FLIPR Penta

High-Throughput Cellular Screening System

Hardware and ScreenWorks Software Version 5.1

User Guide

5069582 G
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MOLECULAR DEVICES

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Contents

Safety Information

The safety information section provides information on the safe use of the instrument. It includes the use of user-attention statements in this guide, a key to understanding the safety labels on the instrument, precautions to follow before operating the instrument, and precautions to follow while operating the instrument.

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

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

Warnings, Cautions, Notes, and Tips

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

When warnings and cautions are displayed in this guide, be careful to follow the specific safety information related to them.

The following user-attention statements can be displayed in the text of Molecular Devices user documentation. Each statement implies a particular amount of observation or recommended procedure as described:

WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed. The warning symbol can vary depending on the warning. The definition of the symbol is included in the text of the warning.

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

Note: A note calls attention to significant information.

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

Symbols on the Instrument

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

Symbol	Indication
	The product documentation must be consulted. Indicates a warning for a situation or operation that could cause personal injury if precautions are not followed. There are specific details written next to the warning symbol.
	Potential ultraviolet light hazard.
	Potential pinch hazard.
	Potential heat hazard.
	Power on.
	Power off.
	Instrument manufacture date.
	European technology conformity.
	United Kingdom technology conformity.
	Certification by TUV Rheinland for compliance with US and Canadian product safety standards.
	Compliance with TUV Rheinland product safety for Germany.
	Compliance with Chinese RoHS Pollution Control Requirements.
	Compliance with California Proposition 65, which requires businesses to warn Californians about significant exposures to chemicals that cause cancer, birth defects or other reproductive harm.

Electrical Safety

To prevent electrically related injuries and property damage, inspect all electrical equipment before use and immediately report all electrical deficiencies. Contact Molecular Devices Technical Support to service equipment that requires the removal of covers or panels.

The instrument must be connected to a properly grounded power outlet to protect from the risk of electric shock. The main chassis of the instrument is grounded together with all related electrical components.

Do not remove the fixed covers, as there are no user-serviceable parts inside. All electrical work must be referred to Molecular Devices approved service personnel.

In the event of a liquid spillage into the main cavity of the instrument, disconnect the mains power supply before trying to clean up.

If the external covers on the instrument are removed, the power supply does not automatically stop.

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

Do not try to use the instrument until all covers are replaced.

To provide access for disconnecting power from the instrument, maintain a 66 cm (26 in.) minimum clearance area on the right side of the instrument.

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

Chemical and Biological Safety

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

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

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

Moving Parts Safety

To prevent injury due to moving parts, observe the following:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.
- Keep the interior of the instrument clear to prevent obstruction of the movement.

The motors use high-powered magnets. The linear drive units and encoders are delicate, so be very careful with them. To prevent serious damage to the instrument or its auxiliary parts, follow the preparation instructions in this guide before every process.

The instrument door automatically locks whenever you run a process. The door prevents UV light from passing through during operation.

As a safety measure, if the door is open, an electromagnetic switch prevents the instrument from running. Never tamper with this switch, as it serves two purposes:

- It prevents the motors from running to reduce the potential of physical damage.
- It disables the UV light to prevent the risk of damage from UV radiation.

Motors and their related drives and cabling are sources of electromagnetic fields. Keep magnetic storage devices or strips, such as hard drives and credit cards, away from the instrument covers.

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

Ultraviolet (UV) Light Safety

The instrument door automatically locks whenever you run a process. The door prevents UV light from passing through during operation.

As a safety measure, if the door is open, an electromagnetic switch prevents the instrument from running. Never tamper with this switch, as it serves two purposes:

- It prevents the motors from running to reduce the potential of physical damage.
- It disables the UV light to prevent the risk of damage from UV radiation.

Chapter 1: Introduction

The FLIPR® Penta High-Throughput Cellular Screening System provides an automated solution to identify early leads in the drug discovery process and to evaluate drug efficacy and toxicity. The system supports fast kinetic cellular assays with simultaneous pipette and read function and can be configured based on library size, detection mode, screening format, assay and target. A fully integrated solution from assay development to lead optimization.

The FLIPR Penta System includes:

- Simultaneous 96-well, 384-well, or 1536-well liquid or cell transfer
- Expanded wavelength support
- User-configurable pipettors and optics
- FLIPR® Cycler, internal robotic plate handler option that works in conjunction with external robotic software
- Choice of camera:
  - EMCCD camera for fluorescence applications
  - HS EMCCD high-speed, high-sensitivity camera for fluorescence and luminescence
- Compact platform with minimal facilities requirements
- ScreenWorks® Software and optional ScreenWorks® Peak Pro™ Software modules for analysis

Workflow

The FLIPR Penta High-Throughput Cellular Screening System uses the following sequence of processes:

1. Prepare the cells.
2. Power on the system.
3. Run a system check.
4. Load cell dye.
5. Prepare source plates.
6. Set up an assay protocol.
7. Run the experiment.
8. Analyze the data.

Before You Start

CAUTION! Before you use the instrument, read and understand all the safety instructions. Then follow the procedures in Powering On the System on page 16.

System Requirements

Electrical

The FLIPR Penta System consumes 5 A continuous and 9 A peak of 110 V power and requires 90-240 VAC power source at 50-60 Hz, which equates to 2.5 A continuous and up to 4.5 A peak at 240 VAC/50 Hz. The system ships with a power cord appropriate for the receiving country.

Note: The power supply has a circuit breaker rated at 15 A because the inrush current has been measured to be as high as 13 A at 110–120 VAC. Customers with 240 VAC should inform their facilities engineers that a breaker may have to be installed for their electrical lines to handle an inrush current 2x the 13 A current. Molecular Devices recommends a D curve circuit breaker for this equipment. Additional shared outlets are required for computer and monitor. A power strip, not included, is acceptable for providing the additional outlets for the computer and monitor.

Minimum Lab Space

System dimensions are approximately 99 cm wide x 69 cm deep x 1780.44 mm high (39 inches wide x 27 inches deep x 70.25 inches high).

Note: To open the stage access door you need more than 76 cm (30 inches) additional space in front of the instrument.

The external ScreenWorks Software host computer mounts on the right side of the instrument cabinet. The monitor, keyboard, and mouse install on an adjustable arm attached to the front right side of the instrument cabinet. Combined, they require an additional minimum amount of space of 102 cm (40 inches) wide x 102 cm (40 inches) deep for maneuverability.

A chiller with dimensions 33 cm (L) x 28 cm (W) x 28 cm (H) (13 inches L X 11 inches W by 11 inches H) connects to the right side of the instrument with a 91.4 cm (3 feet) long tube. Place the chiller within the 91.4 cm (3 feet) radius with access to the chiller on/off button.

A minimum 81 cm (32 inch) square footprint is required on the right side of the instrument for tip wash bottles.

The instrument cabinet should have service access space of 122 cm (48 inches) in front, 61 cm (24 inches) behind, and 25.4 cm (10 inches) on the left.

The FLIPR Penta System has rolling castors to move it for necessary adjustments and perform maintenance. The lower instrument chassis has foot levelers to establish a uniform instrument deck level in situations where the lab floor is not flat and to stabilize the instrument when you integrate a robot. Lower and level the instrument feet when you run an experiment.

WARNING! The FLIPR Penta System can weigh as much as 390 kg (860 lbs.). Follow all necessary safety precautions and use proper moving techniques.

Computer System

The FLIPR Penta System uses two computers, an embedded computer and a host computer.

Embedded Computer

The embedded computer is in the lower chamber of the instrument cabinet. The embedded computer controls the FLIPR Penta System basic functions that the ScreenWorks Software initiates. The ScreenWorks Software is installed on the host computer. This allows separate data processing and instrument control to ensure maximum productivity during an experiment.

Note: External or local IT interference with the embedded PC is not recommended. Contact support for any assistance.

Host Computer

You use the ScreenWorks Software that runs on the external host computer to interact with the FLIPR Penta System.

Computer Specifications

Item	Description
CPU	2.5 GHz, quad processor or faster
Operating system	Windows 10 (64-bit), or Windows 11
Memory	32 GB RAM (8 GB RAM minimum)
Data Connection	10/100 Ethernet port
Storage	500 GB hard drive
Display	27 in., 2560 x 1440 resolution maximum, Custom scaling set to not exceed 107%

Note: Do not change the Display settings to those that exceed the stated specifications.

Do not install anti-virus software that blocks ports or applications which could slow down the performance of software. Do not upload anti-virus software to avoid blocking the software.

Avoid blocking the following ports:

- 5001 - Used for communication between the embedded computer and the host computer.
- 3389 - Used by service engineers to access the embedded computer system.
- 1947 - Used for software license activation.

Operating System Disclaimer

Microsoft continuously updates the Windows operating system. Molecular Devices tests the compatibility of the ScreenWorks Software with a selection of the current Windows operating system releases that are available when the ScreenWorks Software is in development.

The following Windows operating systems are known to be incompatible with the ScreenWorks Software and are not supported: Windows 10/11 Home edition, Mobile edition, and Enterprise IoT edition.

Please contact Molecular Devices for guidance before you select or update your Windows operating system. Although not tested, other Windows operating systems are compatible with the ScreenWorks Software.

Software Installation

Your Molecular Devices representative sets up the FLIPR Penta System in your lab. Use the following software installation procedures when you update the ScreenWorks Software on the host computer. You must have administrative privileges on the computer.

Installing ScreenWorks Software

The installer uninstalls the old software version as long as it is the same major release (5.x to 5.x+x). If you update to the next major release (4.x to 5.x), you must manually uninstall the old software. See Uninstalling ScreenWorks Software on page 15.

To install the software:

1. Double-click the **ScreenWorks_x.x.exe** ScreenWorks Software installation file to display the Welcome to the ScreenWorks Setup Wizard.
2. Click **Next** to display the License Agreement page.
3. Select the **I Accept the Terms of the License Agreement** check box.
4. Click **Next** to display the Online/Offline Mode page.
5. Select a default mode in which to start the software:
   - Select **Online** to have the software look for a connected instrument when the software starts.
   - Select **Offline** to have the software not look for a connected instrument.

   See Online Mode vs. Offline Mode on page 39.
6. Click **Next** to display the Destination Folder page.
7. Note the default installation directory. To change the installation directory, click **Change**, navigate to the destination directory, then click **OK**.
8. Click **Next** to display the Select Program Folder page.
9. Leave the default Program Folder settings and select the **Anyone Who Uses This Computer** check box to make the software available to all users on the FLIPR Penta System host computer.
10. Click **Next** to display the Configuring the ScreenWorks Installation page.
11. Click **Next** to start the installation.
12. When the Completing the Installation Process page displays, click **Finish**.

Activating the ScreenWorks Peak Pro License

The ScreenWorks® Peak Pro™ Software is license protected. The software license activation allows the Peak Pro analysis functionality after the trial period expires. The following Peak Pro analysis licenses are available for purchase:

- ScreenWorks Peak Pro Software version 1.0 adds additional Kinetic Reduction Configuration types.
- ScreenWorks Peak Pro Software version 2.0 adds a new Peak Pro 2.0 Analysis dialog and support for high-speed and high-sensitivity data.

Note: There is a 14-day Peak Pro trial usage option in the Help menu. When the trial period expires, the Peak Pro 1.0 analysis functionality disappears, the Peak Pro 2.0 analysis functionality disables, and the Help menu trial option is inactive. The rest of the ScreenWorks Software remains functional.

To activate the ScreenWorks Peak Pro Software license:

1. Start the ScreenWorks Software.
2. Select the **Help** tab and click **Software License**.
3. If you did not start the 14-day Peak Pro Software trial, either start the 14-day Peak Pro Software trial or enter the product key. After the trial expires, you can only enter the software license product key. Click **Yes**.
4. If the computer has internet access, in the **Product Key** field, enter the product key and click **Activate Online**. Then follow the on-screen instructions.
   If the computer does not have Internet access, click **Activate Offline** and follow the on-screen instructions. You need:
   - Product key
   - A computer with Internet access
   - A USB to transfer files between the computers
5. Restart the ScreenWorks Software to finish the license activation.

Uninstalling ScreenWorks Software

To uninstall the ScreenWorks Software:

1. Open the Control Panel.
2. Depending on the Windows operating system:
   - Windows 7 - Click **Add or Remove Programs**
   - Windows 10 - Click **Programs and Features**
3. Select **ScreenWorks**.
4. Click **Remove** or **Uninstall**.
5. Follow the on-screen instructions to finish the software removal.

Powering On and Powering Off the System

Do the following to power on and power off the FLIPR Penta System, which includes connecting the ScreenWorks Software to the instrument.

Powering On the System

To power on the FLIPR Penta System:

1. Power on the computer and the monitor.
2. Simultaneously press **CTRL+ALT+DELETE** to launch the Windows operating system.
3. Enter your password.

   Note: The first time you log in after system installation, the default password is *fliprtetra*.

4. Power on the external chiller with the switch on the left side of the chiller.

   CAUTION! When the system is idle, the external chiller should remain powered on to protect the camera.

5. Power on the FLIPR Penta System with the switch located on the right side of the instrument.

   Note: The system goes through an initialization cycle to register all of the instrument components. This cycle is not complete until the green Assay Finished (Unlock) light on the upper door is the only light illuminated on the instrument status panel.

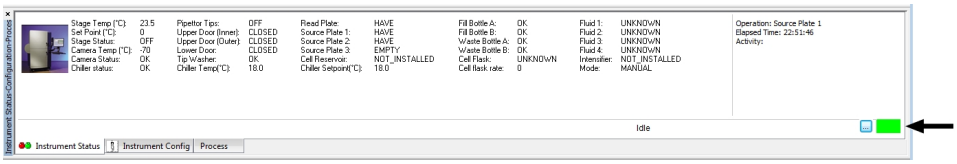
6. On the computer desktop, double-click  to start the ScreenWorks Software.
   Do not repeatedly double-click the software icon. The software can take several seconds to start.

Note: The system is ready for use when the camera temperature is at operating temperature. The camera temperature and status display in the ScreenWorks Software Instrument Status panel. The camera operating temperature depends on the camera installed in the instrument. See CCD Camera Options on page 29.

Connecting the Software to the Instrument

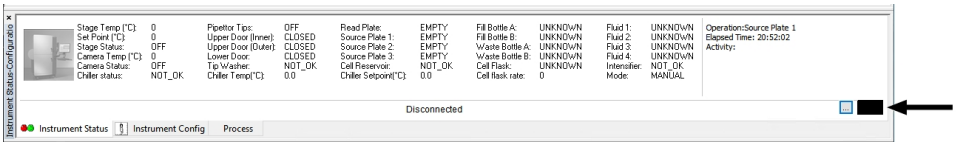
After you power on the instrument, when you start the ScreenWorks Software in Online mode, it should automatically connect to the instrument. In the software, in the Instrument Status panel, the lower right corner of the screen displays a green connection status icon to indicate the connection and 

displays in the toolbar.



If the connection status icon is black, the software is disconnected from the instrument and 

displays in the toolbar.



Click 

to connect the software to the instrument.

Powering Off the System and the Software

To power off the FLIPR Penta System:

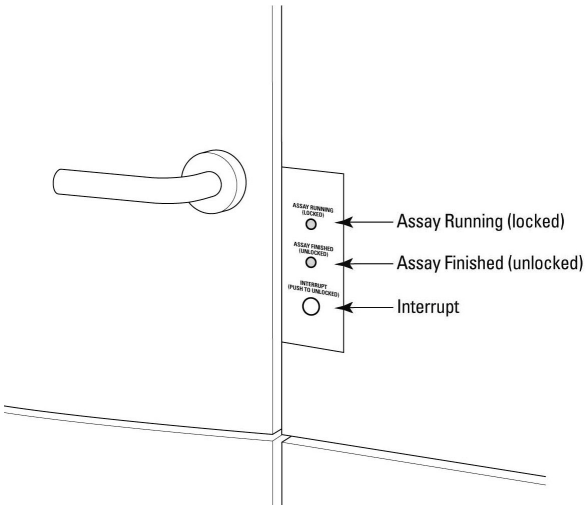
1. Wait for the Assay Finished (Unlocked) light on the upper door, Instrument Status Panel to turn green which indicates the experiment is finished.
2. If the last protocol run did not remove the tips from the pipettor head, use the manual command to remove the tips. Failure to remove tips can result in an error on next start.
3. Click the **File** menu and select **Exit** to exit the ScreenWorks Software.
4. Power off the computer and the monitor.
5. Power off the FLIPR Penta instrument.
6. Power off the chiller.

Checking Instrument Status and Configuration

Check the instrument status on the front of the instrument and from within the ScreenWorks Software. Check the instrument configuration status from within the ScreenWorks Software.

System Status Panel

The system status panel, located next to the upper door handle, indicates if the door is locked or unlocked, depending on the instrument activity. It also includes an emergency Interrupt button to stop any running processes.



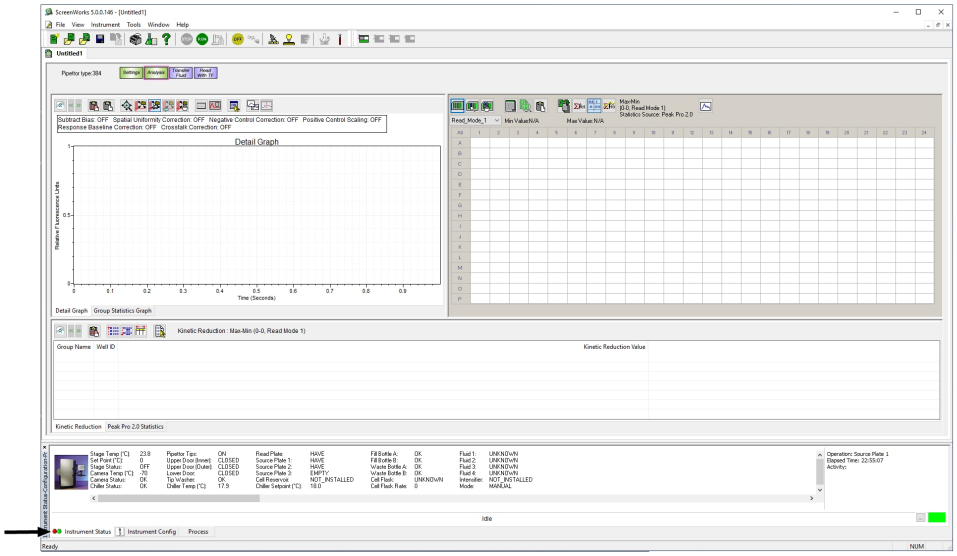
The following are on the System Status panel:

- **Assay Running (Locked)** Yellow light - The system is performing a task. The upper and lower instrument doors are locked until the task finishes or is stopped.
- **Assay Finished (Unlocked)** Green light - The system is not performing a task and it is safe to open the upper and lower instrument doors.
- **Interrupt** Flashing green light - Press to override the system and stop all tasks, so you can access the instrument. When pressed, the light flashes until the system reaches a safe state for you to open the doors.

CAUTION! The Interrupt button immediately ends the experiment and should only be used in emergencies. Before you can use the instrument again, you might need to be reinitialize the system from within the software. Click the Instrument menu and select Reset.

Software Status Tabs

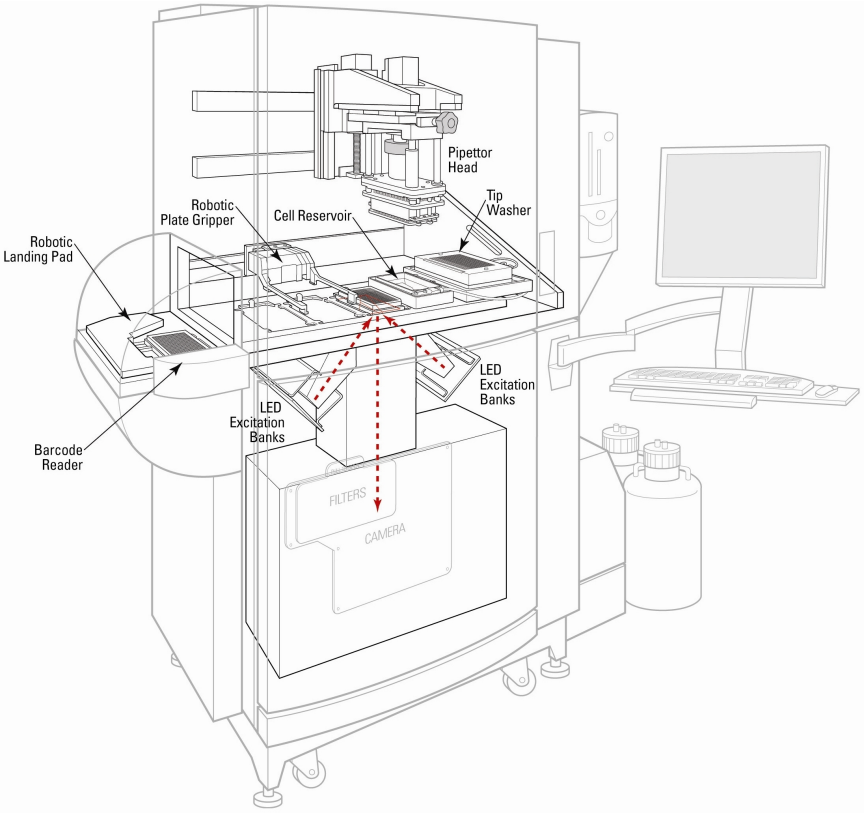
Within the ScreenWorks Software, the Instrument Status Configuration Process tab, located on the bottom of the main screen, reports the status and settings for the FLIPR Penta System hardware and includes the processes you use to create protocols. See Instrument Status Configuration Process on page 55.



Chapter 2: System Hardware Overview

2

The FLIPR Penta System consists of a cabinet 965 mm (39 inches) wide x 686 mm (27 inches) deep x 1780 mm (70 inches) high. There is a top compartment for wet components and bottom compartment for dry components. Outside the cabinet are the wash system fluid bottles, chiller, computer, monitor, keyboard, and mouse.



FLIPR Penta System with Optional Modules

For system component details, see:

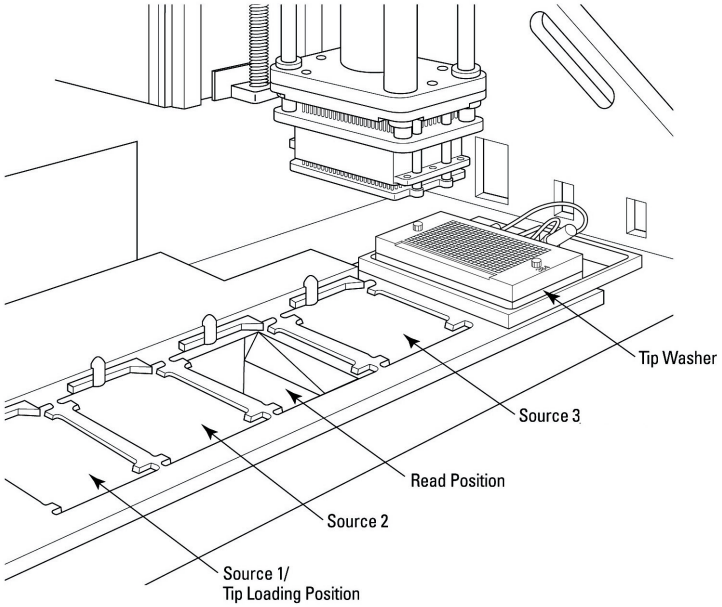
- Five-Position Stage on page 22
- Plate Support on page 23
- Plate Handling System on page 24
- Liquid Handling System on page 26
- Optical System on page 29
- Chiller on page 37

Five-Position Stage

Run experiments from the five-position stage in the upper compartment of the instrument. Manually load plates through the upper compartment door before an experiment or robotically during the experiment using the optional FLIPR® Cycler internal plate handler.

The following are the five plate positions of the stage from left to right:

- Position 1 - Tips and Source Plate 1
- Position 2 - Source Plate 2
- Position 3 - Read Plate
- Position 4 - Source Plate 3
- Position 5 - Tip Washer



Positions 1, 2, and 4 support standard, low-volume, deep-well and reservoir source addition plates.

Position 1 is to load and unload tips and can be used as a source plate position after tips load and the tip box is removed.

Position 3 opens to the optics chamber below for excitation of fluorophores in read plate wells, the read position, and emission reads.

Position 4 is a source plate position.

Positions 1 through 4 have a mechanical plate sensor to identify the presence of plates, tips, or reservoirs.

Position 5 is a dedicated tip wash reservoir and should be configured to match the pipettor heads, such as 96, 384, or 1536. Tip wash reservoirs are included with the purchase of a pipettor head. See Washing Pipettor Tips and Pin Tools on page 139.

Robotic integration allows the optional FLIPR Cycler internal plate handler to automatically exchange up to 12 source plates and tip racks and one read plate in an experiment. See Automating Plate Loading on page 24.

The plate indexers in positions 1 through 4 register plates and tip racks with well A1 in the lower left corner. The indexers serve as mechanical sensors to detect the presence of plates or tips. If the instrument does not sense plates or tips during a Manual mode experiment when the software requests a plate or tips, the instrument stops and ends the experiment. During Remote mode, the system notifies the SynchroMax™ ET plate handler robot or third-party plate handler robot that no plate or tip container is available and stops the instrument until it detects plates or tips. The SynchroMax ET plate handler or third-party plate handler is then responsible to deliver plates or tips to the system. See SynchroMax ET External Plate Handler on page 26.

CAUTION! Sensors can only detect the presence of a plate or a tip container. Sensors cannot identify the type of plate or tips, or if lids are on the plate. It is your responsibility to ensure that the correct plates and tips are loaded into position without lids.

Note: Molecular Devices no longer sells the SynchroMax ET plate handler robot. The FLIPR Penta System becomes dependent on the robot system configuration.

Regulating Stage Temperature

Plate stage positions 1, 2, and 4 for source plates, have temperature control. Stage temperature settings range from ambient 25°C (77°F) to 40°C (104°F). It can take approximately 15 minutes to reach equilibrium temperature.

Configure temperature regulation with the Temperature Control ON/OFF toggle command. Click the **Instrument** menu and select **Manual Operation**.

Plate Support

The FLIPR Penta System supports the use of 96-well, 384-well, and 1536-well plates that conform to the ANSI standards submitted by the Society for Biomolecular Sciences.

Note: Only low-profile 1536-well plates are supported.

Black walled, clear-bottomed read plates provide an optimal imaging environment for fluorescence assays. These plates prevent signal diffraction while allowing excitation and signal access. Use black walled, clear-bottomed plates or white walled, clear-bottomed plates for luminescence assays.

For 96-well read plates, use a slit-shaped mask to minimize saturation and edge effects associated with these plates. Place the mask over the read position. 384-well and 1536-well plates do not require a mask. See Consumables and Accessories on page 179.

Use the 96-pipettor heads and 384-pipettor heads with source or read plates with equal or one order higher well number. The FLIPR Penta System can aspirate or dispense into quadrants of a plate. The following combinations are possible:

- Use the 96-pipettor head with 96-well and 384-well plates.
- Use the 384-pipettor and pin tool head with 384-well and 1536-well plates.
- Use the 1536-pipettor and pin tool head with 1536-well plates.

You can substitute deep-well plates or reservoirs for standard well plates.

When you aspirate or deliver compound to a plate with a greater number of wells than the pipettor head, enter the quadrant number in the protocol configuration for each dispense.

Many compatible source and read plates are available from Molecular Devices. See Consumables and Accessories on page 179.

For compatible plate details, see FLIPR Penta Plate Dimensions on page 189.

Plate Handling System

The plate handling system includes two methods to place plates on the five-position stage to run experiments.

Loading Plates Manually

Before you run an experiment in Manual mode, position the assay components in the five-position stage by hand in the upper compartment. You cannot make additional plate or tip changes after the experiment starts.

Note: Keep the top compartment door closed during system operation. All system functions stop when the door is open.

Automating Plate Loading

Note: Molecular Devices no longer offers the SynchroMax ET external plate handler robot. The following information is for reference only.

Support for automated plate delivery with the FLIPR Penta System is facilitated using optional robot systems.

To increase the number of plates in an experiment and minimize personnel requirements, use the FLIPR Cycler internal plate handler coupled with the external SynchroMax ET plate loader robot or a third-party plate loader robot. One read and up to 12 source plates and tip racks can shuttle in and out of the FLIPR Penta System in one experiment using automated delivery.

When you use automated delivery, the SynchroMax ET plate loader robot or third-party plate loader delivers plates to the landing pad on the outside left of the instrument and picks plates up from the landing pad on the outside left of the instrument. The FLIPR Cycler plate handler then shuttles the plates in and out of the plate stage compartment. The shuttle door over the landing pad opens and closes to maintain a light-tight environment within the compartment.

During automated operation the SynchroMax ET plate loader or third-party plate loader controls the FLIPR Penta System by sending instructions to load protocols, run experiments, and retrieve plates from the landing pad. The instrument executes these commands upon receipt from the SynchroMax ET plate loader or third-party plate loader. Instrument settings cannot be persisted from the remote controlling program. You must configure these settings in the ScreenWorks Software before control passes to the plate-delivery system.

To start Remote Mode:

- To pass control to the plate delivery software, in the ScreenWorks Software, click the **Instrument** menu and select **Set Remote Mode**.

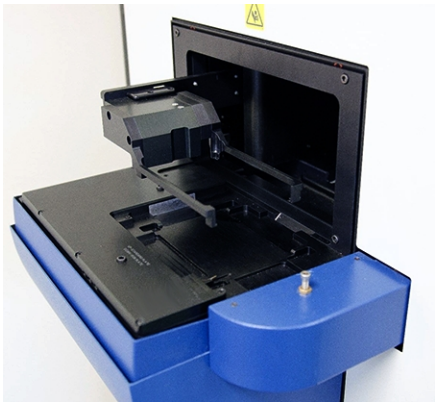
To stop Remote Mode:

- The FLIPR Penta System stays in remote control until you click the **Instrument** menu and select **Set Manual Mode**.

The third-party plate loader software communicates with the FLIPR Penta System computer through the serial communication port. Install the SynchroMax ET software with the ScreenWorks Software on the FLIPR Penta System computer so it can communicate directly with the instrument. See Robotic Integration on page 203.

FLIPR Cycler Internal Plate Handler

The FLIPR Cycler plate handler system is a plate gripper that runs along the back wall of the upper read compartment to give it access to positions 1 through 4 in the five-position stage and the landing pad on the outer left side of the cabinet.



The FLIPR Cycler plate handler shifts source plates and tip containers between these locations when under the control of the external SynchroMax ET plate holder robot or a third-party plate handler.

The FLIPR Cycler plate handler carries standard, low volume and deep well 96-well, 384-well, and 1536-well plates that conform to proposed ANSI standards submitted by the Society for Biomolecular Sciences. The FLIPR Cycler plate handler can move Molecular Devices qualified tips.

Note: You can use reservoirs during robotic integration, however the FLIPR Cycler plate handler cannot move the reservoir. You must manually load the reservoirs before you run an experiment, including the cell reservoir.

CAUTION! Some plates may not be handled as reliably by the FLIPR Cycler plate handler because of their low plate weight. During robotic integration, you should evaluate plate and tip handling by the FLIPR Cycler plate handler for handling reliability before you start a screen. The FLIPR Penta System is validated for use with the Molecular Devices tips and there is no guarantee regarding performance or troubleshoot any instrument issues with any other tips.

The upper and lower door of the FLIPR Penta System must remain closed for the duration of the experiment. Plates are transported in and out of the instrument through the FLIPR Cycler plate handler robotic landing pad door.

WARNING! Do not place your fingers in the FLIPR Cycler shuttle door as this can cause injury.

SynchroMax ET External Plate Handler

Note: The SynchroMax ET plate handler robot is no longer available for new purchases. Other robotic systems are supported.

The SynchroMax ET robot is a six-stack plate handler that was available as an optional purchase with the FLIPR Penta System. It delivers plates to and from the landing pad and integrates with the FLIPR Cycler internal plate handler, which ferries the plates to and from their locations in the five-position stage. The configuration interface of the SynchroMax ET software opens from within ScreenWorks Software.

Liquid Handling System

The liquid handling system includes four components to move liquids to and from the five-position stage.

Standard Pipettor Head

Standard pipettor heads are available in 96-tip, 384-tip, and 1536-tip formats. The 96-pipettor head and 384-pipettor head both use disposable plastic tips.

The 1536-pipettor head uses a stainless-steel tip block with a disposable 1536-tip gasket. Wash the plastic tips, replace them between each compound addition, or replace them at the end of an experiment. Was the 1536-tip block at specified times.

The ScreenWorks Software protocols control pipettor operations. You can use commands in the Instrument menu to load tips. Connectors on the back of the pipettor head identify the head format as 96-tips, 384-tips, or 1536-tips and the ScreenWorks Software offers valid plate formats and pipetting parameters for protocol setup.

The standard pipettor head uses air displacement to control aspiration and dispense speed and volume. Use the software to configure the volume of compound to transfer. You can configure the protocol to draw compound from multiple source plates to dispense into one destination plate or to aspirate from one plate and dispense to multiple well plates or quadrants.

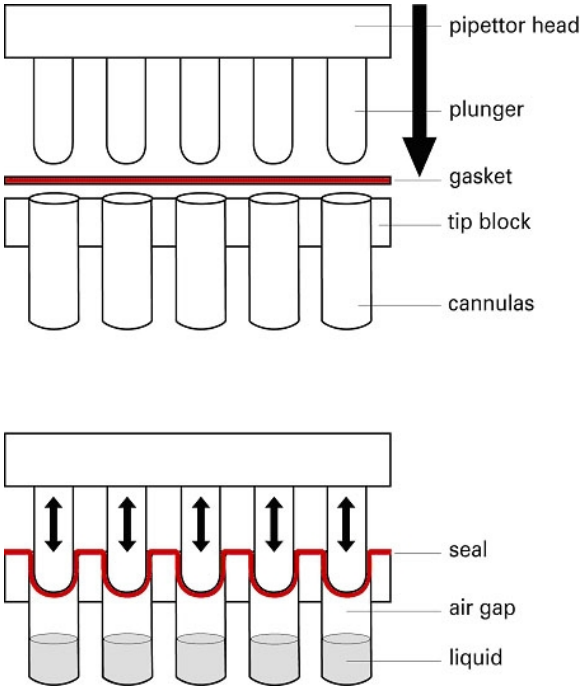
The instrument can use 384-pin and 1536-pin pipettor heads with pin tool and the software has protocols to aspirate, dispense, and wash. These protocols activate when you install the appropriate head.

FLIPR Penta Fluidics Specifications

Wells	Pipettor Head Range	Pipetting Increments	Precision
96	5–200 µL	1 µL	3% CV @ 75 µL
384	1–25 µL	0.5 µL	4% CV @ 25 µL
1536	0.5–3 µL	0.1 µL	6% CV @ 3 µL

The 96-pipettor head and 384-pipettor head displace air in the disposable pipette tips.

The 1536-pipettor head contains a plunger for each of the 1536 tips that presses against an elastic gasket seated on the tip block. When the plungers move down, they create an initial seal between the gasket and tip-block. After creating the seal, further plunger movement causes air displacement in the tip-block.



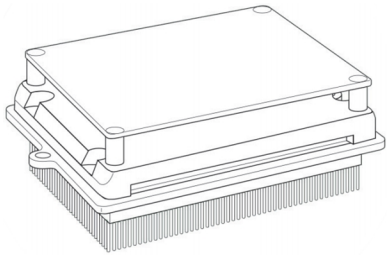
Seal Creation in the 1536-Pipettor Head

CAUTION! Performance depends on tip-block and gasket seating and can be compromised if the seal breaks.

Use only Molecular Devices recommended tip-blocks and gaskets to ensure the highest accuracy and to reduce the possibility of damage to the pipettor and tips. See Consumables and Accessories on page 179.

Pin Tool Head

Pin tools are blocks of solid or slotted pins, where the pins replace the hollow tips you use with a standard pipettor. The pins use capillary action to pick up and transfer liquid from one plate to another. The ability to accurately and reliably transfer compounds in nanoliter volumes allows you to supply test compounds in 100% DMSO solution, which removes the need to prepare intermediate dilution plates.



The volume that each pin picks up is determined by the size of the pin (and, if slotted, the size of the slot) and the withdrawal speed of the pin from the liquid. A faster removal speed leaves more liquid on the pin. Enter the pick-up volumes and tip removal speeds during assay development.

You can use 384 pin tool heads or 1536 pin tool heads and you can exchange the pin tools to change the pin size. The 384 pin tool has four different pin sizes to give a total range (across all the sizes) from 84 nL to 656 nL. The 1536 pin tool has seven different pin sizes to give a total range from 19 nL to 117 nL. The pin tools are available with a hydrophobic and lipophobic coating to prevent or reduce the nonspecific binding of proteins and lipids to the pins. See Exchanging Pin Tools on page 148.

Each pin size has a volume range that it carries:

- The lowest reported volume is for a tip removal speed of 7.8 mm/s.
- The highest reported volume is for a tip removal speed of 57.0 mm/s.

To ensure uniform compound pick-up across the entire pin tool, configure the pins to float in source plate wells. Individual pins are not rigidly attached to the pin block and have a small amount of vertical movement up into the block. When set to float, the pin head moves down very low so that all pins sit on the bottom of the well and push up a little into the block. This ensures that all pins are equally immersed in the wells. This outcome cannot be guaranteed if the pins were rigidly fixed to the block, given that plate bottoms are often not completely flat.

Pipettor Tip and Pin Tool Washer

The washer consists of a reservoir top for the pipettor format that is mounted over a wash basin. The wash basin connects to two solvent-supply carboys and two waste carboys located on the floor beneath the computer monitor. A basin beneath the tip washer base drains to a waste carboy to safely remove any solvent that overflows from the reservoir.

Wash solution fills the reservoir for a calibrated amount of time. Solvent then drains from the reservoir after each wash cycle. You can configure up to five wash cycles within a single wash process in the protocol. See Wash Cell Reservoir Process on page 95, and Creating New Protocols on page 96.

For pipettor tips, you define the volume of solvent to draw up, how long to hold, the volume to expel, and to repeat up to 20 times.

For pins, vertical motion of the tip block agitates the wash solvent around the pins.

You can wash tips or pins in up to two solutions before you reuse the tip washer. When you require additional wash solutions, you can wash pipettor tips or pins a boat or reservoir in one of the source plate positions with a protocol Mix Fluid process configuration. See Mix Fluid Process on page 87.

WARNING! High volumes of volatile, flammable solvents in the read chamber can cause explosive conditions. Use of 100% isopropanol, etc., in the tip washer is discouraged without additional ventilation. Consult your facilities expert to determine the appropriate ventilation to avoid explosive conditions.

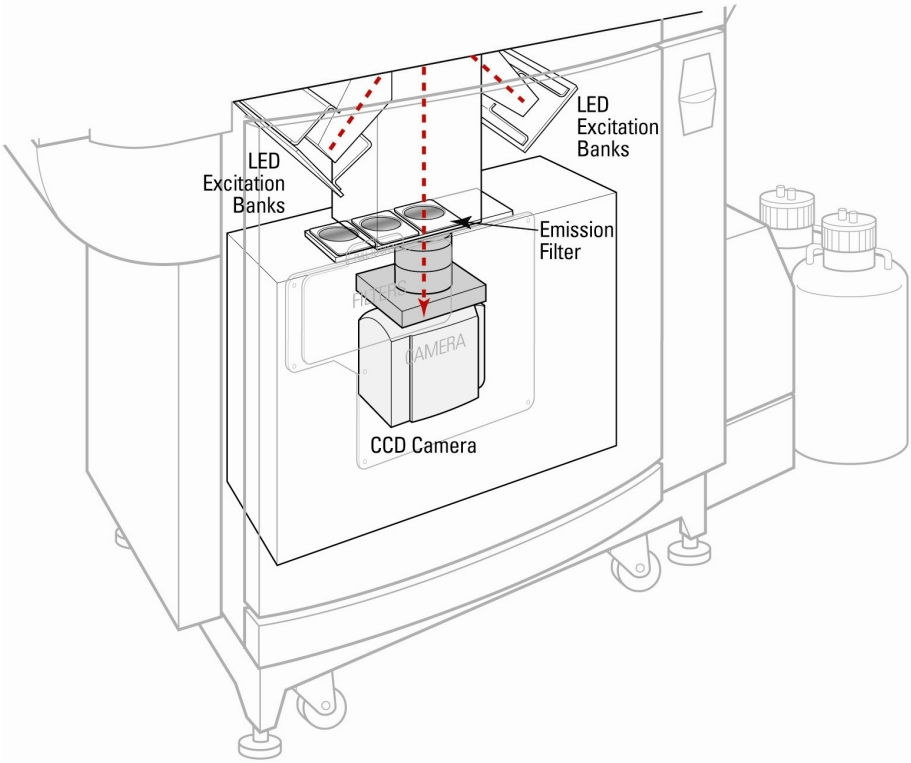
Pin tools are supplied with blotting stations that you load in one of the plate positions on the stage, such as Position 4 (Source 3). Configure blot pin steps in the protocol to remove fluid from the pins.

Note: A waste bottle sensor override (P/N 0700-0827) is available in the FLIPR Penta System accessory kit to bypass the waste sensor and dispose waste in containers other than the dedicated waste carboys.

To exchange the wash reservoir top, see Exchanging Pipettor Tips and Pin Tool Wash Reservoir Top on page 145.

Optical System

The FLIPR Penta System optics are housed in the bottom compartment of the main cabinet. In fluorescence assays, where excitation is required, light from light-emitting diodes (LEDs) is directed at the base of the read plate exposed in position 3 in the 5-position stage above. Light emitted from the plate travels down through emission filters before being captured in the CCD camera.



CCD Camera Options

The FLIPR Penta System supports the following types of CCD cameras:

- EMCCD (Electron Multiplying CCD) - Camera for fluorescence only experiments.
- HS EMCCD (High-Speed, High-Sensitivity Electron Multiplying CCD) - Camera for both high-speed, high-sensitivity, fluorescence, and luminescence assays.

EMCCD Camera

The camera is located directly beneath the read plate on the five-position stage. The camera is thermoelectrically cooled and requires about five minutes to reach its operating temperature of -70°C (-94°F) ± 2°C.

CAUTION! The instrument does not let you use the camera before it reaches the proper operating temperature. Check the camera temperature on the Instrument Status panel before you start an experiment.

The camera uses frame-transfer technology that shifts pixel values from the exposed portion of the chip to the data processing portion. This allows a high acquisition rate and eliminates the possibility of camera shutter failure.

The camera is an integrating-type detector that uses temporal integration to build up the signal-to-noise ratio. Depending on the intensity of the emitted light (reliant on dye efficiency and LED power), you may need to vary camera exposures. This prevents the measured fluorescence signal from being dominated by detector noise.

Images are taken of the bottom of the entire plate for the time you specify in the protocol and exposure time can be from 0.05 to 30 seconds. In ratiometric experiments where two or more wavelengths are measured, the number of images captured increases so that an image is taken for each wavelength at the rate you specify. The software calculates a relative light value from each image for each well. The FLIPR Penta System reports relative fluorescence units (RFUs) in a range from zero to approximately 12,000.

Use the Gain setting in the ScreenWorks Software to amplify the light intensity detected by each pixel on the CCD chip. This parameter has a range from one to 240. Amplification is exponential, increments increase as you go higher up the range. Fluorescence assays typically use a gain of 80 whereas luminescence assays should use 200 as a starting point. Gain optimization should be done during assay development to determine optimal conditions for the protocol.

When a luminescence experiment follows a fluorescence experiment, you should cycle the camera temperature to eliminate ghost images that may be created during the fluorescence assay. Click the **Instrument** menu and select **Manual Operation** and then select **Cycle Camera Temperature**. The camera warms up to room temperature to release ghost images, before cooling back down to approximately -70°C (-94°F).

Note: Luminescence readings are available with the EMCCD camera option, however camera sensitivity is not optimal for this type of experiment.

HS EMCCD Camera

The camera is located directly beneath the read plate on the five-position stage. The camera is thermoelectrically cooled and requires about five minutes to reach its operating temperature range between -70°C (-94°F) ± 2°C.

CAUTION! The instrument does not let you use the camera before it reaches the proper operating temperature. Check the camera temperature on the Instrument Status panel before you start an experiment.

The camera uses frame-transfer technology that shifts pixel values from the exposed portion of the chip to the data processing portion. This allows a high acquisition rate.

The camera is an integrating-type detector that uses temporal integration to build up the signal-to-noise ratio. Depending on the intensity of the emitted light, which is reliant on dye efficiency and LED power, you may need to use shorter or longer camera exposures. This prevents the measured fluorescence signal from being dominated by detector noise.

Images are taken of the bottom of the entire plate for a time you specify in the protocol. There are a variety of exposure settings available depending on the mode in which the camera is run. In ratiometric experiments for example, where two or more wavelengths are measured, the number of images captured increases so that an image is taken for each wavelength at the specified rate. From each image a relative light value is calculated for each well. The FLIPR Penta System reports relative fluorescence units (RFU) in a range from zero to approximately 64,000 RFU.

Use the Gain setting in the ScreenWorks Software to amplify the light intensity detected by each pixel on the CCD chip. The parameter for the Fluorescence mode has a range from 1.5 to 128. In the Camera Mode > Normal, fluorescence assays typically use a gain of 8 for bright calcium assays depending on the exposure time and LED power. Gain optimization should be done during assay development to determine optimal conditions for the protocol.

Tip: When you use the Camera mode > High-Speed, for optimal performance, use a camera gain of 20 or higher and adjust the Exposure Time and Excitation Intensity for your experiment.

When a luminescence experiment follows a fluorescence experiment, you should cycle the camera temperature to eliminate ghost images that may be created during the fluorescence assay. Click the **Instrument** menu and select **Manual Operation** and then select **Cycle Camera Temperature**. The camera warms up to room temperature to release ghost images, before cooling back down to approximately -70°C (-94°F).

When you use the High-Speed camera mode or the Sensitivity camera mode, the camera behaves differently than in the Normal camera mode. The High-Speed camera mode and the Sensitivity camera mode use 2x2 pixel binning in the camera, which combines 4 pixels into one. This increases sensitivity to smaller signals, as well as for speed. The effect of overexposure in the camera sensor when you use this binning mode is different than for a non-binned exposure, due to the design of the sensor. See Determining Protocol Saturation Levels for the HS EMCCD Camera on page 106.

Stabilizing the HS EMCCD Camera

When you use the HS EMCCD camera, you can run the optional stabilization process before the experiment starts to avoid camera signal drift. The Stabilizing camera process takes 50 seconds to complete.

Tip: The software may prompt you to run camera stabilization, but if camera stabilization is not important for the protocol, you can skip the stabilization process.

If the biology is time-sensitive, you can ready the instrument before you run the experiment. Click the **Instrument** menu and select **Manual Operation** and then select **Pre-Stabilize**.

If the biology is not time-sensitive, select **Auto Stabilize** in the protocol settings to start stabilization before the experiment begins. See Edit Read Mode on page 61.

Note: When you save auto stabilize as part of the protocol, if the camera is not already stabilized and the protocol has multiple reads, auto stabilize only runs the first time.

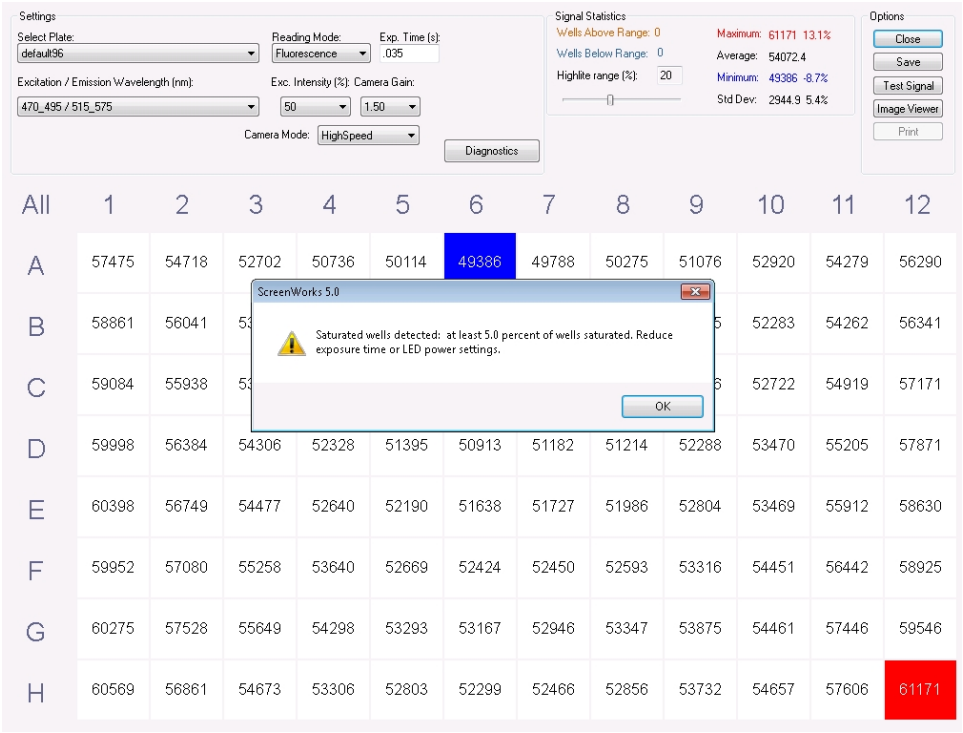
The instrument maintains the camera stability for a limited time. The Instrument Status tab displays a stabilization timer countdown. As long as the protocol is unchanged, and the instrument door is closed, camera stability is sustained for two hours between experiment runs. The two-hour expiration countdown restarts after every experiment runs.

When you open the instrument door, you have up to 15 minutes with the instrument door open before you have to run the camera stabilization process again. Within the 15-minute time limit, after you close the door, the timer returns to the two-hour expiration countdown.

Understanding HS EMCCD Camera Protocol Saturation

When you set up a protocol, you must define the read exposure settings avoid over saturation on the HS EMCCD camera sensor. If well signals hit saturation, they cannot be quantitatively compared. The camera provides a saturation warning when 5% of well values on the plate exceed 90% of the camera sensor dynamic range. Keep the maximum signals below this level. The HS EMCCD camera threshold is about 45000 counts; but it can vary a few thousand counts between cameras. Use the Protocol Signal Test dialog to determine the camera saturation level and to adjust the exposure for the biology.

As the exposure increases past the saturation threshold, the software displays a saturation warning and the raw image in the Protocol Signal Test dialog shows the wells becoming bright white, as before with Normal camera mode as noted in the following images.



High-Speed Mode Signal Test Saturation with Warning

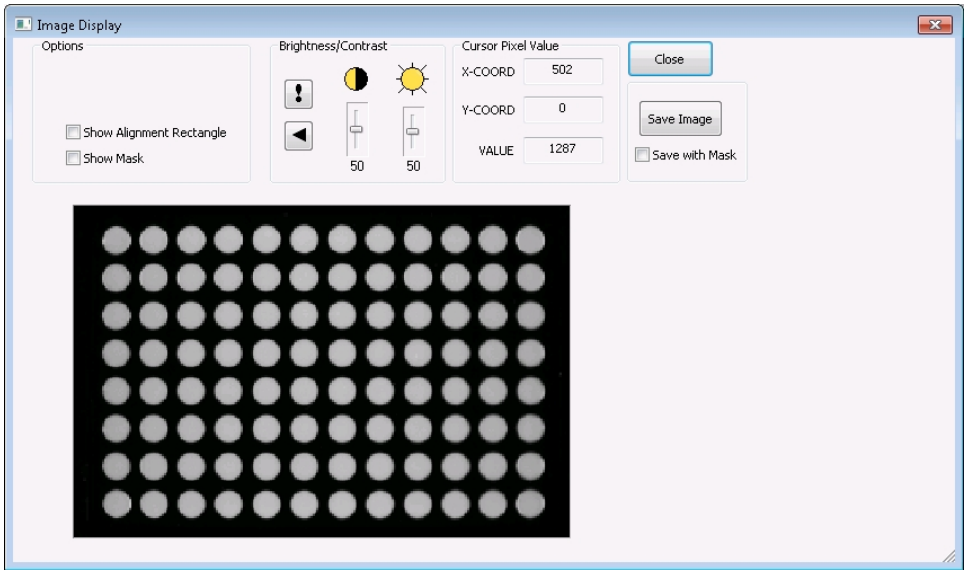


Image Display of High-Speed Mode Signal Test Saturation

For a short range of over exposure, the saturation warning works. As the exposure increases further, the brightest pixel values start to get darker in the center of the well areas, as shown in the following image.

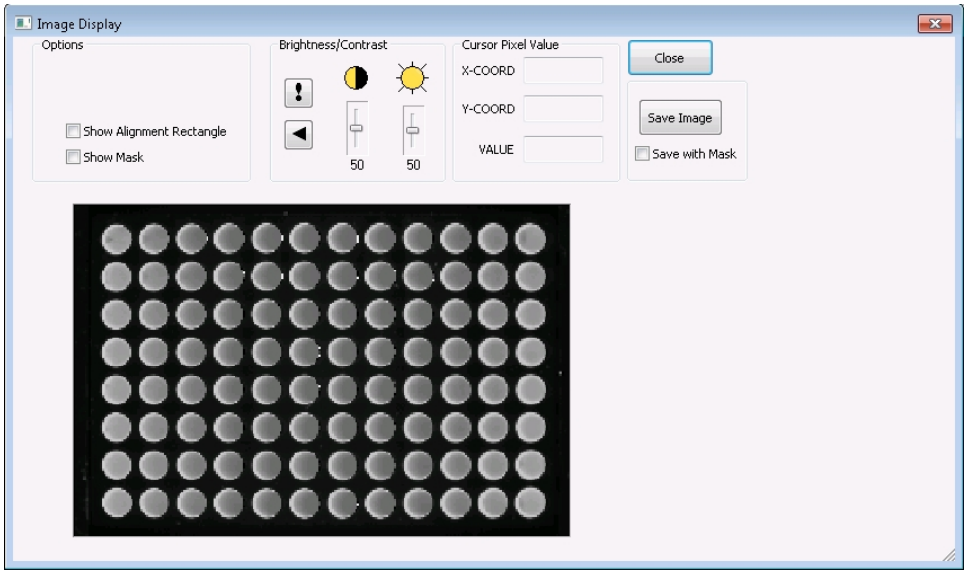


Image Display of High-Speed Mode Signal Test Saturation Reversing

As the brightest pixels get darker, the pixel values drop lower, instead of staying at the maximum saturation value. This reversal effect happens when the binned image pixels are overexposed. The overall average for the image decreases, and after about a 4x overexposure increase, no saturation warning appears. As the exposure increases further, the wells start to bloom and flood across the image as before, except that the center of the streaks are darker, and no saturation warning appears, as shown in the following image.

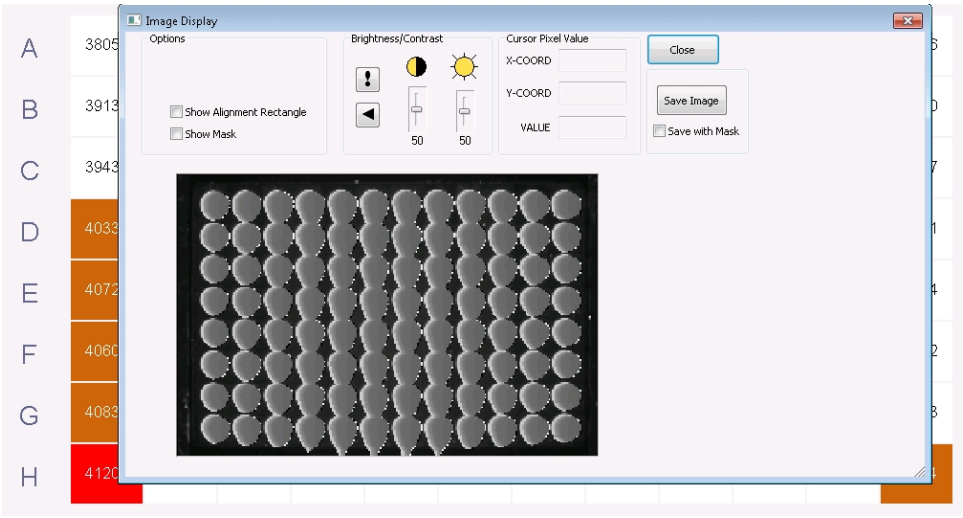


Image Display of High-Speed Mode Signal Test Blooming Without Saturation Warning

This is a problem to detect when the image measurements are saturated, and the system cannot give a reliable saturation warning for grossly overexposed well signals when you use the High-Speed camera mode. See Determining Protocol Saturation Levels for the HS EMCCD Camera on page 106.

