channel read heads support coaxial excitation and emission beams and can service top and bottom read requirements. Internal compensation for detector sensitivity, photomultiplier tube voltage, and excitation source intensity help eliminate instrument based, day-to-day and instrument-to-instrument variations in measured fluorescence values.

The optical system includes a xenon flash lamp, monochromators, excitation bandpass filters, emission cut-off filters, photomultiplier tubes (PMT), and photodiodes. There are several cables and fibers that exit the optics bench and enter the read chamber:

- The excitation fibers are thin and black or red and have a collar and pins.
- The emission fibers are black and fatter than the excitation fibers and have an attached electrical cord.
- The electrical connector to the read head is green and has a brass fitting.



**CAUTION!** Optical fibers are very fragile, especially the excitation fiber. Handle cables with extreme care. Do not flex, twist, bend, or stretch the optical cables.

## Read Chamber

The read chamber includes the plate carriage that holds the assay plate in the read chamber and the top and bottom read heads. Temperature controls enable you to keep the read chamber at an elevated temperature. The instrument uses a plate sensor to make sure that the assay plate is present in the read chamber before a read starts.

The software enables you to switch between a bottom read and a top read with no manual interaction with the instrument. Bottom reads allow for well scanning ability to maximize the sample area for 6, 12, 24, 48, 96, and 384-well plates. Bottom reads enable concurrent reagent addition to monitor fast kinetic reactions such as calcium mobilization.

You must use clear bottomed plates for bottom reads. Bottom reads are intended for cell based assays.

## Temperature Control

The instrument contains electric heaters, a fan, efficient insulation, and temperature sensors to regulate and control the temperature of the read chamber. Seven sensors are mounted inside the read chamber to measure the air temperature and read chamber temperature. The temperature feedback closed-loop control algorithms measure the read chamber air temperature, compare it to the temperature set point, and use the difference to calculate the regulation of the heating cycles. This technique results in accurate and precise control of the read chamber temperature with a temperature variation of the air across the entire assay plate of less than 1°C. Temperature uniformity within the assay plate itself depends on its design, materials, and configuration.

The instrument can maintain the temperature inside the read chamber at 2°C (3.6°F) above ambient to 45°C (113°F). You can set and control the temperature from the control panel on the front of the instrument or from the software. The instrument does not regulate the temperature of the fluidics module. You should warm the compound plate and tips to the desired temperature before you place them inside the instrument.



**Note:** The instrument temperature sensors detect the temperature of the air inside the chamber, not the temperature of the samples in the plate. If you use the instrument to warm the samples, you should use a seal or lid on the plate to prevent evaporation of the sample. A seal or lid helps to maintain uniform temperature. Letting the prepared sample equilibrate inside the plate chamber can take an hour or more. You can speed up equilibration by pre-warming the sample and the assay reagents before you place the plate in the chamber. Validate the data quality to determine whether the seal or lid can stay on the plate for the read.

---

## Chapter 2: Setting Up the Instrument

# 2

Install the SoftMax Pro Software on the computer that operates the microplate reader. See the *SoftMax Pro Data Acquisition and Analysis Software Installation Guide*.



**CAUTION!** Instrument installation must be done by qualified Molecular Devices personnel or a service-trained user. Do not attempt to install and setup the instrument if you have not been trained properly by appropriate Molecular Devices personnel.

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**WARNING!** Always turn the power switch off and disconnect the power cord from the main power source before you set up or relocate the instrument.

---



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

---



**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.

---



**CAUTION!** Do not attempt to move, maintain, or service the instrument with the pipette tips, compound plate, or assay plate in place. Spillage or damage to the pipette tips, plates, or the instrument can occur.

---

## Package Contents

The following accessories are included with the instrument:



**CAUTION!** Do not use parts and accessories that are not authorized by, specified by, or provided by Molecular Devices. Use of unauthorized parts can damage the instrument.

Part Number	Description
SoftMax Pro Software	Latest version, Software installation guide, and product key
4400-0002	Power cord, U.S.
4400-0036	Power cord, Europe
9000-0149	USB computer connection cable, 3 meter (9.8 foot)
0310-9336	Plate adapter (read chamber drawer)
2500-1029	Compound baseplate (compound plate drawer)
4600-0029	Fuse (6.3A 5 mm × 20 mm Slo-Blo IEC)
0310-3992	Pipettor head, 8-channel (if ordered)
0310-5025	Pipettor head, 16-channel (if ordered)
	Yellow plate for each included pipettor head
9100-0051	Hex key, 3/32 inch
9000-0911	*Pipette tips, 96-well (black)
9000-0912	*Pipette tips, 96-well (clear)
9000-0763	*Pipette tips, 384-well (black)
9000-0764	*Pipette tips, 384-well (clear)

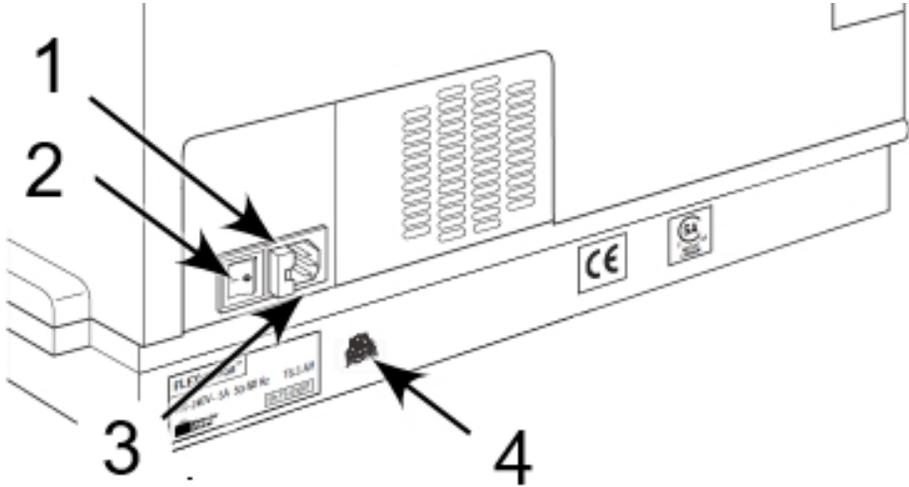
\*One box (10 racks) of pipette tips is shipped with the system pipettor head.

### Connecting Instrument Cables

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

Illustration	Part Number	Description
	9000-0149	USB computer connection cable, 3 meter (9.8 foot)
	4400-0002 or 4400-0036	Power cord, 1 meter (3.3 foot)

To connect the instrument cables:



Item	Description
1	Fuse box cover
2	Power switch
3	Power cord receptacle
4	USB port

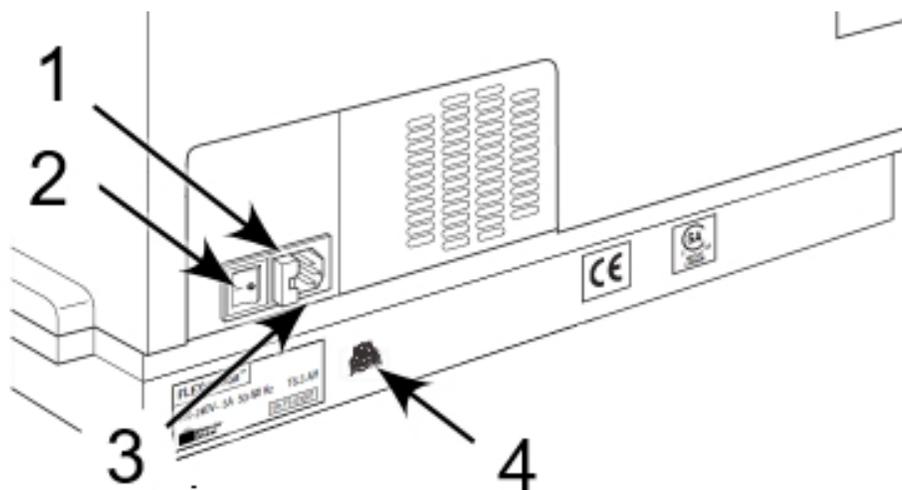
1. Make sure that the power switch on the rear of the instrument is in the Off position.
2. Connect one end of the supplied USB cable to the USB port on the rear of the instrument and the other end to a USB port on the computer.
3. Connect the supplied power cord to the power port on the rear of the instrument and then connect the other end to a grounded electrical wall outlet.

 **CAUTION!** Make sure that no cables or cords run under the instrument.

## Chapter 3: Getting Started

# 3

Now that you installed the SoftMax Pro Software on the computer, the instrument is set up, and the cables are connected, it is time to get started. The power switch and power connection are on the rear of the instrument.



Item	Description
1	Fuse box cover
2	Power switch
3	Power cord receptacle
4	USB port

To ensure sufficient ventilation and provide access to disconnect power from the instrument, maintain a 10 cm to 20 cm (3.9 in. to 7.9 in.) gap between the rear of the instrument and the wall.

To turn the instrument on or off, press the power switch to place the rocker in the on or off position.

When you power on the instrument, it does diagnostic checks to make sure that it is functioning correctly. All three drawers open and close. After about four minutes, the control panel displays the temperature inside the read chamber. When the diagnostic check is complete, the read chamber drawer opens. After about five minutes, the instrument is initialized and ready to use.

When the instrument is initialized and ready, power on the host computer and start the software.

Generally, you do not need to power off the instrument at the end of the day. However, if you do not plan to use the instrument for more than a day, you should power off the instrument.

Before you power off the instrument, make sure that all three drawers are empty. See [Loading and Unloading Drawers on page 25](#).

### In Case of Power Failure

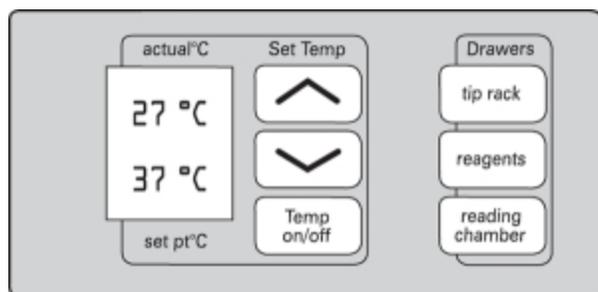
Interacting properly with the instrument following a power failure is very important.

If there is a power failure while the instrument is running, you must manually turn off the power switch on the rear of the instrument.

To avoid instrument damage if a power failure occurs during a fluidics read, see [Recovery Procedure on page 93](#).

## Control Panel

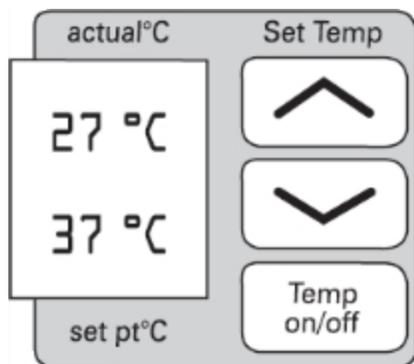
The instrument control panel has hardware controls to view and set the temperature in the read chamber and to open and close the drawers. You can also use the software to control these functions. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.



## Temperature Settings

The instrument can maintain the temperature inside the read chamber at 2°C (3.6°F) above ambient to 45°C (113°F). The instrument control panel and the software enable you to set the read chamber temperature. For how to control the temperature with the software, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

Two temperatures display on the instrument control panel.



The first number displays the temperature inside the read chamber in real time. When the incubator is off, this is the ambient temperature. The second number displays the temperature set point you define for the current experiment when the incubator is on.

When you power on the instrument and when the incubator is off, the temperature in the read chamber is ambient and isothermal. The instrument begins to warm the read chamber when you turn on the incubator.



**Note:** If you want to elevate the temperature in the read chamber for your experiment, turn on the incubator at least 30 minutes before you plan to start your plate read.

---

Press the **Temp on/off** button on the instrument control panel to turn on the incubator. The control panel displays the temperature set point in the lower half of the LCD to indicate that temperature control is on. The instrument sets the read chamber to the default temperature of 37°C.

Press the up arrow button or the down arrow button until the lower number on the control panel displays the required temperature. The instrument maintains the read chamber temperature at the set point until you turn off the incubator.

Press the **Temp on/off** button again to turn off the incubator. When the incubator is off, the temperature in the read chamber starts to return to ambient.

If the power is shut off to the instrument for any reason, you need to turn on the incubator again and allow at least 10 minutes for the control algorithm to fully stabilize the read chamber temperature.



**Note:** The read chamber is warmed to the temperature set point. However, the temperature of the fluidics module might be lower than the set point.

---

The accuracy of the temperature set point is guaranteed only if the set point is at least 2°C above ambient. If the temperature set point is lower than the ambient temperature, the read chamber temperature remains at ambient. Temperature regulation is controlled by heaters only and cannot cool the temperature to a setting lower than ambient.



**Note:** The instrument temperature sensors detect the temperature of the air inside the chamber, not the temperature of the samples in the plate. If you use the instrument to warm the samples, you should use a seal or lid on the plate to prevent evaporation of the sample. A seal or lid helps to maintain uniform temperature. Letting the prepared sample equilibrate inside the plate chamber can take an hour or more. You can speed up equilibration by pre-warming the sample and the assay reagents before you place the plate in the chamber. Validate the data quality to determine whether the seal or lid can stay on the plate for the read.

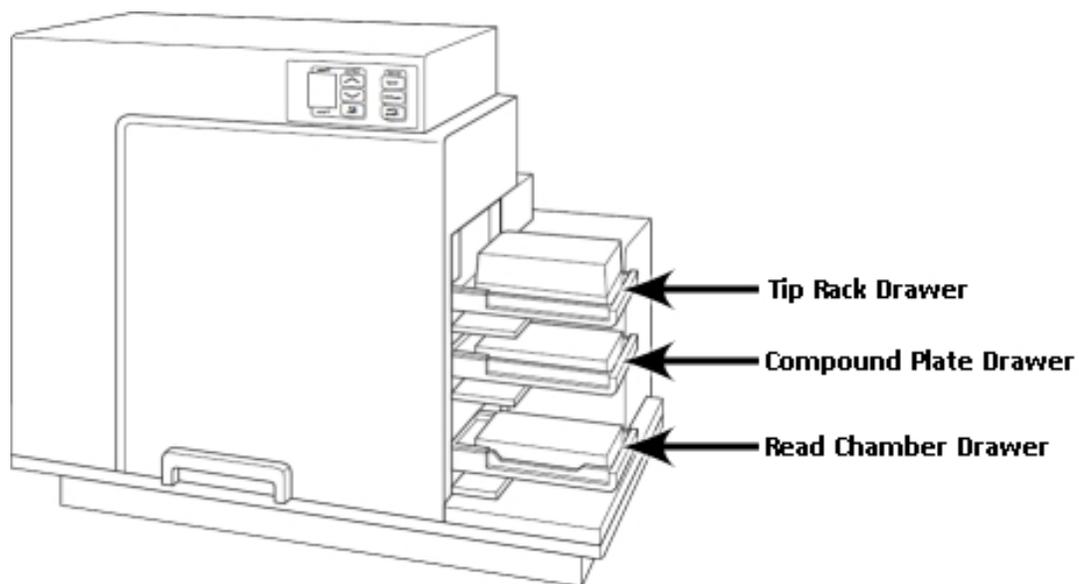
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## Loading and Unloading Drawers

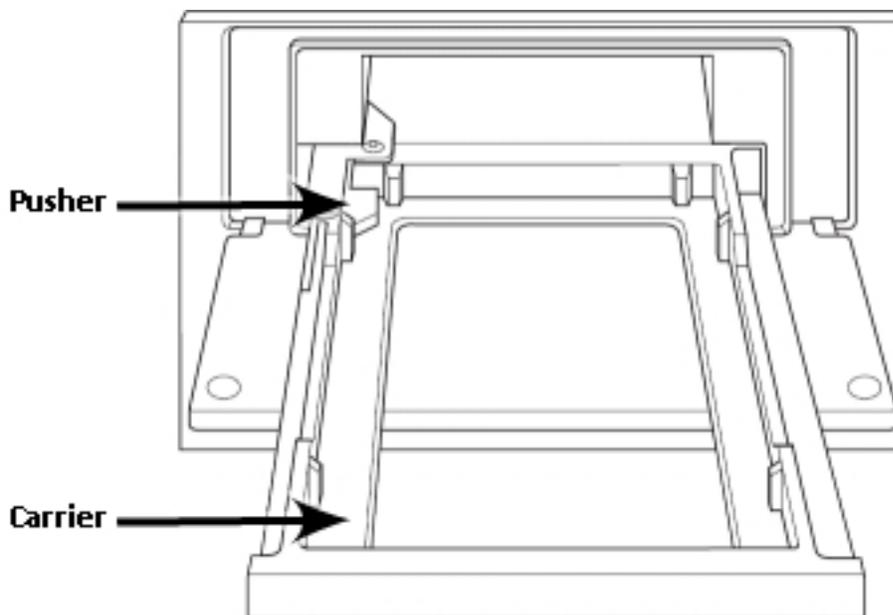
The instrument has three drawers that open on the right side. The top two drawers in the fluidics module open and close to move the pipette tip rack and compound plate (or troughs) into and out of the instrument. The read chamber drawer in the detection module moves the assay plate into and out of the read chamber.



**CAUTION!** Do not obstruct the movement of the drawers. To retrieve a plate after an error condition or power outage when the drawer does not open, you can open the drawer manually. See [Manually Open Drawers on page 79](#).



Small plastic pushers in the front left corner of each drawer hold the plates, racks, or reservoirs in place when the drawers close.



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 **CAUTION!** Do not use parts and accessories that are not authorized by, specified by, or provided by Molecular Devices. Use of unauthorized parts can damage the instrument.

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 **CAUTION!** Do not attempt to move, maintain, or service the instrument with the pipette tips, compound plate, or assay plate in place. Spillage or damage to the pipette tips, plates, or the instrument can occur.

