

# FlexStation® 3 Benchtop Multi-Mode Microplate Reader

## User Guide

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## Description

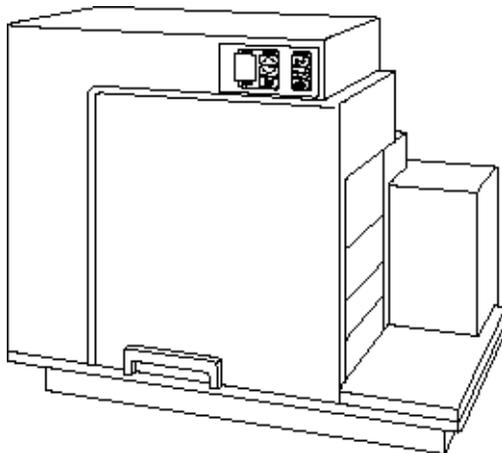
This chapter provides background information on the system, including descriptions of the principal components and overviews of how the system functions. It is divided into the following sections:

- [Introduction, see page 7](#)
- [How To Use This User Guide, see page 9](#)
- [Safety Information, see page 9](#)
- [System Overview, see page 13](#)
- [System Components, see page 15](#)
- [Overview of Operation, see page 32](#)
- [Theory of Operation, see page 33](#)
- [SoftMax Pro Software, see page 41](#)

## Introduction

The FlexStation® 3 Benchtop Multi-Mode Microplate Reader combines the performance of the Molecular Devices SpectraMax® M5e Multi-Mode Microplate Reader with an integrated 8-channel or 16-channel pipettor into one compact benchtop reader. This integrated system provides users with a multi-detection platform capable of increasing the liquid handling throughput and flexibility for biochemical-based and cell-based assays. Using an integrated 8-channel or 16-channel pipettor increases assay flexibility by transferring reagents from 96 or 384 distinct wells in a source plate to the read plate. This method enables users to define individual reagents or compound concentrations to be delivered to each well during the assay. The direct transfer from a source microplate reduces consumption and allows more assay conditions to be explored in a single microplate, making the system equally amenable to agonist and antagonist assay formats. These additions can either occur concurrently with kinetic analysis of reactions or before an assay to automate reagent additions. Combined fluid transfer with multi-detection optics provides a single reader capable of performing a broad span of applications that pass through drug discovery and research environments.

Based on the SpectraMax M5e instrument platform, the FlexStation 3 instrument can address detection modalities including absorbance, fluorescence intensity, fluorescence polarization, time-resolved fluorescence, and luminescence. Dual monochromators allow users to target the optimal assay excitation and emission wavelengths, while eliminating the need to change expensive band pass filters between experiments. Dual PMTs are integrated into the system to provide flexibility to detect multiple detection modes, while a separate PMT provides additional 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 enable the system performance to be comparable to a top-of-the-line dedicated spectrophotometer or spectrofluorometer with no trade-off between instrument performance and the number of read modes.



**Figure 1-1:** FlexStation 3 Instrument

## How To Use This User Guide

This user guide was written to ensure safe and proper use of the system. Before use, read this user guide carefully to realize the full capabilities of the system. Also, if something is unclear during daily use or if a problem occurs, please refer to this user guide.

### User Guide Organization

This user guide is organized as follows:

- [Description on page 7](#) provides background information on the system, including component descriptions, functional overviews, and safety information.
- [Installation on page 49](#) provides instructions for setting up the reader.
- [Operating Procedures on page 65](#) provides instructions for starting up the reader, setting parameters for the various read modes, and reading a microplate.
- [Maintenance on page 113](#) provides instructions for cleaning the fan filter, changing the fuses, and moving the system to a new location.
- [Troubleshooting Procedures on page 129](#) provides instructions for diagnosing and solving common problems, as well as a list of error conditions.
- [Parts and Accessories on page 153](#) provides a list of spare parts.
- [Performance Specifications on page 155](#) provides the technical specifications for the instrument.
- [Glossary on page 163](#) provides a list of terms and definitions.

## Safety Information

When operated properly in a safe environment and according to the instructions in this user guide, there are no known hazards associated with the FlexStation 3 instrument. However, proper use requires an understanding of situations that are potentially dangerous and can result in serious injury. All users must be familiar with the guidelines in this section before working with the system.

## Conventions Used for Precautionary Information

This user guide uses the following conventions to provide technical and safety information of special interest.



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**Note:** A note gives background information that is provided to clarify a particular step or procedure. A note can also provide an instruction to ensure correct results and optimal performance.

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**CAUTION!** A caution is an instruction that, if not followed, can result in damage to the system or in loss of data.

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**WARNING!** A warning is an instruction that, if not followed, can result in potential injury to a person working with the system.

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**WARNING! BIOHAZARD.** A biohazard warning indicates a condition involving potentially infectious biological agents requiring that proper handling precautions be taken.

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## Electrical Safety



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**WARNING!** Follow all instructions in this user guide and on system labels. If you use the system in a manner not specified by Molecular Devices, then any protections provided by the system might be impaired.

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## Service-Trained Users

There are two types of users described in this user guide. Most procedures required for operating and troubleshooting can be performed 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 perform the procedure to ensure user safety and to prevent instrument damage.

Example:



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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 been trained properly by appropriate Molecular Devices personnel.**

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## Avoiding Mechanical Problems During Fluid Transfer

Because of the complex mechanical nature of the FlexStation 3 instrument, including both fluidics and optical reading, smooth and reliable operation of the system depends on both good design and operator knowledge.

To prevent problems of a mechanical nature, be sure to read all sections of this user guide before attempting a reading with fluidics. See [Understanding Potential Mechanical Problems on page 133](#).

## Safety Messages

Observe the following warnings and precautions:

**High internal voltages.** Always turn off the power switch and unplug the system power cord before removing labeled covers or panels.

**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.

**Electrical grounding.** Never use a two-prong plug or extension cord to connect primary power to the system. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to a three-prong receptacle with a functional ground.

**Spilled liquids.** Avoid spilling liquids on the system. Fluid spilled into internal components creates a potential shock hazard. Wipe up all spills immediately. Do not operate the system if internal components have been exposed to spilled fluid. Unplug instrument if there is a fluid spill in the instrument and contact Technical Support.

**Replacement fuses.** Use replacement fuses with the required current rating and specification. Improper fuses or short-circuiting the fuse holders can cause fire or damage the instrument.

**Power rating.** Ensure the system is connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle can create electrical shock and fire hazards.

**Remove watches and jewelry** before removing any panels from the instrument.

**Warning labels.** There are several labels affixed to the instrument covers and inside panels. The purpose of these labels is to alert the user to use caution when servicing a component or the instrument. The user should be aware that ignoring the instructions on any instrument label can result in a hazardous condition that can cause injury.

**Identification labels:** The following label, among others, appears on the instrument.



**Figure 1-2:** FlexStation 3 Instrument Label

## System Overview

The FlexStation 3 Benchtop Multi-Mode Microplate Reader is a monochromator-based microplate reader that has 6-well, 12-well, 24-well, 48-well, 96-well, and 384-well microplate reading capability for absorbance, fluorescence intensity, fluorescence polarization, time-resolved fluorescence, and luminescence. When using the integrated pipettor, the instrument offers 96-well and 384-well microplate-to-microplate fluid transfer, 8 or 16 wells at a time.

The top portion of the instrument, the fluidics module, uses an 8-channel or 16-channel pipettor, to expand assay flexibility by transferring reagents from a 96-well or 384-well source plate to the assay plate. When transferring reagents from distinct wells of a microplate, users can define individual reagents or concentrations to be delivered in each well during their assay. This direct transfer allows more assay conditions to be explored in a single microplate, reducing reagent consumption as well as making the system more amenable to both agonist and antagonist assay formats. In addition, kinetic cell-based assay throughput (for example, calcium mobilization) is increased when 8 or 16 wells of a microplate are analyzed in conjunction rather than individually.

Integrated pipetting provides flexibility, and also offers users parameters to optimize assay robustness. Trituration, mixing via aspiration and dispense of the pipettor, encourages mixing to either resuspend source plate compounds or spontaneously mix reagents to promote a rapid response with minimal assay variability. Dispense parameters can also be optimized for each experiment to accommodate cells with different adherence characteristics to prevent cell dislodging. Furthermore, the integrated fluidics platform uses disposable pipette tips to minimize reagent cross contamination between wells or experiments.

The fluidics module interfaces with the lower reading chamber which encloses a high-powered Xenon flash lamp as the light source. Sensitivity or read-speed can be optimized by varying the number of lamp flashes per read.

The two holographic diffraction grating monochromators allow selection of any wavelength between 200 nm and 1000 nm in absorbance; 250 nm and 850 nm in fluorescence intensity, time-resolved fluorescence (TRF), or luminescence; and 400 nm and 750 nm for readings in fluorescence polarization. Excitation and emission wavelengths are optimized using the mirrored optics to focus light into the sample volume, and cutoff filters to reduce stray light and minimize background interference.

The system has five assay types available which include Flex, Endpoint, Kinetic, Spectrum, and multi-point Well Scan. Flex mode assay enables users to transfer fluid from the source read plate while immediately reading a fast kinetic assay that uses absorbance, fluorescence, or luminescence detection technologies. Alternatively, the pipettor head can be used to transfer fluid before an endpoint or kinetic assay to automate fluid transfer and minimize user interaction.

In addition to fluid transfer, well contents can be mixed automatically by shaking before each read cycle, making it possible to perform kinetic analysis of solid-phase, enzyme-mediated reactions.

Top detection in microplates is available for all fluorescence and luminescence readings. Bottom detection is available for all assay types except fluorescence polarization. When reading absorbance at wavelengths below 340 nm, special UV-transparent, disposable or quartz microplates that allow transmission of the far ultraviolet spectra must be used.

A plate drawer adapter is provided with the reader. The adapter is required for optimum performance when reading from the top or bottom of standard 96-well and 384-well microplates in all read types, including absorbance.

Variations in measured fluorescence values are virtually eliminated by internal compensation for detector sensitivity, photomultiplier tube voltage, and excitation source intensity.

Using the FlexStation 3 instrument with the PathCheck® Pathlength Measurement Technology allows normalization of variable well volumes to absorbance readings. In addition PathCheck technology allows for pipettor validation, including the online 8-channel and 16-channel pipettors, and to compare experiments from different days.

Temperature in the microplate chamber is isothermal, both at ambient and when the incubator is turned on. When the incubator is on, the reading chamber temperature can be controlled from 2°C above ambient room temperature to 45°C. Please note that the temperature of the fluidics module is not regulated and that it is recommended that any microplates or tips should be at the desired temperature before placing them inside the instrument.

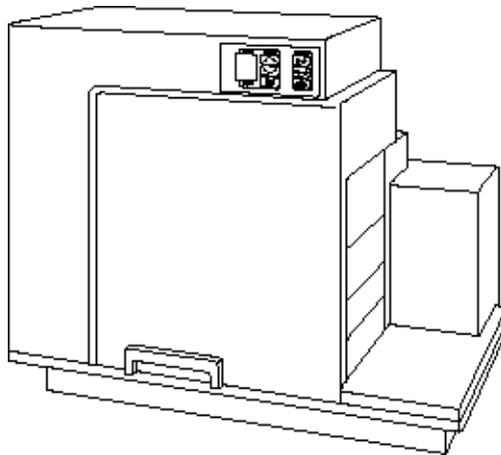
The FlexStation 3 instrument is controlled by an external computer running the SoftMax® Pro Microplate Data Acquisition and Analysis Software, which provides integrated instrument control, data display, and statistical data analysis. The on-board microprocessor calculates and reports the absorbance, percent transmittance, fluorescence, or luminescence for each well of a microplate. Data from multiple wavelengths can be acquired and ratio analysis can be performed during a single reading, if desired. In addition, different calculations can be made based on this data using the SoftMax Pro software, including the subtraction of blanks, quantitation from standard curves, calculation of IC<sub>50</sub> values, and more.

The extreme flexibility and high sensitivity of the FlexStation 3 reader makes it appropriate for applications within the fields of biochemistry, cell biology, immunology, toxicology, molecular biology, and microbiology. Online fluidic integration expands the capabilities of the instrument to include fast fluorescence (calcium mobilization), luminescence, and absorbance assays in addition to typical applications which include ELISA, nucleic acid and protein quantitation, homogeneous and heterogeneous enzyme-activity assays, and microbial growth, endotoxin testing, and reporter-gene assays.

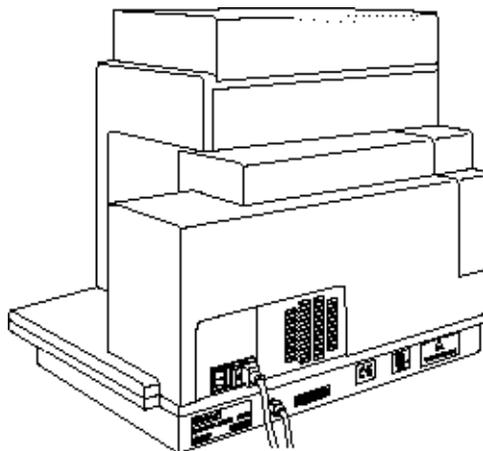
## System Components

This section describes the major system components listed below.

- [Covers and Instrument Panels, see page 17](#)
- [Drawers, see page 21](#)
- [Fluidics Module, see page 24](#)
- [Detection Module, see page 26](#)
- [Computer, see page 28](#)
- [Accessories, see page 28](#)
- [Consumables, see page 30](#)



**Figure 1-3:** Instrument, Front View



**Figure 1-4:** Instrument, Rear View

## Covers and Instrument Panels

### Top Cover

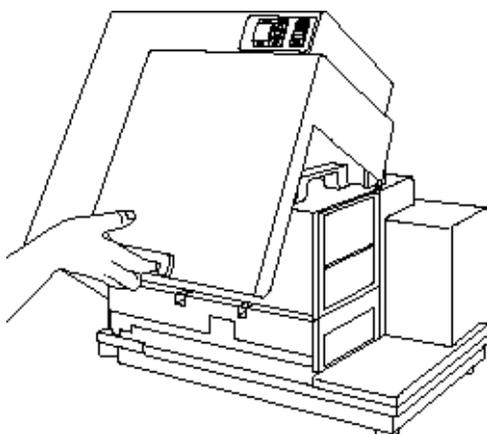
The instrument is protected by a molded plastic housing. The large top cover protects the fluidics module and the exposed portions of the detection module.



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**Note:** The top cover can be lifted back, as shown in the figure below, for certain limited troubleshooting procedures. See [Opening the Instrument on page 137](#).

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**Figure 1-5:** Lifting Off the Top Cover



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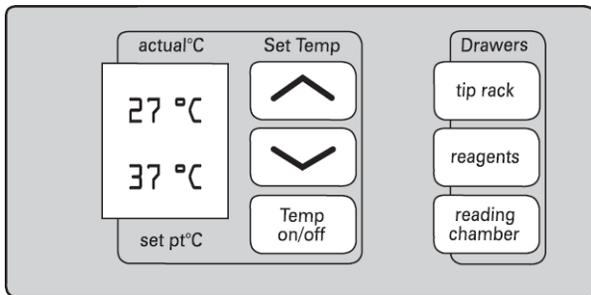
**Note:** To achieve optimal performance during readings, you must operate the system with the top cover in place.

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## Control Panel

The control panel consists of an LCD and six pressure-sensitive membrane keys which can be used to initiate and regulate the temperature and to open and close the drawers.

A 2×3-character liquid crystal display (LCD) shows the current instrument temperature at all times, and the set point temperature when the incubator is on.



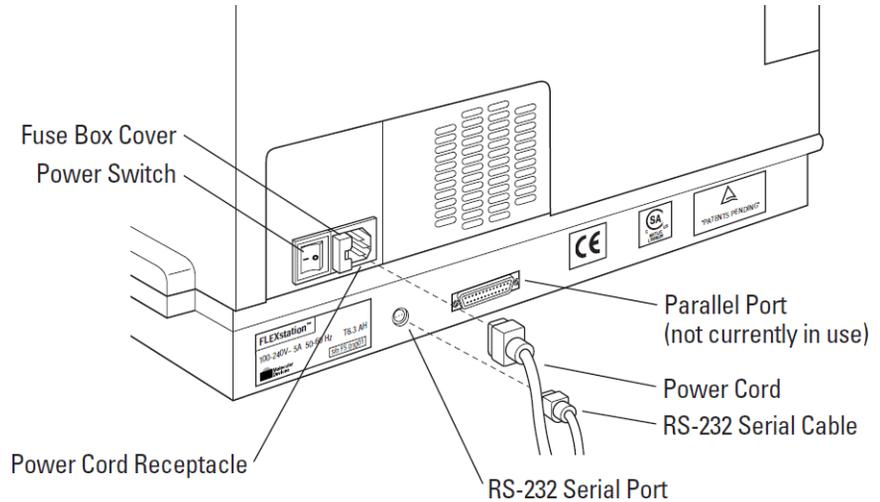
**Figure 1-6:** Control Panel

See [Starting Up the System on page 67](#) and [Setting the Temperature on page 72](#).

## Input/Output Panels

There are two input/output panels on the rear of the instrument.

- The upper input/output panel, on the back cover, consists of a power switch, fuse box cover, and power cord receptacle.
- The lower panel consists of an RS-232 serial port and parallel port (not currently active). There are also a number of identification labels.



**Figure 1-7:** Input/Output Panels.

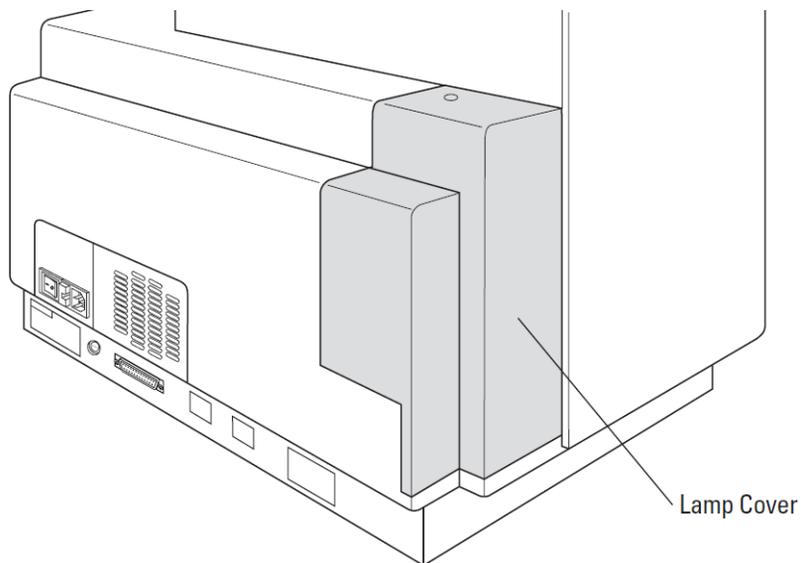
For information about attaching the computer cable and power cords to the instrument, see [Connecting the Cables on page 61](#).

## Lamp Cover

The plastic lamp cover provides access to the flash lamp on the right side of the instrument (as viewed from the rear).



**CAUTION!** Flash lamp access and maintenance are restricted to service-trained users.

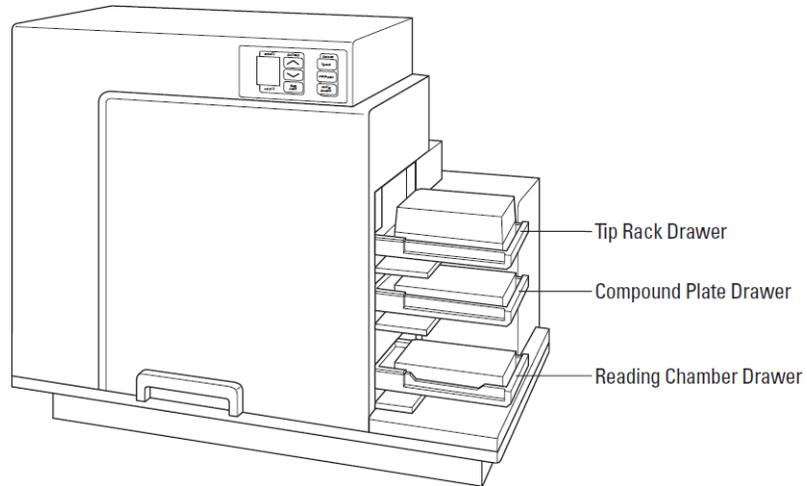


**Figure 1-8:** Rear View

See [Replacing the Flash Lamp](#) on page 123.

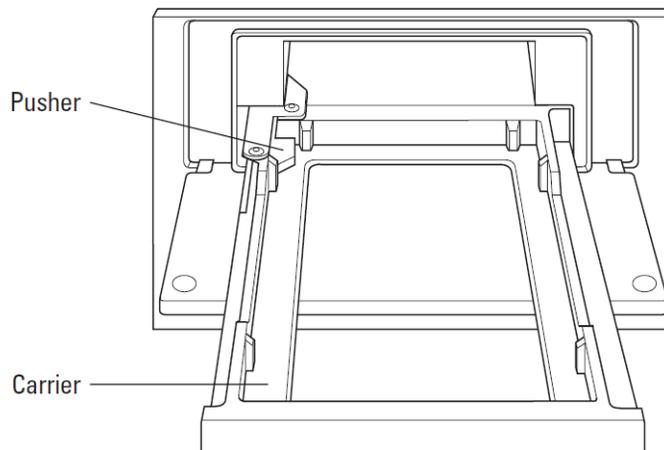
## Drawers

The instrument has three drawers that open on the right side. The two drawers in the fluidics module open and close to move the pipette tip rack and compound plates (or reservoirs) into and out of the instrument. The reading chamber drawer in the detection module transports the assay microplate into the reading chamber.



**Figure 1-9:** Instrument with Drawers Open and Carriages Accessible

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

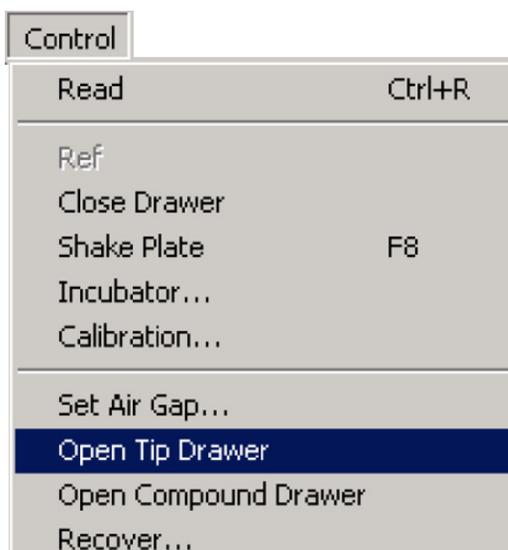


**Figure 1-10:** Drawer Detail



**CAUTION!** Do not obstruct the movement of any of the drawers. If you must retrieve a plate after an error condition or power outage, and if the drawer will not open, it is possible to open the drawer manually. See [Opening a Drawer Manually on page 132](#).

You can open and close the drawers using either the SoftMax Pro Software or by pressing the drawer keys on the instrument control panel. Using the SoftMax Pro Software, open the **Control** menu and click **Tip Drawer** for the tip rack drawer, **Compound Drawer** for the compound plate drawer, or **Open Drawer** for the reading chamber drawer.



**Figure 1-11:** SoftMax Pro Software Control Menu

You can also open or close the reading chamber drawer with the Drawer button on the Status Bar.



**Figure 1-12:** SoftMax Pro Software Drawer Button

### Tip Rack Drawer

The top drawer holds the pipette tip rack.

Only tips specified by Molecular Devices for use with the FlexStation 3 instrument can be safely used. See [Parts and Accessories on page 153](#).



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

---

### Compound Plate Drawer

The compound plate drawer holds a 96-well or 384-well microplate. 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



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**Note:** Be sure to install the compound baseplate before placing a compound plate in the drawer.

---

### Reading Chamber Drawer

The reading chamber drawer opens to accept a 96-well and 384-well microplate for analysis in the reading chamber. It is the lowest of the three drawers.

The reading chamber drawer operation varies, depending on the incubator status. When the incubator is off, the reading chamber drawer is open at power up and after a read. When the incubator is on, the drawer closes automatically to maintain the temperature of the reading chamber.



