FLIPR® Tetra

High Throughput Cellular Screening System

User Guide

MOLECULAR DEVICES

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Contents

Chapter 1: System Overview

This chapter provides an overview of the FLIPR® Tetra High Throughput Cellular Screening System requirements.

FLIPR Tetra systems are fluorescence- and luminescence-based microplate readers with an integrated 1536-, 384- or 96-pipettor. They perform rapid throughput cell-based assays while providing accurate and precise kinetic data. Primary applications include intracellular calcium mobilization and membrane potential. In addition, an expanded choice of wavelengths enables you to utilize a broad range of fluorescent dyes.

The FLIPR Tetra system includes:

- Simultaneous 96-, 384- or 1536-well liquid or cell transfer
- Expanded wavelength support
- User-configurable pipettors and optics
- Agile internal plate handling
- Standard, (EMCCD) camera for fluorescence applications or Aequorin, (ICCD) camera for fluorescence and luminescence
- Cell suspension option
- Slim platform with minimal facilities requirements.

An overhead pipettor delivers compound to all wells of the read plate simultaneously. A protocol file configured in ScreenWorks® Software—the system-control and analysis program for the FLIPR Tetra system—coordinates timing of compound delivery with multiple time-point exposures so that the resulting sequence of data points spans compound addition.

ScreenWorks Software displays relative light units versus time for all 1536-, 384- or 96-wells on the system's monitor. Updates occur in real-time as allowed by processing speed. Data can be exported as relative light units over time (time sequence), or as a single value per well (statistic). Export data files are in ASCII text file format for input into spreadsheet or database programs.

Fluorescence Mode

In fluorescence mode the system's LEDs illuminate the bottom of a 1536-, 384- or 96-well 'read' plate containing 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 binding of particular ions (for example, Ca2+, H+ or Na+) can be tracked.

Light-emitting diodes (LEDs) in the FLIPR Tetra system produce light at distinct wavelength ranges to excite the fluorescent dye that has been added to the cells in the read plate wells. The entire well plate bottom is illuminated. Fluorescent light emitted by the dye—again, for the entire plate—passes through an emission filter before being captured in a CCD camera, standard EMCCD or Aequorin ICCD. Fluorescence is measured from each well independently, and converted into a numerical value. The FLIPR Tetra system can be configured with two LED banks and three emission filters, allowing the software configuration of up to four excitation/emission wavelength combinations ('read modes'). Up to four different fluorescence effects can be measured within a single experiment.

Luminescence Mode

The FLIPR Tetra system also provides luminescence mode with the Aequorin, ICCD camera option. The instrument has a light-tight enclosure so that it can operate in luminescence mode and a specialized high-sensitivity ICCD camera can be installed in place of the standard EMCCD camera. The ICCD camera is mounted directly beneath the read plate, so images are taken of the entire bottom of the plate. For cell suspension experiments, an integrated Cell Reservoir allows uniform cell suspension to be pipetted directly into the read plate. From the 3 filter positions available on FLIPR Tetra system, it is recommended to have one open position, so no filter will be used during luminescence assay.

As with fluorescence, luminescence is measured from each well independently, and converted into a numerical value.

System Requirements

This section provides a brief overview of electrical, physical and environmental requirements of the FLIPR Tetra system.

Electrical

The FLIPR Tetra 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 is supplied with a power cord appropriate for the country it was shipped to. Additional shared outlets are required for computer and monitor. A power strip is acceptable for providing the additional outlets for the computer and monitor.

Minimum Space

System dimensions are as follows:

- Without Cell Suspension Module or TETRAcycler™: approximately 39 inches wide x 27 inches deep x 70 inches high (991 mm wide x 686 mm deep x 1780 mm high).
- With Cell Suspension Module and TETRAcycler: approximately 53 inches wide x 27 inches deep x 70 inches high (1346 mm wide x 686 mm deep x 1780 mm high).

The FLIPR Tetra system is designed with rolling castors so it can be readily moved to make necessary adjustments and perform maintenance. Leveling feet are also installed on the lower instrument chassis. These feet are typically used for stabilizing the instrument when integrated with a robot, but can also be used to establish a uniform instrument deck level in situations where the lab floor is not flat. When running an experiment, please make sure the instrument's feet are lowered and leveled.

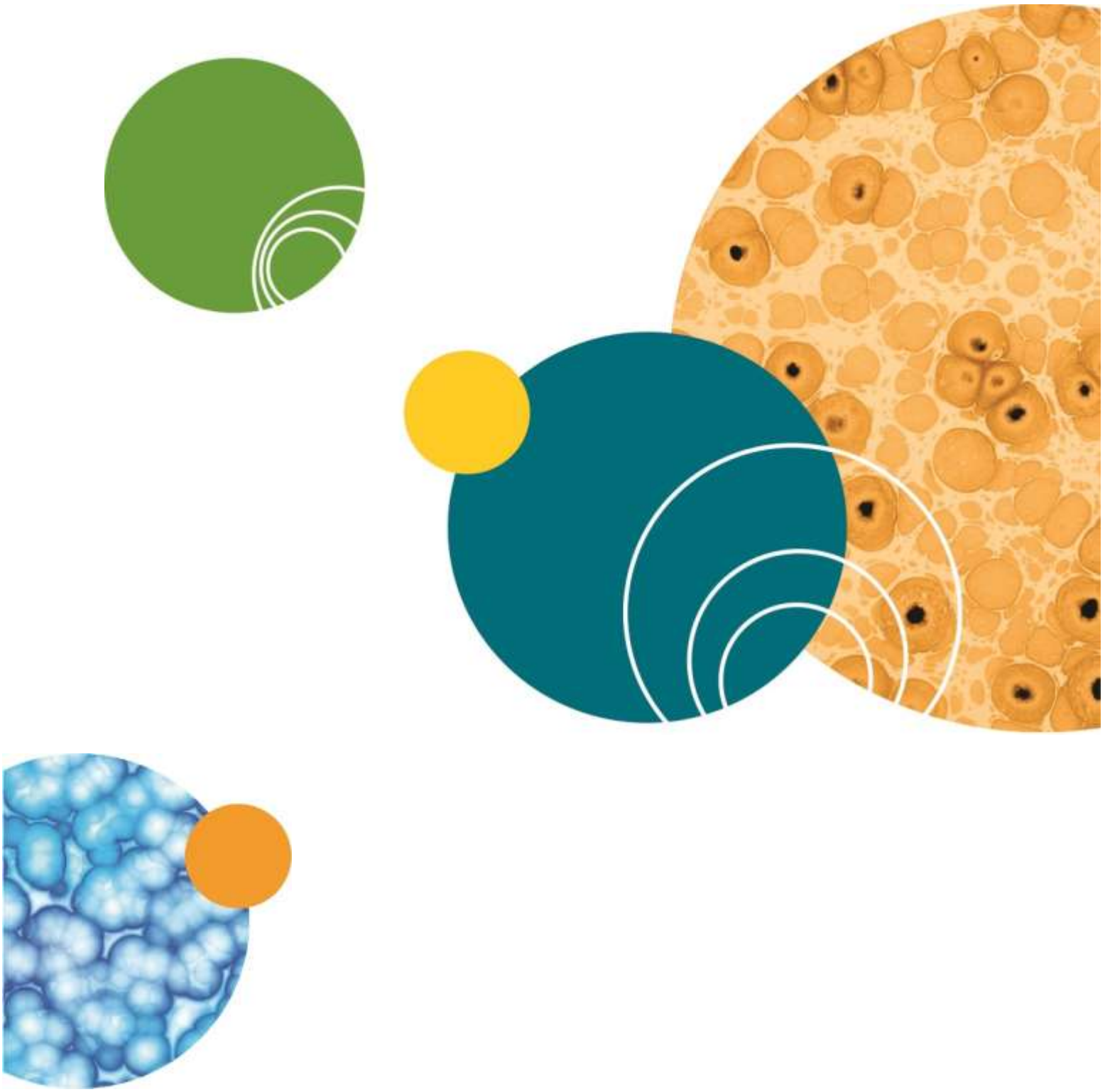
The computer and monitor are mounted to the right front side of the instrument with the included clamp, requiring a minimum lab space of 73 inches (1.85 m) wide x 82 inches (2.08 m) deep for maneuverability.

A chiller with dimensions 11 inches wide by 13 inches deep x 13 inches high (279 mm x 330 mm x 330 mm) is connected via a 3-foot (914 mm) long tube to the right side of the instrument. It can be placed anywhere within that 3-foot radius as long as the user has access to the on/off button on the chiller.

A minimum 25 inch (635 mm) square footprint for tip wash bottles is required to the right side of the instrument.

The cabinet should have a user access space of 48 inches (1.22 m) in front x 24 inches (610 mm) behind x 10 inches (254 mm) to the left for servicing the instrument.

WARNING! The FLIPR Tetra system can weigh as much as 860 lbs (390 kg). Ensure adequate personnel are present when installing or moving the system. Follow all necessary safety precautions and use proper moving techniques.



Chapter 2: System Hardware Features

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The FLIPR Tetra system consists of a cabinet 39 inches (965 mm) wide x 27 inches (686 mm) deep x 70 inches (1780 mm) high, with a number of components, including wash bottles, Cell Suspension module, chiller, host computer and monitor, outside the cabinet.

The cabinet has two compartments, top and bottom, accessed by manual doors on the front of the cabinet.

A 'five-position stage' located in the top compartment is where read and source plates are positioned during an experiment. There are also positions for tips and tip washing, as well as the Cell Suspension reservoir.

The TETRAcycler™ plate shuttle on the back wall of the top compartment can be used for robotically controlled carriage of compound or read plates and tips in and out of the cabinet during experiments. Plates are delivered to and from a landing pad outside the cabinet on the left hand side.

Mounted on the back wall of the top compartment, above the TETRAcycler, is the pipettor. The pipettor transfers compounds from source plates to the read plate, and accesses the tip loading and tip washing positions. When the Cell Suspension option is installed, the pipettor also transfers cells in suspension from the Cell Reservoir to the read plate.

The bottom, 'dry', compartment, houses the FLIPR Tetra system optics and an embedded computer for control of basic system functions. Two LED excitation modules, to the left and right, direct light up onto the base of the read plate, in the five-position stage above. Light emitted from the read plate passes down through emission filters directly below the plate to the camera (either the Standard EMCCD camera or the Aequorin ICCD camera).

The system computer, running ScreenWorks Software, through which all user interaction with the system occurs, is attached to the outside right-hand side of the cabinet. Monitor and keyboard are on an adjustable arm attached on the right-hand side of the cabinet front.

The Cell Suspension module, if installed, is also mounted on the lower right side of the instrument. This external module keeps the cells in suspension and is connected via internal tubing to a Cell Reservoir that is installed in Position 4 (Source Plate 3). The cells are kept in suspension via stirring and are pumped into the reservoir for transfer to the read plate. Up to 4 additional fluid bottles can also be connected to the reservoir for cleaning purposes.

Containers for tip washer fill fluid and waste are placed outside the cabinet beneath the computer.

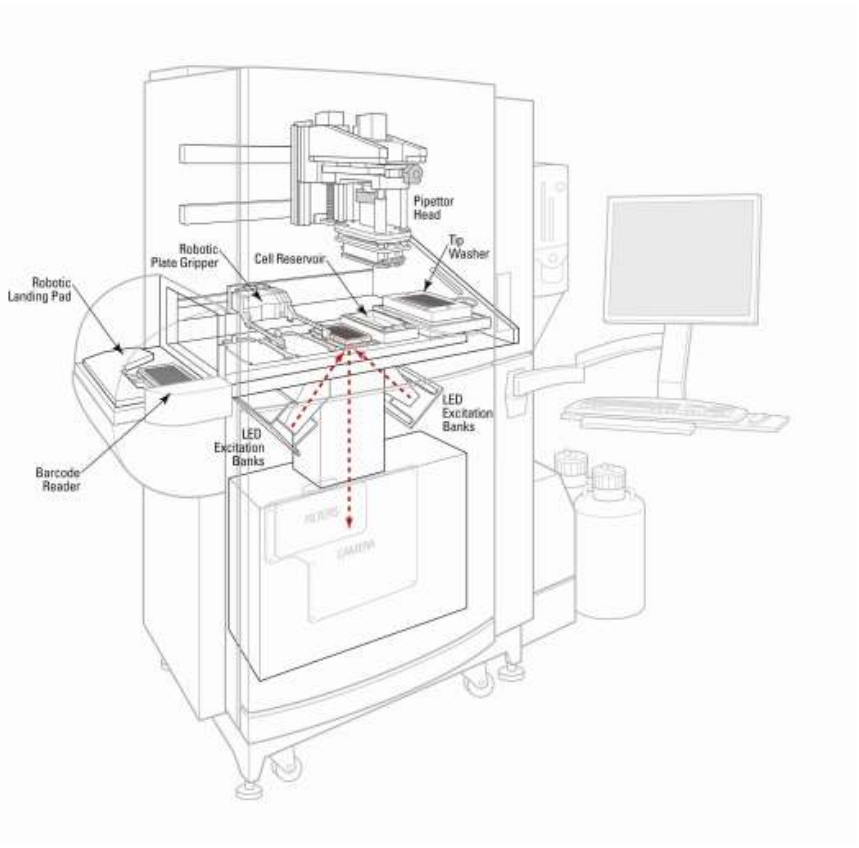


Figure 2-1: Diagram of the complete FLIPR Tetra system

Further information on these subsystems is presented in the following sections.

Plate-Handling System

Five-Position Stage

For an experiment, read and source plates are placed in the five-position stage in the upper compartment of the FLIPR Tetra system, where the pipettor is able to transfer compound between them. Plates can be loaded manually through the upper compartment door, prior to an experiment, or robotically, as the experiment proceeds , using the TETRAcycler.

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

- Position 1: Tips and/or Source Plate 1
- Position 2: Source Plate 2
- Position 3: Read Plate
- Position 4: Cell Reservoir and/or Source Plate 3
- Position 5: Tip Washer

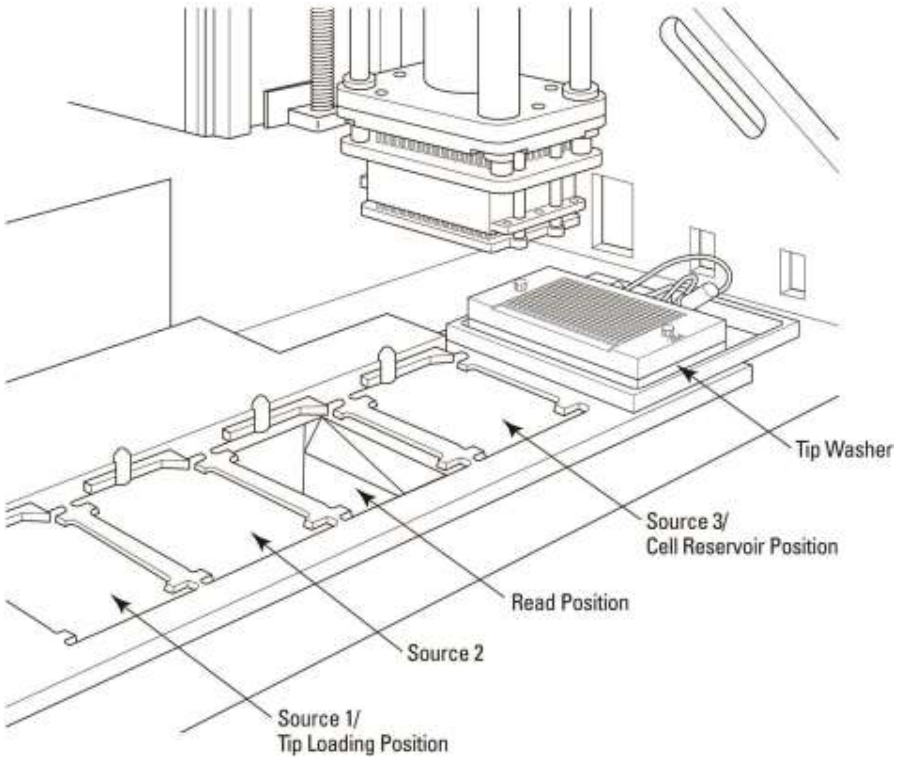


Figure 2-2: The five-position stage

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

Tip loading and unloading occurs in Position 1, but this position can double as a source plate position once tips are removed.

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

Position 4 can be used for the Cell Reservoir included with the Cell Suspension option. A single Cell Reservoir is compatible with all of the FLIPR Tetra system pipettor heads. When the Cell Reservoir is not present, this position can be used as a source plate position.

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

Robotic integration enables the TETRAcycler to exchange up to 12 source plates and tip racks, and one read plate, in an experiment.

A dedicated tip wash reservoir is located in Position 5 and should be configured to match respective pipettor heads (96, 384 or 1536). Appropriate tip wash reservoirs are included in the purchase of a pipettor head. Specific hardware components associated with tip washing are described in Tip and Pin Tool Washing on page 24.

Plates and tip racks are registered with well A1 in the lower left-hand corner using a plate indexer found in Positions 1–4. The indexers also serve as mechanical sensors to detect plate or tip presence. If plates or tips are not present in a Manual Mode experiment, but requested by software, the instrument stops and ends the experiment. During Remote Mode, the system notifies the SynchroMax™ ET or third-party plate-handler that no plate or tip container is present and stops the instrument until plates or tips are detected. It is then the responsibility of the SynchroMax ET or third-party plate-handler to deliver plates or tips to the system.

Note: Sensors can only detect plate or tip container presence. They cannot identify the type of plate or tips. It your responsibility to ensure that the correct plates and tips are loaded into position.

Temperature Regulation

Positions 1, 2 and 4, for source plates, have optional temperature control. Temperature settings range from ambient 5 °C (41°F) to 40 °C (104°F) . Equilibrium temperature may take approximately 15 minutes to reach the set temperature.

Configure temperature regulation with the **Temperature Control** ON/OFF toggle command in the **Instrument > Manual Operation** menu or corresponding button.

Note: The FLIPR Tetra system does not have humidified air flow.

Plates

FLIPR Tetra system accepts 96-, 384- and 1536-well plates that conform to the proposed ANSI standards submitted by the Society for Biomolecular Sciences. A sample of suitable source and read plates is provided in Consumables and Accessories on page 245.

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. Black walled, clear-bottomed plates or white walled plates can be used for luminescence assays.

For 96-well read plates, an optional slit-shaped mask can be used to minimize saturation and edge effects associated with these plates. Simply place the mask over the read position. See Consumables and Accessories on page 245 for types of masks available.

No mask is required for 1536- and 384-well plates.

Instrument Status Panel

The Instrument Status panel, located next to the upper door handle, indicates whether or not the instrument is running and safe to open. It includes an emergency Interrupt button to stop any processes.

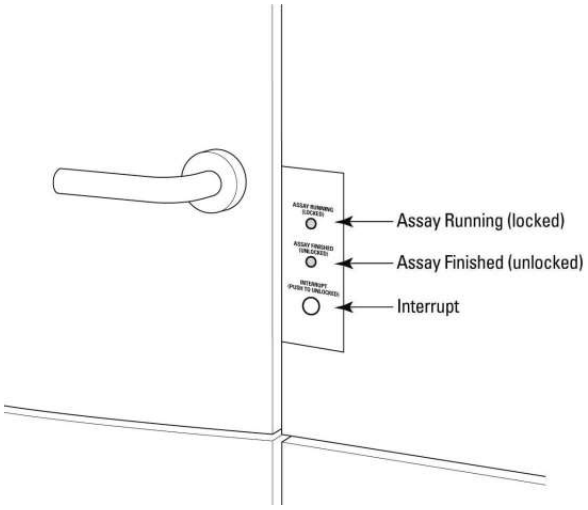


Figure 2-3: The Instrument Status panel

The panel has two lights as well as the Interrupt button. From the top of the panel these are:

- Assay Running (Locked)—Yellow light
  The FLIPR Tetra system is performing a task. The upper and lower doors are locked until the task finishes or is stopped 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.
- Interrupt—Flashing yellow light then green light
  A system override to stop all tasks, so you can access the instrument. If pressed the yellow light flashes until the system has reached a safe state to open the doors, when the green light comes on.

CAUTION! The Interrupt button immediately ends the experiment and should only be used in emergencies. The system might need to be reinitialized by selecting **Reset** from the **Instrument** menu prior to resuming normal instrument function.

Manual Mode

In manual operation all assay components must be positioned in the five-position stage by hand, through the upper manual door, prior to running an experiment. Once the experiment starts no further plate or tip changes can be made. If you need to exchange plates or tips during an experiment then you must run the FLIPR Tetra system in Remote Mode, using the TETRAcycler to replace used tips or plates.

The FLIPR Tetra system always starts in **Manual Mode**. Toggle between manual and remote modes with the **Set Manual Mode** and **Set Remote Mode** commands in the Instrument menu, or use the software buttons available.

In Manual Mode, the TETRAcycler gripper parks itself on the plate-landing pad.

Note: The top compartment door should remain closed during normal system operation. Do not operate the instrument if the door is open. All system functions halt when the door is open.

Robot Integration

To increase the number of plates you can use in an experiment (and minimize personnel requirements), use the TETRAcycler internal plate handler coupled with the SynchroMax ET or a third-party plate handler (for example, stacker system or robotic arm). One read and up to 12 source plates and tip racks can be shuttled in and out of the FLIPR Tetra system in one experiment with this method.

When using automated delivery the SynchroMax ET or third-party plate handler delivers plates to, and picks them up from, the landing pad on the outside left of the instrument, from where the TETRAcycler shuttles them in and out of the read 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 or third-party plate handler controls the FLIPR Tetra system by sending instructions to load protocols, run experiments, and retrieve plates from the landing pad. These commands are executed immediately upon receipt by the instrument. Persisting instrument settings cannot be made from the remote controlling program—these must be configured in ScreenWorks Software before control is passed to the plate-delivery system.

To pass control to the plate-delivery software select **Set Remote Mode** in the **Instrument** menu in ScreenWorks Software. The FLIPR Tetra system remains in remote control until **Set Manual Mode** is pressed.

The third-party plate handler software communicates with theFLIPR Tetra system computer via the serial communication port using TCP/IP. SynchroMax ET software is installed with ScreenWorks Software on the FLIPR Tetra system computer so it is able to communicate directly with the instrument. See Robotic Integration on page 189 for remote control syntax.

TETRAcycler™

The TETRAcycler is a plate gripper that runs along the back wall of the upper read compartment, giving it access to Positions 1–4 in the five-position stage and the landing pad on the outer left-hand side of the cabinet. It shifts source plates and tip containers between these locations when under the control of the SynchroMax ET or a third-party plate handler (see above).

The TETRAcycler carries standard, low volume and deep well 96-, 384- and 1536-well plates that conform to propose ANSI standards submitted by the Society for Biomolecular Sciences. In addition, the TETRAcycler handles Molecular Devices qualified tips. Reservoirs can be used during robotic integration, however the TETRAcycler is not able to move these. All reservoirs must be loaded manually prior to running an experiment, including the Cell Reservoir.

Note: While the system is compatible with plates that conform to proposed ANSI standards submitted by the Society for Biomolecular Sciences, some plates may not be handled as reliably by the TETRAcycler due to their low plate weight. During robotic integration, it is recommended that handling of the plates and tips by the TETRAcycler be evaluated for plate handling robustness prior to starting a screen.

The upper and lower door of the FLIPR Tetra system must remain closed for the duration of the experiment. Plates are transported in and out of the instrument only by the TETRAcycler system robotic landing pad door.

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

SynchroMax ET™

The SynchroMax ET is a six-stack plate handler available as an optional purchase with the FLIPR Tetra system. It delivers plates to and from the landing pad, integrating with the TETRAcycler, which ferries the plates to and from their appropriate locations in the five-position stage. The configuration interface of the SynchroMax ET software is opened directly from within ScreenWorks Software, making experiment configuration straightforward.

Observation Panel

In order to view hardware movements in the upper top compartment while troubleshooting the FLIPR Tetra system, use the observation panel. Under normal operating conditions the upper door must be closed in order to run an experiment, ensuring no light enters the chamber. When the observation panel is mounted to the chamber, however, the door can be left open, allowing you to view movements of the pipettor and TETRAcycler. Normal instrument control is performed via ScreenWorks Software, SynchroMax ET, or third-party plate handling software. For the Aequorin ICCD camera test images are displayed.

The observation panel is stored attached to the inside of the upper door. To mount the panel, remove it from the door and attach it with the four captive thumbscrews to the top compartment frame.

To acquire quality data, reaffix the observation panel to the inner door prior to running an experiment.

WARNING! If pretending to run in luminescence mode with the Aequorin ICCD camera, DO NOT touch the white door switches. Room light will damage the Intensifier. The door switches detect the open door to protect the camera.

Note: The observation panel should only be used to view internal pipettor movement. It should not be used during experiments when data is being accumulated. Test data (in the case of the Aequorin ICCD camera) or compromised data (with the Standard camera) shows if not collected under dark conditions with closed doors.

Liquid-Handling System

Compounds are transferred from source plates or reservoir to read plates by the pipettor mounted on the rear wall of the top compartment, above the TETRAcycler. The pipettor assembly can be fitted with a standard pipettor head, to use disposable tips, or a pin tool head, which uses solid or slotted pins to carry compound.

All 1536, 384, or 96 tips or pins operate at the same time, simultaneously picking up compound from all the wells in a source plate (or a quarter of the wells; see Compatible Plate Configurations on page 23) or Cell Reservoir, and similarly dispensing these simultaneously to the read plate. Fluid mixing steps can be configured for source plates before compound is picked up, and for read plates once it has been dispensed.

Pipettor heads are user-installable and can be interchanged in approximately less than 5 minutes; see Exchanging Pipettor and Pin Tool Heads on page 105.

Standard Pipettor Head

Standard pipettor heads are available in 1536-, 384- and 96-tip formats.

The 384- and 96-pipettor heads both use disposable plastic tips. In contrast, the 1536-pipettor head uses a stainless steel tip block with a disposable 1536-tip gasket.

Plastic tips can be washed or replaced between each compound addition or at the end of an experiment. The 1536-tip block is washed at specified times.

Pipettor operations are controlled from within ScreenWorks Software protocols, or some operations, for example, loading tips, can be performed individually, directly through commands in the **Instrument** menu. Connectors on the back of the pipettor head identify the head format as 1536, 384, or 96 tips, so ScreenWorks Software only 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. The volume of compound to be transferred is configured in the software, and it is possible 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 96- and 384-pipettor heads displace air in the disposable pipette tips. In the 1536-pipettor head a plunger for each of the 1536 tips 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. Once the seal is created, further plunger movement causes air displacement in the tip block.

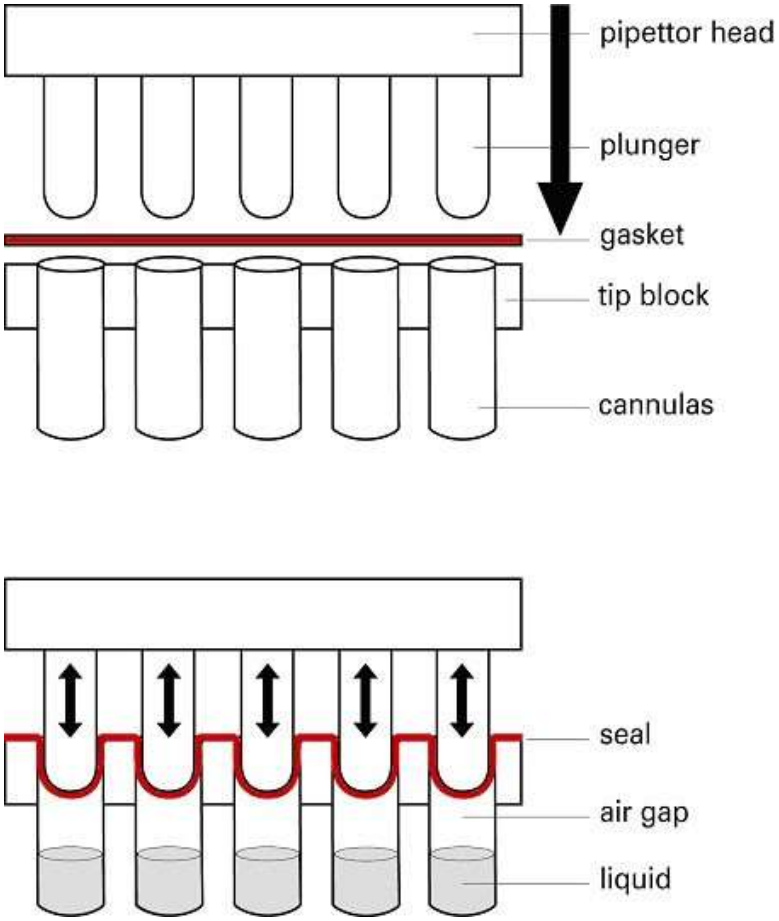


Figure 2-4: Seal creation in the 1536-pipettor head

Minimum pipettor precision is as follows:

- 3% for 75 µL additions (96-well).
- 4% for 25 µL additions (384-well)
- 6% for 3 µL additions (1536-well)

Performance is dependent on tip/gasket seating and can be compromised if the seal is broken. Use only Molecular Devices recommended tips and gaskets to ensure the highest accuracy and to reduce the possibility of damaging the pipettor. See Consumables and Accessories on page 245 for recommended tips.

Cell Suspension

The Cell Suspension option consists of two components:

- The Cell Reservoir installable in Position 4 (Source 3) in the 5 position stage.
- The Cell Suspension module located externally on the right side of the instrument.

The Cell Reservoir (see Figure 2-5) is user installable. The Cell Suspension module (see Figure 2-6) consists of a shelf with a magnetic motor mounted underneath it, a cell flask with a magnetic stirrer, up to four fluid bottles for automated cleaning, and a removable cover for keeping cells in a dark environment.

Figure 2-7 demonstrates how the system is connected and shows all the possible combinations for protocol development.

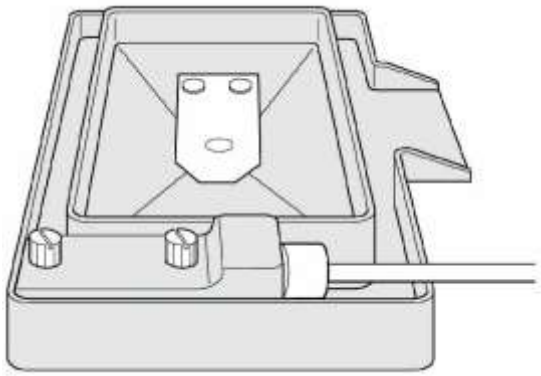


Figure 2-5: Cell Reservoir

The Cell Reservoir, which the user places in a source plate location, is filled from any of the bottles in the external Cell Suspension module by a pump with adjustable speed and direction.

The Cell Reservoir is a special plate type that has one fluid line used for both input and output, and an electronic plate ID that is part of the electrical/fluidic connector which identifies the reservoir to the system. In the case of an overflow, an overflow trough catches excess fluid and pipes it to the tip washer overflow trough, which directs it to the overflow bottle. The reservoir is also autoclavable for cleaning purposes.

The spinner flask contains a stirrer, which is driven by a magnetically coupled motor mounted in the lower part of the Cell Suspension module. The Cell Suspension stir speed can be set in a protocol or with manual instrument controls. Stir speed of 5 equals approximately 1 revolution per second. From the uniform suspension in the spinner flask cells are pumped into the Cell Reservoir, where the pipettor head in 96, 384, or 1536 format removes the appropriate amount and cells are automatically pumped to a specified destination. A protocol in ScreenWorks Software controls the stir speed and source/destination of cell suspension activity.

The cell valve selects the source for filling, or the destination for draining. There are 8 valve positions: flask fill (for filling the reservoir), flask drain (for draining the reservoir without causing air bubbles), Waste Bottle A, Waste Bottle B, and Fluid 1–4.

Fluid 1–4 are user specifiable, and can be cleaning solutions, water or buffer. The user can choose to pump cells back into the cell flask or to any other fluid bottles. Bottles for Fluid 1 and 2 are automatically included with the Cell Suspension option.

Cell Reservoir can be washed by either adding the Wash Reservoir process to the protocol (see Constructing Protocols Using FLIPR Tetra Processes on page 57), selecting **Wash Reservoir** in the **Instrument > Manual Operation** menu, or manually removing the reservoir and autoclaving it.

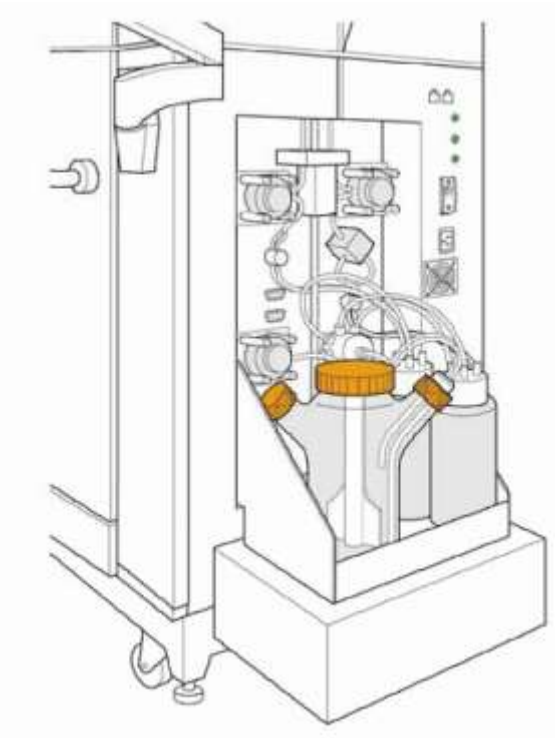


Figure 2-6: Cell Suspension Module

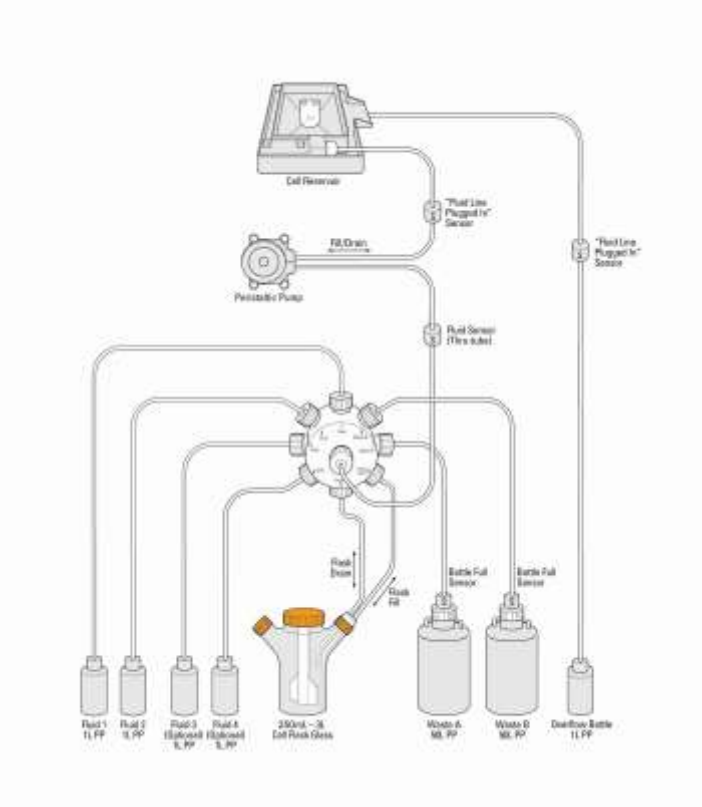


Figure 2-7: Cell Suspension Module connections

Pin Tool Head

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

Pins for the 384 pin tool are supplied in four sizes, giving a total range (across all these sizes) from 84 nL to 656 nL. The 1536 pin tool has seven different pin sizes, giving a total range from 19 nL to 117 nL.

Each pin size has a specified 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.

The precise volumes that will be picked up at given tip removal speeds should be determined by users in assay development.

The FLIPR Tetra system can be fitted with 384- or 1536-pin tool heads. Pin tools themselves, in the appropriate 384 or 1536 format, can be easily and rapidly replaced to change the pin size.

All the pin tools used with the FLIPR Tetra system are available with a hydrophobic and lipophobic coating to prevent or reduce the nonspecific binding of proteins and lipids to the pins.

In order to ensure uniform compound pick-up across the entire pin tool, pins can be configured to 'float' in source plate wells. Individual pins are not rigidly attached to the pin block, having 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 their wells, for example, sitting on the bottom. This outcome could not be guaranteed if the pins were rigidly fixed to the block, given that plate bottoms are often not completely flat.

Compatible Plate Configurations

The 96- and 384-pipettor heads can be used with source or read plates with equal or one order higher well number. This is because the FLIPR Tetra system can aspirate or dispense into quadrants of a plate. The following combinations are possible:

- The 96-pipettor head can be used with 96- and 384-well plates.
- The 384-pipettor and pin tool head can be used with 384- and 1536-well plates.
- The 1536-pipettor head can be used only with 1536-well plates.

Deep-well plates or reservoirs can be substituted for standard well plates.

When compound is aspirated or delivered to a plate with a greater number of wells than the pipettor head, the quadrant number (1 to 4) must be entered in the protocol configuration in ScreenWorks Software for each dispense.

Tip and Pin Tool Loading

For all pipettor and pin tool heads, a sensor informs the software whether or not tips or a pin tool are loaded. If desired, at the start of an experiment, tips or a pin tool can be automatically loaded onto the pipettor head by selecting **Load Tips Position** in the Settings process. Otherwise, tip and pin tool loading or unloading must be requested as independent operations from the **Instrument > Manual Operation** menu. Directions for installation of the 1536 tip gasket are given in Exchanging the 1536 Tip Gasket on page 113.

Tip and pin tool loading steps can be programmed to occur between fluid transfers within experiment protocols.

Tip and Pin Tool Washing

Tip or pin tool washing is controlled with the Wash Tips or Wash Pins process in ScreenWorks Software protocols and can be performed between fluid transfers within an experiment, or after the last fluid transfer, to prepare tips for the next experiment. Disposable tips, as well as the 1536-tip head, and 384- and 1536-pin tools, can be washed.

The washer consists of a reservoir top of the selected pipettor format, mounted over a wash basin. Detailed instructions for exchanging the reservoir top are located in Uninstalling Wash Reservoir Top on page 111. 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.

Wash solution fills the reservoir for a calibrated amount of time. Solvent is then drained from the reservoir after each wash cycle. Up to five wash cycles can be configured within a single wash process. For tips, a user-set volume of solvent is drawn up, optionally 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. The option is available to wash tips or pins in up to two solutions before reusing the tip washer. When additional wash solutions are required, tips or pins can be washed in a boat or reservoir, located in one of the source plate positions, using the **Mix Fluid** process.

WARNING! High volumes of volatile, flammable solvents in the reading chamber may cause explosive conditions. Use of 100% isopropanol, etc., in the tip washer is particularly 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 can be loaded into one of the plate positions. Blot pin steps can be configured in the protocol to remove fluid from the pins, for example, following pin washing.

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

Optical System

The FLIPR Tetra 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.

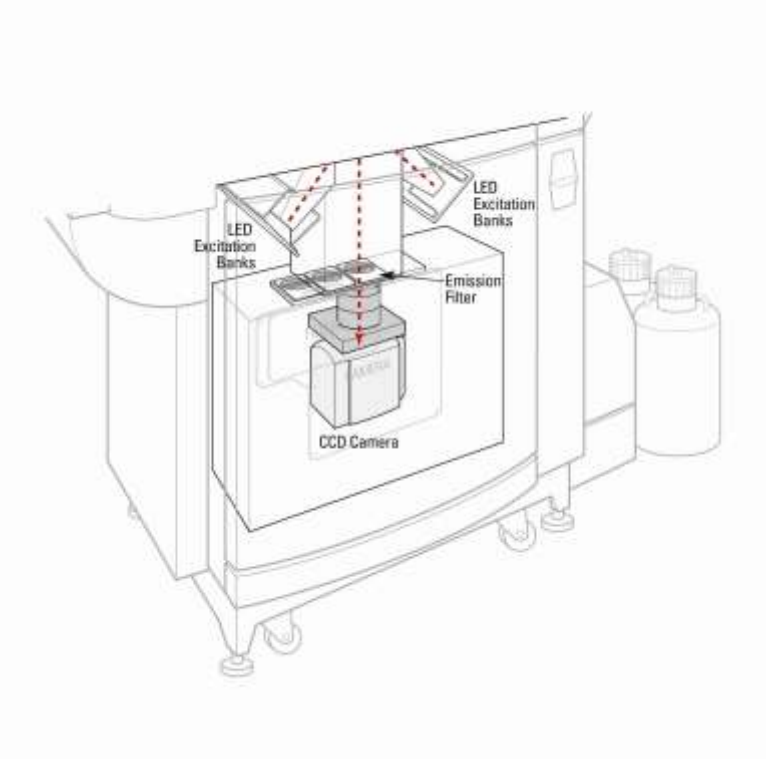


Figure 2-8: System Optics

CCD Camera Options

Two camera options are available for purchase with the FLIPR Tetra system. A standard, EMCCD (Electron Multiplying CCD) camera is recommended for fluorescence only experiments, while the more sensitive Aequorin ICCD (Intensified CCD) camera is designed for both fluorescence as well as luminescence assays.

Standard EMCCD Camera

The CCD 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 -60°C (-76°F) ± 2 °C (35.6°F).

WARNING! Do not use the camera before it has reached its operating temperature—this will result in noisy data. Check the camera temperature on the Instrument Status panel before starting 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 method allows a high acquisition rate and eliminates the possibility of camera shutter failure.

The camera is an integrating-type detector using 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), it may be necessary to use 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 specified in the ScreenWorks Software protocol; exposure time can be set from 0.05 to 30 seconds. 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 Tetra system reports relative light units (RLUs) in a range from zero to approximately 12,000.

Note: Relative light units in the FLIPR Tetra system do not have the same value as those of previous FLIPR Tetra systems.

The light intensity detected by each pixel on the CCD chip can be amplified using the Gain setting in ScreenWorks Software (**Settings Process**). This parameter has a range from zero to 240. Amplification is exponential, with increments increasing as you go higher up the range. Fluorescence assays typically use a gain of 130 whereas luminescence assays should use 200 as a starting point. Gain optimization should be done during assay development to determine optimal conditions for your screen.

When a luminescence experiment follows a fluorescence experiment we recommend that you cycle the camera temperature to eliminate ghost images that may have been created during the fluorescence assay. Select **Cycle Camera Temperature** from the **Instrument > Manual Operation** menu to choose this option. The camera warms up to room temperature to release ghost images, prior to cooling back down to approximately -60 °C (-76°F).

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

Aequorin ICCD Camera

When this option is selected instead of the Standard camera, the ICCD camera is mounted directly beneath the read plate on the five-position stage, although at a slightly different height then the Standard EMCCD camera. This camera operates at -20 °C (-4°F) and requires about 5 minutes to reach that temperature.

Note: The instrument will detect an error if the user tries to operate the instrument with the camera out of its recommended temperature range of -20 °C (-4°F) ± 5 °C (-41°F).

Similar to the Standard camera, the Aequorin camera also uses frame-transfer technology that shifts pixel values from the exposed portion of the chip to the data processing portion. In this camera, however, the signal is enhanced prior to reaching the CCD chip: it is amplified in the intensifier. Using this method there is less noise and therefore the camera is significantly more sensitive then the Standard EMCCD camera.

When using the Aequorin ICCD camera images are taken of the bottom of the plate, amplified in the intensifier and transferred via a fiber optic taper to the CCD chip. The gain control of the intensifier allows for bright signal from fluorescence as well as dim signal from luminescence to be enhanced accordingly, so as to provide the best signal and not saturate the CCD chip below. In Fluorescence mode the Gain is preset to 2000, whereas in Luminescence mode the Gain defaults to 280,000, but can be lowered in the event that the luminescence assay is very bright.

This camera has a Gate Open % feature, adjustable in Fluorescence mode only. This feature controls how long the intensifier is on for each of the frames collected during the exposure time. This controls the signal intensity of the assay.

The recommended value range for the ICCD camera is 40,000 to 50,000 (at maximum signal). This camera does not require temperature cycling when changing between **Fluorescence** and **Luminescence** modes.

Both Cameras

Once calculated, RLUs are displayed in real time in the ScreenWorks Software **Analysis** process window (within the limits of computer processing speed). Data for one wavelength (for example, **Read** mode), for all 1536, 384 or 96 wells of the read plate, are displayed in the Multi-Well Graph on the right side of this view. The **Detail Graph** can be populated by selecting wells in the Multi-well graph. When an experiment is completed, post-assay analysis can be done via ScreenWorks Software, or data can be exported.

In normal operation images are discarded once RLU values have been measured. However, for quality control purposes, users can define the number of images per **Read with Transfer Fluid** step. Up to 100 images per experiment can be retained. These images can be useful for troubleshooting problems, such as cells lifting from well bottoms during compound addition. Images are saved as *.tif files with the same name and to the same directory as the data file. They can be viewed by clicking the **Image** button in the Analysis process page when the resulting data file is open in ScreenWorks Software.

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 for determining assay robustness is the Z' factor equation.

LED Modules

The FLIPR Tetra system has a total of four LED banks providing illumination for plate reading. The LED banks pulse on, two at a time, only when an image is to be captured, protecting cells from possible 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, helping to 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. Ratiometric experiments can be set up to use both of these 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 Tetra system will not operate without the full complement of LED banks installed, however blank LED banks can be used for one pair if only one excitation wavelength is available. Unless additional LED banks are ordered with a purchase, FLIPR Tetra systems are shipped with a set of default calcium LED banks (470–495 nm) and a set of blank LED banks.

Configuration of LEDs for an experiment is mostly carried out in ScreenWorks Software in the Settings window; see Setup Read Mode on page 59 for details. LED banks can be changed by the user in approximately 10 minutes; refer to Exchanging LED Modules on page 116 for instructions.

LEDs do not need time to warm up prior to running an experiment. Startup time is only dependent on the time it takes for the camera to cool down and for the stage to heat up, if this option is used.

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

Flat field calibration is automatically applied to the read plate to adjust for non-uniformity of illumination across the plate. Refer to Optical Calibration on page 127 for instructions on how to manually calibrate the system.

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

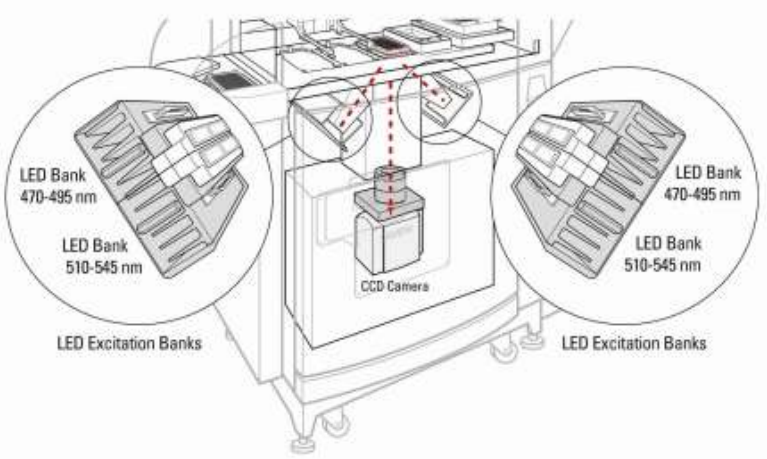


Figure 2-9: LED configuration

Emission Filters

A three-position filter slider holding up to three 60 mm diameter interference filters 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 being used. These filters can be used with a single excitation wavelength or can be 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 that each image taken by the camera matches the right emission filter with the excitation LED bank. The most common FLIPR Tetra system configuration is a LED excitation wavelength of 470–495 nm with a 515–575 nm bandpass emission filter. For luminescence experiments it is also possible to run without the filter.

Emission filters are user-changeable in approximately 5 minutes; see Changing Filters on page 120 for instructions. Once installed, filters are mechanically sensed and the filter configuration can be viewed in the **Instrument Configuration** panel in ScreenWorks Software. The instrument will prompt you to calibrate a new emission filter with respect to an LED module if they are intended to be used in an experiment.

Note: If a desired filter is not available, three custom filter cassettes (P/N 0200-6221) are available for purchase to place filters created by an outside vendor. Once installed, these filters are displayed as Custom 1, Custom 2 and Custom 3 in ScreenWorks Software.

Chiller

Because the FLIPR Tetra system is a light-tight instrument there is very limited airflow inside the instrument enclosure. To provide a suitable operating environment for both the Standard EMCCD and Aequorin ICCD cameras, an external chiller is required. The chiller uses a special cooling liquid supplied with the instrument and is controlled via the embedded computer and instrument firmware. The chiller sits outside of the instrument and is connected via cable and tubing to the FLIPR Tetra system.