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You can use the instrument control panel to open and close the drawers or you can use the software. For how to open and close drawers with the software, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

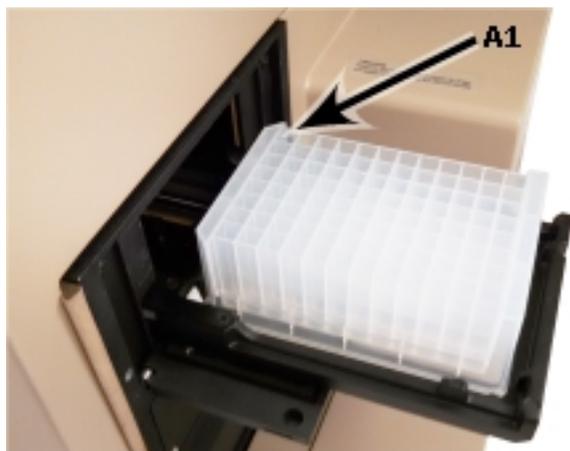
### Tip Rack Drawer

The top drawer holds the pipette tip rack.

To insert a tip rack:

1. Power on the instrument.
2. Press **tip rack** on the instrument front panel to open the tip rack drawer.

3. Insert the filled tip rack into the drawer, place well A1 into the upper left corner of the drawer.



### Compound Plate Drawer

The compound plate drawer holds a 96-well or 384-well plate, or a trough that has the same footprint as a standard plate. The instrument can simultaneously transfer a column of fluids from the compound plate:

- Eight fluids from a 96-well compound plate to a 96-well assay plate
- Sixteen fluids from a 384-well compound plate to a 384-well assay plate

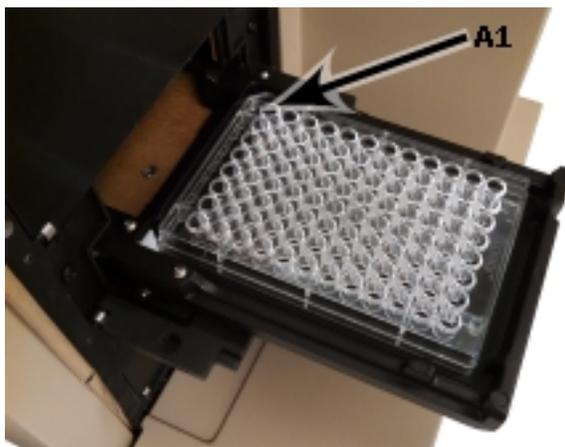
You must insert the compound baseplate in the compound plate drawer before you place the compound plate or trough in the compound plate drawer to reduce stray light.

To insert the compound baseplate and the compound plate:

1. Power on the instrument.
2. Press **reagents** on the instrument front panel to open the compound plate drawer.
3. Hold the baseplate so that its cutout corner faces the front left corner of the drawer and then lower the baseplate into the compound drawer.



4. Insert the filled compound plate into the drawer, with well A1 in the upper left corner of the drawer. Make sure the compound plate is flat against the compound baseplate.



### Read Chamber Drawer

The read chamber drawer opens to load and unload the assay plate from which you want to acquire data. When the temperature control is off, the read chamber drawer opens when you power on the instrument and after a read. When the temperature control is on, the read chamber drawer closes automatically to maintain the temperature in the read chamber.

If you read standard 96-well or 384-well plates, you must insert the black plate adapter in the read chamber drawer before you insert the assay plate. The black plate adapter elevates the assay plate in the drawer for optimal performance with standard 96-well or 384-well plates for bottom read and top read mode.

If you read 6-well, 12-well, 24-well, or 48-well plates and the plate adapter is in the read chamber drawer, you must remove the black plate adapter before you insert the assay plate.



**CAUTION!** Incorrect insertion or removal of the plate adapter can cause damage to the read chamber drawer.

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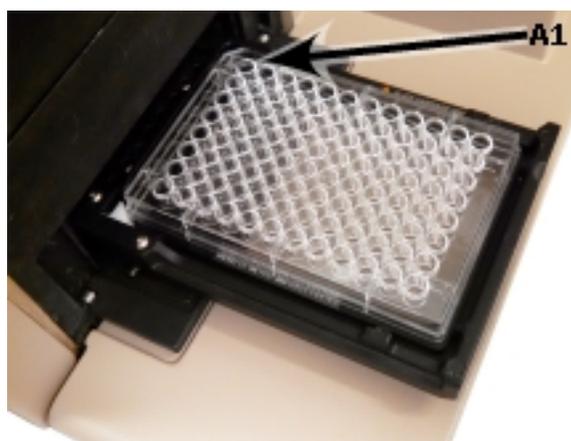
To insert the black plate adapter and the assay plate:

1. Power on the instrument.
2. Press **reading chamber** on the instrument front panel to open the read chamber drawer.

3. For a 96-well plate or a 384-well plate, lower the black adapter plate into the read chamber drawer with the cutout corner facing the front left corner of the drawer.



4. For 6-well, 12-well, 24-well, and 48-well plates, remove the black plate adapter.
  - a. Hold the plate adapter at the front (long side of the drawer) and push toward the back (Row A).
  - b. Lift the front (Row H or Row P) of the plate adapter and remove it from the drawer.
5. Insert the filled assay plate into the drawer with well A1 in the upper left corner of the read chamber drawer.
  - For a 96-well plate or a 384-well plate, make sure the assay plate is flat against the black plate adapter.
  - For 6-well, 12-well, 24-well, or 48-well plates, make sure the assay plate is flat against the read chamber drawer bottom.



## Plate Recommendations

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well plates and strip wells. For micro-volume measurements, the instrument supports SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates.

For the Absorbance read mode at wavelengths below 340 nm, you must use special UV-transparent, disposable, or quartz plates that allow transmission of the far ultraviolet spectra.



**Note:** Use 96-well or 384-well plates for reads that use fluidic integration.

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If you read standard 96-well or 384-well plates, you must insert the black plate adapter in the read chamber drawer before you insert the assay plate. The black plate adapter elevates the assay plate in the drawer for optimal performance with standard 96-well or 384-well plates for bottom read and top read mode.

If you read “high profile” 6-well, 12-well, 24-well, or 48-well plates, you must remove the plate adapter before you insert the assay plate. See [Loading and Unloading Drawers on page 25](#).

The SoftMax Pro Software includes a plate list and you can use the software to define additional plate types with the manufacturer's specifications for well size, spacing, and distance from the plate edge.

The type of plate and the way you handle it can have an affect the measurement performance of the instrument. Select a plate type with properties suited for the application.

General plate handling guidelines:

- Never touch the clear well bottom of plates.
- Visually inspect the bottom and rim of the plate before use to make sure that they are free of dirt and contaminants.
- Keep unused plates clean and dry.
- Make sure that the strips on strip plates are inserted correctly and level with the frame.
- Do not use V-bottom plates unless the performance has been tested and validated with this instrument. Irregular plastic density in the tip of the well can cause inaccurate measurements.

For the Fluorescence Intensity read mode, you should use solid black plates for top reads, and black walled, clear bottomed plates for bottom reads.



**Note:** Not all plates are made with the same materials. Some plastics, most notably polystyrene, have significant native fluorescence and can cause moderate to severe background fluorescence, especially in the UV range. If your fluorescence experiments require high sensitivity, it might be appropriate to use plates designed and designated by the manufacturer to reduce background fluorescence.

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For the Luminescence read mode, you should use white plates.



**Note:** White plates provide significantly higher signal for luminescence than black plates, and are recommended if high sensitivity is required. However, white plates can exhibit some detectable phosphorescence that increases background after being exposed to light (in particular under neon lights). For maximum sensitivity, you should prepare plates under reduced ambient light conditions, and place the plate inside the instrument for 1 to 10 minutes to adapt the plates to darkness before you start the read.

---

## Pipette Tips

Tips are available in black and clear options. Black tips are generally used for the Fluorescence read mode when auto-fluorescent properties of clear tips can interfere with your response. You should evaluate both black and clear tips during assay development to determine which tip version is appropriate to your assay.

- For 96-well assays, Molecular Devices specifies the use of 96-well, FlexStation Pipette Tips. These 200  $\mu$ L tips are available in black (P/N 9000-0911) and clear (P/N 9000-0912) and can be purchased in 10 racks per box quantities.
- For 384-well assays, Molecular Devices specifies the use of 384-well, FLIPR and FlexStation Pipette Tips. These 30  $\mu$ L tips are available in black (P/N 9000-0764) and clear (P/N 9000-0763) and can be purchased in 50 racks per case quantities. To purchase partial cases, contact your local sales representative.

## Chapter 4: SoftMax Pro Software Protocol Settings

Use the SoftMax Pro Software to define the protocol for your assay, to define the instrument data acquisition settings, and to analyze the data the instrument acquires. You define acquisition settings for each Plate section in the protocol. This document describes settings that are for the FlexStation 3 reader. For more detailed information, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

Select the Plate section in the Navigation Tree and click **Acquisition Settings** to display the Settings dialog. On the Settings dialog, select the read mode and read type. The settings for the read mode/read type combination you select appear in categories on the left side of the dialog.

The Settings dialog for the FlexStation 3 includes settings that are available for use with the fluidics module. Several fluidics module settings must correlate with one another. In particular, the settings in the Plate Type category for the assay plate, the Read Area category, the Compound Source category, the Compound Transfer category, and the Compound and Tip Columns category are all dependent upon each other. The SoftMax Pro Software cross checks these settings as you define them. If the settings do not correlate, a message appears, and you must correct the settings before you can continue.

The following topics describe the settings in each category on the Settings dialog.

### Wavelength Settings

The Wavelengths category enables you to define which wavelengths to use for the read. Your read mode and read type selection determines the available settings.

#### Endpoint, Kinetic, Well Scan, and Flex Read Types

For the Absorbance read mode, you can select up to six wavelengths and for all other read modes you can select up to four wavelengths.

Click the **Number of Wavelengths** drop-down and select the number of wavelengths to read. Enter the wavelength value for each wavelength.

#### Spectrum Read Type

For the Spectrum read type, enter the **Start** wavelength value and enter the **Stop** wavelength value. In the **Step** field, enter the value for how much the wavelength should increment between reads.

The minimum **Step** increment is 1 nm.

#### Cutoff

In addition to the emission monochromator, for the Fluorescence, Time-Resolved Fluorescence, and Fluorescence Polarization read modes, fluorescent quality cutoff filters help reduce background signal. The term cutoff refers to the filters the instrument uses to block unwanted residual excitation light and minimize background interference.

- Select the **Auto Cutoff** check box to have the software choose the long pass emission filter to use based on the excitation wavelength you enter.
- Clear the **Auto Cutoff** check box, then click the corresponding drop-down and select the cutoff filter for each wavelength. For the Spectrum read type you must select a cutoff filter (or None).

When you clear the Auto Cutoff check box, to determine the manual setting for a cutoff filter, you need to know the value of the Stokes shift, which is the difference between the wavelengths of the excitation and emission maxima. If the Stokes shift is small, choose an excitation wavelength that is as far as possible from the emission maximum while still being capable of exciting the fluorophore. This causes less of the excited light to overlap the emission spectrum which permits better selection and quantitation of the emitted light.

## Plate Type Settings

The Plate Type category enables you to select the type of assay plate to read. The assay plate contains the sample to read. Your plate type selection determines the display of the wells in the Plate section and the alignment of the read head with respect to the plate. Your plate type selection should match the plate type, the number of wells, the clarity or opaqueness of the wells, and the round or square shape of the wells in the plate to read. You select the plate type for the plate from which any compounds are to transfer in the Compound Source category. See [Compound Source Settings on page 41](#).



**Note:** If you change the plate type, the software discards any well assignments you make for related templates. Group sections remain and you can select new wells to assign to the existing Group sections.

---

The software includes a plate library that contains plates of various sizes with standard dimensions. If you are uncertain which plate to use, select the Standard plate definition.

- Click the **Plate Format** drop-down and select the number of wells in the plate.
- In the **Select Specific** list, select the specific plate to read.

If the plate you use is not in the Select Specific list, select a similar plate and click **Edit Plate** to display the Plate Editor dialog. The Plate Editor dialog enables you to add a new plate type to the plate library. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.



**Note:** If you change the plate type you select in the Plate Type category for the assay plate after you select the plate type in the Compound Source category for the compound plate or after you define the Compound & Tips Columns category settings, those settings might automatically change to reflect the new assay plate type and well layout. Be sure to check the settings in the Compound Source and Compound & Tips Column categories to make sure they remain correct for your assay. See [Compound and Tips Columns Settings on page 43](#).

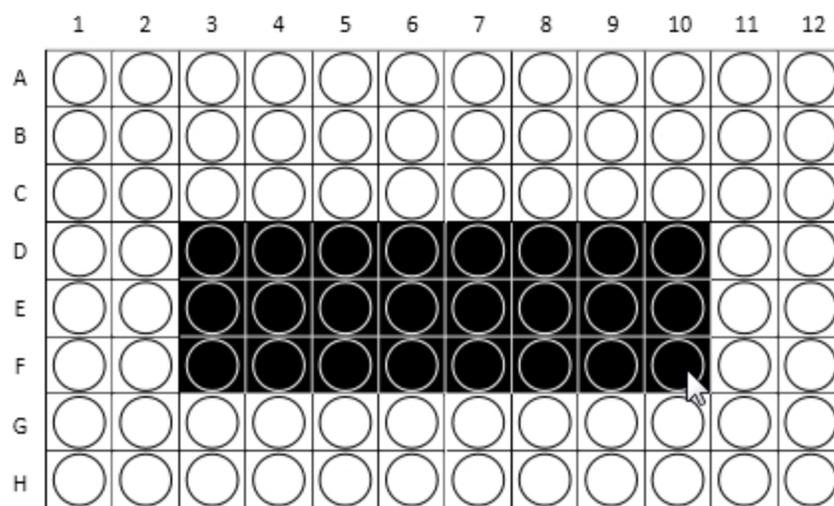
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For more information about plate selection, see [Plate Recommendations on page 29](#).

## Read Area Settings

The Read Area category enables you to select the wells in the assay plate to read. You can select to read a partial plate. Partial plate reads can significantly reduce the time required for Kinetic reads since the instrument does not have to read the entire plate.

To select a contiguous, rectangular region on the plate, drag the mouse pointer to select the wells to read.



Columns do not need to start with “1” but must be contiguous.

To read all the wells in the plate, select the **Select All** check box.

The instrument makes fluid transfers and reads one column at a time. When planning your experiment, you should use partial rows (A through H) rather than partial columns (1 through 12 or 1 through 24) for most situations.

If you select a partial plate read, only the wells to be read are visible in the data display, to indicate that no data will be collected for the other wells. However, all wells display on the Template Editor.

## PathCheck Settings

For the Absorbance read mode with the Endpoint read type, the Pathcheck category enables you to use the PathCheck Technology. See [PathCheck Pathlength Measurement Technology on page 53](#).

- Select the **PathCheck** check box to use the PathCheck Technology Water Constant correction.
- Clear the **PathCheck** check box to not use the PathCheck Technology Water Constant correction.

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:

$$A = \epsilon cL$$

where  $A$  is the absorbance,  $\epsilon$  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.

## TRF Settings

For the Time-Resolved Fluorescence read mode, the TRF Settings category enables you to define the integration delay and the integration time. Time-Resolved Fluorescence measures fluorescence as a function of time after excitation.

- Click the **Integration Delay** drop-down and select the amount of time to elapse between the flash of the lamp (excitation) and the beginning of data acquisition from the well (emission).
- Click the **Integration Time** drop-down and select the amount of time for the instrument to read the well.

## PMT and Optics Settings

The PMT and Optics category enables you to define sensitivity settings for all read modes except Absorbance.

Click the **PMT Gain** drop-down:

- Select **Automatic** to have the instrument automatically adjust the PMT voltage for varying concentrations of sample in the plate. Available for the Fluorescence read mode with Endpoint, Spectrum, and Well Scan read types. Luminescence and Time-Resolved Fluorescence read modes use this setting and the following options are not available.
- Select **High** for samples that have a lower concentration (dim samples).
- Select **Medium** for samples that have a medium concentration.
- Select **Low** for samples that have a higher concentration (bright samples)
- Select **Manual** to enter a specific voltage.

For the Fluorescence Intensity, Time-Resolved Fluorescence, and Fluorescence Polarization read modes, in the **Flashes Per Read** field, enter the number of flashes per read, between 1 and 100.

For the Luminescence read mode, the instrument takes a single reading, as indicated in the **Number of Reads** field. This field is not editable. Click the **Integration Time** drop-down and select the measurement time per well in milliseconds.

For the Fluorescence, Luminescence, and Time-Resolved Fluorescence read modes the instrument can do bottom reads. Not available for the Flex read type.

- Select the **Read From Bottom** check box to have the instrument read up through the bottom of the plate.
- Clear the **Read From Bottom** check box to have the instrument read down from the top.

## Timing Settings

For the Kinetic and Flex read types, the Timing category enables you to define the total run time and the interval between reads.

- In the **Total Run Time** field, enter the amount of time for the total run.
- In the **Interval** field, enter the amount of time to wait between reads.

The software calculates the minimum interval when calibrating based on the total run time and the interval you enter and displays the interval before the first read, before each read, and the number of reads. This calculation depends on many factors including the number of wavelengths, the number of wells to read, and the distance the instrument filter wheel must move. A message appears if you enter an incorrect read interval.

OR

Click **Single Point Read** to set the Total Run Time and the Interval to the same value. Use this to read only one time point.

---

 **Tip:** In some cases, choosing wavelengths in ascending order can get the shortest possible interval.

---

## Well Scan Settings

For the Well Scan read type, the Well Scan Settings category enables you define the pattern and density for the reads within each well. The fill pattern is either round or square to match the well shape. The image in the Well Scan settings shows the shape of the well based on the definition of the assay plate you select. The number of points that are available depend on the well size of the plate you select.