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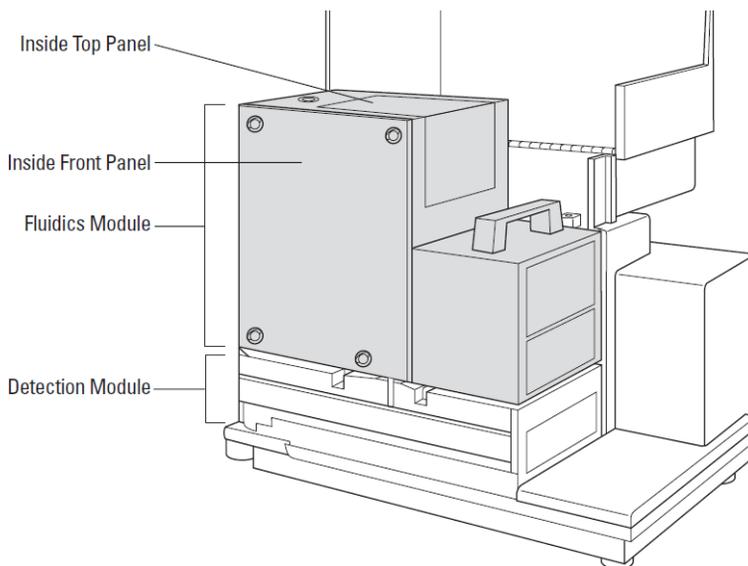
**Note:** Be sure to install the black microplate adapter before placing an assay plate in the drawer for standard 96-well and 384-well microplates. See [Installing the Drawer Adapters on page 62](#).

---

## Fluidics Module

The fluidics module houses the pipettor head, several motors, and all the fluidics components. There are two horizontally-moving carriers, one for the pipette tip rack and the other for the compound plate. The pipettor head moves vertically between the drawers.

The fluidics module can be opened, by service-trained users, from the inside front panel, if necessary for maintenance, or from the inside top panel to install or remove the pipettor head. The entire fluidics module can be removed for maintenance or to transport the system to another location.

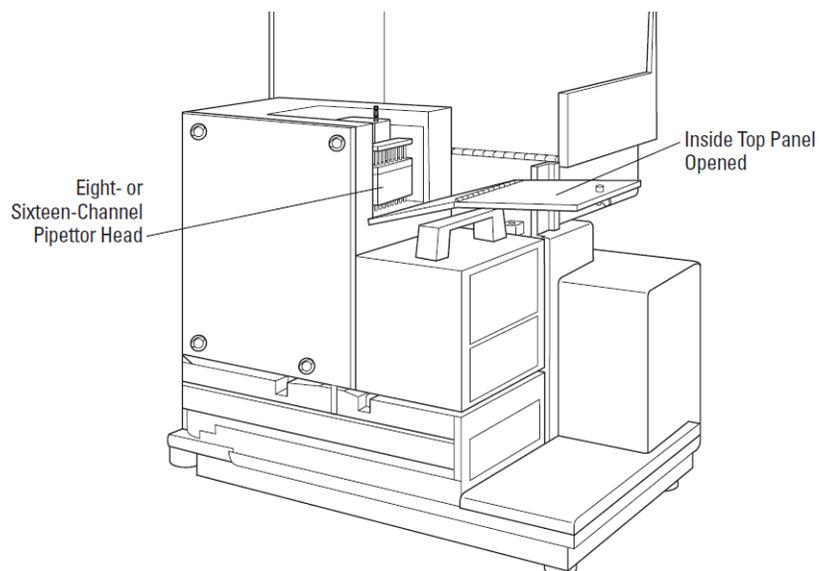


**Figure 1-13:** Fluidics Module

See [Installing the Fluidics Module on page 53](#) or See [Troubleshooting Procedures on page 129](#).

## Pipettor Head

The instrument is configured with an 8-channel pipettor head for use with 96-well microplates or a 16-channel pipettor head for use with 384-well microplates.



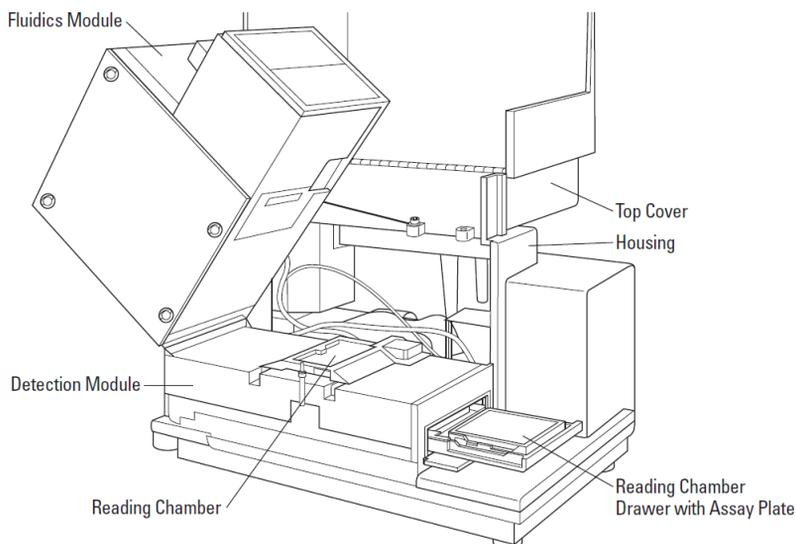
**Figure 1-14:** Pipettor Head

See [Installing the Pipettor Head on page 56](#).

The barrels on the pipettor head require periodic cleaning to remove silicone lubricant, dust, and other miscellaneous contamination. See [Cleaning the Barrels on the Pipettor Head on page 117](#).

## Detection Module

The detection module is the lower portion of the instrument. This module houses the reading 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 and can be tilted off to the side, to provide access to the optical system for troubleshooting or maintenance. The detection module is contained in a molded plastic housing, to which the top cover is attached at the back of the instrument.



**Figure 1-15:** Detection Module Detail

### Reading Chamber

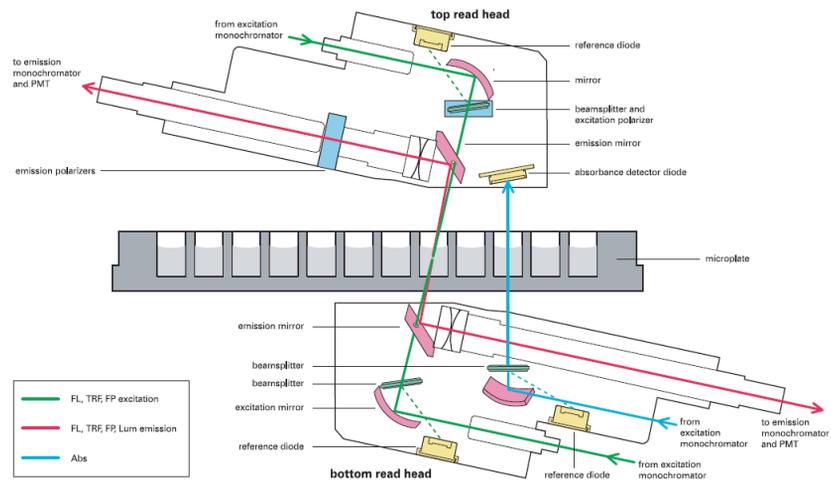
The reading chamber includes the assay plate carriage that holds the assay microplate in the reading chamber during read cycles. The reading chamber can be maintained at an elevated temperature. It contains both top and bottom read heads that can be selected in the software.

The instrument uses a plate sensor to assure that an assay plate is present in the reading chamber before a reading begins.

## Optical System

The optical system includes a xenon flash lamp, monochromators, excitation bandpass filters, emission cut-off filters, PMTs, and photodiodes.

There are a number of cables and fibers that exit the optics bench and enter the reading chamber. They are the excitation fibers (thin and black or red, has a collar and pins), emission fibers (black and fatter, with attached electrical cord), electrical connector to the read head (green with brass fitting).



**Figure 1-16:** Optical System



**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.

## Computer

The FlexStation 3 instrument works as a system with the SoftMax Pro Software. The SoftMax Pro Software must be installed on a dedicated computer to communicate with and control instrument functions.

The instrument is equipped with an 8-pin DIN RS-232 serial port for connecting to a computer.

SoftMax Pro Software, version 5.1 or later, is required to control the FlexStation 3 instrument. The FlexStation 3 instrument is not currently supported in SoftMax Pro Software, version 6.x.

The minimum computer configuration includes a Pentium processor with 2.8 GHz, 1 GB hard drive.

See [Installing SoftMax Pro on page 63](#) and [Setting Up the Software on page 75](#).

## Accessories

The following accessories are included with the system:

- Black microplate adapter (for use in reading chamber drawer)
- Compound baseplate (for use in the compound plate drawer)
- Computer cable
- Power cord, USA/Canada
- Power cord, ECI
- Fuses (2 each)
- User Guide
- Pipettor head, 8-channel and/or 16-channel
- Pipette
- Yellow plate for the respective pipettor head
- Hex key

All necessary accessories are shipped with the system. [Parts and Accessories on page 153](#).

Fuses are rated slow-blow (United States/Canada/Metric: 6.3 amp time delay). See [Replacing Fuses on page 121](#).

## Cables

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

**Serial Interface Cable:** The serial interface cable used to connect the instrument to the computer is a custom cable designed and built by Molecular Devices. Use the cable supplied by Molecular Devices, or contact Molecular Devices for specific pin-out requirements: Male DB8 to Female DB9 (custom cable made by Molecular Devices, P/N 9000-0149).

**USB Adapter Cable:** Many newer computers do not have a serial port. You can connect a serial cable between these computers and the instrument using a USB-to-serial adapter. Molecular Devices has tested many third-party USB-to-serial adapter cables and has found Keyspan USA-19HS (Molecular Devices, P/N 9000-0938) to be the most reliable. It is the only one we recommend.



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**Note:** For specific pin-out requirements, contact Molecular Devices Technical Support.

---

## Microplate Adapters

The black microplate adapter fits in the assay plate carriage, in the reading chamber drawer, to elevate standard microplates for both top reads and bottom reads. Remove the adapter when using high-profile, 6-well, 12-well, 24-well, or 48-well microplates.

## Compound Baseplate

Molecular Devices provides a metal baseplate that must be placed in the compound plate drawer under the compounds plate to reduce stray light.

## Consumables

The system consumables include:

- Microplates
- Pipette tips

One box (10 racks) of pipette tips is shipped with the system pipettor head. See [Parts and Accessories on page 153](#).



---

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

---

## Microplates

The FlexStation 3 instrument can accommodate standard 6-well to 384-well microplates and strip wells. When reading absorbance at wavelengths below 340 nm, special UV-transparent, disposable or quartz microplates allowing transmission of the deep UV spectra must be used.

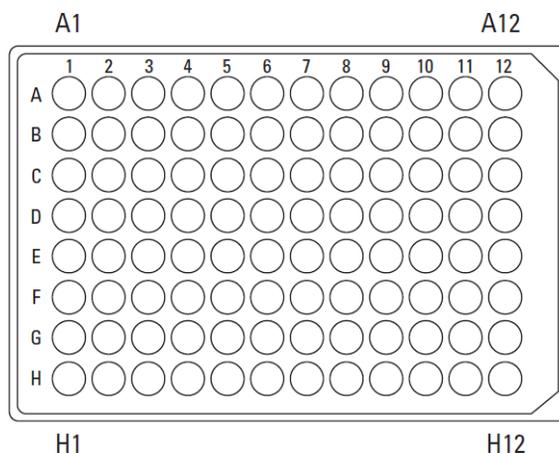
Not all manufacturers' microplates are the same with regard to design, materials, or configuration. Temperature uniformity within the microplate can vary depending on the type of microplate used.

Microplates supported for use in this reader are:

- 6-well, 12-well, 24-well, 48-well, 96-well, and 384-well standard formats
- 96-well half area
- 96-well and 384-well low volume

Read plate formats not already loaded as defaults in the SoftMax Pro Software can be added by manually entering the dimensions using the Plate Editor in the software.

The instrument can accommodate standard 6-well, 12-well, 24-well, 48-well, 96-well, and 384-well microplates. In Flex or other assay types which you intend on using fluidic integration you can only use 96-well or 384-well formatted assay plates.



**Figure 1-17:** Top View of a 96-Well Microplate

For fluorescence, Molecular Devices generally recommends black-walled, clear-bottom microplates for bottom reading, and all-black microplates for top reading, because they have lower backgrounds than clear plates. For luminescence, white microplates can optimize light collection.



---

**Note:** Not all microplates 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 microplates designed and designated by the manufacturer to reduce background fluorescence.

---

## Pipette Tips

- For 96-well assays, Molecular Devices specifies using 96-well, FlexStation Pipette Tips. These 200  $\mu$ L tips are available in black (P/N 9000-0911) and clear (P/N 9000-0912) versions and can be purchased in 10 racks per box quantities.
- For 384-well assays, Molecular Devices specifies using 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) versions and can be purchased in 50 racks per case quantities. Please ask your local sales representative for details regarding purchasing partial cases.

Tips are available in both black and clear options. Black tips are generally used during fluorescence assays when auto-fluorescent properties of clear tips can interfere with your response. Molecular Devices recommends that you evaluate both black and clear tips during assay development to determine which tip version is most appropriate to your assay.

## Overview of Operation

Using the FlexStation 3 instrument is a process in five stages:

- [Choosing an Experiment](#)
- [Preparing the Instrument](#)
- [Preparing the Software](#)
- [Running the Experiment](#)
- [Analyzing the Data](#)

### Choosing an Experiment

- New or repeated experiment?
- Does the protocol exist?

### Preparing the Instrument

- Turning on the power
- Setting temperature, if needed
- Preparing and loading tips, plates, and compounds

## Preparing the Software

- Entering software preferences
- Selecting instrument settings
- Defining templates, reduction parameters, and display parameters
- Confirming hardware and software setup

## Running the Experiment

- Initiating the operation (detection or fluidics plus detection)
- Saving the data file

## Analyzing the Data

- Modifying the template or parameters as desired
- Saving the data file
- Analyzing the data
- Exporting data to another software application as desired

## Theory of Operation

This section includes the following topics:

- [Instrument Design, see page 33](#)
- [Assay Read Types, see page 38](#)

## Instrument Design

### Fluidics

The instrument is designed with a fluidics module that transfers liquids to the assay plate during a fast kinetic read or before an experiment.

The fluidics module incorporates an 8-channel or 16-channel pipettor that automatically changes tips and transfers reagents to the plate that is read in the system. Pipette height and dispensing rate are adjustable. The instrument can add reagents within milliseconds of a column being read, enabling fast kinetic assays of transient responses.

As many as three compounds can be transferred from columns in a compound plate to a single column in an assay plate, at different points during or before the total read time.

## Mixing

Mixing can be accomplished in one of two ways, using either the Trituration or Automix functions. Use of these functions are dependant on the assay performed and the read mode used.

- Trituration is mixing of the well contents in either the compound or assay plates. This is accomplished by fluid being alternately aspirated from and dispensed back into a well using the pipettor. Trituration is available only during assays that include fluid transfer, including Flex, Endpoint, or Kinetic modes, and can be performed in either the compound or assay plate. Within the compound plate, trituration can be used to resuspend compounds that might have crashed out of solution. Alternatively, it can be used to promote prompt mixing in the assay plate when delivering small reagent volumes for fast kinetic cell-based assays, such as calcium mobilization.



---

**Note:** Trituration in the assay plate well may agitate cells, causing responses not associated with the compound addition. Assay development should be performed to determine if trituration is necessary for the assay.

---

- The Automix function permits automatic shaking of the microplate at preset intervals, thereby mixing the contents within each well. Automix must be selected before beginning a reading. Automix settings vary with assay type.
  - ◆ For Endpoint assays, enabling Automix shakes the plate for a definable number of seconds and then reads at all selected wavelengths.
  - ◆ For Kinetic assays, two types of Automix can be enabled. You can set Automix to shake the plate for a definable number of seconds before the initial reading or for a definable number of seconds before each subsequent reading.

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

## Temperature Regulation

The instrument regulates the temperature of the microplate reading chamber from 2°C above ambient to 45°C. On power up, when the incubator is off, the temperature in the reading chamber is ambient and isothermal. Turning on the incubator by pressing the **Temp on/off** key causes the instrument to begin warming the reading chamber and the fluidics module.



---

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

---

The temperature set point defaults to 37°C at startup. With the incubator on, the temperature of the reading chamber can be set and regulated from 2°C above ambient to 45°C. Accuracy of the temperature set point is only guaranteed if the set point is at least 2°C above ambient. If the temperature set point is lower than the ambient temperature, the chamber temperature remains at ambient. Temperature regulation is controlled by heaters only and, therefore, cannot cool the temperature to a setting lower than ambient.

Temperature regulation and control of the reading chamber is achieved through electric heaters, a fan, efficient insulation, and temperature sensors. The heaters are located within the instrument, which is insulated to maintain the temperature set point. The seven sensors are mounted inside the reading chamber and measure the air temperature and chamber temperature. The temperature feedback closed-loop control algorithms measure the chamber air temperature, compare it to the temperature set point, and use the difference to calculate the regulation of the heating cycles. This technique results in accurate, precise control of the reading 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.



---

**Note:** Temperature of samples in all assay plates are affected by evaporation.

---

## Time-Tagged Data

The FlexStation 3 instrument is a single-channel reading system. Although the scan time is very fast (8 wells in about 1.0 seconds; 1 well in about 50 ms), the difference in the exact time each well is read is dependent on the number of rows selected in a column. This difference is an important factor in fast kinetic assays.

For this reason, all readings are tagged with an exact read time, and when multiple-well fast kinetic responses are plotted, the curves overlaid each other as plotted by the SoftMax Pro Software. If kinetic data are to be exported, you can choose either time-interpolated data or raw time-tagged data. Molecular Devices recommends that you select time-interpolated data. This option is explained in more detail in the SoftMax Pro user guide.

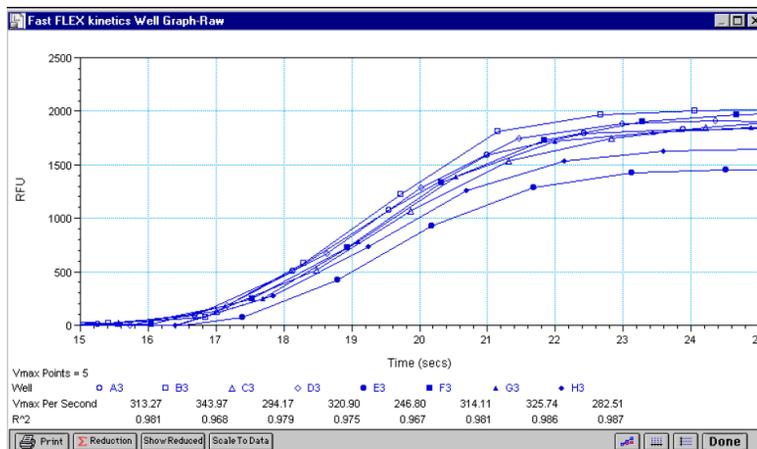


Figure 1-18: Time-Tagged Data Example

## Optical System

The instrument uses excitation and emission filter wheels to decrease interference by stray light, thus augmenting the wavelength selection that is provided by the monochromators. Two independent, single-channel reading heads can service top and bottom reading requirements. Both the top reading head and bottom reading head support coaxial excitation and emission beams.

The instrument's electrical, firmware, and optical designs incorporate many features that work together to virtually eliminate instrument-based day-to-day and instrument-to-instrument variations in measured fluorescence values.

For more detail of the optical design and an illustration of the optical system, see [Detection Module on page 26](#).

## Bottom and Top Reading

Switching to bottom or top reading capability is activated through software. No manual positional switching of the read-head is required. Bottom reading allows for well scanning ability maximizing the sampling area for 6-well, 12-well, 24-well, 48-well, 96-well, and 384-well microplates. In addition, bottom reading enables concurrent reagent addition to monitor fast kinetic reactions such as calcium mobilization. Availability of top or bottom reading functions varies depending on the detection mode.



---

**Note:** Clear-bottom plates must be used for bottom reading. Bottom reading is intended for cell-based assays.

---

## Assay Read Types

The instrument operates in three integrated fluidics/read (Flex, Endpoint, and Kinetic) read and two read types. The Table 1-1 on page 39 compares the types of operation and features that are available for the different read types.



---

**Note:** This user guide describes instrument behavior for Flex read type primarily. For instructions on other read types, see the SoftMax Pro user guide.

---

### Flex Reads

The fluidics module is designed to aspirate fluids from a compound source plate and dispense them into an 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.

In Flex reads, one to eight or one to sixteen wells in one column of the assay plate are read repeatedly for a selected total experimental time. At a preselected 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 preselected time intervals before and after each fluid transfer. After completion of reading the column (or partial column) for a preselected time, the instrument can repeat this cycle with other columns. All the data is collected in one data file represented as a 96-well or 384-well microplate.

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

$$\begin{aligned}\text{Run time} \times \text{Number of columns} &= \text{Plate time} \\ 2 \text{ minutes} \times 12 \text{ columns} &= 24 \text{ minutes}\end{aligned}$$

**Table 1-1:** Operation Modes and Features

Operation Modes	Endpoint	Kinetic	Spectrum	Well Scan	Flex
Operation Type	Fluidics + Detection	Fluidics + Detection	Detection	Detection	Fluidics + Detection
Read Modes	Absorbance, Fluorescence, Fluorescence Polarization, Luminescence, and Time-Resolved Fluorescence	Absorbance, Fluorescence, Fluorescence Polarization, Luminescence, and Time-Resolved Fluorescence	Absorbance, Fluorescence, Fluorescence Polarization, Luminescence, and Time-Resolved Fluorescence	Absorbance, Fluorescence, Luminescence, and Time-Resolved Fluorescence	Absorbance, Fluorescence, and Luminescence
<b>System Settings</b>					
Top read	Yes or No	Yes or No	Yes or No	Yes or No	No
Bottom read	Yes or No	Yes or No	Yes or No	Yes or No	Yes
Wavelength	Yes	Yes	Yes	Yes	Yes
Automix before	Yes	Yes	Yes	Yes	No
Automix between	No	Yes	No	No	No
Timing	No	Yes	No	No	Yes
Wells to read	Yes	Yes	Yes	Yes	Yes
AutoCalibrate	Yes	Yes	Yes	Yes	Yes
Compound source	Yes	Yes	No	No	Yes
Compound transfer	Yes	Yes	No	No	Yes
Triturate	Yes	Yes	No	No	Yes
Compound and tips columns	Yes	Yes	No	No	Yes
AutoRead	Yes	Yes	Yes	Yes	Yes
Well Scan Editor	No	No	No	Yes	No
PMT sensitivity	Yes	Yes	Yes	Yes	Yes

**Table 1-1:** Operation Modes and Features (cont'd)

Operation Modes	Endpoint	Kinetic	Spectrum	Well Scan	Flex
Assay plate type	Yes	Yes	Yes	Yes	Yes
Compound plate type	Yes	Yes	No	No	Yes

### Endpoint Reads

In Endpoint reads, as well as in Kinetic and Flex reads, you can select from one to four excitation/emission pairs to obtain readings for each well of a microplate.

In Endpoint reads, one to eight or one to sixteen wells in one column of the assay plate are delivered before starting the read. At a preselected point or points before the read, the pipettor can transfer up to three reagents from the compound plate to the assay plate. After reagents are transferred, the read initiates for the entire read area. Unlike Flex, the read area is not limited to one column at a time in an Endpoint read. All the data are collected in one data file represented as a 96-well or 384-well microplate.

For more information on this read type, please review the appropriate section in the SoftMax Pro user guide.

### Kinetic Reads

Kinetic analysis can be performed for a total run time of up to 99 hours. The kinetic read interval depends upon the instrument setup parameters selected in the SoftMax Pro Software, but is limited to 2 hours and 45 minutes (165 minutes). At the end of a reading, rates are reported as each well. Kinetic analysis has many advantages when determining the relative activity of an enzyme in different types of assays, including the purification and characterization of enzymes and enzyme conjugates.

In Kinetic reads, one to eight or one to sixteen wells in one column of the assay plate can be delivered before starting the read. At a preselected point or points before the read, the pipettor can transfer up to three reagents from the compound plate to the assay plate. After reagents are transferred, the read initiates for the entire read area. Unlike Flex, the read area is not limited to one column at a time in Kinetic reads. All the data are collected in one data file represented as a 96-well or 384-well microplate.

For more information on this read type, please review the appropriate section in the SoftMax Pro user guide.

## Spectrum Reads

Spectral analysis measures across a spectrum of wavelengths (excitation 250 nm to 850 nm, emission 360 nm to 850 nm). When reading using a specific detection mode, such as fluorescence, 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. All spectrum readings are made using scanning monochromators.

In Spectrum reads, the fluidics module is not enabled.

For more information on this read type, please review the appropriate section in the SoftMax Pro user guide.

## Well Scan Reads

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

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

In Well Scan reads, the fluidics module is not enabled.

For more information on this read type, please review the appropriate section in the SoftMax Pro user guide.