Computer System

Host Computer

Apart from exchanging hardware and manual loading of plates, all normal user interaction with the FLIPR Tetra system is mediated through the ScreenWorks Software run on an external host computer, supplied with the system. The minimum configuration required is as follows:

- 3.4 GHz processor
- Windows XP Professional or Windows 7 operating system
- 2 GB of SDRAM
- 160 GB hard drive
- Ethernet adapter
- DVD-CDRW drive
- 1 PCI expansion slot

Embedded Computer

An embedded computer located in the lower chamber controls basic FLIPR Tetra system functions. These functions are initiated through the ScreenWorks Software control software installed on the host computer and sent to the embedded computer to execute the function. This setup allows data processing and instrument control to be performed separately to ensure the greatest productivity during an experiment.

Chapter 3: Startup and Shutdown

3

This chapter provides procedures for starting up and shutting down the FLIPR Tetra High Throughput Cellular Screening System These procedures should be followed closely in order to ensure proper communication between ScreenWorks Software and the hardware.

Starting Up the System

To start the FLIPR Tetra system:

1. Turn on the computer and monitor.
2. Simultaneously press the **CTRL+ALT+DELETE** keys to launch the Windows operating system.
3. At the prompt enter your password.

Note: After installation, the default password is **FLIPR**.
Wait for the operating system to finish starting-up before proceeding.

4. Turn on the external chiller with the switch located on the left side of the chiller.
5. Turn on the FLIPR Tetra system power switch located on the right side of the instrument. The system goes through an initialization cycle to register all instrument components. This cycle is not complete until the green Assay Finished (Unlock) light is the only light illuminated on the instrument status panel.
6. Start the ScreenWorks Software by double-clicking on the desktop icon.

Note: Starting the software can take several seconds. Do not repeatedly double-click the software icon.

Note: The system is ready for use when the camera temperature in the instrument status window displays -60°C (-76°F) ± 2 °C (-35.6°F) for the Standard EMCCD camera, or -20 (-4°F) ± 5 °C (-41°F) for the Aequorin ICCD camera.

Shutting Down the System

To shut down the FLIPR Tetra system:

1. At the end of a programmed experiment, wait for the **Assay Finished (Unlocked)** light on the **Instrument Status Panel** to turn green, indicating the experiment is finished.
2. If the last protocol did not remove the tips from the pipettor head, Molecular Devices recommends making sure that you remove them using the manual command. Failure to remove tips can result in an error on next start.
3. Exit ScreenWorks Software by selecting **File > Exit**.
4. Turn off the computer and monitor.
5. Turn off the FLIPR Tetra system power switch.
6. Turn off the chiller power switch.

Chapter 4: Software Installation

This chapter describes how to install ScreenWorks System Control Software.

You must have administrative privileges on the computer to install ScreenWorks Software.

Installing ScreenWorks Software

As of version 3.1, the installer automatically uninstalls the old software version as long as it is same major release (3.1 to 3.2). If you are replacing ScreenWorks Software version 3.2, with version 4.0, manually uninstall the software. See Uninstalling ScreenWorks Software on page 36.

1. Double-click the **ScreenWorks_4_0_x.exe** ScreenWorks Software installation file.A **Welcome to the ScreenWorks Setup Wizard** dialog is displayed.
2. Click **Next**.
3. In the **License Agreement** dialog box, select **I accept the terms of the license agreement**, and click **Next**.
4. In the **Online/Offline Mode** dialog, designate the default mode in which you want the software to start.
   - In **Online**, ScreenWorks Software automatically looks for a connected instrument when the software is started.
   - In **Offline**, ScreenWorks Software does not automatically look for a connected instrument.

   See Online vs. Offline Installation on page 34 for details.
5. Click **Next**.
6. In the **Destination Folder** dialog, the **Install ScreenWorks 4.0 to** field displays the default installation directory. To change the installation directory, click **Change**, navigate to the desired directory, then click **OK**.
7. Click **Next**.
8. In the **Select Program Folder** dialog, leave the displayed default **Program Folder** settings. Select **Anyone who uses this computer** to make ScreenWorks Software available to all users on the FLIPR Tetra system host computer, then click**Next**.
9. In the **Configuring the ScreenWorks** installation dialog, if you want to make any changes, click **Back** to go to the previous screen, otherwise click **Next** to start the installation.
10. When the installation is complete, the **Completing the installation process** dialog appears. Click **Finish** to exit the wizard.

Activating the ScreenWorks Peak Pro License

The ScreenWorks Peak Pro™ functionality is license-protected. The software license activation enables the Peak Pro functionality any time after the trial period expires.

Note: There is a one time 14-day Peak Pro trial usage option in the Help menu. When the trial period expires, the Peak Detection functionality disappears, and the Help menu trial option is inactive. The rest of the ScreenWorks Software version 4.0 remains functional.

To activate the ScreenWorks Peak Pro Software license:

1. Start the ScreenWorks Software application.
2. Click the **Help** tab.
3. Click **Software License**.
4. If you have not started the available 14-day Peak Pro trial, the option to start the 14-day Peak Pro trial, or enter the product key appears. Click **Yes**.
   If the trial has expired, only the option to provide a software license product key appears.
5. If you have internet connectivity, type the provided **Product Key** in the field and click **Activate Online**, and then follow the on-screen instructions.
6. If you do not have Internet connectivity, click **Activate Offline** and follow the on-screen instructions. Activate Off line requires the following:
   - Your product key
   - A separate computer with Internet connectivity
   - A USB drive for transferring files between the computers

Online vs. Offline Installation

The software has two start-up modes:

- Offline (Desktop)
- Online (Instrument)

The default start-up mode is determined during software installation in the **Online/Offline** dialog.

After the software is open, you can switch modes by selecting from the **Instrument** menu, **Go Online** or **Go Offline**.

Note: Switching modes after the software is opened does not change the software startup mode chosen at the time of installation. To change the default startup mode the ScreenWorks Software must be reinstalled.

Online (Instrument) Mode

When launched in this mode, ScreenWorks Software checks for instrument connections. If no connections are sensed, you are notified. You may then either check the connections and attempt to connect again, or choose to run the software in Offline mode.

If you create a protocol in Online mode, only the current instrument settings are allowed. Protocols created in Offline mode with hardware settings that do not match current hardware settings are flagged. You must change the hardware settings to match those in the protocol in order to run it.

When ScreenWorks Software is launched 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 are running in Online mode and then switch to Offline mode, the instrument setup configuration will be the last Online configuration.

Note: To be able to select any configuration to generate protocols, you must install the software in Offline (desktop) mode.

Offline (Desktop) Mode

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

- Camera Type
- Excitation Wavelengths
- Emission Wavelengths
- Pipettor (automatically selects matching tip washer type)
- Cell suspension
- TETRAcycler™ (automatically sets bar code reader status)

Note: Regardless of the start-up mode, pipettor head and tip washer type must always match. If the TETRAcycler is installed, it is assumed the bar code reader is also connected.

Uninstalling ScreenWorks Software

1. Click **Start > Control Panel** and double-click on **Add or Remove Programs** from the Windows **Control Panel** dialog.
2. Find **ScreenWorks** in the list of currently installed programs and click **Remove** to initiate the uninstall process.
3. When prompted to **Uninstall** ScreenWorks, click **Next** .
4. In the **Configuring the ScreenWorks installation** dialog, Click **Next**.
5. When the installation is complete, the **Completing the installation process** dialog appears. Click **Finish** to exit the wizard.

Chapter 5: ScreenWorks SoftwareOverview

5

This chapter describes windows, menus, dialog boxes, and toolbar icons of the ScreenWorks Software.

Main Screen

The ScreenWorks Software main screen includes title, menu, and toolbars across the top, and a status bar at the bottom. The main working area in the middle can have up to two sections:

- Experiment window— central proportion of the main window, for protocol configuration, data viewing and analysis.
- Instrument Status Panel— at the bottom of the main work area for displaying instrument information. This panel includes instrument status and configuration in addition to the Process Explorer where processes used in the protocol definition are located.

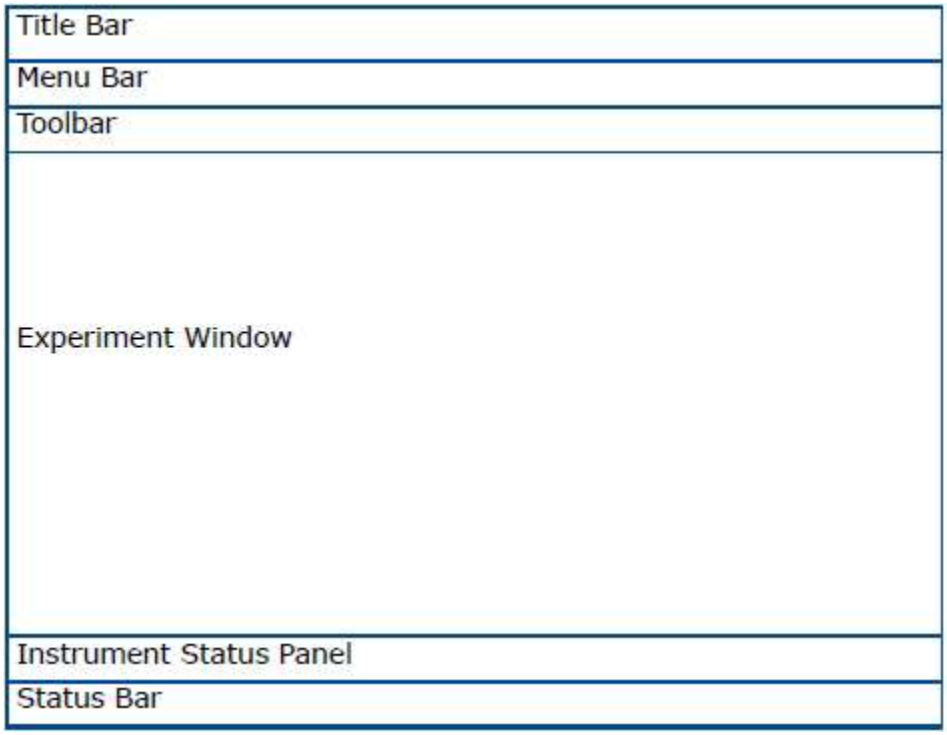


Figure 5-1: Main Interface Screen Diagram

Title Bar

The title bar extends across the top of the ScreenWorks Software window. It reports the application name—**ScreenWorks** and version number—followed by the name of the currently active protocol or data file, open in the Experiment window.

Title bar color indicates whether or not the window is active: the title bar of an active window is typically a different color from (and usually brighter than) other window title bars for programs that are inactive (which might be dimmed).

Dragging the title bar repositions the window on the screen (in window view only; if the window has been maximized, dragging does not work). Buttons are displayed at the right end of the title bar can be used to minimize the window so it appears only on the task bar, maximize the window to full screen, or to close the window.

Menu Bar

The menu bar, beneath the title bar, contains six menus that group together related commands. Click on a menu name to display the commands in the menu.

See Menu Bar on page 41 for a full description of each menu command.

Toolbar

The toolbar, beneath the menu bar, contains tool button shortcuts for a number of main-menu commands.

The toolbar can be hidden or shown from the **View > Toolbar** toggle command.

The table below lists the toolbar commands. See Menu Bar on page 41 for more detailed descriptions of the commands.

Button	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	Opens the **Open File** dialog to browse and open protocol (*.fmp) files.
	Open Data File	Opens the **Open File** dialog to browse and open data (*.fmd) or image files.
	Save File	Opens the **Save File** dialog that allows you to save the current file in the desired location.

Button	Name	Description
	Export File	Opens the **Export File** dialog that allows you to manually export the current file in the desired location.
	Print	Opens the **Print** dialog.
	Experiment Summary	Displays protocol contents (process steps and correction) for the current protocol or data file. This button toggles with **Experiment Setup**.
	Experiment Setup	Opens the Experiment Setup window to edit the current protocol, view or analyze data in a data file. This button toggles with **Experiment Summary**.
	Help	Opens the *FLIPR® Tetra System Users Guide* in PDF format.
	Stop	Stops the experiment currently running.
	Run	Starts the protocol selected in the Experiment window.
	SynchroMax Automation	Opens the SynchroMax™ Automation window to select the desired template to run the SynchroMax ET plate handler.
	Online Mode	This button is displayed when System Control Software is in **Offline Mode**. Click to connect to the instrument and go into Online Mode. This button toggles with **Offline Mode**.
	Offline Mode	This button is displayed when ScreenWorks Software is in Online Mode. Click to disconnect from the instrument and go into **Offline Mode**. This button toggles with **Online Mode**.
	Remote Mode	InstructsScreenWorks Software to disable manual connection and to only receive Remote commands from a third-party robot. This button toggles with **Manual Mode**.
	Manual Mode	Instructs ScreenWorks Software to disable remote connection and to only receive Manual commands from the ScreenWorks Software user interface. This button toggles with **Remote Mode**.

Button	Name	Description
	Calibration	Opens the **Calibration** dialog where **Flat Field** calibrations can be performed.
	Yellow Plate Signal Test	Opens the **Yellow Plate Signal Test** dialog to display the numerical results using the Yellow Plate.
	Protocol Signal Test	Opens the **Protocol Signal Test** dialog to display the numerical results prior to running an experiment. Settings defined in this signal test can be saved to the protocol *.fmp file.
	Set Spinner Flask Stirring Rate	Opens the **Spinner Flask Control** dialog where the stirring rate can be set.
	Set Chamber Temperature	Opens the **Set Temperature** dialog where the chamber temperature can be set in degrees Centigrade, or disabled.
	Restore Layout 1	Restores the Experiment window layout to those defined as Save To Layout 1 in the **View** menu.
	Restore Layout 2	Restores the Experiment window layout to those defined as Save To Layout 2 in the **View** menu.
	Restore Layout 3	Restores the Experiment window layout to those defined as Save To Layout 3 in the **View** menu.
	Restore Layout 4	Restores the Experiment window layout to those defined as Save To Layout 4 in the **View** menu.

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 is displayed in the status bar.

The status bar can be hidden or shown from the **View > Status Bar** toggle command.

Menu Bar

This section lists and explains the commands available in the **Menu Bar** menus.

Some menu items can be opened with a keyboard shortcut using the **ALT** key to underline the letter in each menu title that is used to open the menu; for example, when you click **ALT** the **I** in the **Instrument** menu is underlined. Click the **I** key and the **Instrument** menu opens.

File Menu

The **File** menu contains commands that enable you to open, close, save and print FLIPR Tetra system data and protocol files.

Option	Description	Keyboard Shortcut
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.	Ctrl+N
Open	Opens an **Open File** dialog to open a saved Protocol (*.fmp), Data (*.fmd) or Image (*.png) file, as selected in the submenu. By default, the dialog opens in the folder set in **Tools > Set Default Directories**. **Note**: Only data files created with ScreenWorks Software can be opened in ScreenWorks Software. Data files from previous FLIPR Tetra system (versions 1.X through 2.X) cannot be opened.	Ctrl+O Ctrl+Shift+O
Close	Closes the currently active protocol or data file, displayed in the foreground of the Experiment window. If modifications have been made to the file, you are prompted to save the modifications.	Alt+F,C
Save	Saves the currently active protocol or data file, displayed in foreground of the Experiment window. Also saves If the displayed file is a default protocol that is untitled, the **Save As** dialog box is displayed so that you can name the file.	Crtl+S
Save As	Opens the **Save As** dialog and allows you to save a protocol or data file under a new name or format (for example, save a data file as a protocol). Also allows you to save a protocol or data file as an earlier version of the ScreenWorks Software. When changing formats, select the file type from the **Save as type** drop-down list.	Alt+F,S
Save All Files	Saves all of the opened protocol and data files. If the default protocol file was opened, the **Save As** dialog is displayed so that you can name the files.	

Option	Description	Keyboard Shortcut
Close All Files	Closes all of the opened protocol and data files. If any of the files had changes since they were opened or the default protocol file was opened, the **Save** or **Save As** dialog is displayed so that you can name the files.	
Export	Opens the **Export File** or **Batch Export** dialog, as selected in the submenu, to manually export data from the open data file, or other data files on disk; see Exporting Data on page 82.	
Page Setup	Opens the **Page Setup** dialog to configure the printer and print settings for the document.	Alt+F,G
Print Preview Report	Displays how the document will look when printed.	Alt+F,V
Print Report	The **Print Report** dialog allows you to select the graphs or reports to print from the current data file. Having made the selection you can open **Print Preview Report** to check before printing, or open the **Print** dialog to print.	Crtl+P
1–6 Data Files	Lists the six most recently opened data files, with the most recently opened at the top.	
7–10 Protocol Files	Lists the four most recently opened protocol files, with the most recently opened at the top.	
Exit	Closes ScreenWorks Software. If you have unsaved data, you will be prompted to save it before closing.	Alt+F,X

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 amended protocol and run it. Protocol information stored in data files cannot be edited, nor used to run a new experiment, however it is possible to extract this information to a new protocol file. Experiments run using this file will have exactly the same steps as the steps used to create the data file.

To store a data file as a protocol file, select **Save As**, assign a protocol name, select **.fmp* as **File Type** and click **Save**. The stored file is stripped of all data and only associated protocol information is stored in the protocol file.

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

View Menu

View menu commands enable you to select which displays to show or hide in the FLIPR Tetra system main window.

The keyboard shortcut is **ALT+V**.

Option	Description
Experiment Setup	Displays the **Experiment Setup** view of a protocol or data file, showing processes and associated dialogs.
Experiment Summary	Displays in the Experiment window the **Experiment Summary** for the current protocol or data file. To return to the normal view, select **View > Experiment Setup**.
Save Layout	Saves the proportions displayed in the Experiment window (including **Multi-Well** and **Detail Graphs** as well as **Group Statistics** window) and **Instrument Status** panel to one of four layouts. These layouts can be toggled between when the appropriate **Restore Layout** selection is made.
Restore Layout	Restores the screen layout to one of four proportions saved in the **Saved Layout** selection.
Instrument Status	Toggles the **Instrument Status** panel in and out of view (see Instrument Status Tab on page 53).
Toolbar	Toggles the **Toolbar** in and out of view (see Toolbar on page 38).
Status Bar	Toggles the **Status Bar** at the bottom of the main window in and out of view (see Status Bar on page 40).

Instrument Menu

Instrument menu commands enable you to access instrument communication and manual dialogs.

Options	Description
Go Online/Offline	A toggle that switches the instrument between Online and Offline modes (see Online vs. Offline Installation on page 34). **Go Online**—This is displayed when ScreenWorks Software is offline. Click to connect the software to the instrument, giving ScreenWorks Software control of the instrument. **Go Offline**—This is displayed when ScreenWorks Software is connected to the instrument. Press to disconnect the software from the instrument.
Run Experiment	Instructs ScreenWorks Software to start an experiment using the uppermost protocol file in the experiment.
Stop Experiment	Instructs ScreenWorks Software to stop the experiment. **Note**: **Stop Experiment** should only be used in emergencies to halt an experiment. If used, an instrument reset may be required.

Options	Description
Manual Operation	Commands in this submenu control specific hardware operations. **Note**: The commands are disabled if the associated hardware is not available. **Load Tip**s—Instructs pipettor to load tips or pin tool. **Unload Tips**—Instructs pipettor to unload tips or pin tool to the load tips/source 3 position. It is recommended that you unload tips to a tip rack. If you choose to unload without one, make sure there is a container in the appropriate position to receive tips. **Wash Tips**—Instructs pipettor to wash tips or pin tool. A dialog box opens for you to configure the wash. **Drain Wash Basin**—In case of an instrument error, allows the user to drain the wash basin to the waste bottles. **Yellow Plate Signal Test**—Instructs the instrument to take a reading of the read plate for the current protocol and to display the numerical results in the Signal Test dialog box. For detailed information on the features and functions of the signal test, see Signal Test on page 46. **Protocol Signal Test**—Instructs the instrument to take a reading of the read plate and to display the numerical results in the Signal Test dialog box. Instrument settings can be saved to the open protocol. For detailed information on the features and functions of the protocol signal test, see Signal Test on page 46. **Change Head**—Instructs the pipettor head to move over the read plate position. In this position, the pipettor head can be exchanged to a new pipettor format (see Exchanging Pipettor and Pin Tool Heads on page 105). **Cycle Camera Temperature**—Only used with EMCCD camera. Cycles the camera when you want to run a low-fluorescence or luminescence experiment immediately after running a high-fluorescence experiment. In the approximately 15 minute cycle period the camera is raised to room temperature (20°C—25°C) (68°F—77°F) and then cooled to -60°C (-76°F) ± 2°C (35.6°F). **Temperature Control**—A toggle that turns heating on and off for positions Source Plate 1, 2 and 3. When heating is turned on a dialog box is displayed to enter the desired temperature. **Cell Flask Stirring Control**—Allows the user to set the stir speed rate for the cell flask. **Wash Cell Reservoir**—Enables the user to wash the Cell Reservoir by selecting the Fluid Source, Fluid Destination, Fill Speed, Drain Speed, along with the number of Wash Cycles and Hold Time. It also allows the user to pre-coat the tubes, which is recommended for the first run with any cells in suspension. **Drain Cell Reservoir**—In case of an instrument error or manually poured cells into the Cell Reservoir, allows the user to drain the Cell Reservoir to a specified destination. **Note**: Resetting the instrument automatically drains the Cell Reservoir to the waste bottle.

Options	Description
Set Remote/ Manual Mode	A toggle that switches the instrument between manual and remote modes. **Set Remote Mode**—Enables you to integrate a third-party robotics system with FLIPR Tetra system. See Robotic Integration on page 189. ScreenWorks automatically goes into **Remote Mode** if you open **SynchroMax Automation**, however **Remote Mode** is not used to initiate communication with the SynchroMax ET. **Set Manual Mode**—Disables SynchroMax ET or third-party robotics control. All instrument commands must be initiated through ScreenWorks Software.
SynchroMax Automation	Opens the SynchroMax dialog for configuration of plate handling with the SynchroMax ET. See SynchroMax™ Automation on page 48.
Reset	Reinitializes the system to clear any fatal errors as designated in red at the bottom of the **Instrument Status** panel.
Clear Error	Clears minor system errors as designated in yellow at the bottom of the **Instrument Status** panel.
Calibration	Opens the **Calibration** dialog where **Flat Field Calibration Calibrations** can be performed. See Optical Calibration on page 127.
Refresh Configuration	Refreshes the instrument configuration. Use this command after hardware settings have changed.

Signal Test

The signal test has two functions:

- Checking the state of the overall system: This function is typically performed using the yellow test plate with the respective plate format for your assay and can be accessed through the **Yellow Plate Signal Test** option in the main menu. Outlined in the table below are the default settings used when performing the yellow plate signal test. These settings cannot be saved to a protocol for use at a later time.
- Checking initial fluorescence of a plate prior to running an assay: This function is typically performed to evaluate the assay plate prior to running an experiment and can be accessed through the **Protocol Signal Test** option in the main menu. Settings outlined in the table below will default to the settings in the currently open protocol when performing the protocol signal test. These settings can be saved to the open protocol for use at a later time.

The table below describes the settings in the **Yellow Plate Signal Test** and **Protocol Signal Test** dialog:

Options	Description
Select Plate	Choose the plate type from the drop-down list.
Excitation/Emission Wavelength	Select the appropriate excitation/emission wavelength pair for the signal test from the drop-down list. **Note**: Only calibrated excitation/emission wavelength pairs are displayed in the drop-down list.
Reading Mode	Select **Fluorescence** or **Luminescence** from the list.
Camera Gain	Select the camera gain for the signal test from the list. Typical Camera Gain is 1 for the EMCCD camera and 2000 for the ICCD camera, for the 470–495/515–575 nm excitation/emission pair when reading a yellow test plate.
Gate Open	This option is available only for the Aequorin ICCD camera. Typical Gate Open values for Fluorescence are around 6% but can be set to 100%. Luminescence gate is always 100%, and not user-adjustable.
Excitation Intensity	Select the LED intensity for the signal test from the dropdown list. Excitation intensity is scaled as a percentage of the total LED output (0–100%). Typical Excitation Intensity is 80 for the 470–495/515–575 nm excitation/emission pair when reading a yellow test plate.
Exposure Time	Enter the amount of time (in seconds) to keep the camera shutter open during the Signal Test. Typical Exposure Time is 0.4 seconds for the EMCCD camera and 0.53 seconds for the ICCD camera, for the 470–495/515–575 nm excitation/emission pair when reading a yellow test plate.
Highlight Range	Highlights well values that lie within the set statistical range. Set the statistical range by using the slider.
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.
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	When clicked, initiates a new signal test.

Options	Description
Save	When clicked, saves the signal test as an ASCII text file (*.sig).
Print	When clicked, prints the signal test.
Image Viewer	Displays the CCD image showing where RLU values were derived for the signal test. **Show > Hide Mask**—Shows or hides the mask used in the test, indicating the pixel area used to derive the RLU value for each well. **View Image**—Refreshes signal test image.
Diagnostics	For Technical Support use only.

SynchroMax™ Automation

The **SynchroMax Automation** command is only enabled if you have a SynchroMax ET installed.

When you open the **SynchroMax** dialog from **Instrument > SynchroMax Automation**, ScreenWorks Software automatically goes into **Remote Mode**, giving control of the instrument to the SynchroMax ET software. ScreenWorks Software returns to **Manual Mode** when the **SynchroMax** dialog closes.

To run a series of experiments with the SynchroMax ET, first open the protocol file that you want to use in ScreenWorks Software. Only one protocol is used in a run, as many times as the SynchroMax ET supplies plates for it.

Having prepared the protocol, open the **SynchroMax** dialog and select a **Stack Layout Template**. These files specify, for each plate location in the five-position stage, how many plates will be loaded to that position, from which stack, and where they will be delivered to after use. A number of **Stack Layout Templates** are supplied—select the one that fits your assay.

When a **Stack Layout Template** is opened in the dialog the graphic in the dialog displays which stacks plates should be loaded in. You can use this as a guide for loading the plates. All plates in a single stack should be of the same type.

If there are any stage positions that will use the same plate throughout the duration of the run, these should be loaded manually before the run begins. Stage positions that will have plates brought to them during the run should be empty at the start of the run.

Before selecting **Run**, make sure all output racks are empty prior to beginning the experiment. When plates are in position and the FLIPR Tetra system ready, click **Run** in the **SynchroMax** dialog. After checking that the plate configuration is compatible with the protocol **Settings** configuration, the instrument runs until all plates have been used. During this time the **Run** button changes to **Stop**, so you can stop the run before it completes if necessary.

Note: Stop should only be used in emergencies to halt an experiment. If used, an instrument reset may be required.

When the SynchroMax ET is active, the system will clear all plates at the end of an experiment. The SynchroMax remembers only those plates it loaded during the experiment and checks if any of those plates remain when the experiment completes. If so, they are removed. A reagent reservoir can be used in a source position within the FLIPR Tetra system as long as it is manually loaded and the SynchroMax template does not include the loading or removal of plates to that position.

On completion, the **Done** button is enabled. Unless you want to run another set of experiments, press this to close the dialog and return ScreenWorks Software to **Manual Mode**.

Tools Menu

Options	Description
Set Default Directories	Opens 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 folders set here, as do the **File > Export** dialogs. In addition, **Group Templates *.fmg** can be exported and imported from the default folder defined in the directory. 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**: It is recommended that you save all protocol and data files on the local hard drive to ensure instrument function or data is not lost if your server fails during an experiment.
Plate Library	Opens the **Plate Process Definition** dialog, which lists current plate definitions and allows you to add additional plate definitions to the system. See Plate Process Definition on page 50 for details.

Options	Description
Open Error Log	Opens an error log generated by the FLIPR Tetra system. This feature is for technical support and requires a password.
Save Error Log	Save error logs. Logs are saved as ***.fel** (FLIPR Tetra system Error Log) encrypted files. The files can be forwarded to Molecular Devices Technical Support.
Assay Log	For data files only, opens a dialog reporting when the protocol steps were applied. **Note**: This log can be accessed by selecting **CTRL+SHIFT+A**.

Plate Process Definition

All plates currently in the system are listed in the **Plate Process Definition** dialog, opened from **Tools > Plate Library**. They are categorized first into read and source plates and then by well-number format.

A description of commands in the Plate Process Definition and Define Camera Parameters, both accessed via **Tools > Plate Library** is provided below. For instructions on adding a new read or source plate, see Data Processing Algorithms on page 237. The Plate Library is camera-specific, so if any plates are added with one camera type, they will not be available once the camera type is changed.

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

Fifteen default plates are included with the system software:

- 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
- Default Cell Reservoir 96

- Default Cell Reservoir 384
- Default Cell Reservoir 1536

Options	Description
Close	Closes the **Plate Process Definition** dialog.
Open Plate	Opens the **Define Basic Plate Parameters** dialog for the plate selected in the tree view.
Copy Plate	Opens the **Define Basic Plate Parameters** dialog allowing you to add a new plate (see Define Basic Plate Parameters Dialog on page 51). **Note**: When copying a plate, make sure that you select a plate with a similar well format, as the number of wells and plate mask will be transferred to the new plate.
Delete Plate	Deletes the plate displayed in the **Plate Process Definition** field.
Collapse All	Collapses the **Plate Process Definition** tree.
Expand All	Displays all items in the **Plate Process Definition** tree.

Define Basic Plate Parameters Dialog

This dialog opens when you select **Open Plate** or **Copy Plate** in the **Plate Process Definition** dialog. Use the dialog to inform the FLIPR Tetra system of the critical dimensions of the plates you are using.

Note: Dimensions of default plates cannot be modified. However, the plate mask can be realigned.

Options	Description
Plate Name	Enter the name you want assigned to the new plate (maximum number of characters is 50).
Plate Type	Select **Source Plate** or **Read Plate** for the new plate. **Source Plate**—Creates a plate that does not include a plate mask. These plates are typically used for compound storage. **Read Plate**—Use for all plates that are read in the read position and require a plate mask.

Options	Description
Plate Specifications	Area for entry of physical plate dimensions. Refer to the diagram in the upper right corner of the dialog. For best results, obtain the plate dimensions from the plate manufacturer. **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 appropriate shape from the drop-down list. 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 Dialog Box

When **Read Plate** is identified as the **Plate Type** in the **Define Basic Plate Parameters** dialog, the **Finish** button is enabled. Prior to selecting Finish, a read plate with 10-8 M fluorescein should be placed in the read position. When selected, the instrument will read the plate and define a plate mask to the plate definition created. If a plate is present, but no mask can be defined, the plate definition will be saved as a source plate.

Window Menu

The commands in the **Window** menu allow you to change the way the Experiment window is viewed. The keyboard shortcut is **ALT+W**.

Options	Description	Keyboard Shortcuts
Cascade	Aligns the open windows so that they are overlapped and staggered with the title bars visible.	Alt+W,C
Tile	Divides the screen into as many segments as there are windows and aligns them so that they are displayed side-by-side. Each file occupies a segment and is visible. However, each of the file images will be scaled according to the number of files being displayed.	Alt+W,T
1–10 Data Files	Lists the open files (up to 10)	

Help Menu

The **Help** menu provides access to the user guide and information about the software. The keyboard shortcut is **ALT+H**.

Options	Description
FLIPR Tetra system User Guide (PDF)	Opens a PDF version of this manual that is appropriate for the version of software installed.
ScreenWorks Release Notes	Opens a PDF version of the *ScreenWorks Software Release Notes*.
About ScreenWorks	Opens the **About ScreenWorks** dialog. This reports the version numbers for ScreenWorks Software, the Firmware EC, Firmware Motion and Remote Interface.
MDC on the Web	Displays links to a number of Molecular Devices support pages on the Web, such as theScreenWorks Software Update page, the Technical Support page, and Technical Support Request page. Selecting one of these commands opens your default web browser to the selected page.
Show Update Reminder	When this option is selected an Update Reminder is displayed when ScreenWorks Software is launched, reminding the user to check for an update to the software.

Instrument Status

The **Instrument Status** panel, by default located on the bottom of the main screen, reports the status of and settings for the FLIPR Tetra system hardware in addition to including the processes used to create protocols. The panel has three tabs: **Instrument Status**, **Instrument Configuration** and **Process Explorer**.

The panel can be moved to different locations within the main ScreenWorks Software window, and can be hidden entirely, using the **View** menu command.

Instrument Status Tab

The current status of the system hardware components is reported in the **Instrument Status** tab. Fields below this report information about the step the instrument is at within an experimental run. The color-coded communication field at the bottom-right of the tab reports status with respect to communication between ScreenWorks Software and the instrument.

Status messages and faults are reported at the bottom of the tab as well. Click the button here to see a list of the last thousand messages.

Options	Description
Stage Temp (°C)	Displays the source plate position temperature of the five-position stage as well as the temperature it is set for, **Set Point**. **Stage Status**—Whether the heated stage is turned on or not.
Camera Temp (°C)	Displays the camera temperature. Operating temperature for the camera is -60 ± 2 °C (-76°F ± 35.6°F) for the Standard EMCCD camera and -20°C ± 5°C (68°F ± 41°F) for the Aequorin ICCD camera. **Camera Status**—Indicates whether the camera is turned on or off. **Intensifier**—Indicates the status of the intensifier.
Pipettor Tips	Reports when tips are loaded on the pipettor head.
Upper Door (Inner)	Reports whether the inner-upper door (observation panel) is open or closed. **Note**: The system will run as long as the inner door is closed, however data may not be valid if outer door is open.
Upper Door (Outer)	Reports whether the outer-upper door is open or closed.
Lower Door	Reports whether the lower door is open or closed.
Tip Washer	Indicates the status of the tip washer.
Chiller Status	Indicates whether the status of the chiller.
Read Plate	Reports when a plate is present in Position 3 (Read Plate position).
Source Plate 1	Reports when a plate is present in Position 1 (Source Plate 1 or Tip Loading position).
Source Plate 2	Reports when a plate is present in Position 2 (Source Plate 2).
Source Plate 3	Reports when a plate is present in Position 4 (Source Plate 3 or Cell Reservoir).
Cell Reservoir	Indicates when the Cell Reservoir is installed.
Chiller Temp (°C)	Reports the current temperature in the chiller.
Fill Bottle A	Reports when bottle A is empty of wash solution.
Fill Bottle B	Reports when bottle B is empty of wash solution.
Waste Bottle A	Reports when waste bottle A is full.
Waste Bottle B	Reports when waste bottle B is full.
Mode	Reports whether ScreenWorks Software in **Manual** or **Remote** mode.
Cell Flask Rate	Reports the set stir rate of the cell flask. If the **Cell Suspension** option is installed and the stir rate is 0, an exclamation sign is displayed.
Chiller Setpoint (°C)	Reports the set point from the chiller.
Fluid 1	Reports the last known state of the Fluid 1 bottle. At start the state will be Unknown until that Fluid is used.

Options	Description
Fluid 2	Reports the last known state of the Fluid 2 bottle. At start the state will be Unknown until that Fluid is used.
Fluid 3	Reports the last known state of the Fluid 3 bottle. At start the state will be Unknown until that Fluid is used.
Fluid 4	Reports the last known state of the Fluid 4 bottle. At start the state will be Unknown until that Fluid is used.
Cell Flask	Reports the last known state of the stir Cell Flask. At start the state will be Unknown until the Cell Flask is used.
Status Message and History[1]	Current instrument status, or fault conditions, are reported at the bottom of the tab. To see the full text of the message in a timed list of the last thousand status messages, press the button beside the colored **Communication** field.
1. The Status History dialog can be copied to the clipboard to paste into another application if desired.	

Instrument Configuration Tab

The **Instrument Configuration** tab indicates the current instrument configuration of the LED banks, emission filters, pipettor head and TETRAcycler. If the system is Offline, you can configure these settings to your preference in order to define protocols.

Note: If a protocol created offline does not match instrument configuration when opened online, the protocol will not run until the configuration of the protocol and instrument match.

Options	Description
Excitation Wavelengths	Displays the excitation wavelengths installed on the system. **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. Up to three filters can be installed at the same time.

Options	Description
Pipettor	Displays the type of pipettor head (96, 384, 1536, 384 pin tool, or 1536 pin tool) installed on the system. **Note:** The pipettor head format must agree with the tip wash reservoir format. A warning is issued if these are different.
Tip Washer	Displays the type of tip washer (96, 384 or 1536) installed on the system.
Camera Type	Select from EMCCD or ICCD camera.
Chiller	Reports when a chiller is installed.
TETRAcycler	Reports when the TETRAcycler is installed.
Bar code Reader	Reports when a bar code reader is installed. on the TETRAcycler.
Cell Reservoir	Use the check box to indicate whether or not the Cell Reservoir is installed.

Process Explorer Tab

The Process Explorer is used to create new protocols.

The Process Explorer displays processes that can be incorporated into experiment protocols. To add a process step to a protocol, drag the process into the protocol setup at the top of the Experiment window. See Constructing Protocols Using FLIPR Tetra Processes on page 57.

Note: The processes available on the screen may change based on the instrument status, for example, the **Wash Tips** process is not shown if a tip wash malfunction has occurred.

Experiment Window

The Experiment window is the main working interface in ScreenWorks Software. It is used to create, view and edit protocols, used to control the instrument in an experiment, and to view data files generated in experiments.

This section gives an overview of the Experiment window; following sections provide details of the configuration options for each of the process types used to construct protocols in the Experiment window.

The Experiment window can have one protocol and multiple data files open at once, with options to view these one at a time, cascaded, or tiled (**Window** menu). File windows can be maximized, to occupy the entire Experiment window, minimized, reducing to a small section of title bar in the bottom left of the Experiment window, or arbitrarily sized and located within the Experiment window.

Only one file is active at once. If the active file is a protocol file, it will control the experiment if the **Instrument > Run Experiment** command is given.

Protocol (*.fmp) and data (*.fmd) files both show the processes incorporated in them at the top of the file window, ordered from left to right. Clicking on the process icons brings forward the 'page' for that particular process, displaying its configuration settings. Data files differ from protocol files only in having recorded data to display when the Analysis process is selected, otherwise retaining all the protocol setup information contained in the protocol file that was used to generate the data.

Besides showing the settings for the particular processes incorporated into a file, a one-page protocol summary can be viewed in the Experiment window with **View > Experiment Summary**. Revert to the experiment setup view with **View > Experiment Setup**.

ScreenWorks Software always opens with a default protocol file, Untitled 1, containing four processes. This can be used to start construction of a new protocol, or closed or ignored if you wish to work with existing protocol or data files.

Constructing Protocols Using FLIPR Tetra Processes

Protocols are comprised of combinations of the following processes:

- Settings
- Analysis
- Transfer Fluid
- Mix Fluid
- Wash Reservoir
- Wash Tips or Pins
- Blot Pins (Pin Tool only)
- Pause Pipettor
- Finish with Source
- Read

The **Settings** and **Analysis** processes are required for every protocol. They are automatically included as the first two processes in every new protocol file you create, and cannot be removed.

Transfer Fluid, **Mix Fluid**, **Wash Tips or Pins**, **Blot Pins**, **Pause Pipettor**, **Finish with Source** and **Read** are all available to drag into a protocol from the Process Explorer based on the pipettor head type installed. **Wash Reservoir** is only available when the **Cell Suspension** option is installed.

There are two processes additional to the basic set:

- Mix with TF
- Read with TF

These perform the same functions as **Mix Fluid** and **Read**, but are created as options within a **Transfer Fluid** step, and are more closely integrated with the step, for example, a **Read with TF** is timed precisely to coincide with compound addition to the read plate.

New protocols always include a **Transfer Fluid** step with an associated **Read with TF**, but these can be removed or reordered, and of course additional steps can be dragged across from the **Process Explorer**.

Processes can be added to the end of a protocol, or, using the right edge of a new process you drag over, inserted between other processes already in the protocol, providing you do not attempt to insert between processes that are internally linked, for example, a **Transfer Fluid** and associated **Mix with TF**.

As each new process is dragged to the Experiment window, a new page with configuration options for the process is added to the file. The new page is automatically displayed.

Similarly, as you click on the processes above, the sheet associated with that process, containing its configuration settings, comes forward.

Deleting Processes from the Protocol

To delete processes that are not connected, click on the process icon in the Experiment window and click the **Delete** key.

To delete a connected process, **Mix with TF** or **Read with TF**, go to the **Transfer Fluid** process that it is associated with and deselect the option for the process.

Process Icon Colors

The **Settings** and **Analysis** processes' icons are green to indicate that they are required to run an experiment. These processes' are always present and cannot be deleted.

The **Transfer Fluid**, **Mix Fluid**, **Wash Tips or Pins**, **Wash Reservoir**, **Blot Pins**, **Pause Pipettor** and **Finish with Source** processes icons are blue to indicate that they are **liquid** handling steps. These steps are always in series with each other; no two of them occur simultaneously.

The **Read** process icon is orange to indicate that it will occur in parallel with any liquid handling process, if possible. However, a read can be added through the **Transfer Fluid** process to indicate the liquid handling will occur at a defined time in the experiment. These linked processes will not begin until all read and liquid handling steps prior to the **Transfer Fluid** linked with **Read with TF** are complete.

When **Mix with TF** or **Read with TF** processes are created (from the options in a **Transfer Fluid** step), the icons of all the connected processes change to purple, to show the linkage.

Settings Process

Settings is the first process in all protocols. Use it to define read modes, plate positions, and data directories. You can also configure automatic printing of selected results, automatic file naming, and set plate temperature.

Select the green **Settings** icon in the Experiment window to view the **Settings** page where the settings are made.

Setup Read Mode

The **Setup Read Mode** table at the top of the **Settings** page displays settings for up to four read modes that the FLIPR Tetra system can perform in a single experiment.

Each read mode defines:

- Reading mode (fluorescence or luminescence)
- Excitation and emission wavelengths (i.e., a pair of LED banks and emission filter)
- Camera gain
- Camera exposure time
- Excitation intensity
- Gate open (ICCD camera only)

Molecular Devices FLIPR® Calcium and Membrane Potential Assay Kits use a single read mode, but ratiometric assays, such as the Voltage Sensor Probes, require two read modes. In these cases the instrument alternates between the two read modes for each time point, outputting two distinct sets of readings, one for each mode. ScreenWorks Software can then automatically calculate the ratio of the readings from each mode, for each time point.

If you open a protocol file that uses read modes not available under the present configuration of your instrument, the unavailable read mode rows are displayed in red.

Edit Read Mode

To enable a read mode, click the check box in the far left of the **Set Read Mode** table. Then double-click in the row or select the row and press the **Edit Mode** button below the table to configure it. The **Edit Read Mode** dialog has the following fields:

Options	Description
Read Mode Name	Enter a name for the selected read mode, if desired.
Reading Mode	Select **Fluorescence** or **Luminescence**.
Excitation/Emission Wavelength	Select the desired excitation LED and emission filter wavelength pair from the list. **Note**: Only calibrated wavelength pairs are available when the software is in Online mode; see Calibrating the Optics on page 128. NONE instructs the instrument to not turn on any LEDs or use an empty emission filter position. These are typically used when performing luminescence experiments.
Excitation Intensity	Select a value from the list to regulate the intensity of light emitted by the LED bank for a given Fluorescence read. The range is 20–100%.

Options	Description
Exposure Time	Enter a time (in seconds) to regulate the time that light is collected and measured by the camera. A longer exposure increases signal intensity. Exposure time affects the read interval for data collection. For instance, an exposure time of 0.4 s, added to a required 0.1 s camera integration time, makes the highest update frequency 0.5 s. The range is 0.05 s to 30 s.
Camera Gain	Select a value from the list to regulate the amplification of the camera power. Increasing the camera gain increases the signal. The range for the Standard EMCCD camera is 1–240. With the Aequorin ICCD camera installed, the Fluorescence read mode is preset to 2000, whereas in Luminescence mode the range is 2000–280,000.
Camera Gate	This option is only available with the Aequorin ICCD camera in Fluorescence mode. It further regulates the signal intensity by selecting the percentage of each frame captured.

Note: Differences in **Camera Gain** or **Exposure Time** between read modes drastically increase the minimum update time. For fastest update time, use the same gain and exposure time for all read modes.

Assign Plate to Position

The five-position stage has four positions for plates: one for the read plate and three for compound source plates. In order for the TETRAcycler and pipettor to address each position, a plate type must be assigned to each of the positions that will be used in your experiment. Current plate assignments are reported in the **Assign Plate to Position** table.

For each plate position, the table reports:

- **Plate Name**—the type of plate, as configured in **Tools > Plate Library**.
- **Bar Code Expect**—Whether or not you want the system to read a bar code from the plate. Bar code numbers can optionally be incorporated into output data file names.
- **Bar Code List**—in data files only, the bar codes of plates used in the experiment, in the given plate position.

Change these settings if required with the **Edit Plate** dialog.

If Position 1 is to be used only for tips, and not to hold a source plate, check the **Load Tips Position** check box below the table. This removes Source Plate 1 from the table.

If the Cell Reservoir is installed in the system, Source 3 is automatically dedicated to **defaultcellres384**.

Edit Plate

To change the plate configuration for a particular position, double-click on the relevant row or select it and click the **Edit Plate** button, to open the **Edit Plate** dialog.

Options	Descriptions
Position	Displays the plate position you are editing.
Plate Name	Select the plate type from the drop-down list. The list contains default plates and any additional plates that have been added using **Tools > Plate Library**. **Note**: Only plates with the same number of wells or one order of complexity higher than the pipettor head format are displayed.
Bar Code Expected	Check this option if using a bar code for the plate. The bar code number can be incorporated into the file names of data files generated by the protocol. In **Manual Mode**, you are prompted to enter the bar codes prior to starting an experiment. In **Remote Mode** the bar code number is automatically scanned on the TETRAcycler landing pad or is passed to ScreenWorks by the third-party plate handler.
Cell Flask Spinning Rate	When the Cell Reservoir from the **Cell Suspension** option is installed, spinning rate can be set here or **User Manual** can be selected and the manual settings will be applied.

CAUTION! Each plate position has a mechanical sensor to detect the presence of a plate, tip rack or boat in each position. However, the instrument does not distinguish the type of apparatus placed in a position.

Edit Plate

To change the plate configuration for a particular position, double-click on the relevant row or select it and click the **Edit Plate** button, to open the **Edit Plate** dialog.

Options	Descriptions
Position	Displays the plate position you are editing.
Plate Name	Select the plate type from the drop-down list. The list contains default plates and any additional plates that have been added using **Tools > Plate Library**. **Note**: Only plates with the same number of wells or one order of complexity higher than the pipettor head format are displayed.

Options	Descriptions
Bar Code Expected	Check this option if using a bar code for the plate. The bar code number can be incorporated into the file names of data files generated by the protocol. In **Manual Mode**, you are prompted to enter the bar codes prior to starting an experiment. In **Remote Mode** the bar code number is automatically scanned on the TETRAcycler landing pad or is passed to ScreenWorks by the third-party plate handler.
Cell Flask Spinning Rate	When the Cell Reservoir from the **Cell Suspension** option is installed, spinning rate can be set here or **User Manual** can be selected and the manual settings will be applied.

CAUTION! Each plate position has a mechanical sensor to detect the presence of a plate, tip rack or boat in each position. However, the instrument does not distinguish the type of apparatus placed in a position.

Data File Name

The **Data File Name** group box is used to configure a file-naming protocol for data files created in experiments. The file-naming protocol applies to ***.fmd** (data) and ***.png** (image) files.

Options	Description
Include Date	Check to include the date in the file name. It is checked by default. **Note**: To ensure that data files are not overwritten, we recommend that this option remains checked.
Include Bar Code	When selected, the bar codes of the first five plates used in the experiment are included in the file names. If there are fewer than five bar codes, then only those available are used. **Note**: **Bar Code Expected** must be selected for the desired plate position in order for the bar code to be incorporated.
User Defined Name	Enter a string of characters (up to 25) to be added to the file name.

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 crash or other error occurs during a run that interferes with normal instrument operation, then a data file is automatically stored as:

InterruptedExperiment_Date_Time_NNN.fmd

Folders

Use the two fields here to designate directories into which data and export files will be saved.

By default, data, image and export files are stored under:

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

Change—An option that allows the user to pick a different directory.

Open Folder—An option which opens the Windows Explorer dialog box directly to the directory designated.

Temperature Control

The **Temperature Control** field allows you to set the plate-stage heaters to a desired temperature for an experiment. Temperature range is from ambient 25 °C to 40 °C (77°F to 104°F). Select **Manual Settings** to control the heaters manually from ScreenWorks Software at the time the experiment is run.

If, at the start of an experiment, the stage temperature is different from the selected value a dialog informs you of this. You can wait until the stage reaches the desired temperature or run the experiment anyway.

Note: When the temperature is manually turned on through the **Instrument** menu, a setting of “0” in the protocol does not affect the temperature setting. If the user prefers to run off the temperature, this must be manually done through **Instrument > Manual Operations > Temperature Control Off**.

Auto Print Options

In the **Auto Print Options** section, select information to be automatically printed at the end of each experiment run with the protocol.

Note: These are print options only. To save files of data generated in experiments, use the **Auto-Export** option (see Exporting Data on page 82).