Select from the following read patterns:



Click the **Density** drop-down and select the density of the reads.

The software uses the pattern and density you select to calculate the point spacing and the number of points per well. The minimum number of points is 3 for most plate sizes, while the maximum number of points depends on the well diameter. Only one read per well is allowed for 384-well plates.

The software excludes all points that land within 2.8 mm of an edge.

## Shake Settings

The Shake category enables you to define how to shake the assay plate in the read chamber. Shake is not available for the Flex read type.

- Select the **Before First Read** check box to shake the plate before the first wavelength read. Enter the number of seconds to shake the plate. The plate can shake for 1 second to 999 seconds.
- Clear the **Before First Read** check box to not shake the plate.

For the Kinetic read type you can shake the plate between reads. For single wavelength reads, this shakes the plate before each read at that wavelength. For multiple wavelength reads, this shakes the plate before each read at the first wavelength only.

- Select the **Between Reads** check box to shake the plate between reads. Enter the number of seconds to shake the plate.
- Clear the **Between Reads** check box to not shake the plate between reads.

## Speed Read Settings

For the Absorbance read mode with the Endpoint, Kinetic, and Spectrum read types, the Speed Read category enables you to read the assay plate faster by decreasing the number of lamp flashes. The Speed Read option is particularly useful in Spectrum scans and can greatly reduce the time needed to run the protocol.

Speed Read turns off calibration and uses the air calibration values stored in the instrument firmware. As a result, Speed Read might not provide as accurate an absorbance measurement at each wavelength of a scan as with the normal read.

- Select the **Speed Read** check box to use speed read.
- Clear the **Speed Read** check box to not use speed read.

## Calibrate Settings

The More Settings category enables you to use air calibration. With calibration on, the instrument runs calibration for each wavelength. The new calibrations overwrite the calibration setting stored in the instrument firmware for the current data acquisition read, and for any subsequent read with calibration turned off. When you power off the instrument, the calibrations revert to those stored in the firmware. You should calibrate at your assay wavelengths for at least one plate before you turn off calibration. Turn off calibration to allow the instrument to begin or complete reads more quickly.

- Select the **Calibrate** check box to use air calibration.
- Clear the **Calibrate** check box to not use air calibration.

For the Absorbance read mode, the instrument makes an air-calibration measurement at each wavelength before the plate read. If you turn calibration off, the software uses the air-calibration values stored in the firmware, or the values from the previous calibration. You can update the air-calibration values stored in the firmware for the Absorbance read mode. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

For the Fluorescence and Luminescence read modes, the instrument makes a reference read at each wavelength before the plate read. If you turn calibration off, the software uses the default values stored in the firmware, or the values from the previous calibration.

For the Kinetic read type, the calibration measurement is made between reads during a run, unless the read interval is too short to allow calibration.

## Read Order Settings

For the Endpoint and Kinetic read types, the More Settings category enables you to choose the order in which the assay plate is read in multi-wavelength reads.

Click the **Read Order** drop-down:

- Select **Column** to have the instrument read the entire plate (or chosen number of strips) at the first wavelength and then go back and read the plate at the second and other wavelengths.
- Select **Wavelength** to have the instrument read all wavelengths for the first column of wells in the plate first and then read all wavelengths for the second column, third column, and so on.

## Settling Time Settings

The More Settings category enables you to define a settling time for all read types except for the Flex read type. Settling time is the delay between the motion of the instrument and the start of the read. This delay allows time for the meniscus motion to cease, which potentially improves precision, especially in the low density, high volume plates with fewer than 96 wells.

- Select the **Settling Time** check box to add a delay between the read of each column. In the **Duration** field enter the delay time between 100 and 1000 milliseconds. There is no delay between each well in a column.
- Clear the **Settling Time** check box to not have a delay.

## Compound Transfer Settings

For the Endpoint, Kinetic, and Flex read types, the Compound Transfer category enables you to define settings for the fluidics module. For the Endpoint and Kinetic read types, transfers are done to all the wells you select before the first read. For the Flex read type, transfers are done for a column while the instrument reads that column. All transfers and reads are done for that column before the instrument moves to the next column.

Keep in mind the actual maximum volume of the wells as you define the fluidics settings. These settings enable you to define precise fluid transfers for the protocol and help prevent flooding of the assay plate. The maximum cumulative volume depends on the assay plate type you select. See [Plate Type Settings on page 33](#).

You can enable up to three compound transfers in each column during a read.

Click the **No. of Compound Transfers** drop-down:

- Select **0** to not do compound transfers for the read. No other options display and no additional settings are required.
- Select **1** to do one compound transfer.  displays with additional settings.
- Select **2** to do two compound transfers.  displays with additional settings.
- Select **3** to do three compound transfers.  displays with additional settings.

You must configure the compound transfer settings correctly. In addition to the settings in the Compound Transfer Before Read category, four new categories appear in the Settings dialog.

### Initial Volume

In the **Initial Volume** field, enter a value that equals the largest initial volume in the wells of the assay plate before the compound transfers. If there is no fluid volume in the assay plate before the compound transfers, enter **0**.

- For a 96-well plate, this value can be 0  $\mu\text{L}$  to 269  $\mu\text{L}$ . Typical values are about 10  $\mu\text{L}$  to 200  $\mu\text{L}$ .
- For a 384-well plate, this value can be 0  $\mu\text{L}$  to 120  $\mu\text{L}$ . Typical values are about 5  $\mu\text{L}$  to 80  $\mu\text{L}$ .

The software assigns this initial volume to all wells and uses this value to compute the total volume in each well after all fluids are dispensed. As you define the volume for each transfer in the Compounds & Tips category, the software uses the Initial Volume value to warn you of the potential for overflow of fluid from the wells. See [Compound and Tips Columns Settings on page 43](#).

### Transfer Settings

You define the following settings for each compound transfer. Select the compound transfer to define. The compound transfer image you select displays with a light blue background.



**Note:** This is the only indication for which transfer you are defining.

- In the **Pipette Height** field, enter a value between 1  $\mu\text{L}$  to 300  $\mu\text{L}$  for a 96-well plate or 1  $\mu\text{L}$  to 130  $\mu\text{L}$  for a 384-well plate. This setting determines the volume of fluid in microliters, measured from the bottom of the assay plate well, above which the tip of the pipette is

to remain during the dispense portion of the transfer event. Use this setting to place the tip of the pipette below the surface of the liquid at the end of the transfer to minimize the possibility that undispensed drops remain on the tips.



**Note:** As you define subsequent compound transfers, calculate the amount of fluid added and set the pipette height accordingly. Example: If the initial volume is 15  $\mu\text{L}$  and the first transfer dispenses 20  $\mu\text{L}$ , set the pipette height for the first transfer to 25  $\mu\text{L}$ . If the second transfer dispenses 15  $\mu\text{L}$ , set the pipette height for the second transfer to 45  $\mu\text{L}$ .

- In the **Volume** field, enter the volume of liquid to dispense to each well of the assay plate that you select to receive the transfer.
  - For a 96-well plate, the range is 1  $\mu\text{L}$  to 200  $\mu\text{L}$ .
  - For a 384-well plate, the range is 1  $\mu\text{L}$  to 30  $\mu\text{L}$ .



**Note:** Keep in mind the maximum total volume each well can hold as you accumulate volumes with multiple transfers.

- In the **Rate** field, enter a value of 1 to 8. This setting determines the rate at which the instrument dispenses the fluid into the well of the assay plate.
  - In a 96-well plate, a setting of 1 is equal to  $\sim 16$   $\mu\text{L}$  per second and each subsequent number increases in increments of  $\sim 16$   $\mu\text{L}$  per second, so a setting of 2 is equal to 31  $\mu\text{L}$  per second.
  - In a 384-well plate, a setting of 1 is equal to 4  $\mu\text{L}$  per second and each subsequent number increases in increments of 4  $\mu\text{L}$  per second, so a setting of 2 is equal to 8  $\mu\text{L}$  per second.

A setting of 3 or 4 can help minimize cell damage or dislodgment. For non-contact dispensing, use a rate of 8 to make sure that all liquid dispenses from the pipette tip.

- In the **Time Point** field, enter the point in time after the read starts when the fluid is to begin being dispensed. This value must be equal to or greater than the value the instrument displays for the **Minimum Time**, up to 9999 seconds. This time point becomes the baseline time. This is not the time interval between transfers. For the Endpoint and Kinetic read types, Time Point is the time at which the first dispense occurs. The Endpoint or Kinetic read begins after all transfers are made.



**Note:** The Time Point value cannot be less than the Minimum Time the software identifies for each transfer.

- The **Minimum Time** field displays the minimum time required before a pipetting event can occur. This minimum time value is cumulative, not an interval between pipetting events. The value is the minimum number of seconds of elapsed time from the beginning of the read. It takes into account the mechanical speed of the pipette head and the time needed to load and unload tips, aspirate and dispense fluids, triturate fluids, and shake the plate.

The minimum time for the second pipetting event depends on when the first pipetting event occurs. The calculation for the second event starts at the end of the first event and adds the total time necessary to load pipette tips, aspirate new fluids from the compound plate, and dispense them into the assay plate.

### Leave Tips On Between Columns

For the Flex read type, the pipettor returns the tips to the tip rack after all the transfers for a column are complete and then installs tips from the tip rack before the first transfer for the next column.

- Select the **Leave Tips On Between Columns** check box to use tips from the same position in the tip rack for the first and last transfers in each column. This can reduce the cycle time between columns.
- Clear the **Leave Tips On Between Columns** check box to have the instrument install tips from the tip rack before the first transfer for the next column.

You define tip assignments in the **Compound & Tips Columns** category. See [Compound and Tips Columns Settings on page 43](#).

### Avoid Problems

Time Point calculations are based on the number of wells you select in the Read Area category and the Pipette Tips and Columns category settings you define for the transfer. If your Time Point entry is not long enough to be compatible with the volumes and transfer speed you define, a message instructs you to increase your Time Point entry.

If you enter transfer volumes that add up to more than the maximum that can fit in the assay plate, an overflow message indicates that you have exceeded the volume limit of the assay plate.



**Note:** The Minimum Time value that displays below the Time Point field is different for each transfer you define.

---

## Compound Source Settings

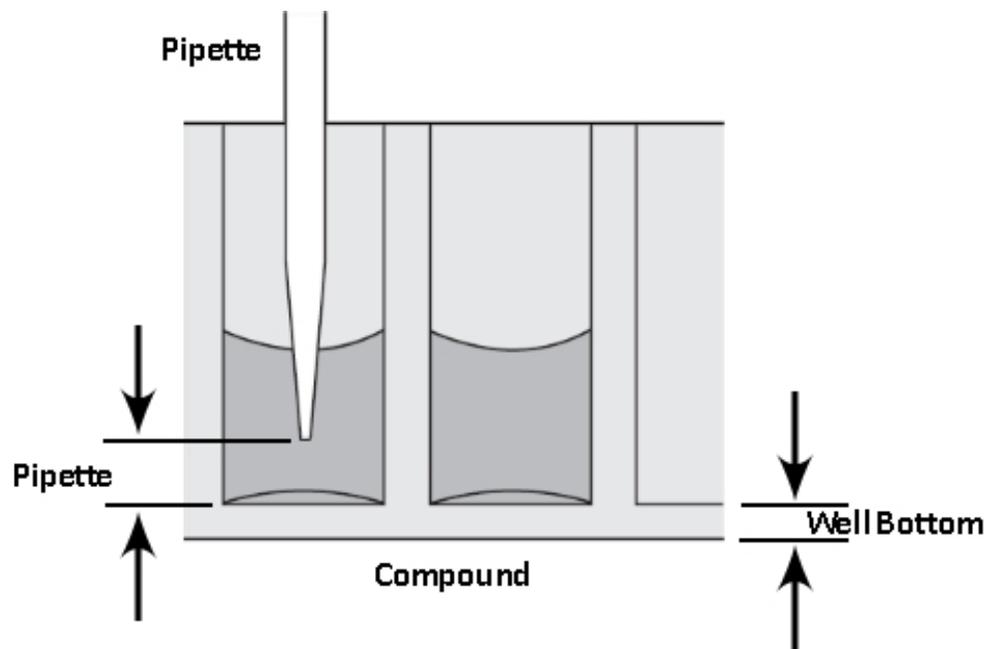
The Compound Source category enables you to select the plate type for the compound plate. The instrument aspirates (withdraws) the fluids from the compound plate and then dispenses (injects) the fluids into the assay plate during the run.

The plate type you select in the Compound Source category must match the type and well configuration of the actual compound plate you use and it must be compatible with the number of wells in the plate type you select for the assay plate in the Plate Type category. See [Plate Type Settings on page 33](#).

The instrument automatically sets the pipette height for the compound plate. The well bottom height is different for different types of plates. Select the plate type correctly to prevent jamming the pipette tips into the bottom of the well.

- For 96-well plates, the instrument aspirates using a pipette height of 20  $\mu\text{L}$  above the bottom of the well.
- For 384-well plates, the instrument aspirates using a pipette height of 10  $\mu\text{L}$  above the bottom of the well.

You define the pipette height for the assay plate in the Compound Transfer category. See [Compound Transfer Settings on page 38](#).

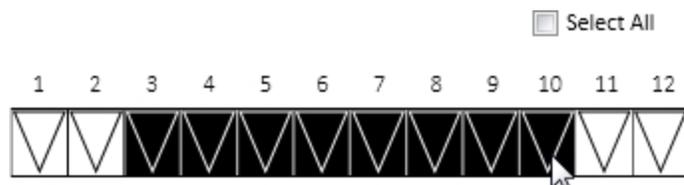


**CAUTION!** Selection of an incorrect compound plate type can result in the pipette tips jamming into the wells and damaging the plate, the tips, and the instrument.

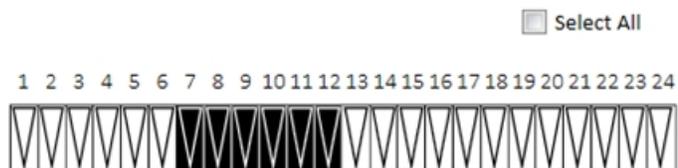
## Pipette Tips Layout Settings

The Pipette Tips Layout category enables you to define which pipette tips to use. If you use a partial rack of tips, this category enables you to have the software instruct the instrument to pull tips from the locations of the tips you insert in the tip drawer.

When you select a 96-well assay plate in the Plate Type category, the Pipette Tips Layout category displays a row of 12 tip columns. See [Plate Type Settings on page 33](#).



When you select a 384-well assay plate in the Plate Type category, the Pipette Tips Layout category displays a row of 24 tip columns.



To use all the tips in the rack, select the **Select All** check box.

To select a contiguous set of tips, drag the mouse pointer to select the tips to use. The tips selection must be contiguous.

If you change the assay plate type in the Plate Type category to a plate with a different number of wells (for example, from 96-wells to 384-wells), the Pipette Tips Layout category defaults to the selection of the full rack of tips.



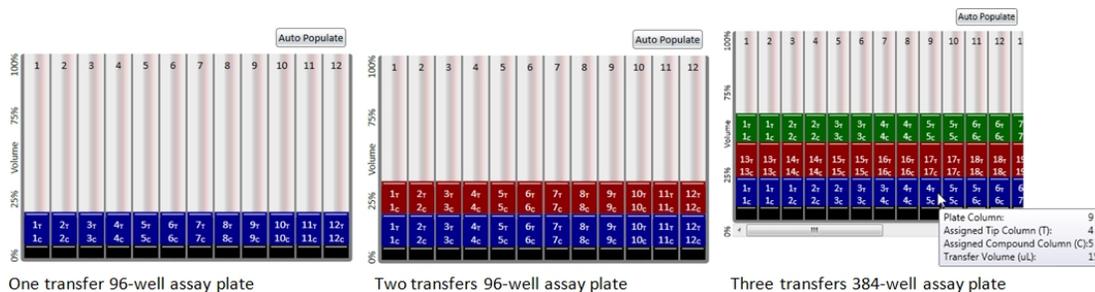
**CAUTION!** You should use a full rack of tips each time you do a fluid transfer. If you enable a pipetting function from a tip that is not present, your samples do not receive the intended compound. There is potential of damage to the plate, the tips, and the instrument.

## Compound and Tips Columns Settings

The Compound & Tips Columns category enables you to define the tips and compounds to use for each transfer. These settings are dependent on the selection you make in the Plate Type category for the assay plate and the number of transfers you define in the Compound Transfer category. The following describes the SoftMax Pro Software version 7.0 and higher. See [Plate Type Settings on page 33](#) and [Compound Transfer Settings on page 38](#).

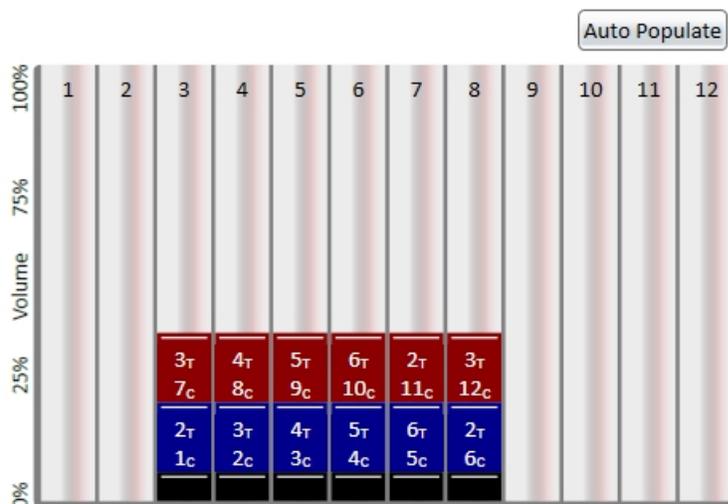
- For a 96-well assay plate, 12 columns display.
- For 384-well assay plate, a scroll bar enables you to view the additional columns.

Each transfer appears as a color coded row.