## SoftMax Pro Software

The Molecular Devices SoftMax Pro Software is a highly integrated program that can be used to:

- Control the reader
- Collect data
- Analyze data

SoftMax Pro software is easy to use, yet is powerful and flexible, and is necessary to access the full capabilities of the FlexStation 3 instrument.

SoftMax Pro Software allows you to:

- [Set Up the Reader and Software Parameters, see page 42](#)
- [Acquire Data from the Reader, see page 43](#)
- [Perform Complex Data Analysis, see page 44](#)

## Set Up the Reader and Software Parameters

- Read microplates in using Flex, Endpoint, Kinetic, Spectrum, or Well Scan read types.
  - ◆ Use up to four wavelengths for Flex, Endpoint, Kinetic, and Well Scan reads.
  - ◆ Perform absorbance and percent transmittance readings in the 200 nm to 1000 nm range.
  - ◆ Perform fluorescence readings in the 250 nm to 850 nm range.
  - ◆ Perform luminescence readings in two ways: wavelength nonspecific (all wavelengths between 360 nm and 630 nm) or wavelength selectable (250 nm to 850 nm).
  - ◆ Read the whole plate or a subset of microplate wells.
  - ◆ Specify kinetic run times up to 99 hours.
  - ◆ Select your own read intervals for kinetic runs.
  - ◆ Specify the duration for Automix before or between reads. Automix shakes the microplate at preset intervals, thereby mixing the contents of each well (highly recommended for ELISAs and other solid-phase, enzyme-mediated reactions).

---

**Note:** Automix is not intended to be used continuously for several hours.

---

- Use PathCheck technology to normalize the absorbance readings in each microplate well to a 1 cm pathlength.
- Design microplate templates to simplify data reduction.
  - ◆ Identify groups of wells with labels of your choice.
  - ◆ Identify individual wells with unique names.
  - ◆ Blank the entire plate, groups, or individual wells.
- Save reader settings, template formats, and data analysis parameters as assay protocol files and recall them for later use.
  - ◆ Rapid reader and analysis setup for repeated assays.
  - ◆ Uniform analysis for equivalent microplates.
- Turn the incubator on or off to control the temperature in the read plate drawer.

- Integrate fluid transfer with Endpoint, Kinetic, and Flex reads.
  - ◆ Transfer fluid during the experiment for fast kinetic fluorescence, luminescence, and absorbance assays.
  - ◆ Transfer fluid before the beginning of an experiment for endpoint and kinetic applications.
  - ◆ Define the reagent source and tip columns to be used for each fluid transfer.
  - ◆ Optimize dispense parameters by specifying the volume, height, and speed of addition.
  - ◆ Specify the number of strokes used during Trituration to mix the contents of the source and read plates. Trituration uses the pipettor to mix the contents of a well by aspirating and dispensing, for both the source and read plates.

## Acquire Data from the Reader

- Pre-read microplates.
- Analyze kinetic and spectrum data as it is collected.
- Save data files for in-depth analysis at a later time.
- Save multiple microplates with individual template and data analysis parameters in one or more experiments in a single data file.
- Display data on screen.
  - ◆ Raw values, reduced number, or raw values with reduced number.
  - ◆ Raw microplate data in a microplate format.
  - ◆ Ranged data as integers from 0 to 9 in a microplate format.
  - ◆ Threshold data as being above, below, or between set limits in a microplate format.
  - ◆ Grayscale data in eight shades of gray corresponding to high and low limits in a microplate format.
  - ◆ Kinetic or spectrum plots of all microplate wells.
  - ◆ Enlarge the display of individual well plots and overlay multiple well plots.

## Perform Complex Data Analysis

- Calculate maximum kinetic rates on non-linear data.
- Assign plate, group, or sample blanks.
- Customize data analysis for each group in the template.
- Create graphs with multiple plots.
- Pick from nine curve-fitting routines.
- Analyze unknown samples against a standard curve.
- Analyze and compare data within a plate, between plates, and between experiments.
- Customize your print formats.
  - ◆ Print all or individual sections of the data file.
  - ◆ Define and print a report containing only selected sections.
  - ◆ Customize the order of data file sections.
- Export data in tab-delimited ASCII format.

For complete information on the current SoftMax Pro Software, see the SoftMax Pro User Guide and Formula Reference Guide included with your FlexStation 3 instrument.

## User Interface

This section briefly presents the basic features of the SoftMax Pro Software user interface. More instructions regarding how to use the interface appear throughout these instructions during relevant steps. You can control the instrument by using either buttons and icons in the windows and along the tool bars, or by using the menus. You can use either your mouse or keystrokes to make selections.



**Note:** For complete details about the SoftMax Pro Software and user interface, refer to your SoftMax Pro User Guide.

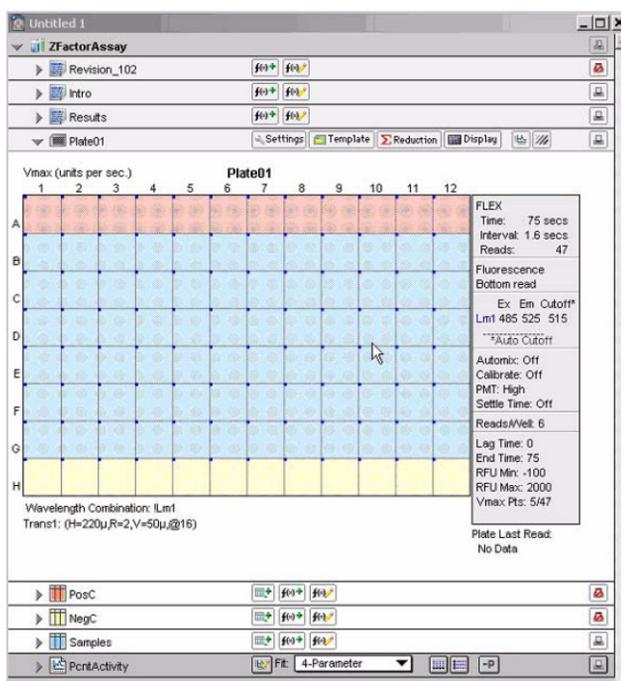


Figure 1-19: Plate Section, Flex Mode

The Status bar allows you to monitor instrument status and access several functions with the click of a button. You can verify communication with SoftMax Pro, and monitor the reading chamber temperature. The Status bar also provides buttons used to begin a reading, open the Incubator dialog box, shake the microplate (Automix), and open or close the instrument drawers. The Status bar can be hidden by selecting **Hide Status** from the View menu.



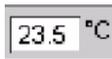
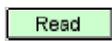
**Figure 1-20:** SoftMax Pro Software Status Bar

The following icons are present in the Status bar and are used to set up the instrument or interact with it during operation.



**Note:** Different Molecular Devices systems have different icons.

**Table 1-2:** SoftMax Pro Software Status Bar Icons

Icon	Description
	The Instrument Status icon provides visual confirmation that SoftMax Pro is communicating with the instrument. Double-click this icon to display the Preferences dialog.
	The temperature icon displays the current temperature inside the instrument. Click this icon to display the Preferences dialog.
	Click to begin reading. It changes to Stop during a reading. Clicking this button also closes any open drawers.
	Click the Incubator button to open the Incubator dialog to change temperature settings.
	Click the Automix button to manually shake the assay plate. <b>Note:</b> The manual shaking that occurs when you click this button differs from the Automix that can be selected as an instrument setting within the protocol settings.
	Click the Drawer button to open or close the reading chamber drawer.

One SoftMax Pro Software file contains at least one experiment, and can contain a section for Notes and one or more Plates. You can enter Notes and edit Plates using the tool bars shown below.



**Figure 1-21:** SoftMax Pro Software Plate Section Toolbar

The following icons appear on the Plate Section tool bar.

**Table 1-3:**

Icon	Description
	Double-click the Plate icon to open the Plate section in a new window.
Plate#1	Double-click the Name of Plate icon to open the Section dialog.
	Click the Settings button to open the Instrument Settings dialog for this plate.
	Click the Template icon to open the Template dialog, where you can create or edit the template. This is used to setup groups for defining areas of the assay plate.
	Click the Reduction icon to configure settings for data analysis and graph reduction.
	Click the Display icon to open the Display dialog and change your display properties.
	Click the Graph icon to enlarge sections of the display into graphic form.
	Click the Mask icon to mask selected wells.
	Click the Printer icon to include or exclude a section from a printed report.

The SoftMax Pro Software provides other icons and tool bars. For example, you can keep Notes on the experiment in the Notes section. Groups are also contained in experiments when you define a template. You can create Graph sections as desired. For details, see your SoftMax Pro Software User Guide.



This chapter provides information about how to install the FlexStation 3 instrument in your laboratory. Installation must be done by qualified Molecular Devices personnel or a service-trained user.



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**CAUTION!** The following procedures must be completed by a service-trained user. Do not attempt the installation procedures if you have not been trained properly by appropriate Molecular Devices personnel.

---

The following sections describe the installation procedure:

- [General Precautionary Information, see page 49](#)
- [Unpacking the System, see page 50](#)
- [Installing the Fluidics Module, see page 53](#)
- [Installing the Pipettor Head, see page 56](#)
- [Setting Up the Computer, see page 61](#)
- [Connecting the Cables, see page 61](#)
- [Installing the Drawer Adapters, see page 62](#)
- [Installing SoftMax Pro, see page 63](#)

## General Precautionary Information



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**WARNING!** Always make sure the power switch on the instrument is in the OFF position and remove the power cord from the back of the instrument before any installation or relocation of the system.

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**WARNING!** Do not install or operate the system in an environment where potentially damaging liquids or gases are present.

---



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

---



---

**CAUTION!** Do not attempt to assemble or disassemble the instrument with the pipette tips or compound and read plates in place. Spillage or damage to the pipette tips, plates, or the instrument can occur.

---

## Unpacking the System

This section provides instructions on how to unpack the system safely.



---

**WARNING!** The instrument weighs approximately 50 pounds and should be lifted with care. To prevent injury, use at least two people to lift the instrument.

---

Each FlexStation 3 instrument comes with the following components.

- Fluidics module and accessories
- Detection module (main instrument body) in housing
- Computer (can be user-supplied)
- Computer monitor (can be user-supplied)
- SoftMax Pro Software package

Please retain the cartons, all boxes, and any significant packing materials. If the system needs to be moved to a different location, use the original packing materials and cartons whenever possible. If the cartons have been damaged in transit, it is particularly important that you retain them for inspection by the carrier in case there has also been damage to the instrument.

As you unpack the system components, examine the packing list that accompanies the system to be sure all items are present.

## Unpacking the Fluidics Module and Accessories

1. Remove the fluidics module from the cardboard box inside the wooden crate and take it out of the protective bag. Set it in a safe place.
2. Remove the box (containing the pipettor head) and the bags of accessories.
3. Open the accessories bags and remove cables and the hex key. You will need them later in the assembly procedure.
4. Set packaging aside.

## Unpacking the Detection Module




---

**Note:** Keep the system in a location that is dedicated to its use, on a level surface, away from direct sunlight, dust, drafts, vibration, and moisture.

---

### Tools Needed

- Hex key, 3/32 inch ball drive, L (provided)
- Phillips screwdriver (not provided)

To unpack the detection module:

1. Use two people to unlatch the midsection of the crate (on top and bottom) and then move that midsection aside. Due to the size of the crate, this step requires two people.
2. Slide the plastic bag enclosing the instrument out of the way, around the base of the detection module.
3. Use two people to reach inside the bag and under the instrument and then lift the instrument out of its shipping tray and place it on the bench.




---

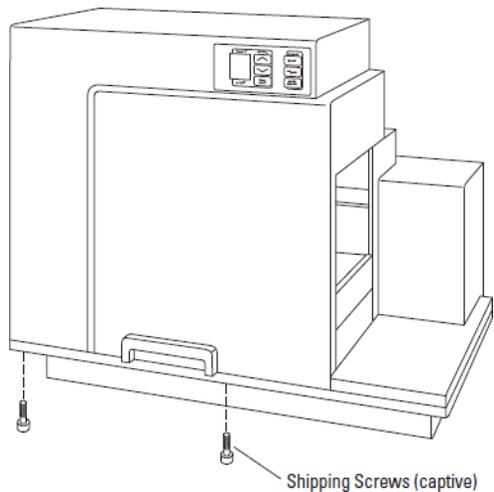
**WARNING!** The instrument weighs approximately 50 pounds and should be lifted with care. To prevent injury, use at least two people to lift the instrument.

---

4. Set the shipping materials out of the way.

## Removing the Shipping Screws

1. Locate the two stainless steel shipping screws under the front flange of the detection module that hold down the cover of the instrument.

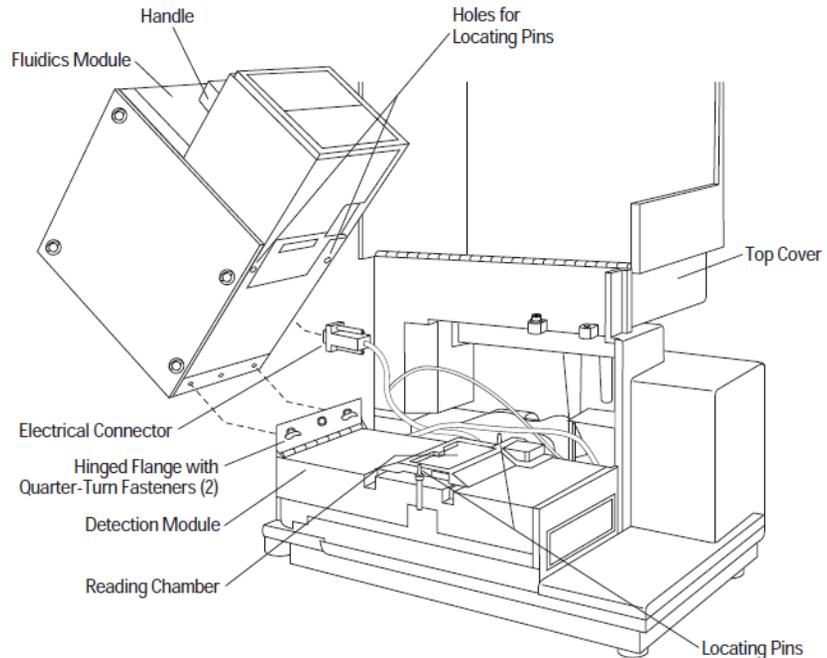


**Figure 2-1:** Location of Shipping Screws

2. Use the 3/32 inch hex key to unscrew the shipping screws. It might be necessary to move the instrument to the front of the bench to reach the screws from below. The screws remain attached to the base.
3. Press the latch in the handle and pivot the top cover up and back.

## Installing the Fluidics Module

After removing the shipping screws and opening the top cover, you can see the location for the fluidics module hardware. There is a hinged flange (metal plate) to the left side of the exposed reading chamber. There are also two quarter-turn fasteners (Zeus screws) attached to the flange, and two locating pins near the middle of the reading chamber.



**Figure 2-2:** Positioning the Fluidics Module



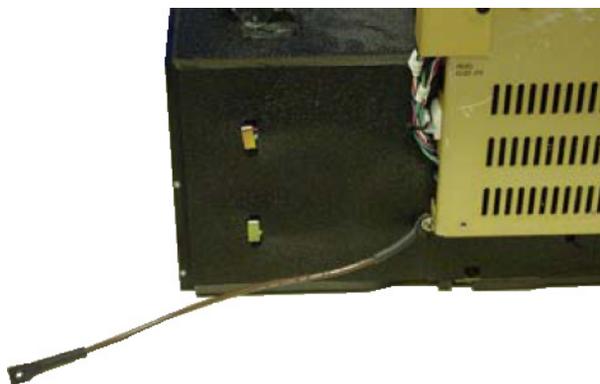
**CAUTION!** To avoid damage to the instrument, follow these instructions and any instruction labels on the instrument exactly.



**WARNING!** Do not remove cover until power is disconnected. Do not operate instrument unless all covers are in place.

To install the fluidics module:

1. Grasp the handle on the fluidics module and use a second person to help to lift the module into position over the detection module.
2. Tilt module up at about 90° and have one person hold it in position while the other person aligns the quarter-turn fasteners in the flange to the holes in the bottom of the fluidics module.
3. Connect the quarter-turn fasteners to the bottom of the fluidics module and lock the fasteners into place.
4. Attach the restraining cable from fluidics module to the right-most mounting tab on the top cover of FlexStation 3 instrument. The restraining cable comes attached to the fluidics module.



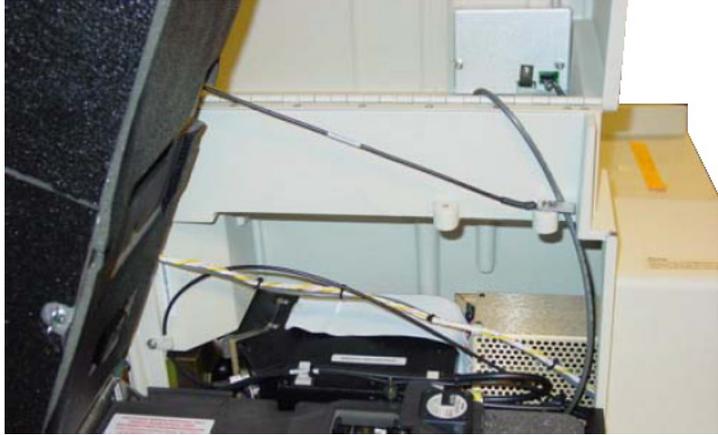
**Figure 2-3:** Restraining cable attached to the fluidics module

Attach the other end of the restraining cable the right-most mounting tab on the top cover.



**Figure 2-4:** Restraining cable attached to the top cover

A finished installation should look like Figure 2-5.



**Figure 2-5:** Restraining cable installation finished

The restraining cable for the fluidics module prevents the module from pivoting too far, and prevents the accidental detachment of the serial communication cable. The restraining cable also holds the module in the open position so that the instrument can be serviced without first detaching the communication or power cable.

5. With the fluidics module tilted back, connect the 15-pin sub-D electrical connector into the communication port on the far bottom edge of the fluidics module. It must be aligned properly to fit.




---

**Note:** The labels on the fluidics connector (**Fluidics Connector**) and near the communication port (**Connect Fluidics Here**) help identify these ports.

---



**Figure 2-6:** Fluidics connection labels

6. Press the connector in firmly.

7. Use your fingers to lightly tighten the two communication port thumbscrews.

Molecular Devices recommends tightening the thumbscrews on the Sub-D connector to firmly attach the communication and power cable to the connector on the fluidics module. This prevents the accidental disconnection of the cable from the fluidics module due to vibration of the FlexStation 3 instrument.

8. Ensure that all cables and wires are tucked out of the way.
9. Gently lower the fluidics module by the handle down over the detection module and onto the locating pins.



---

**CAUTION!** Be careful when lowering the fluidics module that you do not trap or compress any of the optical fibers coming up from the detection module.

---

10. Ensure that the fluidics module is firmly seated on the detection module.

## Installing the Pipettor Head

After you install the fluidics module, you can place the pipettor head into the fluidics module. Use this same procedure for both the 8-channel and 16-channel pipettor heads.

The barrels on the pipettor head require periodic cleaning to remove silicone lubricant, dust, and other miscellaneous contamination. See [Cleaning the Barrels on the Pipettor Head on page 117](#).



---

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

---

1. Remove the pipettor head from its carton.

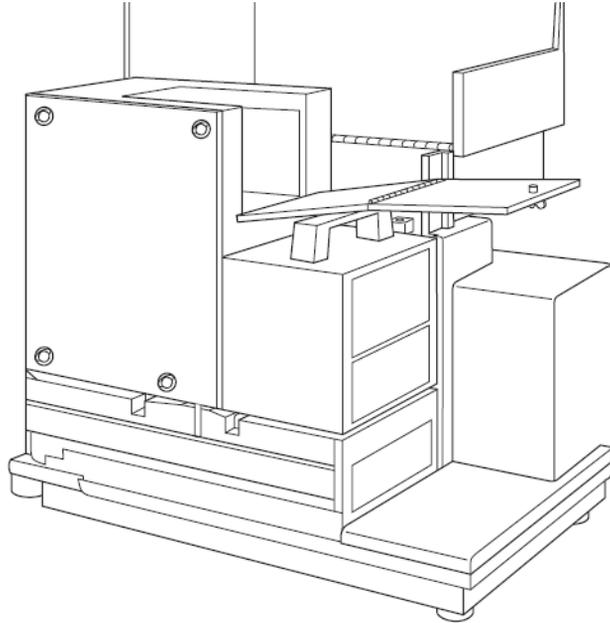


---

**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.

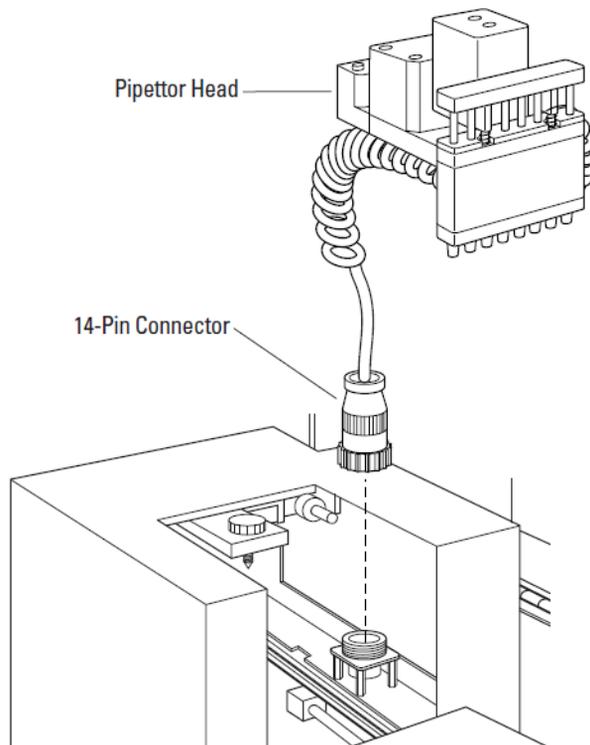
---

- 2 Turn the quarter-turn fastener on the inside top cover and then unfold the cover off the fluidics module.



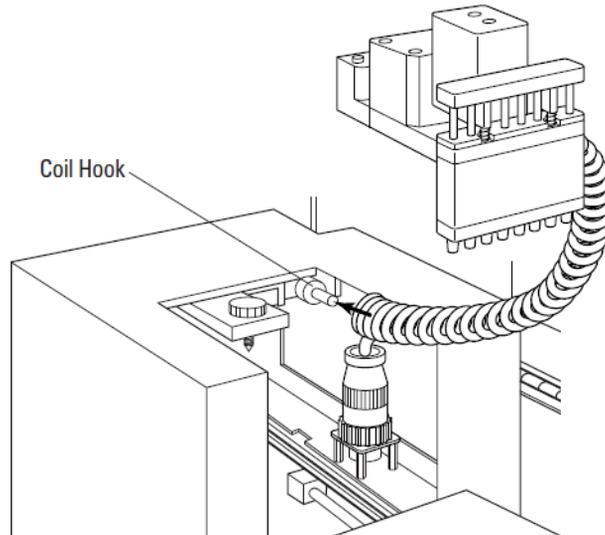
**Figure 2-7:** Opening the Inside Top Cover

3. Hold the pipettor head in one hand and the round, black, 14-pin connector in the other.
4. Move the pipettor head into its approximate position under the z-stage plate and red mounting knob.
5. Maneuver the cable down toward the back of the cavity and align the connector over the receptacle.
6. Press the connector in place and screw down the black outer collar over the pins.



**Figure 2-8:** Securing Spiral Cord to Hook

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



**Figure 2-9:** Positioning Pipettor Head

8. With one hand, pull up on the red knob.
9. With the other hand, align the metal plate at the back of the pipettor head, with the screw hole and the two locating pins, underneath the red knob.
10. Slide the plate up into place.
11. Screw down the red knob, securing the pipettor head so that it hangs in place from the black bar.