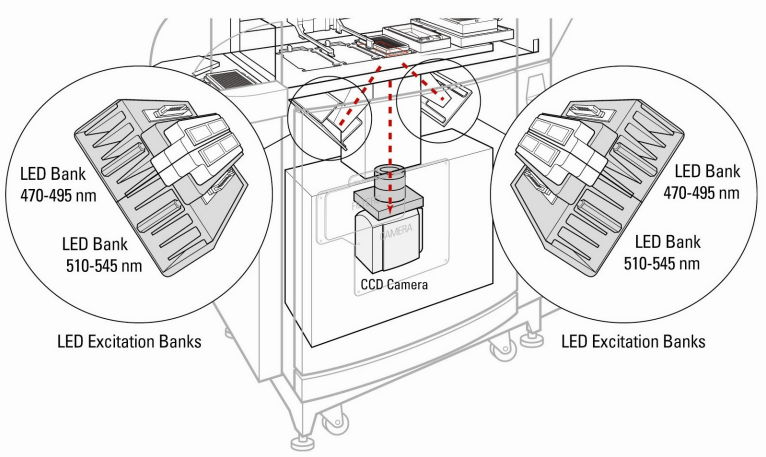
All Cameras

When calculated, relative luminescence units (RLU) and relative fluorescence units (RFU) display in real time in the Analysis process window (within the limits of computer processing speed). Data for one wavelength for all 96-wells, 384-wells, or 1536-wells of the plate, display in the Multi-Well graph on the right. Select wells in the Multi-Well graph to populate the Detail graph. You do post-assay analysis in the ScreenWorks Software when the experiment completes, and you can export the data.

In normal operation, the software discards images after relative luminescence units (RLU) and relative fluorescence units (RFU) values are measured. You can define the number of images to save per Read with Transfer Fluid step for quality purposes, up to 6,000 images per experiment. These images are useful to troubleshoot problems, such as cells lifting from well bottoms during compound addition. Images save as *.tif files with the same name and to the same directory as the data file. To view the image, click **Image** on the Analysis process page when the result data file is open.

Note: The robustness of an assay is not dependent on the size of the signal. It is better determined by the signal-to-noise ratio. A commonly used calculation to determine assay robustness is the Z' factor equation.

LED Modules



The FLIPR Penta System has four LED banks to provide illumination for plate reads. When an image is to be captured, the LED banks pulse on, two at a time to protect cells from dye photo-bleaching.

The LED banks are divided between two modules, one on either side of the read plate position above. LED banks in corresponding positions in either module are paired. The paired LED banks pulse simultaneously during an experiment, so that light strikes the read plate base from two directions to help ensure that the entire plate is maximally illuminated.

Typically, the two LED bank pairs are set up with LEDs of different wavelengths, for example, one pair might have LEDs of range 470 nm to 495 nm, while the other pair might be 510 nm to 545 nm. You can set up Ratiometric experiments to use both wavelengths, in which case the paired banks fire alternately.

Note: Despite the two excitation wavelengths firing alternately, output data files show time points for each as occurring simultaneously.

Deflectors around the LED banks direct all light from the LEDs through excitation bandpass filters that further refine the wavelength. The light is then funneled into light pipes that focus it onto the base of the read plate.

The FLIPR Penta System does not operate without the full complement of LED banks installed. You can use blank LED banks for one pair if only one excitation wavelength is available. You can change the LED banks. See Exchanging LED Modules on page 150.

The FLIPR Penta System includes one set of default calcium LED banks (470 nm – 495 nm) and one set of blank LED banks. Additional LED banks are available for purchase. See FLIPR Penta Instrument LED and Filter Combinations on page 195.

Use the Protocol Builder Settings screen to configure the LEDs for the experiment. See Setup Read Mode on page 61.

The system does not require an LED warmup time before you run an experiment. Start time is only dependent on the time it takes for the camera to cool down and for the stage to heat up.

The LEDs are air-cooled by fans. The light output varies slightly as the LEDs heat up. To help with the heat transfer, a piece of foam in inserted in the back of the LED bank on each side. To counteract the temperature change, a temporal correction is applied to the LED feedback circuit to normalize the system.

The software applies flat field calibration to the read plate to adjust for non-uniformity of illumination across the plate. To manually calibrate the system, see Calibrating Optics on page 101.

WARNING! Do not look into LED banks when turned on, especially at intensities over 30% or in the UV spectrum. If you see light escaping the instrument when the LED modules are turned on, shut the instrument down immediately and call Molecular Devices Technical Support.

Emission Filters

A three-position filter slide holds up to three 60 mm diameter interference filters and is located in front of the CCD camera. The slider can be alternated in front of the camera to separate out the emission band of the dye you use. Use these filters with a single excitation wavelength or paired up with additional emission filters and LEDs in a ratiometric experiment, for example, as excitation LEDs alternate between two different wavelengths, filters change at the same time so each image the camera takes matches the right emission filter with the excitation LED bank. The most common configuration is a LED excitation wavelength of 470 – 495 nm with a 515 – 575 nm bandpass emission filter. You can run without the filter for luminescence experiments.

You can change the emission LEDs and emission filters. The instrument mechanically senses the filters you install, and the Instrument Configuration panel displays the filter configuration. The instrument prompts you to calibrate a new emission filter to correspond to an LED module when are used in an experiment. See Exchanging Filters on page 154.

Note: You can purchase a custom filter cassette (P/N 5018841) for use with filters created by an outside vendor. These filters appear in the ScreenWorks Software as Custom 1, Custom 2 and Custom 3.

Fluorescence Mode

In Fluorescence mode the system LEDs illuminate the bottom of a 96-well, 384-well, or 1536-well read plate that contains cells loaded with fluorescent dye and measure the fluorescence in each well. By taking a sequence of measurements in conjunction with compound application, changes in fluorescence emission characteristics due to the change in ion concentration, for example, Ca^{2+}, Tl^{+}, or Membrane Potential.

Light-emitting diodes (LEDs) produce light at distinct wavelength ranges to excite the fluorescent dye that you add to the cells in the read plate wells. The instrument illuminates the entire well plate bottom. Fluorescent light emitted by the dye (for the entire plate) passes through an emission filter before being captured in a CCD camera, high-speed, high-sensitivity HS EMCCD or EMCCD. The software measures fluorescence from each well independently and converts it into a numerical value. You can configure the FLIPR Penta System with two LED banks and three emission filters to allow the software configuration of up to four excitation/emission wavelength combinations (read modes). You can measure up to four different fluorescence effects within a single experiment.

Luminescence Mode

The FLIPR Penta System provides a Luminescence mode with the HS EMCCD camera option. The instrument has a light-tight enclosure to operate in Luminescence mode. You can have a specialized high-sensitivity HS EMCCD camera installed in place of the standard EMCCD camera to detect signal at both high-speed Fluorescence mode and Aequorin (luminescence) mode. The camera is mounted directly beneath the read plate, so images are taken of the entire bottom of the plate. From the 3 filter positions available on FLIPR Penta System, you should have one open position, so no filter is used during the luminescence assay.

The software measures luminescence from each well independently and converts it into a numerical value.

Chiller

The chiller sits outside of the FLIPR Penta instrument and connects with a cable and tubing. The chiller uses a special cooling liquid, and the instrument embedded computer and firmware controls the chiller.

Because the FLIPR Penta System is a light-tight instrument, there is very limited airflow inside the instrument enclosure. The chiller provides the operating environment requirements for all available camera options.

Chapter 3: ScreenWorks Software Overview

3

Online Mode vs. Offline Mode

The software has two startup modes:

-  Online - The software is connected to the instrument.
-  Offline - The software is disconnected from the instrument.

You determine the default startup mode when you install the software. You must reinstall the software to change the default startup mode. Regardless of the startup mode, the pipettor head and tip washer type must always match.

Note: To allow the selection of either mode when you generate protocols, you must install the software to default to Offline mode.

To switch modes: click the **Instrument** menu and select either **Go Online** or **Go Offline**. This does not change the default startup mode.

Online Mode

When the ScreenWorks Software starts in Online mode, the software checks for instrument connections. A message displays if no connections are sensed. Check the connections and attempt to connect again or select to run the software in Offline mode.

Create new protocols in Online mode.

The software flags the protocols you create in Offline mode with instrument hardware settings that do not match current instrument hardware. You must change the hardware settings to match the protocol before you can run the protocol.

When the software starts in Online mode and connects to the instrument, the default installation configuration file is overwritten using the current instrument settings and plate library information.

If you work in Online mode and then switch to Offline mode, the instrument setup configuration is remembered as the last setting.

Offline Mode

When the ScreenWorks Software starts in Offline mode, you can configure the following hardware options:

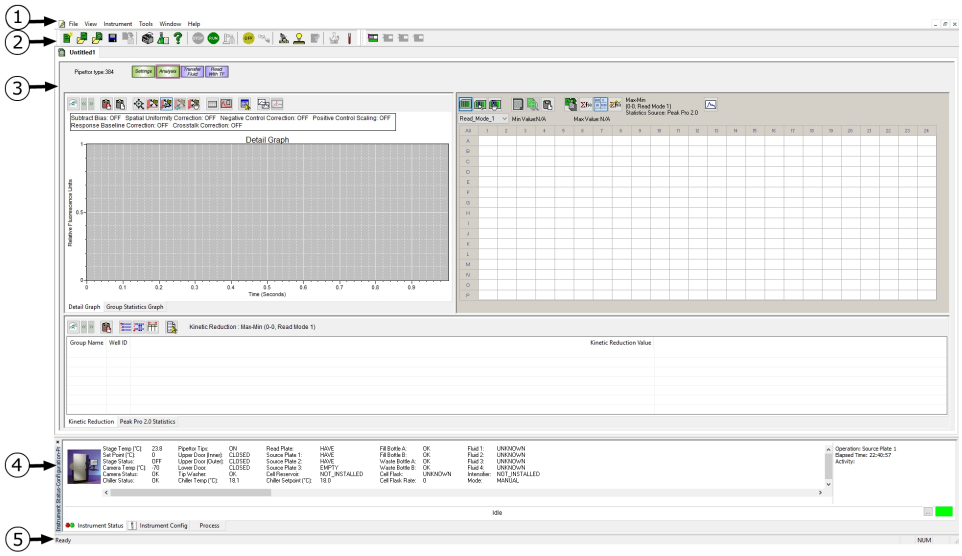
- Camera Type
- Pipettor Format

When the default ScreenWorks Software start is set to Online mode and no instrument connection registers, a message displays. You can check the connections and attempt to connect again in Online mode, or you can select to run the software in Offline mode.

User Interface Overview

The ScreenWorks Software main screen includes a menu and a toolbar across the top and a status bar at the bottom. The main area in the middle has two sections:

- Experiment Window - Use the central proportion of the main window to configure protocols, view data, and perform analysis.
- Instrument Status Configuration Process Panel - Use the bottom of the main work area to view instrument information and create protocols. This panel contains three tabs: Instrument Status, Instrument Config, and Process described in the following sections.



Main Window Layout

Item	Description
1	Menu Bar
2	Toolbar
3	Experiment Window
4	Instrument Status Configuration Process Panel
5	Status Bar

Menu Bar

The menu bar contains sub-menus that group related functions.

File Menu

Use the File menu to select the following options:

File Menu Options

Option	Description
New	Opens a new protocol (*.fmp) in the Experiment window, with default settings for one read with fluid transfer. New protocols are named *Untitled[n]*, where *n* is a number.
Open	Displays the Open File dialog to open a saved protocol (*.fmp), data (*.fmd) or image (*.png) file. Click the Tools menu and select Set Default Directories to set the default folder. **Note**: Only data files you create with ScreenWorks Software can be opened in ScreenWorks Software. You cannot open data files from previous FLIPR Tetra Systems (versions 1.X through 2.X).
Close	Closes the active protocol or data file that displays in the Experiment window. If you modify the file, a message prompts you to save the modifications.
Save	Saves the active protocol or data file that displays in the Experiment window. The Save As dialog displays if the file is a default protocol that is untitled.
Save As	Displays the Save As dialog to save a protocol or data file with a new name or format (for example, save a data file as a protocol). Allows you to save a protocol or data file as an earlier version of the ScreenWorks Software. Select a file type from the Save as Type drop-down to change the format.
Save All Files	Saves all open protocol and data files. The Save As dialog displays if the default protocol file is open.
Close All Files	Closes all open protocol and data files. The Save As dialog displays if a file changed or the default protocol file is open.
Export	Displays the Export File dialog or the Batch Export dialog to export data from the open data file, or other data files on the disk. See Exporting Data on page 117.
Page Setup	Displays the Page Setup dialog to configure the printer and print settings for the document.
Print Preview Report	Displays how the document looks before you print.
Print Report	Displays the Print Report dialog to select the graphs or reports to print. Click Print Preview Report to display how the document looks before you print or click Print to print.
1–6 Data Files	Displays the six most recently opened data files with the most recently opened at the top.
7–10 Protocol Files	Displays the four most recently opened protocol files with the most recently opened at the top.
Exit	Closes the ScreenWorks Software.

Saving Data Files as Protocol Files

When the active file is a protocol file, you can add, remove, or change processes in the file, then save the protocol and run it. You cannot edit the protocol information stored in data files nor used to run a new experiment, however you can extract this information to a new protocol file. Experiments run using this file have exactly the same steps as the steps used to create the data file.

To store a data file as a protocol file, click **Save As**, enter the protocol name, select ***.fmp** as the File Type, and click **Save**. The software strips all data the file and stores the protocol information.

Note: Saved changes only affect the protocol. The data file from which the protocol was derived remains intact.

View Menu

Use the View menu to select the following options:

View Menu Options

Option	Description
Experiment Setup	Displays the Experiment Setup view of a protocol or data file that includes the processes and associated dialogs in the Experiment window.
Experiment Summary	Displays the Experiment Summary for the current protocol or data file in the Experiment window. To return to the Experiment Setup view, click the View menu and select Experiment Setup.
Save Layout	Saves the proportions that display in the Experiment window including; Multi-Well graphs, Detail graphs, Group Statistics window, and Instrument Status panel to one of four layouts. Click Restore Layout to toggle between the layout.
Restore Layout	Restores the screen layout to one of four layouts you save.
Instrument Status	Toggles the Instrument Status panel in and out of view. See Instrument Status on page 56.
Toolbar	Toggles the Toolbar in and out of view. See Toolbar on page 52.
Status Bar	Toggles the Status Bar at the bottom of the main window in and out of view. See Status Bar on page 59.

Instrument Menu

Use the Instrument menu to access instrument communication and manual dialogs.

Instrument Menu Commands

Option	Description
Go Online/Offline	Select **Go Online** to change to Online where you can connect the software to the instrument and give the ScreenWorks Software control of the instrument. Select **Go Offline** to change to Offline mode and disconnect the software from the instrument.
Run Experiment	Starts the experiment that uses the uppermost protocol file in the experiment.
Stop Experiment	Stops the experiment. Only use in emergencies to halt an experiment. An instrument reset may be required.
Manual Operation has the following sub-menu that control hardware operations. A command is not available when the associated hardware is not installed.	
Load Tips	Instructs the pipettor to load tips or pin tool from the stage Position 1.
Unload Tips	Instructs the pipettor to unload tips or pin tool to the stage Position 1. Unload tips to a tip rack, otherwise, make sure there is a container at stage Position 1 to receive tips.
Wash Tips	Displays the Wash Tips dialog to configure how the pipettor is to wash tips or pin tool.
Drain Wash Basin	Drains the wash basin to the waste bottles when there is an instrument error.
Yellow Plate Signal Test	Coordinates the read of the stage Position 3 read plate for the protocol and displays the numerical results in the Signal Test dialog. See Signal Test Dialog on page 45.
Protocol Signal Test	Coordinates the read of the stage Position 3 read plate and displays the numerical results in the Signal Test dialog. Instrument settings can be saved to the open protocol. See Signal Test Dialog on page 45.
Change Head	Instructs the pipettor head to move over stage Position 3 to exchange to a new head format. See Exchanging Pipettor or Pin Tool Head on page 140.
Cycle Camera Temperature	Cycles the camera temperature to run a low-fluorescence or luminescence experiment immediately after you run a high-fluorescence experiment. The temperature cycle runs for about 15 minutes, warms to room temperature, 20°C to 25°C (68°F to 77°F), and then cools to operating temperature: EMCCD camera: -70°C (-94°F) ± 2°C HS EMCCD camera: -70°C (-94°F) ± 2°C
Temperature Control	Turns heat on and off for stage source plate Positions 1, 2, and 3, and allows you to enter the temperature.
Cell Flask Stirring Control	Sets the stir speed rate for the cell flask.

Instrument Menu Commands (continued)

Option	Description
Additional Instrument Menu Commands	
Set Remote/ Manual Mode	Select **Set Remote Mode** to integrate a third-party robotic system with the FLIPR Penta System. The ScreenWorks Software goes into Remote Mode when you use SynchroMax Automation. See Robotic Integration on page 203. Select **Set Manual Mode** to switch instrument communication to the ScreenWorks Software and prevent robotic system control.
SynchroMax Automation	Displays the SynchroMax dialog to configure plate handling with the SynchroMax ET. See SynchroMax Automation on page 47.
Reset	Reinitializes the system to clear any fatal errors that display in red at the bottom of the Instrument Status panel.
Clear Error	Clears minor system errors that display in yellow in the Instrument Status panel. See Instrument Status Colors on page 159.
Calibration	Displays the Calibration dialog where you do flat field calibrations. See Calibrating Optics on page 101.
Refresh Configuration	Refreshes the instrument configuration after you change hardware settings.

Signal Test Dialog

The signal test has two functions:

- Check the state of the overall system: This test is run using the yellow test plate with the plate format for the assay. Open the dialog with the Yellow Plate Signal Test option in the main menu. The table below outlines the default settings to use when you perform the yellow plate signal test. These settings cannot be saved in the protocol for later use.
- Check initial fluorescence of a plate before you run an assay: This test is run to evaluate the assay plate before you run an experiment. Open the dialog with the Protocol Signal Test option in the main menu. The table below outlines the settings that reflect the Normal camera mode and default to the settings in the open protocol when you run the protocol signal test. These settings can be saved in the protocol for later use.

Yellow Plate Signal Test and Protocol Signal Test Setting Options

Option	Description
Select Plate	Select the plate type.
Excitation/Emission Wavelength	Select the excitation/emission wavelength pair for the signal test. Only calibrated excitation/emission wavelength pairs display in the list.
Reading Mode	Select Fluorescence or Luminescence.
Camera Gain	Select the camera gain for the signal test. For the 470–495/515–575 nm excitation/emission pair with a yellow test plate: * Standard EMCCD Camera - 50 * HS EMCCD camera - 8 for Camera Mode Normal, 20 for Camera Mode High-Speed
Camera Mode	In Read Mode > Florescence: Normal, Sensitivity, and High-Speed for the HS EMCCD camera only In Read Mode > Luminescence: Normal and Sensitivity
Excitation Intensity	Select the LED intensity for the signal test. Excitation intensity scales as a percentage of the total LED output (0–100%). For the 470–495/515–575 nm excitation/emission pair with a yellow test plate: * Standard EMCCD Camera - 80 * HS EMCCD camera - 50
Exposure Time	Enter the amount of time (in seconds) to keep the camera shutter open during the Signal Test. For the 470–495/515–575 nm excitation/emission pair with a yellow test plate: * Standard EMCCD Camera - 0.4 * HS EMCCD camera - 0.005
Highlight Range	Highlights well values that lie within the set statistical range. Use the slider to set the statistical range.
Wells Above Range	Displays the number of wells above the statistical range as determined by the Highlight Range.
Wells Below Range	Displays the number of wells below the statistical range determined by the Highlight Range.
Maximum	Displays the largest value on the signal test plate.

Yellow Plate Signal Test and Protocol Signal Test Setting Options (continued)

Option	Description
Average	Displays the average value on the signal test plate.
Minimum	Displays the smallest value on the signal test plate.
Std. Dev	Displays the standard deviation of the signal test plate.
Test Signal	Initiates a new signal test.
Save	Saves the signal test as an ASCII text file (*.sig).
Print	Prints the signal test.
Image Viewer	Displays the CCD image that shows where relative fluorescence units (RFU) values derived for the signal test. Show > Hide Mask - Shows or hides the mask used in the test to indicate the pixel area used to derive the RLU value for each well. View Image - Refreshes signal test image.
Diagnostics	For Technical Support only.

For procedures, see Signal Tests on page 104.

SynchroMax Automation

Note: Molecular Devices no longer offers the SynchroMax ET external plate handler robot. The following information is for reference only.

The SynchroMax™ Automation option is available when you install a SynchroMax ET external plate handler.

When you access the SynchroMax dialog from Instrument > SynchroMax Automation, the ScreenWorks Software goes into Remote mode and the SynchroMax ET software controls the instrument. The ScreenWorks Software returns to Manual mode when you close the SynchroMax dialog.

To run a series of experiments with the SynchroMax ET plate handler, first open the protocol file to use in the ScreenWorks Software. Only one protocol is used in a run for as many times as the SynchroMax ET plate handler delivers plates.

Prepare the protocol, then open the SynchroMax dialog, and then select a Stack Layout Template. Stack Layout Template files specify, for each plate location in the five-position stage, how many plates load to that position, from which stack, and where they move after use. A number of Stack Layout Templates are included in the software.

When you open a Stack Layout Template, the graphic in the dialog displays on which stacks the plates load. Use this as a guide to load the plates. All plates in a single stack should be the same type.

If any stage positions use the same plate throughout the duration of the run, you should manually load the stage positions before you begin the run. Stage positions that have plates brought to them during the run must be empty at the start of the run.

Before you click Run, make sure all output racks are empty before you run the experiment. When plates are in position and the FLIPR Penta System is ready, click Run in the SynchroMax dialog. After the instrument checks that the plate configuration is compatible with the protocol settings, the instrument runs until all plates are used. The Run button changes to Stop so you can stop the run before it completes.

Note: Use Stop only in emergencies to halt an experiment. An instrument reset may be required.

When the SynchroMax ET plate handler is active, the system clears all plates at the end of an experiment. The SynchroMax plate handler remembers only the plates it loads during the experiment and checks if any of those plates remain when the experiment completes. Plates are removed when they remain. You can use a reagent reservoir in a source position within the FLIPR Penta System as long as you load it manually and the SynchroMax template does not include the loading or removal of plates to that position.

Tools Menu

Use the Tools menu to select the following options:

Tools Menu Options

Option	Description
Set Default Directories	Displays the Set Default Directories dialog to designate directories in which to store protocol and data files, signal tests, group templates and to which export files are written. The Open > Protocol, Data, and Image File commands in the File menu open to the default folder you set here, as do the File > Export dialogs. Group templates *.fmg export and import from the default folder. The default protocol directory is: C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyProtocols [MyData, MySignalTests, or MyGroupTemplates] The default data and export directories are: C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyData \ The default signal test directory is: C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MySignalTests The default group template directory is: C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyGroupTemplates **Note**: Save all protocol and data files on the local hard drive to ensure instrument function or data is not lost if the server fails during an experiment.
Plate Library	Displays the Plate Process Definition dialog that lists current plate definitions and allows you to add plate definitions to the system. See Plate Process Definition on page 49.
Open Error Log	Displays an error log generated by the FLIPR Penta System. This is for technical support and requires a password.
Save Error Log	Saves error logs. Logs save as *.fel (FLIPR Penta System error log) encrypted files to forward to Molecular Devices Technical Support.
Assay Log	Displays a dialog that reports when the protocol steps were applied. For data files only.

Plate Process Definition

The Plate Process Definition dialog lists the plates in the system. Click the **Tools** menu and select **Plate Library**. Plates are categorized first into read plates and source plates and then by well-number format. The Plate Library is camera specific. If you add plates with one camera type, they are unavailable after you change the camera type.

The Plate Process Definition dialog is accessible only to administrators and is password protected. The default username is *fliprtetra* and password is *flipr*.

The software includes the following plates:

- Default 96
- Default 96 small volume
- Default 96 no slit mask
- Default 96 boat
- Default 384
- Default 384 small volume 5X3 mask
- Default 384 small volume 3X3 mask
- Default 384 boat
- Default 384 blot
- Default 1536
- Default 1536 boat
- Default 1536 blot

Plate Process Definition Options

Option	Description
Close	Closes the Plate Process Definition dialog.
Open Plate	Displays the Define Basic Plate Parameters dialog for the plate you select.
Copy Plate	Displays the Define Basic Plate Parameters dialog to add a new plate. **Note**: When you copy a plate, select a plate with a similar well format, as the number of wells and plate mask transfer to the new plate.
Delete Plate	Deletes the plate that displays in the Plate Process Definition field.
Collapse All	Collapses the Plate Process Definition tree.
Expand All	Displays all plates in the Plate Process Definition tree.

Define Basic Plate Parameters

Click Open Plate or Copy Plate in the Plate Process Definition dialog to define the plate dimensions. You cannot modify the dimensions of the default plates, but you can realign the plate mask.

Define Basic Plate Parameters Options

Option	Description
Plate Name	Enter the plate name (maximum 50 characters).
Plate Type	Select **Source Plate** to create a plate that does not include a plate mask. These plates are used for compound storage. Select **Read Plate** to create plates that are read in the read position and require a plate mask.
Plate Specifications	Refer to the diagram in the upper right corner of the dialog and for best results, obtain the plate dimensions from the plate manufacturer. See FLIPR Penta Plate Dimensions on page 189. **Note**: Molecular Devices is not responsible for instrument malfunctions if plate specifications are not correct. **Rows** - Enter the number of rows on the plate. **Columns** - Enter the number of columns on the plate. **Well Shape** - Select the shape of the well. **Well Volume (µL)** - Enter the maximum well volume in microliters. **X (mm)** - Enter the distance (in mm) from the left side of the plate to the center of well A1. **Y (mm)** - Enter the distance (in mm) from the top of the plate to the center of well A1. **Bottom (mm)** - Enter the distance (in mm) from the bottom of the plate skirt to the inside of the well. **Top (mm)** - Enter the distance (in mm) from the bottom of the plate skirt to the top of the well. **Well Offset (mm)** - Enter the distance (in mm) from the center of one well to the center of adjacent well.

Define Camera Parameters

The Finish button is available for the Read plate type. Before you click Finish, place a read plate with 10^{-8} M fluorescein in the read position. When you select this plate, the instrument reads the plate and defines a plate mask for the plate definition. If the plate is in position and the instrument cannot define a mask, the plate definition is saved as a source plate.

Window Menu

The Window menu options allow you to change the way you view the Experiment window.

Window Menu Options

Option	Description
Cascade	Aligns the open windows so that they overlap and stagger with the title bars visible.
Tile	Divides the screen into as many segments as there are windows and aligns them so that they display side-by-side. Each file occupies a segment and is visible. Each file image scales according to the number of files that display.
1–10 Data Files	Lists the open files (up to 10)

Help Menu

The Help menu provide access to user guides and information about the software.

Help Menu Options

Option	Description
FLIPR Penta System User Guide (PDF)	Displays a .pdf version of this manual.
Release Notes (PDF)	Displays the *ScreenWorks Software Release Notes*.
Protocol Guide (PDF)	Displays a guide that provides detailed information, procedures, and optimization guidelines to run common types of experiments.
Software License	Displays the Software License dialog for the ScreenWorks Peak Pro Software activation.
Contact Us	Displays the Molecular Devices website.
About ScreenWorks	Displays the About ScreenWorks dialog with the software version number, the Firmware EC, Firmware Motion, and Remote Interface.

Toolbar

The toolbar provides shortcuts for main menu commands. To hide or display the toolbar, click the **View** menu and select **Toolbar**. See Menu Bar on page 41.

Toolbar Icons

Icon	Name	Description
	New Document	Opens a new protocol file (*.fmp) with default settings for an assay with a single read with fluid transfer. Files are named *Untitled[n]* where *n* is a number.
	Open Protocol File	Displays the Open File dialog to open protocol (*.fmp) files.
	Open Data File	Displays the Open File dialog to open data (*.fmd) or image files.
	Save File	Displays the Save File dialog to save the current file.
	Export File	Displays the Export File dialog to export the current file.
	Print	Displays the Print dialog.
	Experiment Summary	Displays the process steps and correction for the current protocol or data file. This button toggles with Experiment Setup.
	Experiment Setup	Displays the Experiment Setup window to edit the current protocol or analyze data in a data file. This button toggles with Experiment Summary.
	Help	Displays help documentation.
	Stop	Stops the current experiment.
	Run	Starts the protocol.
	SynchroMax Automation	Displays the SynchroMax Automation window to select the template to run the SynchroMax ET plate handler.
	Online Mode	Displays when System Control is in Offline mode. Click to connect to the instrument and go into Online mode. This button toggles with Offline Mode.
	Offline Mode	Displays when the ScreenWorks Software is in Online mode. Click to disconnect from the instrument and go into Offline mode. This button toggles with Online Mode.

Toolbar Icons (continued)

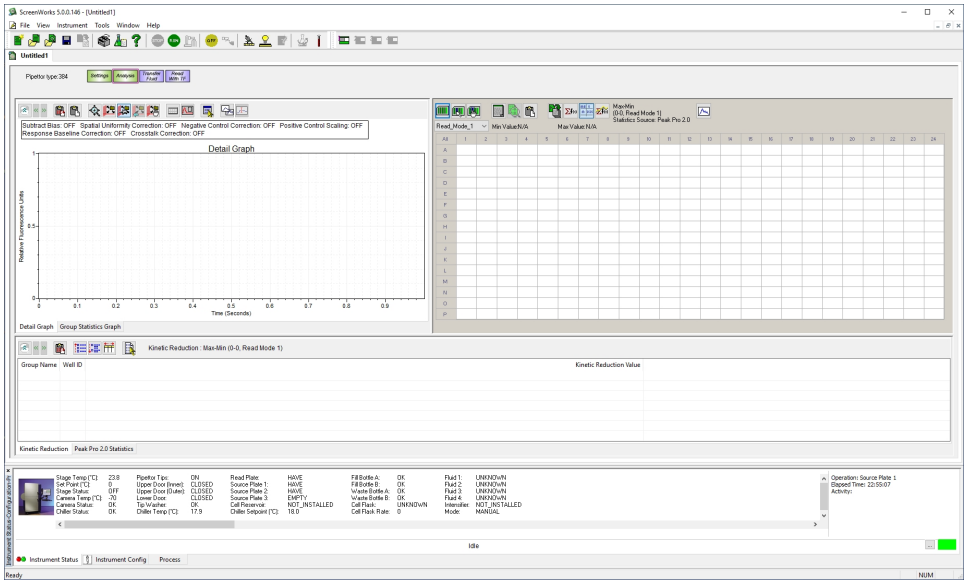
Icon	Name	Description
	Remote Mode	Instructs the ScreenWorks Software to disable the manual connection and to receive Remote commands from a third-party robot. This button toggles with Manual Mode.
	Manual Mode	Instructs the ScreenWorks Software to disable the remote connection and to receive manual commands from the ScreenWorks Software user interface. This button toggles with Remote Mode.
	Calibration	Displays the Calibration dialog to set flat field calibrations.
	Yellow Plate Signal Test	Displays the Yellow Plate Signal Test dialog to view the numerical results using the yellow plate.
	Protocol Signal Test	Displays the Protocol Signal Test dialog to view the numerical results before you run an experiment. You can save the settings in the signal test to a protocol file.
	Set Spinner Flask Stirring Rate	Displays the Spinner Flask Control dialog to set the stirring rate.
	Set Stage Temperature	Displays the Set Temperature dialog to set the stage temperature in degrees Centigrade.
	Restore Layout 1	Changes the Experiment window layout to the layout 1 you define in the View menu.
	Restore Layout 2	Changes the Experiment window layout to the layout 2.
	Restore Layout 3	Changes the Experiment window layout to the layout 3.
	Restore Layout 4	Changes the Experiment window layout to the layout 4.

Experiment Window

The Experiment window is the main area in the ScreenWorks Software. The Experiment window can have one protocol file and multiple data files open simultaneously. Select a file pane to make it active. Use the options in the Windows menu to view the file panes individually, cascaded, or tiled. See Window Menu on page 51.

Experiment Setup

The ScreenWorks Software opens the View > Experiment Setup screen in the Experiment window, by default.

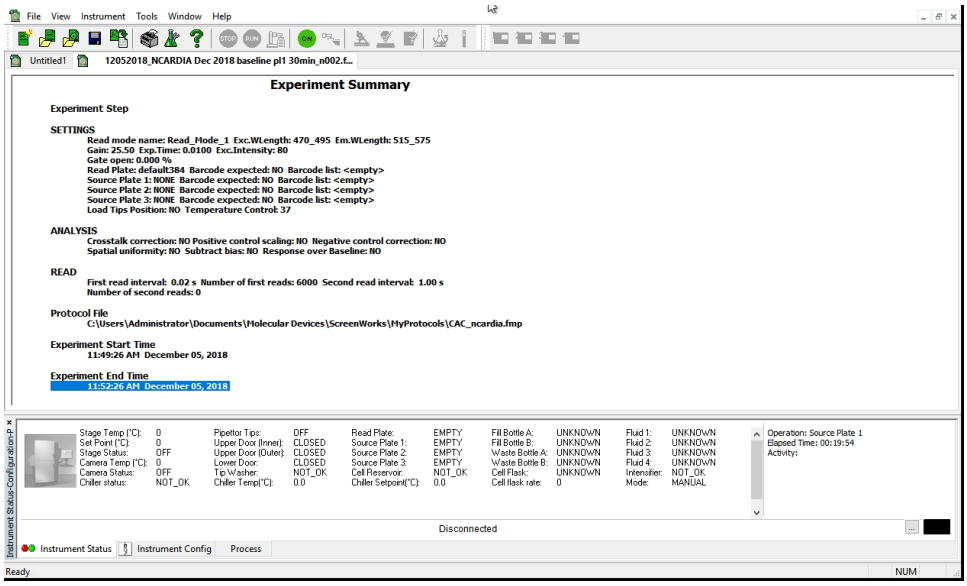


A new protocol file displays as a tab named Untitled1 that contains four default processes. Use the default protocol file to create a new protocol file. Close or ignore the default file to work with an existing protocol file or data file. See Understanding Processes on page 60.

Protocol (*.fmp) and data (*.fmd) files display the process steps to use at the top of the window, sequenced from left to right. Click the process icons to open the Settings dialog. A data file is a protocol file that also includes acquired data in the Analysis process dialog.

Experiment Summary

The Experiment Summary page is a text-based protocol summary.



To display the Experiment Summary page, click the **View** menu and select **Experiment Summary**.

Instrument Status Configuration Process

The Instrument Status Configuration Process panel opens and docks to the bottom of the main screen, by default. See User Interface Overview on page 40.

To undock and relocate the panel, drag the title bar on the left of the panel to where you want it.

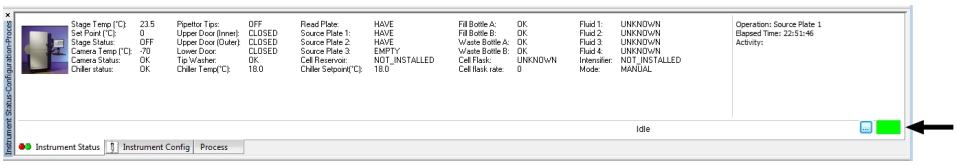
Do one of the following to hide the panel:

- Click the **X** in the title bar.
- Click the **View** menu and select **Instrument Status**.

To show the panel when hidden, select the **View** menu and select **Instrument Status**.

Instrument Status

Use the Instrument Status tab to view the status of the system hardware components.



Instrument Status Options

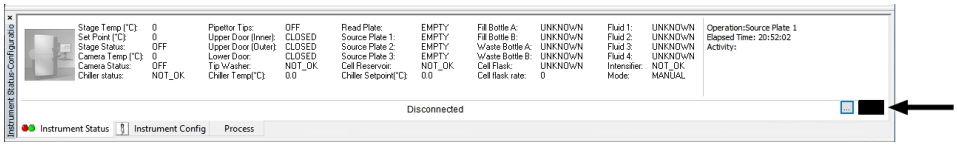
Option	Description
Stage Temp (°C)	Displays the stage temperature.
Set Point	Displays the stage temperature setting.
Stage Status	Displays whether the heated stage is turned on or off.
Camera Temp (°C)	Displays the camera temperature. Operating temperature for the camera depends on the camera. EMCCD camera: -70°C (-94°F) ± 2°C HS EMCCD camera: -70°C (-94°F) ± 2°C
Camera Status	Indicates whether the camera is turned on or off.
Chiller status	Indicates whether the chiller is available or not.
Pipettor Tips	Indicates when tips are loaded on the pipettor head.
Upper Door (Inner)	Indicates whether the inner-upper door is open or closed. **Note**: The system runs as long as the inner door is closed. Data may not be valid if the outer door is open.
Upper Door (Outer)	Indicates whether the outer-upper door is open or closed.
Lower Door	Indicates whether the lower door is open or closed.
Tip Washer	Indicates the status of the tip washer.
Chiller Temp (°C)	Displays the temperature in the chiller.
Read Plate	Indicates when a plate is present in position 3 (read plate position).
Source Plate 1	Indicates when a plate is present in position 1 (source plate 1 or tip loading position).
Source Plate 2	Indicates when a plate is present in position 2 (source plate 2).
Source Plate 3	Indicates when a plate is present in position 4 (source plate 3).
Chiller Setpoint (°C)	Displays the set point from the chiller.
Fill Bottle A	Indicates when bottle A is empty of wash solution.
Fill Bottle B	Indicates when bottle B is empty of wash solution.
Waste Bottle A	Indicates when waste bottle A is full.
Waste Bottle B	Indicates when waste bottle B is full.

Instrument Status Options (continued)

Option	Description
Cell Flask	Displays the last known state of the stir cell flask. At start the state is Unknown until you use the cell flask.
Cell flask rate	Displays the stir rate of the cell flask.
Fluid 1	Displays the last known state of the fluid 1 bottle. At the start the status is Unknown until the fluid is used.
Fluid 2	Displays the last known state of the fluid 2 bottle.
Fluid 3	Displays the last known state of the fluid 3 bottle.
Fluid 4	Displays the last known state of the fluid 4 bottle.
Intensifier	Indicates if the intensifier is installed or not installed.
Mode	Indicates if the software is in Manual mode or Remote mode.

Identify Connection Status

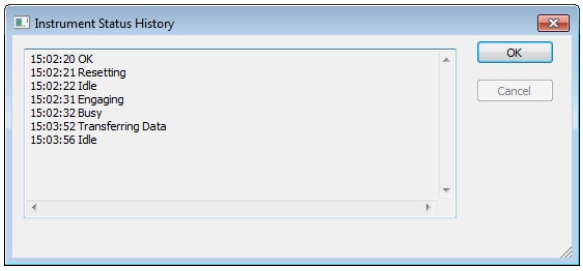
The connection status displays in the bottom-right corner of the panel to report the connection status between the ScreenWorks Software and the instrument. Green means online (connected) and black means offline (disconnected). See Instrument Status Colors on page 159.



Instrument Status Tab Panel with Offline Status Indicator

View Messages and Faults

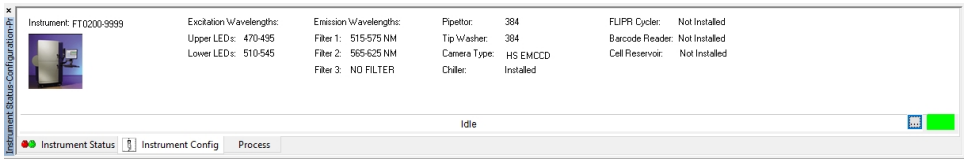
To view status messages and faults click ... next to the connection status icon to display the Instrument Status History dialog that lists the last one thousand messages.



Tip: Copy the text to the computer clipboard to paste elsewhere.

Instrument Configuration

Use the Instrument Configuration tab to view the configuration of the LED banks, emission filters, pipettor head, and optional FLIPR Cycler.



When the system is Offline, you can configure these settings to define protocols. For camera type details, see the *CCD Camera Options* section of the *FLIPR Penta High-Throughput Cellular Screening System User Guide*. See Online Mode vs. Offline Mode on page 39.

CAUTION! If you create a protocol offline that does not match the instrument configuration, when you open the protocol online, the protocol does not run until the configuration of the protocol and instrument match.

Instrument Configuration Options

Option	Description
Excitation Wavelengths	Upper LEDs - Displays the wavelength range of the top set of LED banks in the LED modules. Lower LEDs - Displays the wavelength range of the lower set of LED banks in the LED modules.
Emission Wavelengths	Displays the emission filter wavelengths installed on the system. You can install up to three filters.
Pipettor	Displays the pipettor head type (96, 384, 1536, 384 pin tool, or 1536 pin tool) installed in the system. **Note**: The pipettor head format must agree with the tip wash reservoir format. A message displays if these are different.
Tip Washer	Displays the tip washer type (96, 384, or 1536) installed in the system.
Camera Type	Select from EMCCD or HS EMCCD.
Chiller	Indicates when a chiller is installed.
FLIPR Cycler	Indicates when the FLIPR Cycler is installed.
Barcode Reader	Indicates when a barcode reader is installed.

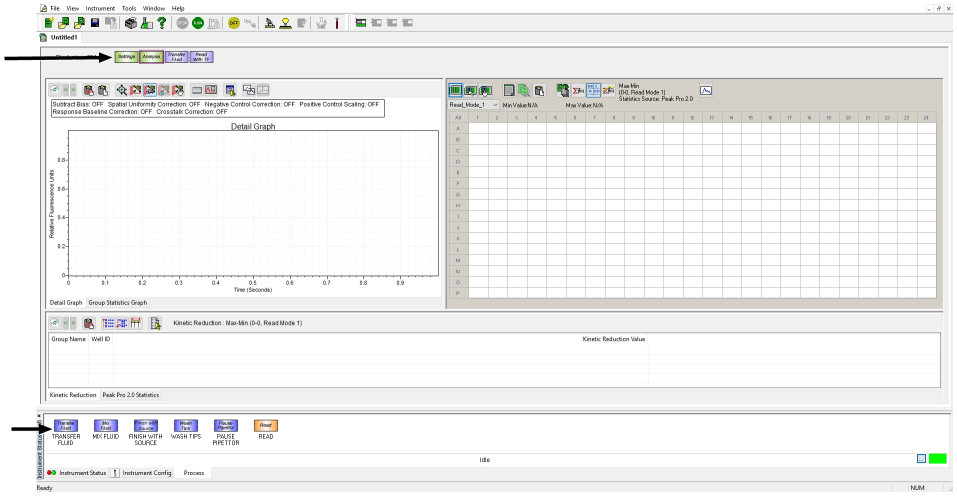
Process Tab

Use the Process tab to create new protocols and edit existing protocols.

The Process tab displays processes that you can incorporate into experiment protocols. See Using Protocols on page 60. The color of the process icon depends on the function in the protocol. See Protocol Process Icon Colors on page 59.

The list of processes is dependent upon the instrument configuration. For example, different processes appear when the instrument uses a pin tool instead of a pipettor. See Understanding Processes on page 60.

Protocol Process Icon Colors



- Green - Indicates the process is required to run an experiment and cannot be deleted.
- Blue - Indicates the process involves liquid handling. The steps run in series with each other and never run simultaneously.
- Orange - Indicates the process runs simultaneously with any liquid handling process.
- Purple - Indicates linked processes that are connected to the Transfer Fluid process, for example Mix with TF and Read with TF.

Status Bar

The status bar, across the bottom of the main window provides tool tips for commands in the main menu. When you open a menu from the menu bar and place the cursor over a command, a description of the command displays in the status bar.

To hide or display the status bar, click the **View** menu and select **Status Bar**.

Using Protocols

The protocols you create determine how the system functions while you run an experiment. Protocols consist of a series of process configurations. As you create the protocol, you define each process. See Creating New Protocols on page 96. Process options are located on the Process tab in the Instrument Status Configuration Process panel. See Process Tab on page 59.

Tip: Refer to the *FLIPR Penta High-Throughput Cellular Screening System Protocol Guide* for specific assay protocols.

To save a data file as a protocol file, see Saving Data Files as Protocol Files on page 99.

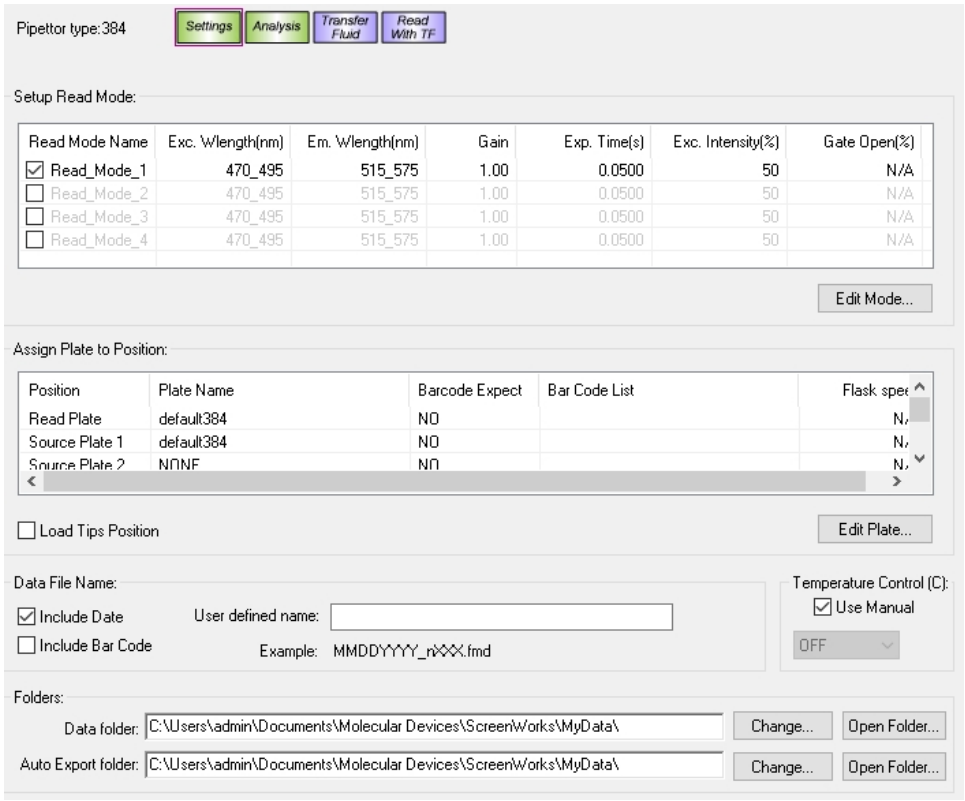
Understanding Processes

The following sections explain how to use the process options.

Settings Process

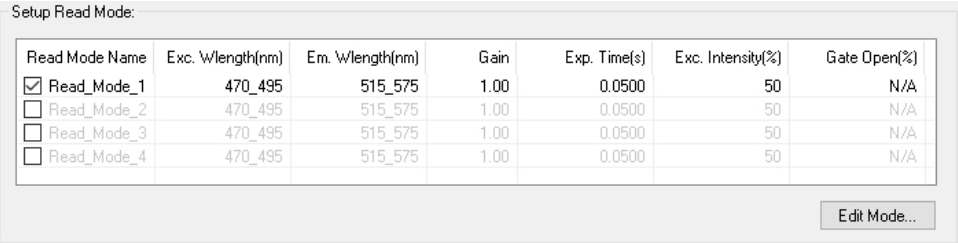
Settings is the first process in all protocols. Use settings to define the read mode, plate position, data directory, automatic file naming, and plate temperature.

Click **Settings** in the Experiment window to display the Settings page.



Setup Read Mode

Use the Setup Read Mode table on the Settings page to display settings for up to four read modes that can run in a single experiment.



Each read mode displays:

- Read Mode
- Excitation and Emission Wavelength
- Excitation Intensity
- Camera Mode (EMCCD camera and HS EMCCD camera)
- Exposure Time
- Camera Gain
- Auto Stabilize (HS EMCCD camera only)

For protocols that use multiple read modes, the instrument alternates between the read modes for each time point and outputs distinct sets of reads, one for each mode. The software calculates the ratio of the reads from each mode for each time point.

Protocol files that use read modes not supported by the instrument configuration display in red.

In the Setup Read Mode table:

- Select the check box for each read mode to use.
- Clear the check box for each read mode to not use.

Edit Read Mode

To edit read mode settings:

1. Select a **Read Mode Name** row and click **Edit Mode** or double-click a **Read Mode Name** row to display the Edit Read Mode dialog.
2. Change the following settings, then click **OK** to finish.

Note: Available options depend on the camera installed in the instrument.

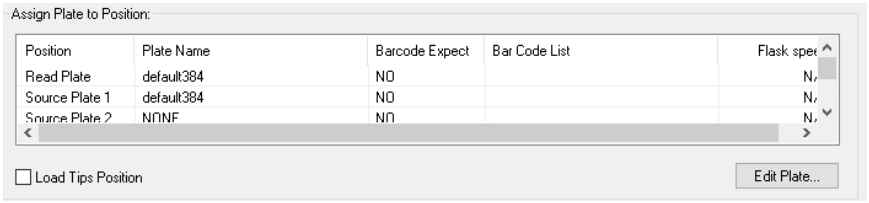
Edit Read Mode Options

Option	Description
Read Mode Name	Enter the read mode name.
Reading Mode	Select Fluorescence or Luminescence.
Excitation/Emission Wavelength	Select the excitation LED and emission filter wavelength pair. Select NONE to not turn on the LEDs or to use an empty emission filter position. These are used in luminescence experiments. **Note**: Only calibrated wavelength pairs display when the software is in Online mode. See Calibrating the Optics on page 101.
Excitation Intensity	Select the value to regulate the intensity of light emitted by the LEDs for a Fluorescence read. The range is 20% to 100%.
Camera Mode	Select the High-Speed, Sensitivity, or Normal camera mode. **Note**: Available with the HS EMCCD camera. See Understanding HS EMCCD Camera Protocol Saturation on page 32.
Exposure Time	Enter the time to regulate the light that the camera collects and measures. A longer exposure increases signal intensity. Exposure time affects the read interval for data collection. For example, in Camera mode Normal, an exposure time of 0.4 seconds added to a required 0.1 second camera integration time makes the highest update frequency 0.5 seconds. Depending on the camera, the range is 0.01 second to 30 seconds. For example, the range for the HS EMCCD camera in Camera mode High-Speed is 0.001 second to 30 seconds.
Camera Gain	Select the value to regulate the amplification of the camera power. Increase the camera gain to increase the signal. Ranges depend on the camera in use: * EMCCD camera: 1–240 * HS EMCCD camera: Read mode Fluorescence: Camera mode Normal: 1-128 or Camera mode High-Speed or Sensitivity: 20-128. Luminescence mode: 1–500
Auto Stabilize	Automatically adjusts the camera for 50 seconds for optimal functionality before the protocol starts. **Note**: Available with the HS EMCCD camera. See Stabilizing the HS EMCCD Camera on page 31.

Note: Differences in camera gain or exposure time between read modes significantly increase the minimum update time. For fastest update time, use the same gain and exposure time for all read modes.

Assign Plate to Position

Current plate assignments display in the Assign Plate to Position table. The pipettor head must have a plate type assignment for each of the positions to use in the experiment.



Click **Edit Plate** to change plate settings.

For each plate position:

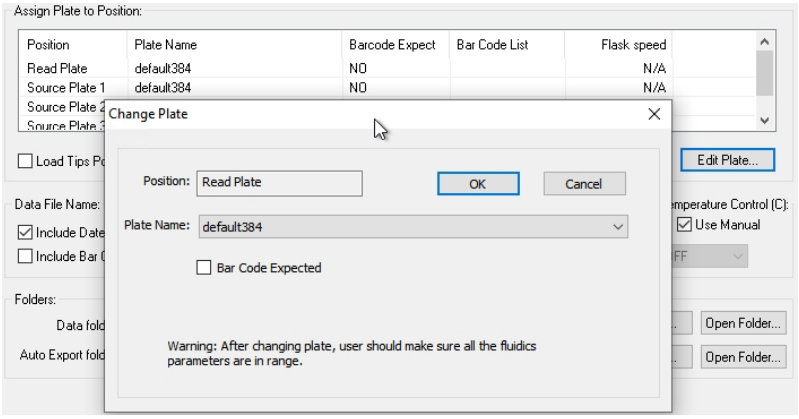
- **Plate Name** - The type of plate from the Plate Library.
- **Bar Code Expect** - Does the system to read a bar code from the plate. Bar code numbers can be incorporated into output data file names.
- **Bar Code List** - The bar codes on the plates in the experiment, in the given plate position (data files only).

If you use position 1 only for tips and to not hold a source plate, select the **Load Tips Position** check box. This removes Source Plate 1 from the assignment table.

Edit Plate

To change a plate position assignment:

1. Select a **Position** row and click **Edit Plate** or double-click a **Position** row to display the Change Plate dialog.
2. Change the following settings and then click **OK**.



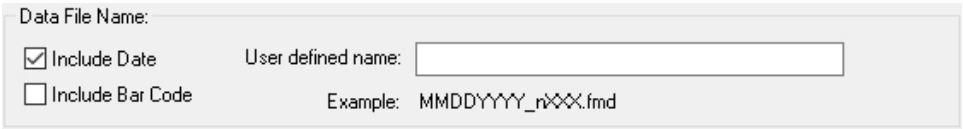
Change Plate Options

Option	Descriptions
Position	Displays the plate position to edit.
Plate Name	Select the plate type from the list of default plates and the plates you add to the Plate Library. **Note**: Only plates with the same number of wells or one order of complexity higher than the pipettor head format display.
Bar Code Expected	Select this check box to use a bar code for the plate. You can include the bar code number in the data file name that the protocol generates. In Manual mode, the software prompts you to enter the bar codes before you start the experiment. In Remote mode, the FLIPR Cycler automatically scans the barcode on the landing pad, or the third-party plate handler passes it to the ScreenWorks Software.
Cell Flask Spinning Rate	Enter the spin rate.

Note: Each plate position has a mechanical sensor to detect the presence of a plate, tip rack, or boat in each position. The instrument does not distinguish the type of apparatus you place in a position.

Data File Name

Use the Data File Name options to configure a file naming protocol for the data files the experiment creates. The file naming protocol applies to *.fmd (data) and *.png (image) files.



Date File Name Options

Option	Description
Include Date	Select this check box to include the date in the file name. **Note**: Select this check box to ensure that data files are not overwritten.
Include Bar Code	Select this check box to include the bar codes of the first five plates in the experiment in the file names. If there are fewer than five bar codes, then only those are used. **Note**: You must also select the Bar Code Expected check box for the plate position to incorporate the bar code.
User Defined Name	Enter the file name (up to 25 characters).

The base structure for file names is as follows:

Date_UserDefinedName_Barcode1_Barcode2_Barcode3_Barcode4_ Barcode5_NNN.fmd

where NNN is an integer from 1 to 999. The integer value NNN starts at 001 and increments for each data file generated with the same base data file name.

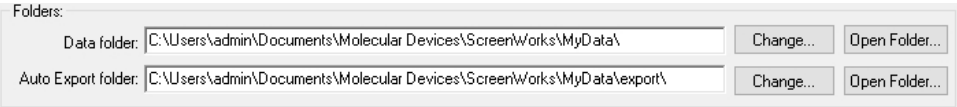
The date uses format: MMDDYYYY, where M = month, D = day, and Y = year.

If a software error occurs during a run that interferes with normal instrument operation the data file is stored as:

InterruptedExperiment_Date_Time_NNN.fmd

Folders

Use the Folders fields to define the directories to save the data and export files.



- Click **Change** to select a different directory.
- Click **Open Folder** to display the Windows Explorer dialog with the directory you designate.

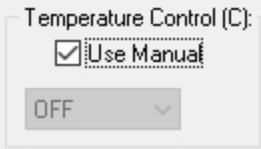
Data, image, and export files save in the following location by default:

C:\Documents and Settings\[your user name]\My Documents\Molecular Devices\ScreenWorks\MyData\

Temperature Control

Use the Temperature Control field to set the plate stage heaters to the temperature for the experiment. Temperature range is from ambient 25°C (77°F) to 40°C (104°F).

Select the **Use Manual** check box to control the heaters manually when the experiment runs.



When the experiment starts, a message displays if the stage temperature is different from the value you set. Wait until the stage reaches the temperature or run the experiment anyway.

Note: When you manually turn the temperature on through the Instrument menu, a setting of “0” in the protocol does not affect the temperature setting. To turn off the temperature manually, click the **Instrument** menu and select **Manual Operations** and then select **Temperature Control Off**.

Analysis Process

The Analysis process is included in all protocols and is always the second process after Settings.

Viewing Data

View acquired data in the ScreenWorks Software on the main Analysis process page in the Multi-Well graph or in the larger Detail graph.

Multi-Well Graph

The Analysis process page displays a grid that represents the wells of the read plate you use in the protocol or data file in the Multi-Well graph. This graph is empty for protocol files because no data is acquired. For data files each cell displays a trace of one of the measurements taken from the corresponding read plate well. The maximum and minimum relative light units value of the display data displays above the Multi-Well graph.

The Multi-Well graph displays one trace of relative light units versus time for each well. If the software records two or more read modes, select the mode to view from the display list above the graph. To see the ratio of two read modes, activate Ratiometric Options in the Configure Corrections > Corrections dialog, and select Ratio in the list.

The software applies the read mode you define in the Multi-Well graph to any traces that display in the Detail graph.

Use the Corrections dialog to adjust the traces that display in the Multi-Well graph. You can scale the traces relative to the average positive control response. See Corrections on page 75.

If you define groups for the assay, the Multi-Well graph color-codes the wells to represent each group. See Grouping on page 72.

You can include the kinetic reduction value below each well trace in the Multi-Well graph.

Use the icons above the Multi-Well graph to access various data analysis options; some relevant to protocol set up, some to already-acquired data in data files, and some to both.