Print options are:

- **Notes**—Comments you have added to the protocol using the Notes option opened from the **Analysis** process page.
- **Experiment Summary**—The experiment summary automatically created for each protocol, viewed in ScreenWorks Software with the **View > Experiment Summary** command.

Note: Read Mode and **Kinetic Reduction** need to be defined for display in the following graphs.

- **Multi-Well Graph**—The graph showing the selected **Read Mode** time-course traces for all wells independently, as displayed in the **Analysis** process page. The option to include kinetic reduction values in each well of the graph is available.
- **Detail Graph**—A single graph with a single average trace for each group and read mode combination configured in the protocol. The option to include error bars on the graph is available.

Note: Group statistic and the option to include effective or inhibition concentration need to be defined for the following options.

- **Group Statistic Graph**—A single graph with statistics acquired from the group statistics table for each group plotted and fit to a four-parameter curve.
- **Group Statistic Table**—A table utilizing the kinetic reduction data to define statistics (for example, mean and standard deviation) for each group defined on the plate.

Analysis Process

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

- Viewing Data, see page 64
- Grouping, see page 73
- Correction, see page 76
- Exporting Data, see page 82

Viewing Data

Acquired data can be viewed in ScreenWorks Software on the main **Analysis** process page, in the **Multi-Well Graph**, or in larger **Detail Graph**. The options are described in this section.

Multi-Well Graph

The major part of the **Analysis** process page consists of a grid representing the wells of the read plate used in the protocol or data file (for example, with 96, 384, or 1536 wells). This is called the **Multi-Well Graph**. For protocol files this graph is empty, as no data have been acquired, but in data files each cell of the graph has a trace of one of the measurements taken from the corresponding read plate well. The maximum and minimum RLU values of the displayed data are reported above the Multi-Well Graph.

The Multi-Well Graph displays, for each well represented, one trace of relative light units (RLUs) versus time. If two or more read modes were recorded, select the mode to view from the display list box above the graph. To see the ratio of two read modes, enable **Ratiometric Options** in the **Configure Corrections > Corrections** dialog, and select **Ratio** in the display list.

The **Read Mode** defined in the Multi-Well Graph is also applied to any traces displayed in the **Detail Graph**.

The traces displayed in the Multi-Well Graph can be adjusted by the application of different data-correction options available in the **Correction** dialog, for example, the traces can be scaled relative to the average positive control response (see Correction on page 76).

If groups have been defined for the assay, wells in the Multi-Well Graph are color-coded to represent these (see Grouping on page 73). In addition, there is the option to include the kinetic reduction value below each well trace in the Multi-Well Graph.

Buttons, just above the Multi-Well Graph, open dialogs that offer 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 buttons and descriptions include:

Buttons	Name	Description
	Configure Groups	Only available for protocol files, this button opens the **Auto-Export** dialog allowing configuration of statistics and time sequence files to be automatically created whenever the protocol is run. For data files the button changes to Export, and opens the **File Export** dialog, also opened from the **File > Export** command. See Exporting Data on page 82.
	Select Groups	Opens the **Select Groups** dialog to classify which groups are quickly displayed in the **Detail Graph** from a predefined list based on the groups defined in the Grouping dialog. **Note**: Hold down the **SHIFT** or **CTRL** key to select multiple groups.
	Group Selection Mode	Button toggles between **Group Selection Mode** and **Well Selection Mode**. During **Group Selection Mode**, wells selected within a predefined group displayed in the Multi-Well Graph will cause all wells within those groups to be displayed in the **Detail Graph**.

Buttons	Name	Description
	Well Selection Mode	Button toggles between **Group Selection Mode** and **Well Selection Mode**. **Well Selection Mode** only displays wells selected in the Multi-Well Graph on the **Detail Graph** with their respective group colors assigned.
	Notes	In protocol files, use this dialog to write comments that will be stored with all data files generated by the protocol. In the data files, view the comments (now read-only) in the same dialog.
	Images	For data files only, where images have been saved during an experiment (by checking **Save Images** in a **Read with TF** step) these can be viewed by clicking the **Images** button.
	Copy	Copies data in the Multi-Well graph to the clipboard so it can be pasted in a different program such as Microsoft Word.
	Configure Auto-Export	Only available for protocol files, this button opens the **Auto-Export** dialog allowing configuration of statistics and time sequence files to be automatically created whenever the protocol is run. For data files the button changes to Export, and opens the **File Export** dialog, also opened from the **File > Export** command. See Exporting Data on page 82.
	Configure Correction	Opens the **Corrections** dialog box to apply various corrections to modify data display. There is also the option to view ratiometric data. Settings made here in protocol files will affect how data are viewed when the protocol is run, but since all raw data are stored in data files, these options all remain available for acquired data as well. See Correction on page 76.
	Hide Kinetic Reduction Value	Hides the kinetic reduction values that are displayed in the Multi-Well Graph.
	Show Kinetic Reduction Value	Shows the kinetic reduction value in each well of the Multi-Well Graph.

Buttons	Name	Description
	Configure Kinetic Reduction	Opens the **Kinetic Reduction Configuration** dialog to define the parameters used to define the kinetic reduction. **Reduction Type**—Defines the reduction to be applied to the kinetic data traces displayed in the Multi-Well Graph. See Kinetic Reduction Types on page 67. **Start Read**—Define the first read to be used to determine the kinetic reduction. **End Read**—Define the last read used to determine the kinetic reduction. **Read Mode**—Select the read mode to apply the kinetic reduction to.

Kinetic Reduction Types

The **Reduction Type** field is located in the **Kinetic Reduction Configuration** dialog. Available options depend on the software version in use. A separate license for ScreenWorks Peak Pro™ adds advanced peak detection and characterization measurements to the field of standard measurement options (see Figure 5-2). Contact your Molecular Devices Sales Representative for details.

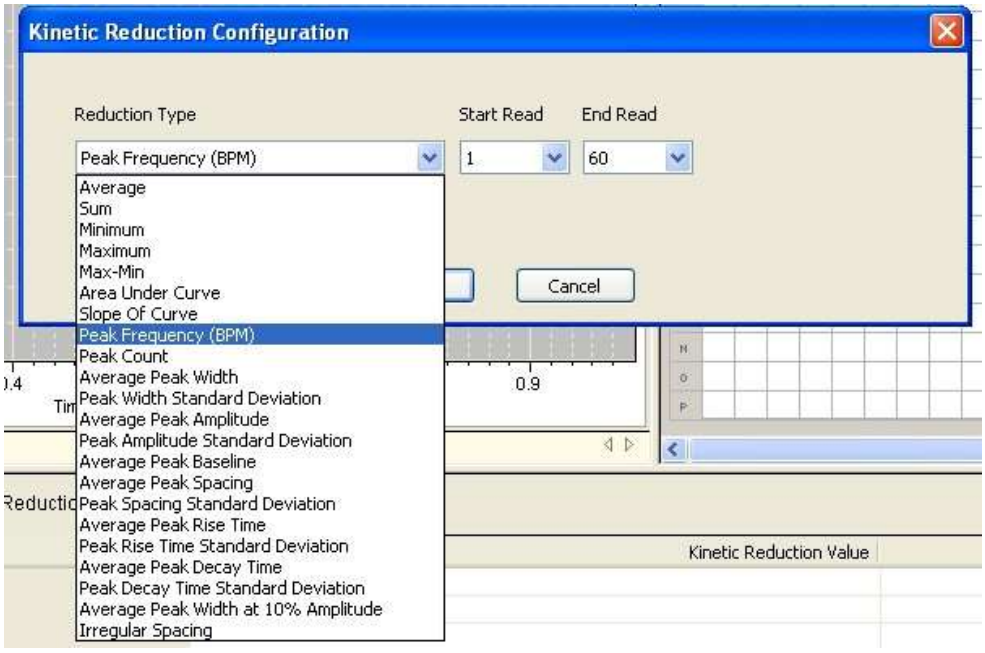


Figure 5-2: Additional ScreenWorks Peak Pro Kinetic Reduction Types

Standard measurement options and definitions include:

- **Average**—Numerical average of RLU counts of the selected reads.
- **Maximum**—Highest detected count (a single number) of all the selected reads.

- **Maximum-Minimum**—Result of subtracting the minimum count (a single number) from the maximum count (a single number).
- **Minimum**—Lowest detected count (a single number) of all the selected reads.
- **Sum**—Numerical sum of RLU counts of the selected reads.
- **Area Under Curve**—Numerical calculation of the area under the curve for the selected reads.
- **Slope of Curve**—Result of calculating the slope between two selected reads.

(With optional license) Peak Pro measurement options and definitions include:

- **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.
- **Peak Count**—Number of peaks or action potentials detected within the read data that meet the specifications in the Configure Peak Detection dialog settings.
- **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 are marked as **OK**. Wells with missing peaks are marked as **MISS**. Wells with extra peaks are marked as **EXTRA**. Wells with peak spacing characteristics of both missing and extra are marked as **IRREG**.

The **Configure Peak Detection** button activates when a Peak Pro measurement is selected from the **Reduction Type** field (see Figure 5-3). The **Configure Peak Detection** dialog allows the Peak Pro measurement settings to be adjusted and optimized (see Figure 5-4).

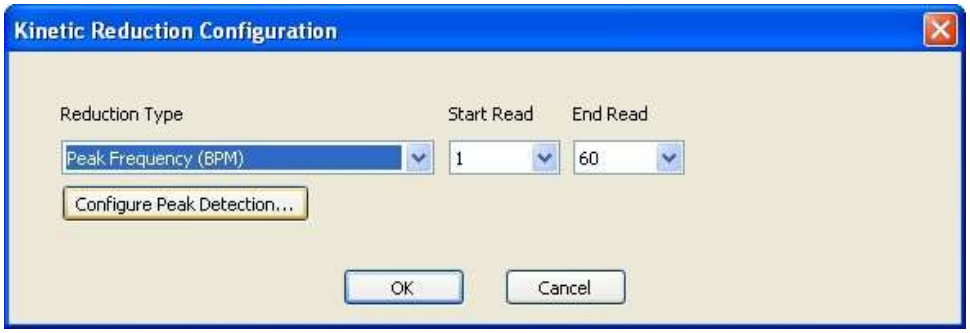


Figure 5-3: Configure Peak Detection button activation

Configure Peak Detection dialog options include:

- Smooth Width
- Fit Width
- Slope Threshold
- Amplitude Threshold Dynamic
- Amplitude Threshold Fixed

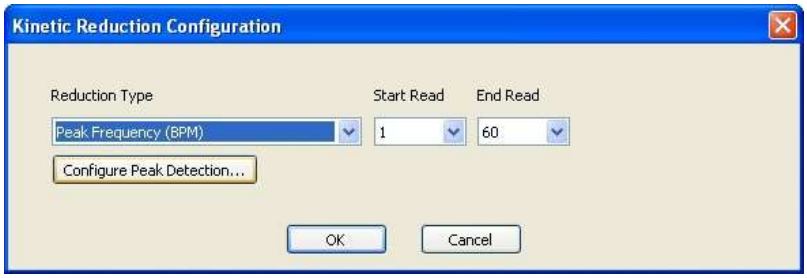


Figure 5-4: Configure Peak Detection dialog

Detail Graph

All the read modes for wells selected in the multi-well graph can be displayed enlarged in a detail graph. You can select individual or multiple wells to view, or select by group.

Note: When the FLIPR Tetra system is configured for two or more read modes, it cycles through each mode alternating between them for each reading. Although readings from each mode within a cycle occur at different times, they are represented as occurring simultaneously.

The data displayed in the **Detail Graph** have the same corrections applied as the data displayed in the Multi-Well Graph. The **Detail Graph** reports these settings at the top of the graph window:

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

These correspond directly to options in the **Correction** dialog, opened from the **Analysis** process page. To change these settings, change them in the **Correction** dialog. This will change all data in the **Multi-Well Graph** and **Detail Graph** to reflect the new correction applied. **Detail Graph** allows a number of data-selection options:

Buttons	Name	Description
	Copy Graph	Copies Graph in the Detail Graph to the clipboard so it can be pasted in a different program such as Microsoft Word.
	Copy Graph Data	Copies graph data in the **Detail Graph** to the clipboard so it can be pasted in a different program such as Microsoft Excel.
	Zoom Mode	Click to enable zoom capability in the graph. Once the **Zoom** button is pressed, drag over a region in the graph to view just that area enlarged to the full size of the graph. Alternatively, drag the cursor along a part of the x or y axis to zoom in on just that axis.
	Auto Scale	Manual prompt to automatically scale the **Detail Graph** and Multi-Well Graphs to include all datapoints of the desired traces.
	Auto Scale Always	Automatically scales the **Detail Graph** and Multi-Well Graph to include all data points of the desired traces without manual prompting.
	Undo Zoom	Rescales a graph to the original settings.
	Manual Scale Graph	Opens the **Manual Scale Graph** dialog to set the maximum and minimum values for the X- and Y-axis of the **Detail Graph** and Multi-Well Graph.
	Show/Hide Data	Toggle button that shows or hides the RLU values for the selected traces at each read.

Buttons	Name	Description
	Show Point Labels	Show Point Labels writes the value of each data point (for example, RLU value) beside the point on the graph.
	Show/Hide Legends	A toggle button that displays or hides the **Detail Graph** legend.
	Average/Overlay Trace	A toggle button that displays the average or overlay traces for selected groups. **Average Group**—Displays average time point values for all wells in the selected group. A separate trace is generated for each read mode. **Note**: When Well Selection Mode selected in the Multi-Well Graph, all traces displayed in the Detail Graph will be averaged regardless of the group they are assigned. **Overlay Group**—Displays traces for all the wells in selected groups. **Note**: Traces are color-coded by group.
	Show/Hide Standard Deviation	A toggle button that enables or hides the standard deviation for the average group trace. **Note**: **Average Group** must be enabled to access this function. When **Well Selection Mode** is selected in the Multi-Well Graph, all traces displayed in the **Detail Graph** will be averaged regardless of the group they are assigned, and the standard deviation for this average will be displayed.

Individual Well Selections

To view a single well in the **Detail Graph**, double-click or drag the mouse cursor inside the well of interest.

To view the traces of multiple wells overlaid in the **Detail Graph**, drag the cursor over the wells you want to view. Data from any rectangular block of wells within the plate can be viewed in this way. Additional wells can be added to the overlay by double-clicking or dragging the mouse cursor over the wells of interest.

Group Selections

You can also select data to view in the **Detail Graph** using group membership (see Grouping on page 73). This can be achieved using **Select Group** or **Group Selection Mode** functions found in the Multi-Well Graph.

Detail Graph Options

Detail graphs have a number of visualization options available in addition to the shortcut buttons found at the top of the graph. When you right-click on the detail graph, a menu of visualization tools is displayed. These are summarized in the following table.

Options	Description
Properties	Opens the **Graph Properties** dialog to define **Detail Graph** properties including: title of graph, X- or Y-axis, graph scaling and background color.
Zoom	Select to enable zoom capability in the graph. Once the **Zoom** is selected, drag over a region in the graph to view just that area enlarged to the full size of the graph. Alternatively, drag the cursor along a part of the X- or Y-axis to zoom in on just that axis.
Undo Zoom	Rescales the graph to the original settings.
Full Scale	Scales the X- and Y-axis of the **Detail Graph** to the parameters defined in the **Manual Scale Graph** option.
Auto Scale	Manual prompt to automatically scale the **Detail Graph** to include all data points of the desired traces.
Auto Scale Always	Automatically scales the **Detail Graph** to include all data points of the desired traces without manual prompting.

Analyzing Data

The **Grouping** and **Correction** dialogs, opened from the **Analysis** process page, are used to analyze data produced during an experiment. **Settings** made in these dialogs in a protocol file affect the way that data, in data files generated with the protocol, are displayed when first viewed in ScreenWorks Software. They also affect data in automatic output options that occur when the protocol is run: automatic print output options (see Settings Process on page 58, and Auto Print Options on page 63) and **Auto Export** (see Exporting Data on page 82). However data files created by ScreenWorks Software retain all raw data readings, so the analysis options in the **Grouping** and **Correction** dialogs can be applied to acquired data, modifying the display of these, irrespective of the analysis settings configured in the original protocol.

Grouping and **Correction** are explained in the following separate sections.

Grouping

Define groups in the **Grouping** dialog, opened from the **Configure Groups** button on **Analysis** process page.

A group is a selection of wells with a common characteristic—usually in the type of compound added to the wells. Three groups—background fluorescence correction, positive controls and negative controls—are defined by default, but no wells are assigned to them. The well assignment for these groups is left to the user.

Users can create additional groups to represent wells with a specific ligand, or ligand concentration, or any other characteristic they want to classify data by. Group definition includes specific input for compound concentration, as this is commonly used for group definition. Additionally, the **Series** option allows users to create a series of groups, where a number of different concentrations are automatically assigned to each group. This can be useful for creating groups for an IC50 or EC50 experiment.

Groups are used to select data to be viewed in the detail graph, and the data itself can be corrected relative to responses to positive and/or negative control groups, in the **Correction** dialog.

The **Grouping** dialog contains a grid representing the 96-, 384- or 1536-well read plate, and above this a list box where groups are defined and selected. The basic mode of operation is to select a group from the list box, then drag the cursor over the wells in the grid that belong to that group. Additional groups can be defined to add to the list.

The following table describes the settings and options in the **Grouping** dialog:

Options	Description
Groups	Displays the list of defined groups, including group name (typically compound name), concentration and notes associated with the group. Wells assigned to a group in the grid below have the same color as the group in the list. **Positive Controls**—Use this group to assign positive control wells, typically the maximum response to a concentration of an agonist. **Note**: **Positive Control** wells need to be defined in order to use the **Positive Control Scaling Correction**. **Negative Controls**—Use this group to assign negative control wells, typically buffer addition controls. **Note**: Negative control wells need to be defined in order to use the **Negative Control Correction** feature. **BF Controls**—Use this group to assign wells in which to measure background fluorescence. Measurements for each time point from these wells are used for Background Fluorescence Correction for ratiometric data (see Ratiometric Options on page 77). **_add new group**—Use this to define a new individual group or a series of groups that are user-defined.

Options	Description
Add/Edit Group	Opens the **Edit Group** dialog to edit the group selected in the **Groups** list. This could be an existing group, or create a new group by selecting the **Add New Group** row. Shortcut: Open the **Edit Group** dialog by double-clicking on a group in the Groups list.
Delete Group	Deletes the group selected in the **Groups** list. Any wells assigned to the group lose their coloring, indicating that they no longer belong to any group.
Delete All Groups	Deletes all user-defined groups. **Note**: Positive, negative and background fluorescence control groups cannot be deleted, however this command deletes all wells assigned to these groups.
Clear All Selections	Removes all group assignments from all wells. Group names in the top list are not affected.
Undo Last Selection	Removes group membership from the last wells that were assigned to a group.

Changing Wells Assigned to Groups

To assign wells to a group:

1. Select the appropriate group from the **Groups** list, for example, **Positive Controls**.
2. Drag the cursor over the wells from the plate layout that you want to include in the selected group. Alternatively, the row or column title can be selected to include all the respective wells in the group.

   Note: Wells in a group do not need to be contiguous. Multiple areas of the plate may be selected.

   You can deselect individual or neighboring wells by right clicking in one corner of a well to be deselected, then dragging over the well or wells to deselect.
3. Repeat Steps 1–2 to assign other wells to different groups.

Adding a New Group

To add a new group:

1. Select **_add new group** from the **Groups** list and click the **Add/Edit Group** button (or double-click on the group). The **Select Type of Group** dialog opens.
2. You can choose to configure a single group with specific settings on the **Group** tab, or configure a series of groups, with incrementing concentration values, on the **Series** tab.

3. To assign a single group concentration, click the **Group** tab and do thefollowing:
   - Enter a name for the group (for example, compound name) in the **Group Name** field.
   - Select or enter the concentration in the **Concentration** box.
   - Select the concentration units in the **Units** drop-down list. The choices are nM, µM, mM, M or Log.
   - In the **Notes** area, type in any notes that you want associated with the specified group and data file.
4. To assign a series of concentrations, click the Series tab and do the following:

   Note: The **Series** option is typically used for assigning a dose-response curve with regular increment steps.

   - Enter a name for the group (for example, compound name) in the **Group Name** field.
   - Select or enter the lowest or highest concentration of the series in the **Starting Value** field.
   - Select or enter the change in concentration between concentrations in the series in the **Step Increment** field.
   - Select the concentration units in the **Units** drop-down list. The choices are nM, µM, mM or M.
   - Decide how the **Step Increment** is applied to the **Starting Value** to create the series, for example, Plus, Minus, Multiply or Divide, and select it from the **Operation** drop-down list.
   - If you have replicates, select whether they are aligned in row 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 selected rows will have same concentration). If two rows are to include the same concentration, indicate “2” replicates. If there are no replicates, select **None**.
   - Select the direction in which the series will increment, for example, Left, Right, Top or Bottom.
   - In the Notes area, type in any notes that you want associated with the specified group and data file.
5. When finished, click **OK** to save the definedgroup.

When a series is created, assign wells to it as described above. Successive rows or columns are assigned different concentrations and group color automatically, as configured in the series.

Correction

Use the **Correction** dialog to apply data-correction algorithms, and to view ratiometric data. These changes affect:

- Data displayed in the Multi-Well Graph and **Detail Graph**.
- Data displayed in group statistic table and graphs.
- Data in files are exported manually or automatically (see Exporting Data on page 82).
- Data in printed output configured in the **Settings** process page (see Auto Print Options on page 63).

Plainly, once data are exported, they cannot be changed, so settings made in this dialog in protocol files are important for these output options. For viewing data in ScreenWorks Software, however, since raw data are always kept, correction options selected in protocol files can be altered when the data are viewed in resulting data files.

The following table includes brief descriptions of the options in the Correction dialog; for a full descriptions, see Data Processing Algorithms on page 237 :

Options	Description
Crosstalk Correction	Only available with Aequorin, ICCD camera. Check this option to correct all wells for crosstalk from neighboring wells. The crosstalk function is based on well-to-well distance and the measured percentage of crosstalk.
Positive Control Scaling	Check to average each maximum RLU value from the positive control group and normalize all samples to this value (set at 100%). This function is useful when graphing a dose-response curve or when comparing data between experiments.
Negative Control Correction	Check this option to have an average negative control well value and ratio-to-well value calculated for each sample interval (time point). This ratio is calculated for each sample interval and applied to all wells. This function provides a good correction for signal drift and artifacts.
Spatial Uniformity Correction	Check this option to use the initial signal to normalize signal in each well, removing fluctuations due to well-to-well variation in cell density and dye loading. The processing algorithm averages the initial signal from all wells together. It then scales each individual well relative to the average. This correction is particularly recommended when you want to normalize cell number, type and dye-loading conditions throughout a plate. **Note**: Spatial uniformity should only be used when all wells in a plate are treated the same prior to an experiment (for example, dye loading, cell numbers, etc.).

Options	Description
Subtract Bias Based On Sample	Check this option to subtract the RLU measured at a selected read number from all other time points in each well. This option enables you to set the Y-axis scale so that at the time point specified, the Y-axis values for all data graphs is zero. Select the read number to be subtracted in the field to the right of the check box. The default is read number 1.
Response Over Baseline	Check this option to display the trace as a ratio of the response to the average of a set of predefined reads. **Baseline Start**—First read to be included in the averaged baseline value. **Baseline End**—Last read to be included in the average baseline value. **Show As Percentage**—Displays response as a percent increase over the average baseline value.

Each of the correction options can be applied alone or in combination with the others. When a combination is selected they are applied in the following order:

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

Note: Spatial uniformity correction and percent baseline cannot be applied at the same time.

Ratiometric Options

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

When this option is enabled, traces of the calculated ratio can be displayed in the multi-well and detail graphs.

Prior to calculating the ratio, ratiometric data is corrected for fluorescence background by subtracting estimated background values from both numerator and denominator for each time point. Once the fluorescence background is subtracted, the ratio is then calculated. Additional user-selected 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 disabled when **Ratiometric Options** are active.

Ratiometric parameter settings are described in the following table:

Options	Description
Ratiometric Options	Enable ratiometric data viewing by checking the check box. Specify how the ratio is defined by selecting the read modes to be used as numerator and denominator.
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**—Constant values to subtract from the numerator and denominator for each time-point ratio are entered by the user. You must enter these constant values in the numerator and denominator fields when this option is selected.

Group Statistics

Below the Multi-Well and Detail Graphs on the **Analysis** process page, the **Group Statistics Table** is used to analyze data produced during an experiment. Groups defined in the Grouping dialog can only be analyzed in this section. In addition, settings made in the Correction dialog influence the group statistics that are reported.

To analyze data in the **Group Statistics Table**, groups must first be defined in the **Grouping** dialog. When defined, the **Configure Kinetic Reduction** button is selected in Multi-Well Graph to define the reads and reduction type to be analyzed.

Features of the **Group Statistics Table** are explained in separate sections.

Buttons	Name	Description
	Copy Table Data	Copies data in the **Group Statistic Table** to the clipboard so it can be pasted in a different program such as Microsoft Excel.
	Collapse All Groups	Collapses the **Group Statistics Table** to only display the group name and relative statistics. This works as a toggle button with **Expand All Groups** which displays the individual wells and kinetic reduction values that comprise each group statistic.

Buttons	Name	Description
	Expand All Groups	Expands the **Group Statistics Table** to display all the individual wells and kinetic reduction values that comprise each group statistic. This works as a toggle button with **Collapse All Groups**, which displays only the group name and individual statistics.
	Auto Fit All Columns	This is used to collapse the column width so it is no wider than the title of the column or largest data point.
	Select Statistics	Opens the **Choose Statistics** dialog box which enables users to define groups statistics (for example, Average, Standard Deviation and Z-score) are displayed in the table. Group statistics available include the following: **Concentration**—Numerical concentration values assigned to each group in the **Grouping** dialog. **Units**—Concentration units (for example, uM) assigned to each group in the **Grouping** dialog. **Notes**—Comments assigned to each group in the Grouping dialog. **Average**—Numerical average of the kinetic reduction values for a given group. **Maximum**—Highest value (a single number) 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 associated 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.

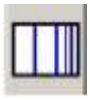
Buttons	Name	Description
	Select Statistics (continued)	**Z Score**—Used to evaluate quality or performance of the assay and is dependent on the concentration evaluated[1]. **Z-factor**—Used to evaluate the quality or performance of the assay at a given concentration. This is typically used for all concentrations, 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. This is 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.		

The statistics defined in the **Group Statistic Table** can be plotted in the **Group Statistic Graph**, located behind the **Detail Graph**, to quickly view a dose-response curve. All groups are included in the graph, unless the user right-clicks on the desired group and selects **Exclude Groups From Statistic Chart**. Groups not used in the graph will have a green line through the entire row of the group. To include the group in the graph, right-click the group and select **Include Groups In Statistic Chart**. Data displayed in the **Group Statistic Graph** is defined by selecting the desired group statistic from the **Group Statistic Table**. Therefore, if the standard deviation is the desired data to be evaluated, select the **Standard Deviation** column header to highlight this data in the **Group Statistic Table** and it will be displayed in the graph.

Features of the **Group Statistic Graph** are explained in separate sections.

Buttons	Name	Description
	Copy Graph	Copies data in the **Group Statistic Graph** to the clipboard so it can be pasted in a different program such as Microsoft Word.
	Copy Graph Data	Copies Graph Data in the **Group Statistic Graph** to the clipboard so it can be pasted in a different program such as Microsoft Excel.

Buttons	Name	Description
	Zoom Mode	Press to enable zoom capability in the graph. Once the **Zoom** button is pressed, drag over a region in the graph to view just that area enlarged to the full size of the graph. Alternatively, drag the cursor along a part of the X- or Y-axis to zoom in on just that axis.
	Auto Scale	Manual prompt to automatically scale the **Group Statistic Graph** to include all data points of the desired traces.
	Auto Scale Always	Automatically scales the **Group Statistic Graph** to include all data points of the desired traces without manual prompting.
	Undo Zoom	Rescales graph to the original settings.
	Manual Scale Graph	Opens the **Manual Scale Graph** dialog to set the maximum and minimum values for the X- and Y-axis graph.
	Show/Hide Data	Toggle button that shows or hides the data for the selected traces at each read.
	Show Point Labels	**Show Point Labels** writes the value of each data point (for example, RLU value) beside the point on the graph.
	Show/Hide Legends	A toggle button that displays or hides the detail graph legend.
	Show/Hide Smoothed Curve	A toggle button that displays or hides the 4-parameter curve fit. This feature can be displayed at the same time as **Show/Hide Original Trace**.
	Show/Hide Original Trace	A toggle button that displays or hides the trace of the graph, which connects each data point. This feature can be displayed at the same time as **Show/Hide Smooth Curve**.

Buttons	Name	Description
	Show/Hide Real Data Points	A toggle button that displays or hides the data points to create the graph. **Note**: It is recommended that either the **Original Trace** or **Smoothed Curve** features are activated when the data points are hidden.
	Display X-Axis in Log Scale	A toggle button that converts the X-axis from a concentration to a log concentration scale.
	Show/Hide EC/ICXX Value	A toggle button that displays or hides the effective/inhibition concentration from being displayed. When activated, the user defines the percentage of activation/inhibition that the graph will plot.

Exporting Data

ScreenWorks Software has two means of exporting data:

- Automatic export when a protocol is run, configured in protocol files.
- Export from already acquired data, in data files.

Export options are accessed in different ways in the two cases:

- In protocol files use the **Auto Export** button in the **Analysis** process page.
- For data files, use **File > Export**, **File** or **Batch Export**, or the **Export** button on the **Analysis** page. The **Export** button allows export only of the current data file, while the **File** menu has options to export the current file or batches of files on disk.

Data is exported as ASCII text format files, with a separate file exported for each measurement configuration you ask for. When data is exported from data files you must enter a folder to write the output files to. In the **Auto-Export** option, files go to the folder defined in the Settings process. The default export folder is:

C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyData

The **Auto-Export** dialog has three tabs:

- **Time Sequence**—Exports time-point measurements for selected read modes. The measurement values that are exported have any corrections configured in the **Correction** dialog applied. If there are two read modes and **Ratiometric Options** is selected in the **Correction** dialog, you can also export the ratio for each time point. Files have a *.seq*n* extension, where n increments for each file generated in the export.
- **Statistics**—Exports averages, maximums, and other kinetic reduction values for selected numbers of reads for each well.
  Files have a *.stat*n* extension, where n increments for each file generated in the export.

- **Group Statistics**—Exports the group statistical values for selected numbers of reads for each group and are based on user-defined kinetic reduction settings.
  Files have a *.gstat*n* extension, where n increments for each file generated in the export.

Further details about these tabs are provided in the sections below.

When exporting data files the dialog also has a **Files** tab where you can set the export directory and select batch files to export if this option is selected.

Note: Data created in previous versions of the FLIPR Tetra system operating software cannot be viewed or exported by ScreenWorks Software.

Exporting Time Sequence

Configure parameters for export of time-sequence data in the **Export Time Sequence** tab. The exported files contain a value for every read interval for each well, for the read mode or ratio selected. This contrasts with the Statistics file, which contains one kinetic reduction per well.

The following table describes the settings and options of this dialog:

Options	Description
Enable Time Sequence	Check this option to have a time sequence export file created.
File Name Options	Choose a name for the exported time sequence file. **Use name of data file**—Export files with the data or protocol file name, with *.seq*n* extension, where *n* is an integer ≥ 1. **Use user-defined name**—Enter your own name for the export files (maximum of 25 characters). Files are given *.seq*n* extension, where *n* is an integer ≥ 1.
Data Format	Format the output from this group of options: **Show Labels**—Check to include information about the processing options selected (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. **Order of Well Data**—Order the data by column (for example, A1, A2, A3) or by row (for example, A1, B1, C1). Set this option in accordance to the way your spreadsheet or database handles well data. **Order Time Data**—Select vertical or horizontal ordering of the data.
Misc. Parameters	Select experimental parameters and group statistics to export with the data.

Options	Description
Individual File Parameters	Use the bottom section of the dialog to choose the number and type of time sequence files to be created from the same data set. **Number of Files**—Click the **Add File** button to add to the number of statistic files to be created from the same data set. **Read Mode**—Enter the read mode (or ratiometric data) from which you want to export data. **From Quadrant**—You can choose a specific quadrant from which to export, or export data from all quadrants. This enables you to export data from one plate into four different spreadsheet files, if desired. **Note**: This option is only available for data created in 384- or 1536-well formats.

Exporting Statistics

Use the **Export Statistics** tab to configure kinetic reductions, for example, averages and maximum and minimum values, for selected numbers of reads within each well. Multiple kinetic reductions can be configured, each resulting in another export file. Within each file only one value is reported per well.

The following table describes the settings and options of this dialog:

Options	Description
Enable Statistics	Check this box to have a statistic file created.
File Name Options	Choose a name for the exported statistic file. **Use name of data file**—Export files with the data or protocol file name, with *.stat*n* extension, where *n* is an integer ≥ 1. **Use user-defined name**—Enter your own name for the export files (maximum of 25 characters). Files are given *.stat*n* extension, where *n* is an integer ≥ 1.
Data Format	Format the output from this group of options: **Show Labels**—When checked, the output file contains information about the processing options selected (for example, name of *.fmd file and well labels). Any corrections done prior to exporting the data (e.g., negative control correction) are indicated in text format at the top of the exported ASCII file. **Order of Well Data**—Order the data by column (for example, A1, A2, A3) or by row (e.g., A1, B1, C1). Set this option in accordance to the way your spreadsheet or database handles well data.
Misc. Parameters	Select experiment parameters and group statistics to export with the data.

Options	Description
Individual File Parameters	Use the bottom section of the dialog to designate the number and type of statistic files to be created from the same data set. **Number of Files**—Click the **Add File** button to add to the number of statistic files to be created from the same data set. **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**—Enter the last read number to be included in kinetic reduction. This can equal the Start Read, if you want to extract values from a single read. **Read Mode**—Enter the read mode (or ratiometric data) from which the data will be processed. **From Quadrant**—You can choose a specific quadrant from which to export, or export data from all quadrants. This enables you to export data from one plate into four different spreadsheet files, if desired. **Note**: This option is only available for data created in 384- or 1536-well formats.

Exporting Group Statistics

Use the **Export Group Statistics** tab to configure group statistics (for example, average, standard deviation and z-scores) based on the kinetic reduction defined for a select number of reads.

The following table describes the settings and options of this dialog:

Options	Description
Enable Group Statistics	Check this box to have a group statistic file created.
File Name Options	Choose a name for the exported group statistic file. **Use name of data file**—Export files with the data or protocol file name, with *.gstat*n* extension, where *n* is an integer ≥ 1. **Use user-defined name**—Enter your own name for the export files (maximum of 25 characters). Files are given *.gstat*n* extension, where *n* is an integer ≥ 1.

Options	Description
Kinetic Reduction	Configures the parameters used to define the kinetic reduction. **Reduction Type**—Defines the reduction to be applied to the kinetic data traces exported. See Kinetic Reduction Types on page 67 for details. **Start Read**—Define the first read to be used to determine the kinetic reduction. **End Read**—Define the last read used to determine the kinetic reduction. **Read Mode**—Select the read mode to apply the kinetic reduction.
Misc. Parameters	Select experiment parameters to export with the data.

Batch Exporting

Manual export of files is accessed through **File > Export**, **File** or **Batch Export**. **Manual Export** allows the same files as defined above to be exported. In addition to individually exporting files, **Batch Export** enables batches of data files to be exported to individual or single export files.

The following table describes the settings and options of the **Batch Export** dialog:

Options	Description
Export Directory	User defines where export files are to be sent. The default export folder is: **C:\Documents and Settings\[your_user_name]\My Documents\Molecular Devices\ScreenWorks\MyData**
Batch Export Following Files	Selecting **Add** opens the **Open File** dialog to choose data files to be exported. **Note**: Hold down the **SHIFT** or **CTRL** key to select data files.

Options	Description
Correction Options	During export, either the existing or new corrections can be applied. **Use corrections already applied in each data file**—Applies the existing corrections saved with each data file during export. **Use same corrections for all files**—Applies and saves new corrections to all data files. Useful when exporting all data files with same parameters or saving the same correction to multiple data files. Details regarding available corrections can be found under Correction on page 76.
If an error occurs while trying to export a file, do the following	**Put up an error message and let me decide what to do**—Enables the user to decide how to proceed when an error is encountered. **Skip the file**—Does not include the data in the export, but continues to export data. The option to write the error to a log file is available.

When batch exporting data, the export files produced will vary depending on the options you select in the individual export statistic, time sequence and group statistic sections. If a single export file is desired for each data file exported, select **Use name of data file** in the respective section you want to export. Export files created using this method will be labeled with the name of the data file it was created from. However, if a single export file is desired that contains information for multiple data files, select **Use user-defined name** in the respective section. In this instance, all exported information will be combined into one file labeled with the desired user-defined name. When the file is open, the individual data file names which the information was exported from are used as the header for each data set within the export file.

Image Display

Use the **Image Display** dialog, opened from the **Analysis** process page, to view images saved in data files when **Save Images** was enabled during a **Read with TF** step. This option stores a total of 100 images per experiment; the number of images per dispense is user-defined. The default is set to one image before and nineteen images after the fluid addition is initiated. Saved ***.tif** files can be played back in sequence, frame-by-frame or as a video.

Image Display is useful for diagnosing problems. For example, if cells are blown off during a fluid dispense, you will 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

In protocol files, open the **Protocol Notes** dialog where you can enter comments that you want kept with all data files generated with the protocol. The dialog contains a simple text editor for you to format your comments.

In data files the same dialog shows the comments that were added in the protocol file, now in read-only format, so they cannot be edited.

Transfer Fluid Process

This process enables you 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 (which will also have a purple icon).

The **Transfer Fluid** process has different options for pin tools and standard pipettors. In each case you must configure aspiration and dispense of compound separately.

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

With a standard pipettor selected, aspiration and dispense steps are configured by selecting the appropriate row in the aspiration or dispense table and pressing the **Edit** button to open a configuration dialog. With the Pin Tool, aspiration and dispense configuration is done directly in the **Transfer Fluid** page.

To enable additional dispense steps with a standard pipettor, select the multiple dispense option in the **Fluid Transfer Type**. Then configure the first dispense with a volume less than the volume aspirated. An additional dispense is automatically enabled for the remaining volume. Similarly, 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)

The following table describes configuration options for the upper, **Aspirate**, section on the **Transfer Fluid** process page when a standard pipettor is selected.

Option	Description
Fluid Transfer Type	Select a type of fluid transfer: **Single Aspirate–Single Dispense**—Aspirates one liquid volume and dispenses that entire liquid volume in one action into a plate. **Single Aspirate–Multiple Dispense**—Aspirates one liquid volume and dispenses 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. **Note**: The last dispense expels all liquid remaining in the tip. Tips to improve **Single Aspirate–Multiple Dispense** (by quadrature) pipetting precision include: * When pipetting do not use hold and expel volumes. In addition 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 dispensing 25 µL into four quadrants. * Include a mix step with 3 strokes in the desired 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. **Multiple Aspirate–Single Dispense—Aspirates** up to four liquid volumes from up to four aspirate plates and dispenses entire tip contents into a single plate. To add additional aspirates, make the aspirate volume less than the dispense volume.
Read	Check this box to link a **Read** process to the **Transfer Fluid** process. This automatically adds a **Read with TF** step to the protocol, and opens the configuration page for that step. See Read Process on page 97 for more information about read process options. **Note**: This option is required if you intend to read a plate while transferring liquid during a kinetic cell-based assay (for example, calcium mobilization).
Aspirate	Check the check box at the start of a row to activate an aspirate sequence. If **Multiple Aspirate** has been selected as **Fluid Transfer Type**, enter an aspirate volume less than the dispense volume to add another aspirate step. **Note**: Only checked aspirate sequences are active in the experimental protocol.

Option	Description
Edit Aspirate	Configure an aspirate step in the table by selecting it and pressing the **Edit Aspirate** button, or double-click on the row. This opens the **Edit Aspirate** dialog. Use this dialog to define aspiration parameters reported in the table. **Source Plate**—Select the plate from which you want an aliquot of fluid removed. Typically, this is a plate containing an agonist, antagonist or if the **Cell Suspension** option is installed, cells in suspension. **Quadrant**—Select quadrant from which volume is aspirated. **Note**: **Quadrant** selection is only available when liquid is aspirated from a plate using a pipettor head of lower density, for example, 384-well plate using a 96 pipettor head. **Volume**—Enter volume (in µL) to aspirate. **Speed**—Enter speed at which to aspirate (in µL/s). **Fill Reservoir Speed**—Select the speed with which fill the Cell Reservoir from the spinner flask.
Edit Aspirate (continued)	**Drain Reservoir Speed**[1]—Select the speed with which to drain the cells from Cell Reservoir to drain destination. **Drain Destination**[1]—Select a destination where cells should be drained from Cell Reservoir after pipettor aspiration. **Height**—Enter distance (measured in µL) from the bottom of the well the tips should start aspirating. **Note**: Tips move downward full volume of aspiration, for example, aspirate 10 µL with a start height of 35 µL will put the pipettor height at 25 µL after aspiration. Pipettor head movement helps prevent well overflow when tips are submerged in the well. If downward pipettor motion is not desired, set tip height to minimum value. Tip movement downward halts when the tips reach 1/30th well volume. **Hold Volume**—Enter size of 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 tips are pulled from well immediately after aspiration. This value is adjusted depending on fluid viscosity or volume of fluid in the well. **Note**: For low volume transfers, a slow up-speed may improve precision.
Put Tips in Well Before Read	Check this option to place tips in the read plate prior to beginning read intervals. This option is suggested for assays in which placing tips below the fluid surface can prevent excessive turbulence. Enabling this option helps ensure accurate timing of fluid dispense relative to reads.
1. These options are only available when Cell Reservoir is installed and selected in Source Plate 3 settings.	

Dispense Configuration (Standard Pipettor)

The following table describes **Dispense** configuration on the **Transfer Fluid** process page when a standard pipettor is selected.

Options	Description
Dispense	Check the check box at the start of a row to activate a dispense sequence. If **Multiple Dispense** has been selected as **Fluid Transfer Type**, enter a dispense volume less than the aspirate volume to add another dispense step. **Note**: Only checked dispense sequences are active in the experimental protocol.
Edit Dispense	Configure a dispense step in the table by selecting it and pressing the **Edit Dispense** button, or double-click on the row. This opens the **Edit Dispense** dialog. Use this dialog to define dispense parameters reported in the table. **Target Plate**—From the list, select the plate to receive the fluid dispense. Typically, this is the Read Plate. **Quadrant**—Select quadrant from which volume is dispensed. **Note**: **Quadrant** selection is only available when liquid is aspirated from a plate using a pipettor head of lower density for example, 384-well plate using a 96-pipettor head. **Volume**—Enter volume (in µL) to dispense. **Note**: In the last dispense, the entire contents in the tip is dispensed. **Height**—Enter the distance (measured in µL) from the bottom of the well the tips should be inserted prior to dispensing. **Note**: Tips move upward the full volume of dispense during dispense, for example, a 10 µL dispense with a start height of 35 µL will put the pipettor height at 45 µL after dispensing. This movement helps prevent well overflow when tips are submerged in the well. Tips will not move higher than the full volume of the well. Height value will need to be adjusted when transferring from previous FLIPR Tetra systems.

Options	Description
Edit Dispense (continued)	**Speed**—Enter speed at which to dispense (in µL/s). Ideal dispense speed takes into account 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. Typically, this value is equal to the aspirate Hold Volume value. In a multiple dispense, only the last dispense can have an **Expel Volume**. If **Mix Fluid After Dispense** is selected, **Expel Volume** automatically changes to zero for the dispense associated with the fluid transfer, to ensure that tips remain in the well prior to the mix step. However, the expel volume designated in the dispense is transferred to the **Mix with TF** step, to help ensure liquid is properly expelled from the tips after mixing. **Removal Speed**—Enter rate (in mm/s) at which tips are pulled from well immediately after the dispense. This value is adjusted for fluid viscosity, fluid volume in the well or if the transfer is performed to a dry well. **Pause in Well**—Enter time (in seconds) to pause with the tips in the wells after dispensing and mixing (if specified) before the next pipettor move. Read intervals may occur simultaneously with this step. Typically, the pipettor is paused during a read to prevent disturbances in the imaging process.
Mix Fluid After Dispense	Check this box to link a **Mix Fluid** process to the **Transfer Fluid** process. This automatically adds a Mix with TF step to the protocol, and opens the configuration page for that step. See Mix Fluid Process on page 95 for more information about mixing options. **Note**: This option is only available when **Fluid Transfer Type** has a single dispense.

Options	Description
Unload Tips after Fluid Transfer	Instructs the instrument to unload tips after the Fluid Transfer process. Typically, this is used when changing the tips during an experiment or when reducing down-time between experiments. **Note**: This option is only available when **Load Tips Position** is selected on the **Settings** process page. Using this feature also requires that no source plate is placed in plate position 3 during the experiment.

Aspirate Configuration (Pin Tool)

The following table describes aspiration configuration options when a pin tool is selected.

Options	Description
Source Plate	Select the plate from which you want an aliquot of fluid removed. Typically, this is a plate containing an agonist or antagonist.
Height	Enter distance (measured in µL) from the bottom of the well to place the pins. The distance equivalent in millimeters is displayed as well. **Float pins**—Moves 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 volume is aspirated. **Note**: **Quadrant** selection is only available when liquid is aspirated from a plate using a pipettor head of lower density, for example, 1536-well plate using a 384 pin tool head.
Down Speed	Enter the speed (in mm/s) at which the pin tool is inserted into the source plate.
Removal Speed	Enter the speed (in mm/s) at which the pin tool is removed from the source plate. The speed of removal determines the volume of fluid remaining on the pins. **Note**: Increasing the removal speed increases the volume of compound on the pin after aspiration. To decrease the fluid volume, slow down the removal speed. It is recommended that assay development be done to determine the volume transferred 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)

The following table describes dispense configuration options when a Pin Tool head is selected.

Options	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 the tips should be inserted. The distance equivalent in millimeters is displayed to the right of the µL value.
Quadrant	Select the quadrant of the target plate into which compound is to be dispensed. **Note**: **Quadrant** selection is only available when liquid is aspirated from a plate using a pipettor head of lower density, for example, 1536-well plate using a 384 pin tool head.
Down Speed	Enter the speed (in mm/s) at which the pin tool is inserted into the target plate.
Removal Speed	Enter the speed (in mm/s) at which the pin tool is removed from the target plate. The speed of removal determines the volume of fluid remaining on the pins.
Pause in Well	Enter a duration (in seconds) to hold the pin tool in the dispense plate after full insertion and before removal.
Read	Check this box to link a **Read** process to the Transfer Fluid process. This automatically adds a **Read with TF** step to the protocol, and opens the configuration page for that step. See Read Process on page 97 for more information about read process options. **Note**: This option is required if you intend to read a plate while transferring liquid during a kinetic cell-based assay (for example, calcium mobilization).
Mix Fluid	Check this box to link a **Mix Fluid** process to the **Transfer Fluid** process. This automatically adds a **Mix with TF** step to the protocol, and opens the configuration page for that step. See Mix Fluid Process on page 95 for more information about mixing options.
Unload Pin Tool	Automatically unload the pin tool after the dispense.

Mix Fluid Process

Fluid mixing steps can be added to protocols as independent protocol steps, **Mix Fluid** or linked to a **Transfer Fluid** step, **Mix with TF**. Independent Mix Fluid steps can be configured to mix fluid in source or read plates, while Mix with TF steps immediately follow the Transfer Fluid step they are linked to, mixing fluid in the read plate where compound was dispensed.

Different mix options are offered for standard pipettors and the pin tool, described in the following sections.

Mix Fluid (Standard Pipettor)

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

The following table describes the options available on the **Mix Fluid** process page:

Options	Description
Mix Plate	Select the plate that has the fluid to be mixed. Typically, it is a source plate from which you will be transferring fluid.
Quadrant	If appropriate, select a quadrant from which fluid is mixed. **Note:** Quadrant selection is only available when liquid is dispensed to a plate using a pipettor head of lower density, for example, 384-well plate using a 96 pipettor head.
Volume	Enter volume (in µL) to mix.
Speed	Enter speed (in µL/s) to mix the fluid.
Height	Enter distance (in µL) from the bottom of the well that the tips will be inserted prior to mixing. **Note:** Tips move up and down with aspirate and dispense commands. These movements help to prevent well overflow when the tips are submerged.
Expel Volume	Enter an additional volume (in µL) the pipettor dispenses, beyond the volume entered in the Volume field above. This helps to ensure that all fluid is expelled from the tips after mixing.
Strokes	Enter number of times to aspirate and dispense mix volume. One stroke is equal to one aspiration and one dispense.
Removal Speed	Enter rate (in mm/s) at which tips are pulled from well immediately after the mix. This value is adjusted for fluid viscosity or fluid volume in the well.
Pause In Well	Enter time (in seconds) to pause with the tips in the well after mixing and before the next pipettor head move.
Unload Tips After Mix	Automatically unloads 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.