---

**CAUTION!** Tighten the red knob (the pipettor retaining nut) as firmly as possible.

---

12. Remove the cap at the completion of the pipettor installation.

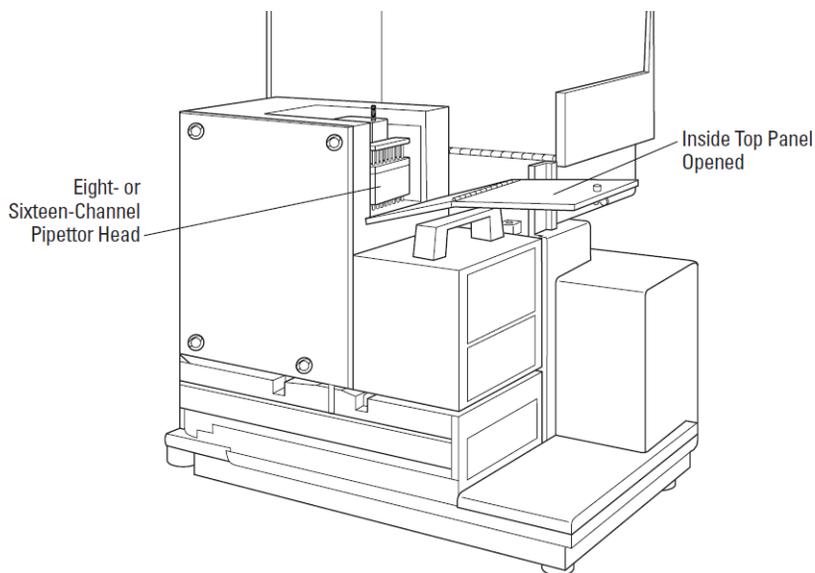


---

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

---

The pipettor head is now installed (see Figure 2-10).



**Figure 2-10:** Pipettor Head Installed

13. Fold the inside top panel back over the pipettor head and lock the quarter-turn screw in place.
14. Bring the top cover back over the fluidics module and snap it into place at the handle on the detection module. Make sure the latch clicks shut.

## Setting Up the Computer

Set up the computer and monitor, according to the instructions that come in their packaging. Place them close to the instrument on the bench.

The power cords for the computer and monitor are provided in the computer packaging. Connect them to the computer hardware as described in [Connecting the Cables on page 61](#). Do not connect the power cables to the power outlet at the wall.



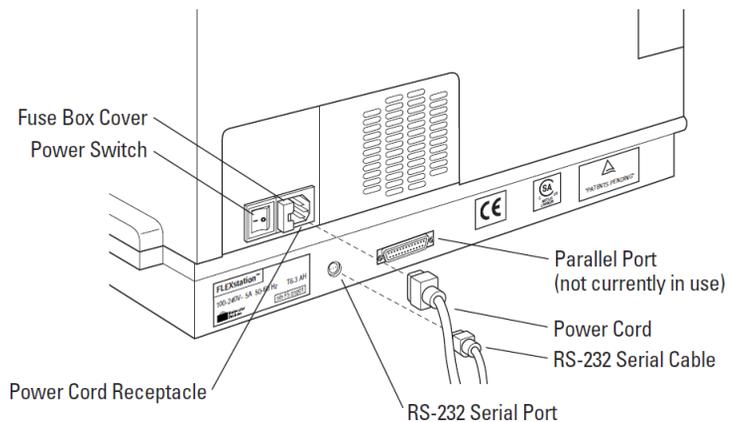
**CAUTION!** Do not attach the computer to a power outlet until after the computer and the instrument are connected.

## Connecting the Cables

After the instrument is assembled and the computer is set up, you can connect computer cable and the power cord.



**CAUTION!** Make sure that all assembly is completed before connecting the power cord to a power outlet.



**Figure 2-11:** Computer Cord and Power Cord Locations

1. Locate the instrument power cord (P/N 4400-0002) and the computer serial cable (P/N 9000-0149) in the FlexStation 3 instrument accessories kit.

2. Insert the 8-pin DIN round end of the serial cable into the RS-232 serial port receptacle on the back panel of the instrument.
3. Attach the other end to the COM serial port to the back of the computer.
4. Insert the female end of the power cord into the power receptacle at the rear of the instrument.
5. Connect the instrument power cord to a grounded power outlet of the appropriate voltage.  
Molecular Devices recommends that you use a surge protector between the power cord and the grounded power outlet.
6. Connect the computer hardware power cords to similarly grounded power outlets.



---

**CAUTION!** Make sure that no cables run beneath the instrument. Leave at least three inches between the back of the instrument and the nearest objects or surfaces to ensure proper ventilation and cooling.

---

## Installing the Drawer Adapters

The drawer adapters include the black microplate adapter and metal compound baseplate.

### Microplate Adapter Installation

To bottom read or top read a standard 96-well or 384-well microplate, you must install the black microplate adapter in the reading chamber drawer. The black adapter elevates the plate in the drawer.



---

**CAUTION!** Incorrect insertion or removal of the adapter can cause damage to the microplate drawer or to the pipettor head.

---

1. Turn the power to the instrument on.
2. Press the **Reading Chamber** button on the front panel. The reading chamber drawer opens.
3. Hold the adapter so that its cutout corner is facing the front left corner of the drawer, and then lower the adapter into the read plate drawer.

## Compound Baseplate Installation

The metal compound baseplate installs in the bottom of the compound plate drawer.

1. Turn on the power to the instrument.
2. Press the **Reagents** button on the front panel. The compound plate drawer opens.
3. Lower the baseplate into the compound drawer with its cutout corner facing the front left corner of the drawer.



---

**CAUTION!** Always remove any microplates and adapters from the instrument drawers before moving the instrument or before any service or maintenance procedures. Microplates and adapters can easily become jammed inside the instrument, causing damage. For instructions on removing adapters, see [Using the Microplate Adapters on page 120](#).

---

## Installing SoftMax Pro

Install the SoftMax Pro Software on the computer according to the instructions in the SoftMax Pro Software User Guide.



This chapter explains how to start up the system and how to use the control panel and the SoftMax Pro Software to configure instrument settings, define experiment protocols, and run an analysis, as described in the following sections.

- Overview, see page 66
- Starting Up the System, see page 67
- Setting the Temperature, see page 72
- Setting Up the Software, see page 75
- SoftMax Pro Software Parameters, see page 78
- SoftMax Pro Software Parameters for Fluid Transfer, see page 86
- Other Software Settings, see page 99
- Reading a Microplate, see page 99
- Shutting Down the System, see page 103
- Optimizing Fluorescence Assays, see page 103
- Optimizing Time-Resolved Fluorescence Assays, see page 110
- Optimizing Fluorescence Polarization Assays, see page 111
- Optimizing Luminescence Assays, see page 112



---

**Note:** The information in this chapter assumes that the instrument and computer are properly installed and connected. See [Installation on page 49](#).

---

## Overview

The following list provides an overview of the basic operating procedures required for using the system.

1. Turn on the power to the instrument and then the computer, if they are not already on, and then start the SoftMax Pro Software. See [Starting Up the System on page 67](#).
2. View the control panel and note the temperature inside the reading chamber, and use the control panel on the front of the instrument to turn on the incubator, if it is required by your experiment. It can take a while for the temperature to stabilize, so do this before configuring other instrument parameters. See [Setting the Temperature on page 72](#).



---

**Note:** Incubator settings can also be set using the SoftMax Pro Software.

---

3. Use the SoftMax Pro Software to configure the read mode, type of analysis, template, and so on, as desired. You can also create sections, such as Notes and Plates, as needed in the Experiment section of the software window. See [Setting Up the Software on page 75](#), [SoftMax Pro Software Parameters on page 78](#), [SoftMax Pro Software Parameters for Fluid Transfer on page 86](#), and [Other Software Settings on page 99](#).
4. Load the prepared pipette tip rack and microplates into their drawers. Use drawer adapters as needed. See [Loading Tips and Microplates on page 99](#).
5. Use the SoftMax Pro Software to start the reading. See [Starting the Reading on page 100](#).

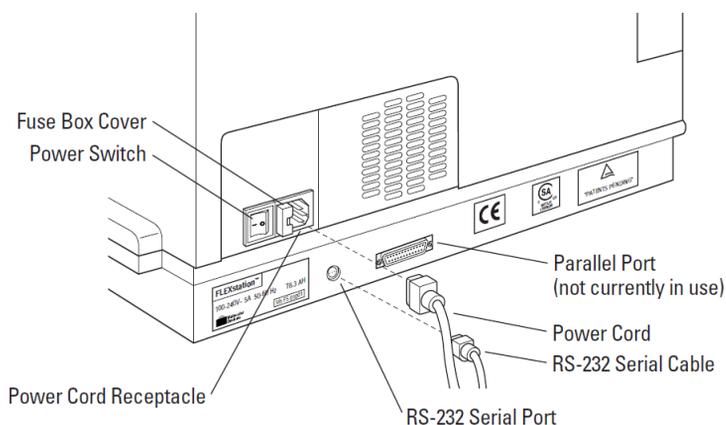
## Starting Up the System

Normally, you do not need to switch off power at the end of the day. If the system will not be used for more than a day, it is best to turn off the instrument. Use the following procedure only if the system has been switched off.



**Note:** These instructions assume that the SoftMax Pro Software has been installed and you are ready to begin an experiment. For software installation instructions, see the SoftMax Pro Software User Guide.

1. Locate the power switch on the back of the instrument.

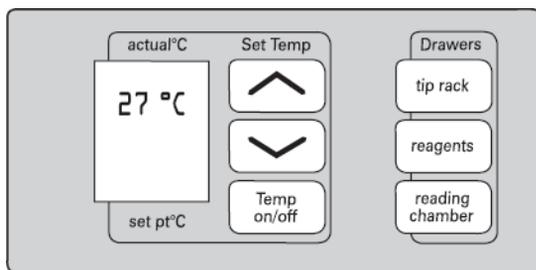


**Figure 3-1:** Power Switch Location

2. Press the rocker switch to the **ON** position (I).

The instrument automatically performs diagnostic checks to ensure that it is functioning correctly. All three drawers open and close. After about four minutes, the control panel displays the temperature inside the reading chamber. The reading chamber drawer automatically opens.

After about five minutes, the instrument is warmed up and ready.



**Figure 3-2:** Control Panel Ready



---

**Note:** No set point temperature appears at this time, since the incubator has not been turned on.

---

3. Turn on the host computer and allow Windows to start up.
4. Double-click the SoftMax Pro Software icon to start the program.

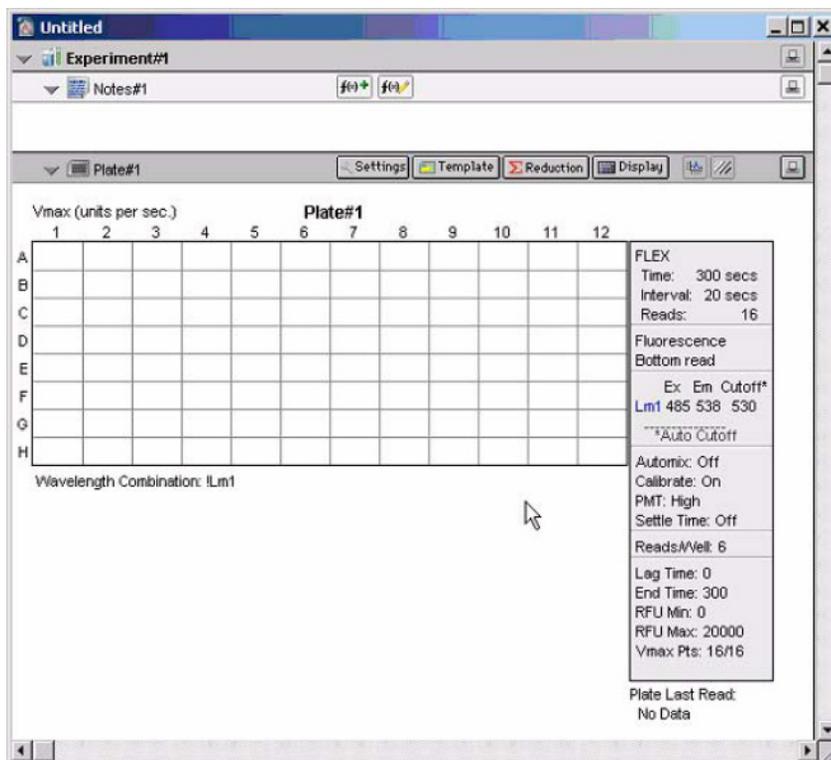


---

**Note:** If you get an error message while the software is starting up, see [Troubleshooting Procedures on page 129](#).

---

The SoftMax Pro Software opens with an Untitled window in Flex mode, with a default template selected.



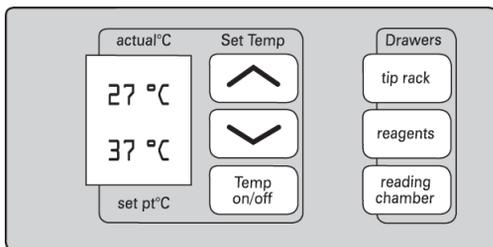
**Figure 3-3:** SoftMax Pro Software New Untitled Window

- For users new to SoftMax Pro Software:  
If you are new to SoftMax Pro Software, familiarize yourself with the software by reading about the default protocol, and running the tutorial described in this window. Also, refer to the SoftMax Pro Software User Guide.
- For users familiar with SoftMax Pro Software:  
If you are already familiar with SoftMax Pro Software, you can close the **Notes** section and open the **Plate** section. You are now ready to begin setting up your experiment protocols.

For information on adjusting software settings, see [Setting Up the Software on page 75](#).

## Using the Control Panel

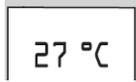
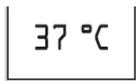
You can interact with the instrument by using the buttons on the instrument control panel. All control panel button functions can also be controlled in the software.



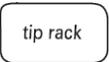
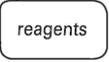
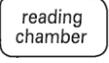
**Figure 3-4:** Detail of Control Panel with LCD

The following tables describe the indicators and buttons in the control panel.

**Table 3-1:** Indicators in the Control Panel

Indicator	Description
actual°C 	The <b>actual°C</b> provides the actual temperature inside the reading chamber at any given time.
 set pt°C	The <b>set pt°C</b> provides the set point temperature you select for the current experiment. This number is displayed only when the incubator is on.

**Table 3-2:** Buttons in the Control Panel

Button	Description
	the <b>Temp on/off</b> button enables or disables the incubator. When the incubator is on, both the set temperature and measured internal temperature are shown on the front panel LCD display.
 	The arrow buttons allow you to enter a set point for the temperature in the instrument reading chamber. Press these buttons to adjust the temperature up or down, starting at the previous temperature setting, or the default of 37°C if no setting has been made. Press either arrow once to increase or decrease the temperature shown in the display by an increment of 0.1°C; press and hold to scroll the temperature.
	The <b>tip rack</b> button opens or closes the tip rack drawer.
	The <b>reagents</b> button opens or closes the compound plate drawer.
	The <b>reading chamber</b> button opens or closes the reading chamber drawer. Whether or not the drawer remains open depends on the incubator setting. <ul style="list-style-type: none"> <li>• If the incubator is off, the drawer remains open.</li> <li>• If the incubator is on, the drawer closes after approximately 10 seconds to assist in maintaining temperature control within the microplate chamber.</li> </ul> <hr/> <p><b>Note:</b> If the feature is selected in Instrument Setup, the drawer remains closed after the assay plate reading.</p>

## Setting the Temperature

If you want an elevated temperature within the instrument for your experiment, turn on the incubator at least 30 minutes before you plan to start plate reading. Up to 30 minutes can be required for the temperature within the chamber to reach the set point. Turning on the incubator and choosing a temperature set point can be done using the software or the front panel of the instrument.

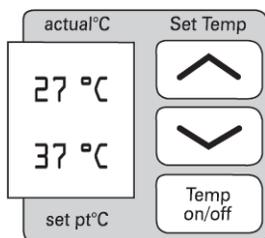
The instrument will operate with a reading chamber temperature from 2°C above ambient to 45°C. The temperature cannot be regulated at a set point that is outside this range.



**Note:** It is possible to enter a temperature setting that is outside the operational range, either with the software or with the control panel. However, the instrument will not respond with a temperature outside the allowable range.

## Displaying the Temperature

Two temperatures are displayed in the LCD on the control panel.

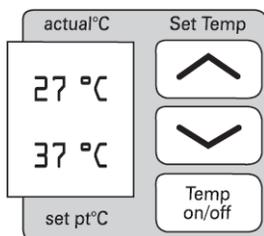


**Figure 3-5:** Temperature Control and Display

The upper reading is the temperature measured inside the reading chamber. When the incubator is off, this upper number is the ambient temperature. The lower reading is the set point, that is, the temperature you desire for the current experiment, and it is displayed only when the incubator is enabled.

## Setting the Temperature with the Control Panel

To enable the incubator, press the **Temp on/off** button on the control panel. The display indicates that temperature control is on by displaying numbers in the lower half of the LCD. The instrument sets the reading chamber to the default temperature, 37°C.



**Figure 3-6:** Temperature Control Enabled

To change the temperature set point, press the up or down arrow keys until the desired temperature set point appears on the display.

The reading chamber temperature is maintained at the set point until you disable temperature control by touching the **Temp on/off** button again. When the incubator is off, the temperature within the reading chamber returns to ambient.



---

**Note:** If the power is shut off to the instrument for any reason, you need to turn on the incubator again and allow sufficient time (at least 10 minutes) for the control algorithm to fully stabilize the reading chamber temperature.

---

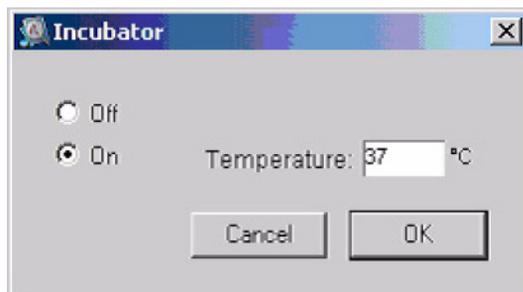
## Setting the Temperature with SoftMax Pro Software

You can turn on the incubator with software by selecting **Incubator** from the **Control** menu. You can also click the **Incubator** button on the instrument Status bar.



**Figure 3-7:** Incubator Button

The Incubator dialog appears.



**Figure 3-8:** Incubator Dialog

You can leave the temperature setting at the default value or you can type a different value into the **Temperature** field.



---

**Note:** The incubator setting is independent of the protocol being run. Running an experiment does not automatically select the temperature set point. After a reading, the temperature set point, range, and average actual temperature are recorded in the saved file.

---

## Setting Up the Software

Use the following procedure to check the instrument status and settings.

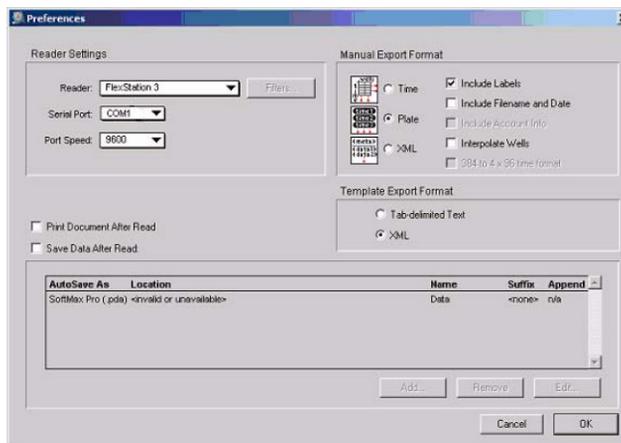
1. Observe the Instrument Status icon in the left corner of the SoftMax Pro Software Status bar. The icon is purple when the SoftMax Pro Software correctly recognizes the instrument.
2. Observe the temperature displayed in the Temperature display field.



**Figure 3-9:** Instrument Status Bar

If there is a red **X** in front of the Instrument Status icon, if there is no temperature in the Temperature display field, or if you see other problems, you might need to adjust instrument preferences.

- ◆ To adjust instrument preferences, follow [Step 3](#) through [Step 5](#).
  - ◆ Otherwise, skip to [Step 6](#).
3. Click **Edit > Preferences** or double-click on the instrument icon. The **Preferences** dialog appears.



**Figure 3-10:** Preferences Dialog

4. Make sure that the serial port setting agrees with the actual port the computer cable (RS-232 cable) is connected to. This is generally Com1.

5. Make sure that the serial comm speed is 9600 Baud.



**Note:** After you read an assay plate in Flex mode, the serial communication speed changes to 57600 Baud.

If you have correctly configured the settings in the Preferences dialog as described in [Step 3](#) through [Step 5](#) and you are still observing problems (a red 'X' over the Flex icon, no temperature in the temperature display box, or other problems), then you must take further steps to establish communication between the computer and the instrument, or to resolve a different instrument problem. See [Troubleshooting Procedures on page 129](#).

6. The SoftMax Pro application defaults to Flex mode every time you start the software. You can confirm this mode, if desired, by clicking **Control > Instrument Setup** or click the **Settings** button on the Plate section tool bar to view the Instrument Settings screen.



Figure 3-11: Settings Button

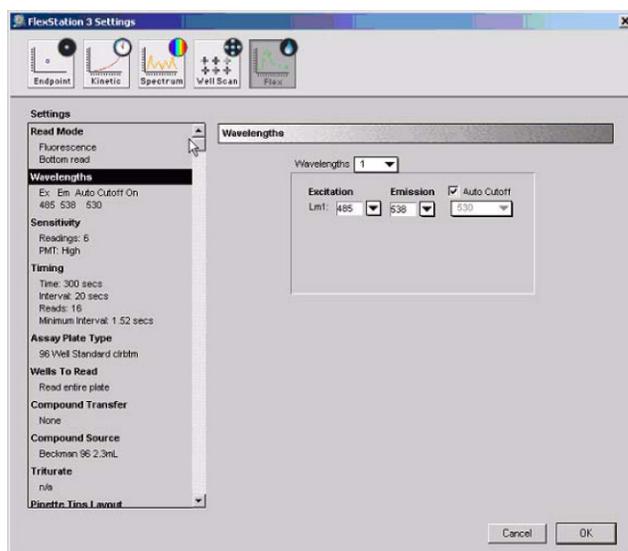


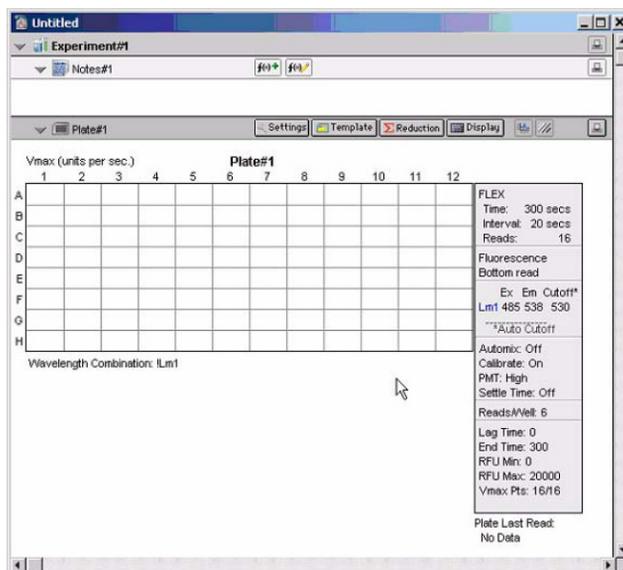
Figure 3-12: Instrument Settings Dialog

7. Make sure that the **Flex** button on the right is selected.



**Note:** You can select a different read type from the Instrument Settings screen by clicking on one of the other four buttons at the top of the window. The rest of these instructions assume you are remaining in Flex mode.

8. Click **OK** to return to the Untitled window.



**Figure 3-13:** Untitled Window in Flex Mode

9. Before continuing with other software settings, create or edit the Plate sections you will need. To create more Plate sections, click **Experiment > New Plate**.

If you want to create a plate section with settings identical to a particular existing plate section, select that plate section and then click **Edit > Duplicate**.

Double click on the word **Plate** in the Plate Section tool bar to open a dialog to name the Plate section. See the SoftMax Pro Software User Guide.

## SoftMax Pro Software Parameters

In addition to the settings discussed in [Setting Up the Software on page 75](#), there are several other parameters to select when reading a microplate. Listed under **Setup** in the SoftMax Pro Software, these reader parameters can and should be adjusted to get the best data from your particular assay. Some of the parameters are discussed here in the FlexStation 3 instrument User Guide, and they are discussed in more detail in the SoftMax Pro Software User Guide. All possible combinations of type, mode, and parameter are not presented here, but Molecular Devices technical support is always available to help you achieve the best possible results.

Refer to the following sections for considerations when setting up for a specific read.

- [Read Types, see page 79](#)
- [Read Modes, see page 79](#)
- [Wavelengths, see page 80](#)
- [Sensitivity, see page 80](#)
- [Timing, see page 81](#)
- [Assay Plate Type, see page 82](#)
- [Wells to Read, see page 83](#)
- [Automix, see page 84](#)
- [AutoCalibrate, see page 85](#)
- [Settling Time, see page 85](#)
- [AutoRead, see page 85](#)

## Read Types

Choose from five read types. The reading can occur in a each well of a microplate unless otherwise noted.