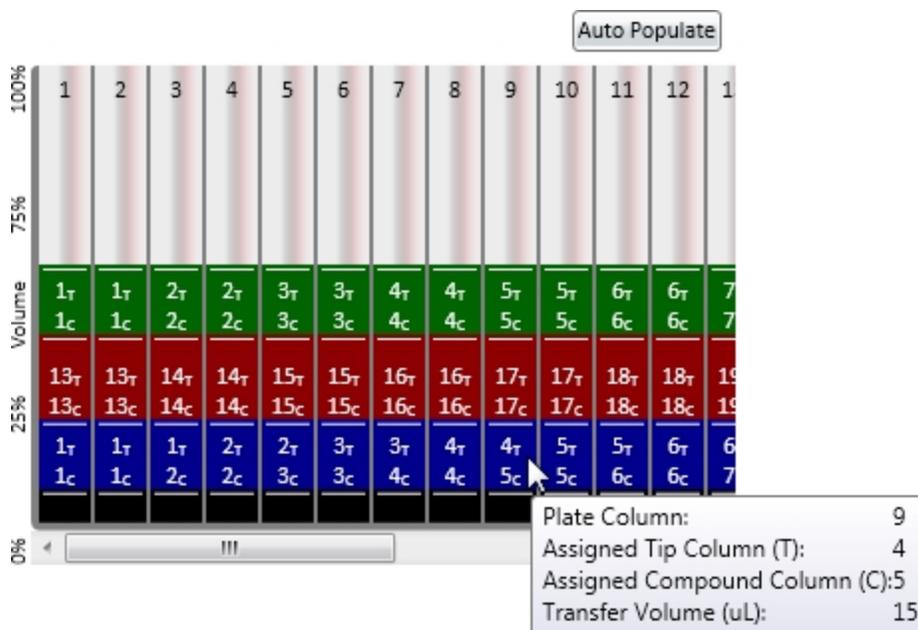
The black row at the bottom represents the initial volume in the wells based on your Initial Volume entry in the Compound Transfer category.

Your Read Area category setting also affects these settings. See [Read Area Settings on page 34](#).



### Display for a Read Area That Includes Columns 3 - 8 on a 96-Well Assay Plate

The left axis displays the dispensed compound volume as a percentage of the total well volume on the assay plate.



Each assay plate column has two indicators for each transfer:

- #T = Tips: Displays the column in the tip rack drawer from which the instrument will pull the tip for the transfer. You define available tips in the Pipette Tips Layout category. See [Pipette Tips Layout Settings on page 42](#).
- #C = Compound: Displays the column in the compound plate from which the instrument will aspirate the fluid to transfer to the assay plate for each pipetting event.
- Hover the mouse over a cell to display details including the transfer volume.

## Auto Populate

To have the software automatically assign the tips and columns, click **Auto Populate**.

The software assigns fluid to aspirate from the compound plate starting with the first available column. The instrument then dispenses the aspirated fluid to the first available column in the assay plate based on your Read Area category settings.

The software makes the assignments based on the following conditions:

- All columns you select in the Read Area category are to receive fluid.
- The fluids transfer from left to right. The read-transfer-read sequence in each column is initiated only after the read-transfer-read event from the previous column completes (the total read time for that column).
- The fluid transfer targets are cumulative from transfer to transfer. That is, the targets for the second transfer start with the next available clean tip and untargeted compound column rather than reusing tips and compound columns targeted by the preceding fluid transfer.
- Each fluid transfer uses new tips.

Example: You define the following settings for three fluid transfers:

- Read Area category: Columns 1 through 4
- Compound Source category: 12-column compound plate, such as a 96-well plate
- Pipette Tips Layout category: Full rack of tips

The software instructs the instrument to select tips from columns 1 - 4 in the tip rack drawer, to aspirate compound from columns 1 - 4 in the compound plate in the compound plate drawer, and to dispense the compound into the wells of columns 1 - 4 on the assay plate in the read chamber drawer. The second transfer uses the next four columns (5 through 8) of tips, and the third transfer uses the remaining four unused columns (9 through 12) of tips.

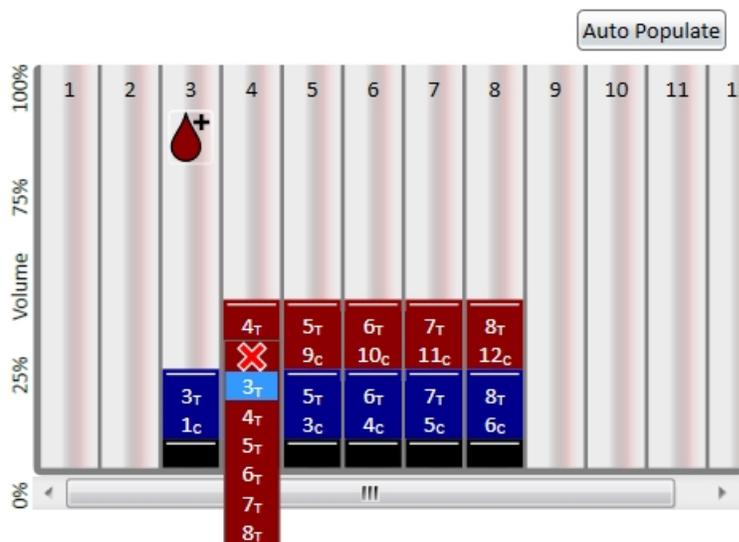


**Note:** The software does not assign targets beyond the limitations of the available tips and compound source columns. After all available tips and compound source columns are assigned, the software stops assigning targets.

---

## Manually Populate

For more complex assays, you can manually define the tips to use and the columns from which to aspirate compound.

**Image Example Displays:****Read Area: columns 3 - 8 on a 96-well assay plate****Compound Transfers: 2****Selecting a tip with Pipette Tips Layout: columns 3 - 8****No fluid transfer for compound 2 into column 3**

To assign each transfer a set of tips from the tip rack and a column from the source plate:

- Click **T** in a column to display a drop-down list of the pipette tip columns you made available in the Pipette Tips Layout category. Select the column from which to have the instrument pull the tip from the instrument tip drawer.
- Click **C** in a column to display a drop-down list of the number of columns in the compound plate from which the instrument can aspirate the compound. Select the column on the source plate from which to aspirate the compound.

If the assay requires that no fluid is to transfer to the assay plate for a column during one or more transfer operations, click **X** at the top of the drop-down list.  displays for the columns to skip.

To enable a transfer for a skipped column, click , then assign the tip and source columns for the transfer.

You can manually assign tips and source columns for each and every transfer.

---

**\* Tip:** To leave the tips on between columns for the Flex read type, select the Leave Tips On between Columns check box in the Compound Transfer category.

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## Triturate Settings

The Triturate category enables you to define how to mix the contents of the wells in a plate by using the pipettor to alternately aspirate the fluid from a well and then dispense it back into the well. Trituration is recommended when you need to resuspend the compound in the source plate before it is added, when you transfer low volumes of fluid to the assay plate, or when you transfer fluid before or during an absorbance read to make sure that the well contents are properly mixed.



Select the compound transfer to define and repeat the following steps for each applicable transfer.

- Select the **Compound Source** check box to do trituration in the compound plate.
- Select the **Assay Plate** check box to do trituration in the assay plate.
- Select both check boxes to do trituration in both plates.

In the **Volume** field, enter the volume of fluid in microliters to withdraw from the well.

In the **Cycles** field, enter the number of times to aspirate and dispense the fluid in the well.

For the Assay Plate, enter a value for the **Height** at which the trituration occurs. The height setting must take into account the trituration volume you enter and the total volume in the well to have the tips remain below the liquid surface and not aspirate air.

If you enter a Time Point that is incompatible with the Triturate settings, a message appears. You modify the Time Point in the Compound Transfer category. See [Compound Transfer Settings on page 38](#).

## Preparing to Read a Plate

Before you read the plate for your assay, you can define more options in the software.

- Plate Template
- Data Display Options
- Data Reduction Options

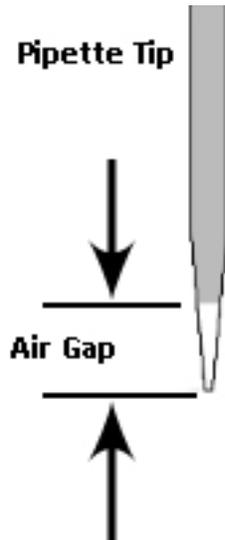
You should define a plate template and set the data display and data reduction parameters before you read the assay plate, because these parameters determine how data displays and is analyzed. However, you can define these options after you run the assay, since the values from the instrument are raw values and are not affected by these settings. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

For experiments with multiple Plate sections, use Auto Read to automatically advance to the next Plate section after a read in the sequence that the Plate sections display within a single experiment. When you start a read for a Plate section that is enabled to automatically advance with Auto Read, the Auto Read feature advances to the next Plate section in the experiment and then reads that plate. You can set an interval, or delay time, between plate reads.

If you use the fluid transfer feature, prepare the tips and the compound plate before you start the read. If you run an assay that does not use the fluid transfer feature, you can load the assay plate in the read chamber and then click **Read** in the software.

### Set the Pipette Tip Air Gap

The pipette air gap is the volume between the end of the pipette tip and the bottom of the liquid in the tip.



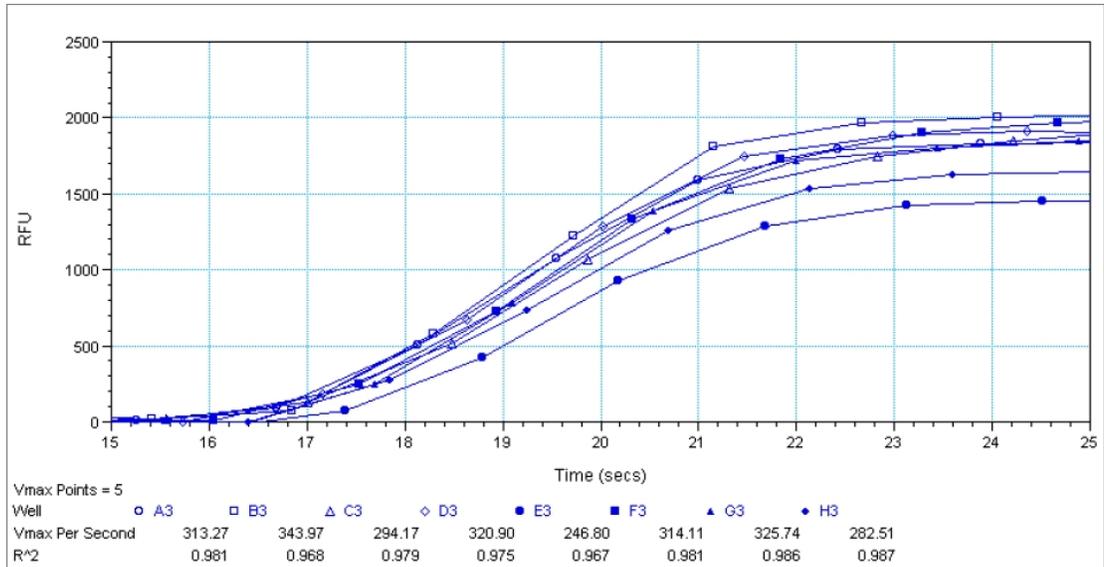
To set the pipette tip air gap in the software, select the Operations tab and click **Air Gap** to display the Air Gap Settings dialog.

In the **Air Gap** field, enter the air gap in microliters between 0  $\mu\text{L}$  and 10  $\mu\text{L}$ .

### Time Tagged Data

The FlexStation 3 is a single channel reader system. Although the scan time is very fast (8 wells in about 1.0 seconds, or 1 well in about 50 ms), the difference in the exact time each well is read depends on the number of rows you select in a column. This difference is an important factor in fast kinetic assays.

For this reason, all reads are tagged with an exact read time. When you plot multiple well fast kinetic responses, the curves overlies each other as plotted by the software. When you export Kinetic data, you can choose either time interpolated data or raw time tagged data. You should select time interpolated data. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.



**Time Tagged Data Example**

## Chapter 5: Read Modes and Read Types

The instrument can measure samples in Absorbance, Fluorescence Intensity, Luminescence, Time-Resolved Fluorescence, and Fluorescence Polarization read modes.

Application notes with specific application protocol suggestions are in the Information Center and Knowledge Base on the Molecular Devices web site at [www.moleculardevices.com](http://www.moleculardevices.com).

### Read Types

The instrument supports the following read types.

#### Endpoint Read Type

For the Endpoint read type, a read of each plate well is taken in the center of each well, at a single wavelength or at multiple wavelengths.

For the Endpoint read type with fluidics transfer, one to eight or one to sixteen wells in one column of the assay plate are delivered before the read starts. At a predesignated point or points before the read, the pipettor can transfer up to three reagents from the compound plate to the assay plate. After reagents transfer, the read initiates for the entire read area. Unlike the Flex read type, the read area is not limited to one column at a time for the Endpoint read type. All data is collected in one data file that the software represents as a 96-well or 384-well plate.

#### Kinetic Read Type

For the Kinetic read type, the instrument collects data over time with reads taken in the center of each well at regular intervals.

The values calculated based on raw kinetic data include VMax, VMax per Sec, Time to VMax, Onset Time, and more. Kinetic reads can be single-wavelength or multiple-wavelength reads. Kinetic analysis can collect data points in time intervals of seconds, minutes, hours, or days. Kinetic analysis has many advantages when determining the relative activity of an enzyme in different types of assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis can provide improved dynamic range, precision, and sensitivity relative to endpoint analysis.

For the Kinetic read type with fluidics transfer, fluid can be transferred to one to eight or one to sixteen wells in one column of the assay plate before the read starts. At a predetermined point or points before the read, the pipettor can transfer up to three reagents from the compound plate to the assay plate. After reagents transfer, the read is initiated for the entire read area. Unlike the Flex read type, the read area is not limited to one column at a time for the Kinetic read type.

### **Well Scan Read Type**

The Well Scan read type takes reads at more than one location within a well. The Well Scan read type takes multiple reads of a single well of a plate on an evenly spaced pattern inside of each well at single or multiple wavelengths.

Some applications involve the detection of cells in large area tissue culture plates. Use the Well Scan read type with such plates to permit maximum surface area detection in cell-based protocols. Since many cell lines tend to grow as clumps or in the corners of plate wells, you can choose from several patterns and define the number of points to scan in order achieve the best results for your application.

Depending on the read mode you select, the software reports values as optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative light units (RLU).

### **Spectrum Read Type**

Depending on the read mode you select, the Spectrum read type measures optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU) across a spectrum of wavelengths.

Spectral analysis measures across a spectrum of wavelengths. Fluorescent intensity reads scan excitation wavelengths between 250 nm to 850 nm and emission wavelengths between 360 nm to 850 nm. You can set a fixed wavelength for excitation and scan the emission wavelengths, or set a fixed wavelength for emission and scan the excitation wavelengths.

### **Flex Read Type**

The fluidics module aspirates fluids from the compound plate and dispenses them into the assay plate. Fluid transfer is made possible with an 8-channel or 16-channel pipettor that is fully automated, including changing the tips from a tip rack.

For the Flex read type, one to eight or one to sixteen wells in one column of the assay plate are read repeatedly for the total experimental time you define. At a predetermined point or points during that time sequence, the pipettor can transfer up to three reagents from the compound plate to the assay plate. The instrument continues to read at the predesignated time intervals before and after each fluid transfer. When the instrument completes a read of the column (or partial column) for a predesignated time, the instrument can repeat this cycle with other columns.

For example, an experiment with a two-minute run time accommodates a 96-well plate in about 24 minutes.

$$\text{Run time} \times \text{Number of columns} = \text{Plate time}$$

$$2 \text{ minutes} \times 12 \text{ columns} = 24 \text{ minutes}$$

## 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.

### **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:

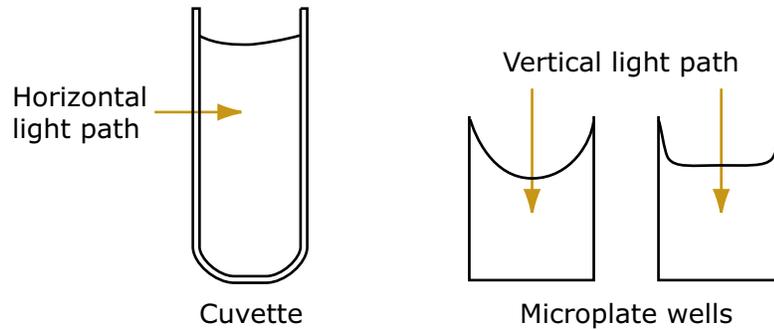
$$A = \epsilon cL$$

where  $A$  is the absorbance,  $\epsilon$  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  $\pm 4\%$  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 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.



**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.

The Cuvette Reference data does not display in a Cuvette Set section. The software uses the Cuvette Reference data for PathCheck Pathlength Measurement Technology calculations on the data the instrument collects from the plate. You can use the accessors in 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.

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## 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.

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## 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.