Options	Description
Mix Plate	Select the plate that has the fluid to be mixed. Typically, it is a source plate from which you will be transferring fluid.
Top Height	Enter the height (in µL) for the top of the stroke during mixing.
Quadrant	If appropriate, select a quadrant in which fluid will be mixed. **Note:** Quadrant selection is only available when liquid is dispensed to a plate using a pipettor head of lower density, for example, 1536-well plate using a 384 pipettor 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 rate (in mm/s) at which pins are pulled from the well immediately after the mix. This value is 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 will be removed from the well.
Pause in Well	Enter a duration to hold the pin tool in the well plate once mixing is completed, before withdrawal.
Unload Pin Tool	Check to have the pin tool unloaded after mixing.

Mix With TF

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

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

Note: Any **Expel Volume** configured in the dispense step prior to a mix is locked if **Mix Fluid After Dispense** is selected. Tips are raised during Expel Volume, but this could lead to inadequate mixing, so mixing occurs with the tips at the level they finish the basic dispense at, and **Expel Volume** occurs after mixing is completed.

Mixing parameters for a **Mix with TF** are similar to a **Mix Fluid** process, however **Mix Plate**, **Quadrant**, **Hold Volume** and **Tip Up Speed**, are not configurable. These options can only be accessed in the **Transfer Fluid** process to which the mix is linked.

To remove a **Mix with TF** process, deselect it in the **Transfer Fluid** process dialog.

Read Process

Read steps can be added to protocols as independent protocol steps, **Read** or linked to a **Transfer Fluid** step, **Read with TF**. Configuration options for the two types of step is similar, except **Read with TF** allows you to time the read precisely with the time that compound is dispensed, and it also gives you the option to save the images used to measure the response.

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 opens when the icon is released. When added to the protocol, the icon is orange, indicating 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. A single Read process can be divided into two intervals with different read rates. Typically this is used to take images at a high rate while compound is being added, and shortly thereafter, to best capture kinetic effects, then images can be taken at a less frequent rate as cells settle and less change occurs.

Note: The read rate you configure for the Read process is the same for all the read modes you have enabled. For example, if you set the Read Interval to 1 s, and have two read modes, two images are taken every second—one for each read mode.

The following table describes the options available in the Read process page:

Options	Description
First Interval	Configure the first series of reads taken during the **Read** process. **Read Interval**—Enter time (in seconds) between reads. The same interval is used for each read mode (for example, the instrument takes images twice as fast if a second read mode is enabled). **Note:** Time should be no less than exposure time plus read-out time (see Setup Read Mode on page 59 for more information). If it is less, then the experiment speed is determined by the capability of the computer. **Number of Reads**—Enter the total number of reads to be taken in the first interval.
Second Interval	Configure the second series of reads taken during the **Read** process. If you do not want a second series with a different read interval, make **Number of Reads** zero. **Read Interval**—Enter time (in seconds) between reads. The same interval is used for each read mode (for example, the instrument takes images twice as fast if a second read mode is enabled). **Note:** Time should be no less than exposure time plus read-out time (see Setup Read Mode on page 59 for more information). If it is less, then the experiment speed will be determined by the capability of the computer. **Number of Reads**—Enter the total number of reads to be taken in the second interval.

Read With TF

Plate reading 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. Inserting a read this way turns the **Read** process icon purple, indicating that the read occurs concurrently with liquid addition.

Read with TF configuration is similar to that of an independent Read process, but with the addition of two more options; **Number of Reads Before Dispense** and **Save Images**.

Note: To remove a **Read With TF** process, uncheck the **Read** option in the **Transfer Fluid** process dialog box.

The following table describes the additional options in the **Read with TF** dialog:

Options	Description
Number of Reads Before Dispense	Enter the number of reads to be taken before fluid is added to the plate. This provides a cellular response baseline prior to addition of agonist or antagonist. Number of **Reads Before Dispense** is included in the total number of first interval reads entered 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 will be 55 reads after dispense is initiated.
Save Images	When checked, up to 100 images are saved in an image file (*.png) associated with an experiment. These images can be used for more than one dispense as long as the total images saved do not exceed 100 for the experiment. **Number of Images Before Dispense**—Enter the total number of images to be saved prior to initiating the dispense. **Number of Images After Dispense**—Enter the total number of images to be saved after initiating the dispense. By default one image prior to and nineteen after dispense are saved for review in Image Display on page 87. **Note:** Images may be useful to troubleshoot problems with your cell plate such as cells detaching during fluid dispense.

Wash Tips or Pins Process

This process is normally used to eliminate unwanted agonist or antagonists prior to another addition within an experiment or between experiments. In many cases, it reduces consumable costs and downtime associated with replacing tips.

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 repeated 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. It is recommended that pins are blotted at a blotting station to remove excess liquid from the pins between washes.

Wash Tips (Standard Pipettor)

The following table describes the options available in the **Wash Tips** process page, for a standard pipettor:

Options	Description
Fluid Type	Select the fluid in the wash reservoir during tip washing, from wash fluid container A or B.
Pump Speed	Select the speed (slow or fast) you want the pump to fill and empty the wash reservoir. **Note:** Molecular Devices recommends you use Fast except with volatile solvents.
Wash Cycles	From the list, 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	From the list, select the number of times to aspirate and dispense wash fluid.
Volume/Stroke	Enter the volume (in µL) of fluid to be aspirated and then expelled for each stroke.
Hold Time	Enter the length of time (in seconds) to pause between aspirating and expelling fluid.
Aspirate Speed	From the list, select the speed (in µL/s) to aspirate fluid.
Dispense Speed	From the list, select the speed (in µL/s) to dispense fluid.
Unload Tips After Washing	Check this option to have tips removed from the pipettor head after washing is complete.

Wash Pins (Pin Tool)

The following table describes the options available in the **Wash Pins** process page, for a **Pin Tool**:

Options	Description
Fluid Type	Select the fluid in the wash reservoir during tip washing, from wash fluid container A or B.
Pump Speed	Select the speed (slow or fast) you want the pump to fill and empty the wash reservoir.  **Note:** Molecular Devices recommends you use Fast except with volatile solvents.
Wash Cycles	From the list, 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	From the list, select the number of times to raise and lower the pins. One stroke is equal to one upstroke and one down-stroke.
Down Speed	Set the speed of the pin tool during the downstroke.
Stroke Distance	Set the height of one stroke that the pin tool travels.
Up Speed	Set the speed of the pin tool during the up-stroke.
Removal Speed	Set the speed that the pin tool is withdrawn from the wash location. The faster the withdrawal speed the more fluid remains on the pins.
Pause in Well	Set a time to hold the pin tool at the bottom of the wells in the wash solution after the wash is completed.
Unload Pin Tool After Wash	Check to have the pin tool removed after washing is complete.

Blot Pins Process

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

Options	Description
Target Plate	Select the plate.
Height	Enter distance (measured in µL) from the bottom of the well to place the pins. The distance equivalent in millimeters is displayed as well. **Float pins**—Moves 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 a duration during which the pin tool is held motionless at the set height.
Unload Pin Tool After Blot	Automatically unload the pin tool once blotting is complete.

Pause Pipettor Process

Pause Pipettor steps are included in a protocol to prevent future pipettor motion until the pause is complete. **Read** processes can continue to execute, unless stopped by the linked action “read with transfer”. This can be useful to stop the pipettor from picking up compound if it will then sit for an extended period in the air. Also, it can be used to provide time for solvents (such as EtOH) to evaporate off the pins after a wash.

This process has only one configurable setting, the duration of the pause, entered in seconds.

Wash Cell Reservoir Process

This process is only available when the Cell Reservoir is installed and is recommended for use before and after using cells in suspension.

This process can be used as a way of priming the reservoir with cells prior to an assay or for cleaning purposes.

No pipetting is associated with this step.

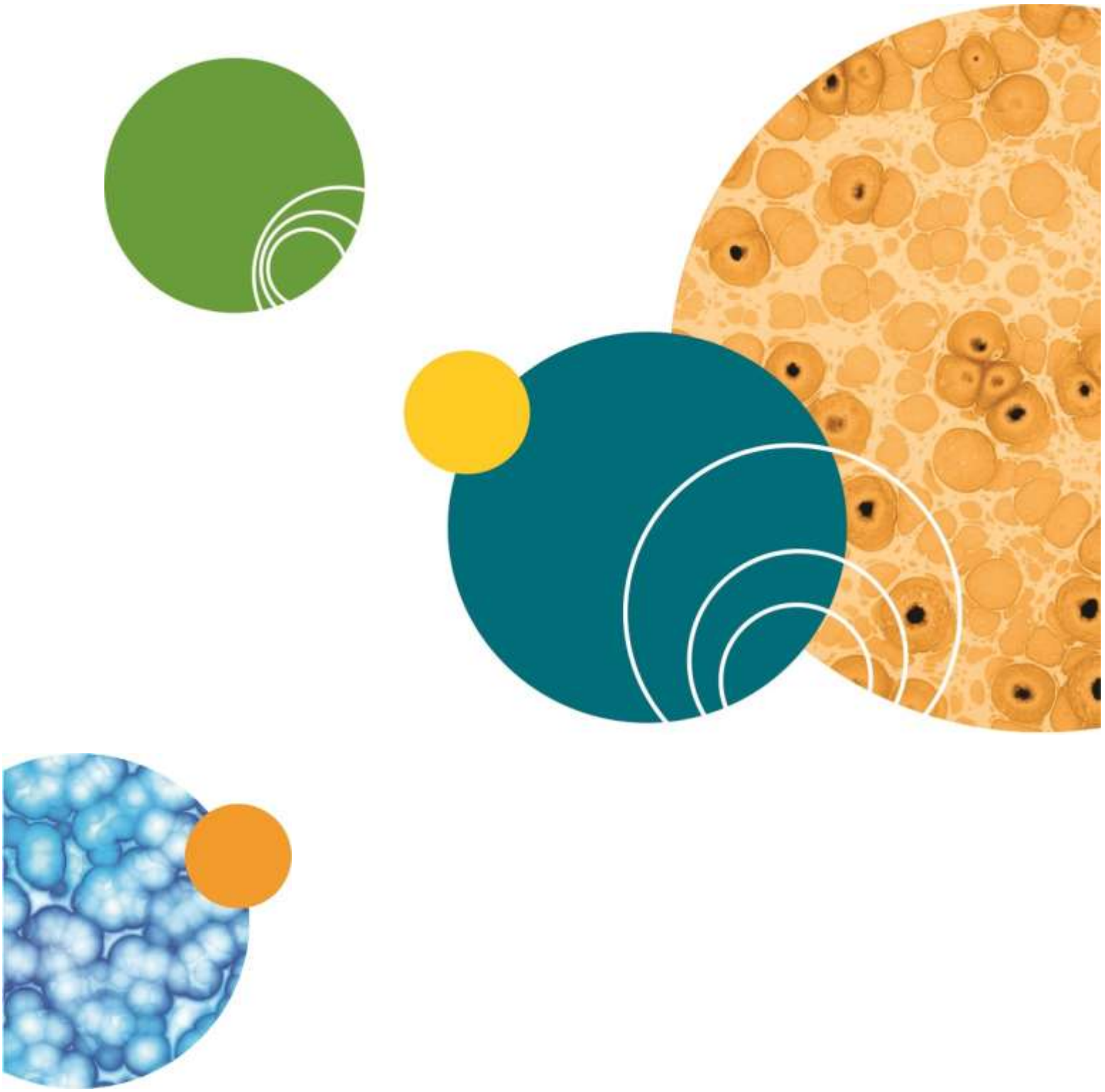
The following table describes the options available in the **Wash Cell Reservoir** process page.

Options	Description
Pre-coat Tubes	Select to pump the fluid up to the sensor before filling the reservoir.
Fluid Source	Select which fluid to use for the wash process. All Fluid 1–4 bottles are available as well as the Cell Flask.
Drain Reservoir Destination[1]	Select a 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 a pump rate for filling the Cell Reservoir; the range is 1–10.
Drain Reservoir Speed[1]	Select a pump rate for draining the Cell Reservoir; the range is 1–10.
Wash Cycles	Set how many wash cycles with the above settings should be done.
Hold Time	This option will soak the reservoir in the pumped fluid by leaving the fluid in the reservoir for a specified time; the range is 0–300 seconds (0–5 minutes).
1. This option is only available when the Cell Reservoir is installed and selected as the source plate for aspiration.	

Finish With Source

In **Remote Mode**, the **Finish with Source** process instructs the instrument to notify the robot controller to remove the desired source plate. This option is required when using multiple plates in a single source location.

Note: This process is not active in **Manual Mode** or for the read plate position.



Chapter 6: Exchanging Hardware

6

The following procedures instruct you on how to exchange pipettor and pin tool heads and their respective wash reservoirs on the FLIPR Tetra system.

Exchanging Pipettor and Pin Tool Heads

All pipettor and pin tool heads must be installed and calibrated on their intended instrument for the first time by a certified Molecular Devices Field Service Engineer. When calibrated, each head can be easily exchanged but only on the instrument for which they were calibrated.

Note: If you have any questions or concerns regarding the procedures, contact Molecular Devices Technical Support. See Obtain Support.

Uninstalling a Pipettor or Pin Tool Head

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

To uninstall a 384 pipettor head:

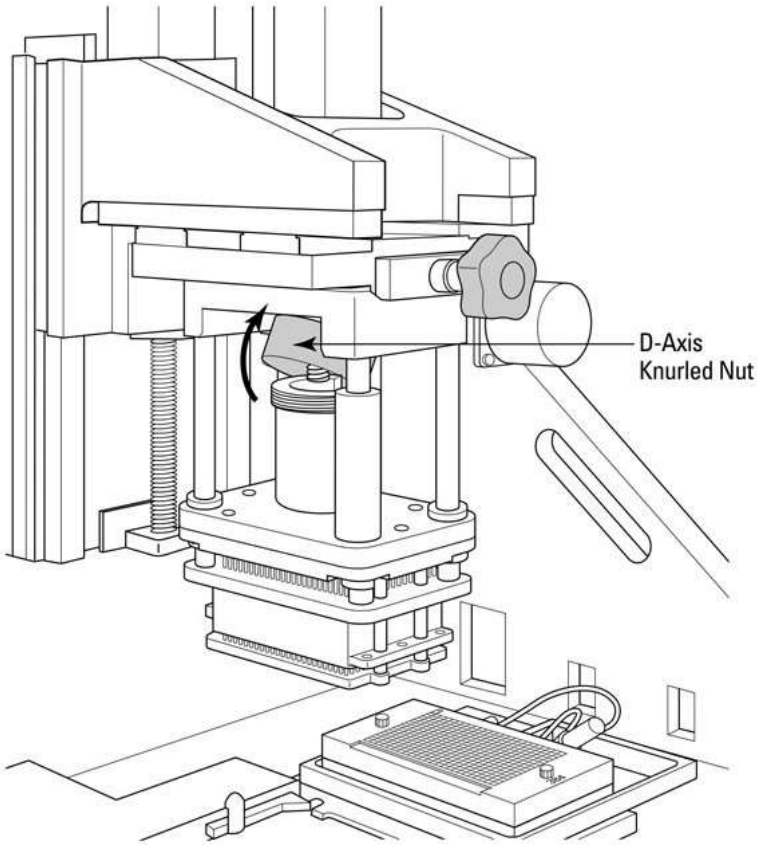
1. In the ScreenWorks Software, unload all tips on the pipettor head by selecting **Instrument > Manual Operation > Unload Tips**.
2. Select **Instrument > Manual Operation > Change Head**. This command instructs the pipettor head to move over the Read Plate position.

   CAUTION! DO NOT select **DONE** in the dialog that opens until you have completed work on the pipettor.

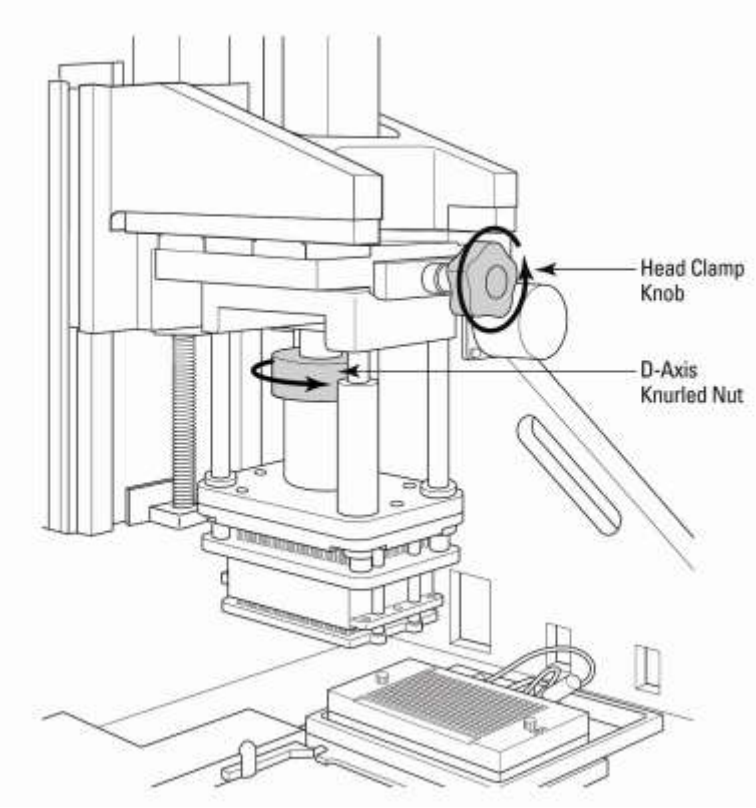
3. Open the upper front door to access the pipettor head.

4. Turn the **D-Axis Knurled Nut** counter-clockwise until it loosens and can be lifted.

   For 1536 pipettor heads only: After loosening the **D-Axis Knurled Nut**, install the **Pipettor Head Guard**, which seats over the 1536-pipettor head plungers, by tightening the two thumb screws. This guard protects the plungers from being damaged while the pipettor head is being handled outside the instrument.

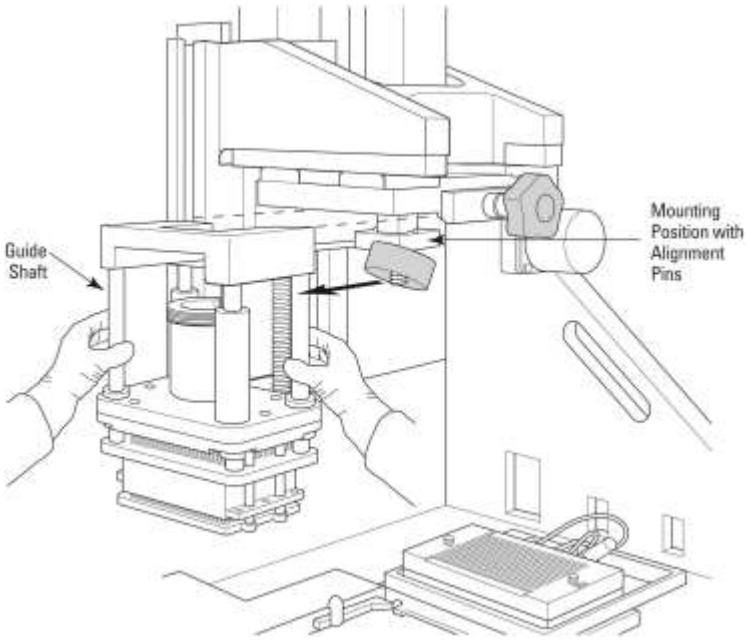


5. Turn the **Head Clamp Knob** counter-clockwise until the head is loose and can be lifted off its mounting position.



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 pipettormount.

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



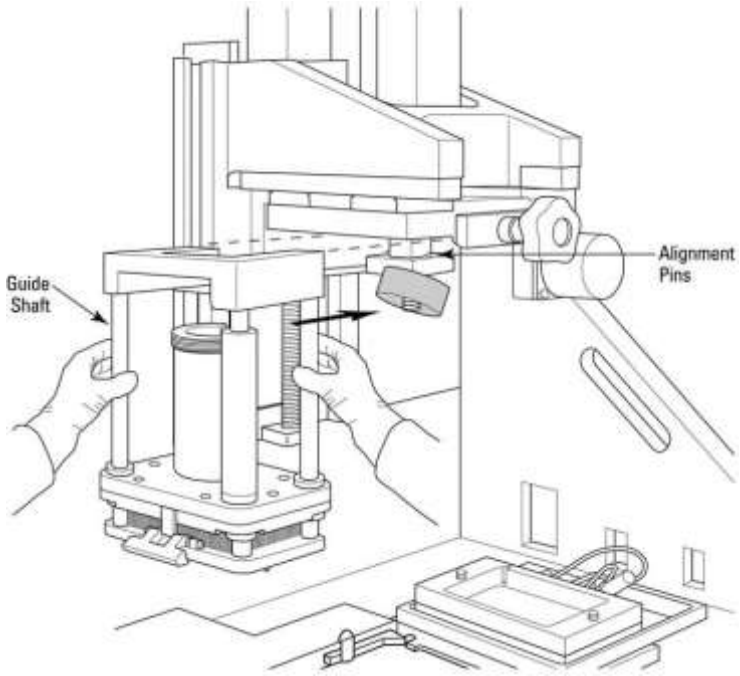
7. Store the pipettor head in a safe location. A recommended safe location should include placing the pipettor head in its plastic storage bag to guard for particulates.

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 seats on the pipettor mount position.

Installing the Pipettor Head

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

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.

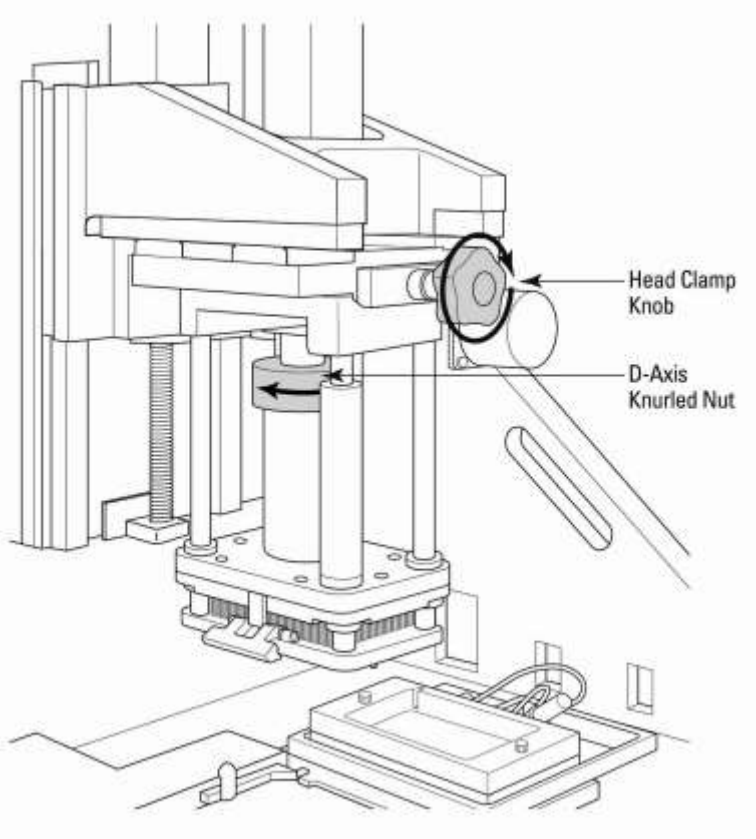


2. Tighten the **Head Clamp** knob by turning it clockwise.

Note: For 1536 pipettor heads only: 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 prior to resetting the instrument, the 1536 pipettor head may be damaged.

3. 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.

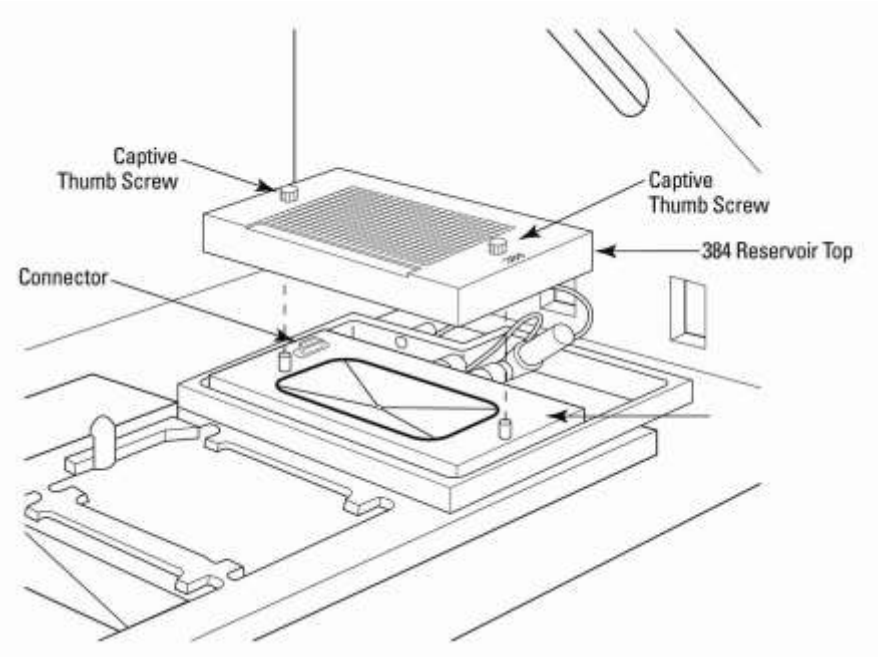


4. After the pipettor head is fastened, see the following procedure to exchange the tip wash reservoir to match the pipettor head format.

Uninstalling Wash Reservoir Top

The following procedure uninstalls a 1536 wash reservoir top as an example.

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

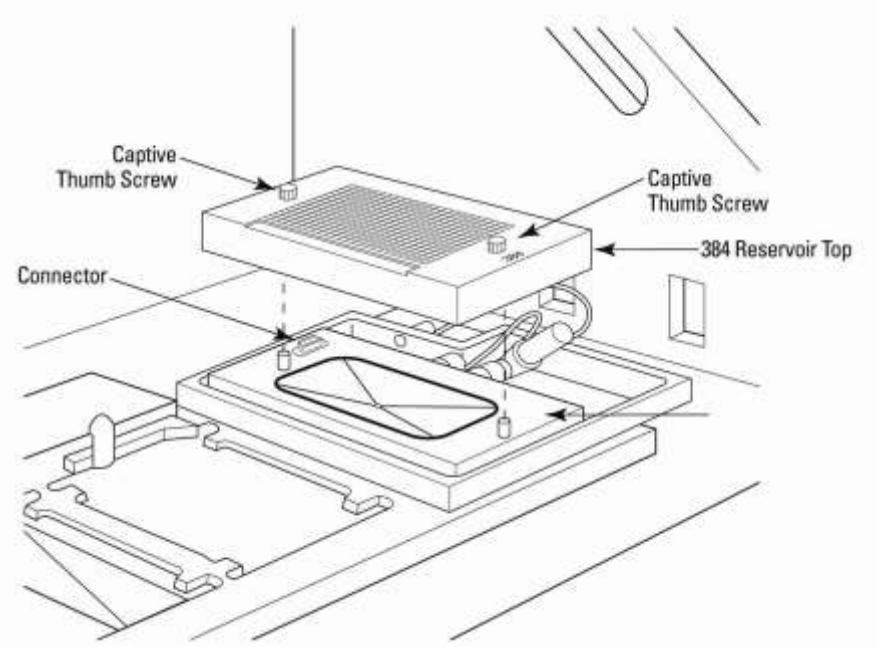


2. Lift reservoir top off of the washer base.
3. Ensure the **Blue O-Ring** on the washer base remains properly seated. If not reinstall the o-ring into its o-ring groove.
4. Store the reservoir top in a safe location.

Installing Wash Reservoir Top

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

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 theinstrument.



2. Once aligned, press the top into position, using the **Alignment Pins** to guide the placement of the top.
3. Tighten both **Captive Thumb Screws** to ensure the reservoir top is fastened to the base.
4. Go to the following procedure to reset theinstrument.

Resetting the System After Changing Pipettor Heads

To reset the FLIPR Tetra system:

1. Make sure the pipettor head and tip wash reservoir top formats match before proceeding. If they do not match, the instrument faults and will not let you run any experiments.
2. When the change is complete, click **DONE** in the dialog box that displayst in the ScreenWorks Software. 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.

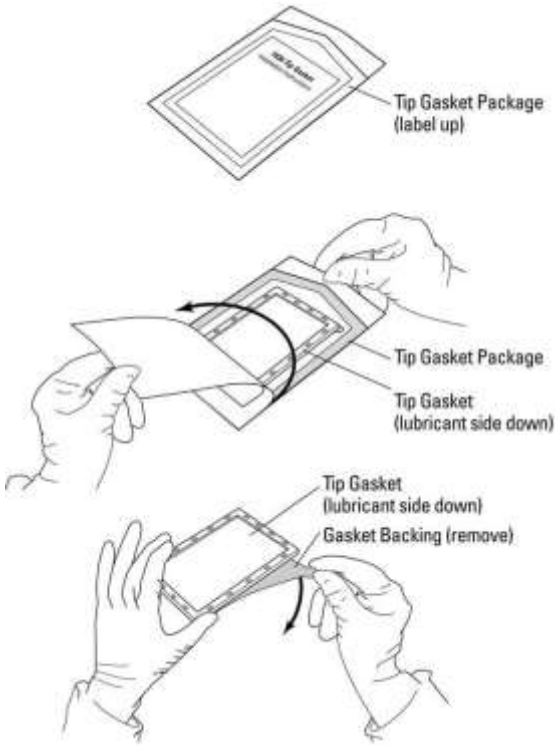
Exchanging the 1536 Tip Gasket

Gaskets should be replaced approximately every 200 plates (~3200 strokes). Gasket lifetime varies depending on the number of aspiration/dispense steps performed in your experiment and should be monitored.

Installing the Gasket

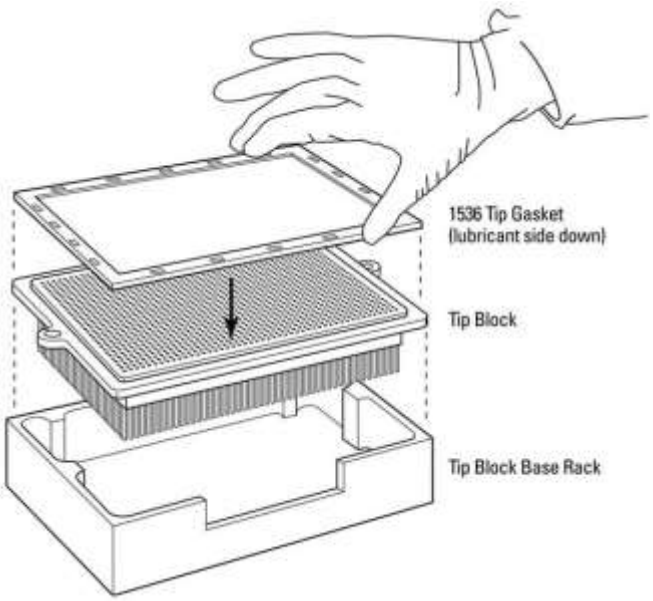
To install the 1536 tip gasket:

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



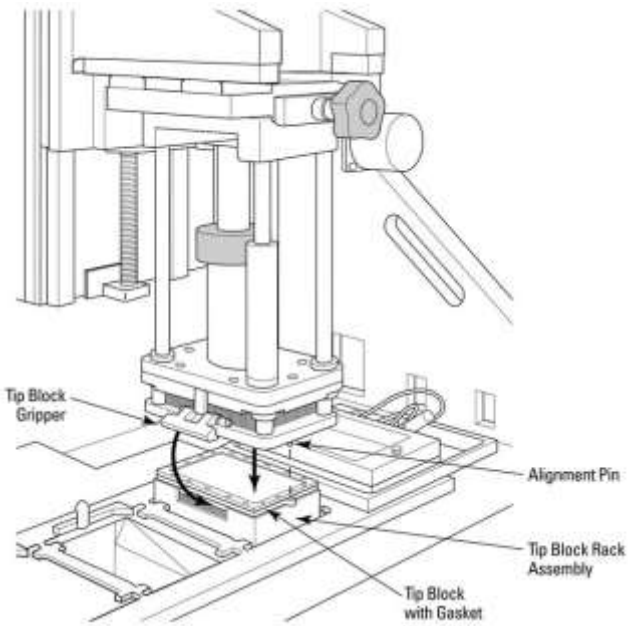
2. Place the lubricated side of the gasket down on the tip block, using the gasket recess to guide the frame position.

   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. Ordering information for disposable gaskets can be found in Consumables and Accessories on page 245.



To use **Position 1** for a source well plate:

- Load tips manually and then remove the tip rack before starting an experiment.

  When loaded, a source plate can be 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 rack.

Exchanging Pin Tools

Pin tools are blocks loaded with pins, which are loaded onto a pin tool head to transfer fluid. Two pin tool heads, 1536-well and 384-well formats, are available for the FLIPR Tetra system. Each pin tool head is compatible only with the pin tools of its respective format. Pin tool heads will not pick up pin tools whose magnetic ID does not match the respective head format. Therefore the 384-well pin tool head will not be able to pick up 1536-well pin tools or vice versa. This is designed for the safety of the pin tool.

Pin tools for a single head type are user-exchangeable. Up to eight or fourteen pin tools are available for the 384-well or 1536-well pin tool heads respectively.

To load or unload a pin tool:

- In the ScreenWorks Software, select **Instrument > Manual Operation > Load Tip and Unload Tip**.

  The pin tool is picked up and clamped to the head using pin tool block grippers similar to the 1536 pipettor head.

To use **Position 1** for a source well plate:

- Load tips manually and then remove the tip rack before starting an experiment.

  When loaded, a source plate can be 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 rack.

Exchanging LED Modules

WARNING! During the exchange procedure, you will have access to precision optical components. Use appropriate care not to damage them. Specifically, do not touch the surfaces of the LED filters with your bare fingers or other objects.

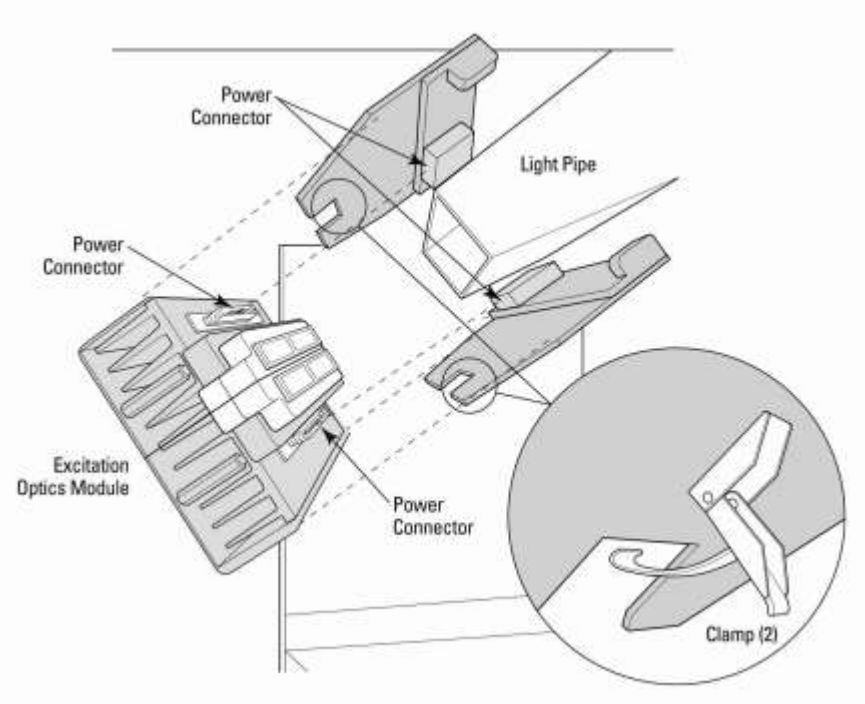
Uninstalling LED Modules

To uninstall LED modules:

1. Open the lower front door of the FLIPR Tetra system.

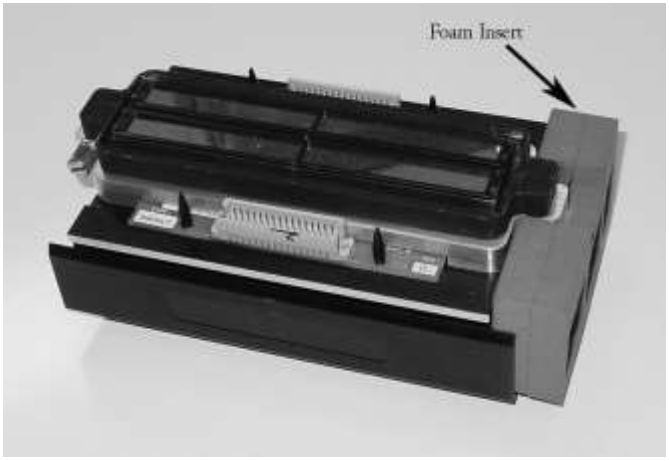
   When the lower door is open, power to the LEDs is disabled.

2. Loosen the two silver **Latching Clamps** securing the excitation optics module to the left light pipe.

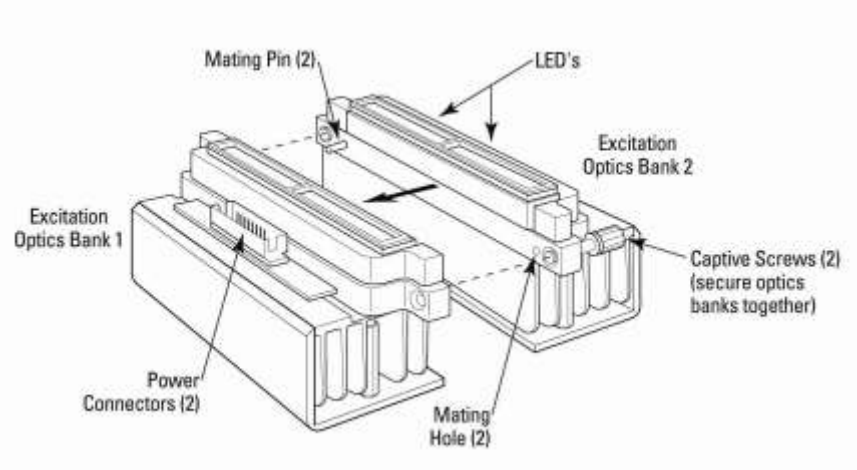


3. Pull the excitation optics module down and away from light pipe to disengage the **Power Connectors** on the LED modules from the light pipe. Be careful not to scratch the bandpass filters when pulling LED modules out of the instrument.

   Remove the **Foam Insert** found on the rear side of the excitation optics module.



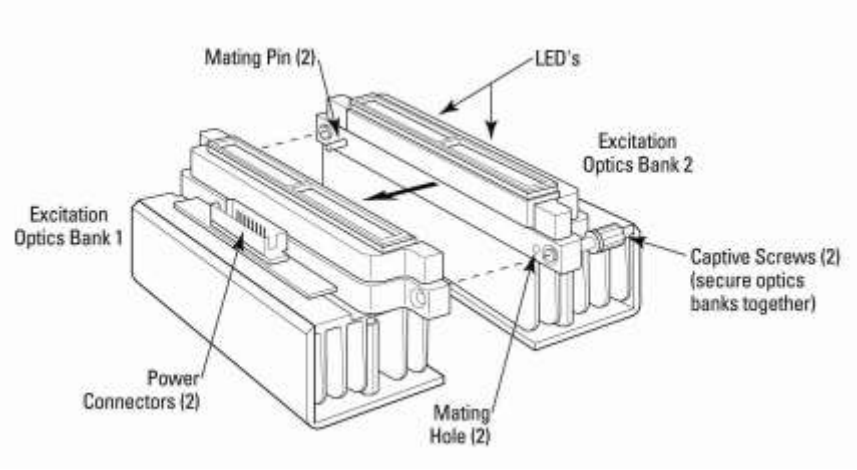
4. Loosen the two **Captive Thumb Screws** holding the LED modules together, and then pull the modules apart so they can be exchanged for new wavelengths.



5. Repeat for the LED module mounted to the right light pipe.
6. When separated, LED modules can be exchanged using the following installation procedure.

Installing LED Modules

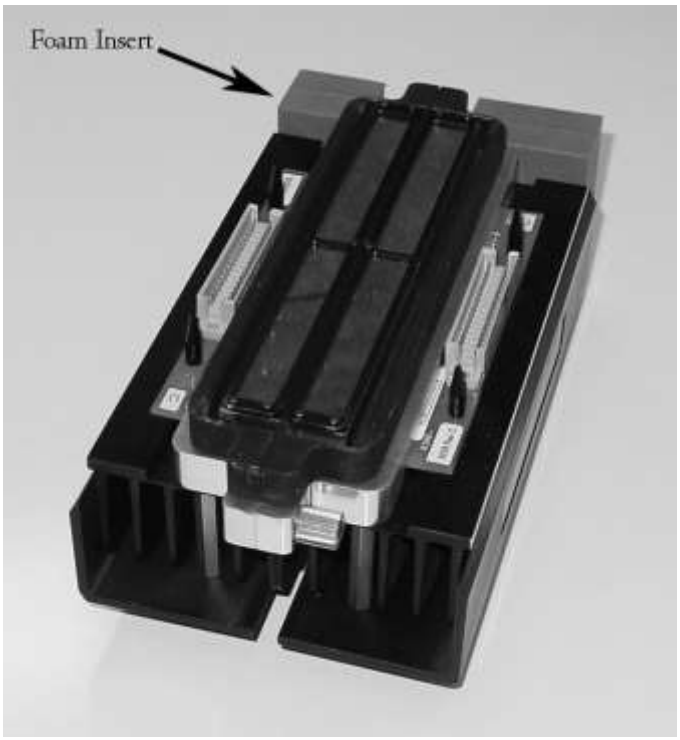
1. To create an Excitation Optics Module, you combine two LED banks of different wavelengths. Align the Mating Pins to their respective Mating Hole in the LED module that you are securing to each other.



2. When aligned, push the two LED banks of different wavelengths together and tighten the two **Captive Thumb Screws** with firm finger pressure.

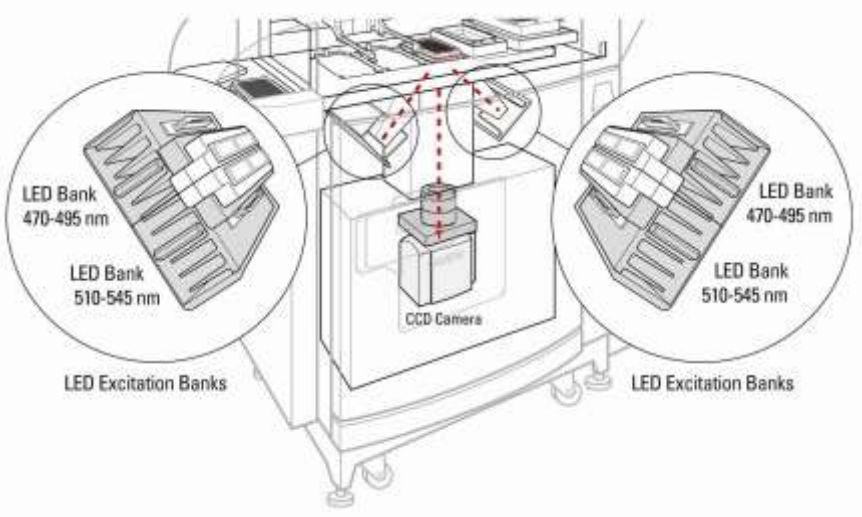
3. Place the **Foam Insert** on the rear end of the excitation optics module.

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 applying mating pressure.
5. After the connectors are mated, engage and tighten the two **Latching Clamps** that secure each module to the **Light Pipe**.

6. Repeat the above procedure for the right **Excitation Optics Module**, being sure the same wavelength modules occupy the corresponding position (upper/lower) on each side.



7. If also changing filters, continue to Changing Filters on page 120. If you are finished, close the lower front door.
8. When the lower front door closes, and the message displays **The lower door has been opened, do you want to update optics?** Click **Yes**.
   - Filters and LEDs are identified.
   - LEDs are tested.
   - ScreenWorks Software **Instrument Status Configuration** is updated.
   - The process finishes when the progress dialog closes.

Changing Filters

Filters are mechanically sensed and the filter configuration can be viewed in the instrument configuration window of the FLIPR Tetra system ScreenWorks Software.

To uninstall and then install the needed filter, use the following procedures.

If the needed filter is not available, three custom filter holders are available for purchase to place filters by an outside vendor. See Consumables and Accessories on page 245 for purchasing information. When installed, these filters appear as 1_1, 2_2, and 3_3 in the software protocol.

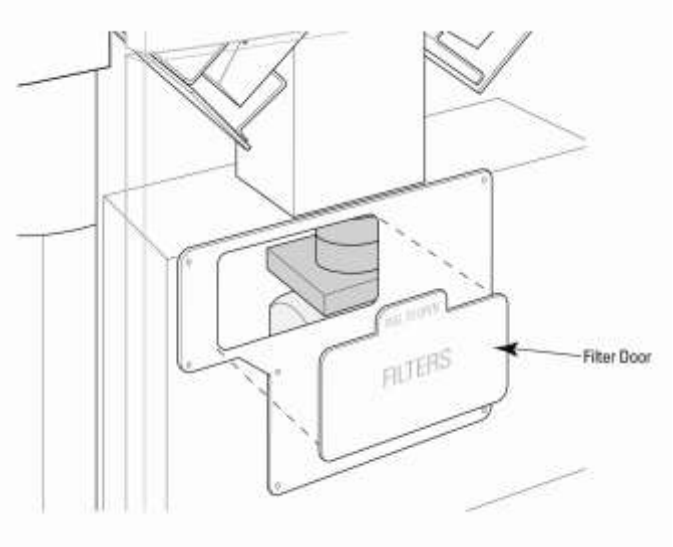
Uninstalling a Filter

To uninstall a filter:

1. Open the lower front door of the FLIPR Tetra system.

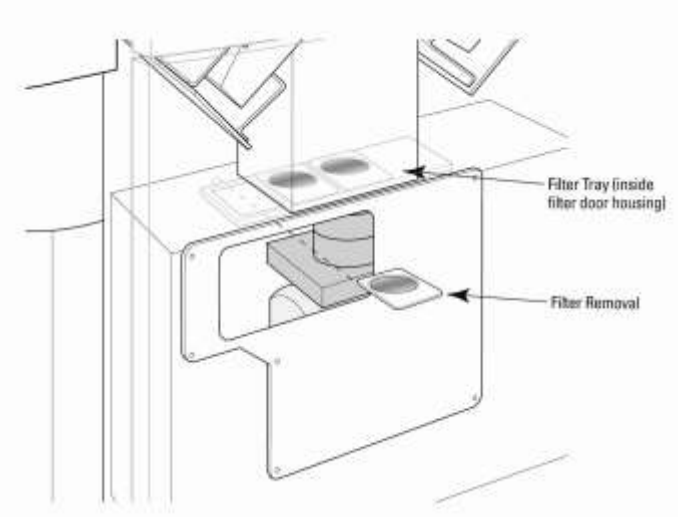
> **Note:** When the lower front door is open, system power is disengaged.

2. Remove the **Filter Cover**, located below the left LED module.



3. Using the filter tab that protrudes slightly from the filter tray, push the filter up out of its position and remove it from the instrument. Only to grasp the filter by the tab.

> **CAUTION!** DO NOT touch the filter surface.



4. Repeat Step 3 for the remaining emission filters you want to remove.

5. After needed emission filters are removed, replace the **Filter Cover**.
6. To install new emission filters into the system, continue to Installing an Emission Filter on page 122.

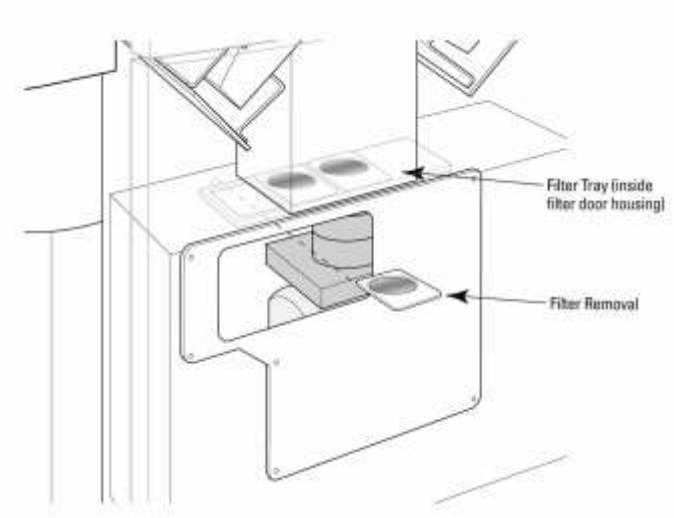
Installing an Emission Filter

The emission filter wavelengths are automatically identified by the system and can be selected from the ScreenWorks Software menu.