- **Flex:** Data is collected over a specified period of time at regular intervals and uses integrated fluid transfer to delivery reagents while the wells are being read.
- **Endpoint:** A single reading is taken. Fluid transfer can occur only before the read.
- **Kinetic:** Data is collected over a specified period of time at regular intervals and fluid transfer can occur only before the first read.
- **Spectrum:** The scanning monochromator is used to take readings of the same sample at many different wavelengths within the range specified. No fluid integrated transfer is available.
- **Well Scan:** Multiple readings are taken in different areas of each well in a 6-well to 96-well microplate. No integrated fluid transfer is available.

## Read Modes

The detection modes available for each experiment will vary depending on the read type selected. Select from up to five possible Read Modes:

- Fluorescence (RFUs)
- Absorbance (ODs)
- Luminescence (RLUs)
- Time-Resolved Fluorescence (RFUs)
- Fluorescence Polarization (RFUs)

If bottom reading is possible for the selected read mode, a check box appears in the window that says **Well Bottom Read**. If you want to read through the bottom of a clear-bottom plate, select the **Well Bottom Read** check box.

## Wavelengths

Up to four wavelengths can be read per well. Although the arrows open a window showing the six most commonly-used wavelengths, the tunable monochromator allows any excitation or emission wavelength to be entered between a certain range for the particular read mode. In addition to an emission monochromator, fluorescent-quality cutoff filters help reduce background signal.

You can either manually choose from 15 emission filters or allow the software to choose which long pass emission filter to use based on the excitation wavelength. See [Table 3-4: Emission Cutoff Filter Default Settings on page 109](#).

## Sensitivity

Adjust sensitivity either by varying the number of readings that are performed in each well or by changing the PMT sensitivity (voltage). In general, a higher number of readings results in better reader precision. In fluorescence intensity reading, the sample can photobleach if a high number of flashes are used, so if the plate will be re-read (especially for kinetic assays or multiwavelength reads), Molecular Devices recommends using 10 readings or fewer. During Flex mode, Molecular Devices recommends that you begin with 6 readings to supply enough light for these fast kinetic assays. For low-signal endpoint assays, such as some FP or TRF assays, 100 readings per well can be optimal.

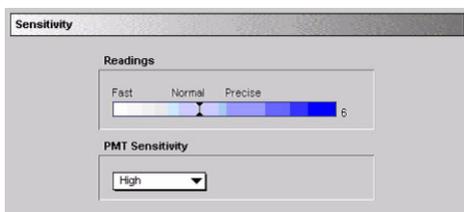
The Automatic PMT sensitivity setting allows the reader to adjust the PMT voltage automatically for varying signals coming from samples on the plate. In modes that allow manual setting of the PMT, a setting of High is best for low signal assays, including assays that require Flex mode. The PMT sensitivity is not adjustable for all read types, including Kinetic and Flex reads.



---

**Note:** Increasing the number of reads also increases the total read time.

---



**Figure 3-14:** Sensitivity Settings

The photomultiplier tube (PMT) is a photon detector that detects light, through the use of photoemission and successive instances of secondary emission, to produce enough electrons to generate a strong signal.

## Timing

The Timing setting depends on several settings which appear after it in the list. To obtain the minimum read interval, you must return to Timing after completing all the settings.

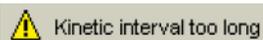
To adjust the Timing for readings, enter the total run time and the time interval between readings.

**Figure 3-15:** Timing Settings

To change the default values, click in the appropriate box and then type the desired run time or interval. The acceptable run time range is from 0 seconds to 1000 seconds.

After you have entered the values, the total Number of Reads calculated by the instrument is displayed automatically. Depending on what sensitivity you have selected in the sensitivity settings, the Minimum Interval and the Minimum Run Time automatically adjusts. The Minimum Interval is also dependent on the Compound Transfer settings and the number of wells to be read per column.

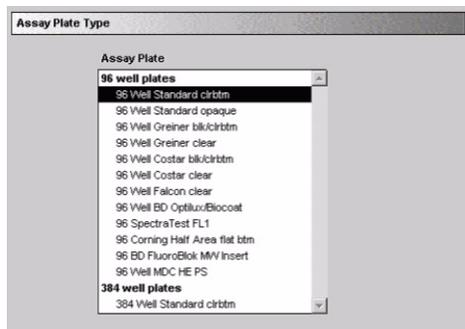
If you select a time that is out of range or an inappropriate interval, an error message appears at the bottom of the window.



**Figure 3-16:** Timing Error Message

## Assay Plate Type

Assay Plate Type determines the alignment of the read head with respect to the microplate. For 384-well microplates, it is important that the correct assay plate be chosen to match the microplate that the reader will read. If the microplate you are using is not listed in the selection, a new plate type can be added under the Plate menu option **Edit Plate Type**.



**Figure 3-17:** Assay Plate Types

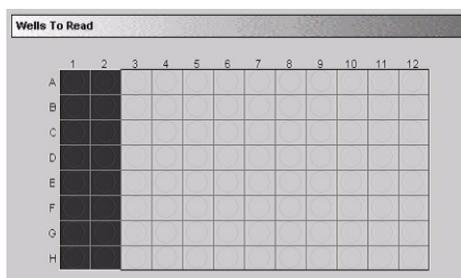


**Note:** If you change the Assay Plate Type setting for a Plate section, the well assignments stored in the previous template are discarded. The previously created groups remain, however, so that you can select new wells and apply existing groups to them. The Assay Plate Type setting takes precedence over all other fluidics module settings and affect the correctness of other settings.

If you go back and change this setting after you have selected settings that follow (for example, Compound Source, Compound and Tip Columns) the earlier settings might be automatically reconfigured to reflect the new assay plate type and well layout. Be sure to check any earlier assignments to ensure they remain correct.

## Wells to Read

You can choose which plate wells to read in your experiment. You can choose a combination of wells, from one well only to all the wells in a plate. Partial-plate reading can significantly reduce the time required for certain types of readings because the instrument does not have to read the entire plate.



**Figure 3-18:** Wells to Read Setting

Select the wells you want read by dragging the pointer. Wells must be contiguous, and in a rectangular arrangement, but do not need to start in the column 1. You can choose a partial row or column, or a single well.

When planning your experiment, remember that the instrument makes fluid transfers and readings one column at a time. You might want to use partial rows (A through H) rather than partial columns (1 through 12 or 1 through 24) for most situations.

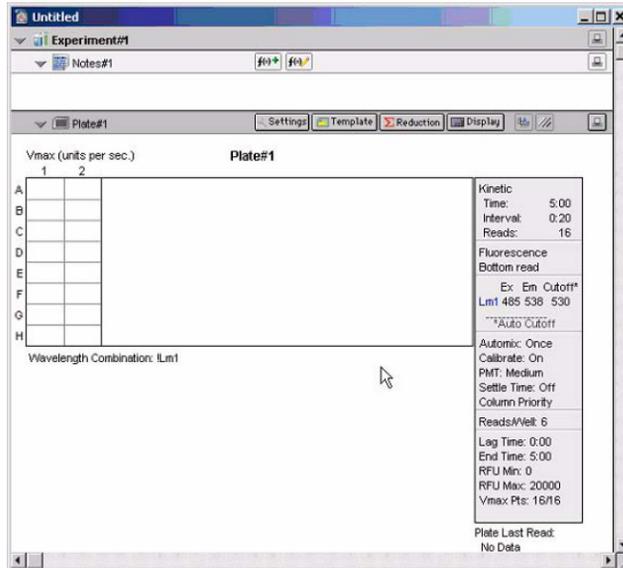



---

**Note:** The selected wells must be contiguous and in a rectangular arrangement.

---

If you select a partial-plate for reading, only those wells selected will be visible in the data display. In the figure below, the Plate section reflects that wells A1 through H2 have been selected for reading.



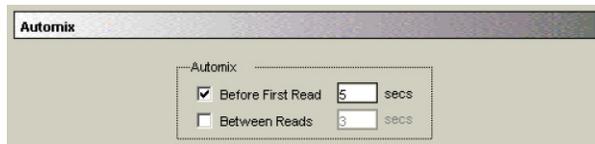
**Figure 3-19:** Plate Section with Selected Wells to Read

## Automix

The Automix function is a patented feature that allows you to set automated shaking of the microplate before and between readings.

Select **Before First Read** to shake the microplate before the first wavelength reading only. You can also set Automix to shake the plate **Between Reads**.

Select the check box next to the type of shaking you want and then type a value in the field on the right to indicate how long shaking should last. Automix time can last from 1 second to 999 seconds.



**Figure 3-20:** Automix Settings

## AutoCalibrate

This AutoCalibrate check box allows you to disable or enable automatic calibration. The default is enabled. Turn automatic calibration off to allow the instrument to begin or complete readings more quickly.

The instrument maintains the most recent automatic calibration settings in memory (NVRAM) until another automatic calibration is performed.



**Figure 3-21:** AutoCalibrate Selection



---

**Note:** Allow the instrument to perform an automatic calibration of at least one microplate before you disable this function.

---

## Settling Time

Settling Time is the delay time between reader motion and initiation of reading. In Fluorescence Polarization, the settling time happens before each well is read. However, in all other read modes, the settling time happens only after the microplate has been moved to begin the read of another column. This delay allows time for the meniscus motion to cease, potentially improving precision, especially in the low-density, high-volume microplates with fewer than 96 wells.

## AutoRead

AutoRead, if checked, causes the reader to read the same microplate over and over, without user intervention. Several different Plate sections, with the same or different setups, can be defined in one experiment protocol. Starting the read in the first protocol results in the microplate being read until it reaches the last Plate section, after which it automatically stops.



---

**CAUTION!** To prevent data loss, make sure that you turn off the AutoRead function for the last Plate section.

---

## SoftMax Pro Software Parameters for Fluid Transfer

In addition to defining the parameters to be used during a read, the FlexStation 3 instrument includes distinct parameters only available for use with the integrated pipettor. It is essential that settings for the fluidics operations are correct and correlate with one another. In particular, layouts and settings for Assay Plate Type, Wells to Read, Compound Source, Compound Transfer, and Compound & Tip Columns must correlate.

The SoftMax Pro Software cross-checks these settings as you move through the configuration windows, and after you click **OK** to close the Instrument Settings dialog. If settings do not correlate, an error message appears, and you must correct the settings before continuing.

The following sections describe the available fluid-transfer settings.

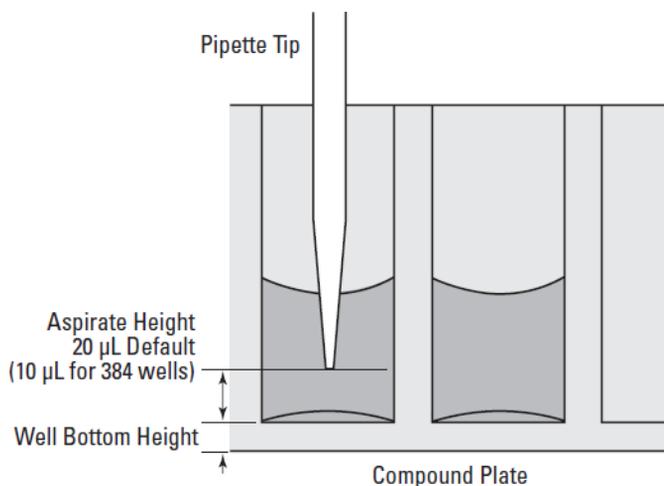
- [Compound Source, see page 87](#)
- [Compound Transfer, see page 88](#)
- [Triturate Selection, see page 92](#)
- [Compound and Tip Columns, see page 93](#)
- [Pipette Tip Air Gap, see page 97](#)

## Compound Source

This setting allows you to select a compound source plate. Compound plates store fluids that are aspirated (withdrawn from the compound plate) and then injected (dispensed) into the assay plate during the run.

Make sure that you select a plate type that matches the type and well configuration of the actual compound plate you are using and the number of wells selected in the Assay Plate Type setting.

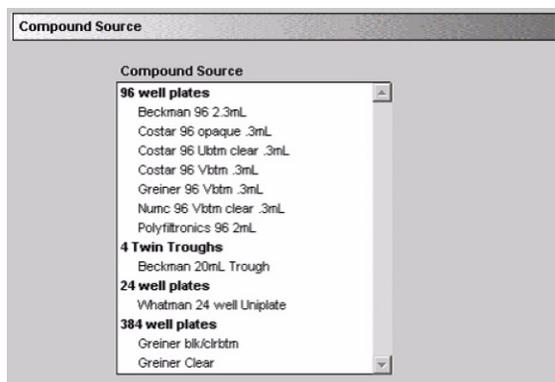
In particular, the well bottom height is different for different types of plates, and selecting the plate type correctly is important to prevent jamming pipette tips into the bottom of the well. The instrument assumes a 20  $\mu\text{L}$  pipette height when aspirating from a 96-well compound plate.



**Figure 3-22:** Well Bottom Height



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

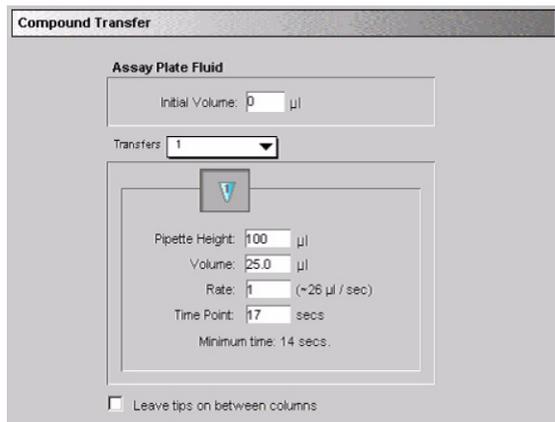


**Figure 3-23:** Compound Source Settings

## Compound Transfer

Compound Transfer is an important setting to configure correctly. In addition to configuring precise fluid transfers for your experiments, this setting also helps prevent flooding of the assay plate.

The fields in this dialog allow you to set volumes for up to three transfers. However, you must be careful to keep in mind the actual maximum volume allowed in the wells you are using as you move through the settings. The maximum cumulative volume depends on the assay plate type you select.



**Figure 3-24:** Compound Transfer Settings

## Assay Plate Fluid Initial Volume

Type a value in the **Initial Fluid** field that equals the largest initial volume before compound transfers for any well in the assay plate. For example, this value can be set from 0  $\mu\text{L}$  to 269  $\mu\text{L}$ , although typical values are about 10  $\mu\text{L}$  to 200  $\mu\text{L}$  for a 96-well microplate.

The SoftMax Pro Software assumes all wells hold the same initial volume. The software uses this value to compute the total volume in each well after all fluids have been dispensed. The software makes this calculation to warn you in the Compound and Tip Columns setting of the potential for overflow of fluid from the wells.

If there is no fluid volume in the assay plate before compound transfers, do not type a value in this field.

Any value entered is saved with the file but the value is not displayed anywhere except in the Compound Transfer setting.

Values for a 384-well microplate can be set from 0  $\mu\text{L}$  to 120  $\mu\text{L}$ .

## Transfers

You can enable up to three compound transfers in a single well during a run time. The default value for the number of transfers is one (1). As you enable transfers, color-coded transfer buttons appear next to the **Transfers** field.

When you select one of the **Transfer** buttons, that button appear with a darker gray background. You can then enter the parameters for that particular transfer in the **Transfer Settings** portion of this dialog.



---

**Note:** There are no other indications in the Transfer Settings for which transfer you are configuring. Pay close attention to which transfer settings you are modifying.

---

## Transfer Settings

- **Pipette Height:** (1  $\mu\text{L}$  to 999  $\mu\text{L}$ ) This setting determines the volume of fluid (in microliters, measured from the bottom of the assay plate microplate well) above which the tip of the pipette will remain during the dispensing portion of the transfer event. This setting helps ensure that the tip of the pipette is below the surface of the liquid at the end of the transfer, minimizing the possibility that undispensed drops remain on the tips.



---

**Note:** As you configure subsequent transfers you must calculate the amount of fluid added and set the pipette height accordingly.

---

- **Rate:** (1 to 8) This setting determines the rate at which the fluid is dispensed into the well of the assay microplate. In a 96-well microplate, a setting of 1 is equal to 26  $\mu\text{L}$  per second and each subsequent number increases in increments of 26 microliters. Therefore, a setting of 2 is equal to 52  $\mu\text{L}$  per second. A setting of 3 or 4 can help minimize cell damage or dislodgment. In 384-well microplates, the speed is set to 6  $\mu\text{L}$  per second and increases in increments of 6  $\mu\text{L}$ .



---

**Note:** For non-contact dispensing, use a rate of 8 to ensure all liquid is dispensed from pipette tip.

---

- **Volume:** During Flex mode, this setting determines the volume of material to be dispensed from the source to each individual well chosen to receive that transfer.
  - ◆ For a 96-well microplate, the range is 1  $\mu\text{L}$  to 200  $\mu\text{L}$ .
  - ◆ For a 384-well microplate, 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.

---

- **Time Point:** (Minimum Time to 9999 seconds) This setting determines the time after the start of the reading when the fluid is scheduled to begin being dispensed. Your time point will eventually be your baseline time. This is not the time interval between transfers. In Endpoint and Kinetic reads, Time Point reflects the time at which the first dispense occurs. The Endpoint or Kinetic read begins after all transfers are made.




---

**Note:** The Time Point cannot be smaller than the Minimum Time identified by the software for each transfer.

---

- **Minimum Time:** This information line automatically 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, trituration, and Automix.

The minimum time for the second pipetting event depends on when the first pipetting event occurred. 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.

### Possible Problems

Time point calculations are based on the number of wells read, the tip column and compound column used during transfer. If you select time points that are not long enough (incompatible with the selected volumes and transfer speed), the system notifies you that the time point needs to be increased.

If you select transfer volumes that add up to more than the maximum that can be accommodated by the assay plate, an overflow warning appears at the bottom of the settings screen indicating you have exceeded the volume limits of the read plate.

Pay attention to the minimum time value displayed under the time point field for each transfer, as the minimum time values are different depending on which transfer you are configuring.

The Compound Transfer setting works in conjunction with the Compound & Tip Columns setting. Until well assignments are made in the Compounds & Tip Columns setting, the Compound Transfer setting displays **No targets assigned**.

## Triturate Selection



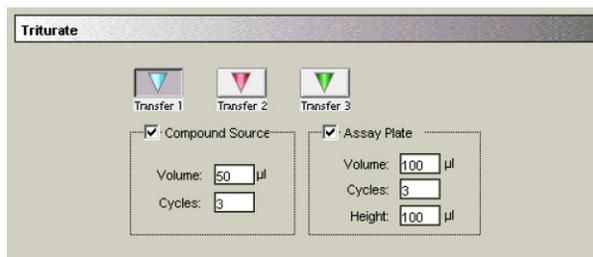
**Note:** The choices shown in this section are based on the number of compound transfers you specified in the Compound Transfer settings. If no transfers are enabled, no triturate settings are applicable.

Trituration is mixing of the contents of the wells in either or both the compound source and assay plates. Trituration is accomplished by fluid being alternately aspirated from and dispensed back into a well. Applications for which trituration is recommended include when compound in the source plate needs to be resuspended before addition, transferring low volumes to the read plate, or when transferring fluid before or during an absorbance read to ensure that there is proper mixing of the well contents.

Select the transfer (if more than one is enabled) during which you wish to perform trituration, and then select the check box for either **Compound Source** or **Assay Plate**, or both.

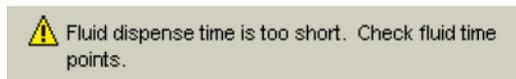
You can set the **Volume** of fluid to be withdrawn from the well and the number of times (Cycles) to be aspirated and dispensed into that well.

In addition, for assay plates, you must also enter a value for the **Height** at which the trituration occurs. The height setting should take into account the volume selected, so that the tips remain below the liquid surface and do not draw air.



**Figure 3-25:** Triturate Settings

If you choose dispense times that are incompatible with the settings, an error message appears.



**Figure 3-26:** Triturate Error Message

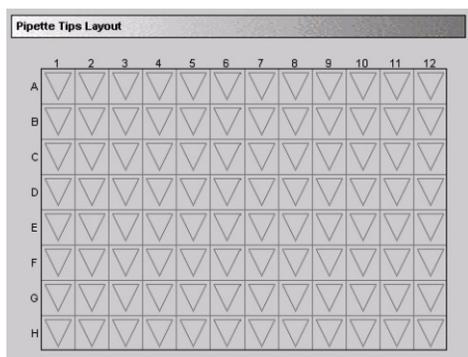
To modify the dispensing time, return to the Transfer Settings in the Compound Transfer settings.

## Pipette Tips Layout

The Pipette Tips Layout setting displays the format of tips, 96-well or 384-well, that need to be used for the respective Assay Plate Type that is chosen.



**CAUTION!** Molecular Devices recommends using a full rack of tips each time you perform a fluid transfer to ensure proper pipetting. If you mistakenly enable a pipetting function from a tip that is not present, the instrument can malfunction, potentially causing serious damage.



**Figure 3-27:** Pipette Tips Layout Settings

## Compound and Tip Columns

The choices for these settings depend upon the number of transfers chosen in the Compound Transfer window. When one or more transfers are enabled, these settings allow you to choose the tips and compounds to be used for transfers.

The settings are either automatically assigned by the software, or you can manually assign them into any configuration you require.

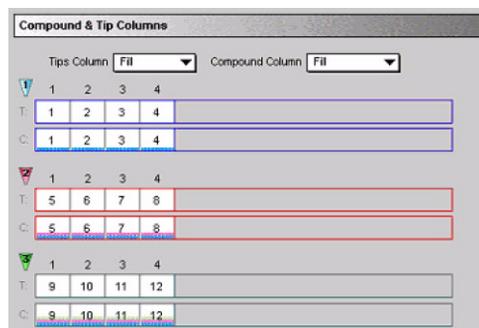
## Compound and Tip Columns Automatically Assigned

The SoftMax Pro Software automatically enters information in the Compound & Tip Columns window taking into consideration the number of transfers and the Wells to Read section already selected. The software assumes fluid will be aspirated from the compound plate starting with the first available column. Aspirated fluid will then be dispensed to the first available column indicated in the Wells to Read selection.

The software assumes the following conditions:

- All columns in the Wells to Read selection will receive fluid.
- The fluids are transferred from left to right. The read-transfer-read sequence in each column is initiated only after the previous column's read-transfer-read event is completed (the total read time for that column).
- The fluid transfer targets are cumulative from transfer to transfer. That is, the second transfer's targets 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 will use a new tip.

The software assumes that all of the columns in the Wells to Read area will receive fluid during the initial transfer. The Tips Target grid is filled left to right starting with the first available tip column and incrementing the tip target by one until all columns in the Wells to Read selection have been filled.



**Figure 3-28:** Compound & Tip Columns Window with Three Transfers

In the previous illustration, the instrument is configured for three fluid transfers as follows:

- Wells to Read selection of columns 1 through 4.
- Compound Source selection of a 12-column compound plate.
- Pipette Tips Layout selection of a full rack of tips.

From those settings, the SoftMax Pro Software selects tip and compound columns 1 through 4 for the first transfer. The second transfer uses the next four columns (5 through 8) and the third transfer employs the remaining four unused columns (9 through 12).

This settings show one, two, or three transfers, with blue, pink, and green color-coded tips to match the setting made in the Compound Transfer window.

There are two menus: Tips Column and Compound Column.

- The Tips Column menu displays a sequence of numbers that match the Pipette Tip Layout settings.
- The Compound Column menu displays numbers from 1 through the number of columns of the compound plate or the number of troughs selected in the Compound Source tab.

A tips or compound row displays 12 columns to represent the number of columns in a 96-well assay plate. The actual number of columns and their placement further depends on the number of columns and their locations as selected in the Wells to Read settings window.

## Manually Assigning Tips and Compounds

Alternatively to automatic assignment, you can assign any tip column and any compound column to correspond to any assay plate column.

To assign a tip to an assay plate well, select one or more tip cells and select one of the items available in the **Tips Column** menu. The tip column selected in the menu is shown as a number in the selected tip rows. Choosing the **Fill** option at the top of the menu fills the selected cells with the tip numbers that correspond to the same column numbers (tip 3 with column 3, and so on). Similarly, to assign a compound, select one or more compound cells and select one of the items from the **Compound Column** menu.



---

**Note:** To leave tips on between columns in Endpoints and Kinetic reads, designate the same Tip Column to be used for multiple read columns in the Tip Target grid.

---

The wells shown graphically convey the volumes of liquid in the assay plate for each pipetting event. Using the color associated with each event, the compound settings display the dispensed compound volume as a percentage of the total assay well volume. If you entered an initial volume in the Compound Transfer settings, that is shown as a gray fill. As the liquid volumes are cumulative, the first event's volume is shown above the initial liquid volume (if any), the second event's volume is shown above the first, and the third event's volume above the second.