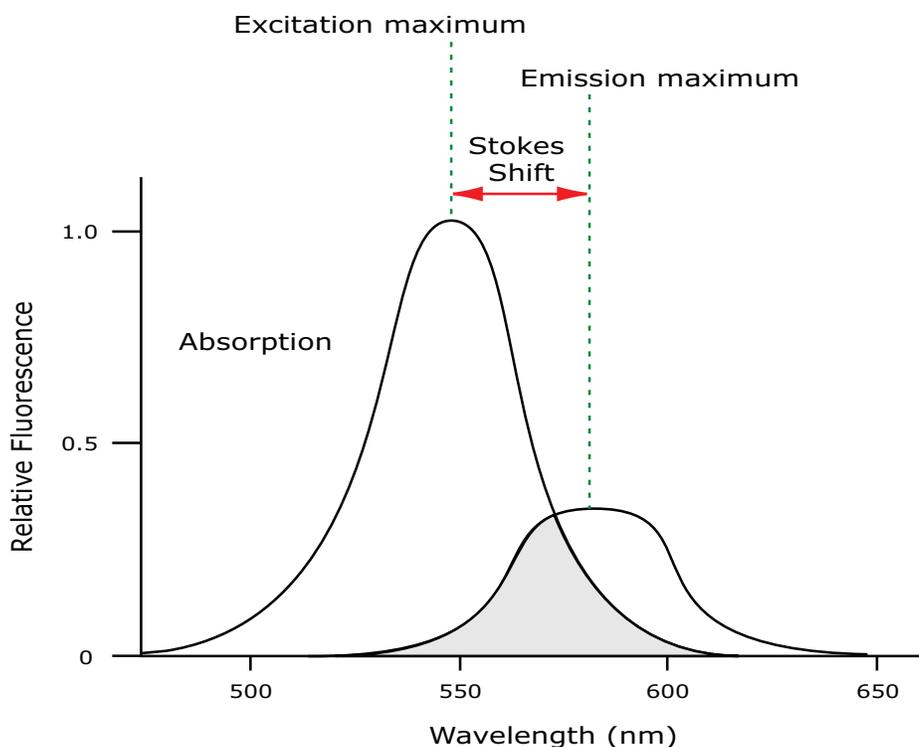
## Fluorescence Intensity Read Mode

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:

$$\text{Fluorescence} = \text{extinction coefficient} \times \text{concentration} \times \text{quantum yield} \times \text{excitation intensity} \times \text{pathlength} \times \text{emission collection efficiency}$$

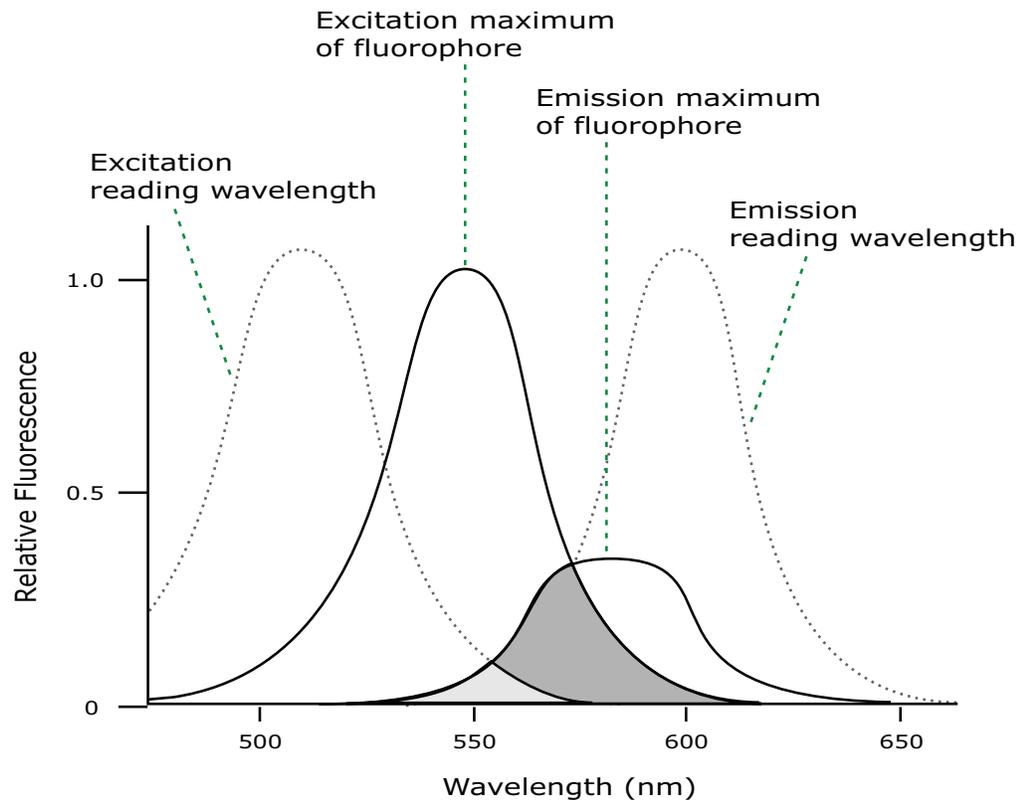
Fluorescent materials absorb light energy of a characteristic wavelength (excitation), undergo an electronic state change, and instantaneously emit light of a longer wavelength (emission). Most common fluorescent materials have well-characterized excitation and emission spectra. The following figure 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.



### 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.

## Optimizing Fluorescence Intensity Assays

The optimal instrument settings for detection of a particular fluorophore depend on a number of different factors. Settings you can adjust for assay optimization include the excitation and emission wavelengths, emission cutoff filter, reads per well, the PMT voltage, and the temperature of the read chamber.

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

- **Excitation and Emission Wavelengths**

The excitation and emission wavelengths can be set in 1 nm increments between 250 nm and 850 nm.

- **Emission Cutoff Filter**

The emission cutoff filters assist in reducing background. Sources of background include stray excitation light and native fluorescence of plate materials, sample constituents, and solvents (including water). The default setting lets the instrument and the software determine which cutoff filter to use for the Endpoint and Kinetic read types. The Spectrum read type default uses no cutoff filter.

- **Reads Per Well**

The number of reads per well can vary between 1 (for a quick estimate) and 100 (for very precise measurements). The default number of reads per well varies with the read mode: for Fluorescence, the default is 6, and for Luminescence the instrument does 1 read per well.

- **PMT Voltage**

The voltage of the photomultiplier tube can be set to low (for higher concentration samples), medium, or high (for lower concentration samples) for all read modes. For the Endpoint and Spectrum read types, you can select Automatic to have the instrument automatically adjust the PMT voltage for varying concentrations of sample in the plate.

- **Temperature Control**

The chamber of the instrument is isothermal at ambient as well as at elevated temperatures.

The temperature inside the read chamber can be maintained at 2°C (3.6°F) above ambient to 45°C (113°F).

### **Spectral Scanning**

This procedure optimizes excitation and emission wavelengths for Fluorescence assays using spectral scanning.

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

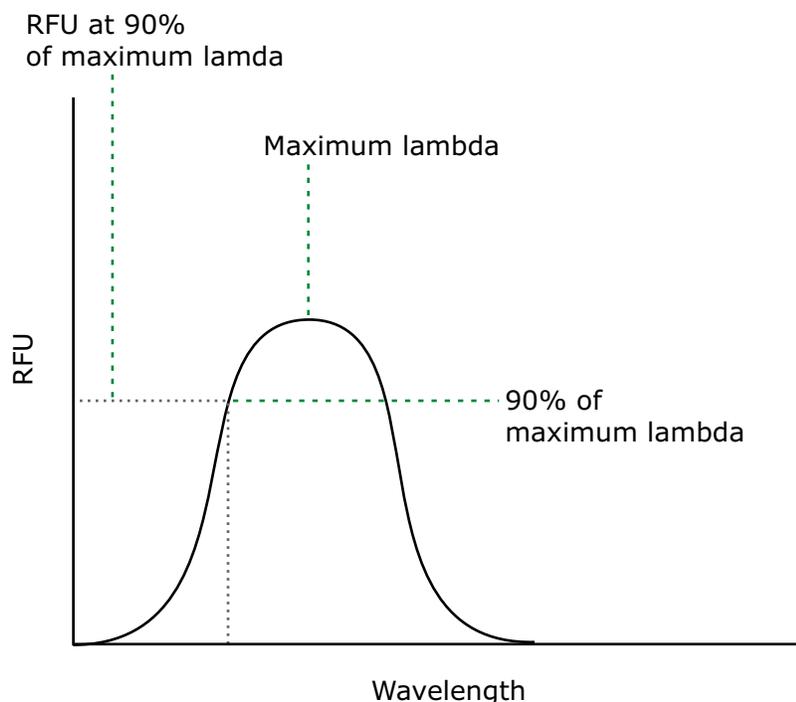
### **Excitation Scan**

1. Set up a Plate section for the Fluorescence read mode, Spectrum read type, Em Fixed/Ex Scan, with no cutoff filter (default), and medium PMT.
2. Set the emission wavelength based on the tentative value from the literature (or from a customary filter set used to measure the fluorophore). If the emission wavelength is not known, select a tentative emission wavelength about 50 nanometers greater than the absorbance maximum of the fluorophore. If necessary, the absorbance maximum can be determined by performing an optical density spectral scan first.
3. Set the excitation scan to start/stop approximately 50 nm below/above the tentative excitation value from the literature (or the customary excitation filter).
4. Set the step increment to 2 nm or 3 nm. You can choose to do a preliminary scan with a 10 nm increment to determine the approximate peak location, and then repeat the scan over a narrower wavelength range with a 2 nm or 3 nm increment.
5. Do the scan and view the results as a plot of emission fluorescence vs. excitation wavelength. Note the excitation wavelength at the emission peak and the maximum RFU value.

If a Missing Data Points message occurs, it can be due to possible saturation reported by the software at the end of the spectral scan. Reset the PMT to Low and rescan the sample (scan the buffer blank with the PMT set to Medium or High). If the error occurs after you scan with the PMT set to Low, you may need to dilute the sample.

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

6. Select the optimal excitation wavelength.
  - If the excitation peak wavelength and emission wavelength are separated by more than 80 nm, use the excitation peak wavelength value.
  - If the excitation and emission wavelengths are less than 80 nm apart, use the shortest excitation wavelength that gives 90% maximal emission. Follow the plot to the left of the peak until the RFU value falls to approximately 90% of the maximum, and then drop a line from the 90% point on the plot to the x-axis as shown in the following figure.



**Plot of RFU and Wavelength**

### Emission Scan #1

1. Set up a second Plate section for the Fluorescence read mode, Spectrum read type, Ex Fixed/Em Scan, with no cutoff filter (default), and Medium PMT.
2. Set the excitation wavelength to the value you determine in the Excitation Scan section above.
3. Set the emission scan to start/stop approximately 50 nm below or above the tentative emission value from the literature (or existing filter pair).



**Note:** If the Stokes shift is less than 50 nm, then start the emission scan above the excitation wavelength.

4. Set the step increment to 2 nm to 3 nm (or do a preliminary scan with a 10 nm increment to determine the approximate peak location and then repeat the scan over a narrower wavelength range using a 2 nm to 3 nm increment.)
5. Do the scan and view the results as a plot of fluorescence vs. emission wavelength.

### Choose the Emission Filter

Select an emission cutoff filter that blocks as much of the residual excitation light as possible without unduly reducing the fluorescence signal. The cutoff wavelength choices are 325, 420, 435, 475, 495, 515, 530, 550, 570, 590, 610, 630, 665, or 695 nm. The cutoff value should be near the maximum emission wavelength (preferably between the excitation wavelength and the maximal emission wavelength) but a minimum of 10 nm less than the emission wavelength. If you have questions, contact Molecular Devices Technical Support and speak to an application scientist.

### Emission Scan #2

1. Set up a third Plate section for an emission scan as specified in Emission Scan #1, except select the Manual Cutoff Filter and set the wavelength to the value you determine in the Choose the Emission Filter section above.
2. Do the scan and view the results as a plot of fluorescence vs. emission wavelength. Note the wavelength that gives the maximum emission (the optimal emission wavelength).
3. Compare the spectra of the sample that contains the fluorophore to the spectra of the buffer blank to get an estimate of the signal-to-noise ratio. If there is significant background interference, repeat the Choose the Emission Filter steps and the steps in this section with a different choice of cutoff filter.

### Results

The optimal excitation and emission wavelengths are those you determine in the Excitation Scan section and the Emission Scan #2 section.

### Comments

- For the Fluorescence read mode with the Endpoint or Kinetic read type, the **AutoCutoff** feature generally selects the same cutoff filter wavelength as the previously described optimization method. However, you can specify the cutoff filters manually.
- For emission wavelengths less than 325 nanometers, experimental iteration is usually the best method to determine the optimal emission and excitation wavelengths. Start the previously described optimization procedure. Try emission and excitation wavelength combinations with the 325 cutoff or with no cutoff filter. For excitation wavelengths greater than 660 nanometers, try emission and excitation wavelength combinations with the 695 cutoff or with no cutoff filter.

The following table lists default settings for the emission cutoff filters.

#### Emission Cutoff Filter Default Settings

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

### Background Correction and Quantification

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.

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label and is generally subtracted before data reduction is done.

Within the linear detection range, the blank-subtracted raw data are proportional to the quantity of label in a sample such that the label concentration is quantified by the following equation.

$$\text{conc}_{\text{label}} = \frac{(\text{sample-blank})}{\left(\frac{\text{std-blank}}{\text{conc}_{\text{std}}}\right)}$$

where  $conc_{std}$  is the concentration of the *standard*, and *sample*, *blank*, and *std* are average values of replicates for the sample, blank, and standard wells. In the general case where the standard curve covers a concentration range of more than a few linear logs,  $(std - blank) / conc_{std}$  is equivalent to the slope of the standard curve, and so the concentration of the label is determined by  $(sample - blank) / (slope\ of\ standard\ curve)$ .

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.

## Detection Limit

The detection limit is the smallest sample concentration that can be detected reliably above the blank. Determining the detection limit requires taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection threshold is defined as the average blank plus three standard deviations. If the average sample value measures above the threshold, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{Det Limit} = \frac{3 \text{ StDev}_{\text{blank}}}{\left( \frac{\text{std-blank}}{\text{conc}_{\text{std}}} \right)}$$

where  $conc_{std}$  is the concentration of the standard,  $StDev_{Blank}$  is the standard deviation of the blank replicates, and *blank* and *std* are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks to calculate their standard deviation.

## Linearity and the Linear Dynamic Range

Within a wide range at moderately high concentrations, blanked raw data is proportional to the quantity of label in a sample.

The linear dynamic range (LDR) is defined by:

$$\text{LDR} = \log_{10} \left( \frac{\text{max conc lin}}{\text{detection limit}} \right)$$

where *LDR* is expressed as a log, and *max conc lin* is the highest concentration in the linear range that can be quantified.

When the standard curve after blank reduction is not linear in concentration at the lower end, there might be an incorrect or contaminated blank.

When the standard curve levels are off at the highest concentrations, this can be addressed to the inner filter effect: excitation does not reach as deep into the sample for lower concentrations, without being more significantly attenuated (absorbance) layer by layer.

## Luminescence Read Mode

In luminescence read mode, no excitation is necessary as the measured species emit light naturally. For this reason, the lamp does not flash, so no background excitation interference occurs.

For the Luminescence (LUM) read mode, the instrument provides measurements in Relative Light Units (RLUs).

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. Luminescence detection relies on the production of light from a chemical reaction in a sample.

To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

You can choose the wavelength where peak emission is expected to occur. Also, multiple wavelength choices let species with multiple components be differentiated and measured easily.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls.

### Applications of Luminescence

Chemiluminescent or bioluminescent reactions can be induced to measure the quantity of a particular compound in a sample. Examples of luminescent assays include the following:

- Reporter gene assays (the measurement of luciferase gene expression)
- Quantitation of adenosine triphosphate (ATP) as an indication of cell counts with cell-proliferation, cytotoxicity, and biomass assays
- Enzyme measurements with luminescent substrates, such as immunoassays

### Optimizing Luminescence Assays

Luminescence can be read from the top or the bottom of a plate. You should use solid white plates or white plates with clear bottoms for luminescence reads.

For standard luminescence a separate light path without monochromators carries the emitted light to a dedicated PMT. The optimal emission wavelength is between 360 nm and 630 nm.

For wavelength-selectable luminescence, the instrument uses the emission monochromator to differentiate the wavelengths being emitted from the well. You can define multiple emission wavelengths. The available wavelength range for the FlexStation 3 is 250 nm to 850 nm.

To read only one luminescent event in the well, you can get the best sensitivity using the standard luminescence measurement, without a wavelength selected.

Luminescence read times are not designated by multiple reads per well, but rather by the total integration time you enter (between 1 ms and 1500 ms). Typical luminescence assays require between 500 ms and 1000 ms integration.

If wells are incubated for a long period of time, you should mix the plate before the read. This can be done using the Shake setting.

If it seems that the signal is always higher in the first wells read (for example, column A), you should dark adapt the plate to reduce the auto-luminescence of the white plastic. To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

## Analyzing Luminescence Data

The conversion rate of photons to counts is individual for each reader. Therefore, raw data from the same plate can seem significantly different from one instrument to the next. Also, the data format used by other manufacturers might not be counts per second and can be different by several orders of magnitude. It is important to know that the number of counts and the size of figures is not a benchmark of sensitivity.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls. The raw data can then be expressed in equivalent concentration of a reference label. The raw data is normalized to counts per second by dividing the number of counts by the read time per well.

## Background Correction

The light detected in a luminescent measurement generally has two components: specific light from the luminescent reaction and an approximately constant level of background light caused by various factors, including the plate material and impurities in the reagents. The background can be effectively measured using blank replicates. Blanks should include the luminescent substrate (chemical energy source) but not the luminescence agent (generally an enzymatic group which makes the substrate glow).

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.

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label and is generally subtracted before data reduction 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.

To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

## Sample Volumes and Concentration of Reactants

The concentration of the luminescent agent impacts the quantity of light output in a luminescent reaction. Light is emitted as a result of a reaction between two or more compounds. Therefore, the quantity of light output is proportional to the quantity of the limiting reagent in the sample.

For example, in an ATP/luciferin-luciferase system, when total volume is held constant and ATP is the limiting reagent, the blanked light output is proportional to the concentration of ATP in the sample. Even if the reaction begins with a high concentration of ATP, as it gets used up it can become rate-limiting. In this case, the non-linearity is an effect of the assay and not caused by the microplate reader.

## Data Optimization

Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when the read time is increased. Therefore, it is important to specify the read time when you compare measurements.

All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

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.