To install an emission filter:

1. If needed, open the lower front door of the FLIPR Tetra system, and remove the **Filter Cover**, located below the left LED module.
2. To install emission filters, grasp the filter you wish to install by its Tab, located where the filter table is, and place it into the appropriate filter position. Typically, this is the rightmost Position 1 in the slider. Insert the rear of the filter first, making sure it fits into the specific filter position. Push the tab down, so that 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. Subsequent filters can be installed by repeating step 2 in Positions 2 and 3.

Note: If a needed filter is not available, three custom filter cassettes are available for purchase (seeOptics Consumables on page 246 to place filters created by an outside vendor. When installed, these filters appear as 1_1, 2_2, 3_3 in the software protocol.

4. The emission filter wavelengths are identified by the system and passed to the ScreenWorks Software where they can be selected from menus.

CAUTION! When using all 3 filter positions, do not duplicate wavelengths, because this will cause a fault in the software.

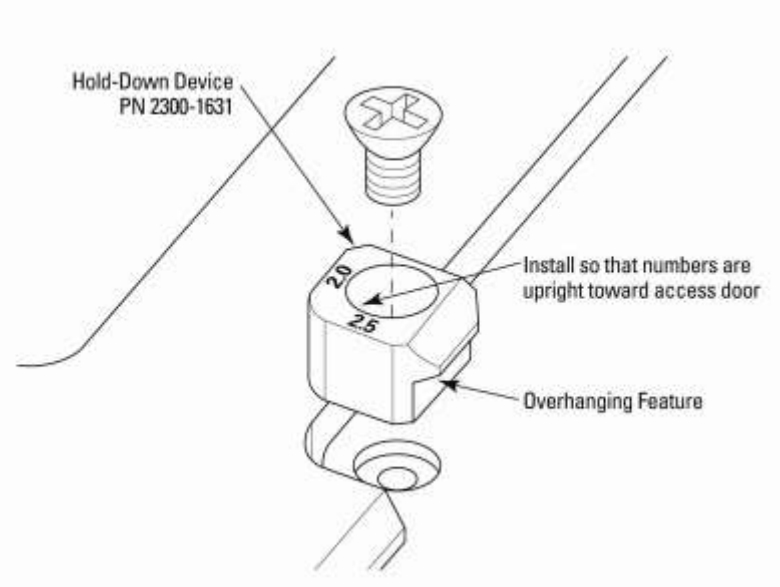
5. After desired emission filters are installed, replace the **Filter Cover**.
6. When you are finished, close the lower front door.
7. When the lower front door closes, and the message displays **The lower door has been opened, do you want to update optics?** Click **Yes**.
   - Filters and LEDs are identified.
   - LEDs are tested.
   - ScreenWorks Software **Instrument Status Configuration** is updated.
   - The process finishes when the progress dialog closes.

Changing Plate Hold-Down Devices

The plate hold-down devices, part numbers 2300-1631 and 2300-1633, are designed to keep plates or tip racks from lifting after aspirating, dispensing and tip loading. Each device has an overhang feature that contacts the skirt of a plate to prevent upward movement when the pipettor is removed from the plate. Three different plate hold-down gaps (2 mm, 2.5 mm and 6 mm) are provided that correspond to three identified plate skirt thicknesses. The 2.0 mm and 2.5 mm are incorporated on one plate hold down device that may be rotated 90×. A second plate hold-down has a 6.0 mm gap and can be found in the accessory kit. Numbers corresponding to the plate skirt thicknesses are marked on the top of the devices. The device is properly oriented for a particular flange thickness when the digits can be read as upright from the service door of the instrument.

Selecting a 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 thee Greiner 1536-well plates. To determine which hold-down device is most appropriate for your plate, use the lowest hold-down device that allows the plate skirt to slide under the overhanging feature freely. It is important the overhanging feature is clearly above the skirts on all plates and tip racks that will be used in a particular plate position or a misalignment with the pipette tips will occur. Test the configuration as described below after installation.



Installating a Plate Hold-Down Device

To install or reposition the plate hold-down:

1. Place the device in its proper orientation so that the numbers appear upright when viewed looking through the main user access door.
2. Insert one of the flat head screws that was originally securing the device or one 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, therefore no additional alignment is necessary.
3. If plates or tip blocks with different flange heights are to be used in the same position, use the device specified for the largest flange thickness.
4. Test the plates or tip blocks to be used by inserting them into the plate pockets at the location in which they will be used.
5. Observe that when the plate is released it is pushed 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: A definite retaining force should be felt. A sideways movement during lifting can cause the plate to slide out from under the retaining device and be lifted upward without retention. This does not happen during instrument operation but might require attention to be avoid when lifting a plate by hand.

Removing a Plate Hold-Down Device

To remove the existing plate hold-down:

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

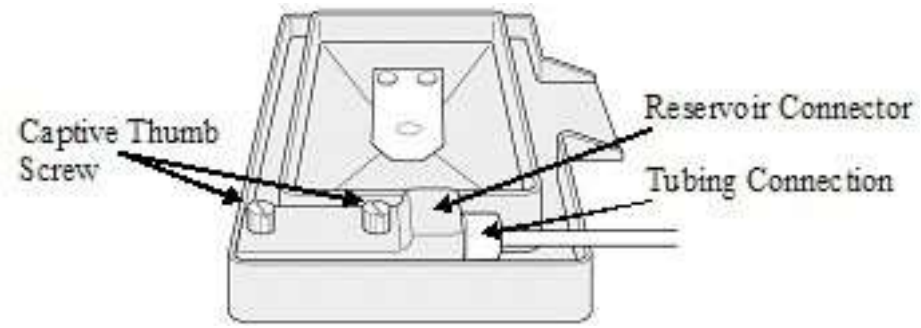
Cell Reservoir

Initial installation of the cell reservoir is done by a Molecular Devices certified field service engineer.

Installing the Cell Reservoir

If the cell reservoir was removed following the instructions in Uninstalling the Cell Reservoir on page 126, reinstall it as follows:

1. Ensure the tubing connection to the connector is tight.
2. Align the **Reservoir Connector** to the Cell Reservoir and tighten the two **Captive Thumb Screws**.



3. Insert the Cell Reservoir into the **Source Plate 3** position.
4. Go to the **Instrument** menu and select **Refresh Configuration**. The **Instrument Status** tab displays **Cell Reservoir: OK**.

   This status indicates that only the Cell Reservoir can be selected for the **Source Plate 3** position.
5. Run a **Manual Wash** (see Wash Cell Reservoir Process on page 103) and inspect the cell reservoir area to ensure that there is no fluid leakage.

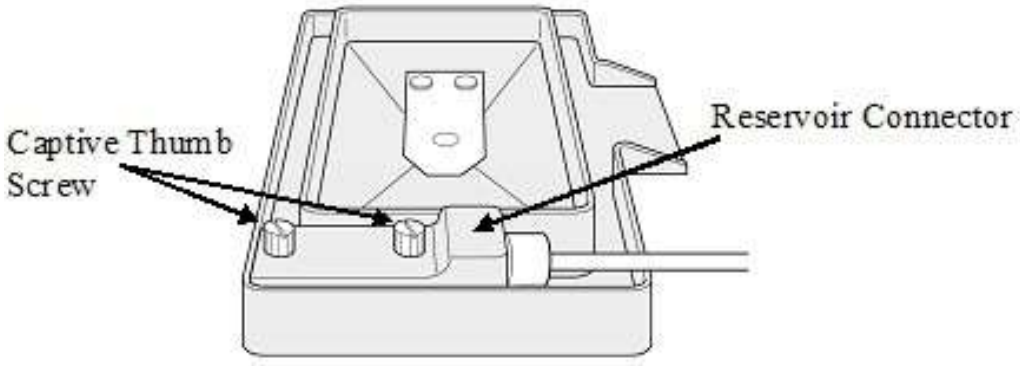
Uninstalling the Cell Reservoir

When the Cell Reservoir is configured and detected by the instrument, it is the only available choice as the Source Plate 3 selection. ScreenWorks Software displays **Cell Reservoir: OK** in the Instrument Status tab.

To use the Source 3 position for a different source plate, the Cell Reservoir must be uninstalled.

To uninstall the Cell Reservoir:

1. Remove the Reservoir Connector by loosening both Captive Thumb Screws.



2. Place the Reservoir Connector by the Tip Washer.
3. Remove the Cell Reservoir.

> **Tip:** You can now autoclave the Cell Reservoir.

4. Click **Instrument > Refresh Configuration**.
   The Instrument Status tab displays **Cell Reservoir: NOT_OK**.
5. Select another plate for the Source Plate 3 position.

Chapter 7: Calibration and Signal Test

The following procedures and information are required to calibrate the optics and run a signal test of the FLIPR Tetra system.

Optical Calibration

Calibrating the optics of FLIPR Tetra system requires taking an image of the 96-, 384-or 1536-well plate and using it as a mask to locate the wells. Typically, calibrations are run with the following procedures:

- After LED banks or pipettor head are exchanged.
- After a new plate is added to the plate library.
- If no filters are in the instrument, install them at this time (see Changing Filters on page 120).

Adding a Read Plate to Plate Library

The FLIPR Tetra system has default plate masks for 96-, 384-, and 1536-well read plate types. If you want to use a plate type that varies significantly from the default formats, you must create new dimensions and mask for that plate type. Use the description of the Plate Library menu item, (see Plate Process Definition on page 50), as a reference guide for options and parameters available when adding a plate to the software.

To add a plate and mask to the software:

1. Prepare a 1×10^{-8} M fluorescein solution.
2. Ensure the microplate 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 typically a safe volume with which to work. Use the FLIPR Tetra system pipettor.
4. Place the microplate containing fluorescein in the read position and select **Plate Library** from the **Tools** menu.
5. From the **Plate Process Definition** list, select a plate with a similar well format.
6. Click **Copy Plate**.
7. Enter the **Plate Specifications**:
   - Enter a **Plate Name**.
   - Select **Read Plate** as the **Plate Type**.
   - Type in the plate dimensions.
8. Click **Finish** to create a new read plate with mask.

Plate mask definition is complete when the green **Assay Finished (Unlocked)** light is illuminated.

Flat-Fielding

Microplate illumination during a fluorescence assay does not result in perfectly uniform excitation distribution on the microplate surface. In the FLIPR Tetra system, light distribution is more intense in the middle of the microplate than the edges, therefore a flat-fielding algorithm is applied to the microplate image to compensate for variations in light intensity across the microplate.

During luminescence assays LEDs are not used. Flat-field corrections are preset during installation and do not require any user adjustment.

Calibrating the Optics

FLIPR Tetra system identifies the serial number, wavelength range and position of each LED bank present in the instrument. This information, in addition to the wavelength range for the emission filters, is stored with the system's calibration files. Any time LEDs or emission filters are changed in the FLIPR Tetra system, you need to recalibrate the Flat-Field Calibration files for the new LED/Filter pairs. The following procedure steps you through the process of calibrating the system optics.

Tip: Due to the improved light tightness of the new FLIPR Tetra system, the background correction calibration available in the previous versions of this software (used with black bottom plates) is no longer required.

If you have any questions or concerns regarding the calibration procedure, contact Molecular Devices Technical Support. See Obtain Support.

The **Excitation LED Calibration** is needed only if the **LED Calibration Status** shows **NotDone** when you select to use the **335–345 nm/475–535 nm** (340 LED Module).

Excitation LED Calibration

To use Excitation LED Calibration:

1. Select **Instrument > Calibration**, or click on the **Calibration** icon.

   The **Calibration** dialog appears. This dialog includes a list of the **Excitation/Emission Wavelength** (LED/Filter) combinations for the installed modules, and corresponding status, **LED Calibration**, either **Done** or **NotDone**, and **Flat-Field Calibration**, either **Done** or **NotDone**.

2. Select **335–345 nm/475–535 nm**.

   The **Excitation LED Calibration** button activates.

3. Click **Excitation LED Calibration**.

   When the progress bar dialog closes, continue with Flat-Field Calibration.

Flat-Field Calibration

To use Flat-Field Calibration:

1. Select the **Calibration** option from the **Instrument** menu, or click on the **Calibration** icon.
2. The **Calibration** dialog appears. This dialog includes a list of the **Excitation/Emission Wavelength** (LED/Filter) combinations for the installed modules, and corresponding calibration status, either **Done** or **NotDone**, for **Flat-Field Calibration**, and **LED Calibrartion** of the selected **Plate Format**.

   Note: None indicates either no LEDs are illuminated or the instrument used a blank emission filter position to create the calibration file.

3. Select a **Plate Format** (96, 384, or 1536).
4. Select the **Excitation/Emission Wavelength** pair from the **Calibration Status** list with the **NotDone** status.
5. Place the **Flat-Field Calibration Plate** into the **Read Position**.
   - 335–345 nm/475–535 nm
   - 380–390 nm/475–535 nm
   - 390–420 nm/565–625 nm
   - 420–455 nm/475–505 nm
   - 420–455 nm/515–575 nm
   - 470–495 nm/515–575 nm
   - 470–495 nm/565–625 nm
   - 495–505 nm/526–586 nm
   - 510–545 nm/565–625 nm
   - 610–626 nm/646–706 nm

   The following dye should be used to calibrate the respective **Excitation/Emission Wavelength** (LED/Filter) pair.
   - 360–380 nm/400–460 nm with MQAE (detailed instructions for handling MQAE are outlined in the MQAE section).
   - 390–420 nm/440–480 nm with Coumarin (detailed instructions for handling Coumarin are outlined in the Coumarin section).
6. Click **Flat-Field Calibration**.

   The calibration completes when the **Assay Finished (Unlock)** light illuminates on the instrument, and the **Excitation/Emission Wavelength** status shows **Done** in the **Flat-Field Calibration** column.

7. When complete, click **Close** to exit.
8. Place your yellow signal test plate of the appropriate well format into your system.
9. Perform a signal test by selecting **Yellow Plate Signal Test** from the **Instrument> Manual Operation** menu, or using the corresponding button. The yellow plate standard deviation should be 5% or less.

Coumarin

Required material:

- Black clear-bottom plate of needed format and brand for assay (96, 384, or 1536)
- Coumarin (7-diethylaminocoumarin-3-carboxylic acid, Cat# D-1421, Molecular Probes, Eugene, OR)

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 (-68°F).
3. Within one hour of calibration, thaw an aliquot and make 20 mL 10^{5}M solution in the same buffer to be used 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 microplate. The volume must be adequate to cover the bottom of the well uniformly. The following quantities are recommended:
   - 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 microplate to dislodge any bubbles. Keep the Coumarin plate covered and in the dark until used.
6. Use the Coumarin plate as the **Flat Field Calibration Plate** in Flat-Field Calibration on page 129.

MQAE

Required material:

- Black clear-bottom plate of desired format and brand for assay (96, 384, or 1536).
- MQAE (N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide) Catalog # E-3101, Molecular Probes, Eugene, OR)

1. Make a 0.02 M stock solution of MQAE in distilled water. Mix by vortexing tube.
2. Store small aliquots at -20 °C (-68°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 be used 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 microplate. The volume must be adequate to cover the bottom of the well uniformly. The following quantities are recommended:
5. Check the plate visually to make sure there are no bubbles or unfilled wells. Shake or tap the microplate to dislodge any bubbles. Keep the MQAE plate covered and in the dark until used.
   - 96-well plate, 100 µL/well
   - 384-well plate, 40 µL/well
   - 1536-well plate, 6 µL/well
6. Use the MQAE plate as the Flat Field calibration plate in Flat-Field Calibration on page 129.

Running a Signal Test

When to run a Signal Test:

- Once a day to check the system.
- Before running an experiment to check the protocol settings.

System Check

Once a day, before running your first assay plate, run a signal test to ensure the system is performing according to specifications. A yellow signal test plate is available for each corresponding pipettor head supplied with the system.

Although both Fluorescence and Luminescence are available in the signal test window, currently there is no easy way of testing signal strength in Luminescence mode. It is therefore recommended to run daily checks of Yellow Plate Signal Test in Fluorescence to check optical performance of the instrument and use the Protocol Signal Test only for Fluorescence experiments only.

Note: To ensure proper system functioning, make sure that the system is turned on and the standard EMCCD camera is cooled to -60 °C ± 2 °C (-76 °F ± 35.6 °F), or the Aequorin ICCD camera is cooled to -20 °C ± 5 °C (-4 °F ± 41 °F).

Store the signal test plate in a safe place away from bright light on an even surface. Avoid scratching the plate bottom as scratches can affect the standard deviation.

Running the Yellow Plate Signal Test

To run a Yellow Plate Signal Test:

1. Select **Yellow Plate Signal Test** from the **Instrument> Manual Operation** menu or click on the appropriate button.

2. Depending on the camera type installed in your instrument, the settings will vary for the Fluorescence signal test. We recommend using the default values set in your software.
3. Place the signal test plate on the stage in the read position.
4. Take a picture by clicking Test Signal. Acceptable test plate results are a standard deviation less than 5% when the instrument is calibrated with the yellow test plate for the appropriate plate format. If the test results are outside of the range of acceptable values, look at the Image Display to see if any anomalies can be found on the plate. For additional information see Troubleshooting on page 169.
5. Print the results and keep them in a maintenance folder by the instrument to track the standard deviation of the test plate over time. Alternatively, you can save the files on the hard drive.

Note: The **Yellow Plate Signal Test** and **Image Display** results are not saved within a data file. You do not have to run a signal test more than once a day.

The standard deviation should be less than 5% if the flat field calibration was performed using the Flat Field Calibration Plate of the respective plate format (96, 384 or 1536).

Running a Plate Prior to an Experiment

This option is only recommended for Fluorescence experiments.

Run the Protocol Signal Test of your cell plate using the camera parameters in your protocol as described in Optimizing Optics Hardware on page 163.

Depending on the range of values obtained, you may choose either to run the experiment or alter the **Excitation Intensity**, **Exposure Time**, **Camera Gain**, or **Camera Gate**. A suggested starting point for a calcium mobilization assay is 200–1,500 counts.

Settings may be changed in the **Protocol Signal Test**. Depending on the installed camera and the read mode, different parameters are available for adjusting the signal strength. See Signal Test on page 46 for the exact description of each of these parameters.

Change the settings as desired, and then click Test Signal to recheck the settings. Settings defined in the Protocol Signal Test can be saved to the open protocol when **OK** is clicked to close the dialog.

Note: Signals displayed in the **Protocol Signal Test** dialog are not saved as part of the data file.

Chapter 8: Running an Experiment

8

This chapter provides a starting point for setting up and running a kinetic cell-based assay on the FLIPR Tetra system. Running an assay requires performing the following tasks:

- Preparing the cells
- Powering-up the system
- Running a system check
- Dye-loading the cells
- Preparing source plates
- Setting up an assay protocol

These tasks are explained in detail in the remainder of this chapter. Following the general procedures for running a FLIPR Tetra system experiment, protocols are outlined for four types of assays:

- Fluorescence-based calcium mobilization
- Fluorescence-based membrane potential
- Fluorescence ratiometric membrane potential using Voltage Sensor Probes
- Luminescence-based detection of calcium mobilization

After the presentation of the assay protocols, guidelines for assay optimization are provided.

Preparing Cells for Adherent Assays

Location of Cells in the Plate

The excellent signal-to-background noise ratio of the FLIPR Tetra 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. It is recommended to 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 1,500 to 5,000 cells per well for 1536-well plates, 5,000 to 20,000 for 384-well plates and 20,000 to 80,000 cells per well for 96-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- or under-confluent cell monolayer may result in reduced cellular response to the test compounds.

Cells that are normally maintained in culture at subconfluent levels should be seeded 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. Assay development needs to be performed to determine the optimal seeding density and plate environment for each cell line.

Cell Seeding

Cells can be seeded in plates using a multi-channel pipettor or a liquid dispensing system, such as the AquaMax® DW4 Dispenser from Molecular Devices. We recommend seeding a 1536-well or 384-well cell plate using an automatic instrument rather than seeding manually. Thin needles used in automatic liquid-dispensing instruments prevent air bubble formation in well bottoms—a problem commonly encountered when cells are seeded with a manual pipettor. 96-well plates can be seeded by either manual or automatic methods. Refer to your specific instrument user guide for instructions on how to dispense cell suspension into wells.

Cells are seeded in clear, flat-bottom, black-wall 1536-well, 384-well, or 96-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. All steps are carried out in the same black-wall 96-well, 384-well, or 1536-well plate.

Note: Flat-bottom, clear-wall tissue culture plates may also be used with the FLIPR® Calcium Assay Kit and Membrane Potential Assay Kit. Depending on signal intensity, either black-wall or white-wall plates can be used when running aequorin assays.

Preparing Cells for Suspension Assays

Location of Cells in the Plate

Suspension cells can be used for luminescence experiments. In this case, cells are kept in uniform suspension by the Cell Suspension option, which consists of a cell suspension module and reservoir system. Suspended cells are first pumped to the reservoir and then delivered to the read plate by the FLIPR Tetra system pipettor head.

These assay methods are also described in the literature, for example, in Boie et al., Eur. J. Pharmacol., 340(2-3):227-241 (1997), and in United States Patent 6,872,536 and European Patent 1,145,002. Users interested in the patented methods may wish to consult legal counsel in evaluating these patents.

Cell Densities

Cell densities in luminescence suspension assays vary. Depending on the bioluminescence signal from the cells, the density can be adjusted to reach appropriate signal window. Bright cells can be diluted, while dim cells might require a higher concentration. The suggested cell densities are 1,000–5,000 cells per well for 1536-well plates, 2,500–10,000 cells per well for 384-well plates, and 5,000–40,000 cells per well for 96-well plates.

Cell Seeding

The size of the experiment will dictate the number of cells required. A typical CHO-based 100-plate suspension cell assay requires approximately 250–300 × 106 cells in the suspension cell flask. This can be accomplished by culturing cells in multiple layer flasks. Cells should be split 2–3 days prior to the assay and not be more than 80–90% confluent prior to antibiotic removal. All flasks should be incubated at 37 °C (-34.6 °F) and 5% CO_2. One day prior to testing, spent media from each flask should be replaced with growth media that does not contain selection antibiotics.

Powering-Up the System

To ensure proper system function, power the instrument well before you begin an experiment. The camera requires about five minutes to reach its operating temperature, and if you are heating the plate stage (**Instrument > Manual Operation > Temperature Control**), allow 15 minutes for complete temperature equilibration (check the **Instrument Status** window for stage temperature).

Follow the start-up procedure in Startup and Shutdown on page 31. Make sure the appropriate pipettor head, wash reservoir, LED banks and filters are in place prior to starting the instrument. If incorrect components are installed, see the appropriate section in Exchanging Hardware on page 105 for installation instructions.

CAUTION! Do not attempt to change pipettor heads, wash reservoirs, LED banks and filters while the instrument is running. All features must be exchanged in the appropriate instrument mode. Optics calibration will need to be performed when the instrument is started for the first time after re-configuration of LEDs or emission filters.

Checking the System

Once a day, before running your first assay plate, run a Yellow Plate Signal Test to ensure the system is performing according to specifications. A yellow signal test plate is available for each corresponding pipettor head supplied with the system.

Note: Make sure that the system is turned on and the CCD camera is cooled to -60 °C ± 2 °C (-76 °F ± 35.6 °F) when the EMCCD camera is present or - 20 °C ± 5 °C (-4 °F ± 41 °F) when the ICCD camera is installed to ensure proper system functioning.

CAUTION! Avoid scratching the plate bottom as scratches can affect the standard deviation. Store the yellow signal test plate in a safe place away from bright light on an even surface. A signal test plate does not need to be run more than once a day unless system components such as pipettor heads or LEDs are changed.

Running the Yellow Plate Signal Test

To run a Yellow Plate Signal Test:

1. Select **Yellow Plate Signal Test** from the **Instrument > Manual Operation** menu or click the **Yellow Plate signal test** button.
2. Set the following parameters:
   a. Select the correct plate from the **Select Plate** list.
   b. Select appropriate **Read Mode**.
   c. Select the appropriate **Excitation/Emission Wavelengths**.
   d. Set the **Excitation Intensity** to 50.
   e. Set the **Exposure Time** to 0.1 seconds.
   f. Set the **Camera Gain** to 80 (EMCCD camera).
   g. Set the **Gate Factor** to 3% (ICCD camera).
3. Place the yellow signal test plate on the stage in the read position.
4. Take a picture by clicking **Yellow Plate Signal Test**. Acceptable test plate results are a standard deviation less than 5% when the instrument is calibrated with the yellow test plate for the appropriate plate format. If the test results are outside of the range of acceptable values, look at the **Image Display** to see if any anomalies can be found on the plate. For additional information see, Troubleshooting on page 169.
5. Print the results and keep them in a “Maintenance” folder by the instrument to track the standard deviation of the test plate over time. Alternatively, you can 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.

Note: The **Yellow Plate Signal Test** and **Image Display** results are not saved within a data file.

You do not have to run a yellow plate signal test more than once a day, unless you recalibrate or change the optics.

This Fluorescence signal test also checks the Luminescence optics, since the optics path is the same in both modes.

Dye-Loading the 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 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 30 min–1 hour (at either room temperature or 37 °C (98.6 °F).

Loading Duration and Temperature

Optimal loading time depends on the cell type and presence of an anion exchange inhibitor. It is recommended not to exceed the optimal loading time. If anion exchange inhibition does not enhance dye-loading conditions, it should not be used. A 60 minute loading time at 37 °C (98.6 °F) is usually effective for most cell lines and is the recommended starting point for calcium mobilization assay development.

Note: If loading for 30 min yields an acceptable fluorescence signal, as has been observed for some cell lines, use the shorter loading time. In some cases, incubation at room temperature may work as well or better than 37° C (98.6 °F).

Preparing a Source and Compound Plate

Preparation Time for the Source Plate

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

Recommended Source Plates

Compound conservation can be achieved 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. Polypropylene plates are most commonly used for this purpose because they are solvent-resistant, can withstand repeated freeze-thaws, and have a low retention. In addition, proteins are less likely to adhere to polypropylene plates rather then 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 are using.

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 final 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. Assay development should always be performed 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) will 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 always recommended to determine the proper assay conditions prior to running a screen.

If addition parameters are not optimized, artifacts will occur affecting 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. Cell displacement can be reduced 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. However, small sample volumes are less disruptive to a cell monolayer and reduce compound consumption. To avoid dislodging weakly adherent cells, compounds should be added in small volumes and at relatively low dispense speeds.

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

For temperature-controlled assays, cell and source plates should be brought 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. Compounds are suggested to be prepared at 2X concentration in the same diluent as used for cells, since equal volumes of cells will be added to the read plate containing the compound.

In 384-well assay 25 µL cells are added to 25 µL 2X compound; in a 1536-well assay 2 µL cells are added to 2 µL compound. Plates should be stored covered to prevent evaporation.

Setting Up an Assay Protocol

An effective way to reduce time and ensure consistent screening parameters is to preconfigure protocol (***.fmp**) files. Some users define and save protocol files for each cell line, project or user. Saved protocol files are opened from **File > Open > Protocol File**. In addition, the last 10 files (6 data and 4 protocol) opened in ScreenWorks Software are listed in the bottom portion of the **File** menu.

Creating a Protocol File

A protocol file defines 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.

Protocols are created by an easy drag-and-drop operation that moves experimental processes from the Process Explorer to the Experiment window.

Defining the Settings Process

In the **Settings** process of the Experiment window, select the appropriate parameters for **Read Mode**, **Plate Position**, **Data File Name** and **Folders**. For a detailed explanation of the Settings process and its options, Settings Process on page 58.

Creating the Assay Sequence

Select the desired processes from the **Process Explorer** and drag them to the desired sequence position in the Experiment window.

Click on the process icon you want to define (for example, **Transfer Fluid**), and then enter the desired parameters. For more information on the available protocol processes and their options, see ScreenWorks SoftwareOverview on page 37.

Note: The protocol processes are executed in a left-to-right order until there is a combined process of **Transfer Fluid** and **Read with TF**. After baseline imaging, all pipetting actions and imaging occurs in parallel.

Choosing Analysis Options

In the **Analysis** process, click on the **Grouping**, **Correction** or **Export** buttons to define the analysis parameters to be applied to the experimental data. For more information about these options see Analysis Process on page 64.

When Finished Creating the Protocol

When you have finished defining the experiment parameters of the processes in the Experiment window, click **Save As** and save the file as an ***.fmp** file.

Optimizing the Optics and Fluid Dispensing

Optimizing the Hardware Settings

Prior to screening, the following hardware components should be optimized for signal output and detection:

- Excitation intensity
- Exposure time
- Number of reads
- Camera gain for EMCCD camera
- Gate Open for ICCD camera
- Pipettor height
- Dispense speed
- Dispense volume
- Cell parameters for suspension assays.

Optimizing Optics Hardware

Optimization is available to help amplify weak signals or reduce saturation. Modifying read settings may not alter the quality of your data because it only regulates the amount of light emitted or detected by the system. For instance, doubling the excitation intensity doubles the RLUs detected by the system, but your 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. Start with the excitation intensity, camera gain and exposure time settings shown below. These settings are the most frequently used to measure basal fluorescence signal.

Camera Type	Calcium Assay		Membrane Potential Assay	
	EMCCD	ICCD	EMCCD	ICCD
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	50	50	50
Exposure Time (s)	0.4	0.533	0.4	0.533
Camera Gain	130	2000	50	2000
Gate Open	—	6%	—	6%

3. Perform a **Protocol Signal Test** to evaluate the basal fluorescence signal. The desirable fluorescence signal for intracellular calcium assays is 200–1,200 fluorescence counts for EMCCD camera and 500–5,000 for ICCD camera.
4. If the basal fluorescence signal is substantially out of these ranges, the excitation intensity, exposure time and gain or gate can be adjusted. Use the suggestions below to adjust the basal fluorescence.

Basal fluorescence too low:

- Increase the **Excitation Intensity** if the basal fluorescence signal is too low. During the assay, the **Excitation Intensity** should range between 20% and 100%.
- Increase the **Exposure Time**. If you increase the **Exposure Time**, the read interval will have to be increased 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 can vary between 1 (extremely bright fluorescence) to 240 (luminescence).
- Increase the **Gate Open** (ICCD camera). The gate can vary between 0.06% (extremely bright fluorescence) and 35% (very dim fluorescence).

Basal fluorescence too high:

- Decrease the **Excitation Intensity**.
- Decrease the **Exposure Time**.
- Decrease the **Camera Gain** (EMCCD camera).
- Decrease the **Gate Open** (ICCD camera).

Adjusting the Pipettor Height

To ensure prompt compound delivery, the pipettor height can be adjusted. It 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 doesn't leak out during pipettor movement. The hold volume will be the first item to leave the pipette tip during dispense and enters the target well as a bubble. These bubbles may cause random light reflections and spurious signals. To avoid bubbles, fluid dispensing should occur with tips just above, but touching the initial well fluid level. This will leave 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 resulting in reproducibility problems. Therefore, the pipettor height should be set just above, but touching the starting fluid volume in the wells.

The fluid surface in a 384- or 1536-well plate is a deeply curved meniscus. Therefore, we recommend setting the pipettor height somewhere below the starting fluid volume for the 384- or 1536-pipettor.

Adjusting the Fluid Dispensing Speed

Default dispense speed is 50 µL/s, 20 µL/s and 2 µL/s when dispensing to 96-well, 384-well, and 1536-well plates respectively. The table below provides recommended fluid dispense speeds for all three plate formats.

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

These values must be experimentally determined for each cell type, but it is generally preferable to 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 as to dislodge 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 ones. However, smaller sample volumes cause less disruption to the cell layer and allow the same source plate to be used for multiple cell plates. To avoid dislodging weakly adherent cells, smaller compound volumes should be added 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 your target plate, we recommend you evaluate the following factors during assay development. Controlling and standardizing these factors for a given application will help you 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

For more information regarding optimization of pin tools, please refer to http://www.vp-scientific.com/molecular_devices.htm or contact:

V&P SCIENTIFIC, INC.

9823 Pacific Heights Boulevard, Suite T

San Diego, CA 92121

Toll Free: +1-800.455.0644

Phone: +1-858.455.0643

Fax: +1-858.455.0703

Email: sales@vp-scientific.com

Optimizing Cell Delivery

During suspension assays, cells are pumped from the external Cell Flask to the Cell Reservoir and pipetted to the read plate. There are several user adjustable settings for suspension cell optimization. These parameters need to be optimized for each cell line as each cell line is different. The settings include:

- Stir speed in the Cell Flask
- Fill pump speed
- Drain pump speed
- FLIPR Tetra system aspirating/dispensing parameters

Start the Assay Run

To start the assay:

- Check the Experiment window to verify that the experiment synopsis is accurate and that the appropriate ***.fmp** file is being used.
- Verify the plates and tips are loaded in the instrument.
- Click the Run button on the toolbar to start the program.

  The upper and lower doors automatically lock before any part in the FLIPR Tetra system tower moves. These doors must remain closed until the end of the program including the duration of the pipettor tip wash.

FLIPR Calcium Assay Kit Protocol

This section provides the following information and procedures:

- Materials required to run the calcium mobilization using the FLIPR Calcium Assay Kit
- Cell preparation guidelines
- Dye loading procedure
- Troubleshooting tips for intracellular calcium assays

Required Materials

The following items are required for running the FLIPR Calcium Assay Kit:

Item	Source
FLIPR® Tetra system with Calcium Optics Kit installed (default with instrument): FLIPR® Tetra system LED Module 470–495 nm FLIPR® Tetra system Emission Filter 515–575 nm	Molecular Devices See Consumables and Accessories on page 245
FLIPR® Calcium Kits (one of the following): FLIPR® Calcium Assay Kit, explorer FLIPR® Calcium Assay Kit, bulk FLIPR® Calcium 3 Assay Kit, express FLIPR® Calcium 3 Assay Kit, explorer FLIPR® Calcium 3 Assay Kit, bulk FLIPR® Calcium 4 Assay Kit, express FLIPR® Calcium 4 Assay Kit, explorer FLIPR® Calcium 4 Assay Kit, bulk FLIPR® Calcium 5 Assay Kit, express FLIPR® Calcium 5 Assay Kit, bulk FLIPR® Calcium 5 Assay Kit, explorer FLIPR® Calcium Evaluation Kit	Molecular Devices P/N R8041 P/N R8033 P/N R8108 P/N R8091 P/N R8090 P/N R8143 P/N R8142 P/N R8141 P/N R8187 P/N R8186 P/N R8185 P/N R8172
Clear, flat-bottom, black-wall or clear-wall 1536-, 384- or 96-well plates	See Consumables and Accessories on page 245
Clear, polypropylene source plate	See Consumables and Accessories on page 245
FLIPR® pipette tips, 96, 384, or 1536 gasket	See Consumables and Accessories on page 245
Cells in suspension	—
Test compounds	Specific to receptor
Growth medium	Major laboratory supplier (MLS)
Incubator (5% CO_2, 37 °C / 98.6 °F)	MLS
Centrifuge	MLS
Pipettor and sterile tips suitable for use with microplates	MLS
Probenecid in powder form	Sigma
1 N NaOH	Sigma

About the Fluorescent Dye

Contents of the FLIPR® Calcium Assay Kit are optimized to give robust results when used on a FLIPR Tetra system according to protocol provided in the kit. Benefits include:

- Increased assay signal
- Reduced background
- Increased z-factor
- Drastically reduced addition artifact (“dip”).

Dye is excited using the FLIPR Tetra system LED Module, 470–495 nm. Signal is detected using the standard 515–575 nm emission filter included with every FLIPR Tetra system. Additional emission filters are not required.

Note: The Kit includes masking dye technology covered by issued and pending patents including US 6,420,183, US 7,063,952 and EP 0,906,572, licensed exclusively from Bayer AG to Molecular Devices.

About Exchange Protein Inhibitors

Some cell types use mechanisms such as anion exchange proteins to export anionic molecules from cells, including anionic forms of fluorescent dyes. This will result in poor dye loading. Therefore, it may be critical to inhibit the anion exchange protein to produce a successful intracellular calcium assay on the FLIPR Tetra System.

Probenecid is an anion exchange protein inhibitor. When added to loading buffer, it may increase dye retention in cells. CHO-K1 is an example of a cell type known to require probenecid. Although probenecid can be useful in slowing dye leakage from cells, it is toxic to cells, and therefore inclusion and duration of dye loading step should be kept to a minimum.

Calcium Assay Kit Experimental Protocol

Cell Handling

The FLIPR Calcium Assay Kit is designed to work with many cell types, both adherent and non-adherent. We recognize that a variety of cell handling conditions might be adopted at the discretion of the user, based on standard operating procedures in the laboratory. In this section we provide guidelines on how to prepare the cells for use with the assay kit.

Adherent cells are the most frequently used cells with the kits. They are typically plated the day prior to an experiment and then incubated in a 5% CO_2, 37 °C (98.6 °F) incubator overnight. See the table below for suggested plating volumes and seeding densities to create an 80–90% confluent cell monolayer before placing the plates in the FLIPR Tetra Instrument.

For non-adherent cells, we recommend centrifuging cells from culture medium and re-suspending the pellet in culture medium on the day of the experiment. It is recommended after the cells are plated to centrifuge the plates at 100 g for up to 4 minutes (with brake off). Alternatively, non-adherent cells can be treated like adherent cells, plating the day before the assay using the same plating volumes and seeding densities, as long as the cells are seeded onto coated plates (for example, poly-D-lysine or collagen) to ensure good attachment to the plate bottom.

Cell Type (cells/well)	96-well Plate (100 µL growth medium)	384-well Plate (25 µL growth medium)	1536-well Plate (4 µL growth medium)
Adherent cells	20,000–80,000	5,000–20,000	1,500–5,000
Non-adherent cells	40,000–200,000	10,000–50,000	3,000–10,000

Preparation of Loading Buffer

The following procedure is designed for preparation of the Loading Buffer per vial of the Explorer Kit, the Bulk Kit, or the Express Kit.

1. To prepare the 1X HBSS Buffer plus 20 mM HEPES for the Bulk and Express Kits only, (Explorer Kit contains ready to use HBSS buffer plus 20 mM HEPES pH 7.4 in Component B), pipette 100 mL of 10X Hank's Balanced Salt Solution and 20 mL of 1M Hepes buffer pH 7.4 into 880 mL cell culture treated water.
2. Remove one vial of FLIPR Calcium Assay Reagent (Component A) and equilibrate to room temperature.
3. Dissolve contents of Component A vial by adding the appropriate amount of 1X HBSS Buffer plus 20 mM HPES as outlined in the table below. Mix by vortexing (~1–2 min) until contents of vial are dissolved. It is important that contents are completely dissolved to ensure reproducibility between experiments.

Table 8-1: Quantities of 1X HBSS Required to Dissolve Component A

Plate Format	Explorer Kit (R8142)	Bulk Kit (R8141)	Express Kit (R8143)
96-Well or 384-Well	10 mL	10 mL	20 mL
1536-Well	6.5 mL	10 mL	20 mL

4. Prepare the Loading Buffer by diluting the Component A vial mixture with an additional volume of 1X HBSS plus 20 mM HEPES Buffer as outlined in table below. Multiple washes of the vial are necessary to completely transfer the contents.

Table 8-2: Quantities of 1X HBSS Plus 20 mM HEPES Required to Perform Second Dilution of Component A.

Plate Format	Explorer Kit (R8142)	Bulk Kit (R8141)	Express Kit (R8143)
96-Well or 384-Well	NA	90 mL	480 mL
1536-Well	NA	55 mL	300 mL

Note: If your cells require probenecid, then a stock solution should be prepared in 1 M NaOH and added fresh to 1X HBSS buffer plus 20 mM HEPES (adjust pH to 7.4 after addition of probenecid) for preparation of the Loading Buffer so that the final in-well working concentration is 2–2.5 mM. Do not store frozen aliquots of Loading Buffer with probenecid and always prepare fresh probenecid on the day of the experiment.

CAUTION! The components supplied are sufficient for proper cell loading. For optimum results it is important NOT to add any additional reagents or change volumes and concentrations.

Loading the Cells Using the Loading Buffer

CAUTION! Do NOT wash the cells after dye loading.

1. Remove cell plates from the incubator or centrifuge. Do not remove the supernatant. Add an equal volume of Loading Buffer to each well (100 µL per well for 96-well plates, 25 µL for 384-well plate).

Note: Add 2 µL per well for 1536-well plate by using anAquaMax® DW4 or equivalent device.

Note: Although Molecular Devices does not recommend washing cells before dye loading growth medium and serum may interfere with certain assays. In this case, the supernatant can be aspirated and replaced with an equal volume of serumfree HBSS plus 20 mM HEPES buffer before adding the Loading Buffer. Alternatively, cells can be grown in serum-free conditions.

2. Incubate cell plates for 1 hour at 37 °C (98.6 °F) and then keep the plates at room temperature until used (loading time should be optimized for your cell line).

Note: Some assays perform optimally when the plates are incubated at room temperature.

Running the FLIPR Calcium Assay Kit

To start the calcium assay:

1. After incubation, acclimate the plates to room temperature for approximately 10–20 minutes prior to transferring them to the FLIPR Tetra system. Please refer to “Recommended Setup Parameters” on page 136 for running the Calcium assay kit on FLIPR Tetra system.
2. When performing a signal test prior to an experiment, typical average baseline counts range from 800–1,200 RFU for the EMCCD camera and 500–5000 for the ICCD camera on FLIPR Tetra system.
3. Suggested experimental setup parameters for each FLIPR Tetra system are as follows. Fast addition speeds close to the cell monolayer are recommended to ensure better mixing of compounds and lower signal variance across the plate. However, further assay development, adjustment of the volume, height and speed of dispense, is recommended to optimize your cell response.

Recommended Setup Parameters

Recommended experimental setup parameters are as follows.

Experimental Setup Parameters	EMCCD Cameras	ICCD Camera
Read Mode	Fluorescence	Fluorescence
Excitation Wavelength	470–495 nm	470–495 nm
Emission Wavelength	515–575 nm	515–575 nm
Excitation Intensity	80%[1]	50%[1]
Camera Gain	50–130	2000
Gate Open	—	6 %[1]
Exposure Length	0.4 s[1]	0.53 s[1]
Read Interval	1 s	1 s
1. Can be adjusted to increase or decrease the signal if low RFUs or saturation problems occur.		

Addition Parameters	96-Well Plate	384-Well Plate	1536-Well Plate
Compound concentration (fold)	5X	5X	7X
Addition volume (µL)	50	12.5	1
Addition height (µL)	210–230	25–45	1–2
Tip up speed (mm/s)	10	10	5
Dispense speed (µL/s) Adherent cells	50–100	30–40	4–7
Dispense speed (µL/s) Non-adherent cells	10–20	10–20	1–5

Troubleshooting Guide

This section presents solutions to problems that users may encounter when running calcium flux assays.

Fluorescence Drop Upon Compound Addition

This may be the result of dislodging cells from the well bottom during addition. Lowering the addition/dispense speed or adjusting the addition height or both should solve the problem in this case.

Another potential reason is the dilution of the non-fluorescent compound into a plate with media containing fluorescent components (like DMEM media). This Ca4 kit mediates this issue compared to earlier developed Ca-kits.

Adding volumes greater than recommended may increase the initial fluorescence drop. In these cases it may be necessary to adjust the volumes of the components. The recommended volume of the Loading Buffer is 100 µL for 96-well plates, 25 µL for 384-well plates and 2 µL for 1536-well plates.

CAUTION! Decreasing the final in-well concentration of the Loading Buffer can decrease the response of the assay. If only one addition is required, then adding a higher concentration of compound in low volume could help reduce any fluorescence drop upon addition.

Serum-Sensitive Cells or Targets

Some cells are serum-sensitive resulting in oscillations of intracellular calcium that could interfere with results. Also, some target receptors or test compounds may interact with serum factors. In these cases, serum-containing growth medium should be removed prior to addition of loading buffer. The volume of growth medium removed should be replaced with an equal volume of 1X HBSS plus 20 mM HEPES buffer before loading. Alternatively cells could be incubated overnight in lower concentrations of FBS and not washed prior to the addition of Dye Loading Buffer.

Cells with DMSO Show a Calcium Response

Buffer used for the negative control wells should contain the same final concentration of DMSO as is present in the wells containing the test compounds. However, this concentration of DMSO could cause a calcium flux. In these cases, add DMSO to the Loading Buffer such that the final concentration of DMSO in the wells does not change after buffer addition.

Precipitation in the Reagent Buffer

The FLIPR® Calcium Assay Kit is compatible with numerous buffers. Use buffers shown to work in previously established assays, if available.

Response is Smaller than Expected

The agonist and antagonist may stick to the tips and trays. Use up to 1% BSA in all compound buffer diluents and presoak tips in compound buffer with up to 1% BSA.

Note: Do not use the same source plate for presoaking and compound addition when using a 384 Pipettor head in the FLIPR® Tetra system. Instead, use a 'Boat' for the presoak.

Apparent Well-to-Well Variation is Observed

AquaMax DW4 or equivalent dispenser is recommended for use with additions of cells or dye prior to the assay on the FLIPR Tetra system if apparent well-to-well variation is observed. In some cases allowing the plates to stand at room temperature prior to use or adding a single mix cycle in the compound or assay plate can decrease well-to-well variation.

FLIPR Membrane Potential Assay Kit Protocol

This section provides the following information and procedures:

- Materials required for the assay
- Cell preparation guidelines
- Dye loading procedure
- Troubleshooting tips for membrane potential assays

Required Materials

The following materials are required for running a membrane potential assay:

Item	Source
FLIPR® Tetra system with Membrane Potential Optics Kit installed: FLIPR® Tetra system LED Module 510–545 nm FLIPR® Tetra system Emission Filter 565–625 nm	Molecular Devices See Consumables and Accessories on page 245
One of the following Membrane Potential Assay Kits: Evaluation Kit MP Blue, explorer MP Blue, bulk MP Red, explorer MP Red, bulk	Molecular Devices R8128 R8042 R8034 R8126 R8123
Clear, flat-bottom, black- or clear-wall 1536-, 384- or 96-well plates	See Consumables and Accessories on page 245
Clear polypropylene source plates	See Consumables and Accessories on page 245
FLIPR® Tetra system pipette tips, 96, 384 or 1536 gasket	See Consumables and Accessories on page 245
Cells in suspension	—
Test compounds	Specific to receptor
Growth medium	Major Laboratory Supplier (MLS)
Incubator	MLS
Centrifuge	MLS
Pipettor and sterile tips suitable for use with microplates	MLS
1 N NaOH	MLS

Note: The Kit includes masking dye technology covered by issued and pending patents including US 6,420,183, US 7,063,952 and EP 0,906,572, licensed exclusively from Bayer AG to Molecular Devices.

About FLIPR Membrane Potential Assay Kit

The FLIPR® Membrane Potential Assay Kits from Molecular Devices provide a fast, simple and reliable fluorescence-based assay for detecting changes in voltage across the cell membrane. These kits are designed to work in association with many receptors and ion channels as well as both adherent and non-adherent cell lines. The assay is a mix-and-read procedure in which cells are incubated with the kit reagents for 30 minutes after which the signal is detected using a FLIPR Tetra system.

There are no intermediate wash steps involved and, typically, the assay is complete one minute after addition of the agonist.

Conventional protocols for evaluating changes in membrane potential are technique-sensitive and multi-step procedures consisting of preparing large batches of dye and introducing dye into the compound plate. Several problems are routinely encountered with the conventional methods, all of which add to experimental variability.

Problems in a conventional assay protocol include:

- Slow response time of traditional dyes used
- Extensive pre-read soaking procedure
- Requirement for precise temperature control
- Variation in fluorescence according to ionic concentrations

Molecular Devices has developed the FLIPR® Membrane Potential Assay Kits to maximize cell line/channel/compound applicability while eliminating causes of variability in the data and reducing the number of steps in the conventional protocol.

Advantages of the FLIPR® Membrane Potential Assay Kits include:

- More reproducible data
- Faster response time
- No cumbersome pre-assay preparation
- Ease of use at room temperature to physiological temperatures
- Fewer steps in the assay resulting in higher sample throughput

FLIPR Membrane Potential Assay Cell Preparation

Cell Densitiess

A variety of cell handling conditions may be adopted at your discretion based on standard operating procedures in the laboratory. Non-adherent cells are typically plated on the day of experiment on a coated plate (for example, poly-d-lysine or collagen) to assist cell adherence to the plate bottom. We recommend you then centrifuge the plates at 1000 rpm for up to 4 min with the brake off. Adherent cells are seeded the day prior to an experiment and incubated in a 5% CO_2, 37 °C incubator overnight. To create an 80%–90% confluent cell monolayer, it is recommended the cells be seeded at densities shown in the table below.