To deselect a tip or compound assignment, first select the appropriate cells and then press **Backspace** on your keyboard. To change an assignment, select the wells and choose new values or type a value.



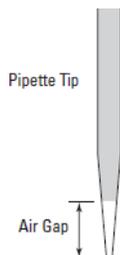
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**Note:** If you have multiple wells selected, and you type a value, that value is shown in the first selected well and the subsequent wells increment to the next higher value.

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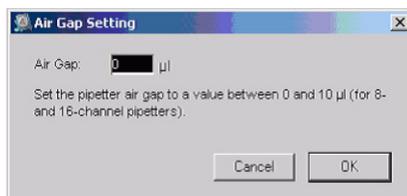
## Pipette Tip Air Gap

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



**Figure 3-29:** Pipette Tip Air Gap

To set the pipette tip air gap, click Control > **Set Air Gap**.



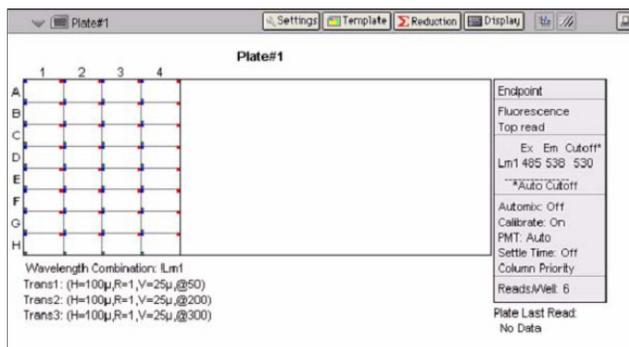
**Figure 3-30:** Setting Pipette Tip Air Gap

In the Air Gap Settings dialog, the allowed values are 0 µL to 200 µL for 96-well microplates and 0 µL to 30 µL for 384-well microplates.

## Settings Displayed in Plate Sections

The Plate section provides visual feedback on all instrument settings in the Instrument Settings section, the gray area to the far right of the Plate section. Information about wells to read and transfer settings are also displayed in the Data Display section.

For example, the following figure shows a Plate section with three fluid transfers. The blue squares in their upper-left corners represent the first transfer, the red squares in the upper-right corners represent the second transfer, and the green squares in the lower-left corners represent the third transfer.



**Figure 3-31:** Plate Section with Wells Selected for Injection

The Plate section also provides information on the transfer settings and the compound sources. The information is displayed below the plate table.

## Other Software Settings

There are other software settings that deal with data calculation and display. In contrast to instrument setup parameters, these data settings can be configured before, during, or after running an experiment. These include:

- Setting reduction parameters
- Setting data display parameters
- Using the Template Editor

Molecular Devices strongly recommends that you define a template and set the reduction and display parameters before reading the assay plate, because these parameters determine how data is displayed and analyzed. You can set up or modify templates, reduction, and display parameters after collection, but this can be complicated or confusing.

For information about using the software to continue to prepare for an experiment, see the SoftMax Pro Software User Guide.



---

**Note:** While it is strongly recommended that you use the Template Editor before running an experiment, it is not strictly necessary. The values received from the instrument are raw values, and are not affected by the settings in the Template Editor.

---

## Reading a Microplate

### Loading Tips and Microplates

Prepare your tip rack, compound plate, and the assay plate you want to analyze and load them into the instrument.



---

**CAUTION!** Make sure that the underside of the assay plate is dry before you place it in the reading chamber drawer. Damage to the lower read head can occur from liquids that come into contact with it. If the microplate has fluid on the underside, dry it using a paper towel, or equivalent, before placing the microplate in the drawer.

---

To load tips and microplates:

1. Open the appropriate drawer by either pressing the appropriate drawer button on the instrument control panel, or by using the SoftMax Pro Software.
2. Insert the filled tip rack or plates into the drawer, placing well A1 into the upper-left corner of the drawer as you look at it.
  - ◆ Make sure the compound plate is flat against the compound baseplate.
  - ◆ Make sure the assay plate is flat against the black adapter (for 96-well and 384-well microplates) or the drawer bottom (for 6-well, 12-well, 24-well, or 48-well microplates).

For more information, see [Using the Microplate Adapters on page 120](#) and [Using the Compound Baseplate on page 121](#).

3. Close drawer by either pressing the appropriate drawer button on the instrument control panel, or by using the SoftMax Pro Software.

## Starting the Reading



---

**Note:** Make sure that you have completed all required settings and configurations before starting the read. You cannot change settings during a read, or after the read and data collection are complete.

---

You can start reading at any time after defining instrument settings.

To read the microplates, click the **Read** button on the SoftMax Pro Software tool bar or click **Control > Read**. You can also press **Ctrl+R** on your keyboard.



**Figure 3-32:** The SoftMax Pro Software Read Button

## Selecting a Plate

If you have created more than one Plate section, and no Plate section is active, choosing the **Read** command will display a dialog box requiring you to choose which section to read.

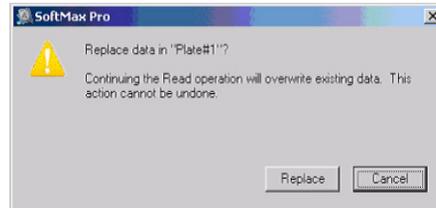


**Figure 3-33:** Selecting a Plate Section

Select the correct section and click **OK**. If a Plate section is active, starting a read will read the active plate.

## Replacing Data in a Plate

If you select a Plate section that already contains data and select the Read command, an alert appears requiring you to confirm that you want to replace the data with data from the new reading.

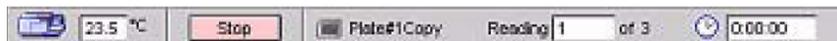


**Figure 3-34:** Replace Data Warning

- ◆ Click **Replace** to proceed with the reading and replace any previous data with the new data.
- ◆ Click **Cancel** to prevent the reading from starting, preserving the current data.

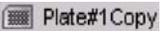
## Viewing Experiment Progress

During the read, the Status bar displays information about the current reading.



**Figure 3-35:** The SoftMax Pro Software Status Bar During a Read

**Table 3-3:** Status Bar Reading Information

Item	Description
	The <b>Read</b> button changes to <b>Stop</b> . Clicking the <b>Stop</b> button halts the reading.
	The plate name and icon appear to indicate which Plate section is currently being read.
	The <b>Reading</b> field displays the portion of the read that is currently under way.
	The time display shows the amount of time elapsed since the beginning of the read.

## Data Display

You can observe the gradual accumulation of data in the Plate section of the SoftMax Pro Software window. The values read by the instrument appear in the data display of the Plate section as they are received from the instrument to the software in real time.

For information about how to change the data display, see the SoftMax Pro User Guide.

## Shutting Down the System

When a read is completed, you can perform more reads, or shut down the instrument.

Shutting down the FlexStation 3 instrument includes the following steps:

1. Save any data files desired in the SoftMax Pro Software.
2. Make sure all three drawers are empty of pipette tips and plates.
3. Clean up any spills that may have occurred during the day's experiments.
4. Turn off the instrument and the computer according to your laboratory's practice.



---

**Note:** Generally, you do not need to switch off the power at the end of the day. If the system will not be used for more than one day, it is best to turn off the instrument.

---

## Optimizing Fluorescence Assays

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

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

### Excitation and Emission Wavelengths

The excitation and emission wavelengths can be set in 1 nm increments between 250 nm and 850 nm. A procedure to optimize excitation and emission wavelengths for a given assay is outlined in [Using Spectral Scanning to Optimize Excitation and Emission Wavelengths for Fluorescence Assays](#) on page 105.

### Emission Cutoff Filter

The emission cutoff filters assist in reducing background. Sources of background include stray excitation light and native fluorescence of microplate materials, sample constituents, and solvents (including water). The default setting allows the instrument and the SoftMax Pro Software to determine which cutoff filter should be used in Endpoint and Kinetic reads. For default settings, see [Table 3-4 on page 109](#). The spectral scan read default uses no cutoff filter.

### Readings Per Well

The number of readings per well may vary between 1 (used for a quick estimate) and 100 (for very precise measurements). The default number of readings per well varies with the read mode. For fluorescence, the default is 6, and for luminescence, the default is 30.

### PMT Voltage

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

### Temperature Control

The reading chamber of the FlexStation 3 instrument is isothermal at ambient as well as at elevated temperatures. The temperature in the reading chamber can be adjusted from 2°C above ambient to 45°C.



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**Note:** Assay optimization requires the use of a computer and SoftMax Pro Software.

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## Using Spectral Scanning to Optimize Excitation and Emission Wavelengths for Fluorescence Assays

Put 200  $\mu\text{L}$  of sample that includes the fluorophore and 200  $\mu\text{L}$  of a buffer control into separate wells of a microplate.

### Perform the Excitation Scan

1. Using the SoftMax Pro Software, set up a Plate section for a fluorescence read, spectrum mode, Em Fixed/Ex Scan, with no cutoff filter (default), and medium PMT.
2. Set the emission wavelength based on the tentative value from the literature, or from a customary filter set used to measure your fluorophore. If the emission wavelength is not known, select a tentative emission wavelength about 50 nanometers greater than the absorbance maximum of the fluorophore. If necessary, the absorbance maximum can be determined by performing an optical density spectral scan first.
3. Set the excitation scan to start/stop approximately 50 nm below/above the tentative excitation value obtained from the literature, or the customary excitation filter.
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. Perform the scan and view the results as a plot of emission fluorescence vs. excitation wavelength.

Note the excitation wavelength at the emission peak and the maximum RFU value.

If an error message reporting missing data points occurs, it might be due to possible saturation reported by the SoftMax Pro Software at the end of the spectral scan. Reset the PMT to **Low** and re-scan the sample. Scan the buffer blank with the PMT set to **Medium** or **High**. If the error occurs after scanning with the PMT set to **Low**, it might be necessary to dilute the sample.

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

Select the optimal excitation wavelength.

- ◆ If the excitation peak wavelength and emission wavelength are separated by more than 80 nm, use the excitation peak wavelength value.
- ◆ If the excitation and emission wavelengths are less than 80 nm apart, use the shortest excitation wavelength that gives 90% maximal emission. Follow the plot to the left of the peak until the RFU value falls to approximately 90% of the maximum, and then drop a line from the 90% point on the plot to the x-axis. See [Figure 3-36](#).

### Perform Emission Scan #1

1. In the SoftMax Pro Software, set up a second Plate section for a fluorescence read, spectrum read type, Ex Fixed/Em Scan, with no cutoff filter (default), and medium PMT.
2. Set the excitation wavelength to the value determined in [Step 5](#) in [Perform the Excitation Scan on page 105](#).
3. Set the emission scan to start/stop approximately 50 nm below or above the tentative emission value obtained from the literature, or existing filter pair.



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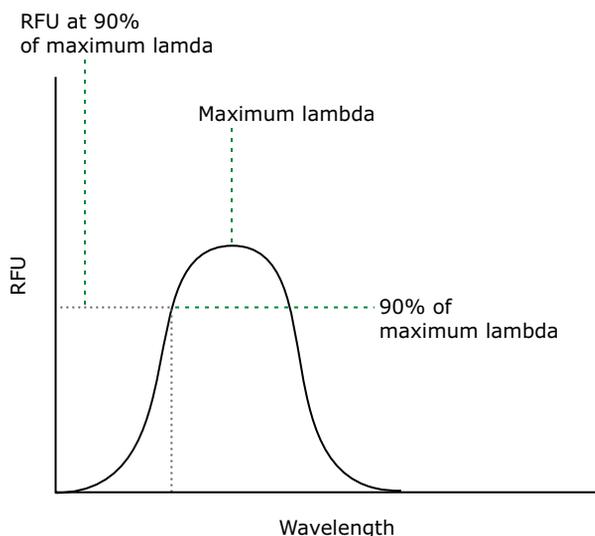
**Note:** If the Stokes shift is less than 50 nm, then start the emission scan above the excitation wavelength.

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4. Set the step increment to 2 nm to 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. Perform the scan and view the results as a plot of fluorescence vs. emission wavelength.
6. 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, 455, 475, 495, 515, 530, 550, 570, 590, 610, 630, 665, or 695 nm. The cutoff value should be near the maximum emission wavelength, preferably between the excitation wavelength and the maximal emission wavelength, but at least 10 nm less than the emission wavelength.

If you have questions about this procedure please contact Molecular Devices Technical Support and ask to speak to an applications scientist.



**Figure 3-36:** Plot of RFU vs. Wavelength

### Perform Emission Scan #2:

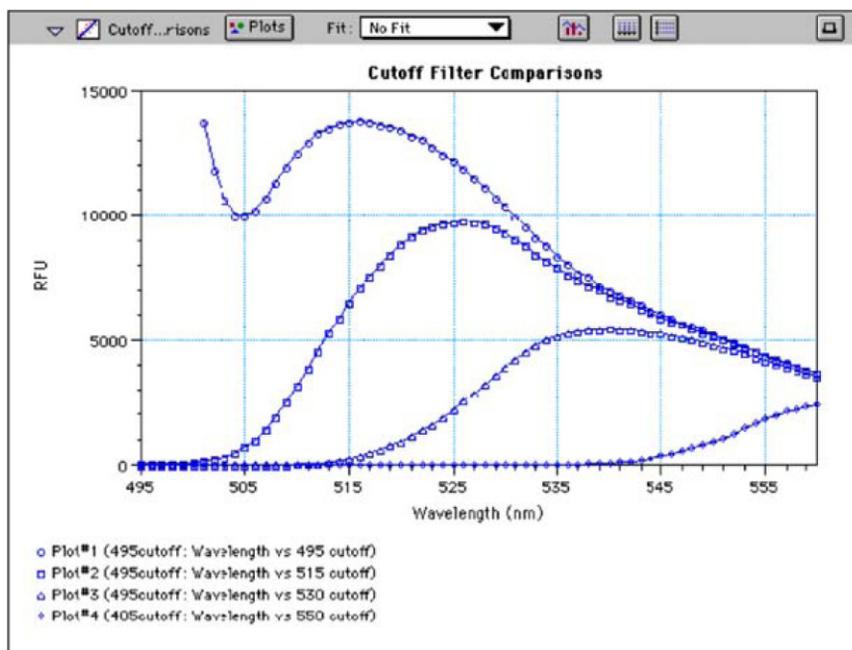
1. In the SoftMax Pro Software, set up a third Plate section for an emission scan as specified in [Perform Emission Scan #1 on page 106](#), except selecting **Manual Cutoff Filter** and setting the wavelength to that determined in [Step 6](#).
2. Perform the scan and view the results as a plot of fluorescence vs. emission wavelength.  
Note the wavelength giving the maximum emission (the optimal emission wavelength).
3. Compare the spectra of the sample containing the fluorophore to the spectra of the buffer blank to get an estimate of the signal-to-noise ratio. If there is significant background interference, repeat [Step 1](#) and [Step 2](#) with another choice of cutoff filter.

## Results

The optimal excitation and emission wavelengths are those determined in [Step 5 in Perform the Excitation Scan on page 105](#) and in [Step 2 in Perform Emission Scan #2: on page 107](#).

## Comments

- ◆ In Endpoint or Kinetic fluorescence reads, the Autofilter feature generally selects the same cutoff filter wavelength as the above optimization method. If desired, however, you can specify the cutoff filters manually.
- ◆ For emission wavelengths less than 325 nm, experimental iteration is usually the best method of determining the optimal emission and excitation wavelengths. Begin optimization by performing the previous procedures. Try emission and excitation wavelength combinations with the 325 nm cutoff or with no cutoff filter. Similarly, for excitation wavelengths greater than 660 nm, try emission and excitation wavelength combinations with the 695 nm cutoff or with no cutoff filter.



**Figure 3-37:** Effects of cutoff filters on fluorescein. Emission was scanned from 490 nm to 560 nm; excitation was fixed at 485 nm.

Figure 3-37 on page 108 shows the effects of different cutoff filters on a scan of fluorescein where excitation was fixed at 485 nm and emission was scanned from 490 nm to 560 nm. Buffer blanks are not shown in this plot. Table 3-4 lists default settings for the emission cutoff filters.

**Table 3-4:** Emission Cutoff Filter Default Settings

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

For Spectrum reads, the default is “manual” (no automatic cutoff).

## Optimizing Time-Resolved Fluorescence Assays

Time-resolved fluorescence (TRF) assays can be read from the top or bottom of a microplate. Solid white plates are recommended for top time-resolved fluorescence reads, and white plates with clear bottoms are recommended for bottom reads.

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

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

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

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

## Optimizing Fluorescence Polarization Assays

Fluorescence polarization (FP) can be read only from the top of a microplate. The plastic from a microplate affects the light polarization, precluding bottom reads and reading a covered plate.

Solid black plates are recommended for fluorescence polarization reads. If the assay components seem to bind to the microplate, as evidenced by poor mP dynamic range (small difference between bound and unbound tracer), we suggest using polypropylene plates or adding a very small amount of detergent, such as Tween-20, to the assay buffer. Background wells, containing all assay components minus the fluorophore, should be tested. If the signal in the background wells is more than 1/10 the signal in the wells containing fluorophore, then background wells should be run on each assay plate. The average raw signal from the parallel and perpendicular readings of the background should be subtracted from the raw parallel and perpendicular readings of each sample well before the mP calculation is performed. For setup of background subtraction in fluorescence polarization, see the SoftMax Pro Software User Guide.

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

## Optimizing Luminescence Assays

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

For standard luminescence, a separate light path without monochromators carries the emitted light to a dedicated PMT. The optimum emission wavelength is between 360 nm and 630 nm. Under reader setup the emission says **All**.

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

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

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

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

This chapter provides procedures for regular maintenance that the FlexStation 3 instrument needs.

- [Obtaining Support, see page 114](#)
- [Moving the Instrument, see page 114](#)
- [Cleaning the Instrument, see page 115](#)
- [Using the Microplate Adapters, see page 120](#)
- [Using the Compound Baseplate, see page 121](#)
- [Replacing Fuses, see page 121](#)
- [Replacing the Flash Lamp, see page 123](#)
- [Long-Term Shutdown, see page 128](#)



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

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

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**Service-Trained Users:** Whenever the following warning message appears, a service-trained user must perform the procedure to ensure user safety and to prevent instrument damage.

Example:



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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 been trained properly by appropriate Molecular Devices personnel.

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## Obtaining Support

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

Our support web site, [www.moleculardevices.com/support](http://www.moleculardevices.com/support), has a link to the Knowledge base with technical notes, software upgrades, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000. Please have the system ID number, system serial number, software version number, and the system owner's name available when you call.

## Moving the Instrument

If you need to relocate the FlexStation 3 instrument, follow these steps.



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**WARNING!** The instrument weighs approximately 50 pounds and should be lifted with care. To prevent injury, use at least two people to lift the instrument.

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1. Ensure that the new location meets the proper specifications as described in [Installation on page 49](#).
2. Remove any microplates, adapters, reservoirs, and tips from all three drawers, and then close the drawers.



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**CAUTION!** Do not attempt to assemble or disassemble the instrument with the pipette tips or compound and read plates in place. Spillage or damage to the pipette tips, plates, or the instrument can occur.

---

3. Be sure reading chamber screws are still in place. Contact Technical Support if reading chamber screws have been removed.
4. Be sure all covers are closed and securely fastened.

5. Turn off the power switch and unplug the power cord from the source and from the receptacle on the back of the instrument.
6. Disconnect the instrument from the computer and any printer.
7. Remove the fluidics module. For related instructions, see [Installing the Fluidics Module on page 53](#).
8. Depending on the distance that you will be moving the instrument, you might want to repackage the FlexStation 3 instrument in its original shipping cartons. Otherwise, carry the instrument or place it on a rolling cart to transport it.

## Cleaning the Instrument



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**WARNING! BIOHAZARD.** Wear gloves and other personal protection equipment during any cleaning procedure that could involve contact with either hazardous or biohazardous materials or fluids.

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Periodically clean the outside surfaces of the FlexStation 3 instrument using a cloth or sponge that has been dampened with water. Do not use abrasive cleaners. If required, clean the surfaces using a mild soap solution diluted with water or a glass cleaner and then wipe with a damp cloth or sponge to remove any residue.



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**CAUTION!** Do not spray cleaner directly onto the instrument or into any openings.

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If needed, clean the reading chamber drawer using a cloth or sponge that has been dampened with water. Never clean the inside of the instrument. Do not allow excess water or other fluids to drip inside the instrument.

## Cleaning Up Spills



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**WARNING!** Always make sure the power switch on the instrument is in the **OFF** position and remove the power cord from the back of the instrument before opening any covers or panels.

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If fluids spill into the bottom of the reading chamber when the reading chamber drawer is outside the instrument, the fluid is directed to a tray at the bottom of the instrument, from which it exits to the bench or counter beneath the instrument. Wipe up spills immediately. Clean the exterior of the unit and the drawer as necessary.

## Cleaning the Fan Filter

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

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



---

**CAUTION!** Do not attempt to assemble or disassemble the instrument with the pipette tips or compound and read plates in place. Spillage or damage to the pipette tips, plates, or the instrument can occur.

---

2. Turn the power to the reader **OFF** and then remove the power cord and cables from the back of the reader.
3. Turn the reader over so that it rests flat on the bench.
4. Remove the black fan cover and then remove the filter.
5. Clean the filter by blowing canned air through it or by rinsing it first with water and then with alcohol, and allowing it to dry completely.
6. Place the clean, dry filter over the fan and replace the black cover.
7. Turn the reader back over.
8. Reconnect the power cord and cables to the reader and return the plate adapters to their respective positions.

## Cleaning the Barrels on the Pipettor Head

The barrels on the pipettor head require periodic cleaning to remove silicone lubricant, dust, and other miscellaneous contamination.

Molecular Devices recommends that you clean the barrels at least once every three months. The frequency of cleaning depends on how dusty your particular lab is and how often the pipettor head is used. The frequency and methods of cleaning are the same for both the 8-channel and the 16-channel pipettor heads.

Remove the pipettor head and then clean the barrels using cotton swabs and OS-2 silicone cleaner or alcohol. Do not touch the barrels with your hands during cleaning.



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

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

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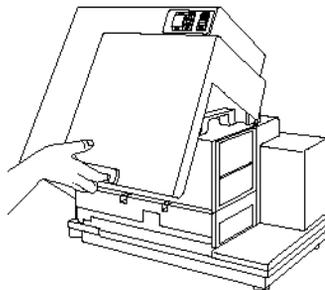
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**WARNING! BIOHAZARD.** This procedure includes steps involving potentially infectious biological agents requiring that proper handling precautions be taken.

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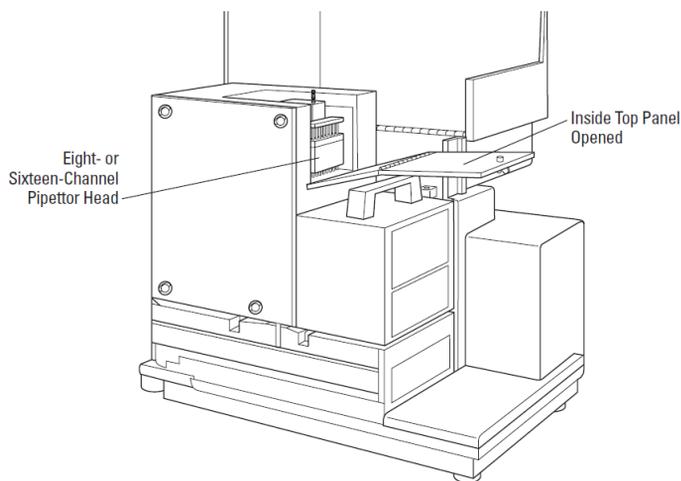
To clean the barrels on the pipettor head:

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



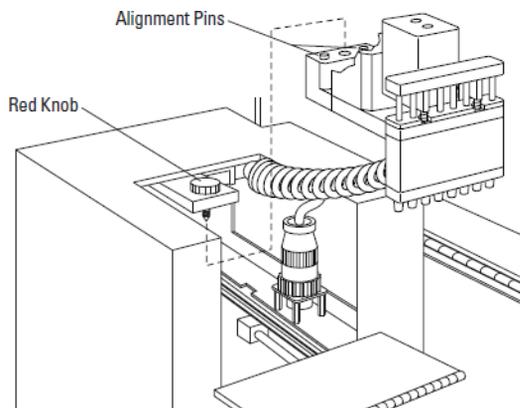
**Figure 4-1:** Opening the Instrument Cover

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



**Figure 4-2: Opening of the Top Panel**

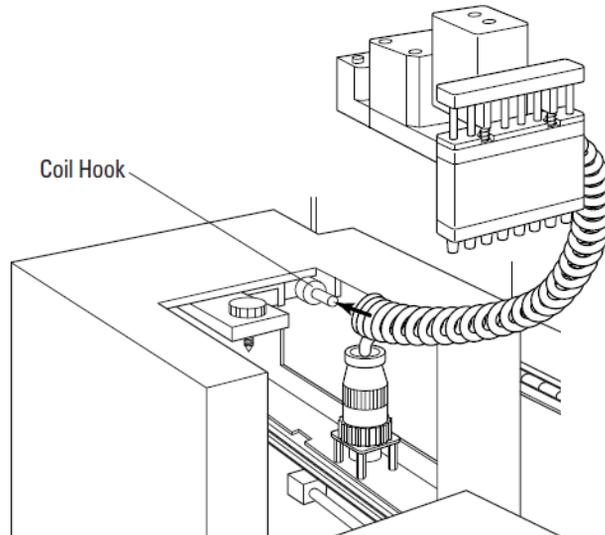
4. Inside the open chamber, loosen the red retainer nut.