Multi-Well Graph Icons

Icon	Name	Description
	Configure Groups	Displays the Grouping dialog to classify wells in the plate into groups. Groups in protocol files transfer to the data files the protocol generates. In data files, you can create new groups or edit existing groups. See Grouping on page 72.
	Select Groups	Displays the Select Groups dialog to classify which groups display in the Detail graph based on the groups you define in the Grouping dialog. **Note**: Shift + Click or Ctrl + Click to multi-select.
	Group Selection Mode	Group Selection mode displays the wells you select within a group in the Multi-Well graph in the Detail graph. Toggles between Group Selection Mode and Well Selection Mode.
	Well Selection Mode	Well Selection mode displays only wells you select in the Multi-Well graph on the Detail graph with the assigned group colors. Toggles between Group Selection Mode and Well Selection Mode.
	Notes	Displays the Notes dialog. For protocol files, enter the comments to store with the data files that the protocol generates. For data files, view the comments (now read-only).
	Images	Displays the images for data files when images save in an experiment.
	Copy	Copies data in the Multi-Well graph to the computer clipboard to paste into a different software application.

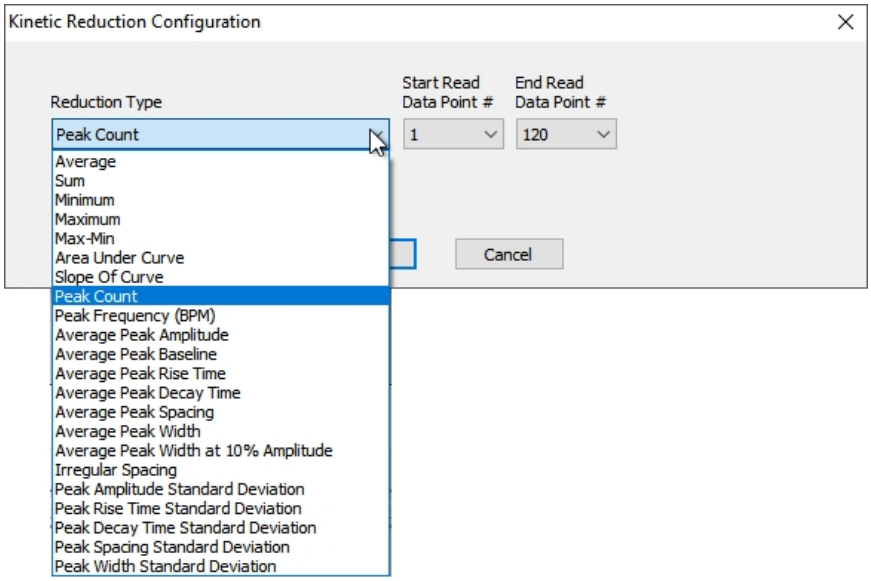
Multi-Well Graph Icons (continued)

Icon	Name	Description
	Configure Auto-Export	For protocol files, displays the Auto-Export dialog to configure the statistics and time sequence files to automatically create whenever the protocol runs. This button also exports the data after data file creation. For data files the button changes to Export and displays the File Export dialog. See Exporting Data on page 117.
	Configure Correction	Displays the Corrections dialog to apply corrections that modify the data display or to view ratiometric data. Settings made to protocol files affect how data displays when the protocol runs, but since all raw data stores in data files, these options remain available for acquired data. See Corrections on page 75.
	Hide Kinetic Reduction Value	Hides the kinetic reduction values that display in the Multi-Well graph.
	Show Kinetic Reduction Value	Displays the kinetic reduction value in each well of the Multi-Well graph.
	Configure Kinetic Reduction	Displays the Kinetic Reduction Configuration dialog to define the kinetic reduction parameters. **Reduction Type** - Select the reduction for the kinetic data traces in the Multi-Well graph. **Start Read** - Select the first read to use to determine the kinetic reduction. **End Read** - Select the last read to use to determine the kinetic reduction. **Read Mode** - Select the read mode for the kinetic reduction.

Kinetic Reduction Types

The options in the Reduction Type field in the Kinetic Reduction Configuration dialog depend on the software version. A separate license for the ScreenWorks® Peak Pro™ Software adds advanced peak detection and characterization measurements to the field of standard measurement options. This functionality is optimal for relatively fast events that are acquired with long sampling intervals. Contact your Molecular Devices Sales Representative for details.

CAUTION! High-speed camera data is not compatible with Peak Pro 1.0. You must use the ScreenWorks Peak Pro Software version 2.0 for the high-speed camera data analysis. See ScreenWorks Peak Pro Software Version 2.0 on page 123.



Standard measurement options:

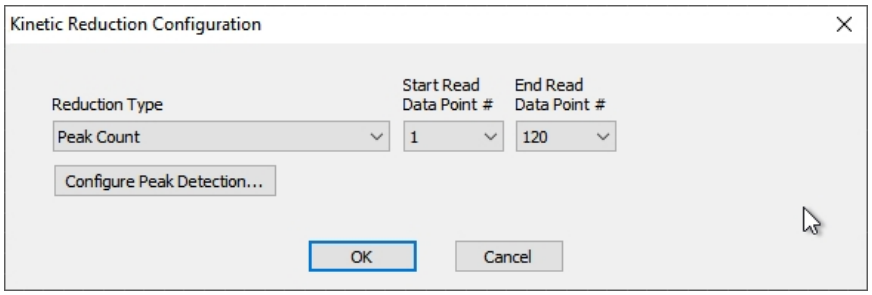
- **Average** - Numerical average of relative light units counts of the reads.
- **Sum** - Numerical sum of relative light units counts of the reads.
- **Minimum** - Lowest detected count (a single number) of the reads.
- **Maximum** - Highest detected count (a single number) of the reads.
- **Maximum-Minimum** - Result of subtracting the minimum count (a single number) from the maximum count (a single number).
- **Area Under Curve** - Numerical calculation of the area under the curve for the reads.
- **Slope of Curve** - Result of calculating the slope between two reads.

The ScreenWorks Peak Pro Software version 1.0 measurement options and definitions include (requires optional license):

- **Peak Count** - Number of peaks or action potentials detected within the read data that meet the specifications in the Configure Peak Detection dialog settings.
- **Peak Frequency (BPM)** - The beats per minute frequency of the peaks detected based on the inverse of the peak temporal spacing. At least two peaks are required for this measurement.
- **Average Peak Width** - Average peak width measurement in seconds from the 50% peak amplitude from baseline.
- **Peak Width Standard Deviation** - Standard deviation of average peak widths.
- **Average Peak Amplitude** - The average peak amplitude relative to the Average Peak Baseline measurement.
- **Peak Amplitude Standard Deviation** - Standard deviation of peak amplitudes.
- **Average Peak Baseline** - Average baseline amplitude measurement from the base of each peak detected.
- **Average Peak Spacing** - Average peak spacing in seconds. At least two peaks are required for this measurement.
- **Peak Spacing Standard Deviation** - Standard deviation of peak spacing.
- **Average Peak Rise Time** - Average time measured on the rising edge for each peak. Equal to the time between the 10% and 90% peak amplitudes.
- **Rise Time Standard Deviation** - Standard deviation of rise times.

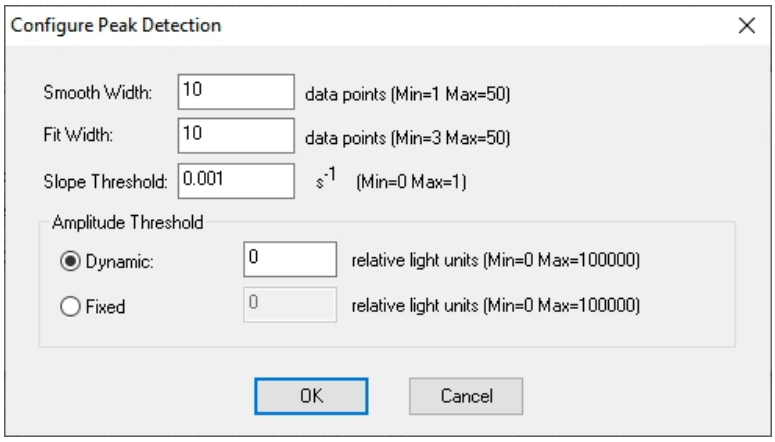
- **Average Peak Decay Time** - Average time measured on the falling edge for each peak. Equal to the time between the 90% and 10% peak amplitudes.
- **Peak Decay Time Standard Deviation** - Standard deviation of decay times.
- **Average Peak Width at 10% Amplitude** - Average peak width in seconds at the 10% peak amplitude from baseline.
- **Irregular Spacing** - Identifies wells that have irregular spacing between peaks. Wells with regular peak temporal spacings display OK. Wells with missing peaks display MISS. Wells with extra peaks display EXTRA. Wells with peak spacing characteristics of both missing and extra display IRREG.

The Configure Peak Detection button is available when you select a Peak Pro measurement in the Reduction Type field. Use the Configure Peak Detection dialog to adjust and optimize the Peak Pro measurement settings.



Configure Peak Detection dialog options:

- **Smooth Width** - Running average to reduce noise (between 5 to 10).
- **Fit Width** - Number of data points that are fit for each peak (between 5 to 15).
- **Slope Threshold** - Distinguishes between cardio beats and noise (set to 0.001).
- **Amplitude Threshold Dynamic** - Value setting is relative to the data average.
- **Amplitude Threshold Fixed** - Value setting is relative to zero.



Detail Graph

The read modes for the wells you select in the Multi-Well graph can display enlarged in a Detail graph. You can select individual or multiple wells to view or select by group.

Note: When you configure the FLIPR Penta System for two or more read modes, it cycles through each mode and alternates between the read modes for each read. Although reads from each mode within a cycle occur at different times, they are represented as occurring simultaneously.

The data that displays in the Detail graph have the same corrections applied as the data that displays in the Multi-Well graph. The Detail graph settings display at the top of the graph window:

- Subtract Bias
- Spatial Uniformity Correction
- Negative Control Correction
- Positive Control Scaling
- Response Over Baseline
- Crosstalk Correction

These settings correspond to options in the Correction dialog that you access from the Analysis process page. Use the Corrections dialog to change the settings. This changes the data in the Multi-Well graph and the Detail graph.

Detail Graph Icons

Icon	Name	Description
	Copy Graph	Copies the Detail graph to the computer clipboard to paste into a different software application.
	Copy Graph Data	Copies graph data in the Detail graph to the computer clipboard to paste into a different software application.
	Zoom Mode	Zooms in the graph. Drag over a region in the graph to enlarge the region to the full size of the graph. Drag the cursor along a part of the X or Y axis to zoom in on that axis.
	Auto Scale	The software prompts you to use the Auto Scale feature to automatically scale the Detail graph and Multi-Well graph to include all data points of the traces.
	Auto Scale Always	Automatically scales the Detail graph and Multi-Well graph to include all data points of the traces without a prompt.
	Undo Zoom	Rescales a graph to the original settings.
	Manual Scale Graph	Displays the Manual Scale Graph dialog to set the maximum and minimum values for the X-axis and Y-axis of the Detail graph and Multi-Well graph.
	Show/Hide Data	Toggles to display or hide the relative light unit values for the traces at each read.
	Show Point Labels	Writes the value of each data point beside the point on the graph.

Detail Graph Icons (continued)

Icon	Name	Description
	Show/Hide Legends	Toggles to display or hide the Detail graph legend.
	Average/Overlay Trace	Toggles to display either the average or overlay traces for groups. **Average Group** - Displays average time point values for all wells in the group. Generates a separate trace for each read mode. When you select Well Selection Mode in the Multi-Well graph, all traces in the Detail graph are averaged regardless of the group to which they are assigned. **Overlay Group** - Displays traces for all wells in the group. Traces are color-coded by group.
	Show/Hide Standard Deviation	Toggles to display or hide the standard deviation for the average group trace. **Note**: You must select Average Group to access this function. When you select Well Selection Mode in the Multi-Well graph, traces that display in the Detail graph are averaged regardless of the group to which they are assigned and the standard deviation for this average displays.

Individual Well Selections

To view a single well in a Detail graph, double-click or drag the cursor inside the Multi-Well graph over a well.

To view the traces of multiple wells overlaid in the Detail graph, drag the cursor inside the Multi-Well graph over multiple wells. This allows you to view data from rectangular block of wells within the plate. To add additional wells to the overlay, double-click or drag the cursor over the wells.

Group Selections

Use group membership to select the data to view in a Detail graph. Use Select Group or Group Selection Mode functions found in the Multi-Well graph. See Grouping on page 72.

Analyzing Data

Use the Grouping dialog and the Corrections dialog to analyze data produced during an experiment. The settings in these dialogs affect the way that data, in data files generated by the protocol, display when first viewed. They affect data in the automatic output option Auto Export that occur when you run the protocol. See Exporting Data on page 117.

Data files that the ScreenWorks Software create retain all raw data readings, so the analysis options in the Grouping dialog and the Correction dialog apply to acquired data, modifying the display of these, irrespective of the analysis settings configured in the original protocol. See Grouping on page 72 and Corrections on page 75.

Grouping

Use groups to select data to view in the Detail graph. Use in the Correction dialog to correct the data relative to responses to positive and/or negative control groups.

Define groups in the Analysis >  Configure Groups > Grouping dialog.

A group is a selection of wells with a common characteristic, usually in the type of compound you add to the wells. Three groups (background fluorescence correction, positive controls, and negative controls) are defined by default, but no wells are assigned to them. You specify the well assignment for these groups.

Create additional groups to represent wells with a specific ligand, ligand concentration, or any other characteristic to classify data. Group definition includes input for compound concentration. Use the Series option to create a series of groups where a number of different concentrations are automatically assigned to each group. This is useful to create groups for an IC_{50} or EC_{50} experiment.

The Grouping dialog contains a grid that represents the 96-well, 384-well, or 1536-well read plate. The basic steps are to select a group from the list then drag the cursor over the wells in the grid to assign to the group.

Grouping Options

Option	Description
Groups	Displays the list of groups, group name, concentration, and notes. Wells you assign to the group have the same color in the grid below and in the group list. **Positive Controls** - Use this group to assign positive control wells, typically the maximum response to a concentration of an agonist. **Note**: You must define Positive Control wells to use the Positive Control Scaling Correction. **Negative Controls** - Use this group to assign negative control wells, typically buffer addition controls. **Note**: You must define Negative control wells to use the Negative Control Correction feature. **BF Controls** - Use this group to assign wells in which to measure background fluorescence. The software uses the measurements for each time point from these wells background fluorescence correction of ratiometric data. See Ratiometric Options on page 77. **Add New Group** - Use this to define a new group or a series of groups.
Add/Edit Group	Displays the Edit Group dialog to edit the group. Select the Add New Group row to add a new group. See Adding New Groups on page 73.
Delete Group	Deletes the group you select in the list. Wells assigned to the group lose their color to indicate that they no longer belong to a group.
Delete All Groups	Deletes all user defined groups. You cannot delete positive, negative, and background fluorescence control groups, but this command deletes all wells you assign to these groups.
Clear All Selections	Removes all group assignments from all wells. This does not affect the group names in the list.
Undo Last Selection	Removes group membership from the last wells that you assign to a group.

Assigning Wells to Groups

To assign wells to a group:

1. Click the **Groups** drop-down and select a group.
2. In the plate layout, drag the cursor over the wells to add to the group. Click the row or column title to include the respective wells in the group.

> **Note:** Wells in a group do not need to be contiguous. You can select multiple areas of the plate.

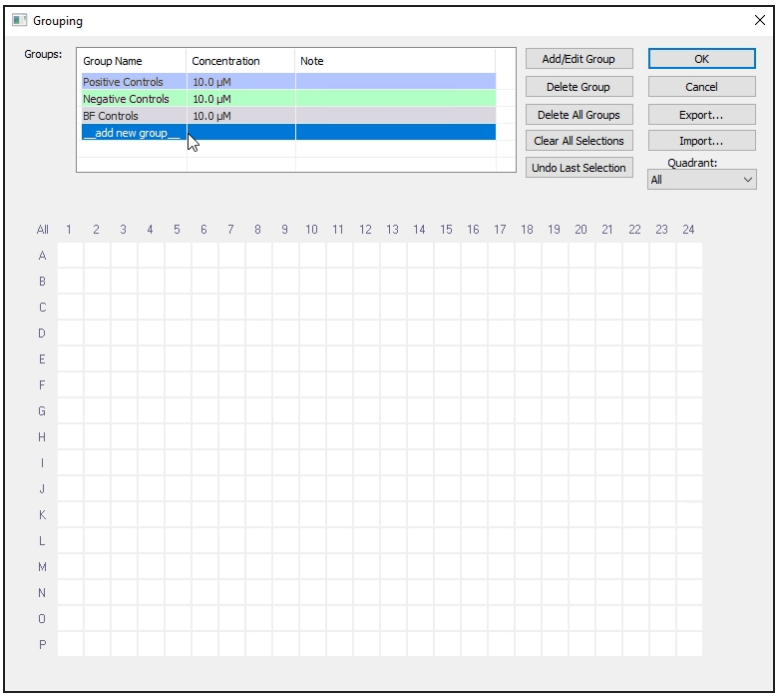
To deselect an individual well or neighboring wells, right-click in one corner of the well, then drag over the well or wells to deselect.

Adding New Groups

Use the Grouping dialog to add and edit groups.

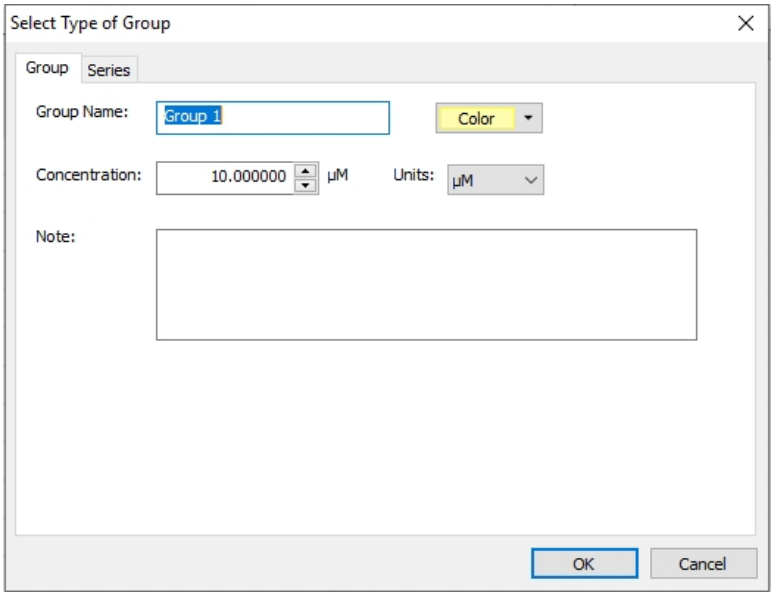
To add a new group:

1. Click the **Analysis** menu and select **Configure Groups** to display the Grouping dialog.



2. Click **Add/Edit Group** or double-click a group name to display the Select Type of Group dialog.
3. Select a tab:
   - Select the **Group** tab to configure a single group with specific settings.
   - Select the **Series** tab to configure a series of groups with incrementing concentration values.

Group Tab

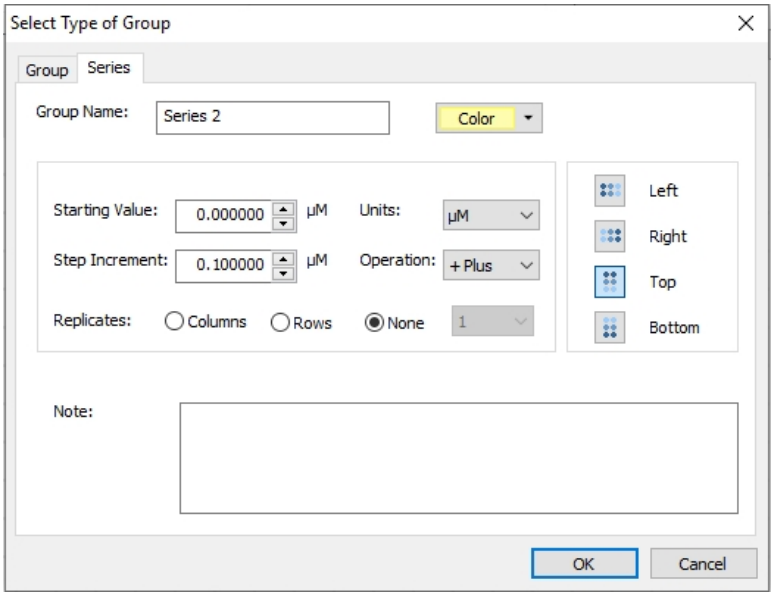


To add a group:

1. Select the **Group** tab.
2. In the **Group Name** field, enter the group name.
3. In the **Concentration** field, enter the concentration value.
4. Click the **Units** drop-down and select **nM**, **µM**, **mM**, **M**, or **Log**.
5. In the **Notes** field, enter a note to associated with the group.
6. Click **OK**.

Series Tab

Use a series to assign a dose response curve with regular increment steps. The software assigns successive rows or columns different concentrations. See Assigning Wells to Groups on page 73.

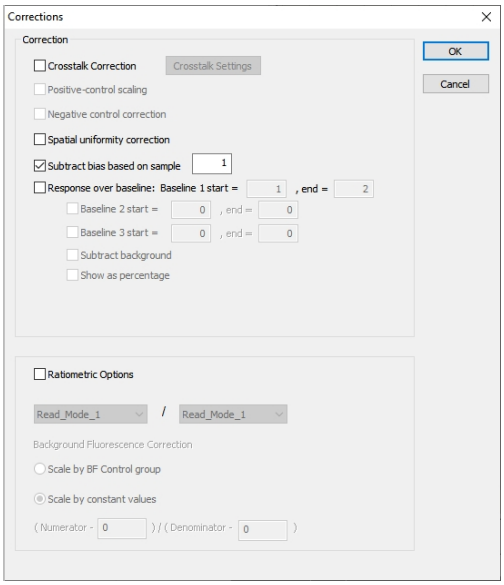


To add a series of groups:

1. Select the **Series** tab.
2. In the **Group Name** field, enter the group name.
3. In the **Starting Value** field, enter the value for the lowest or highest concentration of the series.
4. In the **Step Increment** field, enter the value for the change in concentration between concentrations in the series.
5. Click the **Units** drop-down and select **nM**, **µM**, **mM**, or **M**.
6. Click the **Operation** drop-down and select **Plus**, **Minus**, **Multiply**, or **Divide**.
7. Select a **Replicates** option: select whether replicates align in **Row** format or **Column** format on the plate.
   - If replicates align in both row and column formats, indicate the number of replicates, for example, replicates in rows indicates all wells in the rows that have the same concentration.
   - If two rows include the same concentration, enter **2**.
   - If there are no replicates, select **None**.
8. Select the direction in which the series increments, either **Left**, **Right**, **Top**, or **Bottom**.
9. In the **Notes** field, enter notes to associate with the group.
10. Click **OK**.

Corrections

Use the Corrections dialog to apply data correction algorithms and to view ratiometric data.



Changes affect:

- Data display in the Multi-Well graph and Detail graph.
- Data display in group statistic table and graphs.
- Data in file export. See Exporting Data on page 117.

The settings you make in the Correction dialog for protocol files are important for the output options because you cannot change exported data. The ScreenWorks Software keeps the raw data, so the settings you change in the Correction dialog simply change how you view the data in resulting data files.

Corrections Options

Option	Description
Positive Control Scaling	Select this check box to average each maximum relative light units value from the positive control group and normalize all samples to this value (set at 100%). Use this to graph a dose response curve or to compare data between experiments.
Negative Control Correction	Select this check box to calculate an average negative control well value and ratio-to-well value for each sample interval (time point). The software calculates this ratio for each sample interval and applies it to all wells. This provides a good correction for signal drift and artifacts.
Spatial Uniformity Correction	Select this check box to use the initial signal to normalize signal in each well, remove fluctuations due to well-to-well variation in cell density, and dye loading. The algorithm averages the initial signal from all wells together and then scales each individual well relative to the average. Use this to normalize cell number, type, and dye-loading conditions throughout a plate. **Note**: Use spatial uniformity only when all wells in a plate are treated the same prior to an experiment (for example, dye loading, cell numbers, etc.).
Subtract Bias Based on Sample	Select this check box to subtract the relative light units measured at a read number from all other time points in each well. Use this to set the Y-axis values for all data graphs to zero at the time point you enter. Enter the read number to subtract in the corresponding field. The default is read number 1.
Response Over Baseline	Select this check box to display the trace as a ratio of the response to the average of a set of reads. **Baseline Start** - First read to include in the averaged baseline value. **Baseline End** - Last read to include in the average baseline value. **Show As Percentage** - Displays response as a percent increase over the average baseline value.

For details, see Data Processing Algorithms on page 197.

You can apply each of the correction options alone or in combination with the others. When you select a combination, they apply in the following sequence:

1. Crosstalk
2. Spatial Uniformity Correction or Response Over Baseline
3. Negative Control Correction
4. Positive Control Scaling
5. Subtract Bias

Note: You cannot apply spatial uniformity correction and percent baseline at the same time.

Ratiometric Options

Where you configure two read modes you can view the data as a ratio of one read mode to the other, for each data point.

When you activate this option, traces of the calculated ratio display in the Detail graph.

Before calculating the ratio, the software corrects ratiometric data for fluorescence background by subtracting estimated background values from both numerator and denominator for each time point. After the fluorescence background is subtracted, the ratio is then calculated. Additional corrections are applied to the ratio, beginning with negative control correction, positive control scaling, followed by subtract bias.

Note: Spatial uniformity correction and response over baseline are not available when Ratiometric Options are active.

Ratiometric Options

Option	Description
Ratiometric Options	Select this check box to allow ratiometric data viewing. Select the read modes to use as numerator and denominator to specify how the ratio is defined.
Background Fluorescence Correction	**Scale by BF Control Group** - For each time point and read mode in the ratio, the average value for that time point in the background fluorescence control wells (BF Controls, in the Grouping dialog) is subtracted. The ratio is calculated after subtraction of the average value from both numerator and denominator. **Scale by Constant Values** - Enter the constant values to subtract from the numerator and denominator for each time-point ratio. You must enter these constant values in the numerator and denominator fields.

Group Statistics

Use the Group Statistics table to analyze data from an experiment. You can analyze only groups you define in the Grouping dialog in this section. See Grouping on page 72. Settings you make in the Correction dialog influence the group statistics that are reported. See Corrections on page 75.

Click **Configure Kinetic Reduction** in a Multi-Well graph to define the reads and reduction type to analyze.

Group Statistics Table Icons

Icon	Name	Description
	Copy Table Data	Copies data in the Group Statistic table to the computer clipboard to paste into a different software application.
	Collapse All Groups	Collapses the Group Statistics table to display the group name and statistics. Toggles with Expand All Groups.

Group Statistics Table Icons (continued)

Icon	Name	Description
	Expand All Groups	Expands the Group Statistics table to display the individual wells and kinetic reduction values that comprise each group statistic. Toggles with Collapse All Groups.
	Auto Fit All Columns	Collapses the column width so it is no wider than the title of the column or largest data point.
	Select Statistics	Displays the Choose Statistics dialog to define groups statistics. **Concentration** - Numerical concentration values you assign to each group. **Units** - Concentration units you assign to each group. **Notes** - Comments you assign to each group. **Average** - Numerical average of the kinetic reduction values for a group. **Maximum** - Highest value (single #) of all kinetic reductions within a group. **Maximum-Minimum** - Result of subtracting the minimum kinetic reduction value from the maximum count kinetic reduction value for a single group. **Minimum** - Lowest kinetic reduction value within a group. **Sum** - Numerical sum of all kinetic reduction values within a group. **Standard Deviation** - Defines the numerical value you associate with one standard deviation from the average. **Standard Deviation+1** - Equals the average group value plus one standard deviation. **Standard Deviation-1** - Equals the average group value minus one standard deviation. **Z Score** - Evaluates quality or performance of the assay and is dependent on the concentration evaluated[1]. **Z-factor** - Evaluates the quality or performance of the assay at a given concentration. Typically used for all concentrations, not including the positive control. Calculation used: Z = 1 - [3*(std dev of GROUP + std dev of neg ctrls)/abs(mean of GROUP - mean of neg ctrls)] **Z'-factor** - A characteristic parameter for the quality of the assay itself. Typically performed using data only from the positive control concentration. Calculation used: Z' = 1 - [3*(std dev of pos ctrls + std dev of neg ctrls)/abs(mean of pos ctrls - mean of neg ctrls)].

1. Zhang, J., Chung, T. D. Y. and Oldenburg, K. R. (1997). A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Journal of Biomolecular Screening 4(2):67:73.

You can plot the statistics you define in the Group Statistic table in the Group Statistic graph, located behind the Detail graph, to view a dose-response curve. All groups are included in the graph, unless you right-click on the group and select Exclude Groups From Statistic Chart. Groups not used in the graph display a green line through the row. To include the group in the graph, right-click and select Include Groups In Statistic Chart. Select a group statistic in the Group Statistic table to display the data in the Group Statistic graph.

Group Statistic Graph Icons

Icon	Name	Description
	Copy Graph	Copies data in the Group Statistic graph to the computer clipboard to paste into a different software application.
	Copy Graph Data	Copies the graph data in the Group Statistic graph to the computer clipboard to paste into a different software application.
	Zoom Mode	Zooms in the graph. Drag over a region in the graph to enlarge the region to the full size of the graph. Drag the cursor along a part of the X or Y axis to zoom in on that axis.
	Auto Scale	The software prompts you to use the Auto Scale feature to automatically scale the Detail graph and Multi-Well graph to include all data points of the traces.
	Auto Scale Always	Automatically scales the Detail graph and Multi-Well graph to include all data points of the traces without a prompt.
	Undo Zoom	Rescales the graph to the original settings.
	Manual Scale Graph	Displays the Manual Scale Graph dialog to set the maximum and minimum values for the X-axis and Y-axis.
	Show/Hide Data	Toggles to show or hide the data for the traces at each read.
	Show Point Labels	Writes the value of each data point next to the point on the graph.
	Show/Hide Legends	Toggles to display or hide the detail graph legend.
	Show/Hide Smoothed Curve	Toggles to display or hide the 4-parameter curve fit. Can display at the same time as Show/Hide Original Trace.
	Show/Hide Original Trace	Toggles to display or hide the trace of the graph, which connects each data point. Can display at the same time as Show/Hide Smooth Curve.
	Show/Hide Real Data Points	Toggles to display or hide the data points to create the graph. When you hide the data points, you should activate either the Original Trace or Smoothed Curve function.
	Display X-Axis in Log Scale	Toggles to convert the X-axis from a concentration to a log concentration scale.
	Show/Hide EC/ICXX Value	Toggles to display or hide the effective/inhibition concentration. You define the percentage of activation/inhibition that the graph plots.

Image Display

Use the Image Display dialog, from the Analysis process page, to view images you save in data files when you activate Save Images during a Read with TF step. This stores a total of up to 6,000 images per experiment and you define the number of images per dispense. The default is set to one image before and nineteen images after the fluid addition initiates. You can play back saved *.tif files in sequence, frame-by-frame or as a video.

Use Image Display to diagnose problems. For example, if cells are blown off during a fluid dispense, you see dark holes in the middle of the cell layer after the fluid addition. If the entire well decreases in relative light units, it is likely that extracellular dye has been diluted.

Notes

Use the Protocol Notes dialog to enter comments to keep with the data files that the protocol generates. In data files the same dialog displays the comments that you add in the protocol file in read-only format.

Transfer Fluid Process

Use the Transfer Fluid process to program reagent or cell additions and to execute them in conjunction with other processes. The default protocol has one Transfer Fluid process.

A blue Transfer Fluid icon indicates that the process occurs in series with other blue colored processes in the protocol. A purple icon indicates that the execution of the process is synchronized to a Read or Mix Fluid process.

The Transfer Fluid process has options for pin tools and standard pipettors. You must configure aspiration and dispense of compound in each case separately.

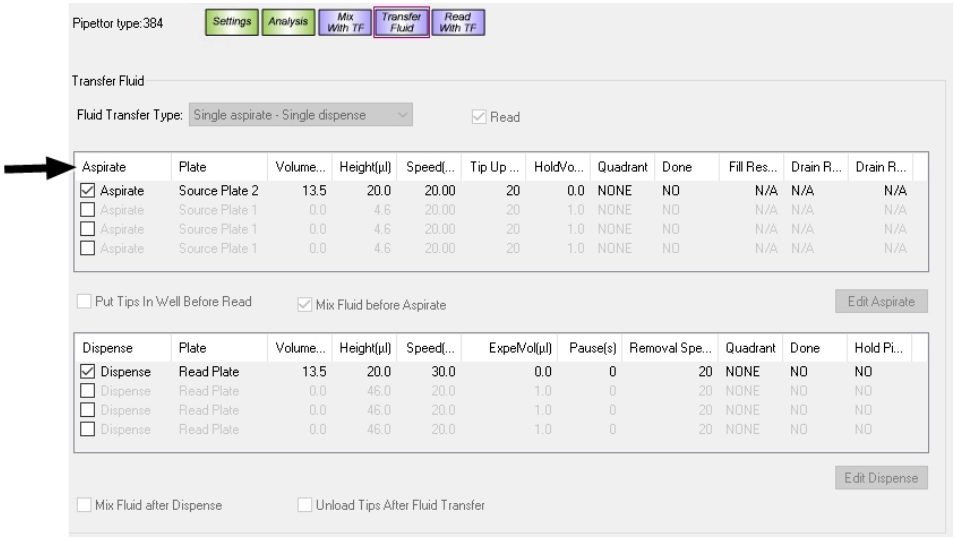
For a standard pipettor you can configure more than one aspiration or dispense (but not both) within the process, with the multiple Fluid Transfer type.

When you select a standard pipettor, you configure aspiration and dispense steps by selecting the row in the aspiration or dispense table and then click Edit to display a configuration dialog. With the Pin Tool, aspiration and dispense configuration is done on the Transfer Fluid page.

Select a multiple dispense Fluid Transfer type to allow additional dispense steps with a standard pipettor. Then configure the first dispense with a volume less than the volume aspirated. An additional dispense is automatically activated for the remaining volume. For multiple aspirates, configure the first aspirate with less volume than the dispense.

Note: The entire volume of fluid aspirated is always dispensed.

Aspirate Configuration (Standard Pipettor)



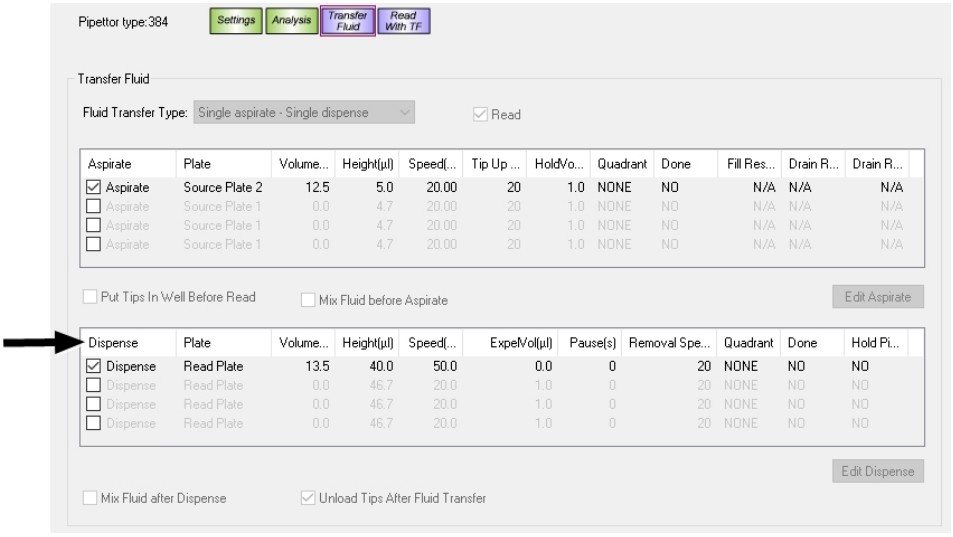
Aspirate Configuration Options for Standard Pipettor Use

Option	Description
Fluid Transfer Type	Select **Single Aspirate – Single Dispense** to aspirate one liquid volume and dispense that entire liquid volume in one action into a plate. Select **Multiple Aspirate – Single Dispense** to aspirate up to four liquid volumes from up to four aspirate plates and dispense entire tip contents into a single plate. To add additional aspirates, make the aspirate volume less than the dispense volume. Select **Single Aspirate – Multiple Dispense** to aspirate one liquid volume and dispense up to four distinct liquid volumes into four distinct quadrants or plates. Add additional dispenses by making the dispense volume less than the aspirate volume. The last dispense expels all liquid that remains in the tip. Tips to improve Single Aspirate – Multiple Dispense (by quadrature) pipetting precision include: * When pipetting, do not use hold and expel volumes and use the slowest removal speed (2 mm/s). * Aspirate 5 –10% more than is needed from the source plate (for example, aspirate 110 µL when you dispense 25 µL into four quadrants. * Include a mix step with 3 strokes in the source plate to pre-wet tips. Mixing of fluid in the source plate primes the internal walls of the tips, reducing surface tension between the fluid and tip plastic.
Read	Select this check box to link a Read process to the Transfer Fluid process. This automatically adds a Read with TF step to the protocol and displays the configuration page for that step. See Read Process on page 89. **Note**: This is required if you intend to read a plate while transferring liquid during a kinetic cell-based assay.
Aspirate	Select the check box at the start of a row to activate an aspirate sequence. If you select the Multiple Aspirate Fluid Transfer type, enter an aspirate volume less than the dispense volume to add another aspirate step. Only the aspirate sequences you select are active in the protocol.

Aspirate Configuration Options for Standard Pipettor Use (continued)

Option	Description
Edit Aspirate	Select the check box for an aspirate row and click Edit Aspirate or double-click on a row to display the Edit Aspirate dialog. **Source Plate** - Select the plate from which to remove an aliquot of fluid. **Quadrant** - Select the quadrant from which to aspirate the volume. Available when you aspirate liquid from a plate using a pipettor head of lower density, for example, 384-well plate using a 96 pipettor head. **Volume** - Enter the volume (in µL) to aspirate. **Speed** - Enter the speed at which to aspirate (in µL/s). **Height** - Enter the distance (measured in µL) from the bottom of the well where the tips should start aspiration. **Note**: Tips move downward full volume of aspiration, for example, aspirate 10 µL with a start height of 35 µL moves the pipettor height to 25 µL after aspiration. Pipettor head movement helps prevent well overflow when tips submerge in the well. To prevent downward pipettor motion, set the tip height to minimum value. Tip movement downward halts when the tips reach 1/30th well volume. **Hold Volume** - Enter the size of the air gap (in µL) following aspiration. Air gaps prevent liquid from leaking out of the tip before dispensing. **Removal Speed** - Enter rate (in mm/s) at which to pull tips from the well after aspiration. Adjust this value depending on fluid viscosity or volume of fluid in the well. For low volume transfers, a slow up-speed may improve precision.
Put Tips in Well Before Read	Select this check box to place the tips in the read plate prior to beginning read intervals. Use this for assays where placing tips below the fluid surface can prevent excessive turbulence. This helps ensure accurate timing of fluid dispense relative to reads.

Dispense Configuration (Standard Pipettor)



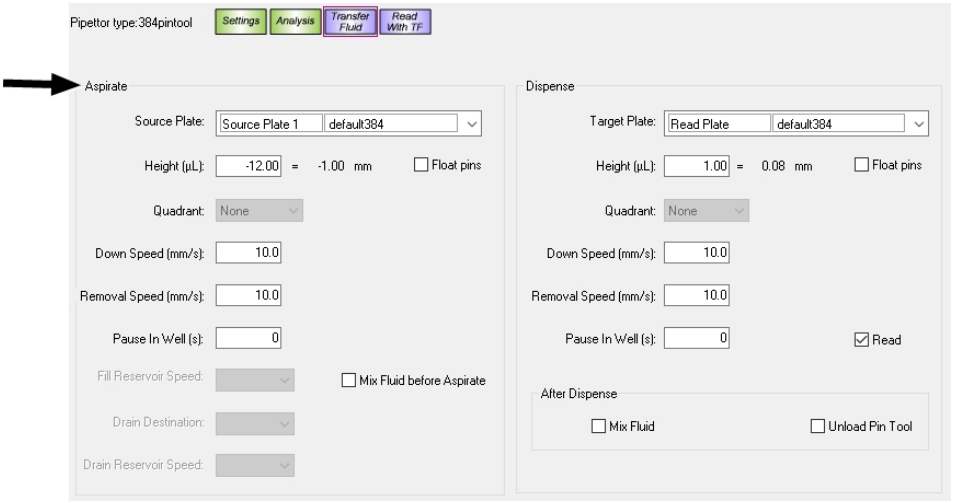
Dispense Configuration Options for Standard Pipettor Use

Option	Description
Dispense	Select this check box for a row to activate a dispense sequence. If you select the Multiple Dispense Fluid Transfer type, enter a dispense volume less than the aspirate volume to add a dispense step. Only dispense sequences you select are active in the protocol.
Mix Fluid After Dispense	Select this check box to link a Mix Fluid process to the Transfer Fluid process. This adds a Mix with TF step to the protocol and displays the configuration page for that step. Available when the fluid transfer type has a single dispense. See Mix Fluid Process on page 87.
Unload Tips after Fluid Transfer	Unloads tips after the Fluid Transfer process. Use this to change the tips during an experiment or to reduce down time between experiments. Available when you select Load Tips Position on the Settings process page. This feature also requires that no source plate is placed in plate Position 3 during the experiment.

Dispense Configuration Options for Standard Pipettor Use (continued)

Option	Description
Edit Dispense	Displays the Edit Dispense dialog to define dispense parameters. **Target Plate** - Select the plate to receive the fluid dispense. This is the Read Plate. **Quadrant** - Select the quadrant from which to dispense the volume. Available when you aspirate liquid from a plate using a pipettor head of lower density for example, 384-well plate using a 96-pipettor head. **Volume** - Enter the volume (in µL) to dispense. In the last dispense, the entire content in the tip dispenses. **Height** - Enter the distance (measured in µL) from the bottom of the well to insert the tips prior to dispense. Tips move upward the full volume of dispense during dispense, for example, a 10 µL dispense with a start height of 35 µL moves the pipettor to a height of 45 µL after dispensing. This movement helps prevent well overflow when tips submerge in the well. Tips do not move higher than the full volume of the well. **Speed** - Enter the speed to dispense (in µL/s). Ideal dispense speed accounts for the volume added, how fast the signal increases in response to compounds, and strength of the attached cells at the bottom of the plate. **Expel Volume** - Enter an additional volume (in µL) the pipettor dispenses over the dispense volume. This is equal to the aspirate hold volume. In a multiple dispense, only the last dispense can have an expel volume. If you select Mix Fluid After Dispense, expel volume changes to zero for the dispense for the fluid transfer to ensure that tips remain in the well prior to the mix step. The expel volume is transferred to the Mix with TF step to help ensure liquid is properly expelled from the tips after mixing. **Removal Speed** - Enter the rate (in mm/s) at which to pull the tips from the well after the dispense. Adjust this value for fluid viscosity, fluid volume in the well, or if the transfer performs to a dry well. **Pause in Well** - Enter the time (in seconds) to pause with the tips in the wells after the dispense and mix before the next pipettor move. **Hold Pipettor During Dispense** - Select this check box to hold the pipettor at the dispense height. Read intervals might occur simultaneously with this step. The pipettor pauses during a read to prevent disturbances in the imaging process.

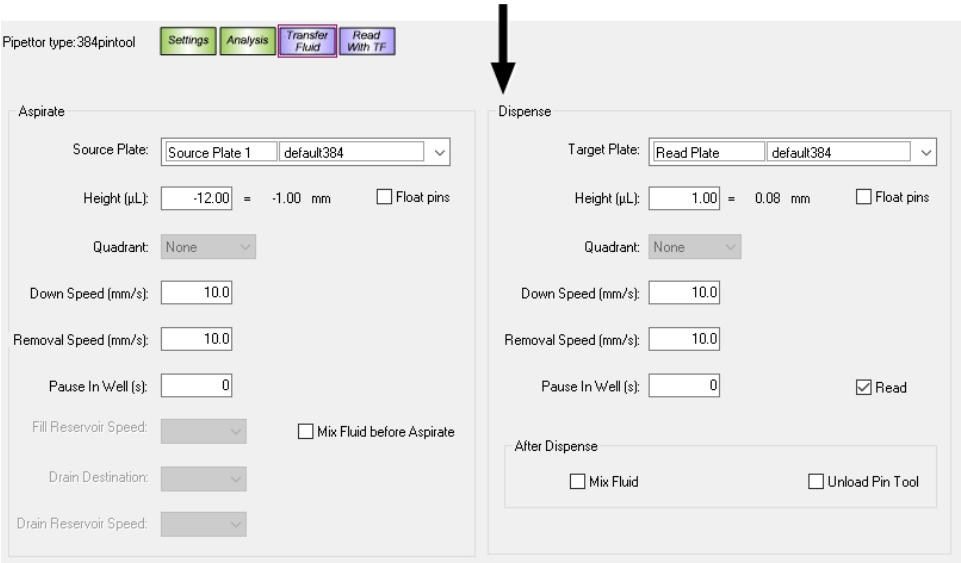
Aspirate Configuration (Pin Tool)



Aspirate Configuration Options for Pin Tool Use

Option	Description
Source Plate	Select the plate from which to remove an aliquot of fluid. This is a plate that contains an agonist or antagonist.
Height	Enter the distance (measured in µL) from the bottom of the well to place the pins. The distance equivalent in millimeters displays. Select the **Float Pins** check box to move the pipettor down to a position at which the pins should be 1 mm below the bottom of the wells. As pins come to rest on the well bottoms additional downward motion of the tip block is absorbed by the tips moving into the block. This ensures that all pins are submerged in the same volume of fluid across the plate. The Height is designated as -1.00 mm.
Quadrant	Select the quadrant from which to aspirate volume. Available when you use a pipettor head of lower density to aspirate liquid from the plate, for example, 1536-well plate using a 384 pin tool head.
Down Speed	Enter the speed (in mm/s) at which to insert the pin tool into the source plate.
Removal Speed	Enter the speed (in mm/s) at which to remove the pin tool from the source plate. The speed of removal determines the volume of fluid remaining on the pins. Increase the removal speed to increase the volume of compound on the pin after aspiration. Slow the removal speed to decrease the fluid volume. Assay development should be done to determine the volume transfer based on removal speed.
Pause in Well	Enter a duration (in seconds) to hold the pin tool in the source plate after full insertion and before removal.

Dispense Configuration (Pin Tool)



Dispense Configuration Options for Pin Tool Use

Option	Description
Target Plate	Select the plate to receive the fluid dispense; typically, the Read Plate.
Height	Enter the distance (measured in µL) from the bottom of the well to insert the tips. The distance equivalent in millimeters displays to the right of the µL value.
Quadrant	Select the quadrant of the target plate into which to dispense compound. Available when you use a pipettor head of lower density to aspirate liquid from the plate, for example, 1536-well plate using a 384 pin tool head.
Down Speed	Enter the speed (in mm/s) at which to insert the pin tool into the target plate.
Removal Speed	Enter the speed (in mm/s) at which to remove the pin tool from the target plate. The speed of removal determines the volume of fluid that remains on the pins.
Pause in Well	Enter the duration (in seconds) to hold the pin tool in the dispense plate after full insertion and before removal.
Read	Select this check box to link a Read process to the Transfer Fluid process. This adds a Read with TF step to the protocol and displays the configuration page for that step. See Read Process on page 89. **Note**: This is required if you read a plate while transferring liquid during a kinetic cell-based assay (for example, calcium mobilization).
Mix Fluid	Select this check box to link a Mix Fluid process to the Transfer Fluid process. This adds a Mix with TF step to the protocol and opens the configuration page for that step. See Mix Fluid Process on page 87.
Unload Pin Tool	Select this check box to unload the pin tool after the dispense.

Mix Fluid Process

You add fluid mixing steps to protocols as independent protocol steps, Mix Fluid or linked to a Transfer Fluid step, Mix with TF. You configure independent Mix Fluid steps to mix fluid in source or read plates, while Mix with TF steps immediately follow the Transfer Fluid step to which they are linked to mix fluid in the read plate where compound dispenses.

Different mix options are available for standard pipettors and the pin tool.

Mix Fluid (Standard Pipettor)

Fluid mixing with a standard pipettor consists of a series of aspirations and dispenses in and out of the tips.

Mix Fluid Options (Standard Pipettor)

Option	Description
Mix Plate	Select the plate that contains the fluid to mix. Typically, it is a source plate from which you transfer fluid.
Quadrant	Select a quadrant from which to mix fluid. Available when you use a pipettor head of lower density to aspirate liquid from the plate, for example, 1536-well plate using a 384 pin tool head.
Volume	Enter the volume (in µL) to mix.
Speed	Enter the speed (in µL/s) to mix the fluid.
Height	Enter distance (in µL) from the bottom of the well that the tips will insert prior to mixing. Tips move up and down with aspirate and dispense commands. These movements help prevent well overflow when the tips submerge.
Expel Volume	Enter an additional volume (in µL) the pipettor dispenses, beyond the volume you enter in the Volume field. This helps ensure that all fluid expels from the tips after mixing.
Strokes	Enter the number of times to aspirate and dispense mix volume. One stroke is equal to one aspiration, and one dispense.
Removal Speed	Enter the rate (in mm/s) at which to pull the tips from the well after the mix. Adjust for fluid viscosity or fluid volume in the well.
Pause In Well	Enter the time (in seconds) to pause with the tips in the well after mixing and before the next pipettor head move.
Unload Tips After Mix	Select this check box to unload the tips after the mix step.

Mix Fluid (Pin Tool)

Fluid mixing with a pin tool consists of a sequence of up and down motions of the pin block.

Mix Fluid Options (Pin Tool)

Option	Description
Mix Plate	Select the plate that has the fluid to mix. Typically, it is a source plate from which you transfer fluid.
Top Height	Enter the height (in µL) for the top of the stroke during mixing.
Quadrant	Select a quadrant in which to mix fluid. Available when you use a pipettor head of lower density to aspirate liquid from the plate, for example, 1536-well plate using a 384 pin tool head.
Bottom Height	Enter the height (in µL) for the bottom of the stroke during mixing.
Strokes	Enter the number of times the pins move within the mix step. One stroke is equal to one up-stroke and one down-stroke.
Down Speed	Enter the speed (in mm/s) of the down stroke.
Removal Speed	Enter the rate (in mm/s) at which to pull pins from the well after the mix. Adjusted for fluid viscosity or fluid volume in the well. The speed of removal determines the volume of fluid remaining on the pins.
Up Speed	Enter the speed (in mm/s) of the up stroke. This is not the rate which pins remove from the well.
Pause in Well	Enter a duration to hold the pin tool in the well plate once the mix completes, before withdrawal.
Unload Pin Tool	Select this check box to unload the pin tool after mixing.

Mix With TF

Fluid can be mixed immediately after the dispense to ensure proper mixing of agonists and antagonists with the cell monolayer. This is necessary when you transfer fluids one-tenth or less of the total fluid volume of a well.

To add a mix after the dispense, select the Mix After Fluid Dispense (Mix Fluid for the Pin Tool) check box in the Transfer Fluid dialog. This inserts a mix step, and the Mix Fluid process icon turns purple to indicate that mixing occurs immediately after the dispense and concurrently with a Read process.

Note: Any expel volume you configure in the dispense step prior to a mix is locked if you select Mix Fluid After Dispense. Tips are raised during expel volume which can lead to inadequate mixing, so mixing occurs with the tips at the level at which they finish the basic dispense and expel volume occurs after mixing completes.
Mixing parameters for a Mix with TF process are similar to a Mix Fluid process, however you cannot configure Mix Plate, Quadrant, Hold Volume, and Tip Up Speed. These options are available in the Transfer Fluid process to which you link the mix.
To remove a Mix with TF process, deselect it in the Transfer Fluid dialog.

Read Process

You can add Read steps to protocols as independent protocol steps, read or linked to a Transfer Fluid step Read with TF. Configuration options for the two types of steps are similar, except Read with TF allows you to time the read precisely with the time that compound dispenses, and it allows you to save the images used to measure the response. You can save up to 6,000 images per experiment.

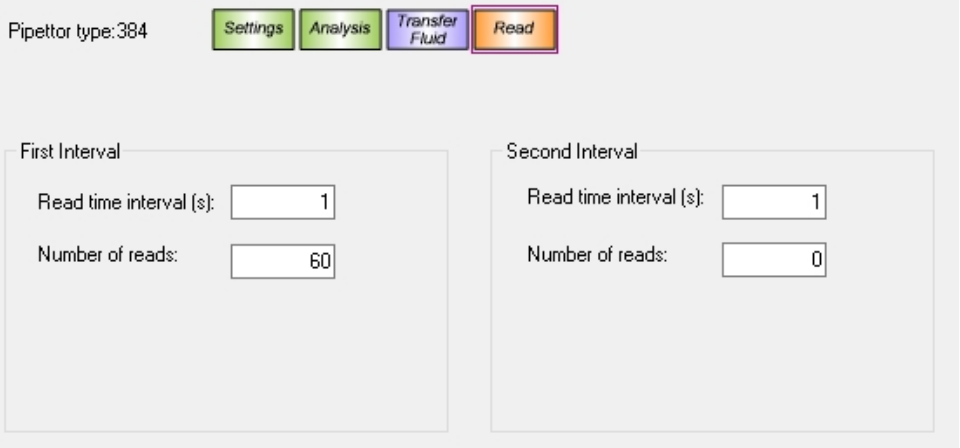
Note: When you use the HS EMCCD camera, depending on the Protocol > Settings > Edit Read Mode > Camera Mode setting, when you use a fast read time interval value, the plate matrix panel update might be delayed until the end of the data acquisition rather than updating during the data acquisition.
Specifically, the following settings cause the plate matrix panel to update at the end of the data acquisition:
For Camera mode High-Speed - A Read Time Intervals setting of 0.018 seconds or shorter.
For Camera mode Normal or Sensitivity - A Read Time Intervals setting of 0.029 or shorter.
For Camera Mode setting details, see Edit Read Mode on page 61.

Read

To add a Read process to a protocol that is independent of a liquid transfer, drag the Read process icon from the Process Explorer to the Experiment window. The Read process page displays when you release the icon. When you add a Read process to a protocol the icon is orange to indicate it is an optics function and occurs in parallel with fluid processes during the experiment.

A Read consists of a sequence of images taken at a predefined rate from which relative light values for each well are measured. You can divide a single Read process into two intervals with different read rates to take images at a high rate while compound is added, and shortly thereafter, to best capture kinetic effects. Images can then be taken at a less frequent rate as cells settle and less change occurs.

Note: The read rate you define for the Read process is the same for all the read modes you select. For example, if you set the Read Interval at one second, and have two read modes, two images are taken every second, one for each read mode.



Read Options

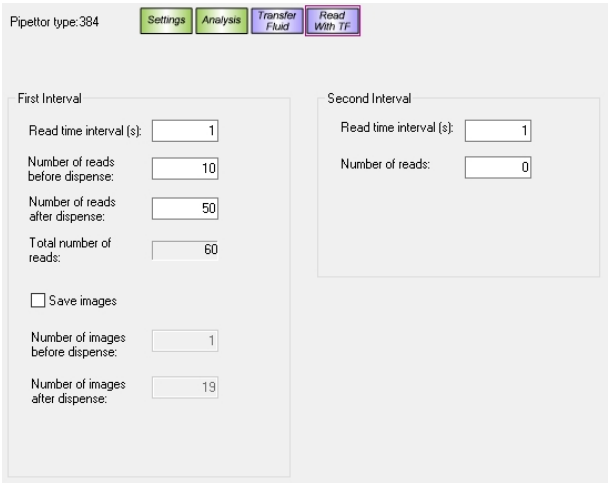
Option	Description
First Interval	Configure the first series of reads in the Read process. **Read Interval** - Enter the time (in seconds) between reads. The software uses the same interval for each read mode (for example, the instrument takes images twice as fast if you select a second read mode). **Note:** Time should be no less than exposure time plus read-out time. If it is less, the experiment speed is determined by the capability of the computer. See Setup Read Mode on page 61. **Number of Reads** - Enter the total number of reads to take in the first interval.
Second Interval	Configure the second series of reads in the Read process. To skip a second series with a different read interval, enter zero for the Number of Reads. **Read Interval** - Enter time (in seconds) between reads. Must be a multiple of the first read time (for example, if read interval 1 is 1 second, then read interval 2 should be 2 seconds or 3 seconds). **Note:** Time should be no less than exposure time plus read-out time. If it is less, the experiment speed is determined by the capability of the computer. See Setup Read Mode on page 61. **Number of Reads** - Enter the total number of reads to take in the second interval.

Read With TF

Plate reads concurrent with fluid transfer is necessary to collect data for kinetic cell-based assays as the response time is very rapid. To link a Read process to a Transfer Fluid process, select Read in the Transfer Fluid page. When you insert a read in the Transfer Fluid page, the Read process icon turns purple to indicate that the read occurs concurrently with liquid addition.

Read with TF configuration is similar to an independent Read process, but with the addition of Number of Reads Before Dispense and Save Images.