Cell Type (cells/well)	96-well Plate (100 µL growth medium)	384-well Plate (25 µL growth medium)	1536-well Plate (4 µL growth medium)
Adherent cells	20,000–80,000	5,000–20,000	1,500–5,000
Non-adherent cells	40,000–200,000	10,000–50,000	3,000–10,000

FLIPR Membrane Potential Assay Kit Dye Loading

Preparation of Loading Buffer

The following procedure is designed for preparation of the Loading Buffer for either of the Membrane Potential Assay Kits, BLUE (R8034) or RED (R8123) in the Bulk format.

Blue or Red

To prepare loading buffer:

1. To prepare a 1X Reagent Buffer dilute 10 mL of 10X Reagent Buffer (Component B) to 100 mL with distilled water. Adjust to pH 7.4 with 1 N NaOH.

   Note: Occasionally, a white precipitate forms in the 10X Reagent Buffer bottle. This is normal and will not affect the assay.

   Depending upon the cell type and application, the Assay Buffer as provided with the FLIPR® Membrane Potential Assay Kit may not be the ideal choice. If so, alternative buffers may be used at your discretion in order to achieve optimal results.

2. Remove a vial of FLIPR® Membrane Potential Assay Reagent (Component A).

3. Dissolve contents of vial completely by adding 100 mL of 1X (96 and 384) or 67 mL of 3X (1536) Assay Buffer. Mix by repeated pipetting until the contents are completely dissolved.

   Note: For best results, dissolve the contents of the vial with 10 mL of 1x Assay Buffer, and then perform several washes of the vial using 1x Assay Buffer to yield a total volume of 100 mL (96 or 384) or 67 mL (1536).

   Note: Components supplied are sufficient for proper cell loading. For optimum results, it is important NOT to add any additional reagents or change volumes and concentrations.

Loading the Cells Using Loading Buffer

To load the cells:

1. Remove the cell plates from the incubator or centrifuge. Do not remove the supernatant. Add Loading Buffer to each well (100 µL per well for 96-well plates, 25 µL for 384-well or 2 µL for 1536-well plates).

   Note: Although Molecular Devices does not recommend washing cells before dye loading, growth medium and serum factors can be washed away before adding the Loading Buffer, provided residual volumes after the wash step are as described. Alternatively, cells can be grown in serum-free conditions.

2. Incubate the cell plates for 30 minutes at 37 °C (98.6 °F).

   Note: In some cases, incubation at room temperature may improve results.

CAUTION! Do not wash the cells after dye loading.

Running the FLIPR Membrane Potential Assay

Before incubation, ensure that the FLIPR Tetra system is equipped with the 510–545 nm LED module and 565–625 nm emission filter. See LED module and emission filter installation instructions in Exchanging Hardware on page 105 for a picture diagram can be faxed to you by contacting Molecular Devices Technical Support. See

1. Choose the 510–545/565–625 excitation/emission wavelengths respectively from the **Settings** process in ScreenWorks Software. After incubation transfer the plates directly to FLIPR Tetra system and begin the Membrane Potential assay.
2. Place the filter holder in its correct position in the FLIPR Tetra system. For a signal test, a starting average count of 1,500-2,500 RLU is recommended.

Recommended Setup Parameters

Recommended experimental setup parameters are provided below. Note that the addition speeds are faster than in the conventional protocol because of the increased robustness of the cells after the newloading procedure.

Faster addition speeds can lead to better mixing of compounds and lower signal variance across the plate.

Detection Parameters	EMCCD Camera	ICCD Camera
Read Mode	Fluorescence	Fluorescence
Excitation Wavelength	510–545 nm	510–545 nm
Emission Wavelength	565–625 nm	565–625 nm
Excitation Intensity	50 %[1]	50 %[1]

Detection Parameters	EMCCD Camera	ICCD Camera
Camera Gain	50	2000
Gate Open	—	6 %[1]
Exposure Length	0.4 s[1]	0.53 %[1]
Read Interval	1 s	1 s
1. Can be adjusted to increase or decrease the signal if low RFUs or saturation problems occur.		

Addition Parameters	96-Well Plate	384-Well Plate	1536-Well Plate
Compound Addition Volume (µL)	50	12.5	1
Compound Concentration (Fold)	5X	5X	X
Dispense Speed (µL/s) Adherent Cells	50–100	10–50	5–10
Dispense Speed (µL/s) Nonadherent Cells	10–50	5-20	1-6

Troubleshooting the FLIPR Membrane Potential Assay Kit

This section presents solutions to problems that users may encounter when running membrane potential assays.

Fluorescence Drop Upon Compound Addition

This may result from dislodging cells from the wells during addition. Shortening incubation times, plating cells on poly-D-lysine plates or slowing the dispense speed should solve the problem in this case.

Adding volumes greater than recommended may increase the initial fluorescence drop. In these cases it may be necessary to adjust the volumes of the components. The recommended volume of the Loading buffer is 100 µL for 96 well plate, 25 µL for 384 well plate or 2 µL for 1536-well plates.

CAUTION! Increasing the final in-well concentration of the Loading Buffer may decrease the response of the assay. If only one addition is required, then add the appropriate volume of buffer before addition one.

Fluorescence Increase

An increase of fluorescence may be observed upon buffer only challenge. This increase is most likely due to a response of endogenous ion channels to increasing ionic strength. Patch clamping data supports this observed change. The choice of cells and expression levels of endogenous channels can greatly influence resting and changing membrane potentials. Match the compound addition buffer to the buffer in the cell plate (culture medium plus dye loading buffer) so there is no change in ion concentration upon compound addition.

No Response

Not all assays work well with all cell lines or channels. To address this problem, we have developed two different assays to maximize the opportunity for a successful assay. If you have not tried both FLIPR® Membrane Potential Assay Kit formulations, Blue and Red, then first try the alternate kit to determine if your cell line is compatible. The FLIPR® Membrane Potential Evaluation Assay Kit (R8128) contains both formulations in an Explorer Kit format to facilitate testing of cell lines, channels, and compounds. If you do not see a change in fluorescence with either formulation, then we suggest changing some of the assay conditions.

Recommendations include: replacing media before dye loading, longer incubation and assay times; preparing compounds in the loading buffer; and choosing different buffers such as Tyrode's or specific ion-free buffers. For example, if studying a calcium channel, dye load cells using a calcium-free buffer and prepare your compound plate using a calcium containing buffer.

Effect of DMSO on Membrane Potential Assays

High concentrations of DMSO are toxic to many cell lines and affect compound mixing. Also, DMSO can induce physiological changes in cells (for example, differentiation). If the test compound preparation contains DMSO, it is important to titrate the DMSO to determine the maximum concentration that can be used with each indicator cell line and test compound. With some cell lines that have been tested, there was no effect on signal level up to 1% DMSO final concentration.

Voltage Sensor Probes Assay Protocol

This section provides the following information and procedures:

- Materials required for the assay
- Cell preparation guidelines
- Dye loading procedure

Required Materials

The following materials are required for running a membrane potential assay:

Item	Source
FLIPR® Tetra system with Voltage Sensor Probes Optics Kit installed: FLIPR® Tetra system LED Module 390–420 nm FLIPR® Tetra System Emission Filter 440–480 nm FLIPR® Tetra System Emission Filter 565–625 nm	Molecular Devices See Consumables and Accessories on page 245

Item	Source
Voltage Sensor Probes: DisBAC2(3) and CC2-DMPE VABSC-1 Background Suppression Dye	Invitrogen K1016 K1019
Clear, flat-bottom, black- or clear-wall 384- or 96-well plates	See Consumables and Accessories on page 245
Clear polypropylene source plates	See Consumables and Accessories on page 245
FLIPR® Tetra system pipette tips, 96 or 384	See Consumables and Accessories on page 245
Cells in suspension	—
Test compounds	Specific to receptor
Growth medium	Major Laboratory Supplier (MLS)
Incubator	MLS
Centrifuge	MLS
Pipettor and sterile tips suitable for use with microplates	MLS
1 N NaOH	MLS

About the Fluorescent Dye

Voltage Sensor Probes are a Fluorescence Resonance Energy Transfer (FRET) based assay technology used for high-throughput ion channel drug discovery. The membrane-bound, coumarinphospholipid (CC2- DMPE) FRET donor binds only to the exterior of the cell membrane. The FRET acceptor is a mobile, negatively charged, hydrophobic oxonol [either DiSBAC2(3) or DiSBAC4(3)], which binds to either side of the plasma membrane in response to changes in membrane potential. Resting cells have a relatively negative potential. Therefore, the two probes associate with the cell membrane exterior, resulting in efficient FRET. Exciting the CC2-DMPE donor probe (at ~405 nm) generates a strong red fluorescence signal (at ~580 nm) from the oxonol acceptor probe. When the membrane potential becomes more positive, as occurs with cell depolarization by KCl, the oxonol probe rapidly translocates (on a sub second time scale) to the other membrane face. Thus, each oxonol probe "senses" and responds to voltage changes in the cell. This translocation separates the FRET pair, and exciting the CC2-DMPE donor probe now generates a strong blue fluorescence signal (at ~460 nm) from the CC2-DMPE probe.

Cell Preparation for the voltage Sensor Probe Assay

Cell Densities

A variety of cell handling conditions may be adopted at your discretion based on standard operating procedures in the laboratory. Nonadherent cells are typically plated on the day of experiment on a coated plate (for example, poly-d-lysine or collagen) to assist cell adherence to the plate bottom. We recommend you then centrifuge the plates at 1000 rpm for up to 4 min with the brake off. Adherent cells are seeded the day prior to an experiment and incubated in a 5% CO_2, 37° C (98.6 °F)incubator overnight. To create an 80–90% confluent cell monolayer, it is recommended the cells be seeded at densities shown in the following table.

Cell Type (cells/well)	96-Well Plate (100 µL growth medium)	384-Well Plate (25 µL growth medium)
Adherent cells	20,000–50,000	5,000–30,000
Non-adherent cells	40,000–100,000	10,000–60,000

Preparation of Loading Buffer

To prepare loading buffer:

1. CC2-DMPE: A 5 mM stock solution can be prepared in DMSO and stored at -20° C (-4 °F).

   On the day of the assay, a working solution needs to be prepared. An equal volume of 10% Pluronic acid is mixed with CC2-DMPE and subsequently diluted to 5 µM in VSP-1 solution. Vigorously mix the buffer and protect from light prior to use.

2. DisBAC2(3): A 12 mM stock solution can be prepared in DMSO and stored at -20° C (-4 °F).

   A 200 mM stock solution of VABSC-1 can be prepared in water and stored at RT.

   On the day of the assay, a 4 µM working solution of DisBAC2(3) needs to be prepared including 0.21 µM VABSC-1 quencher in VSP-1 solution.

Loading the Cells Using Loading Buffer

To load the cells:

1. Remove media from all wells of the 384-well plate and replaced with 50 µL/well VSP-1 solution.
2. Remove VSP-1 immediately and replace with 25 µL/well CC2-DMPE loading buffer and incubate at room temperature for 30 minutes, covered and protected from light.
3. After 30 minutes, remove the CC2-DMPE loading buffer and wash wells with 50µL/well VSP-1 solution.
4. Immediately remove VSP-1 and replace with 4 µM DisBAC2(3) and 0.21 µM VABSC-1 loading buffer, then incubate for 30 minutes at room temperature protected from light.

Running the Voltage Sensor Probe Assay

Before incubation, ensure that the FLIPR Tetra system is equipped with the 390–420 nm LED module plus the 440-480 nm and 565-625 nm emission filter. See LED module and emission filter installation instructions in Exchanging Hardware on page 105.

Choose the 390–420/440–480 excitation/emission wavelengths respectively for Read Mode 1 from the Settings process in ScreenWorks Software. Also select the 390–420/565–625 excitation/emission wavelengths respectively for Read Mode 2. After incubation transfer the plates directly to FLIPR Tetra system and begin the Voltage Sensor Probes assay.

The assay may be performed at room temperature up to physiological temperature.

Recommended Setup Parameters

Recommended experimental setup parameters are provided in the following tables.

Faster addition speeds can lead to better mixing of compounds and lower signal variance across the plate.

Detection Parameters	Standard, EMCCD Camera		Aequorin, ICCD Camera	
	Read Mode 1	Read Mode 2	Read Mode 1	Read Mode 2
Read Mode	Fluorescence		Fluorescence	
Excitation Wavelength	390–420 nm	390–420 nm	390–420 nm	390–420 nm
Emission Wavelength	440–480 nm	440–480 nm	440–480 nm	565–625 nm
Excitation Intensity	50–100 %[1]		50 %[1]	
Camera Gain	80		2000	
Gate Open	—		6 %[1]	
Exposure Length	0.25 s each mode		0.25 s each mode	
Read Interval	1 s for both modes		1 s for both modes	

1. Can be adjusted to increase or decrease the signal if low RFUs or saturation problems occur.

Addition Parameters	96-Well Plate	384-Well Plate
Compound Addition Volume (µL)	50	25
Dispense Speed (µLs) Adherent Cells	50–100	10–50
Dispense Speed (µL/s) Non-adherent Cells	10–50	5–20

Luminescence Assay Protocol

This section provides the following information and procedures:

- Materials required for the assay
- Cell preparation guidelines
- Coelentrazine loading procedure
- Running the assay on FLIPR® Tetra System
- Optimizing tips for Luminescence assays

Note: The references to the instrument assume the Aequorin ICCD camera option is present along with the Cell Suspension option.

Required Materials

The following materials are required for running a luminescence assay:

Table 8-3: Cell Preparation for Aequorin Assays

Item	Source
FLIPR® Tetra system with Aequorin, ICCD camera option and Cell Suspension option	Molecular Devices
Clear, flat-bottom, black wall 96-, 384-, 1536-well plates	See Consumables and Accessories on page 245
Clear polypropylene source plates (for adherent protocols)	See Consumables and Accessories on page 245
Black FLIPR® System pipette tips, 96- or 384-well or 1536 tip block and gaskets	See Consumables and Accessories on page 245
Test compounds	Specific to receptor
Cells in suspension	Optional
Coelenterazine	Specific to Photoproteins
Growth medium	Major Laboratory Supplier (MLS)
Incubator	MLS
Centrifuge	MLS
Pipettor and sterile tips suitable for use with microplates	MLS

Cell Densities

A variety of cell handling conditions may be adopted at your discretion based on standard operating procedures in the laboratory. Non-adherent cells are typically plated on the day of experiment on a coated plate (for example, poly-d-lysine or collagen) to assist cell adherence to the plate bottom. We recommend you then centrifuge the plates at 100g for up to 4 min with the brake off. Adherent cells are seeded the day prior to an experiment and incubated in a 5% CO_2, 37 °C (98.6 °F) incubator overnight. To create an 80%–90% confluent cell monolayer, it is recommended the cells be seeded at densities shown in the table below. For suspension experiments, cells are loaded into a cell flask on the day of the experiment and are pipetted via the instrument into the read plate, so the flask concentration as well as the volume pipetted controls the final concentration.

The recommended concentrations are listed in the following table.

Cell Type (cells/well)	96-Well Plate (100 µL growth medium)	384-Well Plate (25 µL growth medium)	1536-Well Plate (2–3 µL growth medium)
Adherent cells	5,000–50,000	1,250–15,000	1,000–2,500
Non-adherent cells	40,000–100,000	10,000–60,000	2500–-15,000
Suspension cells	10,000–40,000	2,500–10,000	1,000–5,000

Coelenterazine Loading For Adherent Assays

To load the cells for an adherent assay:

Before the day of the assay, remove culture medium from wells.

Wash cells in 'BSA Medium' containing DMEM/HAM's F12 with HEPES, without phenol red, catalog# 11039-021 (Invitrogen) + 0.1% BSA.

In reduced lighting conditions, make up a solution of coelenterazine appropriate for your cell type, in 'BSA Medium' and add a volume of 100 µL (96-well plates), 25 µL (384-well plates), or 3 µL (1536-well plate) per well. Final concentration in the well should be 5–10 µM.

Incubate the cells for 4–6 hrs at room temperature (22 °C/71.6 °F or lower). Cover the plates with aluminum foil to protect from light and to avoid degradation of coelenterazine.

Coelenterazine Loading for Suspension Cell Assays

To load the cells for a suspension assay:

1. Before the day of the assay, remove the selection Antibiotics from cells.
2. On the day of the assay, wash cells in seeding flask with 20 mL DPBS (without Calcium and Magnesium).
3. Dislodge the cells by adding 15 mL Versene, incubating for 5 minutes in 37 °C (98.6 °F) 5% CO_2 and immediately adding 20 mL of growth media without selection antibiotics.

4. Centrifuge in 50 mL tube @ 1000 RPM or 168 g for 2.5–3 minutes.
5. With supernatant removed and cells re-suspended in 40 mL BSA-medium, count cells and adjust density to 5.0x 106 cells/mL.
6. Under reduced lighting conditions, recommended type of coelenterazine should be added to reach a final concentration of 5 µL.
7. Cells should be placed in 15 mL aluminum wrapped tubes on rotating wheel (~7–8 RPM) at room temp (below 22 °C/71.6 °F) for 4 hours to overnight.
8. After incubation, cell density should be adjusted while protecting from light and cells should be placed in the cell stir flask and stirred on FLIPR Tetra system at speed 6.
9. Cells should spin protected from light for 1–2 hours prior to the start of the assay.

Preparation of Cell Reservoir and Running the Assay

1. The Cell Reservoir must have been cleaned at the end of the previous day's assay. If this was done, rinse with DI H_2O, several times.
2. If the Cell Reservoir was not cleaned after previous assay, follow instructions for making AquaMax Sterilant Kit Procedure (Cat# R8156, Molecular Devices). See Consumables and Accessories on page 245.
3. Fill a flask with the sterilant solution and run 3–4 Cell Reservoir wash cycles including a 2 minute soak (Hold Time =120 s).
4. Physically wipe the Cell Reservoir with Kimwipes moistened in DI water.
5. Rinse the Cell Reservoir 5 times with DI water and follow the steps for preparation in #6.
6. Prior to start of assay, Cell Reservoir and lines to and from spinner flask should be rinsed 5X each with the following:
   - 70% Ethanol
   - Endotoxin-free DI water
   - Media containing BSA-Medium 0.1% BSA
7. Prior to start of screening, insert the hard plastic tubes from the Cell Flask into the lines running from the 8-way valve by pinching the lines and sliding the tubing inside.
8. Cycle the cells through to the Cell Reservoir two to three times prior to starting assay.
9. Use 2–3 plates to prior to screening and insure that the camera settings are within range of the signal.

If cells are recirculated back to the cell flask, it is strongly recommended that the tips be washed each time an assay is run to reduce risk of contamination of the cells by toxic or antagonist compounds.

Instrument Setup

Luminescence assays require no excitation source, but do require optimal instrument conditions to collect the maximum amount of light emitted from the assay. These settings are specific to the Aequorin camera option.

In order to provide optimal light collection by the FLIPR Tetra system, we recommend you leave an empty emission filter position in the filter slider to run aequorin. In addition, you must calibrate the instrument to use no LEDs and emission filters. This state is identified as the NONE/NONE Excitation/Emission Wavelengths. Detailed instructions outlining the removal of the emission filters and calibration are found in Exchanging Hardware on page 105.

Experimental Setup Parameter

Parameter	Setting
Read Mode	Luminescence
Excitation Wavelength	None
Emission Wavelength	None
Camera Gain[1]	70,000–280,000
Exposure Time[1]	0.53 s
LED Excitation Intensity	NA
Gate Open	100%
Read Interval	1 s
Reads before pipetting	5
Reads during first interval	50–90
Save Assay Images	Possible, but creates very large files
1. Can be adjusted to compensate for cell brightness or saturation problems.	

Fluid Transfer Settings—Aspirate

Parameter	Suspension Setting (pipette cells)	Adherent Settings (pipette compound)
Cell Flask Spinning Rate	Manual setting, Speed 6	NA
Fluid Transfer Type	Single Aspirate, Single Dispense	Single Aspirate, Single Dispense
Source Plate Fill Reservoir speed Drain Reservoir speed Drain destination	defaultcellres384 5 4 Cell flask	Source Plate 2 NA NA NA
Aspiration Volume	25 µL	25 µL

Parameter	Suspension Setting (pipette cells)	Adherent Settings (pipette compound)
Aspiration Height	70 µL	5 µL
Aspiration Speed	20 µL/s	20 µL/s
Tip Up Speed	10 mm/s	10 mm/s
Hold Volume	0 µL	0 µL

Fluid Transfer Settings—Dispense

Parameter	Suspension Setting	Adherent Settings
Target Plate	Read Plate	Read Plate
Volume	25 µL	25 µL
Height	15 µL	30 µL
Speed	30 µL/s	20 µL/s
Removal Speed	10 mm/s	10 mm/s
Expel Volume[1]	0 µL	0 µL
Pause in Well	None	None
Hold pipettor during dispense	None	None
1. A hold or expel volume will introduce air bubbles into the well causing false spikes in signal.		

Recommended Assay Settings

Parameter	Setting
Reduction Type	Area under the curve, from or shortly after addition point to the end of the assay
Correction	Subtract bias based on Sample 1

Post Assay Cell Suspension Cleaning

CAUTION! The suspension cell system should never be left without cleaning at the end of an assay day or between assays with different cells.

1. Remove cell spinner flask and insert tubing from flask into DI watersolution.
2. Insert return line tubing to a waste flask making sure that the tubing does not reach into the waste fluid. It will need to be left as clean as the inlet tubing.
3. Perform a manual reservoir wash with water and follow by wiping out the reservoir with a Kimwipe.

4. Build a FLIPR Tetra system protocol to wash the reservoir and all tubing in the following order:
   - 5 times with Endotoxin-free DI water.
   - 5 times with Cytoclense Sterilant wash and soak (2 min).
   - 5 times with Endotoxin-free DI water.
   - 5 times with 70% Ethanol and leave system and all tubing dry.
5. The cell flask also must be washed each time after use:
   - Rinse flask, spinner, caps, and all tubing with Endotoxin-free DI water.
   - Use glassware detergent safe for use with cell culture vessel.
   - Scrub with bottle brush and flush lines.
   - Rinse with Endotoxin-free DI water.
   - Soak flasks in Cytoclense sterilant (see Appendix D).
   - Rinse with Endotoxin-free DI Water.
   - Rinse with 70% Ethanol and leave all components to air dry or bake in oven.
   - In addition, reservoir and spinner flask may be autoclaved.

Optimizing an Assay

The majority of the FLIPR Tetra system assay optimization is related to cell treatment prior to and during the assay. Cell and assay conditions to check include the following:

- Cell Culture on page 166
- Cell Seeding on page 167
- Loading Coelenterazine on page 167
- Source Plate on page 167

Cell Culture

Conditions to check:

- Cell passage number.
- Cell growth conditions, for example confluency in flasks and/or in wells.
- Expression induction time for transfected cells—concentration of selection antibiotics and overall cell viability.

Cell Seeding

Conditions to check:

- Seeding density. Do not allow cells to become over-confluent.
- Type of black, clear-bottom white or clear plate.
- Volume of growth medium in each well.
- Growth medium replacement during seeding period.

Loading Coelenterazine

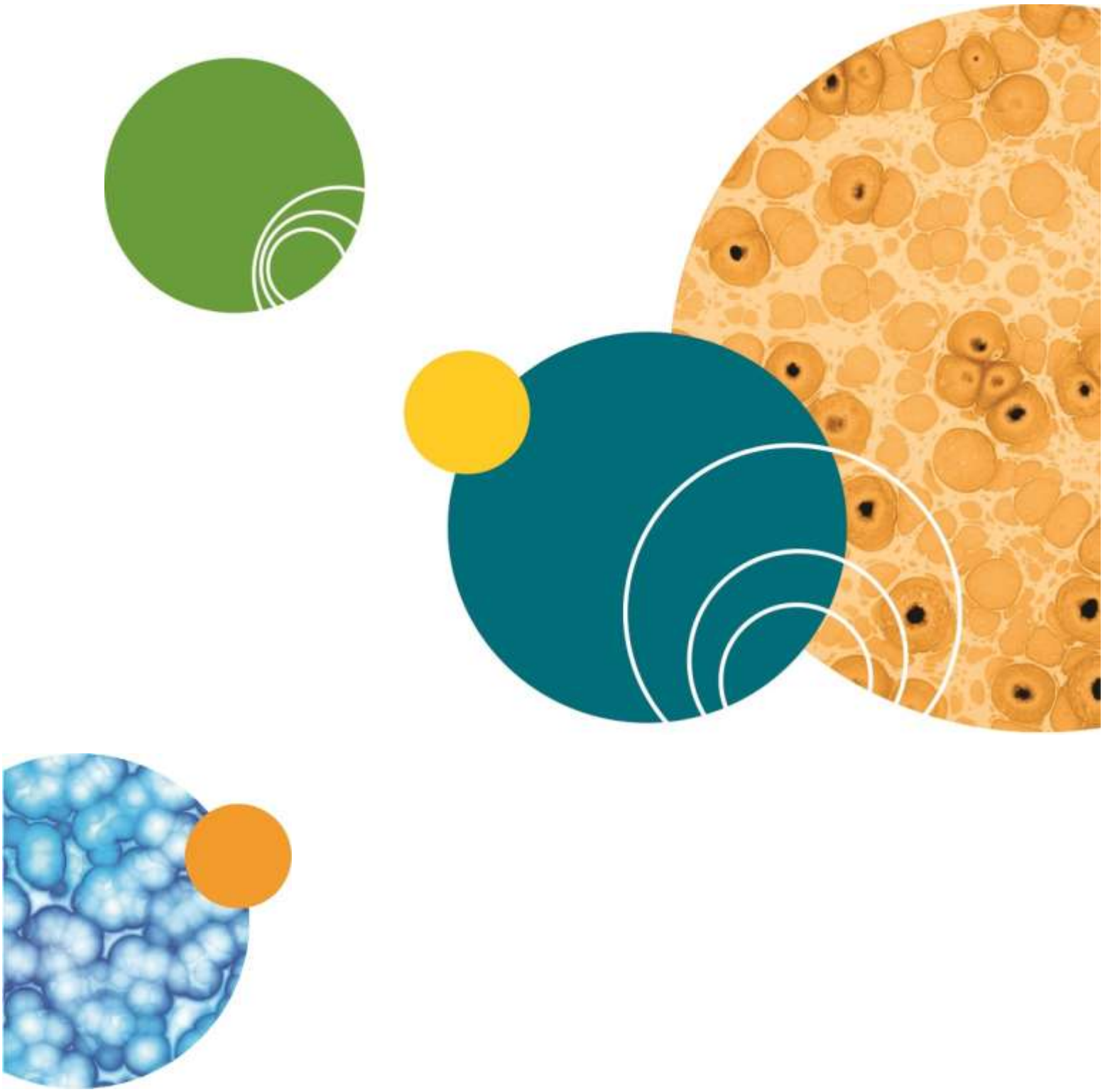
Conditions to check:

- Seeding density. Do not allow cells to become over-confluent.
- Type of black, clear-bottom white or clear plate.
- Volume of growth medium in each well.
- Growth medium replacement during seeding period.

Source Plate

Conditions to check:

- Type of buffer used should match coelenterazine buffer.
- For suspension assay, prepare 2x compound directly in read plate.
- For adherent assays, prepare a polypropylene plate with 2x compound.
- Volume of fluid in source plate in relation to required transfer volume and plate dead volume.



Chapter 9: Troubleshooting

This chapter contains tables of symptoms and solutions to help you diagnose and repair problems with the FLIPR Tetra system. In some cases, you will need to contact Molecular Devices Technical Support. See Obtain Support.

Instrument Status Colors

ScreenWorks Software is designed to handle most errors that occur when running the FLIPR Tetra system. The status of the system is reported in the **Instrument Status** window. Depending on the state of the instrument, a different color block (green, yellow, red or black) is displayed at the bottom of the **Instrument Status** window.

If the Instrument Status Color is...	Then there is...
Green	Proper and open communication.
Yellow	Minor communication error. Clear error by selecting **Clear Error** from the **Instrument** menu.
Red	Instrument failure. Instrument must be reset by selecting **Reset** from the **Instrument** menu.
Black	No instrument communication available. This is typically shown when working in Offline mode.

Troubleshooting Start-Up

Symptom or Error Message	Possible Causes	Solutions
“Connecting to the instrument, please wait. Will enter offline if the instrument is not connected or if this dialog is closed. Dialog may not close immediately on pressing the close button while connection is being attempted.”	ScreenWorks Software is waiting for the FLIPR Tetra system to complete initialization prior to connecting.	Wait until **Assay Finished (Unlocked)** light is the only light illuminated on the front instrument panel before the software and instrument will connect.
"Camera chiller subsystem not functional. Please contact Technical Support. The instrument may have degraded read performance until it is repaired: Can't communicate with chiller."	Instrument is not communicating with the chiller.	Make sure the chiller is turned ON. Contact Technical Support. See Obtain Support.

Symptom or Error Message	Possible Causes	Solutions
409:“Initialization halted because tips are loaded. Please verify that the appropriate tip rack (96,384,1536) 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 3/tip loading position to unload tips and select **Reset** from the **Instrument** menu.
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 and select **Reset** from the **Instrument** menu.
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.	Select **Reset** from the Instrument menu.
“Disconnected” is displayed in the bottom of the **Instrument Status Window**.	ScreenWorks Software is in **Offline Mode**.	Select **Go Online** from the **Instrument** drop-down menu.
	Multiple software copies are open.	Close all versions of ScreenWorks Software before starting software.
	Instrument timed-out.	Select **Go Online** from the **Instrument** drop-down menu.
Instrument Configuration does not show desired components installed on instrument.	Instrument does not have proper components installed.	Install proper components and restart system.
	ScreenWorks Software is in **Offline Mode**.	Select **Go Online** from the **Instrument** drop-down menu.
“Camera Temp” not at -60 °C ± 2 °C (-76 °F ±35.6 °F) for EMCCD camera or -20 °C ± 5 °C (-4 °F ±41 °F) for ICCD camera.	Camera is not cooled down to temperature.	Allow 10 minutes for camera temperature to cool. Make sure the chiller is working properly.
		Contact Technical Support. See Obtain Support.

Symptom or Error Message	Possible Causes	Solutions
"Chamber Temp" not at desired temperature.	Heated stage is not warming to desired temperature.	Allow 15 minutes for the chamber to warm to the desired temperature.
		Contact Technical Support. See Obtain Support.
	Temperature control is not ON.	Turn on heated stage by selecting **Temperature Control ON** from the **Instrument > Manual Operation** drop-down menu.
Pipettor head is in the plate after the instrument initializes.	Instrument did not initialize properly.	Select **Reset** from the **Instrument** drop-down menu.
		Contact Technical Support. See Obtain Support.

General Troubleshooting

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 appropriate plate in the Read position.
205: "Source 1 plate position empty".	No plate is present in the Source 1 position.	Place appropriate plate in the Source 1 position.
206: "Source 2 plate position empty".	No plate is present in the Source 2 position.	Place appropriate plate in the Source 2 position.
207: "Source 3 plate position empty".	No plate is present in the Source position.	Place appropriate plate in the Source 3/Tip Loading position.

Symptom or Error Message	Possible Causes	Solutions
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.	Convert from a source only to a read plate using the plate library.
100: “Command not found.”	Input command is not valid. See appended string on the error message for details.	Contact Technical Support. See Obtain Support.
101: “Invalid argument.”	Input argument is not valid. See appended string on the error message for details.	Contact Technical Support. See Obtain Support.
102: “Invalid number of arguments.”	Number of input arguments is not valid. See appended string on the error message for details.	Contact Technical Support. See Obtain Support.
103: “Invalid start of command.”	Start of command is not valid. See appended string on the error message for details.	Contact Technical Support. See Obtain 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. See Obtain Support.
“Data buffer full”.	The data buffer is full. See appended string on the error message for details.	Contact Technical Support. See Obtain Support.

Symptom or Error Message	Possible Causes	Solutions
106: “Option not installed, command unavailable”.	Pipettor head, LED, Emission Filter or TETRAcycler™ is not installed.	Install option you want to use.
		Change protocol to use available instrument options.
107: “Command failed”.	General failure.	Contact Technical Support. See Obtain Support.
108: “Command failed, system in an error state".	Minor communication error occurred.	Select **Clear Error** command from **Instrument** drop-down menu.
109: “Command failed, system not initialized “	Communication failed between software and instrument.	Select **Reset** from the **Instrument** drop-down menu.
		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 appropriate user name 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.	Select **Reset** command from the **Instrument** drop-down menu to reset instrument to see if communication can be reconnected.
		Contact Technical Support. See Obtain Support.
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.	Clean off hard drive.

Symptom or Error Message	Possible Causes	Solutions
"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.	*.tif files must be saved in same folder as data to retain **Image** button option.
309: "Unable to modify or delete selected plate. It does not exist."	Plate does not exist.	Contact Technical Support. See Obtain 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. See Obtain 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. See Obtain 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.	Select **Reset** from the **Instrument** menu.
		Contact Technical Support. See Obtain 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.	Select **Reset** from the **Instrument** menu.
		Contact Technical Support. See Obtain Support.
405: "Motion communication fault. Please select Reset. If this error repeats, please contact Technical Support."	Motion communication fault.	Select **Reset** from the **Instrument** menu.
		Contact Technical Support. See Obtain 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. See Obtain Support.
407: "LCB read/write test failed. Please select Reset. If this error repeats, please contact Technical Support."	LCB read/write test failed.	Select **Reset** from the **Instrument** menu.
		Contact Technical Support. See Obtain Support.

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.	Select **Reset** from the **Instrument** menu.
		Contact Technical Support. See Obtain Support.
ScreenWorks indicates the user-defined name of the data file is invalid.	Illegal characters were used in the file name.	Eliminate illegal characters from userdefined name (for example, “ , ’ : ? ;) .
	User-defined name includes too many characters.	Use 25 characters or less in the user-defined 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 will prevent you from reading the plate. It is at the discretion of the user to 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 the Pipettor

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.

Symptom or Error Message	Possible Causes	Solutions
231: “Pipettor head not detected. Please verify proper installation and select Reset.”	No pipettor head is present on pipettor mount.	Install appropriate 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 appropriate pipettor onto instrument.
		Change plate format used in the software to match the pipettor head.
208: “Unable to load tips. Tips are already on pipettor.”	Tips are already present on pipettor.	If you would like to change tips, select **Unload Tips** from the **Instrument > Manual Operation** drop-down menu. Once tips are exchanged, select Load Tips from the same drop-down menu.
		If want to keep the present tips on, clear error and proceed with your experiment.
209: “Unable to unload tips. There are no tips loaded on the pipettor.”	No tips are present on the pipettor head.	Clear error.
233: Unable to dispense. No tips loaded on pipettor.”	No tips are present on the pipettor head.	Load tips manually on the pipettor head using the **Load Tips** command which is found in the **Instrument > Manual Operation** drop-down menu.
		Change 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.	Load tips manually on the pipettor head using the **Load Tips** command which is found in the **Instrument > Manual Operation** drop-down menu.
		Change 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.	Load tips manually on the pipettor head using the **Load Tips** command which is found in the **Instrument > Manual Operation** drop-down menu.
		Change protocol to include loading of tips at the beginning of the experiment

Symptom or Error Message	Possible Causes	Solutions
236: “Unable to mix. No tips loaded on pipettor.”	No tips are present on the pipettor head.	Load tips manually on the pipettor head using the **Load Tips** command which is found in the **Instrument > Manual Operation** drop-down menu.
		Change 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.	Load tips manually on the pipettor head using the **Load Tips** command which is found in the **Instrument > Manual Operation** drop-down menu.
		Change 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.	Unload tips manually from the pipettor head using the **Unload Tips** command which is found in the **Instrument > Manual Operation** drop-down menu.
Some tips are left in the tip rack after loading the tips.	Pipettor calibration failure.	Contact Technical Support. See Obtain Support.
Some tips are left on the pipettor after unloading.	Pipettor calibration failure.	Contact Technical Support. See Obtain Support.
The tip rack is attached to the tips.	Tip box failure.	Remove tip box from tips. Choose **Instrument > Manual Operation > Unload** Tips to unload tips to tip loading position.
		Contact Technical Support. See Obtain 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 appropriate pipettor head type.
		Change protocol to include 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 appropriate pipettor head type.
		Change protocol to include Source 1 plate format that is compatible with pipettor head.

Symptom or Error Message	Possible Causes	Solutions
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 appropriate pipettor head type.
		Change 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 volume that you are aspirating/dispensing so it is 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 volume that you are aspirating/dispensing so it is 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 speed that you are aspirating/dispensing so it is 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 speed that you are aspirating/dispensing so it is within the specified range.
“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 height that you are aspirating/dispensing so it is 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 height that you are aspirating/dispensing so it is within the specified range.

Symptom or Error Message	Possible Causes	Solutions
"Done with plate after aspiration" is not present.	TETRAcycler is not present on instrument.	Must install TETRAcycler on instrument for command to be available.
"Done with plate after dispense" is not present.	TETRAcycler is not present on instrument.	Must install TETRAcycler on instrument for command to be available.
	Dialog does not appear because you are dispensing to the Read plate position.	Read plate is automatically changed 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 the Optics

Symptom or Error Message	Possible Causes	Solutions
240: "Attempting to use a LED module that is not installed. Please install the desired LED banks."	Specified LED module in protocol is not installed.	Install the appropriate LED banks.
		Select the appropriate LED module that is already installed on the instrument.
241: "Attempting to use an emission filter that is not installed. Please install the desired emission filter."	Specified emission filter in the protocol is not installed.	Install the appropriate emission filter.
		Select the appropriate emission filter that is already 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 desired 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.

Symptom or Error Message	Possible Causes	Solutions
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. See Obtain 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 are unable to recalibrate LED bank.
		Contact Technical Support. See Obtain 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. See Obtain Support.

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 instrument with 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 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. See Obtain 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. See Obtain Support.
304: “Gain calibration failed. Please contact Technical Support.”		Contact Technical Support. See Obtain Support.

Symptom or Error Message	Possible Causes	Solutions
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 Excitation/Emission pair for which you are looking.
“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 so it is 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 so it is 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.
“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 so it is 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 so it is 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 so it is 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 dispense so it is within the specified range.
238: “Protocol requests greater than 800 reads. This is not permitted.”	Sum of all reads for all read actions and configurations must not exceed 800.	Reduce the number of reads in the experiment to less than 800 for all combined read modes.

Symptom or Error Message	Possible Causes	Solutions
239: “Protocol requests greater than 50 images. This is not permitted.”	Sum of all raw images for all read actions and configurations must not exceed 50.	Reduce the number of raw images in the experiment to less than 50 for all combined dispenses.
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. See Obtain Support.

Troubleshooting the Yellow Plate

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, for ICCD gate to 3%. If condition repeats, lower excitation intensity.
	Incorrect LED and/or emission filter choice.	Check to make sure the appropriate LED and emission filters are selected.
	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. See Obtain 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. See Obtain Support.
	Signal test was not initiated.	Select **Test Signal** in the Signal Test dialog box.

Symptom or Error Message	Possible Causes	Solutions
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 instrument was calibrated using the yellow boat, 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. See Obtain Support.
	Calibration plate failure— plate is dirty or scratched.	Clean calibration plate with lens paper. DO NOT use lab wipes. If cleaning does not work, replace the calibration plate.

Troubleshooting the Tip Washer

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 appropriate pipettor head to match tip wash reservoir top.
		Install appropriate 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 appropriate 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 with tip wash solvent.	Empty tip wash solvent from waste bottle A.
224: "Check Waste Bottle B - sensor indicates full."	Waste bottle B is full with tip wash solvent.	Empty tip wash solvent from waste bottle B.
225: "Check TipWasher - 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. See Obtain Support.

Symptom or Error Message	Possible Causes	Solutions
226: "TipWasher: Check Wash Fluid Bottle A - fill sensor dry."	Fill Bottle A is empty.	Place tip wash solvent into Fill Bottle A.
227: "TipWasher: Check Wash Fluid Bottle B - fill sensor dry."	Fill Bottle B is empty.	Place tip wash solvent into Fill Bottle B.
250: "TipWasher: 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: "TipWasher: Check Wash fluid. Fill sensor dry after fill."	The selected wash bottle might be running low on fluid.	Check the wash bottles.
252: "TipWasher: 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: "TipWasher: Check tubing at Fill Pump - fill sensor wet after draining line."	Fluid sensor is detecting fluid when it should not.	Check for any unconnected tubing. Contact Technical Support. See Obtain 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.”	Can’t communicate with the Tip Washer.	Select **Reset** command from the **Instrument** drop-down menu to reset instrument to see if communication can be reconnected.

Troubleshooting the Cell Reservoir

Symptom or Error Message	Possible Causes	Solutions
255 “CellReservoir: Reservoir not detected. Please install it at Source 3 position and select Refresh Configuration from Instrument menu bar.”	Instrument cannot detect the cell reservoir.	Check to make sure the Cell Reservoir is installed.
		Check the connection of the reservoir sensor inside the instrument.
256 “CellReservoir: prime sensor dry during prime/fill. Please check Fluid Bottle 1”.	Fluid sensor is not detecting fluid when pumping from Fluid 1.	Check to make sure that Fluid 1 bottle is not empty.
257 “CellReservoir: prime sensor dry during prime/fill. Please check Fluid Bottle 2”.	Fluid sensor is not detecting fluid when pumping from Fluid 2.	Check to make sure that Fluid 2 bottle is not empty.
258 “CellReservoir: prime sensor dry during prime/fill. Please check Fluid Bottle 3”.	Fluid sensor is not detecting fluid when pumping from Fluid 3.	Check to make sure that Fluid 3 bottle is not empty.

Symptom or Error Message	Possible Causes	Solutions
259 “CellReservoir: prime sensor dry during prime/fill. Please check Fluid Bottle 4”.	Fluid sensor is not detecting fluid when pumping from Fluid 4.	Check to make sure that Fluid 4 bottle is not empty.

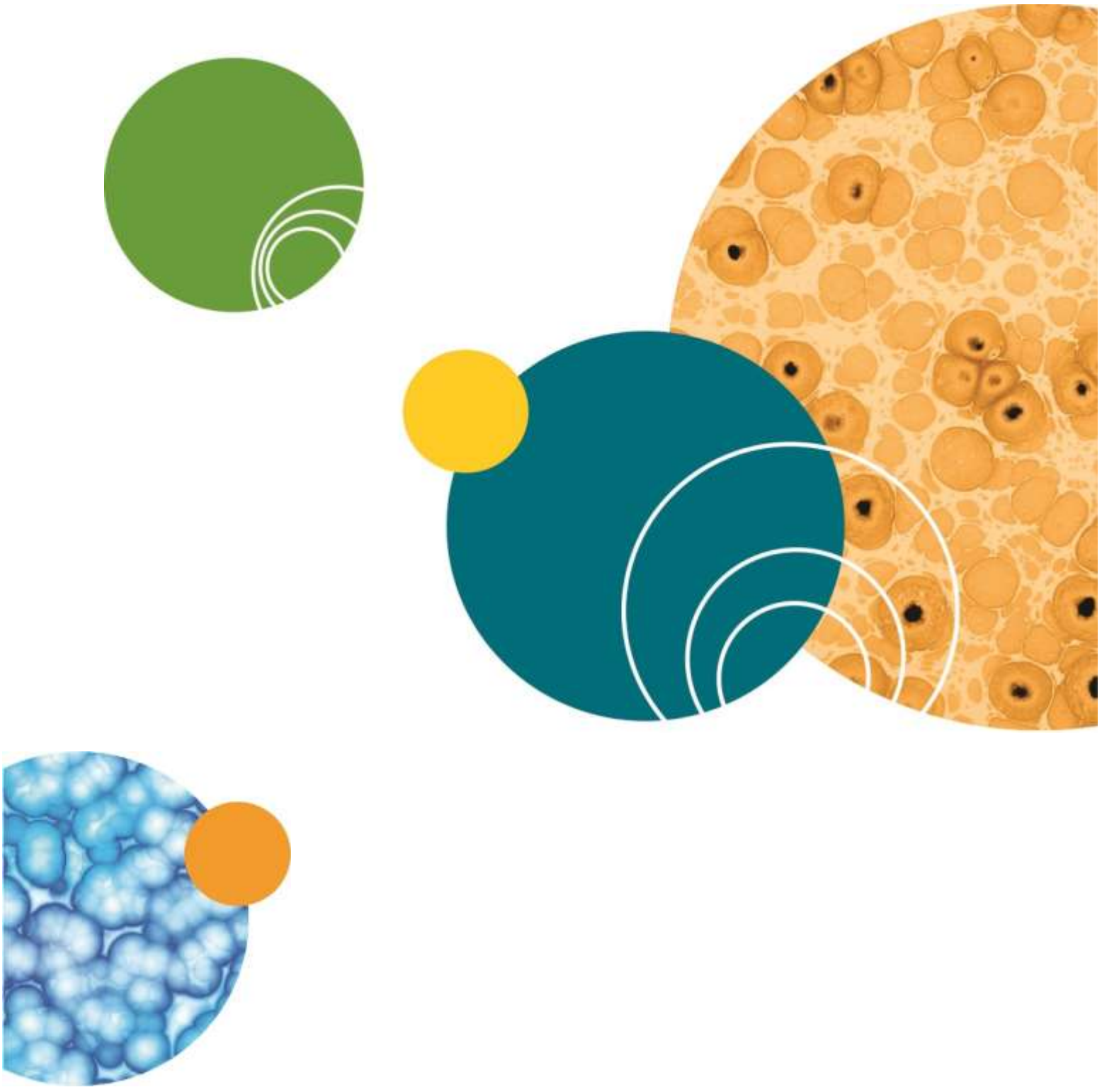
Troubleshooting Data

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.	Refer to Data Processing Algorithms on page 237.
Data parameters need to be reset (subtract bias, spatial uniformity correction, positive control, etc.) for each experiment.	*.vamp file parameters not set up correctly.	Select the Grouping or Correction buttons to adjust the parameters as necessary.
False negative wells.	Pipettor adjustment problem.	See Optimizing an Assay on page 166.
	Tip problem.	Contact Technical Support. See Obtain Support.

Troubleshooting Robotic Integration

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. See Obtain Support.
		If plate not positioned properly by robot, contact Technical Support for robot manufacturer.
	No plate was delivered to landing pad by 3rd party robot.	Contact Technical Support for robot manufacturer.
221: “Unable to load plate. Plate already detected at requested location.”	Plate already at requested position.	Unload plate currently in the desired position prior to delivering new plate.
		If robot is attempting to load multiple plates to one position without retrieving plates, contact Technical Support for robot manufacturer.

Symptom or Error Message	Possible Causes	Solutions
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 reading. Place label as low as possible. Use labels with numbers on top.



Appendix A: Robotic Integration

This document describes the interface between a FLIPR Tetra system and a robotic instrument. The intention of the document is to provide a general overview of the interface between the instrument and robot and a detailed description of the communication protocol between the ScreenWorks Software, which will run on the host computer of the FLIPR Tetra system instrument, and the robotic control software.

Information contained in this document might change as new development occurs—we recommend checking 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 FLIPR Tetra system firmware. The interface now provides a command so it can be queried for its version number. This command is described in detail later in this document.

Instrument Overview

Basic Function

FLIPR Tetra system is a fluorescence microplate reader used primarily for kinetic live cell-based fluorescence and luminescence assays. A typical assay involves the following steps:

- Introduction of a read plate containing 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 Tetra system. Between additions, the pipette tips used for fluid transfer may be replaced or washed.

Cell response in a typical assay typically begins within 2 seconds of fluid addition and is monitored for 1–3 minutes. Fluorescence or luminescence signals are monitored at wavelengths and frequencies selected by the user within the physical constraints of the instrument.