**Figure 4-3: Removing the Pipettor Head**

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. Lift the pipettor head out of the fluidics module carefully, as it is still connected inside the instrument.
7. Hold the pipettor head over a tray or something into which you can catch the liquid used for cleaning the barrels.

8. Clean the barrels using cotton swabs and OS-2 silicone cleaner or alcohol. Do not touch the barrels with your hands during cleaning.
9. Slide the pipettor head back under its mounting plate inside the fluidics module, making sure that the white spiral cord is secured on its retaining hook.



**Figure 4-4:** Pipettor Head Coil Hook

10. Tighten the red retainer nut to secure the pipettor head. Make sure the knob is as tight as possible.
11. Replace the inside top panel and then lower the cover back onto the instrument.
12. Reattach the power cord to the instrument and reconnect the instrument to the computer.

## Using the Microplate Adapters

### Microplate Adapter Installation

If you are reading standard 96-well or 384-well microplates, you need to install a microplate adapter in the reading chamber drawer. The black adapter elevates the plate in the drawer for optimum performance with standard 96-well or 384-well microplates in bottom-read and top-read mode.



---

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

---

1. Turn power to the instrument on.
2. Press the **reading chamber** button on the front panel, or click **Control > Open Drawer** in the software.
3. Lower the black adapter plate into the reading chamber drawer with its cutout corner facing the front-left corner of the drawer.

### Removing the Microplate Adapter

If you are reading “high profile” 6-well, 12-well, 24-well, or 48-well microplates and the adapter is in the drawer, you must remove the adapter.

1. Turn power to the instrument on.
2. Press the **reading chamber** button on the front panel, or click **Control > Open Drawer** in the software.
3. Hold the adapter at the front (long side of the drawer) and push toward the back (Row A).
4. Lift the front (Row H) of the adapter and remove it from the drawer.

## Using the Compound Baseplate

To use the compound baseplate, place the metal baseplate into the bottom of the compound plate drawer so that a compound plate can be inserted on top of the baseplate.

1. Turn power to the instrument on.
2. Press the **reagents** button on the front panel, or click **Control > Open Compound Drawer** in the software.
3. Lower the compound baseplate into the compound drawer with its cutout corner facing the front-left corner of the drawer.

To remove the compound baseplate, open the compound drawer and lift the baseplate out.



---

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

---

## Replacing Fuses

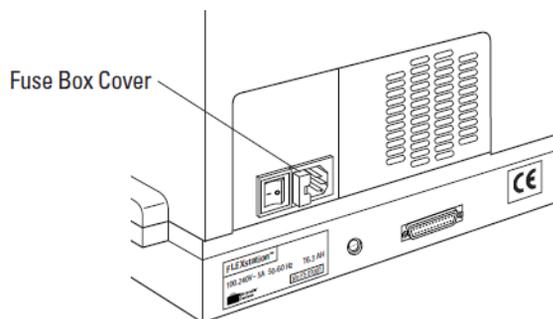
Fuses burn out occasionally and must be replaced. If the instrument is not getting power after switching it on (the LCD shows no display), check to see whether the power cord is securely plugged in to a functioning power outlet and to the receptacle at the rear of the instrument. If power failed while the instrument was already on, check that the power cord is not loose or disconnected and that power to the power outlet is functioning properly. If these checks fail to remedy the loss of power, follow the steps listed to replace the fuses. Spare fuses (two U.S. and two metric) are shipped with the reader in the original carton. The U.S. and metric fuses are identical except for physical size. They can be taped to the back of the FlexStation 3 instrument.

If you no longer have spare fuses, you can obtain new fuses from Molecular Devices (part number 4601-0029) or from a local hardware store. Make sure fuses are rated slow-blow (6.3A 5 × 20 mm Slo-Blo IEC).

To replace the fuses:

1. Turn off power to the instrument and remove the power cord from the outlet and from the instrument power cord receptacle.
2. Remove the printer cable and computer cable (if connected) from the back of the instrument.
3. If necessary, turn the instrument to access the rear panel.

On the left side of the rear panel, viewed from the back, is the power switch, fuse box, and power cord receptacle.



**Figure 4-5:** Fuse Box Location

4. Press to the right of the black plastic cover of the fuse box to release it.
5. Pull the fuse box cover away from the instrument. The fuse box begins to slide forward.
6. Continue gently pulling the fuse box forward until it is free of the instrument.

After the fuse holder is out of the instrument, you can see that it contains two fuses. It is possible that only one of the fuses has blown. Molecular Devices recommends that you replace both fuses, however, to ensure continued proper operation.

7. Pull both fuses out of the holder and discard them.
8. Insert new slow-blow rated fuses into the fuse holder. Either end of the fuse can be forward.
9. Insert the fuse box into the opening in the instrument, making sure that the fuses are on the right side toward the power receptacle.
10. Press the fuse box into place, making sure the cover snaps closed.
11. Plug the power cord into the instrument and then into the wall outlet.
12. Reconnect other cables previously disconnected.

## Replacing the Flash Lamp

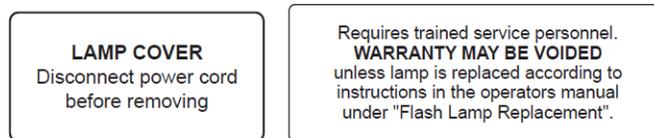
The flash lamp should be replaced when an error message appears in the SoftMax Pro Software to indicate replacement is necessary.



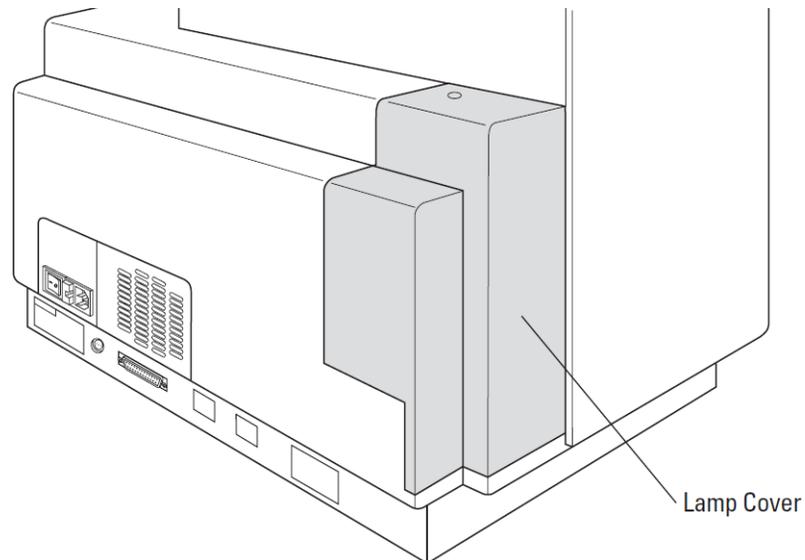
**WARNING!** The following procedures must be completed by a service-trained user. Do not attempt the following procedures if you have not been trained properly by appropriate Molecular Devices personnel.



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



**Figure 4-6:** Labels Near the Lamp Cover



**Figure 4-7:** Flash Lamp Location

## Required Tools

- Hex keys: 3/32 inch, 9/64 inch, and 5/32 inch
- #2 Phillips screwdriver

To replace the flash lamp:

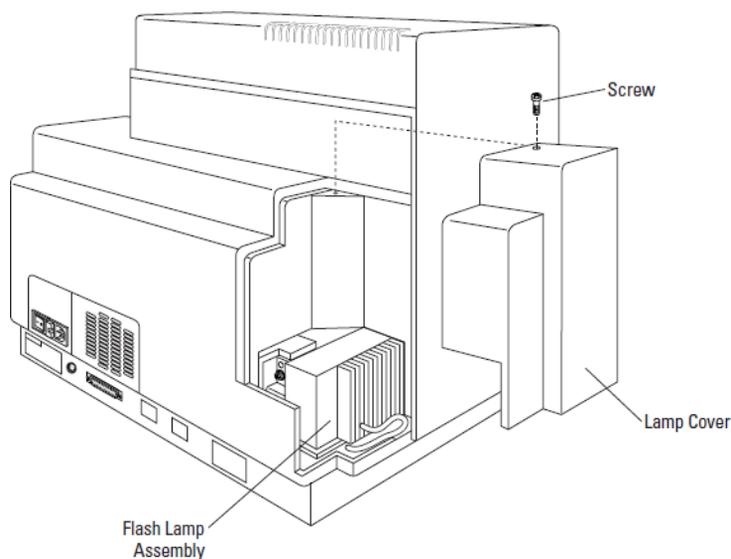
1. Turn off the instrument power switch and unplug the power cord and the computer cable from the rear of the instrument.
2. Remove the Phillips head screw on the top of the cover and lift the cover off vertically.
3. Set the cover aside, and keep track of the screw.



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**Note:** An interlock switch, held closed by the lamp cover, interrupts power to the flash lamp automatically when the cover is removed.

---



**Figure 4-8:** Removing the Lamp Cover

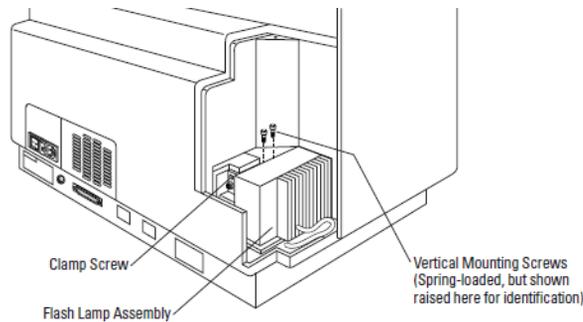
4. Use a 9/64 inch hex key to loosen the clamp screw, located on the lamp mounting block and facing the rear of the instrument. Leave the clamp screw in place.
5. Use a 5/32 inch hex key to loosen the two vertical-mounting cap screws that are holding the Litepac mounting block to the baseplate. They are located toward the inside of the lamp's mounting box. The two cap screws are spring-loaded and stay in a raised position so that you can slide out the entire Litepac without getting the screws caught in the holes.



---

**Note:** You might need a flashlight to see the cap screws.

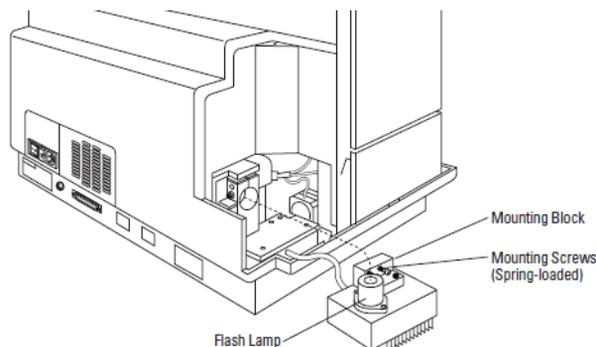
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**Figure 4-9:** Removing Litepac Mounting Block Screws

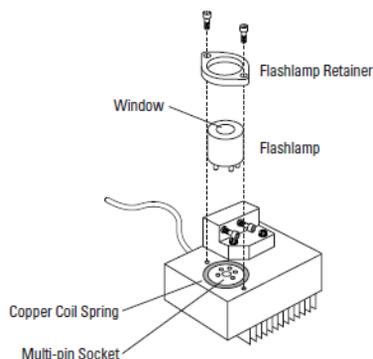
6. Open the top cover of the instrument to provide better access and clearance to the lamp's mounting box. This makes it easier to remove the lamp assembly.

7. Slide the lamp assembly horizontally out the side of the instrument and lay it on its heat sink fins, leaving the orange electrical cord connected.



**Figure 4-10:** Removing the Flash Lamp Assembly

8. Use a 3/32" hex key to remove the two screws that hold down the diamond shaped retainer. Be careful not to lose the screws.



**Figure 4-11:** Withdraw the Lamp

9. Withdraw the silver-colored lamp from its multi-pin socket by gently rocking it and pulling it upward.
10. Notice the location of the copper coil spring and keep it at hand.
11. Remove the new lamp from its packaging, being careful not to touch the lamp window.



---

**CAUTION!** Do not touch the lamp window at any time.

---

12. With the copper coil spring in place around the socket, carefully align the lamp pins in the proper orientation according to the pin pattern. Again, do not touch the lamp window.
13. Install the lamp with a gentle rocking motion and steady downward pressure.
14. Replace the diamond shaped lamp retainer over the new lamp, and use the 3/32 inch hex key to install the two screws.
15. Tighten the two screws securely and evenly, compressing the coil spring until the lamp is seated.
16. Tilt up the lamp assembly and line up the lamp with the bore of the mounting block.
17. Slide the lamp all the way into the block, orienting the assembly to clear the baseplate. Slide it in until it stops.
18. Tuck the orange power cord inside, behind the assembly.
19. Close the instrument top cover.
20. Use the 5/32 inch hex key to firmly tighten the two vertical mounting screws.
21. Tighten the clamp screw snugly but not tightly. Use caution not to over-tighten.



---

**CAUTION!** Do not over-tighten the clamp screw or the lamp could crack inside.

---

22. Replace the plastic lamp cover over the opening. Be careful not to pinch the orange power cord if it is not tucked away.
23. Replace and install the screw.
24. Plug in the power cord and the computer cord.

## Long-Term Shutdown

If you will not be using the FlexStation 3 instrument for an extended period of time, clean the external surfaces of the instrument before storage. See [Cleaning the Instrument on page 115](#).

Be sure the instrument is emptied of all plates and tips before storage.

Keep all cables and accessories with the instrument during storage.

This chapter provides instructions for troubleshooting problems that can occur with the FlexStation 3 instrument.

- [Problems During Startup](#), see page 130
- [Opening a Drawer Manually](#), see page 132
- [Understanding Potential Mechanical Problems](#), see page 133
- [Recovering from Mechanical Problems in Flex Mode when Using Fluidics](#), see page 135
- [General Error Messages](#), see page 147
- [Tilting or Removing the Fluidics Module](#), see page 149



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**Note:** These troubleshooting instructions address only those problems that can occur with the fluidics operation of the instrument. They do not address problems with the flash lamp or the detector, or error messages related to missing data or saturated data points. If you need to contact Molecular Devices Technical Support, see [Obtaining Support on page 114](#).

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## Problems During Startup



**Note:** Before starting the SoftMax Pro Software, make sure that the instrument is powered on.

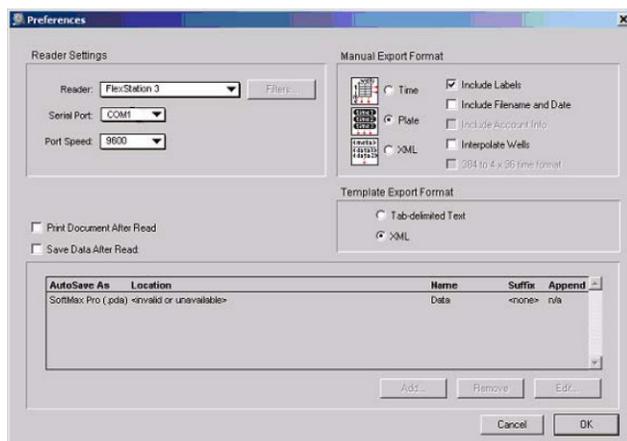
After you start up the SoftMax Pro Software, the Instrument Status bar should appear as in the illustration on the left, with a temperature displaying in the temperature box.



**Figure 5-1:** Status Bar Detail

If you see a red X in front of the instrument icon, or if no temperature is displayed in the temperature box (as in the illustration on the right) there is either a communication problem between the instrument and the software or the instrument has experienced a fatal error.

1. Make sure that the FlexStation 3 instrument is plugged in and turned on.
2. Make sure that the instrument has finished its warming up sequence. Wait until the temperature is displayed on the front panel of the FlexStation 3 instrument.
3. Click **Edit > Preferences** in the software to view the Preferences dialog.



**Figure 5-2:** Preferences Dialog

There are two settings in the Preferences dialog that must be correct for the instrument to operate.

4. Make sure the Serial Port selected is the same as the one on the computer to which the instrument is physically connected. It might be COM1 or COM2.
5. Make sure the Serial Comm Speed is correctly selected. The communication speed is 9600 during the instrument's initial warm up sequence, while it is first communicating with the software.



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**Note:** The Serial Comm Speed automatically changes to 57,600 when you select Flex mode from the Settings window.

---

If you continue to see the red X after confirming the Serial Comm Speed, see [Recovery Procedure on page 146](#).

If you follow these steps and are still having trouble starting up the system, contact Technical Support. See [Obtaining Support on page 114](#).

## Opening a Drawer Manually

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

1. If the power is not already off (as can occur with a power failure, or if you are already troubleshooting the instrument with the covers open), turn off the power. Disconnect the power cord from the instrument, and the computer cables from the computer.



---

**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 could suddenly move, resulting in operator injury.

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



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**CAUTION!** Never force a carriage out of the instrument. If it does not come out reasonably easily, contact Molecular Devices Technical Support. See [Obtaining Support on page 114](#).

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## Understanding Potential Mechanical Problems



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**Note:** Read these instructions before attempting to use the FlexStation 3 instrument for Flex reads or Endpoint and Kinetic reads.

---

### Background

The intended function of the FlexStation 3 instrument requires it to have many moving parts. The operator interacts extensively with the instrument by introducing various types of plastic disposable plates, tips, and troughs. Molecular Devices has made every effort to ensure smooth and reliable operation of the FlexStation 3 instrument. However, problems can occur. For example:

- 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 accidentally released from the nose cones during operation, they might fall outside of the rack. To help prevent frequent tip dropping, periodically clean the pipettor barrels. See [Cleaning the Barrels on the Pipettor Head on page 117](#).
- If the power fails during a pipetting step, serious damage can result to the instrument if appropriate corrective steps are not taken.

As noted, there are several opportunities for mechanical problems within the fluidics module. Fortunately, these problems will be rare if you understand how to avoid them. The design of the FlexStation 3 instrument provides the means for successfully recovering from most problems.

### Before Using the Instrument

Molecular Devices recommends that you disconnect the power and open up the instrument to become familiar with the mechanical parts of the fluidics module. Observe that it is possible to manually (and always gently) move the drawers, doors, and pipettor head. Moving the parts manually is important for recovering from some mechanical problems. For further information, see [Opening a Drawer Manually on page 132](#).

## Avoiding Mechanical Problems

Following a few simple steps helps avoid many potential problems.

- Make sure that the red knob that holds the pipettor head in place is securely tightened.
- Use only tips specified for the FlexStation 3 instrument in a 96-well or 384-rack configuration.

**Table 5-1:** 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

- To help prevent frequent tip dropping, periodically clean the pipettor barrels. See [Cleaning the Barrels on the Pipettor Head on page 117](#).
- Make sure that you maintain appropriate well formats. For example, the 16-channel pipettor uses 384 tips with a 384-well compound plate and a 384-well assay microplate.
- Remove the lid of the tip rack before placing it in the tip rack drawer.
- Make sure that you seat the tip rack, plates, and trough (if applicable) securely in the correct drawers.
- Make sure that the layout of the tips that you specify in your SoftMax Pro Software protocol accurately represents what you have in the in the rack.
- Make sure that the compound and assay plates that you specify in your SoftMax Pro Software protocol accurately represent the microplates you are actually using.




---

**Note:** The software has no way of confirming the presence, absence, or configuration of tips or plates in the instrument drawers.

---

## 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 at the back of the instrument. To avoid instrument damage, follow the instructions in [Recovering from Mechanical Problems in Flex Mode when Using Fluidics](#) on page 135.

## Recovering from Mechanical Problems in Flex Mode when Using Fluidics

The procedures in this section are intended for cases in which tips have jammed, or the power fails while you are using the instrument with fluidics, and you see an error message on the computer screen indicating a problem with the instrument. You might need to open the instrument to locate and correct the problem. The procedures in this section include:

- [Assessing a Mechanical Problem](#), see page 136
- [Opening the Instrument](#), see page 137
- [Evaluating the Tip Rack](#), see page 138
- [Inspecting Inside the Fluidics Module](#), see page 139
- [Removing the Pipettor Head](#), see page 142
- [Expelling Undispensed Fluid from Tips](#), see page 145
- [Recovery Procedure](#), see page 146



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**WARNING!** Always make sure the power switch on the instrument is in the OFF position and remove the power cord from the back of the instrument before removing any panels.

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

---

## Assessing a Mechanical Problem

Depending on when the problem occurs during the fluidics operation, one of several error messages can appear in the SoftMax Pro Software.

The error message might not provide complete information about what has occurred. The software cannot confirm the presence, absence, or exact position of tips or plates. Whenever you experience 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 might occur if the fluidics module is not correctly installed.
- **Pipettor failure. There may be undispensed fluid in the pipette tips.**  
This message might occur if the tip rack is jammed. Tips might be missing. To help prevent frequent tip dropping, periodically clean the pipettor barrels. See [Cleaning the Barrels on the Pipettor Head on page 117](#).
- **Pipettor failure. Undispensed fluid in the tips.**  
This message occurs if fluid remains in the tips. The instrument needs to be manually recovered at this point.



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**Note:** If you need to contact technical support during the following procedures, be sure to note which error message you observed.

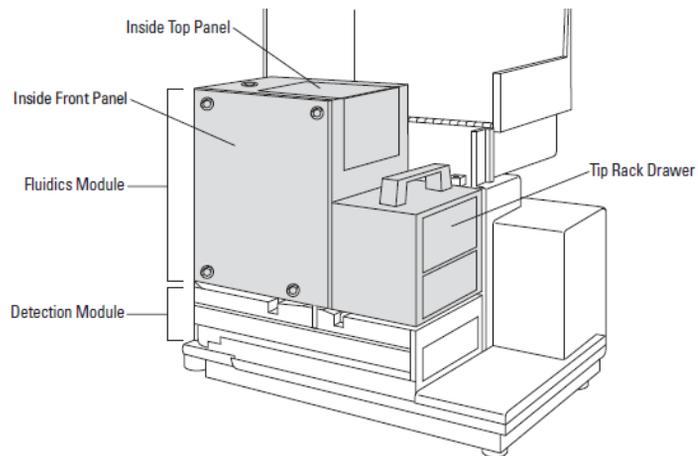
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## Opening the Instrument



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

1. Turn the instrument power off and disconnect the power cords from the instrument and the cables connected to the computer.
2. Press in the handle at the bottom of the top cover and lift the cover back off the detection module, revealing the fluidics module.



**Figure 5-3:** Location of Inside Panels and Tip Rack Drawer

3. Turn the four quarter-turn screws and lift the front panel out.  
You might need to inspect the interior of the 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 needs to be removed. Instructions for these procedures are in the following topics.

## 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 fluidics module to the right. Remove the tip rack cover and reposition the tip rack in the carriage.
- Tip rack is improperly positioned.  
If the tip rack is jammed because it was not positioned securely in the drawer, pull the 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 preventing the drawer from opening, you might be able to remove the rack and 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, you must attempt to retrieve them from inside the instrument. See [Inspecting Inside the Fluidics Module on page 139](#).

After correcting a tip rack issue, replace the inside front panel and top cover. Reattach the power cord and computer cable to the instrument and 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.

---

To help prevent frequent tip dropping, periodically clean the pipettor barrels. See [Cleaning the Barrels on the Pipettor Head on page 117](#).

## Inspecting Inside the Fluidics Module

If there is any chance at all that there could be fluid in the pipette tips, you must remove the pipettor head from the instrument and dispel the fluid or remove the tips outside the instrument. This must be done to avoid spilling fluid in the reading chamber. See [Removing the Pipettor Head on page 142](#) and [Expelling Undispensed Fluid from Tips on page 145](#).



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



**WARNING! BIOHAZARD.** This procedure includes steps involving potentially infectious biological agents requiring that proper handling precautions be taken.