Note: To remove a Read with TF process, deselect the Read option in the Transfer Fluid process dialog.



Read With TF Options

Option	Description
Number of Reads Before Dispense	Enter the number of reads to take before fluid is added to the plate. This provides a cellular response baseline before the addition of agonist or antagonist. Number of Reads Before Dispense is included in the total number of first interval reads you enter in the Number of Reads field, for example, if the Number of Reads is 60 and the number of Reads Before Dispense is five, there are 55 reads after the dispense initiates.
Number of Reads After Dispense	Enter the number of reads to take after fluid is added to the plate. This captures the cellular response after the addition of agonist or antagonist. Number of Reads After Dispense is included in the total number of first interval reads you enter in the Number of Reads field
Save Images	Select this check box to save up to 6,000 images an image file (*.tif) for the experiment. Use these images for more than one dispense as long as the total images saved do not exceed 6,000 for the experiment. **Number of Images Before Dispense** - Enter the total number of images to save prior to the dispense initiation. **Number of Images After Dispense** - Enter the total number of images to save after the dispense initiates. By default, one image prior to and nineteen after the dispense save for review. See Image Display on page 80. **Note:** Use images to troubleshoot problems with the cell plate such as cells detaching during fluid dispense.

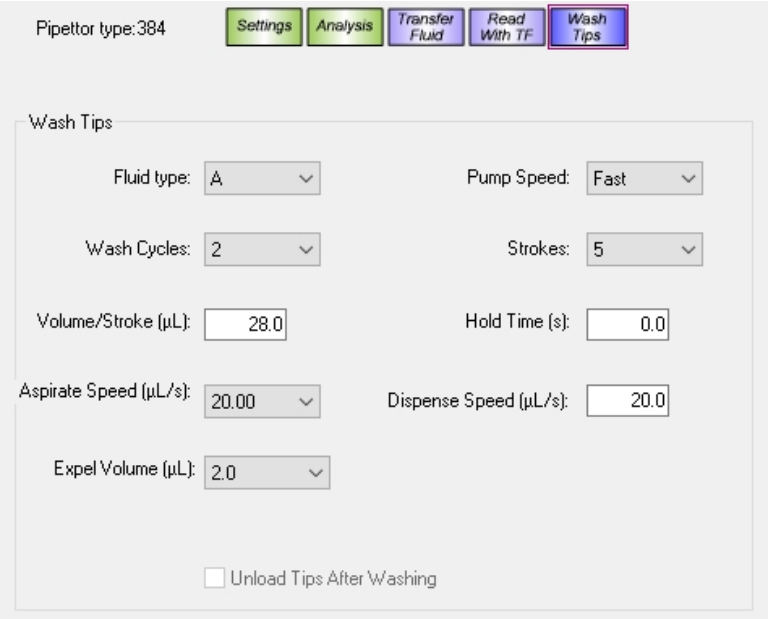
Wash Tips or Pins Process

Use the Wash Tips or Pins process to eliminate unwanted agonist or antagonists prior to another addition within an experiment or between experiments to reduce consumable costs and downtime associated with tips replacement.

For standard pipettors, tips are inserted into the wash location and wash fluid aspirated and dispensed. This can be done a number of times (strokes) in the same wash fluid, then the whole process can repeat up to five times (wash cycle) with fresh wash fluid.

For the pin tool, up and down motion is used to force fluid in and out of the capillary slot of the pins. A typical wash protocol uses an initial solvent to remove compound from the pins, followed by a second solvent to remove the first solvent. You should blot pins at a blotting station to remove excess liquid from the pins between washes.

Wash Tips (Standard Pipettor)



Wash Tips Options for Standard Pipettor Use

Option	Description
Fluid Type	Select the fluid in the wash reservoir during tip washing, from wash fluid container A or B.
Pump Speed	Select the pump speed (slow or fast) to fill and empty the wash reservoir. Select Fast except with volatile solvents.
Wash Cycles	Select the number of times that fluid cycles through the wash reservoir. For each cycle, the pipettor executes the number in the Strokes field.
Strokes	Select the number of times to aspirate and dispense wash fluid.
Volume/Stroke	Enter the volume (in µL) of fluid to aspirate and then expel for each stroke.
Hold Time	Enter the length of time (in seconds) to pause between aspirating and expelling fluid.
Aspirate Speed	Select the speed (in µL/s) to aspirate fluid.
Dispense Speed	Enter the speed (in µL/s) to dispense fluid.
Expel Volume	Select the volume to expel.
Unload Tips After Washing	Select this check box to remove tips from the pipettor head after the wash completes.

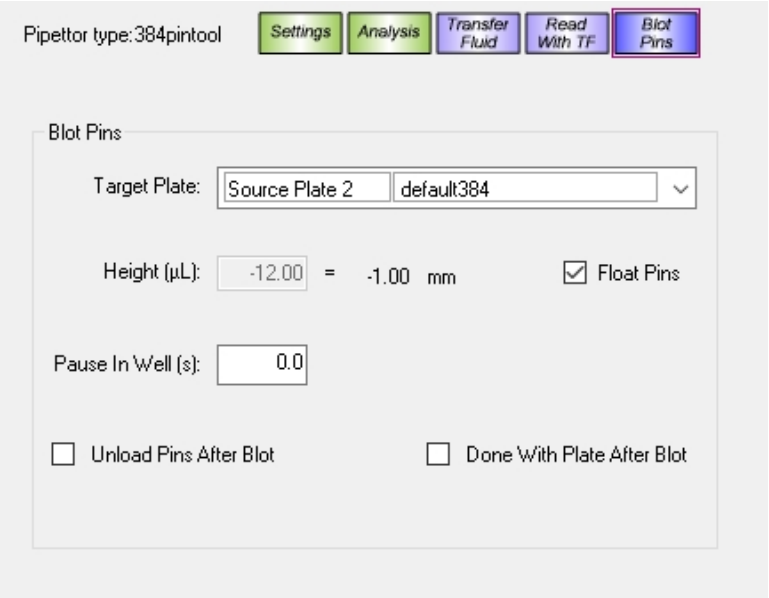
Wash Pins (Pin Tool)

Wash Pins Option for Pin Tool Use

Option	Description
Fluid Type	Select the fluid in the wash reservoir during tip washing, from wash fluid container A or B.
Pump Speed	Select the pump speed (slow or fast) to fill and empty the wash reservoir. Select Fast except with volatile solvents.
Wash Cycles	Select the number of times that fluid cycles through the wash reservoir (up to 5). For each cycle, the pipettor executes the number in the Strokes field.
Strokes	Select the number of times to raise and lower the pins. One stroke is equal to one upstroke and one down stroke.
Down Speed	Enter the speed of the pin tool during the down stroke.
Stroke Distance	Enter the height of one stroke that the pin tool travels.
Up Speed	Enter the speed of the pin tool during the up stroke.
Removal Speed	Enter the speed of the pin tool withdrawal from the wash location. The faster the withdrawal speed the more fluid remains on the pins.
Pause in Well	Enter the time to hold the pin tool at the bottom of the wells in the wash solution after the wash completes.
Unload Pin Tool After Wash	Select this check box to remove the pin tool after the wash completes.

Blot Pins Process

The Blot Pins process is only available for the pin tool to dry the pins between transfers or washes, to prevent carry-over.



Blot Pins Options

Option	Description
Target Plate	Select the plate.
Height	Enter the distance (measured in µL) from the bottom of the well to place the pins. The distance equivalent in millimeters is displays. Select the Float Pins check box to move the pipettor down to a position at which the pins would be 1 mm below the bottom of the wells. As pins come to rest on the blotting surface additional downward motion of the tip block is absorbed by the tips moving into the block. This ensures that all pins are in contact with the blotting surface. The Height is designated as -1.00 mm.
Pause in Well	Enter the duration during which the pin tool is held motionless at the set height.
Unload Pin Tool After Blot	Select this check box to unload the pin tool once blotting completes.
Done with Plate After Blot	Select this check box to indicate that you are done with the plate after blotting.

Pause Pipettor Process

Include Pause Pipettor steps in a protocol to prevent future pipettor motion until the pause completes. Read processes can continue to execute, unless stopped by the linked action read with transfer. Use this to stop the pipettor from picking up compound if it sits for an extended period in the air and to provide time for solvents (such as EtOH) to evaporate off the pins after a wash.

Enter the duration of the pause in seconds.

Wash Cell Reservoir Process

Use this process to prime the reservoir with cells prior to an assay or for cleaning purposes and is recommended for use before and after you use cells in suspension. No pipetting is associated with this process. This is available only when the cell reservoir is installed.

Wash Cell Reservoir Options

Option	Description
Pre-coat Tubes	Select to pump the fluid up to the sensor before filling the reservoir.
Fluid Source	Select the fluid to use for the wash process. Fluid 1 – 4 bottles are available as well as the cell flask.
Drain Reservoir Destination[1]	Select the drain destination for the fluid left in the reservoir after the wash. Waste bottles, cell flask, or any of the fluid bottles are available.
Fill Reservoir Speed[1]	Select the pump rate to fill the cell reservoir (range 1 – 10).
Drain Reservoir Speed[1]	Select the pump rate to drain the cell reservoir (range 1 – 10).
Wash Cycles	Enter the number of wash cycles to run.
Hold Time	Soaks the reservoir in the pumped fluid by leaving the fluid in the reservoir for this length of time (range 0 – 300 seconds or 0 – 5 minutes).
1. Available when the cell reservoir is installed and selected as the source plate for aspiration.	

Finish With Source Process

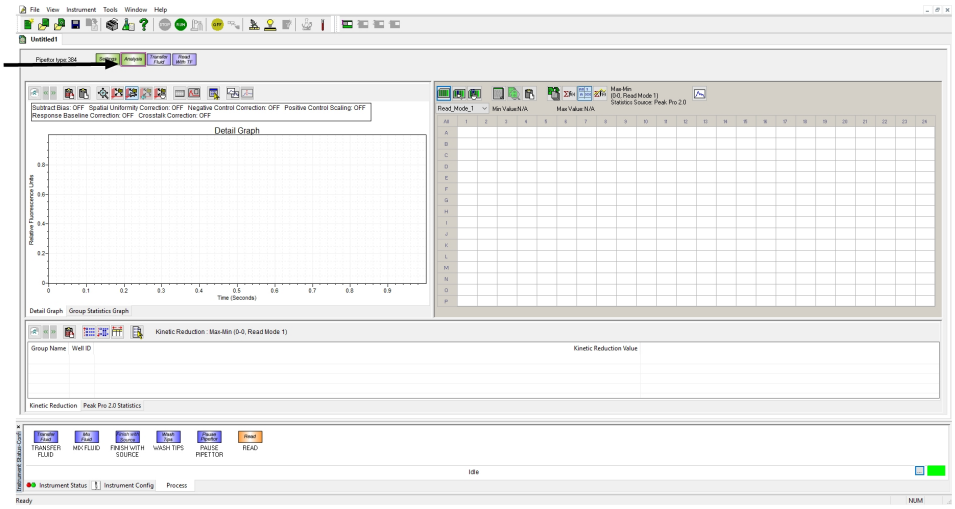
In Remote mode, the Finish With Source process instructs the instrument to notify the robot controller to remove the source plate. This is required when you use multiple plates in a single source location.

Note: This process is not available in Manual mode or for the read plate position.

Creating New Protocols

In the ScreenWorks Software, creating a protocol involves combining processes represented in the protocol builder as colored block icons at the top of the Experiment window. The available processes depend on your instrument configuration. See Instrument Configuration on page 58.

The color of the process icon depends on the function in the protocol. A red box around the process in the protocol list indicates the process selection and indicates which configuration settings dialog is open. See Protocol Process Icon Colors on page 59.



Protocols contain combinations of the following processes:

- **Settings** - Required for every protocol, included at the beginning of every new protocol file, and cannot be removed.
- **Analysis** - Required for every protocol, included at the beginning of every new protocol file, and cannot be removed.
- **Transfer Fluid** - Included in new protocols and has the following linked processes:
  - **Read with TF** - Included in new protocols after Transfer Fluid and timed precisely to coincide with compound addition to the read plate. You can deselect this setting from the Transfer Fluid settings dialog.
  - **Mix with TF** - Optional selection within the Transfer Fluid settings dialog.
- **Mix Fluid**
- **Wash Tips** - Available with pipettor configuration.
- **Wash Pins** - Available with pin tool configuration.
- **Blot Pins** - Available with pin tool configuration.
- **Pause Pipettor**
- **Finish with Source**
- **Read**

To create a new protocol:

1. Select the **Process** tab. See Process Tab on page 59.
2. Do one of the following to add the assays steps:
   - To place the new process at the end of the protocol list, drag and drop the process anywhere in the Experiment window.
   - To place the new process in the protocol list, drag and drop the process where you want it. The software prompts you if the position is invalid.

3. When the Configuration screen displays, adjust the settings. See Understanding Processes on page 60.
4. Repeat the previous steps until you finish building the protocol.
5. Click the **Analysis** process and define the analysis parameters See Analysis Process on page 65:
   - Click **Grouping** to define grouping parameters.
   - Click **Correction** to define correction parameters.
   - Click **Export** to define export parameters.
6. Click on other process icons to open the Configuration screen and make changes.

Note: When you use the HS EMCCD camera with a fast read time interval value, depending on the Protocol > Settings > Edit Read Mode > Camera Mode setting, the plate matrix panel update may delay until the end of the data acquisition.
Specifically, the following settings cause the plate matrix panel to update at the end of the data acquisition:
For Camera mode High-Speed with a Read Time Intervals setting of 0.018 seconds or shorter.
For Camera mode Normal or Sensitivity with a Read Time Intervals setting of 0.029 or shorter.

7. Click the **File** menu and select **Save** to display the Save As dialog.
8. In the **File Name** field, enter the protocol name.
9. Click **Save**. Protocol files save as a *.fmp file. The default Save In location for the protocols is C:\Documents\Molecular Devices\ScreenWorks\MyProtocols.
10. Run the experiment.

Refer to the *FLIPR Penta High-Throughput Cellular Screening System Protocol Guide* for details.

Deleting Processes from Protocols

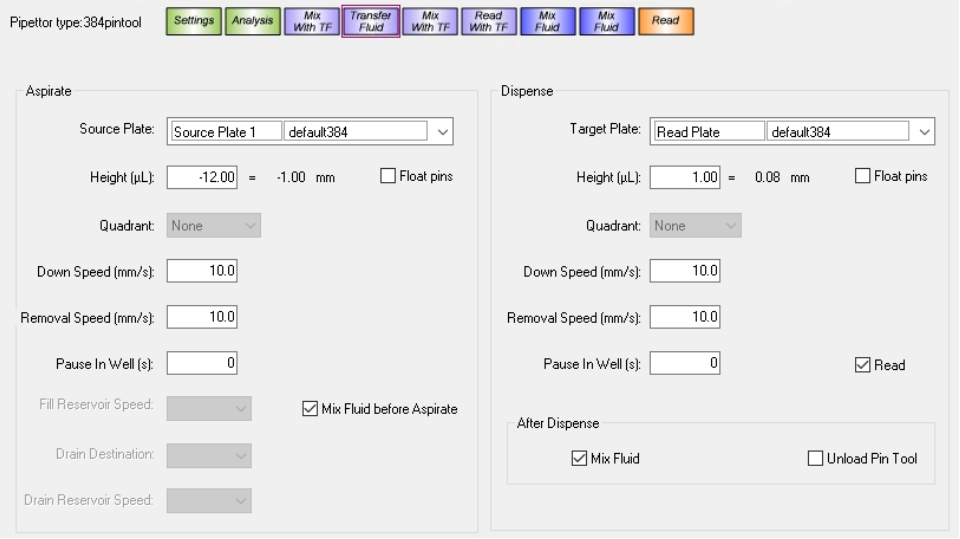
You can link Protocol processes to the Transfer Fluid process.

To delete processes that are not linked:

- In the protocol list, select a non-purple process icon, and then on the keyboard, press **DELETE**.

To delete a linked Transfer Fluid process:

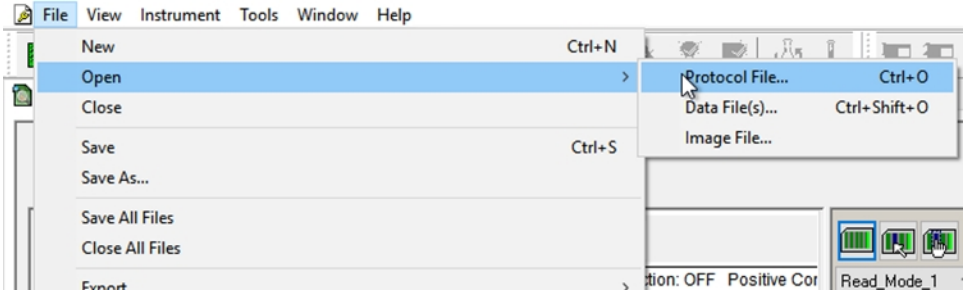
1. In the protocol list, select the purple **Transfer Fluid** icon to display the settings dialog.
2. Deselect the linked process to delete, either **Read** or **Mix Fluid**.



Opening Protocols

To open a protocol file:

1. Click the **File** menu and select **Open** and then select **Protocol File** to display the Open dialog.



2. Select the protocol file name and click **Open**.

Deleting Protocols

You cannot delete a protocol from within the ScreenWorks Software.

To delete a protocol:

1. In the Windows File Explorer navigate to where the protocols save. The default location is C:\Documents\Molecular Devices\ScreenWorks\MyProtocols.
2. Select the *.fmp file to remove and on the keyboard, press **DELETE**.

Saving Data Files as Protocol Files

You cannot edit or use the protocol information stored in data files (*.fmd) to run a new experiment. You can extract the information to a protocol file. Experiments you run using the new protocol file have the same protocol steps as the original data file.

Tip: When you use a protocol file (*.fmp), you can add, remove, or change processes and then save the amended protocol to run it.

To store a data file as a protocol file:

1. Click the **File** menu and select **Save As** to display the Save As dialog.
2. In the **File name** field, enter the protocol name.
3. In the **Save as Type** field, select **Protocol Files (*.fmp)**.
4. Click **Save**. The software strips the data from the new file and stores associated protocol information in the protocol file.

Note: Saved changes only affect the protocol. The data file from which the protocol was derived remains intact.

Chapter 4: Calibrating and Signal Testing

Calibrating Optics

Adding Read Plates to Plate Library

The FLIPR Penta System has default plate masks for 96-well, 384-well, and 1536-well read plate types. If you use a plate type that varies from the default formats, you must create new dimensions and mask for that plate type. See Plate Process Definition on page 49.

To add a plate and mask to the software:

1. Prepare a 1 x 10^{-8} M fluorescein solution.
2. Ensure the plate bottom is clean and free of scratches and dust.
3. Dispense enough fluorescein solution into each well so as to cover the bottom. Dispensing half of the total well volume is a safe volume. Use the FLIPR Penta System pipettor.
4. Place the plate that contains fluorescein in the read position
5. Click the **Tools** menu and select **Plate Library**.
6. From the **Plate Process Definition** list, select a plate with a similar well format.
7. Click **Copy Plate**.
8. Enter a **Plate Name**.
9. Click the **Plate Type** drop-down and select **Read Plate**.
10. Enter the plate dimensions.
11. Click **Finish**. The plate mask definition is complete when the green Assay Finished (Unlocked) light illuminates on the instrument door.

Calibrating the Optics

The FLIPR Penta System identifies the serial number, wavelength range, and position of each LED bank in the instrument. This information, in addition to the wavelength range for the emission filters, is stored with the system calibration files. When you change LEDs or emission filters you must recalibrate the Flat Field Calibration files for the new LED/Filter pairs.

Due to the improved light tightness, the background correction calibration in previous software versions that used black bottom plates is no longer required.

Excitation LED

The Calibration dialog displays the list of the excitation/emission wavelength (LED/Filter) combinations for the installed modules. The corresponding status for LED calibration displays Done or Not Done. The status for the flat field calibration displays either Done or Not Done.

Use the excitation LED calibration only when the LED calibration status displays *Not Done* after you select to use the 335–345 nm/475–535 nm (340 LED Module).

To run an excitation LED calibration:

1. Click the **Instrument** menu and select **Calibration** or click the **Calibration** icon to display the Calibration dialog.
2. Select **335–345 nm/475–535 nm**.
3. Click **Excitation LED Calibration**.
4. When the progress bar closes, continue with flat field calibration.

Flat Field Calibration

An accurate flat field calibration is required before you adjust exposure settings. Use this to correct for light falloff at the corners of the camera lens and to correct gradients or falloff in the coverage of the light module. When you apply an accurate flat field correction, the well values for all wells give the same count value for the same stimulus.

When you test for HS EMCCD saturation in the Signal Test dialog, keep the flat field correction in mind because the central values hit saturation first, while the outer values continue to get larger as the exposure increases to beyond the maximum camera sensor limit, because they are being multiplied by the correction factor.

During a fluorescence assay, plate illumination results in imperfect, non-uniform excitation distribution on the plate surface. Light distribution is more intense in the middle of the plate than the edges. The software applies a flat field algorithm to the plate image to compensate for variations in light intensity across the plate. LEDs are not used for luminescence assays so flat field corrections do not require any adjustment.

The Calibration dialog displays the excitation/emission wavelength (LED/filter) combinations for the installed modules. The calibration status for flat field calibration displays either Done or Not Done and the LED calibration of the plate format. None indicates either no LEDs are illuminated, or the instrument used a blank emission filter position to create the calibration file.

Note: Molecular Devices does not sell LEDs for this assay.

To run flat-field calibration:

1. Click the **Instrument** menu and select **Calibration** to display the Calibration dialog.
2. Select a **Plate Format** (96, 384, or 1536).
3. Select an **Excitation/Emission Wavelength** pair that displays the **Not Done** status.
4. Place the flat field calibration plate into the Read position. Use the provided yellow plate to flat field calibrate the following excitation/emission wavelength pairs:
   - 335–345 nm/475–535 nm
   - 380–390 nm/475–535 nm
   - 390–420 nm/515-575 nm
   - 390-420 nm/565–625 nm
   - 420–455 nm/475–535 nm
   - 470–495 nm/515–575 nm
   - 470–495 nm/565–625 nm
   - 495–505 nm/526–586 nm
   - 495-505 nm/565-625 nm
   - 510–545 nm/565–625 nm
   - 610–626 nm/646–706 nm

   Use the following dye to calibrate the excitation/emission wavelength (LED/filter) pair:
   - 360–380 nm with MQAE. See MQAE on page 103.
   - 390–420 nm/440–480 nm with Coumarin. See Coumarin on page 103.
5. Click **Flat-Field Calibration**.
6. Wait for the Assay Finished (unlock) light to illuminate on the instrument and the excitation/emission wavelength status to display **Done** in the Flat-Field Calibration column.
7. Click **Close**.
8. Place the yellow signal test plate with the correct well format into the instrument. Click the **Instrument** menu and select **Manual Operation** and then select **Yellow Plate Signal Test** or click  . The yellow plate standard deviation should be 5% or less.

Coumarin

Required material:

- Black clear bottom plate with the correct format and brand for assay (96, 384, or 1536)
- Coumarin (7-diethylaminocoumarin-3-carboxylic acid)
  Catalog # 36799-100MG-F, Sigma-Aldrich

To run a Coumarin calibration:

1. Make a 0.02 M stock solution of Coumarin in Dimethyl Sulfoxide (DMSO). Mix by vortexing tube.
2. Store small aliquots at -20°C (-4°F).
3. Within one hour of calibration, thaw an aliquot and make 20 mL 10^{-5} M solution in the same buffer to use for dye loading. Adjust pH to 7.4. Mix by vortexing.
4. Pipette a uniform quantity of the Coumarin solution into each well of the plate. The volume must be adequate to cover the bottom of the well uniformly. Recommended quantities:
   - 96-well plate, 100 µL/well
   - 384-well plate, 40 µL/well
   - 1536-well plate, 6 µL/well
5. Check the plate visually to make sure there are no bubbles or unfilled wells. Shake or tap the plate to dislodge bubbles. Keep the Coumarin plate covered and in the dark until used.
6. Use the Coumarin plate as the flat field calibration plate. See Flat Field Calibration on page 102.

MQAE

Required material:

- Black clear bottom plate with the correct format and brand for assay (96, 384, or 1536)
- MQAE (N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide)
  Catalog # 46123-100MG-F, Sigma Aldrich

To run a MQAE calibration:

1. Make a 0.02 M stock solution of MQAE in distilled water. Mix by vortexing tube.
2. Store small aliquots at -20°C (-4°F).
3. Within one hour of calibration, thaw an aliquot and make 20 mL 10^{-5} M solution in water or in a chloride-free buffer to use for dye loading. Adjust pH to 7.4. Mix by vortexing.
4. Pipette a uniform quantity of the MQAE solution into each well of the plate. The volume must be adequate to cover the bottom of the well uniformly. Recommended quantities:
   - 96-well plate, 100 µL/well
   - 384-well plate, 40 µL/well
   - 1536-well plate, 6 µL/well
5. Check the plate visually to make sure there are no bubbles or unfilled wells. Shake or tap the plate to dislodge bubbles. Keep the MQAE plate covered and in the dark until used.
6. Use the MQAE plate as the flat field calibration plate. See Flat Field Calibration on page 102.

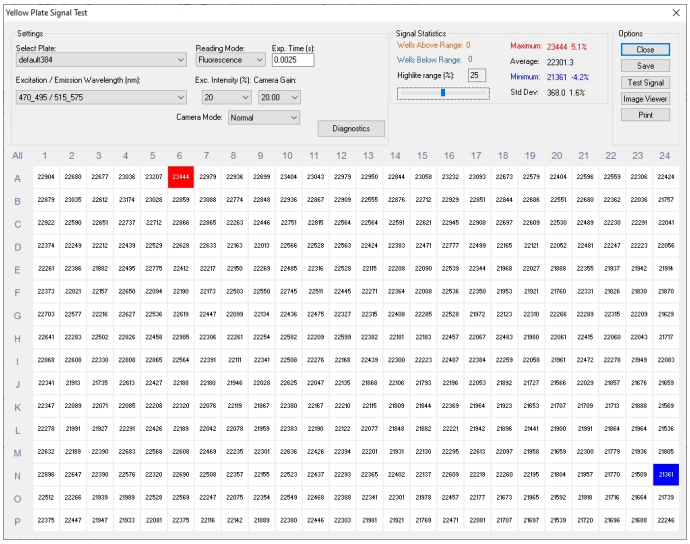
Signal Tests

Running the Yellow Plate Signal Test

Use the Yellow Plate Signal test once a day to verify that the system optics are calibrated. Additionally use the Yellow Plate Signal test after you recalibrate or change the optics.

To run a Yellow Plate Signal test:

1. Place the yellow signal test plate on the stage in the read position.
2. Click the **Instrument** menu and select **Manual Operation** then select **Yellow Plate Signal Test** or click  **Yellow Plate Signal Test**.



3. Set the following parameters:
   a. Click the **Select Plate** drop-down and select the correct plate.
   b. Click the **Reading Mode** drop-down and select **Fluorescence**.

> **Note:** Although both Fluorescence and Luminescence are available, there is no easy way to test signal strength in the Luminescence read mode. Do not run a Luminescence signal tests.

   c. Click the **Excitation/Emission Wavelengths** drop-down and select the correct wavelengths.
   d. Click the **Excitation Intensity** drop-down and select 50.
   e. In the **Exposure Time** field, enter 0.1 seconds.
   f. Adjust the following variable camera settings:
      - For the EMCCD camera, set the **Camera Gain** to 80.
      - For the HS EMCCD camera:
        - In Camera mode Normal, set the **Camera Gain** to 1.5 and the **Exposure Time** to 0.005 seconds.
        - In Camera mode High-Speed, set the **Camera Gain** to 20 and the **Exposure Time** to 0.001 seconds.

4. Click **Test Signal** to take a picture. When you calibrate the instrument with the yellow test plate for the appropriate plate format and optics, normal test plate results are a relative standard deviation less than 5%.
   If the test results are abnormal values, look at the image display for any anomalies on the plate. See Troubleshooting on page 159.
5. Print the results and keep the printout in a folder by the instrument to track the standard deviation of the yellow signal test plate over time or save the files on the hard drive in the signal test directory (C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MySignalTests) as a *.sig file to open in third-party spreadsheet software.

   Note: The Yellow Plate Signal test and image display results are not saved within a data file.

Note: The relative standard deviation should be less than 5% if you perform the flat-field calibration using the flat field calibration plate of the respective plate format (96-wells, 384-wells, or 1536-wells). See Flat Field Calibration on page 102.

Running Protocol Signal Tests

Use the Protocol Signal test to optimize the camera settings you use in florescence assay protocols. You can save the adjustments made to the settings in this test within the protocol. See Optimizing Optics Hardware on page 114.

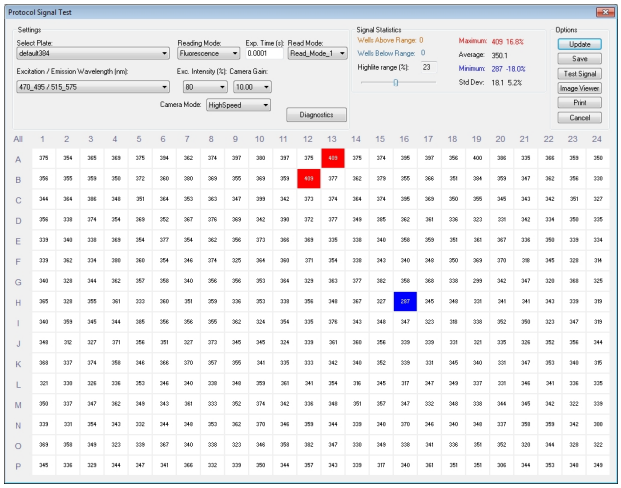
Tip: For the HS EMCCD camera using the High-Speed or the Sensitivity camera mode, run a Protocol Signal test to determine the saturation level for the biology and adjust the exposure settings accordingly. This is important because, after a 4 times overexposure increase, there are no over saturation warning messages from this camera. Start with average RFU numbers 2,000 to 10,000. Any higher could risk saturation.

Note: Although both Fluorescence and Luminescence are available, there is no easy way to test signal strength in the Luminescence read mode. Do not run a Luminescence signal tests.

To run a fluorescence protocol signal test:

1. Place the cell plate on the stage in the read position.
2. Open the fluorescence assay protocol to test.
3. Click  **Protocol Signal Test**.
4. Click **Test Signal**.

5. Review the results and adjust the settings in the Protocol Signal Test dialog. Depending on the camera and the read mode, different parameters are available to adjust the signal strength. See Signal Test Dialog on page 45.



6. Depending on the range of values in the results, decide either to run the experiment or change the Excitation Intensity, Exposure Time, or Camera Gain settings.

Tip: For a calcium mobilization assay, you should start between 200 to 1,500 counts.

Note: For the HS EMCCD camera, after a 4 times overexposure increase, there are no warning messages.

7. Click **Update** to apply the new settings and click **Test Signal** to recheck them.
8. Click **Save**.

Determining Protocol Saturation Levels for the HS EMCCD Camera

When you use the HS EMCCD camera High-Speed camera mode and Sensitivity camera mode, after about a 4x overexposure level increase, no saturation warning appears. This is a problem to detect when the image measurements are saturated, and the system cannot give a reliable saturation warning for grossly overexposed well signals. See Understanding HS EMCCD Camera Protocol Saturation on page 32.

While you create a protocol that uses the HS EMCCD camera High-Speed camera mode or Sensitivity camera mode, run a Protocol Signal test using a cells test plate to determine the optimal exposure levels.

To determine the optimal exposures for the biology using High-Speed and Sensitivity camera mode:

1. Prepare a test plate to check for saturation of the maximum signals with the Protocol Signal test. See Running Protocol Signal Tests on page 105.
2. Start with an initial estimated exposure setting, based on experience or as recommended in the *FLIPR Penta High-Throughput Cellular Screening System Protocol Guide*, then adjust the exposure to keep the maximum signals below saturation.

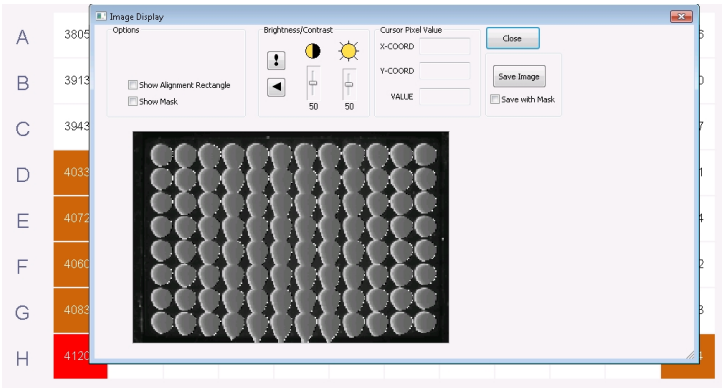
Tip: The gain factors are approximately linear, so reducing the gain by half is similar to reducing exposure time by half.

3. Click **Test Signal** to see the exposure setting results.
4. If a saturation message displays, reduce the exposure by a factor of 4x and test again.

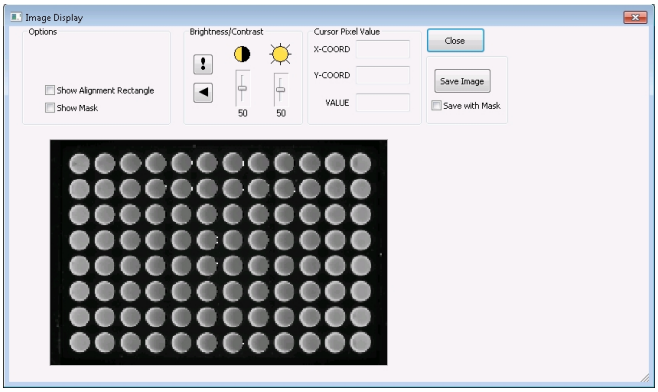
5. If a saturation message does not display, click **Image Display**, and do the following:
   - See if the image does not show bright wells, which confirms that you are below saturation.

     Tip: If you are not sure, reduce the exposure by 2x and test again to verify.

   - See if the image is blooming. If yes, reduce the exposure by a factor of 8x and test again.



   - See if the image is reversing, showing wells with dark centers and bright outlines. If yes, reduce the exposure by a factor of 4x and test again.



6. When the test which is below the saturation level, adjust the exposure linearly to give an average value that matches the target exposure. Keep the maximum signal below 43,000 counts.

   Tip: If the experiment could have brightness that exceeds the signal test sample plate, then reduce the exposure accordingly so that the brightest signal does not exceed 43,000 counts.

7. Leave enough room above the starting levels to measure the experiment without camera saturation.

   Tip: If you do not expect the signal to drop during the experiment, start as low as you can to give enough room for the signal to rise without saturation.

8. Click **Save**.

Chapter 5: Running Experiments

The following sections provide a starting point to set up and run a kinetic cell-based assay. Refer to the *FLIPR Penta High Throughput Cellular Screening System Protocol Guide* for details.

Preparing Cells for Adherent Assays

Location of Cells in the Plate

The excellent signal-to-background noise ratio of the FLIPR Penta System is made possible by the instrument's bottom illumination and detection system. The read plate is illuminated by the LED modules at an angle approximately 45. You should have cells located at the bottom of the wells during an experiment. Adherent cells are typically grown overnight in the cell plate, whereas non-adherent cells are seeded either the night before, on a coated plate, or the day of an experiment and then centrifuged.

Cell Densities

Cell densities used in fluorescence assays vary because each cell type has different requirements. Cell densities range from 20,000 to 80,000 cells per well for 96-well plates, 5,000 to 20,000 for 384-well plates, and 1,500 to 5,000 cells per well for 1536-well plates. Non-adherent assays require higher cell densities.

It is necessary to optimize the cell seeding density so that a uniform, 80–90% confluent monolayer is formed on the day of the experiment. Over confluent or under confluent cell monolayer may result in reduced cellular response to the test compounds.

You should seed cells that are maintained in culture at sub-confluent levels at relatively low densities. Depending on the individual cell line, attachment matrix-coated plates (such as the poly D-lysine coated plate) might be required to improve adherence and minimize cellular blow-off during compound addition. Perform assay development to determine the optimal seeding density and plate environment for each cell line.

Cell Seeding

Use a multi-channel pipettor or a liquid dispensing system to seed cells in plates. Seed 96-well plates by either manual or automatic methods. Use an automatic instrument to seed a 384-well or 1536-well cell plate rather than seeding manually. Thin needles used in automatic liquid-dispensing instruments prevent air bubble formation in well bottoms which is a problem commonly encountered when cells are seeded with a manual pipettor.

Cells are seeded in clear, flat-bottom, black-wall or 96-well, 384-well, or 1536-well, tissue culture treated plates. A flat plate bottom ensures that cellular fluorescence is localized to a single horizontal plane. Adherent cells typically are seeded one day before an experiment. Non-adherent cells are either plated one day before on a coated plate or the day of an experiment. Carry out all steps in the black-wall 96-well, 384-well, or 1536-well plate.

Note: You can use flat-bottom, clear-wall tissue culture plates with the FLIPR® Calcium Assay Kit and Membrane Potential Assay Kit. Depending on signal intensity, you can use either black-wall or white-wall plates when you run aequorin assays.

Starting the System

To ensure proper system function, power the instrument well before you begin an experiment. The camera requires about five minutes to reach operating temperature. If you heat the plate stage, click the Instrument menu and select Manual Operation and then select Temperature Control. Allow 15 minutes for complete temperature equilibration. View the Instrument Status panel for the stage temperature.

To start the system:

1. Verify that the appropriate pipettor head, wash reservoir, LED banks, and filters are in place before you power on the instrument. See Exchanging Hardware Components on page 140.

   CAUTION! Do not attempt to change pipettor heads, wash reservoirs, LED banks and filters while the instrument is running. You must exchange all features in the appropriate instrument mode. When the instrument is started for the first time after a re-configuration of LEDs or emission filters, optics calibration is required.

2. Follow the procedure in Powering On the System on page 16.

Checking the System

A yellow signal test plate is provided with each corresponding pipettor head supplied with the system. A signal test plate only needs to be run once a day unless you change system components such as pipettor heads or LEDs.

CAUTION! To avoid damaging the yellow signal test plates. Avoid scratching the plate bottom because scratches can affect the standard deviation. Store the plates in a safe place away from bright light on an even surface.

Once a day, do the following system checks before you run the first assay plate:

1. Make sure that the system is turned on and the camera is cooled to proper operating temperature.
   - EMCCD camera: -70°C (-94°F) ± 2°C
   - HS EMCCD camera: -70°C (-94°F) ± 2°C
2. Run a Yellow Plate Signal test to ensure the system is operating according to specifications. See Running the Yellow Plate Signal Test on page 104.

Dye Loading Cells for Fluorescence Assays

Many cell-based responses require fluorescent dye loaded in cells to bind or change conformation in the presence of a ligand. These changes are monitored through a shift in emission wavelengths and captured by a CCD camera. Calcium sensitive dyes, such as the ones used in the Molecular Devices FLIPR Calcium Assay kits, monitor intracellular calcium flux assays, or environmentally sensitive for membrane potential assays. Depending on the application evaluated, dye loading typically lasts from 30 minutes to 2 hours, at either room temperature or 37°C (98.6°F).

Dye Loading Duration and Temperature

Optimal dye load time depends on the cell type and presence of an anion exchange inhibitor. For most cell lines, especially for calcium mobilization assay development, start with a 60 minute to 120 minute load time at 37°C (98.6°F).

CAUTION! Avoid exceeding the optimal load time. If anion exchange inhibition does not enhance dye load conditions, it should not be used.

Tip: If loading for 30 minutes yields an acceptable fluorescence signal, as has been observed for some cell lines, use a shorter load time. Incubation at room temperature can work as well or better than 37°C (98.6°F).

Preparing Source and Compound Plates

Preparation Time for the Source Plate

Depending on assay complexity, source plate preparation time may vary. To avoid conflicts, plan the experiment carefully to ensure source plates are ready before dye loading is complete.

Recommended Source Plates

Achieve compound conservation by reducing dead volumes and minimizing source plate adherence. Reducing source plate adherence help to ensure proper compound concentrations are delivered to a read plate. Use polypropylene plates for this purpose because they are solvent-resistant, can withstand repeated freeze-thaws, and have a low retention. Proteins are less likely to adhere to polypropylene plates rather than polystyrene surface.

Manufacturers offer a wide selection of plate bottom shapes (U-, V- or flat bottom) to lessen dead volumes. Dead volumes decrease as you move from a flat to U- to V-bottom plates. Contact the plate manufacturer for the dead volume in the plates you use.

Concentration of Compounds in the Source Plate

Compound concentrations are prepared based on ratio of addition to initial well volume of the read plate. Common concentrations are 3X, 4X, or 5X because ratio of addition to read plate volumes are 1:3, 1:4, or 1:5, respectively. Volume of addition is dependent on compound mixing efficiency, cell adherence, and kinetics of cellular response. Perform assay development to determine optimal addition volume and concentrations prior to screening.

Addition and Mixing of Compounds to the Cell Plate

Prompt mixing is required to initiate robust cellular kinetics associated with signal transduction assays. Proper addition parameters (for example, dispense speed, height, and volume) initiate a rapid response upon addition. Slow signal increases or variation in signal between wells may be signs that mixing is too slow or not uniform. Optimal addition parameters typically consist of a fast dispense speed, low pipettor height, and large addition volume. Assay optimization is recommended to determine the proper assay conditions before you run a screen.

If you do not optimize addition parameters, artifacts occur that affect data quality. One common problem is adding compounds too rapidly. Weakly adherent cells may dislodge from the monolayer, causing a decrease in fluorescence upon addition. you can reduce cell displacement by slowing dispense speed while decreasing addition volume and increasing height. Larger dispense volumes mix rapidly because they encounter less surface tension as compared to smaller volumes. Small sample volumes are less disruptive to a cell monolayer and reduce compound consumption. To avoid dislodging weakly adherent cells, add compounds in small volumes and at relatively low dispense speeds.

Tip: Mixing is not typically used during addition in calcium or membrane potential assays. Signal artifacts, such as additional noise in the fluorescent trace, can be attributed to cell disruption induced by rapid pipettor movement. It is not uncommon to use mixing before aspirating, to resuspend reagents that have fallen out of solution in the source plate.

For temperature-controlled assays, bring cell and source plates to temperature outside of the instrument. It takes approximately 45 to 60 minutes in a 95% humid, CO_2 incubator (15 minutes in a heating block) to warm plates to equilibrium at 37°C (98.6°F).

Compound Plates for Suspension Assays

Unlike adherent assays, suspension cell assays require cell addition directly to the compound in the read plates. Prepare compounds at 2X concentration in the same diluent you use for cells, since equal volumes of cells are added to the read plate that contains the compound.

In 384-well assay add 25 µL cells to 25 µL 2X compound; in a 1536-well assay 2 µL add cells to 2 µL compound. Store plates covered to prevent evaporation.

Setting Up Assay Protocols

Pre-configure the protocol (*.fmp) files to reduce time and ensure consistent screening parameters. Define and save protocol files for each cell line, project, or user. The ScreenWorks Software lists the last 10 files (6 data and 4 protocol) in the bottom portion of the File menu.

A protocol file defines the following experiment parameters:

- Instrument conditions, for example, read mode, plate positions, file names, and file storage
- Assay steps, for example, fluid transfer and reads
- Analysis options, for example, groups, corrections, and export parameters

To create a new protocol:

1. Select the **Process** tab. See Process Tab on page 59.
2. Do one of the following to add the assays steps:
   - To place the new process at the end of the protocol list, drag and drop the process anywhere in the Experiment window.
   - To place the new process in the protocol list, drag and drop the process where you want it. The software prompts you if the position is invalid.

3. When the Configuration screen displays, adjust the settings. See Understanding Processes on page 60.
4. Repeat the previous steps until you finish building the protocol.

5. Click the **Analysis** process and define the analysis parameters See Analysis Process on page 65:
   - Click **Grouping** to define grouping parameters.
   - Click **Correction** to define correction parameters.
   - Click **Export** to define export parameters.
6. Click on other process icons to open the Configuration screen and make changes.

   Note: When you use the HS EMCCD camera with a fast read time interval value, depending on the Protocol > Settings > Edit Read Mode > Camera Mode setting, the plate matrix panel update may delay until the end of the data acquisition.
   Specifically, the following settings cause the plate matrix panel to update at the end of the data acquisition:
   For Camera mode High-Speed with a Read Time Intervals setting of 0.018 seconds or shorter.
   For Camera mode Normal or Sensitivity with a Read Time Intervals setting of 0.029 or shorter.

7. Click the **File** menu and select **Save** to display the Save As dialog.
8. In the **File Name** field, enter the protocol name.
9. Click **Save**. Protocol files save as a *.fmp file. The default Save In location for the protocols is C:\Documents\Molecular Devices\ScreenWorks\MyProtocols.
10. Run the experiment.

Refer to the *FLIPR Penta High-Throughput Cellular Screening System Protocol Guide* for details.

Optimizing Optics and Fluid Dispensing

Optimizing Hardware Settings

Prior to screening, optimize the following hardware components for signal output and detection:

- Excitation intensity
- Exposure time
- Number of reads
- Camera gain for EMCCD cameras
- Pipettor height
- Dispense speed
- Dispense volume
- Cell parameters for suspension assays

Optimizing Optics Hardware

Optimization helps amplify weak signals or reduce saturation. Modifications to read settings might not alter the quality of the data because it only regulates the amount of light emitted or detected by the system. For instance, doubling the excitation intensity doubles the RLUs the system detects, but the signal-to-noise ratio and Z-factor can remain the same.

To optimize the fluorescence signal:

1. Place the cell plates on the read positions.
2. For Camera mode Normal, start with the following excitation intensity, camera gain, and exposure time settings to measure basal fluorescence signal. The read interval is 1.0 second.

Basal Fluorescence Signal Camera Settings

Camera Type	Calcium Assay		Membrane Potential Assay	
	EMCCD	HS EMCCD	EMCCD	HS EMCCD
Excitation wavelength (nm)	470–495	470–495	510–545	510–545
Emission Wavelength (nm)	515–575	515–575	565–625	565–625
Excitation Intensity (%)	50	40	50	50
Exposure Time (s)	0.4	0.05	0.4	0.02
Camera Gain	130	6.5	50	4.0

3. Click  **Protocol Signal Test** to evaluate the basal fluorescence signal. The fluorescence signal for intracellular calcium assays varies depending on the camera and settings. Look for the following fluorescence counts:
   - EMCCD camera: 200 – 1,200
   - HS EMCCD camera: 4,000 – 6,000
4. If the basal fluorescence signal is substantially out of these ranges, adjust the excitation intensity, exposure time, or gain.

To adjust the basal fluorescence:

Basal fluorescence too low:

- Increase the **Excitation Intensity** - During the assay, the excitation intensity ranges between 20% and 100%.
- Increase the **Exposure Time** - When you increase the exposure time, you must increase the read interval to a minimum of exposure time + 0.1 s. It takes 0.1 s to integrate the data.
- Increase the **Camera Gain**
  - EMCCD camera: The gain varies between 1, extremely bright fluorescence, to 240, luminescence.
  - HS EMCCD camera: The gain varies between 1 and 128.

Basal fluorescence too high:

- Decrease the **Excitation Intensity**
- Decrease the **Exposure Time**
- Decrease the **Camera Gain** (EMCCD and HS EMCCD camera)

Adjusting Pipettor Height

To ensure prompt compound delivery, adjust the pipettor height. Pipettor height is based on pipettor mechanics and the physics of liquid. For fluorescence an air gap, called the hold volume, is drawn into the tips after fluid aspiration from the source plate. This air gap ensures fluid does not leak out during pipettor movement. The hold volume is the first item to leave the pipette tip during dispense and enters the target well as a bubble. These bubbles can cause random light reflections and spurious signals. To avoid bubbles, dispense fluid with tips just above, but touching the initial well fluid level. This leaves the tips touching the meniscus after the addition has been completed to ensure complete sample dispensing.

Note: For luminescence suspension assays, no hold volume is recommended along with low and fast addition.

If pipette tips are in the air at the end of fluid delivery, a drop can form on the tip end due to surface tension which results in reproducibility problems. Set the pipettor height to just above but touching the starting fluid volume in the wells.

The fluid surface in a 384-well or 1536-well plate is a deeply curved meniscus. Set the pipettor height somewhere below the starting fluid volume for the 384-pipettor. There is no air gap with the 1536-pipettor.

Adjusting Fluid Dispensing Speed

Default dispense speed is 50 µL/s, 20 µL/s, and 2 µL/s when you dispense to 96-well, 384-well, and 1536-well plates respectively.

Recommended Fluid Dispense Speeds

Cell Conditions	96-Well Plate (µL/s)	384-Well Plate (µL/s)	1536-Well Plate (µL/s)
Slow dispense speed for weakly adherent cells or non-adherent cells.	10–50	5–20	1–6
Fast dispense speed for strongly adherent cells.	100–200	25–50	5–10

You must determine these values for each cell type, but you should dispense as fast as possible to enhance mixing of the compounds in the wells. The trade-off is that the pipetting speed must not be so forceful that it dislodges cells from the well.

Optimizing Fluid Volume

The fluid volume parameters have the following range:

- 96-well plate = 5 – 200 µL
- 384-well plate = 1 – 25 µL
- 1536-well plate = 0.5 – 3 µL

Large volumes mix more rapidly into the wells than smaller volumes. Smaller sample volumes cause less disruption to the cell layer and allow you to use the same source plate for multiple cell plates. To avoid dislodging weakly adherent cells, you should add smaller compound volumes to the cell plate and the pipettor should dispense the compounds at a relatively low speed.

Optimizing Pin Tool Delivery

To ensure proper transfer of compound using a pin tool to the target plate, evaluate the following factors during assay development. Control and standardize these factors for an application to help deliver reproducible volumes:

- Pin diameter
- Surface tension of the liquid being transferred
- Surface tension of the pin
- Speed of removal from source liquid
- Speed of pin striking recipient plate
- Depth to which the pin is submerged in the source plate
- Depth to which the pin is submerged in the target plate
- Volume of slot in pin
- Surface tensions of plate and dwell time

Note: The FLIPR Penta instrument and the FLIPR Tetra instrument use the same pin tools.

For more information regarding optimization of pin tools, contact:

V&P SCIENTIFIC, INC. 9823 Pacific Heights Boulevard, Suite T San Diego, CA 92121

800.455.0644

sales@vp-scientific.com

Starting Assay Runs

To start the assay:

1. Check the Experiment screen to verify that the experiment summary is accurate and that the correct protocol file is used.
2. Verify the plates and tips are loaded in the instrument.
3. Save the protocol.
4. Click **Run** on the toolbar to start.

CAUTION! The upper and lower doors lock before any part in the FLIPR Penta System moves. These doors must remain closed until the end of the run, including through the duration of the pipettor tip wash.

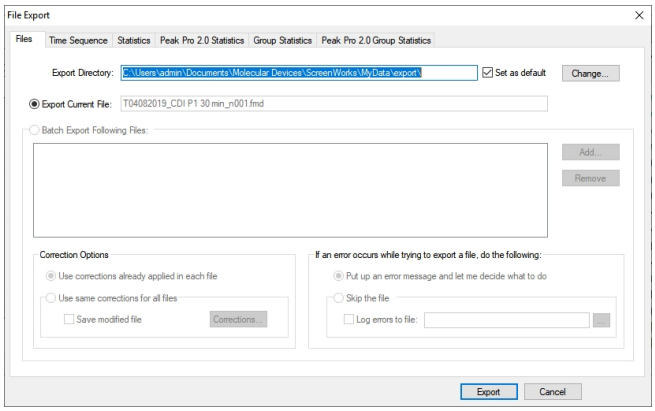
Chapter 6: Exporting Data

You can automatically export data when a protocol runs, and export already acquired data. Data exports as ASCII text format files with a separate file for each measurement configuration. You specify the folder in which to write the output files. The default export folder is: C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyData

To configure the data export:

- In protocol files, on the Analysis screen, click **Configure Auto Export**.
- For data files, click the **File** menu and select **Export**, and then select either **File** or **Batch Export**.

Note: Auto-Export and Batch Export do not support the ScreenWorks® Peak Pro™ Software Version 2.0.



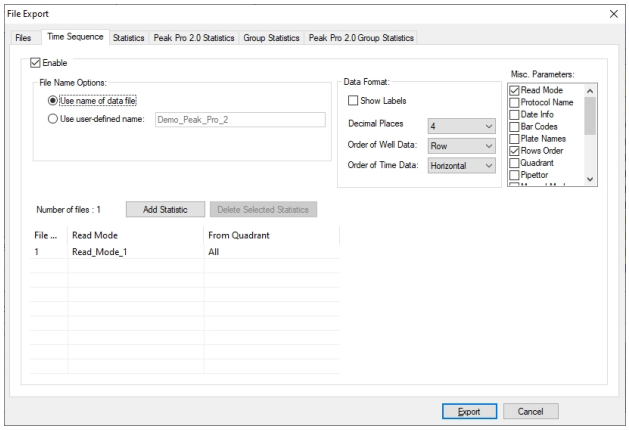
The File Export dialog has the following tabs:

- **Files** - Export directory setting or batch files selections.
- **Time Sequence** - Exports time point measurements for each read mode. The measurement values apply the corrections you configure in the Correction dialog. If there are two read modes and you select ratiometric options in the Correction dialog, you can export the ratio for each time point. Files have a *.seq*n* extension, where n increments for each export file.
- **Statistics** - Exports averages, maximums, and other kinetic reduction values for the numbers of reads for each well. Files have a *.stat*n* extension, where n increments for each export file.
- **Peak Pro 2.0 Statistics** - You must run the analysis in the Peak Pro 2.0 software module before the export. This export works the same way as statistics. See Exporting Data with Peak Pro 2.0 Software Module on page 135.
- **Group Statistics** - Exports the group statistical values for the numbers of reads for each group and are based on the kinetic reduction settings you define. Files have a *.gstat*n* extension, where n increments for each export file.
- **Peak Pro 2.0 Group Statistics** - You must run the analysis in the Peak Pro 2.0 software module before the export. After you run the analysis, this export works the same way as Group Statistics. See Exporting Data with Peak Pro 2.0 Software Module on page 135.

CAUTION! Data you create in ScreenWorks Software Version 2.0 cannot be viewed or exported by ScreenWorks Software Version 5.x.

Exporting Time Sequence

Use the Export Time Sequence tab to configure parameters for export of time sequence data. The export files contain a value for every read interval for each well, for the read mode or ratio you select. This contrasts with the Statistics file, which contains one kinetic reduction per well.

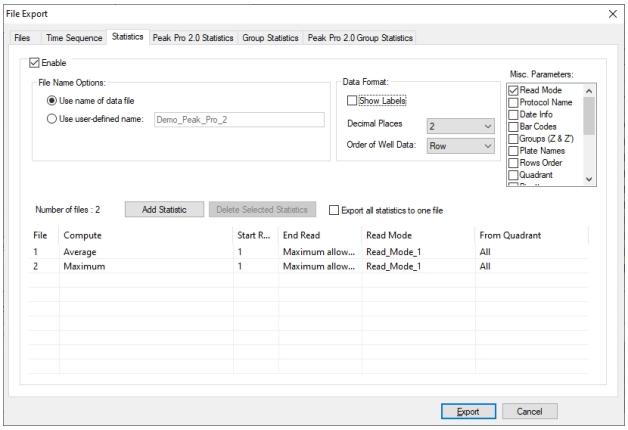


Time Sequence Options

Option	Description
Enable	Select this check box to create a time sequence export file.
File Name Options	Select **Use Name of Data File** to export files with the data or protocol file name, with *.seq*n* extension, where *n* is an integer. Select **Use User Defined Name** to enter the name of the export files (maximum of 25 characters). Files have a *.seq*n* extension, where *n* is an integer.
Data Format	**Show Labels** - Select this check box to include information about the processing options you select (for example, name of *.fmd file and well labels). Any corrections prior to export (e.g., negative control correction) are reported at the top of the exported ASCII file. **Decimal Places** - Select 2, 3, or 4 decimal places for the data export. This setting applies only if that number of decimal places exist in the data. **Order of Well Data** - Select Column to sequence data by column or select Row to sequence data by row. Set this in accordance with the way the spreadsheet or database handles well data. **Order Time Data** - Select vertical or horizontal order of the data.
Misc. Parameters	Select the check box for each parameter and group statistic to export with the data.
Number of Files	**Add Statistic** - Adds a statistic file to create from the same data set. For data created in 384-well or 1536-well formats. **Read Mode** - Enter the read mode (or ratiometric data) from which to export data. **From Quadrant** - Select the quadrant from which to export or select to export data from all quadrants. This allows you to export data from one plate into four different spreadsheet files.

Exporting Statistics

Use the Export Statistics tab to configure kinetic reductions, averages, maximum, and minimum values, for the number of reads within each well. You can configure multiple kinetic reductions. Each creates a separate export file where only one value reports per well.