## Time-Resolved Fluorescence Read Mode

The Time-Resolved Fluorescence read mode is a secondary read mode.

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  $\mu\text{s}$ ), samarium (lifetime of about 70  $\mu\text{s}$ ), or terbium (lifetime of about 1000  $\mu\text{s}$ ).

### 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<sup>®</sup> (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 Assays

For the Time-Resolved Fluorescence read mode, the instrument flashes the excitation lamp, then, after the lamp is off, collects the delayed emission for a period of time before the lamp flashes again. Long-lifetime rare-earth lanthanide dyes are generally used to provide a long-lived fluorescent signal that persists after the lamp turns off. Background fluorescence fades, while lanthanide chelates and cryptates have fluorescent lifetimes between 100  $\mu\text{s}$  and 2 ms. Time-Resolved Fluorescence assays can be read from the top or bottom of a plate. You should use solid white plates for top reads, and white plates with clear bottoms for bottom reads.

To optimize data collection for a particular assay, you define when to start and end data acquisition. The minimum is 50  $\mu\text{s}$  after the lamp turns off and the maximum is 1450  $\mu\text{s}$ , in 50  $\mu\text{s}$  or 200  $\mu\text{s}$  steps.

If the assay you use has low signal or gives results with high %CV, use 100 reads per well. If you require a faster read speed, experiment with fewer flashes per well until you achieve acceptable precision and speed.

The important settings to get the best results in Time-Resolved Fluorescence assays are integration delay and integration time:

- Click the **Integration Delay** drop-down and select the amount of time to elapse between the flash of the lamp (excitation) and the beginning of data acquisition from the well (emission).
- Click the **Integration Time** drop-down and select the amount of time for the instrument to read the well.

The integration delay and integration time are usually specified in the package insert of commercially available Time-Resolved Fluorescence reagent kits. If you do not use a kit, start with a delay of 50  $\mu\text{s}$  and try different delays up to 400  $\mu\text{s}$  with a fixed integration time of 400  $\mu\text{s}$ . After you choose the optimal 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 generally between 400  $\mu\text{s}$  and 1000  $\mu\text{s}$ .

Some examples of TRF assays are:

- IMAP<sup>®</sup> TR-FRET
- Cisbio HTRF
- LanthaScreen TR-FRET
- LANCE TR-FRET
- DELFIA TRF

## Analyzing Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of pulses. Each pulse consists of turning the light source on, then off (Excitation Time), pausing for a specified length of time (Measurement Delay), and measuring the fluorescence intensity of the sample for a specified length of time (Integration Time). The pulses are repeated several times, as specified in the protocol parameters.

### Blank Correction

Although background is significantly lower than with fluorescence intensity measurements, you should use blanks or assay controls.

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.

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label and is generally subtracted before data reduction 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 Normalization

TRF raw data changes in magnitude when the timing parameters are changed. However, TRF data are normalized for a number of 1000 pulses. This means that the sample raw data does not change when only the number of pulses is changed.

### Data Optimization

There are two timing parameters which can be optimized to adjust the performance of the measurement: time per well and integration time per cycle.

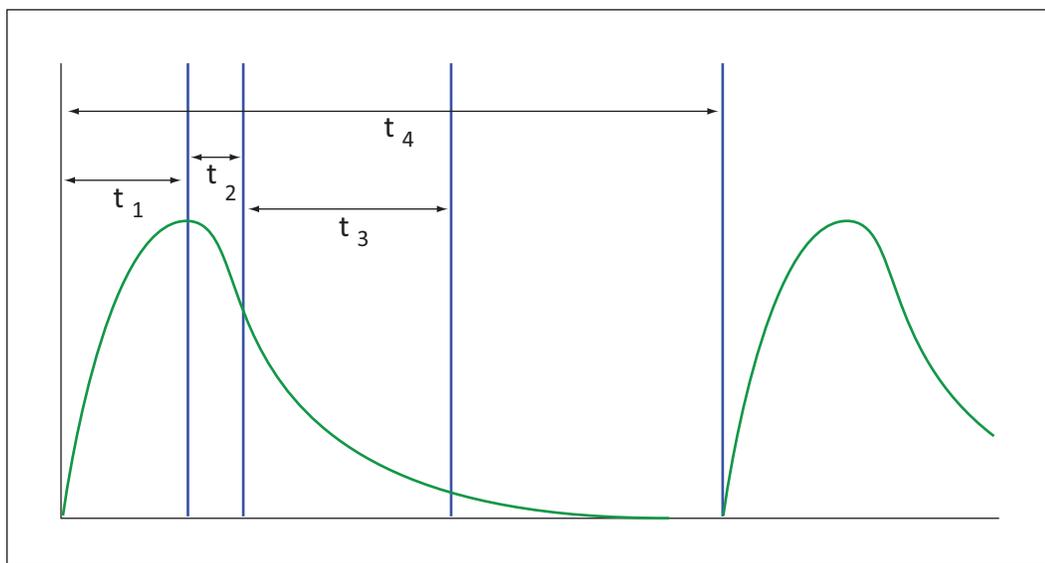
Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when the read time is increased. Therefore, it is important to specify the read time when you compare measurements. For TRF, the read time per well increases with the selected number of pulses. The time between pulses and the intensity of each pulse, however, can be different on various systems.

All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

To further optimize measurement results, optimize the timing parameters. Use the following table and figure as guidelines for the selection of timing parameters.

**Time-Resolved Fluorescence Timing Parameters Example**

Parameter	Value	Comment
Pulse length	0.100 ms	The period for excitation of the sample, shown as $t_1$ in the following figure.
Measurement delay	0.010 ms	The delay to ensure the excitation pulse is no longer detectable, shown as $t_2$ in the following figure.
Integration time per cycle (pulse)	1.890 ms	The period for accumulating the signal, shown as $t_3$ in the following figure.
Total cycle time		The total cycle time is shown as $t_4$ in the following figure.

**Timing Parameters For Time-Resolved Fluorescence**

When neglecting the time delay  $t_2$  compared to the integration time window  $t_3$ , the accumulated signal  $A$  can be approximated with the following equation:

$$A / A_{\max} = (1 - \exp(-M)) \times 100\%$$

In the equation above,  $M$  is the size of the time window (or integration time) divided by the exponential decay time constant (or the fluorescence lifetime of the label).

$$M = (\text{integration time}) / (\text{fluorescence lifetime})$$

For example, using Europium, which has a fluorescence lifetime of  $700 \mu\text{s}$ , and the suggested integration time per cycle of  $1.890 \text{ ms}$  (or  $1890 \mu\text{s}$ ),  $M = 1890 / 700 = 2.7$ . Inserting this value of  $M$  into the first equation yields  $A / A_{\max} = 93\%$ .

To optimize the integration time per cycle (pulse), the integration time should be set such that the value of  $M$  produces the desired signal. For example, to get more than 86% signal, select an integration time such that  $M$  is greater than 2.0. Using the previous Europium example and solving for the integration time, the integration time can be set to  $M$  (2.0) times the fluorescence lifetime ( $700 \mu\text{s}$ ), or  $1400 \mu\text{s}$  ( $1.4 \text{ ms}$ ).

**Achievable Accumulated Signal Percentage Compared to M**

M	0.25	0.50	0.75	1.00	1.25	1.50	2.00	3.00
A / A <sub>max</sub> [%]	22	39	53	63	71	78	86	95

M can be technically limited by the time between pulses. Further gain in signal above some value of M can be negligible to improve results.

## Fluorescence Polarization Read Mode

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

Fluorescence Polarization can be read from the top of a plate. The plastic from which a plate is formed has an effect on the light polarization, precluding bottom reads and reads of a covered plate from the top.

You should use solid black plates for 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), you should use plates treated to minimize binding, or polypropylene plates, and add a very small quantity of detergent to the assay buffer.

Background wells, which contain all assay components minus the fluorophore, should be run alongside samples. 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 should be subtracted from the raw parallel and perpendicular reads of each sample well before the mP calculation is done.

For best precision in assays that uses a low quantity of fluorophore (for example, <5 nM fluorescein), set the PMT sensitivity to High and the number of reads to 100. If a faster read speed is required, experiment with fewer flashes per well until acceptable precision and speed are achieved. Fewer flashes result in a higher speed, but with less precision.

## 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:

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

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:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{| \text{Mean}_{c+} - \text{Mean}_{c-} |}$$

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.

## Chapter 6: Maintenance and Troubleshooting

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 94](#).

Before operating the instrument or performing maintenance operations, make sure you are familiar with the safety information in this guide. See [Safety Information on page 5](#).



**WARNING!** 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.

---

### Service Trained Users

Most procedures required to operate and troubleshoot the instrument can be done by any user who has read the instructions in this user guide and is familiar with the system. However, all installation procedures and some more complex service and troubleshooting procedures require the expertise of a service trained user. Whenever the following warning message appears, a service trained user must do the procedure to ensure user safety and to prevent instrument damage.

Example:



**WARNING!** The following procedures must be completed by a service trained user. Do not attempt the following procedures if you have not received proper training by Molecular Devices personnel.

---

The following topics describe maintenance procedures that can be done by users or service trained users to ensure optimal operation of the instrument.



**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](#).
- 



**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
  - Instrument top
  - Cover edges
  - Underneath the instrument, between the feet
  - Rear edges (Do not damage the warranty seal.)
- If required, clean the surfaces using a mild soap solution diluted with water or a glass cleaner and then wipe with a damp cloth or sponge to remove all residue.
- If needed, clean the plate drawer using a cloth or sponge that has been lightly dampened with water.

## Replacing Fuses

Fuses burn out occasionally and must be replaced.

Fuses are rated slow-blow (United States/Canada/Metric: 6.3 amp time delay).

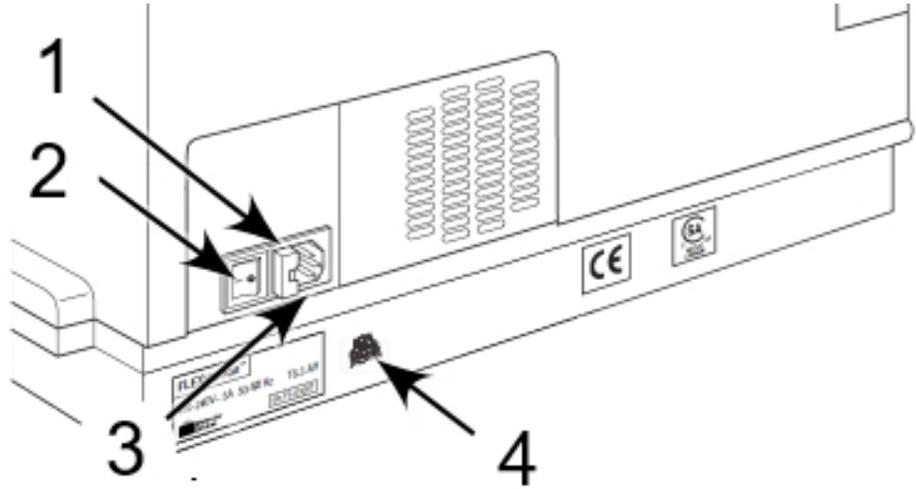
If the instrument does not seem to get power after you switch it on, check to see whether the power cord is securely plugged into a functioning power outlet and to the power port on the rear of the instrument.

If the power failed while the instrument was on, check that the power cord is not loose or disconnected and that power to the power outlet is functioning properly.

If these checks fail to remedy the loss of power, replace the fuses. You can obtain replacement fuses from Molecular Devices. For fuse specifications, see [Parts and Accessories on page 101](#).

**⚠ 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 on the rear of the instrument.



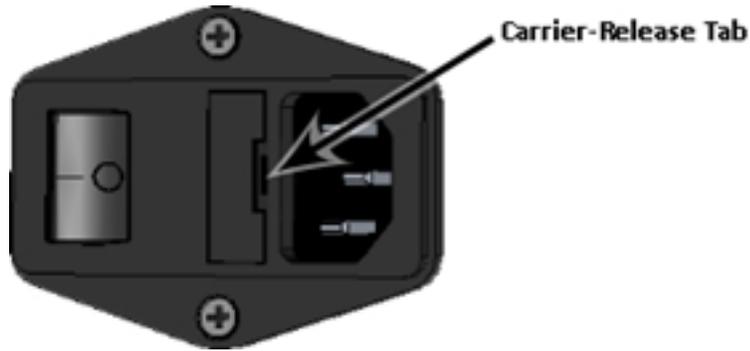
Item	Description
1	Fuse box cover
2	Power switch
3	Power cord receptacle
4	USB port

To replace the fuses:

1. Turn off the power to the instrument and unplug the power cord and the computer cable from the rear of the instrument.

**⚡ WARNING!** Power off the instrument and disconnect the power cord before you do this maintenance procedure.

2. Use a small flat head screwdriver to gently press on the carrier release tab and then pull the fuse carrier to remove it from the instrument.



3. Gently pull the old fuses from the carrier by hand. It is possible that only one of the fuses has blown but you should replace both fuses to ensure continued proper operation.
4. Gently place new fuses into the carrier by hand.
5. Press the fuse carrier into the instrument until the carrier snaps into place.
6. Reattach the power cord to the instrument and reconnect the instrument to the computer.
7. 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.

## Manually Open Drawers

Under some circumstances it might be necessary to open an instrument drawer manually.

1. Power off the instrument and unplug the power cord and the computer cable from the rear of the instrument.



**WARNING!** The power must be off for this procedure. If the power is not disconnected, and a jammed carriage or drawer is suddenly freed, the instrument can suddenly move, resulting in operator injury.

2. Use your fingernail or a narrow blade to open the drawer door to its horizontal position.
3. Slowly, and as gently as possible, pull out the carriage.



**CAUTION!** Never force a carriage out of the instrument. If the carriage does not come out reasonably easily, contact Molecular Devices Technical Support.

## Error Messages

Messages do not stop a read and are logged in the error buffer. They indicate a situation that requires attention, but is not sufficient to stop or prevent a read. Examples of situations that might cause messages are low memory, entries being out of range, or operations that could result in loss of data. The messages are generally self-explanatory.

If a problem occurs during operation that causes an unrecoverable error, the instrument stops and an error code number appears in the display on the front panel. The LCD displays Fatal Error codes when a situation arises that requires attention. Any read in progress stops.

Error Code Numbers	Possible Cause
100 to 199	Errors possibly caused by unrecognized commands being sent from the computer to the instrument.
200 to 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 to 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 to 499	Errors caused by a motor motion failure. Most of these errors require the assistance of Technical Support.
500 to 599	Errors due to failure or improper initialization of the instrument non-volatile memory (NVRAM). All of these errors require the assistance of Technical Support.



**Note:** For error messages specific to fluidics operation failures, see [Assessing Mechanical Problems on page 82](#).

If you see the following message, make sure you correctly placed an assay plate in the read chamber drawer and start the read again.

- There is no assay plate.

If you see any one of the following messages, attempt your read again before you continue with troubleshooting.

- Unexpected response. Result Code = 105
- Instrument communication problem. Response too late.
- Measurement aborted.
- Result code = Instrument reported error.

If you see the following message, try to determine if there are tips jamming the read head. See [Inspecting Inside the Fluidics Module on page 85](#). If this is not the case, contact Molecular Devices Technical Support.

- Instrument reported a fatal error

If a message persists, if you cannot return the instrument and software to normal operation, for a fatal error, and for all error messages or codes not listed above, contact Molecular Devices Technical Support. See [Obtaining Support on page 94](#).

## Troubleshooting the Fluidics System

The following sections address problems that can occur with the fluidics operation of the instrument. They do not address problems with the flash lamp or the detector, or with error messages related to missing data or saturated data points.

The procedures in these sections are intended for cases in which tips have jammed, the power fails while you use the instrument with fluidics, or an error message on the computer screen that indicates a problem with the instrument.



**WARNING! HIGH VOLTAGE** 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.

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**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.

---

## Opening the Instrument



**CAUTION!** To avoid damage to the instrument, follow these instructions and any instruction labels on the instrument exactly.

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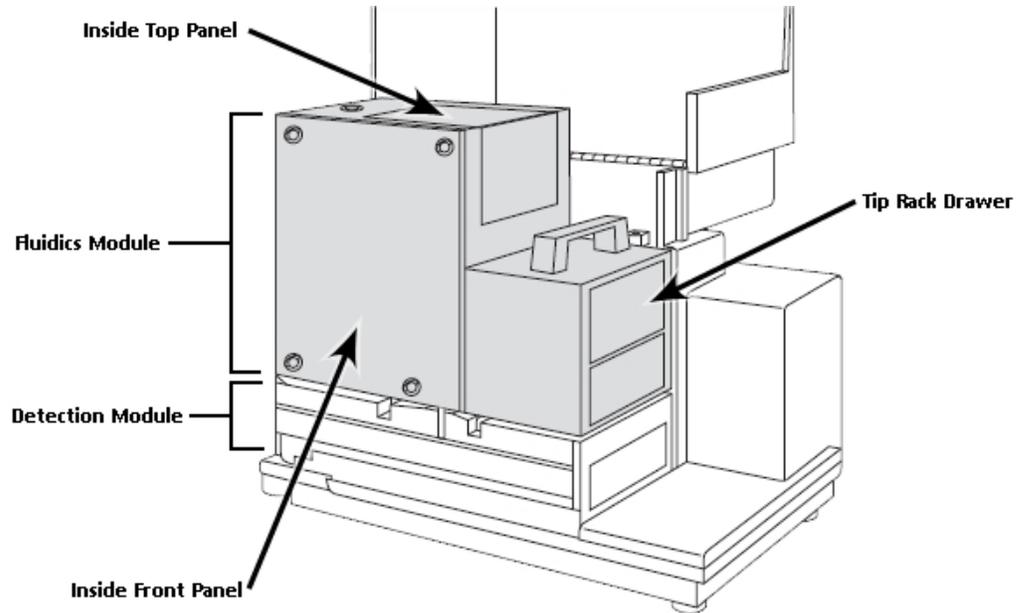
Power off the instrument and unplug the power cord and the computer cable from the rear of the instrument.