Hardware Introduction

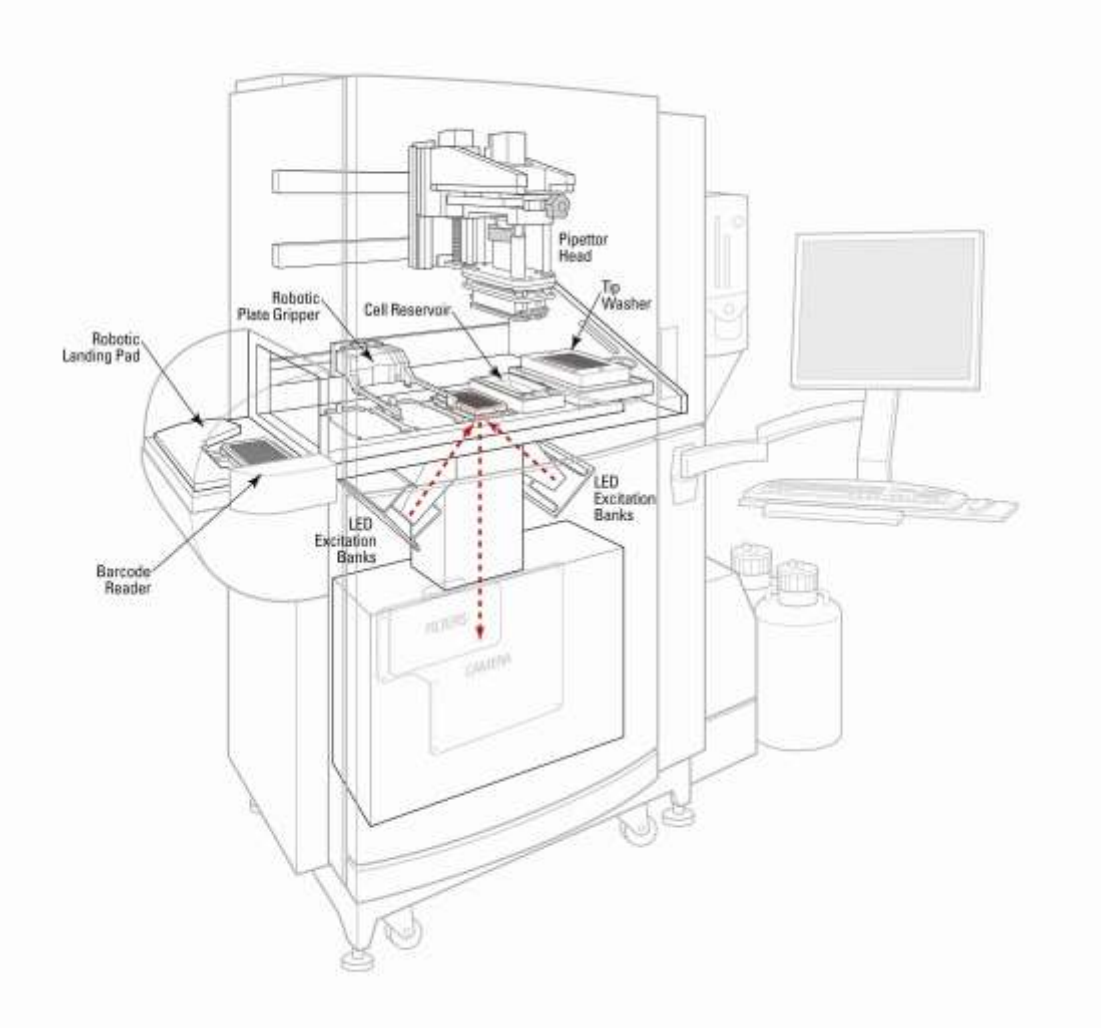
To accomplish the assays described, the instrument includes the following subsystems:

- Embedded control system — The instrument contains an embedded control system for use in controlling the instrument. This control system is accessed only by 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. This system is used to read both fluorescence and luminescence assays.
- Pipettor — In order to accomplish kinetic assays, the instrument contains a pipettor which can add fluids to the read plate during a series of reads. The pipettor, equipped with a 96-, 384-, or 1536-well head, can be used for general purpose fluid transfer between any of the plates in the instrument. Each head type uses pipette tips, or a pin tools. For 96- and 384-well pipettor heads, this is a disposable plastic pipette tip. For 1536 this is a metal tip block. Alternatively, 384- and 1536-well pin tool heads have replaceable pin tools.

- Tip and pin washer — Each instrument includes a washer which can be used to wash tips or pins between fluid transfers. The washer consists of two parts—a washer control module and a wash reservoir. Installed on the right side of the instrument, the sensors, and control valves. These components connect to the wash reservoir located in position 5 of the system stage.
- FLIPR TetraCycler Plate Handler — An optional plate handler is available that is capable of moving 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 TetraCycler Plate Handler is an automated door system that allows plates to be exchanged while an assay is in progress.

Layout

The basic layout of the instrument subsystems is demonstrated in the following illustration:



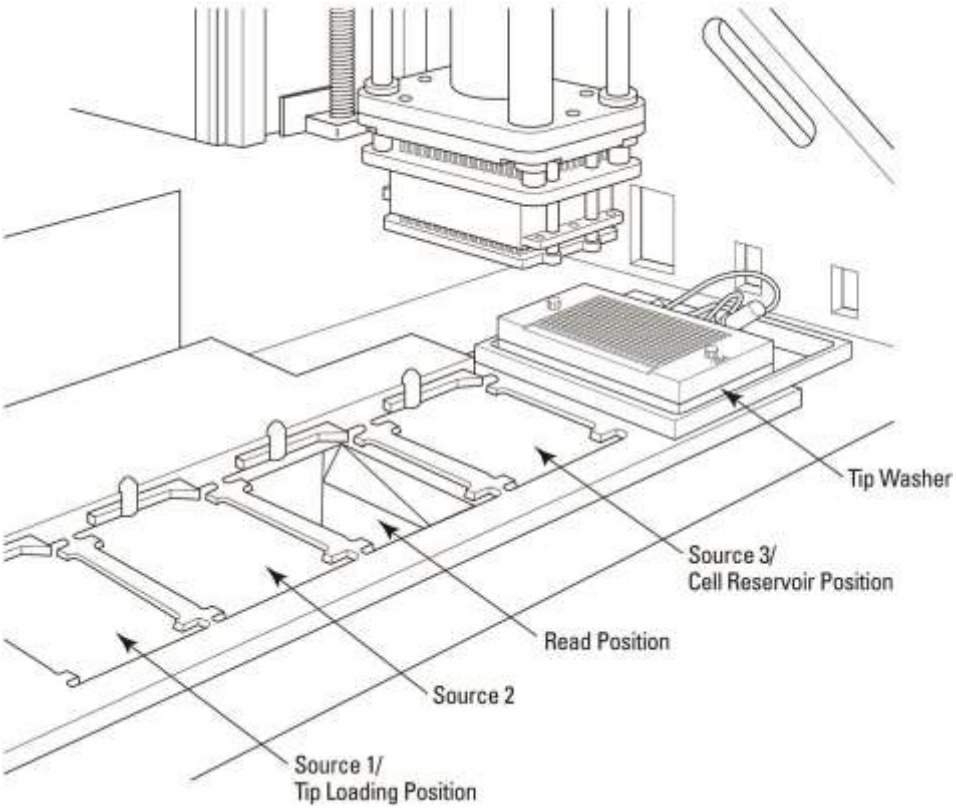
As the illustration indicates, the top instrument compartment contains the pipettor and plate platform (stage). Plates are placed in the read position of the 5-position platform in order to be read by the plate reader, which is located in the optics compartment below the platform.

Plate Layout

The stage has 4 positions where plates and/or tips may be placed. These may be:

- Position 1: Source Plate 1 or Tip-loading
- Position 2: Source Plate 2
- Position 3: Read Plate
- Position 4: Source Plate 3 or Cell Reservoir

A tip wash reservoir for tips or pins is available in position 5.



Users may place either tip racks or plates in the tip-loading position (position 1) and Cell Reservoir or plates in Cell Reservoir position (position 4). The wash reservoir is not disposable but is replaced by users when the head type is changed.

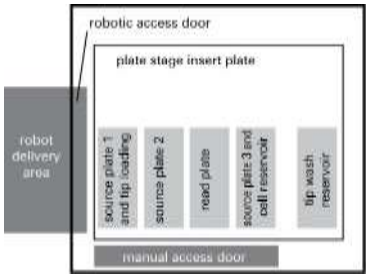
Plate-Handling System

Users may manually place plates or tip racks in the instrument or deliver them automatically using a robot or stacker.

In manual mode, users 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. An observation panel is built into this door and may be removed by users to attach to the top instrument compartment. In this arrangement, users may observe assays in progress (data quality will be poor or simulated) in order to debug assay setup issues.

At the start of an assay, the door latches to prevent users from accidentally interrupting an assay in progress or injuring themselves. The door also latches for most other 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 automatically disengages at the end of the assay.

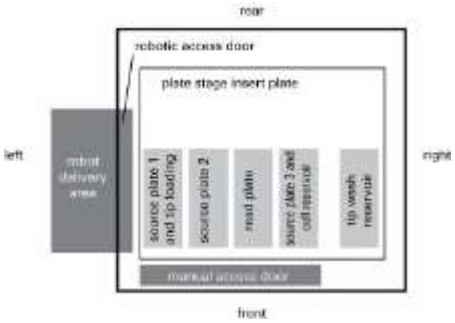
For robotic integration, plates are delivered to a single location outside of the instrument. The placement area for robotic delivery of plates to the instrument is to the left of the instrument. The locations for manual and robotic access to the instrument are shown below:



A further illustration of this arrangement shows the relation of the plate handler to the pipettor system.

Terminology

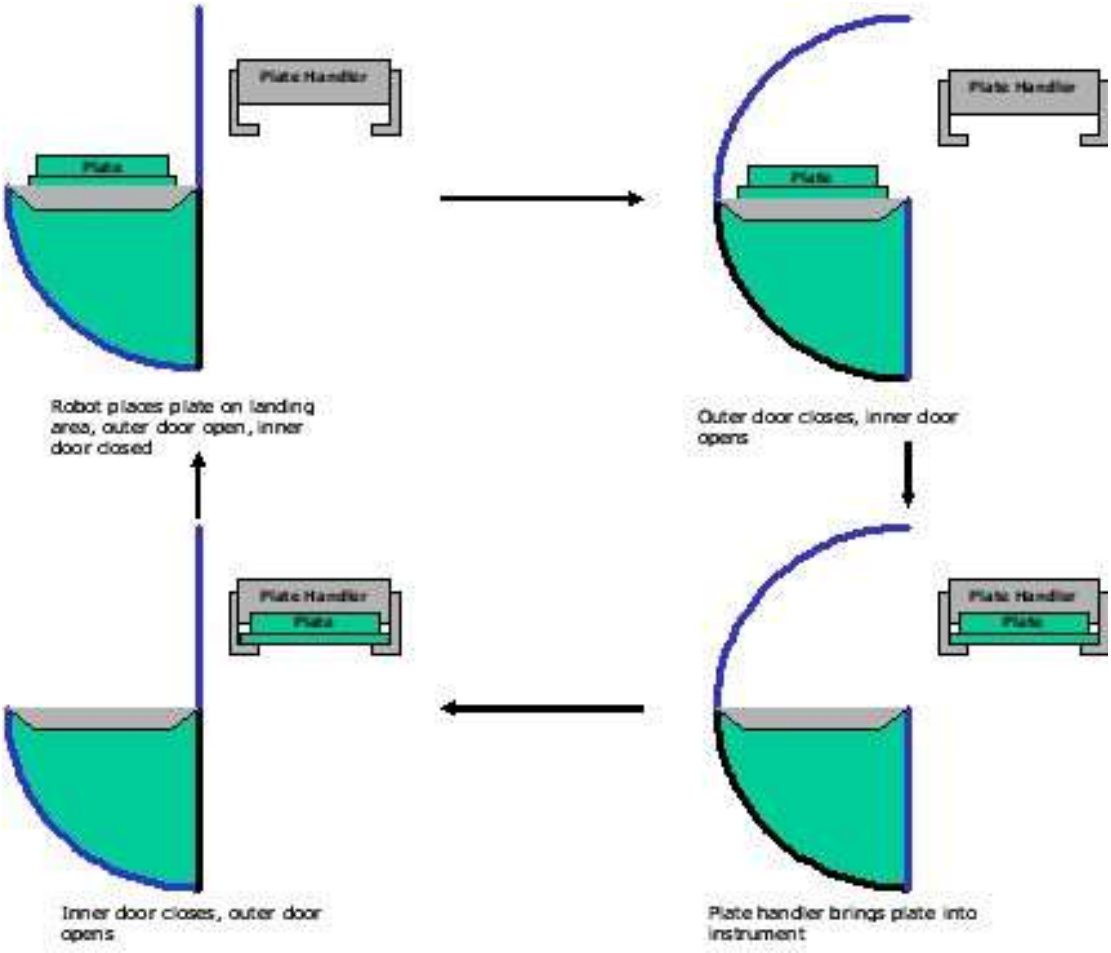
The manual access door side of the instrument is called the front of the instrument. From this, the following illustrates the named sides of the instrument:



Robotic Plate Loading

Plates are shuttled into the instrument by a plate handling subsystem called the FLIPR TetraCycler Plate Handler. To lessen dead time between assays and to handle more source plates in an assay than can be accommodated on the plate stage, the robotic access door(s) and FLIPR TetraCycler Plate Handler are able to exchange plates and tips while an assay is running.

The following diagram illustrates the steps involved in loading 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 is provided to the optics compartment for users to change emission filters and LED modules. This door is also latched when an assay is in progress to prevent users from interrupting assays or injuring themselves.

Washer Placement

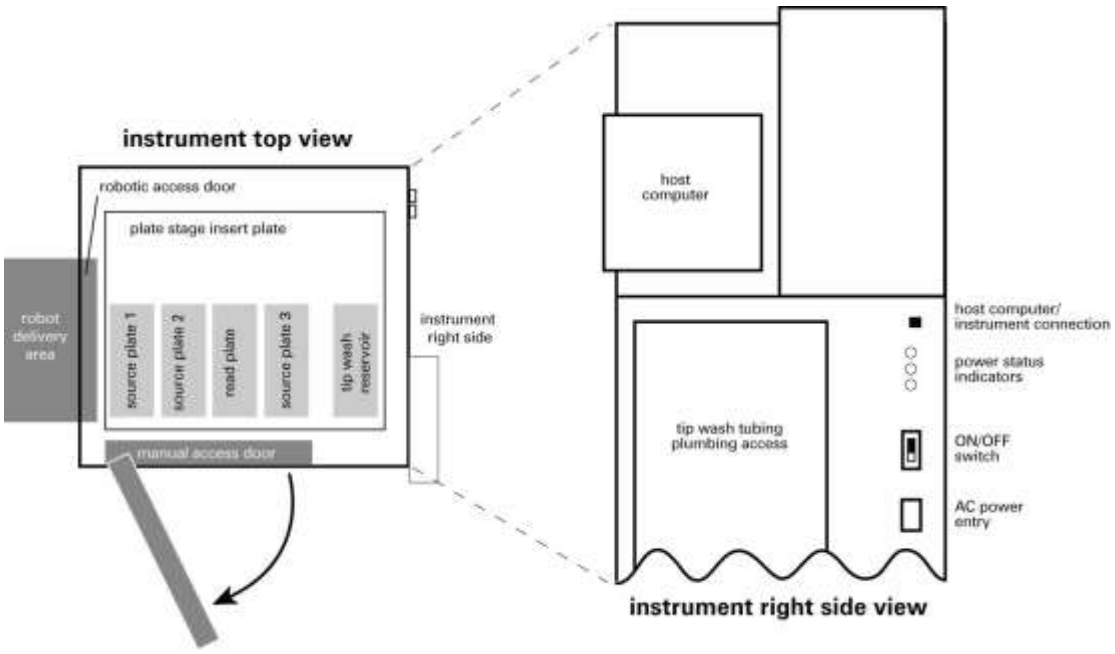
A tip/pin washer is included with the system and is placed on the lower right side of the instrument, towards the front. Extensive work has been done to isolate plumbing for the washer from other optical or power components. Access for tubing to the washer is required in any installation.

Cell Suspension Placement

The Cell Suspension module option is available for the FLIPR Tetra system. When installed, the Cell Suspension module is mounted on the lower right side of the instrument and the Cell Reservoir is inside of the instrument. Access to the module is necessary during testing.

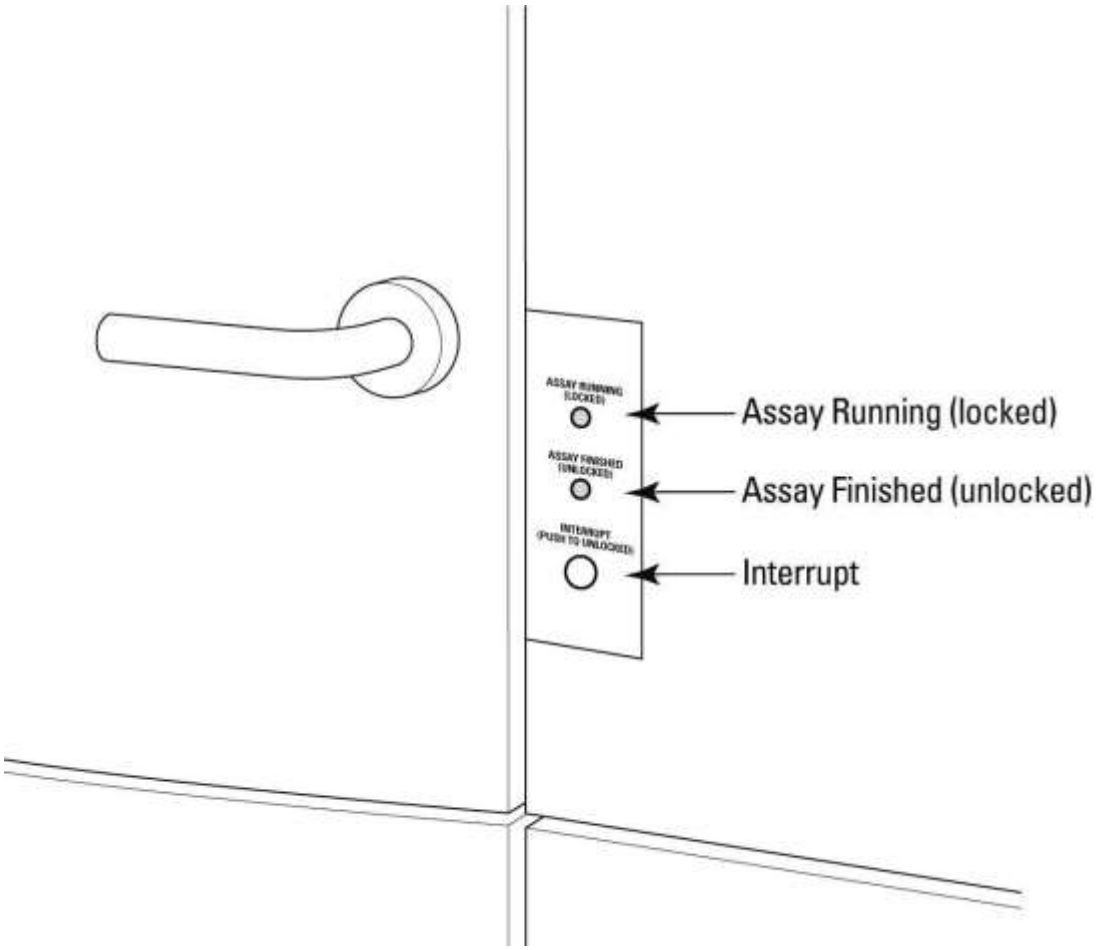
Other Instrument Access Areas

The following diagram illustrates other important access areas in the instrument.



On the right rear of the instrument, AC power enters. Directly above this entry is the main power switch. The power switch should be accessible in both robotic and manual modes. There are three indicator lamps near the power switch. Access to view these indicators can be useful for debugging instrument power problems. The main communication connection from the instrument to the host computer is located immediately above these power indicators.

The instrument status panel, located next to the upper door handle, indicates whether or not the instrument is running and safe to open. It includes an emergency Interrupt button to stop any processes.



The panel has two lights as well as the Interrupt button. From the top of the panel these are:

- Assay Running (Locked)—Yellow light

  The FLIPR Tetra system is performing a task. The upper and lower doors are locked and cannot be opened until the task finishes or is halted using the Interrupt button.

- Assay Finished (Unlocked)—Green light

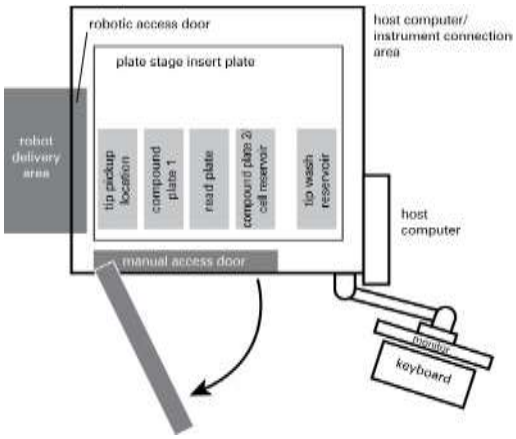
  No tasks are being run and it is safe to open the upper and lower instrument doors.

The Interrupt button is an override button to halt all tasks, so you can access the instrument. If pressed the yellow light flashes until the system has reached 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 will almost always result in the loss of the plate being run. If this button is pressed, the yellow 'busy' light will flash until the instrument is safely unlatched. The system may need to be reinitialized by selecting Reset from the Instrument menu prior to resuming normal instrument function.

Monitor and Keyboard Placement

The host computer provided with FLIPR Tetra system is mounted to the instrument's right side. This computer provides the only direct user interface and path to control the instrument. Monitor and keyboard for this computer are mounted to an adjustable arm on the instrument's front right. The following image illustrates this installation.



The host computer, keyboard, and monitor may be moved to a different location at the user's discretion. Wherever the host computer is placed, 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. A short communication cable is provided between the computer and instrument. In order to move the computer, the user may need to provide an alternate cable. This cable must be a shielded crossover Category 5 cable.

Required Access Areas

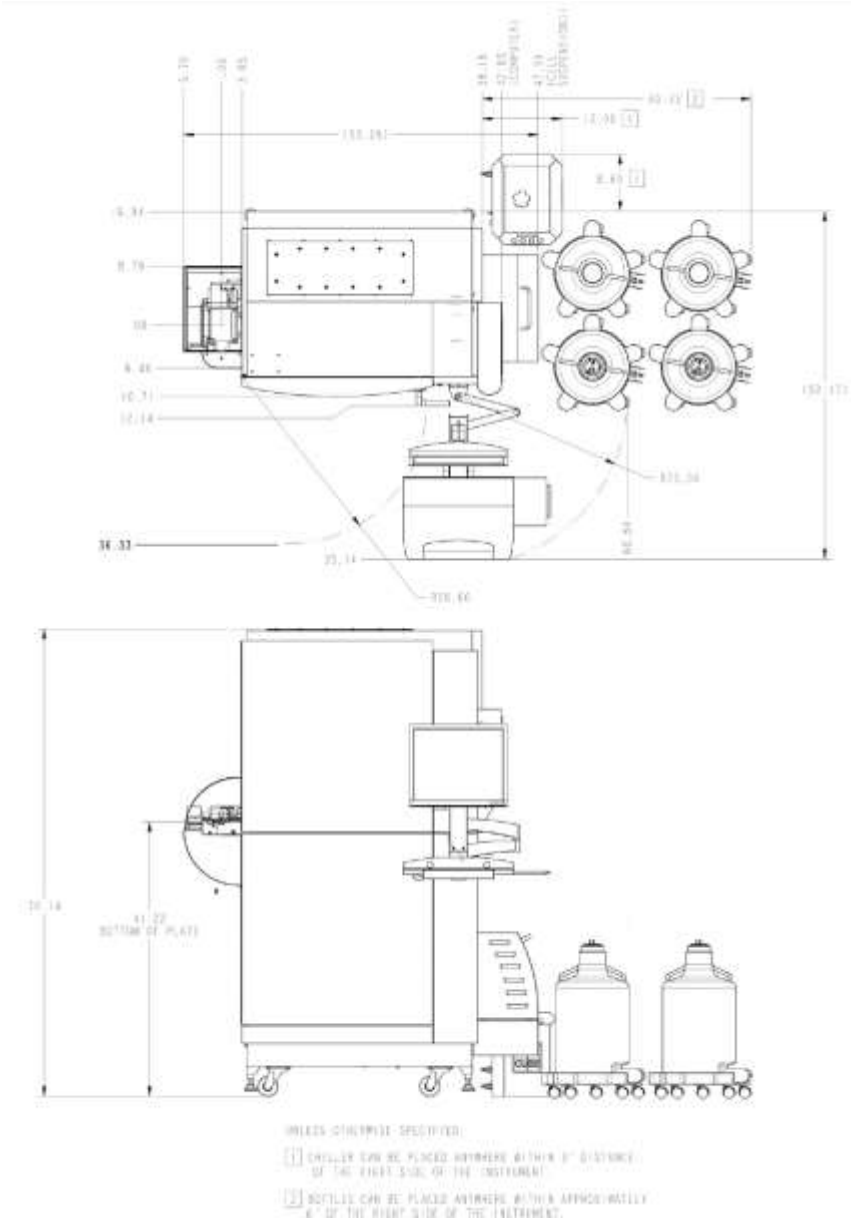
The all inclusive system dimensions (with the FLIPR TetraCycler Plate Handler and Cell Suspension module) are approximately 53 inches (1346 mm) wide by 29 inches (737 mm) deep by 70 inches (1787 mm) tall. The FLIPR Tetra system is designed with rolling castors so it can be readily moved to make necessary adjustments and perform maintenance. Leveling feet are also installed on the lower instrument chassis. These feet are typically used for stabilizing the instrument when integrated with a robot, but can also be used to establish a uniform instrument deck level in situations where the lab floor is not flat. When running an experiment, please make sure the instrument's feet are lowered and leveled.

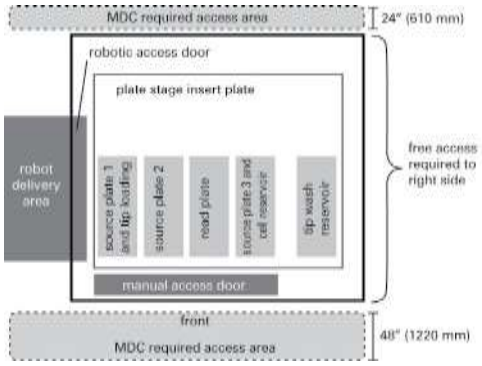
The computer and monitor are mounted to the right front side of the instrument with the included clamp, requiring a minimum lab space of 85 inches (2159 mm) wide by 61 inches (1549 mm) deep for maneuverability. A minimum 28 inch (711 mm) square footprint for tip wash bottles and chiller is required to the right side of the instrument. This space is also recommended to allow access to the main power-switch, washer tubing and communication connections.

The cabinet should have a user access space of 48 inches (1219 mm) in front for users to exchange tips, plates and reservoirs. An additional 24 inches (610 mm) behind and 10 inches (254 mm) to the left should be left available for servicing the instrument.

WARNING! The FLIPR Tetra systemcan weigh as much as 860 lbs (390 kg). Ensure adequate personnel are present when installing or moving the system. Follow all necessary safety precautions and use proper moving techniques.

The following drawings illustrate these requirements:





FLIPR Tetra System Control Architecture

General Description

The FLIPR Tetra system consists of a host computer and a stand-alone instrument.

ScreenWorks Software is installed on the host computer supplied with the instrument. This software is the primary user interface. ScreenWorks Software is used to setup protocols, run assays, analyze and export data. ScreenWorks Software communicates to the standalone instrument with a dedicated connection using a communication protocol proprietary to Molecular Devices. The status of the instrument and assay is monitored by ScreenWorks Software, which then displays data as it is collected.

A stand-alone 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.

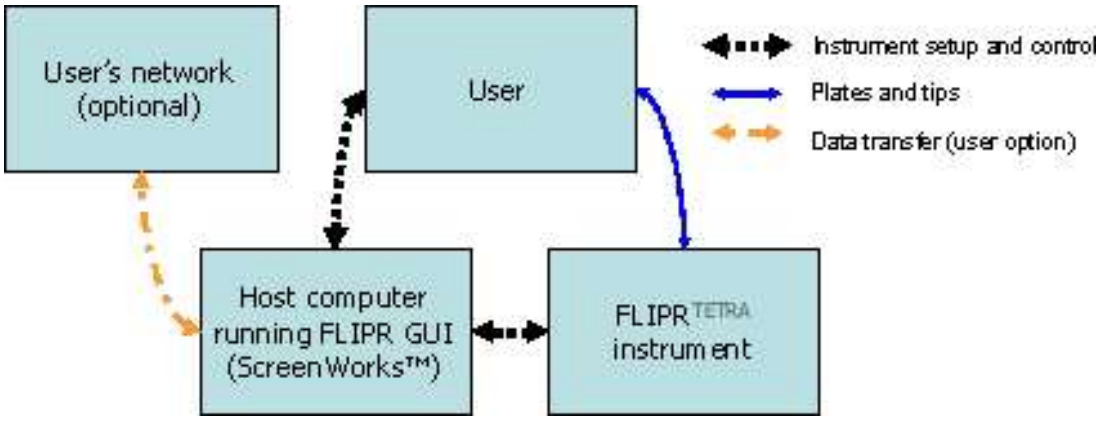
The host computer supplied with the FLIPR Tetra system is configured with two Network Interface Cards (NICs). The first NIC is used to communicate with the instrument using a fixed IP address - this connection cannot be used for any other purpose. The second NIC is provided to connect the host to the Local Area Network (LAN) or to other devices (for example, router, hub, scheduling workstation).

ScreenWorks Software and the instrument have two operating modes. The first of these is Manual mode. The second mode is Remote mode. ScreenWorks Software must be placed in **Remote Mode** in order to be controlled externally by third-party software via the automation interface.

Manual Mode

By default, the instrument starts in Manual mode.

In Manual mode, configure assays in 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 Tetra system host computer for storage or analysis elsewhere.

Remote Mode

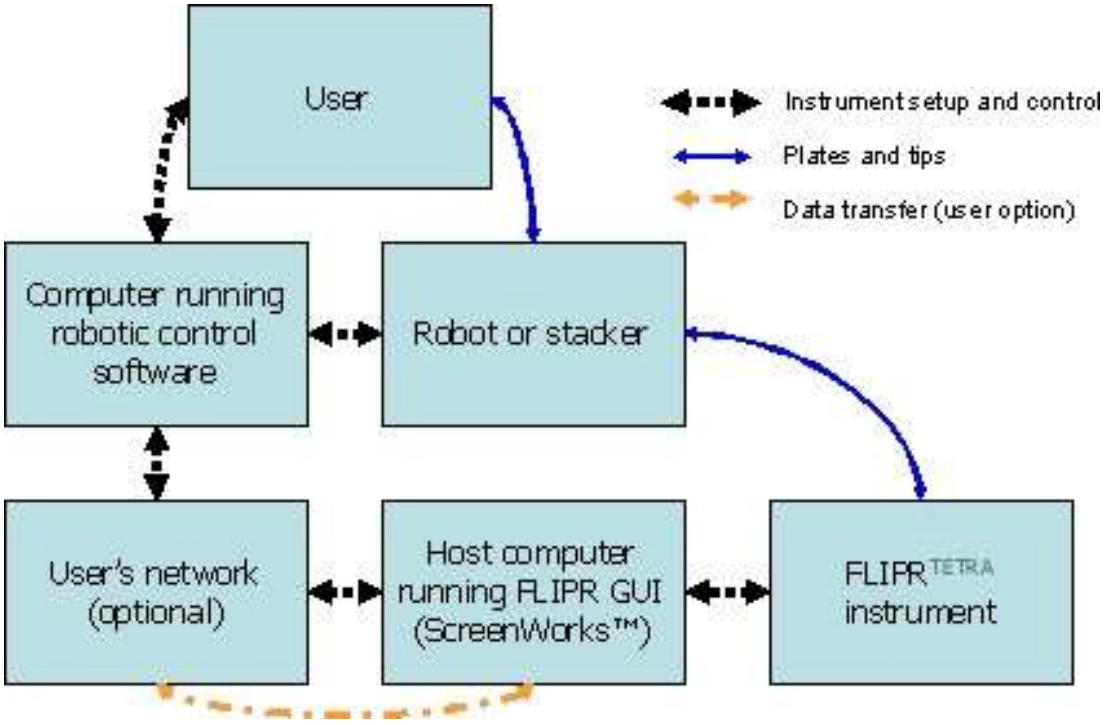
By transitioning into Remote Mode, ScreenWorks Software can be configured to communicate with external devices such as robots and stackers supplied by third parties. These devices can be used to supply plates and tips to the FLIPR Tetra system instrument.

When in remote mode, third-party software is given full control over the internal plate handler, FLIPR TetraCycler Plate Handler. The FLIPR Tetra system software does not initiate any plate handling events. The general philosophy here is to put the third party software in charge and provide status information to allow it to make decisions on when the FLIPR Tetra system should load or unload plates and start assays. Third-party software monitors the assay through the ScreenWorks Software automation interface and provides additional plates and tips to the instrument as needed. External devices might not communicate directly with the FLIPR Tetra system instrument but only with the automation interface.

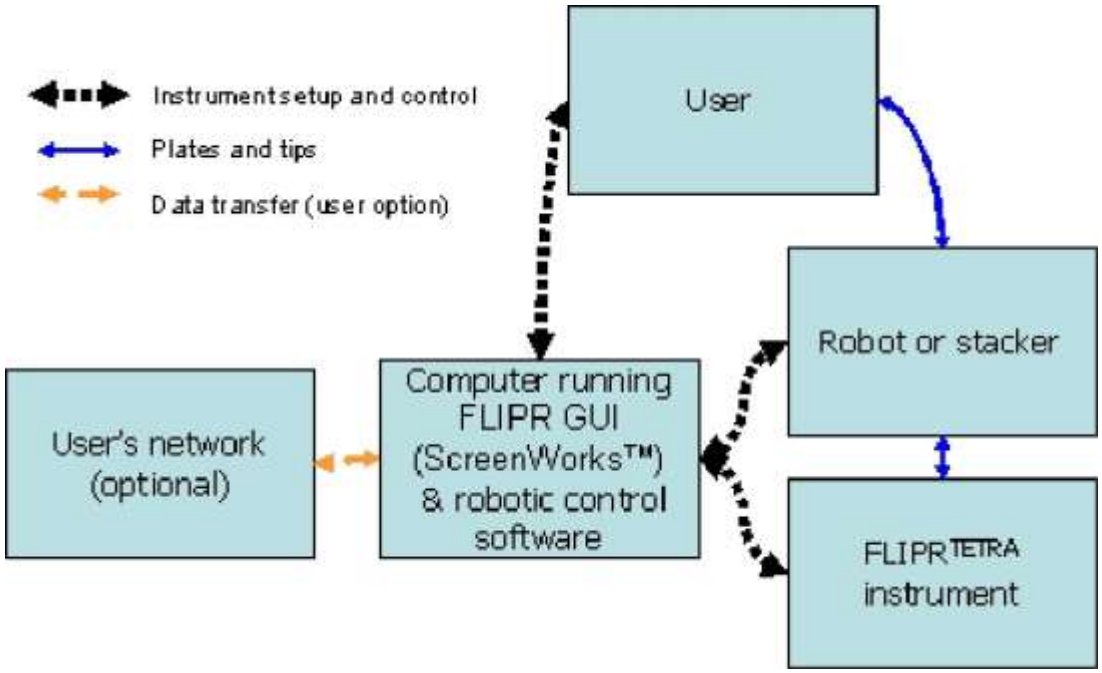
Interact with the third-party software to select a protocol or preload the protocol in ScreenWorks Software before entering remote mode. The automation interface does not provide the functionality to create assay protocols. Interact with ScreenWorks Software while assays are running remotely to QA data and monitor progress.

The control system to communicate between ScreenWorks Software and robotic controller can be configured in several ways. The following illustrations are provided to illustrate the range of options available. These examples are not exhaustive.

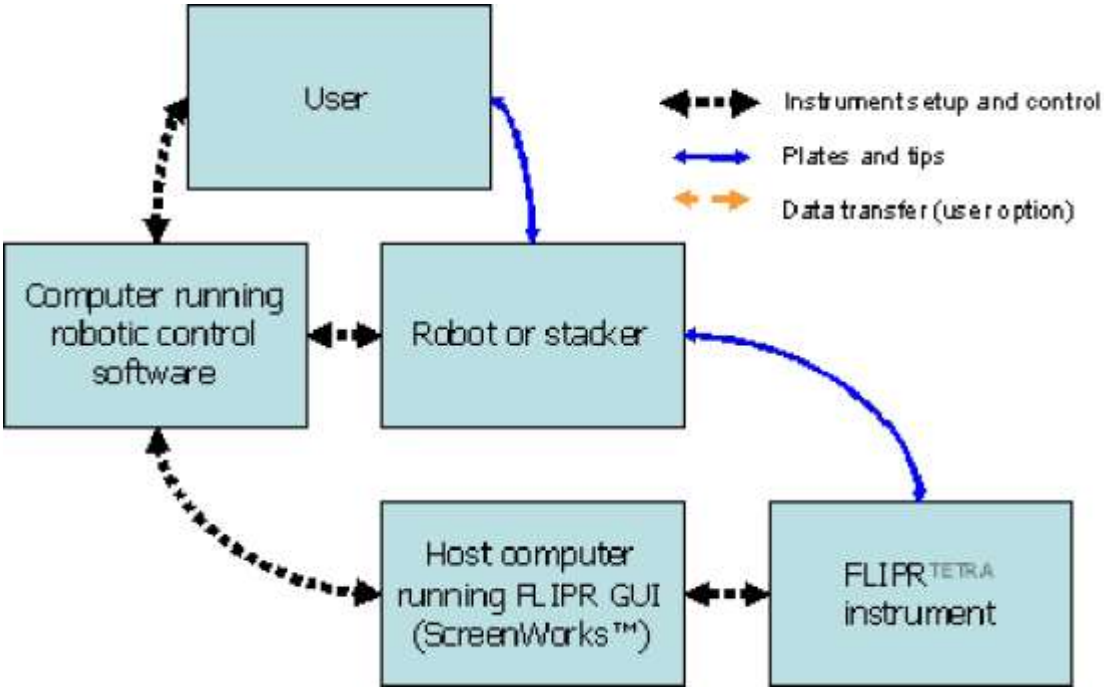
Third-Party Software via Network



Third-Party Software on FLIPR Tetra system Host Computer



Direct Connection From Third-Party Software Host to FLIPR Tetra system host



Communication Protocol and Address

Communication between third-party software and ScreenWorks Software uses the TCP/IP protocol. The third-party software can be resident on another computer outside of the FLIPR Tetra 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, ScreenWorks Software sends and receives commands through TCP/IP port 7.

General Remote Mode Use

This section provides a brief overview of running a FLIPR Tetra system in remote mode. Later sections describe some detailed issues in more detail. This section will provide some context for the introduction of the interface command set in the following section.

Instrument Startup

When the instrument is powered on, it performs a series of initialization steps which may take several minutes. These steps include:

- Configuring the camera
- Initializing the pipettor
- Initializing the plate handler and automatic door (if included)
- Determining and checking the installed LEDs
- Determining the installed emission filters
- Initializing the tip washer
- Communicating with the chiller

By default, the instrument is in manual mode upon startup. In this mode, the main manual access door is unlocked, the outer automated door is closed, and the FLIPR TetraCycler Plate Handler is moved outside the instrument, above the automation landing pad. As long as 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 ScreenWorks Software must be run. ScreenWorks Software establishes communication with the instrument and determine the instrument status and configuration. The instrument status and configuration can be determined by looking at the **Instrument Status** toolbar. This toolbar has two tabs. The **Instrument Status** tab shows the current instrument status including the status of assays in progress. The **Instrument Config** tab shows which LED modules, emission filters, and heads are installed.

Protocol Creation

The user must create assay protocols in manual mode. They do not need to load these protocols prior to placing the instrument into remote mode, but any protocol that is used in remote mode must be created and saved with a valid protocol file name prior to entering remote mode.

Users should test all protocols in manual mode prior to attempting to run them in remote mode.

Instrument Setup

Prior to placing the instrument into remote mode, the user may want to load tips or plates into the instrument. These actions can be done in remote mode but in certain conditions it is advisable to do this through the main manual door prior to entering remote mode.

If tips are used for an extended period of time and the tips/source 1 position is used as a source position, it is best to load the tips manually before placing the instrument into remote mode.

If boats or reservoirs are used as source plates, these should be loaded by hand in manual mode. The FLIPR TetraCycler Plate Handler must not be used to load reservoirs because the plate handler moves rapidly and large wells will spill liquid within the instrument.

Instrument Mode Setting

Prior to running an assay in remote mode, users must place the instrument into remote mode by selecting **Instrument > Set Remote Mode** from the main menu.

After the user requests transition to 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. This is the default position for remote mode. 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 it transitions to remote mode are acceptable for use until a protocol has been run, which indicates that the plate is no longer needed.

Loading Protocols

In remote mode, protocols are loaded using the **openprotocol** command. This command loads the requested protocol and closes any other files open at the time that the command is sent. Protocols open regardless of whether they are valid. This command is described in more detail later in this document.

Running Protocols

After the instrument has been placed in remote mode and a protocol is opened, the protocol may be run by sending the **runprotocol** command. When a protocol begins to run in remote mode, any data files currently open close. This command is described in more detail later in this document.

Instrument Status Updates

The instrument can be queried at any time for instrument status, even when in manual mode. The status command is described in detail later in this document.

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 that can be determined is the plate assigned to the plate position for the protocol to be run. This will be the plate name assigned in ScreenWorks Software for a particular plate type and assigned to that position for the current protocol. This information could be used by the robot to confirm that the correct plate type is delivered to the required position. ScreenWorks Software and the FLIPR Tetra system instrument have no method to determine whether the plate delivered is of the required type and they will always assume that the plate type delivered is correct.

The status response also indicates whether a plate is present in each possible location. This indication 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 in the indicated positions have completed. Plates complete in different ways. If the plates are source plates, ScreenWorks Software indicates that they are finished when an aspiration from the plate is completed and that aspiration is checked to show that the plate is finished after the aspiration. For tip racks, they are marked as complete as soon as tips have been loaded or unloaded to/from them. 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 previously marked as finished.

Bar Code Tracking

Bar codes can be tracked in an assay in two ways.

First, 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 will record the bar code values of the plates delivered in the appropriate data file in which they are used.

Second, the instrument attempts to read a bar code for all 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, all bar code information might not be present when an assay is started, since all required plates might not be present in the instrument. Since the software can be configured to name the data file based on the bar codes of the plates used, it is possible that the data file name will change as an assay is completed.

Setting Up Protocols for Remote Control Use

Protocols must be created in manual mode before entering remote mode.

Protocols should also be tested in manual mode to ensure that they work as expected.

Setting up protocols is described in the user guide and you should be familiar with this process before reading further in this section. See Setting Up an Assay Protocol on page 139. This section describes particular items you should be aware of when setting up protocols to be run in remote mode.

Settings Screen

Read Mode Settings

In the settings screen, configure the read modes. The settings for the read mode are dependent on the assay dye, plate type, cell type, and condition. These settings are determined during assay development and no changes are required when switching into remote mode.

Note: It is not possible to automatically vary these settings based on the particular cell plate loaded.

Assign Plate Settings

Assign plates to the required positions for the assay in the **Assign Plate to Position** group box. The assignment of plates can be critical to the smooth functioning of the assay.

Only one type of plate can be assigned to a position throughout an assay. For example, it is not possible to 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.

The read plate is always assigned to the read position. There are no special suggestions in remote mode for this assignment.

The assignment of source plates and tips should be carefully considered. 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 if necessary to prevent collisions. The pipettor and plate handler are never allowed to be above the same plate position at the same time. Plates should be arranged to minimize potential interactions between the plate handler and the pipettor.

The following guidelines can be helpful in assigning plate positions for automation:

Open reservoirs with very large wells cannot be handled by the plate handler. These plates must be placed in the instrument manually prior to entering manual mode. Replacing these plates will require the user to halt remote control and place the instrument in manual mode to manually replace or refill the reservoir. Deep well plates are a good alternative to using these plates.

If tips are loaded in the experiment, they must be assigned to the Source Plate 1 location. When tips are assigned to this position, the position cannot be used for source plates. Unless the tips are going to be changed for every plate, it is a good idea to load the tips prior to placing the instrument into remote mode. Alternatively, the user could create a tip configuration protocol which load and wash the tips. This assay can be run and then the empty tip rack can be removed by the robot to allow the source 1 position to be used for other plates. As of v1.1 of the automation interface, tip loading and tip washing is available.

Plates that are switched frequently should be placed closer to the left side of the instrument (near the automated landing pad) than plates which are not switched frequently.

Deep well plates (or reservoirs) which can be used for a series of assays before being replaced can be placed in the source 3 location, as long as it is not being used for Cell Reservoir. This minimizes the interaction between the pipettor and the plate handler when switching plates during the majority of assays.

It is only possible to RESET the instrument if a tip rack is present in the instrument. A RESET is required if the instrument enters a fault condition. If automated recovery from fault conditions is desired, the tips/source 1 position should be used for tips only and an empty tip rack should be left in this position at all times.

Data File Name Settings

Configure the desired data file name in the **Data File Name** group box.

If the **Include Bar Code** box is selected, the bar codes for all of the plates which have been checked as requiring them 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. Care should be taken when using this command to ensure that data file names are always unique to prevent the overwriting of data files.

If the ScreenWorks Software is allowed to create the data file name, it will always end in _ ###.fmd, where ### is the plate number with this base name. Using this option ensures that no data is lost due to file overwriting.

Folder Settings

This area is used to assign the directories used in saving the data and export files. There is no difference between settings for these directories in remote control mode and in manual mode.

Running in remote control can produce large amounts of data. ScreenWorks Software will not allow users to begin new assays if less than 50MB of hard disk space is available. Hard disk capacity should be monitored and adequate space to save data should be maintained.

Saving data across a network connection is not recommended in any case. If a protocol is created which saves data across a network connection, you may be unable to run the protocol if the network connection becomes unavailable. If a protocol which saves across the network is running when a network connection is interrupted, data may be lost and the software may encounter problems.

Temperature Control Settings

This area allows the user to set the temperature control set point for the protocol. The instrument must already be at this set point prior to running the assay.

If a protocol requiring temperature control is loaded 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 responds with an error. If the temperature has been reached, the experiment runs.

Auto Print Options Settings

In remote mode, problems with the printer can disrupt the continued operation of the system. No automatic printing is recommended in remote mode.

Analysis Screen

There are no special notes regarding the Analysis screen in remote control. Setup this screen as you would for any manual assay.

It is very important to take care to set up groups, display, and reduction settings prior to running a large number of plates. These settings should be set in the protocol so that they are automatically replicated in each data file as it is created. It will be very tedious to go back to each data file and change these settings manually.

Transfer Fluid Processes

In general, settings for transferring fluids in remote control are the same as in manual mode, with three exceptions. These are in aspiration height over multiple plates, and the **Pause In Well** edit box.

Aspiration Height over Multiple Plates

When running in remote mode, a user might want to use a common addition source plate over a series of plates. The protocol(s) for this can be done in a couple of ways. The simplest way to do this is to create a single protocol which aspirates from the lowest allowed point in the source well. When doing this, you have 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 can be used 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 a user 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 replacing it. The FLIPR Tetra system a instrument 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 than this can spill liquid when the tip is fully inserted. The single protocol begins aspiration at 8 µL height. Then, run this assay 9 times before replacing the plate; leaving 10–20 µL of liquid in the wells after the source plate is removed.

In the more complex implementation, fill the wells to 230–240 µL. Then, set up an assay that would start aspiration at ~240 µL in height, a second assay to start at ~220 µL, a third 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, 11 plates can be run before the plates need to be changed.

The simple method is simpler to setup and run. The more complex method allows source plates to be switched 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 the protocols are created to retrieve liquid from progressively lower heights all the way through the well height.

Pause in Well Edit Box

The **Pause in Well** edit box on the **Edit Dispense** screen allows you to require that the pipettor remain in the well after dispensing. This pipettor operation leaves the tips in the well for the time specified. When the tips are in the well, it might not be possible to exchange plates from any position, and certainly not from the read or source 3 positions. For this reason, use of this option is not recommended in remote mode.

Wash Tips Processes

No special requirements are needed for the **Wash Tips** process.

Note: 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 during protocol creation.

Note: The same warning that applies to **Pause in Well** for dispensing also applies here.

Also, mixing in any well may interfere with plate handling, so use of this function may require some experimentation.

Read Processes

No special requirements are needed for **Read** processes.

Finish with Source Processes

This notifies the plate handler to remove the plates in remote mode.

Wash Cell Reservoir Processes

No special requirements are need for the **Wash Cell Reservoir** process.

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 ScreenWorks Software has one of the following forms:

```
ok<CR>
errorcode, error string<CR>
status<CR>
```

- **ok<CR>** indicates the command was started successfully or command is executed successfully.
- **errorcode, error string<CR>** indicates command failed because of either an indicated condition or an instrument error. Error codes will be followed by a comma, a space, and then a string describing the error of up to 300 characters. Error strings are entirely lower case.
- **status<CR>** is 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 only 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 associated 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 made to the instrument after making a TCP/IP connection to ScreenWorks Software. To ensure problem free operation of your application for FLIPR Tetra 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 has occurred, this field will contain an error code and an error string of less than 300 characters. The error code and error string will be 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 also reports whether the instrument is remote or manual mode. In manual mode, only status information is available through this interface. All other commands will not function. Finally, 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 is finished.
- 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.
- Camera temperature. Status returns “not_ok” while camera CCD temperature is cycling and/or out of range as defined by factory firmware settings, “off” if camera temperature control is disabled, or “ok” otherwise. Typically factory setpoint is -60 °C ± 2 °C (-76 °F ± 35.6 °F) for EMCCD camera or -20 °C ±5 °C (-4 °F ± 41 °F) for ICCD camera. Also returns actual CCD temperature and setpoint.
- 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.
- Waste/fill bottle status.