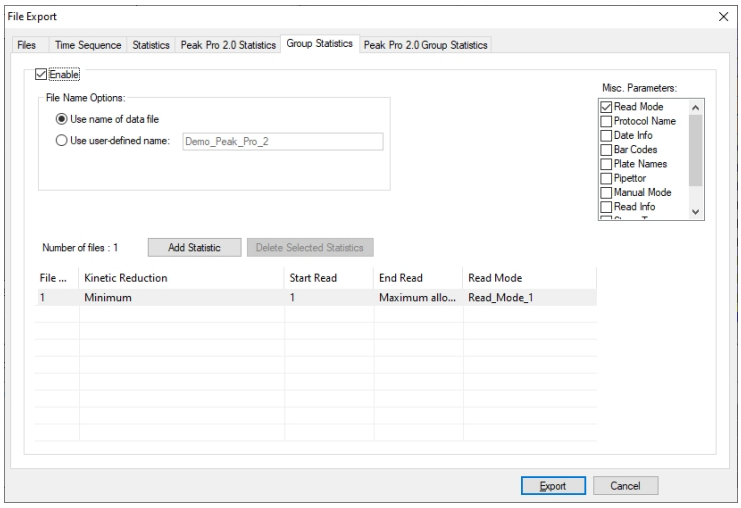
Statistics Options

Option	Description
Enable	Select this check box to create a statistic export file.
File Name Options	Select **Use Name of Data File** to export files with the data or protocol file name, with *.stat*n* extension, where *n* is an integer. Select **Use User Defined Name** to enter the name of the export files (maximum of 25 characters). Files have a *.stat*n* extension, where *n* is an integer.
Data Format	**Show Labels** - Select this check box to include information about the processing options you select (for example, name of *.fmd file and well labels). Any corrections prior to export (e.g., negative control correction) are reported at the top of the exported ASCII file. **Decimal Places** - Select 2, 3, or 4 decimal places for the data export. Your setting applies only if that number of decimal places exist in the data. **Order of Well Data** - Select column to sequence data by column or select Row to sequence data by row. Set this in accordance with the way the spreadsheet or database handles well data.
Misc. Parameters	Select the check box for each parameter and group statistic to export with the data.
Number of Files	**Add Statistic** - Adds a statistic file to create from the same data set. For data created in 384-well or 1536-well formats. **Compute** - Select a kinetic reduction type. See Kinetic Reduction Types on page 67 **Start Read** - Enter the first read number to be included in the kinetic reduction. **End Read** - Select the last read to include in kinetic reduction. This can equal the Start Read to extract values from a single read. **Read Mode** - Enter the read mode (or ratiometric data) from which to process the data. **From Quadrant** - Select the quadrant from which to export or select to export data from all quadrants. This allows you to export data from one plate into four different spreadsheet files.

Exporting Group Statistics

Use the Export Group Statistics tab to configure a group statistics export report, for example, average, standard deviation, and z-scores, based on the kinetic reduction types you define for the number of reads you set up on the Analysis screen.

Note: If you do not define groups on the Analysis screen, an empty report exports.



Export Group Statistics Options

Option	Description
Enable	Select this check box to create a group statistic file.
File Name Options	Select **Use Name of Data File** to export files with the data or protocol file name, with *.gstat*n* extension, where *n* is an integer. Select **Use User Defined Name** to enter the name of the export files (maximum of 25 characters). Files have a *.gstat*n* extension, where *n* is an integer.
Misc. Parameters	Select the check box for each experiment parameter to export with the data.
Number of Files	**Add Statistic** - Adds a statistic file to create from the same data set. **Kinetic Reduction** - Select the reduction to apply to the kinetic data traces to export. See Kinetic Reduction Types on page 67. **Start Read** - Enter the first read number to use to determine the kinetic reduction. **End Read** - Select the last read to use to determine the kinetic reduction. **Read Mode** - Select the read mode to apply the kinetic reduction.

Batch Exporting

Note: Batch export does not support the separately licensed analysis module ScreenWorks Peak Pro Software Version 2.0.

Click the **File** menu and select **Export** and then select **File** or **Batch Export**. Manual export allows you to export the same files you define above.

Batch Export Options

Option	Description
Export Directory	Define where to send export files. The default export folder is: C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyData
Batch Export Following Files	Click **Add** to display the Open File dialog to select the data files to export. Shift + Click or Ctrl + Click to multi-select.
Correction Options	Select **Use Corrections Already Applied in Each Data File** to apply the corrections you save with each data file during export. Select **Use Same Corrections for All Files** to apply and save new corrections to all data files. Useful when you export all data files with same parameters or save the same correction to multiple data files. See Corrections on page 75.
If an error occurs when you export a file, do the following	Select **Put Up an Error Message and Let Me Decide What to Do** to allow you to decide how to proceed when an error occurs. Select **Skip the File** to not include the data in the export and continue to export data. The option to write the error to a log file is available.

When you batch export data, the export files vary depending on the options you select in the individual export statistic, time sequence, and group statistic sections. For a single export file from each data file to export, select Use Name of Data File in the section to export. Export files you create with this method are labeled with the name of the data file from which it was created. For a single export file that contains information from multiple data files, select Use User Defined Name in the section. All exported information combines into one file labeled with the name you enter. When you open the file the individual data file names from which the information was exported display as the header for each data set within the export file.

Chapter 7: ScreenWorks Peak Pro Software Version 2.0

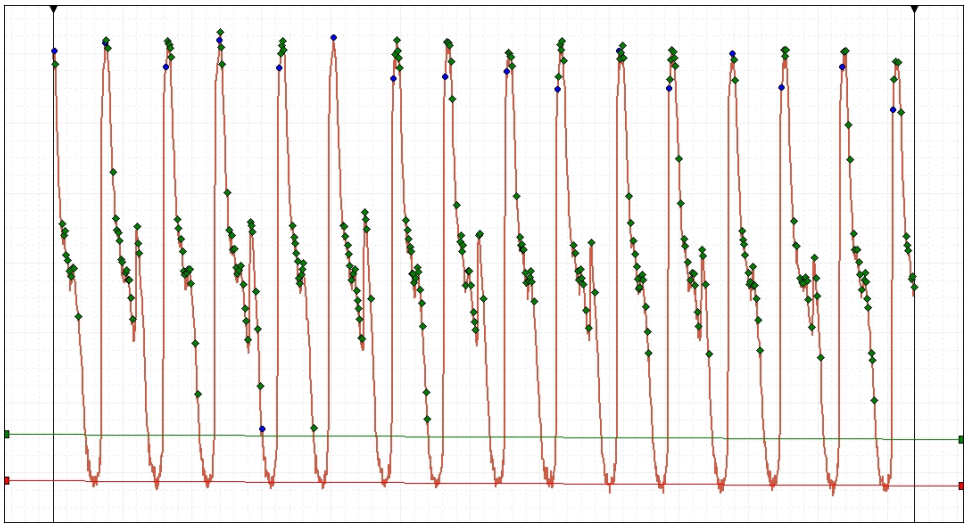
The ScreenWorks Peak Pro Software Version 2.0 requires the purchase of a separate module license after a 14-day trial, which adds advanced peak detection and event characterization measurements and analysis functionality. This data analysis capability is useful with cardiomyocyte and neuronal protocols. Contact your Molecular Devices Sales Representative to purchase. Detect and measure the activity of biological events that exhibit several components of interest, for example, multiple excitations, regular or irregular anomalous events such as early after depolarization-like events (EAD), irregular amplitudes or frequencies, and migrating threshold levels.

Note: For relatively fast events that you acquire with long sampling intervals, use ScreenWorks Peak Pro Software Version 1.0. See Kinetic Reduction Types on page 67.

The Peak Pro 2.0 software module algorithm reduces false detections without the need to condition the raw data. Use search vectors to optimize the lengths of the vectors for specific data sets. They are generated with the midpoint of the vector at each point in the data set and are fitted by linear regression such that the slope at each point is known and the ups and downs of the adjacent noise events are dampened, which reduces the contribution of random noise to the overall movement of the vector.

The following screen shots display how adjusting the search vector length and specifying the minimum noise and amplitude values causes very few false events to appear.

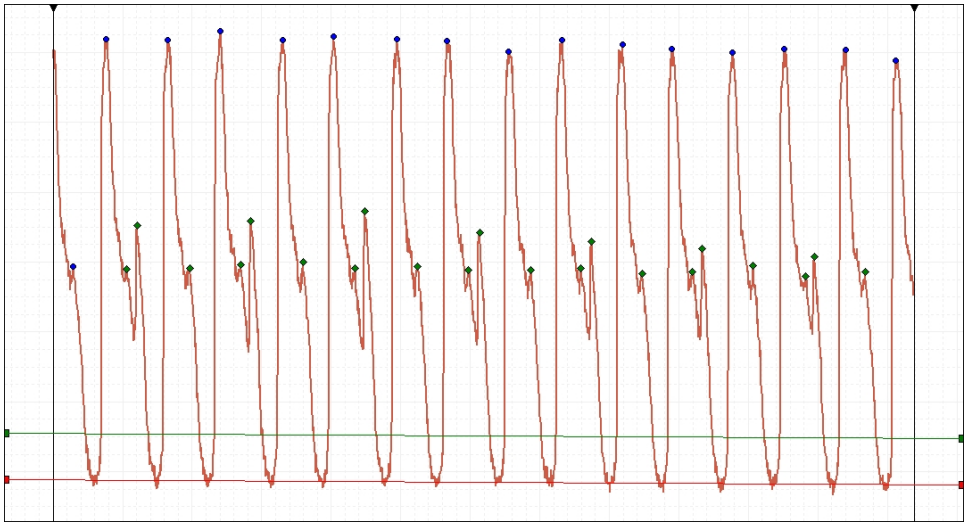
In a moderately noisy data set with a search vector length of 3 the results display a lot of false peaks.



Search Vector Length of 3 is Noisy

Each marker represents a peak. The first peak of each event is a blue dot, and the subsequent peaks are green diamonds.

Change the search vector length to 11 and run the analysis again for more precise data.



Search Vector Length of 11 Reduces Noise

The vector length did not need to be increased by much to see a substantial noise reduction improvement. The major peaks and minor peaks now appear to be real biological events.

Interface Overview

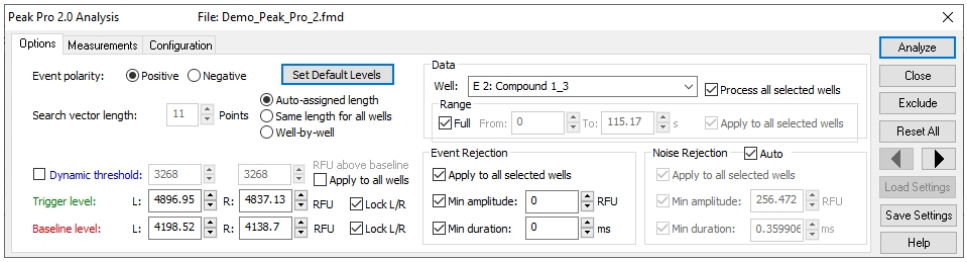
When you start the ScreenWorks Peak Pro Software version 2.0, a dialog displays with the open data file name in the title bar and three tabs.

To open the Peak Pro 2.0 Analysis dialog:

1. Click the **File** menu and select **Open** then select **Data Files** to display the Open dialog.
2. Select the data file name and click **Open**.
3. In the Multi-Well graph, select individual wells, rows, or columns to analyze.
4. Click  to display the Peak Pro 2.0 Analysis dialog. See Running Peak Pro 2.0 Software Module on page 130.

Options Tab

Use the Options tab to define peak detection search settings and data range settings.



- **Event Polarity** - Specifies the direction of the signal for detection, either Positive or Negative.

  Tip: Negative polarity is rarely needed.

- **Set Default Levels** - Resets the threshold, trigger, and baseline values to the default positions.

- **Search Vector Length** - Determines the sensitivity of the peak detection. Long vector lengths filter out noise. Short vector lengths detect lower amplitude transitions. The software generates the search vector from a linear regression across forward and backward points, centered on the mid-point of the vector, and computed for each data point. This vector slides along the data to check the slope at each point and determines peaks and valleys by noting the direction of the slope.

  Tip: This is the most important variable for good peak detection. You can enter a different vector length for each well.

  - Select **Auto Assigned Length** to use the sampling rate and an estimate of the noise amplitude to compute a vector length for each data point. This determines the vector length suitable for most data sets.
  - Select **Same Length For All Wells** to enter one set vector length for the analysis of all the wells you select.
  - Select **Well-By-Well** to enter multiple set vector lengths for the analysis of wells. Each well can use a different vector length setting.
- **Dynamic Threshold** - Select this check box to set a lower limit for peak detection and reject all events below this threshold. Amplitude is relative to the baseline.
  - **Apply to All Wells** - Select this check box to apply the threshold you enter to all wells. Clear this check box to use the value for an individual well.
- **Trigger Level** - Enter when detection starts, with the first crossing of the level, and when the event ends, with the level re-crossing. By default, the level is positioned at 10% above the baseline, relative to the data range. Events that peak below the trigger level are ignored. You can set this for each well. The signal values are relative fluorescence units (RFU).
  - **L** - Enter the left end of the trigger level line.
  - **R** - Enter the right end of the trigger level line.
  - **Lock L/R** - Select this check box to lock the left and right ends so the level line moves as a unit when you change the left or right value.

  Note: An event is the signal crossing the trigger level in both directions. The amplitude of the event is measured relative to the baseline. The detection of the left and right antipeaks of the event is not limited by the trigger level and these measurements can extend as far as the baseline. The event duration is measured as the distance between the two antipeak positions.

- **Baseline Level** - Enter the absolute end of the detection range. Amplitudes are measured relative to the baseline level and not the antipeak value. The signal values are relative fluorescence units (RFU). The algorithm adapts to sloping baselines. In software version 5.1, the new estimation algorithm takes into account the general slope of the baseline by detecting and fitting presumptive baseline points from several segments of the data. The new detection algorithm assigns a search vector length that is based on a number of characteristics of the data.
  - **L** - Enter left end of the baseline level line.
  - **R** - Enter the right end of the baseline level line.
  - **Lock L/R** - Select this check box to lock the left and right ends so the level line moves as a unit when you change the left or right value.

Data

- **Well** - Lists the wells you select in the plate matrix from the Analysis dialog. This selects the graph that displays in the Detail Graph pane.
- **Process All Selected Wells** - Select this check box to include the analysis of the wells you select in the Well list. Clear this check box to analyze only the well visible in the Well field. Select wells in the Well list and click **Exclude** for exceptions before you analyze all wells.
- **Full** - Select this check box to select the whole data range. Clear this check box to analyze the data in the wells listed between the From and To fields.
- **From** - Enter the beginning point of the data range to analyze.
- **To** - Enter the end point of the data range to analyze.
- **Apply to All Selected Wells** - Select this check box to apply the data range you enter to all wells in the list.

Event Rejection

Use Event Rejection to have the software reject the amplitudes relative to the baseline that are lower than what you specify. The software also rejects events with trough-to-trough times that are shorter than the duration you specify.

- **Apply to All Selected Wells** - Select this check box to apply the amplitude and duration values you enter to the data from all the wells you select. Clear this check box to use the value for each individual well.
- **Min Amplitude** - Select this check box to enter the lowest amplitude RFU value, measured from the baseline, for the acceptance of an event.
- **Min Duration** Select this check box to enter the shortest duration ms value, duration, as measured between the left and right antipeak values.

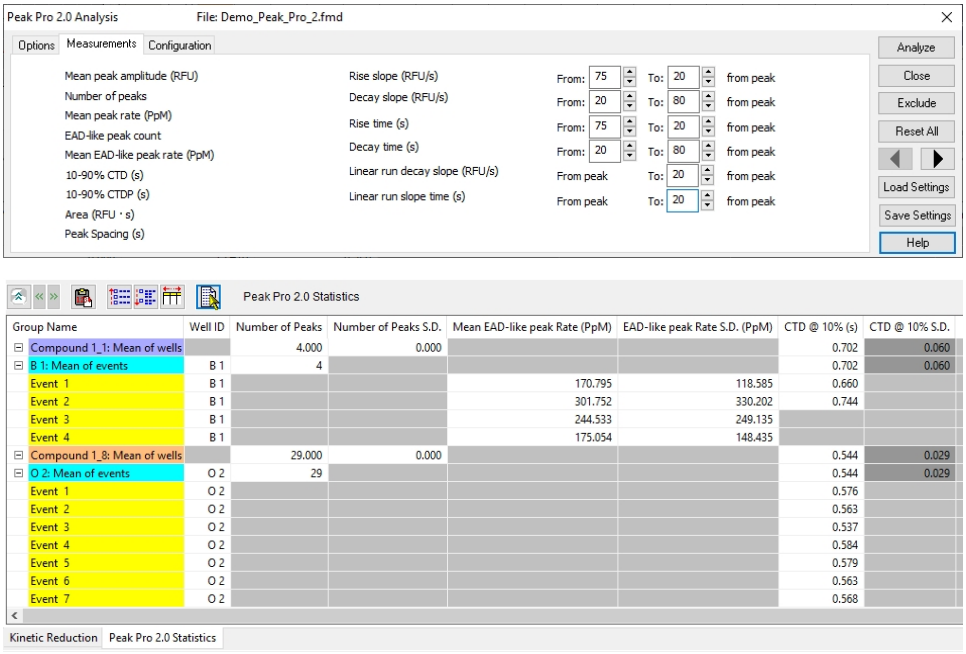
Noise Rejection

Use noise rejection to have the amplitude that is relative to the right trough on the rising phase and the left trough on the decay phase. The software rejects events with amplitudes lower than what you specify and events with right to left trough times that are less than the noise duration.

- **Auto** - Select this check box to have the software measure the noise level of the data and average the difference between sequential amplitude values of all amplitudes within a distance of 10% of the baseline. The software rejects outliers using interquartile ranges. The software uses noise estimate to determine an approximate default noise level.
- **Apply to All Selected Wells** - Select this check box to apply the amplitude and duration values to the data from the wells you select. Clear this check box to use the value for each individual well.
- **Min Amplitude** - Select this check box to enter the lowest amplitude RFU value before the software rejects an event as noise. The measurement is taken between the lowest antipeak amplitude and the peak amplitude.
- **Min Duration** - Select this check box to enter the lowest duration time value before the software rejects an event as noise. The measurement is taken between the antipeak time at the lowest antipeak and the time at the equivalent amplitude opposite the peak.

Measurements Tab

Use the Measurements tab to define the properties of an event that you can display in the Peak Pro 2.0 Statistics pane.

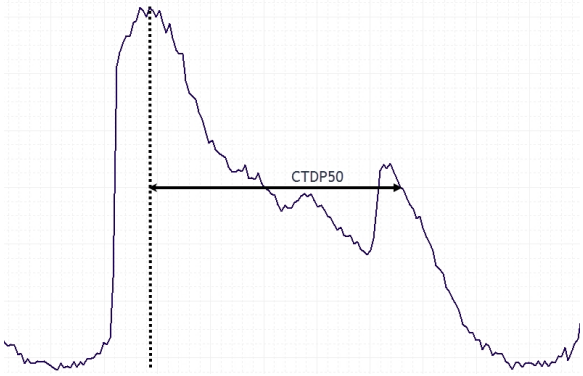


Peak Pro 2.0 Statistics Pane After Analysis With Expanded Group Data

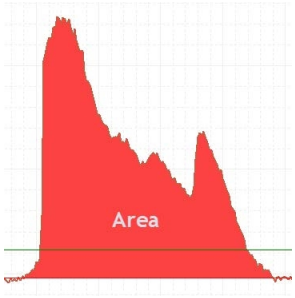
When you run an analysis, the following measurements are calculated:

- **Mean Peak Amplitude** - The average amplitude of the main peaks of the events detected in the well data, measured from the peak to the baseline and expressed in relative fluorescence units (RFU).
- **Number of Peaks** - The total number of peaks.
- **Mean Peak Rate** - The number of peaks per minute (PpM). For cardiac data, this is equivalent to beats per minute (BPM).
- **EAD Like Count** - The early after depolarization like event (EAD like) in an event. Includes number of EAD like events, mean number of EAD like events, and number of EAD like events S.D.
- **Mean EAD Like Rate** - The average rate of early after depolarization like event of the events detected in the well data, expressed in peaks per minute (PpM).
- **10-90% CTD** - The Calcium Transient Duration is the width of a peak in seconds at various peak heights between 10% at the top and 90% at the bottom.

- **10-90% CTDP** - The Calcium Transient Duration from peak position measured as a straight line from the peak to a percentage of the distance from the peak.

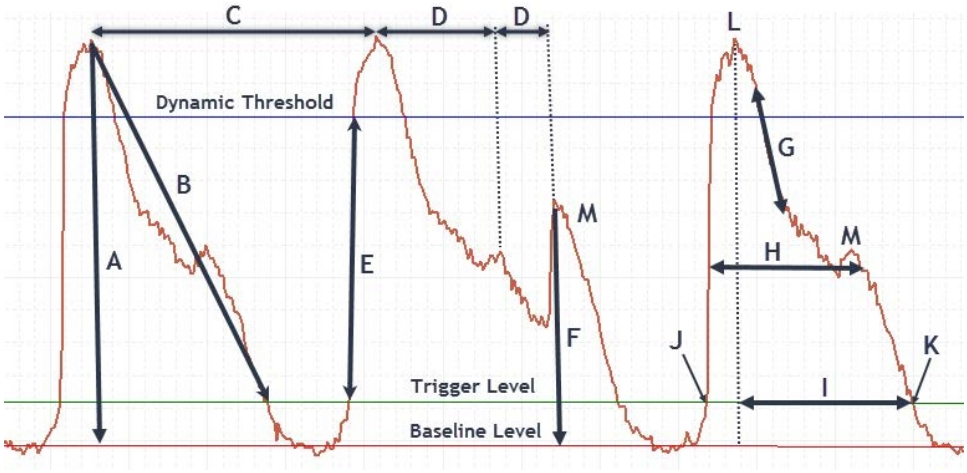


- **Area** - The area below the event measured from the baseline to the start and end of the event as defined by the left and right antipeaks, expressed in relative fluorescence unit seconds.



- **Peak Spacing** - The approximate regularity of the peak spacing, expressed in seconds. The measurement reports either uniform spacing of peaks (OK), or irregular spacing (IRREG).
- **Rise Slope** - The rate of rise over the range, expressed in relative fluorescence units per second.
- **Decay Slope** - The rate of decay over the range, expressed in relative fluorescence units per second. Computed on if there is no early after depolarization like event.
- **Rise Time** - The time to rising phase over the range.
- **Decay Time** - The time to decay over the range.
- **Linear Run Decay Slope** - The slope of a straight line from the peak to a percentage of the peak, expressed in relative fluorescence units per second.
- **Linear Run Slope Time** - The run slope time from the peak, expressed in seconds.

The following illustrates the measurement options and terminology:

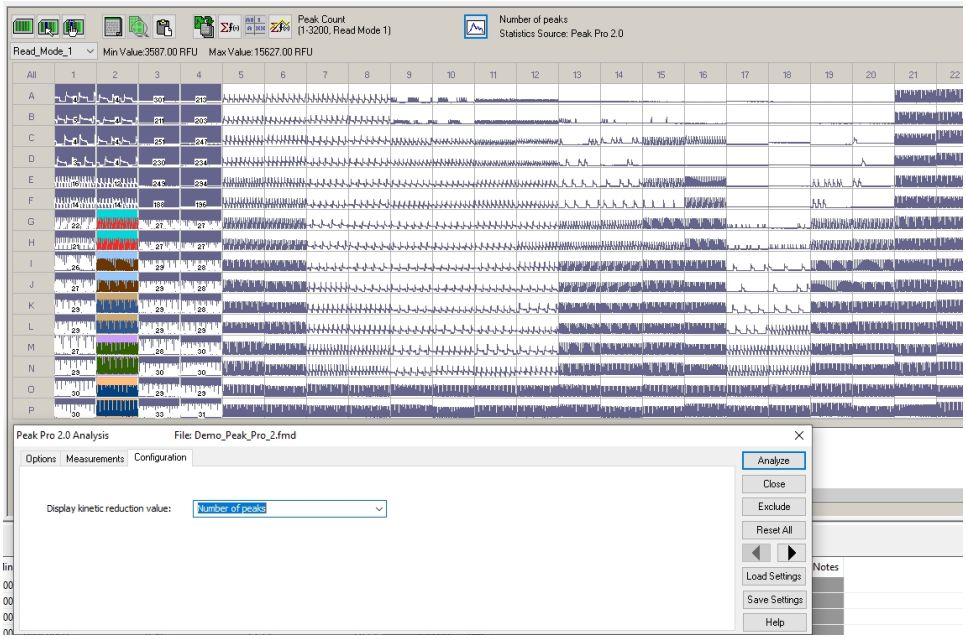


Measurement Terminology Illustrated

Item	Description
A	Main peak amplitude
B	Linear decay slope
C	Main peak interval used to calculate the peak rate, expressed in peaks per minute (PpM)
D	Early after depolarization like event (EAD like) intervals used to calculate the EAD like event rate, expressed in peaks per minute (PpM)
E	Rise slope
F	EAD like event amplitude
G	Decay slope
H	Calcium transient duration (CTD)
I	Calcium transient duration from peak position (CTDP90)
J	Start of an event
K	End of an event
L	Main peak
M	Early after depolarization like event (EAD like)

Configuration Tab

Use the Configuration tab to display one kinetic reduction value in the Multi-Well graph for the wells you select.

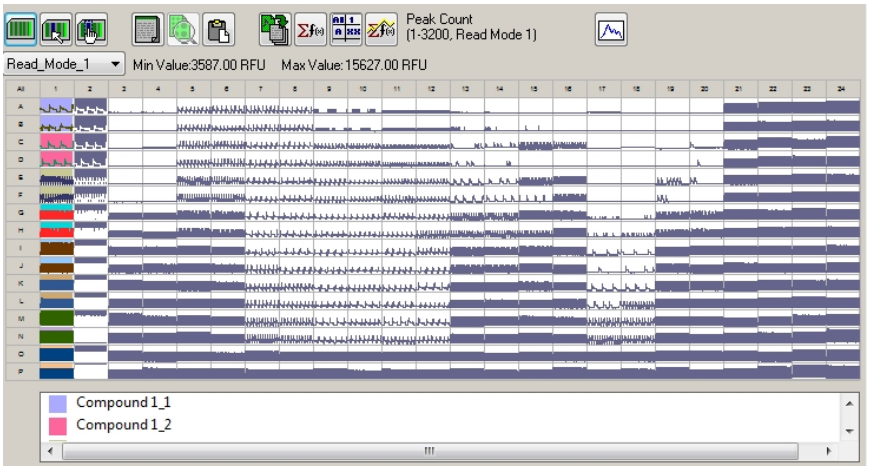


Running Peak Pro 2.0 Software Module

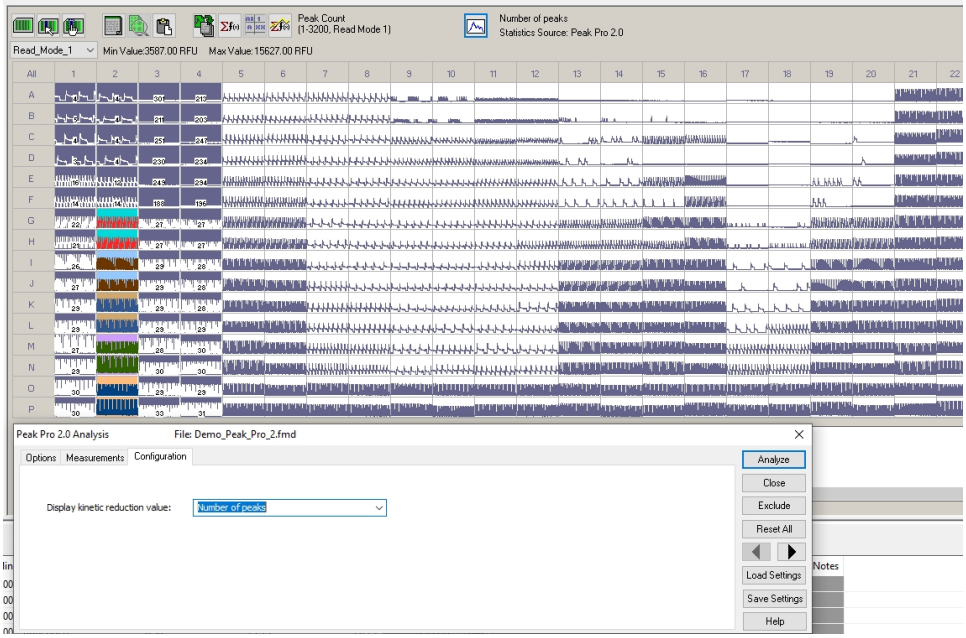
After you save experiment data files you can run an analysis with the ScreenWorks Peak Pro Software version 2.0 license. The Peak Pro 2.0 software module functionality is best suited for cardiomyocyte, neuronal and similarly fluxing assay data.

To run the Peak Pro 2.0 software module:

1. Click the **File** menu and select **Open** and then select **Data Files** to display the Open dialog.
2. Select a data file and click **Open**.
3. In the Multi-Well graph, select the wells, rows, or columns to analyze.



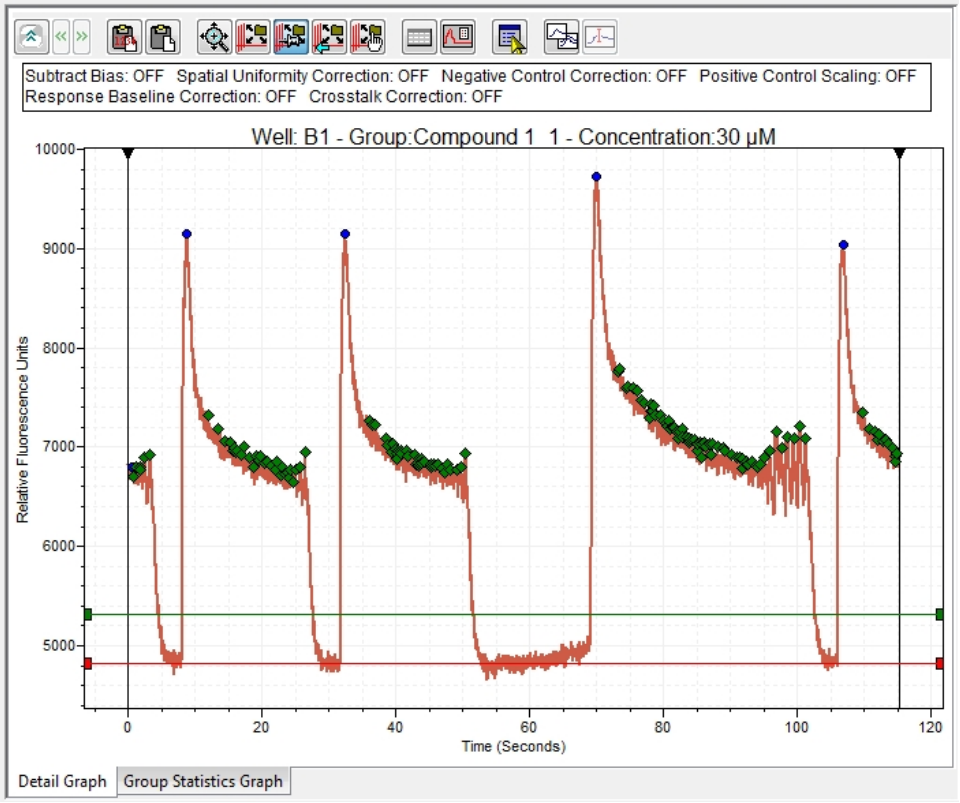
4. Click  to display the Peak Pro 2.0 Analysis dialog where you collate the data, compute baselines, thresholds, optimal search vector length, and format the results for subsequent analysis, sorting, and recording.
5. Select the **Options** tab to run the default settings or adjust the settings. See Options Tab on page 124.
6. Select the **Measurements** tab to adjust ranges. See Measurements Tab on page 127.
7. Select In the **Configurations** tab and then select a statistic to **Display Kinetic Reduction Value** in the Multi-Well graph for the wells, for example, display **Number of Peaks**.



8. Click **Analyze** to start the analysis.

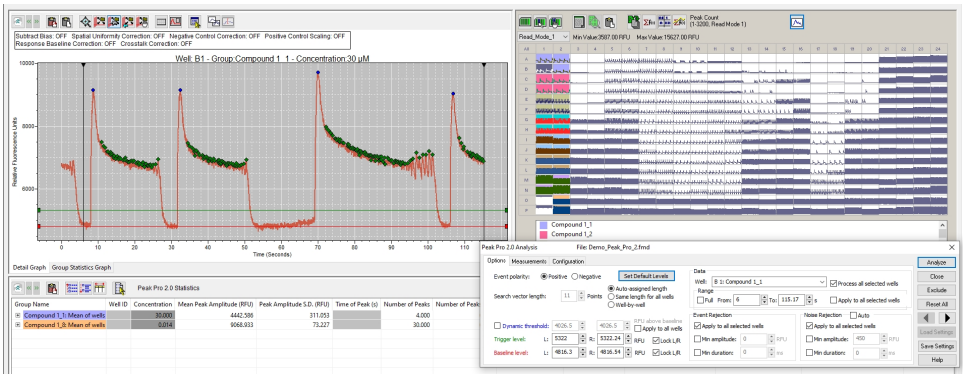
9. Review the results in the Detail graph. Each major peak displays a blue dot. Secondary peaks display a green diamond.

Tip: When there are many green diamonds the secondary markers are questionably significant. Adjust the settings so there is one major peak and few secondary peaks per event. The major peak is the first peak in an event above the trigger level. An event is that portion of a signal that crosses the trigger level in both directions.

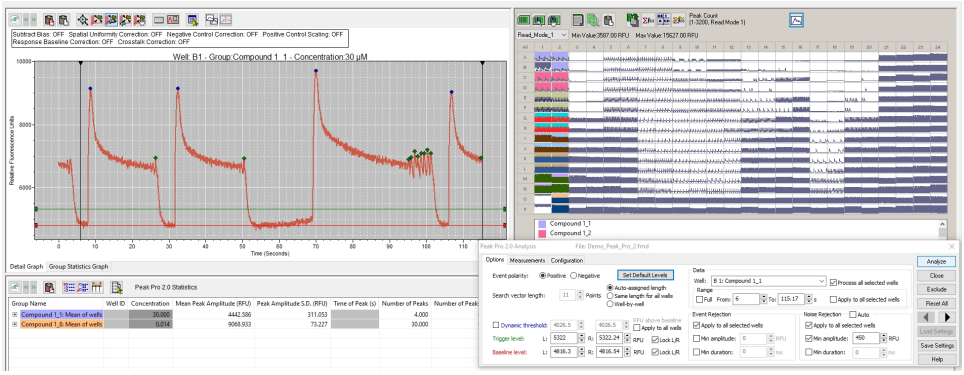


Use the arrow keys to scroll through each well graph or select wells in the Well list to examine the result.

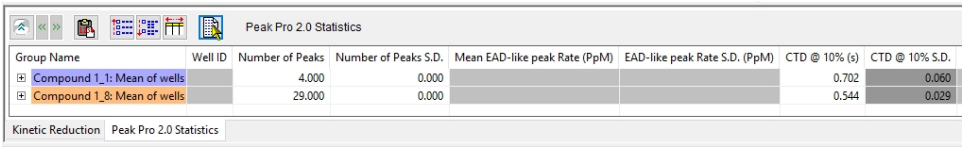
10. If the first marked event is a partial signal that you want to skip adjust the analysis data time range.
    a. Do one of the following:
        - Move the vertical start time range line in the graph.
        - On the **Options** tab, in the **Data** section, clear the **Full** check box and enter the **From** starting value.
    b. On the **Options** tab, click **Analyze**.



11. To reduce the number of secondary peaks, which are likely noise and not biologically significant, adjust settings on the **Options** tab, click **Analyze**, and review the results again in the Detail graph.

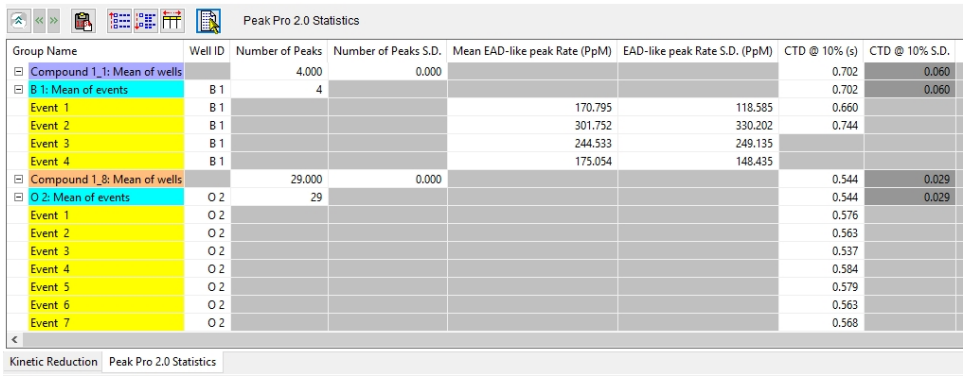


12. After you reduce the noise and identify the real biological events, you are finished with the analysis. Click **Save Settings** to save the settings with data file. When you reopen the Peak Pro 2.0 Analysis dialog, click  to display the last settings as long as the data file stays open.
13. View the results in the Peak Pro 2.0 Statistics pane where the results sort by group name. See Grouping on page 72.



Note: Group Statistics graph functionality is not supported in ScreenWorks Software version 5.1.

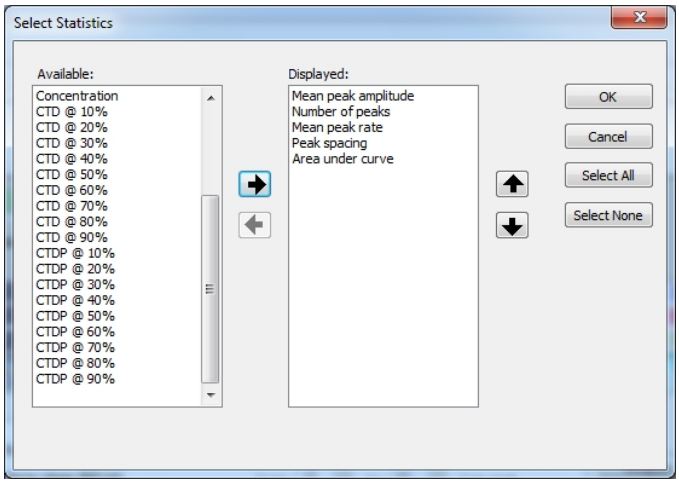
14. Expand the Group rows and the Mean rows to display the Event rows of data.



Group Name	Well ID	Number of Peaks	Number of Peaks S.D.	Mean EAD-like peak Rate (PpM)	EAD-like peak Rate S.D. (PpM)	CTD @ 10% (s)	CTD @ 10% S.D.
Compound 1_1: Mean of wells		4.000	0.000			0.702	0.060
B 1: Mean of events	B 1	4				0.702	0.060
Event 1	B 1			170.795	118.585	0.660	
Event 2	B 1			301.752	330.202	0.744	
Event 3	B 1			244.533	249.135		
Event 4	B 1			175.054	148.435		
Compound 1_8: Mean of wells		29.000	0.000			0.544	0.029
O 2: Mean of events	O 2	29				0.544	0.029
Event 1	O 2					0.576	
Event 2	O 2					0.563	
Event 3	O 2					0.537	
Event 4	O 2					0.584	
Event 5	O 2					0.579	
Event 6	O 2					0.563	
Event 7	O 2					0.568	

Each main row contains the average of measurements of each event and each event row contains the metrics of the individual peaks and measurements.

15. click **Select Statistics** to display the Select Statistics dialog where you select the statistics to display and in which sequence.



Note: The Peak Pro 2.0 Statistics pane data does not save with the data file you use for analysis.

16. To save the analysis data, do one of the following:
    - In the Multi-Well graph, click **Export**. See Exporting Data with Peak Pro 2.0 Software Module on page 135.
    - In the Statistics pane, click **Copy Table Data to Clipboard** and then paste the delimited data into a different software application.

Exporting Data with Peak Pro 2.0 Software Module

Exporting data from the Peak Pro 2.0 software module is similar to the standard the ScreenWorks Software export process, except that you must run an analysis before you can export the Peak Pro 2.0 software module data. For the standard export process, see Exporting Data on page 117.

Note: A message displays when you click Export before you run the analysis in the Peak Pro 2.0 software module.

CAUTION! Because this analysis is a manual process Auto-Export and Batch Export are not supported.

To export data with the Peak Pro 2.0 software module:

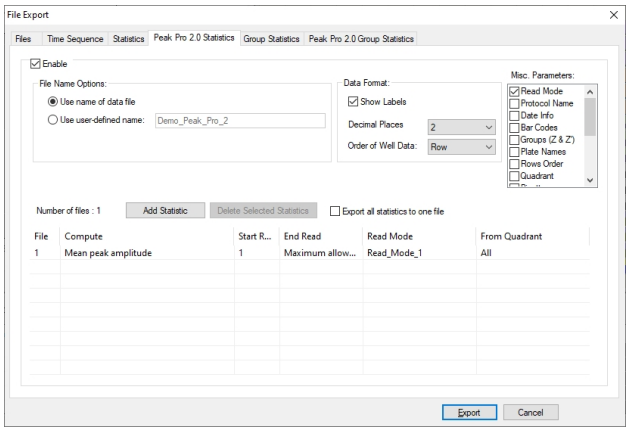
1. Run the analysis. See Running Peak Pro 2.0 Software Module on page 130.
2. Click  to display the File Export dialog.
3. Select the tab for the type of data export to do, **Peak Pro 2.0 Statistics** or **Peak Pro 2.0 Group Statistics**.
4. Select **Enable**.

   Note: After the 14-day trial expires, if you did not install the ScreenWorks Peak Pro Software Version 2.0 license, the Peak Pro 2.0 Statistics and Peak Pro 2.0 Group Statistics tab are not available.

5. Configure the data export on the tab. See Exporting Peak Pro 2.0 Statistics on page 136 or Exporting Peak Pro 2.0 Group Statistics on page 137.
6. Click **Export** to generate the data files from the active tabs.

Exporting Peak Pro 2.0 Statistics

After you run the analysis in the Peak Pro 2.0 software module, use the Peak Pro 2.0 Statistics tab to configure kinetic reductions for the numbers of reads within each well.

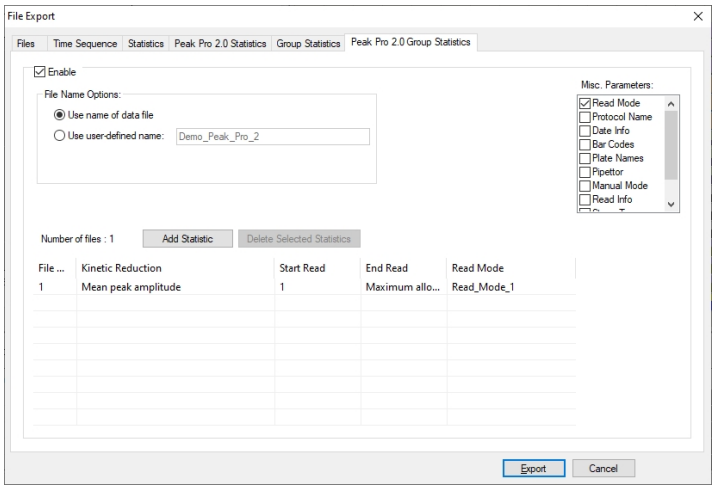


Peak Pro 2.0 Statistics File Export Options

Option	Description
Enable	Select this check box to create a statistic file.
File Name Options	Select **Use Name of Data File** to export files with the data or protocol file name, with *.stat*n* extension, where *n* is an integer. Select **Use User Defined Name** to enter the export file name (maximum of 25 characters). Files have a *.stat*n* extension, where *n* is an integer.
Data Format	**Show Labels** - Select this check box to include information about the processing options you select (for example, name of *.fmd file and well labels). Any corrections prior to export (e.g., negative control correction) are reported at the top of the exported ASCII file. **Decimal Places** - Select 2, 3, or 4 decimal places for the data export. This applies only if the number of decimal places exist in the data. **Order of Well Data** - Select column to sequence the data by column or select Row to sequence data by row. Set this in accordance with the way the spreadsheet or database handles well data.
Misc. Parameters	Select the check box for each experiment parameter and group statistic to export with the data.
Number of Files	**Add Statistic** - Adds a statistic file to create from the data set. For data created in 384-well or 1536-well formats. **Compute** - Select a kinetic reduction type. See Measurements Tab on page 127. **Start Read** - Enter the first read number to use to determine the kinetic reduction. **End Read** - Select the last read to use to determine the kinetic reduction. This can equal the Start Read to extract values from a single read. **Read Mode** - Select the read mode (or ratiometric data) from which to process the data. **From Quadrant** - Select the quadrant from which to export or select to export data from all quadrants. This allows you to export data from one plate into four different spreadsheet files.

Exporting Peak Pro 2.0 Group Statistics

After you run the analysis in the Peak Pro 2.0 software module, in the File Export dialog, use the Peak Pro 2.0 Group Statistics tab to configure group statistics, for example, average and standard deviation, based on the kinetic reduction you define for a number of reads.



Peak Pro 2.0 Group Statistics File Export Options

Option	Description
Enable	Select this check box to create a group statistic file.
File Name Options	Select **Use Name of Data File** to export files with the data or protocol file name, with *.gstat*n* extension, where *n* is an integer. Select **Use User Defined Name** to enter the export file name (maximum of 25 characters). Files have a *.gstat*n* extension, where *n* is an integer.
Misc. Parameters	Select the check box foe each experiment parameter to export with the data.
Number of Files	**Add Statistic** - Adds a statistic file to create from the data set. For data created in 384-well or 1536-well formats. **Kinetic Reduction** - Select the reduction to apply to the kinetic data traces to export. See Measurements Tab on page 127. **Start Read** - Enter the first read to use to determine the kinetic reduction. **End Read** - Select the last read used to determine the kinetic reduction. **Read Mode** - Select the read mode to apply the kinetic reduction.

Chapter 8: Maintaining the Instrument

Do only the maintenance tasks this guide describes. Maintenance procedures other than those specified in this guide must be done by Molecular Devices. See Obtaining Support on page 176.

Before you operate the instrument or perform the following maintenance procedures, make sure you are familiar with the safety information in this guide. See Safety Information on page 7.

Washing Pipettor Tips and Pin Tools

The Wash Tips or Wash Pins process in the ScreenWorks Software protocols controls the washing of the pipettor tip and pin tool you perform between fluid transfers within an experiment, or after the last fluid transfer, to prepare tips for the next experiment. You can wash disposable tips, as well as the 1536-tip head, and 384- and 1536-pin tools.

The washer consists of a reservoir top for the pipettor format that mounts over a wash basin. The wash basin is connected to two solvent supply carboys and two waste carboys located on the floor beneath the computer monitor. A basin beneath the tip washer base drains to a waste carboy to safely remove any solvent that overflows from the reservoir. See Uninstalling Wash Reservoir Top on page 145.

Wash solution fills the reservoir for a calibrated amount of time. Solvent then drains from the reservoir after each wash cycle. You can configure up to five wash cycles within a single wash process. For tips, the volume of solvent is drawn up, held for a time, and then expelled, up to 20 times. For pins, vertical motion of the tip block is used to agitate the wash solvent around the pins. You can wash tips or pins in up to two solutions before you reuse the tip washer. When you require additional wash solutions, use the Mix Fluid process to wash tips or pins in a boat or reservoir, located in one of the source plate positions.

WARNING! High volumes of volatile, flammable solvents in the read chamber may cause explosive conditions. Use of 100% isopropanol, etc., in the tip washer is discouraged without additional ventilation. Consult your facilities expert to determine the appropriate ventilation to avoid explosive conditions.

Pin tools are supplied with blotting stations that you load into one of the plate positions. Configure blot pin steps in the protocol to remove fluid from the pins.

Note: A waste bottle sensor override (P/N 0700-0827) is available in the FLIPR Penta System accessory kit to bypass the waste sensor and dispose waste in containers other than the waste carboys.

Exchanging Hardware Components

The following topics describe how to exchange hardware components in the FLIPR Penta System.

Exchanging Pipettor or Pin Tool Head

A certified Molecular Devices Field Service Engineer does the initial installation of all pipettor and pin tool heads. After the initial installation and calibration, you can exchange the pipettor and pin too heads on the instrument for which they were calibrated.

Note: If you have any questions or concerns regarding the procedures, contact Molecular Devices Technical Support support.moleculardevices.com/.

Uninstalling Pipettor or Pin Tool Heads

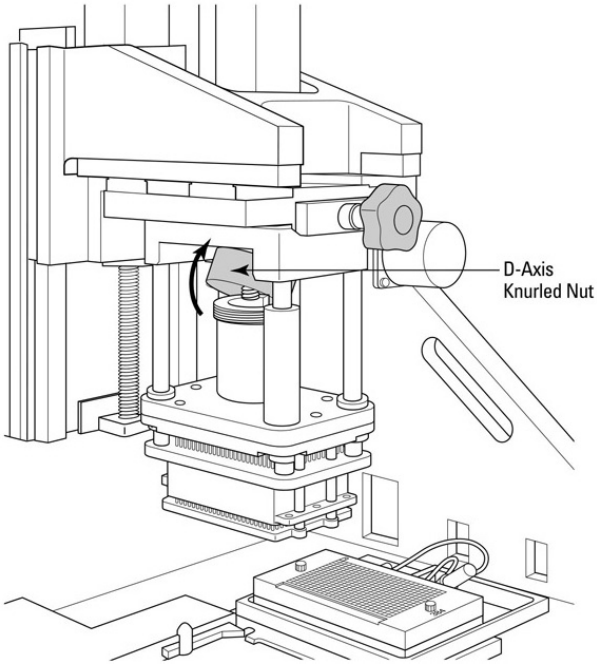
The following procedure uninstalls a 384 pipettor head as an example.

To uninstall a 384 pipettor head:

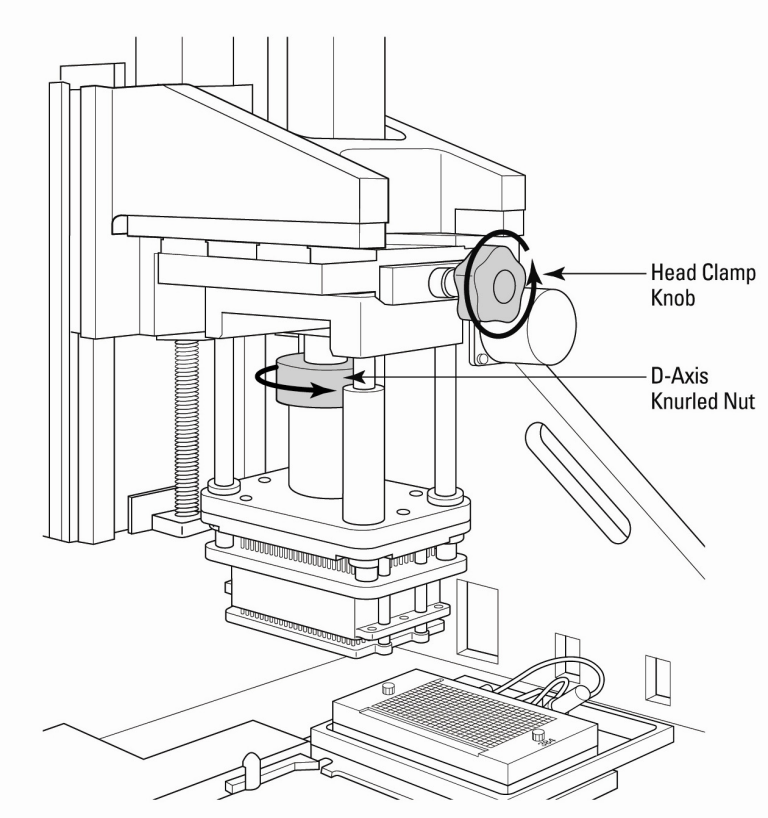
1. In the ScreenWorks Software, click the **Instrument** menu and select **Manual Operation** and then select **Unload Tips** to unload the tips on the pipettor.
2. Click the **Instrument** menu and select **Manual Operation** and then select **Change Head** to instruct the pipettor head to move over the Read Plate position.

   CAUTION! Do not click Done in the dialog that opens until you complete the work on the pipettor.

3. Open the upper front door to access the pipettor head.
4. Turn the **D-Axis Knurled Nut** counterclockwise until it loosens and can be lifted.
   For 1536 pipettor heads only: After you loosen the D-Axis Knurled Nut, install the Pipettor Head Guard that seats over the 1536 pipettor head plungers, by tightening the two thumb screws. This guard protects the plungers from being damaged when you handle the pipettor head outside the instrument.

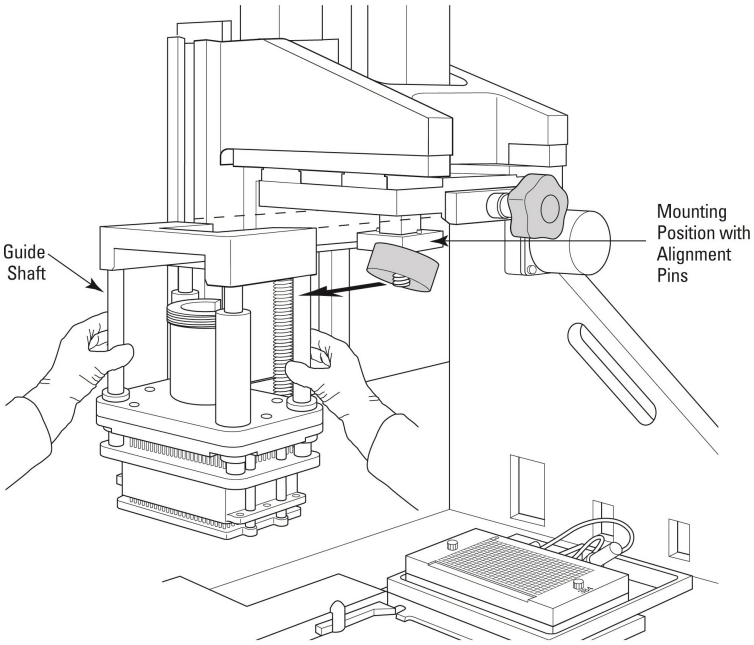


5. Turn the **Head Clamp Knob** counterclockwise until the head is loose and can be lifted off its mounting position.



6. To remove pipettor head, grasp the pipettor head silver **Guide Shaft**, lift upward (to unseat from **Alignment Pins** and to lower plunger pins allowing a lift gap) and then slide to the left off the pipettor mount.

   Note: If the pipettor head does not easily slide off, try grabbing the D-axis knurled nut receiver and press it downward to give the pipettor head additional clearance from the D-axis knurled nut prior to trying again.



7. Store the pipettor head in a safe location. A recommended safe location should include placing the pipettor head in its plastic storage bag to guard for particulates.

   CAUTION! Do not place pipettor heads on nose cone surfaces because this can damage the nose cones. Store pipettor heads inverted, resting on the pipettor head top that seat on the pipettor mount position.

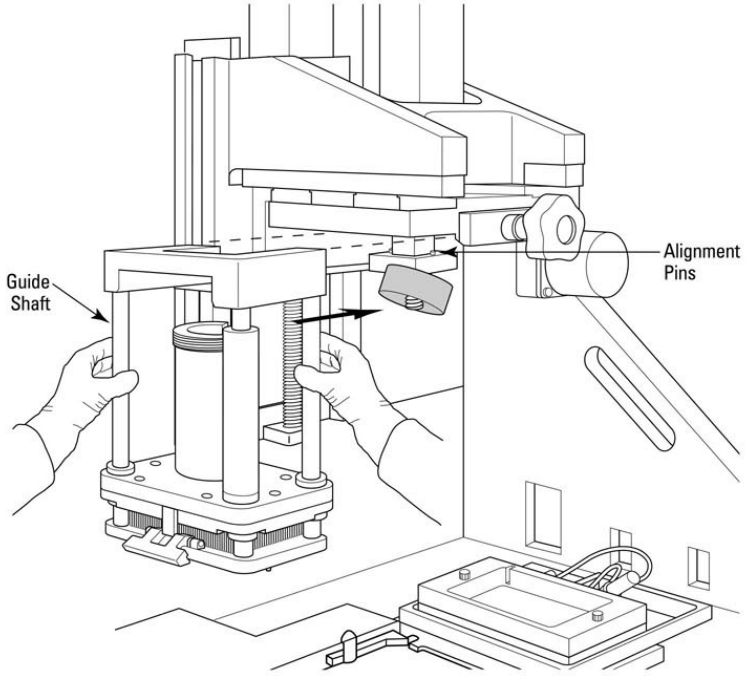
Installing the Pipettor or Pin Tool Head

The following procedure installs a 1536-pipettor head as an example.

CAUTION! Only install a head that has been calibrated for the instrument by a Molecular Devices Field Service Engineer.