**WARNING! HIGH VOLTAGE** 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.

---

1. Press in the handle at the bottom of the top cover and lift the cover back off the detection module, to reveal the fluidics module.



2. Turn the four quarter turn screws on the front panel.
3. Lift the front panel out.

If you are not a service trained user, you should not do anything else to the instrument.

The following maintenance and troubleshooting topics describe how to inspect the interior of the fluidics module to determine if the pipettor was in the process of a fluid transfer when the power was interrupted. The pipettor head should be at rest in the uppermost “home” position. If not, there might be fluid in the tips that you need to remove.

## Assessing Mechanical Problems

Problems during a fluidics operation can result in the display of several error messages in the SoftMax Pro Software. The error message might not provide complete information. The software cannot confirm the presence, absence, or exact position of tips or plates. When there is a mechanical failure of the fluidics operation, follow the entire procedure until you can pinpoint the source of the problem.

- **Fluidics module not detected.**

This message can display if the fluidics module is not correctly installed.

- **Pipettor failure. There may be undispensed fluid in the pipette tips.**

This message can display if the tip rack is jammed. Tips might be missing. To help prevent frequent tip drops, periodically clean the pipettor barrels. See [Pipettor Head Cleaning, Maintenance, and Troubleshooting on page 87](#).

- **Pipettor failure. Undispensed fluid in the tips.**

This message displays if fluid remains in the tips. The instrument needs to be manually recovered at this point. See [Recovery Procedure on page 93](#).



**Note:** If you contact technical support during the following procedures, note which error message displays.

---

## Evaluating the Tip Rack

Inspect the tip rack drawer. The problem might be obvious and visible from the outside if the tip rack drawer is partially open and jammed. Possible scenarios include:

- Tip rack cover was not removed.

If the cover was not removed from the tip rack, gently pull the tip rack carriage out of the instrument to access the rack. Remove the tip rack cover and reposition the tip rack on the carriage.

- Tip rack is improperly positioned.

If the tip rack is jammed because it was not positioned securely in the drawer, gently pull the tip rack carriage out of the instrument to access the rack. If all the tips are accounted for, reposition the rack in the carriage.

- Tips are not seated properly.

If the tip rack is jammed because one or more tips are not seated properly in the rack and prevent the drawer from opening, you might be able to remove the rack and the problematic tips from outside the fluidics module by pulling the tip rack all the way out. If not, gently push the tip rack back inside the instrument. Reach in from the front and remove the tip rack by lifting it up and out toward you. Be careful not to spill tips inside the instrument.

- Tips are missing.

If the tip rack is jammed and there are tips missing, see [Inspecting Inside the Fluidics Module on page 85](#).

To help prevent frequent tip drops, periodically clean the pipettor barrels. See [Pipettor Head Cleaning, Maintenance, and Troubleshooting on page 87](#).

After you correct a tip rack issue, replace the inside front panel and top cover. Reattach the power cord and computer cable to the instrument and then restart the instrument. Save your SoftMax Pro Software file to prevent loss of data, and then close and restart the software.



**Note:** Failure to restart the SoftMax Pro Software can result in communication problems between the instrument and computer.

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## Tilting the Fluidics Module



**WARNING!** The following procedures must be completed by a service trained user. Do not attempt the following procedures if you have not received proper training by Molecular Devices personnel.

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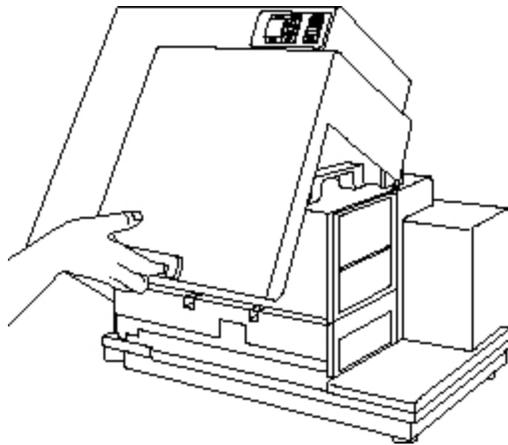
**WARNING! BIOHAZARD.** This procedure includes steps that involve potentially infectious biological agents that require you to follow proper handling precautions.

1. Power off the instrument and unplug the power cord and the computer cable from the rear of the instrument.

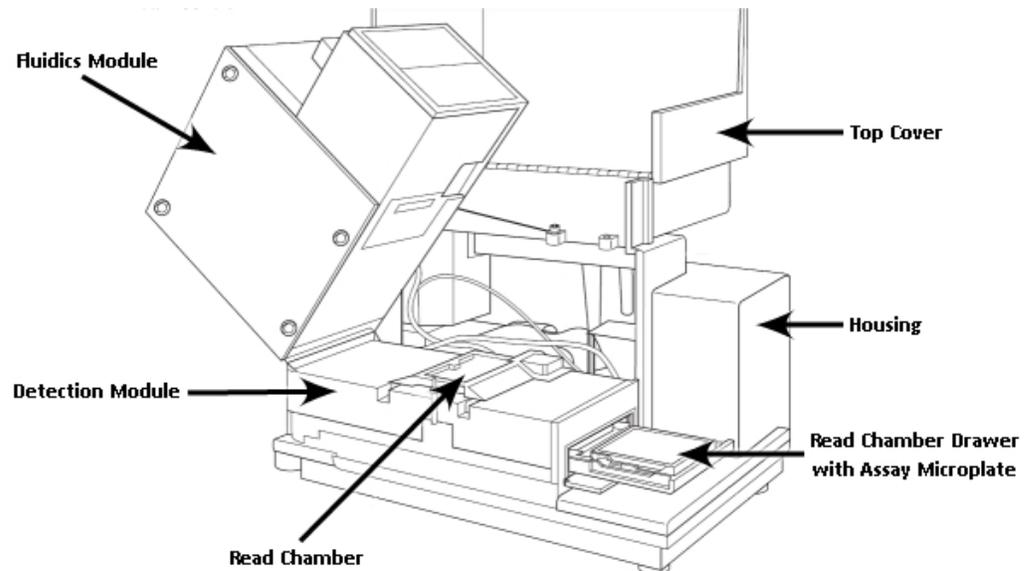


**WARNING! HIGH VOLTAGE** Power off the instrument and disconnect the power cord before you do maintenance procedures that require removal of a panel or cover or disassembly of an interior instrument component.

2. Open the instrument top cover.



3. Use the handle to tilt the fluidics module to the left.



4. If you can clean up a spill or remove the tips without removing the fluidics module, then do so at this time.
  - You can attempt to remove any obvious tips on top or around the sides of the read chamber.
  - If you see tips all the way down in the bottom tray of the instrument, you can safely leave them there. They will be out of the way of the operation of the read heads.
  - If you look through the openings in the read head, you might be able to see tips inside. If you see tips lodged inside the read chamber, contact Molecular Devices Technical Support. See [Obtaining Support on page 94](#).



**CAUTION!** Do not attempt to tilt or remove the read heads as this can damage fragile optical components and can void the warranty.

---

5. When you are done, use the handle to carefully lower the fluidics module onto the detection module until it is completely seated.
6. Lower the top cover, reconnect the computer, and power on the instrument.

## Inspecting Inside the Fluidics Module

If there is any chance that there could be fluid in the pipette tips, you must remove the pipettor head from the instrument and dispel the fluid or you must remove the tips from outside the instrument. Do not spill fluid into the read chamber. See [Pipettor Head Cleaning, Maintenance, and Troubleshooting on page 87](#).



**CAUTION!** Failure to dispel fluid outside of the instrument can result in damage to the optical components.

---



**WARNING! BIOHAZARD.** This procedure includes steps that involve potentially infectious biological agents that require you to follow proper handling precautions.

---

Determine the position of the pipettor head and the location of all the tips that were originally in the tip rack.

- Is the pipettor head loose? Is the red knob tightened securely?
- Is the pipettor head up and out of the way (in home position) or is it down in the tip rack area, the compound plate area, or the read chamber?
- Are there any pipette tips on the pipettor head?
- Is there a chance that liquid remains in the tips? If so, remove the head and expel the liquid safely.



**CAUTION!** Do not inadvertently dispel fluid before the pipettor head is outside of the instrument.

---

- Can you account for all tips or is there a possibility that one or more have dropped out of sight?
- Do all the drawers move freely to gentle pressure, or is there a plate or tip rack jamming a drawer?

After you determine the status of the pipettor head and tips, the following procedures should resolve the problem.

- Pipettor head is in the home position, without tips.  
If the pipettor head is up and out of the way (without tips) but the tip rack is jammed, one or more tips may not be seated properly and block the drawer movement.  
Reach into the fluidics module from the front panel and remove the tip rack by lifting it up and out toward you. If the tip rack drawer is not all the way inside the instrument (in its left-most position), gently push it inside (to the left) to get the rack out.
- Pipettor head is in home position, without tips, but tips are missing from the rack.  
You must locate and recover the missing tips before you proceed.  
Retrieve any tips you can easily see and reach through the front panel.  
You may need to raise the fluidics module to gain access to the read chamber. See [Tilting the Fluidics Module on page 83](#).
- Pipettor head is in lowest position, without tips, and all tips are accounted for.  
If the pipettor head is all the way down its carrier bar (not in home position), without tips on the nose cones, and all tips are safely in the rack, gently remove the tip rack. The compound plate might also be stuck inside the fluidics module.  
Gently push the tip rack and compound plate (or trough) drawers all the way outside to the right.
- Pipettor head is not in home position, with tips.  
If the pipettor head is somewhere other than home position, and has tips on it, you must proceed carefully. The pipettor head might or might not be obviously jammed. Tips could be jammed in the tip rack, in the compound plate, or in the assay plate.  
Carefully raise the pipettor head to home position by manually moving the belt.  
If there is any chance that there could be fluid in the pipette tips, you must remove the pipettor head from the instrument and dispel the fluid or you must remove the tips from outside the instrument. Do not spill fluid into the read chamber.



**CAUTION!** Do not attempt to remove the pipettor head through the front inside panel. See [Pipettor Head Cleaning, Maintenance, and Troubleshooting on page 87](#).

---

If you are certain there is no liquid in the tips, remove the tips from the pipettor head manually. Reach into the fluidics module and gently pull the tips off the cones.  
Gently push the tip rack and compound plate (or trough) drawers all the way outside to the right.

After you secure all tips and return the tip rack and compound plate drawers to their outside, right-most position, replace the inside front panel and top cover. Reattach the power cord and computer cable to the instrument and then restart the instrument. Save your SoftMax Pro Software file to prevent loss of data, and then close and restart the software.



**Note:** Failure to restart the SoftMax Pro Software can result in communication problems between the instrument and computer.

---

When communication between the instrument and computer is interrupted, as with a power failure, a red X appears over the instrument icon in the SoftMax Pro Software. After you correct the problem, and the instrument completes the startup cycle, the red X over the instrument icon should disappear to indicate that communication has been successfully reestablished.

## Pipettor Head Cleaning, Maintenance, and Troubleshooting

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**WARNING!** The following procedures must be completed by a service trained user. Do not attempt the following procedures if you have not received proper training by Molecular Devices personnel.

---

When the Molecular Devices representative installs the instrument, it is set up with either the 8-channel pipette head or the 16-channel pipette head. Your laboratory's service trained users might need to remove the pipettor head to clean the barrels, to remove empty tips, to dispel liquid that remains in tips after a mechanical failure, or to switch to the other pipette head channel set up.

You should clean the barrels to remove silicone lubricant, dust, and other miscellaneous contamination at least once every three months. The frequency depends on how dusty your lab is and how often you use the pipettor head. The frequency and methods of cleaning are the same for both the 8-channel and the 16-channel pipettor heads.

---



**CAUTION!** To avoid damage to the instrument, follow these instructions and any instruction labels on the instrument exactly.

---



**WARNING! BIOHAZARD.** This procedure includes steps that involve potentially infectious biological agents that require you to follow proper handling precautions.

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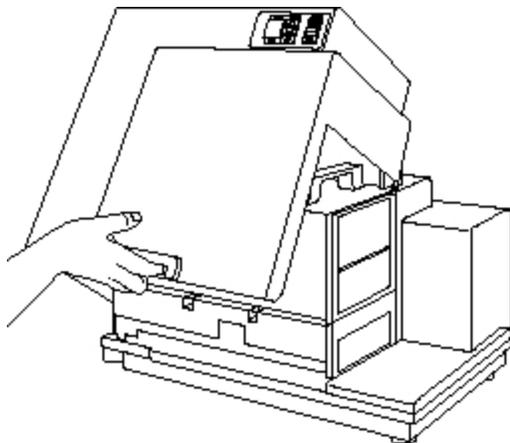


**WARNING! HIGH VOLTAGE** Power off the instrument and disconnect the power cord before you do maintenance procedures that require removal of a panel or cover or disassembly of an interior instrument component.

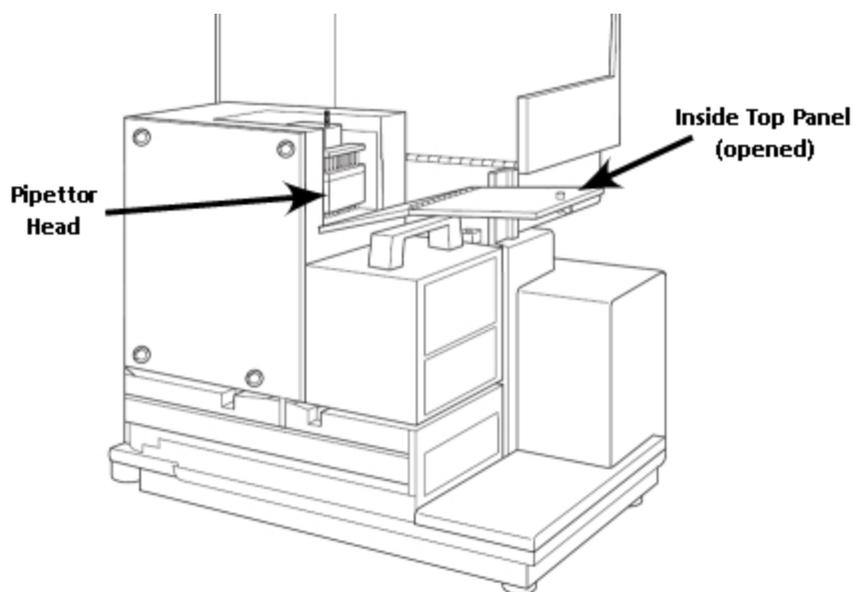
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To remove, clean, and install the pipettor head:

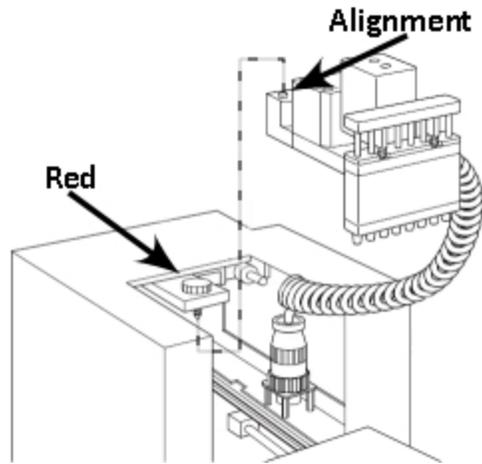
1. Power off the instrument and unplug the power cord and the computer cable from the rear of the instrument.
2. Open the instrument top cover.



3. Turn the quarter turn fastener on the inside top panel and unfold the panel off the fluidics module to the right.



4. Inside the open chamber, loosen the red knob.



5. Slide the pipettor head part way out from under the metal mounting plate, and of the top panel opening, supporting it from underneath.
6. Carefully lift the pipettor head out of the fluidics module. It is still connected inside the instrument.
7. To clean the barrels or to expel undispensed fluid from the pipettor:
  - a. Hold the pipettor head over a tray or something into which you can expel the liquid and remove the tips.



**CAUTION!** Do not spill or drip liquid inside of the instrument, as this can damage the optical components.

---

- b. With your other hand, rotate the black pulley on the bottom of the pipettor motor clockwise. This moves the plungers to expel the liquid and to eject the tips.
- c. Clean the outside and inside of barrels using cotton swabs and Dow Corning OS-2 silicone cleaner or alcohol.

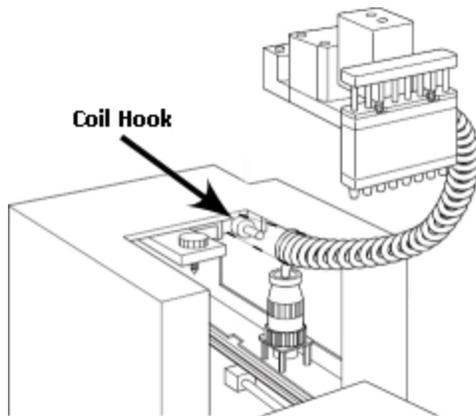


**CAUTION!** Do not touch the barrels with your hands during cleaning.

---

You can skip to step 17 if you just want to clean the pipette head or expel fluid. Do the next ten steps to completely remove the pipettor head for replacement.

8. To completely remove and or replace the pipettor head, pull the spiral cord off the coil hook behind the pipettor head. This frees the mechanism for easier removal.



9. Unscrew the black connector at the end of the white spiral cord from its secure position around the receptacle.
10. Pull the connector out of the receptacle and take the pipettor head the rest of the way out of the fluidics module.
11. Remove the new pipettor head from its carton.