Determine the position of the pipettor head and the location of all the tips that were originally in the tip rack. Things to consider include:

- 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 compounds plate area, or the reading chamber?
- Are there any pipette tips on the pipettor head?
- Is there is any chance there is liquid remaining in the tips? If so, remove the head and expel the liquid safely. See procedures in [Removing the Pipettor Head on page 142](#) and [Expelling Undispensed Fluid from Tips on page 145](#).



**CAUTION!** Take care that you 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 some have dropped out of sight?
- Do all the drawers move freely to gentle pressure or is there a plate or tip rack jamming them?

After you have determined the status of the pipettor head and tips, there are a number of procedures you might need to follow to resolve the problem. Possible scenarios include:

- Pipettor head is in home position, without tips.
  - ◆ If the pipettor head is up and out of the way (without tips) but the tip rack is jammed, it might be because one or more tips are not seated properly and are blocking 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), you might need to gently push it inside (to the left) to get the rack out.
- Pipettor head is in home position, without tips, but tips are missing.
  - ◆ If the pipettor head is up and out of the way (without tips) but there are tips missing from the rack, then you must locate and recover the missing tips before proceeding.
  - ◆ Retrieve any tips you can easily see and reach through the front panel.
  - ◆ It might be necessary to raise the fluidics module, to gain access to the reading chamber. See [Tilting or Removing the Fluidics Module on page 149](#).
- Pipettor head is in lowest position, without tips, 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, you can remove the tip rack easily. 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 there is liquid in the tips, you must then remove the pipettor head and dispel the liquid safely outside the instrument as described in [Removing the Pipettor Head on page 142](#) and [Expelling Undispensed Fluid from Tips on page 145](#).




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**CAUTION!** Do not attempt to remove the pipettor head through the front inside panel. See instructions in [Removing the Pipettor Head on page 142](#).

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- ◆ 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 have secured all tips and returned 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 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 on the SoftMax Pro Software status bar. After you correct the problem, and the instrument has completed its startup cycle again, the red X over the instrument icon in the status bar should disappear, indicating that communication has been successfully reestablished. See [Problems During Startup on page 130](#).

## Removing the Pipettor Head

You might need to remove the pipettor head to remove empty tips or to dispel liquid remaining in tips after a mechanical failure.

To remove the pipettor head, follow the instructions in this procedure. To reinstall the pipettor head, see instructions in [Installing the Pipettor Head on page 56](#).



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

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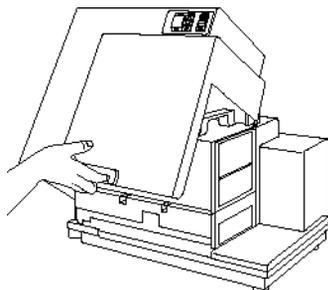
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**WARNING! BIOHAZARD.** This procedure includes steps involving potentially infectious biological agents requiring that proper handling precautions be taken.

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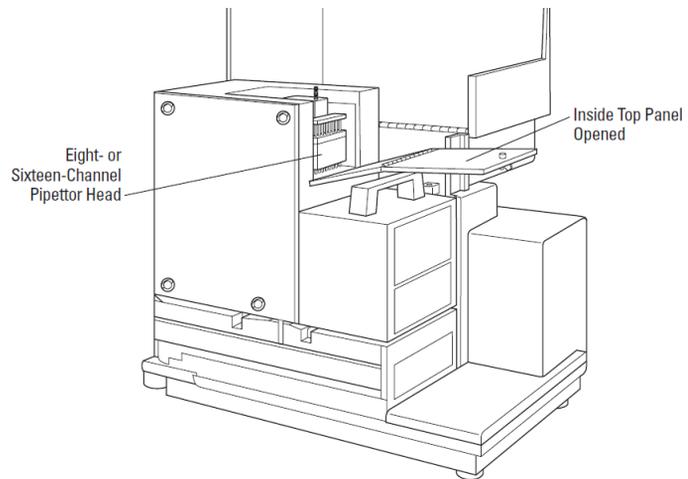
To remove the pipettor head:

1. Confirm that all power to the instrument is turned off.
2. Unplug the power cord and the computer cable from the rear of the instrument.
3. Open the instrument top cover.



**Figure 5-4:** Opening the Instrument Cover

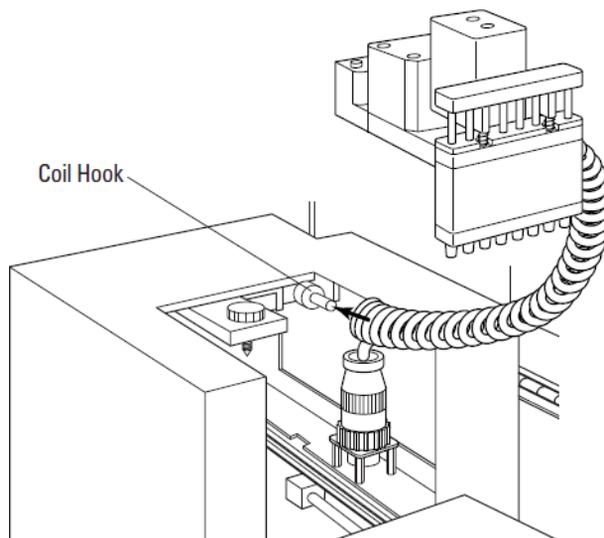
4. Turn the quarter-turn fastener on the inside top panel and unfold the panel off the fluidics module to the right.
5. Inside the open chamber, loosen the red retainer nut.



**Figure 5-5:** Location of the Pipettor Head Inside the Fluidics Module

6. Slide the pipettor head part way out from under the metal mounting plate, and of the top panel opening, supporting it from underneath.

7. Pull the spiral cord off the coil hook behind the pipettor head. This will free the mechanism for easier removal.



**Figure 5-6:** Pipettor Head Coil Hook

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**Note:** Note: If you want to dispense liquid from the tips, skip the next two steps and see next [Expelling Undispensed Fluid from Tips on page 145](#).

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8. To completely remove the pipettor head, unscrew the black connector at the end of the white spiral cord from its secure position around the receptacle.
9. Pull the connector out of the receptacle and take the pipettor head the rest of the way out of the fluidics module.

## Expelling Undispensed Fluid from Tips



**WARNING! BIOHAZARD.** This procedure includes steps involving potentially infectious biological agents requiring that proper handling precautions be taken.

This procedure assumes that you have opened the instrument and accessed the pipettor head as described in the previous procedures.

1. Lift the pipettor head out of the fluidics module carefully, as it is still connected inside the instrument.
2. Hold the pipettor head over a tray or something into which you can expel liquid and remove tips.
3. With your other hand, rotate the black pulley on the bottom of the pipettor motor clockwise. This moves the plungers so that any liquid will be expelled and any tips will be ejected.

## Replace the Pipettor Head



**Note:** For a more complete version of these next steps with illustrations, see [Installing the Pipettor Head on page 56](#).

1. Slide the pipettor head back under its mounting plate inside the fluidics module, making sure to secure the white spiral cord back on its retaining hook.
2. Tighten the red retainer nut to secure the pipettor head. Make sure the knob is as tight as possible.
3. Replace the inner front panel and lower the cover back onto the instrument.
4. Reattach the power cord to the instrument and reconnect the instrument to the computer.
5. Turn on the instrument power. It takes about two minutes for the instrument to complete its startup sequence.
6. Save your SoftMax Pro Software file to prevent loss of data, and then close and restart the software.

## Recovery Procedure

When there is an instrument malfunction in which fluid was left in the tips, rebooting the instrument does not automatically reset the fluidics module. An additional recover cycle is necessary 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.

1. After the instrument completes the restart sequence, observe the instrument display.
  - ◆ If the temperature display on the front panel of the instrument is blank and a red X is covering the instrument icon on the SoftMax Pro Status bar, then see [Problems During Startup on page 130](#).
  - ◆ If the temperature display on the front panel of the instrument displays the actual current temperature, but the red X is still covering the instrument icon on the SoftMax Pro Status bar, then continue to the next step.
2. Click **Control > Recover**.

The recover process is essentially a second startup cycle that restores the fluidics module to operating conditions and restarts the instrument.
3. Click **Continue** to recover the software.

If the red X still remains after the Recover procedure, view the Preferences dialog in the software and recheck that the serial comm rate is set to 9600. For instructions, see [Problems During Startup on page 130](#).

If you cannot establish communication between the instrument and the computer with the Recover procedure, contact Technical Support. See [Obtaining Support on page 114](#).

## General Error Messages

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. To correct the problem, contact your local Molecular Devices representative for assistance.

### Error Messages

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

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

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

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

**Table 5-2:** FlexStation 3 Instrument Error Code Ranges

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

## Other Error Messages

Follow any instructions in the message. Any time a message persists, or if you cannot return the instrument and software to normal operation, contact Technical Support and be sure to note which error message you observed. See [Obtaining Support on page 114](#).



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**Note:** For error messages specific to fluidics operation failures, see [Assessing a Mechanical Problem on page 136](#).

---

If you see the following message, make sure that there is an assay plate correctly placed in the reading chamber drawer and then start the reading again.

- **There is no assay plate.**

If you see any one of the following messages, attempt your reading again before continuing 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 (follow procedure in section 6.7.1). If this is not the case, contact Technical Support. See [Obtaining Support on page 114](#).

- **Instrument reported a fatal error**

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

## Tilting or Removing the Fluidics Module

If you need to tilt or remove the fluidics module to clean up after a large spill, or to recover tips, follow these instructions.



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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 been trained properly by appropriate Molecular Devices personnel.

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

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**WARNING! BIOHAZARD.** This procedure includes steps involving potentially infectious biological agents requiring that proper handling precautions be taken.

---

### Tilting the Fluidics Module

1. Turn the power off to the instrument. Disconnect the power cords from the instrument and the cables connected to the computer.

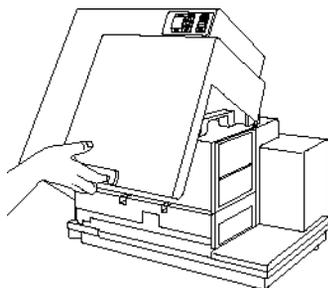


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**CAUTION!** Never operate the instrument with the covers removed. Damage to the instrument can occur. Read and obey all warning labels.

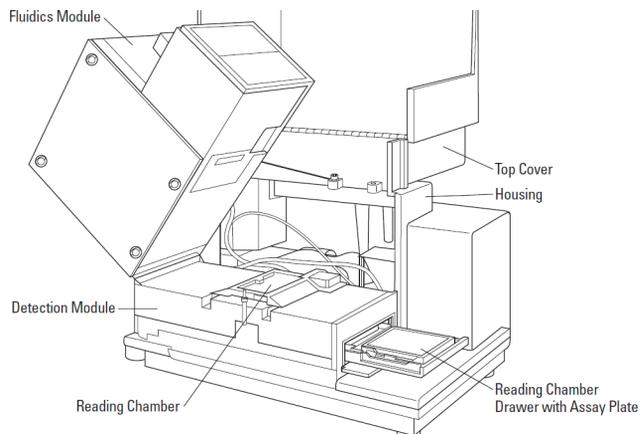
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2. Press in the handle at the bottom of the top cover and lift the cover back off the detection module, revealing the fluidics module.



**Figure 5-7: Opening the Top Cover**

3. Using the handle, tilt the fluidics module to the left.



**Figure 5-8: Tilting the Fluidics Module**

- ♦ If you can clean up the spill, or remove the tips, without removing the fluidics module, do so now.
  - ♦ If you need to remove the fluidics module completely, see [Removing the Fluidics Module on page 151](#).
4. Using the handle, lower the fluidics module carefully back over the detection module, and make sure it is firmly seated.
  5. Lower the top cover over the instrument.
  6. Reattach the power cord and computer cables.

## Removing the Fluidics Module



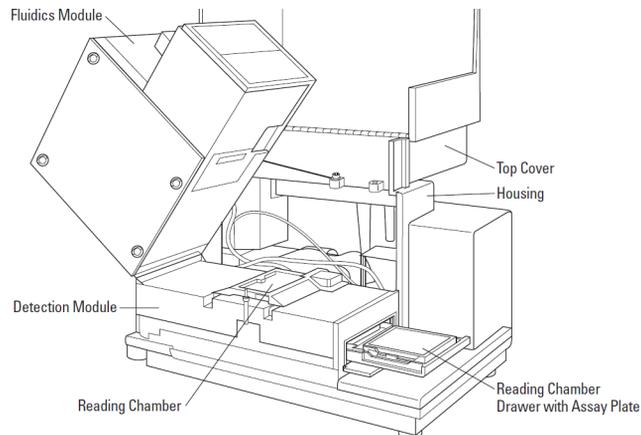
**CAUTION!** To avoid damage to the instrument, follow these instructions and any instruction labels on the instrument exactly.

1. Turn the power off to the instrument. Disconnect the power cords from the instrument and the cables connected to the computer.



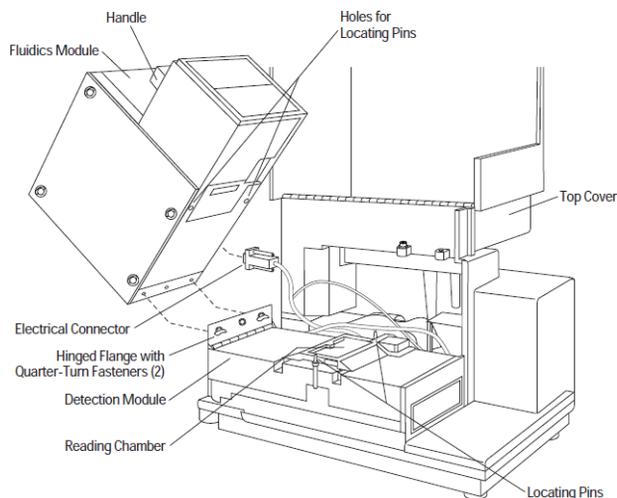
**CAUTION!** Never operate the instrument with the covers removed. Damage to the instrument can occur. Read and obey all warning labels.

2. Press in the handle at the bottom of the top cover and lift the cover back off the detection module, revealing the fluidics module.
3. Using the handle, tilt the fluidics module to the left.



**Figure 5-9:** Tilting the Fluidics Module

4. Locate the electrical connector between the bottom of the fluidics module and the housing, and then unscrew the retaining screws and unplug the connector.
5. Locate the fluidics module restraint (a green cable) and disconnect the restraint from the housing.



**Figure 5-10:** Removing the Fluidics Module

6. Locate and loosen the quarter-turn fasteners in the metal plate where the bottom of the fluidics module is attached to the detection chamber.
7. Lift the fluidics module carefully off the detection chamber and set it on the bench.
8. Clean the spill or remove tips. If you are attempting to remove tips, remove any obvious ones on top or around the sides of the reading chamber. If you look through the openings in the read head, you might be able to see tips inside.  
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.
9. Reattach the fluidics module by following the steps above in the reverse order, or see [Installing the Fluidics Module on page 53](#).
10. Lower the top cover over the instrument.
11. Reattach the power cord and computer cables.

If you see tips lodged inside the reading chamber, contact Technical Support. Do not attempt to tilt or remove the read heads on your own.



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



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

**Table A-1:** Part Numbers and Descriptions

Part Number	Description
4400-0002	Power cord, US
4400-0036	Power cord, European
9000-0149	Computer cable
0310-9336	Bottom read microplate adapter (reading chamber)
2500-1029	Compound Baseplate (compound plate drawer)
4600-0029	Fuse
0310-3992	Pipettor head, 8-channel
0310-5025	Pipettor head, 16-channel
0112-0127	FlexStation 3 Instrument User Guide
9100-0051	Hex key, 3/32 inch
9000-0911	96-Well, FlexStation Pipette tips (Black)
9000-0912	96-Well, FlexStation Pipette tips (Clear)
9000-0763	384-Well, FLIPR Tetra Pipette Tips (Clear)
9000-0764	384-Well, FLIPR Tetra Pipette Tips (Black)

## Ordering Parts and Accessories

To obtain parts and accessories, contact Molecular Devices Customer Service or your authorized service representative.

Molecular Devices

1311 Orleans Drive

Sunnyvale, California 94089

Telephone: +1-408-747-1700

Fax: +1-408-747-3601

U.S. and Canada Toll Free: +1-800-635-5577

Online: [www.moleculardevices.com](http://www.moleculardevices.com)

## Performance Specifications

Thermal specifications for microplates used in the FlexStation 3 instrument apply to flat-bottom microplates with isolated wells. All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

When pathlength compensation is applied to microplate absorbance measurements, the solution volume in the microplate well is between 100  $\mu\text{L}$  and 300  $\mu\text{L}$ .

Technical specifications are subject to change without notice.

**Table B-1:** Fluidics Specifications for the 8-Channel Pipettor

Item	Specification
Volume range	1 $\mu\text{L}$ to 200 $\mu\text{L}$
Dispensing precision	8% CV at 5 $\mu\text{L}$ 2% CV at 50 $\mu\text{L}$
Maximum dispense rate	208 $\mu\text{L}$ per second

**Table B-2:** Fluidics Specifications for the 16-Channel Pipettor

Item	Specification
Volume range	1 $\mu\text{L}$ to 30 $\mu\text{L}$
Dispensing precision	3% CV at 10 $\mu\text{L}$ 5% CV at 1 $\mu\text{L}$
Maximum dispense rate	52 $\mu\text{L}$ per second

**Table B-3:** Absorbance Photometric Performance

Item	Specification
Wavelength range	200 nm to 1000 nm
Wavelength selection	Monochromator tunable in 1 nm increments
Wavelength bandwidth	≤ 4.0 nm
Wavelength accuracy	± 3.0 nm
Wavelength repeatability	± 0.2 nm
Photometric range	0.0 OD to 4.0 OD
Photometric resolution	0.001 OD
Photometric accuracy/linearity, 0 OD to 2.0 OD	± 0.006 OD ± 1.0%
Photometric precision (repeatability), 0 OD to 2.0 OD	± 0.003 OD ± 1.0%
Stray light	≤ 0.05% at 230 nm
Photometric stabilization	Instantaneous
Photometric drift	None Continuous referencing of monochromatic input
Calibration	Automatic before first kinetic read and before every endpoint read
Optical alignment	None required
Light source	Xenon flash lamp (50 Watts)
Average lamp lifetime	1 billion flashes
Photodetectors	Silicon photodiode

**Table B-4:** Fluorescence Intensity Performance

Item	Specification
Sensitivity	Top Read < 5 pM FITC, 1 fmol/200 $\mu$ L (96) < 20 pM, 2 fmol/100 $\mu$ L (384)
Wavelength range	250 nm to 850 nm
Wavelength selection	Monochromators, tunable in 1 nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Number of excitation/emission pairs per plate	4
Dynamic range	106 in 96-well black plates: auto gain circuitry
System validation	Self-calibrating with built-in fluorescence calibrators
Light source	Xenon flash lamp (1 joule/flash)
Average lamp lifetime	2 years normal operation
Detector	Photomultiplier

**Table B-5:** Fluorescence Polarization Performance

Item	Specification
Wavelength range	400 nm to 750 nm
Wavelength selection	Monochromators, tunable in 1 nm increments
Bandwidth (excitation, emission)	9 nm, 15 nm
Precision	< 5 mP standard deviation at 1 nM fluorescein in 96-well and 384-well microplates

**Table B-6:** Time-Resolved Fluorescence Performance

Item	Specification
Sensitivity	100 fM europium in 96-well or 384-well microplates (top read)
Wavelength range	250 nm to 850 nm
Bandwidth (excitation, emission)	9 nm, 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 B-7:** Luminescence Photometric Performance

Item	Specification
Sensitivity	Sensitivity < 2 fg/well for firefly luciferase in 96-well and 384-well microplates (top read)
Wavelength range	250 nm to 850 nm
Crosstalk	< 0.3% in 96-well and 384-well microplates.

**Table B-8:** Photometric Analysis Mode Using SoftMax Pro Software

Item	Specification
Data expression	Absorbance: OD or %Transmittance Fluorescence: Relative Fluorescence Units (RFU) Luminescence: Relative Luminescence Units (RLU)
Wavelengths per read	1 to 4 wavelengths per microplate read
Read types	Endpoint Kinetic and kinetic graphics Spectral scan: 200 nm to 1000 nm Well scan (absorbance or fluorescence)

**Table B-9:** Measurement Time (Calibration Off)

Item	Specification
Absorbance, single wavelength	96 wells: 18 seconds 384 wells: 49 seconds
Fluorescence, single wavelength	96 wells: 17 seconds 384 wells: 48 seconds

**Table B-10:** Temperature Regulation

Item	Specification
Reading chamber	Isothermal when temperature regulation is not enabled
Range	2°C above ambient to 45°C when temperature regulation is enabled Ambient temperature must be >20°C to achieve temperature regulation at 45°C
Resolution	± 0.1°C
Accuracy	± 1.0°C for reading chamber
Temperature uniformity at equilibrium	± 1.0°C at 37°C
Chamber warm-up time	30 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	4 sensors
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling
Recommended microplate	Flat-bottom microplates with isolated wells and lid
Control	Reports reading chamber temperature only

**Table B-11:** Automix with SoftMax Pro Software

Item	Specification
Plate mixing modes	Selectable: off, once before any reading, or once before and between kinetic readings
Plate mixing duration	Selectable: 0 to 999 seconds (three-second default)

**Table B-12:** Microplate Compatibility

Item	Specification
All modes	Standard 6-well to 384-well, flat-bottom microplates low-volume 384-well microplates
Absorbance reads above 340 nm	Polystyrene microplates
Absorbance reads above 220 nm	UV-transparent microplates
Absorbance reads above 200 nm	Quartz microplates
Black adaptor plate	Use with 96-well and 384-well microplates only

**Table B-13:** General Instrument Specifications

Item	Specification
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism and reading chamber assembly protected from accidental spillage by drainage ports
Computer interface	8-pin DIN RS-232 serial (double shielding required).
Printer interface	Parallel 25-pin to Centronics (double shielding required).
Microplates supported	All 6-well to 384-well and strip-well microplates, including lids.

**Table B-14:** Robotics and Automation

Item	Specification
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 B-15:** Environmental Specifications

Item	Specification
Operating temperature	15°C to 60°C
Operating humidity	0% to 70%, non-condensing
Storage temperature	-20°C to 65°C

**Table B-16:** Physical Specifications

Item	Specification
Size (H x W x D)	48 cm × 58 cm × 41 cm )19 in. × 23 in. × 16 in.)
Weight	22.7 kg (50 lbs.)
Power consumption	500 VA
Line voltage and frequency	90 to 240 VAC, 50 to 60 Hz



# Glossary

## Absorbance

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

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

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

## Automix

The Automix function in the SoftMax Pro Software determines if and how often automated shaking of the microplate is performed during a reading.

## Emission Cutoff Filter

An emission cutoff filter is a long pass filter used to condition the emission light before detection by the 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

In an Endpoint read, a reading of each microplate well is taken in the center of each well, at a single wavelength or at multiple wavelengths. Depending on the read mode, raw data values are reported as optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

## **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 excitation filter is automatic. In excitation spectrum scans, the user has the choice of “no excitation filter” (for smoother scans) or “auto excitation filter” in which case 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.

## **Fluorophore**

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

## **Gain**

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

## **Incubator**

In the SoftMax Pro Software, clicking Control > Incubator or clicking the incubator button opens a dialog allowing you to start or stop temperature regulation and to select an elevated temperature for the reading chamber.

## **Instrument Setup**

The instrument setup defines the parameters (mode, wavelengths, automatic mixing, run time, read interval, and so on) used to read the microplate.

## **Kinetic**

In a Kinetic read, the instrument collects data over time with multiple readings taken at regular intervals. To achieve the shortest possible interval for Kinetic readings, choose wavelengths in ascending order. The values calculated based on raw kinetic data include VMax, VMax per Sec, Time to VMax, and Onset Time. Kinetic readings can be single-wavelength or multiple-wavelength readings.

## **Luminescence**

Luminescence is the emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation. Luminescence detection relies on the production of light from a chemical reaction in a sample.

## **Photomultiplier Tube (PMT)**

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

## **Read Mode**

The read mode is type of reading performed: absorbance, fluorescence intensity, time-resolved fluorescence, fluorescence polarization, or luminescence.

## **Read Type**

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

## **Readings per Well**

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

## **SoftMax Pro Software**

The SoftMax Pro Software is an integrated software program from Molecular Devices that is used to control and collect data from Molecular Devices microplate readers.

## Stokes Shift

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

## Time-Resolved Fluorescence

Time-resolved fluorescence (TRF) is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the amount 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 cut off of each light pulse and the start of signal accumulation. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the microplate) 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.

## Trituration

Trituration is mixing of the contents of the wells in either or both the compound source and assay plates. Trituration is accomplished by fluid being alternately aspirated from and dispensed back into a well using the pipettor head.

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