To install a 1536-pipettor head:

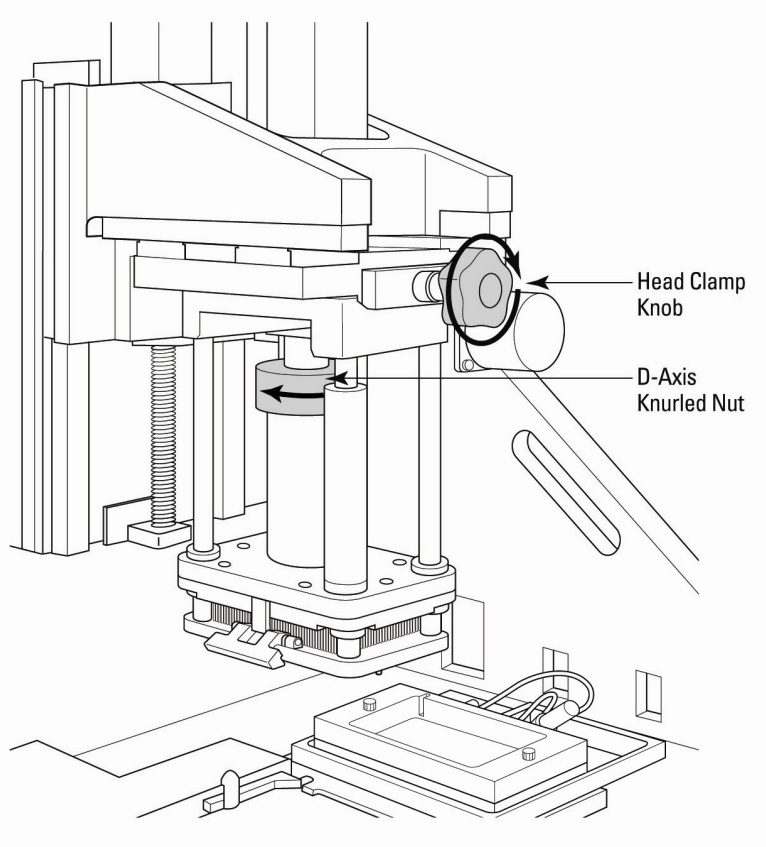
1. Hold the pipettor head by its silver **Guide Shafts** and slide it to the right over the **Pipettor Mount**.
   When the pipettor head is in position, it should be seated on the **Alignment Pins** and should not slide off the pipettor mount without lifting upwards.



2. Tighten the **Head Clamp** knob by turning it clockwise.
3. For 1536-pipettor heads, remove the guard covering the 1536-pipettor head plungers by loosening the two thumb screws.
   This allows the D-axis to move freely so it can be attached to the D-axis knurled nut in the next step.

CAUTION! If the guard is not removed before you reset the instrument, the 1536-pipettor head can be damaged.

4. Tighten the **D-Axis Knurled Nut** by turning it clockwise until tight.
   If the nut does not reach its receiver threads, lift the D-axis receiver to engage the threads and tighten the nut.



5. After the head is fastened, exchange the tip wash reservoir top to match the head format.
   See Exchanging Pipettor Tips and Pin Tool Wash Reservoir Top on page 145.

Exchanging Pipettor Tips and Pin Tool Wash Reservoir Top

After exchanging the pipettor tips or pin tool head, exchange the wash reservoir to match the new head format.

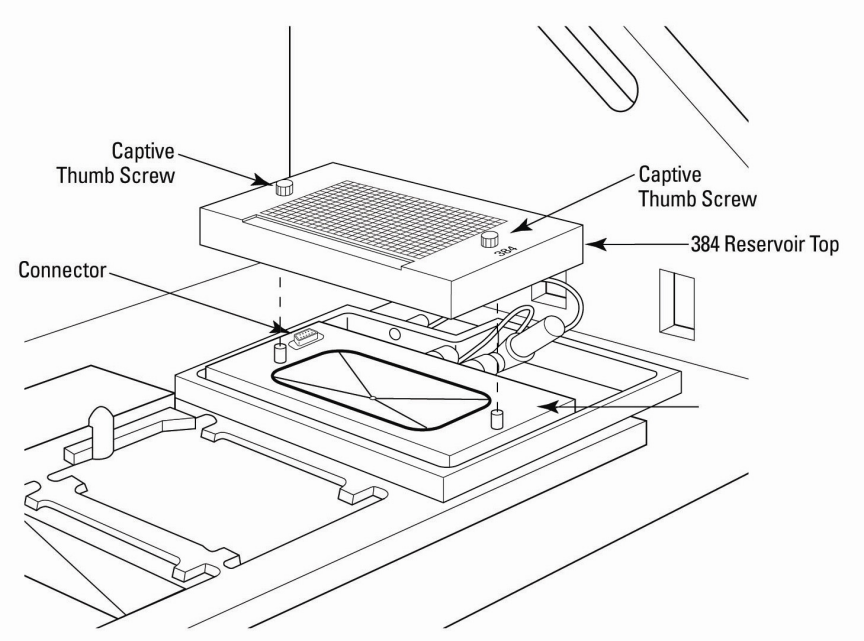
CAUTION! Failure to match head format and wash reservoir top causes damage during instrument operation.

Uninstalling Wash Reservoir Top

The following procedure uninstalls a 384-wash reservoir top as an example.

To uninstall a wash reservoir top:

1. Loosen both **Captive Thumb Screws** on reservoir top.



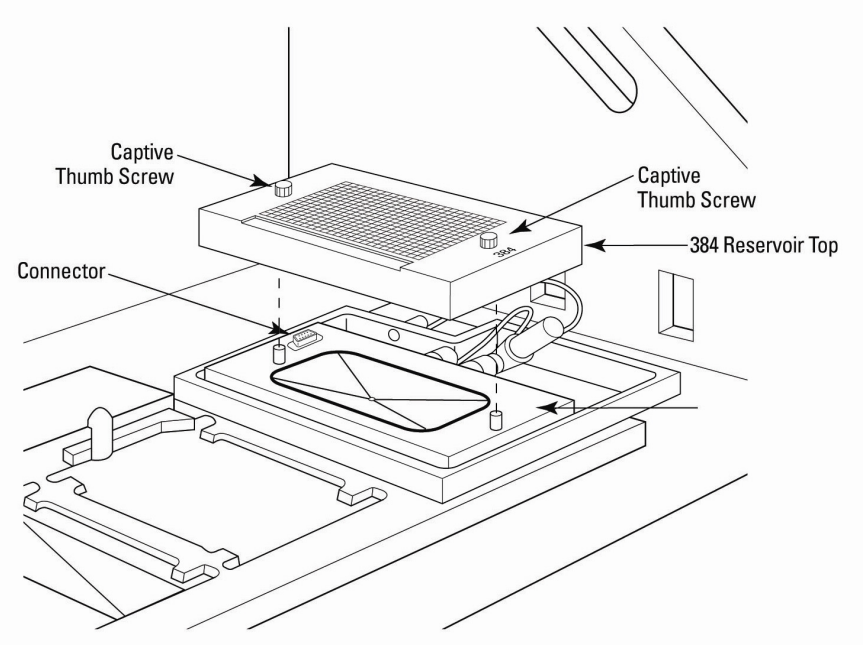
2. Lift the reservoir top off of the washer base.
3. Ensure the **Blue O-Ring** on the washer base remains properly seated. If not, reinstall the O-ring into its O-ring groove.
4. Store the reservoir top in a safe location.

Installing Wash Reservoir Top

The following procedure installs a 384 wash reservoir top as an example.

To install a wash reservoir top:

1. Align the appropriate format reservoir top so the **Connector** matches the washer base connector. When aligned correctly, the reservoir top name (96, 384 or 1536) is located towards the front of the instrument.



2. When aligned, use the **Alignment Pins** to guide the placement of the top and press the top into position.
3. Tighten both **Captive Thumb Screws** to ensure the reservoir top is fastened to the base.
4. Reset the instrument.

Resetting the System After Exchanging Pipettor or Pin Tool Heads

To reset the FLIPR Penta System after exchanging a head and a wash reservoir top:

1. Make sure the head and tip wash reservoir top formats match before continuing.

> **CAUTION!** If there is a head and wash reservoir top format mismatch, the instrument faults and prevents all experiment runs until corrected.

2. To finish the exchanges, click **DONE** in the software dialog that opens when you begin the head exchange. The instrument resets itself.
3. When the reset cycle is complete, the green **Assay Finished (Unlock)** light is the only indicator light illuminated on the front of the instrument.

Exchanging Pipettor Tips

The 96 pipettor head and the 384 pipettor head both use disposable plastic tips to transfer fluid. The 1536 pipettor head uses a stainless-steel tip block with a disposable 1536 tip gasket to transfer fluid. See Exchanging the 1536 Microplate Tip Gasket on page 149.

Each pipettor head is compatible only with the tip racks of matching format. The pipettor head can only pick up a matching tip rack. For example, the 384-well pipettor head should not try to pick up pipettor tips from a 96-well tip rack.

CAUTION! Verify that there is a matching tip rack in the Tip Load position on the instrument deck before you run the experiment. A mismatched pipettor head and tip rack causes a fatal error and can damage the equipment.

CAUTION! The FLIPR Penta System is only validated for use with the Molecular Devices tips. Instrument performance and troubleshooting is not guaranteed with any other tips.

To automate pipettor tip loading for 96-well and 384-well pipettor tips:

- In the protocol configuration, click the **Settings** menu select **Assign Plate to Position** and then select **Load Tips Position**. See Settings Process on page 60 and Creating New Protocols on page 96.

To load pipettor tips for 96-well and 384-well pipettor tips:

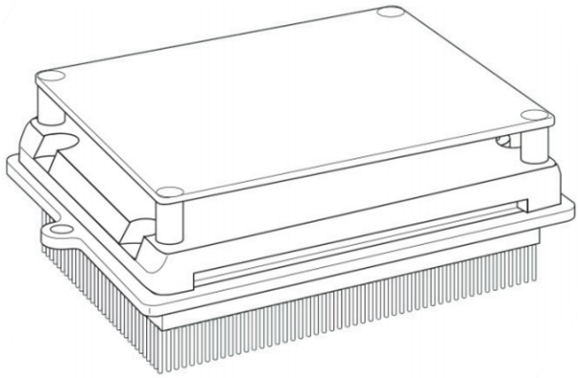
- In the ScreenWorks Software, click the **Instrument** menu and select **Manual Operation** and then select **Load Tip and Unload Tip**. The pipettor tips attach to the pipettor head and the pipettor tips rack stays on the stage in Position 1.

To unload pipettor tips for 96-well and 384-well pipettor tips:

- In the ScreenWorks Software, click the **Instrument** menu and select **Manual Operation** and then select **Unload Tip**. The pipettor tips are removed from the pipettor head in to the pipettor tip rack on the stage in Position 1.

Exchanging Pin Tools

Pin tools are blocks of pins that load onto a pin tool head to transfer fluid.



Two pin tool heads, 384-well and 1536-well formats, are available for the FLIPR Penta System. Each pin tool head is compatible only with the pin tool blocks of matching format. The pin tool head only picks up a matching pin tool block according to the attached magnetic ID. For example, the 384-well pin tool head does not pick up a 1536-well pin tool block. This is designed for the component safety.

To automate pipettor tips or a pin tool block loading:

- In the protocol configuration, click the **Settings** menu and select **Assign Plate to Position** and then select **Load Tips Position**. See Settings Process on page 60 and Creating New Protocols on page 96.

To load a pin tool block:

- In the ScreenWorks Software, click the **Instrument** menu and select **Manual Operation** and then select **Load Tip and Unload Tip**. The pin tool block is picked up and clamped to the head using pin tool block grippers.

To unload a pin tool block:

- In the ScreenWorks Software, click the **Instrument** menu and select **Manual Operation** and then select **Unload Tip**. The pin tool block is placed in Position 1 of the stage and unclamped from the pin tool head grippers.

Alternating Position 1 Use

Stage Position 1 is primarily for tip loading and unloading, but you can use this position as a source plate position after tips are removed.

Note: Sensors indicate only the presence of an object in plate position and do not indicate whether it is a source plate or tip rack.

Use the following procedure in both manual and robotic modes.

To use a source well plate in the stage Position 1:

1. Load tips or pins manually.
2. Remove the tip rack before you start an experiment and place a source plate in Source 1.

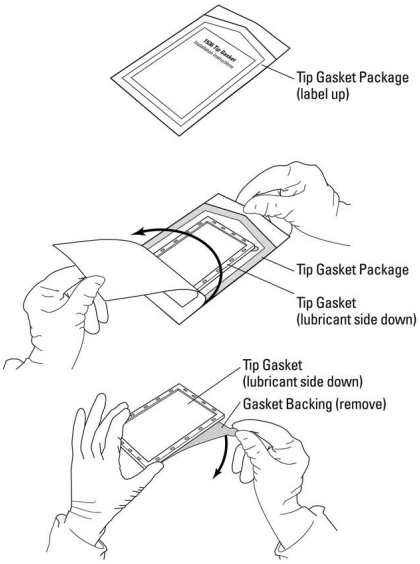
Exchanging the 1536 Microplate Tip Gasket

Replace gaskets approximately every 200 cycles (1 cycle = 1 stroke down + 1 stroke up). Monitor the gasket lifetime because it varies on the number of aspirate and dispense steps you run in the experiment.

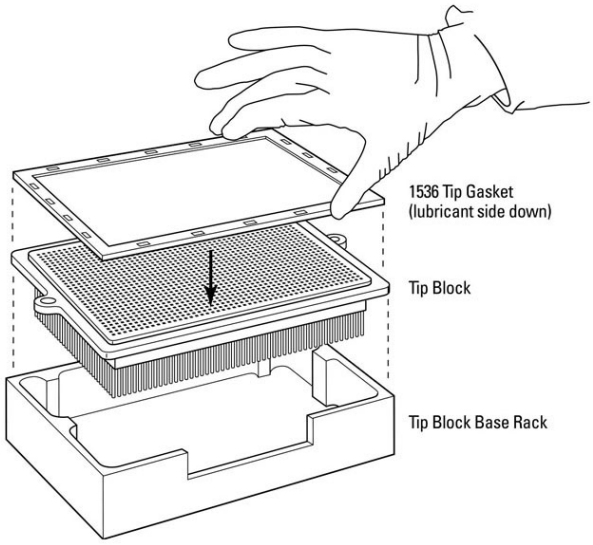
Installing the Gasket

To install the 1536 tip gasket:

1. Remove the gasket from its package and discard the gasket backing.



2. Place the lubricated side of the gasket down on the tip block, using the gasket recess to guide the frame position.
   When aligned, the tip block and gasket are sandwiched together until the tip block grippers clamp into place.



Removing the Tip Block and Gasket

During the unloading, the tip block grippers unclamp and release the gasket and tip block into the rack assembly. When unloaded, dispose the gasket and use a new one the next time the block is loaded. See Consumables and Accessories on page 179.

To load from Position 1 for a source well plate:

- Load tip block manually and then remove the tip block before you start the experiment. When loaded, you can place a source plate placed in Source 1 in both manual and robotic modes.

Note: Sensors indicate only the presence of an object in plate position and do not indicate whether it is a source plate or tip block.

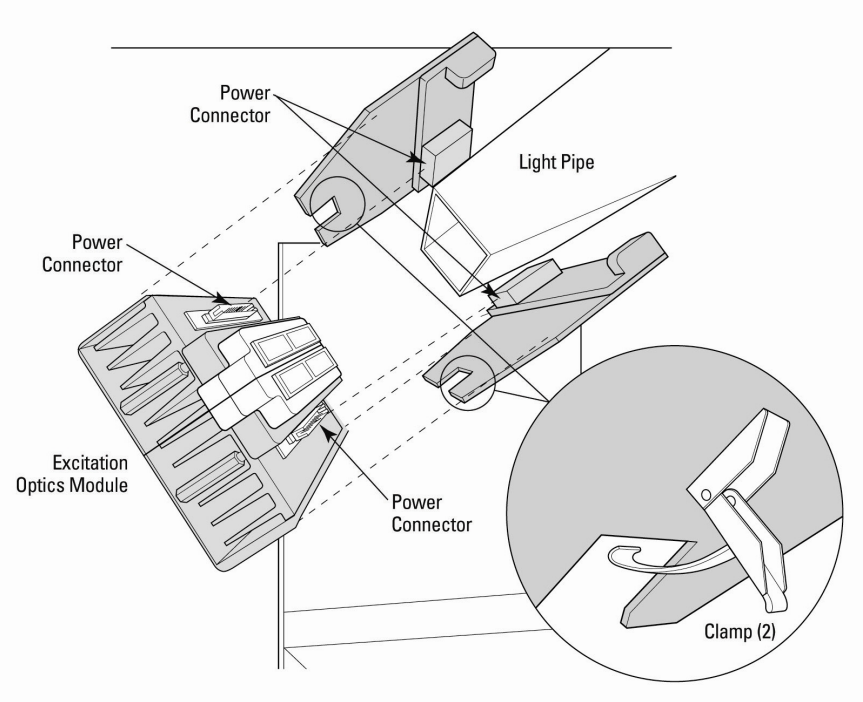
Exchanging LED Modules

WARNING! During the LED exchange procedure, you have access to precision optical components. Do not touch the surfaces of the LED filters with your bare fingers or other objects because you can damage them.

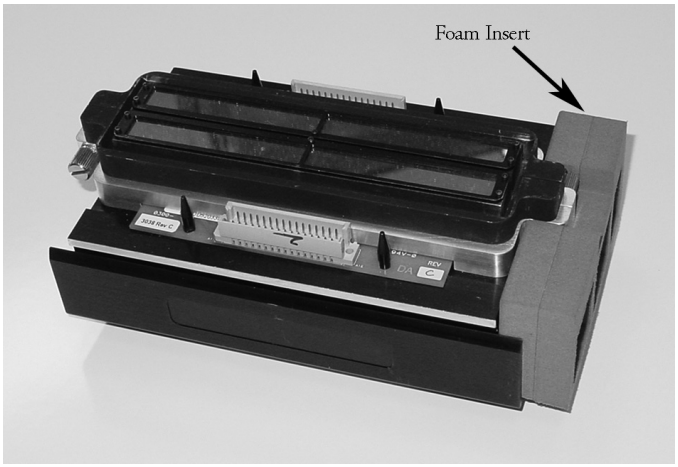
Uninstalling LED Modules

To uninstall LED modules:

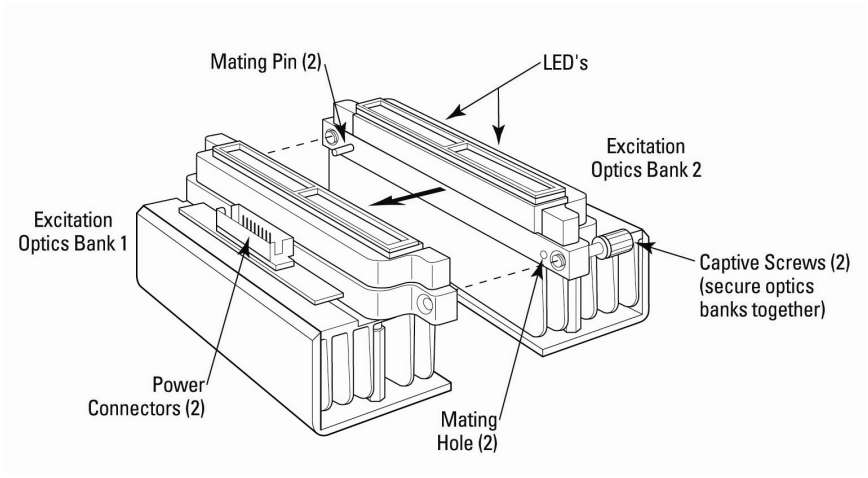
1. Open the lower front door of the instrument. When the lower door is open, power to the LEDs is disabled.
2. Loosen the two silver **Latching Clamps** that secure the excitation optics module to the left light pipe.



3. Pull the excitation optics module down and away from light pipe to disengage the **Power Connectors** on the LED modules from the light pipe. Be careful not to scratch the bandpass filters when you pull the LED modules out of the instrument.
   Remove the **Foam Insert** found on the rear side of the excitation optics module.



4. Loosen the two **Captive Thumb Screws** that hold the LED modules together and then pull the modules apart so you can exchange them for new wavelengths.

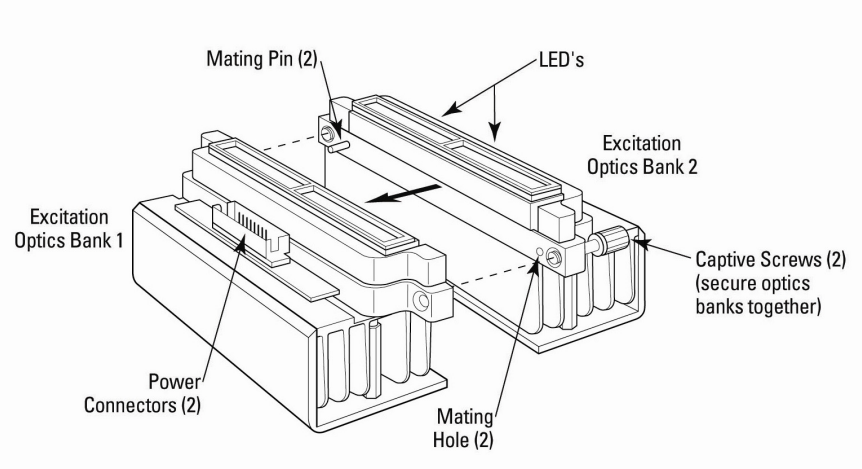


5. Repeat for the LED module mounted to the right light pipe.
6. When separated, use the Installing LED Modules procedure to exchange LED modules. See Installing LED Modules on page 152.

Installing LED Modules

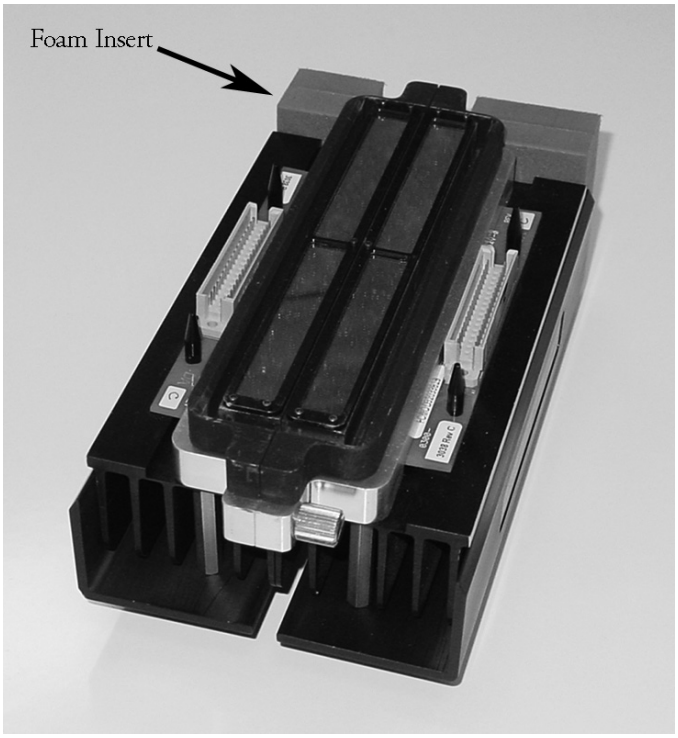
To install LED modules:

1. To create an excitation optics module, combine two LED banks of different wavelengths. Align the mating pins to the respective mating hole in the LED modules you secure to each other.

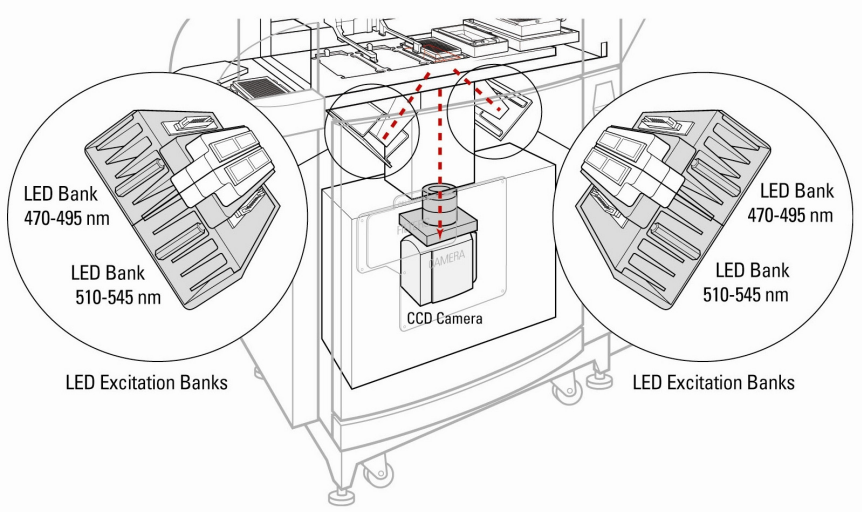


2. When aligned, push the two LED banks of different wavelengths together and tighten the two **Captive Thumb Screws** with firm finger pressure.
3. Place the **Foam Insert** on the rear end of the excitation optics module.

Note: Make sure that the foam piece fits inside the LED Frame.



4. Open the lower front instrument door and slide the left **Excitation Optics Module** into place. Each module is coarsely self-aligning but observe engagement of the two **Power Connectors** and confirm they are aligned before you apply mating pressure.
5. After you mate the connectors, engage and tighten the two **Latching Clamps** that secure each module to the **Light Pipe**.
6. Repeat this procedure for the right **Excitation Optics Module**, being sure the same wavelength modules occupy the corresponding position (upper/lower) on each side.



7. If finished, close the lower front door. If you also change filters, continue to Exchanging Filters on page 154.
8. When you close the lower front door, a message displays "*The lower door has been opened, do you want to update optics?*" Click **Yes**. The instrument and software:
   - Identifies filters and LEDs
   - Tests LEDs
   - Updates the instrument status configuration
   - Closes the Progress dialog when the process finishes

Exchanging Filters

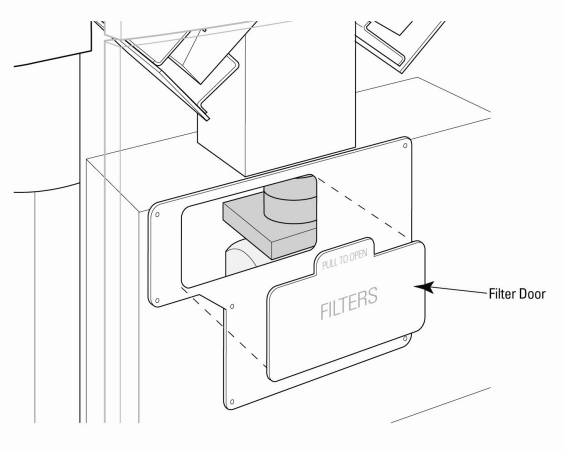
The instrument senses the filters and the Instrument Configuration window in the software displays the filter configuration.

Three custom filter holders are available for purchase if you use filters from an outside vendor. See Consumables and Accessories on page 179. When installed, these filters appear as 1_1, 2_2, and 3_3 in the software protocol.

Uninstalling Filters

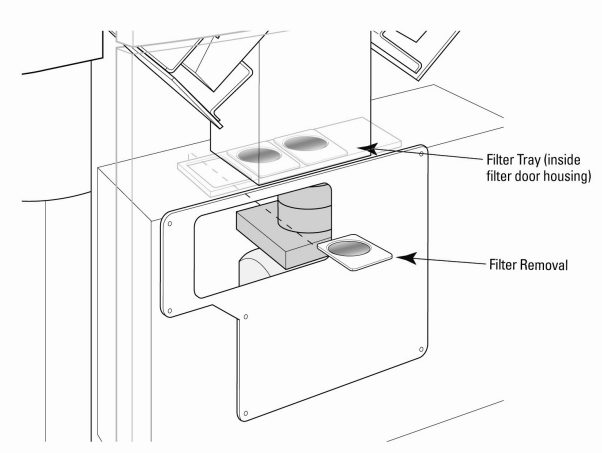
To uninstall a filter:

1. Open the lower front door of the instrument. When the lower front door is open, system power is disengaged.
2. Remove the **Filter Cover**, located below the left LED module.



3. Use the filter tab that protrudes slightly from the filter tray to push the filter up out of its position and remove it from the instrument. Grasp the filter by the tab.

CAUTION! DO NOT touch the filter surface.



4. Repeat Step 3 to remove the remaining emission filters.
5. After you remove the emission filters replace the **Filter Cover**.
6. Install new emission filters into the system. See Installing Emission Filters on page 155.

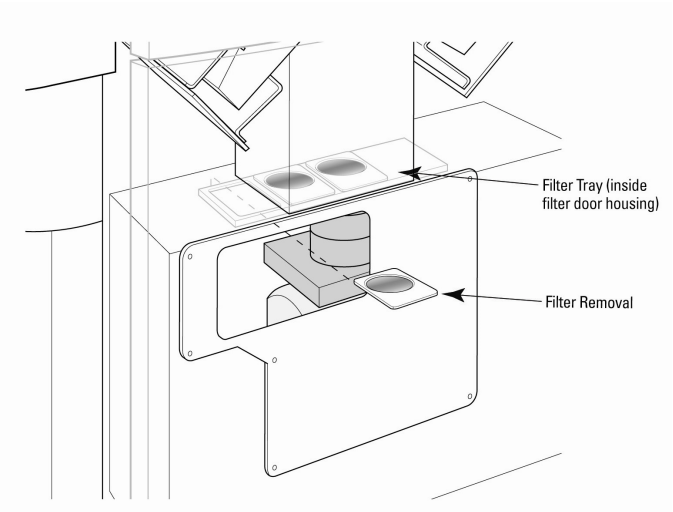
Installing Emission Filters

The system automatically identifies the emission filter wavelengths you can select from the ScreenWorks Software menu.

To install an emission filter:

1. Open the lower front door of the instrument and remove the **Filter Cover** located below the left LED module.
2. Grasp the filter by the tab located where the filter table is and place the filter into the filter position. Typically, this is the rightmost position 1 in the slider. Insert the rear of the filter first to make sure it fits into the filter position. Push the tab down until the filter indexer clicks into the position. A notch on front of the tab should align with one of the lines on the **Filter Tray**.

   CAUTION! DO NOT touch the filter surface.



3. Repeat to install subsequent filters into positions 2 and 3.

   Note: If a filter is not available, you can purchase three custom filter cassettes. See Optics Consumables on page 181. When installed, these filters appear as 1_1, 2_2, 3_3 in the software protocol.

4. The system identifies the emission filter wavelengths and passes the information to the ScreenWorks Software for you to select.

   CAUTION! When you use all 3 filter positions, do not duplicate wavelengths because this causes a fault in the software.

5. After you install emission filters replace the **Filter Cover**.
6. Close the lower front door when you finish.
7. When you close the lower front door, a message displays: *The lower door has been opened, do you want to update optics?* Click **Yes**. The instrument and software:
   - Identifies filters and LEDs
   - Tests LEDs
   - Updates the instrument status configuration
   - Closes the Progress dialog when the process finishes

Exchanging the Plate Hold Down Devices

The plate hold down devices, part numbers 2300-1631 and 2300-1633, keep plates or tip racks from lifting after aspirating, dispensing, and tip loading. Each device has an overhang that extends over the skirt of a plate to prevent upward movement when the pipettor is removed from the plate. The provided devices fit three different plate hold down gap sizes, 2 mm, 2.5 mm, and 6 mm, which correspond to three plate skirt thicknesses.

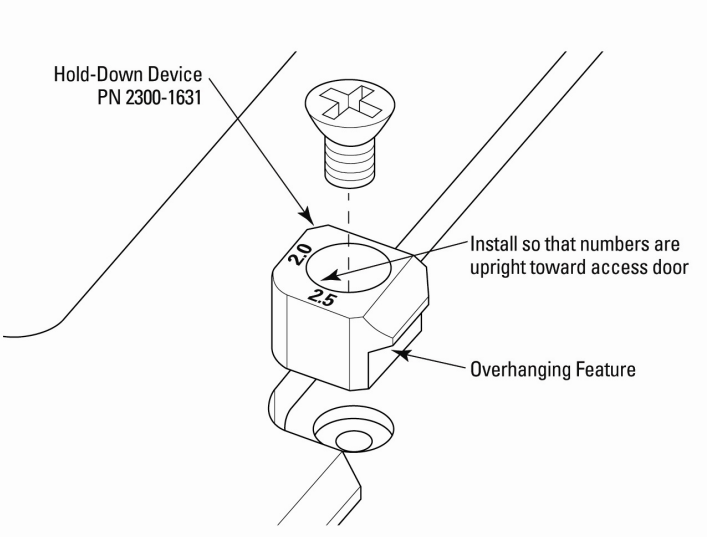
The 2.0 mm and 2.5 mm are incorporated in one device that rotates 90 degrees.

A second plate hold down device in the accessory kit has a 6.0 mm gap. Numbers that correspond to the plate skirt thickness are marked on the top of the device. The device is properly oriented when you can read the digits upright from the instrument service door.

Selecting Plate Hold Down Devices

Most plates and tip racks use the 2.5 mm device setting. Exceptions among approved plates are the Corning/Costar plates that have a very thick 6.0 mm plate skirt and the thin 2.0 mm plate skirt of the Greiner 1536-well plates. To determine which hold down device is most appropriate, use the lowest hold down device that allows the plate skirt to slide under the overhang feature freely. It is important that the overhang feature is clearly above the skirts on all plates and tip racks that you use in a plate position or a misalignment with the pipette tips can occur.

Test the configuration after installation.



Removing Plate Hold Down Devices

To remove a plate hold down:

1. Clear the system of plate or tips that can interfere with the position from which to remove the plate hold down device.
2. Use a Phillips #2 or #3 screwdriver to loosen and remove the screws from the center of the device.
3. Remove the plate hold down device.

Installing Plate Hold Down Devices

To install or reposition the plate hold down:

1. Place the device in the orientation so that the numbers appear upright when you look through the main user access door.
2. Insert the flat head screw that originally secured the device (or a screw included in the accessory kit) through the hole in the device and into the screw threads on the left side of the plate retaining pockets. Tighten the screw firmly. The device is self-locating so no additional alignment is necessary.
3. If you use plates or tip blocks with different flange heights in the same position, use the device with the largest flange thickness.
4. Test the plates or tip blocks to use by inserting them into the plate pockets at the location in which they are used.
5. Observe that when the plate releases it pushes to the left under the retaining device. It should come to rest against the edge locating surfaces on the left and nearest the door.
6. Pull up on the plate gently.

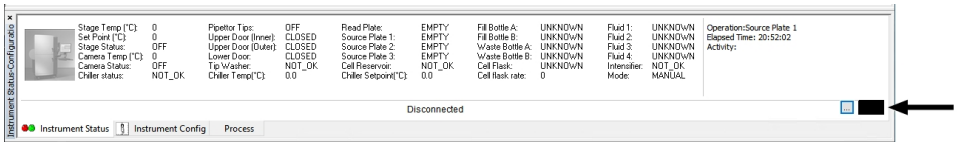
Tip: You should feel a definite retaining force. A sideways movement during lifting can cause the plate to slide out from under the retaining device and lift upward without retention. This does not happen during instrument operation but might require attention to avoid when you lift a plate by hand.

Chapter 9: Troubleshooting

This chapter contains tables of symptoms and solutions to help you diagnose and correct problems with the FLIPR Penta System. To contact Molecular Devices Technical Support. See Obtaining Support on page 176.

Instrument Status Colors

The ScreenWorks Software handles most errors that occur when you run the FLIPR Penta System. The status of the system is displays in the Instrument Status panel. See Software Status Tabs on page 19.



Depending on the state of the instrument, a color icon displays at the bottom right of the Instrument Status window.

Instrument Status Colors

Status Color	Indication
Green	Proper and open communication.
Yellow	Minor communication error. To clear the error, click the Instrument menu and select Clear Error.
Red	Instrument failure. Instrument must be reset. Click the Instrument menu and select Reset.
Black	No instrument communication available. This typically displays when you work in Offline mode.

Troubleshooting Start Up

Start Up Error Messages

Symptom or Error Message	Possible Causes	Solutions
Failed to connect to the instrument	Embedded computer is not communicating with the host computer	Restart the instrument
Connecting to the instrument, please wait. Will enter offline if the instrument is not connected or if you close this dialog. Dialog may not close immediately when you press the Close button while the connection is attempted.	The ScreenWorks Software is waiting for the FLIPR Penta System to complete initialization prior to connecting.	Wait until the Assay Finished (Unlocked) light is the only light illuminated on the front instrument panel before the software and instrument connect.
Camera chiller subsystem not functional. Please contact Technical Support. Can't communicate with chiller.	Instrument cannot communicate with the chiller.	Make sure the chiller is turned on. Contact Technical Support.
409: Initialization halted because tips are loaded. Please verify that the appropriate tip rack (96 or 384 tip rack, 1536 tip block, or 384, 1536 pin tool holder) is loaded and select RESET.	Tips are on the pipettor head and need to be unloaded during instrument initialization/reset.	Place tip rack in source 1/tip loading position to unload tips. Click the Instrument menu and select Reset.
410: Initialization halted. Either the upper or lower door is open. Please shut both doors and select RESET.	The door is open during instrument initialization/reset.	Close the upper and lower doors. Click the Instrument menu and select Reset.
411: Front Panel Interrupt button was pushed while initializing. Initializing was not completed. Select Reset when ready to continue.	The front panel interrupt button was pushed, stopping instrument initialization.	Click the Instrument menu and select Reset.
Disconnected displays in the bottom of the Instrument Status Window.	The ScreenWorks Software is in Offline Mode.	Click the Instrument menu and select Go Online.
	Multiple software copies are open.	Close all versions of the ScreenWorks Software before you start the software.
	Instrument timed out.	Click the Instrument menu and select Go Online.
Instrument Configuration does not show correct components installed on instrument.	Instrument does not have proper components installed.	Install proper components and restart the system.
	The ScreenWorks Software is in Offline Mode.	Click the Instrument menu and select Go Online.
Camera Temp not in its operating temperature range EMCCD camera: -70°C (-94°F) ± 2°C HS EMCCD camera: -70°C (-94°F) ± 2°C	Camera is not cooled down to temperature.	Allow 10 minutes for the camera temperature to cool. Make sure the chiller is working properly.
		Contact Technical Support.

Start Up Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
Chamber Temp not at correct temperature.	Heated stage is not warming to temperature.	Allow 15 minutes for the chamber to warm to the temperature.
		Contact Technical Support.
	Temperature control is not ON.	Turn on heated stage. Click the Instrument menu and select Manual Operation and then select Temperature Control ON.
Pipettor head is in the plate after the instrument initializes.	Instrument did not initialize properly.	Click the Instrument menu and select Reset.
		Contact Technical Support.

General Troubleshooting

General System Error Messages

Symptom or Error Message	Possible Causes	Solutions
201: Upper door open	Upper door (outer) is not closed.	Close outer (upper) door.
202: Both upper and lower doors open	Both upper (outer) and lower doors are not closed.	Close outer (upper) and lower doors.
203: Lower door open	Lower door (outer) is not closed.	Close lower door.
204: Read plate position empty	No plate is present in the Read position.	Place a plate in the Read position.
205: Source 1 plate position empty	No plate is present in the Source 1 position.	Place a plate in the Source 1 position.
206: Source 2 plate position empty	No plate is present in the Source 2 position.	Place a plate in the Source 2 position.
207: Source 3 plate position empty	No plate is present in the Source position.	Place a plate in the Source 3.
229: Attempting to read a source only plate. Please assign a read plate to the read position, or if it is a read plate, go to TOOLS, PLATE LIBRARY and define the mask positions using the appropriate yellow plate.	The plate selected for the read position cannot be read because no mask has been identified.	Use the Plate Library to convert the plate from a source plate to a read plate.
100: Command not found	Input command is not valid. See appended string on the error message for details.	Contact Technical Support.
101: Invalid argument	Input argument is not valid. See appended string on the error message for details.	Contact Technical Support.

General System Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
102: Invalid number of arguments	Number of input arguments is not valid. See appended string on the error message for details.	Contact Technical Support.
103: Invalid start of command	Start of command is not valid. See appended string on the error message for details.	Contact Technical Support.
104: Command invalid, system busy	The system cannot act upon the command, as the system is busy. See appended string on the error message for details.	Contact Technical Support.
Data buffer full	The data buffer is full. See appended string on the error message for details.	Contact Technical Support.
106: Option not installed, command unavailable	Pipettor head, LED, Emission Filter or FLIPR Cycler is not installed.	Install the option to use.
		Change the protocol to use the available instrument options.
107: Command failed	General failure.	Contact Technical Support.
108: Command failed, system in an error state	Minor communication error occurred.	Click the Instrument menu and select Clear Error.
109: Command failed, system not initialized	Communication failed between software and instrument.	Click the Instrument menu and select Reset.
		Turn the instrument power switch off then on to cycle power.
Password does not match	User password is incorrect.	Contact your instrument administrator for the username and password.
Input not accepted	Input value is not valid (e.g., select greater than 16 time sequence files in the Export menu). See appended string on the error message for details.	Change the input value so it is within the specified range.
302: Temperature system not functional. Please contact Technical Support. You will not be able to run protocols with temperature control until it is repaired.	Can't read the temperature sensors for the heated stage.	Click the Instrument menu and select Reset to see if communication can be reconnected.
		Contact Technical Support.

General System Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
Data files cannot be found		Use Windows' Find utility to search for file names and determine where files were saved. Default location for ScreenWorks files is C:\Program Files\Molecular Devices\ScreenWorks\ Data
Hard drive fills up with data files	Files unnecessarily saved with *.tif file.	Discard unwanted *.tif files.
	Data not archived.	Create free space on the hard drive.
Image option in Experiment window is grayed out.	Save Images not selected in Read with TF process.	Save subsequent data with Save Images selected in protocol.
	*.tif files not found (deleted or moved) in data folder.	You must save *.tif files in same folder as the data to display the Image button.
309: Unable to modify or delete selected plate. It does not exist.	Plate does not exist.	Contact Technical Support.
401: Input/Output PCB not functional. Please contact Technical Support. You will not be able to run protocols until it is repaired.	Input/Output PCB not functional.	Contact Technical Support.
402: Motion reports a fatal error. Please select Reset. If this error repeats, please contact Technical Support.	Motion reports a fatal error.	Contact Technical Support.
403: Motion timeout waiting for done. Please select Reset. If this error repeats, please contact Technical Support.	Instrument motion timed out prior to receiving "done" command from instrument.	Click the Instrument menu and select Reset.
		Contact Technical Support.
404: Motion timeout waiting for echo. Please select Reset. If this error repeats, please contact Technical Support.	Instrument motion timed out while waiting for an echo.	Click the Instrument menu and select Reset.
		Contact Technical Support.
405: Motion communication fault. Please select Reset. If this error repeats, please contact Technical Support.	Motion communication fault.	Click the Instrument menu and select Reset.
		Contact Technical Support.
406: Invalid DONE response from motion. Please select Reset. If this error repeats, please contact Technical Support.	Invalid DONE response from motion.	Turn the instrument off. Wait 15 seconds. Turn instrument on.
		Contact Technical Support.
407: LCB read/write test failed. Please select Reset. If this error repeats, please contact Technical Support.	LCB read/write test failed.	Click the Instrument menu and select Reset.
		Contact Technical Support.

General System Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
408: Plate format file fault. Please contact Technical Support. You will be unable to use this plate until repaired.	Plate format file fault.	Click the Instrument menu and select Reset.
		Contact Technical Support.
ScreenWorks indicates the user-defined name of the data file is invalid.	Illegal characters were used in the file name.	Remove illegal characters from the file name (for example, " , ' : ? ;) .
	User defined name includes too many characters.	Enter 25 characters or less in the file name.
Saturation detected	One or more wells in the microplate saturated the camera.	A saturation warning in either the signal test dialog or on a plate prevent you from reading the plate. You decide whether to address the saturation warning or proceed forward. If multiple wells within a plate are saturating the camera, decrease the Excitation Intensity, Exposure Time, or Camera Gain to prevent saturation.

Troubleshooting Pipettors

Pipettor Error Messages

Symptom or Error Message	Possible Causes	Solutions
Cannot uninstall pipettor head	The D-Axis Knurled Nut is caught on the D-Axis receiver.	Grasp the D-axis knurled nut receiver and press downward to give the pipettor head additional clearance from the D-axis knurled nut.
	Pipettor head is caught on the alignment pins.	Lift the pipettor head up prior to moving left to lift the pipettor head off of the pipettor mount alignment pins.
Cannot install pipettor head	The Head Clamp Knob is not tightening all the way down.	Slide the pipettor head all the way to the right over the pipettor mount. When installed properly on the alignment pins, the pipettor head should not slide left without lifting.
	D-Axis Knurled Nut does not reach receiver threads.	Lift D-axis receiver to engage threads and then tighten the D-axis knurled nut.
231: Pipettor head not detected. Please verify proper installation and select Reset.	No pipettor head is present on pipettor mount.	Install the correct pipettor onto instrument.
	Pipettor head is not installed properly.	Head clamp knob may not be secured tightly for instrument to identify pipettor head. Reinstall pipettor head.
	The open protocol may not use the pipettor on the instrument.	Install the correct pipettor onto instrument.
		Change plate format you select in the software to match the pipettor head.
208: Unable to load tips. Tips are already on pipettor.	Tips are already present on pipettor.	To change tips, click the Instrument menu and select Manual Operation and then select Unload Tips. Exchange the tips. Then click the Instrument menu and select Manual Operation and then select Load Tips.
		Clear the error and proceed with the experiment if want to keep the present tips on.
209: Unable to unload tips. There are no tips loaded on the pipettor.	No tips are present on the pipettor head.	Clear the error.

Pipettor Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
233: Unable to dispense. No tips loaded on pipettor.	No tips are present on the pipettor head.	Click the Instrument menu and select Manual Operation and then select Load Tips.
		Change the protocol to include loading of tips at the beginning of the experiment.
234: Unable to aspirate. No tips loaded on pipettor.	No tips are present on the pipettor head.	Click the Instrument menu and select Manual Operation and then select Load Tips.
		Change the protocol to include loading of tips at the beginning of the experiment
235: Unable to place tips in well. No tips loaded on pipettor.	No tips are present on the pipettor head.	Click the Instrument menu and select Manual Operation and then select Load Tips.
		Change the protocol to include loading of tips at the beginning of the experiment
236: Unable to mix. No tips loaded on pipettor.	No tips are present on the pipettor head.	Click the Instrument menu and select Manual Operation and then select Load Tips.
		Change the protocol to include loading of tips at the beginning of the experiment
237: Unable to wash. No tips loaded on pipettor.	No tips are present on the pipettor head.	Click the Instrument menu and select Manual Operation and then select Load Tips.
		Change the protocol to include loading of tips at the beginning of the experiment
246: Unable to change head. Please unload tips and select Change Head again.	“Change Head” action cannot be performed unless tips are removed.	Click the Instrument menu and select Manual Operation and then select Unload Tips.
Some tips are left in the tip rack after loading the tips.	Pipettor calibration failure.	Contact Technical Support.
Some tips are left on the pipettor after unloading.	Pipettor calibration failure.	Contact Technical Support.

Pipettor Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
The tip rack is attached to the tips.	Tip box failure.	Remove tip box from tips. Click the Instrument menu and select Manual Operation and then select Unload Tips.
		Contact Technical Support.
242: The selected read plate has fewer wells than the pipettor head. Please select a plate with a matching format.	The pipettor head and read plate format is incompatible (e.g., 1536 pipettor head with 96-well plate).	Load the correct pipettor head type.
		Change the protocol to include a read plate format that is compatible with pipettor head.
243: The selected source 1 plate has fewer wells than the pipettor head. Please select a plate with a matching format.	The pipettor head and Source 1 plate format is incompatible (e.g., 1536 pipettor head with 96-well plate).	Load the correct pipettor head type.
		Change protocol to include Source 1 plate format that is compatible with pipettor head.
244: The selected source 2 plate has fewer wells than the pipettor head. Please select a plate with a matching format.	The pipettor head and Source 2 plate format is incompatible (e.g., 1536 pipettor head with 96-well plate).	Load the appropriate pipettor head type.
		Change protocol to include Source 2 plate format that is compatible with pipettor head.
245: The selected source 3 plate has fewer wells than the pipettor head. Please select a plate with a matching format.	The pipettor head and Source 3 plate format is incompatible (e.g., 1536 pipettor head with 96-well plate).	Load the correct pipettor head type.
		Change the protocol to include Source 3 plate format that is compatible with pipettor head.
Volume is too large	Volume is too large for the pipettor head or plate format you are using. See appended string on the error message for details.	Reduce the aspirate/dispense volume to within the specified range.
Volume is too small	Volume is too small for the pipettor head or plate format you are using. See appended string on the error message for details.	Increase the aspirate/dispense volume to within the specified range.
Speed is too large	Speed is too large for the pipettor head or plate format you are using. See appended string on the error message for details.	Reduce the aspirate/dispense speed to within the specified range.
Speed is too small	Speed is too small for the pipettor head or plate format you are using. See appended string on the error message for details.	Increase the aspirate/dispense speed to within the specified range.

Pipettor Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
Height is too large	Height is too large for the pipettor head or plate format you are using. See appended string on the error message for details.	Reduce the aspirate/dispense height to within the specified range.
Height is too small	Height is too small for the pipettor head or plate format you are using. See appended string on the error message for details.	Increase the aspirate/dispense height to within the specified range.
Done with plate after aspiration is not present.	FLIPR Cycler is not present on instrument.	Must install FLIPR Cycler on instrument for command to be available.
Done with plate after dispense is not present.	FLIPR Cycler is not present on instrument.	Must install FLIPR Cycler on instrument for command to be available.
	Dialog does not appear because you are dispensing to the Read plate position.	Read plate automatically changes at the end of an experiment when in Remote mode.
Mix Fluid After Dispense is not present.	Command is not available when Single Aspirate – Multiple Dispense fluid transfer type is selected.	Select either Single Aspirate – Single Dispense or Multiple Aspirate – Single Dispense fluid transfer types.

Troubleshooting Optics

Optics System Error Messages

Symptom or Error Message	Possible Causes	Solutions
240: Attempting to use a LED module that is not installed. Please install the correct LED banks.	Specified LED module in protocol is not installed.	Install the correct LED banks.
		Select the correct LED module that is installed on the instrument.
241: Attempting to use an emission filter that is not installed. Please install the correct emission filter.	Specified emission filter in the protocol is not installed.	Install the correct emission filter.
		Select the correct emission filter that is installed on the instrument.
210: Unable to read plate. Calibration not valid for emission/excitation pair.	A calibration file is not available for the LED/emission filter combination selected.	Calibrate instrument for LED/emission filter pair.
214: The 2 upper LED banks are not identical wavelengths. Please install identical wavelengths to both upper positions.	The upper pair of LED banks has mismatched LED module wavelengths.	Make sure LEDs of the same wavelength are located in the upper LED bank position on either side of the read plate.
215: The 2 lower LED banks are not identical wavelengths. Please install identical wavelengths to both lower positions.	The lower pair of LED banks has mismatched LED module wavelengths.	Make sure LEDs of the same wavelength are located in the lower LED bank position on either side of the read plate.
217: One or more LED banks not detected. Please verify all four banks firmly installed.	Cannot detect one of the LED modules.	Make sure LED module is properly connected.
		Contact Technical Support.
216: LED module configuration/position has changes. Please recalibrate.	LED module position changed from where it was flat-field calibrated.	Calibrate instrument for current LED configuration.
		Place LEDs in previous calibrated position.
303: LED modules not calibrated	None of the installed LED banks have been flat-field calibrated.	Calibrate instrument with installed LED banks.
	A LED inside the LED bank burned out.	Calibrate instrument with installed LED banks.
		Replace LED bank if you cannot recalibrate the LED bank.
		Contact Technical Support.
306: Flat field calibration failed. Please verify that the correct plate is loaded. Please repeat flat field calibration.	Flat Field calibration failed; see appended string on the error message for details.	Calibrate using the correct flat field calibration plate in the Read position.
		Contact Technical Support.

Optics System Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
218: Unable to define plate mask. Please install 515–575 nm emission filter.	The 515–575 nm emission filter is not installed. Thus, mask alignment cannot be performed.	Install the 515–575 nm emission filter to match the calibration file.
		Calibrate instrument with new emission filter.
219: Unable to define plate mask. Please install 470–495 nm LED banks.	The 470–495 LED banks are not installed. Thus, mask alignment cannot be performed.	Install the 470–495 nm LED banks to match the calibration file.
		Calibrate the instrument with the new LED bank.
307: Unable to complete plate mask definition. Incorrect number of wells found. Please verify that the correct plate is loaded and repeat.	Mask alignment failed because it didn't find the correct number of wells (e.g., must find 384 wells for a 384 well plate).	Correct read plate format must be in Read position.
308: Unable to complete plate mask definition. Found overlapping wells. Please contact Technical Support. You will not be able to run protocols using this plate until the instrument is repaired.	Plate is tilted/popped out of position.	Make sure the read plate is properly aligned in Read position.
310: Unable to perform gain curve. Please insert 515–575 nm emission filter.	515–575 nm emission filter is not installed on system.	Install the 515–575 nm emission filter prior to performing gain curve.
		Contact Technical Support.
311: Unable to perform gain curve. Please insert 470–495 nm LED banks.	470–495 nm LED banks are not installed on system.	Install the 470–495 nm LED banks prior to performing gain curve.
		Contact Technical Support.
304: Gain calibration failed. Please contact Technical Support.		Contact Technical Support.
Excitation/Emission wavelength is not available in the edit read mode dialog.	Instrument was not calibrated for the excitation/emission pair for which you are looking.	Calibrate instrument for the correct excitation/emission pair.
Exposure time is too large	The exposure time for the camera is too large. See appended string on the error message for details.	Decrease the exposure time to within the specified range.
Exposure time is too small	The exposure time for the camera is too small. See appended string on the error message for details.	Increase the exposure time to within the specified range.
200: Read interval too short	Read interval is shorter than the sum of exposure lengths for each read interval in addition to the camera processing time.	Set a longer read interval.

Optics System Error Messages (continued)

Symptom or Error Message	Possible Causes	Solutions
First interval is too small	The time for the first interval is too short. Interval time must always be longer than the sum of all selected read modes plus the computer processing time. See appended string on the error message for details.	Increase the read interval to within the specified range.
First interval is too large	The time for the first interval is too large. See appended string on the error message for details.	Decrease the read interval to within the specified range.
First interval number of reads is too large	The number of reads in the first interval is too large. See appended string on the error message for details.	Decrease the number of reads to within the specified range.
Number of reads before dispense are too large	Number of reads prior to dispense are larger than the number of reads in the first interval. See appended string on the error message for details.	Decrease the number of reads before the dispense to within the specified range.
400: Camera is not functional. Please contact Technical Support. You will not be able to run protocols until it is repaired.	Camera is not functional.	Contact Technical Support.

Troubleshooting Yellow Plates

Yellow Plate Testing Error Messages

Symptom or Error Message	Possible Causes	Solutions
Saturation detected, data may be invalid	Incorrect settings.	Set excitation intensity to 80%, exposure length to 0.1 s and for EMCCD gain to 80. If condition repeats, lower excitation intensity.
	Incorrect LED and/or emission filter choice.	Select the correct LED and emission filters.
	Emission optics are dirty.	Make sure no dust is on emission filters.
	Bottom of Read plate is dirty.	Make sure no dust or fluorescent compounds are on the bottom of the Read plate.
Wells are cut off in Image Viewer	Plate not properly placed in Read position	Make sure plate is properly placed and indexed in the Read position.
	Dirty/misaligned optics.	Contact Technical Support.
Fluorescence counts are 0	LED power is off.	Increase excitation intensity of LEDs.
		Make sure excitation/emission wavelength in the read mode does not have NONE for the excitation wavelength.
	Camera failure.	Contact Technical Support.
	Signal test was not initiated.	Select Test Signal in the Signal Test dialog.
Standard deviation for the signal test is greater than 5% and fluorescence counts may or may not be in the correct range.	Instrument was calibrated with yellow boat.	If you use the yellow boat to calibrate the instrument, the standard deviation can be 5% or less.
	Camera not fully cooled down.	Wait approximately 10 minutes for the camera temperature to cool down.
	Optical correction out of specifications	Perform flat field calibration.
	Plate is not aligned properly in Read position.	Make sure plate is properly aligned and indexed in the Read position.
	Optics is dirty or failing.	Contact Technical Support.
	Calibration plate failure— plate is dirty or scratched.	Clean the calibration plate with lens paper. DO NOT use lab wipes. If cleaning does not work, replace the calibration plate.