- Tips on|off.
- Outer auto door, open/closed—if it is closing it is reported 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 specified location from landing pad. The plates or tips must have been placed on the instrument landing pad prior to the command being issued. Up to 13 plates (1 source plate, 12 compound plates) can be used in single experiment.

Additional parameters are available to provide the bar code for the delivered plate and 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 available for use 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 the bar code parameter is used then the plate order parameter must also be used.

Location parameter must be one of following:

- re–which indicates read plate location. s1–
- which indicates source plate 1 location.
- s2–which indicates source plate 2 location.
- s3–which indicates source plate 3 location.
- ti–which indicates tips loading location.

The tips and source 1 location are the same position. Either the “ti” or “s1” parameter may be used to indicate this position.

Bar code parameter must be:

- String up to 20 characters.
- String can have letters, numbers, underscore, and spaces.

The instrument will always attempt to read a bar code while loading a plate. If a bar code is supplied through the loadplate command, any bar code actually found on the actual plate loaded will be ignored.

The last plate parameter indicates whether or not the plate being loaded is the last plate in the current experiment. This parameter must be “yes” or “no”. The last plate parameter is no longer used by the FLIPR Tetra 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**, confirming command started execution or Condition code related to command, that confirming command could not start execution.
4. Monitor plate handler status for completion, and error status for any other kind of error.

Command Specific Error Responses

- Instrument is not functionalCondition code is C00
- Invalid plate locationCondition code is C05
- Plate handler busyCondition code is C10
- Landing pad does not have a plateCondition code is C20
- Plate location already has a plateCondition code is C15

Removeplate<TAB>location <CR>

Command Description

Removes plate or tips from the specified location. If source plate3 location is set for tips in the current experiment, the instrument will assume tips are present in that position

Command Parameters

There is one parameter, location, for this command.

Location parameter must be one of following:

- re–which indicates read plate location. s1–
- which indicates source plate 1 location.
- s2–which indicates source plate 2 location.
- s3–which indicates source plate 3 location.
- ti–which indicates tips loading location.

Note: The tips and source 1 location are the same position. Either the “ti” or “s1” parameter may be used 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 confirming command started execution or Condition Code related to command, that confirming command could not start execution.
3. Monitor plate handler status for completion, and error status for any other kind of error.

Command Specific Error Responses

- Instrument is not functionalCondition code is C00
- Invalid plate locationCondition code is C05
- Plate handler busyCondition code is C10
- Landing pad has a plateCondition code is C21
- Plate location doesn’t have plateCondition code is C16

Openprotocol<TAB>File Name<CR>

Command Description

This command opens the specified protocol. Any documents open before this command is sent will be closed automatically. Only one protocol may be open at the time.

Command Parameters

There is one parameter, file name, for this command.

File name parameter will be the complete file name of the protocol to be opened, 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 confirming command executed or Condition code related to command, that confirming command could not execute.

Command Specific Error Responses

- Experiment is runningCondition code is C25
- Protocol not foundCondition code is C30
- Protocol could not be openCondition code is C35
- Invalid protocol nameCondition code is C36

Findprotocols<TAB>Folder<CR>

Command Description

This command returns all the protocols in the specified folder. The command will return a comma-delimited list containing all of the *.fmp type files saved in the indicated directory. Each of these files will NOT be checked to confirm that the protocols that they contain 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 will be returned. 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 Condition code related to command, that confirming command could not execute.

Command Specific Error Responses

- Folder does not existCondition code is C45
- Invalid folder nameCondition code is C46
- Current document is not valid protocolCondition code is C50
- No protocols found in dirCondition code is C56

Runexperiment<TAB>[Data File Name]<CR>

Command Description

This command will run the currently open protocol, if it is valid for the current instrument configuration.

Running any protocol will create a data file and shift the software focus to this data file for the duration of the experiment. When running in Remote mode, the data file will be closed automatically when experiment is done and focus will automatically return to the protocol file.

Command Parameters

There is one optional parameter, data file name, for this command.

If a data file name parameter is used with this command, the data file created by running the current protocol will be named data file name. The filename will be appended to the **Data** folder path specified in the protocol Settings screen in the ScreenWorks Software. Never specify data file name containing a full path.

If the data file name parameter is not given, the data file created will be saved with auto save name as indicated in the Settings screen in the ScreenWorks Software.

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 notrunning.

3. Open protocol first by sending **openprotocol** command.
4. Send **runexperiment** command.

   Response is **OK**, that confirming command started execution or Condition code related to command, that confirming command could not start execution.
5. Monitor status for completion, and error status for any other kind of error.

Command Specific Error Responses

- Instrument is not functionalCondition code is C00
- Experiment is runningCondition code is C25
- Current document is not valid protocolCondition code is C50
- Invalid data file nameCondition code is C47

Stopexperiment<CR>

Command Description

This command will stop the current action being performed by the instrument.

The **stopexperiment** command will stop the experiment immediately. This can halt the instrument pipettor in the midst of a motion. In this case, the instrument immediately enters a fault state and an instrument reset is required. In addition, the pipettor might 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. Regardless, an instrument reset is recommended following the use of any **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**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
4. Monitor status for completion, and error status for any other kind of error.

Command Specific Error Responses

- Instrument is not functionalCondition code is 00
- No experiment is runningCondition code is 55

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 isoccurred.
3. Send **clearerror** command.
   Response is **OK** confirming 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 when commanded if tips are not already loaded and any plate is in the tips position. The instrument is not capable of confirming that the plate in the tip load position is actually a tip rack. It is the responsibility of the user 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 tiprack in tipload position via the **loadplate** command.
4. Send **loadtips** command.

   Response is **OK**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
5. Monitor status for completion, and error status for any other kind of error.

Command Specific Error Responses

- Can't load tips, tips on pipettorCondition code is C58

Unloadtips<CR>

Command Description

This command unloads the pipette tips from the pipettor head into an empty tip rack placed into the tip load position.

This command will execute when commanded if tips are loaded and any plate is in the tips position. The instrument is not capable of confirming that the plate in the tip load position is actually an empty tip rack. It is the responsibility of the user 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 will likely result in an instrument fault and may 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**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
5. Monitor status for completion, and error status for any other kind of error.

Command Specific Error Responses

- Can't unload tips, tips not on pipettorCondition code is C59

Cyclecameratemp<CR>

Command Description

This operation cycles the camera temperature by setting the camera setpoint to 20 °C (68 °F), waiting for the camera to reach this setpoint, resetting the camera to -45 °C (-49 °F), and then waiting for the camera to return to this temperature.

This command may be useful when transitioning 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**, confirming that command execution has begun, or a Condition code

related to the command indicating that execution could not begin.

3. Monitor status for completion, and error status for any other kind of error.

Tempcontrolonoff<TAB>Temp<CR>

Command Description

This command sets the temperature setting for the instrument chamber. After sending this command, the robotic controller should monitor the instrument temperature to confirm that the instrument reaches the setpoint. Some protocols will 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**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
3. Monitor status until the instrument reports that it has reached the requested temperature.

Command Specific Error Responses

- Temperature Control System is not functioningC60

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 selecting **Instrument> Manual Operation > Wash Tips** from the main menu of the ScreenWorks Software. This selection washes the pipette tips with the parameters provided.

Command Parameters

There are eight required parameters for this command. All eight parameters must be supplied for this command to properly execute. For some of the parameters, acceptable values can vary depending on the head type currently installed 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 be used.	a, b		
Wash Cycles	Number of wash reservoir fill/drain cycles to complete. The requested # of strokes will be repeated for each cycle.	1,2,3,4,5		
Volume/stroke	Volume to be drawn 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. Slow is recommended for use with wash solvents which tend to bubble.	fast, slow		
Strokes	Number of times which the pipettor will fill and empty with the requested volume 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 aspiration 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**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
4. Monitor status until the wash is complete.

Command Specific Error Responses

- Tips not loadedC68

Configuration<CR>

Command Description:

The **configuration** command returns the configure of the instrument. The configuration response is an ASCII text string which includes:

- Information about the instrument type and firmware version.
- Pipettor head's type.
- Tip washer's type.
- Bar code reader installed or not.
- Plate handler installed or not.
- Cell Reservoir 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>
cell_reservoir<SP>installed|not installed<TAB>
camera_installed<SP>emccd|iccd<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>
cell_reservoir<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 details than the status command. The **statusex** response is an ASCII text string which includes all status command's response information plus:

- Tip washer status
- Cell Reservoir 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<T
AB>
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>
cell_reservoir<SP>ok|not_ok|off|busy|fault,stir rate<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>
cell_reservoir<SP>not_ok,0<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 sending 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 a value of zero is used for the rate parameter, the cell flask control will stop stirring. If a valid value for rate is used, the cell flask control will be turned on and stir 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**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
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. Range is …

Washcellreservoir<TAB>Fluid Type, Fill Speed, Drain Destination, Drain Speed, Wash Cycles, Hold Time, Volume Level<CR>

Command Description

This command is the equivalent of selecting **Instrument > Manual Operation > Wash Cell Reservoir** from the main menu of the ScreenWorks Software. This selection washes the Cell Reservoir with the parameters provided.

Command Parameters

There are seven required parameters for this command. All seven parameters must be supplied for this command to properly execute. The following table lists the parameters, their purpose, and the appropriate range for each parameter.

Parameter	Type	Description	Acceptable values or range
Fluid type	string	Indicates the fluid bottle to be used.	Fluid 1, fluid 2, fluid 3, fluid 4, cell flask.
Fill speed	integer	Speed to run the pumps which fill the Cell Reservoir. Range from 1–10 where 1 is very slow, and 10 is very fast.	1–10
Drain destination	string	Indicate the bottle to drain fluid to.	Fluid 1, fluid 2, fluid 3, fluid 4, cell flask, waste a, waste b.

Parameter	Type	Description	Acceptable values or range
Drain speed	integer	Speed to run the pumps which drain the Cell Reservoir. Range from 1–10 where 1 is very slow, and 10 is very fast.	1–10
Wash cycles	integer	Number of wash reservoir fill/drain cycles to complete.	1,2,3,4,5
Hold time	float	Pause time in seconds, leaving the fluid in the reservoir for desired period of time before draining it.	0–300
Volume	string	This indicate the level of fluid to be filled in the Cell Reservoir.	low, high

Example Command Line

```
washcellreservoir<TAB>fluid 2, 6, waste a, 8, 1, 0, high<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 **washcellreservoir** command.
   Response is **OK**, confirming that command execution has begun, or a Condition code related to the command indicating that execution could not begin.
4. Monitor status until the wash is complete.

Command Specific Error Responses

- C77–Parameter is out of range. The range is ...
- C78–The number of parameters for wash Cell Reservoir commands is invalid.
- C79–Invalid volume parameter. It must be 'low' or 'high'.
- C72–Hold time is too small.
- C73–Hold time is too large.

Error Handling and Reporting

Interface Errors

Interface errors are errors that are generated by issuing a command. These errors are detected upon receipt of the command by 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. Specific errors are listed with the command that can generate them in Table A-1.

Table A-1: Interface Errors

Error Code	Description
C01	Instrument is not in Remote Mode. Only ‘status’ command can be sent while instrument is in Manual Mode.
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.S
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.

Table A-1: Interface Errors (continued)

Error Code	Description
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 runtime.
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.
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.
C78	The number of parameters for wash Cell Reservoir commands is invalid.
C79	Invalid volume parameter. It must be 'low' or 'high'.

Table A-2 documents the errors one might encounter when sending the **runexperiment** command. The errors explain how the currently opened protocol does not match instrument configure or instrument is not ready to run current protocol.

Table A-2: runexperiment Command Errors

Error Code	Description
E100	Invalid LED filter combination! Calibrate it or choose valid combination.
E110	Plate does not exist in plate library. Choose existing plate or add it into plate library.
E111	Plate is not present or is not in the proper location.
E112	Door(s) are opened. Close the doors before start your experiment.
E113	Load Tips position not checked! Check Load Tips position for auto load tips or manually load tips before running experiment.
E114	The chamber temperature is not in range.
E115	Experiment wash tips and there is no tip washer.

Table A-2: runexperiment Command Errors (continued)

Error Code	Description
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. Please empty the bottle to continue.
E119	Waste bottle B is full. Please empty the bottle to continue.
E120	Remove plates redundant.
E121	Error relate to the data folder. The path is not valid, or cannot be accessed. Or there are not enough disk space.
E122	Error relate to the export folder. The path is not valid, or cannot be accessed. Or there are not enough disk space.
E123	The read interval is too short. Please check settings in Read views.
E124	Invalid aspirate parameters [in the protocol].
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) reading in one protocol. This is not allowed.
E129	Invalid Read Mode configuration. Camera gain or gate time is out of range. Please check your protocol settings.
E130	The protocol has transfer cells and/or wash Cell Reservoir operations even though the Cell Reservoir is not installed.
E131	Cell Reservoir is not ready.
E132	Cell flask spinning rate could not be set. Please check your instrument status.

Instrument Errors

Instrument errors are errors that are generated by executing a command. These errors are detected during the execution of the command by the instrument. They are reported in the status response in the function line. These errors consist of a three-digit numeric code.

Instrument errors fall into two general categories, recoverable and fatal errors. When fatal errors occur, the instrument will not be able to continue to do any operation. It aborts any pending operations. When recoverable errors occur with user interaction, the instrument can continue.

The **status** command returns `ERRORCODE` or `FATALCODE`.

- Error codes 100–199 are call Molecular Devices Tech support errors.
- Error codes 200–299 are instrument recoverable errors.

- Error codes 300–399 are instrument non-fatal errors.
- Error codes 400–499 are instrument fatal errors.
- Error codes 500–599 are instrument diagnostic errors.

A full list of these errors can be found in Troubleshooting on page 169.

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
- washcellreservoir

Note: Tips location has changed from the **Source 3** location to the **Source 1** location.

Barcode Specifications

About Bar Codes

The FLIPR Tetra system bar code scanner is an OEM module with decode capability to most commonly used bar code symbols in industry worldwide. The list includes:

- 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

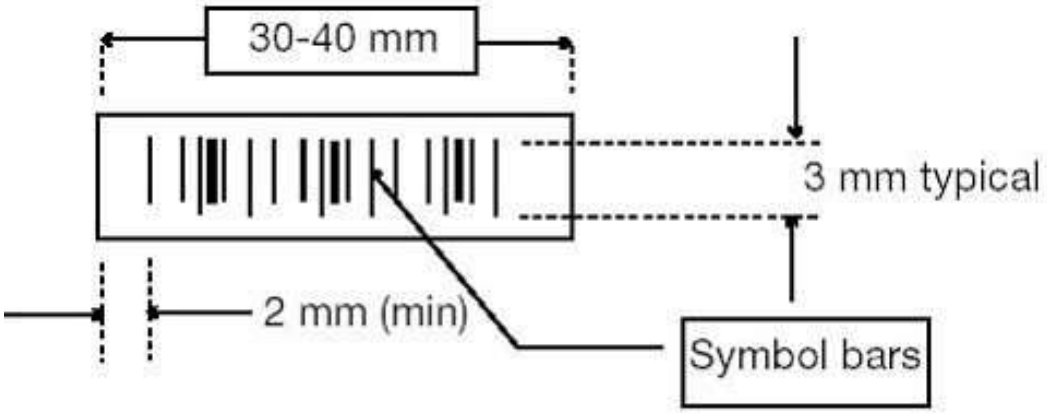
The bar code reader implementation allows a bar code read down to 7.5 mil (0.20 mm) on photographic-quality symbols and ambient light < 450 ft-candles.

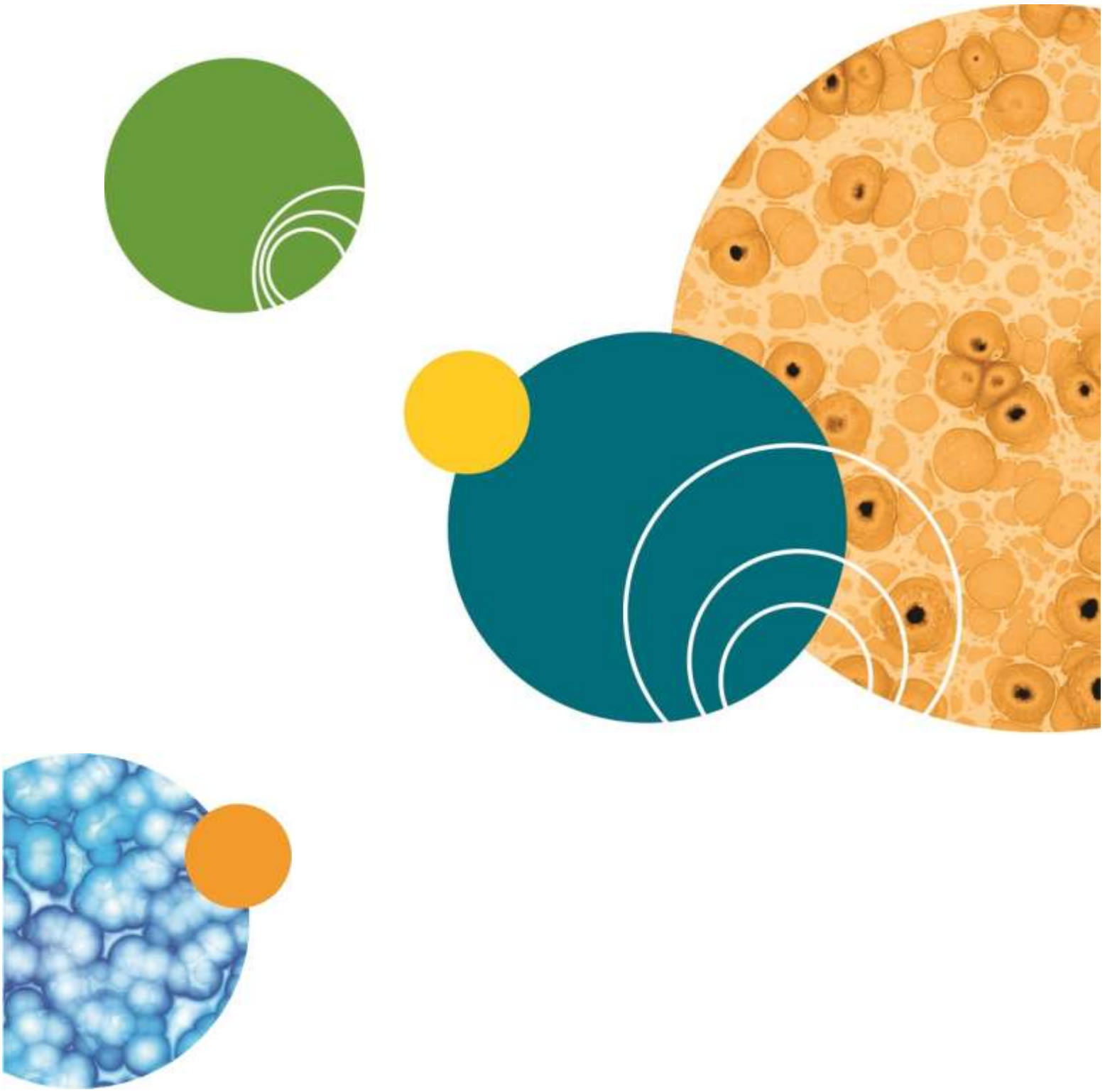
We recommend, however, the user stay above 7.5 mil (0.26 mm) since most bar code printers might not have the resolution needed to match the required aspect ratio for a minimum of 3 mm-high symbol lines. Before adjusting the bar code scanner to the bar code label location on the microplate, some general guidelines to the preparation and quality of the bar code label need to be followed for optimum performance:

- The minimum height or size of the symbol bars depends on the code symbol and the density used. Try to 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 your 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 your 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 using a new color or surface material.

Bar Code Specifications





Appendix B: Data Processing Algorithms

B

This appendix uses results of a hypothetical experiment to describe the algorithms that can be used to adjust data. These options are all applied from the Correction dialog.

Hypothetical Experiment

Consider the results from an experiment consisting 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. This time 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 Tetra system.

The data presented is in the same format as the export data files generated using the FLIPR Tetra system software. In this example, only nine wells are considered.

In Table B-1, the results of the experiment are presented without Spatial Uniformity Correction, Negative Control Correction, Positive Control Scaling, or Subtract Bias Value.

Table B-1: 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

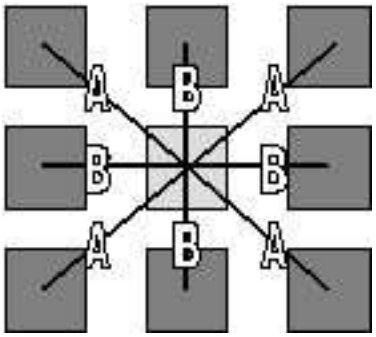
An inspection of the fluorescence counts taken at Sample 1 (time = 0) shows some inter-well variability (range 8000–10000). Variability can be the result of differences in cell density or well positions (so-called 'edge effects' where cells on the outside of the plate are less dense than their internal neighbors), dye loading, non-uniform illumination, buffer variations or any other effect which is constant throughout the experimental run. These variations can complicate data interpretation.

Determining Crosstalk Correction

The crosstalk correction algorithm compensates for light from one well affecting the read out from a neighboring well.

If the middle well in the drawing below was empty and the eight wells surrounding it would have signal, the middle well would show up as having a certain value based on the amount of light crossing over from each of it's neighbors. The percentage of crosstalk from wells directly next to (B) the well of interest is assumed to be different from the percentage of the crosstalk from the wells diagonal (A) from the well of interest, therefore these two values are entered for the crosstalk correction. In this calculation, only the immediate neighboring wells are assumed to impact the middle well.

When the crosstalk correction is applied, the Horizontal/Vertical factor and the Diagonal Factor are applied in a matrix, which automatically subtracts the percentage of each of the neighboring well's value from the middle well's value. If the crosstalk correction option is selected it will be the first correction applied to the data. Crosstalk correction is done in real time and no resulting data will be stored. it is recommended to test this correction during assay optimization.



Determining Spatial Uniformity Correction

The spatial uniformity correction algorithm compensates, to a certain extent, for of the above-mentioned 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:

Table B-2: 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: In actual practice, data from all 96 wells are included in the calculations, but in this example, data from only nine wells are presented, to simplify the example.

Each well-specific spatial uniformity correction factor is calculated by dividing the mean fluorescence counts of all wells by the fluorescence counts of each well (taken at Sample 1.) The table above also presents the correction factor for wells A1–A9.

All samples taken from a particular well are multiplied 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 results of applying the spatial uniformity correction factor are presented in the table below. 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).

Table B-3: 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

Table B-3: Spatial Uniformity Correction factor (continued)

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
5	25	9632	9240	8648	40330	40740	35700	47170	47000	51500
6	30	9856	8925	8930	32700	33950	29580	44500	47940	51500

If the spatial uniformity correction factor is applied to plates with empty wells, non dye-loaded cells, or a panel of cells containing different dyes and/or dye concentrations, the well-specific fluorescence counts are skewed by the correction factor. However, the EC50 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 negative control correction factor is derived 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 mean of Sample 1 is divided by the mean of each of the samples to give the sample-specific correction factor. Each sample is multiplied 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

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
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 also 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 difference in fluorescence counts between Sample 1 and all of the samples is calculated. The greatest difference is determined. 100 is divided by the greatest difference in fluorescence counts to give the positive control correction factor, for example, 100/42296 = 0.0024. All samples are multiplied 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

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
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. The user determines 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 our example, the percent positive fluorescence at Sample 1 is around 20% for all wells. Subtracting the background at Sample 1 would make 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.

Time	Well	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 only 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, to a certain extent, for variations within a single well.

The correction factor is derived by calculating the mean fluorescence counts in each well between the baseline start and end samples.

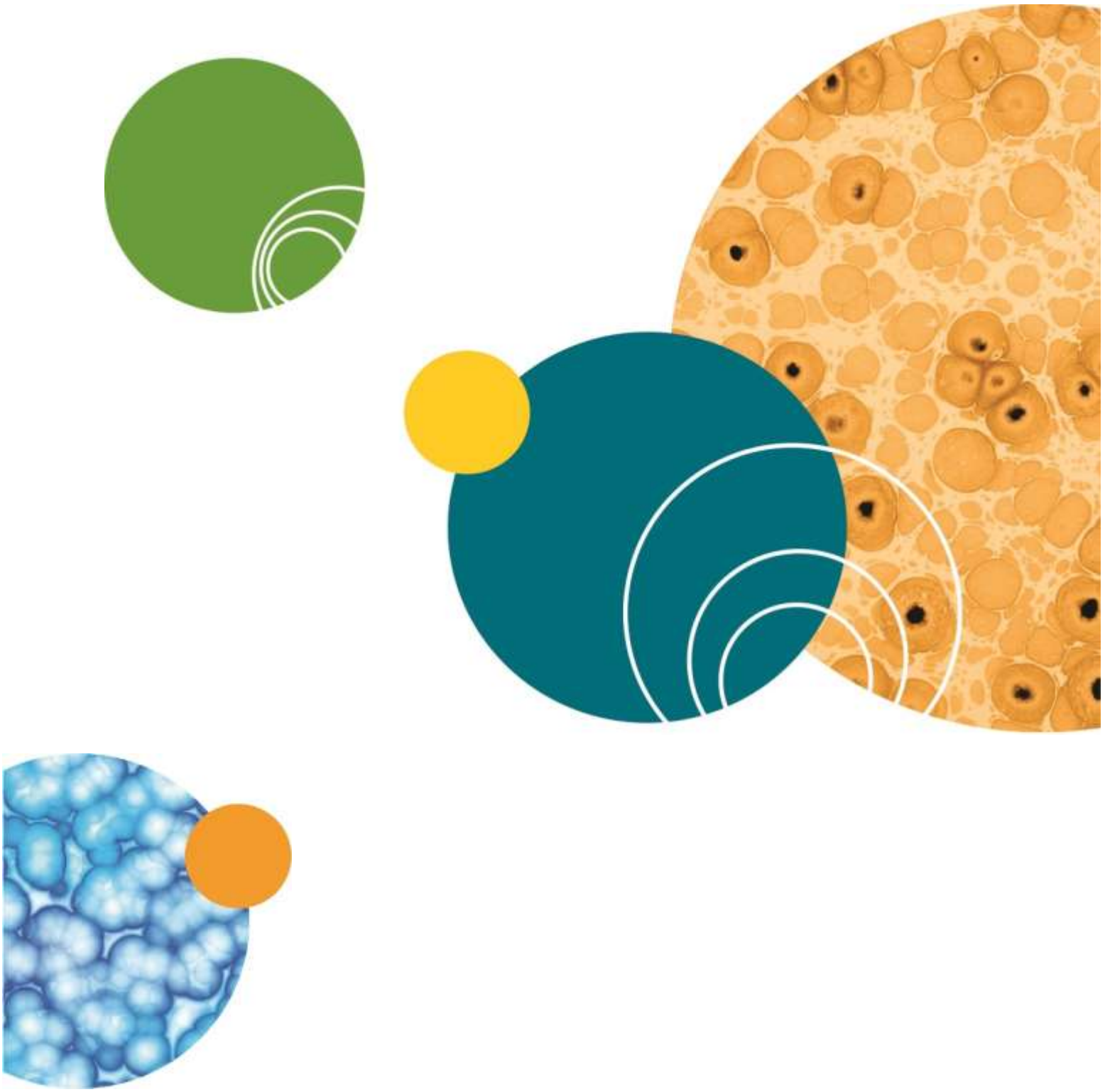
Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exp	A5 Exp	A6 Exp	A7 + Ctrl	A8 + Ctrl	A9 + Ctrl
1	0	8000	8500	9500	8200	9200	8800	10000	9500	8700
2	5	8200	8600	9400	8300	9000	9000	9500	9700	9000
3	10	8400	8500	9200	8500	9000	9100	9700	9700	9400
Mean		8200	8533	9367	8333	9067	8967	9733	9633	9033

Note: In actual practice, data from all 96-wells are included in the calculations, but in this example, data from only nine wells are presented, to simplify the examples.

All samples taken from a particular well are divided 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 results of applying the response over baseline correction factor are presented in the table below. Note the data is displayed is displayed as a fold increase of the response compared to the baseline with Sample values ranging 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 C: Consumables and Accessories

This following catalogs consumables used with the FLIPR Tetra system, including:

System Accessories

Field Installations

Table C-1:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Tetra system Camera Conversion Kit, EMCCD to ICCD	Molecular Devices	+1-800-635-5577	0310-5285
FLIPR® Tetra system Cell Suspension, Field Installation Kit	Molecular Devices	+1-800-635-5577	0310-5339
FLIPR® Tetra system TETRAcycler	Molecular Devices	+1-800-635-5577	Tetra+Cycler

Pipettor Heads

Table C-2:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Tetra system Pipettor Head Kit, 96	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6071
FLIPR® Tetra system Pipettor Head Kit, 384	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6072
FLIPR® Tetra system Pipettor Head Kit, 1536	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6073
[1] FLIPR® Tetra system Pin Tool Head Kit, 384	Molecular Devices	+1-800-635-5577 +1-408-747-1700	384 Pin Tool Head Kit
[1] FLIPR® Tetra system Pin Tool Head Kit, 1536	Molecular Devices	+1-800-635-5577 +1-408-747-1700	1536 Pin Tool Head Kit
1. Contact your local Molecular Devices Sales Representative for details regarding this configure-to- order item.			

Optics Consumables

Table C-3:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Tetra system Calcium Optics Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6206
FLIPR® Tetra system Membrane Potential Optics Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6207
FLIPR® Tetra system Voltage Sensor Probes (VSP) Optics Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6208
FLIPR® Tetra system Fura-2 Optics Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6271
FLIPR® Tetra system LED Module, 335–345 nm (UV)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6272
FLIPR® Tetra system LED Module, 360–380 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6178
FLIPR® Tetra system LED Module, 380–390 nm (UV)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6273
FLIPR® Tetra system LED Module, 390–420 nm (VSP)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6135
FLIPR® Tetra system LED Module, 420–455 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6148
FLIPR® Tetra system LED Module, 470–495 nm (Calcium)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6128
FLIPR® Tetra system LED Module, 495–505 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6175
FLIPR® Tetra system LED Module, 510–545 nm (Membrane Potential)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6127
FLIPR® Tetra system LED Module, 610–626 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6150
FLIPR® Tetra system Emission Filter, 400–460 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6213
FLIPR® Tetra system Emission Filter, 440–480 nm (VSP)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6205
FLIPR® Tetra system Emission Filter, 475–535 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6211

Table C-3: (continued)

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Tetra system Emission Filter, 515–575 nm (Calcium)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6203
FLIPR® Tetra system Emission Filter, 526–586 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6212
FLIPR® Tetra system Emission Filter, 565–625 nm (Membrane Potential and VSP)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6204
FLIPR® Tetra system Emission Filter, 646–706 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6214
FLIPR® Tetra system Custom Filter Set (3)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6221
FLIPR® Tetra system Single Custom Filter Holder (1)	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6276

Pipetting Consumables

Table C-4:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Tetra system pipette tips, black, non-sterile, 96-well, 50 racks/case	Molecular Devices	+1-800-635-5577 +1-408-747-1700	9000-0762
FLIPR® Tetra system pipette tips, clear, non-sterile, 96-well, 50 racks/case	Molecular Devices	+1-800-635-5577 +1-408-747-1700	9000-0761
FLIPR® Tetra system non-sterile, 384-well, 50 racks/case	Molecular Devices	+1-800-635-5577 +1-408-747-1700	9000-0764
FLIPR® Tetra system pipette tips, clear, non-sterile, 384-well, 50 racks/case	Molecular Devices	+1-800-635-5577 +1-408-747-1700	9000-0763
FLIPR® Tetra system 1536 tip gasket, nonsterile, 40 racks/case	Molecular Devices	+1-800-635-5577 +1-408-747-1700	9000-0746
FLIPR® Tetra system 1536 tip block	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6112
[1] FLIPR® Tetra system Pin Tool, 384	Molecular Devices	+1-800-635-5577 +1-408-747-1700	384 Pin Tool

Table C-4: (continued)

Item	Suggested Supplier	Phone Number	Item Number
[1] FLIPR® Tetra system Pin Tool, 1536	Molecular Devices	+1-800-635-5577 +1-408-747-1700	1536 Pin Tool
1. Contact your local Molecular Devices Sales Representative for details regarding this configure-to- order item.			

Cell Reservoir Consumables

Table C-5:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Tetra system Cell Suspension Reservoir	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6222
FLIPR® Tetra system 250 mL Spinner Flask Assembly	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6223
FLIPR® Tetra system 500 mL Spinner Flask Assembly	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6224
FLIPR® Tetra system 1 L Spinner Flask Assembly	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6225
FLIPR® Tetra system 3 L Spinner Flask Assembly	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6226
FLIPR® Tetra system 1 L Spinner Flask Assembly	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6227

Plates

96-Well Read Plates

Table C-6:

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

Table C-6: (continued)

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

96-Well Read Plate Masks

Table C-7:

96-Well Read Plate Masks	Manufacturer	Item #
96-well slit mask	Becton Dickinson	0200-6143
	Corning/Costar	2300-1362
	Nalge/Nunc	0200-6142

96-Well Source Plates

Table C-8:

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

Table C-8: (continued)

96-Well Source Plates	Suggested Supplier	Phone Number	Item Number
Deep-well plate, 96-well	Becton Dickinson	+1-800-343-2035	353966 polypropylene
	Greiner (distributed by E&K)	+1-408-378-2013	780270 polypropylene
	Nalge/Nunc	+1-800-766-7000	278752 polypropylene

384-Well Read Plates

Table C-9:

384-Well Read Plates	Suggested Supplier	Phone Number	Item Number
Black, clear, tissue culture treated, sterile	Becton Dickinson	+1-800-343-2035	353262
	Corning/Costar	+1-800-492-1110	3712
	Greiner with lids (distributed by E&K)	+1-408-378-2013	781091
	Matrical	+1-509-343-6225	MGB101-1-2
	Matrical	+1-509-343-6225	MGB101-1-3 0.17 mm-thick glass
	Nalge/Nunc	+1-800-766-7000	142761
Black, clear bottom, tissue culture treated, sterile, poly-D-lysine coated	Becton Dickinson	+1-800-343-2035	356663
	Corning/Costar		3664
Black, clear, tissue culture treated, sterile, collagen coated	Becton Dickinson	+1-800-343-2035	356667
White, clear, tissue culture treated, sterile	Becton Dickinson	+1-800-343-2035	353963
	Corning/Costar	+1-800-492-1110	3707
	Greiner (distributed by E&K)	+1-408-378-2013	781098
	Nalge/Nunc	+1-800-766-7000	142762
Low volume black, clear, tissue culture treated, sterile	Corning/Costar	+1-800-492-1110	3542
	Greiner (distributed by E&K)	+1-408-378-2013	788092

384-Well Source Plates

Table C-10:

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

1536-Well Read Plates

Table C-11:

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

1536-Well Source Plates

Table C-12:

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

Source Reservoirs

Table C-13:

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

Assays

Calcium Flux Consumables

Table C-14:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Calcium 5 Assay Kit: Bulk Kit Explorer Kit Express Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	 R8186 R8185 R8187
FLIPR® Calcium 4 Assay Kit: Bulk Kit Explorer Kit Express Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	 R8141 R8142 R8143
FLIPR® Calcium 3 Assay Kit: Bulk Kit Explorer Kit Express Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	 R8090 R8091 R8108
FLIPR® Calcium Assay Kit: Bulk Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	 R8033
FLIPR® Tetra system Calcium Optics Kit: FLIPR® Tetra system LED Module, 470-495 nm FLIPR® Tetra system Emission Filter, 515-575 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200- 6206
Hank's Balanced Salt Solution (10X stock)	Gibco	+1-800-828-6686	14065- 056
HEPES buffer solution 1X	Irvine Scientific	+1-800-437-5706	9319
Probenecid, crystalline	Sigma	+1-800-325-3010	P8761
Carbachol (receptormediated positive control)	Sigma	+1-800-325-3010	C4382

Table C-14: (continued)

Item	Suggested Supplier	Phone Number	Item Number
UTP, Na salt (receptormediated positive control)	Sigma	+1-800-325-3010	U6625
Ionomycin (positive control)	CalBiochem	+1-800-854-3417	407950

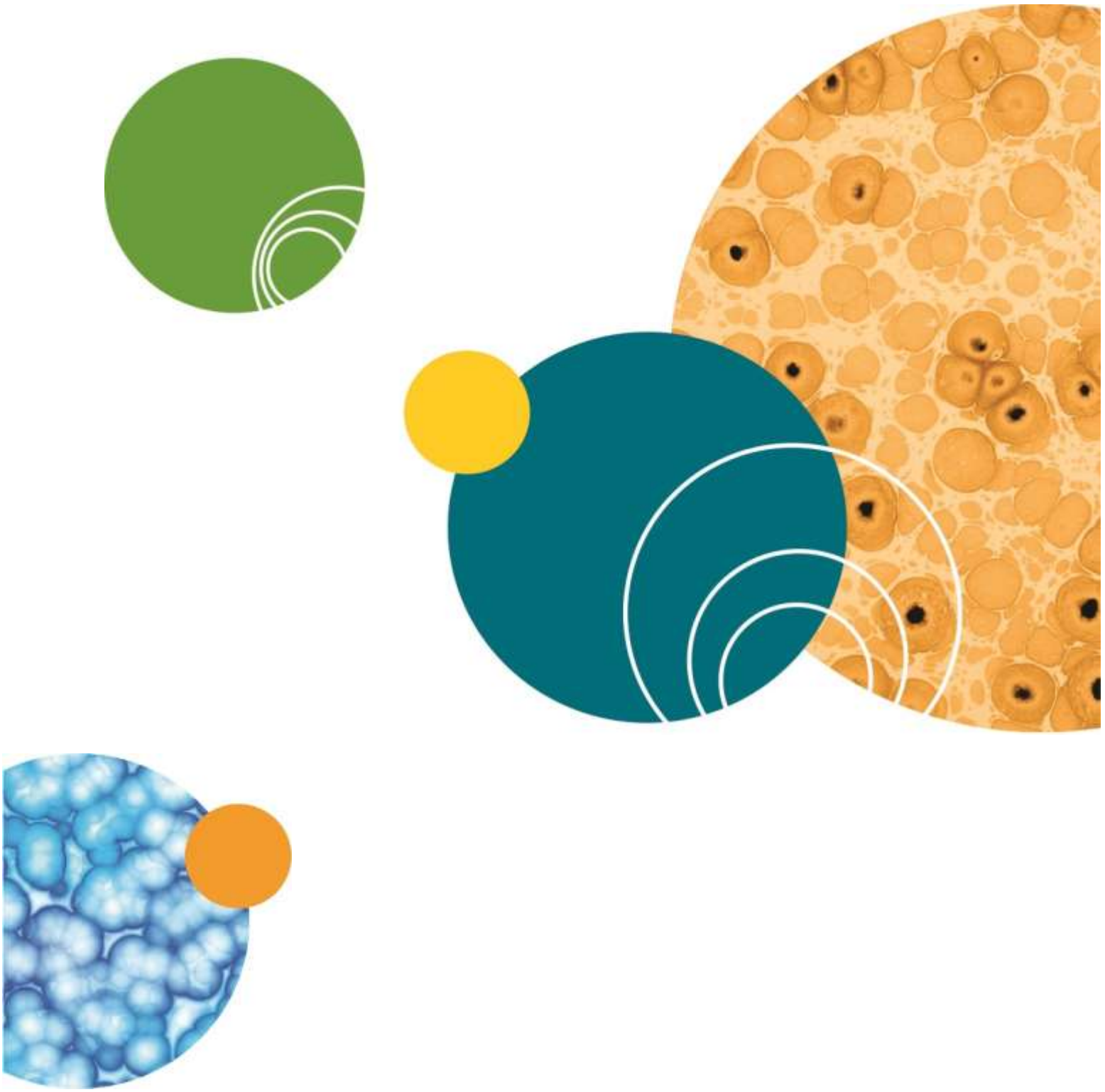
Membrane Potential Assay Kit Consumables

Table C-15:

Item	Suggested Supplier	Phone Number	Item Number
FLIPR® Membrane Potential Assay Kit	Molecular Devices	+1-800-635-5577 +1-408-747-1700	R8034 (BLUE) R8123 (RED)
FLIPR® Tetra system Membrane Potential Kit: FLIPR® Tetra system LED Module, 470–495 nm FLIPR® Tetra system Emission Filter, 515–575 nm	Molecular Devices	+1-800-635-5577 +1-408-747-1700	0200-6207
Hank's Balanced Salt Solution (10X stock)	Gibco	+1-800-828-6686	14065-056
HEPES buffer solution 1X	Irvine Scientific	+1-800-437-5706	9319
Carbachol (receptor-mediated positive control)	Sigma	+1-800-325-3010	C4382

Recommended Assay Equipment and Supplies

- AquaMax® DW4 96/384/1536 Dispenser/Washer, 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 D: Using AquaMax Sterilant

D

AquaMax Sterilant can be used to clean and sterilize the fluid paths in the Cell Reservoir and Cell Suspension module.

Principle of Use

Accumulated residues and contaminating microorganisms have the potential for causing significant adverse effects on the operation of liquid handling equipment. Proteins in cell culture media, salts and minerals in buffers and other reagent components used in liquid handling equipment can build up on fluid path surfaces, clogging channels and probes resulting in incomplete or inaccurate dispensing and aspiration of liquids. In addition, microorganisms such as bacteria, fungi and molds can also accumulate in the fluid path creating similar fouling problems. Simply flushing the fluid path with water or ethanol/water solutions is often ineffective in the removal of residue.

AquaMax Sterilant was developed for the purpose of removing organic and inorganic material, and killing microorganisms, including endospores, in liquid handling instrument fluid paths. In addition, AquaMax simultaneously removes endotoxin from the fluid paths. Endotoxin is a substance produced by Gram negative bacteria that is known to cause a variety of significant cellular reactions in vitro, including cell activation and death. Thus minimizing the amount of endotoxin in fluid paths is necessary in order to avoid detachment and adverse effects on mammalian cells during the course of subsequent experiments. A two-hour treatment with AquaMax will substantially remove endotoxin adhering to interior fluid path surfaces.

Contents

- 4X Concentrated Solution A: detergent in buffered aqueous solution (2 x 1 L bottles)
- Solution B: sodium hypochlorite in aqueous solution (2 x 14 mL bottles) [16 500 mL DW4 washes]

Materials Required but Not Provided

- Deionized water
- 250 mL graduated cylinder
- 1 L beaker, bottle or flask
- 2 mL pipettor

Storage

The unopened kit package can be stored at room temperature (between 10 °C/50 °F and 40 °C/104 °F).

After opening the kit, store 4X Concentrated Solution A and 1X Solution A (prepared by mixing 1 volume of 4X Concentrate Solution A with 3 volumes of deionized water) between 10 °C/50 °F and 40 °C/104 °F.

After opening the kit, store the tightly capped bottle of Solution B between 2 °C/35.6 °F and 8 °C/46.4 °F. Avoid freezing any of the solutions.

Reagent Preparation

500 mL Sterilant is sufficient to run the automated cleaning cycle on the AquaMax DW4 liquid handling system.

1X Solution A can be stored for 30 days in a capped bottle between 10 °C/50 °F and 40 °C/104 °F.

Preparation of 500 mL 1X Solution A

Combine 125 mL of 4X Concentrate Solution A and 375 mL deionized water in a 1 L beaker, bottle or flask and mix thoroughly.

For other needed volumes:

Add 1 part of 4X Concentrate Solution A and 3 parts deionized water.

Preparation of Complete Sterilant:

Within 1 hour of use, add 0.2% (final v/v) Solution B to 1X Solution A and mix thoroughly.

For 500 mL of complete Sterilant:

Use 1 mL of Solution B.

Reagent Use

Follow the recommended cleaning procedures in the user guide for the specific liquid handling instrument to be cleaned.

For stubborn cleaning situations, the Sterilant must sit static in the fluid path for 30 minutes half-way through the cleaning cycle before continuing to completion.

For endotoxin removal, the Sterilant must sit static in the fluid path for 2 hours half-way through the cleaning cycle before continuing to completion.

Rinse the fluid path with at least twice the volume of deionized water immediately after cleaning.

Warnings, Precautions and Limitations

For research use only.

When handling these reagents, gloves and eye protection must be worn.

Do not ingest.

Use in a well ventilated environment.

For more safety information, see the Material Safety Data Sheet for Solutions A and B.

As supplied, Solution A might not be sterile.

The following information is being provided in compliance with Worker and Community Right-To-Know Regulations:

Solution A, 4X Concentrate

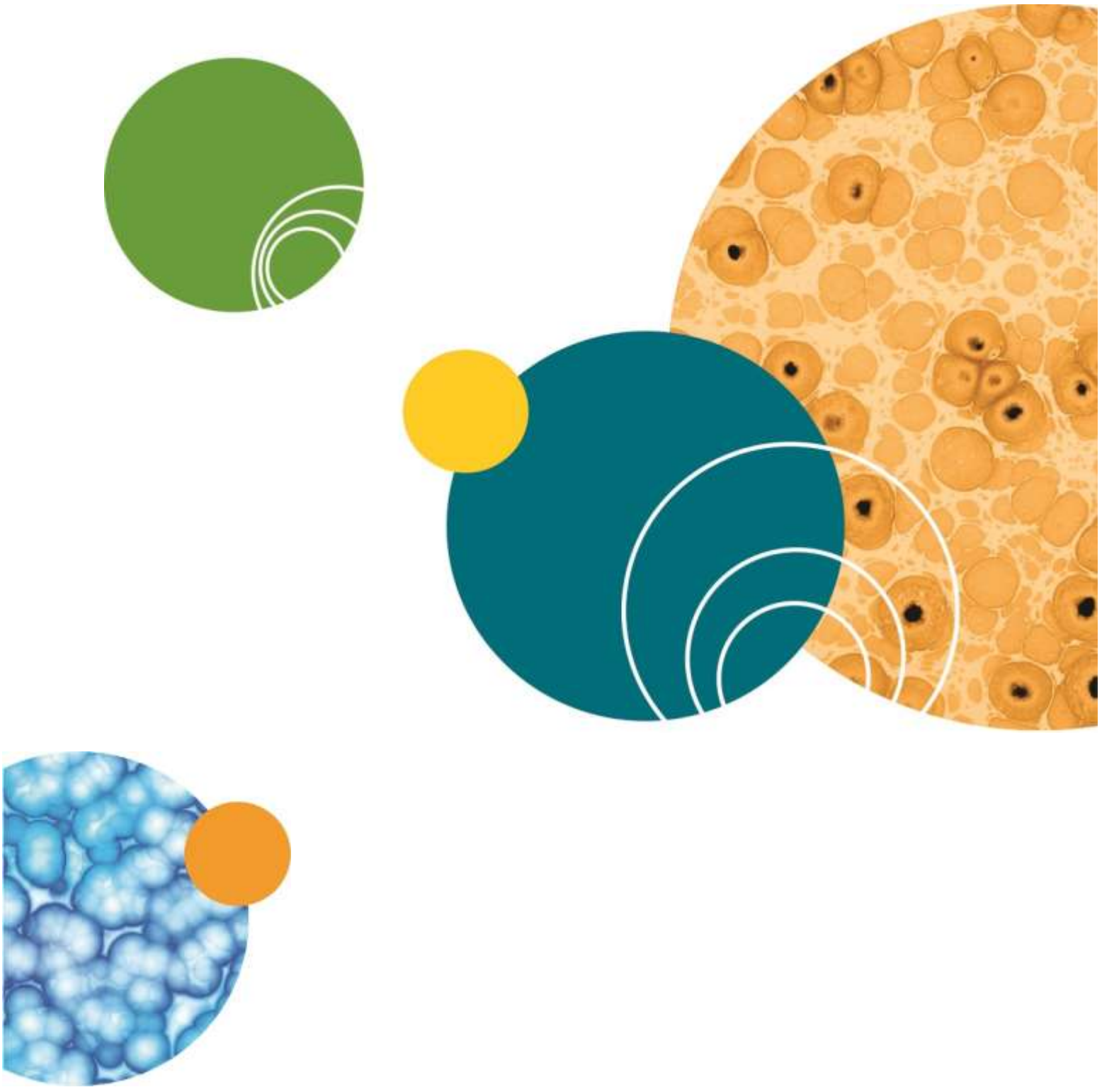
Chemical Composition

The chemical composition of this solution is a trade secret and proprietary property of Molecular Devices.

Solution B

Chemical Composition, CAS #

Sodium Hypochlorite, Solution, 7681-52-9



Appendix E: Electromagnetic Compatibility

E

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

This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

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

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.

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