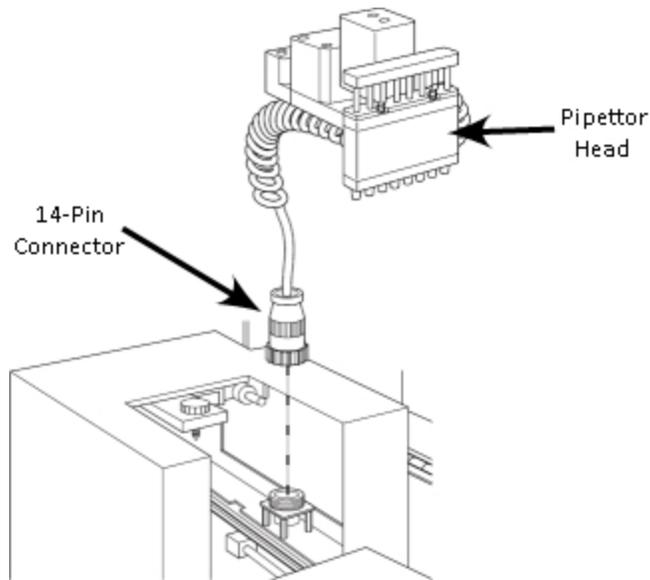


**CAUTION!** During the pipettor installation process, make sure that the pipettor cap remains on the 16-channel pipettor. This minimizes any potential damage to the pipettor nose cones.

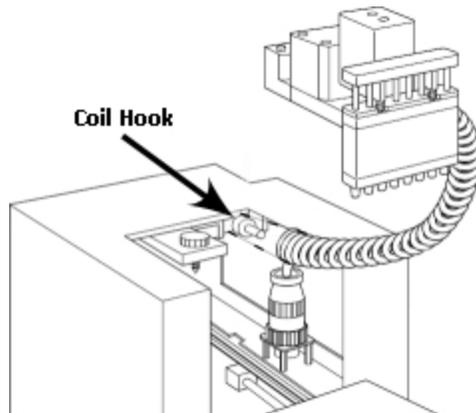
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12. Hold the pipettor head in one hand and the round, black, 14-pin connector in the other.
13. Move the pipettor head into its approximate position under the z-stage plate and red mounting knob.
14. Maneuver the spiral cord down toward the back of the cavity and align the connector over the receptacle.

15. Press the connector in place and tighten the black outer collar over the pins.

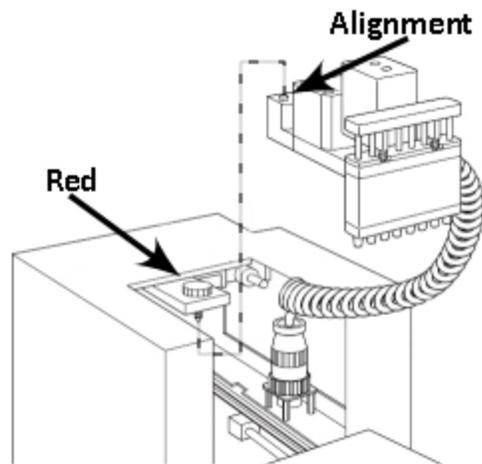


16. Press the first four loops of white spiral cord onto the coil hook in the upper left corner of the top panel opening to secure the cable up out of the way of the pipettor head when it moves in the fluidics module during operation.



17. With one hand, pull up on the red knob (the pipettor retaining nut).

18. With the other hand, align the metal plate at the back of the pipettor head, with the screw hole and the two alignment pins, under the red knob.



19. Slide the plate up into place.
20. Tighten the red knob, securing the pipettor head so that it hangs in place from the black bar.

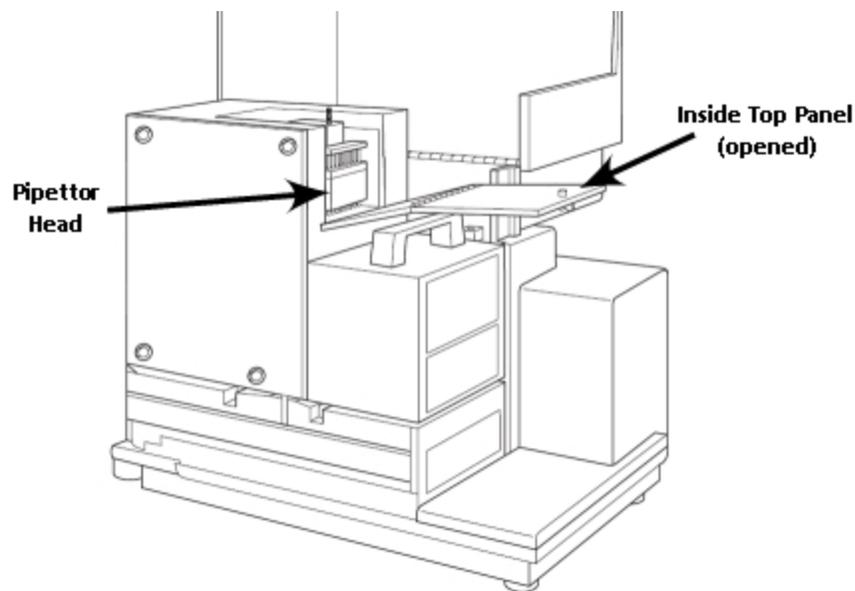


**CAUTION!** Tighten the red knob as firmly as possible.

21. Remove the pipettor cap from the nose cone after you install the pipettor.



**CAUTION!** Do not discard the nose cone cap. Always store the pipettor with the nose cone cap on.



22. Fold the inside top panel back over the pipettor head and lock the quarter-turn screw in place.

23. Bring the top cover back over the fluidics module and snap it into place at the handle on the detection module.

Make sure that the latch clicks shut.

## Recovery Procedure

When there is an instrument malfunction in which fluid was left in the tips, an instrument restart does not automatically reset the fluidics module. An additional recover step is required to restore successful communication between the instrument and the computer. This procedure is a precautionary step, designed to minimize the potentially very serious consequences of liquid spillage in the instrument. The recover process restores the fluidics module to operating conditions and restarts the instrument.

1. After the instrument completes the restart sequence, view the instrument display.
  - If the temperature display on the instrument front panel is blank and the software displays a red X over the instrument icon, use the software to try to reconnect the computer to the instrument.
  - If the temperature display on the instrument front panel displays the actual current temperature, but the software displays a red X over the instrument icon, then continue to the next step.
2. In the SoftMax Pro Software, select the Operations tab and click **Recover**.
3. Remove the pipette head and dispense any liquids from the instrument. See [Pipettor Head Cleaning, Maintenance, and Troubleshooting on page 87](#).
4. Click **OK** to recover the software.

If the Recover procedure does not establish communication between the instrument and the computer, contact Technical Support.

## Before You Move the Instrument



**WARNING! LIFTING HAZARD.** The instrument weighs approximately 22.7 kg (50 lbs.). To prevent injury, use a minimum of two people to lift the instrument.

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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. See [Physical Specifications on page 99](#).

Use the following procedure to move the instrument to a nearby location, such as another workbench in the same laboratory. If you need to move the instrument a longer distance, some disassembly might be required. For assistance, contact technical support.

To move the instrument:

1. Remove any plates, adapters, reservoirs, and tips from all three drawers, and then close the drawers.



**CAUTION!** Do not attempt to move, maintain, or service the instrument with the pipette tips, compound plate, or assay plate in place. Spillage or damage to the pipette tips, plates, or the instrument can occur.

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2. Be sure that the read chamber screws are still in place. If the read chamber screws have been removed, contact technical support.
  3. Be sure that all covers are closed and securely fastened.
  4. Turn off the power switch and unplug the power cord from the instrument and the wall outlet.
- 



**WARNING!** Always turn the power switch off and disconnect the power cord from the main power source before setting up or relocating the instrument.

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5. Disconnect the instrument from the computer.

## 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](http://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 your instrument serial number or Work Order number, and your software version number available when you call.

## Appendix 7: Instrument Specifications

Thermal specifications for plates used in the instrument apply to flat-bottomed plates with isolated wells. All other plate specifications apply to standard 96-well polystyrene flat-bottomed plates. When pathlength compensation is applied to plate absorbance measurements, the solution volume in the plate well is between 100  $\mu$ L and 300  $\mu$ L.

Technical specifications are subject to change without notice.

### Measurement Specifications

The following tables list the instrument measurement specifications.

**Table 7-1: Absorbance Measurement Specifications**

Item	Description
Wavelength range	200 nm to 1000 nm
Wavelength selection	Monochromator tunable in 1 nm increments
Wavelength bandwidth	$\leq 4.0$ nm
Wavelength accuracy	$\pm 3.0$ nm
Wavelength repeatability	$\pm 0.2$ nm
Photometric range	0.0 OD to 4.0 OD
Photometric resolution	0.001 OD
Photometric accuracy/linearity, 0.0 OD to 2.0 OD	$< \pm 0.006$ OD $\pm 1.0\%$
Photometric precision (repeatability), 0.0 OD to 2.0 OD	$< \pm 0.003$ OD $\pm 1.0\%$
Stray light	$\leq 0.05\%$ 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 (Based on specifications from the manufacturer. Individual average lamp lifetime can vary.)	1 billion flashes or 2 years normal operation
Photodetectors	Silicon photodiode

**Table 7-2: Fluorescence Intensity Measurement Specifications**

Item	Description
Sensitivity (top read) For properly functioning, operating, and maintained equipment	<b>96-well</b> <5 pM FITC, 1 fmol/200 $\mu$ L <b>384-well</b> <20 pM FITC, 2 fmol/100 $\mu$ L
Wavelength range	250 nm to 850 nm
Wavelength selection	Monochromator tunable in 1 nm increments
Bandwidth	EX: 9 nm EM: 15 nm
Number of excitation/emission pairs per microplate	4
Dynamic range	>10 <sup>6</sup> in 96-well black plates: auto gain circuitry
System validation	Self calibrating with built-in fluorescence calibrators
Light source	Xenon flash lamp (1 joule per flash)
Average lamp lifetime	1 billion flashes or 2 years normal operation
Detector	Photomultiplier tube (PMT)

**Table 7-3: Luminescence Measurement Specifications**

Item	Description
Sensitivity (top read) For properly functioning, operating, and maintained equipment	<b>96-well</b> <2 fg per well for firefly luciferase <b>384-well</b> <2 fg per well for firefly luciferase
Wavelength range	250 nm to 850 nm
Crosstalk	<0.3% in white 96-well or white 384-well microplate

**Table 7-4: Time-Resolved Fluorescence Measurement Specifications**

Item	Description
Sensitivity (top read) For properly functioning, operating, and maintained equipment	<b>96-well</b> 100 fM europium <b>384-well</b> 100 fM europium
Wavelength range	250 nm to 850 nm
Bandwidth	EX: 9 nm EM: 15 nm
Precision data collection	1 to 100 flashes; delay of 0 $\mu$ s to 600 $\mu$ s before read; integration time selectable 50 $\mu$ s to 1500 $\mu$ s.

**Table 7-5: Fluorescence Polarization Measurement Specifications**

Item	Description
Wavelength range	300 nm to 750 nm
Wavelength precision	<5 mP standard deviation at 1 nM fluorescein in 96-well and 384-well microplates
Wavelength selection	Monochromator tunable in 1 nm increments
Bandwidth	EX: 9 nm EM: 15 nm

**Table 7-6: Photometric Analysis Mode Using the SoftMax Pro Software**

Item	Specification
Data expression	Absorbance: OD or %Transmittance Fluorescence: Relative Fluorescence Units (RFU) Luminescence: Relative Light Units (RLU)
Wavelengths per read	Up to 6
Read types	Endpoint Kinetic and kinetic graphics Spectral scan: 200 nm to 1000 nm Well scan (absorbance or fluorescence) Flex (simultaneous read and fluid transfer)
Measurement time, Absorbance, single wavelength (Calibration off)	<b>96-well</b> 18 seconds <b>384-well</b> 49 seconds
Measurement time, Fluorescence, single wavelength (Calibration off)	<b>96-well</b> 17 seconds <b>384-well</b> 48 seconds
Plate mixing modes	Off Once before a read Once before and between kinetic reads
Plate mixing duration	0 seconds to 999 seconds (default = 3 seconds)

## Physical Specifications

**Table 7-7: Physical Specifications**

Item	Description
Environment	Indoor use only
Power requirements	90 VAC to 240 VAC, frequency range: 50 Hz to 60 Hz, Max. 500 VA
Dimensions	58 cm W x 48 cm H x 41 cm D (23 in. W x 19 in. H x 16 in. D)
Weight	22.7 kg (50 lbs.)
Power disconnect and ventilation clearance (rear)	20 cm to 30 cm (7.9 in. to 11.8 in.) between the rear of the instrument and the wall
Ambient operating temperature	15°C to 60°C (59°F to 140°F)
Ambient storage temperature	-20°C to 65°C (-4°F to 149°F)
Humidity restrictions	0% to 70% (non-condensing)
Installation category	II
Pollution degree	2
Fuses (2)	6.3A 5 mm × 20 mm Slo-Blo IEC (part number: 4600-0029)
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism and read chamber assembly protected from accidental spillage by drainage ports
Data connection	One (1) USB 2.0 port
Plate formats	6, 12, 24, 48, 96, and 384-well plates, ANSI/SBS conformant, SpectraDrop Micro-Volume Microplate, and SpectraCuvette Adapter
Robot-compatible drawer	Positioning and plate gripping as drawer closes
Integrated automation interface	SoftMax Pro Software automation interface integrated with robot partners For more information, visit the Molecular Devices web site: <a href="http://www.moleculardevices.com">www.moleculardevices.com</a>

**Table 7-8: Fluidics Specifications**

Item	Description
Volume range	<b>8-channel pipettor:</b> 1 $\mu$ L to 200 $\mu$ L <b>16-channel pipettor:</b> 1 $\mu$ L to 30 $\mu$ L
Dispensing precision	<b>8-channel pipettor:</b> 8% CV at 5 $\mu$ L, 2% CV at 50 $\mu$ L <b>16-channel pipettor:</b> 3% CV at 10 $\mu$ L, 5% CV at 1 $\mu$ L
Maximum dispense rate	<b>8-channel pipettor:</b> 208 $\mu$ L per second <b>16-channel pipettor:</b> 52 $\mu$ L per second

**Table 7-9: Temperature Controls**

Item	Description
Read chamber	Isothermal when temperature regulation is not enabled
Temperature range	2°C (3.6°F) above ambient to 45°C (113°F) Ambient must be >20°C (68°F) to achieve temperature regulation at 45°C (113°F).
Resolution	$\pm$ 0.1°C (0.2°F)
Accuracy	$\pm$ 1.0°C (1.8°F) in the read chamber
Temperature uniformity at equilibrium	$\pm$ 1.0°C (1.8°F) at 37°C (98.6°F)
Chamber warm-up time	30 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	4 sensors
Evaporation	Microplate lid required to minimize evaporation
Recommended microplate	Flat-bottomed plates with isolated wells and a lid
Control	Reports read chamber temperature only

## Parts and Accessories



**CAUTION!** Do not use parts and accessories that are not authorized by, specified by, or provided by Molecular Devices. Use of unauthorized parts can damage the instrument.

Part Number	Description
4400-0002	Power cord, U.S.
4400-0036	Power cord, Europe
9000-0149	Computer cable
0310-9336	Plate adapter (read chamber drawer)
2500-1029	Compound baseplate (compound plate drawer)
4600-0029	Fuse (6.3A 5 mm × 20 mm Slo-Blo IEC)
0310-3992	Pipettor head, 8-channel
0310-5025	Pipettor head, 16-channel
9100-0051	Hex key, 3/32 inch
9000-0911	Pipette tips, 96-well (black)
9000-0912	Pipette tips, 96-well (clear)
9000-0763	Pipette tips, 384-well (black)
9000-0764	Pipette tips, 384-well (clear)

## Electromagnetic Compatibility

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

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est conforme 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.

## E

### **Emission Cutoff Filter**

An emission cutoff filter is a long-pass filter used to condition the emission light before detection by the photomultiplier tube (PMT). In automatic mode, the instrument sets the cutoffs automatically based on the wavelengths chosen for reading. In manual mode, you can choose the filter wavelength.

### **Emission Spectrum Scan**

An emission spectrum scan measures fluorescence or luminescence across a spectrum of wavelengths. Fluorescent reads use the emitted light from a fixed excitation wavelength. The default value reported for each well is the wavelength of maximum emission in either RFU for fluorescence mode or RLU for luminescence mode.

### **Endpoint**

For the Endpoint read type, a read of each plate well is taken in the center of each well, at a single wavelength or at multiple wavelengths.

### **Excitation Filter**

An excitation filter is a band-pass filter that reduces the amount of extraneous lamp excitation light before the excitation monochromator. In endpoint reads and emission spectral scans, selection of the excitation filter is automatic. In excitation spectrum scans, you can choose “no excitation filter” (for smoother scans) or “auto excitation filter” where there might be slight glitches in the spectrum at the wavelengths where filter changes occur.

### **Excitation Spectrum Scan**

An excitation spectrum scan measures fluorescence at a single emission wavelength for the emitted light across a spectrum of excitation wavelengths. The default value reported for each well is the wavelength of maximum fluorescence excitation in RFU.

## F

### **Fluorophore**

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

See [Fluorescence Intensity Read Mode on page 57](#).

## G

### Gain

Gain is the quantity of increase in signal power expressed as the ratio of output to input for a photomultiplier tube (PMT).

See [PMT and Optics Settings on page 35](#).

## P

### Photomultiplier Tube (PMT)

A Photomultiplier Tube (PMT) is a vacuum tube that can detect light from dim sources through the use of photo emission and successive instances of secondary emission to produce enough electrons to generate a useful current.

## S

### Stokes Shift

The Stokes shift is the difference between the wavelengths of the excitation and emission maxima, or peaks.

See [Emission Spectrum Scan on page 102](#) and [Excitation Spectrum Scan on page 102](#).

## T

### Trituration

Trituration uses the pipettor to alternately aspirate the fluid from a well and then dispense it back into the well to mix the contents of the well in a plate.

## Contact Us

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