Troubleshooting Tip Washers

Tip Washer System Error Messages

Symptom or Error Message	Possible Causes	Solutions
221: Tip wash reservoir format (96/384/1536) does not match pipettor head. Please install matching reservoir and select Reset.	Pipettor head and tip washer reservoir top do not match (e.g., a 384 Pipettor head with a 96-well reservoir top).	Install the correct pipettor head to match tip wash reservoir top.
		Install the correct tip wash reservoir top to match pipettor head.
222: Tip wash reservoir top not detected. Please install matching reservoir and select Reset.	No reservoir top is installed.	Install the correct tip wash reservoir top.
	Tip wash reservoir top is not properly installed.	Tip wash reservoir top connectors may not be properly connected.
223: Check Waste Bottle A - sensor indicates full.	Waste bottle A is full of tip wash solvent.	Empty tip wash solvent from waste bottle A.
224: Check Waste Bottle B - sensor indicates full.	Waste bottle B is full of tip wash solvent.	Empty tip wash solvent from waste bottle B.
225: Check Tip Washer - sensor indicates overflow.	Waste bottles are full.	Empty tip wash solvent from waste bottles A and B and select Reset.
	Fluid level sensor malfunction.	Contact Technical Support.
226: Tip Washer: Check Wash Fluid Bottle A - fill sensor dry.	Fill Bottle A is empty.	Place tip wash solvent into fill bottle A.
227: Tip Washer: Check Wash Fluid Bottle B - fill sensor dry.	Fill Bottle B is empty.	Place tip wash solvent into fill bottle B.
250: Tip Washer: Check Wash fluid. Fill sensor dry during fill after prime.	The selected wash bottle might be running low on fluid.	Check the wash bottles.
251: Tip Washer: Check Wash fluid. Fill sensor dry after fill.	The selected wash bottle might be running low on fluid.	Check the wash bottles.
252: Tip Washer: Check Wash Fluid Bottles A and B - sensor indicates empty.	Both of the wash bottles are detected empty.	Add fluid to both wash bottles.
254: Tip Washer: Check tubing at Fill Pump - fill sensor wet after draining line.	Fluid sensor is detecting fluid when it should not.	Check for unconnected tubing. Contact Technical Support.
301: Tip wash module not functional. Please contact Technical Support. You will not be able to run protocols with tip washing until it is repaired.	Cannot communicate with the Tip Washer.	Click the Instrument menu and select Reset.

Troubleshooting Data

Data Processing Error Messages

Symptom or Error Message	Possible Causes	Solutions
Negative control wells show a response.	Wash buffer components are different from the compound buffer.	Make sure wash buffer and compound buffer are the same. Check for a DMSO stimulation, if present.
Statistical results do not conform to the expected results.	Data analysis has not been optimally set up.	See Data Processing Algorithms on page 197.
Data parameters need to be reset (subtract bias, spatial uniformity correction, positive control, etc.) for each experiment.	*.vamp file parameters not set up correctly.	Click Grouping or Correction to adjust the parameters.
False negative wells.	Tip problem.	Contact Technical Support.

Troubleshooting Robotic Integration

Robotic Integration System Error Messages

Symptom or Error Message	Possible Causes	Solutions
220: Unable to load plate. No plate on landing pad.	Plate present on plate landing pad, but plate presence was not detected.	Contact Technical Support.
		If plate not positioned properly by robot, contact robot manufacturer Technical Support.
	No plate was delivered to landing pad by 3rd party robot.	Contact robot manufacturer Technical Support.
221: Unable to load plate. Plate already detected at requested location.	Plate already at requested position.	Unload plate currently in the position prior to delivering new plate.
		If robot attempts to load multiple plates to one position without retrieving plates, contact robot manufacturer Technical Support.
222: Unable to unload plate. Plate already detected at landing pad.	Plate already present on landing pad.	Remove plate on landing pad prior to unloading a new plate.
223: Unable to unload plate. No plate detected at requested position.	No plate present in position.	Clear error and proceed with experiment.
	Plate may have popped out of position.	Clear plate that is out of position prior to resuming experiment.
Bar code reader misreads the bar code and file name reads Bad_bar_code.	Misplacement of bar code or incorrect type of bar code.	Bar code must be on the side of the plate where the bar code is read. Place label as low as possible. Use labels with numbers on top.

Obtaining Support

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

Our Support website—support.moleculardevices.com/—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Please have your instrument serial number or Work Order number, and your software version number available when you call.

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

Appendix A: Technical Specifications

The following table lists the technical specifications for the FLIPR Penta High-Throughput Cellular Screening System.

Technical Specifications

Item	Description
Instrument Dimensions	
Size	Centimeters - 99 (W) x 178.44 (H) x 69 (D) Inches - 39 (W) x 70.25 (H) x 27 (D)
Weight (approx.)	386 kg (850 lbs.)
Throughput	
Temperature control	Ambient, +25°C to 40°C
Throughput	20 plates/hour (2-minute read time)
96-well	1,920 data points/hour
384-well	7,680 data points/hour
1536-well	30,720 data points/hour
Bar code manual mode 1	Keyboard entry
Bar code manual mode 2	Short or long side
EMCCD Camera Option	
Camera type	EMCCD
Detection	Fluorescence
Mode	Cooled CCD camera
Fluorescence sensitivity with Z' ≥ 0.5 (whole plate)	1.25 nM Fluorescein
Data acquisition rate	7 Hz
Ratiometrics	Up to 4 EX/EM pairs per experiment
Data acquisition rate for ratiometric emission	2 Hz (2EX/EM pairs)
HS EMCCD Camera Option	
Camera type	HS EMCCD
Detection	Luminescence and Fluorescence
Mode	High Speed, High Sensitivity CCD Camera
Fluorescence sensitivity with Z' ≥ 0.5 (whole plate)	0.625 nM Fluorescein
Luminescence sensitivity	4.1 nM ATP (Promega Cell Titer Glow Assay)
Data acquisition rate	100 Hz

Technical Specifications (continued)

Item	Description	
Ratiometrics	Up to 4 EX/EM pairs per experiment	
Data acquisition rate for ratiometric emission	2 Hz (2EX/EM pairs)	
Excitation LEDs and Emission Filters		
Wavelength range	335-626 nm	400-750 nm
Calcium (default)	470-495 nm	515-575 nm
Membrane potential	510-545 nm	565-625 nm
Voltage sensor probes	390-420 nm	440-480 nm 565-625 nm
Fura-2	335-345 nm 380-390 nm	475-535 nm
Instrument holds 2 pairs of LEDS, one for each side to cross-illuminate the bottom of the plate		
Instrument has a 3 position filter slider that identifies the filter to the instrument		
Custom filter holders are available		
Fluidics		
Simultaneous transfer	96, 384 and 1536	
Range-96 pipettor head	5-200 µL	
Precision @75 µL	3% CV	
Pipetting increments-96 pipettor head	1 µL	
Range - 384-Well pipettor	1-25 µL	
Precision @ 25 µL	4% CV	
Pipetting increments - 384-Well pipettor	0.5 µL	
Range - 1536-Well pipettor	0.5-3 µL	
Precision @ 3 µL	6% CV	
Precision @ 1 µL	10% CV	
Pipetting increments - 1536-Well pipettor	0.1 µL	
96 pipettor head - Quadrant transfer	384-well plate	
384 pipettor head - Quadrant transfer	1536-well plate	

Appendix B: Consumables and Accessories

B

System Accessories

Optional Software Modules

Supplier: Molecular Devices, call +1-800-635-5577.

Optional Software Modules

Item	Part Number
ScreenWorks® Peak Pro™ 1.0 Software (For ScreenWorks Software Versions 3.2 and 4)	5016900
ScreenWorks® Peak Pro™ 1.0 Software (For ScreenWorks Software Version 5)	5075630
ScreenWorks® Peak Pro™ 2.0 Software (For ScreenWorks Software Version 5)	5074843

Field Installations

Supplier: Molecular Devices, call +1-800-635-5577.

Field Installations Kits

Item	Part Number
FLIPR® Camera Conversion Kit or EMCCD to HS EMCCD	5069580
Windows 10 Host PC with ScreenWorks® Software Version 5 *for select** EMCCD Systems	5075062
Windows 10 Host PC with ScreenWorks® Software Version 4.2 *for select** EMCCD Systems	5074979
FLIPR® Cycler	5074848
* Contact Molecular Devices to identify applicable kit	

Pipetting Accessories

Supplier: Molecular Devices, call +1-800-635-5577.

Pipetting Accessories

Item	Part Number
FLIPR® 1536 Tip Block	0200-6112
[1]FLIPR® Pin Tool, 384	5075203
[1]FLIPR® Pin Tool, 1536	5075194
[1]FLIPR® Pin Tool Kit, 384 and/or 1536	FLIPR PIN TOOLS OC
1. Contact your Molecular Devices Sales Representative for details regarding this configure-to- order item.	

Pipetting Consumables

Supplier: Molecular Devices, call +1-800-635-5577.

Pipetting Consumables

Item	Part Number
FLIPR® pipette tips, black, non-sterile, 96-well, 50 racks/case	9000-0762
FLIPR® pipette tips, clear, non-sterile, 96-well, 50 racks/case	9000-0761
FLIPR® non-sterile, black, 384-well, 50 racks/case	9000-0764
FLIPR® pipette tips, clear, non-sterile, 384-well, 50 racks/case	9000-0763
FLIPR® 1536 tip gasket, nonsterile, 40 racks/case	9000-0746

Pipettor Heads

Supplier: Molecular Devices, call +1-800-635-5577.

Pipettor Head Kits

Item	Part Number
FLIPR® Pipettor Head Kit, 96	0200-6071
FLIPR® Pipettor Head Kit, 384	0200-6072
FLIPR® Pipettor Head Kit, 1536	0200-6073

Optics Consumables

Supplier: Molecular Devices, call +1-800-635-5577.

Optics Kits

Item	Part Number
FLIPR® Calcium Optics Kit	0200-6206
FLIPR® Membrane Potential Optics Kit	0200-6207
FLIPR® Voltage Sensor Probes (VSP) Optics Kit	0200-6208
FLIPR® Fura-2 Optics Kit	5302739
FLIPR® LED Module, 335–345 nm (UV)	5201196
FLIPR® LED Module, 360–380 nm	0200-6178
FLIPR® LED Module, 380–390 nm (UV)	0200-6273
FLIPR® LED Module, 390–420 nm (VSP)	0200-6135
FLIPR® LED Module, 420–455 nm	0200-6148
FLIPR® LED Module, 470–495 nm (Calcium)	0200-6128
FLIPR® LED Module, 495–505 nm	0200-6175
FLIPR® LED Module, 510–545 nm (Membrane Potential)	0200-6127
FLIPR® LED Module, 610–626 nm	0200-6150
FLIPR® Emission Filter, 400–460 nm	0200-6213
FLIPR® Emission Filter, 440–480 nm (VSP)	0200-6205
FLIPR® Emission Filter, 475–535 nm	0200-6211
FLIPR® Emission Filter, 515–575 nm (Calcium)	0200-6203
FLIPR® Emission Filter, 526–586 nm	0200-6212
FLIPR® Emission Filter, 565–625 nm (Membrane Potential and VSP)	0200-6204
FLIPR® Emission Filter, 646–706 nm	0200-6214
FLIPR® Custom Filter Set (3)	0200-6221
FLIPR® Single Custom Filter Holder (1)	0200-6276

Plates

The following conforming plates can be purchased from Molecular Devices.

96-Well Read Plates

96-Well Read Plates

96-Well Read Plates	Part Number	Suggested Supplier	Phone Number
Black, clear, tissue culture treated, sterile	353948	Becton Dickinson	+1-800-343-2035
	3603	Corning/Costar	+1-800-492-1110
	655090	Greiner (distributed by E&K)	+1-408-378-2013
	165305	Nalge/Nunc	+1-800-766-7000
Black, clear, tissue culture treated, sterile, poly-D-lysine coated	356640	Becton Dickinson	+1-800-343-2035
	3667	Corning/Costar	+1-800-492-1110
Black, clear, tissue culture treated, sterile, collagen coated	356649	Becton Dickinson	+1-800-492-1110
White, clear, tissue culture treated, sterile	353947	Becton Dickinson	+1-800-343-2035
	3903	Corning/Costar	+1-800-492-1110
	655098	Greiner (distributed by E&K)	+1-408-378-2013
	165306	Nalge/Nunc	+1-800-766-7000

96-Well Read Plate Masks Made by Molecular Devices

96-Well Read Plate Masks Made by Molecular Devices

96-Well Read Plate Masks	Part Number	Plate Manufacturer
96-well slit mask	0200-6143	Becton Dickinson
	2300-1362	Corning/Costar
	0200-6142	Nalge/Nunc
	0200-6144	Greiner

96-Well Source Plates

96-Well Source Plates

96-Well Source Plates	Part Number	Suggested Supplier	Phone Number
V-bottom plate, 96-well	353263 polypropylene	Becton Dickinson	+1-800-343-2035
	651201 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013
	249944	Nalge/Nunc	+1-800-766-7000
U-bottomed plate, 96-well	351190 polypropylene	Becton Dickinson	+1-800-343-2035
	3365 polypropylene	Corning/Costar	+1-800-492-1110
	267245	Nalge/Nunc	+1-800-766-7000
	650201 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013
Deep-well plate, 96-well	353966 polypropylene	Becton Dickinson	+1-800-343-2035
	780270 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013
	278752 polypropylene	Nalge/Nunc	+1-800-766-7000

384-Well Read Plates

384-Well Read Plates

384-Well Read Plates	Part Number	Suggested Supplier	Phone Number
Black, clear, tissue culture treated, sterile	353262	Becton Dickinson	+1-800-343-2035
	3712	Corning/Costar	+1-800-492-1110
	781091	Greiner with lids (distributed by E&K)	+1-408-378-2013
	MGB101-1-2	Matrical	+1-509-343-6225
	MGB101-1-3 0.17 mm-thick glass	Matrical	+1-509-343-6225
	142761	Nalge/Nunc	+1-800-766-7000
Black, clear bottom, tissue culture treated, sterile, poly-D-lysine coated	356663	Becton Dickinson	+1-800-343-2035
	3664	Corning/Costar	+1-800-492-1110
Black, clear, tissue culture treated, sterile, collagen coated	356667	Becton Dickinson	+1-800-343-2035

384-Well Read Plates (continued)

384-Well Read Plates	Part Number	Suggested Supplier	Phone Number
White, clear, tissue culture treated, sterile	353963	Becton Dickinson	+1-800-343-2035
	3707	Corning/Costar	+1-800-492-1110
	781098	Greiner (distributed by E&K)	+1-408-378-2013
	142762	Nalge/Nunc	+1-800-766-7000
Low volume black, clear, tissue culture treated, sterile	3542	Corning/Costar	+1-800-492-1110
	788092	Greiner (distributed by E&K)	+1-408-378-2013

384-Well Source Plates

384-Well Source Plates

384-Well Source Plates	Part Number	Suggested Supplier	Phone Number
Flat bottom plate	353265 polypropylene	Becton Dickinson	+1-800-343-2035
	3702 polystyrene	Corning/Costar	+1-800-492-1110
	781201 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013
	265496 polystyrene	Nalge/Nunc	+1-800-766-7000
U/V-bottom plate	3657 polypropylene	Corning/Costar	+1-800-492-1110
	781280 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013
	264573 polypropylene	Nalge/Nunc	+1-800-766-7000
Deep-well plate	353996 polypropylene	Becton Dickinson	+1-800-343-2035
	3965 polypropylene	Corning/Costar	+1-800-492-1110
	781270 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013
	MP102 polypropylene	Matrical	+1-509-343-6225

1536-Well Read Plates

1536-Well Read Plates

1536-Well Read Plates	Part Number	Suggested Supplier	Phone Number
Black/Clear, tissue culture treated, sterile	783092	Greiner with lids (distributed by E&K)	+1-408-378-2013
White/Clear, tissue culture treated, sterile	781098	Greiner (distributed by E&K)	+1-408-378-2013

1536-Well Source Plates

1536-Well Source Plates

1536-Well Source Plates	Part Number	Suggested Supplier	Phone Number
Deep-well plate	782270 polypropylene	Greiner (distributed by E&K)	+1-408-378-2013

Source Reservoirs

Source Reservoirs

Source Reservoirs	Part Number	Suggested Supplier	Phone Number
Omni tray	2428110 polystyrene	Nalge/Nunc	+1-800-786-7000

Assays

Calcium Flux Consumables and Accessories

Calcium Flux Consumables and Accessories

Item	Part Number	Suggested Supplier	Phone Number
FLIPR® Calcium 6 Assay Kit: Bulk Kit Explorer Kit Express Kit	 R8190 R8191 R8195	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Calcium 6 QF Assay Kit: Bulk Kit Explorer Kit Express Kit	 R8192 R8193 R8196	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Calcium 5 Assay Kit: Bulk Kit Explorer Kit Express Kit	 R8186 R8185 R8187	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Calcium 4 Assay Kit: Bulk Kit Explorer Kit Express Kit	 R8141 R8142 R8143	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Calcium 3 Assay Kit: Bulk Kit Explorer Kit Express Kit	 R8090 R8091 R8108	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Cardiocytoxicity Assay Kit: Bulk Kit Explorer Kit	 R8211 (10 plates) R8210 (2 plates)	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Fura-2 QBT Calcium Assay Kit: Bulk Kit Explorer Kit	 R8197 R8198	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Penta System Calcium Optics Kit: LED Module, 470-495 nm Emission Filter, 515-575 nm	0200- 6206	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Penta System Fura-2 Optics Kit: LED Module 333-345 nm LED Module 380-390 nm Emission Filter 475-535nm	5302739	Molecular Devices	+1-800-635-5577 +1-408-747-1700
Hank's Balanced Salt Solution (10X stock)	14065- 056	Thermo Fisher	1-800-828-6686

Calcium Flux Consumables and Accessories (continued)

Item	Part Number	Suggested Supplier	Phone Number
HEPES buffer solution 1X	9319	Irvine Scientific	+1-800-437-5706
Probenecid, crystalline	P8761	Sigma	+1-800-325-3010
Carbachol (receptormediated positive control)	C4382		
UTP, Na salt (receptormediated positive control)	U6625		
Ionomycin (positive control)	407950	CalBiochem	+1-800-854-3417

Membrane Potential Assay Kit Consumables and Accessories

Membrane Potential Assay Kit Consumables and Accessories

Item	Part Number	Suggested Supplier	Phone Number
FLIPR® Membrane Potential Assay Kit	R8034 (BLUE) R8123 (RED)	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Penta System Membrane Potential Optics Kit: FLIPR® Penta System LED Module, 510–545 nm FLIPR® Penta System Emission Filter, 565–625 nm	0200-6207	Molecular Devices	+1-800-635-5577 +1-408-747-1700
Hank's Balanced Salt Solution (10X stock)	14065-056	Thermo Fisher	1-800-828-6686
HEPES buffer solution 1X	9319	Irvine Scientific	+1-800-437-5706
Carbachol (receptor-mediated positive control)	C4382	Sigma	+1-800-325-3010

Potassium Assay Kit Consumables and Accessories

Potassium Assay Kit Consumables and Accessories

Item	Part Number	Suggested Supplier	Phone Number
FLIPR® Potassium Assay Kit Evaluation Kit Explorer Kit Bulk Kit	 R8330 R8222 R8223	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® Penta System Calcium Optics Kit: FLIPR® Penta System LED Module, 470-495 nm FLIPR® Penta System Emission Filter, 515-575 nm	0200-6206	Molecular Devices	+1-800-635-5577 +1-408-747-1700
Hank's Balanced Salt Solution (10X stock)	14065-056	Thermo Fisher	1-800-828-6686
HEPES buffer solution 1X	9319	Irvine Scientific	+1-800-437-5706
DMSO	D8418	Sigma	+1-800-325-3010
Probenecid	P8761	Sigma	+1-800-325-3010

Recommended Assay Equipment and Supplies

The following recommended assay equipment and supplies are not available through Molecular Devices:

- 5 mL, 10 mL, 25 mL sterile serological pipettes
- Rechargeable pipettor for 2–25 mL pipettes
- Sterile tissue culture water
- Gloves
- Culture medium to grow cells
- EDTA and Trypsin/EDTA to lift cells
- Hemacytometer and counter
- Sterile test tubes 15 mL and 50 mL or smaller tubes for compounds dilutions
- 1 N NaOH solution to dissolve probenecid

Appendix C: FLIPR Penta Plate Dimensions

Note: Molecular Devices is not responsible for instrument malfunctions if the provided dimensions are incorrect.

The following dimensions derive from specifications distributed by the individual vendors.

Becton Dickinson Plate Dimensions

Plate Type	Part #	Rows	Columns	Type	Well Volume (µL)	A1 Column Offset "Y" (mm)	A1 Row Offset "X" (mm)	Bottom (MM)	Top (MM)	Well Offset (mm)
96-well Black/Clear	353948	8	12	read	349	14.28	11.23	3.50	14.53	9.01
96-well Black/Clear, Collagen-Coated	356649	8	12	read	349	14.28	11.23	3.50	14.53	9.01
96-well Black/Clear, PDL-Coated	356640	8	12	read	349	14.28	11.23	3.50	14.53	9.01
96-well White/Clear	353947	8	12	read	349	14.42	11.19	3.50	14.66	9.00
96-well U bottom, polypropylene	351190	8	12	source	340	14.23	11.33	2.49	14.62	8.99
96-well V bottom, polypropylene	353263	8	12	source	340	14.24	11.35	3.70	14.61	8.99
96-well 2mL Deep Well, polypropylene	353966	8	12	source	2000	13.88	10.62	—	44.24	9.04
384-well Black/Clear	353962	16	24	read	120	12.21	9.05	2.90	14.32	4.49
384-well Black/Clear, Collagen-Coated	356667	16	24	read	120	12.21	9.05	2.90	14.32	4.49
384-well Black/Clear, PDL-coated	356663	16	24	read	120	12.21	9.05	2.90	14.32	4.49

Becton Dickinson Plate Dimensions (continued)

Plate Type	Part #	Rows	Columns	Type	Well Volume (µL)	A1 Column Offset "Y" (mm)	A1 Row Offset "X" (mm)	Bottom (MM)	Top (MM)	Well Offset (mm)
384-well White/Clear	353963	16	24	read	120	12.26	9.06	2.90	14.34	4.49
384-well flat bottom polypropylene	353265	16	24	source	140	12.15	9.27	2.90	14.40	4.50
384-well Deep Well, polypropylene	353996	16	24	source	400	11.83	8.83	—	43.69	4.51
1536-well Black/Clear	353255	32	48	read	12	11.04	7.85	3.80	10.41	2.25
1536-well White/Clear	353258	32	48	read	12	11.03	7.84	3.80	10.42	2.25

Corning/Costar Plate Dimensions

Plate Type	Part #	Rows	Columns	Type	Well Volume (µL)	A1 Column Offset "Y" (mm)	A1 Row Offset "X" (mm)	Bottom (MM)	Top (MM)	Well Offset (mm)
96-well Black/Clear	3603	8	12	read	360	14.30	11.20	3.57	14.22	9.00
96-Well Black/Clear, PDL-Coated	3667	8	12	read	360	14.30	11.20	3.57	14.22	9.00
96-well White/Clear	3903	8	12	read	360	14.30	11.20	3.57	14.22	9.00
96-well U bottom, polypropylene	3365	8	12	source	360	14.30	11.20	2.92	14.22	9.00
384-well Black/Clear	3712	16	24	read	112	12.12	8.99	2.79	14.22	4.50
384-Well Black/Clear, PDL-coated	3664	16	24	read	112	12.12	8.99	2.79	14.22	4.50
384-well White/Clear	3707	16	24	read	112	12.12	8.99	2.79	14.22	4.50
384-well flat bottom polystyrene	3702	16	24	source	112	12.12	8.99	2.79	14.22	4.50
384-well U bottom, polypropylene	3657	16	24	source	95	12.12	8.99	2.65	14.20	4.50
384-well block, polypropylene	3965	16	24	source	180	12.12	8.99	2.69	27.80	4.50

Greiner Plate Dimensions

Plate Type	Part #	Rows	Columns	Type	Well Volume (µL)	A1 Column Offset "Y" (mm)	A1 Row Offset "X" (mm)	Bottom (MM)	Top (MM)	Well Offset (mm)
96-well Black/µClear	655090	8	12	read	392	14.38	11.24	3.50	14.40	9.00
96-well White/µClear	655098	8	12	read	392	14.38	11.24	3.50	14.40	9.00
96-well U bottom, polypropylene	650201	8	12	source	310	14.38	11.24	3.70	14.60	9.00
96-well V bottom, polypropylene	651201	8	12	source	340	14.38	11.24	3.70	14.60	9.00
96-well 2mL Deep Well, polypropylene	780270	8	12	source	2420	14.38	11.24	3.00	44.00	9.00
384-well Black/µClear	781091	16	24	read	138	12.13	8.99	2.90	14.40	4.50
384-well White/µClear	781098	16	24	read	138	12.13	8.99	2.90	14.40	4.50
384-well flat bottom polypropylene	781201	16	24	source	152	12.13	8.99	2.90	14.40	4.50
384-well V bottom, polypropylene	781280	16	24	source	145	12.13	8.99	2.90	14.40	4.50
384-well Deep Well, polypropylene	781270	16	24	source	240	12.13	8.99	2.70	22.00	4.50
1536-well Black/µClear, Low Base	783092	32	48	read	13	11.01	7.87	1.80	10.40	2.25
1536-well Deep Well, polypropylene	782270	32	48	source	18	11.01	7.87	2.50	10.10	2.25

Matrical Plate Dimensions

Plate Type	Part #	Rows	Columns	Type	Well Volume (µL)	A1 Column Offset "Y" (mm)	A1 Row Offset "X" (mm)	Bottom (MM)	Top (MM)	Well Offset (mm)
384-well Black/.17mm-thick glass	MGB101-1-2	16	24	read	120	12.13	8.99	2.85	14.40	4.50
384-well Black/Clear polystyrene	MGB101-1-3	16	24	read	120	12.13	8.99	2.85	14.40	4.50
384-well Deep well polypropylene	MP102	16	24	source	225	12.13	8.99	4.90	27.40	4.40

Nalge/Nunc Plate Dimensions

Plate Type	Part #	Rows	Columns	Type	Well Volume (µL)	A1 Column Offset "Y" (mm)	A1 Row Offset "X" (mm)	Bottom (MM)	Top (MM)	Well Offset (mm)
96-well Black/Clear	165305	8	12	read	400	14.48	11.20	2.30	14.80	9.00
96-well White/Clear	165306	8	12	read	400	14.48	11.20	2.30	14.80	9.00
96-well U bottom	267245	8	12	source	500	14.30	11.30	2.20	14.30	9.00
96-well V bottom	249944	8	12	source	450	14.48	11.20	3.40	14.40	9.00
96-well 2mL Deep Well, polypropylene	278752	8	12	source	2000	14.30	11.30	2.50	44.00	9.00
384-well Black/Clear	142761	16	24	read	120	12.10	9.00	2.70	14.40	4.50
384-well White/Clear	142762	16	24	read	120	12.10	9.00	2.70	14.40	4.50
384-well flat bottom polystyrene	265196	16	24	source	120	12.10	9.00	2.70	14.40	4.50
384-well U bottom	264573	16	24	source	120	12.10	9.00	2.70	14.40	4.50

Appendix D: FLIPR Penta Instrument LED and Filter Combinations

FLIPR Penta Instrument LED and Filter Combinations

Target Assay	LED (nm)	LED Part #	Emission Filter (nm)	Penta Filter Part #	Penta Kit Part #
Fura-2	335-345 and 380-390 (UV)	5201196 0200-6273	475-535	0200-6211	5302739
MQAE	360-380 (UV)	N/A	N/A	N/A	N/A
Voltage Sensor Probe (VSP)	390-420 (UV)	0200-6135	440-480 565-625	0200-6205 0200-6204	0200-6208
Tango™ GPCR Assay System (FRET)	390-420 (UV)	0200-6135	440-480 515-575	0200-6205 0200-6203	N/A
CFP, eCFP/YFP	420-455	0200-6148	475-535	0200-6211	N/A
FLIPR Calcium Assay Kits	470-495 (Blue)	0200-6128	515-575	0200-6203	0200-6206
Calcium, Fluo-4	470-495 (Blue)	0200-6128	515-575	0200-6203	0200-6206
Calcium, Fluo-3	495-505	0200-6175	526-586	0200-6212	N/A
JC-1	495-505	0200-6175	565-625	0200-6204	N/A
FLIPR Membrane Potential Assay Kits	510-545 (Green)	0200-6127	565-625	0200-6204	0200-6207
Rhod-2 & Rhod-4	510-545	0200-6127	565-625	0200-6204	N/A
Alexa 633 & Bodipy	610-626	0200-6150	646-706	0200-6214	N/A
BRET 2 luminescence assay with HSEMCCD Camera	N/A	N/A	440-480 526-586	0200-6205 0200-6212	N/A
Three Custom Filter Holders	N/A	N/A	N/A	0200-6221	N/A

Appendix E: Data Processing Algorithms

This appendix uses results of a hypothetical experiment to describe the algorithms you use to adjust data. Apply these options from the Correction dialog.

Hypothetical Experiment

Consider the results from an experiment that consists of wells A1–A9. The wells are classified into three groups:

- A1–A3: Negative Control Wells (–Ctrl). These wells contain the same dye-loaded cells and buffers as the other wells yet are not exposed to a stimulus.
- A4–A6: Experimental Wells (Exp). These cells receive the experimental stimulus.
- A7–A9: Positive Control Wells. (+Ctrl). These cells either (1) receive a stimulus known to elicit a predetermined response or (2) demonstrate the maximal activity from an agonist.

In the following experiment, six samples, also known as pictures, readings, or frames, were taken at five second intervals. After Sample 3 was taken, buffer, with or without stimulus, was added to the wells.

Note: The longer time between readings 3 and 4 includes the time necessary for the 96-well pipettor to transfer liquids from the source plate to the cell plate and is automatically corrected for by the FLIPR Penta System.

The data presented is in the same format as the export data files you generate from the FLIPR Penta System software. In this example, nine wells are considered.

The results of the experiment are presented without Spatial Uniformity Correction, Negative Control Correction, Positive Control Scaling, or Subtract Bias Value.

Hypothetical Experiment

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	8000	8500	9500	8200	9200	8800	1000	9500	8700
2	5	8200	8600	9400	8300	9000	9000	9500	9700	9000
3	10	8400	8500	9200	8500	9000	9100	9700	9700	9400
4	20	8600	8400	9200	45000	50000	42000	57000	52000	54000
5	25	8600	8800	9200	37000	42000	35000	53000	50000	50000
6	30	8800	8500	9500	30000	25000	29000	50000	51000	50000

An inspection of the fluorescence counts taken at Sample 1 (time = 0) shows some inter-well variability (range 8000–10000). Variability is the result of differences in cell density or well positions (so-called edge effects where cells on the outside of the plate are less dense than their internal neighbors), dye loading, non-uniform illumination, buffer variations, or any other effect which is constant throughout the experiment run. These variations can complicate data interpretation.

Determining Spatial Uniformity Correction

The spatial uniformity correction algorithm compensates for of the variations using a correction factor applied to all plate wells and samples. The correction factor is derived by calculating the mean fluorescence counts of all wells at Sample 1, for example:

Correction Factor

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	8000	8500	9500	8200	9200	8800	10000	9500	8700
Mean (A1-A9)	8933									
Spatial Uniformity Correction Factor Calculation (Mean/Well)										
Well- Specific Correction Factor		A1	A2	A3	A4	A5	A6	A7	A8	A9
		1.12	1.05	0.94	1.09	0.95	1.02	0.89	0.94	1.03

Note: Data from all 96 wells are included in the calculations, but the table displays data from nine wells to simplify the example.

The software calculates each well-specific spatial uniformity correction factor by dividing the mean fluorescence counts of all wells by the fluorescence counts of each well (taken at Sample 1.) The table above presents the correction factor for wells A1–A9.

The software multiplies all samples taken from a particular well by their well-specific correction factor. For example, all samples from A1 are multiplied by 1.12, A2 by 1.05, and so on.

The following table displays the results of applying the spatial uniformity correction factor. Note the decreased variability range of wells A1–A9 in Sample 1 (8900–8976) as compared to the same data prior to applying the correction algorithm (8000–10000).

Spatial Uniformity Correction factor

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	8960	8925	8930	8938	8924	8976	8900	8930	8961
2	5	9194	9030	8836	9047	8730	9180	8455	9118	9270
3	10	9408	8925	8648	9265	8730	9282	8633	9118	9682
4	20	9632	8820	8648	49050	48500	42840	50730	48880	55620
5	25	9632	9240	8648	40330	40740	35700	47170	47000	51500
6	30	9856	8925	8930	32700	33950	29580	44500	47940	51500

If the software applies the spatial uniformity correction factor to plates with empty wells, non dye-loaded cells, or a panel of cells that contain different dyes and/or dye concentrations, the well-specific fluorescence counts are skewed by the correction factor. The EC_{50} of the agonists tested are not affected.

Determining Negative Control Correction

The negative control correction algorithm corrects for changes in fluorescence that occur in all wells over the course of the experiment. Causes for these changes in fluctuations in fluorescence include dye leakage from cells, fluid addition artifacts, changes in illumination power, dye photo-bleaching, and temperature drifts.

The software derives the negative control correction factor by first calculating the mean fluorescence of each of the samples of the negative control wells.

Sample	Time	Well				
		A1 – Ctrl	A2 – Ctrl	3 – Ctrl	Mean	Correction Factor
1	0	8960	8925	8930	8938	1.00
2	5	9194	9030	8836	9017	0.99
3	10	9408	8925	8648	8994	0.99
4	20	9632	8820	8648	9033	0.99
5	25	9632	9240	8648	9173	0.97
6	30	9856	8925	8930	9237	0.997

The software divides the mean of Sample 1 by the mean of each of the samples to give the sample-specific correction factor. The software multiplies each sample by its sample-specific correction factor.

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	8960	8925	8930	8938	8924	8976	8900	8930	8961
2	5	9194	8940	8748	8957	8643	9088	8370	9027	9177
3	10	9314	8836	8562	9172	8643	9189	8547	9027	9585
4	20	9536	8732	8562	48560	48015	42412	50223	48391	55064
5	25	9343	8963	8389	39120	39518	34629	45755	45590	49955
6	30	9560	8657	8662	31719	32932	28693	43165	46502	49955

For example, A1 Sample 1 is multiplied by 1.00.

Note: The negative control correction factor wells contain the same cells and dyes as the experimental and positive control wells.

Determining Positive Control Scaling

The positive control algorithm compares the percent change in fluorescence counts of the positive control wells with all wells. This algorithm facilitates comparisons of results between data runs, such as different plates, by controlling factors such as cell density, cell response, laser power, or exposure time. This algorithm makes EC_{50} comparisons easier.

Calculate the means of all samples of the positive control wells.

Sample	Time	Well			Mean	Difference from Sample 1S
		A7 + Ctrl	A8 + Ctrl	A9 + Ctrl		
1	0	8900	8930	8961	8930	0
2	5	8370	9027	9177	8858	-72
3	10	8547	9027	9053	9053	123
4	20	50223	48391	55064	51226	42296
5	25	45755	45590	49955	47100	38170
6	30	43165	46502	49955	46541	37610

The software calculates the difference in fluorescence counts between Sample 1 and all of the samples. The software determines the greatest difference. 100 is divided by the greatest difference in fluorescence counts to give the positive control correction factor, for example, 100/42296 = 0.0024. The software multiplies all samples by the correction factor 0.0024.

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	21	21	21	21	21	21	21	21	21
2	5	21	21	21	21	20	21	20	21	23
3	10	22	21	20	22	20	22	20	21	23
4	20	23	21	20	115	113	100	119	114	130
5	25	22	21	20	92	93	82	108	108	118
6	30	23	20	20	75	78	68	1021	110	118

Note: All wells, at samples 1–3, range from 20 to 23%. The positive control wells at Sample 4 range from 114 to 130% while the negative control wells remain at around 20% throughout the experiment.

Determining Subtract Bias

The subtract bias algorithm determines the change in fluorescence over background fluorescence. You determine the sample number chosen as background. This algorithm, by default, is always switched on and subtracts from Sample 1. The choice in the sample number to use as background depends on the assay conditions. For example, the addition of agonist can dilute the fluorescence signal in the supernatant, which would appear as a drop in fluorescence. In this case, the best choice for background is the sample taken at the bottom of this drop in fluorescence.

In the example, the percent positive fluorescence at Sample 1 is around 20% for all wells. Subtracting the background at Sample 1 makes the data easier to interpret.

Sample 1 (or any sample chosen by the operator) from each well is subtracted from the samples from the same well.

Sample	Time	A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0									
2	5									1
3	10	1			1					1
4	20	1			94	92	79	98	93	109
5	25	1			71	72	61	87	87	97
6	30	1			54	57	47	81	89	97

The results show numbers above zero. The subtract bias algorithm places the data in a readily interpretable form. The positive control wells at Sample 4 range from 93–109% positive. In contrast, the negative control wells remain close to zero percent positive throughout the experiment. The experimental wells show two wells with about 90% activity and one with 79% activity at Sample 4.

Determining Response Over Baseline

The response over baseline correction algorithm compensates for variations within a single well.

The software derives the correction factor by calculating the mean fluorescence counts in each well between the baseline start and end samples.

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	8000	8500	9500	8200	9200	8800	10000	9500	8700
2	5	8200	8600	9400	8300	9000	9000	9500	9700	9000
3	10	8400	8500	9200	8500	9000	9100	9700	9700	9400
Mean		8200	8533	9367	8333	9067	8967	9733	9633	9033

Note: Data from all 96 wells are included in the calculations, but the table displays data from nine wells to simplify the example.

The software divides all samples taken from a particular well by their well-specific mean baseline correction factor. For example, all samples from A1 are divided by 8200, A2 by 8533, and so on.

The following table displays the results of applying the response over baseline correction factor. Note the data displays as a fold increase of the response compared to the baseline with Sample values that range in the single digits (0–6).

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	0.98	1.00	1.01	0.98	1.01	0.98	1.03	0.99	0.96
2	5	1.00	1.01	1.00	1.00	0.99	1.00	0.98	1.01	1.00
3	10	1.02	1.00	0.98	1.02	0.99	1.00	0.98	1.01	1.00
4	20	1.05	0.98	0.98	5.4	5.51	4.68	5.86	5.40	5.98
5	25	1.05	1.03	0.98	4.44	4.63	3.90	5.45	5.195	5.54
6	30	1.07	1.00	1.01	3.60	2.76	3.23	5.14	5.29	5.54

Appendix F: Robotic Integration

This section describes the interface between the FLIPR Penta System and a robotic instrument. The intention is to provide a general overview of the interface between the instrument and robot and a description of the communication protocol between the ScreenWorks Software, which run on the host computer of the FLIPR Penta System instrument, and the robotic control software.

The following Information might change as new development occurs. Check with your Molecular Devices sales representative for updated information.

Conventions

- Bold text is used for commands
- Italic text is used for parameters
- Parameters in bracket [parameter1] are optional
- Parameters are separated by comma
- <CR> is the ACSII code for ‘carriage return’ which indicates end of the command or end of the response
- <TAB> represents a tab
- <SP> represents a space
- | represents OR

Interface Versioning

As of version 1.1 of the automation interface, the interface is versioned separately from the ScreenWorks Software and the FLIPR Penta System firmware. The interface now provides a command to query the version number.

Instrument Overview

Basic Function

The FLIPR Penta System is a fluorescence microplate reader you use for kinetic live cell-based fluorescence and luminescence assays. A typical assay involves the following steps:

- Introduction of a read plate that contains cells in solution or compounds into the instrument.
- Acquisition of a baseline read (or series of reads) to determine the background fluorescence or luminescence signal from the read plate.
- Introduction of a potentially activating (or potentially inactivating) compound into the well or cells in suspension.
- Monitoring the changes in the fluorescence or luminescence signal from the read plate for a period of time immediately following the introduction of the compound.
- Further additions of different compounds to the well are typical in an assay on a FLIPR Penta System. Between additions, you can replace or wash the pipette tips you use for fluid transfer.

Cell response typically begins within 2 seconds of fluid addition and is monitored for 1–3 minutes. The software monitors the fluorescence or luminescence signals at the wavelengths and frequencies you select within the physical constraints of the instrument.

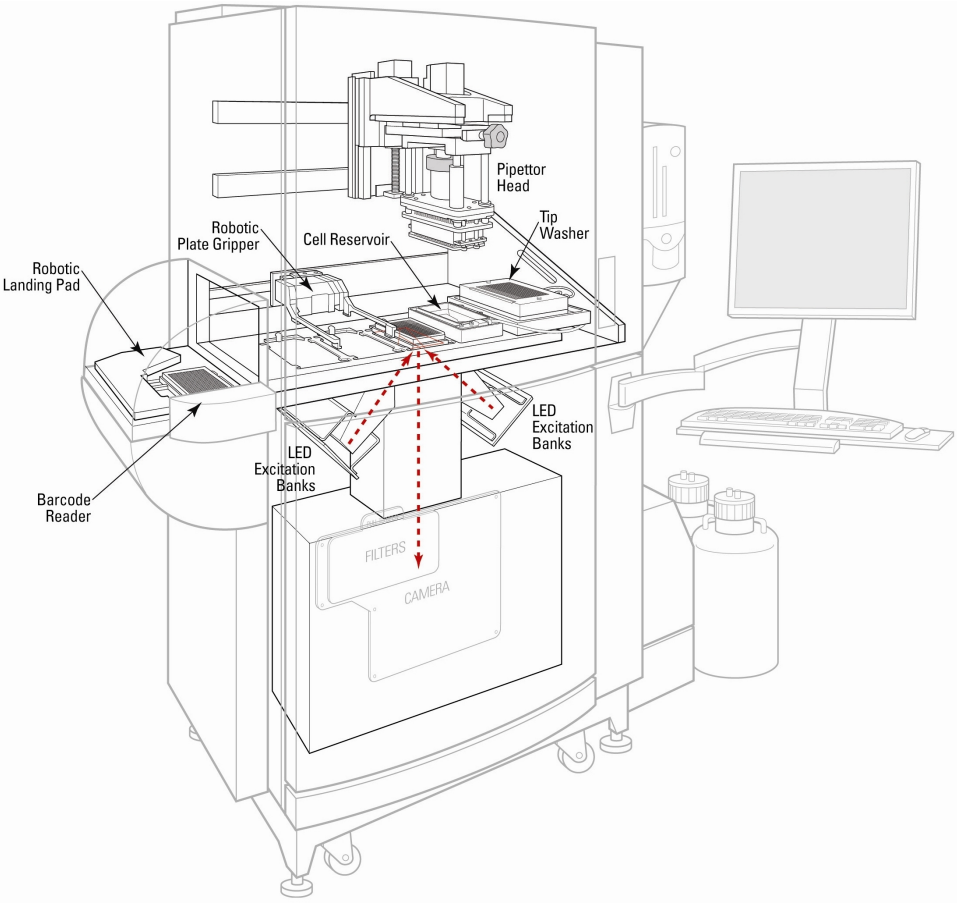
Hardware Introduction

The instrument includes the following subsystems to accomplish the assays described:

- Embedded Control System - The instrument contains an embedded control system to control the instrument. Access this system with the ScreenWorks Software, which is supplied with the instrument.
- Plate Reader - The optics subsystem includes an illumination source with excitation filters, emissions filters in a three-position filter changer, and a CCD camera. Use this system to read both fluorescence and luminescence assays.
- Pipettor - The instrument contains a pipettor to add fluids to the read plate during a series of reads for kinetic assays. Equip the pipettor with a 96-well, 384-well, or 1536-well head for general purpose fluid transfer between the plates in the instrument. Each head type uses pipette tips or pin tools. The 96-well pipettor heads and 384-well pipettor heads use a disposable plastic pipette tip. The 1536-well pipettor heads use a metal tip block. The 384-well and 1536-well pin tool heads have replaceable pin tools.
- Tip and Pin Washer - Each instrument includes a washer to wash tips or pins between fluid transfers. The washer consists of a washer control module and a wash reservoir. The sensors and control valves are installed on the right side of the instrument and connect to the wash reservoir located in position 5 of the system stage.
- FLIPR Cycler Plate Handler - A plate handler is available to move plates from a location outside of the instrument to any of the first four stage positions and vice versa. The second component of the FLIPR Cycler Plate Handler is an automated door system that allows plates to be exchanged while an assay is in progress.

Layout

The following displays the basic layout of the instrument subsystems:



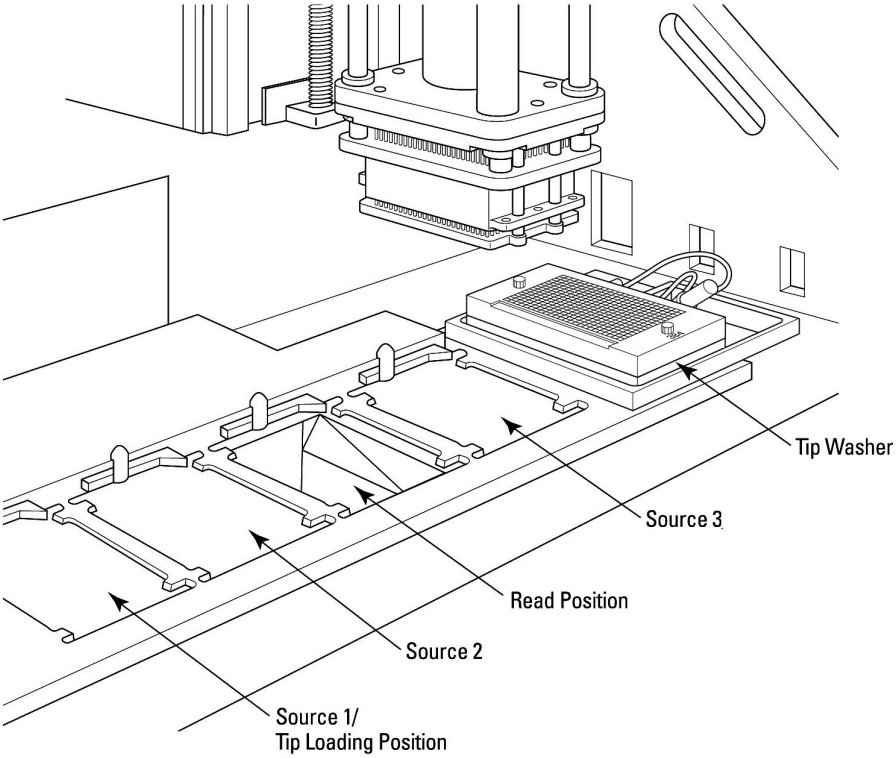
The top instrument compartment contains the pipettor and plate platform (stage). Plates are placed in the read position of the 5-position platform to be read by the plate reader, which is located in the optics compartment below the platform.

Plate Layout

The stage has 4 positions where you place plates and/or tips:

- Position 1: Source Plate 1 or Tip-loading
- Position 2: Source Plate 2
- Position 3: Read Plate
- Position 4: Source Plate 3

A tip wash reservoir for tips or pins is available in position 5.



Place either tip racks or plates in the tip-loading position (position 1) and the source plates in position 4. The wash reservoir is not disposable, but you can replace the wash reservoir when you change the head type.

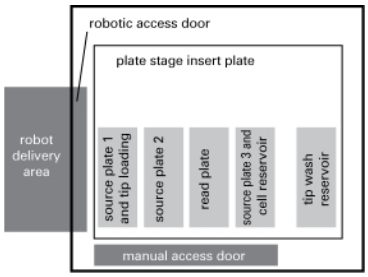
Plate Handling System

Manually place plates or tip racks in the instrument or use a robot or stacker to deliver plates automatically.

In manual mode, you access the plate stage through a large, manually operated door. This door opens on a vertical hinge, mounted to the left side of the door.

At the start of an assay, the door latches to prevent interruption of the assay in progress or injury. The door latches for most instrument actions not associated with an assay, for example, load tips, unload tips, and signal test. This latch remains engaged throughout the assay or operation. In manual mode, this latch disengages at the end of the assay.

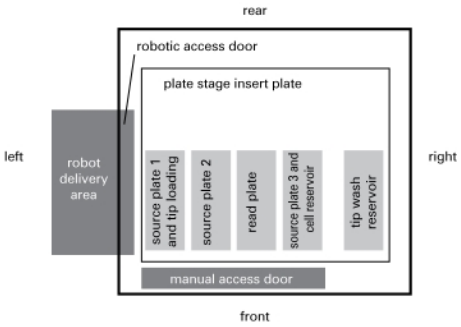
For robotic integration, plates are delivered to a single location outside of the instrument. The placement area for robotic delivery of plates to the instrument is to the left of the instrument. The following image shows the locations for manual and robotic access to the instrument:



A further illustration of this arrangement shows the relation of the plate handler to the pipettor system.

Terminology

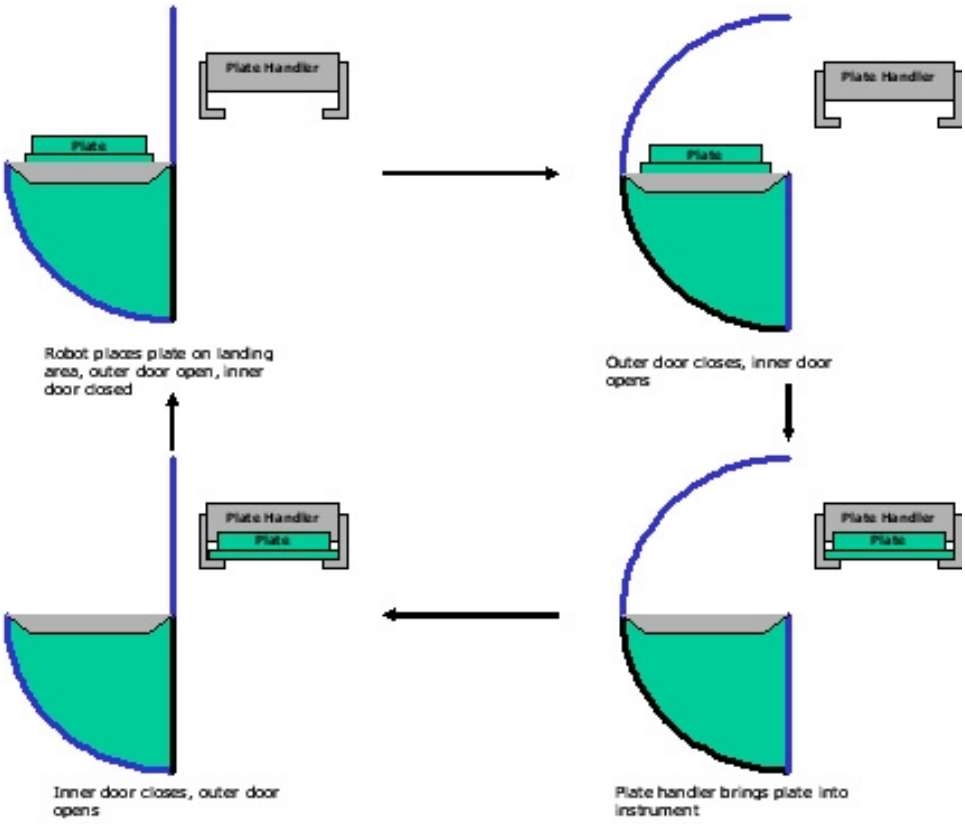
The manual access door side of the instrument is the front of the instrument.



Robotic Plate Loading

Plates can shuttle into the instrument by a plate handling subsystem called the FLIPR Cycler Plate Handler. The robotic access doors and the FLIPR Cycler plate handler can exchange plates and tips while an assay runs to lessen dead time between assays and to handle more source plates in an assay than the plate stage can accommodate.

The following diagram illustrates the steps to load plates in robotic mode:



A reverse process is used when unloading plates in Remote mode.

Optics Access Door

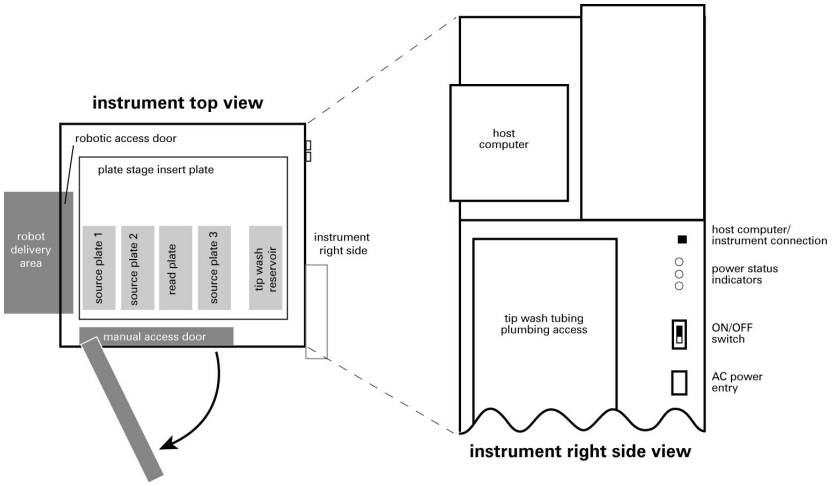
The plate reader optics are mounted to the plate stage directly below the read plate position. A door to the optics compartment allows you to change emission filters and LED modules. This door latches when an assay is in progress to prevent users assay interruption or injury.

Washer Placement

A tip/pin washer is located on the lower right side of the instrument, towards the front. Plumbing for the washer is isolated from the optical and power components. Access is provided for the washer tubing.

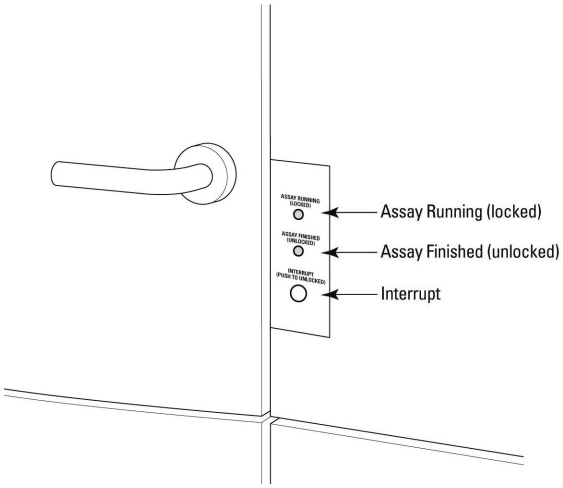
Other Instrument Access Areas

The following diagram illustrates the instrument access areas.



AC power enters on the right rear of the instrument. Directly above this entry is the main power switch. The power switch is accessible in both robotic and manual modes. There are three indicator lamps near the power switch. View these indicators to debug instrument power problems. The main communication connection from the instrument to the host computer is located above the power indicators.

The instrument status panel is located next to the upper door handle to indicate whether or not the instrument is running and safe to open. It includes an emergency Interrupt button to stop processes.



The panel has two lights and the Interrupt button:

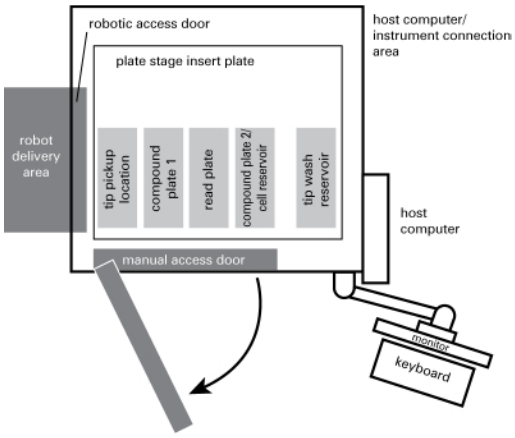
- Assay Running (Locked) yellow light - The FLIPR Penta System is performing a task. The upper and lower doors lock until the task finishes or is halted using the Interrupt button.
- Assay Finished (Unlocked) green light - No tasks are being run and it is safe to open the upper and lower instrument doors.

The Interrupt button is an override button to halt all tasks. When you press the Interrupt button, the yellow light flashes until the system reaches a safe state to open the doors, then the green light comes on.

CAUTION! The Interrupt button immediately ends the experiment and should only be used in emergencies. Interrupting the instrument while an assay in progress almost always results in the loss of the plate being run. The yellow light flashes until the instrument is safely unlatched. You may need to reinitialize the system by clicking the Instrument menu and selecting Reset before you resume normal instrument interactions.

Monitor and Keyboard Placement

The host computer for the FLIPR Penta System mounts to the instrument right side. This computer provides the user interface and path to control the instrument. The computer monitor and keyboard mount to an adjustable arm on the instrument front right.



You can move the host computer, keyboard, and monitor to a different location but it must be directly connected to the instrument. It is not possible to communicate between the host computer running the ScreenWorks Software and the instrument across a network.

Required Access Areas

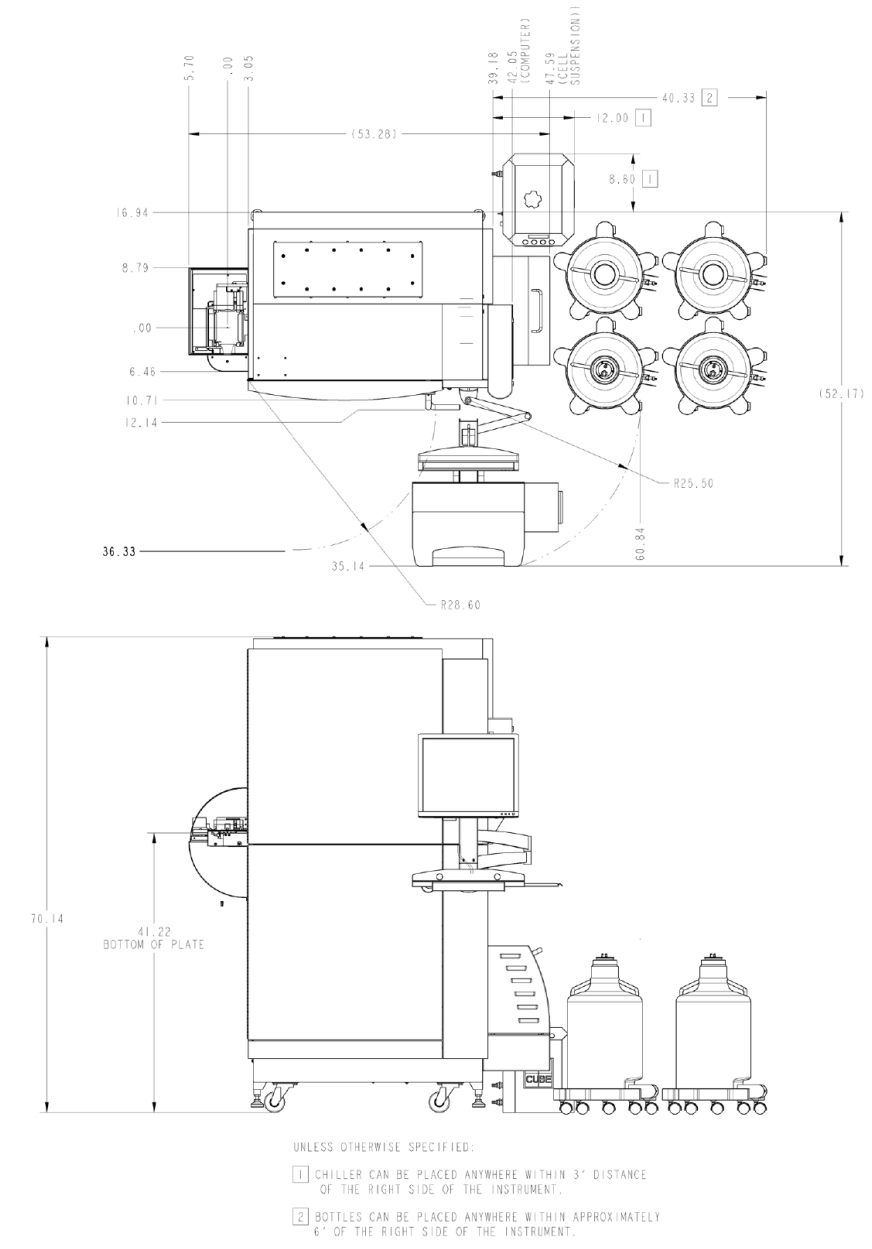
The system dimensions for the FLIPR Cycler Plate Handler is approximately 1346 mm (53 inches) wide by 737 mm (29 inches) deep by 1787 mm (70 inches) tall. The FLIPR Penta System has rolling castors to move for adjustments and to perform maintenance. Leveling feet are installed on the lower instrument chassis to stabilize the instrument and to establish a uniform instrument deck level where the lab floor is not flat.

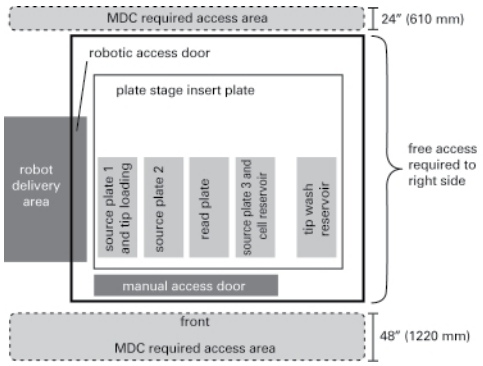
The computer and monitor mount to the right front side of the instrument and require a minimum space of 2159 mm (85 inches) wide by 1549 mm (61 inches) deep. The tip wash bottles and chiller require a minimum 711 mm (28 inch) square footprint on the right side of the instrument. This space allows access to the main power switch, washer tubing, and communication connections.

The cabinet should have access space of 1219 mm (48 inches) in front to exchange tips, plates, and reservoirs. An additional 610 mm (24 inches) behind and 254 mm (10 inches) to the left should be left available to service the instrument.

WARNING! The FLIPR Penta System can weigh as much as 390 kg (860 lbs.). Ensure adequate personnel are present when you install or move the system. Follow all safety precautions and use proper move techniques.

The following drawings illustrate these requirements:





FLIPR Penta System Control Architecture

General Description

The FLIPR Penta System consists of a host computer and a stand-alone instrument.

The ScreenWorks Software installs on the host computer supplied with the instrument. This software is the primary user interface. Use the ScreenWorks Software to setup protocols, run assays, analyze data, and export data. The ScreenWorks Software communicates to the instrument with a dedicated connection using a communication protocol proprietary to Molecular Devices. The status of the instrument and assay is monitored by the ScreenWorks Software, which then displays data as it is collected.

The instrument has all the hardware and firmware components required to perform an assay and report the results. It does not have the ability to display the results of experiments directly to a user. The user may not directly communicate with the stand-alone instrument in order to obtain data or to configure and run assays.

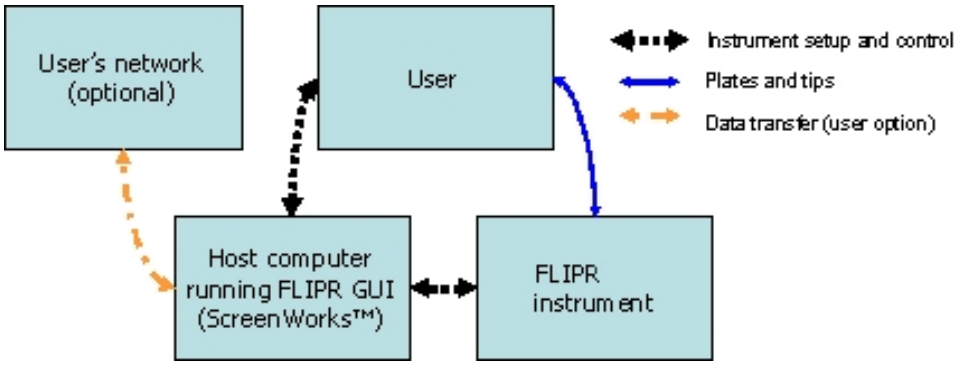
The host computer has two Network Interface Cards (NICs). The first NIC communicates with the instrument using a fixed IP address and cannot be used for any other purpose. The second NIC connects the host to the Local Area Network (LAN) or to other devices (for example, router, hub, scheduling workstation).

The ScreenWorks Software and the instrument have two operating modes: Manual mode and Remote mode. Place the ScreenWorks Software Remote mode for control by third-party software via the automation interface.

Manual Mode

By default, the instrument starts in Manual mode.

In Manual mode, configure assays in the ScreenWorks Software and manually load plates and tips into the instrument. The following simple diagram illustrates this interaction:



In Manual mode, a second network connection on the host computer is not monitored. It is provided primarily to connect to your network to transfer data from the FLIPR Penta System host computer for storage or analysis elsewhere.

Remote Mode

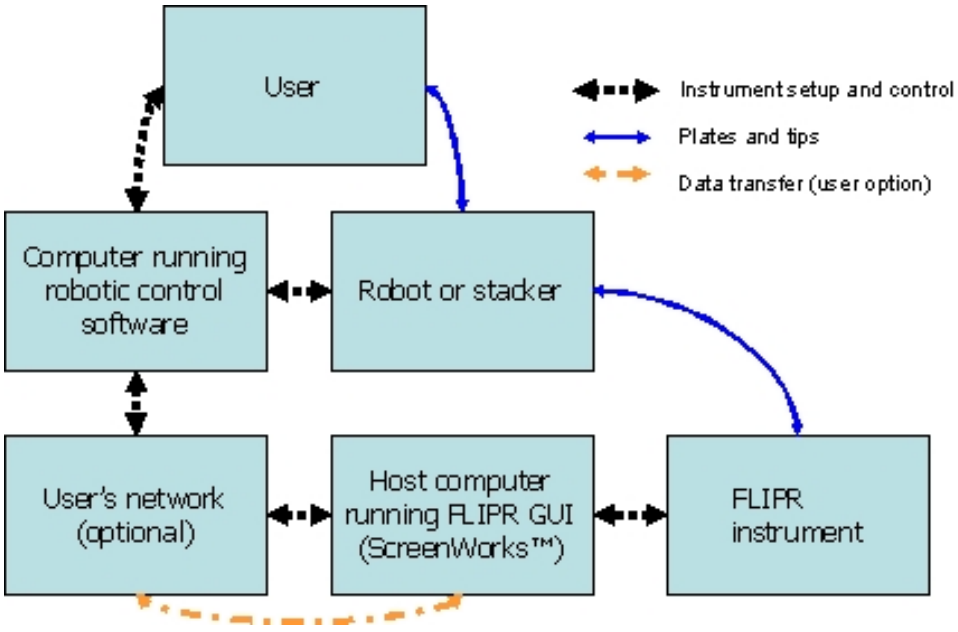
Remote mode allows the ScreenWorks Software to communicate with external devices such as robots and stackers supplied by third parties. Use these devices to supply plates and tips to the FLIPR Penta instrument.

When in Remote mode, third-party software has full control over the FLIPR Cycler plate handler. The FLIPR Penta System software does not initiate any plate handling events. Third-party software monitors the assay through the ScreenWorks Software automation interface and provides additional plates and tips to the instrument as needed.

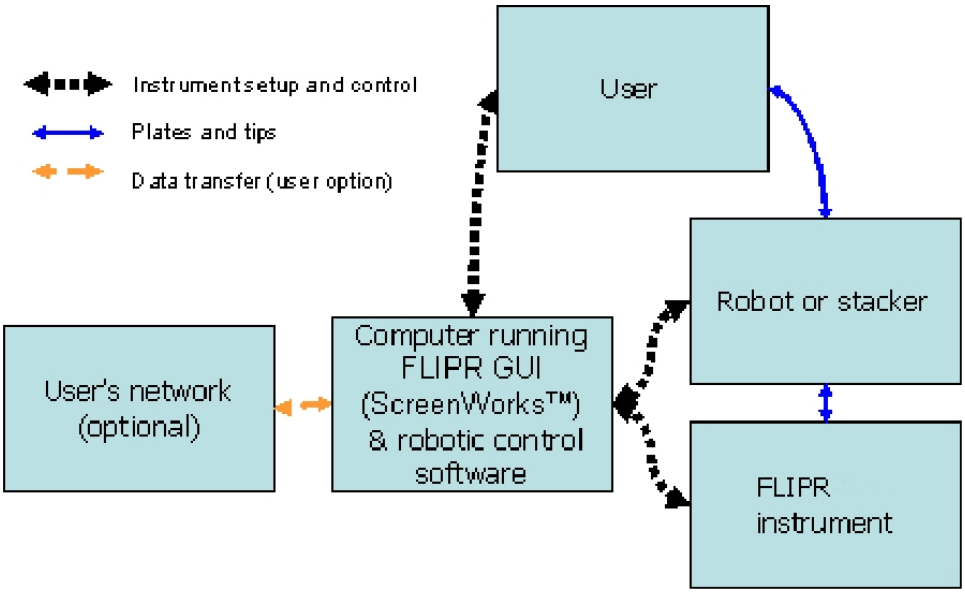
Interact with the third-party software to select a protocol or preload a protocol in the ScreenWorks Software before you enter Remote mode. The automation interface does not create assay protocols. Interact with ScreenWorks Software while assays run remotely to QA data and monitor progress.

You can configure the control system to communicate between the ScreenWorks Software and robotic controller in several ways. The following illustrate a range of options but are not exhaustive.

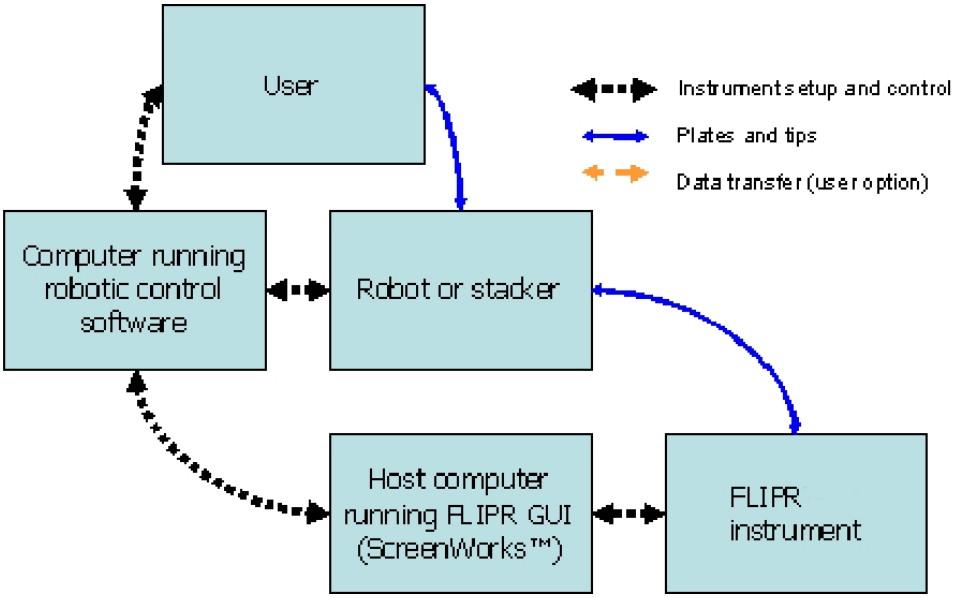
Third-Party Software via Network



Third-Party Software on the FLIPR Penta System Host Computer



Direct Connection From a Third-Party Software Host to the FLIPR Penta System host



Communication Protocol and Address

Communication between third-party software and the ScreenWorks Software uses the TCP/IP protocol. The third-party software can be resident on another computer outside of the FLIPR Penta System or on the instrument host computer.

If the third-party software is remote to the host computer, the ScreenWorks Software receives communication through the IP address of the host computer on which it is running.

If the third-party software is run on the instrument host computer, the ScreenWorks Software receives communication through IP address 127.0.0.1 (localhost).

In all cases, the ScreenWorks Software sends and receives commands through TCP/IP port 7.

General Remote Mode Use

This section provides an overview to run the FLIPR Penta System in Remote mode.

Instrument Startup

When the instrument is powered on, it performs a series of initialization steps which can take several minutes.

- Configure the camera
- Initialize the pipettor
- Initialize the plate handler and automatic door
- Determine and check the installed LEDs
- Determine the installed emission filters
- Initialize the tip washer
- Communicate with the chiller

By default, the instrument is in Manual mode upon startup. The main manual access door is unlocked, the outer automated door is closed, and the FLIPR Cycler plate handler is moved outside the instrument, above the automation landing pad. When the instrument is in Manual mode, the outer automated door and plate handler do not move from these positions, except during an instrument reset.

Software Startup

To use the instrument, the host computer must be started, and the ScreenWorks Software must be run. The ScreenWorks Software establishes communication with the instrument and determines the instrument status and configuration which displays in the Instrument Status toolbar. This toolbar has an Instrument Status tab that displays the instrument status including the status of assays in progress and an Instrument Config tab that displays which LED modules, emission filters, and heads are installed.

Protocol Creation

You create assay protocols in Manual mode. You do not need to load the protocols prior to placing the instrument into Remote mode, but any protocol you use in Remote mode must be created and saved with a valid protocol file name before you enter Remote mode.

Test protocols in Manual mode prior to running them in Remote mode.

Instrument Setup

You can load tips or plates into the instrument before you place the instrument in Remote mode. These actions can be done in Remote mode but in certain conditions you should do this through the main manual door before you start Remote mode.

If you use tips for an extended period of time and the tips/source 1 position is used as a source position, you should load the tips manually before you place the instrument in Remote mode.

If you use boats or reservoirs as source plates, you should load these by hand in Manual mode. Do not use the FLIPR Cycler plate handler to load reservoirs because the plate handler moves rapidly and large wells spill liquid within the instrument.

Instrument Mode Setting

Prior to running an assay in Remote mode, click the Instrument menu and select Set Remote Mode to place the instrument in Remote mode.

After the you start Remote mode, the instrument locks the main manual doors, moves the plate handler inside of the instrument, closes the inner automated door, and opens the outer automated door. At the end of any plate handling operation, the instrument returns to this position. The outer manual doors remain locked as long as the instrument is in Remote mode.

The instrument assumes that any plates in the instrument when you start Remote mode are acceptable for use until a protocol runs and the plate is no longer needed.

Loading Protocols

The **openprotocol** command loads protocols when the instrument is in Remote mode. This command loads the protocol and closes any other open files. Protocols open regardless of whether they are valid.

Running Protocols

After you place the instrument in Remote mode and a protocol opens, the protocol may be run by sending the runprotocol command. When a protocol begins to run in Remote mode, open data files close.

Instrument Status Updates

The status command queries the instrument at any time for instrument status, even when in Manual mode.

Plate Status Tracking

The most important information in the status command is the status of the plates in the instrument. From the status response, four possible information items may be determined about the plates in each of the four plate positions.

The first item is the plate assigned to the plate position for the protocol. This is the plate name you assign in the ScreenWorks Software for the plate type, and you assign to a position for the protocol. A robot can use this information to confirm that the correct plate type is delivered to the required position. The ScreenWorks Software and the FLIPR Penta System instrument have no method to determine whether the plate delivered is of the required type and always assumes that the plate type delivered is correct.

The status response indicates whether a plate is present in each location. This is a direct readout of the status of the plate presence sensors in the instrument. This readout does not depend on whether a plate is required in the position or if the plate present is the correct plate.

The *REMOVEPLATES* line in the status signals that the plates have completed. Plates complete in different ways. If the plates are source plates, the ScreenWorks Software indicates that they are finished when an aspiration from the plate is complete, and that aspiration is checked to show that the plate is finished after the aspiration. For tip racks, they are marked complete as soon as tips load or unload to/from the rack. Read plates are marked as complete at the end of the last read in the assay in which they are used.

The *NEEDPLATES* line indicates plates that are needed for the assay in progress to continue. The instrument indicates that it needs a plate if it gets to an operation that requires a plate in a particular location and that location is either empty or contains a plate that was marked as finished.

Bar Code Tracking

The bar code can be supplied to the software by the robot controller when the robot controller instructs the ScreenWorks Software to load a plate. The software records the bar code values of the plates delivered in the data file in which they are used.

The instrument can attempt to read a bar code for the plates it loads using the plate handler. If this value is not overridden by one supplied by the robot controller, this bar code is recorded in the data file when the plate is used.

Regardless of the method used to deliver bar code information to the instrument, bar code information might not be present when an assay starts, since all required plates might not be present in the instrument. You can configure the software to name the data file based on the bar codes of the plates used, so the data file name can change as the assay completes.

Setting Up Protocols for Remote Control Use

You must create protocols in Manual mode before you start Remote mode, and you should test protocols in Manual mode to ensure that they work as expected.

The user guide describes how to set up protocols and you should be familiar with this process before you read further in this section. See Setting Up Assay Protocols on page 112.

The following sections describe items you should be aware of when you set up the protocols you plan to run in Remote mode.

Settings Screen

Read Mode Settings

In the settings screen, configure the read modes. The settings for the read mode depend on the assay dye, plate type, cell type, and condition. You determine these settings during assay development and no changes are required when you switch to Remote mode.

Note: It is not possible to vary these settings based on the cell plate you load.

Assign Plate Settings

Assign plates to the required positions for the assay in the Assign Plate to Position field. The assignment of plates is critical to the smooth functioning of the assay.

You can assign only one type of plate to a position throughout an assay. For example, you cannot assign a normal plate to a position for the first part of an assay and then assign a deep-well plate to that position for the rest of the assay.

Always assign the read plate to the read position. There are no special suggestions in Remote mode for this assignment.

Carefully consider the assignment of source plates and tips. The pipettor and the plate handler are both used in remote assays and must share the space above the plate locations. The instrument monitors the locations of the pipettor and the plate handler and moves them to prevent collisions. The pipettor and plate handler are never allowed to be above the same plate position at the same time. You should arrange plates to minimize potential interactions between the plate handler and the pipettor.

Use the following guidelines to assign plate positions for automation:

The plate handler cannot handle open reservoirs with very large wells. You must place these plates in the instrument manually prior to entering Manual mode. You must halt remote control to replace these plates and place the instrument in Manual mode to replace or refill the reservoir. Deep well plates are a good alternative to these plates.

If tips load in the experiment, you must assign the tips to the Source Plate 1 location. When you assign tips to this position, you cannot use the position for source plates. Unless the tips are to be changed for every plate, you should load the tips before you place the instrument in Remote mode. Alternatively, if you create a tip configuration protocol that loads and washes the tips, you can run the assay and the empty tip rack can be removed by the robot to allow you to use the source 1 position for other plates. As of v1.1 of the automation interface, tip loading and tip washing is available.

You should place plates that switch frequently closer to the left side of the instrument (near the automated landing pad) than plates which do not switch frequently.

Place deep well plates (or reservoirs) that you use for an entire series of assays in the source 3 location to minimize the interaction between the pipettor and the plate handler when switching plates during the assay.

You can only RESET the instrument if a tip rack is present in the instrument. A RESET is required if the instrument enters a fault condition. If the system requires an automated recovery from fault conditions, use the tips/source 1 position for tips only and leave an empty tip rack in this position at all times.

Data File Name Settings

Configure the data file name in the Data File Name field.

If you select Include Bar Code, the bar codes for all of the plates you require are included in the data file name. Bar codes are separated by an underscore (_). If a bar code cannot be read, or one has not been provided during the loadplate command, the software records *Bad_bar_code*.

The settings for the data file name are ignored in Remote mode when the data file name is included with the runexperiment command line. Take care when you use this command to ensure that data file names are unique to prevent the overwriting of data files.

If you allow the ScreenWorks Software to create the data file name, it ends in _###.fmd, where *###* is the plate number with this base name. Use this option to ensure that no data is lost due to file overwriting.

Folder Settings

Use this to assign the directories in which to save the data and export files. There is no difference between settings for these directories in Remote mode and in Manual mode.

Remote mode can produce large amounts of data. The ScreenWorks Software does not allow new assays to begin if less than 50MB of hard disk space is available.

Saving data across a network connection is not recommended. If a protocol saves data across a network connection, you cannot run the protocol if the network connection is unavailable. If a protocol that saves across the network runs when the network connection is interrupted, data can be lost, and the software can encounter problems.

Temperature Control Settings

Use temperature control settings to set the temperature for the protocol. The instrument must be at the set point before you run the assay.

If you load a protocol that requires temperature control and the runexperiment command is sent, the software confirms that the instrument has achieved this temperature. If the instrument has not reached the temperature, the software displays a message. If the temperature has been reached, the experiment runs.

Auto Print Options Settings

Problems with the printer can disrupt the operation of the system. You should not use automatic printing in Remote mode.

Analysis Screen

There are no special notes regarding the Analysis screen in Remote mode. Setup this screen as you would for a manual assay.

Set up groups, display, and reduction settings prior to running a large number of plates. Define these in the protocol so that they are automatically replicated in each data file as the data file is created.

Transfer Fluid Processes

Settings to transfer fluids in Remote mode are the same as Manual mode, with three exceptions. These are in aspiration height over multiple plates, and the Pause In Well field.

Aspiration Height over Multiple Plates

When you run in Remote mode, you should use a common addition source plate over a series of plates. The simplest way to do this is to create a single protocol which aspirates from the lowest allowed point in the source well. You need to determine the maximum amount of liquid in the well so that the liquid displaced during insertion of the tip into the well is not pushed out of the well. This limits the amount of liquid that you can use in the source well. Alternatively, you can choose to create a series of protocols at successively lower aspiration start levels.

To provide an example of this situation, consider using a 384-well plate with a 250 µL well. This source plate is used to add a common agonist to all wells. In the assay, 20 µL of this agonist is added. Leave the plate in the instrument for a series of plates before you replace it. The FLIPR Penta System allows protocols that begin aspiration as low as 8 µL for this plate. Inserting a 384-well tip all the way to the bottom of this plate displaces 50 µL of liquid.

In the simple method described above, do not start with more than 190-200 µL of liquid in the plate because any more can spill liquid when the tip is fully inserted. The single protocol begins aspiration at 8 µL height. Run this assay 9 times before you replace the plate, leaving 10-20 µL of liquid in the wells after the source plate is removed.

In the more complex method, fill the wells to 230–240 µL. Then, set up an assay that starts aspiration at ~240 µL in height, a second assay to start at ~220 µL, and a third assay to start at ~200 µL. Continue to create separate assays at different heights or use the third assay for the remaining plates. In this case, you can run 11 plates before you need to change the plates.

The simple method is simpler to setup and run. The more complex method allows you to switch the source plates less often. It also decreases the proportion of wasted common reagents. Finally, it can decrease carryover of compounds sticking to the outside of the tips, especially if you create the protocols to retrieve liquid from progressively lower heights all the way through the well height.

Pause in Well Edit Box

The Pause in Well field in the Edit Dispense dialog allows you to have the pipettor remain in the well after the dispense. This pipettor operation leaves the tips in the well for the time you specify. When the tips are in the well, it might prevent the exchange of plates from any position, and certainly not from the read or source 3 positions. For this reason, you should not use this option in Remote mode.

Wash Tips Processes

No special requirements are needed for the Wash Tips process for Remote mode. The pipettor and the plate handler do not share the tip reservoir position. For this reason, plate handling can proceed unhindered during wash operations.

Mix Fluid Processes

The Mix Fluids process does not require any special changes for Remote mode. The same warning that applies to Pause in Well for dispensing also applies here. Mixing in any well can interfere with plate handling, so use of this function might require some experimentation.

Read Processes

No special requirements are needed for the Read processes in Remote mode.

Finish with Source Processes

This notifies the plate handler to remove the plates in Remote mode.

Command Set

Command Syntax

All commands and parameters must be sent in lower case and end with <CR>.

All responses are given in lower case and end with <CR>.

Response from the ScreenWorks Software has one of the following forms:

```
ok<CR>
errorcode, error string<CR>
status<CR>
```

- ok<CR> - Command started successfully or command executed successfully.
- errorcode, error string<CR> - Command failed because of either an indicated condition or an instrument error. Error codes are followed by a comma, a space, and then a string that describes the error of up to 300 characters. Error strings are entirely lower case.
- status<CR> - The formatted status text which was requested.

Version <CR>

Command Description

This command returns the version string for the automation interface command set. This command is supported for versions 1.1 and newer. Older versions return the error *c69, badly formed or unrecognized command* in response to this command.

Command Parameters

There are no parameters with this command.

Example Command Line

```
version<CR>
```

Example Response

```
1.1<CR> or c69, badly formed or unrecognized command.<CR>
```

Suggested Steps in Command Usage

This should be the first call you make to the instrument after making a TCP/IP connection to ScreenWorks Software. To ensure problem free operation of the application for FLIPR Penta System customers, the robotic integrator must consider limiting use of the application to interface versions they have tested.

Status <CR>

Command Description

The status command returns the status of the instrument. The status response is an ASCII text string which includes:

- Information about the instrument type and firmware version.
- Whether the instrument is functioning normally, or an error occurred. If an error occurs, this field contains an error code and an error string of less than 300 characters. The error code and error string are separated by a comma and space.
- The instrument status field reports a summary of status information including whether the instrument is busy, faulted, error, resetting, aborted, or idle. It reports whether the instrument is in Remote mode or Manual mode. In Manual mode, only status information is available. All other commands do not function. This line reports whether the software is online (connected to the instrument) or not.
- Whether an experiment is running or not. Do not open or start another experiment until the first finishes.
- Name of the plates assigned to each position.
- Whether plates are present or not on plate locations.
- Whether plates need to be loaded or removed.
- Whether the plate handler is busy or idle.
- Whether the landing pad has a plate or not.
- Chamber temperature. Status reports *not_ok* while chamber is not at setpoint temperature (typical factory tolerance of ±0.5 °C/± 41°F, *fault* if sensor does not return a reading, *off* if chamber temperature control has been disabled, or *ok* otherwise.

- Camera temperature. Status returns *not_ok* while camera CCD temperature cycles and/or out of range as defined by factory firmware settings, *off* if camera temperature control is disabled, or *ok* otherwise. The factory setpoint depends on the camera:
  EMCCD camera: -70°C (-94°F) ± 2°C
  HS EMCCD camera: -70°C (-94°F) ± 2°C
  Also returns actual CCD temperature and setpoint.
- Waste/fill bottle status.
- Tips on|off.
- Outer auto door, open/closed. If the door is closing, it reports as closed.

Example Command Line

```
status<CR>
```

Response Format

```
instrument <SP>instrument name<TAB>
version<SP>mm/dd/yyyy<TAB>
function<SP>ok|errorcode|fatalcode,[errorstring]<TAB>
inst_status<SP>busy|fault|error|resetting|abort|idle,remote|manual,o
ffline|online<TAB>
exp_running<SP>yes|no<TAB>
readplate<SP>platename|none,have|empty<TAB>
sourceplate1<SP>platename|none,have|empty<TAB>
sourceplate2<SP>platename|none,have|empty<TAB>
sourceplate3<SP>platename|none,have|empty<TAB>
loadplates<SP>re[,s1,s2,s3,ti]|none<TAB>
removeplates<SP>re[,s1,s2,s3,ti]|none<TAB>
platehandler<SP>idle|busy_load|busy_remove<TAB>
plate_on<SP>yes|no<TAB>
camera_temp<SP>ok|not ok|off,temp,setpoint<TAB>
chamber_temp<SP>ok|not_ok|fault|off<TAB>
waste_bottlea<SP>ok|full<TAB>
waste_bottleb<SP>ok|full<TAB>
fill_bottlea<SP>ok|empty<TAB>
fill_bottleb<SP>ok|empty<TAB>
tips<SP>on|off<TAB>
outer_auto_door<SP>open|closed<CR>
```

Example Response

```
instrument<SP> tetra<TAB>
version<SP> sep 30 2004<TAB>
function<SP> d70, initialization halted because tips are loaded. please
verify that the appropriate tip rack (96,384,1536)is loaded and select
reset <TAB>
inst_status<SP> idle,remote,online<TAB>
exp_running<SP>no<TAB>
```

```
readplate<SP> default384,have<TAB>
sourceplate1<SP>default384,have<TAB>
sourceplate2<SP>none,have<TAB>
sourceplate3<SP>none,have<TAB>
loadplates<SP>none<TAB>
removeplates<SP>none<TAB>
platehandler<SP>idle<TAB>
plate_on<SP>no<TAB>
camera_temp<SP>ok,-35,-35<TAB>
chamber_temp<SP>off,0,0<TAB>
waste_bottlea<SP>ok<TAB>
waste_bottleb<SP>ok<TAB>
fill_bottlea<SP>ok<TAB>
fill_bottleb<SP>ok<TAB>
tips<SP>on<TAB>
outer_auto_door<SP>closed<CR>
```

Suggested Steps in Command Usage

- Send `status` command.
  Response is the instrument status.

Loadplate<TAB>Location, Last Plate[, BAR CODE] <CR>

Command Description

Loads plate or tips to the location you specify from landing pad. The plates or tips must be placed on the instrument landing pad prior to issuing the command. You can use up to 13 plates (1 source plate, 12 compound plates) in a single experiment.

Additional parameters are available to provide the bar code for the plate and to identify the final plate for an assay.

The bar codes of up to 5 plates can be recorded in the data file.

Command Parameters

There are four parameters with this command.

The parameters are location, bar code, and last plate. The location parameter is required. The bar code and last plate parameters are optional. If you use the bar code parameter, you must also use the plate order parameter.

Location parameter must be one of following:

- re – Read plate location
- s1 – Source plate 1 location
- s2 – Source plate 2 location
- s3 – Source plate 3 location
- ti – Tips loading location

The tips and source 1 location are the same position. Use either the *ti* or *s1* parameter to indicate this position.

Bar code parameter must be:

- String up to 20 characters.
- String can have letters, numbers, underscore, and spaces.

The instrument always attempts to read a bar code while loading a plate. If a bar code is supplied through the loadplate command, any bar code found on the plate loaded is ignored.

The last plate parameter indicates whether or not the plate being loaded is the last plate in the experiment. This parameter must be *yes* or *no*. The last plate parameter is no longer used by the FLIPR Penta System and ScreenWorks Software but continues to be required for compatibility reasons.

Example Command Line

```
loadplate<TAB>re, no, abcd1234<CR>
```

Example Response

```
ok<CR> or c10, error string<CR>
```

Suggested Steps in Command Usage

1. Check if instrument status is acceptable, such as no error state, no plate on landing pad, no plate in target location, plate handler is idle.
2. Put plate on landing pad.
3. Send `loadplate` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
4. Monitor plate handler status for completion or errors.

Command Specific Error Responses

- C00 - Instrument is not functionalCondition
- C05 - Invalid plate locationCondition
- C10 - Plate handler busyCondition
- C20 - Landing pad does not have a plateCondition
- C15 - Plate location already has a plateCondition

Removeplate<TAB>location <CR>

Command Description

Removes plate or tips from the location you specify. If you set the source plate3 location for tips in the experiment, the instrument assumes tips are present in that position.

Command Parameters

There is one parameter, location, for this command.

Location parameter must be one of following:

- re – Read plate location
- s1 – Source plate 1 location
- s2 – Source plate 2 location
- s3 – Source plate 3 location
- ti – Tips loading location

The tips and source 1 location are the same position. Use either the *ti* or *s1* parameter to indicate this position.

Example Command Line

```
removeplate<TAB>s1<CR>
```

Example Response

```
ok<CR> or c20, error string <CR>
```

Suggested Steps in Command Usage

1. Check if instrument status is acceptable (no error state, no plate on landing pad, plate present in target location, plate handler is idle).
2. Send `removeplate` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
3. Monitor plate handler status for completion or errors.

Command Specific Error Responses

- C00 - Instrument is not functionalCondition
- C05 - Invalid plate locationCondition
- C10 - Plate handler busyCondition
- C21 - Landing pad has a plateCondition
- C16 - Plate location doesn't have plateCondition

Openprotocol<TAB>File Name<CR>

Command Description

This command opens the protocol you specify. Any documents that are open before you send this command close automatically. Only one protocol can be open at the time.

Command Parameters

There is one parameter, file name, for this command.

The filename parameter is the complete file name of the protocol to open, including path and file type identifier (*.fmp).

Example Command Line

```
openprotocol<TAB>c:\screenworks\protocols\myprotocol.fmp<CR>
```

Example Response

```
ok<CR> or c30, error string <CR>
```

Suggested Steps in Command Usage

1. Make sure experiment is not running.
2. Send `openprotocol` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.

Command Specific Error Responses

- C25 - Experiment is runningCondition
- C30 - Protocol not foundCondition
- C35 - Protocol could not be openCondition
- C36 - Invalid protocol nameCondition

Findprotocols<TAB>Folder<CR>

Command Description

This command returns all the protocols in the folder you specify. The command returns a comma-delimited list that contains all *.fmp type files saved in the folder. These files are not checked to confirm that the protocols are valid for the current instrument configuration.

Command Parameters

There is one parameter, folder, for this command.

The folder parameter identifies the folder for which a list of all *.fmp type files return. The folder must be complete and in the correct Windows format, including the drive identifier and a closing backslash(\).

Example Command Line

```
findprotocols<TAB>c:\screenworks\protocols\<CR>
```

Example Response

```
[myprotocol1.fmp,myprotocol2.fmp,myprotocol3.fmp]<CR> or c45, error string
<CR>
```

Suggested Steps in Command Usage

- Send `findprotocol` command at any time.
  Response is a list of protocols separated by comma or a Condition code that confirms the command did not execute.

Command Specific Error Responses

- C45 - Folder does not existCondition
- C46 - Invalid folder nameCondition
- C50 - Current document is not valid protocolCondition
- C56 - No protocols found in dirCondition

Runexperiment<TAB>[Data File Name]<CR>

Command Description

This command runs the open protocol, if it is valid for the current instrument configuration.

Running a protocol creates a data file and shifts the software focus to this data file for the duration of the experiment. When you run in Remote mode, the data file closes when the experiment is done and the focus returns to the protocol file.

Command Parameters

There is one optional parameter, data file name, for this command.

If you use a data file name parameter with this command, the data file created by running the protocol is named from the data file name. The file name appends to the Data folder path you specify in the protocol Settings dialog in the ScreenWorks Software. Never specify a data file name that contains a full path.

If the data file name parameter is not given, the data file saves with the auto save name as indicated in the Settings dialog.

Example Command Line

```
runexperiment<TAB>mydata10312003.fmd<CR>
```

Example Response

```
ok<CR> or c50, error string <CR>
```

Suggested Steps in Command Usage

1. Check if instrument is functioning fine.
2. Make sure the experiment is not running.
3. Open protocol first by sending `openprotocol` command.
4. Send `runexperiment` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
5. Monitor status for completion or errors.

Command Specific Error Responses

- C00 - Instrument is not functionalCondition
- C25 - Experiment is runningCondition
- C50 - Current document is not valid protocolCondition
- C47 - Invalid data file nameCondition

Stopexperiment<CR>

Command Description

This command stops the current action being performed by the instrument.

The `stopexperiment` command stops the experiment immediately. This can halt the instrument pipettor in the midst of a motion causing the instrument to enter a fault state which requires an instrument reset. The pipettor may not be in motion but might have tips that contain fluid or are in a source or target plate. In these cases, it might be possible to recover without resetting by washing the tips. An instrument reset is recommended following the use of the `stopexperiment` command.

Command Parameters

There are no parameters associated with this command.

Example Command Line

```
stopexperiment<CR>
```

Example Response

```
ok<CR> or c55, error string <CR>
```

Suggested Steps in Command Usage

1. Check if instrument is functioning fine.
2. Make sure experiment is running.
3. Send `stopexperiment` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
4. Monitor status for completion or errors.

Command Specific Error Responses

- 00 - Instrument is not functionalCondition
- 55 - No experiment is runningCondition

Clearerror<CR>

Command Description

Cleans error in case recoverable error occurred.

Command Parameters

There are no parameters associated with this command.

Example Command Line

```
clearerror<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command Usage

1. Check instrument status.
2. Check if error is occurred.
3. Send `clearerror` command.
   Response is *OK* that confirms the software has attempted to clear the error.
4. Monitor status for error is cleared.

Loadtips<CR>

Command Description

This command loads the tips onto the pipettor head from position s1.

This command executes if tips are not already loaded and a plate is in the tips position. The instrument cannot confirm that the plate in the tip load position is actually a tip rack. You are responsible to ensure that the plate in that position is actually a tip rack. Attempting to load tips when a plate, rather than a tip rack, is in the tip load position results in an instrument fault and can result in damage to the plate, the instrument, or both.

Command Parameters

There are no parameters associated with this command.

Example Command Line

```
loadtips<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command Usage

1. Make sure an experiment is not running.
2. Check the instrument status to make sure tips are not already loaded.
3. Place tip rack in tip load position via the `loadplate` command.
4. Send `loadtips` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
5. Monitor status for completion, and error status for any other kind of error.

Command Specific Error Responses

- C58 - Cannot load tips, tips on pipettorCondition

Unloadtips<CR>

Command Description

This command unloads the pipette tips from the pipettor head into an empty tip rack you place in the tip load position.

This command executes if you load tips and any plate is in the tips position. The instrument cannot confirm that the plate in the tip load position is actually an empty tip rack. You are responsible to ensure that the plate in that position is actually an empty tip rack. Attempting to load tips when a plate or full tip rack, rather than an empty tip rack, is in the tip load position likely results in an instrument fault and can result in damage to the plate, the instrument, or both.

Command Parameters

There are no parameters associated with this command.

Example Command Line

```
unloadtips<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command Usage

1. Make sure an experiment is not running.
2. Check the instrument status to make sure tips are currently loaded.
3. Ensure that an empty tip rack is in tip load position.
4. Send `loadtips` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
5. Monitor status for completion, and error status for any other kind of error.

Command Specific Error Responses

- C59 - Cannot unload tips, tips not on pipettorCondition

Cyclecameratemp<CR>

Command Description

This operation cycles the camera temperature. It sets the camera setpoint to 20°C (68°F), waits for the camera to reach this setpoint, resets the camera to -45°C (-49°F), and then waits for the camera to return to this temperature. Use this command when you transition from fluorescence to luminescence experiments. Cycling the camera temperature allows the camera CCD chip to recover from the high light levels present during fluorescence experiments and improves data quality in subsequent luminescence experiments.

This command requires up to 15 minutes to complete execution.

Command Parameters

There are no parameters associated with this command.

Example Command Line

```
cyclecameratemp<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command Usage

1. Make sure an experiment is not running.
2. Send `cyclecameratemp` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
3. Monitor status for completion or errors.

Tempcontrolonoff<TAB>Temp<CR>

Command Description

This command sets the temperature for the instrument chamber. After you send this command, the robotic controller monitors the instrument temperature to confirm that the instrument reaches the setpoint. Some protocols do not execute until the required temperature is reached.

Example Command Line

```
tempcontrolonoff<TAB>12<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command Usage

1. Make sure an experiment is not running.
2. Send `tempcontrolonoff` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
3. Monitor status until the instrument reports that it reaches the requested temperature.

Command Specific Error Responses

- C60 - Temperature Control System is not functioning

Washtips<TAB>Fluid Type, Wash Cycles, Volume/Stroke, Aspirate Speed, Pump Speed, Strokes, Hold Time, Dispense Speed<CR>

Command Description

This command is the equivalent of clicking the Instrument menu and selecting Manual Operation and then selecting Wash Tips. This selection washes the pipette tips with the parameters you provide.

Command Parameters

There are eight required parameters for this command. You must supply all eight parameters for this command to properly execute. For some of the parameters, acceptable values can vary depending on the head type you install on the instrument. The following table lists the parameters, their purpose, and the appropriate range for the commands.

Parameter	Description	Acceptable values or range		
		96-well head	384-well head	1536- well head
Fluid type	Indicates which wash fluid bottle to use.	a, b		
Wash Cycles	Number of wash reservoir fill/drain cycles to complete. The requested # of strokes is repeated for each cycle.	1,2,3,4,5		
Volume/stroke	Volume to draw into the pipette tip in each stroke.	5–206 (double)	1–28 (double)	.1–3 (double)
Aspirate speed	Pipettor aspiration speed in micro liters per second.	5.00, 10.00, 20.00, 30.00, 40.00, 50.00, 75.00, 100.00, 125.00	0.50, 1.00, 2.00, 5.00, 7.00, 10.00, 15.00, 20.00, 25.00, 30.00	0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 3.00, 5.00, 10.00
Pump speed	Speed to run the pumps which fill and drain the wash reservoir. Two speeds, fast and slow, are available. Use slow for wash solvents which tend to bubble.	fast, slow		
Strokes	Number of times which the pipettor fills and empties in each wash cycle.	1–20 (integer)		
Hold time	Pause time in seconds at the top of the stroke of the pipettor	0–15		
Dispense speed	Pipettor dispense speed in micro liters per second.	5–200 (double)	1–50 (double)	.1–10 (double)

Example Command Line

```
washtips<TAB>a,2,28.0,20.00,fast,5,0.0,20.0<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command Usage

1. Make sure an experiment is not running.
2. Ensure that tips are loaded.
3. Send `washtips` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
4. Monitor status until the wash completes.

Command Specific Error Responses

- C68 - Tips not loaded

Configuration<CR>

Command Description:

The `configuration` command returns the configuration of the instrument. The configuration response is an ASCII text string which includes:

- Information about the instrument type and firmware version.
- Pipettor head type
- Tip washer type
- Bar code reader installed or not
- Plate handler installed or not
- Camera type installed
- Chiller installed or not
- LEDs installed
- Emission filters wavelength

Example Command Line

```
configuration<CR>
```

Response format

```
instrument<SP>name<TAB>
version<SP>version#<TAB>
pipettor<SP>96|384|1536|384pintool| 1536pintool|no<TAB>
tip_washer<SP>96|384|1536|no<TAB>
bar_code<SP>installed|not installed<TAB>
plate_handler<SP>installed|not installed<TAB>
camera_installed<SP>emccd|<TAB>
chiller_installed<SP>installed|not installed<TAB>
wave1<SP>no led|470-495 nm|510-545 nm|390-420 nm|420-455
nm|610-626 nm|360-380 nm|525-550 nm|525-570 nm|570-595
nm|590-614 nm|620-648 nm|495-505nm|360-380 nm <TAB>
wave2<SP>no led|470-495 nm|510-545 nm|390-420 nm|420-455
nm|610-626 nm|360-380 nm|525-550 nm|525-570 nm|570-595
nm|590-614 nm|620-648 nm|495-505 nm|360-380 nm <TAB>
```

```
emfilt1<SP>no filter|515-575 nm|565-625 nm|440-480 nm|475-535
nm|646-706 nm|400-460 nm|570-630 nm|590-650 nm|615-675
nm|634-694 nm|668-728 nm|526-585 nm|400-460 nm<TAB>
emfilt2<SP>no filter|515-575 nm|565-625 nm|440-480 nm|475-535
nm|646-706 nm|400-460 nm|570-630 nm|590-650 nm|615-675
nm|634-694 nm|668-728 nm|526-585 nm|400-460 nm<TAB>
emfilt3<SP>no filter|515-575 nm|565-625 nm|440-480 nm|475-535
nm|646-706 nm|400-460 nm|570-630 nm|590-650 nm|615-675
nm|634-694 nm|668-728 nm|526-585 nm|400-460 nm<TAB>
<CR>
```

Example Response

```
Instrument<SP>tetra<TAB>
version<SP>2.1.0.0<TAB>
pipettor<SP>96<TAB>
tip_washer<SP>96<TAB>
bar_code<SP>installed<TAB>
plate_handler<SP>installed<TAB>
camera_installed<SP>emccd<TAB>
chiller_installed<SP>installed<TAB>
wave1<SP>470-495 nm<TAB>
wave2<SP>510-545 nm<TAB>
emfilt1<SP>515-575 nm<TAB>
emfilt2<SP>515-575 nm<TAB>
emfilt3<SP>515-575 nm<CR>
```

Suggested Steps in Command Usage

- Send `configuration` command.
  Response is instrument configuration.

Statusex<CR>

Command Description

The `statusex` command is an extension of status command. It returns the status of the instrument in more detail than the status command. The `statusex` response is an ASCII text string which includes all status command response information plus:

- Tip washer status
- Camera chiller status
- Camera intensifier status

Example Command line

```
statusex<CR>
```

Response format

```
instrument <SP>instrument name<TAB>
version<SP>mm/dd/yyyy<TAB>
function<SP>ok|errorcode|fatalcode,[errorstring]<TAB>
```

```
inst_
status<SP>busy|fault|error|resetting|abort|idle,remote|manual,offline|online
<TAB>
exp_running<SP>yes|no<TAB>
readplate<SP>platename|none,have|empty<TAB>
sourceplate1<SP>platename|none,have|empty<TAB>
sourceplate2<SP>platename|none,have|empty<TAB>
sourceplate3<SP>platename|none,have|empty<TAB>
loadplates<SP>re[,s1,s2,s3,ti]|none<TAB>
removeplates<SP>re[,s1,s2,s3,ti]|none<TAB>
platehandler<SP>idle|busy_load|busy_remove<TAB>
plate_on<SP>yes|no<TAB>
camera_temp<SP>ok|not ok|off|busy|fault,temp,setpoint<TAB>
chamber_temp<SP>ok|not_ok|fault|off|busy<TAB>
waste_bottlea<SP>ok|full<TAB>
waste_bottleb<SP>ok|full<TAB>
fill_bottlea<SP>ok|empty<TAB>
fill_bottleb<SP>ok|empty<TAB>
tips<SP>on|off<TAB>
outer_auto_door<SP>open|closed<TAB>
tip_washer<SP>ok|not_ok|off|busy|fault<TAB>
chiller_status<SP>ok|not_ok|off|busy|fault,temp,setpoint<TAB>
intensifier_status ok|not_ok|off|busy|fault<CR>
```

Example Response

```
instrument<SP> tetra<TAB>
version<SP>2.1.0.0<TAB>
function<SP>ok<TAB>
inst_status<SP>idle,remote,online<TAB>
exp_running<SP>no<TAB>
readplate<SP>default96,have<TAB>
sourceplate1<SP>default96,have<TAB>
sourceplate2<SP>default96,have<TAB>
sourceplate3<SP>default96,have<TAB>
loadplates<SP>none<TAB>
removeplates<SP>none<TAB>
platehandler<SP>idle<TAB>
plate_on<SP>no<TAB>
camera<SP>temp ok,0,0<TAB>
chamber_temp<SP>off,0,0<TAB>
waste_bottlea<SP>ok<TAB>
waste_bottleb<SP>ok<TAB>
fill_bottlea<SP>ok<TAB>
fill_bottleb<SP>ok<TAB>
```

```
tips<SP>on<TAB>
outer_auto_door<SP>open<TAB>
tip_washer<SP>ok<TAB>
chiller_status<SP>ok,18.0, 18.0<TAB>
intensifier_status<SP>ok<CR>
```

Cellflaskcontrol<TAB>Rate<CR>

Command Description

This command sets the stir rate for the cell flask control. After you send this command, the robotic controller should monitor the instrument stir rate to confirm that the instrument reaches the setpoint.

Command Parameters

There is one required parameter, rate, for this command.

If you use a value of zero for the rate parameter, the cell flask control stops stirring. If you use a valid value for rate, the cell flask control turns on and stirs at this rate. Valid values for rate are integers between 1–25.

Example Command Line

```
cellflaskcontrol<TAB>22<CR>
```

Example Response

```
ok<CR>
```

Suggested Steps in Command usage

1. Make sure an experiment is not running.
2. Send `cellflaskcontrol` command.
   Response is *OK* that confirms the command executed or a Condition code that confirms the command did not execute.
3. Monitor status until the instrument reports that it has reached the requested stir-rate.

Command Specific Error Responses

- C76 – Cell flask stirring rate parameter was not sent
- C77 – Parameter is out of range

Error Handling and Reporting

Interface Errors

Interface errors are errors generate when you issue a command. These errors are detected upon receipt of the command by the ScreenWorks Software and are reported in the response to the command. These errors consist of a single alpha character and a two or three-digit code.

Interface Errors

Error Code	Description
C01	Instrument is not in Remote mode. You can send only the status command when the instrument is in Manual mode.

Interface Errors (continued)

Error Code	Description
C05	Invalid plate location.
C10	Plate handler is busy.
C15	Plate location already has a plate.
C16	Plate location has no plate.
C20	Landing pad has no plate.
C21	Landing pad has plate.
C25	Experiment is running.
C35	Protocol could not be opened.
C36	Invalid protocol name.
C45	Folder does not exist.
C46	Invalid folder name.
C47	Invalid data file name.
C50	Current document is not valid protocol.
C55	No experiment is running.
C56	No protocols found in dir.
C57	The temperature parameter (int) was not sent.
C58	Can't load tips, tips on pipettor.
C59	Can't unload tips, no tips on pipettor.
C60	The temperature control system is not functioning.
C61	The aspirate speed is out of range.
C62	The pump speed can only be Slow or Fast.
C63	Invalid number of wash strokes.
C64	Invalid number of parameters for the wash tips command.
C65	Invalid number of wash cycles.
C66	Invalid protocol parameters discovered at run time.
C67	Invalid wash fluid parameter.
C68	Can't wash tips, tips are not on.
C69	Unrecognized or badly formatted command.
C70	Volume stroke too large.
C71	Volume stroke too small.
C72	Hold time is too small.

Interface Errors (continued)

Error Code	Description
C73	Hold time is too large.
C74	Dispense speed is too large.
C75	Dispense speed is too small.
C76	Cell flask stirring rate parameter was not sent.
C77	Parameter is out of range.
C79	Invalid volume parameter. It must be 'low' or 'high'.

The following table lists the errors for the runexperiment command. The errors explain how the protocol does not match instrument configuration or the instrument is not ready to run the protocol.

runexperiment Command Errors

Error Code	Description
E100	Invalid LED filter combination! Calibrate the LED filter combination or select a valid combination.
E110	Plate does not exist in Plate Library. Choose an existing plate or add this plate to the Plate Library.
E111	Plate is not present or is not in the proper location.
E112	Doors are opened. Close the doors before you start the experiment.
E113	Load Tips position not checked! Check the Load Tips position for auto load tips or manually load tips before you run the experiment.
E114	The chamber temperature is not in range.
E115	Experiment requires wash tips and there is no tip washer.
E116	Bottle A is empty! Refill it or use another bottle.
E117	Bottle B is empty! Refill it or use another bottle.
E118	Waste bottle A is full. Empty the bottle to continue.
E119	Waste bottle B is full. Empty the bottle to continue.
E120	Remove redundant plates.
E121	Error related to the data folder. The path is not valid, cannot be accessed, or there is not enough disk space.
E122	Error related to the export folder. The path is not valid, cannot be accessed, or there is not enough disk space.
E123	The read interval is too short. Check settings in Read views.
E124	Invalid aspirate parameters in the protocol.

runexperiment Command Errors (continued)

Error Code	Description
E125	Invalid dispense parameters in the protocol.
E126	The wash tips parameters are invalid.
E127	Invalid fluid bottle specified in wash tips process.
E128	Invalid Read Mode configuration. You tried to take fluorescence (with excitation) and luminescence (without excitation) read in one protocol. This is not allowed.
E129	Invalid Read Mode configuration. Camera gain is out of range. Check the protocol settings.
E132	Cell flask spinning rate could not be set. Check the instrument status.

Instrument Errors

The instrument errors that generate when you execute a command are detected during the execution of the command. They are reported in the status response in the function line and display a three-digit numeric code. Instrument errors are either recoverable or fatal. When fatal errors occur, the instrument cannot continue to do any operations and aborts any pending operations. You can provide instructions to the instrument for recoverable errors so that the instrument can continue.

The status command returns `ERRORCODE` or `FATALCODE`.

Instrument Errors

Error Code	Description
100-199	Contact Technical Support
200-299	Recoverable errors
300-399	Non-fatal errors
C15	Fatal errors
C16	Diagnostic errors

For a full list of these errors, see Troubleshooting on page 159.

Remote Interface Revision History

V1.0

First release.

V1.1

Added the following new commands:

- version
- loadtips
- unloadtips
- cyclecameratemp
- tempcontrolonoff
- washtips

Error code response include text description after code, for example, `c45, folder does not exist`.

V1.2

Added Error Code:

`C01, Instrument is not in Remote Mode`. Only status command can be sent while instrument is in Manual mode.

Bug Fix:

Commands made to the remote interface could sometimes take a long time and cause the remote interface to not respond to the client application within an acceptable timeframe. Client applications were able to work around this bug with massive TCP/IP timeouts (minutes) which made a true hang or network dropout undetectable. This workaround is no longer required.

The interface is unchanged, and if the client is correctly parsing the `status` response, no changes should be required in the client application, with the exception of reducing the TCP/IP timeout back to an acceptable value, for example, < 10 seconds. As always, rigorous regression testing of client code is encouraged.

V1.3

Added the following new commands to support new functionality in the Tetra Plus:

- statusex
- configuration
- cellflaskcontrol

Note: Tips location has changed from the Source 3 location to the Source 1 location.

Barcode Specifications

About Bar Codes

The FLIPR Penta System bar code scanner is an OEM module with decode capability to most commonly used bar code symbols:

- WPC (UPC /EAN/JAN
- IATA
- Code 39
- Interleaved 2 of 5
- Industrial 2 of 5
- Code 128
- Code 93
- MSI/Plessey
- Codabar (NW-7)

Bar Code Recommendations

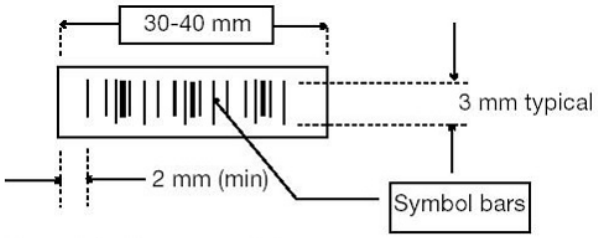
The bar code reader allows a bar code read down to 7.5 mil (0.20 mm) on photographic-quality symbols and ambient light < 450 ft-candles.

We recommend that the label stay above 7.5 mil (0.26 mm) since bar code printers might not have the resolution needed to match the aspect ratio for a minimum of 3 mm-high symbol lines. Before you adjust the bar code scanner to the bar code label location on the plate, follow these general guidelines for the preparation and quality of the bar code label:

- The minimum height or size of the symbol bars depends on the code symbol and the density you use. Keep the height of the symbol bars to more than 0.12 in. (3.0 mm).
- Do not change the aspect ratio required by the label printer. Do not force the bar code symbol size to fit on the label space i.e., maintain the aspect ratio free of distortion.
- Align the bars horizontally (side-to-side) and vertically (top-to-bottom) if possible, to keep space available on the bar code label.
- Keep a few millimeters (2–4) of empty dead or silent zone on both ends of the code-symbol lines. Keep all symbol lines away from the label corners and lifted edges. Optimize the characters so there is no need to cram a lot of lines on the label.
- Keep the longitudinal dimension of the labels/symbols to less that 1.25–1.50 in. (30–40 mm).

- Choose the media (label substrate material or paper) print or transfer ink to give good contrast and optimal reflection. Metalized, very shiny or clear background labels may be less reliable. Test a sample if you use a new color or surface material.

Bar Code Specifications



Appendix G: Electromagnetic Compatibility

Regulatory for Canada (ICES/NMB-001:2020)

This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

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

Information to the User (FCC Notice)

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at their own expense. Changes or modifications made to this equipment not expressly approved by the party responsible for compliance may void the FCC authorization to operate this equipment.

Contact Us

Phone: +1-800-635-5577
Web: moleculardevices.com
Email: info@moldev.com

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